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Melonoside A: An ω-Glycosylated Fatty Acid Amide from the Far Eastern Marine Sponge *Melonanchora kobjakovae*

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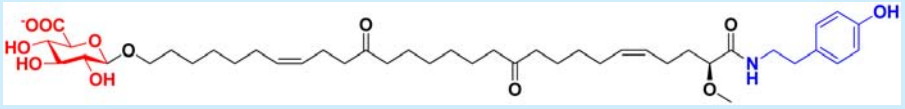
Supporting Information



ABSTRACT: Melonoside A (**1**), the first representative of a new class of ω-glycosylated fatty acid amides, was isolated from the Far Eastern marine sponge *Melonanchora kobjakovae*. The structure of **1**, including absolute configuration, was established using detailed analysis of 1D and 2D NMR, CD, and mass spectra as well as chemical transformations. Compound **1** induces autophagy of human cisplatin-resistant germinal tumor cells NCCIT-R.

Marine organisms have been recognized as a rich source of diverse bioactive metabolites with unusual chemical structures.[1] Among them, glycolipids are emerging as an important class of structurally diverse sponge metabolites that frequently have interesting biological activities.[2,3] A few representatives of glycosylated fatty acid amides, isolated from marine organisms, have been previously described,[4,5] including compounds from sponges,[6−11] a marine sediment bacterium,[12,13] and a fungus.[14] The corresponding secondary metabolites exhibit cytotoxic[10,11] and antifungal activities[12] and inhibit the binding of IL-6 to its receptor.[6,7]

In continuation of our search for bioactive natural products from the Far Eastern marine sponges,[15,16] herein, we report the isolation, structure elucidation, and bioactivity of a glycosylated fatty acid amide, melonoside A (**1**), from the marine sponge *Melonanchora kobjakovae* (Figure 1). Melonoside A possesses a new skeleton system differing from other known lipid derivatives in the structure of the lipid chain (for a list of some previously reported glycosylated fatty acid amides, see the Supporting Information (SI)). The compound contains the 2-methoxy-28-hydroxy fatty acid moiety with two ketone groups, two double bonds, and glucuronic acid and tyramine termini. Previously, metabolites from the *M. kobjakovae* were not studied, except for fatty acid composition.[17]

The EtOH extract of *M. kobjakovae* (dry weight 5.3 g) was concentrated and partitioned between aqueous EtOH and *n*-hexane. The EtOH-soluble materials were separated by ODS flash chromatography, further by Sephadex LH-20 and reversed-phase HPLC to give a difficult to separate mixture of closely related compounds and individual **1**, being one of the main constituents of the fraction, as a colorless amorphous solid (6.3 mg, 0.11% of dry weight; $[\alpha]_D$ −37 (*c* 0.18, EtOH).

The molecular formula of **1** was established as $C_{43}H_{67}NO_{12}$ on the basis of NMR and HRESIMS data (*m*/*z* 788.4590 $[M - H]^-$, calcd 788.4591 $C_{43}H_{66}NO_{12}$).

In the negative MS/MS spectrum of the parent ion at *m*/*z* 788 $[M - H]^-$, a fragment ion of *m*/*z* 612 was observed, corresponding to a loss of 176 mass units. The unit was identified as glucuronic acid after methanolysis of **1** followed by GLC of the obtained peracetylated methyl glycoside mixture in comparison to standard samples.

NMR data (CD_3OD, Table 1) of **1** supported the presence of a glucuronic acid unit attached by a β-glycoside bond (anomeric center δ_C 105.1; δ_H 4.27 d, *J* = 8.0 Hz) to a long hydrocarbon chain with two disubstituted double bonds (δ_H 5.30, 5.38; δ_C 129.8, 132.5; δ_H 5.35, 5.36; δ_C 130.5, 132.0), two ketone groups (δ_C 214.1 and 214.8), flanked by four CH_2 groups (δ_H 2.43 (6H), 2.46; δ_C 43.94, 43.96, 44.0, 44.1), and one OMe group (δ_H 3.27; δ_C 59.1). In addition, a *para*-disubstituted benzene ring (δ_H 6.69 (2H) and δ_C 116.9; δ_H 7.03 (2H) and δ_C 131.4) connected with a pair of mutually coupled methylene groups (δ_H 2.72 (2H) and 3.41(2H)) revealed a tyramine unit.

Substructures **a**−**c** were established by COSY, HSQC, and HMBC experiments (Figure 2). Substructure **a** contains a tyramine unit attached to the C-1 by an amide bond. $^1H-^{13}C$ correlation between H_2-1′ (δ_H 3.41) and C-1 (δ_C 175.8) confirmed the attachment of the tyramine residue to C-1. HMBC cross-peak $OC\underline{H}_3$/C-2 confirmed the placement of the *O*-methyl ether unit at C-2. Location of the double bond at C-5/C-6 was assigned through COSY experiments and HMBC correlations.

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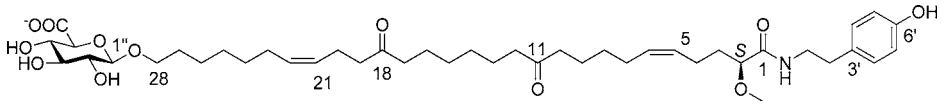

Figure 1. Structure of melonoside A (**1**).

Table 1. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR Data of Melonoside A (1)

position	δ_C[a]	δ_H mult (J in Hz)	COSY	HMBC	position	δ_C[a]	δ_H mult (J in Hz)	COSY	HMBC
1′	42.3	3.41, t (7.3)	2′	1, 2′, 3′	13	25.3[c]	1.52, m	12, 14	11, 12, 14
2′	36.3	2.72, m	1′	1′, 4′, 8′	14	30.6	1.27, m	13	12, 13, 15
3′	131.6				15	30.6	1.27, m	16	14, 16, 17
4′, 8′	131.4	7.03, d (8.5)	5′, 7′	2′, 5′, 6′, 7′	16	25.1[c]	1.52, m	15, 17	15, 17, 18
5′, 7′	116.9	6.69, d (8.5)	4′, 8′	3′, 4′, 6′, 8′	17	44.0[c]	2.43, m	16	15, 16, 18
6′	157.6				18	214.1			
NH		7.89[d]	1′		19	44.1	2.46, t (7.3)	20	18, 20, 21
1	175.8				20	23.3	2.27, m	19, 21	18, 19, 21, 22
2	83.5	3.54, dd (4.4, 7.5)	3a, 3b	1, 3, 4, −OCH$_3$	21	129.8	5.30, m	20, 22	19, 20
OCH$_3$	59.1	3.27, s		2	22	132.5	5.38, m	21, 23	23, 24
3a	34.7	1.60, m	4a, 4b	1, 2, 4, 5	23	28.7[c]	2.04, m	22, 24	21, 22, 24
3b		1.66, m	4a, 4b	1, 4, 5	24	30.8[c]	1.34, m	23	22
4a	24.3	2.03, m	3a, 3b, 4b, 5	2, 3, 5, 6	25	30.6	1.27, m		
4b	2.11, m	3a, 3b, 4a, 5	2, 3, 5, 6		26	27.6	1.38, m	27	27
5	130.5	5.35, m	4a, 4b	3, 4	27	31.4	1.61, m	26, 28a, 28b	25, 27, 28
6	132.0	5.36, m	7	4, 7	28a	71.6	3.52, dt (6.9, 9.3)	27, 28b	26, 27, 1″
7	28.5	2.03, m	6, 8	8, 9	28b		3.91, dt (6.9, 9.3)	27, 28a	26, 27, 1″
8	30.9[c]	1.32, m	7, 9	6, 9, 10	1″	105.1	4.27, d (8.0)	2″	28, 3″
9	25.2[c]	1.54, m	8, 10	7, 8, 10	2″	75.5	3.21, t (8.0)	1″, 3″	1″, 3″
10	43.96[c]	2.43, m	9	7, 11	3″	78.4	3.38, m	2″, 4″	4″
11	214.8				4″	74.2	3.47, t (8.7)	3″, 5″	
12	43.94[c]	2.43, m	13	11, 13, 14	5″	75.6	3.65, m	4″	
					6″	nd[b]			

[a]^{13}C NMR assignments supported by HSQC and HMBC data. [b]Not detected. [c]Signals may be interchangeable. [d]Exchanged with a deuterium oxide.

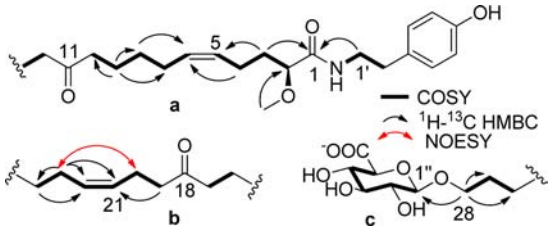

Figure 2. Partial structures of **1** with selected COSY, HMBC, and NOESY correlations.

Substructure **b** consists of one keto group separated from the disubstituted double bond by two CH_2 groups.

The *Z*-geometry of double bonds in both substructures was inferred from the carbon chemical shifts of the adjacent allylic carbons, which have chemical shifts at lower values than 29 ppm[18,19] and correlation between H-20 and H-23 in the NOESY spectra (Table 1).

Substructure **c** contains a glucuronic acid residue attached by a *β*-*O*-glycosidic bond to C-28. Characteristic NMR chemical shifts of the anomeric H-1″ (δ_H 4.27) and C-28 (δ_C 71.6), together with HMBC correlations between the two signals, confirmed the location of glucuronic acid at the end position. However, the assembly of these substructures was difficult due to several NMR overlapping methylene groups.

Initial attempts to determine the position of ketone groups in **1** by either MS analysis of Baeyer−Villiger oxidation products or reductive ozonolysis of **1** and **2** were unsuccessful. Unambiguous data were obtained when **1** was subjected to ozonolysis in methanol at −70 °C. This reaction yielded **3**, which was analyzed by HRESIMS. The peak of the deprotonated molecule $[M-H]^-$ was observed at m/z 353.1456 (calcd for **3** ($C_{14}H_{26}O_{10}$), 353.1453), which permitted determination of the position of one double bond at C-21.

The exact location of the ketone group and double bond positions was achieved by the following chemical transformations (Figure 3). Compound **1** was transformed into 11,18-diaminomelonoside A (**2**) by reductive amination with sodium cyanoborohydride in ammonium acetate solution. Then,

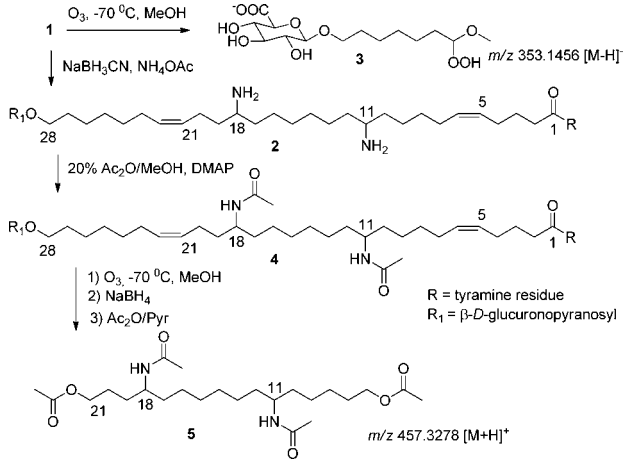

Figure 3. Derivatization and ozonolysis of **1**.

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amino groups in **2** were acetylated with 20% Ac_2O in MeOH in the presence of catalytic amounts of DMAP. *N*,*N*′-Diacetamide of melonoside A (**4**) was subjected to ozonolysis followed by reductive workup and acetylation. These reactions yielded **5**, which was confirmed by HRESIMS and GC−MS (Figure 4).

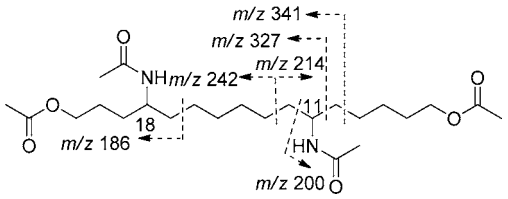

Figure 4. EIMS fragmentations (70 eV) of **5**.

The HRESIMS of **5** exhibited a peak of the protonated molecule at 457.3278 $[M + H]^+$ (calcd for $C_{24}H_{45}N_2O_6$, 457.3272), confirming the location of the double bonds at C-5 and C-21. The position of carbonyl groups at C-11 and C-18 was determined on the basis of mass spectral fragmentation of **5** by EIMS (Figure 4).

Determination of the absolute configuration of the sugar unit was carried out after acid hydrolysis of **1** with 2 M TFA followed by preparation of acetylated (*R*)-octyl glycosides. GC analysis of these compounds and comparison with authentic samples revealed the D-configuration of the glucuronic acid.

Absolute configuration of the C-2 asymmetric center in **1** was assigned by analysis of the CD spectra of the aglycone melonoside A (**6**), model compounds **7a**, **7b**, **8a**, and **8b**, and literature data for CD spectra of 2-methoxy fatty acids (Figure 5).[18,20,21] CD data for (*R*)-2-methoxypropionic acid (**8a**) and

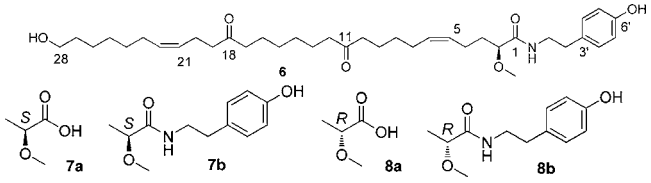

Figure 5. Structures of tyramine derivative **6** and model compounds **7a**, **7b**, **8a**, and **8b**.

(*R*)-2-methoxy fatty acid methyl esters[20,21] demonstated a strong negative Cotton effect at 210−212 nm and a weaker positive one at 240 nm. We attempted to hydrolyze the amide bond in **6** and to isolate a fatty acid moiety, but these efforts were unsuccessful. Model compounds **7b** and **8b** with tyramine residue were synthesized (SI). Tyramine derivatives (**7b**, **8b**) have opposite Cotton effects when compared with acids (**7a**, **8a**). These data showed that the sign of a Cotton effect is independent of the length of the hydrocarbon chain in 2-methoxy acids but is highly sensitive to amidation by tyramine.

The CD spectrum of **6** displayed a negative Cotton effect at λ_{max} = 200 nm ($\Delta\varepsilon$ = −2.53) and 220 nm ($\Delta\varepsilon$ = −2.13), similar to the CD spectrum of **7b**, indicating the *S*-configuration of C-2 in **1** (SI). The proposed configuration is opposite in comparison to that reported for naturally occurring 2-methoxy fatty acids, which are known to possess the *R*-configuration.[18,20−22] So far, 2*S*-hydroxylated fatty acids have been detected only in some bacterial lipopolysaccharides.[23−26]

Melonoside A (**1**) induces autophagy of human cisplatin-resistant germinal tumor cells NCCIT-R, decreasing the expression of autophagy-related proteins LC3B-II and SQSTM1/p62 at a concentration of 10 μM (SI).

In summary, **1** represents a new class of glycolipid derivatives with an unprecedented C_{43} skeleton consisting of glucuronic acid, multifunctionalized fatty acids, and tyramine. The most unique structural feature of **1** is the presence of (2*S*,5*Z*,21*Z*)-28-hydroxy-2-methoxy-11,18-dioxooctacosa-5,21-dienoic acid, which was never described before from natural sources. The novel structure of **1**, combined with its ability to induce autophagy, should make it a useful chemical biology tool.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01678.

Experimental details, copies of 1D and 2D NMR, CD spectra of **1**, and bioassay results (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

§In memory of Vladimir B. Krasokhin.

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