A SYNTHETIC STUDY OF SOME TRIQUINANE NATURAL PRODUCTS AND MICROBIAL REDUCTION OF PROCHIRAL SPIRODIKETONES

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A SYNTHETIC STUDY OF SOME TRIQUINANE NATURAL PRODUCTS AND MICROBIAL REDUCTION OF PROCHIRAL SPIRODIKETONES

by

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ABSTRACT

The first half of this thesis describes an approach to the synthesis of a group of triquinane sesquiterpenes, with (\pm) deoxypentalenic acid as the particular target. The synthesis started from readily available isophorone. The central quaternary center of the natural product was established in an early step by a double acylation reaction on a cyclohexene derivative. Cleavage of the double bond was followed by reclosure to a fivemembered ring. The relative stereochemistry at a stereogeni methine was controlled by catalytic hydrogenation. An intramolecular aldol reaction was used to cyclize the third ring of the triquinane moiety. The remaining steps to (\pm)-deoxypentalenic acid were modeled in reactions leading to β keto esters and in reductions of the ketone moiety of a β -keto ester.

The second part of the thesis provides the results of chiral reductions of 1,3-cyclopentanedione derivatives using baker's yeast. The series of diketones that was examined included 7,9,9-trimethylspiro[4.5]dec-7-ene-1,4-dione. The yeast reduction of this compound to (4S,5R)-4-hydroxy-7,9,9-trimethylspiro[4.5]dec-7-en-1-one (123) proceeded with high facial selectivity and enantioselectivity, as determined by an analysis of the corresponding Mosher's ester [(+)- α -methoxy- α -trifluoromethylphenylacetate derivative]. The facial selectivity was compared with that of the chemical reduction using sodium borohydride. The absolute stereochemistry was established from CD spectra and an X-ray structure. The transformation of compound 123 into (4R,5S)-4-hydroxy-4,7,9,9tetramethylspiro[4.5]dec-7-en-1-one, an optically active form of an intermediate in the triquinane synthesis, required four steps.

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Glossary of Abbreviations

| APT | Attached proton test |
|---------|---|
| CD | Circular dichroism |
| COSY | H-H Correlation spectrum |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| DIBAL | Diisobutylaluminum hydride |
| GCMS | Gas chromatography-mass spectrometry |
| HMPA | Hexamethylphosphoramide |
| IR | Infrared (spectroscopy) |
| LDA | Lithium diisopropylamide |
| Me | Methyl |
| mp | Melting point |
| MS | Mass spectrometry |
| NBS | N-Bromosuccinimide |
| NMR | Nuclear magnetic resonance (spectroscopy) |
| nOe | Nuclear Overhauser effect |
| Nu | Nucleophile |
| PCC | Pyridinium chlorochromate |
| pTSA | para-Toluenesulphonic acid |
| TBDMSCI | tert-Butylchlorodimethylsilane |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| TMSCI | Chlorotrimethylsilane |
| UV | Ultraviolet (spectroscopy) |

To my dear parents

A SYNTHETIC STUDY OF SOME TRIQUINANE NATURAL PRODUCTS

I. Introduction

Our ultimate goal was the synthesis of deoxypentalenic acid (1b), which is a member of a large class of metabolites containing the tricyclo[$6.3.0.0^{4}.8$]undecane skeleton, closely related molecules are deoxypentalenic acid glucuron (1a), ¹ pentalenene (2), ², ³ and pentalenic acid (3), ⁴, ⁵









1b R = H

This group of angularly fused triquinanes also includes isocomene (4),6, 7 silphinene (5),8,9 5-oxosilphiperfolene (6),10, 11 subergorgic acid (7),12, 13 the unique diterpene laurenene (8)14, 15 (the only known



naturally occurring fenestrane), the unusual sesterterpene retigeranic acid (9), 16, 17 and crinipellin (10), 18, 19 Intensive research on pentalenene (2), pentalenic acid (3), deoxypentalenic acid (1b), and deoxypentalenic

acid glucuron (1a) has resulted from their demonstrated role in the biosynthesis of the sesquiterpene antibiotic pentalenolactone (11),20, 21 Seto et al.², ⁴ isolated pentalenic acid (3) from Streptomyces chromofuscus and pentalenene (2) from S. griseochromogenes. Takahashi et al. 1 obtained deoxypentalenic acid glucuron (1a) from S. omivaensis, S. albofaciens, and S. viridifaciens. Deoxypentalenic acid glucuron (1a) displayed antitumor activity against Sarcoma 180 in mice. Pentalenolactone (11) was isolated from several species of Streptomyces²⁰ such as S, chromofuscus, S, griseochromogenes, S. baarnensis, S. arenae and S. roseogriseus. These are rare examples of cyclic terpenoid antibiotics produced by prokaryotic organisms. Pentalenolactone (11) was reported to exhibit potent and specific antiviral activity.²² Studies in Cane's²³ laboratory have shownthat pentalenolactone (11) is a time-dependent, irreversible inactivator of glyceraldehyde-3-phosphate dehydrogenase, whose inhibitory action is due to a specific reaction with all four active-site cysteines of the tetrameric enzyme. They also carried out extensive studies on the biosynthesis of 2, 3, 1a, 1b and 11 as shown in Scheme 1.24 Farnesyl diphosphate (12) was enzymatically cyclized to pentalenene (2), the parent sesquiterpene. Several oxidative steps from pentalenene (2) led to deoxypentalenic acid glucuron (1a), deoxypentalenic acid (1b) and pentalenolactone (11).

Additional interest has recently been directed toward the synthesis of these structurally intriguing molecules.

In 1986, Crimmins and coworkers³⁷ reported the total synthesis of (\pm) -deoxypentalenic acid (1b) (Scheme 2) together with (\pm) -pentalenene 2), and (\pm) -pentalenic acid (3). A key step in the (\pm) -deoxypentalenic acid



Scheme1 Biosynthesis of pentalenene derivatives

(1b) synthesis was a novel conjugate addition-cycloacylation sequence on an acetylenic diester 16. Treatment of 16 with the appropriate Grignard reagent in the presence of tetramethylethylenediamine (TMEDA) and copper(I) iodide led to diene 17. Conversion of 17 to diene diester 18 was readily accomplished by selective ozonolysis of the electron-rich trisubstituted double bond to yield an aldehyde, which was immediately condensed with (carbethoxy-methylene)-triphenylphosphorane to provide 18. Irradiation of diene 18 with UV light resulted in smooth cycloaddition to produce 19 as a 10:3:1 (19a:19b:19c) mixture. Reductive cleavage of the cyclobutane ring of 19 was accomplished by treatment with lithium in liquid ammonia to produce B-keto esters 20a and 20b in a ratio of 13:1. Following hydrolysis-decarboxylation of B-keto ester 20b, esterificationand cyclization in base generated the triguinane skeleton 21. Selective ketalization of 21 gave compound 22. After reducing the carbonyl function of 22 with lithium in ammonia, hydrolysis of the ketal provided keto alcohol 23. Mesylation of this compound 23 ketone followed by treatment with 1.8-diazabicvclo[5.4.0]undec-7-ene (DBU) produced enone 24. Enone 24 was treated with excess LDA and CO₂ followed by esterification with diazomethane (CH2N2) to give keto ester 25. Sodium borohydride reduction of 25 proceeded to give allylic alcohol 26, which was catalytically hydrogenated to alcohol 27. Mesylation of the alcohol and elimination with DBU yielded methyl deoxypentalenate 28. The total synthesis of deoxypentalenic acid (1b) was achieved by hydrolysis of 28 in aqueous potassium hydroxide. Crimmins' synthesis was accomplished in a total of twenty steps.



Scheme 2 Crimmins' synthesis of (±)-deoxypentalenic acid (1b)





In 1988 Fukumoto and coworkers³¹ reported the total synthesis of (±)-deoxypentalenic acid (1b), using a different approach (Scheme 3). The key step in this synthesis was an intramolecular double Michael reaction. Thus, treatment of bis-enone 29 with chlorotrimethylsilane, triethylamine, and zinc chloride gave two separable diastercomers 30 and 31. Both the vield and the ratio of those two isomers depended on the reaction temperature and the solvent. Compound 30 was transformed into compound 32 with ethyl formate in the presence of sodium methoxide. Diazo-exchange using p-toluene sulphonyl azide and triethylamine gave diazo-ketone 33. Irradiation of 33 in methanol produced a mixture of the two separable keto esters 34 and 35 in a ratio of 3.6:1. Reduction of the ketone group of 34 to a methylene was achieved by dithioacetal formation and desulfurization with Raney nickel. Selenenvlation of 36 followed by oxidative elimination furnished methyl deoxypentalenate (28). Hydrolysis of the methyl ester completed the synthesis of deoxypentalenic acid (1b). This synthesis was finished in a total of fifteen steps including six steps to 29. During the synthesis, two steps each produced diastereomeric mixtures of compounds.

The challenges in the synthesis of this group of natural products are to establish the angular triquinane skeleton itself and to obtain the correct relative sterechemistry at C-9. There are a number of approaches to the synthesis of angular triquinane skeleton. Recently, a radical-mediated approach was reported by Yadav and coworkers²⁵ (Scheme 4). The required bromoacetal 40 was prepared from 2-methoxycyclopent-2-en-1one (37). A Grignard reagent, prepared from butenyl bromide, was added















1b





to **37** to give 2-(4-butenyl)cyclopent-2-en-1-one (**38**). Reduction of **38** with DIBAL produced dienol **39**, which was further converted to the desired bromoacetal **40** using NBS and ethyl vinyl ether. Treatment of **40** with sodium cyanoborohydride in the presence of a catalytic amount of tributylin hydride led to the expected mixed cyclic acetal **41**. Hemiacetal **42** was obtained by hydrolysis of ethyl acetal **41** with 90% trifluoroacetic acid. Reaction of **42** with methyl magnesium bromide produced diol **43**, which was converted to diketone **44** by Jones oxidation. The desired 9-methyl- tricyclo[6.3.0.0^{1,5}]undec-4-en-3-one (**45**) was obtained by treatment of **44** with 20% potassium hydroxide in ethanol.

Our synthetic approach to the triquinane skeleton was based on the successful synthesis of the (\pm)-pentalenene (2) by Wu and Burnell³⁰ (Scheme 5). Ketalisation of 3-ethyl-5,5-dimethylcyclohex-2-en-1-one (46) with ethylene glycol provided ketal 47. Treatment ketal 47 with 1,2-bis(trimethylsilyloxy)cyclobutene (48)²⁶ and boron trifluoride etherate afforded the spiro-diketone 49. The mono- alcohol 50 was obtained by addition of methyllithium to 49. Ozonolysis of 50, then cyclization with *p*/TSA as the catalyst provided 51. The conjugated double bond was reduced using Birch conditions. Hydrogenation and aldol condensation gave a mixture of the tricyclic products 53 and 54, which were separated. Starting with 53, catalytic hydrogenation, sodium borohydride reduction and acid catalysed dehydration resulted in product 2.

Even though our approach to deoxypentalenic acid (1b) was designed to parallel the route to pentalenene (2), it could also lead to pentalenene (2) itself by a slight modification of the last steps. We



Scheme 5 Burnell's synthesis of (±)-pentalenene (2)

concentrated on the synthesis of compound 55, which would be only a few steps from the natural product. The results of our work toward the total synthesis of deoxypentalenic acid (1b), including some model studies for the final steps, are presented in this chapter of the thesis.



II. Results and Discussion

Scheme 6 Formation of 57 from 56



The synthesis started from the readily available starting material isophorone (56). Ketalisation was carried out with ethylene glycol in the presence of *p*-toluenesulphonic acid (*p*TSA) (Scheme 6). The reaction produced a mixture of two poorly separable ketals 57 and 58. The desired isomer 57 was the major component as determined by gas thromatography-mass spectrometry (GCMS). Some starting material always remained no matter how long the reaction was run, and some oligomeric material was formed when the reaction time was increased. We finally opted to run the reaction for 12-14 hours. The solvent was evaporated and the products were distilled under vacuum to remove the yellow color. The crude colorless oil was then flash chromatographed. The ketal 57 was always a major isomer, but the ratio of the two isomers varied with the reaction time. GCMS analysis indicated that the some of the ketal product was hydrolysed back to the starting material 56, and some of the ketal 57 isomerized to ketal 58 during chromatography. The isomeric ketals could be clearly distinguished by their mass spectra (Figures 1 and 2).







Figure 2 MS of compound 58

The ketal **57** had its base peak at m/z 86 (C4H6O2.⁺), which arose via the homolytic retre-Diels-Alder reaction of **57**. Likewise, the base peak at m/z 126 in the mass spectrum of **58** could be assigned to a fragment with the formula C7H10O2.⁺ (Scheme 7). In the ¹H NMR spectra, the vinyl proton (H-8) of the ketal **57** resonated at δ 5.17, while the vinyl proton (H-6) of the ketal **58** was at δ 5.34.

Scheme 7 MS fragmentation of 57 and 58



Treatment of this mixture of ketals with three equivalents of 1,2bis(trimethylsilyloxy)cyclobutene **48** and a large excess of boron trifluoride etherate proceeded, via an intermediate cyclobutanone **59**, to afford in a single operation the rearranged, spiro-annulated diketone **60** (Scheme **8**). Scheme 8 Formation of 60 from 57



This reaction has now been extensively studied in our laboratory. However, Kuwajima and co-workers²⁷ first reported this reaction. They demonstrated that under Lewis acid catalysis a ketal reacts with 1,2bis(trimethylsilyloxy)cyclobuten (48) to provide the cyclobutanone, which can be rearranged in the presence of trifluoroacetic acid (TFA) to yield a spirodiketone. Our method employs a single step and leads to a superior yield by prolonging treatment of the ketal with 48 and by using a large excess of boron trifluoride etherate.²⁸⁻³⁰

With the diketone **60** in hand, we introduced the final methyl group. At -78°C addition of methyllithium to the spiro diketone **60** produced monoalcohol **61** as the major product (Scheme 9). From previous studies in our laboratory,³¹ we knew that methyllithium would attack only one ketone, no matter how many equivalents of methyllithium were used. The 1^{3} C NMR spectrum of the product showed only one carbonyl signal at δ 220.5, and there was a resonance for a quaternary carbon bearing an hydroxy at δ 77.7.

Ozonolysis of **61** cleaved the double bond, and reduction of the ozonide with dimethylsulfide yielded the aldehyde **62**. Because compound

• •



62 was not very stable, without any purification *p*-TSA was added to induce smooth cyclization and concomitant dehydration giving the diketone 63. The double bond that resulted from dehydration did not move into conjugation with the ring ketone. The ¹H NMR spectrum of 63 showed two alkenic resonances: one for an unconjugated double bond proton at δ 5.76 (1H, dd, H-3) and the other for a conjugated double bond proton at δ 6.69 (1H, s, H-7). The H-3 signal was coupled to two H-2 signals (*J* = 3.3 Hz) and to 4-CH3 (*J* = 1.8 Hz). The fact that one double bond in 63 remained unconjugated was probably for two reasons. In this arrangement

the double bond is more substituted, and this shape involves less steric compression, since C-4 is sp² the 4-CH3 does not eclipse either C-6 or C-9.

Scheme 10 Formation of 66 and 67 from 63







The conjugated double bond of **63** was reduced using Birch conditions to furnish an exclusive product, the enedione **64** (Scheme **10**). In this reaction, a stereogenic center at C-6 was produced. Nuclear Overhauser effect (nOe) measurements were not successful in indicating the relative stereochemistry, because in its ¹H NMR spectrum the chemical shifts of 4-CH₃, H-9 and H-7 were very similar. Different solvents, such as C₆D₆ and C₅D₅N, were tested, but they all failed to separate these signals. However, the relative stereochemistry of **64** was determined after the next step. Ketone **64** could not be cyclized to **65** in either acid or base due to the acidity of the C-2 hydrogens. Thus, the unconjugated double bond was also reduced by catalytic hydrogenation, and in the process another stereogenic center at C-4 was generated. We obtained two products, which. could have been the pair **66** and **67**, or the pair **68** and **69**. These two products were separated by careful chromatography.



Only the minor product showed a significant nOe on H-6 on saturation of the 4-CH3 signal. Thus, this minor product could only have
been 67. As the two products were epimeric only at C-4, therefore the major product was 66.

In the ¹H NMR spectrum of this major product **66** (Figure 3), the 4-CH₃ signal lay between the two 8-CH₃ singlets, so $\frac{1}{6}$ was difficult to saturate only 4-CH₃. Also, the chemical shift of the 4-CH₃ for **66** was δ 1.04 (d, *J* = 6.4 Hz), and in compound **67** (Figure 4) it was at δ 0.76 (d, *J* = 7.2 Hz) suggesting that in **67** the 4-CH₃ was in both the carbonyl and the acetyl groups' shielding regions, consistent with 4-CH₃ on the *xyn* face. Noted from the ¹³C NMR spectral data, the chemical shifts of 4-CH₃ (δ 17.4) and C-9 (δ 42.6) in **66** were at higher field than the 4-CH₃ in **67** was γ-anti to C-9, but in **66**, it e-Clipsed C-9.





Figure 4 ¹H NMR spectrum of compound 67

Most important, the major reduction product 66 had the correct relative stereochemistry at C-4 for the target sesquiterpenes. The ratio of compound 66 to 67 was 2:1. The spectral data of both 66 and 67 were assigned in detail based on nOe difference spectra, attached proton test (APT), $^{1}H^{-1}H$, and, $^{1}H^{-13}C$ two dimensional (2D) NMR spectra. The assignments are listed in Tables 1, 2 and 3.

Table 1 ¹H NMR data for rel-(4R,5R,6R)-6-acetyl-4,8,8-

trimethylspiro[4.4]nonan-1-one (66)



| position | chemical shift | |
|----------|------------------------------------|--|
| H-2 | 2.45 (1H, ddd, J = 1.2, 9, 19.2 Hz | |
| | 2.15 (1H, m) | |
| H-3 | 1.35 (1H, m) | |
| | 1.89 (1H, m) | |
| H-4 | 1.87 (1H, m) | |
| H-6 | 3.75 (1H, dd, J = 6.2, 13.6 Hz) | |
| H-7 | 1.55 (1H, dd, J = 6, 12.3 Hz) | |
| | 1.99 (1H, m) | |
| H-9 | 1.20 (1H, d, J = 13.8 Hz) | |
| | 1.75 (1H, d, J = 13.8 Hz) | |
| H-11 | 2.00 (3H, s) | |
| 4-CH3 | 1.04 (3H, d, <i>J</i> = 6.4 Hz) | |
| 8-CH3 | 1.03 (3H, s) | |
| 8-CH3 | 1.10 (3H, s) | |

Table 2 ¹H NMR data for *rel-(4R,5S,6S)-6-acetyl-4,8,8*trimethylspiro[4.4]nonan-1-one (67)



| position | chemical shift | |
|----------|-----------------------------------|--|
| H-2 | 2.20-2.42 (2H, m) | |
| H-3 | 1.60 (1H. m) | |
| | 2.07 (1H, m) | |
| H-4 | 2.56 (1H, ddq, J = 1.5, 7.2, 14.4 | |
| | Hz) | |
| H-6 | 3.15 (1H, dd, J = 4.5, 9.1 Hz) | |
| H-7 | 1.71 (1H, dd, J = 4.5, 13.5 Hz) | |
| | 2.24 (1H, m) | |
| H-9 | 1.74 (1H, d, J = 13.5 Hz) | |
| | 1.91 (1H, d, J =13.5 Hz) | |
| H-11 | 2.16 (3H, s) | |
| 4-CH3 | 0.76 (3H, d, J = 7.2 Hz) | |
| 8-CH3 | 0.98 (3H, s) | |
| 8-CH3 | 1.02 (3H, s) | |

 Table 3
 13C NMR data for rel-(4R.5R.6R)-6-acetyl-4.8,8-trimethylspiro[4.4]nonan-1-one (66) and rel-(4R.5S.6S)-6-acetyl-4,8,8-trimethylspiro[4.4]nonan-1-one (67)

| position* | 66 | 67 |
|-----------|-----------|-------|
| C-1(0) | 220.4 | 221.3 |
| C-2 (2) | 35.9 | 32.9 |
| C-3 (2) | 27.1 | 26.4 |
| C-4 (1) | 38.2 | 39.2 |
| C-5 (0) | 61.9 | 65.0 |
| C-6 (1) | 55.5 | 55.1 |
| C-7 (2) | 43.1 | 44.0 |
| C-8 (0) | 37.4 | 37.9 |
| C-9 (2) | 42.6 | 51.2 |
| C-10 (0) | 208.7 | 210.8 |
| C-11 (3) | 31.1 | 30.1 |
| 8-CH3 (3) | 29.4 29.2 | 31.9 |
| 4-CH3 (3) | 14.8 | 17.4 |

* Number of attached protons in parentheses





Catalytic hydrogenation of 64 appeared to take place with moderate facial selectivity. Since its acetyl group was at some distance from the double bond, hydrogenation could proceed mainly from the direction shown in Scheme 11. Previously, Wu^{31} obtained isomer 69, a C-4 and C-6 epimer of 66, by direct hydrogenation of compound 63. In this case the unconjugated double bond was reduced first. In the intermediate compound 70 one face of the enone was blocked by 4-CH₃, so reduction of the conjugated double bond was mainly from the opposite face. But this method compound 69 was the major isomer, and compound 68, which had the desired relative stereochemistry at C-4, was the minor isomer. Hence, the reduction of the enonevia the Birch conditions gave the better facial selectivity and provided the desired compound **66** as the major isomer.



Scheme 12 Formation of 71 from 66 and 72 from 67

Conversions of 66 to 71 and 67 to 72 were carried out in an intramolecular aldol ring-closure fashion using potassium *tert*-butoxide as the base (Scheme 12). When the reaction was kept at room temperature, no cyclization product was detected from both TLC and GCMS. However, when this reaction was carried out at reflux, The TLC revealed a new spot that 'vas very clearly visible under UV. The reaction was complete in 10-20 minutes. Careful TLC monitoring was required. The MS showed a strong peak at *m*/z 43 for the acetyl group in the starting materials. This

fragment was not present in the products, and a molecular ion at m/z 204 implied cyclization to 71 and 72.

Both the major isomer **71** and minor isomer **72** could be distinguished from their ¹H and ¹³C NMR spectra data. In the ¹H NMP spectra, the chemical shift of 9-CH3 in **72** (Figure **5**) was at δ 0.76, whereas in **71** (Figure **6**) it was at δ 1.26. Also noted from the ¹³C NMR spectral data, the chemical shifts of 9-CH3 (δ 14.6) and C-7 (δ 39.4) in **71** were at higher field than 9-CH3 (δ 16.6) and C-7 (δ 51.0) in compound **72**. This was due to 9-CH3 being y-anti to C-7 in **72**, and y-eclipsed with C-7 in **71**. The nOe data confirmed the relative stereochemistry of compound **72** with H-4 syn to 9-CH3. When 9-CH3 was saturated, H-4 had a 10% nOe.



The nOe difference experiments failed on compound 71, because in its ¹H NMR spectrum the 9-CH3 signal was between the two 6-CH3 signals. It was difficult to saturate 9-CH3. In C6D6 (Figure 7) the 9-CH3 signal did separate from the two 6-CH3 peaks, but H-4 was then overlapped with other proton signals. Thus, it was confirmed that compound 71 had the correct stereochemistry at C-9.

The complete assignments of 1H and ^{13}C NMR data of 71 and 72 based on $^1H^{-1}H$ and $^1H^{-13}C$ 2D NMR spectra are compiled in Tables 4, 5 and 6.



Figure 5 ¹H NMR spectrum of compound 72.



Table 4 ¹H NMR data for *rel-*(4*R*.8*R*.9*R*)-6,6,9trimethyltricyclo[6.3.0.0⁴.8]undec-1-en-3-one (71)



| position | chemical shift | |
|----------|----------------------------|--|
| H-2 | 5.77 (1H, s) | |
| H-4 | 2.40 (1H, d, $J = 9.6$ Hz) | |
| H-5 | 1.71 (1H, m) | |
| | 1.94 (1H, m) | |
| H-7 | 1.26 (1H, d, J = 13.5 Hz) | |
| | 1.63 (1H, d, J = 13.5 Hz) | |
| H-9 | 1.75 (1H, m) | |
| H-10 | 1.56 (1H, m) | |
| | 2.03 (1H, m) | |
| H-11 | 2.56-2.65 (2H, m) | |
| 6-CH3 | 0.89 (3H, s) | |
| | 1.03 (3H, s) | |
| 9-CH3 | 1.06 (3H, d, J = 13.5 Hz) | |

Table 5 ¹H NMR data for *rel-(4R,8R,9S)-6,6,9*trimethyltricyclo[6.3.0.0^{4,8}]undec-1-en-3-one (**72**)



| position | chemical shift | |
|----------|-----------------------------------|--|
| H-2 | 5.77 (1H, br s) | |
| H-4 | 2.69 (1H, dd, $J = 5.7$, 8.4 Hz) | |
| H-5 | 1.77-1.80 (2H, m) | |
| H-7 | 1.47 (1H, d, J = 12.9 Hz) | |
| | 1.61 (1H, d, $J = 12.9$ Hz) | |
| H-9 | 2.11 (1H, dq, J = 7.2, 14.1 Hz) | |
| H-10 | 1.60 (1H, m) | |
| | 2.27 (1H, m) | |
| H-11 | 2.54 (2H, m) | |
| 6-CH3 | 0.93 (3H, s) | |
| | 1.02 (3H, s) | |
| 9-CH3 | 0.76 (3H, d, J = 7.2 Hz) | |

 Table 6
 I3C NMR data for rel-(4R,8R,9R)-6.6.9

 trimethyltricyclo[6.3.0.0^{4,8}]undec-1-en-3-one (71)
 and rel-(4R,8R,

 95)-6.6.9-trimethyltricyclo[6.3.0.0^{4,8}]undec-1-en-3-one (72)

| | and the second s | |
|-----------|--|-------|
| position* | 71 | 72 |
| C-1(0) | 194.4 | 192.2 |
| C-2 (1) | 123.9 | 123.8 |
| C-3 (0) | 214.7 | 215.2 |
| C-4 (1) | 57.8 | 53.9 |
| C-5 (2) | 42.9 | 42.6 |
| C-6 (0) | 41.0 | 42.5 |
| C-7 (2) | 39.4 | 51.0 |
| C-8 (0) | 64.9 | 66.7 |
| C-9 (1) | 41.3 | 40.1 |
| C-10 (2) | 32.6 | 32.3 |
| C-11 (2) | 23.5 | 23.7 |
| 6-CH3 (3) | 31.5 | 30.1 |
| 6-CH3 (3) | 29.2 | 28.9 |
| 9-CH3 (3) | 14.6 | 16.6 |

* Number of attached protons in parentheses

Catalytic hydrogenation reduced the double bonds in 71 and 72 (Scheme 13). Both products, 73 and 74, showed carbonyl absorption at 1736 cm⁻¹ for a carbonyl group in their IR spectra. Both the ¹H and the ¹3C NMR spectra showed no olefinic resonances. Detailed ¹3C NMR assignments are reported in Table 7.

Scheme 13



Table 7 ¹³C NMR data of rel-(1R.45,8R.9K)-6,6,9trimethyltricyclo[6.3.0.04.8]undecan-3-one (74) and rel-(1R.45,8R.9S)-6,6.9-trimethyltricyclo[6.3.0.04.8] undecan-3-one (73)

| position* | 73 | 74 |
|-----------|------------|------------|
| 1(1) | 45.7 | 46.0 |
| 2 (2) | 46.8 | 47.0 |
| 3 (0) | 223.0 | 224.8 |
| 4(1) | 59.4 | 53.6 |
| 5 (2) | 31.3 | 32.7 |
| 6 (0) | 41.3 | .39.6 |
| 7 (2) | 47.9 | 56.3 |
| 8 (0) | 62.7 | 60.9 |
| 9(1) | 42.9 | 45.4 |
| 10 (2) | 34.5 | 33.2 |
| 11 (2) | 44.6 | 45.8 |
| 6-CH3 | 29.5, 29.2 | 29.7, 28.1 |
| 9-CH3 | 15.5 | 14.0 |

* Number of attached protons in parentheses

At this point, the triquinane skeleton had been efficiently established. The next four planned steps to deoxypentalenic acid (1b) are shown in Scheme 14.





The last two steps to compounds 28 and 1b are the same as in Crimmins' synthesis. Two steps, to compounds 55 and 27, remained to be studied.

The first reaction was designed to furnish the β -keto ester 55. A couple of methods were studied. Fallis³² reported some acylation success (61%) on cyclopentanone, when it reacted with dicthyl dicarbonate with potassium hydride in benzene to afford a β -keto ester product. Mander^{33,34} used methyl cyanoformate with LDA as the base at -78°C, and the β -keto ester product was obtained in 71% yield. In this reaction there was a competition between *O*-acylation and *C*-acylation, especially for more sterically hindered ketones. However, in a more recent study Mander³⁵ discovered that *O*-acylation may be almost completely suppressed by the use of diethyl ether in the place of tetrahydrofuran as solvent, and this resulted in predominant formation of only the β -keto ester. We examined these methods in model studies. Fallis' method was tested with norcamphor (Scheme 15).





We found that the reaction proceeded properly in THF to yield the β -keto ester. In contrast, in benzene the reaction did not work. The product 77 was a mixture of stereoisomers in a ratio of 8:5. IR absorption maxima at 1760 and 1724 cm⁻¹ were assigned to two carbonyls. No hydroxyl absorption was found. Also there was no double bond resonance in the ¹³C NMR spectrum. Therefore compound 77 was in the keto form only, and no enol compound exist. Both Fallis' and Mander's methods were applied to compound 74, and the results are shown in Scheme 16.

Scheme 16 Formation of \beta-keto ester from 74 and 72



The unidentified compounds, which were obtained in some of these reactions, were not very stable under chromatographic conditions. For all these compounds ¹H NMR revealed the presence of major skeletal pieces, such as an ester group, 9-CH3 and gem-dimethyl group at C-6. However, no clear H-1, H-2, or H-4 resonances. These products were present as mixtures, but it was not understood whether or not the mixtures were the enol and keto forms, or *O*-acylation products. It is worth mentioning that the unidentified compound A had a similar ¹H NMR spectrum to that of unidentified compound C except for a different ester group. However the IR results were not consistent. Compound A had absorption maxima at 3475, 1747, 1660, and 1621 cm⁻¹, and compound B at 3432, 1735, 1662, and 1660 cm⁻¹.





In a further study involving reduction of β -keto esters, it was revealed that none of these unidentified compounds could be reduced by NaBH4. Nevertheless, the reduction of simpler β -keto ester derivatives was well studied with model compounds **77** and **78** (Scheme **17**). The ratio of the two isomeric products was found to change upon the addition of metal chlorides (MCl₂) (Table **8**).

| Substrate | Time | NaBH4/MCl2 | Yielda | Ratiob |
|-----------|------|-------------------------|--------|--------|
| | min | molar equivalents | | |
| 0 | 30 | NaBH4 (1.0) | 98 | 1:2 |
| CO2CH3 | 15 | NaBH4 (1.3)/ | 100 | . 2:1 |
| 78 | | CaCl ₂ (2.0) | | |
| | 10 | NaBH4 (1.0)/ | 74 | 2:1 |
| | | CaCl ₂ (2.0) | | |
| | 10 | NaBH4 (1.3)/ | 70 | 5:1 |
| | | MnCl ₂ (2.0) | | |
| | 5 | NaBH4 (1.0)/ | 39 | 5:1 |
| | | MnCl ₂ (2.0) | | |
| CO2Et | 30 | NaBH4 (1.0) | 90 | 7:1 |
| 77 | | | | |

Table 8 Reduction of B-ketoesters by NaBH4/MCl2

a yield of reduced products as a mixture of stereoisomers.

b as determined by IH NMR.

Some of these reductions were fairly stereoselective. For compound **78**, NaBH4 gave a 1:2 ratio of hydroxy esters, but with MnCl₂ a 5:1 ratio was obtained. A mechanism has been proposed^{36,37} that involves a metal complex with two carbonyls, and NaBH4 choosing the *svn* or *anti* face:



The reaction conditions were varied with respect to the nature of metal chloride and the reaction time. Addition of MnCl₂:4H₂O led to the best facial selectivity. Changing the reaction time did not affect the product ratio, but it was important in determining the yield.

The final reactions that will furnish the ester group in good yield will require further study. Nevertheless, our approach to the triquinane skeleton, which was presented here, was short (8 steps) and with high yields in each step. Furthermore, the correct stereochemistry at C-9 was obtained. Indeed, while the final stages of the synthesis of (\pm) deoxypentalenic acid (1b) have yet to be realized, Wu³¹ has converted ketone 73 into (\pm) -pentalenene (2) in three straightforward steps. Both the work described here towards (\pm)-deoxypentalenic acid (**1b**) and Wu's synthesis of (\pm)-pentalenene (**2**) demonstrate the utility of the geminal acylation reaction in the total synthesis of triquinane natural products.

III. EXPERIMENTAL

General

Both high and low resolution electron impact mass spectra (HRMS, and LRMS) were recorded on a V.G. Micromass 7070HS mass spectrometer. Data are reported as m/z (relative intensity). Gas chromatography-mass spectrometry (GCMS) data were recorded on a Hewlett-Packard 5890 gas chromatograph coupled to a model 5970 mass selective detector, which was equipped with a 12.5 m fused-silica capillary column with cross-linked dimethylsilicone as the liquid phase. Fourier transform infrared (IR) spectra were recorded on a Mattson FT instrument. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were measured (in CDCl3 unless otherwise noted) on a GE 300-NB (300 MHz for 1 H and 75 MHz for ^{13}C) spectrometer. Chemical shifts (δ) are reported in parts per million from tetramethylsilane (TMS). Chloroform (unless otherwise noted) was used as the internal standard, ¹H: § 7.27; ¹³C: § 77.0 relative to TMS. TMS was used as the internal stardard when the solvent shift spectra were recorded. Coupling constants, J, are expressed in Hertz (Hz) and are reported to within \pm 0.2 Hz. The following abbreviations are used: m=multiplet, s=singlet, d=doublet, t=triplet, g=quartet, br=broad, The 13C NMR shift are followed by the number of attached protons, as determined by Attached Proton Test (APT) and heteronuclear 2D experiments. Nuclear Overhauser effect (nOe) difference experiments were done on degassed solutions on the GE 300-NB spectrometer.

Flash chromatography was carried out with E. Merck silica gel 60 (230-400 mesh) according to the method described by Still.³⁸ Thin layer chrom-ttograms (TLC) were examined under ultraviolet light (254 nm). The TLC plates were visualized with iodine vapor, or sprayed with a solution of phosphomolybdic acid (10 g of MoO3·H3PO4, 1.25 g of Ce(SO4)2, 12 mL concentrated H2SO4, diluted to 250 mL with H2O), then the TLC plate was heated on a hot pl te.

Reagent grade solvents were distilled prior to use. Analytical grade diethyl ether (ether) and benzene (ACS) were used without further purification. Dry dichloromethane and toluene were distilled from calcium hydride. Dry tetrahydrofuran (THF) was distilled from sodium benzophenone.

Most reactions were carried out under a positive pressure of nitrogen gas. Reactions which required anhydrous conditions were performed in oven-dried glassware, which was assembled and allowed to cool while being purged with an inert gas. All reactions were monitored by analytical thinlayer chromatography (TLC). All compounds reported gave a single spot on TLC and were judged to be >95% pure on the basis of both ¹H NMR and GCMS.

7,9,9-Trimethyl-1,4-dioxaspiro[4.5]dec-7-ene (57) and 7,9,9-Trimethyl-1,4-dioxaspiro[4.5]dec-6-ene (58)



A mixture of isophorone (5.0 g, 36 mmol), ethylene glycol (8.5 mL, 155 mmol), and pTSA (0.9 g) in benzene was heated at reflux overnight. As the water was formed, it was removed by a Barrett water-separator. Solid then saturated aqueous sodium bicarbonate were added. The aqueous layer was extracted with ether four times. The combined organic lavers were washed with brine, dried over anhydrous potassium carbonate, filtered, and concentrated. The residue was distilled under vacuum to give a coloriess liquid (4.7g). Further purification was carried out using flash chromatography (12-16% ethyl acetate in hexane) to give a colorless oil composed of a mixture of 7.9.9-trimethyl-1,4-dioxaspiro[4,5]dec-7-ene (57) and 7,9,9-trimethyl-1,4-dioxaspiro[4.5]dec-6-ene (58) in a ratio of 10:1 (1.52 g, in 30% vield, but 64% based on the consumed starting material). For 57: ¹H NMR: 8 1.04 (6H, s. 9-CH3), 1.60 (2H, s. H-10), 1.67 (3H, s. 7-CH3), 2.14 (2H, s, H-6), 3.95 (4H, s, H-2 and H-3), 5.17 (1H, s, H-8); 13C NMR: § 131.2 (1), 128.((0), 109.2 (0), 64.0 (2, 2C), 43.5 (2), 39.8 (2), 31.6 (0), 30.3 (3, 2C), 23.4 (3); MS from GCMS m/z (%): 182 (27, M+), 167 (16, M+-CH3), 154 (1), 139 (7), 123 (8), 96 (34), 86 (100), 67 (8), 41 (13). The ¹H NMR of the 6-ene isomer (58) is different from that of 57 at H-8 (δ 2.14, 2H, s) and H-6 (8 5.34, 1H, s); MS of **58** from GCMS *m/z* (%): 182 (4, M⁺), 167 (6, M⁺-CH₃), 149 (1), 137 (12), 126 (100), 99 (9), 82 (13), 67 (3), 41 (6).

7,9,9-Trimethylspiro[4.5]dec-7-ene-1,4-dione (60)



Boron trifluoride etherate (7.0 mL, 57 mmol) and 1,2-bis(trimethylsilyloxy)cyclobutene (3.0 mL, 11 mmol) in 10 mL of dichloromethane were added to a solution of 7.9.9-trimethyl-1.4-dioxaspiro[4.5]dec-7-ene (622 mg, 3.42 mmol) in dichloromethane (60 mL). The mixture was stirred at -78°C under nitrogen overnight during which time the mixture was allowed to attain room temperature, and then it was poured into an ice-cold saturated sodium bicarbonate solution. The aqueous layer was extracted with dichloromethane three times. The combined organic layers were washed with saturated sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (10%-16% ethyl acetate in hexane) to give the title compound as a colorless oil (601 mg, 85%); ¹H NMR; 8 0.94 (6H, s, 9-CH3), 1.66 (2H, s, H-10), 1.76 (3H, s, 7-CH3), 2.03 (2H, s, H-6), 2.59-2.68 (2H, m, H-2 or H-3), 3.02-3.11 (2H, m, H-2 or H-3), 5.20 (1H, s, H-8); 13C NMR: § 214.2 (0, 2C), 129.6 (1), 128.7 (0), 59.0 (0), 43.3 (2), 34.7 (2, 2C), 32.8 (0), 30.1 (3, 2C), 29.1 (2), 23.6 (3); MS from GCMS m/z (%): 206 (100, M⁺), 191 (38, M⁺-CH₃), 178 (13), 163 (73), 145 (59), 107 (35), 91 (37), 77 (19), 41 (21).

rel-(4R,5S)-4-Hydroxy-4,7,9,9-tetramethylspiro[4.5]dec-7-en-1-one (61)



To a solution of 7,9,9-trimethylspirol4.5]dcc-7-ene-1,4-dione (897 mg, 4.35 mmol) in 30 mL of dry ether at -78°C under nitrogen was added 1.4 M methyllithium in ether (16 mL, 22 mmol). The solution became cloudy. After stirring for 2 hours, the solution was slowly poured into ice-cold brine. The aqueous layer was extracted with ether three times. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (12%-20% ethyl acetate in hexane) to give the title compound as a colorless oil (803 mg, 83%): ¹H NMR: δ 0.90 (3H, s, 9-CH3), 1.00 (3H, s, 9-CH3), 1.15 (3H, s. 4-CH3), 1.71 (2H, d, J = 4.5 Hz, H-10), 1.74 (3H, s, 7-CH3), 1.79 (2H, s, H-6), 1.96 (1H, m), 2.21 (2H, m), 2.56 (1H, m), 5.19 (1H, s, H-8); ¹3C NMR: δ 220.5(0), 130.4 (1), 128.7 (0), 77.7 (0), 55.9 (0), 37.9 (2), 34.0 (2), 33.3 (2), 32.7 (3), 32.0 (0), 30.2 (2), 28.4 (3), 24.1 (3), 23.7 (3); MS from GCMS *m/z* (%): 222 (2, M⁺), 189 (1), 164 (4), 149 (7), 123 (13), 99 (32), 83 (13), 43 (100).



rel-(4R.5S)-4-Hvdroxy-4.7.9.9-tetramethylspiro[4.5]dec-7-en-1-one (321 mg, 1.45 mmol) in 20 mL of dichloromethane was cooled to -78°C. Ozone was passed through the solution until the solution turned blue. Excess ozone was removed by bubbling the solution with oxygen. Dimethyl sulfide (ca. 6 mL) was added, and the mixture was stirred under nitrogen overnight. The dichloromethane was evaporated. The residue was redissolved in benzene (20 mL) and a catalytic amount of pTSA (ca. 100 mg) was added. The mixture was heated at reflux for 2 hours. The water formed was removed by a Barrett water-separator. After the solution had cooled, saturated sodium bicarbonate was added. The aqueous layer was extracted with ether three times. The compined organic layers were washed with saturated sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. 'he residue was separated by flash chromatography (2% acetone in hexane) to provide 63 as a vellow oil (214 mg, 71%); ¹H NMR; δ 1.26 (3H, s, 8-CH3), 1.30 (3H, s, 8-CH3), 1.63 (3H, dd, J = 1.8, 2.4 Hz, 4-CH3), 1.71 (1H, d, J = 13.9 Hz, H-9), 1.91 (1H, d, J = 13.9 Hz, H-9), 2.25 (3H, s, acetyl), 2.88 (1H, ddg, J = 2.4, 3.3, 22.6 Hz, H-2), 3,18 (1H, ddg, J = 2.4, 3,3, 22,6 Hz, H-2), 5,76 (1H, dd, J = 1.8, 3,3 Hz, H-3), 6.69 (1H, s, H-7); ¹³C NMR; 8 219.2 (0), 195.2 (0), 157.1 (1), 142.6 (0), 141.6 (0), 121.4 (1), 68.3 (0), 46.5 (2), 45.5 (0), 41.9 (2), 29.6 (3), 29.0
(3), 26.5 (3), 14.6 (3); MS from GCMS m/z (%): 218 (37, M⁺), 203 (10, M⁺-CH₃), 175 (96, M⁺-COCH₃), 161 (23), 147 (44), 133 (43), 119 (18), 105 (22), 91 (25), 77 (16), 43 (100).

rel-(5R, jR)-6-Acetyl-4,8,8-trimethylspiro[4.4]non-3-en-1-one (64)



Lithium (ca. 60 mg) was added to liquid ammonia (ca. 50 mL) at -78°C. 6-Acetyl-4,8,8-trimethylspiro[4.4]nona-3,6-dien-1-one (205 mg, 0.939 mmol) in THF (15 mL) was added when the bath temperature reached -35°C. The mixture was stirred for 35 minutes and then it was quenched by addition of solid ammonium chloride until the blue color disappeared. The ammonia was allowed to evaporate at room temperature. The residue was partitioned between ether and water. The aqueous layer was extracted with ether three times. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was dissolved in dichloromethane (40 mL) and pyridinium chlorochromate (PCC, ca. 500 mg, 2.3 mmol) was added. The mixture was stirred at room temperature overnight. Ether was added and the mixture was filtered through a pad of silica gel. Separation of the residue by flash chromatography (2% acetone in hexane) provided the title compound as a

vellow oil (139 mg, 67%); ¹H NMR; δ 1.14 (3H, s, 8-CH3), 1.15 (3H, s, 8-CH3), 1.58 (1H, d, AB, J = 14.1 Hz, H-9), 1.66-1.71 (5H, m, 4-CH3, H-7, H-9), 1.93 (3H, s, acetyl), 2.04 (1H, t, J = 13.1 Hz, H-7), 2.85 (1H, ddg, J = 2.4, 4.8, 23.4 Hz, H-2), 2.98 (1H, ddg, J = 2.4, 5.1, 23.4 Hz, H-2), 3.51 (1H, dd, J = 6.3, 13.5 Hz, H-6), 5.70 (1H, br s, H-3); ¹H NMR (C6D6); $\delta 0.94$ (3H, s, 8-CH3), 1.10 (3H, s, 8-CH3), 1.30 (1H, d, AB, J = 14.1 Hz, H-9), 1.48-1.55 (5H, m, 4-CH3, H-7, H-9), 1.59 (3H, s, H-11), 2.02 (1H, t, J = 13.0 Hz, H-7), 2.52 (2H, dq, J = 2.1, 4.5 Hz, H-2), 3.43 (1H, dd, J = 6.3, 13.5 Hz, H-6), 5.19 (1H, br s, H-3); ¹H NMR (C5D5N): 8 1.02 (3H, s, 8-CH3), 1.10 (3H, s, 8-CH3), 1.47 (1H, d, AB, J = 14.1 Hz, H-9), 1.60-1.65 (5H, m, 4-CH3, H-7, H-9), 1.90 (3H, s, H-11), 2.06 (1H, t, J = 12.9 Hz, H-7), 2.90 (2H, dq, J = 2.4, 4.8 Hz, H-2), 3.57 (1H, dd, J = 6.3, 13.5 Hz), 5.59 (1H, br s, H-3); ¹³C NMR: δ 220.9 (0), 207.7 (0), 144.2 (0), 121.0 (1), 64.0 (0), 60.1 (1), 49.4 (2), 43.5 (2), 41.5 (2), 37.8 (0), 29.7 (3), 29.2 (3), 28.7 (3), 15.4 (3); MS from GCMS m/z (%): 220 (11, M+), 177 (41, M+-COCH3), 159 (9), 149 (13), 121 (23), 107 (61), 77 (31), 43 (100),

rel-(4R,5R,6R)-6-Acetyl-4,8,8-trimethylspiro[4.4]nonan-1-one (66) and rel-(4R,5S,6S)-6-Acetyl-4,8,8-trimethylspiro[4.4]nonan-1-one (67)





rel-(5R,6R)-6-Acetyl-4,8,8-trimethylspirol-4.4]non-3-en-1-one (98 mg. 0.45 mmol) was dissolved in dry methanol (39 mL) and 5% palladium on carbon (ca. 250 mg) was added. The mixture was shaken under 50 psi pressure of hydrogen for 1.5 hours and then filtered through a pad of silica gel. The residue was separated on a silica gel column with 4% acetone in hexane providing as a colorless oil rel-(4R,5S,6S)-6-acetyl-4.8.8trimethylspiro[4.4]-nonan-1-one (67) (36 mg, 36%) and rel-(4R,5R,6R)-6acetyl-4,8,8-trimethylspirof4,41-nonan-1-one (66) (63 mg, 64%). For the minor epimer (67); IR (film) Umax: 1733, 1707 cm⁻¹; ¹H and ¹³C NMR see Table 2 and 3; MS from GCMS m/z (%): 222 (8, M+), 207 (7, M+-CH3), 179 (19, M⁺-COCH₃), 161 (33), 152 (60), 137 (30), 123 (35), 110 (17), 109 (23), 95 (36), 81 (27), 55 (31), 43 (100). For the major epimer (66); IR (film) Umax: 1735, 1708 cm⁻¹; ¹H and ¹³C NMR see Tables 1 and 3; MS from GCMS m/z (%): 222 (3, M+), 207 (5, M+-CH3), 179 (4, M+-COCH3), 161 (13), 152 (99), 137 (56), 123 (21), 110 (64), 109 (25), 95 (22), 81 (24), 55 (29), 43 (100).

rel-(4R,8R,9R)-6,6,9-Trimethyltricyclo[6.3.0.04,8]undec-1-en-3-one (71)



Potassium tert-butoxide (76 mg, 0.67 mmol) was added to a solution of rel-(4R,5R,6R)-6-acetyl-4,8,8-trimethylspiro[4.4]nonan-1-one (70 mg, 0.32 mmol) in 10 mL of benzene. The mixture was heated at reflux for 20 minutes. It had a red color. When the solution had cooled, 10% HCl in water was added. The aqueous layer was extracted with ethyl acetate three times. The combined organic layers were washed with saturated sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (2% acetone in hexane) to provide the title compound as a colorless oil (45 mg, 70%): IR (film) v_{max} : 1704, 1630 cm⁻¹; 1H and ¹³C NMR see Tables 4 and 6; MS from GCMS m/z (%): 204 (61, M⁺), 189 (19, M⁺-CH₃), 176 (9, M⁺-CO), 148 (100), 133 (58), 107 (94), 91 (78), 77 (49), 41 (60).

rel-(4R,8R,9S)-6,6,9-Trimethyltricyclo[6.3.0.04,8]undec-1-en-3-one (72)



Potassium tert-butoxide (121 mg, 1.07 mmol) was added to a solution of rel-(4R,5S,6S)-6-acetyl-4,8,8-trimethylspiro[4.4]nonan-1-one (110 mg, 0.500 mmol) in 10 mL of benzene. The mixture was heated at reflux for 15 minutes. It had a red color. When the solution had cooled, 10% HCl in water was added. The aqueous layer was extracted with ethyl acetate three times. The combined organic layers were washed with saturated sodium bicarbonate and brine, dried over anhydrous magnesium sulfate, filtered, and concentratei. The residue was separated by flash chromatography (2% acetone in hexane) to provide the title compound as a colorless oil (64 mg, 63%): IR (film) umax: 1703, 1630 cm⁻¹; ¹H and ¹³C NMR see Tables 5 and 6; MS from GCMS *m/*2 (%): 204 (7, M⁺), 189 (2, M⁺-CH₃), 176 (1, M⁺-CO), 148 (32), 133 (28), 107 (69), 91 (68), 77 (58), 41 (100).

rel-(1R,4S,8R,9S)-6,6,9-Trimethyltricyclo[6.3.0.04,8]undecan-3-one (73)



To a solution of *rel*-(4*R*,8*R*,9*R*)-6,6,9-trimethyltricyclo]6.3.0.0^{4,8}]undec-1-en-3-one (43 mg, 0.21 mmol) in dry methanol (25 mL) was added 5% palladium on carbon (*ca.* 200 mg), and this was shaken under 50 psi pressure of hydrogen for 1 hour. The mixture was filtered through a pad of silica gel, and the filtrate was evaporated. The residue was purified on a silica gel column with 4% acetone in hexane providing the title compound as a colorless oil (43 mg, 98%): IR (film) ν_{max} : 1736 cm⁻¹; ¹H NMR: δ 0.97 (3H, d, *J* = 6.7 Hz, 9-CH3), 0.985 (3H, s, 6-CH3), 1.01 (3H, s, 6-CH3), 1.32-1.37 (3H, m), 1.59-1.92 (5H, m), 2.05-2.14 (2H, m), 2.41-2.46 (2H, m), 2.78 (1H, dd, *J* = 9.2, 18.5 Hz); ¹³C NMR: δ 223.0 (0), 62.7 (0), 59.4 (1), 47.9 (2), 46.8 (2), 45.7 (1), 44.6 (2), 42.9 (1), 41.3 (0), 34.5 (2), 31.3 (2), 29.5 (3), 29.2 (3), 15.5 (3); MS from GCMS *m/z* (%): 206 (44, M⁺), 191 (37, M⁺-CH3), 173 (6), 163 (44, M⁺-COCH3), 135 (30), 107 (62), 95 (71), 79 (49), 55 (52), 41 (100).



To a solution of *rel*-(4*R*,8*R*,9*S*)-6,6,9-trimethyltricyclo[6.3.0.0^{4,8}]undec-1-en-3-one (40 mg, 0.19 mmol) in dry methanol (25 mL) was added 5% palladium on carbon (*ca.* 200 mg), and this was shaken under 50 psi pressure of hydrogen for 1 hour. The mixture was filtered through a pad of silica gel and the filtrate was evaporated. The residue was purified on a silica gel column with 4% acetone in hexane providing the title compound as a colorless oil (39 mg, 98%): IR (film) v_{max}: 1736 cm⁻¹; ¹H NMR: 8 0.79 (3H, s, 6-CH3), 0.98 (3H, d, *J* = 6.6 Hz, 9-CH3), 1.03 (3H, s, 6-CH3), 1.17 (1H, dd, *J* = 6.1, 12.1 Hz), 1.43-1.65 (4H, m), 1.70-1.94 (4H, m), 2.05 (1H, ddd, *J* = 1.9, 7.3, 19.1 Hz, H-2), 2.43-2.50 (2H, m), 2.74 (1H, dd, *J* = 11.6, 19.1 Hz, H-2); ¹3C NMR: 8 224.1 (0), 60.9 (0), 56.3(2), 53.6 (1), 47.0 (2), 46.0 (1), 45.8 (2), 45.4 (1), 39.6 (0), 33.2 (2), 32.7 (2), 29.7 (3), 28.1 (3), 14.0 (3); MS from GCMS *m*/₂ (%): 206 (24, M⁴), 191 (16, M⁴-CH3), 163 (39, M⁴-COCH3), 150 (51), 124 (23), 107 (62), 95 (53), 81 (48), 41 (100). 3-Carboxyethylbicyclo[2.2.1]heptan-2-one (77)



A solution of norcamphor (126 mg, 1.14 mmol) and diethyl dicarbonate (0.34 mL, 2.3 mmol) in 4.0 mL of THF was added to a stirred mixture of potassium hydride (500 mg, 4.36 mmol, 35% oil dispersion). After reflux for 1.5 hours, 10% HCl in water was added at 0°C. The aqueous layer was extracted with ethyl acetate three times. The combined organic layers were washed with saturated sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (5% acetone in hexane) to give the title compound as a yellow oil (153 mg, 74%): IR (film) umax: 1760, 1724 cm-1; ¹H NMR (8:5 mixture): δ 1.28 (3H, t, J = 7.2 Hz), 1.45-1.95 (12H, m), 2.19 (1H, t, J =1.8 Hz), 2.23 (1H, t, J = 1.8 Hz), 2.65 (1H, m), 2.70 (1H, m), 2.84 (1H, d, J = 3.6 Hz), 2.91 (1H, m), 2.96 (1H, m), 3.05 (1H, dd, J = 0.6, 4.5 Hz), 4.17 (2H, q, J = 7.2 Hz); ¹³C NMR (8:5 mixture); δ 209.7(0), 168.5 (0), 167.5 (0), 61.1 (2), 60.8 (2), 59.1 (1), 57.7 (1), 50.0 (1), 48.6 (1), 39.2 (1), 39.3 (1), 36.4(t), 35.7 (2), 27.0 (2), 24.1 (2), 23.7 (2), 22.9 (2), 14.0 (3); GC-MS m/z (%): 182 (9, M+), 154 (74, M+-CO), 137 (50, M+-OCH2CH3), 126 (92), 108 (57), 81 (10), 67 (49), 41 (56); HRMS calcd. for C10H14O3: 182.0942; found: 182.0929.

Reduction of 78 with NaBH4 in the presence of calcium chloride



Anhydrous calcium chloride (228 mg, 2.05 mmol) was added to a solution of methyl 2-oxocyclopentanecarboxylate (78) (140 mg, 0.99 mmol) in methanol (10 mL). The mixture was stirred at room temperature for 30 minutes, then cooled to 0°C. Sodium borohydride (50 mg. 1.3 mmol) was added. Vigorous gas evolution occurred. After stirring for 15 minutes, the mixture was poured into 1M HCl (10 mL). The aqueous layer was extracted with ethyl acetate five times. The combined organic layers were washed with saturated sodium bicarbonate, dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (15-21% ethyl acetate in hexane) providing a minor isomer (41 mg, 29%) and a major isomer (101 mg, 71%) in the ratio 1:2.4. For the minor isomer: IR (film) υmax: 3461, 1736, 1437 cm⁻¹; ¹H NMR: δ 1.60-1.69 (1H, m), 1.76-1.82 (2H, m), 1.91-2.05 (3H, m), 2.70 (1H, dt, J = 4.2, 9.9 Hz, H-1), 3.03 (1H, d, J = 3 Hz, OH), 3.73 (3H, s, COOMe), 4.45 (1H, dq, J = 3.3, 6.9 Hz, H-2); 13C NMR: 8 175,3 (0), 73,7 (1), 51,8 (3), 49,4 (1), 33,9 (2), 26,3 (2), 22,0 (2); MS m/z (%): 144 (2, M+), 127 (3, M+-OH), 116 (29), 95 (18), 87 (100), 67 (50), 55 (96), 41 (6). For the major isomer: IR (film) umax: 3430, 1733, 1436 cm⁻¹; ¹H NMR; δ 1.61-1.85 (4H, m), 1.95-2.09 (2H, m), 2.47 (1H, s, OH), 2.67 (1H, m, H-2), 3.71 (3H, s, COOMe), 4.38 (1H, q, J = 6.6 Hz, H-2); ¹³C NMR; 8 175.5 (0), 76.3 (1), 54.4 (3), 51.8 (1), 34.1 (2), 27.1 (2),
22.0 (2); MS *m/z* (%): 145 (1, M⁺+1), 126 (3, M⁺-H₂O), 116 (20), 113 (26), 95 (8), 87 (98), 67 (56), 55 (100), 41 (6).

Reduction of 78 with NaBH4 in the presence of manganese(11) chloride



MnCl2·4H2O (525 mg, 2.65 mmol) was added to a solution of methyl 2-oxocyclopentanecarboxylate (189 mg, 1.33 mmol) in methanol (10 mL). The mixture was stirred at room temperature for 30 minutes, then it was cooled to 0°C. Sodium borohydride (64 mg, 1.7 mmol) was added. Vigorous gas evolution occurred. After stirring for 10 minutes, the mixture was poured into 1M HCl (10 mL). The aqueous layer was extracted with ethyl acetate five times. The combined organic layers were washed with saturated sodium bicarbonate, dried over sodium sulfate, filtered, and concentrated, The residue was purified by flash chromatography (15-21% ethyl acetate in hexane) providing a colorless oil composed of a 5:1 mixture of two products (133 mg, 70%): IR (film) umax: 3461, 1736 cm-1. For the minor isomer: 1H NMR: § 2.67 (1H, m, H-1), 3.15 (1H, br s, OH), 3.73 (3H, s, OCH3), 4.45 (1H, m, H-2). For the major isomer: ¹H NMR: § 2.28 (1H, br s, OH), 2.67 (1H, m, H-1), 3.71 (3H, s, OCH3), 4.38 (1H, m, H-2). Other signals were evident at: δ 1.61-2.08 (6H, m); ¹³C NMR: δ 175.4 (0), 175.3 (0), 76.3 (1), 73.7 (1), 52.5 (3), 51.8 (1), 49.4 (1), 34.1 (2), 33.9 (2), 27.1 (2), 26.3 (2), 22.0 (2).

2α -Hydroxy- 3α -carboxyethylbicyclo[2.2.1]heptane (80) and 2β -Hydroxy- 3β -carboxyethylbicyclo[2.2.1]heptane (81)



NaBH4 (21 mg, 0.56 mmol) was added to a solution of 3carboxyethylbicyclo[2.2.1]heptan-2-one in methanol (10 mL). The mixture was stirred for 30 minutes at room temperature. Then the mixture was poured into 1M HCl (10 mL). The aqueous layer was extracted with ethyl acetate five times. The combined organic layers were washed with saturated sodium bicarbonate, dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (10-15% ethyl acetate in hexane) providing a vellow oil composed of a 7:1 mixture of two products (89 mg, 90%): IR (film) vmax: 3462, 1731, 1704 cm⁻¹. For the minor isomer (80); ¹H NMR; δ 1.28 (3H, t, J = 7.2 Hz), 1.37 (2H, m), 1.40-1.50 (2H, m), 1.82-2.00 (2H, m), 2.39 (1H, m), 2.51 (1H, br s), 2.74 (1H, dd, J = 4.8, 9.9 Hz), 4.17 (2H, q, J = 7.2 Hz), 4.74 (1H, d, J = 6.3 Hz); ¹³C NMR: δ 174.8 (0), 71.5 (1), 60.6 (2), 45.5 (1), 42.9 (1), 41.0 (1), 35.8 (2), 24.5 (2), 19.5 (2), 14.2 (3). For the major isomer (81): ¹H NMR: δ 1.27 (3H, t, J = 7.2 Hz), 1.39 (1H, m), 1.59 (3H, m), 1.88 (1H, m), 1.77 (1H, d, J = 3.0 Hz), 1.99 (1H, dd, J = 2.7, 3.9 Hz), 2.33 (1H, m), 2.46 (1H, m), 4.14 (2H, q, J = 7.2)Hz), 4.43 (1H, dd, J = 3.9, 8.1 Hz); ¹³C NMR; δ 174.9 (0), 75.8 (1), 60.5 (2), 55.7 (1), 42.0 (1), 41.3 (1), 35.7 (2), 29.7 (2), 19.3 (2), 14.2 (3); MS m/z (%): 156 (45, M⁺-CO). 139 (18), 128 (16), 110 (23), 101 (55), 88 (28), 81 (37), 67 (100), 43 (77), 41 (92); HRMS caled. for C9H₁₆O₂ (M⁺-CO): 156.1149; found: 156.1151.

MICROBIAL REDUCTION OF PROCHIRAL SPIRODIKETONES

I. Introduction

Enzymes acting as specific and chiral catalysts have been recognized for many years.³⁹⁻⁴³ Biochemical procedures that utilize enzymes are becoming accepted as routine procedures in organic synthesis. Recently the application of enzyme-mediated reactions has increased in both academic and industrial laboratories, particularly in the pharmaceutical area, to meet the requirements for enantio- and diastereodifferentiation. This is because enzymatic reactions give products of higher optical purity than do the corresponding chemical reactions. Enzymatic reactions also produce less chemical waste and are therefore environmentally safer.

Baker's yeast is one of the most efficient reagents for the enantioselect, ve reduction of many prochiral ketones. The yeast is easy to use, since it requires only tap water together with the substrate; the yeast itself contains enough nutrients to support its dehydrogenase enzyme. Yeast reduction of 1,3-cyclopentanediones, which have two prochiral carbonyls, has interesting synthetic potential. Kosmol *et al.*⁴⁴ first investigated this enzymatic reduction of 2,2-dialkyl-substituted 1,3-cyclopentanedione (82) (Scheme 18), and they applied the reaction as the chirality-introducing step in an industrial total synthesis of estradiol. With the appropriate choice of the microbiological system, the reduction could be carried out with nearly complete stereoselectivity. Only one (83) of the four possible stereoisomers (83-86) was obtained.

Scheme 18 Yeast reducton of 2,2-dialkyl 1,3-cyclopentanedione by Kosmol



Many enzymes are able to make prochiral distinctions. Their selectivity is based on the formation of a preferred enzyme-substrate complex, which leads to a favored catalytic reaction of only one of the two enantiotopic reaction sites. Microbial reductions of several 2,2-disubstituted 1,3-diones have been reported with a variety of microorganisms to provide chiral products.^{45, 46} In more recent work, Brooks *et al.*^{47, 48} carried out an investigation with baker's yeast designed to establish the relationship between stereoselectivity and the differences in size between the two substituents attached at C-2 of the 1,3-cyclopentanedione (Scheme 19).







In Table 9 the results of the yeast reduction are compared with the monoreduction products formed by reduction with NaBH4. It is important to point out that only the 25,35 (90a, 92a, 94a) and 2R,35 (91a, 93a, 95a) diastereoisomers vere formed by yeast reduction. The enantiomeric purity of each chiral ketoalcohol was over 98% ee, as determined by the ¹H NMR spectrum of the corresponding Mosher's ester of the produci.⁴⁹

| dione | . Baker's yeast | NaBH4 |
|-------|-----------------|--------------|
| 87 | 90a (100%) | 90a,b (85%) |
| | | 91a,b (15%) |
| 88 | 92a (90%) | 92a,b (75%) |
| | 93a (10%) | 93a,b (25%) |
| 89 | 94a (67%) | 94a,b (67%) |
| | 95a (33%) | 95 a,b (33%) |

Table 9. Reduction of prochiral 2,2-disubstituted 1,3cyclopentanediones by baker's yeast and by NaBH4

The baker's yeast reduction was more stereoselective than NaBH₄, and, like NaBH₄ reduction, the microbial system was very sensitive to changes in the substituent changes. Both the microbial and NaBH₄ reductions had the same trend of decreasing stereoselectivity: propyl> allyl> propynyl.

In conjunction with the stereoselectivity studies, Brooks and coworkers^{50, 51} used actively fermenting baker's yeast in a key step to synthesize optically pure trichothecene derivatives like anguidine (96) (Scheme 20). Baker's yeast reduced one of the two enantiotopic carbonyl groups of 2-allyl-2-methyl-1,3-cyclopentanedione (88), providing the keto-alcohols 92a (25,35) and 93a (27,35) in a ratio of 9:1 (Table 9). Examination of the C-ring of the anguidine (96) reveals that keto-alcohol 92a has the correct absolute configuration at the quaternary carbon C5, but the configuration of the hydroxy group in 92a is opposite to that required at C4. Therefore all the other chiral centers must be elaborated in the course of

the synthesis. Inversion of the 4-OH group in 92a was effected in two steps. Compound 92a was reacted with *p*-toluenesulphonyl chloride in pyridine. and the corresponding tosylate was transformed to (2R,3R)-keto-alcohol 97 with potassium nitrite. After protection of the hydroxy group and the ketone, oxidative cleavage of the double bond provided the carboxylic acid, which was converted to the corresponding methyl ester 98 with diazomethane. Bromination of 98 with trimethylphenylammonium tribromide followed by treatment with DBU gave compound 99. Osmium tetroxide oxidation gave a separable mixture (5:1) of two isomeric cis-vicinal diols 100 and 101. Lactonization of 100 by treatment of the carboxylate salt with excess acetic anhydride, and protection of the remaining hydroxy group gave a 3:1 mixture of bicyclic lactones, which could be separated as the benzoates 102 and 103. The synthesis of ring A was completed by introducing a hydroxymethylene group on C6 to provide compound 104. Compound 104 was converted into 105 by stereocontrolled Robinson spiroannulation. Reaction of 105 with methyllithium provided an allylic alcohol. The lactone was opened reductively with LiAlH4 followed by triacetylation. Selective deacetylation gave 106. The 2-hydroxy function was then used for closure of ring B via an acid-induced SN2'-reaction. Finally, 107 was converted into 96 via six routine steps.

Mori and coworkers used baker's yeast reduction of 2,2-dimethylcyclohexane-1,3-dione (108) as the key steps to synthesize (1S,5R)-karahana ether (109),52 (1S,5R)-karahana lactone (110),52 and both enantiomers of ancistrofuran (111a) and (111b),53



Scheme 20 Brooks' synthesis of anguidine (96)





111b

Scheme 21 shows the route to (15,5R)-karahana ether (109). Baker's yeast reduction of the prochiral diketone 108 gave (35)-keto-alcohol 112 in 99% ee. Compound 112 was treated with HCO₂Et and NaOMe to give 113. Reaction of 113 with *n*-BuSH, and *p*TSA yielded 114b along with a considerable amount of 114a (114a:114b=1:2). Reprotection of 114a with dihydropyran and *p*TSA smoothly regenerated 114b. Reduction of 114b with NaBH₄ and removal of the sulfur with HgCl₂ and CdCO₃ furnished 115. Compound 116 was obtained by reducing the aldehyde with NaBH₄, followed by alkylation with (*n*-Bu)₃SnCH₂[, and a [2,3]- sigmatropic rearrangement. (15,5R)-Karahana ether (109) was reached after a further four steps from 116.



Scheme 21 Mori's synthesis of (15,5R)-karahana ether (109)

We were interested in the chiral reduction of our spirodiketone systems. During the total synthesis of (±)-pentalencie (2), Wu31 found remarkable facial selectivity in the NaBH4 reduction of the spirodiketone 60. This gave a single (racemic) product 117a, and further reduction gave trans diol 117b. Reaction of methyllithium with 60 produced a 63:1 mixture of 61a and 61b, respectively (Scheme 22). Also, in a synthesis of prezizaene, Liu54 reported that NaBH4 reduction of spirodiketone 118 gave 120a and 120b in a 2.5:1 ratio, and addition of methyllithium to 118 gave 119a and 119b in a 4:1 ratio, respectively (Scheme 23). It was clear that there was dramatically less facial selectivity of the purely chemical reactions with spirodiketone 118. Baker's yeast reductions on these spirodiketones and others of synthetic interest were to be studied, and the facial selectivities were to be compared with those by NaBH4 reduction. The enzymatic reduction was expected to proceed not only with facial selectivity, but also enantiospecifically, 48, 55, 56 Thus, the baker's yeast reduction was to provide an entry to the synthesis of optically pure triguinanes, and this will also be reported in this chapter.



Scheme 22 Methyllithium addition and NaBH₄ reduction of 60

Scheme 23 Methyllithium addition and NaBH4 reduction of 118



120a

120b

I. Results and Discussion

Scheme 24 Baker's yeast reduction of 121



We first applied baker's yeast reduction to the simple spirodiketone **121** (Scheme **24**). The typical procedure was developed, based largely on the procedure of Mori,⁵² that involved initial fermentation of some sucrose in water at 31°C by the yeast, then spirodiketone **121** in 0.2% Triton X-100 and 95% ethanol was added, and the fermentation was continued for 48 hours. Reduction of compound **121** required only a small amount of yeast. The starting material **121** appeared to be completely transformed into a keto-alcohol **122**. The mass spectrum of the product showed a molecular ion at *m*/*z* 168. Absorption maxima in the IR for the ring carbonyl and the hydroxyl were found at 1722 and 3425 cm⁻¹, respectively. The ¹H NMR spectrum of **122** was more complicated than that of **121**, since **122** was no longer symmetrical. A one-proton signal at δ 4.27 indicated a proton attached to the carbon bearing the hydroxy group, confirming that only one carbonyl was reduced. The ¹³C NMR spectrum showed a carbonyl resonance at δ 222.1 and another signal for the carbon bearing the hydroxy

group at δ 75.0 (C-4). The specific rotation of **122** ([α ln = +80°) verified that enantioselective reduction of a prochiral ketone in compound **121** had occurred. Both the ¹H and the ¹⁹F NMR spectra of the Mosher's ester [(+)- α -methox y- α -trifluoromethylphenylacetate] of **122** demonstrated that the optical purity of **122** was over 98% ee.

Compound 121 was a simple symmetrical compound with no substituent on the cyclohexane ring. A series of spirodiketones with different substituents on the six-membered ring was then examined. Reduction could be to either face of the five-membered ring, which would lead to diastercomers (Scheme 25). Reduction at one carbonyl or the other, on the same face of the five-membered ring, would lead to two enantiomers.

Scheme 25 Principle of yeast reduction: facial selectivity and



enantioselectivity

Facial Selectivity --- Diastereomers



Enantioselectivity --- Enantiomers

Whereas diastereoselectivity might be expected in a purely chemical (achiral) process, the yeast reductase is a chiral reducing agent so both diastereoselectivity and enantioselectivity could result.

The spirodiketone **60** was an important intermediate in the synthesis of (±)-pentalenene (2) and in our route to (±)-deoxypentalenic acid (1b). Unlike in **121**, the spirocenter (C-5) of compound **60** is prochiral. Nevertheless, yeast reduction of **60** produced only one product (**123**), but "ome starting material **60** was also recovered (Scheme **26**).

Scheme 26 Baker's yeast reduction of 60



The reduction required roughly four times more baker's yeast than did the reduction of **121.** A larger excess of yeast did not improve yields and some diol was also formed. A small amount of starting material was always recovered. The gross structure and the relative stereochemistry of **123** were evident from the ¹H NMR and ¹³C NMR data. The chemical shift of II-4 was δ 4.24, and that of C-4 was δ 74.7. The remaining carbonyl carbon was at δ 221.7. The reduction of **60** had very high facial selectivity, since only one product was obtained. The ¹H NMR and ¹³C NMR spectra of compound **123** were the same as those of compound **117a** (racemic), which was obtained by reduction with NaBH4. The stereochemistry of **117a** was established by nOe difference spectra. The addition of hydride was clearly from the face of the five-membered ring *syn* to the double bond. Therefore, the yeast reduction was also from the *syn* face.

The specific rotation of **123** was $[\alpha]_D = +45^\circ$. Initially we used a chiral shift reagent Eu(hfc)₃ {tris[3-(heptafluoropropylhydroxymethylene)*d*-camphorato] europium (III)} to determine the enantioselectivity by ¹H NMR (Figure 8). The racemic compound **117a** led to unequal shifts for the enantiomeric carbinol resonances such that two peaks separated, in a ratio of 1:1. Compound **123** gave only a single peak, for the carbinol hydrogen at a very similar concentration of the shift reagent, therefore the optical purity of **123** was over 95% ee. However, ¹H and ¹⁹F NMR spectra of the Mosher's ester of **123** more unequivocally determined the optical purity of **123** to be at least 98% ee (Figure 9). For this ester, the H-4 was shifted to δ 5.41 and the vinyl proton was at δ 5.20.

With the high facial selectivity and the high enantioselectivity, the yeast reduction of compound **60** to **123** might be exploited as a key step to synthesize triquinanes such as (+)-pentalenene^{3h} in an optically pure form. A detailed discussion of this synthesis is presented later.

Spirodiketone 118 was used in a route to prezizaene. Yeast reduction of 118 produced two isomers with a diastereoselectivity of 2.5:1, which was no better than in the NaBH₄ reduction (2.5:1) (Scheme 27).





Figure 9 ¹H NMR spectrum of 123 Mosher's ester





The reduction used four times more yeast than did the reduction of **121**. No starting material remained and the isolated yield was 74%. The ratio

of the two diastereomers was determined by integration of the ¹H NMR spectrum. The chemical shift of the vinyi proton (H-7) of the major isomer was δ 5.28 and for the minor isomer it was δ 5.44. In the ¹³C NMR spectrum of the mixture, signals at δ 134.7 and δ 117.2 (C-7) were assigned to the double bond carbons of the major isomer, and signals at δ 145.0 and δ 119.2 (C-7) were attributed to the double bond carbons of the minor isomer. Both the ¹H NMR and the ¹³C NMR spectra of 124a and 124b were the same as those of 120a and 120b, which were obtained by NaBH₄ reduction of 118. The mixture of the keto-alcohol epimers 124a and 124b proved very difficult to separate, but the mixture showed optical activity ($(\alpha)_D = +89^{\circ}$). The enantiomeric purity of each diaztereomer was over 98% ec, which was determined from the ¹H and ¹⁹F NMR spectra of the Mosher's esters of 124a and 124b (Figure 10). The ratio of the two ester isomers was 1.61.

The results of the reductions of spirodiketones **60** and **118** showed that the facial selectivity was greatly influenced by substituents on the sixmembered ring. To determine how these substituents affected the selectivity, reduction of more spirodiketones **125**, **126**, **127** and **128** with baker's yeast was investigated (Scheme **28**). Yeast ieduction of **125** gave an exclusive, optically active product **129**. Its ¹H NMR spectrum showed a carbinol appeared at δ 4.48 (H-4), and in the ¹³C NMR spectrum the carbinol appeared at δ 79.5 (C-4). The signal at δ 222.0 was due to the remaining carbonyl carbon. The spectra of **compound 129** were the same as those of the major isomer obtained by reduction **125** with NaBH₄.

Yeast reduction of compound **126** gave one optically active product too. Both the ¹H NMR and the ¹³C NMR spectra confirmed that the product



130 was same as the major compound obtained by reduction of 126 with NaBH4. Yeast reduction of compound 127 provided a diastereomeric mixture 13'a and 131b, in a ratio of 1:1. The two isomers were clearly evident from the ¹H NMR and ¹³C NMR spectra. Vinyl doublets were found at δ 5.12 (J = 9.9 Hz), δ 5.37 (J = 10.2 Hz), δ 5.73 (J = 9.9 Hz), and δ 5.88 (J = 10.2 Hz). The chemical shifts for H-4 were δ 4.11 and δ 4.15 (in a ratio of 1:1). The result was the same for the yeast reduction of 128, which produced a 1:1 diastereomeric mixture. In the ¹H NMR spectrum of the product, the vinyl signals at 8 5.44 and 8 5.49 were in a ratio of 1:1. In the 13C NMR spectrum of the product, there were two carbonyl resonances, at 8 221.6 and δ 220.7. Nevertheless, the mixture of 131a and 131b and the mixture of 132a and 132b were both optically active, which showed that in spite of a lack of locial selectivity, the enzyme still exhibited enantio-selectivity in its action. All compounds 125, 126, 127 and 128 were efficiently reduced by baker's yeast to keto-alcohols with over 98% ee, which was assessed by 111 NMR of the corresponding Mosher's esters. The 19F NMR spectrum of the two diastereomeric Mosher's esters of 131a and 131b gave a single signal at δ -71.83. Also, there was only one signal at δ -71.55 in the ¹⁹F NMR spectrum for the two diastereomeric Mosher's esters of 132a and 132b. However, both the ¹H NMR and the ¹³C NMR spectra of these Mosher's esters showed two, and only two, sets of signals. The methine protons H-4 of the Mosher's esters were at δ 5.64 (1H, d, J = 3.6 Hz) for 129, δ 5.19 (1H, t, J = 3.9 Hz) for 130, δ 5.43 (2H, m) for 131a and 131b, δ 5.43 (2H, m) for 132a and 132b.









130











*These spirodiketones were kindly supplied by P.Y. Liu (compounds 125 and 128), and T.J. Jenkins (compounds 126 and 127).

Table 10 summarizes the results of the yeast reduction of spirodiketones 60, 118, 125, 126, 127 and 128. Table 11 compares the facial selectivity of the yeast reduction with that of NaBH₄ reduction. Liu^{5,4} studied the reduction of several spirodiketones with NaBH₄. The donation of electron density by a suitable substituent to C-7 or C-9, or withdrawal of electron density relative to bond *a* (Scheme 29). This correlated directly with the facial selectivity of the NaBH₄ reductions these spirodiketones. Thus, the facial selectivity of the NaBH₄ reductions was controlled largely by a stereoelectronic effect. The facial selectivity of yeast reductions was quite different from that of the NaBH₄ reductions. In contrast, facial selectivity in the yeast reductions must have depended mainly on the binding of the substrate in the active site of the reductase enzyme. As the planenonsymmetric moieties of the substrates are nonpolar, binding was probably controlled by steric factors.





| Compound Structure | [α] ^D | Ratio of the Diastereomer | Isolated Yield s (%) | Recovered Starting Material (%) |
|-----------------------|--|------------------------------|-------------------------|------------------------------------|
| | +45 ⁰ (McOH) | exclusive | 62 | 23 |
| | +82 ⁰ (McOH) | exclusive | 79 | 11 |
| | +7 ⁰ (CHCl ₃) | exclusive | 50 | 20 |
| ÷ | +89 ⁰ (McOH) | 2.5:1 | 74 | 0 |
| | +42 ⁰ (CHCl ₃) | 1:1 | 52 | 43 |
| 000 | +112 ⁰ (McOH) | 1:1 | 61 | 19 |

Table 10 Baker's Yeast Reduction

| Compound Number | Gross Structure | Baker's Yeast | NaBH ₄ |
|--------------------|-----------------|---------------|-------------------|
| 123 | оСон | >100:1 | >100:1 |
| 129 | о ОН | >100:1 | 6.5:1* |
| 130 | отон | >100:1 | 18:1* |
| 124a/b | отор | 2.5:1 | 2.5:1* |
| 131a/b | отон | 1:1 | 3:1 |
| 132a/b | о | 1:1 | 9:1* |

Table 11 Comparison of facial selectivities in yeast and chemical reductions (product ratios)

* From the Ph.D. thesis on P-Y. Liu54

Not all spirodiketones could be reduced by the yeast. Yeast reduction of spirodiketone 133 failed, which suggested that the bulky substituents on the six-membered ring prevented the binding of 133 to the active site of the enzyme.



133

Brooks et al.⁵⁵ studied several 2,2-disubstituted 1,3-diones, and they concluded that reduction by baker's yeast provides only the (S) configuration at the carbinol carbon, including in compound **122**. Thus, the absolute configurations of our spirodiketones were established relative to this carbinol's configuration, by comparsion of some circular dichroism (CD) spectra,⁵⁷ and this absolute stereochemistry was confirmed by two X-ray structures,

Compounds 122 and 130 both had only one chiral center (C-4). The CD spectra of compound 122 and of 130 (Figure 11) had opposite Cotton effects. Compound 122 (S)-enantiomer displayed a positive Cotton effect at 310 nm, and compound 130 had a negative Cotton effect at 300 nm. (Both compounds showed observable signals in 240-340 nm region.) Compound 130 was different from other diketones in that it had only one significant



b) CD spectrum of compound 130 (CHCl3)

conformational form. From the studies of NaBH₄ reduction and methyllithium addition on 126.5^{44} it was conformed that the equatorial carbonyl was the more reactive one. An X-ray structure (Figure 12) of the Mosher's ester of the product of yeast reduction 130 demonstrated conclusively that the yeast also chose to reduce the equatorial carbonyl, and, consistent with reductions on other spirodiketones, the configuration at the new stereogenic center was *S* (Scheme **30**).

Scheme 30 Baker's yeast reduction on 126



126





Figure 12 X-Ray structure 130 Mosher's ester

The CD spectrum of compound **129** was consistent with that of compound **122** (Figure **13**), they both showed a positive Cotton effect with maxima at 310 nm. Thus, **129** had the (45,55) configuration. The diastereomeric mixtures of compounds **131a:131b, 132a:132b,** and **124a:124b** all showed a positive Cotton effect with maxima around 300 nm. The NaBH4 reductions and baker's yeast reductions on spirodiketones both showed a preference for reduction of a ketone on its face *syn* to the double bond. Nevertheless, when the diastereomeric mixtures of yeast reduction products were derivatized to the corresponding mixtures of Mosher's esters the number of sets of signals in the NMR spectra did not double. This is consistent with high enantioselectivity in the reduction to give the *S* sconfiguration at the carbinol, even when the diastereomeric mixtures were composed only of the (45,55) and (45,58) isomers.

Compound 123 was produced by yeast reduction with very high facial and enantioseiectivity. This compound has potential as a key intermediate for the production of optically pure triquinanes via the strategy described in the first chapter of this thesis. In order to produce a compound that would link into the previously described synthetic route we explored the reaction sequence shown in Scheme 31. The hydroxy group of 123 was protected with chlorotrimethylsilane to give 134. The X-ray structure of 134 was obtained (Figure 14) which confirmed its relative stereochemistry. Methyllithium addition to 134 was initially difficult because of competing enolate formation. However, employing several cycles of methyllithium addition-quench in one pot, as Corey³⁸ had done, overcame this problem



Figure 13 CD spectrum of compound 129 (McOH)



Figure 14 X-ray structure 134





and 135 was obtained in good yield. Since our reaction was conducted at reflux, a minor amount of compound 136, in which the trimethylsilyl group had been lost, was detected, so without further purification 135 was deprotected with tetrabutylammonium fluoride. A diastereomeric mixture of 136a and 136b (7:1, respectively) was obtained, but it should be emphasized that each isomer of 136 had an enantiomeric excess of over 98%, as determined by ¹H NMR of the corresponding Mosher's esters. Compound 137 was generated by oxidation of the mixture of 136a and 136b. The relative stereochemistry of 137 is shown in the X-ray structure in Figure 15. The ¹H NMR and ¹³C NMR spectra of compound 137 were identical with those of compound 61 (racemic).



Figure 15 X-ray structure of 137

It is important to realize that the chirality of the spiro-center (C-5) in 123 is *apposite* to the chirality of the central quaternary center in the triquinane sesquiterpenes that were investigated in Chapter 1.

After oxidation, the spiro center (C-5) of **137** was effectively inverted with respect to compound **123**, which was (4S,5R). Therefore compound **137** was assigned the (4R,5S) stereo-chemistry, and **137** had the chirality at C-5 that would lead to the "correct" enantiomer of deoxypentalenic acid or pentalenene. Racemic **61** was an intermediate in our approach to (\pm) deoxypentalenic acid **(1b)** and in Wu's route to (\pm) -pentalenene **(2)**. Therefore, it is now obvious that the corresponding optically pure compound **137** could be used in the place of **61** to yield optically pure triquinanes with the same absolute stereochemistry as it is found in Nature.

III. EXPERIMENTAL

General

For general information and a detailed description of the instruments, see the Experimental Section of Chapter 1. Baker's yeast reactions were carried out in a water-bath shaker at about 32°C. The yeast was Fleischmann's "Traditional" brand purchased in local grocery stores. All reactions were monitored by analytical thin-layer chromatography (TLC). Optical rotations were recorded on a Perkin Elmer 141 polarimeter. Circular dichroism (CD) measurements were made on a Jasco J40A instrument using a cell of 0.05 cm path length.

(4S)-4-Hydroxyspiro[4.5]decan-1-one (122)



A mixture of dry baker's yeast (2.0 g) and sucrose (4.5 g) in water (30 mL) was stirred at 31°C for 10 minutes. Diketone **121** (197 mg, 1.18 mmol) in 95% ethanol (0.3 mL) and 0.2% Triton X-100 (1.2 mL) was added. The mixture was stirred for 48 hours. Ether (2.4 mL) was added, and the solution was allowed to stand overnight at room temperature. After precipitation of the yeast cells, the mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The aqueous layer was re-extracted with
ethyl acetate. and the combined organic solutions were washed with brine, dried over magnesium sulfate, filtered, and concestrated. The residue was separated by flass: chromatography (10-16% ethyl acetate in hexane) to provide compound 122 as a colorless oil (134 mg, 68%): $[\alpha]_D = +80^{\circ}$ (c = 0.0069, MeOH); IR ν_{max} ; 3425, 1722 cm⁻¹; ¹H NMR: δ 1.26-1.42 (4H, m), 1.46-1.63 (4H, m), 1.94 (1H, m), 2.09-2.27 (2H, m), 2.34-2.46 (2H, m), 4.27 (1H, hr s); ¹³C NMR: δ 222.1 (0), 75.0 (1), 54.2 (0), 34.1 (2), 30.8 (2), 27.8 (2), 25.5 (2), 22.5 (2), 21.9 (2), NS from GCMS *m/z* (%): 168 (44, M⁺), 150 (65, M⁺+12O), 124 (16), 108 (98), 93 (41), 81 (100), 67 (62), 55 (39), 41 (35); HRMS calcel, for C10H16O2: 168.1149; found: 168.1152.

(4S,5R)-4-Hydroxy-7,9,9-trimethylspiro[4.5]dec-7-en-1-one (123)



A mixture of dry baker's yeast (8.0 g) and sucrose (18.0 g) in water (100 mL) was shaken in a 32°C water bath for 10 minutes. After brisk fermentation had started, a solution of diketone 60 (158 mg, 0.780 mmol) in 95% ethanol (3.0 mL) and 0.2% Triton X-100 (12 mL) was added. The mixture was shaken for 48 hours. Ether (15 mL) was added, and the solution was allowed to stand overnight at room temperature. After precipitation of the yeast cells, the mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The aqueous layer was re-extracted with ethyl acetate, and the combined organic solutions were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (12-18% ethyl acetate in hexane) to provide compound **123** as a colorless oil (100 mg, 62%), and 36 mg (23%) of the starting material was recovered. For **123**: $[\alpha_{115} + 45^{\circ} (c = 0.0063, MeOH); IR v_{max}: 3447, 1729 cm⁻¹; ¹H NMR; <math>\delta$ 1.03 (3H, s), 1.05 (3H, s), 1.64 (3H, s), 1.66-1.73 (2H, m), 1.81 (1H, d, *J* = 17.2 1Iz), 2.05 (1H, m), 2.17-2.25 (2H, m), 2.32 (1H, dd, *J* = 2.4, 9.0 Hz), 2.50 (1H, m), 4.24 (1H, br s, H-4); ¹³C NMR; δ 221.7 (0), 131.8 (1), 127.4 (0), 74.7 (1), 54.5 (0), 35.1 (2), 35.0 (2), 33.3 (2), 32.1 (3), 31.3 (0), 30.3 (3), 28.0 (2), 23.8 (3); MS *m/z* (%): 208 (32, M⁺), 175 (95), 149 (100), 133 (46), 121 (61), 119 (40), 91 (40), 79 (25), 41 (41); HRMS caled. for C 1₃H₂₀O₂: 208.1462; found: 208.1460.

rel-(4R,5S)-4-Hydroxy-7,9,9-trimethylspiro[4.5]dec-7-en-1-one (117a) and *trans*-7,9,9-trimethylspiro[4.5]dec-7-ene-1,4-diol (117b)



7,9,9-Trimethylspiro[4.5]dec-7-ene-1,4-dione (85 mg, 0.40 mmol) was dissolved in methanol (10 mL), and NaBH4 (ca. 7.0 mg, 0.19 mmol) was added to the solution in several small portions. The mixture was stirred at room temperature for 1 hour. Water was added. The aqueous layer was extracted with ether four times, and the combined organic solutions were

washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (5% acetone in hexane) to give 117a (60 mg, 70%), and 117b (22 mg, 26%). For 117a: IR υmax: 3447, 1729 cm⁻¹; ¹H NMR: δ 1.03 (3H, s), 1.05 (3H, s), 1.64 (3H, s), 1.66-1.73 (2H, m), 1.81 (1H, d, J = 17.2 Hz, AB), 2.05 (1H, m), 2.17-2.25 (2H, m), 2.32 (1H, dd, J = 2.4, 9.0 Hz), 2.50 (1H, m), 4.24 (1H, br s, H-4), 5.23 (1H, s, H-8); ¹³C NMR: δ 221.8 (0), 131.8 (1), 127.4 (0), 74.7 (1), 54.4 (0), 35.0 (2), 34.9 (2), 33.3 (2), 32.1 (3), 31.2 (0), 30.3 (3), $28(1(2), 23, 8(3); MS m/z (\%); 208(13, M^+), 175(46), 149(52), 133(60),$ 131 (22), 123 (15), 121 (34), 119 (35), 107 (35), 105 (28), 91 (45), 81 (24), 77 (31), 65 (22), 57 (26), 55 (49), 43 (79), 41 (100); HRMS calcd. for C13H20O2: 208.1462; found: 208.1462. For 117b: IR umax: 3340 cm-1; H NMR: δ 1.03 (3H, s), 1.06 (3H, s), 1.38-1.63 (5H, m), 1.67 (3H, s), 2.11-2.19 (4H, m), 3.97 (1H, m), 4.10 (1H, t, J = 7.9 Hz), 5.19 (1H, s, H-8); ¹³C NMR: § 131.4 (1), 129.4 (0), 77.4 (1), 76.8 (1), 48.5 (0), 38.6 (2), 2.1 (2C, () and 3), 31, 1 (2C, 2 and 3), 29,7 (2), 29,0 (2), 24,1 (3); MS m/z (%); 210 (6, M+), 195 (26, M+-Me), 177 (36), 159 (70), 149 (21), 148 (20), 136 (29), 135 (32), 133 (100), 123 (35), 121 (25), 119 (33), 109 (20), 107 (38), 105 (39), 93 (27), 91 (38), 81 (29), 79 (23), 77 (20), 55 (32), 43 (64), 43 (64), 41 (70); HRMS calcd. for C12H19O2 (M+-Me): 195.1384; found: 195.1379.

(4S,5S)-4-Hydroxy-7,7-dimethylspiro[4.5]decan-1-one (129)



A mixture of dry baker's yeast (6.0 g) and sucrose (16.0 g) in water (50 mL) was shaken in a 32°C water bath for 10 minutes. After brisk fermentation started, a solution of diketone 125 (47 mg, 0.24 mmol) in 95% ethanol (2.0 mL) and 0.2% Triton X-100 (8.0 mL) was added, and another 50 mL of water was added. The mixture was shaken for 48 hours. Ether (10) mL) was added, and the solution was allowed to stand overnight at room temperature to precipitate the yeast cells. The mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The aqueous layer was re-extracted with ethyl acetate, and the combined organic solutions were washed with brine, dried over magnesium salfate, filtered, and concentrated. The residue was separated by flash chromatography (16-20% ethyl acetate in hexane) to provide 129 as colorless crystals (37 mg, 79%), and some starting material (5 mg, 11%) was recovered. For 129: $\alpha_{ln} = +82^{\circ}$ (c = 0.0045, MeOH); mp 82-85°C; IR ν_{max} ; 3456, 1736 cm⁻¹; ¹H NMR; δ 0.98 (3H, s), 1.00 (3H, s), 1.24 (1H, m), 1.44-1.56 (7H, m), 2.00-2.46 (4H, m), 4.48 (1H, br s, H-4); ¹³C NMR: δ 222.0 (0), 74.9 (1), 55.1 (0), 38.7 (2), 37.0 (2), 33.4 (3), 33.1 (2), 31.0 (2), 29.6 (0), 27.9 (2), 26.7 (3), 19.2 (2); MS m/z (%); 196 (11, M⁺), 181 (66), 149 (14), 127 (13), 121 (27), 109 (84), 95 (24), 81 (37), 69 (100), 57 (24), 41 (64); HRMS calcd. for C12H20O2: 196.1462; found: 196.1462.

(4S)-8-tert-Butyl-4-hydroxyspiro[4,5]decan-1-one (130)



A mixture of dry baker's yeast (1.0 g) and sucrose (2.5 g) in water (75 mL) was shaken in a 32°C water bath for 10 minutes. After brisk fermentation started, a solution of diketone 126 (51 mg, 0.23 mmol) in 95% ethanol (0.40 mL) and 0.2% Triton X-100 (1.2 mL) was added. The mixture was shaken for 48 hours. Ether (0.5 mL) was added, and the solution was allowed to stand overnight at room temperature to precipitate the yeast cells. The mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The aqueous layer was re-extracted with ethyl acetate, and the combined organic lavers were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (14-20% ethyl acetate in hexane) to provide 130 as colorless crystals (26 mg, 50%), and some starting material (10 mg, 26%) was recovered. For 130: $[\alpha]_{D} = +7^{\circ}$ (c = 0.0086, CHCl₃); mp 135-137°C; IR υmax: 3408, 1729 cm⁻¹; ¹H NMR: δ 0.86 (9H, s), 1.26-1.45 (3H, m), 1.56-1.71 (5H, m), 1.81-1.92 (2H, m), 2.19-2.28 (2H, m), 2.42 (1H, m), 3.88 $(1H, t, J = 4.8 \text{ Hz}); \frac{13}{2} \text{C} \text{NMR}; \delta 219.8 (0), 79.5 (1), 51.9 (0), 47.7 (1), 34.6$ (2), 32.5 (0), 31.5 (2), 27.6 (3, 3C), 27.1 (2), 26.3 (2), 22.5 (2), 22.1 (2); MS

n/z (%): 224 (8, M⁺), 206 (9), 167 (32), 150 (29), 126 (13), 107 (34), 81 (32), 67 (24), 57 (100), 41 (58); HRMS caled, for C₁₄H₂₄O₂; 224,1775; found: 224,1777.

(45,5R) and (45,5S)-4-Hydroxy-8-methylspiro[4.5]dec-7-en-1-one (2.5:1 mixture) (124a and 124b)



A mixture of dry baker's yeast (8.0 g) and sucrose (18.0 g) in water (100 mL) was shaken in a 31°C water bath for 10 minutes. After brisk fermentation started, a solution of diketone **118** (119 mg, 0.67 mmol) in 95% ethanol (3.0 mL) and 0.2% Triton X-100 (12 mL) was added, and another 100 mL of water was added. The mixture was stirred at 32°C for 48 hours. Ether (15 mL) was added and the solution was allowed to stand overnight at room temperature to precipitate the yeast cells. The mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The aqueous layer was re-extracted with ethyl acetate, and the combined organic solutions were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The flash chromatography of the residue (12-20% ethyl acetate in hexane) provided a mixture of **124a** and **124b** (89 mg, 74%) as a colorless oil: $[\alpha]_{D=} + 89^\circ$ (c = 0.0033, MeOH); IR vmax: 3443, 1727 cm⁻¹; ¹H NMR for the major product: δ 1.65 (3H, s), 1.77 (1H, m), 1.802.40 (8H, m), 2.48 (1H, m), 5.28 (1H, s); for the minor one: δ 1.65 (3H, s), 1.80-2.40 (9H, m), 2.48 (1H, m), 4.25 (1H, m), 5.44 (1H, br s); ¹³C NMR for the major product δ 222.0 (0), 134.7 (0). 117.2 (1), 75.1 (1), 52.6 (0), 34.3 (2), 31.1 (2), 28.4 (2), 27.1 (2), 23.3 (3), 22.7 (2); for the minor one: δ 222.0 (0), 145.0 (0), 119.2 (1), 75.2 (1), 52.6 (0), 34.1 (2), 27.9 (2), 27.8 (2), 26.8 (2), 25.5 (2), 23.3 (3); MS of the mixture *m/z* (%): 180 (54, M⁺), 147 (17), 136 (100), 120 (49), 93 (54), 91 (45), 79 (43), 43 (40); HRMS calcel. for C1 [1H]6O2: 180.1147.

(4*S*,5*R*) and (4*S*,5*S*)-4-Hydroxy-8,8-dimethylspiro[4.5]dec-6-en-1-one (1:1 mixture) (131a and 131b)



A mixture of dry baker's yeast (10.0 g) and sucrose (22.0 g) in water (100 mL) was shaken in a 31°C water bath for 10 minutes. After brisk fermentation started, this system was poured into the solution of diketone 127 (187 mg, 0.97 mmol) in 95% ethanol (4.0 mL) and 0.2% Triton X-100 (16 mL). Another 100 mL of water was added, The suspension was shaken at 32°C for 48 hours. Ether (15 mL) was added, and the solution was allowed to stand overnight at room temperature to precipitate the yeast cells. The mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The combined organic solutions were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The flash chromatography of the residue (12-20% ethyl acetate in hexane) provided an inseparable mixture of **131a** and **131b** as a colorless oil (97 mg, 52%), and some starting material (81 mg, 43%) was recovered. For **131a** and **131b**: [α]_D = +42° (c = 0.0048, CHC13): IR vmax: 3472, 1723 cm⁻¹: ¹H NMR of the mixture: δ 0.99 (3H, s), 1.00 (3H, s), 1.03 (3H, s), 1.03 (3H, s), 1.42-1.69 (8H, m), 1.84 (1H, m), 1.97-2.10 (3H, m), 2.23-235 (4H, m), 2.45-2.53 (2H, m), 4.11-4.15 (2H, m, H-4), 5.12 (1H, d, J = 9.9 Hz), 5.37 (1H, d, J = 10.2 Hz), 5.37 (1H, d, J = 9.9 Hz), 5.88 (1H, d, J = 10.2 Hz); 5.37 (NMR of the mixture: δ 219.5 (0), 218.2 (0), 143.8 (1), 142.5 (1), 122.6 (1), 119.6 (1), 78.2 (1), 77.0 (1), 56.3 (0), 55.8 (0), 34.3 (2), 34.0 (2), 33.0 (2), 32.8 (2), 31.4 (0, 2C), 29.3 (3, 2C), 29.0 (3), 28.9 (3), 27.8 (2), 27.0 (2), 26.0 (2), 19.8 (2); MS of the mixture m/z (%): 194 (39, M⁺), 161 (32), 135 (100), 107 (80), 105 (42), 93 (44), 91 (36), 41 (47); HRMS caled. for C12H 1802: 19.4.1308.

rel-(4R,5S) and rel -(4R,5R)-4-Hydroxy-8,8-dimethylspiro[4.5]dec-6-en-1-one (3 :1 mixture) (131a' and 131b')



8,8-Dimethylspiro[4.5]dec-6-ene-1,4-dione (127) (78 mg, 0.40 mmol) was dissolved in 10 mL of methanol, and NaBH4 (4.0 mg, 0.10 mmol) was added to the solution in several small portions. The mixture was stirred at

room temperature for 1 hour. Water was added. The aqueous layer was extracted with ether four times, and the combined organic layers were washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (10%-16% ethyl acetate in hexane) to give the keto-alcohol mixture (78 mg, 98%); IR umax: 3472, 1723 cm⁻¹; ¹H NMR of the mixture: δ 1.00 (3H, s), 1.01 (3H, s), 1.04 (6H, s), 1.44-1.48 (2H, m), 1.62-1.72 (4H, m), 1.82-2.05 (4H, m), 2.20-2.33 (4H, m), 2.45-2.57 (2H, m), 4.11-4.15 (2H, m, H-4), 5.12 (1H, d. J = 9.9 Hz, H-6), 5.36 (1H, d, J = 10.2 Hz), 5.74 (1H, d, J = 9.9 Hz, H-7). 5.90 (1H, d, J = 10.2 Hz); nOe data for 131a': irradiate δ 5.12 (H-6), 1.5% nOe at δ 4.15 (H-4); irradiate δ 4.15 (H-4), 1.9% nOe at δ 5.12 (H-6); 13C NMR for the major product: § 217.8 (0), 142.7 (1), 122.6 (1), 77.2 (1), 56.3 (0), 34.3 (2), 32.9 (2), 31.5 (0), 29.4 (3), 29.1 (3), 27.0 (2), 19.9 (2); for the minor product; § 219.3 (0), 144.2 (1), 119.5 (1), 78.3 (1), 55.9 (0), 34.1 (2), 33.1 (2), 31.5 (0), 29.4 (3), 28.9 (3), 27.9 (2), 26.1 (2); MS of the mixture m/z (%): 194 (60, M⁺), 179 (17, M⁺-CH₃), 161 (32), 135 (100), 121 (27), 119 (30), 107 (78), 105 (33), 93 (35), 85 (21), 79 (22), 55 (19), 43 (28); HRMS calcd. for C12H18O2: 194.1306; found: 194.1320.

(45,5R) and (45,55)-4-Hydroxy-7-methylspiro[4.5]dec-7-en-1-one (1:1 mixture) (132a and 132b)



A mixture of dry baker's yeast (8.0 g) and sucrose (18.0 g) in water (100 mL) was stirred at a 31°C water bath for 10 minutes. After brisk fermentation started, a solution of diketone 128 (140 mg, 0.79 mmol) in 95% ethanol (3.0 mL) and 0.2% Triton X-100 (12 mL) was addded, and another 100 mL of water was added. The mixture was stirred at 32°C for 48 hours. Ether (15 mL) was added, and the solution was allowed to stand overnight at room temperature to precipitate the yeast cells. The mixture was filtered through Celite, and the filter cake was extracted with ethyl acetate. The aqueous layer was re-extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was separated by flash chromatography (14-20% ethyl acetate in hexane) to provide a mixture of 132a and 132b as a colorless oil (87 mg, 61%), and some starting material (27 mg, 19%) was recovered. For 132a and 132b: $|\alpha|_{D} = +|12^{\circ}|_{C} = 0.0072$. MeOH); IR υmax: 3450, 1729 cm⁻¹; ¹H NMR of the mixture: δ 1.65 (61), s), 1.71 (2H, s), 1.95-2.60 (18H, m), 4.19-4.25 (2H, m), 5.44 (1H, s), 5.49 (1H, s); ¹³C NMR of the mixture: δ 221.6 (0), 220.7 (0), 133.0 (0), 130.4 (0), 121.4 (1), 119.9 (1), 75.6 (1), 75.3 (1), 53.3 (0), 53.1 (0), 35.3 (2), 34.3 (2), 34.1 (2), 29.9 (2), 28.3 (2), 27.9 (2), 27.4 (2), 23.7 (3, 2C), 22.3 (2), 22.1 (2), 22.0 (2); MS of the mixture m/z (%); 180 (55, M+), 147 (15), 124 (20), 121 (100), 105 (36), 93 (74), 91 (50), 79 (46), 77 (38), 43 (31); HRMS calcd. for C11H16O2: 180.1150; found: 180.1152.

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(45,5R)-7,9,9-Trimethyl-4-trimethylsilyloxyspiro[4.5]dec-7-en-1-one (134)



(45,5R)-4-Hydroxy-7,9,9-trimethylspiro[4.5]dec-7-en-1-one (123) (82 mg, 0.39 mmol) was dissolved in pyridine (2.0 mL). Chlorotrimethylsilane (0.15 mL, 1.2 mmol) was added to the solution of ketc-alcohol at 0°C under nitrogen. Then the ice bath was removed, and the mixture was stirred at room temperature for 1 hour. Carbon tetrachloride was added, and the mixture was filtered, and concentrated. The crude sample was separated by flash chromatography (1% ethyl acetate in hexane) to provide 134 as a colorless solid (106 mg, 97%); $[\alpha]_{D} = +39^{\circ}$ (c = 0.0064, MeOH); mp 75-77°C; IR υmax: 1742 cm-1; ¹H NMR: δ0.13 (9H, s), 0.96 (6H, s), 1.54 (1H, d, J = 14.1 Hz, AB), 1.65 (3H, s), 1.69-1.76 (3H, m), 2.01-2.17 (2H, m), 2.24 (1H, dd, J = 4.1, 8.3 Hz), 2.40 (1H, m), 4.12 (1H, t, J = 3.7 Hz), 5.20 (1H, s); ¹³C NMR: 8 221.6 (0), 132.1 (1), 127.5 (0), 75.9 (1), 54.6 (0), 34.9 (2), 34.1 (2), 33.3 (2), 31.5 (0), 31.3 (3), 31.1 (3), 27.9 (2), 23.8 (3), 0.45 (3, 3C); MS m/z (%); 280 (90, M+), 175 (58), 164 (19), 149 (23), 133 (24), 129 (29), 107(16), 91 (18), 75 (28), 73 (100), 41 (19); HRMS calcd. for C16H28O2Si: 280.1857; found: 280.1858.

(15,45,5R) and (1R,45,5R)-1,7,9,9-Tetramethylspiro[\$.5]dec-7-ene-1,4diol (7:1 mixture) (136a and 136b)



(4S.5R)-7.9.9-Trimethyl-4-trimethylsilyloxyspiro[4.5]dec-7-en-1-one (134) (348 mg, 1.24 mmol) was dissolved in hexane-ether (1:1, 30 mL). The solution was heated under nitrogen, and 1.4 M methyllithium (2.70 mL, 3.78 mmol) was added. After 10 min, 0.15 mL of methanol was added. Then 1.4 M methyllithium (2.70 mL, 3.78 mmol) was added again. After 10 min, methanol (0.15 mL) was added. The methyllithium addition continued in this way for a total of six cycles. After the mixture was cooled, water was added. The aqueous layer was extracted with ether four times, and the combined organic solutions were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was redissolved in dichloromethane (5.0 mL), and 1.0 M tetrabutylammonium fluoride (2.0 mL. 2.0 mmol) was added. The mixture was stirred at room temperature for 3 hours. The water was added. The aqueous layer was extracted with dichloromethane four times, and the combined organic solutions were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was separated by flash chromatography (14%-18% ethyl acetate in hexane) to provide a colorless product that was a 7:1 epimeric mixture (221 mg, 80%). For the major isomer 136a: $|\alpha|_{D} = +98^{\circ}$ (c = 0.022, CHCl₃); IR υmax: 3361 cm⁻¹; ¹H NMR: δ 1.03 (3H, s), 1.10 (3H, s), 1.19 (3H, s), 1.56 (2H, s), 1.62 (3H, s), 1.66-1.99 (5H, m), 2.19 (1H, m), 2.58 (1H, d, J = 6.3 Hz, OH), 2.88 (1H, s, OH), 4.04 (1H. t. J = 6.3 Hz, H-4), 5.21 (1H, s, H-8); ¹³C NMR; δ 131.6 (1), 128.1 (0), 84.7 (0), 78.0 (1), 51.4 (0), 37.7 (2), 35.4 (2), 33.9 (2), 33.3 (3), 31.9 (0), 31.1 (2), 30.3 (3), 24.1 (3), 21.5 (3); MS m/z (%): 206 (56, M+-H2O), 191 (51), 188 (3, M+-2 H2O), 173 (49), 163 (12), 149 (48), 148 (39), 147 (20), 133 (51), 121 (44), 107 (38), 105 (16), 85 (63), 67 (13), 43 (100); HRMS calcd. for C14H21O (M^+-H_2O) : 206, 1670; found: 206, 1675. For the minor isomer 136b; $[\alpha]_D =$ +36° (c = 0.029, CHCl₃); IR v_{max} : 3354, 1736 cm⁻¹; ¹H NMR: δ 0.95 (3H, s), 0.96 (3H, s), 1.11 (3H, s), 1.79 (3H, s), 1.63-1.70 (4H, m), 1.85-1.94 (2H, m), 2.21-2.32 (2H, m), 2.85 (1H, d, J = 6.9 Hz, OH), 2.92 (1H, s, OH), 4.19 (1H. t. J = 6.6 Hz, H-4), 5.64 (1H, s, H-8); ¹³C NMR; δ 138.1 (0), 117.3 (1), 85.9(0), 79.3(1), 53.5(0), 44.1(2), 40.8(2), 37.4(2), 32.5(3), 31.4(2),30.8 (0), 27.2 (3), 25.0 (3), 21.7 (3); MS m/z (%): 206 (56, M+-H2O), 191 (32) 173 (29) 163 (24) 149 (100) 133 (30) 121 (56) 107 (51) 93 (30) 85 (38), 69 (23), 55 (34), 43 (98); HRMS calcd. for C14H21O (M+-H2O): 206.1670; found: 206.1649.

(4R,5S)-4-Hydroxy-4,7,9,9-tetramethylspiro[4.5]dec-7-en-1-one (137)



The mixture of (1S,4S,5R) and (1R,4S,5R)-1,7,9,9-tetramethylspiro[4.5]dec-7-ene-1,4-diol (136) (221 mg, 0.99 mmol) was dissolved in dichloromethane (20 mL). PCC (250 mg, 1.16 mmot) was added. The mixture was stirred for six hours at room temperature. The mixture was filtered through a pad of silica gel, which was extracted with ether. The combined organic solutions were concentrated. The residue was separated by flash chromatography (12%-20% ethyl acetate in hexane) to provide the title compound as a yellow oil (107 mg, 49%), and some spirodiketone 60 (40.8 mg, 19%) was recovered. For compound 137: $[\alpha]_D = +12^\circ$ (e = 0.099, CHCl₃); IR v_{max}: 3426, 1730 cm⁻¹; ¹H NMR: 8 0.90 (3H, s, 9-CH₃), 1.15 (3H, s, 4-CH₃), 1.71 (2H, d, J = 3.9 Hz, H-10), 1.74 (3H, s, 7-CH₃), 1.79 (2H, s), 1.96 (1H, m), 2.21 (2H, m), 2.56 (1H, m), 5.19 (1H, s, H-8); ¹³C NMR: 8 219.9 (0), 130.6 (1), 128.9 (0), 78.1 (0), 55.9 (0), 38.0 (2), 34.1 (2), 33.6 (2), 32.8 (3), 32.0 (0), 30.5 (2), 28.6 (3), 24.4 (3), 23.9 (3); MS m/z (%): 222 (43, M⁺), 189 (14), 164 (20), 149 (19), 123 (26). 99 (50), 84 (17), 55 (16), 43 (100); HRMS caled. for C1₄H₂₂O₂: 222.1619; found: 222.1612.

Preparation of (R)-(+)-MTPA Derivatives (Mosher's esters)

The reaction was carried out in a dried vial fitted with a rubber septum. The reagents were injected into the vial, to which was added about 0.1 mmol of the keto-alcohol, in the following order: dry pyridine (300μ L), (+)-MTPA-chloride (52μ L), and dry CCl4 (300μ L). The mixture was allowed to stand at room temperature for 3 days with occasional stirring. After the reaction was complete by TLC, 3-dimethylamino-1-propylamine (*ca.* 50 μ L) was added, and the mixture became a yellow color. After 5 minutes, the mixture was diluted with ether, then it was washed with icc cold 10% aqueous HCl, cold saturated Na₂CO₃, and cold brine. It was dried over anhydrous magnesium sulfate, filtered, and concentrated. Carbon tetrachloride was added to the residue, and the solution was reconcentrated. This was repeated several times in order to remove the traces of ether. Each sample was analysed by ¹H NMR and, in many cases, by ¹⁹F NMR, also.

For the Mosher's ester of **122**: ¹H NMR: δ 1.26-1.54 (10H, m), 2.10-2.39 (4H, m), 3.53-3.54 (3H, m, OCH₃), 5.63 (1H, m), 7.41-7.55 (5H, m); ¹⁹F NMR: δ -71.68.

For the Mosher's ester of **123**: ¹H NMR: δ 0.64 (3H, s, CH3), 0.93 (3H, s, CH3), 1.59 (1H, m), 1.63 (3H, s, CH3), 1.67 (1H, m), 1.81 (1H, m), 2.11-2.31 (5H, m), 3.49 (3H, m, OCH3), 5.21 (1H, s), 5.41 (1H, m), 7.40-7.53 (5H, m); ¹⁹F NMR: δ -70.73.

For the Mosher's ester of **124**: ¹H NMR (1.6:1): δ 1.42-1.60 (2H, m), 1.61 (3H, s, CH₃), 1.62 (3H, s, CH₃), 1.65-2.10 (4H, m), 2.18-2.50 (4H, m), 3.51-3.53 (3H, m, OCH₃), 5.14 (1H, br s), 5.26 (1H, br s), 5.39 (1H, d, *J* = 3.6 Hz), 5.46 (1H, d, *J* = 3.6 Hz), 7.41-7.50 (5H, m); ¹⁹F NMR: δ -71.92 (minor isomer), -71.40 (major isomer).

For the Mosher's ester of **129**: ¹H NMR: $\delta 0.61$ (3H, s, CH3), 0.84 (3H, s, CH3), 1.02-1.56 (8H, m), 2.08-2.39 (4H, m), 3.51 (3H, d, J = 1.2 Hz, OCH3), 5.65 (1H, d, J = 3.6 Hz), 7.41-7.55 (5H, m).

For the Mosher's ester of **130**: ¹H NMR: **8** 0.83 (9H, s), 1, 15-1.35 (4H, m), 1.51-1.75 (5H, m), 2.03 (1H, m), 2.34-2.40 (3H, m), 3.53 (3H, d, *J* = 1.2 Hz, OCH3), 5.19 (1H, m), 7.41-7.55 (5H, m).

For the Mosher's ester of **131**: ¹H NMR (1:1): δ 0.90 (3H, s, CH₃), 0.95 (3H, s, CH₃), 0.99 (3H, s, CH₃), 1.00 (3H, s, CH₃), 1.22-1.62 (4H, m), 2.02-2.41 (4H, m), 3.52-3.54 (3H, m, OCH₃), 4.98 (1H, d, J = 10.2 Hz), 5.11 (1H, d, J = 10.2 Hz), 5.40-5.44 (2H, m), 5.64 (1H, d, J = 10.2 Hz), 5.70 (1H, d, J = 10.2 Hz), 7.41-7.50 (5H, m); ¹⁹F NMR: δ -71.83.

For the Mosher's ester of **132**: ¹H NMR (1:1): δ 1.45 (3H, s, CH₃), 1.64 (3H, s, CH₃), 1.51-1.70 (3H, m), 1.90-2.48 (7H, m), 3.51-3.53 (3H, m, OCH₃), 5.29 (1H, br s), 5.42-5.43 (2H, br s), 7.41-7.50 (5H, m); ¹⁰F NMR: δ-71.55.

For the Mosher's ester of **136**a: ¹H NMR: **8** 0.61 (3H, s, CH₃), 0.92 (3H, s, CH₃), 1.19 (3H, s, CH₃), 1.24-1.28 (2H, m), 1.62 (3H, s, CH₃), 1.75-1.93 (6H, m), 2.22 (1H, m), 2.67 (1H, s, OH), 3.52 (3H, s, OCH₃), 5.15 (1H, s), 5.22 (1H, d, *J* = 5.4 Hz), 7.41-7.56 (5H, m).

For the Mosher's ester of **136**b: ¹H NMR: δ 0.96 (6H, s), 1.10 (3H, s, CH₃), 1.55 (2H, s), 1.61 (3H, s, CH₃), 1.62-1.99 (5H, m), 2.40 (1H, m), 3.56 (3H, d, J = 1.2 Hz, OCH₃), 5.19 (1H, br s), 5.47 (1H, dd, J = 2.1, 7.8 Hz), 7.41-7.56 (5H, m); ¹⁹F NMR: δ -71.76.

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APPENDIX

The selected ¹H NMR spectra are arranged according to the order in which they appear in the text.






















































Mdo































