Tetrahedron Vol. 42, No. 14, pp. 3789 to 3792, 1986
Printed in Great Britain.

0040-4020/86 $3.00+.00
Pergamon Journals Ltd.

ISOLATION OF (10*R*,11*R*)-(+)-SQUALENE-10,11-EPOXIDE FROM THE RED ALGA *LAURENCIA OKAMURAI* AND ITS ENANTIOSELECTIVE SYNTHESIS

HIDEO KIGOSHI, MAKOTO OJIKA, YOSHIKAZU SHIZURI, HARUKI NIWA, and KIYOYUKI YAMADA*

Department of Chemistry, Faculty of Science, Nagoya University,
Chikusa, Nagoya 464 Japan

(*Received in Japan* 13 *March* 1986)

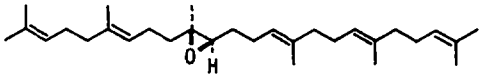
Abstract - (10*R*,11*R*)-(+)-Squalene-10,11-epoxide 1 has been isolated from the red alga *Laurencia okamurai*. On the basis of the spectral data and comparison to the racemic compound prepared from squalene, assignment of the planar structure was made. The enantioselective synthesis of 1 was performed, which determined the absolute stereochemistry of 1.

As part of our chemical studies on marine algae, we have investigated the constituents of the red alga *Laurencia okamurai* collected in July at Goza, Mie Prefecture, Japan, and isolated an epoxy compound, (10*R*,11*R*)-(+)-squalene-10,11-epoxide 1.[1] This paper deals with isolation, characterization, and enantioselective synthesis of 1 in details.

Isolation and Characterization.

The benzene-EtOAc soluble material from the acetone extract of fresh *L. okamurai* was separated by chromatography on silica gel with eluants of increasing polarity from hexane-benzene (4:1) through hexane-benzene (1:1) to benzene. The material eluted with benzene was further separated by TLC on silica gel to obtain an epoxy compound 1, $[\alpha]_D^{25}$ +10.4° (*c* 0.80, $CHCl_3$) (3 x 10^{-4}%) as a colorless oil. The molecular formula $C_{30}H_{50}O$ was determined by the high resolution mass spectral measurement on the molecular ion peak at m/z 426.

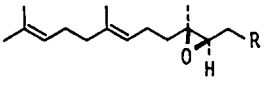
The ^1H NMR spectrum showed three kinds of Me signals: a signal due to the Me group on carbon bearing an oxygen atom at δ 1.26 (3H, s), signals arising from five vinyl Me groups at δ 1.60 (15H, br s), and signals due to two vinyl Me groups at δ 1.68 (6H, br s). In addition, there were observed a signal due to a methine hydrogen on carbon bearing an oxygen function at δ 2.73 (1H, t, J = 6 Hz) and signals of five vinyl hydrogens at δ 4.9 - 5.3 (5H, m) in the ^1H NMR spectrum of 1. The signal at δ 2.73 described above in the ^1H NMR spectrum and the resonances at δ 59.9 (s) and 62.7 (d) in the ^{13}C NMR spectrum revealed the presence of a trisubstituted epoxide group. These ^1H NMR spectral data together with the ^{13}C NMR and mass spectral information (see Experimental Section) suggested that the epoxy compound 1 was squalene-10,11-epoxide. This inference was verified by comparison of the spectral data of the epoxy compound 1 with those of (±)-squalene-10,11-epoxide prepared from squalene with *m*-chloroperbenzoic acid according to the procedure[2] of Katayama and Marumo. Further, enantioselective synthesis of 1 was executed for the purpose of establishing the absolute stereochemistry of the epoxy moiety in 1.



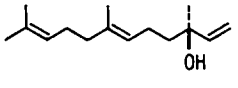
1

Enantioselective Synthesis.

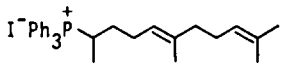
The Sharpless asymmetric epoxidation[3] of *trans, trans*-farnesol employing (-)-diethyl tartrate gave (2*R*,3*R*)-epoxyfarnesol 2 (84%). The ^{1}H NMR spectral analysis of the derived acetate 3 in the presence of a chiral shift reagent Eu(hfc)$_3$ gave an enantiomeric excess (ee) of 96%. The absolute stereochemistry of 2*R* and 3*R* was assigned to epoxyfarnesol 2 as depicted on the basis of the observations reported by Sharpless.[3] This assignment was confirmed by converting epoxyfarnesol 2 to (*R*)-(-)-nerolidol 8[4] by a series of the following reactions: tosylation of epoxyfarnesol 2 with TsCl in pyridine gave the tosylate 4 (96%), which on reaction with NaI in acetone was led to the iodide 5 (quantitative) and subsequently reduction of 5 with zinc in AcOH - ether afforded (*R*)-(-)-nerolidol 8, $[\alpha]_D^{20}$ -17.9° (*c* 1.15, EtOH) (97%) [lit.[4] $[\alpha]_D^{22}$ +15.1° for (*S*)-(+)-nerolidol]. Bishomologation of the C_{15}-skeleton of the iodide 5 was achieved by reacting the iodide 5 with *t*-butyl lithioacetate[5,6] in THF - HMPA to afford the ester 6 (84%). Reduction of the ester 6 with DIBAL in toluene yielded the aldehyde 7 (81%), the Wittig reaction of which with the phosphorane generated from the phosphonium salt 9[7] in THF provided a 4:3 mixture of 1 and its isomer 10. Separation of the mixture by chromatography on silica gel impregnated with $AgNO_3$ afforded the epoxy compound 1 (24%) and the isomer 10 (16%), respectively. Synthetic 1 exhibited an optical rotation, $[\alpha]_D^{22}$ +10.7° (*c* 1.19, $CHCl_3$), comparable to the value of natural 1, $[\alpha]_D^{25}$ +10.4° (*c* 0.80, $CHCl_3$). The spectral (IR, ^{1}H NMR, ^{13}C NMR, and MS) properties as well as chromatographic behaviors of synthetic 1 were in accord with those of natural 1. Thus, the present synthesis has unambiguously determined the absolute structure of the epoxy compound 1 isolated from *L. okamurai* to be (10*R*,11*R*)-(+)-squalene-10,11-epoxide.



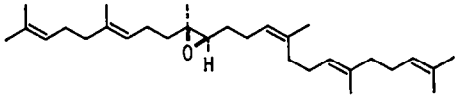
2 R = OH
3 R = OAc
4 R = OTs
5 R = I
6 R = CH_2COO^tBu
7 R = CH_2CHO



8



9



10

Discussion.

Natural occurrence of squalene-10,11-epoxide was first reported in 1976 by Katayama and Marumo:[2] it was isolated as a fungal metabolite from the mycelia of *Sclerotinia fructicola*, but the amount isolated was too small to be sufficient for determination of the stereochemistry. In 1982, Fattorusso and associates isolated (10*S*,11*S*)-(-)-squalene-10,11-epoxide from a marine green alga *Caulerpa prolifera*.[8] It is worthy of note that the (10*S*,11*S*)-enantiomer of squalene-10,11-epoxide was isolated from the green alga,[8] whereas the (10*R*,11*R*)-enantiomer in the present study was obtained from the red alga. Isolation of 1 would be interesting in view of its possible biogenetic significance in connection with the structurally related squalene-2,3-epoxide,[9,10] the important role of which in the biosynthesis of terpenoids is well-known.

EXPERIMENTAL

IR spectra were recorded on a JASCO Model IRS spectrophotometer in $CHCl_3$ solution unless otherwise stated. ^{1}H NMR spectra were obtained on a JEOL FX-90QE (90 MHz) spectrometer in $CDCl_3$: chemical shifts (δ) are reported in ppm downfield from internal TMS and coupling constants in Hz. ^{13}C NMR spectra were measured at 22.5 MHz on a JEOL FX-90QE spectrometer in C_6D_6: chemical shifts (δ) are reported in ppm downfield from internal TMS. Mass spectra were recorded on Hitachi RMU-6C and JEOL JMS-DX300 instruments. Optical rotations were measured on a JASCO DIP-4 polarimeter. Fuji-Davison silica gel BW-80 was used for column chromatography. Merck precoated silica gel $60F_{254}$ plates were employed for analytical thin layer chromatography (TLC) and Merck

silica gel PF_{254} for preparative TLC. For HPLC a JASCO TRI ROTAR-II apparatus equipped with a UV detector (JASCO UVIDEC-100-II) and a refractive index detector (Shodex RI SE-11) was used. Organic solutions in the synthetic operations were dried over anhydrous Na_2SO_4 and concentrated by vacuum rotary evaporator.

Isolation of (10*R*,11*R*)-(+)-squalene-10,11-epoxide 1. The alga (*L. okamurai*, 3.2 kg wet weight) was collected in July at Goza, Mie Prefecture, Japan, and extracted with acetone (6 ℓ) at room temperature. After filtration the filtrate was concentrated under reduced pressure to obtain an aqueous suspension (*ca.* 2 ℓ), which was extracted with benzene (2 x 1 ℓ) and subsequently with EtOAc (1 ℓ). The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford an oily material (12.5 g), a portion of which (11 g) was chromatographed on silica gel (330 g) with 4:1 hexane-benzene (3 ℓ), 1:1 hexane-benzene (3 ℓ), and benzene (9.5 ℓ) successively. The early fractions eluted with benzene contained 1, which was further separated by preparative TLC (85:15 hexane-Et_2O) to give 1 (9.3 mg, 3 x 10^{-4}%).
1: $C_{30}H_{50}O$: colorless oil; $[\alpha]_D^{23}$ +10.4° (*c* 0.80, $CHCl_3$); IR (CCl_4) 1660 (broad, weak) cm^{-1}; ^1H NMR δ 1.26 (3H, s), 1.60 (3H x 5, br s), 1.68 (3H x 2, br s), 1.3-1.8 (4H, m), 1.9-2.3 (16H, m), 2.73 (1H, t, J = 6), 4.9-5.3 (5H, m); ^{13}C NMR δ 16.1(Me x 3, q), 16.9(q), 17.8(Me x 2, q), 24.2(t), 25.4(t), 25.9(Me x 2, q), 27.0(t), 27.1(t), 27.2(t), 29.5(t), 39.3(t), 40.1(CH_2 x 3, t), 59.9(s), 62.7(d), 124.0(d), 124.4(d), 124.6(d), 124.8(CH x 2, d), 131.3(C x 2, s), 135.1(s), 135.2(s), 135.7(s); MS m/z 426 (M^+), 411, 408 [HRMS. Found: 426.3853 (M^+). $C_{30}H_{50}O$ requires: 426.3860].

(2*R*,3*R*)-Epoxyfarnesol 2. To a stirred solution of diethyl (-)-tartrate (0.18 ml, 1.06 mmol) and $Ti(O\text{-}i\text{-}Pr)_4$ (0.30 ml, 1.02 mmol) in CH_2Cl_2 (10 ml) cooled at -50 °C under nitrogen were added a solution of *trans, trans*-farnesol (210 mg, 0.95 mmol) in CH_2Cl_2 (2 ml) and a 4.8 M solution of *t*-butyl hydroperoxide in dichloroethane (0.43 ml, 2.06 mmol) sequentially. The mixture was stirred at -50 - -45 °C for 1 h, diluted with a 10% aqueous tartaric acid solution (2.5 ml), and stirred at -50 °C for further 30 min. The reaction mixture was warmed to room temperature and the stirring was continued for 1 h. The aqueous layer separated was extracted with CH_2Cl_2 (2 x 5 ml), and the CH_2Cl_2 extracts were combined with the organic layer. The combined organic solution was washed with H_2O, dried, and concentrated. To a solution of the residual oil in Et_2O (9 ml) was added aqueous 1 M NaOH (3 ml) and the mixture was stirred at 0 °C for 30 min. The aqueous layer was extracted with Et_2O (3 x 5 ml), and the Et_2O extracts were combined with the ethereal layer. The combined ethereal solution was washed with saturated NH_4Cl solution and saturated NaCl solution, dried, and concentrated. The crude product was purified by chromatography on silica gel (4:1 hexane-EtOAc) to obtain 2 (188 mg, 84%) as a colorless oil: $[\alpha]_D^{26}$ +6.53° (*c* 4.21, $CHCl_3$); IR 3600, 3430, 1025 cm^{-1}; ^1H NMR δ 1.31 (3H, s), 1.60 (3H x 2, br s), 1.68 (3H, br s), 1.9-2.3 (6H, m), 2.97 (1H, dd, J = 5, 6, the M part of an ABM pattern), 3.77 (2H, m, the AB part of an ABM pattern), 5.10 (2H, br t, J = 7); MS m/z 238 (M^+), 223, 220, 217 [HRMS. Found: 238.1949 (M^+). $C_{15}H_{26}O_2$ requires: 238.1933].

(2*R*,3*R*)-Epoxyfarnesyl acetate 3. A solution of 2 (8 mg, 0.034 mmol) in Ac_2O (0.2 ml) and pyridine (0.5 ml) was stirred at room temperature for 1.5 h and concentrated. The crude product was purified by preparative TLC (8:1 hexane-EtOAc) to give 3 (10 mg, quantitative) as a colorless oil: $[\alpha]_D^{18}$ +25.1° (*c* 1.01, $CHCl_3$); IR 1740, 1385, 1230 (broad), 1035 cm^{-1}; ^1H NMR δ 1.31 (3H, s), 1.60 (3H x 2, br s), 1.68 (3H, br s), 1.9-2.2 (6H, m), 2.10 (3H, s), 2.99 (1H, dd, J = 4, 6), 4.02 (1H, dd, J = 6, 12), 4.33 (1H, dd, J = 4, 12), 5.05 (2H, m); MS m/z 280 (M^+), 262, 221, 220.

(2*R*,3*R*)-Epoxyfarnesyl tosylate 4. A mixture of 2 (306 mg, 1.29 mmol) and TsCl (342 mg, 1.80 mmol) in pyridine (0.45 ml) was stirred at 0 °C for 5.5 h. A small amount of ice was added to the mixture. The mixture was stirred at room temperature for 20 min, diluted with H_2O (3 ml), and extracted with Et_2O (4 x 5 ml). The ethereal extracts were washed with H_2O, saturated $CuSO_4$ solution, H_2O, and saturated NaCl solution, dried, and concentrated. Chromatography of the crude product on silica gel (6:1 hexane-EtOAc) gave the tosylate 4 (482 mg, 96%) as a colorless oil: $[\alpha]_D^{27}$ +21.4° (*c* 5.67, $CHCl_3$); IR 1650, 1595, 1370, 1180, 1100 cm^{-1}; ^1H NMR δ 1.21 (3H, s), 1.59 (3H x 2, br s), 1.68 (3H, br s), 1.9-2.2 (6H, m), 2.45 (3H, s), 2.98 (1H, t, J = 6, the X part of an ABX pattern), 4.12 (2H, m, the AB part of an ABX pattern), 5.06 (2H, m), 7.35 (2H, br d, J = 8), 7.81 (2H, br d, J = 8); MS m/z 392 (M^+), 374 [HRMS. Found: 392.2019 (M^+). $C_{22}H_{32}O_4S$ requires: 392.2019].

(2*R*,3*R*)-Epoxyfarnesyl iodide 5. A mixture of 4 (150 mg, 0.38 mmol) and NaI (181 mg, 1.21 mmol) in acetone (1.5 ml) was stirred at room temperature for 10 h and diluted with Et_2O (8 ml). The precipitates were removed by passing the mixture through a short column of Florisil and the resulting Et_2O solution was concentrated. The crude product was chromatographed on silica gel (20:1 hexane-EtOAc) to give 5 (133 mg, quantitative) as a colorless oil: $[\alpha]_D^{22}$ -27.2° (*c* 4.45, $CHCl_3$); IR 1660 cm^{-1}; ^1H NMR δ 1.28 (3H, s), 1.60 (3H x 2, br s), 1.68 (3H, br s), 1.9-2.3 (6H, m), 3.05 (2H, m, the AB part of an ABM pattern), 3.36 (1H, dd, J = 3, 6, the M part of an ABM pattern), 5.12 (2H, m); MS m/z 348 (M^+), 221 [HRMS. Found: 221.1885 (M^+ - I). $C_{15}H_{25}O$ requires: 221.1903].

Ester 6. (1) Preparation of a 0.2 M $LiCH_2COO^tBu$ solution. To a stirred solution of $i\text{-}Pr_2NH$ (0.45 ml, 3.22 mmol) in THF (9.6 ml) cooled at -78 °C was added a 1.63 M solution of *n*-BuLi in hexane (1.8 ml, 2.93 mmol) under nitrogen. The mixture was stirred at -78 °C for 50 min. To this mixture was added a solution of CH_3COO^tBu (0.40 ml, 3.04 mmol) in THF (2.8 ml) over 5 min and the mixture was kept at -78 °C for 40 min. (2) To a solution of 5 (190 mg, 0.55 mmol) in THF (2.5 ml) - HMPA (0.5 ml) cooled at -78 °C under nitrogen was added the 0.2 M $LiCH_2COO^tBu$ solution (3.0 ml, 0.60 mmol), and the mixture was stirred for 30 min at -78 °C. Then the 0.2 M $LiCH_2COO^tBu$ solution (2.0 ml, 0.40 mmol) was further added to the mixture and the stirring was

continued for further 30 min. The mixture was diluted with saturated NH_4Cl solution (4 ml) and the temperature was raised to room temperature. The mixture was extracted with Et_2O (3 x 10 ml). The ethereal extracts were washed with saturated NaCl solution, dried, and concentrated. The crude product was purified by chromatography on silica gel (20:1 hexane-EtOAc) to obtain 6 (154 mg, 84%) as a colorless oil: $[\alpha]_D^{22}$ +8.44° (*c* 3.78, $CHCl_3$); IR 1720, 1380, 1230 (broad), 1150 cm^{-1}; ^1H NMR δ 1.27 (3H, s), 1.45 (3H x 3, s), 1.60 (3H x 2, br s), 1.68 (3H, br s), 1.7-2.2 (6H, m), 2.38 (2H, t, J = 7), 2.75 (1H, t, J = 6), 5.09 (2H, br t, J = 7); MS m/z 280 ($M^+ - C_4H_8$), 237 [HRMS. Found: 280.2026 ($M^+ - C_4H_8$). $C_{17}H_{28}O_3$ requires: 280.2036].

Aldehyde 7. To a stirred solution of 6 (161 mg, 0.48 mmol) in toluene (3 ml) cooled at -110 °C under nitrogen was added a 1.76 M solution of *i*-Bu_2AlH in toluene (0.41 ml, 0.72 mmol). The mixture was stirred at -110 °C for 2 h, diluted with MeOH (1.5 ml), kept with stirring at -110 °C for 10 min, and warmed to room temperature. To the mixture was added a saturated aqueous solution of potassium sodium tartrate (10 ml) and the mixture was extracted with EtOAc (4 x 10 ml). The combined organic extracts were washed with saturated NaCl solution, dried, and concentrated. The crude product was purified by chromatography on silica gel (3:1 hexane-Et_2O) to give 7 (102 mg, 81%) as a colorless oil: $[\alpha]_D^{21}$ +11.3° (*c* 4.41, $CHCl_3$); IR 2720, 1725 cm^{-1}; ^1H NMR δ 1.28 (3H, s), 1.60 (3H x 2, br s), 1.68 (3H, br s), 1.8-2.3 (6H, m), 2.5-2.9 (3H, m), 5.10 (2H, m), 9.82 (1H, t, J = 1); MS m/z 264 (M^+), 246 [HRMS. Found: 264.2068 (M^+). $C_{17}H_{28}O_2$ requires: 264.2088].

(*R*)-(-)-Nerolidol 8. Activated zinc powder was prepared by washing commercially available zinc powder with 1 M HCl for 2 min, H_2O, EtOH, and Et_2O successively, which was dried under reduced pressure (3 mmHg). A mixture of 5 (22 mg, 0.075 mmol) and activated zinc powder (27 mg, 0.42 mmol) in Et_2O (0.5 ml) - AcOH (0.05 ml) was stirred at room temperature for 1 h and then passed through a short column of Florisil with Et_2O. The ethereal solution was concentrated and the crude product was purified by chromatography on silica gel (10:1 hexane-EtOAc) to afford 8 (18 mg, 97%) as a colorless oil: $[\alpha]_D^{20}$ -17.9° (*c* 1.15, EtOH) (lit.[4] $[\alpha]_D^{22}$ +15.1° for (*S*)-(+)-nerolidol); IR 3600, 3400, 1640, 1100, 1000, 920 cm^{-1}; ^1H NMR δ 1.28 (3H, s), 1.60 (3H x 2, br s), 1.68 (3H, br s), 1.9-2.2 (6H, m), 5.05 (1H, dd, J = 1, 10), 5.10 (2H, m), 5.20 (1H, dd, J = 1, 17), 5.93 (1H, dd, J = 10, 17); MS m/z 207 (M^+ - Me), 204 ($M^+ - H_2O$).

Synthesis of (10*R*,11*R*)-(+)-squalene-10,11-epoxide 1 and the isomer 10. To a stirred mixture of the phosphonium iodide 9[7] (262 mg, 0.46 mmol) in THF (3 ml) cooled at -78 °C was added a 1.56 M solution of *n*-BuLi in hexane (0.25 ml, 0.39 mmol) under nitrogen, and the mixture was stirred at -78 °C for 30 min. To the mixture was added a solution of 7 (102 mg, 0.39 mmol) in THF (2 ml) at -78 °C. The mixture was stirred at -78 °C for 40 min and then at room temperature for 5 h, diluted with saturated NH_4Cl solution (10 ml), and extracted with Et_2O (4 x 10 ml). The combined organic extracts were washed with saturated NaCl solution, dried, and concentrated. The oily residue was chromatographed on silica gel (20:1 hexane-EtOAc) to give a 4:3 mixture of 1 and 10 (99 mg, 60%). The ratio of 1 and 10 was determined by HPLC analysis using a Develosil ODS-5 column (0.46 x 25 cm) with MeCN. The mixture was further separated by chromatography on 13% $AgNO_3$ impregnated silica gel (20:1 benzene-EtOAc) to give 1 (39 mg, 24%) and 10 (26 mg, 16%) together with a mixture of 1 and 10 (26 mg).
1; colorless oil; $[\alpha]_D^{22}$ +10.7° (*c* 1.19, $CHCl_3$) ($[\alpha]_D^{25}$ +10.4° (*c* 0.80, $CHCl_3$) for natural 1). The IR, ^1H NMR, ^{13}C NMR, and mass spectra and chromatographic behaviors of synthetic 1 proved identical with those of natural 1. 10: colorless oil; $[\alpha]_D^{21}$ +5.87° (*c* 2.81, $CHCl_3$); IR (CCl_4) 1660 (broad, weak) cm^{-1}; ^1H NMR δ 1.25 (3H, s), 1.3-1.7 (4H, m), 1.60 (3H x 4, br s), 1.68 (3H x 3, br s), 1.9-2.2 (16H, m), 2.73 (1H, t, J = 6), 5.0-5.2 (5H, m); ^{13}C NMR δ 16.0(q), 16.1(q), 16.9(q), 17.7(Me x 2, q), 23.6(q), 24.2(t), 25.4(t), 25.8(Me x 2, q), 27.0(t), 27.2(CH_2 x 2, t), 29.8(t), 32.3(t), 39.3(t), 40.2(CH_2 x 2, t), 60.0(s), 62.7(d), 124.4(d), 124.6(d), 124.8(CH x 2, d), 124.9(d), 131.2(C x 2, s), 135.4(C x 2, s), 135.9(s); MS m/z 426 (M^+), 411, 408 [HRMS. Found: 426.3855. $C_{30}H_{50}O$ requires: 426.3860].

Acknowledgements - We are grateful to Professor W. Kida, Mie University, for identification of the alga. We thank the Ministry of Education, Science, and Culture (Grant-in-aid for Scientific Research No. 59470019) for partial support of this work.

REFERENCES

1. Preliminary communication: H. Kigoshi, M. Ojika, Y. Shizuri, H. Niwa, and K. Yamada, *Tetrahedron Lett.*, 23, 5413 (1982).
2. M. Katayama and S. Marumo, *Tetrahedron Lett.*, 1293 (1976).
3. T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, 102, 5974 (1980).
4. P. Vlad and M. Soucek, *Collect. Czech. Chem. Commun.*, 27, 1726 (1962).
5. M. W. Rathke and A. Lindert, *J. Am. Chem. Soc.*, 93, 2318 (1971).
6. W. Bos and H. J. J. Pabon, *Rec. Trav. Chim. Pays-Bas*, 99, 141 (1980).
7. R. M. Coates and W. H. Robinson, *J. Am. Chem. Soc.*, 93, 1785 (1971).
8. L. De Napoli, E. Fattorusso, S. Magno, and L. Mayol, *Phytochemistry*, 21, 782 (1982).
9. E. J. Corey, W. E. Russey, and P. R. O. de Montellano, *J. Am. Chem. Soc.*, 88, 4750 (1966).
10. E. E. van Tamelen, J. D. Willet, R. B. Clayton, and K. E. Lord, *J. Am. Chem. Soc.*, 88, 4752 (1966).