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Melophluosides A and B, new triterpene galactosides from the marine sponge *Melophlus sarasinorum*

Yusaku Sadahiro [a], Yuki Hitora [a], Arina Fukumoto [a], Yuji Ise [b], Esther D. Angkouw [c], Remy E.P. Mangindaan [c], Sachiko Tsukamoto [a,*]

[a] Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan
[b] Centre for Marine & Coastal Studies (CEMACS), Universiti Sains Malaysia, 11800 USM Penang, Malaysia
[c] Faculty of Fisheries and Marine Science, Sam Ratulangi University, Kampus Bahu, Manado 95115, Indonesia

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ABSTRACT

Two new triterpene galactosides, melophluosides A and B, were isolated from a marine sponge *Melophlus sarasinorum* collected in Indonesia. Their structures were determined by the analysis of spectroscopic data and chemical reactions. The absolute configuration of cyclohexenone moiety was determined by the calculated ECD spectrum of a simplified model. Melophluosides A and B exhibited moderate cytotoxicity against HeLa cells (IC_{50}, 11.6 and 9.7 μM, respectively).

Introduction

Marine invertebrates are rich sources of diverse secondary metabolites. Although triterpene glycosides in plants have been well studied and their various pharmacological activities investigated, only a few examples have so far been reported from marine invertebrates. Holotoxins [1], holothurins [2,3], andechinosides [4] were isolated from the sea cucumbers *Stichopus japonicus*, *Holothuria leucospilota*, and *Actinopyga echinites*, respectively. Sarasinosides [5,6] and pouosides [7,8] were isolated from marine sponges of the genera *Asteropus, Melophlus,* and *Lipastrotethya*. These triterpene glycosides also showed a wide range of biological activities including cytotoxicity [7,8], antimicrobial activity [1–4,6], inhibition of development of fertilized starfish eggs [5], and antifouling activity [9].

In our continuing study of biologically active secondary metabolites from marine organisms, the extract of the marine sponge *M. sarasinoside* collected in Indonesia showed cytotoxic activity in HeLa cells. Its purification yielded two new cytotoxic triterpene glycosides, melophluosides A and B (**1** and **2**), which were found to be pouoside congeners (Fig. 1). Pouosides A–E were initially isolated from a marine sponge *Asteropus* sp. [7], and pouosides F–I and pouogenins A–E were more recently isolated from a marine sponge *Lipastrotethya* sp. [8]. We here report the isolation, structure elucidation, and cytotoxicity of **1** and **2**.

Results and discussion

The marine sponge *M. sarasinorum* collected in Indonesia [10] was extracted with EtOH and MeOH. The extract was partitioned between EtOAc and water, and the EtOAc layer was partitioned between *n*-hexane and 90% MeOH–H_2O. The 90% MeOH–H_2O fraction, which showed cytotoxic activity with a 7% survival ratio at 5 μg/mL in HeLa cells. A portion (7.0 g) of the fraction (8.0 g) was subjected to silica gel column chromatography with stepwise gradient elution using *n*-hexane/CH_2Cl_2/MeOH (10:19:1), CH_2Cl_2/MeOH (19:1, 9:1, and 4:1), and CH_2Cl_2/MeOH/H_2O (6:4:1). Chemical analysis of these fractions by LC-MS indicated that the fractions eluted with CH_2Cl_2/MeOH (19:1, 9:1, and 4:1) contained many pouoside derivatives. Among them, the presence of new metabolites was indicated in the first and third fractions. The third fraction (940 mg) eluted with CH_2Cl_2/MeOH (4:1) was fractionated by ODS column chromatography followed by ODS HPLC and phenyl-hexyl HPLC to yield **1** (3.0 mg) [11]. Another pouoside-rich fraction (689 mg), yielded by the first silica gel column chromatography with CH_2Cl_2/MeOH (19:1), was fractionated by ODS column chromatography followed by ODS HPLC and C30 HPLC to yield **2** (11.0 mg) as a major compound [11].

* Corresponding author.
E-mail address: sachiko@kumamoto-u.ac.jp (S. Tsukamoto).

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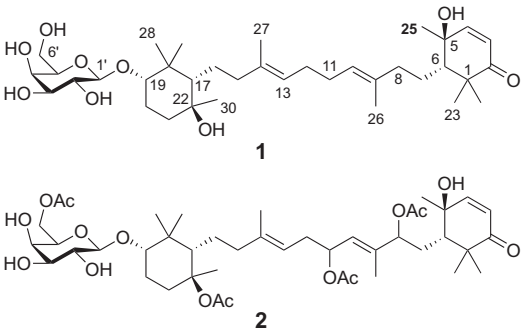

Fig. 1. Structures of **1** and **2**.

The molecular formula of **1** was determined to be $C_{36}H_{60}O_9$ by HRESIMS. The ^{1}H NMR data of **1** (Table 1) showed four olefin protons at δ_H 6.73 (d, *J* = 10.1 Hz, H-4), 5.79 (d, *J* = 10.1 Hz, H-3), 5.20 (br t, *J* = 6.2 Hz, H-10), and 5.17 (br t, *J* = 6.2 Hz, H-13), an oxygen bearing methylene at δ_H 3.71 (2H, d, *J* = 6.1 Hz, H_2-6′), six oxygen bearing methines at δ_H 4.27 (d, *J* = 7.7 Hz, H-1′), 3.83 (d, *J* = 3.1 Hz, H-4′), 3.51 (dd, *J* = 9.5, 7.7 Hz, H-2′), 3.49 (t, *J* = 6.1 Hz, H-5′), 3.43 (dd, *J* = 9.5, 3.1 Hz, H-3′), and 3.24 (dd, *J* = 11.5, 4.0 Hz, H-19), sixteen aliphatic protons at δ_H 2.30–1.14, and eight methyl signals at δ_H 1.65 (s, H_3-26), 1.63 (s, H_3-27), 1.34 (s, H_3-25), 1.16 (s, H_3-23), 1.13 (s, H_3-30), 1.09 (s, H_3-28), 1.03 (s, H_3-24), and 0.84 (s, H_3-29). ^{13}C NMR and HSQC spectra of **1** revealed a carbonyl carbon at δ_C 206.9 (C-2), six olefinic carbons at δ_C 158.1 (C-4), 137.4 (C-14), 136.8 (C-9), 125.5 (C-10), 125.2

Table 1
^{13}C and ^{1}H NMR data for **1** and **2.**

no.	1			2	
	δ_C, type	δ_H (*J* in Hz)	HMBC	δ_C, type	δ_H (*J* in Hz)
1	47.1, C			46.6, C	
2	206.9, C			206.1, C	
3	124.9, CH	5.79, d (10.1)	1, 5	125.2, CH	5.80, d (10.1)
4	158.1, CH	6.73, d (10.1)	2, 6	158.1, CH	6.74, d (10.1)
5	72.3, C			72.0, C	
6	54.4, CH	1.90, dd (6.2, 4.3)	1, 5, 8, 23, 24, 25	50.2, CH	2.02, m
7a	26.1, CH_2	1.78, m	5, 6, 8	30.8, CH_2	1.90, m
7b		1.57, m	5, 8		1.80, m
8a	42.4, CH_2	2.30, ddd (13.0, 12.8, 5.0)	7, 9, 10, 26	80.0, CH	5.46, dd (9.3, 4.8)
8b		2.06, m	6, 9, 10, 26		
9	136.8, C			139.6, C	
10	125.5, CH	5.20, br t (6.2)	8, 11, 26	127.1, CH	5.36, br d (9.0)
11	29.2, CH_2	2.04, m	9, 10, 12, 13	72.4, CH	5.41, m
12	29.2, CH_2	2.03, m	10, 11, 13, 14	34.1, CH_2	2.40, q (7.0)
					2.27, q (7.0)
13	125.2, CH	5.17, br t (6.2)	12, 15, 27	119.6, CH	5.12, br t (7.0)
14	137.4, C			140.4, C	
15a	44.3, CH_2	2.19, ddd (13.0, 12.6, 4.8)	13, 14, 16, 27	43.8, CH_2	2.15, ddd (13.2, 12.5, 5.2)
15b		1.99, m	13, 14		2.00, m
16a	26.2, CH_2	1.56, m	17, 22	26.4, CH_2	1.56, m
16b		1.34, m			1.48, m
17	57.1, CH	1.14, m	15, 16, 18, 22	54.3, CH	1.63, m
18	41.9, C			42.0, C	
19	89.8, CH	3.24, dd (11.5, 4.0)	18, 28, 29, 1′	89.1, CH	3.26, dd (11.5, 3.8)
20α	28.9, CH_2	1.57, m		28.2, CH_2	1.58, m
20β		2.01, m	22		2.02, m
21α	41.6, CH_2	1.68, m		36.2, CH_2	2.49, br dt (12.0, 4.2)
21β		1.47, m	20		1.78, m
22	74.1, C			88.1, C	
23	25.4, CH_3	1.16, s	1, 2, 6, 24	25.2, CH_3	1.19, s
24	22.0, CH_3	1.03, s	1, 2, 6, 23	22.0, CH_3	1.02, s
25	23.3, CH_3	1.34, s	4, 5, 6	23.2, CH_3	1.34, s
26	16.4, CH_3	1.65, s	8, 9, 10	13.1, CH_3	1.75, s
27	16.3, CH_3	1.63, s	13, 14, 15	16.6, CH_3	1.67, s
28	28.7, CH_3	1.09, s	17, 18, 19, 29	28.5, CH_3	1.09, s
29	16.3, CH_3	0.84, s	17, 18, 19, 28	16.6, CH_3	0.89, s
30	23.0, CH_3	1.13, s	17, 21, 22	20.5, CH_3	1.46, s
1′	107.3, CH	4.27, d (7.7)	19	107.1, CH	4.27, d (7.6)
2′	73.1, CH	3.51, dd (9.5, 7.7)	1′, 3′	72.8, CH	3.50, dd (9.7, 7.7)
3′	75.1, CH	3.43, dd (9.5, 3.1)	2′	74.9, CH	3.45, dd (9.7, 3.2)
4′	70.2, CH	3.83, d (3.1)	2′, 3′	70.2, CH	3.78, d (3.2)
5′	76.4, CH	3.49, t (6.1)	1′, 6′	73.7, CH	3.71, dd (7.7, 4.9)
6′	62.4, CH_2	3.71 (2H), d (6.1)	4′, 5′	64.7, CH_2	4.34, dd (11.3, 7.7)
					4.14, dd (11.9, 4.9)
8-OAc				171.9, C	
				23.2, CH_3	2.02, s
11-OAc				172.1, C	
				23.2, CH_3	2.01, s
22-OAc				172.4, C	
				20.7, CH_3	1.97, s
6′-OAc				172.5, C	
				23.2, CH_3	1.93 s

(C-13), and 124.9 (C-3), nine heteroatom substituted carbons at δ_C 107.3 (C-1′), 89.8 (C-19), 76.4 (C-5′), 75.1 (C-3′), 74.1 (C-22), 73.1 (C-2′), 72.3 (C-5), 70.2 (C-4′), and 62.4 (C-6′), two methine carbons at δ_C 57.1 (C-17) and 54.4 (C-6), two quaternary carbons at δ_C 47.1 (C-1) and 41.9 (C-18), eight methylene carbons at δ_C 44.3 (C-15), 42.4 (C-8), 41.6 (C-21), 29.2 (C-11), 29.2 (C-12), 28.9 (C-20), 26.2 (C-16), and 26.1 (C-7), and eight methyl carbons at δ_C 28.7 (C-28), 25.4 (C-23), 23.3 (C-25), 23.0 (C-30), 22.0 (C-24), 16.4 (C-26), 16.3 (C-27), and 16.3 (C-29) (Table 1). The presence of a sugar unit was indicated by characteristic ^{1}H and ^{13}C signals δ_H 3.43–4.27 and δ_C 62.4–107.3. Two proton spin systems H-1′/H-2′/H-3′/H-4′ and H-5′/H_2-6′ shown by COSY and HMBC correlations from H-5′ to C-1′ and from H_2-6′ to C-4′ showed a pyranose unit (Fig. 2a). The structure of the aglycone moiety of **1** was determined by a detailed analysis of 2D NMR data. The COSY spectrum revealed five spin systems (H-3/H-4, H-6/H_2-7/H_2-8, H-10/H_2-11/H_2-12/H-13, H-15/H_2-16/H-17, and H-19/H_2-20/H_2-21). HMBC correlations from H_3-23 and H_3-24 to C-1, C-2, and C-6, from H-4 to C-2, and from H_3-25 to C-4, C-5, and C-6 showed the structure of a 5-substituted 4-hydroxy-4,6,6-trimethylcyclohex-2-en-1-one (C-1–C-8) moiety (Fig. 2a). Meanwhile, HMBC correlations from H_3-28 and H_3-29 to C-17, C-18, and C-19, from H_3-30 to C-17, C-21, and C-22, and from H-1′ to C-19 indicated the structure of a 2-substituted 1,3,3-trimethylcyclohexan-1-ol (C-15–C-22) moiety with a *β*-D-galactose unit at C-19 (Fig. 2a). HMBC correlations from H_3-26 to C-8, C-9, and C-10, and from H_3-27 to C-13, C-14, and C-15 connected the cyclohexane and cyclohexenone moieties through a methylene unit (C-11–C-12) to complete the planar structure of **1** (Fig. 2a). *E* geometries of the double bonds at C-9 and C-13 were determined by the NOE correlations of H_2-8/H-10 and H-13/H_2-15.

The relative and absolute configurations were elucidated as follows. Large coupling constants, H-1′/H-2′ (*J* = 7.7 Hz) and H-2′/H-3′ (*J* = 9.5 Hz), and small coupling constants, H-3′/H-4′ (*J* = 3.4 Hz), together with NOE correlations of H-1′/H-3′, H-1′/H-5′, and H-3′/H-5′ indicated *β*-glycosylated galactose as a pyranose unit (Fig. 2b). The absolute configuration of galactose was determined from the ECD spectra. Galactose liberated from **1** by acid hydrolysis was benzoylated, and the ECD spectrum of the product was identical to that of penta-*O*-benzoylgalactopyranoside prepared from a commercially available D-galactose [12] (Fig. 3). Galactose in **1** was therefore assigned as D-series.

The 17*S**,19*S**, 22*S** configuration of the cyclohexane unit was determined by the NOE correlations (Fig. 2c). Namely, H-17, H-19, and H-21β are on β face and H-20α, H_3-29, and H_3-30 are on α face. To determine the absolute configuration of the cyclohexane unit, the modified Mosher's method [13] was used with the acid hydrolysate of **1**, but this attempt failed because of decomposition of the aglycone moiety by the acid hydrolysis.

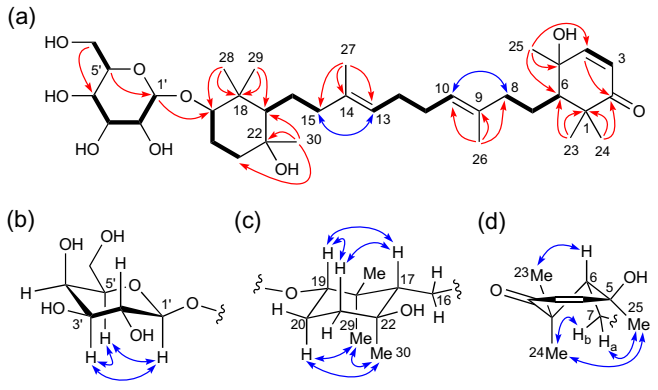

Fig. 2. COSY (bold lines), key HMBC (red arrows), and key NOESY (blue arrows) correlations of compound **1** (a) and substructures (b), (c), and (d).

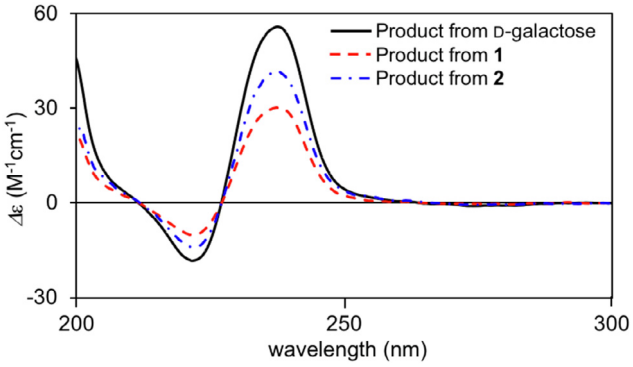

Fig. 3. ECD spectra (MeCN) of pentabenzoates prepared from a commercially available D-galactose and galactose liberated from **1** and **2**.

The relative configuration of the cyclohexenone unit was established to be 5*S**,6*S**, which was determined by the NOE correlations H-7b/H_3-24, H-7a/H_3-25, and H_3-24/H_3-25 on one side and H-6/H_3-23 on the opposite site (Fig. 2d). The absolute configuration of the cyclohexenone moiety was determined by the experimental ECD spectrum compared with the calculated spectrum of a simplified model 5*S*,6*S*-**3b** [14]. The positive cotton effect at 236 nm of **1** matched with that of 5*S*,6*S*-**3**. Thus, the absolute configuration of the cyclohexenone moiety of **1** was established (Fig. 4).

The molecular formula of compound **2**, $C_{44}H_{68}O_{15}$, was determined by the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1** (Table 1), except for the presence of four acetoxy groups (δ_H 2.02 (8-OAc), 2.01 (11-OAc), 1.97 (22-OAc), and 1.93 (6′-OAc); δ_C 171.9–172.5 (4C)). Analysis of 2D NMR data indicated the same carbon framework as **1**. HMBC correlations, δ_H 5.41 (H-11)/δ_C 172.1 and δ_H 4.14 and 4.34 (H_2-6′)/δ_C 172.5, clearly indicated that two acetoxy groups were attached to C-11 (δ_C 72.4) and C-6′ (δ_C 64.7). Although no HMBC correlation was observed from H-8 to an acetoxy carbonyl carbon, the downfield H-8 (δ_H 5.46, dd, *J* = 9.3, 4.8 Hz) and C-8 (δ_C 80.0) indicated the presence of an acetoxy group at C-8. These characteristic shifts matched well with those of pouoside G (H-8: δ_H 5.45, dd, *J* = 9.0, 5.0 Hz; C-8: δ_C 80.0) [8], containing the same 5-substituted 4-hydroxy-4,6,6-trimethylcyclohex-2-en-1-one (C-1–C-8) moiety. The remaining acetoxy group was attached to a quaternary carbon C-22 (δ_C 88.1) by the downfield shift, compared to that of **1** (δ_C 74.1). Pouoside A contains three acetoxy groups at C-8 (δ_C 80.0), C-11 (δ_C 73.6), and C-22 (δ_C 89.9) [7], and these shifts match with

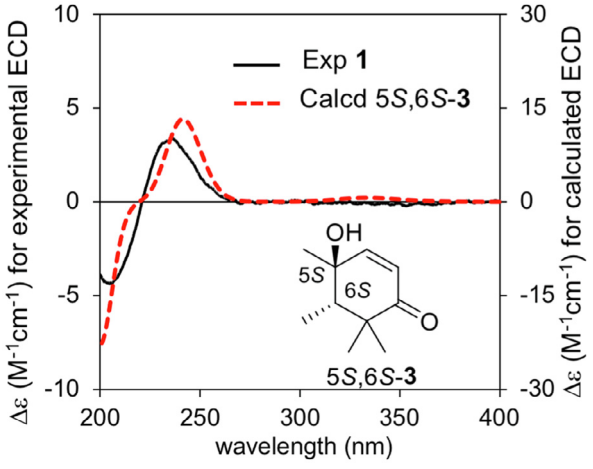

Fig. 4. Experimental ECD spectrum (MeCN) of **1** and calculated ECD spectrum of 5*S*,6*S*-**3** with B3LYP/TZVP level.

those of **2**. The NOESY spectrum indicated that the relative configurations of cyclohexane, cyclohexenone, and sugar moieties were identical to those of **1**. The absolute configurations of galactose and cyclohexenone moieties were determined in the same way as described for **1** [12]. While the 8*S*,11*R* configurations of pouosides F and G were determined by the Mosher's method and NOESY analyses, respectively [8], those of **2** remained unknown, i.e., the NOESY spectrum of **2** showed neither 8*R* nor 8*S*.

Compounds **1** and **2** showed weak cytotoxicity against HeLa cells, with IC_{50} values of 11.6 and 9.7 μM, respectively. Although pouoside A was initially reported to be cytotoxic against P388 cells with an EC_{50} value of 1.5 μg/mL (1.9 μM) [7], weaker activity was subsequently reported against K562 cells (IC_{50}, 17.0 μM) [8]. Compounds **1** and **2** showed no antimicrobial activities against bacteria (*Escherichia coli* and *Bacillus cereus*) or yeast (*Candida albicans*), even at 20 μM.

Conclusion

In summary, two new triterpene galactosides, melophluosides A and B (**1** and **2**), were isolated from the marine sponge *M. sarasinorum* collected in Indonesia. Their planar structures were elucidated by the interpretation of NMR and mass spectral data. The absolute configuration of the cyclohexenone moiety was established by comparison of the experimental and calculated ECD spectra, and that of the galactose moiety was determined by the ECD spectra after chemical derivatization. Melophluoside A is the first compound in the pouoside class found to lack an oxygenated group at C-11. Melophluosides A and B exhibited moderate cytotoxic activity against HeLa cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.151852.

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[10] The marine sponge (wet weight 1.2 kg) was collected at a depth of 10 m in Siladen, Indonesia, in September 2014 and immediately soaked in EtOH. The sponge was identified as *Melophlus sarasinorum* by one of the authors (Y.I.). A voucher specimen (14M217) has been deposited at the Department of Natural Medicines, Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan.
[11] Melophluoside A (**1**): Colorless oil; $[\alpha]_D^{20}$ −2.9 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 296 (4.3) and 220 (4.9); ECD (MeCN) λ_{max} (Δε) 236 (+3.8), 204 (-4.3) nm; IR (film) ν_{max} 3396, 2932, 2115, 1663, 1387, and 1059 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESITOFMS $[M + Na]^+$ *m/z* 659.4126 (calcd for $C_{36}H_{60}O_9Na$, 659.4130). Melophluoside B (**2**): Colorless oil; $[\alpha]_D^{20}$ −16.5 (c 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 296 (4.0) and 220 (4.9); ECD (MeOH) λ_{max} (Δε)233 (+8.7), 205 (-8.4) nm; IR (film) ν_{max} 3425, 2929, 2114, 1724, 1674, 1368, and 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESITOFMS $[M + Na]^+$ *m/z* 859.4457 (calcd for $C_{44}H_{68}O_{15}Na$, 859.4450).
[12] Compounds **1** (1.0 mg) and **2** (1.5 mg) were each hydrolyzed in 1 mL of 3 M HCl (200 μL) at 80 °C for 5 h. The water-soluble fraction of the product was reacted with benzoyl chloride (50 μL) in pyridine (250 μL) at room temperature for 20 h and purified by silica gel HPLC to afford penta-*O*-benzoyl-D-galactopyranoside. Penta-*O*-benzoyl-D-galactopyranoside prepared from **1**: ECD (MeCN) λ_{max} (Δε) 238 (+30), 223 (-10). Penta-*O*-benzoyl-D-galactopyranoside prepared from **2**: ECD (MeCN) λ_{max} (Δε) 238 (+41), 223 (-13). Commercially available D-galactose (5.0 mg) was treated in the same way to afford penta-*O*-benzoyl-D-galactopyranoside: ^1H NMR ($CDCl_3$) δ_H 8.12 (2H, d, *J* = 7.3 Hz), 8.10 (2H, d, *J* = 7.4 Hz), 7.95 (2H, d, *J* = 7.4 Hz), 7.86 (2H, d, *J* = 7.4 Hz), 7.81 (2H, d, *J* = 7.4 Hz), 7.64 (2H, t, *J* = 7.6 Hz), 7.54–7.44 (7H, overlapping signals), 7.38 (2H, t, *J* = 7.8 Hz), 7.30 (2H, d, *J* = 7.4 Hz), 7.26 (2H, d, *J* = 7.9 Hz), 6.94 (1H, d, *J* = 3.6 Hz), 6.18 (1H, d, *J* = 3.6 Hz), 6.12 (1H, dd, *J* = 3.3, 10.7 Hz), 6.02 (1H, dd, *J* = 3.6, 10.7 Hz), 4.82 (1H, t, *J* = 6.8 Hz), 4.63 (1H, dd, *J* = 6.5, 11.4 Hz), 4.42 (1H, dd, *J* = 7.1, 11.4 Hz); ECD (MeCN) λ_{max} (Δε) 238 (+55), 223 (-18) nm.
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[14] Conformational analysis and ECD calculations were conducted as previously described [15]. Stable conformers were investigated using the Merck molecular force field method and further optimized using the Hartree–Fock/3-21G and B3LYP/6-31G*. ECD calculation was conducted at the B3LYP/TZVP level and wavelengths of the spectra were corrected (-10 nm).
[15] M. Torii, H. Kato, Y. Hitora, E.D. Angkouw, R.E.P. Mangindaan, N.J. de Voogd, S. Tsukamoto, J. Nat. Prod. 80 (2017) 2536–2541.