Journal of Natural Products
Vol. 48, No. 5, pp. 751-755, Sep-Oct 1985

STARFISH SAPONINS, PART 21.[1] STEROIDAL GLYCOSIDES FROM THE STARFISH *OREASTER RETICULATUS*

ROSABEL SEGURA DE CORREA,[2] RAFFAELE RICCIO, LUIGI MINALE,

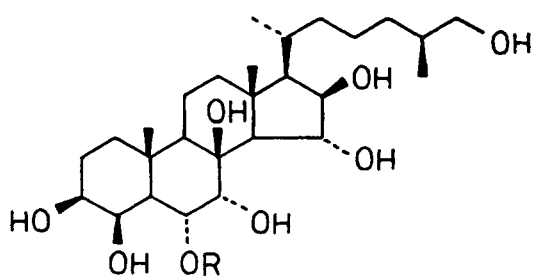
Dipartimento di Chimica delle Sostanze Naturali, Università, Via L. Rodinò, 22, 80138 Napoli, Italy

and CARMENZA DUQUE

Departamento de Quimica, Universidad Nacional de Colombia, Bogotà, Colombia

ABSTRACT.—A new sulfated steroidal glycoside (**3**), 3′-*O*-sulfate 24-*O*-(α-arabinofuranosyl)-5α-cholestane-3β,6α,8,15α,24-pentol, has been isolated from the starfish *Oreaster reticulatus*. It co-occurs with asterosaponin-P-1 (**2**), previously isolated from *Patiria pectinifera*, and 5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octol 6-*O*-sulfate (**1**).

Continuing with our investigation of biologically active steroidal glycosides from echinoderms, we have examined the polar extracts of the starfish *Oreaster reticulatus* L. and have isolated a novel sulfated steroidal glycoside (**3**) along with asterosaponin P-1 (**2**), previously isolated from *Patiria pectinifera* M.Tr. (1), and the 6-*O*-sulfate derivative of the (25*S*)-5α-cholestane-3β,4β,6α,7α,8,15α, 16β,26-octol, previously isolated as the nonsulfated steroid from *Protoreaster nodosus* L. (2,3).



1 R=$SO_3^-Na^+$
1a R=H

EXPERIMENTAL

INSTRUMENTATION.—The following instruments were used: nmr, Brüker WM-250; mass spectrometer, Kratos MS 50 mass spectrometer equipped with Kratos fab source; hplc, Waters Model 6000A pump equipped with a U6K injector and a differential refractometer, model 401 detector; dccc, DCCC Büchi equipped with 300 tubes. The fab-mass spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with Argon atoms of energy of 2-6 kv.

EXTRACTION AND ISOLATION.—*O. reticulatus* was collected in the Bay of Cartagena, Colombia, in April, 1984, and identified by Sven Zea, M.S., Biologist, National University of Colombia, Bogotà. A reference specimen of the organism is deposited at the Universidad de Bogotà, Jorge Tadeo Lozano, Museo del Mar., collection code: MM-5069 ECHIN. 249.

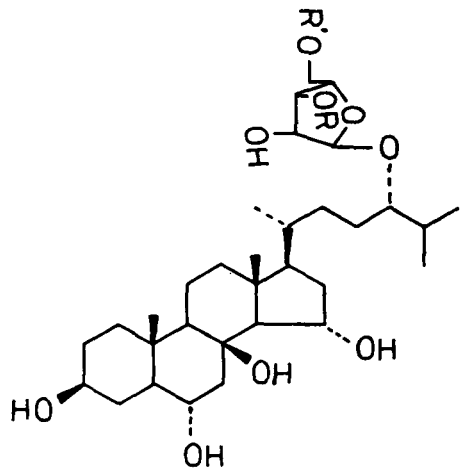
The animals (300 g) were extracted with $CHCl_3$-MeOH-H_2O (4:2:1) (3×1 liter). The MeOH-H_2O extracts were evaporated on a rotary evaporator and then lyophilized to give 5.1 g of material. This material was chromatographed on a column of silica gel (60 Merck, 200 g) using $CHCl_3$-MeOH (7:3) and increasing amounts of MeOH. The sulfated steroids fraction eluted with $CHCl_3$-MeOH (1:1). This fraction submitted to dccc with *n*-BuOH-Me_2CO-H_2O (3:1:5) (ascending mode; flow 20 ml/h). The eluants were col-

[1]For Part 20, see C. Pizza, P. Pezzullo, L. Minale, E. Breitmaier, J. Pusset, and P. Tirard, *J. Chem. Res.* (in press).

[2]On leave of absence from Departamento de Quimica, Universidad Nacional de Colombia, Bogotà, Colombia.

lected in 5 ml fractions and monitored by tlc on silica with *n*-BuOH-HOAc-H_2O (12:3:5). Fractions 64-102 contained a mixture of **2** and **3** which was resolved by hplc on a C_{18} μbondapak column (30 cm×7.8 mm i.d.) with MeOH-H_2O (1:1) (flow 5 ml/min). The band with elution time of 12 min gave 16 mg of compound **3**, [α]D +0.2 (c, 1; MeOH), fab-ms *m/z* 709 (M+Na), 607 (M+H−SO_3), 589 (M+H−H_2SO_4). The band with elution time of 14 min gave 17 mg of asterosaponin P-1 (**2**), [α]D=+0.4° (c, 1; MeOH (Lit [1] [α]D +3.0°), fab-ms *m/z* 739 (M+K), 723 (M+Na). Fractions 111-138 contained mainly compound **1**, which was further purified by hplc on the same column as before with MeOH-H_2O (55:45) to give 10 mg of pure **1**, [α]D ±0°, fab-ms *m/z* 625 (M+Na).

SOLVOLYSIS OF COMPOUNDS **1, 2,** AND **3** TO GIVE THE DESULFATED **1a, 2a,** AND **3a**.—A solution of each sulfated compound (ca. 5 mg) in dioxane (0.4 ml) and pyridine (0.4 ml) was heated at 120° for 4 h in a stoppered vial. After the solution had cooled, H_2O was added, and the solution was extracted with *n*-BuOH (three times). The combined extracts were washed with H_2O, evaporated, and subjected to hplc on a C_{18} μ-bondapak column (30 cm×7.8 mm) with MeOH-H_2O (65:35) to afford the desulfated compounds.



2 R=CH_3, R'=$SO_3^-Na^+$
2a R=CH_3, R'=H
3 R=$SO_3^-Na^+$, R'=H
3a R=R'=H

Compound **1a**.—[α]D=+9° (lit. [2] [α]D +10°), fab-ms *m/z* 523 (M+Na), ^{1}H nmr identical with that of 5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octol (2).

Compound **2a**.—Fab-ms *m/z* 621 (M+Na), 435 (M+H−146−H_2O), 417 (M+H−146−2H_2O), 399 (M+H−146−3H_2O), 381 (M+H−146−4H_2O); ^{1}H nmr δ (aglycone) 0.93 (9H, d, *J*=6.7 Hz, 21-, 26-, and 27-H_3) 1.00 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 2.42 (1H, dd, *J*=12.5, 4.5 Hz, 7β-H), 3.53 (1H, m, 3α-H), 3.65 (1H, dt, *J*= 3.5, 9 Hz, 6β-H), 4.23 (1H, dt, *J*=3.5, 10 Hz, 15β-H); δ (3-*O*-methyl arabinose), 3.45 (3H, s, OCH_3), 3.58 (1H, dd, *J*=5.0 and 2.5 Hz, 3-H), 3.68 (1H, dd, *J*=12.5 and 5.0 Hz, 5-H), 3.75 (1H, dd, *J*=12.5 and 3.5 Hz, 5-H), 4.05-4.08 (2H, m, 2-H and 4-H), and 4.98 (1H, bs, 1-H).

Compound **3a**.—Fab-ms *m/z* 607 (M+Na), 435 (M+H−132−H_2O), 417 (M+H−132−2H_2O), 399 (M+H−132−3H_2O); ^{1}H nmr, aglycone identical to that of compound **2a**, δ (arabinose) 3.67 (1H, dd, *J*=12.5 and 5 Hz, 5-H), 3.76 (1H, dd, *J*=12.5 and 2.5 Hz, 5-H), 3.87 (1H, dd, *J*=6.5 and 3.5 Hz, 3-H), 4.04 (2H, m, 2- and 4-H), 4.95 (1H, d, *J*=1.5 Hz, 1-H).

RESULTS AND DISCUSSION

Fast atom bombardment (fab) mass spectrometry of compound **1** gave molecular ion species at *m/z* 625 (M+Na). M is the molecular weight of the sodium salt. The intense peak at *m/z* 523 was interpreted as due to the loss of SO_3 from M+H. Solvolysis, using a dioxane-pyridine mixture (4), afforded (25*S*)-5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octol (**1a**), fab-ms *m/z* 523 (M+Na), previously described from *Proto-*

reaster nodosus (2,3). The sulfate group was located at C-6 on comparing the ^{1}H and ^{13}C nmr of **1** with that of the desulfated analogue (**1a**). In the ^{1}H-nmr spectrum of **1** the resonance frequency of the protons at positions 6β and 7β have moved downfield relative to **1a** (δ 5.03 vs. 4.25 and 4.18 vs. 3.88). The sulfation at 6α-OH also resulted in a downfield shift of the C-19 methyl protons (δ 1.30 vs. 1.21). The ^{13}C nmr frequencies of C-6, C-5, and C-7 in the spectrum of (**1**) are shifted by +9.3, −1.2, and −1.2 ppm, respectively, relative to **1a**.

Compound **2** was identified as 5′-*O*-sulfate 24-(α-3-*O*-methyl-L-arabinofuranosyl)-5α-cholestane-3β,6α,8β,15α-24-pentol, previously isolated from the starfish *Patiria pectinifera*, on the basis of the fab mass spectral data and comparison of ^{1}H- and ^{13}C-nmr data with those reported in the literature (1). Tables 1 and 2 contain the spectral data of **2**.

TABLE 1. ^{1}H-nmr Data in δ (Hz) (d_4-MeOH).[a]

H at C	Compounds					
	1	**1a**	**2**	**2a**	**3**	**3a**
3	3.52 m (W½ 22)		3.52 m		3.52 m	
4	4.26 m (W½ 7)	4.22 m				
6	5.03 dd (11.5,2.5)	4.25 dd (11.5,2.5)	3.64 dt[c] (3.5,10)		3.66 dt (3.5,10)	
7	4.18 d (2.5)	3.88 d (2.5)	2.41 dd (1H) (11.5,4)		2.41 dd (11.5,4)	
15	4.18 dd (10,2.5)	4.17 dd (10.5,2.5)	4.25[d]	4.23 dt (3.5,9.5)	4.25[f]	4.23 dt (3.5,9.5)
16	4.02 dd (7,2.5)					
18	1.15 s		1.00 s		1.00 s	
19	1.30 s	1.21 s	1.05 s		1.05 s	
21	0.95 d (6.7)		0.93 d (6.5)		0.93 d (6.5)	
26	3.46 dd (1H)[b] (12.0,6.5)		0.93 d (6.5)		0.93 d (6.5)	
27	0.95 d (6.7)		0.93 d (6.5)		0.93 d (6.5)	
1′			4.97 s	4.98 s	5.02 s	4.95 d (1.5)
2′			4.07 d (2)	4.06 m	4.27 m	4.04 m
3′			3.62 dd (7,5)	3.58 dd (6,3.5)	4.51 dd (5.5,3)	3.87 dd (6.5,3.5)
4′			4.15[e]	4.06 m	4.27 m	4.04 m
5′			4.22 m	3.75 dd (12.5,3.5)	3.87 dd (12,3)	3.75 dd (12.5,3.5)
				3.68 dd (12.5,5)	3.76 dd (12,5)	3.68 dd (12.5,5)

[a]The peaks due to compounds **1a, 2a,** and **3a**, which are shifted relative to **1, 2,** and **3**, respectively, are shown in this Table. Also shown are the peaks due to 15-H, which in **2a** and **3a** resonate as well-separated signals. In all 24-*O*-glycosidated compounds 24-H signal is under solvent.

[b]The B portion of the ABX system is under the MeOH signal.

[c]Partially overlapped with 3′-H.

[d]Overlapped with 5′-H.

[e]Overlapped with 5′-H and 15-H.

[f]Overlapped with 2′-H and 4′-H.

TABLE 2. ^{13}C-nmr Data (δ/ppm)[a]

Carbons	Compounds		
	1	**2**	**3**
1	39.6	39.1	39.0
2	26.7	32.3	32.0
3	72.9	71.2	71.2
4	69.3	32.9	32.7
5	46.7	53.5	53.4
6	75.4	66.6	66.5
7	75.4	50.6	50.4
8	78.0	75.5	75.4
9	52.0	56.6	56.5
10	38.8	37.1	37.0
11	18.7	19.1	18.9
12	43.0	42.2	42.1
13	45.6	44.7	44.5
14	59.5	66.6	66.5
15	79.8	69.1	68.9
16	82.7	41.4	41.4
17	61.4	55.4	55.1
18	16.8	15.5	15.3
19	16.7	14.3	14.2
20	30.5	35.5	35.4
21	18.3	18.7	18.7
22	37.1	31.8	31.6
23	24.8	28.5	28.1
24	35.0	83.5	83.3
25	37.0	31.3	30.6
26	68.6	18.2	18.1
27	17.2	18.0	17.9
1′		109.4	108.4
2′		81.2	82.3
3′		89.2	83.7
4′		81.7	82.8
5′		68.3	62.6
OCH_3		57.8	

[a]Spectra were recorded in d_4-MeOH solution for **1** and in d_5-pyridine solution for **2** and **3** at 62.9 MHz.

The fab mass spectrum of compound **3** showed molecular ion species at *m/z* 709 (M+Na), 14 mass units shifted relative to **2**; M is the molecular weight of the sodium salt. Intense peaks at *m/z* 607 and 589 were interpreted as due to the losses of SO_3 and H_2SO_4, respectively, from M+H. ^{1}H- and ^{13}C-nmr spectra of **3** indicated that it contained the same steroidal aglycone as compound **2**, and one sulfated α-arabinofuranosyl unit (Tables 1 and 2). ^{13}C nmr also indicated C-24 to be the site of glycosidation, as the chemical shifts of side chain carbons were identical to those of compound **2**. Solvolysis of **3** to the desulfated derivative **3a** [fab-ms *m/z* 607 (M+Na)] produced changes in the chemical shifts of the protons in the ^{1}H-nmr spectrum at positions 3′ (δ 3.87 dd vs. 4.51), 2′ and 4′ (δ 4.02 m vs. 4.22) and 1′ (δ 4.95 d vs. 5.02) of the arabinofuranosyl unit in agreement with the sulfate group being at 3′. The ^{13}C-nmr frequencies of C-3′, C-2′, and C-4′ in the spectrum of **3** are shifted by +5.1, −1.5, and −2.5 ppm, respectively, relative to steroidal 24-*O*-α-arabinofuranosides, isolated from the starfish *Hacelia attenuata* Gray (5). This supported the location of the sulfate group at 3′.

The stereochemistry at C-24 of compounds **2** and **3** is tentatively assigned as 24*S* by analogy with nodososide, a steroidal glycoside from *Protoreaster nodosus* (3,6).

ACKNOWLEDGMENTS

Fab mass spectra were provided by Servizio di Spettrometria di massa del CNR e dell'Università di Napoli. The assistance of the staff is gratefully acknowledged. One of us (RSDC) thanks COLCIENCIAS, OEA and Departamento de Quimica de la Universidad Nacional de Colombia, for financial support of her traveling and living expense abroad.

LITERATURE CITED

1. A.A. Kicha, A.I. Kalinovsky, E.V. Levina, V.A. Stonik, and G.B. Eliakov, *Tetrahedron Lett.*, **24**, 3893 (1983).
2. R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron*, **38**, 3615 (1982).
3. M.V. D'Auria, L. Minale, C. Pizza, R. Riccio, and F. Zollo, *Gazz. Chim. It.*, **114**, 469 (1984).
4. J. McKema, J.K. Norymberski, *J. Chem. Soc. (C)*, 3889 (1957).
5. L. Minale, C. Pizza, R. Riccio, and F. Zollo, *Experientia*, **39**, 569 (1983).
6. R. Riccio, L. Minale, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron Lett.*, **23**, 2899 (1982).

Received 28 December 1984