Two New Hydroxy Sterol Xylosides from the Sea Cucumber *Synapta Muculata*†

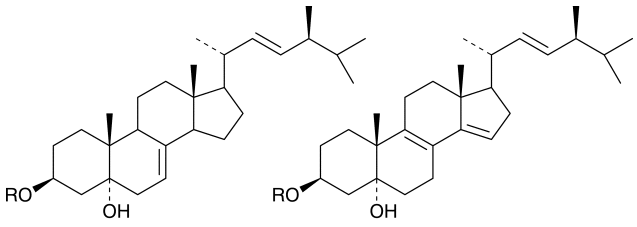
J. Chem. Research (S), 1998, 404–405†

S. V. A. S. Pradeep Kumar,[a] N. Dhananjaya[a] and G. B. S. Reddy*[b]

[a]*School of Chemistry, Andhra University, Visakhapatnam-530 003, India*
[b]*Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India*

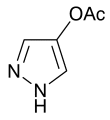
Two new steroidal xylosides (**1**–**2**) and a new 4-acetoxy pyrazole (**3**) along with two known dihydroxy sterols and a known 22,25-oxidoholothurinogenin are isolated from the cucumber *Synapta muculata*, their structures are elucidated by spectral data.

Sea cucumbers occur widely in the intertidal rocky regions of tropical as well as subtropical seas and are known to convert dietary Δ^6-sterols into Δ^7-sterols as a shield against their own toxic saponins.[1] Methanolic extract of the sea cucumber results in the isolation of two new sterol xylosides (**1**–**2**) and a new pyrazole derivative (**3**), two known dihydroxy sterols (24*S*)-24-methyl-5α-cholestane-7-ene-3β,5-diol[2] and (24*S*)-24-methyl-5α-cholestane-22-ene-3β,5-diol[2] and a known 22,25-oxidoholothurinogenin.[3]



1 R = xylose
1a R = H
1b R = triacetylxylose

2 R = xylose
2a R = H
2b R = triacetylxylose



3

Compounds **1** and **2** were recognized as a sterol xylosides from their ^{1}H NMR spectra. **1** and **2** were formed triacetyl derivatives which resonated signals at δ_H 1.98, 2.01 and 2.07 for acetoxy groups (IR showed tertiary OH band). The anomeric protons (1′-H) resonated at δ 4.90 and 4.91 (d, J 7 Hz) respectively, indicating the diaxial relation and hence β-glycosidic nature. Further evidence by a ^{13}C NMR signal at δ 106.3 supported the assignment. Xylose moiety was attached to C^3 of the aglycones (**1a** and **2a**) as evidenced[2] by ^{13}C NMR of the compound. The C3 carbon was at ~2.0 ppm downfield shift (Table 2). Xylose was identified by comparison with the authentic sample by descending co-paper chromatography. The ^{13}C NMR values for 3β,5α-diol[4] were in good agreement with the reported values by direct comparison. A signal at δ 3.75 (1H, m, $w_{1/2} - 16$ Hz) was assigned to the ubiquitous α-axial proton at C^3. Acid hydrolysis of **1** and **2** afforded **1a** [(24*S*)-24-methyl-5α-cholestane-7,22-diene-3β,5-diol, 12 mg, $[\alpha]_D^{25}$ −22.0, mp 133–5 °C, MS: 414] and **2a** [(24*S*)-24-ethyl-5α-cholestane-8,14,22-triene-3β,5-diol, 20 mg, $[\alpha]_D^{25}$ −29.0, mp 140–2 °C, MS: 426] respectively. A signal at δ 5.41 in the ^{1}H NMR spectrum indicates the presence of a double bond. On comparison of the ^{13}C NMR of compound **1** with cholest-7-ene,[5] the double bond was fixed at Δ^7. The ^{13}C NMR data of the side chain in **1** is in agreement with that of a 24*S*-methyl-22-cholestene side chain[6,7] and a signal at δ 5.17 (2 H, m) suggests Δ^{22}-unsaturation.[6,7] The FABMS of **1** showed the molecular ion at m/z 546, in addition to the ions at m/z 449, 421 and 413. The EIMS of **1a** showed a peak at m/z 299 ($M^+ - H_2O - C_7H_{13}$) supporting the presence of unsaturation in the side chain. Thus **1** was identified as (24*S*)-5-hydroxy-24-methyl-5α-cholesta-7,22-dien-3β-yl-D-xylopyranoside.

The UV spectrum showed an absorption maximum at 248 nm suggesting conjugation in **2**. The ^{1}H NMR spectrum of **2a** exhibited a multiplet at δ 5.32 (brd, 1H) and direct comparison of the ^{13}C NMR data[8] suggested unsaturation between trisubstituted and disubstituted carbon atoms possibly at $\Delta^{14(15)}$ implying the presence of conjugation. This could be supported by ^{13}C NMR values of **2a** at δ 150.68 (lit. 151.0[8] of C^{14}) and 116.78 (lit. 116.7[8] of C^{15}) and the signals at δ 122.95 (lit. 122.9[8] of C^8) and 140.96 (lit. 141.0[8] of C^9) suggests a double bond at $\Delta^{8(9)}$ leading to the conjugation in the structure. The ^{1}H NMR spectrum showed a signal at δ 5.17 (2 H, m) which limits the existence of a double bond between two disubstituted carbon atoms. This suggested unsaturation at Δ^{22} when comparing with ^{13}C NMR at δ 135.91 (lit. 136.05[5–7] of C^{22}) and 131.91 (lit. 131.84[5–7] of C^{23}). The configuration at C^{24} was assigned as 24(*S*) on comparison of ^{13}C NMR[6,7] data. The EIMS of **2a** exhibits a peak at m/z 287 ($M^+ - H_2O - C_8H_{15}$) also indicating the presence of unsaturation in the side chain. The FABMS of **2** showed a molecular ion m/z 558 and ions at m/z 447, 419 and 286. Hence, **2** was identified as (24*S*)-5-hydroxy-24-ethyl-5α-cholesta-8,14,22-trien-3β-yl-D-xylopyranoside.

The IR spectrum of compound **3** showed absorption at 3550, 3150 and 3096 cm^{-1} of C—NH and C=N groups respectively. The absorption bands at 1699, 1600, 1386, 1183, 1025 and 855 cm^{-1} indicate the presence of unsaturation. The UV absorption at 263 nm indicates the presence of conjugation. In the ^{13}C NMR spectrum signals appear at δ 168.0 (C=O), 13.1 ($OCOCH_3$), 119.0 and 138.6 (two olefinic carbons of the pyrazole moiety) and 154.0 (acetoxy-bearing tertiary olefinic carbon). The ^{1}H NMR spectrum showed signals at δ 2.0 (3H, s, $OCOCH_3$) and 5.15 (2H, s, olefinic protons). From the data **3** was characterised as 4-acetoxy pyrazole.

Experimental

Extraction and Isolation of Compounds **1**–**3**.—The sea cucumber was collected at the Andaman and Nicobar Islands. The extraction of the material was carried out at room temperature by percolating with methanol repeatedly until no residual material was found. The solvent was stripped off and the residue (100 g) separated by chromatography with silica gel using solvents with increasing polarity [light petroleum (bp 40–45 °C) and methanol] and yielded the six pure compounds **1**–**3**.

*To receive any correspondence (*e-mail:* vasavi-vizag/bangalore@-dartmail.dartnet. com).

†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1998, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

Table 1 ^{1}H NMR (200 MHz) data for **1**, **2**, **1a** and **2a**

Assignment	Chemical shift, δ (multiplicity, proton integration, J in Hz)			
	1[a]	**2**[a]	**1a**[b]	**2a**[b]
H_3^{19}	1.12 (s, 3H)	1.13 (s, 3H)	0.86 (s, 3H)	0.86 (s, 3H)
H_3^{28}	0.90 (d, 3H, 6.6)	–	0.91 (d, 3H, 6.6)	–
H_3^{29}	–	1.03 (t, 3H, 6.8)	–	0.92 (t, 3H, 7)
$H_3^{2'}$	0.96 (d, 3H, 6.8)	0.96 (d, 3H, 6.7)	1.06 (d, 3H, 6.8)	1.06 (d, 3H, 6.6)
H_3^{26}	1.02 (d, 3H, 6.7)	1.06 (d, 3H, 6.6)	0.83 (d, 3H, 6.7)	0.83 (d, 3H, 6.6)
H_3^{27}	1.06 (d, 3H, 6.6)	1.06 (d, 3H, 6.8)	0.81 (d, 3H, 6.6)	0.81 (d, 3H, 6.8)
$H^{3\alpha}$	3.75 (m, 1H)	3.75 (m, 1H)	3.6 (m, 1H)	3.62 (m, 1H)
H^7	5.41 (br d, 1H, 7.2)	–	5.03 (br, d, 1H, 7)	–
H^{15}	–	5.36 (br d, 1H, 7.1)	–	5.32 (br d, 1H, 7)
H^{22} and H^{23}	5.17 (m, 2H)	5.20 (m, 2H)	5.11 (m, 2H)	5.17 (m, 2H)
$H^{1'}$	4.90 (d, 1H, 7)	4.91 (d, 1H, 7)		
$H^{2'}$	4.21 (m, 1H)	4.20 (m, 1H)		
$H^{3'}$	4.39 (m, 1H)	4.39 (m, 1H)		
$H^{4'}$	4.01 (m, 1H)	4.00 (m, 1H)		
$H_2^{5'}$	3.90 (m, 2H)	3.90 (m, 2H)		

[a]In C_5D_5N. [b]In $CDCl_3$.

Table 2 ^{13}C NMR (50 MHz) date for **1**, **2**, **1a** and **2a**

C No.	Chemical shift, δ			
	1[a]	**2**[a]	**1a**[b]	**2a**[b]
1	37.3	37.2	36.82	36.82
2	32.2	32.21	31.46	31.42
3	73.4	73.38	71.05	71.45
4	36.8	36.80	35.47	35.47
5	75.8	75.9	73.46	73.45
6	32.4	30.10	29.62	29.50
7	118.59	32.30	117.43	32.36
8	141.0	123.88	139.57	122.95
9	50.2	141.91	50.61	140.96
10	38.8	38.90	39.30	39.36
11	21.23	22.40	21.38	21.38
12	39.45	36.06	39.46	36.08
13	41.9	41.80	40.02	40.02
14	55.2	151.60	55.00	150.68
15	22.92	116.99	22.96	116.78
16	27.41	26.70	27.47	26.64
17	55.30	55.97	55.03	55.03
18	12.24	16.10	12.24	15.54
19	20.6	20.42	17.02	18.40
20	39.49	40.50	40.52	40.62
21	18.4	21.20	21.16	21.46
22	135.55	135.50	135.91	135.91
23	132.10	132.00	131.87	131.91
24	45.40	51.30	43.24	51.35
25	35.90	35.80	33.78	33.78
26	18.23	18.23	19.60	19.60
27	24.1	24.50	19.72	21.09
28	18.92	26.10	18.97	25.52
1′	106.3	12.90		12.26
2′	75.3	106.30		
3′	78.21	75.40		
4′	70.9	78.40		
5′	67.29	70.15		
		68.00		

[a]In C_5D_5N. [b]In $CDCl_3$.

(24S)-5-*Hydroxy*-24-*methyl*-5α-*cholesta*-7,22-*dien*-3β-*yl*-D-*xylopyranoside* **1**.—Yield 35 mg, mp 233–235 °C; $[\alpha]_D^{28}$ +10.5° (*c* 0.2, MeOH); (Found: C, 72.7; H, 10.3. $C_{33}H_{54}O_6$ requires C, 72.5; H, 10.0), MS: *m*/*z* 546 (M^+, 4%) 449 (M^+–C_7H_{13}, 10%), 421 (M^+ – side chain, 8), 413 (M^+ – xylose, 20) and 269 (M^+ – xylose – side chain – H_2O – H, 40).

(24S)-5-*Hydroxy*-24-*ethyl*-5α-*cholesta*-8,14,22-*trien*-3β-*yl*-D-*xylopyranoside* **2**.—Yield 28 mg, mp 245–247 °C; $[\alpha]_D^{25}$ +16.8° (*c* 0.2 MeOH); UV: 248 nm (EtOH, ε 18000); (Found: C, 73.6; H, 9.4. $C_{34}H_{54}O_6$ requires C, 73.1; H, 9.7%), MS: *m*/*z* 558 (M^+, 3%) 447 ($M^+ - C_8H_{15}$, 11%), 425 (M^+ – xylose, 23), 419 (M^+ – side chain, 10) and 286 (M^+ – xylose – side chain, 34).

4-*Acetoxypyrazole* **3**.—Yield 20 mg, mp 243–245 °C; UV: 263 nm (MeOH, ε 14500); M^+ (126) analysed for $C_5H_6N_2O_2$, 83 (60%), 71 (32%), 55 (100%); ^{1}H NMR (400 MHz, C_5D_5N): δ 2.0 (3H, s, $OCOCH_3$), 5.15 (2H, s, H^3 and H^5), 8.1 (1H, s, NH); ^{13}C NMR (100 MHz, C_5D_5N): δ 168.0 ($COCH_3$), 154 (C^4), 138.6 (C^3), 119.0 (C^5) and 13.1 ($OCOCH_3$).

Acetylation of **1**: *formation of* (24S)-5-*hydroxy*-24-*methyl*-5α-*cholesta*-7,22-*dien*-3β-*yl*-D-*triacetoxy xylopyranoside* **1b**.—To a solution of **1** (3 mg) in pyridine (1 ml), Ac_2O (0.5 ml) was added and kept overnight at room temperature. Usual work-up afforded a triacetyl derivative (**1b**, 3 mg), $C_{39}H_{60}O_9$, mp 200–202 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.98 (s, 3H, $C^{4'}$OMe), 2.02 (s, 3H, $C^{2'}$OMe) and 2.07 (s, 3H, $C^{3'}$OMe).

Acetylation of **2**: *formation of* (24S)-5-*hydroxy*-24-*ethyl*-5α-*cholesta*-8,14,22-*trien*-3β-*yl*-D-*triacetoxy xylopyranoside* **2b**.—To a solution of **2** (3 mg) in pyridine (1 ml), Ac_2O (0.5 ml) was added and kept overnight at room temperature. Usual work-up afforded a triacetyl derivative (**2b**, 3 mg), $C_{40}H_{60}O_9$, mp 205–207 °C, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.96 (s, 3H, $C^{4'}$OMe), 2.01 (s, 3H, $C^{2'}$OMe), and 2.07 (s, 3H, $C^{3'}$OMe).

Received, 5th August 1997; Accepted, 2nd February 1998
Paper E/7/05682F

References

1 P. J. Scheuer, *Bioorg. Nat. Prod.*, 1988, **2**, 43.
2 J. W. Blunt and J. B. Stothers, *Org. Magn. Reson.*, 1977, **9**, 439.
3 N. S. Sarma, A. S. R. Anjaneyulu and Y. Venkateswarlu, *Indian J. Chem., Sect. B*, 1987, **26**, 715.
4 H. Eggert, C. L. VanAntwerp, N. S. Bhacca and C. Djerassi, *J. Org. Chem.*, 1976, **41**, 71.
5 M. Kobayashi, M. M. Krishna and V. Anjaneyulu, *Chem. Pharm. Bull.*, 1992, **40**, 2845; M. Kobayashi, M. M. Krishna, B. Haribabu and V. Anjaneyulu, *Chem. Pharm. Bull.*, 1993, **41**, 87.
6 J. L. C. Wright, A. G. McInnes, S. Shimizu, D. G. Smith, J. A. Walter, D. Idler and W. Khalil, *Can. J. Chem.*, 1978, **56**, 1898.
7 I. Rubinstein, J. L. Goad, A. D. H. Clague and J. Mulheirn, *Phytochemistry*, 1976, **15**, 195.
8 L. Minale, R. Riccio, F. De Simone, A. Dini, C. Pizza and E. Ramundo, *Tetrahedron Lett.*, 1978, **29**, 2609.