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BIOSYNTHETIC STUDIES OF MARINE LIPIDS 10.[1] DOUBLE SIDE CHAIN EXTENSION IN THE TRIPLY ALKYLATED SPONGE STEROL XESTOSTEROL

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Abstract: Xestosterol, a triply alkylated marine sponge sterol, with a symmetrically extended side chain is shown to be efficiently biosynthesized by the sponge Xestospongia testudinaria via a nonstereoselective SAM biomethylation and 1,2-hydrogen shift. De novo biosynthesis via mevalonate, rarely demonstrated in sponges, has also been demonstrated.

Numerous sterols with unusual alkylation patterns have been isolated from marine sponges.[2] Recent studies[3] of their biosynthesis have shown that only two double bond isomers in the side chain of a C_{28} sterol, namely 24-methylenecholesterol (1) and (epi)codisterol (2a,b), derived from a single common parent, desmosterol (3), are the precursors of a variety of unusual sponge sterols. Depending on the regioselectivity of sponge methyltransferases, side chain extension or branching can occur at C28 (Calyx niceansis,[3a] Pseudaxinyssa sp.,[3b,c] and Petrosia ficiformis[3d]), at C26 (Aplysina fistularis[3e]), or at both C28 and C26 (Strongylophora durissima[3f]).

A unique symmetrical double extension at C26 and C27 has been encountered in the triply alkylated sterol xestosterol (5), the principal (up to 85%) sterol constituent of Carribean[4] and Great Barrier Reef[5] Xestospongia species. The likely involvement of this unusual sterol in cell membrane function[6] prompted the present study of its biosynthesis. The most plausible biosynthetic pathway (Scheme 1) involves codisterol (2a) or epicodisterol (2b), derived from desmosterol (3), and 25(27)-dehydroaplysterol (4a)[7] or its epimer 4b. These precursors, as well as additional candidates (cf. Table 1) were introduced in labelled form into separate specimens of Australian Xestospongia testudinaria via 11-14 hours aquarium incubations and then returned to their natural marine environment (Pandora Reef, Great Barrier Reef) for 22-46 days during October 1984 and 1985 according to our standard procedure.[3b]

The high incorporation (cf. Table 1), in increasing order, of the incubated precursors desmosterol (3), (epi)codisterol (2a,b) and 25(27)-dehydro(epi)aplysterol (4a,b)[7] confirms the proposed sequence. Contrary to the high stereospecificity encountered in the recent SAM alkylation step in the biosynthesis of 4b[3e] and strongylosterol (6),[3f] a remarkably high incorporation was encountered with both epimers 2a and 2b of codisterol, thus suggesting an unexpected lack of stereospecificity in this step of bioalkylation. Consequently, the demonstrated high incorporation (cf. Table 1) of the 24-epimeric mixture of 25(27)-dehydroaplysterol (4a,b)[7] in the third SAM bioalkylation step was not repeated through separate incorpo-

ration of the two individual epimers. A similar lack of stereospecificity was demonstrated[3f] with the pure isomers of 24(28)-dehydroaplysterol (7) in the third SAM bioalkylation step of strongylosterol (6).

Table 1. Incorporation of Labelled Precursors in Xestospongia testudinaria

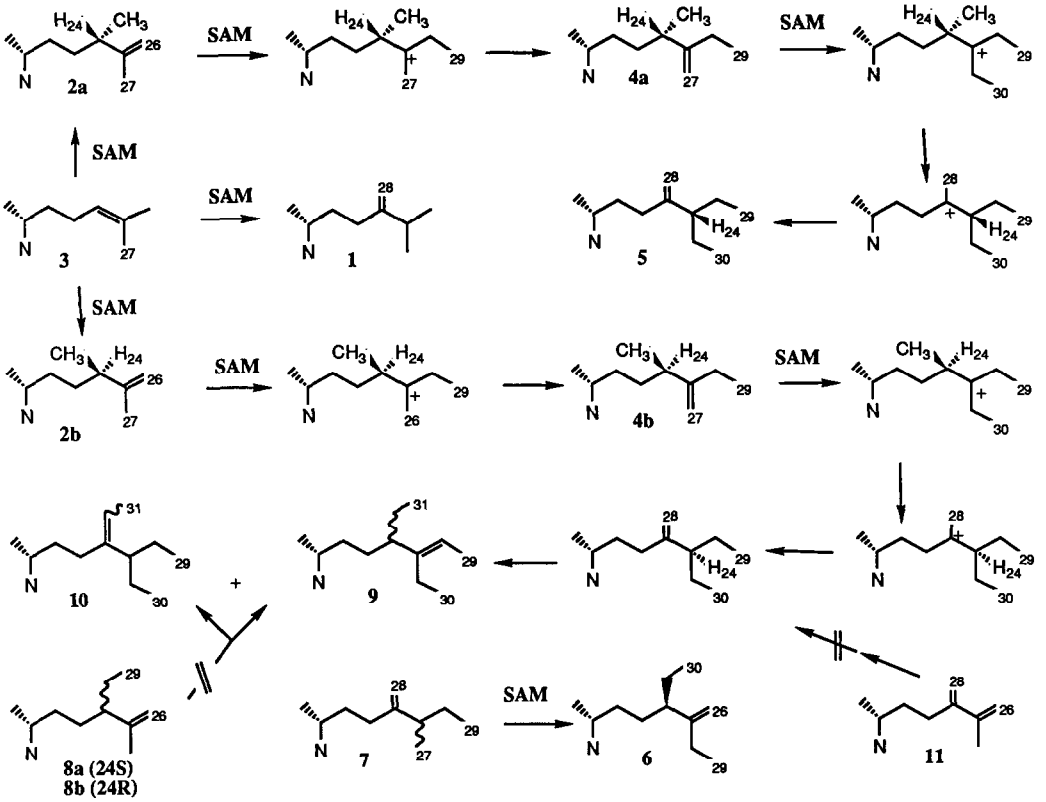
Labelled Sterol	Precursor Label*	Total Activity	Sponge dry wt	Recovered Radioactivity in Xestosterol		Precursor	
		[Ci]	[g]	[dpm]	2 (%)[8]	[dpm]	2 (%)[8]
3	[24-^{14}C][9]	16.7	11.8	960,000	0.9	---	
2a	[26-^{14}C][3e]	20	12.3	3,890,620	0.4	9,000	6.3
2b	[26-^{14}C][3e]	20	13.1	2,562,000	0.5	260,000	1.3
4a,b	[27-^{14}C]**	20	13.8	9,514,300	0.2	240,000	1.3
8a	[26-^{14}C][3f]	20	14.1	cold		980,000	0.9
8b	[26-^{14}C][3f]	20	15.2	cold		490,000	1.1
1	[28-^{14}C][3e]	20	14.7	cold		700,400	1.0
11	[28-^{14}C][10]	21	18.5	cold		---	
Mevalonate	[2-^{14}C]***	17.8	14.1	182,000	1.5	---	

* Reference citations refer to synthesis.

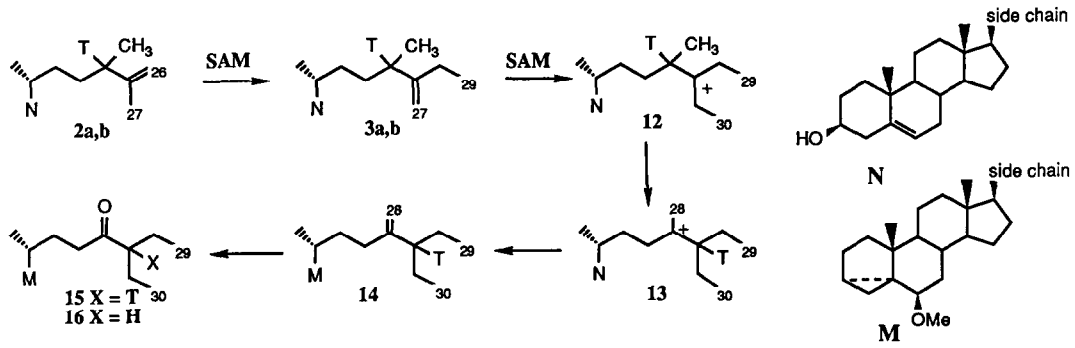
** Prepared by Wittig condensation using the corresponding ketone (ref.11) and radioactive methyl iodide (ref. 3).

*** Purchased from Amersham Corporation (DBED-salt).

This unexpected lack of stereospecificity in the bioalkylation of codisterol (2a) and epicodisterol (2b) prompted an incorporation of their 24-ethyl homologs, clerosterol (8a) and epiclerosterol (8b), to determine whether a lack of structural specificity also operates. We would expect transformation of these "abnormal" precursors into the C_{30} sterols 26-methyl(epi)strongylosterol (9) or 28-methylxestosterol (10), which have been isolated[12] as minor sterols from the sponge Strongylophora durissima. Although not detected in X. testudinaria, these sterols, even if produced by the sponge in trace amounts, can readily be isolated in pure form by adding the cold co-carriers due to their relatively long HPLC retention times. However, no such incorporation (cf. Table 1) was detected, thus demonstra-



SCHEME 1



SCHEME 2

ting a high substrate selectivity with respect to an ethyl substituent at C24. This is further confirmed by lack of incorporation (Table 1) of [28-^{14}C]-24-methylenecholesterol (1) or 24-methylene-25-dehydrocholesterol (11).[10]

The above established sequence (2a,b --> 4a,b --> 5) of side chain extension requires a 1,2-hydrogen shift (12 --> 13, Scheme 2), which was demonstrated by incorporating a mixture of codisterol (2a) and epicodisterol (2b), specifically labelled with T at C24 (Scheme 2), into the sponge. The resulting T-labelled xestosterol (14) was protected as the i-methyl-ether (M); ozonolysis to the ketone 15 did not change its specific activity, but all radio-activity was lost after base-exchange (15 --> 16).

In contrast to earlier unsuccessful attempts[13] to demonstrate de novo sterol biosynthesis with mevalonate, significant incorporation was encountered (Table 1) in X. testudinaria. Work with sponge cell isoaltes would be required to determine whether such de novo synthesis occurs in the sponge or in microorganism symbionts.

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