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MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES 52.[1]

ISOLATION, STRUCTURE ELUCIDATION AND PARTIAL SYNTHESIS OF 24-PROPYL-24,28-METHYLENECHOLEST-5-EN-3β-OL

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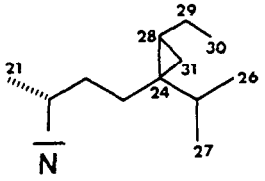
Abstract: The isolation and structure elucidation of the first cyclopropyl-containing sterol (1) resulting from quadruple bioalkylation of the sterol side chain is reported.

Recently we reported[3] the discovery of several new sterols in a Pseudaxinissa species from the Australian Great Barrier Reef. This sponge had been reported to contain only 24-isopropylcholesterol and its 22-dehydro analog.[4] We now communicate the discovery of a novel cyclopropane-containing sterol arising from quadruple biomethylation of the cholesterol sidechain.

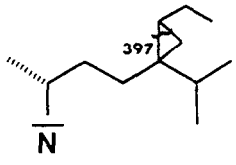
Fractionation of the sponge sterol mixture (700 mg) by reverse phase HPLC provided, along with previously described components, several new sterols[5] among which was a cyclopropyl sterol (readily identifiable by ^{1}H NMR signals δ 0.5 - δ 0.2). Repeated reverse phase HPLC (2x Altex Ultrasphere^R mobile phase MeOH or MeCN/EtOAc/MeOH 3/1/1) eventually gave the pure sterol 1. The molecular weight of 440 ($C_{31}H_{52}O$) coupled with the mass spectral fragments at 271, 255, 229 and 213 typical[6] of a conventional cholesterol nucleus indicated that all the extra carbons had to be in the side chain. A fragment ion of mass 397 ($M-C_3H_7$) resulting from cleavage of the three membered ring (2) pointed to the presence of an ethyl-substituted cyclopropane. The 300 MHz ^{1}H NMR spectrum (Table 1) showed three one-proton resonances at low field indicative of a tri-substituted cyclopropyl ring system. Signals due to three secondary methyl groups (C21, C26 and C27), one ethyl group (C30) and the C18 and C19 methyl substituents were also apparent. Irradiation of the secondary methyl groups confirmed that none was attached to the cyclopropane ring. These data, along with basic biosynthetic considerations[7] (viz., its probable origin via multiple biomethylation of 24-methylenecholesterol) led to the formulation of this new sterol as 24-propyl-24,28-methylenecholesterol (1).

In order to prove this structure and obtain some information regarding the relative dispositions of the cyclopropane ring substituents the following synthetic studies were carried out (Scheme 1). E-24-Propylidenecholesterol (3)[8] as the i-methyl ether 4[9] was subjected to dichlorocarbene addition.[10] The resulting dichlorocyclopropanes[11] 5 and 6 were separated by reverse phase HPLC. Each was then transformed to the corresponding cyclopropyl sterol by standard methods. The mass spectral data of these synthetic sterols 7 and 8 corresponded well to those of the natural compound although significant chemical shift differences were apparent in the ^{1}H NMR data (Table 1). This suggested that the natural sterol possessed the other relative stereochemistry between C24 and C28.

The above synthetic sequence was repeated with i-methyl ether of Z-24-propylidene-



1



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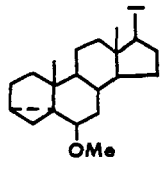

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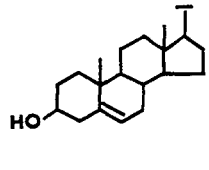

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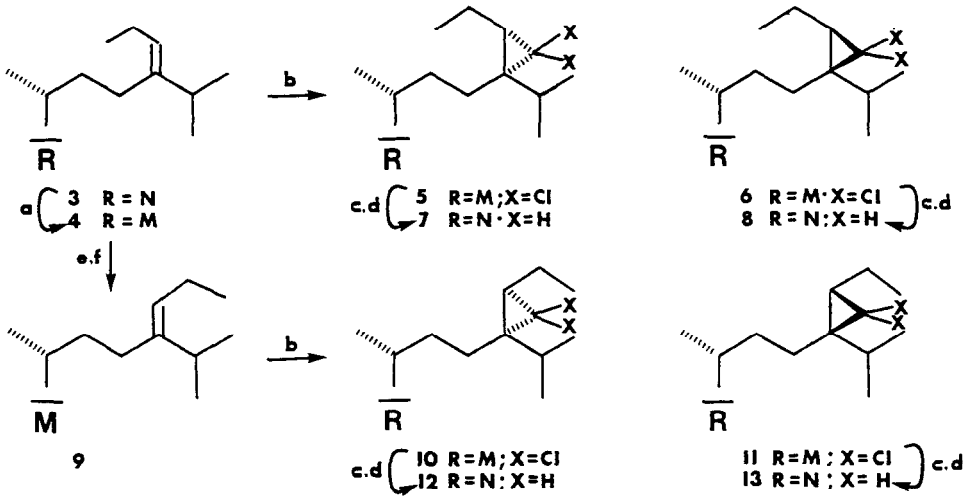
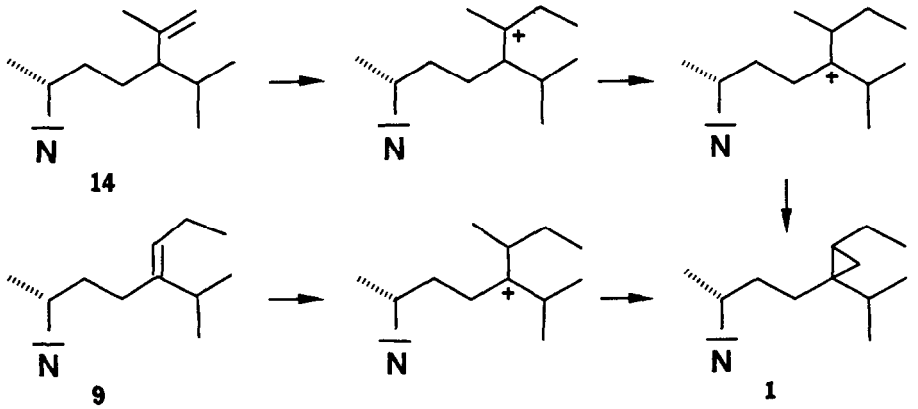
SCHEME 1



(a) p-TsCl, pyridine ; KOAc, MeOH, reflux. (b) $CHCl_3$, BTEAC, 50% NaOH. (c) Li / NH_3. (d) p-TsOH, dioxan, H_2O. (e) m-CPBA, CH_2Cl_2. (f) Ph_2PLi, THF, MeI.

SCHEME 2



cholesterol[9](obtained by olefin inversion of the E-isomer by the method of Vedejs and Fuchs[12]). The cyclopropyl sterols 12 and 13 obtained by this pathway displayed very similar ^{1}H NMR data (Table 1). However only the spectrum of 13 was superimposable upon that of the natural product. The crucial distinction is the difference (Δ) between the signals of the ethyl triplet (C31 in Table 1) and the C26 methyl doublet [Δ = 0.020 for 12 vs. 0.024 for 13 vs. 0.024 for 1].

Table 1. 300 MHz ^{1}H NMR data ($CDCl_3$), J values in Hertz

Sterol	C18	C19	C21	C26[c]	C27[c]	C30	C28	C31	
7[a]	0.669	1.004	0.810	0.850	0.902	0.955	0.458	0.352	-0.025
	s	s	d,6.9	d,6.8	d,6.5	t,7.3	m	dd	dd
8[a]	0.668	1.003	0.786	0.858	0.910	0.993	0.449	0.378	-0.188
	s	s	d,6.9	d,6.8	d,6.2	t,7.1	m	dd	dd
12[b]	0.659	1.000	0.877	0.952	0.973	0.972	0.513	0.388	-0.235
	s	s	d,6.4	d,6.5	d,6.4	t,n.a.	m	dd	dd
13[b]	0.659	1.001	0.879	0.951	0.974	0.975	0.517	0.388	-0.236
	s	s	d,6.5	d,6.9	d,6.9	t,7.0	m	dd	dd
1	0.661	1.002	0.880	0.952	0.976	0.976	0.520	0.391	-0.236
	s	s	d,6.5	d,7.0	d,7.0	t,7.0	m	dd	dd

a,b,c. These assigments may be reversed. n.a. Not available.

Although in the course of this study we were able to determine the relative configuration of the C24 and C28 substituents of the natural product (i.e. 12 or 13 and not 7 or 8), it was not possible to assign the absolute stereochemistry of the cyclopropane ring, i.e. structure 12 or 13 since there are no known compounds suitable for chemical correlation.

Biosynthetically, this cyclopropyl sterol may arise from two precursors as shown in Scheme 2. We favor 24-isopropylidenecholesterol (14) as the precursor because it, but not 24-propylidenecholesterol (9), is known[35] to occur in this Pseudaxinissa species.

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11. Satisfactory mass and ^{1}H NMR spectra were obtained for new compounds. Further spectroscopic and physical data for 1,12,7 and 8. Compound 1: m.p. 126-7° C (MeOH); 300 MHz ^{1}H NMR δ (C_6D_6) 1.104 (3H,d J = 6.8 Hz,C26 or C27),1.066 (3H, t J = 7.5 Hz,C30), 1.077 (3H, d J = 6.8 Hz, C26 or C27), 0.974 (3H, d J = 6.4 Hz, C21), 0.944 (3H, s, C19), 0.646 (3H, s, C18); mass spectrum, m/z (relative intensity), 440 (M^+, 24), 425 (3), 407 (4), 397 (2), 379 (4), 355 (2), 314 (40), 299 (22), 271 (20), 255 (6), 229 (10), 213 (12), 69 (100). Compound 12: m.p. 111-113° C (MeOH); 300 MHz ^{1}H NMR δ (C_6D_6) 1.103 (3H, d J not available, C26 or C27), 1.078 (3H, d J = 6.8 Hz, C26 or C27), 1.067 (3H, t J = 7.9 Hz, C30), 0.974 (3H, d J = 6.5 Hz, C21), 0.943 (3H, s, C19), 0.646 (3H, s, C18); high resolution mass spectrum, m/z (relative intensity) 440.4096 ($M^{+\cdot}$ $C_{31}H_{52}O$, 81), 425.3751 ($C_{30}H_{49}O$, 11), 422.3884 ($C_{31}H_{50}$, 18), 397.3503 ($C_{28}H_{45}O$, 11), 379.3315 ($C_{28}H_{43}$, 15), 314.2535 ($C_{22}H_{34}O$, 65), 299.2384 ($C_{21}H_{31}O$, 36), 271.2045 ($C_{19}H_{27}O$, 47), 255.2153 ($C_{19}H_{27}$, 17), 253.2046 ($C_{19}H_{25}$, 15), 229.1585 ($C_{16}H_{21}O$, 13), 213.1680 ($C_{16}H_{21}$, 28), 69.0682 (C_5H_9, 100). Compound 7: m.p. 130-32° C (CH_3CN); mass spectrum m/z (relative intensity) 440 (M^+, 17), 425 (1), 422 (1), 407 (3), 379 (3), 355 (1), 314 (12),299 (9),271 (10),255 (4),253 (3), 229 (4), 213 (9), 69 (100). Compound 8: m.p. 117-19° C (CH_3CN); mass spectrum m/z (relative intensity) 440 (M^+, 100), 425 (14), 422 (14), 407 (14), 397 (5), 379 (14), 355 (5), 314 (43), 299 (38), 271 (33), 255 (10), 229 (24), 213 (43).
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