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BIOSYNTHETIC STUDIES OF MARINE LIPIDS. 6.[1] EVIDENCE FOR AN UNPRECEDENTED BIOMETHYLATION PATHWAY IN THE BIOSYNTHESIS OF THE CYCLOPROPYL-CONTAINING MARINE STEROL, PETROSTEROL

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Abstract: Petrosterol, an unusual cyclopropane-containing marine sponge sterol, is shown, unexpectedly, to be derived by SAM biomethylation of 24-methylenecholesterol via a complex rearrangement process.

Petrosterol (**1**),[3] one of an ever-enlarging number of marine steroidal-cyclopropanes,[4] is the major sterol of Petrosia ficiformis and is likely to be a membrane component. Its biosynthesis was postulated to proceed through epicodisterol (**2**) by SAM biomethylation as shown in Scheme 1.[5] However, feeding experiments with 26-^{14}C-epicodisterol[6] (**2**) and 26-^{14}C-codisterol[6] (**3**) failed to incorporate any label, whereas 28-^{14}C-24-methylenecholesterol[6] (**4**) was found to be a very efficient precursor, with up to 19% of the recovered radioactivity being incorporated into **1** (cf. Table 1.

In order to account for the results of these incorporation experiments, we postulated the mechanism,[4,7] shown in Scheme 2, whereby biomethylation of **4** leads, via the series of rearrangements shown, to petrosterol (**1**). A crucial feature of this proposed mechanism is that the ^{14}C label at C28 in **4** appears in the product **1** at the original position of C24, and therefore provides an opportunity to test this scheme.

The degradation to determine the position of the label followed the pathway shown in Scheme 3. The 3β-OH-Δ^5 nucleus (**N**) was protected as the 5α-dihydro-3β-OMe system (**S**). Acid-catalyzed cyclopropane ring opening[8] gave, among other olefins, **6** and **7**, which were separated by reverse phase HPLC and diluted with cold material (10 mg and 1.7 mg, respectively). Ozonolysis of **7** yielded aldehyde **8** (or, on one occasion, its dimethoxyacetal derivative) which retained no radioactivity. The radiolabel is therefore among carbons 24-29. Epoxidation of olefin **6**, followed by isomerization with aluminum isopropoxide[9] yielded a mixture of allylic alcohols **9** and **10** which were separated by reverse phase HPLC on the basis of the stereochemistry around the hydroxyl group. The alcohols in each case were obtained as a 1:1 mixture (by NMR integration of the olefin regions) of double bond regio-isomers. Acetylation and micro-ozonolysis[10] gave **11** and **12** from **9**, and **13** and **14** from **10**.

TABLE 1. FEEDING EXPERIMENTS IN PETROSIA FICIFORMIS

PRECURSOR	INCUBATION PERIOD (DAYS)	TOTAL STEROLS (mg)	(DPM/mg)	TOTAL FED (DPM)	% RECOVERED RADIOACTIVITY	DPM/mg IN PETROSTEROL	% INCORPORATED[a] IN PETROSTEROL
Control	27	14.7	21	----	----	23	----
3 (56 mCi/mmol)	27	13.3	25.3×10^3	41.8×10^6	0.8	29	----
2 (57 mCi/mmol)	27	231.0	48.5×10^3	46.2×10^6	24.2	92	----
	29	219.0	21.3×10^3	46.2×10^6	10.1	43	----
4 (54 mCi/mmol)	27	28.5	95.6×10^3	46.2×10^6	5.9	11300	7.7
	29	163.2	42.8×10^3	46.2×10^6	15.1	12700	19.3

[a]Based on recovered radioactivity and assuming that petrosterol accounts for 65% of the sterol mixture as determined by gas-chromatography.

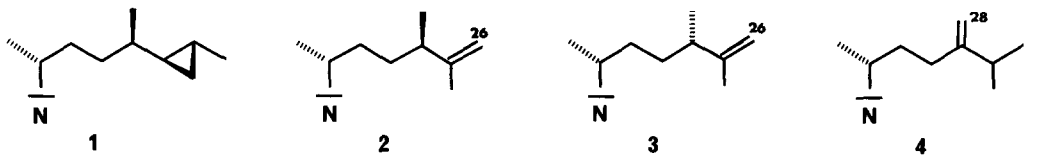
TABLE 2. COMPARISON OF ^{14}C LABEL RETAINED IN 11, 12, 13 AND 14.

COMPOUND	FROM 6					
	DPM[a]	RATIO DPM	RATIO[b] PEAK AREA	DPM[a,c]	RATIO DPM	RATIO[b,c] PEAK AREA
11	226	1	1	245	1	1
12	267	1.18	1.14	277	1.13	1.21
13	206	1	1	187	1	1
14	220	1.07	1.05	196	1.05	1.01

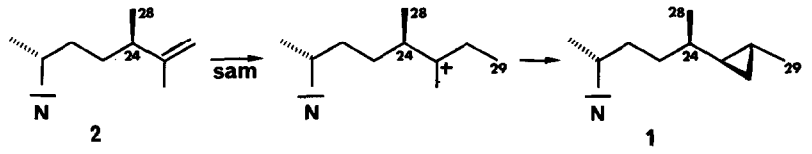
[a]Background counts (20-25 dpm) have been subtracted.

[b]Peak areas were obtained by cutting and weighing the HPLC traces, obtained under identical conditions.

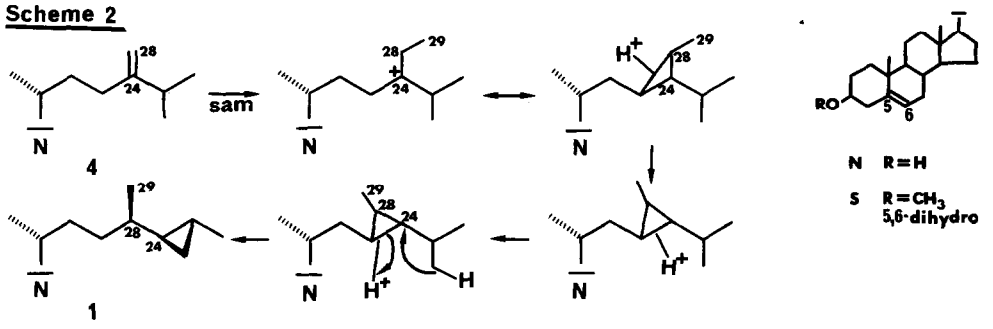
[c]These results were obtained after reinjection on HPLC using CH_3CN/EtOAc (3/1) as the mobile phase.



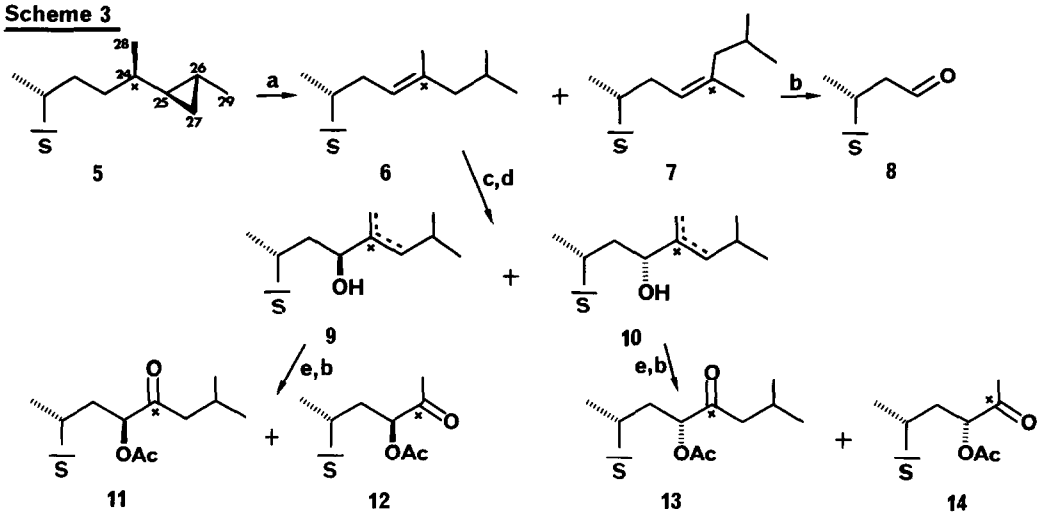
Scheme 1



Scheme 2



Scheme 3



Yields are based on recovered radioactivity unless otherwise stated.

(a) CF_3COOH / C_6H_6 , 48h ; 6 , 6% ; 7 , 2.5% (b) O_3 , CH_2Cl_2 / CH_3OH , -78°C , 8 , 65% (chemical yield) (c) m-$ClC_6H_4CO_3H$, CH_2Cl_2 (d) $Al(O^iC_3H_7)_3$, $CH_3C_6H_5$, reflux 48h , 9 + 10 , 56% (e) $(CH_3CO)_2O$, C_6H_5N , overall (e) , (b) average 60% , 11 +12 or 13 + 14.

Compounds **12** and **14**, since they contain both C28 and C24 of the original 24-methylene-cholesterol, are expected to be "hot" regardless of the operative mechanism, and act as an internal standard for compounds **11** and **13** which will be "hot" only if the mechanism proposed in Scheme 2 acts. Table 2 summarizes the results obtained.

Because of the small amounts of material obtained, actual specific activities were not obtained. It can be seen however that the specific activities of the pairs **11/12** and **13/14** must be the same since the ratios of disintegration per minute (dpm) and amount of material counted are in very close agreement. Thus all the compounds **11**, **12**, **13** and **14** retain the ^{14}C label of the starting material.[11] Remembering the result of the ozonolysis (**7** ⟶ **8**), the only position for the ^{14}C label consistent with these results is C24 of petrosterol which is exactly as predicted by our proposed mechanism in Scheme 2.

We are currently in the process of elaborating further details of this unprecedented mechanism of sterol side chain biosynthesis.

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