SYNTHESIS OF TRITIUM-LABELLED CEMBRENE-A

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SUMMARY

The synthesis of [16-^{3}H]-cembrene-A from unlabelled cembrene-A is achieved in four steps *via* selective hydroboration-oxidation of the isopropenyl double bond, borotritide reduction of the 16-aldehyde, and *o*-nitroselenoxide elimination to regenerate the labelled olefin. This sequence is of general utility in labelling polyisoprenoid hydrocarbons for metabolic studies. Cembrene-A is a putative intermediate in the conversion of geranylgeranyl pyrophosphate to the polycyclic trinervitane and kempane diterpenes of nasute termite soldiers.

Keywords: diterpene hydrocarbon, cembrenoids, termite defense secretion

Cembrenoid diterpenes (1) are non-uniformly distributed in nature, with the four primary sources being tobacco (2), *Commiphora mukul* sap (3), soft corals (4), termite soldiers (5-8), termite workers (9-10), and ants (11). Neither the biosynthesis of cembrene-A (presumably from geranylgeranyl pyrophosphate) nor its conversion into any simple epoxide, diol, ether, lactone, or complex polycyclic structure has yet been demonstrated *in vivo* or *in vitro*. One impediment to evaluating the intermediacy of cembrene-A in the biogenesis of cembrenoids has been the absence of a radiolabelled isotopomer for metabolic studies. We report herein a mild and selective process for introducing a high specific activity tritium label at C-16 (the methylidene carbon). The method has general applicability to the labelling of polyolefinic terpene hydrocarbons bearing an isopropenyl side chain.

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Cembrene-A is not readily obtainable from any natural source in gram quantities, although isolation from termite soldiers (12,13), corals (14,15), and Commiphora mukul (3) is possible. For this work, racemic cembrene-A (1) was prepared in six steps from (E,E)-geranyllinalool via intramolecular addition of the allylic thiophenyl ether anion to the terminal epoxide by the method of Itô (16). Reaction of cembrene-A (Fig. 1) with 1 equivalent of 9-borabicyclononane (9-BBN) followed by treatment with basic peroxide gave the 16-hydroxylated cembrene-A (2). This was readily separated from the unreactive isopropylidene isomer which is present as a hard-to-remove minor component in the synthetic cembrene-A. Oxidation with pyridinium chlorochromate (PCC) in CH_2Cl_2 afforded the 16-aldehyde (3).

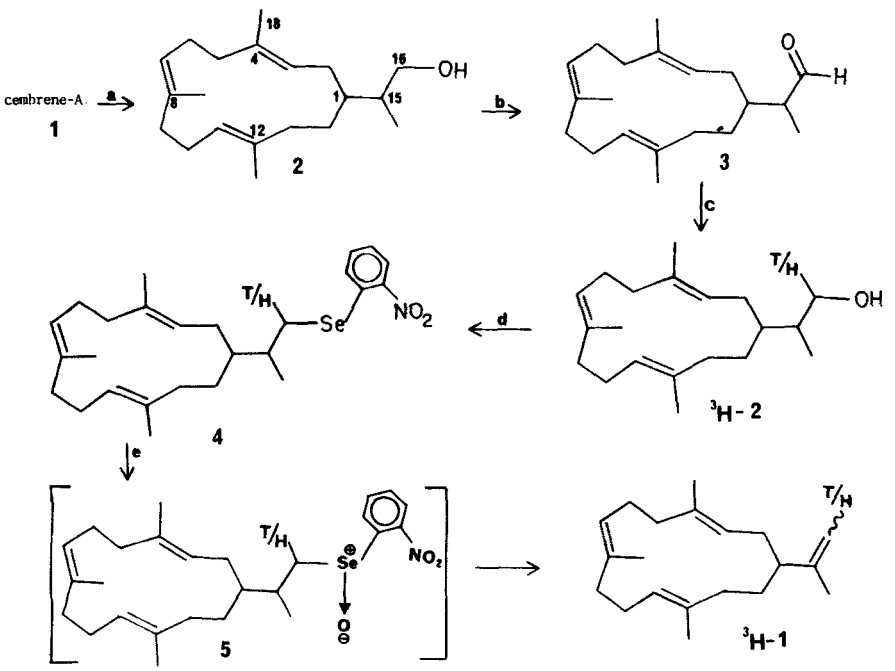

Figure 1. Synthesis of ^{3}H-Cembrene-A. Reagents: (a) 9-BBN, THF; H_2O_2, NaOH; (b) PCC, CH_2Cl_2; (c) $NaBH_3T$, C_2H_5OH; (d) o-$NO_2PhSeCN$, Bu_3P, THF; (e) H_2O_2.

Reduction of the aldehyde with [^{3}H]-$NaBH_4$ (100 mCi, specific activity 5.7 Ci/mmol) in ethanol gave an essentially quanitative yield of the

[16-^{3}H]-16-hydroxy compound (^{3}H-2). After chromatography, the alcohol was treated with recrystallized o-nitrophenylselenocyanate (17,18) and tri-n-butyl phosphine in THF, and the intermediate selenide 4 was oxidatively eliminated via 5 to regenerate the [16-^{3}H]-labelled isopropenyl side chain. After purification by flash silica chromatography and $AgNO_3$-silica chromatography, 37% radiochemical yield of ^{3}H-cembrene-A (specific activity 1.5 Ci/mmol) was realized from the aldehyde. This material was stored in degassed hexane-benzene (90:10) at -20° C; nonetheless, slow decomposition was a severe problem (half life < 4 weeks) even in 10 mM solutions.

Preliminary experiments using Nasutitermes corniger soldiers (5), and workers were performed to detect incorporation of [16-^{3}H]-cembrene-A into biosynthesized trinervitanes (e.g., 6, Fig. 2). Neither topical application in vivo nor in vitro incubation with homogenates provided any soldier-specific metabolites with trinervitane mobilities on TLC (15) (autoradiographic detection). Either ^{3}H-cembrene-A is not a free intermediate in the pathway, or the hydrophobic material does not have access to the enzymatic sites under these assay conditions. Both in vitro and in vivo techniques had proven successful earlier in Nasutitermes using [^{14}C]-labelled acetate and mevalonate (19).

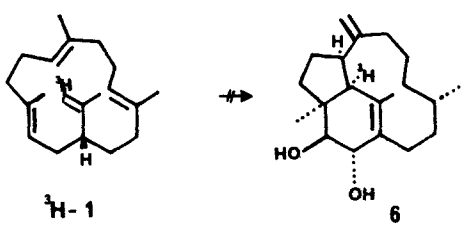

Figure 2. Putative intermediacy of cembrene-A in diterpene biogenesis in Nasutitermes spp.

EXPERIMENTAL

16-Hydroxy-cembrene-A (2). To a solution of cembrene-A (1) (140 mg, 0.5 mmol) in THF (10 ml) was added 0.5 M 9-BBN in THF (1.2 ml, 0.6 mmol) at

room temperature. The reaction mixture was refluxed for 20 min, cooled, treated with ethanol (3 ml), 3 M NaOH (3 ml) and 30% H_2O_2 (2 ml), and stirred for 4 h at 20° C. After saturation with anhydrous K_2CO_3, the mixture was extracted with ether. The combined organic layers were washed (satd. NaCl), dried (anhydrous $MgSO_4$) and evaporated. Flash column chromatography (20) (2% ethyl acetate/hexane) gave the 16-hydroxy-cembrene-A (2) (90 mg, 62%, R_f 0.49, 20% ethyl acetate/hexane) as an inseparable mixture of diastereoisomers: ^1H NMR (80 MHz, $CDCl_3$): δ0.94 (d, 3H, H-17, J = 7.2 Hz), 1.56 (br s, 9H, H-18, 19, 20), 3.54 (m, 2H, H-16), 5.02 (m, 3H, H-3, 7, 11).

Cembrene-A-aldehyde (3). To a solution of the above alcohol (86 mg, 0.3 mmol) in CH_2Cl_2 (5 ml) was added PCC (77 mg, 0.36 mmol) in one portion at room temperature. The black reaction mixture was stirred for 2 h and then anhydrous ether (25 ml) was added. The black residue was decanted out and washed several times with ether. The combined organics were filtered through Celite and evaporated. Flash chromatography (1% ethyl acetate/hexane) gave the aldehyde (3) as an oil (70 mg, 81%, R_f 0.56, 30% ethyl acetate/hexane): IR (film) 2675 cm^{-1}, 1710 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$): δ0.99 (d, 3H, H-17, J = 6.95 Hz), 1.58 (br s, 9H, H-18, 19, 20), 5.00 (br m, 3H, H-3, 7, 11), 9.66 (d, 1H, H-16, J = 1.14 Hz) (chemical shifts shown for one of the diastereomers.

[16-^{3}H]-16-Hydroxy-cembrene-A (^{3}H-2). This was conducted after successful execution of a microscale "cold" run with $NaBH_4$. A solution of the aldehyde (3) (26 mg, 0.08 mmol) in ethanol (2 ml) was added to an ampoule containing a small stirring bar and [^{3}H]-$NaBH_4$ (0.8 mg, 0.02 mmol, specific activity = 5.7 Ci/mmol) at room temperature. After stirring for 1 h, 0.5 M acetic acid (0.5 ml) was added and the reaction mixture was transferred to a round bottom flask, washing the ampoule several times with ether. Solvents were removed *in vacuo* and 1:1 ether-hexane (8 ml) was added. The organic phase was washed (satd. $NaHCO_3$), dried ($MgSO_4$) and concentrated. Purification of the crude labelled alcohol by chromatography in a Pasteur pipette (2% ethyl acetate/hexane) afforded 9.6 mg of slightly impure and 6 mg of the homogeneous tritiated 16-hydroxy-cembrene-A (^{3}H-2).

[16-^{3}H]-Cembrene-A (^{3}H-1). To a stirred solution of tritiated alcohol (^{3}H-2) (6 mg, 0.02 mmol) in THF (2 ml) was added o-NO_2PhSeCN (9.4 mg, 0.04 mmol). After 10 min, $(n\text{-Bu})_3P$ (10.5 µl, 0.04 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. It was then cooled to 0° C, 30% H_2O_2 solution (22 µl, 0.2 mmol) was added, and the reaction mixture was stirred for 3 h at room temperature. Saturated $NaHCO_3$ solution (1 ml) was added and the reaction mixture was extracted with 10% ethyl acetate/hexane. The combined organic layers were washed (satd. NaCl), dried (Na_2SO_4, $MgSO_4$). Solvents were removed in vacuo to afford a yellow residue which was dissolved in minimum amount of CH_2Cl_2 and loaded on to a silica-gel column. Elution with hexane gave pure [16-^{3}H]-cembrene-A (3 mg, 64% yield, specific activity = 1.5 Ci/mmol).

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