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SYNTHESIS OF ENANTIOMERICALLY PURE (10R,11S)-(+)-JUVENILE HORMONES I AND II†

KENJI MORI* and **MITSUHIKO FUJIWHARA**

Department of Agricultural Chemistry, The University of Tokyo,
Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

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Abstract -- Enantiomerically pure (+)-juvenile hormone I [methyl (2E,6E,10R,11S)-10,11-epoxy-3,11-dimethyl-7-ethyl-2,6-tridecadienoate; Cecropia JH = C_{18} JH = JH I] and (+)-juvenile hormone II [methyl (2E,6E,10R,11S)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate; C_{17} JH = JH II] were synthesized employing the enantioselective reduction of 2-ethyl-2-methyl-1,3-cyclohexanedione by a yeast (Pichia terricola KI 0117) as the key-step.

Since Röller's discovery of juvenile hormone I (JH I) in 1967,[1] four additional juvenile hormones were isolated and identified as shown in Fig. 1 (JH II,[2] JH III,[3] JH 0,[4] and 4-Me JH I[5]). Importance of the juvenile hormones in both basic and applied entomological research was well appreciated from the beginning, and an enormous amount of works were published in the past by chemists and entomologists.[6] Due to the limited amount of the juvenile hormones available from insects, their absolute configuration was difficult to be determined. Indeed still at present the absolute configuration of both JH 0 and 4-Me JH I remains obscure. In 1970 Meyer and Hanzmann were able to isolate 1.372 mg of a mixture of JH I and JH II (90.2:9.8 mol%) and showed it to be dextrorotatory, $[\alpha]_D \simeq +7°$ ($CHCl_3$).[7] They then proposed 10R,11S configuration to JH I and JH II by the application of Horeau's method of determination of absolute configuration.[8] Subsequently two independent syn-

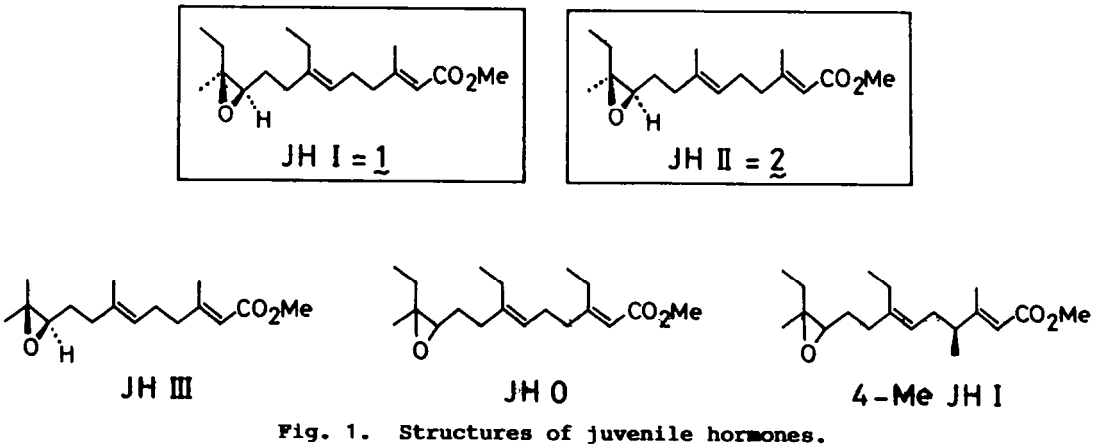

Fig. 1. Structures of juvenile hormones.

†Synthesis of compounds with juvenile hormone activity -- 26. Part 25, K. Mori and H. Mori, Tetrahedron, in the press. The experimental part of this work was taken from the forthcoming M. Sc. thesis of M. F. (March, 1988).

theses of the optically active form of JH I were achieved by Loew and Johnson[9] and by Faulkner and Petersen.[10] By using Johnson's sample of (+)-JH I, Nakanishi *et al.* proposed its 10R,11S configuration basing on physiochemical measurements.[11] The definite proof of the proposed 10R,11S configuration was provided by Faulkner's synthesis, because it started from (S)-(+)-2,2-dimethoxy-3-methyl-3-pentanol to furnish (10R,11S)-(+)-JH I.[10] Although these syntheses were decisive in settling the stereochemical argument on (+)-JH I, they did not give enantiomerically pure (+)-JH I. In Johnson's case their enantiomers of JH I were of 80-90% e.e.,[9] while in the case of Faulkner the products were neither diastereomerically nor enantiomerically pure.[10] In 1985 Prestwich and Wawrzeńczyk achieved the third synthesis of (+)-JH I by employing the Sharpless asymmetric epoxidation.[12] Their product was of high enantiomeric purity (~95% e.e.), but the Sharpless epoxidation, in our experience, never gave a product of 100% e.e. In the case of (+)-JH II, there exists only one synthesis of it, utilizing a microbial kinetic resolution process.[13] The enantiomeric purity of the product, however, was not determined.[13] Herein we describe the synthesis of both (+)-JH I and (+)-JH II in enantiomerically pure state.

Preparation of the chiral building block (2S,3S)-5a.

Fig. 2 shows our synthetic plan, which is an extension of the strategy employed in our recent synthesis of (R)-(+)-JH III.[14] If it is possible to prepare pure hydroxy ketone **A**, then Baeyer-Villiger oxidation of **A** after protecting the OH group will give lactone **B**. Conversion of **B** to acetonide ester **C** will be followed by its modification to alkyne **D**. The alkyne **D** will give diene esters **E** (R=Et and R=Me) by the sequence developed for the synthesis of (+)-JH III.[14] Finally the diene esters **E** will give (+)-JH I and (+)-JH II, respectively, by the standard procedure.[14]

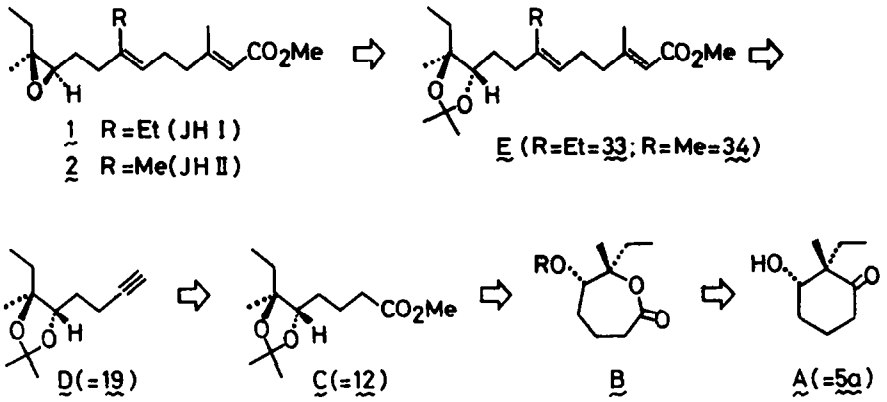

Fig. 2. Synthetic plan for (+)-JH I and (+)-JH II.

The first problem to be solved was therefore to find an efficient preparative method for enantiomerically pure **A**. In Fig. 3 is summarized our preparation of the chiral building block **A** [=(2S,3S)-**5a**]. Ethylation of 2-methylcyclohexane-1,3-dione (**3**) with EtI in the presence of Triton B in MeOH gave a mixture of the desired **4a** and ethyl enol ether of **3**. After hydrolyzing the enol ether with dil HCl, the product **4a** (15% yield) was separated from the recovered **3** (76% recovery). By repeating this process, **4a** was prepared in 63% yield from **3**.[cf.15] Our task was then to find a method to realize an efficient conversion of **4a** to **5a** (=**A**). Reduction of **4b** with baker's yeast was known to give (S)-3-hydroxy-2,2-dimethylcyclohexanone (98-99% e.e.) in good yield.[16] In the case of **4c**, however, its reduction with baker's yeast gave a diastereomeric mixture of **7A** and **7B**.[17] It was therefore difficult to predict the steric course of the biochemical reduction of **4a** beforehand, and we began the screening experiments to find an appropriate strain of microorganism. The final result is summarized in Table 1, which deserves further explana-

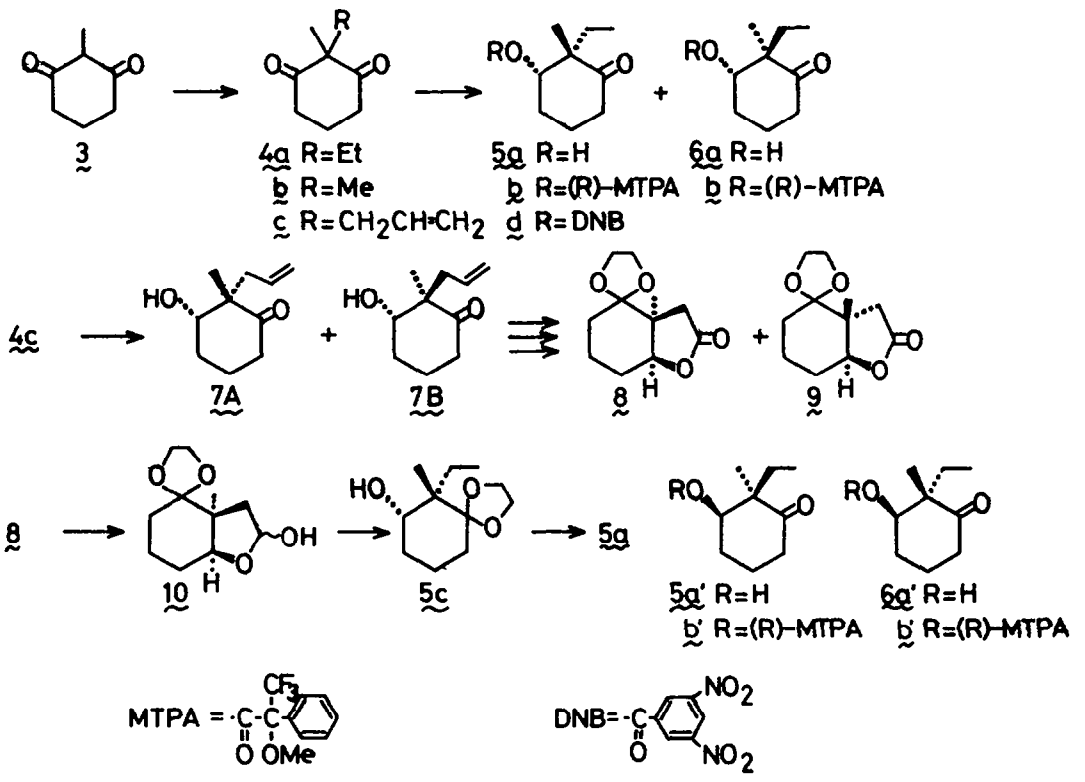

Fig. 3. Preparation of the chiral building block (2S,3S)-5a.

Table 1. Reduction of the prochiral ketone **4a** with yeast to give (2S,3S)-**5a**.

Yeast strain	Ratio of the generated hydroxy ketones 5a : 6a	Enantiomeric purity at C-3 (% e.e.)
Saccharomyces cerevisiae (baker's yeast)	1 : 2.7	99
Saccharomyces bailii KI 0116	1 : 1.5	73
Pichia terricola KI 0117	>99 : <1	99

tion as detailed below.

Our first choice was to employ baker's yeast, Saccharomyces cerevisiae. The reduction yielded a mixture of two ketols in a ratio of 1:2.7 as judged by the ^{1}H NMR analysis where a pair of Me singlets was observed. In analogy with Brooks's result,[17] these two ketols were assumed to be **5a** and **6a**. Because **5a** was inseparable from **6a** by conventional TLC, the mixture was converted to the corresponding mixture of ethylene acetals **5c** and **6c** by treatment with ethylene glycol and p-TsOH in benzene under reflux. The acetals **5c** and **6c** were separable by SiO_2 chromatography. The two acetals **5c** and **6c** gave back the parent

ketones when treated with p-TsOH in acetone. The ^{1}H NMR spectra of the two ketones were readily distinguishable. It was difficult, however, to tell which was which (**5a** or **6a**). Unambiguous derivation of **5a** from a compound of known absolute configuration must therefore be the only way to assign absolute configuration to the reduction products generated by baker's yeast. The hydroxy ketone **7A** was used for this purpose, because of its known absolute configuration.[17] A mixture of **7A** and **7B** was submitted to a multi-step conversion to give a mixture of lactones **8** and **9**.[18] These two lactones were separated by chromatography, and their stereochemistries were unambiguously assigned on the basis of the magnitude of the J-value of the signal due to the angular -CHOCO- proton.[18†] Reduction of **8** with $(i\text{-Bu})_2$AlH gave lactol **10**, which was reduced with N_2H_4 and KOH under the Huang Minlon condition to give **5c**. Treatment of **5c** with p-TsOH in acetone yielded **5a**, whose ^{1}H NMR spectrum as well as the sign of its specific rotation was identical to that of the ketone derived from the less polar ethylene acetal. That ketone **5a** turned out to be the minor product of the yeast reduction. Thus the less polar ethylene acetal was **5c**, while the more polar one was **6c**. It was now clear that the reduction with baker's yeast gave the desired **5a** only as the minor product.

Although both of the above mentioned samples of **5a** were dextrorotatory, the magnitude of their $[\alpha]_D$ values was different. Namely, the hydroxy ketone **5a** derived from **8** exhibited $[\alpha]_D^{23}$ +77.1° ($CHCl_3$), while the $[\alpha]_D$ value of **5a** derived from **5c** of yeast-reduction origin was only +32.7°. The both samples of **5a** were converted to the corresponding α-methoxy-α-trifluoromethylphenylacetates (MTPA esters),[19] and analyzed by HPLC. The MTPA ester **5b** of **5a** originating from **8** was shown to be of 98.3% diastereomeric purity. However, the MTPA ester **5b** of **5a** derived from **5c** (of yeast-reduction origin) was a mixture of two diastereomers (**5b** and **5b'**) in a ratio of 7:3, indicating the enantiomeric purity of this sample of **5a** to be 40% e.e. In the course of acetalization of **5a** to **5c** employing p-TsOH and ethylene glycol in refluxing benzene, partial racemization apparently took place at C-3 due to a retroaldol-aldol process. After directly acylating the hydroxy ketone mixture (**5a** and **6a**) produced by the reduction of **4a** with baker's yeast, the resulting mixture of MTPA esters **5b**, **5b'**, **6b** and **6b'** was analyzed by HPLC. Both **5a** and **6a** were found to be of 99% e.e. in accord with the similar study published by Brooks *et al.*[17] The rather tedious and troublesome work as described above taught us the method how to estimate the enantio- and diastereoselectivity of the biochemical reduction of **4a**.

As the result with baker's yeast was not so promising, we tried other yeasts. *Saccharomyces bailii* KI 0116 was known to reduce ethyl acetoacetate to ethyl (S)-3-hydroxybutanoate of 96% e.e.[20] When this yeast was employed for the reduction of **4a**, neither the diastereoselectivity nor the enantioselectivity could be improved. Thus the ratio **5a** to **6a** in this reduction was 1:1.5 with the enantiomeric purity of 73% e.e. at C-3.

The next choice was *Pichia terricola* KI 0117.[21] Fortunately, this yeast achieved the reduction with satisfactory selectivity. Reduction of the diketone **4a** with *P. terricola* KI 0117 gave in 86% yield only the desired hydroxy ketone **5a** of 99.5% e.e. To enhance the enantiomeric purity of **5a** to 100% e.e., it was converted to the corresponding 3,5-dinitrobenzoate **5d**. Highly crystalline nature of **5d** allowed us to purify it by recrystallization giving pure **5d**. Regeneration of **5a** from **5d** by hydrolysis was apt to accompany partial racemization at C-3 by the retroaldol-aldol mechanism. It was therefore impossible to check directly the enantiomeric purity of **5d** at this stage. The enantiomeric purity of our intermediate was checked in a later stage on **14** in Fig. 4. The most important problem of selective reduction of **4a** to **5a** was thus solved by the help of the yeast, *P. terricola* KI 0117.

† In the case of **8**, a 1H signal was observed at δ= 4.35 with $W_{H/2}$= 8 Hz (eq H), while in the case of **9**, a 1H signal was observable at δ= 4.03 with $W_{H/2}$= 18 Hz (ax H).

Synthesis of the common intermediate 21 leading to both JH I and II.

The next phase of this work was to convert pure **5d** to the common intermediate **21**, which would lead to both JH I and JH II. Baeyer-Villiger oxidation of **5d** with MCPBA in CH_2Cl_2 gave crystalline lactone **11** quantitatively. Treatment of **11** with K_2CO_3 in MeOH to effect methanolysis of **11** to the corresponding dihydroxy ester was followed by acetonide formation with $Me_2C(OMe)_2$ and p-TsOH to furnish acetonide ester **12** in 90% yield from **5d**. To check the diastereomeric purity of **12**, it was analyzed by capillary GLC to show a single peak. In its ^1H NMR spectrum, the 3H singlet due to C-6 Me appeared at δ 1.06.[cf.22] To be sure about the satisfactory diastereomeric purity of **12**, a reference sample was prepared as follows, which contained both (±)-**12** and its diastereomer (±)-**13**. The crude **5a** obtained by the reduction with *P. terricola* KI 0117 was treated with aq KOH-MeOH to effect retroaldol-aldol reaction. The resulting mixture of (±)-**5a** and (±)-**6a** was acetylated and the mixture of acetoxy ketones was converted to a mixture of (±)-**12** and (±)-**13** as described above for the conversion of **5d** to **12**. These two diastereoisomers [(±)-**12** and (±)-**13**] were inseparable by capillary GLC. Thus the GLC analysis of **12** could not be the proof of its homogeneity. However, in the ^1H NMR spectrum of the mixture of (±)-**12** and (±)-**13**, two signals due to C-6 Me were observable, one at δ 1.06 and the other at δ 1.18.[cf.22] The optically active **12** was therefore thought to be diastereomerically pure within the limit of the experimental error of the ^1H NMR measurement.

The next step was the reduction of **12** with LAH to furnish **14** in 90% yield. The enantiomeric purity of **14** was rigorously estimated as follows. Deprotection of the acetonide group of **14** with hot aq AcOH yielded the parent triol, which was acylated with (*R*)- or (*S*)-MTPA-Cl. The resulting bis-(*R*)- and bis-(*S*)-MTPA esters **15** were clearly distinguishable by HPLC analysis. Each of them showed a single peak upon HPLC, confirming the ~100% enantiomeric purity of **14**.

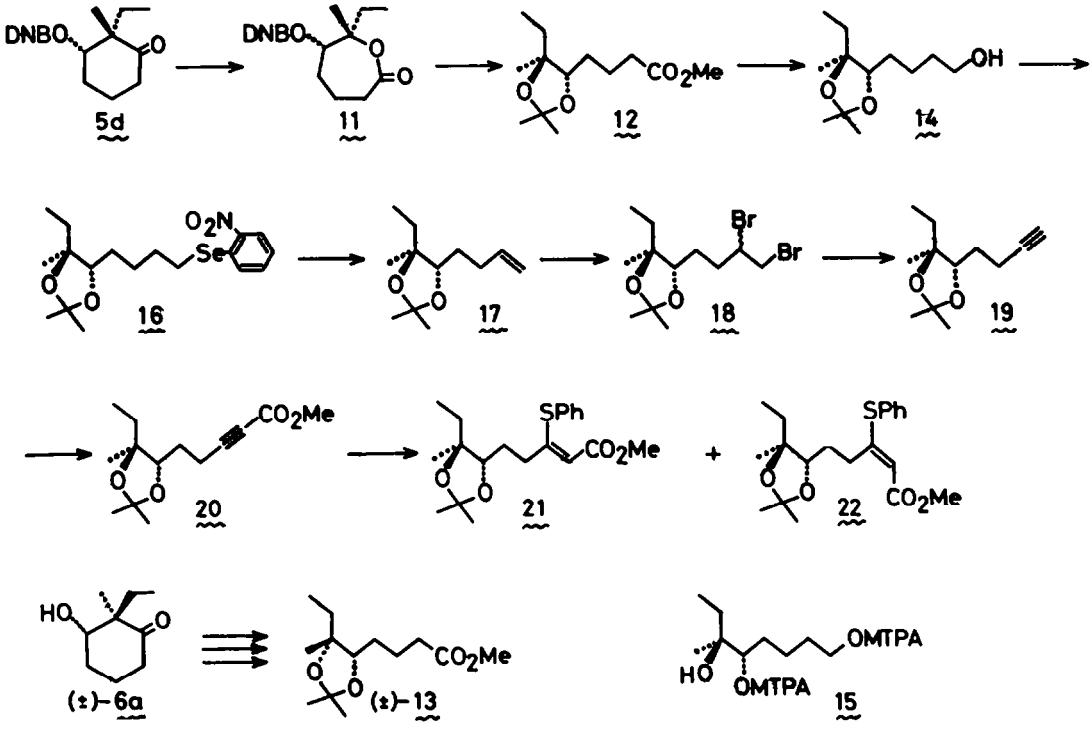

Fig. 4. Synthesis of the common intermediate 21.

The remaining steps from **14** to **21** were almost same as those employed in our synthesis of (+)-JH III.[14] Treatment of **14** with o-$(O_2N)C_6H_4SeCN$ and $(n\text{-}Bu)_3P$ in THF[23] gave selenide **16**, which was immediately oxidized with H_2O_2 to give olefin **17** in 79% yield from **14**. Bromination of **17** with Br_2 in CCl_4 furnished dibromide **18**, which was dehydrobrominated with $NaNH_2$ in liq NH_3 to alkyne **19** in 50% yield from **17**. According to Mukaiyama et al.,[24] **19** was methoxycarbonylated with n-BuLi and $ClCO_2Me$ in THF to give **20** in 86% yield. Treatment of **20** with PhSNa in MeOH afforded, after chromatographic separation, the desired common intermediate **21** in 76% yield together with the unwanted **22** (11% yield).

Synthesis of juvenile hormones I and II.

The third stage of this work was the preparation of (+)-JH I and (+)-JH II from the common intermediate **21**. Substitution of the PhS group of **21** with Et would lead to JH I **1**, and that with Me would yield JH II **2**. Accordingly, treatment of **21** with EtMgBr or MeMgBr in the presence of CuI in THF at -65°C[24] gave **23** (80% yield) or **24** (81% yield). These esters **23** and **24** were separately reduced with DIBAH to give alcohols **25** (quantitative) and **26** (90% yield), respectively. These were converted quantitatively to the corresponding bromides **27** and **28** by the method of Stork et al.[25] Alkylation of the dianion[26] of $MeCOCH_2CO_2Me$ with **27** and **28** gave **29** and **30**, respectively. Their corresponding enol phosphates **31** and **32** were treated with Me_2CuLi to give **33** and **34**.[14, 26]

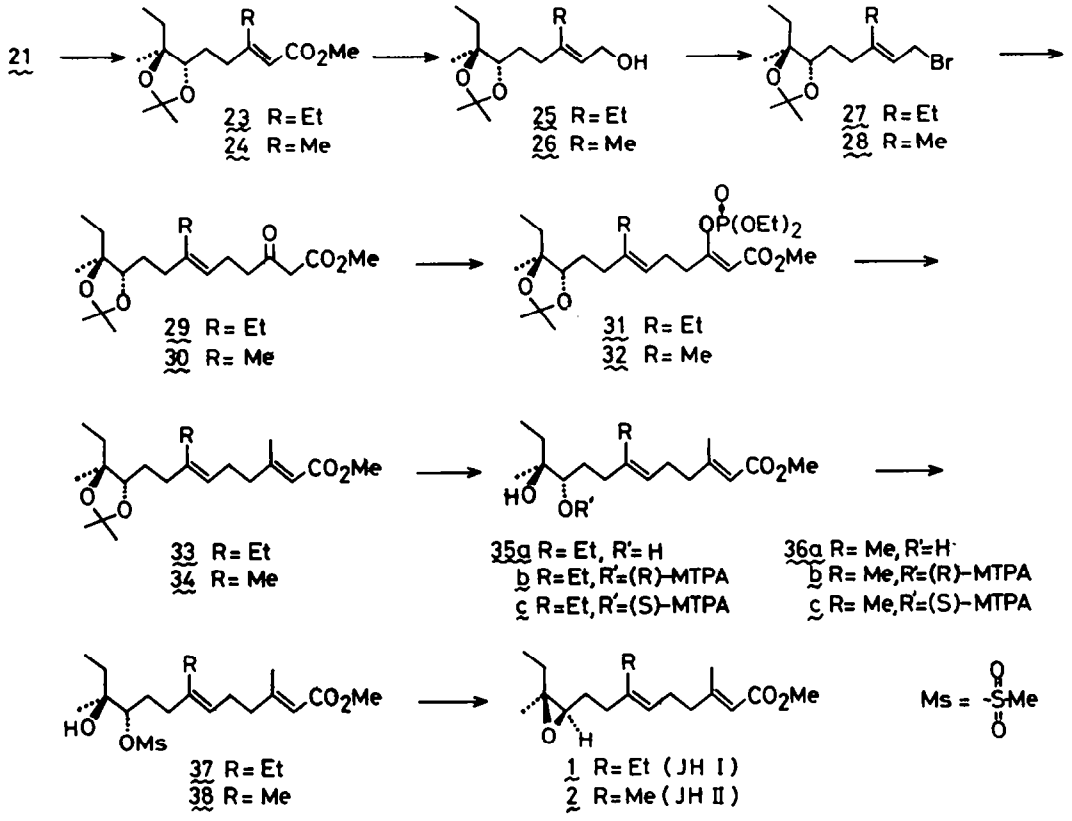

Fig. 5. Synthesis of JH I and JH II.

The final task of the epoxy-ring formation was executed as reported by us in the synthesis of (+)-JH III[14] so as to avoid partial racemization at C-10. Hydrolytic removal of the acetonide protective group of **33** and **34** by treatment with hot aq AcOH gave **35a** (99% yield) and **36a** (quantitative), respectively. The enantiomeric purity of both **35a** and **36a**

was estimated to be ~100% by the HPLC analysis of the corresponding MTPA esters, **35b**, **35c**, **36b** and **36c**. Mesylation of **35a** and **36a** with methanesulfonic anhydride and Et_3N[14] gave **37** and **38**, respectively. These were treated with NaOMe in MeOH to give (+)-JH I [**1**, 78% from **35a**; $[\alpha]_D^{23}$ +14.9° ($CHCl_3$); $[\alpha]_D^{23}$ +14.5° (MeOH); lit.[9] $[\alpha]_D^{20}$ +12.2° ($CHCl_3$)] and (+)-JH II [**2**, 66% yield from **36a**; $[\alpha]_D^{24.5}$ +17.6° (MeOH); lit.[13] $[\alpha]_D$ +11.7° (MeOH)], respectively.

As we rigorously checked the 100% enantiomeric purity of the dihydroxy esters **35a** and **36a**, the enantiomeric purity of our **1** and **2** must be 100%. Any racemization at C-10 would generate the diastereomers of JH I and JH II. To prove the high diastereomeric purity of our (+)-JH I and (+)-JH II, their ^1H NMR (400 MHz) and ^{13}C NMR (25 MHz) spectra were carefully examined. Fig. 6 shows the NMR spectra of our JH I and JH II. These are the first published 400 MHz ^1H NMR and ^{13}C NMR spectra of **1** and **2**. The spectra clearly supported their 100% diastereomeric purity.[27,28] Comparison of the 400 MHz ^1H NMR spectra of JH I and JH II as shown in Fig. 6 with the published ^1H NMR chart of JH I[29] and that of JH II[2a] is quite instructive to show the remarkable improvement in NMR techniques in these twenty years.

In summary, the pure naturally occurring enantiomers of JH I and JH II were synthesized in 19 steps from the chiral building block **5a** in 2.7% and 1.2% overall yield, respectively. Biochemical reduction of a prochiral diketone was again proved to be a useful method in preparing a chiral building block.

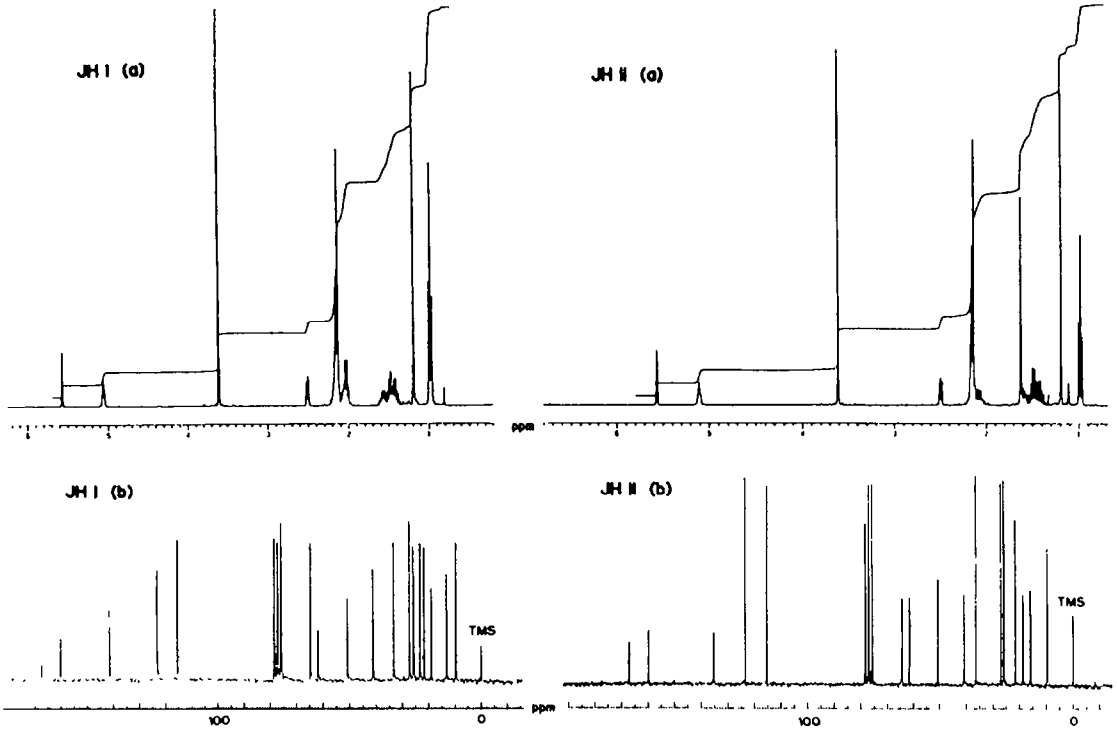

Fig. 6. NMR spectra of (+)-JH I and (+)-JH II.
(a) ^1H NMR Spectra (400 MHz, CCl_4).
(b) ^{13}C NMR Spectra (25 MHz, $CDCl_3$).

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra were measured as films for oils or as KBr discs for solids on a Jasco IRA-102 spectrometer. ^1H NMR spectra were recorded with TMS as an internal standard at 60 MHz on a Hitachi R-24A spectrometer or at 100 MHz on a JEOL JNM FX-100 spectrometer or at 400 MHz on a JEOL JNM GX-400 spectrometer. ^{13}C NMR spectra were measured with TMS as an internal standard as $CDCl_3$ soln at 25 MHz on a Jeol JNM FX-100 spectrometer. Optical rotations were measured on a Jasco DIP 140 polarimeter. Merck Kieselgel 60 Art 7734 (particle size 0.063-0.200 mm) was

used for column chromatography. HPLC analyses were performed on Nucleosil® 50-5 (25 cm × 4.6 mm) as a column by the detection at 254 nm. The water used for the cultivation of yeast was deionized.

2-Ethyl-2-methylcyclohexane-1,3-dione **4a**.[cf.15] To a soln of **3** (37.8 g, 300 mmol) in MeOH (390 ml) was added a soln of Triton B (126 ml, 40% soln in MeOH) followed by EtI (51 g, 327 mmol) dropwise at room temp. The mixture was stirred and heated under reflux for 5 h. It was then cooled to room temp and MeOH was almost removed by evaporation. The residue was poured into N HCl soln and stirred for 10 min at room temp. Then the mixture was filtered and the residual solid on the filter was washed with EtOAc and dried under reduced pressure to give 28.6 g (75.7% recovery) of **3**. The combined filtrate and washings were diluted with EtOAc and washed with 5% $Na_2S_2O_3$ soln, 20% K_2CO_3 soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was chromatographed over SiO_2 (100 g). Elution with n-hexane-EtOAc (20:1) gave 7.1 g (0.046 mmol, 15% yield, 63% yield based on the consumed **3**) of **4a**, νmax 3440 (w), 1730 and 1700 (s) cm^{-1}; δ (CCl_4) 0.73 (3H, t, J=7 Hz), 1.10 (3H, s), 1.40-2.20 (4H, m), 2.38-2.75 (4H, m).

Cultivation of Saccharomyces bailii KI 0116 or Pichia terricola KI 0117.[20] Two loops of S. bailii KI 0116 or P. terricola KI 0117 were inoculated into a 100 ml of medium containing malt extract (20 g), peptone (1 g), glucose (20 g) in water (1000 ml) placed in a 500 ml-shaking flask. This was cultivated at 37°C for 36 h on a reciprocal shaker (100 cpm). Two batches of the seed culture were added into a 1800 ml of medium with the same ingredient in a 5000 ml-Erlenmeyer cultivating flask with two internal projections for aeration. After cultivating at 37°C for 36 h on a gyrorotary shaker (100 rpm), the wet cells of yeast (ca. 10 g of S. bailii KI 0116 or ca. 22 g of P. terricola KI 0117) were harvested by centrifugation (3000 rpm) for 5 min.

Reduction of **4a** with baker's yeast. An emulsion of 2-ethyl-2-methylcyclohexane-1,3-dione **4a** (0.5 g) in Triton X-100 (0.2%, 10 ml) was treated with the dry cells of baker's yeast (Oriental Yeast Industries, 7 g) in sucrose soln (total 25g/100 ml water) at 30°C for 24 h. A usual workup and purification gave 0.26 g (1.7 mmol, 51%) of ketol; δ (100 MHz, $CDCl_3$) 1.12 and 1.15 (total 3H, each s, ca. 2.7:1). This was shown to be a ca. 1:2.7 mixture of two diastereomers.

Preparation of authentic samples of **5a** and **6a**. A mixture of **5a** and **6a** [obtained by the reduction with baker's yeast as above (0.70 g, 4.5 mmol)], ethylene glycol (1.5 g, 24 mmol) and p-TsOH·H_2O (8 mg) in benzene (15 ml) was stirred and heated under reflux for 15 h. After cooling, the mixture was poured into sat $NaHCO_3$ soln and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give 0.93 g (quantitative) of a diastereomeric mixture of **5c** and **6c**. This was chromatographed over SiO_2 (70 g). Elution with n-hexane-EtOAc (20:1) gave 0.30 g of the less polar isomer, TLC [SiO_2, Merck Art 5715; developed with n-hexane-EtOAc (3:1)] Rf 0.42; νmax (film) 3550 (s), (CCl_4 soln) 3580 (m) cm^{-1}: the IR data suggested the presence of the intramolecular hydrogen bonding. Further elution gave a diastereomeric mixture (0.15 g). Further elution gave 0.40 g of the more polar isomer; TLC (same condition) Rf 0.33; νmax (film) 3475 (br,m), (CCl_4 soln) 3580 (s) cm^{-1}: this IR data showed the lack of intramolecular hydrogen bonding. In the case of **6c**, internal hydrogen bonding causes an unfavorable 1,2-diaxial orientation of ethyl and hydroxyl group. Therefore the less polar isomer was tentatively assigned to be **5c**, while the more polar isomer was to be **6c**.

5c and **6c** were separately deprotected to give **5a** and **6a**, respectively. **5a**, $[\alpha]_D^{25}$ +32.7° (c=1.00, $CHCl_3$); δ (100 MHz, $CDCl_3$) 0.89 (3H, t, J=8 Hz), 1.15 (3H, s), 3.72 (1H, dd, J=6, 9 Hz). **6a**, $[\alpha]_D^{23.5}$ -25.0° (c=1.00, $CHCl_3$); δ (100 MHz, $CDCl_3$) 0.87 (3H, t, J=8 Hz), 1.12 (3H, s), 3.92 (1H, m). The product obtained by the reduction with baker's yeast was therefore supposed to be a 1:2.7 mixture of **5a** and **6a**.

Determination of the optical purity. A mixture of **5a**, **5a'**, **6a** and **6a'** was prepared as follows; to a soln of **5a** (25 mg, 0.16 mmol) in MeOH (5 ml) was added 2 N KOH (0.1 ml) and the mixture was stirred for 2 h at room temp. Then the mixture was poured into water and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give 20 mg (80%) of a mixture of **5a**, **5a'**, **6a** and **6a'**. This mixture was converted to the corresponding (R)-MTPA esters. HPLC analysis [eluent: n-hexane-THF (30:1), 1.1 ml/min] showed three peaks; Rt 24.0 min (47.6%), 26.8 min (28.6%), 28.0 min (23.8%).

Authentic **5a** and **6a** were converted to the corresponding MTPA esters, respectively. HPLC analysis of MTPA ester of **5a**: Rt 24.0 min (30.0%), 26.8 min (70.0%). MTPA ester of **6a**: Rt 24.0 min (25.6%), 28.0 min (74.4%). Therefore the optical purity of authentic **5a** and **6a** was determined to be 40.0% e.e and 48.8% e.e, respcetively.

In the same manner, reduction product with baker's yeast was converted to the corresponding MTPA esters. HPLC analysis: Rt 24.0 min (0.5%), 26.8 min (26.6%), 28.0 min (72.9%). The optical purity was determained to be 99.0 % e.e. The discrepancy of the optical purities as above was supposed to be due to the partial racemization or epimerization during acetalization.

Reduction of **4a** with S. bailii KI 0116 and P. terricola KI 0117.

S. bailii KI 0116: Diketone **4a** (0.5 g) was reduced with the wet cells of S. bailii KI 0116 (10 g) in glucose soln (total 15 g/100 ml water) at 37°C for 24 h to give 0.2 g of ketol; δ (100 MHz, $CDCl_3$) 1.12 and 1.15 (total 3H, each s, ca. 1.5:1). This was shown to be a ca. 1:1.5 mixture of **5a** and **6a**.

HPLC: (same condition); Rt 24.0 min (13.5%), 26.8 min (35.0%), 28.0 min (51.5%).

Pichia terricola KI 0117: Diketone **4a** (0.5 g) was reduced with the wet cells of P. terricola KI 0117 (22 g) in glucose soln (total 15 g/100 ml water) at 37°C for 24 h to give 0.3 g of ketol; δ (100 MHz, $CDCl_3$) 1.15 (3H, s). Therefore reduction with P. terricola gave only one diasteroemer.

HPLC: (same condition); Rt 24.0 min (0.3%), 26.8 min (99.5%), 28.0 min (0.2%). Therefore the ketol **5a** obtained by P. terricola KI 0117 reduction was almost both enantiomerically (99.4% e.e.) and diastereomerically (99.6% d.e.) pure.

Determination of the absolute configuration of **5a**.

1) (2RS,3bS,7bS)-4,4-Ethylenedioxy-2-hydroxy-3b-methyloctahydrobenzofuran **10**. According to Brooks et al.,[17] (2RS,3S)-2-allyl-3-hydroxy-2-methylcyclohexanone (a mixture of **7A** and **7B**) was prepared by baker's yeast reduction of 2-allyl-2-methylcyclohexane-1,3-dione **4c**. From this ketol, lactones (3bS,7bS)-4,4-ethylenedioxy-3b-methyl-2-oxo-octahydrobenzofuran **8** and (3bR,7bS)-isomer **9** were obtained via several steps. **8**: m.p. 90-91°C; $[\alpha]_D^{21}$ -27.9° (c=1.97, $CHCl_3$); νmax 1785 (s) cm^{-1}; δ (60 MHz, $CDCl_3$) 1.25 (3H, s), 4.35 (1H, deformed t, $W_{H/2}$=8 Hz); TLC [SiO_2, developed with n-hexane-EtOAc (1:1)] Rf 0.3. **9** (oil): $[\alpha]_D^{21}$ -27.5° (c=2.03, $CHCl_3$); νmax 1790 (s) cm^{-1}; δ (60 MHz, $CDCl_3$) 1.05 (3H, s), 4.03 (1H, dd, $W_{H/2}$=18 Hz); TLC (same condition) Rf 0.4. Comparison of NMR spectra revealed that **8** had cis-configuration as well as **9** had trans-configuration, respectively. To a soln of **8** (540 mg, 2.55 mmol) in dry toluene (20 ml) was added a soln of DIBAL-H (3.5 ml, 1 mol/l

in toluene, 3.5 mmol) at -50°C under Ar and the mixture was stirred for 30 min at -60°C. Then the reaction was quenched by adding sat potassium sodium tartrate tetrahydrate soln (5 ml) and the mixture was stirred for 30 min at room temp. Then the mixture was poured into brine and extracted with ether. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give 589 mg (quantitative) of **10**, νmax 3450 (s), 1740 (m) cm^{-1}. This was employed in the next step without further purification.

2) (2*S*,3*S*)-2-Ethyl-3-hydroxy-2-methylcyclohexanone ethylene acetal **5c**. To a soln of **10** (589 mg) in diethylene glycol (50 ml) was added $N_2H_4 \cdot H_2O$ (460 mg, 9.2 mmol) and KOH (462 mg, 85%, 7.01 mmol), and the mixture was stirred and heated at 120°C for 30 min. Then the temp was raised to 200°C and the stirring was continued for 1 h. After the mixture was cooled to room temp, this was poured into water and extracted with ether. The ether soln was washed with sat NH_4Cl soln and brine, dried ($MgSO_4$) and concentrated in vacuo to give 300 mg (58.8% from **8**) of **5c**. Its NMR and IR spectra were identical to those of the less polar isomer obtained by the acetalization of **5a** and **6a**. This was employed in the next step without further purification.

3) Deprotection of ethylene acetal. *p*-TsOH·H_2O (5 mg) was added to a soln of **5c** (45 mg, 0.23 mmol) in acetone (20 ml) at room temp and the mixture was stirred for 2 h at room temp. Then this was poured into sat $NaHCO_3$ soln and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give 32 mg (91%) of **5a**, $[\alpha]_D^{23}$ +77.1° (c=0.626, $CHCl_3$); νmax 3450 (s), 1695 (s), 1060 (s), 990 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.89 (3H, t, J=8 Hz), 1.15 (3H, s), 1.35-2.20 (7H, m), 2.37 (2H, deformed t, J=7 Hz), 3.72 (1H, dd, J=6, 9 Hz). The optical purity was determined as 98.3% e.e. in the same manner as above. The sign of optical rotation and ^1H NMR spectrum of this **5a** was identical with that of **5a** obtained by the reduction with *P. terricola*. Therefore the absolute configuration of **5a** from *P. terricola* was unambiguously determined to be (2*S*,3*S*).

Reduction with *P. terricola* KI 0117 in a preparative scale. **4a** (8.2 g, 53 mmol) in Triton X-100 (0.2 %, 80 ml) was reduced with the wet cells of *P. terricola* KI 0117 (87 g) in glucose soln (total 175 g/1000 ml) at 37°C for 36 h to give 7.1 g (46 mmol, 86%) of ketol; b.p. 109-114°C/5 Torr; n_D^{24} 1.4743; $[\alpha]_D^{24}$ +65.6° (c=1.07, $CHCl_3$); νmax 3450 (s), 1695 (s), 1060 (s), 990 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.89 (3H, t, J=8 Hz), 1.15 (3H, s), 1.35-2.20 (7H, m), 2.37 (2H, deformed t, J=7 Hz), 3.72 (1H, dd, J=6, 9 Hz); MS *m/z* 156 (M^+).

(2*S*,3*S*)-2-Ethyl-2-methyl-3-(3',5'-dinitro)benzoyloxycyclohexanone **5d**. 3,5-Dinitrobenzoyl chloride (65 g, 0.28 mol) was added to a stirred and cooled soln of **5a** (40 g, 0.26 mool) in pyridine (60 ml) and CH_2Cl_2 (120 ml). After the mixture was stirred for 3 h at 0°C, the reaction was quenched by adding ice and the stirring was continued for 10 min. Then the mixture was poured into dilute HCl and extracted with CH_2Cl_2. The extract was washed with $CuSO_4$ soln, water, sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo to give 120 g of crude **5d**. This was recrystallized from n-hexane-EtOAc (1:1) to give 65 g (72%) of pure **5d** as pale yellow needles, m.p. 150.5-151.4°C; $[\alpha]_D^{24}$ +72.3° (c=1.01, $CHCl_3$); νmax 1725 (s), 1710 (s), 1630 (w), 1550 (s), 1345 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.84 (3H, t, J=8.2 Hz), 1.20 (3H, s), 1.60-2.62 (8H, m), 5.26 (1H, dd, J=5.0, 7.0 Hz), 9.13 (2H, d, J=2.1 Hz), 9.25 (1H, dd, J=2.1, 2.2 Hz). (Found: C, 54.94; H, 5.39; N, 8.14. Calc for $C_{16}H_{18}O_7N_2$: C, 54.85; H, 5.18; N, 8.00%).

A small portion of it was carefully saponified (0°C, 5 min) to give almost pure **5a** (>99% e.e.), $[\alpha]_D^{24}$ +79.2° (c=1.00, $CHCl_3$). This value was in good accord with that (+77.1°) of **5a** obtained from the lactone **8**. Therefore **5a** freshly obtained by the action of *P. terricola* which showed smaller $[\alpha]_D$ value ($[\alpha]_D$ +65.6°) must have been contaminated with a small amount of impurities of microbial origin.

(5*S*,6*S*)-6-Methyl-5-(3',5'-dinitro)benzoyloxy-6-ocatnolide **11**. To a stirred suspension of MCPBA (80%, 2.6 g, 12 mmol) and $NaHCO_3$ (1.0 g, 12 mmol) in dry CH_2Cl_2 (100 ml) was added dropwise a soln of **5d** (3.5 g, 10 mmol) in dry CH_2Cl_2 (50 ml) and the mixture was stirred for 72 h at room temp. Then the mixture was filtered through a pad of celite and filter-cake was washed with CH_2Cl_2. The combined filtrate and washings were washed with 10% $NaHSO_3$ soln, sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo to give 4.0 g (quantitative) of crystalline crude **11**. This was employed in the next step without further purification. A small portion was recrystallized from n-hexane-EtOAc (2:1) to give an analytical sample as pale yellow needles, m.p. 160.0-161.0°C; $[\alpha]_D^{24}$ +8.35° (c=1.05, $CHCl_3$); νmax 1720 (s), 1635 (w), 1555 (s), 1350 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 1.01 (3H, t, J=8.0 Hz), 1.56 (3H, s), 1.60-2.33 (6H, m), 2.70-2.92 (2H, m), 5.42 (1H, t, J=5.0 Hz), 9.12 (2H, d, J=2.1 Hz), 9.25 (1H, dd, J=2.1, 2.2 Hz). (Found: C, 52.64; H, 4.88; N, 7.74. Calc for $C_{16}H_{18}O_8N_2$: C, 52.45; H, 4.95; N, 7.65%).

Methyl (5*S*,6*S*)-5,6-isopropylidenedioxy-6-methyloctanoate **12**. K_2CO_3 (40 mg, 0.29 mmol) was added to a stirred soln of crude **11** (4.0 g, 10 mmol) in MeOH (10 ml) and CH_2Cl_2 (30 ml) and the mixture was stirred for 30 min at room temp. To this was added *p*-TsOH until the color of the soln changed from red to yellow. Then most of the solvent was removed by evaporation, the residue was diluted with acetone and filtered through a pad of celite. The filter-cake was washed with acetone and the combined filtrate and washings were concentrated in vacuo until the volume was ca. 20 ml. To this was added 2,2-dimethoxypropane (5 ml, 41 mmol) and the mixture was stirred for 3 h at room temp. Then a half of the solvent was removed by evaporation and the residual soln was poured into sat $NaHCO_3$ soln and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give a mixture of **12** and crystalline methyl 3,5-dinitrobenzoate. The following operation was repeated three times to remove crystalline methyl 3,5-dinitrobenzoate; dilution of the mixture with n-hexane, filtration, concentration of the filtrate. The oily residue was distilled to give 2.2 g (90% from **5d**) of **12**, b.p. 83°C/0.55 Torr; n_D^{24} 1.4333; $[\alpha]_D^{24}$ -13.9° (c=1.07, MeOH); νmax 1740 (s), 1260 (s), 1220 (s), 1180 (br.s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.95 (3H, t, J=7.6 Hz), 1.06 (3H, s), 1.33 (3H, s), 1.43 (3H, s), 1.25-2.05 (6H, m), 2.38 (2H, deformed t, J=7.0), 3.68 (3H,s), 3.70-3.82 (1H, m); GLC (PEG 0.2 mm x 50 m, 1.2 kg/cm^2, 170°C) Rt 13.6 min. (Found: C, 63.68; H, 9.78. Calc for $C_{13}H_{24}O_4$: C, 63.90; H, 9.90%).

A mixture of (±)-**12** and its diastereomer (±)-**13** was prepared from a mixture of **5a**, **5a'**, **6a** and **6a'**. 100 MHz ^1H NMR spectra of this mixture showed signals at 1.06 ppm and 1.18 ppm due to C-6 Me singlet.[22] The ester **12** obtained above showed only 1.06 ppm singlet, therefore **12** was revealed to be diastereomerically pure.

(5*S*,6*S*)-5,6-Isopropylidenedioxy-6-methyl-1-octanol **14**. To a stirred and cooled suspension of LAH (0.76 g, 20 mmol) in dry ether (10 ml) was added dropwise a soln of **12** (3.9 g, 16 mmol) in dry ether (20 ml) at 0°C and the mixture was stirred for 2.5 h at room temp. It was again cooled to 0°C and the reaction was quenched by the addition of water (0.76 ml), 15% NaOH soln (0.76 ml) and water (2.28 ml) dropwise. Anhydrous Na_2SO_4 was added to the mixture and it was stirred for 12 h at room temp, then filtered through a pad of celite and the filter-cake was washed with THF. The combined filtrate and washings were concentrated in vacuo and the residual oil was distilled to give 3.1 g (90%) of **14**, b.p. 92-94°C/0.45 Torr; n_D^{24}

1.4441; $[\alpha]_D^{24}$ -7.55° (c=1.02, $CHCl_3$); νmax 3450 (br.s), 1250 (s), 1210 (s), 1190 (s), 1110 (s), 1060 (s), 1020 (br.s) cm^{-1}; δ (60 MHz, CCl_4) 0.90 (3H, t, J=7.2 Hz), 0.98 (3H, s), 1.22 (3H, s), 1.31 (3H, s), 1.20-2.30 (9H, m), 3.30-3.72 (3H, m). (Found: C, 66.35; H, 11.17. Calc for $C_{12}H_{24}O_3$: C, 66.63; H, 11.18%).

A small portion of this was treated with aq AcOH (75 v/v%) at 50°C for 2 h to remove acetonide protective group to give a triol, which was converted to the corresponding bis (R)- and (S)-MTPA esters **15**. HPLC analysis; eluent: n-hexane-THF (5:1), 1.0 ml/min; Rt 11.3 min [(S)-MTPA ester], 14.2 min [(R)-MTPA ester]; bis (R)-MTPA ester of **15**, Rt 14.2 min (100%). Therefore **14** was revealed to be enantiomerically pure.

(3S,4S)-3,4-Isopropylidenedioxy-3-methyl-7-octene **17**. To a stirred and cooled soln of **14** (2.5 g, 12 mmol) and o-nitrophenyl selenocyanate (3.2 g, 14 mmol) in dry THF (30 ml) was added dropwise $(n\text{-Bu})_3P$ (3.5 ml, 2.8 g, 14 mmol) under Ar at 0°C. The mixture was stirred for 5 h at room temp and the solvent was removed by evaporation. The residue was chromatographed over SiO_2 (80 g). Elution with C_6H_6-ether (10:1) gave 5.4 g of crude **16**. This was dissolved in dry THF (25 ml) and to this was added dropwise 35% H_2O_2 (8.7 ml) at 0-5°C with stirring. After stirring for 2.5 h at room temp, the mixture was poured into sat $NaHCO_3$ soln and extracted with ether. The ether soln was washed with 5% $Na_2S_2O_3$ soln, 10% Na_2CO_3 soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was filtered through a column of neutral Al_2O_3 (grade IV). The fraction eluted with n-pentane was concentrated in vacuo followed by distillation to give 1.8 g (79%) of **17**, b.p. 130-150°C(bath)/15 Torr; n_D^{24} 1.4286; $[\alpha]_D^{26}$ -1.25° (c=1.02, $CHCl_3$); νmax 1640 (w), 1250 (s), 1210 (s), 1190 (s), 920 (s), 870 (s) cm^{-1}; δ (60 MHz, CCl_4) 0.90 (3H, t, J=6.3 Hz), 0.96 (3H, s), 1.20 (3H, s), 1.30 (3H, s), 1.15-2.40 (6H, m), 3.55 (1H, dd, J=4.1, 8.7 Hz), 4.70-5.12 (2H, m), 5.75 (1H, ddt, J=10.3, 18.0, 6.0 Hz). (Found: C, 72.49; H, 10.86. Calc for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18%).

(3S,4S)-3,4-Isopropylidenedioxy-3-methyl-7-octyne **19**. Br_2 (1.3 g, 8.1 mmol) was added to a stirred and cooled soln of **17** (1.6 g, 8.1 mmol) in CCl_4 (20 ml) and the mixture was stirred for 2 h at 0-10°C. Then it was poured into 5% $Na_2S_2O_3$ soln and extracted with ether. The ether soln was washed with 5% $Na_2S_2O_3$ soln, sat $NaHCO_3$ soln and brine, dried ($CaCl_2$) and concentrated in vacuo to give 2.8 g of dibromide **18**. This was dissolved in dry THF (20 ml) and this was added to a suspension of $NaNH_2$ (from 2.3 g of Na; 11 eq) in liq NH_3 (100 ml) dropwise at -60°C. After stirring for 3 h at -60--70°C, the temp was gradually raised to ca. -33°C with stirring, then the reaction was quenched by adding solid NH_4Cl (5.0 g, 93 mmol) followed by sat NH_4Cl soln and stirred overnight. The mixture was extracted with ether and the ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was distilled to give 0.80 g (50%) of **19**, b.p. 86-91°C/15 Torr; n_D^{24} 1.4396; $[\alpha]_D^{24.5}$ -33.5° (c=1.03, $CHCl_3$); νmax 3320 (m), 2120 (w), 1255 (s), 1215 (s), 1000 (s) cm^{-1}; δ (60 MHz, CCl_4) 0.92 (3H, t, J=6.4 Hz), 0.98 (3H, s), 1.22 (3H, s), 1.30 (3H, s), 1.10-2.50 (7H, m), 3.70 (1H, dd, J=4.0, 8.7 Hz). (Found: C, 73.07; H, 10.01. Calc for $C_{12}H_{20}O_3$: C, 73.43; H, 10.27%).

Methyl (6S,7S)-6,7-isopropylidenedioxy-7-methyl-2-nonynoate **20**. To a stirred and cooled soln of **19** (3.8 g, 19 mmol) in dry THF (60 ml) was added a soln of n-BuLi (1.6 N in n-hexane, 16 ml, 26 mmol) dropwise at 5°C and the mixture was stirred for 1 h at 0°C under Ar. Then the soln was cooled to -78°C and a soln of $ClCO_2Me$ (2.5 g, 26 mmol) in dry THF (6 ml) was added dropwise and the mixture was stirred for 1 h at -78°C. It was then stirred at room temp for additional 2.5 h. The reaction was quenched by adding sat NH_4Cl soln, and the mixture was extracted with ether. The ether soln was washed with sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was chromatographed over SiO_2 (70 g). Elution with n-hexane-ether (20:1) gave recovered **19** (0.45 g). Further elution with n-hexane-ether (20:1-10:1) gave 3.7 g (86% based on the consumed **19**) of **20**. A small portion of it was distilled to give an analytical sample, b.p. 115-118°C/5 Torr; n_D^{25} 1.4536; $[\alpha]_D^{26}$ -38.7° (c=0.995, $CHCl_3$); νmax 2250 (m), 1720 (s), 1255 (s) cm^{-1}; δ (60 MHz, CCl_4) 0.92 (3H, t, J=6.4 Hz), 0.99 (3H, s), 1.24 (3H, s), 1.30 (3H, s), 1.12-2.70 (6H, m), 3.65, (3H, s), 3.55-3.85 (1H, m). (Found: C, 65.62; H, 8.42. Calc for $C_{14}H_{22}O_4$: C, 66.11; H, 8.72%).

Methyl (2Z,6S,7S)-6,7-isopropylidenedioxy-7-methyl-3-phenylthio-2-nonenoate **21** and its (2E,6S,7S)-isomer **22**. NaOH (0.69 g, 17 mmol) was added to a stirred soln of C_6H_5SH (1.9 g, 17 mmol) in MeOH (30 ml) and the mixture was stirred for 30 min at room temp. To this was added dropwise a soln of **20** (3.7 g, 15 mmol) in MeOH (10 ml) and the stirring was continued for 4 h at room temp. A soln of AcOH (1 g, mmol) in water (5 ml) was added to quench the reaction. The reaction mixture was diluted with ether and it was washed with 4% NaOH soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil (5.0 g) was repeatedly chromatographed over SiO_2. Elution with n-hexane-ether (40:1) gave 0.58 g (11%) of **22**, n_D^{23} 1.5253; $[\alpha]_D^{23}$ +18.3° (c=1.10, MeOH); νmax 3100 (w), 1710 (s), 1600 (s), 1370 (m), 1340 (m), 1170 (br.s), 750 (m), 710 (s), 690 (m), 680 (m) cm^{-1}; δ (100 MHz, $CDCl_3$)0.98 (3H, t, J=7.8 Hz), 1.12 (3H, s), 1.34 (3H, s), 1.46 (3H, s), 1.14-2.20 (4H, m), 3.63 (3H, s), 3.88 (1H, dd, J=4.5, 8.4 Hz), 5.17 (1H, s), 7.30-7.53 (5H, m). (Found: C, 65.86; H, 7.83. Calc for $C_{20}H_{28}O_4S$: C, 65.90; H, 7.74 %). Further elution gave 4.0 g (76%) of **21**, $n_D^{24.5}$ 1.5264; $[\alpha]_D^{24.5}$ +9.25° (c=1.02, MeOH); νmax 3100 (w), 1720 (s), 1590 (s), 1380 (s), 1200 (br.s), 760 (m), 710 (m), 700 (m) cm^{-1}; δ (100 MHz, CDCl) 0.85 (3H, t, J=7.8 Hz), 0.92 (3H, s), 1.20 (3H, s), 1.33 (3H, s), 1.00-1.82 (4H, m), 2.00-2.64 (2H, m), 3.30 (1H, dd, J=4.5, 8.4 Hz), 3.65 (-OMe singlet of the trace of the desconjugated ester), 3.78 (3H, s), 5.94 (1H, s), 7.20,-7.65 (5H, m). (Found: C, 65.70; H, 7.59. Calc for $C_{20}H_{28}O_4S$: C, 65.90; H, 7.74%).

Methyl (2E,6S,7S)-3-ethyl-6,7-isopropylidenedioxy-7-methyl-2-nonenoate **23**. To a stirred and cooled suspension of CuI (2.1 g, 11 mmol) in dry THF (3 ml) was added a soln of EtMgBr (27 mmol in 20 ml THF) at -60--70°C and the stirring was continued for 10 min under Ar. To the mixture was added a soln of **21** (2.0 g, 5.5 mmol) in dry THF (30 ml). After stirring for 2 h at -78°C, the reaction was quenched by adding sat NH_4Cl soln. The stirring was continued for additional 1 h at room temp. Then the mixture was extracted with ether. The ether soln was washed with sat NH_4Cl soln and 5% NaOH soln. The precipitated inorganic material was removed by filtration. The ether layer was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was chromatographed over SiO_2 (40 g). Elution with n-hexane-ether (20:1) gave 1.3 g (83%) of **23**. A small portion of it was distilled to give an analytical sample, b.p. 68-70°C/0.25 Torr; n_D^{22} 1.4576; $[\alpha]_D^{22}$ -11.7° (c=0.890, MeOH); νmax 1720 (s), 1645 (m), 1380 (m), 1210 (s), 1150 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.97 (3H, t, J=7.1 Hz), 1.08 (3H, s), 1.11 (3H, t, J=7.1 Hz), 1.33 (3H, s), 1.43 (3H, s), 1.40-1.90 (4H, m), 2.00-2.84 (4H, m), 3.60-3.85 (1H, m), 3.70 (3H, s), 5.66 (1H, br.s); GLC (5% PEG-20M; 5 mm x 1.5 m; 150°C; 1.0kg/cm^2): Rt 6.1 min (99.7%). (Found: C, 67.51; H, 9.91. Calc for $C_{16}H_{28}O_4$: C, 67.57; H, 9.93%).

Methyl (2E,6S,7S)-6,7-isopropylidenedioxy-3,7-dimethyl-2-nonenoate **24**. In the same manner as above, **21** (2.0 g, 5.5 mmol) was converted to **24** (1.2 g, 81%), 89-92°C/1 Torr; $n_D^{24.5}$ 1.4562; $[\alpha]_D^{24.5}$ -21.3° (c=0.960, MeOH); νmax 1720 (s), 1650 (m), 1375 (m), 1220 (s), 1155 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.96 (3H, t, J=7.8 Hz), 1.08 (3H, s), 1.32 (3H, s), 1.43 (3H, s),

1.45-1.90 (4H, m), 2.19 (3H, d, J=1.2 Hz), 1.99-2.60 (2H, m), 3.70 (3H, s), 3.60-3.80 (1H, m), 5.73 (1H, q, J=1.2 Hz); GLC (10% PEG-20M; 5 mm x 2 m; 150°C + 3°C/min; N_2, 1.1 kg/cm^2): Rt 14.2 min (100%). (Found: C, 66.24; H, 9.59. Calc for $C_{15}H_{26}O_4$: C, 66.63; H, 9.69%).

(2E,6S,7S)-3-Ethyl-6,7-isopropylidenedioxy-7-mehyl-2-nonen-1-ol **25.** To a stirred and cooled soln of **23** (562 mg, 1.98 mmol) in dry toluene (5 ml) was added dropwise a soln of DIBAL-H (1 M in n-hexane, 4.2 ml, 4.2 mmol) at 0°C under Ar. After stirring for 30 min at 0°C, the reaction was quenched by adding MeOH (2 ml). The stirring was continued for 10 min at room temp. Then the mixture was filtered through a pad of celite and the filter-cake was washed with MeOH. The combined filtrate and washings were concentrated in vacuo to give 523 mg (quantitative) of **25**, n_D^{24} 1.4610; $[\alpha]_D^{24}$ -3.20° (c=0.950, $CHCl_3$); νmax 3400 (br.m), 1660 (w), 1210 (s), 1000 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.96 (3H, t, J=7.8 Hz), 1.00 (3H, t, J=7.8 Hz), 1.08 (3H, s), 1.33 (3H, s), 1.43 (3H, s), 1.45-2.50 (9H, m), 3.72 (1H, dd, J=3.7, 8.3 Hz), 4.19 (2H, br.d, J=6.9 Hz), 5.40 (1H, br.t, J=6.9 Hz). (Found: C, 69.78; H, 10.89. Calc for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01%).

(2E,6S,7S)-6,7-Isopropylidenedioxy-3,7-dimethyl-2-nonen-1-ol **26.** In almost the same manner as above, **24** (1.15 g, 4.26 mmol) was converted to **26** (930 mg, 90.2%), n_D^{25} 1.4584; $[\alpha]_D^{25}$ -5.42° (c=0.985, $CHCl_3$); νmax 3400 (br.m), 1660 (s), 1250 (m), 1210 (s), 1000 (br.s) cm^{-1}; δ (60 MHz, CCl_4) 0.90 (3H, t, J=6.4 Hz), 0.98 (3H, s), 1.20 (3H, s), 1.30 (3H, s), 1.65 (3H, s), 1.10-2.40 (7H, m), 3.55 (1H, dd, J=4.8, 8.2 Hz), 4.00 (2H, br.d, J=7.1 Hz), 5.35 (1H, br.t, J=7.1 Hz). (Found: C, 69.28; H, 10.91. Calc for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81%).

(3S,4S,7E)-9-Bromo-7-ethyl-3,4-isopropylidenedioxy-3-methyl-7-octene **27.** n-BuLi (1.53 N in n-hexane, 0.61 ml, 0.93 mmol) was added dropwise to a stirred and cooled soln of **25** (238 mg, 0.93 mmol) and Ph_3CH (3 mg) in dry ether (1 ml) and dry HMPA (1 ml) at 0°C under Ar until the characteristic red color persisted. After stirring for 5 min at 0°C, a soln of p-TsCl (190 mg, 1.0 mmol) in dry ether (1.2 ml) was added dropwise and the mixture was stirred for 45 min at 0°C. Then anhydrous LiBr (450 mg, 5.1 mmol) was added to the mixture and it was stirred overnight at room temp. The mixture was poured into sat $NaHCO_3$ soln and extracted with n-hexane. The n-hexane soln was washed with water, sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo to give 315 mg (quantitative) of **27**. This was employed in the next step without further purification. νmax 1655 (w), 1250 (m), 1200 (br.s), 1000 (m) cm^{-1}.

(3S,4S,7E)-9-Bromo-3,4-isopropylidenedioxy-3,7-dimethyl-7-octene **28.** In the same manner as above, **26** (485 mg, 2.00 mmol) was converted to **28** (722 mg, quantitative). νmax 1660 (w), 1260 (m), 1210 (s), 1000 (m) cm^{-1}.

Methyl (6E,10S,11S)-7-ethyl-10,11-isopropylidenedioxy-11-methyl-3-oxo-6-tridecanoate **29.** To a stirred and cooled suspension of NaH (66 mg, 1.7 mmol, 60% dispersion in mineral oil) in dry THF (1 ml) was added dropwise a soln of methyl acetoacetate (174 mg, 1.5 mmol) in dry THF (1 ml) at 0°C under Ar. The mixture was stirred for 15 min at 0°C and n-BuLi (1.53 N in n-hexane, 0.98 ml, 1.5 mmol) in dry THF (1 ml)was added dropwise to it below 5°C. The mixture was stirred for 20 min at 0°C and a soln of **27** (300 mg, 0.94 mmol) in dry THF (2 ml) was added dropwise to it at 0°C. After stirring for 2 h at -5°C, the reaction was quenched by adding sat NH_4Cl soln, and the mixture was extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was chromatographed over SiO_2 (6 g). Elution with n-hexane-ether (10:1-5:1) gave 245 mg (78% from **25**) of **29**, n_D^{23} 1.4633; $[\alpha]_D^{23}$ -4.68° (c=1.08, MeOH); νmax 1755 (s), 1725 (s), 1255 (s), 1215 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.96 (3H, t, J=8.0 Hz), 0.98 (3H, t, J=8.0 Hz), 1.08 (3H, s), 1.33 (3H, s), 1.44 (3H, s), 1.30-2.68 (12H, m), 3.60-3.80 (1H, m), 3.46 (2H, s), 3.78 (3H, s), 5.07 (1H, t, J=7.5 Hz). (Found: C, 67.85; H, 9.63. Calc for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67%).

Methy (2E,6E,10S,11S)-7-ethyl-10,11-isopropylidenedioxy-3,11-dimethyl-2,6-tridecadienoate **33.** To a stirred and cooled suspension of NaH (20 mg, 0.50 mmol, 60% dispersion in mineral oil) in dry THF (1 ml) was added dropwise a soln of **29** (140 mg, 0.395 mmol) in dry THF (1.5 ml) at 0°C under Ar. The mixture was stirred at 0°C for 15 min and $(EtO)_2P(O)Cl$ (72 μl, 86 mg, 0.50 mmol) was added dropwise at 0°C. After stirring for 2 h at room temp, the mixture was poured into sat NH_4Cl soln and extracted with ether. The ether soln was washed with sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo to give 233 mg of crude phosphoric ester **31**. This was dissolved in dry ether (1.5 ml) and the ether soln was added dropwise to a soln of Me_2CuLi (1.40 mmol) which was prepared according to the reported procedure[26] at -60°C under Ar. The mixture was stirred for 1.5 h at -70°C, and quenched by adding sat NH_4Cl soln. The stirring was continued for additional 5 min. Then the mixture was extracted with ether. The ether soln was washed with sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was chromatographed over SiO_2 (3 g). Elution with n-hexane-ether (20:1) gave 83.0 mg of crude **33**. The purity of it was 78.1% by GLC analysis. (5% PEG-20M; 5 mm x 1.5 m; 200°C; N_2; Rt 4.9 min). Further purification was carried out by using Merck Lobar® column. Pure **33** (118 mg, 35%) was obtained from 199 mg of crude **33**. GLC (same condition): Rt 4.9 min (94.7 %); HPLC [eluent: n-hexane-THF (30:1)]: Rt 12.9 min (100%). n_D^{23} 1.4677; $[\alpha]_D^{23}$ -4.15° (c=1.11, MeOH); νmax 1720 (s), 1645 (m), 1370 (m), 1250 (m), 1220 (s), 1140 (s), 1000 (m) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.96 (3H, t, J=7.8 Hz), 0.98 (3H, t, J=8.3 Hz), 1.07 (3H, s), 1.33 (3H, s), 1.44 (3H, s), 1.35-1.70 (4H, m)2.18 (3H, d, J=1.3 Hz), 1.80-2.32 (8H, m), 3.65-3.80 (1H, m), 3.70 (3H, s), 5.00-5.18 (1H, m), 5.68 (1H, br.s). (Found: C, 71.84; H, 10.17. Calc for $C_{21}H_{36}O_4$: C, 71.55; H, 10.30%).

Methyl (2E,6E,10S,11S)-10,11-isopropylidenedioxy-3,7,11-trimethyl-2,6-tridecadienoate **34.** In almost the same manner as above, **26** (485 mg, 2.00 mmol) was converted to **34** (110 mg, 16.3%). n_D^{24} 1.4718; $[\alpha]_D^{24.5}$ -7.33° (c=1.02, MeOH); νmax 1720 (s), 1645 (m), 1370 (m), 1250 (m), 1220 (s), 1145 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.96 (3H, t, J=7.8 Hz), 1.07 (3H, s), 1.32 (3H, s), 1.44 (3H, s), 1.63 (3H, d, J=1.2 Hz), 1.26-1.70 (4H, m), 1.80-2.30 (6H, m), 3.70 (3H, s), 3.62-3.82 (1H, m), 5.05-5.25 (1H, m), 5.68 (1H, br.s); GLC (5% PEG-20M, 5 mm x 1.5 m; 170°C; N_2, 1.0 kg/cm^2; Rt 12.4 min (1.7%), 16.5 min (98.3%). (Found: C, 70.53; H, 9.86. Calc for $C_{20}H_{34}O_4$: C, 70.97; H, 10.13%).

Methyl (2E,6E,10S,11S)-7-ethyl-10,11-dihydroxy-3,11-dimethyl-2,6-tridecanoate **35a.** A soln of **33** (112 mg, 0.318 mmol) in 75%(v/v) aq AcOH (4 ml) was stirred and heated for 6 h at 50°C. After the mixture was cooled to 0°C, this was diluted with EtOAc and a soln of NaOH (2.00 g, 50.0 mmol) in water (10 ml) was added dropwise to this. This was poured into brine and extracted with EtOAc. The EtOAc soln was washed with sat $NaHCO_3$ soln and brine, dried (Na_2SO_4) and concentrated in vacuo to give 98.0 mg (98.8%) of **18a**. This was employed in the next step without further purification. $[\alpha]_D^{25}$ -17° (c=0.99, MeOH); νmax 3450 (br.m), 1720 (s), 1645 (m), 1220 (s), 1150 (s) cm^{-1}. This was revealed to be enantiomerically pure by the HPLC analysis of the corresponding MTPA esters: eluent: n-hexane-THF (5:1); **35b** and **35c**, Rt 6.84 and 7.61 min; **35b**, 7.61 min (100%).

Methyl (2E,6E,10S,11S)-10,11-dihydroxy-3,7,11-trimethyl-2,6-tridecadienoate **36a**. In the same manner as above, **34** (106 mg, 0.314 mmol) was converted to **36b** (100 mg, quantitative), $[\alpha]_D^{24}$ -20° (c=0.96, MeOH); νmax 3455 (br,m), 1720 (s), 1650 (m), 1220 (s), 1150 (s) cm^{-1}. This was revealed to be enantiomerically pure by the HPLC analysis of the corresponding MTPA esters: eluent: n-hexane-THF (7:1); **36b** and **36c**, Rt 10.4 and 12.5 min; **36b**, 12.5 min (100%).

Methyl (2E,6E,10R,11S)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecanoate (JH I) **1**. To a stirred and cooled soln of **35a** (50 mg, 0.16 mmol) in dry CH_2Cl_2 (1.5 ml) and Et_3N (24.0 mg, 0.238 mmol) was added solid Ms_2O (36 mg, 0.21 mmol) at 0°C. After stirring for 1 h at 0°C, the mixture was poured into sat $NaHCO_3$ soln and extracted with EtOAc. The EtOAc soln was washed with brine, dried (Na_2SO_4) and concentrated in vacuo to give 55.7 mg of **37**. This was dissolved in MeOH (1 ml) and to this was added NaOMe (28.9 mg, 0.150 mmol, 28 % in MeOH) in MeOH (1 ml) dropwise at 0°C with stirring. After the mixture was stirred for 10 min at 0°C, it was poured into sat $NaHCO_3$ soln, and extracted with ether. The ether soln was washed with $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residual oil was purified by prep TLC (Merck Kiesel gel 60 F-254) to give 37 mg (79%) of pure **1**, n_D^{24} 1.4732; $[\alpha]_D^{23}$ +14.9° (c=0.935, $CHCl_3$), $[\alpha]_D^{22.5}$ +14.5° (c=0.78, MeOH); νmax 2990 (s), 2950 (m), 2895 (m), 1720 (s), 1650 (m), 1470 (m), 1450 (m), 1435 (m), 1380 (m), 1360 (m), 1325 (w), 1280 (w), 1250 (w), 1225 (s), 1150 (s), 1060 (w), 1030 (w), 920 (w), 880 (m), 860 (m), 820 (m), 785 (w), 730 (w) cm^{-1}; δ (400 MHz, CCl_4) 0.98 (3H, t, J=7.8 Hz), 0.98 (3H, t, J=7.8 Hz), 1.19 (3H,s), 1.35-1.63 (4H, m), 2.13 (3H, d, J=1.2 Hz), 1.99-2.23 (8H, m), 2.50 (1H, dd, J=5.1, 7.3 Hz), 3.61 (3H, s), 5.05 (1H, br.t, J=6.4 Hz), 5.57 (1H, br.d, J=1.2 Hz); ^{13}C NMR δ 9.65, 13.10, 18.84, 21.59, 23.17, 25.57, 25.80, 27.26, 33.29, 41.19, 50.72, 61.78, 64.65, 115.31, 122.91, 141.28, 159.83, 167.14. HPLC: eluent, n-hexane-THF (30:1): Rt 12.9 min (100%). (Found: C, 73.25; H, 10.08. Calc for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27 %).

Methly (2E,6E,10R,11S)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate (JH II) **2**. In the same manner as above, **36a** (80 mg, 0.27 mmol) was converted to **2** (50 mg, 66%), $n_D^{24.5}$ 1.4774; $[\alpha]_D^{24.5}$ +17.6° (c=0.590, MeOH); νmax 3000 (s), 2950 (s), 2900 (m), 2850 (m), 1725 (s), 1650 (s), 1470 (m), 1450 (m), 1440 (m), 1420 (w), 1385 (m), 1360 (m), 1325 (w), 1280 (w), 1225 (s), 1150 (s), 1110 (w), 1060 (m), 1030 (m), 1000 (w), 980 (w), 920 (w), 880 (m), 865 (m), 800 (w), 740 (w) cm^{-1}; δ (400 MHz, CCl_4) 0.98 (3H, t, J=7.8 Hz), 1.19 (3H, s) 1.62 (3H, s), 1.34-1.65 (4H, m), 2.14 (3H, d, J=1.3 Hz), 2.01-2.21 (6H, m), 2.49 (1H, dd, J=5.1, 7.4 Hz), 3.61 (3H, s), 5.11 (1H, br.t, J=5.9 Hz), 5.57 (1H, br.s); ^{13}C NMR δ 9.65, 16.03, 18.78, 21.59, 25.80, 25.92, 27.03, 36.51, 40.78, 50.72, 61.72, 64.53, 115.31, 123.44, 135.38, 159.89, 167.20. HPLC: eluent: n-hexane-THF (60:1), Rt 21.6 min (100%). (Found: C, 72.93; H, 9.89. Calc for $C_{17}H_{28}O_3$: C, 72.82; H, 10.06%).

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