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BIOSYNTHETIC STUDIES OF MARINE LIPIDS 7[1]. EXPERIMENTAL DEMONSTRATION OF A DOUBLE ALKYLATION AT C-28 IN THE BIOSYNTHESIS OF 24-ISOPROPYLCHOLESTEROLS IN A SPONGE

Ivan L. Stoilov,[a] Janice E. Thompson,[a,b] and Carl Djerassi[a*]

[a]Department of Chemistry, Stanford University, Stanford, CA 94305 USA
and
[b]Australian Institute of Marine Science, Townsville, Queensland, 4810 Australia

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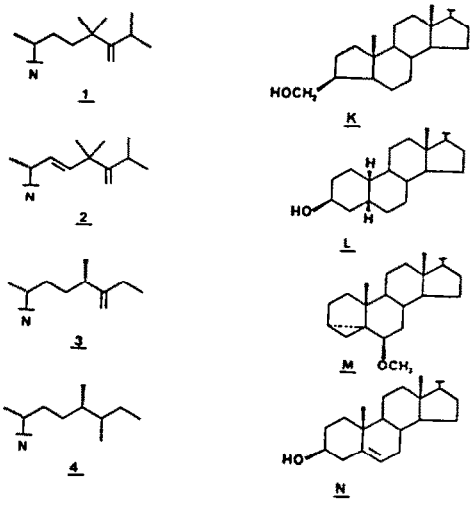
Abstract - The biosynthesis of 24-isopropyl sterols comprising 99% of the sterols of the Great Barrier Reef sponge Pseudaxinyssa sp. was studied. The major biosynthetic pathway was established by incorporation of 24-methylenecholesterol-[28-^{14}C], fucosterol-[22-T], isofucosterol-[22-T], (24R)-24-isopropenylcholesterol-[29-^{14}C], (24S)-24-isopropenylcholesterol-[29-^{14}C], DL-mevalonate-[2-^{14}C] and L-methionine-[methyl-^{14}C]. Very efficient side chain branching through double alkylation at C28 was demonstrated in contrast to relatively poor de novo biosynthesis. A stereoselective mode for hydrogen migration is observed and a regioselective mode is proposed for this migration.

INTRODUCTION

Intensive studies on sterols from marine organisms during the last two decades have furnished a bewildering variety of new sterols[2] with unusual nuclei[3,4] and side chain alkylation patterns.[5] The recent isolation of sterols 1 and 2 (Scheme 1) with an intriguing new pattern of side chain alkylation[6] demonstrates the ever expanding range of unprecedented sterols in marine organisms, suggesting that, as predicted earlier,[7] other examples are awaiting discovery.



SCHEME 1

Biosynthetic studies on sponge sterols are largely limited to the unusual A-nor- (K) and 19-nor-sterol (L) nuclei (Scheme 1),[8,9] while the biosynthesis of sterols with unusual side chain alkylation patterns still remains unexplored.[10] Only recently did we report[11] the first experimental demonstration of the course of side chain extension in marine sponges through a study of the biosynthesis of 25,26-dahydroaplysterol (3) and aplysterol (4) in the sponge Aplysina fistularis. Methodological problems that have hindered progress on the biosynthesis of sponge sterols have now been largely solved in our laboratory, thus allowing rapid progress in this field. The techniques developed[12] for separation of trace sterols, which often provide "missing links" in predicted biosynthetic pathways, as well as techniques developed[11] for microscale synthesis of radioactive labelled sterol precursors made possible a systematic approach to the biosynthesis of these intriguing sterols. Finally, a new field method for the incorporation

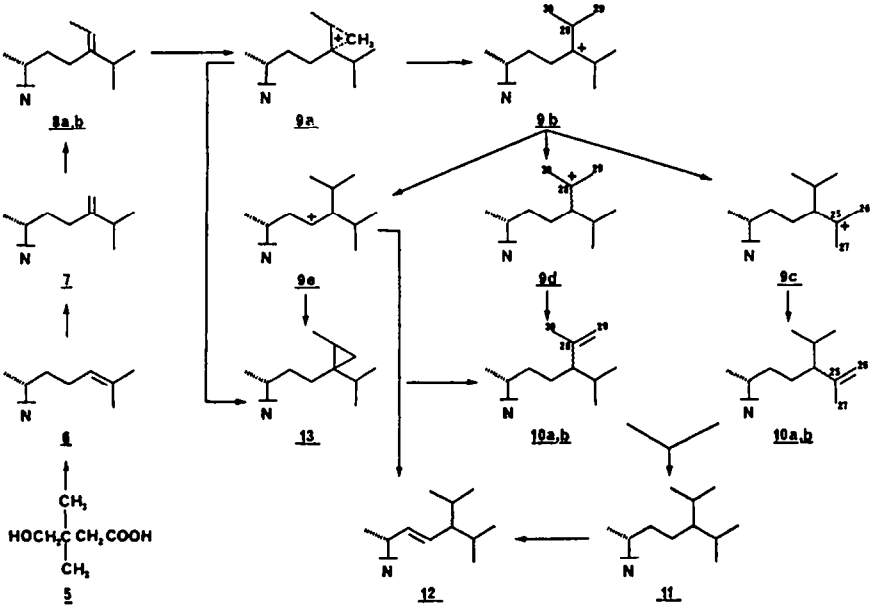
of labelled precursors, tested simultaneously in the fatty acid biosynthesis of sponges,[13] allows incorporation experiments with sponges that cannot be maintained under laboratory conditions or which occur in regions without access to running sea water or electricity.

The Great Barrier Reef sponge Pseudaxinyssa sp. (Axinellida)[14] is of particular interest since its sterol mixture consists of the unconventional sterols 10a, 11 and 12, with sterols 11 and 12 accounting for 98% of this mixture.[12,15,16] Analysis of the membrane fractions isolated from this sponge confirmed that these unconventional sterols are almost exclusively associated with membranes,[17] which suggests a role in membrane function.[18]

In order to define the precise biosynthetic course by which an isopropyl substituent at C-24 is introduced, we undertook a systematic biosynthetic study with appropriately labelled sterol precursors, based on plausible, but hitherto unproven, biosynthetic pathways as outlined in Scheme 2. The following seven labelled precursors were incorporated separately into each sponge specimen using the earlier described incorporation procedure:[13] [2-^{14}C]-DL-mevalonic acid DBED salt (5), [methyl-^{14}C]-L-methionine (30), [28-^{14}C]-24-methylenecholesterol (7), [22-^{3}H]-[24Z]-24-ethylidenecholesterol (fucosterol) (8a), [22-^{3}H]-[24E]-24-ethylidencholesterol (iso-fucosterol) (8b), [30-^{14}C]-(24R)-24-isopropenylcholesterol (10a), and [30-^{14}C]-(24S)-24-isopropenylcholesterol (10b). The five precursors 7, 8a, 8b, 10a and 10b were synthesized as outlined in Scheme 3.

RESULTS AND DISCUSSIONS

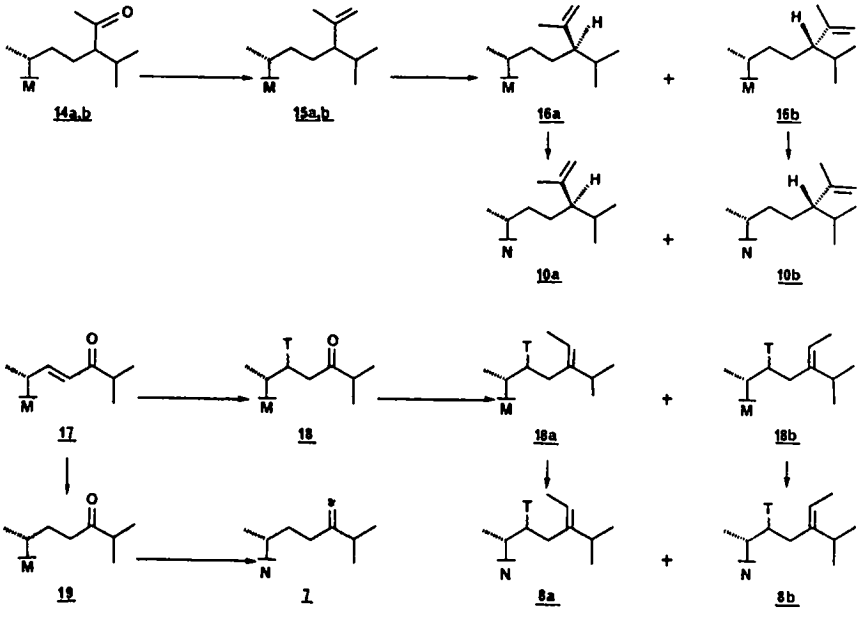
The results of the incorporations for each radiolabelled precursor in Pseudaxinyssa sp. are summarized in Table 1. Each sterol precursor tested was taken up by the sponge and was either effectively incorporated (>70%) into the major sterols of the sponge, transformed into other minor sterols of the sponge, or recovered unchanged, depending on precursor fitness in the biosynthetic transformations. The biosynthesis (Scheme 2) from desmosterol (6) to (iso)fucosterol (8a,8b) presumably proceeds along a similar pathway established for plants.[19] In addition we established the further alkylation at C-28 (8a,8b --> 9a --> 9b) which, after proton migration from C-28 (9b --> 9d) or C-25 (9b --> 9c), followed by proton abstraction from either of the adjacent methyl groups, gives rise to an isopropenyl substituent (9c,9d --> 10a,10b) at C-24 (Scheme 2). Lack of substrate specificity towards the Z and E isomers of 24-ethylidenecholesterol (8a,8b) was observed in this key alkylation step. This is probably due to the fact that both isomers have the 29-methyl group in the same plane as the alkylated double bond (sp2); as a result, the two flat structures at C-24, C-28 and C-29 are indistinguishable for the enzyme. An easy approach of Me^{+} from either side of the ethylidene double bond is thus equally possible.



SCHEME 2

The most intriguing point in the biosynthesis is the fate of the key intermediate 9b, which will determine whether the isopropenyl group encompasses carbon atoms 28,29 and 30 (10a) or 25,26 and 27 (10b). The alkylation at C-28 gives rise to the formation of two equivalent isopropyl substituents at C-24 in 9b, whereupon C-24 is no longer prochiral. Nevertheless the involvement of any of these isopropyl groups in a subsequent H25 --> C24 (9c) or H28 --> C24 (9d) hydrogen migration creates an asymmetric center at C-24. If this migration, as well as the subsequent proton abstraction from C-26/27 or C-29/30, is not regioselective, the resulting 24-isopropenyl sterols should be a 1:1 mixture of the 24(R) 10a and (24S) 10b isomers.

Two major problems are relevant in revealing the detailed mechanism in this key biosynthetic step: (1) the stereochemical purity of the natural sterol (10a or 10b) from Pseudaxinyssa sp.; and (2) the absolute configuration of the two C-24 epimers. 24-Isopropenylcholesterol (10a) was reported by Kokke et al.[20] from Aplysina fistularis (originally classified as Verongia cauliformis) as a 23:77 mixture of the C24-epimers 10a and 10b, based on the single difference of the C-18 methyl shifts at 0.666 ppm and 0.672 ppm. Later, in a separate study with the same sponge, Catalan et al.[11] reported that only one epimer was present. Since the 300 MHz NMR spectra of the two C-24 epimers are very similar, a peak attributed to a second epimer in a mixture could be misidentified if due to an impurity. Two separate studies report the isolation of this sterol from the sponge Peudaxinyssa sp.,[15,16] and another one[21] describes the occurrence of this rare sterol for the first time in a terrestrial source. No investigator, however, had adequately treated the problem of its stereochemical purity, a matter which is of crucial importance for a discussion of its biosynthesis. Therefore, we synthesized both isomers and separated them by HPLC in the form of their i-methyl ethers 16a and 16b (Scheme 3). Deprotection



SCHEME 3

furnished the two epimeric sterols 10a and 10b which displayed very distinctive 500 MHz NMR spectra (Fig.1). The NMR spectrum of the sterol isolated in the present study from the sponge Pseudaxinyssa sp. displayed a single peak at 0.661 ppm for the 19 methyl group and no peak at 0.670 ppm for the other epimer. The natural compound was identical (in terms of HPLC mobility) with the more polar epimer of the synthetic mixture. In the meantime, the absolute configuration of the two epimers was established as 24(R)-24-isopropenylcholesterol (10a) and 24(S)-24-isopropenylcholesterol (10b) by Kikuchi et al.[22] We were thus able to correlate their stereochemical data with ours and assign a (24S) stereochemistry (10b) to the natural 24-isopropenylcholesterol from Pseudaxinyssa sp., which turned out to be the same as the natural one from the orchid Nervila purpurea.[22] On the basis of the reported NMR data,[11] we can now extend the assignment of the absolute stereochemistry to the natural sterol isolated from Aplysina fistularis. Therefore all three natural sources contain one and the same sterol: (24S)-24-isopropenylcholesterol (10b).

The existence of only one epimer (10b) of 24-isopropenylcholesterol in Pseudaxinyssa sp. is extremely important for the interpretation of the biosynthetic mechanism in the last alkylation

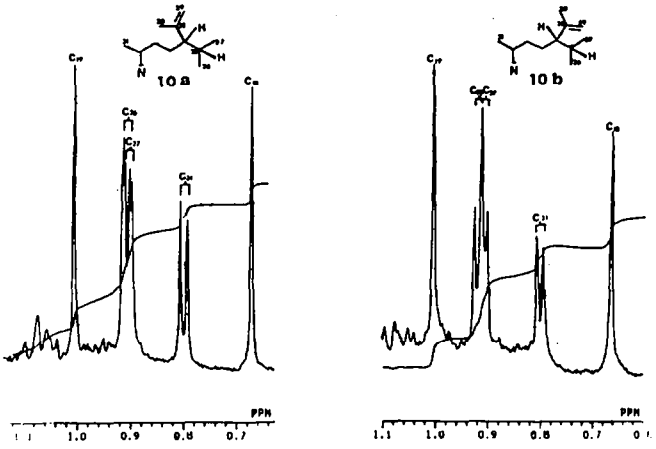


Fig 1

step. This result rules out a stereoselective hydrogen migration with only two possibilities of regioselectivity in 9b: H28 or H25 --> C24. There are four possible conformers, A, B, C and D (Scheme 4), with the most favorable orientation of the H-28 and H-25 perpendicular to the plane of the carbocation 9b. Structures A and B correspond to alkylation from the α-face, while C and D result from β-face attack. α-Face alkylation determines a β-face migration of the proton and vice versa (concerted mechanism).[23] Therefore routes B_2 and C_2 are prohibited, establishing a regioselective B_1 and C_1 mode of proton migration (28H --> 24C). Furthermore B_1, but not C_1, will furnish the isomer with the natural (24S) stereochemistry at C-24. Structures A and D in a non-regioselective hydrogen migration would afford a 1:1 mixture of the two isomers, which is not the case. Therefore, a regioselective hydrogen migration can be predicted for A and D along paths A_1 and D_2. To determine the preferred regioselectivity (9c vs.9d), the above alternative paths A_1 and B_1 must be distinguished from D_2 proton migration. A high degree of specificity in the proton migration requiring substrate/enzyme intermediates with conformations A, B, C and D (Scheme 4) of the isopropyl substituents at C-24 are likely to exist since the two isopropyl groups, and hence their methyl substituents, are enantiotopic.

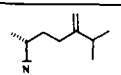
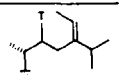
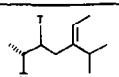
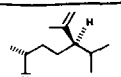
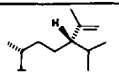
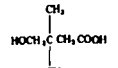
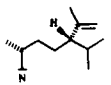
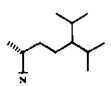
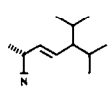
In contrast to the lack of specificity (see Table 1) in the original alkylation step (8a,8b --> 9b), a striking stereoselective preference for (24S)-24-isopropylcholesterol (10b) as compared to (24R)-24-isopropylcholesterol (10a) is observed in the next step (10 --> 11) of the biosynthetic pathway. The resulting 24-isopropylcholesterol (11) in a final step of 22(23)-dehydrogenation gives 24-isopropyl-22-dehydrocholesterol (12). These latter two sterols (11 and 12) accumulate in the sponge in an approximate ratio of 1:1, and comprise 98% of the total sterol mixture, which suggests that they equally share a membrane function in this sponge.

The validity of the biosynthetic sequences outlined in Scheme 2 is verified by the order of decrease in specific activity (cf Table 1) in the isolated sterol intermediates. The big drop in specific activity in the sequence 10a --> 11 --> 12 is apparently due to the high factor of dilution in each subsequent biosynthetic pool. The sequential order of decrease in radioactivity shown in Table 1 serves to exclude the possible alternative biosynthetic intermediate 9e and the corresponding 9b --> 9e --> 12 --> 11 --> 10a pathway for which a reversed ratio of decrease in radioactivity should exist. Another alternative sequence, 8 --> 9a --> 13 --> 10 was also excluded based on the lack of incorporation of the radioactive labelled intermediate 13[24] into any of the sterols 10a, 11 and 12.

An analogous sequence of decreasing radioactivity was observed with the incorporation of mevalonate when de novo sterol synthesis was tested. However, contrary to the results for the labelled sterol precursors 7, 8a, 8b, 10a and 10b, a relatively low efficiency of incorporation into the sponge sterols was detected (cf. Table 1). This result is in agreement with the earlier hypothesis that filter-feeding invertebrates are characterized by poor de novo sterol biosynthesis due to an efficient feedback suppression by abundant dietary sterols.[10] An alternative explanation for our result could be difficulty in uptake of low molecular weight, water-soluble compounds by the filter feeding-sponge. The extremly poor incorporation of [methyl-^{14}C]-L-methionine (30) (Table 1), which has also been encountered earlier,[25] may be due to the same reason or to the amino acid's much more rapid and efficient utilization for other non-steroid biosynthetic processes.

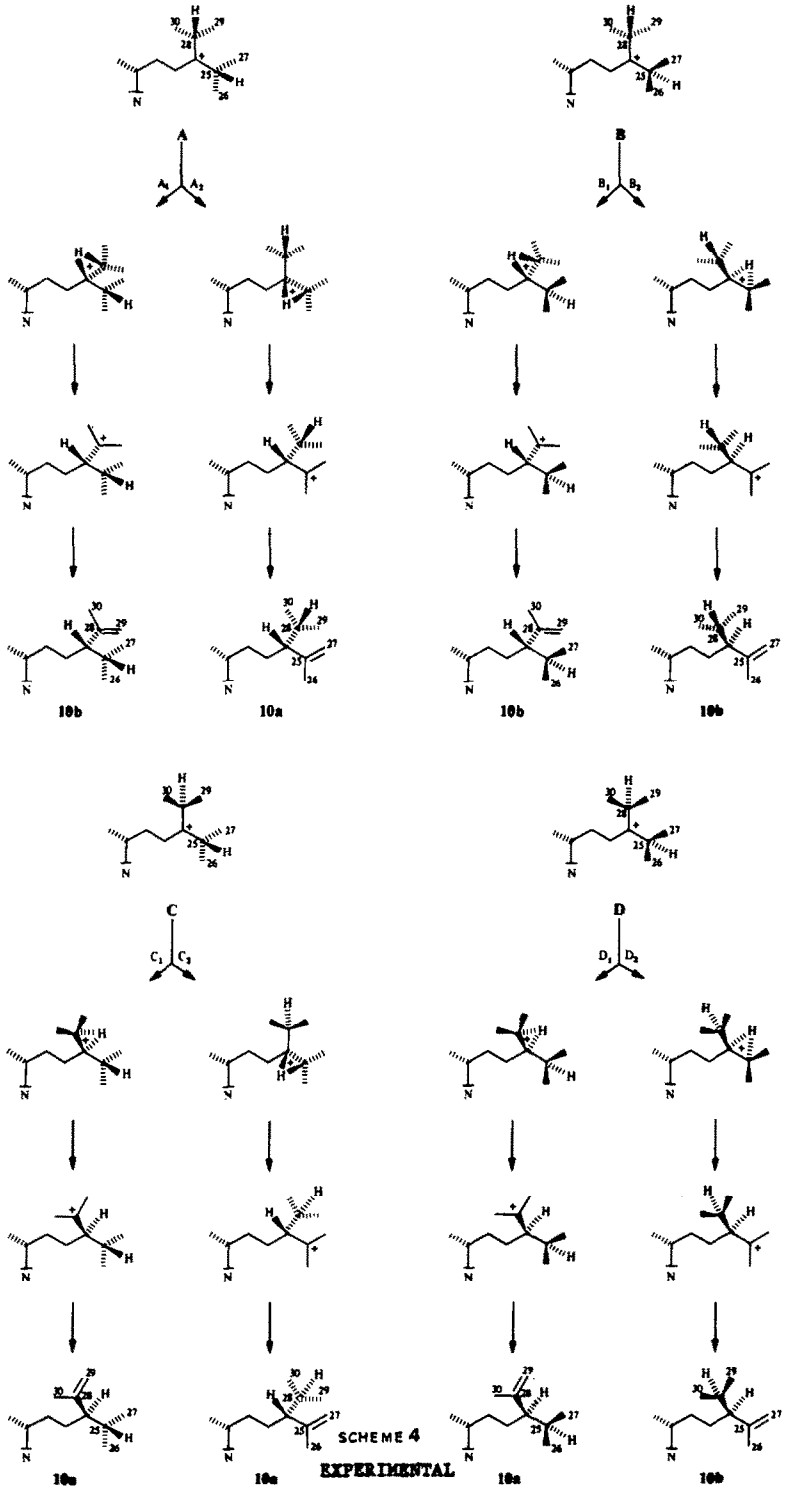
We can assume that all the above biosynthetic transformations are exclusively performed by the sponge Pseudaxinyssa sp. since its symbionts are primarily bacteria and Cyanobacteria.[26] These are known to be devoid of sterols and are incapable of sterol synthesis.[27]

TABLE 1. INCORPORATION EXPERIMENTS WITH PSEUDAXINYSSA SP.

Precursors		 7 24-Methylene-cholesterol [28-^{14}C]	 8a Fucosterol [22T]	 8b Isofucosterol [22T]	 10a (24R)-24-Iso-propylidene-cholesterol [29-^{14}C]	 10b (24S)-24-Iso-propylidene-cholesterol [29-^{14}C]	 5 Mevalonate [2-^{14}C]	L-Methionine [methyl-^{14}C]
Sponge, freeze-dry weight (gr)		6.37	3.6	4.65	3.8	4.5	5.17	7.1
Incubation period (days)		30	41	41	41	41	30	23
Total activity fed (Ci)		20	28	26	13.7	14.3	20	20
Total free sterols	(mg)[a]	30.2	21.5	26.3	25.1	25.9	34.5	46.4
	(dpm)[b]	1,356,806	3,420,640	3,866,350	5,589,804	5,372,226	133,377	6,400
	(dpm/mg)[c]	44,927	159,100	147,010	222,701	207,422	3,866	138
	(%)[d]	3.1	5.6	6.8	18.6	17.1	0.3	0.014
 10b	(mg)[a]	0.41	0.29	0.36	0.34	0.36	0.5	0.63
	(dpm)[b]	366,403	912,242	1,113,004	4,771,310	1,423,876	28,000	1,800
	(dpm/mg)[c]	893,665	3,145,662	3,091,678	14,033,264	3,955,211	56,000	2,857
	(%)[e]	27.0	26.0	28.8	85.4	26.5	21.0	28.0
 11	(mg)[a]	12.9	9.2	11.2	10.5	11.1	15.2	19.9
	(dpm)[b]	768,247	2,400,000	2,655,620	704,394	3,800,320	89,300	3,400
	(dpm/mg)[c]	59,554	260,870	237,109	67,085	342,371	5,875	171
	(%)[e]	56.6	70.2	68.7	12.6	70.7	67.0	53.0
 12	(mg)[a]	16.3	11.6	14.3	13.6	14.1	19.3	25.1
	(dpm)[b]	208,722	74,530	97,726	58,756	94,840	16,077	1,200
	(dpm/mg)[c]	12,805	6,425	6,834	4,320	6,726	833	48
	(%)[e]	15.4	2.2	2.5	1.0	1.9	12	18.8

[a]Weight of sterols.
[b]Total radioactivity.
[c]Specific radioactivity.
[d]Percent original incorporated radioactivity.
[e]Percent recovered radioactivity in total sterol mixture.

The experiments described herewith, together with the accompaning report[28] demonstrating a regioselective mode of the proton migration, settle the question of sterol side chain biosynthesis of these marine sterols arising from a triple bioalkylation. What has not yet been established is the nature and origin of the earlier sterol biosynthetic precursors. Are these sterols of dietary origin or does de novo synthesis play a significant role? We hope to answer this question through additional experiments.



SCHEME 4

EXPERIMENTAL

General. Waters HPLC equipment (M6000 A and M45 pumps, U6K injector, R401 differential

refractometers) as well as Rheodyne model 7120 and a Valco model CV-6-UHPa-N60 injector were used for separation of sterol mixtures. The columns for isolation and further purification were two Altex Ultrasphere ODS columns (10mm i.d. x 25cm) connected in series. Retention times are relative to retention time of cholesterol, co-injected in a separate run with cold sterol mixture with the point of injection, rather than the beginning of the solvent peak, used to calculate the RRT in HPLC. The purity of HPLC fractions was checked by GC using a Hewlett-Packard model 402 gas chromatograph with FID (3% SP2250 column, 2mm i.d. x 1.80m. 260° C). Low resolution mass spectra were recorded with a Hewlett Packard 5995 spectrometer in either DI or GC-MS mode (capillary SE54 column, 15m, 260° C); 300 MHz ^{1}H NMR spectra on Nicolet NT 300 WB spectrometer and 500 MHz ^{1}H NMR spectra on a JEOL JNM-GX 500 FT NMR spectrometer. Radioactivity was determined with a Beckman LZ7500 liquid scintillation counter.

[28-^{14}C]-24-methylenecholesterol (7). This sterol was prepared according to the previous described procedure.[11] The specific activity of the product was 54 mCi/mmol.

[22Tl]-6β-Methoxy-3α,5-cyclocholestan-24-one (18). 2.8 mg (0.074 mmol) of $NaBT_4$ (25 mCi, dissolved in 1.5 ml of dry pyridine in a small round bottom flask with a stirring bar and seale with a rubber stopper and 30.5 mg (0.074 mmol) of ketone 17[29] was added via syringe. After stirring for 1 hour the reaction was quenched with water and, after workup, 31.5 mg (10.58 mCi, 42% based on radioactivity of starting $NaBT_4$) of crude ketone fraction was obtained. After purification by TLC, 8.6 mg of pure ketone 18 (28.1% yield based on starting ketone) with 2.03 mCi total radioactivity (8.12% based on radioactivity of starting $NaBT_4$) was obtained.

[22Tl]-24-E,Z-24-ethylidenecholesterol (8a,8b). The phosphonium salt $[Ph_3PC_2H_5]I$ (18.2 mg, 0.044 mmol) was placed in a small vial with a magnetic stir bar, sealed with a rubber stopper and flushed with argon. Anhydrous ether (1.5 ml) was introduced via syringe followed by 21 ml (0.05 mmol) n-BuLi in hexane (2.3-2.4 M). The mixture was stirred for 2.5 hours at room temperature and then transferred via syringe into another small vial containing the ketone 18 (3.6 mg, 0.0087 mmol, specific radioactivity 97.7 mCi/mmol) in 1 ml of anhydrous ether. The reaction mixture after stirring for 48 hours at room temperature and usual workup gave 1.7 mg, 47% yield, of a mixture (24% and 76%, respectively, by GC) of the i-methyl ethers of fucosterol (18a) and (iso)fucosterol (8b). After deprotection with p-toluene-sulphonic acid in aqueous dioxane the mixture of the free sterols 8a and 8b was separated on an Altex column using 50 mmol/l $AgNO_3$ in 2% aqueous methanol (rt 77.5 and 73 min respectively). The fractions containing the sterols were evaporated to dryness under N_2 and the sterols were purified by column chromatography on silica gel containing 10% NaCl for complete removal of $AgNO_3$.

[29-^{14}C]-(24R)-24-isopropenylcholesterol (18a) and [29-^{14}C]-(24S)-24-isopropenylcholesterol (18b) i-methyl ethers. The procedure was similar to that described for 7 with ketone 14a,14b instead of ketone 19 for the Wittig reaction. From 7.7 mg (17 mmol) of the $[Ph_3P^{14}CH_3]I$ and 14.7 mg (33 mmol) of the ketone 14a,14b, 5.9 mg (80% of crude) of 16a,16b were obtained as an oil. The epimeric mixture was purified by elution in hexane on a small silica gel column. The use of pure hexane for elution allowed a better separation of the i-methyl ether from the triphenyl-phosphine (yield of pure 16a,16b 4.5 mg, 60% or 58% based on the activity of the phosphonium salt used). The epimeric mixture was further separated by reverse phase HPLC (Altex columns, MeOH-H_2O 98:2, flow 3.0 ml/min, injection vol. 0.5 ml, 2.2 mg/ml). A purity of 96% and 91% could be obtained for the (24R) and (24S) epimers, respectively, after the first HPLC run based on parallel separation of a "cold" sample. By rechromatographing each peak the purity could be further increased to >98% (no detectable peaks in 500 MHz NMR spectra) and 96%, respectively, again determined by rechromatography of the "cold" peaks. The i-methyl ethers 16a and 16b were deprotected separately in 10% aqueous dioxane (3 ml) containing 5% p-toluenesulfonic acid by refluxing for 1h. After cooling, 0.4 g of anhydrous Na_2CO_3 and 3 ml of hexane were added to each reaction vial to absorb all the water and the acid. The mixture was then stirred for 15 min and filtered with the precipitate being washed 3 times with 2 ml of hexane. After evaporation of solvents, the combined solutions were purified on small silica gel columns by elution with 30% ether in hexane and HPLC (Altex column, flow 3 ml/min MeOH). Thus 2 mg (44% yield, spec. act. 60 mCi/mmol) of the (29-^{14}C)-(24R)-24-isopropenylcholesterol (10a) was obtained (shorter rt in HPLC), MS: 426 (78, M^+), 411 (11), 393 (12), 328 (14), 314 (32), 299 (54), 271 (66), 213 (39), 55 (100). NMR (500 MHz): 0.6704 (s, 3H); 0.7973 (d, J=5.8, 3H); 0.9016 (d, J=6.4, 3H); 0.9053 (d, J=6.4, 3H); 1.0035 (s, 3H); 1.5592 (s, 3H); 4.62 (br.s, 1H); 4.74 (br.s, 1H); 5.36 (br.d, 1H); 3.53 (m, 1H). The other epimer (longer rt in HPLC) furnished 2.1 mg (46% yield, spec. act. 60 mCi/mmol) of the (29-^{14}C)-(24S)-24-isopropenylcholesterol (10b). MS: identical with the above for 10a. NMR: 0.6631 (s, 3H); 0.8010 (d, J=6.4, 3H); 0.9078 (d, J=5.7, 3H); 0.9200 (d, J=7.1, 3H); 1.0035 (s, 3H); 1.5641 (s, 3H); 4.60 (br.s, 1H); 4.72 (br.s, 1H); 5.35 (br.d, 1H); 3.54 (m, 1H).

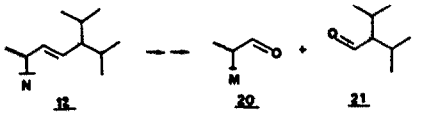
Incorporation experiments. Pseudaxinyssa sp.[14] were collected and transplanted in situ onto plastic plaques one month prior to the experiments.[13] The precursors were then introduced into separate specimens via 11 (methionine only) or 17 hour aquarium incubations[11] and finally returned to the sea (15m) for approximately one month before collection and analysis.[13] The experiments were done in three groups at three different mid-shelf reefs in the central portion of the Great Barrier Reef, Australia. Date, site, and duration of incubation for each precursor were as follows: mevalonate and 24-methylenecholesterol: 15 February 1984, Davies Reef, 30 d; fucosterol, isofucosterol, (24R)-24-isopropenylcholesterol and (24S)-24-isopropenylcholesterol: 31 October 1984, Rib Reef, 41 d; methionine, 9 October 1985, John Brewer Reef, 23 d. Transfer of precursors to the aquaria was in EtOH or ether for the sterols, warm seawater for mevalonate, and 70% EtOH/H_2O for methionine.

Isolation of the sterols. Sponge samples were freeze-dried, broken into small pieces and extracted three times by adding hot chloroform and setting them in the freezer for 12 h with

occasional shaking. Extracts were combined and the solvent removed by evaporation. This procedure reduces loss of radioactive materials and avoids unnecessary contamination of glassware, in particular separatory funnels for water-organic solvent partition and associated emulsions thereafter. After the final extraction, residual material was discarded as homogenation with chloroform in a Waring Blender did not yield substantial additional radioactivity in a test experiment (>1%). The lipid extract was enriched in sterols, as evidenced by crystallization, was practically free from salts and other more polar compounds, and was readily soluble in relatively small amounts of hexane. Thus no water/chloroform partition was necessary. The green extract contained chlorophyls, contributed by symbiotic Cyanobacteria. The chlorophyls were removed by column chromatography on silica gel-florisil 3:1 mixture and by elution with 25% ether in hexane. This procedure was used in preference to saponification as the sterols are obtained free from the chlorophyls which elute together if silica gel is used alone; also the intact sterol esters can be isolated and analyzed separately. Further purification of the sterol fraction was done by saponification, rechromatography on silica gel and recrystallization yielding pure white crystals.

Separation and purification of sterols of Pseudaxinyssa sp. Conditions for isolation of the sterols and establishment of their purity were first established with cold reference material before workup of the radioactive material. Three HPLC fractions, corresponding to the only three detectable peaks, were collected as well as short interval baseline fractions. The three fractions were further purified to constant specific radioactivity by rechromatography in different solvent systems: MeOH, CH_3CN-MeOH-EtOAc 22:9:9, and THF-MeOH-H_2O 3:3:1. Fractions from the baseline were reinjected with corresponding cocarriers to locate and recover starting precursors. Baseline fractions containing less than 0.001% of the total recovered radioactivity (<1000 dpm/mg total radioactivity) were not analyzed further. Purification was also terminated for cold fractions (< 200 dpm/mg specific radioactivity).

Chemical degradation experiments. 10 mg of 24-isopropyl-22-dehydrocholesterol (12) from the incorporation experiment with [28-^{14}C]-24-methylenecholesterol after protection as i-methyl ether and ozonolyzis in CH_2Cl_2 gave a mixture of the aldehydes 20 and 21 (Scheme 5). They were converted to the corresponding 2,4-dinitrophenylhydrazones and separated by TLC. The hydrazone of 20 (7.2 mg, 64% from 12) was cold and was not purified further. The hydrazone of 21 (4.8 mg, 60% from 12) was recrystallized to constant specific radioactivity of 5,442,125. dpm/mmol (specific radioactivity of 12: 5,454,930. dpm/mmol). Analogous chemical degradation of the sterol 12 from the incorporation experiment with isofucosterol 8b gave a cold hydrazone of 21 and hydrazone of 20 with specific radioactivity of 2,892,964. dpm/mmol (specific radioactivity of starting 12: 2,911,284. dpm/mmol).



12 20 21

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