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A Triterpene Tetrasaccharide, Formoside, from the Caribbean Choristida Sponge *Erylus Formosus*

Marcel Jaspars and Phillip Crews*

Department of Chemistry and Biochemistry and Institute for Marine Sciences
University of California, Santa Cruz, California 95064, USA.

Abstract: A new triterpene tetrasaccharide, formoside (1) was isolated in 25% yield from the butanolic extract of the marine sponge *Erylus formosus*. The structure of the aglycone was identical to that of penasterol while the glycoside was composed of two arabinose and two galactose units with Ara-I as the branch point for Gal-I and the Ara-II-Gal-II unit.

We have a continuing interest in the chemistry of Indo-Pacific Choristida order sponges as they seem to be a source of structurally diverse compounds.[1,2] An opportunity to examine a Caribbean Choristid specimen arose during an expedition to the Bahamas when a habitat with abundant amounts of *Erylus formosus*[3] (family, Geodiidae) was located. Both Indo-Pacific and Caribbean members of the genus *Erylus* have been examined in the past. An interesting unifying theme in the chemistry reported for these organisms are complex carbohydrates such as the Erylosides (from *E. lendenfeldi*,[4] *E.* sp.[5] and *E. goffrilleri*[6]) and the Erylusamines (from *E. placenta*[7]). In addition, a series of unusual fatty acids were detected in the extract of *E. formosus*.[8] Our investigation of a polar solvent partition extract fraction of *E. formosus* seemed warranted as a plethora of peaks were observed in the ^{13}C NMR spectrum. Surprisingly, approximately half of these resonances matched those of triterpene, penasterol[9] rather than the aglycone of eryloside. Most of the remaining resonances were characteristic of four carbohydrate moieties but not immediately evident were the exact substructures and their relative interconnectivity. To be described in this communication is the solution of this problem which was achieved when a pure compound, formoside (1) was obtained and extensively examined by FABMS and two dimensional NMR techniques.

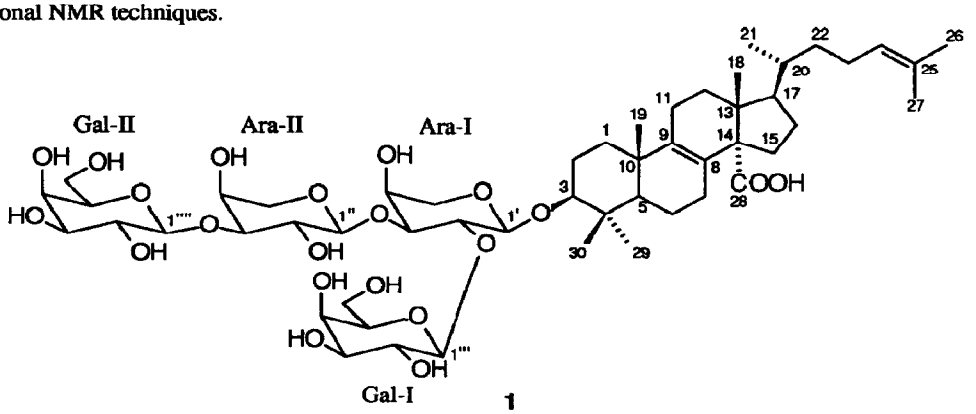

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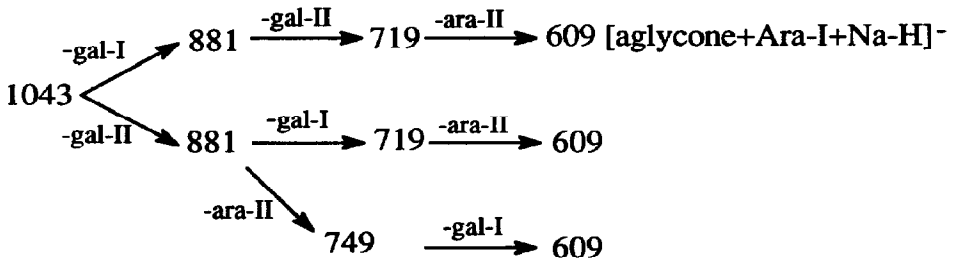
Table 1. NMR Data for Formoside in CD_3OD at 125 (^{13}C) and 500 (^1H) MHz.

Aglycone			Oligosaccharide		
	^{13}C	^1H	Sugar	^{13}C	^1H
1	36.5 t	1.72 m, 1.26 m	Ara-I		
2	27.7 t	1.87 m, 1.71 m	1'	105.9 d	4.38 (d, *J*=7.5)
3	91.2 d	3.12 (dd *J*=11.5, 4.0)	2'	77.2 d	3.95 (dd, *J*=9.0, 7.5)
4	40.6 s		3'	83.5 d	3.76 m
5	51.7 d	1.08 (d, *J*=12.5)	4'	69.8 d	3.98 bs
6	19.3 t	1.66 m, 1.49 m	5'	66.1 t	3.83 m, 3.51 (d,
7	28.9 t	2.04 m, 1.53 m			*J*=12.0)
8	131.8 s		Ara-II		
9	141.2 s		1"	105.3 d	4.56 (d, *J*=7.5)
10	38.5 s		2"	71.7 d	3.77 m
11	23.3 t	2.10 m	3"	83.8 d	3.63 m
12	28.8 t	2.04 m, 1.92 m	4"	69.5 d	4.08 bs
13	48.0 s		5"	66.6 t	3.85 (dd, *J*=12.0, 2.0),
14	63.9 s				3.56 (d, *J*=12.0)
15	32.8 t	2.20 m, 1.66 m	Gal-I		
16	30.2 t	2.03 m, 1.35 m	1'''	104.5 d	4.71 (d, *J*=7.5)
17	52.1 d	1.50 m	2'''	73.5 d	3.43 m
18	18.3 q	0.76 s	3'''	75.2 d	3.40 m
19	20.0 q	1.02 s	4'''	70.2 d	3.79 m
20	36.9 d	1.40 m	5'''	76.9 d	3.51 m
21	19.1 q	0.91 (d, *J*=6.5)	6'''	62.4 t	3.69 m, 3.61 m
22	37.2 t	1.40 m, 1.00 m			
23	25.7 t	1.98 m, 1.84 m	Gal-II		
24	126.1 d	5.05 (t, *J*=7.5)	1''''	106.0 d	4.47 (d, *J*=7.5)
25	128.9 s		2''''	72.9 d	3.59 m
26	17.7 q	1.57 s	3''''	74.6 d	3.47 m
27	25.8 q	1.64 s	4''''	70.2 d	3.79 m
28	179.9 s		5''''	76.8 d	3.42 m
29	28.3 q	1.04 s	6''''	62.5 t	3.69 m
30	16.7 q	0.87 s			

The sponge (coll. no. 93358, 55 g dry wt) was obtained in the Bahamas at a depth of 60 ft by SCUBA. It was immersed in MeOH/H_2O (1:1) and after 24 h at rt this liquid was decanted and discarded. The sponge was returned to the home lab at ambient temperature, where it was then extracted with MeOH (3x). The concentrated oil (4.5 g) was partitioned between water and CH_2Cl_2 and the water layer was next extracted with *sec*-BuOH, yielding 1.8 g of a yellow amorphous solid. Purification by ODS HPLC (7% aq MeOH) gave a pale yellow amorphous pure compound (25% of the BuOH fraction, 0.8% yield based on dry sponge weight), which exhibited an intense $[M-H]^-$ ion in the HRFAB (negative) mass spectrum at 1043.5427, corresponding to a molecular formula of $C_{52}H_{84}O_{21}$ (Δ 4.2 mmu of calcd). Analysis of the ^{13}C and ^1H NMR spectra was divided into two phases. The resonances of the aglycone were first located and comparison of these data, shown in Table 1, to that in the literature indicated this residue was identical in structure to that of penasterol[9] $C_{30}H_{47}O_3H$. The remaining count of $C_{22}H_{37}O_{18}$, plus the presence of four anomeric carbons (^{13}C NMR δ 106.0, 105.9, 105.3, 104.5), intimated four carbohydrate subunits which could be further designated as two hexoses and two pentoses based on the NMR data of Table 1. There were many ^1H and ^{13}C resonances which

were badly overlapping between, δ 3.6 - 4.1 and δ 73 - 77, respectively. Unambiguous assignments of these proton and carbon signals (Table 1) were first made by obtaining and interpreting HMQC and HMQC-TOCSY[10] NMR spectra. With this information in hand, a TOCSY NMR spectrum, together with a ^{1}H-^{1}H COSY NMR spectrum allowed the identification of four separate carbohydrate ring spin systems (Table 1) further confirming the saccharide chain was composed of two hexoses and two pentoses. Next, a ^{1}H NOESY NMR spectrum (t_{mix} = 200 ms) was used to define the relative stereochemistry of each of the carbohydrate units. Each of the pentoses were concluded, from the NOE data, to be an arabinose and not a xylose as the OH at C4 was in the axial position versus equatorial for xylose. Additional verification came from experimental J constants observed between H5'$_{ax}$-H4' (~0 Hz), H5"$_{ax}$-H4" (~0Hz) and H5"$_{eq}$-H4" (2.0 Hz). These data matched values calculated for the molecular mechanics energy minimized structure (using PC MOD™) of arabinose ($J_{H5ax-H4}$ = 1.0 Hz and $J_{H5eq-H4}$ = 2.6 Hz) and were different than J values for the minimized structure of xylose ($J_{H5ax-H4}$ = 10.6 Hz and $J_{H5eq-H4}$ = 6.2 Hz). The J = 7.5 Hz for each of the four anomeric protons indicated that all of the carbohydrate units were connected by β-glycosidic linkages.

The difficult problem of how to link these carbohydrate units together was approached using a combination the LRFAB (negative) mass spectral peaks, ^{13}C shifts and NOE data. That the tetrasaccharide was branched was initially suggested from the FABMS (negative) fragmentation pattern shown in Scheme 1. Of extreme importance were the *m/z* fragments at both 749 and 719 which were consistent with the separate loss of either a Gal and Gal-Ara or Ara and Gal-Gal moieties, indicating the tetrasaccharide was branched. These two possibilities could be distinguished by the strong NOE correlations observed from H2' (δ 3.95) to H1''' (δ 4.71), from H3' (δ 3.76) to H1" (δ 4.56) and H3" (δ 3.63) to H1'''' (δ 4.47). This NOE data clearly supported the connections shown in **1** for the carbohydrate units. Also, each of the galactoses could be concluded as being in a terminal position by comparison of chemical shift values to that of known terminal galactoses.[5] Substantiation of the link between the triterpene and Ara-I, via C3 to C1', was provided by the strong NOE observed from H3 to H1'. This connectivity relationship also explained the downfield shift of C3 in **1** (δ 91.2) versus that reported for penasterol (δ 79).[9]



Scheme 1. Interpretation of the LRFAB (negative) Mass Spectrum.

Currently, one of the best known marine sources of triterpene saponins are taxa of the phylum echinodermata.[11] In addition to this, an interesting new pattern seems to be emerging regarding the occurrence of these compounds from sponges. To date triterpene saponins or their related aglycones appear to be restricted to the Choristida (a.k.a. Astrophorida) order sponges and they are present in three of its genera, *Erylus*[4-7] (family, Geodiidae), *Asteropus*[12] (family Stellettidae), and *Penares*[9] (family Stellettidae).

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