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STARFISH SAPONINS, PART 49.[1] NEW CYTOTOXIC STEROIDAL GLYCOSIDES FROM THE STARFISH *FROMIA MONILIS*

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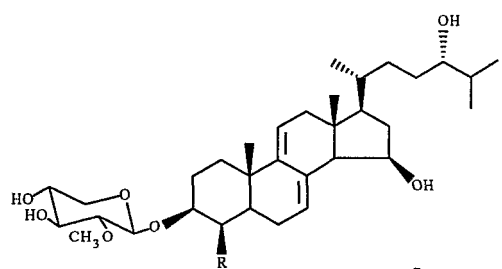
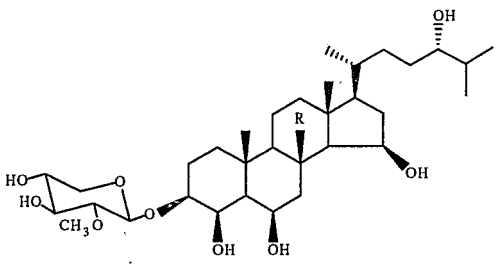
ABSTRACT.—Nine new cytotoxic steroidal glycosides **1–9** have been isolated from the starfish *Fromia monilis* collected off New Caledonia. Structures of these compounds, which include four mono- (**1–4**), two di- (**5**, **6**), and three tri-glycosides (**7–9**), were elucidated through spectral interpretation. Monilosides G [**7**], H [**8**], and I [**9**] are the first tri-glycosides to be found among the group of glycosides of polyhydroxilated steroids from starfishes.

Starfishes contain steroidal glycosides which are responsible of their general toxicity. According to their chemical structures the steroidal glycosides were subdivided into three main groups: the asterosaponins, which are sulfated glycosides (usually penta- and hexa-glycosides) based on the $\Delta^{9(11)}$-3β, 6α-dioxysteroidal aglycone with a sulfate at C-3 and the oligosaccharide moiety at C-6; the cyclic steroidal glycosides, only found in two species of the genus *Echinaster*; and the glycosides of polyhydroxylated steroids, which consist of a polyhydroxylated steroidal aglycone linked to one or two sugar units and can be found in both sulfated and non-sulfated form (1,2). Recently a new class of saponins, in which the polyhydroxylated steroids present a phosphate conjugation to which the sugars are glycosidically attached, has been isolated from *Tremaster novaecaledoniae* (3). As a part of our continuing study of the biologically active compounds from echinoderms we have analyzed the Me_2CO extracts of the starfish *Fromia monilis* Perrier (family Ophidiasteridae, order Valvatida), which showed marked anti-Herpes activity at a dose of 3 μg/ml as well as strong cytotoxicity on KB cells, 100% at 10 μg/ml. Bioassay-guided fractionation of the extract yielded nine cytotoxic steroidal glycosides **1–9**, designated as monilosides A–I. Three of them, monilosides G



1 R=H
2 R=OH

[1]For part 48, see M. Iorizzi, L. Minale, R. Riccio, and T. Yasumoto, *J. Nat. Prod.*, **55**, 866 (1992).



3 R=H
4 R=OH

[7], H [8], and I [9], are the first tri-glycosides of polyhydroxylated steroids to be isolated from the starfishes. This paper deals with the isolation and structure elucidation of these substances.

RESULTS AND DISCUSSION

The Me_2CO extract of the fresh animals was partitioned between MeOH and hexane. The MeOH layer, which retained the major part of the biological activity, was chromatographed on Sephadex LH-20 with MeOH. The toxic fractions (26–35; 10 ml each) were then subjected to dccc with $CHCl_3$-MeOH-H_2O (7:13:8) followed by chromatography on Lichroprep Si-60 with $CHCl_3$ and $CHCl_3$/MeOH and hplc on μ-Bondapak C_{18} with 80% aqueous MeOH and 72% aqueous MeOH to obtain monilosides A–I (**1–9**). The first fractions eluted from the Sephadex LH-20 column (fractions 13–25) retained most of the antiviral activity and are still under investigation. The results of our analyses are shown in Table 1.

Moniloside A [**1**] gave a negative ion mode fabms with a quasi molecular ion peak at *m/z* 561 $[M-H]^-$ (100%). Examination of ^1H- and ^{13}C-nmr spectra indicated the presence of the common 2-*O*-methyl-β-xylopyranosyl unit. The presence of a steroid aglycone was evident from methyl proton signals (Table 2). Uv absorptions at λ max 235, 242 (ε = 12300) and 250 nm are typical of $\Delta^{7,9(11)}$-sterols (4). This structural de-

TABLE 1. Chemical Constituents of the Starfish *Fromia monilis*.

Glycoside	Amount[a] (mg)	Rotations[b] $[\alpha]_D$	Hplc Rt (min.)	Cytotoxic activity[c] Dose in μg/5 × 10^5 cells
Moniloside A [**1**]	6.8	−30.2	7.0[d]	3
Moniloside B [**2**]	0.9	−24.2	7.8[d]	3
Moniloside C [**3**]	30.0	−33.0	9.2[d]	3
Moniloside D [**4**]	4.4	−18.4	7.8[d]	3
Moniloside E [**5**]	1.7	+0.0	19.4[e]	10
Moniloside F [**6**]	2.5	−9.1	22.2[e]	10
Moniloside G [**7**]	4.1	−17.3	18.6[e]	10
Moniloside H [**8**]	4.6	+23.5	17.0[e]	10
Moniloside I [**9**]	1.8	−3.5	44.0[f]	1

[a]From 0.5 kg fresh animal collected off Noumèa, New Caledonia in January 1986.
[b]In MeOH (*c* ranging from 1 to 0.1).
[c]Cytotoxic activity was valued with the neutral red test uptake on Vero cells (19).
[d]On a Waters C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) in 80% aqueous MeOH.
[e]In 72% aqueous MeOH.
[f]In 70% aqueous MeOH.

TABLE 2. Nmr Data for **1** and **2** in CD_3OD.

Position		**1**			**2**		
		δ_C	δ_H[a]	$J_{H\text{-}H}$/Hz	δ_C	δ_H[a]	$J_{H\text{-}H}$/Hz
Steroidal	1	37.4	1.25–1.47 m		37.1	1.47 m	
	2	30.7	1.60–1.97 m		24.8	1.78–2.08 m	
	3	79.6	3.64 m		81.1	3.64 m	
	4	35.6	1.35 m		71.2	3.97 brs	
			1.87 dt	12.2; 3.2			
	5	40.5	1.37 m		44.3	1.42 m	
	6	30.9	1.98 m		27.7	1.95 dt	15.5; 4.8
			1.60 m			2.67 brt	15.5
	7	123.0	5.79 d	5.4	123.4	5.87 brd	4.8
	8	133.1			132.4		
	9	145.5			146.9		
	10	37.1			37.4		
	11	119.2	5.52 d	6.1	118.1	5.45 d	6.8
	12	44.7	2.08 m		44.4	2.06	
			2.32 dd	16.6; 6.1		2.30 dd	16.9; 6.78
	13	42.6			42.4		
	14	57.6	2.06 m		57.4	2.08 m	
	15	70.5	4.52 t	5.4	70.3	4.52 m	
	16	42.3	1.45 m		42.1	1.48 m	
			2.55 m			2.54 m	
	17	57.2	1.25 m		57.0	1.25 m	
	18	14.7	0.88 s		14.7	0.87 s	
	19	19.5	1.01 s		23.0	1.19 s	
	20	36.1	1.60 m		36.3	1.66 m	
	21	19.2	1.02 d	6.7	19.2	1.01 d	6.4
	22	33.5	1.05 m		33.3	1.05 m	
			1.75 m			1.75 m	
	23	31.6	1.30 m		31.6	1.30 m	
			1.47 m			1.70 m	
	24	78.2	3.28 m		78.0	3.26 m	
	25	34.5	1.65 m		34.3	1.65 m	
	26	17.5	0.93 d	6.7	17.3	0.93 d	6.4
	27	19.4	0.95 d	6.7	18.9	0.95 d	6.8
2-OMe-Xylose	1	103.6	4.42 d	7.4	102.2	4.46 d	7.5
	2	84.9	2.84 dd	9.8; 7.4	84.5	2.92 dd	8.9; 7.5
	3	77.5	3.37 t	9.8	77.5	3.37 dd	9.5; 8.9
	4	71.4	3.50 m		71.3	3.51 m	
	5	66.8	3.18 t ax.	11.5	66.6	3.20 t ax.	11.5
			3.83 dd eq.	11.5; 5.4		3.85 dd eq.	11.5; 5.4
	OMe	60.9	3.61 s		60.7	3.65 s	

[a]Assignments made by ^1H-^1H 2D cross correlation spectroscopy (COSY) at 500 MHz, which delineated the correlation of almost all the protons.

duction was supported by the presence of two olefinic proton signals at δ 5.52 (d, $J = 6.1$ Hz, H-11) and 5.79 (d, $J = 5.4$ Hz, H-7) and confirmed by the results of COSY experiments, which established the J connectivity between H_2-6 (ca. δ 2.00) and H-7 and between H_2-12 (δ 2.32, dd, $J = 16.6$ and 6.1 Hz and 2.10, brd, $J = 16.6$ Hz) and H-11. The ^1H-nmr spectrum also showed three 1H isolated signals for hydroxymethine protons of the steroid at δ 3.62 with the complexity normally observed for a 3β-hydroxy group, at δ 4.52 in the form of an apparent triplet with $J = 5.4$ Hz characteristic of a 15β-hydroxy group (5), and at δ 3.28 as a multiplet, already seen in the spectra of the

many 24-hydroxysteroids isolated from starfishes (6). Analysis of a COSY experiment (Table 2), which allowed the determination of the proton connectivity from C-1 to C-5, from C-14 to C-16, and from C-24 to C-26 and C-27, definitively confirmed the hydroxylic substitution pattern in the $\Delta^{7,9(11)}$ steroidal structure. The chemical shift of the anomeric proton (δ 4.42) was strongly indicative for the location of the xylose unit at C-3 of the aglycone (7); in steroidal 24-*O*-xylopyranosides the signal is found regularly at δ 4.25 (8). The ^{13}C-nmr spectrum (Table 2) confirmed the location of the xylose at C-3 of the aglycone and also indicated the *S* configuration at C-24. In our sample the shift values of the side-chain carbons are identical with those reported for (24*S*)-24-hydroxysteroids (6,9). Thus, the structure of moniloside A can be defined as (24*S*)-3-*O*-(2-*O*-methyl-β-xylopyranosyl)-5α-cholesta-7,9(11)-diene-3β,15β,24-triol [**1**].

Moniloside B [**2**] is related to the previous moniloside A [**1**] by introduction of an additional hydroxyl group at C-4β of the steroid. The negative ion mode fabms gave a quasi molecular ion peak at *m/z* 577 $[M-H]^-$ (100%) and a fragment at *m/z* 431 (40%) corresponding to the loss of the 2-*O*-methylxylosyl unit. The location of the "extra" hydroxyl group at C-4β was established by a COSY experiment, which indicated that the new signal at δ 3.97 (1H, brs) gives a cross peak with the H-3 signal at δ 3.64. Continuing this *J*-connectivity path, it was possible to locate H-5 at δ 1.42 m and H_2-6 at δ 1.95 (dt, $J=15.5$ and 4.8 Hz) and 2.67 (brt, $J=15.5$ Hz), which in turn are coupled with H-7 at δ 5.87 (brd, $J=4.8$ Hz).

Moniloside C [**3**] is the major steroidal glycoside isolated from *F. monilis*. The negative ion mode fabms gave a quasi molecular ion peak at *m/z* 597 $[M-H]^-$ (100%) and a strong fragment (70%) at *m/z* 451 corresponding to the loss of a methoxylated pentose unit, which was recognized as the common 2-*O*-methyl-β-xylopyranosyl unit by ^{1}H- and ^{13}C-nmr. The sugar residue accounts for $C_6H_{11}O_4$ out of the $C_{33}H_{58}O_9$ molecular formula, as deduced from DEPT ^{13}C-nmr and fabms data, leaving $C_{27}H_{47}O_5$ for the aglycone. A saturated steroid with five hydroxy groups (all secondary from DEPT measurements) was thus a plausible candidate for a structural assignment. An analysis of the 500 MHz ^{1}H-nmr spectrum of **3** (Table 3) and comparison with the many polyhydroxysteroids and steroidal glycosides isolated in our laboratory allowed the 5α-choles-

TABLE 3. Selected 500 MHz ^{1}H-nmr Signals (CD_3OD) of the Steroidal Protons of the Glycosides **3**, **4**, **5**, and **6**. Multiplicities and *J* (Hz) are shown in Parentheses.

Proton	Compound			
	3	**4**	**5**[a]	**6**[b]
H-3	3.69 m	3.69 m	3.45 m	3.45 m
H-4	4.29 brs	4.31 brs	4.29 brs	4.29 brs
H-6	4.19 brs	4.31 brs	4.21 (ddd, 10.5, 10.5, 4)	
H-7		2.43 (dd, 12.5, 5)	2.50 (dd, 12.3, 5.1)	2.50 (dd, 12.3, 5.1)
H-15	4.19 brs	4.44 (dd, 6.7, 5.6)	4.41 (dd, 6.5, 5.6)	4.41 (dd, 6.5, 5.6)
H-16	2.42 m	2.34 m	4.25 (t, 6.5)	4.25 (t, 6.5)
H-18	1.01 s	1.31 s	1.27 s	1.31 s
H-19	1.40 s	1.48 s	1.19 s	1.20 s
H-21	0.97 (d, 6.5)	0.98 (d, 6.5)	0.98 (d, 6.5)	1.07 (d, 6.5)
H-24		3.23 m		
H-26	0.93 (d, 6.5)	0.93 (d, 6.5)	0.88 (d, 6.5)	0.88 (d, 6.5)
H-27	0.95 (d, 6.5)	0.94 (d, 6.5)	0.91 (d, 6.5)	0.93 (d, 6.5)

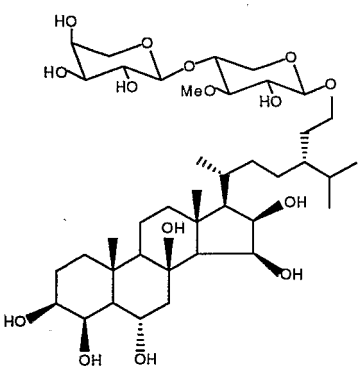
[a]Chemical shift values of the steroid protons of **7** and **9** virtually identical with those of **5**.

[b]Chemical shift values of the steroid protons of **8** virtually identical with those of **6**. Olefinic H's at δ 5.35 dd (15.7 Hz)–5.48 dd (15.7 Hz) ppm.

tane-3β,4β,6β,15β,24-pentaol structure to be established. This structure is similar to that of the steroidal aglycone of laeviuscoloside F but differs from it in the stereochemistry at C-15, which in **3** is 15β-OH. The major differences observed in their ^{1}H-nmr spectra deal with H-15 and H_3-18 resonances, which in laeviuscoloside F are shifted upfield to δ 3.92 (dt, J = 10.3 Hz) and 0.77 (s), respectively (10). The ^{13}C-nmr spectrum (Table 4) confirmed C-3 to be the site of glycosidation and supported the configuration at C-24 to be *S* (6). Differences in the ^{13}C-nmr spectra of (24*R*)- and (24*S*)-hydroxycholesterol are quite small (9), and we prefer, when possible, to apply Mosher's method (11) for the determination of the absolute configuration of 24-hydroxysteroids. Thus, **3** was converted into the diastereomeric (+) and (−) MTPA esters, which showed the signals of the isopropyl methyl protons significantly upfield (δ 0.86 and 0.88) in the nmr spectrum of (+) MTPA ester and downfield (0.91 and 0.93) in that of the (−) MTPA, in agreement with the 24*S* configuration (8). [The term (+) or (−) MTPA esters refers to an ester prepared using acid chloride derived from *R*-(+)- or *S*-(−)-α-methoxy-α(trifluoromethyl)-phenylacetic acid, respectively.] Thus, moniloside C can be defined as (24*S*)-3-*O*-(2-*O*-methyl-β-xylopyranosyl)-5α-cholestane-3β,4β,6β,15β,24-pentaol [**3**].

Moniloside D [**4**], fabms (negative ion mode), *m/z* 613 $[M-H]^-$, is the 8-hydroxy derivative of the previous moniloside C [**3**], as determined by ^{1}H- and ^{13}C-nmr spectra (Tables 3 and 4) and comparison between the two compounds. The introduction of an 8-hydroxy group into the 3β,4β,6β,15β-tetrahydroxycholestane skeleton causes a downfield shift of H-15, H_3-18, and H_3-19 in the ^{1}H-nmr spectrum. In the ^{13}C-nmr it gives rise to a decreased shielding γ-gauche effect at C-11 (−2.1 ppm) relative to that expected (12) and to equally unexpected deshielding effects at the γ-carbons C-6 (+1.8 ppm) and C-15 (+0.7 ppm). The large deviations from additivity must be associated with skeletal deformations taking place to relieve the severe steric hindrance caused by the 1,3 syn-diaxial interactions suffered by the 8β-hydroxyl with C-18 and C-19 methyl groups and with 6β- and 15β-hydroxyl groups (13).

Examination of the spectral data (^{1}H and ^{13}C nmr, Tables 3 and 4) of moniloside E [**5**] indicated that **5** contained a (24*R*)-24-ethyl-5α-cholestane-3β,4β,6α,8,15β,16β,29-heptaol aglycone, already found as the aglycone of halityloside A, the major glycoside from *Halityle regularis* (14), and in many other starfish glycosides. A detailed ^{1}H-nmr analysis using COSY and 2D homonuclear Hartmann-Hahn (HOHAHA) (15) experiments also identified the presence of two monosaccharide residues as 3-*O*-methyl-β-xylopyranosyl and α-arabinopyranosyl units, and the complete assignments were



5
6 Δ^{22E}

TABLE 4. ^{13}C-nmr Shifts (62.9 MHz, CD_3OD) of the Steroid Carbons in **3**, **4**, **5**, and **7**.

Carbon	Compound			
	3	**4**	**5**	**7**
C-1	39.4	40.9	39.7	39.7
C-2	25.4	25.2	26.2	26.2
C-3	80.8	80.6	73.7	73.7
C-4	74.8	74.7	69.1	69.2
C-5	50.6	50.8	57.4	57.4
C-6	74.5	76.3	64.8	64.8
C-7	41.7	44.1	u.m.	u.m.
C-8	28.0	79.1	77.2	77.2
C-9	56.5	57.6	58.5	58.6
C-10	37.1	37.1	38.2	38.2
C-11	21.5	19.4	18.9	19.0
C-12	39.4	43.2	43.6	43.6
C-13	43.4	44.4	44.6	44.6
C-14	62.3	61.9	61.4	61.4
C-15	70.7	71.4	71.3	71.3
C-16	42.6	41.9	72.9	72.9
C-17	57.6	58.1	63.0	63.1
C-18	15.7	16.5	17.9	17.9
C-19	18.2	18.5	16.9	16.9
C-20	37.0	36.4	31.5	31.5
C-21	19.5	19.1	18.5	18.5
C-22	33.5	33.3	34.8	34.8
C-23	31.7	31.7	28.7	28.8
C-24	78.2	78.2	42.5	42.6
C-25	34.5	34.5	30.8	30.8
C-26	17.5	17.5	19.1	19.1
C-27	19.5	19.4	20.0	20.0
C-28			31.8	31.9
C-29			70.0	70.1

achieved (Table 5). The negative ion mode fabms showed a quasi molecular ion peak at *m/z* 789 $[M-H]^-$ and fragments at *m/z* 657 and 511, corresponding to the sequential loss of an arabinose unit (132 mass units) and of a 3-*O*-methylxylose unit (146 mass units), indicating the sequence arabinose-3-*O*-methylxylose-aglycone. ^{13}C-nmr data (Table 5) and comparison with those of 3-*O*-methyl-β-D-xylopyranosides (16) established the 1↦4 interglycosidic linkage. ^{13}C-nmr data (Table 4) also clarified that the disaccharide moiety is attached at C-29 of the steroidal part of **5**. Thus moniloside E can be defined as (24*R*)-29-*O*-[α-arabinopyranosyl-(1↦4)-3-*O*-methyl-β-xylopyranosyl]-24-ethyl-5α-cholestane-3β,4β,6α,8,15β,16β,29-heptaol [**5**]. Arabinose is a common sugar in polyhydroxysteroid glycosides from starfishes but has not been found previously in the pyranose form in this group of compounds.

Moniloside F [**6**] is the Δ^{22E} analogue of moniloside E [**5**]. The structure was derived by fabms, *m/z* 787 $[M-H]^-$, 655 $[M-H-132]^-$, and 509 $[M-H-132-146]^-$, ^{1}H nmr (Tables 3 and 5), and comparison with **5**.

Examination of ^{1}H and ^{13}C nmr spectra of moniloside G [**7**] immediately showed that moniloside G had the same steroid aglycone as the previous moniloside E [**5**]. The presence in the ^{1}H-nmr spectrum of three doublets ($J=7.5$ Hz) at δ 4.21, 4.35, and 4.39, assigned to anomeric protons, indicated the existence in this compound of three

TABLE 5. Assignments of Nmr Signals (CD_3OD) of the Disaccharide Moiety of **5**[a] and **6**.

Position	α-L-arabinopyranosyl		3-*O*-methyl-β-D-xylopyranosyl		
	^1H (mult., *J*, Hz)	^{13}C	^1H (mult., *J*, Hz)	^{13}C	Lit.[b]
1	4.32 (d, 7.5)	104.9	4.28 (d, 7.5)	103.3	104.9
2	3.54–3.60[c]	72.4	3.33[b]	74.3	75.0
3	3.54–3.60[c]	74.2	3.30[b]	85.2	87.6
4	3.84 m	69.5	3.78 m	76.3	71.0
5	3.93 (dd, 12.5; 3)	66.8	4.05 (dd, 11.5; 5)	64.0	66.5
	3.55 (dd)[c]		3.22 (t, 11.5)[c]		
OCH_3			3.64 s	60.5	60.8

[a]^1H assignments based on ^1H-^1H 2D correlation spectroscopy (COSY and HOHAHA). ^{13}C assignments based on δ chemical shift arguments and comparison with references.

[b]Values in this column are from Iorizzi *et al.* (16).

[c]Overlapping signals.

monosaccharide residues, as supported by the signals at 105.0, 103.8, and 103.6 ppm for the anomeric carbons in the ^{13}C-nmr spectrum. On acid methanolysis, moniloside G [7] liberated methyl xylosides (glc) and a second group of methyl glycosides with glc retention times corresponding with those of methyl-3-*O*-methylxylosides, as obtained by acid methanolysis of borealoside C, a steroidal 3-*O*-methyl-β-*O*-xylopyranoside from *Solaster borealis* (16). The negative ion mode fabms gave a quasi molecular ion at *m/z* 935 $[M-H]^-$ in agreement with a trisaccharide chain made up from one xylosyl and two 3-*O*-methyl xylosyl units. The fabms fragments at *m/z* 789 (loss of 146 mass units from $[M-H]^-$), 643 (loss of 146 mass units from *m/z* 789) and 511 (loss of 132 mass units from *m/z* 643), corresponding to the sequential loss of two *O*-methylxylosyl and one xylosyl units, indicated the sequence. An accurate analysis of the ^{13}C-nmr spectrum (Table 6) and comparison with reference sugars (16,18), established the (1↦4) nature of the interglycosidic linkages. The ^{13}C-nmr spectrum of **7** (Table 4) also clarified the attachment of the saccharide chain at C-29 of the steroid. Permethylation, followed by acid methanolysis and *p*-bromobenzoylation of the hydrolysis mixture, afforded methyl 4-*O*-(*p*-bromobenzoyl)-2,3-di-*O*-methyl-α-D-xylopyranoside as identified by ^1H nmr, confirming the 1↦4 glycosidic linkages. The region of the ^1H-nmr spectrum for the resonances of the sugar portions and the hydroxymethine and -methylene protons of the steroids was rather crowded and some signals overlapped. Only selected chemical shifts of the sugar protons could be determined: i.e., doublets with $J=7.5$ Hz at δ 4.21, 4.35, and 4.39 for the anomeric H's; double doublets ($J=5$, 11.0 Hz) at δ 3.88 for H-

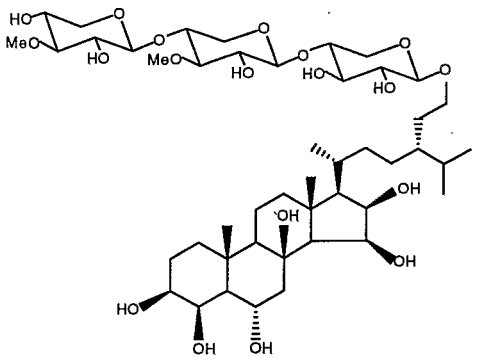
TABLE 6. Assignments of ^{13}C Nmr (62.9 MHz, CD_3OD) of the Trisaccharide Moiety of **7**[a].

Sugar carbon	Lit.[b]	Xyl.	Internal 3-OMe-xyl	T-3-OMe-xyl	Lit.[c]
1	104.9	105.0	103.6	103.8	104.9
2	75.4	74.8	74.3	73.8	75.0
3	78.0	75.9	85.1	87.2	82.6
4	71.4	78.2	76.6	70.8	71.0
5	66.7	64.4	64.4	66.9	66.7
OMe			60.6	60.6	60.8

[a]Assignments based on chemical shift arguments and references comparison.

[b]Values in this column are from Gorin and Mazureck (18).

[c]Values in this column are from Iorizzi *et al.* (16).

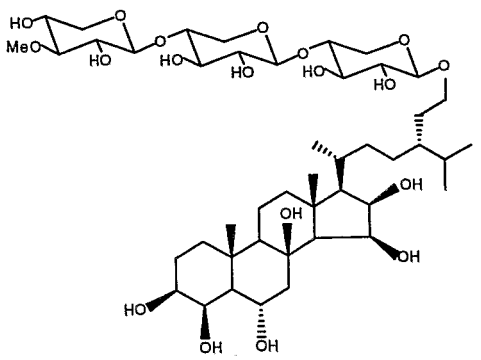


7
8 Δ^{22E}

5eq of the terminal unit and at δ 4.03 and 4.09 for the H-5eq of the 4-substituted internal units, and a one-proton triplet ($J = 9.0$ Hz) shifted upfield to δ 3.08 for H-3 of the terminal 3-*O*-methylxylose unit. Thus moniloside G could be defined as (24*R*)-29-*O*-[3-*O*-methyl-β-xylopyranosyl-(1↦4)-3-*O*-methyl-β-xylopyranosyl-(1↦4)-β-xylopyranosyl]-24-ethyl-5α-cholestane-3β,4β,6α,8,15β,16β,29-heptaol [**7**].

Moniloside H [**8**] is the Δ^{22E} analogue of moniloside G [**7**]. The structure was derived from fabms, *m/z* 933 $[M-H]^-$, 777 (loss of 3-*O*-methylxylose), 641 (loss of 3-*O*-methylxylose from *m/z* 777) and 509 (loss of xylose from *m/z* 641), ^1H nmr, and comparison with **7**.

Moniloside I [**9**] is related to moniloside G [**7**] but lacks the methyl group at the 3-OH position of the internal xylosyl residue. The negative ion fabms gave a quasi molecular ion peak at *m/z* 921 $[M-H]^-$, fourteen mass units less than **7**, and fragments at *m/z* 775 corresponding to the loss of the 3-*O*-methylxylosyl residue (146 mass units) and at *m/z* 643 and 511 corresponding to the sequential loss of two xylosyl residues (132 mass units), thus indicating the sequence with the 3-*O*-methylxylosyl residue as the terminal unit. The presence of a 3-*O*-methylxylopyranosyul unit is indicated by the one-proton signal in the 500 MHz ^1H nmr shifted upfield to δ 3.08 (t, $J = 10$ Hz), characteristic of H-3 in 3-*O*-methylxylopyranosyl unit. In addition, the 1↦4 glycosidic linkages were derived from the appearance of two clearly separated double doublets ($J = 11.5$, 5 Hz) shifted downfield to δ 4.08 and 4.01 ppm, characteristic of



9

the equatorial proton at C-5 in 4-substituted β-xylopyranose units (17); in unsubstituted β-xylopyranosyl units this signal is observed in the range 3.84 to 3.90 ppm. Thus, moniloside I can be defined as (24*R*)-29-*O*-[3-*O*-methyl-β-xylopyranosyl-(1↦4)-β-xylopyranosyl-(1↦4)-β-xylopyranosyl]-24-ethyl-5α-cholestane-3β,4β,6α,8,15β,16β,29-heptaol [**9**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Bruker AMX-500 spectrometer equipped with Bruker X-32 computer, using the UXNMR software package, was used for ^{1}H nmr; Bruker WM-250 spectrometer for ^{13}C nmr. The 2D homonuclear proton chemical shift correlation (COSY) experiment was measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set ($t_1 \times t_2$) of 1024 × 1024 points for a spectral width of 1165 Hz (relaxation delay 1 sec). 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 1.13 Hz/pt). The 2D HOHAHA experiment was performed in the phase-sensitive mode (TPPI) using an MLEV-17 sequence for mixing (15). The spectral width (t_2) was 1012 Hz; 512 experiments of 40 scans each (relaxation delay 1.5 sec, mixing time 100 msec) acquired in 2K data points. For processing, an unshifted sine bell window function was applied in both dimensions before transformation. Resulting digital resolution in F2 was 0.48 Hz/pt. For mass spectra, VG ZAB mass spectrometer equipped with fab source [in glycerolthioglycerol (3:1) matrix; Xe atoms of 2–6 Kv] was used; for optical rotations, Perkin-Elmer model 241 polarimeter; for glc, Carlo Erba Fractovap 2900 for capillary column on SE-30, 25 m, 125°, helium carrier flow 2 ml/min, for reversed-phase hplc, C_{18} μ-Bondapak column (30 cm × 8 mm i.d.; flow rate 5 ml/min), Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401; and for dccc, DCC-A apparatus manufactured by Tokio Rikakikai Co., equipped with 250 tubes.

EXTRACTION AND ISOLATION.—The animals, *F. monilis*, were collected off Noumèa, New Caledonia, and identified at the ORSTOM, Centre de Noumèa, New Caledonia. A voucher specimen is preserved there under the reference EA 13. The animals (1.6 kg) were chopped and soaked in H_2O; the aqueous extracts were decanted and the solid mass was extracted with Me_2CO. The Me_2CO extracts (13.6 g) were evaporated in vacuo, and part of the extract (3.8 g) was partitioned between hexane and MeOH. The MeOH layer (2.7 g), which retained almost all the antiviral and cytotoxic activity, was chromatographed on a Sephadex LH-20 column (4 × 100 cm) with MeOH as eluent. Fractions of 15 ml were collected. Fractions 26–35 gave 1.2 g of a solid residue, cytotoxic at a dose of 3 μg/5 × 10^4 cells, which was fractioned by dccc using $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the upper phase was the stationary phase). Fractions of 5 ml were collected and combined, after tlc on SiO_2 analysis, into four main fractions: A (21–34), B (35–48), C (77–104), and D (126–164). Fraction A (183 mg) was chromatographed on Si gel (Kieselgel 60, 70–230 mesh ASTH) with C_6H_6/EtOAc step gradient, followed by hplc (C_{18} μ-Bondapak) with 80% aqueous MeOH to give pure moniloside A [**1**] (6.8 mg). Fraction B (89 mg) was subjected to hplc (C_{18} μ-Bondapak) with 80% aqueous MeOH to give two peaks. The last one eluted contained pure moniloside C [**3**] (30 mg). The residue from the first peak eluted was chromatographed on Lichroprep Si-60 with $CHCl_3$/MeOH step gradient to give moniloside D [**4**] (4.9 mg) and moniloside B [**2**]. Moniloside B [**2**] was further purified by hplc (C_{18} μ-Bondapak) with 80% aqueous MeOH to give 0.9 mg of pure sample. Fraction C (19 mg) was subjected to hplc (C_{18} μ-Bondapak) with 72% aqueous MeOH to give moniloside F [**6**] (2.5 mg). Fraction D (64 mg) was chromatographed on Lichroprep Si-60 with $CHCl_3$/MeOH step gradient to give two main fractions. The first one eluted was subjected to hplc (C_{18} μ-Bondapak) with 72% aqueous MeOH to give moniloside H [**8**] (4.6 mg), moniloside G [**7**] (4.1 mg), and moniloside I [**9**] (1.8 mg). The second eluted fraction after further purification by hplc (C_{18} μ-Bondapak) with 72% aqueous MeOH gave moniloside E [**5**] (1.7 mg). Physicochemical data (^{1}H and ^{13}C nmr, fabms, and optical rotations) are reported in the text and in Tables 2–6. ^{1}H-nmr signals of the steroid protons of the glycosides **7** and **9** are superimposable with those of **5**, whereas those of the glycoside **8** correspond with those of **6**.

CYTOTOXIC ACTIVITY ON VERO CELLS IN VITRO.—The isolated fractions were dissolved in MeOH and added to Vero cells cultivated in RPMI 1640 (Biochrom KG, Germany) with the addition of 5% inactivated fetal calf serum (FCS), 2 mM glutamine, 1% of an antibiotic mixture containing 10,000 U penicillin and 10,000 mg/ml streptomycin. Decreasing quantities of the fractions were added to 5 × 10^5 cells in Falcon wall plates and incubated at 37° for 24 h in 5% CO_2. Controls were performed with the solvent only. After 3 washes with RPMI 1640 the vitality of the cells was determined with the neutral red test (19). In brief, cells were stained with neutral red diluted in PBS (1/10,000), and were incubated at 37° in 5% CO_2 for 2 h in darkness, after which they were washed with PBS three more times to eliminate the excess

dye. In order to extract the stain incorporated in the cells, 0.1 ml of lysing solution [EtOH/ citrate buffer pH 4.2 (54 mM citric acid and 38 mM of tri-sodium citrate)] was then added. Plates were read at 550 nm with a Bahring Elisa Processor II using a test wavelength of 570 nm and a reference wavelength of 650 nm. The tests were carried out in triplicate.

METHANOLYSIS OF MONILOSIDE G [7].—A solution of **7** (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 being 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) for 5 min at room temperature. Glc analysis gave peaks that co-eluted with those of methylxyloside standards and peaks with a shorter retention time that co-eluted with those of methyl 3-*O*-methylxylosides obtained by methanolysis and silylation of borealoside C, (24*S*)-24-*O*-(3-*O*-methyl-β-D-xylopyranosyl)-5α-cholestane-3β,6α,8,15α,24-pentaol (16).

METHYLATION OF THE MONILOSIDE G [7] FOLLOWED BY METHANOLYSIS AND *p*-BROMOBENZOYLATION.—A solution of moniloside G [7] (7.2 mg) in DMF (0.5 ml) was slowly added under N_2 to a stirred mixture of NaH (60 mg) in dry DMF (0.5 ml), cooled in an ice bath. The mixture was stirred for 15 min, and MeI (0.5 ml) was added. The reaction mixture was kept for a further 4 h at room temperature. The excess of NaH was destroyed by a few drops of MeOH, and after addition of H_2O the mixture was extracted twice with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was methanolyzed in anhydrous 2 M HCl/MeOH (0.3 ml) at 80° in a stoppered reaction vial for 8 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under reduced pressure to give a residue of 3.8 mg. A solution of this residue (3.8 mg) in dry pyridine (1.5 ml) was treated with *p*-bromobenzoyl chloride (10 mg) and a few mg of 4-dimethylaminopyridine, the mixture was stirred overnight at 60° under nitrogen, and chilled H_2O was added to the solution, which, after 30 min, was extracted with $CHCl_3$. The extract was washed successively with saturated aqueous $NaHCO_3$ and H_2O, and the solvent was evaporated off under reduced pressure. The residue (ca. 2 mg) purified by hplc [Whatman Partisil PXSM910/25; Et_2O-hexane (25:75), flow rate 3 ml/min; 260 nm detection] gave methyl-4-*O*-(*p*-bromobenzoyl)-2,3-di-*O*-methyl-α-D-xylopyranoside: δ_H ($CDCl_3$) 3.12 (1H, dd, J = 7.5, 6.5 Hz, H-2), 3.35 (1H, t, J = 11.0 Hz, H-5ax), 3.45 (1H, t, J = 9.6 Hz, H-3), 4.15 (1H, dd, J = 11.0, 6.0 Hz, H-5eq), 4.30 (1H, d, J = 6.5 Hz, H-1), 5.06 (1H, ddd, J = 10.5, 9.6, 6.0 Hz, H-4), 7.90 (2H, d, J = 7 Hz, H-Ar), 7.60 (2H, d, J = 7 Hz, H-Ar).

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