

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

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Received 00 March, 2008; received in revised form 00 May, 2008

Available online 00 May, 2008

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Abstract

Sixteen compounds were isolated from the MeOH extract of leaves of *Myoporum bontioides*. Of the compounds isolated, five were found to be new. Their structures 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.

Keywords: iridoid; iridoid glucoside; acetogenin glucoside; monoterpene glucoside;

Myoporum bontioides; Myoporaceae; myopochlorin; myobontioside

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 it 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, $[\alpha]_{\text{D}}^{25} -54.9$ (c=6.35, MeOH) (Burger et al., 1987), cimidahurine (**7**), amorphous powder, $[\alpha]_{\text{D}}^{26} -40.7$ (c=0.21, MeOH) (Sugiyama and Kikuchi, 1992), verbascoside (**8**), amorphous powder, $[\alpha]_{\text{D}}^{25} -83.3$ (c=9.61, MeOH) (Birkofer et al., 1968; Nishimura et al., 1991), isoverbascoside (**9**), amorphous powder, $[\alpha]_{\text{D}}^{26} -28.2$ (c=0.17, MeOH) (Miyase et al., 1982), and oxoverbascoside (**10**), amorphous powder, $[\alpha]_{\text{D}}^{25} -68.6$ (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), tricetin (**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.

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**). The details and yields are given under **Experimental**. The structures of the new compounds (**1-5**) 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 , $J = 6\text{Hz}$)] (Table 1). According to these spectral data, **1** was considered to be a C-9 iridoid, and judging from the three degrees of unsaturation, it must have a tricyclic skeleton. The ^1H - ^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 inspected (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, the 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, H₂-3, and H₂-4 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) 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-oxira-5,6,7-triol.

--Table 1--

--Fig. 1--

--Fig. 2--

--Fig. 3--

--Fig. 4--

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]⁺ (C₁₅H₂₃³⁵ClO₉Na), and 407.0886 [M+Na]⁺ (C₁₅H₂₃³⁷ClO₉Na) 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 signals ($\delta_{\text{C}} 93.3$ with $\delta_{\text{H}} 5.44$), and a *cis*-double bond [$\delta_{\text{C}} 105.7$ with $\delta_{\text{H}} 4.94$ (*dd*, $J = 6, 3\text{ Hz}$) and $\delta_{\text{C}} 140.9$ with $\delta_{\text{H}} 6.22$ (*dd*, $J = 6, 2\text{ Hz}$)] 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 revealed 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 ($\delta_{\text{C}} 52.9$). Therefore, the structure of myobontioside A (**2**) was elucidated to be as shown in Fig. 1.

--Fig. 5--

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 signals (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 H₂-6' 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 ¹³C-NMR spectrum showed six signals assignable to a β-glucopyranose and ten signals for an aglycone moiety, consisting of seven methylenes, one oxygenated methylene (δ_C 70.8) signal, one methine (δ_C 83.1) signal, 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 signals 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**), [α]_D²³ -27.7, was also isolated as an amorphous powder and its elemental composition was determined to be C₁₆H₃₀O₇. The one-dimensional NMR spectra exhibited six signals assignable to a β-glucopyranose, and the remaining ten signals 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 ¹H-¹H COSY spectrum, which showed a correlation sequence from H₂-1 through H-6, and H₃-10 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 H₂-8 (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. Conclusion

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 its derivatives (Sudo et al., 1997), 7-chlorodeutzol (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

Mp. 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. ^1H - and ^{13}C -NMR spectra were taken on a JEOL JNM α -400 spectrometer at 400 MHz 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 75C₁₈-OPN (Nacalai Tesque, Kyoto, Japan) [$\Phi = 50$ mm, $L = 25$ cm, linear gradient: MeOH-H₂O (1:9, 1 L) \rightarrow (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 ($\Phi = 2$ mm, $L = 40$ cm), and the lower and upper layers of a solvent mixture of CHCl₃-MeOH-H₂O-*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; $\Phi = 6$ 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 one 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 water (3 l) and then extracted with EtOAc (3 l) to give 114 g of an EtOAc-soluble fraction. 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 221 g of a water-soluble fraction.

The 1-BuOH-soluble fraction (90.0 g) was subjected to a Diaion HP-20 column ($\Phi = 50$ mm, $L = 50$ cm) using H₂O–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 20% MeOH eluent was subjected to silica gel (500 g) CC, with elution with CHCl₃ (2 l) and CHCl₃–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 47.3 mg of **1** in fractions 35–43. Combined 10% MeOH 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 (H₂O-MeOH, 9:1) to afford 1.36 mg of **4**. Combined 20% MeOH eluate 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 (H₂O-CH₃CN, 19:1) to give 3.2 mg of **7**, 9.6 mg of **6** and 2.2 mg of **2**. 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 H₂O-MeOH (3:2) to give 36.7 mg of **8** and the latter with H₂O-CH₃CN (5:1) to give 5.15 mg of **3**. An aliquot (4.41 g) of the residue (6.62 g) of 25% MeOH 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, 29.9 mg of **11** 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 CHCl₃ (2 l) and CHCl₃-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 (H₂O-MeOH, 7:3) to give 6.58 mg of **12** and 5.23 mg of **5**. The residue (9.53 g) of 15–30% MeOH 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 (H₂O-MeOH, 7:3) to give 5.18 mg of **10** and 145 mg of **8**.

Insoluble matter (94.0 mg) in 60–80% eluate fractions 14–21, obtained on Diaion HP-20 CC, was dissolved in DMSO and purified by HPLC (H₂O-MeOH, 7:3) to furnish 1.83 mg of **14**, 4.01 mg of **13**, 17.5 mg of **16** and 19.2 mg of **15**. The remaining residue (20.6 g) was subjected to silica gel CC in the same manner as 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 (H₂O-MeOH, 7:3) to afford 22.8 mg of **8** and 3.94 mg of **9**.

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 (CD₃OD, 400 MHz) δ : 5.41 (1H, d, $J = 6$

Hz, H-1), 4.53 (1H, *dd*, $J = 11, 2$ Hz, H-7), 4.46 (1H, *d*, $J = 10$ Hz, H-10a), 3.74 (1H, *ddd*, $J = 13, 13, 2$ Hz, H-3a), 3.71 (1H, *d*, $J = 11$ Hz, H-6), 3.66 (1H, *ddd*, $J = 13, 6, 2$ Hz, H-3b), 3.40 (1H, *dd*, $J = 10, 2$ Hz, H-10b), 2.15 (1H, *d*, $J = 6$ Hz, H-9), 1.87 (1H, *ddd*, $J = 13, 2, 2$ Hz, H-4a), 1.62 (1H, *ddd*, $J = 13, 13, 6$ Hz, H-4b); (DMSO- d_6 , 400 MHz) δ : 5.70 (1H, *s*, 8-OH), 5.33 (1H, *d*, $J = 6$ Hz, H-1), 5.31 (1H, *br s*, 6-OH), 4.75 (1H, *s*, 5-OH), 4.46 (1H, *dd*, $J = 11, 2$ Hz, H-7), 4.24 (1H, *d*, $J = 10$ Hz, H-10a), 3.57 (2H, *m*, H₂-3), 3.54 (1H, *m*, H-6), 3.30 (1H, *dd*, $J = 10, 2$ Hz, H-10b), 2.04 (1H, *d*, $J = 6$ Hz, H-9), 1.74 (1H, *br dt*, $J = 13, 2$ Hz, H-4a), 1.45 (1H, *ddd*, $J = 13, 13, 6$ Hz, H-4b); HR-FAB-MS (negative-ion mode) m/z : 235.0335 [M-H]⁻ (Calcd for C₉H₁₂³⁵ClO₅: 235.0339), m/z : 237.0349 [M-H]⁻ (Calcd for C₉H₁₂³⁷ClO₅: 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 (CD₃OD, 400 MHz) δ : 6.22 (1H, *dd*, $J = 6, 2$ Hz, H-3), 5.44 (1H, *d*, $J = 4$ Hz, H-1), 4.94 (1H, *dd*, $J = 6, 3$ Hz, H-4), 4.65 (1H, *d*, $J = 8$ Hz, H-1'), 3.96 (1H, *m*, H-6), 3.90 (1H, *dd*, $J = 12, 2$ 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*, $J = 12, 6$ Hz, H-6'b), 3.38 (1H, *m*, H-3'), 3.29 (2H, *m*, H-4' and 5'), 3.21 (1H, *dd*, $J = 9, 8$ Hz, H-2'), 2.84 (1H,

dd, $J = 9, 3$ Hz, H-5), 2.64 (1H, *dd*, $J = 9, 4$ Hz, H-9), 2.34 (1H, *dd*, $J = 14, 6$ Hz, H-7a), 1.81 (1H, *dd*, $J = 14, 4$ Hz, H-7b); HR-ESI-MS (positive-ion mode) m/z :405.0912 [M + Na]⁺ (Calcd for C₁₅H₂₃³⁵ClO₉Na: 405.0922), m/z :407.0886 [M+Na]⁺ (Calcd for C₁₅H₂₃³⁷ClO₉Na: 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 (CD₃OD, 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*, $J = 8, 2$ 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*, $J = 12, 2$ Hz, H-6'a), 4.43 (1H, *dd*, $J = 12, 6$ 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*, -OCH₃), 3.61 (1H, *d*, $J = 13$ Hz, H-10b), 3.53 (1H, *d*, $J = 1$ Hz, H-7), 3.53 (1H, *m*, H-3'), 3.40 (2H, *m*, H-4' and 5'), 3.28 (1H, *dd*, $J = 9, 8$ Hz, H-2'), 2.55 (1H, *d*, $J = 8$ Hz, H-9); HR-ESI-MS (positive-ion mode) m/z :577.1518 [M+Na]⁺ (Calcd for C₂₅H₃₀O₁₄Na: 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; $^1\text{H-NMR}$ (CD_3OD , 400 MHz) δ : 4.54 (1H, *dddd*, $J = 8, 8, 7, 6$ Hz, H-4), 4.25 (1H, *d*, $J = 8$ Hz, H-1'), 3.90 (1H, *dt*, $J = 10, 7$ Hz, H-10a), 3.86 (1H, *dd*, $J = 12, 2$ Hz, H-6'a), 3.66 (1H, *dd*, $J = 12, 5$ Hz, H-6'b), 3.55 (1H, *dt*, $J = 10, 7$ Hz, H-10b), 3.34 (1H, *m*, H-3'), 3.26 (1H, *m*, H-5'), 3.25 (1H, *m*, H-4'), 3.17 (1H, *dd*, $J = 9, 8$ Hz, H-2'), 2.57 (1H, *ddd*, $J = 18, 10, 9$ Hz, H-2a), 2.50 (1H, *ddd*, $J = 18, 9, 5$ Hz, H-2b), 2.34 (1H, *dddd*, $J = 13, 9, 7, 5$ Hz, H-3a), 1.86 (1H, *dddd*, $J = 13, 10, 9, 8$ Hz, H-3b), 1.64 (1H, *m*, H-5a), 1.61 (3H, *m*, H-5b and H₂-9), 1.41 (6H, *m*, H₂-6, 7 and 8); HR-ESI-MS (positive-ion mode) m/z : 371.1674 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{16}\text{H}_{28}\text{O}_8\text{Na}$: 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; $^1\text{H-NMR}$ (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*, H₂-8), 3.86 (1H, *dd*, $J = 12, 2$ Hz, H-6'a), 3.85 (1H, *m*, H-1a), 3.67 (1H, *dd*, $J = 12, 5$ Hz, H-6'b), 3.64 (1H, *m*, H-1b), 3.31 (3H, *m*, H-3', 4' and 5'), 3.16 (1H, *dd*, $J = 9, 8$ Hz, H-2'), 2.07 (2H, *m*, H₂-5), 1.71 (1H,

m, H-2a), 1.65 (3H, *s*, H₃-9), 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, H₃-10); HR-ESI-MS (positive-ion mode) *m/z*:357.1877 [M+Na]⁺ (Calcd for C₁₆H₃₀O₇Na: 357.1883).

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

(*I*) A solution of **1a** (1.5 mg) in 1 ml of dry CH₂Cl₂ was reacted with (*R*)- and (*S*)-MTPA (43.2 mg and 41.3 mg, respectively) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (28.7 mg and 34.5, respectively) and *N,N*-dimethyl-4-aminopyridine (23.3 mg and 19.8 mg, respectively), and then the mixture was occasionally stirred at 25 °C for 30 min. After the addition of 1 ml of CH₂Cl₂, the solution was washed with H₂O (1 ml), 5% HCl (1 ml), NaHCO₃-saturated H₂O, and then brine (1 ml), successively. The organic layer was dried over Na₂SO₄ and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, developed with CHCl₃-(CH₃)₂CO (9:1) for 9 cm, and then eluted with CHCl₃-MeOH (9:1)] to furnish esters, **1a** (0.37 mg) and **1b** (0.73 mg). Myopochlorin *R*-MTPA ester (**1a**) Amorphous powder, ¹H-NMR (CDCl₃, 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*, $J = 12, 12, 2$ Hz, H-3a), 3.51 (3H, *br s*, -OCH₃), 3.79 (1H, *ddd*, $J = 12, 5, 2$ Hz, H-3b), 3.50 (1H, *dd*, $J = 12, 2$ Hz, H-10b), 2.34 (1H, *d*, $J = 5$ Hz, H-9), 1.77 (1H, *ddd*, $J = 14, 14, 5$ Hz, H-4a), 1.59 (1H, *ddd*, $J = 14, 2, 2$ Hz, H-4b); HR-ESI-MS (positive-ion mode) m/z :475.0741 [M+Na]⁺ (Calcd for C₁₉H₂₀O₇F³⁵ClNa: 475.0741), 477.0729 [M+Na]⁺ (Calcd for C₁₉H₂₀O₇F³⁷ClNa: 477.0712). Myopochlorin *S*-MTPA ester (**1b**) Amorphous powder, ¹H-NMR (CDCl₃, 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*, $J = 12, 12, 2$ Hz, H-3a), 3.58 (3H, *br s*, -OCH₃), 3.77 (1H, *ddd*, $J = 12, 5, 2$ Hz, H-3b), 3.50 (1H, *dd*, $J = 12, 2$ Hz, H-10b), 2.30 (1H, *d*, $J = 5$ Hz, H-9), 1.69 (1H, *ddd*, $J = 14, 14, 5$ Hz, H-4a), 1.49 (1H, *ddd*, $J = 14, 2, 2$ Hz, H-4b); HR-ESI-MS (positive-ion mode) m/z :475.0747 [M+Na]⁺ (Calcd for C₁₉H₂₀O₇F³⁵ClNa: 475.0741), 477.0722 [M+Na]⁺ (Calcd for C₁₉H₂₀O₇F³⁷ClNa: 477.0712).

4.6. Analyses of the sugar moiety

About 500 μ g each of myobontiosides A (**2**), B (**3**), C (**4**) and D (**5**) were hydrolyzed with 1N 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 water layers were analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Shodex Asahipak NH2P-50 4E, CH₃CN-H₂O (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.

Acknowledgements

The authors are grateful for access to the superconducting NMR instrument at the Analytical Center of Molecular Medicine of the Hiroshima University Faculty of Medicine. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, Culture and Technology of Japan, the Japan Society for the Promotion of Science, and the Ministry of Health, Labour and Welfare. Thanks are also due to the Astellas Foundation for Research on Medicinal Resources and the Takeda Science Foundation for the financial support.

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Table 1. ^{13}C -NMR Data for Compounds **1-5** (CD_3OD , 100 MHz).

C No.	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		

^aChemical shifts in parentheses are for $\text{DMSO}-d_6$.

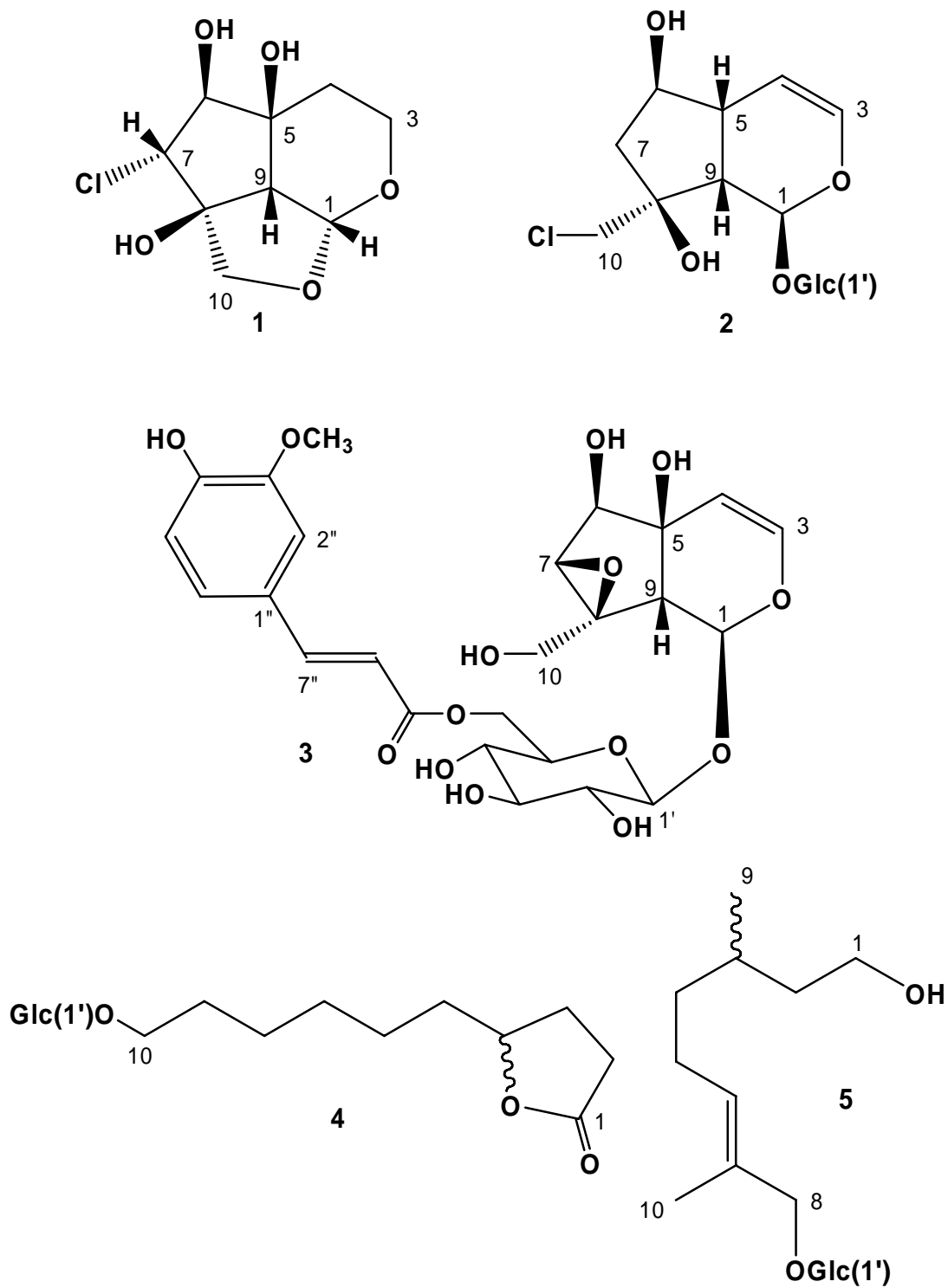
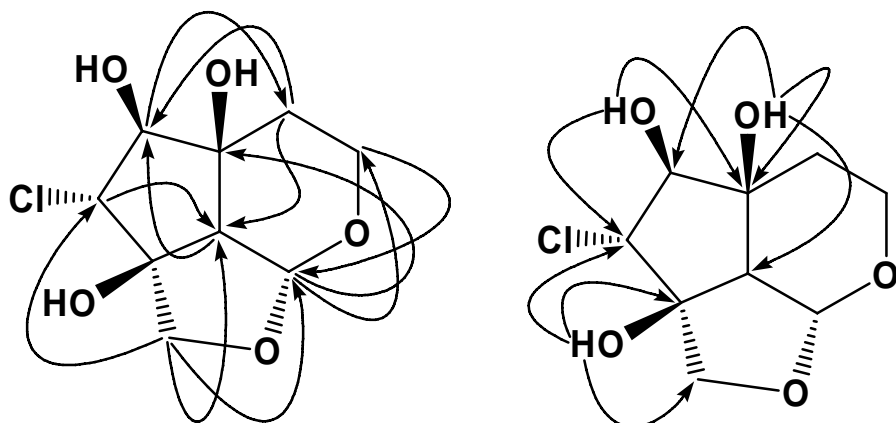


Fig. 1. Structures.



(a) for CD_3OD solution

(b) for $\text{DMSO-}d_6$ solution

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

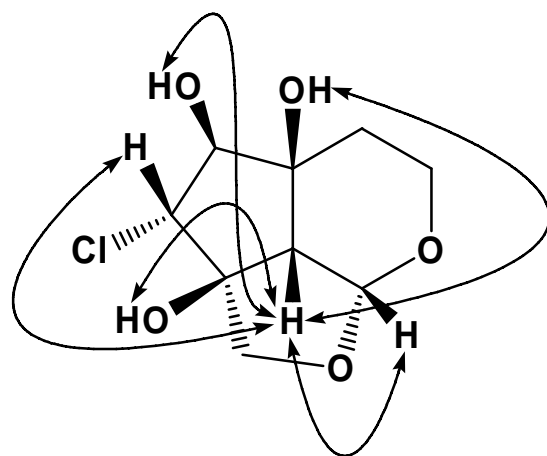


Fig. 3. Diagnostic NOESY correlations of **1** ($\text{DMSO-}d_6$).

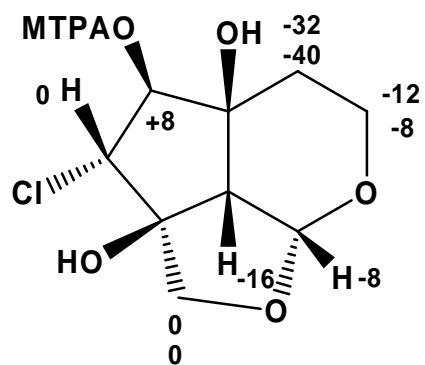


Fig. 4. Results of modified Mosher's method of myopochlorin (1) ($\Delta\delta$ values in Hz, $\delta_S - \delta_R$ at 400 MHz).

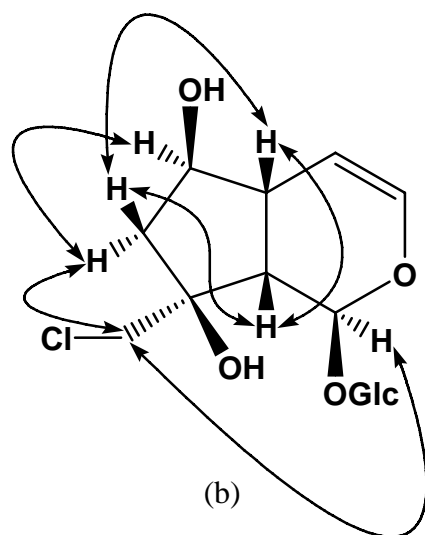
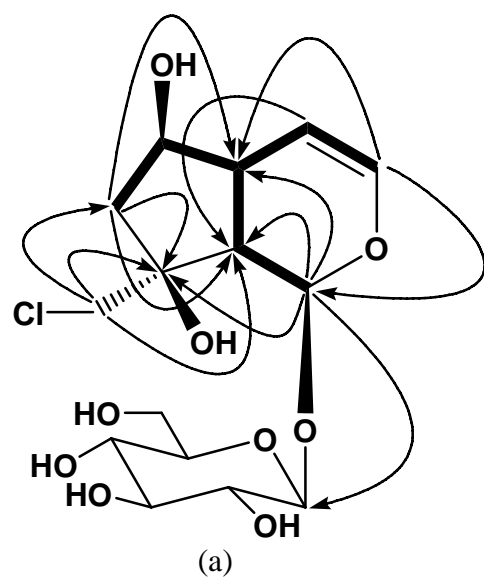


Fig. 5. Two-dimensional correlations for myobontinoside A (2).
 (a) ^1H - ^1H COSY correlations (**—**) and HMBC correlations from H to C.
 (b) NOESY correlations.