BIOSYNTHETIC STUDIES OF MARINE LIPIDS 8.[1] COURSE OF THE STEREOSELECTIVE ALKYLATION AND REGIOSELECTIVE HYDROGEN MIGRATION IN THE BIOSYNTHESIS OF THE SPONGE STEROL 24(S)-24-ISOPROPENYLCHOLESTEROL

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Abstract - The detailed mechanism of the formation of (24S)-24-isopropenylcholesterol, the key intermediate in the biosynthesis of 24-isopropylcholesterol, was studied in a Pseudaxinyssa sponge species. A stereoselective alkylation at C28 of (iso)fucosterol was demonstrated involving a regioselective hydrogen migration.

INTRODUCTION

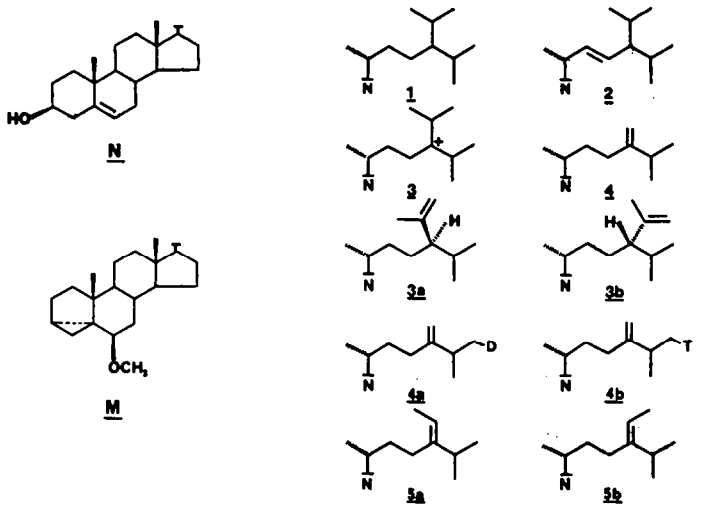
In our previous investigation of the biosynthesis of 24-isopropyl sterols in a Pseudaxinyssa sponge species we established the major biosynthetic pathway whereby 24-isopropylcholesterol[1] is formed through triple bioalkylation. Although this sterol (1), together with its 22-dehydro analog (2), comprises 98% of the total sponge sterol mixture, no advantage can be taken of the efficient incorporation of precursors into these sterols to study the stereochemistry and regioselectivity in the earlier steps of their biosynthesis. This is due to a lack of functional groups close to the site of bioalkylation that can be utilized in an appropriate chemical degradation.

(24S)-24-Isopropenylcholesterol (3b), which meets these requirements, is only present to the extent of 1% of the total sterol mixture. To overcome the problem of its low abundance, a synthetic co-carrier has to be added. Such dilution with a cold co-carrier will not result in a problem of low specific activity since the specific activity of this sterol (3a) was the highest of all biosynthetic intermediates in our previous[1] experiment with [28-^{14}C]-24-methylenecholesterol (4) and [22-T]-24-ethylidenecholesterol (5). Therefore, incorporation of a specifically labelled [26(27)-T]-24-methylenecholesterol (4b) could offer insight into the detailed mechanism of regioselective hydrogen migration.

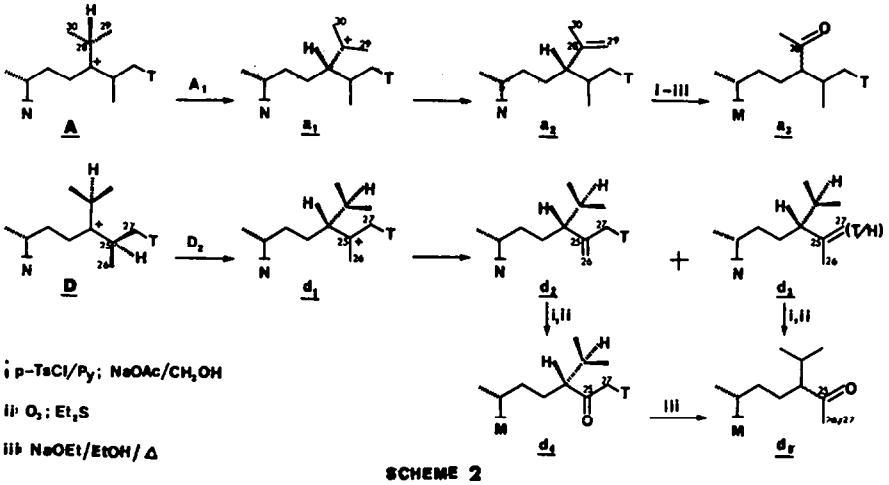
In the preceding paper,[1] we considered the four possible conformations with the migrating hydrogen perpendicular to the plane of the key carbocation intermediate 3; we concluded that only two of these conformations (presented as A and D in Scheme 2) can afford the natural 24(S)-24-isopropenylcholesterol (3b). Hydrogen migration along path A_1 will give an olefin a_2 which will retain all the tritium label through the sequences i, ii and iii of chemical transformation. The alternative migration along path D_2, which will afford the natural 3b, will give a mixture of olefins (d_2,d_3) with about 17% loss of the initial radioactivity (ignoring possible isotope effects). The radioactivity of this sterol mixture will not change when subjected to transformation i (protection). Loss of radioactivity should occur in the next step ii (ozonolysis) due to component d_3 and a complete loss of activity is to be expected at the final step iii (base exchange). Intermediate values of loss of radioactivity should correspond to a partial regioselectivity involving hyrogen migrations from both positions 25 and 28 in a ratio which could be presented as the difference between loss of activity at step ii (ozonolysis) and step iii (hydrogen exchange). The establishment of the regioselectivity would further allow the establishment of the site of the final bioalkylation, since the corresponding conformation A arises from α-face alkylation, while conformation D is the result of an attack from the β-face.

RESULTS AND DISCUSSION

For the synthesis (Scheme 3) of [26(27)-T]-24-methylenecholesterol (4b), the iodide 6 was obtained via a literature procedure[2] and was treated with the dianion 7 from ethyl acetoacetate,



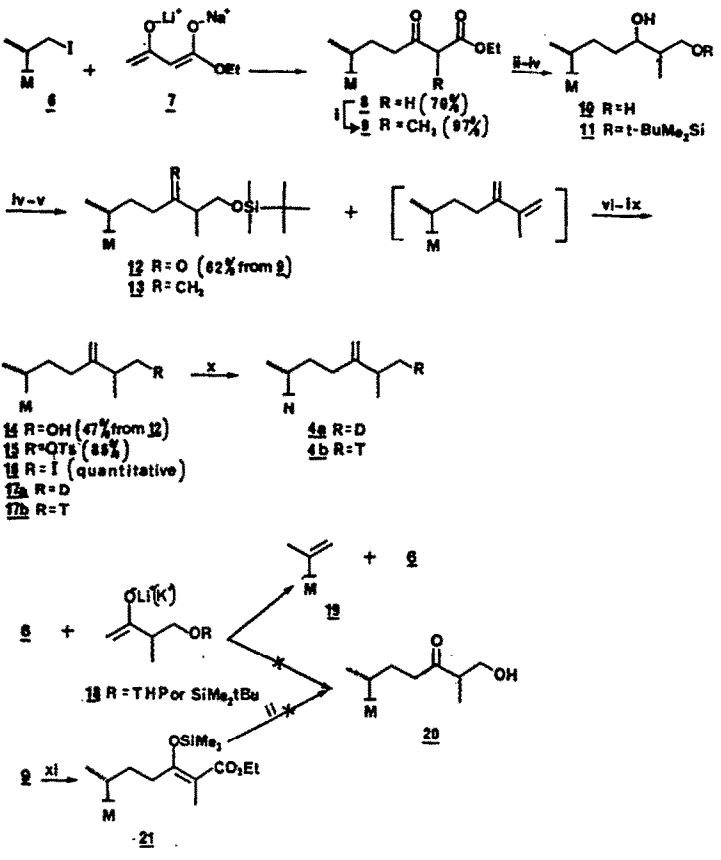
SCHEME 1



SCHEME 2

generated by the method of Weiler,[3] to produce the keto ester 8. Contrary to expected results, the attempted alkylation of the dianion from methyl 2-methylacetoacetate with iodide 6 under similar conditions failed, necessitating a separate alkylation step to convert 8 into 9. After reduction of the keto ester 9 with excess lithium aluminium hydride, the diol 10 was selectively protected as the monosilyl ether 11[4] and then oxidized to the ketone 12 with pyridinium dichromate in DMF.[5] The conversion of ketone 12 to the 24-methylene derivative 13 was effected by means of a Wittig reaction which was accompanied by partial elimination to the corresponding conjugated diene. The latter product was most conveniently removed by chromatography after desilylation of the crude product 13 to the more polar alcohol 14. NMR evidence suggests that alcohol 14 is formed as a mixture of diastereomers in roughly equal amounts. The corresponding tosylate 15 was cleanly reduced to 17a with lithium aluminium deuteride. Alternatively, tosylate 15 was first converted to the iodide 16, which reacted slowly with sodium borodeuteride in DMSO at room temperature[6] to furnish a mixture of 17a and unreacted iodide. The remaining starting material was then reduced to completion with lithium aluminium deuteride. Acid-catalyzed hydrolysis of the i-methyl ether 17a then completed the synthesis of 4a. The final product was identified by its NMR and mass spectra. No significant isomerization of the double bond to produce desmosterol occurred during this sequence. Finally, the conversion of the iodide 16 to the tritiated product 4b was performed under identical conditions to those employed for 4a, except that tritium-labelled sodium borohydride was used, followed by cold lithium aluminium hydride to remove unreacted iodide.

Two alternative syntheses of the key intermediate 12 were also investigated (Scheme 2), but



i – KOt-Bu; MeI; ii – $LiAlH_4$; H_3O^+; iii – t-$BuMe_2SiCl$/imidazole; iv – PDC/DMF; v – $CH_2{=}PPh_3$; vi – $nBu_4N^+F^-$; vii – TsCl/Py; viii – NaI; ix – NaBD(T)$_4$; x – TsOH/H_2O; xi – $(Me_3Si)_2NH$/imidazole.

SCHEME 3

proved fruitless. All attempts to react iodide 6 (or the corresponding tosylate) with enolate 18 failed, even under forcing conditions (THF-HMPA, 18-crown-6, reflux). The only identifiable products, apart from unreacted starting material 6 were the olefin 19 and self-condensation products of the enolate. Furthermore, attempts to selectively reduce the ester moiety of the keto ester 9 by prior protection of the ketone function as its enol silyl 21 failed as only complex product mixtures ensued.

Incubation of the sponge Pseudaxinyssa sp. with [26(27)-T]-24-methylenecholesterol (4b) according to an earlier described field method[1] furnished the desired labelled (24S)-24-isopropenylcholesterol (3b). A cold co-carrier of a mixture of (24R) (3a) and (24S) (3b) sterols was added, followed by protection of the free sterols in the form of i-methyl ethers (M) and ozonolysis. By monitoring the specific activity along the sequence of performed chemical transformation i --> ii, it was established that the specific activity of 3.83×10^8 dpm/mmol remained constant during the chemical transformations, although the total activity dropped from 3.6×10^5 dpm to 2.8×10^5 dpm in agreement with an overall yield of 78% for the above sequence. No change was detected after an attempted hydrogen exhange (cf. iii in Scheme 2) in the resulting ketone 8$_3$.

Therefore we conclude that there is no tritium bound directly to the double bond (d_1) in (24S)-24-isopropenylcholesterol (3b) nor is there a tritium bound to the methyl group (C26(27)) adjacent to the double bond (d_2). This confirms a regioselective (H28 --> C24) H-migration along pathway A_1 rather than D_2 and leads us to the conclusion that Me^+ attacks the double bond of (iso)fucosterol (3a,3b) from the α-face of the molecule in the final bioalkylation step.

EXPERIMENTAL SECTION

Synthesis of [26(27)-T]-24-methylenecholesterol (17b).

Keto ester 8. A dispersion of NaH in mineral oil (1.29 g of 57% content, 31 mmol) in 45 ml of dry THF was cooled to 0° C. Ethyl acetoacetate (3.19 g, 25 mmol) was added in 10 ml of THF, followed after 5 min by n-butyllithium (19 ml of 1.3 M solution, 25 mmol). After stirring the resulting dianion solution for 10 min at 0° C, iodide 6 (0.78 g, 1.71 mmol) was added in 5 ml of THF and stirring was continued for 2 h at room temperature. The reaction was then quenched with water, diluted with 50 ml of ether, washed several times with aqueous NaCl, dried with $MgSO_4$, and evaporated to dryness. Flash chromatography over silica gel (elution with 5% EtOAc-hexane) provided 0.55 g (70%) of the keto ester 8, Rf 0.64 (20% EtOAc-hexane), NMR (60 MHz) 4.1 (q, 2H), 3.33 (s, 2H), 3.20 (s, 3H), 1.20 (t, 3H), 0.95 (s, 3H), 0.65 (s, 3H).

Keto ester 9. Keto ester 8 (0.46 g, 1.00 mmol) and freshly sublimed potassium t-butoxide (118 mg, 1.05 mmol) were stirred in 5 ml of dry THF for 5 min. Methyl iodide (0.075 ml, 1.2 mmol) was added and the mixture was allowed to stir 24 h at room temperature. Workup as for keto ester 8 afforded, after flash chromatography, 0.46 g (97%) of the desired product 9, Rf 0.70 (20% EtOAc-hexane); IR (film) 1735, 1707 cm^{-1}; NMR (300 MHz) 4.19 (q, J=7.1 Hz, 2H), 3.52 (crude q, 1H), 3.32 (s, 3H), 1.33 (d, J=7.0 Hz, 3H), 1.27 (t, J=7.1 Hz, 3H), 1.02 (s, 3H), 0.90 (d, J=6.4 Hz, 3H), 0.71 (s, 3H).

Silyl ether 12. Keto ester 9 (114 mg, 0.24 mmol) and $LiAlH_4$ (100 mg, 2.6 mmol) were stirred for 1.5 h in 5 ml of dry THF. The mixture was treated with excess EtOAc, then with a minimum quantity of water and was filtered through a short column containing a layer of $MgSO_4$ between two layers of Celite. The filtrate was evaporated in vacuo to provide the crude diol 10, Rf ca 0.1 (20% EtOAc-hexane), (no C=O stretch in the IR spectrum). The above diol, t-butyldimethylsilyl chloride (38 mg, 0.25 mmol) and imidazole (34 mg, 0.50 mmol) were stired for 3 h at room temperature in 2 ml of dry DMF. The mixture was then diluted with ether, washed 3 times with aqueous NaCl, dried with $MgSO_4$ and evaporated to dryness to afford the crude monosilyl ether 11, Rf 0.64 (20% EtOAc-hexane). The above silyl ether was stirred for 6.5 h with freshly prepared pyridinium dichromate (376 mg, 1.00 mmol) in 5 ml of DMF. The mixture was then diluted with ether, filtered through Celite, washed 3 times with aqueous NaCl, dried with $MgSO_4$ and flash chromatographed over silica gel (elution with 5% EtOAc-hexane) to furnish 81 mg (62% overall) of the silyl ether 12, Rf 0.79 (20% EtOAc-hexane); IR (film) 1709 cm^{-1}; NMR (300 MHz) 3.32 (s, 3H), 1.02 (s superimposed on d, total 6H), 0.90 (d, J=6.3 Hz, 3H), 0.87 (s, 9H), 0.71 (s, 3H), 0.03 and 0.02 (s, total 6H).

Alcohol 14. Silyl ether 12 (81 mg, 0.15 mmol) and the phosphorane freshly prepared from 1.0 mmol of methyltriphenylphosphonium iodide and n-butyllithium were stirred for 3 h in 7 ml of dry THF. The solution was evaporated to dryness and flash chromatographed over silica gel (elution with 20% benzene-hexane) to give the olefin 13 together with the diene elimination product, as ascertained by NMR. The above product was dissolved in 5 ml of THF and 0.7 ml of 1 M tetrabutylammonium fluoride in THF was added. After 6 h, the solution was diluted with ether, washed 3 times with aqueous NaCl, dried with $MgSO_4$ and evaporated in vacuo. Flash chromatography over silica gel (elution with 10% benzene-hexane) gave first the diene and then (elution with 10% EtOAc-hexane) 30 mg (47% overall) of the desired alcohol 14, Rf 0.41 (20% EtOAc-hexane); IR (film) 3400, 1640 cm^{-1}; NMR (300 MHz) 4.88 (s, 1H), 4.82 (s, 1H), 3.32 (s, 3H), 1.05 (two closely overlapping d, 3H), 1.02 (s, 3H), 0.94 (d, J=6.3 Hz, 3H), 0.72 (s, 3H); mass spectrum, m/e (relative intensity, %) 428 (4, M^+), 373 (13), 296 (10), 253 (18), 161 (22), 159 (29), 147 (26), 145 (40).

Tosylate 15. The alcohol 14 (20 mg, 0.047 mmol) and freshly recrystallized p-toluenesulfonyl chloride (50 mg, 0.26 mmol) were stirred in 0.5 ml of pyridine for 21 h at room temperature. The mixture was then diluted with ether, washed 3 times with aqueous NaCl, dried over $MgSO_4$ and evaporated in vacuo to afford 23 mg (85%) of the desired tosylate 15, Rf 0.65 (20% EtOAc-hexane); NMR (300 MHz) 7.78 (d, J=8 Hz, 2H), 7.34 (d, J=8 Hz, 2H), 4.77 (s, 1H), 4.70 and 4.67 (s, total 1H), 3.32 (s, 3H), 1.02 (s overlapping with d, total 6H), 0.88 (d, J=6.3 Hz, 3H), 0.70 (s, 3H).

Reduction of Tosylate 15 with $LiAlD_4$. The tosylate 15 (7 mg) and $LiAlD_4$ (3 mg) were stirred for 21 h in 1 ml of dry THF. Ethyl acetate was then added, followed by two drops of water. The mixture was evaporated to dryness in a stream of N_2 and the residue was triturated with hexane and dried with $MgSO_4$. The hexane solution was chromatographed over a short column of silica gel (elution with hexane) to afford 4 mg (80%) of the 26(27)-deuterio derivative 17a, Rf 0.52 (50% benzene-hexane); NMR (300 MHz) 4.71 (s, 1H), 4.65 (s, 1H), 3.32 (s, 3H), 1.02 (s, 3H), 0.94 (d, J=6.3 Hz, 3H), 0.86 (d, J=7.2 Hz, 3H), 0.72 (s, 3H); mass spectrum, m/e (relative intensity, %) 413 (16, M^+), 398 (23), 358 (34), 328 (21), 285 (20), 253 (25).

Reduction of Iodide 16 with $NaBD_4$. The tosylate 15 was refluxed with a ca 10-fold excess of NaI in acetone for 25 h. The mixture was evaporated to dryness, triturated with hexane and chromatographed over a short column of silica gel (elution with hexane) to afford the iodide 16 quantitatively, Rf 0.44 (50% benzene-hexane); NMR (300 MHz) 4.84 (s, 1H), 4.78 (s, 1H), 3.32 (s, 3H), 1.17 (d, J=6.9 Hz, 3H), 1.02 (s, 3H), 0.94 (d, J=6.6 Hz, 3H), 0.72 (s, 3H). The above iodide (6 mg) and $NaBD_4$ (2 mg) were stirred for 15 h in 0.15 ml of dry DMSO at room temperature. Several drops of water were added and the mixture was extracted with 3 portions of chloroform and passed through a short column of silica gel. NMR and TLC analysis indicated the presence of roughly equal amounts of the starting iodide 16 and product 17a. The crude mixture was dissolved in 1 ml of dry THF and treated for 1.5 h with $LiAlD_4$ (10 mg). Workup as in the reduction of 15 with $LiAlD_4$ then furnished the pure 26(27)-deuterio derivative 17a, identical to that from the

previous procedure.

26(27)-Deuterio-24-methylidenecholesterol 4a. Product 17a, produced from iodide 16 in the previous procedure, was dissolved in 0.5 ml of dioxane and 0.1 ml of water containing a crystal of p-toluenesulphonic acid. The mixture was heated for 1 h at 80 C, then concentrated in a stream of nitrogen, triturated with hexane, dried over $MgSO_4$ and chromatographed over a short column of silica gel (elution with 20% EtOAc-hexane) to afford the pure title compound 4a, Rf 0.45 (20% EtOAc-hexane); NMR (300 MHz) 5.35 (crude d, 1H), 4.71 (s, 1H), 4.65 (s, 1H), 1.01 (s overlapping with d, total 6H), 0.95 (d, J=6.3 Hz, 3H), 0.68 (s, 3H); mass spectrum, m/e (relative intensity, %) 399 (14, M^+), 315 (25), 314 (90), 300 (17), 299 (27), 281 (25), 272 (24), 271 (54), 229 (31).

26(27)-Tritio-24-methylidenecholesterol 4b. The title compound was prepared from iodide 16 in the same manner as the deuterio derivative 4a, using the tritium-labelled $NaBH_4$ (ca. 1 mg, ca 10 mCi) followed by cold $LiAlH_4$ to reduce any unreacted iodide. The product was identical to 4a on TLC and contained 92 mCi of incorporated tritium (ca. 1% incorporated radioactivity).

Incorporation experiment.

Pseudaxinyssa sp. (Australian Museum specimen number Z4988) was collected at John Brewer Reef, Great Barrier Reef, and was transplanted onto plastic plaques one month prior to use. The precursor was incorporated in duplicate specimens via 12 h aquarium incubations and the specimens were returned to the sea for 27 days before collection and analysis.[1]

ACKNOWLEDGEMENTS

We thank M. J. Garson for field assistance and D. Tarker for provision of facilities. Financial support was provided by NIH grants GM06840 and GM28352. Use of the 300 MHz ^1H NMR facility at Stanford was made possible by NSF grant CHE81-09064. We thank Ruth Records for recording low resolution mass spectra and Prof. R.D. Simoni for the use of his liquid scintillation counter. T.G. Back thanks the Natural Sciences and Engineering Research Council of Canada for assistance in the form of an International Collaborative Grant during his sabbatical leave from the University of Calgary to Stanford University (January -June, 1985). This is contribution number 320 from the Australian Institute of Marine Science.

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