Microtropiosides A−F: *ent*-Labdane-type diterpenoid glucosides from the leaves of *Microtropis japonica* (Celastraceae)

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ABSTRACT

From a 1-BuOH-soluble fraction of a MeOH extract of the leaves of *M. japonica*, collected in the Okinawa islands, six new *ent*-labdane glucosides, named microtropiosides A-F, were isolated together with one known acyclic sesquiterpene glucoside. The structures of the new compounds were elucidated by a combination of spectroscopic analyses, and their absolute configurations by application of the β-D-glucopyranosylation-induced shift-trend rule in the ^{13}C NMR spectroscopy and the modified Mosher's method.

Keywords: Microtropis japonica, Celastraceae, *ent*-labdane glucoside, microtropioside

1. Introduction

Microtropis japonica Hallier f. (Celastraceae) is an evergreen tree of about 5 m in height, and has a distinct distribution in restricted southern parts of Kanto and Kyushu, Japan, Okinawa islands and Taiwan. Only three reports have been published concerning the constituents of *M. japonica* (Chen et al., 2008; Chou et al., 2008; Chen et al., 2009). In a continuing study on Okinawan resource plants, the chemical constituents of *M. japonica*, collected in Okinawa, were investigated.

From a 1-BuOH-soluble fraction of a MeOH extract of leaves of *M. japonica*, six new *ent*-labdane diterepene glucosides (**1**-**6**) were isolated together with one acyclic sesquiterpene glucoside (**7**) (Fiorentino et al., 2006). This paper deals with structural elucidation of the six *ent*-labdane diterpene glucosides.

2. Results and discussion

Air-dried leaves of *Microtropis japonica* 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 seven compounds (**1**-**7**). The details and yields are given in the **Experimental**. The structures of the new *ent*-labdane glucosides (**1**–**6**) were elucidated on the basis of spectroscopic evidence, and the known compound was identified as amarantholidoside IV by comparison of its spectroscopic properties with those reported in the literature (Fiorentino et al., 2006) (Fig. 1).

-- Fig. 1 –

Microtropioside A (**1**), $[\alpha]_D$ −34.2,1 was isolated as an amorphous powder whose elemental composition was determined by HR-ESI-TOF-MS as $C_{26}H_{44}O_9$. Its spectroscopic properties (see experimental) indicated the presence of a β-glucopyranose unit and a diterpenoid unit consisting of four tertiary methyls, eight methylenes, two methines and four quaternary carbons. Their carbon chemical shift values clearly showed that two methylenes, two methines and two quaternary carbons were all oxygenated. Acetylation afforded a pentaacetate (**1a**) in which only one secondary hydroxyl group in the aglucone was acetylated [δ_H 3.84 (H-14) which shifted downfield to δ_H 5.07]. Thus the other oxygenated carbons were involved in ether linkages. HMBC correlations (see Table 1 for chemical shifts) from Me-18, Me-19 and Me-20 to C-5, H-5, Me-20 and H_2-17 to C-9 and H_2-17 to C-15 suggested that microtropiosdie A was a glucoside of a labdane diterpenoid with two oxirane rings as in **1**

(Fig. 2). Comparison of the carbon shifts of C-2 and C-3 of **1** with those of the labdane 3-*O*-glucoside, tricalysioside U (**8**) (Otsuka et al., 2007) and the *ent*-labdane 3-*O*-glucoside (**9**) (Shen et al., 2006) (Table 1) clearly showed that microtropioside A belonged to the *ent*-labdane series. The configuration of the C-14 hydroxyl group was deduced to be β on the basis of significant NOE interactions between H-11 axial and H-14. H-11 axial also showed a NOE with one of the C-17 methylene protons thus confirming the configuration at C-8 (Fig. 3). The glucose moiety was shown to belong to the D series by chirality analysis of a hydrolysate of **1** and the coupling constants of H-3 indicated it was axial. Thus microtropioside A (**1**) is *ent*-8*R*,13*S*;15,17-diepoxy-3β-D-glucopyranosyloxylabdan-14a-ol.

Microtropioside B (**2**), $[\alpha]_D^{24}$ −52.1, was isolated as colorless needles and its elemental composition was determined to be $C_{26}H_{46}O_9$ by HR-ESI-TOF-MS. The IR and NMR spectra were similar to those of **1**. However, in the ^{1}H NMR spectrum, signals for five singlet methyls were observed and based on the elemental composition, the structure of the aglycone of **2** was presumed to possess a tricyclic labdane skeleton. The ^{13}C NMR spectrum also supported the presumption that one of the oxymethylene carbons observed in that of **1** was replaced by a methyl signal [δ_C 25.7 with δ_H 1.28 (3H, s)]. Six signals assignable to β-glucopyranose were also observed in the ^{13}C NMR spectrum. The absolute configuration of the glucose was determined to be in the D-series using a chiral detector. Therefore, the seven

membered oxirane ring in **1** must be cleaved to generate a primary alcohol and a methyl group, and this was further confirmed by acetylation of **2** gave a hexaacetate (**2a**). Since the ^{13}C NMR data for the A ring were essentially the same as those for **1**, the aglycone of microtropioside B (**2**) must also belong to the *enantio*-series. This was confirmed by application of the ^{13}C-β-D-glucosylation-induce shift-trend rule (Kasai et al., 1977), namely ongoing from the aglycone (**2b**) to the glucoside (**2**), C-2 shifted up field by 3.9 ppm, whereas C-4 only shifted by 1.2 ppm (Table 1). Thus, the absolute configuration at the 3-position was determined to be *R*, i.e., the cyclic part of the aglycone was found to be in the *enantio* form. The absolute configuration at the C-14 position was expected to be the same as that of co-occurring **1**. To confirm this, 15-*O*-pivaloyl-3,14-di-*O*-α-methoxy-α-trifluoromethylphenylacetic acid (MPTA) esters were prepared. The results with the modified Mosher's method clearly showed that the absolute configuration at the 3-position was *R* and that of the 14-position *S*, which were the same as those in **1** (Fig. 4) (Ohtani et al., 1991). Accordingly, the aglycone of microtropioside B was found to be a synthetically known compound (Garcia-Alvarez et al., 1982). Therefore, microtropioside B has structure **2**, as shown in Fig. 1.

Microtropioside C (**3**), $[\alpha]_D^{24}$ −73.0, was isolated as colorless needles and its elemental composition was determined to be $C_{32}H_{56}O_{14}$ by HR-ESI-TOF-MS. Spectral data

indicated that **3** was an analogous compound to **2**. In the ^{1}H NMR spectrum, two anomeric protons [δ_H 4.32 (2H, d, J = 8 Hz)] were observed and in the ^{13}C NMR spectrum, 12 signals were assigned to two β-glucopyranose sets. NMR spectra including two-dimensional ones suggested the microtropioside C (**3**) was the β-D-glucopyranoside of **2**. The second glucopyranosyloxy group was clearly attached to C-14, judging from a significant low field shift of C-14 (δ_C 77.8 in **2** and δ_C 88.9 in **3**) in the ^{13}C NMR spectrum. The HMBC correlation cross peaks between H-1' (δ_H 4.32) and C-3 (δ_C 85.8) and C-14 (δ_C 88.9) further substantiated the structure of **3**.

Microtropioside D (**4**), $[\alpha]_D^{24}$ −46.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_{32}H_{56}O_{14}$, which was the same as that of **3**. Other spectral data were similar to those of **3**, except for significant up field (δ_C 88.9 in **3** and δ_C 75.5 in **4**) and down field (δ_C 64.0 in **3** and δ_C 72.6 in **4**) shifts of C-14 and C-15 in the ^{13}C NMR spectrum, respectively. In the HMBC spectrum, one (δ_H 4.31) of the anomeric protons showed a correlation cross peak with C-3 (δ_C 85.9) and the other (δ_H 4.27) with C-15 (δ_C 72.6). Thus microtropioside D has structure **4**.

Microtropioside E (**5**), $[\alpha]_D^{24}$ −32.5, was also isolated as an amorphous powder and its elemental composition was determined to be $C_{32}H_{54}O_{13}$. In the ^{1}H NMR spectrum, signals for two anomeric protons (δ_H 4.32 and 4.33) and three olefinic protons [δ_H 6.30 (1H, dd, J =

18, 11 Hz), 5.44 (1H, dd, *J* = 18, 2 Hz) and 5.11 (1H, dd, *J* = 11, 2 Hz)] were observed. Supporting the above evidence, ^{13}C NMR exhibited 12 carbon signals assignable to two β-glucopyranose sets and two *sp*2 carbon signals [δ_C 116.2 (CH_2) and 143.4 (CH)]. The 13C NMR spectrum confirmed the presence of two glucose moieties and a vinyl group. The latter can be reasonably attached to C-13 and this was by the HMBC correlation of H-15 (δ_H 5.44 and 5.11) with C-13 (δ_C 77.2). Since the oxymethine proton [δ_H 3.50 on C-12 (δ_C 89.1)] showed correlation peaks with C-13, C-14 (δ_C 143.4), and C-16 (δ_C 29.8) in the HMBC spectrum, an oxygen atom must be attached to C-12 (δ_C 89.1), to which one of the anomeric proton (δ_H 4.33) was correlated. H-12 is placed axial (β), namely the β-orientation in case of the *ent*-labdane skeleton, judging from its coupling constant values (*J* = 12, 5 Hz). PS-NOESYcorrelation of H-12 with Me-16 and H-9 confirmed its axial configuration.

Microtropioside F (**6**), $[\alpha]_D^{25}$ −82.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{32}H_{54}O_{13}$. Its spectroscopic properties were very similar to those of **5** apart from the coupling. Thus, the orientation of the H-12 proton must be at the equatorial position, namely the α-orientation in the case of the *ent*-labdane skeleton. Thus H-12 must be equatorial (α). PS-NOESY correlations of H-12 with 2H-11 and H-14 confirmed this assignment. Thus microtropioside F has structure **6**.

3. Conclusion

From the leaves of *M. japonica*, six new *ent*-labdane glucosides, named microtropiosides A–F (**1**–**6**), were isolated. Microtropioside A (**1**) possesses unusual two oxyrane rings between C-8 and C-17, and side chain. The primary alcohol of the aglycone (**2a**) of microtropioside B (**2**) was first protected as pivaloyl ester and then the modified Mosher's method was applied to determine the absolute structure. Chen et al. (2008) and Chou et al. (2008) isolated highly acylated dihydroagarofuranoid sesquiterpenes from the EtOAc-soluble fraction of the stem of *M. japonica*, while cytotoxicic triterpenoids were isolated from the same plant by the same group (Chen et al., 2009). Dihydroagarofuranoid sesquiterpenes (Chen at al., 2006a) and triterpenes (Chen et al., 2006b) were also isolated from a related species, *M. fokienensis* and *M. triflora* (Wang et al., 2007). Whereas, our phytochemical investigation led an isolation of diterpenoids for the first time from *M. japonica*.

4. Experimental

4.1. General

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 polarimeter. IR spectra

were measured on a Horiba FT-710 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, and a JEOL ECA-600 spectrometer at 600 MHz and 150 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HR-ESI-TOF-MS were measured with an Applied Biosystem QSTAR® XL system.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecylsilyl silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan), 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. Preparative (prep.) 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.

Emulsin and, (*R*)- and (*S*)-MTPA were purchased from Tokyo Chemical Industries Co., Ltd. (Tokyo, Japan), and crude hesperidinase was a gift from Tokyo Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan).

4.2. Plant material

Leaves of *M. japonica* Hallier f. (Celastraceae) were collected in Kunigami Village, Kumigami County, Okinawa, Japan, in July 1997, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (97-MJ-Okinawa-0716).

4.3. Extraction and isolation

Dried leaves of *M. japonica* (3.25 kg) were extracted three times with MeOH (15 l) at 25 ^{o}C for one week and then concentrated to 3 l *in vacuo*. The extract was washed with *n*-hexane (3 l, 17.9 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 171 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 l) to give a 1-BuOH-soluble fraction (32.1 g), and the remaining water-layer was concentrated to furnish 136 g of a water-soluble fraction.

The 1-BuOH-soluble fraction (32.0 g) was subjected to a Diaion HP-20 column (Φ = 30 mm, L = 50 cm) using H_2O–MeOH (4:1, 2 l), (2:3, 2 l), (3:2, 2 l), and (1:4, 2 l), and MeOH (2 l), 500 ml fractions were being collected. The residue (6.16 g in fractions 6–12) of the 60% MeOH eluent was subjected to silica gel (150 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(49:1, 1 l), (24:1, 1 l), (23:2, 1 l), (9:1, 1 l), (17:3, 1 l), (4:1, 1 l), (3:1, 16 l), and (7:3, 1 l)], 200 ml fractions being collected. The residue (439 mg) in fractions 25−28 was separated by ODS CC [Φ = 25 mm, L = 25 cm, linear gradient: MeOH-H_2O (1:9, 1 l) → (1:1, 1 l), 5 g fractions being collected] and the residue (36.0 mg) in 184−192 was purified by prep. HPLC (MeOH-H_2O, 1:1) to give 5.0 mg of **1** from the peak at 11 min. The residue (891 mg) in fraction 29−33 obtained on silica gel CC separated by ODS CC [Φ = 50 mm, L = 25 cm, linear gradient: MeOH-H_2O (1:9, 2 l) → (1:1, 2 l), 5 g fractions being collected] to give 74.3 mg of **2** in fractions 175−181 and 29.6 mg of **7** in fractions 227−233.

The residue (594 mg) in fractions 39−41 obtained on silica gel CC was separated by ODS CC [Φ = 25 mm, L = 25 cm, linear gradient: MeOH-H_2O (1:9, 1 l) → (1:1, 1 l), 5 g fractions being collected] to give residues, 24.3 mg in fractions 125−132, 37.3 mg in fractions 137−143, 29.3 mg in fractions 189−195 and 47.6 mg in fractions 211−219. The first one was subjected to DCCC and the residue (5.0 mg) in fractions 17−22 was purified by prep. HPLC (MeOH-H_2O, 2:3) to give 2.2 mg of **4** from the peak at 10 min. The second one was subjected

to DCCC and the residue (7.1 mg) in fractions 58−66 was crystallized from MeOH to give 3.0 mg of **3** as colorless needles. The third one was also subjected to DCCC and the residue (5.6 mg) in fractions 59−68 was purified by prep. HPLC (MeOH-H_2O, 11:9) to give 2.5 mg of 5 from the peak at 18 min. The fourth residue was purified by prep. HPLC (MeOH-H_2O, 3:2) to give 31.8 mg of **6** from the peak at 15 min.

*4.4. Microtropioside A (**1**)*

Amorphous powder; $[\alpha]_D^{24}$ −34.2 (MeOH; c 0.33); IR ν_{max} (film) cm^{-1}: 3362, 2936, 1456, 1368, 1159, 1055, 1020; ^1H NMR (CD_3OD, 400 MHz): δ 4.32 (1H, *d*, *J* = 8 Hz, H-1'), 3.98 (1H, *d*, *J* = 14 Hz, H-17a), 3.86 (1H, *dd*, *J* = 12, 10 Hz, H-15a), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6'a), 3.84 (2H, *m*, H-14 and H-15b), 3.81 (1H, *d*, *J* = 14 Hz, H-17b), 3.67 (1H, *dd*, *J* = 12, 6 Hz, H-6'b), 3.35 (1H, *dd*, *J* = 9, 9 Hz, H-3'), 3.33 (1H, *dd*, *J* = 11, 4 Hz, H-3), 3.29 (1H, *m*, H-4'), 3.23 (1H, *ddd*, *J* = 9, 6, 2 Hz, H-5'), 3.16 (1H, *dd*, *J* = 9, 8 Hz, H-2'), 1.97 (1H, *dddd*, *J* = 13, 13, 13, 5 Hz, H-11a), 1.89 (1H, *ddd*, *J* = 14, 7, 2 Hz, H-12a), 1.80 (1H, m, *dddd*, *J* = 14, 4, 4, 4 Hz, H-2a), 1.70 (2H, *m*, H-6a and 7a), 1.69 (1H, *m*, H-1a), 1.63 (1H, *m*, H-2b), 1.61 (1H, *m*, H-12b), 1.56 (1H, *m*, H-11b), 1.28 (1H, *dd*, *J* = 9, 3 Hz, H-9), 1.26 (1H, *m*, H-7b), 1.19 (3H, *s*, H_3-16), 1.02 (3H, *s*, H_3-18), 1.00 (1H, *m*, H-1b), 0.98 (1H, *dd*, *J* = 12, 2 Hz, H-5), 0.90 (3H, *s*, H_3-20), 0.81 (3H, *s*, H_3-19); ^1H NMR (C_5D_5N, 400 MHz): δ 4.91 (1H, *d*, *J* = 8 Hz,

H-1'), 4.58 (1H, *br d*, *J* = 12 Hz, H-6'a), 4.37 (1H, *dd*, *J* = 12, 5 Hz, H-6'b), 4.25 (1H, *dd*, *J* = 9, 9 Hz, H-3'), 4.22 (1H, *m*, H-4'), 4.20 (1H, *dd*, *J* = 9, 9 Hz, H-14), 4.08 (1H, *d*, *J* = 15 Hz, H-17a), 4.10 (1H, *dd*, *J* = 9, 5 Hz, H-15a), 4.07 (1H, *m*, H-15b), 4.02 (1H, *br d*, *J* = 9 Hz, H-2'), 3.98 (1H, *d*, *J* = 15 Hz, H-17b), 3.97 (1H, *m*, H-5'), 3.55 (1H, *dd*, *J* = 12, 5 Hz, H-3), 2.06 (1H, *dddd*, *J* = 13, 3, 3, 3 Hz, H-2a), 1.98 (2H, *m*, H-6a and 12a), 1.88 (1H, *ddd*, *J* = 13, 3, 3 Hz, H-7a), 1.74 (1H, *m*, H-2b), 1.70 (1H, *m*, H-12b), 1.62 (1H, *m*, H-1a), 1.54 (3H, *s*, H_3-16), 1.38 (1H, *m*, H-11a), 1.31 (1H, *m*, H-9), 1.30 (1H, *m*, H-7b), 1.28 (1H, *m*, H-11b), 1.18 (3H, *s*, H_3-18), 0.90 (1H, *m*, H-5), 0.88 (1H, *m*, H-6b), 0.86 (3H, *s*, H_3-19), 0.85 (1H, *m*, H-1b), 0.84 (3H, *s*, H_3-20); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 523.2870 $[M + Na]^+$ (calculated for $C_{26}H_{44}O_9Na$, 523.2877).

*4.5. Microtropioside B (**2**)*

Colorless needles, mp 265−267 ºC (MeOH); $[\alpha]_D^{24}$ −52.1 (MeOH; c 0.19); IR ν_{max} (KBr) cm^{-1}: 3396, 2938, 2868, 1383, 1074, 1025; ^{1}H NMR (CD_3OD, 400 MHz): δ 4.31 (1H, *d*, *J* = 8 Hz, H-1'), 3.89 (1H, *dd*, *J* = 12, 3 Hz, H-15a), 3.85 (1H, *dd*, *J* =12, 2 Hz, H-6'a), 3.67 (1H, *dd*, *J* = 12, 6 Hz, H-6'b), 3.66 (1H, *dd*, *J* = 8, 3 Hz, H-14), 3.44 (1H, *dd*, *J* = 12, 8 Hz, H-15b), 3.35 (1H, *dd*, *J* = 9, 9 Hz, H-3'), 3.34 (1H, *dd*, *J* = 11, 4 Hz, H-3), 3.30 (1H, *m*, H-4'), 3.22 (1H, *ddd*, *J* = 10, 6, 2 Hz, H-5'), 3.16 (1H, *dd*, *J* = 9, 8 Hz, H-2'), 2.17 (1H, *ddd*, *J* = 13, 7,

Hz, H-12a), 1.79 (1H, *dddd*, *J* = 14, 4, 4, 4 Hz, H-11a), 1.74 (1H, *dd*, *J* = 9, 3 Hz, H-7a), 1.67 (1H, *m*, H-6a), 1.65 (2H, *m*, H-1a and 2a), 1.62 (1H, *m*, H-11b), 1.42 (1H, *m*, H-12b), 1.36 (1H, *dd*, *J* = 11, 5 Hz, H-9), 1.35 (1H, m, H-6b), 1.30 (1H, *m*, H-2b), 1.28 (3H, *s*, H_3-17), 1.27 (1H, *m*, H-7b), 1.05 (3H, *s*, H_3-16), 1.03 (3H, *s*, H_3-18), 0.98 (1H, *m*, H-5), 0.97 (1H, *ddd*, *J* = 11, 6, 2 Hz, H-1b), 0.85 (3H, *s*, H_3-20), 0.81 (3H, *s*, H_3-19); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 525.3012 $[M + Na]^+$ (calculated for $C_{26}H_{46}O_9Na$, 525.3034).

*4.6. Microtropioside C (**3**)*

Colorless needles, mp 263−264 ºC (MeOH); $[\alpha]_D^{24}$ −73.0 (MeOH; c 0.20); IR ν_{max} (KBr) cm^{-1}: 3366, 2960, 1370, 1161, 1076, 1029; ^{1}H NMR (CD_3OD, 400 MHz): δ 4.32 (2H, *d*, *J* = 8 Hz, H-1' and 1"), 4.00 (1H, *dd*, *J* = 12, 1 Hz, H-15a), 3.90 (1H, *dd*, *J* = 12, 2 Hz, H-6"a), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6'a), 3.78 (1H, *dd*, *J* = 7, 1 Hz, H-14), 3.67 (1H, *dd*, *J* = 12, 6 Hz, H-6'b), 3.61 (1H, *dd*, *J* = 12, 7 Hz, H-15b), 3.39−3.21 (6H, *m*, H-3', 4', 5', 3", 4" and 5"), 3.35 (1H, *m*, H-3), 3.22 (1H, *dd*, *J* = 9, 8 Hz, H-2"), 3.16 (1H, *dd*, *J* = 9, 8 Hz, H-2'), 2.41 (1H, *ddd*, *J* = 14, 5, 5 Hz, H-12a), 1.78 (1H, *m*, H-2a), 1.77 (1H, *m*, H-7a), 1.72 (1H, *m*, H-6a), 1.69 (1H, *m*, H-1a), 1.62 (1H, *m*, H-11a), 1.57 (1H, *m*, H-11b), 1.43 (1H, *m*, H-12b), 1.39 (1H, *m*, H-7b), 1.29 (3H, *s*, H_3-17), 1.27 (1H, *dd*, *J* = 12, 4 Hz, H-9), 1.06 (3H, *s*, H_3-16), 1.03 (3H,

s, H_3-18), 0.98 (1H, *dd*, *J* = 14, 3 Hz, H-5), 0.96 (1H, *dd*, *J* = 12, 2 Hz, H-1b), 0.93 (1H, *m*, H-2b), 0.85 (3H, *s*, H_3-20), 0.81 (3H, *s*, H_3-19), H-6b could not be assigned; ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 687.3560 [M + Na]$^+$ (calculated for $C_{32}H_{56}O_{14}Na$, 687.3562).

*4.7. Microtropioside D (**4**)*

Amorphous powder; $[\alpha]_D^{24}$ −46.5 (MeOH; c 0.14); IR ν_{max} (film) cm^{-1}: 3368, 2936, 1532, 1446, 1384, 1074, 1026; ^{1}H NMR (CD_3OD, 400 MHz): δ 4.31 (1H, *d*, *J* = 8 Hz, H-1'), 4.27 (1H, *d*, *J* = 8 Hz, H-1"), 4.08 (1H, *dd*, *J* = 11, 8 Hz, H-15a), 3.89 (1H, *dd*, *J* = 8, 2 Hz, H-14), 3.87 (1H, *dd*, *J* = 12, 3 Hz, H-6"a), 3.85 (1H, *dd*, *J* = 12, 3 Hz, H-6'a), 3.76 (1H, *dd*, *J* = 11, 8 Hz, H-15b), 3.66 (2H, *dd*, *J* = 12, 6 Hz, H-6'b and 6"b), 3.37−3.22 (6H, *m*, H-3', 4', 5', 3", 4" and 5"), 3.35 (1H, *dd*, *J* = 11, 2 Hz, H-3), 3.21 (1H, *dd*, *J* = 9, 8 Hz, H-2"), 3.16 (1H, *dd*, *J* = 9, 8 Hz, H-2'), 2.23 (1H, *ddd*, *J* = 13, 6, 6 Hz, H-12a), 1.79 (1H, *m*, H-2a), 1.75 (1H, *m*, H-7a), 1.68 (1H, *m*, H-1a), 1.66 (1H, *m*, H-6a), 1.57 (1H, *m*, H-11a), 1.56 (1H, *m*, H-6b), 1.40 (1H, *m*, H-11b), 1.38 (1H, *m*, H-12b), 1.35 (1H, *m*, H-7b), 1.32 (1H, *dd*, *J* = 11, 4 Hz, H-9), 1.28 (1H, *s*, H_3-17), 1.05 (3H, *s*, H_3-16), 1.02 (3H, *s*, H_3-18), 1.01 (1H, *m*, H-5), 1.00 (1H, *m*, H-1b), 0.85 (3H, *s*, H_3-20), 0.81 (3H, *s*, H_3-19), H-2b could not be assigned; ^{13}C NMR (CD_3OD, 100 MHz): Fig. 1; δ HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 687.3560 [M +

Na]$^+$ (calculated for $C_{32}H_{56}O_{14}Na$, 687.3562).

*4.8. Microtropioside E (**5**)*

Amorphous powder; $[\alpha]_D^{24}$ −32.5 (MeOH; c 0.17); IR ν_{max} (film) cm^{-1}: 3386, 2932, 1456, 1363, 1162, 1074, 1024; ^{1}H NMR (CD_3OD, 400 MHz): δ 6.30 (1H, *dd*, *J* = 18, 11 Hz, H-14), 5.44 (1H, *dd*, *J* = 18, 2 Hz, H-15a), 5.11 (1H, *dd*, *J* = 11, 2 Hz, H-15b), 4.33 (1H, *d*, *J* = 8 Hz, H-1"), 4.32 (1H, *d*, *J* = 8 Hz, H-1'), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6'a), 3.84 (1H, *dd*, *J* = 12, 2 Hz, H-6"a), 3.69 (1H, *dd*, *J* = 12, 6 Hz, H-6"b), 3.67 (1H, *dd*, *J* = 12, 6 Hz, H-6'b), 3.50 (1H, *dd*, *J* = 12, 5 Hz, H-12), 3.37−3.12 (6H, *m*, H-3', 4', 5', 3", 4" and 5"), 3.34 (1H, *dd*, *J* = 9, 4 Hz, H-3), 3.17 (1H, *dd*, *J* = 9, 8 Hz, H-2"), 3.16 (1H, *dd*, *J* = 9, 8 Hz, H-2'), 2.12 (1H, *ddd*, *J* = 12, 4, 1 Hz, H-11a), 1.81 (1H, *dddd*, *J* =14, 4, 4, 4 Hz, H-2a), 1.75 (2H, *m*, H-1a and 7a), 1.69 (3H, *m*, H-2b, 6a and 11b), 1.35 (3H, *s*, H_3-16), 1.32 (2H, *m*, H-6b and 7b), 1.23 (3H, *s*, H_3-17), 1.23 (1H, *m*, H-9), 1.02 (3H, *s*, H_3-18), 0.98 (1H, *m*, H-1b), 0.93 (1H, *dd*, *J* = 12, 2 Hz, H-5), 0.82 (3H, *s*, H_3-20), 0.79 (3H, *s*, H_3-19); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 669.3483 [M + Na]$^+$ (calculated for $C_{32}H_{54}O_{13}Na$, 669.3456).

*4.9. Microtropioside F (**6**)*

Amorphous powder; $[\alpha]_D^{25}$ −82.0 (MeOH; c 0.38); IR ν_{max} (film) cm^{-1}: 3395, 2939, 1383, 1075, 1036, 1018; ^1H NMR (CD_3OD, 400 MHz): δ 6.06 (1H, *dd*, *J* = 18, 11 Hz, H-14), 5.04 (1H, *d*, *J* = 18 Hz, H-15a), 4.97 (1H, *d*, *J* = 11 Hz, H-15b), 4.34 (1H, *d*, *J* = 8 Hz, H-1"), 4.30 (1H, *d*, *J* = 8 Hz, H-1'), 4.25 (1H, *dd*, *J* = 3, 3 Hz, H-12), 3.87 (1H, *dd*, *J* = 12, 2 Hz, H-6"a), 3.84 (1H, *dd*, *J* = 12, 2 Hz, H-6'a), 3.68 (1H, *dd*, *J* = 12, 5 Hz, H-6'b), 3.66 (1H, *dd*, *J* = 12, 5 Hz, H-6"b), 3.42−3.20 (6H, *m*, H-3', 4', 5', 3", 4" and 5"), 3.32 (1H, *m*, H-3), 3.25 (1H, *dd*, *J* = 9, 8 Hz, H-2"), 3.16 (1H, *dd*, *J* = 8, 8 Hz, H-2'), 1.89 (1H, *m*, H-11a), 1.87 (1H, *m*, H-9), 1.83 (1H, *m*, H-6a), 1.79, (1H, *m*, H-2a), 1.72 (1H, *m*, H-11b), 1.71 (1H, *dddd*, *J* = 14, 14, 14, 2 Hz, H-6b), 1.70 (1H, *m*, H-7a), 1.65 (1H, *m*, H-1a), 1.46 (1H, *ddd*, *J* = 14, 14, 2 Hz, H-7b), 1.38 (1H, *dddd*, *J* = 13, 13, 13, 2 Hz, H-2b), 1.27 (3H, *s*, H_3-17), 1.19 (1H, *m*, H-1b), 1.18 (3H, *s*, H_3-16), 1.07 (1H, *m*, H-5), 1.02 (3H, *s*, H_3-18), 0.79 (6H, *s*, H_3-19 and 20); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 669.3429 [M + Na]$^+$ (calculated for $C_{32}H_{54}O_{13}Na$, 669.3456).

*4.10. Acetylation of microtropioside A (**1**) to its pentaacetate (**1a**)*

Microtropioside A (**1**) (1.8 mg) was acetylated with 100 μL each of Ac_2O and pyridine at 25ºC for 18 h. The reagents were removed with a stream of N_2 to give a pentaacetate (**1a**), which was crystallized from MeOH-$CHCl_3$. Pentaacetate (**1a**): Colorless

needles, mp 170-172 ºC; $[\alpha]_D^{22}$ -16.8 ($CHCl_3$; c 0.16); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.21 (1H, *dd*, *J* = 10, 10 Hz, H-3'), 5.08 (1H, *dd*, *J* = 10, 10 Hz, H-4'), 5.07 (1H, *dd*, *J* = 4, 4 Hz, H-14), 4.97 (1H, *dd*, *J* = 10, 8 Hz, H-2'), 4.55 (1H, d, *J* = 8 Hz, H-1'), 4.23 (1H, dd, *J* = 12, 5 Hz, H-6'a), 4.16 (1H, dd, *J* = 12, 3 Hz, H-6'b), 4.06 (1H, *d*, *J* = 14 Hz, H-17a), 3.92 (1H, *dd*, *J* = 13, 4 Hz, H-15a), 3.83 (1H, *dd*, *J* = 13, 4 Hz, H-15b), 3.80 (1H, *d*, *J* = 14 Hz, H-17b), 3.67 (1H, *ddd*, *J* = 10, 5, 3 Hz, H-5'), 3.20 (1H, *dd*, *J* = 12, 4 Hz, H-3), 2.13, 2.08, 2.03, 2.03, 2.01 (each 3H, each s, C*H*$_3$CO × 5), 1.17 (3H, *s*, H_3-16), 0.97 (3H, *s*, H_3-18), 0.91 (3H, *s*, H_3-20), 0.73 (3H, *s*, H_3-19); HR-ESI-MS (positive-ion mode) *m*/*z*: 733.3414 $[M + Na]^+$ (calculated for $C_{36}H_{54}O_{14}Na$, 733.3405).

*4.11. Acetylation of microtropioside B (**2**) to its hexaacetate (**2a**)*

Microtropioside B (6.6 mg) (**2**) was similarly acetylated with Ac_2O and pyridine to give a hexaacetate (**2a**). Hexaacetate (**2a**): amorphous powder, $[\alpha]_D^{25}$ −17.2 ($CHCl_3$; c 1.01); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.40 (1H, dd, *J* = 9, 2 Hz, H-14), 5.20 (1H, dd, *J* = 9, 9 Hz, H-3'), 5.07 (1H, dd, *J* = 9, 9 Hz, H-4'), 4.95 (1H, dd, *J* = 9, 8 Hz, H-2'), 4.59 (1H, dd, *J* = 12, 2 Hz, H-15a), 4.53 (1H, d, *J* = 8 Hz, H-1'), 4.23 (1H, dd, *J* = 12, 5 Hz, H-6'a), 4.18 (1H, dd, *J* = 12, 9 Hz, H-15b), 4.15 (1H, dd, *J* = 12, 3 Hz, H-6'b), 3.64 (1H, ddd, *J* = 9, 5, 3 Hz, H-5'), 3.18 (1H, dd, *J* = 12, 4 Hz, H-3), 2.071, 2.069, 2.020, 2.018, 2.016, 2.00 (3H each, each s, C*H*$_3$CO

×6), 1.30 (3H, s, H_3-17), 1.12 (3H, s, H_3-16), 0.96 (3H, s, H_3-18), 0.78 (3H, s, H_3-20), 0.71 (3H, s, H_3-19); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.0, 170.63, 170.58, 170.4, 169.5, 169.2 (CH_3*C*O- ×6), 98.5 (C-1'), 85.4 (C-3), 75.6 (C), 74.2 (CH), 73.5 (C), 73.0 (CH), 71.62 (CH), 71.56 (CH), 69.0 (CH), 64.0 (CH_2), 62.2 (CH_2), 56.9 (CH), 56.3 (CH), 43.4 (C-7), 38.2 (C-4), 37.1 (C-1), 36.6 (C-11), 33.1 (C-12), 27.9 (C-18), 25.3 (C-17), 24.8 (C-16), 22.9 (C-2), 20.9, 20.73, 20.68, 20.64, 20.6 (*C*H_3CO- ×6), 19.7 (C-6), 15.9 (C-19), 15.6 (C-20), 14.9 (C-11); HR-ESI-MS (positive-ion mode) *m*/*z*: 777.3685 $[M + Na]^+$ (calculated for $C_{38}H_{58}O_{15}Na$, 777.3667).

*4.12. Enzymatic hydrolysis of microtropioside B (**2**)*

Microtropioside B (**2**) (18 mg) in 100 μL of DMSO was mixed with a solution of emulsin (10 mg) in H_2O (2 mL), followed by incubation at 37 ºC for 18 h. Further amounts of emulsion (10 mg) and crude hesperidinase (20 mg) were added, followed by keeping at 37 ºC for 72 h. The reaction mixture was evaporated to dryness and the resulting residue was separated by silica gel CC (*Φ*= 20 mm, L = 20 cm), with elution with $CHCl_3$ (100 ml), $CHCl_3$-MeOH [(19:1, 100 ml), (9:1, 100 ml), (17:3, 100 ml) and (7:3, 300 ml)] and MeOH (300 ml), 5 ml fractions being collected. An aglycone (**2b**) (3.5 mg) was recovered in fractions 31–35 and 0.7 mg of a sugar moiety in fractions 91–92.

Aglycone (**2b**), colorless needles, mp 110−112 ºC (MeOH); $[\alpha]_D^{24}$ −15.0 (MeOH; c 0.23); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.76 (1H, dd, *J* = 11, 3 Hz, H-15a), 3.59 (1H, dd, *J* = 11, 7 Hz, H-15b), 3.55 (1H, dd, *J* = 7, 3 Hz, H-14), 3.24 (1H, dd, *J* = 12, 5 Hz, H-3), 2.07 (1H, ddd, *J* = 14, 8, 8 Hz, H-12a), 1.80 (1H, dd, *J* = 12, 3 Hz, H-7), 1.68 (2H, m, H-2a and 6a), 1.61 (1H, m, H-1a), 1.60 (1H, m, H-2b), 1.59 (1H, m, H-9), 1.57 (1H, m, H-11a), 1.48 (1H, m, H-11b), 1.45 (1H, m, H-12b), 1.39 (1H, m, H-6b), 1.33 (1H, m, H-6b), 1.24 (3H, s, H_3-17), 1.20 (3H, s, H_3-16), 1.05 (1H, m, H-1b), 1.00 (3H, s, H_3-18), 0.94 (1H, br d, *J* = 12 Hz, H-5), 0.83 (3H, s, H_3-20), 0.79 (3H, s, H_3-19); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; 13C NMR ($CDCl_3$, 150 MHz): δ 78.9 (C-3), 77.3 (C-4), 75.5 (C-13), 75.3 (C-8), 63.2 (C-15), 55.6 (C-5), 52.4 (C-9), 44.0 (C-7), 38.9 (C-4), 37.3 (C-1), 37.1 (C-10), 28.1 (C-18), 27.2 (C-2 and 12), 26.0 (C-17), 25.0 (C-16), 19.9 (C-6), 15.4 (C-19), 15.1 (C-20), 14.1 (C-11); HR-ESI-MS (positive-ion mode) *m*/*z*: 363.2503 $[M + Na]^+$ (calculated for $C_{20}H_{36}O_4Na$, 363.2505).

The sugar was analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH2P-50 4E, CH_3CN-H_2O (3:1), 1 ml/min]. The sugar moiety, obtained on hydrolysis gave a peak for D-glucose at the retention time of 9.5 min (positive optical rotation sign). The peak was identified by co-chromatography with authentic D-glucose.

*4.13. Preparation of 15-*O*-pivalate (**2c**) from **2b***

Aglycone (**2b**) (3.5 mg) was dissolved in 1 mL of pyridine and 15 μL of pivaloyl chloride was added. The reaction mixture was stirred for 1 h at 25 ºC. To the reaction mixture, 1 mL of H_2O was added, followed by extracting with 2 mL of $CHCl_3$ three times. The organic layer was washed with 1 mL of brine and then dried over Na_2SO_4. The residue was purified by prep. TLC [$CHCl_3$-$(CH_3)_2CO$, 20:1] to give 1.1 mg of pivalate (**2c**).

15-*O*-Pivalate (**2c**): Amorphous powder, $[\alpha]_D^{25}$ −49.4 (MeOH; c 0.11); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.39 (1H, dd, *J* = 12, 2 Hz, H-15a), 4.07 (1H, dd, *J* = 12, 8 Hz, H-15b), 3.73 (1H, dd, *J* = 8, 2 Hz, H-14), 3.24 (1H, dd, *J* = 12, 5 Hz, H-3), 2.17 (1H, m, H-12a), 1.80 (1H, ddd, *J* = 12, 3, 3 Hz, H-7a), 1.68 (2H, m, H-2a and 6a), 1.62 (1H, m, H-1a), 1.60 (2H, m, H-2b and 11a), 1.49 (1H, m, H-9), 1.48 (1H, m, H-11b), 1.41 (1H, m, H-12b), 1.39 (1H, br dd, *J* = 12, 12 Hz, H-7b), 1.32 (1H, m, H-6b), 1.23 (9H, s, CH_3 × 3), 1.22 (3H, s, H_3-17), 1.18 (3H, s, H_3-16), 1.04 (1H, m, H-1b), 1.00 (3H, s, H_3-18), 0.94 (1H, dd, *J* = 12, 3 Hz, H-5), 0.81 (3H, s, H_3-20), 0.78 (3H, s, H_3-19); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 179.4 (C=O), 78.9 (C-3), 75.4 (C-14), 75.2 (C-13), 74.8 (C-8), 66.4 (C-15), 55.5 (C-5), 53.9 (C-9), 43.9 (C7), 38.9 [C-4 and $(CH_3)_3C$-], 37.4 (C-1), 37.0 (C-10), 28.7 (C-12), 27.2 (C-2), 25.6 (C-17), 25.1 (C-16), 28.1 (C-18), 27.3 (CH_3-×3), 19.9 (C-6), 15.4 (C-19), 15.3 (C-20), 14.3 (C-11); HR-ESI-MS (positive-ion mode) *m*/*z*: 447.3075 $[M + Na]^+$ (calculated for $C_{25}H_{44}O_5Na$, 447.3080).

*4.14. Preparation of (R)- and (S)-MTPA (**2d** and **2e**, respectively) from 15-O-pivalate (**2c**)*

A solution of **2c** (0.5 mg) in 1 ml of dry CH_2Cl_2 was reacted with (*R*)-MTPA (9.2 mg) in the presence of EDC (17.3 mg) and 4-DMAP (11.6 mg), and then the mixture was occasionally stirred at 25 ºC for 2 days. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 4N HCl (1 ml), $NaHCO_3$-saturated H_2O, and then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by prep. TLC [silica gel (0.25 mm thickness), being applied for 18 cm and developed with $CHCl_3$-$(CH_3)_2CO$ (19:1) for 9 cm, and then eluted with $CHCl_3$-MeOH (9:1)] to furnish an MTPA ester, **2d** (0.3 mg). Through a similar procedure, an (*S*)-MTPA ester (**2e**) of 15-*O*-pivalate (0.2 mg) was prepared from **2c** (0.5 mg) using (*S*)-MTPA (11.6 mg), EDC (22.1 mg), and 4-DMAP (7.4 mg).

15-*O*-Pivaloyl-3,14-di-(*R*)-MPTA ester (**2d**): Amorphous powder, ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.60−7.52 (4H, m, aromatic protons), 7.42−7.38 (6H, m, aromatic protons), 5.63 (1H, br d, *J* = 8 Hz, H-14), 4.87 (1H, dd, *J* = 12, 1 Hz, H-15a), 4.68 (1H, dd, *J* = 12, 5 Hz, H-3), 4.14 (1H, dd, *J* = 12, 8 Hz, H-15b), 3.59 (3H, br s, CH_3O-), 3.53 (3H, br s, CH_3O-), 1.81 (1H, m, H-7a), 1.80 (1H, m, H-2a), 1.66 (2H, m, H-6a and 7b), 1.65 (1H, m, H-2b), 1.67−1.60 (2H, m, H-11a and 12 a), 1.64 (1H, m, H-1a), 1.46 (1H, m, H-9), 1.32 (1H, m, H-6b), 1.31 (3H, s, H3-17), 1.26−1.18 (2H, m, H-11b and 12b), 1.07 (1H, m, H-1b), 1.04 (3H,

s, H_3-16), 1.02 (1H, br d, *J* = 12 Hz, H-5), 0.90 (3H, s, H_3-18), 0.81 (3H, s, H_3-20), 0.80 (3H, s, H_3-19); HR-ESI-MS (positive-ion mode) *m*/*z*: 879.3880 $[M + Na]^+$ (calculated for $C_{45}H_{58}O_9F_6Na$, 879.3877).

15-*O*-Pivaloyl-3,14-di-(*S*)-MPTA ester (**2e**): Amorphous powder, ^1H NMR ($CDCl_3$, 600 MHz): δ 7.59−7.54 (4H, m, aromatic protons), 7.43−7.38 (6H, m, aromatic protons), 5.60 (1H, br d, *J* = 8 Hz, H-14), 4.78 (1H, dd, *J* = 12, 1 Hz, H-15a), 4.71 (1H, dd, *J* = 12, 5 Hz, H-3), 4.08 (1H, dd, *J* = 12, 8 Hz, H-15b), 3.57 (3H, br s, CH_3O-), 3.49 (3H, br s, CH_3O-), 1.86 (1H, m, H-2a), 1.81 (1H, m, H-7a), 1.79−1.73 (2H, m, H-11a and 12a), 1.76 (1H, m, H-2b), 1.69 (1H, m, H-1a), 1.65 (2H, m, H-6a and 7a), 1.50 (1H, m, H-9), 1.36−1.27 (2H, m, H-11b and 12b), 1.31 (1H, m, H-6b), 1.30 (3H, s, H_3-17), 1.10 (1H, m, H-1b), 1.08 (3H, s, H_3-16), 1.02 (1H, br d, *J* = 12 Hz, H-5), 0.84 (3H, s, H_3-20), 0.82 (3H, s, H_3-18), 0.79 (3H, s, H_3-19); HR-ESI-MS (positive-ion mode) *m*/*z*: 879.3872 $[M + Na]^+$ (calculated for $C_{45}H_{58}O_9F_6Na$, 879.3877).

4.15. Chirality analyses of sugar moieties

About 500 μg each of **1** and **3**−**7** was hydrolyzed with 1N HCl (0.1 ml) at 100 ºC for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 ml), and the water layers were analyzed under the same conditions as above.

4.16. Known compound isolated

Amarantholidoside IV (**7**), amorphous powder, $[\alpha]_D^{25}$ −6.7 (MeOH; c 1.97) (Fiorentino et al., 2006).

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Table 1

^{13}C NMR data for microtropiosides A-F (**1**−**6**), reference compounds (**8** and **9**), and an aglycone (**2b**) of **2** (100 MHz, CD_3OD).

C	**1**	**1**[a]	**8**[a,b]	**9**[a,c]	**2**	**2b**	**3**	**4**	**5**	**6**
1	38.3	37.3	38.3	37.0	38.4	38.7	38.5	38.5	38.5	37.8
2	24.2	23.8	26.6	24.3	24.1	28.0 (−3.9)[d]	24.1	24.1	24.2	24.2
3	85.7	84.8	89.0	84.8	85.8	79.7 (+6.1)[d]	85.8	85.9	85.9	85.9
4	39.3	38.6	39.7	38.9	39.3	40.5 (−1.2)[d]	39.3	39.3	39.3	39.3
5	57.4	56.1	55.8	55.1	57.5	57.0	57.2	57.3	57.2	56.8
6	20.8	20.0	20.5	24.3	20.9	20.9	20.9	20.9	20.7	20.7
7	39.2	38.6	45.1	38.4	45.0	45.0	44.9	45.0	43.6	43.4
8	77.4	76.2	73.0	148.3	76.6	76.5	76.4	76.7	77.7	78.1
9	58.3	57.2	61.9	56.3	56.7	56.8	58.0	57.4	59.1	49.6
10	37.6	36.6	38.9	39.8	38.0	38.1	37.9	37.9	37.8	37.3
11	17.8	17.1	24.6	22.3	15.7	15.7	16.0	15.8	25.7	20.2
12	35.9	36.6	43.7	25.1	32.8	32.8	34.6	33.4	89.1	74.7
13	77.0	76.2	138.6	134.2	76.8	76.8	76.9	76.3	77.2	77.9
14	75.4	74.4	125.4	145.7	77.8	77.8	88.9	75.5	143.4	148.6
15	71.7	71.4	59.0	70.8	64.3	64.3	64.0	72.6	116.2	111.3
16	28.4	28.5	16.7	174.8	24.6	24.6	25.2	24.9	29.8	28.2
17	73.3	72.2	24.5	107.2	25.7	25.9	25.6	25.8	26.0	25.0
18	28.7	28.5	16.8	28.8	28.8	28.7	28.7	28.8	28.7	28.7
19	16.8	16.7	28.3	17.0	16.9	16.1	16.9	16.9	16.8	16.8
20	15.7	15.4	15.9	14.7	16.0	16.0	16.2	16.1	16.7	16.5
1'	102.0	102.4	106.9	102.5	102.0		102.0	102.0	102.0	101.3
2'	75.2	75.3	75.8	75.2	75.2		75.2	75.2	75.2	75.1
3'	78.3	78.8	78.8	78.7	78.3		78.3	78.4	78.4	78.3
4'	72.0	72.2	71.9	72.1	72.0		72.0	72.0	72.0	72.0
5'	77.7	78.4	78.3	78.5	77.8		77.8	77.8	77.8	77.7
6'	63.1	63.3	63.0	63.3	63.1		63.1	63.1	63.1	63.0
1"							105.9	104.4	106.5	102.0
2"							75.4	75.2	75.5	75.0
3"							78.1	78.1	78.4	78.1
4"							71.9	72.0	71.8	71.9
5"							77.9	78.0	77.8	77.9
6"							62.9	62.8	62.9	63.0

[a]Data for C_5D_5N.

[b]Data from lit. Otsuka et al.

[c]Data from lit. Shen et al.

[d]$\Delta(\delta_{\mathbf{2}}-\delta_{\mathbf{2a}})$.

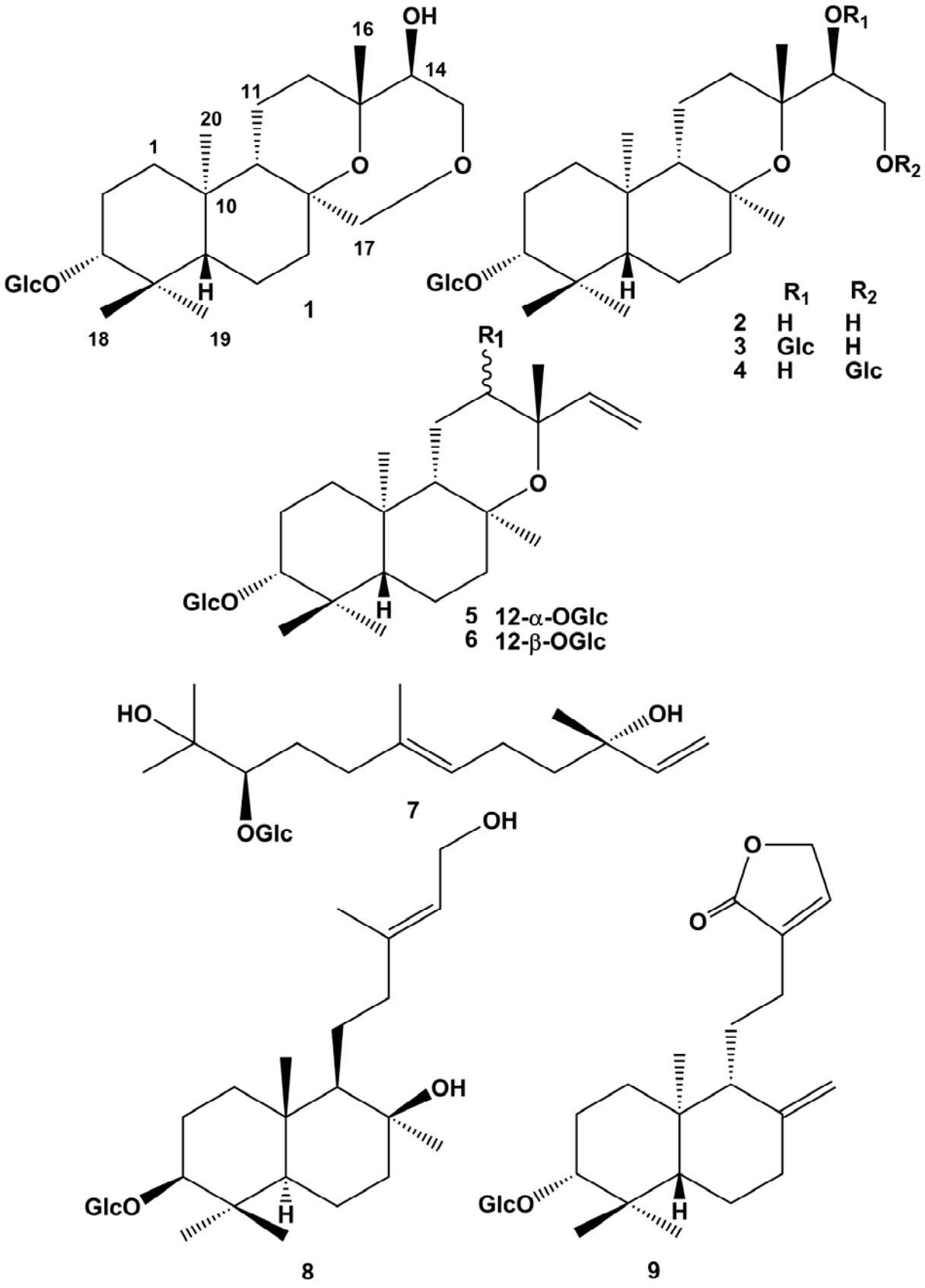

Fig. 1. Structures of compounds of interest.

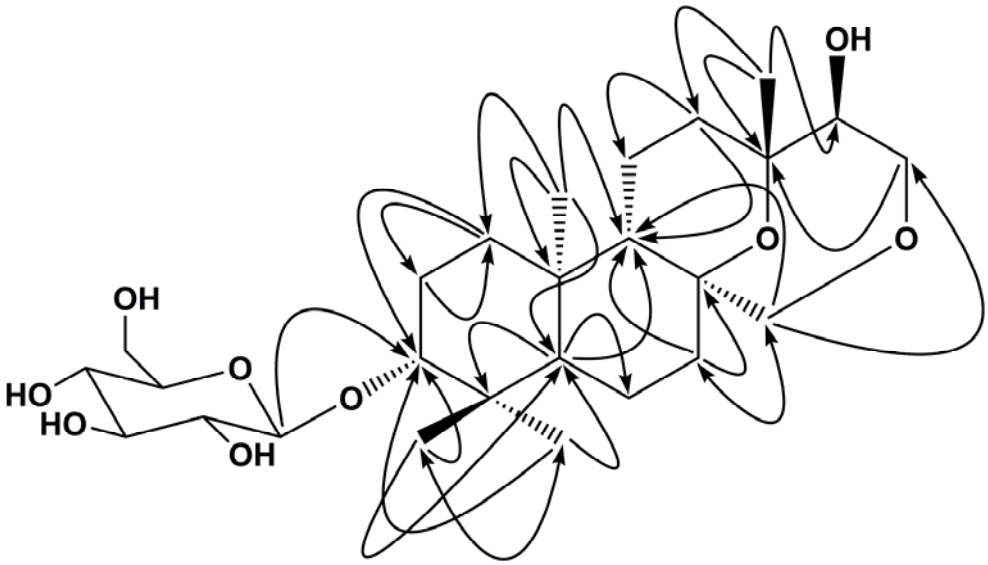

Fig. 2. Diagnostic HMBC correlations of **1**.
Dual arrow curves denote HMBC correlations were observed in both ways.

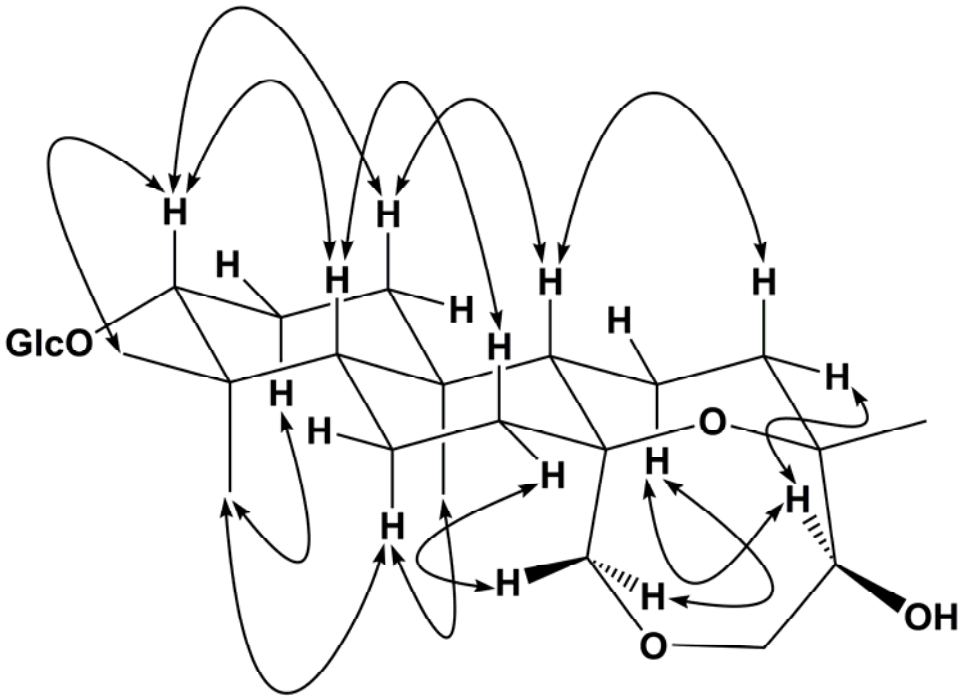

Fig. 3. Phase-sensitive NOESY correlations of **1**.

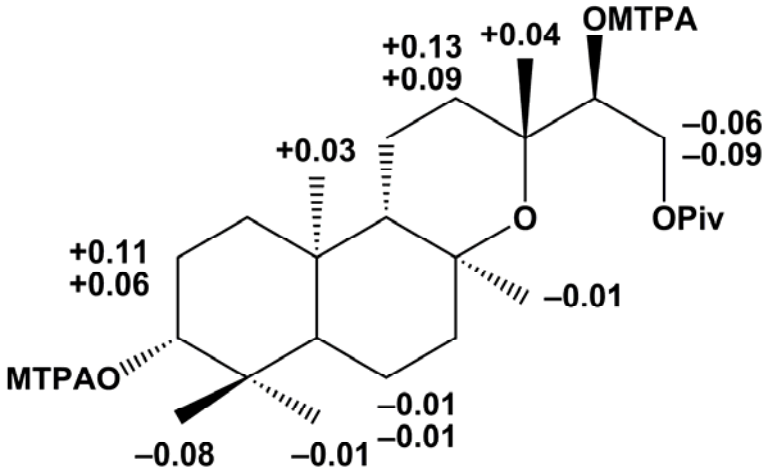

Fig. 4. Results with the modified Mosher's method ($\Delta\delta_S$-δ_R) for **2**.