PERGAMON

Phytochemistry 50 (1999) 135–138

PHYTOCHEMISTRY

A triterpenoid saponin from *Cucumaria frondosa*

Nurettin Yayli [a,*], John A. Findlay [b]

[a] *Department of Chemistry, Karadeniz Technical University, 61080 Trabzon, Turkey*
[b] *Department of Chemistry, University of New Brunswick, Fredericton, NB, Canada E3B 6E2*

Received 12 May 1998

Abstract

The structure of a new triterpenoid saponin, frondoside D, isolated from *Cucumaria frondosa* has been determined principally by high field 1D and 2D NMR and FAB-MS spectrometry. Frondoside D was shown to be 3β-{3-*O*-methyl-*O*-β-D-glucopyranosyl-(1-3)-*O*-β-D-xylopyranosyl-(1-4)-[*O*-β-D-xylopyranosyl-(1-2)]-*O*-β-D-quinovopyranosyl-(1-2)-*O*-β-D-4-sulfonatoxylo-pyranosyl}-16β-acetoxy-23*S*-hydroxy-holost-7-ene, sodium salt.

Keywords: *Cucumaria frondosa*; Triterpene saponin; Holost-7-ene; 18 → 20 lactone

1. Introduction

Recently we reported the structure of two novel oligosaccharides isolated from the common sea cucumber *Cucumaria frondosa* Gunnerus (Findlay, Yayli & Radics, 1992). One of these was a saponin designated frondoside B, (**3**) comprising a 3β-hydroxyholosta-7,24-diene aglycone and a disulfated pentasaccharide sidechain. The major saponin from this source, frondoside A, (**2**) (Girard et al., 1990; Yayli, 1993) features a 16β acetoxy-holosta-7-ene aglycone and a monosulfated pentasaccharide sidechain which differs from that of frondoside B (**3**) by the presence of a xylose unit in place of the (1 → 3) attached glucose-6-sulfate feature. Of particular interest was the isolation of the dimeric pentasaccharide frondecaside (Findlay, Yayli & Radics, 1992; Yayli, 1993) featuring six sulfate moieties.

2. Results and discussion

From *C. frondosa* we have now characterized an additional saponin designated frondoside D (**1**). Frondoside D (**1**) is closely related to frondoside A (**2**) and frondoside A_1 (**4**) (Avilov et al., 1993), differing only by the presence of an hydroxyl group at C-23. The structure of frondoside D was deduced from 1D and 2D high field NMR data and supported by positive FAB-MS.

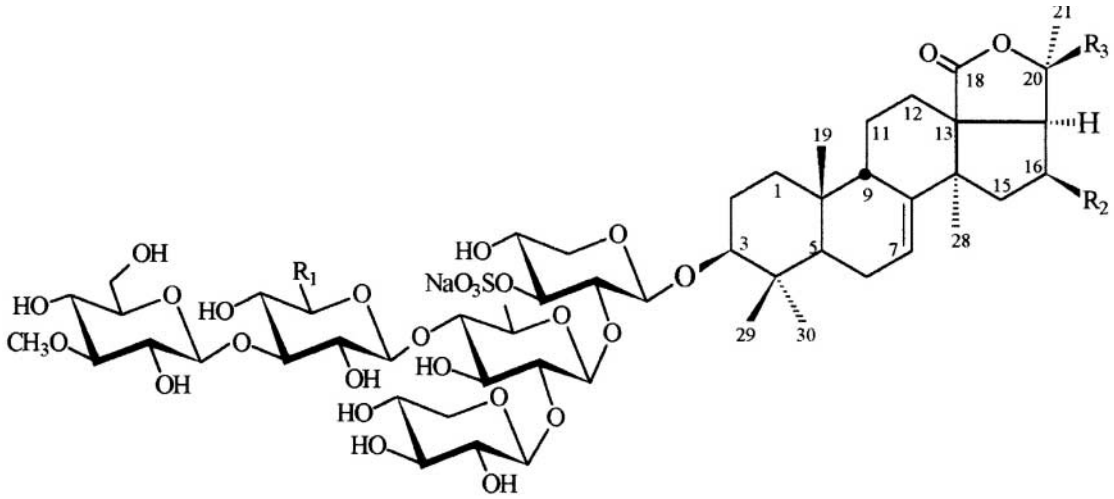
Conventional ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra, combined with DEPT data, afforded the structure of the oligosaccharide moiety which is identical with the monomeric oligosaccharide part of frondoside A (**2**) and frondoside A_1 (**4**) (Girard et al., 1990; Yayli, 1993; Avilov et al., 1993).

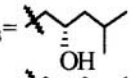
Analysis of the spectral data for frondoside D and comparison with those published for related saponin aglycones (Findlay, Yayli & Radics, 1992; Girard et al., 1990; Yayli, 1993; Avilov et al., 1993; Kitagawa et al., 1981; Kalinin, Stonik, Kalinovskii & Isakov, 1989; Stonik et al., 1982; Stonik, Mal'tsev, Kalinovskii & Elyakov, 1982) shows that the aglycone part of frondoside D is a holostane skeleton featuring a hydroxyl group at C-23 (Avilov et al., 1993; Kitagawa et al., 1981; Kalinin, Stonik, Kalinovskii & Isakov, 1989; Stonik et al., 1982; Stonik, Mal'tsev, Kalinovskii & Elyakov, 1982).

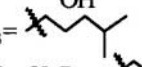
The positive FAB-mass spectrum (MNBA) of frondoside D (**1**) displayed a single peak in the higher mass range at m/z 1373 corresponding to $[M + Na]^+$ ($M = C_{60}H_{95}O_{30}SNa$), a composition differing from that of frondoside A (**2**) by the presence of an ad-

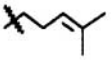
* Author to whom correspondence should be sent.

0031-9422/98/$ - see front matter
PII: S0031-9422(98)00463-4



1 R_1=H, R_2=OAc, R_3= 

2 R_1=H, R_2=OAc, R_3= 

3 R_1=CH_2OSO_3Na, R_2=H, R_3= 

4 R_1=H, R_2=OAc, R_3= 

ditional oxygen. The ^{13}C NMR chemical shift inventory of **1** (Table 1) closely parallels that of frondoside A except for signals assigned to C-22 (46.78), C-23 (66.24) and C-24 (48.49) which are shifted downfield by δ 7.65, 43.48 and 8.86 ppm, respectively, in agreement with the presence of an OH group at C-23. The aglycone sidechain is thus comparable to that of stichlorogenol (Kitagawa et al., 1981; Kalinin, Stonik, Kalinovskii & Isakov, 1989; Stonik et al., 1982; Stonik, Mal'tsev, Kalinovskii & Elyakov, 1982), a C-23-hydroxy aglycone from the sea cucumber *Stichopus chloronotus* stereochemistry have been confirmed by X-ray crystallography (Kitagawa et al., 1981). Comparable signals in the ^{13}C NMR spectrum (pyridine-d_5) of the latter are observed at δ 47.62, 65.70 and 49.27 (Kitagawa et al., 1981), suggesting the 23S configuration in frondoside D. The ^{13}C NMR chemical shifts inventory (Table 1) for the oligosaccharide chain of frondoside D are virtually identical with those of frondoside A and A_1 (Girard et al., 1990; Yayli, 1993; Avilov et al., 1993). Thus we conclude that frondoside D possesses structure **1**, that is 3β-{3-*O*-methyl-*O*-β-D-glucopyranosyl-(1-3)-*O*-β-D-xylopyranosyl-(1-4)-[*O*-β-D-xylopyranosyl-(1-2)]-*O*-β-D-quinovopyranosyl-(1-2)-*O*-β-D-4-sulfonato-xylopyranosyl}-16β-acetoxy-23*S*-hydroxy-holost-7-ene, sodium salt.

3. Experimental

3.1. Instrumentation

Mass spectra were recorded with a Kratos MS50 instrument. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were obtained at 20°C with a Bruker AMX-500 spectrometer and are referred to internal tetramethylsilane.

3.2. Isolation of saponins

A crude glycoside containing mixture (2.50 g) obtained from *C. frondosa* as previously described (Findlay, Yayli & Radics, 1992) was chromatographed on a silica gel 60 (100 g, 230–400 mesh) column eluting with a discontinuous gradient of $CHCl_3$–MeOH (4:1–4:4) and $CHCl_3$–MeOH–H_20 (4:3:1) to give 26 fractions (~20 ml each) which were combined on the basis of TLC analysis to provide four sub-fractions *a*, *b*, *c* and *d*.

3.3. Frondoside D, 1

Fraction *c* (9–13, 410 mg) was rechromatographed on a reversed-phase silica column (6 g, LiChroprep RP-18) eluting with a discontinuous gradient of

Table 1
^{13}C NMR data for frondoside D, (**1**) in pyridine-d_5–D_2O (5:2)

Aglycone of **1**[a]			Sugar moiety **1**[a]		
C	^{13}C (δ, ppm)	DEPT	C	^{13}C (δ, ppm)	DEPT
1	36.04	CH_2	Xyl I, 1	104.44	CH
2	26.84	CH_2	2	81.69	CH
3	89.19	CH	3	76.15	CH
4	39.47	C	4	76.30	CH
5	47.90	CH	5	64.18	CH_2
6	24.10	CH_2			
7	120.41	CH	Qui, 1	102.20	CH
8	145.91	C	2	82.66	CH
9	47.71	CH	3	75.12	CH
10	35.43	C	4	85.23	CH
11	23.27	CH_2	5	71.20	CH
12	31.54	CH	6	17.99	CH_3
13	59.30	C			
14	47.31	C	Xyl II, 1	104.66	CH
15	44.35	CH_2	2	73.59	CH
16	75.20	CH	3	86.07	CH
17	55.27	CH	4	68.89	CH
18	180.87	C	5	65.90	CH_2
19	24.30	CH_3			
20	86.42	C	MGlc, 1	104.36	CH
21	30.33	CH_3	2	74.58	CH
22	46.78	CH_2	3	86.91	CH
23	66.24	CH	4	70.52	CH
24	48.49	CH_2	5	77.45	CH
25	28.10	CH	6	61.85	CH_2
26	21.77	CH_3		60.97	OCH_3
27	23.93	CH_3			
28	32.79	CH_3	Xyl III, 1	105.33	CH
29	17.46	CH_3	2	74.90	CH
30	28.78	CH_3	3	76.54	CH
31	171.09	C	4	70.09	CH
32	21.60	CH_3	5	66.54	CH_2

[a] Chemical shifts (ppm) are relative to internal TMS.

CH_3COCH_3–MeOH–H_20 (2:2:3–2:2:4) solvent system to give 42 fractions (1–3 ml each) which were combined on the basis of TLC analysis to provide 5 subfractions. Sub-fraction *cc* (9–19, 0.320 mg) was further purified on a reversed-phase silica column (6 g, LiChroprep RP-18) eluting with CH_3COCH_3–MeOH–H_2O (2:2:4) to give 74 fractions (1–3 ml each). On the basis of TLC, fractions were combined to provide 7 subfractions. Sub-fraction *ccc* (17–29, 121 mg wet) was chromatographed by prep. TLC (1 mm, 20 × 20 cm, 2 plates) using $CHCl_3$–MeOH–H_2O (3:2.5:0.5) solvent system to give 3 major bands. The least polar band *ccca* (13.4 mg, $R_f = 0.64$) was finally purified by a reversed-phase silica column (5 g, LiChroprep RP-18) eluting with CH_3COCH_3–CH_3OH–H_20 (2:2:5) to give frondoside D [7.0 mg, $R_f = 0.25$, rpTLC, CH_3COCH_3–MeOH–H_2O (2:3:4)]; m.p. 217–220°, $[\alpha]_D^{23} = -22.9°$ [$c = 0.0013$, pyridine–H_20 (1:4)]; ^1H NMR d (ppm) [pyridine-d_5–D_2O (5:2), 500 MHz] 0.92 (H-27), 0.95 (H-26), 1.12 (H-29), 1.20 (H-19), 1.27 (H-28), 1.29 (H-30), 1.68 (H-21), 2.10 (H-32), 3.30 (H-3), 3.72 (H-23), 4.89 ($J = 7.0$ Hz, Xyl I H-1), 4.90 ($J = 7.6$ Hz, Xyl II H-1), 5.31 ($J = 7.7$ Hz, Qui H-1), 5.32 ($J = 7.3$ Hz, Xyl III H-1), 5.38 ($J = 7.6$ Hz, MGlc H-1), 5.70 (H-7), 5.95 (H-16); ^{13}C NMR [pyridine-d_5–D_2O (5:2), 125 MHz] d (ppm) see Table 1; positive FAB-mass (MNBA) *m/z* (rel. int): 1373 (1.8) $[M + Na]^+$, 1351 (0.9) $[M + H]^+$, 838 (1.2) [sugar moiety + H]$^+$, 609 (23) [MGlc–*O*-Xyl II–*O*-Qui–*O*-Xyl III]$^+$, 530 (09) [M-aglycone + H]$^+$, 493 (8.6) [MGlc–*O*-Xyl II–*O*-Qui(*O*)-*O*]$^+$, 325 (9.2) [MGlc–*O*-Xyl II]$^+$, 177 (100) [MGlc]$^+$.

Acknowledgements

Thanks are due to Donald M. Leek, Institute for Marine Biosciences, MRC of Canada, for recording NMR data to process, Professor Isao Kitagawa for supplying the ^{13}C NMR spectrum of stichlorogenol

and to Dan Drummond for assistance with FAB-Mass spectra. This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada and the Turkish Government through a grant via Karadeniz Technical University to N.Y.

References

Avilov, S. A., Kalinin, V. I., Drozdova, O. A., Kalinovskii, A. I., Stonik, V. A., & Gudimova, E. N. (1993). *Khimiya Prirodnykh Soedinenii, 2,* 260.

Findlay, J. A., Yayli, N., & Radics, L. (1992). *Journal of Natural Products, 55,* 93.

Girard, M., Hélanger, J., ApSimon, J. W., Garneau, F. X., Narvey, C., & Brisson, J. R. (1990). *Canadian Journal of Chemistry, 68,* 11.

Kalinin, V. I., Stonik, V. A., Kalinovskii, A. I., & Isakov, V. V. (1989). *Khimiya Prirodnykh Soedinenii, 5,* 678.

Kitagawa, I., Kobayashi, M., Inamoto, T., Yasuzawa, T., Kyogoku, Y., & Kodo, M. (1981). *Chemical and Pharmaceutical Bulletin, 29,* 1189.

Stonik, V. A., Mal'tsev, I. I., Kalinovskii, A. I., Conde, C., & Elyakov, G. B. (1982). *Khimiya Prirodnykh Soedinenii, 2,* 194.

Stonik, V. A., Mal'tsev, I. I., Kalinovskii, A. I., & Elyakov, G. B. (1982). *Khimiya Prirodnykh Soedinenii, 2,* 200.

Yayli, N. (1993). Ph.D. Thesis, University of New Brunswick, Fredericton, NB.