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SYNTHESIS OF 10,15-[$^{13}C_2$]-SQUALENE AND -DL-SQUALENE OXIDE

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SUMMARY

[3-^{13}C]-Ethyl farnesoate was prepared by alkylating 3-^{13}C-ethyl acetoacetate with geranyl bromide, followed by hydrolysis, decarboxylation and treatment with the anion of triethyl phosphonoacetate. The ester was reduced with LAH, brominated with CBr_4/ϕ_3P, and the farnesyl bromide coupled with CuI/Li-pyrrolidine to produce [10,15-$^{13}C_2$]-squalene. Epoxidation was effected by treatment with NBS in aqueous THF, followed by K_2CO_3-mediated HBr elimination. The overall yield of DL-10,15-[$^{13}C_2$]-squalene-2,3-oxide was 1.6%.

Keywords: squalene oxide, NMR, lanosterol, ^{13}C.

INTRODUCTION

Squalene oxide occupies an important position in the biochemical pathway leading to steroid production and details of the mechanism by which it is converted to lanosterol by squalene-oxide lanosterol cyclase are still under study. We wished to utilize ^{13}C labeled squalene in studies of this enzymatic conversion and in preparations of labeled steroids and analogs but found no commercial source for the compound. The synthetic procedures described were developed, using a mixture of new procedures and the best earlier methods to produce material in high purity and reasonable overall yield.

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RESULTS AND CONCLUSIONS

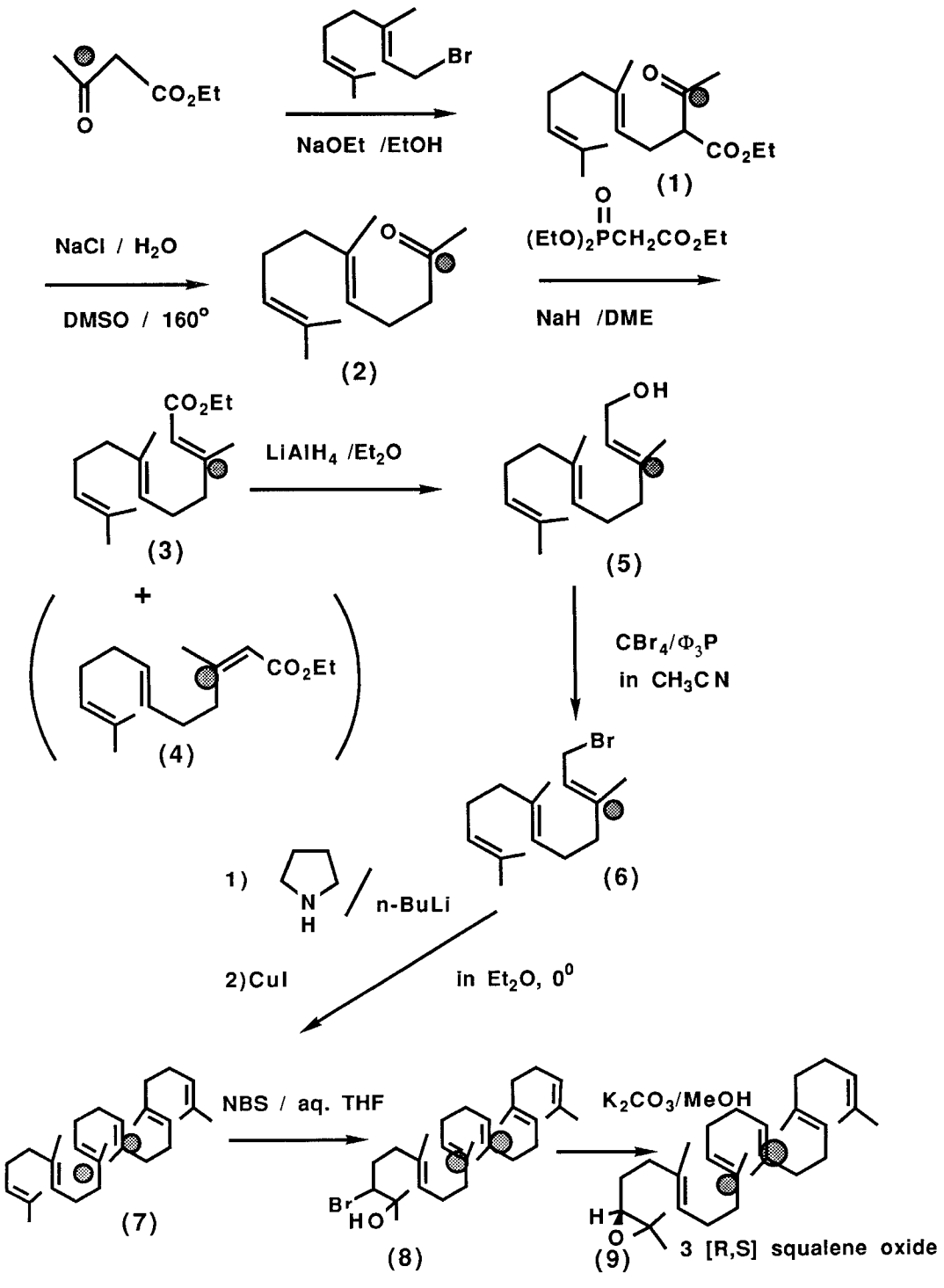
Previous chemical syntheses of labeled squalene and squalene oxide suffered from poor overall yields and/or lack of stereoselectivity and in the case of Wittig reaction produced final mixtures which were extremely difficult to purify. Our attempts to prepare squalene from mevalonate using enzyme from bovine liver extract (6) which would have produced stereochemically pure product were unsuccessful due to low and widely varying yields of product which was not easily isolated from the incubation matrix. The synthetic procedures we developed after our initial studies as shown in the reaction scheme used a reasonably inexpensive labeled starting material and produced sufficient labeled steriochemically pure product for our future research. The major advantages of our methods are the high yield decarboxylation and bromination steps, the separation of isomers at the unsaturated ester stage which is much easier than it would be at any other point, and the symmetrical coupling reaction which leads stereospecifically to squalene. Difficulties which had to be overcome before utilization of the labeled starting material included optimization of conditions to prevent a second alkylation in the first reaction and optimization of yields in the coupling reaction. A problem which still exists in the procedure is lack of an enantioselective reaction which epoxidizes only the terminal double bond of squalene. An enzymatic procedure for performing this final step in an enantioselective manner would increase the utility of our synthesis and is under study.

EXPERIMENTAL

NMR spectra were measured in deuterio-chloroform except for squalene oxide which was solubilized in acetone-d_6 due to acid sensitivity. Bruker WM 300 or AM 500 Fourier Transform instruments were used. Chemical shifts were referenced to the solvent peak; 7.24 ppm for ^1H NMR and 77.0 ppm for ^{13}C NMR, except for squalene oxide, in which solvent reference peaks were at 2.04 ppm for ^1H and 29.8 ppm for ^{13}C. 3-^{13}C-Ethyl acetoacetate was purchased from Isotec Inc. Geranyl bromide and triethyl phosphonoacetate were purchased from Aldrich Chemical Co.

Preparation of carbonyl-labeled geranylacetone (2) [1]

Freshly prepared sodium ethoxide (9.5 ml 1.56 M, 14.8 mmol) was added dropwise to a solution of commercial 3-^{13}C-ethyl acetoacetate (1.90 g,14.48 mmol) in 10 ml of ethanol at room


CO2Et
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NaH /DME
CO2Et
LiAlH4 /Et2O
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in CH3CN
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2)CuI
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K2CO3/MeOH
(7)
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(8)
H
O
(9)
3 [R,S] squalene oxide

temperature. The salt thus obtained was added slowly at 0° C to a vigorously stirred solution of geranylbromide (4.35 g, 20.0 mmol) in 6.5 ml of ethanol. The solution was then gradually warmed to room temperature and allowed to stand for 2 hrs. The mixture was diluted with 50 ml of water, extracted twice with 75 ml of ether and the ethereal solution was washed with water and dried over sodium sulfate. The residue after evaporation of ether was subjected to column chromatography on SiO_2. Elution with *n*-hexane: ethyl acetate (100:5) removed excess geranylbromide, yielding the monoalkylated and dialkylated products. The yield of monoalkylated product (**1**) was 2.70 g (69.8%), the dialkylated product was 0.31 g (8.0%). Compound (**1**): ^{13}C NMR 203.2; ^{1}H NMR 4.99 (2H br t, J=6.2 Hz), 4.15 (2H, q, J=7.1 Hz), 3.40 (1H, t, J=6.3 Hz), 2.51 (2H, br t, J=6.3 Hz), 2.18 (3H, CH_3-, d, J=4.4 Hz, $^{2}J_{CH}$), 1.99 (2H, t-like, J=7.0 Hz), 1.93 (2H, t-like, J=7.1 Hz), 1.63 (3H, s, CH_3-), 1.58 (3H, s, CH_3-), 1.54 (3H, s, CH_3-), 1.22 (3H, s, CH_3-). The dialkylated product: ^{13}C NMR 205.0; ^{1}H NMR 5.01 (2H, t, J=6.0 Hz), 4.87 (2H, t, J=7.3 Hz), 4.14 (2H, q, J=7.1 Hz), ~2.54 (4H, m, $2CH_2$), 2.07 (3H, CH_3-, d, J=5.2 Hz, $^{2}J_{CH}$), ~2.0 (8H, m, 4 x CH_2), 1.65 (3H, s, 1xCH_3-), 1.56 (3H, s, 5xCH_3-), 1.22 (3H, t, J=7.1 Hz).

Compound (**1**), 3-carboethoxy-6,10-dimethyl-*trans*-5,9-undecadien-2-one, was decarbethoxylated to give geranylacetone according to the method of A. P. Krapcho and A. J. Lovey [2]. To a three necked flask fitted with a condenser and a trap containing a saturated aq. solution of barium hydroxide was added a solution of (**1**) [2.70 g, 10.1 mmol in dimethylsulfoxide (8.1 ml)] followed by the addition of water (0.4 ml, 22.2 mmol) and sodium chloride (0.71 g, 12.15 mmol). The mixture was stirred and heated at 160°-170° C for 6 hrs under nitrogen. The reaction mixture was diluted with 25 ml of water, then extracted with *n*-hexane and dried over sodium sulfate. After evaporation of the solvent, the residue was distilled, b.p. 122-124° C (5 mm), yield 1.73 g (87.8%). The product (**2**) was chromatographically pure (Hewlett Packard 5890A GC with FID detector, Grob type injection, 25 m x 0.22 mm ID BP1 capillary column (SGE), temperature programmed 55° for 1 min, then to 300 ° at 15°/min). ^{13}C NMR 208.8; ^{1}H NMR 5.04 (2H, t, J=7.0 Hz), 2.44 (2H, m, due to $^{2}J_{CH}$), 2.24 (2H, m, due to $^{3}J_{CH}$), 2.11 (3H,CH_3-, d, J=5.6 Hz due to $^{2}J_{CH}$),~2.0 (2H,CH_2-,t-like), ~1.94 (2H,CH_2-,t-like), 1.65 (3H,CH_3-,s), 1.56 (6H, 2xCH_3-, s).

Preparation of ethyl 3,7,11-trimethyl-trans-2,6,10-dodecatrien-1-oate (3)

Compound (**3**) was synthesized by a modification of the method of O.P. Vig *et al.* [3]. To a suspension of sodium hydride (0.570 g, 60% NaH in parafin, 14.25 mmol) in 15 ml of dimethoxymethane was added dropwise 2.24 g (9.99 mmol) of triethyl phosphonoacetate at room temperature. The mixture was stirred for 30 min. after which the evolution of hydrogen gas was complete and a clear solution was obtained. The resulting solution was cooled to 0° C with an ice-bath, a solution of 1.70 g (8.7 mmol) of geranylacetone (**2**) in 13 ml of dimethoxymethane was slowly added and then the mixture was gradually warmed to room temperature and stirred for 5 hrs, the reaction being monitored by SiO_2 TLC. The reaction mixture was diluted with water and extracted with ether. TLC on SiO_2 showed three spots; the isomers of trans-2, trans-6 (**3**) and cis-2, trans-6 (**4**), and starting material. The two isomers were separable using preparative TLC by developing three times with n-hexane:ethylacetate (100:3). The yield of (**3**) was 1.05 g (45.5%), while the yield of (**4**) was 0.38 g (16.5 %).

Compound (**3**): ^{13}C NMR 159.8; ^{1}H NMR 5.64 (1H,s), 5.06 (2H, br),4.11 (2H, q, J=7.0 Hz), around ~2.15 (4H, 2xCH_2-,m, due to $^{2}J_{CH}$ and $^{3}J_{CH}$), 2.12 (3H, CH_3-,d, $^{2}J_{CH}$=11.5 Hz), 2.02 (2H,CH_2-,t-like), 1.95 (2H,CH_2-,t-like), 1.63 (3H, CH_3-,s), 1.57 (6H,2x CH_3-), 1.24 (3H, t, J=7.0 Hz).Compound (**4**): ^{13}C NMR 160.02; ^{1}H NMR (unlabeled) 5.62 (1H, CH=C, s), 5.13 (1H, CH=C, t, J=7.2 Hz), 5.05 (1H, CH=C,t, 6.8 Hz), 4.10 (2H, q, J= 7.1 Hz), 2.62 (2H, t, J=7.7 Hz), 2.14 (2H,CH_2-,q-like), 2.02 (2H,CH_2-,t-like), 1.94 (2H,CH_2-,t-like), 1.85 (3H, CH_3-,s), 1.64 (3H, CH_3-,s), 1.58 (3H, CH_3-,s), 1.56 (3H, CH_3-,s), 1.23 (3H, t, J=7.0 Hz).

Preparation of trans-trans-farnesol (5)[3].

To a suspension of lithium aluminum hydride (93.8 mg, 2.47 mmol) in anhydrous ether (26 ml) was added dropwise with stirring a solution of compound (**3**) (1.0 g, 3.77 mmol) in ether (3.8 ml) and the mixture was stirred for 5 hrs at room temperature. The metal complex was decomposed with saturated sodium sulfate, the organic layer was taken up in ether and dried over sodium sulfate. The ^{13}C NMR of the reaction mixture showed two strong signals at 139.9 and 29.2 ppm. The two compounds were separated by chromatography on a SiO_2 column chromatography impregnated with silver nitrate (7%), by eluting with *n*-hexane:ethyl acetate (100:10). The yield of farnesol (**5**), 3,7,11,-trimethyl-trans-2,6,10-dodecatrien-1-ol, was 0.58 g (68.3%). Another compound, which showed at 29.17 ppm, was 3,7,11-trimethyl-trans-6,10-dodecadien-1-ol (76 mg) deduced from ^{1}H NMR assignment. Compound (**5**): ^{13}C NMR 139.9; ^{1}H NMR 5.39 (1H,t,

J=6.6 Hz,$^2J_{CH}$ =~0 Hz),5.08 (2H,2xCH=C, q-like),4.13 (2H,-CH_2OH,t, J=5.9 Hz,$^3J_{CH}$=5.8 Hz), 2.1-1.9 (8H, m), 1.65 (6H, 2xCH_3,s), 1.57 (6H, 2xCH_3,s).

Preparation of farnesylbromide (6) [4]

To the solution of farnesol (5) in acetonitrile (0.577 g/6.6 ml) was added slowly with stirring solid triphenylphosphine (0.747 g, 2.85 mmol). The triphenylphosphine solid gradually dissolved, and then a solution of carbon tetrabromide (0.946 g,2.85 mmol) in acetonitrile (5.77 ml) was added dropwise at 4° C and the resulting solution was stirred for 3 hrs at room temperature. The reaction mixture was concentrated to a small volume, 50 ml of pentane was added, and triphenylphosphine oxide produced in the reaction was precipitated. This procedure was repeated to remove traces of triphenylphosphine oxide. No further purification was carried out. Compound (6): ^{13}C NMR 143.6;^1H NMR 5.51 (1H, t,J=6.6 Hz), 5.06 (2H, 2xCH=C, t-like,br signal),4.00 (2H,-CH_2OH,dd, J=5.9,$^3J_{CH}$=5.6 Hz),2.15-1.9 (8H, m), 1.71 (3H, CH_3-, d, $^2J_{CH}$ =11 Hz), 1.66 (3H, CH_3-, s), 1.58 (6H, 2xCH_3-,s).

Synthesis of Squalene (7)

Squalene (7) with all trans-configuration was stereospecifically synthesized according to the method of Y. Kitagawa et al. [5]. An ethereal solution of pyrrolidine (527 mg/16 ml) was treated with *n*-butyllithium (1.45M, 6.88 ml) at 0° C for 15 min, then cuprous iodide 713 mg was added to the lithium amide and the mixture was stirred 30 min at 0° C. Freshly prepared farnesyl bromide in anhydrous ether (11 ml) was added slowly. The reaction was stirred for 4 hrs at 0° C. The reaction mixture was then diluted with hexane, filtered using a glass filter, washed with 0.1N HCl and then concentrated *in vacuo*. The product was purified by SiO_2 column chromatography (hexane:ethylacetate=100:3). Yield 150 mg (27.7 %) from farnesol (5). ^{13}C NMR 135.1. ^1H NMR ~5.1 (6H, 6xCH=C, m), ~2.0 (20H, 10xCH_2, m), 1.84 (6H, 2xCH_3,s), 1.57 (18H,6xCH_3,s). ^1H NMR of the labeled squalene was nearly identical to that of natural material with added ^{13}C coupling.

Synthesis of 3-[R,S]Squalene oxide(9)[6]

To the solution of 120 mg (0.28 mmol) of squalene (7) in tetrahydrofuran (8 ml) was added 2.0 ml of water at 0° C, the solution becoming cloudy. A small amount of tetrahydrofuran was

added to clear the solution. *N*-bromosuccinimide (56.3 mg,0.31 mmol) was added in small portions over a period of 15 min and the mixture was stirred for 25 min under nitrogen. Water was added and the reaction mixture was extracted with hexane. After the evaporation of hexane, the residue was subjected to SiO_2 column chromatography using hexane:ethylacetate (100:8) to yield pure bromohydrin (**8**) (51 mg,34.6 %). ^{13}C NMR 134.95, 135.19; ^1H NMR 5.2-5.05 (5H, 5xCH=C, m), 3.95 (1H, dd, J= 11.2,1.35 Hz), 2.2-1.9 (20H, 10xCH_2, m), 1.66 (3H, CH_3-,s), 1.58 (15H, 5xCH_3,s), 1.32 (3H,CH_3-,s), 1.30 (3H,CH_3-,s).

Compound (**8**) was dissolved in 4 ml of methanol, potassium carbonate (20.1 mg) was added and the mixture was stirred for 2 hrs at room temperature under nitrogen. The reaction mixture was then diluted with water, extracted with hexane and dried over sodium sulfate. After evaporation of hexane, pure squaleneoxide (40 mg) was obtained in 93% yield. This compound was pure chromatographically and NMR spectroscopically. ^{13}C NMR in $(CD_3)_2CO$ 135.5; ^1H NMR in $(CD_3)_2CO$ 5.2-5.1 (5H, 5xCH=C,m), 2.62 (1H, t, J=3.6 Hz), 2.2-1.9 (CH_2 groups,), 1.63 (3H,CH_3-,s), 1.60 (3H,CH_3-,s), 1.59 (6H, 2x CH_3-,s), 1.57 (3H,CH_3-,s), 1.26 (3H,CH_3-,s), 1.20 (3H,CH_3-,s).

Synthesis of Lanosterol

Labeled squalene oxide (9) (1.8 mg in 0.25 ml hexane) and 0.7 mg of Triton X-100 in 0.025 ml acetone were placed in a 10 mm NMR tube and blown dry with a stream of nitrogen. To the oily residue was added 0.9 ml 0.6M phophate buffer (pH 7.4) and 0.3 ml D_2O, the mixture was sonnicated until clear, and then mixed well with 1.5 ml of partially purified yeast squalene oxide cyclase solution containing 2.2 mg protein and 0.06% w/v Triton X-100 (7). The resulting solution was degassed by bubbling with nitrogen for 5 min, a nitrogen aerating/mixing apparatus was inserted into the tube, nitrogen flow was adjusted to produce approximately 5 bubbles/sec and the tube was lowered into the magnet of a 300 MHz NMR instrument. The reaction mixture was maintained at 30° and ^{13}C spectra were recorded in one hour blocks for 12 hours. At the end of this period approximately 40% of the original DL-squalene oxide had been consumed and lanosterol whose labeled peaks were identical to those of a standard crystallized from solution as a white powder. Extraction with hexane and HPLC (Altech silica gel, 25 cm x 4.7 mm ID, hexane:isopropanol 100:2, uV detection at 210 mm) analysis indicated only unreacted squalene oxide and lanosterol were present.

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REFERENCES

1 Dauben W.G. and Bradlow H.L. - J. Am. Chem. Soc. 74: 5204-5206 (1952).

2 Krapcho A.P. and Lovey A. J. - Tetrahedron Lett., 957-960 (1973).

3 Vig O.P. , Kapur J.C., Khurana C.K. and Vig B. - J. Indian Chem. Soc. 46: 505-511 (1969).

4 Axelrod E.H., Milne G.M., van Tamelen E.E. - J. Am. Chem. Soc. 92: 2139-2141 (1970).

5 Kitagawa Y., Oshima K., Yamamoto H. and Nozaki H. - Tetrahedron Lett., 1859-1862 (1975).

6 Methods in ENZYMOLOGY, Academic Press, 15: 346-349, (1969). Edited by Clayton R.B.

7. Hoshino T., Williams H.J., Chung Y., Scott A.I., In preparation.