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THE STRUCTURE OF ERYLOSIDE A, A NEW ANTITUMOR AND ANTIFUNGAL 4-METHYLATED STEROIDAL GLYCOSIDE FROM THE SPONGE *ERYLUS LENDENFELDI*

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ABSTRACT.—The structure of a new glycoside, eryloside A [**1**], isolated from the Red Sea sponge *Erylus lendenfeldi*, has been determined by 1D and 2D nmr techniques.

In search of biologically active marine natural products (1) we have isolated two new oligoglycosides named eryloside A and B from the Red Sea sponge *Erylus lendenfeldi* (Geodiidae) Sollas, and we herewith report the structure of eryloside A [**1**], the major component, which is responsible for the antitumor and antifungal activity of the crude extract.

The 15% MeOH/$CHCl_3$ extract of the sponge contains up to 3% eryloside A. Reversed-phase chromatography on an RP-18 column eluted with decreasing percentages of H_2O in MeOH afforded compound **1** which precipitated from H_2O as a white amorphous powder.

Eryloside A [**1**] showed 40 resonance lines in the ^{13}C-nmr spectrum, of which 12 could readily be assigned to two sugar units (two anomeric carbon atoms at 102.96 and 103.28 ppm). Two anomeric protons also were observed in the ^1H-nmr spectrum (Table 1). In addition, the uv spectrum, λ max (MeOH) (ε) 249 (19500) nm, together with the chemical shifts of four sp^2 carbons in the ^{13}C-nmr spectrum (Table 1) suggest a penta-substituted diene. The high degree of overlapping in certain regions of the ^1H-nmr spectrum and the relatively low solubility of **1**, which resulted in a poor long-range H-C correlation map, prevented the full structure elucidation of **1**. Nevertheless, the C-1 to C-4, C-11 to C-18, and C-20 to C-27 fragments could have been established by 2D homo- and heteronuclear experiments. Furthermore, a COSY experiment (2) together with the proton *J*-values (3) of the methinoxy groups enabled the determination of two β-galactopyranoside units in **1** (Table 2).

Acetylation of eryloside A gave an octaacetate **2**. Comparison of the ^1H-nmr data of the sugar portion of **2** with those of **1** (Table 2) and the ^4J connectivity between H-1″ and H-2′ observed in a COSYLR experiment (4) elucidated the connections between the two galactose moieties and to the aglycone; that is, the two sugar units are

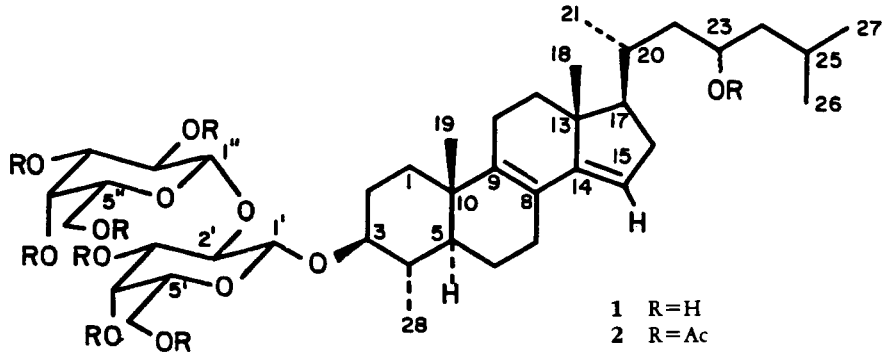

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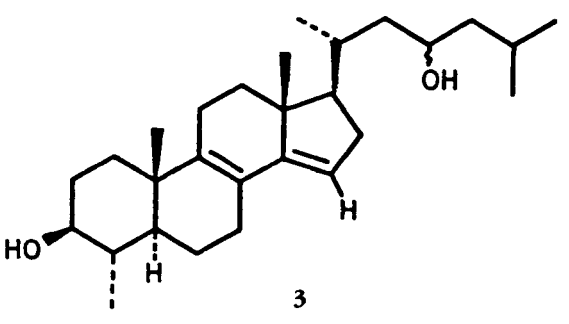
TABLE 1. ^{1}H- and ^{13}C-nmr Data of Compounds **1** and **3**.[a]

Carbon	Compound					
	1		3			
	δ_C ppm, mult	δ_H ppm (H;H′)	δ_C ppm, mult	δ_H ppm (H;H′)	Long Range H-C Correlations	
					^{2}J	^{3}J
1	35.16 t	1.77, 1.21	35.20 t	1.80, 1.20	H-2	H_3-19
2	29.50 t	2.00, 1.56	30.92 t	1.80, 1.46		
3	86.54 d	3.06	75.98 d	3.00		
4	37.62 d	1.43	38.90 d	1.29	H_3-28	H-2
5	47.52 d	1.02	47.09 d	1.03		H_3-19
6	20.44 t	1.78, 1.20	20.54 t	1.78, 1.78	H-5	
7	21.56 t	2.15, 2.08	21.64 t	2.20, 2.12		
8	122.82 s		122.83 s			
9	140.49 s		140.79 s			H-12, H_3-19
10	36.60 s		36.98 s		H_3-19	H-2
11	26.53 t	2.20, 2.05	26.74 t	2.10, 2.01		
12	36.93 t	1.98, 1.32	36.98 t	1.95, 1.34		H-17, H_3-18
13	44.94 s		45.01 s		H_3-18	H-15
14	150.83 s		150.87 s			H_3-18, H-16′
15	116.92 d	5.27	117.02 d	5.27	H-16, H-16′	
16	35.67 t	2.30, 2.01	35.87 t	2.25, 2.01	H-15	
17	57.79 d	1.41	57.78 d	1.42		H-15, H_3-18, H_3-21, H-22
18	15.39 q	0.76	15.63 q	0.77		H-12′
19	18.93 q	0.92	19.25 q	0.94		H-1, H-1′, H-5
20	30.48 d	1.83	30.58 d	1.82	H_3-21, H-22′	
21	18.55 q	0.91	18.68 q	0.92		H-22, H-22′
22	44.13 t	1.43, 0.99	44.16 t	1.42, 1.00		H_3-21
23	66.78 d	3.67	66.77 d	3.72		
24	47.79 t	1.33, 1.11	47.86 t	1.34, 1.12		H_3-26, H_3-27
25	24.46 d	1.67	24.55 d	1.65	H_3-26, H_3-27	
26	22.77 q	0.829	23.06 q	0.83		H_3-27
27	22.00 q	0.834	22.21 q	0.84		H_3-26
28	14.72 q	0.98	14.90 q	0.94		
1′	102.96 d	4.34				
2′	77.85 d	3.78				
3′	73.13 d	3.62				
4′	68.58 d	3.90				
5′	74.16 d	3.42				
6′	60.98 t	3.72, 3.67				
1″	103.28 d	4.50				
2″	70.74 d	3.64				
3″	73.13 d	3.53				
4″	68.89 d	3.82				
5″	75.31 d	3.51				
6″	61.20 t	3.73, 3.68				

[a]Both compounds were dissolved in CD_3OD-$CDCl_3$ (1:3). The field strengths were 360.13 MHz for ^{1}H and 90.53 MHz for ^{13}C.

connected through C-1″ to C-2′, and C-1′ of the disaccharide is linked to C-3 of the aglycone. The structure of the octaacetate (seven of the acetates belonging to the sugar moieties) also confirmed the C-23 hydroxyl location first suggested from the structure of aglycone **3**.



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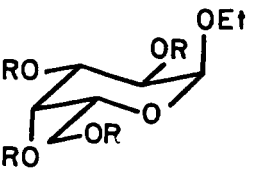

4 R=Ac
5 R=H

TABLE 2. ^{1}H-nmr Chemical Shifts (ppm) and *J*-values (Hz) of the Sugar Units of Compounds **1** and **2**.

Compound	Sugar Unit	H-1	$J_{1,2}$	H-2	$J_{2,3}$	H-3	$J_{3,4}$	H-4	$J_{4,5}$	H-5	H-6	H-6′
1	galactose′	4.34	8.0	3.78	≈9	3.62	3.5	3.90	<1	3.42	3.67	3.72
	galactose″	4.50	8.0	3.64	≈9	3.53	3.8	3.82	<1	3.51	3.68	3.73
2	galactose′	4.48	7.6	3.97	10.5	4.99	3.3	5.30	<1	3.90	4.08	4.15
	galactose″	4.75	7.9	5.11	10.4	4.96	2.8	5.37	<1	3.95	4.10	4.18

Hydrolysis of **1** with concentrated HCl-C_6H_6-EtOH (1:1:48) solution at 65° for 3 h (5) yielded two major compounds, namely, the aglycone **3** and a mixture of ethyl galactosides. Two other minor compounds, 3β-*O*-[β-D-galactopyranosyl]-23ξ-hydroxy-4α-methyl-5α-cholesta-8,14-diene and ethyl β-D-galactopyranoside, were also isolated from this reaction mixture. The structure of compound **3** was fully established by a series of hetero (^1J and long range) (6) and homonuclear correlation spectra (Table 1). Compound **3** possesses the 4α-methyl substituent, a group which is well known in zooxanthellae sterols (7). In addition, **3** embodies the naturally rare 8,14-diene (8) and 23-hydroxyl moieties.

The 4α-methyl configuration was deduced from the 11.2-Hz diaxial coupling constant between H-3α (3.00, dt, J = 5.4, 11.2 Hz) and H-4β (1.29, dd, J = 10.8, 11.2 Hz). The 10.8-Hz diaxial coupling constant between H-4β and H-5 (1.03, dddd, J = 2.3, 2.8, 10.8, 13.7 Hz) established the α configuration of the latter proton. Furthermore, the 8,14-diene moiety was suggested on the basis of the uv absorption (8), the carbon chemical shifts, and the long range CH-correlations of the vinylic carbons with the neighbor protons (Table 1). The 23-hydroxylated side chain which was suggested by both the COSY and the H-C correlation experiments (Table 1) was in full agreement with the mass spectrum fragments at *m/z* $[MH-CH_2CH(OH)CH_2CHMe_2-H]^+$ 313 (30%) and $[MH-MeCHCH_2CH(OH)CH_2CHMe_2-H]^+$ 285 (25%).

Compound **5**, the major C-1 ethyl galactoside epimer, was purified after acetylation (Ac_2O/pyridine) on a Si gel column to afford the tetraacetyl derivative **4**. Removal of the acetate groups with NH_3 followed by acid hydrolysis of the ethoxy group furnished D-galactose.

The above data suggest the 3β-*O*-[β-D-galactopyranosyl-(1,2)-β-D-galactopyranosyl]-23ξ-hydroxy-4α-methyl-5α-cholesta-8,14-diene structure for **1**.

Recently we have isolated from the sponge *Siphonochalina siphonella* another triterpene glycoside designated sipholenoside A (9). It can be expected that in the future more glycosides will be revealed from polar extracts of other sponges.

EXPERIMENTAL

Ir spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 2.5 cm microcell. Low-resolution mass spectra were recorded on a Finnigan-4021 mass spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-360 spectrometer, equipped with an Aspect 3000 computer and operated at 360.1 MHz and 90.5 MHz for ^{1}H and ^{13}C, respectively. All chemical shifts are reported with respect to TMS (δ = 0).

ISOLATION OF ERYLOSIDE A [**1**].—A sample of the sponge (YK 1396, School of Chemistry, Tel Aviv University), which was collected in the southern part of the Gulf of Eilat in July 1984 and deep-frozen immediately after collection, was lyophilized to give 100 g of dry material. Extraction of the dry material (50 g) with 15% MeOH in $CHCl_3$ solution afforded 5.1 g of crude material. The crude extract was flash chromatographed through an RP-18 column eluted with solvent of decreasing polarity from H_2O through MeOH. Eryloside A [**1**] (1.5 g, 3% dry wt) was eluted with 90% MeOH in H_2O. Precipitation from H_2O afforded a white amorphous powder, mp 214–219°, [α]D +11° (*c* = 1.5, $CHCl_3$). Found C 64.90, H 9.15; $C_{40}H_{66}O_{12}$ requires C 65.02, H 9.00. Ir (KBr) 3250 br, 2870, 1640, 1380, 1070 cm^{-1}; λ max MeOH (ε) 249 (19500) nm; cims (NH_3) *m/z* (rel. int.) 445 (12), 415 (15), 406 (33), 355 (20), 315 (33),

264 (100); ^{1}H and ^{13}C nmr see Table 1. Biological activity: antitumor P388, IC_{50} = 4.2 μg/ml; antifungal, *Candida albicans*, MIC = 15.6 μg/ml.

ACETYLATION OF **1** TO GIVE COMPOUND **2**.—Compound **1** (150 mg) was treated overnight at room temperature with Ac_2O-pyridine (1:1) (2 ml). Evaporation of the reaction mixture afforded compound **2**, an oil; ir ($CHCl_3$) 2930, 2870, 1730, 1640, 1380, 1240, 1050 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.38 brs (1H), 5.37 brd (2.8, 1H), 5.30 brd (3.3, 1H), 5.13 m (1H), 5.11 dd (10.4, 7.9, 1H), 4.99 dd (10.5, 3.3, 1H), 4.96 dd (10.4, 2.8, 1H), 4.75 d (7.9, 1H), 4.48 d (7.6, 1H), 4.18, 4.15, 4.10, 4.08 m (4H), 3.97 dd (10.5, 7.6, 1H), 3.95 brdd (6.8, 6.0, 1H), 3.90 brdd (6.7, 6.3, 1H), 3.09 brdt (4.8, 10.8, 1H), 2.16 s (3H), 2.15 s (3H), 2.06 s (3H), 2.055 (3H), 2.04 (3H), 2.01 s (3H), 1.98 s (3H), 1.12 d (6.3, 3H), 1.02 s (3H), 0.98 d (6.9, 3H), 0.92 d (6.3, 3H), 0.91 d (6.3, 3H), 0.81 s (3H).

ACID HYDROLYSIS OF COMPOUND **1** TO GIVE AGLYCONE **3** AND ETHYL GLYCOSIDE **5**.—Compound **1** (100 mg) was treated with concentrated HCl-C_6H_6-EtOH (1:1:48) (10 ml) at 65° for 3 h. After neutralization of the acid with Ag_2CO_3 (0.56 g), the slurry was filtered and the eluent evaporated under vacuum to afford a residue (115 mg) which was applied to a Sephadex LH-20 column. The fast-moving fractions contained compound **3** and the slow-moving fractions compound **5**. Compound **3**: white amorphous solid; mp 186–188°; [α]D +6° (*c* = 2, $CHCl_3$); ir ($CHCl_3$) 3450, 2930, 1980, 1650, 1280, 1200, 1050 cm^{-1}; cims (NH_3) *m/z* (rel. int.) $[MH]^+$ 415 (100), $[MH-H_2O]^+$ 397 (9), $[MH-C_6H_{14}O]^+$ 313 (3), $[MH-C_8H_{18}O]^+$ 285 (3); ^{1}H- and ^{13}C-nmr see Table 1. Compound **5**: an oil; ^{1}H nmr ($CDCl_3$) δ 4.89 d (3.3, H-1), 4.00 brs (H-4), 3.79 m (4H), 3.60–3.50 m (3H), 1.24 t (7.0, OCH_2CH_3); cims (NH_3) *m/z* (rel. int.) $[MNH_4]^+$ 226 (100), $[MH]^+$ 209 (2), $[MNH_4-EtOH]^+$ 180 (20).

ACETYLATION OF COMPOUND **5** TO GIVE COMPOUND **4**.—Compound **5** (50 mg) was treated overnight with Ac_2O-pyridine (1:1) (1 ml) to give upon evaporation under vacuum 65 mg of the crude acetylation mixture. The reaction mixture was chromatographed on a silica H column eluted with petroleum ether-EtOAc (9:1) to give pure **4** (40 mg): an oil; ^{1}H nmr ($CDCl_3$) δ 5.39 dd (0.8, 3.0, H-4), 5.30 ddd (10.4, 3.5, 1.5, H-2), 5.07 d (3.5, H-1), 5.05 dd (10.4, 3.0, H-3), 4.18 dt (0.8, 6.1, H-5), 4.05 d (6.1, H-6,6′), 3.68 dq (9.8, 7.0), 3.47 dq (10.0, 7.0, -OCH_2CH_3), 2.07 s (OAc), 2.01 s (OAc), 1.97 s (OAc), 1.92 s (OAc), 0.81 t (7.0, OCH_2CH_3); cims (NH_3) *m/z* (rel. int.) $[MNH_4]^+$ 394 (100), $[MH-EtOH]^+$ 331 (35).

HYDROLYSIS OF COMPOUND **4** TO GIVE D-GALACTOSE.—Compound **4** (40 mg) was treated for 1 h with a 10% NH_4OH/MeOH solution. The solvent was then evaporated and the residue refluxed in 10% concentrated HCl/MeOH solution overnight to give upon neutralization and evaporation a crude material (50 mg) which was applied to an RP-18 column eluted first with H_2O and then with H_2O/MeOH (1:1). Pure D-galactose was recovered from the second fraction: [α]D +83° (*c* = 10, H_2O).

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