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Mycaloside A, a new steroid oligoglycoside with an unprecedented structure from the Caribbean sponge *Mycale laxissima*

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Abstract—The structure of mycaloside A (**1**) isolated from the Caribbean sponge *Mycale laxissima* has been established as (22*E*,20*R*,24*S*)-3-*O*-{α-D-Galp(1→2)-β-D-Arap(1→3)-[β-D-Galp(1→4)]-β-D-Glcp}-3β,4β,15α,21-tetrahydroxy-24-methylcholesta-5,22-diene by interpretation of spectral data and chemical transformations. © 2002 Elsevier Science Ltd. All rights reserved.

In the animal kingdom, steroid and triterpene glycosides are the predominant metabolites of starfishes and sea cucumbers, respectively.[1–3] More recently, this type of glycoside has been isolated from some sponges. To the best of our knowledge, about 20 sponge glycosides have been reported to date, including sarasinosides from *Asteropus* spp.[4–7] and *Melophlus isis*,[8] erylosides and formosides from *Erylus* spp.,[9–12] ulososides from *Ulosa* sp.[13,14] pachastrelloside A from a *Pachastrella* sp.,[15] wondosterols from unidentified sponges and[16] ectyoplasides and feroxosides from *Ectyoplasia ferox*[17,18] The majority of these glycosides belong to nor-lanostane-triterpenoid saponins, derived from lanosterol or related triterpenes as a result of oxidative elimination of one or two methyl groups.

In the course of our continuing interest in marine oligoglycosides,[19] we have isolated a steroid oligoglycoside, mycaloside A (**1**) from the Caribbean sponge *Mycale laxissima* (Demospongiae, order Poecilosclerida,

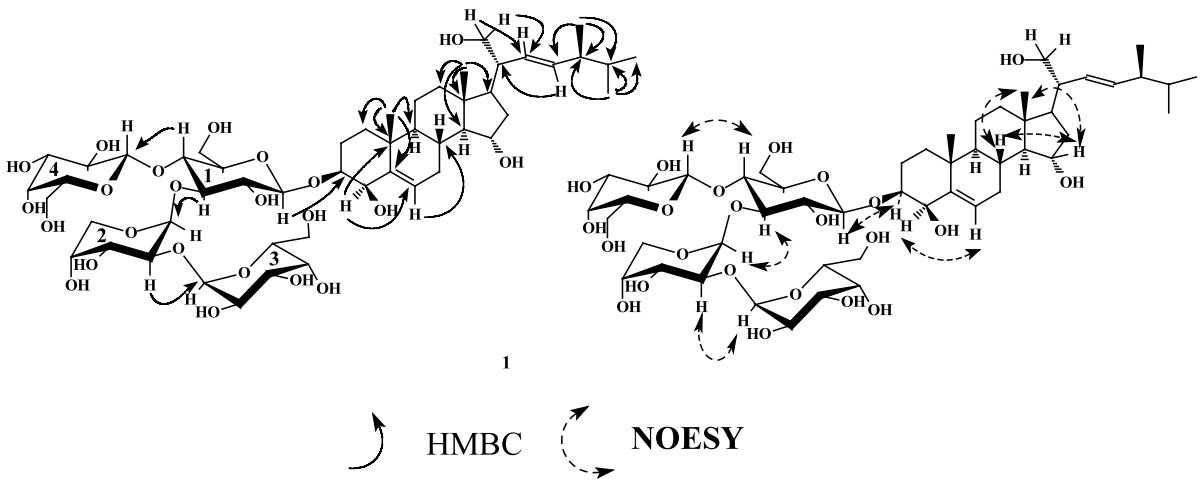

Figure 1. Key HMBC and NOESY correlations of Mycaloside A.

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family Mycalidae). The sponge was collected by hand near San-Felipe Island, Cuba. The lyophilized specimens (0.3 kg) were macerated and sequentially extracted with ethyl acetate (2×2 L) and ethanol (3×2 L). Compound **1** (28 mg) was obtained from ethanol-soluble materials by partition between water and *n*-butanol followed by column chromatography of the butanol extract on Sephadex LH-20 ($CHCl_3$–EtOH, 1:1), Polychrome-1 (50% EtOH), silica gel L ($CHCl_3$–EtOH, 5:4) and by HPLC on Zorbax ODS (45% EtOH) and Zorbax-Si ($CHCl_3$–EtOAc–MeOH, 4:6:5) columns. Mycaloside A (**1**), mp 217–220°C, $[\alpha]_D^{25}$ −23.0 (*c* 0.5, MeOH), analysed for $C_{51}H_{84}O_{23}$ by combined HRMALDI TOF MS (positive mode) and ^{13}C NMR analyses. A quasi-molecular ion peak at *m*/*z* 1087.5250 $(M+Na)^+$ ($C_{51}H_{84}NaO_{23}$ requires *m*/*z* 1087.5297) was detected. Hydrolysis of **1** with 2 M HCl (2 h, 100°C) gave glucose (1), arabinose (1) and galactose (2), identified by GLC as the corresponding aldononitrile peracetates. The monosaccharides themselves were isolated from the hydrolysate by HPLC on Separon™ SGX NH_2 column (90% acetonitrile) and shown to be of the D-series by measurement of their optical rotations.

The sequence of monosaccharides, interglycosidic linkages and configurations of glycosidic bonds in **1** were determined by NMR including HMBC and NOESY (see Fig. 1) and various ^1H–^1H COSY experiments (Table 1). In addition, the structure of the carbohydrate chain was confirmed by methylation of **1** (DMSO, NaH, CH_3I) followed by methanolysis and acetylation, which gave methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside, methyl 2-*O*-acetyl-3,4-di-*O*-methylarabinopyranoside and methyl 3,4-di-*O*-acetyl-2,6-di-*O*-methylglucopyranoside, identified by comparison with standard samples using GLC and GLC-MS. Only D-glucose was not destroyed upon periodate oxidation followed by acid hydrolysis. It was evident from consideration of

Table 1. NMR data of carbohydrate moiety in **1** (DRX500,[a] $C_5D_5N + 5\%$ CD_3OD)

C	DEPT	δ H (*J*, Hz)	NOESY	C	DEPT	δ H (*J*, Hz)	NOESY
1^1	101.8, d	4.89, d (7.8)	H-3; H-4; $H^1{}_3$; $H^1{}_5$	1^3	103.3, d	5.77, d (3.6)	$H^2{}_2$
2^1	76.3, d	4.20, dd (7.8; 9.1)	$H^1{}_4$	2^3	71.0, d[b]	4.60, dd (3.5; 10.0)	
3^1	77.4, d	4.47, t (9.2)	$H^1{}_5$	3^3	71.2, d	4.58, dd (3.1; 9.9)	
4^1	74.5, d	4.71, t (9.6)	$H^1{}_2$	4^3	70.9, d[b]	4.38[c]	
5^1	77.6, d	3.75, dt (9.8; 2.8)	$H^1{}_3$	5^3	73.1, d	4.81,ddd (1.3; 4.7; 7.3)	$H^3{}_4$; $H^3{}_3$
6^1	60.6, t	4.44[c]; 4.72[c]		6^3	62.9, t	4.27, dd (4.6; 11.3) 4.39[c]	
1^2	99.3, d	6.61, d (3.5)	$H^1{}_3$; $H^2{}_2$	1^4	104.2, d	5.31,d (7.7)	$H^1{}_4$; $H^4{}_3$; $H^4{}_5$
2^2	80.2, d	4.73, dd (3.5; 10.0)	$H^2{}_1$	2^4	72.7, d	4.41, dd (7.7; 9.4)	
3^2	69.1, d	5.19, dd (3.4; 10.0)	$H^2{}_4$	3^4	74.8, d	4.10, dd (9.5; 3.4)	$H^4{}_4$; $H^4{}_5$
4^2	70.7, d	4.84, brd (3.3)	$H^2{}_3$, $H^2{}_5$	4^4	69.2, d	4.47, brd (3.4)	$H^4{}_5$
5^2	64.4, t	3.99, dd (1.9; 12.0) 5.51, brd (12.0)	$H^2{}_4$	5^4	76.2, d	3.95, brt (6.5)	$H^4{}_4$
				6^4	61.6, t	4.33, dd (6.1; 10.8); 4.37[c]	

[a] 500 MHz for ^1H and 125.8 MHz for ^{13}C, assignments were based on COSY45, COSY RCT, DEPT and HMQC experiments.
[b] Values can be interchanged.
[c] Coupling constants were not determined as a result of overlapping of signals.

Table 2. NMR data of aglycone part in **1**[a]

C	DEPT	δ H (*J*, Hz)	NOESY	C	DEPT	δ H (*J*, Hz)	NOESY
1	37.6, t	1.77, m (e) 1.02, m (a)	H-11 H-3	15	73.4, d	4.21, td (9.0; 3.2)	H-8; CH_3-18
2	23.9, t	1.79, m (e) 2.28, m (a)	H-3	16	41.0, t	2.04, m 2.18, m	 H-15
3	80.1, d	3.84, dt (12.2; 3.0)	H-1, H-4	17	48.7, d	2.04, m	H-22
4	74.4, d	4.57, d (2.7)	H-3, H-6	18	13.9, q	0.83, s	H-8; H-11; H-20
5	141.9, s			19	21.1, q	1.40, s	H-8
6	128.7, d	5.73, dd (2.1; 5.2)	H-4	20	48.3, d	2.36, m	H-18
7	32.9, t	2.70, dt (18.9; 5.4) 2.27, m	H-6; H-8, H-15 H-6; H-9; H-14	21	64.9, t	4.01, dd (4.4; 10.5) 3.78, dd (7.0; 10.5)	H-12
8	32.1, d	1.87, m	H-15	22	132.3, d	5.48, dd (8.6; 15.4)	H-17
9	50.8, d	1.02, m		23	135.2, d	5.45, dd (7.6; 15.3)	H-20
10	36.5, s			24	43.5, d	1.95, m	
11	20.6, t	1.45, m	H-1	25	33.4, d	1.50, m	
12	39.6, t	2.01, m (e) 1.39, m (a)	H-21	26	19.7, q	0.86, d (7.0)	
13	43.2, s			27	20.2, q	0.86, d (7.0)	
14	63.4, d	1.43, m	H-9	28	18.1, q	0.96, d (7.0)	

[a] 500 MHz for ^1H and 125.8 MHz for ^{13}C, assignments were based on COSY45, COSY RCT, DEPT and HMQC experiments.

mass and NMR data, that the aglycone in **1** must be a C_{28} tetrahydroxylated steroid, probably similar to 3β,4β-dihydroxy-pregn-5-en-20-one 3-sulfate from the sponge *Stylopus australis*[20] in respect of the A and B ring structures. The 5(6)-position of the double bond and 3β,4β-dihydroxy-substitution were confirmed by ^{1}H–^{1}H COSY, HMBC and values of coupling constants (see Fig. 1 and Table 2). The presence of a hydroxy group at C-15 was determined from HMQC and COSY 45 experiments, while its α-configuration followed from H-15 coupling constants and NOESY. Further analysis of NMR data revealed the presence of an additional hydroxyl at C-21, the 22*E* double bond ($J_{22,23}=15.3$ Hz) and a C-24 methyl group in the side chain. The *S* configuration at C-24 was suggested based on comparison of the ^{13}C NMR spectrum of **1** with those of related steroids.[21] NOESY and especially HMBC data indicated that the carbohydrate chain was attached to C-3 in **1**.

Mycaloside A is the first steroid oligoglycoside isolated from sponges. So far, only nor-triterpene oligoglycosides[4–14,17,18] and a few steroid biosides with aglycones oxidized at positions 2α,3β,4β and 7α[15,16] were isolated from these animals.

Carbohydrate chains such as that of **1** have also not been found. Therefore, **1** has an unprecedented structure consisting of both carbohydrate and aglycone moieties and is unique when compared with all the previously known sponge glycosides.

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