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UNIVERSITY OF ALBERTA

I. METABOLITES OF SIROCOCCUS AND GODRONIA SPECIES
II. STUDIES RELATED TO THE SYNTHESIS OF STERPURIC ACID

BY

YU-TING MA 

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUREMENTS FOR THE DEGREE OF

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THE UNDERSIGNED CERTIFY THAT THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED I. METABOLITES OF SIROCOCCUS AND GODRONIA SPECIES II. STUDIES RELATED TO THE SYNTHESIS OF STERPURIC ACID SUBMITTED BY YU-TING MA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT

Chapter I:

The metabolites of three strains of a *Sirococcus* fungus (UAMH 5401, 5402, and 5394) which cause Sirococcus shoot blight, have been studied. In addition to the known compounds, 9(11)-dehydroergosterol (**1**), ergosta-4,6,8(11),22-tetraen-3-one (**2**), sclerodin (**3**), Scleroderris blue (**5**), the acetone adduct of atrovenetinone (**8**), lactone **9**, and trypethelone (**11**), three new natural products, sirocodilide (**12**), sirocodin (**18**), and sirocodinine (**36**) were isolated. The structures of the new compounds were determined by a combination of spectroscopic techniques, the formation of chemical derivatives, and biogenetic considerations.

The structure and absolute stereochemistry of **12** was established by chemical transformation of **12** to the known compound (S)-β-hydroxy-n-caprohydrazide (**14**). Twelve derivatives of **18** and **36** were prepared. The central ring joining the sterol part and the pigment portion of **18** and **36** could not be opened under various reaction conditions. We also found that the culture medium (PDY and wort) has an effect on the optical purity of sclerodin (**3**).

The metabolites of two other fungi, *Godronia mytilli* T256 and *G. cassandrae*, were also examined. It was found that the metabolites produced by these two fungi are similar to those isolated from the *Sirococcus* species.

Several ergosterol derivatives were prepared during the determination of the structure of sirocodin (**18**) and sirocodinine (**36**). 6α-Hydroxyergosta-4,7,22-trien-3-one (**52**), a metabolite isolated from the fungus *Ganoderma lucidum*, was synthesized from ergosterol. The earlier incorrect stereochemical assignments of **52** and related compounds have been corrected.

Chapter II:

Studies towards the total synthesis of sterpuric acid (**1**), a compound isolated from the fungus *Stereum purpureum* which causes the so-called "silver leaf" disease, have been carried out. An intermediate **86** was constructed in 16 steps using the approach developed in our laboratories. An alternative route to sterpuric acid was also investigated. Bicyclic compound **131** was synthesized efficiently from commercially available material.

Ring B was constructed via a Diels-Alder reaction of acetate **55** and maleic anhydride. The resulting adduct **56** was transformed to the bicyclic compounds **72** and **73** by a cycloalkylation reaction utilizing dimethyl malonate. Transformation of **72** and **73** to **86** was easily achieved.

Attempts to improve the yield in the protection of triol **57** and the alkylation of tosylate **60** were unsuccessful. An alternative approach to the synthesis was then developed and the AB ring intermediate **131** was prepared. Diels-Alder reaction of acetate **55** and 4-oxobutenate **112** or **113** provided monocyclic compounds **114** and **119**. Conversion of **114** and **119** to ester **128** was achieved in good yield by chemical transformations. The bicyclic compound **131** was readily obtained using the previously developed methods. This approach circumvents some of the problems of the previous approach.

ACKNOWLEDGEMENTS

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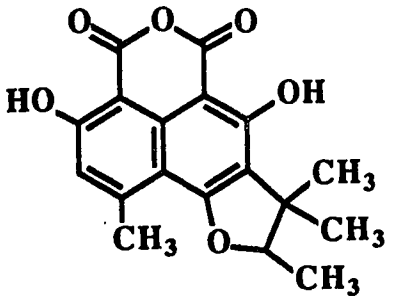
INTRODUCTION

Sirococcus strobilinus Preuss (syn.*Ascochyta piniperda* Lindau), the fungus associated with shoot blight of conifers, has been reported on pine, spruce, true fir, and western hemlock in Europe and North America.[1] In Canada, the pathogen was found in British Columbia[2] and the Maritime provinces.[3] Disease symptoms resemble those caused by winter injury; its typical appearance is characterized by the death of the leader, the youngest branch whorl, and the upper part of the last year's internodium. Not all shoots on a tree are attacked in a single year, but the injury is cumulative. The lower branches of infected large trees die first, and the pathogen usually advances further upward each year. Eventually, the tree will be killed. The disease, known for a century, is similar to the Scleroderris canker disease caused by the Ascomycetous fungus *Gremmeniella abietina*.[4] The metabolites produced by *G.abietina* were well studied by Ayer and coworkers. Several phenalenones derivatives including sclerodin (I), atrovenetinone (II), sclerodione (III), scleroderolide (IV), Scleroderris blue (V) , and Scleroderris green (VI) were isolated from *G.abietina*.[5-8] It was suggested that the greenish discoloration of the wood of Scleroderris infected pine may be due to the presence of Scleroderris green (VI). Our interest in the Scleroderris metabolites led us to examine several strains of a fungus which was believed to belong to the related genus *Sirococcus*. This work was prompted by a request by Professor Otto Kandler, Botanical Institute, University of Munich, to determine whether there was a chemotaxonomical relationship between these two groups of fungi.

Three strains, strain 20 (UAMH 5402), strain 35B (UAMH 5401), and STM8 (UAMH 5394) were isolated by Professor Kandler from Norway spruce in the mountains and in the Alps of southern Germany.[9] Since we began our studies, it has been suggested that the fungus may be *Godronia cassandrae* rather than



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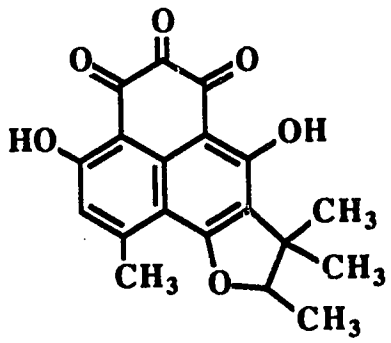

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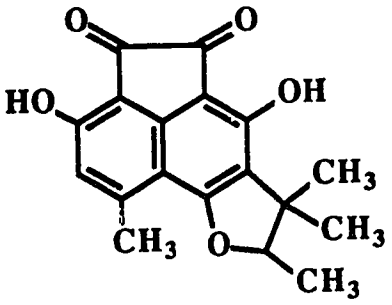

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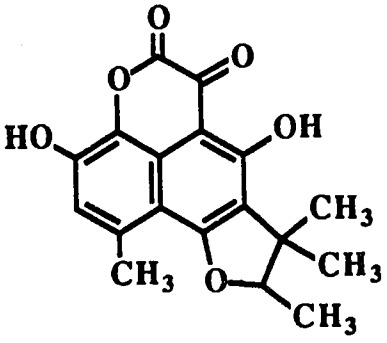

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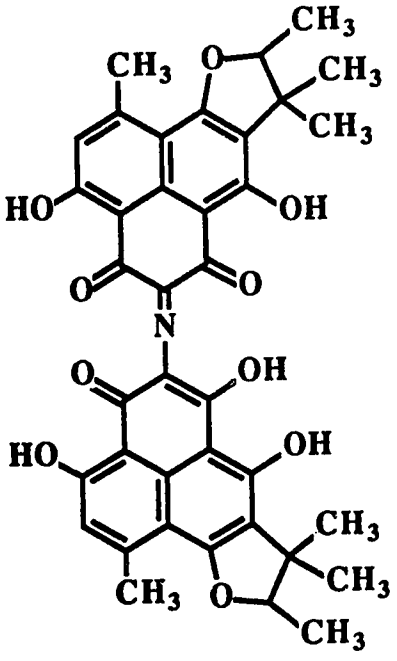

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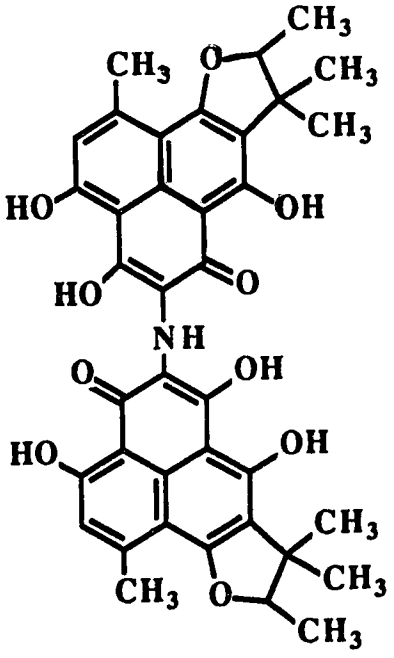


VI

Sirococcus strobilinus.[10] However, the identity of these strains is still not clear. Studies at the University of Alberta Microfungus Collection and Herbarium

(UAMH) have suggested that strain 20 (UAMH 5402) is morphologically related to *G. abietina*, while strain 35B (UAMH 5401) is morphologically closer to *Sirococcus strobilinus*.[11] Because of the identification problem with these strains, we have undertaken a comprehensive chemical study to compare the metabolites produced by these strains. For comparison purposes, two true Godronia species from West Germany, *G. myrtilli* T256 and *G. cassandrae*, were also included in our investigation. *G. cassandrae* has been reported as a pathogen of blueberries in the United States[12] and Canada.[13] The fungus is also associated with dying of seedlings of trembling aspen and gray, white, and yellow birch. On speckled alder and willow, it causes mortality of branches and tops of trees of all ages. The disease symptoms are very similar to those of *Sirococcus strobilinus* which in turn are similar to those of *Gremmeniella abietina*. The metabolites produced by these various fungi are reported herein.

RESULTS AND DISCUSSION

Strain 20 (UAMH 5402), strain 35B (UAMH 5401), strain STM8 (UAMH 5394), *Godronia myrtili* T256, and *G. cassandrae* were grown in liquid culture on both potato dextrose-yeast (PDY) and Wort media, since both media had been used by Professor O. Kandler for these strains. After growing the fungus in still culture for about 60 days, the mycelium was separated from the broth. Soxhlet extraction (CH_2Cl_2) of the mycelium provided a large amount of metabolites. Extraction of the culture broth provided relatively small amounts of metabolites. In the cases of strain STM8 and *G. cassandrae*, neither fungus produced pigments. The methylene chloride extracts obtained from both PDY and Wort media are similar by thin layer chromatography (TLC) and thus we studied only the metabolites produced by strain STM8 in PDY medium and by *G. cassandrae* in Wort medium. Strain 20 (UAMH 5402), strain 35B (UAMH 5401), and *G. myrtillis* T256 all produced a colorful array of metabolites in both PDY and Wort media. Careful studies of the metabolites produced in both media by these fungi were undertaken.

Since there were much less metabolites in the broth, we did not investigate these further except in one case. The methylene chloride extract of broth from strain 35B in PDY medium was red in colour which prompted us to study it further (as discussed later the purple compound trypethelone was isolated).

The metabolites from different extracts were separated by repeated silica gel chromatography. Triglycerides and fatty acids are the major components making up 50~90% of the original extracts. *G. cassandrae* produced only triglycerides; no other metabolites were detected. Ergosterol and ergosterol endoperoxide are the only two other compounds isolated from strain STM8 (UAMH 5394). This result was surprising to us since strain STM8 was believed to be a true *Sirococcus strobilinus*.

G. cassandrae and strain STM8 fail to produce any pigments or any metabolites possessing the phenalenone skeleton. A similar result was encounted previously with strain C-656 of *G. abietina* (Lagerb). Strain C-656 only produced fatty acids, glycerides, and sterols.[7] It is not clear to us why these strains do not produce the typical pigments. Are they misidentified? From the viewpoint of our study, are *G. cassandrae*, strain STM8, and strain C-656 of *G. abietina* closely related?

Ergosterol was also isolated from strain 35B (PDY medium) and *G. myrtilli* T256 (Wort medium); ergosterol endoperoxide from strain 20 (Wort medium) and *G. myrtilli* T256 (PDY medium). Ergosterol is the most commonly occurring fungal sterol. Ergosterol endoperoxide, which may be an artifact, is formed from ergosterol by photooxygenation.[14]

Several known compounds have been isolated in this study. These include 9(11)-dehydroergosterol endoperoxide (**1**), ergosta-4,6,8(14),22-tetraen-3-one (**2**), sclerodin (**3**), Scleroderris blue (**5**), the acetone adduct of atrovenetinone (**8**), lactone **9**, and trypethelone (**11**). In addition, three new natural products were obtained. The identification of the known compounds and the structure elucidation of the new compounds is discussed below.

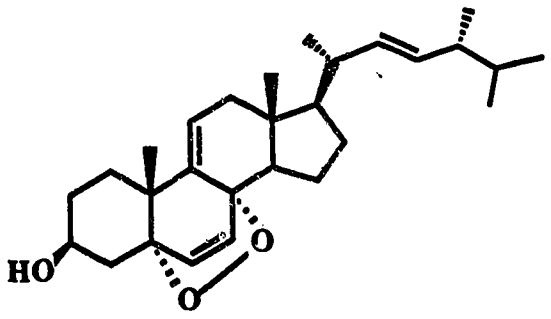
9(11)-dehydroergosterol endoperoxide (1)

and ergosta-4,6,8(14),22-tetraen-3-one (2)

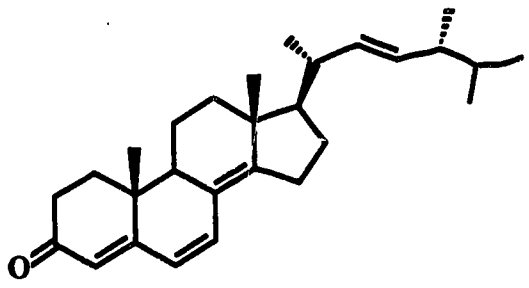
We isolated both 9(11)-dehydroergosterol endoperoxide (**1**) and ergosta-4,6,8(14),22-tetraen-3-one (**2**) from strain 35B (PDY) and from *G. myrtillis* T256 (PDY); the former compound was also isolated from strain 20 (PDY), the latter compound was also obtained from strain 20 (Wort medium) and from *G. myrtilli* T256 (Wort medium).

The molecular formula of 1 is $C_{28}H_{42}O_3$ (MW 426) as determined from its high resolution mass spectrum. A detailed analysis of the major fragments suggests 1 is a dehydroergosterol endoperoxide[15,16]: m/z 408 (M-H_2O), 394 (M-O_2), 376 (M-H_2O-O_2), 299 (M-$C_7H_{11}O_2$), 269 (M-H_2O-side chain), 251 (M-H_2O-O_2-side chain). It displays a broad hydroxyl absorption in the infrared (ir) spectrum at 3400 cm^{-1}. The ^{1}Hnmr spectrum shows six methyl groups at δ1.22(s), 1.12 (d, J=6), 1.06 (d, J=7), 0.95 (d, J=7), 0.92 (d, J=7), and δ0.85 (s) ppm and a carbinolic hydrogen at δ4.08 ppm. The chemical shifts of the vinylic hydrogens at δ6.58 (d, J=8), 6.27 (d, J=8), 5.22(dd), and 5.13 (dd) ppm are consistent with those of ergosterol endoperoxide. Another alkenic hydrogen at δ5.53 (dd, J= 6, 1.5Hz) ppm suggested 1 is 9(11)-dehydroergosterol endoperoxide. The nuclear Overhauser effect (nOe) experiment results are also consistent with structure 1. Irradiation of the 19-methyl group (δ1.22 ppm) gave a 2.3% nOe enhancement of a hydrogen at δ5.53 ppm (H-11), while irradiation of 18-methyl group at δ0.85 ppm and 6-H at δ6.27 ppm gave 6% and 7.7% enhancements of H-7 at δ6.58 ppm, respectively.

9(11)-Dehydroergosterol endoperoxide is a compound previously reported from natural sources.[17,18] The spectroscopic data are in agreement with those reported.



1



2

Compound **2** is an ultraviolet (UV) active substance. From the high resolution mass spectrum (hrms), the molecular formula of **2** is $C_{28}H_{40}O$. The ir spectrum displays a carbonyl absorption at 1645 cm^{-1} and double bond absorption at 1638 cm^{-1}. The ^{1}Hnmr shows one signal assigned to the olefinic hydrogens on the side chain at δ5.22 ppm and signals for three more olefinic hydrogens at δ5.73 (s), 6.04 (d, J=5.4), and 6.62 (d, J=5.4)ppm. Considering the chemical shifts and the coupling pattern, the compound was assigned structure **2**. Ergosta-4,6,8(14)22-tetraen-3-one (**2**) has been isolated from several other fungi.[14] A comparison of spectroscopic data with those reported[19] confirms its identity.

Sclerodin (3)

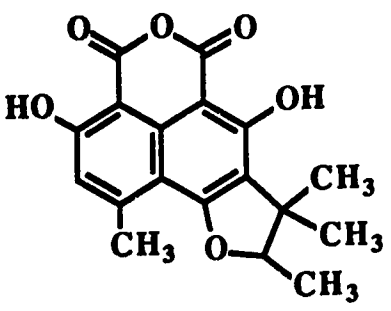
Sclerodin, a strongly fluorescent compound, was isolated from strain 20 (PDY and Wort media), from strain 35B (PDY and Wort media), and from *G. myrtillis* T256 (PDY and Wort media). Compound **3**, mp. 256-257^{0}C, shows carbonyl absorption in the ir at 1710 and 1666 cm^{-1}. The hrms suggested the molecular formula $C_{18}H_{16}O_6$ (MW 398). The ^{1}Hnmr spectrum shows signals for 16 hydrogens: two hydrogen-bonded phenolic hydrogens (δ11.78 ppm,s; δ12.44 ppm, s), an aromatic hydrogen (δ6.80 ppm, s), a methine hydrogen α to oxygen (δ4.66 ppm, q, J= 7), an aromatic methyl (δ2.76 ppm, s), a doublet methyl (δ1.44 ppm, d, J=7), and two singlet methyls (δ1.24, 1.58 ppm).

Comparison of **3** with an authentic sample verified its structure. The spectroscopic data are identical with those of the authentic sample. However, the optical rotations of **3** isolated from these fungi are quite different from the reported value.[6] The variation of optical purity of sclerodin (**3**) is show in table I-1.

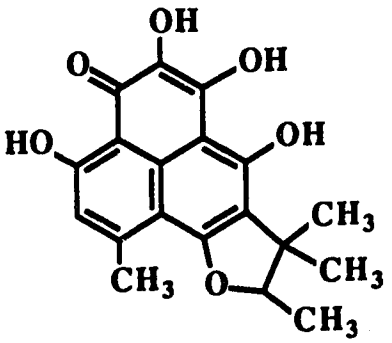
Table I-1 Variation in optical purity of sclerodin (3)

Fungus (medium)	$[\alpha]_D$
G. abietina (V8G)	-73^0 (reference 6)
Sirococcus 20 (PDY)	$+2.3^0$ (c, 0.43, $CHCl_3$)
Sirococcus 20 (Wort)	-28.1^0 (c, 0.41, $CHCl_3$)
Sirococcus 35B (PDY)	-1.1^0 (c, 0.28, $CHCl_3$)
Sirococcus 35B (Wort)	-34.0^0 (c, 0.35, $CHCl_3$)
G. myrtillis T256 (PDY)	-30.0^0 (c, 0.26, $CHCl_3$)

In the case of strain 20 and 35B, the optical rotations of sclerodin produced in PDY medium are nearly zero, indicating that sclerodin is almost totally racemic. However, sclerodin produced in Wort medium is only partially racemic. It is interesting to note that the culture medium (PDY and Wort) affects the optical purity of sclerodin. The reason for this is not clear to us.



3



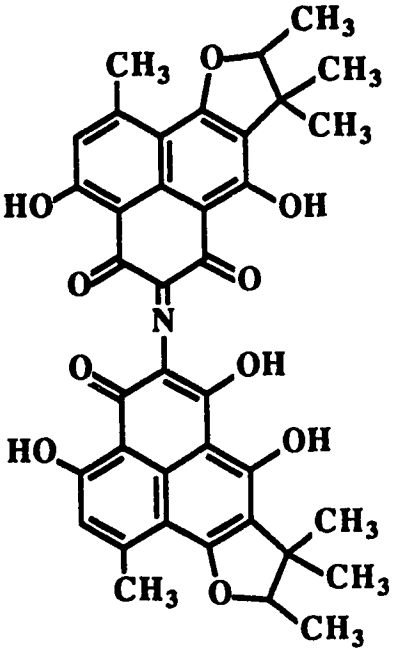
4

Sclerodin (3) had been isolated from *G. abietina* by Ayer and coworkers.[5] Sclerodin is the enantiomer (S configuration at C-2') of the so-called naphthalic anhydride which was obtained from atrovenetin (4) by oxidation.[20,21] The absolute configuration of the (+) form of the anhydride has been determined by X-ray crystallography.[22] The (+) form has been obtained from *Penicillium herqui*,[23] *Roesleria pallida*[24] and *Aspergillus silvaticus*.[22] The (-) form has only been reported from *G. abietina*.[5] We find the different optical purity of sclerodin in our cases puzzling. In order to understand these unusual results, further studies are necessary.

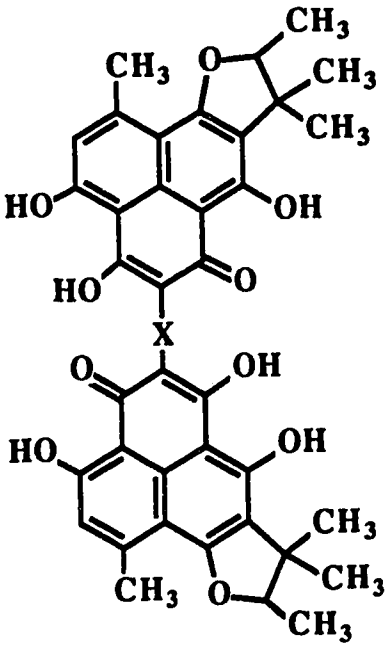
Scleroderris Blue (5), the Acetone Adduct of Atrovenetinone (8), Lactone (9), and Trypethelone (11)

Scleroderris blue (5), a rather unstable compound, was isolated from strain 20 (PDY and Wort media), strain 35B (PDY and Wort media), and *G. myrtillis* T256 (PDY and Wort media). Compound 5 appears deep blue when it is isolated (CH_2Cl_2/MeOH/HOAc, 94:2:2), but it changes color gradually to green at room temperature in solution. A pure sample of 5 is identical with an authentic sample. Scleroderris blue was previously isolated from *G. abietina* by Ayer *et al.*[5] and from *Roesleria hypogea* by Bachmann *et al.*[25] Ayer and coworkers prepared 5 from atrovenetinone (6) and an amino acid by a ninhydrin-like reaction. Thus 5 was obtained by treatment of a buffered solution of glycine with 6 in aqueous dioxane. It was suggested that Scleroderris blue (5) may be an artifact derived from Scleroderris green (7) which was also isolated from *G. abietina*.[8] In our cases, we believed Scleroderris green (7) was also produced by strain 20, strain 35B, and *G. myrtillis* T256, since a green colored compound had changed to the blue colored compound when the separation was carried out using acidic solvent (CH_2Cl_2/MeOH/HOAc,

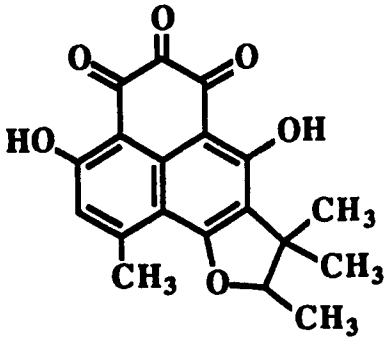
96:2:2). Structurally, Scleroderris blue (5) and Scleroderris green (7) are closely related to Scleroderris yellow (7a) whose structure has been determined recently by us.[26] Scleroderris yellow (7a) was isolated from a Sirococcus strain 33B (UAMH 5400) in our laboratory. It has been suggested that the greenish discoloration of the wood of pines suffering from Scleroderris canker may be due to the compound 5 and/or 7. It is possible that compounds 5 and 7 may also play a role in the disease of Sirococcus shoot blight or the disease caused by *G. myrtillis* T256.



5



7 X=NH
7a X=CH_2



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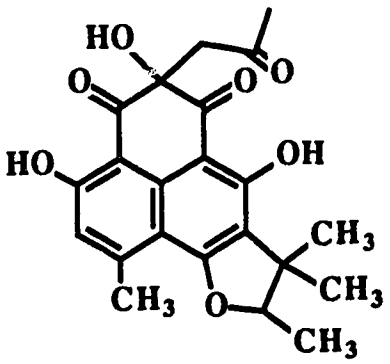
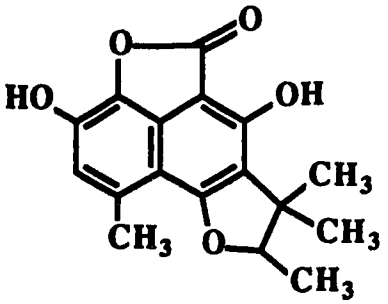


8

The acetone adduct of atrovenetinone (**8**), an artifact formed during isolation (acetone as eluant), was isolated from strain 35B (Wort medium). The hrms suggested the molecular formula $C_{22}H_{22}O_7$ (MW 398). In the ^{1}Hnmr and ^{13}Cnmr spectra, most of the peaks are doubled. This indicates that **8** is a mixture of epimers (ratio *ca.*1:1). The ^{1}Hnmr spectrum is similar to that of sclerodin (**3**) with most signals doubled except for three additional signals attributable to the acetone moiety (δ2.18, 6H,s; δ3.26, 2H, s; δ3.28, 2H,s). The spectroscopic data of compound **8** are identical with that of an authentic sample previously obtained in our laboratory.[5] Ayer *et al.* have verified that compound **8** is an artifact by treatment of **6** with acetone in the presence of a catalytic amount of acetic acid. Since compound **6** is very unstable, formation of its acetone adduct has proved to be a better way to trap and isolate it.

Lactone **9**, an oxidative product of sclerodione (**10**), has been isolated from strain 20 (PDY medium). The ir spectrum of **9** shows the ester absorption at 1725 cm^{-1}. The ^{1}Hnmr spectrum of **9** is very similar to that of **10**. The hrms spectrum indicates a molecular formula $C_{17}H_{16}O_5$ (MW 300) which is one carbon less than sclerodione (**10**). This evidence suggested that the compound has structure **9**. This is the first time that compound **9** has been isolated from a natural source. Compound **9** was previously obtained by alkaline peroxide oxidation of sclerodione (**10**).[5] Our sample of lactone **9** is identical with an authentic sample.



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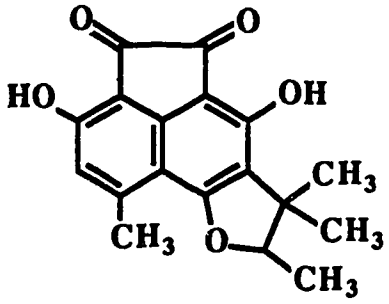
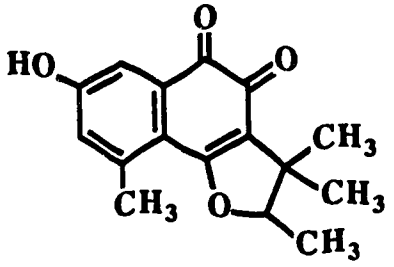


10

Trypethelone (11), the only compound obtained from the broth, was isolated from strain 35B (PDY). The purple compound 11 has a molecular formula $C_{16}H_{16}O_4$ (MW 272) according to the high resolution mass spectrum. In the ^{1}Hnmr spectrum, two doublets for meta-substituted aromatic hydrogens are present at δ6.87 (d, J=2Hz) and δ7.43 (d, J=2Hz). The aromatic methyl signal appears at δ2.58 ppm. The remaining signals are very similar to those of the modified isoprene residual in compounds 3-10. A search of the literature revealed that this compound is trypethelone. Trypethelone was previously isolated from the tropical cortical lichen *Trypethelium eluteriae* Sprengel and has been shown to have antibiotic activity.[27] The spectral data for compound 11 are in agreement with those reported.



11

Sirocodilide (12)

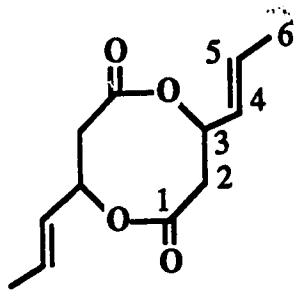
We have isolated from strain 35B (PDY medium) a crystalline compound which we named sirocodilide.

Sirocodilide is an optically active ($[\alpha]_D$+40.8^0 (*c* 0.5, $CHCl_3$)), UV inactive compound, with a melting point of 121-123^{0}C (from methylene chloride-ethanol). The ir spectrum shows the presence of carbonyl at 1745 cm^{-1} and double bond at 1680 cm^{-1}. The hrms suggested a molecular formula $C_{12}H_{16}O_4$ (MW 224) which requires 5 unsaturations in the molecule. The mass spectrum shows two

fragments accounting for all of the molecule at m/z 129 ($C_6H_9O_3$, 13.4%) and at m/z 95 (C_6H_7O, 100%). The latter may be assigned to a sorbyl fragment.

The ^{13}Cnmr spectrum of sirocodilide shows 6 signals, indicating an element of symmetry in the molecule. There is a lactone carbon at δ168.72 ppm (s), two sp^2 carbon doublets at δ130.59 and δ127.91 ppm, one oxygenated carbon at δ71.28 ppm (d), a methylene carbon at δ39.59 ppm (t), and a methyl carbon at δ17.70 ppm (q). The signals in the ^{1}Hnmr also indicate the symmetry. A doublet of doublets methyl group at δ1.72 ppm (dd, J=1.5, 6.5Hz) is coupled to a hydrogen at δ5.41 ppm (ddq, J=1.5, 7, 15.5Hz) and a hydrogen at δ5.81 ppm (dq, J=6.5, 15.5Hz). The latter two hydrogens are coupled to each other with a large coupling constant of 15.5Hz indicating they are olefinic hydrogens *trans* to each other. The hydrogen at δ5.41 ppm is also coupled to a hydrogen at δ5.65 ppm (ddd, J=4.5, 7, 9Hz) which is coupled to two other hydrogens at δ2.57 ppm (dd, J=4.5, 16Hz) and δ2.68 ppm (dd, J=9, 16Hz). The hydrogens at δ2.57 and 2.68 ppm are geminal hydrogens adjacent to a carbonyl or double bond group because these two hydrogens are coupled to each other with a large coupling constant of 16Hz.[28] Considering the chemical shift of the hydrogen at δ5.65 ppm, we assigned it α to an oxygen and adjacent to a double bond.

Since sirocodilide is dimeric, all the carbons and hydrogens are accounted for. Four unsaturations may be attributed to the two double bonds and two lactones, leaving one unassigned unsaturation, indicating that sirocodilide is monocyclic. Structure **12** was proposed based on the above evidence.



12

The complete ^{1}Hnmr and ^{13}Cnmr assignments are compiled in Table I-2.

Table I-2 Assignment of ^{1}H and ^{13}Cnmr data for sirocodilide (12)

Position	^{13}Cnmr data	^{1}Hnmr data
1	168.72 (s)	
2	39.59 (t)	2.57 (dd, 9, 16)
		2.68 (dd, 4.5, 16)
3	71.28 (d)	5.65 (ddd, 4.5, 7, 9)
4	127.91 (d)	5.41 (ddq, 1.5, 7, 15.5)
5	130.59 (d)	5.81 (dq, 6.6, 15.5)
6	17.60 (q)	1.72 (dd, 1.5, 6.5)

Decoupling experiments (Table I-3) and a COSY-90 2Dnmr confirmed the connectivity of all hydrogens. When the methyl signal at δ1.72 ppm is irradiated, the signal at δ5.81 ppm (dq) becomes a doublet and the signal at δ5.41 ppm (ddq) changes to a doublet of doublets.

In the COSY-90 2Dnmr, the methyl group correlated with two olefinic hydrogens at δ5.41 and δ5.81 ppm and the CH_2 group correlated with a hydrogen at δ5.65 ppm which is also correlated with the hydrogen at δ5.41 ppm (Figure I-1).

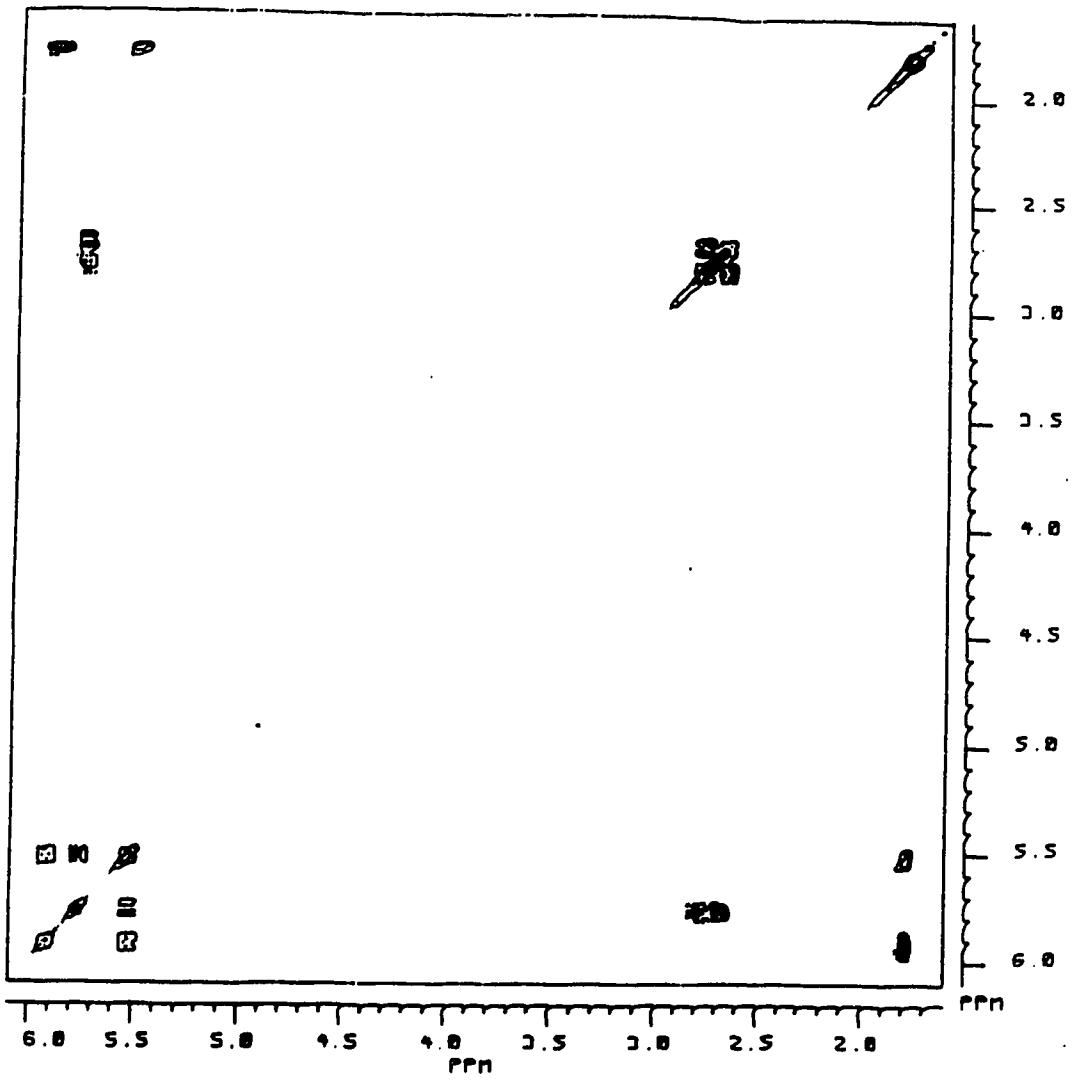


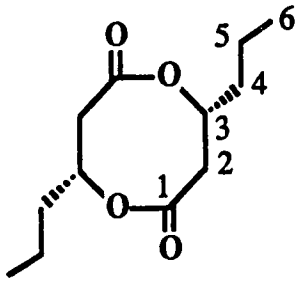
Figure I-1 $^{1}H/^{1}H$ COSY spectrum of sirocodilide (12) ($CDCl_3$, 360MHz, COSY 90, contour plot)

Table I-3 Spin decoupling data for sirocodilide (12)

Signal irradiated	Observed change
H-6 1.72	H-5 5.81 dq→d (15.5Hz)
	H-4 5.41 ddq→dd (15.5, 7Hz)
H-5 5.81	H-6 1.72 dd→d (1.5Hz)
	H-4 5.41 ddq→dq (1.5, 7Hz)
H-2 2.57	H-3 5.65 ddd→dd (4.5, 7Hz)
	H-2 2.68 dd→d (4.5Hz)

Since sirocodilide (12) is optically active, the element of symmetry in the molecule must be a proper axis of symmetry. Comparing the optical rotation with that of (S)-3-hydroxyhexanoic acid ($[\alpha]_D+30^0$ (*c* 2, $CHCl_3$)),[29] we assigned both chiral centers in sirocodilide (12) as S.

In order to confirm the assigned stereochemistry of sirocodilide, the following transformations have been carried out. Hydrogenation of sirocodilide with platinum oxide or 10% palladium on carbon in ethanol provided tetrahydrosirocodilide (13) after separation by flash chromatography.



13

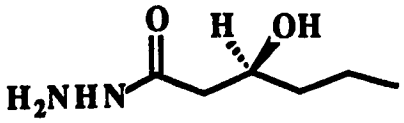
In the ir spectrum, 13 shows lactone absorption at 1742 cm^{-1}. The low resolution mass spectrum afforded the expected molecular ion at m/z 228 (6.4%).

^{1}Hnmr displays all 10 hydrogen signals: a triplet methyl at δ0.89 ppm (J=7Hz), two multiplets of two hydrogens at δ1.31 and δ1.54 ppm, a hydrogen α to oxygen at δ 5.26 ppm (ddt, J=5, 6, 7Hz), two geminal doublet of doublets hydrogens at δ2.57 ppm (J=7, 15Hz) and δ2.49 ppm (J=5, 15Hz). The hydrogen at δ5.26 ppm is coupled to the two geminal hydrogens. Decoupling experiments (Table I-4) confirmed the assigned structure **13**.

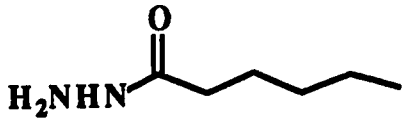
Table I-4 Spin decoupling data for tetrahydrosirocodilide (**13**)

Signal irradiated	Observed change
H-6 0.89	H-5 1.31 simplify
H-5 1.31	H-6 0.89 t→s
	H-4 1.54 simplify
H-4 1.54	H-5 1.31 simplify
	H-3 5.26 ddt→dd (5, 7Hz)
H-3 5.26	H-2 2.49 dd→d (15Hz)
	H-2' 2.57 dd→d (15Hz)
	H-4 1.54 simplify
H-2, H-2' 2.57, 2.49	H-3 5.26 ddt→t (6Hz)

The hydrogenation products without separation (from 3.9mg **12**) were heated on the hot water bath with 2 drops of anhydrous hydrazine for a half hour. Ethanol was then added and the solution was refluxed for two hours. The products were separated by silica gel chromatography providing 1.2mg of (S)-3-hydroxy-*n*-caprohydrazide (**14**) and 0.5mg of *n*-caprohydrazide (**15**).



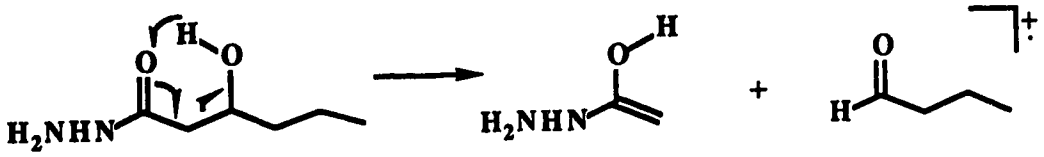
14



15

The ir of **14** shows NH and OH absorptions at 3300, 3217, 3209, and 3201 cm^{-1} and hydrazide absorption at 1645 and 1620cm^{-1}.[30] All hydrogens of **14** were observed in the ^{1}Hnmr spectrum. Three broad NH signals appeared at δ7.02, 3.92, and 3.14 ppm and the hydroxyl appeared at δ1.59 ppm as a broad signal. The two hydrogens at 2 position are coupled to each other at δ2.35 ppm (dd, J=2.7, 14Hz) and 2.25 ppm (dd, J=8.3, 14Hz) and also coupled to the hydrogen geminal to the hydroxy at δ4.03 ppm (ddt, J=2.7, 6.7, 8.3Hz). The remaining hydrogens are at δ1.42 ppm (4H, m) and 0.95 ppm (3H, t, J=6.5Hz). The decoupling experiments verified the structure: when the hydrogen at 4.03 ppm is irradiated, both hydrogens at δ2.35 ppm and 2.25 ppm change from a doublet of doublets to a doublet. Upon irradiation of the multiplet hydrogens at δ1.42 ppm, the methyl signal changes to a singlet and the hydrogen at δ4.03 ppm collapses to a broad doublet. The ^{13}Cnmr spectrum also supports the structure and shows all six carbons at δ172.94, 68.32, 40.89, 39.13, 18.67, and 13.91 ppm.

In the mass spectrum of **14**, the peak corresponding to the molecular ion (MW 146) was absent. However, a peak at m/z 72 (M-$C_2H_6N_2O$) was the base peak which may be derived by a McLafferty rearrangement (equation I-1).[31]



m/z 72 (100%) I-1

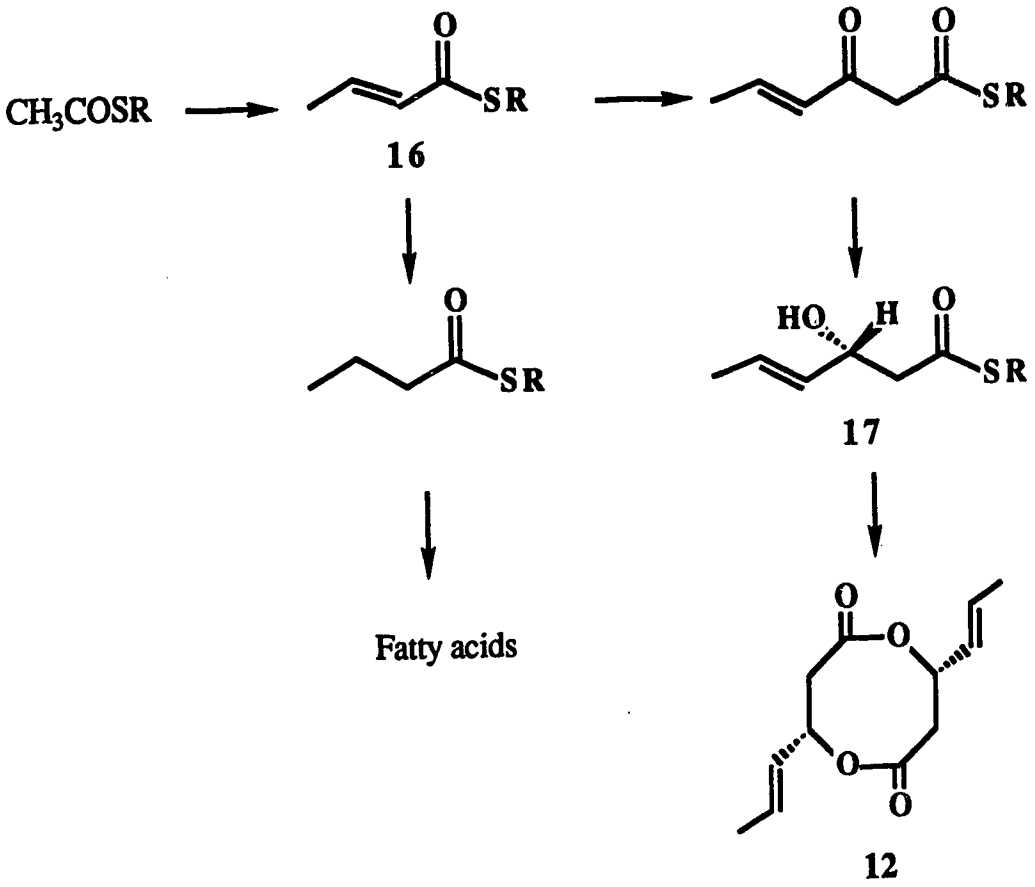
The peaks corresponding to M-NH_2, M-H_2O, M-NH_2-OH, and M-N_2H_3-H_2O were also observed in the mass spectrum of **14**.

The optical rotation of (S)-3-hydroxy-*n*-caprohydrazide (**14**) derived from sirocodilide is $+16.7^0$ (*c* 0.12, $CHCl_3$) which is consistent with the reported value of $+15.9^0$ (*c* 0.75, H_2O).[32] Therefore, the absolute configuration of the two chiral centers in sirocolidide (**12**) is unambiguously determined as (S).

n-Caprohydrazide (**15**) may be derived from the hydrogenolysis of sirocodilide (**12**) and subsequent reaction with hydrazine. The crystalline compound **15** shows in the ir spectrum the hydrazide absorption at 1630 cm^{-1} and NH absorptions at 3313, 3292 cm^{-1}. The ^{1}Hnmr spectrum displays two broad singlets at δ6.64 ppm (1H) and 3.90 ppm (2H) for NH, a methylene triplet at δ2.16 ppm, a methylene quintet at δ1.67 ppm, two methylenes at δ1.33 ppm, and a methyl triplet at δ0.91 ppm. The molecular ion (MW 130) was observed in the mass spectrum, together with peaks corresponding to M-NH_2 and M-N_2H_3.

Since the polyketide-derived compounds, sclerodin (**3**) and Scleroderris blue (**5**) as well as fatty acids were isolated from the same fungus, sirocodilide (**12**) may be biogenetically synthesized at an early stage of polyketide synthesis (at the three acetate unit level). Condensation of **16** with another molecule of malonyl-CoA, followed by reduction of the β-ketone to alcohol, gives the monomer **17**. Dimerization of **17** leads to sirocodilide (Scheme I-1).

Scheme I-1 Biogenetic formation of sirocodilide (12)

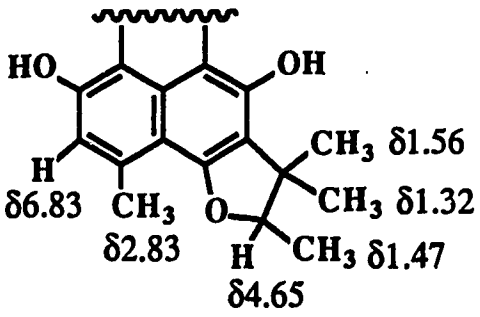


Sirocodin (18)

Sirocodin (18), a yellow compound, is isolated from strain 35B (PDY medium) and from *G. myrtillis* T256 (PDY and Wort media). When sirocodin was initially isolated, we obtained its ^{1}Hnmr spectrum. It seemed to us that it might be a mixture of a sterol and a phenalenone type compound (pigment), since we isolated several sterols and sclerodin-type compounds from the same fungus. Another reason for doubting the purity of 18 was that the hrms and chemical ionization mass spectrum (cims) did not show a clear molecular ion. Therefore, we tried further purification of it by using different methods. Repeated silica gel chromatography

using different solvent systems proved unsuccessful. We then methylated the compound with diazomethane and hoped to separate the sterol and the pigment. Again the supposed two compounds could not be separated! We finally realized that this compound is an adduct of a sterol and a pigment. Many attempts to crystallize this substance failed. The ir spectrum of **18** shows the presence of hydroxyl groups at 3390, 3384 $cm^{-1}$and a strongly chelated carbonyl group at 1611 cm^{-1}.[33] Hrms and cims did not provide the molecular ion. However, the fast atom bombardment mass spectrum (fabms) shows an ion at 737.48 ($M+H^+$, 2.67%) which corresponds to a molecular formula of $C_{47}H_{60}O_7$.

The ^{1}Hnmr spectrum of **18** is very similar to that of a mixture of a sterol and a pigment. The signals for the pigment part are shown as follows. Two downfield signals at δ17.16 ppm and δ9.56 ppm indicate that there are two phenols, one is strongly chelated and the other is not. Both signals disappear upon the addition of D_2O. An aromatic hydrogen and an aromatic methyl group appear at δ6.83 ppm and δ2.83 ppm. The signals at δ4.65 (1H), 1.56 (3H, s), 1.47 (3H, d, J=6), and δ1.32 (3H, s) are typical of the modified isoprene residual in compounds **3-11**. These data indicate the presence of partial structure **19** in sirocodin.



19

There are six methyl groups attributed to the sterol part in the ^{1}Hnmr spectrum of **18** at δ1.14 (s), 1.00 (d, J=6 Hz), 0.88 (d, J=6 Hz), 0.81 (d, J=5.3 Hz), 0.80 (d, J=5.3 Hz), and δ0.57 (s) ppm. Two olefinic hydrogens at δ5.15ppm are

characteristic of the double bond hydrogens on the side chain of the sterol. There are two secondary carbinol methines at δ4.05 (m) and δ4.91 (brs) ppm. The broad methine multiplet at δ4.05ppm has the normal complexity of a 3α-carbinol hydrogen of an A/B trans-steroid. This unusually downfield signal is typical of 3β-hydroxysterols bearing a 5α-oxygen substituent.[34] A solvent shift experiment also confirmed the 3α-hydrogen in compound **18.** A downfield shift of 0.11 ppm was observed for the hydrogen when the spectrum was recorded in pyridine-d_5/chloroform-*d* compared to that in chloroform-*d*.The ^{1}Hnmr also shows an olefinic hydrogen at δ4.99 ppm (brs) which correlates with the broad singlet hydrogen at δ4.91 ppm in the COSY-90 2Dnmr (two-dimensional correlation spectroscopy) spectrum of methyl sirocodin (Figure I-2 and Figure I-3). The 18"-methyl resonance at δ0.57 ppm is in agreement with the value expected for a 7-ene sterol. These data suggested the sterol part has a structure with 7-ene, 3β, 5α, 6-substituents. In order to assign the stereochemistry of C-6", a series of nOe experiments were performed on compound **18** (Table I-5). On irradiation of a signal at δ1.14 ppm (19"-methyl), the broad signal at δ4.91 ppm (6"-H) shows 7.5% nOe indicating that H-6" is β. This configuration can also be deduced from the multiplicity of that signal based on the Karplus rule.[35] The broad singlet of H-6" means that the dihedral angle between H-6" and H-7" approaches almost 90^0. This can only be achieved when H-6" is β.

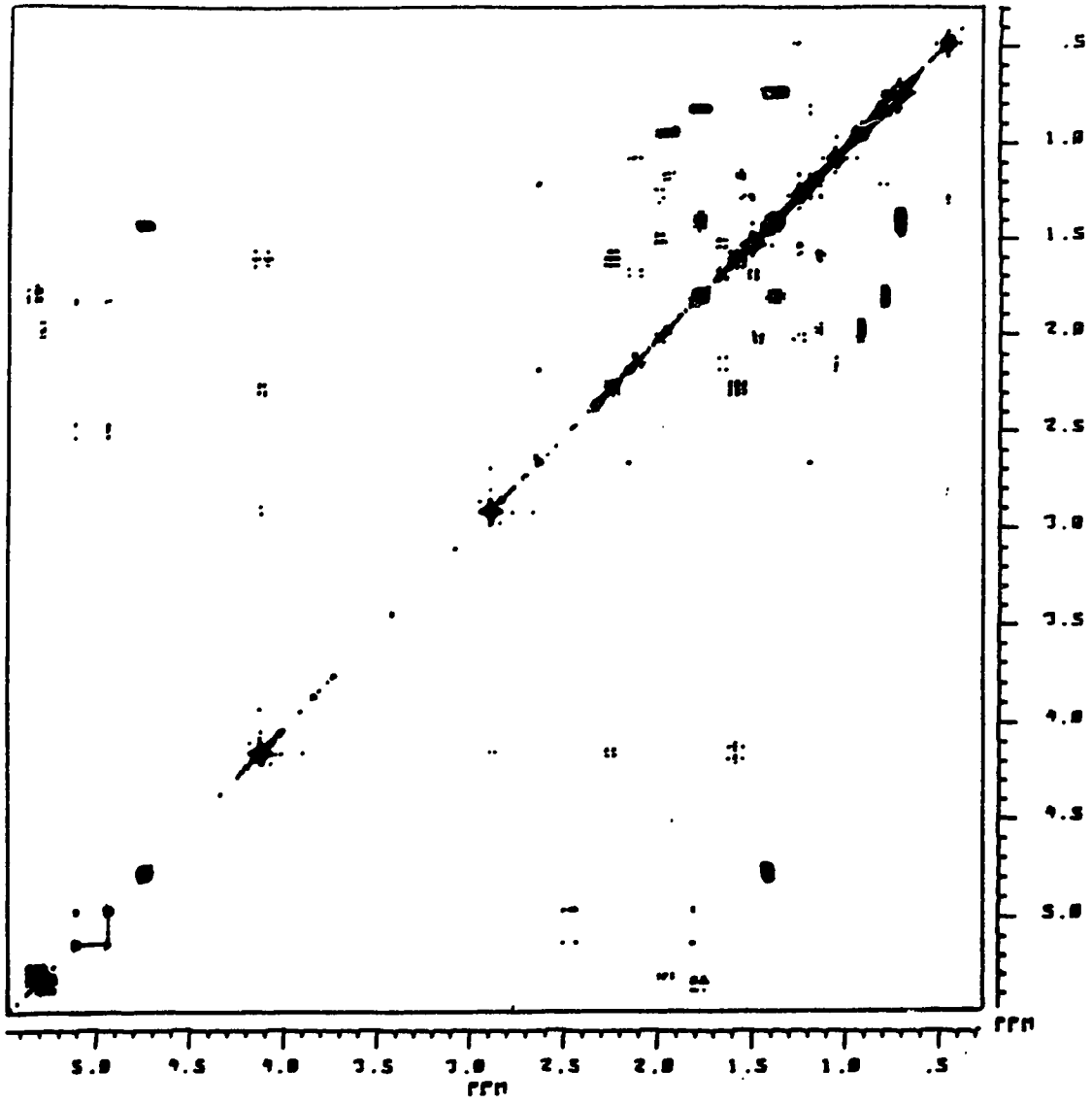


Figure I-2 The $^{1}H/^{1}H$ COSY spectrum of 24 ($CDCl_3$, 360Hz, COSY 90, contour plot)

Figure I-3 The $^{1}H/^{1}H$ COSY spectrum of 24 ($CDCl_3$, 360MHz, COSY 90, stacked plot)---see next page

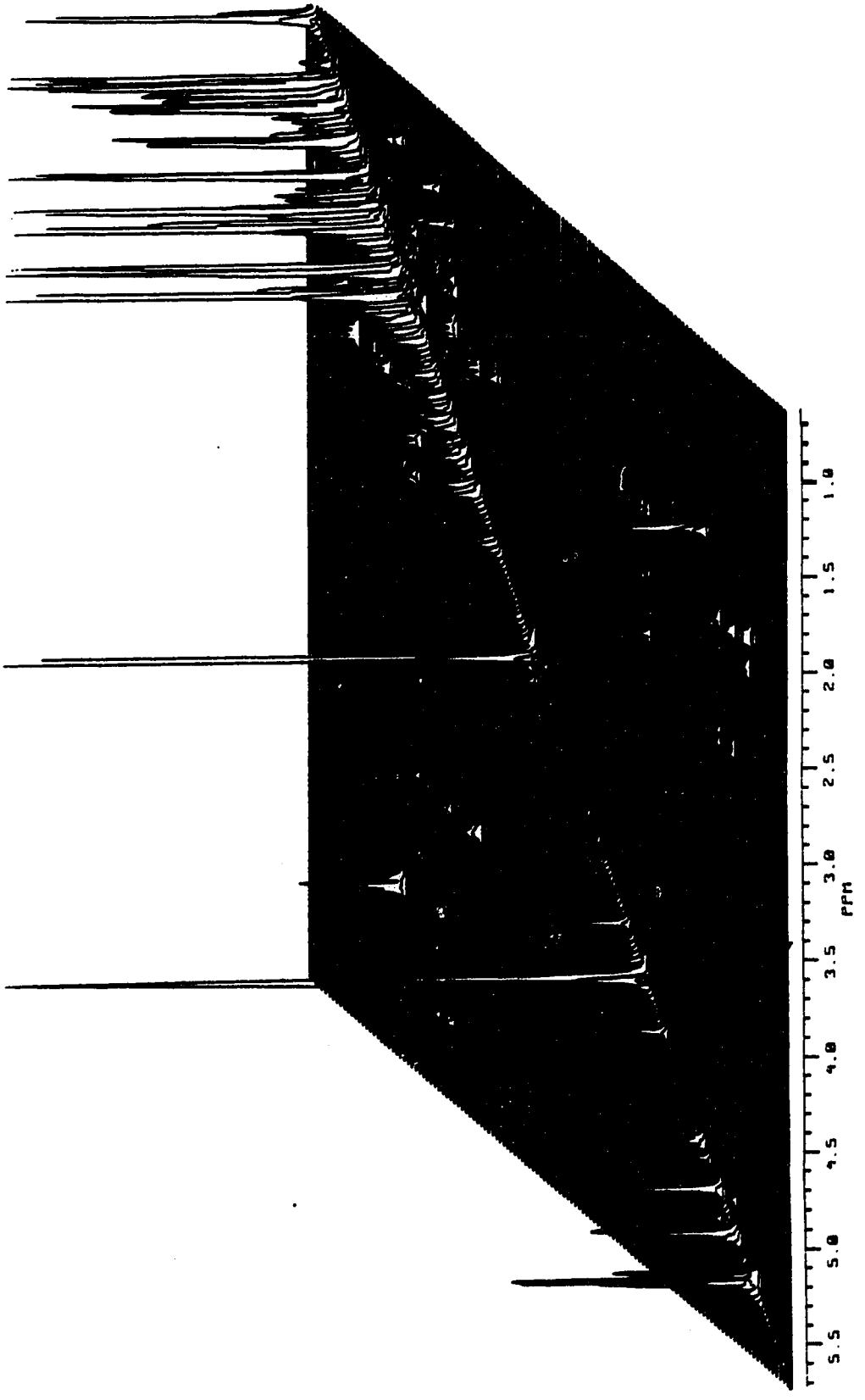
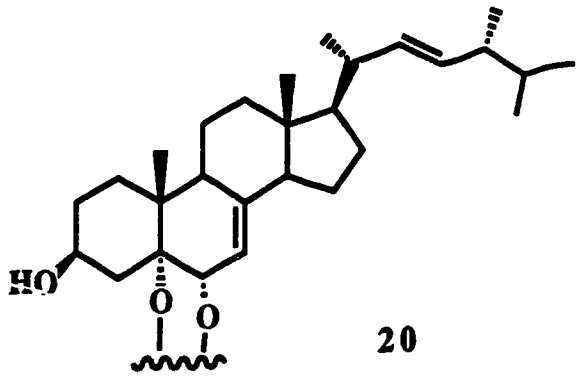

5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
PPM

Table I-5 The ^{1}Hnmr nOe data for sirocodin (**18**) ($CDCl_3$, 360MHz)

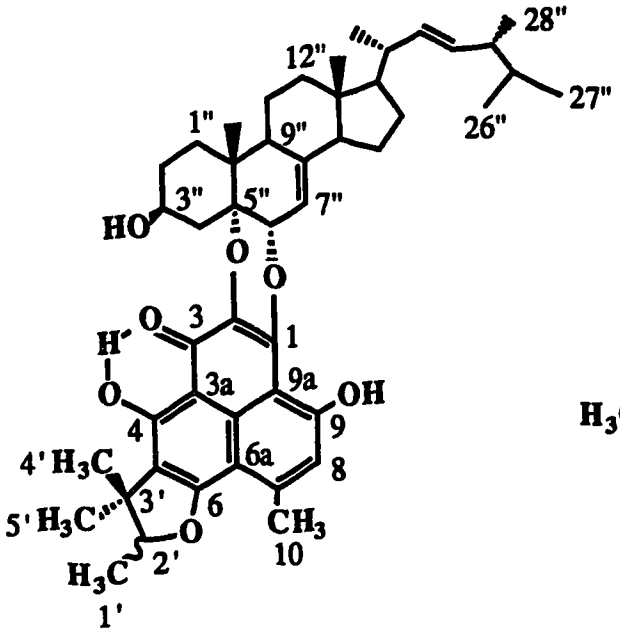
Signal saturated	Observed nOe
H-19", 1.14	H-6", 4.91, 7.5%
H-8, 6.83	H-10, 2.82, 3.4%
H-10, 2.82	H-8, 6.83, 15.5%

Thus, the structure of the sterol part of sirocodin was formulated as **20**. The stereochemistry of the side chain methyls is based simply on analogy with ergosterol.

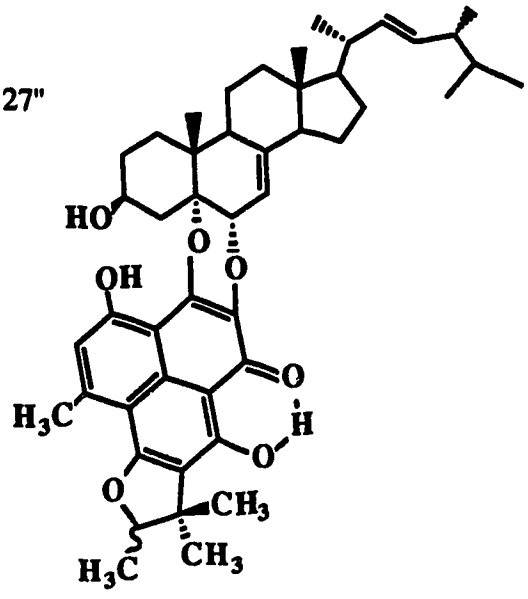


20

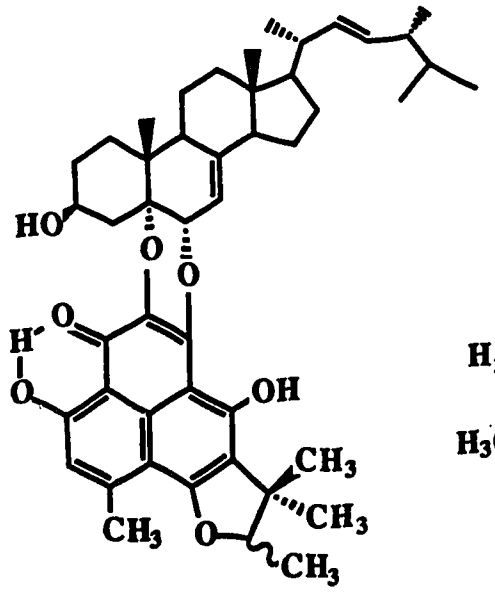
Considering the molecular formula $C_{47}H_{60}O_7$ and the two partial structures **19** and **20**, we considered four structures, **18**, **21**, **22**, and **23** for sirocodin.



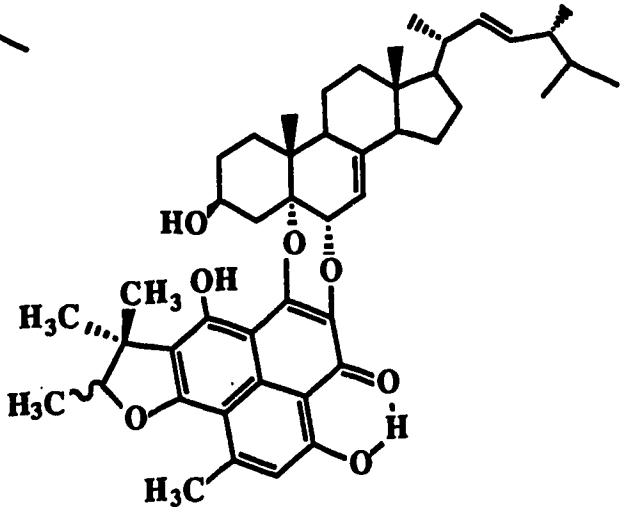
18



21



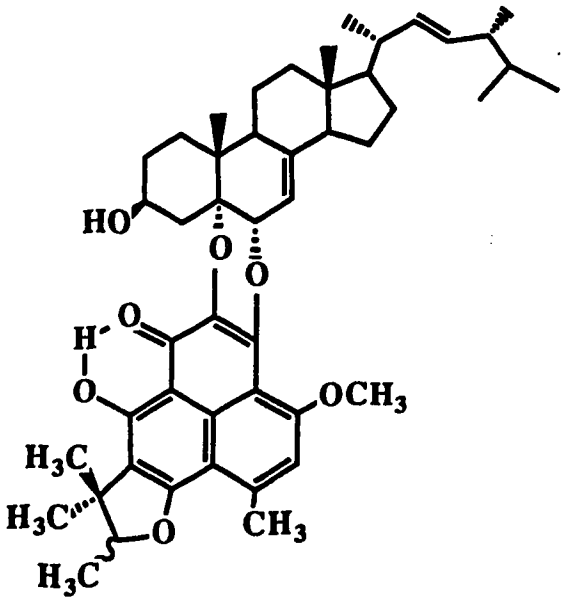
22



23

In order to gather further evidence for the structure of sirocodin, several derivatives were prepared.

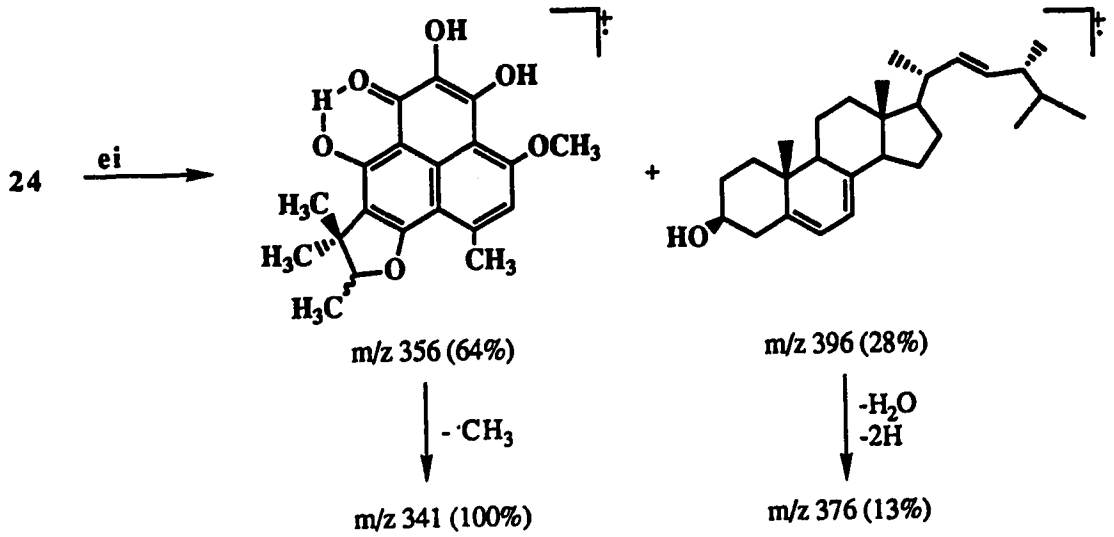
Sirocodin was treated with diazomethane in ether for 24 hours. A single monomethyl ether, **24**, was obtained after purification by flash chromatography.



24

The ir spectrum of the monomethyl ether of sirocodin indicates the presence of hydroxyls at 3300 cm^{-1} and a strongly chelated carbonyl at 1611 cm^{-1}. The fabms provided the expected molecular ion at m/z 751.64 ($M+H^+$, 10.1%) corresponding to a molecular formula $C_{48}H_{62}O_7$. One of the cims showed an intense peak at m/z 377 (90.1%). A second cims gave a base peak at m/z 357 (100%) which may be derived from the cleavage of the center ring between sterol part and the pigment part.The hrms did not reveal the molecular ion. However, it shows two strong fragment ions corresponding to both parts of the molecule at m/z 396 ($C_{28}H_{44}O$, 27.94%) and at m/z 356.1281 ($C_{20}H_{20}O_6$, 63.51%). Loss of a methyl radical from the latter fragment give rise to the most intense peak at m/z 341 ($C_{19}H_{17}O_6$, 100%) in the spectrum (Scheme I-2).

Scheme I-2 Mass fragments of monomethyl ether of sirocodin (24)



The ^{1}Hnmr of **24** shows the methyl ether signal at δ4.05 ppm. One of the phenolic OH signals (δ9.56 ppm) in compound **18** has disappeared in the spectrum of **24**. The signal for the strongly chelated phenol remains at δ17.95 ppm, indicating that only the non-chelated phenol has been methylated. All the remaining signals are similar to that of **18**, that is, signals for pigment part : δ6.85 (ArH), 4.65 (2'-H), 2.89 ($ArCH_3$), 1.57 (5'-CH_3), 1.48 (1'-CH_3), and δ1.32 (4'-CH_3), and signals for sterol part: δ5.14 (22",23"-H), 4.99 (7"-H), 4.83 (6"-H), 4.05 (3"-H), 1.13 (19"-CH_3), 1.02 (21"-CH_3), 0.89 (28"-CH_3), 0.82 (26"-CH_3), 0.81 (27"-CH_3), and δ0.57 (18"-CH_3).

A series of nOe esperiments was carried out in order to establish the location of the methoxyl, that is , the position of the non-chelated phenol. The results are shown in Table I-6.

Table I-6 The ^{1}Hnmr nOe data for methyl ether of sirocodin (24)

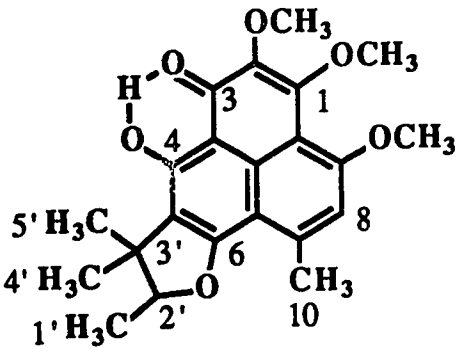
Signal saturated	Observed nOe
8-H 6.85	9-OCH_3 4.05 4%
	10-H 2.89 3.4%
7"-H 4.99	6"-H 4.83 4%
6"-H 4.83	7"-H 4.99 3.1%
9-OCH_3 4.05	8-H 6.85 15.6%
10-H 2.89	8-H 6.85 10.5%
19"-H 1.13	6"-H 4.83 10.4%

When the methoxyl (δ4.05) and aromatic methyl (δ2.89) signals are irradiated, the aromatic hydrogen (δ6.85) has 15.6% and 10.5% nOe, respectively. On irradiation of the aromatic hydrogen, the methoxyl has 4% nOe and the aromatic methyl has 3.4% nOe. These results strongly suggested that the methoxyl is at C-9, thus, the non-chelated phenol is at C-9 in sirocodin (**18**). Therefore, structures **22** and **23** are ruled out for sirocodin.

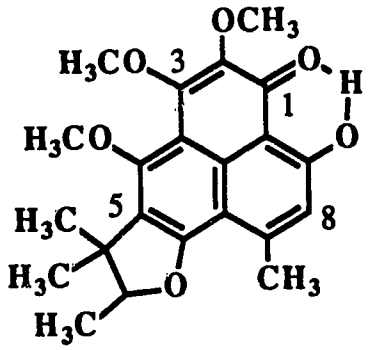
The nOe results also confirmed the sterochemistry at C-6" in the sterol part. When the 19"-methyl at δ1.13 ppm is irradiated, the signal at δ4.83 ppm (H-6") has 10.4% nOe which is consistent with the 6"β-H. Upon irradiation of the signal at δ4.83 ppm, the hydrogen at δ4.99 ppm has 3.1% nOe; when the hydrogen at δ4.99

ppm is irradiated, the hydrogen at δ4.83 ppm has 4% nOe. These data also support that the two broad singlet hydrogens at δ4.83 ppm and δ4.99 ppm are adjacent to each other.

The ^{13}Cnmr spectrum of the methyl ether of sirocodin is consistent with the assigned structure **24**. The ^{13}Cnmr data for the pigment part are very similar to that of atrovenetin trimethyl yellow (**25**) and different from that of atrovenetin trimethyl orange (**26**). Both compounds **25** and **26** were prepared previously by methylation of atrovenetin (**4**) with diazomethane. The assignment of ^{13}C chemical shifts of **25** and **26** has been made.[8] Comparison of the ^{13}Cnmr data of the pigment moiety of **24** with those of compounds **25** and **26** is shown in Table I-7.



25



26

Table I-7 Comparison of ^{13}Cnmr data of **24** with those of **25** and **26** ($CDCl_3$)

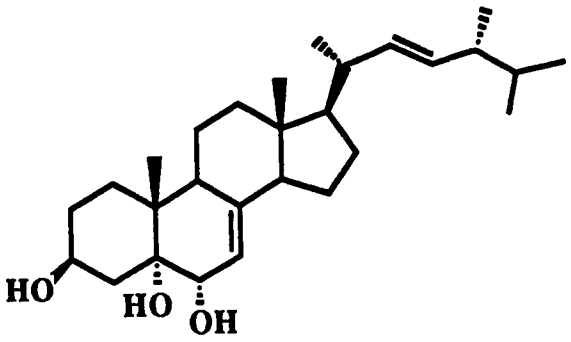
Compound	24[a]	25[b]	26[b]
C-1	145.69	157.28	177.31
C-2	130.26	142.37	140.35
C-3	173.96	176.01	160.48
C-3a	107.09	108.19	109.93

C-3b	125.49	127.38	127.85
C-4	172.09	173.93	157.60
C-5	118.99	119.42	126.97
C-6	166.07	166.99	163.34
C-6a	109.20	109.42	108.35
C-7	144.87	144.70	149.96
C-8	111.28	110.66	121.30
C-9	161.06	161.19	174.75
C-9a	105.93	109.23	109.34
C-10	24.06	24.07	24.53
C-1'	14.69	14.65	14.39
C-2'	91.18	91.40	90.48
C-3'	43.38	43.30	44.35
C-4'	25.89	25.87	26.19
C-5'	20.57	20.49	22.09
OCH_3	56.69	56.49, 60.80, 61.61	60.97, 62.29, 64.38

[a] Measured at 75 MHz in APT (attached proton test).

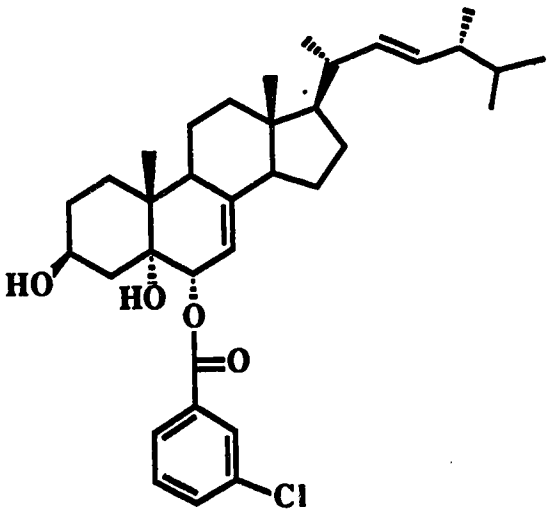
[b] Obtained at 100 MHz.

The data in Table I-7 also support that the non-chelated phenol in sirocodin is at C-9 as shown in structure **18**. In order to assign the ^{13}Cnmr data of the sterol part, it was necessary to synthesize a reference compound. Triol **27** [34] would be an ideal compound for this purpose.With this idea in mind, we carried out the preparation of this compound.

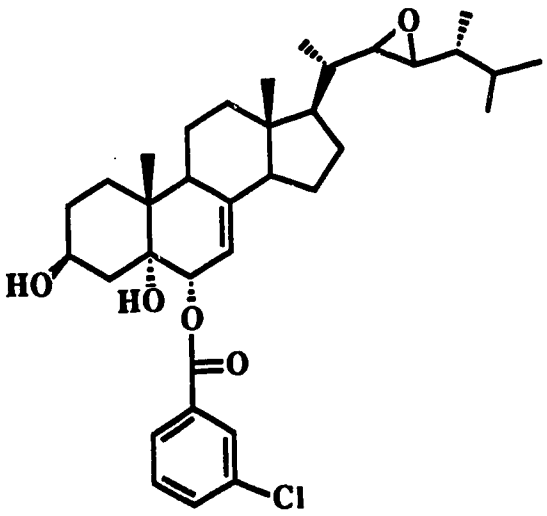


27

Oxidation of ergosterol with 1.25 eq.of *m*-chloroperbenzoic acid[36] at rt for 24 hours provided ester **28** in 45% yield, along with a small amount of epoxide **29**.



28



29

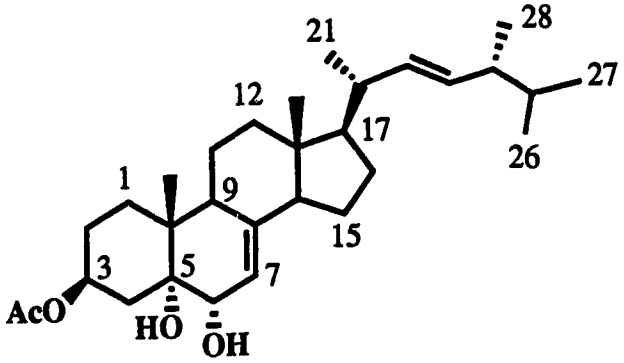
Compound **28** is a crystalline compound with a melting point of 196-198^{0}C. The ir spectrum of **28** shows absorptions at 3560, 3320, and 1705 cm^{-1}, indicating the

presence of hydroxy and ester groups. The formation of the ester was further confirmed by the ^{1}Hnmr spectrum in which the signals corresponding to the *m*-chlorobenzoate were observed at δ8.03 (dd, J=2, 2Hz), 7.95 (ddd, J=8, 2, 2Hz), 7.55 (ddd, J=8, 2, 2Hz), and 7.40 (dd J=8, 8Hz). The H-3 signal was shifted downfield from δ3.62 ppm in ergosterol to δ4.03 ppm in **28** suggesting the presence of a 5α-hydroxy group. Both H-6 and H-7 are broad singlets and appear at δ5.55 ppm and δ5.03 ppm, respectively. The resonance signals in the ^{13}Cnmr spectrum (Table I-8) at δ75.69 and 75.40 ppm also reveal the existence of the 5α-hydroxy and 6α ester groups. The hrms did not display the molecular ion, however, the fragments at m/z 412 (M-$C_7H_5O_2Cl$), 394 (M-$C_7H_5O_2Cl$-H_2O), 376 (M-$C_7H_5O_2Cl$-$2H_2O$), and 156 ($C_7H_5O_2Cl$) are in agreement with structure **28**.

Compound **29** is a mixture of stereoisomers of epoxide. The ^{1}Hnmr spectrum of **29** resembles that of **28** with the following differences. The signals for two olefinic hydrogens on the side chain at δ5.19 ppm in compound **28** have disappeared in the spectrum of **29**. Two groups of doublet of doublets signals appear at δ2.74 (J=2, 8Hz) and δ2.46 (J=2, 8Hz) for compound **29**. The ir spectrum of **29** displays the presence of hydroxyl (3440, 3240 cm^{-1}) and ester (1719, 1706 cm^{-1}) groups. The hrms fragments at m/z 428 (M-$C_7H_5O_2Cl$), 410 (M-$C_7H_5O_2Cl$-H_2O), 392 (M-$C_7H_5O_2Cl$-$2H_2O$), and 156 ($C_7H_5O_2Cl$) also support the structure.

Conversion of the ester **28** to the triol **27** is readily achieved by hydrolyzing the ester with 10% potassium hydroxide in methanol. An acetate **30** was also obtained when the work-up was done as follows: evaporation of methanol, addition of water, extraction of the aqueous solution with ethyl acetate, washing, drying, evaporation of the solvent, and separation by silica gel chromatography.

The ir spectrum of triol 27 displays hydroxyl absorption at 3323 and 3273 cm^{-1}. The ^{1}Hnmr spectrum shows two broad singlets at δ5.03 and δ3.98 ppm for H-7 and H-6. Three downfield signals at δ67.44, 76.05, and 70.35 ppm (Table I-8) also support the triol structure 27. A series of nOe experiments have secured the stereochemistry at C-6. When the signal of the 19-methyl (δ0.98 ppm) is irradiated, H-6 (δ3.98 ppm) has 7.5% nOe. Upon irradiation of H-6, H-7 (δ5.03 ppm) has 2.1% nOe; when H-7 is irradiated, H-6 has 4.3% nOe. These results clearly indicate that H-6 is β.



30

Compound 30 is a transesterification product formed during the work up. Its ir spectrum indicates the presence of an ester at 1731 cm^{-1}. The methyl group of the acetate is at δ2.04 ppm in the ^{1}Hnmr spectrum. H-3 is shifted downfield to δ5.09 ppm. H-7 is at δ5.01 ppm as a doublet (J=2Hz), while H-6 is at δ3.95 ppm as a broad doublet (J=8Hz). However, the signal for H-6 changes to a doublet (J=2Hz) after D_2O exchange.The fragments at m/z 454 (M-H_2O), 394 (M-H_2O-$C_2H_4O_2$), and 376 (M-2H_2O-$C_2H_4O_2$) in the hrms of 30 are consistent with the assigned structure. The nOe result supports the configuration assignment at C-6. When the signal of the 19-methyl (δ0.98 ppm) is irradiated, H-6 (δ3.95 ppm) has 8.7% nOe.

The comparion of the ^{13}Cnmr data of the sterol part of sirocodin methyl ether (**24**) and compounds **27**, **28**, and **30** is compiled in Table I-8.

Table I-8 Comparion of ^{13}Cnmr data of **24** with those of compounds **27**, **28**, and **30** ($CDCl_3$)

Compound	24[a]	27[b]	28[c]	30[b]
C-1	27.97*	30.68*	30.96*	29.66*
C-2	30.61	31.60	31.74	31.32
C-3	66.90	67.44	67.29	70.58
C-4	31.16	38.72	39.51	34.99
C-5	75.96	76.05	75.69	75.46
C-6	73.42	70.35	75.40	70.26
C-7	115.35	119.52	115.70	119.38
C-8	143.42	142.08	144.48	142.08
C-9	42.86	42.81	43.95	42.82
C-10	35.43	38.51	39.42	38.56
C-11	21.23	21.41	21.42	21.35
C-12	39.03	39.23	39.91	39.20
C-13	43.72	43.33	43.82	43.19
C-14	54.29	54.70	55.08	54.68
C-15	22.67	22.70	22.95	22.70

C-16	29.75*	28.04*	28.40*	27.99*
C-17	55.75	55.89	56.05	55.92
C-18	12.30	12.19	12.45	12.19
C-19	17.54	17.77	17.95	17.60
C-20	40.42	40.39	40.81	40.34
C-21	21.17	21.11	21.52	21.09
C-22	135.57	135.40	136.00	135.40
C-23	132.07	132.14	132.35	132.17
C-24	42.99	43.76	43.30	43.73
C-25	33.13	33.08	33.43	33.06
C-26	19.97	19.94	20.21	19.90
C-27	19.67	19.64	19.88	19.63
C-28	17.67	17.58	17.95	17.60
C-1' or CO			165.16	170.45
C-2' or CH_3			132.55	26.90
C-3'			130.11	
C-4'			134.75	
C-5'			133.06	
C-6'			129.92	

C-7'	129.85

* Signals are interchangeable in the same column.

a Measured at 75MHz.

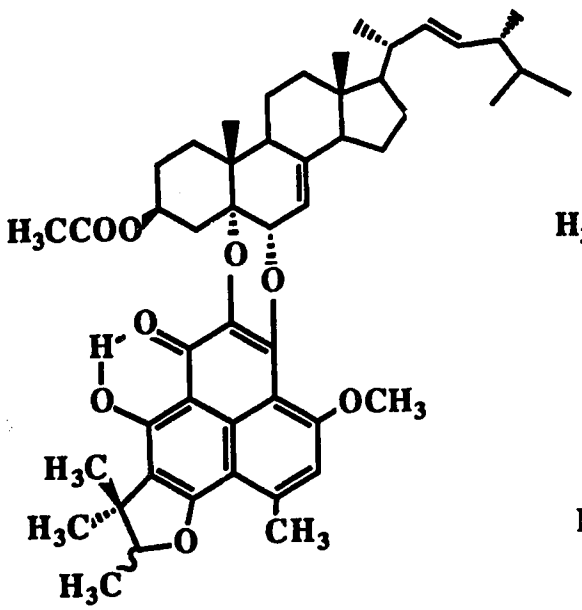
b Recorded at 90MHz.

c Obtained at 100MHz in C_6D_6.

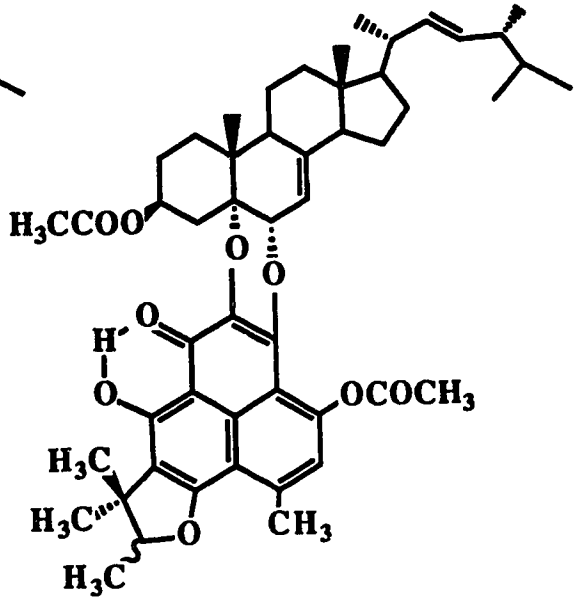
As shown in the table, the major differences of ^{13}C chemical shifts are at C-1, C-4, C-7, and C-10 in compound **24** compared with the other componds. The chemical shift of C-1" of methyl sirocodin (**24**) is shifted upfield compared with triol **27** by 2.71ppm which is consistent with a γ-gauche effect. The upfield shifts of C-4, C-10 may be also due to a steric effect.[37]

Treatment of sirocodin methyl ether (**24**) with acetic anhydride and pyridine at room temperature for 18 hours produced monomethyl monoacetyl sirocodin (**31**). The ir spectrum of **31** suggested the presence of an ester group at 1736 cm^{-1}. In the ^{1}Hnmr spectrum, the methyl group of the acetate appears at δ1.91 ppm; H-3" is shifted downfield to δ5.05 ppm. This indicates the acetate is at C-3". The fabms gave a peak at m/z 793.59 ($M+H^+$, 2.46%) which corresponds to a molecular formula $C_{50}H_{64}O_8$.

The diacetate of sirocodin (**32**) was obtained under standard acetylation conditions. The ir spectrum of **32** displays weak phenol absorption at 3400 cm^{-1} and two ester absorptions at 1753 and 1734 cm^{-1}. The ^{1}Hnmr spectrum shows two acetyl methyl groups at δ2.38 and 1.96 ppm. The signal for the chelated phenol hydrogen appears at δ17.41 ppm. The fabms also confirmed the structure of the diacetate (m/z 826.56, $M+H^+$, 0.87%).



31

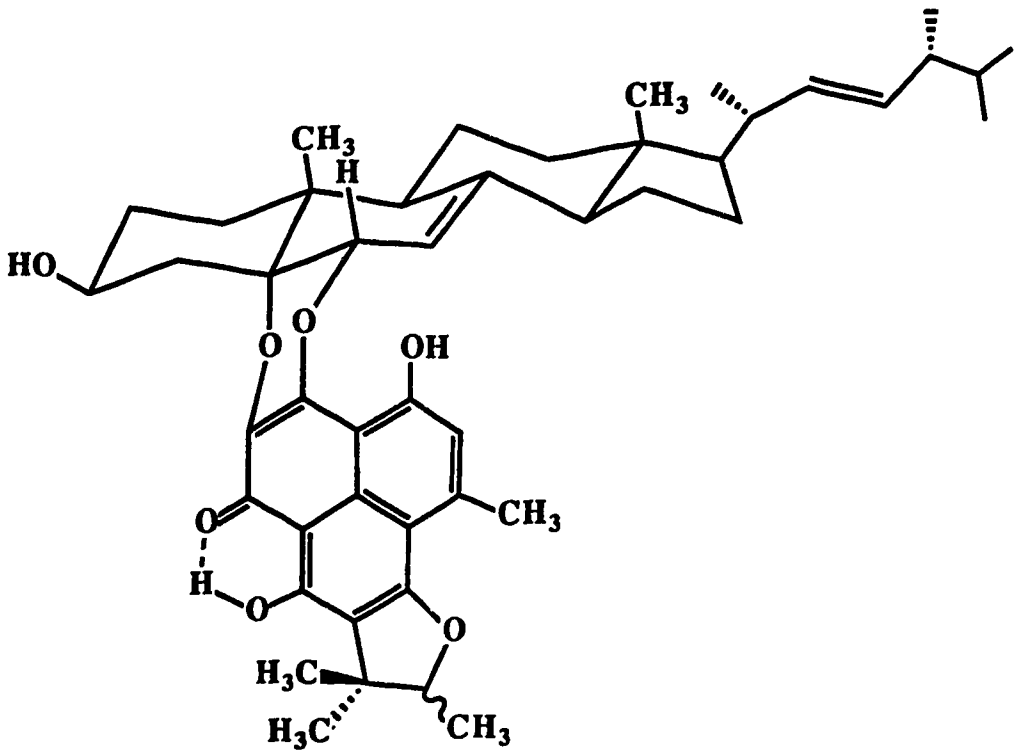


32

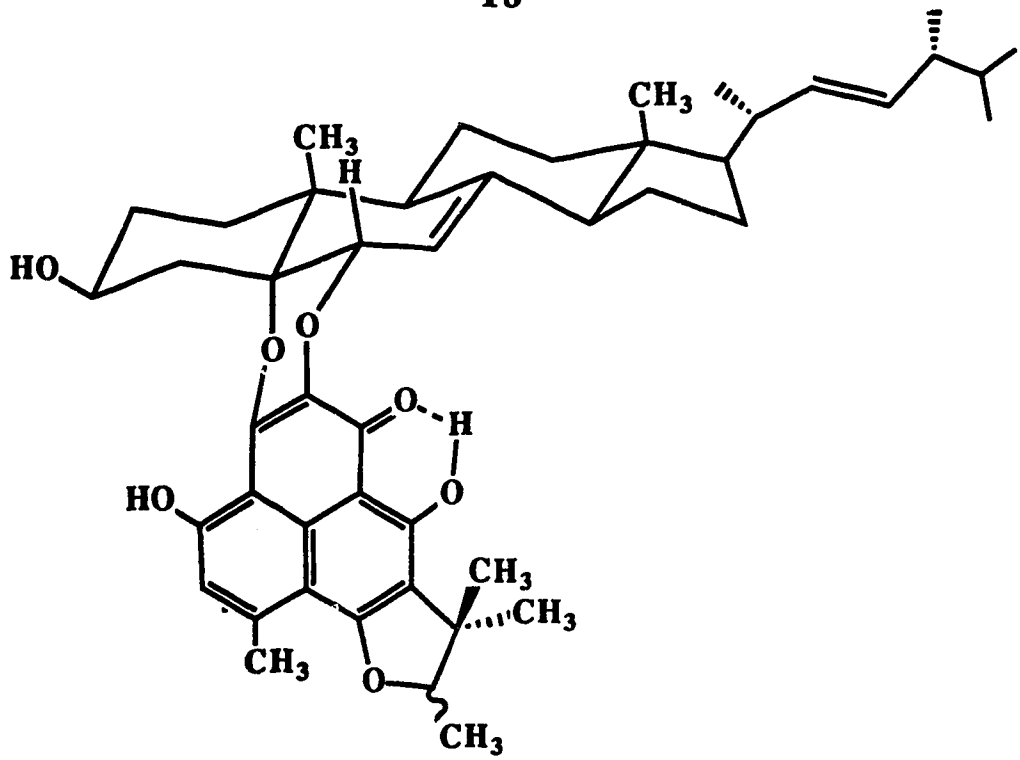
At this point, with three derivatives, **24**, **31**, and **32**, in hand, a careful comparison of the ^{1}Hnmr data of sirocodin (**18**) and its derivatives was conducted in order to differentiate structures **18** and **21**. The chemical shifts of H-6" and H-7" in compounds **18**, **24**, **31**, and **32** are compiled in Table I-9.

Table I-9 Comparison of ^{1}Hnmr data of H-6" and H-7" in compounds **18**, **24**, **31**, and **32** ($CDCl_3$, 360MHz)

Compound	18	24	31	32
H-6"	4.91	4.83	4.82	4.68
H-7"	4.99	4.99	4.96	4.91



18



21

It is observed that H-7" and H-6" in diacetate **32** are shifted upfield by 0.08 ppm and 0.23 ppm, respectively, compared with the parent compound **18**. For the methyl ether **24**, H-6" is also shifted upfield by 0.08 ppm. Since both methylation and acetylation of the non-chelating phenol shift the H-6" and H-7" upfield, it is reasonable to assume that the phenol must be close to H-6" and H-7" in space. This can only be achieved in the structure **18** as shown in its conformational structure, not in the conformation **21**.

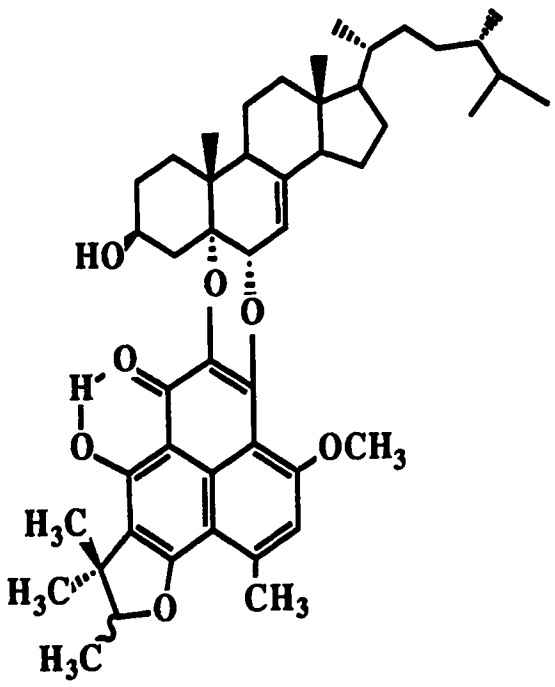
As seen from conformation **18**, the 9-OH is much closer to H-6" and H-7" than that in conformation **21**. The acetate at position 9 in compound **32** shields H-6" and H-7" and shifts them upfield. The upfield shifts of H-6" and H-7" can not be caused by the 3"-acetate because the chemical shifts of H-6 and H-7 do not change in acetate **30** compared to those in triol **27**. Thus, we favor the structure **18** for sirocodin. The isolation and structure determination of a similar compound, sirocodinine (discussed later), from the same fungi also support this structure.

Since the 9-acetate in **32** is close to H-7" and H-6", we reasoned that if the signal of the methyl group of the acetate is irradiated, H-6" or H-7" might show nOe. With this idea in mind, we carried out a series of nOe experiments. When the signal of the aromatic methyl (δ2.86 ppm) is irradiated, the aromatic hydrogen (δ6.94 ppm) has 12.9% nOe. Upon irradiation of the 19"-methyl (δ1.15 ppm) signal, H-6" (δ4.68 ppm) has 4.8% nOe. Disappointingly, when the methyl signal of the aromatic acetate (δ2.38 ppm) was irradiated, no detectable nOe was observed. We believe the reason for this is that since the 9-acetate is freely rotating and, the lack of an nOe to H-6" or H-7" is not completely unexpected.

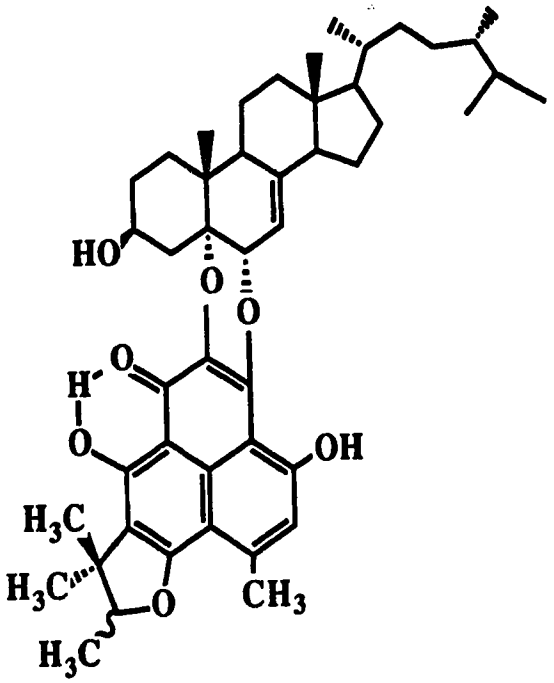
The sterol part and the pigment part of sirocodin (**18**) are united by a 6-membered ring. Usually, ethers of this type are not reactive (e.g. 1,4-dioxane) and

this is the case for sirocodin. The sirocodin methyl ether (24) is stable under the following conditions: 1) refluxing with 0.5M potassium hydroxide in water and acetone solution for 18 hours; 2) rt or refluxing in trifluoroacetic acid/water/acetone/methylene chloride (2:2:1:1) solution; 3) refluxing with 0.2M sodium hydroxide in ethanol for 5 hours; 4) refluxing with 50% sulfuric acid in acetone for 24 hours; 5) stirring with *p*-toluenesulfonic acid in dry acetone for 5 hours.

Hydrogenolysis of benzyl and allyl alcohols and ethers over palladium or platinum under acidic conditions are well documented.[38] Since one ether linkage between the sterol part and the pigment part in sirocodin is allylic, we attempted to cleave this bond (C-6"-O) by hydrogenolysis. Hydrogenation of sirocodin methyl ether (24) with platinum dioxide and a few drops of concentrated hydrogen chloride in ethanol yielded a dihydro compound 33. No trace of compound with C_6"-O bond cleavage was observed.The fabms spectrum of 33 shows the expected molecular ion at m/z 753.51 ($M+H^+$, 11.24%) which corresponds to a molecular formula $C_{48}H_{64}O_7$. The signals for the olefinic hydrogens on the side chain have disappeared in the ^{1}Hnmr spectrum of 33.



33

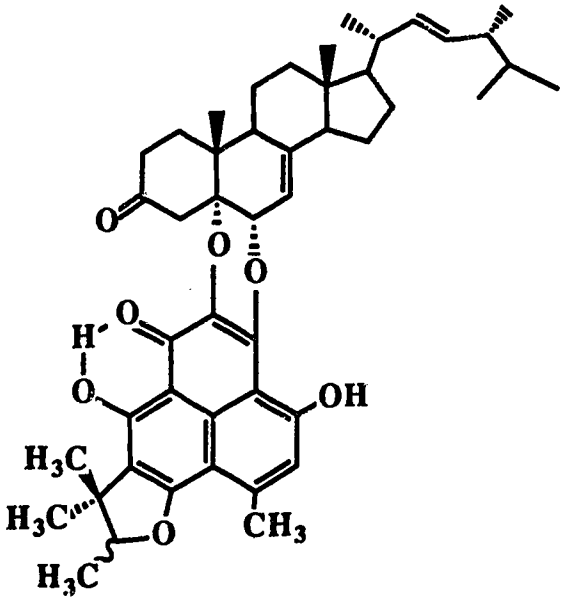


34

In another reaction, hydrogenation of diacetate **32** with 10% palladium on carbon and a small amount of 3M HCl in methanol was carried out. A mixture was detected after 22 hours reaction (partial deacetylation had occurred). A few drops of concentrated HCl was added and the hydrogenation was allowed to continue for 20 hours. Dihydrosirocodin (**34**) was obtained. Once again, the C_6"-O bond cleavage did not occur! The ir spectrum of **34** shows hydroxy absorption at 3400 cm^{-1}. Fabms gave a molecular ion at m/z 739.60 ($M+H^+$, 4.20%) which matches the molecular formula $C_{47}H_{62}O_7$. The ^{1}Hnmr spectrum displays two phenolic hydrogens at δ17.18 and 9.55 ppm and a carbinol methine hydrogen at δ4.04 ppm, indicating that the two acetates had hydrolyzed under the acidic conditions. The peaks corresponding to the olefinic hydrogens on the side chain are absent in the ^{1}Hnmr spectrum of **34**. The remaining signals are very similar to those of **33**.

Because the attempts to cleave of C_6"-O bond were unsuccessful, we turned our attention to cleaving the C_5"-O bond. Oxidation of the 3"-hydroxyl to a ketone

and β-elimination under basic condition would produce an α,β-unsatured ketone. Among many oxidation conditions, Swern oxidation[39] seemed the most desirable method for our purpose because this procedure uses triethylamine as a reagent. In our case, triethylamine might also served as the base for β-elimination. A model study proved to be successful with compound **28** (this will be discussed later). However, oxidation of sirocodin (**18**) using the Swern procedure [DMSO, $(CF_3CO)_2O$, then Et_3N] yielded 3"-ketosirocodin (**35**) exclusively, no α,β-unsaturated ketone was detected.



35

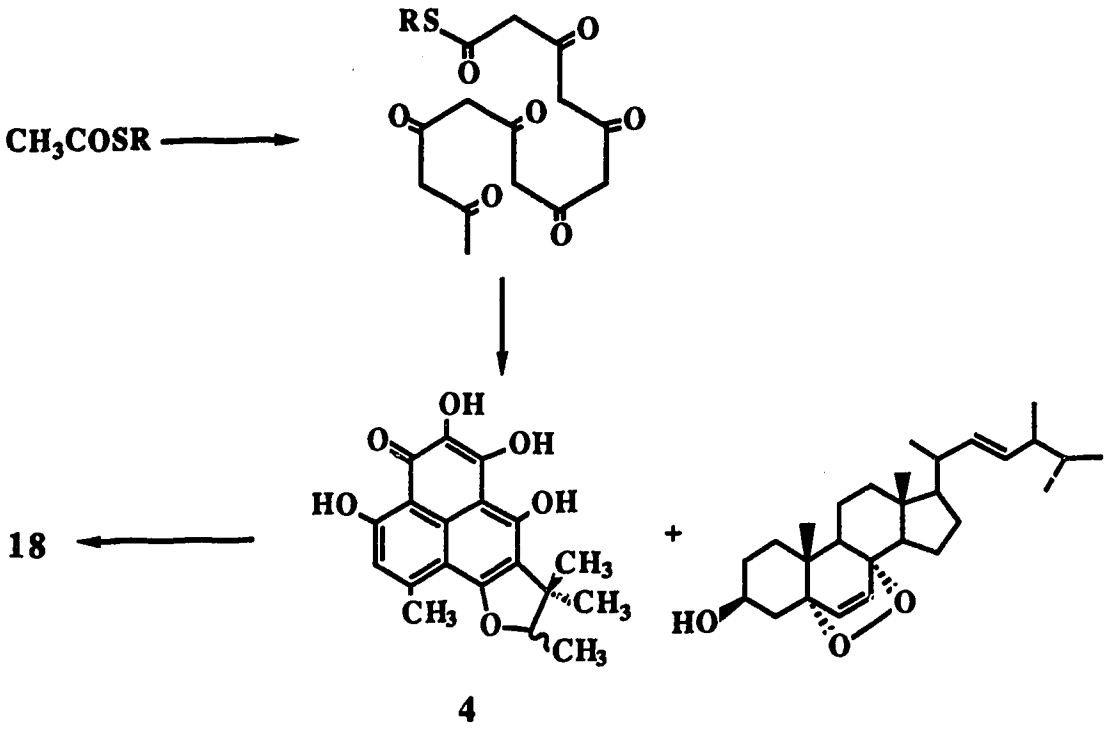
In the fabms, a molecular ion at 735.17 ($M+H^+$, 5.83%) was observed for a molecular formula $C_{47}H_{58}O_7$.The ir spectrum displays absorption for phenols at 3390 cm^{-1}, a ketone at 1722 cm^{-1}, and a chelated carbonyl at 1610 cm^{-1}. The ^{1}Hnmr spectrum shows signals for two phenols (δ17.03 and 9.43 ppm), an aromatic hydrogen (δ6.83 ppm), two olefinic hydrogens of the side chain of the sterol (δ5.18 ppm), H-6" and H-7" (δ5.03 ppm), a methine hydrogen geminal to an oxygen (δ4.65 ppm), an aromatic methyl (δ2.82 ppm), and nine methyl groups

(δ1.56, 1.49, 1.34, 1.31, 1.02, 0.90, 0.83, 0.81, and 0.60 ppm). The signal for H-3" at δ4.05 ppm in sirocodin (**18**) has disappeared in compound **35** indicating the 3"-hydroxy has been oxidized to the ketone. It appears that the 2H-1,4-dioxine ring between the sterol part and pigment part is very stable and β-elimination is not favored.

Sirocodin (**18**) is a mixture of diastereoisomers at C-2' (*ca.*2:1) as indicated by the multiplicity of H-2' (two q, J=6Hz) in the ^{1}Hnmr spectrum of sirocodin (**18**) and its derivatives. The signals for the phenolic hydrogens in sirocodin and its derivatives sometimes appear as two close singlets which also indicates the presence of two epimers. This is consistent with the observation that sclerodin isolated from these fungi is partially racemic. Due to the small amount of sirocodin (**18**) available, we did not attempt to separate these epimers.

Since ergosterol or ergosterol endoperoxide is isolated from the same fungus, the isolation of sirocodin is very interesting. Sirocodin (**18**) might be synthesized biogenetically from atrovenetin (**4**) and a sterol such as ergosterol endoperoxide or its derivatives (Scheme I-3). A partially racemic **4** would produce diastereoisomers of sirocodin (**18**) at C-2'.

Scheme I-3 Biogenetic formation of sirocodin (18)



Sirocodinine (36)

A second pigment-sterol adduct, which we named sirocodinine, was isolated from strain 35B (PDY medium) and from *G.myrtillis* T256 (PDY and Wort media).

Sirocodinine is a yellow compound. Its ir spectrum shows the presence of OH and NH absorptions (3398, 3388, 3380 cm^{-1})[40] and a strongly chelated carbonyl group (1610 cm^{-1}). A molecular ion was obtained from fabms at m/z 734.55 ($M+H^+$, 0.72%) which matches a molecular formula $C_{47}H_{59}O_6N$. The ^{1}Hnmr spectrum of sirocodinine displays the signals for the pigment part : two phenols (δ17.84, 9.76 ppm, D_2O exchangeable), one aromatic hydrogen (δ6.84 ppm), a hydrogen geminal to oxygen (δ4.65 ppm,) an aromatic methyl (δ2.82 ppm), and three aliphatic methyls (δ1.57, 1.48, and 1.30 ppm). These signals are

very similar to those of the pigment part in sirocodin (**18**) indicating that sirocodinine has the same pigment part as sirocodin (Table I-10).

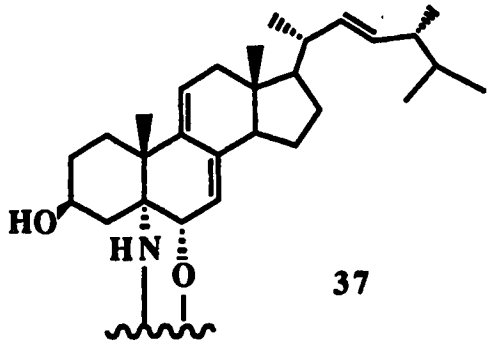
Table I-10 Comparison of ^{1}Hnmr data of pigment part in sirocodin (**18**) and sirocodinine (**36**) ($CDCl_3$, 360MHz)

Compound	**18**	**36**
4-OH (s)	17.16	17.84
9-OH (s)	9.56	9.76
8-H (s)	6.83	6.84
10-H (s)	2.82	2.82
1'-H (d)	1.47	1.48
2'-H (q)	4.65	4.65
4'-H (s)	1.56	1.57
5'-H (s)	1.32	1.30

The sterol part of the molecule shows the following signals: four olefinic hydrogens (δ5.73, 5.16(2H), and 5.04 ppm), one hydrogen geminal to oxygen (δ4.89 ppm) and six methyls (δ1.29, 1.00, 0.89, 0.82, 0.80, 0.55 ppm). There is also a singlet signal for one hydrogen at δ4.01 ppm which disappears after addition of D_2O. This signal is not readily assigned to either the sterol part or the pigment portion. Comparing the ^{1}Hnmr spectra of sirocodin (**18**) and sirocodinine (**36**), we realized that both compounds are similar in structure. The following signals appear in the ^{1}Hnmr spectrum of sirocodinine but not sirocodin: δ5.73 ppm (1H, d, J=6Hz) and

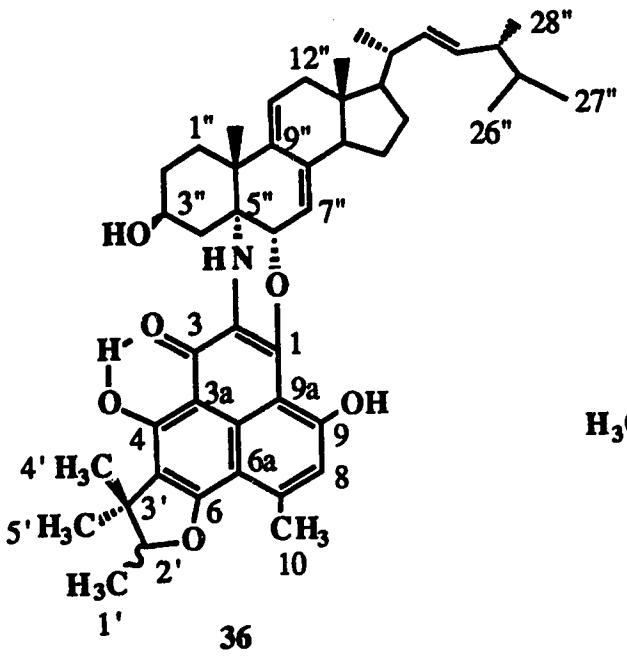
4.01 ppm (1H, s). Since all the hydrogens of the pigment part are accounted for, the hydrogen at δ5.73 ppm must be from the sterol part. At this point, we recalled the isolation of 9(11)-dehydroergosterol endoperoxide (**1**) from this same fungus. The H-11 of **1** appears at δ5.53 ppm (1H, dd, J=1.5, 6Hz) which is similar to the signal in sirocodinine(**36**). Decoupling experiments were performed on both compound **1** and **36**. When the signal of H-11 (δ5.53 ppm) of **1** is irradiated, a doublet of doublets hydrogen of H-12 (δ2.18 ppm) becomes a doublet. On irradiation of the signal of H-11" (δ5.73 ppm) of **36**, a doublet of doublets hydrogen of H-12" (δ2.38 ppm) collapses to a doublet; when the hydrogen at δ2.38 ppm (H-12") is irradiated, the signal for the hydrogen at δ5.73 ppm (H-11") becomes a singlet. This suggests that the sterol part of **36** is similar to **1**.

The ^{13}Cnmr spectrum of sirocodinine reveals all 47 carbons. Signals at δ117.05, 124.81, 139.17, and 140.61 ppm suggested the presence of a conjugated diene system in sirocodinine. The two secondary carbons at δ75.73 ppm and 66.84 ppm can be assigned to the allylic carbon (C-6") and the carbon at C-3". A quaternary carbon downfield at δ55.66 ppm may be attached to a nitrogen. The exchangeable hydrogen at δ4.01 ppm in the ^{1}Hnmr spectrum may then be assigned to NH. The above information suggests the sterol portion may have structure **37**.

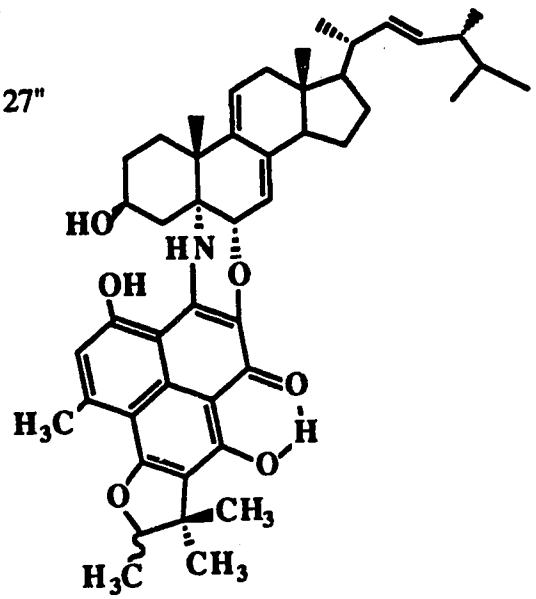


37

There are two possible structures, **36** and **38**, if we put the pigment part and the sterol part together.



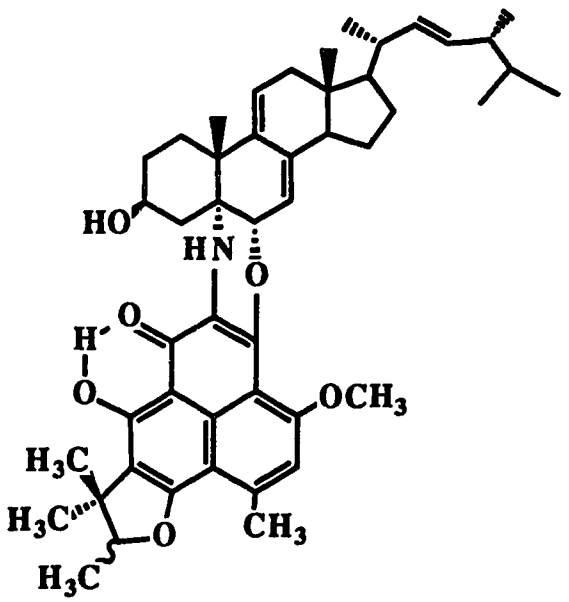
36



38

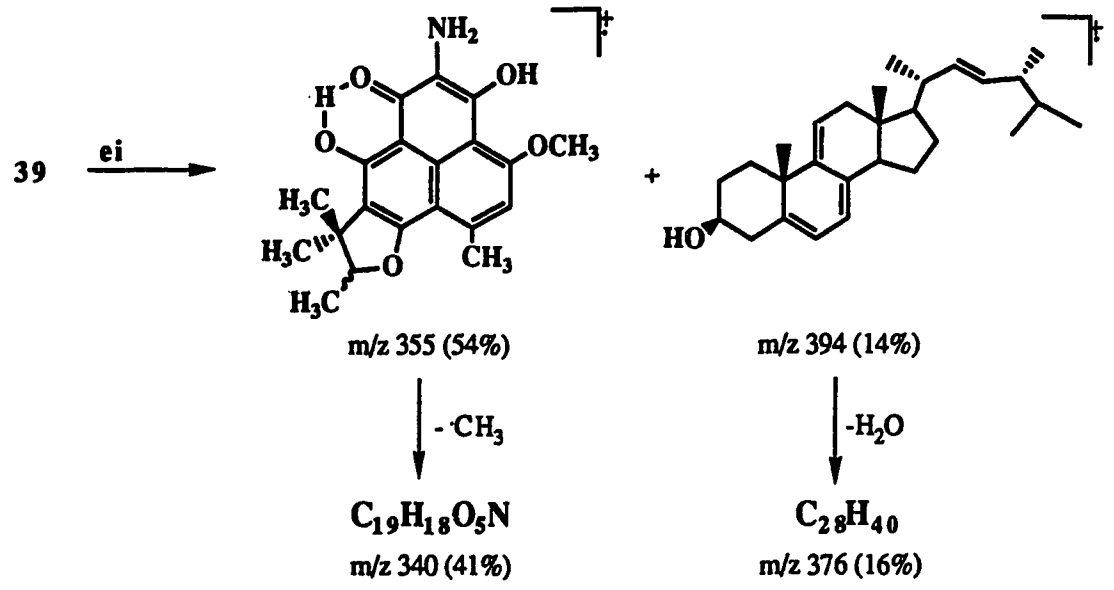
We favor structure **36** for sirocodinine over structure **38** based on arguments presented later.

Methylation of sirocodinine with diazomethane provided the monomethyl ether **39**. The methoxy signal appears at δ4.07 ppm in the ^{1}Hnmr spectrum. The phenol signal at δ9.76 ppm in **36** has disappeared in the spectrum of **39**, the remaining signals are similar to those of **36**. The fabms gave an ion at m/z 748.33 ($M+H^+$, 7.27%) corresponding to a molecular formula $C_{48}H_{61}O_6N$. Hrms does not show a molecular ion, but it displays ions corresponding to both parts of the molecule at m/z 394.3231 ($C_{28}H_{42}O$, 14.35%) and at m/z 355.1419 ($C_{20}H_{21}O_5N$, 54.05%). Loss of water from the former ion gave a fragment at m/z 376 (16.26%); Another strong ion was observed at m/z 340 (40.90%) resulting from 355 ion (Scheme I-4).



39

Scheme I-4 Fragmentation of monomethyl ether of sirocodinine (39)



In the COSY 2Dnmr spectrum (Figure I-4 and Figure I-5), a hydrogen at δ5.67 ppm (H-11") is weakly correlated with a hydrogen at δ5.03 ppm (H-7") which is coupled to a hydrogen at δ4.81 ppm (H-6"). The latter hydrogen is also

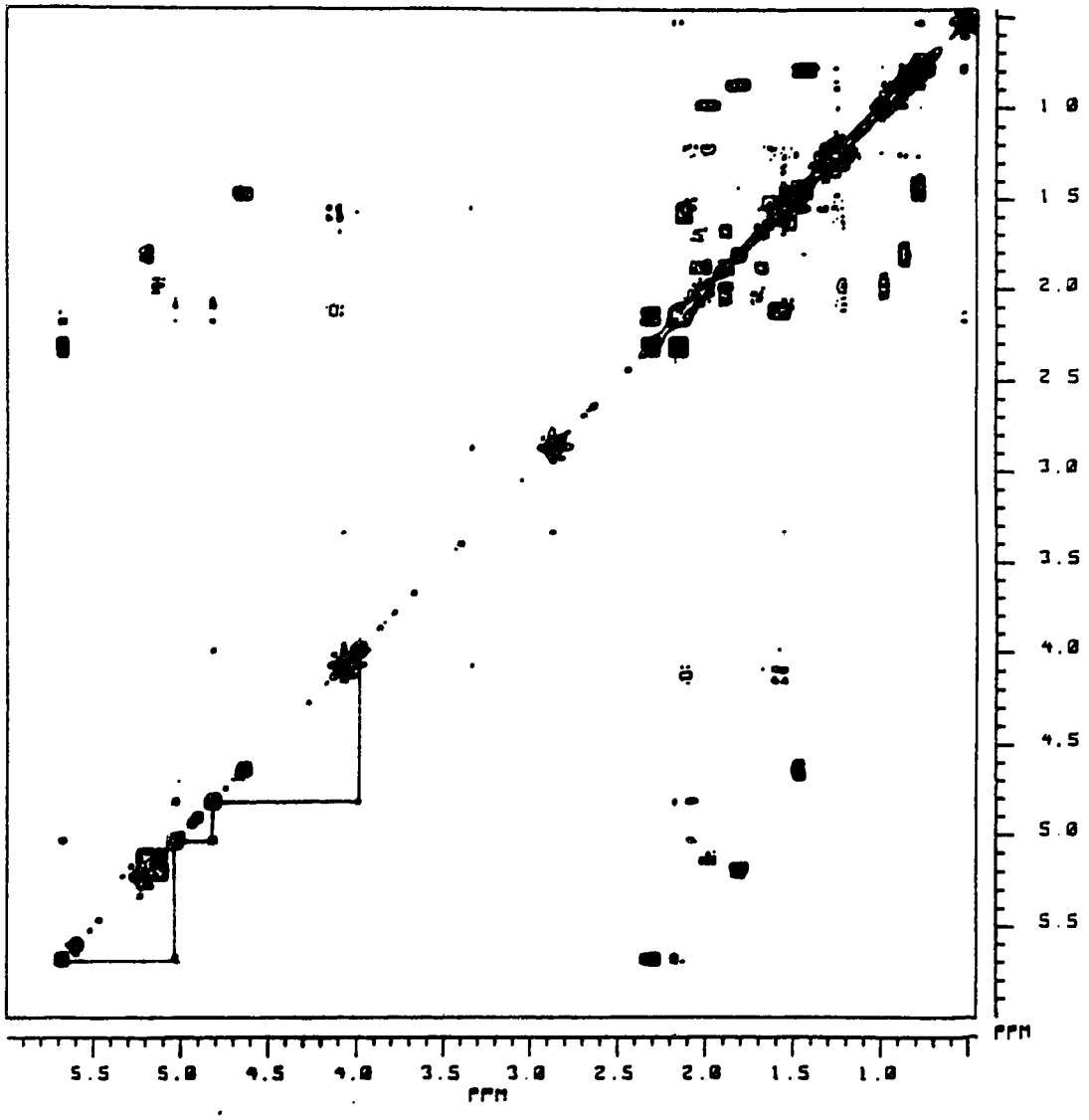
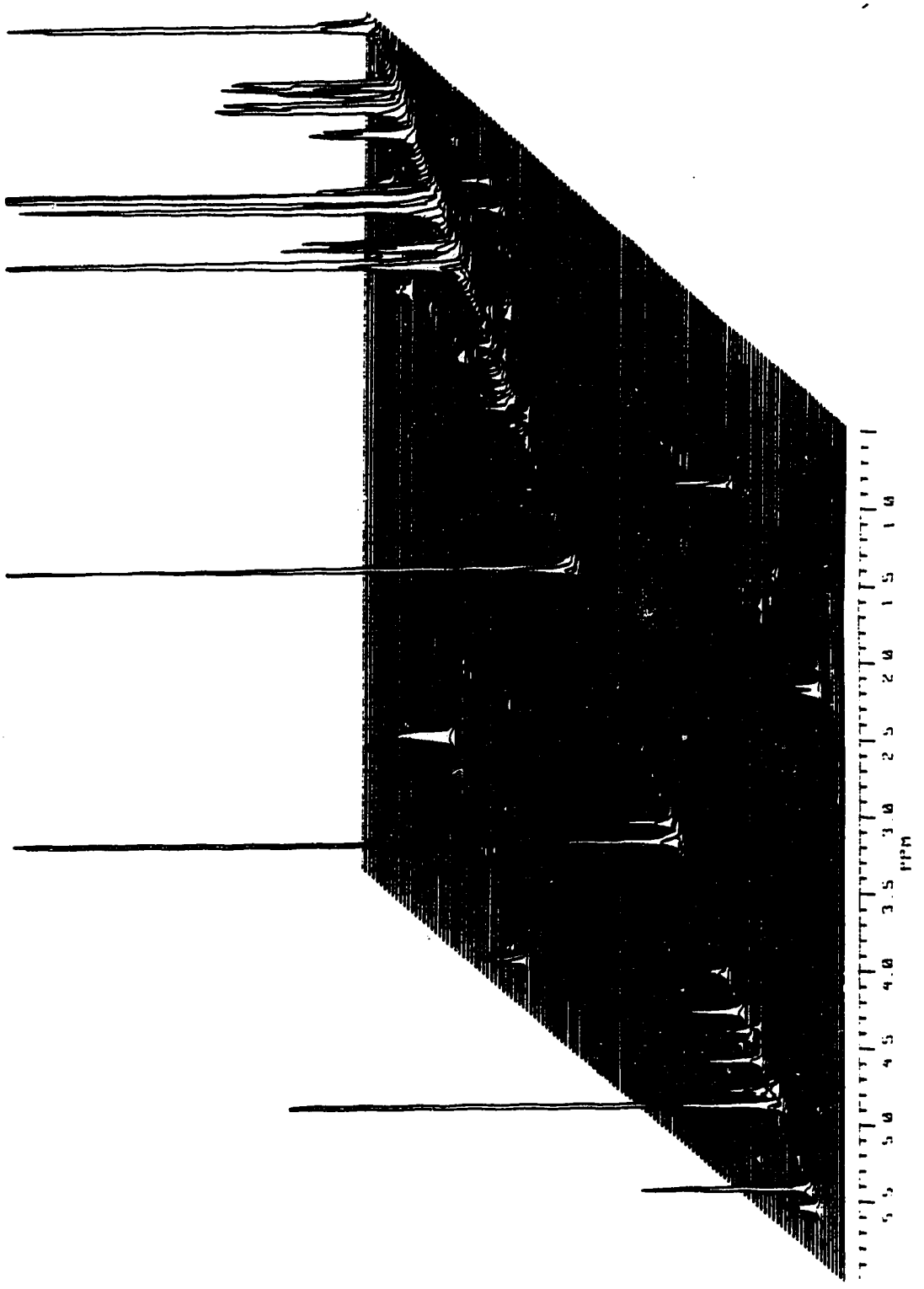


Figure I-4 The $^{1}H/^{1}H$ COSY spectrum of 39 ($CDCl_3$, 360MHz, COSY 90, contour plot)

Figure I-5 The $^{1}H/^{1}H$ COSY spectrum of 39 ($CDCl_3$, 360MHz, COSY 90, stacked plot)---see next page


PPM

weakly correlated with the NH hydrogen. These correlations are four bond couplings and support the structure **39**.

We also performed nOe experiments on sirocodinine methyl ether (**39**) (Table I-11). These confirmed the stereochemistry of the sterol part and the position of the non-chelated phenol. When the signal of 19"-methyl (δ1.27 ppm) is irradiated, H-6" (δ4.81 ppm) shows a 4% nOe; on irradiation of H-6", the hydrogens at δ1.29 ppm and δ5.03 ppm (H-7") give 2.1% and 5% nOes, respectively. 6.1% nOe is observed for the hydrogen at δ4.81 ppm when the signal for the hydrogen at δ5.03 ppm is saturated. No nOe was observed when the signal for the hydrogen at δ5.67 ppm (H-11") and the signal for NH at δ3.99 ppm were irradiated. These nOe results indicate the stereochemistry of the sterol part of sirocodinine is the same as that of sirocodin. The same substitution pattern for the pigment part of sirocodinine and sirocodin also was confirmed by nOe studies. The aromatic hydrogen (δ6.90 ppm) has 20% and 11.9% nOes, respectively, when the signals of methoxyl (δ4.07 ppm) and aromatic methyl (δ2.88 ppm) are irradiated.

Table I-11 The ^{1}Hnmr nOe data for sirocodinine methyl ether (**39**) ($CDCl_3$, 360MHz)

Signal saturated	Observed nOe
H-11" 5.67	no nOe
H-7" 5.03	H-6" 4.81 6.1%
H-6" 4.81	H-7" 5.03 5%
	H-19" 1.27 2.1%
9-OCH_3 4.07	H-8 6.90 20%

NH 3.99	no nOe
H-10 2.88	H-8 6.90 11.9%
H-19" 1.27	H-6" 4.81 4%

The ^{13}Cnmr spectrum of **39** shows the signal for methoxy group at δ56.86 ppm. The ^{13}Cnmr data of **39** along with that of **36** are shown in Table I-12.

Table I-12 Comparison of ^{13}Cnmr data of sirocodinine (**36**) and **39**.

Compound	**36**[a]	**39**[b]
C-1	140.61	141.73
C-2	124.18	123.75
C-3	174.89	172.56
C-3a	105.50	104.96
C-3b	121.04	121.65
C-4	170.07	172.39
C-5	118.29	119.09
C-6	166.23	165.76
C-6a	108.58	108.17
C-7	142.37	142.73
C-8	116.43	111.76
C-9	158.51	160.34

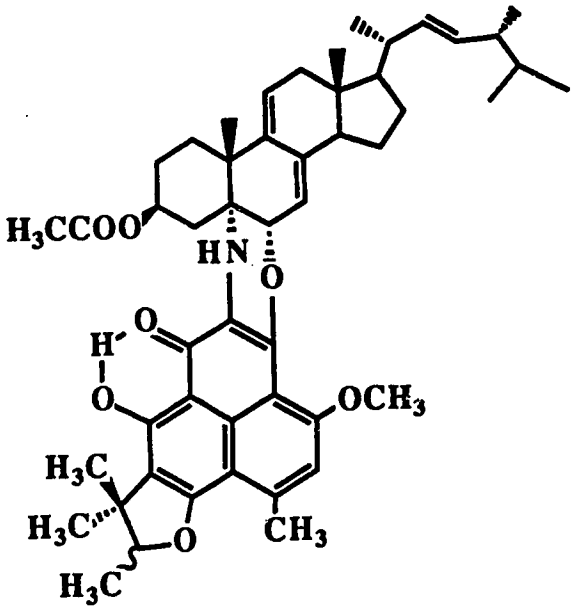
C-9a	104.17	109.32
C-10	23.49	23.69
C-1'	14.68	14.62
C-2'	91.27	91.12
C-3'	43.29	3.32
C-4'	25.85	5.86
C-5'	20.65	20.57
OMe		56.86
C-1"	28.65	28.63
C-2"	30.36	30.39
C-3"	66.84	67.11
C-4"	42.03	42.08
C-5"	55.66	55.37
C-6"	75.73	74.17
C-7"	117.05	118.06
C-8"	139.17	139.17
C-9"	140.61	139.70
C-10"	37.17	37.59
C-11"	124.81	124.32

C-12"	40.22	40.15
C-13"	42.43	42.33
C-14"	51.03	50.95
C-15"	22.99	23.02
C-16"	29.45	29.53
C-17"	55.85	55.84
C-18"	11.63	11.58
C-19"	23.67	23.89
C-20"	40.33	40.30
C-21"	20.75	20.70
C-22"	135.31	135.40
C-23"	132.30	132.15
C-24"	42.86	42.82
C-25"	33.10	33.08
C-26"	19.66	19.60
C-27"	19.98	19.92
C-28"	17.67	17.64

[a] APT spectrum measured at 75MHz.

[b] Obtained at 90 MHz.

Acetylation of **39** with acetic anhydride and pyridine provided a mono-methyl monoacetyl **40**. The ir spectrum of **40** shows the presence of ester absorption at 1729 cm^{-1}. In the ^{1}Hnmr spectrum, H-3" is shifted downfield to δ5.15 ppm and the methyl group of the acetate appears at δ1.94 ppm. Fabms provided an ion at m/z 790.77 ($M+H^+$, 2.56%) which corresponds to a molecular formula $C_{50}H_{63}O_7N$.

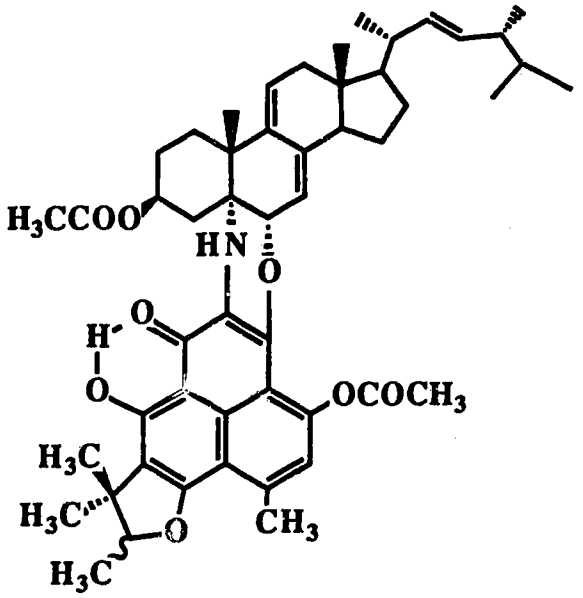


40

Since compound **40** contains a NH functional group, we decided to make a N-methyl derivative. Thus, compound **40** was refluxed with formaldehyde and formic acid for 6 hours.[41] However, only starting material was recovered, no N-methyl compound was detected.

Diacetate **41** was obtained when **36** was treated with acetic anhydride and pyridine. The ir spectrum of **41** displays phenol acetate (1770 cm^{-1}) and aliphatic acetate (1739 cm^{-1}). The acetates appear at δ2.40 ppm and δ1.96 ppm in the ^{1}Hnmr spectrum. The chelating phenol is observed at δ17.01 ppm. All other signals are

similar to those of the parent compound. Fabms confirmed the molecular formula $C_{51}H_{63}O_8N$ (m/z 818.80, $M+H^+$, 0.99%)



41

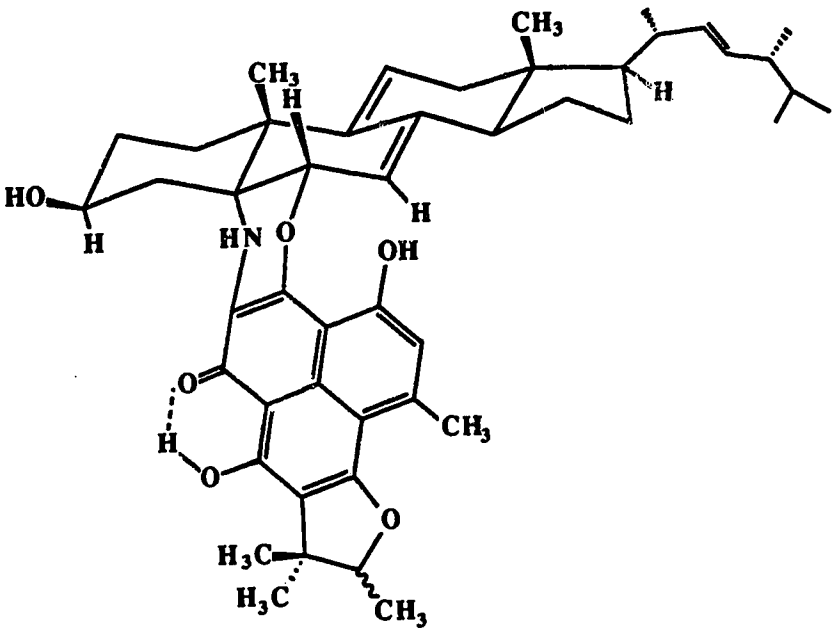
A comparison of ^{1}Hnmr data for sirocodinine **36** and its derivatives **39**, **40**, and **41** was undertaken in order to differentiate structures **36** and **38**. The chemical shifts of H-6" and H-7" in compounds **36**, **39**, **40**, and **41** are shown in Table I-13.

Table I-13 Comparison of ^{1}Hnmr data of H-6" and H-7" in compounds **36**, **39**, **40**, and **41** ($CDCl_3$, 360MHz)

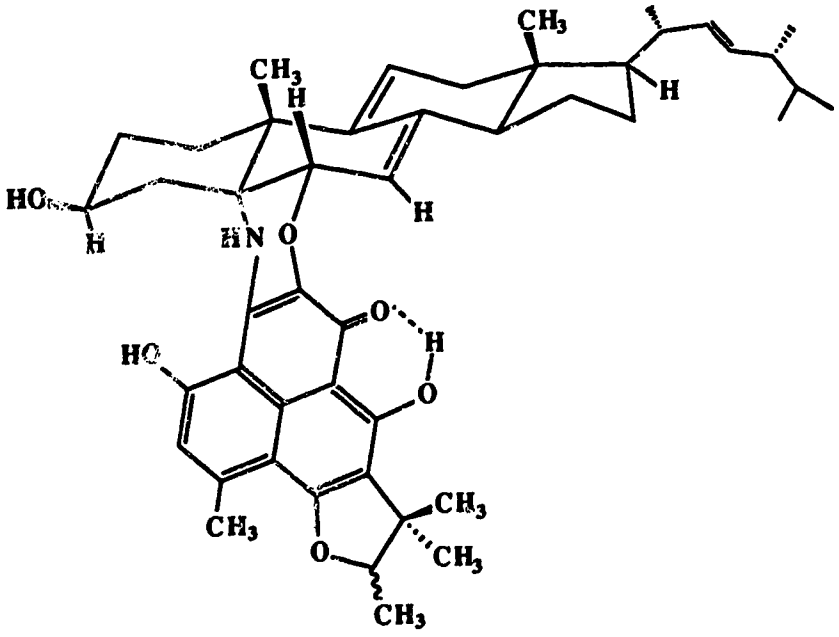
Compound	36	39	40	41
H-6"	4.89	4.81	4.81	4.64
H-7"	5.04	5.03	5.01	4.99

As shown in Table I-13, when the 9-phenol is methylated, the H-6" is shifted upfield in both compounds **39** and **40**. The upfield shift is even larger (0.25 ppm)

for H-6" in diacetate **41**. H-7" is also shifted upfield although the shifts are small. The above information indicates the non-chelated phenol is close to H-6" and H-7". This is best achieved in structure **36**, not in **38**, as illustrated in the conformational drawings.



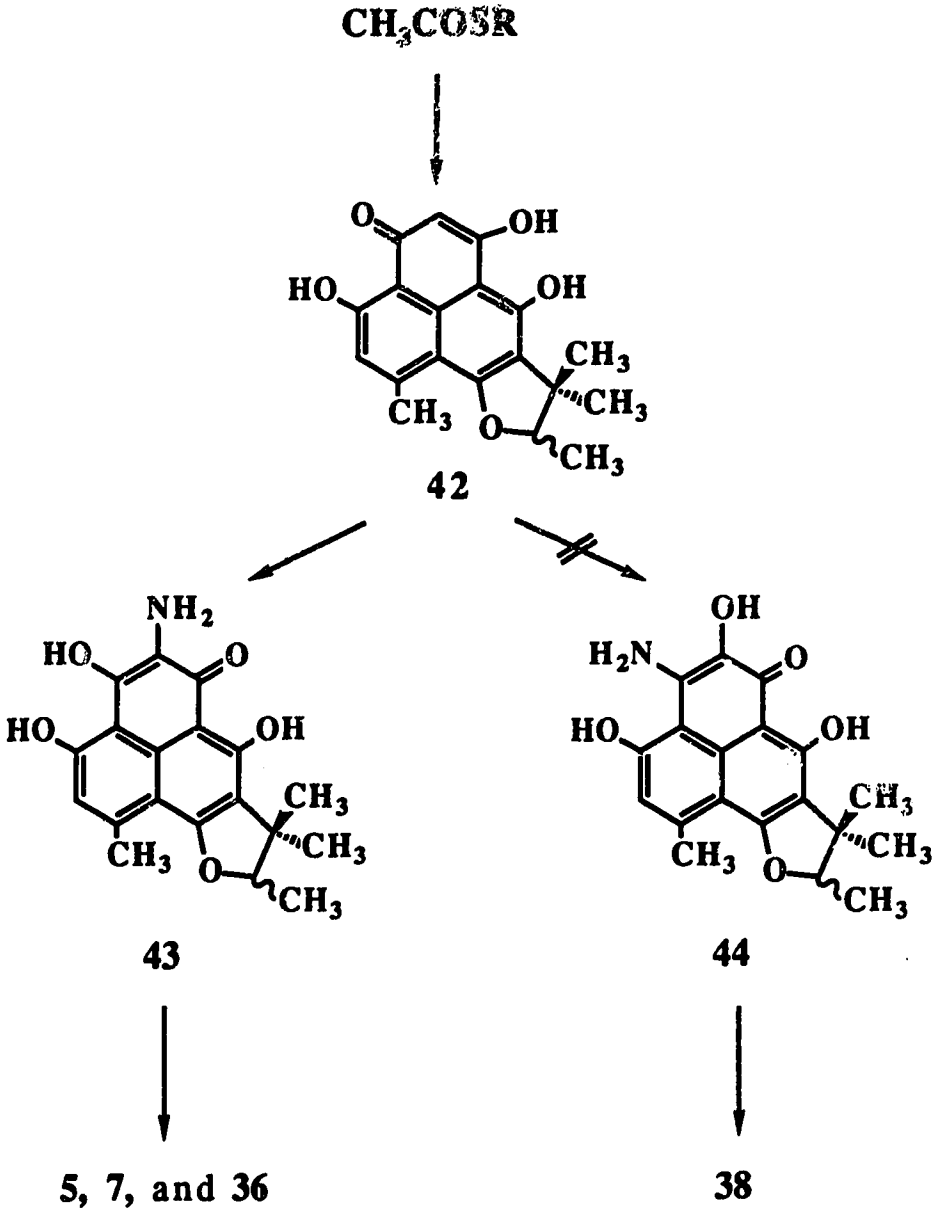
36



38

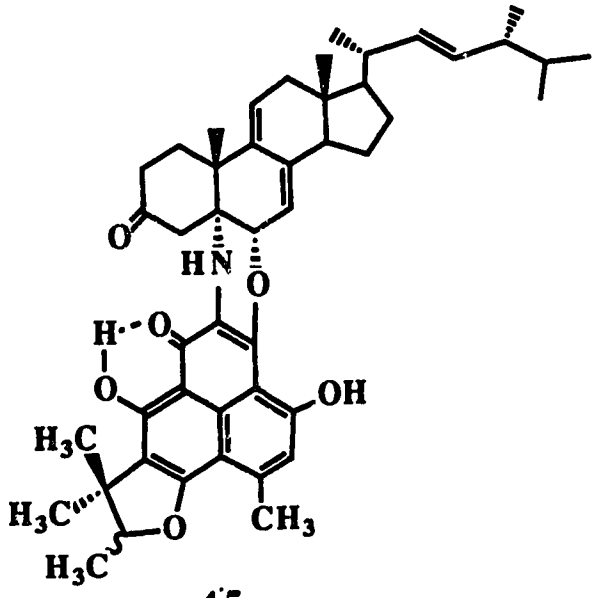
Biogenetic considerations also favor structure **36** over **38** for sirocodinine. Since Scleroderris blue (**5**) and Scleroderris green (**7**) were isolated from the same fungus, a biogenetic intermediate such as **43** (Scheme I-5), may well be involved.

Scheme I-5 Biogenatic consideration for sirocodinine (**36**).



The argument is as follows. Biogenetically, an intermediate such as **42** may be the first cyclized heptaketide. C ·idative amination would give either **43** or **44**. We believe that formation of **43** is the actual process because Scleroderris blue (**5**) and Scleroderris green (**7**), which derive from intermediate **43**, are isolated from the fungus. Reaction of intermediate **43** with a sterol such as 9(11)-dehydroergosterol endoperoxide (**1**) or one of its derivatives could lead to sirocodinine (**36**).

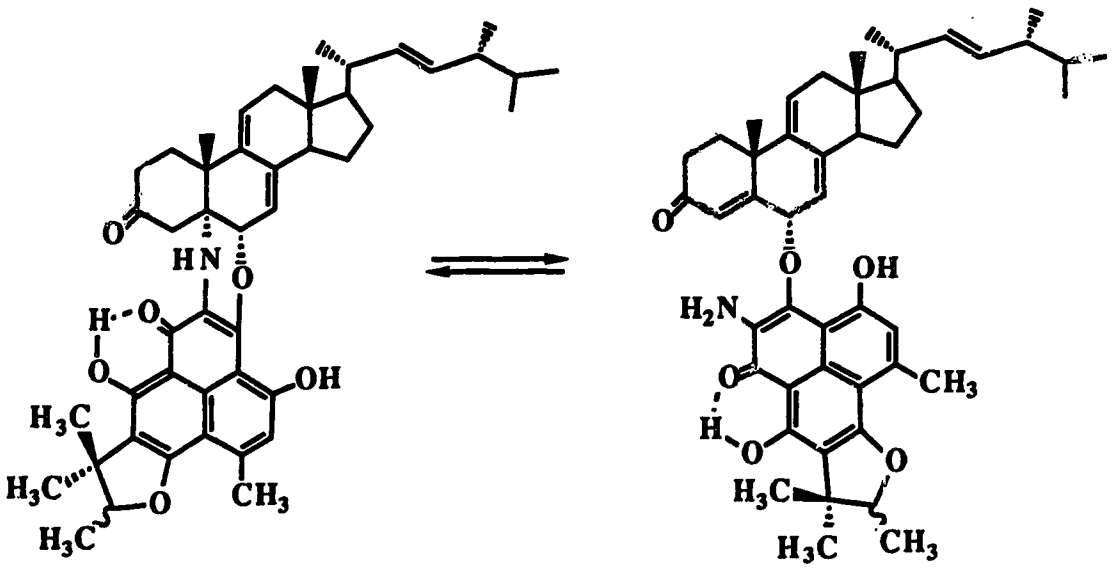
In the case of sirocodin (**18**), we have tried to cleave both the C_6"-O bond and C_5"-O bond by hydrogenolysis or oxidation-elimination without success. Since there is a C_5"-N bond in sirocodinine (**36**), we decided to attempt to cleave this bond using an oxidation-elimination approach. Swern oxidation was our first choice. Oxidation of sirocodinine (**36**) using the Swern procedure [DMSO, $(CF_3CO)_2O$; then Et_3N] yielded 3"-keto sirocodinine (**45**) without cleavage of the C_5"-N bond.



45

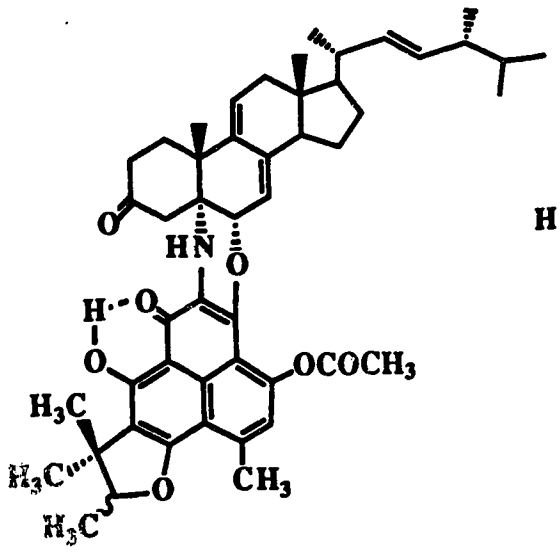
The ir spectrum of **45** shows a ketone absorption at 1720 cm^{-1}. Fabms displays an ion at m/z 732.62 ($M+H^+$, 0.67%) corresponding to a molecular formula

$C_{47}H_{57}O_6N$. The signal for H-3" is not observed in the ^{1}Hnmr spectrum of **45**. Two phenol signals appear at δ16.70 and 9.63 ppm and the NH signal is at δ4.00 ppm. H-6" and H-7" are noted at δ5.01 and 5.10 ppm.

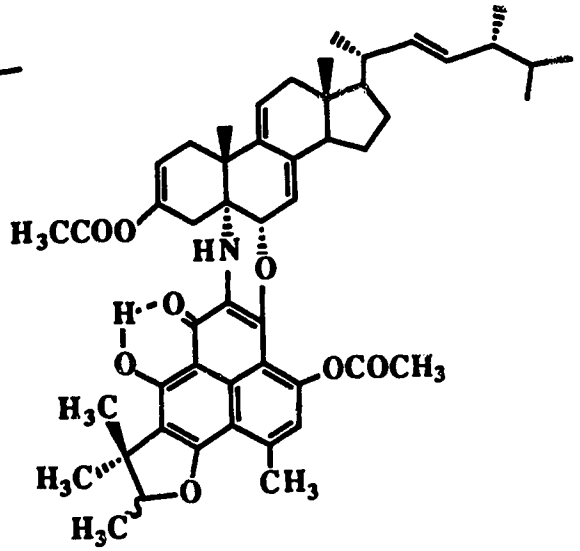


I-2

Considering that there might be an equilibrium between the open form and the closed form (equation I-2), we attempted trapping the open form. One way to achieve it would be to acetylate the amino group in the open form. Thus, compound **45** was refluxed with acetic anhydride and triethylamine for two hours. A monoacetate **46** and a diacetate **47** were obtained, but no trace of a compound with the C5"-N bond cleaved was detected.



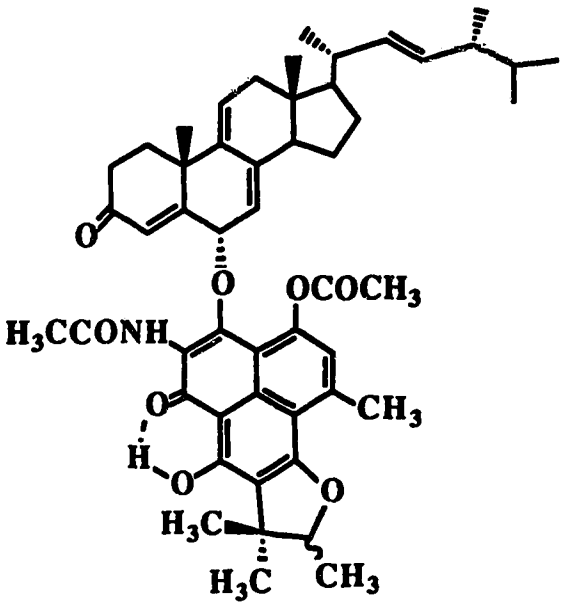
46



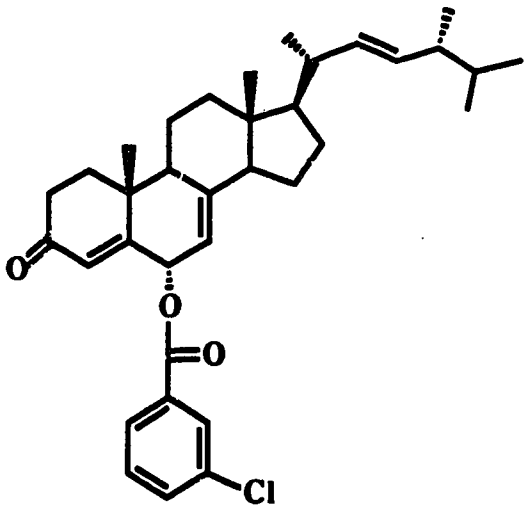
47

The monoacetate is a phenol acetate of **45**. Its ir spectrum shows acetate and ketone absorptions at 1767, 1744, and 1717 cm^{-1}. The signal for the methyl group of the acetate appears at δ2.38 ppm. The expected molecular formula $C_{19}H_{59}O_7N$ was confirmed by fabms (m/z 774.53, $M+H^+$, 2.15%). The ketone absorption is absent in the ir spectrum of diacetate **47**, which shows acetate absorption at 1753 cm^{-1}. In the ^{1}Hnmr spectrum, a number of changes are observed. A new olefinic hydrogen signal appears at δ5.50 ppm (d, J=5Hz) and an enol acetate methyl appears at δ2.04 ppm. The NH signal is shifted downfield to δ4.48 ppm. The fabms confirmed it is a diacetate with the molecular formula $C_{51}H_{61}O_8N$ (m/z 816.67, $M+H^+$, 0.28%).

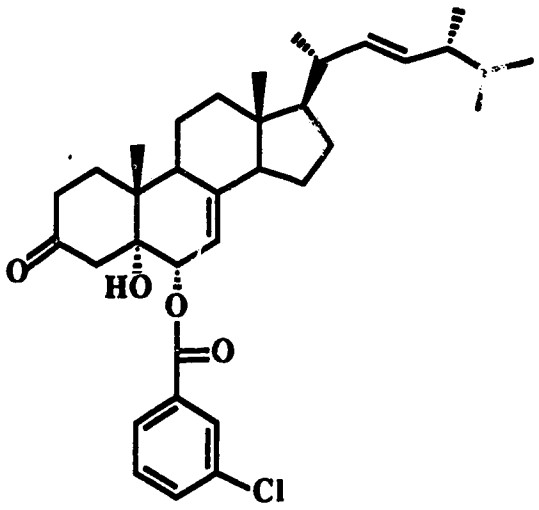
To ascertain that the diacetate has structure **47** instead of open form **48**, we synthesized a model compound of **48**. Oxidation of compound **28** with the Swern procedure gave enone **49** and ketone **50** in a *ca* 1:1 ratio.



48



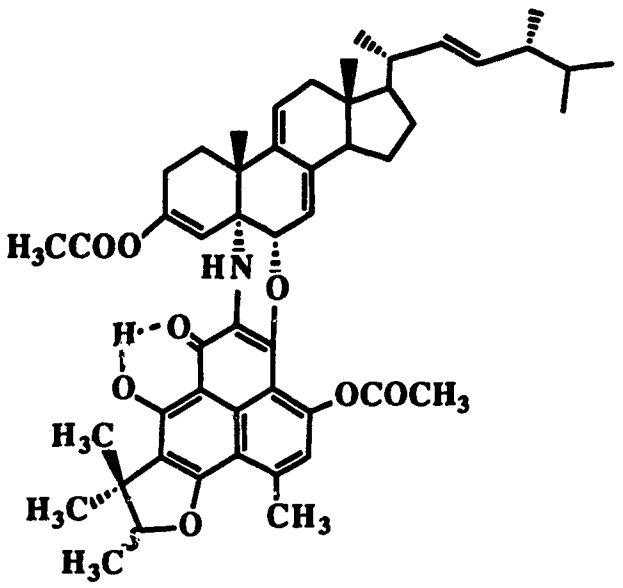
49



50

The ir spectrum of **49** clearly shows absorption for an ester at 1725 cm^{-1} and an α,β-unsaturated ketone at 1682 cm^{-1} which is not observed in the ir spectrum of diacetate **47** and compound **50**. Instead compound **50** displays absorption for a ketone and an ester at 1718 cm^{-1} and a hydroxyl at 3400 cm^{-1}. The ^{1}Hnmr spectra of **49** and **50** are very similar except for the following difference. H-6 appears at

δ6.38 ppm as a broad singlet in compound **49**, at δ5.66 ppm in compound **50**. The assignment was verified by an nOe experiment with compound **49**. When the signal of the 19-methyl (δ1.31 ppm) is irradiated, H-6 has 5% nOe. The much lower field shift of H-6 in compound **49** compared with that of **50** indicates the presence of an enone adjacent to it. The olefinic hydrogen (H-4) on the α,β-unsaturated ketone appears at δ5.95 ppm as a doublet (J=2Hz) which is a result of a coupling between H-4 and H-6. The decoupling experiment confirmed this long range coupling: when H-6 is irradiated, H-4 collapses to a singlet. H-7 is also observed to have different chemical shifts: at δ5.21 ppm for **49** and at δ5.03 ppm for **50**. The coupling constant and chemical shift of H-2" (δ5.50 ppm, J=5Hz) in diacetate **47** are different from those of H-4 (δ5.95, J=2Hz) in compound **49**. Thus, the open form structure **48** was ruled out for the diacetate. Based on the large coupling constant of the hydrogen at δ5.50 ppm (H-2"), the alternative structure **51** which is a double bond isomer of **47** was discarded because H-4" in compound **51** should have a small coupling constant due to the long range coupling.The assignment of structure **47** to the diacetate is also consistent with the observations found in the steroid field.[42] The enolization of 3-ketones has been well studied and the results indicate that the prefered enolic form of the 5α-series (A/B ring is *trans*) is the 2-ene, whereas a 3-ketone of the 5β-series (A/B ring is *cis*) enolizes preferentially to the 3-ene. Since the sterol part of **45** belongs to 5α-series, the formation of 2-ene **47** is very reasonable.



51

Like sirocodin (**18**), sirocodinine (**36**) is a mixture of diastereoisomers at C-2' as indicated by two pairs of signals for the phenolic hydrogens at δ17.84ppm and δ9.76ppm. This also occurs in the derivatives of sirocodinine. The ratio of the two epimers for sirocodinine (*ca* 5:1) is different from that for sirocodin (*ca* 2:1).

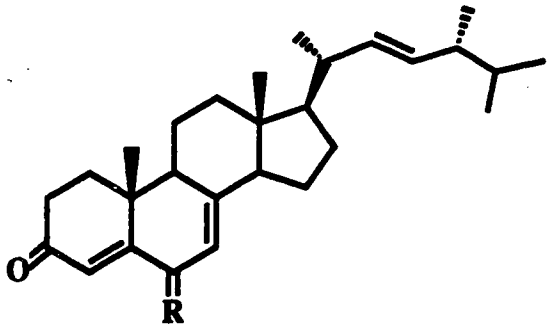
The biological function of steroids is well known. It is believed that the long-lived (not metabolized further) sterols have a vital role in maintaining the structural integrity of most membraneous structures in organisms. They also appeared to assist in the regulation of the permeability of these membranes to various ions.[43] The isolation of sirocodin (**18**) and sirocodinine (**36**) from the fungi is of special interest possibly suggesting the participation of this type of pigment in the plant disease. Formation of the pigment sterol adducts of type **18** and **36** could inhibit the function of the steroid and possibly have some role in the disease of Sirococcus shoot blight or the disease caused by *G. myritillis* T256.

Synthesis of 6α-hydroxyergosta-4,7,22-trien-3-one (52)

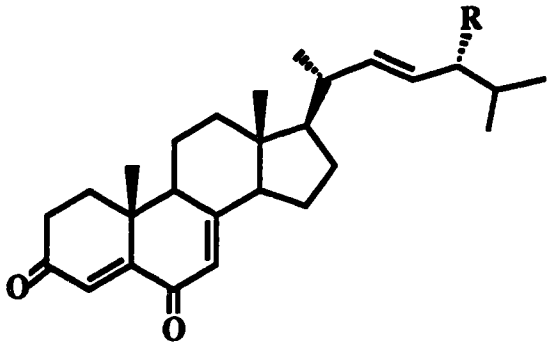
Although we did not isolate 6α-hydroxyergosta-4,7,22-trien-3-one from our fungi, the synthesis of this compound is closely related to our work.

In 1988, Nishitoba and coworkers isolated both 6α- and 6β-hydroxyergosta-4,7,22-trien-3-one, (52) and (53), from the fungus *Ganoderma lucidum*.[44] They reported 52 as a novel steroid and 53 as a natural product for the first time. That compounds 52 and 53 are epimers at C-6 was confirmed by oxidation of 52 and 53 to 54 with pyridinium dichromate (PDC). The stereochemistry of C-6 of 53 was assigned as β-hydroxy based on a comparison of the spectral data with the "same compound" in the literature. Thus 52 was assigned as 6α-hydroxyergosta-4,7,22-trien-3-one. However, no further spectral or chemical evidence was available to support these structures.

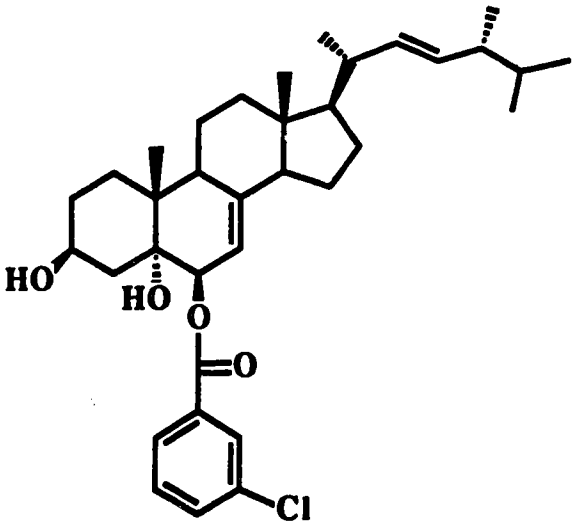
Malorni and coworkers isolated 4,7,22-ergostatrien-3,6-dione (54) from the sponge *Raphidostila incisa* as a mixture of three compounds 54, 55, and 56 in 1978.[45] In order to confirm the presence of 54, the authors synthesized 54 from ergosterol. Oxidation of ergosterol with *m*-chloroperbenzoic acid (MCPBA) gave a triol monoester. Based on the ir, ^{1}Hnmr, and ms spectral data, structure 57 was assigned to this compound. Jones oxidation of 57 yielded the corresponding ketone ester 58 which was hydrolyzed with potassium hydroxide in methanol providing compound 53 in 10% yield. Oxidation of 53 with manganese dioxide gave compound 54 in 60% yield. Thus, structure 54 for 4,7,22-ergostatrien-3,6-dione was confirmed. However, the stereochemistry of C-6 in compounds 53, 57, and 58 was not assigned unambiguously.



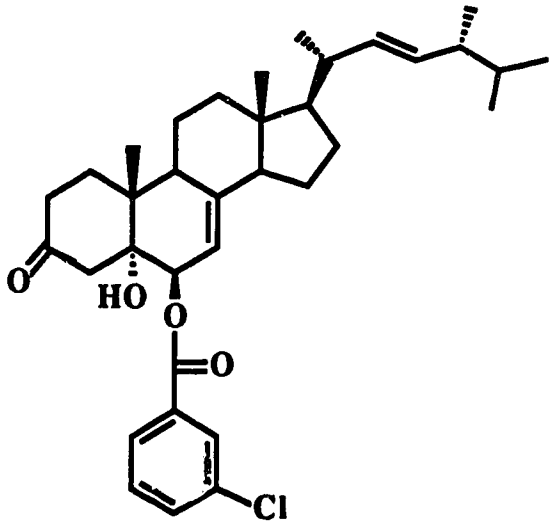
52 R=α-OH, β-H
53 R= β-OH, α-H



54 R=CH_3
55 R=H
56 R=CH_3CH_2-



57



58

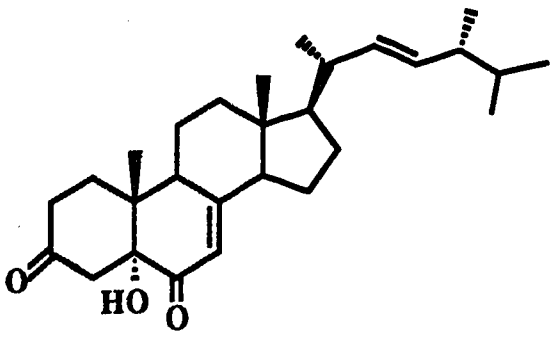
In our structure determination of sirocodin (**18**) and sirocodinine (**36**), we had to prepare compounds (**27**) and (**49**) in order to assign the ^{1}Hnmr and ^{13}Cnmr data of sirocodin, sirocodinine, and their derivatives. With several derivatives of ergosterol in hand, we find that the assignment of the structures of **57**, **58**, and **53** in the literature is incorrect. The configuration at C-6 in the three compounds should

be 6β-H. The spectral data for compounds **52** and **53** is also misassigned. In order to clarify the points, we decided to prepare compound **52.**

The stereochemistry of C-6 in compounds **28** and **49** has been secured by nOe experiments. Hydrolysis of compound **49** with 1M KOH/MeOH in methylene chloride for 5 minutes gave 6α-hydroxyergosta-4,7,22-trien-3-one (**52**). It is important to carry out this reaction for a short time period (5~10 minutes). If the reaction time is longer, the initial product **52** gives a mixture of different compounds. An attempt to hydrolyze compound **50** and eliminate water to give **52** was unsuccessful. The ester **50** was refluxed with 1M KOH/MeOH in methanol for 1.5 hours. The resulting mixture was the same as that from compound **48** using long reaction time. This lack of success was probably due to the long reaction time. The ir spectrum of **52** indicates the presence of a hydroxy (3364 cm^{-1}) and an enone system (1661 cm^{-1}). The molecular formula of **52** is $C_{28}H_{42}O_2$ (MW 410) as determined by hrms. The ^{1}Hnmr spectrum of **52** shows a doublet at δ6.13ppm (J=2Hz, H-4), a broad singlet at δ5.23ppm (H-7), two multiplet hydrogens at δ5.20ppm (H-22, 23), a broad doublet at δ4.98ppm (J=2Hz, H-6), and six methyl groups at δ1.19 (19-CH_3), 1.04 (J=6.5Hz, 21-CH_3), 0.92 (J=7Hz, 28-CH_3), 0.85 (J=6Hz, 27-CH_3), 0.83 (J=6Hz, 26-CH_3), and δ0.62ppm (18-CH_3). The long range coupling between H-4 and H-6 is confirmed by a decoupling experiment. When the signal at δ4.98ppm (H-6) is irradiated, the signal at δ6.13ppm (H-4) changes to a singlet. The nOe experiment verified the assigned stereochemistry at C-6 of **52**.On irradiation of the signal at δ1.19ppm (19-CH_3), the signal at δ4.98ppm (H-6) shows 14.9% nOe strongly indicating that H-6 has β-configuration. The spectral data (ir, ^{1}Hnmr, ms) of **52** are almost identical with the reported data for compound **53**. Thus, structures **52** and **53** were misassigned by Nishitoba and

coworkers. This was mainly because of the incorrect assignment of structure **53** made by Malorni and associates.

We also attempted to transform triol **27** to **54**. Oxidation of triol **27** with pyridinium chlorochromate (PCC)[46] yielded diketone **59**. However, elimination of water from **59** in refluxing methylene chloride with triethylamine for 30 minutes was unsuccessful. Due to the small amount of sample of **59** available, we did not study this further.



59

The ir spectrum of **59** shows absorption of a hydroxyl (3200 cm^{-1}), a ketone (1710 cm^{-1}), and an enone (1672 cm^{-1}). In the ^{1}Hnmr spectrum of **59**, the H-7 signal appears at δ5.72ppm as broad singlet. The doublets at δ2.83ppm and δ2.58ppm (J=16Hz) are attributed to H-4 geminal hydrogens. The ^{13}Cnmr spectrum and hrms also support structure **59**.

In summary, we have synthesized 6α-hydroxyergosta-4,7,22-trien-3-one from ergosterol, and the incorrect stereochemical assignments in the literature have been clarified.

EXPERIMENTAL

General

High resolution electron impact mass spetra (hrms) were recorded on an AEI MS-50 mass spectrometer coupled to a DS-50 computer. Data are reported as m/z (relative intensity) except for the molecular ion, which is reported as m/z found (m/z calculated, relative intensity). Unless diagnostically significant, peaks with intensity less than 20% of the base peak are omitted. Low resolution electron impact mass spectra (lrms) were recorded on a AEI MS-12 mass spectrometer. Chemical ionization mass spectra (cims) were obtained using an AEI MS-12 mass spetrometer with ammonia as the reagent gas. The data were processed using DS-55 software and a Nova-4 computer. Fast atom bombardment mass spectra (fabms) were recorded on a AEI MS-9 mass spectrometer. The sample was suspended in matrix of 5 parts of dithiothreitol (Cleland's reagent) and 1 part of dithioerythritol. Fourier transform infrared (ir) spectra were recorded (as a cast from $CHCl_3$ solution unless otherwise noted) on a Nicolet FT 7199 interferometer. Optical rotations were recorded on a Perkin Elmer 141 polarimeter. ^1H and ^{13}C nuclear magnetic resonance (nmr) spectra were measured (in $CDCl_3$ unless otherwise noted) on Bruker WH-300 (300 MHz for ^1H and 75 MHz for ^{13}C), WM-360 (360 MHz for ^1H and 90 MHz for ^{13}C), or WH-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometers with either Aspect 2000 or Aspect 3000 computer systems. Chemical shifts are reported in parts per million (δ value from tetramethylsilane (TMS)). Chloroform (unless otherwise noted) was used as the internal standard, ^1H: δ7.27; ^{13}C: δ77.00 relative to TMS. TMS was used as the internal stardard when the solvent shift spectra were recorded. Coupling constants, J, are expressed in hertz

(Hz) and are reported to within ± 0.2Hz. The ^{13}C multiplicities were derived from attached proton test (APT) experiments. The following abbreviations are used: m=multiplet, s=singlet, d=doublet, t=triplet, q=quartet, br=broad. Difference nuclear Overhauser effect (nOe) experiments were done on degassed solutions on the Brucker WM-360 spectrometer. Melting points are uncorrected and were determined on a Thomas model 40 melting point apparatus.

Reagent grade solvents were distilled prior to use. Skellysolve B (SKB) refers to Skelly oil company light petroleum, bp 62-70^{0}C. Analytical grade diethyl ether (ACS 288) was used without further purification. Dry methylene chloride was distilled from phosphous pentoxide. Dry dimethyl sulfoxide (DMSO) was distilled from calcium hydride under reduced pressure.

Flash chromatography was carried out with E. Merck silica gel 60 (230~400 mesh), according to the method described by Still.[47] E. Merck precoated glass plates of silica gel 60F-254 were used for preparative thin-layer chromatography (ptlc). Analytical thin-layer chromatography (tlc) was performed on cut sections of E. Merck precoated aluminum sheets of silica gel 60F-254. The chromatograms were examined under ultraviolet light (254nm). The tlc plates were then dipped in a solution of phosphomolybdic acid (10g of $MoO_3 \cdot H_3PO_4$, 1.25g of $Ce(SO_4)_2$, 12ml concentrated H_2SO_4, diluted to 250ml with H_2O) for several seconds, and the spots were visualized by charring on a hot plate (300^{0}C).

Culturing of the fungi and isolation of the metabolites

Source of the fungi

All the stock cultures used in our study were provided by Professor O. Kandler, Botanical Institute, University of Munich. The Sirococcus strains are deposited at the University of Alberta Microfungus Herbarium (UAMH) under the following accession numbers: Sirococcus 35B (UAMH 5401), Sirococcus 20 (UAMH 5402), and Sirococcus STM8 (UAMH 5394).

Growth and harvesting of the fungi

Stock cultures of each strain of Sirococcus and of Godronia were maitained in slant tube culture on potato dextrose agar at 4^0C. To initiate large-scale cultures, small fragments of agar containing the mycelium were aseptically transfered to Erlenmeyer flasks containing sterile culture medium (200ml). The medium was either Difco potato dextrose broth containing 0.04% (w/v) yeast extract or Wort medium (malt extract 15g, peptone 0.78g, maltose 12.75g, dextrin 2.75g, glycerol 2.35g, K_2HPO_4 1.0g, NH_4Cl 1.0g in 1L H_2O; pH adjusted to 4.8). The cultures were shaken for 10 days at 15~17^{0}C. The content of each inoculation flask was used to inoculate five 2.8-L Fernbach flasks containing the same liquid medium (1L, still culture) and the flasks were kept at 15~17^{0}C. After 50~60 days, the mycelium was separated from the broth by gravity filtration through cheese cloth. The broth was concentrated to one tenth of its original volume and extracted with methylene chloride. The solvent was removed under reduced pressure to give the crude broth extracts. The air-dried mycelium was extracted with methylene chloride in a Soxhlet extractor for 2 days. Concentration of the methylene chloride extracts gave the crude mycelial extracts. Most of the metabolites were present in the mycelial extract and only a small amount in the broth extract.

Separation of metabolites of Sirococcus 20

The methylene chloride extract (2.8g) of strain 20 (UAMH 5402) grown on PDY medium was separated into non-polar, medium polar, and polar fractions by silica gel chromatography (gradient elution; SKB-toluene-ethyl acetate-methanol). Further chromatographic separation (gradient elution; SKB-ether) of the non-polar fraction (1.16g) gave sclerodin (3, 67mg), 9,11-dehydroergosterol endoperoxide (1, 2mg), and a mixture of fatty acids and triglycerides. Further chromatographic separation (gradient elution; SKB-ether, then methylene chloride-methanol-acetic acid 96:2:2) of the medium polarity fraction (0.48g) gave compound 3 (61mg), lactone 9 (5mg), and Scleroderris blue (5, 3mg). Further separation of the polar fraction (1.17g, gradient elution; SKB-ethyl acetate, then methylene chloride-methanol-acetic acid 96:2:2) gave compound 3 (5.5mg).

The methylene chloride extract (1.42g) obtained from the culture of Sirococcus 20 grown on Wort medium was separated as described above. The following compounds were isolated: sclerodin (3, 11mg), ergosterol endoperoxide, ergosta-4,6,8(14),22-tetraen-3-one (2), and Scleroderris blue (5).

Separation of the metabolites of Sirococcus 35B

The methylene chloride extract (2.99g) of strain 35B (UAMH 5401) grown on PDY was separated into non-polar, medium polar, and polar fractions by silica gel chromatography (gradient elution; SKB-toluene-ethyl acetate-methanol). The non-polar and medium polar fractions were separated by repeated flash chromatography over silica gel. Fatty acids, triglycerides, sclerodin (3, 140mg), ergosterol (36mg), 9(11)-dehydroergosterol endoperoxide (1, 3mg), ergosta-4,6,8(14),22-tetraen-3-one (2, 3mg), sirocodilide (12, 17mg), sirocodin (18, 5mg), and sirocodinine (36, 2mg) were isolated. Scleroderris blue (5, 3mg) was isolated from the polar fraction by flash chromatography (methylene chloride-

methanol-acetic acid 96:2:2). The methylene chloride extract (39mg) obtained from broth of Sirococcus 35B grown on PDY medium was separated by silica gel chromatography (gradient elution; SKB-acetone). A purple compound, trypethelone (**11**, 1mg) was obtained.

The methylene chloride extract (5.7g) obtained from mycelium of Sirococcus 35B grown on Wort medium was separated into 3 portions by chromatography over silica gel (gradient elution; SKB-acetone). Further separation of portion 1 gave fatty acids and triglycerides. Chromatography of portion 2 using gradient elution (toluene-ethyl acetate) led to the isolation of sclerodin (**3**, 38mg) and the acetone adduct of atrovenetinone (**8**, 4mg). Further separation of portion 3 by silica gel chromatography (eluent: SKB-acetone-acetic acid 15:4:1) led to the isolation of Scleroderris blue (**5**, 6mg).

Separation of metabolites of Sirococcus STM8

The methylene chloride extract (0.85g) obtained from mycelium of Sirococcus STM8 (UAMH 5394) grown on PDY was separated using similar procedures to those described above. The following metabolites were isolated: fatty acids, triglycerides, ergosterol (106mg), and ergosterol endoperoxide (20mg).

Separation of metabolites of *Godronia myrtilli* T256

The following compounds were isolated from the methylene chloride extract (7.7g) obtained from mycelium of *G. myrtillis* T256 grown on PDY: sclerodin (**3**), ergosterol endoperoxide, triglycerides, fatty acids, 9(11)dehydroergosterol endoperoxide (**1**), ergosta-4,6,8(14),22-tetraen-3-one (**2**), sirocodin (**18**), and sirocodinine (**36**). In addition to the above compounds, Scleroderris blue (**5**) was

also obtained from the methylene chloride extract of *G. myrtillis* T256 grown on Wort medium.

Separation of metabolites of *Godronia cassandrae*

Only triglycerides were isolated from the methylene chloride extract (8.3g) of *G. cassandrae* grown on Wort medium. The metabolites of *G. cassandrae* grown on PDY medium were similar to that grown on Wort medium as determined by TLC. Further separation of the metabolites was not undertaken.

Characterization of the metabolites and their derivatives

9(11)-Dehydroergosterol endoperoxide (1)

Ir υ_{max}: 3400, 2958, 2932, 2872, 1459, 1373, 1075 1035 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.85 (3H, s), 0.92 (3H, d, 7Hz), 0.95 (3H, d, 7Hz), 1.06 (3H, d, 7.2Hz), 1.12 (3H, d, 6Hz), 1.22 (3H, s), 4.08 (1H, m), 5.13 (1H, dd, 8, 15.5Hz), 5.22 (1H, dd, 8, 15.5Hz), 5.53 (1H, dd, 1.5, 6Hz), 6.27 (1H, d, 8Hz), 6.58 (1H, d, 8Hz); Hrms: m/z 426.3130 (calcd for $C_{28}H_{42}O_3$, 426.3134, 24%), 408 (8), 394 (40), 376 (29), 269 (51), 251 (46).

Ergosta-4,6,8(14),22-tetraen-3-one (2)

Ir υ_{max}: 2958, 2924, 2871, 2855, 1645, 1638, 1461, 1376, 1356, 1270, 1216, 1196 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.22 (2H, m), 5.73 (1H, s), 6.04 (1H, d, 5.4Hz), 6.62 (1H, d, 5.4Hz); Hrms: m/z 392.3068 (calcd for $C_{28}H_{40}O$, 392.3079, 19%), 268 (92), 253 (25).

Sclerodin (3)

mp. 256-257^{0}C (methanol-methylene chloride); $[\alpha]_D$ see Table I-1; Ir υ_{max}: 2993, 2965, 1710, 1666, 1611, 1461, 1387, 1303, 1186, 1038, 811, 761 cm^{-1}; ^{1}Hnmr (360MHz): δ 1.24 (3H, s), 1.44 (3H, d, 7Hz), 1.58 (3H, s), 2.76 (3H, s), 4.66 (1H, q, 7Hz), 6.80 (1H, s), 11.78 (1H, s), 12.44 (1H, s); Hrms: m/z 328.0951 (calcd for $C_{18}H_{16}O_6$, 328.0947, 35%), 313 (100).

Scleroderris blue (5)

Ir υ_{max}: 2950, 2930, 1609, 1551, 1457, 1392, 1384, 1345, 1333, 1304 cm^{-1}; ^{1}Hnmr (400MHz): δ 1.22 (6H, brd, 7Hz), 1.28 (6H, brs), 1.52 (6H, brs), 2.76 (6H, brs), 4.62 (2H, brq, 7Hz), 6.89 (2H, brs), 13.50 (2H, brs), 14.50 (2H, brs); Fabms: m/z 664.34 ($M+H^+$, calcd for $C_{38}H_{34}O_{10}N$, 664.2183, 1.28%).

The acetone adduct of atrovenetinone (8)

Ir υ_{max}: 3400, 2965, 2928, 1710, 1631, 1606, 1458, 1382, 1336, 1311cm-1; ^{1}Hnmr (360MHz): δ 1.24 (3H, s), 1.26 (3H, s), 1.46 (6H, d, 6Hz), 1.56 (3H, s), 1.58 (3H, s), 2.18 (6H, s), 2.75 (6H, s), 3.26 (2H, s), 3.28 (2H, s), 4.64 (2H, m), 6.74 (2H, s), 12.74 (1H, s), 12.76 (1H, s), 13.24 (1H, s), 13.30 (1H, s); ^{13}Cnmr (75MHz): δ14.47 (q), 14.73 (q), 24.25 (q), 25.52 (q), 25.85 (q), 30.98 (q), 31.09 (q), 43.33 (s), 43.38 (s), 52.16 (t), 51.80 (t), 91.63 (d), 91.71 (d), 102.69 (s), 105.52 (s), 109.78 (s), 118.00 (d), 118.45 (s), 118.57 (s), 137.50 (s), 137.53 (s), 149.25 (s), 165.39 (s), 165.45 (s), 166.23 (s), 166.29 (s), 197.01 (s), 197.09 (s), 199.28 (s), 205.70 (s), 205.93 (s); Hrms: m/z 398.1378 (calcd for $C_{22}H_{22}O_7$, 398.1365, 42%), 355 (23), 313 (100), 297 (24).

Lactone 9

mp 213-215^{0}C (toluene); Ir υ_{max}: 3200, 1725, 1647, 1627, 1382, 1371, 1319, 1284, 1166 cm^{-1}; ^{1}Hnmr (360MHz): δ 1.40 (3H, s), 1.60 (3H, d, 7.2Hz), 1.63 (3H, s), 2.78 (3H, d, 2.5Hz), 4.76 (1H, q, 7.2Hz), 6.78 (1H, brs); Hrms: m/z 300.1005 (calcd for $C_{17}H_{16}O_5$, 300.0998, 61%), 285 (100).

Trypethelone (11)

Ir υ_{max}: 3040, 2963, 2927, 1735, 1682, 1647, 1602, 1585, 1541, 1460, 1407, 1383, 1363, 1319 cm^{-1}; ^{1}Hnmr (360MHz): δ 1.25 (3H, s), 1.40 (3H, s), 1.46 (3H, d, 6Hz), 2.58 (3H, s), 4.58 (1H, q, 6Hz), 6.87 (1H, d, 2Hz), 7.43 (1H, d, 2Hz); Hrms: m/z 272.1049 (calcd for $C_{16}H_{16}O_4$, 272.1048, 6%), 257 (9), 252 (18), 229 (18).

Sirocodilide (12)

mp 121-123^{0}C (ethanol-methylene chloride); $[\alpha]_D$ +40.8^0 (*c* 0.5, $CHCl_3$); Ir υ_{max}: 1745, 1680, 1378, 1309, 1269, 1250, 1171, 1125, 965, 924 cm^{-1}; ^{1}Hnmr (360MHz) see Table I-2; ^{13}Cnmr (75MHz) see Table I-2; Hrms: m/z 224.1042 (calcd for $C_{12}H_{16}O_4$, 224.1048, 5%), 129 (13), 113 (64), 95 (100), 67 (75).

Tetrahydrosirocodilide (13)

Sirocodilide (5mg), 10% Pd-C (5mg), ethanol (5ml) were placed in a three-necked flask. The mixture was hydrogenated at rt. for 2 hours. The catalyst was filtered off and the filtrate was evaporated. The residue was separated by flash chromatography (SKB-acetone 5:1) giving 4mg of 13. In another reaction, PtO_2 was used as catalyst

and compound 13 was obtained in good yield. Ir υ_{max}: 2959, 2923, 2872, 1742, 1173, 1117 cm^{-1}; ^{1}Hnmr (360MHz): δ0.89 (3H, t, 7Hz), 1.31 (2H, m), 1.54 (2H, m), 2.49 (1H, dd, 5, 15Hz), 2.57 (1H, dd, 7,15Hz), 5.26 (1H, ddt, 5, 6, 7Hz); Lrms: m/z 228 (calcd for $C_{12}H_{20}O_4$, 228.1361, 6.4%), 174 (17), 131 (34), 115 (57), 114 (66), 99 (100), 97 (47), 96 (23), 68 (21).

(S)-3-Hydroxy-*n*-caprohydrazide (14) and *n*-caprohydrazide (15)

The hydrogenation product (from 3.9mg 12) was heated on a hot water bath with 2 drops of anhydrous [illegible] for a half hour. Ethanol, 1ml, was then added, and the solution was refluxed for two hours. The solvent was evaporated and the residue was separated by silica gel chromatography (SKB-acetone 10:1, 5:1, 2:1, acetone) providing 1.2mg of (S)-3-hydroxy-n-caprohydrazide (14) and 0.5mg of n-caprohydrazide (15). 14: $[\alpha]_D$ $+16.7^0$ (*c* 0.12, $CHCl_3$); Ir υ_{max}: 3300, 3217, 3209, 3201, 3197, 2956, 2925, 1645, 1620, 1538, 1128, 1013 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.95 (3H, t, 6.5Hz), 1.42 (4H, m), 1.59 (1H, s), 2.55 (1H, dd, 8.3, 14Hz), 2.35 (1H, dd, 2.7, 14Hz), 3.14 (1H, brs, NH), 3.92 (1H, brs, NH), 4.03 (1H, ddt, 2.7, 6.7, 8.3Hz), 7.02 (1H, brs, NH); ^{13}Cnmr (90MHz): δ 13.91, 18.67, 39.13, 40.89, 68.32, 172.94; Lrms: m/z 130 (M-NH_2, 9%), 128 (M-H_2O, 4), 113 (M-NH_2-OH, 15), 97 (M-N_2H_3-H_2O, 18), 99 (17), 72 (M-$C_2H_6N_2N$, 100), 55 (62), 43 (31). 15: mp 68-70^0C (ethanol); Ir υ_{max}: 3313, 3292, 2955, 2923, 2858, 1630, 1595 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.91 (3H, t, 6Hz), 1.33 (4H, m), 1.67 (1H, quint, 5.3Hz), 2.16 (2H, t, 5.3Hz), 3.90 (2H, brs, NH), 6.64 (1H, brs, NH); Lrms: m/z 114 (M-NH_2, 4.2%), 99 (M-N_2H_3, 40), 71 (M-N_2H_3-CO, 36), 43 (100), 32 (100).

Sirocodin (18)

Ir υ_{max}: 3390, 3384, 1730 (w), 1660 (w), 1611, 1576, 1444, 1416, 1375, 1339, 1307, 1281, 1239, 1190 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.57 (3H, s, 18"-CH_3), 0.80 (3H, d, 5.3Hz, 26"-CH_3), 0.81 (3H, d, 5.3Hz, 27"-CH_3), 0.88 (3H, d, 6Hz, 28"-CH_3), 1.00 (3H, d, 6Hz, 21"-CH_3), 1.14 (3H, s, 19"-CH_3), 1.25 (1H, brs, OH), 1.32 (3H, s, 4'-CH_3), 1.47 (3H, d, 6Hz, 1'-CH_3), 1.56 (3H, s, 5'-CH_3), 2.82 (3H, s, ArCH_3), 4.05 (1H, m, H-3"), 4.65 (1H, q, 6Hz, H-2'), 4.91 (1H, brs, H-6"), 4.99 (1H, brs, H-7"), 5.15 (2H, m, H-22", 23"), 6.83 (1H, s, ArH), 9.56 (1H, s, 9-OH), 17.16 (1H, s, 4-OH); ^{1}Hnmr ($CDCl_3$+C_5D_5N, 360MHz): δ 0.57 (3H, s, 18"-CH_3), 0.80 (3H, d, 5.3Hz, 26"-CH_3), 0.82 (3H, d, 5.3Hz, 27"-CH_3), 0.90 (3H, d, 6Hz, 28"-CH3), 1.02 (3H, d, 6Hz, 21"-CH_3), 1.14 (3H, s, 19"-CH_3), 1.27 (1H, brs, OH), 1.31 (3H, s, 4'-CH_3), 1.48 (3H, d, 6Hz, 1'-CH_3), 1.59 (3H, s, 5'-CH_3), 2.83 (3H, s, ArCH_3), 4.16 (1H, m, H-3"), 4.66 (1H, q, 6Hz, H-2'), 4.90 (1H, brs, H-6"), 5.05 (1H, brs, H-7"), 5.20 (2H, m, H-22", 23"), 6.88 (1H, s, ArH), 9.82 (1H, s, 9-OH), 17.55 (1H, s, 4-OH); Fabms: m/z 737.48 ($M+H^+$, calcd for $C_{47}H_{61}O_7$, 737.4417, 2.67%), 341 (6), 309 (9).

Methyl sirocodin (**24**)

Sirocodin (5mg) was treated with an excess of diazomethane in ether solution at rt. for 24 hours. The solvent was evaporated and the residue was purified by flash chromatography (SKB-acetone 3:1) giving 5mg of **24**. $[\alpha]_D$ $+79.1^0$ (*c* 0.22, $CHCl_3$); Ir υ_{max}: 3300, 2956, 2932, 1718(w), 1642(w), 1611, 1444, 1375, 1220, 1181, 1162, 1106, 1060, 1037, 1012 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.57 (3H, s, 18"-CH_3), 0.81 (3H, d, 5.3Hz, 26"-CH_3), 0.82 (3H, d, 5.3Hz, 27"-CH_3), 0.89 (3H, d, 6Hz, 28"-CH_3), 1.09 (3H, d, 6Hz, 21"-CH_3), 1.13 (3H, s, 19"-CH_3), 1.26 (1H, brs, OH), 1.32 (3H, s, 4'-CH_3), 1.48 (3H, d, 6Hz, 1'-CH_3), 1.57 (3H, s, 5'-CH_3), 2.89 (3H, s, ArCH_3), 4.05 (1H, m, H-3"), 4.05 (3H, s, OCH_3), 4.65

(1H, q, 6Hz, H-2'), 4.83 (1H, brs, H-6"), 4.99 (1H, brs, H-7"), 5.14 (2H, m, H-22",23"), 6.85 (1H, s, ArH), 17.95 (1H, s, 4-OH); ^{13}Cnmr (75MHz) see Table I-7 and I-8; Hrms: m/z 396.3385 (calcd for $C_{28}H_{44}O$, 396.3392, 28%) 376 ($C_{28}H_{40}$, 13), 356 ($C_{20}H_{20}O_6$, 63), 341 ($C_{19}H_{17}O_6$, 100), 323 (20), 251 (33); Cims1: m/z 413 (4%), 397 (10), 377 (90); Cims2: m/z 377 (9%), 357 (100); Fabms: m/z 751.64 ($M+H^+$, calcd for $C_{48}H_{63}O_7$, 751.4574, 10.1%), 377.53 (1.97), 357.14 (8.94), 341.07 (5.19).

Esters **28** and **29**

m-Chloroperbenzoic acid (MCPBA, 81mg, 80%, 0.376mmol) was added to a solution of ergosterol (149mg, 0.376mmol) in 12ml of methylene chloride.The mixture was stirred at rt. for 5 hours. Excess of MCPBA (20mg) was added and the mixture was allowed to stir for 19 hours. The solution was washed twice with sodium thiosulfate solution, then twice with sodium bicarbonate solution. The organic layer was dried and evaporated. The residue was separated by flash chromatography (SKB-ethyl acetate 80:30) giving 95.3mg of **28** and 17mg of **29**. **28**: mp 196-198^{0}C (SKB/ethyl acetate); Ir υ_{max}: 3560, 3220, 2956, 2870, 1705, 1660, 1575, 1457, 1427, 1381, 1371, 1279, 1263, 1130, 1074, 1050, 1025, 968, 903, 749 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.60 (3H, s, 18-CH_3), 0.83 (3H, d, 6Hz, 26-CH_3), 0.84 (3H, d, 6Hz, 27-CH_3), 0.92 (3H, d, 6.5Hz, 28-CH_3), 1.04 (3H, d, 6.5Hz, 21-CH_3), 1.09 (3H, s, 19-CH_3), 4.03 (1H, m, H-3), 5.03 (1H, brs, H-7), 5.19 (2H, m, H-22,23), 5.55 (1H, brs, H-6), 7.40 (1H, dd, 8, 8Hz), 7.55 (1H, ddd, 2,2,8Hz), 7.95 (1H, ddd, 2,2,8Hz), 8.03 (1H, dd, 2,2Hz); ^{1}Hnmr (C_6D_6, 360MHz): δ 0.47 (3H, s, 18-CH_3), 0.84 (9H, m), 0.91 (3H, d, 7Hz, 28-CH_3), 1.00 (3H, d, 6Hz, 21-CH_3), 4.07 (1H, m, H-3), 5.11 (1H, brs, H-7), 5.18 (2H, m, H-22,23), 5.72 (1H, brs, H-6), 6.80 (1H, dd, 8,8Hz), 7.12 (1H, brd,

8Hz), 7.89 (1H, d, 8Hz), 8.18 (1H, brs); ^{13}Cnmr (C_6D_6, 100MHz) see Table I-8; Hrms: m/z 412.3348 (M-$C_7H_5O_2Cl$, calcd for $C_{28}H_{44}O_2$, 412.3341, 4%), 394 (M-$C_7H_5O_2Cl$-H_2O, 12), 376 (M-$C_7H_5O_2Cl$-$2H_2O$, 18), 251 (36), 158 (17) 156 (53), 139 (58), 69 (100). **29:** Ir υ_{max}: 3440, 3240, 2957, 2872, 1719, 1706, 1665, 1575, 1459, 1427, 1280, 1258, 1128, 1073, 1051, 1023, 1006, 966, 904, 750 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.62 (3H, s, 18-CH_3), 0.90~1.00 (9H, m), 1.02 (3H, d, 6.5Hz, 21-CH_3), 1.12 (3H, s, 19-CH_3), 2.46 (1H, dd, 2,8Hz), 2.74 (1H, dd, 2,8Hz), 4.08 (1H, m, H-3), 5.07 (1H, brs, H-7), 5.58 (1H, brs, H-6), 7.44 (1H, dd, 8,8Hz), 7.58 (1H, ddd, 2,2,8Hz), 7.98 (1H, ddd, 2,2,8Hz), 8.06 (1H, dd, 2,2Hz); Hrms: m/z 428.3279 (M-$C_7H_5O_2Cl$, calcd for $C_{28}H_{44}O_3$, 428.3290, 2%), 410 (M-$C_7H_5O_2Cl$-H_2O, 20), 392 (M-$C_7H_5O_2Cl$-$2H_2O$, 21), 251 (44), 158 (32), 156 (93), 139(100).

Triol **27** and ester **30**

Ester **28** (61.2mg) and 10ml 10% KOH/MeOH were dissolved in 10ml of methanol. The mixture was stirred at rt. for 24 hours. Evaporation of the methanol, addition of 10ml water, extraction with ethyl acetate, washing the organic layer with brine, drying over sodium sulfate, and evaporation provided the crude products. Triol **27** (32.6mg) and ester **30** (6.4mg) were obtained after separation of the crude products by flash chromatography (methylene chloride-acetone 50:50). **27:** mp 227-229^{0}C (methanol); $[\alpha]_D$ +13.3^0 (*c* 0.18, $CHCl_3$); Ir υ_{max}: 3323, 3273, 2889, 2870, 2850, 1660, 1380, 1370, 1052, 1043, 1035, 973, 868 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.57 (3H, s, 18-CH_3), 0.83 (3H, d, 6Hz, 26-CH_3), 0.84 (3H, d, 6Hz, 27-CH_3), 0.93 (3H, d, 7Hz, 28-CH_3), 0.98 (3H, s, 19-CH_3), 1.03 (3H, d, 6.5Hz, 21-CH_3), 3.98 (1H, brs, H-6), 4.01 (1H, m, H-3), 5.03 (1H, brs, H-7), 5.20 (2H, m, H-22,23); ^{13}Cnmr (90MHz) see Table I-8; Hrms: m/z 412.3341 (M-

H_2O, calcd for $C_{28}H_{44}O_2$, 412.3341, 100%), 394 (M-$2H_2O$, 13), 379 (26), 269 (11), 251 (17). **30**: Ir υ_{max}: 3500, 3420, 3280, 2955, 2935, 2870, 2854, 1731, 1658, 1458, 1443, 1371, 1262, 1247, 1045, 1035, 970 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.58 (3H, s, 18-CH_3), 0.81 (3H, d, 6Hz, 26-CH_3), 0.83 (3H, d, 6Hz, 27-CH_3), 0.91 (3H, d, 6.5Hz, 28-CH_3), 0.98 (3H, s, 19-CH_3), 1.02 (3H, d, 6.5Hz, 21-CH_3), 2.04 (3H, s, $COCH_3$), 3.95 (1H, brd, 8Hz, H-6), 5.01 (1H, d, 1.5Hz, H-7), 5.09 (1H, m, H-3), 5.19 (2H, m, H-22, 23); Upon addition of D_2O, signal at δ3.95ppm changes to δ3.95 (1H, d, 1.5Hz); ^{13}Cnmr (90MHz) see Table I-8; Hrms: m/z 454.3447 (M-H_2O, calcd for $C_{30}H_{46}O_3$, 454.3447, 4%), 394 (M-H_2O-$C_2H_4O_2$, 18), 376 (M-$2H_2O$-$C_2H_4O_2$, 12), 251 (13), 69 (100).

Methyl acetyl sirocodin (31)

Acetic anhydride (1ml) and pyridine (1ml) were added to a solution of methyl sirocodin (1mg) in 2ml of methylene chloride. The mixture was stirred at rt. for 18 hours. The solvent was evaporated and the residue was purified by flash chromatography (SKB/acetone 4:1) to give 1mg of **31**. Ir υ_{max}: 3320, 2957, 2936, 2933, 2870, 1736, 1614, 1590, 1460, 1376, 1349, 1305, 1246, 1222, 1201, 1184, 1167, 1104, 1063, 1035, 1029 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.56 (3H, s, 18"-CH_3), 0.80 (3H, d, 5.3Hz, 26"-CH_3), 0.81 (3H, d, 5.3Hz, 27"-CH_3), 0.89 (3H, d, 6Hz, 28"-CH_3), 1.01 (3H, d, 6Hz, 21"-CH_3), 1.15 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.47 (3H, d, 6Hz, 1'-CH_3), 1.57 (3H, s, 5'-CH_3), 1.91 (3H, s, $COCH_3$), 2.90 (3H, s, $ArCH_3$), 4.04 (3H, s, OCH_3), 4.63 (1H, q, 6Hz, H-2'), 4.82 (1H, brs, H-6"), 4.96 (1H, brs, H-7"), 5.05 (1H, m, H-3"), 5.16 (2H, m, H-22",23"), 6.85 (1H, s, ArH), 17.97 (1H, s, 4-OH); Fabms: m/z 793.59 ($M+H^+$, calcd for $C_{50}H_{65}O_8$, 793.4679, 2.46%), 356.85 (2.72), 340.80 (1.62), 308.80 (5.62).

Diacetyl sirocodin (32)

Sirocodin (0.5mg) was dissolved in acetic anhydride (1ml), pyridine (1ml), and methylene chloride (2ml). The mixture was stirred at rt. for 17 hours. The solvent was co-evaporated with ethanol and the residue was purified by flash chromatography (SKB-acetone 5:1) providing 0.5mg of 32. Ir υ_{max}: 3400, 2975, 2927, 1753, 1734, 1620, 1593, 1456, 1443, 1376, 1366, 1265, 1242, 1226, 1195, 1176 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.56 (3H, s, 18"-CH_3), 0.80 (3H, d, 5.3Hz, 26"-CH_3), 0.81 (3H, d, 5.3Hz, 27"-CH_3), 0.88 (3H, d, 6Hz, 28"-CH_3), 1.00 (3H, d, 6Hz, 21"-CH_3), 1.15 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.47 (3H, d, 6Hz, 1'-CH_3), 1.57 (3H, s, 5'-CH_3), 1.96 (3H, s, $COCH_3$), 2.38 (3H, s, $COCH_3$), 2.86 (3H, s, $ArCH_3$), 4.66 (1H, q, 6Hz, H-2'), 4.68 (1H, brs, H-6"), 4.91 (1H, brs, H-7"), 5.02 (1H, m, H-3"), 5.16 (2H, m, H-22",23"), 6.94 (1H, s, ArH), 17.41 (1H, s, 4-OH); Fabms: m/z 821.56 ($M+H^+$, calcd for $C_{51}H_{65}O_9$, 821.5107, 0.87%).

Dihydro methyl sirocodin (33)

Methyl sirocodin (0.8mg) in 3ml of ethanol was hydrogenated in the presence of PtO_2 (5mg) and 3 drops of concentrated HCl for 12 hours. The catalyst was filtered off and the solvent was evaporated. The residue was purified by flash chromatography (SKB-acetone 3:1) giving 0.7mg of 33. Ir υ_{max}: 3400, 2920, 1740(w), 1613, 1560, 1463, 1457, 1376, 1348, 1304, 1268, 1221, 1185, 1168, 1104, 1062, 1037, 758 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.56 (3H, s, 18"-CH_3), 0.74~0.89 (12H, m), 1.12 (3H, s, 19"-CH_3), 1.22 (1H, s, OH), 1.30 (3H, s, 4'-CH_3), 1.46 (3H, d, 6Hz, 1'-CH_3), 1.56 (3H, s, 5'-CH_3), 2.89 (3H, s, $ArCH_3$), 4.06 (1H, m, H-3"), 4.06 (3H, s, OCH_3), 4.64 (1H, q, 6Hz, H-2'), 4.83 (1H,

brs, H-6"), 4.99 (1H, brs, H-7"), 6.87 (1H, s, ArH), 17.96 (1H, s, 4-OH); Fabms: m/z 753.51 ($M+H^+$, calcd for $C_{48}H_{65}O_7$, 753.4730, 11.24%), 397.39 (3), 379 (6), 357 (13), 341 (12), 327 (21).

Dihydrosirocodin (34)

Diacetylsirocodin (32, 1mg) in 3ml methanol was hydrogenated in the presence of 5% Pd-C (5mg) and 3 drops 3M HCl for 22 hours. Concentrated HCl (3 drops) was then added and the hydrogenation was allowed to continue for a further 20 hours. The catalyst was filtered off and the solvent was evaporated. The residue was purified by preparative thin layer chromatography (SKB-ethyl acetate 3:1) giving 0.9mg of 34. Ir υ_{max}: 3400, 2955, 2923, 2852, 1610, 1463, 1446, 1377, 1280, 1261, 1225, 1190, 1157, 1100, 1056 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.52 (3H, s, 18"-CH_3), 0.72-0.91 (12H, m), [illegible] (3H, s, 19"-CH_3), 1.29 (3H, s, 4'-CH_3), 1.43 (3H, d, 6Hz, 1'-CH_3), 1.61 (3H, s, 5'-CH_3), 2.80 (3H, s, ArCH_3), 4.04 (1H, m, H-3"), 4.63 (1H, q, 6Hz, H-2'), 4.90 (1H, brs, H-6"), 4.99 (1H, brs, H-7"), 6.82 (1H, s, ArH), 9.55 (1H, s, 9-OH), 17.18 (1H, s, 4-OH); Fabms: m/z 739.61 ($M+H^+$, calcd for $C_{47}H_{63}O_7$, 739.4574, 4.2%), 379.40 (6), 341.02 (22).

3"-Ketosirocodin (35)

Trifluoroacetic anhydride (20µl) was added dropwise to a stirred solution of dimethyl sulfoxide (20µl) in 3ml of methylene chloride at -78^{0}C. After 10 minutes, sirocodin (1mg) in 2ml methylene chloride was added and the solution was stirred for 40 minutes at the same temperature. Triethylamine (50µl) was then added and the reaction was kept for another 40 minutes at -78^{0}C. The reaction mixture was allowed to warm to rt. and 3ml of water was added. The aqueous layer was separated and extracted with methylene chloride twice. The combined organic

extracts were washed, dried, and evaporated. The residue was purified by flash chromatography (SKB-ethyl acetate 7:3) providing 0.8mg of **35**. Ir υ_{max}: 3390, 2957, 2927, 2871, 2855, 1722, 1610, 1454, 1445, 1377, 1306, 1280, 1237, 1223, 1189, 1171, 1157, 1135, 1101, 1063, 1036 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.60 (3H, s, 18"-CH_3), 0.81 (3H, d, 6Hz, 26"-CH_3), 0.83 (3H, d, 6Hz, 27"-CH_3), 0.90 (3H, d, 6.5Hz, 28"-CH_3), 1.02 (3H, d, 6.5Hz, 21"-CH_3), 1.31 (3H, s, 19"-CH_3), 1.34 (3H, s, 4'-CH_3), 1.49 (3H, d, 6.5Hz, 1'-CH_3), 1.56 (3H, s, 5'-CH_3), 2.82 (3H, s, ArCH_3), 4.65 (1H, q, 6.5Hz, H-2'), 5.03 (2H, brs, H-6" and H-7"), 5.18 (2H, m, H-22",23"), 6.83 (1H, s, ArH), 9.43 (1H, s, 9-OH), 17.03 (1H, s, 4-OH); Fabms: m/z 735.17 ($M+H^+$, calcd for $C_{47}H_{59}O_7$, 735.4261, 6%), 394.16 (2), 340.68 (29).

Sirocodinine (**36**)

Ir υ_{max}: 3398, 3388, 3380, 2956, 2869, 1610, 1569, 1455, 1428, 1384, 1371, 1296, 1249, 1226, 1193, 1134, 1101, 1048, 1032 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.55 (3H, s, 18"-CH_3), 0.80 (3H, d, 6Hz, 26"-CH_3), 0.82 (3H, d, 6Hz, 27"-CH_3), 0.89 (3H, d, 6Hz, 28"-CH_3), 1.00 (3H, d, 6.5Hz, 21"-CH_3), 1.29 (3H, s, 19"-CH_3), 1.30 (3H, s, 4'-CH_3), 1.48 (3H, d, 6.5Hz, 1'-CH_3), 1.57 (3H, s, 5'-CH_3), 2.82 (3H, s, ArCH_3), 4.01 (1H, s, NH), 4.12 (1H, m, H-3"), 4.89 (1H, brs, H-6"), 5.04 (1H, brs, H-7"), 5.16 (2H, m, H-22",23"), 5.73 (1H, d, 6Hz, H-11"), 6.84 (1H, s, ArH), 9.76 (1H, s, 9-OH), 17.84 (1H, s, 4-OH); ^{13}Cnmr (75MHz) see Table I-12; Fabms: m/z 734.55 ($M+H^+$, calcd for $C_{47}H_{60}O_6N$, 734.4421, 0.72%), 733.55 (0.81), 375.30 (2), 339.89 (9).

Methyl sirocodinine (**39**)

Sirocodinine (**36**, 5mg) was treated with an excess of diazomethane in ether at rt. for 24 hours. Evaporation of the solvent and purification of the residue by flash chromatography (SKB-ethyl acetate 5:1) provided 5mg of **39**. Ir υ_{max}: 3400, 2957, 2924, 2850, 1612, 1590, 1552, 1457, 1370, 1304, 1264, 1175, 1121, 1055, 1008 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.54 (3H, s, 18"-CH_3), 0.80 (3H, d, 6Hz, 26"-CH_3), 0.81 (3H, d, 6Hz, 27"-CH_3), 0.89 (3H, d, 6Hz, 28"-CH_3), 1.00 (3H, d, 6Hz, 21"-CH_3), 1.27 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.48 (3H, d, 6Hz, 1'-CH_3), 1.55 (3H, s, 5'-CH_3), 2.88 (3H, s, ArH), 3.99 (1H, s, NH), 4.07 (3H, s, OCH_3), 4.13 (1H, m, H-3"), 4.64 (1H, q, 6Hz, H-2'), 4.81 (1H, brs, H-6"), 5.03 (1H, brs, H-7"), 5.16 (2H, m, H-22",23"), 5.67 (1H, d, 6Hz, H-11"), 6.90 (1H, s, ArH), 17.65 (1H, s, 4-OH); ^{13}Cnmr (90MHz) see Table I-12; Hrms: m/z 394.3231 ($C_{28}H_{42}O$, 14%), 376 ($C_{28}H_{40}$, 16), 355.1419 ($C_{20}H_{21}O_5N$, 54), 340 ($C_{19}H_{18}O_5N$, 41), 251 (74), 57 (100); Fabms: m/z 748.33 ($M+H^+$, calcd for $C_{48}H_{62}O_6N$, , 748.4577, 7.27%), 747.32 (6.5), 354.19 (15).

Methyl acetyl sirocodinine (**40**)

Methyl sirocodinine (1mg) was dissolved in 2ml of acetic anhydride, 1ml of pyridine, and 2ml of methylene chloride. The mixture was stirred at rt. for 22 hours. The solvent was co-evaporated with ethanol. The residue was purified by flash chromatography (SKB-ethyl acetate 5:1) providing 0.9mg of **40**. Ir υ_{max}: 3398, 2956, 1729, 1610, 1590, 1454, 1414, 1301, 1263, 1166, 1050 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.53 (3H, s, 18"-CH_3), 0.79 (3H, d, 5.3Hz, 26"-CH_3), 0.81 (3H, d, 5.3Hz, 27"-CH_3), 0.88 (3H, d, 6Hz, 28"-CH_3), 0.99 (3H, d, 6Hz, 21"-CH_3), 1.26 (3H, s, 19"-CH_3), 1.30 (3H, s, 4'-CH_3), 1.48 (3H, d, 6.5Hz, 1'-CH_3), 1.56 (3H, s, 5'-CH_3), 1.94 (3H, s, $COCH_3$), 2.87 (3H, s, $ArCH_3$), 4.03 (1H, s, NH), 4.06 (3H, s, OCH_3), 4.62 (1H, q, 6.5Hz, H-2'), 4.81 (1H, brs, H-6"), 5.01 (1H,

brs, H-7"), 5.15 (2H, m, H-22",23"), 5.68 (1H, d, 6Hz, H-11"), 6.88 (1H, s, ArH), 17.66 (1H, s, 4-OH); Fabms: m/z 790.77 ($M+H^+$, calcd for $C_{50}H_{64}O_7N$, 790.4683, 2.56%), 789.74 (2.36), 355.98 (7), 353.95 (8).

Diacetyl sirocodinine (41)

Acetic anhydride (1ml) and pyridine (1ml) were added to a solution of sirocodinine (0.5mg) in 1ml of methylene chloride. The mixture was stirred at rt. for 16 hours. The solvent was evaporated and the residue was redissolved in methylene chloride. The organic phase was washed with 5% HCl, and then evaporated to dryness. The residue was purified by passing through a short florisil column giving 0.4mg of **41**. Ir υ_{max}: 3400, 2955, 2870, 1770, 1739, 1619, 1596, 1463, 1366, 1337, 1293, 1242, 1201, 1182, 1139, 1122, 1099, 1065, 1030 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.53 (3H, s, 18"-CH_3), 0.78 (3H, d, 6Hz, 26"-CH_3), 0.80 (3H, d, 6Hz, 27"-CH_3), 0.91 (3H, d, 6Hz, 28"-CH_3), 0.99 (3H, d, 6.5Hz, 21"-CH_3), 1.31 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.48 (3H, d, 6.5Hz, 1'-CH_3), 1.57 (3H, s, 5'-CH_3), 1.96 (3H, s, $COCH_3$), 2.40 (3H, s, $COCH_3$), 2.85 (3H, s, $ArCH_3$), 4.06 (1H, s, NH), 4.64 (1H, brs, H-6"), 4.66 (1H, q, 6.5Hz, H-2'), 4.99 (1H, brs, H-7"), 5.15 (2H, m, H-22",23"), 5.68 (1H, d, 6Hz, H-11"), 6.93 (1H, s, ArH), 17.01 (1H, s, 4-OH); Fabms: m/z 818.80 ($M+H^+$, calcd for $C_{51}H_{64}O_8N$, 818.4632, 0.99%), 817.78 (1.06), 382.33 (6), 368.37 (11), 340.12 (12).

3"-Ketosirocodinine (45)

Trifluoroacetic anhydride (30µl) was added dropwise to a stirred solution of dimethyl sulfoxide (30µl) in 3ml of methylene chloride at -78^{0}C. After 10 minutes, sirocodinine (2mg) in 2ml of methylene chloride was added and the solution was allowed to stir at the same temperature for 40 minutes. Then, triethylamine (100µl)

was added and the mixture was kept at -78^{0}C for another 40 minutes. The reaction mixture was allowed to warm up to rt. Water (3ml) was added and the aqueous layer was separated and extracted with methylene chloride twice. The combined organic extracts were washed with brine, dried, and evaporated. Purification of the crude product by flash chromatography (SKB-ethyl acetate 70:30) yielded 1.2mg of **45**. Ir υ_{max}: 3420, 2958, 2927, 2871, 1720, 1607, 1571, 1452, 1427, 1371, 1332, 1298, 1224, 1191, 978 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.57 (3H, s, 18"-CH_3), 0.80 (3H, d, 6Hz, 26"-CH_3), 0.82 (3H, d, 6Hz, 27"-CH_3), 0.89 (3H, d, 6.5Hz, 28"-CH_3), 1.01 (3H, d, 6.5Hz, 21"-CH_3), 1.30 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.47 (3H, d, 6.5Hz, 1'-CH_3), 1.54 (3H, s, 5'-CH_3), 2.82 (3H, s, ArCH_3), 4.00 (1H, s, NH), 4.66 (1H, q, 6.5Hz, H-2'), 5.01 (1H, brs, H-6"), 5.10 (1H, brs, H-7"), 5.20 (2H, m, H-22",23"), 5.83 (1H, d, 6Hz, H-11"), 6.84 (1H, s, ArH), 9.63 (1H, s, 9-OH), 16.70 (1H, s, 4-OH); Fabms: m/z 732.62 ($M+H^+$, calcd for $C_{47}H_{58}O_6N$, 732.4264, 0.67%), 731.63 (1.02), 341.44 (11), 340.43 (11), 339.43 (21).

Monoacetate **46** and diacetate **47**

3"-Ketosirocodinine (**45**, 1.2mg) was refluxed with 2ml of acetic anhydride and 2ml of triethylamine for 2 hours. The mixture was poured onto crushed ice. The aqueous layer was extracted with methylene chloride twice and the organic layer was washed with brine, dried, and evaporated. The residue was separated by silica gel chromatography (SKB-ethyl acetate 70:30) giving 0.5mg of monoacetate **46** and 0.6mg diacetate **47**. **46**: Ir υ_{max}: 3400, 2929, 1767, 1744, 1717, 1620, 1594, 1428, 1369, 1226, 1200, 1185 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.57 (3H, s, 18"-CH_3), 0.80 (3H, d, 6Hz, 26"-CH_3), 0.81 (3H, d, 6Hz, 27"-CH_3), 0.88 (3H, d, 6.5Hz, 28"-CH_3), 1.00 (3H, d, 6.5Hz, 21"-CH_3), 1.30 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.45 (3H, d, 6.5Hz, 1'-CH_3), 1.50 (3H, s, 5'-CH_3), 2.38 (3H,

s, $OCOCH_3$), 2.85 (3H, s, $ArCH_3$), 4.10 (1H, s, NH), 4.67 (1H, q, 6.5Hz, H-2'), 4.78 (1H, brs, H-6"), 5.05 (1H. brs, H-7"), 5.20 (2H, m, H-22",23"), 5.79 (1H, d, 6Hz, H-11"), 6.94 (1H, s, ArH), 16.88 (1H, s, 4-OH); Fabms: m/z 774.53 ($M+H^+$, calcd for $C_{49}H_{60}O_7N$, 774.4370, 2.15%), 773.55 (1.65), 339.86 (6), 308.85 (9). **47**: Ir υmax: 3400, 2957, 2925, 2869, 2853, 1753, 1621, 1596, 1456, 1368, 1291, 1204, 1183, 1174 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.55 (3H, s, 18"-CH_3), 0.80 (3H, d, 6Hz, 26"-CH_3), 0.82 (3H, d, 6Hz, 27"-CH_3), 0.89 (3H, d, 6.5Hz, 28"-CH_3), 1.00 (3H, d, 6.5Hz, 21"-CH_3), 1.24 (3H, s, 19"-CH_3), 1.32 (3H, s, 4'-CH_3), 1.48 (3H, d, 6.5Hz, 1'-CH_3), 1.54 (3H, s, 5'-CH_3), 2.04 (3H, s, $COCH_3$), 2.41 (3H, s, $COCH_3$), 2.85 (3H, s, $ArCH_3$), 4.48 (1H, s, NH), 4.67 (1H, q, 6.5Hz, H-2'), 4.83 (1H, brs, H-6"), 5.09 (1H, brs, H-7"), 5.16 (2H, m, H-22",23"), 5.50 (1H, d, 5Hz, H-2"), 5.66 (1H, d, H-11"), 6.95 (1H, s, ArH), 16.96 (1H, s, 4-OH); Fabms: m/z 816.67 ($M+H^+$, calcd for $C_{51}H_{62}O_8N$, 816.4475, 0.28%), 815.68 (0.35), 339.89 (2).

Ketoesters **49** and **50**

Trifluoroacetic anhydride (0.9ml, 134g, 6.4mmol) was added dropwise to a stirred solution of dimethyl sulfoxide (0.8ml, 0.88g, 11.3mmol) in 5ml of methylene chloride at -78^{0}C. After 10 minutes, ester **28** (800mg, 1.41mmol) in 12ml of methylene chloride was added and the mixture was stirred at the same temperature for 1 hour. Triethylamine (2ml, 1455mg, 14.4mmol) was then added and the mixture was stirred overnight. Water (10ml) was added and the aqueous layer was separated and extracted with methylene chloride twice. The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was separated by silica gel chromatography (SKB-ethyl acetate 10:1 and 5:1) providing 300mg of **49** and 250mg of **50**. **49**: Ir υmax:

2956, 2871, 1725, 1682, 1290, 1279, 1251, 1126, 748 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.63 (3H, s, 18-CH_3), 0.82 (3H, d, 6Hz, 26-CH_3), 0.84 (3H, d, 6Hz, 27-CH_3), 0.91 (3H, d, 6.5Hz, 28-CH_3), 1.03 (3H, d, 6Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 5.20 (2H, m, H-22,23), 5.21 (1H, brs, H-7), 5.95 (1H, d, 2Hz, H-4), 6.38 (1H, brs, H-6), 7.41 (1H, dd, 8,8Hz), 7.57 (1H, ddd, 2,2,8Hz), 7.98 (1H, ddd, 2,2,8Hz), 8.07 (1H, dd, 2,2Hz); Hrms: m/z 408.3026 (M-C_7H_5OCl, calcd for $C_{28}H_{40}O_2$, 408.3028, 1.03%), 392 (M-$C_7H_5O_2Cl$, 100), 377 (48), 268 (69), 253 (44). **50**: Ir υ_{max}: 3440, 2956, 2871, 1718, 1580, 1280, 1256, 750 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.62 (3H, s, 18-CH_3), 0.82 (3H, d, 6Hz, 26-CH_3), 0.84 (3H, d, 6Hz, 27-CH_3), 0.91 (3H, d, 6.5Hz, 28-CH_3), 1.03 (3H, d, 6.5Hz, 21-CH_3), 1.28 (3H, s, 19-CH_3), 5.03 (1H, brs, H-6), 5.19 (2H, m, H-22,23), 5.66 (1H, brs, H-6), 7.39 (1H, dd, 8,8Hz), 7.55 (1H, ddd, 2,2,8Hz), 7.93 (1H, ddd, 2,2,8Hz), 8.02 (1H, dd, 2,2Hz); Hrms: m/z 548.3068 (M-H_2O, calcd for $C_{35}H_{45}O_3Cl$, 548.3058, 4%), 410 (M-$C_7H_5O_2Cl$, 14), 392 (M-$C_7H_5O_2Cl$-H_2O, 40), 268 (26).

6α-Hydroxyergosta-4,7,22-trien-3-one (**52**)

Enone ester (**49**, 10mg) was dissolved in 10ml of methylene chloride and 5 drops of 1M KOH/MeOH was added. The mixture was stirred at rt. for 5 minutes. Acetic acid (1ml, 80%) and 5ml of water was added. The aqueous layer was extracted twice with methylene chloride and the combined organic extracts were washed successively with saturated $NaHCO_3$ solution and brine, dried, and evaporated. The residue was purified by flash chromatography (SKB-ethyl acetate 4:1)giving 6mg of **52**. Ir υ_{max}: 3364, 2956, 2871, 1661, 1623, 1455, 1381, 1372, 1229, 1173, 1118, 1007 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.62 (3H, s, 18-CH_3), 0.83 (3H, d, 6Hz, 26-CH_3), 0.85 (3H, d, 6Hz, 27-CH_3), 0.92 (3H, d, 7Hz, 28-CH_3), 1.04 (3H, d, 6.5Hz, 21-CH_3), 1.19

(3H, s, 19-CH_3), 4.98 (1H, brd, 2Hz, H-6), 5.20 (2H, m, H-22,23), 5.23 (1H, brs, H-7), 6.13 (1H, d, 2Hz, H-4); Hrms: m/z 410.3212 (calcd for $C_{28}H_{42}O_2$, 410.3185, 43%), 392 (M-H_2O, 31), 286 (79), 283 (25), 268 (45).

Diketone **59**

Pyridinium chlorochromate (76mg) was added to a solution of triol **27** (23mg) in 10ml of methylene chloride. The mixture was stirred at rt. for 21 hours and then diluted with 20ml of ether. The precipatate was filtered through a pad of celite. Evaporation of the solvent and separation by flash chromatography (SKB-ethyl acetate 9:3) provided 6mg of diketone **59**. Ir υ_{max}: 3320, 2954, 2870, 1710, 1672, 1620, 1369, 1232, 1141, 1124, 967, 873 cm^{-1}; ^{1}Hnmr (360MHz): δ 0.63 (3H, s, 18-CH_3), 0.83 (3H, d, 6Hz, 26-CH_3), 0.85 (3H, d, 6Hz, 27-CH_3), 0.93 (3H, d, 6.5Hz, 28-CH_3), 1.05 (3H, d, 6Hz, 21-CH_3), 1.16 (3H, s, 19-CH_3), 2.58 (1H, d, 16Hz, H-4), 2.83 (1H, d, 16Hz, H-4), 5.17 (1H, dd, 8,15Hz, H-22 or H-23), 5.26 (1H, dd, 7,15Hz, H-22 or H-23), 5.72 (1H, brs, H-7); ^{13}Cnmr (90MHz): δ 12.74 (q, C-18), 15.90 (q, C-19), 17.57 (q, C-28), 19.63 (q, C-26), 19.94 (q, C-27), 21.11 (q, C-21), 22.09 (t, C-11), 22.50 (t, C-15), 27.83 (t, C-16), 31.93 (t, C-1), 33.05 (d, C-25), 37.35 (t, C-2), 38.75 (t, C-12), 40.25 (d, C-20), 40.85 (s, C-10), 42.81 (t, C-4), 43.75 (d, C-24 and C-9), 44.73 (s, C-13), 55.85 (d, C-17), 56.04 (d, C-14), 79.91 (s, C-5), 119.52 (d, C-7), 132.59 (d, C-23), 134.91 (d, C-22), 165.82 (s, C-8), 196.81 (s, C-6), 209.67 (s, C-3); Hrms: m/z 426.3127 (calcd for $C_{28}H_{42}O_3$, 426.3134, 16%), 408 (M-H_2O, 27), 299 (34), 69(100).

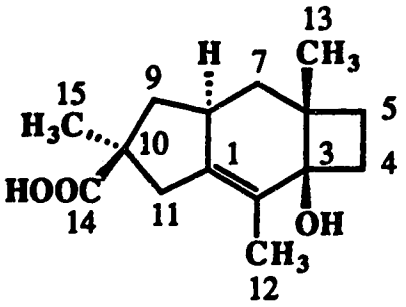
Chapter II Studies Related to the Synthesis of Sterpuric Acid

INTRODUCTION

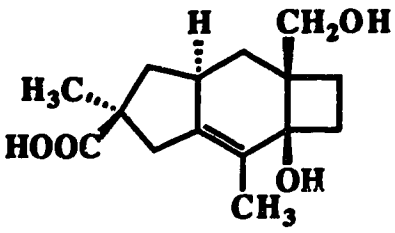
The fungus *Stereum purpureum* causes the so-called silver leaf disease on plum, apple, and other fruit trees.[1] In Alberta it is also found on mountain ash, cotoneaster, and aspen. The fungus enters through wounds, grows first in the heartwood, then kills the sapwood and bark. Infected trees develop foliage with a dull leaden of metallic lustre, thus the name silver leaf disease. The metabolites produced by *S. purpureum* grown in liquid culture were well studied by Ayer and coworkers.[2-4]

S. purpureum was grown in malt extract-dextrose-peptone liquid culture. Extraction of the culture broth with ethyl acetate provided crude metabolites which cause "silvering" in mountain ash seedlings. The crude metabolites were separated into neutral and acidic fractions and the latter fractions were further separated by silica gel chromatography to give sterpuric acid (**1**). Esterification (diazomethane) of the remaining acidic fractions followed by further chromatographic purification led to the isolation of hydroxysterpuric acid (**2**) and hydroxysterpuric acid ethylidene acetal (**3**) in the form of their methyl esters. Sterpurene-3,12,14-triol (**4**) was obtained from the neutral fraction by flash chromatography. The parent hydrocarbon sterpurene (**5**) was isolated from the chloroform extract of the mycelium.

The structures of **1**, **2**, **3**, **4**, and **5** were established by a combination of spectroscopic analysis and formation of chemical derivatives. The relative stereochemistry of sterpuric acid was unequivocally confirmed by X-ray crystallographic studies.



1



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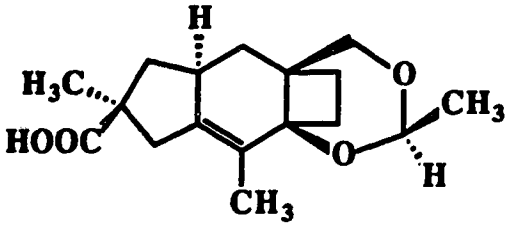

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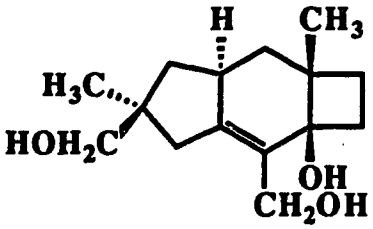

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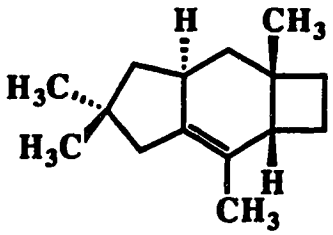
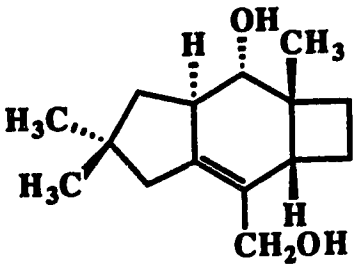


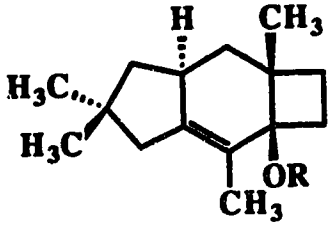
5

In 1987, Abell and Leech [5] also examined the metabolites produced by *S. purpureum* grown on a malt extract broth. A major compound, 7,12-dihydroxysterpurene (**6**), was isolated and identified. Sterpurenes were obtained only from the fungus *S. purpureum* until Cimino and coworkers reported the isolation of 3-acetoxysterpurene (**7**) from the alcyonacean *Alcyonum acaule* in

1989.[6] Reduction of 7 with lithium aluminum hydride ($LiAlH_4$) produced 3-hydroxysterpurene (8). Compound 7 is the first sterpurene sesquiterpenoid isolated from marine organisms.

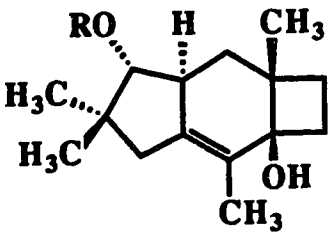


6

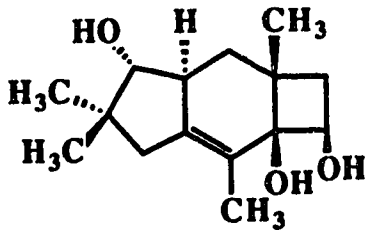


7 R=$COCH_3$
8 R=H

Two sterpurenes, tremediol (9) and tremetriol (10), were isolated recently from a culture of the fungus *Merulius tremellosus*.[7] The structures of 9 and 10 were confirmed by X-ray crystallographic analyses of chloroacetyltremediol (11).



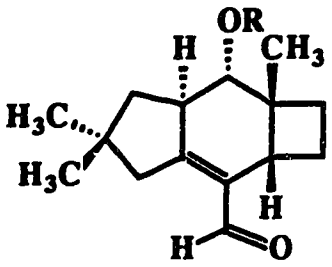
9 R=H
11 R=$COCH_2Cl$



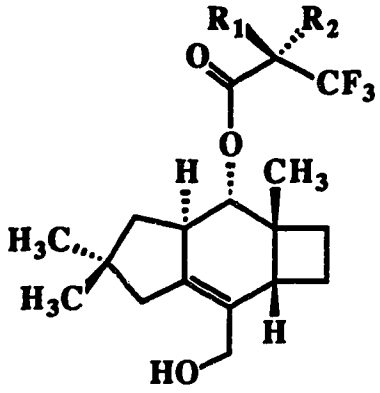
10

The absolute stereochemistry of the sterpurene sesquiterpenes has been determined by Abell and Leech.[8] The exciton chirality method was employed on compound 12 which was obtained from 7,12-dihydroxysterpurene (6). A positive Cotton effect at 256nm for 12 caused by the interaction of the two chromophores

(the enone and the *p*-bromobenzoyl group) indicates a positive chirality of the two chromophores, consistent with the S configuration at C-7. The same absolute configuration was also suggested by using the ^{19}Fnmr method on Mosher's esters **13** and **14**. A total synthesis of (+)-sterpurene (**5**)[9,10] confirmed the assigned absolute stereochemistry of the sterpurenes.



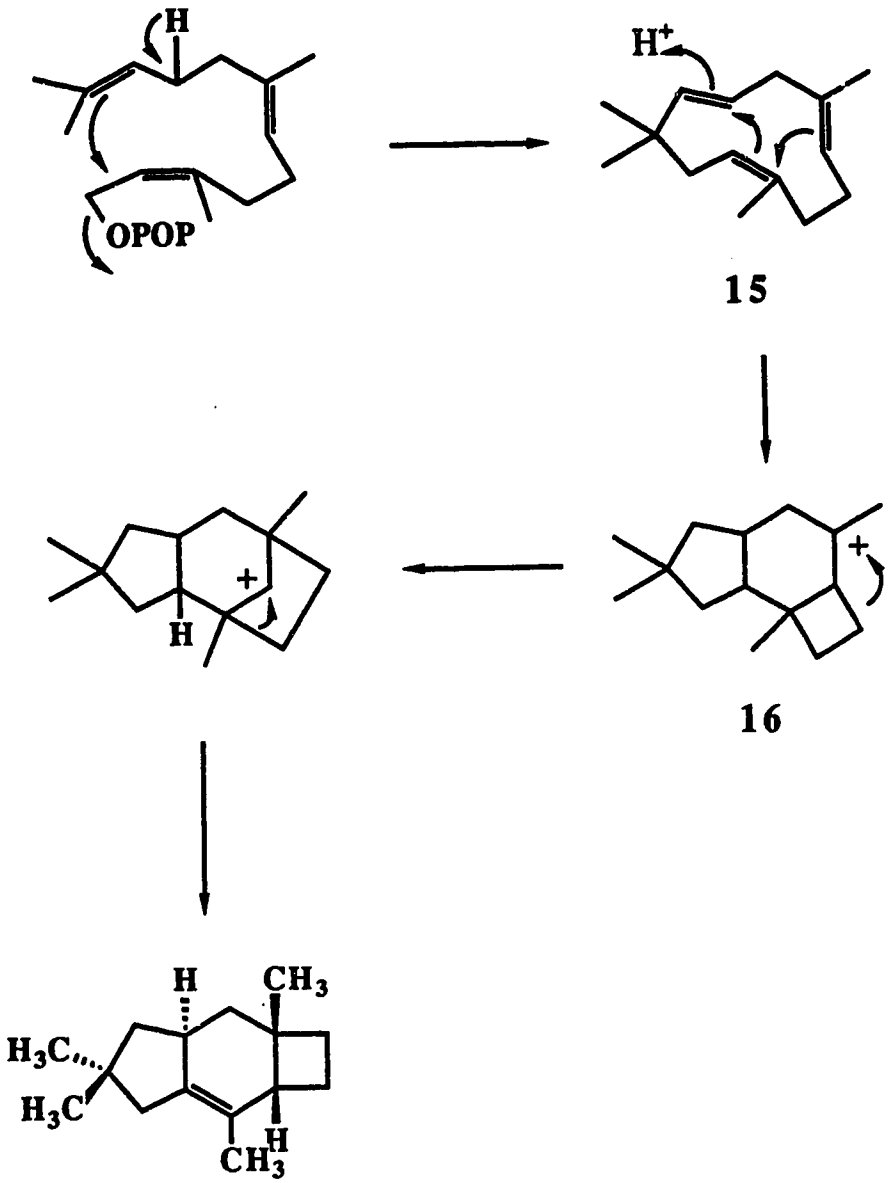
12 R=BrC_6H_5CO-



13 R_1=Ph, R_2=OCH_3
14 R_1=OCH_3, R_2=Ph

The sterpurenes, which are constructed of contiguously fused five-, six-, and four-membered carbocyclic rings, constitute natural products of a new structural type.[11] Biosynthetic studies indicate that the sterpurenes are derived from farnesyl pyrophosphate via humulene (**15**) and the protoilludyl cation **16** (Scheme II-1).[4,5]

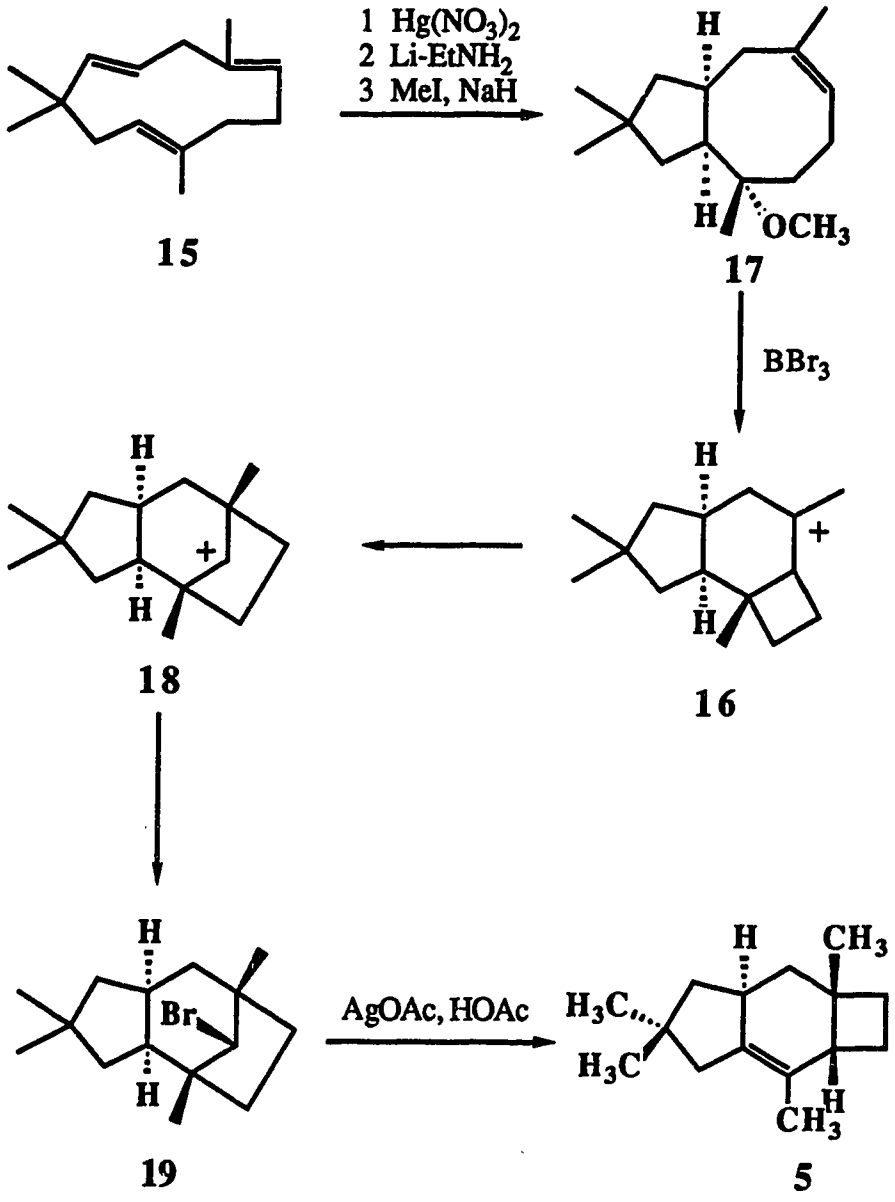
Scheme II-1 Biosynthetic pathway of sterpurenes



The unique structure of sterpurenes with a 4/6/5 tricyclic system provided a challenge to synthetic organic chemists. Three total syntheses of sterpurene (**5**) have been reported. Sterpuric acid (**1**) and sterpurene-3,12,14-triol (**4**) have also been synthesized.

The first synthesis of sterpurene was reported by Murata and coworkers.[12] The synthesis is patterned after the biosynthesis of sterpurene (Scheme II-2).

Scheme II-2 Synthesis of sterpurene (5) from humulene (15)



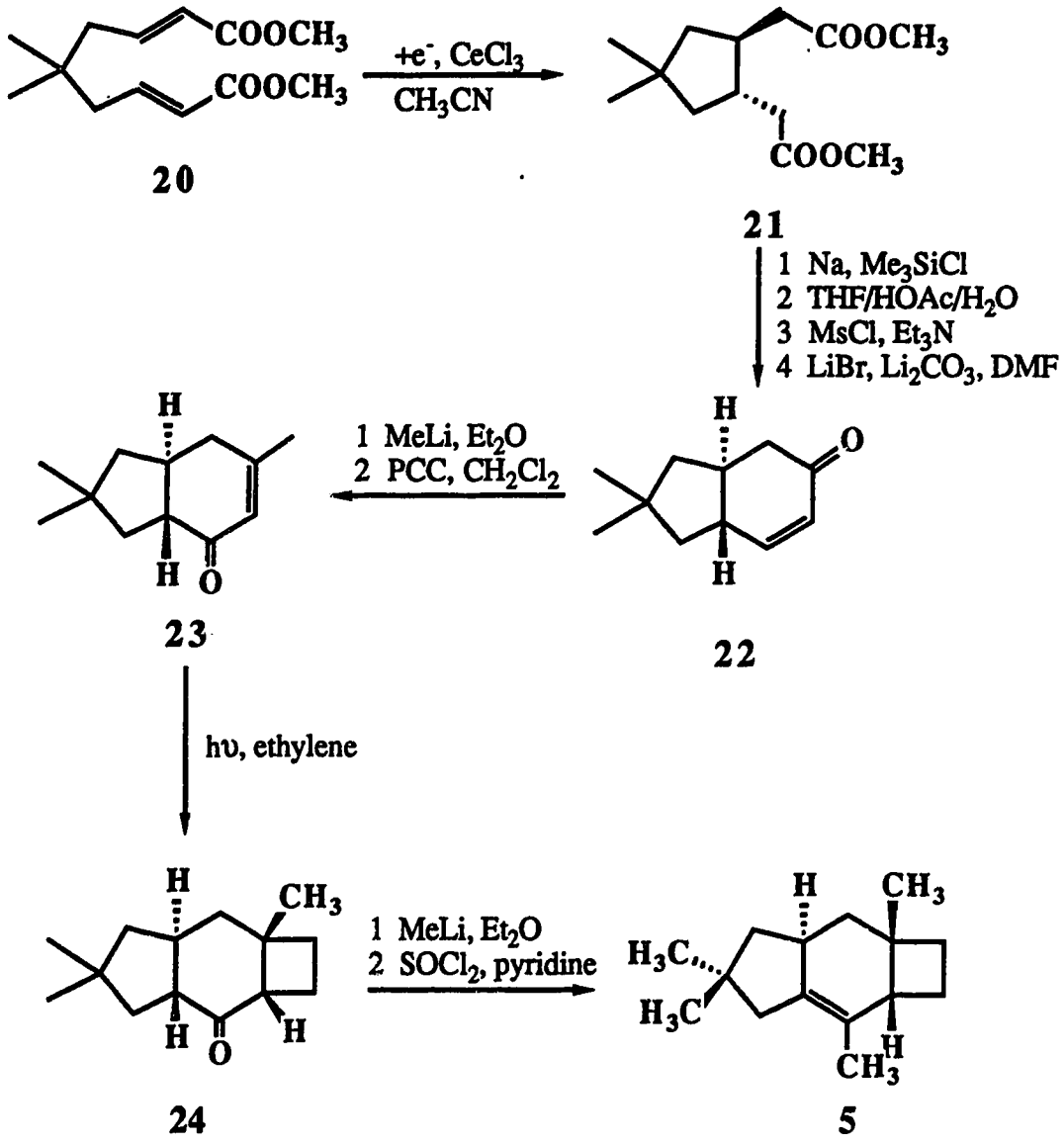
Methyl ether 17, produced from humulene (15), was treated with 2 eq. of boron tribromide (BBr_3) in methylene chloride for 30 minutes to give the bridged bromide 19 in 21% yield. The formation of bromide 19 may be interpreted as

follows: the methyl ether **17** provided the protoilludanyl cation **16** which rearranged to **18** to provide the bromide **19**. Treatment of **19** with 2 eq. of silver acetate in acetic acid at 90^0C for 7 hours afforded racemic sterpurene (**5**) in 61% yield.

In 1986, Little and coworkers reported the synthesis of sterpurene using a different approach (Scheme II-3).[13] A key step in this synthesis was the intramolecular electrochemically induced cyclization of bisenoate **20** in the presence of cerium (III) chloride ($CeCl_3$) leading to the cyclopentane diester **21**. The required six-membered ring was generated by a modified acyloin condensation. Hydrolysis of the initially formed bis(silyl enol)ether with THF/AcOH/H_2O (2:2:1), mesylation, and elimination provided enone **22**. Conversion of **22** to enone **23** was achieved by treatment of **22** with methyllithium and subsequent oxidation of the resulting tertiary allylic alcohol using pyridinium chlorochromate (PCC). Photoaddition of ethylene to enone **23** proceeded smoothly to give cyclobutane **24**. Finally, racemic sterpurene (**5**)was obtained by treatment of ketone **24** with methyllithium followed by elimination.

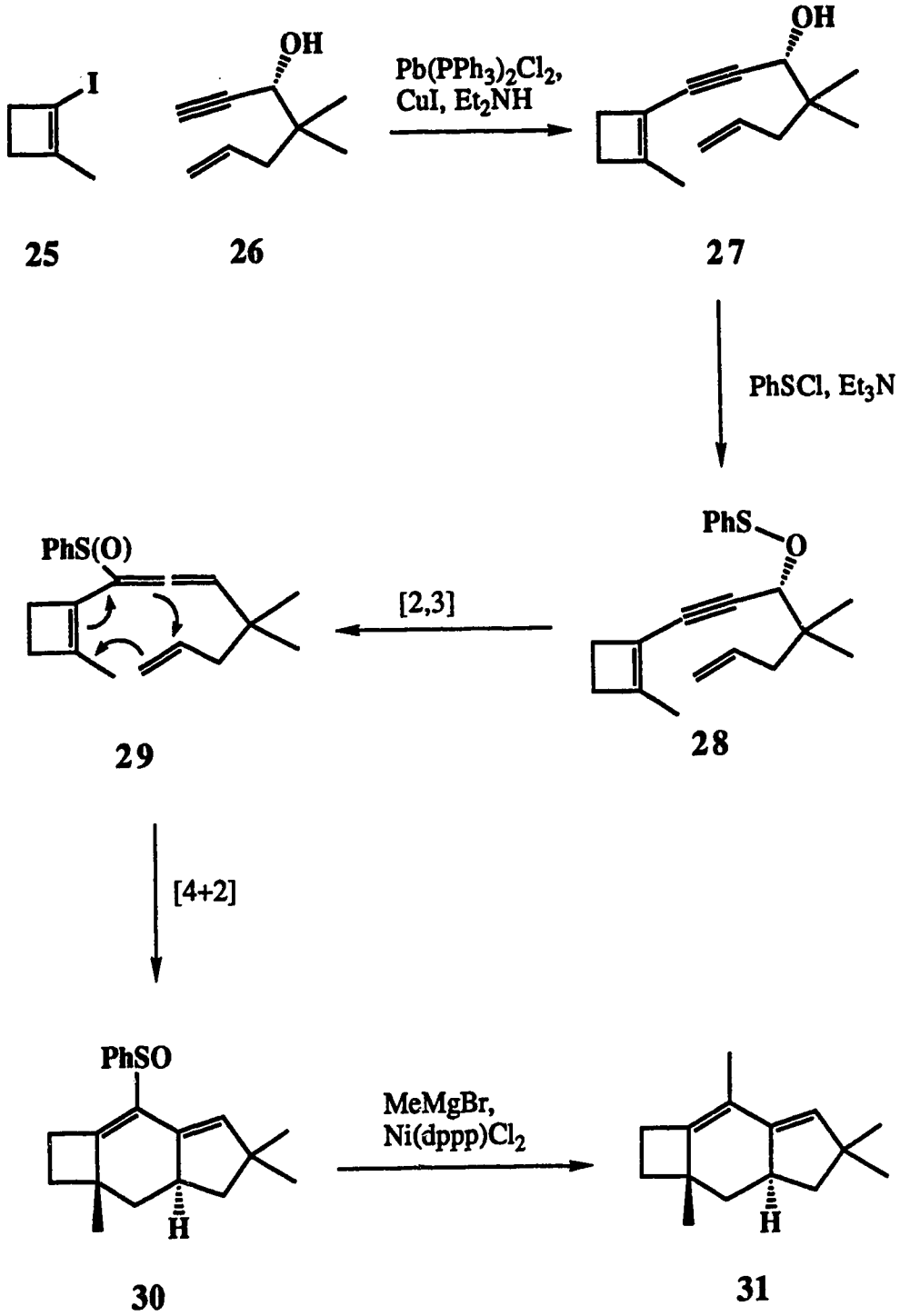
An enantioselective synthesis of (+)-sterpurene was achieved by a stereoselective vinylallene intramolecular Diels-Alder reaction (Scheme II-4).[9,10] Coupling of propargyl alcohol **26** with vinyl iodide **25** afforded the enyne **27** in 77% yield. Treatment of dienynol **27** with benzenesulfenyl chloride (PhSCl) furnished the tricyclic sulfoxide **30** in 70% yield. This step consists of a [2,3]-sigmatropic shift and an intramolecular Diels-Alder reaction. The chiral element in dienynol **27** was transferred to the allene **29** and subsequently to the two chiral elements of **30** in an entirely enantio- and diastereoselective fashion. Transformation of sulfoxide **30** to diene **31**, followed by dissolving metal reduction afforded

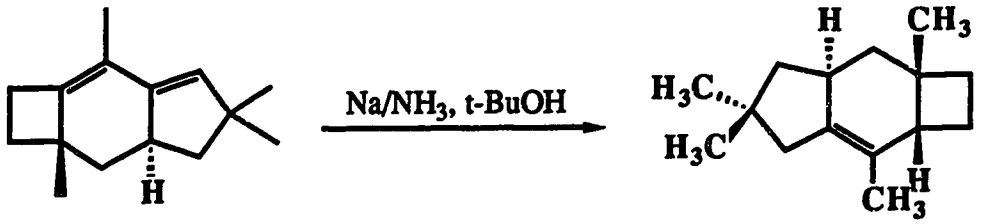
Scheme II-3 Little's synthesis of sterpurene (5)



(+)-sterpurene (5). This was the first enantioselective synthesis of 5 and thus, the absolute stereochemistry of sterpurenes was confirmed as is shown.

Scheme II-4 Okamura's synthesis of (+)-sterpurene (5)





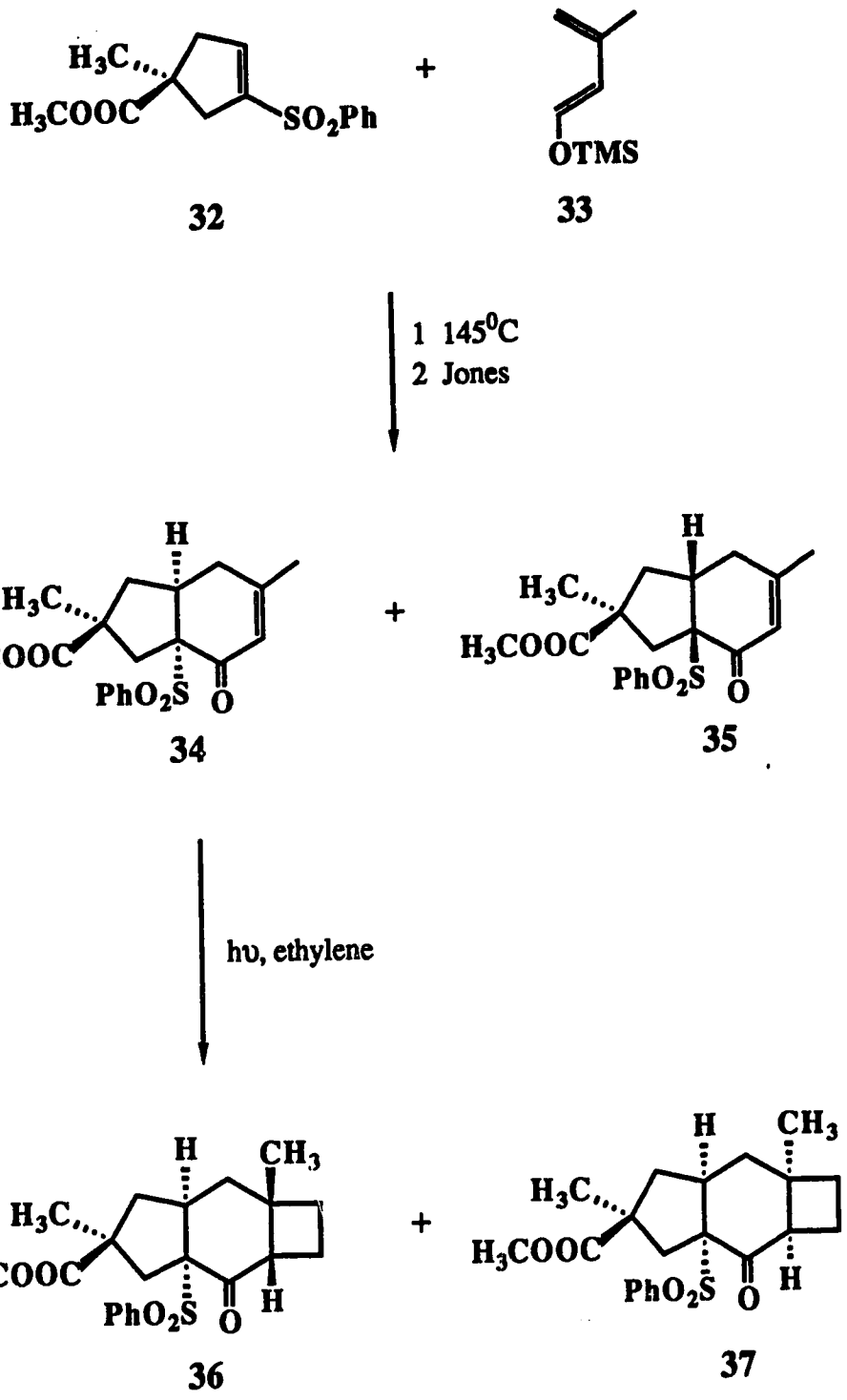
31 (+)-5

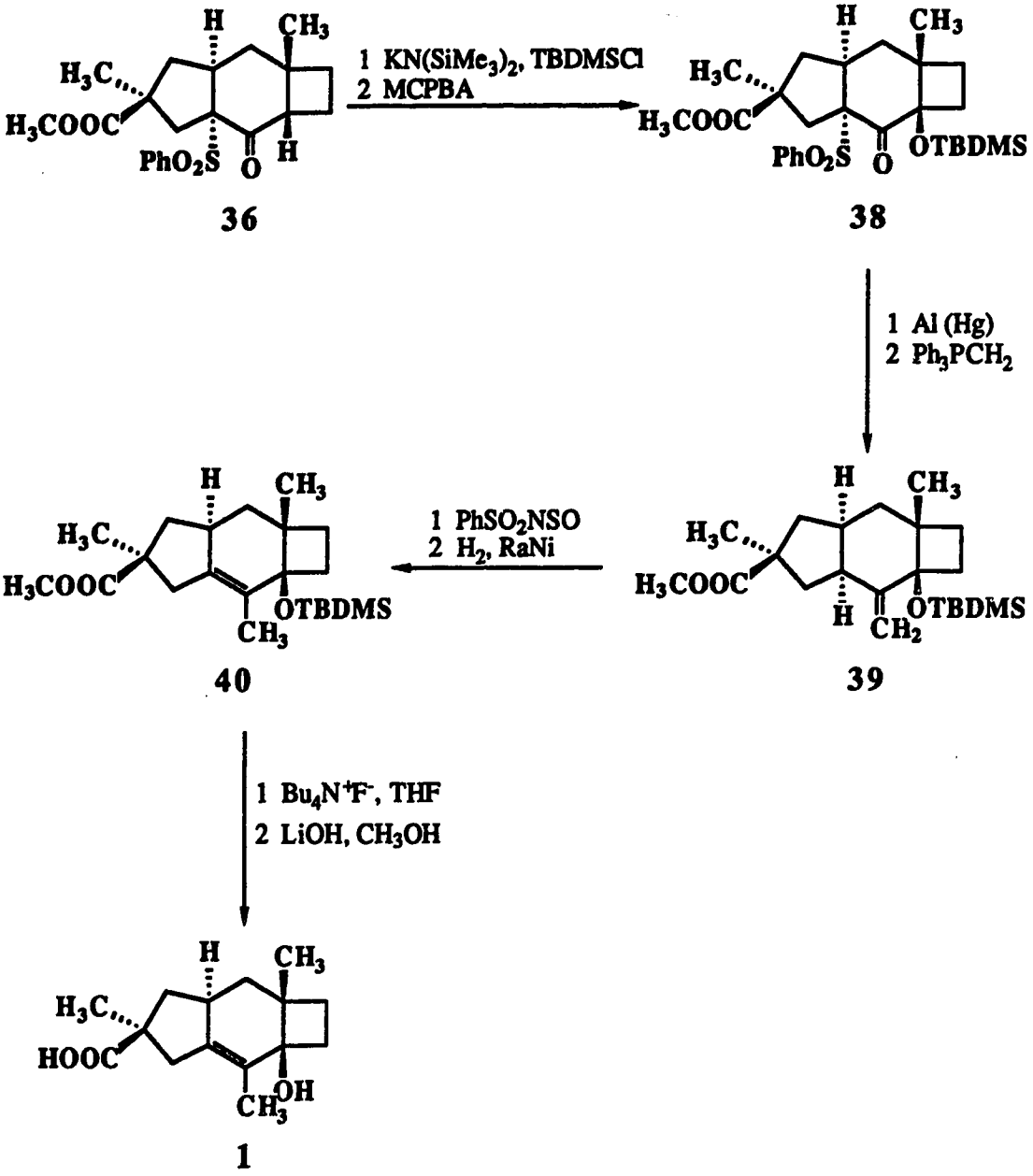
The synthesis of racemic sterpuric acid (1) and sterpurene-3,12,14-triol (4) was reported by Paquette and coworkers (Scheme II-5 and Scheme II-6).[14,15]

Diels-Alder reaction of vinyl sulfone **32** and diene **33** at 145^{0}C for 5 days, followed by direct Jones oxidation, provided **34** and **35** in a ratio of 2.1:1. Irradiation of the major enone **34** in the presence of ethylene furnished **36** and **37** in 71% and 23% yield, respectively (irradiation was interrupted when **34** was half-consumed). Ketone **36** was converted to its silyl enol ether which was treated with MCPBA to provide ketosulfone **38** as the major product. Chemspecific removal of the phenylsulfonyl group in **38** and subsequent Wittig olefination afforded **39**. Migration of the double bond to give **40** was efficiently achieved by first subjecting **39** to an ene reaction with N-sulfinylbenzenesulfonamide and then carrying out reductive desulfurization. The synthesis of sterpuric acid (1) was completed by sequential exposure of **40** to tetra-n-butyl ammonium fluoride and saponification.

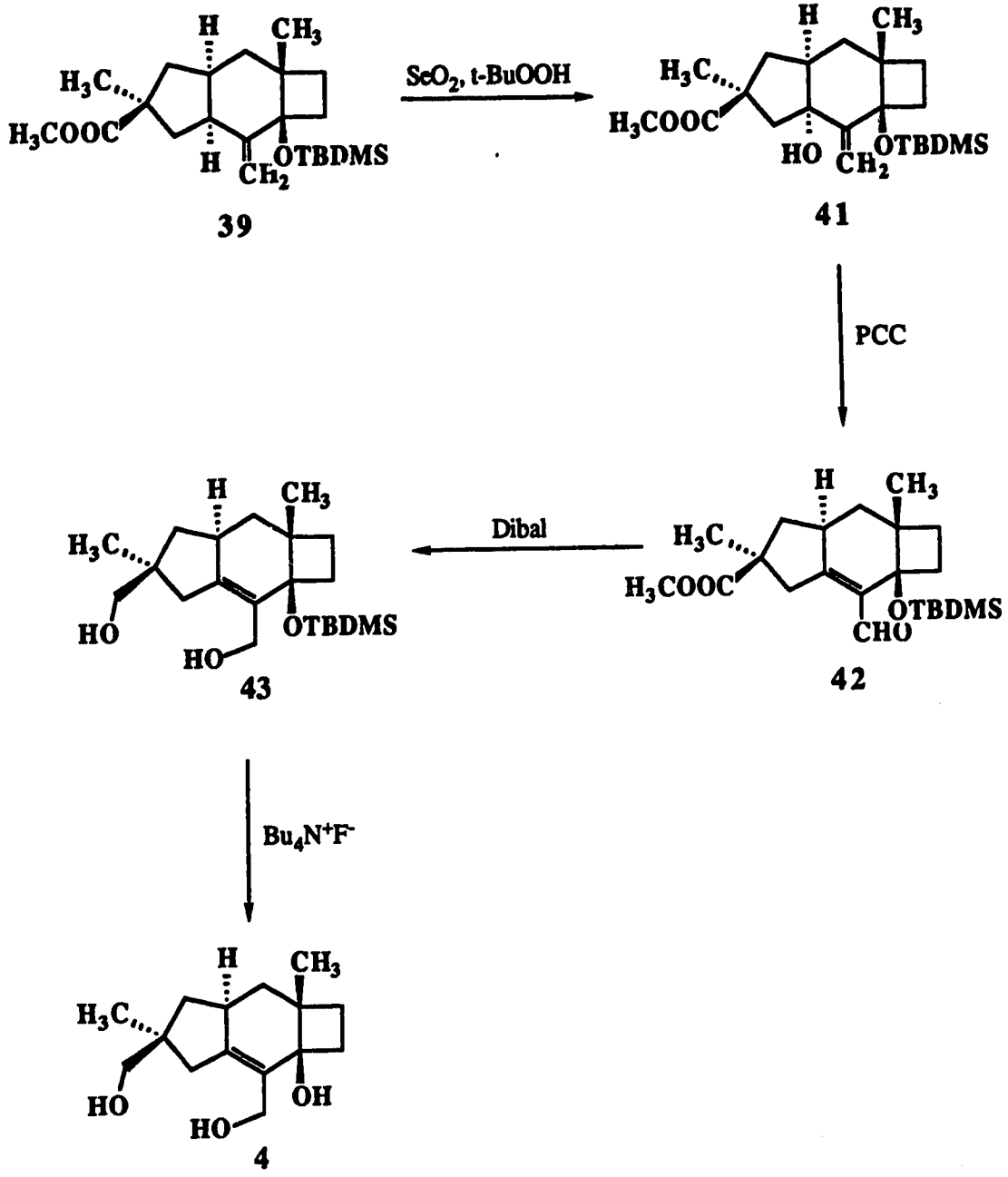
The synthesis of sterpurene-3,12,14-triol (4) proceeded from intermediate **39** (Scheme II-6). Oxidation of **39** with a combination of selenium dioxide and *tert*-butyl hydroperoxide furnished allylic alcohol **41** in 47% yield. Oxidative rearrangement and subsequent reduction provided protected triol **43**. The triol **4** was obtained by deprotection of **43**.

Scheme II-5 Paquette's synthesis of sterpuric acid (1)



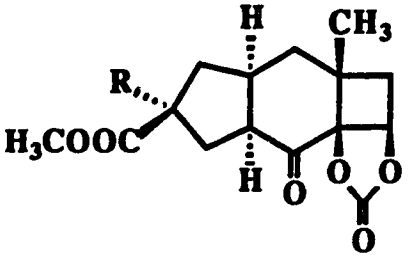

1 KN(SiMe3)2, TBDMSCl
2 MCPBA
1 Al (Hg)
2 Ph3PCH2
1 PhSO2NSO
2 H2, RaNi
1 Bu4N+F-, THF
2 LiOH, CH3OH
36
38
39
40
1

Scheme II-6 Paquette's synthesis of sterpurene-3,12,14-triol (4)

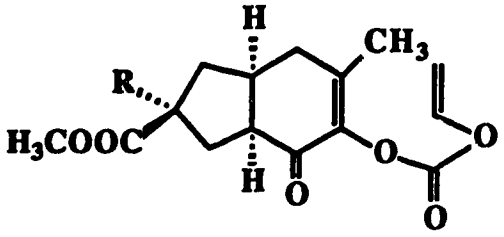


We became interested in the synthesis of sterpuric acid because of its unusual 4/6/5 tricyclic system. The synthesis will enable us to study in more detail the biological properties of this compound and will allow us to produce analogs, if required. This on-going project has been carried out in our laboratory for several

years. Tricyclic compounds **44** and **45** were synthesized from carbonates **46** and **47**, respectively.[16]

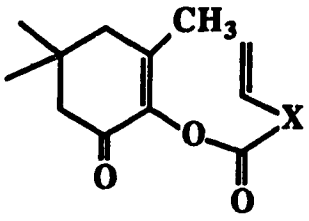


44 R=$COOCH_3$
45 R=CH_3

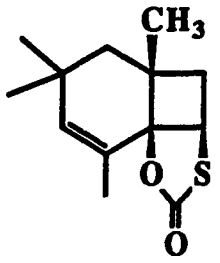


46 R=$COOCH_3$
47 R=CH_3

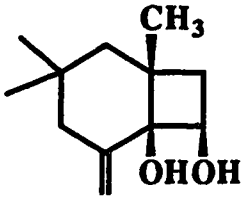
A model study had also shown that vinylthio carbonate **48** is more reactive towards photocycloaddition than the corresponding oxygen analogue **49**[17]. Desulfurization of **50** (Raney nickel reduction) proved to be much easier than the deoxygenation of **51** (1. Swern oxidation 2. Ac_2O 3. $TsNHNH_2$ 4. catecholborane).[17]



48 X=S
49 X=O

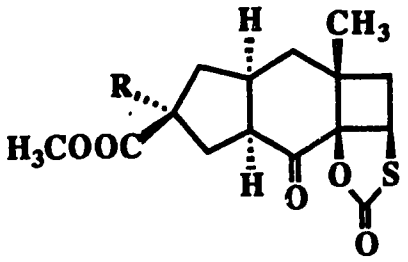


50



51

Thus, we were interested in synthesizing compounds **52** and **53**, the sulfur analogues of **44** and **45**, and eventually sterpuric acid.



52 R=$COOCH_3$
53 R=CH_3

In order to facilitate the discussion of these synthetic studies, the results are presented under the following three headings:

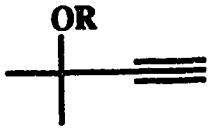
1. Synthesis of compound **86**, a close examination of the approach.
2. Some unsuccessful results.
3. Synthesis of compound **131**, a precursor of the AB ring system.

RESULTS AND DISCUSSION

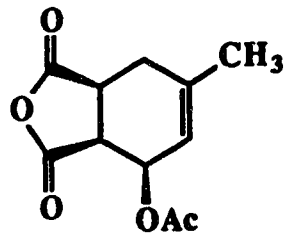
1. Synthesis of Compound 86, a Close Examination of the Approach

Most of the reactions have been investigated previously in our laboratory. [16] The known compounds will not discussed further, only the unreported compounds will be discussed. Acetate **55** was readily obtained by acetylation of the commmercially available alcohol **54** (Ac_2O, Et_3N, reflux). Diels-Alder reaction of acetate **55** [18] and maleic anhydride in refluxing benzene in the presence of silver trifluoroacetate ($AgOCOCF_3$) afforded adduct **56** as a single stereoisomer [19] in quantitative yield. The crude **56** was readily reduced with lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF) to give triol **57**. Selective protection of triol **57** with dimethoxypropane and trifluoroacetic acid [20] furnished two compounds **58** and **59**. The yield of the desired primary alcohol **58** has never been more than 60%. Separation of the two compounds also proved difficult when the reaction scale is up to ~20g.

Primary alcohol **58** was readily converted to tosylate **60** with *p*-toluenesulfonyl chloride (*p*-TsCl) and triethylamine (Et_3N).[21] Alkylation of **60** with the anion of dimethyl malonate ($CH_2(COOCH_3)_2$, NaH)[22] in hexamethylphosphoramide (HMPA) at 55~60^{0}C for 6 days afforded the esters **61** and **62**. The yield of the desired ester **61** was in the range of 10~65%. The isolation of **61** from the reaction mixture is extremely difficult since the excess dimethyl malonate and the residual HMPA are not easily removed. The ir spectrum of **62** shows the presence of esters (1754 and 1731 cm^{-1}). The ^{1}Hnmr spectrum displays methoxyl (δ3.79, s) and a methylene (δ3.45, s) signals indicating the presence of a malonate moiety in the molecule. The signals for methyl (δ1.73, s), a methylene



54 R=H
55 R=Ac



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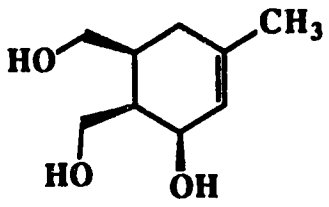

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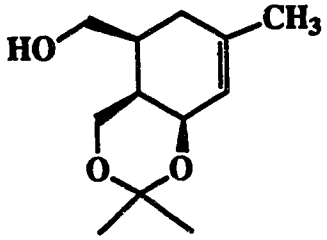
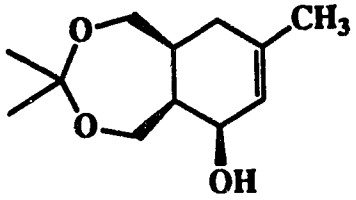
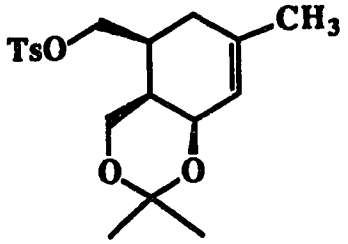


58

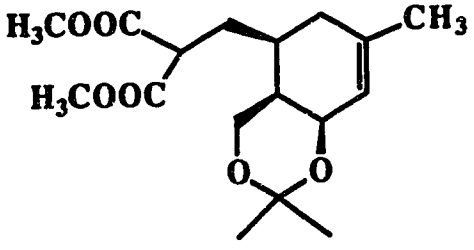


59

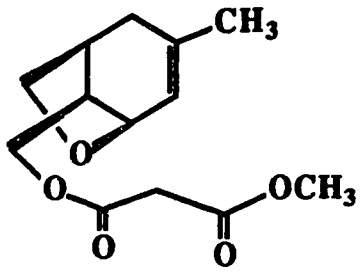
(δ2.34, dd, J=7, 17Hz; 2.10, d, J=17Hz), and olefinic hydrogen (δ5.74, brd, J=5Hz) suggest the presence of the intact six-membered ring. There are signals for five hydrogens geminal to oxygen (δ4.19, d, J=5Hz; δ4.12, dd, J=7,11Hz; δ4.08, dd, J=8,11Hz; δ4.01, m; δ3.65, d, J=8Hz) indicating intramolecular substitution has occured. Two ester carbons (δ166.75 and δ166.29) and four oxygenated carbons (δ72.42, 71.05, 65.36, and δ52.29) in the ^{13}Cnmr spectrum also support structure **62**. The formation of **62** can be explained as follows (Scheme II-7).



60



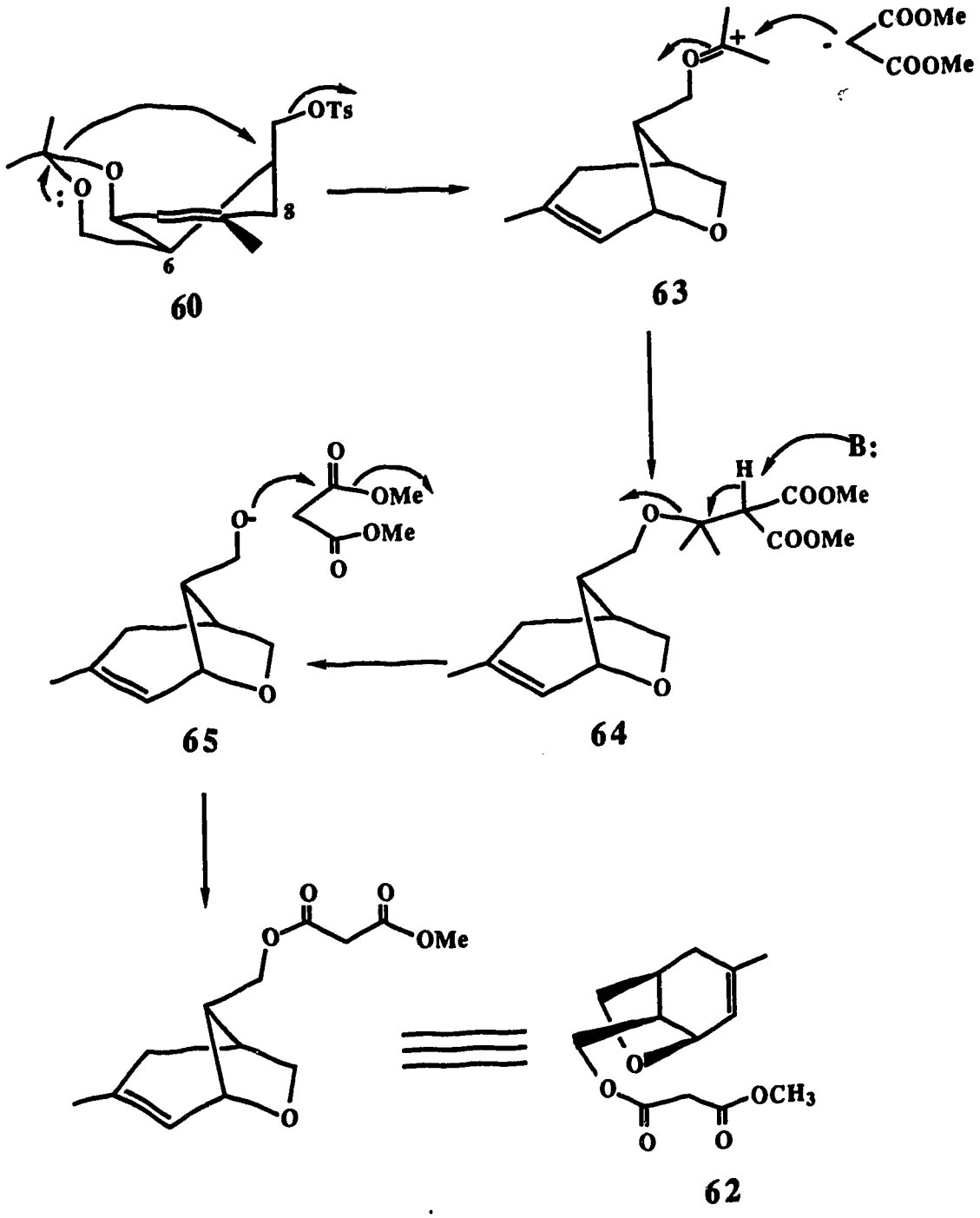
61



62

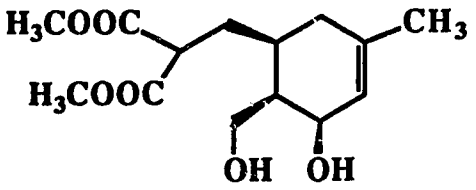
The departure of the tosylate was promoted by a 1,3-pseudodiaxial interaction with an oxygen of the acetonide to generate oxonium ion **63**. Reaction of **63** with malonate anion provided **64**. Base-catalyzed β-elimination produced anion **65** which reacted with a second dimethyl malonate leading to the ester **62**. In support of this mechanism, ester **64** and alcohol **65** were isolated from the same reaction mixture in the previous studies.[16]

Scheme II-7 Formation of **62** from tosylate **60**.

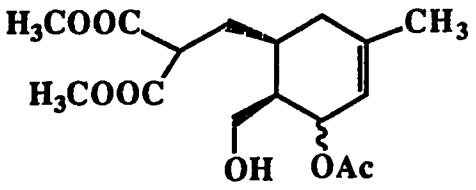


Deprotection of acetonide **61** under acidic conditions (80% HOAc in methylene chloride)[23] afforded diol **66** in good yield. When the reaction was scaled

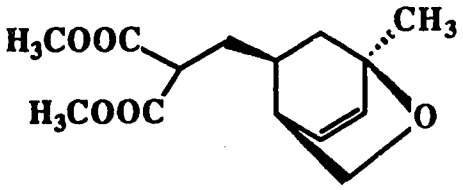
up, compounds **67** and **68** were also obtained. Compound **67** was assigned structure **69** in the previous study.[16] The ^{13}Cnmr spectrum of **67** shows only two olefinic carbons (δ137.72 and 119.68) indicating that structure **69** is incorrect. Compound **67** is a mixture of epimers at C-1 (*ca.* 3:1). The methyl signal of the acetyl group at δ2.18 ppm was previously assigned to impurity leading to the incorrect structure **69**.



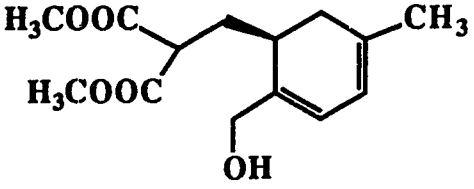
66



67



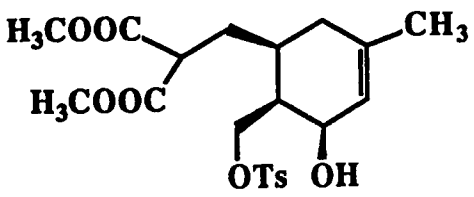
68



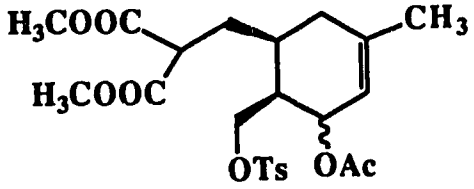
69

The molecular formula of **68** is $C_{14}H_{20}O_5$ as determined by hrms. The ir spectrum displays ester absorption at 1754, 1736 cm^{-1}. The ^{1}Hnmr spectrum shows two olefinic hydrogen signals (δ6.46, dd, J=7, 7Hz; δ6.11, d, J=7Hz), two methoxy signals (δ3.68,s; δ3.67,s), a methine hydrogen (δ3.35, t, J=7Hz), two hydrogens geminal to oxygen (δ3.98, d, J=8Hz; δ3.14, d, J=8Hz), and a singlet methyl (δ1.39). The ^{13}Cnmr spectrum shows two olefinic carbons at δ136.30 (d) and δ134.66 (d) and four oxygenated carbons at δ69.98 (s), 61.69 (t), 52.30 (q), and δ50.20 (q). All the data are consistent with structure **68**. Compound **68** may be derived from **66** or **67** by ionization and internal addition.

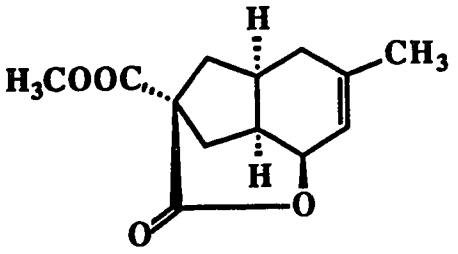
Tosylation (*p*-TsCl, Et3N) of the primary hydroxyl group of **66** and **67** provided **70** and **71**, respectively, in good yield. Compound **71** shows ester absorption (1734 cm^{-1}) in the ir spectrum. Its ^{1}Hnmr spectrum shows aromatic hydrogens at δ7.83 (2H, d, J=8Hz) and δ7.40 (2H, d, J=8Hz) and an aromatic methyl at δ2.60 (s).



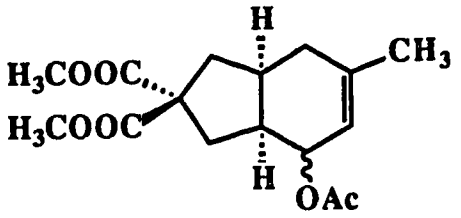
70



71



72



73

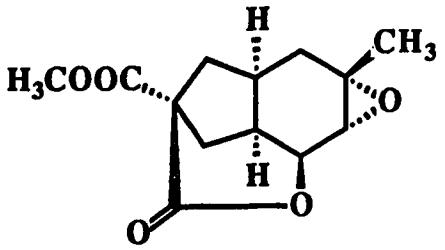
Formation of the five-membered-ring compounds **72** and **73** was readily achieved, respectively, when tosylates **70** and **71** were treated with NaH in dry THF at room temperature. The ir spectrum of **73** shows the ester absorption at 1734 cm^{-1}. Its ^{1}Hnmr spectrum displays signals for an olefinic hydrogen (δ5.55, brs), a hydrogen geminal to the acetoxy (δ5.43, brs), two methoxyls (6H, δ3.84, s), an acetyl methyl (δ2.19, s), and an olefinic methyl (δ1.84, s).

Epoxidation of **72** and of **73** was carried out using *m*-chloroperbenzoic acid (MCPBA, CH_2Cl_2, rt.)[24] to afford quantitative yields of **74** and **75**, respectively.

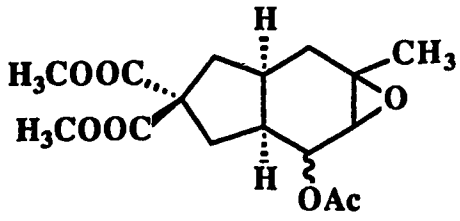
The ^{1}Hnmr spectrum of **75** shows a signal at δ5.02 (dd, J=3, 5Hz) for the methine hydrogen geminal to acetoxy. This hydrogen is coupled to another methine hydrogen at δ3.38 which may be assigned to the hydrogen of the epoxide ring. The acetyl methyl appears at δ2.25 and an aliphatic methyl is at δ1.48 as a singlet.

Hydrogenolysis[25] of epoxides **74** and **75** using first Pd/C and then PtO_2 (each for 2 days) provided alcohols **76** and **77**, respectively, in good yield.

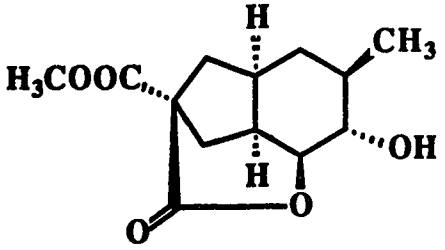
The ir spectrum of **77** shows the hydroxyl group at 3500 cm^{-1}. The ^{1}Hnmr spectrum displays signals for the hydrogen geminal to acetoxy (δ4.46, d, J=2Hz), a hydrogen geminal to oxygen (δ3.52, d, J=8Hz), and an aliphatic methyl (δ1.04, d, J=6Hz).



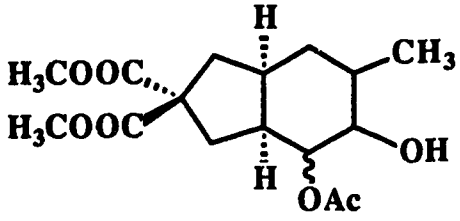
74



75



76

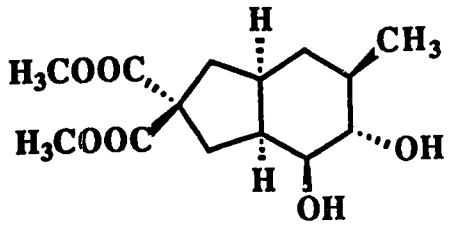


77

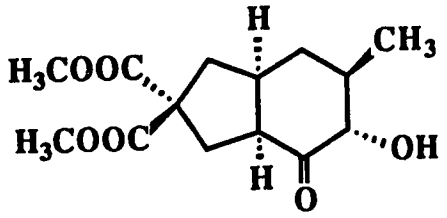
Treatment of alcohol **76** with KOH in methanol[26] afforded diol **78** which is also the major product from the hydrolysis of alcohol **77** under the same reaction conditions.

Oxidation of vicinal diols to α-dicarbonyl compounds by trifluoroacetic anhydride "activated" dimethyl sulfoxide was reported recently. Conversion of 1,2-cyclohexanediol to 2-hydroxy-2-cyclohexen-1-one was achieved in 80% yield.[27,28] Encouraged by this report, we applied this modified Swern procedure[29] to diol **78**. Oxidation (3 eq. $(CF_3CO)_2O$, 3.5 eq. DMSO, -78^{0}C, then 7 eq. Et_3N) of diol **78** provided α-hydroxy ketones **79** and **80** in a ratio of 9:2 without any diketone **81**. However, diketone **81** was obtained by a second oxidation of **79** and **80** under the same reaction conditions.

Diketone **81** was also obtained from **76** by an alternative route. Oxidation of **76** using a modified Swern procedure (DMSO, $(CF_3CO)_2O$, then Et_3N) or with PCC[30,31] furnished ketone **82** in good yield. Basic hydrolysis (KOH/MeOH) of **82** provided α-hydroxy ketone **80** which was converted to diketone **81** by Swern oxidation.



78



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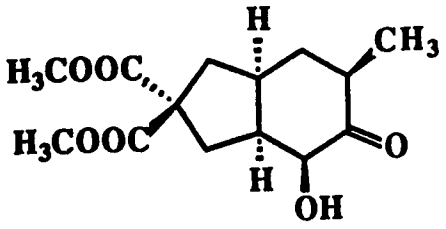
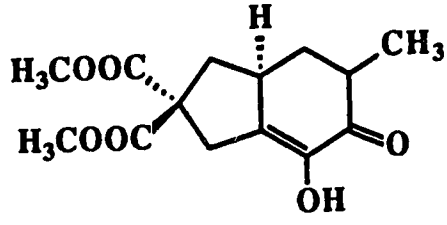
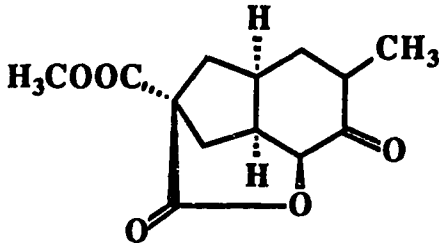


80

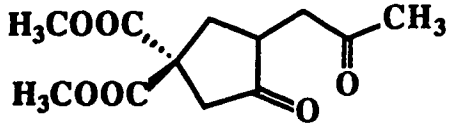


81

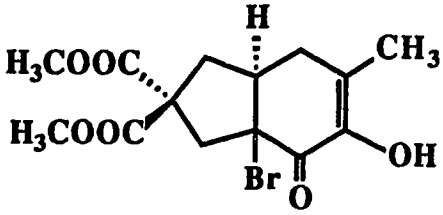


82

It is interesting to note that oxidation of diol **78** and α-hydroxy ketone **80** with PCC provides diketone **83**. The hrms indicates the molecular formula of **83** is $C_{12}H_{16}O_6$. This is also confirmed by a cims (m/z 274, $M+NH_4^+$, 100%). There is ketone absorption (1733 cm^{-1}) in the ir spectrum of **83**. The ^{1}Hnmr spectrum displays the methyl group at δ2.18 ppm. All twelve carbon signals appear in the ^{13}Cnmr spectrum. The signal at δ214.26 is typical of ketone on a five-membered ring and the signal at δ205.56 is assigned to aliphatic ketone.[32]



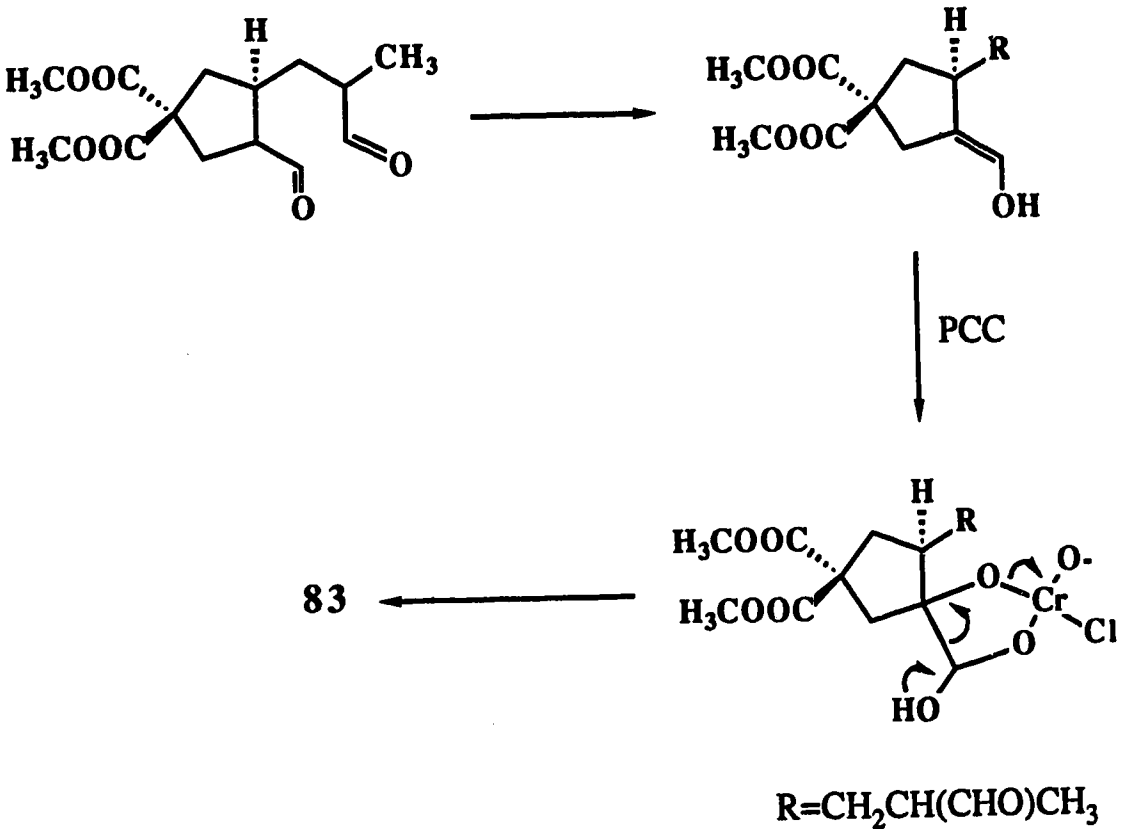
83



84

A possible reaction mechanism for formation of **83** could involve the cleavage of vicinal diol or α-hydroxyketone to dialdehyde or aldehyde carboxylate.[33] Attack on the enol derived from the aldehyde by PCC afforded an unstable cyclic intermediate (Scheme II-8). Heterolytic cleavage of the chromium-oxygen bond can then give the diketone **83**. Other reaction mechanisms are possible. However, since we do not have any evidence for these mechanisms, further speculation is deferred.

Scheme II-8 A possible mechanism for formation of 83

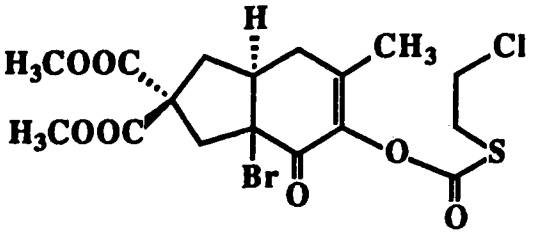


R=$CH_2CH(CHO)CH_3$

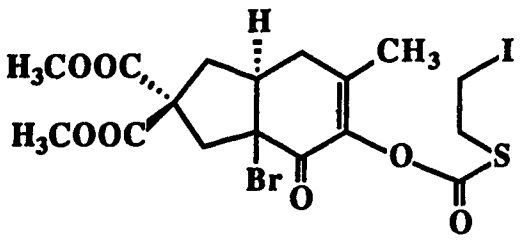
Bromination of diketone **81** with N-bromosuccinimide (NBS, 1.2eq, THF, -20^0C)[34] provided bromoketone **84** in good yield. The thiocarbonate **85** was obtained in 60% yield when compound **84** was treated with S-(β-chloroethyl)chlorothioformate[35,36] and pyridine.

The ^{1}Hnmr spectrum of **85** shows four triplets at δ3.70 (2H, t, J=7Hz) and δ3.23 (2H, t, J=7Hz) consistent with the structure. The ^{13}Cnmr spectrum displays all seventeen carbon signals. The signals at δ168.10, 42.33, and δ33.37 are attributed to the thioformate moiety.[37]

Conversion of **85** to iodothiocarbonate **86** was readily achieved by refluxing of **85** with sodium iodide in methyl ethyl ketone.[38] The four hydrogen signals on the thioformate moiety of **86** have changed to a multiplet at δ3.34 in the ^{1}Hnmr spectrum. The corresponding carbon signals shift to δ167.94, 33.88, and δ29.66 ppm in the ^{13}Cnmr spectrum.



85



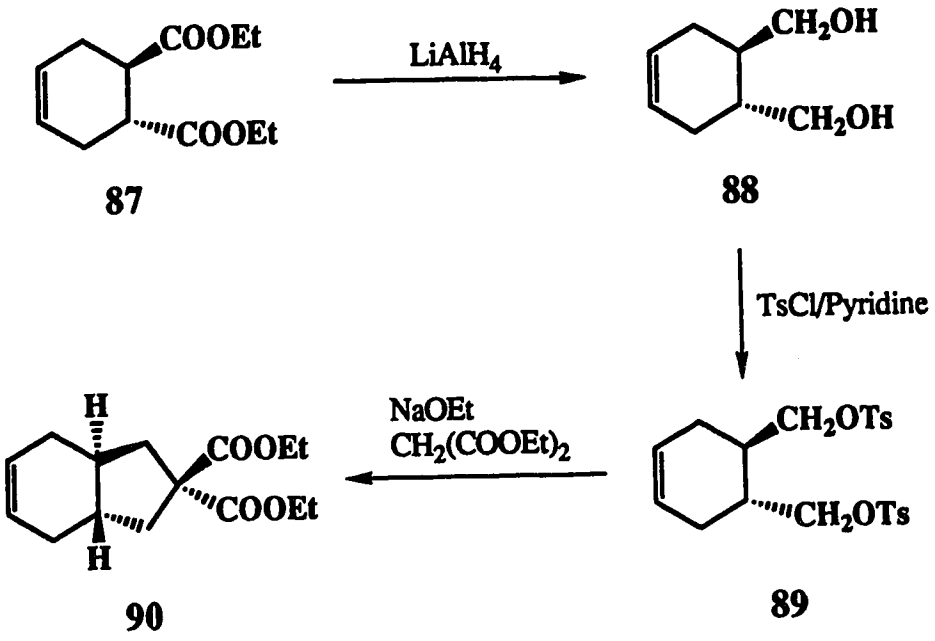
86

At this point, the small amount of **86** available prevented us from continuing our synthesis. On a close examination of each step in our synthesis, the formations of **58** and **61** are found to be the major low-yield steps. The transformation of tosylate **60** to ester **61** not only occurred in low yield but also a long reaction time (6 days) and extensive separations were required. Thus, we decided to re-investigate these two steps to see if they could be improved. If not, alternative approaches might be in order. These investigations are discussed below.

2. Some unsuccessful results

As discussed earlier, reaction of triol **57** with dimethoxypropane in the presence of trifluoroacetic acid provided desired alcohol **58** and undesired alcohol **59**. Since the yield of **59** is about 30%, we attempted to convert **59** to an intermediate which could be used in our synthesis. A report of the synthesis of compound **90** from diester **87** (Scheme II-9)[39] attracted our attention.

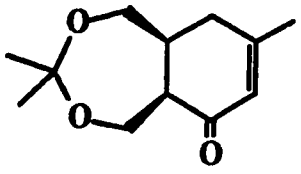
Scheme II-9 Formation of bicyclic compound **90**



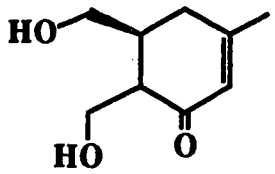
In principle, the secondary hydroxy group in compound **59** could be oxidized to a ketone. Hydrolysis under acidic condition may generate a *trans* diol and then following the steps **88**→**89**→**90** in Scheme II-9 could provide the bicyclic compound. If this works, the diol could be obtained from triol **57** by oxidation with manganese dioxide. With this idea in mind, we carried out the reactions. Oxidation of **59** with PCC furnished the corresponding ketone **91**. The molecular formula of **91** was determined as $C_{12}H_{18}O_3$ by hrms. The ir spectrum shows the absorption of α,β-unsaturated ketone (1659 cm^{-1}). The signal for the olefinic hydrogen was shifted downfield to δ5.82 ppm from δ5.30 ppm in compound **59**.

Hydrolysis of acetonide **91** to diol **92** was carried out under acidic conditions (80% HOAc). Diol **92** was also obtained by oxidation of triol **57** with maganese dioxide.[40] The ir spectrum of **92** shows the hydroxy groups (3428, 3377 cm^{-1}) and an α,β-unsaturated ketone (1658 cm^{-1}). The molecular formula $C_9H_{14}O_3$ was obtained from hrms. The methyl signals for acetonide **91** disappear

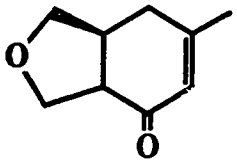
in the ^{1}Hnmr spectrum of **92**. With the spectral data available, we were unable to assign the configuration of C-6. Nevertheless, we carried on the next reaction. Tosylation of diol **92** (*p*-TsCl, Et_3N, CH_2Cl_2 or *p*-TsCl, pyridine) provided compounds **93** and **94** in 42% and 31% yield, respectively.



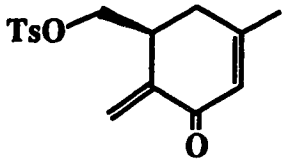
91



92



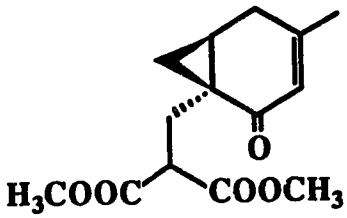
93



94

The molecular formula of **93** is $C_9H_{12}O_2$ as determined by hrms. The ir spectrum of **93** shows the absorption of an α,β-unsaturated ketone (1662 cm^{-1}) The ^{1}Hnmr spectrum displays an olefinic hydrogen (δ5.95, brs), four hydrogens geminal to oxygen (δ4.14, dd, J=5,8Hz; δ4.03, dd, J=8,8Hz; δ3.95, dd, J=7,8Hz; δ3.55, dd, J=6,8Hz), two geminal hydrogens (δ2.61, dd, J=5,19Hz; δ2.34, dd, J=3,19Hz), and a methyl (δ2.02, s). The hrms of **94** shows the molecular ion at m/z 306.0923 which corresponds to a molecular formula $C_{16}H_{18}O_4S$. Its ^{1}Hnmr spectrum displays signals for aromatic hydrogens (δ7.81, d, J=8Hz; δ7.39, d, J=8Hz), an aromatic methyl (δ2.46, s), three olefinic hydrogens (δ6.15, s; δ6.03, s; δ5.33, brs), and two hydrogens geminal to oxygen (δ4.09, dd, J=8,10Hz; δ4.01, dd, J=10,10Hz).

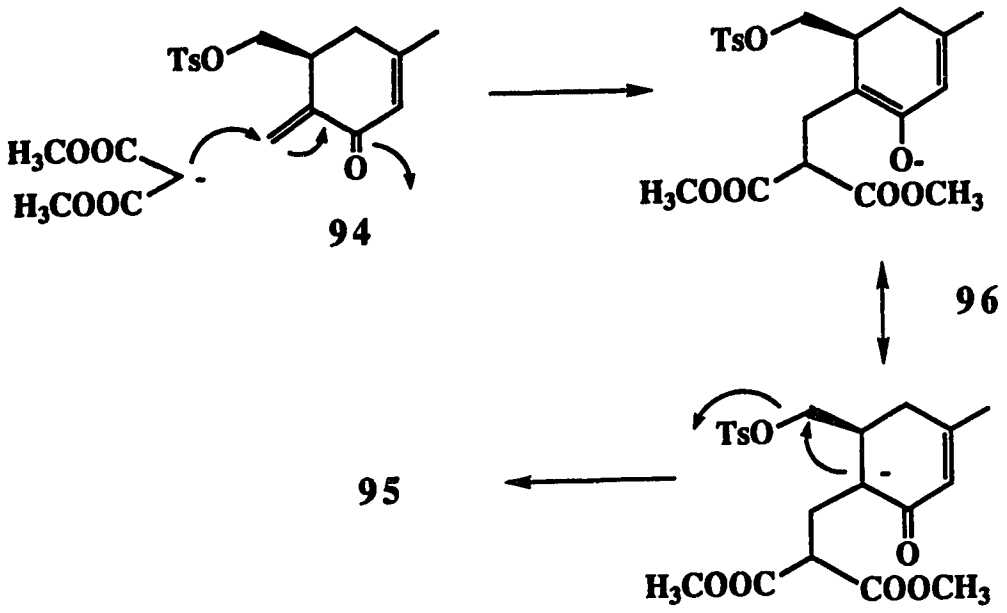
Although the desired ditosylate was not obtained and the yield for the formation of **94** was low, we were interested in the alkylation of the tosylate **94**. This was carried out with dimethyl malonate anion ($CH_2(COOCH_3)_2$, NaH, THF-HMPA) in refluxing THF for 12 hours. The bicyclic compound **95** was obtained in 96% yield.



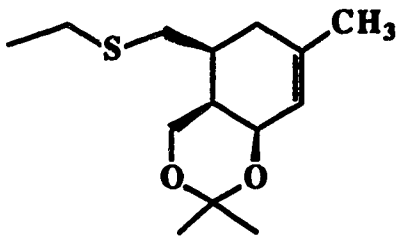
95

The molecular formula of **95** is $C_{14}H_{18}O_5$ as determined by hrms. Its ^{1}Hnmr spectrum displays the signals for an olefinic hydrogen (δ5.73, brs), a methine hydrogen (δ3.88, dd, J=6,8Hz), two methoxy (δ3.75,s; δ3.74, s), and a methyl (δ1.87, s). The signals at δ2.61 (dd, J=5.5, 21Hz) and δ2.49 (d, J=21Hz) with a large geminal coupling constant of 21Hz indicate these two hydrogens are adjacent to a double bond and a three-membered ring.[41] The high field signals at δ1.08 (dd, J=5,6Hz) and δ0.93 (dd, J=4,6Hz) are typical geminal hydrogens on a three-membered ring.[42] All fourteen carbons appear in the ^{13}Cnmr spectrum which also supports the structure **95**. The formation of **95** can be explained as follows (Scheme II-10). Michael addition of dimethyl malonate anion to the exocyclic α,β-unsaturated ketone produced anion **96**. Compound **95** is obtained by internal alkylation of enolate **96**.

Scheme II-10 Formation of **95** from tosylate **94**.



Because of the lack of success in the conversion of **59** to a suitable intermediate, we turned our attention to the alkylation of tosylate **60**. We tried different bases and alkylating agents and the results are as follows. Displacement with S,S'-diethyl dithiomalonate[43] was investigated. It has been reported that the thioester is easily reduced by sodium borohydride to the alcohol.[44] This could, in principle, simplify the later stages of our synthesis. When the alkylation was carried out in refluxing THF ($CH_2(COSCH_2CH_3)_2$, NaH, THF-HMPA) with tosylate **60**, no reaction was observed. However, sulfide **97** was obtained in 71% yield when excess of sodium hydride was added.



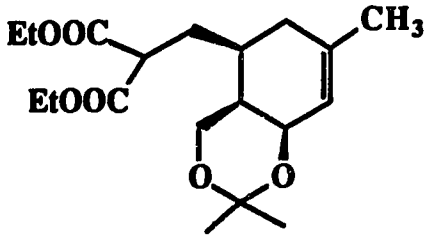
97

The hrms of **97** shows the molecular ion at m/z 256.1491 which corresponds to a molecular formula $C_{14}H_{24}O_2S$. The signals at δ3.05 (1H, dd, J=4,14Hz), δ2.64 (1H, dd, J=10.5,14Hz), and δ2.51 (2H, q, J=7Hz) in the ^{1}Hnmr spectrum are assigned to the four hydrogens geminal to the sulfur.

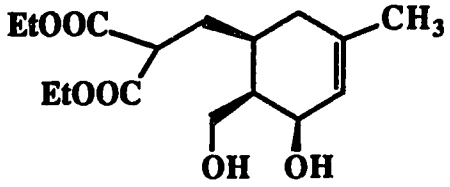
The failure to produce the desired alkylation product may be due to the very hindered tosylate **60** and the bulky S,S'-diethyl dithiomalonate. However, when the alkylation was carried out using diethyl malonate in HMPA at 55~60^{0}C for 6 days (NaH, $CH_2(COOEt)_2$, HMPA), the alkylation product **98** was obtained in 11% yield. The ^{1}Hnmr spectrum shows the signals for ethoxy groups (δ4.15, 4H, m; δ1.20, 6H, m). All other signals are consistent with the assigned structure. Hydrolysis of **98** with 80% HOAc in methylene chloride yielded diol **99** which also supports the structure **98**. The ir spectrum of **99** shows the presence of hydroxyl (3480 cm^{-1}) and esters (1747, 1731 cm^{-1}). The signals for the methyl groups of the acetonide in compound **98** disappear in the ^{1}Hnmr spectrum of **99**. A broad signal appears at δ2.30 which is assigned to the hydroxyl hydrogens.

We have also tried to use different bases with or without sodium iodide as a catalyst. When potassium hydride was used as a base (KH, $CH_2(COOCH_3)_2$, HMPA, 55~60^{0}C, 6 days), no alkylation product was isolated from the reaction

mixture. The ester **61** was obtained with or without sodium iodide as a catalyst (NaH, $CH_2(COOCH_3)_2$, HMPA, 55~60^{0}C, 6 days).

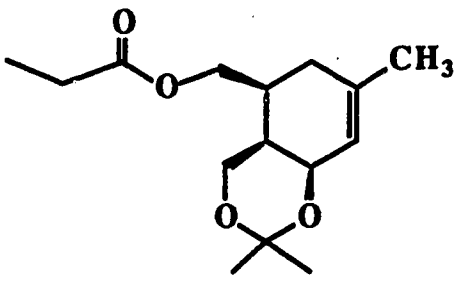


98

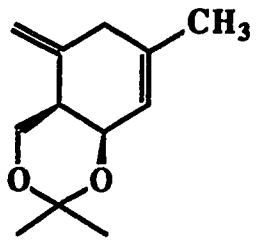


99

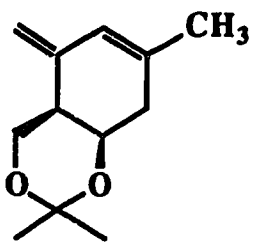
Since dimethyl malonate, diethyl malonate, and S,S'-diethyl dithiomalonate are bulky, we decided to investigate the smaller alkylating agents methyl propionate and propionitrile. Treatment of tosylate **60** with methyl propionate anion (LDA, HMPA, rt, 17 hours) did not bring about any reaction. However, when tosylate **60** was treated with methyl propionate anion (n-BuLi, HMPA, NaI, 6 days)[45] at 55~60^{0}C, two compounds, **100** and **101**, were obtained in low yield. When lithium bis(trimethylsilyl)amide[46] was used as a base {$[(CH_3)_3Si]_2NLi$, 55~60^{0}C, ~6 days, HMPA or THF-HMPA (1:1)}, compounds **100** and **102** were isolated from the reaction mixture.



100



101

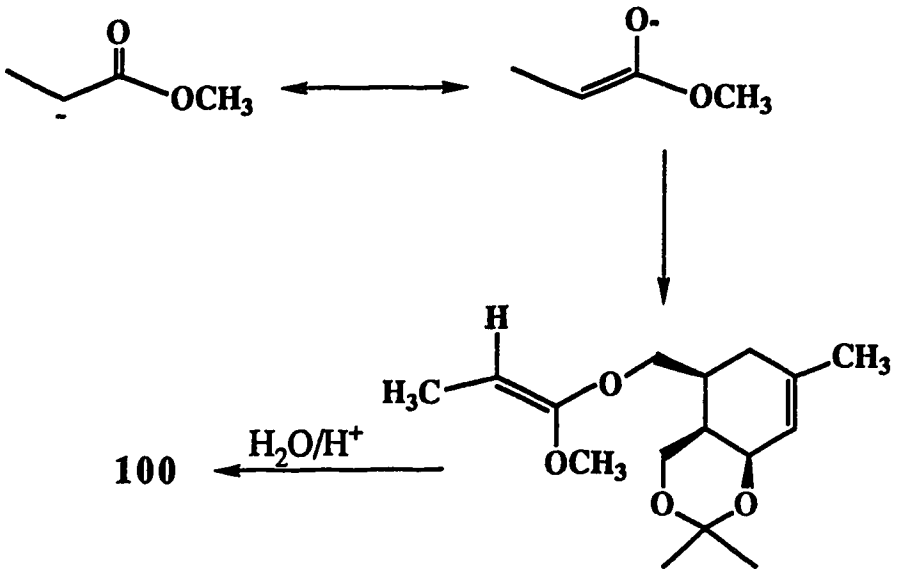


102

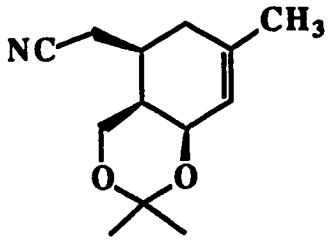
The ir spectrum of **100** shows ester absorption (1735 cm^{-1}). The signals at δ1.14 (3H, t, J=8Hz) and δ2.33 (2H, q, J=8Hz) in the ^{1}Hnmr spectrum of **100** suggest the propionate moiety in the molecule. The ^{13}Cnmr spectrum displays all fifteen carbons. The molecular formula of **101** was determined to be $C_{12}H_{18}O_2$ by hrms. The ^{1}Hnmr spectrum of **101** displays signals for three olefinic hydrogens (δ5.53, brs; δ5.16, brd, J=2Hz; δ5.10, brs). Two geminal hydrogens at δ2.85 (brd, J=19Hz) and δ2.76 (d, J=19Hz) with a large coupling constant suggest they are adjacent to two double bonds.[41] Four olefinic carbons are also revealed by ^{13}Cnmr spectrum (δ140.65, s; δ140.02, s; δ120.97, d; δ110.21, t). The ^{1}Hnmr spectrum of **102** also shows signals for three olefinic hydrogens (δ6.08, s; δ5.12, 2H, s), however one hydrogen is shifted downfield. The ^{13}C chemical shifts of the olefinic carbons are at δ140.28 (s), δ132.96 (s), δ125.39 (d), and δ110.44 (t). This information suggests the conjugated diene structure **102**.

Compound **100** was formed possibly by an O-alkylation of tosylate **60** as shown in Scheme II-11. Another possible mechanism involves propionate anion which might be formed by attacking the methyl propionate with iodide. Replacement of the tosylate with the carboxylate leads to compound **100**.

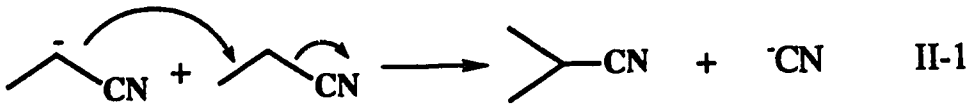
Scheme II-11 A possible mechanism for formation of **100**



Alkylation with propionitrile was then carried out. Treatment of tosylate **60** with propionitrile anion (n-BuLi, CH_3CH_2CN, HMPA, 55~60^{0}C, 6 days) furnished compounds **101**, **102**, and **103** in low yield. The molecular formula $C_{13}H_{19}O_2N$ of **103** was obtained from hrms. The molecular ion was also confirmed by cims (m/z 460, $2M+NH_4^+$, 29.2%; 239, $M+NH_4^+$, 100%). The ir spectrum shows the presence of a nitrile group at 2240 cm^{-1}.[47] The ^{13}C signal at δ120.48 in the ^{13}Cnmr spectrum is typical of a nitrile carbon.[48] Hydrogen signals at δ3.10 (brd, J=17Hz) and δ2.86 (dd, J=12, 17Hz) in the ^1Hnmr spectrum are assigned to the hydrogens geminal to the nitrile group.



103



II-1

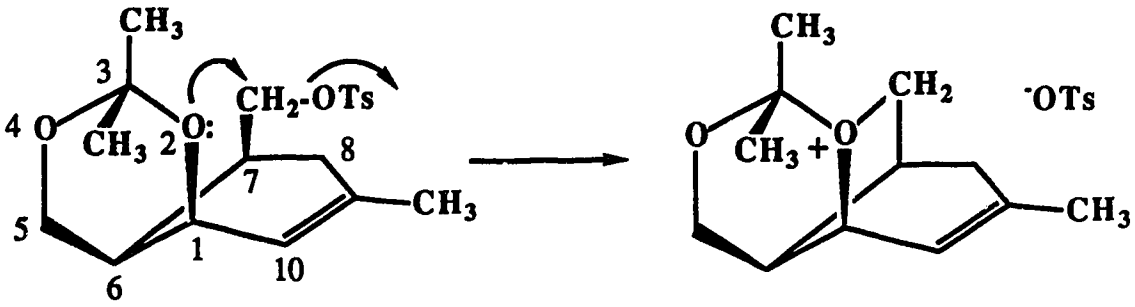
The formation of nitrile **103** may be explained as follows. The propionitrile anion attacks a second molecule of propionitrile to give a nitrile anion (equation II-1) which displaces the tosylate group in **60** to yield compound **103**. The nitrile group as a leaving group in an alkylation reaction is known.[49,50]

Since our attempts to improve the yield or the methods in both the protection and the alkylation steps were unsuccessful, we decided to investigate a different approach where these steps are avoided.

3. Synthesis of 131, a precursor of the AB ring system.

As discussed in parts 1 and 2, the alkylation of tosylate **60** gave a low yield and side reactions. This may be due to the hindered nature of the tosylate group. Molecular models of **60** (see Figure II-1) show that the approach of the alkylating agent to the carbon bearing the leaving group is highly hindered by the acetonide moiety. In this conformation, the acetonide oxygen 2 is in a position to participate in the displacement (ionization) process (Figure II-1), further complicating the course of some reactions.

Figure II-1 Conformation of tosylate 60



However, if the tosylate substituent at C-7 is *trans* to the acetonide substituents (at C-1 and C-6), the steric interactions with the incoming alkylating agent are reduced, and hence, the alkylation might occur more readily. In order to make compound with the tosylate *trans* to C-1 and C-6, the Diels-Alder reaction [51] of acetate **55** has to be carried out with a *trans* dienophile. The best dienophile would bear a leaving group which could be displaced in the alkylation step. We considered dienophile **104** as our first choice since the aldehyde could be reduced selectively in the presence of the acetate ester. However, compound **104** is not commercially available. A literature search indicated **104** can be prepared from crotonaldehyde.[52]

Refluxing crotonaldehyde, acetic anhydride, and triethylamine for 21 hours provided *cis*- and *trans*-1-acetoxybutadiene (**105**) and (**106**) in 88% yield. The coupling constant for the *cis* diene is 6 Hz, while for the *trans* diene it is 12 Hz. 4-bromocrotonaldehyde (**104**) was obtained in 42% yield by treatment of **105** and **106** with bromine, followed by sodium bicarbonate.

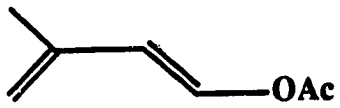
The ^{1}Hnmr spectrum of **104** shows signals for aldehyde hydrogen (δ9.60, d, J=8Hz), a methylene (δ4.12, dd, J=1,7Hz), and two *trans* olefinic hydrogens (δ6.26, bdd, J=8,15Hz; δ6.88, dt, J=7,15Hz).

104

CH_2=CH-CH=CHOAc

105 *cis*
106 *trans*

With compound **104** in hand, the Diels-Alder reaction was carried out. Thus, a mixture of compound **104** and acetate **55** was refluxed in benzene in the presence of silver trifluoroacetate for 4 days and for 9 days. Disappointingly, the desired Diels-Alder adduct was not obtained, instead diene **107** [16] and aldehyde **104** were recovered. An attempt to combine **104** and **107** at higher temperature in refluxing xylene for 21 hours was also unsuccessful. The ^{1}Hnmr spectrum of **107** displays signals for two methyls (δ1.90, brs; δ2.19, s), two *trans* olefinic hydrogens (δ6.15, d, J=12.5Hz; δ7.38, d, J=12.5Hz), and two geminal olefinic hydrogens (δ4.94, brs; δ4.97, brs).



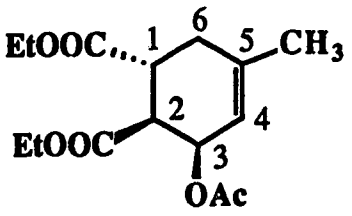
107

Methyl 4-bromocrotonate (**108**) was then investigated as the dienophile. However, heating **108** and acetate **55** in benzene in the presence of silver trifluoroacetate for 3 days provided only the diene **107** and recovered **108**. Combining **107** and **108** in refluxing xylene did not bring about the Diels-Alder reaction. Reaction of **107** and **108** in benzene in the presence of boron triflouride etherate (BF_3·OEt_2)[53] also failed to produce the desired product.

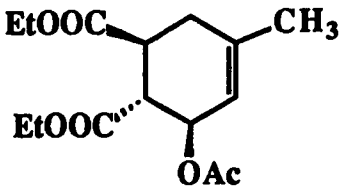
108

109

At this point, we felt that the lack of success of the Diels-Alder reaction of acetate **55** with **104** and **108** might be due to the low reactivity of the dienophiles. Diethyl fumarate (**109**) is a dienophile with two electron-withdrawing groups and should be reactive enough to give the Diels-Alder product. This is the case. When a mixture of **109** and acetate **55** was refluxed in benzene in the presence of silver trifluoroacetate, adducts **110** and **111** were obtained in a ratio of 1:1.



110



111

The molecular formulas of **110** and **111** were $C_{15}H_{22}O_6$ as determined by hrms. The assignment of ^{1}Hnmr data for both compounds was achieved by decoupling and nOe experiments. The signals for H-3 and H-4 of compound **110** appear at δ5.56 (brs) and δ5.33 (brs), while for compound **111**, they are at δ5.56 (brs) and δ5.65(brs). The signal for H-2 in compound **110** is at δ3.00 (dd,

J=4,12Hz), in compoud **111** is at δ2.89 (dd,J=9,11Hz). A 11% nOe between H-2 and H-3 of **110** indicates that this two hydrogens are *cis* to each other. The *cis* relation between H-1 and H-3 of **111** is also obtained from the nOe experiments (Table II-1).

Table II-1 The ^{1}Hnmr nOe data for **110** and **111** ($CDCl_3$, 360MHz)

110		**111**	
Signal saturated	Observed nOe	Signal saturated	Observed nOe
5-CH_3 1.74	H-4 5.33 1%	5-CH_3, 1.74	H-4, 5.65 2%
H-3 5.56	H-4 5.33 1.6%	H-4 5.65	5-CH_3 1.74 8%
	H-2 3.00 6.4%	H-3 5.56	H-4 5.65 1.6%
H-2 3.00	H-3 5.56 11%		H-1 3.10 3.2%

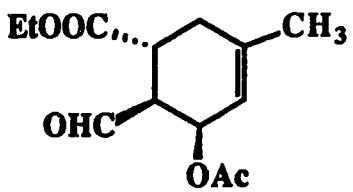
Although dienophile **109** is reactive enough to give **110** and **111**, the reaction is not suitable for our purposes since this reaction produces two products in equal amount. Another reason is that reduction of the three esters in **110** and **111** would produce triols and selective protection of two hydroxyl groups of the three would be troublesome. Thus, two different electron-withdrawing groups on the dienophile are desired. Ethyl and methyl 4-oxobutenoate (**112**) and (**113**) were chosen. Compouds **112** and **113** were prepared from ethyl and methyl crotonate. Refluxing the crotonates with selenium dioxide (SeO_2) in dioxane provided **112** and **113** in 7% (yield is not optimized) and 27% yield.[54,55] The combination of

selenium dioxide and *tert*-butyl hydroperoxide (TBHP)[56] did not lead to the desired product.

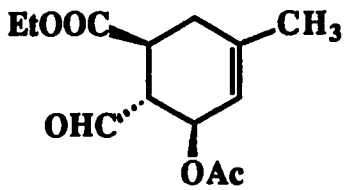
112 R=CH_2CH_3
113 R=CH_3

The ^{1}Hnmr spectrum of **112** shows the signals for an aldehyde hydrogen (δ9.64, d, J=8Hz), two olefinic hydrogens (δ6.83, dd, J=8,16Hz; δ6.65, d, J=16Hz), and an ethoxy group (δ4.17, q, J=6Hz; δ1.22, t, J=6Hz). The ^{1}Hnmr data of **113** is consistent with the structure, displaying signals for aldehyde hydrogen (δ9.74, d, J=7Hz), two olefinic hydrogens (δ6.92, dd, J=7,16Hz; δ6.73, d, J=16Hz), and a methoxy (δ3.74, s). With both dienophiles **112** and **113** in hand, we were ready to carry out the Diels-Alder reactions.

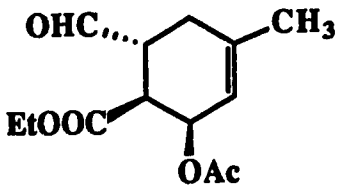
Acetate **55** was refluxed with **112** in benzene in the presence of silver trifluoroacetate for 3 days. Compounds **114**, **115**, **116**, and **117** were isolated from the reaction mixture in 63% yield in a ratio of 5:2:1:1 which was obtained by integration of the aldehyde signal in the ^{1}Hnmr spectrum. An attempt to change the ratio to favor **114** by using Lewis acid (BF_3·OEt_2) catalysis was unsuccessful. That fact that acyloxydienes polymerize too quickly to be useful in the presence of Lewis acids in the Diels-Alder reaction is known.[57]



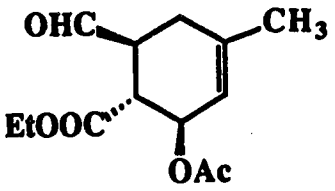
114



115



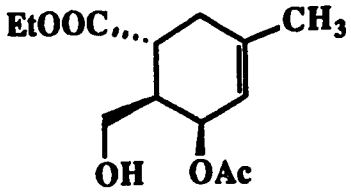
116



117

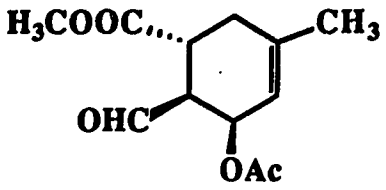
The hrms of mixture **114, 115, 116,** and **117** suggests the molecular formula $C_{13}H_{18}O_5$ (MW 254). The ^{1}Hnmr spectrum is complex due to the four isomers. However, major isomer **114** shows the signals for aldehyde hydrogen (δ9.65, s), an olefinic hydrogen (δ5.59, brs), a hydrogen geminal to acetoxy (δ5.43, brs), ethoxy (δ4.15, q, J=7Hz; δ1.23, t, J=7Hz), a hydrogen geminal to the ester (δ3.07, m), a hydrogen geminal to the aldehyde (δ2.98, m), two geminal hydrogens (2H, m), and two methyls (δ2.02, s; δ1.74, s).

Reduction of the mixture of **114, 115, 116,** and **117** with sodium borohydride in methanol ($NaBH_4$, MeOH, 3 hours) provided, in 84% yield, a mixture with **118** as the major product. The ir spectrum of **118** shows the presence of hydroxyl (3470 cm^{-1}) and ester (1735 cm^{-1}). The molecular formula $C_{13}H_{20}O_5$ was determined by hrms. In the ^{1}Hnmr spectrum of **118,** the aldehyde hydrogen signal has disappeared and two hydrogen signals at δ4.42 (dd, J=9,11Hz) and δ4.06 (dd, J=5,11Hz) are assigned to the hydrogens geminal to the hydroxyl.

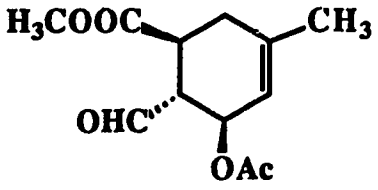


118

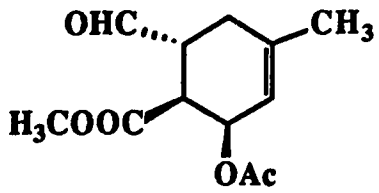
Similarly, Diels-Alder reaction of the methyl ester **113** with acetate **55** under the same conditions ($AgOCOCF_3$, benzene, refluxing, 3 days) provided **119**, **120**, **121**, and **122** in 76% yield in a ratio of 5:2:1:1.



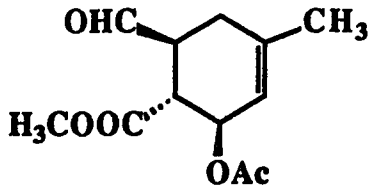
119



120

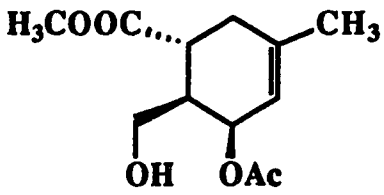


121



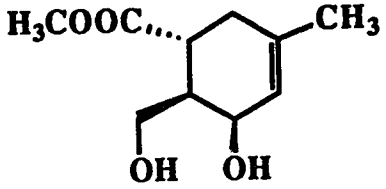
122

Reduction of the mixture of **119-122** ($NaBH_4$, MeOH, 3 hours) furnished a mixture (85% yield) containing **123** as the major compound. The ir spectrum of **123** shows the presence of hydroxyl (3480 cm^{-1}) and ester (1735 cm^{-1}). The hrms of **123** suggests the molecular formula is $C_{12}H_{18}O_5$. Two signals at δ4.37~4.04 in the ^{1}Hnmr spectrum of **123** indicates the methylene group geminal to the hydroxyl.



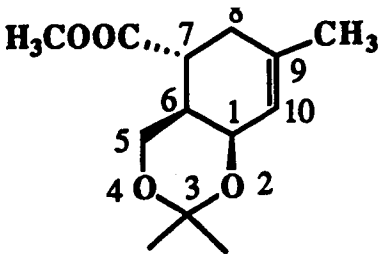
123

Hydrolysis of compounds **118** and **123** with 1M KOH/MeOH provided diol **124** as the major component, along with a small amount of isomers, in 61% and 44% yield, respectively. The hrms of **124** displays the molecular ion peak at m/z 200.1048 which is consistent with a molecular formula $C_{10}H_{16}O_4$. The hydrogen at C-3 appears at δ4.34 (brs) in the ^{1}Hnmr spectrum of **124**. A broad signal, integrating for two hydrogens, at δ2.72 is assigned to the hydroxyl hydrogens. The primary hydrogens geminal to hydroxyl are at δ3.80.

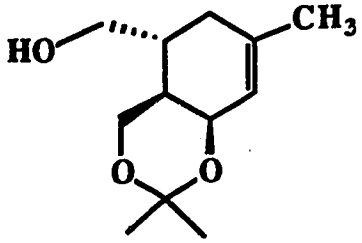


124

Treatment of crude diol **124** with dimethoxypropane in the presence of trifluoroacetic acid (CF_3COOH, CH_2Cl_2, rt, 17 hours) furnished pure acetonide **125** in 77% yield after chromatography. The molecular formula of **125** is $C_{13}H_{24}O_4$ (MW 240) as determined by hrms. The ir spectrum shows the presence of an ester (1735 cm^{-1}) and no hydroxyl absorption. The ^{1}Hnmr spectrum of **125** displays all the hydrogen signals: δ5.52 (1H, brs, H-10), 4.38 (1H, brs, H-1), 4.14 (1H, dd, J=4,12Hz, H-5), 3.72 (3H, s, OMe), 3.70 (1H, dd, J=3,12Hz, H-5), 3.20 (1H, m, H-7), 2.32 (1H, dd, J=6, 17Hz, H-8), 2.17 (1H, brd, J=17Hz, H-8), 1.76 (3H, s, 9-CH_3), 1.74(1H, m, H-6), and two aliphatic methyls at δ1.51 (3H, s) and δ1.41 (3H, s). The relative stereochemistry of **125** is determined by nOe experiments. On irradiation of the signal of H-1 (δ4.38), both olefinic hydrogen (δ5.52) and H-6 (δ1.74) have 10% nOe, respectively. When H-7 (δ3.20) is irradiated, the hydrogen at δ2.32 (Hβ-8) has 3.4% nOe, no nOe was observed to H-6. These results confirmed the assigned relative configuration of **125**.



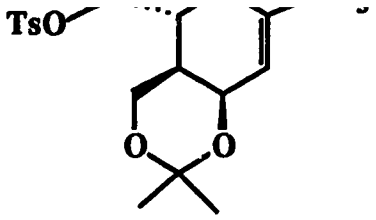
125



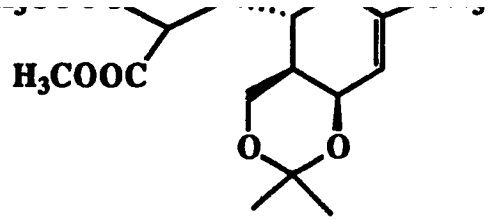
126

Reduction of ester **125** to alcohol **126** was readily achieved in 98% yield by refluxing **125** with lithium aluminum hydride ($LiAlH_4$) in THF for 2 hours. The ir spectrum of **126** shows the presence of hydroxyl (3446 cm^{-1}). The molecular formula is $C_{12}H_{20}O_3$ as determined by hrms. The signals at δ3.73 (2H, m) and δ1.66 (1H, brs, OH) in the ^{1}Hnmr spectrum of **126** indicate the reduction of ester to alcohol. The ^{13}Cnmr spectrum displays all twelve carbons and an additional oxygenated carbon signal at δ64.33ppm also supports structure **126**.

Alcohol **126** was converted in 83% yield to tosylate **127** (*p*-TsCl, Et_3N, DMAP, CH_2Cl_2). The ir spectrum of **127** shows absorption at 1360 and 1177 cm^{-1}, typical of sulfonate groups.[58] A molecular ion at m/z 366.1502 which corresponds to a molecular formula $C_{19}H_{26}O_5S$ was observed in the hrms of **127**. The aromatic hydrogens appear at δ7.79 (2H, d, J=8Hz) and δ7.36 (2H, d, J=8Hz) and the aromatic methyl appears at δ2.48 (s) in the ^{1}Hnmr spectrum. The methylene hydrogens geminal to the tosyloxy group are shifted downfield to δ4.23 (dd, J=4,10Hz) and δ4.07 (dd, J=2,10Hz).



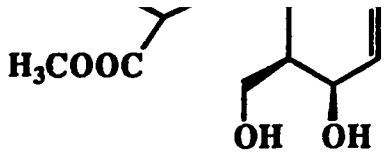
127



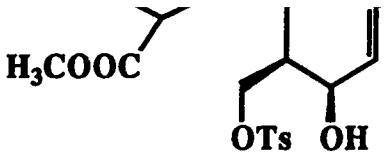
128

With tosylate **127** in hand, we were ready to study the alkylation with malonate anion. To our delight, the desired product **128** was obtained in 99% yield when tosylate **127** was refluxed with the anion of dimethyl malonate ($CH_2(COOCH_3)_2$, NaH, THF) in THF for 24 hours. This reaction not only gives a high yield of the desired product but also eliminates the use of HMPA which is highly toxic and difficult to remove. The ir spectrum of **128** shows the presence of esters (1753, 1736 cm^{-1}). A molecular ion at m/z 326.1733 suggests the molecular formula of **128** is $C_{17}H_{26}O_6$. The signals at δ3.59 (1H, dd, J=5,7Hz) and δ3.78 (6H, s) indicate the diester group. The ^{13}Cnmr signals at δ169.53 (s), δ49.71 (d), and δ52.51 (q) also support structure **128**.

With the desired ester **128** in hand, we were concerned about the formation of the *trans*-fused 5-, 6-membered bicyclic compound. Thus, we carried on to the synthesis of the bicyclic compound. Deprotection of acetonide **128** under mild acidic condition (80% HOAc, CH_2Cl_2) afforded diol **129** in 69% yield. The ir spectrum of **129** shows strong hydroxyl (3480, 3470 cm^{-1}) and carbonyl (1734 cm^{-1}) absorptions. The ^{1}Hnmr spectrum displays two hydroxyl hydrogens at δ3.01 and δ2.40 as broad singlets. The ^{13}Cnmr signals at δ69.05 and δ63.09 indicate the carbinol carbons. The cims shows a peak at m/z 304 ($M+NH_4^+$, 100%) which supports the proposed molecular formula $C_{14}H_{22}O_6$.



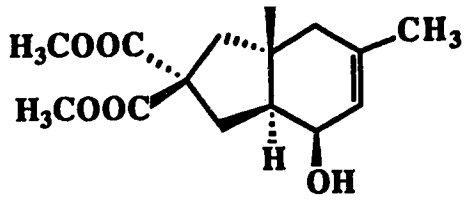
129



130

Selective tosylation (*p*-TsCl, Et3N, CH_2Cl_2) of the primary hydroxyl group of diol **129** provided compound **130** in 78% yield. This compound shows hydroxyl absorption at 3480 cm^{-1} and ester absorption at 1750 and 1734 cm^{-1} in the ir spectrum. Its ^{1}Hnmr spectrum displays the signals for aromatic hydrogens (δ7.82, d, J=8Hz; δ7.38, d, J=8Hz) and an aromatic methyl (δ2.48, s). The signals for the hydrogens of the methylene bearing the tosylate appear at δ4.22 (d, J=7Hz) and δ3.77 (1H). The olefinic hydrogen and the hydrogen geminal to the hydroxyl group are noted at δ5.56 (brs) and δ4.28 (brs).

The conversion of tosylate **130** to bicyclic compound **131** was easily achieved in 79% yield by treatment of **130** with NaH in THF (rt, 14 hours). The ir spectrum of **131** shows the presence of hydroxyl (3460 cm^{-1}) and ester (1733 cm^{-1}). The hrms displays a molecular ion at m/z 268.1307 corresponding to a molecular formula $C_{14}H_{20}O_5$. The signal at δ3.76 (6H, s) in the ^{1}Hnmr spectrum of **131** suggests the dimethyl esters. The broad singlet at δ1.16 for the hydroxyl hydrogen is consistent with the observation of hydroxyl absorption in the ir spectrum. The olefinic hydrogen and the hydrogen geminal to the hydroxyl are shifted to δ5.69 and δ4.14ppm. The ^{13}Cnmr spectrum shows all the carbons. The signals for methyl ester carbons appear at δ169.35 and δ52.69ppm. The two olefinic carbons are at δ139.74 and δ123.86ppm.



131

It is worth mentioning that the yields for the last three steps were not optimized. In summary, we have developed an efficient approach to bicyclic compound **131.** Compound **131** contains the AB ring of our target molecule. Using this approach the two unsatisfactory steps of the initial approach are avoided and the road is paved for the preparation of sterpuric acid, utilizing the procedures developed early in our laboratory.[16,17]

EXPERIMENTAL

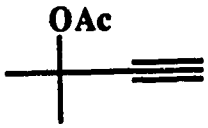
General

For a detailed description, see chapter I, Experimental Section. Most reactions were carried out under a positive pressure of argon or nitrogen gas. Reactions which required anhydrous conditions were performed in oven-dried glassware which was assembled and allowed to cool while being purged with an inert gas. All reactions were monitered by analytical thin-layer chromatography (TLC). All compounds reported gave a single spot on TLC and were judged to be >95% pure on the basis of ^{1}Hnmr spectroscopy. Names in brackets after some compounds are named in accordance with IUPAC standards. Numbering used in text and experimental corresponds to first name given.

Materials

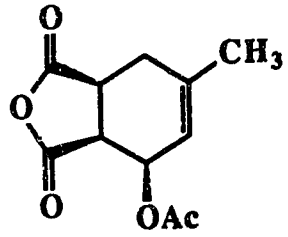
The anhydrous solvents used for reactions were purified by distillation from appropriate drying agents. Tetrahydrofuran (THF) was distilled from potassium-sodium or from lithium aluminum hydride ($LiAlH_4$) and diethyl ether was obtained by distillation from sodium. Benzene, xylene, triethylamine (Et_3N), pyridine, dimethyl sulfoxide (DMSO), and hexamethylphosphoramide (HMPA) were distilled from calcium hydride. Acetone was distilled from $CaSO_4$ and methylene chloride was obtained by distillation from P_2O_5. Silver trifluoroacetate was freshly prepared following a reported procedure.[59] Dimethyl malonate, diethyl malonate, methyl propionate, propionitrile, crotonaldehyde, methyl 4-bromocrotonate, ethyl crotonate, methyl crotonate, ethyl methyl ketone, and boron trifluoride etherate ($BF_3{\cdot}OEt_2$) were all distilled prior to use.

3-Acetoxy-3-methyl-1-butyne (55)



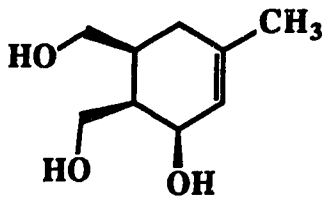
A mixture of 2-methyl-3-butyn-2-ol (54, 100ml, 1.03mol), acetic anhydride (118ml, 1.25mol), triethylamine (175ml, 1.25mol), and 4-N,N-dimethylaminopyridine (DMAP, 0.5g) was refluxed for 9 hours. The resulting solution was diluted with methylene chloride (200ml) and ice-water (200ml), washed successively with 5% hydrochloric acid (HCl), saturated $NaHCO_3$ solution, and brine, dried, and evaporated. Distillation of the residue afforded 55 (128.11g, 98%) as a colourless liquid; bp. 124-126^{0}C. Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 169.13 (s, CO), 84.66 (s, C-3), 72.12 (d, C-1), 71.46 (s, C-2), 28.72 (q, C-4 and 3-$\underline{C}H_3$), 21.72 (CO$\underline{C}H_3$).

(1S*,2S*,6R*)-6-Acetoxy-4-methyl-4-cyclohexene-1,2-dicarboxylic anhydride (56)



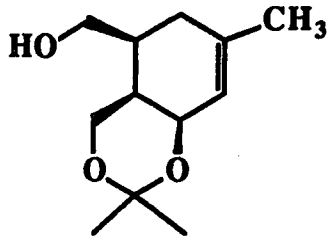
A solution of acetate **55** (40g, 0.32mol), maleic anhydride (31.1g, 0.32mol), and silver trifluoroacetate (7g, 0.032mol) in dry benzene (750ml) was refluxed for 3 days. The solvent was removed and then diethyl ether (350ml) was added to precipitate the salts. The mixture was filtered and the solvent was removed to provide crude **56** (66.87g, 94%) as an oil. Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 173.13 (s, CO), 169.75 (s, CO), 169.39 (s, CO), 140.17 (s, C-4), 120.06 (d, C-5), 65.42 (d, C-6), 43.28 (d, C-1), 37.79 (d, C-2), 26.48 (t, C-3), 23.32 (q, 4-CH_3), 20.64 (q, CO$\underline{C}H_3$).

(1R*,2R*,3S*)-2,3-Bis(hydroxymethyl)-5-methyl-5-cyclohexen-1-ol (57)



A solution of crude **56** (66.87g) in THF (300ml) was added dropwise to a suspension of $LiAlH_4$ (25g) in THF (300ml) at 0^0C. This mixture was refluxed for 10 hours and then ice-water (200ml) was slowly added. The precipitate was filtered through a pad of celite and washed with acetone (300ml). The solvent was evaporated at the reduced pressure to provide **57** (46.26g, 90%). For ir, ^{1}Hnmr, and hrms data see ref.16.

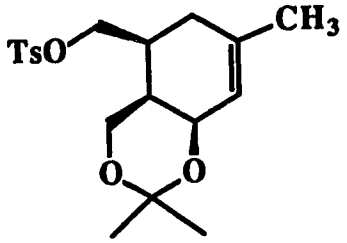
(1R*,6R*,7S*)-7-Hydroxymethyl-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (58) { (4aR*, 5S*, 8aR*)-4a,5,6,8a-tetrahydro-5-hydroxymethyl-2,2,7-trimethyl-1,3-benzodioxan (58) }



A solution of triol **57** (30g), dimethoxypropane (100ml), acetone (50ml), trifluoroacetic acid (5ml), and acetic acid (1ml) in methylene chloride (190ml) was stirred at rt for 23 hours.The resulting mixture was washed with saturated $NaHCO_3$ and brine, dried, and concentrated. The residue was separated by silica gel chromatography (SKB/ethyl acetate 50:50) to give **59** (14.75g, 39%). Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 139.25 (s, C-9), 119.32 (d, C-10),

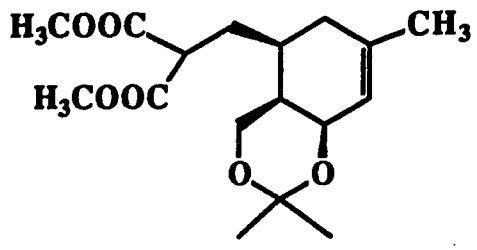
98.57 (s, C-3), 64.54 (C-1 and C-12), 63.36 (t, C-5), 38.37 (d, C-6), 34.38 (t, C-8), 33.87 (d, C-7), 29.55 (q, 3-CH_3), 23.66 (q, 3-CH_3), 18.98 (q, 9-CH_3).

(1R*,6R*,7S*)-3,3,9-Trimethyl-7-*p*-tolylsulfonyloxymethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (60) { (4aR*, 5S*, 8aR*)-4a,5,6,8a-tetrahydro-2,2,7-trimethyl-5-*p*-tolylsulfonyloxymethyl-1,3-benzodioxan (60) }

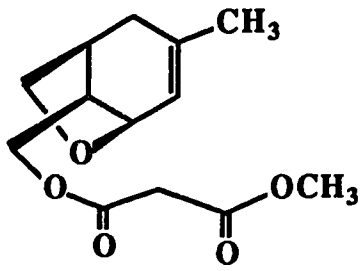


p-Toluenesulfonyl chloride (3.3g, 17.4mmol) was added in small portions to a solution of alcohol **58** (3.20g, 15.4mmol), triethylamine (6.4ml, 46.2mmol), and DMAP (0.2g) in methylene chloride (120ml) at 0^0C. The mixture was stirred at rt for 34 hours and then poured onto ice-water (100ml). The organic layer was separated, washed with saturated NaHCO3 and brine, dried, and concentrated. The crude product was purified by flash chromatography (SKB/ ethyl acetate 70:30) to give **60** (4.1g, 74%) as a viscous oil. Ir, ^{1}Hnmr, and cims data: see ref.16; ^{13}Cnmr (90MHz): δ 144.32 (Ar-C), 136.91 (s, C-9), 133.16 (Ar-C), 129.57 (Ar-C), 127.69 (Ar-C), 120.32 (d, C-10), 97.71 (s, C-3), 71.26 (t, C-12), 63.41 (d, C-1), 62.87 (t, C-5), 36.15 (d, C-6), 33.62 (t, C-8), 31.70 (d, C-7), 29.01 (q, 3-CH_3), 23.46 (q, 3-CH_3), 21.46 (q, ArCH_3), 18.82 (q, 9-CH_3).

(1R*,6R*,7S*)-7-[2,2-Bis(methoxycarbonyl)ethyl]-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (61) and by-product 62 { (4aR*, 5S*, 8aR*)-4a,5,6,8a-tetrahydro-5-[2,2-bis(methoxycarbonyl)ethyl]-2,2,7-trimethyl-1,3-benzodioxan (61) }



61

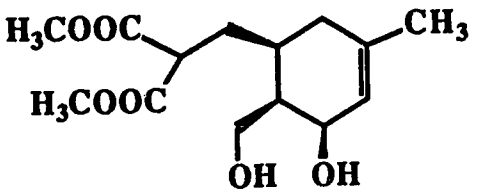


62

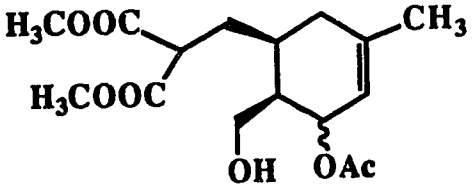
Sodium hydride (3.169g, 60% in mineral oil, 79 mmol) was placed in a dry three-necked flask, washed twice with SKB, then suspended in dry HMPA (100ml). Dimethyl malonate (11.9ml, 95mmol) was added dropwise to the suspension at 0^0C and the mixture was stirred at rt for 2 hours. A solution of tosylate **60** (6g, 16.4mmol) in dry HMPA (50ml) was added. The resulting mixture was warmed to 55~60^0C (oil bath) with stirring for 7 days. The cooled mixture (ice bath) was neutralized with acetic acid and then partitioned between ice-water (100ml) and ether (100ml). The aqueous layer was extracted with ether (7×200ml) and the combined organic extracts were washed with brine, dried, and concentrated. The residue was separated by repeated silica gel chromatography to afford **61** (3g, 56%) and **62** (234mg). **61**: Ir, ^{1}Hnmr, hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 169.86 (s, CO), 169.69 (s, CO), 135.13 (s, C-9), 123.18 (d, C-10), 97.89 (s, C-3), 67.46 (d, C-1), 59.60 (t, C-5), 51.62 (q, OCH_3), 49.96 (d, C-13), 34.23 (t, C-8), 33.79 (d, C-6), 33.44 (d, C-7), 30.52 (t, C-12), 26.19 (q, 3-CH_3), 23.49 (q, 9-CH_3). **62**: Ir υ_{max}: 2954, 1754, 1737, 1437, 1334, 1275, 1226, 1197, 1151, 1021 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.74 (1H, brd, 5), 4.19 (1H, d, 5), 4.12 (1H, dd, 7,11), 4.08 (1H, dd, 8,11), 4.01 (1H, m), 3.79 (3H, s, OCH_3), 3.65 (1H, d, 8), 3.45 (2H, s), 2.46 (2H, m), 2.34 (1H, dd, 7,17), 2.10 (1H, d, 17), 1.73 (3H, s); ^{13}Cnmr (90MHz): δ 166.75 (s, CO), 166.29 (s, CO), 137.32, 124.52, 72.42, 71.05, 65.36, 52.29, 45.34, 41.20, 40.92, 35.76, 22.00; Hrms: m/z 171.0659

($C_8H_{11}O_4$, 10.41%), 136 (84), 106 (100); Cims: m/z 272 ($M+NH_4^+$, 39%), 255 ($M+H^+$, 29), 137 (100).

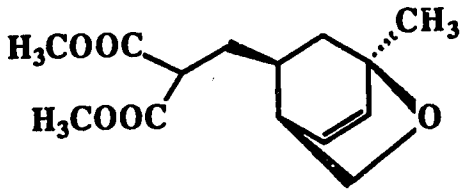
(1R*,2R*,3S*)-2-Hydroxymethyl-3-[2,2-bis(methoxycarbonyl)ethyl]-5-methyl-5-cyclohexen-1-ol (66) and (2R*, 3S*)-1-acetoxy-2-hydroxymethyl-3-[2,2-bis(methoxycarbonyl)ethyl]-5-methyl-5-cyclohexene (67) and by-product 68



66



67

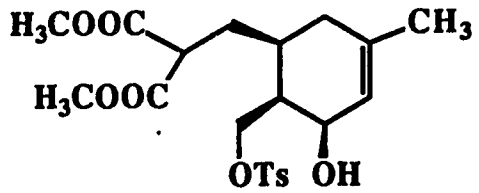


68

A solution of acetonide **61** (167mg) in methylene chloride (15ml) and 80% aqueous acetic acid (10ml) was stirred at rt for 9 hours. The solvent was removed in the rotavap first at water aspirator pressure then using the vacuum pump. The residue was separated by flash chromatography (methylene chloride/acetone 70:30) to give **66** (128mg, 87%). In another reaction, **66** (541mg), **67** (514mg), and **68** (98mg) were obtained from **61** (2g). **66**: Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 169.81 (s, CO), 169.75 (s, CO), 135.38 (s, C-5), 124.24 (d, C-6), 71.22 (d, C-1), 59.76 (t, C-8), 52.68 (q, OCH_3), 52.64 (q, OCH_3), 49.74 (d, C-10), 42.76 (d, C-2), 33.72 (t, C-4), 32.84 (t, C-9), 31.48 (d, C-3), 22.86 (q, 5-CH_3). **67**: Ir υ_{max}: 3533, 2954, 1734, 1437, 1373, 1331, 1243, 1155, 1050

cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 5.46 (1H, brs, H-6), 5.40 (1H, brs, H-1), 3.80 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.75 (1H, dd, 5,14, H-8), 3.60 (1H, dd, 7,14, H-8), 3.50 (1H, t, 5, H-10), 2.68 (1H, brs, OH), 2.20 (3H, m, H-4 and H-2), 2.18 (3H, s, OAc), 1.94 (3H, m, H-9 and H-3), 1.80 (3H, s, 5-CH_3); ^{13}Cnmr (90MHz, major isomer): δ 170.62 (s, CO), 169.63 (s, CO), 169.22 (s, CO), 137.72 (s, C-5), 119.68 (d, C-6), 69.89 (d, C-1), 59.77 (t, C-8), 52.27 (q, OCH_3), 52.22 (q, OCH_3), 50.00 (d, C-10), 42.01 (d, C-2), 38.96 (t, C-4), 31.41 (t, C-9), 29.47 (d, C-3), 23.06 (q, 5-CH_3), 20.89 (q, $CO\underline{C}H_3$); Hrms: m/z 285.1342 (M-C_2H_3O, calcd for $C_{14}H_{21}O_5$, 285.1338, 3.48%), 268 (6), 252 (18), 232 (18), 187 (28), 178 (24), 136 (100); Cims: m/z 346.179 ($M+NH_4^+$, 64%), 286 (23), 251 (100). **68**: Ir υ_{max}: 2969, 2869, 1754, 1734, 1436, 1377, 1350, 1338, 1319, 1286, 1262, 1226, 1208 cm^{-1}; ^{1}Hnmr (360MHz): δ 6.46 (1H, dd, 7,7), 6.11 (1H, d, 7), 3.98 (1H, d, 8), 3.68 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 3.35 (1H, t, 7), 3.14 (1H, d, 8), 2.41 (1H, d, 7), 2.16 (1H, dd, 7,7), 1.70 (1H, d, 11), 1.60 (1H, m), 1.39 (3H, s, CH_3); ^{13}Cnmr (90MHz): δ 169.51 (s, CO), 136.30 (d), 134.66 (d), 69.98 (s), 61.69 (d), 52.30 (q, OCH_3), 50.20, 40.39, 35.07, 33.03, 31.84, 24.00; Hrms: m/z 268.1308 (calcd for $C_{14}H_{20}O_5$, 268.1311, 2%), 187 (3), 96 (100).

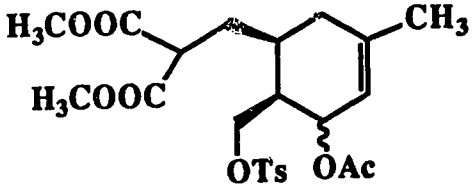
(1R*,2R*,3S*)-3-[2,2-Bis(methoxycarbonyl)ethyl]-5-methyl-2-*p*-tolylsulfonyloxymethyl-5-cyclohexen-1-ol (70)



p-Toluenesulfonyl chloride (269mg, 1.42mmol) was added to a solution of diol **66** (187mg, 0.65mmol), triethylamine (0.45ml, 3.27mmol), and a catalytic amount of DMAP in methylene chloride (40ml) at 0^0C. The mixture was stirred at rt

for 24 hours and then ice-water (20ml) was added. The aqueous layer was extracted with methylene chloride (2×20ml) and the combined organic extracts were washed with saturated $NaHCO_3$ solution and brine, dried, and concentrated. The crude was separated by flash chromatography (methylene chloride/acetone 95:5) to give **70** (226mg, 78%). Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 169.77 (s, CO), 169.47 (s, CO), 144.81 (Ar-C), 135.66 (s, C-5), 132.95 (Ar-C), 129.87 (Ar-C), 127.99 (Ar-C), 123.49 (d, C-6), 68.16 (t, C-8), 66.87 (d, C-1), 52.58 (q, OCH_3), 49.95 (d, C-10), 41.44 (d, C-2), 33.64 (t, C-4), 32.06 (t, C-9), 30.37 (d, C-3), 23.11 (q, $ArCH_3$), 21.64 (q, 5-CH_3).

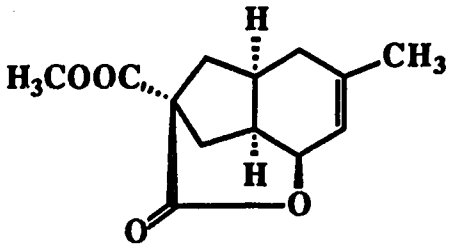
(2R*,3S*)-1-Acetoxy-3-[2,2-bis(methoxycarbonyl)ethyl]-5-methyl-2-*p*-tolylsulfonyloxymethyl-5-cyclohexene (71)



p-Toluenesulfonyl chloride (133mg, 0.7mmol) was added to a solution of alcohol **67** (140mg, 0.43mmol), triethylamine (0.31ml, 2.13mmol), and a catalytic amount of DMAP in methylene chloride (20ml) at 0^0C. The mixture was stirred at rt for 43 hours. Ice-water (20ml) was added and the aqueous layer was separated and extracted with methylene chloride (2×20ml). The combined organic extracts were washed successively with saturated $NaHCO_3$ and brine, dried, and concentrated. Separation of the residue by flash chromatography (SKB/ethyl acetate 70:30) provided **71** (173mg, 84%). Ir υ_{max}: 1734, 1436, 1363, 1236, 1189, 1177, 1155, 1019, 969 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 7.83 (2H, d, 8, ArH), 7.40 (2H, d, 8, ArH), 5.42 (1H, brs, H-6), 5.36 (1H, brs, H-1), 4.31 (1H, dd, 6,10, H-8), 4.14 (1H, dd, 6,10, H-8), 3.87 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.52 (1H, t, 6.5, H-10), 2.60 (3H, s, $ArCH_3$), 2.50 (1H, m, H-4), 2.13 (3H, s, OAc),

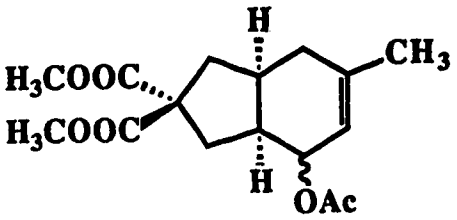
2.20~2.00 (4H, m, H-4, H-2, and H-9), 1.90 (1H, m, H-3), 1.82 (3H, s, 5-CH_3); Hrms: m/z 251.1281 (M-$C_7H_9O_4S$-C_2H_2O, 21%), 232 (11), 172 (53), 91 (100).

(1S*,2S*,6R*,8S*)-8-Methoxycarbonyl-4-methylbicyclo[4.3.0]non-3-en-8,2-carbolactone (72) { (2S*,3aS*,4S* 7aR*)-3a,4,7,7a-tetrahydro-2-methoxycarbonyl-6-methylindan-2,4-carbolactone (72) }



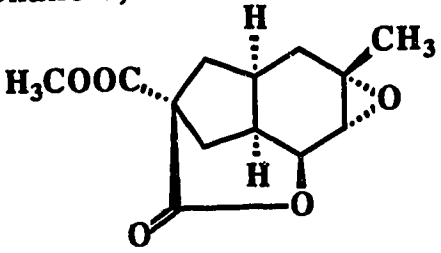
Sodium hydride (113mg, 60% in mineral oil, 2.8mmol) was added to a solution of tosylate **70** (230mg, 0.52mmol) in dry THF (30ml). The mixture was stirred at rt for 7 hours and then acetic acid (4 drops) was added at 0^0C. The mixture was filtered and the precipitate was washed with diethyl ether. Evaparation of the solvent and separation of the crude by flash chromatography (methylene chloride/acetone 98:2) gave **72** (121mg, 70%) as white prisms. Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 171.47 (s, CO), 170.99 (s, CO), 138.99 (s, C-4), 118.61 (d, C-3), 75.00 (d, C-2), 56.85 (s, C-8), 52.70 (q, OCH_3), 36.46, 36.00, 35.37, 33.48, 31.41, 23.79(q, 4-CH_3).

(1S*,6R*)-8,8-Bis(methoxycarbonyl)-4-methylbicyclo[4.3.0]non-3-en-2-acetate (73) { Dimethyl (3aS*,7aR*)-4-acetoxy-3a,4,7,7a-tetrahydro-6-methyl-2-indandicarboxylate (73) }



Sodium hydride (62.4mg, 60% in mineral oil, 1.56mmol) was added to a solution of tosylate **71** (173mg, 0.36mmol) in dry THF (30ml). The mixture was stirred at rt for 2.5 hours and then neutralized with acetic acid at 0^0C. Water (20ml) was added and the aqueous layer was extracted with diethyl ether (2×20ml). The combined organic extracts were washed successively with saturated $NaHCO_3$ and brine, dried, and concentrated. Purification of the crude product by flash chromatography (first with SKB, then methylene chloride/acetone 95:5) gave **73** (101mg, 92%). Ir υ_{max}: 2953, 2855, 1734, 1684, 1435, 1371, 1239, 1200, 1162, 1118, 1094, 1075, 1054, 1023 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 5.55 (1H, brs, H-3), 5.43 (1H, brs, H-2), 3.84 (6H, s, OCH_3), 2.70~2.14 (7H, m), 2.19 (3H, s, OAc), 1.95 (1H, dd, 7,18, H-5), 1.84 (3H, s, 4-CH_3); Hrms: m/z 279.1232 (M-OCH_3, calcd for $C_{15}H_{19}O_5$, 279.1232, 0.64%), 267 (3), 250 (8), 219 (10), 208 (15), 145 (100).

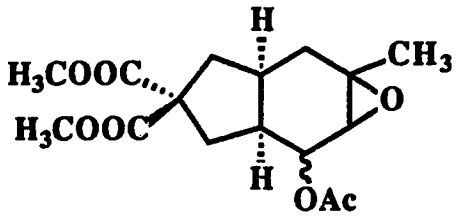
(1S*,2S*,3R*,4S*,6S*,8S*)-8-Methoxycarbonyl-4-methyl-3,4-epoxybicyclo[4.3.0]nonano-8,2-carbolactone (74)



m-Chloroperbenzoic acid (168mg, 80%, 0.78mmol) was added to a solution of methyl ester **72** (94mg, 0.4mmol) in methylene chloride (40ml) at 0^0C. The mixture was stirred at rt for 47 hours and then washed succesively with 5% $NaHSO_3$, saturated $NaHCO_3$, and brine, dried, and concentrated to give **74**

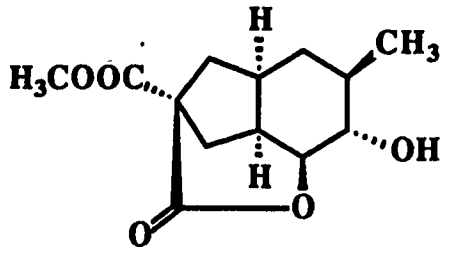
(100mg, 100%). Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 170.42 (s, CO), 170.23 (s, CO), 77.58 (d, C-2), 68.18 (s, C-4), 58.97 (d, C-3), 55.88 (s, C-8), 52.71 (q, OCH_3), 38.90, 35.55, 33.70, 30.80, 30.43, 28.83 (q, 4-CH_3).

Dimethyl (1S*,6S*)-2-acetoxy-3,4-epoxy-4-methylbicyclo[4.3.0]nonano-8,8-dicarboxylate (75)



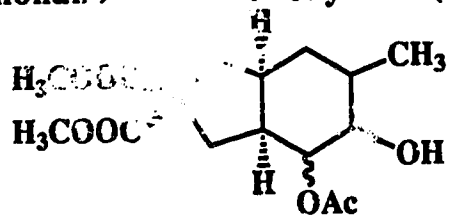
m-Chloroperbenzoic acid (160mg, 80%, 0.74mmol) was added to a solution of ester **73** (95mg, 0.31mmol) in methylene chloride (20ml) at 0^{0}C. The mixture was stirred at rt for 24 hours and then washed successively with 5% $NaHSO_3$ solution (20ml), saturated $NaHCO_3$ (20ml), and brine (20ml). The organic layer was dried and concentrated to give **75** (99mg, 99%). Ir υ_{max}: 2955, 1733, 1435, 1369, 1328, 1272, 1241, 1203, 1161, 1138 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 5.02 (1H, dd, 3,5, H-2), 3.83 (6H, s, OCH_3), 3.38 (1H, d, 3, H-3), 2.88 (1H, dd, 11,14, H-9), 2.70~1.70 (7H, m), 2.25 (3H, s, OAc), 1.48 (3H, s, 4-CH_3); Hrms: m/z 295.1183 (M-OCH_3, calcd for $C_{15}H_{19}O_6$, 295.1181, 11.25%), 284 (4), 268 (28), 240 (22), 226 (100), 166 (88).

(1S*,2S*,3S*,4R*,6R*,8S*)-3-Hydroxy-8-methoxycarbonyl-4-methylbicyclo[4.3.0]nonano-8,2-carbolactone (76)



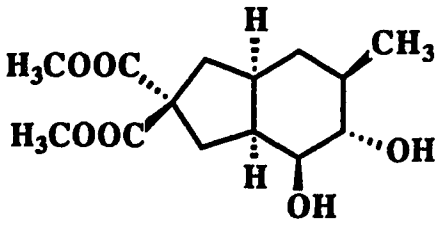
Epoxide 74 (104mg), 10% Pd-C (13mg), ethyl acetate (20ml), and acetic acid (1ml) was mixed in a three-necked flask. The mixture was hydrogenated for 2 days. Then, PtO_2 (12mg) was added and the reaction was continued for another 2 days. The catalysts were removed by filtration and the filtrate was evaporated to provide 76 (105mg, 99%). Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 170.31 (s, CO), 170.23 (s, CO), 87.04 (d, C-2), 76.57 (d, C-3), 57.20 (q, C-8), 52.61 (q, OCH_3), 40.39, 35.67, 34.73, 33.86, 33.57, 32.22, 19.33 (q, 4-CH_3).

Dimethyl (1S*,6R*-8-acetoxy-3-hydroxy-4-methylbicyclo[4.3.0]nonano-8,8-dicarboxylate (77)



Epoxide 75 (47mg, 0.14mmol), 10% Pd-C (16mg), ethyl acetate (20ml), and acetic acid (0.5ml) was mixed in a three-necked flask. The mixture was hydrogenated for 2 days. PtO_2 (21mg) was then added and the reaction was continued for another 2 days. The catalysts were filtered off and the filtrate was evaporated. Purification of the crude product by flash chromatography (SKB/ethyl acetate 50:50) gave 77 (41mg, 87%). Ir υ_{max}: 3500, 2954, 1734, 1458, 1436, 1371, 1332, 1295, 1277, 1268, 1178, 1161, 1151, 1101, 1074 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 4.46 (1H, d, 2, H-2), 3.76 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.52 (1H, d, 8, H-3), 2.64~0.80 (10H, m), 2.04 (3H, s, $OCOCH_3$), 1.04 (3H, d, 6, 4-CH_3); Hrms: m/z 297.1336 (M-CH_3, calcd for $C_{15}H_{21}O_6$, 297.1338, 4.97%), 268 (25), 208 (64), 145 (100).

Dimethyl (1S*,2S*,3S*,4R*,6R*)-2,3-dihydroxy-4-methylbicyclo[4.3.0]nonano-8,8-dicarboxylate (78)



Hydrolysis of **76:**

A 1M solution of KOH in methanol (0.5ml, 0.5mmol) was added to a solution of alcohol **76** (42mg, 0.165mmol) in methanol (10ml) at 0^0C. After 10 minutes, the ice-water bath was removed and the mixture was stirred at rt for 1 hour. The mixture was neutralized with acetic acid and the solvent evaporated. The residue was redissolved in methylene chloride. The precipitate was filtered off and the filtrate was concentrated. The crude product was purified by flash chromatography (SKB/ethyl acetate 5:95) to give **78** (34mg, 72%).

Hydrolysis of **77:**

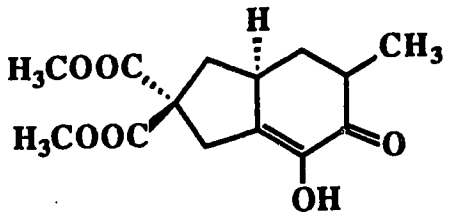
A 1M KOH/MeOH solution (1ml, 1mmol) was added to a solution of acetate **77** (40mg, 0.12mmol) at 0^0C. After 10 minutes, the ice-water bath was removed and the mixture was stirred at rt for 1 hour. The reaction mixture was neutralized with acetic acid and the solvent was evaporated. The residue was redissolved in ethyl acetate. The precipitate was filtered off and the filtrate was concentrated. Purification of the crude product by flash chromatography (SKB/ethyl acetate 20:80) provided **78** (28mg, 80%) as a major product. Ir, ^{1}Hnmr, hrms, and cims data: see ref.16; ^{13}Cnmr (90MHz): δ 173.37 (s, CO), 76.10 (d, C-2), 74.77 (d, C-3), 58.07 (s, C-8), 52.81 (q, OCH_3), 52.77 (q, OCH_3), 45.67, 39.49, 38.37, 36.88, 35.72, 33.15, 17.72 (q, 4-CH_3).

Swern oxidation of diol 78

Trifluoroacetic anhydride (50μl, 0.35mmol) was added to a stirred solution of dimethyl sulfoxide (29μl, 0.41mmol) in methylene chloride (3ml) at -78^0C. After 10

minutes, a solution of diol **78** (33.6mg, 0.12mmol) in methylene chloride (2ml) was added to the mixture. After 1.5 hours, the reaction mixture was treated with triethylamine (0.114ml, 0.82mmol), stirred for 1.5 hours, allowed to warm up to rt and then treated with water (5ml). The aqueous layer was separated and extracted with methylene chloride (2×10ml). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4. Evaporation of the solvent provided **79** and **80** (32mg, 96%) in a ratio of 9:2. For spectral data (ir, ^{1}Hnmr, hrms) for **79** and **80** see ref.16.

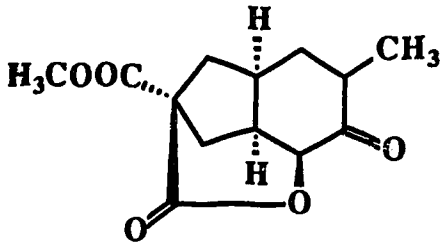
(4R*,6R*)-2-Hydroxy-8,8-bis(methoxycarbonyl)-4-methylbicyclo[4.3.0]non-1(12)-en-3-one (81) { Dimethyl (6R*,7aR*)-5,6,7,7a-tetrahydro-4-hydroxy-6-methyl-5-oxo-2-indandicarboxylate (81) }



Trifluoroacetic anhydride (25µl, 0.18mmol) was added to a stirred solution of dimethyl sulfoxide (15µl, 0.21mmol) in methylene chloride (2ml) at -78^{0}C. After 10 minutes, a solution of hydroxyketone **79** and **80** (15mg) in methylene chloride (2ml) was added to the reaction mixture. After 1.5 hours, the mixture was treated with triethylamine (57µl, 0.41mmol), stirred for another 1.5 hours, and then allowed to warm up to rt. Water (5ml) was added and the mixture was stirred for 5 minutes. The aqueous layer was separated and extracted with methylene chloride (2×5ml). The combined organic extracts were washed with brine, dried, and concentrated. Purification of the residue by flash chromatography (SKB/ethyl acetate 70:30) gave **81** (8mg, 54%). For ir, ^{1}Hnmr, and hrms data see ref.16; ^{13}Cnmr

(90MHz): δ 196.81 (s, C-3), 171.98 (s, CO), 171.50 (s, CO), 140.80 (s, C-2), 135.48 (s, C-1), 59.14 (s, C-8), 53.05 (q, OCH_3), 52.96 (q, OCH_3), 41.03, 39.96, 39.65, 38.15, 35.72, 15.37 (q, 4-CH_3).

(1S*,2S*,4R*,6R*,8S*)-8-Methoxycarbonyl-4-methyl-3-oxobicyclo[4.3.0]nonano-8,2-carbolactone (82)



PCC oxidation:

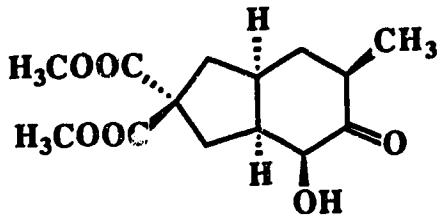
PCC (28.3mg, 0.13mmol) was added to a solution of alcohol **76** (10.7mg, 0.042mmol) in methylene chloride (10ml). The mixture was stirred at rt for 11 hours, diluted with diethyl ether, and filtered through a pad of celite. The filtrate was evaporated and the residue was purified by flash chromatography (SKB/ethyl acetate 100:40) to give **82** (8.1mg, 76%).

Swern oxidation:

Trifluoroacetic anhydride (300µl, 2.12mmol) was added to a solution of dimethyl sulfoxide (200µl, 2.82mmol) in methylene chloride (5ml) at -78^{0}C. After 10 minutes, alcohol **76** (174.7mg, 0.69mmol) in methylene chloride (14ml) was added and the mixture was stirred for 1 hour. Then, triethylamine (0.5ml, 3.6mmol) was added. After another 1 hour, the mixture was allowed to warm up to rt and water (15ml) was added. The aqueous layer was separated and extracted with methylene chloride (2×15ml). The combined organic extracts were washed and dried. Evaporation of the solvent provided crude **82** (173mg, 100%). Ir, ^{1}Hnmr, and hrms data see ref.16; ^{13}Cnmr (90MHz): δ 204.27 (s, C-3), 169.58 (s, CO),

169.01 (s, CO), 81.92 (d, C-2), 57.01 (s, C-8), 52.80 (q, OCH_3), 40.85, 40.55, 36.14, 35.23, 35.00, 31.14, 14.30 (q, 4-CH_3).

Dimethyl (1S*,2S*,4R*,6R*)-2-hydroxy-4-methyl-3-oxobicyclo[4.3.0]nonano-8,8-dicarboxylate (80)

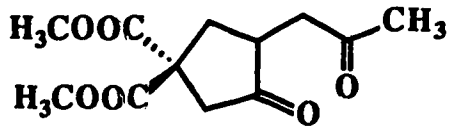


A 1M solution of KOH in methanol (1.5ml, 1.5mmol) was added to a solution of ketone **82** (173mg) in methanol (30ml) at 0^0C. The mixture was stirred at 0^0C for 10 minutes, at rt for 1 hour, and then neutralized with acetic acid. The solvent was evaporated and the residue was redissolved in methylene chloride. The precipitate was filtered off and the filtrate was evaporated to afford **80** (195mg, 100%). Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 212.79 (s, C-3), 172.95 (s, CO), 172.85 (s, CO), 74.57 (d, C-2), 57.82 (s, C-8), 52.89 (q, OCH_3), 49.77, 41.20, 39.01, 38.33, 37.48, 33.33, 13.56 (q, 4-CH_3).

Swern oxidation of 80 to 81

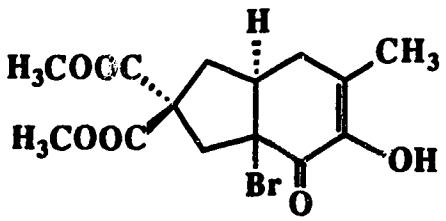
Trifluoroacetic anhydride (200μl, 1.42mmol) was added to a stirred solution of DMSO (1.28ml, 18mmol) in methylene chloride (5ml) at -78^0C. After 10 minutes, hydroxyketone **80** (128mg, 0.45mmol) was added and the mixture was stirred for 40 minutes. Triethylamine (0.5ml, 3.6mmol) was then added and the reaction was kept for another 40 minutes. Water (10ml) was added and the mixture was stirred for 5 minutes. The aqueous layer was separated and extracted with methylene chloride (3x10ml). The combined organic extracts were washed with brine, dried, and evaporated. The residue was purified by flash chromatography (SKB/ethyl acetate 70:30) to give **81** (94mg, 74%).

Oxidation of diol 78 and hydroxyketone 80 with PCC to 83



PCC (49mg, 0.23mmol) was added to a solution of diol **78** (6.5mg) in methylene chloride (5ml). The mixture was stirred at rt for 15 hours, diluted with diethyl ether, and filtered through a pad of celite. Evaporation of the filtrate and purification of the crude extract by passing through a short column of silica gel provided **83** (2.5mg). Under the same condition (PCC, CH_2Cl_2), hydroxyketone **80** (9mg) provided diketone **83** (4.2mg). Ir υ_{max}: 2955, 2924, 2871, 1733, 1457, 1436, 1274, 1258, 1202, 1162, 1143 cm^{-1}; ^{1}Hnmr (360MHz): δ 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.05~2.70 (6H, m), 2.19 (1H, dd, 10, 14), 2.18 (3H, s, $COCH_3$); ^{13}Cnmr (90MHz): δ 214.26 (s, C)), 205.56 (s, CO), 171.52 (s, CO), 170.96 (s, CO), 55.03 (s), 53.22 (q, OCH_3), 53.09 (q, OCH_3), 44.50, 43.05, 42.98, 35.55, 29.68 (q, CH_3); Hrms: m/z 256.0949 (calcd for $C_{12}H_{16}O_6$, 256.0947, 31%), 225 (22), 199 (26), 172 (33), 165 (29), 140 (100).

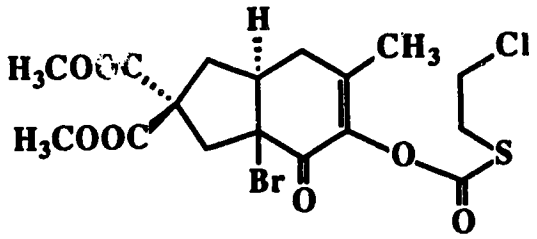
(6R*)-1-Bromo-3-hydroxy-8,8-bis(methoxycarbonyl)-4-methylbicyclo[4.3.0]non-3-en-2-one (84) { Dimethyl (7aR*)-3a-bromo-3a,4,7,7a-tetrahydro-5-hydroxy-6-methyl-4-oxo-2-indandicarboxylate (84) }



N-Bromosuccinimide (20.7mg, 0.116mmol) was added to a solution of diketone **81** (27mg, 0.096mmol) in dry THF (5ml) at -20^{0}C. The mixture was

stirred at the same temperature for 1 hour and then concentrated. The crude was purified by flash chromatography (methylene chloride/acetone 98:2) to give **84** (25.4mg, 73%) as a slightly yellow oil. Ir, ^{1}Hnmr, and hrms data: see ref.16; ^{13}Cnmr (90MHz): δ 186.62 (s, C-2), 171.79 (s, CO), 171.02 (s, CO), 140.42 (s, C-3), 127.74 (s, C-4), 59.79 (s, C-8), 56.71 (s, C-1), 53.26 (q, OCH_3), 53.19 (q, OCH_3), 46.61, 44.81, 36.31, 28.65, 17.39 (q, 4-CH_3).

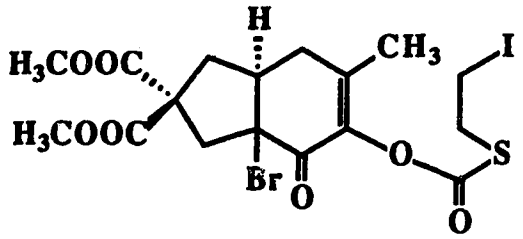
(6R*)-1-Bromo-8,8-bis(methoxycarbonyl)-4-methyl-2-oxo-[4.3.0]non-3-en-3-yl-(β-chloroethyl)thioformate (85) { Dimethyl (7aR*)-3a-bromo-5-[(β-chloroethyl)thioformate]-3a,4,7,7a-tetrahydro-6-methyl-4-oxo-2-indandicarboxylate (85) }



S-(β-Chloroethyl)chlorothioformate (3 drops) was added to a solution of bromoketone **84** (26mg, 0.072mmol) and pyridine (4 drops) in methylene chloride (10ml) at rt. The mixture was stirred at rt for 12 hours and then washed with 5% hydrochloric acid, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and concentrated. Purification of the crude by flash chromatography (methylene chloride/acetone 98:2) gave **85** (21.3mg, 61%) as a slightly yellow oil. Ir υ_{max}: 1734, 1680, 1650, 1430, 1270, 1103 cm^{-1}: ^{1}Hnmr (360MHz): δ 3.76 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.70 (2H, t, 7, CH_2Cl), 3.67 (1H, d, 14.4, H-9), 3.23 (2H, t, 7, CH_2S), 2.99 (1H, brdd, 6, 20, H-5), 2.84 (1H, m, H-6), 2.83 (1H, d, 14.5, H-9), 2.39 (1H, dd, 7.5, 14, H-7), 2.38 (1H, brd, 20, H-5), 2.26 (1H, dd, 13,14, H-7), 1.93 (3H, s, 4-CH_3); ^{13}Cnmr (90MHz): δ 182.95 (s, C-2), 171.91 (s, CO), 170.62 (s, CO), 168.10 (s, SCO), 144.73 (s, C-3), 129.68

(s, C-4), 60.30 (s, C-1), 56.59 (s, C-8), 53.31 (q, OCH_3), 53.22 (q, OCH_3), 46.19, 44.11, 42.33 (t, CH_2Cl), 36.15, 33.37 (t, SCH_2), 29.65, 18.24 (q, 4-CH_3); Cims: m/z 502 ($M+NH_4^+$, 57.7%), 500 (52), 422 (100), 326 (74), 324 (64).

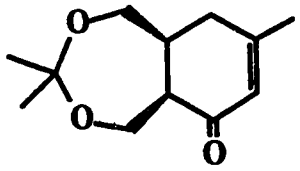
(6R*)-1-Bromo-8,8-bis(methoxycarbonyl)-4-methyl-2-oxo-[4.3.0]non-3-en-3-yl-(β-iodoethyl)thioformate (86) { Dimethyl (7aR*)-3a-bromo-3a,4,7,7a-tetrahydro-5-[(β-iodoethyl)thioformate]-6-methyl-4-oxo-2-indandicarboxylate (86) }



A mixture of chloride **85** (9.4mg, 0.019mmol) and sodium iodide (26mg, 0.173mmol) in methyl ethyl ketone (10ml) was heated under reflux for 22 hours. The solvent was evaporated and methylene chloride (10ml) and water (5ml) were added. The aqueous layer was separated and extracted with methylene chloride (2×5ml). The combined organic extracts were washed, dried, and evaporated. Purification of the crude by flash chromatography (methylene chloride 98:2) provided **86** (7.5mg, 67%) as a yellow oil. Ir υ_{max}: 1734, 1685, 1650, 1435, 1260, 1099 cm^{-1}; ^{1}Hnmr (360MHz): δ 3.75 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.34 (4H, m, SCH_2CH_2I), 2.98 (1H, ddd, 1,6,19, H-5), 2.83 (1H, m, H-6), 2.82 (1H, d, 14.5, H-9), 2.39 (1H, dd, 8,14, H-7), 2.38 (1H, brd, 19, H-5), 2.25 (1H, dd, 13,14, H-7), 1.94 (3H, s, 4-CH_3); ^{13}Cnmr (90MHz): δ 182.95 (s, C-2), 171.91 (s, CO), 170.62 (s, CO), 167.94 (s, SCO), 144.72 (s, C-4), 128.84 (s, C-4), 60.31 (s, C-1), 56.59 (s, C-8), 53.31 (q, OCH_3), 53.25 (q, OCH_3), 46.20,

44.13, 36.15, 33.88, 29.68, 29.66, 18.25 (q, 4-CH_3); Cims: m/z 594 ($M+NH_4^+$, 30%), 592 (30), 514 (100).

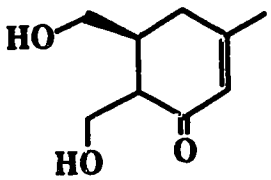
Oxidation of alcohol 59 to ketone 91



PCC (2.16g, 10mmol) was added to a solution of alcohol **59** (940mg, ~40%) in methylene chloride (25ml). The mixture was stirred at rt for 45 hours and then diluted with diethyl ether (25ml). The precipitate was filtered off and the filtrate was evaporated. The residue was separated by flash chromatography (SKB/ethyl acetate 100:20) to give **59** (192mg). Ir υ_{max}: 2988, 2940, 1659, 1637, 1385, 1377, 1291, 1252, 1218, 1170, 1090, 1077, 1057, 980, 832 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.82 (1H, brs, H-2), 4.01 (1H, dd, 8,12, H-7), 3.78 (1H, dd, 2,12, H-7), 3.67 (2H, m, H-8), 2.65 (1H, dd, 8,18, H-4), 2.55 (1H, m, H-6), 2.39 (1H, m, H-5), 2.26 (1H, dd, 5,18, H-4), 2.08 (3H, s, 3-CH_3), 1.46 (3H, s, CH_3), 1.42 (3H, s, CH_3); Hrms: m/z 210.1255 (calcd for $C_{12}H_{18}O_3$, 210.1256, 11.28%), 153 (30), 152 (52), 149 (59), 122 (100), 79 (49).

(5S*)-5,6-Dihydroxymethyl-3-methyl-2-cyclohex-1-one (92)

{ (3S*)-2,3-dihydroxymethyl-5-methyl-5-cyclohexen-1-one (92) }



Hydrolysis of enone **91**

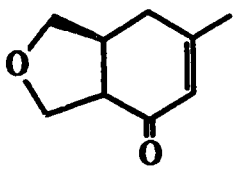
Enone **91** (20mg, 0.09mmol) was dissolved in methylene chloride (10ml) and 80% aqueous HOAc (10ml). The mixture was stirred at rt for 16 hours and the

solvent was co-evaporated with ethanol. Water (5ml) and methylene chloride (10ml) were added. The aqueous layer was separated and extracted with methylene chloride 3×10ml). The combined organic extracts were washed, dried, and evaporated. The residue was separated by flash chromatography (SKB/ethyl acetate 3:1) to give **92** (14.6mg, 94%).

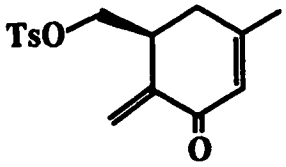
Oxidation of triol **57**

A mixture of triol **57** (120mg, 0.7mmol) and active magnesium dioxide (608mg, 7mmol) in hexane (16ml) and ethyl acetate (5ml) was stirred at rt. for 3 days. The mixture was filtered and the filtrate was evaporated. The residue was separated by flash chromatography (methylene chloride/acetone 80:20) to give **92** (84mg, 71%). Ir υ_{max}: 3428, 3410, 3393, 3384, 3377, 1658, 1022 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.85 (1H, brs, H-2), 4.00 (1H, dd, 6,12, H-7), 3.77 (1H, dd, 5,12, H-8), 3.71 (1H, dd, 7,12, H-7), 3.55 (1H, dd, 5,12, H-8), 2.71 (1H, dd, 5,10, H-6), 2.48 (3H, m), 1.96 (3H, s, 3-CH_3); ^{13}Cnmr (90MHz): δ 200.72 (s, C-1), 161.61 (s, C-3), 126.04 (d, C-2), 62.98 (t, C-7), 60.93 (t, C-8), 50.85 (d, C-6), 40.41 (t, C-4), 34.00 (d, C-5), 24.37 (q, 3-CH_3); Hrms: m/z 170.0941 (calcd for $C_9H_{14}O_3$, 170.0943, 1.7%), 139 (71), 121 (25), 109 (87), 82 (100).

Tosylation of 92 to give 93 and 94



93

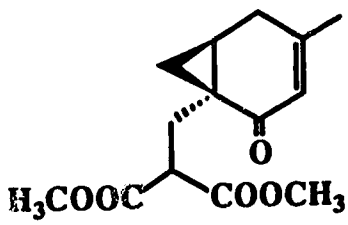


94

p-Toluenesulfonyl chloride (35mg, 0.186mmol) was added to a solution of diol **92** (15.8mg, 0.093mmol), triethylamine (0.039ml, 0.28mmol), and a catalytic amount of DMAP in methylene chloride (5ml) at 0^0C. The mixture was stirred at rt for 42 hours, diluted with methylene chloride (10ml), washed successively with

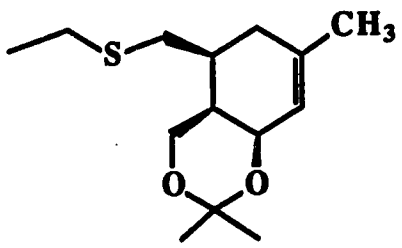
water (2×10ml), saturated $NaHCO_3$ (10ml), and brine (10ml), dried, and evaporated. The residue was separated by silica gel chromatography (SKB/ethyl acetate 70:30) to give **93** (6mg, 42%) and **94** (8.8mg, 31%). The reaction was also carried out with pyridine instead of triethylamine and compounds **93** and **94** were obtained. **93**: Ir υ_{max}: 2951, 2926, 1662, 1379, 1359, 1189, 1176, 1096, 1082, 1060, 1053, 1018, 952, 901, 829, 816, 665, 555 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.95 (1H, brs, H-2), 4.14 (1H, dd, 5,8, H-7), 4.03 (1H, dd, 8,8, H-8), 3.95 (1H, dd, 7,8, H-7), 3.55 (1H, dd, 6,8, H-8), 2.87 (1H, m, H-6), 2.61 (1H, dd, 5,19, H-4), 2.34 (1H, dd, 3,19, H-4), 2.02 (3H, s, 3-CH_3), 0.89 (1H, m, H-5); Hrms: m/z 152.0835 (calcd for $C_9H_{12}O_2$, 152.0837, 34%), 122 (62), 82 (100). **94**: Ir υ_{max}: 1669, 1632, 1613, 1598, 1381, 1360,1190, 1176, 1097, 967, 928, 832, 815 cm^{-1}; ^{1}Hnmr (360MHz): δ 7.81 (2H, d, 8, ArH), 7.39 (2H, d, 8, ArH), 6.15 (1H, s, H-8), 6.03 (1H, s, H-8), 5.33 (1H, brs, H-2), 4.09 (1H, dd, 8,10, H-9), 4.01 (1H, dd, 10,10, H-9), 3.17 (1H, m, H-5), 2.55 (1H, dd, 6,18, H-4), 2.46 (3H, s, $ArCH_3$), 2.39 (1H, dd, 5,18, H-4), 1.97 (3H, s, 3-CH_3); Hrms: m/z 306.0923 (calcd for $C_{16}H_{18}O_4S$, 306.0926, 9.27%), 155 (34), 134 (100), 121 (53), 91 (76).

(5R*,6R*)-6-[2,2-Bis(methoxycarbonyl)ethyl]-3-methylbicyclo[4.1.0]hept-2-en-1-one (95) { **(1R*,6R*)-1-[2,2-Bis(methoxycarbonyl)ethyl]-4-methylbicyclo[4.1.0]hept-3-en-2-one (95)** }



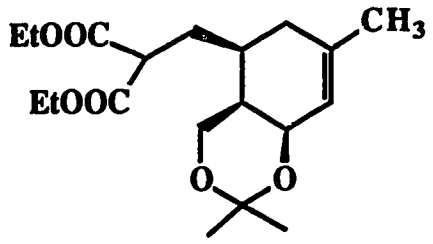
Dimethyl malonate (0.03ml, 0.263mmol) was added to a suspension of sodium hydride (9mg, 50% in mineral oil, 0.188mmol) in THF (6ml) and HMPA (0.1ml) at 0^0C and the mixture was stirred at rt for 10 minutes. Tosylate **94** (6.8mg, 0.022mmol) in THF (4ml) was added, followed by a catalytic amount of sodium iodide. The mixture was refluxed for 12 hours and water (5ml) was added. The aqueous layer was separated and extracted with diethyl ether (3×5ml). The combined organic extracts were washed, dried, and concentrated. The residue was purified by flash chromatography (SKB/ethyl acetate 80:20) to give **95** (5.7mg, 96%). Ir υ_{max}: 1751, 1735, 1656, 1437, 1226, 1192, 1155 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.73 (1H, brs, H-2), 3.88 (1H, dd, 6,8, H-10), 3.75 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 2.78 (1H, dd, 5.5,14, H-9), 2.61 (1H, dd, 5.5,21, H-4), 2.49 (1H, d, 21, H-4), 1.87 (3H, s, 3-CH_3), 1.67 (2H, m, H-5 and H-9), 1.08 (1H, dd, 5,6, H-8), 0.93 (1H, dd, 4,6, H-8); ^{13}Cnmr (90MHz): δ 197.57 (s, C-1), 170.13 (s, CO), 169.80 (s, CO), 153.96 (s, C-3), 123.76 (d, C-2), 52.44 (q, OCH_3), 52.28 (q, OCH_3), 49.42 (d, C-10), 32.46 (d, C-5), 30.72 (t, C-9), 29.11 (s, C-6), 23.73 (q, 3-CH_3), 20.79 (t, C-4), 18.98 (t, C-8); Hrms: m/z 266.1152 (calcd for $C_{14}H_{18}O_5$, 266.1154, 9.45%), 235 (35), 234 (36), 219 (21), 206 (34), 203 (72), 202 (48), 187 (30), 175 (92), 147 (84), 145 (46), 134 (100), 121 (38), 91 (62).

(1R*,6R*,7S*)-7-Ethylthiomethyl-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (97) { (4aR*,5S*,8aR*)-5-ethylthiomethyl-4a,5,6,8a-tetrahydro-2,2,7-trimethylbenzodioxan (97) }



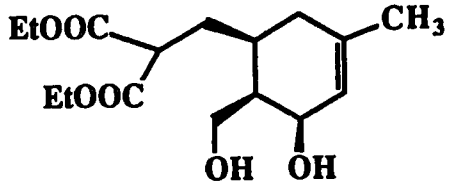
S,S'-diethyl dithiomalonate (0.168ml, 289mg, 1.5mmol) was added to a suspension of sodium hydride (60.6mg, 50% in mineral oil, 1.26mmol) in THF (10ml) and HMPA (1ml) and the mixture was stirred at rt for 20 minutes. Tosylate **60** (154.1mg, 0.42mmol) in THF (10ml) was added, followed by a catalytic amount of sodium iodide. The mixture was refluxed for 9 hours and no reaction was detected. Sodium hydride (60mg, 50% in mineral oil, 1.25mmol) was added and the mixture was refluxed for 9 hours. Water (10ml) was added and the aqueous layer was extracted with diethyl ether (2×10ml). The combined organic extracts were washed, dried, and evaporated. The residue was separated by flash chromatography (SKB/ethyl acetate 5:1) to give **97** (76.7mg, 71%). Ir υ_{max}: 2966, 2929, 1674, 1560, 1456, 1447, 1406, 1378, 1293, 1272, 1244, 1230, 1195, 1173, 1152, 1142, 1110, 1090, 1078, 1054, 1030, 1007 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.46 (1H, brs, H-10), 4.39 (1H, brs, H-1), 4.08 (1H, dd, 5,12, H-5), 3.79 (1H, dd, 5,12, H-5), 3.05 (1H, dd, 4,14, H-12), 2.64 (1H, dd, 10.5,14, H-12), 2.51 (2H, q, 7, SCH_2), 2.17 (1H, dd, 4,18, H-8), 2.03 (1H, dd, 5,18, H-8), 1.96 (1H, m, H-7), 1.84 (1H, m, H-6), 1.73 (3H, s, 9-CH_3), 1.43 (3H, s, 3-CH_3), 1.36 (3H, s, 3-CH_3), 1.23 (3H, t, 7, CH_3); ^{13}Cnmr (90MHz): δ 136.52 (s, C-9), 121.77 (d, C-10), 97.81 (s, C-3), 65.91 (d, C-1), 61.44 (t, C-5), 36.28 (t, C-12), 34.53 (t, SCH_2), 33.53 (d, C-7), 33.43 (t, C-8), 27.61 (q, 3-CH_3), 26.29 (q, 3-CH_3), 23.73 (q, 9-CH_3), 14.79 (q, CH_3); Hrms: m/z 256.1491 (calcd for $C_{14}H_{24}O_2S$, 256.1494, 41.67%), 241 (27), 198 (67), 169 (56).

(1R*,6R*,7S*)-7-[2,2-Bis(ethoxycarbonyl)ethyl]-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (98) { (4aR*,5S*,8aR*)-4a,5,6,8a-tetrahydro-5-[2,2-bis(methoxycarbonyl)ethyl]-2,2,7-trimethylbenzodioxan (98) }



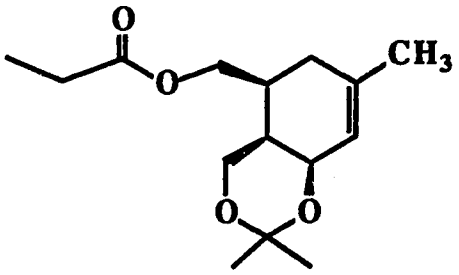
Sodium hydride (210mg, 50% in mineral oil, 4.38mmol) was placed in a dry three-necked flask, washed twice with SKB, then suspended in dry HMPA (10ml). Diethyl malonate (0.685ml, 6mmol) was added dropwise to the suspension at 0^0C and the mixture was stirred at rt for 40 minutes. A solution of tosylate **60** in HMPA (10ml) was added, followed by a catalytic amount of sodium iodide. The resulting mixture was warmed to 55~60^0C with stirring for 7 days. The cooled mixture (ice-bath) was neutralized with acetic acid and partitioned between ice-water (50ml) and methylene chloride (50ml). The aqueous layer was extracted with methylene chloride (5x50ml) and the combined organic extracts were washed with brine, dried, and evaporated. Separation of the crude extract by flash chromatography (methylene chloride/acetone 98:2) provided **98** (53mg, 11%). Ir υ_{max}: 2989, 1748, 1732, 1379, 1370, 1261, 1239, 1196, 1173, 1154, 1113, 1094, 1032 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.47 (1H, brs, H-10), 4.39 (1H, brs, H-1), 4.15 (4H, m, OCH_2), 3.92 (1H, dd, 5,12, H-5), 3.82 (1H, dd, 5,12, H-5), 3.44 (1H, dd, 7,8, H-13), 2.36~1.70 (6H, m), 1.65 (3H, s, 9-CH_3), 1.35 (3H, s, 3-CH_3), 1.34 (3H, s, 3-CH_3), 1.20 (6H, m, CH_3); Hrms: m/z 339.1796 (M-CH_3, calcd for $C_{18}H_{27}O_6$, 339.1807, 1.51%), 279 (3), 239 (4), 233 (5).

(1R*,2R*,3S*)-2-Hydroxymethyl-3-[2,2-bis(ethoxycarbonyl)ethyl]-5-methyl-5-cyclohexen-1-ol (99)

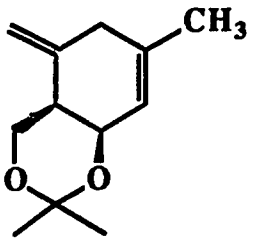


A solution of acetonide **98** (44mg, 0.12mmol) in methylene chloride (12ml) and 80% aqueous acetic acid (5ml) was stirred at rt for 3 hours. The solvent was removed in the rotavap first at water aspirator pressure, then using the vacuum pump. The residue was redissolved in methylene chloride (20ml) and washed with saturated $NaHCO_3$ and brine, dried, and evaporated. The crude was separated by flash chromatography (methylene chloride/acetone 100:25) to give **99** (16.9mg, 44%). Ir υ_{max}: 3480, 1747, 1731, 1370, 1299, 1267, 1236, 1180, 1152, 1029 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.43 (1H, brs, H-6), 4.52 (1H, brs, H-1), 4.23 (4H, m, OCH_2), 3.92 (1H, dd, 9,11, H-8), 3.77 (1H, dd, 4,11, H-8), 3.50 (1H, dd, 7,8, H-10), 2.30 (2H, brs, OH), 2.22~1.60 (6H, m), 1.75 (3H, s, 5-CH_3), 1.33 (6H, m, CH_3); Hrms: m/z 296.1624 (M-H_2O, calcd for $C_{16}H_{24}O_5$, 296.1624, 0.97%), 214 (2), 161 (17), 96 (100).

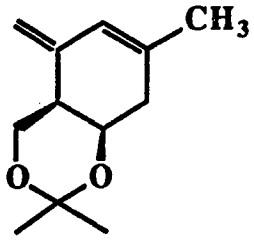
Alkylation of tosylate 60 with methylpropionate to give 100, 101, and 102



100



101



102

n-BuLi as a base

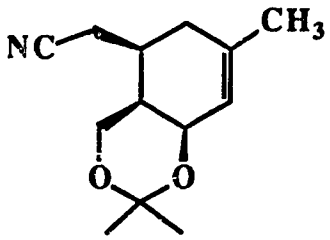
n-Butyllithium (1.8ml, 1.6M in hexane, 2.66mmol) was added to 10ml HMPA in a three-necked flask. Methyl propionate (0.36ml, 3.73mmol) was added dropwise at 0^0C and the mixture was stirred at rt for 50 minutes. A solution of tosylate **60** (160mg, 0.44mmol) in 10ml HMPA was added, followed by a catalytic amount of sodium iodide. The mixture was stirred at rt for 3 hours, then warmed up to 55~60^0C for 5 days. The cooled mixture was neutralized with acetic acid and partitioned between water (50ml) and SKB/ether (1:1, 50ml). The aqueous layer was separated and extracted with SKB/ether (1:1, 50ml), and ether (4x50ml). The combined organic extracts were washed with brine, dried, and evaporated. Repeated separations (CH_2Cl_2/acetone 98:2, SKB/ethyl acetate 5:1) by silica gel chromatography provided compounds **100** (59.6mg, 51%) and **101** (5mg).

$[(CH_3)Si]_2NLi$ as a base

Methyl propionate (0.3ml, 3mmol) was added to a solution of lithium bis(trimethylsilyl)amide (2ml, 1M in THF) in THF (10ml) at -78^{0}C and the mixture was stirred for 45 minutes. A solution of tosylate **60** (145mg, 0.4mmol) in dry HMPA (10ml) and THF (5ml) was added at -78^{0}C. The mixture was allowed to warm up to rt, then warmed to 55~60^{0}C with an oil bath for 6 days. The cooled mixture was neutralized with acetic acid and partitioned between water (50ml) and SKB/ether (1:1, 50ml). The aqueous layer was separated and extracted with diethyl ether (4x50ml). The combined organic extracts were washed with brine, dried, and evaporated. Separation of the crude product by repeated silica gel chromatography (methylene chloride/acetone 99:1, SKB/ethyl acetate 5:1) provided compounds **100** (10mg, 9%) and **102** (5mg). **100:** Ir υ_{max}: 2989, 2972, 1735, 1676, 1463, 1436, 1379, 1358, 1344, 1327, 1279, 1260 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.50 (1H, brs, H-10), 4.59 (1H, dd, 5,11, H-12), 4.38 (1H, brs, H-1), 4.15 (1H, dd, 9,11, H-12), 4.13 (1H, dd, 5,7, H-5), 3.86 (1H, dd, 7,12, H-5), 2.33 (2H, q, 8, CH_2), 2.18 (1H, m, H-7), 2.05 (2H, brs, H-8), 1.78 (1H, m, H-6), 1.72 (3H, s, 9-CH_3), 1.45 (3H, s, 3-CH_3), 1.38 (3H, s, 3-CH_3), 1.14 (3H, t, 8, CH_3); ^{13}Cnmr (90MHz): δ 174.30 (s, CO), 136.33, 121.84, 97.97, 65.20, 64.97, 61.72, 35.70, 33.67, 31.92, 28.04, 27.66, 23.73, 21.55, 9.17; Hrms: m/z 253.1438 (M-CH_3, calcd for $C_{14}H_{21}O_4$, 253.1440, 4.16%), 210 (3), 136 (31), 119 (100); Cims: m/z 554 (2M+NH_4^+, 27%), 286 (M+NH_4^+, 100). **101:** Ir υ_{max}: 2990, 2928, 1640, 1436, 1379, 1273, 1261, 1248, 1233, 1222, 1196, 1172, 1141, 1111 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.53 (1H, brs), 5.16 (1H, brd, 2), 5.10 (1H, brs), 4.57 (1H, dd, 4,4), 4.22 (1H, dd, 3,12), 4.16 (1H, dd, 4,12), 2.85 (1H, brd, 19), 2.76 (1H, d, 19), 2.29 (1H, m), 1.82 (3H, s), 1.56 (3H, s), 1.48 (3H, s); ^{13}Cnmr (90MHz): δ 140.65, 140.02, 120.97, 110.21, 98.65, 67.09, 60.46, 39.52, 38.63, 28.61, 23.14, 20.78; Hrms: m/z 194.1301 (calcd for $C_{12}H_{18}O_2$, 194.1307, 1.92%), 179 (10), 136 (38), 91 (100). **102:** Ir υ_{max}: 2925, 2872, 1379, 1234, 1196, 1170,

1142, 1137, 1108, 1092 cm^{-1}; ^{1}Hnmr (360MHz): δ 6.08 (1H, s), 5.12 (2H, s), 4.43 (1H, brdd, 2,2), 4.32 (1H, dd, 2,12), 4.18 (1H,dd, 3,12), 2.50 (1H, brd, 18), 2.26 (1H, brd, 2), 2.16 (1H, d, 18), 1.88 (3H, s), 1.58 (3H, s), 1.43 (3H, s); ^{13}Cnmr (90MHz): δ 140.28, 132.96, 125.39, 110.44, 98.82, 66.58, 61.19, 36.29, 35.79, 29.77, 23.49, 19.41; Cims: m/z 228.21 ($M+2NH_3$, 46%), 211 ($M+NH_3$, 51), 169 (100).

Alkylation of 60 with propionitrile to give 103



Propionitrile (0.23ml, 3.2mmol) was added dropwise to a solution of n-butyllithium (1.5ml, 1.6m in hexane, 2.4mmol) in dry HMPA (10ml) at 0^0C and the mixture was stirred at the same temperature for 45 minutes. A solution of tosylate **60** (145mg, 0.4mmol) in HMPA (5ml) was added. The mixture was stirred at rt for 4 hours, then warmed up to 55~60^0C with an oil bath for 7 days. The cooled mixture was neutralized with acetic acid and partitioned between water (60ml) and SKB/ether (1:1, 100ml). The aqueous layer was separated and extracted with diethyl ether (2x50ml, 2x100ml). The combined organic extracts were washed with brine, dried, and evaporated. Repeated flash chromatographic separation of the crude product provided **101** (6mg), **102** (8mg), and **103** (22mg, 25%). **103**: Ir υ_{max}: 2963, 2240, 1454, 1433, 1379, 1249, 1235, 1192, 1171, 1154, 1138, 1109, 1076 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.51 (1H, brs, H-10), 4.39 (1H, brs, H-1), 4.30 (1H, dd, 5,13, H-5), 3.80 (1H, d, 13, H-5), 3.10 (1H, brd, 17, H-12), 2.86 (1H, dd, 12,17, H-12), 2.28 (3H, brs, H-8 and H-7), 1.78 (3H, s, 9-CH_3), 1.59 (1H, m, H-6), 1.49 (3H, s, 3-CH_3), 1.34 (3H, s, 3-CH_3); ^{13}Cnmr (90MHz): δ 136.46 (s,

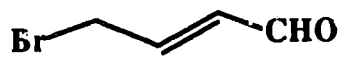
C-9), 120.54 (d, C-10), 120.48 (s, CN), 98.19 (s, C-3), 63.71 (d, C-1), 63.18 (t, C-5), 34.95, 33.78, 33.53, 29.24, 23.82 (q, 3-CH_3), 19.25 (q, 3-CH_3), 18.88 (q, 9-CH_3); Hrms: m/z 221.1413 (calcd for $C_{13}H_{19}O_2N$, 221.1416, 12.79%), 206 (12), 163 (17), 146 (89), 119 (100); Cims: m/z 460 ($2M+NH_4^+$, 29%), 239 ($M+NH_4^+$, 100), 221 (M, 33).

cis- and _trans_-1-Acetoxybutadiene (105) and (106)

$$CH_2=CH-CH=CHOAc$$

A solution of crotonaldehyde (6ml, 5g, 0.07mol) , acetic anhydride (10ml, 0.11mmol), and triethylamine (15ml, 0.11mol) was refluxed for 21 hours. The resulting mixture was poured onto ice-water (50 ml) and the aqueous layer was separated and extracted with methylene chloride (3x50ml). The combined organic extracts were washed, dried, and evaporated. Distillation of the crude at 25^0C/10 mmHg provided **105** and **106** (7g, 88%) as a colorless liquid. ^{1}Hnmr (360MHz): **105**: δ 7.00 (1H, d, 6, H-1), 6.65 (1H, ddd, 8,11, 17, H-3), 5.46 (1H, dd, 6,16, H-2), 5.24 (1H, dd, 2,17, H-4), 5.06 (1H, dd, 2,8, H-4), 2.09 (3H, s, $COCH_3$); **106**: δ 7.34 (1H, d, 12, H-1), 6.22 (1H, ddd, 8,11,16, H-3), 5.99 (1H, dd, 11,12, H-2), 5.18 (1H, dd, 2,16, H-4), 5.03 (1H, dd, 2,8, H-4), 2.16 (3H, s, $COCH_3$).

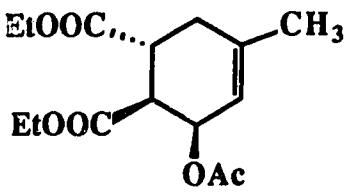
4-Bromocrotonaldehyde (104)



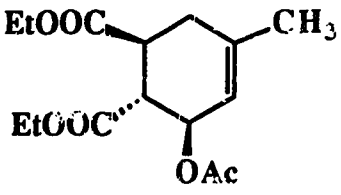
Bromine (11.83g, 0.074mol) in carbon tetrachloride (50ml) was added to a hexane solution of 1-acetoxybutadiene **105** and **106** (13.8g, 0.123mol) containing 29mg of barium carbonate. Throughout the addition (1 hour), the solution was maintained at -78^0C. After 10 minutes, the solution was allowed to warm to rt. Sodium bicarbonate (20.7g, 0.246mol) and water (100ml) were added and the

heterogeneous mixture was stirred for 19 hours. The aqueous layer was separated and extracted with methylene chloride (3×100ml). The combined organic extracts were washed, dried, and evaporated. Distillation of the crude product at 48~50^{0}C/20mmHg gave a liquid (9.54g, 52%). ^{1}Hnmr (360MHz): δ 9.60 (1H, d, 8, H-1), 6.88 (1H, dt, 7,15, H-3), 6.26 (1H, brdd, 8,15, H-2), 4.12 (2H, dd, 1,7, H-4).

Diethyl 3-acetoxy-5-methyl-4-cyclohexene-1,2-dicarboxylate (110) and (111) { Diethyl 6-acetoxy-4-methyl-4-cyclohexene-1,2-dicarboxylate }



110

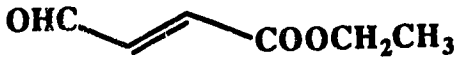


111

A mixture of acetate **55** (1.45g, 11.51mmol), diethyl fumarate (1.88ml, 11.51mmol), and silver trifluoroacetate (324mg, 1.47mmol) in dry benzene (60ml) was refluxed for 5 days. The solvent was evaporated and diethyl ether (100ml) was added. The precipitate was filtered off and the filtrate was concentrated. Separation of the crude by flash chromatography (SKB/acetone 5:1) gave a mixture of **110** and **111** (1.91g, 56%) in a ratio of 1:1. Ir υ_{max}: 2981, 1738, 1734, 1466, 1446, 1372, 1335, 1308, 1241, 1232, 1183, 1140, 1096 cm^{-1}; ^{1}Hnmr (360MHz): **110**: δ 5.56 (1H, brs, H-3), 5.33 (1H, brs, H-4), 4.16 (4H, m, OCH_2), 3.10 (1H, m, H-1), 3.00 (1H, dd, 4,12, H-2), 2.10~2.40 (2H, m, H-6), 1.99 (3H, s, $COCH_3$), 1.74 (3H, s, 5-CH_3), 1.24 (6H, m, CH_3); **111**: δ 5.65 (1H, brs, H-4), 5.56 (1H, brs, H-3), 4.16 (4H, m, OCH_2), 3.10 (1H, m, H-1), 2.89 (1H, dd, 9,11, H-2), 2.10~2.40 (2H, m, H-6), 2.07 (3H, s, $COCH_3$), 1.74 (3H, s, 5-CH_3), 1.24 (6H,

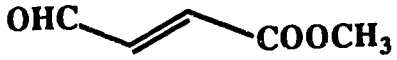
m, CH_3); Hrms: m/z 298.1420 (calcd for $C_{15}H_{22}O_6$, 298.1416, 3.56%), 255 (23), 209 (22), 193 (41), 182 (70), 165 (100).

Ethyl 4-oxo-butenate (112)



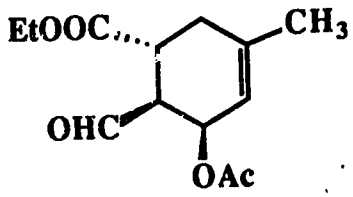
A mixture of ethyl crotonate (50ml, 04mol) and selenium dioxide (28.49g, 0.257mol) in dioxane (300ml) was refluxed for 17 hours. The precipitate was filtered off and the filtrate was concentrated. Distillation of the residue at 50~60^{0}C/4.5mmHg provided **112** (3.6g, 7%). ^{1}Hnmr (360MHz): δ 9.64 (1H, d, 8, H-4), 6.83 (1H, dd, 8,16, H-3), 6.65 (1H, d, 16, H-2), 4.17 (2H, q, 6, OCH_2), 1.22 (3H, t, 6, CH_3).

Methyl 4-oxo-butenate (113)



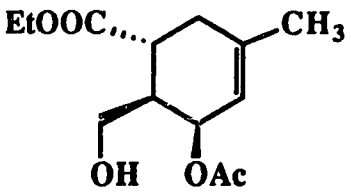
A mixture of methyl crotonate (50ml, 0.472mol) and selenium dioxide (31.67g, 0.285mol) in dioxide (200ml) was refluxed for 19 hours. The precipitate was filtered off and the filtrate was evaporated. Distillation of the crude product at 45~60^{0}C/12mmHg gave **113** (14.3g, 27%). ^{1}Hnmr (360MHz): δ 9.74 (1H, d, 7, H-4), 6.92 (1H, dd, 7,16, H-3), 6.73 (1H, d, 16, H-2), 3.74 (3H, s, OCH_3).

Ethyl (1R*,2S*,3R*)-3-acetoxy-2-formyl-5-methyl-4-cyclohexene-1-carboxylate (114) { Ethyl (1R*,5R*,6S*)-5-acetoxy-6-formyl-3-methyl-3-cyclohexene-1-carboxylate (114) }



A mixture of acetate **55** (2.68g, 0.02mol), ethyl 4-oxo-butenate **112** (2.72g, 0.02mol), and silver trifluoroacetate (582mg, 2.63mmol) in dry benzene (100ml) was refluxed for 3 days. The solvent was evaporated and diethyl ether (100ml) was added. The precipitate was filtered off and the filtrate was concentrated. The residue was purified by flash chromatography (SKB/acetone 5:1) to give a mixture of **114**, **115**, **116**, and **117** (3.39g, 63%) in a ratio of 5:2:1:1. Ir υ_{max}: 1733, 1373, 1237, 1194, 1181, 1024 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 9.65 (1H, s, CHO), 5.59 (1H, brs, H-4), 5.43 (1H, brs, H-3), 4.15 (2H, q, 7, OCH_2), 3.07 (1H, m, H-1), 2.98 (1H, m, H-2), 2.24 (2H, m, H-6), 2.02 (3H, s, $COCH_3$), 1.74 (3H, s, 5-CH_3), 1.23 (3H, t, 7, CH_3); Hrms: m/z 254.1151 (calcd for $C_{13}H_{18}O_5$, 254.1154, 0.64%), 211(32), 165 (45), 149 (23), 121 (74), 109 (55), 93 (100).

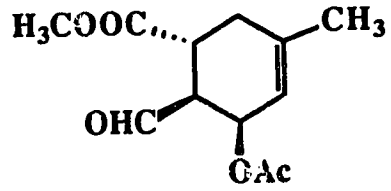
Ethyl (1R*,2S*,3R*)-3-acetoxy-2-hydroxymethyl-5-methyl-4-cyclohexene-1-carboxylate (118) { Ethyl (1R*,5R*,6S*)-5-acetoxy-6-hydroxymethyl-3-methyl-3-cyclohexene-1-carboxylate (118) }



Sodium borohydride (481mg, 12.66mmol) was added to a solution of the mixture of aldehydes **114**, **115**, **116**, and **117** (1.41g, 5.55mmol) in methanol (40ml) at 0^0C. The mixture was stirred at rt for 3 hours. The solvent was evaporated and water (20ml) and methylene chloride (20ml) were added, together with several drops of 3M HCl. The aqueous layer was separated and extracted with methylene chloride (3x20ml). The combined organic extracts were washed, dried, and concentrated to provide **118** as a major product (1.2g, 84%). A analytical sample was obtained by flash chromatographic purification of the crude (SKB/ethyl acetate 5:1). Ir υ_{max}: 3470, 2935, 1735, 1445, 1372, 1302, 1240, 1177, 1113 cm^{-1};

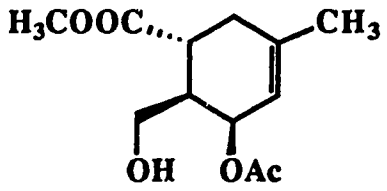
^{1}Hnmr (360MHz, major isomer): δ 5.66 (1H, brs, H-4), 5.58 (1H, brs, H-3), 4.42 (1H, dd, 9,11, H-7), 4.19 (2H, q, 7, OCH_2), 4.06 (1H, dd, 5,11, H-7), 3.62 (1H, brs, OH), 2.70 (1H, m, H-1), 2.40~2.20 (3H, m, H-2 and H-6), 2.10 (3H, s, $COCH_3$), 1.78 (3H, s, 5-CH_3), 1.30 (3H, t, 7, CH_3); Hrms: m/z 256.1318 (calcd for $C_{13}H_{20}O_5$, 256.1311, 0.57%), 213 (5), 196 (7), 167 (18), 123 (100), 105 (43).

Methyl (1R*,2S*,3R*)-3-acetoxy-2-formyl-5-methyl-4-cyclohexene-1-carboxylate (119) { Methyl (1R*,5R*,6S*)-5-acetoxy-6-formyl-3-methyl-3-cyclohexene-1-carboxylate (119) }



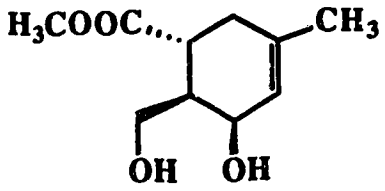
A mixture of acetate **55** (2.4g, 19 mmol), methyl 4-oxo-butenate **112** (1.8g, 15.8 mmol), and silver trifluoroacetate (900mg, 4 mmol) in dry benzene (100ml) was refluxed for 3 days. The solvent was evaporated and diethyl ether (200ml) was added. The precipitate was filtered off and the filtrate was concentrated. The crude products were purified by flash chromatography (SKB/ethyl acetate 70:30) to give a mixture of **119**, **120**, **121**, and **122** (2.88g, 76%) in a ratio of 5:2:1:1. Ir υmax: 2936, 1736, 1437, 1374, 1304, 1240, 1198, 1170, 1122, 1062 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 9.54 (1H, s, CHO), 5.72~5.54 (2H, H-3 and H-4), 3.67 (3H, s, OCH_3), 3.06 (1H, m, H-1), 2.80 (1H, m, H-2), 2.44 (2H, m, H-6), 1.99 (3H, s, $COCH_3$), 1.77 (3H, s, 5-CH_3); Hrms: m/z 240.0998 (calcd for $C_{12}H_{16}O_5$, 240.0998, 1.35%),209 (5), 197 (46), 181 (29), 180 (25), 165 (23), 149 (25), 139 (22), 137 (33), 121 (100).

Methyl (1R*,2S*,3R*)-3-acetoxy-2-hydroxymethyl-5-methyl-4-cyclohexene-1-carboxylate (123) { Methyl (1R*,5R*,6S*)-5-acetoxy-6-hydroxymethyl-3-methyl-3-cyclohexene-1-carboxylate (123) }



Sodium borohydride (946mg, 24.9mmol) was added to a solution of the mixture of aldehydes **119**, **120**, **121**, and **122** (2.3g, 12mmol) in methanol (70ml) at 0^0C. The mixture was stirred at rt for 3 hours. The solvent was evaporated and water (30ml) and 3M HCl (10ml) were added. The aqueous layer was extracted with methylene chloride (3×40ml). The combined organic extracts were washed with brine, dried, and concentrated to provide **123** as a major product (2.41g, 85%). An analytical sample was obtained by flash chromatographic purification of the crude (SKB/acetone 4:1). Ir υ_{max}: 3480, 2953, 1735, 1437, 1373, 1305, 1236, 1208, 1172, 1097, 1072, 1020, 974 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 5.68 (1H, brs, H-4), 5.37 (1H, brs, H-3), 4.37~4.04 (2H, H-7), 3.73 (3H, s, OCH_3), 3.60 (1H, brs, OH), 2.62 (1H, m, H-1), 2.16 (2H, m, H-6), 2.08 (1H, m, H-2), 2.04 (3H, s, $COCH_3$), 1.73 (3H, s, 5-CH_3); Hrms: m/z 242.1141 (calcd for $C_{12}H_{18}O_5$, 242.1154, 1.13%), 211 (5), 199 (21), 182 (40), 167 (83), 164 (35), 151 (49), 123 (40), 109 (66), 105 (50).

Methyl (1R*,2S*,3R*)-3-hydroxy-2-hydroxymethyl-5-methyl-4-cyclohexene-1-carboxylate (124) { Methyl (1R*,5R*,6S*)-5-hydroxy-6-hydroxymethyl-3-methyl-3-cyclohexene-1-carboxylate (124) }



From compound **118**:

1M KOH/MeOH (5ml) was added to a solution of acetate **118** (800mg, 3.13mmol) in methanol (40ml) at 0^0C and the mixture was stirred overnight at rt. The resulting mixture was neutralized with acetic acid, evaporated, and redissolved in ethyl acetate. The precipitate was filtered off and the filtrate was evaporated. The crude products were separated by flash chromatography (SKB/acetone 70:30) to

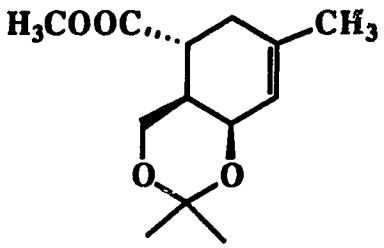
- a major product (380mg, 61%).

mpound **123**:

Acetate **123** (2.4g, 9.9mmol) and 1M KOH/MeOH (20ml, 20mmol) in methanol (70ml) were stirred at 0^0C for 2.5 hours. The mixture was neutralized with acetic acid, evaporated, and redissolved in methylene chloride. The precipitate was filtered off and the filtrate was concentrated. Separation of the crude products by flash chromatography (SKB/acetone 60:40) provided **124** as a major product (870mg, 44%). Ir υ_{max}: 3399, 2949, 1733, 1677, 1437, 1379, 1267, 1198, 1170, 1063, 1029, 1014 cm^{-1}; ^{1}Hnmr (360MHz, major isomer): δ 5.62 (1H, brs, H-4), 4.34 (1H, brs, H-3), 3.80 (2H, m, H-7), 3.71 (3H, s, OCH_3), 2.87 (1H, m, H-1), 2.72 (2H, brs, OH), 2.24 (2H, m, H-6), 1.92 (1H, m, H-2), 1.73 (3H, s, 5-CH_3); Hrms: m/z 200.1048 (calcd for $C_{10}H_{16}O_4$, 200.1048, 0.31%), 182 (2), 168 (10), 153 (15), 123 (100).

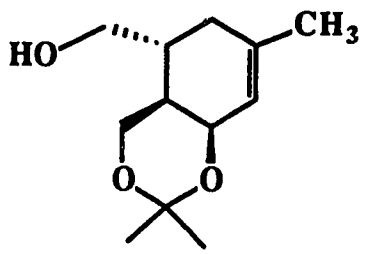
(1R*,6R*,7R*)-7-Methoxycarbonyl-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (125) **{ (4aR*,5R*,8aR*)-4a,5,6,8a-**

tetrahydro-5-methoxycarbonyl-2,2,7-trimethyl-1,3-benzodioxan (125)}



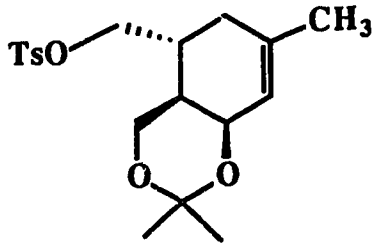
A mixture of diol **124** (140mg, 0.7mmol), 2,2-dimethoxypropane (2ml), and trifluoroacetic acid (0.3ml) in methylene chloride (30ml) was stirred at rt for 17 hours. The resulting solution was washed carefully with saturated $NaHCO_3$, brine, then dried and evaporated. The residue was separated by flash chromatography (SKB/ethyl acetate 5:1) to give pure **125** (168mg, 77%). Ir υ_{max}: 2990, 2914, 1735, 1682, 1436, 1381, 1338, 1265, 1238, 1224, 1195, 1167, 1024, 975 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.52 (1H, brs, H-10), 4.38 (1H, brs, H-1), 4.14 (1H, dd, 4,12, H-5), 3.72 (3H, s, OCH_3), 3.70 (1H, dd, 3,12, H-5), 3.20 (1H, m, H-7), 2.32 (1H, dd, 6,17, H-8), 2.17 (1H, brd, 17, H-8), 1.76 (3H, s, 9-CH_3), 1.74 (1H, m, H-6), 1.51 (3H, s, 3-CH_3), 1.41 (3H, s, 3-CH_3); Hrms: m/z 240.1357 (calcd for $C_{13}H_{20}O_4$, 240.1361, 20.30%), 225 (43), 206 (10), 182 (60), 165 (78), 151 (31), 123 (60), 105 (99).

(1R*,6R*,7R*)-7-Hydroxymethyl-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (126) { (4aR*,5R*,8aR*)-4a,5,6,8a-tetrahydro-5-hydroxymethyl-2,2,7-trimethyl-1,3-benzodioxan (126)}



A solution of ester **125** (223mg, 0.93mmol) in THF (30ml) was added to a suspension of $LiAlH_4$ (124mg, 3.26mmol) in THF (10ml) at 0^0C. The mixture was refluxed for 2 hours and ice-water was added slowly at 0^0C. The precipitate was filtered through a pad of celite and washed with ethyl acetate. The aqueous layer was separated and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried, and evaporated to give **126** (194mg, 98%). Ir υ_{max}: 3446, 2989, 2912, 2876, 1678, 1436, 1380, 1344, 1319, 1271, 1224, 1196, 1163, 1102, 1077, 1071, 1026 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.46 (1H, brs, H-10), 4.33 (1H, brs, H-1), 4.04 (1H, dd, 4,12, H-5), 3.94 (1H, dd, 3,12, H-5), 3.73 (2H, m, H-12), 2.30 (1H, m, H-7), 2.08 (1H, dd, 5,17, H-8), 1.94 (1H, dd, 12,17, H-8), 1.72 (3H, s, 9-CH_3), 1.66 (1H, s, OH), 1.46 (3H, s, 3-CH_3), 1.36 (3H, s, 3-CH_3); ^{13}Cnmr (90MHz): δ 140.82 (s, C-9), 120.29 (d, C-10), 99.06 (s, C-3), 65.43 (d, C-1), 64.33 (t, C-12), 60.90 (t, C-5), 35.64 (d, C-6), 33.81 (t, C-8), 32.38 (d, C-7), 28.77 (q, 3-CH_3), 23.59 (q, 3-CH_3), 20.08 (q, 9-CH_3); Hrms: m/z 212.1411 (calcd for $C_{12}H_{20}O_3$, 212.1412, 33.51%), 197 (16), 154 (22), 137 (34), 119 (45), 107 (100).

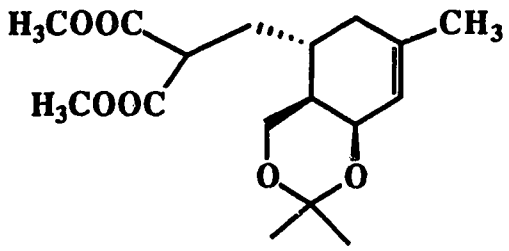
(1R*,6R*,7R*)-3,3,9-Trimethyl-7-*p*-tolylsulfonyloxymethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (127) { (4aR*,5R*,8aR*)-4a,5,6,8a-tetrahydro-2,2,7-trimethyl-5-*p*-tolylsulfonyloxymethyl-1,3-benzodioxan (127)}



p-Toluenesulfonyl chloride (246mg, 1.29mmol) was added to a solution of alcohol **126** (100mg, 0.47mmol), triethylamine (0.33ml, 2.38mmol), and a

catalytic amount of DMAP in methylene chloride (20ml) at 0^0C. The mixture was stirred at rt for 33 hours and ice-water (15ml) was added. The aqueous layer was separated and extracted with methylene chloride (2×15ml). The combined organic extracts were washed successively with saturated $NaHCO_3$ and brine, dried, and concentrated. The residue was separated by flash chromatography (SKB/ethyl acetate 80:20) to give **127** (144mg, 83%). Ir υ_{max}: 2990, 2913, 1360, 1265, 1236, 1224, 1189, 1177, 1101, 1080, 976, 966, 937, 916 cm^{-1}; ^{1}Hnmr (360MHz): δ 2.29 (2H, d, 8, ArH), 7.36 (2H, d, 8, ArH), 5.47 (1H, brs, H-10), 4.35 (1H, brs, H-1), 4.23 (1H, dd, 4,10, H-12), 4.07 (1H, dd, 2,10, H-12), 3.99 (1H, dd, 4,13, H-5), 3.68 (1H, dd, 2,13, H-5), 2.48 (3H, s, $ArCH_3$), 2.38 (1H, m, H-7), 2.06 (2H, m, H-8), 1.73 (3H, s, 9-CH_3), 1.53 (1H, m, H-6), 1.48 (3H, s, 3-CH_3), 1.36 (3H, s, 3-CH_3); Hrms: m/z 366.1502 (calcd for $C_{19}H_{26}O_5S$, 366.1501, 0.33%), 308 (0.51), 172 (4), 155 (7), 136 (6).

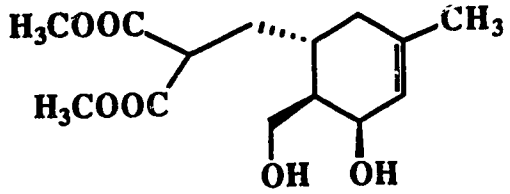
(1R*,6R*,7R*)-7-[2,2-Bis(methoxycarbonyl)ethyl]-3,3,9-trimethyl-2,4-dioxabicyclo[4.4.0]dec-9-ene (129) { (4aR*,5R*,8aR*)-4a,5,6,8a-tetrahydro-5-[2,2-bis(methoxycarbonyl)ethyl]-2,2,7-trimethyl-1,3-benzodioxan (128) }



Dimethyl malonate (0.1ml, 0.87mmol) was added to a suspension of sodium hydride (25mg, 60% in mineral oil, 0.63mmol) in dry THF (10ml) at 0^0C and the mixture was stirred at rt for 20 minutes. A solution of tosylate **127** (75mg, 0.2mmol) in dry THF (15ml) was added, followed by a catalytic amount of sodium iodide. The solution was refluxed for 24 hours and water (15ml) was added. The

aqueous layer was separated and extracted with diethyl ether (2×20ml). The combined organic extracts were washed with brine, dried, and evaporated. Purification of the crude product by flash chromatography (first SKB, then methylene chloride/acetone 98:2) provided **128** (66mg, 99%). Ir υ_{max}: 2989, 2954, 1753, 1736, 1680, 1436, 1380, 1372, 1346, 1290, 1268, 1223, 1210, 1197, 1164, 1103 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.49 (1H, brs, H-10), 4.32 (1H, brs, H-1), 4.06 (1H, dd, 5,12, H-5), 3.90 (1H, dd, 4,12, H-5), 3.78 (6H, s, OCH_3), 3.59 (1H, dd, 5,9, H-13), 2.28 (1H, ddd, 4,9,15, H-8), 2.17 (1H, dd, 5,15, H-8), 2.05 (1H, m, H-7), 1.76 (3H, s, 9-CH_3), 1.68 (2H, m, H-12), 1.50 (3H, s, 3-CH_3), 1.41 (3H, s, 3-CH_3), 1.40 (1H, m, H-6); ^{13}Cnmr (90MHz): δ 169.53 (s, CO), 140.11 (s, C-9), 120.41 (d, C-10), 99.35 (s, C-3), 65.06 (d, C-1), 60.94 (t, C-5), 52.51 (q, OCH_3), 49.71 (d, C-13), 39.39 (d, C-6), 36.14 (t, C-8), 32.06 (t, C-12), 28.75 (d, C-7), 27.81 (q, 3-CH_3), 23.66 (q, 3-CH_3), 20.86 (q, 9-CH_3); Hrms: m/z 326.1733 (calcd for $C_{17}H_{26}O_6$, 326.1729, 3.05%), 268 (12), 251 (9), 219 (21), 187 (100), 159 (28), 136 (39), 132 (33).

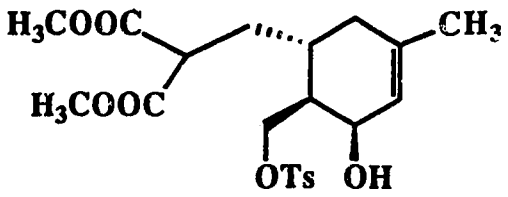
(1R*,2R*,3R*)-2-Hydroxymethyl-3-[2,2-bis(methoxycarbonyl)ethyl]-5-methyl-5-cyclohexen-1-ol (129)



A solution of acetonide **128** (60mg, 0.18mmol) in methylene chloride (20ml) and 80% aqueous HOAc (20ml) was stirred at rt for 4 hours. The solvent was removed first at water aspirator pressure then using the vacuum pump. The residue was separated by flash chromatography (methylene chloride/acetone 70:30) to give **129** (36.1mg, 69%). Ir υ_{max}: 3480, 3470, 2955, 1734, 1663, 1436, 1322, 1262, 1241, 1200, 1155 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.58 (1H, brs, H-6), 4.35

1241, 1200, 1155 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.58 (1H, brs, H-6), 4.35 (1H, brs, H-1), 3.97 (2H, m, H-8), 3.76 (6H, s, OCH_3), 3.61 (1H, dd, 6,9, H-10), 3.01 (1H, brs, OH), 2.40 (1H, brs, OH), 2.33 (1H, ddd, 4,8,17, H-4), 2.17 (1H, dd, 5,17, H-4), 1.93 (1H, m, H-3), 1.70 (3H, s, 5-CH_3), 1.66 (2H, m, H-9), 1.48 (1H, m, H-2); ^{13}Cnmr (90MHz): δ 170.07 (s, CO), 170.00 (s, CO), 138.35 (s, C-5), 122.77 (d, C-6), 69.05 (d, C-1), 63.09 (t, C-8), 52.69 (q, OCH_3), 52.64 (q, OCH_3), 49.46 (d, C-10), 44.17 (d, C-2), 36.34 (t, C-4), 31.98 (t, C-9), 27.86 (d, C-3), 23.28 (q, 5-CH_3); Hrms: m/z 268.1310 (M-H_2O, calcd for $C_{14}H_{20}O_5$, 268.1311, 6.33%), 189 (23), 187 (26), 173 (22), 152 (46), 145 (39), 136 (100), 133 (90), 123 (97); Cims: m/z 304 ($M+NH_4^+$, 100%).

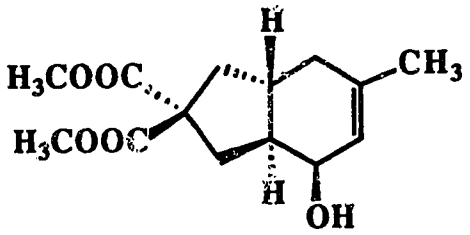
(1R*,2R*,3R*)-3-[2,2-Bis(methoxycarbonyl)ethyl]-5-methyl-2-*p*-tolylsulfonyloxymethyl-5-cyclohexen-1-ol (130)



p-Toluenesulfonyl chloride (23.8mg, 0.125mmol) was added to a solution of diol **129** (13mg, 0.045mmol), triethylamine (0.05ml, 0.36mmol), and a catalytic amount of DMAP in methylene chloride (15ml) at 0^0C. The mixture was stirred at rt for 40 hours and ice-water (10ml) was added. The aqueous layer was separated and extracted with methylene chloride (2×10ml). The combined organic extracts were washed successively with saturated $NaHCO_3$ and brine, dried, and evaporated. The residue was separated by flash chromatography (methylene chloride/acetone 95:5) to give **130** (15.5mg, 78%). Ir υ_{max}: 3480, 1750, 1734, 1437, 1356, 1254, 1247, 1235, 1210, 1189, 1176, 961 cm^{-1}; ^{1}Hnmr (400MHz): δ 7.82 (2H, d, 8, ArH), 7.38 (2H, d, 8, ArH), 5.56 (1H, brs, H-6), 4.28 (1H, brs, H-1), 4.22 (1H, d, 7, H-8), 3.77 (1H, H-8), 3.76 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.46 (1H, dd,

5,10, H-10), 2.48 (3H, s, $ArCH_3$), 2.16 (2H, m, H-4), 1.78 (1H, m, H-3), 1.68 (3H, s, 5-CH_3), 1.62 (1H, m, H-2), 1.60 (2H, m, H-9), 1.28 (1H, brs, OH); Hrms: m/z 251.1272 (M-H_2O-$C_7H_7O_3S$, calcd for $C_{14}H_{19}O_4$, 251.1283, 13%), 187 (37), 172 (62), 119 (44), 107 (30), 91 (100).

(1S*,2S*,6S*)-8,8-bis(methoxycarbonyl)-4-methylbicyclo[4.3.0]non-3-en-2-ol (131) { Dimethyl (3aS*,4S*,7aS*)-3a,4,7,7a-tetrahydro-4-hydroxyl-6-methyl-2-indandicarboxylate (131) }



Sodium hydride (3mg, 60% in mineral oil, 0.075mmol) was added to a solution of [illegible]ylate **130** (10mg, 0.02mmol) in THF (10ml). The mixture was stirred at rt for 14 hours and then acetic acid (2 drops) was added at 0^0C. The solvent was evaporated and water (10ml) was added. The aqueous layer was separarted and extracted with methylene chloride (2×10ml). The combined organic extracts were washed, dried, and concentrated. Purification of the crude product by [illegible]h chromatography (methylene chloride/acetone 95:5) provided **131** (4.8mg, [illegible]6). Ir υ_{max}: 3460, 2954, 2925, 1733, 1436, 1256, 1196, 1177, 1164, 1148, 1139 cm^{-1}; ^{1}Hnmr (360MHz): δ 5.69 (1H, brs, H-3), 4.14 (1H, brs, H-2), 3.76 (6H, s, OCH_3), 2.73 (1H, dd, 6,13, H-9), 2.50 (1H, dd, 7,14, H-7), 2.22 (2H, m, H-5), 1.84 (2H, m, H-9 and H-1), 1.76 (1H, m, H-6), 1.75 (3H, s, 4-CH_3), 1.62 (1H, m, H-7), 1.16 (1H, brs, OH); ^{13}Cnmr (90MHz): δ 169.35 (s, CO), 139.74 (s, C-4), 123.86 (d, C-3), 64.62 (d, C-2), 58.26 (s, C-8), 52.69 (q, OCH_3), 46.43 (t, C-7), 40.17 (d, C-6), 36.75 (d, C-1), 34.51 (t, C-5), 29.68 (t,

C-9), 23.51 (q, 4-CH_3); Hrms: m/z 268.1307 (calcd for $C_{14}H_{20}O_5$, 268.1311, 11.4%), 250 (13), 219 (15), 208 (92), 191 (20), 176 (42), 161 (22), 149 (86), 145 (59), 136 (25), 131 (60), 123 (50), 118 (24), 113 (46), 109 (100).

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