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STARFISH SAPONINS, PART 37.[1] STEROIDAL GLYCOSIDE SULFATES FROM STARFISHES OF THE GENUS *PISASTER*

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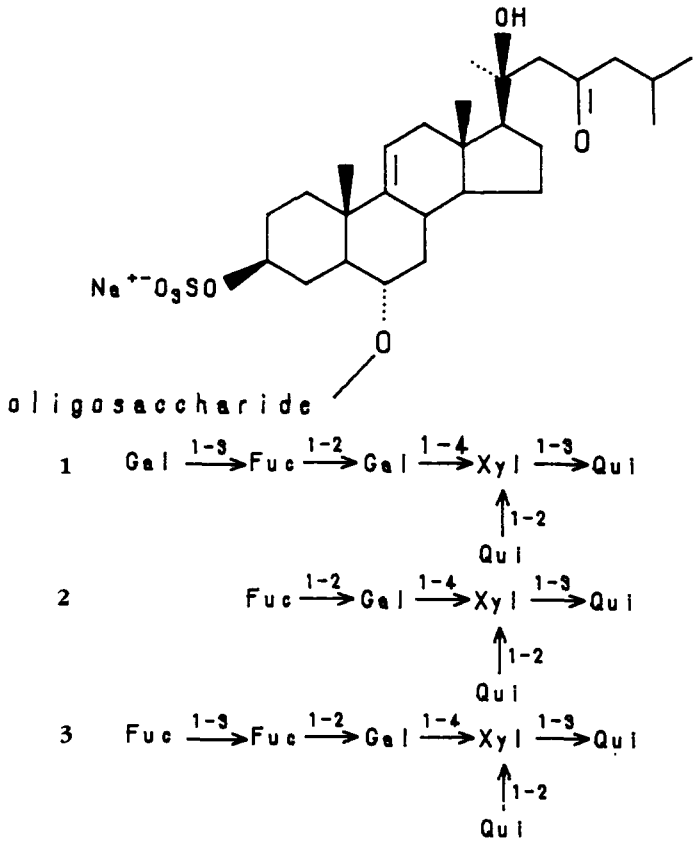
ABSTRACT.—An investigation of the extracts from two starfishes of the genus *Pisaster* has led to the isolation of three new steroidal monoglycoside sulfates, designated pisasterosides A [**4**], B [**5**], and C [**6**], along with major amounts of known asterosaponins (steroidal penta- and hexaglycoside sulfates) versicoside A [**1**], thornasteroside A [**2**], and marthasteroside A_1 [**3**]. The structures of the new metabolites were determined on the basis of spectral data; the stereochemistry at C-24 of pisasteroside A [**4**], 28-*O*-β-D-glucopiranosyl(6′-*O*-sulfate)-24-methyl-5α-cholest-22-ene-3β,6α,8,15β,16β,28-hexol, has been determined by comparison of the spectral data of the aglycone with those of model compounds.

In the course of our continuing studies of oligoglycoside and polyhydroxy steroidal constituents of starfishes, we have been working on starfish species collected off the Gulf of California, and in this note we report three new steroidal glycoside sulfates isolated from *Pisaster ochraceus* Brandt, i.e., pisasterosides A [**4**] and B [**5**], and from *Pisaster brevispinus* Stimpson, i.e., pisasterosides A [**4**] and C [**6**].

In addition, *P. ochraceus* and *P. brevispinus* Asteriidae contained major amounts of



[1]For Part 36, see R. Riccio, O. Squillace Greco, L. Minale, S. La Barre, and D. Laurent, *J. Nat. Prod.*, **51**, 1003 (1988).

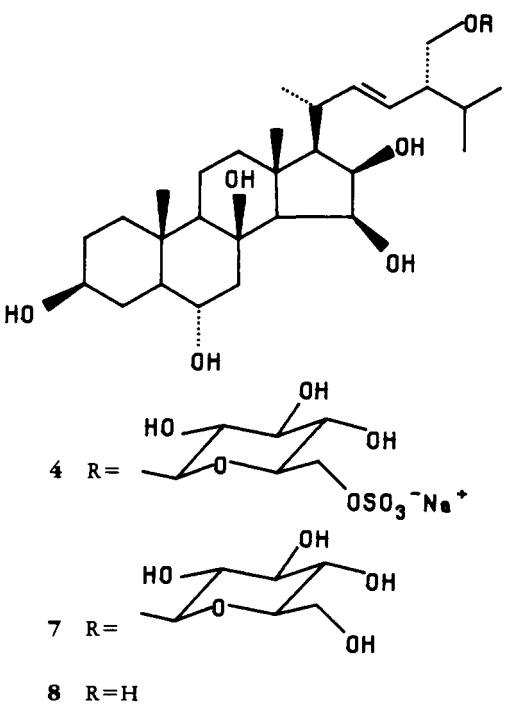
[2]R. Riccio, E. Finamore, M. Santaniello and F. Zollo, manuscript in preparation.

the known asterosaponins, versicoside A [**1**] (1) and thornasteroside A [**2**] (2); *P. brevispinus* also contained marthasteroside A_1 [**3**] (3).

Pisasteroside A [**4**], $[\alpha]^{25}D$ +4°, fabms *m/z* $[M]^-$ 721 is isomeric with coscinasteroside C, previously isolated from *Coscinasterias tenuispina* (4), the only difference being the location of the sulfate group at C-6′ of the glucosyl moiety in **4** instead of C-4′ as in coscinasteroside C. Upon solvolysis using dioxane/pyridine, compound **4** yielded the desulfated glycoside **7**, identical with the desulfated coscinasteroside C. The 500 MHz ^1H nmr of **4** and double resonance experiments showed the signals for the methylene protons due to -CH_2-O- of glucose downfield shifted to δ 4.18 (dd, 5, 12 Hz) and 4.35 (dd, 2.5, 12 Hz), (δ 3.70 and 3.90 in **7**), and the ^{13}C nmr showed the signal for C-6′ of glucose downfield shifted to 68.2 ppm. This clarified that C-6′ bears the sulfate in pisasteroside A [**4**].

The only feature needed to establish the structure of both coscinasteroside and pisasteroside A [**4**] is the stereochemistry at C-24. This problem has now been solved with compound **4**, after the stereoselective synthesis of (24*R*)- and (24*S*)-hydroxymethylcholesta-5,(22*E*)-diene-3β-ol.[2] These model compounds showed very similar nmr spectra, but they could be differentiated by ^1H-nmr spectra of their (+)-α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA). The most noticeable feature in the ^1H-nmr spectra of these MTPA derivatives was the large difference in the chemical shift of the C-21 methyl signal, which in the 24*S* synthetic model was upfield shifted to 0.96 ppm (CD_3OD), while in the 24*R* synthetic model it resonated at δ 1.04 (CD_3OD), virtually unshifted relative to the free alcohols (δ 1.08–1.07 ppm).

The ^1H-nmr spectra of the MTPA derivatives also showed significative differences in the shape and chemical shift of the C-28 methylene signal. This appeared as a doublet (J = 7 Hz) at δ 4.34 in the spectrum of the 24*R* synthetic model and as two double doublets centered at δ 4.25 (J = 14, 8 Hz) and 4.39 (J = 14, 7 Hz) in that of the 24*S* synthetic model.



8 R=H

These ^{1}H-nmr measurements of the MTPA derivatives were a highly reliable means for stereochemical assignments in Δ^{22}, 24-hydroxy methyl steroids.

Enzymic hydrolysis with *Charonia lampas* glycosidase mixture (Scikagaku Kogyo Co., Ltd., Tokyo, Japan) of **7** removed glucose to yield the steroidal aglycone **8**, which was then treated with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to give the corresponding 3,6,28-(+)MTPA triester. Its ^{1}H-nmr spectrum (CD_3OD) showed signals at δ 1.06 (3H, d, J = 7 Hz) for H_3-21 and at δ 4.37 (2H, d, J = 7 Hz) for H_2-28 in excellent agreement with the corresponding signals in the spectrum of the 24*R* model compound. On this basis we support the 24*R* configuration for compound **8** and accordingly for the natural glycoside **4**.

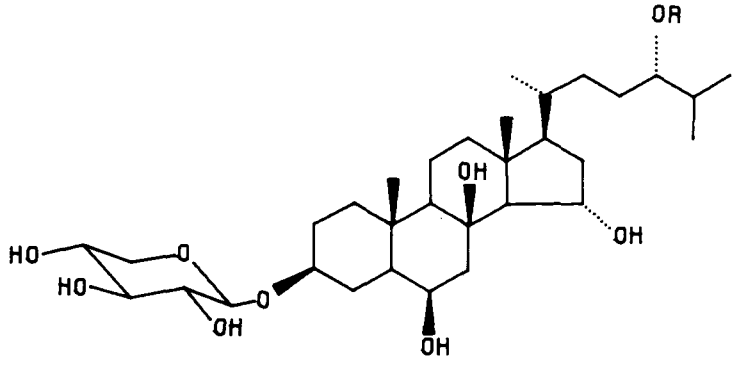
Pisasteroside B [**5**], $[\alpha]^{25}D$ +6°, is isomeric with coscinasteroside B (4), the differences being the location of the sulfate group at C-24 in **5** instead of C-15 and the location of the β-xylopyranosyl residue at C-3 in **5** instead of C-24 in coscinasteroside B.

The negative ion fabms showed the molecular anion peak at *m/z* 663 $[M]^-$ and a fragment at *m/z* 531 (loss of 132 mu = xylose).

Upon solvolysis, **5** was desulfated to **9**, which gave a quasi molecular ion at *m/z* $[M-H]^-$ 583 and a major fragment at *m/z* 451 $[M-H-132]^-$. These data indicated that the natural compound is a glycoside of a sulfated steroidal aglycone. The mol wt of the steroid is 452, corresponding to a pentahydroxycholestane.

Examination of the ^{1}H- and ^{13}C-nmr (Table 1) spectra (see Experimental) indicated that the molecule bears a β-xylopyranosyl moiety. The ^{1}H-nmr signals (see Experimental) for the steroidal aglycone and comparison with those of coscinasteroside B (4) were suggestive of a 3β,6β,8,15α,24-pentahydroxycholestane structure with the sulfate group located at C-24. Upfield shift of the H-24 signal from 4.14 (q, J = 6.5 Hz) in **5** to 3.24 ppm in **9** confirmed the location of the sulfate. Analysis of the ^{13}C-nmr spectrum (Table 1) of **5** and comparison with spectra of the many steroid xylopyranosides isolated from starfishes established the location of the xylopyranosyl residue at C-3. Significantly the signal for C-3 in **5** was shifted downfield by 7.5 ppm to 80 ppm, while those for C-2 and C-4 were shifted upfield by 1.7 and 5.8 ppm to 30.0 and 30.7 ppm, respectively, relative to the 24-*O*-xylopyranosyl-6β,8,15α,24-pentol, 15-sulfate (i.e., coscinasteroside B) (4) [cf. glycosidation shift (7–9)]. The 24*S* configuration was suggested for **5** by analogy with the many (24*S*)-hydroxysteroids isolated from starfishes (10–13).

Pisasteroside C [**6**], $[\alpha]^{25}D$ +12°. Negative ion fabms exhibited an intense molecular anion peak at *m/z* 705 and no further fragmentation was observed. Upon solvolysis, **6** yielded **10**, which gave a quasi molecular ion peak at *m/z* 625 $[M-H]^-$



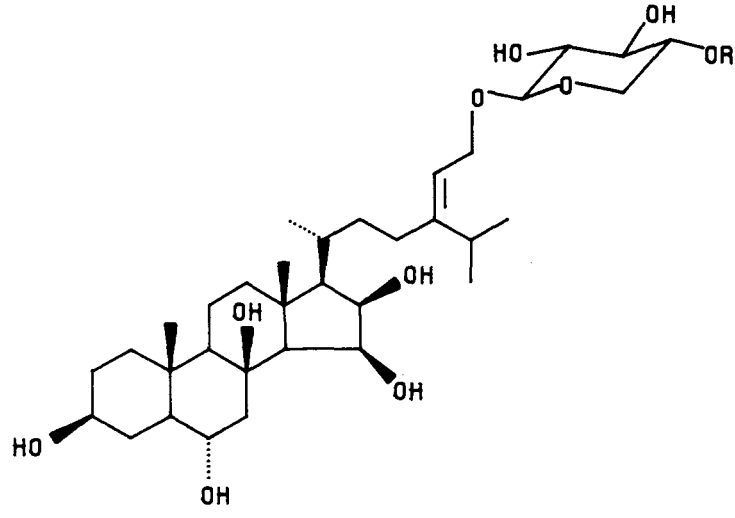
5 R=$SO_3^-Na^+$
9 R=H

TABLE 1. ^{13}C-Nmr Shifts (δ/ppm) of Glycosides in CD_3OD.

Carbon	Compound		
	4	**5**	**6**
C-1	39.4	41.4	39.5
C-2	31.5	30.7	31.5
C-3	72.2	80.0	72.3
C-4	32.3	33.0	32.4
C-5	53.8	49.0	53.9
C-6	67.7	74.2	67.7
C-7	49.6	45.5	49.7
C-8	77.5	77.2	77.3
C-9	57.4	57.2	57.6
C-10	38.0	37.9	38.0
C-11	19.5	19.8	19.5
C-12	43.5	42.8	43.7
C-13	44.5	44.5	44.7
C-14	60.8	66.6	61.2
C-15	71.5	70.1	71.4
C-16	73.2	41.5	72.9
C-17	62.9	55.7	63.0
C-18	18.1	15.3	17.9
C-19	14.1	15.8	14.0
C-20	35.8	36.1	31.5
C-21	20.8	18.9	18.5
C-22	140.9	32.2	36.6
C-23	129.5	28.7	28.9
C-24	51.1	85.9	151.8
C-25	30.2	31.7	30.9
C-26	19.4	18.6	21.7
C-27	21.3	17.8	21.6
C-28	74.2	—	120.2
C-29	—	—	66.0
C-1′	105.0	103.2	103.4
C-2′	75.1	75.4	74.8
C-3′	77.6	77.9	76.0
C-4′	71.5	71.3	77.5
C-5′	76.0	66.8	64.7
C-6′	68.2	—	—

accompained by strong fragments at *m/z* 493 $[M-H-132]^-$ and *m/z* 475 $[M-H-132-H_2O]^-$. Those data suggested a sulfated glycoside of a steroid aglycone with the sulfate located on the sugar moiety.

The ^{1}H-nmr spectrum of **6** and double resonance experiments showed four methine protons at 4.29 (d, 7 Hz), 3.28 (dd, 9, 7 Hz), 3.51 (t, 9 Hz), and 4.19 (ddd, 5, 9, 10 Hz) ppm, which couple to their neighbors in this order. The last methine proton is further coupled to methylene protons at 3.30 (t, 10 Hz) and 4.15 (dd, 10, 5 Hz). The coupling constants between these protons indicated that molecule has a moiety equivalent to β-xylopyranoside. Upfield shift of H-4′ from 4.19 in **6** to 3.50 in **10** clarified that C-4′ bears the sulfate. The above data together with the ^{13}C-nmr spectrum (Table 1) indicated that the natural compound corresponds to the xyloside 4′-*O*-sulfate of a C_{29} monounsaturated steroid with six hydroxyl groups. In addition to the sugar moiety, readily recognized from the nmr data, were two tertiary methyls δ 1.03 (s) and 1.29 (s) and four methine protons at δ 4.41 (dd, 5, 7 Hz), 4.24 (t, 7 Hz), 3.75 (td, 12, 3 Hz), and 3.53 (m), already observed in the spectrum of pisasteroside A [**4**] and assigned to



6 R=$SO_3^-Na^+$
10 R=H

H_3-19, H_3-18, H-15, H-16, H-6, and H-3 of the steroid, respectively. Moreover, the ^{13}C-nmr spectrum of **6** (Table 1) showed signals virtually identical with those of the corresponding signals of **4**. Thus the sugar moiety is attached to the remaining oxygenated site of the side chain.

In the ^{1}H-nmr spectrum of **6** the isopropyl methyls were observed as two doublets downfield-shifted to δ 1.04 (6.5 Hz) and 1.07 (6.5 Hz); in addition a 2H doublet (7 Hz) and one proton triplet (7 Hz), coupled to each other, appeared at 4.32 and 5.37 ppm, respectively, indicating a 29-hydroxyethyl-24(28)-ene structure. The stereochemistry of the side chain double bond was supposed to be *Z* on the basis of the chemical shift value of the C-25 methine proton at δ 2.88 (septet; in the *E* isomer it would be expected to give a signal upfield shifted to δ 2.3) (14–16). The *Z* stereochemistry is also supported by ^{13}C-nmr data for C-25 (upfield-shifted to 30.9 ppm) and for C-23 (downfield-shifted to 28.9 ppm) when compared with corresponding data for isofucosterol and fucosterol (17).

EXPERIMENTAL

INSTRUMENTAL.—For instruments used, see Zollo *et al.* (18).

EXTRACTION AND ISOLATION.—The animals (*P. ochraceus*, 9.7 kg, *P. brevispinus* 3.2 kg) were collected in 1985 off the Gulf of California and identified by zoologists of the Scripps Institution of Oceanography, La Jolla, California; a voucher specimen is preserved at Dipartimento di Chimica delle Sostanze Naturali, Università, Napoli. The animals were chopped and extracted with distilled H_2O (*P. ochraceus* 5 liters, *P. brevispinus* 3 liters) for 3 h at room temperature. The extraction was repeated two times.

The aqueous extracts were passed through a column of Amberlite XAD-2 (1 kg) eluting with H_2O and then MeOH. The H_2O fractions were re-chromatographed on the same Amberlite XAD-2 column.

The combined MeOH eluates were dried on a rotary evaporator to give a glassy material (*P. ochraceus* 9.1 g, *P. brevispinus* 3.4 g) which was then chromatographed on a column of Sephadex LH-60 (80 cm × 4 cm i.d.; 100 g) using MeOH-H_2O (2:1) as eluent.

The saponins were eluted in the first fractions to give 2.8 g (*P. ochraceus*), 1.4 g (*P. brevispinus*) of material. The further fractions contained glycoside sulfates (*P. ochraceus* 0.6 g, *P. brevispinus* 0.9 g).

The crude asterosaponin fraction from *P. ochraceus* was further purified by dccc with *n*-BuOH–Me_2CO–H_2O (45:15:75) [descending mode, the upper phase was the stationary phase, flow 24 ml/h]; 6-ml fractions were collected and monitored by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5) to give two main fractions.

Fractions 90–112 (32 mg) contained mainly versicoside A [**1**] and smaller amounts of thornasteroside A [**2**]; fractions 113–150 (22 mg) contained major amounts of **2** together with **1**.

All fractions were finally separated by hplc on C-18 μ-Bondapak column (30 cm × 7.8 mm i.d.) using MeOH-H_2O (9:11). The saponins were dissolved in H_2O (ca. 0.5 ml/100 mg) and the solution added to an equal volume of saturated NaCl solution. This solution was applied to the column (ca. 20 mg saponin mixture for each injection). The total yields of saponins were: versicoside A [**1**] 20 mg, thornasteroside A [**2**] 8 mg.

The crude saponin fraction from *P. brevispinus* was purified by dccc with *n*-BuOH–Me_2CO–H_2O (45:15:75) (descending mode) to give three main fractions. Fractions 99–128 (74 mg) contained mainly versicoside A [**1**] and smaller amounts of marthasteroside A_1 [**3**]; fractions 129–151 (90 mg) contained marthasteroside A_1 [**3**] as major component in admixture with **1** and **2**; fractions 152–194 (150 mg) contained thornasteroside A [**2**] as major component in admixture with **3**.

The total yields of saponins purified by hplc (see *P. ochraceus*) were: versicoside A [**1**] 53 mg, thornasteroside A [**2**] 41 mg, marthasteroside A_1 [**3**] 20 mg. Versicoside A [**1**] was identified by comparison of its fabms and ^{13}C-nmr spectra with those published (1); thornasteroside A [**2**] and marthasteroside A_1 [**3**] were identified by fabms, ^1H-nmr spectra, and authentic sample comparison by hplc and SiO_2 tlc.

The crude glycoside sulfate fraction from *P. ochraceus* was purified by dccc with *n*-BuOH–Me_2CO–H_2O (45:15:75) (ascending mode); the lower phase was used as stationary phase; flow 24 ml/h; 6-ml fractions were collected and checked by tlc on silica with *n*-BuOH–HOAc–H_2O (12:3:5) to give two main fractions. Fractions 9–12 contained compound **5**; fractions 13–18 contained compound **4**.

All fractions were finally separated by hplc on C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) using MeOH-H_2O (1:1). The glycoside sulfates were dissolved in MeOH (ca. 0.5 ml/100 mg) and the solution added to an equal volume of saturated NaCl solution. This solution was applied to the column (ca. 20 mg glycoside sulfate mixture for each injection).

The total yields of glycoside sulfates were: **4**, 3 mg; **5**, 11 mg.

The crude glycoside sulfate fraction from *P. brevispinus* was purified by dccc with *n*-BuOH–Me_2CO–H_2O (45:15:75) (ascending mode) to give two main fractions. Fractions 77–80 (23 mg) contained mainly **4** in admixture with **6**; fractions 80–109 (200 mg) contained major amounts of **4** in admixture with **6**.

The total yields of glycoside sulfates, purified by hplc (see *P. ochraceus*) were: **4**, 12 mg; **6**, 5 mg.

Compound **4**.—$[\alpha]^{25}D$ +4 (*c* = 0.8, MeOH); negative ion fabms *m/z* $[M-Na]^-$ 721 (100%); ^1H nmr δ (aglycone) 0.88 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.93 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 1.02 (3H, s, H_3-19), 1.07 (3H, d, *J* = 6.5 Hz, H_3-21), 1.31 (3H, s, H_3-18), 1.64 (1H, m, H-25), 2.18 (1H, m, H-24), 2.43 (1H, dd, *J* = 5, 12 Hz, H-7), 2.64 (1H, m, H-20), 3.50 (1H, m, H-3α), 3.54 (1H, t, *J* = 10 Hz, H-28), 3.73 (1H, dd, *J* = 5, 15 Hz, H-6β), 4.00 (1H, dd, *J* = 5, 10 Hz, H-28), 4.25 (1H, t, *J* = 6.5 Hz, H-16α), 4.43 (1H, dd, *J* = 5.6, 6.7 Hz, H-15α), 5.33 (1H, dd, *J* = 9, 15 Hz, H-23), 5.49 (1H, dd, *J* = 9, 15 Hz, H-22); (sugar) 3.20 (1H, dd, *J* = 7, 9 Hz, H-2′), 3.35 (1H, under solvent signal H-3′), 3.48 (2H, m, H-4′ and H-5′), 4.18 (1H, dd, *J* = 2.5, 12 Hz, H-6′), 4.31 (1H, d, *J* = 7 Hz, H-1′), 4.35 (1H, dd, *J* = 5, 12 Hz, H-6′).

Compound **5**.—$[\alpha]^{25}D$ +6 (*c* = 1, MeOH); negative ion fabms, *m/z* $[M-Na]^-$ 663 (100%), $[(M-Na)-132]^-$ 531 (10%); ^1H nmr δ (aglycone) 0.95 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.96 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 0.97 (3H, s, H_3-18), 0.98 (3H, d, *J* = 6.5 Hz, H_3-21), 1.20 (3H, s, H_3-19), 2.43 (1H, dd, *J* = 5, 12 Hz, H-7), 3.73 (1H, m, H-3α), 3.90 (1H, broad s, H-6α), 4.14 (1H, q, *J* = 6.5 Hz, H-24), 4.30 (1H, td, *J* = 10, 3 Hz, H-15β); (sugar) 3.17 (1H, dd, *J* = 7, 9 Hz, H-2′), 3.20 (1H, dd, *J* = 9, 11 Hz, H-5′ax), 3.35 (1H, under solvent signal H-3′), 3.50 (1H, m, H-4′), 3.85 (1H, dd, *J* = 5, 11 Hz, H-5′eq), 4.39 (1H, d, *J* = 7 Hz, H-1′).

Compound **6**.—$[\alpha]^{25}D$ +12 (*c* = 1, MeOH); negative ion fabms *m/z* $[M-Na]^-$ 705 (100%); ^1H nmr δ (aglycone) 1.00 (3H, d, *J* = 6.5 Hz, H_3-21), 1.03 (3H, s, H_3-19), 1.04 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 1.07 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 1.29 (3H, s, H_3-18), 2.43 (1H, dd, *J* = 5, 12 Hz, H-7), 2.88 (1H, m, H-25), 3.53 (1H, m, H-3α), 3.75 (1H, td, *J* = 3, 12 Hz, H-6β), 4.24 (1H, t, *J* = 7 Hz, H-16α), 4.32 (2H, d, *J* = 6.5 Hz, H_2-29), 4.41 (1H, dd, *J* = 5, 7 Hz, H-15α), 5.37 (1H, t, *J* = 7.5 Hz, H-28); (sugar) 3.28 (1H, dd, *J* = 7, 9 Hz, H-2′), 3.30 (1H, under solvent signal, H-5′ax), 3.51 (1H, t, *J* = 9 Hz, H-3′), 4.15 (1H, dd, *J* = 11, 5 Hz, H-5′eq), 4.19 (1H, ddd, *J* = 5, 9, 10 Hz, H-4′), 4.29 (1H, d, *J* = 7 Hz, H-1′).

SOLVOLYSIS OF SULFATED COMPOUNDS **4–6**.—A solution of each compound (from 2 to 4 mg) in dioxane (0.25 ml) and pyridine (0.25 ml) was heated at 130° for 2 h in a stoppered reaction vial. After the solution had cooled, H_2O (1 ml) was added, and the solution was extracted with *n*-BuOH (3 × 0.5 ml). The combined extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residues were submitted to fabms and 250 MHz ^1H-nmr (CD_3OD) measurements, without purification.

Spectral data for desulfated compounds are given below.

Compound **7**.—Negative ion fabms, *m/z* $[M-H]^-$ 641 (100%), $[M-H-162]^-$ 479 (40%); ^1H nmr δ (aglycone) 0.89 (3H, d, $J=6.5$ Hz, H_3-26 or H_3-27), 0.94 (3H, d, $J=6.5$ Hz, H_3-27 or H_3-26), 1.03 (3H, s, H_3-19), 1.07 (3H, d, $J=6.5$ Hz, H_3-21), 1.31 (3H, s, H_3-18), 2.23 (1H, m, H-24), 2.43 (1H, dd, $J=5$, 12 Hz, H-7), 2.66 (1H, m, H-20), 3.50 (1H, m, H-3α), 3.52 (1H, t, $J=9$ Hz, H-28), 3.73 (1H, dd, $J=5$, 15 Hz, H-6β), 4.05 (1H, dd, $J=5$, 10 Hz, H-28), 4.25 (1H, t, $J=6.5$ Hz, H-16α), 4.43 (1H, dd, $J=5.6$, 6.7 Hz, H-15α), 5.37 (1H, dd, $J=9$, 15 Hz, H-23), 5.48 (1H, dd, $J=9$, 15 Hz, H-22), (sugar) 3.20 (1H, dd, $J=7$, 9 Hz, H-2′), 3.35 (1H, under solvent signal, H-3′), 3.48 (1H, t, $J=9$ Hz, H-4′), 3.50 (1H, ddd, $J=2.5$, 5, 9 Hz, H-5′), 3.70 (1H, dd, $J=2.5$, 12 Hz, H-6′), 3.90 (1H, dd, $J=5$, 12 Hz, H-6′), 4.31 (1H, d, $J=7$ Hz, H-1′).

Compound **9**.—Negative ion fabms *m/z* $[M-H]^-$ 583 (100%), $[M-H-132]^-$ 451 (50%); ^1H nmr δ 0.92 (3H, d, $J=6.5$ Hz, H_3-26 or H_3-27), 0.94 (3H, d, $J=6.5$ Hz, H_3-27 or H_3-26), 0.98 (3H, d, $J=6.5$ Hz, H_3-21), 0.99 (3H, s, H_3-18), 1.20 (3H, s, H_3-19), 2.43 (1H, dd, $J=5$, 12 Hz, H-7), 2.44 (1H, m, H-16), 3.73 (1H, m, H-3α), 3.90 (1H, broad s, H-6α), 4.30 (1H, td, $J=10$, 3 Hz, H-15β), the other signals for the sugar indistinguishable from those in the spectrum of **5**.

Compound **10**.—Negative ion fabms *m/z* $[M-H]^-$ 625 (100%), $[M-H-132]^-$ 493 (30%), $[M-H-132-H_2O]^-$ 475 (70%); ^1H nmr δ 3.17 (1H, dd, $J=7$, 9 Hz, H-2′), 3.20 (1H, dd, $J=9$, 11 Hz, H-5′ax), 3.35 (1H, under solvent signal, H-3′), 3.50 (1H, m, H-4′), 3.85 (1H, dd, $J=5$, 11 Hz, H-5′eq), 4.29 (1H, d, $J=7$ Hz, H-1′), the other signals for the aglycone indistinguishable from those in the spectrum of **6**.

ENZYMIC HYDROLYSIS OF **7**.—The glycoside sulfate (10 mg), after solvolysis, in citrate buffer (2.0 ml, pH 4.5) was incubated with a glycosidase mixture (10 mg) of *C. lampas* at 37°. After reaction for 24 h, the tlc analysis [SiO_2 with *n*-BuOH–HOAc–H_2O (60:15:25)] showed that the starting material had disappeared. The mixture was then extracted with *n*-BuOH and evaporated, and the residue was fractionated by hplc on a C-18 μ-Bondapak column (30 cm × 3.9 mm i.d.) using MeOH-H_2O (70:30) as eluent.

Compound **8**.—Negative ion fabms *m/z* $[M-H]^-$ 479; ^1H nmr δ 0.88 (3H, d, $J=6.5$ Hz, H_3-26 or H_3-27), 0.94 (3H, d, $J=6.5$ Hz, H_3-27 or H_3-26), 1.03 (3H, s, H_3-19), 1.09 (3H, d, $J=6.5$ Hz, H_3-21), 1.32 (3H, s, H_3-18), 1.64 (1H, m, H-25), 2.18 (1H, m, H-24), 2.43 (1H, dd, $J=5$, 12 Hz, H-7), 3.46 (1H, dd, $J=9$, 12.5 Hz, H-28), 3.50 (1H, m, H-3α), 3.67 (1H, dd, $J=5$, 10 Hz, H-28), 3.73 (1H, td, $J=3$, 12 Hz, H-6β), 4.16 (1H, t, $J=6.5$ Hz, H-16α), 4.39 (1H, dd, $J=5.6$, 6.7 Hz, H-15α), 5.29 (1H, dd, $J=9$, 15 Hz, H-23), 5.49 (1H, dd, $J=9$, 15 Hz, H-22).

Compound **8** (1 mg) was treated with freshly distilled (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (3 μl) in 0.1 ml dry pyridine at room temperature for 3 h. After solvent removal, the product was eluted through a Pasteur pipet filled with a slurry of Si gel in $CHCl_3$ to give (+)-MPTA triester: negative ion fabms *m/z* $[M-H]^-$ 1127; ^1H nmr δ 0.88 (3H, d, $J=6.5$ Hz, H_3-26 or H_3-27), 0.92 (3H, d, $J=6.5$ Hz, H_3-27 or H_3-26), 1.06 (3H, d, $J=6.5$ Hz, H_3-21), 1.12 (3H, s, H_3-19), 1.31 (3H, s, H_3-18), 2.64 (1H, m, H-20), 4.37 (2H, dd, $J=6.5$ Hz, H_2-28), 5.37 (1H, dd, $J=9$, 15 Hz, H-23), 5.79 (1H, dd, $J=9$, 15 Hz, H-22).

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