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NOVEL SULFATED OLIGOSACCHARIDES FROM THE SEA CUCUMBER *CUCUMARIA FRONDOSA*

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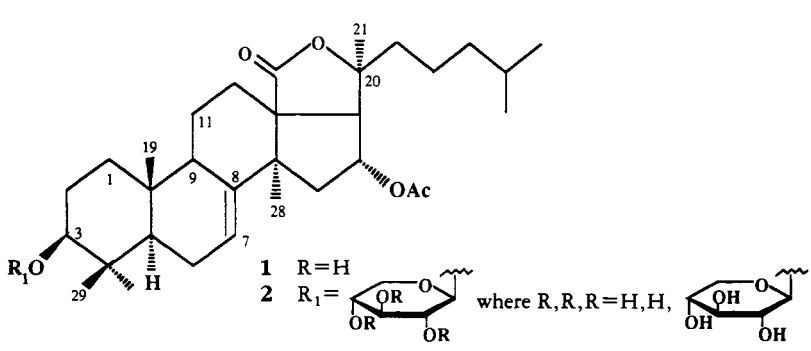
ABSTRACT.—The structures of a novel sulfated saponin, frondoside B ($C_{59}H_{92}O_{31}S_2M_2$) [**5**], and frondecaside ($C_{58}H_{92}O_{63}S_6M_6$) [**6**], a unique hexasulfated decasaccharide, have been deduced by nmr (500 MHz) methods. Based on ^{1}H COSY, relay COSY, NOESY, and ^{13}C-nmr data, frondoside B was shown to have the structure 3β-*O*- { 3-*O*-methyl-β-D-glucopyranosyl-(1↦3)-*O*-β-D-6-sulfonatoglucopyranosyl-(1↦4)-*O*-[β-D-xylopyranosyl-(1↦2)]-*O*-β-D-quinovopyranosyl-(1↦2)-*O*-β-D-4-sulfonatoxylopyranosyl } -holost-7-ene sodium (or potassium) salt. From ^{1}H-COSY, relay COSY, ^{13}C-nmr data, T_1 measurements, and comparison with nmr spectra of frondoside B, the structure of frondecaside was established as 3-*O*-methyl-6-sulfonato-β-D-glucopyranosyl-(1↦3)-*O*-β-D-6-sulfonatoglucopyranosyl-(1↦4)-*O*-[β-D-xylopyranosyl-(1↦2)]-*O*-β-D-quinovopyranosyl-(1↦2)-*O*-β-D-4-sulfonatoxylopyranosyl-(1↦1)-3-*O*-methyl-6-sulfonato-β-D-glucopyranosyl-(1↦3)-*O*-β-D-6-sulfonatoglucopyranosyl-(1↦4)-*O*-[β-D-xylopyranosyl-(1↦2)]-*O*-β-D-quinovopyranosyl-(1↦2)-*O*-β-D-4-sulfonatoxylopyranoside sodium (or potassium) salt.

Earlier we presented preliminary findings on the structure of a triterpenoid lactone aglycone **1** (1) obtained by hydrolysis of the saponin fraction of the sea cucumber *Cucumaria frondosa* Gunnerus (Cucumariidae). We also reported on the structures of two partial hydrolysis products of the saponin fraction, one of which featured a disaccharide chain comprising a quinovose and a xylose unit as in **2**; however, the location of the quinovose unit was not established and the stereochemistry of the C-16 acetoxy group was tentatively assigned the α configuration. Recently, Girard *et al.* (2) have reported the complete structure of frondoside A [**3**], a holothurin obtained from *C. frondosa*. A structurally related triterpene glycoside lefevreioside A_2 [**4**] featuring a 16α-acetoxy group and lacking the xylose III unit has been reported by Rodriguez and Riguera (3) from *Cucumaria lefevrei*.

Continuing our studies on the polar constituents of *C. frondosa*, we have isolated a novel triterpenoid pentaglycoside, frondoside B, which differs from **3** by the absence of



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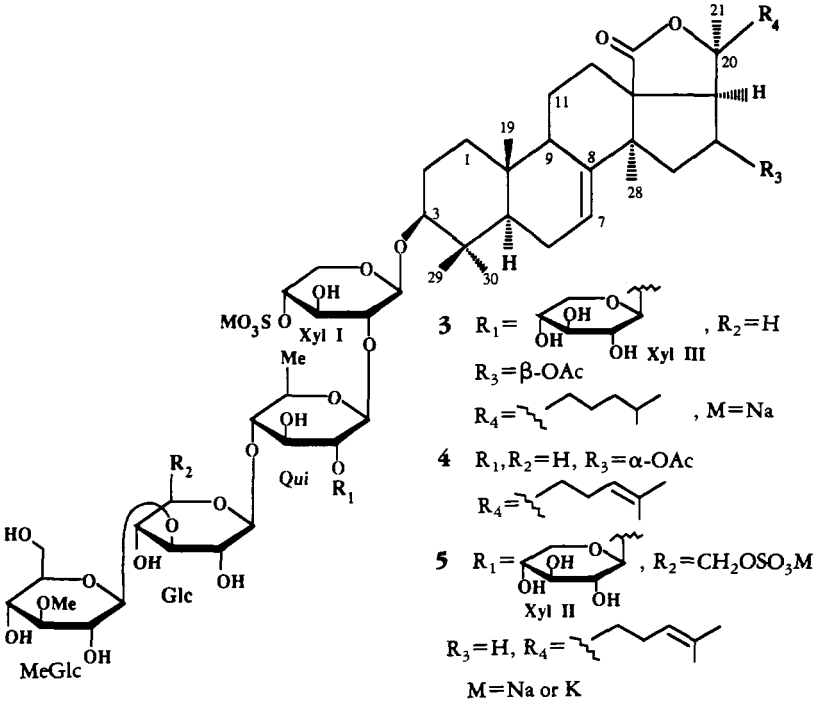
a C-16 substituent and features a Δ^{24} double bond and a glucose-6-sulfate moiety in place of the xylose II unit. We have also encountered a novel oligosaccharide, frondecaside [**6**] whose structure is closely related to the frondoside B pentasaccharide chain. We formulate frondoside B as **5** on the basis of analysis of 1D and 2D high field nmr data.

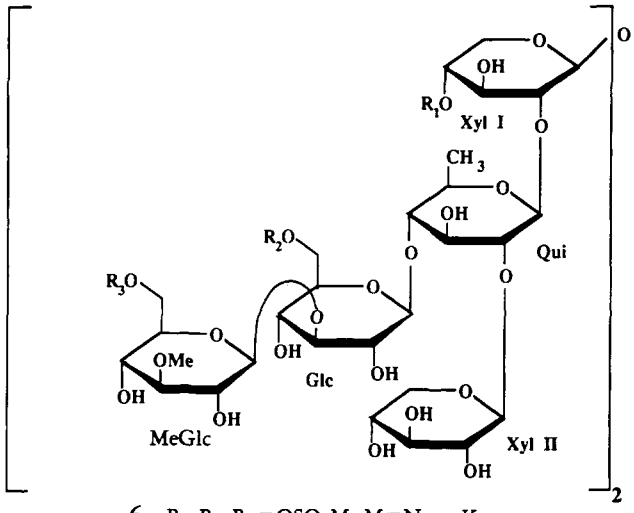


Recently, we demonstrated the power of the systematic application of 1D and 2D high field nmr to deduce the complete structure and stereochemistry of complex astreosaponins without resorting to derivatization/degradation (4–6). This method has subsequently been applied by Girard *et al.* (2) in deducing the structure of frondoside A, and we now provide an account of the structure elucidation of the saponin frondoside B [**5**] and the oligosaccharide frondecaside [**6**] using this approach.

Standard 1D and 2 D nmr procedures were employed to elucidate the structure of frondoside B. Conventional ^1H (500 MHz) and ^{13}C (125 MHz) spectra combined with multiplicity-selected [APT (7)] ^{13}C data have yielded the gross structure of the molecule and shown it to consist of a triterpenoid aglycone ($C_{30}H_{45}O_3$) and a pentasaccharide (C_{29}) sugar moiety. COSY, relayed COSY [RECSY, with one and two relay steps (8)], and NOESY (9) maps have afforded a comprehensive description of through-bond and through-space proton-proton connectivities which were then interpreted in terms of constitution and relative stereochemistry of the molecule. Corroborative evidence for the molecular structure thus derived was gleaned from ^{13}C-^1H chemical shift correlation [C,H-COSY (10)] experiments.

The assigned ^1H and ^{13}C resonances for the aglycone moiety of **5** are shown in Table 1, and nmr data for the sugar moieties of **5** and **6** are in Table 2. The relevant dipolar contacts identified in the NOESY maps are summarized in Table 3. A combined NOESY/RECSY map is shown in Figure 1.

Comparison of the spectral data in Table 1 with those published for related saponin aglycones (2,3) shows that the aglycone part of frondoside B is of the lanosterol type



6 $R_1,R_2,R_3=OSO_3M$, M=Na or K
7 $R_1,R_2,R_3=H$

TABLE 1. Nmr Data for the Aglycone Moiety of Frondoside B [**5**].

Carbon	δ_C	Proton	δ_H
C-1	36.04	H-1α, -1β	1.42 m, 1.42 m
C-2	26.86	H-2α, -2β	2.04 m, 1.87 m
C-3	88.94	H-3α	3.27 dd, J = 4 and J = 11.6
C-4	39.46		
C-5	47.91	H-5α	1.07 m
C-6	23.22	H-6α, -6β	2.08 m, 2.08 m
C-7	119.79	H-7	5.742 m
C-8	146.65		
C-9	47.24	H-9β	3.50 bd, J = 14
C-10	35.41		
C-11	22.77	H-11α, -11β	1.82 m, 1.60 m
C-12	30.22	H-12α, -12β	2.02 m, 2.02 m
C-13	58.59		
C-14	51.22		
C-15	24.41	H-15α, -15β	1.96 m, 1.81 m
C-16	34.17	H-16α, -16β	1.84 m, 1.64 m
C-17	53.00	H-17α	2.07 dd, J = 5 and J = 9
C-18	180.24		
C-19	23.89	H-19	1.204 s, 3H
C-20	84.10		
C-21	25.92	H-21	1.472 s, 3H
C-22	39.19	H-22, -22′	1.84 m, 1.84 m
C-23	22.94	H-23, -23′	2.06 m, 2.06 m
C-24	123.34	H-24	5.14 m
C-25	131.81		
C-26	25.50	H-26	1.702 s, 3H
C-27	17.82	H-27	1.614 s, 3H
C-28	30.78	H-28	1.205 s, 3H
C-29	17.33	H-29	1.191 s, 3H
C-30	28.61	H-30	1.292 s, 3H

[a]In C_5D_5N-D_2O (8:2) at 300°K. Chemical shifts (ppm) are relative to internal TMS.

TABLE 2. Nmr Data for the Oligosaccharide Moiety of Frondoside B [**5**] and ^{13}C nmr Data for Frondecaside [**6**].

Unit		Compound			
		5[a]			**6**[b]
		δ_C	δ_H	$J_{H,H}$	δ_C
Xyl I	1	104.69	4.813	$J_{1,2}=7.9$	104.80
	2	81.71	4.036	$J_{2,3}=9.0$	81.69
	3	75.33	4.302	$J_{3,4}=0.2$	75.32
	4	75.77	5.480	$J_{4,5e}=4.2, J_{4,5e}=9.2$	75.74
	5	64.18	4.869 (5e)	$J_{5e,5a}=-11.4$	64.18
			3.964 (5a)		
Qui	1	102.16	5.280	$J_{1,2}=7.6$	102.87
	2	83.87	3.848	$J_{2,3}=9.4$	83.87
	3	74.66	3.980	$J_{3,4}=9.1$	74.66
	4	87.09	3.425	$J_{4,5}=9.3$	87.09
	5	70.56	3.648	$J_{5,6}=6.1$	70.56
	6	17.47	1.700		17.47
Glc	1	104.33	4.847	$J_{1,2}=7.2$	104.24
	2	73.37	3.940	$J_{2,3}=8.4$	73.55
	3	86.41	4.067	$J_{3,4}=9.0$	86.58
	4	69.65	3.027	$J_{4,5}=8.6$	69.95
	5	74.85	4.968	$J_{5,6}$ 1.4, $=J_{5,6'}=7.8$	75.03
	6	67.64	5.078 (6)	$J_{6,6'}=-11.2$	67.54
			4.625 (6')		
MeGlc	1	104.90	5.314	$J_{1,2}=7.9$	104.99
	2	74.71	3.898	$J_{2,3}=9.1$	74.96
	3	87.45	3.738	$J_{3,4}=8.8$	87.87
	4	77.95	4.064	$J_{4,5}=9.4$	76.59
	5	70.35	3.887	$J_{5,6}=1.6, J_{5,6'}=5.7$	70.18
	6	61.79	4.483 (6)	$J_{6,6'}=-11.3$	66.88
			4.168 (6')		
	OMe	60.65	3.882		60.65
Xyl II	1	105.93	5.041	$J_{1,2}=7.4$	105.68
	2	75.19	3.990	$J_{2,3}=8.8$	75.11
	3	76.56	4.137	$J_{3,4}=8.7$	76.03
	4	69.65	4.022	$J_{4,5e}=5.2, J_{4,5a}=7.4$	69.34
	5	64.18	4.264 (5e)	$J_{5e,5a}=-11.6$	64.18
			3.600 (5a)		

[a]In C_5D_5N-D_2O (8:2) at 300°K. Chemical shifts (ppm) are relative to internal TMS; mutual coupling constants (Hz) are given only once, at their first occurrence in the Table.

[b]In C_5D_5-D_2O (5:1) at 300°K. Chemical shifts (ppm) are relative to internal TMS.

featuring the characteristic ring-D-fused γ-lactone function. Distinctive features of the new aglycone are: (a) the lack of substituent group at C-16 evidenced, inter alia, by the double doublet ($J=3$ and 9 Hz) of resonance due to H-17 (2.070 ppm) and (b) the presence of a double bond between C-24 and C-25 attested to by the olefinic carbon resonances at 123.34 (C-24) and 131.81 ppm (C-25).

Inspection of the nOe data for the aglycone part of the molecule (Table 3) reveals that the relative stereochemistries at the common chiral centers are identical with those reported recently for frondoside A (2). In particular, we find that the axially oriented H-3 ($J=4$ and 11.5 Hz) is in spatial proximity with H-5, which requires an A/B ring junction with H-5α; the resonance due to H-9 shows strong dipolar contacts with pro-

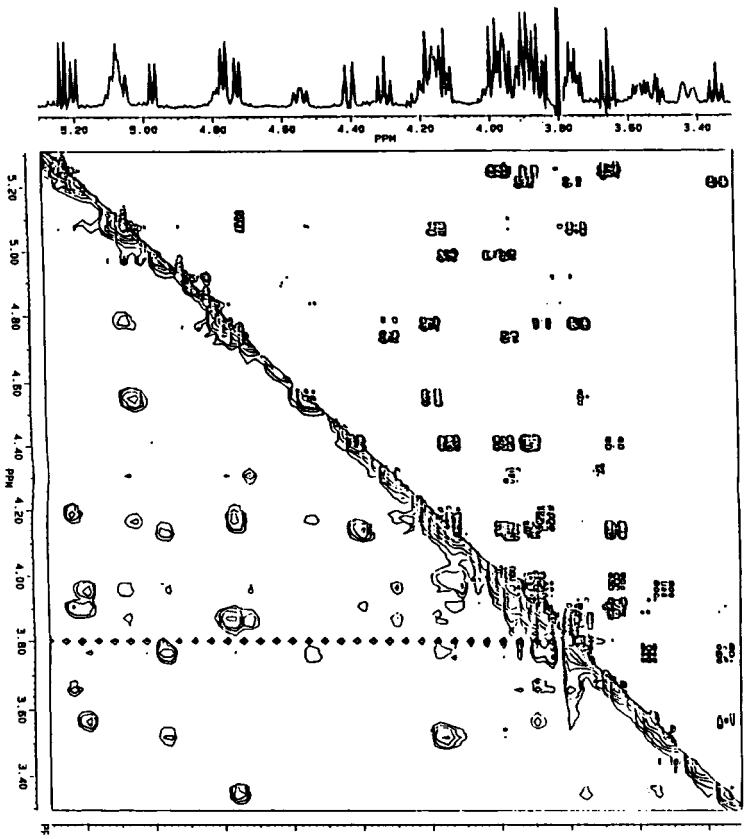


FIGURE 1. Pentasaccharide region of the combined RECSY/NOESY spectrum of frondoside B [**5**]. Upper triangle: two step relayed COSY with $\tau_1 = \tau_2 = 50$ msec. Lower triangle: phase sensitive NOESY with $\tau_{mix} = 400$ msec.

TABLE 3. Relevant nOe Data[a] for Frondoside B [**5**].

Proton	Resonances showing nOe cross peaks
H-1αβ	H-2α, H-2β, H-3α
H-3α	H-1α, H-2α, H-3α, H_3-30, Xyl I H-1
H-5α	H-1α, H-3α, H-6α, H_3-30
H-6αβ	H-5α, H_3-19, H_3-30
H-7α	H-6α, H-6β, H-15α, H-15β, H_3-28
H-9β	H-1β, H_3-19
H-17α	H-12α, H-16α, H_3-21, H_3-28
H-24	H_3-26
Xyl I H-1	Xyl I H-2, Xyl I H-3, Xyl I H-5, H-3α
Qui H-1	Qui H-2, Qui H-3, Qui H-5, Xyl I H-2
Glc H-1	Glc H-2, Glc H-3, Glc H-5, Qui H-4
MeGlc H-1	MeGlc H-2, MeGlc H-3, MeGlc H-5, Glc H-3
Xyl II H-1	Xyl II H-2, Xyl II H-3, Xyl II H-5, Qui H-2

[a]Identified as medium to strong nOe cross peaks in 2D NOESY map with $\tau_{mix} = 400$ msec.

tons H-1 and H_3-19, effects that can be brought about only if the B/C ring junction features H-9β; and, finally, the occurrence of dipolar contact between C-17α and the two methyl groups H_3-21 and H_3-28 requires that H_3-28 is α at the C/D ring junction.

Summarized in Table 2 are the ^{1}H- and ^{13}C-nmr data obtained for the oligosaccharide portion of frondoside B [**5**]. The pentasaccharide nature of the sugar moiety followed from the occurrence of five glycosidically linked anomeric CH resonances in the ^{1}H (5.4–4.8 ppm) and ^{13}C (106–102 ppm) spectra. Thanks to moderate spectral overlap in the 4.2–3.2 ppm region of the ^{1}H spectrum, sequential assignment of ring proton resonances within individual monosaccharide units was readily available from ^{1}H chemical shift correlation experiments. These experiments have also revealed that vicinal couplings between sugar ring protons assumed values between 7 and 10 Hz, a range characteristic for trans diaxial disposition of pertinent protons. Furthermore, intraresidue nOe contacts inferred from 2D NOESY maps showed that each anomeric proton was in 1,3 syn diaxial relationship with its respective H-3 and H-5 partners, a finding that requires that the anomeric carbon atoms were in β configuration and the sugar rings assumed a preferred chair conformation. Thus, the individual monosaccharides comprise two xyloses (Xyl I, Xyl II), one quinovose (Qui) and two glucoses (Glc), one of which (MeGlc) has a methyl ether substituent at C-3, a feature common to several holothurins (11,12). Inspection of data in Table 2 shows that resonances due to Xyl I H-4 and Glc H_2-6 experience a 1.0 to 1.4 ppm downfield shift compared to the values found for the respective resonances in Xyl II and MeGlc, while their adjacent ring carbon atoms, Xyl I C-4, Glc C-6, have their resonances shifted 5 to 6 ppm to lower fields. These effects can be readily accounted for by assuming that the hydroxyl functions in these positions feature sulfate moieties.

Sequential arrangement of the monosaccharide units and the sites of glycosidic linkages followed from strong interresidue nOe contacts identified in the 2D NOESY maps. As shown in Figure 1, these contacts appear without spectral overlap and occur at the following chemical shift coordinates: 5.280 (Qui H-1), 4.036 (Xyl H-2); 5.041 (Xyl II H-1), 3.848 (Qui H-2); 4.847 (Glc H-1), 3.425 (Qui H-4) and, 5.314 (MeGlc H-1), 4.067 (Glc H-3). The assumption that these contacts reflect spatial proximities associated with formation of glycosidic linkages is fully supported by the ^{13}C-nmr data. Formation of a glycosidic bond is known to result in a 6 to 8 ppm downfield shift of the resonance due to carbon atoms involved in the glycosidic linkage, a fact clearly reflected by the ^{13}C chemical shift data in Table 2.

As attested to by the nOe cross-peak occurring at the coordinate corresponding to the chemical shifts of Xyl I H-1 and H-3α, the sugar moiety is attached to the aglycone at a location characteristic for this class of natural products. While the negative fabms (magic bullet) did not provide any distinctive molecular ions, the major high mass fragments can be interpreted as in Table 4.

Assuming that the sugars belong to the D configuration family as is the case in related holothurins (2,3), we conclude frondoside B has the structure 3β-*O*-{3-*O*-

TABLE 4. Negative Fabms Fragmention of Frondoside B [**5**].

Ion (rel. int.)[a]	Composition
1337 (17)	$M_{H,H}$ − MeGlc − H + matrix
1260 (17)	$M_{K,K}$ − MeGlc − H
1224 (43)	$M_{K,K}$ − MeGlc − K − 2H
1190 (38)	$M_{Na,Na}$ − MeGlc − O − Na

[a]Relative to base peak *m/z* 40.

methyl-β-D-glucopyranosyl-(1↦3)-*O*-β-D-6-sulfonatoglucopyranosyl-(1↦4)-*O*-{β-D-xylopyranosyl-(1↦2)]-*O*-β-D-quinovopyranosyl-(1↦2)-*O*-β-D-4-sulfonatoxylopyranosyl}-holost-7-ene sodium (or potassium) salt [**5**].

The ^{13}C-nmr spectrum of frondecaside [**6**] was remarkably similar to that of frondoside B in the low field. Thus all the pentasaccharide chain carbons of the latter had their chemical shift counterparts in the frondecaside spectrum except for the signal at δ 61.79 ppm for C-6 of MeGlu which appeared to have been replaced by a signal at δ 66.88 ppm. Examination of the COSY and relayed COSY spectra of frondecaside permitted assignment of all protons as shown in Table 2. Thus, it was evident that four of the five sugars in **3** and **6** were the same and enjoyed the same glycosylation sites. The fifth and terminal sugar in **6** appeared to be a 3-methyl-6-sulfate glucose, in view of the downfield chemical shift of the protons located at C-6. A heteronuclear chemical shift correlation spectrum permitted assignment of all carbon resonances to their respective sugars as in Table 2. Thus, the oligosaccharide **6** appears to be identical to the pentasaccharide chain of **5** except for the location of an additional sulfate. The ^{13}C chemical shift of Xyl I C-1 (δ 104.80 ppm) is consistent with C-1 glycosylation; however, the only significant signal in the high field of the spectrum is the single methyl signal at δ 17.47 assigned to the quinovose unit. Thus **6** appears to have no organic aglycone, and no inorganic aglycone is evident. We thus conclude that frondecaside has a dimeric structure **6** in which two identical trisulfated pentasaccharide moieties are β linked via their Xyl I anomeric sites. This structure **6** is thus consistent with all the nmr data including the ^{1}H and ^{13}C shifts for the Xyl I units.

In the absence of definitive mass spectral evidence (vide infra) for the composition of **6** we have obtained strong support for the dimeric nature of frondecaside from T_1 experiments. It is to be expected that carbon nuclei of individual monosaccharide units in a linearly extended olgisaccharide chain anchored at one end will exhibit different T_1 values according to the distance of that unit from the anchor. Larger distance means more motional freedom, hence longer T_1. In frondosides A and B where the anchor is provided by the aglycone, the T_1 values of Xyl I and Qui carbons are substantially shorter than those in the terminal MeGlc units. The same motional behavior, hence the same gradation of T_1, values is expected to occur if the pentasaccharide is dimerized. In this event the anchor is provided by the center of gravity of the dimer. Should, by contrast, frondecaside constitute a pentasaccharide with a "light" (organic or inorganic) substituent at the reducing terminus, the T_1 values are expected to be uniformly distributed over the entire oligosaccharide. As it follows from the experimentally observed NT_1 data collected in Table 5, the relaxation results favor the dimer picture.

TABLE 5. Relaxation Time Data for Frondecaside [**6**].[a]

Carbon	Xyl I	Qui	Glc	MeGlc	Xyl II
C-1	0.21	0.20	0.26	0.31	0.25
C-2	0.19	0.19	0.23	+[b]	0.23
C-3	0.17	+[b]	0.23	+	+
C-4	0.20	0.21	0.26	0.33	+
C-5	0.18	0.19	0.23	0.28	0.24
C-6	–	n.m.[c]	0.24	0.26	0.24
OMe	–	–	–	2.23	

[a]NT_1 (in sec). N = number of protons directly bonded to the given carbon atom.

[b]The sign + indicates signal overlap.

[c]n.m. = not measured.

Attempts to obtain molecular ion or pseudomolecular ion data for **6** via fabms in both negative and positive modes employing various matrices were unsuccessful. However, the negative fabms (glycerol) did provide evidence that the parent molecule was substantially larger than the trisulfated pentasaccharide moiety deduced on the basis of the interpretation of the initial nmr data. While several ions can be interpreted in terms of fragmentation of the dimeric structure **6**, we have found more convincing corroboration of the dimeric pentasaccharide nature of frondecaside [**6**] by recording the negative fabms spectrum of the desulfated product **7** obtained by treatment of **6** with pyridine/dioxane at 120° for 49 h. The strongest high field ions in this spectrum correspond to $[M+H]^+$ and $[M-H]^+$ for **7** while several major fragments are accounted for by fragmentations at glycosidic linkages accompanied by loss of molecules of H_2O. We thus conclude that frondecaside [**6**] has the structure 3-*O*-methyl-6-sulfonato-β-D-glucopyranosyl-(1↦3)-*O*-β-D-6-sulfonatoglucopyranosyl-(1↦4)-*O*-[β-D-xylopyranosyl-(1↦2)]-*O*-β-D-quinovopyranosyl-(1↦2)-*O*-β-D-4-sulfonatoxylopyranosyl-(1↦1)-3-*O*-methyl-6-sulfonato-β-D-glucopyranosyl-(1↦3)-*O*-β-D-6-sulfonatoglucopyranosyl-(1↦4)-*O*-[β-D-xylopyranosyl-(1↦2)]-*O*-β-D-quinovopyranosyl-(1↦2)-*O*-β-D-4-sulfonatoxylopyranoside sodium (or potassium) salt. This is the first report of a complex dimeric oligosaccharide closely related to an echinoderm saponin.

EXPERIMENTAL

INSTRUMENTATION.—All nmr spectra of frondoside B were recorded on a Bruker 5M 500 instrument in 0.5 ml pyridine-d_5–D_2O (8:2). The probe temperature was set to 300°K. COSY and RECSY spectra were recorded in the absolute value mode using a spectral window of 3 KHz, 1K complex data points in t_2 and 512 t_1 increments. The coherence transfer time periods in the RECSY experiments were set to 50 nsec. NOESY spectra were obtained in the phase sensitive mode using time proportional phase increment (TPPI) method (13) for quadrature detection in F_1; the mixing time was set to 200 and 400 msec. Prior to Fourier transformation the time domain data matrices were extended to yield 2K × 2K real matrices in the frequency domain. Heteronuclear chemical shift correlation (C,H-COSY) experiment was performed with a 12,500 × 2800 Hz spectral window, acquired in a 2K × 0.5K complex time domain data matrix. Prior to Fourier transformation this was multiplied with Gaussian window function in both dimensions and extended to yield a 4K × 2K frequency domain real matrix. The nmr spectra of frondecaside were recorded with a Varian VXR 400 instrument using 28 mg of sample in pyridine-d_5–D_2O (5:1) with probe temperature at 30°. T_1 data for frondecaside were obtained with the inversion recovery method, taking eight samples of the recovering signals with a recycling time of 2.8 sec, 2560 scans per FID. The data were analyzed by the three-parameter non-linear least squares routine of the Varian software. Optical rotation was measured with a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a Kratos MS50 instrument using gylcerol matrix.

EXTRACTION.—Whole specimens (30.4 kg, wet wt) of *C. frondosa* collected in Passamaquoddy Bay, New Brunswick, Canada (October 1988) were deep frozen until use. A voucher specimen is retained at the Chemistry Department, University of New Brunswick. The animals were thawed, chopped into small pieces, placed in vats and extracted (×4) with MeOH (total 6000 ml) for 24 h. The extract (4000 ml) obtained after removal of MeOH in vacuo at 30–35° was introduced onto an Amberlite XAD-2 (1 kg) column which was eluted with H_2O until Cl^- was absent. Elution with MeOH (4000 ml) gave, after evaporation, a crude glycoside-containing mixture (17 g) which was treated with Me_2CO (200 ml). The Me_2CO-insoluble portion was filtered off, air-dried (12 g), and chromatographed on a Kielsegel 60 (360 g, 230–400 mesh) column with discontinuous gradient of CH_2Cl_2-MeOH-H_2O (6.5:3.8:1.1↦5:5:1) to give 32 fractions (100 ml each) from which frondoside B and frondecaside were isolated.

ISOLATION OF FRONDOSIDE B [**5**].—Evaporation of fractions 20–21 gave an amorphous material (100 mg) showing R_f 0.4–0.45 on Si gel tlc using CH_2Cl_2-MeOH-H_2O (6.5:3.8:1.1) which was further purified on a column of Kieselgel 60 (50 g, 230–400 mesh) eluting with discontinuous gradient of $CHCl_3$-MeOH-H_2O (6:3:0.5↦4:3:1.0) to give 16 fractions (25–30 ml each). Evaporation of fractions 5–16 gave an amorphous material (80 mg), which was further purified on a column of reversed-phase silica (10 g, Lichroprep RP-18) eluting first with MeOH (5 ml), then MeOH-CH_2Cl_2-H_2O (4:0.5:0.5) and MeOH-EtOH-CH_2Cl_2 (4:0.5:0.5) to give 20 fractions (5 ml each). Evaporation of fractions 4–12 gave an amorphous material, frondoside B (22.7 mg): mp 216–218° (dec), $[\alpha]^{23}D$ −34.7° [c = 0.0017, pyridine-H_2O (1:4)].

ISOLATION OF FRONDECASIDE [**6**].—Evaporation of fractions 22–28 gave an amorphous material (ca. 600 mg wet) showing R_f 0.3–0.35 on Si gel tlc using CH_2Cl_2-MeOH-H_2O (6.5:3.8:1.1). This material was further purified on a column of Kieselgel 60 (60 g, 230–400 mesh) eluting with discontinuous gradient of $CHCl_3$-MeOH-H_2O (6.5:3:0.5→4:3:3) to give 85 fractions (10–15 ml each). Evaporation of fractions 1–16 gave an amorphous material (250 mg wet wt) which was further purified on a column of reversed-phase silica (15 g Lichroprep RP-18) eluting with a discontinuous gradient of MeOH-H_2O (1:1→3:1) to give 25 fractions (5 ml each). Evaporation of fractions 5–25 gave amorphous material (215 mg) which was further purified by preparative tlc (Kieselgel F_{254}, 1 mm, 20 × 20 cm (4 plates), using *n*-BuOH–EtOAc–H_2O–MeOH (4:1:2.2:0.5). The lower band (R_f 0.3–0.4) was recovered and rechromatographed by preparative tlc [Kieselgel F_{254}, 0.5 mm, 20 × 20 cm (8 plates)] using $CHCl_3$-MeOH-H_2O-EtOAc (4:3:0.5:0.5). The recovered lower band (R_f ca. 0.3–0.4, 80 mg) was finally purified on a column of reversed-phase silica (7 g, Lichroprep RP-18) using MeOH-H_2O-EtOH (6:4:2) solvent system under pressure to give 27 fractions (1 ml each). Evaporation of fractions 14–22 gave amorphous frondecaside (28.1 mg), mp 251–252°, $[\alpha]^{23}D$ −19.2° [c = 0.0013, pyridine-H_2O (1:4)]; negative fabms (glycerol) 1653 (3.5), 1568 (4.7), 1484 (12.4), 1458 (10.9), 1431 (12.1), 1405 (8.5), 1347 (8.3).

DESULFATION OF FRONDECASIDE.—A solution of frondecaside [**6**] (2.6 mg) in pyridine-dioxane (1:1) (6 ml) was heated at 120° for 49 h in a reflux system. After evaporation to dryness, the residue was dissolved in H_2O (3 ml) and filtered, and the filtrate was freeze-dried to provide the desulfated amorphous frondecaside which was used directly for negative fabms (glycerol) 1515 $[M+H]^+$ (1.7), $[M-H]^-$ 1513 (1.5), $[M-H-4H_2O]^-$ 1441 (9), 1385 (5), $[M-Xyl]^-$ 1381 (4), 1367 (5.1), 1340 (8.8), 1323 (9.1), 1312 (10.1), $[M-MeGlc-3H_2O]^-$ 1283 (15.4).

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