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MARINE STEROLS IV.[1] C_{21} STEROLS FROM MARINE SOURCES. IDENTIFICATION OF PREGNANE DERIVATIVES IN EXTRACTS OF THE SPONGE HALICLONA RUBENS.

James A. Ballantine* and Kevin Williams
Department of Chemistry, Institute of Marine Studies, University College of Swansea, U.K.
Basil A. Burke
Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica.

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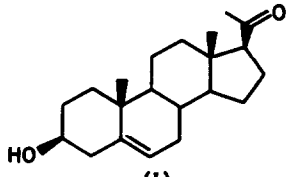
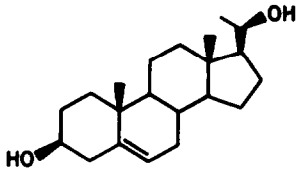
As part of our investigations into marine invertebrate sterol profiles and their relation to the marine food web, we wish to report the occurrence of a number of pregnane derivatives in the sterol mixture isolated from a sponge Haliclona rubens. This animal has been found to contain 3β-hydroxy-17β-pregn-5-ene-20-one (I), 3β,20β-dihydroxy-17β-pregn-5-ene (II) and 3β,20α-dihydroxy-17β-pregn-5-ene (III) in addition to other, as yet unidentified, keto sterols.

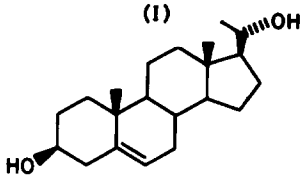
This is the first report of free pregnane derived sterols in the marine environment. In fact the only other C_{21} steroid which has been isolated from marine sources is the aglycone (IV) from the hydrolysis of a complex saponin obtained by a number of workers[2,3,4] from three species of starfish.



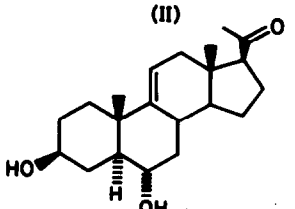
(I)



(II)



(III)



(IV)

The sponge was collected off the north coast of Jamaica and the chopped material was extracted with methanol and the residue from the chloroform soluble portion of the methanol extract was chromatographed on silica. The sterol fraction was crystallised from methanol and examined by GC-MS techniques as previously described.[1,5] A very complex sterol g.l.c. profile was observed (Fig. 1) and temperature programming was employed over the lower end of the chromatogram to increase the resolution. The retention data for each of the peaks is given in Table 1 , accompanied by the masses

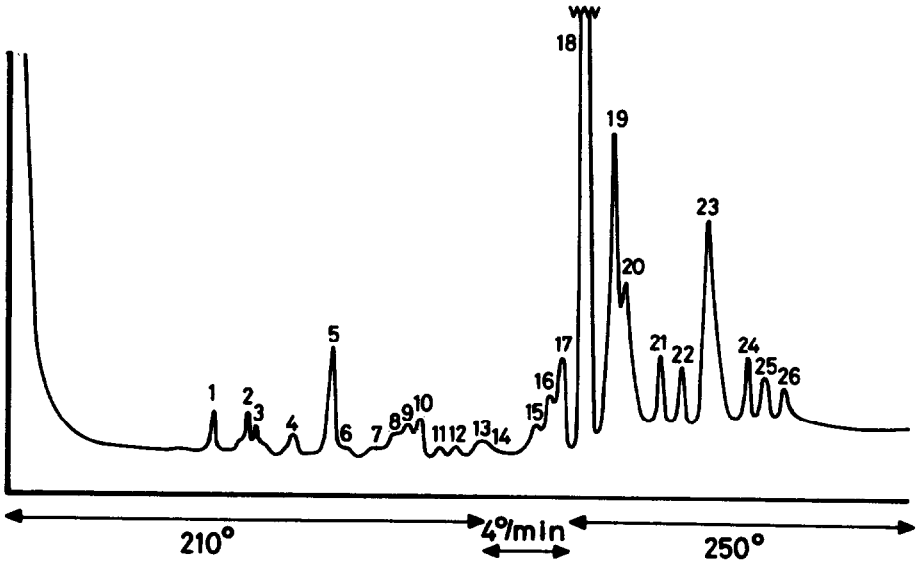


FIGURE 1. Chromatogram of Halicona rubens Sterol TMS Ethers on 1% SE-30 Ultraphase

TABLE 1. Data for Haliclona rubens Sterol TMS Ethers.

Peak No.	RRT	$M^{+\cdot}$	Identification	
1	0.10	346	*	
2	0.17	372,374	*	
3	0.18		*	
4	0.23	344	*	
5	0.30	388	21C 5	3β-hydroxy-17β-pregn-5-ene-20-one
6	0.34	374	*	
7	0.39		*	
8	0.41	402,462	*/ 21C 5	3β,20β-dihydroxy-17β-pregn-5-ene
9	0.43	402,416	*/*	
10	0.45	462	21C 5	3β,20α-dihydroxy-17β-pregn-5-ene
11	0.51		*	
12	0.54		*	
13	0.59	402	*	
14	0.61	402,442	*/ 26C 5,22(E)	24-norcholesta-5,22-dien-3β-ol
15	0.79	490	*	
16	0.85	456	27C 5,22(E)	occelasterol
17	0.88	456	27C 5,22(E)	cholesta-5,22-dien-3β-ol
18	1.00	458	27C 5	cholesterol
19	1.10	470	28C 5,22(E)	brassica- or crinosterol
20	1.17	504	*	
21	1.26	472	28C 5	dihydrobrassica- or campesterol
22	1.36	484	29C 5,22(E)	stigma- or poriferasterol
		486	29C 22(E)	stigma- or poriferastanol
23	1.54	486	29C 5	β-sito- or clionasterol
24	1.64	472	*	
25	1.80	546,484,472	*/*/*	
26	1.94	486,456	*/*	

Footnote * requires further work for identification.

of the molecular ions of the sterol trimethylsilyl ethers which have been identified within each peak.

The common marine sterols were identified by comparison of g.l.c. retention data and mass spectra with those of trimethylsilyl ethers of standard sterols and by multiple mass spectrometric scanning of each g.l.c. peak to identify all overlapping constituents.

The new marine sterols were identified as follows :

PEAK 5: A molecular formula of $C_{21}H_{31}O_2(SiMe_3)$ was established for the TMS ether by accurate mass measurement of the molecular ion during the GC-MS scan. The presence of prominent ions at m/e 129 and $(M-129)^+$ were indicative of a Δ5-3β-trimethylsilyl ether grouping[6] and the ion at m/e 43 suggested the presence of an acetyl function.

The mass spectrum was identical with the published GC-MS data for the TMS ether[7] of the mammalian hormone 3β-hydroxy-17β-pregn-5-ene-20-one and was slightly different from that of the 17α-analogue[8], particularly in the lower abundance of the m/e 213 ion. The published g.l.c. retention data for the 17β-isomer were also in agreement with those observed for Peak 5.

Accordingly the sterol in Peak 5 was compared with an authentic sample of 3β-hydroxy-17β-pregn-5-ene-20-one[9] by GC-MS techniques. The comparison of g.l.c. retention times is given in Table 2 for both the free sterols and their TMS ethers. The GC-mass spectra of the two materials were identical and Peak 5 sterol is formulated as 3β-hydroxy-17β-pregn-5-ene-20-one (I).

PEAKS 8 and 10 : A molecular formula of $C_{21}H_{32}O_2(SiMe_3)_2$ was established for these sterols by accurate mass measurements and corresponds to that required for pregnene diols. The major ion at m/e 117 supported the presence of a 20-hydroxy pregnane system[10] and the ion at m/e 129 was indicative of a Δ5-3β-TMS ether structure[6]. In addition the g.l.c. retention times were comparable with the published data for Δ5-3β-pregnen-3,20-diols .[10]

The compounds were therefore compared with authentic samples of 3β,20β-dihydroxy-17β-pregn-5-ene[9] and 3β,20α-dihydroxy-17β-pregn-5-ene[9] ; epimers which are not distinguishable by mass spectrometry. The g.l.c. retention times are given in Table 2 for both the free sterols and the di-TMS ethers. The mass spectra of Peaks 8 and 10 were similar to the published spectra of the TMS ethers of the diols and are identical with those of authentic samples when run under identical conditions. Peak 8 sterol is therefore formulated as 3β,20β-dihydroxy-17β-pregn-5-ene (II) and Peak 10 sterol is formulated as 3β,20α-dihydroxy-17β-pregn-5-ene (III).

MINOR CONSTITUENTS : There are indications that some other keto-sterols may be present in these extracts. In particular Peaks 15 and 20 have been mass measured at high resolution and correspond to a $C_{22}O_3$-di-TMS ether and a $C_{23}O_3$-di-TMS ether respectively. These constituents and other minor sterols in this extract are being further investigated following large scale extractions of the sponge.

Table 2. Relative Retention Times on SE-30 at 210° (cholestane = 1.00)

3β-hydroxy-17β-pregn-5-ene-20-one[9]	0.62	0.78
Peak 5 sterol	0.63	0.79
3β-hydroxy-17α-pregn-5-ene-20-one[8]	0.56	0.70
3β,20β-dihydroxy-17β-pregn-5-ene[9,10]	0.62	1.09
Peak 8 sterol	0.62	1.08
3β,20α-dihydroxy-17β-pregn-5-ene[9,10]	0.66	1.16
Peak 10 sterol	0.67	1.18

The presence of these 20-keto and 20-hydroxy sterols containing the usual Δ5-3β-OH system is presumably due to oxidative cleavage of the normal marine sterol sidechains at C_{20} perhaps via a 20,22-diol intermediate metabolite.

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