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Chlorine-containing iridoid and iridoid glucoside, and other glucosides from leaves of *Myoporum bontioides*

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ABSTRACT

Sixteen compounds were isolated from the MeOH extract of leaves of *Myoporum bontioides*. The five compounds hitherto unknown, were elucidated to be a chlorine-containing iridoid, named myopochlorin, and an iridoid glucoside, an acylated iridoid glucoside, a linear acetogenin glucoside, and an acyclic monoterpene glucoside, named myobontiosides A–D, respectively, by means of spectroscopic analyses.

1. Introduction

Myoporum bontioides A. Gray is a small evergreen tree of about 1–2 m in height found in coastal areas of the southern Kyushu islands through Okinawa, Taiwan, southern China and Indochina (Hatusima, 1975). In China, a decoction of *M. bontioides* is used as a folk medicine for antidermatosis, and as an antipyretic and antipsychotic. A furanosesquiterpene, (*R*)-myoporone, is a major constituent of the volatile oil of *M. bontioides* and has showed insecticidal activity against *Plutella xylostella* (Gu et al., 2004). In a continuing study on Okinawan plants, the polar constituents of *M. bontioides*, collected in Okinawa, were investigated.

From the 1-BuOH-soluble fraction of a MeOH extract of leaves of *M. bontioides*, five new compounds, myopochlorin (**1**), myobontiosides A (**2**) and B (**3**), acetogenin glucoside (**4**), and monoterpene glucoside (**5**), were isolated together with five phenylethanoids, decaffeoylverbascoside (**6**), amorphous powder, $[\alpha]_D^{25}$ −54.9 (*c* = 6.35, MeOH) (Burger et al., 1987), cimidahurine (**7**), amorphous powder, $[\alpha]_D^{26}$ 40.7 (*c* = 0.21, MeOH) (Sugiyama and Kikuchi, 1992), verbascoside (**8**), amorphous powder, $[\alpha]_D^{25}$ −83.3 (*c* = 9.61, MeOH) (Birkofer et al., 1968; Nishimura et al., 1991), isoverbascoside (**9**), amorphous powder, $[\alpha]_D^{26}$ −28.2 (*c* = 0.17, MeOH) (Miyase et al., 1982), and oxoverbascoside (**10**), amorphous powder, $[\alpha]_D^{25}$ −68.6 (*c* = 0.33, MeOH) (Nishibe et al., 1993), two phenylpropanoids, meliotoside (**11**), colorless prisms, m.p. 239–241 °C (MeOH), $[\alpha]_D^{25}$ −57.3 (*c* = 3.36, MeOH) (Takaishi, 1968; Vasange et al., 1997) and ferulic acid β-D-glucopyranosyl ester (**12**), amorphous powder, $[\alpha]_D^{27}$ −7.56 (*c* = 0.44, MeOH) (Schlenk and Gellerman, 1965; Hashimoto et al., 1992), and four flavonoid glucosides, the 7-*O*-β-D-glucopyranosides of apigenin (**13**), yellow amorphous powder, $[\alpha]_D^{25}$ −36.3 (*c* = 4.98, pyridine) (Harborne and Mabry, 1982a), luteolin (**14**), yellow amorphous powder, $[\alpha]_D^{25}$ −51.0 (*c* = 1.07, pyridine) (Harborne and Mabry, 1982b), tricin (**15**), amorphous powder, $[\alpha]_D^{26}$ −53.3 (*c* = 1.18, MeOH) (Mabry et al., 1984; Wang et al., 2004), and chrysoeriol (**16**), yellow amorphous powder, $[\alpha]_D^{27}$ −44.7 (*c* = 1.16, MeOH) (Harborne and Mabry, 1982c). Structures of the known compounds were identified by comparison of spectroscopic data with those reported in the literature. This paper deals with isolation and structural elucidation of the new compounds.

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2. Results and discussion

Air-dried leaves of *M. bontioides* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The 1-BuOH soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on a highly porous synthetic resin (Diaion HP-20), and then normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC), and high-performance liquid chromatography (HPLC) to afford 16 compounds (**1**–**16**) (details and yields are given in Section 4). The structures of the new compounds (**15**) were elucidated on the basis of spectroscopic evidence, and those of known compounds were identified by comparison of spectroscopic data with those reported in the literature.

Myopochlorin (**1**), ($[\alpha]_D^{21}$ +71.9), was isolated as an amorphous powder, and two quasi-molecular ion peaks were observed at *m*/*z* 235.0335 ($C_9H_{12}{}^{35}ClO_5$) and 237.0349 ($C_9H_{12}{}^{37}ClO_5$) $[M-H]^-$in the ratio of 3:1 on high-resolution (HR)-FAB-mass spectrometry (MS), which proved that **1** contains one chlorine atom. The IR spectrum showed the absorption band of a hydroxyl group (3369 cm^{-1}). The NMR spectra of **1** exhibited three methylenes, two of which bear an oxygen atom, three deshielded (δ_C 72.4 with δ_H 4.53 and 76.8 with δ_H 3.71) and one non-deshielded (δ_C 55.7 with δ_H 2.15) methines, two deshielded quaternary carbons (δ_C 73.1 and 84.3), and one acetal carbon [δ_C 102.3 with δ_H 5.41 (*d*, *J* = 6 Hz)] (Table 1). According to the spectroscopic data, **1** was considered to be a C-9 iridoid, and judging from the 3° of unsaturation, it must have a tricyclic skeleton. The ^{1}H–1H correlation spectroscopy (COSY) spectrum showed three sets of correlations, however, they were not further correlated. Thus, the heteronuclear multiple bond correlation (HMBC) spectrum was examined to confirm the structure (Fig. 2a). The correlation between H-10a (δ_H 4.46) and C-1 (δ_C 102.3) showed the presence of a third cyclic system (C-10–O–C-1) in the iridoid skeleton through acetal formation. The correlation peaks between H-1 (δ_H 5.41) and C-3 (δ_C 58.5), C-5 (δ_C 73.1), and C-9 (δ_C 55.7), those between H-10a and C-1, C-7 (δ_C 72.4), and C-9, those between H-4a (δ_H 1.87) and C-5, C-6 (δ_C 76.8), and C-9, and those between H-4b (δ_H 1.62) and C-3, C-5 and C-6 established the carbon framework of myopochlorin (**1**) as shown in Fig. 1. However, the ^{13}C NMR chemical shifts of C-5, -6, -7 and -8 were too close to determine the position of the chlorine atom. Therefore, the HMBC spectrum for a DMSO-d_6 solution, in which the hydroxyl protons were found to appear at δ_H 4.75, 5.31 and 5.70, was analysed (Fig. 2b). The hydroxy proton appearing at δ_H 5.31 was placed at C-6 based on the cross-peak between the proton and C-5 (δ_C 71.3), and that appearing at δ_H 5.70 at C-8 based on the cross-peak between the proton and C-10 (δ_C 71.2). The remaining hydroxyl group (δ_H 4.75) was placed at C-5 based on the appearance of a cross-peak between the proton and C-5. Therefore, C-7 was the sole remaining position for the chlorine atom. The relative structure of **1** was assessed by phase-sensitive (PS)-nuclear Overhauser exchange (NOESY) spectrometry for a DMSO-d_6 solution, in which H-9 (δ_H 2.04) showed correlation peaks with H-1, H-7, and protons of 5-OH, 6-OH and 8-OH, and thus H-1, H-9 and three hydroxy groups are assigned to locate in the same face and the chlorine atom to the opposite face to H-1, H-9 and the three hydroxyl groups (Fig. 3). The modified Mosher's method was applied to establish the absolute structure (Fig. 4) (Ohtani et al., 1991). The $\Delta\delta$ values of H-1, H_2-3, and H_2-4 were shown to have minus signs, however, those of protons on the other side were uniformly 0, probably due to that the protons on this side were far from the influence of the ring current of the MTPA aromatic moiety. Therefore, the structure of myopochlorin (**1**)

Table 1
^{13}C NMR spectroscopic data for compounds **1**–**5** (CD_3OD, 100 MHz)

	Compound number				
	1[a]	**2**	**3**	**4**	**5**
1	102.3 *d* (100.5)	93.3 *d*	95.5 *d*	180.4 *s*	69.1 *t*
2				29.7 *t*	37.8 *t*
3	58.5 *t* (57.1)	140.9 *d*	142.8 *d*	29.0 *t*	30.6 *d*
4	32.0 *t* (31.1)	105.7 *d*	108.0 *d*	83.1 *d*	38.1 *t*
5	73.1 *s* (71.3)	42.9 *d*	74.5 *s*	36.5 *t*	26.1 *t*
6	76.8 *d* (75.0)	77.7 *d*	78.7 *d*	26.4 *t*	127.1 *d*
7	72.4 *d* (72.6)	46.2 *t*	63.4 *d*	30.3 *t*	135.8 *s*
8	84.3 *s* (82.6)	81.8 *s*	66.8 *s*	27.0 *t*	69.1 *t*
9	55.7 *d* (54.5)	52.7 *d*	51.0 *d*	30.7 *t*	13.8 *q*
10	72.2 *t* (71.2)	52.9 *t*	61.6 *t*	70.8 *t*	20.0 *q*
1′		99.7 *d*	100.0 *d*	104.5 *d*	104.4 *d*
2′		74.9 *d*	74.7 *d*	75.2 *d*	75.2 *d*
3′		78.1 *d*	77.6 *d*	78.3 *d*	78.3 *d*
4′		71.9 *d*	71.7 *d*	71.8 *d*	71.8 *d*
5′		78.4 *d*	76.1 *d*	78.0 *d*	78.0 *d*
6′		63.0 *t*	64.1 *d*	62.9 *t*	62.9 *t*
1″			128.7 *s*		
2″			112.0 *d*		
3″			149.2 *s*		
4″			151.1 *s*		
5″			116.6 *d*		
6″			124.3 *d*		
7″			147.2 *d*		
8″			115.4 *d*		
9″			168.9 *s*		
$-OCH_3$			56.6 *q*		

[a] Chemical shifts in parentheses are for DMSO-d_6.

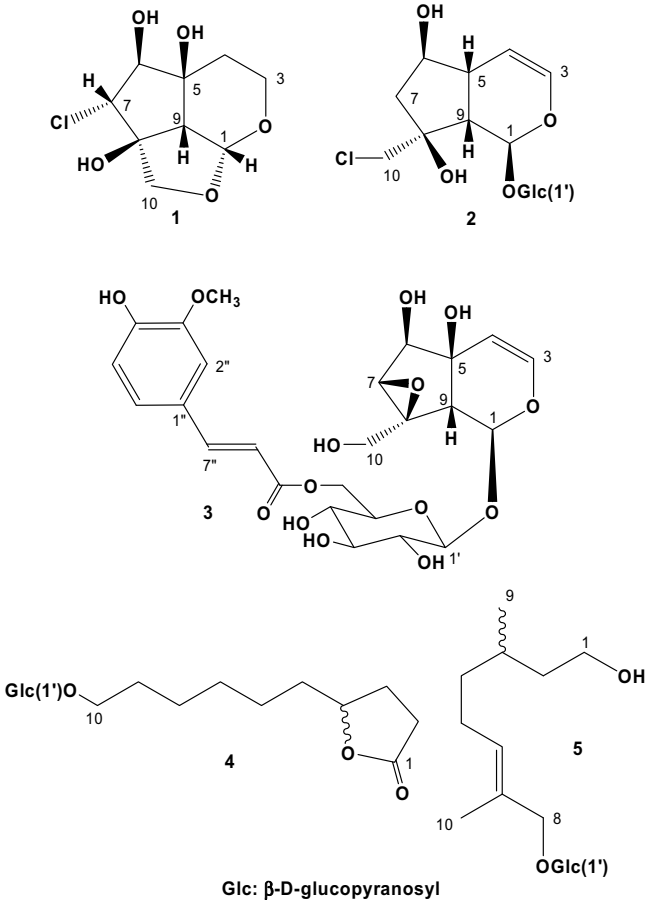
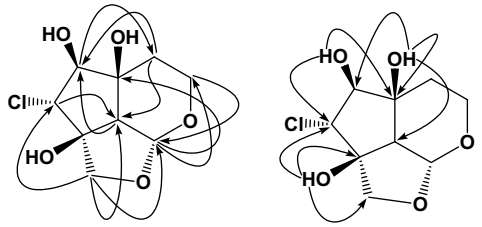


Fig. 1. Structures.



(a) for CD_3OD solution (b) for DMSO-d_6 solution

Fig. 2. Key HMBC correlations from H to C for myopochlorin (**1**).

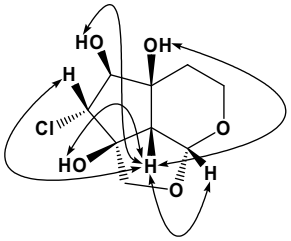


Fig. 3. Diagnostic NOESY correlations of **1** (DMSO-d_6).

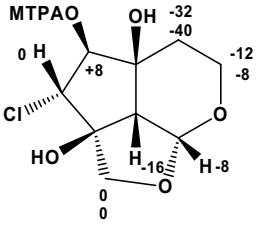


Fig. 4. Results of modified Mosher's method of myopochlorin (**1**) ($\Delta\delta$ values in Hz, $\delta_S-\delta_R$ at 400 MHz).

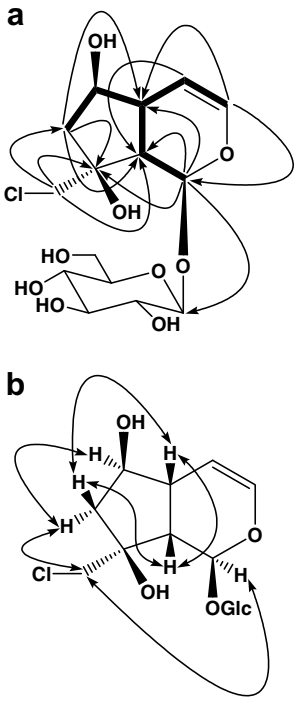


Fig. 5. Two-dimensional correlations for myobontinoside A (**2**). (a) $^1H-^1H$ COSY correlations (▬) and HMBC correlations from H to C. (b) NOESY correlations.

was elucidated to be (1*R*,5*S*,6*S*,7*R*,8*S*,9*S*)-7-chloro-8-(hydroxymethyl)octahydrocyclopenta[*c*]pyran-1,10-oxyra-5,6,7-triol.

Myobontioside A (**2**), ($[\alpha]_D^{23}$ −90.7), was isolated as an amorphous powder, and two quasi-molecular ion peaks were observed at *m*/*z*: 405.0912 $[M+Na]^+$ ($C_{15}H_{23}{}^{35}ClO_9Na$), and 407.0886 $[M+Na]^+$ ($C_{15}H_{23}{}^{37}ClO_9Na$) in the ratio of 3:1 on HR-electrospray-ionization (ESI)-time-of-flight (TOF)-MS, which also suggested that **2** contains one chlorine atom. In the IR spectrum, strong absorption (3362 cm^{-1}) for hydroxyl groups and typical absorption (1229 cm^{-1}) for an enol ether in the dihydropyran ring were observed. In the NMR spectra, six carbon signals assignable to the β-glucopyranosyl unit, an acetal carbon and proton resonances (δ_C 93.3 with δ_H 5.44), and a *cis*-double bond [δ_C 105.7 with δ_H 4.94 (*dd*, *J* = 6 and 3 Hz) and δ_C 140.9 with δ_H 6.22 (*dd*, *J* = 6 and 2 Hz)] in an enol form were observed (Table 1). From this evidence, myobontioside A (**2**) is an iridoid glucoside containing a chlorine atom. The COSY spectrum indicated connections from C-3 to C-7 and C-5 to C-1 via C-9 (Fig. 5a), and two or three bond correlations observed in the HMBC (Fig. 5a) spectrum supported the carbon framework to be as shown as **2** and the ^{13}C NMR spectrum was indeed very similar to that published for bisdeoxycynanchoside (Adriani et al., 1982). The PSNOESY experiment showed that the hydroxy groups at the 5- and 8-positions are both on the β-face (Fig. 5b), and the position of the chlorine atom was clearly demonstrated to be at the methylene group at C-10 from its ^{13}C NMR chemical shift (δ_C 52.9). Therefore, the structure of myobontioside A (**2**) was elucidated to be as shown in Fig. 1.

Myobontioside B (**3**), ($[\alpha]_D^{26}$ −54.6), was isolated as an amorphous powder and its elemental composition was determined to be $C_{25}H_{30}O_{14}$ on HR-ESI-TOF-MS. In the IR spectrum, absorptions for hydroxy groups (3369 cm^{-1}), a conjugated ester (1702 cm^{-1}), an aromatic ring (1517 cm^{-1}) and an enol ether (1277 cm^{-1}) were observed. In the NMR spectra, six carbon signals and two fairly deshielded proton resonances (δ_H 4.30 and 4.50) assignable to a 6-acylated glucopyranose (Matsunami et al., 2006). The remaining part of the ^{13}C NMR spectrum was almost identical to that published for macfadienoside (Davini et al., 1983), allowing for the different solvent. The phenylpropanoid moiety was linked by an ester bond to the hydroxyl group of C-6′, which was confirmed by the low field shifts of carbon (δ_C 64.1) and proton (δ_H 4.43 and 4.50) signals in the one-dimensional spectra, and the correlation peak between H_2-6′ and C-9″ (δ_C 168.9) in the HMBC spectrum. NOESY correlation between methoxyl protons (δ_H 3.90) and an aromatic proton [δ_H 7.20 (*d*, *J* = 2 Hz)] confirmed that the phenylpropanoid was ferulic acid. Therefore, the structure of myobontioside B (**3**) was elucidated to be macfadienoside 6″-ferulic acid ester.

Myobontioside C (**4**), $[\alpha]_D^{28}$ −26.4, was isolated as an amorphous powder and its elemental composition was determined to be $C_{16}H_{28}O_8$ on HR-ESI-TOF-MS. The IR spectrum exhibited absorption for hydroxyl groups (3396 cm^{-1}) and a γ-lactone (1765 cm^{-1}). The ^{13}C NMR spectrum showed six signals assignable to a β-glucopyranose and 10 signals for an aglycone moiety, consisting of seven methylenes, one oxygenated methylene (δ_C 70.8) resonance, one methine (δ_C 83.1) resonance, and a carbonyl carbon signal (Table 1). γ-Lactone was placed at one end of the methylene chain and oxymethylene at the other end based on the HMBC correlation resonances between δ_H 1.86 and 2.34 on δ_C 29.0, and δ_C 180.4 and a sequential ^1H-1H COSY correlation from protons on C-2 (δ_C 29.7) to those on C-3 (δ_C 29.0), and then that on C-4 (δ_C 83.1), and finally to those on C-5 (δ_C 36.5). Therefore, the structure of myobontioside C (**4**) was elucidated to be 4,10-dihydroxylcapric acid 1,4-olide β-D-glucopyranoside, as shown in Fig. 1. The absolute configuration at C-4 remains to be determined.

Myobontioside D (**5**), $[\alpha]_D^{23}$ −27.7, was also isolated as an amorphous powder and its elemental composition was determined to

be $C_{16}H_{30}O_7$. The one-dimensional NMR spectra exhibited six signals assignable to a β-glucopyranose, and the remaining 10 resonances consisted of one doublet methyl and one methyl on a double bond, three methylenes and two oxymethylenes, one methine and one trisubstituted double bond. From the ^{1}H–1H COSY spectrum, which showed a correlation sequence from H_2-1 through H-6, and H_3-10 and H-3, together with other two-dimensional spectra, the structure of the aglycone was established to be 3,7-dimethyloct-6-ene-1,8 or 1,9-diol. On irradiation of H_2-8 (δ_H 3.91), significant enhancement of the H-6 (δ_H 5.40) signal and *vice versa* were observed in the difference NOE experiments, and thus the geometry of the double bond was clarified to be *E*. The position of the glucopyranose unit was determined to be on the hydroxyl group at C-8 from the correlation peak between H-8 and C-1′ (δ_C 104.4) in the HMBC spectrum. Therefore, the structure of myobontioside D (**5**) was elucidated to be 2,6-dimethyl-2-Octene-1,8-diol 1-*O*-β-D-glucopyranoside, as shown in Fig. 1. The absolute configuration at C-3 remains to be determined.

3. Conclusions

Several compounds structurally related to myopochlorin (**1**) have been isolated from *Cistanche salsa* (Orobanchaceae) (Kobayashi et al., 1984), *Rehmannia glutinosa* (Kitagawa et al., 1986), *R. glutinosa* var. *hueihingensis* (Scrophulariaceae) (Morota et al., 1989), *Catalpa ovata* (Bignoniaceae) (Nozaki et al., 1989), etc. It is noteworthy that some of these, such as cistachlorin from *C. salsa*, rehmaglutins B and D from *R. glutinosa*, and jioglutins A and B from R. glutinosa var. *hueihingensis* contain a chlorine atom at the 7-position similar to myopochlorin (**1**). As iridoid glucosides, asystasioside E (Demuth et al., 1989) and it derivatives (Sudo et al., 1997), 7-chlorodeutziol (El-Naggar et al., 1982), mentzofoliol and its derivatives (Catalano et al., 1995), and stegioside I (Nass and Rimpler, 1996) are known to also contain a chlorine atom at the 7-position. Myobontioside A (**2**) is the first example of an iridoid glucoside, chlorinated at the 10-position. Only two iridoids have been found to be chlorinated at the 10-position. They are a seco-iridoid glucoside, eustoside (Uesato et al., 1979), and a valeriana iridoid, valechlorine (Petkov et al., 1974).

4. Experimental

4.1. General experimental procedures

M.p. was measured with a Yanagimoto micro-melting point apparatus and is uncorrected. Optical rotations were obtained on a JASCO P-1030 polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/VIS spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane as an internal standard. Negative-ion HR-MS was performed with a JEOL SX-102 spectrometer in the FAB mode and positive-ion HR-MS with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS.

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecyl silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ = 50 mm, L = 25 cm, linear gradient: MeOH–H_2O (1:9, 1 l) → (1:1, 1 l), fractions of 10 g being collected], respectively. The droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, L = 40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) were used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ = 6 mm, L = 25 cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

(*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) were the products of Wako Pure Chemical Industry Co. Ltd. (Tokyo, Japan).

4.2. Plant material

Leaves of *M. bontioides* (Sieb. et Zuu.) A. Gray (Myoporaceae) were collected in a coastal area of Yaeyama-gun, Okinawa, Japan, in November 2004, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (04-MB-Okinawa-1105).

4.3. Extraction and isolation

Dried leaves of *M. bontioides* (2.08 kg) were extracted three times with MeOH (15 l) at 25 °C for 1 week and then concentrated to 3 l *in vacuo*. On concentration, 238 g of precipitate (NaCl) was formed, which was removed by suction. The extract was washed with *n*-hexane (3 l, 16.6 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in H_2O (3 l) and then extracted with EtOAc (3 l) to give an EtOAc-soluble fraction (114 g). The aqueous layer was then extracted with 1-BuOH (3 l) to give a 1-BuOH-soluble fraction (91.1 g), and the remaining water-layer was concentrated to furnish a water-soluble fraction (221 g).

The 1-BuOH-soluble fraction (90.0 g) was subjected to a Diaion HP-20 CC (Φ = 50 mm, L = 50 cm) using H_2O–MeOH (4:1, 3 l), (2:3, 3 l), (3:2, 3 l), and (1:4, 3 l), and MeOH (3 l), 500 ml fractions being collected. The residue (20.2 g in fractions 4–8) of the H_2O–MeOH (4:1, v/v) eluent was subjected to silica gel (500 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(49:1, 3 l), (24:1, 6 l), (23:2, 3 l), (9:1, 3 l), (17:3, 3 l), (4:1, 3 l), (3:1, 3 l), and (7:3, 3 l)], 500 ml fractions being collected. Combined 8% MeOH eluate fractions 14–17 (95.5 mg) were separated by DCCC to give **1** (47.3 mg) in fractions 35–43. Combined H_2O–MeOH (9:1, v/v) eluate fractions 18–20 (97.1 mg) were separated by DCCC to give a residue in fractions 55–67 (16.8 mg), which was then purified by HPLC (H_2O–MeOH, 9:1, v/v) to afford 1.36 mg of **4**. Combined H_2O–MeOH (1:4, v/v) eluted fractions 23–31 (1.58 g) were subjected to ODS open CC. The residue (59.7 mg) of fractions 66–78 was then purified HPLC (H_2O–CH_3CN, 19:1, v/v) to give **7** (3.2 mg), **6** (9.6 mg) and **2** (2.2 mg). The residue (467 mg) of fractions 134–169 was subjected to DCCC to give two fractions, 259 mg in fractions 11–16 and 120 mg in fractions 17–27. The former was purified by HPLC with H_2O–MeOH (3:2, v/v) to give **8** (36.7 mg) and the latter with H_2O–CH_3CN (5:1, v/v) to give **3** (5.15 mg). An aliquot (4.41 g) of the residue (6.62 g) of H_2O–MeOH (1:3, v/v) eluate fractions 32–52 was subjected to two runs of ODS open CC, and the residues (2.13 g) of fractions 88–132 and 96–118 were separated by two runs of DCCC. From the combined residues of fractions 44–61 and 35–45, **11** (29.9 mg) was obtained as a precipitate.

The residue (21.8 g) of 40% eluate fractions 9–13, obtained on Diaion HP-20 CC, was similarly subjected to silica gel (500 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(49:1, 3 l), (24:1, 6 l), (23:2, 3 l), (9:1, 3 l), (17:3, 3 l), (4:1, 3 l), (3:1, 3 l), and (7:3, 3 l)], 500 ml fractions being collected. The residue (254 mg) of fractions 18–28 was purified by HPLC (H_2O–MeOH, 7:3) to give **12** (6.58 mg) and **5** (5.23 mg). The residue (9.53 g) of H_2O–MeOH (85:15 → 70:30, v/v) eluate fractions 31–58 was separated by two runs of ODS open CC, and the combined residues (3.15 g) of fractions 43–88 and 51–100 were purified by DCCC, fractions

39–46 (1.94 g) of which were finally purified by HPLC (H_2O–MeOH, 7:3, v/v) to give **10** (5.18 mg) and **8** (145 mg).

Insoluble matter (94.0 mg) in H_2O–MeOH (40:60 → 20:80, v/v) eluate fractions 14–21, obtained on Diaion HP-20 CC, was dissolved in DMSO and purified by HPLC (H_2O–MeOH, 7:3, v/v) to furnish **14** (1.83 mg), **13** (4.01 mg), **16** (17.5 mg) and **15** (19.2 mg). The remaining residue (20.6 g) was subjected to silica gel CC in the same manner as for the previous Diaion HP-20 fractions. The residue (4.48 g) of fractions 51–55 was separated by ODS open CC, and then the residue (2.07 g) by DCCC to give the partially purified fraction (636 mg), which was finally purified by HPLC (H_2O–MeOH, 7:3, v/v) to afford **8** (22.8 mg) and **9** (3.94 mg).

4.4. Characterization data

4.4.1. Myopochlorin (*1*)

Amorphous powder, $[\alpha]_D^{21}$ +71.9 (*c* = 2.63; MeOH); IR ν_{max} (film) cm^{-1}: 3369, 2922, 1402, 1347, 1292, 1147, 1033; ^1H NMR (CD_3OD, 400 MHz) δ: 5.41 (1H, *d*, *J* = 6 Hz, H-1), 4.53 (1H, *dd*, *J* = 11 and 2 Hz, H-7), 4.46 (1H, *d*, *J* = 10 Hz, H-10a), 3.74 (1H, *ddd*, *J* = 13, 13 and 2 Hz, H-3a), 3.71 (1H, *d*, *J* = 11 Hz, H-6), 3.66 (1H, *ddd*, *J* = 13, 6 and 2 Hz, H-3b), 3.40 (1 H, *dd*, *J* = 10 and 2 Hz, H-10b), 2.15 (1H, *d*, *J* = 6 Hz, H-9), 1.87 (1H, *ddd*, *J* = 13, 2 and 2 Hz, H-4a), 1.62 (1H, *ddd*, *J* = 13, 13 and 6 Hz, H-4b); (DMSO-d_6, 400 MHz) δ: 5.70 (1H, *s*, 8-O*H*), 5.33 (1H, *d*, *J* = 6 Hz, H-1), 5.31 (1H, *br s*, 6-O*H*), 4.75 (1H, *s*, 5-O*H*), 4.46 (1H, *dd*, *J* = 11 and 2 Hz, H-7), 4.24 (1H, *d*, *J* = 10 Hz, H-10a), 3.57 (2H, *m*, H_2-3), 3.54 (1H, *m*, H-6), 3.30 (1H, *dd*, *J* = 10 and 2 Hz, H-10b), 2.04 (1H, *d*, *J* = 6 Hz, H-9), 1.74 (1H, *br dt*, *J* = 13 and 2 Hz, H-4a), 1.45 (1H, *ddd*, *J* = 13, 13 and 6 Hz, H-4b); for ^{13}C NMR spectroscopic assignments, see Table 1; HR-FAB-MS (negative-ion mode) *m*/*z*: 235.0335 $[M-H]^-$ (Calcd. for $C_9H_{12}{}^{35}ClO_5$: 235.0339), *m*/*z*: 237.0349 $[M-H]^-$ (Calcd. for $C_9H_{12}{}^{37}ClO_5$: 235.0350).

4.4.2. Myobontioside A (*2*)

Amorphous powder, $[\alpha]_D^{23}$ −90.7 (*c* = 0.14; MeOH); IR ν_{max} (film) cm^{-1}: 3362, 2927, 1655, 1514, 1229, 1074, 1050, 1010; ^1H NMR (CD_3OD, 400 MHz) δ: 6.22 (1H, *dd*, *J* = 6 and 2 Hz, H-3), 5.44 (1H, *d*, *J* = 4 Hz, H-1), 4.94 (1H, *dd*, *J* = 6 and 3 Hz, H-4), 4.65 (1H, *d*, *J* = 8 Hz, H-1′), 3.96 (1H, *m*, H-6), 3.90 (1 H, *dd*, *J* = 12 and 2 Hz, H-6′a), 3.83 (1H, *d*, *J* = 12 Hz, H-10a), 3.74 (1H, *d*, *J* = 12 Hz, H-10b), 3.65 (1H, *dd*, *J* = 12 and 6 Hz, H-6′b), 3.38 (1H, *m*, H-3′), 3.29 (2H, *m*, H-4′ and -5′), 3.21 (1H, *dd*, *J* = 9 and 8 Hz, H-2′), 2.84 (1H, *dd*, *J* = 9 and 3 Hz, H-5), 2.64 (1H, *dd*, *J* = 9 and 4 Hz, H-9), 2.34 (1H, *dd*, *J* = 14 and 6 Hz, H-7a), 1.81 (1H, *dd*, *J* = 14 and 4 Hz, H-7b); for ^{13}C NMR spectroscopic assignments, see Table 1; HR-ESI-MS (positive-ion mode) *m*/*z*: 405.0912 $[M+Na]^+$ (Calcd. for $C_{15}H_{23}{}^{35}ClO_9Na$: 405.0922), *m*/*z*: 407.0886 $[M+Na]^+$ (Calcd. for $C_{15}H_{23}{}^{37}ClO_9Na$: 407.0893).

4.4.3. Myobontioside B (*3*)

Amorphous powder, $[\alpha]_D^{26}$ −54.6 (*c* = 0.28; MeOH); IR ν_{max} (film) cm^{-1}: 3369, 2932, 1702, 1517, 1277, 1164, 1072, 1018; UV λ_{max} (MeOH) nm (log ε): 325 (3.95), 235 (3.84), 221 (3.83); ^1H NMR (CD_3OD, 400 MHz) δ: 7.63 (1H, *d*, *J* = 16 Hz, H-7″), 7.20 (1H, *d*, *J* = 2 Hz, H-2″), 7.09 (1H, *dd*, *J* = 8 and 2 Hz, H-6″), 6.82 (1H, *d*, *J* = 8 Hz, H-5″), 6.38 (1H, *d*, *J* = 16 Hz, H-8″), 6.35 (1H, *d*, *J* = 6 Hz, H-3), 4.89 (1H, *d*, *J* = 6 Hz, H-4), 5.22 (1H, *d*, *J* = 8 Hz, H-1), 4.73 (1H, *d*, *J* = 8 Hz, H-1′), 4.50 (1H, *dd*, *J* = 12 and 2 Hz, H-6′a), 4.43 (1H, *dd*, *J* = 12 and 6 Hz, H-6′b), 4.13 (1H, *d*, *J* = 13 Hz, H-10a), 3.95 (1H, *d*, *J* = 1 Hz, H-6), 3.90 (3H, *s*, –OC*H*$_3$), 3.61 (1 H, *d*, *J* = 13 Hz, H-10b), 3.53 (1H, *d*, *J* = 1 Hz, H-7), 3.53 (1H, *m*, H-3′), 3.40 (2 H, *m*, H-4′ and -5′), 3.28 (1H, *dd*, *J* = 9 and 8 Hz, H-2′), 2.55 (1H, *d*, *J* = 8 Hz, H-9); for ^{13}C NMR spectroscopic assignments, see Table 1; HR-ESI-MS (positive-ion mode) *m*/*z*: 577.1518 $[M+Na]^+$ (Calcd. for $C_{25}H_{30}O_{14}Na$: 577.1527).

4.4.4. Myobontioside C (*4*)

Amorphous powder, $[\alpha]_D^{28}$ −26.4 (*c* = 0.08; MeOH); IR ν_{max} (film) cm^{-1}: 3396, 2934, 1765, 1419, 1187, 1077, 1038; ^1H NMR (CD_3OD, 400 MHz) δ: 4.54 (1H, *dddd*, *J* = 8, 8, 7 and 6 Hz, H-4), 4.25 (1H, *d*, *J* = 8 Hz, H-1′), 3.90 (1H, *dt*, *J* = 10 and 7 Hz, H-10a), 3.86 (1H, *dd*, *J* = 12 and 2 Hz, H-6′a), 3.66 (1H, *dd*, *J* = 12 and 5 Hz, H-6′b), 3.55 (1H, *dt*, *J* = 10 and 7 Hz, H-10b), 3.34 (1 H, *m*, H-3′), 3.26 (1H, *m*, H-5′), 3.25 (1H, *m*, H-4′), 3.17 (1H, *dd*, *J* = 9 and 8 Hz, H-2′), 2.57 (1H, *ddd*, *J* = 18, 10 and 9 Hz, H-2a), 2.50 (1H, *ddd*, *J* = 18, 9 and 5 Hz, H-2b), 2.34 (1H, *dddd*, *J* = 13, 9, 7 and 5 Hz, H-3a), 1.86 (1H, *dddd*, *J* = 13, 10, 9 and 8 Hz, H-3b), 1.64 (1H, *m*, H-5a), 1.61 (3H, *m*, H-5b and H_2-9), 1.41 (6H, *m*, H_2-6, -7 and -8); for ^{13}C NMR spectroscopic assignments, see Table 1; HR-ESI-MS (positive-ion mode) *m*/*z*: 371.1674 $[M+Na]^+$ (Calcd. for $C_{16}H_{28}O_8Na$: 371.1676).

4.4.5. Myobontioside D (*5*)

Amorphous powder, $[\alpha]_D^{23}$ −27.7 (*c* = 0.35; MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2926, 1665, 1511, 1268, 1077, 1036; ^1H NMR (CD_3OD, 400 MHz) δ: 5.40 (1H, *t*, *J* = 7 Hz, H-6), 4.25 (1H, *d*, *J* = 8 Hz, H-1′), 3.91 (2H, *s*, H_2-8), 3.86 (1H, *dd*, *J* = 12 and 2 Hz, H-6′a), 3.85 (1H, *m*, H-1a), 3.67 (1H, *dd*, *J* = 12 and 5 Hz, H-6′b), 3.64 (1H, *m*, H-1b), 3.31 (3H, *m*, H-3′, -4′ and -5′), 3.16 (1H, *dd*, *J* = 9 and 8 Hz, H-2′), 2.07 (2H, *m*, H_2-5), 1.71 (1H, *m*, H-2a), 1.65 (3H, *s*, H_3-9), 1.42 (2H, *m*, H-2b and H-4a), 1.26 (2H, *m*, H-3 and H-4b), 0.93 (3H, *d*, *J* = 7 Hz, H_3-10); for ^{13}C NMR spectroscopic assignments, see Table 1; HR-ESI-MS (positive-ion mode) *m*/*z*: 357.1877 $[M+Na]^+$ (Calcd. for $C_{16}H_{30}O_7Na$: 357.1883).

4.5. Syntheses of (*R*)- and (*S*)-MTPA esters (*1a* and *1b*, respectively) of myopochlorin (*1*)

A solution of **1a** (1.5 mg) in 1 ml of dry CH_2Cl_2 was reacted with (*R*)- and (*S*)-MTPA (43.2 and 41.3 mg, respectively) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (28.7 and 34.5 mg, respectively) and *N*,*N*-dimethyl-4-aminopyridine (23.3 and 19.8 mg, respectively), and then the mixture was occasionally stirred at 25 °C for 30 min. After addition of CH_2Cl_2 (1 ml), the solution was successively washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$-saturated H_2O, and then brine (1 ml). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, developed with $CHCl_3$–$(CH_3)_2CO$ (9:1) for 9 cm, and then eluted with $CHCl_3$–MeOH (9:1)] to furnish esters, **1a** (0.37 mg) and **1b** (0.73 mg). Myopochlorin *R*-MTPA ester (**1a**) Amorphous powder, ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.57–7.60 (2H, *m*, aromatic protons), 7.43–7.46 (3H, *m*, aromatic protons), 5.50 (1H, *d*, *J* = 5 Hz, H-1), 5.48 (1H, *d*, *J* = 10 Hz, H-6), 4.79 (1H, *d*, *J* = 10 Hz, H-7), 4.64 (1H, *d*, *J* = 10 Hz, H-10a), 3.99 (1H, *ddd*, *J* = 12, 12 and 2 Hz, H-3a), 3.51 (3H, *br s*, –OC*H*$_3$), 3.79 (1H, *ddd*, *J* = 12, 5, 2 Hz, H-3b), 3.50 (1H, *dd*, *J* = 12 and 2 Hz, H-10b), 2.34 (1H, *d*, *J* = 5 Hz, H-9), 1.77 (1H, *ddd*, *J* = 14, 14 and 5 Hz, H-4a), 1.59 (1H, *ddd*, *J* = 14, 2 and 2 Hz, H-4b); HR-ESI-MS (positive-ion mode) *m*/*z*: 475.0741 $[M+Na]^+$ (Calcd. for $C_{19}H_{20}O_7F^{35}ClNa$: 475.0741), 477.0729 $[M+Na]^+$ (Calcd. for $C_{19}H_{20}O_7F^{37}ClNa$: 477.0712). Myopochlorin *S*-MTPA ester (**1b**) Amorphous powder, ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.57–7.61 (2H, *m*, aromatic protons), 7.43–7.46 (3H, *m*, aromatic protons), 5.50 (1H, *d*, *J* = 10 Hz, H-6), 5.48 (1H, *d*, *J* = 5 Hz, H-1), 4.79 (1H, *d*, *J* = 10 Hz, H-7), 4.64 (1H, *d*, *J* = 10 Hz, H-10a), 3.96 (1H, *ddd*, *J* = 12, 12 and 2 Hz, H-3a), 3.58 (3H, *br s*, –OC*H*$_3$), 3.77 (1 H, *ddd*, *J* = 12, 5 and 2 Hz, H-3b), 3.50 (1H, *dd*, *J* = 12 and 2 Hz, H-10b), 2.30 (1H, *d*, *J* = 5 Hz, H-9), 1.69 (1H, *ddd*, *J* = 14, 14 and 5 Hz, H-4a), 1.49 (1H, *ddd*, *J* = 14, 2 and 2 Hz, H-4b); HR-ESI-MS (positive-ion mode) *m*/*z*: 475.0747 $[M+Na]^+$ (Calcd. for $C_{19}H_{20}O_7F^{35}ClNa$: 475.0741), 477.0722 $[M+Na]^+$ (Calcd. for $C_{19}H_{20}O_7F^{37}ClNa$: 477.0712).

4.6. Analyses of the sugar moiety

About 500 μg each of myobontiosides A (**2**), B (**3**), C (**4**) and D (**5**) were individually hydrolyzed with 1 N HCl (0.1 ml) at 100 °C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 ml) and the H_2O layers were analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Shodex Asahipak NH2P-50 4E, CH_3CN–H_2O (4:1), 1 ml/min]. Hydrolyzates of myobontiosides A (**2**), B (**3**), C (**4**) and D (**5**) gave a peak for D-glucose at the retention time at 14.2 min (positive optical rotation sign). Peaks were identified by co-chromatography with authentic D-glucose.

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