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STARFISH SAPONINS, PART 46.[1] STEROIDAL GLYCOSIDES AND POLYHYDROXYSTEROIDS FROM THE STARFISH *CULCITA NOVAEGUINEAE*

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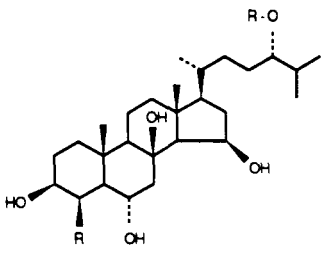
ABSTRACT.—A reinvestigation of the extracts from the starfish *Culcita novaeguineae* has led to the isolation of eleven polyhydroxysteroidal glycosides and five polyhydroxysteroids. One of them has been identified with culcitoside C_1 [**2**], previously isolated from the same organism. Nine are known compounds previously found in starfishes, the majority having been isolated from the related species *Halityle regularis*. Six are new compounds and include one polyhydroxysteroid **13** and five steroid diglycosides, designated culcitosides C_4 [**3**], C_5 [**4**], C_6 [**9**], C_7 [**10**], and C_8 [**14**]. All compounds have been obtained in very small amounts, ranging from 9.2 to 2 mg from 3.8 kg of fresh organism. A second group of compounds isolated from *Cu. novaeguineae* consists of phosphorylglycerylethers.

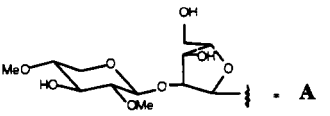
There are three groups of steroid oligoglycosides in starfishes: sulfated steroidal penta- and hexa-glycosides ("asterosaponins"), steroidal cyclic glycosides (found only in the genus *Echinaster*), and glycosides of polyhydroxysteroids consisting of a polyhydroxysteroid with one or two sugar units (1–3). This third group of steroid glycosides, which are found in both sulfated and non-sulfated form and appear to be as widespread as the "asterosaponins" among starfishes, has shown a great structural variability. They usually occur in minute amounts and are present as a very complex mixture.

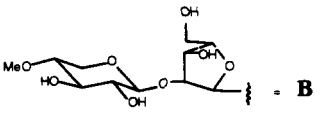
For example, *Coscinasterias tenuispina*, which is characterized by the most complicated mixture of asterosaponins and glycosides of polyhydroxysteroids of all starfishes that have been studied, contains sixteen steroidal glycosides and three polyhydroxysteroids (4). Further examples are represented by the steroid glycosides from the starfish *Halityle regularis* (5,6) which comprise eight biosides with three variants of aglycones and two types of disaccharide moieties, co-occurring with three asterosaponins, and by those from the starfish *Henricia laeviuscola* (7), from which eleven monosides and biosides and one asterosaponin have been isolated.

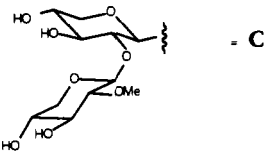
We now report the isolation of eleven steroid glycosides along with five polyhydroxysteroids from the starfish *Culcita novaeguineae* Müller and Troschel (family Oreasteridae), collected at Zampa, Okinawa, which is a further example of the structural variety of steroid glycosides co-occurring in the same organism. Five variants of aglycones and three types of disaccharide moieties have been identified in eleven glycosides from this starfish. One of them has been identified with culcitoside C_1 (=halityloside D) [**2**], previously described from the same organism (8) and from *Hal. regularis* (5); five (**1**, **7**, **8**, **11**, **12**) are known compounds already isolated from the related *Hal. regularis* (5) (family Oreasteridae) and other species, and five (**3**, **4**, **9**, **10**, **14**) are new compounds, designated culcitosides C_4–C_8, respectively. Culcitoside C_2 (=16β-hydroxy culcitoside C_6) and C_3 (its 4-deoxy analogue) have been reported by Kicha *et al.* (9) from *Cu. novaeguineae*, but we failed to isolate them as individual compounds from our sample. Of the five isolated polyhydroxysteroids only one [**13**] is new.

[1]For Part 45, see M. Iorizzi, L. Minale, R. Riccio, and H. Kamiya, *J. Nat. Prod.*, **53**, 1225 (1990).









1 R=**A**, R′=H
A, R′=OH
3 R=**B**, R′=H
4 R=**B**, R′=OH
5 R=H, R′=H
6 R=H, R′=OH

RESULTS AND DISCUSSION

Separation and isolation of the individual compounds from the aqueous extracts of the animals followed the steps described previously (4). The results of our analyses are shown in Table 1. Identification of the known compounds has been achieved by direct comparison (fabms, ^{1}H nmr, hplc) with authentic samples. The occurrence of 1-hexadecyl-*sn*-glycerol-3-phosphoryl choline [**18**] and of its dehydroderivative is also described.

Culcitoside C_4 [**3**], $[\alpha]_D$ −20.3° (*c* = 1.0, MeOH), is closely related to the previously isolated halityloside E [**1**] (5), which is also a component of the glycoside mixture

TABLE 1. Steroidal Glycosides and Polyhydroxysteroids in the Composition of the Starfish *Culcita novaeguineae*.

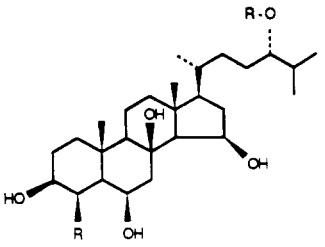
Glycoside	Amount[a] (mg)	Reference
Halityloside E [**1**]	3.5	Iorizzi *et al.* (5)
Culcitoside C_1 (=halityloside D) [**2**]	2.7	Iorizzi *et al.* (5), Kicha *et al.* (8)
Culcitoside C_4 [**3**]	7.5	
Culcitoside C_5 [**4**]	2.9	
Halityloside F [**7**]	9.2	Iorizzi *et al.* (5)
Gomophioside A [**8**]	3.2	Riccio *et al.* (10)
Culcitoside C_6 [**9**]	2.5	
Culcitoside C_7 [**10**]	4.2	
Halityloside A [**11**]	2.8	Iorizzi *et al.* (5)
Halityloside B [**12**]	2.8	Iorizzi *et al.* (5)
Culcitoside C_8 [**14**]	2.0	
Steroids		
5	5.0	Riccio *et al.* (10)
6	3.0	Riccio *et al.* (10)
13	2.0	
15	1.6	Zollo *et al.* (11)
16	2.2	Bruno *et al.* (12)

[a]From starfish (3.8 kg) collected at Zampa, Okinawa in July 1986.

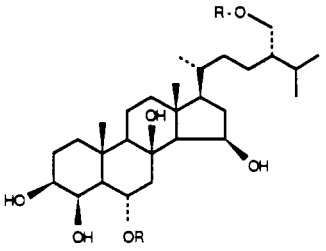
TABLE 2. ^{1}H nmr Data (250 MHz, CD_3OD) for New Compounds in δ [J (Hz) values are shown in parentheses].

Proton	Compound					
	3	**4**	**9**	**10**	**13**	**14**
H-3	3.50 m[a]	3.50 m[a]	3.50 m[a]	3.50 m[a]	3.50 m	3.50 m[a]
H-4		4.29 br s	4.29 br s	4.33 br s	4.29 br s	
H-6	3.70 ddd (10, 10, 4)	4.19 ddd (10.5, 10.5, 4)	4.19 ddd (10.5, 10.5, 4)	4.90 t[a]	4.20 ddd (10.5, 10.5, 4)	3.78 ddd (10.5, 10.5, 4)
H-7	2.41 dd (12, 4)	2.47 dd (12, 3.5)	2.49 dd (12, 4)	2.74 dd (12, 4)	2.50 dd (12, 4)	2.42 dd (12, 3.5)
H-14			1.04 d (5.6)	1.05 d (5.6)		
H-15	4.45 m	4.45 m	4.45 m	4.44 m	4.42 dd (5.6, 6.5)	4.40 dd (5.6, 6.5)
H-16	2.41 m	2.43 m	2.42 m	2.40 m	4.25 t (6.5)	4.27 t (6.5)
H-18	1.30 s	1.29 s	1.29 s	1.29 s	1.27 s	1.27 s
H-19	1.02 s	1.19 s	1.19 s	1.26 s	1.19 s	1.02 s
H-21	0.97 d (6.3)	0.96 d (6.8)	0.98 d (6.8)	0.97 d (7.0)	1.00 d (7.0)	1.00 d (7.0)
H-25				1.82 m	2.34 m	2.44 m
H-26	0.93 d (6.5)	0.94 d (7.0)	0.95 d (7.0)	0.95 d (7.0)	3.60 dd (12, 5)	3.60 m[b]
					3.38 dd (12, 6.5)	3.42 m[b]
H-27	0.95 d (6.5)	0.92 d (7.0)	0.92 d (7.0)	0.92 d (7.0)	1.08 d (7.0)	1.12 d (7.0)
H-28			3.28 dd (10, 5)	3.28 t (10)	4.77 br s	4.77 br s
			3.78 dd (10, 5)	3.78 dd (10, 5)	4.87 br s	4.87 br s
H-1′	5.11 d (1.5)	5.11 d (1.5)	4.99 d (1.5)	4.99 d (7.5)		4.41 d (7.0)
H-2′	4.06 dd (1.5, 4)	4.06 dd (1.5, 4)	4.07 dd (1.5, 4)	4.07 dd (1.5, 4)		3.21 dd (7.0, 9)
H-3′	4.04 m	4.04 m	4.03 m	4.03 m		3.40 t[b]
H-4′	4.00 m	4.00 m	3.94 m	3.93 m		3.50 m[a]
H-5′	3.81 dd (11.2, 3)	3.81 dd (11.2, 3)	3.81 dd (12.5, 3)	3.81 dd (12.5, 3)		3.26 dd[b]
	3.65 dd (11.2, 4.2)	3.65 dd (11.2, 4.2)	3.66 dd (12.5, 4.8)	3.66 dd (12.5, 4.8)		3.92 dd[b]
H-1″	4.37 d (7.5)	4.38 d (7.0)	4.45 d (7.5)	4.44 d (7.5)		4.75 d (7.0)
H-2″	3.18 dd (7.5, 9)	3.19 dd (7.0, 9)	2.90 dd (7.5, 9)	2.90 dd (7.5, 9)		2.97 dd (7.0, 8.6)
H-3″	3.40 t[b] (9.0)	3.40 t[b] (9)	3.42 t (9)	3.40 t[b]		3.34 t[a]
H-4″	3.21 m	3.22 m	3.22 m	3.20 m		3.50 m[a]
H-5″	3.14 t[b]	3.14 dd[b]	3.14 t (10.6)	3.15 t (10.6)		3.19 t[b]
	4.05 dd[b]	4.05 dd[b]	4.05 dd (10.6, 4)	4.01 dd (10.6, 4)		3.85 dd (10.6, 4)
OMe	3.50 s	3.50 s	3.50 s	3.50 s		3.64 s
			3.60 s	3.60 s		

[a]Signal under solvent signal.
[b]Partially overlapping with the sugar signals.

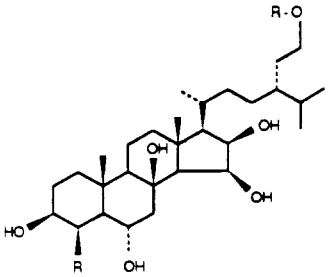


7 R=**A**, R′=H
8 R=**A**, R′=OH

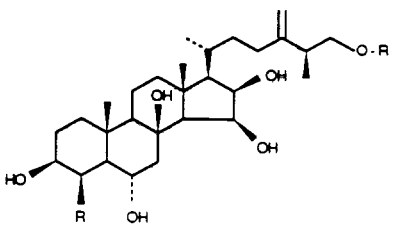


9 R=**A**, R′=H
10 R=**A**, R′=SO_3^-

from this species but lacks the methyl group at the 2″-OH position of the xylosyl residue. The fab (negative ion mode) mass spectrum gave a quasi molecular ion at *m/z* 729 $[M-H]^-$ fourteen mass units shifted relative to **1**, accompanied by fragments at *m/z* 583 and 451 corresponding to the consecutive loss of an O-methylpentose (= 146 mass units) and of a pentose (= 132 mass units) residue. On acid methyanolysis, culcitoside C_4 liberated methyl arabinosides (glc) and a second group of methyl glycosides. Examination of ^{1}H-nmr spectral data (Table 2) of **3** indicated the presence of the same 5α-cholesta-3β,6α,8,15β,24-pentaol aglycone found in **1** (5) and also in attenuatoside A-1 from *Hacelia attenuata* (13). Also present were signals for a α-arabinofuranosyl residue, a moiety equivalent to a β-xylopyranosyl, both determined by decoupling, and one methoxyl group. Upfield shift of the H-4″ to δ_H 3.21 m along with the downfield shifts of Heq-5″ (δ_H 4.05 dd) (cf. δ_H 3.50 and 3.85 ppm in non-substituted β-xylopyranosides) indicated the location of the methoxyl group at C-4″ of the xylosyl residue. 4-*O*-Methylxylopyranosyl units have been already encountered among crossasterosides from the starfish *Crossaster papposus* (14,15). Analysis of the ^{13}C-nmr spectrum (Table 3) confirmed the location of the methyl group at 4″-OH of the terminal xylopyranosyl (C-4″ 80.9, C-5″ 64.6 ppm; cf. δ_C 71.4 and 66.7 in methyl β-D-xylopyranoside) (16,17) and also indicated its attachment at 2′-OH of the arabinofuranose moiety (glycosidation shifts observed for C-2′: 93.0 vs. 81.9 ppm in methyl α-D-arabinofuranoside) (5,13,17). Furthermore, the glycosidation shift observed for C-24 (δ_C 84.6 ppm in **3** vs. 77.2 ppm in the aglycone) (10) established the location there of 4-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinofuranosyl moiety. The 24*S* configuration is proposed by analogy with the many steroid (24*S*)-24-*O*-



11 R=**C**, R′=H
12 R=**C**, R′=OH



13 R=H, R′=OH
14 R=**C**, R′=H

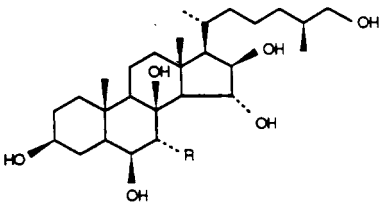
TABLE 3. ^{13}C-nmr Shifts (62.9 MHz, CD_3OD) in ppm.[a]

Carbon	Compound			
	3	**9**	**10**	**14**
C-1	39.5	39.8	39.6	39.5
C-2	31.6	26.3	26.7	31.5
C-3	72.3	73.7	72.9	72.3
C-4	32.5	69.2	69.0	32.4
C-5	54.0	57.5	56.3	53.9
C-6	67.8	64.8	74.5	67.7
C-7	49.5	49.9	47.7	49.7
C-8	77.3	77.1	77.5	77.3
C-9	57.6	58.6	58.4	57.3
C-10	38.0	38.2	38.8	38.0
C-11	19.8	19.2	19.2	19.5
C-12	43.5	43.5	43.4	43.6
C-13	44.4	44.5	44.5	44.5
C-14	62.6	63.0	62.9	61.2
C-15	71.2	71.3	71.2	71.2
C-16	42.7	42.5	42.4	72.8
C-17	58.2	58.1	58.0	63.0
C-18	16.6	16.5	16.5	17.9
C-19	14.1	16.9	16.9	14.1
C-20	36.5	36.6	36.6	30.6
C-21	19.1	19.2	19.2	18.5
C-22	33.0	35.1	35.1	35.6
C-23	28.7	26.2	26.2	32.4
C-24	84.6	46.1	46.1	154.0
C-25	31.6	29.8	29.8	40.0
C-26	18.3	20.1	20.1	74.9
C-27	18.4	19.9	19.9	17.9
C-28		70.4	70.4	109.2
C-1′	107.8	108.4	108.4	103.5
C-2′	93.0	91.7	91.7	81.3
C-3′	77.6	77.7	77.7	76.9
C-4′	83.8	84.4	84.4	71.3
C-5′	62.7	62.9	62.9	66.5
C-1″	105.2	104.7	104.7	104.3
C-2″	75.2	84.9	84.9	84.8
C-3″	77.0	77.7	77.7	77.6
C-4″	80.9	81.0	81.0	71.3
C-5″	64.6	64.5	64.5	66.6
OMe	59.1	59.0	59.0	60.8
		61.0	61.0	

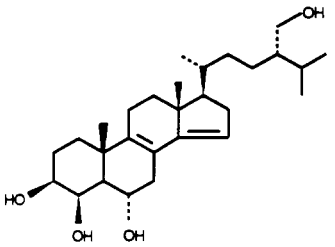
[a]Assignments were aided by DEPT measurement.

arabinofuranosides isolated from starfishes and supported by the chemical shifts of the side chain carbons identical with those of halitylosides D–F and other steroid 24-*O*-xylopyranosyl-(1→2)-arabinosides, e.g, nodososide, for which the 24*S* stereochemistry was determined (18).

Culcitoside C_5 [**4**], $[\alpha]_D$ −22.4° ($c = 0.1$, MeOH), is related to culcitoside C_4 by introduction of an additional hydroxyl group at the 4β position of the steroid aglycone. The structure was derived by fabms, ^{1}H-nmr, and comparison with **3**. The fab (negative ion mode) mass spectrum gave a quasi molecular ion at *m/z* 745 $[M - H]^-$, sixteen mass units shifted relative to **3** (*m/z* 729), and fragments at *m/z* 599 and 467 corre-



15 R=H
16 R=OH



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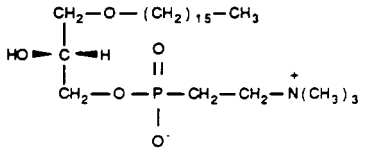

18

sponding to the sequential loss of the 4-*O*-methylxylose (= 146 mass units) and of the arabinose (= 132 mass units) residue. The ^{1}H-nmr spectrum (Table 2) was similar to that of **3** except for the presence of one more signal in the downfield region of the spectrum at δ_H 4.29 (broad singlet), assigned to H-4α and for the expected downfield shifts of the signals for H-6 (δ_H 4.19 vs. 3.70 ppm in **3**) and H_3-19 (δ_H 1.19 vs. 1.02 ppm in **3**).

Culcitoside C_6 [**9**], $[\alpha]_D$ −23.2° (*c* = 0.1, MeOH), gave a fab (negative ion mode) mass spectrum with a quasi molecular ion peak at *m/z* 773 $[M-H]^-$ accompanied by fragments at *m/z* 613 and 481, corresponding to the consecutive loss of a dimethoxylated pentose unit (= 160 mass units) and of a pentose unit (= 132 mass units). Examination of ^{1}H- and ^{13}C-nmr spectra (Tables 2 and 3) indicated the presence of the 2,4-di-*O*-methyl-β-xylopyranosyl-(1→2)-α-arabinofuranosyl moiety already encountered in halityloside E [**1**] (5) and culcitoside C_1 [**2**] (8). In addition to the sugar moiety, the ^{1}H-nmr spectrum showed signals corresponding to the 3β,4β,6α,8,15β-pentahydroxycholestane structure (cf. culcitosides C_1 and C_5) and also two 1 H double doublets at δ_H 3.28 (*J* = 10 and 5 Hz) and 3.78 (*J* = 10 and 5 Hz) coupled to each other by 10 Hz, indicative of an oxymethylene grouping. This was confirmed by the ^{13}C-nmr spectrum, which showed a methylene carbon signal (DEPT technique) at 70.4 ppm. Thus, the aglycone of culcitoside C_6 corresponds to a C_{28} saturated sterol, mol. wt. 482 (fabms measurements), with five hydroxyl groups in the nucleus and one side chain methyl group oxidized to hydroxymethylene. The ^{13}C-nmr spectrum showed signals for the steroid aglycone moiety from C-1 to C-21 virtually identical with those of the corresponding signals of the steroid **6** (10). Thus the sugar moiety is attached to the remaining oxygenated site of the side chain, for which the 24-hydroxymethylene structure was preferred to the alternative 24-methyl-26-hydroxy structure mainly on the

basis of the 26- and 27-methyl carbon signals at 19.9 and 20.1 ppm and comparison with the spectra of the synthetic model 24-hydroxymethyl steroids (19) (in CD_3OD; 24*R* isomers δ_C 19.2, 20.4 ppm; 24*S* isomers δ_C 19.3, 19.9 ppm) and 24-methyl-26-hydroxysteroids (20) (Me-27 signals ranging from δ_C 14.8 to 17.5 and Me-28 signals ranging from δ_C 11.6 to 14.4 ppm depending on the stereochemistry).

The 24*S* configuration of culcitoside C_6 was then assigned by comparison of the ^{1}H-nmr spectrum of the 24-hydroxymethylene steroid **17**, derived from the glycoside **9** on acid treatment with 2 M HCl/MeOH, with those of the synthetic models (24*R*)- and (24*S*)-3α,5-cyclo-6β-methoxy-5α-ergostan-28-ol (19). The ^{1}H-nmr shift values (CD_3OD) for the 28-methylene protons (two double doublets at δ_H 3.46 and 3.56) in **17** were virtually identical with the corresponding signals of the synthetic 24*S* isomer (δ_H 3.47–3.56) and far away from those of the 24*R* isomer (2H broad doublet at δ_H 3.52). Small but significant differences were also observed for the 26- and 27-methyl signals (24*S* δ_H 0.92 d and 0.93 d; 24*R* δ_H 0.91 d and 0.94 d), and the corresponding signals in the spectrum of the steroid **17** (δ 0.92 d, 0.93 d) were superimposable with those of the 24*S* isomer.

Thus the structure of culcitoside C_6 can be defined as (24*S*)-28-*O*-[2,4-di-*O*-methyl-β-xylopyranosyl-(1→2)-α-arabinofuranosyl]-24-methyl-5α-cholesta-3β,4β,6α,8,15β,28-hexaol [**9**].

Culcitoside C_7 [**10**], $[\alpha]_D$ −17.7° (*c* = 0.1, MeOH), is the 6-*O*-sulfate derivative of the culcitoside C_6 [**9**]. The fab (negative ion mode) mass spectrum gave a molecular anion species at *m/z* 853. The ^{1}H-nmr spectrum was very similar to that of culcitoside C_6 [**9**] except the signals for H-6, H-7, and H_3-19, which were shifted downfield to δ_H 4.90 (4.19 in **9**), 2.74 (2.49 in **9**) and 1.26 (1.19 in **9**). These data placed the sulfate at C-6 in **10**. This was supported by ^{13}C-nmr signals for C-6 downfield shifted to 74.5 (64.8 in **9**) ppm and for C-5 and C-7 highfield shifted to 56.3 (57.5 in **9**) and 47.7 (49.9 in **9**) ppm, respectively. On solvolysis using dioxane/pyridine (21), it afforded culcitoside C_6 [**9**].

Culcitoside C_8 [**14**], $[\alpha]_D$ −1.4° (*c* = 0.1, MeOH), gave a fab (negative ion mode) mass spectrum with a quasi molecular ion peak at *m/z* 757 $[M-H]^-$ accompanied by fragments at *m/z* 611 and 479, corresponding to the sequential loss of an *O*-methylpentose (=146 mass units) and of a pentose unit (=132 mass units) residue. On acid methanolysis it gave methylxylosides and methyl 2-*O*-methylxylosides. The shifts of the anomeric carbons by ^{13}C nmr at 103.5 and 104.3 ppm (Table 3) and the coupling constants of the anomeric protons at δ 4.75 (*J* = 7.0 Hz) and 4.41 (*J* = 7.0 Hz) (Table 2) suggested that the glycosidic linkages in **14** are β and the xylosyl units are in their pyranose form. The presence of two low-field signals at 84.8 and 81.3 ppm in the ^{13}C-nmr spectrum (Table 3) suggested location of the interglycosidic linkage of the disaccharide moiety, since the lower-field signal is due to C-2 of the 2-*O*-methylxylose and the second one is assignable to the glycosidated C-2 of the other xylosyl unit (22). We note that the same 2-*O*-methyl-β-xylopyranosyl-(1→2)-β-xylopyranosyl unit was found in the previously described halitylosides A [**12**] and B [**11**], isolated from the starfish *Hal. regularis* (5) and also components of the steroid glycoside mixture of *Cu. novaeguineae*.

In addition to the sugar moiety, the ^{1}H-nmr spectrum showed the presence of a 3β,6α,8,15β,16β-pentahydroxy steroidal tetracyclic nucleus already found in previous steroid glycosides from *Hal. regularis* [e.g., halityloside B [**11**] (5)] and *Co. tenuispina* (4), and signals for the side chain protons at δ_H 1.00 and 1.12 (methyl doublets), 3.60 and 3.42 (partially overlapping with sugar signals, assigned to an hydroxymethylene group) and 4.77 br s and 4.87 br s (1H each, olefinic protons, terminal methylene). A signal for a proton in the allylic position was observed at δ_H 2.44 (C-25).

Irradiation of this multiplet did collapse the methyl doublet at δ 1.12 to a singlet and simplified the signals at δ 3.60 and 3.42. These data indicated a 24-methylene-26-hydroxy side chain structure. The ^{13}C-nmr spectrum showed signals for the aglycone moiety from C-1 to C-21 virtually identical with those of the corresponding signals in the spectrum of the glycoside **11** (5). Thus the sugar moiety is attached at the oxygenated site of the side chain. The ^{13}C-nmr signal at 74.9 ppm for the oxygenated methylene carbon and comparison with the corresponding signal at 67.6 ppm observed in the spectra of 24-methylene-26-hydroxy steroids (10,23,24) definitively confirmed the location of the sugar moiety at C-26. The 25*S* configuration is proposed by analogy with **13** (see below).

(25*S*)-24-methyl-5α-cholest-24(28)-ene-3β,4β,6α,8,15β,16β,26-heptaol [**13**], $[\alpha]_D$ −10.4° (c = 0.1, MeOH), gave a fab (positive ion mode) mass spectrum with a quasi molecular ion species at *m/z* 627 $[M + Na + thioglycerol]^+$ and 605 $[M + H + thioglycerol]^+$. Examination of its ^{1}H-nmr spectrum (Table 2) immediately indicated the presence of the same 3β,4β,6α,8,15β,16β-hydroxylation pattern as in halityloside A [**12**] (5) and of a 24-methylene-26-hydroxy side chain [olefinic signals at δ_H 4.87 brs and 4.77 brs, and signals for H-25, H_2-26, and H_3-27 shifted to δ_H 2.34 m, 3.60 (1H, dd, J = 12, 5 Hz), 3.38 (1H, dd, J = 12, 6.5 Hz), and 1.08 (3H, d, J = 7.0 Hz), respectively].

The 25*S* configuration for **13** is proposed by analogy with the previous (25*S*)-24-methyl-5α-cholest-24(28)-en-3β,4β,6α,7α,8,15β,16β,26-octaol, isolated from the starfish *Patiria miniata* (24). The chemical shifts of the side chain protons were superimposable in both compounds; in the spectra of their 26-(+)MTPA [MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic acid] esters the shifts of the methylene protons at C-26, which are sensitive to the stereochemistry at C-25 (24), were also identical.

The final fraction was shown to consist of phosphorylglycerylethers, and two components were separated by hplc. The first component **18** was assigned the 1-hexadecyl-*sn*-glycerol-3-phosphorylcholine structure on the basis of the fab (positive ion mode) mass spectrum, *m/z* 483 $[M+H]^+$, ^{1}H- and ^{13}C-nmr spectra (see Experimental) and comparison with the data described by Fusetani *et al.* (25), who isolated the same compound from the hydroid *Solanderia secunda*. The specific rotation $[\alpha]_D$ −2.3° (c = 0.1, MeOH) is in numerical agreement with that reported for the sample isolated by Fusetani *et al.* (25), which possesses the 2*S* absolute configuration. The second component showed in its fab (positive ion mode) mass spectrum a molecular ion species peak at *m/z* 481 $[M + H]^+$, two mass units shifted relative to **18**. In addition to signals already observed in **18**, the ^{1}H-nmr spectrum showed signals for two olefinic protons at δ 5.36 and for four allylic methylene protons at δ 2.07 ppm. Thus the second phosphorylglycerylether component is simply a dehydroderivative of **18** with one "in chain" double bond. These compounds are precursors of platelet activating factors (PAF) and are hemolytic with an activity comparable to that of standard saponin (25). Glyceryl ethers are widespread among marine animals, and in echinoderms they are distributed almost evenly between neutral and polar lipids (3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra, Bruker WM-250 (^{1}H at 250 MHz, ^{13}C at 62.9 MHz), δ (ppm), J in Hz, spectra referred to CHD_2OD signal at 3.34 ppm and to central carbon CD_3OD signal at 49.0 ppm; mass spectra, VG ZAB mass spectrometer equipped with fab source [in glycerol or glycerol-thioglycerol (3:1) matrix; Xe atoms of 2–6 kV]; optical rotations, Perkin Elmer model 241 polarimeter; glc, Carlo Erba Fractovap 2900 for capillary column (SE-30, 25 mt, 125°, helium carrier flow 2 ml·min^{-1}); reversed-phase hplc, C_{18} μ-Bondapak column (30 cm × 8 mm i.d.; flow rate 5 ml·min^{-1}), and C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.; flow rate 2 ml·min^{-1}), Waters Model

6000 A pump equipped with a U6K injector and a differential refractometer, model 401; dccc, DCC-A apparatus manufactured by Tokyo Rikakikai Co., equipped with 250 tubes and Büchi apparatus equipped with 300 tubes.

EXTRACTION AND ISOLATION.—The animals, *Cu. novaeguineae*, were collected at Zampa, Okinawa in July 1986 and identified at the Department of Marine Sciences, University of the Ryukyus, Okinawa, Japan; a voucher specimen is preserved there. The animals (3.8 kg) were chopped and soaked in H_2O (twice, 2 liters for 8 h each): the aqueous extracts were decanted and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with distilled H_2O (1 liter) and eluted with MeOH (2 liters) to give, after removal of the solvent, a glassy material (4.12 g). The solid mass was extracted with Me_2CO (2 liters, 24 h twice). The Me_2CO extracts were evaporated in vacuo and partitioned between H_2O and Et_2O. The aqueous residue was then extracted with *n*-BuOH. Evaporation of the *n*-BuOH extracts afforded 5.1 g of an oily residue, which was combined with the above MeOH eluate from Amberlite XAD-2 column and chromatographed on a column of Sephadex LH-60 (4 × 100 cm) with MeOH-H_2O (2:1) as eluent. Fractions (6 ml) were collected and analyzed by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 91–150 (1.15 g) contained a complex mixture of the steroid glycosides and polyhydroxysteroids along with the ether phospholipids. Fractionation was pursued by dccc using $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase). Fraction (5 ml each) were collected and monitored by tlc on SiO_2 with $CHCl_3$-MeOH-H_2O (80:18:2), and the results are summarized in Table 4. Each of the above fractions was then submitted to hplc with MeOH-H_2O (7:3), or MeOH-H_2O (8:2) for the less polar fractions 291–338 and 339–390, on C_{18} column (30 cm × 3.9 mm i.d.) to give pure compounds. The amount of each steroidal constituent is reported in Table 1. Rotation and fabms data are in the text, while ^{1}H- and ^{13}C-nmr data are in Tables 2 and 3.

TABLE 4. Dccc Fractionation[a] of the Mixture of Steroid Glycosides from *Cu. novaeguineae*.

Fractions No.	Amounts (mg)	Compounds
67–75	16	**4, 12, 14**
76–83	21	**4, 14**
84–87	13	**4**
88–97	26.7	**3, 11, 15, 16**
98–107	25	**11**
108–113	8.5	**2**
114–119	10.6	**2, 9, 13**
120–134	23.2	**1, 9, 13**
135–147	19.5	**1, 6**
153–182	35.4	**5**
291–338	82.3	**7, 8**
339–390	70.0	ether phospholipids

[a]Solvent system $CHCl_3$-MeOH-H_2O (7:13:8), ascending mode; 250 tubes; 5 ml fractions collected.

Fractions 339–390, when submitted to reversed-phase hplc, gave two main peaks. The first was identified as 1-hexadecyl-*sn*-glycerol-3-phosphorylcholine [**18**]: $[\alpha]_D$ −2.3° (*c* = 1, MeOH); fabms $[M+H]^+$ 483; ^{1}H nmr (CD_3OD) δ_H 0.96 (3H, t, *J* = 7 Hz, Me), 1.36 (m, -CH_2-), 1.62 (2H, m, -CH_2-CH_2-O), 3.24 (9H, s, N-Me), 3.52 (4H, m, H_2-1, H_2-1′), 3.7 and 4.42 (2H, each, m, -O-CH_2-CH_2-N^+<-), 3.90 (3H, m, H-2′, H_2-3′); ^{13}C nmr δ 14.1 (Me, C-16), 23.7 (CH_2, C-15), 27.2 (CH_2, C-3), 30.4 (CH_2, C-14), 30.7 (10C, CH_2), 33.0 (CH_2, C-2), 54.7 (N-Me), 60.4* (C-1″), 67.6* (C-3′), 68.5* (C-2″), 71.0* (C-2′), 72.7 and 72.9 (C-1 and C-1′) ppm [the signals marked with the asterisk appear as doublets (*J* = 5–7 Hz) because of the coupling with ^{31}P]. The second component had $[\alpha]_D$ −2.36° (*c* = 1, MeOH); fabms $[M+H]^+$ 481; ^{1}H nmr (CD_3OD) δ_H 0.93 (3H, t, Me), 1.36 (m, -CH_2-), 1.45 (4H, m), 1.62 (2H, m), 2.07 (4H, m), 3.24 (9H, s, N-Me), 3.50 (4H, m), 3.70 (2H, m), 3.94 (3H, m), 4.32 (2H, m), 5.36 (2H, m). It was identified as an "in chain" double bond analogue of **18**, with the position of the double bond undefined.

Fractions 79–90 (0.5 g) eluted from the column of Sephadex LH-60 contained more polar compounds (511 mg). This material was combined with the first fractions 11–26 (0.389) derived from the above dccc separation and submitted to dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the ascending mode (the lower

phase was the stationary phase; flow rate 6 ml/h; 3-ml fractions were collected and monitored by tlc). Fractions 49–58 contained culcitoside C_7 **[10]**, which was further purified by hplc on the C_{18} column with MeOH-H_2O (6:4) to give 4.2 mg of pure compound (physical data in the text and in Tables 2 and 3). The subsequent dccc fractions contained a complex mixture of sulfated material, difficult to separate, and tryptophan (370 mg).

METHANOLYSIS OF GLYCOSIDES: SUGAR ANALYSIS.—A solution of glycoside **3**, **9**, or **14** (1 mg) in anhydrous 2 M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. After having cooled, each 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 gave peaks which coeluted with those of methylarabinoside and methyl 4-*O*-methylxyloside (compound **3**), methylarabinoside and methyl 2,4-di-*O*-methylxyloside (compound **9**) and methylxyloside and methyl 2-*O*-methylxyloside (compound **14**).

Methyl 4-*O*-methylxylosides, methyl 2,4-di-*O*-methylxylosides, and methyl 2-*O*-methylxylosides standards were obtained by acid methanolysis of crossasteroside A (14), halityloside E (5), and halityloside A (5).

SOLVOLYSIS OF CULCITOSIDE C_7 **[10]**.—A solution of **10** (2 mg) in pyridine (0.1 ml) and dioxane (0.1 ml) was heated at 160° for 2 h in a stoppered raction vial. After the solution was cooled, H_2O (1 ml) was added and the solution was extracted with *n*-BuOh (3 × 1 ml). The combined extracts were evaporated to dryness under reduced pressure. The residue was purified by hplc [C_{18} μ-Bondapak column, MeOH-H_2O (7:3)] to give the desulfated material: fabms (negative ion) *m/z* 773; ^{1}H nmr identical with that of **9**.

ACID TREATMENT OF **9** TO GIVE THE STEROID **17**.—A solution of **9** (4.5 mg) in 2 M HCl/MeOH (0.8 ml) was heated at 75° in a stoppered reaction vial. After 2 h, tlc analysis [SiO_2 with $CHCl_3$-MeOH-H_2O (80:18:2)] showed that the starting material (R_f 0.3) had disappeared and was replaced by a uv-active spot (R_f 0.7). The reaction mixture was cooled, neutralized with Ag_2CO_3, and centrifuged, and the supernatant was taken to dryness under N_2. The residue was purified by hplc [C_{18} μ-Bondapak (30 cm × 3.9 mm i.d.), MeOH-H_2O (7:3)] to give the steroid **17**: fabms *m/z* $[M - H]^-$ 445; uv λ max 248 (ε = 18.800) nm [in agreement with a $\Delta^{8(9),11}$ steroidal diene (26)]; ^{1}H nmr (CD_3OD) 0.85 (3H, s, H_3-18), 0.92–0.93 (6H, each doublet, J = 7 Hz, H_3-26, -27), 1.01 (3H, d, J = 6.3 Hz, H_3-21), 1.24 (3H, s, H_3-19), 3.45 and 3.56 (each 1H, dd, J = 10, 6.5 Hz, H_2-26), 3.50 (1H, m), 4.21 (1H, dt, J = 3.5, 9 Hz, H-6β), 4.30 (1H, br s, H-4α), 5.40 (1H, br, H-15).

3,26-DI-(+)-MTPA ESTER OF **13**.—The steroid **13** (1 mg) was treated with freshly distilled (+)-methoxytrifluoromethylphenylacetyl chloride (2 μl), prepared from (+)-(*R*)-MTPA acid, in dry pyridine (0.2 ml) for 1 h at room temperature.

After removal of solvent, the product was analyzed by ^{1}H nmr: δ 0.99 (3H, d, J = 7 Hz, H_3-21), 1.13 (3H, d, J = 7 Hz, H_3-27), 1.19 (3H, s, H_3-19), 1.27 (3H, s, H_3-18), 2.53 (1H, dd, J = 12.5, 3.7 Hz, H-7β), 2.65 (1H, m, H-25), 3.58 (6H, s, OMe), 4.23–4.36 (5H overlapping, H-6β, H-16α, H-4α, and H_2-26), 4.42 (1H, t, J = 6 Hz, H-15α), 4.89–4.82 (each H, br, $=CH_2$), 4.96 (1H, m, H-3α), 7.56–7.48 (m, Ph-H's).

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