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# FORBESIN: A NOVEL SULFATED GLYCOLIPID FROM THE STARFISH *ASTERIAS FORBESI*

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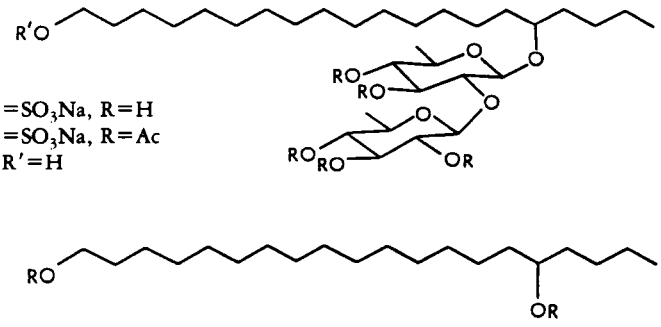
ABSTRACT.—Two novel compounds representing two new classes of marine natural products have been isolated from *Asterias forbesi* together with several nucleosides and deoxynucleosides. The structures of the novel compounds have been determined by spectroscopic means: one, forbesin, a glycolipid, is shown to be 16-*O*-[β-D-quinovopyranosyl-(1↦2)-*O*-β-D-quinovopyranosyl]eicosane-1-sodium sulfate and the other is the disodium salt of eicosane-1,16-disulfate.

In our continuing examination of the polar extracts from the common Atlantic starfish *Asterias forbesi* Desor (family Asteriidae, order Forcipulatida), we have isolated a novel sulfated glycolipid, forbesin [**1**]. We are not aware of any previous reports of this type of compound either from starfish or from other marine organisms. Indeed the closest structural relatives would appear to be the "resin glycosides" from *Ipomoea* species (1,2), which are composed of hydroxy fatty acid oligoglycosides featuring a partially acylated tetrasaccharide chain. We have also isolated the disodium salt **5** of the aglycone **4** of forbesin from *A. forbesi*.

Forbesin [**1**] displays a prominent pseudo molecular ion *m/z* 731 in its positive fabms spectrum (glycerol) corresponding to $[M(C_{32}H_{61}O_{13}SNa)+Na]^+$. Additional fragment ions at *m/z* 629 $[731-NaSO_3+H]^+$, 627 $[731-NaSO_3-H]^+$, 585 $[731-C_6H_{11}O_4+H]^+$, 437 $[731-2\times C_6H_{11}O_4]^+$, 421 $[437-O]^+$, and 333 $[437-H-NaSO_3]^+$ attest to the presence of two hexose units and an $NaSO_4$ moiety. $^1H$, $^1H$-$^1H$ COSY, and $^1H$-$^1H$ RCT COSY data (Table 1) confirm these findings and reveal the nature and stereochemistry of the disaccharide moiety. Thus, the large coupling constants for the ring protons of each of the 6-deoxy sugar units establish that both are β-linked quinovose units.

Acid hydrolysis of **1** with 2 N HCl at 80–90° for 6 h afforded D-quinovose and the aglycone **4** (mp 60–64°). A low resolution ei mass spectrum of **4** displayed a weak molecular ion *m/z* 314 ($C_{20}H_{42}O_2$) and more intense ions at 296 $[M-H_2O]^+$ and 278 $[M-2H_2O]^+$. The location of a hydroxyl group at C-16 was affirmed by the presence of substantial fragments resulting from fission of the C-16–C-17 and C-15–C-16 bonds as shown in Fig-



**1** R′=$SO_3Na$, R=H
**2** R′=$SO_3Na$, R=Ac
**3** R,R′=H

**4** R=H
**5** R=$SO_3Na$

TABLE 1. $^1$H- and $^{13}$C-nmr Data [Pyridine-$d_5$–$D_2O$ (5:1)] for Forbesin [**1**] and Derivatives.

| Atom | Compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | | | **2** | | **3** | |
| | $^1$H[a] (ppm) | $J$ (Hz) | $^{13}$C (ppm) | $^1$H[a] (ppm) | $J$ (Hz) | $^1$H (ppm) | $J$ (Hz) |
| 1 | 4.53 | 6.7 | 67.3 | 4.59 | 6.7 | 3.89 | 6.5 |
| 16 | 3.75 | | 78.8 | 3.87 | | | |
| 20 | 0.95 | 7.0 | 12.7 | 1.11 | 7.3 | 0.94 | 7.3 |
| 1′ | 5.18 | 7.6 | 102.8 | 4.82 | 7.6 | 5.29 | 7.6 |
| 2′ | 4.05[b] | | 81.4 | 4.10 | 9.5, 7.7 | | |
| 3′ | 4.26 | 8.6[d] | 74.3[b] | 5.62 | 9.5[d] | | |
| 4′ | 3.77[c] | | 74.1[b] | 5.08 | 9.6[d] | | |
| 5′ | 3.73[c] | | 70.5[c] | 3.85 | 9.7, 6.2 | | |
| 6′ | 1.63 | 6.2 | 16.4 | 1.42 | 6.2 | 1.62[b] | 5.3 |
| 1″ | 4.81 | 7.6 | 99.6 | 5.25 | 8.0 | 4.28 | 7.6 |
| 2″ | 4.09[b] | | 74.7[b] | 5.41 | 9.6, 8.0 | | |
| 3″ | 4.17 | 9.7[d] | 75.2[b] | 5.69 | 9.6[d] | | |
| 4″ | 3.89[c] | | 74.1[b] | 5.22 | 9.6[d] | | |
| 5″ | 3.80[c] | | 71.3[c] | 4.00 | 9.7, 6.1 | | |
| 6″ | 1.65 | 6.2 | 16.5 | 1.51 | 6.1 | 1.67[b] | 5.6 |

[a]Assignments based on $^1$H-$^1$H COSY experiments.
[b,c]Assignments with the same superscript in the same column may be interchanged.
[d]$J_1 \approx J_2$

ure 1 (3). An hrms confirmed the elemental composition of all the fragments indicated. The $^1$H-nmr spectrum ($CDCl_3$) showed a triplet at δ 3.63 ppm (2H, $J$ = 6.5 Hz, H-1) and a multiplet at δ 3.59 ppm (1H, H-16) in agreement with formulation **4**.

Acetylation of **1** afforded pentaacetate **2** whose 500 MHz $^1$H-$^1$H COSY spectrum revealed that the C-2′ proton was not subject to acetylation shift. Thus C-2′ must be the site of interglycosidic linkage. Irradiation at δ 4.82 ppm (H-1′) afforded a 16% nOe enhancement of the δ

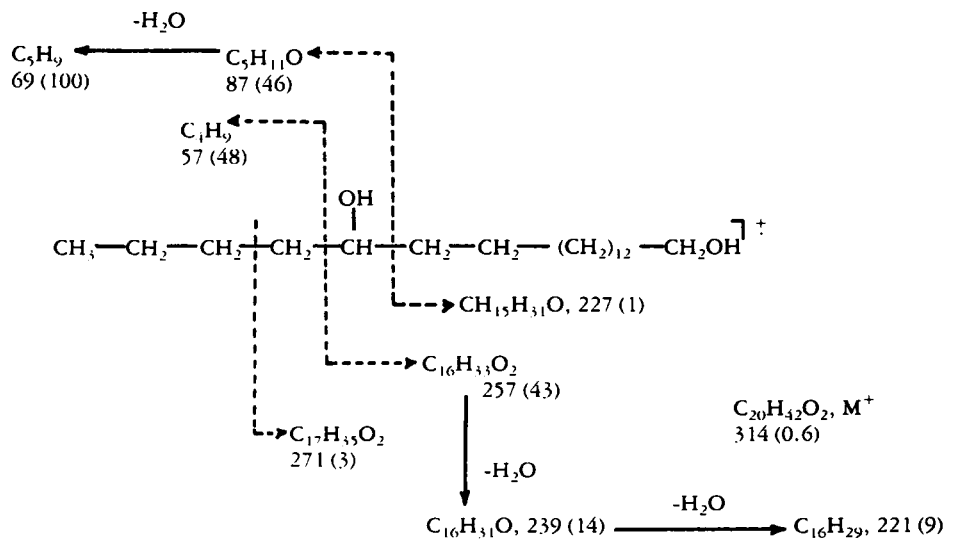



FIGURE 1. Mass spectral analysis of compound **4**.

3.87 ppm (H-16) multiplet, while irradiation at δ 4.59 ppm (H-1) enhanced the δ 1.74 ppm (H-2) multiplet 10% without affecting the signal at δ 4.82 (H-1′). Thus, the disaccharide moiety must be linked at C-16 and the sulfate at C-1. These findings have been further confirmed by a 2D-NOESY spectrum of **2**. The significant peak of the positive fab mass spectrum (glycerol) of **2** at *m/z* 942 corresponds to $[M (C_{42}H_{71}O_{18}SNa) + Na + H]^+$, thus confirming the composition of the pentaacetate. The ei mass spectrum showed no molecular ion; however, major fragments at *m/z* 503 and 273 correspond to fission at the glycosidic linkages.

Solvolysis of forbesin [**1**] in pyridine-$d_5$–dioxane-$d_8$–$D_2O$ (12:3:1) at 120° for 6 h afforded the desulfated glycolipid **3**, whose $^1$H-nmr spectrum (Table 1) displayed the expected upfield shift of the C-1 methylene triplet to δ 3.89 ppm.

Thus, the structure of forbesin is established as 16-*O*-[β-D-quinovopyranosyl-(1↦2)-*O*-β-D-quinovopyranosyl]eicosane-1-sodium sulfate [**1**], with the configuration at C-16 yet to be determined.

Compound **5** displays a relatively intense pseudo molecular ion *m/z* 541 $[C_{20}H_{40}O_8S_2Na_2 + Na]^+$ in the positive mode fab mass spectrum, while the highest mass ions *m/z* 314 $[M - 2NaSO_3 + 2H]^+$ and 278 $[314 - 2H_2O]^+$ of the ei spectrum are consistent with its formulation. Solvolysis of **5** in the same system as employed for **1** afforded the previously characterized eicosane-1,16-diol **4**. The distinguishing features of the $^1$H-nmr of **5** [pyridine-$d_5$–$D_2O$ (5:1)] comprise a triplet (2H, $J = 6.6$ Hz) at δ 4.30 ppm and a multiplet (1H) at δ 4.72 ppm assigned to protons at C-1 and C-16, respectively. Thus, compound **5** is formulated as the disodium salt of eicosane-1,16-disulfate.

Both forbesin [**1**] and the disulfate **5** represent new categories of marine natural products whose biological roles have yet to be ascertained. We have also isolated 2′-deoxythymidine, 2′-deoxyinosine, and 2′-deoxyguanosine, as well as adenosine and inosine, from the crude glycoside mixture from *A. forbesi*. Free deoxynucleosides are not common, and only a few reports of isolation from marine invertebrates exist (4–6). Inosine and 2′-deoxyguanosine were recently found in *Cellaria* spp. (7). We have also isolated forbesin [**1**], inosine, and 2′-deoxyinosine from *Asterias vulgaris* (Verrill) by parallel isolation procedures.

## EXPERIMENTAL

Nmr spectra were recorded on Varian XL200 and Brucker AM500 instruments using TMS as internal standard. Mass spectra were recorded with a Kratos MS50 instrument using xenon as ionizing gas. Ptlc was performed on E. Merck precoated 20 × 20 cm glass plates of Si gel 60 F254 and RP-18 F254.

Specimens of *A. forbesi* (4.5 kg wet wt) were collected at Passamaquoddy Bay, New Brunswick, and identified by staff of the Identification Center, Biological Station, St. Andrews, New Brunswick. A voucher specimen is preserved at the Department of Chemistry, University of New Brunswick.

Forbesin [**1**] and compound **5** were recovered from a fraction obtained by Si gel cc of a crude glycoside mixture (2.5 g) from *A. forbesi* extracted as previously described (8,9). Rechromatography of the second fraction, first on Si gel F254 (1 mm) ptlc plates [$CHCl_3$-MeOH-aqueous $NH_3$ (7:3:0.5)] and then with reversed phase ptlc [MeOH-$H_2O$ (4:1)] afforded pure forbesin [**1**] (18 mg) as a colorless powder and compound **5** (10 mg) as a colorless powder, together with 2′-deoxyguanosine (5 mg), inosine (5 mg), and adenosine (2 mg). Sodium thornasterol 3-*O*-sulfate (9,10) (40 mg), 2′-deoxythymidine (5 mg), and 2′-deoxyinosine (16 mg) were recovered by Si gel pltc [$CHCl_3$-MeOH-aqueous $NH_3$ (10:3:0.5)] of the first Si gel cc fraction. Nucleosides and 2′-deoxynucleosides were identified by comparison of spectral data with those of authentic samples.

FORBESIN [**1**].—Mp 168° (dec); positive fabms (glycerol) 732 (22), 731 (51), 717 (13), 703 (6), 629 (5), 627 (7), 613 (7), 611 (5), 585 (5), 439 (4), 437 (5), 421 (7), 337 (5), 333 (10), 331 (6), 197 (11), 169 (11), 165 (51), 153 (11), 143 (100), 137 (47); $^1$H nmr and $^{13}$C nmr see Table 1.

Compound **5**.—Mp 154° (dec); positive fabms (glycerol) 541 (5), 439 (3), 437 (3), 421 (14), 414 (7), 413 (27), 393 (12), 391 (5), 307 (12), 285 (7), 199 (5), 177 (25), 167 (5), 165 (68), 149 (37), 143 (100); eims 314 (6), 278 (16), 264 (5),

250 (10), 194 (5), 166 (6), 152 (9), 151 (6), 138 (15), 137 (14), 125 (11), 124 (21), 123 (20), 111 (23), 110 (32), 109 (31), 97 (50), 95 (46), 83 (68), 82 (95), 81 (53), 70 (24), 69 (75), 68 (44), 67 (48), 57 (34), 55 (100), 43 (24), 41 (45).

FORBESIN PENTAACETATE [**2**].—Forbesin [**1**] (5 mg) in pyridine (1 ml)/$Ac_2O$ (1 ml) was warmed at 50° for 10 min. The product was purified by ptlc using $CHCl_3$-MeOH-$H_2O$ (20:3:0.5). The oily pentaacetate **2** gave positive fabms (magic bullet) 942 (40), 901 (8), 900 (10), 840 (4), 544 (6), 484 (5), 253 (8), 171 (11), 143 (34); eims 503 (5), 273 (79), 231 (11), 213 (10), 207 (5), 171 (14), 170 (5), 153 (27), 129 (6), 128 (8), 115 (8), 111 (34), 83 (18), 81 (14), 69 (13), 55 (20), 43 (100); $^1H$ and $^{13}C$ nmr see Table 1.

SOLVOLYSIS OF FORBESIN [**1**].—Forbesin [**1**] (5 mg) in pyridine-$d_5$–dioxane-$d_8$–$D_2O$ (12:3:1) (0.5 ml) was heated at 120° for 6 h in an nmr tube, and after cooling to room temperature the $^1H$-nmr spectrum was recorded (Table 1).

HYDROLYSIS OF FORBESIN [**1**].—Forbesin [**1**] (8 mg) in 2 N HCl (15 ml) was heated at 80–90° for 6 h, cooled, and extracted with $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ layer was evaporated and subjected to ptlc [$CHCl_3$-MeOH (20:1)], affording the aglycone **4** (3 mg): mp 60–64°; eims (low resolution) 314 (0.6), 296 (1.0), 278 (5), 271 (3), 257 (43), 239 (14), 221 (9), 87 (46), 69 (100); hrms 278.3007 (9) ($C_{20}H_{38}$), 257.2441 (63) ($C_{16}H_{33}O_2$), 239.2402 (18) ($C_{16}H_{31}O$), 221.2303 (10) ($C_{16}H_{29}$), 87.0795 (60) ($C_5H_{11}O$), 69.0694 (100), ($C_5H_9$), 57.0705 (35) ($C_4H_9$); $^1H$ nmr ($CDCl_3$) δ (ppm) 3.64 (2H, t, $J$ = 6.4 Hz, H-1), 3.60 (1H, bm, H-16), 1.2–1.5 (36H, m), 0.91 (3H, t, $J$ = 6.0 Hz, H-20). Evaporation of the aqueous layer, dissolution in MeOH, and crystallization afforded D-quinovose (2 mg), identified by comparison with an authentic sample.

SOLVOLYSIS OF **5**.—Compound **5** (5 mg) in pyridine-$d_5$–dioxane-$d_8$–$D_2O$ (12:3:1) (0.5 ml) was heated at 120° for 6 h. Evaporation and dissolution in $CHCl_3$ afforded the aglycone **4** (3 mg), which was purified by ptlc and shown to be identical with the aglycone **4** obtained by hydrolysis of forbesin.

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