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ONE NEW MEGASTIGMANE GLYCOSIDE WITH A DOUBLE BOND AT A RARE POSITION FROM THE LEAVES OF *ANTIDESMA PENTANDRUM* VAR. *BARBATUM*

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Abstract – From the 1-BuOH-soluble fraction of a MeOH extract of leaves of *Anitdesma pentandrum* var. *barbatum*, one new megastigmane glycoside, α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside of (6*ξ*,9*R*)-megastigman-4(13)-en-9-ol, was isolated along with four known megastigmane glycosides, (6*R*,9*R*)-megastigma-4,7-dien-9-ol-3-one α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside, foliasalacinoside B, (6*S*,9*R*)-magastigma-4,7-diene-6,9-diol-3-one 9-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside and byzantionoside B.

There are more than 100 species belonging to the genus *Antidesma* in subtropical and tropical areas of the Old World. *Antidesma pentandrum* (Blanco) Merril var. *barbatus* (Presl) Merril (Euphorbiaceae) is a small tree of 2–4 m in height, which grows wild in the southern parts of the Okinawa Islands, Japan, and in Taiwan and Philippines.[1] Although some of *Antidesma* species have medicinal value in some part of Asia, neither the medicinal use of *A. pentandrum* var. *barbatus* nor its chemical constituents has been reported previously. A closely related plant, *A. japonicum*, collected in Okinawa was found to contain several α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosides in its leaves.[2] From the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *A. pentandrum* var. *barbatus*, collected in the Yaeyama area, Okinawa Islands, one new magastigmane glycoside (**1**) along with four known megastigmane glycosides (**2**–**5**) and a phenolic compound (**6**) were isolated using various types of chromatography. The structures of the known compounds were identified as (6*R*,9*R*)-megastigma-4,7-dien-9-ol-3-one

α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (**2**),[2] foliasalacinoside B_2 (**3**),[3] (6*S*,9*R*)-magastigma-4,7-diene-6,9-diol-3-one 9-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (**4**),[4] byzantionoside B (**5**),[5] and eximine (**6**)[6] by comparison of the spectroscopic data with those reported in the literature. This paper deals with structural elucidation of the new compound.

Separation of the 1-BuOH-soluble fraction of a MeOH extract of leaves of *A. pentandrum* var. *barbatus* by various kinds of chromatography resulted in the isolation of one new megastigmane glycoside (**1**) (Figure 1). Its structure was elucidated from spectroscopic evidence, and the partial absolute structure of **1** was confirmed by the modified Mosher's method.[7]

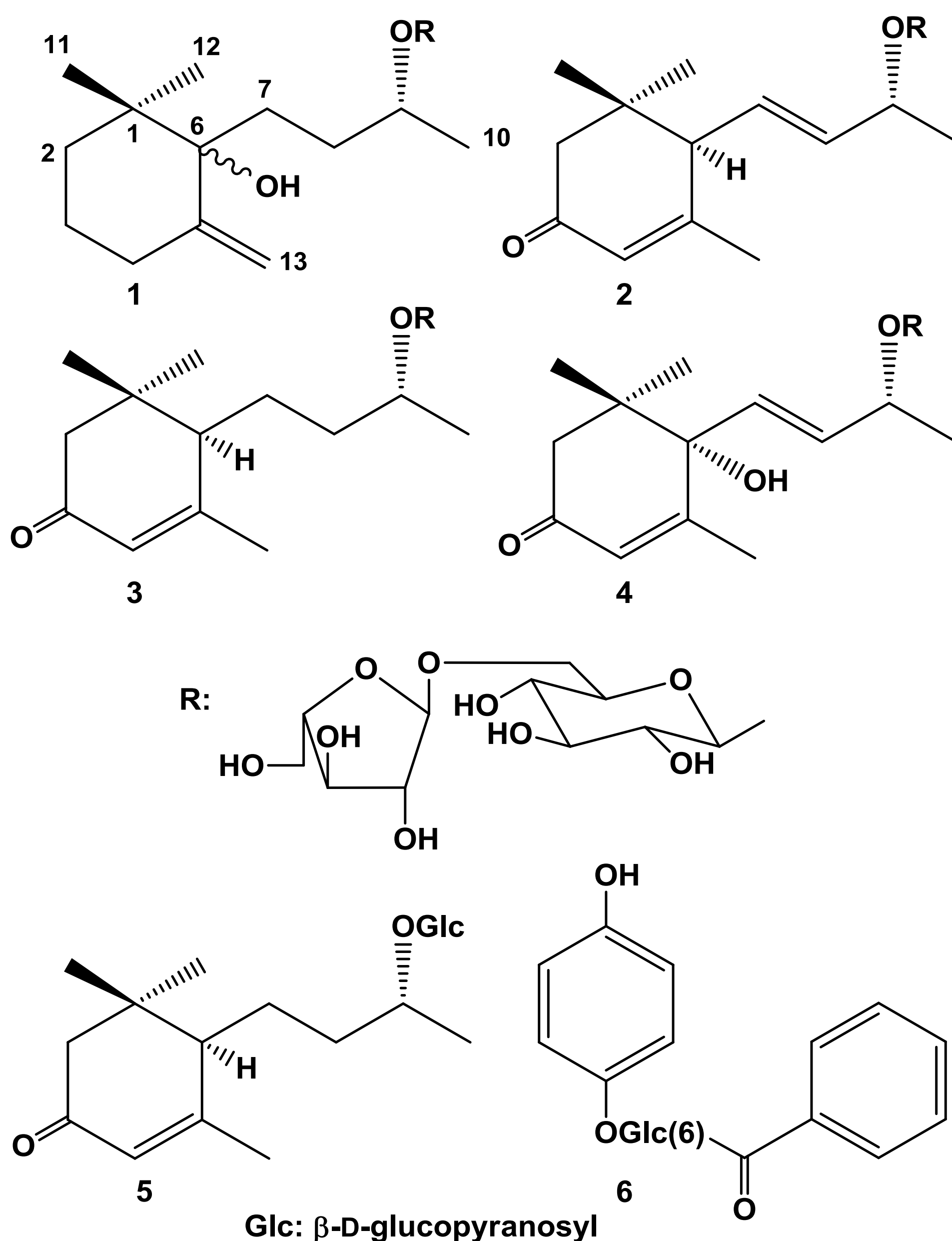

Figure 1. Structures of compounds isolated

Compound **1**, $[\alpha]_D^{25}$ +33.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{24}H_{42}O_{11}$ by observation of a quasi-molecular ion peak at *m/z* 529.2618 ($C_{24}H_{42}O_{11}Na$) on HR-ESI-mass spectrometry. The IR spectrum exhibited absorptions assignable to hydroxy groups (3381 cm^{-1}) and a double bond (1648 cm^{-1}). The ^{13}C-NMR spectrum displayed 24 resonances, which comprised those of two anomeric carbon signals (δ_C 102.4 and 110.1), and the HSQC spectrum revealed that they were associated with the respective anomeric proton signals (δ_H 4.30 and 4.97). In the acid hydrolyzate of **1**, L-arabinose and D-glucose were identified as sugar components on HPLC analysis using a chiral detector. The terminal sugar was expected to be α-L-arabinofuranoside from the NMR spectroscopic data for co-occurring known compounds **2**, **3** and **4**, and thus the inner sugar must be β-D-glucopyranoside, carrying a glycosidic substitutent at the 6-position. The remaining 13 carbon signals represent the aglycone moiety, suggesting a megastigmane skeleton, comprising three methyls, five methylenes, one each of oxygenated tertiary and secondary carbons, one quaternary carbon and a double bond. The HSQC spectrum revealed that one (δ_C 109.1) of the carbons that was involved in the double bond carried two protons (δ_H 4.84 and 4.89), and thus they formed an *exo*-methylene motif, and the ^{1}H-^{1}H COSY spectrum exhibited two proton sequences from H_2-2 to H_2-4 and H_2-7 to H_3-10 (Figure 2). In the HMBC spectrum, since significant correlations from *exo*-methylene protons to C-4, 5 and 6 (δ_C 35.1, 151.8 and 81.4, respectively) were observed, the double bond must be placed between C-5 and C-13 (Figure 2). The HMBC correlations seen in Figure 2 from H_2-7 and H_2-3 to C-5 also supported the structure. Although the absolute configuration at the 9-postion was expected to be *R* from the ^{13}C-NMR chemical shifts around C-9, 10 and 1',[5] it was confirmed by the modified

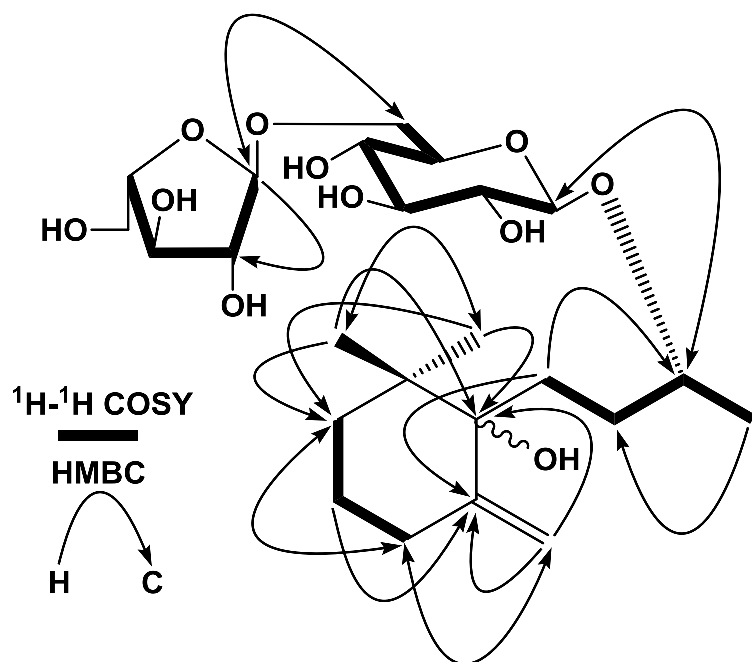

Figure 2. ^{1}H-^{1}H COSY and HMBC correlation of **1**
Dual arrow curves denote HMBC correlation was observed in the both direction.

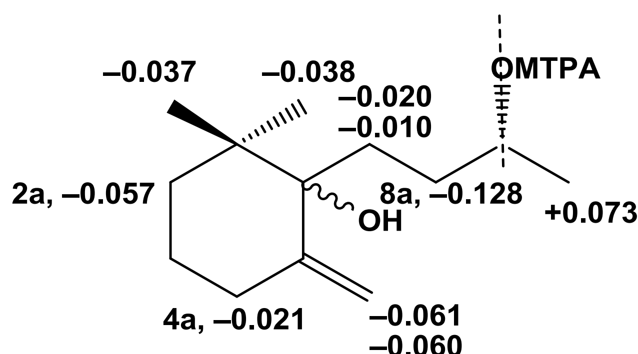

Figure 3. Results of modifed Mosher's method ($\Delta\delta_{S-R}$)

Mosher's method, as shown in Figure 3. The absolute configuration at the 6-postion remains to be determined. Therefore, compound **1** was elucidated to be (6*ξ*,9*R*)-megastigman-5-ene-6,9-diol 9-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside, as shown in Figure 1. Compound **1** exhibits three unusual features, i.e. the double bond is between C-5 and C-13, C-3 does not carry any oxygen functional group and a furanose-type α-L-arabinose is present in the sugar moiety.

Table 1. NMR spectroscopic data for compound **1** (^{13}C: 100 MHz, ^{1}H: 400 MHz; CD_3OD).

	C	H		C	H
1	40.9	-	1'	102.4	4.30 d, 8 Hz
2	39.3	1.70 ddd, 14, 9, 5 Hz	2'	75.2	3.15 dd, 9, 8 Hz
		1.34 ddd, 14, 4, 2 Hz	3'	78.2	3.35 dd, 9, 9 Hz
3	24.1	1.50–1.58 2H m	4'	72.3	3.27 dd, 9, 9 Hz
4	35.1	2.24 ddd, 14, 4, 2 Hz	5'	76.6	3.43 ddd, 9, 6, 3 Hz
		2.05 ddd, 14, 10, 5 Hz	6'	68.6	4.01 dd, 11, 3 Hz
5	151.8	-			3.61 dd, 11, 6 Hz
6	81.4	-	1"	110.1	4.97 d, 1 Hz
7	30.0	2.03 ddd, 13, 5, 2 Hz	2"	83.1	4.00 dd, 3, 1 Hz
		1.53 ddd ,13, 5, 2 Hz	3"	79.0	3.83 dd, 6, 3 Hz
8	32.6	1.46 ddd, 11, 5, 2 Hz	4"	86.0	3.96–4.00 m
		1.28 ddd, 11, 5, 2 Hz	5"	63.2	3.74 dd, 12, 3 Hz
9	76.8	3.82–3.87 m			3.64 dd, 12, 5 Hz
10	20.4	1.16 3H, d, 6 Hz			
11	24.8	0.96 3H, s			
12	23.2	0.90 3H, s			
13	109.1	4.89 dd, 2, 1 Hz			
		4.84 dd, 2, 2 Hz			

EXPERIMENTAL

General Experimental Procedures Optical rotation was measured on a JASCO P-1030 digital polarimeter. An IR spectrum was 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 with tetramethylsilane as an internal standard. Positive-ion HR-ESI-MS was performed with an Applied Biosystems QSTAR XL NanoSprayTM System.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [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]. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, L = 40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$-MeOH-H_2O-*n*-PrOH (9:12:8:2) being 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 = 20 mm, L = 25 cm, 6 mL/ min), and the eluate was monitored with UV (210 nm) and a refractive index monitor. Crude hesperidinase was a generous gift from Tanabe Pharmaceutical Company Ltd. The (*R*)-(+)- and (*S*)-(–)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Plant material Leaves of *A. pentandrum* var. *barbatus* were collected in Yaeyama-gun, Okinawa in November 1997. A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University (Accession No. 97-APB-1107). Plant material was identified by Dr. Takakazu Shinzato, Emeritus Professor of Ryukyu University.

Extraction and isolation Leaves of *A. pentandrum* var. *barbatus* (4.25 kg) were extracted three times with MeOH (45 L × 3) at room temperature for one week and then concentrated to 3 L *in vacuo*. The concentrated extract was washed with *n*-hexane (3 L, 37.3 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 37.5 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 L) to give a 1-BuOH-soluble fraction (61.7 g), and the remaining water-layer was concentrated to furnish 179 g of a water-soluble fraction. The 1-BuOH-soluble fraction (61.0 g) was subjected to Diaion HP-20 CC (Φ =50 mm, L = 50 cm), using H_2O-MeOH (4:1, 3 L), (3:2, 3 L), (2:3, 3 L), and (1:4, 3 L), and MeOH (3 L), 500 mL fractions being collected. The residue (17.2 g) in fractions 5–7 was subjected to silica gel (500 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 L), and $CHCl_3$-MeOH (49:1, 3 L), (19:1, 3 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 (1.22 g) in fractions 29–32 was separated by ODS open CC and then the residue (295 mg) in fractions 161–191 was purified by DCCC to give 267 mg of **3** in fractions 47–65. The residue (1.52 g) in fractions 33–37 was separated by ODS open CC to give three fractions, fractions 64–91 (354 mg), fractions 134–149 (196 mg) and fractions 150–166 (285 mg). The residue of the first fraction was purified by DCCC to give 180 mg of **4** in fractions 30–37. The residue of the second fraction was separated by DCCC, and then the residue (110 mg) in fractions 51–66 was purified by HPLC (H_2O-MeOH, 11:9) to give 6.7 mg of **2** and 10.3 mg of **3** from the peaks at 13 min and 17 min, respectively. The third fraction was separated by DCCC and the residue (222 mg) in fractions 44–58 was purified by HPLC (H_2O-MeOH, 11:9) to give a further amount of **3** from the peak at 17 min.

The residue (6.19 g) in fractions 16–20 obtained on HP-20 CC was subjected to silica gel (150 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$, 2 L], and $CHCl_3$-MeOH (49:1, 1.5 L), (19:1, 1.5 L),

(23:2, 1.5 L), (9:1, 1.5 L), (17:3, 1.5 L), (4:1, 1.5 L), (3:1, 1.5 L), and (7:3, 1.5 L)], 250 mL fractions being collected. The residue (425 mg) in fractions 20–22 was separated by ODS open CC and then the residue (191 mg) in fractions 121–161 was purified by DCCC to give 109 mg of **5** in fractions 175–200. The residue (35.6 mg) in fractions 1–174 was purified by HPLC (H_2O-MeOH, 32:29) to give 4.7 mf of **6** from the peak at 24 min. The residue (479 mg) in fractions 26–28 obtained on silica gel CC was separated by ODS open CC to give 11.9 mg of **1** in fractions 235–243 and the residue (15.7 mg) in fractions 244–252 was then purified by HPLC (H_2O-MeOH, 1:1) to give further amount of **1** from the peak at 17 min.

Compound 1: Amorphous powder; $[\alpha]_D^{25}$ +33.0 (*c* 0.40, MeOH); IR ν_{max} (film) cm^{-1}: 3381, 2929, 1648, 1455, 1071, 900; NMR spectroscopic data (CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m*/*z*: 529.2618 $[M+Na]^+$ (Calcd for $C_{24}H_{42}O_{11}Na$: 529.2619).

Sugar analysis About 500 μg of compound **1** was hydrolyzed with 1M HCl (0.1 mL) at 90 ºC for 2 h. The reaction mixture was partitioned with an equal amount of EtOAc (0.1 mL), and the water layer was analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH_2P-50 4E, Φ = 4.6 mm, L = 25 cm, MeCN-H_2O (3:1), 1 mL/min]. The hydrolyzate of **1** gave peaks for L-arabinose and D-glucose at 6.4 min and 14.1 min, respectively, with positive optical rotation signs.

Enzymatic hydrolysis of compound 1 Compound **1** (5.7 mg in 2 mL) was hydrolyzed with 5 mg of crude hesperidinase at 37 °C for 18 h. The reaction mixture was evaporated to dryness and then subjected to preparative TLC (precoated silica gel, 0.25 mm thickness, Merck) with $CHCl_3$-MeOH (10:1) to give 1.3 mg of aglycone (**1a**).

Aglycone (**1a**) Colorless syrup; $[\alpha]_D^{25}$ +67.7 (*c* 0.06, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 4.89 (1H. dd, J = 2, 2 Hz, H-13a), 4.85 (1H, dd, J = 2, 2 Hz, H-13b), 3.68 (1H, m, H-9), 2.26 (1H, dddd, J = 13, 2, 2, 2 Hz, H-4a), 2.04 (1H, br d, J = 13 Hz, H-4b), 2.02 (1H, m H-7a), 1.68 (1H, ddd, J = 13, 4, 2 Hz, H-2a), 1.51–1.58 (2H, m, H_2-3), 1.50 (1H, ddd, J = 13, 4, 2 Hz, H-7b), 1.46 (1H, ddd, J = 13, 4, 2 Hz, H-8a), 1.36 (1H, ddd, J = 13, 4, 2 Hz, H-2b), 1.34 (1H, ddd, J = 13, 6, 2 Hz, H-8b), 1.14 (3H, d, J = 6 Hz, H_3-10), 0.96 (3H, s, H_3-11), 0.90 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 151.8 (C-5), 109.1 C-5), 81.2 (C-6), 69.2 (C-9), 40.9 (C-1), 39.4 (C-2), 35.1 (C-4), 34.3 (C-8), 30.4 (C-7), 24.6 (C-11), 24.1 (C-3), 23.9 (C-12), 23.1 (C-10). HR-ESI-MS (positive-ion mode) *m*/*z*: 235.1670 $[M+Na]^+$ ($C_{13}H_{24}O_2Na$ requires 235.1674).

Preparation of (*R*)- and (*S*)-MTPA esters (1b and 1c, respectively) from 1a Solutions of **1a** (0.70 mg)

in 1 mL of dehydrated CH_2Cl_2 were reacted with (*R*)- and (*S*)-MTPA (13 mg and 10 mg, respectively) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (EDC) (12 mg and 11 mg, respectively) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (8 mg and 7 mg, respectively), and then the mixture was occasionally stirred at 40 °C overnight. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 mL), 5% HCl (1 mL), saturated aqueous $NaHCO_3$, 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 preparative TLC [silica gel (0.25 mm thickness); the residue being applied for 18 cm, developed with $CHCl_3$-$(CH_3)_2CO$ (20:1) for 9 cm, and then eluted with $CHCl_3$-MeOH (5:1)] to furnish the respective esters, **1b** and **1c** (0.3 mg and 0.3 mg, respectively).

(6*ξ*,9*R*)-Megastigman-5-ene-6,9-diol 9-*O*-(*R*)-MTPA ester (**1b**): Colorless syrup; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.55–7.51 (2H, m, aromatic protons), 7.43–7.38 (3H, m, aromatic protons), 4.08(1H, d, *J* = 1 Hz, H-13a), 4.05 (1H, d, *J* = 1 Hz, H-13b), 3.65 (1H, m, H-9), 2.37 (1H, br d, *J* = 14 Hz, H-4a), 2.35 (1H, m, H-7a), 2.06 (1H, ddd, *J* = 13, 4, 2 Hz, H-2a), 1.94 (1H, m, H-7b), 1.93 (1H, m, H-8a), 1.81 (1H, ddd, *J* = 14, 7, 2 Hz, H-8b), 1.30 (3H, d, *J* = 6 Hz, H_3-10), 1.04 (3H, s, H_3-11), 0.81 (3H, s, H_3-12), H-2b, 3a, 3b and 4b could not be assigned; HR-ESI-MS (positive-ion mode) *m/z*: 451.2077 $[M+Na]^+$ (Calcd for $C_{23}H_{31}O_4F_3Na$, 451.2072).

(6*ξ*,9*R*)-Megastigman-5-ene-6,9-diol 9-*O*-(*S*)-MTPA ester (**1c**): Colorless syrup; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.56–7.52 (2H, m, aromatic protons), 7.44–7.39 (3H, m, aromatic protons), 3.99 (1H, m, H-13a), 3.98 (1H, m, H-13b), 3.66 (1H, m, H-9), 2.37 (1H, br d, *J* = 14 Hz, H-4a), 2.33 (1H, m, H-7a), 1.97 (1H, m, H-2a), 1.93 (1H, m, H-7b), 1.80 (1H, m, H-8a), 1.37 (3H, d, *J* = 6 Hz, H_3-10), 1.00 (3H, s, H_3-11), 0.77 (3H, s, H_3-12), H-2b, 3a, 3b, 4b and 8b could not be assigned; HR-ESI-MS (positive-ion mode) *m/z*: 451.2080 $[M+Na]^+$ (Calcd for $C_{23}H_{31}O_4F_3Na$, 451.2072).

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