**UNIVERSIDAD DE VALENCIA**

**FACULTAD DE CIENCIAS QUÍMICAS**

**DEPARTAMENTO DE QUÍMICA ORGÁNICA**



# SÍNTESIS DE TERPENOS CON ESQUELETO DE ESPONGIANO, ESCOPADULANO Y ESTRANO

TESIS DOCTORAL

Presentada por

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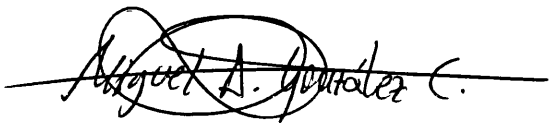

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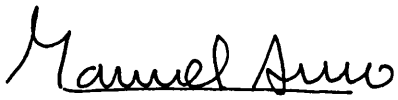

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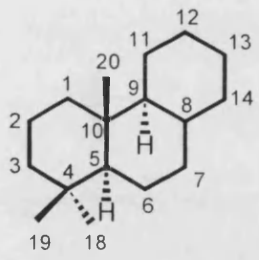


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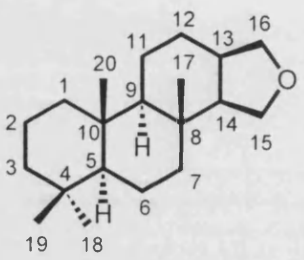


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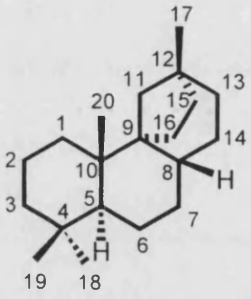


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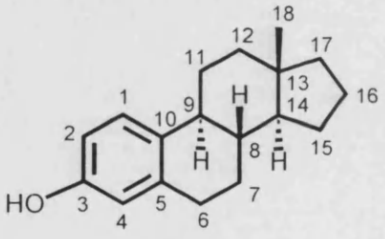


*estrano*

# *1. INTRODUCCIÓN Y OBJETIVOS GENERALES*

# 1.- INTRODUCCIÓN Y OBJETIVOS GENERALES.

La búsqueda de agentes terapéuticos dirigida por la química, y cada vez más por la farmacología y las ciencias clínicas ha participado más en el progreso de la medicina en el último siglo que cualquier otro sector científico. En un siglo y medio, el esfuerzo inicial de químicos y posteriormente fruto del diálogo entre químicos y biólogos ha contribuido a que el hombre durante el último siglo haya luchado con éxito contra numerosas enfermedades.[1]

Desde antiguo, se han aprovechado los recursos naturales para curar diferentes dolencias, y su poder siempre ha atraído la atención de químicos y biólogos a lo largo de la historia, interesándose éstos en la determinación de los principios activos responsables de una determinada actividad terapéutica. Por ejemplo, el estudio de componentes activos en plantas medicinales ha proporcionado que muchos de los tratamientos actuales contra diversas enfermedades, contengan sustancias activas aisladas principalmente de unas cuarenta especies diferentes de plantas superiores. A menudo el descubrimiento de fármacos implica el estudio de la afinidad de diversas moléculas por una proteína dada o la influencia de estas sustancias en los procesos biológicos de organismos o células.

En los últimos años, los nuevos métodos para sintetizar estereoselectivamente las moléculas orgánicas han aumentado la eficacia para preparar estas moléculas. A ello ha contribuido el desarrollo de reactivos específicos, el perfeccionamiento de las técnicas de separación analítica así como la aplicación de un método sistemático surgido en los años sesenta llamado análisis retrosintético.

Todos estos avances se han ido plasmando en un mayor control quimio-, regio-, estereo- y finalmente enantioselectivo de las reacciones orgánicas. Particularmente importante ha sido este último aspecto, el desarrollo de procesos sintéticos que conduzcan a compuestos enantioméricamente puros, debido fundamentalmente a que la mayoría de los productos naturales pertenecen a una serie enantiomérica y que la actividad biológica de

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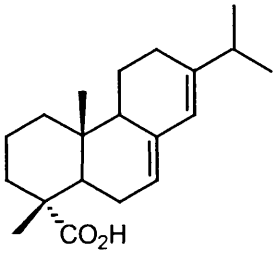
[1] Drews, J *Science*, **2000**, *287*, 1960.

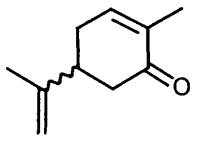
muchos de ellos está relacionada con su estereoquímica absoluta, no siendo infrecuente que enantiómeros de una misma molécula muestren actividad antagónica.

En la síntesis de productos naturales quirales pueden utilizarse dos tipos de estrategias: a) la primera consiste en la síntesis total a partir de productos sencillos, comerciales y habitualmente ópticamente inactivos. Por tanto, para llegar a productos quirales hay que recurrir al empleo de transformaciones enzimáticas, reactivos o catalizadores quirales o resolución de enantiómeros en alguna de las etapas de la secuencia; b) la segunda consiste en utilizar productos naturales quirales como sustancias de partida. El número de productos naturales que han sido utilizados como materiales de partida quirales en síntesis es grande, pero destacan especialmente, debido a su fácil disponibilidad y su bajo costo, dos grupos de productos: los carbohidratos y los terpenos. Muchos terpenos son baratos, disponibles comercialmente y poseen uno o más centros quirales que se aprovechan para inducir quiralidad durante la síntesis y una modesta funcionalización que facilita la elaboración de sistemas más complejos.

Nuestro grupo de investigación lleva varios años dedicado a la síntesis estereoselectiva de terpenos naturales que posean algún tipo de actividad biológica e interés estructural, a partir de productos naturales ópticamente activos. Los productos de partida que se han empleado son terpenos fácilmente obtenibles de la naturaleza en grandes cantidades y enantioméricamente puros, como son los ácidos resínicos del pino (ácido abiético) o la carvona. Los primeros proporcionan una estructura cíclica ya construida que puede incorporarse al esqueleto sintetizado o degradarse para adaptarla a la funcionalización de las moléculas objetivo. La carvona también permite el acceso a estructuras más complejas incorporándose a la estructura final.

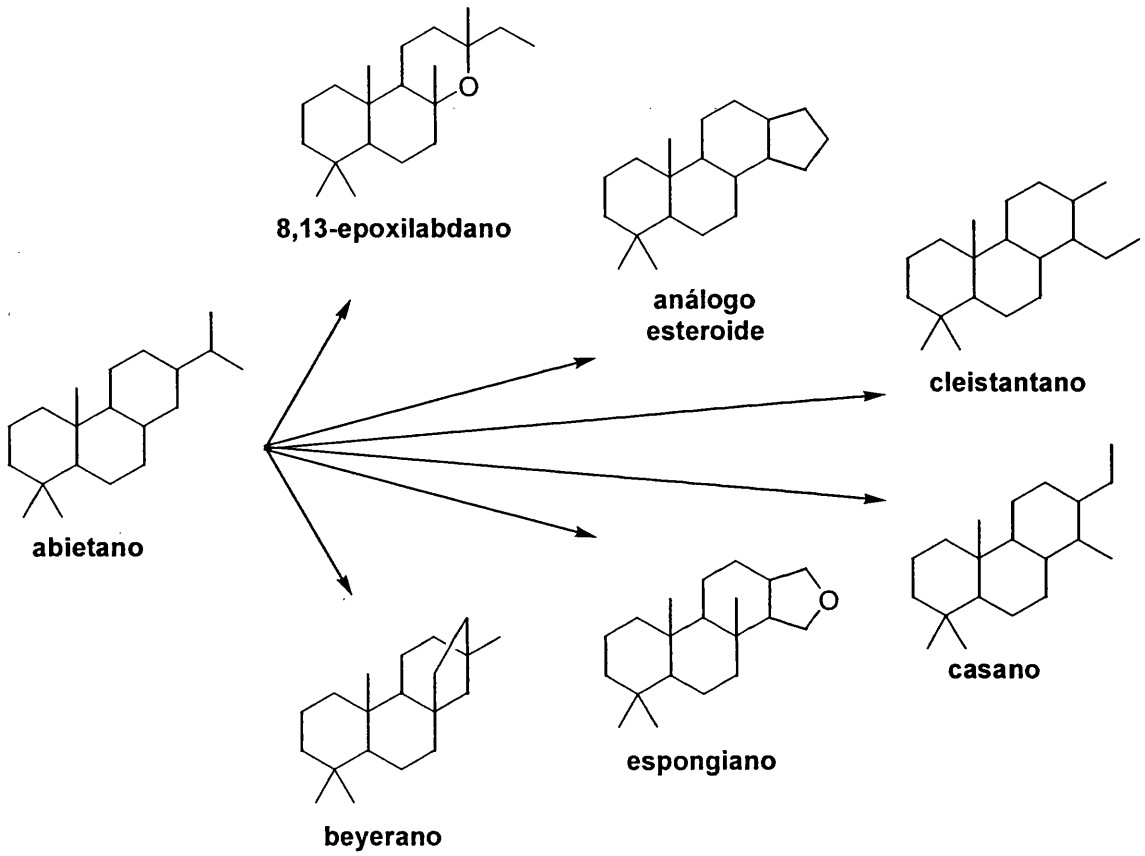



ácido abiético




carvona

En este sentido, en nuestro laboratorio se ha conseguido la transformación del esqueleto de abietano, presente en el ácido abiético comercial y en otros ácidos resínicos obtenibles de la colofonia, en esqueletos diterpénicos de 8,13-epoxilabdano,[2] casano,[3] cleistantano,[4] espongiano,[5] beyerano,[6] y análogos de esteroide[7] entre otros.




[2] a) Abad, A.; Agulló, C.; Arnó, M.; Cuñat, A. C.; Zaragozá, R. J. *J. Org. Chem.* **1992**, *57*, 50. b) Abad, A.; Agulló, C.; Arnó, M.; Cuñat, A. C.; Meseguer, B.; Zaragozá, R. J. *J. Nat. Prod.* **1993**, *56*, 2133.

[3] Abad, A.; Agulló, C.; Arnó, M.; Domingo, L. R.; Zaragozá, R. J. *J. Chem. Soc. Perkin Trans. I,* **1989**, 1875.

[4] Abad, A.; Arnó, M.; Peiró, M.; Zaragozá, R. J. *Tetrahedron* **1991**, *47*, 3829.

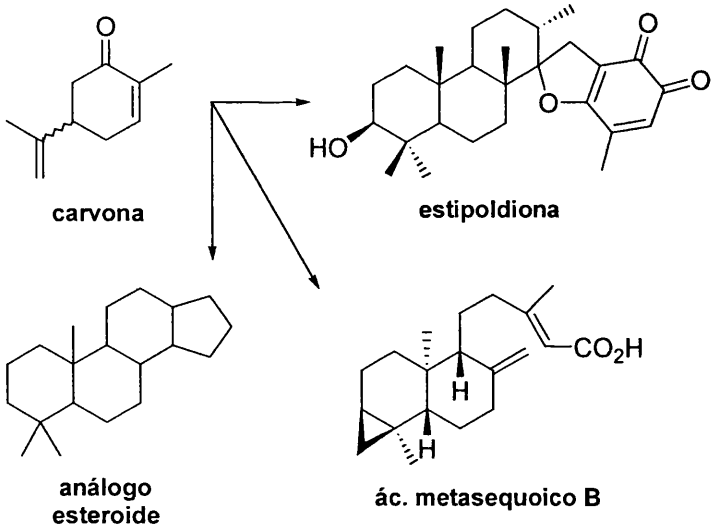
[5] Abad, A.; Agulló, C.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *J. Chem. Soc. Perkin Trans. I,* **1996**, 2193.

[6] Abad, A.; Agulló, C.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *J. Chem. Soc. Perkin Trans. I,* **1994**, 2987.

[7] a) Abad, A.; Agulló, C.; Arnó, M.; Domingo, L. R.; Zaragozá, R. J. *J. Org. Chem.* **1990**, *55*, 2369. b) Abad, A.; Agulló, C.; Arnó, M.; Domingo, R. L.; Rozalen, J.; Zaragozá, R. J. *Can. J. Chem.* **1991**, *69*, 379.

Por otra parte, los denominados monoterpenos, constituidos formalmente por dos unidades de isopreno también han sido muy usados como material de partida, encontrándose en la literatura abundantes ejemplos de síntesis de moléculas de estructuras compleja que se inician en monoterpenos tales como: limoneno, alcanfor, pulegona, citral, carvona, etc.

De esta forma, nuestro equipo de investigación ha utilizado en los últimos años uno de estos monoterpenos, la carvona (en cualquiera de sus dos enantiómeros), resultando ser un excelente punto de partida para realizar la síntesis de sistemas policíclicos funcionalizados, constituyendo el sintón de uno de los anillos. La carvona es un producto comercial, de bajo coste, disponible en ambas formas enantioméricas con elevado grado de pureza óptica, lo que permite la síntesis de productos naturales de cualquiera de las series enantioméricas, permitiendo de este modo confirmar, también, la configuración absoluta de los productos naturales obtenidos. A partir de la carvona ya hemos realizado la síntesis de Estipoldiona,[8] que contiene una unidad spiro-*o*-benzofuránica, la síntesis del ácido metasequoico B,[9] que posee una unidad ciclopropánica en el anillo A y presenta actividad fungicida frente a *Pyricularia oryzae*, además de análogos de esteroide.[10]




[8] Abad, A.; Agulló, C.; Arnó, M.; Meseguer, B.; Zaragozá, R. J. *J. Org. Chem.*, **1998**, 63, 5100.

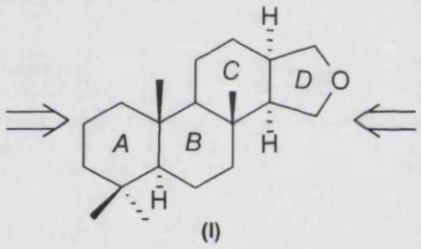
[9] Abad, A.; Agulló, C.; Arnó, M.; Cantín, A.; Cuñat, A. C.; Meseguer, B.; Zaragozá, R. J. *J. Chem. Soc. Perkin Trans. I* **1997**, 1837.

[10] Abad, A.; Agulló, C.; Arnó, M.; Cuñat, A. C.; Meseguer, B.; Zaragozá, R. J. *Synlett* **1994**, 733.

En los últimos veinte años, se ha incrementado el número de compuestos aislados de fuentes marinas confirmándose que los organismos marinos son una rica fuente de metabolitos secundarios, y muchos de éstos presentan estructuras poco comunes, además de interesantes propiedades biológicas.[11]

- Dentro de nuestra línea de investigación, el primer objetivo que nos planteamos en esta Tesis fue la síntesis quiral de *espongianos*. Estos constituyen una familia de diterpenos naturales de origen marino con un esqueleto (**I**) caracterizado por poseer un sistema tetracíclico ABCD. El anillo D es de tipo tetrahidrofuránico y como ya veremos en un próximo capítulo puede encontrarse funcionalizado como anillo lactónico, furánico o hemiacetálico. También existen compuestos en los que el anillo A es de tipo lactónico, así como compuestos pentacíclicos caracterizados por un quinto anillo de tipo hemiacetálico.

Figura **1**




(I)

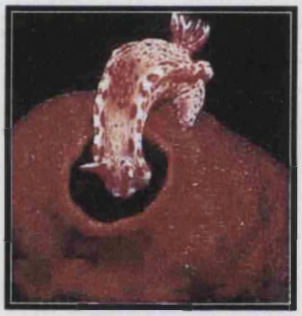


Figura **2**

Los *espongianos* se aislaron por primera vez en 1974 a partir de las esponjas de mar (Fig. **1**), y más tarde se han encontrado también estas sustancias en determinados géneros de babosas de mar que se alimentan de esponjas marinas (Fig. **2**). Estos productos son una defensa natural que las esponjas poseen para preservar su masa corporal generalmente blanda y desprotegida, actuando contra ciertos microorganismos que bloquean la entrada de agua y comida, o contra peces y artrópodos que se alimentan de esponjas. Las babosas de mar son moluscos sin caparazón que también entran en la dieta de peces y artrópodos.

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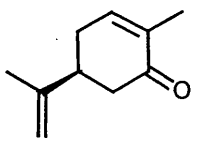
[11] Bergquist, P. R. In *Marine Natural Prod.*, Scheuer, P. J., Academics Press: New York, **1983**, *5*, 1-50.

Curiosamente, los moluscos pertenecientes al orden *Nudibranchia* (subclase Opisthobranchia) son especialmente devoradores de esponjas marinas. Estos moluscos son capaces de superar los metabolitos de defensa generados por las esponjas y a menudo, aprovechan estas sustancias de defensa, concentrándolas (y a veces modificándolas), para defenderse ellos mismos.

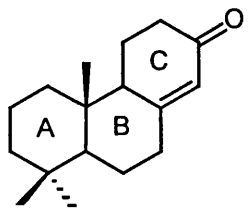
Estas fueron las observaciones iniciales que llevaron a muchos investigadores a estudiar los productos potencialmente tóxicos de este conjunto de organismos marinos. De hecho, muchos de los espongianos aislados han mostrado actividad antiviral contra el virus *Herpes simplex* I (HSV-1) y citotóxica (en células cancerosas como P388, L1210, PS y KB). De entre los espongianos pentacíclicos, algunos han presentado cierta capacidad inhibitoria de la enzima fosfolipasa $A_2$ ($PLA_2$) encargada de la hidrólisis de los fosfolípidos para dar precursores biosintéticos de moléculas responsables del proceso de la inflamación. Por esto, estas sustancias también tienen interés como medicinas potenciales para tratar enfermedades relacionadas con procesos de inflamación.

A pesar de que se ha aislado un considerable número de espongianos, el esfuerzo que se ha realizado por desarrollar síntesis que tengan como objetivo este tipo de compuestos ha sido mínimo. Dado que de muchos de ellos además no se conoce la estereoquímica absoluta, la síntesis estereoselectiva constituiría un método adecuado para establecer su estereoquímica absoluta de manera inequívoca, así como el medio de obtener mayor cantidad de material para poder llevar a cabo un estudio más profundo de sus propiedades biológicas.

Para la síntesis de diterpenos espongiánicos hemos utilizado como materiales de partida el monoterpeno comercial *S*-(+)-carvona (**1**), y la podocarpenona (**2**) fácilmente obtenible del ácido abiético.




*S*-(+)-carvona **1**




**2**

- El segundo objetivo de esta Tesis fue la síntesis quiral de *escopadulanos*. Estos diterpenos policíclicos atrajeron nuestro interés por guardar cierta similitud estructural con los esqueletos carbonados previamente sintetizados por nosotros.


Figura 3

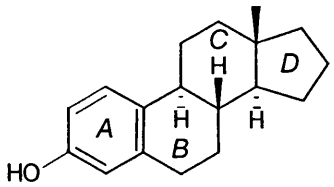
Los *escopadulanos* son una familia de sustancias de origen vegetal que se aislaron por primera vez en 1987 a partir de un extracto medicinal de la planta *Scoparia dulcis* L. (Scrophulariaceae). Esta planta es una hierba que puede llegar a medir medio metro de altura y se encuentra en muchos países tropicales (Fig. **3**). Las poblaciones nativas han utilizado tradicionalmente esta hierba como remedio a distintas enfermedades. Por ejemplo, en Paraguay el extracto crudo preparado a partir de plantas enteras de *Scoparia dulcis*, llamado *Typchá-Kuratû*, se conoce por sus propiedades digestivas y como protector gastrointestinal. En Taiwan se utiliza para tratar la hipertensión y en la India para el dolor de muelas y también para desórdenes gastrointestinales. Durante la investigación de esta medicina popular, se identificaron en los extractos farmacológicamente activos unos ácidos diterpénicos (ácidos escopadúlcicos) que poseen un esqueleto tetracíclico nuevo (**II**), llamado escopadulano.

En el año 1991 se descubrieron dos ácidos diterpénicos más que poseían el mismo esqueleto hidrocarbonado en los extractos de la planta *Calceolaria thyrsiflora* L., y poco más tarde se aislaron nuevos escopadulanos de la planta *Calceolaria dentata* L., ambas pertenecientes al género *Scrophulariaceae*. El interés en estas sustancias se debe a que, junto con alguno de sus derivados, han mostrado actividad antiviral *in vitro* e *in vivo* contra el virus *Herpes simplex 1* (HSV-1), actividad antitumoral tanto *in vitro* como *in vivo* en varias líneas celulares humanas (HeLa 229, HeLa S3, HEp-2), así como la inhibición de los ésteres de forbol como promotores de tumores.

Pero las posibilidades terapéuticas de estas sustancias van más lejos, ya que también inhiben la enzima $H^+$, $K^+$-adenosina trifosfatasa (ATPasa) encargada de la secreción de ácido gástrico, por lo que son candidatos a fármacos para tratar úlceras, gastritis etc...Aunque estas razones se citan a menudo para justificar una síntesis, los nuevos desafíos usualmente encontrados en una síntesis total ofrecen oportunidades para encontrar nuevas reacciones o procesos que pueden ser de gran valor para otros químicos orgánicos. Algunos químicos sintéticos se interesaron rápidamente por estos productos y en pocos años se publicaron un par de secuencias sintéticas racémicas. Durante la realización del presente trabajo, se ha publicado una síntesis enantioselectiva de diterpenos escopadulánicos.[12]

En nuestro trabajo sintético para obtener escopadulanos quirales hemos utilizado como material de partida la podocarpenona (**2**).

- Finalmente, la última parte de esta Tesis se ha centrado en la síntesis de sistemas policíclicos intermedios en la síntesis de *hormonas esteroidales*. Se ha utilizado una metodología poco común en la construcción de sistemas policíclicos, como es la ciclación en cascada de sistemas poliénicos mediante radicales libres. Todo el trabajo de esta Tesis realizado en este campo ha sido realizado íntegramente en el Departamento de Química Orgánica de la Universidad de Nottingham (UK), bajo la supervisión del Profesor Gerald Pattenden.




(III)

Los esteroides con esqueleto de estrano (**III**) son muy importates biológicamente, ya que controlan parte de los mecanismos implicados en la reproducción humana. Además las hormonas esteroideas tienen papeles bien establecidos en la etiología de determinados cánceres como el de pecho y de próstata.

[12] Fox, M. E.; Chi, L.; Marino, Jr J. P.; Overman, L. E. *J. Am. Chem. Soc.* **1999**, *121*, 5467 y referencias citadas allí.

Es de vital importancia la detección en fases tempranas de estos tumores, así como el contenido de receptor de estrógenos para elegir el tratamiento más apropiado. Los tumores con un elevado contenido de este receptor responderán satisfactoriamente a una terapia con antiestrógenos, mientras que los tumores con un bajo contenido de este receptor responderán pobremente a una terapia hormonal, y necesitarán otros tratamientos más agresivos como la cirugía y la quimioterapia.[13]

El contenido del receptor estrogénico se puede determinar aprovechando las características de emisión de ciertos fármacos marcados isotópicamente que se unen a este receptor, como los esteroides marcados con $^{18}F$ o $^{123}I$. Los antiestrógenos son sustancias que compiten con los estrógenos por el receptor estrogénico sin activar la transcripción de los genes sensibles a los estrógenos. Sin embargo, el perfecto bloqueo de la acción de los estrógenos implicaría un compuesto de acción doble, por un lado bloquear el receptor y por otro inhibir la biosíntesis de estradiol, el más potente de los estrógenos.

Así pues, la síntesis de nuevos derivados y otros estereoisómeros de los estrógenos haría posible el estudio y el descubrimiento de estos "antiestrógenos perfectos" y por tanto un avance en la terapia hormonal.[14]

En resumen, el trabajo lo hemos dividido en tres bloques independientes:

1. Espongianos.
2. Escopadulanos.
3. Estranos.

En general, la distribución de ellos comienza con un capítulo de antecedentes bibliográficos de aislamiento y síntesis. A continuación, se introducen y presentan las publicaciones (preprints y reprints) obtenidas por cada campo de estudio. Por último, se presenta un resumen-discusión global de los resultados obtenidos en esta Tesis Doctoral.

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[13] Skaddan, M. B.; Wüst, F. R.; Katzenellenbogen, J. A. *J. Org. Chem.* **1999**, *64*, 8108.

[14] Trembley, M. R.; Poirier, D. *J. Steroid Biochem. Molec. Biol.* **1998**, *66*, 179.

## *2. ESPONGIANOS*

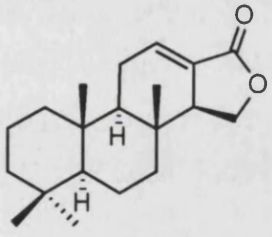
# 2.- ESPONGIANOS

## 2.1.- Compuestos espongiánicos aislados de fuentes naturales y propiedades.

Hasta la fecha, la bibliografía existente en el campo de los espongianos es relativamente reciente. Sin embargo, dado que muchos de los compuestos con esqueleto espongiánico presentan actividad biológica, el interés por aislar y caracterizar nuevos compuestos crece día a día.

El primer compuesto natural (**1**) con esqueleto de espongiano fue aislado en 1974 por Minale y colaboradores de la esponja de mar *Spongia officinalis* (orden Dictyoceratida, familia Spongiidae, Fig. **1**).[1] La mayoría de las especies comerciales pertenecen a la especie *Spongia officinalis* (Linnaeus 1759) o sus subespecies, las cuáles han sido utilizadas desde muy antiguo como esponjas de baño (Fig. **2**) debido a la suavidad y capacidad absorbente del esqueleto que poseen.

**Figura 1**

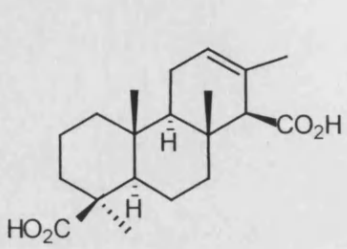


(1)

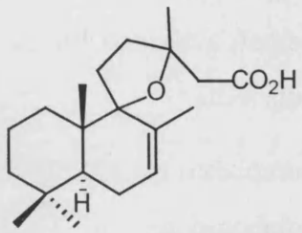
**Figura 2**

[1] Cimino, G.; De Rosa, D.; De Stefano, S.; Minale, L. *Tetrahedron* **1974**, *30*, 645.

El compuesto (**1**) conocido con el nombre común de ***Isoagatolactona***, debido a su similitud con el ácido isoagático (**2**), es una γ-lactona α,β-insaturada cuya estereoquímica absoluta fue determinada por interrelación química con el ácido grindélico (**3**), un diterpeno de la familia de los grindelanos de estereoquímica absoluta conocida. La ***Isoagatolactona*** ha resultado ser inactiva en los tests antimicrobianos ensayados.




(2)




(3)

Pocos años después, se empezó a aislar un grupo creciente de diterpenos espongiánicos tanto tetracíclicos como pentacíclicos de la misma esponja y de otras especies, así como de otros organismos marinos como las babosas de mar.

Para simplificar el estudio de los espongianos vamos a agruparlos en familias basándonos en la funcionalización que poseen en el anillo D. Primeramente, presentaremos aquéllos que poseen el anillo D de tipo lactónico; a continuación, un segundo grupo que tendrá como característica común el anillo D de tipo furánico. La tercera familia agrupará a los espongianos con el anillo D hemiacetálico, y, por último, presentaremos los espongianos pentacíclicos.

Por tanto, tenemos en primer lugar los espongianos relacionados formalmente con la ***Isoagatolactona*** con el anillo D de tipo lactónico. Así por ejemplo, el compuesto (**4**), que presenta actividad biológica, ha sido aislado tanto de las especies (orden Dendroceratida) *Aplysilla rosea*,[2] *Dendrilla rosea*,[3] y *Aplysilla polyrhapis*,[4] como de la babosa de mar *Chromodoris obsoleta* (Fig. **3**).[5]

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[2] Karuso, P.; Taylor, W. C. *Aust. J. Chem.* **1986**, *39*, 1629.

[3] Karuso, P.; Bergquist, P. R.; Cambie, R. C.; Bucketlon, J. S.; Clark, G. R.; Rickard, C. E. F. *Aust. J. Chem.* **1986**, *39*, 1643.

[4] Bobzin, S. C.; Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 3902.

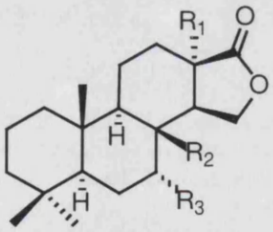
[5] Miyamoto, T.; Sakamoto, K.; Arao, K.; Komori, T.; Higuchi, R; Sasaki, T. *Tetrahedron* **1996**, *52*, 8187.

**Figura 3**. Chromodoris obsoleta

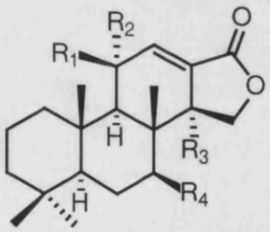
De la primera de estas especies se aisló también el espongiano (**5**), que también ha sido encontrado en el citado molusco del género *Chromodoris* junto al compuesto nuevo (**6**). Del molusco *Ceratosoma brevicaudatum* (Fig. **4**) se aisló el compuesto más sencillo de esta serie con la posición C-17 funcionalizada (**7**).[6]

***Aplyroseol-14*** (**8**) y ***aplyroseol-16*** (**9**) han sido aislados recientemente por Taylor y colaboradores también de la esponja *Aplysilla rosea*;[7] mientras que la 16-espongianona (**10**), cuya única funcionalización es el anillo D lactónico, fue encontrada en las especies (orden Dendroceratida) *Dictyodendrilla cavernosa*,[8] *Chelonaplysilla violacea*,[9] y recientemente en *Aplysilla* var. *sulphurea*.[7]

**Figura 4**. Ceratosoma brevicaudatum




**(4)** $R_1$= H, $R_2$= Me, $R_3$= OAc
**(5)** $R_1$= H, $R_2$= $CH_2OAc$, $R_3$= OAc
**(6)** $R_1$= H, $R_2$= Me, $R_3$= OH
**(7)** $R_1$= H, $R_2$= CHO, $R_2$= H
**(8)** $R_1$= $R_3$= H, $R_2$= $CH_2OAc$
**(9)** $R_1$= OH, $R_2$= $CH_2OAc$, $R_3$= OAc
**(10)** $R_1$= H, $R_2$= Me, $R_3$= H




**(11)** $R_1$= OH, $R_2$= $R_3$= $R_4$= H
**(12)** $R_1$= OAc, $R_2$= $R_3$= $R_4$= H
**(13)** $R_1$= OH, $R_2$= $R_3$= H, $R_4$= OH
**(14)** $R_1$= H, $R_2$= $R_4$= OH, $R_3$= H
**(15)** $R_1$= OAc, $R_2$= H, $R_3$= OH, $R_4$= OAc
**(16)** $R_1$= OAc, $R_2$= $R_4$= H, $R_3$= OH
**(18)** $R_1$= $R_4$= OAc, $R_2$= $R_3$= H

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[6] Ksebati, M. B.; Schmitz, F.J. *J. Org. Chem.* **1987**, *52*, 3766.
[7] Taylor, W. C.; Toth, S. *Aust. J. Chem.* **1997**, *50*, 895.
[8] Kernan, M. R.; Cambie, R. C.; Bergquist, P. R. *J. Nat. Prod.* **1990**, *53*, 724.
[9] Hambley, T. W.; Poiner, A.; Taylor, W. C. *Aust. J. Chem.* **1990**, *43*, 1861.

Asimismo, se han encontrado otros espongianos lactónicos que presentan actividad antimicrobiana. Entre ellos los compuestos (**11**) a (**14**), aislados de la especie *Spongia officinalis*,[10] cuyo extracto es activo frente a *Staphylococus aureus*, *Pseudomonas aeruginosa* y *Bacillus sphaericus*, y también inhibe el crecimiento de células HeLa con valores $ID_{50}$ de 1-5 μg/ml. Las **dorisenonas A** (**15**), **B** (**16**), **C** (**17**), y **D** (**18**) se aislaron del molusco marino japonés *Chromodoris obsoleta*.[5] En concreto, la dorisenona A ha mostrado cierta actividad citotóxica contra ciertas cepas cancerígenas ($IC_{50}$=0.21 μg/ml contra *Murine lymphoma* L1210; $IC_{50}$=0.22 μg/ml contra *Human epidermoid carcinoma* KB).

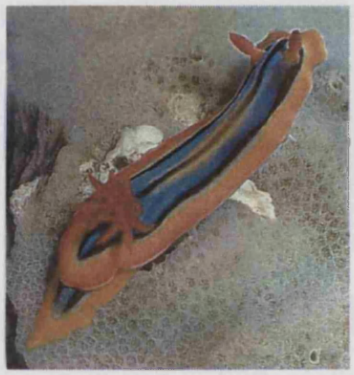
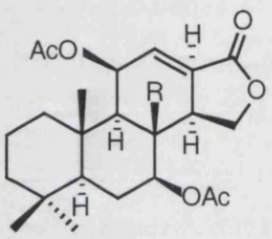


**Figura 5**. Chromodoris hamiltoni




**(17)** $\Delta^{13}$ R= Me
**(19)** $\Delta^{12}$ R= CHO
**(20)** R= CHO

Del molusco africano *Chromodoris hamiltoni* (Fig. **5**)[11] también se han aislado nuevos espongianos que poseen un grupo formilo en C-8 como (**19**) y (**20**), cuya actividad biológica no ha sido investigada.

En los últimos años se han encontrado nuevas lactonas espongiánicas caracterizadas por poseer un grupo ácido en C-19, y al igual que (**17**) un doble enlace entre las posiciones C-13 y C-14. Por ejemplo, (**21**) fue aislado de la esponja *Spongia matamata* (orden Dictyoceratida, familia Spongiidae)[12] junto a la lactona hemiacetálica (**22**) y las lactonas (**24**) a (**26**), éstas últimas con el grupo carbonilo en C-15. Los productos metoxilados podrían ser artefactos resultado del proceso de extracción.

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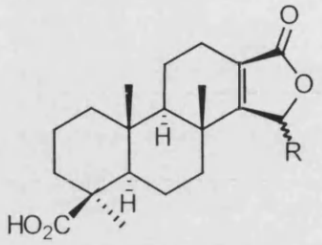
[10] González, A. G.; Estrada, D. M.; Martin, J. D.; Martin, V. S.; Perez, C.; Perez, R. *Tetrahedron* **1984**, *40*, 4109.

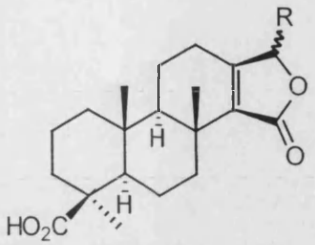
[11] McPhail, K.; Davies-Coleman, M. T. *Tetrahedron* **1997**, *53*, 4655.

[12] Li, C-J.; Schmitz, F. J.; Kelly-Borges, M. *J. Nat. Prod.* **1999**, *62*, 287.

Por otro lado, los metabolitos (**21**) y (**24**) también se han encontrado recientemente en la esponja *Coscinoderma mathewsi* (orden Dictyoceratida, familia Spongiidae)[13] junto al lactol (**23**).

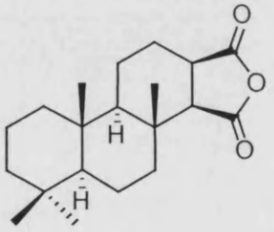


(**21**) R= H
(**22**) R= α-OMe
(**23**) R= ξ-OH

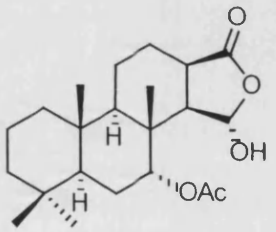


(**24**) R= H
(**25**) R= β-OMe
(**26**) R= α-OMe

Cabe mencionar el compuesto (**27**), aislado de *Dictyodendrilla cavernosa* (orden Dendroceratida, familia Dictyodendrillidae)[8] por ser el primer anhídrido aislado de una esponja marina y el lactol (**28**) llamado ***aplyroseol-15***, aislado de *Aplysilla rosea* (orden Dendroceratida, familia Aplysillidae),[7] por poseer una oxidación del anillo D intermedia entre la lactona y el anhídrido.



(**27**)



(**28**)

De entre los espongianos caracterizados por poseer el anillo D de tipo furánico, cabe destacar los compuestos (**29**) a (**31**) aislados de la esponja *Spongia officinalis*,[14] cuyo extracto presenta actividad fungicida, y que se diferencian en la funcionalización en C-19.

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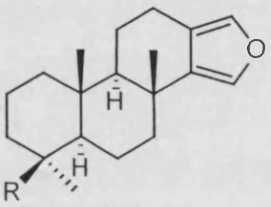
[13] Hyosu, M.; Kimura, J. *J. Nat. Prod.* **2000**, *63*, 422.

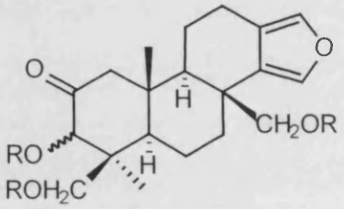
[14] Capelle, N.; Braekman, J. C.; Daloze, D.; Tursch, B. *Bull. Soc. Chim. Belg.*, **1980**, *89*, 399.

Años más tarde, (**29**) se aisló también de la esponja *Hyatella intestinalis* (orden Dictyoceratida. familia Spongiidae),[15] al igual que (**31**) que también ha sido encontrado recientemente en las esponjas *Spongia matamata*[12] y *Coscinoderma mathewsi*.[13]

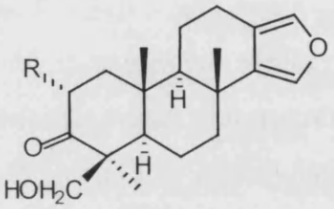
Otros espongianos de tipo furánico se caracterizan por poseer el anillo A altamente funcionalizado, como los compuestos (**32**) a (**46**). Por ejemplo, la serie de compuestos (**32**) ha sido aislada de especies parecidas del género *Spongia*,[16] y su estereoquímica fue confirmada por estudio de difracción de rayos X, además de su configuración absoluta en base a medidas de dicroísmo circular y dispersión óptica rotatoria; mientras que (**33**) a (**35**) se aislaron de *Hyatella intestinalis*.[15]



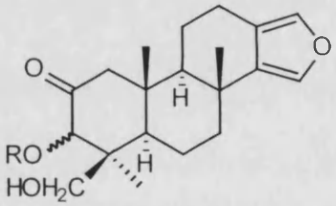
(**29**) R=$CH_3$
(**30**) R=CHO
(**31**) R=COOH



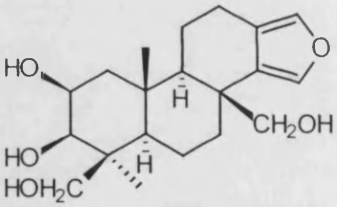
(**32a**) R=H
(**32b**) R=Ac



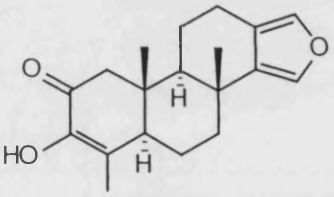
(**33**) R= H
(**34**) R= OH



(**35**) R= β-Ac
(**36**) R= H



(**37**)



(**38**)

Por otra parte, los dos epímeros del compuesto (**36**) (3α y 3β), llamados ***Espongiadiol*** y ***Epiespongiadiol*** respectivamente, fueron encontrados también en *Hyatella intestinalis*[15] y en especies *Spongia*[16] de Australia, que junto a (**34**), llamado comúnmente ***Isoespongiadiol*** y encontrado en especies *Spongia* del caribe[17] son activos frente al virus HSV-1 (Herpes simplex) ($IC_{50}$=0.25, 12.5 y 2 μg/ml, respectivamente) y frente a células de *murina leukemia* P388 ($IC_{50}$= 0.5, 8 y 5 μg/ml, respectivamente).

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[15] Cambie, R. C.; Craw, P. A.; Stone, M. J.; Bergquist, P. R. *J. Nat. Prod.*, **1988**, *51*, 293.

[16] Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Noack, K.; Oberhänsli, W. E.; Schönholzer, P. *Aust. J. Chem.* **1979**, *32*, 867.

[17] Kohmoto, S.; McConnell, O. J.; Wright, A.; Cross, S. *Chem. Lett.* **1987**, 1687.

De una esponja del género *Spongia* recogida en la Gran Barrera de Coral[18] se aislaron los compuestos (**37**) y (**38**), los cuáles fueron inactivos frente a células P388. La funcionalización presente en el anillo A de (**38**) también se ha encontrado en otro metabolito aislado del molusco *Casella atromarginata* (Fig. **6**),[19] el cuál posee en este caso el grupo carbonilo en C-3.

**Figura 6**. Casella atromarginata

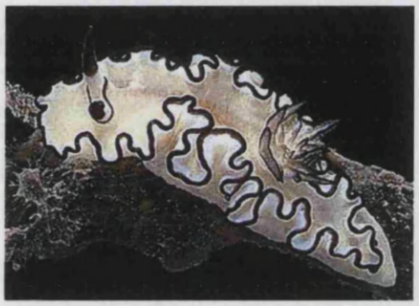
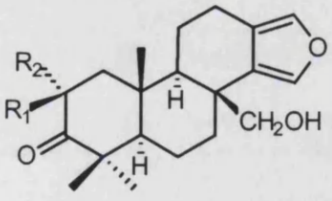
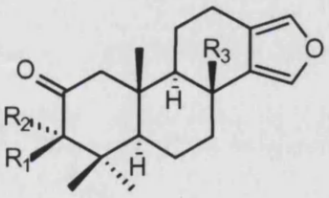


**Figura 7**. Glossodoris atromarginata

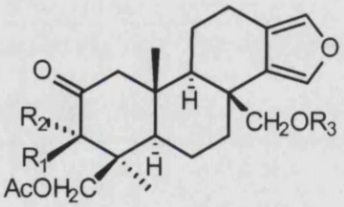
En otras especies australianas *Spongia* se encontraron los compuestos (**39**) a (**43**)[20] y recientemente, Fontana y colaboradores aislaron a partir del molusco *Glossodoris atromarginata* (Fig. **7**)[21] cinco espongianos nuevos como son: (**44**) a (**46**) más (**45**) sin acetilar en C-19 y el derivado tetraacetilado de (**37**).



**(39)** $R_1$= OH, $R_2$= H
**(40)** $R_1$= H, $R_2$= OH



**(41)** $R_1$= OH, $R_2$= H, $R_3$= $CH_2OH$
**(42)** $R_1$= H, $R_2$= OH, $R_3$= $CH_2OH$
**(43)** $R_1$= H, $R_2$= OH, $R_3$= $CH_3$



**(44)** $R_1$= OH, $R_2$= H, $R_3$= Ac
**(45)** $R_1$= OAc, $R_2$= H, $R_3$= H
**(46)** $R_1$= H, $R_2$= OAc, $R_3$= H

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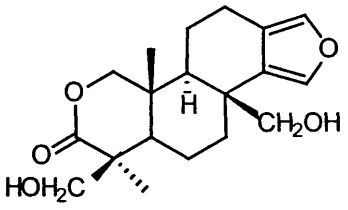
[18] Gunasekera, S.P., Schmitz, F. J. *J. Org. Chem.* **1991**, *56*, 1250.

[19] Dilip de Silva, E.; Scheuer, P. J. *Heterocycles* **1982**, *17*, 167.

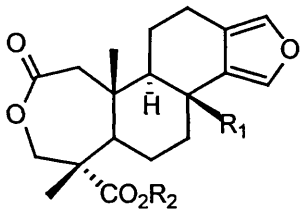
[20] Searle, P. A.; Molinski, T. F. *Tetrahedron*, **1994**, *50*, 9893.

[21] Fontana, A; Mollo, E.; Ricciardi, D.; Fakhr, I.; Cimino, G. *J. Nat. Prod.* **1997**, *60*, 444.

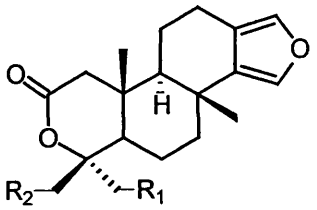
Otro compuesto estructuralmente relacionado con estos últimos como la *spongialactone A* (**49**), ya había sido previamente aislado de *Spongia officinalis* (var. *arabica*).[22] Recientemente se han encontrado dos nuevas lactonas espongiánicas, compuestos (**50**) y (**51**), en la esponja *Spongia zimocca sensu* (orden Dictyoceratida, familia Spongiidae)[23] que fue identificada erróneamente como *Spongia matamata.*[12]



(47)

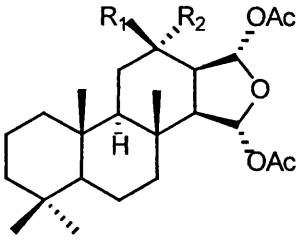


(48) $R_1$= $CH_2OH$, $R_2$= H
(49) $R_1$= $CH_3$, $R_2$= H

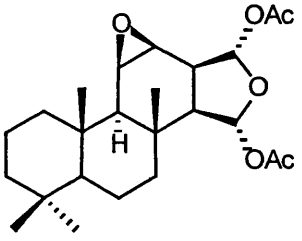


(50) $R_1$= OH, $R_2$= H
(51) $R_1$= H, $R_2$= OH

Siguiendo con la clasificación de los espongianos conocidos, el tercer grupo de ellos se caracteriza por presentar en el anillo D un sistema doblemente hemiacetálico acetilado. El compuesto (**52**) fue aislado de *Spongia officinalis*,[24] de *Chelonaplysilla* violacea,[9] y de *Aplysilla* var. *sulphurea*;[7] el espongiano (**53**) fue aislado tanto de la esponja *Aplysilla rosea*[25] (aunque estudios posteriores indican que se trataba de *Darwinella* sp.)[3] como de *Spongia officinalis*,[10] y es conocido con el nombre común de ***Aplysillin***, el cuál ha sido inactivo en los tests antimicrobianos ensayados.



(52) $R_1$=$R_2$=H
(53) $R_1$=H, $R_2$=OAc
(54) $R_1$=OAc, $R_2$=H
(55) $R_1$=OH



(56)

[22] Hirsch, S.; Kashman, Y. *J. Nat. Prod.* **1988**, *51*, 1243; Kashman, Y.; Carmely, S.; Blasberger, D.; Hirsch, S.; Green, D. **1989**, *61*, 517.

[23] Li, C-J.; Schmitz, F. J.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 546.

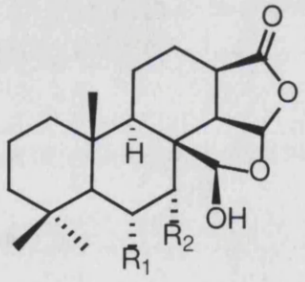
[24] Cimino, G.; Morrone, R.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 4139.

[25] Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Daly, J. J. *Tetrahedron Lett.* **1979**, 903.

El compuesto (**54**), sin embargo, ha sido encontrado en un organismo marino, *Chromodoris luteorosea* (Fig. **8**),[26] junto con el correspondiente 12-hidroxiespongiano (**55**). Ambos han resultado tóxicos para el pez mosquito *Gambusia affinis* a una concentración de 10 μg/ml, por lo que se sugiere que dichos compuestos pueden servir como defensa en estos organismos. Recientemente se ha aislado un nuevo miembro de este grupo, compuesto (**56**), que se caracteriza por poseer un β-epóxido entre las posiciones C-11 y C-12. Este metabolito aislado del molusco *Chromodoris obsoleta*[5] presenta una fuerte actividad citotóxica frente a células de *murine lymphoma* L1210 ($IC_{50}$=0.18 μg/ml) y *human epidermoid carcinoma* KB ($IC_{50}$=0.98 μg/ml).

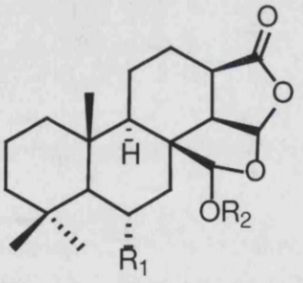
**Figura 8**. Chromodoris luteorosea

El cuarto grupo de esta clasificación lo constituirían los espongianos que presentan un quinto anillo de cinco miembros, de carácter hemiacetálico, que da lugar a una estructura altamente oxigenada (compuestos (**57**) a (**70**)).



**(57)** $R_1$= H, $R_2$= OCOPr
**(58)** $R_1$= H, $R_2$= OAc
**(59)** $R_1$= H, $R_2$= OH
**(60)** $R_1$= OH, $R_2$= OCOPr
**(61)** $R_1$= OAc, $R_2$= OCOPr

**(62)** $R_1$= OCOPr, $R_2$= OH
**(63)** $R_1$= OCOPr, $R_2$= OAc
**(64)** $R_1$= $R_2$= H
**(65)** $R_1$= $R_2$= OAc



**(66)** $R_1$= OCOPr, $R_2$= H, 17-β
**(67)** $R_1$= OAc, $R_2$= H, 17-β
**(68)** $R_1$= OAc, $R_2$= Ac, 17-α
**(69)** $R_1$= COPr, $R_2$= Ac, 17-α
**(70)** $R_1$= H, $R_2$= H, 17-β

Schmitz y colaboradores establecieron la estructura (**57**) por análisis de rayos-X y sugirieron que las agrupaciones oxigenadas del anillo lactónico y tetrahidrofuránico de estos compuestos podrían actuar como complejantes de cationes, y por esto posiblemente

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[26] Cimino, G.; Crispino, A.; Gavagnin, M.; Sodano, G. *J. Nat. Prod.* **1990**, *53*, 102.

bioactivos.[27] Por ejemplo el compuesto (**57**), llamado comúnmente ***Aplyroseol-1*** es ligeramente citotóxico ($ED_{50}$= 6.5 μg/ml en células *lymphocytic leukemia* (PS)). Además, tanto (**57**) como ***Aplyroseol-5*** (**62**) y ***Aplyroseol-6*** (**63**) son inhibidores de la enzima fosfolipasa $A_2$ ($PLA_2$), que es la enzima encargada de hidrolizar fosfolípidos generando precursores de eicosanoides que a su vez intervienen en los procesos de inflamación.[28]

***Aplyroseol-1*** (**57**) y ***Aplyroseol-2*** (**58**) han sido aislados de las esponjas *Igernella notabilis*,[27] de *Aplysilla* rosea (Fig. **9**)[2] y *Dendrilla* rosea;[3] y recientemente (**58**) también se ha aislado del molusco *Chromodoris obsoleta*.[5] El compuesto (**59**) se aisló de *Igernella notabilis*,[27] y desde (**60**) a (**63**) se aislaron de *Aplysilla rosea*[2] y también de *Dendrilla* rosea (excepto (**61**)),[3] aunque ***aplyroseol-3*** (**60**) fue aislado por primera vez de *Aplysilla sp.*[29]

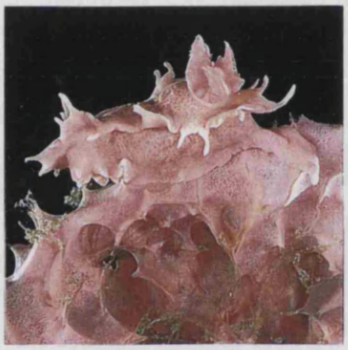

**Figura 9**. Verconia verconis (chromodoris) sobre Aplysilla Rosae

**Figura 10**. Cadlina luteomarginata

***Dendrillol-1*** (**64**) y ***Dendrillol-2*** (**65**) se aislaron de *Dendrilla rosea*,[3] y el primero también se encontró poco después en el molusco identificado como *Ceratosoma brevicaudatum* (Género *Chromodoris*),[6] junto a otros componentes de esta familia ((**66**) a (**69**)). Recientemente se ha aislado el espongiano (**70**) del molusco *Cadlina luteomarginata* (Fig. **10**).[30]

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[27] Schmitz, F. J.; Chang, J. S.; Hossain, M. B.; Van der Helm, D. *J. Org. Chem.* **1985**, *50*, 2862.

[28] Potts, B. C. M.; Faulkner, D. J. *J. Nat. Prod.* **1992**, *55*, 1701.

[29] Molinski, T. F.; Faulkner, D. J. *J. Org. Chem.* **1986**, *51*, 1144.

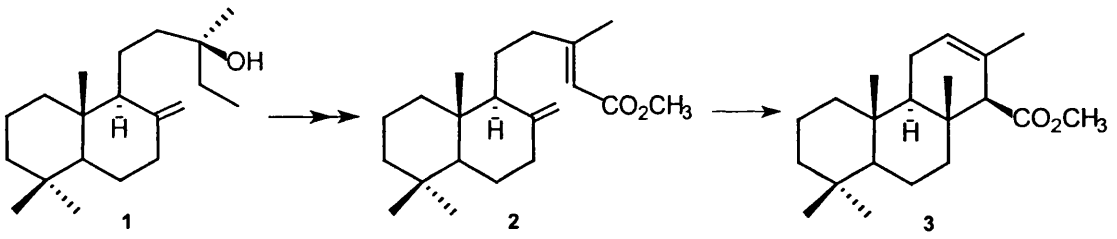
[30] Dumdei, E. J.; Kubanek, J.; Coleman, J. E.; Pika, J.; Andersen, R. J.; Steiner, J. R.; Clardy, J. *Can. J. Chem.* **1997**, *75*, 773.

## 2.2.- Síntesis previas de compuestos espongiánicos.

Tras esta revisión de los espongianos conocidos, donde se ha visto la gran variedad estructural existente dentro de esta familia de compuestos, pasamos a describir los estudios sintéticos que se han realizado para la preparación de alguno de los compuestos de este grupo de productos naturales.

Algunas de estas secuencias utilizan como precursor en la síntesis de los espongianos el (-)-isocopalato de metilo (**3**) (Esquema **1**), ya que posee la configuración adecuada en las posiciones C-5, C-8, C-9, C-10 y C-14. Este producto puede obtenerse mediante una doble oxidación del ***manool*** (**1**) al éster copalato de metilo (**2**), seguida de ciclación.[1]

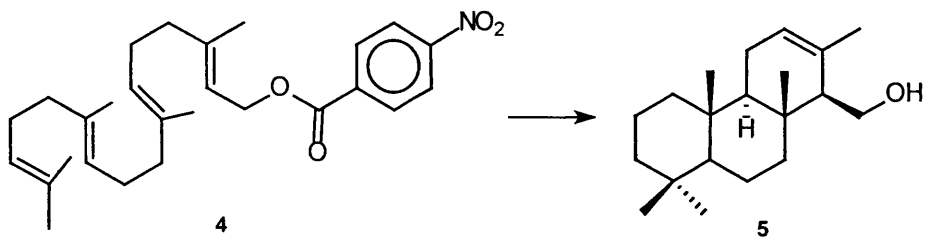
Esquema **1**




**1** **2** **3**

En cambio, Nishizawa y colaboradores,[2] proponen que el precursor mencionado (**1**) puede provenir del p-nitrobenzoato de geranilgeranilo (**4**) (Esquema **2**). La ciclación biomimética de éste usando un complejo de triflato de mercurio (II) y amina, da origen al alcohol (**5**), que mediante oxidación y esterificación conduciría al isocopalato de metilo.

Esquema **2**



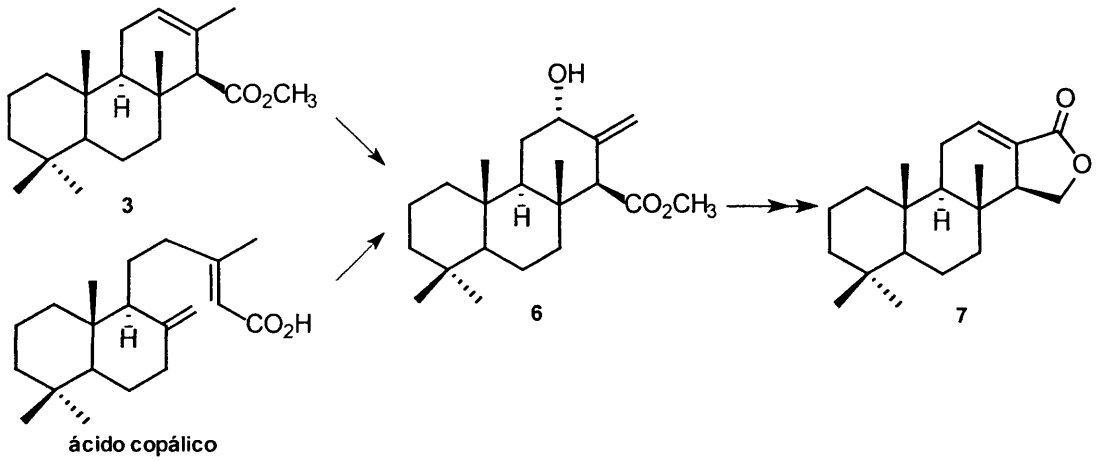

**4** **5**

[1] Imamura, P. M.; González Sierra, M.; Rúveda, E. A. *J. Chem. Soc., Chem. Comm.* **1981**, 734.

[2] Nishizawa, M.; Takenaka, H.; Hayashi, Y. *Chem. Lett.*, **1983**, 1459.

La primera síntesis de un diterpeno espongiánico tuvo como objetivo la (+)-***Isoagatolactona*** (**7**). La ruta sintética desarrollada por Rúveda y colaboradores,[1] utilizaba como material de partida el (-)-isocopalato de metilo mencionado (**3**) (Esquema **3**). La fotooxigenación de este compuesto, con un rendimiento del 20%, condujo al alcohol (**6**). La transposición alílica de (**6**) en medio ácido, con lactonización simultánea, seguida de reducción de la lactona y oxidación con dióxido de manganeso dio la (+)-***Isoagatolactona*** (**7**).

Esquema **3**




Una visión global de esta secuencia nos muestra que, a pesar de que el precursor utilizado es adecuado por la estereoquímica de sus centros, el bajo rendimiento de la reacción fotoquímica, unido a que el producto de partida no es comercial ni fácilmente asequible, hace poco atractiva esta secuencia. Poco más tarde, Nakano y colaboradores utilizaron la misma secuencia sintética para preparar la (±)-***Isoagatolactona***,[3] en este caso, a partir del ácido (±)-copálico y modificando ligeramente las condiciones y los reactivos empleados (Esquema **3**). Rúveda y colaboradores también han realizado, utilizando esta secuencia, la síntesis del enantiómero de la isoagatolactona natural, es decir, la (-)-***Isoagatolactona***[4] utilizando en este caso el (+)-isocopalato de metilo (**8**).

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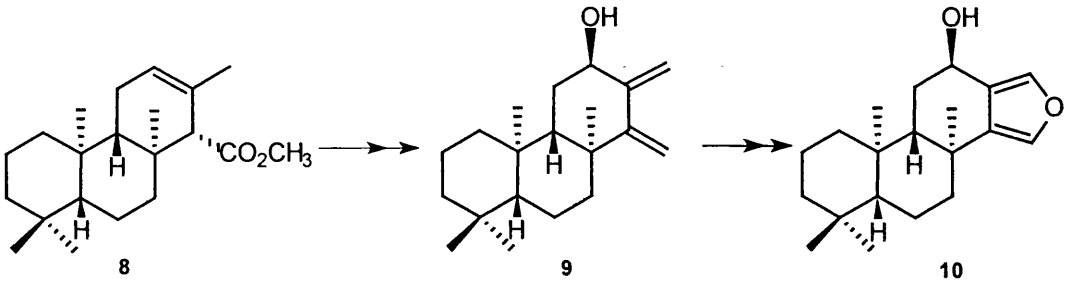
[3] a) Nakano, T.; Hernández, M. I. *Tetrahedron Lett.* **1982**, *23*, 1423; b) Nakano, T.; Hernández, M. I. *J. Chem. Soc., Perkin Trans. I* **1983**, 135.

[4] De Miranda, D. S.; Brendolan, G.; Imamura, P. M.; González Sierra, M.; Marsaioli, A. J.; Rúveda, E. A. *J. Org. Chem.* **1981**, *46*, 4851.

En la aproximación hacia la síntesis de espongianos, también han despertado interés aquéllos que poseen el anillo D de tipo furánico. La primera síntesis descrita fue realizada por Rúveda y colaboradores,[4] ya que partiendo del (+)-isocopalato de metilo (**8**) (Esquema **4**) se obtuvo el furano (-)-***12α-hidroxiespongia-13(16),14-dieno*** (**10**).

Esquema **4**

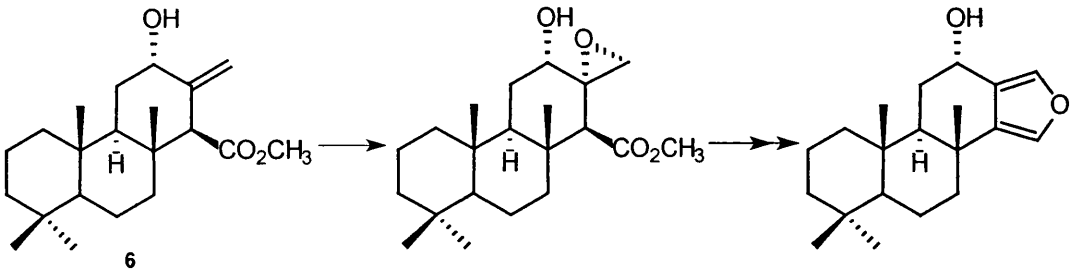



**8** **9** **10**

Por reducción del éster de partida a alcohol seguida de eliminación, fotooxigenación y reducción se obtuvo el alcohol (**9**). Éste fue sometido de nuevo a foto-oxigenación y reducción con sulfato ferroso para dar el compuesto furánico (**10**). En la segunda fotooxigenación se obtiene un rendimiento muy bajo, lo que limita su utilidad sintética.

Posteriormente, aunque el furano (**10**) no es natural, Nakano y colaboradores[4] realizaron la síntesis del enantiómero (Esquema **5**). Para ello, partieron del producto de fotooxigenación (**6**), que ya había sido obtenido con anterioridad por estos mismos autores (ver Esquema **3**). La epoxidación de éste dio el α-epóxido correspondiente, que bajo tratamiento con LDA experimentó apertura del anillo oxiránico y lactonización dando una lactona α,β-insaturada que se transformó, con DIBAL-H, en el compuesto deseado (+)-***12α-hidroxiespongia-13(16),14-dieno***.

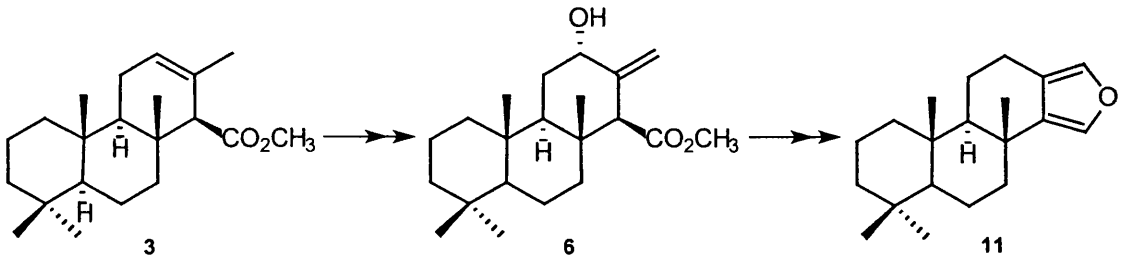
Esquema **5**




**6**

Dado que el mayor inconveniente de esta secuencia radica, de nuevo, en el bajo rendimiento de la reacción fotoquímica y en la etapa final de aromatización, estos autores[5] propusieron para obtener el producto de partida una nueva estrategia. En esta secuencia el isocopalato de metilo racémico (**3**) fue sometido a epoxidación por la cara α, seguida de apertura del anillo oxiránico con isopropóxido de aluminio para obtener el mismo alcohol (**6**) que se obtenía por fotooxigenación de (**3**). Este procedimiento ya había sido utilizado por Rúveda y colaboradores para la síntesis de otros diterpenos tricíclicos derivados del isocopalato de metilo.[6] Así, el alcohol alílico (**6**) se transformó en dos etapas en una lactona α,β-insaturada, que por reducción y posterior oxidación condujo al espongiano (**11**) (Esquema **6**).

Esquema **6**




El furanoditerpeno (-)-***Espongia-13(16),14-dieno*** (**11**), ha sido sintetizado recientemente en nuestro grupo de investigación, utilizando como material de partida la ***podocarpenona*** (**12**) (Esquema **7**). [7] En la ruta utilizada se obtiene, después de 6 etapas, la hidroxicetona (**13**) con un rendimiento global del 47% desde (**12**), la cuál se utiliza como intermedio para sintetizar el espongiano natural (**11**) y también la (+)-***Isoagatolactona*** (**7**) con un rendimiento global del 34% y del 25% respectivamente, a partir de la podocarpenona de partida.

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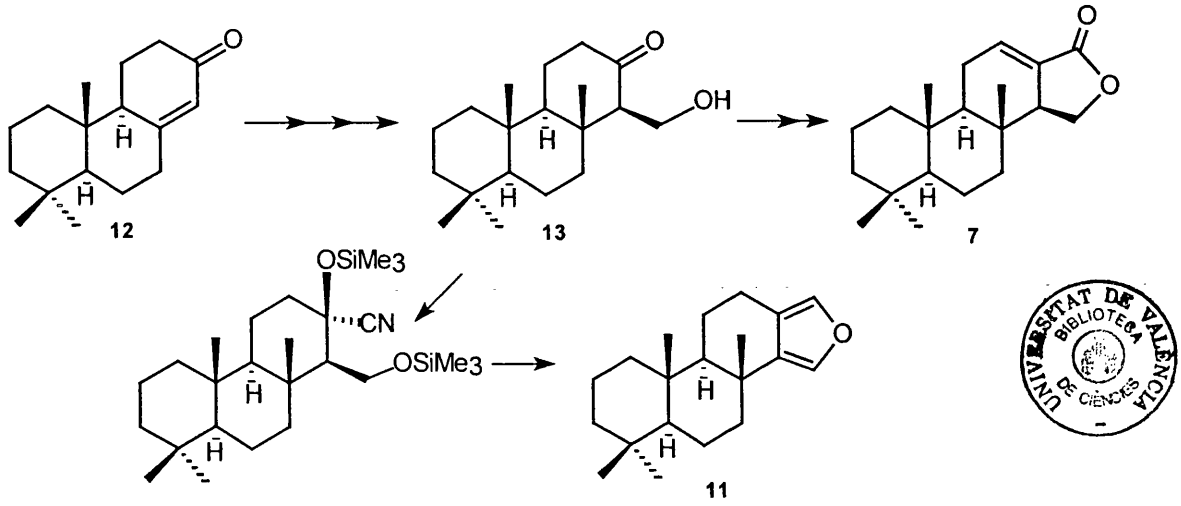
[5] Nakano, T.; Hernández, M. I.; Gomez, M.; Domingo Medina, J. *J. Chem. Research (S)* **1989**, 54.

[6] Mischne, M. P.; González Sierra, M.; Rúveda, E. A. *J. Org. Chem.* **1984**, *49*, 2035.

[7] Abad, A.; Agulló, C.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *J. Chem. Soc., Perkin Trans. 1*, **1996**, 2193.

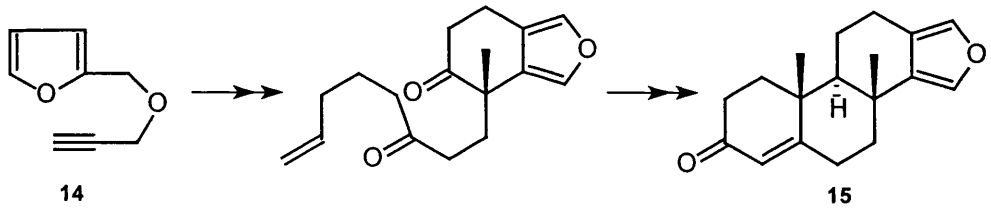
Esquema **7**






En los últimos años se han publicado distintas aproximaciones sintéticas para construir el esqueleto de espongiano e incluso de varios productos naturales, tales como ***Espongia-13(16),14-dieno*** (**11**) , (±)-***Espongiadiosfenol*** (**17**) y (±)-***Isoespongiadiol*** (**20**). Cabe mencionar una secuencia sintética de Kanematsu y colaboradores[8] en la que partiendo del compuesto furánico (**14**) se construyen los anillos A, B y C, mediante una secuencia de reacciones de Diels-Alder y condensaciones aldólicas (Esquema **8**). Se llega así al producto (**15**) que no posee el esqueleto completo de espongiano pero es susceptible de transformarse en un espongiano furánico.

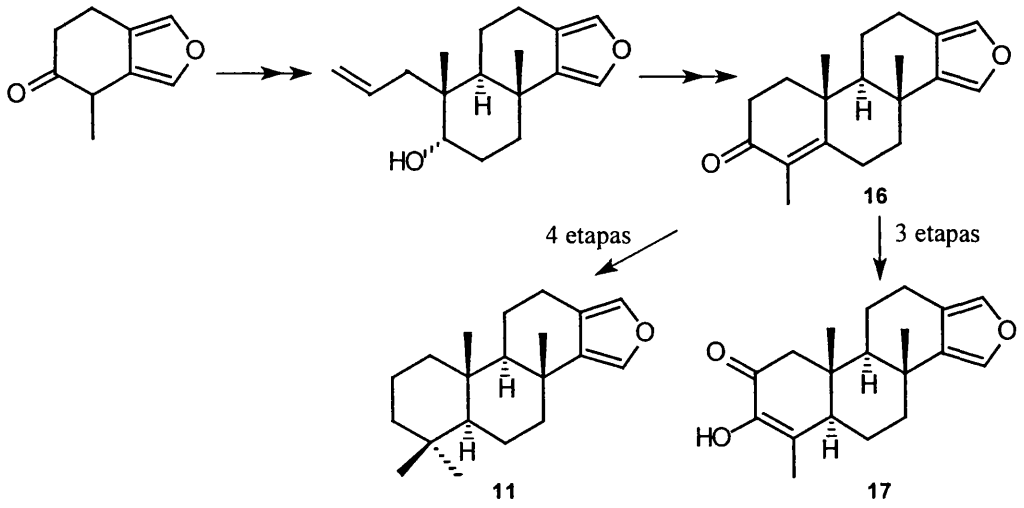
Esquema **8**




Un año más tarde, los mismos autores, reconocen un error cometido en la estereoquímica asignada al C-20 de (**15**) no era la dada en el esquema, es decir, con los metilos C-17 y C-20 en CIS sino que era TRANS por lo que este intermedio no servía para sus objetivos.

Obtuvieron entonces el intermedio (**16**) (Esquema **9**) con la estereoquímica adecuada que transformaron en (±)-***Espongia-13(16),14-dieno*** (**11**) y (±)-***Espongiadiosfenol*** (**17**).[9]

Esquema **9**




Por otro lado, durante los últimos años Zoretic y colaboradores han estado investigando en la construcción de esqueletos de espongiano mediante estrategias biomiméticas que utilizan reacciones de ciclación oxidativa de radicales libres. Este estudio ha resultado en la publicación de trabajos en los que se describe la síntesis de un compuesto con esqueleto de isoespongiano (**18**) (marginatano), y de un espongiano furánico (**19**) que no se encuentran entre los aislados de fuentes naturales, ambos con posiciones oxigenadas en el anillo A (Esquema **10**).[10] Esta estrategia consiste en la formación del sistema poliénico adecuado para llevar a cabo la ciclación utilizando una mezcla de $Mn(OAc)_3 \cdot 2H_2O$ y $Cu(OAc)_2 \cdot H_2O$ en ácido acético, que les ha servido para obtener el producto natural: (±)-***isoespongiadiol*** (**20**)[11] con un rendimiento global del 3.5%. También han utilizado esta estrategia para sintetizar diversos intermedios que les permite obtener espongianos funcionalizados en C-17 (Esquema **11**).[12]

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[8] Baba, Y.; Sakamoto, T.; Kanematsu, K. *Tetrahedron Lett.* **1994**, *35*, 5677.

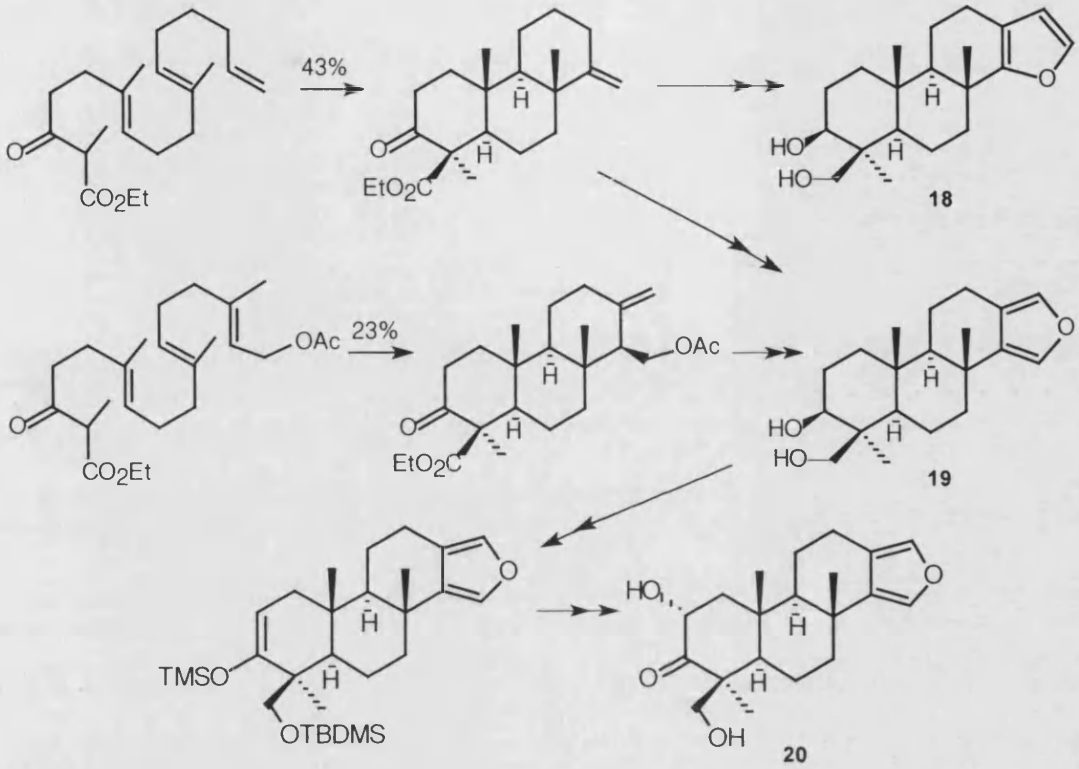
[9] Sakamoto, T.; Kanematsu, K. *Tetrahedron* **1995**, *51*, 5771.

[10] a) Zoretic, P. A.; Shen, Z.; Wang, M.; Ribeiro, A. A. *Tetrahedron Lett.* **1995**, *36*, 2925; b) Zoretic, P. A.; Zhang, Y.; Ribeiro, A. A. *Tetrahedron Lett.* **1995**, *36*, 2929.

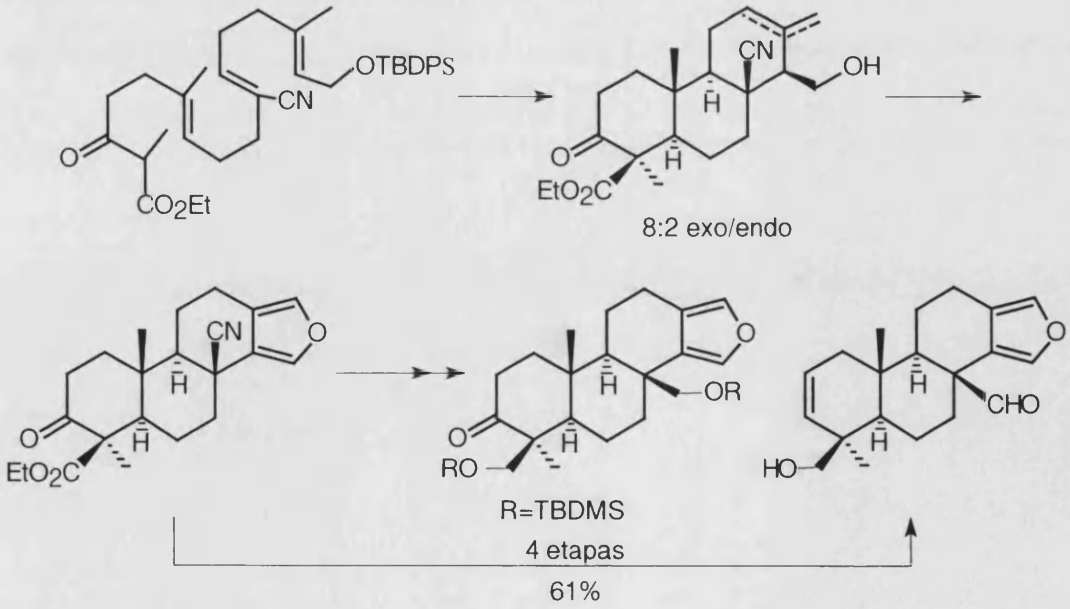
[11] Zoretic, P. A.; Wang, M.; Zhang, Y.; Shen, Z.; Ribeiro, A. A. *J. Org. Chem.* **1996**, *61*, 1806.

[12] Zoretic, P. A.; Zhang, Y.; Fang, H.; Ribeiro, A. A.; Dubay, G. *J. Org. Chem.* **1998**, *63*, 1162.

Esquema **10**

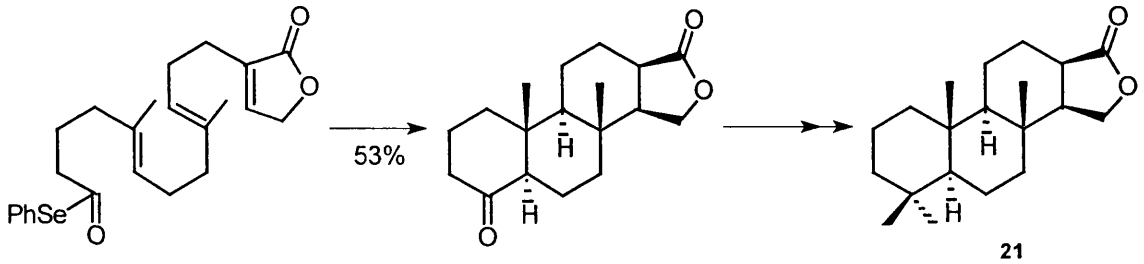



Esquema **11**



Al mismo tiempo, el grupo del profesor Pattenden desarrollaba otra estrategia basada también en la ciclación en cascada de sistemas poliénicos a través de radicales libres. La idea consiste en generar sistemas policíclicos de 6 miembros fusionados a partir de precursores de radicales acilo mediante varias ciclaciones 6-*endo-trig* consecutivas. Con esta metodología se ha obtenido (±)-***espongia-16-ona***[13] (**21**) (Esquema **12**) con un rendimiento global del 5.8%.

Esquema **12**




**21**

Por último, vamos a mencionar la única estrategia que existe hasta la fecha para la obtención de espongianos pentacíclicos, y que ha sido desarrollada en nuestro grupo de investigación. Como material de partida se ha utilizado la ***podocarpenona*** (**12**), utilizada anteriormente en nuestro grupo en la síntesis de otros espongianos.

El paso clave en esta secuencia es la adición fotoquímica de acetileno al doble enlace C(8)-C(14), así se obtiene el sistema ciclobuténico que previa homologación del C-13, genera mediante ozonólisis el sistema dialdehídico latente que sufre *in situ* una lactonización-hemiacetalización para dar el sistema pentacíclico final de forma estereoselectiva. De esta forma se ha sintetizado (-)-***dendrillol-1***[14] (**22**) y además (-)-***aplyroseol-1*** (**23**), (-)-***aplyroseol-2*** (**24**) y (-)-***deacetilaplyroseol-2*** (**25**) si previamente se introduce una función α–hidroxi en C-7 (Esquema **13**).[15]

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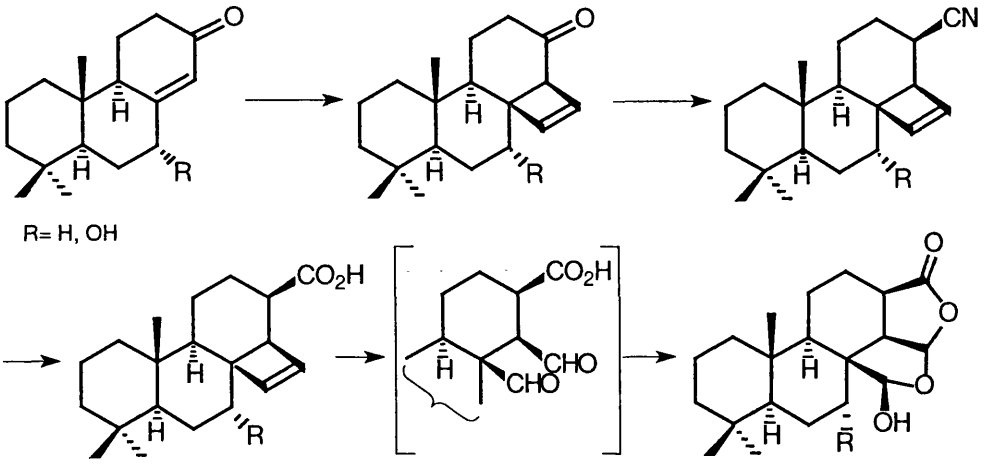
[13] a) Pattenden, G.; Roberts, L. *Tetrahedron Lett.* **1996**, *37*, 4191; b) Pattenden, G.; Roberts, L.; Blake, A. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 863.

[14] a) Abad, A.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *Synlett.* **1991**, 789; b) Abad, A.; Arnó, M.; Cuñat, A. C.; Marín, M. L.; Zaragozá, R. J. *J. Org. Chem.* **1992**, *57*, 6861.

[15] Abad, A.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *J. Chem. Soc., Perkin Trans. 1*, **1993**, 1861.

Esquema **13**

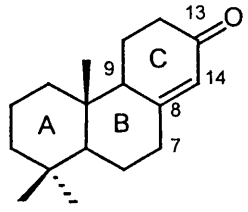



**22** R= H
**23** R= OCOPr
**24** R= OAc
**25** R= OH

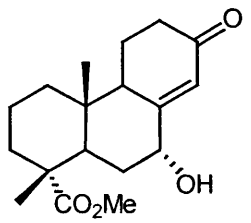
## 2.3.- Síntesis de espongianos a partir de la podocarpenona y carvona.

- **Podocarpenona**

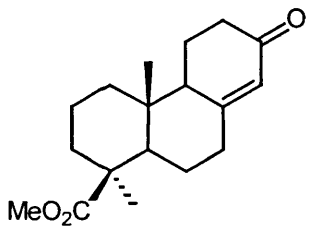
Como se ha descrito en el capítulo anterior, la preparación de compuestos con esqueleto de espongiano[1] a partir de la (+)-podocarpenona (**1**), fácilmente obtenible de los ácidos resínicos,[2] ha resultado ser muy eficaz dado que contiene la mayor parte de su estructura policíclica. La podocarpenona **1** no es un producto natural pero puede ser obtenido en forma enantioméricamente pura por degradación de productos naturales como el manool,[3] el esclareol,[4] el ácido labdanóico.[5] También ha sido descrita su síntesis total,[6] así como la síntesis de otras podocarpenonas funcionalizadas en el anillo A,[7] como (**2**)[8] y (**3**),[9] que podrían utilizarse para la síntesis de espongianos con el anillo A o B funcionalizado.




**1**




**2**




**3**

En la presente Tesis, utilizando la podocarpenona **1** como material de partida decidimos estudiar nuevas estrategias, complementarias a las desarrolladas por nuestro

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[1] Abad, A.; Agulló, C.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *J. Chem. Soc. Perkin Trans. I,* **1996**, 2193.

[2] Abad, A.; Arnó, M.; Domingo, L. R.; Zaragozá, R. J. *Tetrahedron* **1985**, *41*, 4937.

[3] Manh, D.K.; Fetizon, M. ; Flament, P. *Tetrahedron* **1975**, *31*, 1897.

[4] a) Christenson, P.A. *Tetrahedron* **1988**, *44*, 1925; b) Coste-Maniere, I.C.; Zahra, J.P.; Waegell, B. *Tetrahedron Lett.* **1988**, *29*, 1017.

[5] Medina, J.D.; de Santis, V. *J. Nat. Prod.* **1983**, *46*, 462.

[6] a) Matsumoto, T.; Usui, S. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 212; b) Church, R.F.; Ireland, R.E.; Marshall, J.A. *J. Org. Chem.* **1966**, *31*, 2526; c) Skeean, R.W.; Trammell, G.L.; White, J.D. *Tetrahedron Lett.* **1976**, 525.

[7] Total Synthesis of Natural Products, ApSimon, 1984, vol. 6, pag. 85-139.

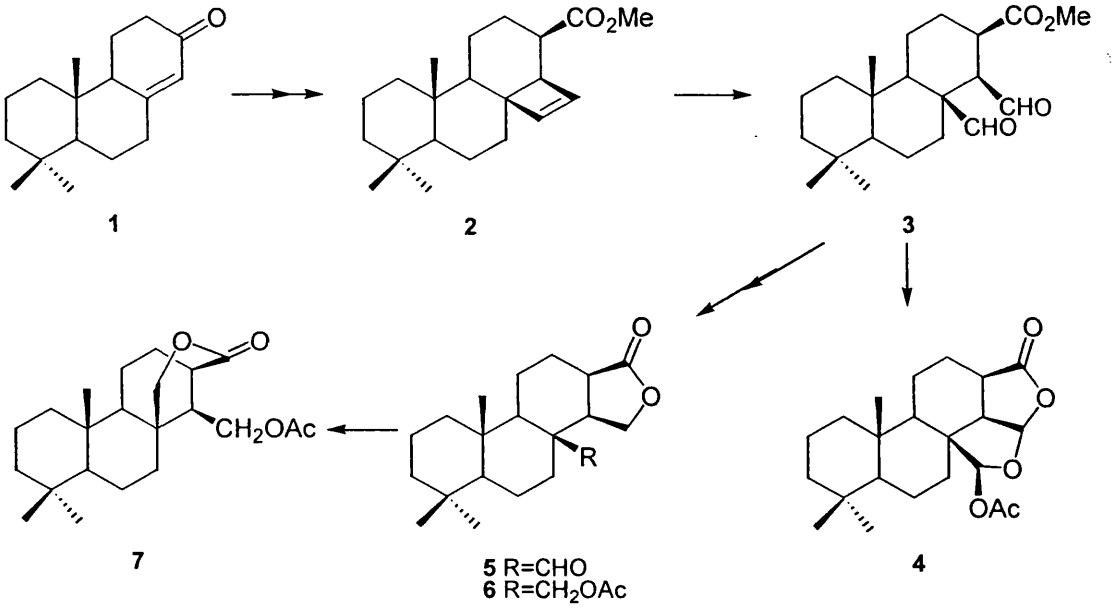
[8] Abad, A.; Arnó, M.; Peiró, M.; Zaragozá, R. J. *Tetrahedron* **1991**, *47*, 3829.

[9] Abad, A.; Agulló, C.; Arnó, M.; Marín, M. L.; Zaragozá, R. J. *J. Chem. Soc. Perkin Trans. I,* **1996**, 2193.

grupo de investigación,[10] para la preparación de espongianos tetracíclicos que contengan una función oxigenada en C-17 (**5**-**6**) (Esquema **1**).

Hasta ahora, aunque se han descrito algunas síntesis de diterpenos espongiánicos, sólo existe una síntesis de diterpenos pentacíclicos con una función hemiacetálica en C-17 que fue desarrollada en nuestro grupo. En la literatura, solamente se ha descrito una aproximación para obtener espongianos tetracíclicos, de tipo furánico, que poseen un grupo hidroxilo en C-17.[11]

Esquema **1**




Nuestra estrategia se basa en la utilización del intermedio **2** (Esquema **1**) preparado según nuestra metodología por adición fotoquímica de acetileno al doble enlace de la enona **1** y posterior homologación del grupo carbonilo en C-13. La clave de esta secuencia está en la posibilidad de aislar el dialdehído **3** obtenido por ozonólisis de **2**. Con el dialdehído **3** se ha obtenido por lactonización y acetilación *in situ* el espongiano pentacíclico **4**, mientras que la reducción selectiva del grupo carbonilo deseado de C-15 y posterior reacción de lactonización condujo al espongiano natural **5**. El aldehido **5** ha sido convertido en la δ-lactona **7**. Esta lactona **7** presenta una estructura desconocida hasta

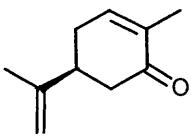
[10] Abad, A.; Arnó, M.; Cuñat, A. C.; Marín, M. L.; Zaragozá, R. J. *J. Org. Chem.* **1992**, *57*, 6861.

ahora dentro de esta familia de diterpenos espongiánicos, sus propiedades espectroscópicas coincidieron con las descritas para el espongiano Aplyroseol-14, al que se le asignó inicialmente una estructura de γ-lactona **6** con el grupo acetoxilo en C-17. La conversión de **5** en esta γ-lactona **6**, mediante un nuevo método sintético de reducción y acetilación *in situ* de grupos formilo, permitió estudiar detalladamente la resonancia magnética nuclear de ambas lactonas **6** y **7**, concluyendo que el Aplyroseol-14 natural tiene la misma estructura de la δ-lactona **7** sintetizada por nosotros.

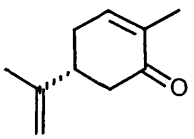
La síntesis del (-)-acetyldendrillol-1 **4** también ha conducido a la revisión de la estereoquímica de C-17 dada para el producto natural, sustituyéndose la configuración de C-17 con el grupo acetoxilo en α por la de su epímero con el grupo acetoxilo en β.

- **Carvona**

La R-(-)-carvona **9** se encuentra principalmente en el aceite de la menta verde, mientras que la S-(+)-carvona **8** se aísla mayoritariamente de los aceites de eneldo y alcaravea, siendo el coste de este último mucho mayor. Aunque actualmente casi toda la carvona disponible se obtiene mediante síntesis a partir del limoneno.[12]



**8** *S*-(+)-carvona



**9** *R*-(-)-carvona

Muchas síntesis están basadas en la preparación de intermedios muy versátiles, cuyo esqueleto y funcionalización permitan obtener tanto el producto natural objetivo como otros miembros de la familia. Existen varias recopilaciones sobre la utilización de los terpenos en síntesis, entre éstas cabe destacar la realizada por T.L Ho.[13] De esta revisión destacamos las síntesis de sistemas tricíclicos tipo fenantreno que ha resultado

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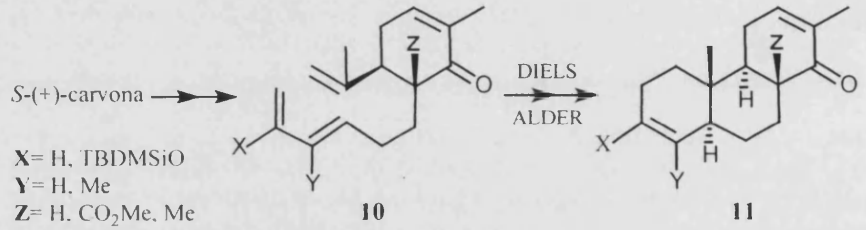
[11] Dubay, G.; Fang, H.; Ribeiro, A. A.; Zhang, Y.; Zoretic, P.A. *J. Org. Chem.* **1998**, *63*, 1162.

[12] Clark, G.S. *Perfurmer & Flavorist* **1989**, *14*, 35.

[13] Ho, T. L. In *Enantioselective Synthesis of Natural Products from Quiral Terpenes*, John Wiley and Sons, Inc., New York, 1992.

poseer una gran versatilidad y eficacia para la preparación de terpenos naturales.[14] En ellas se utiliza una reacción de Diels-Alder intramolecular (DAI) como paso clave para la construcción del esqueleto base del terpeno.

En este sentido, los esfuerzos de nuestro grupo de investigación se centraron en la construcción de varios sistemas tricíclicos (**11**), con diversa funcionalización, a partir de la carvona. Así, se ha desarrollado una ruta en la que partiendo del anillo C proporcionado por la carvona, se han construido los anillos A y B mediante una reacción de DAI de un trieno (**10**). De este modo la elección de la funcionalización sobre la cadena lateral (X,Y) y el grupo (Z) permite la síntesis estereoselectiva de diversos sistemas tricíclicos en función de los esqueletos que pretendamos sintetizar.



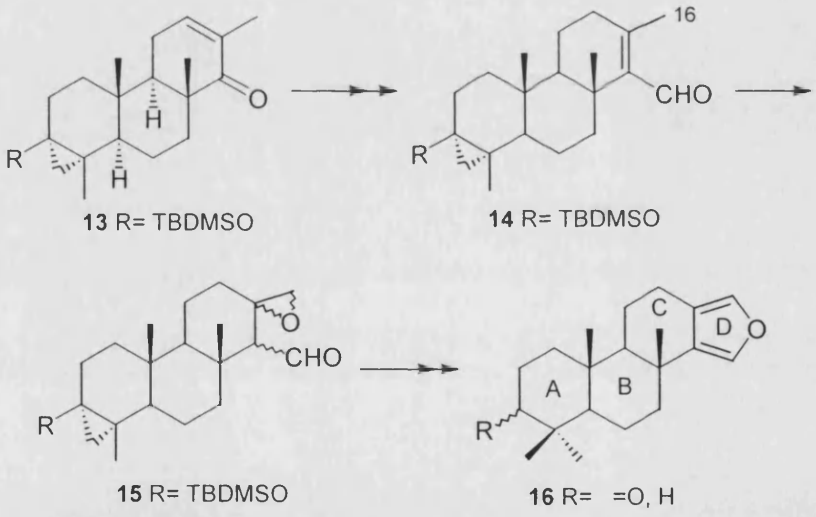

En el presente trabajo, partiendo de la carvona **8** se ha obtenido la fenantrenona **12** (Esquema **2**) en forma enantioméricamente pura. Este compuesto se consideró un buen material de partida para la síntesis de diterpenos espongiánicos ya que contiene el mismo sistema tricíclico ABC, y una funcionalización entorno al anillo C que permite la construcción del cuarto anillo presente en nuestras moléculas objetivo. Además, la presencia del silil-enol-éter en el anillo A facilita tanto la modificación de la funcionalización como la alquilación de C-4 necesaria para completar el esqueleto.

Esquema **2**


[14] Shing, T.K.M.; Jiang, Q. *J. Org. Chem.* **2000**, *65*, 7059 y referencias citadas aquí.

Para la conversión de la fenantrenona **12** en diterpenos espongiánicos con el anillo D de tipo furánico (**16**), se ha seguido el siguiente esquema sintético (Esquema **3**) que mantiene la función oxigenada de C-3 y permitiría obtener derivados funcionalizados en el anillo A. Así, en la transformación de la fenantrenona **12** en el compuesto **13** se introduce un grupo ciclopropánico que puede ser transformado posteriormente en el grupo metilo sobre C-4. Al mismo tiempo, esta transformación introduce una agrupación más resistente que el silil-enol-éter a las condiciones de reacción posteriores. La homologación del grupo carbonilo de **13** conduce al aldehído α,β-insaturado **14**, que por desconjugación del doble enlace seguida de epoxidación proporciona el epoxi-aldehído **15**. Finalmente, la desprotección del silil-éter con apertura del anillo ciclopropánico y formación del anillo furánico se produce simultáneamente en medio ácido, dando primero el espongiano **16** (R= O) y por reducción de su grupo carbonilo el espongiano natural **16** (R= H).

Esquema **3**




**13** R= TBDMSO **14** R= TBDMSO

**15** R= TBDMSO **16** R= =O, H

La obtención de estos productos sintéticos ha permitido realizar un estudio de la actividad biológica de productos naturales que se habían aislado en cantidades insuficientes para realizarlo. En realidad, la mayoría de los productos naturales e intermedios sintetizados en este trabajo se han evaluado como agentes citotóxicos y antivirales fruto de una colaboración con el equipo de inmunovirología de la facultad de Medicina en la Universidad de Antioquía, Medellín (Colombia).

Este estudio biológico ha permitido el establecimiento de ciertas relaciones estructura-actividad y su comparación con los estudios realizados con otras sustancias de esta familia de compuestos. Estos resultados y su discusión se describen en los trabajos que siguen a continuación.

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**TÍTULO:** Diastereoselective Synthesis of Spongian Diterpenes.
Total Synthesis of the Furanoditerpene (-)-Spongia-13(16),14-diene.

**REF. REVISTA:** Tetrahedron **1999**, *55*, 12419-12428.

Pergamon

Tetrahedron 55 (1999) 12419–12428

TETRAHEDRON

# Diastereoselective Synthesis of Spongian Diterpenes.Total Synthesis of the Furanoditerpene (-)-spongia-13(16),14-diene.

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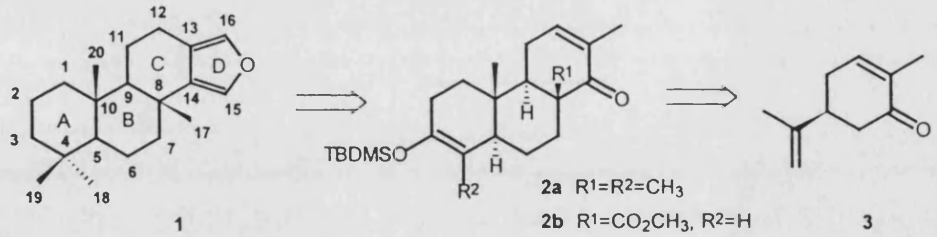
**Abstract:** An effective diastereoselective synthesis of the marine-sponge metabolite (-)-spongia-13(16),14-diene **1** is achieved starting from *S*-(+)-carvone *via* a homochiral phenanthrenone as the key intermediate for the construction of the furan ring system. *S*-(+)-Carvone was transformed into the phenanthrenone **2a** in six steps (53% overall yield), using an intramolecular Diels-Alder reaction as the key step. Conversion of the enone function in **2a** into an epoxyaldehyde function followed by cyclisation and aromatisation in acid conditions completed the construction of ring D. 

Keywords: Marine metabolites; Synthesis; Furanoditerpenes; Sponges.

## INTRODUCTION

An increasing number of diterpenes with the spongian carbon skeleton have been isolated from various marine organisms.[1] Several members of this family have shown many biological activities against a wide range of organisms, including microorganisms, invertebrates and vertebrates.[2] For the past few years our research group has developed several synthetic strategies towards the synthesis of some of these compounds.[3] As a starting material we have used (+)-podocarp-8(14)-en-13-one, which is easily obtained from natural sources.[4] Recently, we have developed a new synthetic approach which, starting from carvone, permits the synthesis of different enantiomerically pure phenanthrenone systems **2** (Scheme 1).[5]




**Scheme 1**

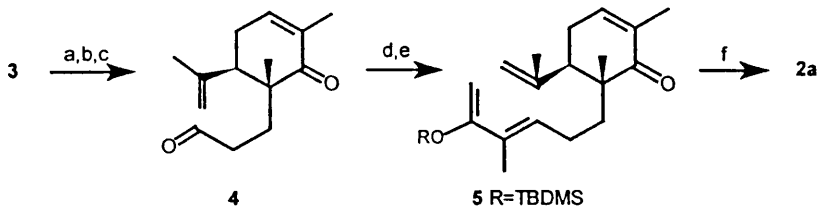
0040-4020/99/$ - see front matter 
*PII:* S0040-4020(99)00725-5

These compounds contain the tricyclic ABC-ring system characteristic of many spongian diterpenes, and a functionalisation which makes them useful as a starting material in the diastereoselective synthesis of spongian systems. They possess an enone group in the C-ring that can be used in the construction of the D-ring present in the spongian structure; in addition, the silyl enol ether in ring A can facilitate the introduction of an appropriate functionalisation in this ring.

In this paper we describe the diastereoselective total synthesis of the simplest member of the furanoditerpene spongian family, (-)-spongia-13(16)-14-diene **1**,[6] using as starting material the phenanthrenone **2a**. This tetracyclic diterpene, isolated from the sponges *Spongia officinalis*[7] and *Hyatella intestinalis*,[8] has been shown to have interesting antifungal activities.

## RESULTS AND DISCUSSION

The synthesis of (-)-spongia-13(16),14-diene starts with the preparation, in which commercial *S*-(+)-carvone **3** is used, of the tricyclic intermediate **2a**,[9] which contains the two necessary methyl groups $R^1$,$R^2$ present in the target molecule (Scheme 2).

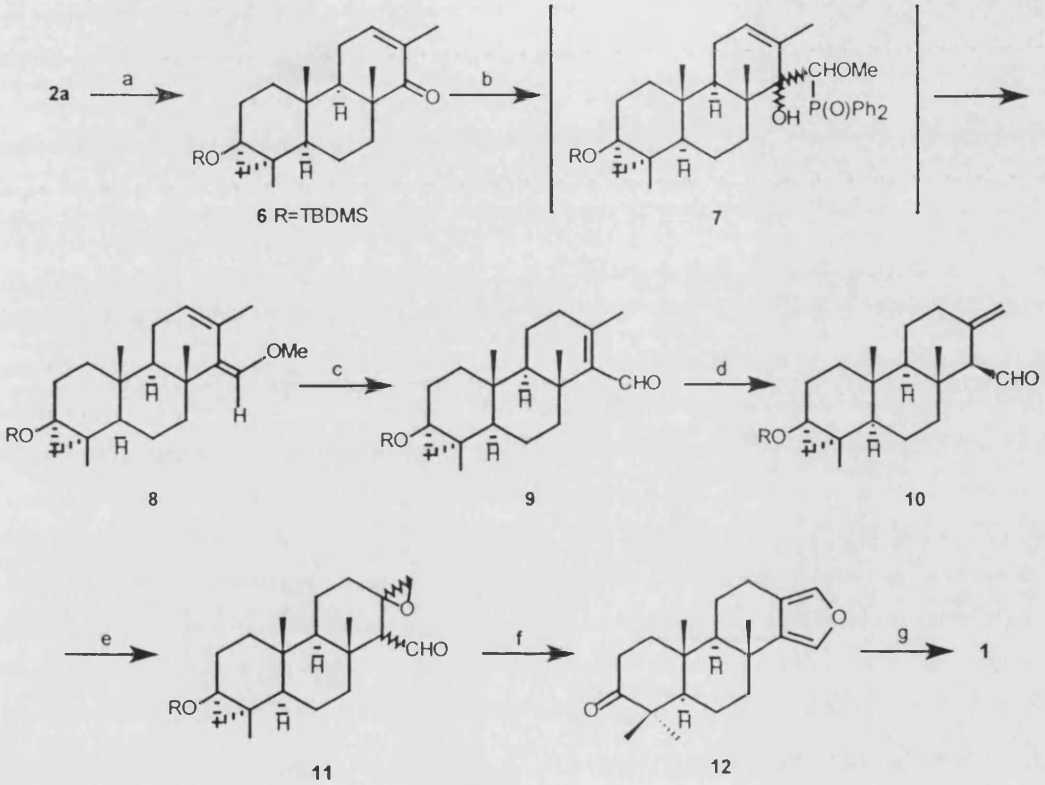



**Scheme 2**

Reagents and Conditions: a) LDA, THF, -10°C then MeI, 87%; b) LDA, THF, HMPA, -78°C then $ICH_2CH_2CH(OEt)_2$, 80%; c) PPTS, acetone-$H_2O$, reflux, 94%; d) $(EtO)_2P(O)C(Na)(Me)COMe$, THF, rt, 86%; e) $Et_3N$, TBDMSTf, $CH_2Cl_2$, -78°C, 98%; f) PhMe, propylene oxide, 190°C, 97%.

*S*-(+)-Carvone was transformed into the aldehyde **4** by double alkylation of its kinetic enolate, first with methyl iodide and then with 3-iodopropanaldehyde diethyl acetal. Subsequent removal of the acetal protecting group through PPTS in aqueous acetone yielded **4** diastereoselectively. Wittig reaction of aldehyde **4** with the $\alpha$-phosphonate carbanion generated from diethyl 2-oxobutane-3-phosphonate and NaH in THF at room temperature gave an (*E*)-enone, which was then converted into the intramolecular Diels-Alder (IMDA) precursor **5** after undergoing treatment with TBDMS triflate and triethylamine in dichloromethane at -78°C. The IMDA reaction of **5** was carried out in toluene solution with a catalytic amount of propylene oxide at 190°C for seven days to give the *trans-anti-trans* fused adduct **2a**.

With the phenanthrenone **2a** in hand, the necessary structural changes for the functionalisation of ring A and the construction of the furan D-ring were made in order to complete the furanospongian skeleton. The synthetic route developed is presented in Scheme 3.




**Scheme 3**

Reagents and Conditions: a) $CH_2I_2$, $ZnEt_2$, toluene, rt, 92%; b) $Ph_2P(O)CH(Li)OCH_3$, THF, -78°C-rt, 90%; c) TMSCl, NaI, $CH_3CN$-$CH_2Cl_2$ 2:3, rt, 90%; d) i). LDA, HMPA, THF, -78°C; ii). $H_2O$-THF 1:3, 50%; e) MCPBA, $CH_2Cl_2$, pH= 8, 5°C, 76%; f) PTSA anhydrous, DMSO-$CH_2Cl_2$ 3:1, 50°C, 53%; g) Hydrazine, di(ethylene glycol), KOH, 120°C-220°C, 75%.

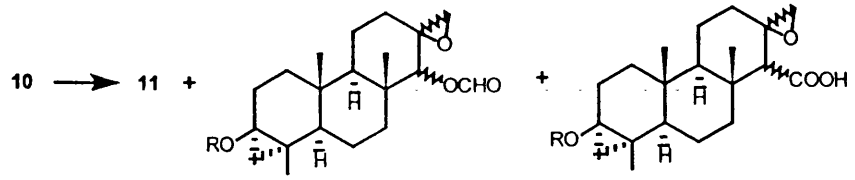
Preliminary attempts at homologation of the C-14 carbonyl group of the enone **2a** showed high instability for the *tert*-butyldimethylsilyl enol ether group present in ring A. For this reason, a cyclopropane ring was introduced stereoselectively and regioselectively into the 3,4-double bond of **2a** by means of cyclopropanation with diiodomethane and diethyl zinc.[10] This gave the enone **6** in 92% yield, as the only stereoisomer to be obtained. We thus obtained a more resistant functionalisation in ring A, which not only allowed us to work on the construction of ring D, but also facilitated the conversion of ring A into the spongian skeleton through cyclopropane ring opening. An NOE enhancement observed between the $\alpha$ hydrogen atom of the methylene

group of the cyclopropane ring at $\delta$ 0.21 ppm and the axial hydrogen atoms at C-5 and C-1 supported the stereochemistry assigned to the cyclopropane ring. A variety of methods were explored for the homologation of enone **6**, but we obtained the best results using the carbanion generated from methoxymethyldiphenylphosphine oxide and LDA/THF at –78°C.[11] This reaction led to a mixture of alcohols **7** in 88% yield. Subsequent standard treatment with NaH/DMF afforded the enol ether **8** in a poor 24% yield. We eventually determined that the yield of this homologation could be notably improved if the reaction was allowed to reach room temperature after the addition of the carbanion. This caused the diphenylphosphine oxide to be eliminated *in situ*, which gave the (*E*)-enol ether **8** as the sole identifiable product in an excellent 90% yield. The assigned stereochemistry at C-15 was supported by the NOE effect observed between the C-16 methyl and the methoxy group.

Once the introduction of the new carbon at C-14 had been accomplished, the functionalisation of C-16 had to be carried out. This proved to be the main difficulty in this synthesis. Treatment of **8** with *tert*-BuOOH and $SeO_2$ supported on silica gel in $CH_2Cl_2$[12] to obtain the allylic alcohol at C-16 led to a complex mixture of products. We eventually determined that the functionalisation at C-16 was only possible through the aldehyde **9** obtained from **8**. Initially, the conversion of the enol ether **8** into the aldehyde **9** was unsuccessful when acid conditions such as HCOOH/$H_2O$ or HCl/ether at room temperature were used; this only yielded recovered starting material. When stronger conditions such as MeOH/HCl 9:1 or Acetone/HCl 9:1 at 60°C were used, the reaction led to mixtures arising from the hydrolysis of the enol ether functionalisation and the *tert*-butyldimethylsilyl group together with products from partial cyclopropane ring opening. We finally completed the synthesis of the aldehyde **9** using nucleophilic conditions; namely, by treatment with trimethylsilyl chloride and NaI[13] at room temperature, obtaining a 90% yield. For this result, however, a very strict control of the time and temperature of reaction was necessary. In all cases the conversion of enol ether **8** into the aldehyde **9** was accompanied by the isomerisation of the 12,13-double bond to 13-14 position, which is a more thermodynamically stable structure. After much experimentation, we were able to introduce a double bond between C-13 and C-16 isomerising **9** to the unconjugated aldehyde **10**.[14] This transformation was carried out through the addition of a mixture of cold water/THF 1:3 to the kinetic enolate of **9**, which had been generated with LDA/HMPA, at –78°C. This afforded **10** in 50% yield and 25% of the starting material. The stereochemistry of aldehyde **10** was confirmed by means of NOE experiments. Irradiation of the hydrogen in the aldehyde group at $\delta$ 9.86 ppm enhanced the signals assigned to the C-17 methyl at $\delta$ 1.17 ppm and H-16 at $\delta$ 4.45 ppm.

After having functionalized C-16, we thought that the epoxidation of the exocyclic double bond and subsequent acid treatment would probably lead to the furan D-ring. But when MCPBA in $CH_2Cl_2$,[15] $CHCl_3$,[16] or ether[17] was used at different temperatures, concentrations and times of reaction, a complex mixture of products was obtained. Among them we identified the epoxides **11**, and the formiates **13** and acids **14** which resulted

from Baeyer-Villiger oxidation (Scheme 4). The use of MCPBA in $CH_2Cl_2$ in a basic medium like 0.5 M $NaHCO_3$[18] decreased the amount of by-products from Baeyer-Villiger oxidation, but the epoxide yield barely reached 50%.




13 R=TBDMS 14

**Scheme 4**

Finally, epoxidation of **10** with MCPBA in $CH_2Cl_2$ at 5°C and pH= 8,[19] gave a mixture of four epoxides **11** in an approximate 55:23:16:6 ratio in 76% yield and with 16% of the starting material. For our purposes, the isolation of the isomeric epoxides was unnecessary since they could be directly transformed into the furan ring.

The next steps involved the cyclisation and aromatisation of the aldehyde and epoxy groups to obtain the furan ring D, followed by hydrolysis and opening of the silyl enol ether and cyclopropane rings, respectively, to complete the *gem*-dimethyl function in ring A. To reduce the number of steps in the sequence, the possibility of carrying out all the processes under the same conditions was explored. After testing various methods, we obtained the optimum yield for the completion of the target furanospongian skeleton by using dry PTSA in DMSO-$CH_2Cl_2$ 3:1 at 50°C for 8 hours, which gave the furanoketone **12** in 53% yield. The spectroscopic data for this compound were identical to those previously reported for the racemic form of **12**.[6b]

We considered carrying out the final conversion of **12** into spongia-13(16)-14-diene **1** by following the procedure described by Kanematsu.[6b] In this process, **12** is first reduced with $LiAlH_4$ to produce an alcohol, which is then converted into the corresponding xanthate, followed by radical reduction with tristrimethylsilylsilane to afford the desired diterpene **1** in 50% overall yield for the three steps. Instead of this elaborate process, however, we chose to use a simple Wolff-Kishner reduction with hydrazine/KOH in di(ethylene) glycol to obtain the pure furanoditerpene **1** as a solid in 75% yield. The spectral and physical data of synthetic **1**, including its optical rotation, were in total agreement with those recorded for the natural product isolated from *Spongia officinalis*.[7]

In conclusion, we have accomplished a diastereoselective synthesis of (-)-spongia-13(16)-14-diene **1** in 9% total yield from *S*-(+)-carvone, using the phenanthrenone **2a**, which was obtained in 53% overall yield from *S*-(+)-carvone in six steps. The utility of the tricyclic intermediates **2** in natural product synthesis has also been well demonstrated. Studies involving the functionalisation of ring A and manipulation of ring D are currently in progress.

## EXPERIMENTAL

*General details*

The melting points were measured with a Büchi 535 apparatus and are uncorrected. Optical rotations were determined on a Schmidt Haensch Polartronic D polarimeter using a 5-cm path-length cell. $[\alpha]_D$-values are given in $10^{-1}$ deg cm$^2$ g$^{-1}$. IR spectra were measured as KBr pellets on a Perkin-Elmer 281 spectrophotometer. NMR spectra were measured on a Varian Unity VXR-300 at 299.95 MHz ($^1$H) and at 75.43 MHz ($^{13}$C), and a Varian Unity VXR-400 at 399.95 MHz ($^1$H). The signal of the deuteriated solvent ($CDCl_3$) was taken as the reference (the singlet at $\delta_H$ 7.24 for $^1$H and the triplet centered at $\delta_C$ 77.00 for $^{13}$C NMR data). Complete assignments of NMR data were made on the basis of a combination of DEPT, HMQC and NOE experiments. *J* values are given in Hz. In all compounds, NMR assignments are given with respect to the numbering scheme shown in structure **1**. Mass spectra (EI) were run on a VG AUTOSPEC SS mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on a silica gel plate (Merck Kieselgel 60 $F_{254}$) and flash chromatography was performed with Merck silica gel 60 (230-400mesh), although the purification by MPLC was carried out on a medium pressure apparatus with a Büchi 688 pump and a Knauer refractometer using Macherey Nagel silica gel 60 Duren (0.015-0.04 mm). Reactions were carried out in an argon atmosphere when necessary. Commercial reagent grade solvents and chemicals were used as obtained unless otherwise noted. THF was distilled from sodium benzophenone ketyl. Organic extracts were washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure on a Büchi rotary evaporator.

*Conversion of the intermediate **2a** into the cyclopropane **6***

To a solution of **2a** (477 mg, 1.23 mmol) in dry toluene (18 mL) at room temperature was added a solution of $Et_2Zn$ in hexane (7.5 mL, 7.38 mmol). $CH_2I_2$ (1.17 mL, 14.70 mmol) was then added dropwise under inert atmosphere. After being stirred for 1 h 40 min at room temperature, saturated aqueous $NH_4Cl$ (6 mL) was added and the mixture was extracted with ether. The extracts were washed, dried, filtered and concentrated. The residue was purified by MPLC (hexane-ethyl acetate 97.5:2.5) to afford the cyclopropane **6** (453 mg, 92%) as a white solid: mp 138-139 °C (from MeOH); $[\alpha]^{25}_D$ +62 (*c* 6.0, $CHCl_3$); $\nu_{max}$/cm$^{-1}$ 3080, 3050, 1660, 1425, 1360, 1260, 1200, 1165, 1010, 910, 840 and 770; $\delta_H$ (400 MHz; $CDCl_3$) 6.61 (1 H, br s, H-12), 1.70 (3 H, br s, H-16), 1.63 (1 H, dd, *J* 12.5 and 7.5, H-9), 1.03 and 1.02 (3 H each, each s, H-19 and H-17), 0.91 (3 H, s, H-20), 0.83 (9 H, s, $Me_3CSi$), 0.53 (1 H, ddd, *J* 14.0, 14.0 and 6.5, H-1α), 0.49 (1 H, d, *J* 4.5, H-18β), 0.21 (1 H, d, *J* 4.5, H-18α), 0.08 and 0.02 (3 H each, each s, $Me_2Si$); $\delta_C$ (75 MHz; $CDCl_3$) 205.81 (C14), 143.38 (C12), 132.92 (C13), 58.23 (C3), 53.73 (C5), 49.90 (C9), 44.59 (C8), 35.68 (C10), 35.05 (C1), 33.83 (C7), 28.84 (C2, C18), 25.73 ($Me_3CSi$), 23.89 (C11), 21.93 (C4), 21.71 (C6), 18.06 (C17), 17.87 ($Me_3CSi$), 16.31 (C16), 15.45 (C19), 13.08 (C20), -3.85 and -3.09 ($Me_2Si$) ; *m/z* (EI) 403 ($M^+$ + 1, 10%), 402 ($M^+$, 40), 346 (25), 345 (90), 317 (5), 288 (7), 253 (12), 211 (100), 159 (10), 119 (14), 95 (11), 82 (13), 75 (45) and 73 (55). HRMS calcd. for

$C_{25}H_{42}O_2Si$ ($M^+$) 402.2954, found 402.2954.

*Preparation of enol ether **8** from cyclopropane **6***

To a suspension of methoxymethyldiphenylphosphine oxide (124 mg, 0.51 mmol) in THF (0.34 mL) at 0°C under an argon atmosphere a 0.6 M solution of LDA in THF (0.67 mL, 0.40 mmol) was added dropwise and stirred until full dissolution (red solution). This solution was cooled to –78 °C, and then a solution of **6** (68 mg, 0.17 mmol) in THF (0.9 mL) was added to it dropwise. The mixture was allowed to warm to room temperature and after being stirred for 1 h at the same temperature, the reaction mixture was quenched by the addition of saturated aqueous $NH_4Cl$ (1 mL), then poured into water and extracted with ether. The diethyl ether solution was washed, dried, filtered and concentrated. The residue was chromatographed (hexane-diethyl ether 9:1) to give the enol ether **8** (66 mg, 90%) as a white solid: mp 124-125 °C (from methanol); $[\alpha]^{25}_D$ +130.3 (*c* 3.7, $CHCl_3$); $\nu_{max}/cm^{-1}$ 3050, 2960, 2930, 2860, 1645, 1620, 1455, 1250, 1190, 1120, 830, 770 and 670; $\delta_H$ (400 MHz; $CDCl_3$) 5.76 (1 H, s, H-15), 5.31 (1 H, br s, H-12), 3.51 (3 H, s, MeO), 1.94 (3 H, br s, H-16), 1.03 (3 H, s, H-19), 0.99 (3 H, s, H-17), 0.84 (9 H, s, $Me_3CSi$ ), 0.82 (3 H, s, H-20), 0.47 (1 H, d, *J* 6.0, H-18β), 0.21 (1 H, d, *J* 6.0, H-18α), 0.08 and 0.02 (3 H each, each s, $Me_2Si$); $\delta_C$ (75 MHz; $CDCl_3$) 141.77 (C15), 130.30 and 128.16 (C13, C14), 124.36 (C12), 59.73 (MeO), 58.47 (C3), 54.38 (C5), 49.43 (C9), 38.52 (C7), 35.91 and 35.44 (C8, C10), 35.50 (C1), 29.04 (C2), 28.82 (C18), 25.85 (*Me*$_3$CSi), 24.08 (C11), 23.74 (C16), 22.60 (C6), 22.10 (C4), 22.01 (C17), 17.87 ($Me_3CSi$), 15.57 (C19), 12.65 (C20), -3.02 and -3.81 ($Me_2Si$); *m/z* (EI) 431 ($M^+$+1, 35%), 430 ($M^+$, 100), 416 (30), 415 (69), 374 (15), 373 (41), 283 (14), 225 (25), 211 (19), 189 (17), 175 (15), 163 (11), 149 (17), 75 (24) and 73 (56). HRMS calcd. for $C_{27}H_{46}O_2Si$ ($M^+$) 430.3267, found 430.3267.

*Conversion of the enol ether **8** into the aldehyde **9***

To a mixture of the enol ether **8** (65 mg, 0.15 mmol) and NaI (23 mg, 0.15 mmol) in $CH_3CN$ (0.9 mL) and $CH_2Cl_2$ (1.35 mL) at 20 °C was added trimethylsilyl chloride (19 μL, 0.15 mmol); the reaction mixture became a dark orange solution. After stirring for only 3 min the reaction mixture was poured into water and extracted with diethyl ether. The residue obtained after usual work-up was purified by column chromatography (hexane-diethyl ether 7:3) to give the aldehyde **9** (57 mg, 90%) as a white solid: mp 156-157 °C (from pentane); $[\alpha]^{25}_D$ +16.1 (*c* 1.2, $CHCl_3$); $\nu_{max}/cm^{-1}$ 3060, 2970, 2940, 2870, 2780, 1670, 1460, 1415, 1260, 840 and 780; $\delta_H$ (300 MHz; $CDCl_3$) 10.00 (1 H, s, H-15), 2.63 (1 H, ddd, *J* 13.0, 3.5 and 3.5), 1.98 (3 H, s, H-16), 1.19 (3 H, s, H-17), 1.00 (3 H, s, H-19), 0.83 (9 H, s, $Me_3CSi$), 0.80 (3 H, s, H-20), 0.48 (1 H, d, *J* 4.5, H-18β), 0.20 (1 H, d, *J* 4.5, H-18α), 0.08 and 0.02 (3 H each, each s, $Me_2Si$); $\delta_C$ (75 MHz; $CDCl_3$) 192.54 (C15), 153.41 (C14), 143.72 (C13), 58.29 (C3), 54.33 (C5), 52.25 (C9), 37.42 (C8), 36.85 and 36.65 (C7, C12), 35.88 (C1), 35.60 (C10), 29.28 (C2), 29.17 (C18), 25.79 (*Me*$_3$CSi), 22.38 (C6), 22.14 (C4), 21.12 (C17), 19.09 (C16), 17.92 ($Me_3CSi$), 17.79 (C11), 15.37 (C19), 12.15 (C20), -3.04 and -3.78 ($Me_2Si$); *m/z* (EI) 417 ($M^+$ + 1, 8%), 416 ($M^+$, 23), 360

(23), 359 (77), 213 (12), 212 (23), 211 (100), 75 (23) and 73 (44). HRMS calcd. for $C_{26}H_{44}O_2Si$ ($M^+$) 416.3110, found 416.3110.

*Isomerisation of 9 to the unconjugated aldehyde 10*

To a cooled (-78 °C) solution of aldehyde **9** (44 mg, 0.11 mmol) in THF (1.25 mL) a solution of 0.6 M LDA in THF (0.26 mL, 0.16 mmol) was added dropwise during 5 min; the mixture became a yellow solution. After 15 min HMPA (92 μL) was added and the mixture was stirred for 20 min more. A mixture of cold water-THF 1:3 (2 mL) was then added and the reaction mixture was poured into cold water (5 °C) and extracted with diethyl ether. The combined extracts were washed, dried, filtered and concentrated. The residue was purified by column chromatography (hexane-diethyl ether 9:1) to afford the aldehyde **10** (22 mg, 50%) as a white solid and 11 mg (25%) of starting material, mp 97-98 °C (from methanol); $[\alpha]^{25}_D$ +69.7 (*c* 2.2, $CHCl_3$); $\nu_{max}/cm^{-1}$ 3060, 2930, 2860, 1720, 1640, 1460, 1430, 1380, 1250, 1190, 900, 830, 770 and 670; $\delta_H$ (300 MHz; $CDCl_3$) 9.86 (1 H, d, *J* 4.8, H-15), 4.86 (1 H, br s, H-16a), 4.45 (1 H, br s, H-16b), 1.17 (3 H, s, H-17), 1.00 (3 H, s, H-19), 0.83 (9 H, s, $Me_3CSi$), 0.81 (3 H, s, H-20), 0.48 (1 H, d, *J* 5.0, H-18β), 0.21 (1 H, d, *J* 5.0, H-18α), 0.08 and 0.02 (3 H each, each s, $Me_2Si$); $\delta_C$ (75 MHz; $CDCl_3$) 205.65 (C15), 144.80 (C13), 109.21 (C16), 67.99 (C14), 58.19 (C3), 54.79 and 54.41 (C5, C9), 40.21 and 36.57 (C7, C12), 38.84 (C8), 35.93 (C1), 35.84 (C10), 29.18 (C2), 29.04 (C18), 25.77 ($Me_3CSi$), 22.42 and 22.21 (C6, C11), 22.09 (C4), 17.90 ($Me_3CSi$), 17.04 (C17), 15.40 (C19), 12.49 (C20), -3.06 and -3.81 ($Me_2Si$); *m/z* (EI) 417 ($M^+$ + 1, 5%), 416 ($M^+$, 19), 360 (17), 359 (57), 213 (11), 212 (23), 211 (100), 155 (10), 75 (29) and 73 (55). HRMS calcd. for $C_{26}H_{44}O_2Si$ ($M^+$) 416.3110, found 416.3119.

*Conversion of the aldehyde 10 into the epoxides mixture 11*

To a solution of aldehyde **10** (120 mg, 0.29 mmol) in $CH_2Cl_2$ (10.2 mL) was added a 0.5 M buffer solution of $Na_2HPO_4/KHPO_4$ (10.2 mL, pH= 8). The mixture was then cooled to 5 °C and MCPBA (41 mg, 0.20 mmol) was added. After being stirred for 16 h MCPBA (39 mg, 0.17 mmol) was added again. The reaction mixture was stirred for 2 days at the same temperature (5°C), and then diluted with diethyl ether. The diethyl ether solution was washed first with 0.5 M $Na_2S_2O_4$, then with saturated aqueous $Na_2CO_3$ and finally with brine. After drying and removal of the solvent, the residue was chromatographed over silica gel using hexane-ethyl acetate (95:5) as eluent to give 19 mg (16%) of starting material and a mixture of four epoxides **11** (94 mg, 76%) in an approximate 55:23:16:6 ratio (determined by integration of $^1H$ NMR signals). $^1H$ NMR data of major product (from the mixture) are given: $\delta_H$ (400 MHz; $CDCl_3$) 9.55 (1 H, d, *J* 4.0, H-15), 3.11 (1 H, dd, *J* 4.1 and 1.9, H-16a), 2.68 (1 H, dd, *J* 4.1 and 1.0, H-16b), 2.41 (1 H, d, *J* 4.0, H-14), 1.23 (3 H, s, H-17), 0.99 (3 H, s, H-19), 0.83 (9 H, s, $Me_3CSi$), 0.81 (3 H, s, H-20), 0.21 (1 H, m, H-18α), 0.08 and 0.02 (3 H each, each s, $Me_2Si$).

*Preparation of spongia-13(16),14-dien-3-one* **12**

To a solution of epoxides **11** (22 mg, 0.051 mmol) in $CH_2Cl_2$ (0.18 mL) and DMSO (0.27 mL) was added a solution of anhydrous PTSA in DMSO (0.27 mL, 330 mg PTSA/mL). The reaction mixture was warmed to 50°C and after being stirred for 8 h the reaction mixture was diluted with diethyl ether and worked up as usual to give the furanoketone **12** (8.1 mg, 53%) as a white solid: mp 116-117 °C (from hexane) (lit.,[6b] 120-121 °C); $[\alpha]^{20}_D$ +11.8 (*c* 3.5, $CHCl_3$); $\nu_{max}/cm^{-1}$ 2950, 2910, 2840, 1690, 1450, 1380, 1030 and 790; $\delta_H$ (400 MHz; $CDCl_3$) 7.08 (1 H, d, *J* 1.7, H-15), 7.04 (1 H, ddd, *J* 3.0, 1.7 and 1.7, H-16), 2.78 (1 H, dd, *J* 15.9 and 6.1, H-12β), 2.58-2.40 (3 H, m), 2.12 (1 H, m), 2.02 (1 H, ddd, *J* 12.9, 7.5 and 4.8), 1.22 (3 H, s, H-17), 1.08 (3 H, s, Me), 1.06 (3 H, s, Me) and 0.98 (3 H, s, Me); $\delta_C$ (100 MHz; $CDCl_3$) 217.77 (C3), 136.92 (C16), 136.86 (C14), 135.15 (C15), 119.56 (C13), 55.35 and 54.84 (C5, C9), 47.38 (C4), 40.17 (C7), 39.15 (C1), 36.90 (C10), 34.06 (C8), 33.91 (C2), 26.78 (C17), 25.71 (C18), 20.91 (C19), 20.69 (C12), 19.78 and 18.73 (C6, C11), 16.19 (C20); *m/z* (EI) 301 ($M^+$+1, 18%), 300 ($M^+$, 80), 286 (21), 285 (100), 267 (15), 147 (18) and 133 (10). HRMS calcd. for $C_{20}H_{28}O_2$ ($M^+$) 300.2089, found 300.2085.

*Spongia-13(16),14-diene* **1**

A solution of the ketone **12** (10 mg, 0.034 mmol), KOH 85% (60 mg, 0.91 mmol), 0.38 mL of di(ethylene glycol) and 0.03 mL of hydrazine monohydrate was stirred at 120 °C for 2 hours. The temperature was then brought to 220 °C while a slow stream of argon was maintained; this process took 30 minutes. The mixture was then stirred at this temperature for 15 additional minutes. The reaction mixture was cooled, poured over saturated aqueous $NH_4Cl$ and extracted with diethyl ether. The organic layer was washed, dried, filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexane-diethyl ether (9:1) as eluent to afford spongian **1** (7.1 mg, 75%) as a colorless solid, mp 112-113 °C (from methanol) (lit.,[7] 115-116 °C) $[\alpha]^{26}_D$ –21.1 (*c* 0.5, $CHCl_3$) (lit.,[7] –32.7, *c* 0.26); $\nu_{max}/cm^{-1}$ 1460, 1390, 1380, 1370, 1040, 895 and 770; $\delta_H$ (300 MHz; $CDCl_3$) 7.06 (1 H, d, *J* 1.6, H-15), 7.02 (1 H, ddd, *J* 1.6, 1.6 and 1.2, H-16), 2.74 (1 H, dddd, *J* 16.2, 6.2, 1.7 and 1.2, H-12β), 2.42 (1 H, dddd, *J* 16.2, 12.0, 7.1 and 1.6, H-12α), 2.07 (1 H, m, H-7β), 1.20 (3 H, d, *J* 0.7, H-17), 0.88 (3 H, s, H-20), 0.85 (3 H, s, H-18), 0.82 (3 H, s, H-19); $\delta_C$ (75 MHz; $CDCl_3$) 137.77 (C14), 136.67 (C16), 134.97 (C15), 119.87 (C13), 56.76 (C5), 56.29 (C9), 42.13 (C3), 41.16 (C7), 39.97 (C1), 37.63 (C10), 34.35 (C8), 33.37 (C4, C18), 26.28 (C17), 21.43 (C19), 20.71 (C12), 18.80 (C11), 18.54 (C2), 18.05 (C6), 16.33 (C20); *m/z* (EI) 287 ($M^+$+1, 13%), 286 ($M^+$, 63), 272 (20) and 271 (100). HRMS calcd. for $C_{20}H_{30}O$ ($M^+$) 286.2297, found 286.2295.

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# Synthesis of spongian diterpenes: (–)-spongian-16-oxo-17-al and (–)-acetyldendrillol-1

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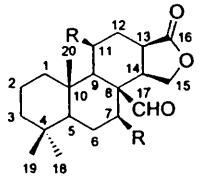
**Abstract**—An efficient diastereoselective synthesis of the spongian diterpenes (–)-spongian-16-oxo-17-al (**1**) and (–)-acetyldendrillol-1 (**13**) is described starting from (+)-podocarp-8(14)-13-one (**6**) via the ester-dialdehyde **11** as key intermediate. The absolute configuration at C-17 in synthetic compound **13** has conclusively been proved by NOE experiments. 

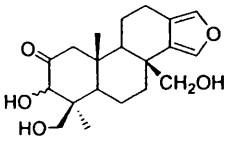
Spongian diterpenes are a family of tetracyclic and pentacyclic metabolites existing in marine organisms such as sponges and nudibranches.[1,2] These compounds have attracted the attention of synthetic chemists and biologists since they were discovered in 1974,[3] because of their unique structural features and wide spectrum of biological activities.[4–6]

Some spongian diterpenes display an oxygenated function at C-17 (**1–5**) (Fig. 1)[6–12] having both tetracyclic and pentacyclic structures. To the best of our knowledge, a complete synthesis of tetracyclic spongians functionalized at C-17 has not yet been developed: only the preparation of an intermediate which already contains a hydroxymethyl group at C-17 has been reported.[13] In the case of the pentacyclic spongians, we have already reported the preparation of (–)-dendrillol-1 and also of three other related diterpenes oxygenated at C-7 (**4**).[14–16] Thus, in connection with our work on spongian diterpene synthesis,[17,18] we report the first diastereoselective synthesis of a 17-oxygenated tetracyclic spongian **1** isolated from the nudibranch *Cerato-soma brevicaudatum*[7] and also the preparation of **13** isolated from the dorid nudibranch *Cadlina luteomarginata*,[12] whose C-17 absolute configuration has wrongly been assigned based on a 17α-acetoxy orientation (see **5**). It is worth to note that both spongian diterpenes have been isolated in such minute amounts that no investigation of their bioactivity has been possible.

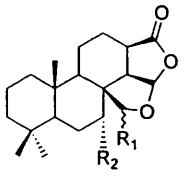
Our synthesis (Scheme 1) starts with podocarpenone **6**,[19] which is converted, in five steps, into the key intermediate **11**. Then, this intermediate is used in separate approaches to the synthesis of (–)-spongian-16-oxo-17-al **1** and (–)-acetyldendrillol-1 **13**.




**1** R=H
**2** R=OAc




**3**




**4** $R_1$=β-OH; $R_2$=H,OH,OAc,OCOPr
**5** $R_1$=α-OAc; $R_2$=H

**Figure 1.**

*Keywords*: diterpenes; marine metabolites; sponges; synthesis; podocarpenone.

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Podocarpenone **6** was transformed into the ester **10** using the methodology previously developed in our laboratory for related compounds.[16] Thus, irradiation of podocarpenone **6** in dry acetone saturated with acetylene at −40°C resulted in the stereoselective formation of the photoadduct **7** in 60% yield. Treatment of cyclobutenone **7** with diethyl phosphorocyanidate [$(EtO)_2P(O)CN$] and LiCN in THF/DMF at 0°C gave a mixture of epimeric cyano phosphates at C-13 (**8**) in essentially quantitative yield. The crude cyano phosphates **8** were reduced by treatment with $SmI_2$ and *t*-BuOH in THF at room temperature to give a mixture of nitriles **9**.[20] Alkaline hydrolysis of both nitriles using potassium hydroxide in ethylene glycol ethyl ether at 110°C, followed by in situ treatment with dimethyl sulfate afforded a 93:7 mixture of methyl ester **10** and the corresponding epimer at C-13. Chromatographic separation of these compounds afforded the isomer **10** in 85% yield from **7**. Ozonolysis of the cyclobutene ring of ester **10** followed by decomposition of the resultant ozonide with $Me_2S$ provided the ester–dialdehyde **11** in 90% yield. This dialdehyde **11** showed a high tendency to internal lactone–hemiacetal formation.

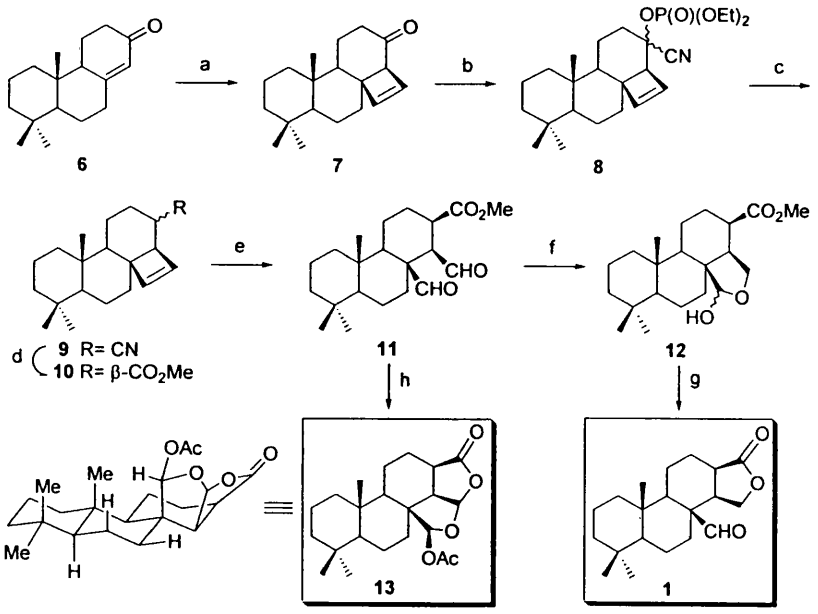
With the 1,4-dialdehyde **11** in hand, our next objective was to accomplish the preparation of the aforementioned spongians **1** and **13**. Toward the preparation of **1**, regioselective reduction of the carbonyl group at C-15 of dialdehyde **11** was carried out using $NaBH_4$/MeOH at 0°C to afford a 2:1 mixture of ester–hemiacetals **12**. Subsequent lactonization of the crude hemiacetals **12** using *p*-toluenesulfonic acid (PTSA) in refluxing benzene for 18 h furnished (−)-spongian-16-oxo-17-al (**1**) in 65% overall yield from **11**. The synthetic tetracyclic spongian **1**[21] had spectroscopic data identical to those recorded for the natural product.[7]

After completion of the synthesis of tetracyclic spongian **1** we studied the utility of the key intermediate **11** for the synthesis of the pentacyclic spongian **13**. A variety of methods were explored for the lactone–hemiacetal formation and in situ acetylation of **11**. In the end this conversion was successfully accomplished by using a catalytic amount of sulfuric acid (1%) in a 9:1 mixture of acetic acid and acetic anhydride at 65°C for 17 h. Thus, **13** was obtained in 85% yield directly from **11**. The synthetic pentacyclic spongian **13**[22] had spectroscopic data identical to those recorded for the natural product.[12] Furthermore, a detailed analysis of the NMR spectra of the synthetic **13** permitted us to unequivocally assign the configuration at C-17 of natural product as 17β-acetoxy instead of the 17α reported by Andersen and co-workers (see **5**). In particular, the NOE enhancement of the signals due to protons H20, H6β and H7β when proton H17 was irradiated conclusively proved this assignment.[23]

Application of our synthetic route to the preparation of other spongians as well as studies of the antiviral and antitumor activities of compounds **1** and **13** are currently under study.

**Acknowledgements**

We are grateful for financial support from DGICYT (Research Program PB95-1088). M.A.G. also thanks




**Scheme 1.** *Reagents and conditions*: (a) $C_2H_2$, acetone, *hv*, −40°C, 60%; (b) $(EtO)_2P(O)CN$, LiCN, THF/DMF, 0°C; (c) $SmI_2$, *t*-BuOH, THF; (d) KOH, $HOCH_2CH_2OEt$, 110°C; then $(CH_3)_2SO_4$, DMF, 85% from **7**; (e) $O_3$, $CH_2Cl_2$, −78°C; then $(CH_3)_2S$, −78 to 5°C, 90%; (f) $NaBH_4$, MeOH, 0°C; (g) PTSA, benzene, reflux, 65% from **11**; (h) $H_2SO_4$ (1%), $AcOH/Ac_2O$ (9:1), 65°C, 85%.

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20. The conversion of ketone **7** to nitriles **9** could also be achieved using tosylmethyl isocyanide (TosMIC), but the yield is lower (see Ref. 15).
21. Compound **1**: white solid, mp 176–178°C (from hexane–$CH_2Cl_2$); $[\alpha]_D^{24}$ −59 (*c* 1.64, $CHCl_3$); IR (KBr): $\nu_{max}/cm^{-1}$ 2943, 2738, 1769, 1708; $\delta_H$ (300 MHz; $CDCl_3$) 9.96 (1H, d, *J* 1.5, H-17), 4.09 (1H, dd, *J* 10, 4.5, H-15), 4.04 (1H, br d, *J* 10, H-15), 2.67 (1H, m, H-13), 2.54 (1H, dt, *J* 13, 3, H-7β), 2.50 (1H, m, H-12β), 2.28 (1H, dd, *J* 7.5, 4.5, H-14), 0.85 (3H, s, H-19), 0.75 (3H, s, H-18), 0.69 (3H, s, H-20); $\delta_C$ (75 MHz; $CDCl_3$) 203.8, 177.7, 66.9, 56.6, 55.9, 50.8, 49.0, 41.8, 39.0, 38.5, 37.8, 35.7, 33.4, 33.3, 23.1, 21.4, 18.8, 18.6, 16.3, 14.9; HRMS (EI): *m/z* calcd for $C_{20}H_{30}O_3$ ($M^+$) 318.2195, found 318.2193.
22. Compound **13**: white solid, mp 218–219°C (from MeOH); $[\alpha]_D^{25}$ −75 (*c* 1.90, $CHCl_3$); IR (KBr): $\nu_{max}/cm^{-1}$ 2930, 1785, 1754, 1223; $\delta_H$ (400 MHz; $CDCl_3$) 6.29 (1H, s, H-17), 6.11 (1H, d, *J* 6, H-15), 2.76 (1H, dd, *J* 11, 7, H-13), 2.65 (1H, dd, *J* 11, 6, H-14), 2.45 (1H, br d, *J* 11.5, H-12β), 2.04 (3H, s, OCO*Me*), 1.94 (1H, dt, *J* 13.5, 3, H-7β), 0.86 (3H, s, H-18), 0.81 (3H, s, H-19), 0.72 (3H, s, H-20); $\delta_C$ (75 MHz; $CDCl_3$) 176.6, 169.1, 104.2, 100.6, 56.8, 55.3, 49.2, 46.2, 41.8, 41.7, 39.0, 38.1, 37.5, 33.3, 33.3, 23.7, 21.5, 21.3, 19.9, 18.7, 16.6, 15.6; HRMS (EI): *m/z* calcd for $C_{22}H_{32}O_5$ ($M^+$) 376.2250, found 376.2240.
23. Once we noted that the stereochemistry at C-17 for compounds **13** and (−)-dendrillol-1 (**4** $R_1$ = β-OH, $R_2$ = H) was the same, we envisaged that (−)-dendrillol-1 could be transformed into **13** under acetylation conditions. However, when (−)-dendrillol-1 was submitted to the usual acetylation conditions only products resulting from acetylation after opening of the hemiacetal system were obtained. Eventually, (−)-dendrillol-1 could be converted into **13** under the conditions described for the conversion of **11** into **13**.

**AUTORES:** Manuel Arnó, Miguel A. González and Ramón J. Zaragozá

**TÍTULO:** Synthesis of C-17-Functionalized Spongian Diterpenes: Diastereoselective Synthesis of (-)-Aplyroseol-14 from (-)-Abietic Acid

**REF. REVISTA:** Manuscript in preparation.

# Synthesis of C-17-Functionalized Spongiane Diterpenes: Diastereoselective Synthesis of (-)-Acetyldendrillol-1, (-)-Spongian-16-oxo-17-al and (-)-Aplyroseol-14 from (-)-Abietic Acid†

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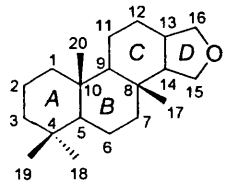
*Abstract:* The diastereoselective synthesis of the spongiane diterpenes (-)-acetyldendrillol-1 (**24**), (-)-spongian-16-oxo-17-al (**2**) and (-)-aplyroseol-14 (**31**) has been completed efficiently starting from (+)-podocarp-8(14)-13-one **16**, easily available from commercial (-)-abietic acid. The key steps in the syntheses were a "one-pot" acetalization-acetylation reaction, a regioselective reduction of a 1,4-dialdehyde unit and a translactonization reaction, respectively. The synthesis of **24** led us to the revision of the absolute configuration at C-17 for natural (-)-acetyldendrillol-1 by means of NOE experiments. We have also initiated a new method for the mild and selective *in situ* reductive acetylation of carbonyl groups, which allowed us to reassign chemically and unambiguously the structural features of aplyroseol-14 **31**. Thus, aplyroseol-14 **31** presents an unprecedented δ-lactone-based structure for spongiane-type diterpenoids.

## Introduction

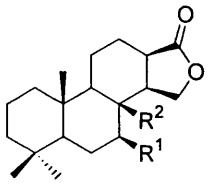
Sponges are the most primitive of the multicellular animals, containing neither true tissues nor organs. They have proved to be a rich source of new bioactive products with ecological or biomedical importance.[1] Sponges face a variety of dangers in their environment, but they have few predators due to the production of allelochemicals which deter feeding and also kill off any attacker microorganism. Nudibranchs, a family of sea slugs has many members that sequester secondary metabolites from sea fans and sponges, on which they feeds.[2] By using the concentrated toxins taken from the sponges, nudibranchs acquire the same protection. This led to the investigation of the "chemical defense" and its mechanism in these organisms.[3]

Among the numerous bioactive substances isolated from sponges and nudibranchs,[1] the spongiane family[4,5] comprises a group of tetracyclic and pentacyclic metabolites characterized by the carbon framework (**1**), named spongiane skeleton. These compounds have attracted the interest of synthetic organic chemists and biologists since they were discovered in 1974 by Minale and co-workers.[6] Since then, these have revealed a wide spectrum of biological activities, including antifungal, antimicrobial, antifeedant, antiviral, and antitumor activities, as well as $PLA_2$ inhibition.[7] To date there are about seventy known members of this family which differ basically in the extent of oxidation at C-17 and C-19, and the oxidation pattern on rings A, B, C and D.[8] Most of them possess a γ-lactone[9] or furan ring D[5a] but also there are examples where the D-ring system contains an anhydride,[10] an acetal or lactol,[5d,9] and even two acetylated acetals (**2-9**).[7d,11] Another interesting subgroup of spongianes presents an extra ring E (**10-12**).[9]

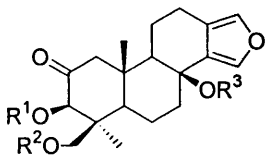
During the past two decades, several syntheses of compounds with the spongiane carbon framework have been reported starting from manool,[12] methyl isocopalate,[13] labda-8(20),13,dien-15-oic acid,[14] and furfuryl alcohol[15] which have led to racemic products in most cases.



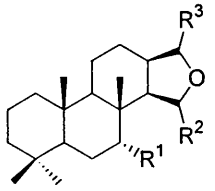

**1** spongiane skeleton



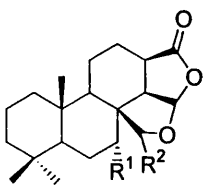

**2** $R^1$= H;$R^2$= CHO
**3** $R^1$= H;$R^2$= $CH_2OAc$
**4** $R^1$= α-OAc;$R^2$= $CH_2OAc$




**5** $R^1$= H;$R^2$= $R^3$= Ac
**6** $R^1$= $R^2$= Ac;$R^3$= H




**7** $R^1$= H;$R^2$= $R^3$= =O
**8** $R^1$= H;$R^2$= $R^3$= α-OAc
**9** $R^1$= OAc;$R^2$= α-OH;$R^3$= =O




**10** $R^1$= H,OH,OAc,OCOPr
$R^2$= β-OH
**11** $R^1$= H;$R^2$= α-OAc
**12** $R^1$= OAc;$R^2$= α-OAc

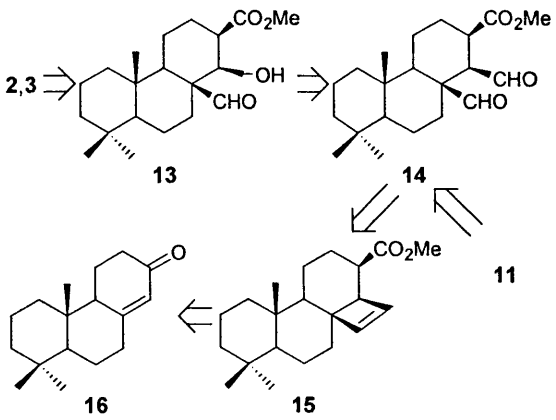
It has also been reported a number of powerful biomimetic-like polyene cyclizations leading to practically the complete spongiane skeleton,[16,17] but none of them afforded the targeted products in optically active form. We also became interested in these fascinating compounds around a decade ago, and since then we have developed several strategies, starting from either (+)-podocarp-8(14)-13-one[18] **16** or commercial *S*-(+)-carvone,[19] to obtain spongiane-type diterpenes in enantiomerically pure form. Podocarpenones like **16** are versatile chiral starting materials, which are easily obtained in quantity from commercially available (-)abietic acid using our method,[20] and have been used to good advantage in terpene or steroid synthesis.[21] In connection with our work on spongiane diterpene synthesis, we sought a convenient route towards the synthesis of tetracyclic spongianes functionalized at C-17, since little synthetic attention had been paid to this group of natural spongianes.[17c]

In this paper, we describe another utility of the chiral synthon **16** for the synthesis of optically active C-17-functionalized tetracyclic spongianes and as an extension an alternative route to obtain pentacyclic spongianes, of which we have already reported the preparation of (-)-dendrillol-1 **10** ($R^1$= H; $R^2$= β-OH) and also of three other related diterpenes oxygenated at C-7 (**10**).[18a-c] In particular, we disclose[22] the strategy to achieve the first diastereoselective synthesis of 17-oxygenated tetracyclic spongianes, such as (-)-spongian-16-oxo-17-al (**2**) isolated from the nudibranch *Ceratosoma brevicaudatum*[23] and (-)-aplyroseol-14 (**31**) isolated from the sponge *Aplysilla Rosea* Barrois,[9] whose reported structure has been revised and reassigned (**3** => **31**). The preparation of the new pentacyclic spongiane (**24**) isolated from the dorid nudibranch *Cadlina luteomarginata*,[24] and the revision of the reported stereochemistry at C-17 for natural (-)-acetyldendrillol-1 (**11** => **24**) is also disclosed.

**Results and Discussion**

The retrosynthetic strategy toward spongianes **2**, **3** and **24** is illustrated in Scheme **1**. The preparation of the tricyclic ester-dialdehyde **14**, which already contains an oxigen functionality at C-17 and the full carbon backbone as required, would allow to obtain either tetracyclic (through **13**) or pentacyclic spongiane diterpenes. From our earlier work we were confident that the intermediate **14** could be obtained from podocarpenone **16** by a sequence of reactions involving (1) introduction of a potential dialdehyde unit by photochemical reaction with acetylene; (2) homolagation at C-13; and (3) ozonolysis of the cyclobutene moiety. However, we were less sure of isolating this intermediate due to the probable internal lactone-hemiacetal formation as it occurs in the corresponding acid-dialdehyde derivative.

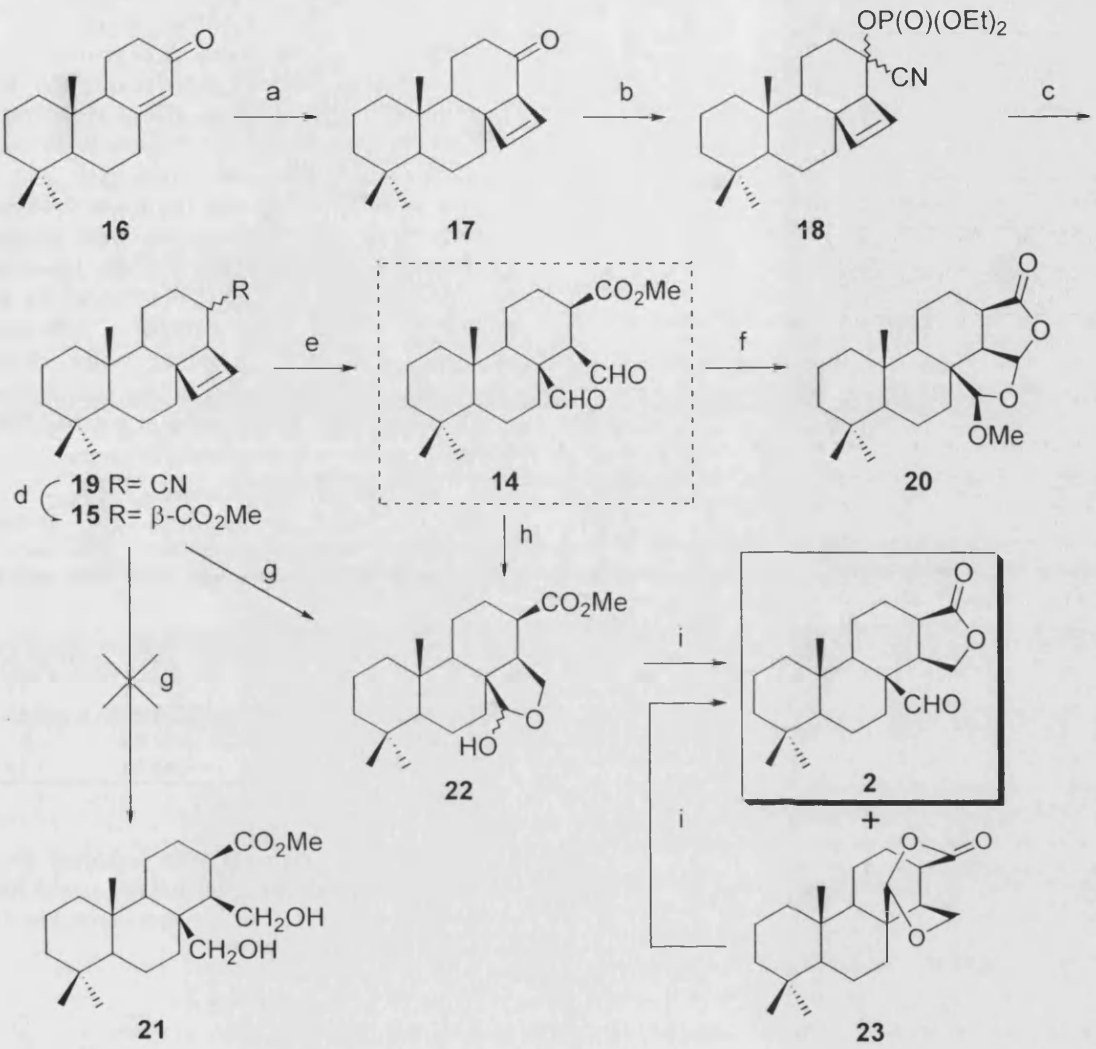
**Scheme 1**




The application of this plan is shown in the Scheme **2**. Our synthetic route started with the readily available podocarpenone (+)-**16**[20] which is converted, in five steps, into the key intermediate **14**. Then, this intermediate is used in separate approaches to the synthesis of the tetracyclic metabolites (-)-spongian -16-oxo-17-al **2** and (-)-aplyroseol-14 **31**, and the pentacyclic diterpene (-)-acetyldendrillol-1 **24**.

Podocarpenone **16** was transformed into the ester **15** using the methodology previously developed in our laboratory for related compounds.[18c] Thus, the photoaddition of acetylene was carried out by irradiation of podocarpenone **16** in dry acetone saturated with acetylene at –40°C to give stereoselectively the photoadduct **17** (60%). To effect the required homologation at the C-13 carbonyl group we initially thought that the reductive cyanation, using tosylmethyl isocyanide (TosMIC),[25] would be the method of choice since we had previously obtained up to a 67% yield of a 7:3 mixture of β- and α- nitriles **18**.[18b] However, the cyanophosphorylation and subsequent removal of the phosphate moiety seemed to us a more efficient method[26] if successful, since the same conversion for the 7-hydroxylated analogue furnishes a 83% yield of the corresponding epimeric nitriles. Considering this, we treated the cyclobutenone **17** with diethyl phosphorocyanidate [$(EtO)_2P(O)CN$] and LiCN in THF/DMF at 0°C and it was found a mixture of epimeric cyano phosphates at C-13 (**18**) in essentially quantitative yield, after working up the reaction mixture.

**Scheme 2**[a]




[a] (a) $C_2H_2$, acetone, hν, -40°C, 60%; (b) $(EtO)_2P(O)CN$, LiCN, THF/DMF, 0°C; (c) $SmI_2$, *t*-BuOH, THF; (d) KOH, $HOCH_2CH_2OEt$, 110°C; then $(CH_3)_2SO_4$, DMF, 85% from **17**; (e) $O_3$, $CH_2Cl_2$, -78°C; then $(CH_3)_2S$, -78°C-5°C, >90%; (f) $NaBH_4$, MeOH, -78°C; (g) $O_3$, MeOH or $CH_2Cl_2$, -78°C; then $NaBH_4$ or $BH_3{\cdot}Me_2S$, -78°C-rt; (h) $NaBH_4$, MeOH, 0°C, quant.; (i) PTSA, benzene, reflux or $CF_3CO_2H$, 2:3 acetone/water, reflux, 65% from **14**.

Subsequent treatment of the crude cyano phosphates **18** with $SmI_2$ and *t*-BuOH in THF at room temperature gave a mixture of nitriles **19**. Alkaline hydrolysis of both nitriles using potassium hydroxide in ethylene glycol ethyl ether at 110°C overnight, followed by *in situ* treatment with dimethyl sulfate afforded a 93:7 mixture of 13β-ester **15** and the corresponding epimer in a 91% overall yield from **17**, after careful chromatographic separation. The minor 13α-isomer can be recycled by equilibration with 2% sodium methoxide in MeOH at 80°C. Therefore, with these optimized conditions we were able to obtain the required ester **15** in an approximately 90% yield from **17** without any separation of epimer intermediates at C-13.

Preliminary attempts to effect the required cleavage of the cyclobutene moiety of **15** were carried out by ozonolysis in either MeOH or $CH_2Cl_2$ at –78°C, followed by decomposition of the resultant ozonides with $NaBH_4$ or $BH_3{\cdot}Me_2S$ (Scheme **2**).[27] These initial experiments to convert directly the ozonides into alcohols **21** led to complex mixtures of products with poor yields of hemiacetals **22**, for example; and showed us the high tendency of the initially formed dialdehyde **14** to lactone-hemiacetal formation on isolating (-)-dendrillol-1 **10** ($R^1$= H; $R^2$= β-OH) and its methoxy derivative

**20** (if MeOH is used as solvent or co-solvent) among the products. Ozonolysis of the cyclobutene ring of ester **15** followed by decomposition of the resultant ozonide with $Me_2S$ provided the crude ester-dialdehyde **14** in essentially quantitative yield (ca. 98% purity), which was used without further purification. For this result, it was necessary the treatment of the ozonide with $Me_2S$ overnight at 5°C. Attempts to purify **14** by chromatography over silica gel or neutral alumina reduced the yield because of the formation of (-)-dendrillol-1.

With the 1,4-dialdehyde **14** in hand, our next objective was to accomplish the preparation of the afore-mentioned spongianes **2**, **3** and **24**. We firstly focused our efforts on the synthesis of the tetracyclic spongiane **2**. To this end, regioselective reduction of the carbonyl group at C-15 of dialdehyde **14** was required (Scheme 2). First attempts of reduction with $NaBH_4$/MeOH at –78°C led to complex mixture of products where the compound **20** was identified as the major product. Reduction with $BH_3$·THF[28] gave **22** in moderate yield (ca. 40%), but common reduction using $NaBH_4$/MeOH at 0°C afforded a 2:1 mixture of ester-hemiacetals **22** in high yield. After chromatographic purification we realized that this step could have been ignored since both epimers were not separated, probably due to a rapid equilibration process between them. In order to obtain the lactone ring present in natural **2** several lactonization conditions were tried on **22** such as 9:1 acetone/HCl, 1% $H_2SO_4$/AcOH, *p*-toluenesulfonic acid (PTSA)/PhH or PTSA/toluene, which normally led to mixtures varying from 1:1 to 3:1 of γ-lactone **2** and the hemiacetalic δ-lactone **23**, respectively. Therefore, after testing different experimental conditions, lactonization of the crude hemiacetals **22** using PTSA in refluxing benzene for 18 h furnished after careful flash chromatography (-)-spongian-16-oxo-17-al (**2**) in an optimized overall yield of 65% from **14**, accompanied by δ-lactone **23** (20%). An identical result was later obtained by the employment of trifluoroacetic acid (TFA) in a 2:3 mixture of acetone/water at reflux for 60 h. The synthetic tetracyclic spongiane **2** had spectroscopic data identical to those recorded for the natural product.[23] The structure shown in Scheme 2 for compound **23** was supported by their physical and spectroscopic data. This minor isomer can be conveniently recycled under the above-mentioned conditions to give a 3:1 mixture of spongiane **2** and recovered starting material **23**, respectively.

After having successfully accomplished the synthesis of the tetracyclic spongiane **2**, we studied the utility of the key intermediate **14** for the synthesis of the new pentacyclic spongiane **24**, and other related marine metabolites. Therefore, it was envisaged that the conversion of **14** into the natural product **24** could be done under simple acetylation conditions (Scheme 3), since it was clear that the internal lactone-hemiacetal formation is highly favoured; although the outcome of the stereochemistry at C-17 was unknown. Predictions from a conformational analysis and molecular mechanics calculations[29] (Fig. **1**, Table **1**) indicated that the formation enthalpy difference for the more stable conformer (chair ring-C conformation, AM1), is only of 1.2 kcal/mol, which means a 85:15 ratio of β:α epimers at the equilibrium at room temperature. This result is in good agreement with the existence of α–epimers in nature.

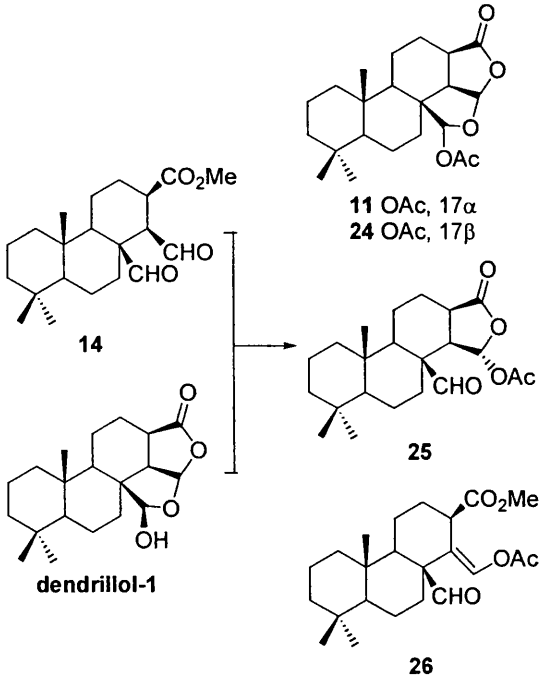
**Table 1**. Molecular mechanics and semiempiric calculations of formation enthalpy (kcal $mol^{-1}$) for compounds **11** and **24** in both chair and boat C-ring conformations.

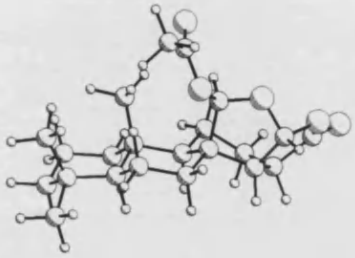
| Compound | PCMODEL | AM1 | Dif.(AM1)* |
|---|---|---|---|
| **11, C-chair** | -259.01 | -250.30 | 1.19 |
| **11, C-boat** | -259.97 | -248.67 | 2.82 |
| **24, C-chair** | -263.83 | -251.49 | 0 |
| **24, C-boat** | -258.97 | -248.34 | 3.15 |

*Energy relative to **24, C-chair.**

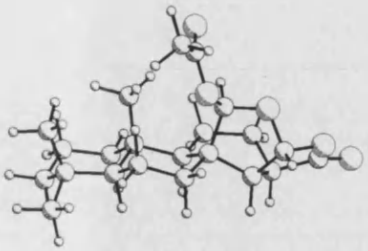
A variety of methods were explored for the lactone-hemiacetal formation and *in situ* acetylation of **14**. Some of the results are summarized in Table **2** (Scheme **3**).

**Scheme 3**

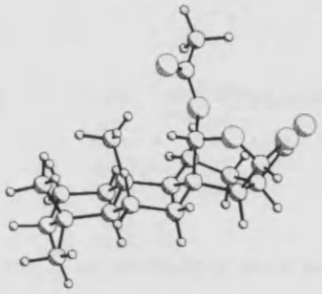




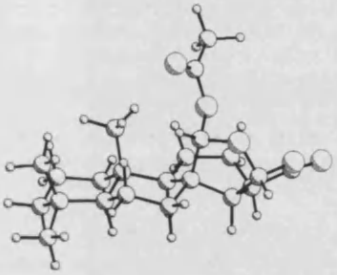
**11, C-chair**



**11, C-boat**



**24, C-chair**



**24, C-boat**

**Fig. 1.** PLUTO drawings for compounds **11** and **24** having the C-ring in either chair or boat conformations.

**Table 2**

| *Starting material* | *Conditions* | *Products* |
|---|---|---|
| 14 | a | no reaction |
| " | b | dendrillol-1, 11, 24 |
| " | c | no reaction |
| " | d | 26 |
| " | e | no reaction |
| " | f | 11, 26 (6:4 mixture), others |
| " | h | 24 (85% purified), 11 (traces) |
| Dendrillol-1 | a | no reaction |
| " | b | no reaction |
| " | f | 11 (traces), 24 (traces) |
| " | g | 24, 11 and dendrillol-1 (traces) |
| " | h | 24, 11 (traces) |
| " | i | 25 |
| " | j | 25 |
| " | k | 25 (90% purified) |



***Reagents and conditions***: a) AcOH, 20°C ,17 h; b) AcOH, 65°C , 24 h; c) AcOH, anhydrous AcONa, 20°C , 17 h; d) $Ac_2O$, anhydrous AcONa, 65°C , 15 h; e) AcOH cat., $Ac_2O$, 40°C , 17 h; f) 4:1 AcOH/$Ac_2O$, 65°C , 17 h; g) AcOH, $H_2SO_4$ cat., 65°C , 17 h; h) AcOH, $Ac_2O$, $H_2SO_4$ cat., 65°C , 17 h; i) $Ac_2O$, pyridine, 35°C , 3 h; j) $Ac_2O$, pyridine, -30°C , 3 days; k) $Ac_2O$, $Et_3N$, 4-pyrrolidinopyridine.

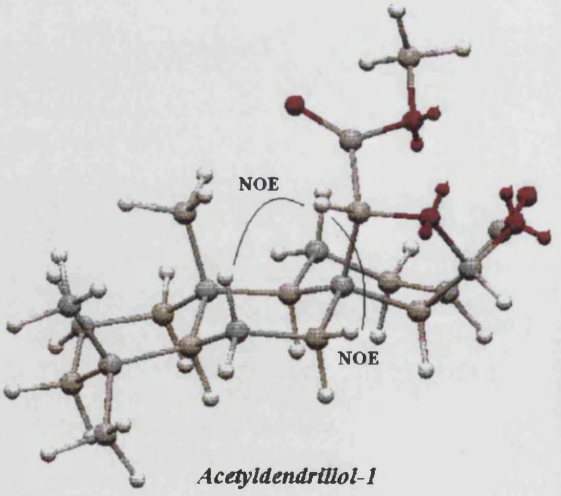
**Table 3**

| *Carbon no.* | Dendrillol-1 | | Compound **11** | | Compound **24** | |
|---|---|---|---|---|---|---|
| | $\delta$ $^1$H | $\delta$ $^{13}$C | $\delta$ $^1$H | $\delta$ $^{13}$C | $\delta$ $^1$H | $\delta$ $^{13}$C |
| 1 | | 39.08 | | 39.0 | | 39.0 |
| 2 | | 18.76 | | 18.7 | | 18.7 |
| 3 | 1.14 (ddd) ax | 41.94 | | 41.8 | | 41.8 |
| 4 | | 33.30 | | a | | 33.3 |
| 5 | 0.94 (dd) | 56.75 | | 55.3 | | 56.8 |
| 6 | 1.35 (dddd) eq | 20.03 | | 19.9 | | 19.9 |
| 7 | 1.85 (ddd) eq | 41.46 | 1.9 (m) | 41.7 | 1.9 (m) | 41.7 |
| 8 | | 46.90 | | a | | 46.1 |
| 9 | 1.23 (dd) | 55.51 | | 56.8 | | 55.3 |
| 10 | | 38.10 | | a | | 38.1 |
| 11 | 1.96 (dddd) eq | 16.52 | | 16.6 | | 16.6 |
| 12 | 2.38 (dddd) eq | 23.85 | 2.45 (br d) | 23.7 | 2.45 (br d) | 23.7 |
| 13 | 2.71 (ddd) | 37.79 | 2.76 (dd) | 37.5 | 2.76 (dd) | 37.5 |
| 14 | 2.57 (dd) | 49.60 | 2.65 (dd) | 49.2 | 2.65 (dd) | 49.2 |
| 15 | 6.09 (d) | 104.40 | 6.10 (d) | 104.2 | 6.10 (d) | 104.2 |
| 16 | | 177.40 | | a | | 176.6 |
| 17 | 5.50 (d) | 103.91 | 6.29 (s) | 100.6 | 6.29 (s) | 100.6 |
| 18 | 0.84 (s) | 33.33 | 0.86 (s) | 33.4 | 0.86 (s) | 33.4 |
| 19 | 0.82 (s) | 21.34 | 0.80 (s) | 21.3 | 0.80 (s) | 21.3 |
| 20 | 0.91 (s) | 15.96 | 0.72 (s) | 15.6 | 0.72 (s) | 15.6 |
| Acetate | | | 2.04 (s) | a | 2.04 (s) | 169.1 |

[a]Signal not seen due to sample size.

It is of interest to note that our predictions coincide with the experimental result of observing both epimers at C-17 under certain reaction conditions. In the end the required conversion was successfully accomplished by using a catalytic amount of sulfuric acid (1%) in a mixture 9:1 of acetic acid and acetic anhydride at 65°C for 17 h. Thus, **24** was obtained in 85% yield directly from **14**. The synthetic pentacyclic spongiane **24** had spectroscopic data identical to those recorded for the natural product.[24] Furthermore, a detailed analysis of the NMR spectra (Table **3**) for synthetic **24** permitted us the unambiguous assignment of the absolute stereochemistry of the natural product as 17$\beta$-acetoxy instead of the 17$\alpha$ reported by Andersen and co-workers.[24]

In particular, comparison of the $^{13}$C NMR data for **24** with the spectrum for (-)-dendrillol-1 **10** ($R^1$= H; $R^2$= $\beta$-OH) reveals that the signal due to C-7 is not affected. In the case we had had an 17$\alpha$-acetoxy function, the shielding effect ($\gamma$-effect) due to that group would have shifted upfield the signal due to C-7 as it occurs in compound **12**.[23] But NOE experiments left no doubt of the stereochemistry, since the NOE enhancement of the signals due to protons H20, H6$\beta$ and H7$\beta$ when proton H17 was irradiated conclusively proved our assignment (Fig. **2**).

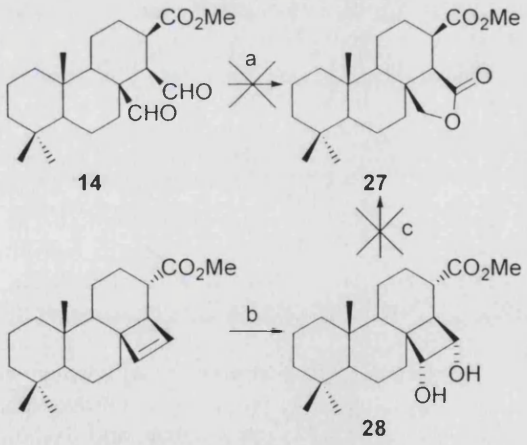



*Acetyldendrillol-1*

**Fig. 2**

Once we noted that the stereochemistry at C-17 for compounds **24** and (-)-dendrillol-1 **10** ($R^1$= H; $R^2$= β-OH) was the same, we envisaged that (-)-dendrillol-1 could also be transformed into **24** under acetylation conditions. However when (-)-dendrillol-1, which was available in our group from a previous work, was submitted to usual acetylation conditions only products resulting from acetylation after opening of the hemiacetal system[30] and traces of the desired acetylated product were obtained in most cases (see Table 2). Eventually, (-)-dendrillol-1 could also be transformed into **24** under the same conditions described for the conversion of **14** into **24**. It should be noted that acetylation of **14** with $Ac_2O$/pyridine led to the formation of the lactone **25**, even at low temperature, probably as a result of a base-promoted hemiacetal ring opening and epimerization at C-15 prior to acetylation. Interestingly, the lactone **25** was obtained in 90% yield by treatment of **14** with $Ac_2O/Et_3N$ and 4-pyrrolidinopyridine as acetylation catalyst.[31] The structure shown for lactone **25** was deduced from its NMR data and by comparison with similar compounds. The stereochemistry of **25** was confirmed with the aid of NOE measurements. Irradiation of the H-15β signal showed enhancements of the H-7β, H-17, and H-14 signals. At this stage, we also thought that other related marine diterpenes as the *ent*-isocopalane dendrillol-3 **27**[10] could be obtained from our key intermediate **14** (Scheme 4).

**Scheme 4**[a]

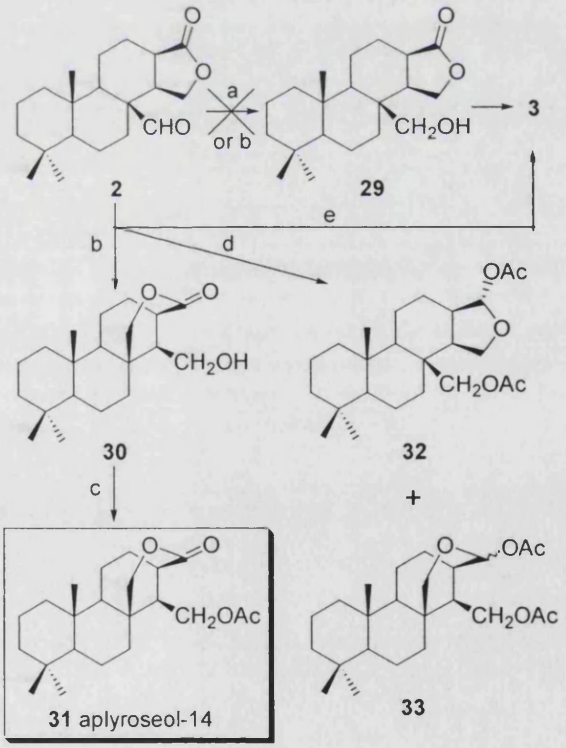



[a] (a) $Al(^iOPr)_3$ or $RuH_2(PPh_3)_4$; (b) $OsO_4$; (c) $NaIO_4$ /MeOH; (d) KOH/MeOH.

Unfortunately, all attempts to produce an intramolecular crossed Cannizzaro-Tishchenko reaction with aluminium isopropoxide or the $RuH_2(PPh_3)_4$ complex were unsuccessful.[32] The formation of the lactone ring present in **27** was also attempted through diol **28** in a similar manner to a reported procedure[33] but also with disappointing results.

Following our research toward the functionalization at C-17, our next objective was to introduce an acetoxy function at C-17 in synthetic **2** to obtain (-)-aplyroseol-14 (Scheme **5**). At first sight, the conversion of **2** into its 17-acetoxy derivative **3** seems to be immediate by reduction and subsequent acetylation, but in practice the preparation of the compound containing the original γ-lactone ring resulted to be really difficult. Reduction of aldehyde (-)-**2** with $NaBH_4$/MeOH at 0°C did not proceed as expected and gave the alcohol **30** in a 87% yield instead of **29**. Compound **30** resulted from reduction and *in situ* translactonization. We soon suspected that we were handling a δ-lactone group instead of the desired γ-lactone group on the basis of IR and $^{13}C$ NMR data for alcohol **30** ($\nu_{CO}$ 1721 $cm^{-1}$; $\delta_{CO}$ 174.7). Attempts to get the equilibrium between lactones **29** and **30** from **30** in either acid (PTSA/PhH or toluene) or basic (KOH, *t*-BuOH) media were unsuccessful.

**Scheme 5**[a]




[a] (a) $NaBH(OAc)_3$, $CF_3CO_2Al(^iOPr)_2$, DIBALH, SMEAH; (b) $NaBH_4$/MeOH, 0°C, 87%; (c) $Ac_2O$, $Et_3N$, 4-pyrrolidinopyridine, 89%; (d) 1) DIBALH, 2) $Ac_2O$, py, DMAP, 65%; (e) $BF_3{\cdot}OEt_2$, AcCl, $Bu_3SnH$, toluene, -78 °C, 45%.

To our surprise, subsequent acetylation of **30** with acetic anhydride, triethylamine and 4-pyrrolidinopyridine as catalyst afforded acetate **31** (89%) having the $^1$H NMR spectrum superimposable on that recorded for the natural product aplyroseol-14.[12] However, its infrared band at 1732 cm$^{-1}$ assignable to a combination of the acetate and a δ-lactone groups, as well as its $^{13}$C NMR spectra ($\delta_{CO}$ 173.6) indicated clearly the presence of a δ-lactone moiety and accordingly, that the proposed structure **3** for aplyroseol-14 was incorrect. It is worth to note that the $^1$H NMR spectrum of **30** showed an AB quartet at $\delta_H$ 4.87 and 4.18, *J* 12.2 Hz assignable to protons H-17, which rested practically unaltered after acetylation. This fact reveals that the hydroxyl group is not located at C-17 but at C-15 in **30** and consequently the acetoxyl group in **31** will be located at C-15. The structural assignment for compound **31** as depicted in Scheme 5 was based on $^1$H and $^{13}$C NMR data as well as NOE difference experiments. We confirmed that the NOE effects observed by Taylor and co-workers were not conclusive to distinguish both lactone structures, since the NOE enhancements on irradiating protons H-20 and H-17 as well as H-19 can be observed independently of the position of the acetoxyl group. In a key NOE experiment, irradiation of the H-13 signal caused the enhancement of both protons H-15, and the acetoxyl group signals providing further support to our assignment. The total synthesis of (-)-aplyroseol-14 **31** had thus been achieved. Comparison with other relevant data ($^{13}$C NMR) has not been possible since the authors did not provide them probably due to sample size.[34]

In order to fully confirm our assignment of the structural features of (-)-aplyroseol-14 and to achieve our objectives, the synthesis of the γ-lactone **3** initially assigned for (-)-aplyroseol-14 was carried out. We observed that reduction of the aldehyde group in **2** with $NaBH_4$/MeOH at 0°C produced the reduction of the lactone moiety to some extent. In front of this finding, we tried several conditions for the selective and mild reduction of aldehydes in the presence of ester groups, and even reductive acetylation conditions to suppress the favorable translactonization process leading to δ-lactone **30**. For instance, when spongianal **2** was subjected to reduction with $NaBH(OAc)_3$ in benzene at reflux,[35] we obtained a complex mixture of products and recovered starting material. The reaction with diisopropoxyaluminium trifluoroacetate[36] also failed. We then tried the preparation of alcohol **29** at low temperature with sodium bis(2-methoxyethoxy)aluminum hydride (SMEAH) in toluene and diisobutylaluminum hydride (DIBALH) in THF as reducing agents, but mixtures of products arising from reduction without selectivity at C-16 and/or C-17, and translactonization of the corresponding aluminum alkoxide *in situ* or during the workup process were obtained. Then, we thought that trapping the aluminum intermediate *in situ* with acetic anhydride would probably lead to our target molecule. Therefore, by using the method of Rychnovsky,[37] for example, it was only obtained acetylation of the products resulting from reduction of the lactone group or reduction at C-17 and translactonization when stoichiometric DIBALH was used, apart from an approximately 50% of unreacted **2**. On increasing the DIBALH amount (2.2 equiv) as well as the amounts of pyridine, 4-(dimethylamino)-pyridine and acetic anhydride for the acetylation reaction *in situ*, we could identify among the reaction products three doubly acetylated lactols, tentatively assigned as depicted in **32** and **33** (7:3 mixture of epimers at C-16) in a 15% and 50% yield, respectively.[38] The use of reductive acetylation conditions on **2** such as those described by Kaplan[39] did not lead to the desired acetate, but unreacted starting material. In view of the poor reactivity of the aldehyde group in **2**, probably due to steric hindrance, which even permitted the lactone reduction taking precedence over the desired reduction in some cases, we decided to use a carbonyl activator to enhance its electrophility. We examined the possibility of using boron trifluoride etherate ($BF_3{\cdot}OEt_2$) as recently reported by Maruoka[40] and co-workers for the reduction of hydroxy carbonyl and dicarbonyl substrates with tributyltin hydride at low temperature. Fortunately, a combination of the methods of Kaplan and Maruoka turned out to be a good reagent system to effect the desired reductive acetylation of the aldehyde group at C-17 in an approximately 45% yield without translactonization. In fact, the combined use of $BF_3{\cdot}OEt_2$, AcCl and $Bu_3SnH$ as reducing agent in toluene at –78°C gave a 1:1 mixture of acetates **3** and **31** in essentially quantitative yield, which were easily separated by preparative normal phase HPLC. As it was expected, compound **3** showed the $^1$H and $^{13}$C data very similar to those of a naturally ocurring 17-acetoxy spongiane diterpenoid as lactone **4**.[41] Isoaplyroseol-14 **3** exhibited carbonyl absorptions due to a γ-lactone ($\nu_{CO}$ 1774 cm$^{-1}$; $\delta_{CO}$ 178.9) and an acetate group ($\nu_{CO}$ 1739 cm$^{-1}$; $\delta_{CO}$ 170.7). Further evidence of the assigned structure for **3** was obtained by NOE difference experiments. In particular, the NOE

**8β,14β-ethylidenepodocarpan-13-one (17).** In a similar manner as reported,[18a] a solution of podocarpenone **16** (100 mg, 0.41 mmol) in dry acetone (160 mL), previously degassified with Helium, was placed in a Pyrex photoreactor and purged with Ar. This solution was bubbled through an dry ice trap with acetylene while cooling to –45 °C with a MeOH-cooled jacket until saturation (ca. 30 min). The mixture was irradiated at this temperature with a 125-W OSRAM high-pressure mercury lamp for 1 h while acetylene was slowly bubbled through the reaction mixture. During this time the reaction temperature reached about –25 °C, after which the reaction mixture was unloaded. The photoreactor was again loaded with the same above-mentioned amounts, and it was proceeded in a similar manner. This cycle was repeated five times more and the combined reaction mixtures were concentrated to give a yellowish oil, which was purified by column chromatography using hexane-ethyl acetate (from 95:5 to 8:2) as eluent, to give photoadduct **17**[18a] (400 mg, 60%) as a white solid followed by unreacted enone **16** (36 mg, 6%).

**Methyl 8β,14β-ethylidenepodocarpan-13β-oate (15).** In a similar manner as described in ref. 21b for the preparation of the 7-hydroxylated analogue of **15**, a solution of ketone **17** (400 mg, 1.47 mmol) in THF (20.6 mL) at 0 °C , LiCN in DMF (0.5 M, 8.8 mL, 4.4 mmol) and DEPC ( 0.72 mL, 4.4 mmol) were successively added. After being stirred for 20 min, the reaction mixture was diluted with diethyl ether. Workup as usual gave the crude product as a yellow oil, wich was dissolved in THF (14.2 mL) and *t*-BuOH (0.14 mL, 1.47 mmol) was added. This mixture was then added to an intense blue solution of $SmI_2$, prepared from Sm (877 mg, 5.83 mmol) and $ICH_2CH_2I$ (1.24 g, 4.4 mmol) in THF (15.5 mL) as follows: The reaction flask containing the solid Sm and $ICH_2CH_2I$ was filled with Ar after doing vacuum on it. After repeating this process three times more, it was cooled to –78 °C and under vacuum the THF was added. Then, the mixture was allowed to warm to rt for 30 min interrupting vacuum, to initiate the reaction, cooled again to –78 °C and stirred overnight warming slowly to reach rt. After 30 min of mixing the crude cyano phosphates with the $SmI_2$ solution, the resulting mixture was diluted with diethyl ether, washed with brine containing diluted HCl and $Na_2S_2O_3$ and brine, dried, filtered and concentrated. The solid residue (480 mg) thus obtained was directly hydrolized and esterified without previous purification.

To a solution of the crude nitriles in a teflon flask in ethylene glycol monoethyl ether (5.6 mL) was added 15 M aqueous KOH (1.44 mL) and the mixture was stirred and heated at 110 °C for 16 h. The mixture was cooled to rt and DMF (7 mL) and $Me_2SO_4$ (2.7 mL) were added. After stirring for 4 h the mixture was poured into 1.5 M aqueous HCl and extracted with diethyl ether. Workup as usual gave a residue which was purified by careful column chromatography, using hexane-diethyl ether (98:2) as eluent, to afford 13β–ester **15**[18a] as a white solid (395 mg, 85% from **17**) and its 13-epimer (28 mg, 6%) which can be equilibrated by treatment with sodium methoxide to a 1:4 mixture of α:β methyl esters.

**Methyl 8β,14β-dioxopodocarpan-13β-oate (14).** Cyclobutene **15** (47 mg, 0.149 mmol) in $CH_2Cl_2$ (9 mL) was cooled to –78 °C , and ozone was passed into the reaction mixture until a light blue color was observed (15-20 min). Argon was then bubbled through the solution to remove excess ozone. Dimethyl sulfide (1.7 mL) was added, and the reaction mixture was slowly allowed to warm to 5 °C in a refrigerator overnight. The reaction mixture was then diluted with diethyl ether, washed with $H_2O$ and brine, dried, filtered and concentrate *in vacuo* to give in essentially quantitative yield the crude dialdehyde **14** (52 mg, 100%, whose $^1H$ NMR was shown to have a purity higher than 95%) as a solid. The dialdehyde **14** was used directly without further purification. A part of this product (20 mg) was filtered through a short silica column eluting with hexane-ethyl acetate (7:3), to afford 11 mg of **14** as a white solid: mp 141-143 °C (from $CH_2Cl_2$); $[\alpha]^{27}_D$ –35.8 (c 0.95); IR (NaCl) 2925, 2865, 1718, 1230 $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 10.02 (1H, s, H-17), 9.75 (1H, d, $J$ = 1.3, H-15), 3.67 (3H, s, $CO_2Me$), 3.21 (1H, ddd, $J$ = 5.6, 5.6, 2.0, H-13), 2.82 (1H, ddd, $J$ = 12.8, 3.3, 3.3, H-7β), 2.51 (1H, m), 2.25 (1H, dd, $J$ = 5.6, 1.3, H-14), 0.85, 0.78, and 0.77 (3H each, each s, H-18, H-19 and H-20); $^{13}C$ NMR (75 MHz) $\delta_C$ 204.76 (d), 201.54 (d), 173.58 (s), 60.67 (d), 59.38 (d), 55.97 (d), 52.13 (q), 49.68 (s), 41.75 (t), 41.16 (d), 38.84 (t), 37.91 (s), 35.72 (t), 33.27 (q), 33.20 (s), 28.30 (t), 21.30 (q), 18.81 (t), 18.59 (t), 17.13 (t), 15.85 (q); MS (EI) *m/z* 348 ($M^+$, 9), 320 (57), 302 (100), 292 (61), 288 (82), 177 (78); HRMS $C_{21}H_{32}O_4$ requires 348.2301, found 348.2304.

**Methyl 15,17-epoxy-17-hydroxy-ent-isocopalan-16-oate (22).** To a stirred solution of crude dialdehyde **14** (52 mg, 0.149 mmol) in MeOH (5.9 mL) at 0 °C , $NaBH_4$ (98%, 45 mg, 1.14 mmol) was added. The reaction mixture was stirred for 45 min and then diluted with diethyl ether. The resulting

solution was washed with 1.5 M aqueous HCl, brine, dried, filtered and concentrated to yield the crude 2:1 mixture of hemiacetals **22** (52 mg, 99%) as an amorphous solid: IR (KBr) 3500-3200, 1732, 1019 $cm^{-1}$; $^1H$ NMR (major isomer, 300 MHz) $\delta$ 5.23 (1H, d, $J$ = 2.4, H-17), 3.64 (3H, s, $CO_2Me$), 2.66 (1H, ddd, $J$ = 13.2, 5.9, 3.2), 0.94, 0.85, and 0.83 (3H each, each s, H-18, H-19 and H-20); $^{13}C$ NMR (major isomer, 75 MHz) $\delta_C$ 175.67 (s), 102.84 (d), 66.44 (t), 56.96 (d), 51.48 (q), 49.71 (d), 48.85 (s), 47.31 (d), 43.67 (t), 42.06 (t), 39.01 (d), 38.83 (t), 38.14 (s), 33.56 (q), 33.17 (s), 21.61 (q), 20.65 (t), 19.57 (t), 18.72 (t), 15.81 (t), 15.19 (q); $^1H$ NMR (minor isomer, 300 MHz) $\delta$ 5.56 (1H, d, $J$ = 3.2, H-17), 3.65 (3H, s, $CO_2Me$), 2.77 (1H, m), 0.93, 0.86, and 0.82 (3H each, each s, H-18, H-19 and H-20); $^{13}C$ NMR (minor isomer, 75 MHz) $\delta_C$ 175.64 (s), 99.18 (d), 66.32 (t), 56.81 (d), 51.54 (q), 50.83 (d), 49.78 (d), 49.42 (s), 41.99 (t), 39.33 (d), 39.22 (t), 38.31 (s), 36.92 (t), 33.66 (q), 33.17 (s), 21.64 (q), 20.87 (t), 19.61 (t), 19.24 (t), 18.55 (t), 14.34 (q). The mixture of epimers at C-17 **22** were inseparable by chromatography and was used directly in the lactone ring formation reaction.

**(-)-Spongian-16-oxo-17-al (2).** A solution of hemiacetals **22** (52 mg, 0.148 mmol) and PTSA (16 mg, 0.084 mmol) in benzene (16.8 mL) was refluxed for 18 h. The reaction mixture was then cooled, diluted with ethyl acetate, and washed with 10% aqueous $NaHCO_3$ and brine. Workup ($Na_2SO_4$) and careful flash chromatography, using hexane-ethyl acetate (from 95:5 to 6:4) as eluent, gave the δ-lactone **23** (9.4 mg, 20% from **14**) followed by the γ-lactone **2** (30.5 mg, 65% overall yield for the three steps from **14**) as a white solid. Alternatively, the same result can be achieved from the residue obtained by treatment of a refluxing solution of hemiacetals (52 mg, 0.148 mmol) in a 2:3 mixture of acetone-water (20 mL) with TFA (1.4 mL) for 60 h, after a similar workup and purification. For spongiane **2**: mp 176-178 °C (from hexane-$CH_2Cl_2$); $[\alpha]^{24}_D$ –59.0 (c 1.64); IR (KBr) 2943, 2738, 1770, 1708, 1131 $cm^{-1}$; $^1H$ NMR (300 MHz) 9.96 (1 H, d, $J$ = 1.5, H-17), 4.09 (1 H, dd, $J$ = 10.0, 4.5, H-15), 4.04 (1 H, br d, $J$ = 10.0, H-15'), 2.67 (1 H, m, H-13), 2.54 (1 H, dt, $J$ = 13.0, 3.0, H-7β), 2.50 (1 H, m, H-12β), 2.28 (1 H, dd, $J$ = 7.5, 4.5, H-14), 0.85 (3 H, s, H-19), 0.75 (3 H, s, H-18), 0.69 (3 H, s, H-20); $\delta_C$ (75 MHz) 203.83 (d), 177.71 (s), 66.87 (t), 56.56 (d), 55.88 (d), 50.81 (s), 49.00 (d), 41.77 (t), 39.03 (t), 38.53 (d), 37.80 (s), 35.65 (t), 33.37 (q), 33.25 (s), 23.08 (t), 21.43 (q), 18.75 (t), 18.62 (t), 16.34 (t), 14.89 (q); MS (EI) *m/z* 318 ($M^+$, 15), 275 (11), 218 (100), 123 (25); HRMS $C_{20}H_{30}O_3$ requires 318.2195, found 318.2193.

**(-)-17β-Acetoxy-15,17-oxidospongian-16-one (acetyldendrillol-1) (24).** The dialdehyde **14** (12 mg, 0.034 mmol) was dissolved in a 1% $H_2SO_4$ solution in acetic acid (0.7 mL), acetic anhydride (42 μL, 0.44 mmol) was added and the resulting mixture was heated at 65 °C for 17 h. The brownish mixture was cooled to rt, poured into water, and extracted with diethyl ether. The combined organic layers were washed with 10% aqueous $NaHCO_3$ and brine, dried, filtered, and concentrated. The resulting residue was purified by column chromatography (7:3 hexane-EtOAc) to afford 10.9 mg (85%) of acetyldendrillol **24** as a white solid: mp 218-219 °C (from MeOH); $[\alpha]^{25}_D$ –74.7 (*c* 1.87); IR (KBr) 2930, 1785, 1754, 1223 $cm^{-1}$; $^1H$ NMR (400 MHz) $\delta$ 6.29 (1 H, s, H-17), 6.11 (1 H, d, $J$ = 6.0, H-15), 2.76 (1 H, dd, $J$ = 11.0, 7.0, H-13), 2.65 (1 H, dd, $J$ = 11.0, 6.0, H-14), 2.45 (1 H, br d, $J$ = 11.5, H-12β), 2.04 (3 H, s, OCO*Me*), 1.94 (1 H, dt, $J$ = 13.5, 3.0, H-7β), 0.86 (3 H, s, H-18), 0.81 (3 H, s, H-19), 0.72 (3 H, s, H-20); $^1H$ NMR (300 MHz; $C_6D_6$) $\delta$ 6.41 (1 H, s, H-17), 5.57 (1 H, d, $J$ = 6.0, H-15), 2.44 (1 H, m), 2.00 (1 H, dd, $J$ = 11.0, 7.0), 1.79 (3 H, s, OCO*Me*), 0.76, 0.65 and 0.55 (3H each, each s, H-18, H-19 and H-20); $\delta_C$ (75 MHz) 176.64 (s), 169.06 (s), 104.22 (d), 100.57 (d), 56.78 (d), 55.25 (d), 49.17 (d), 46.17 (s), 41.82 (t), 41.68 (t), 39.00 (t), 38.06 (s), 37.48 (d), 33.33 (s), 33.33 (q), 23.71 (t), 21.49 (q), 21.31 (q), 19.89 (t), 18.69 (t), 16.57 (t), 15.62 (q); MS (EI) *m/z* 376 ($M^+$, 10), 316 (100), 288 (60), 260 (41); HRMS $C_{22}H_{32}O_5$ requires 376.2250, found 376.2240.

**(-)-15α-Acetoxyspongian-16-oxo-17-al (25).** To a stirred solution of dendrillol-1 **10** ($R^1$= H; $R^2$= β-OH) (15.0 mg, 0.045 mmol), 4-pyrrolidinopyridine (98%, 1.0 mg, 0.007 mmol) in dry $Et_3N$ (0.4 mL) at 0 °C was added acetic anhydride (25 μL, 0.26 mmol) dropwise. The reaction mixture was then allowed to warm to room temperature, stirred for 70 min, and diluted with diethyl ether. The organic phase was washed with 1.5 M HCl and brine, dried, filtered and concentrated. Purification of the residue by flash chromatography, using hexane-ethyl acetate (from 7:3 to 6:4) as eluent, provided spongiane **25** (15.2 mg, 90%) as a white solid: mp 161-162 °C (from hexane-EtOAc); $[\alpha]^{23}_D$ –9.0 (c 0.69); IR (KBr) 2943, 2739, 1790, 1769, 1711, 1214 $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 9.93 (1H, d, $J$ = 1.3, H-17), 6.18 (1H, s, H-15β), 2.97 (1H, dd, $J$ = 7.9, 7.7, H-13), 2.72 (1H, ddd, $J$ = 13.1, 3.2, 3.2, H-

enhancements of the signals due to protons H-12α, H-14 and H-15α , on irradiation of proton H-13 unambigously proved the structure of **3** as depicted. Although the favoured translactonization reaction could not be completely avoided, we have found a new procedure for the mild one-step aldehyde-to-acetate conversion in high yield and without affecting cyclic esters. We do believe that this method may be extended to reductive acetylation of ketones chemoselectively in the presence of other ester groups.

In conclusion, the versatility of the dialdehyde **14** as a key precursor of C-17-functionalized spongiane diterpenes has now been well demonstrated. This compound was obtained in 48% overall yield in five steps from chiral podocarpenone **16**. The utility of our sequence has been proved by preparing (-)-acetyldendrillol-1 **24** (85%, one step), (-)-spongian-16-oxo-17-al **2** (65%, two steps) and (-)-aplyroseol-14 **31** (56%, four steps) from intermediate **14**, which has permitted to reassign the structures of acetyldendrillol-1 and aplyroseol-14 using NOE studies. Confirmation of the incorrect structure assignment to aplyroseol-14 was also obtained chemically by synthesizing the molecule having the proposed structure **3**, whose $^1$H NMR spectrum was similar to, but clearly different from, that of natural aplyroseol-14. To this end, we have developed a novel reaction for conversion of aldehydes to acetates, which may find application in other synthetic problems. Biochemically, it is interesting to note the existence of the δ-lactone **31**, unknown to date, which may be also involved in the biogenetic pathways of other naturally occurring spongiane-related diterpenoids.

**Compound Testing.** It has been investigated the cytotoxic activity *in vitro* of (-)-acetyldendrillol-1 (**24**), (-)-spongian-16-oxo-17-al (**2**) and (-)-aplyroseol-14 (**31**) against HeLa and HEp-2 cells, and the antiherpetic activity on Herpes simplex virus type 2 (HSV-2). As a result, compound **2** was the most toxic ($CC_{50}$= 14.2 and 9.6 μg/mL for HeLa and HEp-2 cells, respectively). Preliminar colorimetric assays of these compunds showed a weak antiherpetic activity, insignificant to be estimated quantitatively. The results of a study on the structure-activity relationship of these spongianes along with other from previous works, and several intermediates will be reported elsewhere in due course.

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**Supporting Information Available**

Representative data and experimental procedures for the preparation of **26** and **33** and representative data for **20** and **23**.$^1$H NMR spectra for **2, 3, 20, 23-26, 30** and **31** (12 pages).

**Experimental Section**

**General experimental details.**
Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were determined using a 5-cm path-length cell, using chloroform as solvent (concentration expressed in g/100 mL). $[\alpha]_D$-values are given in $10^{-1}$ deg·cm$^2$·g$^{-1}$. IR spectra were taken as KBr pellets, liquid films on NaCl plates or solutions in chloroform. NMR spectra were recorded on 250, 300 or 400 MHz spectrometers with tetramethylsilane as an internal standard. All spectra were recorded in $CDCl_3$ as solvent, unless otherwise described. Complete assignments of $^{13}$C NMR multiplicities were made on the basis of DEPT experiments. HMQC and NOE experiments were used in some $^1$H NMR assignments. *J* values are given in Hz. In all compounds, NMR assignments are given with respect to the numbering scheme shown in structure 1. Mass spectra (MS) were run by electron impact (EI) at 70 eV. Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F-254 in 0.25 mm-thick plates. Compounds on TLC plates were detected under UV light at 254 nm and visualized by immersion in a 10% sulfuric acid solution and heating on a hotplate. Purifications were performed by flash chromatography on Merck silica gel (230-400 mesh). HPLC was performed on a Konik instruments KNK-500A LC system. All non-aqueous reactions were carried out in an argon atmosphere in oven-dried glassware. Commercial reagent grade solvents and chemicals were used as received unless otherwise noted. THF was freshly distilled from sodium benzophenone ketyl under argon atmosphere. Combined organic extracts were washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Compound **16** was prepared from commercial (-)-abietic acid (Aldrich Chemical Co., Inc.) using our method.[20]

7β), 2.51 (1H, br d, *J* = 12.1, H-12β), 2.26 (1H, d, *J* = 7.9, H-14), 2.08 (3H, s, OCO*Me*), 0.85, 0.76 and 0.68 (3H each, each s, H-18, H-19 and H-20); $^{13}$C NMR (75 MHz) $\delta_C$ 204.25 (d), 176.31 (s), 168.55 (s), 93.92 (d), 56.40 (d), 55.75 (d), 53.32 (d), 49.36 (s), 41.69 (t), 38.96 (t), 37.80 (s), 35.84 (t), 35.15 (d), 33.34 (q), 33.23 (s), 22.36 (t), 21.42 (q), 20.86 (q), 18.66 (t), 18.55 (t), 16.08 (t), 15.14 (q); MS (EI) *m/z* 376 ($M^+$, 4), 316 (58), 288 (78), 123 (44), 86 (100); HRMS $C_{22}H_{32}O_5$ requires 376.2250, found 376.2249.

**(-)-15,16-Dideoxy-15-hydroxy-16,17-oxidospongian-16-one (30).** To a stirred suspension of synthetic spongianal **2** (15 mg, 0.047 mmol) in MeOH (1.8 mL) at 0 °C was added $NaBH_4$ (17.8 mg, 0.47 mmol). After stirring for 15 min everything was dissolved and this solution was stirred at this temperature for 45 additional minutes, and then diluted with ethyl acetate. This solution was washed successively with 5% HCl, brine, 10% $NaHCO_3$ and brine again. Workup as usual of the resulting organic extract gave a white, glassy solid which was sufficiently pure for synthetic purposes in the next reaction. Although this crude alcohol was purified by flash chromatography with hexane-ethyl acetate (from 5:5 to 3:7) to afford alcohol **30** (13.6 mg, 90%) as a white solid: mp 174-175 °C (from hexane-EtOAc); $[\alpha]^{27}_D$ –15.0 (c 0.5); IR (KBr) 3500-3100, 2925, 1721, 1022 cm$^{-1}$; $^1$H NMR (300 MHz) $\delta$ 4.86 (1H, dd, *J* = 12.2, 1.7, H-17), 4.17 (1H, dd, *J* = 12.2, 1.4, H-17'), 3.85 (1H, dd, *J* = 10.9, 4.2, H-15), 3.73 (1H, dd, *J* = 10.9, 6.4, H-15'), 2.92 (1H, br s, H-13), 1.02, 0.86 and 0.81 (3H each, each s, H-18, H-19 and H-20); $^{13}$C NMR (300 MHz) $\delta_C$ 174.66 (s), 75.36 (d), 61.24 (t), 58.90 (d), 56.40 (d), 51.62 (d), 41.68 (t), 40.87 (d), 39.85 (t), 37.95 (s), 37.25 (t), 35.83 (s), 33.21 (q), 33.21 (s), 31.06 (t), 21.46 (q), 19.83 (t), 18.78 (t), 18.36 (t), 15.90 (q); MS (EI) *m/z* 320 ($M^+$, 100), 302 (31), 290 (50), 123 (27); HRMS $C_{20}H_{32}O_3$ requires 320.2351, found 320.2352.

**(-)-15-Acetoxy-15,16-dideoxy-16,17-oxidospongian-16-one (aplyroseol-14) (31).** To a solution of alcohol **30** (8.1 mg, 0.025 mmol) and 4-pyrrolidinopyridine (98%, 0.5 mg, 0.003 mmol) as a catalyst in dry $Et_3N$ (0.8 mL) cooled in a –20 °C bath, acetic anhydride (7 μL, 0.075 mmol) was added. After stirring for 1 h, the reaction mixture was diluted with diethyl ether. Workup as usual followed by column chromatography, using hexane-ethyl acetate (7:3) as eluent, yielded the acetate **31** (8.4 mg, 92%) as a white solid: mp 146-148 °C; $[\alpha]^{25}_D$ –37.0 (c 2.0); IR (KBr) 1760, 1739, 1232, 1215 cm$^{-1}$; IR (NaCl) 1741, 1231 cm$^{-1}$; IR ($CHCl_3$) 1732, 1237 cm$^{-1}$; $^1$H NMR (400 MHz) $\delta$ 4.89 (1H, dd, *J* = 12.5, 1.5, H-17), 4.35 (1H, dd, *J* = 11.5, 4.4, H-15), 4.09 (1H, dd, *J* = 12.5, 1.0, H-17'), 3.99 (1H, dd, *J* = 11.5, 6.8, H-15'), 2.85 (1H, br s, H-13), 2.14 (1H, m), 2.05 (3H, s, OCO*Me*), 1.02 (3H, s, H-20), 0.86 (3H, s, H-18), 0.81 (3H , s, H-19); $^{13}$C NMR (75 MHz) $\delta_C$ 173.57 (s), 170.85 (s), 74.85 (t), 62.68 (t), 58.82 (d), 56.32 (d), 48.63 (d), 41.63 (t), 40.80 (d), 39.82 (t), 37.95 (s), 37.18 (t), 35.73 (s), 33.19 (q), 33.19 (s), 31.04 (t), 21.43 (q), 20.80 (q), 19.74 (t), 18.70 (t), 18.33 (t), 15.88 (q); MS (EI) *m/z* 362 ($M^+$, 21), 302 (100), 287 (40), 245 (29), 123 (57); HRMS $C_{22}H_{34}O_4$ requires 362.2457, found 362.2462.

**(-)-17-Acetoxyspongia-16-one (Isoaplyroseol-14) (3).** To a solution of aldehyde **2** (9.0 mg, 0.028 mmol) in toluene (0.4 mL) was added $BF_3{\cdot}OEt_2$ (30μL, 0.237 mmol) dropwise at –78 °C under argon and the resulting solution was stirred for 5 min. Then acetyl chloride (120μL, 1.69 mmol) and $Bu_3SnH$ (97%, 80 μL, 0.288 mmol) were successively added dropwise. The reaction mixture was stirred for 3 h at –78 °C and allowed to warm up to –35 °C for 2 h, then diluted with ethyl acetate and washed with 10% $NaHCO_3$. Usual workup afforded a colorless oil containing tin residues which was filtered through a short silica column, using hexane-ethyl acetate (from 95:5 to 7:3) as eluent, to give a mixture of two acetates **3** and **31** (10.2 mg) in an approximate 1:1 ratio (by $^1$H NMR and $^{13}$C NMR analysis) in quantitative yield. This mixture was separated by preparative HPLC (μPORASIL, 7.8 x 300 mm; hexane-ethyl acetate = 8:2) with a flow rate of 1 mL/min. The first compound eluted (4.6 mg, 45%) was identified as the γ-lactone **3**, a colorless oil which solidified upon standing: mp 132-134 °C ; $[\alpha]^{23}_D$ –29.8 (c 0.94); IR (NaCl) 1774, 1739, 1235 cm$^{-1}$; $^1$H NMR (400 MHz) $\delta$ 4.51 (1H, d, *J* = 9.7, H-15), 4.29 (1H, d, *J* = 12.9, H-17), 4.10 (1H, d, *J* = 12.9, H-17'), 4.08 (1H, dd, *J* = 9.7, 5.8, H-15'), 2.59 (1H, dd, *J* = 8.1, 8.1, H-13), 2.38 (1H, m), 2.20 (1H, m), 2.06 (3H, s, OCO*Me*), 0.90 (3H, s, H-20), 0.86 (3H, s, H-18), 0.80 (3H , s, H-19); $^{13}$C NMR (75 MHz) $\delta_C$ 178.88 (s), 170.71 (s), 67.59 (t), 64.24 (t), 56.99 (d), 56.63 (d), 49.84 (d), 41.80 (t), 40.03 (t), 37.85 (t), 37.81 (d), 37.60 (s), 37.48 (s), 33.35 (s), 33.25 (q), 22.28 (t), 21.43 (q), 21.00 (q), 18.49 (t), 18.25 (t), 17.00 (t), 16.38 (q); MS (EI) *m/z* 362 ($M^+$, 2), 302 (100), 287 (52), 246 (29), 123 (44); HRMS $C_{22}H_{34}O_4$ requires 362.2457, found 362.2446. The second material eluted (5.4 mg, 53%) was the δ-lactone **31** (aplyroseol-14, see above).

**References and Notes**

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## Supporting Information

# Synthesis of C-17-Functionalized Spongiane Diterpenes: Diastereoselective Synthesis of (-)-Acetyldendrillol-1, (-)-Spongian-16-oxo-17-al and (-)-Aplyroseol-14 from (-)-Abietic Acid

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**Methyl 15-acetoxy-17-oxo-ent-isocopal-14-en-16-oate (26).** A suspension of dialdehyde **14** (2 mg, 0.0057 mmol) and approximmately 2 mg of sodium acetate in acetic anhydride (50 μL) was heated at 60 °C for 16 h. The reaction mixture was then diluted with diethyl ether, washed with 10% aqueous $NaHCO_3$, brine, filtered and concentrated. The resulting residue was filtered through a short silica gel column eluting with hexane-ethyl acetate (9:1), to give exclusively the enol acetate **26** in essentially quantitative yield as a colorless oil: IR (KBr) 3098, 2947, 1764, 1731, 1215 $cm^{-1}$; $^1H$ NMR (400 MHz) δ 9.76 (1H, s, H-17), 7.21 (1H, s, H-15), 3.92 (1H, br d, J = 6.0, H-13), 3.63 (3H, s, $CO_2Me$), 2.58-2.50 (2H, m), 2.15 (3H, s, COMe), 2.06 (1H, m), 0.85, 0.77, and 0.76 (3H each, each s, H-18, H-19 and H-20). NOE result: Irradiation of the proton H-15 (δ 7.21) showed a enhancement of a couple of signals (δ 2.53 and 1.30) assignable to protons H-7.

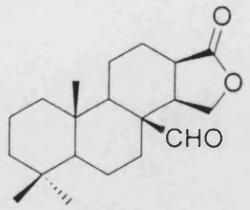
**Preparation of acetates 33 from aldehyde 14.** A slight adaption of the procedure reported by Rychnovsky was employed.[37] To a cooled solution of aldehyde **14** (11.0 mg, 0.034 mmol) in dichloromethane (0.21 mL) at –78 °C DIBALH (1 M in cyclohexanes, 80 μL, 0.08 mmol, 2.3 equiv) was added dropwise. After 45 min, the reaction was treated sequentially with pyridine (13 μL, 0.16 mmol, 4.6 equiv), a solution of DMAP (12.7 mg, 0.10 mmol, 3.0 equiv) in dichloromethane (0.1 mL) via syringe, and acetic anhydride (30 μL, 0.31 mmol, 9.0 equiv) dropwise. The mixture was stirred at – 78°C for 6 h and allowed to warm to 0 °C slowly. After being stirred for 14 h the temperature reached to 0 °C , the mixture was then quenched with ammonium chloride and Rochelle's salt and warmed to

room temperature. The resulting mixture was diluted with dichloromethane and stirred until layer separation occurred. The organic layer was separated and the aqueous one was reextracted with additional dichloromethane. The combined dichloromethane extracts were washed with 10% aqueous $NaHCO_3$ and brine, dried, filtered and concentrated. The residue thus obtained was purified by column chromatography, using hexane-ethyl acetate (from 9:1 to 8:2) as eluent, to afford at least three isomers of reduction and acetylation in approximmately 70-80% overall yield. Mixture of epimers **33** (7 mg, 50%): For major epimer: $^1$H NMR (300 MHz) δ 5.94 (1H, br s, H-16), 4.42-4.17 (3H, m), 3.59 (1H, dd, *J* = 12.7, 1.5), 2.12 (3H, s, OCOMe), 2.06 (3H, s, OCOMe), 1.00, 0.84 and 0.80 (3H each, each s, H-18, H-19 and H-20); $\delta_C$ (75 MHz) 171.03 (s), 169.67 (s), 93.84 (d), 69.81 (t), 61.97 (t), 59.42 (d), 56.58 (d), 49.63 (d), 41.87 (t), 39.94 (t), 37.84 (s), 35.63 (s), 35.14 (t), 33.86 (d), 33.23 (q), 33.18 (s), 26.43 (t), 21.44 (q), 21.24 (q), 21.04 (q), 20.58 (t), 18.67 (t), 18.47 (t), 15.72 (q). For minor epimer: 5.93 (1H, br s, H-16), 4.41 (1H, dd, *J* = 11.2, 8.6), 4.27 (1H, dd, *J* = 11.2, 6.6), 4.12 (1H, d, *J* = 12.3), 3.67 (1H, d, *J* = 12.3), 2.05 (3H, s, OCOMe), 2.05 (3H, s, OCOMe), 1.01, 0.84 and 0.80 (3H each, each s, H-18, H-19 and H-20); $\delta_C$ (75 MHz) 171.13 (s), 169.91 (s), 96.07 (d), 65.49 (t), 63.85 (t), 59.26 (d), 56.68 (d), 46.35 (d), 41.88 (t), 39.93 (t), 37.81 (s), 35.86 (t), 35.48 (s), 33.74 (d), 33.25 (s), 33.17 (q), 30.06 (t), 21.44 (q), 21.35 (q), 21.08 (q), 19.62 (t), 18.48 (t), 18.44 (t), 15.73 (q). For compound **32** (2.1 mg, 15%): $^1$H NMR (300 MHz) δ 6.04 (1H, d, *J* = 5.6, H-16), 4.42 (1H, d, *J* = 12.0, H-17), 4.17 (1H, dd, *J* = 9.0, 2.2, H-15), 4.04 (1H, d, *J* = 12.0, H-17'), 3.96 (1H, dd, *J* = 9.0, 6.0, H-15'), 2.19 (1 H, m), 2.08 (3H, s, OCOMe), 2.05 (3H, s, OCOMe), 0.92, 0.86 and 0.80 (3H each, each s, H-18, H-19 and H-20).

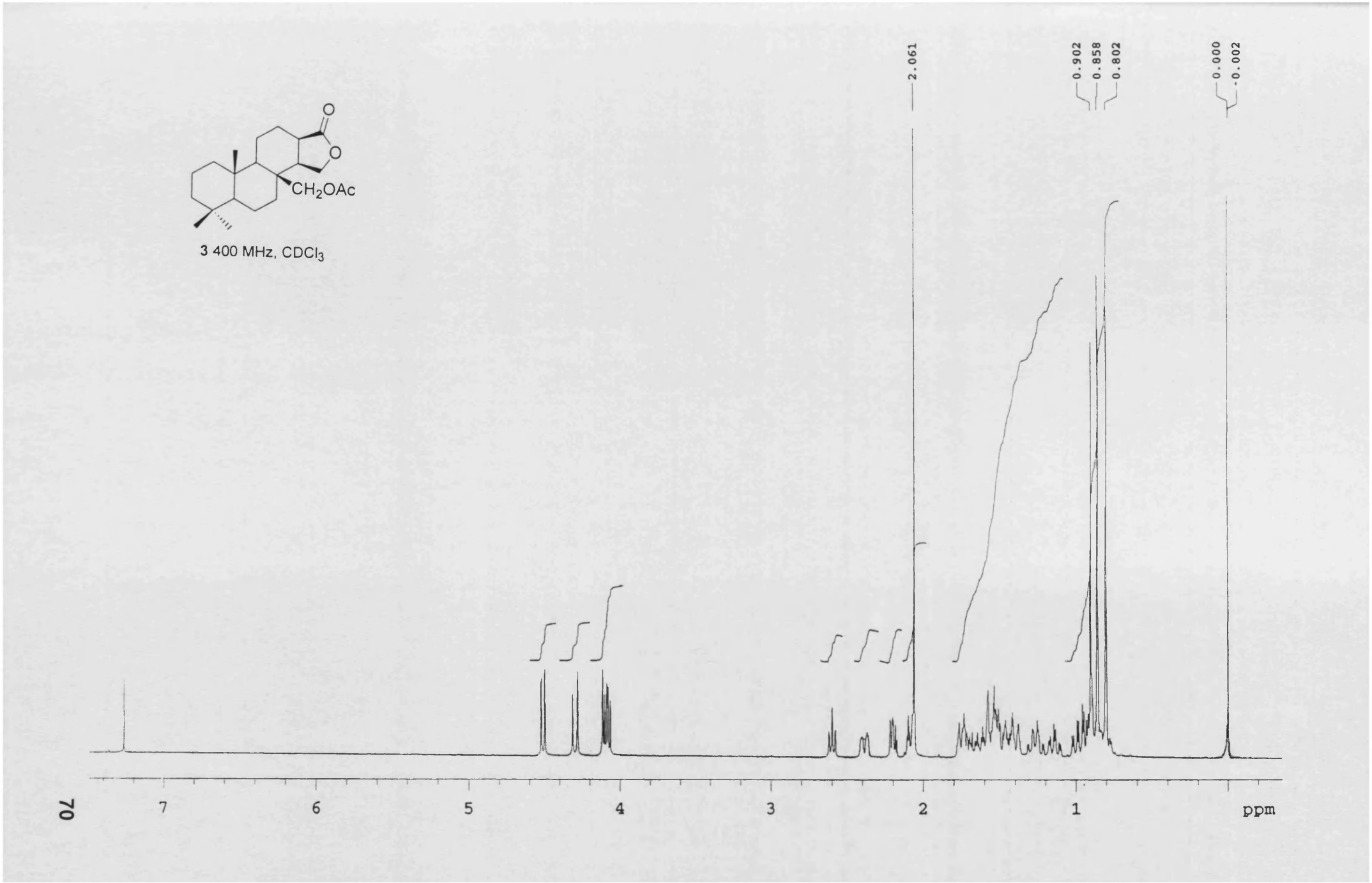
**(-)-17β-methoxy-15,17-epoxyspongian-16-one (20).** Representative data: mp 205-26 °C (from MeOH); $[\alpha]^{25}_D$ –44.8 (*c* 1.25); IR (KBr) 2924, 1770, 1112, 1072 cm$^{-1}$; $^1$H NMR (400 MHz) δ 6.05 (1 H, d, *J* = 6.0, H-15), 4.92 (1 H, s, H-17), 3.28 (3 H, s, OMe), 2.67 (1 H, dd, *J* = 11.0, 7.7, H-13), 2.52 (1 H, dd, *J* = 11.0, 6.0, H-14), 2.36 (1 H, m, H-12β), 1.92-1.78 (2 H, m), 0.85, 0.82 and 0.82 (3H each, each s, H-18, H-19 and H-20); $\delta_C$ (75 MHz) 176.94 (s), 109.99 (d), 104.08 (d), 56.78 (d), 55.43 (d), 53.88 (d), 49.24 (d), 46.76 (s), 41.95 (t), 41.41 (t), 39.09 (t), 38.11 (s), 37.69 (d), 33.35 (s), 33.35 (q), 23.70 (t), 21.36 (q), 20.11 (t), 18.77

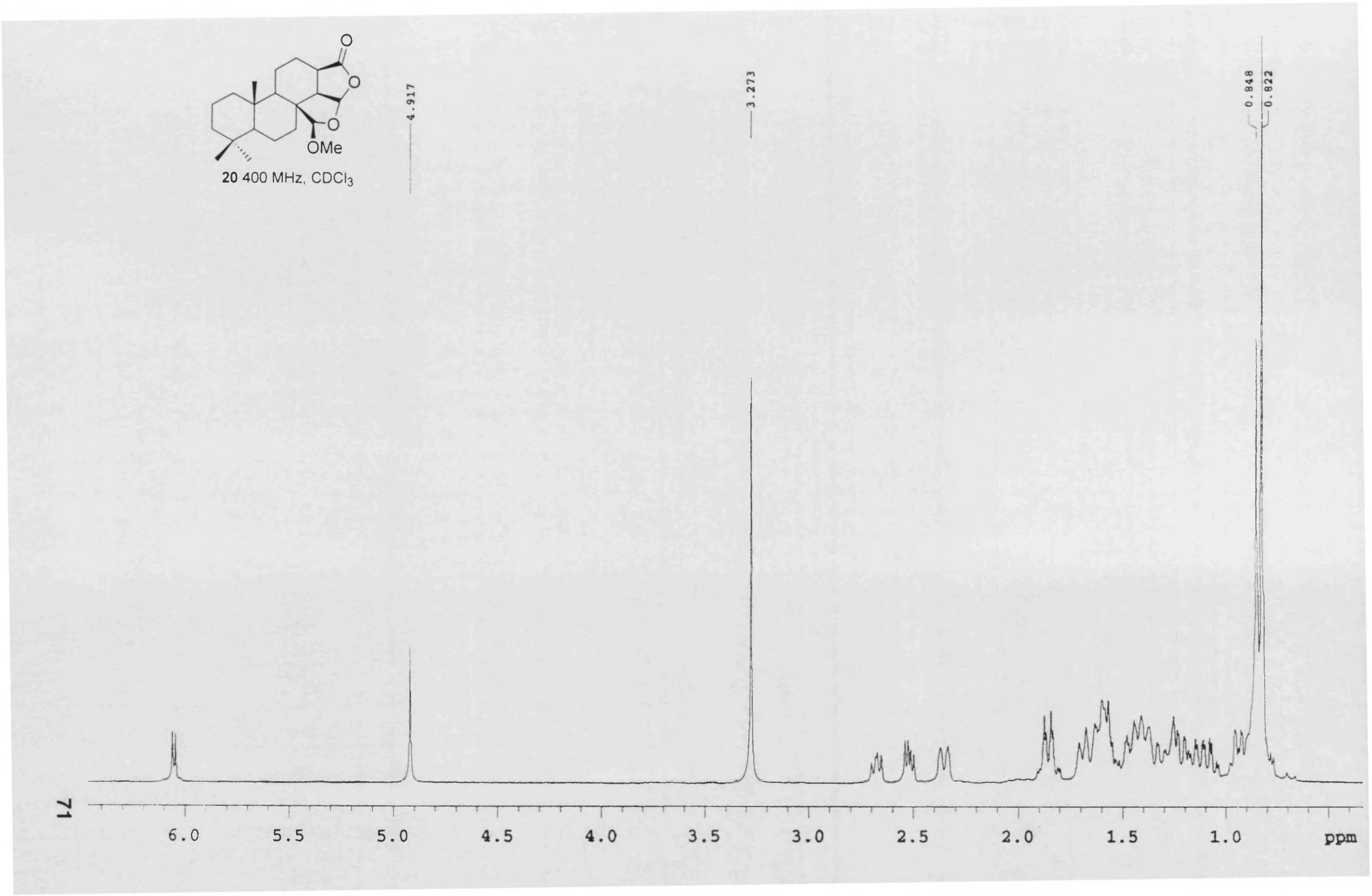
(t), 16.69 (t), 15.53 (q); MS (EI) *m/z* 348 ($M^+$, 30), 317 (20), 288 (100), 137 (23); HRMS $C_{21}H_{32}O_4$ requires 348.2301, found 348.2308.

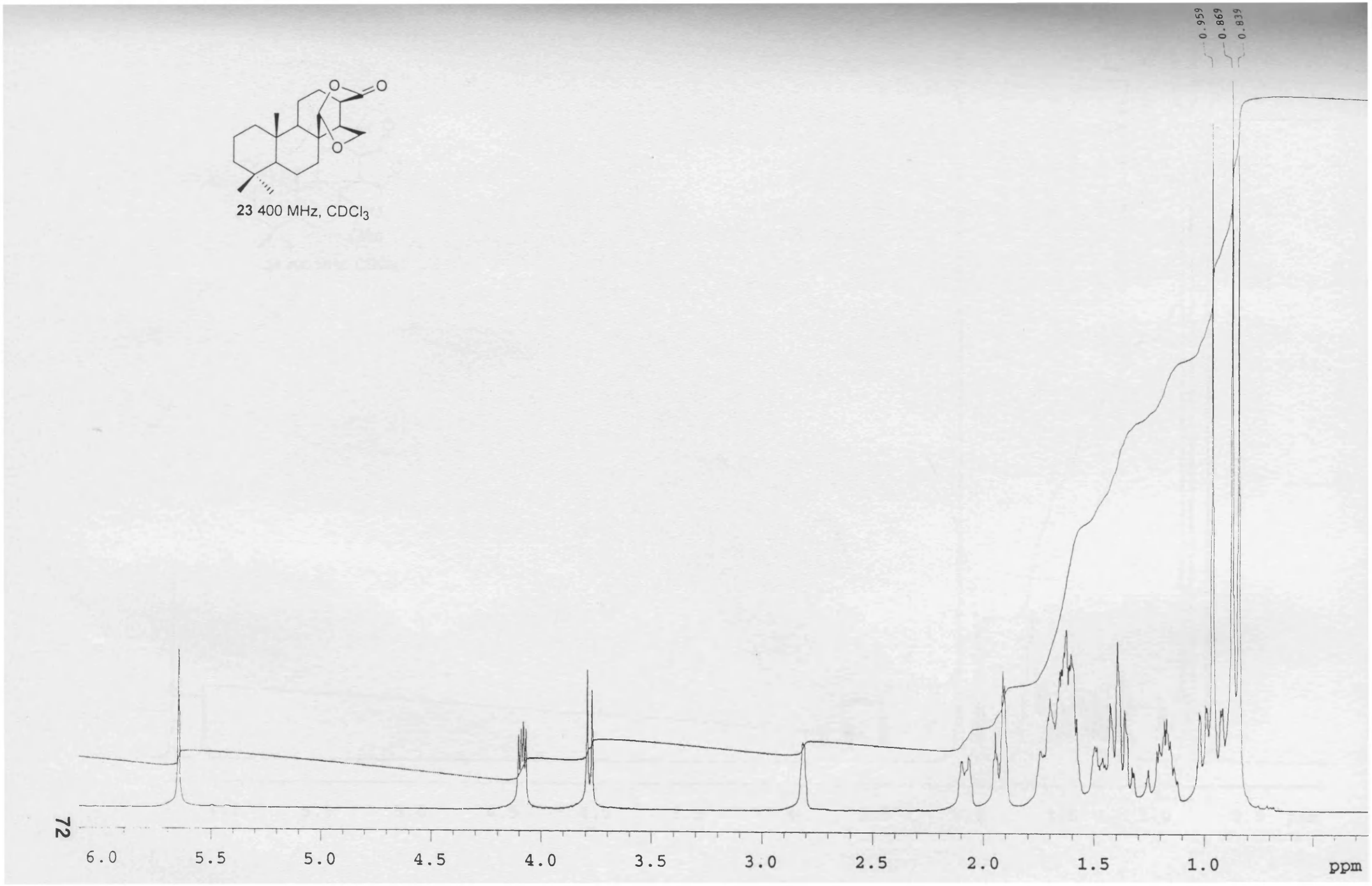
**(-)-15,16-dideoxy-15,17-oxido-16,17-oxidospongian-16-one (23).** Representative data: mp 241-243°C (from $Et_2O$-$CH_2Cl_2$); $[\alpha]^{24}_D$ –65.0 (c 2.0); IR (KBr) 2926, 2865, 1742, 1120, 967 $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 5.65 (1H, d, $J$ = 1.2, H-17), 4.08 (1H, dd, $J$ = 8.8, 4.7, H-15), 3.77 (1H, d, $J$ = 8.8, H-15'), 2.80 (1H, m, H-13), 0.96, 0.87 and 0.84 (3H each, each s, H-18, H-19 and H-20); $^{13}C$ NMR (100 MHz) $\delta_C$ 173.77 (s), 104.50 (d), 70.07 (t), 56.56 (d), 53.81 (d), 46.31 (d), 45.90 (d), 45.71 (s), 41.77 (t), 38.74 (t), 37.86 (s), 34.73 (t), 33.30 (s), 33.08 (q), 29.59 (t), 21.29 (q), 19.38 (t), 19.22 (t), 18.70 (t), 15.33 (q); MS (EI) *m/z* 318 ($M^+$, 5), 274 (12), 218 (100); HRMS $C_{20}H_{30}O_3$ requires 318.2195, found 318.2200.



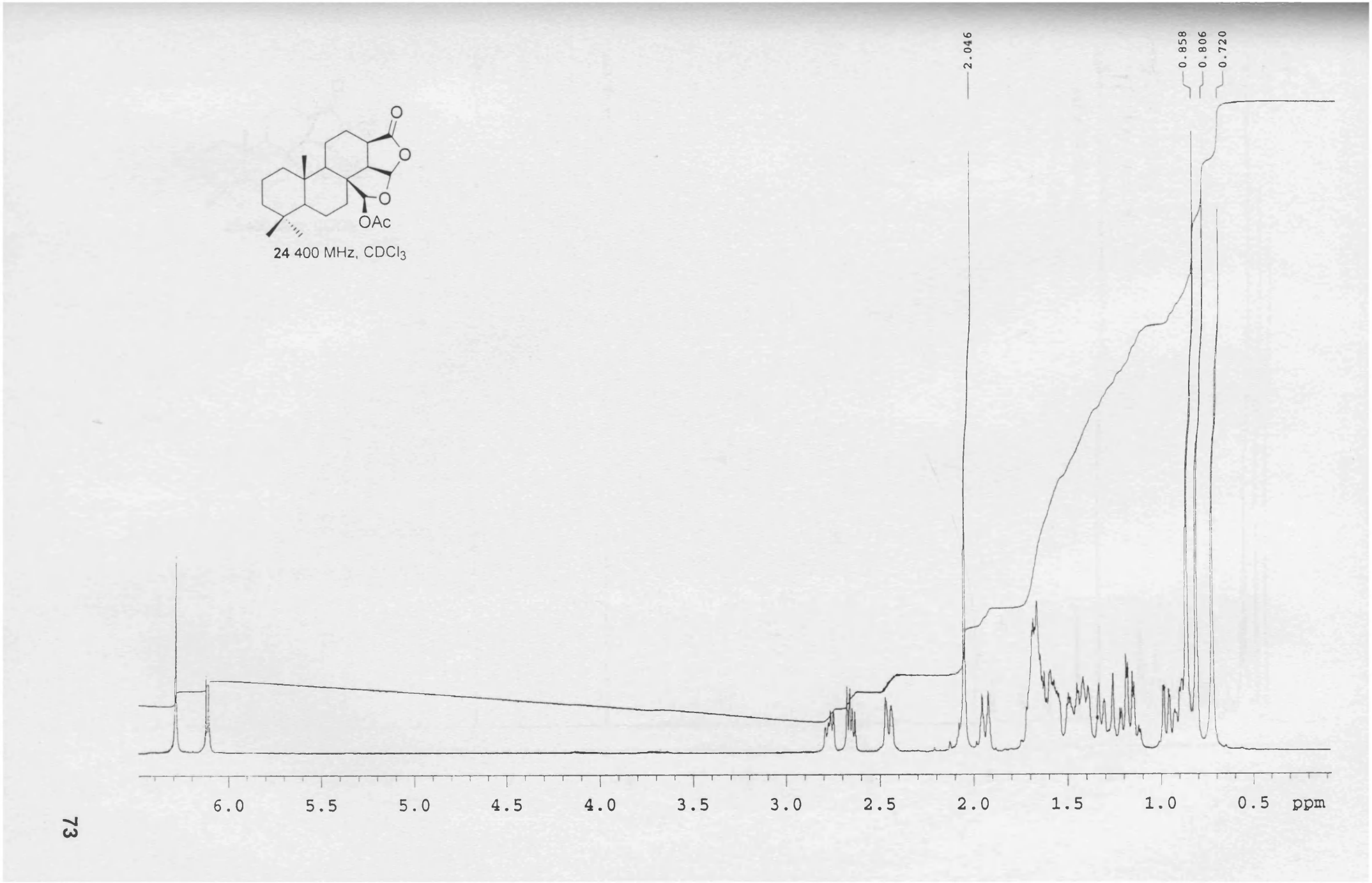

**2** 300 MHz, $CDCl_3$


O
O
CH2OAc
3 400 MHz, CDCl3
2.061
0.902
0.858
0.802
0.000
-0.002
7
6
5
4
3
2
1
ppm
70


OMe
20 400 MHz, CDCl3
4.917
3.273
0.848
0.822
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm




**23** 400 MHz, $CDCl_3$


OAc
24 400 MHz, CDCl3
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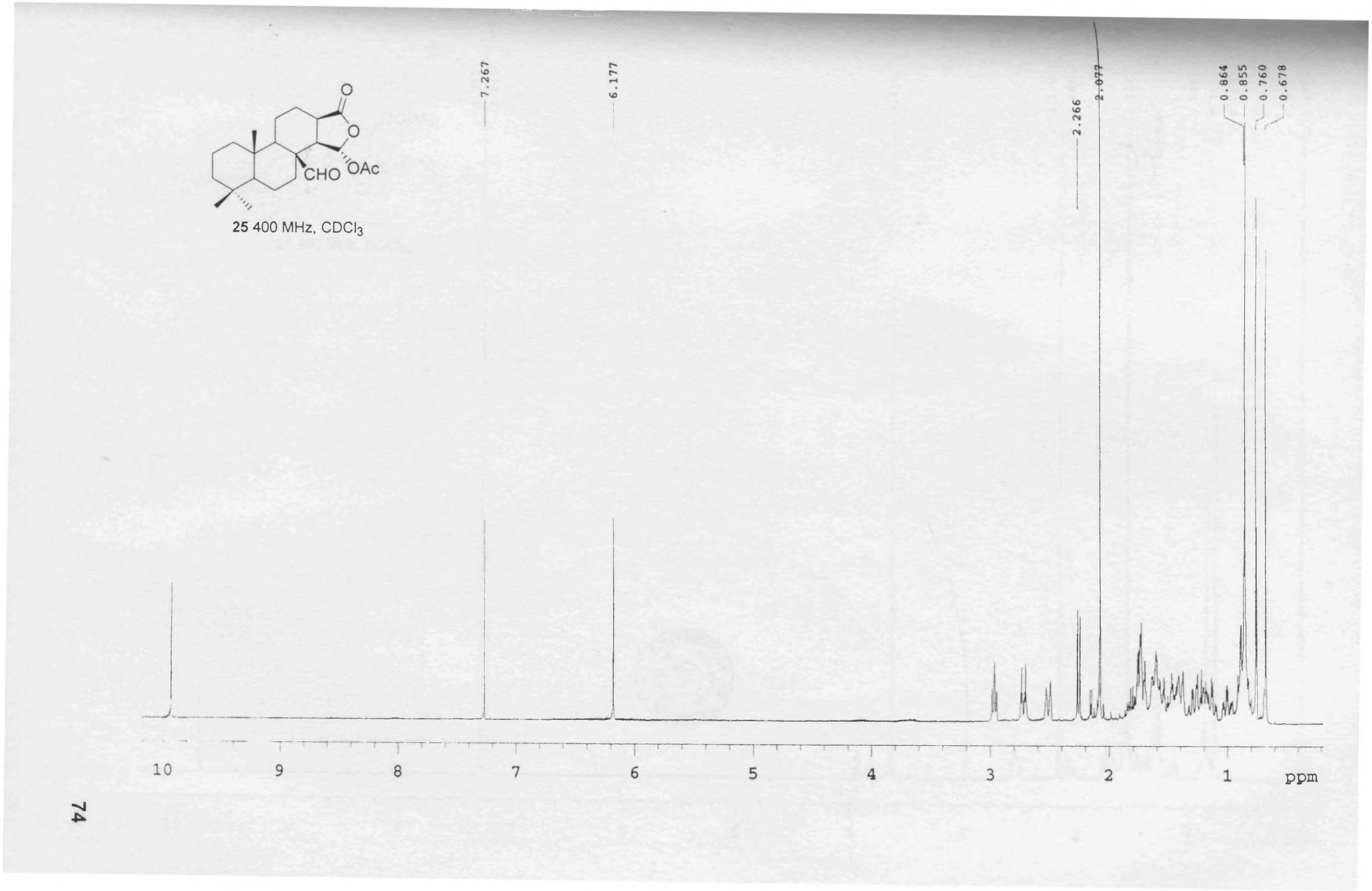
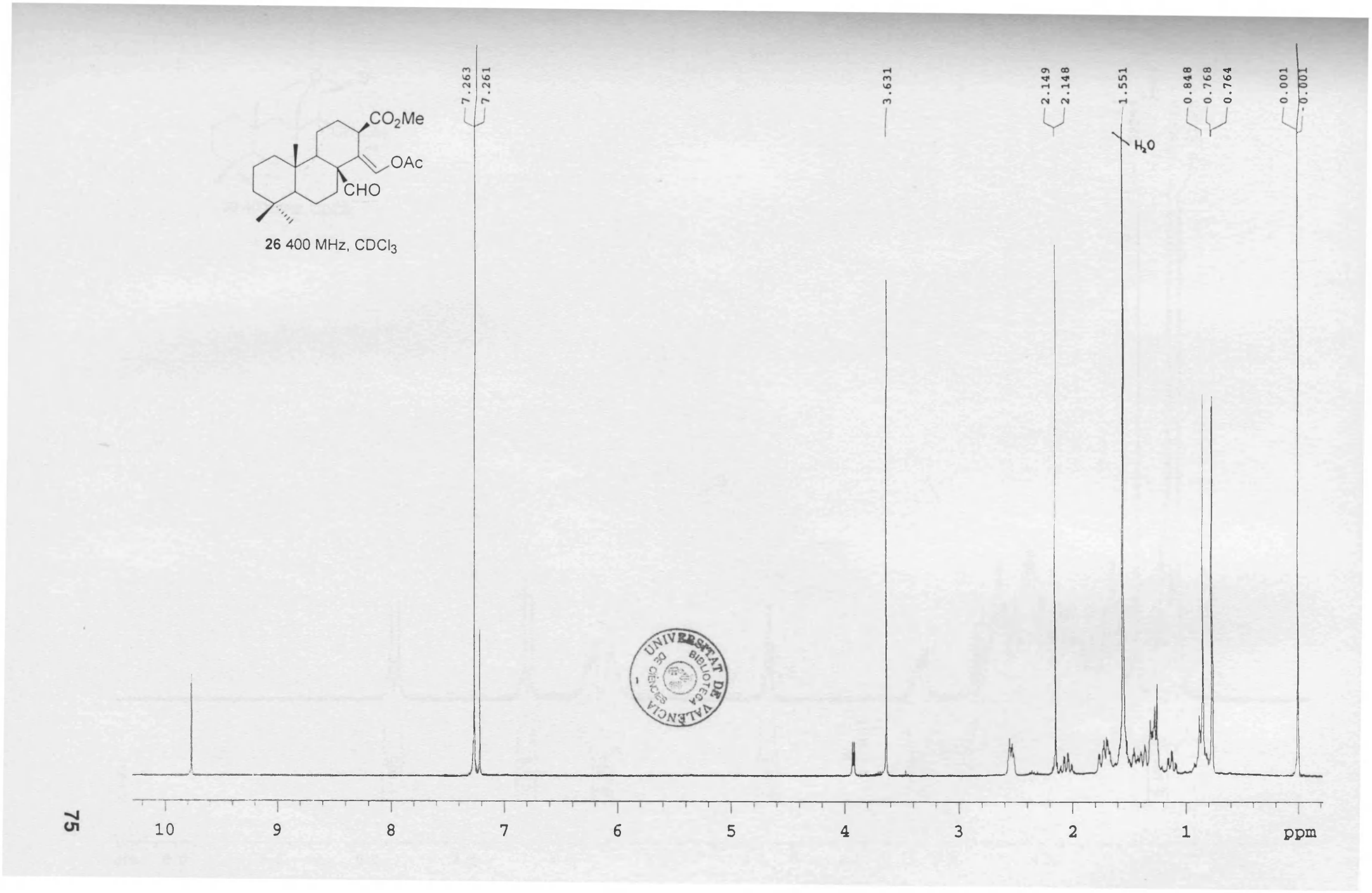
0.858
0.806
0.720
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm




**25** 400 MHz, $CDCl_3$




**26** 400 MHz, $CDCl_3$

30 400 MHz, CDCl3
CH2OH
O
O
Integral
ppm
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.005
0.976
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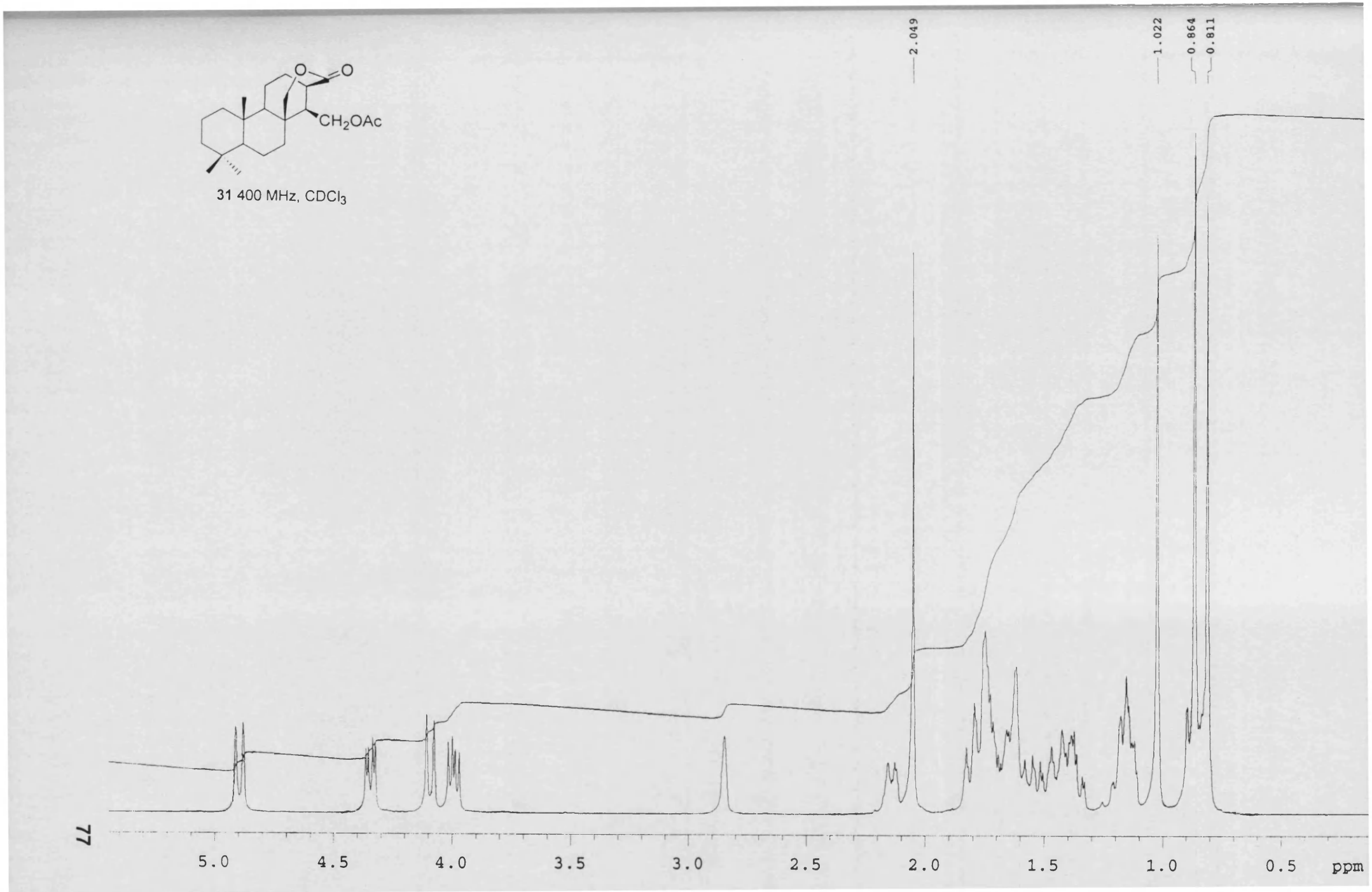
0.965
1.112
13.001
1.02252
0.86216
0.81025


O
O
CH2OAc
31 400 MHz, CDCl3
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1.022
0.864
0.811
77
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

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**TÍTULO:** Evaluación de la actividad antitumoral y antiviral *in vitro* de diterpenos espongiánicos sintéticos

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# EVALUACIÓN DE LA ACTIVIDAD ANTITUMORAL Y ANTIVIRAL *IN VITRO* DE DITERPENOS ESPONGIÁNICOS SINTÉTICOS

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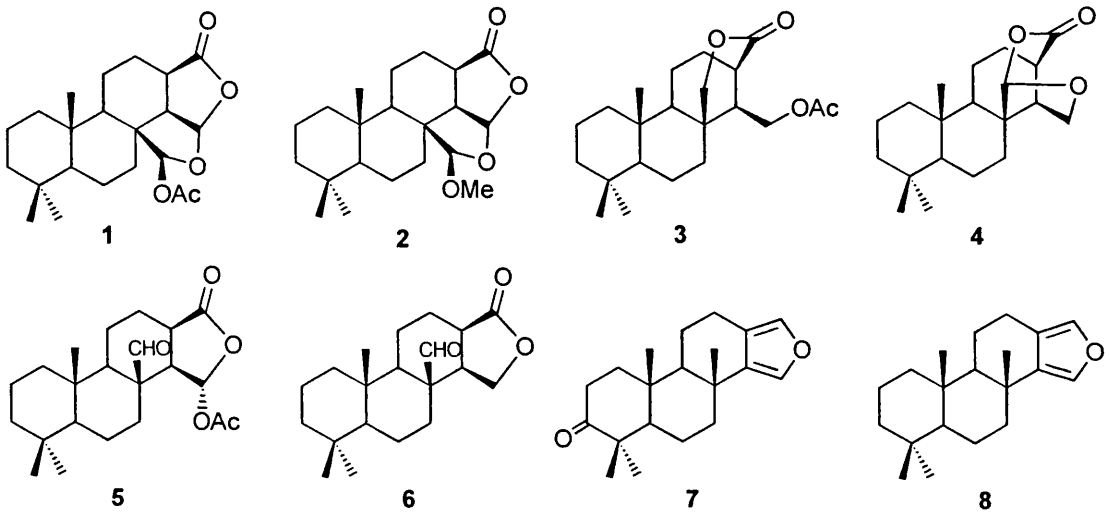
Es de nuestro interés el estudio de la actividad antitumoral y antiviral de compuestos derivados principalmente de productos naturales, y en los últimos años hemos visto que se han incrementado las investigaciones sobre un número de diterpenos aislados de organismos marinos los cuales presentan un amplio rango de actividades biológicas. Los diterpenos espongiánicos son una familia creciente de compuestos diterpenoides tetracíclicos o pentacíclicos aislados de esponjas y moluscos marinos, algunos de los cuáles han mostrado actividad citotóxica y antiviral. Miyamoto y colaboradores han aislado del molusco *Chromodoris obsoleta* varios diterpenos espongiánicos citotóxicos entre ellos el nuevo *15α, 16α-diacetoxy-11, 12β-epoxyspongian* y el ya conocido *7α-acetoxy-17β-hydroxy-15,17-oxidospongian-16-one*, los cuales mostraron valores de $IC_{50}$ en células tumorales KB de 0.18 y 1.9 μg/ml respectivamente[(1)].

En nuestro estudio hemos evaluado, hasta ahora, la actividad antitumoral y antiviral *in vitro* de ocho diterpenos espongiánicos, los cuáles se han sintetizado a partir de *(+)-podocarp-8(14)-en-13-one*,[(2)] y *S-(+)-carvone*[(3)]. Estos compuestos son: *17β-acetoxy-15,17-oxidospongian-16-one* (**1**), *17β-methoxy-15,17-oxidospongian-16-one* (**2**), *15,16-dideoxy-15-hydroxy-16,17-oxidospongian-16-one 15-acetate* (**3**), *15,16-dideoxy-15-17-oxido-16,17-oxidospongian-16-one* (**4**), *15α-acetoxy-spongian-17-al-16-one* (**5**), *spongian-17-al-16-one* (**6**), *spongia-13(16),14-dien-3-one* (**7**) y *spongia-13(16),14-diene* (**8**). Algunos de ellos han sido encontrados en la naturaleza como el compuesto **1**, aislado del molusco *Cadlina luteomarginata*,[(4)] el compuesto **6** aislado del molusco *Ceratosoma brevicaudatum*[(5)] y el furanoditerpeno **8**.[(6)]

*Revista Latinoamericana de Química, Vol. 28, Suplemento Especial, (2000)*




Para evaluar la actividad antitumoral *in vitro* de los anteriores diterpenos espongiánicos se utilizaron las líneas tumorales HeLa y Hep-2, la línea no tumoral CHO, y el cultivo primario Bon-Fib, con el fin de identificar compuestos con posible citotoxicidad selectiva hacía células tumorales, bajo la metodología descrita en la referencia 7; brevemente, las células se siembran en platos de cultivo de 96 pozos y se incuban por 24h, agregando luego los compuestos y dejando nuevamente en incubación por 72h. Para determinar la actividad antiviral se siembran las células MDBK y se dejan en incubación hasta formar monocapa, luego se agregan los compuestos y una hora después se agrega el virus Herpes simplex tipo2 humano en 10 o 1 dosis infecciosas, y se evalúa el efecto antiviral a través del método de reducción de placas[7]. Ambos bioensayos se realizaron por triplicado y se visualizaron por la fijación del microplato utilizando cristal violeta en formalina. Para la actividad antitumoral se reporta la concentración que desprendió el 100% de la momonopa celular, el compuesto con mayor actividad fue el **5**, con un valor de 6.4 μg/ml para células HeLa; en cuanto a la actividad antiviral todos los compuestos mostraron una reducción del titulo de $10^{0.5}$, actividad que se considera débil.

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**TÍTULO:** Structure-Activity Relationship of Cytototoxic effect on Tumor Cells and *In Vitro* Antiviral Activity against Herpes Simplex Virus of Synthetic Spongiane Diterpenes

**REF. REVISTA:** Journal of Natural Products, submitted.

# Structure-Activity Relationship of Cytotoxic effect on Tumor Cells and *In Vitro* Antiviral Activity against Herpes Simplex Virus of Synthetic Spongiane Diterpenes

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Abstract. A series of synthetic spongiane-type diterpenes has been tested in vitro for their potential antitumor and antiherpetic activity. Although the antiviral activity of these compounds against herpes simplex virus type 2 (HSV-2) resulted to be very weak, some compounds have exhibited relevant cytotoxicity in the human tumor cell lines HeLa and HEp-2. The biological activity of formyl spongianes is reported for the first time. With the present study, some structure-activity relationships have been recognized for the cytotoxic activity of these sponge-derived natural products.

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Several spongiane-type diterpenoids showing antifungal, antimicrobial, antifeedant, antiviral, and antitumuor activities, as well as $PLA_2$ inhibition, have been isolated from marine sponges or nudibranchs.[1] To date, the literature has provided many examples of new spongianes but in a relatively short period of time because to their interesting biological properties and various chemotaxonomic studies on marine invertebrates.

Isoagatholactone (**1**)[2] was isolated by Minale and co-workers in 1974 from the sponge *Spongia Officinalis*, and represents the first member known of the spongiane family. Over the years, different members of this family have been isolated and most of them vary in their functionalization in ring D and pattern of oxidation on rings A, B, and C (Fig. 1). Among the numerous spongianes possessing a ring D lactone, dorisenone A (**2**) isolated from the mollusk *Chromodoris obsoleta*[3] has displayed important cytotoxic activity in different tumor cell lines ($IC_{50}$=0.21 and 0.22 μg/ml, on murine lymphoma L1210 and human epidermoid carcinoma KB cells, respectively). Within the group of spongianes having a ring D furan and certain biological activity, spongiadiol (**3**) and its epimer epispongiadiol (**4**) found in several species of sponges should be mentioned. These two compounds along with isospongiadiol (**5**) isolated from Caribbean *Spongia* species have exhibited antiviral ($IC_{50}$=0.25, 12.5 and 2.0 μg/ml, against Herpes simplex virus type 1, respectively) and antitumor properties ($IC_{50}$= 0.5, 8.0 and 5.0 μg/ml on P388 murine leukemia cells, respectively).[4] Another set of bioactive spongiane diterpenes are characterized by structures containing a diacetylated double hemiacetal in ring D. For example, compounds (**6**) and (**7**) have been isolated from the nudibranch *Chromodoris luteorosea*,[5] and both have resulted to be toxic for the mosquito fish *Gambusia affinis*. Recently, another member of this sub-group such as epoxide (**8**) has showed a potent cytotoxic activity on L1210 and KB cells ($IC_{50}$=0.18 and 0.98 μg/ml, respectively).[3] An interesting additional group of spongianes is composed of pentacyclic members typified by the mildly cytotoxic aplyroseol-1 (**9**) ($ED_{50}$= 6.5 μg/ml on lymphocytic leukemia PS cells),[6] in which there is a highly oxygenated structure because of the presence of a hemiacetal forming the extra ring E.

This biological background prompted us to evaluate several synthetic spongiane diterpenoids as well as some derivatives and precursors with the aim of identifying new structure-activity

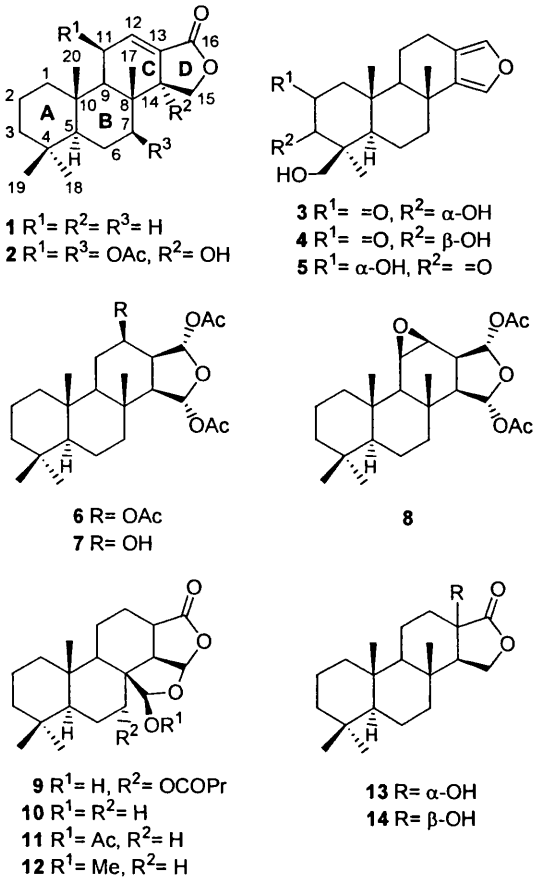


Figure 1

relationships in the spongiane skeleton. In the present work, we describe the *in vitro* antiherpetic and antitumor activities of a number of synthetic natural spongiane diterpenes and some derivatives including tetracyclic structures with lactone, furan and hemiacetal groups in ring D, and also three pentacyclic examples. It is worth to note that some spongiane diterpenes have been isolated in tiny amounts, what has precluded any bioactivity investigation. Consequently, this study signifies the first report on the *in vitro* biological activity of several of these rare marine secondary metabolites.

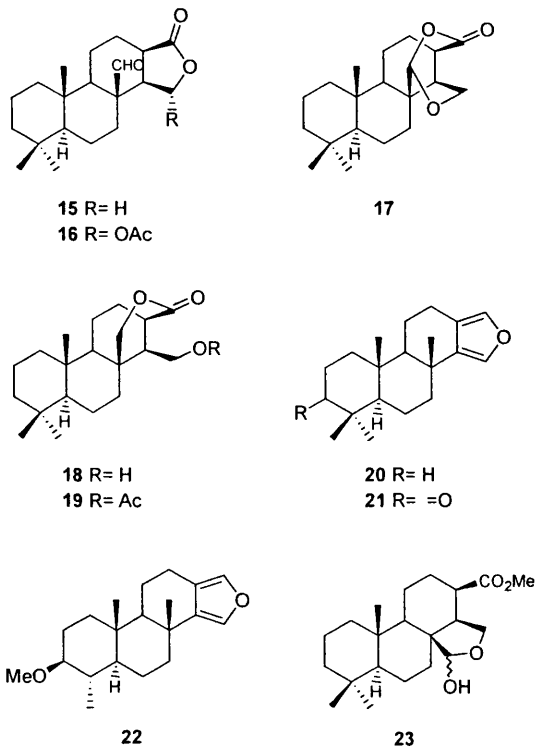


Figure 2

**Results and Discussion**

In this work we have evaluated so far the *in vitro* antitumor and antiviral activity of fourteen synthetic compounds (Fig. 1 and 2) including several naturally occurring spongiane diterpenes (compounds **1**, **10**, **11**, **15**, **19**, **20**).[7] We have also examined a bioactive 2:1 mixture of compounds **10** and **16**, as well as the 2:1 mixture of *ent*-isocopalane epimers **23**. All these compounds were obtained in enantiomerically pure from either (+)-podocarp-8(14)-en-13-one[8] or *S*-(+)-carvone,[9] and are as follows: pentacyclic spongiane compounds as 17β-hydroxy-15,17-oxidospongian-16-one (dendrillol-1) (**10**), 17β-acetoxy-15,17-oxidospongian-16-one (**11**), and 17β-methoxy-15,17-oxidospongian-16-one (**12**); compounds containing lactone and/or hemiacetal groups as spongia-12-en-16-one (**1**), 13α-hydroxyspongian-16-one (**13**), 13β-hydroxyspongian-16-one (**14**), spongian-16-oxo-17-al (**15**), 15α-acetoxyspongian-16-oxo-17-al (**16**), 15,16-dideoxy-15,17-oxido-16,17-oxidospongian-16-one (**17**), 15,16-dideoxy-15-hydroxy-16,17-oxidospongian-16-one (**18**), and 15-acetoxy-15,16-dideoxy-16,17-oxidospongian-16-one (aplyroseol-14) (**19**),[10] whose reassigned structure as depicted has recently been confirmed by means of chemical and spectral evidence;[11] and finally, furanoditerpenes as spongia-13(16),14-diene (**20**), spongia-13(16),14-dien-3-one (**21**), and 3β-methoxy-19-norspongia-13(16),14-diene (**22**).[12]

A preliminary evaluation of the potential biological activities of all these compounds was carried out using the *End-point titration technique* (EPTT),[13] in which the cytotoxic activity and the antiviral effect were simultaneously evaluated (Table **1**). Vlietinck and co-workers have reported that only the compounds with reduction factors of the viral titer over $1x10^3$ show relevant antiviral activity.

**Table 1.** Cytotoxicity and Anti-HSV-2 Activity of Spongiane Diterpenes on Vero Cells[a] determined by End-point titration technique (EPTT)

| Compound | $CC_{100}$ (μg/ml)[b] | Viral Reduction Factor[c] | Antiviral Activity (μg/ml)[d] |
|---|---|---|---|
| 1 | 40 | --- | --- |
| 10 | 8 | --- | --- |
| 11 | >120 | $10^{0.5}$ | 120 |
| 12 | 120 | $10^{0.5}$ | 60 |
| 13 | 50 | $10^{0.5}$ | 25 |
| 14 | 56 | $10^{1}$ | 28 |
| 15 | 28 | $10^{0.5}$ | 14 |
| 16 | 9 | $10^{0.5}$ | 4.5 |
| 10/16 | 7.5 | $10^{1}$ | 1.8 |
| 17 | 58 | $10^{0.5}$ | 29 |
| 18 | 37.5 | $10^{1}$ | 18.7 |
| 19 | 28 | $10^{0.5}$ | 14 |
| 20 | 120 | $10^{0.5}$ | 60 |
| 21 | 28 | --- | --- |
| 22 | 28 | $10^{0.5}$ | 15 |
| 23 | 30 | --- | --- |

[a] VERO, Cercopithecus aethiops african green monkey kidney ATCC No. CCL 81. [b] The minimal toxic dose that detached the100% the cell monolayer. [c] Ratio of the virus titer in the absence over virus titer in the presence of the tested compound. [d] The maximal nontoxic dose that showed the highest viral reduction factor.

As can be seen in Table 1 the compounds **14**, **17**, **18**, and the 2: 1 mixture of **10/16** were found to be slightly active against Herpes simplex virus type II (HSV-2), with a reduction factor of the viral titer of $1x10^1$. In most of these compounds, the nontoxic concentration for obtaining the largest reduction of the viral titer is quite close to the cytotoxic concentration that detached the 100% the cell monolayer ($CC_{100}$), revealing that their antiviral activity is principally due to their cytotoxicity. This very weak antiviral activity is not significant to be

further evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method.[14]

The furanoditerpenoids **20**, **21**, **22** have not exhibited important antiherpetic activity. On comparing the antiviral activity for isospongiadiol (**5**),[4] with the activity of furanoditerpenoids evaluated in this study, it can be concluded that the presence of a hydroxymethyl group at C-19 as well as other oxygenated functions in ring A in this compound class clearly enhances the resultant antiviral activity.

**Table 2.** *In vitro* Activity of Spongiane Diterpenes against Cell growth[a]

| Spongiane Diterpene | Cell lines[b] | | | |
|---|---|---|---|---|
| | HeLa | HEp-2 | CHO | Bon-Fib |
| 10 | 13 | 13 | 13 | 13 |
| 15 | 20 | 20 | 20 | 40 |
| 16 | 12 | 12 | 24 | 24 |
| 10/16 | 10 | 5 | 5 | 10 |
| 19 | 18 | 36 | 36 | 36 |
| 21 | 30 | 40 | 30 | 40 |
| 22 | 18 | 36 | 36 | 36 |
| 23 | 60 | 30 | 30 | >120 |

[a] The minimal toxic dose that detached the100% the cell monolayer ($CC_{100}$ values (μg/ml) in 48 h). [b] HeLa, human cervix epitheloid carcinoma ATCC No. CCL 2; HEp-2, human larynx epidermoid carcinoma ATCC No. CCL 23; CHO, Cricetulus griseus ovary chinese hamster cells ATCC No. CCL 61; Fib-Bon, Bovine ear skin primary culture.

The compounds that showed $CC_{100}$ on Vero cells lower than 30 μg/ml by using *EPTT* (Table 1) were further tested for their cytotoxicity against several tumor cell lines. In Table 2, it is shown $CC_{100}$ values for spongianes **10**, **15**, **16**, **19**, **21**, **22**, the 2:1 mixture of **10/16**, and *ent*-isocopalanes **23** obtained in the human tumor cell lines HeLa and HEp-2, CHO cell line and the Fib-Bon primary cell culture. Compound **16** and the mixture of **10/16** were the most toxic on the tumor cell lines with $CC_{100}$ of 12 and 10 μg/ml, respectively. All these compounds were examined for the *in vitro* antitumoral activity using the MTT method except the compound **21** which presents low values of cytotoxicity for the studied tumor cell lines ($CC_{100}$= 30 and 40 μg/ml, on HeLa and Hep-2, respectively). By using the MTT technique, we were able to obtain in a quantitative way the 50% cytotoxic concentration for cell growth ($CC_{50}$) for every active compound in the different cell lines tested. The $CC_{50}$ values can be used for establishing some structure-activity relationships in the spongiane skeleton (Table 3).

As can be seen in Table 3, again compound **16** was the most cytotoxic with $CC_{50}$ of 6.3±0.3 and 4.4±0.2 μg/ml, on HeLa and HEp-2 cells, respectively. The mixture of **10/16** also exhibits similar values of cytotoxicity. However, the other component of this mixture was found to be individually more selective for Hep-2 cells than in the mixture, and at the same time the most potent with $CC_{50}$ of 3.5±0.6 μg/ml on HEp-2 cells. The $CC_{50}$ values for cell growth inhibition were similar for the rest of compounds, including the natural products **15** and **19**.

**Table 3.** Cytotoxic Activity of Spongiane Diterpenes determined by MTT Technique.[a]

| Spongiane Diterpene | Cell lines[b] | | | |
|---|---|---|---|---|
| | HeLa | HEp-2 | CHO | Bon-Fib |
| 10 | 14.81±1.08 | 3.46±0.64 | 5.10±0.47 | 18.86±0.68 |
| 15 | 14.17±1.03 | 9.56±0.66 | 13.15±0.68 | 18.98±0.57 |
| 16 | 6.27±0.30 | 4.43±0.22 | 4.96±0.45 | 18.3±0.99 |
| 10/16 | 5.79±1.2 | 6.07±0.66 | 6.21±0.39 | 21.96±0.57 |
| 19 | 16.51±0.61 | 10.05±1.66 | 21.52±1.81 | 15.86±0.23 |
| 22 | 13.57±1.15 | 10.42±1.23 | 13.30±0.54 | 17.92±0.28 |
| 23 | 37.58±4.49 | 21.83±0.59 | 24.51±1.14 | >120 |

[a] The minimal toxic dose that detached the100% the cell monolayer ($CC_{100}$ values (μg/ml) in 48 h).[b] HeLa, human cervix epitheloid carcinoma ATCC No. CCL 2; HEp-2, human larynx epidermoid carcinoma ATCC No. CCL 23; CHO, Cricetulus griseus ovary chinese hamster cells ATCC No. CCL 61; Fib-Bon, Bovine ear skin primary culture.

On comparison of the activity of compound **16** and that of compounds **13** and **14**, it can be concluded that the presence of a methyl group at C-8 will decrease the cytotoxic activity, if there is no additional functionalization with suitable polar groups at C-7 and C-14 as it is deduced from Miyamoto's study.[3] For this reason, it can be speculated that in this part of the molecule is necessary the presence of polar substituents to enhance its cytotoxicity. Interestingly, this value of cytotoxicity for compound **16** is quite significant since it was obtained in only 48 h on tumor cell lines of known resistance to chemotherapy; therefore this is the first report on the biological activity of spongiane-type diterpenes possessing a formyl group at C-8.

The pentacyclic spongiane **10** also displays a lactone group in ring D as in the lead compound **16**, but there is an additional hemiacetal ring system. On comparing the activities of pentacyclic compounds **10-12**, it can be concluded that a hydroxyl group at C-17 is essential to maintain the cytotoxic effect. Therefore, it is proved again the importance of polar groups located at position C-17 in the spongiane skeleton, which may be responsible for interacting with certain receptors on tumor cells.

These results encourage us to continue our research of this series by synthesizing further spongiane-type derivatives with the aim of obtaining more potent and selective cytotoxic compounds towards tumor cell lines, which is the main objective in our group.

**Experimental Section**

*Compounds*

The diterpenoids tested were obtained following our procedure.[8,9,11] Chemical structures of the compounds are shown in Fig. 1 and Fig. 2. Stock solutions (7 mg/ml) of these compounds for testing in vitro were prepared in dimethylsulfoxide and stored at 4 °C.

*Cell Culture and Virus* - The lines cells used were: Cricetulus griseus ovary chinese hamster cells (CHO cell line ATCC CCL-61), Human cervix epitheloid carcinoma cells (HeLa cell line ATCC CCL-2), Human larynx epidermoid carcinoma cells (HEp-2 cell line ATCC CCL23), Cercopithecus aethiops african green monkey kidney (VERO cell line ATCC CCL-81). Fib-Bon primary culture cells were obtained in our laboratory from Bovine ear skin biopsies. Briefly the protocol used to obtain primary cell cultures was as follows: the biopsy was washed three times with Phosphate Buffered Saline (PBS) containing 2% penicillin-streptomycin-amphotericine B, the skin was discarded, the cartilage and the subcutaneous tissue was minced finely, the pieces of tissues were placed in 25 $cm^2$ cell culture flasks with just enough growth medium (Eagle minimum essential medium MEM with L-glutamine 2nM, 1% vitamins, 1% non essential amino acids, 1% penicillin-streptomycin-amphotericine B and 10% of fetal bovine serum (FBS) to cover the pieces of tissue. When the fibroblasts proliferated to 30 or 40% confluence, the pieces of tissues were discarded by gently shaking with PBS and again the cells were fed with 50% of used medium and 50% of fresh medium. When 80% confluence was reached, the cells were trypsinized and cultured in 150 $cm^2$ flasks. Once the cells covered about 80% of the surface, they were trypsinized, centrifuged and cryopreserved.

All cells were grown in MEM supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, 20 mg/ml glutamine, 0.14% $NaHCO_3$, and MEM non-essential aminoacid and vitamins solution. The culture were maintained at 37 °C in a humidified 5% $CO_2$ atmosphere.

HSV-2 was obtained from Center for Diseases Control, Atlanta, Georgia. USA. The virus stock was prepared from HSV-2-infected HEp-2 cell cultures. The infected cultures were subjected to three cycles of freezing-thawing, and centrifuged at 2000 rpm for 10 min. The supernatant was collected, titrated and stored at -170 ° C in 1 ml aliquots.

*Antiviral Assays*

*End-point titration technique* (EPTT)- The technique described by Vander Berghe[13] with few modifications was used. Briefly, confluent monolayer of VERO cells were grown in 96-well flat-bottomed plates. Two-fold dilutions of the compounds in maintenance medium, supplemented with 2% serum and antibiotics, were added 1 h before the viral infection. Cells were infected with 0.1 ml of serial tenfold dilutions of the appropriate virus suspension and incubated again at 37 °C in a humidified 5% $CO_2$ atmosphere for a period of 48 h. Virus control, tissue culture control and compound control (in order to determine the cytotoxicity of the compound at each dilution) were included in each test. The antiviral activity is expressed as the virus titer reduction at the maximal nontoxic dose (MNTD) of the tested compound. The virus titer reduction was determined as the reduction factor of the viral titer (ratio of the virus titer in the absence over virus titer in the presence of the compound). Three assays were carried out with at least five concentrations by duplicated.

In vitro *Assay on Cell growth*
Cell monolayers in culture were trypsinized and washed with culture medium. The cells were plated at 5.000 cells per well for HeLa, HEp-2 and CHO cells and at 20.000 cells per well for Fib-Bon cells in a 96-well flat-bottomed plate. After 24 h preincubation, compound dilutions were added to the appropriate wells and the plates were incubated for further 48 h at 37 °C in a humidified incubator with 5% $CO_2$. The cytotoxic activity was expressed as the minimal toxic dose of the compound that detached the 100% the cell monolayer ($CC_{100}$). Three assays were carried out with at least five concentrations by duplicated.
*Cytotoxicity Assay*
By the tetrazolium-dye (MTT)[14] cytotoxicity assays, adherent cell monolayers (HeLa, HEp-2) in culture were trypsinized and washed with culture medium. The cells were plated at 15.000 cells/well in 96-well flat-bottomed plate. After a 24 h preincubation period, compound dilutions were added to the appropriate wells and the plates were incubated for 48 h at 37 °C in a humidified incubator with 5% $CO_2$. The supernatants were removed from the wells and 28 µl of a MTT (Sigma, 2 mg/ml) solution in Phosphate Buffered Saline (PBS) was added to each well. Plates were incubated for 1.5 h at 37 °C, and 130 µl of DMSO was added to the wells to dissolve the MTT crystals. The plates were placed on a shaker for 15 min and absorbency was read at 492 nm on a multiwell spectrophotometer (Titertek Uniskan). The results were obtained from three assays with at least five concentrations by duplicated.
*Data Analysis* - The 50% cytotoxic concentration ($CC_{50}$) for each compound were obtained from dose-effect-curves (not shown).

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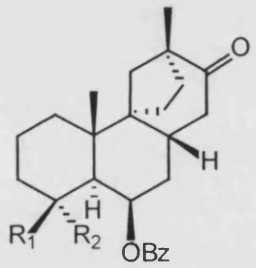
## *3. ESCOPADULANOS*

# 3.- ESCOPADULANOS

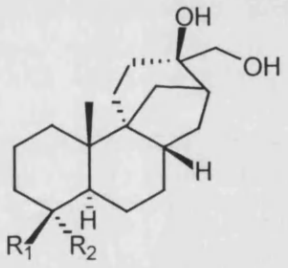
## 3.1.- Compuestos escopadulánicos aislados de fuentes naturales y propiedades.

Los *escopadulanos* son una nueva familia de compuestos diterpénicos de origen vegetal de reciente descubrimiento, y hasta el momento está compuesta por escasamente una decena de compuestos. La planta donde se encuentran estos metabolitos, *Scoparia dulcis*, es conocida tradicionalmente en Paraguay como planta medicinal para mejorar la digestión y proteger el estómago, en Taiwan para tratar la hipertensión y en la India para el dolor de muelas y de estómago.[1]

Actualmente, los estudios para averiguar la biosíntesis de estos nuevos diterpenos tetracíclicos proponen que, contrariamente a la ruta general biosintética de los terpenoides *via* mevalonato (MVA), los *escopadulanos* como el *ácido escopadúlcico A* (SDA) (**1**) se biosintetizan por una ruta independiente del MVA, *via* fosfato de desoxixilulosa, aunque otros diterpenos de estructura similar como ***aphidicolin*** (**4**) se biosintetizan por la ruta del mevalonato.[2]




**1** $R_1$= COOH, $R_2$= $CH_2OH$
**2** $R_1$= Me, $R_2$= COOH
**3** $R_1$= Me, $R_2$= $CH_2OH$




**4** $R_1$= Me, $R_2$= $CH_2OH$

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Durante la búsqueda de sustancias biológicamente activas de plantas medicinales de Paraguay, Hayashi y colaboradores encontraron en el extracto etanólico del preparado medicinal "Typychá kuratû", obtenido de plantas enteras de *Scoparia dulcis* L. (Scrophuraliaceae) (Fig. **1**), dos nuevos diterpenos llamados *ácido escopadúlcico A* (SDA) (**1**) y *ácido escopadúlcico B* (SDB) (**2**).[3] Sus estructuras fueron determinadas, incluyendo su configuración absoluta, con técnicas bidimensionales de RMN y dicroísmo circular. Poco más tarde, la estructura propuesta para el SDA fue confirmada por difracción de rayos X de monocristal.[4]

**Figura 1**. *Scoparia dulcis* y detalle de su flor.

Los mismos autores aislaron más tarde del extracto etanólico de *Scoparia dulcis* de Taiwan otro nuevo metabolito, *Scopadulciol* (**3**),[5] el cuál ya había sido encontrado en otro especimen de *Scoparia dulcis* en Blangladesh, y fue llamado *Dulcinol*.[6]

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Desde el primer aislamiento en 1987, se han realizado varios estudios biológicos para estudiar sus propiedades citotóxicas y posibles aplicaciones terapéuticas, que a continuación describiremos. Por ejemplo, el (SDB) (**2**) ha mostrado su capacidad inhibidora de la enzima $H^+$, $K^+$-adenosin trifosfatasa (ATPasa), responsable del intercambio $H^+/K^+$ y de la secreción de ácido en el estómago.[7] Estos resultados lo convierten en un posible agente farmacológico para tratar la ulcera.

La **actividad antiviral** de SDA (**1**) y SDB (**2**) tanto *in vitro* como *in vivo* contra el virus *herpes simplex 1* (HSV-1) también ha sido estudiada.[8] En concreto, se estudió la capacidad inhibitoria de varios diterpenos aislados de *Scoparia dulcis* en la replicación de HSV-1 en cultivos de células HeLa 229. Por ejemplo, los valores de $ID_{50}$ para inhibir el crecimiento de células HeLa (citotoxicidad) de SDA y SDB fueron de 0.78 y 0.20 μg/mL, respectivamente.

Pero las aplicaciones de estos compuestos aún son más amplias, ya que SDB (**2**) también ha mostrado cierta **actividad antitumoral**. Hasta la fecha, se han estudiado sus propiedades *in vitro* en varias líneas celulares derivadas de tejidos tumorales, e *in vivo* contra células ascitos de Ehrlich inoculadas en ratones.[9] De hecho, SDB (**2**) tiene una estructura muy parecida a la de *aphidicolin* (**4**),[10] el cuál es un diterpeno tetracíclico con reconocida capacidad inhibitoria de la ADN polimerasa α, ya que a concentraciones de 1μM anula el crecimiento celular al inhibir la síntesis de ADN en células cultivadas,[11] aunque carece de actividad antitumoral al administrarse en animales ya que se metaboliza y se excreta rápidamente.



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[7] a) Hayashi, T.; Okamura, K.; Kakemi, M.; Asano, S.; Mizutani, M.; Takeguchi, N.; Kawasaki, M.; Tezuka, Y.; Kikuchi, T.; Morita, N. *Chem. Pharm. Bull.* **1990**, *38*, 2740; b) Asano, S.; Mizutani, M.; Hayashi, T.; Morita, N.; Takeguchi, N. *J. Biol. Chem.* **1990**, *265*, 22167.

[8] a) Hayashi, K.; Niwayama, S.; Hayashi, T.; Nago, R.; Ochiai, H.; Morita, N. *Antiviral Res.* **1988**, *9*, 345; b) Hayashi, T.; Hayashi, K.; Uchida, K.; Niwayama, S.; Morita, N. *Chem. Pharm. Bull.* **1990**, *38*, 239.

[9] a) Hayashi, T.; Kawasaki, M.; Miwa, Y.; Taga, T.; Morita, N. *Chem. Pharm. Bull.* **1990**, *38*, 945; b) Hayashi, K.; Hayashi, T.; Morita, N. *Phytother. Res.* **1992**, *6*, 6.

[10] Síntesis de aphidicolanos ver: Bélanger, G.; Deslongchamps, P. *J. Org. Chem.* **2000**, *65*, 7070 y referencias citadas allí.

[11] Morita, T.; Tsutsui, Y.; Nishiyama, Y.; Nakamura, H.; Yoshida, S. *Int. J. Radiat. Biol.* **1982**, *42*, 471.

Más recientemente, los *escopadulanos* y sus análogos semisintéticos también se han estudiado como inhibidores de los ésteres de forbol en la formación de tumores,[12] e incluso se les ha encontrado aplicación en el tratamiento de la **osteoporosis**,[13] ampliándose más aún el espectro farmacológico de estos compuestos.

Por otro lado, también se han encontrado diterpenos escopadulánicos en otro género de la família *Scrophulariaceae* como es *Calceolaria* (Fig. **2**). En concreto, la especie *Calceolaria thyrsiflora* es una hierba como la *Scoparia* de media altura (0.5 m) que en este caso crece en las colinas del Chile central.[14]

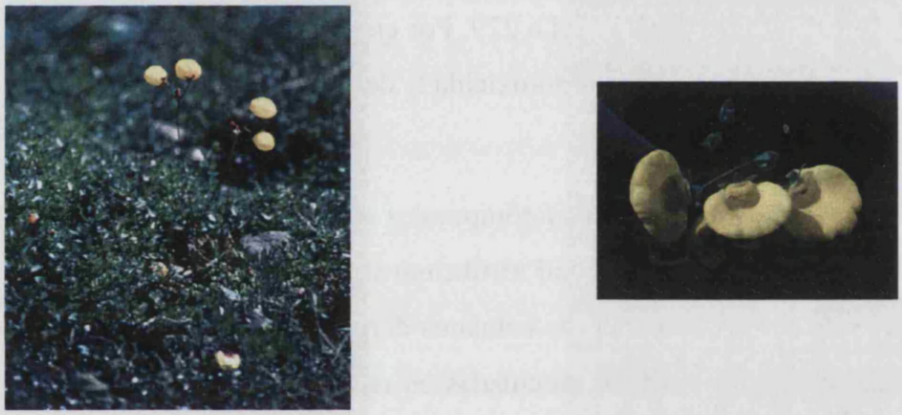

**Figura 2**. Ejemplo de *Calceolaria* de Chile y detalle de su flor.

Del extracto clorofórmico de esta hierba, Garbarino y colaboradores identificaron dos nuevos ácidos con esqueleto de *escopadulano* que fueron aislados a través de sus metil ésteres correspondientes, así como un diol también escopadulánico. Las estructuras de *thyrsiflorin A* (**5**), *thyrsiflorin B* (**6**) y *thyrsiflorin C* (**7**) fueron asignadas mediante espectroscopía, transformaciones químicas y análisis de difracción de rayos X.

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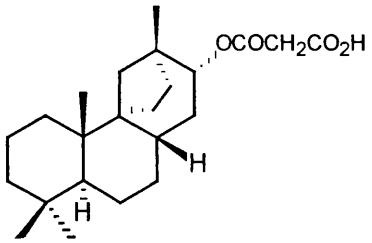
[12] Nishino, H.; Hayashi, T.; Arisawa, M.; Satomi, Y.; Iwashima, A. *Oncology* **1993**, *50*, 100.

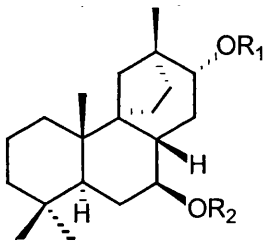
[13] Miyahara, T.; Hayashi, T.; Matsuda, S.; Yamada, R.; Ikeda, K.; Tonoyama, H.; Komiyama, H.; Matsumoto, M.; Nemoto, N.; Sankawa, U. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1037.

[14] Chamy, M. C.; Piovano, M.; Garbarino, J. A.; Miranda, C.; Gambaro, V.; Rodríguez, M. L.; Ruiz Pérez, C.; Brito, I. *Phytochemistry*, **1991**, *30*, 589.

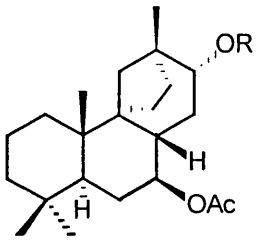
Recientemente, también durante el estudio del género *Calceolaria*, Garbarino y colaboradores identificaron en la planta *Calceolaria dentata* recogida en Chile tres nuevos diterpenos escopadulánicos (**8-10**).[15] Las estructuras de los compuestos aislados se propusieron en base a métodos espectroscópicos, y dos de ellos también fueron caracterizados a través de sus metil ésteres correspondientes.



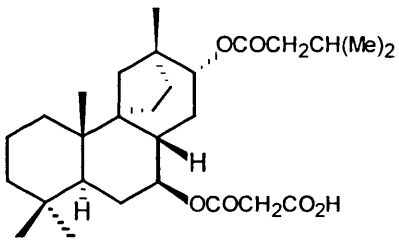

**5**




**6** $R_1$= $COCH_2CO_2H$, $R_2$= H
**7** $R_1$= H, $R_2$= H




**8** R = $COCH_2CO_2H$
**9** R = $COCH_2CH(Me)_2$

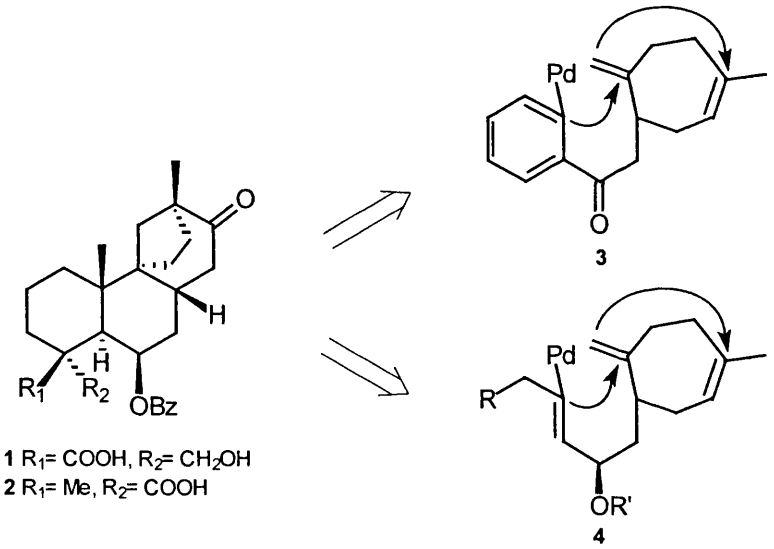



**10**

[15] Chamy, M. C.; Piovano, M.; Garbarino, J. A.; Vargas, C. *Phytochemistry*, **1995**, *40*, 1751.

## 3.2.- Estudios sintéticos previos de compuestos escopadulánicos.

Desde su aislamiento los *escopadulanos* han despertado un gran interés entre numerosos químicos sintéticos, incluyendo también a varias compañías farmacéuticas que han financiado parte de las investigaciones. La información publicada hasta hoy relacionada con la síntesis de estos compuestos tiene poco más de una década de existencia. Dentro de la escasa literatura existente hasta hoy sobre la síntesis de diterpenos escopadulánicos, hay que destacar el esfuerzo de Overman y colaboradores que les ha llevado a conseguir la síntesis del *ácido escopadúlcico A* (SDA) (**1**) a través del precursor **4**, tanto en su forma racémica como en ambas formas enantioméricas, y también del *ácido escopadúlcico B* (SDB) (**2**) racémico a partir de **3**.




**1** $R_1$= COOH, $R_2$= $CH_2OH$
**2** $R_1$= Me, $R_2$= COOH

En 1988, Overman y colaboradores demostraron que los yoduros de arilo pueden ciclar con dienos en presencia de un catalizador de paladio para dar el sistema tetracíclico presente en los *escopadulanos* (Esquema **1**).[1] Esta estrategia se basa en reacciones tandem intramoleculares de tipo Heck de sistemas poliénicos, y ha permitido la construcción estereoselectiva de centros cuaternarios en sustratos altamente sustituidos.[2]

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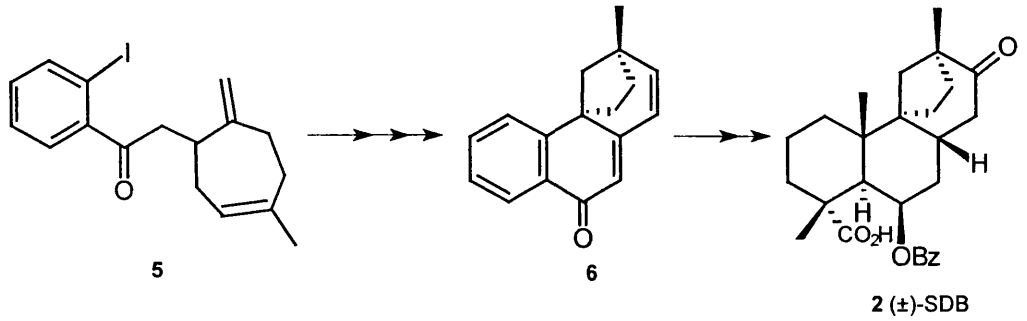
[1] Abelman, M. M.; Overman, L. E. *J. Am. Chem. Soc.* **1988**, *110*, 2328.

[2] a) Overman, L. E. *Pure Appl. Chem.* **1994**, *66*, 1423; b) Gibson, S. E.; Middleton, R. J. *Contemp. Org. Synth.* **1996**, *3*, 447.

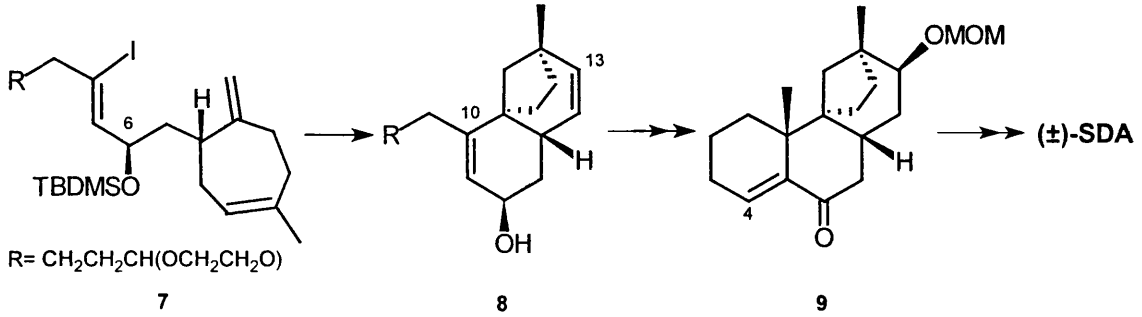
Esquema **1**




Esta idea se ha aplicado satisfactoriamente en la síntesis de (±)-SDB **2** a partir del sistema poliénico **5**, obtenido a partir del 2-yodobenzaldehido con un rendimiento global del 22% (Esquema **1**).[3] El paso clave de la síntesis es la ciclación del yoduro **5**, en presencia de $Pd(OAc)_2$-$Ph_3P$, para dar el sistema tetracíclico **6** con la estereoquímica adecuada en las posiciones C-9 y C-12. El intercambio en la funcionalización de los anillos B y C, seguido de reducción del anillo aromático y metilación en C-10 permitió obtener el SDB (**2**). Esta es la primera síntesis total de un diterpeno escopadulánico, se publicó en 1993,[3a] y consta de 30 pasos para obtener un rendimiento global del 0.5% desde 2-yodobenzaldehido.

En una segunda estrategia (Esquema **2**), el precursor **7** incorpora el sustituyente necesario en C-6 para que la reacción de Heck transcurra de forma regio- y estereoselectiva para dar el sistema tricíclico **8**.

Esquema **2**

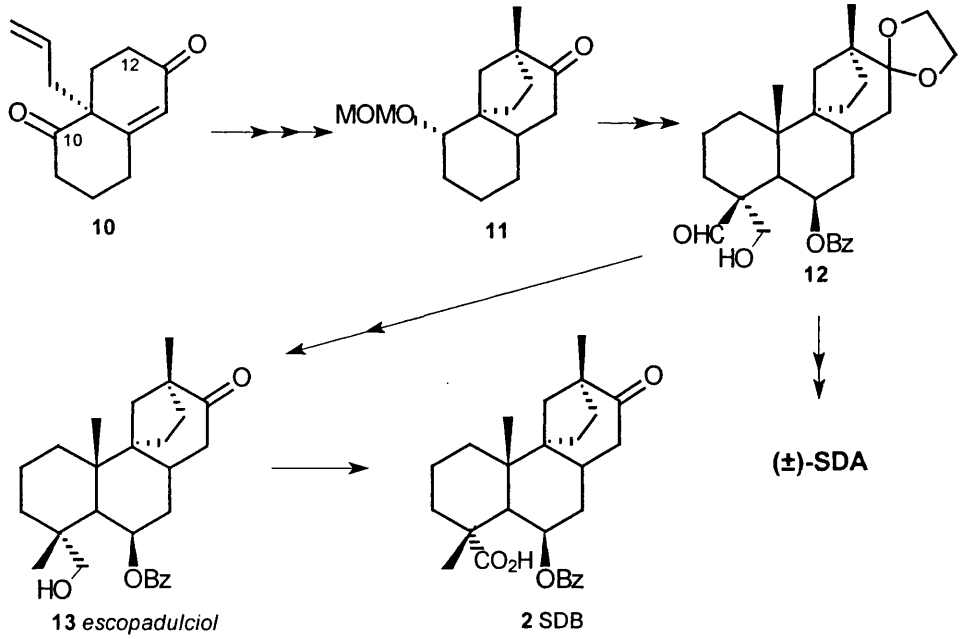



[3] a) Overman, L. E.; Ricca, D. J.; Tran, V. D. *J. Am. Chem. Soc.* **1993**, *115*, 2042; b) Overman, L. E.; Ricca, D. J.; Tran, V. D. *J. Org. Chem.* **1997**, *119*, 12031.

La metilación de C-10 y formación del anillo A por condensación aldólica intramolecular conduce a la enona **9**, que por doble alquilación en C-4 y posterior refuncionalización proporcionó el (±)-SDA **1**.[4] De esta forma, en 27 pasos se pudo obtener (±)-SDA en ~3% desde el 1-bromo-2-metil-2-vinilciclopropano. Recientemente, Overman y colaboradores han obtenido ambas formas enantioméricas del SDA estableciendo así la configuración absoluta de éste.[5] Se ha utilizado básicamente esta misma secuencia pero con el precursor de ciclación **7** enantioméricamente puro.

Por otro lado, la única síntesis que existe de ácidos escopadúlcicos publicada hasta la fecha es la de Ziegler y Wallace en 1995 (Esquema **3**) que utilizando como material de partida la dicetona **10** consiguieron la síntesis del (±)-SDA **1**, (±)-SDB **2** y (±)-*escopadulciol* **13** .[6] La construcción del anillo D se realizó mediante alquilación intramolecular de C-12 dando la cetona **11** que, después de introducir los sustituyentes adecuados en C-10, por condensación aldólica intramolecular y doble alquilación en C-4 dio el intermedio **12** usado para la síntesis de los tres compuestos mencionados.

Esquema **3**




**10** **11** **12**

**13** *escopadulciol* **2** SDB

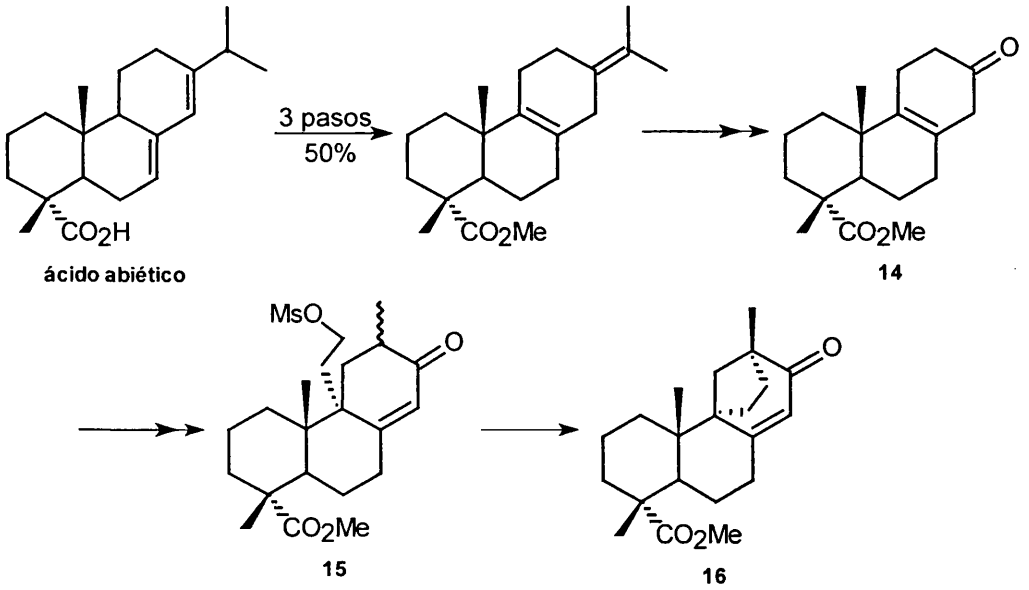
[4] Kucera, D. J.; O'Connor, S. J.; Overman, L. E. *J. Org. Chem.* **1993**, *58*, 5304.

[5] Fox, M. E.; Li, C.; Marino, J. P. Jr.; Overman, L. E. *J. Am. Chem. Soc.* **1999**, *121*, 5467.

[6] Ziegler, F. E.; Wallace, O. B. *J. Org. Chem.* **1995**, *60*, 3626.

También se han descrito otras estrategias para obtener el esqueleto de *escopadulano*, por ejemplo: mediante reacciones de adición a oxazolinas quirales,[7] reacciones de Diels-Alder intramoleculares,[8] y acoplamiento intramolecular de olefinas mediante oxidación anódica,[9] pero todas ellas quedan bastante lejos de completar el esqueleto deseado. Tagat y colaboradores[10] a partir de la podocarpenona **14**, obtenida del ácido abiético siguiendo nuestra metodología,[11] han sintetizado el intermedio **16** con esqueleto de *escopadulano* (Esquema **4**). Básicamente, esta estrategia utiliza la misma alquilación intramolecular de la síntesis de Ziegler para construir el anillo D, que a través del mesilato **15** conduce a la enona **16** (11%; 10 pasos desde ác. abiético). Sin embargo, la funcionalización necesaria en C-6 o C-7 para obtener escopadulanos naturales no fue conseguida.

Esquema **4**




[7] Robichaud, A. J.; Meyers, A. I. *J. Org. Chem.* **1991**, *56*, 2607.

[8] Hall, D. G.; Caillé, A.–S.; Drouin, M.; Lamothe, S.; Müller, R.; Deslongchamps, P. *Synthesis* **1995**, 1081.

[9] Reddy, S. H. K.; Moeller, K. D. *Tetrahedron Lett.* **1998**, *39*, 8027.

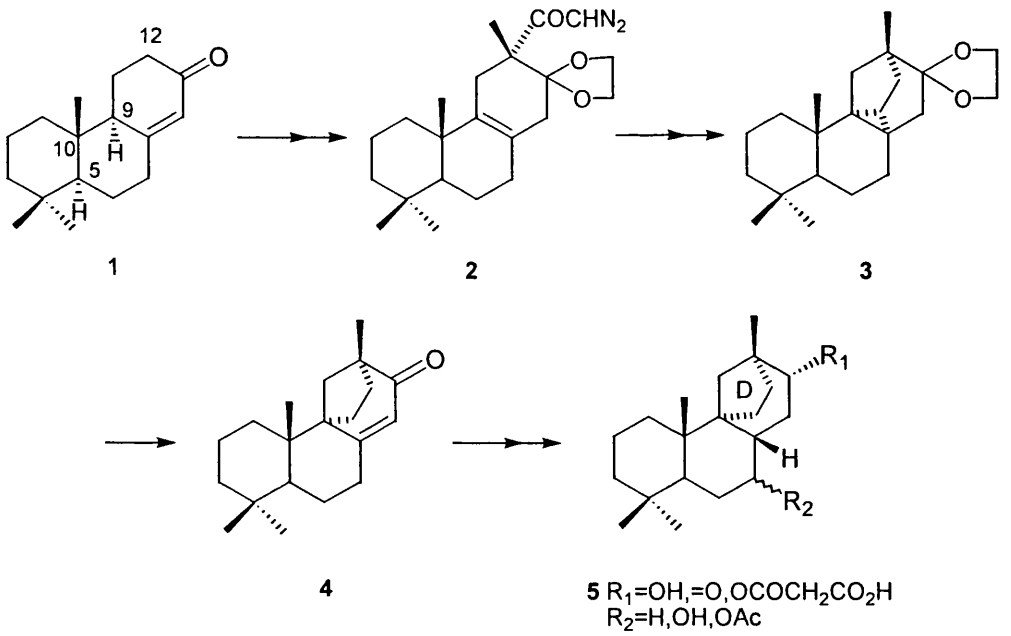
[10] a) Tagat, J. R.; McCombie, S. W.; Puar, M. S. *Tetrahedron Lett.* **1996**, *47*, 8459; b) Tagat, J. R.; McCombie, S. W.; Puar, M. S. *Tetrahedron Lett.* **1996**, *47*, 8463.

[11] Abad, A.; Arnó, M.; Domingo, L. R.; Zaragozá, R. J. *Tetrahedron* **1985**, *41*, 4937.

## 3.3.- Síntesis de Escopadulanos a partir de la podocarpenona.

En esta Tesis también hemos abordado la síntesis de diterpenos escopadulánicos (**5**) funcionalizados en C-7 a partir de la podocarpenona **1** (Esquema **1**). La podocarpenona **1** tiene un esqueleto tricíclico ABC idéntico al que presentan los escopadulanos, así pues la construcción del anillo D y metilación de C-12 ha conducido a este tipo de esqueleto. Además, la funcionalización de las posiciones C-8 y C-14 en la podocarpenona **1** ha facilitado la consecución de la funcionalización en C-7 observada en algunos escopadulanos naturales.

Esquema **1**




**1** **2** **3**

**4**

**5** $R_1$=OH,=O,$OCOCH_2CO_2H$
$R_2$=H,OH,OAc

La facilidad de la desconjugación del doble enlace de la podocarpenona **1** a las posiciones C-8 y C-9, ha permitido construir el anillo D a través de una ciclopropanación intramolecular de la diazocetona **2**, obtenida mediante una doble alquilación estereoselectiva de C-12. El paso clave de esta secuencia es la apertura regioselectiva del anillo ciclopropánico del compuesto **3**, de la cuál no había precedentes en la literatura. Esta apertura regenera el doble enlace entre C-8 y C-14, dando la enona **4**, que posteriormente se desconjuga para funcionalizar C-7 y obtener diversos escopadulanos naturales (**5**).

Hay que destacar que gracias al trabajo realizado en esta Tesis se ha desarrollado una ruta para obtener de manera estereoselectiva el segundo grupo de escopadulanos de reciente aislamiento, llamados *tirsifloranos*, lo que ha supuesto la primera obtención de estas sustancias en forma enantioméricamente pura. A nivel estructural este nuevo grupo de escopadulanos y sus precursores han sido estudiados y caracterizados con detalle mediante resonancia magnética nuclear. De forma similar a lo realizado con los *espongianos*, en colaboración con la Universidad de Antioquía se han evaluado actividades antivirales contra HSV-2 y actividades antitumorales en líneas celulares tumorales HeLa y HEp-2. Este estudio biológico preliminar también ha permitido el establecimiento de ciertas relaciones estructura-actividad y su comparación con los estudios realizados de los ácidos escopadúlcicos. Los resultados obtenidos y su discusión se describen en los trabajos que siguen a continuación.

**AUTORES:** Manuel Arnó, Miguel A. González, M. Luisa Marín and Ramón J. Zaragozá

**TÍTULO:** First Diastereoselective Synthesis of (-)-Methyl Thyrsiflorin A, (-)-Methyl Thyrsiflorin B acetate, and (-)-Thyrsiflorin C

**REF. REVISTA:** The Journal of Organic Chemistry, **2000**, *65*, 840-846.

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# First Diastereoselective Synthesis of (−)-Methyl Thyrsiflorin A, (−)-Methyl Thyrsiflorin B Acetate, and (−)-Thyrsiflorin C

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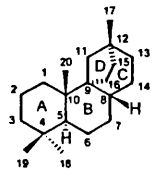
*Received October 7, 1999*

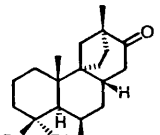
An efficient procedure for the synthesis of scopadulan diterpenes, using (+)-podocarp-8(14)-en-13-one **13** as starting material, is reported. This procedure has been used for the diastereoselective synthesis of (−)-methyl thyrsiflorin A (**8**), (−)-methyl thyrsiflorin B acetate (**9**), and (−)-thyrsiflorin C (**7**). Key steps in our strategy are the intramolecular cyclopropanation of diazoketone **19** and the regioselective cleavage of the cyclopropane ring.

## Introduction

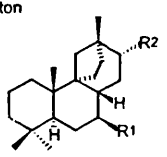
The plant *Scoparia dulcis* has long been used in Paraguay, India, and Taiwan as an alternative medicine for the treatment of stomach disease, hepatosis, and hypertension. Investigating biologically active substances from Paraguayan medicinal plants (*S. dulcis* L., Scrophulariaceae), Hayashi and co-workers isolated a number of structurally unique tetracyclic diterpenes.[1] These diterpenes, which had a novel skeleton (**1**), were named scopadulcic acid A (**2**), scopadulcic acid B (**3**), and scopadulciol (**4**) and revealed interesting antiviral and antitumor properties.[2] During the past several years, new scopadulan diterpenes such as thyrsiflorin A (**5**), thyrsiflorin B (**6**), and thyrsiflorin C (**7**) have been isolated from *Calceolaria thyrsiflora*[3] and *Calceolaria dentata*,[4] and the acids were identified as the corresponding methyl esters.



**1** Scopadulan skeleton

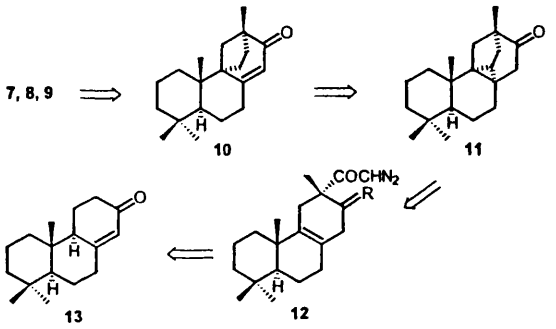


**2** R1=$CH_2OH$, R2=$CO_2H$
**3** R1=$CO_2H$, R2=$CH_3$
**4** R1=$CH_2OH$, R2=$CH_3$



**5** R1=H, R2=$OCOCH_2CO_2H$
**6** R1=OH, R2=$OCOCH_2CO_2H$
**7** R1=OH, R2=OH
**8** R1=H, R2=$OCOCH_2CO_2Me$
**9** R1=OAc, R2=$OCOCH_2CO_2Me$

## Scheme 1



Despite the pharmacological properties shown by these diterpenes and their interesting skeleton structure, only a few cases of synthesis have been reported; most of them were racemic,[5] and only the enantiodivergent total synthesis of (+)- and (−)-scopadulcic acid A has recently been described.[6] This paper reports a diastereoselective approach for the synthesis of these tetracyclic diterpenes, using as starting material (+)-podocarp-8(14)-en-13-one **13**, easily available in optically active form from natural sources.[7]

The utility of this procedure has been proved by preparing (−)-methyl thyrsiflorin A (**8**, MTA),[8] (−)-methyl thyrsiflorin B acetate (**9**, MTBA), and (−)-thyrsiflorin C (**7**, TC).

## Results and Discussion

The retrosynthetic analysis of MTA (**8**), MTBA (**9**), and TC (**7**) is described in Scheme 1. The versatility of our

(1) Hayashi, T.; Kishi, M.; Kawasaki, M.; Arisawa, M.; Shimizu, M.; Suzuki, S.; Yoshizaki, M.; Morita, N.; Tezuka, Y.; Kikuchi, T.; Berganza, L. H.; Ferro, E.; Basualdo, I. *Tetrahedron Lett.* **1987**, *28*, 3693. Hayashi, T.; Kishi, M.; Kawasaki, M.; Arisawa, M.; Morita, N. *J. Nat. Prod.* **1988**, *51*, 360. Hayashi, T.; Asano, S.; Mizutani, M.; Takeguchi, N.; Kojima, T.; Okamura, K.; Morita, N. *J. Nat. Prod.* **1991**, *54*, 802.

(2) Hayashi, K.; Niwayama, S.; Hayashi, T.; Nago, R.; Ochiai, H.; Morita, N. *Antivir. Res.* **1988**, *9*, 345.

(3) Chamy, M. C.; Piovano, M.; Garbarino, J. A.; Miranda, C.; Gambaro, V.; Rodriguez, M. L.; Ruiz-Perez, C.; Brito, I. *Phytochemistry* **1991**, *30*, 589.

(4) Chamy, M. C.; Piovano, M.; Garbarino, J. A.; Vargas, C. *Phytochemistry* **1995**, *40*, 1751.

(5) Overman, L. E.; Ricca, D. J.; Tran, V. D. *J. Am. Chem. Soc.* **1993**, *115*, 2042. Kucera, D. J.; O'Connor, S. J.; Overman, L. E. *J. Org. Chem.* **1993**, *58*, 5304. Ziegler, F. E.; Wallace, O. B. *J. Org. Chem.* **1995**, *60*, 3626. Overman, L. E.; Ricca, D. J.; Tran, V. D. *J. Am. Chem. Soc.* **1997**, *119*, 12031.

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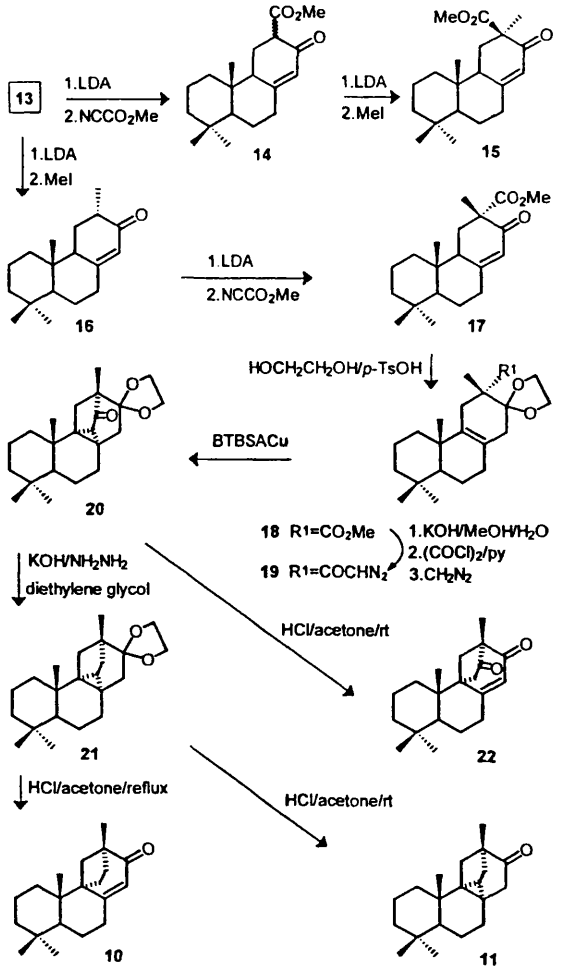
**Scheme 2**




approach is based on the preparation of a tetracyclic intermediate **10**, which already possesses the scopadulan skeleton and a suitable functionality, able to be converted into the three target scopadulan diterpenes. The intermediate **10** would be the result of regioselective cleavage of a cyclopropane ring existing between C-8, C-9 and C-16 in compound **11**. This cyclopropane moiety could arise from an intramolecular cyclopropanation of the convenient diazoketone **12**. Finally, this diazoketone could be obtained by alkylation and acylation of podocarpenone **13**.

The synthetic route begins with the alkylation of podocarpenone **13** using LDA/THF at −25 °C followed by the addition of MeI in order to yield stereoisomer **16** in 94% yield (Scheme 2). Assignment of the stereochemistry of the C-12 Me group was supported by its spectroscopic data, in particular from the *J* values of the signal due to H-12 (*δ* 2.45). This signal collapsed to a double doublet with *J* 5.8 and 5.5 Hz when C-12 Me was irradiated; these coupling constants are consistent with an equatorial (*β*) orientation of H-12 that establishes the α-disposition of C-12 Me group.

By treatment of a solution of **16** in THF at −15 °C with LDA followed by addition of $NCCO_2Me$, using Mander's methoxycarbonylation procedure,[9] introduction of the methoxycarbonyl group occurred stereoselectively from the less hindered α side of the molecule to give **17** in 80% yield. It is interesting to note that reversing the order of the last two steps yielded, via the compound **14**, the keto ester epimer **15** as the sole identifiable product. The ester **14** was obtained as a 7:3 mixture of 12*β*-ester and its 12α-epimer, respectively. The assigned stereochemistries at C-12 in both keto esters **15** and **17** were supported by their spectroscopic data. Of special significance were the NOE enhancements to H-11α and H-11*β* when the C-17 methyl of isomer **17** was irradiated and the NOE effect observed between the C-17 methyl of isomer 15 (irradiated) and protons H-11α and H-9.

After the introduction of the substituents at C-12, our attention was focused on the ring closure between the α-side chain at C-12 and C-9 in order to complete the tetracyclic structure. It was envisaged that the cyclization could be achieved by intramolecular cyclopropanation of the corresponding diazoketone and subsequent regioselective cleavage of the cyclopropane ring. However, before applying this methodology three transformations had to be made. First of all, the methyl ester had to be converted into the corresponding α-diazoketone. In addition, the double bond between C-8 and C-14 had to migrate to positions C-8 and C-9. Furthermore, after completion of the cyclization two carbonyl groups would exist simultaneously in the molecule; therefore, protection of the carbonyl group at C-13 at this stage was considered to be appropriate.

To this end, migration of the double bond and protection of the carbonyl group could be accomplished simultaneously if using the convenient protective group and the suitable acidic catalyst. Therefore, both transformations were achieved using ethylene glycol and *p*-toluenesulfonic acid (*p*-TsOH) as a catalyst,[10] to give **18** in 83% yield and recovering a 9% of starting material. Ester **18** was then converted into diazoketone **19** using a standard procedure[11] in 92% crude yield.

Initial attempts to effect the cyclization of α-diazoketone **19**, $Rh_2(OAc)_4$,[12] $BF_3{\cdot}OEt_2$,[13] trifluoroacetic acid/$HClO_4$,[14] and $HClO_4$,[15] all gave disappointing results. Eventually, the desired intramolecular insertion was successfully carried out by a slow addition of diazoketone **19** over a refluxing solution of bis(*N-tert*-butylsalicylaldiminato)copper(II) (BTBSACu) in toluene[16] to give ketone **20** in 82% yield.

With ketone **20** in hand, we turned our attention to effect the regioselective opening of cyclopropane ring. Ring cleavage of cyclopropyl moieties caused by the enolic form of ketones and assisted by another carbonyl group in the α′-position to the cyclopropane ring has already

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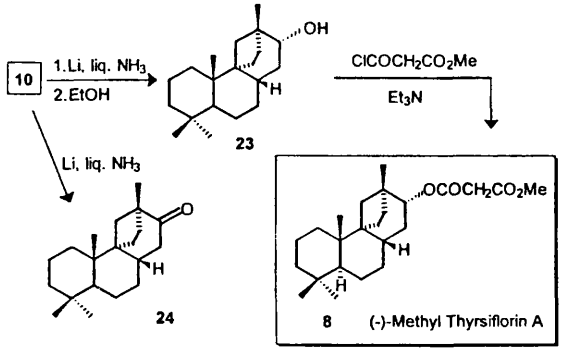
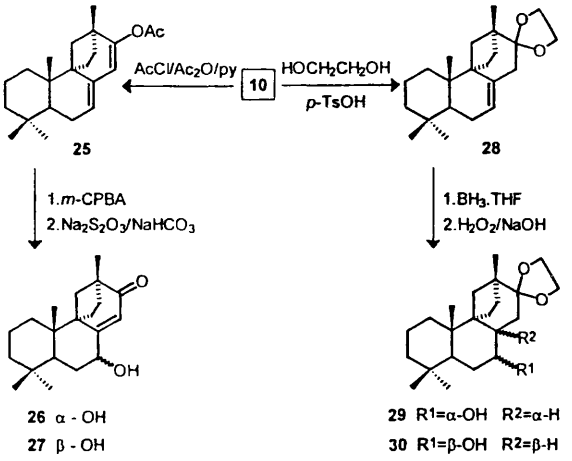
**Scheme 3**




**Scheme 4**




been reported in the literature.[17] After testing different experimental conditions, the enone **22** was obtained in 90% yield by treatment of ketone **20** with acetone/HCl 5:1 at room temperature for 2.5 h.

However, this method results in the existence of two carbonyl groups simultaneously in the molecule. To arrive at target compound **10**, the carbonyl group at C-15 needs to be reduced, which involves two additional steps, the first being protection of the carbonyl group at C-13 selectively and the second being deprotection after the reduction. To reduce the number of steps in the sequence, we explored the possibility of reducing the saturated ketone **20** first and then producing the regioselective cleavage of the cyclopropane ring.

To this end, ketone **20** was subjected to Wolff–Kishner conditions giving **21** in 92% yield. Then treatment of **21** under the ring cleavage conditions used above succeeded only in deprotecting ketal moiety without opening the ring, to give the saturated ketone **11**. This fact confirmed that the absence of carbonyl moiety α′ to the cyclopropane ring makes cleavage more difficult. Fortunately, treatment of **21** under more forcing conditions (refluxing 9:1 acetone/HCl for 3.5 h) provided the desired enone **10** in an optimized 81% yield accompanied by ketone **11** (12%). All efforts to complete the conversion of the intermediate ketone **11** into enone **10**, including longer reaction time, led to poorer yields. It should be noted that to our knowledge this ring opening, without the assistance of a second carbonyl group α′ to the cyclopropane ring, has not been described previously. A mechanism to explain this cleavage is proposed in Scheme 5 (see below).

At this point, we had already prepared the key intermediate **10**, able to be converted into the three target scopadulan diterpenes. Then, to fulfill the synthesis of MTA, MTBA and TC, modification of B and C-ring functionalities was necessary. The transformation of enone **10** into MTA is detailed in Scheme 3. This transformation required stereoselective reduction of the double bond to give the B/C ring trans-juncture characteristic of the scopadulan skeleton and also stereoselective reduction of the carbonyl group followed by esterification of the resulting α-hydroxy group. Birch reduction was chosen to effect both stereoselective reductions. Thus, treatment of **10** under Birch's conditions using a proton donor ($Li/NH_3$–THF/EtOH) caused reduction on both carbonyl and double bond affording alcohol **23** in 86% yield. The assignment of the α-configuration of the hydroxy group at C-13 was based on the $J$ values of the H-13 signal at $\delta$ 3.36 ($J$ = 10.4 and 5.5 Hz) corresponding to an axial-axial and an axial–equatorial couplings with H-14α and H-14β, respectively. When enone **10** was subjected to Birch reduction without using a protic donor ($Li/NH_3$–THF), the carbonyl moiety at C-13 remained unchanged affording **24** in 89% yield. Since this functionalization is present in other scopadulan diterpenes the sequence developed here is also applicable to the preparation of such natural compounds. To reach completion of MTA (**8**), esterification of the secondary alcohol of **23** was accomplished with $ClCOCH_2CO_2CH_3$ /$Et_3N$ in 76% yield.

After having successfully accomplished the synthesis of the simplest member of the three target scopadulan diterpenes, our efforts were next focused on the oxidation of B ring of enone **10** to reach the other goals of the synthesis of MTBA and TC. Preliminary attempts to introduce an additional hydroxyl group at C-7 met with troubles, since direct allylic oxidation of **10** with PDC/*t*-BuOOH on Celite[18] led to complex mixtures of products. An alternative sequence to obtain the 7β-hydroxy enone **27** was conversion of enone **10** into the corresponding 7,13-dienyl acetate **25**, stereoselective epoxidation of the double bond between C-7 and C-8 and subsequent cleavage of the oxirane ring. Thus, treatment of enone **10** under standard conditions (AcCl/py/$Ac_2O$) afforded its dienyl acetate (**25**) (80% yield); however, oxidation of **25** with *m*-CPBA[19] yielded the 7α-hydroxy enone **26** (67% yield) (Scheme 4). Since the opposite stereochemistry was obtained in this epoxidation a different route was sought.

It was envisaged that the hydroxyl group at C-7 could be introduced by hydroboration-oxidation of a double bond existing between C-7 and C-8. This double bond would be the result of the acidic isomerization during transformation of enone **10** into the ketal **28**. To this end, **10** was subjected to similar conditions to those used in the synthesis of ketal **18** (*p*-TsOH, 2.1 × $10^{-3}$ M in benzene); however, only unreacted enone **10** was recovered after long reaction time. This unexpected result can

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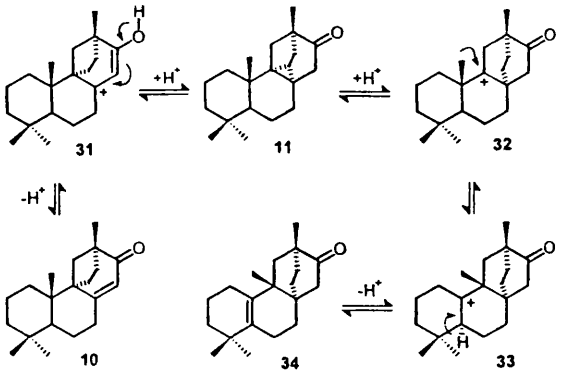
(19) Abad, A.; Agulló, C.; Arno, M.; Domingo, L. R.; Zaragoza, R. J. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1875.

**Scheme 5**

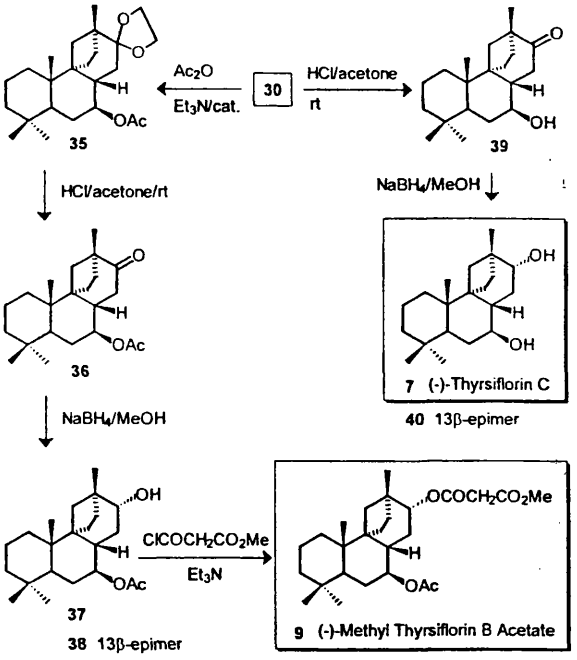



be explained by the low concentration of catalyst, which only permits ketalization of the carbonyl group without isomerization of the double bond. That molecule would be extremely sensitive to hydrolysis and would restore the starting enone during the workup stage.[20] Eventually, this ketalization was best accomplished using *p*-TsOH ($5.5 \times 10^{-3}$ M in benzene) to afford the unconjugated ketal **28** in 72% yield and 20% of starting enone **10**.

Further attempts were made in order to optimize the efficiency of this transformation. However, when higher concentrations of *p*-TsOH ($4.0 \times 10^{-2}$ M in benzene) were used, instead of obtaining the predictable ketal **28**, the reaction followed a different reaction pathway leading to the formation of a new product which was identified, after deketalization, as the rearranged ketone **34** (Scheme 5). To our knowledge, this molecule displays a novel carbon framework, which is a new tetracyclic diterpene skeleton. We confirmed that this new ketone **34** could be obtained directly from enone **10**, using the same conditions, without the presence of ethylene glycol. A reasonable mechanism to explain this rearrangement is proposed in Scheme 5, and it is very related with the cyclopropane ring opening of **11** that produces enone **10**. In fact, this last transformation may be explained through regioselective protonation of C8−C16 bond to give the tertiary carbocation **31**, which assisted by the enolic form of the carbonyl group at C-13 would evolve to the enone **10**. On the other hand, the rearrangement of enone **10** to give ketone **34** implies, in the first stage, the equilibrium between **10** and **11**. Then, regioselective protonation of C9−C16 bond would afford the carbocation **32**, which after 1,2-methyl shift[21] would evolve to the tertiary carbocation **33**. Finally, proton elimination would give the, probably more stable, rearranged ketone **34**. When cyclopropane **11** was treated under the same conditions as enone **10**, ketone **34** was obtained, supporting the contribution of **11** in this proposed reaction pathway. A complete study of this mechanism is currently in progress.

Following our research toward the functionalization at C-7, we investigated the hydroboration−oxidation of the double bond in ketal **28**. Previous hydroboration−oxidation studies on similar compounds[22] had showed preferential hydroboration from the less hindered α-side. However, in this case the presence of the cyclopentane

**Scheme 6**




D-ring increases the hindrance of the α-side. Therefore, hydroboration of the double bond could predominantly occur from the β-side permitting the synthesis of alcohol **30** with the required stereochemistry at C-7 and C-8. Then, treatment of olefin **28** with $BH_3 \cdot SMe_2$, followed by oxidation ($H_2O_2$, NaOH),[23] afforded a complex mixture of compounds, from which the desired β-alcohol **30** was only isolated in 25% yield accompanied by a 20% of a new product which resulted to be, after deketalization, the ketone **24**. This product is the result of an unusual double bond reduction. Fortunately, further experiments with $BH_3 \cdot THF$[24] modifying the amount of hydroborating agent,[25] provided the desired β-alcohol **30** in an optimized 71% yield along with a 22% of the α-alcohol **29**. The splitting pattern showed for H-7 of alcohol **30** (δ 3.40, ddd, $J$ = 10.8, 10.8, and 5.5 Hz) is in agreement with an axial orientation, thus, coupling with two axial (H-8β and H-6β) and an equatorial (H-6α) protons.

Once the correct functionalization at C-7 and stereochemistry at C-8 have both successfully been achieved, our attention was next turned to modify the functionalization present in **30**, completing the synthesis of MTBA and TC as outlined in Scheme 6. Treatment of **30** with $Ac_2O/Et_3N$ and 4-pyrrolidinopyridine[26] yielded the acetate **35** in 92% yield, and then subsequent deketalization of **35** gave ketone **36** in almost quantitative yield. Reduction of **36** using $NaBH_4$/MeOH occurred preferentially on β-side, giving alcohol **37** (77%) accompanied by the minor

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(24) Kabalka, G. W.; Hedgecock, H. C., Jr. *J. Org. Chem.* **1975**, *40*, 1776.
(25) It should be pointed out that 4 mol of $BH_3 \cdot THF$ per mol of olefin was required to effect this conversion. When either longer reaction times or higher concentration of hydroborating agent were used lower yields were obtained, due probably to the reduction of the ketal moiety.[29] Likewise the use of a more bulky hydroborating agent, such as 9-BBN,[30] to increase the stereoselectivity of the reaction, was unsuccessful.
(26) Hassner, A.; Krepski, L. R.; Alexanian, V. *Tetrahedron* **1978**, *34*, 2069.

epimer **38** (21%). Finally, esterification of **37** with $ClCOCH_2CO_2CH_3/Et_3N$ afforded the second scopadulan diterpene (−)-methyl thyrsiflorin B acetate (**9**) in 73% yield.

To obtain the (−)-thyrsiflorin C (**7**), ketal **30** was subjected to deketalization providing ketone **39** in 82% yield. Subsequent treatment of **39** with $NaBH_4$/MeOH furnished as major compound the (−)-thyrsiflorin C (**7**) in 78% yield together with 20% of 13β-epimer **40**.

Synthetic scopadulan diterpenes **7**, **8**, **9** and also alcohol **23** and ketone **24** showed spectroscopic and physical data similar to those previously reported in the literature.[27] Also specific optical rotations recorded here for **7**, **8**, and **9** are in good accord with those reported previously, confirming the absolute stereochemistry of these scopadulan diterpenes.

In conclusion, a diastereoselective synthetic route to the scopadulan diterpenes whose key steps are an intramolecular cyclopropanation and a regioselective cyclopropane ring opening, has been designed and demonstrated, using a chiral podocarpenone **13** as a starting material. This route permits the synthesis of an advanced intermediate such as enone **10**, which already possesses the scopadulan skeleton, in seven steps (31% overall yield) from podocarpenone **13**. The utility of this sequence has been proved by preparing (−)-methyl thyrsiflorin A **8** (65%, two steps), (−)-methyl thyrsiflorin B acetate **9** (27%, six steps), and (−)-thyrsiflorin C **7** (33%, four steps) from the intermediate **10**.

## Experimental Section

**General Experimental Details.** Melting points are uncorrected. Optical rotations were determined using a 5-cm pathlength cell. $[\alpha]_D$ values are given in $10^{-1}$ deg·cm²·g$^{-1}$. IR spectra were measured as KBr pellets or as films on NaCl plates. NMR spectra were recorded on 250, 300 or 400 MHz spectrometers. The signal of the deuterated solvent ($CDCl_3$) was taken as the reference (the singlet at δ 7.24 for $^1H$ and the triplet centered at $\delta_C$ 77.00 for $^{13}C$ NMR data). Complete assignments of $^{13}C$ NMR multiplicities were made on the basis of DEPT experiments. HMQC and NOE experiments were used in some $^1H$ NMR assignments. *J* values are given in Hz. In all compounds, NMR assignments are given with respect to the numbering scheme shown in structure 1. Mass spectra were run by electron impact (EI) at 70 eV. Elemental analyses were performed by Servei de Microanalisi del CSIC (Barcelona). Purifications were performed by flash chromatography on Si gel (230−400 mesh). All nonaqueous reactions were carried out in an argon atmosphere in oven-dried glassware. Commercial reagent grade solvents and chemicals were used as received unless otherwise noted. THF was distilled from sodium benzophenone ketyl under argon atmosphere. Organic extracts were washed with brine, dried over sodium sulfate and concentrated under vacuum.

**12α-Methyl-8(14)-podocarpen-13-one (16).** A solution of LDA in THF (0.5 M, 9.76 mL, 4.88 mmol) was slowly added (ca. 2 h) to a solution of podocarpenone **13** (1.09 g, 4.43 mmol) and *o*-phenanthroline (used as indicator) in THF (35.9 mL) at −25 °C, until persistence of red color. Then, HMPA (0.77 mL, 4.43 mmol) and MeI (0.83 mL, 13.29 mmol) were successively added via syringe, and the resulting yellow solution was allowed to warm to room temperature for 1.25 h. The reaction mixture was quenched with saturated $NH_4Cl$ (5 mL), poured into saturated aqueous $NH_4Cl$ solution, and extracted with ether. Workup afforded an oily residue which was purified by column chromatography, using hexanes−ethyl acetate (from 95:5 to 9:1) as eluent, to give the unreacted enone **13** (35 mg, 3%) and the methyl podocarpenone **16** as a colorless oil (1.088 g, 94%): $[\alpha]^{23}_D$ −25.4 (*c* 2.2, $CHCl_3$); IR (KBr) 1671 cm$^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 5.78 (1H, dd, *J* = 2.1, 1.8, H-14), 2.45 (1H, m, H-12), 1.06 (3H, d, *J* = 7.2, H-17), 0.91, 0.86 and 0.84 (3H each, each s, H-18, H-19 and H-20); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 203.28 (s), 164.54 (s), 123.93 (d), 54.36 (d), 48.38 (d), 41.83 (t), 39.62 (s), 39.57 (d), 39.39 (t), 35.81 (t), 33.65 (q), 33.42 (s), 27.57 (t), 22.52 (t), 21.95 (q), 18.80 (t), 16.70 (q), 15.40 (q); MS (EI) *m/z* 260 ($M^+$, 82), 245 (33), 123 (100); HRMS $C_{18}H_{28}O$ requires 260.2140, found 260.2151.

**12α-Methoxycarbonyl-12β-methyl-8(14)-podocarpen-13-one (17).** In a similar manner as above, a solution of LDA in THF (0.5 M, 9.30 mL, 4.65 mmol) was slowly added (ca. 2.5 h) to a solution of methyl podocarpenone **16** (1.098 g, 4.22 mmol) and a small amount of *o*-phenanthroline in THF (38 mL) at −15 °C. After cooling to −78 °C, HMPA (0.73 mL, 4.22 mmol) and $CNCO_2Me$ (1.0 mL, 12.66 mmol) were successively added via syringe. The reaction mixture was allowed to warm to −30 °C for 2.5 h, then quenched by addition of saturated $NH_4Cl$ (6 mL), poured into aqueous $NH_4Cl$ and extracted with ether. Workup gave a residue, which was purified by chromatography, using hexanes−ethyl acetate (from 9:1 to 8:2) as eluent, to afford the methyl ester **17** as a white solid (1.065 g, 80%): mp 102−103 °C (needles, from hexane); $[\alpha]^{23}_D$ +40.2 (*c* 2.0, $CHCl_3$); IR (KBr) 1729, 1685, 1621 cm$^{-1}$; $^1H$ NMR (400 MHz; $CDCl_3$) δ 5.85 (1H, dd, *J* = 2.1, 1.8, H-14), 3.59 (3H, s, OMe), 2.45 (1H, ddd, *J* = 15.7, 5.3, 1.9, H-7β), 2.35 (1H, dd, *J* = 13.8, 5.3, H-11α), 1.46 (from NOE) (1H, dd, *J* = 13.8, 10.4, H-11β), 1.29 (3H, s, H-17), 0.86 (3H, s, H-18), 0.81 (3H, s, H-19), 0.69 (3H,s, H-20); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 196.08 (s), 173.14 (s), 165.17 (s), 124.84 (d), 53.53 (d), 52.40 (q), 49.56 (d), 41.63 (t), 38.88 (t), 38.71 (s), 35.13 (t), 33.53 (q), 33.34 (s), 32.01 (t), 21.97 (q), 21.86 (t), 21.22 (q), 18.60 (t), 14.66 (q), the signal of a quaternary carbon was hidden by another carbon signal; MS (EI) *m/z* 318 ($M^+$, 73), 303 (40), 243 (23), 137 (100); HRMS $C_{20}H_{30}O_3$ requires 318.2195, found 318.2197. Anal. Calcd for $C_{20}H_{30}O_3$: C, 75.42; H, 9.50. Found: C, 75.54; H, 9.63.

**13,13-Ethylenedioxy-12α-methoxycarbonyl-12β-methyl-8-podocarpene (18).** A mixture of podocarpenone **17** (1.065 g, 3.35 mmol), ethylene glycol (1.48 mL, 26.47 mmol), *p*-TsOH monohydrate (18 mg, 0.095 mmol), and benzene (44 mL) was refluxed in a Dean−Stark system for 31 h. After this time the reaction mixture was diluted with hexane, washed with 10% aqueous $NaHCO_3$ solution and brine, dried and concentrated to give a semisolid. Chromatography of the crude with hexanes−ethyl acetate (from 9:1 to 8:2) gave the ketal **18** as a white solid (1.0 g, 83%) and 95 mg (9%) of starting material. For **18**: mp 96−97 °C (from hexane); $[\alpha]^{24}_D$ +22.2 (*c* 1.4, $CHCl_3$); IR (KBr) 1727 cm$^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 3.98−3.72 (4H, m, ketal), 3.67 (3H, s, OMe), 2.80 (1H, br d, *J* = 17.5, H-14), 1.19 (3H, s, H-17), 0.92 (3H, s, H-20), 0.85 (3H, s, H-18), 0.81 (3H, s, H-19); $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta_C$ 175.07 (s), 135.75 (s), 122.91 (s), 110.13 (s), 64.98 (t), 64.90 (t), 51.88 (q), 51.07 (d), 48.41 (s), 41.55 (t), 39.31 (t), 37.28 (s), 35.94 (t), 33.40 (t), 33.17 (s), 33.05 (q), 31.15 (t), 21.51 (q), 18.93 (q), 18.73 (q), 18.71 (t), 18.49 (t); MS (EI) *m/z* 362 ($M^+$, 97), 347 (100), 315 (28); HRMS $C_{22}H_{34}O_4$ requires 362.2457, found 362.2456.

**Preparation of Diazoketone 19 from Ketal 18.** A mixture of ketal **18** (1.0 g, 2.76 mmol), KOH (85%, 4.0 g, 60.71 mmol), distilled water (8.7 mL), and methanol (51 mL) was refluxed for 18 h. The reaction mixture was then cooled, poured into cold aqueous HCl (1.2 M, 70 mL) and extracted once with $CH_2Cl_2$ and further three times with ether. Workup as usual gave 933 mg of a yellowish solid crude acid which was used in the following step without further purification.

To a solution of the crude acid in dry benzene (23.9 mL), pyridine (337 μL, 4.14 mmol) and $(COCl)_2$ (98%, 1.46 mL, 33.12 mmol) were successively added. When the addition was

(27) For spectroscopic and physical data see refs 3 and 4. Although some typographical errors have been found in the cited references. The corrected values have been given to us by a personal communication from Prof. Garbarino and are as follows. For (−)-methyl thyrsiflorin B acetate: $[\alpha]^{25}_D$ = −4.7 (*c* 0.8) (ref 3) and $[\alpha]^{25}_D$ = −7.63 (*c* 1.0) (ref 4). For (−)-thyrsiflorin C (ref 3): mp 166−168 °C and $[\alpha]^{26}_D$ = −12.3 (*c* 1.2).

complete a white suspension appeared. After the mixture was stirred for 27 h at room temperature, the solvent was removed in vacuo (Teflon vacuum pump). The crude was cooled in an ice bath, and an excess of an ethanol-free $CH_2N_2$/ether solution[28] (0.35 M, 32 mL) was added. The reaction mixture was stirred for 24 h at 4 °C before evaporation of the solvent. The obtained residue was filtered through a pad of neutral alumina, using hexanes−ethyl acetate (9:1) as eluent, to give the α-diazoketone **19** (959 mg, 92%) as a yellowish foam containing a 10% of ketal **18**. This diazoketone was not purified due to its instability and then used directly in the next step. NMR data for crude diazoketone **19**: $^1$H NMR (400 MHz; $C_6D_6$) δ 5.28 (1H, s, $COCHN_2$), 3.48−3.35 (4H, m, ketal), 3.16 (1H, br d, $J$ = 17.3, H-14), 1.16 (3H, s, H-17), 0.91, 0.84 and 0.81 (3H each, each s, H-18, H-19 and H-20); $^{13}$C NMR (75 MHz; $C_6D_6$) $\delta_C$ 196.09 (s), 136.96 (s), 122.54 (s), 110.85 (s), 64.65 (t), 64.66 (t), 53.52 (d), 52.30 (s), 51.31 (d), 41.89 (t), 39.67 (t), 37.73 (s), 36.28 (t), 33.36 (t), 33.32 (q), 33.28 (s), 31.67 (t), 21.77 (q), 19.36 (q), 19.28 (q), 19.14 (t), 18.99 (t).

**Preparation of Cyclopropane 20 from Diazoketone 19.** To a refluxing solution of bis(*N-tert*-butylsalicylaldiminato)-copper(II) (24 mg, 0.06 mmol) in dry toluene (30 mL), a solution of the diazoketone **19** (90%, 237 mg, 0.57 mmol) in dry toluene (10 mL) was slowly added for 6 h (syringe pump). After the addition was complete, the reaction mixture was stirred for a further 30 min before evaporation of the solvent. Chromatography of the crude using hexanes−ethyl acetate (8:2) afforded 23 mg of the unreacted ketal impurity **18** and the ketone **20** as a white solid (162 mg, 82%): mp 185−186 °C (from ether); $[\alpha]^{20}_D$ −17.4 (*c* 1.4, $CHCl_3$); IR (KBr) 1725 cm$^{-1}$; $^1$H NMR (400 MHz; $CDCl_3$) δ 3.98−3.65 (4H, m, ketal), 2.28 (1H, d, $J$ = 14.8, H-14), 2.11 (1H, d, $J$ = 12.3, H-11), 2.04 (1H, d, $J$ = 14.8, H-14′), 1.80 (1H, s, H-16), 1.68 (1H, d, $J$ = 12.3, H-11′), 1.06 (3H, s, H-20), 0.90 (3H, s, H-17), 0.80 (3H, s, H-19), 0.79 (3H, s, H-18); $^{13}$C NMR (100 MHz; $CDCl_3$) $\delta_C$ 210.99 (s), 112.24 (s), 65.24 (t), 64.94 (t), 52.29 (s), 47.98 (s), 47.48 (d), 41.82 (t), 41.59 (t), 39.47 (d), 36.56 (t), 34.06 (s), 33.71 (q), 33.05 (s), 32.20 (s), 31.89 (t), 29.19 (t), 21.37 (q), 18.41 (t), 17.75 (t), 17.53 (q), 10.97 (q); MS (EI) *m/z* 344 ($M^+$, 100), 316 (15); HRMS $C_{22}H_{32}O_3$ requires 344.2351, found 344.2352. Anal. Calcd for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.82; H, 9.45.

**Preparation of Compound 21 from Ketone 20.** A solution of the ketone **20** (190 mg, 0.55 mmol), KOH (85%, 1.2 g, 18.21 mmol), hydrazine monohydrate (98%, 0.6 mL, 12.12 mmol), and di(ethylene glycol) (7.6 mL) was refluxed at 120 °C for 2 h. Then, the resulting yellow solution was heated at 220 °C and the excess of reactants distilled for 2 h. The yellow-brown mixture was cooled to room temperature, poured into saturated aqueous $NH_4Cl$ and extracted with hexane. Workup as usual furnished a solid residue that was purified by column chromatography, using hexane−diethyl ether (from 95:5 to 9:1) as eluent, to afford ketal **21** as a white solid (167 mg, 92%): mp 76−77 °C (from methanol); $[\alpha]^{24}_D$ +23.1 (*c* 1.9, $CHCl_3$); IR (KBr) 3008, 1109 cm$^{-1}$; $^1$H NMR (400 MHz; $CDCl_3$) δ 3.90−3.74 (4H, m, ketal), 1.03 (3H, s, H-17), 0.86 (3H, s, H-20), 0.78 (3H, s, H-19), 0.77 (3H, s, H-18); $^{13}$C NMR (100 MHz; $CDCl_3$) $\delta_C$ 111.34 (s), 64.72 (t), 64.62 (t), 48.18 (d), 43.86 (s), 42.56 (t), 42.00 (t), 41.71 (s), 36.86 (t), 36.14 (t), 35.32 (t), 33.91 (q), 33.64 (s), 33.08 (s), 30.54 (t), 26.53 (d), 21.51 (q), 19.77 (q), 19.70 (s), 18.80 (t), 18.27 (t), 17.72 (q); MS (EI) *m/z* 330 ($M^+$, 100), 316 (7); HRMS $C_{22}H_{34}O_2$ requires 330.2559, found 330.2551. Anal. Calcd for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.96; H, 10.36.

**8(14)-Scopadulen-13-one (10).** A solution of ketal **21** (107 mg, 0.32 mmol) in acetone/HCl 12M (9:1, 6.2 mL) was refluxed for 3.5 h. The mixture was cooled to room temperature, diluted with ether and washed with 10% aqueous $NaHCO_3$ solution. Workup gave a residue, which after chromatography, using hexanes−ethyl acetate (9:1), afforded the cyclopropane **11** (13 mg, 12%) followed by the enone **10** (75 mg, 81%): mp 97−98 °C (from hexane); $[\alpha]^{23}_D$ +164.1 (*c* 1.5, $CHCl_3$); IR (KBr) 3007, 1664, 1601 cm$^{-1}$; $^1$H NMR (300 MHz; $CDCl_3$) δ 5.72 (1H, dd, $J$ = 2.2, 0.5, H-14), 2.56 (1H, dddd, $J$ = 17.7, 5.8, 2.4, 0.5, H-7β), 2.44 (1H, dddd, $J$ = 17.7, 12.1, 6.8, 2.2, H-7α), 1.16 (3H, s, H-17), 0.93, 0.90 and 0.88 (3H each, each s, H-18, H-19 and H-20); $^{13}$C NMR (75 MHz; $CDCl_3$) $\delta_C$ 204.50 (s), 171.12 (s), 124.37 (d), 58.94 (s), 50.39 (s), 47.22 (d), 44.12 (t), 41.91 (t), 37.46 (s), 35.29 (t), 33.76 (q), 33.43 (s), 32.79 (t), 30.80 (t), 29.27 (t), 22.66 (q), 20.97 (t), 20.86 (q), 20.22 (q), 18.40 (t); MS (EI) *m/z* 286 ($M^+$, 68), 271 (24); HRMS $C_{20}H_{30}O$ requires 286.2297, found 286.2297. Anal. Calcd for $C_{20}H_{30}O$: C, 83.85; H, 10.56. Found: C, 83.92; H, 10.61.

**13α-Scopadulanol (23).** To an intense blue solution of Li (20 mg, 2.90 mmol) in $NH_3$ (8 mL) at −35 °C a solution of **10** (37 mg, 0.13 mmol) in THF (1.2 mL) was added. After being stirred for 1 h, a mixture of EtOH/THF (1:4, 0.5 mL) was slowly added for 30 min and the reaction mixture was allowed to stir for further 30 min. The lithium excess was then destroyed by addition of solid $NH_4Cl$ and the $NH_3$ excess was evaporated under argon atmosphere. The crude mixture was poured into water and extracted with ether. Usual workup afforded a solid residue which was purified by chromatography, using hexanes−ethyl acetate (9:1) to give 3.0 mg (8%) of ketone **27** and 32.3 mg (86%) of alcohol **23**: mp 133−134 °C (from hexanes−ethyl acetate) (lit.[3] mp 140−141 °C, from MeOH−$H_2O$); $[\alpha]^{23}_D$ −30.3 (*c* 1.5, $CHCl_3$) (lit.[3] $[\alpha]^{25}_D$ −38.3, *c* 1.0); IR (KBr) 3250 cm$^{-1}$; $^1$H NMR (400 MHz; $CDCl_3$) δ 3.36 (1H, dd, $J$ = 10.4, 5.5, H-13), 0.97 (3H, s, H-17), 0.94 (3H, s, H-20), 0.79 (3H, s, H-18), 0.78 (3H,s, H-19); $^{13}$C NMR (100 MHz; $CDCl_3$) $\delta_C$ 76.32 (d), 52.41 (s), 47.91 (d), 44.44 (t), 44.05 (s), 42.14 (t), 38.46 (s), 38.11 (t), 37.33 (d), 33.56 (q), 33.06 (s), 32.51 (t), 30.63 (t), 30.08 (t), 24.37 (t), 23.24 (q), 21.99 (q), 21.77 (t), 18.73 (t), 17.28 (q); MS (EI) *m/z* 290 ($M^+$, 100), 275 (10), 272 (9), 261 (12); HRMS $C_{20}H_{34}O$ requires 290.2610, found 290.2615.

**(−)-Methyl Thyrsiflorin A (8).** To a solution of alcohol **23** (29.3 mg, 0.102 mmol) in diethyl ether (1.2 mL) cooled in an ice bath, dry $Et_3N$ (143 μL, 1.02 mmol) and $ClOCCH_2CO_2CH_3$ (110 μL, 1.02 mmol) were successively added. After being stirred for 40 min at room temperature, the reaction mixture was diluted with hexane. Workup as usual gave an oily residue, which was purified by flash chromatography with hexanes−ethyl acetate (95:5) to afford (−)-methyl thyrsiflorin A (**8**) as a colorless oil (29.9 mg, 76%): $[\alpha]^{20}_D$ −47.6 (*c* 2.1, $CHCl_3$) (lit.[3] −50.0, *c* 1.5); IR (KBr) 1755, 1734 cm$^{-1}$; $^1$H NMR (400 MHz; $CDCl_3$) δ 4.64 (1H, ddd, $J$ = 11.0, 6.1, 1.2, H-13), 3.71 (3H, s, $COOCH_3$), 3.33 (2H, s, $COCH_2CO$), 0.94 (3H, s, H-20), 0.89 (3H, s, H-17), 0.86 (1H, dd, $J$ = 12.1, 2.8, H-5), 0.79 (3H, s, H-18), 0.77 (3H, s, H-19); $^{13}$C NMR (300 MHz; $CDCl_3$) $\delta_C$ 167.17 (s), 166.21 (s), 80.00 (d), 52.49 (s), 52.34 (q), 47.94 (d), 44.47 (t), 43.05 (s), 42.16 (t), 41.70 (t), 38.52 (s), 37.14 (d), 34.19 (t), 33.60 (q), 33.10 (s), 32.49 (t), 31.94 (t), 29.94 (t), 24.36 (t), 23.11 (q), 22.02 (q), 21.77 (t), 18.72 (t), 17.31 (q); MS (EI) *m/z* 390 ($M^+$, 12), 306 (7), 272 (100); HRMS $C_{24}H_{38}O_4$ requires 390.2770, found 390.2775.

**13,13-Ethylenedioxy-7-scopadulene (28).** A mixture of enone **10** (49 mg, 0.17 mmol), ethylene glycol (238 μL, 4.26 mmol), *p*-TsOH monohydrate (14 mg, 0.074 mmol), and benzene (13.1 mL) was refluxed in a Dean−Stark system for 2.5 h. The reaction mixture was poured into water and extracted with hexane. The combined extracts were washed with 10% aqueous $NaHCO_3$ solution and brine, dried and concentrated to give a solid. Chromatography of the crude using hexanes−ethyl acetate (95:5) gave the ketal **28** as a white solid (41 mg, 72%) followed by 9.9 mg (20%) of starting material. For **28**: mp 124−125 °C (from methanol); $[\alpha]^{24}_D$ −106.4 (*c* 1.9, $CHCl_3$); IR (KBr) 1115 cm$^{-1}$; $^1$H NMR (400 MHz; $CDCl_3$) δ 5.20 (1H, m, H-7), 4.00−3.75 (4H, m, ketal), 2.50 (1H, dddd, $J$ = 16.1, 5.8, 2.9, 2.9, H-14), 2.30 (1H, d, $J$ = 16.1, H-14′), 2.12 (1H, ddd, $J$ = 16.4, 16.4, 5.8), 1.66 (1H, br d, $J$ = 11.5, H-11), 0.97 and 0.93 (3H each, each s, H-17 and H-20), 0.89 (3H, s, H-19), 0.83 (3H,s, H-18); $^{13}$C NMR (100 MHz; $CDCl_3$) $\delta_C$ 140.19 (s), 118.08 (d), 113.05 (s), 65.45 (t), 65.02 (t), 54.80 (s), 46.85 (s), 45.44 (d), 42.56 (t), 41.20 (t), 40.28 (t), 37.16 (s), 34.05 (t), 33.79 (q), 33.21 (t), 32.88 (s), 31.28 (t), 24.08 (t),

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23.16 (q), 19.83 (q), 18.79 (q), 18.72 (t); MS (EI) *m/z* 330 ($M^+$, 100), 315 (6); HRMS $C_{22}H_{34}O_2$ requires 330.2559, found 330.2560.

**13,13-Ethylenedioxy-7β-scopadulanol (30).** A solution of $BH_3$·THF (1 M in THF, 344 μL, 0.344 mmol) was slowly added to the olefin **28** (28.4 mg, 0.086 mmol) cooled in an ice-bath. Once the addition was complete the reaction mixture was allowed to warm to room temperature, stirred for 50 min, and cooled in an ice-bath again. Then, a mixture of NaOH 6M/$H_2O_2$ 35% (1:1.25, 100 μL) was carefully added dropwise. After 35 min at room temperature, the resulting mixture was heated to 60 °C for 1 h, cooled, quenched with saturated aqueous $NH_4Cl$ and diluted with diethyl ether. Usual workup gave an oily residue which was purified by chromatography, using hexanes–ethyl acetate (from 7:3 to 6:4) as eluent, to afford the α-alcohol **29** (6.5 mg, 22%) followed by the β-alcohol **30** (21.2 mg, 71%) as a white solid: mp 161–162 °C (from hexane–diethyl ether); $[\alpha]^{23}_D$ +8.3 (*c* 1.2, $CHCl_3$); IR (KBr) 3398, 1121 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 4.00–3.75 (4H, m, ketal), 3.40 (1H, ddd, *J* = 10.8, 10.8, 5.5, H-7), 2.10 (1H, dd, *J* = 13.9, 5.1, H-14β), 1.00 (3H, s, H-20), 0.91 (3H, s, H-17), 0.82 and 0.81 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta_C$ 112.81 (s), 73.83 (d), 65.30 (t), 64.96 (t), 53.06 (s), 47.16 (s), 46.17 (d), 44.46 (d), 42.58 (t), 41.97 (t), 38.49 (s), 35.22 (t), 35.07 (t), 33.53 (q), 33.05 (s), 32.41 (t), 32.13 (t), 25.24 (t), 22.07 (q), 19.44 (q), 18.67 (t), 17.67 (q); MS (EI) *m/z* 348 ($M^+$, 100), 330 (6), 316 (6); HRMS $C_{22}H_{36}O_3$ requires 348.2664, found 348.2666.

**7β-Acetoxy-13,13-ethylenedioxyscopadulane (35).** To a solution of alcohol **30** (14.7 mg, 0.042 mmol) and 4-pyrrolidinopyridine (98%, 0.6 mg, 0.004 mmol) as a catalyst in dry $Et_3N$ (155 μL) cooled in an ice bath, acetic anhydride (9 μL, 0.084 mmol) was added dropwise. After stirring for 20 min, the reaction mixture was diluted with hexane. Workup as usual followed by column chromatography, using hexanes–ethyl acetate (from 8:2 to 7:3) as eluent, yielded the acetate **35** (15.1 mg, 92%) as an amorphous solid that could not be induced to crystallize: $[\alpha]^{24}_D$ +5.5 (c 2.9, $CHCl_3$); IR (KBr) 1726, 1124 $cm^{-1}$; $^1H$ NMR (250 MHz; $CDCl_3$) δ 4.67 (1H, ddd, *J* = 11.0, 11.0, 5.5, H-7), 4.00–3.72 (4H, m, ketal), 2.02 (3H, s, $COCH_3$), 1.00 (3H, s, H-20), 0.90 (3H, s, H-17), 0.80 and 0.79 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (62.5 MHz; $CDCl_3$) $\delta_C$ 170.89 (s), 112.61 (s), 76.35 (d), 65.37 (t), 65.03 (t), 53.09 (s), 47.24 (s), 45.83 (d), 42.43 (t), 41.90 (t), 41.12 (d), 38.30 (s), 35.07 (t), 34.93 (t), 33.49 (q), 33.13 (s), 32.21 (t), 27.95 (t), 25.10 (t), 21.99 (q), 21.38 (q), 19.48 (q), 18.60 (t), 17.55 (q); MS (EI) *m/z* 390 ($M^+$, 42), 348 (48), 331 (27), 330 (100); HRMS $C_{24}H_{38}O_4$ requires 390.2770, found 390.2762.

**7β-Acetoxy-13-scopadulanone (36).** A solution of ketal **35** (20.5 mg, 0.052 mmol) in acetone/HCl 12 M (9:1, 1.2 mL) was stirred at room temperature for 30 min. The mixture was diluted with diethyl ether and washed with a 10% aqueous $NaHCO_3$ solution. Workup as usual gave a residue, which was purified by chromatography using hexanes–ethyl acetate (8:2) to furnish the ketone **36** (17.8 mg, 98%) as a colorless oil: $[\alpha]^{25}_D$ +32.5 (c 1.5, $CHCl_3$); IR (NaCl) 1733, 1710 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 4.82 (1H, ddd, *J* = 10.7, 10.7, 5.6, H-7), 2.43 (1H, dd, *J* = 15.4, 5.6, H-14β), 2.21 (1H, ddd, *J* = 11.5, 10.5, 5.6, H-8), 2.01 (3H, s, $COCH_3$), 1.04 and 1.02 (3H each, each s, H-17 and H-20), 0.84 and 0.81 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 213.19 (s), 170.82 (s), 76.02 (d), 53.71 (s), 52.39 (s), 46.11 (d), 45.11 (t), 44.19 (d), 41.80 (t), 39.84 (t), 38.69 (s), 36.62 (t), 33.48 (q), 33.17 (s), 32.19 (t), 27.92 (t), 25.47 (t), 21.86 (q), 21.15 (q), 19.60 (q), 18.53 (t), 17.40 (q); MS (EI) *m/z* 346 ($M^+$, 1), 304 (4), 287 (17), 286 (74), 271 (100); HRMS $C_{22}H_{34}O_3$ requires 346.2508, found 346.2507.

**7β-Acetoxy-13α-scopadulanol (37).** To a solution of ketone **36** (17.8 mg, 0.051 mmol) in MeOH (1.9 mL) at 0 °C, $NaBH_4$ (98%, 20 mg, 0.51 mmol) was added. The reaction mixture was stirred for 50 min and then diluted with diethyl ether. Usual workup followed by column chromatography, using hexanes–ethyl acetate (from 8:2 to 6:4) as eluent, yielded the β-alcohol **38** (3.8 mg, 21%) followed by the α-alcohol **37** (13.8 mg, 77%) as a white solid: mp 181–183 °C (from MeOH–$H_2O$); $[\alpha]^{24}_D$ +3.4 (*c* 1.8, $CHCl_3$); IR (KBr) 3306, 1732 $cm^{-1}$; $^1H$ NMR (250 MHz; $CDCl_3$) δ 4.68 (1H, ddd, *J* = 11.0, 11.0, 5.6, H-7), 3.35 (1H, dd, *J* = 10.6, 5.9, H-13), 2.02 (3H, s, $COCH_3$), 0.99 (6H, s, H-17 and H-20), 0.81 and 0.79 (3H, s, H-18 and H-19); $^{13}C$ NMR (62.5 MHz; $CDCl_3$) $\delta_C$ 170.86 (s), 76.28 (d), 76.00 (d), 53.10 (s), 45.86 (d), 44.49 (t), 44.11 (s), 41.99 (d), 41.88 (t), 38.34 (s), 33.99 (t), 33.47 (s), 33.12 (q), 32.27 (t), 30.58 (t), 27.94 (t), 25.83 (t), 23.08 (q), 21.94 (q), 21.30 (q), 18.61 (t), 17.49 (q); MS (EI) *m/z* 348 ($M^+$, 7), 289 (13), 288 (61), 273 (100); HRMS $C_{22}H_{36}O_3$ requires 348.2664, found 348.2651.

**(−)-Methyl Thyrsiflorin B Acetate (9).** In the same manner as described to obtain (−)-methyl thyrsiflorin A (**8**), alcohol **37** (13.6 mg,0.039 mmol) was converted, after chromatography using hexanes–ethyl acetate (from 9:1 to 8:2) as eluent, into (−)-methyl thyrsiflorin B acetate (**9**) (12.8 mg, 73%) as a colorless oil: $[\alpha]^{24}_D$ −12.0 (*c* 0.5, $CHCl_3$) (lit.[27] −7.6, *c* 1.0 and −4.7, *c* 0.8, respectively); IR (NaCl) 1750, 1740, 1735 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 4.75–4.60 (2H, m, H-7 and H-13), 3.72 (3H, s, $CO_2Me$), 3.34 (2H, s, $CH_2CO_2Me$), 2.02 (3H, s, $COCH_3$), 0.99 (3H, s, H-20), 0.91 (3H, s, H-17), 0.81 and 0.79 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 170.95 (s), 167.04 (s), 166.23 (s), 79.31 (d), 75.97 (d), 53.20 (s), 52.37 (q), 45.87 (d), 44.48 (t), 43.13 (s), 41.89 (t), 41.80 (d), 41.65 (t), 38.40 (s), 33.47 (q), 33.14 (s), 32.25 (t), 31.87 (t), 30.30 (t), 27.98 (t), 25.82 (t), 22.90 (q), 21.93 (q), 21.34 (q), 18.58 (t), 17.50 (q); MS (EI) *m/z* 448 ($M^+$, 10), 388 (28), 373 (42), 270 (100); HRMS $C_{26}H_{40}O_6$ requires 448.2825, found 448.2846.

**7β-Hydroxy-13-scopadulanone (39).** In the same manner as described for the synthesis of **36**, ketal **30** (28.4 mg, 0.04 mmol) was converted, after chromatography using hexanes–ethyl acetate (from 7:3 to 5:5) as eluent, into the ketone **39** (20.2 mg, 82%) as a colorless oil: $[\alpha]^{25}_D$ +45.0 (*c* 1.4, $CHCl_3$); IR (NaCl) 3570–3110, 1701 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 3.53 (1H, ddd, *J* = 11.0, 10.5, 5.4, H-7), 2.72 (1H, dd, *J* = 15.6, 5.9, H-14β), 1.87 (1H, ddd, *J* = 12.4, 5.7, 2.5), 1.04 and 1.00 (3H each, each s, H-17 and H-20), 0.85 and 0.83 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 213.77 (s), 73.91 (d), 53.62 (s), 52.46 (s), 47.34 (d), 46.34 (d), 45.19 (t), 41.84 (t), 40.13 (t), 38.81 (s), 36.73 (t), 33.50 (q), 33.08 (s), 32.33 (t), 32.02 (t), 25.57 (t), 21.91 (q), 19.69 (q), 18.59 (t), 17.47 (q); MS (EI) *m/z* 304 ($M^+$, 100), 289 (17), 286 (13), 271 (14); HRMS $C_{20}H_{32}O_2$ requires 304.2402, found 304.2402.

**(−)-Thyrsiflorin C (7).** In the same manner as described for alcohol **37**, ketone **39** (9 mg, 0.0296 mmol) was treated. Chromatography of the crude product, using hexanes–ethyl acetate (from 6:4 to 3:7) as eluent, afforded the dialcohol **40** (1.9 mg, 20%) and (−)-Thyrsiflorin C (**7**) (7.1 mg, 78%) as a white solid: mp 167–169 °C (from MeOH–$H_2O$) (lit.[27] 166–168 °C); $[\alpha]^{24}_D$ −8.8 (*c* 0.8, $CHCl_3$) (lit.[27] −12.3, *c* 1.2); IR (KBr) 3337, 1036 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 3.45–3.35 (2H, m, H-7 and H-13), 2.31 (1H, ddd, *J* = 12.9, 5.9, 5.1), 0.99 and 0.97 (3H each, each s, H-17 and H-20), 0.82 and 0.81 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 76.22 (d), 73.80 (d), 53.04 (s), 46.12 (d), 45.18 (d), 44.59 (t), 44.14 (s), 41.96 (t), 38.53 (s), 34.28 (t), 33.50 (q), 33.06 (s), 32.45 (t), 31.85 (t), 30.67 (t), 25.97 (t), 23.13 (q), 22.01 (q), 18.68 (t), 17.58 (q); MS (EI) *m/z* 306 ($M^+$, 59), 289 (22), 288 (100), 273 (41); HRMS $C_{20}H_{34}O_2$ requires 306.2559, found 306.2549.

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**Supporting Information Available:** Representative data and experimental procedures for the preparation of **15** and **22**. Representative data for **11**, **24–26**, **29**, **34**, **38**, and **40**. $^1H$ NMR spectra for **7–10**, **17**, **20**, and **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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# Supporting Information

## First Diastereoselective Synthesis of (-)-Methyl Thyrsiflorin A, (-)-Methyl Thyrsiflorin B Acetate and (-)-Thyrsiflorin C

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## Experimental section

**12β-Methoxycarbonyl-12α-methyl-8(14)-podocarpen-13-one (15).** It was obtained reversing the order of the two steps used for the synthesis of the keto ester **17**. Thus, to a solution of podocarpenone **13** (50 mg, 0.20 mmol) and o-phenanthroline (used as indicator) in THF (5 mL) at –30 °C, a solution of LDA in THF (0.5 M, 447 μL, 0.22 mmol) was slowly added (ca. 1 h). Then, HMPA (36 μL, 0.20 mmol) and $CNCO_2Me$ (81 μL, 1.0 mmol) were successively added via syringe, and the reaction mixture was stirred at the same temperature for 2.5 h, quenched with saturated $NH_4Cl$ (2 mL), poured into water and extracted with diethyl ether. Workup afforded an orange oil which resulted to be a 7:3 (β:α) mixture of epimeric esters at C-12 **14**. $^1H$ NMR of the mixture (300 MHz; $CDCl_3$); for the β–ester: δ 5.85 (1H, br s, H-14), 3.72 (3H, s, OMe), 3.21 (1H, dd, J = 14.0, 5.7, H-12α), 0.88 , 0.84 and 0.78 (3H each, each s, H-18, H-19 and H-20). For the α–ester: δ 5.88 (1H, br s, H-14), 3.64 (3H, s, OMe), 3.34 (1H, dd, J = 5.5, 4.6, H-12β), 0.87, 0.82 and 0.76 (3H each, each s, H-18, H-19 and H-20). This epimeric mixture was submitted to the following step without further purification. Thus, to a solution of the crude ester and a small amount of o-phenanthroline in THF (5 mL) at –78 °C, a solution of LDA in THF (0.5 M, 447 μL, 0.22 mmol) was slowly added (ca. 25 min). Then, HMPA (36 μL, 0.20 mmol) and MeI (63 μL, 1.0 mmol) were added via syringe. The reaction mixture was slowly allowed to warm to –10 °C for 5 h,

quenched by addition of saturated $NH_4Cl$ (2 mL) and poured into aqueous $NH_4Cl$. Extraction with diethyl ether and workup as usual gave a orange residue, which was purified by chromatography, using hexane-ethyl acetate (from 9:1 to 8:2) as eluent, to afford podocarpenone methyl ester **15** as a solid (46 mg, 72%, two steps): mp 108-110 °C (from hexane-EtOAc); $[\alpha]^{27}_D$ +57.6 (c 2.6, $CHCl_3$); IR (KBr) 2943, 1735, 1671, 1626, 1455, 1388, 1264, 1240, 1105, 1003, 881 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 5.79 (1H, dd, J = 2.2, 2.0, H-14), 3.72 (3H, s, OMe), 2.54 (1H, ddd, J = 15.5, 4.9, 2.0, H-7β), 1.82 (1H, dd, J = 13.8, 5.0, H-11α), 1.30 (3H, s, H-17), 0.91 (3H, s, H-18), 0.87 (3H, s, H-19), 0.83 (3H, s, H-20); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 197.98 (s), 173.86 (s), 164.68 (s), 123.32 (d), 53.81 (d), 52.40 (q), 48.35 (d), 41.62 (t), 39.08 (t), 38.70 (s), 35.02 (t), 33.54 (q), 33.39 (s), 30.06 (t), 22.00 (q), 21.70 (t), 19.55 (q), 18.57 (t), 15.14 (q), the signal of a quaternary carbon was hidden by another carbon signal.

**8(14)-Scopadulen-13,15-dione (22).** A solution of ketal **20** (16 mg, 0.047 mmol) in acetone/HCl 12 M (5:1, 3 mL) was stirred at rt for 2.5 h. Workup as usual followed by chromatography, hexane-ethyl acetate (9:1), gave **22** (12.6 mg, 90%) as a white solid: $^1H$ NMR (400 MHz; $CDCl_3$) δ 5.76 (1H, d, J = 2.4, H-14), 2.63 (1H, ddd, J = 17.4, 5.2, 1.5, H-7β), 2.61 (1H, d, J = 17.5, H-16), 2.39 (1H, dddd, J = 17.4, 12.7, 6.5, 2.4, H-7α), 2.30 (1H, dd, J = 11.9, 3.6, H-11β), 2.22 (1H, dd, J = 17.5, 3.6, H-16'), 1.95 (1H, d, J = 11.9, H-11α), 1.20 (3H, s, H-17), 1.00 (3H, s, H-20), 0.92 (3H, s, H-18), 0.91 (3H, s, H-19); $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta_C$ 211.83 (s), 192.26 (s), 171.42 (s), 125.70 (d), 63.69 (s), 52.57 (s), 47.95 (d), 42.83 (t), 41.64 (t), 41.33 (t), 37.34 (s), 33.71 (q), 33.47 (s), 33.03 (t), 31.03 (t), 22.69 (q), 20.62 (t), 19.10 (q), 18.23 (t), 14.46 (q).

## Spectroscopic and physical data

**Ketone 11.** Representative data: a white solid: mp 91-92 °C (from hexane-EtOAc); $[\alpha]^{26}_D$ –2.3 (c 1.8, $CHCl_3$); IR (KBr) 3020, 2966, 2919, 2860, 1715, 1435, 1374, 1228, 1069, 1037, 921 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 2.42 (2H, s, H-14), 1.04 and 0.99 (3H each, each s, H-17 and H-20), 0.80 (6H, s, H-18 and H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 213.36 (s), 49.79 (s), 48.11 (d), 42.05 (s), 41.86 (t), 41.67 (t), 37.05 (t), 36.92 (t), 36.40 (t), 33.87 (q), 33.56 (s), 33.08 (s), 29.53 (t), 26.49 (d), 21.46 (q), 19.96 (s), 19.86 (q), 18.71 (t), 18.04 (t), 17.32 (q).

**13-Scopadulanone (24).** Representative data: an oil; IR (KBr) 2930, 1710, 1461, 1379, 1261 $cm^{-1}$; $^1H$ NMR (400 MHz; $CDCl_3$) δ 2.21 (1H, dd, J = 14.7, 5.4, H-14), 1.03 (3H, s, H-17), 0.98 (3H, s, H-20), 0.83 (3H, s, H-18), 0.80 (3H, s, H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 214.64 (s), 53.10 (s), 52.44 (s), 47.98 (d), 45.38 (t), 43.68 (t), 42.12 (t), 40.01 (d), 38.83 (s), 36.88 (t), 33.61 (q), 33.13 (s), 32.55 (t), 30.29 (t), 24.09 (t), 21.96 (q), 21.70 (t), 19.81 (q), 18.70 (t), 17.24 (q).

**13-Acetoxy-7,13-scopaduladiene (25).** Representative data: a solid; $^1H$ NMR (300 MHz; $CDCl_3$) δ 5.59 (1H, s, H-14), 5.24 (1H, dd, J = 5.1, 2.7, H-7), 2.13 (3H, s, COMe), 1.07 , 0.92, 0.91 and 0.84 (3H each, each s, H-17, H-18, H-19 and H-20); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 169.43 (s), 155.26 (s), 142.45 (s), 118.75 (d), 116.68 (d), 54.56 (s), 44.57 (t), 44.52 (s), 44.47 (d), 42.57 (t), 42.42 (t), 37.05 (s), 33.84 (t), 33.41 (q), 32.83 (s), 32.25 (t), 24.41 (t), 22.31 (q), 20.71 (q), 19.84 (q), 18.67 (t), 17.87 (q).

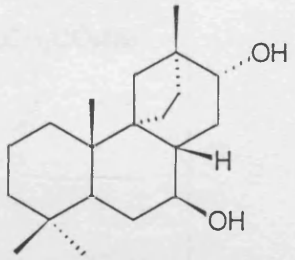
**7α-Hydroxy-8(14)-scopadulen-13-one (26).** Representative data: a solid; $^1H$ NMR (250 MHz; $CDCl_3$) δ 5.96 (1H, s, H-14), 4.50 (1H, dd, J = 3.7, 3.7, H-7), 1.17 (3H, s, H-17), 0.93 and 0.90 (3H and 6H, each s, H-18, H-19 and H-20).

**13,13-Ethylenedioxy-8α-scopadulan-7α-ol (29).** Representative data: a white solid: mp 140-141 °C (from hexane-ether); $[\alpha]^{27}_D$ –7.6 (c 1.3, $CHCl_3$); IR (KBr) 3402, 2951, 1458, 1367, 1123, 1087, 1041 $cm^{-1}$; $^1H$ NMR (300 MHz; $CDCl_3$) δ 4.32 (1H, dd, J = 6.6, 6.6, H-7), 4.00-3.73 (4H, m, ketal), 0.94, 0.88, 0.87, and 0.85 (3H each, each s, H-17, H-18, H-19 and H-20); $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta_C$ 113.19 (s), 68.96 (d), 65.07 (t), 64.82 (t), 50.16 (s), 48.32 (s), 45.09 (d), 44.81 (d), 43.04 (t), 40.87 (t), 38.77 (s), 35.98 (t), 33.87 (q), 33.13 (s), 33.05 (t), 31.90 (t), 31.85 (t), 29.29 (t), 22.53 (q), 20.18 (t), 20.09 (q), 17.94 (q).

**Rearranged ketone 34.** Representative data: a viscous oil; $[\alpha]^{23}_D$ –92.7 (c 1.7, $CHCl_3$); IR (NaCl) 2924, 1721, 1455, 1411, $cm^{-1}$; $^1H$ NMR (400 MHz; $CDCl_3$) δ 2.21 (1H, dd, J = 18.3, 3.0, H-14β), 2.0 (1H, d, J = 18.3, H-14α), 1.75 (1H, d, J = 12.9, H-11α), 0.97 and 0.96 (3H each, each s, H-18 and H-19), 0.92 (3H, s, H-17), 0.86 (3H, s, Me-C9); $^{13}C$ NMR (100 MHz) $\delta_C$ 218.65 (s), 133.00 (s), 132.78 (s), 47.29 (t), 46.69 (t), 44.81 (s), 40.11 (t), 39.22 (s), 36.83 (s), 34.09 (s), 30.94 (t), 28.99 (t), 28.93 (q), 27.96 (q), 26.87 (t), 25.95 (t), 24.16 (q), 21.20 (t), 20.25 (q), 19.95 (t); HRMS $C_{20}H_{30}O$ requires 286.2297, found 286.2300.

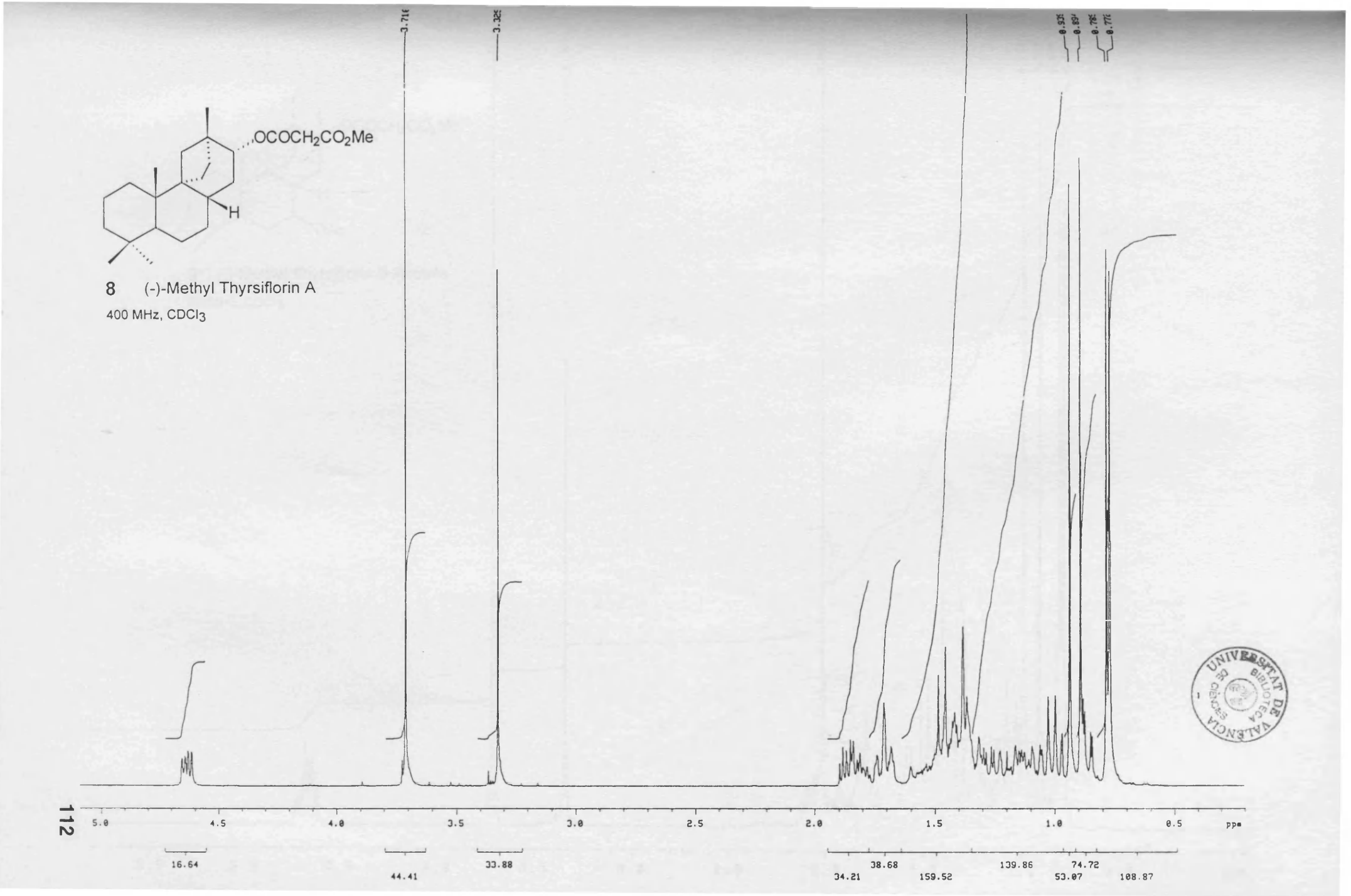
**7β-Acetoxy-13β-scopadulanol (38).** Representative data: a colorless oil; $[\alpha]^{25}_D$ +5.3 (c 0.8, $CHCl_3$); $^1H$ NMR (300 MHz; $CDCl_3$) δ 4.67 (1H, ddd, J = 11.0, 11.0, 5.6, H-7), 3.43 (1H, br s, H-13), 2.02 (3H, s, $COCH_3$), 1.03 and 0.98 (3H each, each s, H-17 and H-20), 0.81 and 0.80 (3H each, each s, H-18 and H-19); $^{13}C$ NMR (75 MHz; $CDCl_3$) $\delta_C$ 171.07 (s), 76.58 (d), 74.49 (d), 54.00 (s), 45.92 (d), 43.73 (s), 41.96 (t), 38.55 (s), 38.30 (d), 37.68 (t), 36.27 (t), 33.51 (q), 33.17 (s), 33.10 (t), 32.11 (t), 28.05 (t), 24.24 (t), 23.85 (q), 22.04 (q), 21.35 (q), 18.63 (t), 17.71 (q).

**7β,13β-Scopadulanediol (40).** Representative data: $^1H$ NMR (300 MHz; $CDCl_3$) 3.48 (1H, br s, H-13), 3.36 (1H, ddd, J = 11.0, 10.0, 5.4, H-7), 2.04 (1H, ddd, J = 14.1, 4.8, 1.9), 1.02 and 0.99 (3H each, each s, H-17 , H-20), 0.83 and 0.82 (3H each, each s, H-18 , H-19).



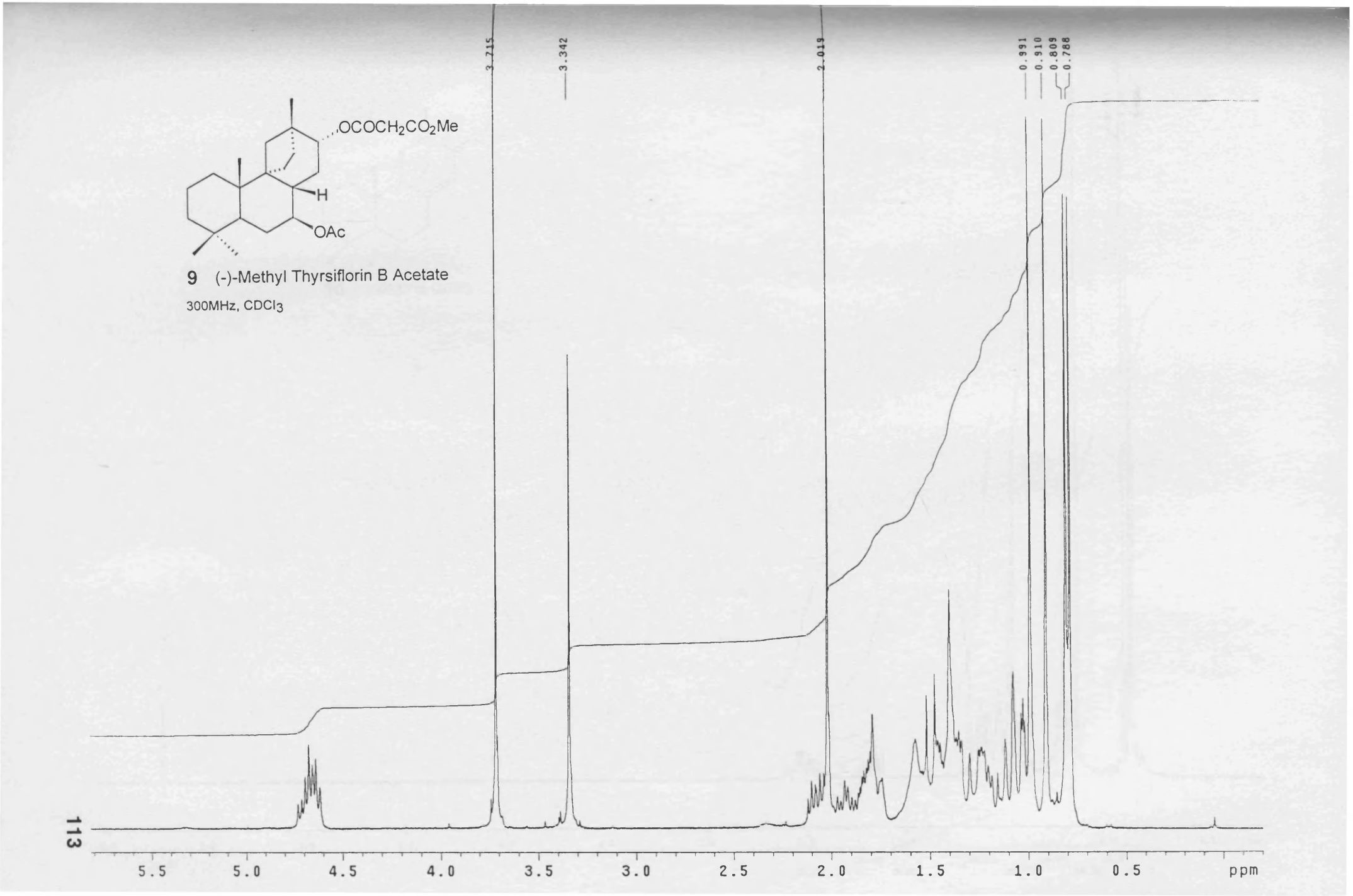

**7** (-)-Thyrsiflorin C

300MHz, $CDCl_3$




8 (-)-Methyl Thyrsiflorin A

400 MHz, $CDCl_3$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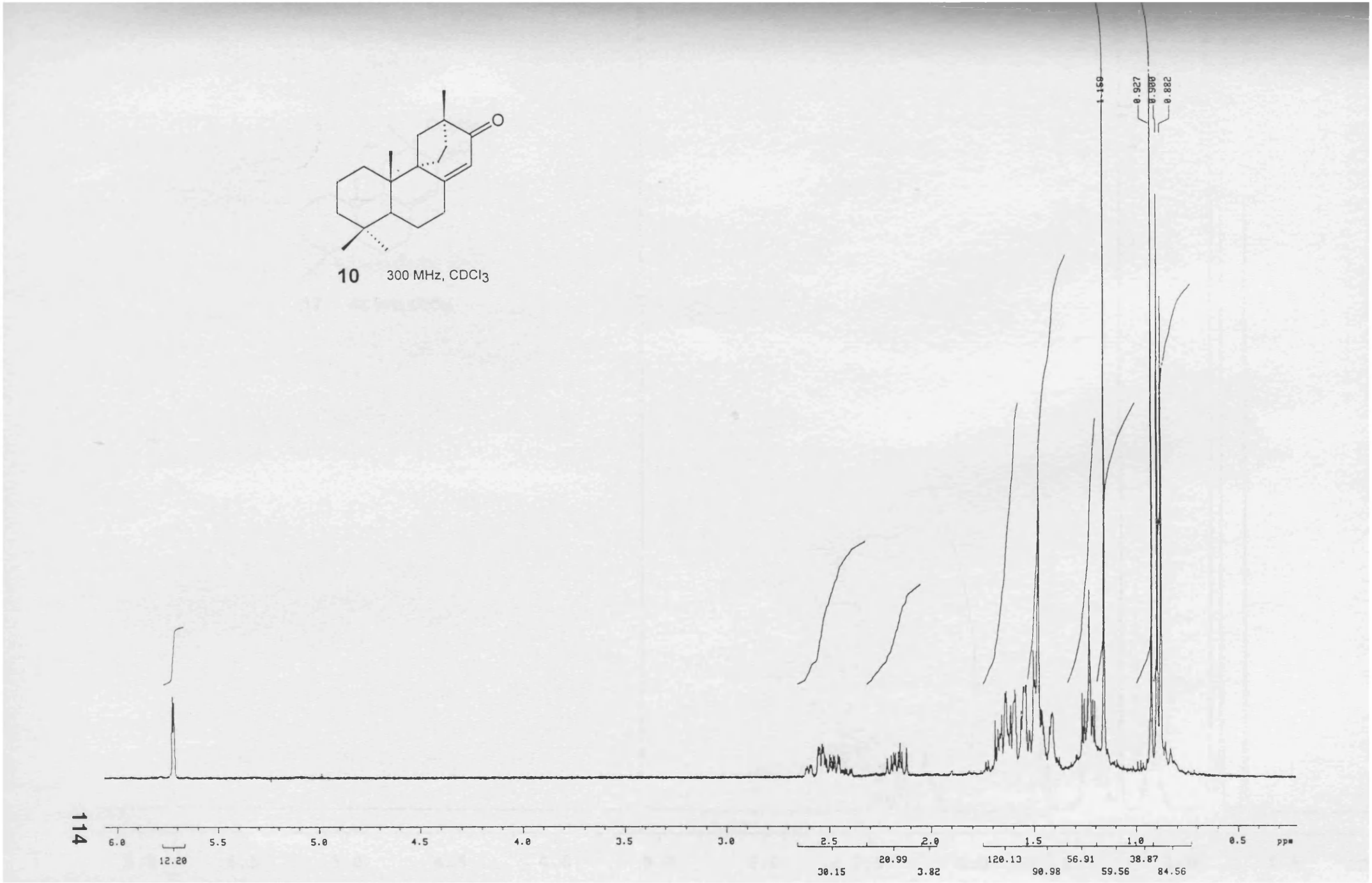
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2.5
2.0
1.5
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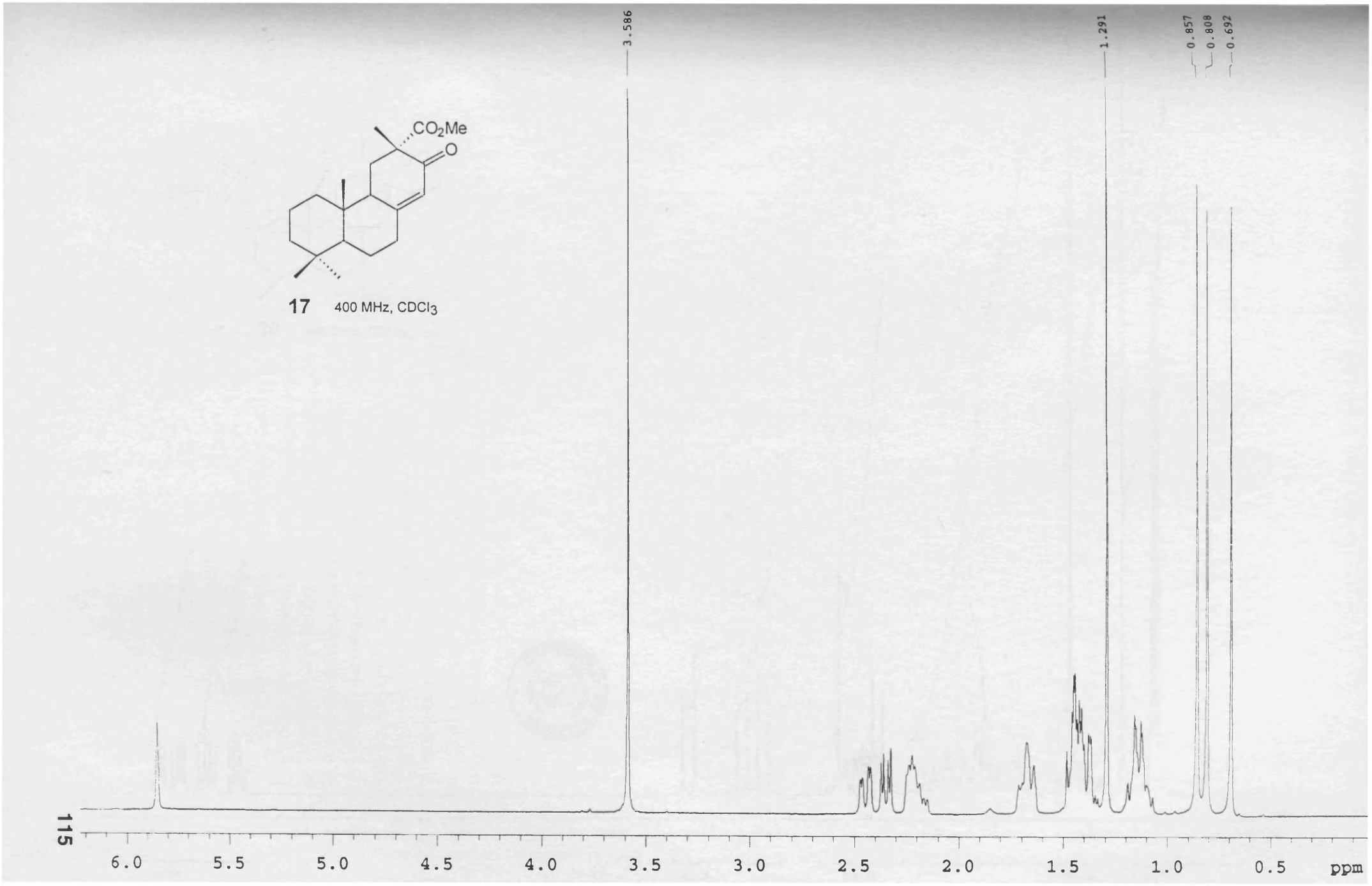
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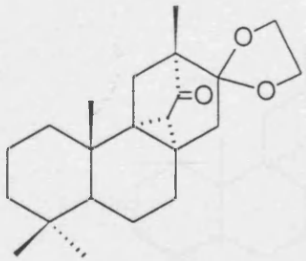
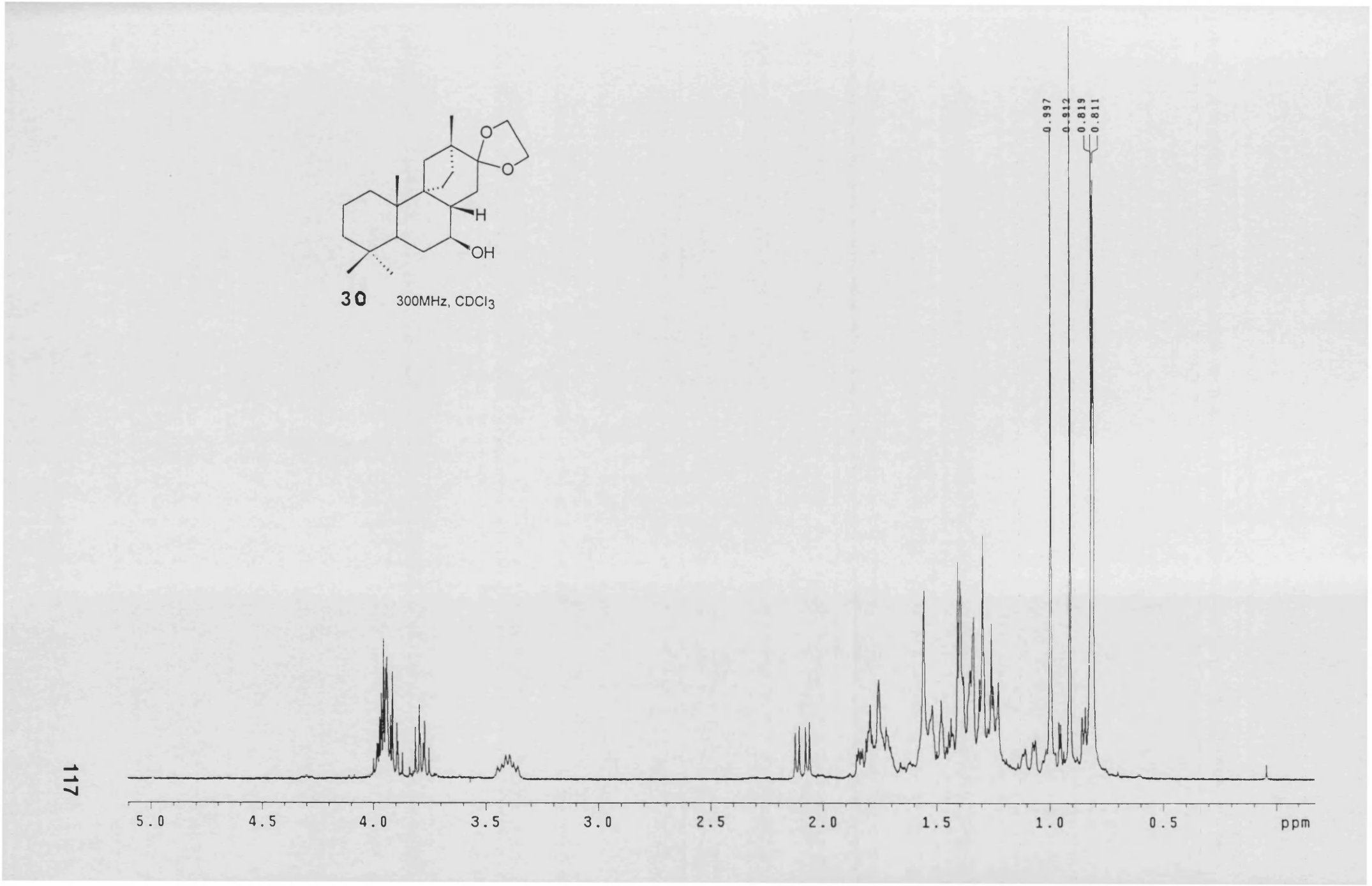
0.857
0.808
0.692
CO2Me
O
17
400 MHz, CDCl3
115
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm



**20** 400 MHz, $CDCl_3$




**30** 300MHz, $CDCl_3$

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**TÍTULO:** $^{1}H$ and $^{13}C$ NMR assignments and conformational analysis of some podocarpene derivatives

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MAGNETIC RESONANCE IN CHEMISTRY
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# $^{1}H$ and $^{13}C$ NMR assignments and conformational analysis of some podocarpene derivatives

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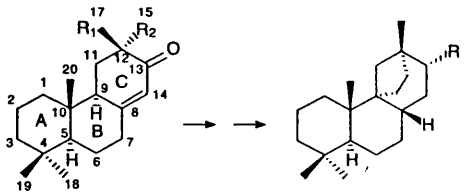
ABSTRACT: This paper reports on the assignment of the $^{1}H$ and $^{13}C$ NMR spectra of five podocarpene derivatives. Resonance assignments were made on the basis of one- and two-dimensional NMR techniques which included $^{1}H$, $^{13}C$, DEPT and HMQC and also 1D NOE difference spectroscopy. The ratio of the different conformers in the six-membered C-ring of the podocarpene system was determined by molecular mechanics calculations and analysis of proton spin–spin coupling constants. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: NMR; $^{1}H$ NMR; $^{13}C$ NMR; conformational analysis; MMX calculations; podocarpene derivatives; scopadulan precursors

## INTRODUCTION

In the past, several scopadulan terpenoids have been isolated from medicinal plants.[1] This new family of tetracyclic diterpenes shows interesting pharmacological properties.[2] Recently, we reported the first diastereoselective synthesis of the simplest member of this group, thyrsiflorin A methyl ester (**6**).[3] Using our synthetic route, several tricyclic podocarpene intermediates (**1**, **2**, **4** and **5**) (Scheme 1) have been prepared to achieve the synthesis of the target **6**.




**1-5**




**6** R = $OCOCH_2CO_2Me$

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| $R_1$ | H | H | Me | Me | $CO_2Me$ |
| $R_2$ | H | Me | H | $CO_2Me$ | Me |

**Scheme 1**

In this paper, we show that the C-ring of these four podocarpene derivatives (**1**, **2**, **4** and **5**) and also of the 14$\beta$-methylpodocarpenone **3**, which was obtained from **2** by basic equilibration (see Experimental), can exist in two different half-chair conformations. Since the energies of the two conformations for podocarpenone **1** differ by only 0.9 kcal mol$^{-1}$ (1 kcal = 4.184 kJ), the presence of substituents at C-12 (compounds **2**–**5**) has a great influence on the conformational equilibrium. The effect of several substituents at C-12 on the ratio of the two conformers was studied using both molecular mechanics calculations and $^{1}H$ NMR coupling constants.

## RESULTS AND DISCUSSION

### Assignments

All $^{13}C$ NMR signals can be separated into different classes of carbon atoms using the editing technique DEPT, and according to their chemical shifts and multiplicities most signals in this study were assigned (Table 1). Complete assignment of the remaining signals was made on the basis of their displacement, the $^{1}H$–$^{13}C$ shift correlation experiment (HMQC) and by comparison with similar compounds. The $^{1}H$ NMR spectrum was assigned using double resonance experiments, $^{1}H$–$^{13}C$ HMQC shift correlation experiments and some 1D NOE difference experiments (NOED). The most relevant signals and coupling constants for the conformational analysis are shown in Table 2.

The assigned stereochemistries at C-12 in **2**–**5** were determined from their spectroscopic data and confirmed by 1D NOE experiments. In the case of **2** and **5**, the NOE effect observed between protons H-15 (irradiated) and H-9 unambigously proved the $\alpha$-orientation of the 12-methyl group. For **3** and **4**, the signals due to protons H-11$\alpha$ and H-11$\beta$ were enhanced when proton H-17 was irradiated, confirming the $\beta$-orientation of the 12-methyl group for both compounds.

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*Contract/grant sponsor*: DGICYT; *Contract/grant number*: PB95-1088.

**Table 1.** $^{13}C$ NMR data for compounds **1–5**[a]

| Carbon | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 39.3 | 39.4 | 39.2 | 38.9 | 39.1 |
| 2 | 18.7 | 18.8 | 18.7 | 18.6 | 18.6 |
| 3 | 41.7 | 41.8 | 41.7 | 41.6 | 41.6 |
| 4 | 33.4 | 33.4 | 33.4 | 33.3 | 33.4 |
| 5 | 53.8 | 54.4 | 53.6 | 53.5 | 53.8 |
| 6 | 21.9 | 22.5 | 21.7 | 21.9 | 21.7 |
| 7 | 35.6 | 35.8 | 35.1 | 35.1 | 35.0 |
| 8 | 165.8 | 164.5 | 164.5 | 165.2 | 164.7 |
| 9 | 51.6 | 48.4 | 52.4 | 49.6 | 48.4 |
| 10 | 38.9 | 39.6 | 38.4 | 38.7 | 38.7 |
| 11 | 20.5 | 27.6 | 29.3 | 32.0 | 30.1 |
| 12 | 36.8 | 39.6 | 40.3 | —[b] | —[b] |
| 13 | 200.0 | 203.3 | 202.1 | 196.1 | 198.0 |
| 14 | 125.8 | 123.9 | 125.5 | 124.8 | 123.3 |
| 15 | | 16.7 | | 173.1 | 19.6 |
| 17 | | | 14.7 | 21.2 | 173.9 |
| 18 | 33.6 | 33.7 | 33.6 | 33.5 | 33.5 |
| 19 | 22.0 | 22.0 | 22.1 | 22.0 | 22.0 |
| 20 | 15.3 | 15.4 | 15.1 | 14.7 | 15.1 |
| $CO_2Me$ | | | | 52.4 | 52.2 |

[a] Spectra measured in $CDCl_3$ at 298 K and referenced relative to TMS.
[b] Signal was hidden.

## Conformational analysis

The conformational study was carried out using molecular mechanics calculations by means of the PCMODEL program. For every compound (**1–5**) a rotational analysis incrementing the dihedral angle C-9—C-11—C-12—C-13 by 5° steps from −85° to 85° using the option D-DRV was performed. In all cases, two minimun energy conformations, half-chair-1 (dihedral angle about −55°) and half-chair-2 (dihedral angle about 40°), were found, the rotational barrier between them being low (1–5 kcal $mol^{-1}$) (see Fig. 1).

The two conformers of each compound were independently minimized. For **4** and **5** containing a $CO_2Me$ group which can occur in different non-equivalent spatial orientations, an independent rotational study for this group was carried out in order to find the most stable conformation.

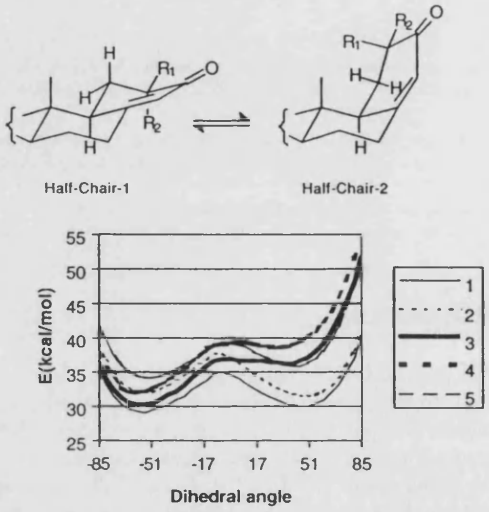


**Figure 1.** Rotational analysis for compounds **1–5**.

**Table 2.** Selected $^1H$ NMR chemical shifts (ppm) and coupling constants (Hz) of compounds **1–5**[a,b]

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| H-7α | 2.28 (ddddd, 15.6, 12.8, 6.6, 2.2, 1.5) | 2.27 (ddddd, 14.5, 12.8, 6.4, 1.8, 1.5) | 2.27 (ddddd, 15.9, 13.0, 6.7, 2.2, 1.5) | 2.27 (ddddd, 15.7, 12.8, 6.5, 1.8, 1.5) | 2.27 (ddddd, 15.8, 12.8, 6.7, 2.2, 1.3) |
| H-7β | 2.54 (ddd, 15.6, 4.9, 2.0) | 2.51 (ddd, 14.5, 4.6, 1.8) | 2.53 (ddd, 15.9, 4.9, 1.9) | 2.52 (ddd, 15.7, 5.3, 1.9) | 2.56 (ddd, 15.8, 5.0, 1.8) |
| H-9 | 2.07 (dddd, 9.5, 5.3, 2.2, 1.5) | 2.11 (dddd, 7.8, 6.0, 2.1, 1.5) | 2.14 (dddd, 10.8, 5.1, 2.2, 1.5) | 2.29 (dddd, 10.4, 5.3, 2.1, 1.5) | 2.11 (dddd, 11.0, 5.0, 2.2, 1.3) |
| H-11α | 2.00 (dddd, 13.3, 5.3, 5.3, 4.2) | 1.73 (ddd, 13.9, 6.0, 6.0) | 1.96 (ddd, 13.3, 5.1, 5.1) | 2.42 (dd, 13.8, 5.3) | 1.84 (dd, 13.4, 5.0) |
| H-11β | 1.71 (dddd, 13.7, 13.3, 9.5, 4.8) | 1.96 (ddd, 13.9, 7.8, 5.2) | 1.40 (ddd, 13.5, 13.3, 10.8) | 1.53 (dd, 13.8, 10.4) | 2.33 (dd, 13.4, 11.0) |
| H-12α | 2.21 (ddd, 15.9, 13.7, 5.3) | | 2.24 (dd[c], 13.5, 5.1) | | |
| H-12β | 2.40 (ddd, 15.9, 4.8, 4.2) | 2.45 (dd[c], 6.0, 5.2) | | | |
| H-14 | 5.88 (dd, 2.2, 2.2) | 5.80 (dd, 2.1, 1.8) | 5.86 (dd, 2.2, 2.2) | 5.92 (dd, 2.1, 1.8) | 5.81 (dd, 2.2, 2.2) |
| H-15 | | 1.07 (d, 6.8) | | | 1.32 (s) |
| H-17 | | | 1.10 (d, 6.8) | 1.36 (s) | |
| H-18 | 0.93 (s) | 0.93 (s) | 0.93 (s) | 0.93 (s) | 0.94 (s) |
| H-19 | 0.88 (s) | 0.88 (s) | 0.88 (s) | 0.88 (s) | 0.89 (s) |
| H-20 | 0.81 (s) | 0.85 (s) | 0.77 (s) | 0.76 (s) | 0.85 (s) |
| $CO_2CH_3$ | | | | 3.66 (s) | 3.74 (s) |

[a] Spectra measured in $CDCl_3$ at 298 K and referenced relative to TMS.
[b] Some coupling constants were determined by analysis of the multiplet in an NOE difference spectrum and double resonance experiments.
[c] When H-15 (for **2**) or H-17 (for **3**) were irradiated.

Results obtained from these calculations are shown in Table 3, together with the conformer population ($n_{HC1}$, $n_{HC2}$) expressed as a molar fraction and calculated using the relationship between the Gibbs free energy ($\Delta G$) and the equilibrium constant ($K$):[4]

$$\Delta G_{HC1-HC2} = -RT \ln(K_{HC1-HC2})$$

where $R = 1.988 \times 10^{-3}$ kcal K$^{-1}$ mol$^{-1}$ and $T = 298$ K. Considering $K_{HC1-HC2} = n_{HC2}/n_{HC1}$, $n_{HC1} + n_{HC2} = 1$ and $\Delta S \approx 0$, so that $\Delta G \approx \Delta H \approx \Delta E_{MMX}$, then

$$n_{HC2} = 1/\{\exp[(E_{HC2} - E_{HC1})/RT] + 1\} \quad (1)$$

and

$$n_{HC1} = 1 - n_{HC2} \quad (2)$$

From the above results, it can be seen that in the podocarpenone **1** the half-chair-1 conformation (82% of the population) is 0.9 kcal mol$^{-1}$ more stable than the half-chair-2 conformation (18% of the population). Also, owing to the small barrier between them, this compound exists at room temperature in a rapid conformational equilibrium between the two half-chair conformations. When a methyl group is present at $R_2$ (**2**), the 1,3-diaxial interaction between H-9 and the methyl group makes the half-chair-1 conformation slightly less stable than before. Hence the energy difference between the two half-chair conformations is reversed, the half-chair-2 conformation being more stable (71% of the population).

In the case of **3–5**, in which a substituent different from H is present at $R_1$, the strong steric interaction between H-20 and the $R_1$ group makes the half-chair-1 conformation clearly more stable. When $R_1$ is a methyl group (see **3** and **4**), the energy difference is about 6 kcal mol$^{-1}$, in this case the half-chair-1 conformation being the preferred form (100% of the population). If $R_1$ is a $-CO_2Me$ group (**5**), the sp$^2$ hybridization of the carbonyl group exerts a smaller steric interaction between this group and protons H-20. Consequently, there is a decrease in the energy difference to 1.6 kcal mol$^{-1}$, thereby increasing the population of molecules in the half-chair-2 conformation until it reaches 6%.

Finally, it is worth noting that the calculated 1.25 kcal mol$^{-1}$ energy difference between the more stable conformers of the epimeric compounds at C-12, **2** and **3**, means that in an equilibration process between the two isomers, at room temperarure, there would exist an 11% population of **2** versus 89% of **3**. This theoretical calculation is in good accordance with the 20 : 80 ratio obtained experimentally (see Experimental).

**Table 3.** Molecular geometry and distribution of the different conformations of compounds **1–5**

| | Half-chair-1 | | | Half-chair-2 | | |
|---|---|---|---|---|---|---|
| Compound | $E_{HC1}$[a] | $n_{HC1}$[b] | Dihedra angle[c] | $E_{HC2}$[a] | $n_{HC2}$[b] | Dihedra angle[c] |
| 1 | 29.24 | 0.82 | −57 | 30.13 | 0.18 | 46 |
| 2 | 32.14 | 0.29 | −55 | 31.62 | 0.71 | 49 |
| 3 | 30.37 | 1.00 | −57 | 36.31 | 0.00 | 35 |
| 4 | 32.11 | 1.00 | −59 | 38.66 | 0.00 | 32 |
| 5 | 34.19 | 0.94 | −53 | 35.79 | 0.06 | 41 |

[a] Steric energy in kcal mol$^{-1}$ obtained from MMX calculations.
[b] Molar fraction calculated from Eqns (1) and (2).
[c] Dihedral angle C-9—C-11—C-12—C-13.

## $^1$H NMR and coupling constants analysis

There are some signals in the $^1$H NMR of **1–5** that are useful for studying the favoured conformations adopted by those compounds, specifically the coupling constants between protons H-9 and H-11$\alpha$/H-11$\beta$. As can be seen in Fig. 1, the dihedral angle between proton H-9 and the two protons H-11 is different enough in the two conformations to permit not only a qualitative but also a quantitative study.

Coupling constant data [$^3J$(HH)] between H-9 and H-11$\alpha$/H-11$\beta$ for **1–5** are shown in Table 4. The $^3J$(HH) ($J_{half-chair-1}$, $J_{half-chair-2}$) values were obtained using the PMR option of the PCMODEL program for each calculated conformation (Table 3). This option makes use of a generalization of the Karplus equation.[5] The average $^3J$(HH) was calculated by including the mole fractions of both conformations from the formula $n_{HC1}J_{half-chair-1}$ +

**Table 4.** Coupling constants $^3J$(HH) (Hz) of H-9 protons of **1–5**

| Compound | Vicinal protons | $J_{half-chair-1}$[a] | $J_{half-chair-2}$[a] | Average[b] | Expt. | Diff.[c] |
|---|---|---|---|---|---|---|
| 1 | 9–11$\alpha$ | 4.6 | 6.8 | 5.0 | 5.3 | 0.3 |
| | 9–11$\beta$ | 12.1 | 1.2 | 10.0 | 9.5 | −0.5 |
| 2 | 9–11$\alpha$ | 4.8 | 7.0 | 6.4 | 6.0 | −0.4 |
| | 9–11$\beta$ | 11.9 | 1.1 | 4.0 | 7.8 | 3.8 |
| 3 | 9–11$\alpha$ | 4.8 | 8.9 | 4.8 | 5.1 | 0.3 |
| | 9–11$\beta$ | 12.0 | 1.0 | 12.0 | 10.8 | −1.2 |
| 4 | 9–11$\alpha$ | 4.6 | 9.5 | 4.6 | 5.3 | 0.7 |
| | 9–11$\beta$ | 12.0 | 1.2 | 12.0 | 10.4 | 1.6 |
| 5 | 9–11$\alpha$ | 4.5 | 8.2 | 4.7 | 5.0 | 0.3 |
| | 9–11$\beta$ | 12.0 | 1.0 | 11.3 | 11.0 | −0.3 |

[a] Coupling constants for the half-chair-1, and half-chair-2 conformations calculated from the option PMR of the PCMODEL program based on Karplus' generalization equation.[7]
[b] Calculated from the formula $n_{HC1}J_{half-chair-1} + n_{HC2}J_{half-chair-2}$. (for $n_{HC1}$ and $n_{HC2}$, see Table 3).
[c] Difference = experimental − average.

$n_{HC2}J_{half-chair-2}$,[6] where $n_{HC1}$ and $n_{HC2}$, are calculated from Eqns (1) and (2).

Reasonable agreement can be seen between the experimental values and the calculated averages. Most of the differences (last column in Table 4) can be explained by the fact that the calculations were performed using only the most stable conformations, without considering the small vibrational variations and the possibility of similar energy rotamers when the —$CO_2$Me group is present. In particular, in the case of **2**, the experimental values could accommodate a higher population for half-chair-1 (about 50–60%). It can be concluded that the mole fractions calculated using molecular mechanics are very similar to the real values, and the study of the $^1$H coupling constants in general confirms the results of the previous MM-based conformational analysis.

## CONCLUSIONS

Podocarpene intermediates in the synthesis of scopadulan diterpenes have a six-membered C-ring that can exist in two different half-chair conformations. From a detailed study of the $^1$H NMR data together with molecular mechanics calculations, the ratio of the two conformers (half-chair-1 and half-chair-2) of those systems was calculated. It can be concluded that when no substituents different from H are present at C-12$\beta$ (**1** and **2**), both conformations have a similar energy. Hence these compounds exist at room temperature in a rapid equilibrium between the half-chair-1 and half-chair-2 conformations. However, when bulkier substituents (such as Me or —$CO_2$Me) at C-12$\beta$ are present (**3–5**), the population in the half-chair-1 conformation increases to the detriment of half-chair-2, owing to the strong steric interaction between protons H-20 and the substituent. When the substituent at C-12$\beta$ is a methyl group (**3** and **4**), the energy difference increases to approximately 6 kcal mol$^{-1}$, in these cases the half-chair-1 conformation being the favoured form.

## EXPERIMENTAL

### Calculations

Molecular mechanics calculations[7] were performed using the MMX force field, which is a derived version of the MM2 program, developed by Allinger and implemented in the PCMODEL (4.0) program (Serena Software, Bloomington, In, USA).

### Spectra

$^1$H and $^{13}$C NMR and DEPT spectra were measured on a Varian XL-300 spectrometer (299.95 MHz for $^1$H and 75.43 MHz for $^{13}$C) operating at a probe temperature of 298 K using a dual $^1$H/$^{13}$C 5 mm probe. The $^1$H measurement conditions were spectral width 4000 Hz, 90° pulse with 18 µs, acquisition time 3.7 s, number of transients 16–64 and 0.1 Hz digital resolution.

$^1$H–$^{13}$C HMQC and NOED spectra were measured on a Varian 400 spectrometer (399.95 MHz for $^1$H and 100 MHz for $^{13}$C) equipped with a 5 mm indirect detection probe operating at 298 K. Sample concentrations were typically in the range 5–20 mg per 0.5 ml of $CDCl_3$. The signal of the TMS was taken as the reference. All these experiments were performed either using standard pulse sequences supplied by the spectrometer manufacturer or slightly modified pulse sequences. NOED experiments were typically acquired with 8K data points covering a spectral width of 3200 Hz and with a 1.5 s presaturation time. Spectra at each presaturation position were interleaved in groups of four scans to minimize artefacts due to instrument inconsistencies and processed with a 1 Hz exponential line broadening to reduce subtraction artefacts. The HMQC spectrum was obtained using a spectral width of 3200 Hz in the $^1$H dimension and 16 000 Hz in the $^{13}$C dimension. A total of 256 increments were collected with eight transients per increment and an acquisition time of 0.1 s.

### Preparation of 12$\beta$-methyl-8(14)-podocarpen-13-one (3)

A solution of NaOMe in MeOH (2 M, 2 ml, 4 mmol) was added to a solution of 12$\alpha$-methyl-8(14)-podocarpen-13-one (**2**) (50 mg, 0.19 mmol) in THF (1 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for a further 30 min. The solution was poured into water and extracted with $CH_2Cl_2$. The organic extract was washed with brine, dried over sodium sulfate and concentrated under vacuum to give an oily residue which resulted in an 8 : 2 ($\beta$ : $\alpha$) mixture (determined by $^1$H NMR) of epimers at C-12. When longer reaction times were used, lower yields of the same 8 : 2 ($\beta$ : $\alpha$) mixture were obtained. Chromatography of the crude with hexane–ethyl acetate (from 9 : 1 to 8 : 2) gave the 12$\beta$ epimer (**3**) as an oil which solidified on standing (36 mg, 72%) and 9 mg (18%) of starting material (**2**).

### Acknowledgments

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**REF. REVISTA:** Magnetic Resonance in Chemistry, **2001**, *39*, in press.

MRC841/1545

MAGNETIC RESONANCE IN CHEMISTRY
*Magn Reson Chem* 2001; 39: 000–000

# Spectral Assignments and Reference Data

## Assignment of $^{1}H$ and $^{13}C$ NMR data for (−)-methyl thyrsiflorin A and some scopadulan precursors

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Received 28 December 2000; Accepted 8 February 2001

The $^{1}H$ and $^{13}C$ NMR spectral analysis of synthetic (−)-methylthyrsiflorin A and 10 scopadulan precursors is reported. Resonance assignments were based on one- and two-dimensional NMR techniques, which included $^{1}H$, $^{13}C$, DEPT and HMQC and also 1D NOE difference spectroscopy. 

KEYWORDS: NMR; $^{1}H$ NMR; $^{13}C$ NMR; scopadulan diterpenes; scopadulan precursors

### INTRODUCTION

A number of structurally unique tetracyclic terpenoids have been isolated from the medicinal plants *Scoparia dulcis* L. and *Calceolaria thyrsiflora* (both belonging to the Scrophulariaceae family).[1] These compounds and some semisynthetic analogues have been shown to be potentially useful for treating disorders such as peptic ulcers, osteoporosis, cancer and some viral infections.[2]

We recently reported the first diastereoselective synthesis of the methyl ester of the scopadulan diterpene (−)-methylthyrsiflorin A (**11**).[3] During this synthesis, we prepared several intermediates whose structures were confirmed by both NMR spectroscopy and physical data. Among these scopadulan precursors, we noted that several tricyclic intermediates possessing a podocarpene skeleton can exist in two different conformations. A complete conformational study using their $^{1}H$ NMR data together with molecular mechanics calculations was published separately.[4]

In this paper, we report the $^{1}H$ and $^{13}C$ NMR chemical shift assignments obtained from one- and two-dimensional NMR techniques for synthetic **11** and the rest of the scopadulan intermediates (**1**–**10**) of our synthesis. The $^{13}C$ NMR data for synthetic **11** were identical with those previously reported, whereas the $^{1}H$ NMR, HMQC and 1D NOE difference data showed that the assignments of protons H-17 and H-20 were reversed in the literature.[1d]

### RESULTS AND DISCUSSION

The structures and numbering system for compounds **1**–**11** are presented in Fig. 1. Assignments of $^{1}H$ and $^{13}C$ NMR chemical shifts for **1**–**11** are listed in Tables 1 and 3, respectively, and the multiplicity and coupling constants of some characteristic $^{1}H$ NMR signals are shown in Table 2. The obvious signal assignments were made from $^{1}H$ and $^{13}C$ NMR and DEPT spectra according to their chemical shifts and multiplicities. The remaining signals were assigned with the aid of double resonance experiments, one-bond heteronuclear ($^{1}H$–$^{13}C$) multiple quantum correlation (HMQC) spectra, and some 1D NOE difference experiments (NOED). When it is indicated, the $\alpha$ and $\beta$ orientations of protons H-6, H-7, H-11 and H-14 were determined unequivocally by NOED experiments.

From the $^{1}H$ NMR data, it is interesting to note the chemical shift of proton H-5 in **4**–**6** (0.65–0.77 ppm). The high shielding of this proton may be attributed to the magnetic anisotropy induced by the cyclopropane ring between C-8 and C-9.

From the $^{13}C$ NMR data, the $\gamma$-effect observed between C-1 and C-16 in **7**–**11** is also of interest. This effect is transmitted through proton H-1$\alpha$, so the distance between this proton and C-16 determines the intensity of the effect, the greatest effect being with the shortest distance. Therefore, the signal due to C-1 in **7**–**11** is upfield (about 3–5 ppm to lower frequency) with respect to **1**–**6** with longer distances between H-1$\alpha$ and C-16, where the latter exists. The $^{13}C$ NMR spectral data for synthetic **11** were found to be identical with those reported for the methyl ester of the natural (−)-methylthyrsiflorin A,[1d] whereas the HMQC spectrum and NOED experiments indicated that the assignments of protons H-17 and H-20 were originally reversed. In particular, the NOE enhancement of the signal located at 0.92 ppm when proton H-13 was irradiated proved that this signal was due to protons H-17.

Using our synthetic route for the synthesis of **11**, we prepared several intermediates with no precedents in the literature, in particular **4**–**6** which have a novel pentacyclic carbon framework. We think that the $^{1}H$ and $^{13}C$ NMR data of these compounds will be useful as reference data for the assignment and characterization of similar compounds.

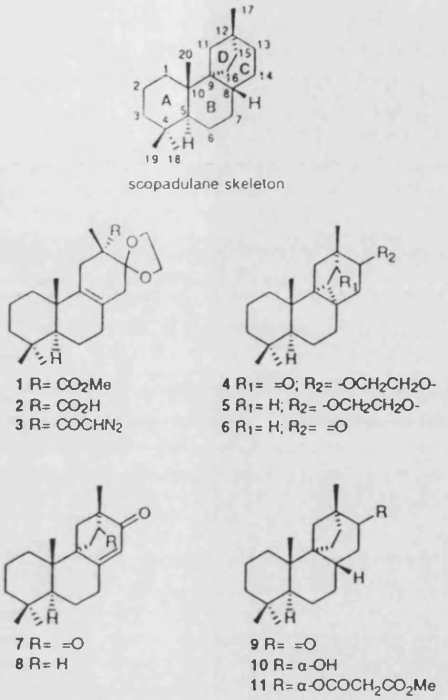


**Figure 1.** Structures and numbering of the compounds investigated.

### EXPERIMENTAL

#### Compounds

All the compounds were prepared as reported previously.[4]

#### Spectra

$^{1}H$, $^{13}C$ NMR and DEPT spectra were measured on a Varian XL-300 spectrometer (299.95 MHz for $^{1}H$ and 75.43 MHz for $^{13}C$) operating at a probe temperature of 298 K using a dual $^{1}H/^{13}C$ 5 mm probe. The $^{1}H$ measurement conditions were spectral width 4000 Hz, 90° pulse with 18 µs, acquisition time 3.7 s, number of transients 16–64 and 0.1 Hz digital resolution.

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## Spectral Assignments and Reference Data

**Table 1.** $^{1}H$ NMR chemical shifts ($\delta$, ppm) for compounds **1–11**

| Proton | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1α | 1.19 | ~1.19 | —[c] | 1.00 | 1.05 | 1.14 | 1.33 | 1.50 | ~1.47 | 1.30–1.50 | ~1.40 |
| 1β | 1.78 | 1.76 | 1.80 | 1.69 | 1.77 | 1.82 | 1.52 | | | | |
| 2α | ~1.45 | 1.40–1.50 | —[c] | 1.38–1.50 | 1.20–1.28 | 1.52 | 1.51 | 1.50 | ~1.30–1.55 | 1.36–1.64 | 1.40–1.60 |
| 2β | 1.58 | 1.58 | 1.55 | | 1.54 | 1.56 | | | | | |
| 3α | ~1.15 | 1.16 | —[c] | 1.06 | 1.12 | 1.15 | 1.16 | 1.18 | 1.14 | 1.09 | ~1.08 |
| 3β | ~1.40 | 1.35–1.45 | —[c] | 1.38 | 1.40 | 1.43 | 1.47 | 1.46 | ~1.40 | ~1.37 | ~1.36 |
| 5 | 1.26 | 1.23 | ~1.25 | 0.65 | 0.70 | 0.77 | 1.14 | 1.26 | 1.00 | 0.90 | 0.88 |
| 6α | ~1.65 | ~1.68 | —[c] | 1.25–1.40 | 1.20–1.28[b] | 1.27–1.36 | 1.69 | 1.64[b] | ~1.56[b] | ~1.51[b] | ~1.48 |
| 6β | 1.42 | 1.38–1.46 | —[c] | | 1.32–1.44[b] | | 1.55 | ~1.55[b] | ~1.32[b] | ~1.20[b] | 1.30 |
| 7α | 1.80–1.95 | 1.84–1.94 | —[c] | 1.65–1.83 | 1.35–1.75 | 1.64–1.76 | 2.39 | 2.48 | 1.60[b] | ~1.50[b] | ~1.50[b] |
| 7β | | | —[c] | | | | 2.64 | 2.59 | ~1.26[b] | ~1.26[b] | ~1.13[b] |
| 8 | — | — | — | — | — | — | — | — | 2.12 | 1.80 | 1.84 |
| 11α | 2.81 | 2.66 | 2.76 | 1.68 | 1.41 | 1.65[b] | 1.94 | 1.54 | 1.70[b] | 1.46[b] | 1.50[b] |
| 11β | 2.02 | 2.16 | ~1.90 | 2.11 | 1.78 | 1.79[b] | 2.30 | 1.63 | 1.39[b] | 0.95[b] | 1.03[b] |
| 13 | — | — | — | — | — | — | — | — | — | 3.38 | 4.66 |
| 14α | 2.02 | 2.10 | 2.00 | 2.04[b] | 1.92[b] | 2.45 | 5.76 | 5.74 | 1.96 | ~0.91[b] | ~1.00 |
| 14β | 2.15 | | 2.10 | 2.28[b] | 1.99[b] | | — | — | 2.23 | 1.82[b] | 1.90 |
| 15 | — | — | — | — | 1.36 | 1.77 | — | 1.64 | ~1.56 | ~1.19 | ~1.18 |
| 15′ | — | — | — | — | 1.96 | | — | 1.71 | ~1.74 | ~1.73 | ~1.73 |
| 16 | 3.68 | — | 5.78 | 1.80 | 0.98 | 1.22 | 2.22 | 2.19 | 2.02 | ~1.38 | ~1.41 |
| 16′ | — | — | — | — | — | — | 2.61 | 1.44 | ~1.72 | ~1.73 | ~1.73 |
| 17 | 1.21 | 1.25 | 1.16 | 0.90 | 0.88 | 1.02 | 1.20 | 1.18 | 1.05 | 1.00 | 0.92 |
| 18 | 0.87 | 0.87 | 0.88 | 0.79 | 0.80 | 0.83 | 0.92 | 0.92 | 0.85 | 0.82 | 0.81 |
| 19 | 0.83 | 0.83 | 0.84 | 0.81 | 0.81 | 0.83 | 0.91 | 0.90 | 0.82 | 0.80 | 0.80 |
| 20 | 0.93 | 0.94 | 0.94 | 1.07 | 1.05 | 1.08 | 1.00 | 0.95 | 1.00 | 0.97 | 0.96 |
| Ketal | 3.75–4.00 | 4.07 | 3.80–4.00 | 3.68–4.00 | 3.75–3.92 | — | — | — | — | — | — |

[a] Additional side-chain ester $^{1}H$ signals in **11** at $\delta$ 3.74 (s) and 3.35 (s).

[b] α and β may be interchanged.

[c] Signal not assigned.

**Table 2.** Selected $^{1}H$ NMR signals for compounds **1–11**: multiplicity and coupling constants (Hz)[a]

| Proton | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H-5 | dd; 11, 2 | dd; 11, 2 | dd; 11, 2 | dd; 12, 4 | dd; 9, 6 | dd; 11, 4 | dd; 13, 3 | dd; 13, 3 | dd; 12, 3 | dd; 12, 3 | dd; 12, 3 |
| H-7α | m | m | m | m | m | m | dddd; 17.5, 13, 6.5, 2.5 | dddd; 17.5, 12, 7, 2 | m | m | m |
| H-7β | m | m | m | m | m | m | ddd; 17.5, 5, 1.5 | dddd; 17.5, 6, 2.5, 0.5 | m | m | m |
| H-11α | br d; 16.5 | br d; 16.5 | br d; 16.5 | d; 12 | d; 12 | d[b]; 12 | d; 12 | d; 11.5 | d[b]; 12 | d[b]; 11.5 | d[b]; 11.5 |
| H-11β | d; 16.5 | d; 16.5 | d; 16.5 | d; 12 | d; 12 | dd[b]; 12, 2 | dd; 12, 3.5 | br d; 11.5 | br d[b]; 12 | br d[b]; 11.5 | br d[b]; 11.5 |
| H-13 | — | — | — | — | — | — | — | — | — | dd; 10.5, 5.5 | ddd; 11, 6, 1 |
| H-14α | d; 16.5 | br s | d; 16.5 | d; 15 | d; 15 | s | d; 2.5 | dd; 2, 0.5 | dd; 15, 12 | m | m |
| H-14β | br d; 16.5 | br s | br d; 16.5 | d; 15 | d; 15 | s | — | — | dd; 15, 5.5 | m | ddd; 12.5, 6, 6 |
| H-15 | — | — | — | — | dd; 12, 3 | m | — | m | m | m | m |
| H-15′ | — | — | — | — | d; 12 | m | — | m | m | m | m |
| H-16 | — | — | — | s | d; 3 | d; 3 | dd; 17.5, 3.5 | ddd; 12.5, 10, 6 | m | m | m |
| H-16′ | — | — | — | — | — | — | d; 17.5 | m | m | m | m |

[a] Some coupling constants were determined by analysis of the multiplet in an NOE difference spectrum and double resonance experiments.

[b] α and β may be interchanged.

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## Spectral Assignments and Reference Data

Table 3. $^{13}C$ NMR chemical shifts ($\delta$, ppm) for compounds 1–11

| Carbon | 1 | 2 | 3[a] | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 35.94 | 35.91 | 36.28 | 36.56 | 36.84 | 37.05 | 33.03 | 32.79 | 32.55 | 32.57 | 32.49 |
| 2 | 18.71 | 18.71 | 18.99 | 18.41 | 18.79 | 18.71 | 18.23 | 18.40 | 18.70 | 18.79 | 18.72 |
| 3 | 41.55 | 41.55 | 41.89 | 41.59 | 41.98 | 41.87 | 41.64 | 41.91 | 42.12 | 42.20 | 42.16 |
| 4 | 33.17 | 33.25 | 33.36 | 33.05 | 33.07 | 33.08 | 33.47 | 33.43 | 33.13 | 33.12 | 33.10 |
| 5 | 51.07 | 51.12 | 51.31 | 47.48 | 48.16 | 48.11 | 47.95 | 47.22 | 47.98 | 47.97 | 47.94 |
| 6 | 18.49 | 18.51 | 19.14 | 17.75 | 18.26 | 18.04 | 20.62 | 20.97 | 21.70 | 21.83 | 21.77 |
| 7 | 31.15 | 31.26 | 31.67 | 29.19 | 30.53 | 29.53 | 31.03 | 30.80 | 30.29 | 30.14 | 29.94 |
| 8 | 122.91 | 122.41 | 122.54 | 32.20 | 19.68 | 19.97 | 171.42 | 171.12 | 40.01 | 37.39 | 37.14 |
| 9 | 135.75 | 135.93 | 136.96 | 52.29 | 43.84 | 42.05 | 52.57 | 58.94 | 52.44 | 52.47 | 52.49 |
| 10 | 37.28 | 37.44 | 37.73 | 34.06 | 33.63 | 33.56 | 37.33 | 37.46 | 38.83 | 38.52 | 38.52 |
| 11 | 33.40 | 33.56 | 33.32 | 31.89 | 35.30 | 36.40 | 42.83 | 44.12 | 45.38 | 44.50 | 44.47 |
| 12 | 48.41 | 47.64 | 52.30 | 47.89 | 41.69 | 49.79 | 63.69 | 50.39 | 53.10 | 44.11 | 43.05 |
| 13 | 110.13 | 110.75 | 110.85 | 112.24 | 111.34 | 213.36 | 192.26 | 204.50 | 214.64 | 76.38 | 80.00 |
| 14 | 39.31 | 37.33 | 39.67 | 41.82 | 42.56 | 41.67 | 125.70 | 124.37 | 43.68 | 38.17 | 34.19 |
| 15 | — | — | — | 210.99 | 36.14 | 36.92 | 211.82 | 35.29 | 36.88 | 30.69 | 31.94 |
| 16 | — | — | — | 39.47 | 26.51 | 26.49 | 41.33 | 29.27 | 24.09 | 24.43 | 24.36 |
| 17 | 18.73 | 19.02 | 19.28 | 10.97 | 17.72 | 17.32 | 14.46 | 20.22 | 19.81 | 23.30 | 23.11 |
| 18 | 33.05 | 33.11 | 33.28 | 33.71 | 33.91 | 33.87 | 33.71 | 33.76 | 33.61 | 33.62 | 33.60 |
| 19 | 21.51 | 21.58 | 21.77 | 21.37 | 21.50 | 21.46 | 22.69 | 22.66 | 21.96 | 22.05 | 22.02 |
| 20 | 18.93 | 19.11 | 19.36 | 17.53 | 19.76 | 19.86 | 19.10 | 20.86 | 17.24 | 17.34 | 17.31 |
| R | 175.07; 51.88 | 176.13 | 196.10; 53.52 | — | — | — | — | — | — | — | — |
| Ketal | 64.98; 64.90 | 64.59; 64.50 | 64.66; 64.66 | 65.24; 64.94 | 64.72; 64.62 | — | — | — | — | — | — |

[a] In $C_6D_6$.

[b] Additional side-chain ester $^{13}C$ signals in **11** at $\delta$ 167.17 (s), 166.21 (s), 52.34 (q) and 41.70 (t).

$^{1}H$–$^{13}C$ HMQC and NOED spectra were measured on a Varian 400 spectrometer (399.95 MHz for $^{1}H$ and 100 MHz for $^{13}C$) equipped with a 5 mm indirect detection probe operating at 298 K. Sample concentrations were typically in the range 5–20 mg per 0.5 ml of $CDCl_3$. The signal of TMS was taken as the reference. All these experiments were performed either using standard pulse sequences supplied by the spectrometer manufacturer or slightly modified pulse sequences. NOED experiments were typically acquired with 8 K data points covering a spectral width of 3200 Hz and with a 1.5 s presaturation time. Spectra at each presaturation position were interleaved in groups of four scans to minimize artefacts due to instrument inconsistencies and processed with a 1 Hz exponential line broadening to reduce subtraction artefacts. The HMQC spectrum was obtained using a spectral width of 3200 Hz in the $^{1}H$ dimension and 16 000 Hz in the $^{13}C$ dimension. A total of 256 increments were collected with eight transients per increment and an acquisition time of 0.1 s.

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**TÍTULO:** Evaluación de la actividad antitumoral y antiviral *in vitro* de diterpenos escopadulánicos sintéticos

**REF. REVISTA:** Revista Latinoamericana de Química, **2000**, *28*, s. 151-152.

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# EVALUACIÓN DE LA ACTIVIDAD ANTITUMORAL Y ANTIVIRAL *IN VITRO* DE DITERPENOS ESCOPADULÁNICOS SINTÉTICOS

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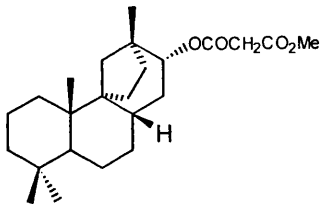
Las especies de la familia *Scrophuraliaceae* como la *Scoparia dulcis* han sido investigadas en los últimos años por el interés farmacológico que despierta los metabolitos secundarios aislados de estas especies. A nivel de la información etnomédica se conoce que la *Scoparia dulcis* ha sido usada en Paraguay, India y Taiwan como remedio herbal para una variedad de desordenes. Recientemente, Hayashi y colaboradores identificaron de los extractos farmacólogicamente activos de esta especie dos diterpenos tetracíclicos nuevos, cuyo sistema de anillos carbonados fue llamado escopadulano, los ácidos escopadúlcicos A y B. Los ácidos escopadúlcicos exhiben un amplio rango de actividades biológicas, los cuales incluyen actividad antiviral contra el herpes simplex tipo1 humano (HSV-1) y actividad antitumoral en varias líneas celulares.[(1)] Años mas tarde, se aislaron nuevos diterpenos escopadulánicos de plantas pertenecientes también a la familia *Scrophuraliaceae*, en concreto de *Calceolaria thyrsiflora* y *Calceolaria dentata*, los cuáles fueron llamados thyrsifloranos.

Nuestro principal interés es el estudio de la actividad antitumoral y antiviral de compuestos derivados principalmente de productos naturales; en este estudio se ha evaluado hasta ahora la actividad antitumoral y antiviral *in vitro* de cinco diterpenos escopadulánicos. Estos compuestos se han sintetizado por un método diastereoselectivo a partir del compuesto *(+)-podocarp-8(14)-en-13-one*[(2)], estos son los siguientes: *(-)-methyl thyrsiflorin A* (**1**), *(-)-methyl thyrsiflorin B acetate* (**2**), *(-)-thyrsiflorin C* (**3**), *(-)-thyrsiflorin C diacetate* (**4**) y el compuesto de transposición **5**.

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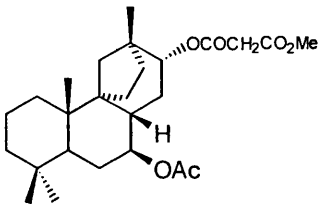


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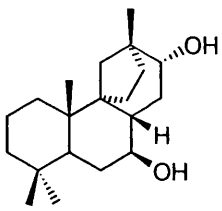


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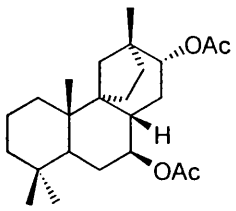


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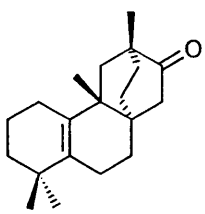


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Para evaluar la actividad antitu moral *in vitro* de los anteriores diterpenos espongiánicos se utilizaron las líneas tumorales HeLa y Hep-2, la línea no tumoral CHO, y el cultivo primario Bon-Fib, con el fin de identificar compuestos con posible citotoxicidad selectiva hacía células tumorales, bajo la metodología descrita en la referencia 3; brevemente, las células se siembran en platos de cultivo de 96 pozos y se incuban por 24h, agregando luego los compuestos y dejando nuevamente en incubación por 72h. Para determinar la actividad antiviral se siembran las células MDBK y se dejan en incubación hasta formar monocapa, luego se agregan los compuestos y una hora después se agrega el virus HSV-2 en 10 o 1 dosis infecciosas, y se evalúa el efecto antiviral a través del método de reducción de placas[3]. Ambos bioensayos se realizaron por triplicado y se visualizaron por la fijación del microplato utilizando violeta cristal en formalina. Para la actividad antitumoral se reporta la concentración que desprendió el 100% de la momonopa celular, el compuesto con mayor actividad fue el **5**, con un valor de 20 μg/ml para células HeLa; en cuanto a la actividad antiviral todos los compuestos mostraron una débil reducción del titulo de $10^{0.5}$, excepto el compuesto **5** que fue de $10^{1}$.

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**TÍTULO:** Structure-Activity Relationship of *In Vitro* Antiviral and Cytototoxic Activity of Synthetic Analogues of Scopadulane Diterpenes

**REF. REVISTA:** Journal of Natural Products, submitted.

# Structure-Activity Relationship of *In Vitro* Antiviral and Cytotoxic Activity of Synthetic Analogues of Scopadulane Diterpenes

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Abstract. Fourteen synthetic compounds derived from the natural scopadulane-type diterpenes thyrsiflorin A (**4**), B (**5**), and C (**6**), including several precursors, have been examined *in vitro* for their antiherpetic activity against herpes simplex virus type II (HSV-2) and cytotoxicity against two human tumor cell lines. Four of these compounds showed moderate antiherpetic activity but none of them exhibited a significant cytotoxicity against the cell lines used. Some structure-activity relationships have been identified for the antiviral activity in these scopadulane derivatives as well as important structural features for the cytotoxic activity.

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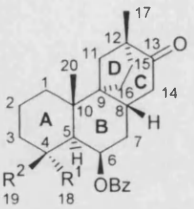
Scopadulane diterpenes are members of a small group of plant metabolites recently discovered in Scrophulariaceae family. Among those plants, *Scoparia dulcis,* has been known in Paraguayan traditional medicine for improving digestion and protecting the stomach, in Taiwan as a remedy for hypertension, and in India for toothaches and stomach disease.[1] In the course of the search for biologically active compounds from Paraguayan medicinal plants, Hayashi et al. reported the isolation and structure of two novel tetracyclic diterpenoids, scopadulcic acids A (SDA, **1**) and B (SDB, **2**),[2] from the 70% ethanol extract of the crude drug "Typchá-Kuratû" (whole plants of *S. dulcis*). The proposed structure of SDA was later confirmed by X-ray crystallography.[3] The same authors isolated a new SDB analogue, scopadulciol (SDC, **3**),[4] from *S. dulcis* collected in Taiwan which is believed to be identical to dulcinol that was found in *S. dulcis* from Bangladesh.[5] The plants of the genus *Calceolaria* (Scrophulariaceae family) available in various parts of Chile have also yielded several new diterpenes with the scopadulane skeleton. For example, thyrsiflorin A (TA, **4**), thyrsiflorin B (TB, **5**), and thyrsiflorin C (TC, **6**) were obtained from the chloroform extract of *Calceolaria thyrsiflora*.[6]

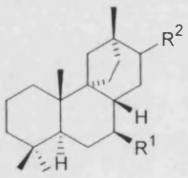
Since the scopadulcic acids were discovered in 1987, several of their pharmacological properties and possible therapeutic applications have been discovered. For example, these bioactive diterpenes inhibit *in vitro* gastric acid secretion and are considered potential antiulcer agents.[4] The *in vitro* antiviral activity of SDA, SDB, SDC, and other diterpenoids against Herpes simplex virus type I (HSV-1) has also been studied. SDB delays the appearance of herpetic lesions and prolongs the survival time of hamsters with HSV-1 corneal infection.[7] In addition, SDB has showed a potent cytotoxic activity against several tumor cell lines and against Ehrlich ascites cells inoculated in mice.[8]

The scopadulane diterpenes have similar structure to that of aphidicolin, a tetracyclic diterpenoid produced by the mould *Cephalosporium aphidicola*, which is a potent inhibitor of eukaryotic DNA polymerase α[9] and DNA polymerases induced by some viruses including HSV.[10] The mechanism through which the scopadulane diterpenes induce their cytotoxic and antiviral activities is unknown, but, these activities may be also related to the inhibition of DNA polymerase(s). However, Hayashi and co-workers have shown that the antiviral effect of SDC could be attributed in part to the inhibition of viral protein synthesis in late steps of viral replication,[11] while SDB interferes at early events of virus growth.[7]

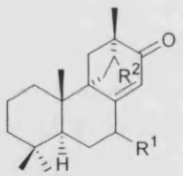
**Scheme 1**



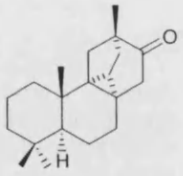
**1** $R^1$= $CH_2OH$, $R^2$= $CO_2H$
**2** $R^1$= $CO_2H$, $R^2$= $CH_3$
**3** $R^1$= $CH_2OH$, $R^2$= $CH_3$



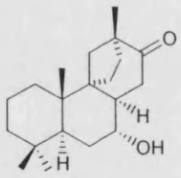
**4** $R^1$= H, $R^2$= α-$OCOCH_2CO_2H$
**4a** $R^1$= H, $R^2$= α-$OCOCH_2CO_2Me$
**5** $R^1$= OH, $R^2$= α-$OCOCH_2CO_2H$
**5a** $R^1$= OAc, $R^2$= α-$OCOCH_2CO_2Me$
**6** $R^1$= OH, $R^2$= α-OH
**6a** $R^1$= OAc, $R^2$= α-OAc
**7** $R^1$= H, $R^2$= =O
**8** $R^1$= H, $R^2$= α-OH
**9** $R^1$= OH, $R^2$= =O
**10** $R^1$= OAc, $R^2$= β-OH



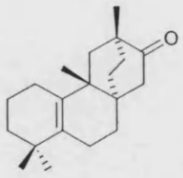
**11** $R^1$= H, $R^2$= H
**12** $R^1$= H, $R^2$= =O
**13** $R^1$= α-OH, $R^2$= H



**14**



**15**



**16**

Up to date, the investigation on the structure-activity relationship of scopadulane diterpenes has mainly focused on inhibition of gastric $H^+$, $K^+$-adenosine triphosphatase (ATPase), which is the enzyme responsible for acid secretion in gastric mucous. Additionally, the studies performed on antiviral activity of SDA, SDB and SDC, have concluded that the carboxyl group at C-18 in ring A, together with the benzoyl group present at C-6 are necessary to maintain a relevant antiviral activity.[8]

In this paper we describe the *in vitro* antiviral and antitumor activity of various synthetic scopadulane-type diterpenoids and other related compounds (Scheme 1). We establish new structure-activity relationships for these molecules, mainly in the C- and D-rings, and contribute to the characterization of their biological activity.

**Results and Discussion**

In this study we have evaluated the *in vitro* antiviral and cytotoxic activity of fourteen synthetic compounds derived principally from the natural diterpenoids TA (**4**), TB (**5**) and TC (**6**) and their precursors (Scheme 1). All the compounds were diastereoselectively synthesized from the chiral starting material (+)-*podocarp-8(14)-en-13-one*,[12] which are as follows: methyl thyrsiflorin A (**4a**, MTA), methyl thyrsiflorin B acetate (**5a**, MTBA), thyrsiflorin C (**6**, TC), thyrsiflorin C diacetate (**6a**), 13-scopadulanone (**7**), 13α-scopadulanol (**8**), 7β-hydroxy-13-scopadulanone (**9**), 7β-acetoxy-13β-scopadulanol (**10**), 8(14)-scopadulen-13-one (**11**), 8(14)-scopadulen-13,15-dione (**12**), 7α-hydroxy-8(14)-scopadulen-13-one (**13**), cyclopropane intermediate (**14**), 7α-hydroxy-8α-scopadulan-13-one (**15**), and the rearranged scopadulane-type diterpene ketone (**16**).

**Table 1.** Cytotoxicity and Anti-HSV-2 Activity of Scopadulane Diterpenes on Vero Cells[a] determined by End-point titration technique (EPTT)

| Compound | $CC_{100}$ (μg/ml)[b] | Viral Reduction Factor[c] | Antiviral Activity (μg/ml)[d] |
|---|---|---|---|
| 4a | 30 | $10^{0.5}$ | 15 |
| 5a | 30 | $10^{0.5}$ | 15 |
| 6a | 50 | $10^{0.5}$ | 10 |
| 6 | 30 | N.A. | N.A. |
| 7 | 28 | $10^{2}$ | 14 |
| 8 | 32 | $10^{2}$ | 7.5 |
| 9 | 48 | $10^{0.5}$ | 10 |
| 10 | 50 | 10 | 25 |
| 11 | 40 | $10^{2}$ | 20 |
| 12 | 60 | 10 | 30 |
| 13 | 28 | 10 | 14 |
| 14 | 40 | 10 | 20 |
| 15 | 60 | $10^{2}$ | 30 |
| 16 | 35 | N.A. | N.A. |

[a] VERO, Cercopithecus aethiops african green monkey kidney ATCC No. CCL 81. [b] The minimal toxic dose that detached the100% the cell monolayer. [c] Ratio of the virus titer in the absence over virus titer in the presence of the tested compound. [d] The maximal nontoxic dose that showed the highest viral reduction factor.. N.A., no activity.

Initially, the cytotoxic activity and antiviral effect of all compounds were evaluated in a primary screening using the *End-point titration technique* (EPTT).[13] As shown in Table 1, compounds **7**, **8**, **11** and **15** exhibited the highest antiviral activity (reduction factor of the viral titer of $10^2$) indicating a moderate activity against HSV-2. In most of these compounds, the non-toxic concentration needed to obtain the largest reduction of the viral titer was approximately the same as the cytotoxic concentration needed to detach the 100% of the cell monolayer ($CC_{100}$), revealing that their antiviral activity is principally due to their cytotoxicity. To clarify this aspect, it was necessary to calculate a selective index or therapeutic index for each compound with antiviral activity. The *in vitro* therapeutic index for each compound was calculated using the 50% cytotoxic concentration for cell growth ($CC_{50}$) and the 50% inhibitory concentration of the viral effect ($IC_{50}$), which was obtained by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method[14] (Table 2).

**Table 2.** Anti-HSV-2 activity of Scopadulane Diterpenes on Vero Cells[a] determined by MTT method

| Compound | $CC_{50}$ (μg/ml)[b] | $IC_{50}$ (μg/ml)[c] | Therapeutic index[d] |
|---|---|---|---|
| 7 | 16.7± 1.9 | 21.03± 0.9 | 0.81± 0.06 |
| 8 | 19.5± 1.2 | 16.5± 0.13 | 1.25± 0.29 |
| 11 | 23.2± 1.2 | 32.4± 4.1 | 0.72± 0.09 |
| 15 | 27.8± 5.9 | 42.1± 7.3 | 0.66± 0.19 |
| Aciclovir | $12.2x10^4$± 1.9 | 4.21± 0.9 | |

[a] VERO, Cercopithecus aethiops african green monkey kidney ATCC No. CCL 81. [b] 50% cytotoxic concentration.[c] 50% inhibitory concentration of the viral effect. [d] The therapeutic is defined as $CC_{50}$ over $IC_{50}$.

These therapeutic index values permitted us to establish accurately some structure-activity relationship as well as to identify important structural features responsible for the biological activity in the molecules. As it is shown in Table 2, compound **8** presented the highest therapeutic index (1.25). A polar substituent at C-13, as a hydroxyl group, is essential to preserve this activity since the presence of a carbonyl group at C-13 (compound **7**) showed a lower antiviral activity (therapeutic index 0.81) and there was complete loss of activity when the hydroxyl group was esterified (compound **4a**).

Furthermore, the substitution at C-7 with a polar group led to either partial loss of activity (compound **10**) or even more to complete loss of activity as it has been observed with the natural product TC (**6**) (Table 1). The combination of the decreasing effects, as is the substitution at C-7 with a polar group and the presence of a carbonyl group at C-13, showed a complete reduction of the activity in compounds **5a**, **6a**, and **9**. The antiviral activity of compound **11** was very similar to that of compound **7**, what reveals that the double bond has little influence on activity. Interestingly, compound **15** showed anti-HSV-2 activity of the same order than compounds **7**, **8**, and **11** in spite of having the stereochemistry at C-8 inverted (8α-scopadulane structure) and a polar group at C-7.

Finally, it is interesting to note that modification in the D-ring, as in compounds **12**, **14** and **16**, also reduces the activity suggesting that the D-ring spatial conformation is important.

Hayashi et al., reported the inhibitory activities of several derivatives of scopadulciol **3** against hog gastric $H^+$, $K^+$-ATPase, and found that the potency of inhibition was higher for those compunds that contain an acetyl or oxime group at C-13. The importance of the substitution at C-13 to enhance biological activity is also confirmed by our results, however, in order to obtain better antiviral activities the functional should be a hydroxyl group instead of an acetyl group. To the best of our knowledge, this is the first study in which the structure-activity relationship in the C-ring of scopadulane diterpenes for their antiviral activity is evaluated. Based on our results and the results of others it can be predicted that the presence of a polar group at C-13 and no polar group at C-7, together with a carboxyl group at C-18 and a benzoyl group at C-6, as found in other studies, may enhance the anti-HSV activity of these molecules.

For the most toxic compounds evaluated in the preliminary screening using the EPTT technique (Table 1), the $CC_{100}$ values were obtained in the human tumor cell lines HeLa and HEp-2, in the CHO cell line and the Fib-Bon primary cell culture. As shown in Table 3, compound **13** was the most toxic, displaying on CHO cells and Fib-Bon culture, $CC_{100}$ values of 20 μg/ml and 10 μg/ml, respectively. In general , all the compounds examined in this study did not show significant cytotoxic activity for the cell lines tested (the compounds with $CC_{50}$> 4 μg/ml are judged as inactive by the criteria established by the U.S. National Cancer Institute).[15] This weak *in vitro* antitumor activity was not important to be evaluated by the MTT quantitative technique,

**Table 3.** Cytotoxicity data for **4a-6a**, **6**, **7**, and **13**.[a]

| Scopadulane Diterpene | Cell lines[b] HeLa | HEp-2 | CHO | Bon-Fib |
|---|---|---|---|---|
| 4a | 28 | 28 | 28 | 28 |
| 5a | 30 | 30 | 30 | 60 |
| 6a | 40 | 40 | 40 | 60 |
| 6 | 45 | 45 | 45 | 60 |
| 7 | 28 | 24 | 24 | 24 |
| 13 | 20 | 20 | 20 | 10 |

[a] The minimal toxic dose that detached the100% the cell monolayer ($CC_{100}$ values (μg/ml) in 48 h).[b] HeLa, human cervix epitheloid carcinoma ATCC No. CCL 2; HEp-2, human larynx epidermoid carcinoma ATCC No. CCL 23; CHO, Cricetulus griseus ovary chinese hamster cells ATCC No. CCL 61; Fib-Bon, Bovine ear skin primary culture.

although, in the light of our results (Table 3) it may be concluded that in contrast with the antiviral activity, for the cytotoxic activity seems to be important a polar substituent at C-7, a α–hydroxyl group, as in compound **13**. About the cytotoxic behavior of this compound against either tumor cell lines or non-tumor cell lines, based on the results reported by Hayashi et al and our results, it can be speculated that the substituents at C-18 and the benzoyl group at C-6 provide to this molecule with certain cytotoxic selectivity against tumor cell lines.[8] Although these observations will need further confirmation for our group.

## Experimental Section

*Compounds*

The diterpenoids tested were obtained following the procedure described for us.[12] Stock solutions (7 mg/ml) of these compounds for testing in vitro were prepared in dimethylsulfoxide and stored at 4 °C

*Cell Culture and Virus* - The lines cells used were: Cricetulus griseus ovary chinese hamster cells (CHO cell line ATCC CCL-61), human cervix epitheloid carcinoma cells (HeLa cell line ATCC CCL-2), human larynx epidermoid carcinoma cells (HEp-2 cell line ATCC CCL23), Cercopithecus aethiops African green monkey kidney (VERO cell line ATCC CCL-81). Fib-Bon primary culture cells were obtained in our laboratory of ear skin biopsies from pure BON cattle. Briefly, the protocol used to obtain primary cell cultures was as follows: the biopsy was washed three times with Phosphate Buffered Saline (PBS) containing 2% penicillin-streptomycin-amphotericine B, the skin was discarded, the cartilage and the subcutaneous tissue was minced finely, the pieces of tissues were placed

in 25 $cm^2$ cell culture flasks with just enough growth medium (Eagle minimum essential medium MEM with L-glutamine 2nM, 1% vitamins, 1% non essential amino acids, 1% penicillin-streptomycin-amphotericine B and 10% of fetal bovine serum (FBS) to cover the pieces of tissue. When the fibroblasts proliferated to 30 or 40% confluence, the pieces of tissues were discarded by gently shaking with PBS and again the cells were fed with 50% of used medium and 50% of fresh medium. When 80% confluence was reached, the cells were trypsinized and cultured in 150 $cm^2$ flasks. Once the cells covered about 80% of the surface, they were trypsinized, centrifuged and cryopreserved.

All cells were grown as a monolayer culture in MEM supplemented with 10% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, 20 mg/ml glutamine, 0.14% $NaHCO_3$, and MEM non-essential aminoacid and vitamins solution. The culture were maintained at 37 °C in a humidified 5% $CO_2$ atmosphere.

HSV-2 was obtained from Center for Diseases Control, Atlanta, Georgia. USA. The virus stock was prepared from HSV-2-infected HEp-2 cell cultures. The infected cultures were subjected to three cycles of freezing-thawing, and centrifuged at 2000 rpm for 10 min. The supernatant was collected, titrated and stored at -170 ° C in 1ml aliquots.

*Antiviral Assays*

*End-point titration technique* (EPTT)- The technique described by Vander Berghe[13] with few modifications was used. Briefly, confluent monolayer of VERO cells were grown in 96-well flat-bottomed plates. Two-fold dilutions of the compounds in maintenance medium, supplemented with 2% serum and antibiotics, were added 1 h before the viral infection. Cells were infected with 0.1 ml of serial ten-fold dilutions of the appropriate virus suspension and incubated again at 37 °C in a humidified 5% $CO_2$ atmosphere for a period of 48 h. Controls consisted of infected cells with HSV-2 serial ten-fold dilutions in the absence of the compounds, treated noninfected and untreated noninfected cells. The antiviral activity was expressed as the maximal non toxic dose (MNTD) of the test compound needed to obtain the virus titer reduction. The virus titer reduction was expressed as the reduction factor Rf (virus titer in the absence of the extract/ virus titer in the presence of it). Rf was obtained by duplicates of at least five dilutions for each of the compounds. The results are expressed as the mean obtained from three different assays.

*Antiviral colorimetric assay* - VERO cell monolayers were grown in 96 well microtiter plates fashion. Dilutions of the compounds, prepared as described above in the EPTT assay, were added 1 h before the viral infection. Ten infectious doses of virus were added to each well and incubated at 37 °C in a humidified 5% $CO_2$ atmosphere for a period of 48 h. Controls consisted of: untreated infected, treated noninfected and untreated noninfected cells. Furthermore all tests were compared with a positive control, Acyclovir, and tested simultaneously under identical conditions as reported previously.[16] The cellular viability was evaluated by the MTT (3-(4,5-dimethylthiazol–2-yl)-2,5-diphenyltetrazolium bromide) colorimetric technique.[14] Briefly, the supernatants were removed from the wells and 28 µl of a MTT (Sigma) solution (2 mg/ml in PBS) was added to each well. Plates were incubated for 1.5 h at 37 °C, and 130 µl of DMSO was added to the wells to dissolve the MTT crystals. The plates were placed on a shaker for 15 min and absorbency was read at 492 nm on a multiwell spectrophotometer (Titertek Uniskan).

*Cytotoxicity Assay* - Cell monolayers were trypsinized and washed with culture medium and then plated at $5x10^3$ cells per well for HeLa, HEp-2 and CHO cells and at $2x10^4$ cells per well for Fib-Bon cells in a 96-well flat-bottomed plate. After 24h of incubation, each diluted compound was added to the appropriate wells and the plates were incubated for further 48 h at 37 °C in a humidified incubator with 5% $CO_2$. The cytotoxic activity was expressed as the minimal toxic dose of the compound that induce 100% detachment of the cell monolayer ($CC_{100}$). The results were obtained by duplicates of at least five dilutions for each of the compounds. The results are expressed as the mean obtained from three different assays.

*Data Analysis* - The 50% cytotoxic concentration ($CC_{50}$) and the 50% inhibitor concentration of the viral effect ($IC_{50}$) for each compound were obtained from dose-effect-curves (not shown). The $CC_{50}$ and $IC_{50}$ are the average of four assays with five concentrations within the inhibitory range of the compounds. The therapeutic index or selective index was defined as $CC_{50}$ /$IC_{50}$.

**Acknowledgement.**

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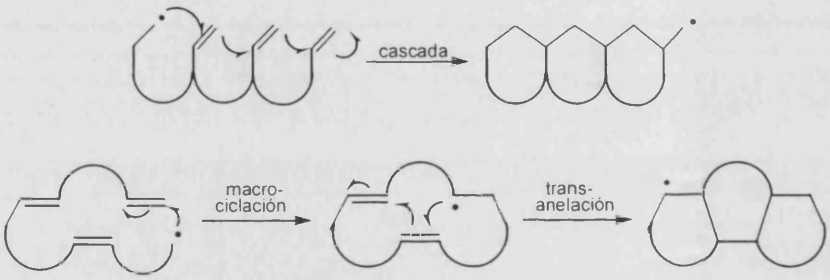
## *4. ESTRANOS*

## 4.1.- Síntesis de esteroides con esqueleto de estrano.

En la última parte de esta Tesis se estudia la síntesis total de esteroides con esqueleto de estrano mediante reacciones de ciclación radicalaria regio- y estereoselectivas. El esqueleto se forma a partir de un intermedio clave, un polieno precursor del radical libre que sufrirá el proceso de ciclación de dos formas: en cascada formando anillos pequeños[1] o una macrociclación seguida de sucesivas transanelaciones[2] hasta que el radical sea atrapado o cese la cascada porque ya no hay más dobles enlaces que atacar (Esquema **1**).

Esquema **1**




En estudios sintéticos previos, Pattenden y colaboradores han demostrado la utilidad de radicales acilo/alquilo poliénicos para la construcción de policiclos fusionados de seis miembros mediante ciclaciones 6-*endo-trig* de forma regio y estereoselectiva.[3]

Mi interés en este tipo de metodología residía en conocer estas técnicas de ciclación biomimética de polienos para obtener terpenos policíclicos, por ello solicité trabajar con el Profesor Pattenden y así profundizar en el conocimiento de la técnica.

La aportación a la investigación que he realizado en el grupo del Profesor Pattenden sobre la síntesis de esteroides, ha consistido en obtener sistemas

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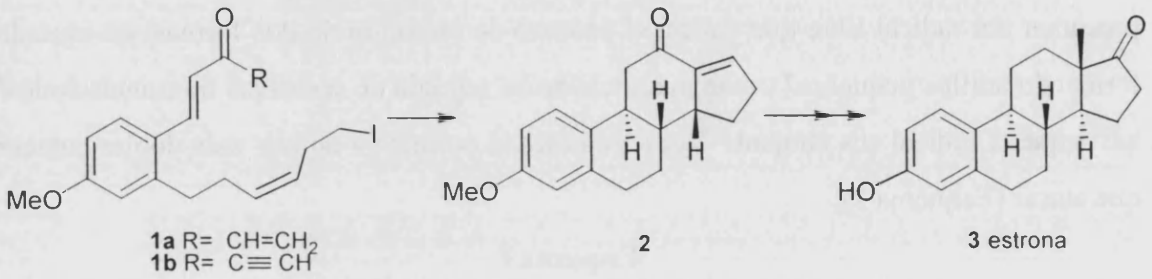
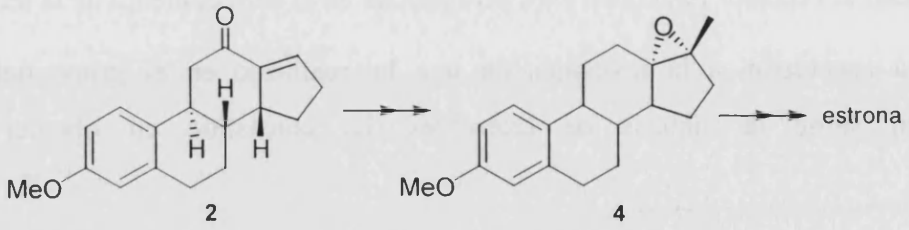
hidrocarbonados susceptibles de ser transformados en *estrona* **3** a partir del correspondiente precursor radicalario, como por ejemplo los sistemas poliénicos **1** (Esquema **2**), así como un estudio preliminar con un modelo bicíclico para su conversión en esta hormona.

Esquema **2**




En este trabajo, se ha comprobado que la presencia de un doble o un triple enlace en los yoduros **1a** ó **1b** conduce a un proceso de macrociclación-transanelación radicalaria con estereoquímica diferente. Cuando el precursor contiene un triple enlace terminal **1b** la cascada radicalaria da el compuesto de fusión trans-sin **2**, con un grupo carbonilo α,β-insaturado que facilita su conversión en esqueleto de estrano. Por ejemplo, basándonos en precedentes de la literatura si podemos introducir un α-epóxido en el doble enlace entre C-13 y C-17 y obtener el epóxido **4** a partir de **2**, se completaría formalmente la síntesis de la estrona ya que es conocida la trasposición de este epóxido con trifluoruro de boro para obtener estrona (Esquema **3**).[4]

Esquema **3**




Los resultados de esta investigación se describen en los dos apartados siguientes.

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[4] a) Bartlett, P. A.; Johnson, W. S. *J. Am. Chem. Soc.* **1973**, *95*, 7501; b) Groen, M. B.; Hindriksen, B.; Zeelen, F. J. *J. R. Neth. Chem. Soc,* **1982**, *101*, 148.

**AUTORES:** Miguel A. González, Stuart McCulloch, Gerald Pattenden.

**TÍTULO:** A Cascade Radical-mediated Macrocyclisation-transannulation Approach to Oestrogen Steroids

**REF. REVISTA:** Comptes rendus de l'Académie des science, Série IIc, submitted.

# *A Cascade Radical-mediated Macrocyclisation-transannulation Approach to Oestrogen Steroids*

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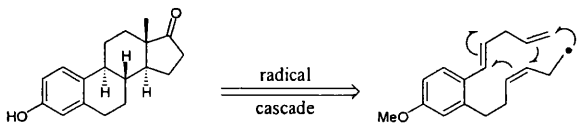
Abstract
A new approach to ring A aromatic steroids, based on cascade 13-*endo-trig/dig* macrocyclisations followed by sequential 5-*exo-trig* and 6-*exo-trig* transannulations, exemplified in the syntheses of the *cis,anti,trans* tetracycle **9** and the *trans,syn* tetracycle **13** from the *ortho*-substituted aryl polyen(yne) precursors, **8** and **12** respectively, is described.

Keywords
Radical, cascade, macrocyclisation-transannulation, steroids.

Steroids are ubiquitous in nature, and an enormous variety of creative designs have been developed for their total synthesis.[1,2] In recent years we have illustrated the scope for cascades of 6-*endo-trig* radical cyclisation reactions from polyene acyl radical precursors in the synthesis of polycyclic systems including steroids,[3,4,5] aza-steroids,[6] spongianans,[7] and even steroid-like heptacycles.[8] We now describe a new approach to ring A aromatic steroids, *eg* oestrone **1**, whereby the B/C/D ring tricyclic system is elaborated by a radical-mediated macrocyclisation in tandem with two radical transannulation reactions[9] from an appropriate *ortho*-substituted aryl polyene radical precursor, *ie* **2**.[10]

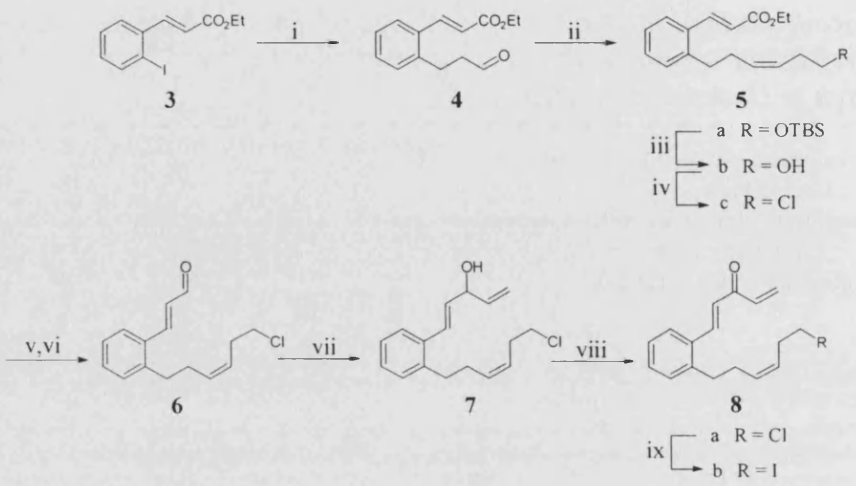



**1** **2**

In order to demonstrate the feasibility of this 13-*endo-trig*, 5-*exo-trig*, 6-*exo-trig* radical cascade, we first examined the iodotrienone system **8** with the expectation that the double enone electrophores in **8** would 'drive' the cascade of radical cyclisations in the anticipated sense. The iodotrienone **8** was synthesised from ethyl 2-iodocinnamate as shown in Scheme 1. Thus, a Heck reaction between the aryl iodide **3** and 2-propenol first led to the aldehyde **4** which then underwent a Wittig reaction to produce the *Z*-alkene **5a**. Deprotection of the TBS ether **5a**, followed by conversion of the resulting alcohol **5b** into the corresponding chloride **5c**, and a reduction-oxidation sequence next led to the cinnamaldehyde **6**. Addition of vinylmagnesium bromide to **6**, followed by oxidation of the resulting carbinol **7** to the dienone **8a** and a Finkelstein reaction finally gave the iodotrienone precursor **8b**.

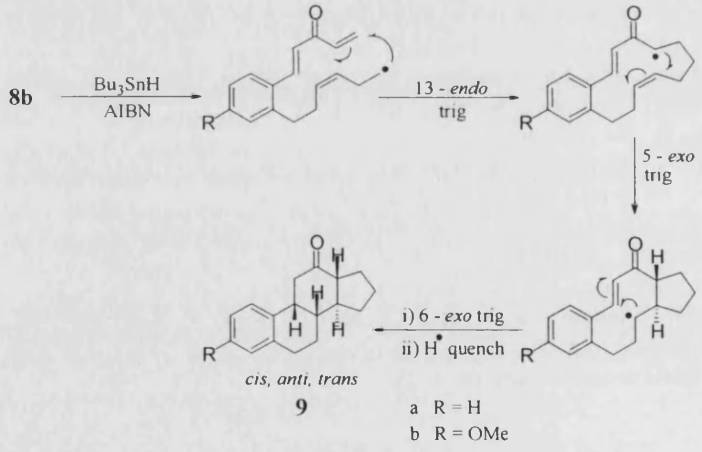
When a solution of the iodotrienone **8b** was treated with $Bu_3SnH$ and catalytic AIBN in degassed benzene over 6 hr under high dilution conditions, followed by heating at reflux for 10 hr, work-up and chromatography resulted in the separation of almost equal amounts of two crystalline isomeric steroidal products in a combined yield of 51%. NMR spectroscopic data for each of the products were consistent with the formation of tetracyclic ring systems resulting from the anticipated macrocyclisation-transannulation cascade, (*viz* Scheme **2**), and the structure **9a** with *cis-anti-trans*-stereochemistry was established for one of the isomers by X-ray crystallographic analysis.[11] When the synthetic sequence shown in Scheme **1** was repeated, starting from ethyl 2-iodo-4-methoxy cinnamate, the corresponding 4-methoxyaryl iodotrienone was elaborated. When this derivative was treated with $Bu_3SnH$-AIBN, work up and chromatography produced a similar yield of two diastereoisomeric tetracycles. Close inspection and comparison of the NMR spectroscopic data for the major product resulting from this reaction, with the ketone **9a**, showed that it had the same *cis, anti, trans* stereochemistry, *ie* **9b**. However, at this stage we remained uncertain of the relative stereochemistry of the diastereoisomeric tetracycles formed in these two cascade radical-mediated cyclisations.

With the ultimate objective of developing a synthesis of oestrone **1**, we next examined a synthesis of the iododienynone **12** corresponding to **8b**, and its cascade radical-mediated triple cyclisation.[12] The dienynone **12** was synthesised in a similar manner to that used to elaborate **8b** with the modification that ethynylmagnesium bromide was added to the aldehyde **6** instead of vinylmagnesium bromide.

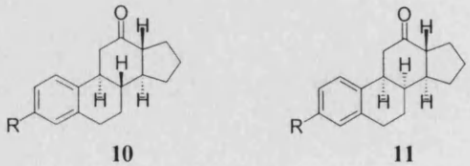



**Scheme 1**

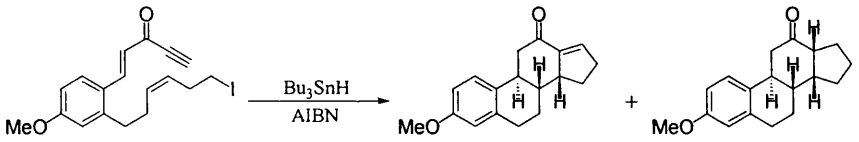
Reagents and conditions: i, $CH_2$=CH-$CH_2OH$, $nBu_4NCl$, $NaHCO_3$, $Pd(OAc)_2$, DMF, 50 °C, 82%; ii, KHMDS, $Br^-Ph_3P^+(CH_2)_3OTBS$, THF, -78 °C, 85%; iii, TBAF, THF, 0 °C, 82%; iv, NCS, $PPh_3$, DMF, 0 °C, 84%; v, DIBALH, DCM, -78 °C, 81%; vi, $MnO_2$, DCM, rt, 82%; vii, $CH_2$=$CHMgBr$, THF, -78 °C, 88%; viii, $BaMnO_4$, DCM, 25 °C, 92%; ix, NaI, $K_2CO_3$, butan-2-one, 92%.




**Scheme 2**



**10** **11**




**12** **13** **14**

When a solution of the iododienynone **12** was treated with $Bu_3SnH$-AIBN under the same conditions to those used with the analogue **8b**, work up and chromatography gave two crystalline tetracylic products in 40% and 20% yield. The structure of the major product, m.p. 95-97°C, was established unambiguously by single crystal X-ray analysis as the *trans, syn* diastereoisomer of the anticipated cyclopentaphenathrenone **13**.[11] The *trans-syn-cis* steroidal structure **14** followed for the minor product of the 13-*endo-dig*, 5-*exo-trig* cyclisation of **12** from 1D NMR, COSY, HMQC and NOE difference experiments, and presumably results from *in situ* reduction of the major product **13** under the $Bu_3SnH$-AIBN radical conditions.

Even with detailed NMR data available for the isomeric tetracycles **9, 10, 11,** and **14**, alongside X-ray data for **9a** and **13** and molecular mechanics calculations, we are unable to distinguish between the two most likely stereochemistries, *ie trans, anti, trans* and *cis,syn,trans*, **10** and **11** respectively, for the other tetracyclic products produced from cascade radical cyclisation of the polyenes **8a** and the 4-OMe derivative. Further studies are now in progress to address this issue and also to develop this method of polycycle constructions in other fused and steroid systems.

**Acknowledgements**

We thank the EPSRC for a studentship (to SM) and Zeneca Specialties for support *via* a CASE award. We also thank Dr Alan Chorlton for his interest in this study and Dr A J Blake for the X-ray crystal structure determinations.

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11. We thank Dr A J Blake of this School for this information, which will be published in the full paper.

*cf:* Hitchcock, S A, Houldsworth, S J, Pattenden, G, Pryde, D C, Thompson, N M and Blake, A J, *J. Chem. Soc., Perkin Trans. 1,* (1998) 3181.

**AUTORES:** Gerald Pattenden, Miguel A. González, Stuart McCulloch
Alexander J. Blake

**TÍTULO:** Cascade radical macrocyclisation-transannulation approach towards steroid hormones

**REF. REVISTA:** Manuscript in preparation.

## Cascade Radical Macrocyclisation-transannulation Approach Towards Steroids Hormones

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### Introduction

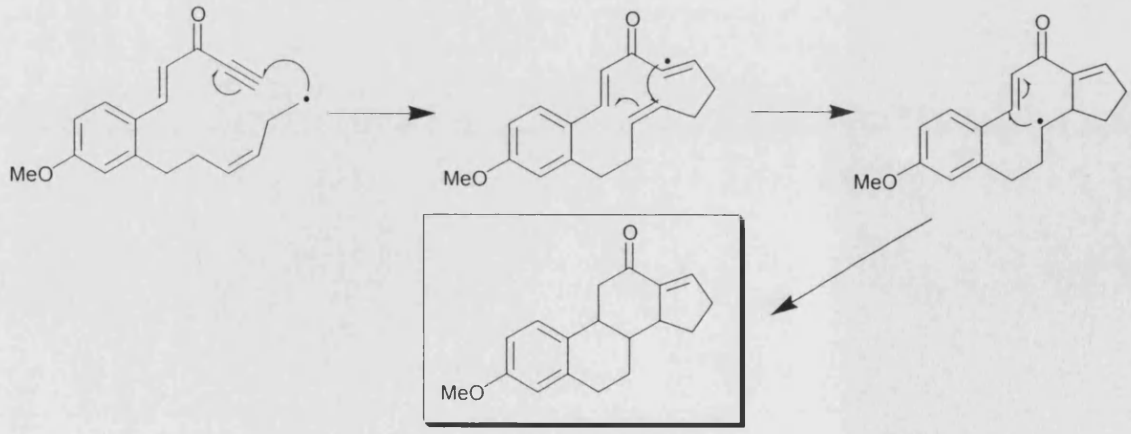
Steroid hormones have well-established roles in hormone-dependent diseases such as breast, prostatic and endometrial cancers.[1,2] Steroids containing the estrane skeleton are very important biologically, since they control part of the mechanisms involved in the human reproduction. Since the first total synthesis of equilenin and estrone in 1939,[3] many different approaches and synthesis of steroid hormones have been reported.[4] The two most recent synthesis of estrone **1** come from Diels-Alder cycloaddition reactions as the key step for the construction of the estrane skeleton.[5,6] The use of biomimetic polyene cyclisations is another useful methodology towards steroid systems. In these cyclisations, normally, a Lewis acid-mediated reaction is involved.[7] But it is also possible an approach based on free radical chemistry, in which a cascade radical-mediated macrocyclisation-transannulation reaction leads to the carbon framework characteristic of steroid hormones (Scheme **1**).[8]

In this work, it was envisaged that the use of an alkyne group in **4** as an electrophore[9] would lead to the 12-ketosteroid system **3**, as shown in the retrosynthetic Scheme **2**. This cyclised system could be later transformed into the intermediate **2**, which has already been converted into the estrone system as reported in the literature.[7,10] Thus, the introduction of an α-epoxide in the 13,17-double bond of the intermediate **2**, would lead via epoxide rearrangement with boron trifluoride etherate to the desired estrane skeleton. The preparation of the olefin **2** would imply an 1,4-additon of lithium dimethylcuprate followed by regeneration of the double bond and reduction of the carbonyl group at C13. The presence of the α,β-unsaturated ketone in **3** extends the number of possibilities to effect its conversion into the estrone system. For example, it was thought on the alkoxylation of the enone to obtain the corresponding 17-oxygenated position, which could be further oxidised to afford the carbonyl group. Completion of the estrone system could be achieved then, by methylation of the angular position and reduction of the carbonyl group. Similarly, the 1,4-addition of a hydroxy-masked group such as dimethyl(phenyl)silyl group and *in situ* methylation, would lead to an intermediate, which by reduction of C13 and conversion of the silicon group into its corresponding hydroxy group would give after oxidation the desired product (Scheme **3**).

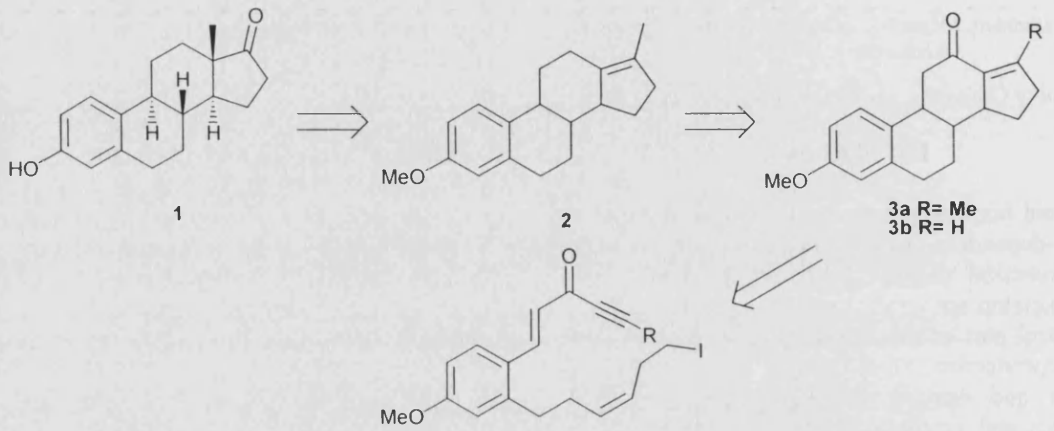
### Results and Discussion

Synthesis of the radical precursor **4a**.

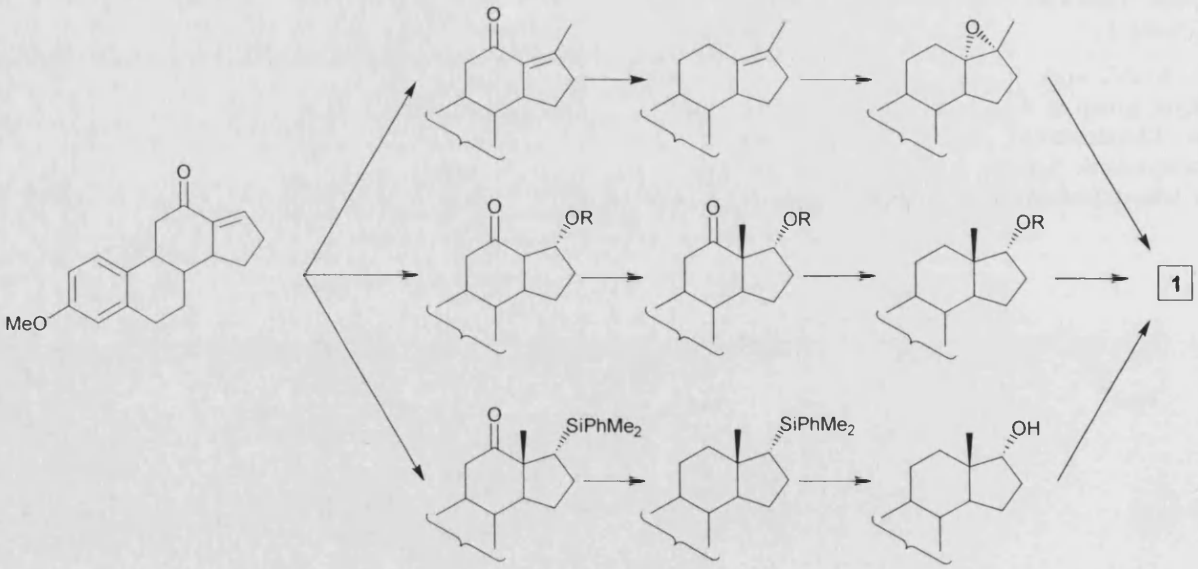
The iodide **5** was used as starting material in the sequence towards **10**, which is the key intermediate for the synthesis of **4a** and **4b**, is described as outlined in Scheme **4**. It is known that the Heck-type reaction of aromatic halides leads to the formation of carbonyl compounds when allylic alcohols are used as the olefinic compounds.



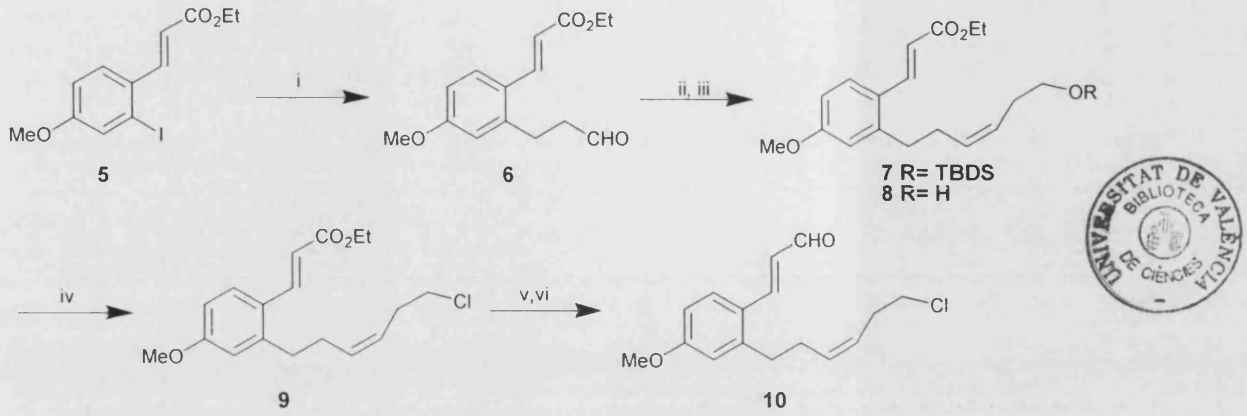

Scheme **1**




Scheme **2**




Scheme **3**



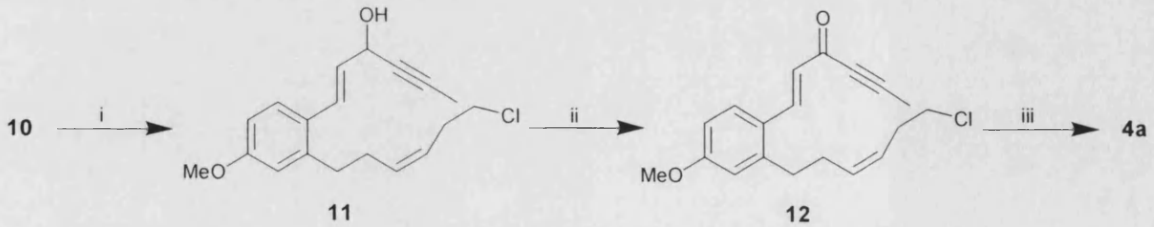



Scheme **4** *Reagents and conditions*: i, $H_2CCCH_2OH$, $Pd(OAc)_2$, $NaHCO_3$, $Bu_4NCl$, DMF, 30°C, 80-85%; ii, $BrPh_3P(CH_2)_3OTBDS$, KHMDS, THF, -78°C, 92%; iii, TBAF, THF, 0°C, 99%; iv, NCS, $Ph_3P$, $K_2CO_3$, $CH_2Cl_2$, 0°C, 95%; v, DIBAL, THF, -78°C, 100%; vi, $MnO_2$, $CH_2Cl_2$, 0°C, 97%.

Thus, the treatment of the halide **5** with allylic alcohol at 30°C in the presence of palladium acetate, sodium hydrogen carbonate as base, and tetrabutylammnonium chloride as a phase transfer agent in DMF led to the aldehyde **6** in 80-85% yield.[11] The wittig reaction between the corresponding phosphonium salt[12] and the aldehyde **6** at -78°C in THF, using sodium hexamehtyldisilylazide as base, produced compound **7** in 92% yield, as the sole isomer isolated. On irradiation at the frequence due to the methylene groups, which are coupling with the olefinic protons, resulted in the collapse of the multiplet of the olefininc protons to a doublet of $J$ = 10.8 Hz, characteristic of a *cis* double bond. The *t*-butyldimethylsilylether in **7** was hydrolysed under usual conditions with TBAF in THF at 0°C to give the corresponding alcohol **8** in 99% yield.

Treatment of this alcohol with *N*-chlorosuccinamide, triphenylphosphine and potassium carbonate in $CH_2Cl_2$ at 0°C, led to the chloride **9** in 95% yield. Reduction of compound **9** with a solution of DIBAL-H (1M; toluene) in THF at -78°C gave the corresponding cinnamyl alcohol in quantitative yield. Subsequent oxidation with activated manganese dioxide[13,14] in $CH_2Cl_2$ at 0°C afforded the intermediate aldehyde **10** in 97% yield.

With the compound **10** in hand, the construction of the top chain was undertaken using the Suffert's methodology[15] (Scheme **5**). Thus, the aldehyde **10** was treated at -78°C in dry THF with the acetylide generated from a mixture of (Z/E)-1-bromopropene and n-BuLi to afford the carbinol **11** in 85% yield. Subsequent oxidation was achieved in only 56% yield using Dess-Martin periodinane/pyridine in DCM at 0°C.[16] At this stage, several repetitions were tried to improve this yield but, it seems that during the working up the sensitive α,β-acetylenic ketone was affected decreasing the yield of the conversion. This oxidation resulted later to be improved by using manganese oxide in DCM when using the carbinol **15**.




Scheme **5** *Reagents and conditions*: i, (*E*/*Z*)-1-Bromopropene, n-BuLi, THF, -78°C, 85%; ii, Dess-Martin periodinane, py, $CH_2Cl_2$, 0°C, 56%; iii, NaI, 2-Butanone, reflux, 92%.

The ketone **12** was then subjected to chlorine-iodine exchange[17] to give in 92% yield the precursor **4a** containing a 10% of an impurity, which I was not able to remove by chromatography and the precursor was used for the radical reaction without further purification.

Synthesis of the radical precursor **4b.**

Initial studies were carried out for the preparation of cinnamoyl α,β-acetylenic ketones. Therefore, addition of ethynylmagnesium bromide[18] to commercially available cinnamaldehyde at 0°C in THF followed by oxidation of the resulting alcohol **13** using manganese oxide[13,14] produced the ketone **14** as desired in 77% overall yield for the two steps. This methodology was then developed in the synthesis of the precursor **4b** (Scheme **6**).

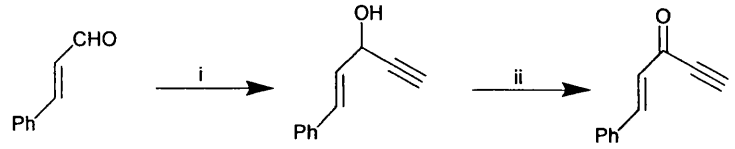
The radical precursor **4b** was readily synthesised using the above-mentioned procedure, from the aldehyde **10** in three steps as outlined in Scheme **7**.

Thus, addition of ethynylmagnesium bromide in dry THF at 0°C yielded the ethynyl carbinol **15** in 90% yield. For synthetic purposes, the reduction of **9**, followed by oxidation with $MnO_2$ and addition of the grignard reagent were carried out without the need of purification of any intermediate affording the carbinol **15** in 86% overall yield for the three steps. Oxidation of the carbinol **15** with manganese oxide[13,14] led to the ethynyl styryl ketone **16** in 72% yield (not optimised), which by Finkelstein halogen exchange[17] gave the required iodide **4b** in essentially quantitative yield.

Cascade radical macrocyclisation-transannulation reaction.

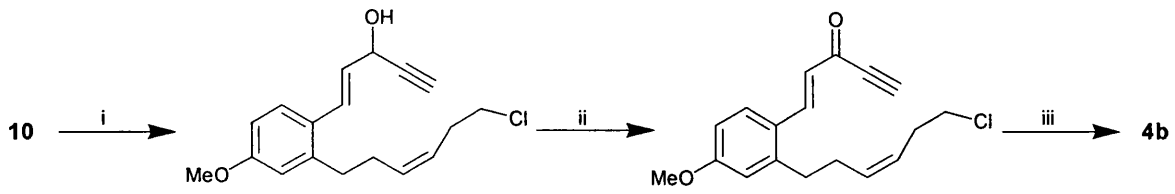
The iodide **4a** was treated with tributyltin hydride (1.1 eq) and AIBN (0.8 eq) in dry degassed benzene (3mM) under reflux, as reported in other radical cyclisations with α,β-ethynyl ketones[19] but changing the duration of the addition time. The study of the $^1H$ NMR spectrum of the crude residue revealed a complex mixture of products since it could be seen several different methoxy and olefinic signals. Purification of the residue led to several impure fractions in very low yield and all containing tin, from which, no pure compound was isolated and clearly identified.

Nevertheless, when the iodide **4b** was treated with tributyltin hydride (1.2 eq) and AIBN (0.8 eq) in dry degassed benzene (2mM) under reflux over 12 h, and extending the time of reaction after completing the addition to 6 h more, a 2:1 mixture of 12-ketosteroids **17** and **18** was obtained in approximmately 60% yield (Scheme **8**). The structure of the steroids **17-18** was deduced from a series of 1D NMR, COSY, HMQC and NOE difference experiments, and mass spectral data.



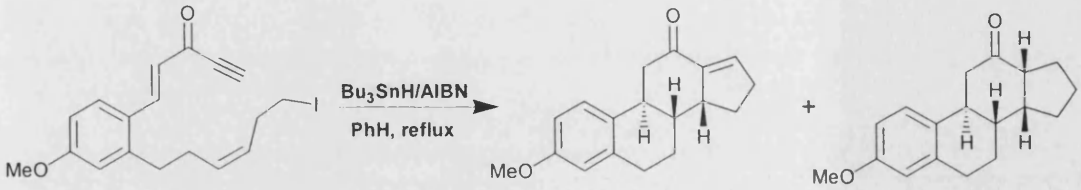

**cinnamaldehyde** **13** **14**

Scheme **6** *Reagents and conditions:* i, HCCMgBr, THF, 0°C to rt, 95%; ii, $MnO_2$, $CH_2Cl_2$, 0°C, 80%.




**15** **16**

Scheme **7** *Reagents and conditions:* i, HCCMgBr, THF, 0°C to rt, 90%; ii, $MnO_2$, $CH_2Cl_2$, 0°C, 72%; iii, NaI, 2-Butanone, reflux, quant.




Scheme 8

It is interesting to note that the coupling constants predicted by molecular mechanincs calculations (MACROMODEL 5.5) were in good agreement with the observed experimental values.

Compound **17** was obtained as a colourless oil with a molecular formula of $C_{18}H_{20}O_2$ as calculated from the EI mass spectrum (*m/z* 268 $[M]^+$). The $^1$H- and $^{13}$C-NMR spectra displayed signals assignable to a steroid with an $\alpha,\beta$-unsaturated ketone moiety ($\delta_H$ 6.67, dd, H-17; $\delta_C$ 200.6, s, C-12; $\delta_C$ 146.6, s, C-13, and $\delta_C$ 140.2, d, C-17). In the $^1$H-$^1$H-COSY experiment the proton H-14 at $\delta_H$ 3.29 showed vicinal couplings to both methylene protons H-15 (α: 1.79, dddd; β: 2.18, dddd), proton H-8 ($\delta_H$ 2.06, dddd) and a cross peak to H-17 ($\delta_H$ 6.67, dd). The stereochemistry of the protons H-8, H-9 and H-14 was determined by NOE experiments, which revealed correlations from H-8 ($\delta_H$ 2.06, dddd, $J$ = 12.0, 11.9, 9.1, 2.7 Hz) to H-14 ($\delta_H$ 3.29, m), H-11β ($\delta_H$ 2.23, dd, $J$ = 18.2, 12.7 Hz) and H-7β ($\delta_H$ 1.96, dddd, $J$ = 12.8, 5.0, 2.7, 2.4 Hz), and from H-11α ($\delta_H$ 3.07, dd, $J$ = 18.2, 3.4 Hz) to H-9 ($\delta_H$ 2.89, m) and H-1 ($\delta_H$ 7.14, d, $J$ = 8.6 Hz). These results established that protons H-8 and H-14 had to be *cis*, whereas H-9 had to be *trans* to proton H-8.

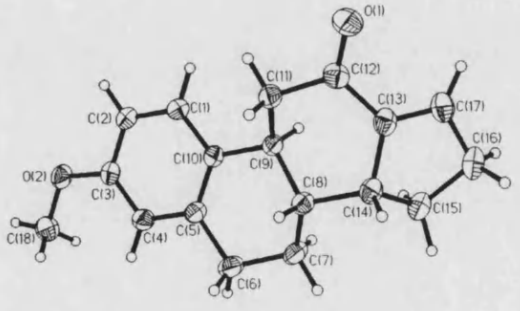
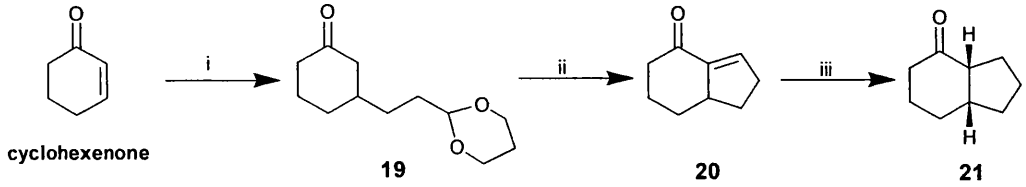



Figure 1. ORTEP drawing of the crystal structure of **17**.

Therefore, the structure **17** was assigned to this compound. Furthermore, the assignment of this structure as derivative of 14β-estrane skeleton (3-methoxy-6,7,8,9,11,14,15,16-octahydro-cyclopenta[*a*]phenanthren-12-one) was later confirmed unambiguously by single crystal X-ray analysis (Figure 1).

Compound **18**, colourless needles, gave the molecular formula $C_{18}H_{22}O_2$ (EIMS, *m/z* 270 $[M]^+$). Its $^1$H-NMR spectrum was quite similar to that of **17**, except that the resonance of the olefinic proton H-17 in **17** disappeared, the signal of proton H-14 was shifted highfield to $\delta_H$ 2.53 (m) and it could be seen a new signal due to the proton H-13 at $\delta_H$ 2.75 (br t). The HMQC experiment revealed the presence of this new methine proton ($\delta_H$ 2.75, br t, $J$ = 8.0 Hz, H-13; $\delta_C$ 53.3, d, C-13) and methylene protons H-17 ($\delta_C$ 24.5, t, C-17). Thus, the $^1$H- and $^{13}$C-NMR assignments by $^1$H-$^1$H-COSY, $^1$H-$^{13}$C-HMQC, DEPT experiments established the constitution of compound **18** as 3-methoxy-6,7,8,9,11,13,14,15,16,17-decahydro-cyclopenta[*a*]phenanthren-12-one.
Furthermore, the *β*–configuration of the proton H-13 has been confirmed by NOE difference experiments. Irradiation at the frequence of the signal due to proton H-8 ($\delta_H$ 2.15, dddd, $J$ = 16.4, 11.7, 4.7, 2.5 Hz) showed a strong enhancement of the signals due to protons H-13 ($\delta_H$ 2.75, br t, $J$ = 8.0 Hz), H-14 ($\delta_H$ 2.53, m) and H-11β ($\delta_H$ 2.28, dd, $J$ = 13.7, 9.8 Hz). It is thought that compound **18** could come from a double-bond reduction after the cascade reaction has finished. Although it was tried to improve the yield of the reaction reducing the amount of tributyltin hydride to 1.1 eq, and the time of reaction to 12 h addition plus one hour further, only an approximately 35% yield was isolated of **17** when the crude residue was not treated with KF aq to remove the tin residues, what is believed reduces the yield of the reaction.




Scheme **9** *Reagents and conditions:* i, 2-(2-bromoethyl)-1,3-dioxane, Mg, CuBr($Me_2S$), $Me_2S$, THF/Ether, -60°C to rt; ii, 2N HCl, THF, 80°C, 70% (overall for the two steps); iii, $Bu_3SnH$, AIBN, PhH, reflux, 10%.

Synthesis of Bicyclo[4,3,0]non-9-en-2-one **20.**

The compound **20** possessing an enone moiety was synthesised, following the procedure of Helquist[20] with some modifications, to carry out model studies for the conversion of **17** into 14-isoestrone[21] and to confirm the latter unexpected reduction during the radical reaction (Scheme **9**).

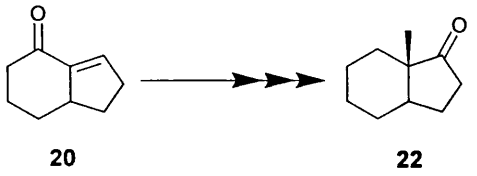
The acetal **19** was prepared by 1,4-addition of the corresponding dioxane cuprate, instead of using the dioxolane cuprate as described. This was added to cyclohexenone in a 2:1 mixture THF-diethyl ether (60 mL) at -60°C. The crude product was hydrolised with 2N HCl in THF at 80°C, *in situ* aldol reaction and elimination afforded the enone **20** in 70% overall yield for the two steps.

The enone **20** was treated with tributyltin hydride (1 eq) and AIBN (0.2 eq) in dry degassed benzene (110mM) under reflux for 7 h. Among the mixture of products obtained, it was isolated a 10% of ketone **21** resulting from the double-bond reduction, together with unreacted starting material. The $^1H$- and $^{13}C$-NMR data for **21** were in completely agreement with the reported ones.[22] This result confirmed us the possibility of reduction of **17** to give compound **18**, under the radical cyclisation conditions.

It is believed that the yield for obtaining **17** can be improved with a slower addition of the tin reagent and with the use of crystallised precursor, since it has been observed that impure precursor leads to more complex mixtures decreasing the yield of the desired steroid.

Studies towards the synthesis of *trans*-8-methyl-1-hydrindanone **22.**

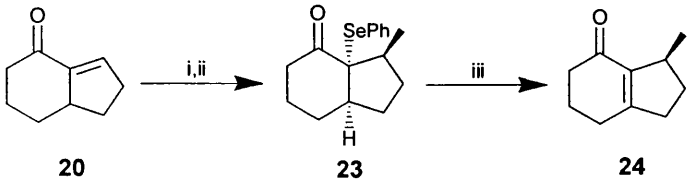
The first of the proposed routes towards **22** from **20** (Scheme **10**)., by analogy for the obtention of 14-iso-**1** from **17** (see Scheme **3**), resulted to be useless since the selenoxide elimination reaction[23] of **23** led to the formation of the enone **24** in essentially quantitative yield (Scheme **11**).




Scheme **10**

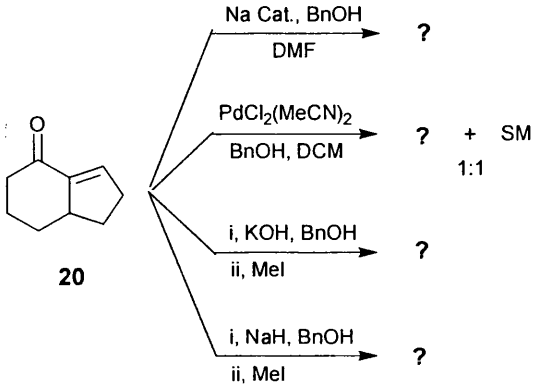
This fact implies that the selenenylketone **23** had the phenylselenenyl group *syn* to the proton H-5, and the addition of the dimethylcuprate occurred from the β–face. Ketone **23** was prepared by treating enone **20** with lithium dimethylcuprate[24] in diethyl ether at -60°C, followed by addition of phenylselenenyl chloride.

The second proposed route, which was based on the alkoxylation of an enone moiety (see Scheme **3**), was attempted with several conditions for the introduction of a benzyloxy group. This group could be later in the sequence directly converted into the carbonyl group by ceric ammonium nitrate-catalysed




Scheme **11** *Reagents and conditions:* i, $Me_2CuLi$/Ether, -78°C; ii, PhSeCl, THF, -78°C to rt, 55% (overall for the two steps); iii, $H_2O_2$, $CH_2Cl_2$, rt, 100%.

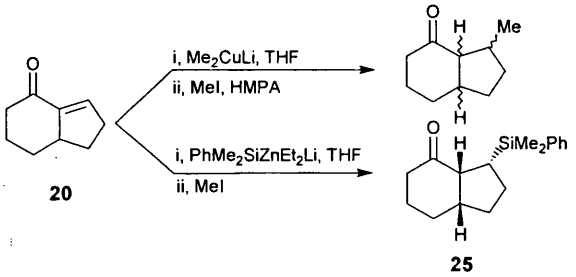
oxidation with sodium bromate.[25] Unfortunately, the treatment of enone **20** with Na as catalyst and benzyl alcohol in DMF,[26] and with $PdCl_2(MeCN)_2$ as catalyst and benzyl alcohol in DCM,[27] led to complex mixtures where the desired product could not be isolated (Scheme **12**).




Scheme **12**

Similar results were obtained on attempting *in situ* methylation with MeI of the possible benzyloxy ketone enolate, under basic conditions with either KOH or NaH in benzyl alcohol.[28]

The last of the proposed strategies towards **22** was based on the 1,4-addition of dimethyl(phenyl)silyllithium (DMPSLi)[29] to the enone **20** and trapping of the enolate intermediate with MeI (Scheme **13**).




Scheme **13**

Thus, treatment of **20** with DMPSLi-Copper (I) at -25°C, followed by addition of MeI/HMPA led to a mixture of products. Among those products, it seemed to be present some compounds resulting from silylation for both sides of the molecule but without any traces of further alkylation. The use of the corresponding silylzincate seems to improve the stereoselectivity, in this particular case, during the silyl-zincation[30] to give with good stereoselectivity one of the four possible diastereomers. But likewise in the silyl-cupration after treating the corresponding enolate intermediate with MeI no traces of alkylation products were detected.

## Conclusions

We have completed the synthesis of the radical precursors **4a** and **4b** in nine steps from iodobenzene **5** in 31% and 46% overall yield, respectively. Although the precursor **4a** led to unidentified products, the precursor **4b** led to the cyclised product **17**, *via* a series of macrocyclisation-transannulation processes, as predicted. The full characterisation of the structure of the product was achieved by a detailed analysis of the NMR data, and later confirmed by X-ray analysis. Thus, it has been successfully demonstrated again the utility of the radical chemistry in steroids synthesis.

Finally, initial studies were carried out, using a model system, for the conversion of the prepared 12-ketosteroid into 14-isoestrone, which is not trivial and needs a more concise study. Unfortunately, not very promising results were obtained.

## Experimental

### General details

All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were obtained using a Perkin-Elmer 1600 series FT-IR instrument as liquid films on NaCl plates. NMR spectra were measured on a Bruker AM 360 (360 MHz) or a Bruker AM 500 (500 MHz) spectrometers. The signal of the deuteriated solvent ($CDCl_3$) was taken as the reference (the singlet at $\delta_H$ 7.27 for $^1H$ and the triplet centered at $\delta_C$ 77.1 for $^{13}C$ NMR data). Complete assignments of NMR data were made on the basis of a combination of DEPT, HMQC and NOE experiments. *J* values are given in Hz. Mass spectra were recorded on AEI MS-902 or MM-701CF spectrometers using either electron ionisation (EI) or FAB techniques.

Flash chromatography was performed on Merck silic gel 60 as the stationary phase and the solvents employed were either of analytical grade or were distilled before use. All reactions were monitored by TLC using Merck silica gel 60 F254 precoated aluminium backed plates which were visualised with ultraviolet light and then with either 10% sulphuric acid in water or acidic alcoholic vanillin solution.

Ether refers to diethyl ether and light petroleum to the fraction of bp 40-60 °C. Routinely, dry organic solvents were stored under nitrogen and/or over sodium wire. Other organic solvents were dried by distillation from the following: THF (sodium benzophenone ketyl), and dichloromethane (calcium hydride). Reactions were carried out in an argon atmosphere when necessary. Commercial reagent chemicals were used as obtained unless otherwise noted. Organic extracts were washed with brine, dried over anhydrous magnesium sulphate and concentrated under reduced pressure on a Büchi rotary evaporator.

**2-iodo-4-methoxy-cinnamic acid methyl ester 5.**
This product was used as starting material and was directly obtained from a previous work.
White solid; $\delta_H$ (360 MHz; $CDCl_3$) 7.87 (1 H, d, *J* 15.8), 7.51 (1 H, d, *J* 8.8), 7.43 (1 H, d, *J* 2.6), 6.91 (1 H, dd, *J* 8.8, 2.6), 6.23 (1 H, d, *J* 15.8), 4.28 (2 H, q, *J* 7.1), 3.83 (3 H, s, MeO), 1.33 (3 H, t, *J* 7.1).

**(E)-3-[4-Methoxy-2-(3-oxo-propyl)-phenyl]-acrylic acid ethyl ester 6.**
To a stirred solution of the halide (816 mg, 2.45 mmol), $NaHCO_3$ (517 mg, 6.15 mmol), $NBu_4Cl$ hydrate (680 mg, 2.45 mmol)and the catalyst (palladium (II) acetate, 13.6 mg, 0.06 mmol) in DMF (12 mL) at 30°C was added allylic alcohol (408 µL, 5.78 mmol) dropwise. After being stirred for 2 h 30 min, it was quenched with saturated ammonium chloride, extracted with ether and once with DCM. The combined extracts were washed with brine, dried and concentrated. The crude was purified by flash chromatography on silica eluting with 30% ehter in light petroleum to give the aldehyde **6** (554 mg, 85%) as a colourless oil; $\delta_H$ (360 MHz; $CDCl_3$) 9.82 (1 H, t, *J* 1.2), 7.88 (1 H, d, *J* 15.8), 7.55 (1 H, d, *J* 8.6), 6.79 (1 H, dd, *J* 8.6, 2.6), 6.75 (1 H, d, *J* 2.6), 6.29 (1 H, d, *J* 15.8), 4.26 (2 H, q, *J* 7.1), 3.82 (3 H, s, MeO), 3.08 (2 H, t, *J* 7.4), 2.75 (2 H, dt, *J* 7.4, 1.2), 1.34 (3 H, t, *J* 7.1).

**(E)-3-{2-[(Z)-6-(*tert*-Butyl-dimethyl-silanyloxy)-hex-3-enyl]-4-methoxy-phenyl}-acrylic acid ethyl ester 7.**
A suspension of the phosphonium salt (1.2 g, 2.33 mmol) in THF (8 mL) was treated at -78°C with potassium bis(trimethylsilyl)amide (4.68 mL; 0.5 M in toluene). After being stirred for 20 min, the resulting orange mixture was allowed to warm to 0°C for 10 min, before cooling to -78°C again. The aldehyde **6** (510 mg, 1.94 mmol) in THF (10.8 mL) was added dropwise with stirring to the ylide solution at -78°C, then it was allowed to warm slowly until -20°C. After 1 h 45 min, the reaction mixture was quenched with water (5 mL) and extracted with ether. The residue obtained after usual work-up was purified by column chromatography eluting with 5% ethyl acetate in light petroleum to afford the title olefin (750 mg, 92%) as a colourless oil; $\delta_H$ (360 MHz; $CDCl_3$) 7.96 (1 H, d, *J* 15.8), 7.55 (1 H, d, *J* 8.6), 6.77 (1 H, dd, *J* 8.6, 2.6), 6.72 (1 H, d, *J* 2.6), 6.28 (1 H, d, *J* 15.8), 5.55-5.35 (2 H, m), 4.26 (2 H, q, *J* 7.1), 3.83 (3 H, s, MeO), 3.54 (2 H, t, *J* 6.8), 2.80 (2 H, t, *J* 7.6), 2.33 (2 H, q, *J* 7.6), 2.20 (2 H, q, *J* 6.8), 1.34 (3 H, t, *J* 7.1), 0.86 (9 H, s, $Me_3CSi$), 0.38 (6 H, s, $Me_2Si$). Irradiation of the multiplet centred at $\delta$2.33 resulted in collapse of a multiplet centred at $\delta$5.45 to a doublet, $J_{CIS}$ 10.9).

**(E)-3-[2-((Z)-6-Hydroxy-hex-3-enyl)-4-methoxy-phenyl]-acrylic acid ethyl ester 8.**
To a solution of the silyl ether (550 mg, 1.26 mmol) in THF (27.5 mL) cooled in an ice-bath, *n*-$BuNF{\cdot}3H_2O$ (1.65 mL; 1 M in THF) was added dropwise. After being stirred for 30 min, it was allowed to warm to rt for 1 h 30 min. Then, water and ether were added and the organic layer was separated. The aqueous phase was re-extracted twice with DCM and once with ether. The combined extracts were washed with brine, dried over magnesium sulphate and concentrated under vacuum. Purification of the residue by chromatography on silica eluting with 50% ethyl acetate in light petroleum gave the alcohol **8** (396 mg, 99%) as a yellowish oil; $\delta_H$ (360 MHz; $CDCl_3$) 8.00 (1 H, d, *J* 15.8), 7.57 (1 H, d, *J* 8.6), 6.77 (1 H, dd, *J* 8.6, 2.6), 6.74 (1 H, d, *J* 2.6), 6.30 (1 H, d, *J* 15.8), 5.61 (1 H, m), 5.44 (1 H, m), 4.26 (2 H, q, *J* 7.1), 3.83 (3 H, s, MeO), 3.63 (2 H, t, *J* 6.7), 2.77 (2 H, m), 2.32 (4 H, m), 1.33 (3 H, t, *J* 7.1).

**(E)-3-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-acrylic acid ethyl ester 9.**
NCS (318 mg, 2.39 mmol) was added in one portion to a solution of the alcohol **8** (530 mg, 1.74 mmol), triphenylphosphine (612 mg, 2.33 mmol) and anhydrous potassium carbonate (46 mg, 0.33 mmol) in dry DCM (32.8 mL) at 0°C. After being stirred for 30 min it was concentrated *in vacuo* to give a residue, which was purified by chromatography eluting with 10% ethyl acetate in light petroleum to afford the title chloride (577 mg, 95%) as a colourless oil; $\delta_H$ (360

MHz; $CDCl_3$) 7.94 (1 H, d, *J* 15.8), 7.56 (1 H, d, *J* 8.6), 6.77 (1 H, dd, *J* 8.6, 2.6), 6.72 (1 H, d, *J* 2.6), 6.29 (1 H, d, *J* 15.8), 5.60-5.55 (1 H, m), 5.46-5.41 (1 H, m), 4.26 (2 H, q, *J* 7.1), 3.83 (3 H, s, MeO), 3.39 (2 H, t, *J* 7.0), 2.81 (2 H, t, *J* 7.3), 2.45-2.31 (4 H, m), 1.34 (3 H, t, *J* 7.1).

**(E)-3-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-prop-2-en-1-ol.**

To a solution of the ester (420 mg, 1.304 mmol) in THF (7.9 mL) at -78°C, a solution of DIBAL-H (2 mL; 1.5 M in toluene) was added dropwise over 10 min. After 30 min, it was quenched with a 5:1 THF-$H_2O$ (3 mL) solution, diluted with ether, and Rochelles salt was added (7 mL). After being stirred for 2 h it was poured into ether/water/brine, and the organic layer was separated and the aqueous layer was re-extracted with DCM.

The combined organic extracts were dried over magnesium sulphate and concentrated under vacuum. The crude alcohol was used directly in the next step without further purification. $\delta_H$ (360 MHz; $CDCl_3$) 7.41 (1 H, d, *J* 8.6), 6.75 (1 H, dd, *J* 8.6, 2.7), 6.22-6.14 (1 H, m), 5.62-5.58 (1 H, m), 5.43-5.38 (1 H, m), 4.32 (2 H, dd, *J* 5.9, 1.5), 3.81 (3 H, s, MeO), 3.40 (2 H, t, *J* 7.1), 2.71 (2 H, t, *J* 7.5), 2.45 (2 H, q, *J* 7.1), 2.34 (2 H, q, *J* 7.5), 1.46 (3 H, br s, OH).

**(E)-3-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-propenal 10.**

A suspension of activated manganese oxide (4.08 g, 46 mmol) in a DCM (16.5 mL) solution of the alcohol (465 mg, 1.66 mmol) was stirred under ice-cooling for 3 h. After this time, the manganese oxide was filtered off through a pad of celite eluting with DCM to give the aldehyde **10** (450 mg, 97%) as a colourless oil which solidified on standing at 3°C; $\delta_H$ (360 MHz; $CDCl_3$) 9.70 (1 H, d, *J* 7.7), 7.74 (1 H, d, *J* 15.6), 7.61 (1 H, d, *J* 8.7), 6.82 (1 H, dd, *J* 8.7, 2.7), 6.77 (1 H, d, *J* 2.7), 6.61 (1 H, dd, *J* 15.6, 7.7), 5.62-5.56 (1 H, m), 5.48-5.42 (1 H, m), 3.84 (3 H, s, MeO), 3.42 (2 H, t, *J* 6.8), 2.85 (2 H, t, *J* 7.4), 2.46-2.33 (4 H, m).

**(E)-1-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-hex-1-en-4-yn-3-ol 11.**

A stirred solution of (Z/E)-1-bromopropene (73 µL, 0.836 mmol) in dry THF (0.7 mL) at -78°C, was treated dropwise with n-BuLi (475 µL; 2.5 M in hexane) over 10 min. After 15 min, the mixture was allowed to warm to rt and stirred for 40 min. Then, this prepared acetylide solution was cooled at -78°C, and a solution of the aldehyde **10** (150 mg, 0.539 mmol) in dry THF (1.3 mL) was added *via* syringe. It was allowed to warm slowly to -15°C and after being stirred for 1 h 45 min it was quenched with saturated ammonium chloride and diluted with ether. The mixture was poured into saturated ammonium chloride/brine and extracted with ether. The combined extracts were washed with brine, dried over magnesium sulphate and concentrated. The crude was filtrated through a pad of silica using 40% ether in light petroleum as eluent to give the title carbinol (145 mg, 85%) as a colourless oil; [Found: $M^+$ (EI), 318.1377. $C_{19}H_{23}O_2Cl$ requires *M*, 318.1387]; $\nu_{max}/cm^{-1}$ 3398, 3011, 2956, 2237, 1606, 1571, 1494, 1298, 1257, 1040, 966; $\delta_H$ (360 MHz; $CDCl_3$) 7.43 (1 H, d, *J* 8.6, H-1), 6.94 (1 H, d, *J* 15.5, ArCH=CH), 6.74 (1 H, dd, *J* 8.6, 2.7, H-2), 6.68 (1 H, d, *J* 2.7, H-4), 6.10 (1 H, dd, *J* 15.5, 6.2, ArCH=CH), 5.62-5.52 (1 H, m), 5.45-5.35 (1 H, m), 5.02 (1 H, br s, CHOH), 3.81 (3 H, s, MeO), 3.40 (2 H, t, *J* 7.0), 2.72 (2 H, t, *J* 7.4), 2.44 (2 H, q, *J* 7.0), 2.35 (1 H, q, *J* 7.4), 1.91 (3 H, d, *J* 2.2, -C≡CMe); $\delta_C$ (90 MHz; $CDCl_3$) 159.4 (s), 141.1 (s), 131.7 (d), 128.7 (d), 128.6 (d), 127.6 (d), 127.6 (s), 126.0 (d), 115.1 (d), 111.9 (d), 82.8 (s), 78.7 (s), 63.5 (d), 55.3 (q), 44.2 (t), 33.4 (t), 30.7 (t), 28.9 (t), 3.7 (q); MS (EI) *m/z* 319 ($M^+$+ 1, 12), 318 ($M^+$, 73), 302 (24), 300 (66), 275 (15), 265 (25), 251 (16), 249 (19), 239 (25), 235 (52), 224 (44), 215 (46), 209 (24), 197 (100), 183 (37), 173 (83).

**(E)-1-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-hex-1-en-4-yn-3-one 12.**

To a solution of the carbinol **11** (129 mg, 0.405 mmol) in dry DCM (7 mL) at 0°C, was added pyridine (98 µL, 1.21 mmol). Whitin 2 min of the addition Dess-Martin periodinane (223 mg, 0.527 mmol) was added in one portion. After being stirred for 1 h, it was diluted with ether and poured into a saturated sodium hydrogencarbonate-5% sodium thiosulphate solution. The organic layer was separated, washed with brine and concentrated. Purification of the residue by chromatography eluting with 20% ether in light petroleum gave the ketone **12** (72 mg, 56%) as a yellow oil; [Found: $M^+$ (EI), 316.1255. $C_{19}H_{21}O_2Cl$ requires *M*, 316.1230]; $\nu_{max}/cm^{-1}$ 3212, 3010, 2956, 2287, 1625, 1597, 1492, 1464, 1298, 1256, 1100, 1033, 975; $\delta_H$ (360 MHz; $CDCl_3$) 8.11 (1 H, d, *J* 15.8, ArCH=CH), 7.59 (1 H, d, *J* 8.6, H-1), 6.80 (1 H, dd, *J* 8.7, 2.6, H-2), 6.75 (1 H, d, *J* 2.6, H-4), 6.64 (1 H, d, *J* 15.8, ArCH=CH), 5.62-5.52 (1 H, m), 5.48-5.40 (1 H, m), 3.86 (3 H, s, MeO),

3.41 (2 H, t, *J* 6.9), 2.86 (2 H, t, *J* 7.4), 2.46-2.34 (4 H, m), 2.12 (3 H, s, -C≡CMe); $\delta_C$ (90 MHz; $CDCl_3$) 178.7 (s), 161.8 (s), 145.3 (d), 144.2 (s), 131.1 (d), 128.7 (d), 127.4 (d), 126.5 (d), 125.2 (s), 115.7 (d), 112.6 (d), 90.3 (s), 78.9 (s), 55.4 (q), 44.1 (t), 33.4 (t), 30.6 (t), 29.5 (t), 4.3 (q); MS (EI) *m/z* 317 ($M^+$+ 1, 2), 316 ($M^+$, 11), 213 (22), 199 (86), 185 (11), 146 (9), 115 (7), 67 (100).

**(E)-1-[2-((Z)-6-Iodo-hex-3-enyl)-4-methoxy-phenyl]-hex-1-en-4-yn-3-one 4a.**
A stirred solution of the chloride **12** (79 mg, 0.25 mmol) and sodium iodide (75 mg, 0.5 mmol) in methyl ethyl ketone (3.7 mL) was heated at reflux for 2 h, then it was cooled and concentrated. The residue was taken into 60 mL of ether and washed with brine. The organic layer was separated, dried, and evaporated *in vacuo* to leave the crude iodide which was purified by chromatography eluting with 20% ether in light petroleum to afford the precursor **4a** (94 mg, 92%; 90% purity) as a yellow oil; $\delta_H$ (360 MHz; $CDCl_3$) 8.10 (1 H, d, *J* 15.8, ArCH=CH), 7.58 (1 H, d, *J* 8.6, H-1), 6.79 (1 H, dd, *J* 8.6, 2.6, H-2), 6.75 (1 H, d, *J* 2.6, H-4), 6.63 (1 H, dd, *J* 15.8, 1.9, ArCH=CH), 5.62-5.50 (1 H, m), 5.48-5.30 (1 H, m), 3.84 (3 H, s, MeO), 3.39 (2 H, t, *J* 6.9), 3.0 (1 H, t, *J* 7.2), 2.84 (2 H, m), 2.51 (1 H, ddd, *J* 14.3, 7.2, 1.1), 2.45-2.30 (3 H, m), 2.11 (3 H, d, *J* 1.9, -C≡CMe); $\delta_C$ mixture of rotamers (90 MHz; $CDCl_3$) 178.6 (s), 161.8 (s), 145.2 (d), 144.2 (s), 131.0 (d), 130.38 (d), 129.3 (d), 128.7 (d + s), 127.3 (d), 126.5 (d), 126.1 (d), 115.7 (d), 112.5 (d), 90.3 (s), 78.9 (s), 55.4 (q), 44.1 (t), 33.3 (t), 32.2 (t), 31.3 (t), 29.4 (t), 5.19 (q), 4.2 (q).

**(E)-1-Phenyl-pent-1-en-4-yn-3-ol 13.**
To an ethynylmagnesium bromide solution (5.6 mL; 0.5 M in THF) under ice cooling and stirring, cinnamaldehyde (250 mg, 1.89 mmol) in dry THF (0.5 mL) was added dropwise. After stirring for 17 h at rt, saturated ammonium chloride was added and the mixture was diluted with ether. The organic fraction was washed with brine, water, dried over magnesium sulphate and concentrated *in vacuo* to give a brown oil which solidified on standing. The crude was purified by chromatography on silica eluting with 50% ether in light petroleum to give the carbinol (290 mg, 95%) as a yellow solid; $\delta_H$ (360 MHz; $CDCl_3$) 7.5-7.2 (5 H, m), 6.82 (1 H, d, *J* 15.8), 6.32 (1 H, dd, *J* 15.8, 5.9), 5.06 (1 H, dd, *J* 5.9, 1.2), 2.66 (1 H, d, *J* 1.2, -C≡CH), 2.20 (1 H, br s, OH); $\delta_C$ (90 MHz; $CDCl_3$) 136.1 (s), 132.3 (d), 128.6 (d), 128.2 (d), 127.5 (d), 126.8 (d) x 3, 82.3 (s), 74.5 (d), 62.7 (d).

**(E)-1-Phenyl-pent-1-en-4-yn-3-one 14.**
A suspension of activated manganese oxide (690 mg, 8 mmol) in a DCM (2.6 mL) solution of the alcohol (40 mg, 0.26 mmol) was stirred under ice-cooling for 2 h. After this time, the manganese oxide was filtered off through a pad of celite eluting with DCM to give the ketone **14** (32 mg, 80%) as a yellow solid; $\delta_H$ (360 MHz; $CDCl_3$) 7.90 (1 H, d, *J* 16.1), 7.61-7.58 (2 H, m), 7.46-7.43 (2 H, m), 6.82 (1 H, d, *J* 16.1), 3.34 (1 H, s, -C≡CH); $\delta_C$ (90 MHz; $CDCl_3$) 177.7 (s), 149.8 (d), 133.9 (s), 131.5 (d), 129.2 (d) x 2, 128.8 (d) x 2, 128.1 (d), 79.9 (s), 79.4 (d).

**(E)-1-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-pent-1-en-4-yn-3-ol 15.**
To an ethynylmagnesium bromide solution (1.22 mL; 0.5 M in THF) under ice cooling and stirring, the aldehyde **10** (113 mg, 0.406 mmol) in dry THF (1.7 mL) was added dropwise. After stirring for 17 h at rt, saturated ammonium chloride was added and the mixture was diluted with ether. The mixture was extracted with ether and the organic fraction was washed with brine, water, dried over magnesium sulphate and concentrated *in vacuo*. The crude was purified by chromatography on silica eluting with 30% ether in light petroleum to give the carbinol (111 mg, 90%) as an orange-brown oil; [Found: $M^+$ (EI), 304.1222. $C_{18}H_{21}O_2Cl$ requires *M*, 304.1230]; $\nu_{max}/cm^{-1}$ 3558-3550, 3289, 3010, 2955, 2115, 1606, 1570, 1493, 1463, 1298, 1257, 1039, 967; $\delta_H$ (360 MHz; $CDCl_3$) 7.42 (1 H, d, *J* 8.6, H-1), 7.02 (1 H, d, *J* 15.5, ArCH=CH), 6.75 (1 H, dd, *J* 8.6, 2.6, H-2), 6.69 (1 H, d, *J* 2.6, H-4), 6.12 (1 H, dd, *J* 15.5, 6.0, ArCH=CH), 5.61-5.55 (1 H, m), 5.44-5.39 (1 H, m), 5.08 (1 H, br s, $W_{h/2}$ 13.4, CHOH), 3.81 (3 H, s, MeO), 3.40 (2 H, t, *J* 7.0), 2.73 (2 H, t, *J* 7.4), 2.66 (1 H, d, *J* 2.1, -C≡CH), 2.44 (2 H, q, *J* 7.0), 2.32 (2 H, q, *J* 7.4), 2.14 (1 H, d, *J* 6.0, OH); $\delta_C$ (90 MHz; $CDCl_3$) 159.5 (s), 141.2 (s), 131.6 (d), 129.5 (d), 127.6 (d), 127.4 (s), 127.3 (d), 126.0 (d), 115.2 (d), 111.9 (d), 83.1 (s), 74.6 (d), 63.0 (d), 55.3 (q), 44.2 (t), 33.4 (t), 30.7 (t), 28.9 (t); MS (EI) *m/z* 305 ($M^+$+ 1, 6), 304 ($M^+$, 65), 251 (15), 249 (18), 241 (15), 237 (64), 235 (26), 223 (34), 201 (52), 187 (35), 173 (31), 159 (100), 147 (93), 115 (35).

**(E)-1-[2-((Z)-6-Chloro-hex-3-enyl)-4-methoxy-phenyl]-pent-1-en-4-yn-3-one 16.**

A suspension of activated manganese oxide (1.6 g, 18.4 mmol) in a DCM (6.5 mL) solution of the alcohol (160 mg, 0.526 mmol) was stirred under ice-cooling for 4 h. After this time, the manganese oxide was filtered off through a pad of celite eluting with DCM to give the ketone **16** (114 mg, 72%) as a orange oil which solidified on standing at 3°C, mp 64-66°C (from ether-light petroleum); [Found: $M^+$ (EI), 302.1071. $C_{18}H_{19}O_2Cl$ requires *M*, 302.1074]; $\nu_{max}/cm^{-1}$ 3274, 3011, 2954, 2096, 1632, 1597, 1493, 1261, 1233, 1099, 1037, 975; $\delta_H$ (360 MHz; $CDCl_3$) 8.20 (1 H, d, *J* 15.9, ArCH=CH), 7.61 (1 H, d, *J* 8.7, H-1), 6.82 (1 H, dd, *J* 8.7, 2.7, H-2), 6.77 (1 H, d, *J* 2.7, H-4), 6.69 (1 H, d, *J* 15.9, ArCH=CH), 5.61-5.55 (1 H, m), 5.48-5.42 (1 H, m), 3.86 (3 H, s, MeO), 3.41 (2 H, t, *J* 6.9), 3.33 (1 H, d, *J* 1.4, -C≡CH), 2.86 (2 H, t, *J* 8.0), 2.46-2.34 (4 H, m); $\delta_C$ (90 MHz; $CDCl_3$) 177.7 (s), 162.2 (s), 146.8 (d), 144.7 (s), 131.0 (d), 128.8 (d), 126.8 (d), 126.6 (d), 124.9 (s), 115.8 (d), 112.7 (d), 80.3 (s), 78.9 (d), 55.5 (q), 44.1 (t), 33.4 (t), 30.7 (t), 29.6 (t); MS (EI) *m/z* 302 ($M^+$, 14), 267 (6), 235 (8), 216 (7), 199 (23), 185 (100), 146 (46), 131 (14).

**(E)-1-[2-((Z)-6-Iodo-hex-3-enyl)-4-methoxy-phenyl]-pent-1-en-4-yn-3-one 4b.**

A stirred solution of the chloride **16** (114 mg, 0.377 mmol) and sodium iodide (111 mg, 0.74 mmol) in methyl ethyl ketone (5.7 mL) was heated at reflux for 2 h, then it was cooled and concentrated. The residue was taken into 100 mL of ether and washed with brine. The organic layer was separated, dried, and evaporated *in vacuo* to leave the crude iodide which was purified by chromatography eluting with 20% ether in light petroleum to afford the precursor **4b** (146 mg, 99%) as a yellow solid, mp 63-64°C (from ether-light petroleum); [Found: $M^+$ (FAB), 395.051286. $C_{18}H_{19}O_2I$ requires *M*, 395.050807]; $\nu_{max}/cm^{-1}$ 3262, 3009, 2945, 2095, 1629, 1597, 1492, 1260, 1232, 1097, 1035, 974; $\delta_H$ (360 MHz; $CDCl_3$) 8.20 (1 H, d, *J* 15.9, ArCH=CH), 7.61 (1 H, d, *J* 8.6, H-1), 6.82 (1 H, dd, *J* 8.6, 2.6, H-2), 6.77 (1 H, d, *J* 2.6, H-4), 6.70 (1 H, dd, *J* 15.9, 1.8, ArCH=CH), 5.60-5.55 (1 H, m), 5.45-5.30 (1 H, m), 3.86 (3 H, s, MeO), 3.41 (1 H, t, *J* 6.9), 3.32 (1 H, d, *J* 1.8, -C≡CH), 2.99 (1 H, t, *J* 7.2), 2.89-2.84 (2 H, m), 2.53 (1 H, q, *J* 7.2), 2.44-2.32 (3 H, m); $\delta_C$ mixture rotamers (90 MHz; $CDCl_3$) 177.6 (s), 162.1 (s), 146.7 (d), 144.6 (s), 130.9 (d), [130.3 (d), 129.4 (d)], 128.8 (d), 126.7 (d), 126.6 (d), 124.8 (s), 115.7 (d), 112.7 (d), 80.2 (s), 79.0 (s), 55.4 (q), 44.1 (t), 33.3 (t), 30.6 (t), 29.5 (t); MS (EI) *m/z* 396 ($M^+$ + 1 , 11), 307 (28), 303 (12), 176 (11), 154 (100), 139 (16), 138 (37), 136 (68), 107 (22).

**(8R,9S,14R)-3-Methoxy-6,7,8,9,11,14,15,16-octahydro-cyclopenta[*a*]phenanthren-12-one 17 and**

**(8S,9S,13R,14R)-3-methoxy-6,7,8,9,11,13,14,15,16,17-decahydro-cyclopenta[*a*] phenanthren-12-one 18.**

A solution of tri-*n*-butyltin hydride (41 μL, 0.152 mmol) and azoisobutyronitrile (12 mg, 0.073 mmol) in dry benzene (4.8 mL) was added dropwise over 12 h to a stirred, refluxing solution of the iodide (50 mg, 0.126 mmol) and azoisobutyronitrile (4 mg, 0.024 mmol) in dry, degassed benzene (57 mL), under argon. The mixture was held at reflux for 6 h more and then cooled and concentrated *in vacuo*. The orange residue was taken up in ether (30 mL), and then stirred at rt for 18 over a 5% KF solution (5 mL). The organic layer was separated and then dried and evaporated *in vacuo* to give an orange oil which was chromatographed on silica, eluting from 2% to 40% ether in light petroleum, to afford the 12-ketosteroid **18** (8 mg, 23%) as a crystalline solid, followed by the 12-ketosteroid **17** (15 mg, 44%) as a colourless oil. Compound **17** was crystallised from ether-light petroleum giving colourless crystals; mp 96-97°C; [Found: $M^+$ (EI), 268.1460. $C_{18}H_{20}O_2$ requires *M*, 268.1463]; $\nu_{max}/cm^{-1}$ 3056, 2923, 2852, 1682, 1609, 1501, 1260, 1038, 811; $\delta_H$ (500 MHz; $CDCl_3$) 7.14 (1 H, d, *J* 8.6, H-1), 6.74 (1 H, dd, *J* 8.6, 2.8, H-2), 6.67 (1 H, dd, *J* 5.6, 3.0, H-17), 6.65 (1 H, d, *J* 2.8, H-4), 3.79 (3 H, s, MeO), 3.29 (1 H, m, H-14), 3.07 (1 H, dd, *J* 18.2, 3.4, H-11α), 2.95-2.82 (3 H, m, 2 x H-6 and H-9), 2.49-2.36 (2 H, m, 2 x H-16), 2.23 (1 H, dd, *J* 18.2, 12.7, H-11β), 2.18 (1 H, dddd, *J* 11.6, 6.5, 6.3, 1.3, H-15β), 2.06 (1 H, dddd, *J* 12.0, 11.9, 9.1, 2.7, H-8), 1.96 (1 H, dddd, *J* 12.8, 5.0, 2.7, 2.4, H-7β), 1.79 (1 H, dddd, *J* 11.7, 11.7, 11.2, 8.6, H-15α), 1.61 (1 H, dddd, *J* 12.8, 12.6, 12.0, 5.4, H-7α); $\delta_C$ (90 MHz; $CDCl_3$) 200.6 (C12), 157.8 (C3), 146.6 (C13), 140.2 (C17), 138.3 (C5[a]), 130.0 (C10[a]), 128.3 (C1), 113.9 (C4), 112.3 (C2), 55.3 (MeO), 47.4 (C11), 44.6 (C14), 39.7 (C8), 35.3 (C9), 32.0 (C16), 31.7 (C15), 30.9 (C6), 25.4 (C7). [a]These signals may be interchanged; MS (EI) *m/z* 269 ($M^+$ + 1 , 16), 268 ($M^+$, 100), 240 (12), 211 (7), 174 (40), 159 (32), 147 (27), 94 (29); Compound **18**, white needles, mp 106-

107°C (from MeOH); [Found: $M^+$ (EI), 270.1620. $C_{18}H_{22}O_2$ requires *M*, 270.1621]; $\nu_{max}/cm^{-1}$ 2918, 2866, 1702, 1609, 1572, 1499, 1452, 1282, 1253, 1132, 1033, 866, 795; $\delta_H$ (360 MHz; $CDCl_3$) 7.12 (1 H, d, *J* 8.6, H-1), 6.74 (1 H, dd, *J* 8.6, 2.7, H-2), 6.66 (1 H, d, *J* 2.7, H-4), 3.79 (3 H, s, MeO), 3.08 (1 H, dd, *J* 13.7, 3.6, H-11α), 3.00-2.84 (3 H, m, 2 x H-6 and H-9), 2.75 (1 H, br t, *J* 8.0, H-13), 2.53 (1 H, m, H-14), 2.37 (1 H, m, H-17β), 2.28 (1 H, dd, *J* 13.7, 9.8, H-11β), 2.15 (1 H, dddd, *J* 16.4, 11.7, 4.7, 2.5, H-8), 1.92 (1 H, dddd, *J* 12.7, 7.9, 4.7, 3.2, H-7β), 1.75-1.50 (5 H, m, H-7α, 2 x H-16, H-15β, H-17α), 1.19 (1 H, dddd, *J* 12.3, 12.0, 8.8, 3.6, H-15α); $\delta_C$ (90 MHz; $CDCl_3$) 212.6 (C12), 157.8 (C3), 137.9 (C5[a]), 130.8 (C10[a]), 127.1 (C1), 114.0 (C4), 112.1 (C2), 55.3 (MeO), 53.3 (C13), 47.9 (C14), 47.0 (C11), 40.3 (C8), 36.7 (C9), 30.7 (C6), 27.9 (C7), 24.7 (C15), 24.5 (C17), 22.9 (C16). [a]These signals may be interchanged; MS (EI) *m/z* 271 ($M^+$ + 1, 19), 270 ($M^+$, 100), 227 (19), 211 (4), 202 (14), 199 (12), 171 (11), 159 (13).

**X-ray crystal structure determination of compound 17.**

A colourless rectangular block was mounted as oil film on a dual stage fibre and transferred to the diffractometer.

**Crystal data**

$C_{18}H_{20}O_2$, *M* = 268.34. Monoclinic, a = 7.0346(5), b = 11.8967(8), c = 16.505(1) Å, *V* = 1374.6(3) $Å^3$, space group *P*2(1)/*c*, *Z* = 4, *D* = 1.297 g $cm^{-3}$, colourless rectangular crystal 0.50 x 0.38 x 0.35 mm.

**(±)-3-(2-[1,3]Dioxan-2-yl-ethyl)-cyclohexanone 19 and Bicyclo[4,3,0]non-9-en-2-one 20.**

Magnesium turnings (1.8 g, 75 mmol; dried overnight in the oven at 110°C) were ground for a few minutes with a mortar and were immediately placed into a nitrogen-filled flask with 20 mL of dry THF. To the slyrry of Mg, at rt, was added 1,2-dibroethane (150 μL, 1.7 mmol) followed by 2-(2-bromoethyl)-1,3-dioxane (3.4 mL, 24.9 mmol) dropwise. After 1 h, it was cooled to -78°C and a $CuBr(Me_2S)$ (1.23 g, 6 mmol) solution in $Me_2S$ (10 mL) was added dropwise to the milky suspension, which was diluted with fresh THF (20 mL). The mixture was stirred at -78°C for 1 h 30 min, then a cyclohexenone (1.95 mL, 20.4 mmol) solution in dry ether (21 mL) was added dropwise over a 15 min period. The mixture was stirred at -78°C for 1 h and allowed to warm to 0°C slowly. After being stirred for 4 h, the resulting grey mixture was quenched with a solution of THF (70 mL) and 0.2N HCl (10 mL). The organic layer was neutralised with saturated sodium hydrogen carbonate, diluted with ether and washed with water and brine, dried and concentrated. The crude compound **19** (4.46 g), colourless oil, was used without purification for the next step; $\delta_H$ (360 MHz; $CDCl_3$) 4.49 (1 H, t, *J* 5), 4.08 (2 H, m), 3.74 (4 H, m), 2.50-1.20 (13 H, m).

The crude acetal **19** was dissolved in a solution of THF (55 mL) and 2N HCl (10 mL) and heated at 80°C for 6 h. The mixture was then neutralised with saturated sodium hydrogen carbonate, diluted with ether and washed with water and brine, dried and concentrated. The dark brown crude was chromatographed eluting with 30% ethyl acetate in light petroleum to afford the enone **20** (1.73 g, 70% overall yield for the two steps) as an orange oil; bp 120°C/20 Torr; $\delta_H$ (360 MHz; $CDCl_3$) 6.62 (1 H, dd, *J* 5.1, 2.5), 2.86 (1 H, m), 2.49-1.24 (10 H, m); $\delta_C$ (90 MHz; $CDCl_3$) 199.7 (s), 145.0 (s), 138.6 (d), 45.8 (d), 40.4 (t), 33.2 (t), 31.9 (t), 31.6 (t), 24.1 (t).

**(±)-*Cis*-bicyclo[4,3,0]nonan-2-one 21.**

The enone **20** (45 mg, 0.33 mmol) was dissolved along with AIBN (11 mg, 0.06 mmol) in benzene (3 mL) and flushed with nitrogen. Then, it was heated until reflux and tri-*n*-butyltin hydride (41 μL, 0.152 mmol) was added dropwise. After being stirred for 7 h, it was cooled and concentrated. The crude residue was chromatographed on silica eluting with 20% ethyl acetate in light petroleum to give the reduced product **21** (6 mg, 10%) along with 15 mg of unreacted starting material. Compound **21**, colourless oil, MS (EI) *m/z* 138.1 ($M^+$, 11); bp 61°C/1 Torr; $\delta_H$ (360 MHz; $CDCl_3$) 2.62 (1 H, dd, *J* 13.0, 8.0), 2.51-2.24 (3 H, m), 2.11-1.25 (10 H, m); $\delta_C$ (90 MHz; $CDCl_3$) 214.6[lit[22] 216.5], 53.2, 43.1, 39.7, 31.1, 27.4, 26.8, 23.9, 23.2.

**(±)- (3S,3aR,7aR)-3-Methyl-3a-phenylselanyl-octahydro-inden-4-one 23.**

To a 250 mL round bottom flask containing a stir bar, CuI (540 mg, 4 mmol) and diethyl ether (35 mL), methyllithium (10 mL; 1.6 M in diethyl ether) was added dropwise *via* syringe to the stirred suspension at 0°C. The yellow colour changed into grey. After 30 min, the resulting colourless solution was cooled to -60°C and the enone **20** (540 mg, 4 mmol) was added dropwise in 4 mL of dry ether over a 15 min period.

The resulting yellow solution was allowed to warm up to -15°C (2 h). Then, it was cooled again to -60°C, before 10 mL of dry THF and phenylselenenyl chloride (2.25 g, 12 mmol) in THF (10 mL) were added. The reaction mixture was allowed to warm to rt (2h 30 min), and then quenched with saturated ammonium chloride. The mixture was poured into a 1:1 mixture of ammonium hydroxide-sat. ammonium chloride. The organic layer was separated, washed successively with 10% ammonium hydroxide, water, sat. ammonium chloride and brine. The normal workup gave a orange residue which was purified by column chromatography eluting with 20% ether in light petroleum to afford the title product (600 mg, 55%; 90% pure by $^1$H NMR) as an orange oil; $\delta_H$ (360 MHz; $CDCl_3$) 7.40-7.16 (5 H, SePh), 3.05 (1 H, m), 2.65 (1 H, m), 2.46 (1 H, m), 2.40-0.88 (9 H, m), 1.06 (3 H, d, *J* 7.2, Me); $\delta_C$ (90 MHz; $CDCl_3$) 207.3, 135.6, 128.8, 69.07, 45.3, 38.0, 30.6, 28.3, 26.4, 21.9, 18.7.

**(±)-3-Methyl-1,2,3,5,6,7-hexahydro-inden-4-one 24.** To a solution of the phenylselenenyl indanone **23** (326 mg, 1.06 mmol) in DCM (9 mL) were added six 0.35 mL portions of 30% $H_2O_2$ at 10 min intervals. Five minutes after the final peroxide addtion, the mixture was transferred to a separatory funnel, and the layers were separated. The organic layer was washed with water and dried over magnesium sulphate and evaporated to give the crude enone **24** (159 mg, quant.) as a colourless oil; $\delta_H$ (360 MHz; $CDCl_3$) 2.90 (1 H, m), 2.62-0.82 (10 H, m), 1.00 (3 H, d, *J* 7.0, Me); $\delta_C$ (90 MHz; $CDCl_3$) 197.6, 164.8, 141.4, 37.9, 37.2, 35.7, 30.6, 26.5, 23.3, 19.8.

**(±)-(3R,3aR,7aS)-3-(Dimethyl-phenyl-silanyl)-octahydro-inden-4-one 25.**
A solution of dimethylphenylsilyl chloride (1.2 mL, 0.007 mmol) in dry THF (12 mL) was added slowly to the cut lithium wire (200 mg, 0.028 mmol). The reaction took some time to be started (colourless suspension). After being stirred for 2 h the colour changed into wine-red. After 16 h more, the deep red solution was filtered through glass wool and kept under nitrogen in a round bottom flask.
From the above dimethyl(phenyl)silyl lithium solution (ca. 0.4 M in THF), 1 mmol was added to a stirred solution of diethylzinc (1 mL; 1 M in THF) in THF (3 mL) at 0°C under nitrogen.
The wine-red solution was stirred 5 min and then brought to -78°C. The enone **20** (95 mg, 0.698 mmol) in THF (1.3 mL) was injected over 5 min. After being stirred for 30 min the reaction mixture was quenched with methyl iodide (270 µL, 4.2 mmol) and the resulting mixture was allowed to warm to rt (2 h). 2N HCl (2mL) was added to dissolve the zinc salts, and the aqueous layer was extracted with ether. The organic layers were combined, washed with brine, dried and evaporated under reduced pressure. The crude residue was chromatographed on silica eluting with 10% ethyl acetate in light petroleum to give the title compound (140 mg, 70%) as a colourless oil; $\delta_H$ (360 MHz; $CDCl_3$) 7.55-7.52 (2 H, m), 7.37-7.34 (3 H, m), 2.47 (1 H, t, *J* 7.6), 2.42-2.20 (3 H, m), 2.02-1.97 (1 H, m), 1.92-1.74 (4 H, m), 1.60-1.22 (4 H, m), 0.26 (6 H, 2 x s, $SiMe_2$); $\delta_C$ (90 MHz; $CDCl_3$) 214.0 (s), 138.2 (s), 134.1 (d) x 2, 129.0 (d), 127.7 (d)x 2, 54.9 (d), 44.9 (d), 40.1 (t), 32.1 (t), 27.3 (t), 26.4 (t), 24.14 (t), 24.08 (d), -4.0 (q), -4.5 (q).

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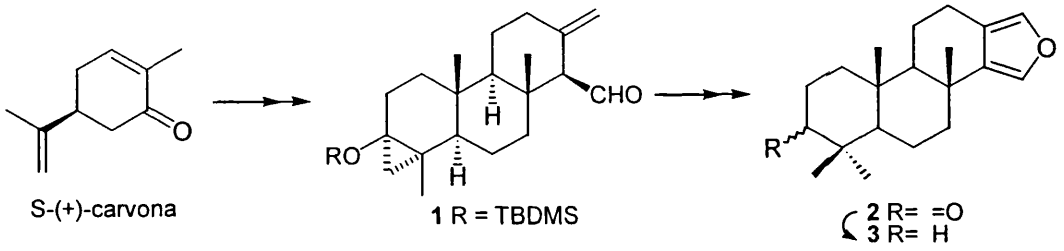
# *5. RESUMEN Y CONCLUSIONES*

## 5.- RESUMEN Y CONCLUSIONES

- En esta Tesis se ha desarrollado una ruta sintética que partiendo de S-(+)-carvona permite la obtención de *espongianos* quirales que contienen el anillo D de tipo furánico. La construcción del anillo D se ha realizado a través del aldehído **1**, cuya epoxidación y ciclación conduce al furano **2** precursor de los furanoditerpenos espongiánicos con el anillo A funcionalizado. También se ha sintetizado, mediante reducción de **2**, el *(-)-espongia-13(16),14-dieno* natural **3** con un rendimiento global del 9%, mejorando la síntesis racémica de **3** realizada por Sakamoto[1] y colaboradores en 20 etapas y con un rendimiento global del 3% (Esquema **1**).

Esquema **1**




- Utilizando como material de partida la podocarpenona quiral **4** hemos desarrollado una secuencia sintética que permite, a través del intermedio clave **5**, tanto la obtención de *espongianos* tetracíclicos funcionalizados en la posición C-17 como la preparación de *espongianos* pentacíclicos. La utilidad de esta ruta sintética se ha demostrado con la síntesis de los productos naturales siguientes: (-)-spongian-16-oxo-17-al **6**, (-)-aplyroseol-14 **7** y (-)-acetyldendrillol-1 **8** (Esquema **2**), con un rendimiento global desde **4** del 30%, 23% y 39%, respectivamente.

La síntesis de (-)-aplyroseol-14 **7** y (-)-acetyldendrillol-1 **8** también ha conducido a la revisión de la estructura asignada inicialmente a Aplyroseol-14, así como la estereoquímica de C-17 dada para el producto natural **8**, sustituyéndose la configuración de C-17 con el grupo acetoxilo en α por la de su epímero con el grupo acetoxilo en β.

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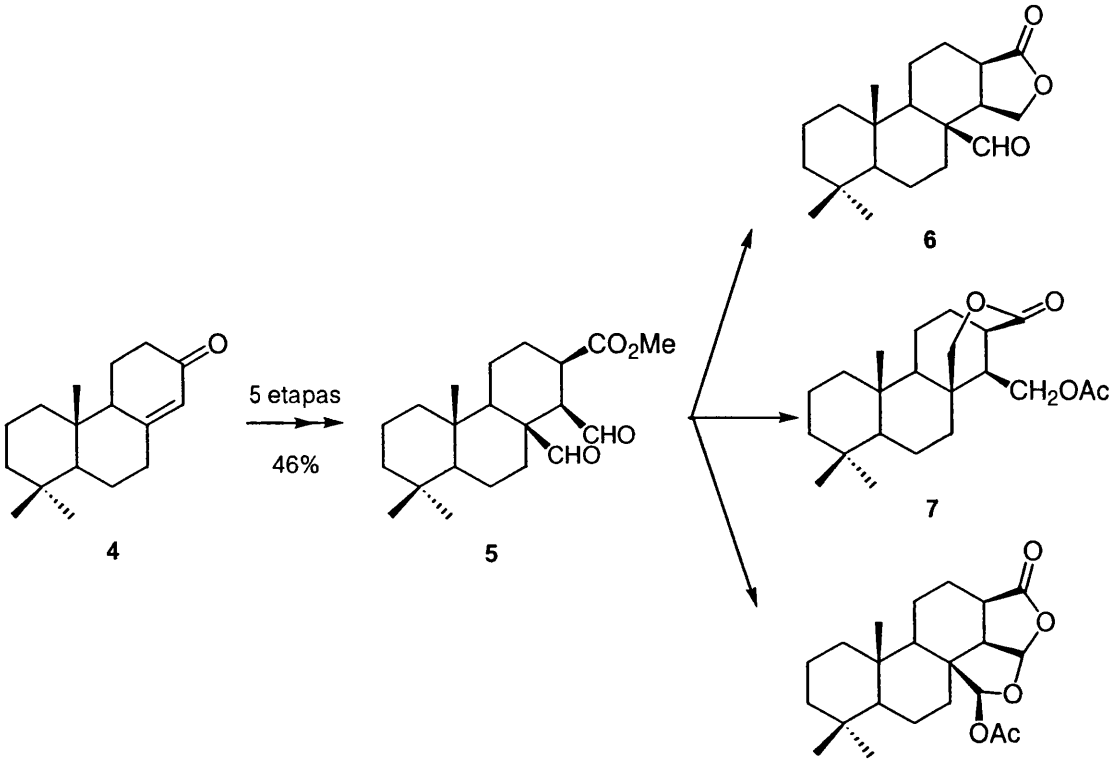
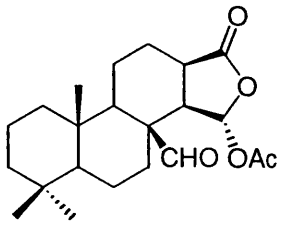
[1] Sakamoto, T.; Kanematsu, K. *Tetrahedron* **1995**, *51*, 5771.

Esquema 2



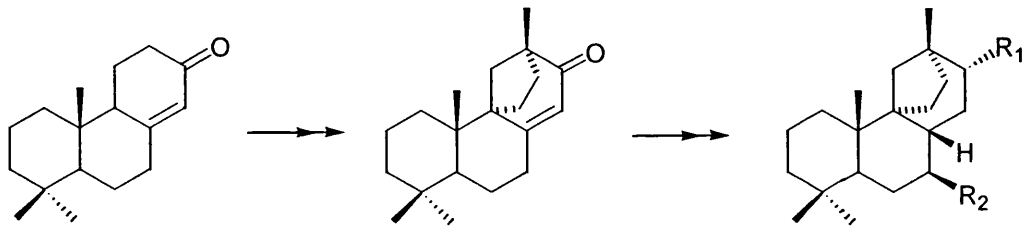

Además, este trabajo sintético ha permitido la evaluación biológica de los productos naturales obtenidos y de algunos derivados. En concreto, se ha evaluado la actividad antiviral contra el virus *Herpes simplex 2* (HSV-2) y la actividad antitumoral en líneas celulares tumorales HeLa y HEp-2. Aunque las actividades antivirales son más bien débiles, algunos de los compuestos ensayados han mostrado cierta toxicidad específica contra las líneas celulares ensayadas. También se han determinado por primera vez las actividades biológicas de *espongianos* que poseen un grupo formilo en C-8 (compuestos **6** y **9**), destacando la actividad del producto sintético **9** que presentó un valor de $CC_{50}$ de 6.3 y 4.4 μg/ml para las líneas celulares HeLa y Hep-2 en 48 h, respectivamente. Este estudio nos ha servido para identificar algunas características estructurales de importancia relacionadas con la actividad citotóxica que presentan estos compuestos.




9

- Partiendo de la podocarpenona quiral **4** también hemos desarrollado una ruta para construir de manera estereoselectiva el esqueleto de *escopadulano*. La secuencia sintética transcurre a través de la enona **10** (Esquema **3**) y ha permitido obtener por primera vez de forma enantioméricamente pura los siguientes escopadulanos: el éster metílico de thyrsiflorin A **11**, el éster metílico del acetato de thyrsiflorin B **12** y thyrsiflorin C **13** con un rendimiento global desde **4** del 20%, 8% y 10%, respectivamente.

Esquema **3**




**4** **10**

**11** $R_1$= $OCOCH_2CO_2Me$;$R_2$= H
**12** $R_1$= $OCOCH_2CO_2Me$;$R_2$= OAc
**13** $R_1$= OH;$R_2$= OH
**14** $R_1$= OH;$R_2$= H



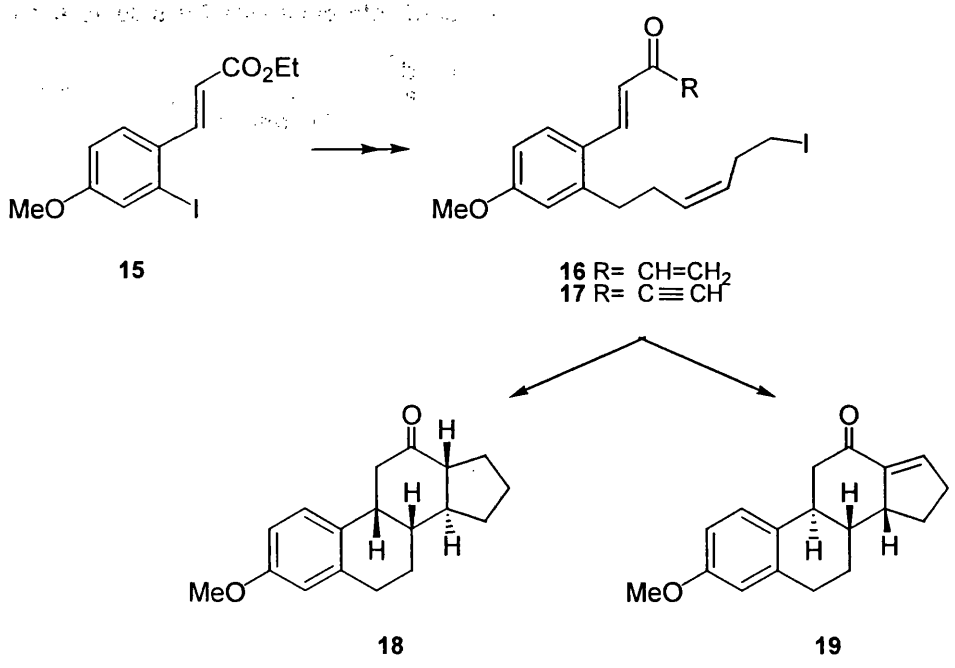
A nivel estructural este nuevo grupo de *escopadulanos* y sus precursores han sido estudiados y caracterizados con detalle mediante resonancia magnética nuclear, lo que ha permitido la asignación de las señales de resonancia de $^1H$ y $^{13}C$ de estos productos. Cabe destacar el estudio realizado del equilibrio conformacional en las podocarpenonas sustituidas en C-12 debido a la existencia de diferentes conformaciones posibles en el anillo C en estas moléculas, lo cuál se refleja en la variación de algunas constantes de acoplamiento en el espectro de $^1H$ según el tipo de sustituyente en C-12 y su orientación.

De forma similar a lo realizado con los *espongianos* y en colaboración con la Universidad de Antioquía (Colombia), se ha evaluado la actividad antiviral contra HSV-2 y la actividad antitumoral en las líneas tumorales HeLa y HEp-2. En este caso, algunos de estos compuestos han mostrado una actividad antiherpética moderada pero de toda la serie de escopadulanos analizados ninguno ha presentado una actividad citotóxica importante en las líneas celulares utilizadas. El compuesto más activo fue el alcohol **14** con un $IC_{50}$= 16.5 μg/ml contra HSV-2.

- En la última parte de esta Tesis se ha desarrollado una estrategia sintética basada en reacciones radicalarias tandem para obtener *esteroides* que poseen el anillo A aromático. Se ha observado que la ciclación radicalaria con los precursores **16** y **17**, obtenidos a partir del yoduro **15** en 9 etapas con un rendimiento del 31% y 46%, respectivamente, transcurre con una estereoquímica diferente (Esquema **4**). Mientras que la vinil-cetona **16** conduce al esqueleto tetracíclico cis-anti-trans **18**, la cetona **17** da el compuesto trans-sin **19** con un rendimiento del 40%. La estructura de este compuesto fue determinada completamente mediante experimentos de resonancia magnética nuclear, y posteriormente fue confirmada por análisis de difracción de Rayos X de monocristal.

Durante la realización de este trabajo también se han realizado, utilizando moléculas bicíclicas como modelo, estudios preliminares para la transformación del producto de ciclación **19** en la hormona estrona.

Esquema **4**



- En conclusión, las rutas sintéticas diseñadas y desarrolladas durante esta Tesis Doctoral permiten la construcción de terpenos naturales que poseen estructuras caracterizadas por esqueletos de *espongiano*, *escopadulano* y *estrano*. La eficacia de estas rutas se ha demostrado con la síntesis de varios productos naturales, algunos de los cuáles fueron aislados en cantidades tan pequeñas que han impedido cualquier evaluación biológica posterior. Fruto de este trabajo sintético se han podido evaluar biológicamente varios de estos productos naturales y derivados, encontrándose diversa actividad biológica. Asimismo se ha demostrado una vez más la versatilidad de la química de radicales en reacciones tandem para la construcción de un esquelet̀o carbonado de forma estereoselectiva.

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Reunido el Tribunal que suscribe en el día de la fecha, acordó otorgar a esta Tesis doctoral de
D. MIGUEL ANGEL GONZALEZ CARDENETE...
la calificación de SOBRESALIENTE CUM LAUDE
Valencia, a 14... de SEPTIEMBRE.... de 2.001
El Secretario,

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