JOURNAL OF NATURAL PRODUCTS

Note

Cite This: J. Nat. Prod. XXXX, XXX, XXX−XXX

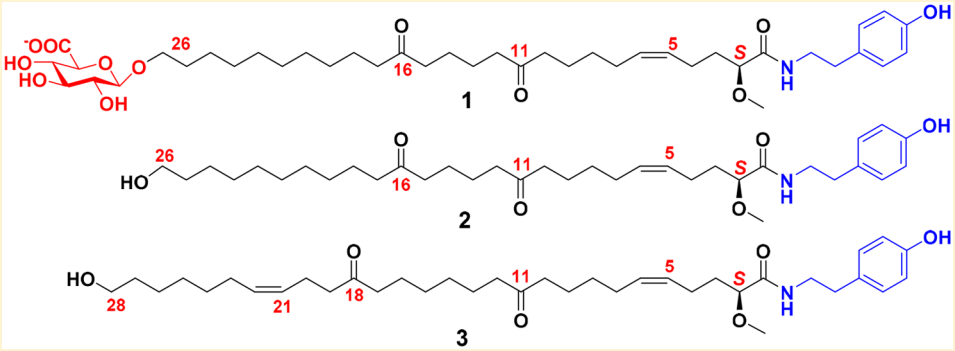
pubs.acs.org/jnp

# Melonoside B and Melonosins A and B, Lipids Containing Multifunctionalized ω-Hydroxy Fatty Acid Amides from the Far Eastern Marine Sponge *Melonanchora kobjakovae*

Alla G. Guzii, Tatyana N. Makarieva,* Vladimir A. Denisenko, Pavel S. Dmitrenok, Roman S. Popov, Alexandra S. Kuzmich, Sergey N. Fedorov, Vladimir B. Krasokhin, Natalya Yu. Kim, and Valentin A. Stonik

G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Prospect 100-let Vladivostoku 159, Vladivostok 690022, Russian Federation

Supporting Information




**ABSTRACT:** Melonoside B (**1**) and melonosins B (**2**) and A (**3**), new lipids based on polyoxygenated fatty acid amides, and known melonoside A (**4**) were isolated from two different collections of the marine sponge *Melonanchora kobjakovae*. The structures of these compounds, including their absolute configurations, were established using detailed analysis of 1D and 2D NMR, ECD, and mass spectra as well as chemical transformations. Melonosins **2** and **3** inhibit AP-1- and NF-kB-dependent transcriptional activities in JB6 Cl41 cells at noncytotoxic concentrations, demonstrating potential cancer preventive activity.

Marine sponges have been reported to be a source of rare oxygenated fatty acid amides with unprecedented chemical structures and biological activities.[1−14] Recently we have isolated and structurally elucidated the first member of a new class of ω-*O*-glycosylated polyoxygenated fatty acid amides, melonoside A (**4**), from the marine sponge *Melonanchora kobjakovae*.[15] Three related compounds, named myxillines A−C, were almost simultaneously isolated from the marine sponge *Myxilla incrustans* by Icelandic chemists.[16] Melonoside A and myxillines A−C represent an unexpected departure from the structures of other lipids from marine as well as terrestrial organisms. Herein we report the structures and biological activities of three new lipids of this series, melonoside B (**1**) and melonosins B (**2**) and A (**3**), containing ω-hydroxylated fatty acid amide cores. Melonoside B (**1**) was isolated from a collection of the sponge *M. kobjakovae* acquired near Urup Island (Kurile Islands, Sea of Okhotsk). The extracts from this sponge contained a complicated mixture of glycosylated lipids, including melonoside A (**4**), that was difficult to separate. Another collection of the sponge in waters of the neighboring Iturup Island contained predominantly non-glycosylated compounds and afforded melonosins A (**3**) and B (**2**).

The EtOH extract of the sponge *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, followed by Sephadex LH-20 column chromatography and reversed-phase HPLC, to give known melonoside A (**4**)[15] and previously unknown melonoside B (**1**, 8.5 mg). Following the extraction and purification methods used for melonosides B and A, another sample of *M. kobjakovae* (dry weight 24.2 g) was processed and afforded two new non-glycosylated derivatives, named melonosin B (**2**, 1.3 mg) and melonosin A (**3**, 2.6 mg).

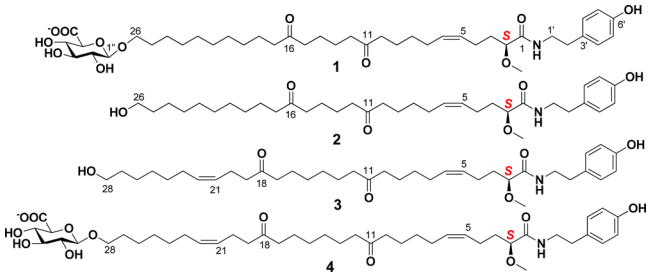
The molecular formula of **1** was determined to be $C_{41}H_{65}NO_{12}$ from the $[M - H]^-$ ion peak at $m/z$ 762.4426 in the (−)HRESIMS spectrum. In the corresponding MS/MS spectrum of the parent ion at $m/z$ 762 $[M - H]^-$, a fragment ion peak at $m/z$ 586 was observed, showing the loss of 176 mass units. The corresponding fragment was identified as D-glucuronic acid after hydrolysis of **1** with 2 M trifluoroacetic acid (TFA) at 100 °C followed by the preparation of

Received: September 13, 2018





DOI: 10.1021/acs.jnatprod.8b00785
J. Nat. Prod. XXXX, XXX, XXX−XXX



acetylated (*R*)-2-octylglycosides and GC of these compounds by comparison with standard samples.

The $^{1}H$ and $^{13}C$ NMR data ($CD_3OD$, Table 1) of **1** supported the presence of a glucuronic acid unit attached by a *β*-glycosidic bond (anomeric atoms $\delta_C$ 105.1; $\delta_H$ 4.26 d, $J$ = 7.7 Hz) to a long hydrocarbon chain with one disubstituted double bond ($\delta_H$ 5.34 and 5.38; $\delta_C$ 130.5 and 132.0), two ketone carbonyls ($\delta_C$ 214.6 and 214.8) flanked by polymethylene chains, and one methoxy group ($\delta_H$ 3.28; $\delta_C$ 59.1). In addition, a *para*-disubstituted benzene ring ($\delta_H$ 6.69 (2H) and $\delta_H$ 7.03 (2H); $\delta_C$ 116.9 and $\delta_C$ 131.4) connected with a

**Table 1. $^{1}H$ (700 MHz) and $^{13}C$ (175 MHz) NMR Spectroscopic Data for 1, 2, and 3 in $CD_3OD$**

| | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| position | $\delta_C$,[a] type | $\delta_H$ mult ($J$ in Hz) | $\delta_C$,[a] type | $\delta_H$ mult ($J$ in Hz) | $\delta_C$,[a] type | $\delta_H$ mult ($J$ in Hz) |
| 1′ | 42.3, $CH_2$ | 3.41, t (7.0) | 42.3, $CH_2$ | 3.41, t (7.3) | 42.3, $CH_2$ | 3.41, t (7.3) |
| 2′ | 36.3, $CH_2$ | 2.72, m | 36.3, $CH_2$ | 2.73, m | 36.3, $CH_2$ | 2.72, m |
| 3′ | 131.6, C | | 131.6, C | | 131.6, C | |
| 4′, 8′ | 131.4, CH | 7.03, d (8.5) | 131.4, CH | 7.04, d (8.5) | 131.4, CH | 7.04, d (8.5) |
| 5′, 7′ | 116.9, CH | 6.69, d (8.5) | 116.9, CH | 6.70, d (8.5) | 116.9, CH | 6.70, d (8.5) |
| 6′ | 157.6, C | | 157.6, C | | 157.6, C | |
| NH | | 7.89[c] | | | | |
| 1 | 175.8, C | | 175.8, C | | 175.8, C | |
| 2 | 83.5, CH | 3.54, m | 83.5, CH | 3.54, m | 83.5, CH | 3.54, m |
| $OCH_3$ | 59.1, $CH_3$ | 3.28, s | 59.1, $CH_3$ | 3.28, s | 59.1, $CH_3$ | 3.28, s |
| 3a | 34.7, $CH_2$ | 1.60 m | 34.7, $CH_2$ | 1.60, m | 34.7, $CH_2$ | 1.60, m |
| 3b | | 1.66 m | | 1.66, m | | 1.66, m |
| 4a | 24.3, $CH_2$ | 2.03, m | 24.3, $CH_2$ | 2.04, m | 24.3, $CH_2$ | 2.04, m |
| 4b | | 2.11, m | | 2.11, m | | 2.11, m |
| 5 | 130.5, CH | 5.34, m | 130.5, CH | 5.35, m | 130.5, CH | 5.35, m |
| 6 | 132.0, CH | 5.38, m | 132.0, CH | 5.37, m | 132.0, CH | 5.37, m |
| 7 | 28.5, $CH_2$ | 2.03, m | 28.5, $CH_2$ | 2.04, m | 28.5, $CH_2$ | 2.04, m |
| 8 | 30.9[b], $CH_2$ | 1.32, m | 30.9[b], $CH_2$ | 1.34, m | 30.9[b], $CH_2$ | 1.34, m |
| 9 | 25.2,[b] $CH_2$ | 1.54, m | 25.2,[b] $CH_2$ | 1.54, m | 25.2,[b] $CH_2$ | 1.54, m |
| 10 | 43.96,[b] $CH_2$ | 2.43, m | 43.9,[b], $CH_2$ | 2.43, m | 43.94,[b] $CH_2$ | 2.43, m |
| 11 | 214.8, C | | 214.8,[b] C | | 214.7, C | |
| 12 | 43.94,[b] $CH_2$ | 2.43, m | 44.1,[b] $CH_2$ | 2.43, m | 44.10,[b] $CH_2$ | 2.43, m |
| 13 | 25.3,[b] $CH_2$ | 1.52, m | 25.5,[b] $CH_2$ | 1.54, m | 25.3,[b] $CH_2$ | 1.54, m |
| 14 | 25.1,[b] $CH_2$ | 1.52, m | 25.1,[b] $CH_2$ | 1.54, m | 30.6,[b] $CH_2$ | 1.28, m |
| 15 | 44.09,[b] $CH_2$ | 2.43, m | 43.8, $CH_2$ | 2.43, m | 30.6,[b] $CH_2$ | 1.28, m |
| 16 | 214.6, C | | 214.6,[b] C | | 25.1,[b] $CH_2$ | 1.52, m |
| 17 | 44.13,[b] $CH_2$ | 2.43, m | 43.8, $CH_2$ | 2.43, m | 43.98,[b] $CH_2$ | 2.43, m |
| 18 | 25.1,[b] $CH_2$ | 1.52, m | 25.1,[b] $CH_2$ | 1.54, m | 214.6, C | |
| 19 | 31.3,[b] $CH_2$ | 1.27, m | 31.2,[b] $CH_2$ | 1.27, m | 43.95,[b] $CH_2$ | 2.47, t (7.3) |
| 20 | 31.2,[b] $CH_2$ | 1.27, m | 31.1,[b] $CH_2$ | 1.27, m | 23.3, $CH_2$ | 2.28, m |
| 21 | 31.1,[b] $CH_2$ | 1.27, m | 31.08,[b] $CH_2$ | 1.27, m | 129.8, CH | 5.31, m |
| 22 | 31.0,[b] $CH_2$ | 1.27, m | 31.02,[b] $CH_2$ | 1.27, m | 132.5, CH | 5.38, m |
| 23 | 30.9,[b] $CH_2$ | 1.27, m | 30.87,[b] $CH_2$ | 1.27, m | 28.7, $CH_2$ | 2.04, m |
| 24 | 27.6, $CH_2$ | 1.38, m | 27.5, $CH_2$ | 1.35, m | 30.8,[b] $CH_2$ | 1.34, m |
| 25 | 31.3, $CH_2$ | 1.60, m | 34.3, $CH_2$ | 1.52, m | 30.6,[b] $CH_2$ | 1.27, m |
| 26a | 71.6, $CH_2$ | 3.52, m | 63.6, $CH_2$ | 3.53, t (6.7) | 27.4, $CH_2$ | 1.35, m |
| 26b | | 3.91, m | | | | |
| 27 | | | | | 34.2, $CH_2$ | 1.53, m |
| 28 | | | | | 63.6, $CH_2$ | 3.53, t (6.7) |
| 1″ | 105.1, CH | 4.26, d (7.7) | | | | |
| 2″ | 75.5, CH | 3.21, t (8.0) | | | | |
| 3″ | 78.4, CH | 3.38, m | | | | |
| 4″ | 74.2, CH | 3.47, t (8.7) | | | | |
| 5″ | 77.0, CH | 3.65, m | | | | |
| 6″ | Nd[d] | | | | | |

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



DOI: 10.1021/acs.jnatprod.8b00785
J. Nat. Prod. XXXX, XXX, XXX−XXX

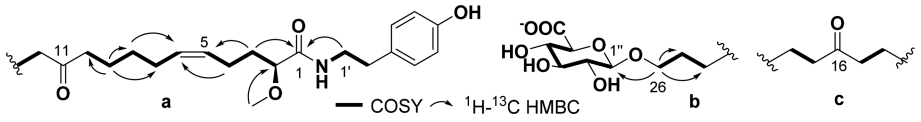
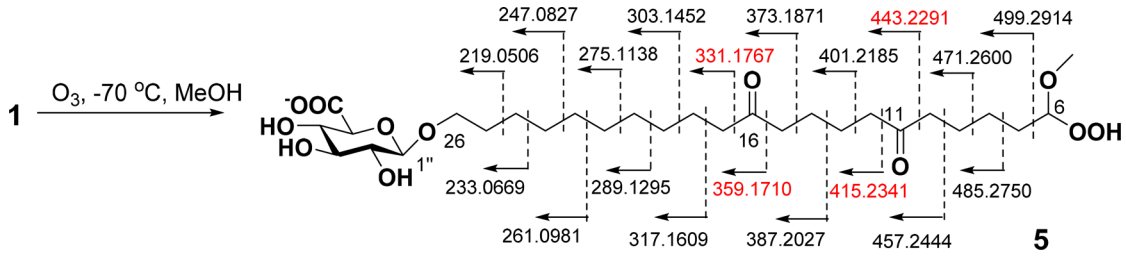
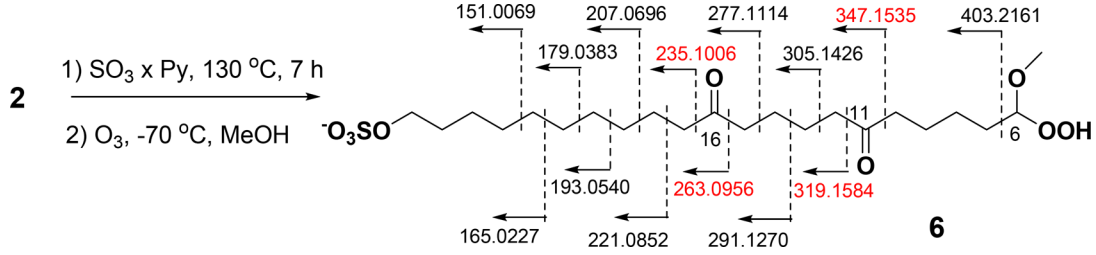



**Figure 1.** Partial structures of **1** with selected COSY and HMBC correlations.

**Scheme 1. Ozonolysis of 1 and Sequential Fragmentation in 5 by (−)HRESIMS/MS**




**Scheme 2. Derivatization of 2 and Key Fragmentation in 6 by (−)HRESIMS/MS**




pair of mutually coupled methylene groups ($\delta_H$ 2.72 (2H) and 3.41(2H)) revealed a tyramine unit.

Substructures **a**−**c** were established by COSY, HSQC, and HMBC experiments (Figure 1). The $^{1}$H−$^{13}$C correlation between H$_2$-1′ ($\delta_H$ 3.41) and C-1 ($\delta_C$ 175.8) confirmed the attachment of the tyramine residue (**a**) to C-1. The HMBC cross-peak OC<u>H</u>$_3$/C-2 confirmed the placement of the *O*-methyl ether unit at C-2. The position of the double bond at C-5/C-6 was assigned through COSY experiments and HMBC correlations (Figure 1). The *Z*-configuration of the double bond was inferred from the chemical shifts of the adjacent allylic carbons at lower values than 29 ppm.[17,18] The tetramethylene spacer between the double bond and a ketone group was established by COSY and HMBC correlations (Table 1).

Substructure **b** contains the glucuronic acid residue attached by a *β*-*O*-glycosidic bond to C-26. Characteristic NMR chemical shifts of an anomeric H-1″ ($\delta_H$ 4.26) and C-26 ($\delta_C$ 71.6), together with an HMBC correlation between these signals, confirmed the location of the glucuronic acid moiety at the terminus of a chain.

Substructure **c** consists of another keto group flanked by polymethylene chains. However, assembling these substructures proved to be difficult due to several overlapping methylene signals in the NMR spectra.

Initial attempts to specify the positions of the keto groups in **1** by ESIMS/MS were unsuccessful using either negative or positive mode spectra. The ESIMS spectra of **1** showed only one interpreted signal, corresponding to the loss of a glucuronic acid unit.

The exact locations of the carbonyl groups and the double bond in the alkyl chain were finally confirmed when **1** was subjected to ozonolysis in MeOH at −70 °C. This reaction yielded the methoxy hydroperoxide **5**, which was analyzed by (−)HRESIMS/MS. The [M − H]$^-$ ion peak was observed at *m*/*z* 577.3231, permitting the determination of the position of a double bond at C-5 (Scheme 1). Sequential fragmentation of this precursor ion in the (−)HRESIMS/MS spectrum (Table S1, Supporting Information) gave fragment ion peaks at *m*/*z* 443.2291 and 415.2341, 359.1710, and 331.1767 and allowed us to place the carbonyl groups at C-11 and C-16. The very weak fragment ion peak observed at *m*/*z* 345.1557, presumably arising from the 17-oxo isomer, was present as a small inseparable impurity in **1**.

In order to determine the absolute configuration of the C-2 stereogenic center, acid hydrolysis of glycoside **1** with 5% HCl in MeOH at 100 °C was carried out. The electronic circular dichroism (ECD) spectrum of the aglycon, obtained from melonoside B, was compared with the ECD spectrum of the melonoside A aglycon, in which the corresponding absolute configuration was established earlier.[15] Both ECD spectra displayed similar negative Cotton effects (Figure S24, Supporting Information), allowing us to establish the same 2*S*-configuration for compound **1**.

Melonosin B (**2**) has the molecular formula $C_{35}H_{57}NO_6$, which was established by (−)HRESIMS measurement of the [M − H]$^-$ peak at *m*/*z* 586.4111. The NMR spectroscopic data of **2** were shown to be related to those of **1** and differed only in the absence of glucuronic acid residue signals, agreeing with the molecular mass difference of 176 amu between **1** and **2**. The signals of the CH$_2$ group at $\delta_H$ 3.52 and $\delta_C$ 63.6 in the NMR spectra indicated a hydroxy group at the non-amidated end of the chain (*ω*-terminus).

Although the NMR and HRESIMS data of natural **2**[19] were essentially the same as those of the aglycon, obtained from melonoside B, these compounds could be positional isomers of the ketone carbonyls.[14] Because the keto groups in **2** are placed in a long methylene chain, it was not possible to determine



DOI: 10.1021/acs.jnatprod.8b00785
J. Nat. Prod. XXXX, XXX, XXX−XXX

their exact locations on the basis of the NMR data. Moreover, the HRESIMS and HRESIMS/MS of **2** practically did not show $\alpha$ or $\beta$ cleavage fragment ions. However, the sequential fragmentation in the (−)HRESIMS/MS spectra of methoxy hydroperoxide **5** with a negative charge at the $\omega$-terminus allowed us to exactly locate the carbonyl groups in the related compound **1**. That is why we have synthesized the methoxy hydroperoxide **6** with a negatively charged sulfate at the $\omega$-terminus (Scheme S1, Supporting Information) and analyzed this compound by negative ion mode HRESIMS/MS (Scheme 2).

The peak of the deprotonated molecule $[M - H]^-$ in the mass spectrum of **6** was observed at $m/z$ 481.2479, permitting the determination of the double-bond position at C-5 (Scheme 2). The fragment ion peaks at $m/z$ 347.1535 and 319.1584 in the (−)HRESIMS/MS spectrum of the $[M - H]^-$ ion at $m/z$ 481.2479 (Table S2) allowed us to locate one carbonyl group at position 11. The ion peaks at $m/z$ 263.0956 and 235.1006 show that **2** contains the second keto group at position 16.

Comparison of the ECD spectra (Figure S24) and the specific rotation of melonosin B (**2**) with those of aglycons obtained from melonosides A and B suggested the same 2*S*-absolute configuration in **2**. All data confirmed that **2** is identical to the aglycon obtained from acid hydrolysis of **1**.

The molecular formula of melonosin A (**3**) was determined to be $C_{37}H_{59}NO_6$ from (−)HRESIMS measurement of the $[M - H]^-$ ion at $m/z$ 612.4267. Its spectroscopic properties were similar to those of **2** (Table 1). The differences between the NMR spectra of **3** and **2** were from the signals belonging to two $CH_2$ groups located between a keto group and one of the disubstituted double bonds. The peak of the deprotonated molecule $[M - H]^-$ in the (−)HRESIMS spectrum was the same as that of the aglycon, obtained earlier from melonoside A as a result of acid hydrolysis.[15]

The corresponding positions of the keto groups and the double bonds in **3** were also confirmed by chemical transformations (Scheme S2). In fact, compound **3** was transformed into a mixture of melonosin A sulfates. Ozonolysis of the mixture and analysis of the degradation products by HRESIMS confirmed the double-bond positions at C-5 and C-21 and the carbonyl groups at C-11 and C-18 in **3** (Figure S2). The ECD spectrum of **3** displayed negative Cotton effects at $\lambda_{max}$ = 200 nm ($\Delta\varepsilon$ = −2.23) and 220 nm ($\Delta\varepsilon$ = −2.13), similar to the ECD spectrum of melonoside A aglycon.[15] This confirmed the *S*-configuration of C-2 in **3** (Figure S24). Therefore, **3** is without a doubt identical to the melonoside A aglycon, obtained by acid hydrolysis of this glycoside.

Thus, melonoside B as well as melonosins A and B proved to be new natural products. Melonosin A (**3**) contains the oxygenated (2*S*,5*Z*,21*Z*)-28-hydroxy-2-methoxy-11,18-dioxooctacosa-5,21-dienoic acid residue previously found only in melonoside A (**4**) from the same sponge *M. kobjakovae*.[15] The most unique structural feature of melonoside B (**1**) and melonosin B (**2**) is the presence of the core (2*S*,5*Z*)-26-hydroxy-2-methoxy-11,16-dioxohexacosa-5-enoic acid, never described before from natural sources. They contain two keto groups at positions 11 and 16, but not 11 and 18 as in **4**[15] or 11 and 17 as in myxillins A−C.[16] The skeletal systems of **1**−**4** and myxillin C include tyramine moieties, while myxillins A and B possess dopamine-derived fragments. Melonosins are most likely biosynthetic precursors of the melonosides.

To assess the biological activities of the compounds studied, their influence on the AP-1, NF-kB, and/or p53 transcriptional activation was investigated in JB6 Cl41 cells stably expressing a luciferase reporter gene controlled by the AP-1, NF-kB, or p53 DNA binding sequences. However, **1** and **4**, which possess glucuronic acid moieties, were not active in these experiments. Conversely, non-glycosylated compounds **2** and **3** inhibited oncogenic AP-1 and NF-$\kappa$B transcriptional activities at noncytotoxic concentrations (25−37 $\mu$M for **2**) and (6−12 $\mu$M for **3**), but did not affect the p53 tumor suppressor protein. In particular, melonosin A (**3**), which has two double bonds, showed $IC_{50}$ values of 7.0 and 7.2 $\mu$M against AP-1 and NF-$\kappa$B nuclear factors, respectively. At the same time melonosin B (**2**), containing only one double bond, was found to be less active, inhibiting AP-1 or NF-$\kappa$B activities only to 87% or 67%, respectively, at the concentration of 25 $\mu$M.

Thus, we propose that the presence of double bonds and/or sugar portions in these bipolar lipids plays a crucial role in their influence on the AP-1 or NF-$\kappa$B nuclear factors. It is interesting that aglycons of some other marine lipids, such as two-headed sphingolipids, were also more bioactive when compared to glycosides. In that case, the sugar elimination was important for the ability to suppress AR-signaling.[20] Melonosin A (**3**) might be considered as a new potential cancer preventive agent, which inhibits oncogenic AP-1 or NF-$\kappa$B nuclear factor activities at noncytotoxic concentrations independently of the tumor suppression protein p53. Taking into consideration that many cancer cell lines contain mutated, inactive p53, this topic is of interest.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured using a PerkinElmer 343 polarimeter. UV spectra were recorded on a Shimadzu UV-1601 PC spectrophotometer. ECD spectra were recorded with an Applied Photophysics Chirascan plus spectropolarimeter. IR spectra were recorded using a Bruker Equinox 55 spectrophotometer. The $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker Avance III-700 spectrometer at 700 and 175 MHz, respectively, with $Me_4Si$ as an internal standard. ESI mass spectra (including HRESIMS) were obtained on a Bruker maXis Impact II Q-TOF mass spectrometer (Bruker Daltonics) by direct infusion in MeOH. GC analysis was conducted on an Agilent 6580 Series apparatus equipped with an HP-5 MS capillary column (30 m × 0.25 mm) with He as carrier gas (1.7 mL/min) using a temperature gradient of 100−270 °C at 5 °C/min. Temperatures of the injector and detector were 250 and 300 °C, respectively. Low-pressure column liquid chromatography was performed using YMC*Gel ODS-A (YMC Co., Ltd.) and Sephadex LH-20 (Sigma) columns. HPLC was performed using a Shimadzu Instrument equipped with an RID-10A differential refractometer and a YMC-ODS-Am (250 × 10 mm) column.

**Animal Material.** The sponge *Melonanchora kobjakovae* (registration number PIBOC O41-135) was collected by dredging during the 41st scientific cruise of the R/V *Academic Oparin*, in July 2011, near Urup Island, Sea of Okhotsk (46°02,1 N; 149°55,3 E, depth 121 m). Another sample of *M. kobjakovae* (registration number PIBOC O47-004) was collected by dredging during the 47th scientific cruise of R/V *Academic Oparin*, in August 2015, near Iturup Island, Sea of Okhotsk (45°44,4 N; 148°33,4 E, depth 263 m). Voucher specimens of the both samples are stored in the marine invertebrate collection of the G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS (Vladivostok, Russia).

**Extraction and Isolation.** The EtOH extract of the sponge *M. kobjakovae* (registration number O41-135, dry weight 5.3 g) was concentrated and partitioned between *n*-hexane and aqueous EtOH. The EtOH-soluble materials were subjected to column chromatography on a reversed-phase YMC*Gel ODS-A with a stepped gradient from $H_2O$ to EtOH. The fraction that eluted with EtOH−$H_2O$



DOI: 10.1021/acs.jnatprod.8b00785
J. Nat. Prod. XXXX, XXX, XXX−XXX

(80:20) was purified by Sephadex LH-20 (EtOH) column chromatography. Preparative HPLC of the obtained mixture (YMC-ODS-Am, 55:45:1% EtOH–$H_2O$–$AcONH_4$) gave pure melonoside A (**4**, 6.3 mg, 0.11% based on dry weight of the sponge)[15] and melonoside B (**1**, 8.5 mg, 0.16% based on dry weight of the sponge).

The EtOH extract of the sponge *M. kobjakovae* (registration number PIBOC O47-004, dry weight 24.2 g) was concentrated and partitioned between *n*-hexane and aqueous EtOH. The EtOH-soluble materials were concentrated and further separated by column chromatography on a reversed-phase YMC*Gel ODS-A with a stepped gradient from $H_2O$ to EtOH. The fraction that eluted with EtOH–$H_2O$ (50:50) was purified by HPLC (YMC-ODS-Am, 60:40:1% EtOH–$H_2O$–$AcONH_4$) to give melonosin B (**2**, 1.3 mg, 0.005% based on dry weight of the sponge) and melonosin A (**3**, 2.6 mg, 0.011% based on dry weight of the sponge).

*Melonoside B (**1**):* colorless glass; $[\alpha]^{20}_D$ −23 (*c* 0.17, EtOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 198 (4.45), 225 (3.77), 279 (2.98) nm; IR ($CHCl_3$) $\nu_{max}$ 2928, 2855, 1726, 1671, 1602 $cm^{-1}$; $^1H$, $^{13}C$ NMR data ($CD_3OD$), Table 1; HRESIMS *m*/*z* 762.4426 $[M - H]^-$ (calcd for $C_{41}H_{65}NO_{12}$, 762.4434); HRESIMS/MS of the ion $[M - H]^-$ at *m*/*z* 762:586 $[M - C_6H_8O_6]^-$.

*Melonosin B (**2**):* colorless glass; $[\alpha]^{20}_D$ −5 (*c* 0.13, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 196 (4.62), 224 (4.00), 278 (3.43) nm; ECD ($1.7 \times 10^{-4}$, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 218 (−4.52) nm; IR ($CHCl_3$) $\nu_{max}$ 2932, 2857, 1706, 1666, 1520 $cm^{-1}$; $^1H$, $^{13}C$ NMR data ($CD_3OD$), Table 1; HRESIMS *m*/*z* 586.4111 $[M - H]^-$ (calcd for $C_{35}H_{57}NO_6$, 586.4113).

*Melonosin A (**3**):* colorless glass; $[\alpha]^{20}_D$ −6 (*c* 0.05, EtOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 196 (4.74), 225 (3.97), 279 (3.21) nm; ECD ($2.0 \times 10^{-4}$, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 200 (−2.23), 220 (−2.13) nm; IR ($CHCl_3$) $\nu_{max}$ 2933, 2858, 1706, 1667, 1526 $cm^{-1}$; $^1H$, $^{13}C$ NMR data ($CD_3OD$), Table 1; HRESIMS *m*/*z* 612.4267 $[M - H]^-$ (calcd for $C_{37}H_{59}NO_6$, 612.4227).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00785.

Experimental details, schemes of derivatization of **3**, copies of 1D and 2D NMR, HRESIMS, ECD spectra of melonoside B aglycon and compounds **2** and **3**, tabulated HRESIMS/MS data for compounds **5** and **6** (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*Tel/Fax: +7-432-231-1168. Fax: +7-432-231-4050. E-mail: makarieva@piboc.dvo.ru.

### ORCID

Tatyana N. Makarieva: 0000-0002-2446-8543


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The work on the isolation and study on the chemical structures was supported by Grant 17-14-01065 from RSF. The study of the biological activities of the compounds was supported by Grant 16-03-00553 from RFBR. The authors are grateful to Professor Z. Dong (Hormel Institute of Minnesota University, USA) who kindly provided JB6 cell lines, which were used in the present study.

## ■ REFERENCES


(1) Blunt, J. W.; Carroll, A. R.; Copp, B. R.; Davis, R. A.; Keyzers, R. A.; Prinsep, M. R. *Nat. Prod. Rep.* **2018**, *35*, 8–53 and other reviews of this series .

(2) Takada, K.; Imae, Y.; Ise, Y.; Ohtsuka, S.; Ito, A.; Okada, S.; Yoshida, M.; Matsunaga, S. *J. Nat. Prod.* **2016**, *79*, 2384–2390.

(3) McCulloch, M. W. B.; Bugni, T. S.; Concepcion, G. P.; Coombs, G. S.; Harper, M. K.; Kaur, S.; Mangalindan, G. C.; Mutizwa, M. M.; Veltri, C. A.; Virshup, D. M.; Ireland, C. M. *J. Nat. Prod.* **2009**, *72*, 1651–1656.

(4) Kubota, T.; Suzuki, H.; Takahashi-Nakaguchi, A.; Fomont, J.; Gonoi, T.; Kobayashi, J. *RSC Adv.* **2014**, *4*, 11073–11079.

(5) Kim, C. K.; Song, I. H.; Park, H. Y.; Lee, Y. J.; Lee, H. S.; Sim, C. J.; Oh, D. C.; Oh, K. B.; Shin, J. *J. Nat. Prod.* **2014**, *77*, 1396–1403.

(6) Emura, C.; Higuchi, R.; Miyamoto, T. *Tetrahedron* **2006**, *62*, 5682–5685.

(7) Ishiyama, H.; Ishibashi, M.; Ogawa, A.; Yoshida, S.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3831–3836.

(8) White, K. N.; Tenney, K.; Crews, P. *J. Nat. Prod.* **2017**, *80*, 740–755.

(9) Takada, K.; Uehara, T.; Nakao, Y.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *J. Am. Chem. Soc.* **2004**, *126*, 187–193.

(10) Gaspar, H.; Cutignano, A.; Grauso, L.; Neng, N.; Cachatra, V.; Fontana, A.; Xavier, J.; Cerejo, M.; Vieira, H.; Santos, S. *Mar. Drugs* **2016**, *14*, 179.

(11) Sata, N.; Asai, N.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1994**, *50*, 1105–1110.

(12) Goobes, R.; Rudi, A.; Kashman, Y.; Ilan, M.; Loya, Y. *Tetrahedron* **1996**, *52*, 7921–7928.

(13) Fusetani, N.; Sata, N.; Asai, N.; Matsunaga, S. *Tetrahedron Lett.* **1993**, *34*, 4067–4070.

(14) Peddie, V.; Takada, K.; Okuda, S.; Ise, Y.; Morii, Y.; Yamawaki, N.; Takatani, T.; Arakawa, O.; Okuda, S.; Matsunaga, S. *J. Nat. Prod.* **2015**, *78*, 2808–2813.

(15) Guzii, A. G.; Makarieva, T. N.; Denisenko, V. A.; Dmitrenok, P. S.; Kuzmich, A. S.; Dyshlovoy, S. A.; von Amsberg, G.; Krasokhin, V. B.; Stonik, V. A. *Org. Lett.* **2016**, *18*, 3478–3481.

(16) Einarsdottir, E.; Liu, H. B.; Freysdottir, J.; Gotfredsen, C. H.; Omarsdottir, S. *Planta Med.* **2016**, *82*, 903–909.

(17) Carballeira, N. M.; Montano, N.; Amador, L. A.; Rodriguez, A. D.; Golovko, M. Y.; Golovko, S. A.; Reguera, R. M.; Alvarez-Velilla, R.; Balana-Fouce, R. *Lipids* **2016**, *51*, 245–256.

(18) Mudianta, I. W.; Skinner-Adams, T.; Andrews, K. T.; Davis, R. A.; Hadi, T. A.; Hayes, P. Y.; Garson, M. J. *J. Nat. Prod.* **2012**, *75*, 2132–2143.

(19) Melonosins B (**2**) and A (**3**) are natural products, but not products of hydrolysis of glycosides at their isolation, because they were obtained by the same experimental procedure (without application of any acids) as the related glycosylated compounds from another collection of the sponge.

(20) Dyshlovoy, S. A.; Otte, K.; Tabakmakher, K. M.; Hauschild, J.; Makarieva, T. N.; Shubina, L. K.; Fedorov, S. N.; Bokemeyer, C.; Stonik, V. A.; von Amsberg, G. *Oncotarget* **2018**, *91*, 16962–16973.




DOI: 10.1021/acs.jnatprod.8b00785
*J. Nat. Prod.* XXXX, XXX, XXX–XXX