Isolation of 20 Glycosides from the Starfish *Henricia downeyae*, Collected in the Gulf of Mexico

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Thirteen new (**1**−**13**) and seven known (**14**−**20**) steroid glycosides were isolated from *Henricia downeyae*, collected from the offshore waters of the northern Gulf of Mexico. Ethanolic extracts of these starfish caused growth inhibition in bacteria and fungi, potent antifouling activity against barnacle and bryozoan larvae, and feeding deterrent activity against a marine fish. The known compounds are typical glycosides found in several species of the family Echinasteridae, *i.e., Echinaster sp.* and *Henricia laeviuscola*. One of the new compounds belongs to this group, whereas the remaining 12 new compounds represent a novel series of steroid glycosides which have aglycons with structural similarities to the "asterosaponins". They possess a $\Delta^{9(11)}$ 3β,6α-dihydroxysteroidal aglycon with 23-oxo or 22,23-epoxy functionalities and often a 20-hydroxyl group in the side chain. The sulfate is located at C-6 and the saccharide moiety at C-3, in contrast with the asterosaponins which have the sulfate at C-3 and the oligosaccaride moiety at C-6. All the new compounds contain a glucuronic acid unit, which is uncommon among steroid glycosides from echinoderms. The structures of the new compounds, isolated in amounts ranging from 3.4 to 0.9 mg, were determined by interpretation of their spectral data and by comparison with spectral data of known compounds.

Continuing with our investigation of the chemical ecology of echinoderms in the northern Gulf of Mexico,[2] we have examined the polar extracts of the starfish *Henricia downeyae* [3] (Clark 1987; family Echinasteridae). Bioactivity against two species of bacteria and two species of fungi, as well as potent antifouling activity against marine larvae of a barnacle and a bryozoan, have been detected in ethanolic extracts of the body wall tissues.[4] In addition, ethanolic body wall extracts caused significant feeding deterrence in a marine fish.[5] Chemical investigation of the polar metabolites associated with this starfish has led to the isolation of 13 new steroid glycosides (**1**−**13**) along with seven known ones (**14**−**20**), previously found in starfish of the family Echinasteridae, *i.e., Echinaster sepositus*,[6] *Echinaster brasiliensis*,[7] and *Henricia laeviuscola*.[8] Most of the known glycosides are characterized by a Δ^4-3β,6β,8,15α,16β-pentahydroxysteroid aglycon with different side chains linked to a β-xylosyl residue. Compound **13**, the 26-nor desulfated echinasteroside A, and compound **15**, the 15-desulfated echinasteroside A, are new additions to the list of this group of naturally occurring steroid glycosides; the known **15** has not previously been isolated as a natural compound.[6] The remaining 12 compounds (Figure 1) represent a novel series among the starfish steroid glycosides with structural similarities to the asterosaponins,[9] having a 3β,6α-dihydroxysteroid aglycon with 23-oxo or 22,23-epoxy functionalities and often a 20-hydroxyl group in the side chain, but with the sulfate at C-6 and the saccharide moiety at C-3. In the asterosaponins the oligosaccharide chain is always linked at C-6 and the sulfate at C-3.[9]

This group of new glycosides, which is named downeyosides A−L (**1**−**12**), contain a glucuronic acid moiety, which is uncommon among echinoderm saponins, having been found only in the "cyclic glycosides" reported from two *Echinaster sp.*, i.e., *E. sepositus*[10] and *E. luzonicus*.[11] We have also isolated cholest-7-en-3β-yl sulfate, which is a common echinoderm metabolite.[12] In the preceding paper, we described the structural elucidation of downeyoside A (**1**) and B (**2**), which have the remarkable feature of a fifth ring linking C-16 to C-22 through an ethereal oxygen.[13] In this paper, we describe the structural elucidation of the remaining new compounds **3**−**13**.

Results and Discussion

Specimens of *H. downeyae* were collected at a depth of 90 m from the northern Gulf of Mexico during July and August 1992. Some of the starfish were dissected into their primary body components and their body wall tissues extracted in ethanol. Ethanolic body extracts were then subjected to a suite of biological assays. Ethanolic extracts caused weak growth inhibition in the bacteria *Staphylococcus aureus* and *Micrococcus roseus* and strong growth inhibition in the fungus *Sordaria fimicola*.[14] Ethanolic extracts tested at concentrations similiar to those in the body wall caused significant attachment in component cyprid larvae of the barnacle *Balanus amphitrite* and competent coronate larvae of the *Bugula neritina*.[4] In addition, ethanol extracts imbedded at ecologically relevant concentrations in alginate feeding pellets containing krill as a feeding

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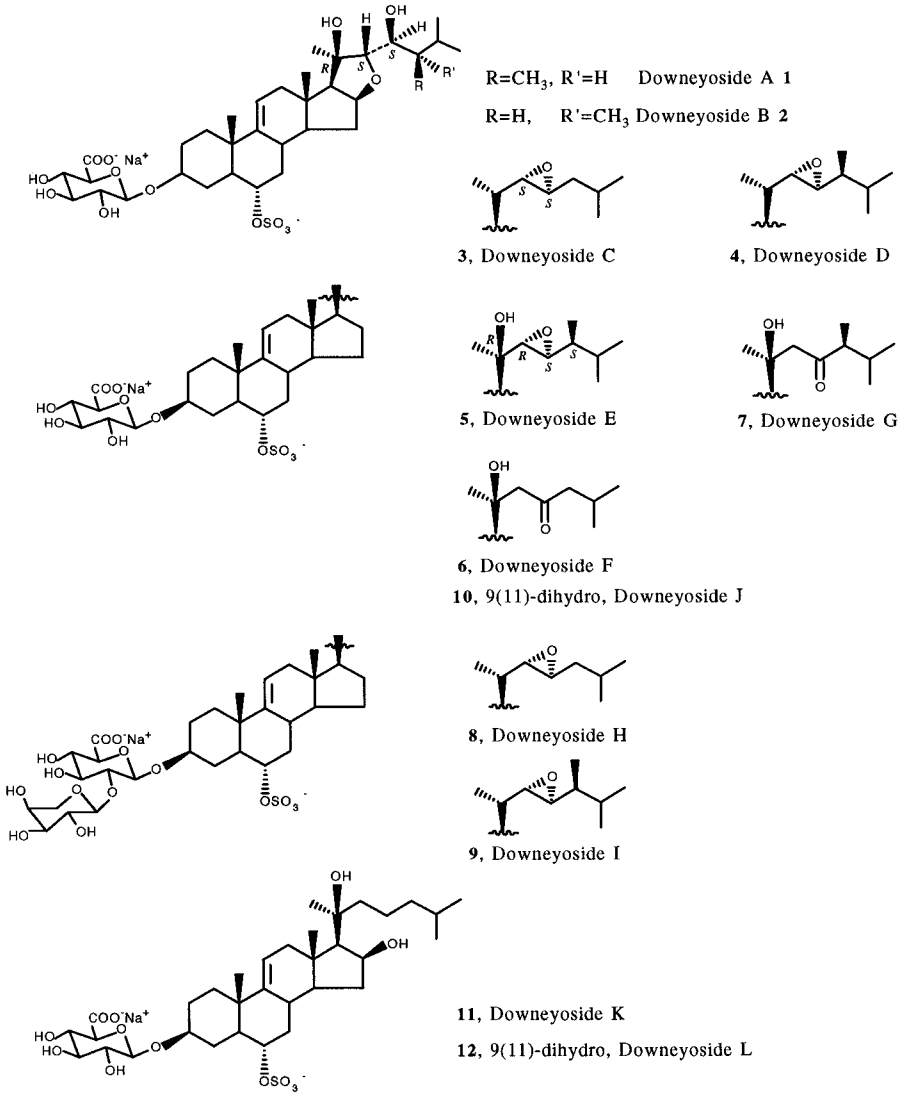

Figure 1. New steroid glucuronides (**1**−**12**) from *H. downeyae*.

stimulant were rejected by the marine pinfish *Logodon rhomboides*.[5] These biological investigations suggest that polar compounds in the body wall tissues may serve as antifoulants or antifeedants. The remainder of the freshly collected starfish were freeze-dried and shipped to Naples for chemical investigation. Separation and isolation of the individual compounds from the aqueous extracts, combined with the *n*-butanol soluble portion from the acetone extracts, followed the steps described previously,[2] *i.e.*, chromatography on Amberlite XAD-2 of the aqueous extracts and, after combination of methanol eluate with the *n*-butanol-soluble portion from the acetone extracts, on Sephadex LH-60, DCCC, and HPLC. The results of our analysis are shown in Table 1.

The known compounds **14**−**21** (Figure 2) were identified by direct comparison (^{1}H NMR and FABMS) with authentic samples.[6,8] The steroid **15**, which corresponds to the 15-desulfated echinasteroside A, was identified by direct comparison with an authentic sample as obtained by solvolysis in dioxane/pyridine of the naturally occurring sulfated product.[6] The new steroid **13** gave a FABMS (negative ion) pseudomolecular ion peak at *m*/*z* 609 [M − H]$^-$, 14 mass units less than compound **15**. Spectral data (^{1}H NMR and ^{13}C NMR) indicated that compounds **13** and **15** possessed identical nuclei including the 3β-(2-*O*-methyl)xylopyranoside linked at C-3 and differed only in the side chain. The ^{1}H NMR spectrum of **13** showed signals for only two secondary methyls at δ 1.01 (3H, d) and 1.04 (3H, d), one CH_2OH group, δ 3.58 (2H, m), and an eight-line pattern centered at δ 5.51 (1H, dd, J = 15.2, 8.3 Hz) and 5.37 (1H, dd, J = 15.2, 8.6 Hz) for the two protons of the Δ^{22} double bond. Each proton was coupled with a methine proton at δ 2.53 (m) and 2.26 (m), respectively, which were both in turn coupled with methyl doublet signals at δ 1.04 and 1.01, respectively. This was suggestive for a Δ^{22E}-27-nor-24-methyl-26-hydroxy side chain, which was confirmed by a ^{1}H−^{1}H COSY spectrum. This showed that the signal at δ 1.58 (2H, m, H_2-25) gave cross peaks with the methine signal at δ 2.26 (H-24) and with the methylene signal at δ 3.58 (H_2-26). The ^{13}C NMR spectral data confirmed this structural assignment. A 27-nor-24-methyl-26-hydroxy side chain has been previously found in polyhydroxysteroid glycosides from *Coscinasterias tenuispina*[15] and *Sphaerodiscus placenta*,[16] which contained the saccharide moiety linked at C-26.

NMR spectral data indicated that compounds **3**−**9** possess identical tetracyclic steroidal nuclei but differ in the side chain or in the saccharide moiety linked at C-3. It was thus only necessary to determine the nature of the side chain and sugar for each glycoside.

The FABMS (negative ion mode) of downeyoside C (**3**) exhibited pseudomolecular anion peaks at *m*/*z* 693

Table 1. Compounds Isolated from the Starfish *H. downeyae* (from 150 g Freeze-Dried)

compd	amount (mg)	$[\alpha]_D$ (deg)	FAB-MS (*m/z*)	ref
new compounds (**1**−**13**)				
downeyoside A (**1**)	3.5	−13.3	739	13
downeyoside B (**2**)	3.0	−18.5	717	13
downeyoside C (**3**)	1.5	−16.6	693	
downeyoside D (**4**)	0.9	−23.3	707	
downeyoside E (**5**)	3.4	−14.7	723	
downeyoside F (**6**)	1.0	−10.0	709	
downeyoside G (**7**)	1.0	−13.4	723	
downeyoside H (**8**)	1.9	−6.3	825	
downeyoside I (**9**)	2.0	−5.5	839	
downeyoside J (**10**)	1.2	−9.5	711	
downeyoside K (**11**)	1.5	−12.0	711	
downeyoside L (**12**)	1.3	−11.5	713	
26-*nor*echinasteroside A desulfated (**13**)	1.3	−5.3	609	
known compounds (**14**−**21**)				
22(23)-dihydroechinasteroside A desulfated (**14**)	0.3		625	7
echinasteroside A desulfated (**15**)	1.2		627	6
echinasteroside B desulfated (**16**)	1.9		639	7
echinasteroside C desulfated (**17**)	0.5		611	8
laeviuscoloside H (**18**)	3.1		625	8
laeviuscoloside I (**19**)	1.7		627	8
laeviuscoloside G (**20**)	4.3	−0.8	759	8
5α-cholest-7-en-3*β*-yl-sulfate (**21**)	1.0	+6.6	465	12

Table 2. ^{13}C-NMR Data (CD_3OD, 125.76 MHz) of the Steroid Carbons of Compounds **3**−**5**

carbon	**3**	**4**	**5**
1	36.9	37.0	36.9
2	30.2	30.2	30.1
3	79.0	79.0	79.0
4	30.2	30.2	30.2
5	49.0	49.1	49.1
6	78.6	78.6	78.6
7	41.0	40.8	40.7
8	36.9	37.0	36.6
9	146.7	146.7	146.4
10	39.9	39.9	39.8
11	117.8	117.8	117.9
12	42.8	42.8	42.1
13	42.5	42.6	42.7
14	54.5	54.5	54.8
15	26.5	26.6	26.0
16	28.4	28.4	23.6
17	57.4	57.5	60.3
18	12.0	12.0	13.5
19	19.7	19.7	19.7
20	39.8	39.6	72.4
21	16.0	16.1	23.5
22	65.3	63.9	65.0
23	56.8	61.2	58.7
24	42.6	43.4	42.9
25	27.8	32.6	32.6
26	23.0	20.7	20.7
27	23.3	19.4	19.4
28		13.1	13.1

Table 3. NMR Data for the Steroid Moiety of Compounds **11** and **12** (CD_3OD) and Assignments Made by ^{1}H−^{1}H COSY Experiments

position	11		12	
	δ_H	δ_C	δ_H	δ_C
1	1.49, 1.73	36.9		38.0
2	1.65, 2.07	30.2		29.7
3	3.73 m	78.9	3.73m	79.0
4	1.32, 2.43	30.1		30.1
5	1.25	49.0		50.8
6	4.36 dt (J = 10.8, 4.4 Hz)	78.5	4.17 dt (J = 10.8, 4.4 Hz)	78.9
7	1.05, 2.59	40.5		39.9
8	2.25	36.3		54.6
9		146.9		55.5
10		39.8		40.5
11	5.40 bd (J = 5.2 Hz)	117.8		21.9
12	2.04, 2.31	41.0		40.9
13		42.5		44.2
14	1.16	53.0		55.1
15	1.31, 2.41	39.3		38.4
16	4.62 m	74.5	4.59 m	74.4
17	1.36	60.7		60.9
18	1.078 s	14.9	1.15 s	13.7
19	1.083 s	19.7	0.94 s	15.3
20		73.8		73.8
21	1.28 s	26.0	1.27 s	26.4
22		45.2		45.4
23		23.4		23.4
24		43.7		41.7
25	1.60	29.0		29.1
26	0.92 d (J = 6.6 Hz)	23.0	0.92 d (J = 6.5 Hz)	23.1
27	0.92 d (J = 6.6 Hz)	23.1	0.92 d (J = 6.5 Hz)	22.9
28				

[100, MNa SO_3]$^-$ and 671 [10, MH SO_3]$^-$ and a strong fragment at *m/z* 495 corresponding to the loss of 198 mass units (sodium glucuronate) from *m/z* 693. The appearance in the FABMS of two pseudomolecular anion peaks was suggestive of the presence of two strong anionic functions, and IR absorptions at 1240 cm^{-1} and 1700 cm^{-1} were indicative for the presence of a sulfate and a carboxylic group (δ_C 176.9). The presence of a glucuronic acid unit in **3** was established by acid methanolysis which liberated methyl glucuronides, while the presence of a sulfate was confirmed by solvolysis in dioxane−pyridine of **3** giving a desulfated material with *m/z* 591 [M − H]$^-$ in FABMS. Subtraction of 176 mass units for the glucuronic acid from the MW of 592 for the desulfated material gave the MW of the aglycon as 416, which corresponds to a C-27 tetracyclic steroid with three oxygen atoms and two additional unsaturations. The NMR spectra of **3** supported the presence of a glucuronic acid unit, whose ^{1}H NMR assignments were made by a COSY experiment (Table 4) and ^{13}C assignments were based on comparison with those reported for methyl glucopyranosiduronic acids.[17] The ^{13}C NMR chemical shifts as well as the ^{1}H NMR coupling constants of the anomeric proton (δ 4.51, d, J = 7.8 Hz) indicated that the sugar is in its pyranose form and the linkage is β. The NMR spectra also showed the presence of a trisubstituted double bond [(1H br d, 5.40, J = 5.2 Hz); ^{13}C δ 117.8 (=CH), 146.7 (s)] and two single proton signals at δ 3.74 (m) with the shape typical for a 3β-*O*-grouping and at δ 4.35 (dt, J = 4.4, 10.8 Hz), already observed in 6α-*O*-sulfoxyster-

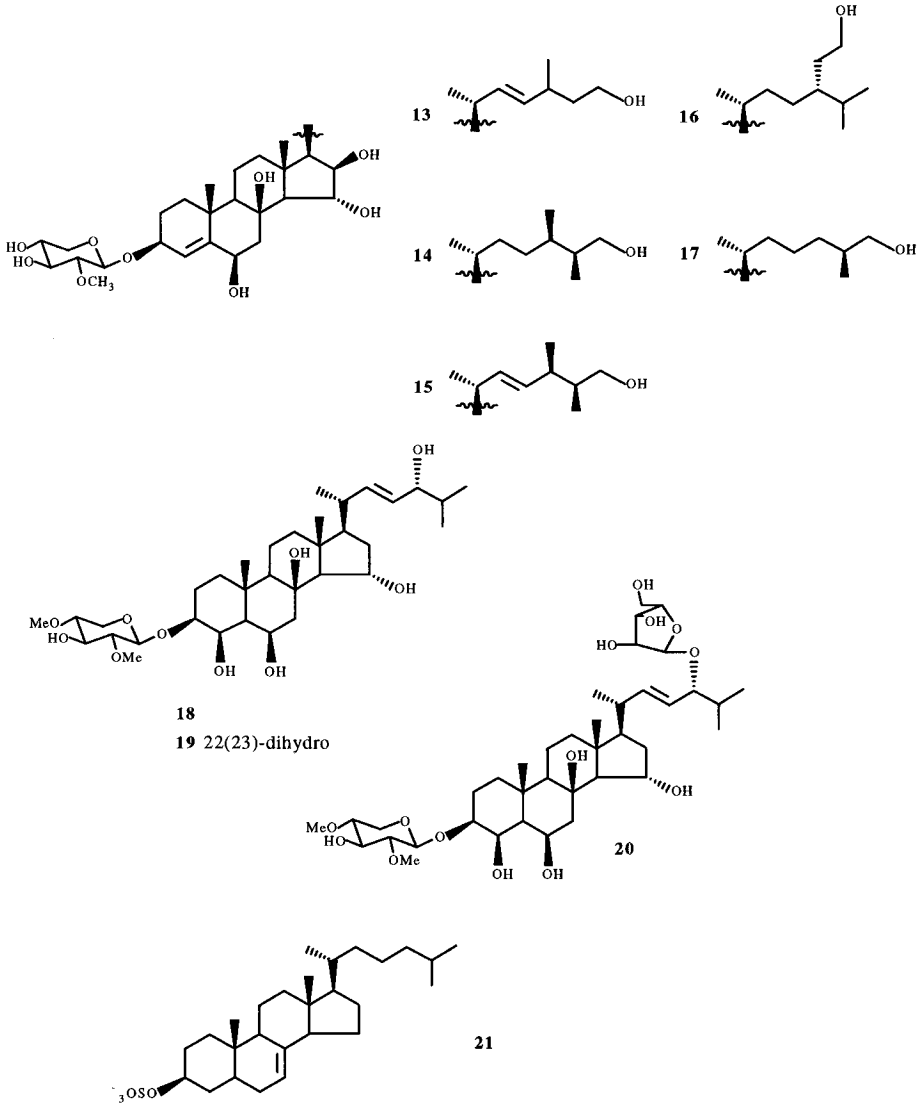

Figure 2. Known steroidal constituents, except one (**13**), from *H. downeyae*, typical of the family *Echinasteridae*.

Table 4. Assignments of NMR Signals (CD_3OD) of the β-Glucuronopyranosiduronic Acid Unit in Compounds **3**−**7**, and **10**−**12**

position	^{1}H (mult, *J*, Hz)	^{13}C
1	4.51 d (7.8)	102.3
2	3.20 t (8.0)	75.1
3	3.45[b]	78.0
4	3.45[b]	73.8
5	3.61	76.0
6		176.9

[a] ^{1}H assignments based on ^{1}H−^{1}H 2D COSY. ^{13}C assignments based on δ chemical shifts arguments and comparison with ref 17. [b] Overlapping signals.

oids.[18] Also present were singlets at δ 0.66 and 1.06 corresponding to Me-18 and Me-19, respectively, as well as doublets at δ 1.04 (3H) and 1.02 (6H) corresponding to Me-21 and Me_2-26,27, respectively. These data supported the presence of a $\Delta^{9(11)}$-5α-cholestene-3β,6α-diol aglycon found in many asterosaponins [9] with the sulfate at C-6 as confirmed by the upfield shift of H-6 observed in the NMR spectrum of the desulfated material (δ 3.58 *vs*. 4.35 in **3**). The saccharide moiety was at C-3 as indicated by ^{13}C NMR [$\delta_{C\text{-}3}$ 79.0 ppm in **3** *vs*. 71.1 ppm in 5α-cholestane-3α,6α-diol [18] *cf*. glycosidation shift].[20−22] Two one-proton signals at δ 2.55 (1H, dd, J = 7.6, 2.1 Hz) and at δ 2.74 (1H, dt, J = 6.4, 2.1 Hz), coupled to each other, were assignable to the epoxide methine protons at C-22 and C-23. These assignments completed the ^{1}H NMR spectrum and account for the remaining oxygen atom and the formal unsaturation of the aglycon. The ^{13}C NMR spectrum supported this conclusion, showing two CH signals at 65.3 and 56.8 ppm consistent with the presence of an epoxide functionality. Comparison with models allowed assignments of the 22*S*(β),23*S*(β)-configuration. The epoxy-*cis* stereochemistry could be eliminated because of the low-field resonance of C-20 and C-24 in the spectrum of **3**. In the model 22,23-*cis*-epoxycholesta-5,7-dien-3β-ol, the resonances of C-20 and C-24 were reported shifted upfield by 6.7 and 4.5 ppm (*i.e.*, C-20 33.1 ppm, C-24 38.1 ppm), respectively,[23] relative to the *trans* models because of the γ-gauche interaction. The resonance of C-17 at δ 57.4 ppm in **3** with respect to the same signal in 22*S*,23*S*- and 22*R*,23*R*-model compounds at 56.3 and 53.8 ppm, respectively,[23] was the key argument supporting the 22*S*(β),23*S*(β) configuration. The chemical shift of the Me-21 protons in **3** at δ 1.04 with respect to the same signal in 22(*S*),23(*S*)-epoxy-24(*R*)-methyl- and 22(*S*),23(*S*)-epoxy-24(*S*)-methylsteroid models at 1.05 and 1.02 and 22(*R*),23(*R*),24(*R*)- and 22(*R*),23(*R*),24(*S*)- at δ 1.12 and 1.07[24] gave support to the assigned stereochemistry. Thus, the structure of downeyoside C was established as 3β-*O*-(β-glucuronopyranosyl)-(22*S*,-23*S*)-22,23-epoxy-5α-cholest-9(11)-en-6α-yl sulfate.[6]

Downeyoside D (**4**), FABMS *m/z* 707 $[MNaSO_3]^-$, 685 $[MHSO_3]^-$, 509 [707 − sodium glucuronate]$^-$, was the 24-methyl analogue of **3**. In the methyl region of the spectrum of **4** an additional methyl doublet was observed at δ 1.01 (^{13}C NMR δ 13.1 ppm), which was accompanied by the shift of the 23-epoxymethine signal upfield to δ 2.55, overlapping with the 22-H signal. A comparison of ^{1}H NMR and ^{13}C NMR data for the side chain of **4** with those of the four stereoisomeric 24-methyl-22,23-epoxy-*trans*-steroids synthetized by Riccio *et al.*[24] allowed the 22*S*,23*S*,24*S*-configuration to be supported for **4**. We note that the NMR data of the stereiosomeric models are significantly different, allowing a clear discrimination of the four possible configurations, and our data (Experimental Section) compare much better with those of the 24*S*-methyl-22*S*,23*S*-epoxy steroid model: [*i.e.*, ^{1}H NMR (CD_3OD) $\delta_{Me\text{-}21}$ 1.02 s, $\delta_{Me\text{-}26,27}$ 0.93 d, 0.96 d, $\delta_{22,23H}$ 2.53 m (2H); ^{13}C NMR (CD_3OD) $\delta_{C\text{-}17}$ 57.5, $\delta_{C\text{-}24}$ 43.3, $\delta_{C\text{-}25}$ 32.6, $\delta_{C\text{-}26,27}$ 19.3, 20.6, $\delta_{C\text{-}28}$ 13.0] than the remaining three models.[24]

Downeyoside E (**5**), FABMS *m/z* 723 $[MNaSO_3]^-$, 525 [707 − sodium glucuronate]$^-$, was the 20-hydroxyl analog of the previous **4**. In the methyl region of the spectrum the Me-21 doublet at 1.03 in **4** is replaced by a methyl singlet at δ 1.28 in **5**. The presence of a hydroxyl group at C-20 was supported by a quaternary carbon singlet at δ 72.4 ppm and by the doublet shape of the 22-epoxymethine proton (J = 2.0 Hz) at δ 2.75. A 24-methyl-20-hydroxy-22,23-epoxycholestane aglycone has already been encountered in regularoside A, an asterosaponin from *Halityle regularis*.[24] All the NMR data for the side chain of **5** match very closely with those of the side chain of the regularoside A for which the 20*R*,22*R*,23*S*,24*S*-configuration was determined.

Downeyoside F (**6**), FABMS *m/z* 709 $[MNaSO_3]^-$, 511 [709 − sodium glucuronate]$^-$, possesses the 20-hydroxy-23-oxocholestane side chain of thornasterol A, the most widely distributed aglycon among asterosaponins.[9] The common 20-hydroxyl functionality is clearly recognized by the shift of the Me-21 singlet at δ 1.37, which also indicates the 20*S*-configuration [*cf.* (20*S*)- and (20*R*)-20-hydroxycholesterol: δ 1.28 and 1.13 ppm, respectively][25] and by the downfield shift of the Me-18 singlet at δ 0.82. Characteristic C-22 and C-24 methylene signals at δ 2.55 (1H, m) and 2.66 (1H, d) with an AB quartet with J = 15 Hz (H_2-22) and at 2.41 (2H, H_2-24) with a doublet with J = 6.5 Hz, were indicative of the 23-oxo functionality. This was confirmed by a major fragment at *m/z* 609 in the FABMS, originated by retroaldol cleavage of the C(20)−C(22) bond and ^{1}H transfer to give a pregnane-type ion.[9,26] The remaining spectral data correspond to the β-glucuronopyranosyl residue attached at C-3.

Downeyoside G (**7**), FABMS *m/z* 723 $[MNaSO_3]^-$, 701 $[MHSO_3]^-$, 526 [723 − sodium glucuronate]$^-$, is the 24-methyl analogue of **6**. This was indicated by the presence in the NMR spectrum of an extra methyl doublet at δ 1.07 relative to **6** and by signals for the C-22 methylene protons resonating as two well-separated doublets at 2.64 and 2.77. The C-24 methyl proton appeared as a quartet at δ 2.40, previously observed in asterosaponins, such as both (24*R*)- and (24*S*)-thornasterol B (*i.e.*, 24(*R*)- and 24(*S*)-methylthornasterol A aglycon.[9] Differentation between the two epimers is possible by the ellipticity [Θ] in the CD spectra, with is much higher in the 24*R*-isomer, [Θ] = −5.780, then in the 24*S*-isomer, [Θ] = −631.[27] The CD (H_2O) spectrum of **7** exhibited [Θ] = −565, in agreement with the 24*S*-configuration.

Table 5. Assignments of NMR Signals (CD_3OD) of the Carbohydrate Moiety of Compounds **8** and **9**[a]

position	α-arabinopiranosyl		β-glucuronopyranosyl	
	^{1}H (mult, *J*, Hz)	^{13}C	^{1}H (mult, *J*, Hz)	^{13}C
1	4.62 d (7.6)	105.3	4.58 d (7.0)	101.7
2	3.42 dd (11, 5.5)	73.1	3.70 t (7.5)	83.0
3	3.63[b]	73.9	3.60[b]	77.7
4	3.85 dd (10.5, 5)	69.2	3.47 dd (10.5, 5)	73.6
5	3.60, 4.09 dd (12.8, 3.8)[b]	67.0	3.60[b]	75.9
6				176.8

[a] ^{1}H assignments based on ^{1}H−^{1}H 2D COSY. ^{13}C assignments based on δ chemical shifts arguments and comparison with references. [b] Overlapping signals.

Downeyoside H (**8**), has FABMS *m/z* 825 [50, $MNaSO_3]^-$, 693 [30, 825−132]$^-$, 495 [100, 693−198 m.u.]$^-$. Examination of its spectral data (Experimental Section) immediately indicated that **8** contains a (22*S*,-23*S*)-22,23-epoxy-5α-cholest-9(11)-ene-3β,6α-diol 6-sulfate aglycon as in compound **3** and also a β-glucopyranosiduronic acid unit at C-3. In addition, the NMR spectra showed signals for a moiety equivalent to an α-arabinopyranoside (Table 5). Acid methanolysis liberated methyl glucuronides and methyl arabinosides. The sequential loss of 132 mass units, corresponding to an arabinosyl residue from the pseudomolecular anion peak at 825, followed by the loss of 198 mass units, corresponding to the sodium glucoronate unit, indicated the sequence arabinoseglucuronic acid. An analysis of the ^{13}C-NMR data of **8** based upon comparison with the glucuronide **3**, and the known glycosidation shifts,[20−22] established the α-arabinopyranoside residue to be attached at C-2 of a β-glucopyranosiduronic acid residue; C-2 of glucuronic acid is found shifted downfield to 83.0 ppm relative to **3** (δ_C 75.1 ppm), and C-1 and C-3 were slightly shifted upfield to 101.7 and 77.7 relative to 102.3 and 78.0 for the same signals in **3**. Thus, the structure of downeyoside H can be determined as 3-*O*-β-[α-arabinopyranosyl-(1→2)-glucuronopyranosyl]-(22*S*,23*S*)-22,23-epoxy-5α-cholest-9(11)-en-6α-yl sulfate.[11]

Downeyoside I (**9**), FABMS *m/z* 839 $[MNaSO_3]^-$, 707 [839 − arabinose]$^-$, 509 [707 − sodium glucuronate]$^-$ is the 24-methyl analogue of **8**. The structure was based on spectral data and acid methanolysis affording methyl glucuronides and methyl arabinosides, and the assignment of the 22*S*,23*S*,24*S*-configuration was based on the arguments used for **4**.

Downeyoside J (**10**), FABMS *m/z* 711 $[MNaSO_3]^-$, is the 9(11)-dihydro derivative of **6**, as conclusively shown by its NMR spectrum (see Experimental Section) and comparison with that of downeyoside F (**6**).

Downeyoside K (**11**) has FABMS *m/z* 711 $[MNaSO_3]^-$, 689 $[MHSO_3]^-$, 513 [711 − sodium glucuronate]$^-$. ^{1}H NMR and ^{13}C NMR spectral data indicated the presence of the same $\Delta^{9(11)}$-6α-sulfoxy-3β-hydroxy tetracyclic steroid nucleus with a glucopyranosiduronic unit β-glycosidically linked at C-3 seen in compounds **3**−**9**. The molecular weight as deduced from FABMS required the presence of two more hydroxyl groups on a cholestane skeleton. The presence in the ^{1}H NMR spectrum of a methyl singlet at δ 1.28 suggested the location of one hydroxyl function at C-20, and this was supported by a quaternary carbon signal at δ 73.8 ppm in the ^{13}C NMR

spectrum. The value of the chemical shift at δ 1.28 was also indicative for the common 20*S*-configuration [*cf.* (20*S*)-and (20*R*)-20-hydroxycholestane: δ 1.28 and 1.13, respectively].[25] The use of the COSY experiment allowed the remaining hydroxyl function to be located at position 16, being the multiplet at δ 4.62 for an hydroxymethine proton coupled to a doublet at δ 1.36 (H-17) and to a pair of geminally coupled protons (H_2-15, δ 1.36, 2.41). The presence of the 16β-hydroxyl group was deduced from the downfield shift of the 18-methyl protons δ 1.078 s, requiring the presence of a 1,3-syn interaction. The small shifts observed in the ^{13}C NMR spectrum of **11** for C-14 (γ effect) and for C-17 (β effect) relative to thornasterol A [*i.e.*, 3β,6α-dihydroxy-5α-cholest-9(11)-en-20-one)[9] (*i.e.*, −1.0 and +1.1 ppm, respectively] are also consistent with a 16β-hydroxy substitution.[28,29] Therefore, on the basis of the above results, downeyoside K has structure **11**, that is (20*S*)-3β-*O*-(β-glucuronopyranosyl)-6β,20-dihydroxy-5α-cholest-9(11)-en-6α-yl sulfate.

Downeyoside L (**12**), FABMS *m*/*z* 713 $[MNaSO_3]^-$, 691 $[MHSO_3]^-$, 515 [713 − sodium glucuronate]$^-$ is the 9(11)-dihydro derivative of **11**, as conclusively shown by its NMR spectra (Experimental Section) and comparison with those of downeyoside K (**11**).

Experimental Section

General Experimental Procedures. NMR measurements were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured by employing the conventional sequence. The COSY spectra were obtained using a data set ($t_1 \times t_2$) of 1024 × 512 points for a spectral data width of 2673.797 Hz (relaxation delay 1 s). The data matrix was processed using an unshifted sine bell window function, following transformation to give a magnitude spectrum with symmetrization (digital resolution in both F2 and F1 dimensions was 2.611 Hz/pt).

Fast atom bombardment mass spectra (FABMS) were recorded in a glycerol matrix in the negative mode on a VG ZAB instrument (argon atom energy of 2−6 kV).

Animal Material. The animals *H. downeyae* were collected in the northern Gulf of Mexico[30] and identified by Dr. T. Hopkins of the University of Alabama; a voucher specimen is preserved at the Department of Biological Sciences, University of Alabama.

Extraction and Isolation. The animals (150 g lyophilized) were chopped and soaked in H_2O (twice, 3 L for 8 h each); the aqueous extracts were decanted and passed through a column of Amberlite XAD-2 (750 g). This column was washed with distilled H_2O (3 L) and eluted with MeOH (6 L). The MeOH eluate was taken to dryness to give a glassy material (2.0 g). The remaining solid mass, after extraction with H_2O, was then reextracted with Me_2CO (2 L) twice, and the Me_2CO extracts were combined, evaporated under vacuum, and partitioned between H_2O and Et_2O. The aqueous residue was then extracted with *n*-BuOH. Evaporation of the *n*-BuOH extracts afforded 500 mg of a glassy material, which was combined with the above MeOH eluate from an Amberlite XAD-2 column and chromatographed on a column of Sephadex LH-60 (4 × 80 cm) with MeOH:H_2O (2:1) as eluent. Fractions (6 mL) were collected and analyzed by TLC on SiO_2 with MeOH−HOAc−H_2O (12:3:5) and $CHCl_3$−MeOH−H_2O (80:18:2). Fractions 17−63 (910 mg) from the column of Sephadex LH-60 were submitted to DCCC using *n*-BuOH−Me_2CO−H_2O (3:1:5) [descending mode; the upper phase was used as the stationary phase; flow rate 12 mL/h; 6 mL fractions were collected and analyzed by TLC on SiO_2 with *n*-BuOH−HOAc−H_2O (12:3:5)] to give three main fractions: 40−47 (57 mg), 48−53 (44.5 mg), and 54−59 (35.8 mg).

Fractions 40−47 were separated by HPLC on a C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.) with MeOH−H_2O (3:7) (flow rate 1 mL min^{-1}) to give pure **5**, **6**, **7**, and **10**. The MeOH eluates from the previous C_{18}-column were then combined and rechromatographed on a C_{18} μ-Bondapak column with MeOH−H_2O (45:55, flow rate 1 mL min^{-1}) to give pure **8** and **9**. Fractions 48−53 were separated by HPLC with MeOH−H_2O ranging from 1:1 to 3:7 to give pure **3**, **11**, and **12**. Fractions 54−59 contained compounds **4** and **11** and were purified by HPLC. Fractions 64−109, from the Sephadex LH-60 column, were purified by DCCC using $CHCl_3$−Me_2CO−H_2O (7:13:8) [ascending mode; the lower phase was used as the stationary phase]; fractions (5 mL each) were collected and analyzed by TLC on SiO_2 with $CHCl_3$−MeOH−H_2O (80:18:2). Fractions 29−55 (mg 21.3) mainly contained compound **21**; fractions 56−85 (mg 21.3) gave pure **14**; and fractions 86−101, 107−129, 130−150, 151−175, and 176−183 contained **13**, **15**, **16**, **17**, **18**, **19**, and **20** in different proportions. All fractions were then purified by HPLC on a C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.) with MeOH−H_2O (75:25) flow rate 2 mL min^{-1} to give pure compounds.

Downeyoside C (**3**): FABMS (−ve ion) and rotation data in Table 1; ^{1}H NMR (aglycon) in the text; ^{13}C NMR (aglycon) in Table 2; ^{1}H and ^{13}C NMR (sugar) in Table 4.

Downeyoside D (**4**): FABMS (−ve ion) in the text; rotation data in Table 1; ^{1}H NMR (aglycon) in the text; ^{13}C NMR (aglycon) in Table 2; ^{1}H and ^{13}C NMR (sugar) in Table 4.

Downeyoside E (**5**): FABMS (−ve ion) in the text; rotation data in Table 1; ^{1}H NMR (aglycon) in the text; ^{13}C NMR (aglycon) in Table 2; ^{1}H and ^{13}C NMR (sugar) in Table 4.

Downeyoside F (**6**): FABMS (−ve ion) in the text; rotation data in Table 1; ^{1}H NMR (aglycon) in the text; ^{1}H NMR (sugar) in Table 4.

Downeyoside G (**7**): FABMS and rotation data in Table 1; ^{1}H NMR (aglycon) in the text; ^{1}H NMR (sugar) in Table 4.

Downeyoside H (**8**): FABMS (−ve ion) and rotation data in Table 1; ^{1}H and ^{13}C NMR data of sugar portion are in Table 5; ^{13}C NMR (CD_3OD, 125.76 MHz) (aglycon) δ 146.8 (C-9), 117.8 (C-11), 80.5 (C-3), 78.5 (C-6), 65.2 (C-22), 57.4 (C-17), 56.8 (C-23), 54.5 (C-14), 49.0 (C-5), 42.8 (C-12), 42.6 (C-13), 42.5 (C-24), 40.8 (C-7), 39.9 (C-10), 39.7 (C-20), 37.0 (C-8), 36.9 (C-1), 30.4 (C-4), 30.2 (C-2), 28.3 (C-16), 27.8 (C-25), 26.6 (C-15), 23.3 (C-27), 23.0 (C-26), 19.8 (C-19), 15.9 (C-21), 12.0 (C-18).

Downeyoside I (**9**): FABMS (−ve ion) and rotation data in Table 1; ^{1}H NMR (CD_3OD, 500 MHz) δ_H (sugars) are in Table 5; (aglycon) δ 2.55 (2H, dt, J = 8.9, 2.0 Hz, H-22 and H-23), 1.03 (3H, d, J = 7.0 Hz, Me-21), 1.00 (3H, d, J = 7.0 Hz, Me-26), 0.96 (3H, d, J = 7.0 Hz, Me-27), 0.93 (3H, d, J = 7.0 Hz, Me-28), other signals are identical to those reported for **3**; ^{13}C NMR (CD_3OD, 125.76 MHz) δ_C (sugars) are in Table 5; (aglycon) δ 63.9

(C-22), 61.2 (C-23), 43.4 (C-24), 39.7 (C-20), 32.6 (C-25), 20.7 (C-26), 19.4 (C-27), 16.1 (C-21), 13.1 (C-28), other signals are identical to those reported for **3**.

Downeyoside J (**10**): FABMS (−ve ion) in Table 1; ^{1}H and ^{13}C NMR (CD_3OD) (sugar portion) are reported in Table 4; ^{1}H NMR (aglycon) δ 4.19 (1H, dt, J = 10.8, 4.4 Hz, H-6), 2.64 (1H, d, J = 15 Hz, H-22), 2.55 (1H, d, J = 15 Hz, H-22), 2.40 (2H, d, J = 6.5 Hz, H-24), 1.35 (3H, s, Me-21), 0.94 (6H, each d, J = 7.0 Hz, Me-26 and -27), 0.92 (3H, s, Me-19),0.87 (3H, s, Me-18).

Downeyoside K (**11**): FABMS (−ve ion) and rotation data in Table 1; ^{1}H and ^{13}C NMR (aglycon) in Table 3; ^{1}H and ^{13}C NMR (sugar) in Table 4.

Downeyoside L (**12**): FABMS (−ve ion) and rotation data in Table 1; ^{1}H and ^{13}C NMR (aglycon) in Table 3; ^{1}H and ^{13}C NMR (sugar) in Table 4.

Compound (**13**): FABMS (−ve ion) and rotation data in Table 1; ^{1}H NMR (CD_3OD, 500 MHz) (aglycon) δ 5.67 (1H, br s, H-4), 4.34 (1H, br t, H-6), 4.23 (1H, m, H-3), 4.19 (1H, dd, J = 11.2, 2.5 Hz, H-15), 3.92 (1H, dd, J = 2.5, 7.4 Hz, H-16), 1.40 (3H, s, Me-19), 1.19 (3H, s, Me-18); ^{1}H NMR (sugar) δ 4.45 (1H, d, J = 7.6 Hz, H′-1), 3.84 (1H, dd, J = 5.6, 11.3 Hz, H′-5), 3.61 (3H, s, OCH_3), 3.51 (1H, m, H′-4), 3.30 (1H, under solvent signal, H′-3), 3.19 (1H, dd, J = 10.0, 11.3 Hz, H′-5), 2.85 (1H, dd, J = 7.6, 8.5 Hz, H′-2); ^{13}C NMR (CD_3OD, 125.76 MHz) δ_C (aglycon) 150.1 (C-5), 136.6 (C-22), 135.2 (C-23), 126.9 (C-4), 83.3 (C-16), 80.3 (C-15), 77.4 (C-3), 76.4 (C-8), 76.2 (C-6), 63.7 (C-14), 61.4 (C-26), 61.1 (C-17), 57.9 (C-9), 45.1 (C-7), 44.4 (C-13), 43.0 (C-12), 40.1 (C-25), 39.7 (C-1), 37.8 (C-10), 35.5 (C-24), 34.4 (C-20), 27.9 (C-2), 22.7 (C-19), 21.7 (C-28); 20.5 (C-21), 19.5 (C-11), 16.9 (C-18); (sugar) δ 104.6 (C-1′), 84.9 (C-2′), 77.5 (C-3′), 71.3 (C-4′), 66.8 (C-5′), 61.0 (OCH_3).

Compound (**21**): FABMS (−ve ion) in Table 1; ^{13}C NMR (CD_3OD, 125.76 MHz) δ 140.6 (C-8), 118.5 (C-7), 79.4 (C-3), 57.4 (C-17), 56.2 (C-14), 50.8 (C-9), 44.5 (C-13), 41.7 (C-5), 40.9 (C-12), 40.3 (C-24), 38.3 (C-1), 37.8 (C-20), 36.1 (C-22), 35.2 (C-10), 35.0 (C-4), 30.8 (C-6), 29.7 (C-25), 29.0 (C-16), 28.6 (C-2), 24.2 (C-23), 24.0 (C-15), 23.0 (C-26), 22.6 (C-11), 20.2 (C-27), 19.4 (C-21), 13.4 (C-19), 12.3 (C-18).

Methanolysis of Sulfated Glycosides: Sugar Analysis. A solution of the glycoside **7** (1.0 mg), in anydrous 2.5 M HCl in MeOH (400 μL) was heated at 80 °C in a stoppered reaction vial for 8 h. After having been cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with TRISIL Z (Pierce Chemical Co.) for 15 min at room temperature. GLC analysis (SBP-1 capillary column, 30 m, i.d. 32 mm, 150 °C, He carrier, flow 2 mL min^{-1}), gave peaks which coeluted with those of methyl glucuronide. The glycosides **8** (1 mg) and **9** (1.0 mg) were similarly treated to give GLC peaks which coeluted with those of methyl arabinoside and methyl-glucuronic acid.

Solvolysis of 3. A solution of the glycoside **3** (0.5 mg) in a mixture of pyridine (100 μL) and dioxane (100 μL) was heated at 160 °C for 2 h in a stoppered reaction vial. The residue was evaporated to dryness and purified by HPLC (C_{18}-μ-Bondapak column 30 cm × 3.8 mm i.d.) with MeOH−H_2O (75:25) as eluent to give only one peak (desulfated **3**). Spectral data: FABMS (negative ion) m/z 591 [MH − H]$^-$; ^{1}H NMR (CD_3OD) δ_H 3.76 (m, 3-H), 3.54 (dt, J = 4, 12 Hz, 6H), 1.02 (s, 19-Me), other signals are identical with those reported for natural **3** (see the text).

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