

ISOLATION OF $\Delta^{9(11)}$ -STEROLS FROM THE SEA CUCUMBER PSOLUS FABRICII.
IMPLICATIONS FOR HOLOTHURIN BIOSYNTHESIS

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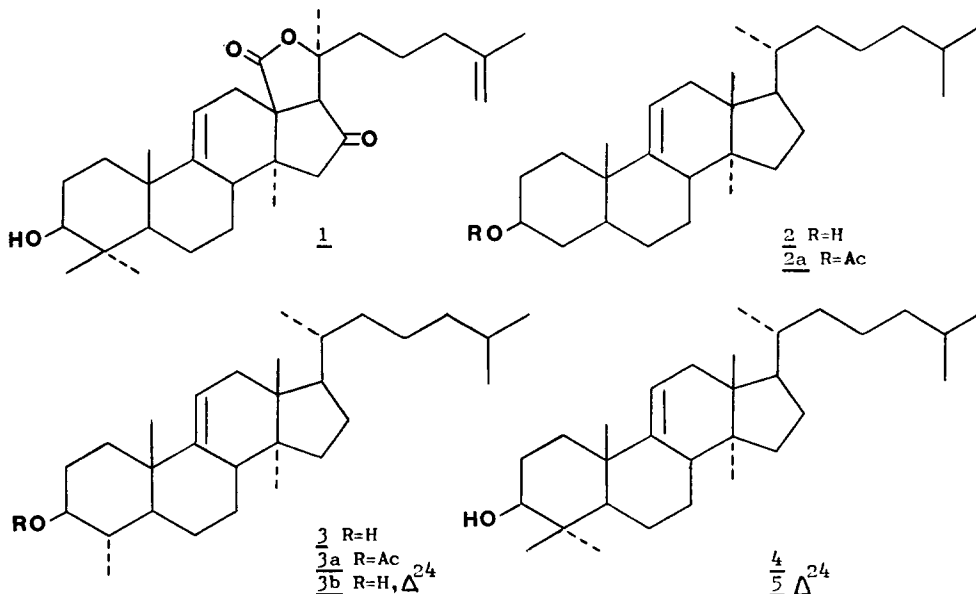
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Abstract - 14 α -Methyl-5 α -cholest-9(11)-en-3 β -ol (2) and 4 α ,14 α -dimethyl-5 α -cholest-9(11)-en-3 β -ol (3) have been isolated from the sea cucumber Psolus fabricii and characterised by ¹H NMR, ¹³C NMR and mass spectrometry. Lanost-9(11)-en-3 β -ol (4) has also been tentatively identified. The relevance of this series of $\Delta^{9(11)}$ -sterols to holothurin biosynthesis is briefly discussed.

The sterols present in marine organisms have received considerable attention and this has resulted in the identification of many new compounds.¹⁻³ Echinoderms have proved interesting as members of the Asteroidea (starfish) and Holothuroidea (sea cucumbers) contain predominantly free Δ^7 -sterols. This is in contrast to the Δ^5 -sterols which are found in Echinoidea (sea urchins), Crinoidea (sea lillies) and Ophiuroidea (brittle stars) and most other marine invertebrate phyla. Moreover, the starfish and sea cucumbers contain saponins.⁴ The holothurins, found in the sea cucumbers, have an aglycone based upon the lanostane skeleton (e.g. holotoxigenol, 1, the aglycone of psolothurin A)⁵ while the asterosaponins, found in starfish, have a cholestane or pregnane steroidal aglycone. Many of these compounds are characterised by a $\Delta^{9(11)}$ -bond and the biosynthetic origin of this structural feature is unknown. To gain an insight into possible biosynthetic pathways to the saponins we have undertaken an analysis of the sterol and steroid conjugate compositions of selected echinoderms. We now report the identification of three $\Delta^{9(11)}$ -sterols from the sea cucumber Psolus fabricii obtained from the North shore of the St. Lawrence river, Canada.

Lipid (5g) extracted from Psolus fabricii with benzene was subjected to medium pressure liquid chromatography on silica gel developed with CHCl₃-MeOH mixtures to yield a free sterol fraction (0.44g). Further chromatography on alumina followed by preparative TLC gave the 4,4-dimethylsterols (3mg), 4 α -methylsterols (29mg) and 4-desmethylsterols (105mg). The 4-desmethylsterol fraction was acetylated and separated into seven bands by preparative TLC on 10% AgNO₃-silica gel. The major compound 2a (34mg) was pure by GLC (RR_t = 1.10) and showed a molecular ion at m/z 442 (18%) and fragmentation ions at m/z 427 [M-CH₃]⁺ (100), 367 [M-acetate-CH₃]⁺ (78), 287 [M-ring D]⁺ (6) and 273 [M-ring D-CH₃]⁺ (13). The facile loss of a methyl group is characteristic of 14 α -methylsterols⁶ while the other fragmentations revealed a saturated C₈-side chain and a double bond in the ring system. The Δ^5 - or Δ^7 - positions for the double bond were apparently eliminated by the fact that compound 2a was

more polar than the Δ^5 - and Δ^7 -steryl acetate compounds of the mixture on AgNO_3 -silica gel TLC.⁷ The ^1H NMR spectrum of 2a (Table 1) showed an olefinic proton signal at δ 5.287 thus revealing a trisubstituted double bond and eliminating a $\Delta^{8(9)}$ -bond. This left the $\Delta^{9(11)}$ -position as the likely location for the unsaturation. A $\Delta^{9(11)}$ -bond was substantiated by the chemical shifts observed for the C-18 (δ 0.658) and C-19 (0.981) methyl protons. These values are considerably higher than those observed for a Δ^7 -sterol and compare with the calculated⁸ values for a $\Delta^{9(11)}$ -sterol (0.583 and 0.967, respectively) and those reported⁹ for another $\Delta^{9(11)}$ -sterol (0.64 and 1.00). A singlet at δ 0.753 was assigned to the protons on C-32 (i.e. a 14α -methyl group). The remaining three doublets had chemical shift values consistent with a saturated iso-octane side chain. The ^{13}C NMR spectrum (Table 2) was assigned on the basis of SFORD measurements and by comparison with literature ^{13}C NMR spectral assignments for other sterols.¹⁰ In particular the signals for C-9 (δ 145.85) and C-11 (116.63) established the presence of the $\Delta^{9(11)}$ -bond.¹⁰ Based upon the spectral evidence compound 2a was thus identified as the acetate of 14α -methyl- 5α -cholest-9(11)-en- 3β -ol (2).



The 4α -methylsterol fraction showed one main component (3) by GLC ($\text{RR}_t = 1.20$) which had a mass spectrum with ions at m/z 414 $[\text{M}]^+$ (19%), 399 $[\text{M}-\text{CH}_3]^+$ (100) and 301 $[\text{M}-\text{side chain}]^+$ (4) indicating a C_{29} -sterol with a C_8 -saturated side chain, a ring double bond and two methyl groups probably at C-4 and C-14. The ^1H NMR spectra (Table 1) of 3 and its acetate 3a (purified by AgNO_3 -silica gel TLC) showed an olefinic proton with a chemical shift (δ 5.29) very similar to that of 2a and the signals for H-18 (0.658) and H-19 (0.990) again revealed a $\Delta^{9(11)}$ -bond. The doublet at δ 0.983 for the protons of a methyl group and the H- 3α multiplet at δ 3.095 for 3 are characteristic for a 4α -methylsterol¹¹. A singlet for three protons at δ 0.738 can be assigned to a 14α -methyl group in 3. The ^{13}C NMR

spectrum for 3 (Table 2) was consistent with the other spectral evidence for the identification of the sterol as 4 α ,14 α -dimethyl-5 α -cholest-9(11)-en-3 β -ol. A very minor component had an M⁺ at m/z 412 (53%) and other ions at m/z 397, 375, 285, 273 and 69 indicating that it may be 4 α ,14 α -dimethyl-5 α -cholesta-9(11), 24(or 25)-diene-3 β -ol (3b). Compound 3 has previously been identified in a plant¹² and significantly as a constituent of another sea cucumber, *Cucumaria frondosa*.¹³

Table 1. ¹H NMR spectral data for *P. fabricii* sterols.

(CDCl₃, 250MHz for 2a, 400MHz for 3 and 3a)

	<u>2a</u>	<u>3</u>	<u>3a</u>
H-18	0.658 _s	0.658 _s	0.652 _s
H-32	0.753 _s	0.738 _s	0.740 _s
H-26	0.868 _d	0.864 _d	0.864 _d
H-27	0.871 _d	0.872 _d	0.872 _d
H-21	0.879 _d	0.877 _d	0.877 _d
H-30	-	0.983 _d	0.843 _d
H-19	0.981 _s	0.990 _s	1.005 _s
Acetate	2.025 _s	-	2.055 _s
H-3 α	4.678 _m	3.095 _{brm}	4.350 _{brm}
H-11	5.287 _m	5.290 _m	5.295 _m

Table 2. ¹³C NMR spectral data for *P. fabricii* sterols

(CDCl₃, 62.89 MHz for 2a and 100.57 MHz for 3).

Carbon	<u>2a</u>	<u>3</u>	Carbon	<u>2a</u>	<u>3</u>	Carbon	<u>2a</u>	<u>3</u>
1	35.30	35.52	11	116.63	116.39	21	18.43	18.46
2	27.24	31.34	12	37.34	37.52	22	36.57	36.65
3	73.60	76.53	13	44.35	44.39	23	24.15	24.17
4	34.29	39.58	14	47.16	47.20	24	39.56	39.63
5	42.88	49.46	15	33.97	34.03	25	28.03	28.09
6	28.54	24.17	16	27.64	27.54	26	22.84	22.83
7	28.03	28.09	17	51.11	51.21	27	22.56	22.57
8	41.87	41.52	18	14.45	14.50	30(4 α)	-	15.34
9	145.85	146.57	19	19.21	20.57	32(14 α)	18.43	18.46
10	38.07	38.76	20	36.19	36.21	CH ₃ CO	21.42;170.60	-

The 4,4-dimethylsterol material was obtained in small amount permitting only GC-MS analysis. One major component (RR_t = 1.41) had M⁺ at m/z 428 (26%) and fragments at 413(100), 395(36) and 273(14). It separated from lanost-8-en-3 β -ol (RR_t = 1.31) and the GLC¹⁴ and MS properties of 4 permitted its identification as lanost-9(11)-en-3 β -ol. A very minor component (RR_t = 1.51) had mass spectral ions at m/z 426 [M]⁺ (19%), 411(50), 393(21) and 69(100) revealing a diene structure. Its GLC

retention time was longer than that of lanosta-8,24-dien-3 β -ol and was consistent with its identification as lanosta-9(11),24-dien-3 β -ol (5).

The amounts of 4 and 5 were insufficient to permit further characterisation studies but the identification of sterols 2 and 3 suggests that the assignment of $\Delta^{9(11)}$ -structures to 4 and 5 is reasonable and would be in accord with the operation of the biosynthetic sequence squalene \rightarrow squalene-2,3-oxide \rightarrow 5 \rightarrow 4 \rightarrow 3 \rightarrow 2. This sequence requires that the squalene-2,3-oxide cyclisation reaction produces an intermediate cation which is stabilised to yield the $\Delta^{9(11)}$ -compound 5 rather than lanosta-8,24-dien-3 β -ol, the usual sterol precursor in other animals. The holothurin aglycones such as 1 would then arise from 5 by appropriate modifications and the $\Delta^{9(11)}$ -bond of the holothurins can thus be envisaged to arise at the triterpene cyclisation step rather than by C-9/C-11 dehydrogenation of an intermediate at a later stage. It is considered that a $\Delta^{8(9)}$ -bond is essential for C-14 demethylation in cholesterol biosynthesis. The presence of a $\Delta^{9(11)}$ -bond in the first cyclic precursors (4, 5) in sea cucumbers may thus result in the failure to remove the 14 α -methyl group and lead to the accumulation of sterols 2 and 3 in the animal. The 'normal' 14-demethyl Δ^7 -sterols found in the animal⁷ can be envisaged to arise by modification of dietary sterol as observed in other echinoderms.²

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- Calculations of RR_t's for sterols 4 and 5 based upon separation factors¹⁵ determined from sterols of known structure gave values in good agreement with the experimental RR_t's of 4 and 5 on a BP-1 capillary column (25m) operated at 280°C.
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