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NOVEL SULFATED STEROL GLYCOSIDES FROM *ASTERIAS FORBESI*

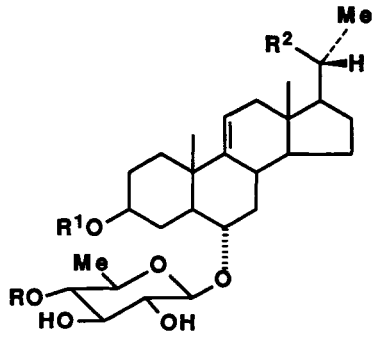
JOHN A. FINDLAY* and ZHENG-QUAN HE

Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E2

ABSTRACT.—The structures of three minor constituents of the saponin fraction of the starfish *Asterias forbesi* have been established as sodium (20*R*)-5α-pregn-9(11)ene-3β-sulfo-oxy-6α-[*O*-β-D-quinovopyranosyl]-20-ol [**2**], sodium (20*R*)-5α-pregn-9(11)ene-6α-[*O*-β-D-sulfo-oxyquinovopyranosyl]-3β,20-diol [**4**], and sodium (20*R*)-5α-pregn-9(11)ene-3β-sulfo-oxy-6α-[*O*-β-D-quinovopyranosyl]-20-one [**5**] by spectroscopic means.

The occurrence of steroidal sulfated glycosides in echinoderms is well established (1). Although their biological role is uncertain, several such compounds are reported to be cytotoxic (2). Recently we reported (3) the occurrence of a novel sulfated sterol glycoside, forbeside E, in the common starfish *Asterias forbesi* Desor (family Asteriidae, order Forcipulatida). Its structure **1** we established principally by nmr methods. We have now succeeded in separating and purifying three structurally related new steroidal sulfated glycosides from *A. forbesi*, which we designate forbesides E1, E2, and E3.

We assign structure **2** to forbeside E1 based on the following evidence. The positive ion fab mass spectrum of forbeside E1 displays prominent ions at *m/z* 604 $[M(C_{27}H_{43}O_{10}SNa)-H+Na]^+$, 627 $[M-H+2Na]^+$, and 563 $[M-H-H_2O]^+$ in support of formulation **2**. The ^{13}C nmr [pyridine-d_5–D_2O (5:1)] shows the presence of 27 carbon atoms whose chemical shifts are entirely consistent with structure **2** (see Table 1). Thus, while the signals for the aglycone are in close agreement with those of forbeside E [**1**], significant differences are seen in the chemical shifts for the carbohydrate moiety. The latter are consistent with those of an unsubstituted β-linked quinovose unit (4). Solvolysis of **2** affords the same product **3** as was obtained by solvolysis of **1**. Thus we formulate forbeside E1 as sodium (20*R*)-5α-pregn-9(11)ene-3β-sulfo-oxy-6α-[*O*-β-D-quinovopyranosyl]-20-ol [**2**].



1 R,R^1=SO_3Na, R^2=OH
2 R=H, R^1=SO_3Na, R^2=OH
3 R,R^1=H, R^2=OH
4 R=SO_3Na, R^1=H, R^2=OH
5 R=H, R^1=SO_3Na, R^2= =O

Forbeside E2 showed an intense pseudo molecular ion at *m/z* 605 $[M(C_{27}H_{43}O_{10}SNa)+Na]^+$ in its positive ion fab mass spectrum. The ^1H-nmr spectrum [pyridine-d_5–D_2O, (5:1)] is generally similar to that of forbeside E in the high field region and closely resembles the low field region of the latter except for the chemical shift for H-3 which is located upfield in forbeside E2 at δ 4.1 ppm, indicating the 3-OH to be unsubstituted. From the 2D COSY (5,6) spectrum it is possible to elucidate the connectivity of proton signals in the sugar moiety; this, together with coupling constant data, establishes it to be a β-linked quinovose. The structure **4**, sodium (20*R*)-5α-pregn-9(11)ene-6α-*O*-β-D-sulfo-oxyquinovopyranosyl]-3β, 20-diol, for forbeside E2 was corroborated by a solvolysis reaction which removed the sulfate moiety and gave compound **3** previously obtained from forbeside E.

TABLE 1. Nmr Data for Forbeside E [1], Forbeside E1 [2], Forbeside E2 [4], Forbeside E3 [5], and Compound 3.[a]

Position	Compound							
	1	2		3		4	5	
	^{13}C	^{13}C	$^{1}H^{b}$ J(Hz)	^{13}C	$^{1}H^{b}$ J(Hz)	$^{1}H^{b}$ J(Hz)	^{13}C	$^{1}H^{b}$ J(Hz)
1	34.80	35.24		35.42			35.79	
2	28.09	28.49		30.66			29.09	
3	77.07	77.83	4.80 m	70.12	3.7 m	4.1 m	78.06	4.8 m
4	29.45	29.87		31.95			30.44	
5	47.84	48.29		48.59			48.74	
6	78.55	79.04	3.8 m	78.92	3.7 m	3.8 m	79.32	3.8 m
7	40.64	41.04		41.12			41.27	
8	34.56	35.05		35.17			35.50	
9	144.03	144.46		144.50			145.79	
10	37.18	37.67		37.92			38.25	
11	116.42	116.96	5.18 bs	117.00	5.18 bs	5.25 bs	116.11	5.23 dd 1.9
12	40.53	40.77		40.81			40.41	
13	40.34	40.77		40.81			42.66	
14	52.37	52.83		52.90			53.56	
15	24.90	25.20		25.20			23.05	
16	25.17	25.65		25.64			25.45	
17	57.39	57.80		57.82			63.36	
18	11.09	11.57	0.90 s	11.58	0.69 s	1.00 s	13.13	0.89 s
19	18.15	18.63	0.77 s	18.82	0.86 s	0.78 s	19.13	0.52 s
20	68.43	68.99	3.8 m	68.99	3.8 m	3.8 m	211.45	—
21	22.83	23.22	1.39 5.9	23.21	1.20 d 5.7	1.39 d 5.8	31.30	2.34 s
1′	103.77	104.56	4.84 d 7.9	104.49	4.75 7.4	4.88 d 7.3	105.19	4.85 d 7.2
2′	74.07	74.70	4.01 dd 9.2[c]	74.83	3.95 9[c]	4.1	75.31	4.04 dd 7, 9
3′	75.34	76.52	4.16 dd 8.9[c]	76.70	4.09 9[c]	4.37 dd 8.5[c]	77.18	4.20 dd
4′	81.05	75.75	3.75 dd 9.0[c]	75.81	3.7 m	4.68 dd 8.6[c]	76.33	3.75 dd
5′	69.71	71.88	3.8 m	71.87	3.7 m	3.8 m	72.44	3.8 m
6′	17.45	17.97	1.64 5.8	17.96	1.50 d 6.4	1.78 d 6.4	18.66	1.65 d 6.0

[a]Chemical shifts (ppm) in pyridine-d_5–D_2O (5:1).
[b]Assignments assisted by COSY data.
[c]$J_1 \simeq J_2$.

The ^{13}C nmr [pyridine-d_5–D_2O, (5:1)] of forbeside E3 is very similar to that of **2** except for the signal for C-20 which appears at δ 211.45 in the former. The ^1H-nmr spectrum of forbeside E3 shows a methyl singlet at δ 2.34 ascribed to a 21-Me. Comparison of ^{13}C chemical shift data (see Table 1) with that for pectinoside D (7) confirmed the presence of the asterone aglycone in forbeside E3. The positive fab mass spectrum of forbeside E3 displays a prominent pseudo molecular ion at *m/z* 603 [M ($C_{27}H_{41}O_{10}SNa$)+Na]$^+$; hence we formulate this compound as sodium (20*R*)-5α-pregn-9(11)ene-3β-sulfo-oxy-6α-[*O*-β-D-quinovopyranosyl]-20-one [**5**].

In the course of isolating these compounds several new complex saponins have been obtained and are currently under investigation.

EXPERIMENTAL

Nmr spectra were recorded on a Varian XL200 instrument using TMS as internal standard. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a Kratos MS50 instrument. Preparative tlc was performed with precoated Si gel F254 (1 mm) plates.

ISOLATION.—The starfish, *A. forbesi*, were collected at Indian Point, Bay of Fundy, Canada, on September 26, 1988, and frozen. A voucher specimen is maintained at the Chemistry Department, University of New Brunswick. The frozen animals (20 kg, wet wt) were defrosted, cut into small pieces, and extracted with cold MeOH (40 liters). The total aqueous MeOH extract was filtered, and the filtrate was concentrated on a rotary evaporator at 30°. The aqueous extract thus obtained (4 liters) was passed through a column of Amberlite XAD-2 (1 kg, 30–50 mesh). The column was washed with distilled H_2O (2 liters) and then eluted with MeOH (4 liters). The MeOH eluate was concentrated to small volume (250 ml) and then treated with cold Me_2CO (2500 ml) to yield a yellow powder of crude glycosides (12 g).

The crude glycoside mixture (10 g) was dissolved in $CHCl_3$-MeOH-H_2O (66:44:5) and filtered, and the filtrate was chromatographed on a Si gel (500 g, 230–400 mesh) column and eluted with the same solvent system, yielding 15 fractions. Fraction 3 (236 mg) was rechromatographed on preparative tlc plates (1 mm, 50 mg/plate) using the solvent system $CHCl_3$-MeOH-H_2O (7:3:0.5) to give compounds **2** (32 mg), **4** (6 mg), and **5** (16 mg), which were further purified by preparative tlc (0.5 mm) using *n*-BuOH–EtOAc–H_2O (5:1:1).

Forbeside E1 [**2**]: mp 218°, $[\alpha]^{28}$ +4.2 (*c* = 0.005, H_2O); fabms (magic bullet) *m/z* 627 (7.4), 604 (19.6), 585 (4.7), 563 (14.4), 542 (4.8), 507 (4.6), 485 (4.5), 465 (5.2), 451 (9.9), 439 (8.1), 422 (13.1), 407 (8.2), 367 (4.6), 332 (5.6), 221 (13.9), 199 (42.1), 177 (25.8), 165 (57).0), 143 (72.0).

Forbeside E2 [**4**]: mp 204°, $[\alpha]^{28}$ +8 (*c* = 0.002, H_2O); fabms (magic bullet) *m/z* 605 (16.9), 590 (3.2), 485 (3.1), 449 (4.3), 329 (4.2), 307 (15.2), 221 (8.7), 199 (17.9), 177 (21.2), 165 (67.2), 143 (72.3).

Forbeside E3 [**5**]: mp 206°, $[\alpha]^{28}$ −3.5 (*c* = 0.004, H_2O); fabms (magic bullet) *m/z* 603 (10.1), 551 (5.5), 507 (3.6), 463 (2.8), 453 (2.8), 425 (4.2), 307 (7.1), 199 (11.5), 177 (24.3), 165 (45.1), 143 (89.6).

Solvolysis of forbesides E1 [**2**] and E2 [**4**] was performed as described (3) for forbeside E and afforded the same product **3**, identified by ^1H-nmr and tlc behavior, in each case.

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LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **4**, 5, 539 (1987).
2. M.V. D'Auria, R. Riccio, L. Minale, S. La Barre, and J. Pusset, *J. Org. Chem.*, **52**, 3947 (1984).
3. J.A. Findlay, Z.-Q. He, and M. Jaseja, *Can. J. Chem.*, **67**, (1989).
4. J.A. Findlay, M. Jaseja, and J.-R. Brisson, *Can. J. Chem.*, **65**, 2605 (1987).
5. A. Bax and R. Freeman, *J. Magn. Reson.*, **44**, 542 (1981).
6. A. Bax, R. Freeman, and G. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
7. M.A. Dubois, Y. Noguchi, R. Higuchi, and T. Komori, *Liebigs Ann. Chem.*, 495 (1988).

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