FRONDOGENIN, A NEW AGLYCONE FROM THE SEA CUCUMBER *CUCUMARIA FRONDOSA*

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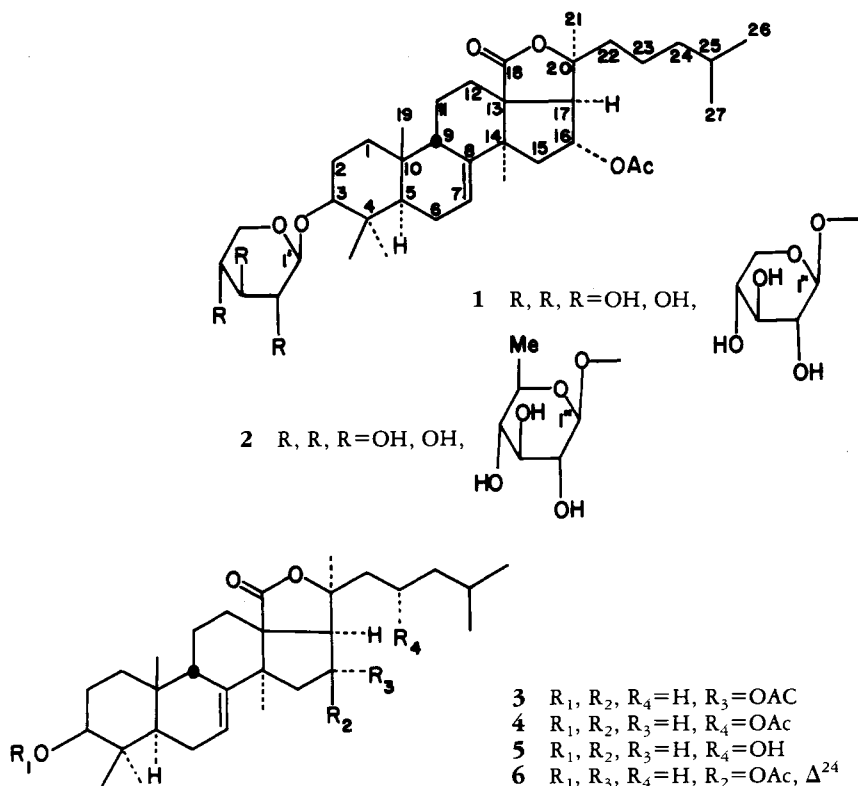
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ABSTRACT.—Partial hydrolysis of the saponin of *Cucumaria frondosa* has afforded several closely related novel triterpenoid lactones with a C_{16} acetate, including two diglycosides whose structures (**1**) and (**2**) and that of the free aglycone (**3**) have been deduced from spectral data.

Recently, we presented (1, 2) preliminary results of our studies on extracts of the orange-footed sea cucumber *Cucumaria frondosa* Gunnerus collected in Passamaquoddy Bay and noted the isolation of a triterpenoid lactone acetate xyloside recovered from partial hydroysis of the saponin fraction. We propose structure **1** for this compound on the basis of the following data and suggest the name frondogenin for the corresponding aglycone (**3**), which we have separately obtained and characterized.

The high resolution mass spectrum (ei) displays a prominent ion at m/z 646.4080 (53, $C_{37}H_{58}O_9$) corresponding to M^+ $[C_{42}H_{66}O_{13}]$-$[C_5H_8O_4$, terminal xylose moiety], while other significant fragments attest to the presence of lactone and acetate features. The ir spectrum (KBr) shows strong absorptions at 3400 (hydroxyl) and 1740 (acetate and γ-lactone) cm^{-1}.

The 360 MHz pmr spectrum (pyridine-D_5) shows six multiplet signals between δ 3.4 and 4.5 ppm, the integration of which confirms the presence of two xylose units which are linked via β glycosidic linkages in view of the large coupling constant ($J=8$

1   R, R, R=OH, OH,

2   R, R, R=OH, OH,

3   R_1, R_2, R_4=H, R_3=OAC
4   R_1, R_2, R_3=H, R_4=OAc
5   R_1, R_2, R_3=H, R_4=OH
6   R_1, R_3, R_4=H, R_2=OAc, Δ^{24}

Hz) for the anomeric protons. The general appearance of the higher field of the pmr spectrum (see Table 1) closely resembles that of known triterpenoid lactone aglycones from other holothurians, and our proposed aglycone structure (**3**) is isomeric with one of the native genins (**4**) from the sea cucumber *Stichopus chloronotus*, differing only in the location of the acetate moiety found at C23 in the latter. The corresponding alcohol, stichlorogenol (**5**), has been subjected to X-ray analysis (3), which confirmed the structure and stereochemistry and established the 9βH configuration and the strained boat conformation for ring C. It has been suggested by Kitagawa *et al.* (3) that these latter constraints are the principal reason for the ready migration of the 7-ene feature to generate isomeric artifacts with 8-ene and 9(11)-ene features during acid catalyzed hydrolysis of the parent saponins. This observation is particularly significant inasmuch as we have also obtained by mild-acid-catalyzed hydrolysis of *C. frondosa* saponin compounds **3, 7,** and **8**. The fact that the secondary acetate is not readily hydrolyzed we attribute to steric hindrance at the C16α position.

TABLE 1.   ¹H-Nuclear Magnetic Resonance Data[a]

Compound:	1	1	2	2	3	7	8
Solvent:	CDCl₃	C₅D₅N	CD₃OD/CDCl₃	C₅D₅N	CDCl₃	CDCl₃	CDCl₃
3αH		3.47 d,d $J=12, J=4$		3.38 d,d $J=12, J=4$	3.22 m	3.22 m	3.22 m
4αCH₃	1.02^+ s	1.23^- s	1.03^+ s	1.27^- s	1.02^+ s	1.01^+ s	0.92^+ s
4βCH₃	1.04^+ s	1.12^+ s	1.07^+ s	1.26^+ s	1.04^+ s	1.06^+ s	0.99^+ s
7H	5.55 m	5.68 m	5.55 m	5.69 m	5.54 m		
8H						3.10 bd	3.10 bd
9H		3.47 bd		3.51 bd	3.20 bd		
10CH₃	1.14 s	1.37 s	1.15 s	1.40 s	1.15 s	1.08 s	1.16 s
11H							5.18 m
12H							2.47
14CH₃	0.88 s		0.86 s	1.17 s	0.88 s	0.83 s	0.83 s
15αH		2.62 d,d $J_{13\alpha,15\beta}=12$ $J_{15\alpha,16}=8$		2.60 d,d $J_{15\alpha,15\beta}=12.5$ $J_{15\alpha,16}=7.5$	2.48 d,d $J_{15\alpha,15\beta}=12$ $J_{15\alpha,16}=8$	2.08 d,d	
15βH		1.77 d,d $J=12, J=8$					
16H	5.64 m	5.98 d,d,d $J_{15\alpha,16}=8$ $J_{15\beta,16}=8$ $J_{16,17}=8$	5.68 m	5.96 d,d,d $J_{15\alpha,16}=7.5$ $J_{15\beta,16}=10$ $J_{16,17}=9$	5.68 d,d,d $J_{15\alpha,16}=8$ $J_{15\beta,15}=10$ $J_{16,17}=8$		
16CH₃CO	2.04 s	2.03 s	2.05 s	2.05 s	2.04 s	2.05 s	
17H		2.64 d $J=10$		2.63 d $J=9$	2.49 d $J=10$	2.47 d $J=10$	
20CH₃	1.48 s	1.50 s	1.50 s	1.51 s	1.48 s	1.47 s	1.44 s
25CH₃	0.88 d $J=6.5$	0.87 d $J=6.8$	0.86 d $J=6.5$	0.87 $J=6.8$	0.87 d $J=6.6$	0.87 $J=6.6$	0.87 $J=6.5$
25CH₃	0.86 d $J=6.5$	0.87 d $J=6.8$	0.85 d $J=6.5$	0.87 $J=6.8$	0.86 d $J=7.5$	0.86 $J=6.6$	0.86 $J=6.5$

[a]Chemical shifts in ppm relative to internal TMS. Couplings in Hz.

It is well established from pmr studies (3) that compounds in this series with a Δ^7 feature show a signal for the vinylic hydrogen near 5.5 ppm while those with a $\Delta^{9,11}$ unsaturation give a signal consistently at lower field (~5.2 ppm). Thus, we conclude that frondogenin (**3**) (vinylic proton signal 5.54 ppm) has a Δ^7 bond.

Confirmation of the location and the configuration of the secondary acetate in **1** comes from 360 MHz ¹H-¹H decoupling experiments. Thus, irradiation near 6.0 ppm (16H) causes simultaneous collapse of the doublet at 2.64 ppm (17H) to a singlet, a doublet of doublets at 2.62 ppm (15Hα) to a doublet ($J=12$ Hz) and another double doublet centered at 1.77 ppm (15Hβ) to a doublet ($J=12$ Hz). Irradiation at ~2.6
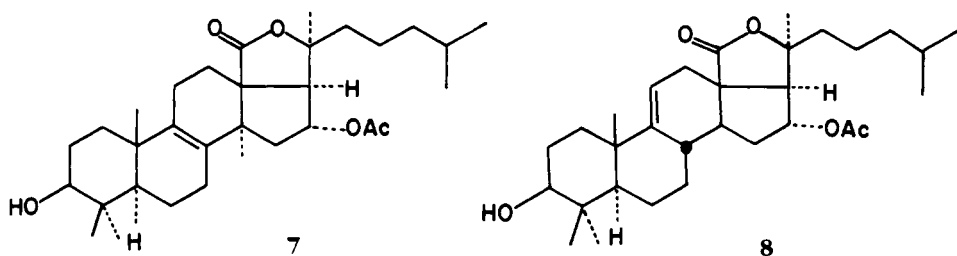
ppm collapses the multiplet signal at 5.98 ppm (16H) to a doublet (J=8 Hz) and the double doublet at 1.77 ppm (15Hβ) to a doublet (J=8 Hz).

It is evident from the chemical shift data that one C15 proton and the 17H experience profound deshielding; this we attribute to their *cis*-relationship to the C16 acetate, and we tentatively assign the α- configuration to the acetate moiety. Elyakov *et al.* (4) have reported the structure **6** for the acetylation product of the native genin from cucumarioside G (*Cucumaria fraudatrix*) and concluded the β-configuration for the C16 acetate in that compound based on analysis of coupling constant data. In **6**, the 17H is located at 2.49 ppm (J=9.05 Hz), but no explanation is given for its anomalous chemical shift.

We have also obtained another diglycoside triterpenoid lactone acetate (**2**) by partial hydrolysis of *C. frondosa* saponin. Analysis of its 360 MHz pmr spectrum (C_5D_5N) indicates the presence of two sugars because 11 protons appear as multiplets in the region δ 3.20-5.63 ppm, including the two anomeric (β linkage) protons, one at δ 5.23 (d, J=8 Hz) and other at δ 4.86 (d, J=8 Hz). A signal (3H, d, J=6 Hz) at δ 1.70 is shifted to δ 1.30 in the $CDCl_3/CD_3OD$ spectrum, and this we have determined is characteristic of the C5 methyl of free quinovose (6-deoxyglucose). Other pmr features closely resemble those of aglycone **3**.

The mass spectrum of **2** does not display a molecular ion, but, like diglycoside **1**, shows the highest mass ion at m/z 646.4073 ($C_{37}H_{58}O_9$) corresponding to loss of the terminal sugar moiety. Other prominent high mass ions m/z 586.3868 (M-quinovose+H-CH_3COOH) and 527.3725 (M-quinovose+H-CH_3COOH-CO_2-CH_3) provide additional support for the acetate and lactone functions.

Because we have determined that hydrolysis of the saponin fraction from *C. frondosa* yields only xylose, quinovose, and 3-O-methylglucose, we conclude that this partial hydrolysis product features a xylose-quinovose side-chain.

In contrast to our findings, other workers (5-7) have proposed structure **4** (or its C23 epimer) for the aglycone of spaonins from *C. frondosa* collected in the Gulf of St. Lawrence and, in addition to xylose, quinovose, and 3-O-methylglucose, have found glucose to be a constituent of the oligosaccharide side-chain of three of the four saponins isolated.

Burnell *et al.*(8) have drawn attention recently to the seasonal and geographical variation in composition of the sterols in the starfish *Asterias vulgaris*, and it is now apparent that variations in the composition of echinoderm saponins is also possible in some species.

<h2 style="text-align:center">EXPERIMENTAL</h2>

High resolution pmr spectra were recorded on a 360 MHz spectrometer at the Department of Medical Genetics, University of Toronto, and low resolution pmr spectra on a Varian T60 instrument. Cmr spectra were obtained with a 400 MHz facility at Laboratoire Regional de RMN de Haut Champ, Département de chimie, Université de Montréal. Gc was performed on a Perkin-Elmer 900 instrument with a $10' \times 2$ mm OV101 (30%) column at $130°$ and using a flame ionization detector.

ISOLATION OF SAPONIN FROM *CUCUMARIA FRONDOSA*.—Whole specimens of mature, freeze-dried *C. frondosa* (collected in Passamaquoddy Bay, June 1980) were defatted by extraction with petroleum ether-$CHCl_3$ in a Soxhlet. A subsequent methanolic extract was evaporated under reduced pressure at room temperature. The residue was dissolved in distilled H_2O (1 liter) and extracted five times with portions (200 ml) of *n*-BuOH. The *n*-BuOH extracts were combined and concentrated under reduced pressure on a steam bath affording crude saponin (2.6 g) displaying an Rf range of 0.3-0.4 on tlc ($CHCl_3$-MeOH-H_2O, 60:30:1) and containing sterol sulphate (Rf~0.8) and an unidentified material (Rf~0.95).

ACID HYDROLYSIS OF SAPONIN.—The crude saponin (2.6 g) was dissolved in HCl (100 ml, 2 N) and heated for 2 h at $100°$. After cooling, the reaction mixture was extracted with $CHCl_3$ (3×200 ml). The extracts were combined, washed with $NaHCO_3$ solution (100 ml, 5%), saturated brine (100 ml), and then dried over anhydrous Na_2SO_4. The evaporated $CHCl_3$ extract (0.98 g) was chromatographed on a silica gel column using a gradient of EtOAc (5 to 100%) in hexane, and fractions were collected as follows: J (10 mg), K (4 mg), L (65 mg), M (55 Mg), N (35 mg), O (23 mg), P (45 mg), Q (10 mg), R (7 mg). Selected fractions provided compounds **1**, **2**, **3**, **7**, and **8** as indicated below.

Compound **1**.—The amorphous fraction Q (compound **1**) proved to be homogeneous on tlc: ir ($CHCl_3$) ν max 3400, 1040 (OH), 1740 (lactone, acetate) cm^{-1}; ms m/z 646.4080 (53, M+H-xylose), 586 (8.5, M+H-xylose-CH_3COOH), 571 (5.5, M+H-xylose-CH_3COOH-CH_3), 542 (10.5, M+H-xylose-CH_3COOH-CO_2), 527 (17.7, M+H-xylose-CH_3COOH-CO_2-CH_3), 514 (18.4, M+H-2 xylose), 499 (66.3, 514-CH_3), 481 (11.9, 499-H_2O), 454 (8.3, 514-CH_3COOH), 439 (27.6, 514-CH_3COOH-CH_3), 421 (60.1, 514-CH_3COOH-CH_3-H_2O), 410 (9.6, 514-CH_3COOH-CO_2), 395 (66.1, 514-CH_3COOH-CO_2-CH_3), 377 (57.1, 514-CH_3COOH-CO_2-CH_3-H_2O), 297 (10, 514-CH_3COOH-CO_2-C_8H_{17}), 279 (11, 514-CH_3COOH-CO_2-C_8H_{17}-H_2O), 255 (21.3), 171 (20.6), 145 (36.6), 122 (21.3), 95 (49.9), 73 (100); pmr ($CDCl_3$) δ_{TMS}, 4.87 (d, 2H, $J=7$, H1', H1''), 3.80 (t, 2H, J 8, H2', H2''), 4.05 (t, 2H, $J=8$, H4', H4''), 4.18 (t, 2H, $J=8$, H3', H3''), 4.25 (m, 2H, H5'$_A$, H5''$_A$), 4,43 (m, 2H, H5'$_B$, H5''$_B$); see Table 1 for other signals.

Compound **2**.—The amorphous fraction R (compound **2**) was homogeneous on tlc; ir (KBr) ν max 3400, 1040 (OH), 1755 (lactone, acetate) cm^{-1}; ms m/z 646.4073 (3.5, M+H-quinovose), 586 (4.4, M+H-quinovose-CH_3COOH), 542, (2.9, M+H-quinovose-CH_3COOH-CO_2), 527 (1.8, M-quinovose-CH_3COOH-CO_2-CH_3), 514 (5.2, M+H-quinovose-xylose), 499 (12.2, 514-H_2O), 481 (5.2, 514-CH_3-H_2O), 454 (17.3, 514-CH_3COOH), 439 (20.6, 514-CH_3COOH-CH_3), 421 (29.0, 514-CH_3COOH-CH_3-H_2O), 410 (10.3, 514-CH_3COOH-CO_2), 395 (21.4, 514-CH_3COOH-CO_2-CH_3), 377 (23.7, 514-CH_3COOH-CO_2-CH_3-H_2O), 297 (9.5, 514-CH_3COOH-CO_2-C_8H_{17}), 279 (8.9, 514-CH_3COOH-CO_2-C_8H_{17}-H_2O), 255 (24.8), 171 (20.8), 157 (23.9), 145 (27.5), 122 (100), 95 (49.8), 73 (54.8).

Compounds **3**, **7**, *and* **8**.—Fraction M, homogeneous on tlc, proved to be a mixture of the three compounds, **3**, **7**, and **8** (10:4:1), from which **3** was separated by hplc; ir ν max (CCl_4) 3500 (OH), 1760 (γ-lactone), 1740 (acetate) cm^{-1}; ms m/z 514 (100), 499 (24), 496 (10), 481 (4), 454 (10), 439 (27), 421 (38), 411 (12), 410 (24), 409 (24), 396 (33), 395 (90), 339 (3), 297 (21), 279 (14), 281 (20), 255 (29), 171 (21), 157 (22), 145 (23), 143 (23), 133 (23), 121 (21), 119 (28), 107 (24), 106 (25), 105 (33), 94 (34), 93 (26), 91 (22), 83 (21), 81 (25), 71 (23), 69 (79), 57 (36), 55 (63).

A mixture of **7** and **8** (4:1) was also obtained from fraction M by hplc; ir (CCl_4) νmax 3500 (OH), 1760 (γ-lactone), 1740 (acetate) cm^{-1}; ms m/z 514.3659 (98.9, M$^+$, $C_{32}H_{50}O_5$ requires 514.3637), 499 (24.4, M-CH_3), 496 (4.4, M-H_2O), 481 (3.1, M-CH_3-H_2O), 454 (8.4, M-CH_3COOH), 439 (21.9, M-CH_3-CH_3COOH), 421 (33.6, M-CH_3-CH_3COOH-H_2O), 409 (18.5, M-CH_3COOH-CO_2-H), 395 (100, M-CH_3COOH-CO_2-CH_3), 339 (1.8, M-CO_2-H_2O-C_8H_{17}), 297 (17.2, M-CO_2-CH_3COOH-C_8H_{17}), 281 (17.3), 279 (4.7, M-CO_2-C_8H_{17}-CH_3COOH-H_2O), 265 (9.9), 255 (14.5), 239 (7.3), 183 (10.9), 171 (14.2), 169 (10.7), 159 (14.0), 157 (18.0), 155 (11), 145 (21.2), 143 (18.6), 133 (13), 129 (12.9), 121 (11), 19 (20.6), 109 (15.4), 107 (17), 105 (25.4), 95 (21.9), 93 (18.8), 69 (43.9); cmr δ_{TMS} ($CDCl_3$) 176.9 (s, 18C), 170.3 (s, acetate C=O), 151.0 (s, 9C, **8**), 110.3 (d, 11C, **8**), 130.1 (s, 8C, **7**), 135.5 (s, 9C, **7**), 85.1 (s, 20C, **7**), 84.6 (s, 20C, **8**), 79.0 (d, 3C, **7**), 75.2 (d, 16C, **7**), 74.9 (d, 16C, **8**), 59.6 (s, 13C, **7**), 58.8 (s, 13C, **8**).

ISOLATION AND IDENTIFICATION OF SUGARS FROM HYDROLYZED SAPONIN.—The aqueous solution resulting from acid hydrolysis of saponin was passed through a column of quaternary ammonium ion exchange resin (3 g). The resulting solution was evaporated under reduced pressure to yield a solid residue

which was silylated and examined by gc using authentic sugar trimethysilyl ethers as controls. Only three sugars (xylose, quinovose, 3-O-methylglucose) were found to be present in substantial quantity and were identified by coinjection with authentic samples. Glucose was absent in the hydrolysis product of the saponin as determined by control sample.

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