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UNIVERSITY OF ALBERTA

# SYNTHETIC STUDIES ON LONGIFOLENE
# AND
# CONSTITUENTS of *GUANG JISHENG*

BY

JOHN E. YULE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA
SPRING, 1990

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submitted by JOHN E. YULE in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

Supervisor
H.J. Liu

W.A. Ayer

Robert B Jordan
R.B. Jordan

E.V. Blackburn

Date: MARCH 30, 1990

To Ang

# ABSTRACT

In the first part of this thesis, some synthetic studies on longifolene were undertaken. Two routes were used each dictated by the starting dienophile. For the first route, maleic anhydride was reacted with 6,6-dimethylfulvene to give the favored *exo* Diels-Alder adduct **51**. Acid induced cyclization and esterification gave **53**. Epimerization of the ester, hydrogenation and conversion of the lactone into the α,β-unsaturated ketone-ester **56** was followed by a 1,4-addition to give **57**. Ring expansion of **57** was done using ethyl diazoacetate in the presence of $BF_3 \cdot OEt_2$ to give a mixture of regioisomers **59a** and **59b**. Decarboxylation using lithium iodide–collidine gave keto-acids **60a** and **60b**. Esterification gave the ring-expanded keto-esters **61a** and **61b**. Several suggestions to complete the synthesis from this point are put forward.

In the second approach, citraconic anhydride was used as the starting dienophile. Reaction with 6,6-dimethylfulvene gave the desired *exo* Diels-Alder adduct **63**. Cyclization induced by strong acid and esterification resulted in the lactone-ester **66**. Hydrogenation of **66** followed by acid induced rearrangement of the lactone gave the α,β-unsaturated keto-ester **69a** and **69b**.

In the second part of this thesis, the petroleum ether extracts of *Loranthus parasiticus* were investigated. Ten known compounds were isolated along with hydrocarbon and long chain ester fractions. The known compounds isolated were β-amyrin acetate (**74**), lupeol acetate (**75**), friedelin (**76**), amyrenone (**77**), lupeone (**78**), β-amyrin (**79**), lupeol (**80**),

β-sitosterol (**81**), stigmasterol (**82**) and 24(*S*)-ethylcholesta-$\Delta^{5,22(E),25}$-triene-3β-ol (**83**). Only β-sitosterol was previously reported for this plant. The identity of these compounds was established by comparison either with authentic samples or with literature information.

# Acknowledgements

I would like to take this opportunity to thank Prof. H.J. Liu for his guidance and outstanding patience and support during the course of this work and for his assistance with the prepartion of this thesis. His financial support during the latter part of my stay was very much appreciated.

To my wife, Angela, a very special thanks for encouraging me take this chance.

Finally to acknowledge the support from friends made here at the U. of A.: to Dan Raymond and Jianliang Xiao, to the members past and present of Dr.Liu's group and in particular to Tricia McKenzie and Gerardo Ulibarri for running many of the nmr spectra used in this thesis; to Dr. Milan Ralitsch for his help with the interpretation of some of the nmr experiments and most of all for his assistance when I was struggling with the computer in its more temperamental moments; to Dr. B.H. Huang and Dr. S.C. Feng a special thanks for all their advice on the isolation work. I feel fortunate that I had the opportunity to share their years of experience. The completion of this thesis would not have been possible without the able assistance of the technical staff of this department: R. Swindlehurst, D. Formanski, J. Hoyle, D. Mahlow and A. Dunn in the Spectral Services and Microanalytical Laboratories, Dr. A.M. Hogg, L. Harrower, D. Morgan, J. Olekszyk and A. Jodhan on the Mass Spectrometry Laboratory, T.T. Nakashima, T. Brisbane, G. Bigam, L. Kong and G. Aarts in the NMR Spectrometry Laboratory and all the personnel in the Electronics, Glass Blowing and Machine Shops.

Last but not least, I would like to thank the University of Alberta for financial support.

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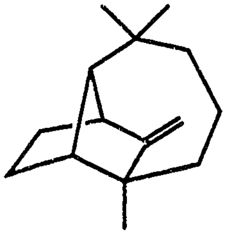
# Part 1

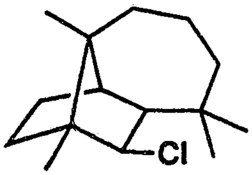
# Synthetic Studies on Longifolene

# INTRODUCTION

Longifolene is a tricyclic sesquiterpene first isolated in 1920 by J.L. Simonsen[1] as one of the constituents of Indian turpentine obtained from *Pinus longifolia* Roxb.. In 1953, the work of two goups disclosed the correct structure of (+)-longifolene (**1**). Moffett[2] completed an X-ray crystallographic study of the hydrochloride **2**, while Ourisson[3] undertook a detailed chemical investigation, from which it was recognized that the hydrochloride structure arose from a Wagner-Meerwein rearrangement during the addition of hydrogen chloride. Such structural rearrangements are one of the most studied aspects of the chemistry of longifolene.[4, 5] The enantiomer (-)-longifolene is much rarer but has been isolated from the liverwort *Scapania undulata* (L.) Dum.[6] and from the fungus *Helminthosporium sativum.*[7]



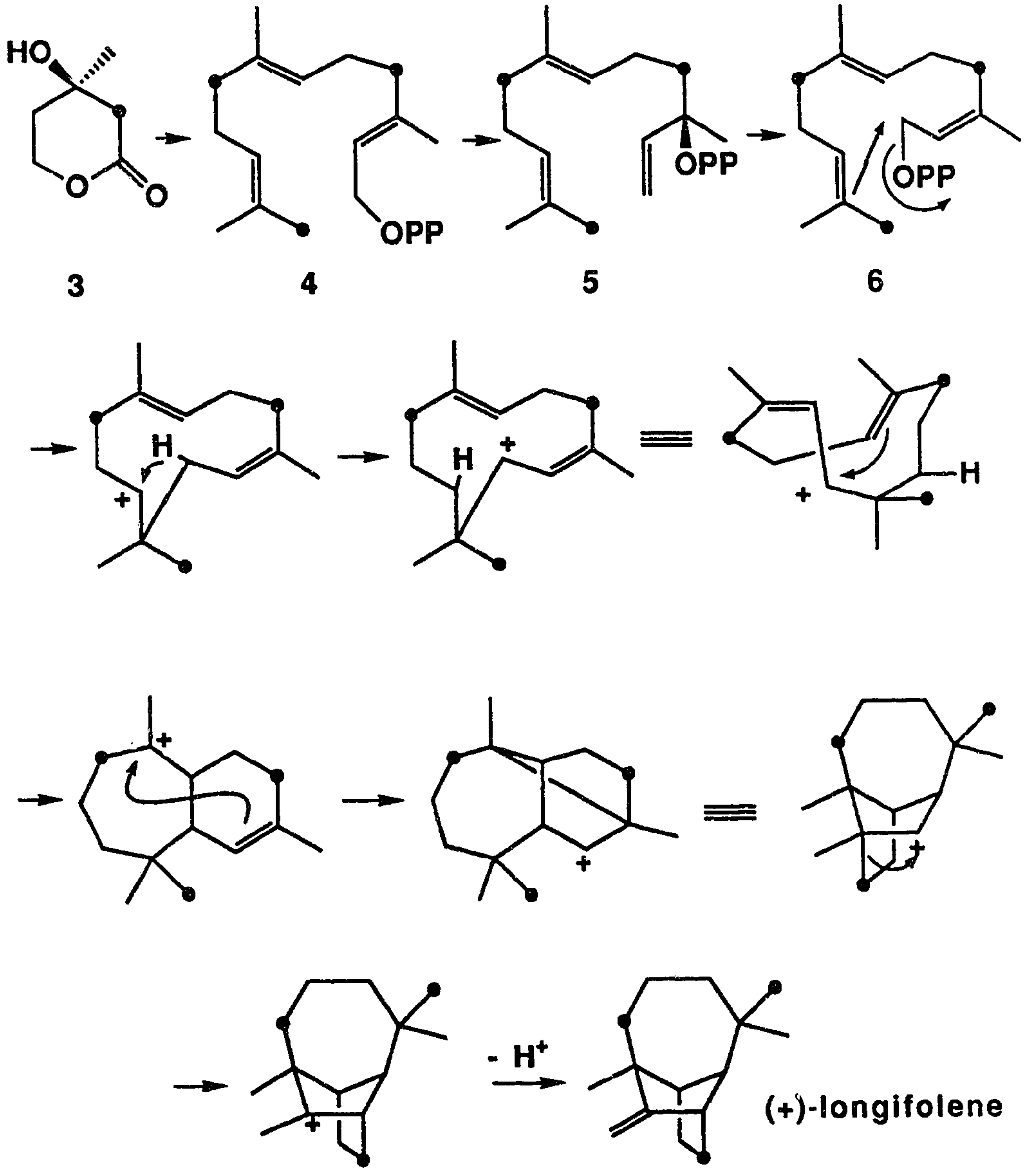
**1**



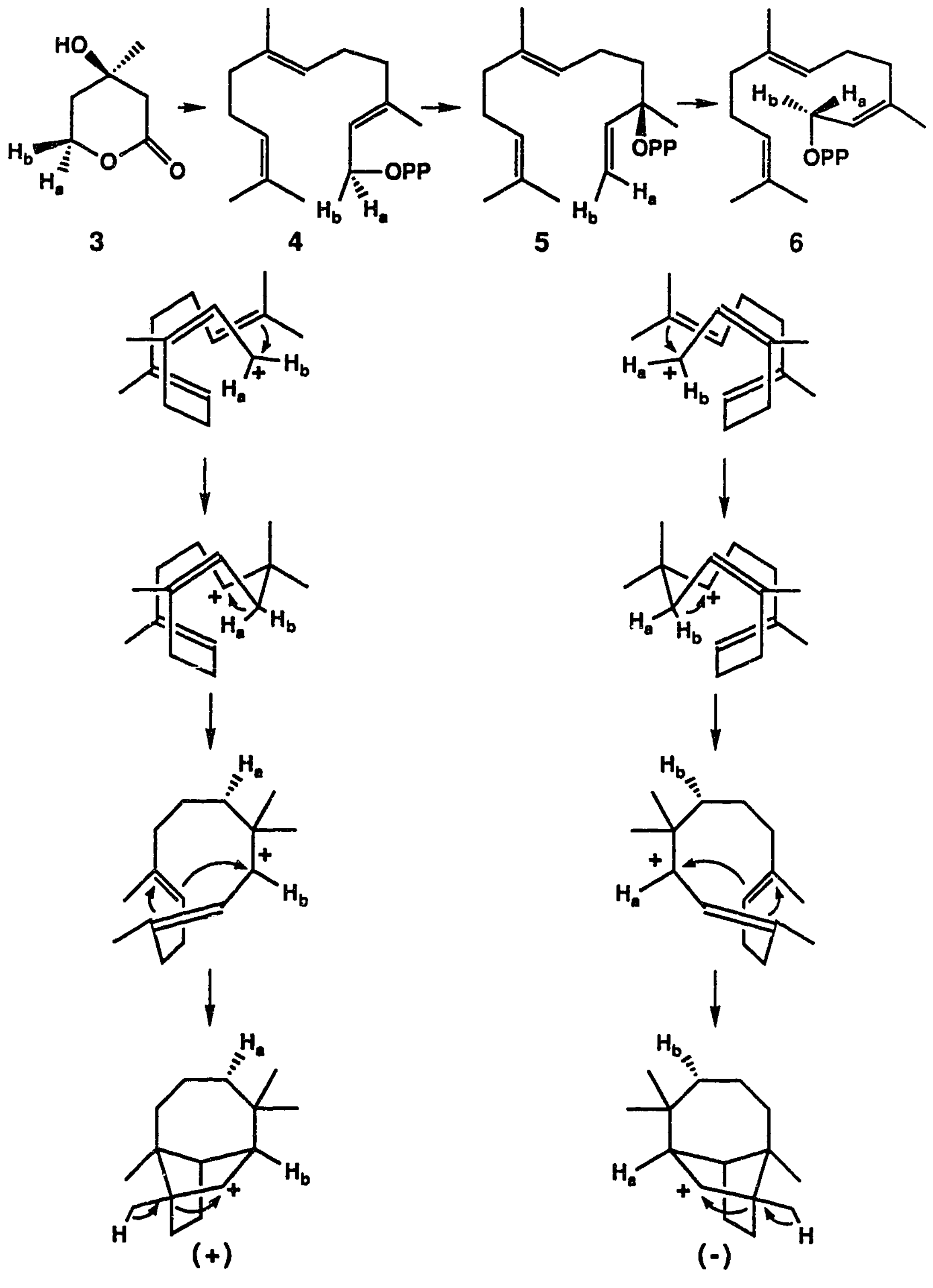
**2**

In order to more closely examine the biosynthesis of (+)- and (-)-**1**, Arigoni and his co-workers[7] administered [2-$^{14}$C]-mevalonate (**3**) to young cuttings of *Pinus ponderosa.* They observed an incorporation of activity of 0.1 to 0.2%. This reasonable incorporation of activity provided evidence for the biosynthetic pathway outlined in Figure 1.[8]

# FIGURE 1



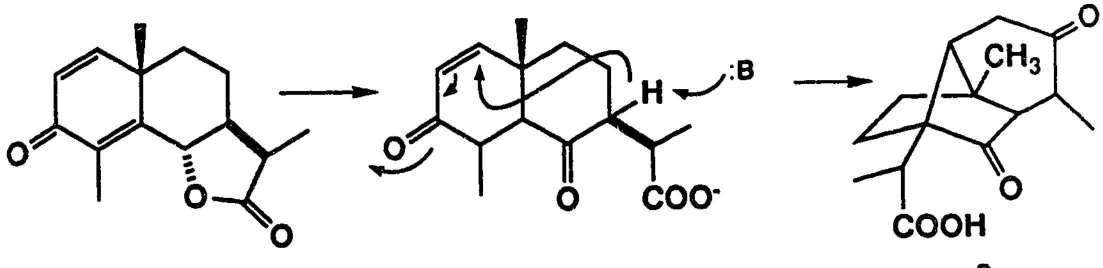
# FIGURE 2


HO
$H_b$
$H_a$
O
O
3
OPP
$H_b$
$H_a$
4
OPP
$H_b$
$H_a$
5
$H_b$
$H_a$
OPP
6
$H_a$
$H_b$
+
$H_a$
$H_b$
+
$H_a$
$H_b$
$H_a$
$H_b$
$H_a$
$H_b$
+
$H_b$
$H_a$
+
$H_a$
$H_b$
+
H
(+)
$H_b$
$H_a$
+
H
(-)

A substantial number of sesquiterpenes require a *cis* carbon-carbon double bond corresponding to the C2–C3 double bond in their *trans, trans*-farnesyl pyrophosphate precursor **4**. This double bond isomerization is thought to occur via a nerolidyl pyrophosphate intermediate **5** with subsequent rotation about the C2–C3 single bond generated in this intermediate.[7, 9] This isomerization scheme was advanced by Arigoni to explain how the folding and stereochemistry of the *cis, trans*-farnesyl pyrophosphate precursor **6** could account for the observed 1,3-hydride shift and the absolute configuration of the product. This is shown in Figure 2.[8]

The tricyclic carbon framework of longifolene has attracted the synthetic attentions of several groups, each elaborating the structure in a unique fashion. The first total synthesis of racemic and enantiomerically pure (+)-**1** was carried out under the direction of E.J.Corey in 1964.[10] The approach adopted stemmed from an observed rearrangement of santonin (**7**) to santonic acid (**8**)[11] under the influence of alkali as illustrated in Figure 3.

**FIGURE 3**



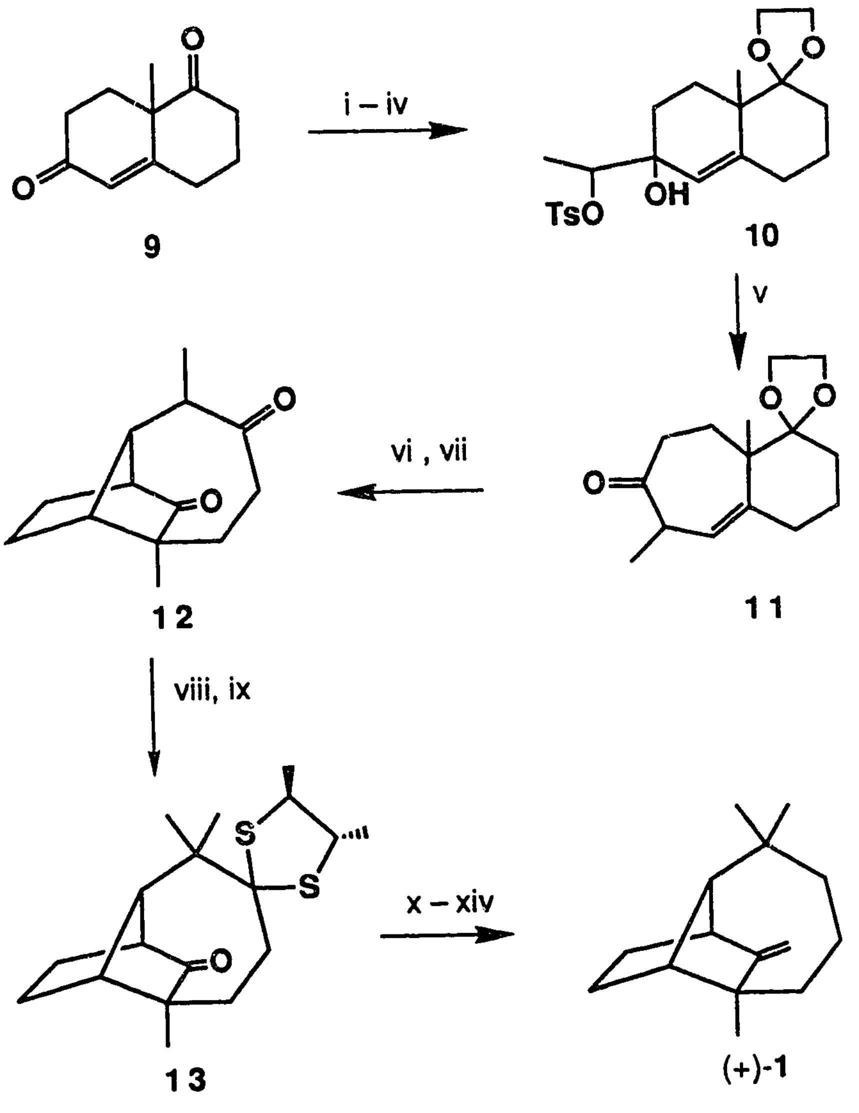
**7** **8**

Starting with the Wieland-Miescher ketone **9** shown in Scheme 1, the desired ring expanded intermediate **11** was produced via a pinacol rearrangement from **10**. Although the intramolecular Michael cyclization of this intermediate proved to be much more difficult than in the santonin case, the required cyclized product **12** was eventually obtained in 10 to 20% yield along with considerable decomposition products.

Using L-(+)-2,3-butanedithiol for protection and resolution of the seven-membered ring ketone **13**, Corey followed up with a sequence of reductions, an oxidation and finally treatment with methyllithium followed by a reaction with thionyl chloride in pyridine to obtain enantiomerically pure (+)-**1** in poor yield in fourteen steps.

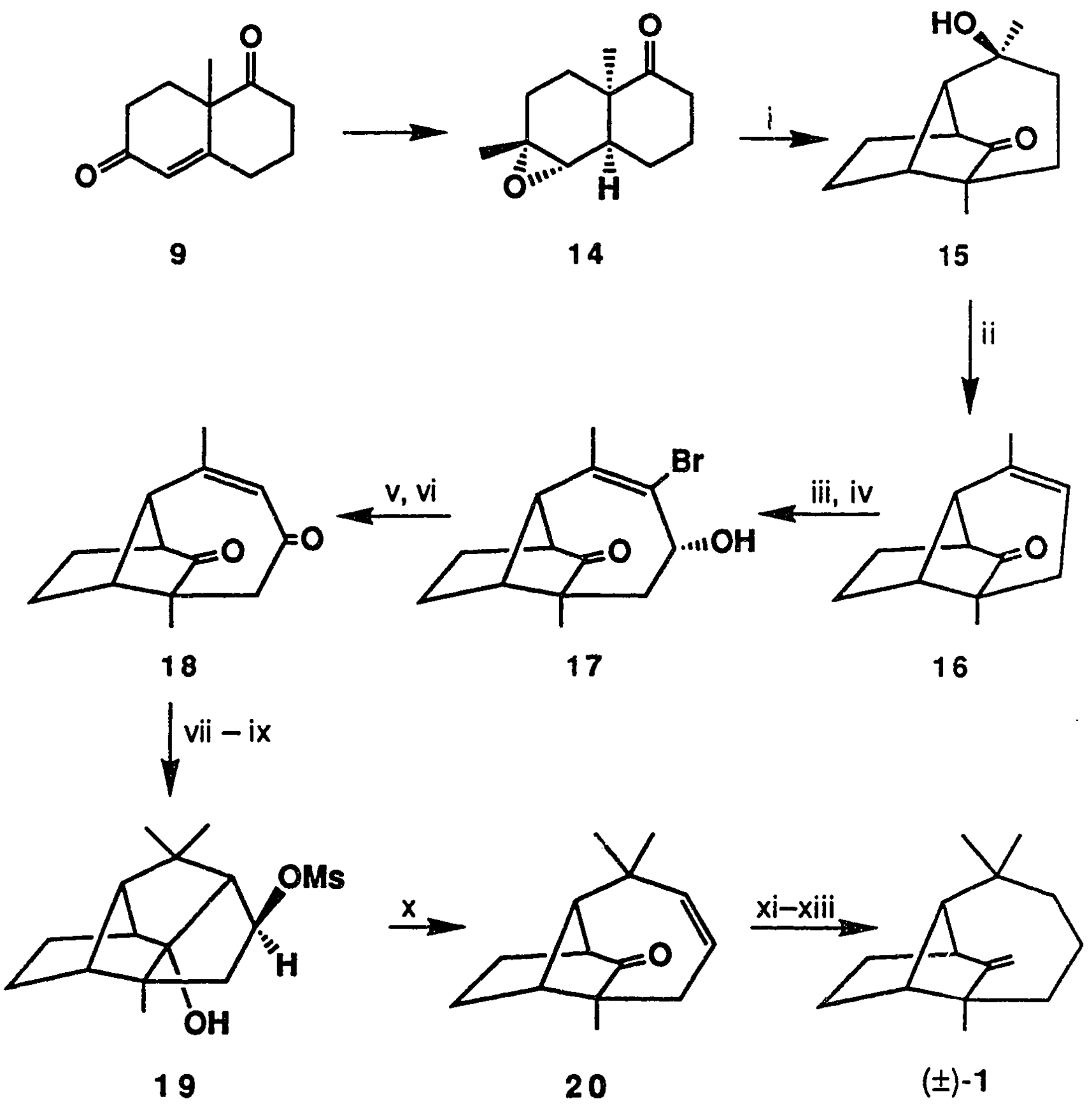
In 1972, McMurry and Isser [12] synthesized (±)-longifolene from the same starting material as used by Corey's group. In the McMurry approach, outlined in Scheme 2, an intramolecular alkylation of the bicyclic keto-epoxide **14** established the tricyclic skeleton **15**. Dehydration of the keto-alcohol **15** produced the keto-olefin **16**. Silver assisted solvolysis of the product, formed on addition of dibromocarbene to **16**, provided the required ring expanded intermediate **17**. Debromination, followed by oxidation of the alcohol functionality of **17**, gave **18**. Treatment of **18** with dimethylcopper lithium produced a tetracyclic ketol which was then reduced and treated with methanesulphonyl chloride to give **19**. Treatment of **19** with strong base returned the required tricyclic system in the form of the keto-olefin **20**. Reduction with Wilkinson's catalyst led to the same ketone intermediate as Corey had obtained, and so the synthesis was completed in the same manner. This seventeen step synthesis had an overall yield of less than 8%.

## SCHEME 1



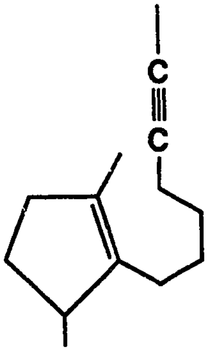
i. $(CH_2OH)_2$, *p*-TsOH ; ii. $Ph_3P{=}CHMe$ ; iii. $OsO_4$; iv. TsCl , Py ; v. $LiClO_4$ , $CaCO_3$ ; vi. aq HCl, 100°C ; vii. $Et_3N$ , $(CH_2OH)_2$, 225°C ; viii. $Ph_3CNa$ , MeI ; ix. L-(+)-2,3-butanedithiol, $BF_3{\bullet}OEt_2$; x. LAH ; xi. Na, $(CH_2OH)_2$, $H_2NNH_2$, 195°C; xii. $CrO_3$, 50% HOAc, rt; aq $MnSO_4$, 55°C; MeOH; xiii. MeLi ; xiv. $SOCl_2$, Py

## SCHEME 2



i. $CH_3S(O)CH_2^-$, DMSO, 60°C; ii. 50% $H_2SO_4$; iii. $CHBr_3$, KO*t*-Bu; iv. $AgClO_4$, $(CH_3)_2CO/H_2O$ (95:5), 45°C; v. Na, 10% MeOH-$NH_3$ (l); vi. $CrO_3$, py; vii. $Me_2CuLi$; viii. $NaBH_4$; ix. MsCl, $NEt_3$, $CH_2Cl_2$; x. KO*t*-Bu, 45°C; xi. $(Ph_3P)_3RhCl$, $H_2$ (1 atm); xii. MeLi; xiii. $SOCl_2$, py

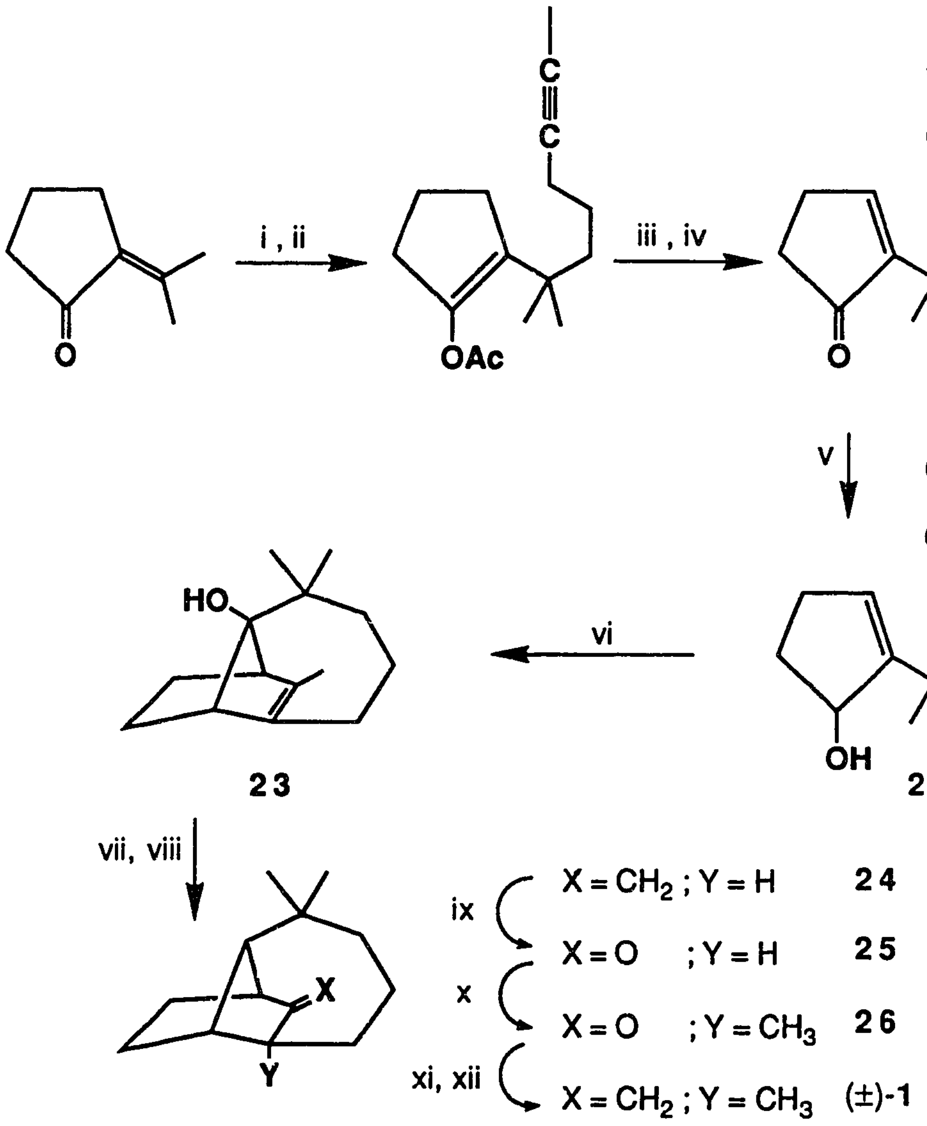
In 1975, the group of W.S.Johnson[13] discovered a more efficient route to racemic longifolene. The discovery came during their work in developing a new hydroazulene synthesis that used a stannic chloride catalysed cyclization of the heptynylmethylcyclopentenol **21**.



**21**

In the Johnson longifolene synthesis, shown in Scheme 3, the key step was the cyclizaton of the enynol **22** using trifluoroacetic acid in ether at 0°C. This step gave the required tricyclic carbon skeleton **23**. Because no further ring expansion steps were needed, this eleven step procedure gave (±)-**1** in 21% overall yield, a major improvement on the methods of Corey and McMurry. Hydrogenolysis of the hydroxyl group of **23**, acidic isomerization of the double bond to the exocyclic position in **24** and ruthenium tetroxide catalysed periodate oxidation of this exocyclic double bond produced the ketone **25**. Since then, this ketone has served as the target for other synthetic studies. Completion of the target molecule was accomplished by treating **25** with lithium diisopropylamide (LDA) and methyl iodide to introduce the angular methyl group in compound **26**. Longifolene was formed from **26** as in the previous two studies.

## SCHEME 3

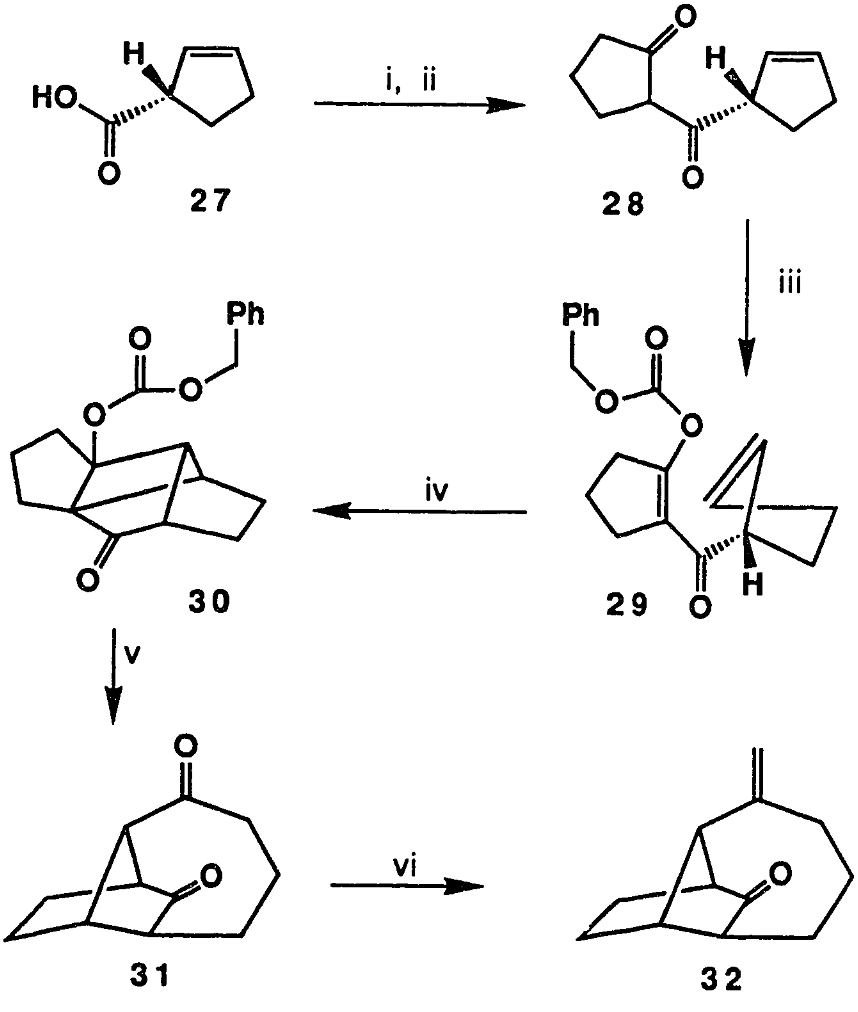


i. CuLi $[(CH_2)_3C{\equiv}CCH_3]_2$ ; ii. $CH_3COCl$ ; iii. $CH_3Li$, $Et_2O$, 0°C; $Br_2$, -78°C, $CH_2Cl_2$; iv. 2,4,6-$(CH_3)_3C_6H_2CO_2N(CH_3)_4$, $(CH_3)_2CO$, 25°C; v. LAH, $Et_2O$, 0°C; vi. $CF_3COOH$, $Et_2O$, 0°C; $K_2CO_3$; vii. $ZnBr_2$, $NaBH_3CN$, $Et_2O$, 25°C, viii. *p*-TsOH, 25°C, ix. $RuO_2$, 50% $H_2O$/*t*-BuOH, $H_5IO_6$–$NaIO_4$, 25°C, x. LDA, THF; MeI, -78° to 25°C; xi. MeLi; xii. $SOCl_2$, py

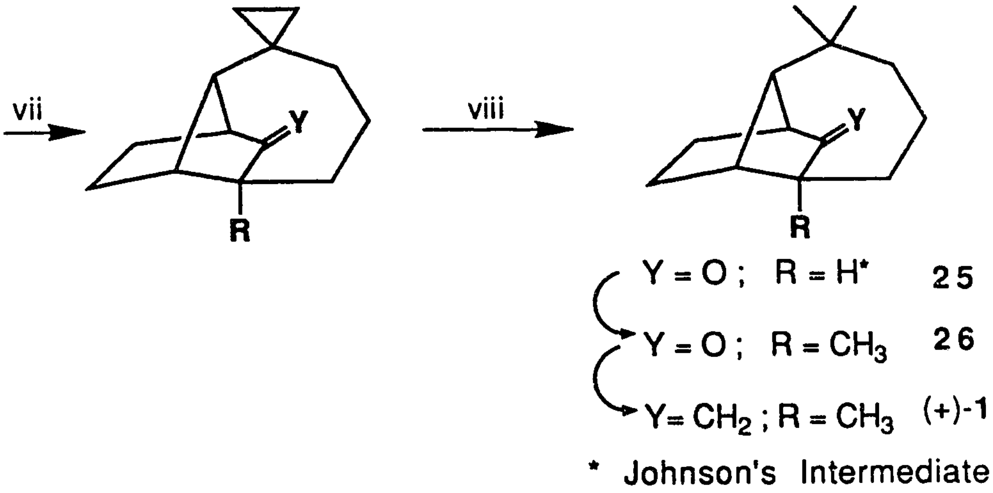
Oppolzer and Godel,[14, 15] in their synthesis, used the enantiomerically enriched (95%) acid *S*-(-)-**27** to effectively induce the correct absolute configuration at the remaining chiral centers of longifolene. Using mild reaction conditions to avoid epimerization, they first converted **27** into its acyl chloride and followed this with enamine acylation and hydrolysis to obtain **28**. Acylation with benzyl chloroformate/pyridine converted **28** into the enol carbonate **29**. Irradiation of **29** caused an intramolecular photoaddition (an intramolecular de Mayo reaction[16]) to produce the key chiral intermediate **30**. Hydrogenolysis ($H_2$, 10% Pd-C) of **30** resulted in a retroaldol reaction producing the tricyclic diketone **31** as outline in Scheme 4. This approach provided a unique two-carbon ring expansion to produce the desired carbon skeleton. A Wittig reaction converted **31** into **32**. Following this, a Simmons-Smith reaction and hydrogenolysis introduced the geminal methyls giving the Johnson intermediate **25** to effectively complete the synthesis. The authors reported a 26% overall yield of (±)-**1**, in nine steps, from the (±)-acyl chloride derivative of **27** and a 16% overall yield of (+)-**1**, in nine steps, from the (*S*)-acyl chloride derivative of **27**.

Based upon the investigations of other groups[17] into the de Mayo reaction, the authors initially used an enol acetate derivative of the the cyclic 1,3-dione **33** (Figure 4), but abandoned this approach because they felt that regioselective geminal dimethylation of the keto olefin **34**, formed using this approach, would impose too many extra steps.

## SCHEME 4

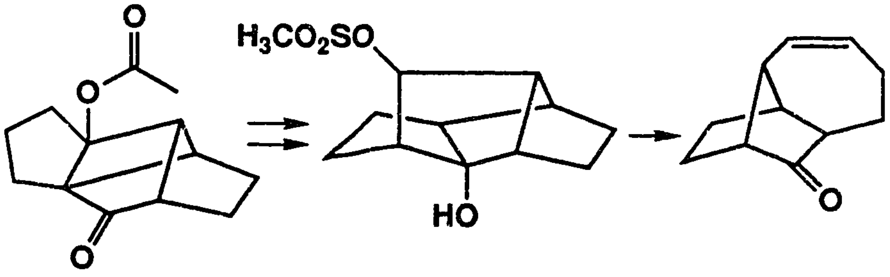


## SCHEME 4 (cont'd)



i. $(COCl)_2$, $CH_2Cl_2$, 0°C →rt; ii. 1-morpholino-1-cyclopentene, rt; 3.5 N HCl, reflux; iii. $ClCO_2CH_2Ph$, py, THF, 5°C; iv. light (Hg); v. $H_2$ (1 atm), 10% Pd-C, HOAc; vi. $PH_3PCH_3Br$, Na *t*-pentylate, $PhCH_3$; vii. $CH_2I_2$, Zn, AgOAc, HOAc; viii. $PtO_2$, $H_2$ (1 atm), HOAc, rt

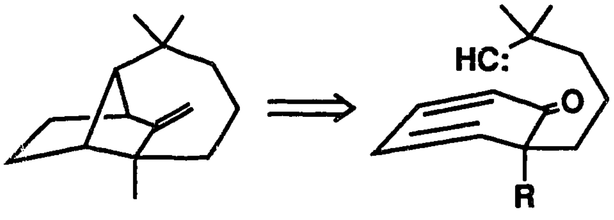
## FIGURE 4



**33** **34**

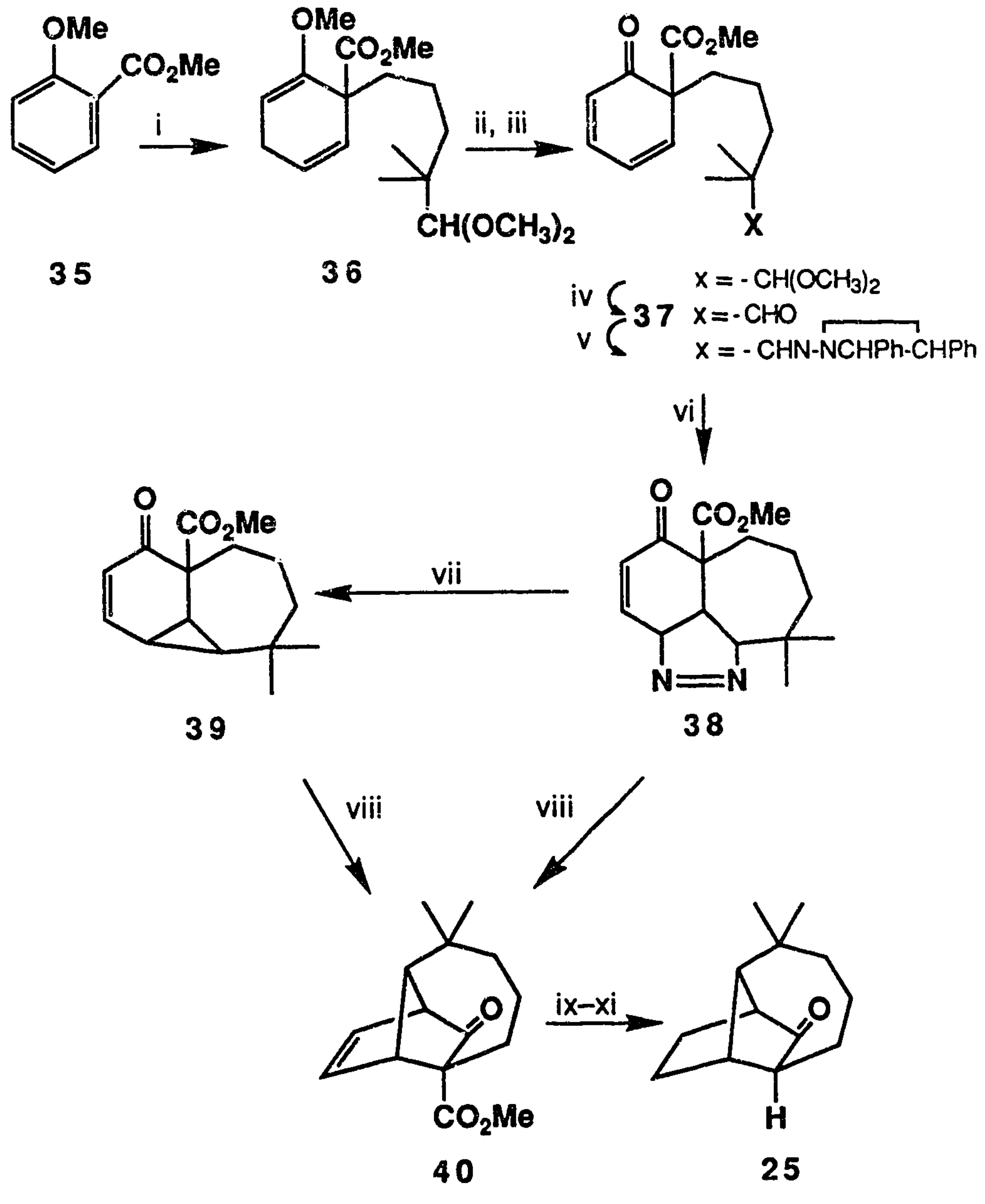
In 1985, Shultz and Puig[18] prepared (±)-longifolene and enantiomerically pure (-)-longifolene using a reaction synthetically equivalent to an intramolecular cycloaddition between a diene and a carbene (Figure 5). Their approach, as illustrated in Scheme 5, led to the direct construction of the seven-membered ring, and saved several ring expansion steps.

## FIGURE 5



Birch reduction of methyl 2-methoxybenzoate **35** followed by alkylation using the dimethyl acetal of 2,2-dimethyl-5-iodopentanal gave the cyclohexadiene **36**.

## SCHEME 5

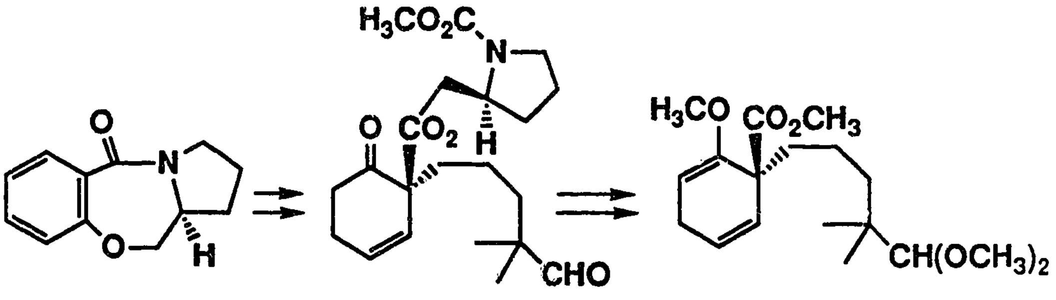


i. Birch reduction; alkylation; ii. *N*-Bromoacetamide, MeOH; iii. DBN, $PhCH_3$, reflux; iv. $(CH_3)$ [illegible] p-TsOH, reflux; v. 1-Amino-*trans*-2,3-diphenylaziridine; vi. $PhCH_3$, reflux; vii. h [illegible] PhH; viii. Xylene, reflux; ix. $H_2$ (1 atm), 5% Pd-C, EtOH; x. KOH, MeOH-[illegible]$_3$, reflux

Treatment of aldehyde **37** with 1-amino-*trans*-2,3-diphenylaziridine in ether at 8°C produced an aziridinyl imine which, on thermolysis in refluxing toluene, produced **38**. Photolysis of **38** in benzene produced the cyclopropane derivative **39**. In refluxing xylene, **38** or **39** gave the tricyclic ketone-ester **40** via what is believed to be the diene-carbene intermediate. Compound **40** was subsequently hydrogenated and decarbomethoxylated to give the Johnson ketone **25** in ten steps and 30% yield from **35**.

Shultz and Puig were also able to synthesize (-)-longifolene starting with the chiral benzoxazepenone **41**, which was used to control the stereochemistry of the intermediate cyclohexadiene **43**. This is outlined in Figure 6.

**FIGURE 6**



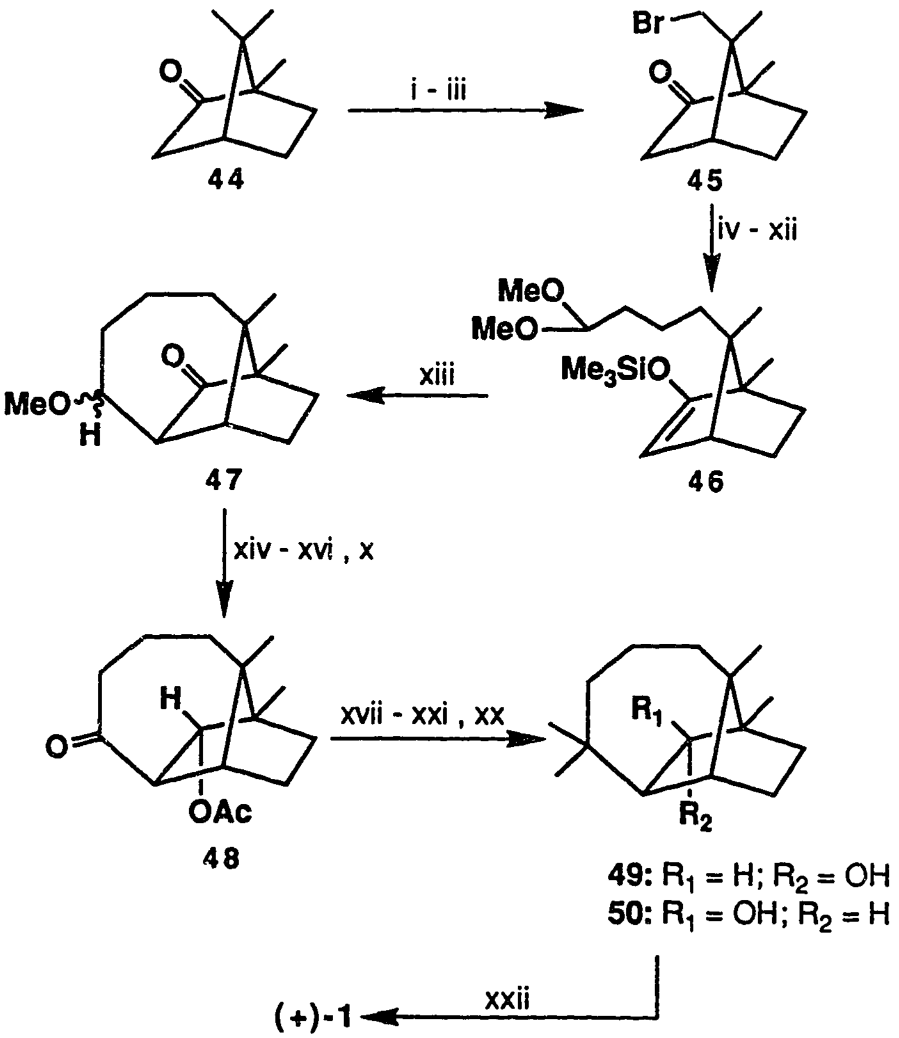
**41** **42** **43**

Reductive alkylation of **41** followed by treatment with methanol-hydrochloric acid and *N*-acylation with methylchloroformate-sodium bicarbonate gave the urethane **42**. Refluxing **42** in methanol-trimethyl orthoformate-hydrogen chloride followed by transesterification of the resulting acetal-enol ether using sodium methoxide in methanol produced optically active **43** in 72% overall yield from **41**.

Money and Kuo's enantiospecific synthesis of (+)-longifolene[19] (Scheme 6) developed from some well studied sesquiterpene chemistry within the Money group involving (+)-camphor (**44**), (-)-camphor or their derivatives, as starting materials.[20, 21, 22, 23, 24] In this case, (+)-8-bromocamphor (**45**) was converted to the chiral trimethylsilyl enol ether acetal **46** and then cyclized under Mukiayama conditions[25, 26] to provide the tricyclic methoxy ketone **47**. Further reduction, acetylation, demethylation and oxidation steps resulted in the keto-acetate **48**. This compound was then converted into (+)-longiborneol (**49**) by first following a sequence similar to that used by Oppolzer (*vide supra*) in order to introduce the geminal methyls and then reducing the ester to give the alcohol. A further oxidation and reduction of **49** produced isolongiborneol (**50**). Upon treatment with methanesulphonyl chloride and 4-dimethylaminopyridine-pyridine, **50** underwent a Wagner-Meerwein rearrangement to provide (+)-**1**.

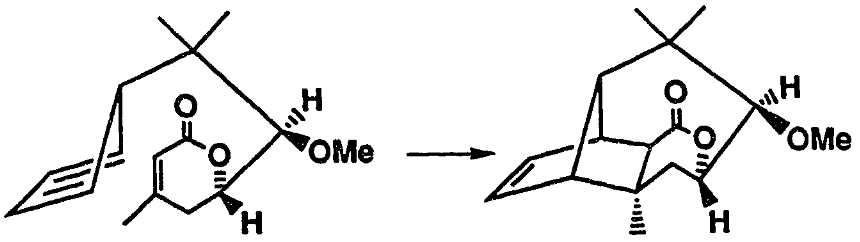
We have recently been made aware of another total synthesis of longifolene by Fallis and co-workers.[27] The key step of this synthesis involved an intramolecular [4+2] cycloaddition that stereoselectively led to the desired longifolene carbon skeleton (Figure 7).

## SCHEME 6

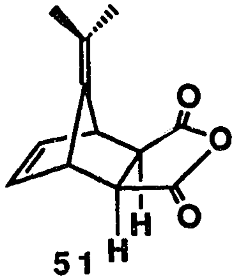


i. $Br_2$, HBr, HOAc, 110°C; ii. $Br_2$, $ClSO_3H$; iii. Zn, HOAc, $Et_2O$, 0°C; iv. KI, DMSO, 110°C; v. $Me_3SiCl$, $(CH_2OH)_2$; vi. NaCN, DMSO, 70°C; vii. LDA, THF, -78°C, $t$-$BuMe_2SiO(CH_2)_3Br$; viii. K, HMPA, $Et_2O$, $t$-BuOH, 0°C ; ix. HCl, $(CH_3)_2CO$; x. PDC, $CH_2Cl_2$ xi. $HC(OMe)_3$, $CeCl_3$, MeOH; xii. LDA, THF, -78°C; $Me_3SiCl$; xiii. $TiCl_4$, -78°C; xiv. Ca/ $NH_3$ (l); xv. $Ac_2O$, DMAP, py; xvi. $BBr_3$, 15-Crown-5, NaI, $CH_2Cl_2$; xvii. $Ph_3MePBr$, BuLi, -78°C to 20°C; xviii. $Et_2Zn$, $CH_2Cl_2$, $PhCH_3$; xix. $H_2$ (2.5 atm)/Pt, HOAc; xx. LAH, THF; xxi. PCC, $CH_2Cl_2$; xxii. $MeSO_2Cl$, py, DMAP, 100°C

FIGURE 7



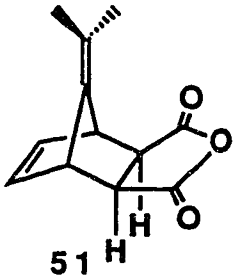
In our synthetic approach to longifolene compound **51**, readily available by the addition of maleic anhydride to 6,6-dimethylfulvene, was selected as the starting material. This compound possesses the required bicyclo[2.2.1]heptane skeleton found in the target molecule. The existing carbonyl group and the tetrasubstituted double bond could be used to elaborate the required tricyclic ring system as detailed in chapter one of this thesis.



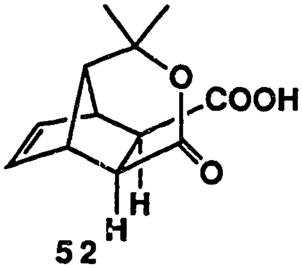
51

# RESULTS AND DISCUSSON

Alder and his co-workers found that when 6,6-dimethylfulvene and maleic anhydride reacted together in refluxing benzene, the *exo*-adduct **51** was formed as the thermodynamically favored product.[28] This was confirmed when Alder found that refluxing the *endo* isomer in benzene also produced **51**.



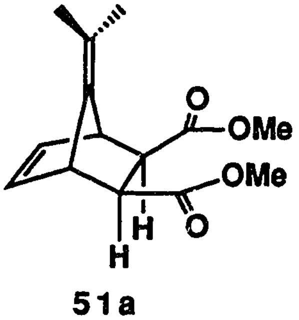
**51**



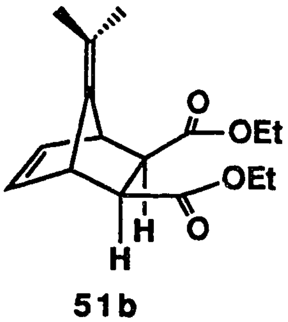
**52**

Following Alder's procedure, we obtained **51** as the major product along with a very small amount of the *endo* isomer. The infrared spectrum of **51** had a strong signal at 1771 cm$^{-1}$ for the carbonyl stretch of the anhydride. A 200 MHz $^1$H nmr spectrum of **51** showed multiplets at $\delta$ 6.45 for the two olefinic protons, at $\delta$ 3.87 for the two *endo* protons and at $\delta$ 3.04 for the two bridgehead protons. A singlet appeared at $\delta$ 1.60 for the two vinylic methyl groups. The *endo* isomer gave a similar pattern for the 200 MHz $^1$H nmr spectrum. However, there were two significant changes in this spectrum. The first change was the downfield shift of the two bridgehead protons to a multiplet at $\delta$ 3.40 and the second change was the downfield shift of the two *exo* protons to a multiplet at $\delta$ 3.94. The multiplet for the olefinic protons appeared at $\delta$ 6.46 while a signal for the six methyl protons appeared as a singlet at $\delta$ 1.61. In the infrared spectrum, the carbonyl stretch for the anhydride appeared at 1783 $cm^{-1}$. Cyclization of the *exo*-adduct **51** followed an established procedure for the production of lactones from unsaturated acids.[29] After purification of the crude solid, by recrystallizing from a mixture of ethyl acetate and petroleum ether, the lactone-acid **52** was obtained in a 55-60 % yield. A strong carbonyl absorption at 1721 $cm^{-1}$ in the infrared spectrum indicated the presence of the δ-lactone and carboxylic acid functionalities. The -OH stretch of the carboxylic acid appeared as a broad absorption around 3000 $cm^{-1}$. Esterification of the lactone acid **52** was accomplished by stirring **52** at room temperature in methanol in the presence of a catalytic amount of sulphuric acid. The methyl ester **53**, that precipitated from this solution, was filtered and washed to give a 92% yield of a white crystalline product. This lactone-ester was identified by two carbonyl stretches in the infrared spectrum at 1748 and 1731 $cm^{-1}$ for the δ-lactone and methyl ester. A singlet at δ 3.49 in the 200 MHz $^1H$ nmr spectrum of **53** corresponded to the methoxy hydrogens. The mass spectrum had a parent peak at m/z 236.1047 in close agreement with the calculated mass of 236.1049 for a molecular formula $C_{13}H_{16}O_4$.

At this point we investigated the possibility of the direct conversion of **51** into **53** in an effort to save time and to improve upon the overall yield. Treatment of **51** with one equivalent of *p*-toluenesulphonic acid in dried methanol produced a mixture from which the only identifiable products were the diester **51a** and the lactone-ester **53** in only a 9% yield. The structure of **51a** was established from the nmr spectrum which showed a singlet at δ 3.62 for the six methoxy protons and a singlet at δ 1.65 for the two geminal vinyl methyl groups. We also tried treating **51** with one equivalent of *d,l*-camphorsulphonic acid in dried ethanol. This led to the same disappointing result, producing **51b** as the only identifiable product.

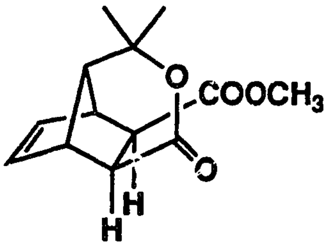


**51a**

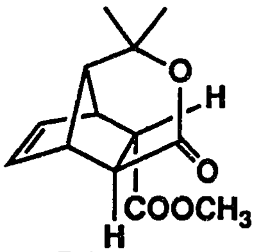


**51b**

Towards the synthesis of longifolene, we planned to convert the δ-lactone present in **53** into a seven-membered ketone in preparation for the ring expansion step. In order to accomplish this conversion we first epimerized the ester functionality of **53** to obtain **54**.

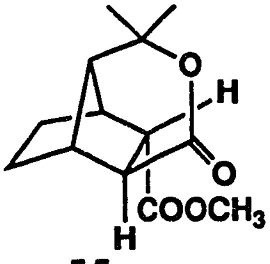


**53**

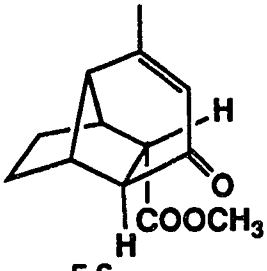


**54**

It was felt that with the ester in the *endo* position any potential interference by the ester function, during the opening of the lactone and the subsequent recyclization to form the enone, would be eliminated. Epimerization was carried out by treating **53** with sodium methoxide in refluxing methanol to give an 82% yield of **54**. The resulting *endo* ester **54** was then hydrogenated under a hydrogen pressure of 15 to 18 p.s.i. using Pd-C as the catalyst and ethyl acetate as the solvent. On completion of the hydrogenation, as determined by tlc, the solution was filtered and concentrated to produce a viscous, colorless liquid that slowly solidified on standing. The disappearance of the olefinic proton signals of **54** at δ 6.25 in the $^1$H nmr spectrum of **55**, confirmed that the hydrogenation was complete. The product **55** was obtained in 98% yield from **54** and was used without further purification in the subsequent step. In 1973, Eaton and co-workers[30] described a method for the preparation of cyclopentenones from their respective lactones. The transformation was effected using a combination of phosphorus pentoxide and methanesulphonic acid. This new reagent was found to be much easier to handle and to work up than polyphosphoric acid traditionally used for this type of transformation. Applying Eaton's procedure, we converted the lactone-ester **55** into the enone-ester **56** in 93% yield. This new product was identified by the appearance, in the $^1$H nmr spectrum, of a multiplet at δ 5.75 for a vinylic hydrogen and a doublet at δ 2.03 for the vinylic methyl group. In the ir spectrum there was a strong absorption at 1678 cm$^{-1}$ for the ketone formed. The mass spectrum displayed a parent ion peak at m/z 220.1099 in agreement with the assigned structure.



**55**

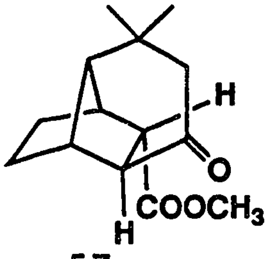


**56**

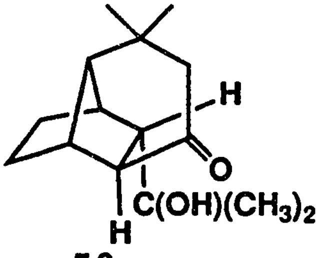
The 1,4-addition reaction to convert **56** into **57** was initially attempted using a dimethylcopper lithium reagent[31] prepared from 1 eq of CuI and

2 eq of methyllithium in dry ether at 0°C and a reaction time of 15 hours. This gave a very poor yield (<10%) of the desired product **57**. In addition to **57**, a small amount of the keto-alcohol **58** was obtained likely as a result of too long a reaction time. The keto-alcohol was identified from its ir spectrum which showed a strong absorption at 3490 $cm^{-1}$ for the hydroxyl group along with a strong band at 1695 $cm^{-1}$ for the ketone. In the $^1H$ nmr spectrum of **58**, the four methyl groups were displayed as singlets at δ 1.28, 1.19, 1.12 and 1.06.

The 1,4-addition reaction was also attempted using methylmagnesium bromide-cuprous iodide without success. In this case a complex mixture of products was obtained from which the keto-alcohol **58** was isolated but none of **57**.



**57**

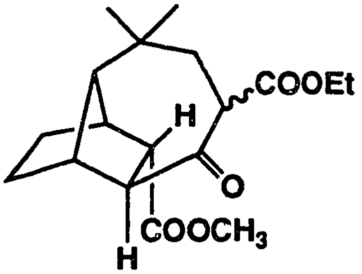


**58**

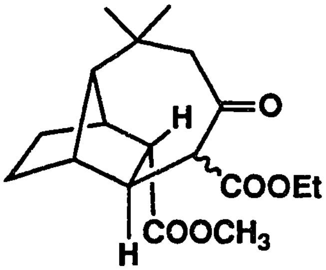
The best results for the 1,4-addition occurred when the dimethylcopper lithium reagent was prepared using 6 eq of methyllithium and 3 eq of cuprous iodide[32] in dry ether at 0°C under argon. In this manner **57** was prepared in 67% yield. The infrared spectrum showed two carbonyl stretches at 1736 $cm^{-1}$ (ester) and 1714 $cm^{-1}$ (ketone). The 400 MHz $^1H$ nmr spectrum of **57** had a singlet at δ 3.68 for the ester methyl, doublets at δ 2.35 and δ 2.21 (J = 18 Hz each) for the methylene hydrogens alpha to the ketone and singlets at δ 1.13 and δ 1.02 for the geminal methyls. A parent ion peak at m/z 236.1407 in the mass spectrum was consistent with a molecular formula of $C_{14}H_{20}O_3$.

With compound **57** in hand, we then explored the ring expansion using a method developed in this laboratory.[33, 34] Thus, the keto-ester **57** was treated with ethyl diazoacetate and boron trifluoride etherate in dried diethyl ether at room temperature for eleven hours. Under these conditions, a 69% yield of ring expanded product was formed on the basis of 83% of consumed starting material. The ring expanded product was found to be a mixture consisting of both regioisomers **59a** and **59b**, with **59b** predominating by a slight amount (1:1.4) (*vide infra*), each existing mainly in the corresponding enol form (**59c**, **59d**). The ir spectrum of the mixture showed carbonyl stretches at 1739 and 1704 $cm^{-1}$, for the ester and ketone respectively. The strong absorption at 1636 $cm^{-1}$ and the medium absorption at 1608 $cm^{-1}$ were characteristic of a $\beta$-hydroxy-$\alpha,\beta$-unsaturated ester moiety. In the $^1H$ nmr spectrum, a singlet was observed at $\delta 13.1$ indicating the presence of a chelated enol hydrogen. The mass spectrum lent support to the overall structural assignment displaying a molecular ion peak at m/z 322.1779 consistent with the expected formula $C_{18}H_{26}O_5$.

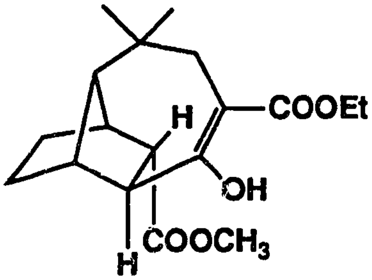
The mixture of $\beta$-ketoesters was subjected to decarboethoxylation using lithium iodide-collidine reagent.[34] The resulting mixture of keto-acids **60a** and **60b** thus obtained, in 43% yield, was esterified using a method developed in this lab.[35] Compounds **60a** and **60b** were converted into the methyl esters **61a** and **61b** by stirring with potassium bicarbonate (or potassium carbonate) and methyl iodide in dry acetone at room temperature. In this manner **61a** and **61b**, which were partially separable by flash chromatography on a silica gel column, were formed



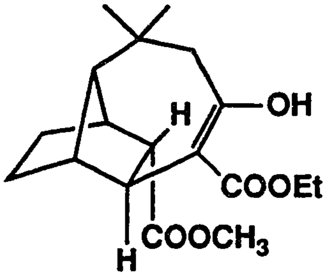
**59a**



**59b**



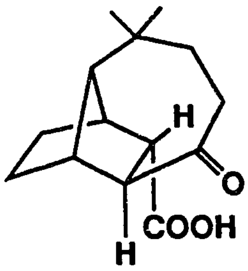
**59c**



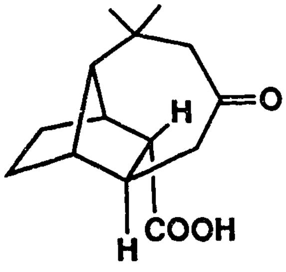
**59d**

in 59% total yield from **60a** and **60b**. The methyl esters were confirmed by the appearance of two carbonyl peaks at 1726 and 1689 cm$^{-1}$ in the infrared spectrum and the singlet for the methoxy protons at δ 3.73 in the $^1$H nmr spectrum.

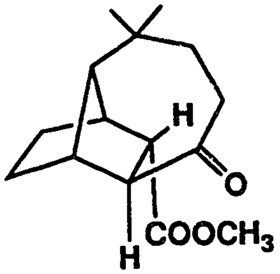
In the course of purification of the ester ketone mixture, two fractions were obtained. In the 300 MHz $^1$H nmr spectrum of **61b**, the pure minor fraction, two low field sets of signals were observed, each with a large coupling constant indicative of geminal couplings for two methylene groups each alpha to the ketone. This made **61b** the structure of choice for the minor fraction. The $^1$H nmr spectrum of **61b** showed a doublet of doublets of doublets (ddd) at δ 3.2 and was assigned to the hydrogen alpha to the ester. A doublet that appeared at δ 2.78 (J = 12 Hz), typical of a geminal coupling, was assigned to one of the hydrogens at C4. The other hydrogen at this position was assigned to a ddd at δ 2.2 (J = 12, 1, 1 Hz). The two smaller coupling constants at δ 2.2 were attributed to small W-couplings to the hydrogens at C6 and C2. Another ddd appeared at δ 2.67 for two hydrogens one of which was thought to be one of the hydrogens at C2. The other C2 hydrogen appears as a dd signal at δ 2.32 (J = 16.5, 3 Hz). Finally there was a multiplet at δ 2.43 for the hydrogen at C1 and a dd signal at δ 2.03 for the lone hydrogen at C6.



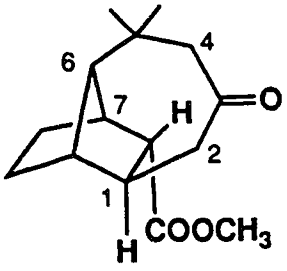
**60a**



**60b**



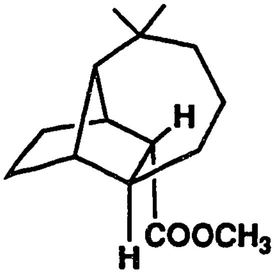
**61a**



**61b**

Decoupling experiments were carried out in order to confirm this assignment. Irradiation of the signal at δ 2.78 saw the ddd signal at δ 2.2 coalesce into a single sharp doublet at δ 2.18 (J = 1 Hz), with loss of the geminal coupling. When the signal at δ 2.67 is irradiated the original doublet of doublets at δ 2.32 are replaced by a doublet at δ 2.32 with loss of the geminal coupling. Such observations would not be expected if **61a** were assigned as this minor portion and a more complex nmr spectrum would be observed for the hydrogens between the ketone and the geminal methyls. Comparison of the pure minor and the mixed fraction bears out this observation.

Although the isomeric ketones **61a** and **61b** were obtained, for the synthesis of the target molecule the location of the ketone carbonyl is of no consequence. Removal of the ketone carbonyl, which could in principle be effected by a thioketalization-desulphurization process, would result in the same intermediate **62**.

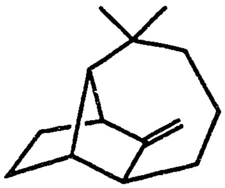


**62**

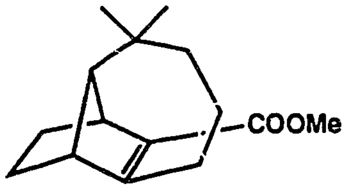
From this point there are several possibile routes to complete the synthesis.

Basically, two operations are required: the angular methyl group must be introduced and the ester function must be converted into an exocyclic double bond. A more obvious approach is the conversion of **62** into the Johnson intermediate **24**. This could be accomplished by a reduction followed by a dehydration.

Alternatively, ester **62** could, in principle, be converted into the α,β-unsaturated ester **62a**. A 1,4-addition followed by subsequent conversion of the ester group into a carbon-carbon double bond would result in completion of the synthesis.

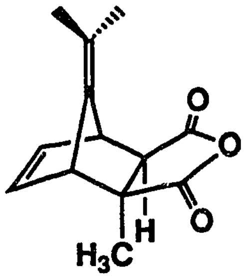


**24**



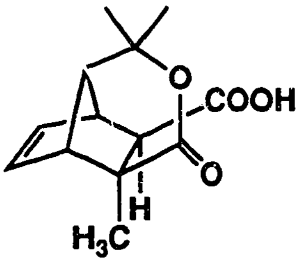
**62a**

We also chose a second route that introduced the angular methyl group of **1** at the beginning of the synthesis. For this purpose we used citraconic anhydride as the starting dienophile. Citraconic anhydride underwent a Diels-Alder reaction with 6,6-dimethylfulvene to produce **63** in 30% yield.

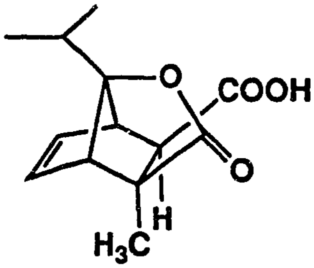


**63**

This route, for all its good intentions, was a disappointment in that it followed the general trend of Diels-Alder reactions toward *endo* products. Attempts to increase the *exo* yield by prolonged refluxing in benzene, using a higher boiling solvent such as toluene, or using Lewis acid catalysts such as $FeCl_3$, $SnCl_4$ or $ZnCl_2$ all failed. The former two approaches merely increased the yield of the *endo* material. The experiments using Lewis acids were carried out by first dissolving the dienophile in dried ether and cooling this solution to 0°C prior to adding the Lewis acid. One equivalent of the Lewis acid was then added followed by addition of the fulvene. In each case a dark brown tar-like material was produced. Subsequent work-up indicated that only decomposition products had been formed. The method settled upon used an excess of dienophile (1.3 eq) with the diene in refluxing benzene for 14 to 16 hours. A 30% yield of the desired product **63** was obtained along with a 13% recovery of 6,6-dimethylfulvene. Cyclization of **63** was carried out under the same conditions as those used to prepare **52** from **51**. In this case both the desired δ-isomer **64** and the other possible γ -isomer **65** were produced in 18 % yield and 8% yield respectively.



**64**

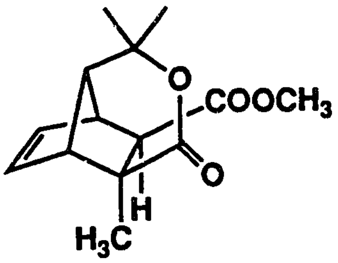


**65**

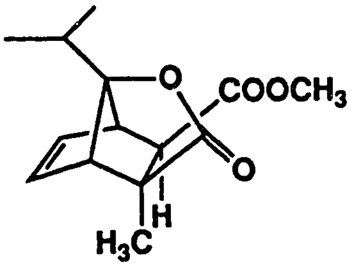
These two isomers were characterized based upon the expected differences in their spectroscopic data. In the infrared spectrum of **64**, two carbonyl peaks were observed at 1737 and 1705 $cm^{-1}$ for the δ-lactone and the carboxylic acid functionality respectively, with a broad peak centered around 2920 $cm^{-1}$ for the carboxylic acid. In the 200 MHz $^1H$ nmr spectrum, **64** had a broad singlet at δ 5.44 for the single acid proton. The three methyl groups appeared as singlets at δ 1.41 for three hydrogens and $\delta$ 1.33 for six hydrogens. The mass spectrum gave a parent peak at m/z 236.1036 consistent with the molecular formula $C_{13}H_{16}O_4$. In contrast to **64**, the ir spectrum for **65** showed carbonyl stretches at 1784 and 1706 $cm^{-1}$ for the $\delta$-lactone and the carboxylic acid respectively. A 300 MHz $^1H$ nmr spectrum pointed out other differences. A multiplet appeared at $\delta$ 2.17 (J = 7 Hz) for the methine hydrogen of the isopropyl group. Doublets at $\delta$ 1.00 (J = 7 Hz) and $\delta$ 0.91 (J = 7 Hz), each for three hydrogens, represented the remaining isopropyl protons. The angular methyl group appeared as a singlet at $\delta$ 1.40. Again the mass spectrum was consistent with the expected molecular formula.

Despite the failure of the previous attempt at a one-step conversion of **51** into **53**, the low yields to this point prompted another try. Compound **63** was treated with *d,l*-camphorsulphonic acid in dried methanol. This met a similar fate giving starting material along with the diester analog of **51a** as the only identifiable product. No further exploration was made on this approach.

Esterification of **64** was carried out using the same procedure as that used to prepare **61a** and **61b**. This method proved much more successful than attempts at using methanol and acid and gave **66** in 75% yield.



**66**

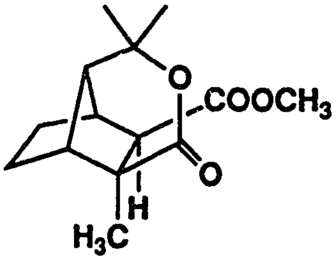


**67**

Esterification of a crude mixture of **64** and **65** produced compound **67** along with **66.** This mixture of the esters **66** and **67** proved much easier to separate than the mixture of the acids **64** and **65**. The ir spectra provided easy distinction between the two lactones. The favored δ-lactone **66** showed a strong carbonyl absorption at 1729 $cm^{-1}$ for the carbonyl of both the ester and lactone, whereas the minor γ-lactone **67** showed two strong carbonyl absorptions, one at 1780 $cm^{-1}$ for the γ-lactone and the other at 1732 $cm^{-1}$ for the ester.

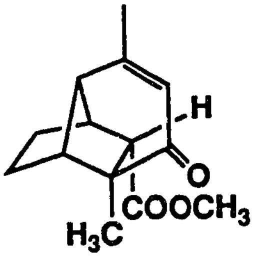
As the next step, epimerization of the methyl ester functionality using sodium methoxide in refluxing methanol failed to give significant amounts of the desired epimer likely as a result of steric interference caused by the angular methyl. The synthesis was then carried through from **66**.

Compound **66** was hydrogenated using the previous conditions to give **68** in 99% yield. The absence of any signals below δ 4.0 in the $^{1}H$ nmr spectrum indicated that olefinic hydrogens were no longer present. The material was used without further purification.

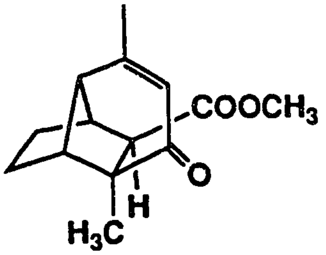


**68**

Treatment of **68** with the phosphorus pentoxide-methanesulphonic acid reagent, as previously carried out on **56**, produced the enone-esters **69a** and **69b**. Unfortunately, mainly decomposition products were obtained suggesting that having the ester in the *exo* position may indeed pose a problem in this step. It appeared from the $^1H$ nmr spectrum, that



**69a**



**69b**

the starting material had at least partially epimerized during the reaction, giving a 13% total yield of two enone-esters **69a** and **69b**, after separation by column chromatography. Compound **69a** (2%) showed two carbonyl stretches in the infrared spectrum at 1737 and 1677 $cm^{-1}$ for the ester and α,β-unsaturated ketone respectively. A 400 MHz $^1H$ nmr spectrum gave a doublet at δ 5.80 (J = 1 Hz) for the vinylic hydrogen, a singlet at δ 3.64 for the methoxy hydrogens, a doublet at δ 2.01 (J = 1.5 Hz) for the vinylic methyl hydrogens and a singlet at δ 1.20 for the angular methyl group. A parent ion peak in the mass spectrum at m/z 234.1256 for a molecular formula of $C_{14}H_{18}O_3$ helped to confirm the assigned structure. By comparison, **69b** (11%) had two carbonyl absorptions, in its infrared spectrum, at 1732 $cm^{-1}$ and 1677 $cm^{-1}$ for the ester and α,β-unsaturated ketone respectively. The 400 MHz $^1H$ nmr spectrum of **69b** was less complex and had significant differences with respect to that of **69a**. The vinylic hydrogen appeared as a doublet at δ 5.68, the signal for the methoxy hydrogens appeared as a singlet at δ 3.53 while the vinylic methyl signal was at δ 1.96. Finally, the angular methyl appeared as a singlet at δ 1.37. The mass spectrum displayed a parent ion peak at m/z 234.1256 in accordance with the assigned structure. Completion of the synthesis from **69a** and **69b** should be straightforward. The 1,4-addition, ring expansion, decarboxylation and re-esterification steps should all follow the procedure established for the first route. Since the angular methyl is already in place, conversion of the ester into the exocyclic double bond would effect the synthesis of longifolene (**1**).

# EXPERIMENTAL

## General

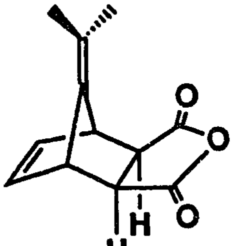
Melting points were recorded on a Köfler hot stage apparatus and are uncorrected. Infrared spectra (ir) were recorded using Nicolet 7199 FTIR and Nicolet MX-1 FTIR spectrometers. High resolution mass spectra (ms) were obtained using a Kratos AEI MS50 high resolution mass spectrometer. Elemental analyses were carried out by the microanalytical laboratory of this department. Proton nuclear magnetic resonance spectra ($^1$H nmr) were obtained using the following spectrometers: Bruker WH-200 (200 MHz), Bruker WH-400 (400 MHz) and Bruker AM-300 (300 MHz). Coupling constants are reported to within ±0.5 Hz. Carbon-13 nuclear magnetic resonance spectra ($^{13}$C nmr) were recorded on a Bruker AM-300 (75.47 MHz). Carbon-13 multiplicities were derived from off-resonance or Carr-Purcel-Meiboom-Gill spin echo J-modulated experiments (APT or Attached Proton Test).[36, 37, 38] Methylene groups and quarternary carbons appear as signals in phase with respect to the deuteriochloroform signal while the signals having an antiphase to that of $CDCl_3$ are for the methyl and methine groups.

The following abbreviations were used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad

## Materials

Still's procedure for flash chromatography[39] using silica gel of mesh 230-400 was routinely used for separations. Thin layer chromatography was perfomed on aluminum-backed plates precoated with silica gel 60 $F_{254}$ as supplied by Merck. Solvents routinely used for chromatography (petroleum ether, skellysolve B, diethyl ether, ethyl acetate and chloroform) were distilled prior to use. Solvents were purified as follows: benzene by distillation from lithium aluminum hydride; diethyl ether by distillation from a blue or purple solution of sodium benzophenone ketyl under argon; dichloromethane by distillation from calcium hydride; acetone by distillation first over potassium permanganate and then over 'Drierite'; methanol by distillation over magnesium/iodine. Pyrrolidine was distilled, under argon, prior to use. Cyclopentadiene was freshly prepared by fractional distillation through a twenty centimeter column packed with glass beads. Cuprous iodide was purified according to the method described by Perrin and Armarego.[40]

**(1*R**, 2*R**, 6*S**, 7*S**)-10-Isopropylidene-4-oxatricyclo-[5.2.1.0$^{2,6}$]non-8-ene-3,5-dione (51) and (1*R**,2*S**, 6*R**, 7*S**)-10-isopropylidene-4-oxatricyclo[5.2.1.0$^{2,6}$]non-8-ene-3,5-dione:**



**51**

A solution of 6,6-dimethylfulvene[41] (10.0 ml, 8.82 g, 0.083 mol) and maleic anhydride (10.58g, 0.108 mol) in 75 ml of benzene was stirred at refluxing temperature under an argon atmosphere for 48 hours. Cooling this solution produced a large amount of yellow-white solid which was filtered and recrystallized from petroleum ether-ether to produce the white crystalline anhydride **51**. The filtrate and mother liquor fractions were combined and concentrated to give more of **51**. This solid was recrystallized as before. The remaining filtrate and mother liquor were then combined, concentrated and then applied to a silica gel column. Elution with a solution of ether (5%) in petroleum ether removed any of the starting fulvene (1.1 g). Increasing the ether concentration to 33% first eluted a small amount of the *endo* isomer of **51** (37 mg, 0.25% based on consumed fulvene) followed by the *exo*-adduct 51. The *exo* product obtained by recrystallization and chromatography gave a combined yield of 10 g (67% based on consumed fulvene).

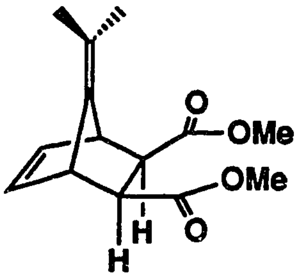
*EXO*-isomer:

mp 138-140°C; ir ($CHCl_3$ cast): 1771 $cm^{-1}$ (C=O); $^1H$ nmr (200 MHz, $CDCl_3$): δ 6.45 (m, 2H), 3.87 (m, 2H), 3.04 (s, 2H), 1.60 (s. 6H); ms calcd. for $C_{12}H_{12}O_3$: 204.0787; found: m/z 204.0779 ($M^+$).

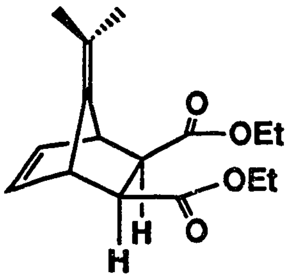
*ENDO*-isomer

ir ($CHCl_3$ cast): 1783 $cm^{-1}$ (C=O); $^1H$ nmr (200 MHz, $CDCl_3$): δ 6.46 (m, 2H), 3.94 (m, 2H), 3.40 (m, 2H), 1.61 (s, 6H); ms calcd. for $C_{12}H_{12}O_3$: 204.0787; found: m/z 204.0788 ($M^+$).

### (1R*, 2R*, 3S*, 4S*)-2,3-Dicarbomethoxy-7-isopropylidene-bicyclo[2.2.1]hept-5-ene (51a) and (1R*, 2R*, 3S*, 4S*)-2,3-Dicarboethoxy-7-isopropylidenebicyclo[2.2.1]hept-5-ene (51b)



**51a**



**51b**

The Diels-Alder adduct **51** (100.8 mg, 0.49 mmol) and *p*-toluenesulphonic acid (93 mg, 0.49 mmol) were dissolved in 1 mL of dried methanol and 0.5 mL of chloroform. This solution was then stirred at room temperature for 60 hours by which time the starting material had been consumed according to a tlc of the reaction mixture. The reaction mixture was then made basic with saturated $NaHCO_3$, and extracted with chloroform (3x15 mL). The chloroform extracts were dried over $MgSO_4$

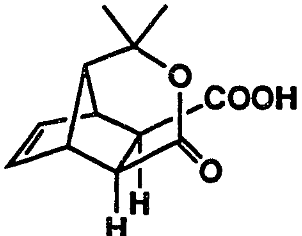
to give a crude oily material (122mg). Chromatography of the extracts on a silica gel column, using a solution of ether (10%) in petroleum ether as the eluant, gave the dimethyl ester **51a** (29 mg, 24% yield). With the ether concentration at 15-20% the desired methyl ester **53** (10 mg, 9% yield) (*vide infra*) was obtained. Further elution with 30% ether in petroleum ether gave 26 mg of a complex mixture of products none of which was the lactone-ester **53**.

$^1$H nmr (200 MHz, $CDCl_3$): δ 6.34 (dd, 2H, -CH=CH-, J = 2.0, 2.0 Hz), 3.62 (s, 6H, $-CO_2CH_3$), 3.57 (dd, 2H, J = 2.0, 2.0 Hz), 2.67 (s, 2H), 1.68 (s, 6H, $=C(CH_3)_2$).

*d,l*-Camphorsulphonic acid (42.2 mg, 0.18 mmol) and the anhydride **51** (37.1 mg, 0.18 mmol) were dissolved in 2 mL of dried ethanol. The resulting solution was stirred at room temperature overnight. At the end of this time the reaction mixture was made slightly basic with saturated sodium bicarbonate solution and extracted with chloroform (4x10 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated to give a crude oily product. Chromatography of this material on silica gel using a solution of ether (15-20%) in petroleum ether gave **51b** (3.7 mg, 7% yield), as the only identifiable producted to be isolated from the complex mixture.

ir ($CHCl_3$ cast): 1728 cm$^{-1}$ (C=O ester); $^1$H nmr (200 MHz, $CDCl_3$): δ 6.30 (dd, 2H, -CH=CH-, J = 2.0, 2.0 Hz), 4.13 (dq, 4H, -COOCH$_2$CH3, J = 2.0, 7.0 Hz), 3.64 (dd, 2H, J = 2.0, 2.0 Hz), 2.74 (s, 2H), 1.70 (s, 3H, $-CH_3$), 1.66 (s, 3H, $-CH_3$), 1.26 (t, 6H, $-COOCH_2CH_3$, J = 7.0 Hz).

**(1*S**, 5*S**, 6*S**, 9*R**, 10*R**)-10-Carboxy-4,4-dimethyl-3-oxatricyclo[4.3.1.0$^{5,9}$]dec-7-en-2-one (52):**

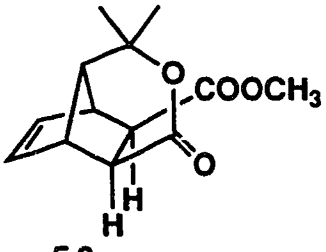


**52**

The Diels-Alder adduct **51** (9.25 g, 45.3 mmol) was slowly dissolved in 50% $H_2SO_4$ (45 mL). The resulting solution was stirred at room temperature under an argon atmosphere for 48 hours. The resulting dark red-brown solution was then diluted with water (50-70 mL) and filtered through Celite. The filtrate was then extracted with ethyl acetate (4x20 mL). The organic portions were combined, dried over $MgSO_4$ and concentrated to produce the acid **52** (5.3 g, 53 % yield) as a white crystalline solid.

mp 174-176.5°C; ir ($CHCl_3$, cast): 3440 (COOH), 1721 cm$^{-1}$ (C=O); $^1H$ nmr (400 MHz, $CDCl_3$): $\delta$ 6.63 (ddd, 1H, -CH=, J = 1.0, 3.0, 6.0 Hz), 6.15 (ddd,1H, -CH=, J = 1.0, 3.0, 6.0 Hz), 3.41 (m, 1H), 3.26 (m, 1H), 2.87 (ddd, 1H, J = 1.5, 1.5, 8.0 Hz), 2.49 (dd, 1H, J = 1.5, 8.0 Hz), 1.98 (m, 1H), 1.40 (s, 3H, -$CH_3$), 1.35 (s, 3H, -$CH_3$); ms calcd. for $C_{12}H_{14}O_4$: 222.0892, found: m/z 222.0877 ($M^+$).

### (1*S**, 5*S**, 6*S**, 9*R**, 10*R**)-10-Carbomethoxy-4,4-dimethyl-3-oxatricyclo[4.3.1.$0^{5,9}$]dec-7-en-2-one (53):

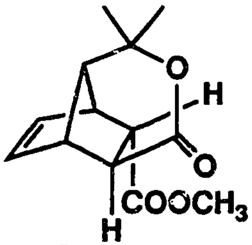


**53**

The acid **52** (5.0 g, 22.5 mmol) was dissolved in dried methanol (29 mL). Concentrated $H_2SO_4$ (12 drops) was then added to this solution and the resulting reaction mixture was left to stir at room temperature overnight. At the end of this time the reaction flask was filled with a white solid. This solid was filtered and redissolved in $CHCl_3$. The chloroform was then washed with a saturated $NaHCO_3$ solution. The $CHCl_3$ layer was then removed, dried, filtered and concentrated to produced the methyl ester **53** (4.5 g, 85% yield) as a white crystalline material. This material was used in the following step without further purification.

mp: 159-160°C; ir ($CHCl_3$ cast): 1748, 1731 cm$^{-1}$ (C=O); $^1$H nmr (200 MHz, $CDCl_3$): $\delta$ 6.61 (m, 1H), 6.14 (m, 1H), 3.70 (s, 3H, -$COOCH_3$), 3.45 (m, 1H), 3.24 (m, 1H), 2.85 (ddd, 1H, J = 1.5, 1.5, 8.0 Hz), 2.43 (m, 1H), 1.97 (m, 1H), 1.39(s, 3H, -$CH_3$), 1.34 (s, 3H, -$CH_3$); ms calcd. for $C_{13}H_{16}O_4$: 236.1049, found: m/z 236.1047 ($M^+$); analysis: calcd. for %C: 66.08, %H: 6.83; found for %C: 65.87, %H: 6.76.

### (1*S**, 5*S**, 6*S**, 9*R**,10*S**)-10-Carbomethoxy-4,4-dimethyl-3-oxatricyclo[4.3.1.$0^{5,9}$]dec-7-en-2-one (54):

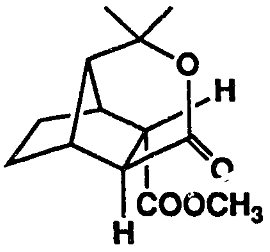


**54**

A solution of NaOMe/MeOH was prepared by dissolving sodium metal (0.45 g, 19.6 mmol) in 20 mL of dried MeOH. Epimerization was carried out by adding the methyl ester **53** (4.09 g, 17.3 mmol) to this solution and refluxing under an atmosphere of argon until the tlc indicated the absence of starting material. The solution was then acidified with 1N HCl and extracted with $CHCl_3$. The organic layer was dried, filtered and concentrated to produce the *endo*-methyl ester **54** (3.06 g, 75% yield) as a white solid. This was used without further purification in the hydrogenation step that followed.

mp: 104-107°C; ir ($CHCl_3$ cast): 1741 $cm^{-1}$ (C=O); $^1H$ nmr (200 MHz, $CDCl_3$): $\delta$ 6.25 (m, 2H, -CH=CH-), 3.66 (s, 3H, $-COOCH_3$), 3.32 (m, 1H), 3.11(m, 1H), 3.02 (dd, 1H, J = 2.0, 4.0 Hz), 2.90 (m, 1H), 2.03 (d, 1H, J = 2.0 Hz), 1.40 (s, 3H,$-CH_3$), 1.36 (s, 3H, $-CH_3$); ms calcd. for $C_{13}H_{16}O_4$: 236.1049, found: m/z 236.1045 ($M^+$); analysis: calcd. for %C: 66.08, %H: 6.83; found for %C: 65.95, %H: 6.71.

## (1S*, 5S*, 6S*, 9R*,10S*)-10-Carbomethoxy-4,4-dimethyl-3-oxatricyclo[4.3.1.$0^{5,9}$]dec-2-one (55):

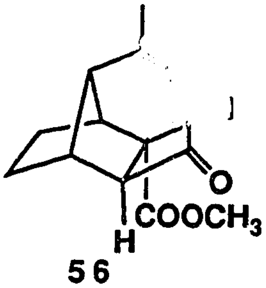


55

Hydrogenation was carried out using a Parr hydrogenation apparatus. In a typical reaction compound **54** (3.06 g, 13 mmol) was dissolved in ethyl acetate (60 mL) and this solution together with 5% Pd-C (35.6 mg) was placed in a Parr bottle. Hydrogenation then took place under a $H_2$ pressure of 16 to 18 p.s.i. over a period of 3 hours. The mixture was then filtered through Celite and the solvent was removed *in vacuo* to give compound **55** (3.05 g, 99% yield) as a white solid.

mp 91-93°C; ir ($CHCl_3$ cast) 1737 $cm^{-1}$ (C=O); nmr (200 MHz, $CDCl_3$): δ 3.74 (s, 3H,-$COOCH_3$), 3.10 (m, 1H), 2.88 (m, 1H), 2.73 (m, 1H), 2.50 (br d, 1H, J = 5.0 Hz), 2.06 (dd, 1H, J = 1.5, 3.0 Hz), 1.49 (s, 3H, -$CH_3$), 1.44 (s, 3H, -$CH_3$); ms calcd. for $C_{13}H_{18}O_4$: 238.1205, found m/z 238.1193 ($M^+$); analysis: calcd. for %C: 65.53, %H 7.61, found for %C: 65.39, %H 7.77.

### (1*S**, 5*R**, 6*S**, 9*R**, 10*S**)-10-carbomethoxy-4-methyltricyclo-[4.3.1.0$^{5,9}$]dec-3-en-2-one (56):

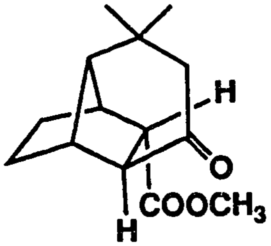


56

In a typical reaction, the reagent required to effect the transformation of the lactone-ester **55** into the enone-ester **56** was prepared by dissolving phosphorus pentoxide (241 mg, 1.7 mmol) in methanesulfonic acid (0.8 mL, 12.3 mmol). The lactone-ester **55** (175 mg, 0.73 mmol) and 0.8 mL of dichloromethane were then added to the acidic mixture. The resulting reaction mixture was then stirred at 60°C for 40 hours under an argon atmosphere. At the end of this time the solution, which was dark brown in color, was diluted with more dichloromethane and poured into 10 ml of water. The organic layer was then removed and washed with saturated sodium carbonate solution, water and then dried and concentrated to produce a dark yellow oil. This oil was purified by column chromatography on silica gel, eluting with ether, to produce **56** (153 mg, 95% yield) as a viscous yellow oil.

ir ($CHCl_3$ cast): 1739 (C=O ester), 1678 cm$^{-1}$ (C=O ketone); $^1H$ nmr (300 MHz, $CDCl_3$): $\delta$ 5.75 (m, 1H, -C($CH_3$)=CH-), 3.71 (s, 3H, -$COOCH_3$), 3.02 (m, 1H), 2.78 (m, 1H), 2.70 (m, 1H), 2.61 (m, 1H), 2.51 (m, 1H), 2.03 (d, 3H, -C($CH_3$)=CH-, J = 1.5 Hz), 1.70 (m, 4H); ms calcd. for $C_{13}H_{16}O_3$: 220.1100, found: m/z 220.1099 ($M^+$).

### (1*S**, 5*R**, 6*S**, 9*R**, 10*S**)-10-Carbomethoxy-4,4-dimethyltricyclo[4.3.1.0$^{5,9}$]decan-2-one (57):

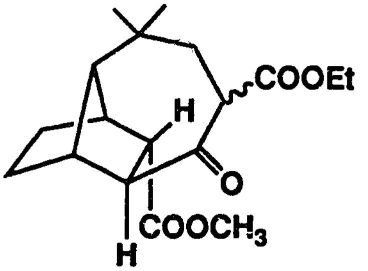


**57**

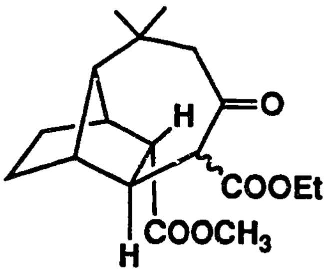
The preparation of the dimethylcopper lithium reagent was carried out as follows: CuI (1.62 g, 8.51 mmol) was added, under an atmosphere of argon, to 60 ml of dried diethyl ether that had been previously cooled to 0°C. Methyllithium (17 mL, 17 mmol) was then added, dropwise, to this stirred suspension. The solution initially turned bright yellow and then became colorless as more methyllithium was added. There was also a yellow-brown solid that began to form around the sides of the flask while the addition was proceeding. Once the addition of methyllithium was completed, the mixture was left to stir at 0°C until all the yellow-brown solid had dissolved, about 30 minutes. A solution of **56** (623 mg, 2.83 mmol) in dried ether (5 mL, 0°C) was slowly added to the dimethylcopper lithium reagent over a period of 15 to 20 minutes. When all of the enone-ester had been added, the reaction was left to stir at 0°C until the reaction was deemed complete by tlc, about 1 to 1.5 hours. The reaction was then quenched by adding 5% HCl followed by chloroform. The chloroform layer was removed and the aqueous portion was extracted with $CHCl_3$ (2x30 ml). The combined organic extracts were then washed with water, dried over $MgSO_4$, filtered and concentrated to produce a light brown solid. Purification by either sublimation (58°C, 1.5mm Hg) or by column chromatography (silica gel, eluting with 5% $Et_2O$ in petroleum ether) produced a white crystalline solid in 67% yield.

mp 93-93.5°C; ir ($CHCl_3$ cast): 1736 (C=O ester), 1714 $cm^{-1}$ (C=O ketone); $^1H$ nmr (400 MHz, $CDCl_3$): $\delta$ 3.68 (s, 3H, $-COOCH_3$), 2.91 (s, 1H), 2.89 (m, 1H), 2.72 (m, 1H), 2.35 (d, 1H, J = 18 Hz), 2.31 (d, 1H, J = 5 Hz), 2.23.(d, 1H, J = 18 Hz), 1.71 (m, 2H), 1.48 (m, 2H), 1.27 (m, 1H), 1.13 (s, 3H, $-CH_3$), 1.02 (s, 3H, $-CH_3$); ms calcd. for $C_{14}H_{20}O_3$: 236.1413, found: m/z 236.1413 ($M^+$).

### (1*S**, 6*R**, 7*S**, 10*R**, 11*S**)-3-Carboethoxy-11-carbomethoxy-5,5-dimethyltricyclo[5.3.1.$0^{6,10}$]undecan-2-one (59a) and (1*S**, 6*R**, 7*S**, 10*R**, 11*S**)-2-Carboethoxy-11-carbomethoxy-5,5-dimethyltricyclo[5.3.1.$0^{6,10}$]undecan-3-one (59b):



**59a**



**59b**

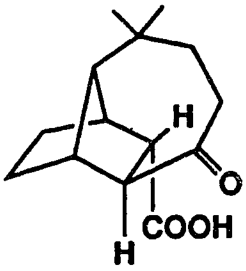
The reaction flask was first wrapped in aluminum foil and flushed with argon. To a stirred solution of ketone-ester 57 (159 mg, 0.67 mmol) in dried ether (5 mL, 0°C) was added $BF_3 \cdot OEt_2$ (128μL, 1.0 mmol). This was followed by the addition of ethyl diazoacetate (105 μL, 1.0 mmol). When all of the ethyl diazoacetate had been added, the reaction mixture was warmed to room temperature and left to stir at this temperature for 11 hours. Reaction times of up to 20 hours were also tried. However, because the tlc results taken after 11 hours and after 20 hours appeared to be the same, we chose the shorter reaction time.

It was found that not only did the starting material **57** and the products **59a** and **59b** have nearly the same $R_f$ but the product was UV active unlike **57**. As a result, this presented a problem in determining when the reaction was completed.

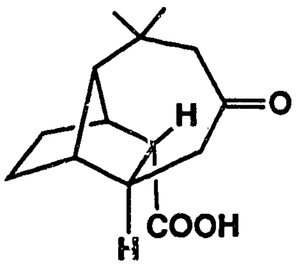
At the end of the 11 hours, the reaction solution was made basic using saturated sodium carbonate solution and then extracted wiith chloroform. The organic extracts were then washed with saturated sodium chloride solution and dried over magnesium sulphate. The solvent was then removed *in vacuo* to give 251 mg of crude product. The crude material was then purified by column chromatography on silica gel eluting with diethyl ether (5 to 30%) in petroleum ether to give a mixture of the product isomers **59a-59d** (124.4 mg, 70% yield based upon consumed **57**), along with starting material **57** (27.9 mg, 18% recovery).

ir ($CHCl_3$ cast): 1739 (C=O ester), 1704 (C=O ketone), 1636 (C=O, enol ester), 1608 $cm^{-1}$ (C=C enol); $^1H$ nmr (300 MHz, $CDCl_3$): $\delta$ 13.1 (s, 1H, -C(COOEt)=C(OH)-), 4.20 (m, 2H, $-COOCH_2CH_3$), 3.7 (s, 3H, $-COOCH_3$), 3.47 (m, 1H), 2.93 (br d, 1H, J = 3.5 Hz), 2.65 (m, 1H), 2.44 (m, 1H), 2.32 (m, 2H), 1.6 (m, 2H), 1.29 (m, 3H, $-COOCH_2CH_3$), 1.06 (s, 3H, $-CH_3$), 0.92 (s, 3H, $-CH_3$); ms calcd. for $C_{18}H_{26}O_5$: 322.1781, found: m/z 322.1779 ($M^+$).

### (1*S**, 6*R**, 7*S**, 10*R**, 11*S**)-11-Carboxylic acid-5,5-dimethyl-tricyclo[5.3.1.$0^{6,10}$]undecan-2-one (60a) and (1*S**, 6*R**, 7*S**, 10*R**, 11*S**)-11-Carboxylic acid-5,5-dimethyl-tricyclo[5.3.1.$0^{6,10}$]undecan-3-one (60b):



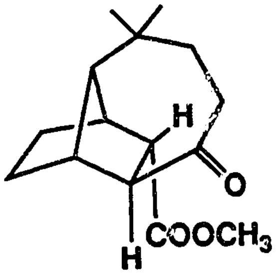
**60a**



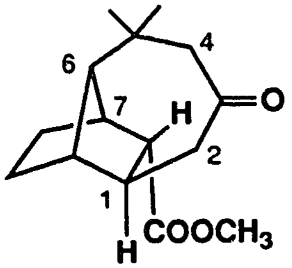
**60b**

Since lithium iodide is light and air sensitive, this decarboxylation reaction was conducted under an atmosphere of argon in a flask wrapped in aluminum foil. In a typical reaction, the mixture of **59a** and **59b** (164 mg, 0.51 mmol) was dissolved in collidine (3.16 mL) followed by the addition of LiI (546 mg, 4.08 mmol) and water (73.4 µL, 4.08 mmol). The resulting mixture was heated to reflux (about 165°C) in a silicone oil bath for 2 hours. At the end of this time the oil bath was removed and the solution was allowed to cool to room temperature. It was then poured into 20 mL of ice-cold 10% HCl, 25 mL of ether was then added and the mixture shaken. The ether layer was removed and washed with cold 10% HCl (5x10 mL) followed by a washing with water and saturated NaCl. The ether layer was removed, dried over magnesium sulphate and finally concentrated to give a light yellow colored oil which became colorless on standing open to the air overnight. The crude mixture of the decarboxylated products **60a** and **60b** (118.2 mg, 43% yield) was recovered as an oily white solid. The $^1$H nmr of the mixture indicated that no starting material remained. The crude material **60a** and **60b** was used without further purification in the next step.

### (1*S**, 6*R**, 7*S**, 10*R**, 11*S**)-11-Carbomethoxy-5,5-dimethyl-tricyclo[5.3.1.$0^{6,10}$]undecan-2-one (61a) and (1*S**, 6*R**, 7*S**, 10*R**, 11*S**)-11-Carbomethoxy-5,5-dimethyl-tricyclo[5.3.1.$0^{6,10}$]undecan-3-one (61b):



**61a**



**61b**

The mixture of compounds **60a** and **60b** (16 mg, 0.07 mmol) and potassium bicarbonate (16.9 mg, 0.17 mmol), were stirred together in dried acetone (1 mL) for two hours. At the end of this time methyl iodide (21µL, 48 mg, 0.34 mmol) was added and the mixture was left to stir at room temperature overnight. At the end of this time the reaction mixture was acidified with 1N HCl and extracted with chloroform (2x10 mL). The chlorofrom extracts were dried over $MgSO_4$ and concentrated to leave a colorless oil (15.5 mg).

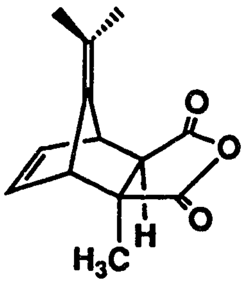
For a larger scale reaction, it was necessary during the work-up of the crude product to wash the extracts with sodium bisulphite solution in order to reduce any free iodine to iodide and thereby eliminate this contaminant.

The crude oil was then placed on a silica gel column. Elution using ether (10%) in petroleum ether gave **61b** (0.9 mg, 5% yield) as a white solid.

ir ($CHCl_3$, cast): 1726 (C=O ester), 1689 $cm^{-1}$ (C=O ketone); $^1H$ nmr (400 MHz, $CDCl_3$): δ 3.76 (s, 3H, $COOCH_3$), 3.2 (m, 1H), 2.78 (d, 1H, J = 12 Hz), 2.67 (ddd, 1H, J = 5, 5, 16.5 Hz), 2.44 (m, 1H), 2.32 (dd, 1H, J = 3, 16.5 Hz), 2.20 (ddd, 1H, J = 1, 1, 12 Hz), 2.03 (d, 1H, J = 4 Hz), 1.12 (s, 3H, $-CH_3$), 1.01 (s, 3H, $-CH_3$); ms calcd. for $C_{15}H_{22}O_3$: 250.1570, found: m/z 250.1560 ($M^+$).

Further elution with ether (10%) in petroleum ether gave a mixture of **61a** and **61b** (8.6 mg, 51% yield) as a colorless oil.

### (1*S**, 2*S**, 6*R**, 7*R**)-10-Isopropylidene-2-methyl-4-oxatricyclo[5.2.1.$0^{2,6}$]non-8-ene-3,5-dione (63):

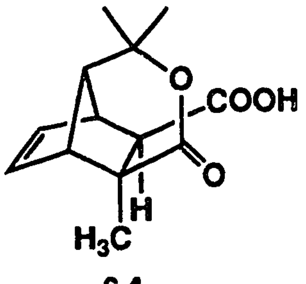


**63**

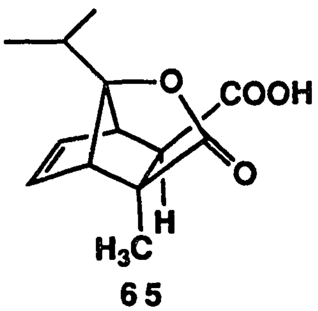
Citraconic anhydride (9.7 mL, 0.108 mol) and 6,6 dimethylfulvene (10 mL, 8.81 g, 0.083 mol) were dissolved in 75 mL of dried benzene. The solution was then stirred under an argon atmsophere at reflux temperature for 14 to 16 hours. The reaction was monitored closely by tlc after about 14 hours for the build up of a second spot on the tlc attributed to the undesired *endo* adduct. Once this began, the reaction was cooled to room temperature and the benzene was removed on a roto-evaporator. At this point a solid appeared coated with a yellow oil of the unreacted fulvene. This mixture was filtered and washed repeatedly with petroleum ether to removed most of the yellow oil. The solid residues were then purified by column chromatography starting with a solution of ether (5%) in petroleum ether to remove any remaining fulvene (1.1g, 17% recovery). Using a mixture of petroleum ether:ether:ethyl acetate [3:4:1] as the eluant, the desired anhydride **63** (4.7 g, 30% yield based on consumed fulvene) was isolated as a white crystalline solid.

mp107-109°C; ir ($CHCl_3$ cast): 1850, 1768 $cm^{-1}$ (C=O); $^1H$ nmr (300 MHz, $CDCl_3$): $\delta$ 6.49 (m, 2H, -CH=CH-), 3.83 (m, 1H), 3.58 (m, 1H), 2.47 (d, 1H, J = 1 Hz), 1.57 (s, 6H, $=C(CH_3)_2$), 1.32 (s, 3H, $-CH_3$); ms calcd. for $C_{13}H_{14}O_3$: 218.0943, found: ms 218.0943 ($M^+$).

### (1*S**, 5*S**, 6*S**, 9*R**, 10*R**)-10-Carboxy-1,4,4-trimethyl-3-oxatricyclo[4.3.1.$0^{5,9}$]dec-7-en-2-one (64) and (1*S**, 4*S**, 5*R**, 8*S**, 9*R**)-9-Carboxy-4-[1-(methyl)ethyl]-1-methyl-3-oxatricyclo[3.3.1.$0^{4,8}$]non-6-en-2-one (65):



**64**



**65**

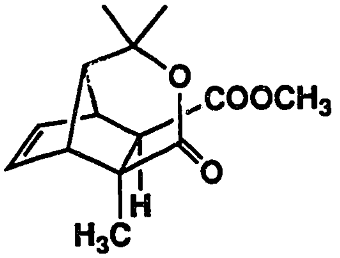
The anhydride **63** (4.0 g, 0.018 mmol) was slowly dissolved in 50% sulphuric acid (20 mL). This mixture was then stirred at room temperature for 48 hours. At the end of this time, the solution was diluted with water (30-50 mL) and filtered through Celite leaving a large amount of a brown solid residue on the Celite. Extraction of the filtrate with ethyl acetate (3x20mL) left the acid **64** (0.79 g, 18% yield) once the ethyl acetate had been dried and concentrated.

mp 214-7°C; ir ($CHCl_3$ cast): 1737 (C=O, lactone), 1705 $cm^{-1}$ (C=O, acid); $^1H$ nmr (200 MHz $CDCl_3$): δ 6.59 (m, 1H, -**CH**=CH-), 6.19 (m, 1H, -CH=**CH**-), 5.44 (br s, 1H, -**COOH**), 3.40 (s, 1H), 3.01 (s, 1H), 2.12 (d, 1H, J = 1.5 Hz), 2.00 (d, 1H, J = 1.5 Hz), 1.41 (s, 3H, -$CH_3$), 1.33 (s, 6H, -$C(CH_3)_2$); ms calcd. for $C_{13}H_{16}O_4$: 236.1049, found: m/z 236.1036 ($M^+$); analysis: calcd. for %C: 66.08, %H 6.83, found for %C 65.70, %H 6.69.

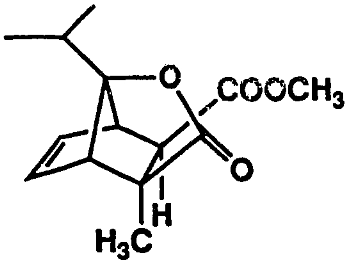
The solid residues from the filter were recovered and extracted with $CHCl_3$. The extracts were then concentrated to give the γ-lactone acid **65** (0.21 g, 5% yield) along with a mixture of **64**and **65**.

mp 208-210°C; ir ($CHCl_3$ cast): 1784 (C=O, lactone), 1706 $cm^{-1}$ (C=O, acid); $^1H$ nmr (400 MHz $CDCl_3$): δ 6.58 (m, 1H, -**CH**=CH-), 5.83 (m, 1H, -CH=**CH**-), 3.18 (dd, 1H, J = 2, 2 Hz), 3.01 (dd, 1H, J = 2, 2 Hz), 2.19 (s, 1H), 2.17 (septet, 1H, J = 7 Hz, -$CH(CH_3)_2$), 1.40 (s, 3H, -$CH_3$), 1.00 (d, 3H, -$CH(CH_3)_2$, J = 7 Hz), 0.91 (d, 3H, -$CH(CH_3)_2$, J = 7 Hz); ms calcd. for $C_{13}H_{16}O_4$: 236.1049, found: m/z 236.1045 ($M^+$).

### (1*S**, 5*S**, 6*S**, 9*R**, 10*R**)-10-Carboxymethyl-1,4,4-trimethyl-3-oxatricyclo[4.3.1.$0^{5,9}$]dec-7-en-2-one (66) and (1*S**, 4*S**, 5*R**, 8*S**, 9*R**)-9-Carbomethoxy-4-[1-(methyl)-ethyl]-1-methyl-3-oxatricyclo[3.3.1.$0^{4,8}$]non-6-en-2-one (67):



**66**



**67**

In order to get a better separation of the acids **64** and **65**, it was felt that conversion of these compounds into their respective less polar methyl esters would be of help. The preparation of the methyl esters was best accomplished by a method developed in this laboratory. In a typical experiment a mixture of **64** and **65** (554 mg, 2.21 mmol) was dissolved in 10 mL of dried acetone. $KHCO_3$ (575 mg, 5.74 mmol) was then added and the resulting suspension was stirred under an atmosphere of argon for 2 hours. At the end of this time $CH_3I$ (0.72 mL, 11.57 mmol) was added and the reaction was left to stir overnight. For the work-up, the reaction mixture was acidified with 5% HCl followed by the extraction of the product into chloroform (3x15 mL). The organic extracts were dried and concentrated to give 541 mg of crude product. Chromatography on silica gel using 15-20% ether in petroleum ether as the eluant gave the γ-lactone-ester **67** (41 mg, 10% yield), a mixture of **66** and **67** (38.6 mg, 10% yield), δ-lactone-ester **66** (185 mg, 33% yield) and a 22% yield of an unidentified product, all as white solids.

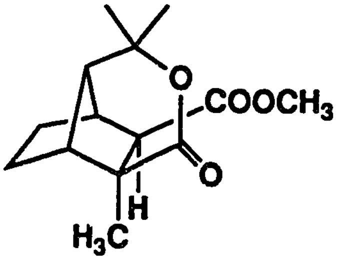
Compound **66** showed the following physical properties:

mp: 171-172°C; ir ($CHCl_3$ cast): 1729 $cm^{-1}$ (C=O); $^1H$ nmr (300 MHz, $CDCl_3$): δ 6.62 (m, 1H), 6.23 (m, 1H), 3.70 (s, 3H, $-COOCH_3$), 3.46 (s, 1H), 3.04 (s, 1H), 2.10 (s, 1H), 2.02 (s, 1H), 1.40 (s, 3H, $-CH_3$), 1.34 (s, 3H, $-C(CH_3)_2$), 1.33 (s, 3H, $-C(CH_3)_2$); $^{13}C$ nmr (75.46 MHz, $CDCl_3$): 175.11 (s), 171.60 (s), 141.61 (d), 133.46 (d), 80.87 (s), 63.98 (d), 56.12 (q), 51.77 (d), 50.70 (d), 47.69 (s), 45.22 (d), 30.29 (q), 24.81 (q), 19.91 (q); ms calcd. for $C_{14}H_{18}O_4$: 250.1205, found: m/z 250.1206 ($M^+$).

Compound **67** gave the following spectral data:

ir ($CHCl_3$ cast): 1780 (C=O lactone), 1732 $cm^{-1}$ (C=O ester); $^1H$ nmr (400 MHz, $CDCl_3$): δ 6.56 (m, 1H), 5.82 (m, 1H), 3.71 (s, 3H), 3.21 (m, 1H), 2.99 (m, 1H), 2.17 (septet, 1H, $-CH(CH_3)_2$, J = 7 Hz), 2.16 (s, 1H), 1.36 (s, 3H, $-CH_3$), 1.01 (d, 3H, $-CH(CH_3)_2$, J = 7 Hz), 0.91 (d, 3H, $-CH(CH_3)_2$, J = 7 Hz); ms calcd. for $C_{14}H_{18}O_4$: 250.1205, found: m/z 250.1208 ($M^+$).

**(1*S**, 5*S**, 6*S**, 9*R**, 10*R**)-10-Carbomethoxy-1,4,4-trimethyl-3-oxatricyclo[4.3.1.0$^{5,9}$]decan-2-one (68):**

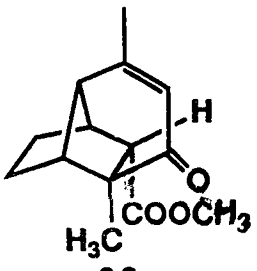


**68**

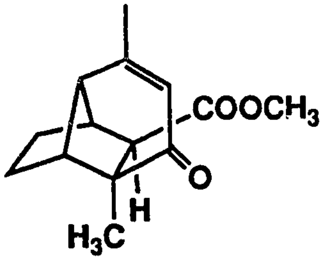
The δ-lactone ester **66** (92.4 mg, 0.37 mmol), dissolved in ethyl acetate (20 mL), was placed in a Parr bottle along with 5% Pd-C (10 mg). The hydrogenation then took place under $H_2$ (18 p.s.i.) over a period of 2.5 hours. At the end of this time the mixture was filtered through Celite and the solvent removed *in vacuo* to give the product **68** (89.4 mg, 99% yield) as a white solid.

mp: 154-156°C; ir ($CHCl_3$ cast): 1734 $cm^{-1}$ (C=O); $^1H$ nmr (300 MHz, $CDCl_3$): δ 3.66 (s, 3H, $-COOCH_3$), 2.96 (s, 1H), 2.38 (m, 1H), 2.13 (s, 1H), 1.88 (d, 1H, J = 1.5 Hz), 1.75 (m, 4H, $-CH_2-CH_2-$), 1.45 (s, 3H, $-CH_3$), 1.43 (s, 3H, $-CH_3$), 1.37 (s, 3H, $-CH_3$); ms calcd. for $C_{14}H_{20}O_4$: 252.1362, found: m/z 252.1364 ($M^+$).

### (1*S**, 5*S**, 6*S**, 9*R**, 10*S**)-10-Carbomethoxy-1,4-dimethyl-tricyclo[4.3.1.0$^{5,9}$]dec-3-en-2-one (69a) and (1*S**, 5*S**, 6*S**, 9*R**, 10*R**)-10-Carbomethoxy-1,4-dimethyl-tricyclo[4.3.1.0$^{5,9}$]dec-3-en-2-one (69b)



**69a**



**69b**

The reagent used to convert the ester-lactone **68** into the enone-ester **69a** and **69b** was prepared by dissolving $P_2O_5$ (259.1 mg, 1.83 mmol) in $CH_3SO_3H$ (0.85 mL, 13.1 mmol). To this reagent was added, under an argon atmosphere, compound **68** (190 mg, 0.75 mmol) and $CH_2Cl_2$ (0.85 mL). The reaction mixture was then stirred at 60°C for 29 hours. At the end of this time the reaction was cooled and a further 5 mL of $CH_2Cl_2$ was added to the reaction flask. Once this was done, the reaction mixture was poured into water (10mL). The $CH_2Cl_2$ layer was separated and the aqueous layer was extracted with further portions of dichloromethane (2x10 mL). The combined organic extracts were then washed with water, saturated $NaHCO_3$ and then dried over $MgSO_4$. Concentration gave a crude oil (67.2 mg). Chromatography of this crude oil on silica gel, eluting with a solution of ether (5-8%) in petroleum ether gave the enone-ester **69a** (1.0 mg, 2% yield).

ir ($CHCl_3$ cast): 1737 (C=O ester), 1677 $cm^{-1}$ (C=O ketone); $^1H$ nmr (400 MHz, $CDCl_3$): $\delta$ 5.80 (d, 1H, =CH-, J = 1.5 Hz), 3.64 (s, 3H, $-COOCH_3$), 2.73 (dd, 1H, J = 1.5, 5.0 Hz), 2.53 (m, 1H), 2.46 (s, 1H), 2.35 (m, 1H), 2.28 (d, 1H, J = 4.5 Hz), 2.01 (d, 3H, $-C(CH_3)=$, J = 1.5 Hz), 1.70 (m, 4H, $-CH_2-CH_2-$), 1.20 (s, 3H, $-CH_3$); ms calcd. for $C_{14}H_{18}O_3$: 234.1256, found: m/z 234.1256 ($M^+$).

Increasing the ether concentration to 10-15% eluted **69b** (6.3 mg, 11% yield).

ir ($CHCl_3$ cast): 1734 (C=O ester), 1678 $cm^{-1}$ (C=O ketone); $^1H$ nmr (400 MHz, $CDCl_3$): $\delta$ 5.68 (d, 1H, =CH-, J = 1.5 Hz), 3.53 (s, 3H, $-COOCH_3$), 2.93 (br d, 1H, J = 4 Hz), 2.36 (m, 2H), 2.30 (s, 1H), 1.96 (d, 3H, $-C(CH_3)=$, J = 1.5 Hz), 1.65 (m, 4H, $-CH_2-CH_2-$), 1.37 (s, 3H, $-CH_3$); ms calcd. for $C_{14}H_{18}O_3$: 234.1256, found: m/z 234.1256 ($M^+$).

# Part 2

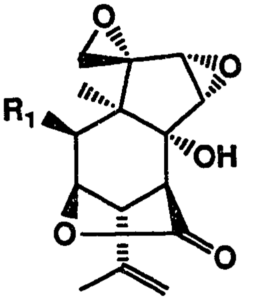
# Constituents of *Guang Jisheng*

# INTRODUCTION

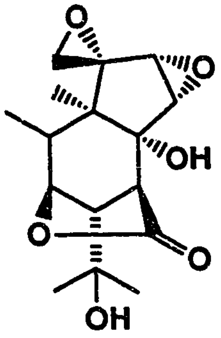
This project was initiated as part of an on-going study, here at the University of Alberta, of various plants used in traditional Chinese medicine. The principle focus of this study was the search for potential agents in the control of hypertension. Our study was directed at the constituents of the traditional medicine known as *Ma sang jisheng.* This name, in fact, refers to four different plants:[42] *Loranthus parasiticus* Merr., *L. yadoriki* Sieb., *L. philippensis* Cham. or *L. sutchuenensis* Lecomte (Loranthaceae), all of which are parasites on the plant *Coriaria sinica* Maxim (Coriariaceae). This plant is known in traditional medicine as *Ma sang* .

*Ma sang jisheng* and *Ma sang* are quite toxic. The toxic principles in both medicines have been narrowed to two sesquiterpene lactones: coriamyrtin (**70**) and tutin (**71**). The isolation of both these compounds from the leaves of *L. parasiticus* and *C. sinica* led to the suggestion that these toxins had initially been made by the host plant and then transported to the parasite where they were accumulated without being metabolized. To date most of the interest in *Ma sang jisheng* has centered on the isolated sesquiterpene lactones noted above and two others: coriatin (**72**) and corianin (**73**)[43] which were found to be non-toxic.

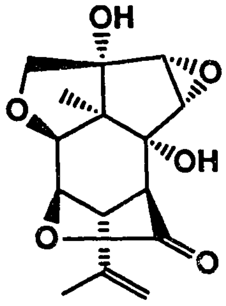
An aqueous extract of *L. parasiticus* or a crystalline mixture of the sesquiterpine lactones has been applied by muscle injection to treat catatonia. The effects of the extract are similar to those of insulin or electric shock and other drugs used for treatment.[43] Its action is deemed



**70**: $R_1$= H
**71**: $R_1$= -OH



**72**



**73**

to be just as fast and because it is cheaper and more widely available, the traditional approach is more attractive.

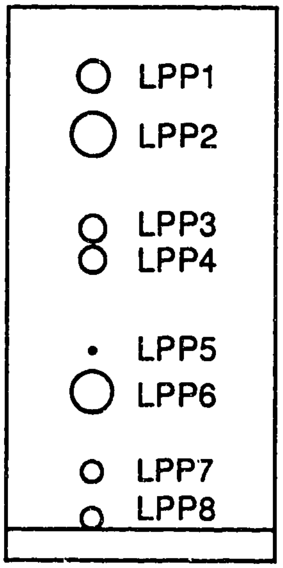
Within the last twelve years a number of reports have been published in China on the use of *Ma sang jisheng* for the treatment of schizophrenia.[42, 43, 44] Because of the toxicity of the medicine this treatment must be carefully monitored and yet it still remains a widely used alternative to previous treatments which are similar to that used to treat catatonia.

In light of the previous discussion it should be pointed out that the traditional name for a plant will vary depending upon the location in which the plant has been collected and, because the plant in question is a parasite, the particular host for the plant. The variety of *L. parasiticus* used for this study was imported from Guangxi Province in southern China and is more correctly referred to as '*guang jisheng*' or '*kwang chi sheng*' since the specific host for the plant is also unknown. Specific from this plant then, the compounds isolated of record to date include the triterpenoids β-amyrin and oleanolic acid and the flavanols quercetin and quercetin-3-arabinoside, a powerful diuretic.[45]

# RESULTS AND DISCUSSION

In this project the petroleum ether extracts of *guang jisheng* were examined. Boxes of the dried plant which included both leaves and twigs, were purchased locally. This material was then separated into leaf and twig portions. The leaves were ground to a fine powder for extraction and the twigs were kept for separate studies. The leaf material was then extracted with 2x4 L of petroleum ether which was then filtered and concentrated. Figure 8 illustrates a tlc of the crude petroleum ether extract.

FIGURE 8



These petroleum ether extracts were placed on a silica gel column made up in petroleum ether. Elution of the components was then carried out using a petroleum ether–diethyl ether solvent system of increasing ether concentration from 0 to 33%. These fractions were then treated with a combination of recrystallization, column chromatography and preparative tlc in order to obtain pure compounds. The flowchart in Figure 9 summarizes the extraction processes carried out.

It should be mentioned at the outset that the petroleum ether extracts were tested for biological activity by other groups involved in this project and these tests proved to be negative.

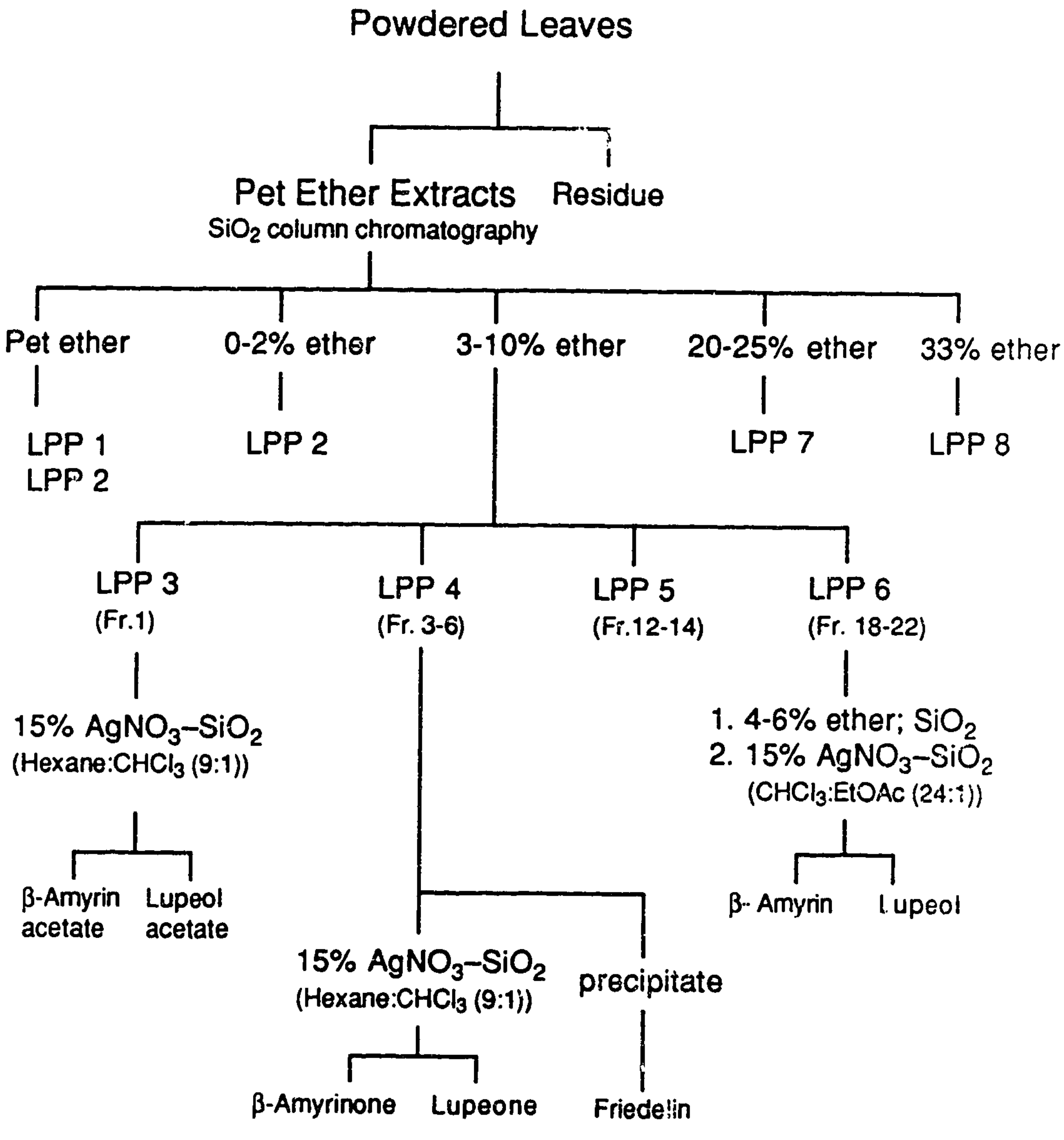
From the ir, nmr and mass spectra results of LPP1 obtained by petroleum ether elution, it was determined that this material was made up of long chain hydrocarbons and nothing further was done with this material.

Elution with petroleum ether-ether (100:1 to 100:2) gave a yellow band from which more of LPP1 was isolated along with LPP2. The compounds from LPP2 consisted of long chain esters, of the form $C_nH_{2n}O_2$, as determined from the ir, nmr and mass spectra of this material. No further studies were done on this material.

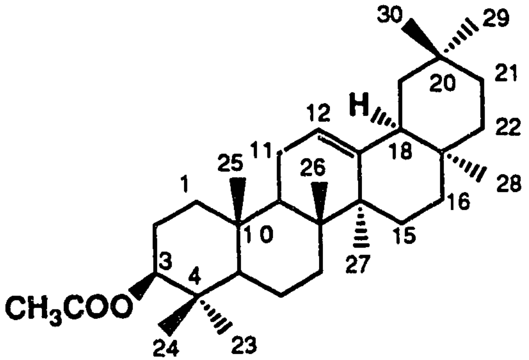
Increasing the ether concentration to 3%–10% resulted in the isolation of the compounds LPP3 to LPP6. Each of these compounds appeared as a single spot on tlc when initially separated from the others. However, the nmr results of several of the LPP3-6 materials indicated the presence of a mixture.

LPP3 was found to be a mixture of β-amyrin acetate (**74**) and lupeol acetate (**75**). This mixture was separated in two ways. In the first method the LPP3 was dissolved in a mixture of petroleum ether and acetone and the solvent was then allowed to partially evaporate at room temperature. Two lots of crystals of different physical appearance were obtained and could be separated by controlling the amount of evaporation that took

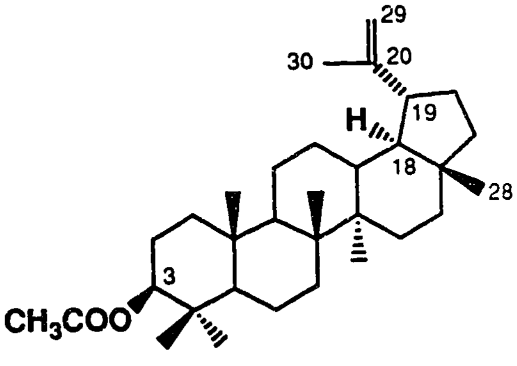
# FIGURE 9


Powdered Leaves
Pet Ether Extracts
Residue
SiO2 column chromatography
Pet ether
0-2% ether
3-10% ether
20-25% ether
33% ether
LPP 1
LPP 2
LPP 2
LPP 7
LPP 8
LPP 3
(Fr.1)
LPP 4
(Fr. 3-6)
LPP 5
(Fr.12-14)
LPP 6
(Fr. 18-22)
15% AgNO3-SiO2
(Hexane:CHCl3 (9:1))
1. 4-6% ether; SiO2
2. 15% AgNO3-SiO2
(CHCl3:EtOAc (24:1))
β-Amyrin acetate
Lupeol acetate
β- Amyrin
Lupeol
15% AgNO3-SiO2
(Hexane:CHCl3 (9:1))
precipitate
β-Amyrinone
Lupeone
Friedelin

place. β-Amyrin acetate (**74**) crystallized first as large prisms. As more solvent evaporated, lupeol acetate (**75**) crystallized as fine needles. In the second approach, the two products were separated by preparative tlc using silica gel impregnated with 15% $AgNO_3$ as the absorbant and a solution of hexane-chloroform (1:9) as the eluant. β-Amyrin acetate had an $R_f$ of 0.59 while lupeol acetate had an $R_f$ of 0.34 under these conditions.



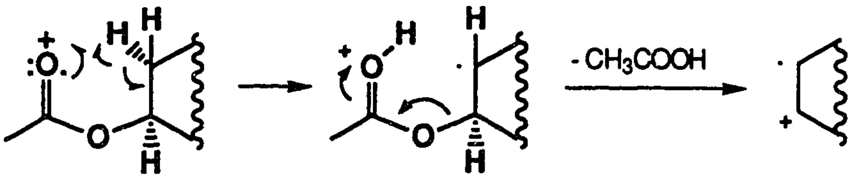
**74**



**75**

The spectroscopic data of **74** and **75** compared favorably with those reported in the literature.[46] The ir spectrum of **74** showed a carbonyl stretch at 1734 $cm^{-1}$. A strong C–O stretch found at 1247 $cm^{-1}$, is characteristic for the acetates of saturated alcohols.[47] The vinylic C-12 hydrogen appeared as a triplet at δ 5.16 in the $^1H$ nmr spectrum. The 3α-H appeared as a doublet of doublets at δ 4.48. The acetoxy methyl hydrogens appeared as a singlet at δ 2.03 and the remaining methyls appeared as singlets in the region δ 1.11 to δ 0.84. A $^{13}C$ nmr spectrum and APT were also run on **74**. In the $^{13}C$ nmr spectrum, a signal for the carbonyl carbon appeared at δ 171.0. Signals appeared at δ 145.3 and δ 121.7 for the C-13 and C-12 carbons respectively. Tori *et al*[48] originally assigned C-15 to a signal at δ 28.3 and C-28 to a signal at δ 27.0. An examination of **74** indicates that the methylene C-15 will appear as a triplet in an APT spectrum while the methyl C-28 will appear as a quartet. In an APT spectrum these signals will be unambiguous. From the APT spectrum it was determined that C-15 should be assigned to δ 26.99 and C-28 should be assigned to δ 28.46, reversing the original proposal. The C-2 and C-30 carbons, were originally assigned by Tori to a peak at δ 23.6. Better resolution of this signal has established the assignment of the methylene C-2 to a triplet at δ 23.63 and the methyl C-30 to a quartet at δ 23.75. Similarly the C-24 and C-26 carbons originally proposed as a single signal at δ 16.8 could be assigned to δ 16.87 and δ 16.76 respectively. The mass spectrum gave a parent peak at m/z 468.3969 corresponding to the molecular formula $C_{32}H_{52}O_2$. Also present was a peak at 408 corresponding to a fragment due to a McLafferty rearrangement.[49] Figure 10 illustrates this process.

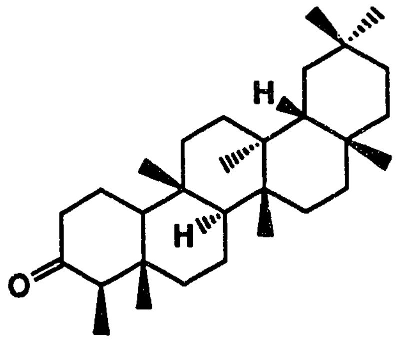
**FIGURE 10**



Finally there was a base peak at m/z 218 arising from a *retro*-Diels-Alder rearrangement of ring C. The 218 peak was due to a fragment consisting of rings D and E and C-11, C-12 and C-27.

The 300 MHz $^1H$ nmr spectrum of lupeol acetate (**75**) showed a doublet at δ 4.68 and a multiplet at δ 4.57 for one hydrogen each. These signals represent the vinylic hydrogens at C-29. The 3α-H appeared at δ 4.47 as a multiplet, the ester methyl at δ 2.04 as a singlet and the vinylic C-20 methyl at δ 1.68 also as a singlet. The remaining methyls gave singlets in the region δ 1.03 to δ 0.79. In the ir spectrum there were strong absorptions at 1733 and 1245 cm$^{-1}$ due to the acetate functionality. The mass spectrum gave a parent peak at m/z 468.3970 for the molecular formula $C_{32}H_{52}O_2$. The fragment at 408, due to the McLafferty rearrangement, was more intense than that found for **74**. As expected, with the absence of a double bond in ring C, the peak at 218 was much less intense in **75** .

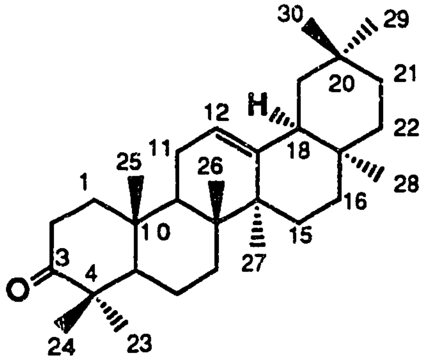
Further elution (fractions 3 to 6), with a solution of 3-10% ether in petroleum ether gave a white solid LPP4. As was the case for the LPP3 fraction, LPP4 was also found to be a mixture, this time of three compounds. On partial concentration compound **76** crystallized out. This compound was further purified by recrystallizing from 98% ethanol.



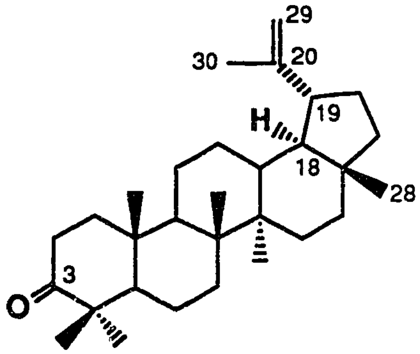
**76**

The spectral data of this compound was found to be consistent with those reported for friedelin.[50,51,52] The 400 MHz $^1$H nmr of this material showed no signals below δ 2.5 and a complex set of signals between δ 2.5 and δ 1.25. A series of sharp singlets at δ 1.18, 1.05, 1.01, 1.00, 0.95, 0.87 and 0.73 along with a doublet at δ 0.89, all corresponding to the methyl groups, are characteristic for friedelin. The identification of **76** was facilitated by these methyl signals which serve as fingerprints often helping to establish the structures for many sterols and triterpenes.[53] In the mass spectrum of **76**, there was a parent peak at m/z 426.3851 corresponding to a molecular formula of $C_{30}H_{50}O$. The ir spectrum gave a strong absorption at 1715 $cm^{-1}$ for the ketone carbonyl.

Separation of the remaining material was carried out using a 15% $AgNO_3$–$SiO_2$ preparative tlc plate. Eluting with a chloroform:hexane (9:1) solution provided the ketone **77** ($R_f$ = 0.45) and the ketone **78** ($R_f$ = 0.15).



**77**



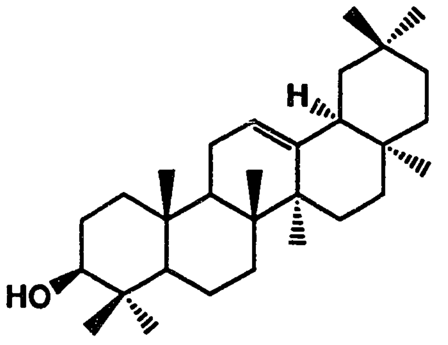
**78**

The ketones **77** and **78** were identified as amyrenone and lupeone respectively, by comparison of the spectroscopic information with that reported in the literature.[54,55] In its 300 MHz $^1H$ nmr spectrum, amyrenone was characterized by a multiplet at δ 5.21 for the single vinylic hydrogen at C-12 and a series of methyl signals between δ 1.15 and δ 0.85. By comparison, lupeone shows a doublet at δ 4.69 and a multiplet at δ 4.58 for the olefinic hydrogens. The vinylic methyl appears as a singlet at δ 1.68 with the remaining methyls as singlets between δ 1.07 and δ 0.80. An examination of the nmr spectral data of ketones **76**, **77** and **78** reveals significant differences. The mass spectral data also point out signifcant differences. The most apparent distinction is in the mass spectrum of amyrenone with its parent peak corresponding to the fragment at m/z 218. This is a result of a *retro*-Diels-Alder fragmentation of the C-ring, which is quite characteristic of triterpenes possessing a C12–C13 double bond. This pattern is absent in the mass spectra of the other two ketones.

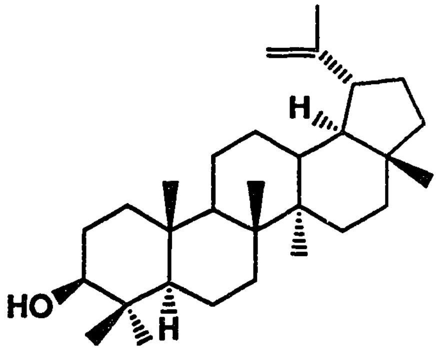
As a further proof of the identity of **77** and **78**, samples of β-amyrin (**79**) and lupeol (**80**), also isolated as part of this project (*vide infra*), were subjected to oxidation with pyridinium chlorochromate (PCC). Lupeol was stirred at room temperature overnight with 1.5 eq of PCC and 0.3 eq of sodium acetate in dichloromethane. Purification by column chromatography, using 2% ethyl acetate in petroleum ether, gave ketone **78** in 80% yield. Similar treatment of β-amyrin gave rise to ketone **77** in 78% yield. The semi-synthetic products obtained were found to be identical with those obtained directly from the natural source.

Further elution with 3-10% ether in petroleum ether gave a very small amount of compound LPP5. This compound was tentatively identified from its nmr spectrum as friedelinol.

The final eluate obtained using 3-10% ether in petroleum ether was again determined to be a mixture. The two components were separated by preparative tlc using a 15% $AgNO_3$-silica gel mixture. The plate was eluted using a solvent system consisting of 24 parts chloroform and one part ethyl acetate. Two bands were observed having a $R_f$ of 0.44 and 0.26. The faster moving compound was identified as β-amyrin (**79**) by comparison with reported literature data.[46] The slower moving compound was found to be lupeol (**80**) which was identical with an authentic sample.



**79**



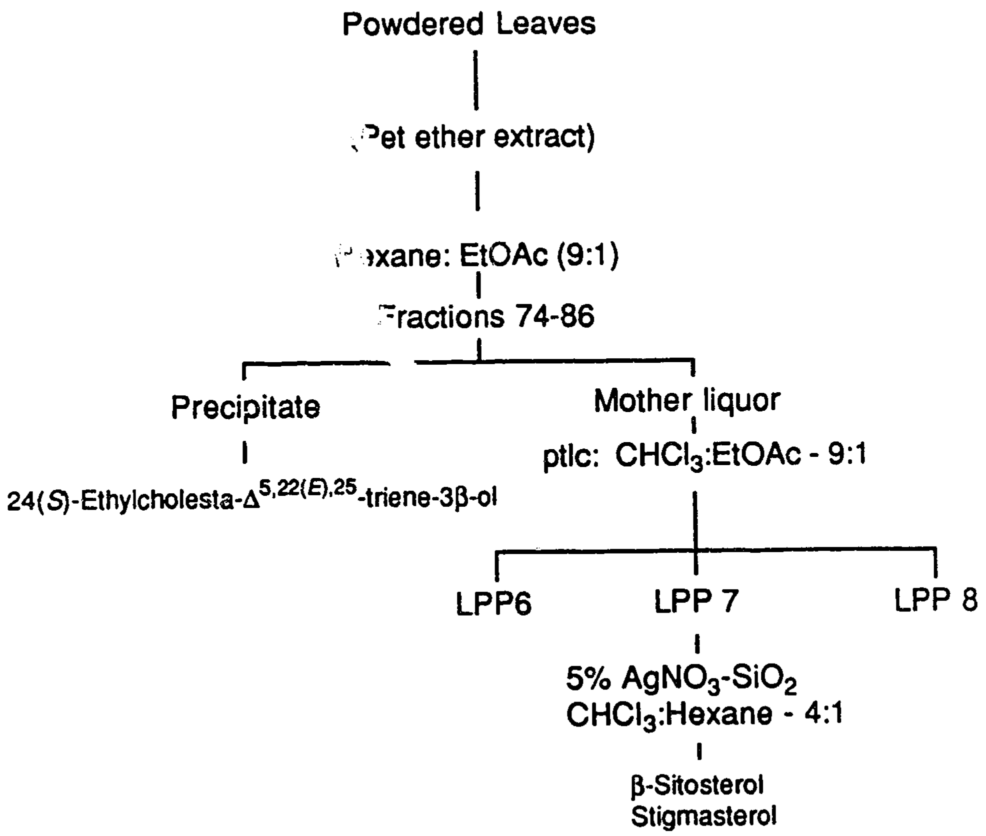
**80**

The 400 MHz $^1$H nmr spectrum of β-amyrin showed a multiplet at δ 5.19 for the vinylic hydrogen at C-12 and another multiplet at δ 3.23 for the α-hydrogen at C-3. The eight methyl groups appeared as seven singlets between δ 0.79 and δ 1.14. The chemical shifts of these signals were in close agreement with those reported by Ito *et al.*[56] In the infrared spectrum, the hydroxyl stretch appeared at 3290 cm$^{-1}$. The mass spectrum showed a parent peak at m/z 426.3858 corresponding to a molecular formula of $C_{30}H_{50}O$. There was also a peak at m/z 218.2034 (100%) corresponding to a molecular formula of $C_{16}H_{26}$ due to a *retro*-Diels-Alder fragmentation.

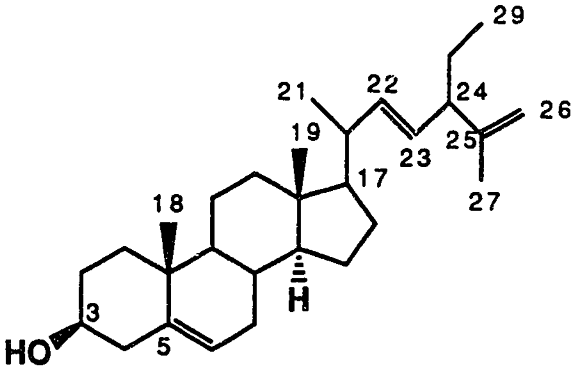
Lupeol (**80**) gave a 300 MHz $^1$H nmr spectrum that was identical with that of an authentic sample. In the ir spectrum the hydroxyl stretch was at 3367 cm$^{-1}$ while a medium absorption appeared at 1640 cm$^{-1}$ for the olefinic stretch. The mass spectrum had a parent peak at m/z 426.3869 for a molecular formula of $C_{30}H_{50}O$.

Increasing the polarity (20-33% ether in petroleum ether) of the solvent system resulted in the isolation of LPP7 and LPP8. Since these were obtained in very small amounts, a second petroleum ether extraction of the leaves of *guang jisheng* was carried out. The crude petroleum ether extract was again subjected to column chromatography on silica gel. In order to facilitate the isolation of the more polar components LPP7 and LPP8, a solvent system consisting of nine parts hexane and one part ethyl acetate was used. This isolation process is illustrated in Figure 11.

**FIGURE 11**

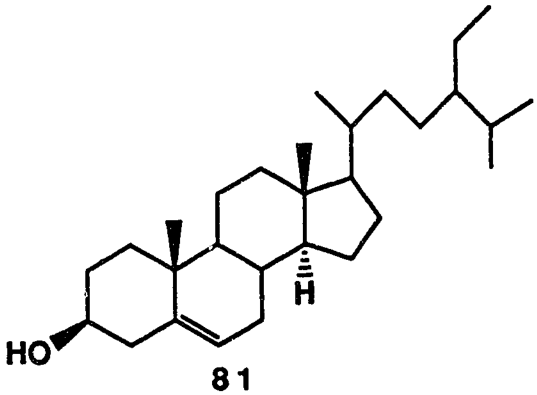


Fractions containing LPP6, LPP7 and LPP8 were combined. Upon partial concentration a solid was obtained. Recrystallization using 98% ethanol afforded long white needles identified, by comparison with the reported literature data,[57, 58] as 24(*S*)-ethylcholesta-$\Delta^{5,22(E),25}$-triene-3β-ol (**83**). From the infrared spectrum three absorptions of importance to this compound were observed: a broad absorption at 3400 $cm^{-1}$ for the hydroxyl stretch, a medium band at 959 $cm^{-1}$ for the C-22 double bond and a medium absorption at 885 $cm^{-1}$ for the C-24 double bond. The 300 MHz $^1H$ nmr spectrum showed a series of multiplets at δ 5.35 (C-6 H), δ 5.2 (C-22, C-23 H's) and δ 4.7 (C-26 H's) for the vinylic hydrogens.

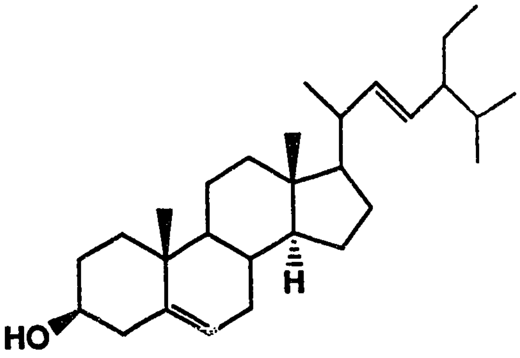


Another multiplet appeared at δ 3.52 for the C-3 α-H. Also in close agreement with the reported values were the chemical shifts of the remaining methyl groups. The mass spectrum of **83** gave a parent peak at m/z 410.3554 consistent with a molecular formula of $C_{29}H_{46}O$. A tlc analysis indicated that **83** had a $R_f$ value identical to that of two closely related sterols obtained later from LPP7.

After removal of **83**, the mother liquor was subjected to preparative tlc from which two additional known sterols were also isolated. These two compounds also appeared as a single spot on a silica gel tlc plate. Even on a silver nitrate impregnated silica gel plate, there was no obvious separation. By converting these products into their acetate esters, using acetic anhydride and pyridine, AgNO$_3$–silica gel preparative tlc techniques allowed easy separation of the mixture. The separated compounds were then hydrolysed, using sodium hydroxide, to the respective alcohols. The spectral data of these two alcohols were then compared with those of authentic samples. In this manner the two sterols were found to be β-sitosterol (**81**) and stigmasterol (**82**). The ir spectrum of β-sitosterol had a broad hydroxyl absorption at 3360 cm$^{-1}$. In the 400 MHz $^1$H nmr spectrum there was a multiplet at δ 5.35 for the C-6 hydrogen and a multiplet at δ 3.53 for the 3α-H. The six methyl groups appeared in the region between δ 1.01 and δ 0.68 in the same fashion as the authentic sample. Finally the mass spectrum gave a parent ion peak at m/z 414.3862 for a molecular formula of C$_{29}$H$_{50}$O.



**81**



**82**

For stigmasterol (**82**), the infrared spectrum had a broad band at 3340 cm$^{-1}$ for the hydroxyl stretch along with a medium absorption at 969 cm$^{-1}$ for the *trans* double bond at C-22. A 300 MHz $^1$H nmr spectrum of **82** revealed a multiplet at δ 5.36 for the single C-6 hydrogen, a multiplet at

$\delta$ 5.08 for the two *trans* hydrogens of the C-22 double bond with J = 17 Hz and a multiplet at $\delta$ 3.52 for the 3$\alpha$-H. The remaining six methyl groups were found in the region between $\delta$ 1.02 and $\delta$ 0.70 as observed for the authentic sample and as reported in the literature.[59] The mass spectrum gave a parent ion at m/z 412.3726 for a molecular formula of $C_{29}H_{48}O$.

The constituents of the petroleum ether extract of *guang jisheng* were found to consist mainly of steroid and triterpenoid compounds. The triterpene components formed an interesting series of the acetate, ketone and alcohol derivatives. Although all of the compounds isolated have been found in other plants, to our knowledge this is the first report of these compounds in this plant.

# EXPERIMENTAL

## General

Melting points were recorded on a Köfler hot stage apparatus and are uncorrected. Infrared spectra (ir) were recorded using Nicolet 7199 FTIR and Nicolet MX-1 FTIR spectrometers. High resolution mass spectra (ms) were obtained using a Kratos AEI MS50 high resolution mass spectrometer. Proton nuclear magnetic resonance spectra ($^1$H nmr) were obtained using the following spectrometers: Bruker WH-200 (200 MHz), Bruker WH-400 (400 MHz) and Bruker AM-300 (300 MHz). Coupling constants are reported to within ±0.5 Hz. Carbon-13 nuclear magnetic resonance spectra ($^{13}$C nmr) were recorded on a Bruker AM-300 (75.47 MHz). Carbon-13 multiplicities were derived from off-resonance or Carr-Purcel-Meiboom-Gill spin echo J-modulated experiments (APT or Attached Proton Test). Methylene groups and quarternary carbons appear as signals in phase with respect to the deuteriochloroform signal while the signals having an antiphase to that of $CDCl_3$ are for the methyl and methine groups. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Mass spectra are reported as m/z (relative intensity as a %). Abbreviations are the same as those used in the first chapter.

## Materials

All separations by flash chromatography employed Still's procedure and used Merck silica gel of mesh 230-400. Thin layer chromatography was perfomed on aluminum-backed plates precoated with silica gel 60 $F_{254}$ as supplied by Merck. Preparative tlc plates were prepared using Merck silica gel 60 $PF_{254}$. Solvents routinely used for chromatography (petroleum ether, skellysolve B, diethyl ether, ethyl acetate and chloroform) were distilled prior to use. Bands on the preparative tlc plates were located using a solution prepared from 6 g of phosphomolybdic acid, 1g of ceric sulphate and 3 ml of concentrated sulphuric acid in 200ml of water.

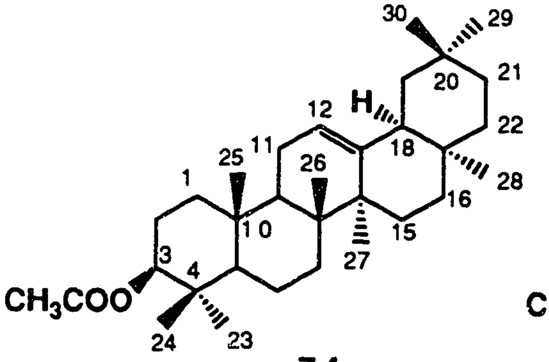
## Preliminary Isolation of Compounds 74 to 80

Compounds **74** to **80** were obtained by the following procedure: 200 g of the leaves of *guang jisheng* were ground to a fine powder and extracted with 4x1.0 L of petroleum ether over a period of 4 days. The extracts were filtered and concentrated to give 3.7 g of a dark green waxy solid. The solid was then placed on a column of silica gel and eluted with a solution of ether (0 to 33%) in petroleum ether. Using only petroleum ether, 137 mg of a white waxy solid (LPP1) was collected. The spectral data on this material identified it as hydrocarbon and no further work was done on this material. The next fraction (LPP2) was isolated when the ether concentration was increased to 2%. A white solid (167 mg) was isolated from this fraction. The spectral data on this material identified it as long chain saturated ester compounds. No further investigation was done into this material.

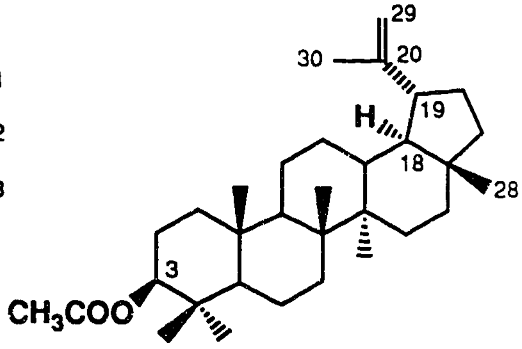
Increasing the concentraton of the eluant to 3 to 10% ether in petroleum ether removed a yellow band as the first fractions. Concentration of the eluate provided 737 mg of an yellow oily solid. Tlc comparison with the previous fractions indicated that this material was more of LPP1 and LPP2. Following the yellow band a further 26 fractions were collected. The first fraction when concentrated gave 77 mg of a yellow material form which **74** and **75** (LPP3) would eventually be isolated. Fractions 3 to 6 provided 88 mg of crude solid from which compounds **76-78** (LPP4) were obtained. Fractions 12 to 14 gave 79 mg of crude material. LPP5 was the compound of interest here. The final fractions with compounds of interest from this isolation were fractons 18-22 from which 699 mg of crude material was recovered. The products of interest were those compounds that made up LPP6 (**79** and **80**). The 34 mg of crude material collected in the final fractions 23 to 26 were found to contain only traces LPP4 and LPP6

Following these fractions, the ether concentration was gradually increased from 10 to 33%. A total of 312 mg of crude solid was collected that consisted mainly of yellow or green pigment. Tlc comparison of this material with that of the crude extract showed that LPP6, LPP7 and LPP8 were present. A later examination of these extracts indicated that the amount of the compounds of interest was too small for study and so another leaf extraction was carried out. The details of this extraction are outlined prior to the discussion of compounds **81** to **83**.

### β-Amyrin Acetate (74) and Lupeol Acetate (75)



**74**



**75**

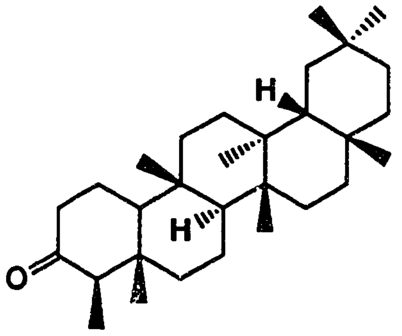
From the 3% ether fractions, the first fraction removed contained 77 mg of a yellow solid. Recrystallization of this solid from acetone left 17.5 mg of a white solid found to be a mixture of two compounds. Separation was effected by preparative tlc using a 15% $AgNO_3$-silica gel mixture. The plates were developed using a 9:1 mixture of chloroform and hexane. From the upper band ($R_f$=0.59), 9.4 mg of compound **74** was isolated.

mp: 236-8°C (lit.$^{60}$ 238-9°C); $[\alpha]_D$=+81.82° (c=0.407,$CHCl_3$); ir ($CHCl_3$ cast): 1734 (C=O), 1247 $cm^{-1}$ (C-O-C); $^1H$ nmr (300 MHz, $CDCl_3$): δ 5.16 (dd, 1H, -C**H**=C-, J = 3.5, 3.5 Hz), 4.48 (dd, 1H, 3α-H, J = 7.5, 9.5 Hz), 2.03 (s, 3H, -OCOC**H**$_3$), 1.11 (s, 3H, -C**H**$_3$), 0.95 (s, 3H, -C**H**$_3$), 0.94 (s, 3H, -C**H**$_3$), 0.84 (br s, 12H,-C**H**$_3$), 0.81 (s, 3H, -C**H**$_3$); $^{13}C$ nmr (75.4 MHz, $CDCl_3$): 171.0 (s, -O**C**OCH$_3$), 145.3 (s, -CH=**C**-), 121.7 (d, -**C**H=C-), 80.99 (d, C-2), 55.32 (d, C-5), 47.62 (d, C-9), 47.30 (d, C-18), 46.85 (t, C-19), 39.87 (s, C-8), 38.33 (t, C-1), 37.78 (s, C-4), 37.20 (t, C-22), 36.90 (s, C-10), 34.79 (t, C-21), 33.39 (q, C-29), 32.65 (t, C-7), 32.55 (s, C-17), 31.14 (s, C-17), 28.46 (q, C-28), 28.10 (q, C-23), 26.99 (t, C-15), 26.20 (t, C-16), 26.01 (q, C-27), 23.75 (q, C-30), 23.63 (t, C-2), 23.59 (t, C-11), 21.36 (q, $-OCOCH_3$), 18.32 (t, C-6), 16.87 (q, C-24), 16.76 (q, C-26), 15.62 (q, C-25) ; ms calcd. for $C_{32}H_{52}O_2$: 468.3970, found: m/z 468.3969 ($M^+$, 5), 408 (1), 218 (100), 203 (28), 189 (11).

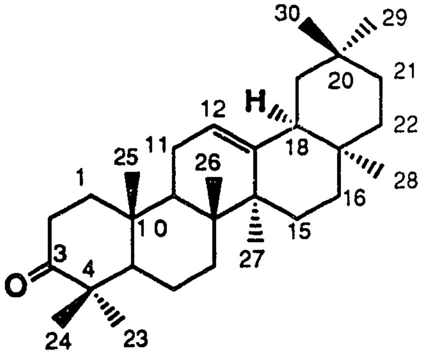
The lower band ($R_f$=0.33) gave a white solid (10 mg) that was identified as **75**.

mp: 213-215°C (lit.[61] 218°C); ir ($CHCl_3$ cast): 1733 $cm^{-1}$ (C=O); $^1H$ nmr (300 MHz, $CDCl_3$): δ 4.68 (d, 1H, $-C=CH_2$, J = 2.5 Hz), 4.57 (m, 1H, $-C=CH_2$), 4.47 (m, 1H, 3α-H), 2.04 (s, 3H, $-OCOCH_3$),1.68 (s, 3H, $H_2C=C(CH_3)-$), 1.03 (s, 3H, $-CH_3$), 0.94 (s, 3H, $-CH_3$), 0.85 (s, 3H, $-CH_3$), 0.84 (s, 3H, $-CH_3$), 0.83 (s, 3H, $-CH_3$), 0.79 (s, 3H, $-CH_3$); ms calcd. for $C_{32}H_{52}O_2$: 468.3970, found: m/z 468.3970 ($M^+$, 79), 453 (16), 408 (11), 218 (48), 204 (46), 190 (48), 189 (100), 108 (49), 107 (68), 82 (17).

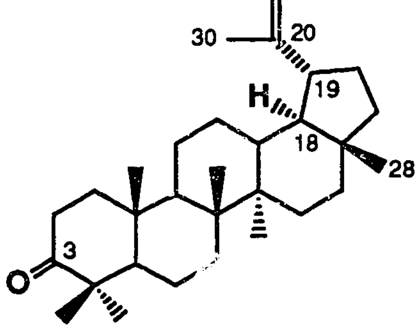
## Friedelin (76), Amyrenone (77) and Lupeone (78)



76



77



78

From fractions 3 to 6, the 88 mg of crude yellow material collected was first recrystallized from acetone to give a white solid. Three compounds were then isolated from this solid. The first precipitated from solution on slow evaporation of the solvent. Recrystallization from 98% ethanol gave of a white solid identified as the saturated triterpene ketone **76** (1.0 mg) by comparison with reported ir, nmr and ms spectral data.[50]

mp 254-7°C (lit.[62] 255-261°, 261-3°(vac)); $[\alpha]_D$= -22° (c=0.0009, $CHCl_3$) (lit.[60] -29.4°); ir ($CHCl_3$ cast): 1715 $cm^{-1}$ (C=O); $^1H$ nmr (400 MHz, $CDCl_3$): $\delta$1.18 (s, 3H, $-CH_3$), 1.05 (s, 3H, $-CH_3$), 1.01 (s, 3H, $-CH_3$), 1.00 (s, 3H, $-CH_3$), 0.95 (s, 3H, $-CH_3$), 0.89 (d, 3H, $-CH_3$,J = 6.5 Hz), 0.87 (s, 3H, -$CH_3$), 0.73 (s, 3H, -$CH_3$); ms calcd. for $C_{30}H_{50}O$: 426.3864, found: m/z 426.3851 ($M^+$, 46), 411 (15), 302 (25), 273 (38), 246 (26), 205 (38), 179 (28), 149 (43), 109 (78), 95 (95), 69 (100).

Amyrenone and lupeone were separated using a 15% $AgNO_3$–$SiO_2$ preparative tlc plate and developing the plate with $CHCl_3$:Hexane (9:1). Amyrenone was the upper band with a $R_f$ of 0.45. This was removed and extracted with chloroform. The extracts were then filtered, dried over $MgSO_4$ and finally concentrated to give amyrenone **77** (3.8 mg) as a white solid.

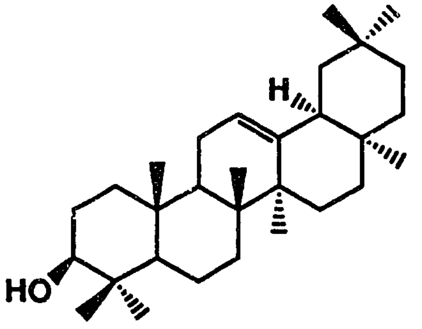
mp 169-169.5°C; $[\alpha]_D$=+83.74° (c=0.123, $CHCl_3$); ir ($CHCl_3$ cast): 1707 $cm^{-1}$; $^1H$ nmr (300 MHz, $CDCl_3$): δ5.21 (m, 1H, -CH=C), 1.15 (s, 3H, -$CH_3$), 1.10 (s, 3H, -$CH_3$), 1.08 (d, 3H, -$CH_3$, J = 1 Hz), 1.06 (s, 3H, -$CH_3$), 1.02 (s, 3H, -$CH_3$), 0.88 (s, 6H, -$CH_3$), 0.85 (s, 3H, -$CH_3$); ms calcd. for $C_{30}H_{48}O$: 424.3707, found: m/z 424.3703 ($M^+$, 16), 409 (7), 218 (100), 203 (33), 189 (9), 95 (11).

Lupeone (**78**) (6.4 mg) was removed as the lower band with an $R_f$ of 0.15 and treated in the same manner as amyrenone to give a white solid.

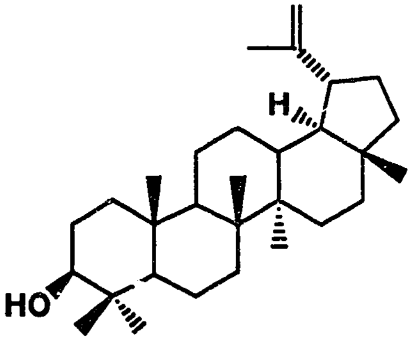
mp 170-171°C (lit.[60] 168-170.5°C); $[\alpha]_D$=+55.08° (c=0.118, $CHCl_3$) (lit.[60] +60.6 (c=0.5, $CHCl_3$)); ir ($CHCl_3$ cast): 1706 $cm^{-1}$ (C=O); $^1H$ nmr (300 MHz, $CDCl_3$): δ 4.69 (d, 1H, $CH_2=C(CH_3)$-, J = 2.5 Hz), 4.58 (dd, 1H, $CH_2=C(CH_3)$-, J = 1, 2 Hz), 1.68 (s, 3H, -$C(CH_3)=CH_2$), 1.07 (s, 6H, -$CH_3$), 1.03 (s, 3H, -$CH_3$), 0.96 (s, 3H, -$CH_3$, J = 1 Hz), 0.93 (s, 3H, -$CH_3$, J = 1 Hz), 0.80 (s, 3H, -$CH_3$); ms calcd. for $C_{30}H_{48}O$: 424.3707, found: 424.3702 ($M^{+\cdot}$, 100), 409 (23), 314 (23), 313 (30), 245 (21), 218 (27), 205 (47), 189 (29), 161 (19), 149 (26), 135 (32), 109 (67), 95 (68).

The crude fractions 12 to 14 were found to contain LPP4, LPP5 and LPP6 along with considerable pigment material. After recrystallization from acetone–petroleum ether, that removed most of the pigment, the remaining material was rechromatographed on silica gel using 5% ether in petroleum ether as the eluant. In this manner LPP5 (<1 mg) was isolated with traces of LPP6 present. The amount of sample isolated was sufficient for a $^1H$ nmr spectrum from which LPP5 was tentatively identified as friedelan-3β–ol (the 3β–OH derivative of **76**). No $^1H$ nmr or mass spectral data has been reported in the literature and we are awaiting a sample of authentic material to confirm our assignment.

**β-Amyrin (79) and Lupeol (80)**



**79**



**80**

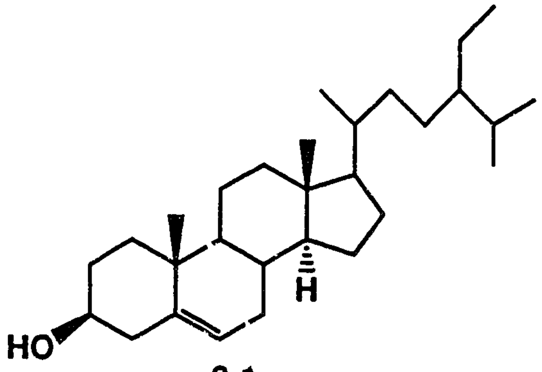
A 15% $AgNO_3$-silica gel mixture in water was evenly applied to a glass plate. It was first air dried and then placed in a warm oven overnight. 30 to 40 mg of the LPP6 mixture, as a chloroform soluton, was then evenly applied to the plate. The plate was developed by eluting twice with a chloroform-ethyl acetate (24:1) mixture. When finished the two bands were found by spraying a narrow strip on each side of the plate with a phosphomolybdic acid solution and warming to locate the compounds as two blue spots. The top band with an $R_f$ of 0.44 was identified as β-amyrin (**79**). Each band was then removed separately and extracted with chloroform. The chloroform was then filtered, dried and concentrated to provide of **79** (12 mg) as a white solid.

mp 193-5°C (lit.[61] 197-197.5°C); $[\alpha]_D$=+66.13° (c=0.186, $CHCl_3$); ir ($CHCl_3$ cast): 3290 $cm^{-1}$ (OH); $^1H$ nmr (300 MHz, $CDCl_3$): δ 5.19 (m, 1H, -CH=C), 3.23 (m, 1H, 3α-H), 1.14 (s, 3H, $-CH_3$), 1.00 (s, 3H, $-CH_3$), 0.97 (s, 3H, $-CH_3$), 0.94 (s, 3H, $-CH_3$), 0.87 (s, 3H, $-CH_3$), 0.83 (s, 3H, $-CH_3$), 0.79 (s, 3H, $-CH_3$); ms calcd. for $C_{30}H_{50}O$: 426.3864, found m/z 426.3858 ($M^+$, 12), 218 (100).

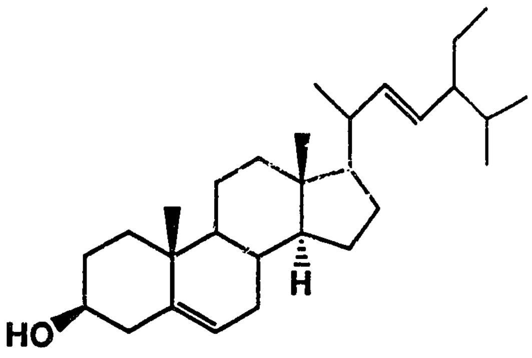
Lupeol (**80**) was removed as the lower band ($R_f$=0.29) from the purification of LPP6. In this manner **80** (3 mg) was obtained.

mp 210-213°C (lit.[63] 215°C); ir ($CHCl_3$ cast): 3367 (OH), 1640 $cm^{-1}$ ($C=CH_2$); $^1H$ nmr (300 MHz, $CDCl_3$): δ 4.69 (d, 1H, $=CH_2$, J = 2.5 Hz), 4.57 (q, 1H, $=CH_2$), 3.19 (dd 1H, 3α-H), 2.38 (m, 1H, -OH), 1.90 (m, 1H), 1.68 (s, 3H, $H_2C=C(CH_3)-$), 1.03 (s, 3H, $-CH_3$), 0.97 (s, 3H, $-CH_3$), 0.94 (s, 3H, $-CH_3$), 0.83 (s, 3H, $-CH_3$), 0.78 (s, 3H, $-CH_3$), 0.76 (s, 3H, $-CH_3$); ms calcd. for $C_{30}H_{50}O$: 426.3864, found: m/z 426.3869 ($M^+$, 26), 411 (8), 408 (2), 393 (2), 218 (20), 208 (11), 207 (36), 204 (14), 203 (21), 189 (45), 109 (58), 81 (84), 69 (89), 55 (100).

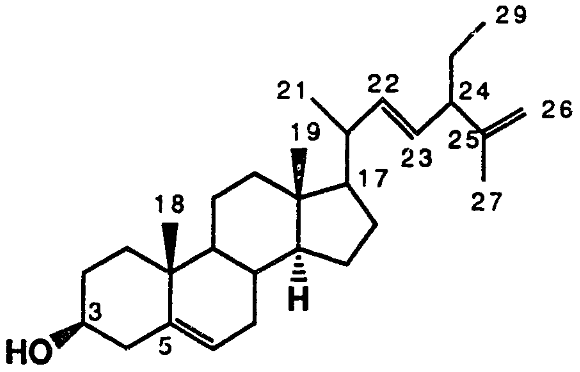
### β-Sitosterol (81), Stigmasterol (82) and 24-Ethylcholesta-$\Delta^{5,22(E),25}$-triene-3β-ol (83)



**8 1**



**8 2**



**8 3**

The three sterols **81**, **82** and **83** were obtained as follows: 832 grams of powdered leaves were extracted by stirring with 4x3 L of Skellysolve B over a period of 4 days. The material was filtered after each extraction and the filtrates were combined and concentrated to give 44.3 grams of a waxy solid. This material was then placed on a 9x15 cm column of silica gel and eluted with 9 L of a 8.6:1.4 mixture of skellysolve B and ethyl acetate, collecting 87 fractions. The three sterols were found in fractions 74 to 86 along with **79** and **80** and a more polar fraction. After recovery of the precipitated sterol **83**, the residues were separated by preparative tlc using $CHCl_3$:EtOAc (9:1) as the solvent. The band containing **81** and **82** was then removed, extracted with chloroform, dried and concentrated. In order to make the separation easier, the mixture was converted into an acetate ester mixture using acetic anhydride and pyridine. This mixture was then separated by preparative tlc using 5% $AgNO_3$-$SiO_2$ and eluting with $CHCl_3$:hexane as a 4:1 mixture. The two bands were located in the same manner as that used for β-amyrin and lupeol. The upper band of β-sitosterol acetate and the lower band of stigmasterol acetate were each scraped off the plate and extracted from the silica with chloroform to give white solids. Since we had authentic samples of **81** and **82** for comparative purposes, each of the acetates were hydrolysed with base to give the respective sterol. Recrystallization from 98% ethanol gave white samples of each compound.

β-Sitosterol (**81**) showed the following spectral data:
ir ($CHCl_3$ cast): 3360 $cm^{-1}$ (OH); $^1H$ nmr (400 MHz, $CDCl_3$): $\delta$5.35 (m, 1H, C6-H), 3.53 (m, 1H, 3α-H), 1.01 (s, 3H, C-19 $CH_3$), 0.92 (d, 3H, C-21 -$CH_3$, J = 10.5 Hz), 0.84 (t, 3H, C-29 -$CH_3$, J = 8.5 Hz), 0.82 (d, 3H, C-26 -$CH_3$, J = 7.0 Hz), 0.80 (d, 3H, C-27 -$CH_3$, J = 6.5 Hz), 0.68 (s, 3H, C-18 -$CH_3$); ms calcd. for $C_{29}H_{50}O$: 414.3864, found: m/z 414.3862 ($M^+$, 52), 399 (12), 396 (17), 329 (14), 303 (17), 273 (17), 255 (21), 231 (16), 213 (25), 159 (29), 121 (27), 109 (26), 107 (56), 95 (57), 93 (43), 67 (40), 55 (100).

Stigmasterol (**82**) showed the following spectral data:
ir (CHCl$_3$ cast): 3340 (OH), 969 cm$^{-1}$; $^1$H nmr (300 MHz, CDCl$_3$): δ5.36 (m, 1H, C6-H), 5.15 (dd, 1H, -C**H**=CH-, J = 8.5, 15 Hz), 5.01 (dd, 1H, -CH=C**H**-, J = 8.5, 15 Hz), 3.52 (m, 1H, 3α-H), 1.02 (d, 3H, J = 6.5 Hz, -CH$_3$), 1.01 (s, 3H, -CH$_3$), 0.84 (d, 3H, J = 6.5 Hz, -CH$_3$), 0.80 (t, 3H, J = 6.5 Hz, -CH$_3$), 0.70 (s, 3H, -CH$_3$); ms calcd. for $C_{29}H_{48}O$: 412.3707, found: m/z 412.3726 (M$^+$, 57), 397 (4), 394 (4), 379 (3), 351 (13), 271 (24), 255 (30), 213 (11), 159 (22), 135 (25), 9[illegible] (27), 67 (30), 55 (100).

The triene **83** was isolated from fractions 74-86 by slow evaporation of the solvent. The solid crystallized from the solution as long needles. These were recrystallized from 98% ethanol to give 5.9 mg of a white product.

ir (CHCl$_3$ cast): 3400 (OH), 959 , 885 cm$^{-1}$; $^1$H nmr (300 MHz, CDCl$_3$): δ5.35 (m, 1H, C**H**=CH), 5.2 (m, 2H, C**H**=C**H**), 4.7 (m, 2H, C=C**H**$_2$), 3.52 (m, 1H, C-3 α-H), δ1.65 (s, 3H, C-26 -CH$_3$), δ1.10 (d, 3H, C-21 -CH$_3$, J = 7.0 Hz), δ1.00 (s, 3H, C-19 -CH$_3$), δ0.83 (t, 3H, C-28 -CH$_3$, J = 7.5 Hz), δ0.68 (s, 3H, C-18 -CH$_3$); ms calcd. for $C_{29}H_{46}O$: 410.3551, found: m/z 410.3554 (M$^+$, 24), 395 (5), 392 (5), 381 (16), 300 (42), 271 (100), 255 (67), 213 (25), 159 (48), 138 (50), 137 (57), 109 (95), 95 (82), 81 (88), 55 (67).

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