Glochidionionosides A—D: Megastigmane Glucosides from Leaves of *Glochidion zeylanicum* (Gaertn.) A. Juss

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Five megastigmane glucosides were isolated from the leaves of *Glochidion zeylanicum*. One of them was a known compound, blumenol C *O*-β-D-glucopyranoside (1), and the structures of the four new compounds, glochidionionosides A—D (2—5), were mainly elucidated by spectroscopic methods, including a modified Mosher's method. The absolute configurations of the six-membered ring of glochidionionoside D (5) were deduced by β-D-glucopyranosylation-induced shift trends in the ^{13}C-NMR spectra and confirmed by X-ray analysis as its *p*-bromobenzoate (5b), and the axis chirality of C-7 was determined to be *R*.

Key words *Glochidion zeylanicum*; Euphorbiaceae; megastigmane glucoside; glochidionionoside; modified Mosher's method; X-ray analysis

In our continuing studies on plants collected in the Okinawan islands, we investigated the constituents of *Glochidion zeylanicum* (Euphorbiaceae). Plants belonging to the Euphorbiaceae are of taxonomic interest, since the family contains various morphologically different plants.

The isolation of butenolide glucosides has been reported from the leaves of *Glochidion zeylanicum* (Gaertn.) A. Juss.[1] Further investigation afforded five megastigmane glucosides, blumenol C glucoside (**1**), and glochidionionosides A—D (**2**—**5**). Megastigmane glycoside was first isolated from *Vinca rosea* as roseoside.[2] The glycosides are a rapidly expanding family and have been isolated from a variety of plant sources since the aglycone of one, vomifoliol, was isolated from *Rauwolfia vomitoria* in 1969.[3,4] This paper deals with the spectroscopic structural elucidation of new megastigmane glucosides and the structure of a compound analogous to grasshopper ketone was analyzed using an X-ray diffraction method.

Results and Discussion

Compounds **1**—**5** were isolated from the *n*-BuOH-soluble fraction of a MeOH extract of the leaves of *G. zeylanicum* by a combination of Diaion HP-20 and normal- and reversed-phase silica gel column chromatography (CC). Droplet counter-current chromatography (DCCC) and HPLC were also used for purification of compounds (Fig. 1, Table 1). The details and yields are given in Experimental.

Blumenol C glucoside (**1**) was isolated as an amorphous powder, and the NMR spectra indicated the presence of a megastigmane skeleton and a glucopyranose unit. From the spectroscopic data including ^{1}H- and ^{13}C-NMR, and circular dichroism (CD), the structure of **1** was identified as (6*S*,9*R*)-megastigman-3-on-4-en-9-ol 9-*O*-β-D-glucopyranoside.[5]

Glochidionionoside A (**2**) was isolated as an amorphous powder, and the elemental composition was determined to be $C_{19}H_{29}O_9$ by high-resolution (HR)-FAB-MS. The ^{13}C-NMR spectrum showed the presence of six signals assignable to β-glucopyranose. The remaining 13 signals comprised those of one carbonyl carbon, di- and trisubstituted double bonds, three methyls, two methylenes, one of which was assigned to be a primary carbinol from its chemical shift, one methine with an oxygen atom, and two quaternary carbons with and without an oxygen functional group, respectively. From the above, together with the ^{1}H-NMR data, the structure of **2** was assumed to be a megastigmane glucoside with hydroxyl functional groups at the C-6, -9, and -13 positions, like roseoside, except for the methyl group on C-5 was oxidized to a primary alcohol. The position of the glucosidic linkage was deduced to be on the hydroxyl group at C-13 from the ^{13}C-NMR chemical shift of C-9 (δ_C 68.8),[6] and this was further confirmed by the heteronuclear multiple-bond correlation spectrum in which δ_H 4.67 (H-1′) crossed δ_C 68.1 (C-13). The absolute configuration of the 6-position was deduced to be *S* by comparison of the CD maxima with those of a known compound.[6] The absolute configuration of C-9 was determined to be *S* by a modified Mosher's method, after hydrolysis of **2** and esterification with *R*- and *S*-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) (Fig. 2, **2b**, **c**).[7] Therefore the structure of glochidionionoside A (**2**) was elucidated to be (6*S*,7*E*,9*S*)-megastigman-3-one-4,7-diene-6,9,13-triol 13-*O*-β-D-glucopyranoside.

Glochidionionosides B (**3**), $C_{19}H_{32}O_8$, and C (**4**), $C_{19}H_{30}O_8$, were also isolated as amorphous powders. The NMR spectra showed that **3** and **4** were compounds analogous to **2**, except for the absence of a hydroxyl group at the 6-position, and the former does not have a double bond between C-7 and -8, whereas the latter does. The absolute stereochemistries at the 6 and 9 positions were determined similarly to the previous compoyund, from their CD spectra[6,8] and the results obtained using the modified Mosher's method, as shown in Fig. 2, respectively (Fig. 2, **3b**, **c**, and **4b**, **c**). Therefore the structures of glochidionionosides B (**3**) and C (**4**) were elucidated

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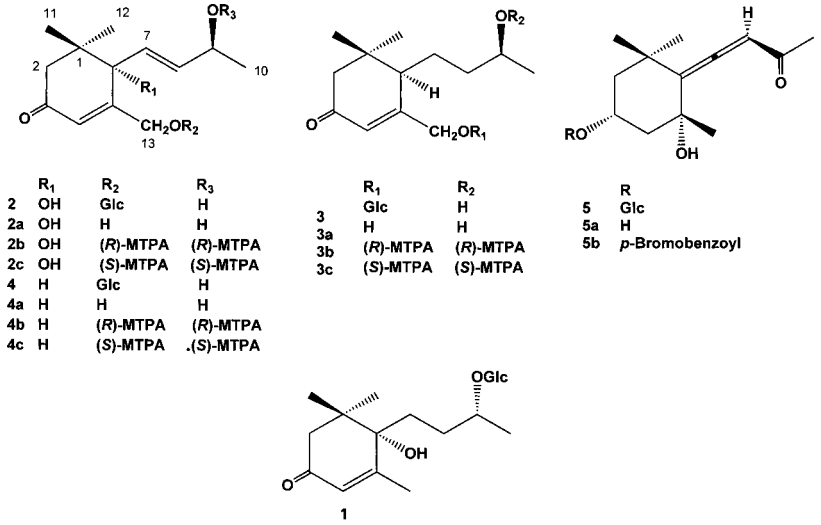

Fig. 1. Structures of Blumenol C Glucoside (**1**) and Glochidionionosides A (**2**)—D (**5**)

Table 1. ^{13}C-NMR Data for Glochidionionosides A—D (**2**—**5**) and **5a** (100 MHz, CD_3OD)

C	2	3	4	5	5a
1	42.8	37.8	37.1	32.7 (32.7)[a]	35.8
2	50.8	48.6	49.0	45.2 (44.8) (−3.5)[b]	48.7
3	201.3	202.2	202.0	73.4 (72.8) (+7.4)	66.0
4	124.8	123.2	124.1	48.1 (48.3) (−1.5)	49.6
5	165.0	167.9	164.0	72.5 (71.5)	72.7
6	79.2	47.8	52.0	123.8 (123.4)	123.8
7	130.2	27.8	127.3	202.0 (198.6)	202.0
8	137.2	39.8	140.5	102.8 (102.8)	102.7
9	68.8	68.9	68.9	211.5 (210.4)	211.5
10	23.9	23.6	23.8	26.7 (26.6)	26.7
11	23.5	27.6	27.6	29.9 (29.7)	30.2
12	24.2	28.9	28.9	32.7 (32.7)	32.7
13	68.1	70.8	70.1	30.5 (30.5)	30.4
1′	103.9	103.5	103.5	102.8 (103.2)	
2′	75.2	75.1	75.0	75.2 (75.3)	
3′	78.2	78.2	78.2	78.1 (78.7)	
4′	71.8	71.7	71.7	71.7 (71.7)	
5′	78.1	78.1	78.1	78.0 (78.6)	
6′	62.9	62.8	62.8	62.9 (62.8)	

a) Data for C_5D_5N. *b*) $\Delta\delta_{\mathbf{5a}-\mathbf{5}}$ (CD_3OD).

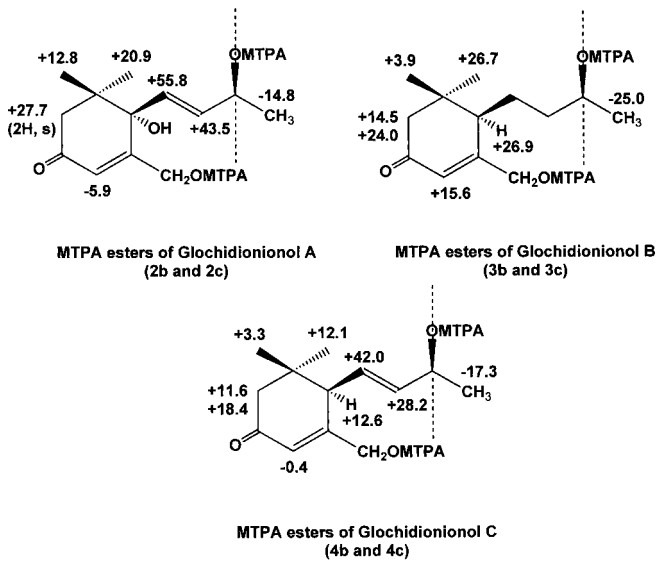

Fig. 2. Results Obtained with the Modified Mosher's Method for Glochidionionosides A—C (**2**—**4**)

The $\Delta\delta$ values are in Hz (δS–δR, 400 MHz).

to be (6*R*,9*S*)-megastigman-3-on-4-ene-9,13-diol and (6*R*,7*E*,9*S*)-megastigman-3-one-4,7-diene-9,13-diol 13-*O*-β-D-glucopyranosides, respectively.

Glochidionionoside D (**5**) was isolated as an amorphous powder, with the elemental composition of $C_{19}H_{32}O_8$ by HR-FAB-MS, and the ^{13}C-NMR spectrum showed the presence of three characteristic signals for an allenic moiety [δ_C 123.8 (s), 202.0 (s) and 102.8 (d)], together with six for β-glucopyranose and 10 other signals, one of which was highly deshielded, appearing at δ_C 211.5. These results and the IR absorption band at 1941 cm^{-1} indicated that the aglycone must have the same planar structure as that of grasshopper ketone[9] and its 3-*O*-β-D-glucopyranoside (**6**) (icariside B_1). 5-*O*-β-D-Glucopyranosides (**7**, **8**) (citrosides A, B), which are isomers at the 8 position, and previously isolated from *Epimedium grandiflorum* var. *thunbergianum*[10] and *Citrus unshu*,[11] respectively. However, the spectroscopic data were not identical to those of **6** or **7** and **8**. Furthermore, the downfield shifts of the axial proton of H-3 (δ_H 4.95 in **6** and 4.14 in **5**) and the axial methyl protons of C-12 (δ_H 1.51 in **6** and 1.23 in **5**) disappeared, due to 1,3-diaxial interaction of the hydroxyl group on C-5. These data imply that one of the absolute configurations at C-3 and -5 must be different from that of **6**, **7**, and **8**. To confirm this, **5** was enzymatically hydrolyzed for determination of the absolute configuration of C-3 using the β-D-glucopyranosylation-induced shift-trend rule for ^{13}C-NMR spectrometry.[12] When the ^{13}C-NMR data of **5** and **5a** were compared, the absolute configuration of C-3 was assigned to be *R*, which is opposite to that of **6**, **7**, and **8** (Table 1). However, the orientation of the hydroxyl substituent was found to be in the equatorial position, which was the same as in other grasshopper ketone glucosides. In turn, the hydroxyl group at the 5-position must be oriented in the equatorial position. These findings were confirmed by the H–H long-range correlation (H–H COSY) spectrum and the results of difference NOE experiments. In the H–H COSY

spectrum, H-13 (δ_H 1.53) crossed H-4ax (δ_H 1.80) in a W-figure correlation, and on irradiation of H-3 (δ_H 4.14), significant enhancement was observed at δ_H 1.28 (H_3-11ax), 1.53 (H_3-13ax), 1.98 (H-2eq), 2.25 (H-4eq), and 4.22 (H-1′). Thus the methyl group (C-13) on C-5 must be in the axial orientation. Spectral determination of the axis chirality of the allenic bond was ambiguous. Thus the aglycone was treated with *p*-bromobenzoyl chloride, the resulting ester (**5b**) was recrystallized from *i*-PrOH, and a suitable crystal was subjected to X-ray analysis using the heavy atom method. The absolute configuration was determined by Vijvoet's anomalous dispersion method (Table 2).[13] The absolute structure of the ring system was the same as that determined by NMR spectroscopy. The axis chirality of the side chain was ascertained by X-ray analysis to be *R*, as shown in Fig. 3.

Table 2. Determination of Absolute Stereochemistry of **5b**

h	*k*	*l*	Obs. (F_o) (+) (−)	Calcd. (F_c) (+) (−)
2	2	−2	61<132	64<132
1	2	0	193>145	193>142
1	−4	−2	149<201	152<204
1	0	−1	202<254	198<249
1	5	1	220>172	220>172
0	10	−1	120<163	116<167
3	8	0	292>239	280>227
3	−5	−1	235>188	235>191
3	−2	−2	204>162	206>167
3	0	−3	118<156	112<154
3	2	1	142<176	139<178
1	−10	−1	49<104	68<110
4	3	−1	111<151	103<141
2	3	1	244<287	241<283
3	5	0	229<271	228<268
0	2	3	64>30	62>22
1	3	0	268>230	264>229
3	−4	−2	151>64	150>62
1	0	2	217<253	208<241
4	−5	−3	98<135	100<135
1	4	1	438>385	441>389
4	1	−3	78<102	73<108
2	1	−3	282<316	275<314
4	10	−1	162>136	168>135
1	10	1	90>60	88>60

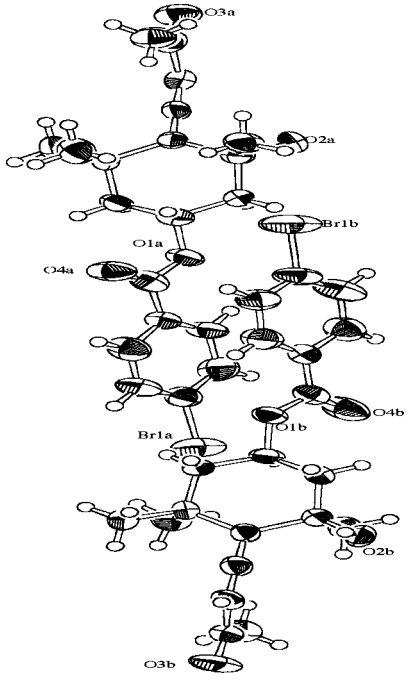

Fig. 3. Perspective ORTEP Drawing of **5b**

There are two independent molecules in an asymmetric unit. Atom numberings were omitted for clarification, except for noncarbon atoms.

Compounds closely related to glochidionionosides A (**2**) and C (**4**) were isolated from *Apocynum venetum*, apocynosides II and I, respectively.[14] On the C-13 methyl groups, they similarly possessed hydroxyl groups to which sugar moieties were attached. However, they are respective epimers at the C-9 position. Therefore the stereochemical structures at the 9-positions, which do not carry glucose moieties, have been determined independently in this study.

Experimental

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel column chromatography (CC) and reversed-phase [octadecyl silica gel (ODS)] open CC (RPCC) were performed on silica gel 60 (E. 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)→(7 : 3, 1 l), fractions of 10 g being collected], respectively. Droplet counter-current chromatography (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 for 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 ODS (Inertsil; GL Science, Tokyo, Japan; Φ=20 mm, L=250 mm), and the eluate was monitored with a UV detector. Hesperidinase was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acids were from Nacalai Tesque. (Kyoto, Japan).

A melting point was determined with a Yanagimoto micromelting point apparatus and is uncorrected. Optical rotations were measured on a Union Giken PM-101 digital polarimeter. IR spectra were measured on a Shimadzu IR-408 spectrophotometer and UV spectra on a Shimadzu UV-160A spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Negative-ion HR-FAB-MS were recorded on a JEOL JMS SX-102 spectrometer. CD spectra were obtained on a JASCO J-720 spectropolarimeter.

Plant Material Leaves of *G. zeylanicum* (Gaertn.) A. Juss (Euphorbiaceae) were collected in Okinawa, Japan, in August 1990, and a voucher specimen was deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University Faculty of Medicine (90-GZ-Okinawa-0822).

Extraction and Fractionation The air-dried leaves of *G. zeylanicum* (4.72 kg) were extracted three times with MeOH. The MeOH extract was concentrated to 1.5 l, and then 75 ml of H_2O was added to form a 95% aqueous solution. This solution was washed with 1.5 l of *n*-hexane and then the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 1.5 l of H_2O, and then extracted with 1.5 l each of EtOAc and *n*-BuOH successively to give 75.7 and 108 g of EtOAc- and *n*-BuOH-soluble fractions, respectively. The *n*-BuOH extract (107 g) was subjected to highly porous synthetic resin (Diaion HP-20) CC (Mitsubishi Chemical Co., Ltd.; Φ=80 mm, L=55 cm), using H_2O–MeOH (4 : 1, 6 l), (2 : 3, 6 l), (3 : 2, 6 l), and (1 : 4, 6 l), and MeOH (6 l), with 2-l fractions collected. Fractions 4—6 were combined (28.7 g) and then subjected to silica gel (530 g) CC with elution with $CHCl_3$ (1.5 l), $CHCl_3$–MeOH [(99 : 1, 3 l), (49 : 1, 6 l), (24 : 1, 6 l), (47 : 3, 6 l), (23 : 2, 6 l), (9 : 1, 6 l), (7 : 1, 6 l), (17 : 3, 4.5 l), (4 : 1, 3 l), and (7 : 3, 3 l)], and $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 3 l), with 500-ml fractions collected. Combined fractions (45—64, 2.35 g) were then separated by RPCC. The residue (480 mg) of fractions 74—90 was subjected to DCCC, and the residue (229 mg) of fractions 35—49 was subjected to HPLC [H_2O–MeOH (7 : 3)], which afforded an allenic compound **5** in a yield of 77 mg. Combined RPCC fractions (91—105, 311 mg) were subjected to DCCC, and the residue (161 mg) of fractions 45—56 was subjected to HPLC [H_2O–MeOH (7 : 3)] to give 59 mg of **3** and 57 mg of **4**. Combined silica gel CC (65—87, 2.17 g) were subjected to RPCC. The residue

(485 mg) of fractions 60—75 was subjected to DCCC, and **2** (25 mg) was purified from the residue (125 mg) of fractions 4—16 by HPLC [H_2O–MeOH (7 : 3)].

From combined fractions (7—8, 25.9 g) obtained on Diaion HP-20 CC, 14 mg of **1** was isolated using similar chromatographic methods.

Blumenol C *O*-*β*-D-Glucopyranoside (**1**): $[\alpha]_D^{28}$ +43.9° (c=0.96, MeOH); CD $\Delta\varepsilon$ (nm): +2.15 (218), +2.84 (239), +0.63 (331) (c=1.05×10^{-4} M, MeOH).

Glochidionionoside A (**2**): Amorphous powder; $[\alpha]_D^{28}$ +29.1° (c=1.48, MeOH); IR ν_{max} (KBr) cm^{-1}: 3407, 2971, 2930, 2882, 1659, 1413, 1372, 1317, 1163, 1076, 1042; UV λ_{max} (MeOH) nm (log ε): 237 (3.86); ^{1}H-NMR (CD_3OD) δ: 1.00 (3H, s, H_3-12eq), 1.05 (3H, s, H_3-11ax), 1.24 (3H, d, J=6 Hz, H_3-10), 2.20 (1H, d, J=17 Hz, H-2a), 2.51 (1H, d, J=17 Hz, H-2b), 3.24 (1H, t, J=8 Hz, H-2′), 3.66 (1H, dd, J=12, 6 Hz, H-6′a), 3.86 (1H, dd, J=12, 2 Hz, H-6′b), 4.27 (1H, d, J=8 Hz, H-1′), 4.29 (1H, dd, J=14, 2 Hz, H-13a), 4.32 (1H, m, H-9), 4.67 (1H, dd, J=14, 2 Hz, H-13b), 5.82 (1H, s, H-7), 5.83 (1H, d, J=4 Hz, H-8), 6.30 (1H, br s, H-4); ^{13}C-NMR (CD_3OD): Table 1; CD $\Delta\varepsilon$ (nm): +8.18 (240), −1.20 (331) (c=1.11×10^{-4} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 401.1829 [M−H]$^-$ (Calcd for $C_{19}H_{29}O_9$: 401.1812).

Glochidionionoside B (**3**): Amorphous powder, $[\alpha]_D^{28}$ +7.4° (c=1.22, MeOH); IR ν_{max} (KBr) cm^{-1}: 3409, 2965, 2930, 2878, 1651, 1371, 1163, 1078, 1045; UV λ_{max} (MeOH) nm (log ε): 239 (3.93); ^{1}H-NMR (CD_3OD) δ: 1.03 (3H, s, H_3-12), 1.11 (3H, s, H_3-11), 1.16 (3H, d, J=6 Hz, H_3-10), 2.02 (1H, t, J=5 Hz, H-6), 2.03 (1H, d, J=18 Hz, H-2a), 2.54 (1H, d, J=18 Hz, H-2b), 3.25 (1H, dd, J=12, 6 Hz, H-6′a), 3.68 (1H, m, H-9), 3.88 (1H, dd, J=12, 2 Hz, H-6′b), 4.35 (1H, d, J=8 Hz, H-1′), 4.38 (1H, dd, J=17, 2 Hz, H-13a), 4.52 (1H, dd, J=17, 2 Hz, H-13b), 6.17 (1H, br s, H-4); ^{13}C-NMR (CD_3OD): Table 1; CD $\Delta\varepsilon$ (nm): +1.47 (217), +1.34 (239), +0.42 (332) (c=9.43×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 387.2016 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

Glochidionionoside C (**4**): Amorphous powder; $[\alpha]_D^{28}$ +112.1° (c=1.42, MeOH); IR ν_{max} (KBr) cm^{-1}: 3395, 2966, 2930, 2878, 1657, 1413, 1370, 1298, 1163, 1076; UV λ_{max} (MeOH) nm (log ε): 236 (4.06); ^{1}H-NMR (CD_3OD) δ: 0.98 (3H, s, H_3-12), 1.04 (3H, s, H_3-11), 1.23 (3H, d, J=6 Hz, H_3-10), 2.09 (1H, d, J=17 Hz, H-2a), 2.49 (1H, d, J=17 Hz, H-2b), 2.75 (1H, d, J=9 Hz, H-6), 3.24 (1H, dd, J=9, 8 Hz, H-2′), 3.68 (1H, dd, J=12, 6 Hz, H-6′a), 3.68 (1H, m, H-9), 3.87 (1H, dd, J=12, 2 Hz, H-6′b), 4.27 (1H, d, J=8 Hz, H-1′), 4.27 (1H, dd, J=17, 2 Hz, H-13a), 4.48 (1H, dd, J=17, 2 Hz, H-13b), 5.61 (1H, ddd, J=15, 9, 1 Hz, H-7), 5.72 (1H, dd, J=15, 6 Hz, H-8), 6.25 (1H, br s, H-4); ^{13}C-NMR (CD_3OD): Table 1, CD $\Delta\varepsilon$ (nm): +20.8 (244), −1.06 (322) (c=1.09×10^{-4} M, MeOH), HR-FAB-MS (negative-ion mode) m/z: 385.1843 [M−H]$^-$ (Calcd for $C_{19}H_{29}O_8$: 385.1862).

Glochidionionoside D (**5**): Amorphous powder; $[\alpha]_D^{28}$ −47.8° (c=1.15, MeOH); IR ν_{max} (KBr) cm^{-1}: 3409, 2967, 1941, 1669, 1366, 1246, 1076 1028; UV λ_{max} (MeOH) nm (log ε): 229 (4.14), 279 (3.19); ^{1}H-NMR (CD_3OD) δ: 1.23 (3H, s, H_3-12eq), 1.28 (3H, s, H_3-11ax), 1.53 (3H, s, H_3-13), 1.56 (1H, dd, J=10, 13 Hz, H-2ax), 1.80 (1H, dd, J=13, 10 Hz, H-4ax), 1.98 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 2.22 (3H, s, H_3-10), 2.25 (1H, ddd, J=13, 4, 1 Hz, H-4eq), 3.16 (1H, dd, J=9, 8 Hz, H-2′), 3.28 (1H, t, J=9 Hz, H-4′), 3.37 (1H, t, J=9 Hz, H-3′), 3.68 (1H, dd, J=12, 6 Hz, H-6′a), 3.88 (1H, dd, J=12, 2 Hz, H-6′b), 4.14 (1H, tt, J=10, 4 Hz, H-3), 4.42 (1H, d, J=8 Hz, H-1′), 5.93 (1H, s, H-8); ^{13}C-NMR (CD_3OD and C_5D_5N): Table 1; CD $\Delta\varepsilon$ (nm): −1.75 (210), +0.29 (242), −0.25 (278), −0.27 (323) (c=8.94×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 385.1841 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 385.1862).

Enzymatic Hydrolysis of Glochidionoionoside A (2) Glochidionoionoside A (**2**) (19 mg) was hydrolyzed with emulsin (20 mg) in 2 ml of H_2O at 37 °C for 18 h. The reaction mixture was concentrated and then subjected to silica gel CC (Φ=15 mm, L=20 cm) with C_6H_6 (40 ml), C_6H_6–$CHCl_3$ (1 : 1, 40 ml), $CHCl_3$ (100 ml), and $CHCl_3$–MeOH (19 : 1, 100 ml, 9 : 1, 100 ml, 17 : 3, 100 ml, 7 : 3, 300 ml), with 10-ml fractions collected. Glochidionionol A (**2a**) and D-glucose were recovered in fractions 28—32 (6.9 mg, 61%) and 44—50 (6.0 mg, 71%), respectively.

Glochionionol A (**2a**): Colorless syrup, $[\alpha]_D^{28}$ +156.7° (c=0.46, MeOH), ^{1}H-NMR (CD_3OD) δ: 0.996 (3H, s, H_3-12eq), 1.04 (3H, s, H_3-11ax), 1.23 (3H, d, J=6 Hz, H_3-10), 2.17 (1H, d, J=17 Hz, H-2a), 2.49 (1H, d, J=17 Hz, H-2b), 4.18 (1H, dd, J=19, 2 Hz, H-13a), 4.30 (1H, qd, J=6, 2 Hz, H-9), 4.38 (1H, dd, J=19, 2 Hz, H-13b), 5.80 (2H, m, H-7, 8), 6.16 (1H, br s, H-4); ^{13}C-NMR (CD_3OD) δ: 23.3, 23.7, 24.0 (C-10, 11, 12), 42.7 (C-1), 50.7 (C-2), 61.3 (C-13), 68.6 (C-9), 79.3 (C-6), 123.1 (C-4), 130.2 (C-7), 136.9 (C-8), 169.4 (C-5), 201.3 (C-3); HR-FAB-MS (negative-ion mode): m/z: 239.1288 [M−H]$^-$ (Calcd for $C_{13}H_{19}O_4$: 239.1283). D-Glucose: $[\alpha]_D^{28}$ +37.5° (c=0.40, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MTPA Esters (2b, c) from Glochidionionol A (2a) A solution of **2a** (3.4 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (50 mg) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (38 mg) (DCC) and 4-dimethylaminopyridine (DMAP) (17 mg), and the resulting mixture was occasionally stirred at 25 °C for 40 min. After the addition of 1 ml each of H_2O and CH_2Cl_2, the solution was washed successively with 5% HCl, $NaHCO_3$-saturated H_2O and brine. 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, spotted for 18 cm, and developed with $CHCl_3$–$(CH_3)_2CO$ (19 : 1) for 9 cm and eluted with $CHCl_3$–MeOH (9 : 1)] to furnish the ester, **2b** (5.7 mg, 63%). Using a similar procedure, **2c** (5.4 mg, 59%) was prepared from **2a** (3.4 mg) with (*S*)-MTPA (46 mg), DCC (38 mg), and 4-DMAP (17 mg).

Glochidionionol A 9,13-Di-(*R*)-MTPA Ester (**2b**): Amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 0.894 (3H, s, H_3-12), 0.999 (3H, s, H_3-11), 1.43 (3H, d, J=7 Hz, H_3-10), 2.23 (2H, s, H_2-2), 3.52—3.54 (6H, m, –OCH_3×2), 4.78 (1H, dd, J=16, 2 Hz, H-13a), 4.93 (1H, dd, J=16, 2 Hz, H-13b), 5.62 (1H, quid, J=7, 1 Hz, H-9), 5.71 (1H, dd, J=16, 1 Hz, H-7), 5.82 (1H, dd, J=16, 7 Hz, H-8), 5.93 (1H, t, J=2 Hz, H-4), 7.37—7.52 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode) m/z 671.2103 [M−H]$^-$ (Calcd for $C_{33}H_{33}O_8F_6$: 671.2080).

Glochidionionol A 9,13-Di-(*S*)-MTPA Ester (**2c**): Amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 0.947 (3H, s, H_3-12), 1.03 (3H, s, H_3-11), 1.39 (3H, d, J=7 Hz, H_3-10), 2.30 (2H, s, H_2-2), 3.52—3.54 (6H, m, –OCH_3×2), 4.67 (1H, dd, J=16, 2 Hz, H-13a), 5.03 (1H, dd, J=16, 2 Hz, H-13b), 5.59 (1H, qui, J=7 Hz, H-9), 5.85 (1H, d, J=16 Hz, H-7), 5.91 (1H, t, J=2 Hz, H-4), 5.93 (1H, dd, J=16, 7 Hz, H-8), 7.37—7.52 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode) m/z 671.2052 [M−H]$^-$ (Calcd for $C_{33}H_{33}O_8F_6$: 671.2080).

Enzymatic Hydrolysis of Glochidionoionosides B (3) and C (4) to Glochidionionols B and C (3a and 4a, Respectively) Glochidionoionosides B (**3**) (21 mg) and C (**4**) (25 mg) were hydrolyzed with emulsin (25, 18 mg, respectively) in 2 ml of H_2O at 37 °C for 15 h. The reaction mixtures were concentrated, and then the aglycones and D-glucose were purified in a similar manner to that for **2a**. The aglycones (**3a**, **4a**) were recovered in fractions 25—29 (5.7 mg, 47%) and 24—28 (10.2 mg, 70%), and D-glucose in fractions 44—50 (5.7 mg, 59%) and 44—51 (9.0 mg, 68%), respectively.

Glochidionionol B (**3a**): Colorless syrup; $[\alpha]_D^{28}$ +89.5° (c=0.38, MeOH); ^{1}H-NMR (CD_3OD) δ: 1.02 (3H, s, H_3-12), 1.11 (3H, s, H_3-11), 1.16 (3H, d, J=6 Hz, H_3-10), 1.46—1.64 (3H, m, H-7a, 7b, 8a), 1.73—1.83 (1H, m, H-8b), 2.04 1H, d, J=18 Hz, H-2a), 2.55 (1H, d, J=18 Hz, H-2b), 3.69 (1H, sextet, J=6 Hz, H-8), 4.15 (1H, dd, J=18, 2 Hz, H-13a), 4.30 (1H, dd, J=18, 2 Hz, H-13b), 6.65 (1H, br s, H-4); ^{13}C-NMR (CD_3OD) δ: 23.5 (C-10), 27.6 (C-11), 28.0 (C-7), 28.8 (C-12), 37.4 (C-1), 39.8 (C-8), 48.0 (C-6), 48.7 (C-2), 65.1 (C-13), 68.8 (C-9), 121.6 (C-4), 172.3 (C-5), 202.3 (C-3); HR-FAB-MS (negative-ion mode) m/z: 225.1506 [M−H]$^-$ (Calcd for $C_{13}H_{21}O_3$: 225.1491). D-Glucose: $[\alpha]_D^{25}$ +44.7° (c=0.38, H_2O, 24 h after being dissolved in the solvent).

Glochidionionol C (**4a**): Colorless syrup; $[\alpha]_D^{26}$ +230.9° (c=0.68, MeOH); ^{1}H-NMR (CD_3OD) δ: 0.991 (3H, s, H_3-12), 1.03 (3H, s, H_3-11), 1.23 (3H, d, J=6 Hz, H_3-10), 2.09 (1H, d, J=17 Hz, H-2a), 2.48 (1H, d, J=17 Hz, H-2b), 2.57 (1H, d, J=8 Hz, H-6), 4.11 (1H, dd, J=12, 2 Hz, H-13a), 4.20 (1H, dd, J=12, 2 Hz, H-13b), 4.26 (1H, qui, J=6 Hz, H-9), 5.60 (1H, dd, J=16, 8 Hz, H-7), 5.67 (1H, dd, J=15, 6 Hz, H-8), 6.14 (1H, t, J=2 Hz, H-4); ^{13}C-NMR (CD_3OD) δ: 23.8 (C-10), 27.4 (C-11), 27.9 (C-12), 37.3 (C-1), 49.2 (C-2), 52.3 (C-6), 64.2 (C-13), 68.8 (C-9), 122.5 (C-4), 127.5 (C-7), 140.2 (C-8), 168.3 (C-5), 202.1 (C-3); HR-FAB-MS (negative-ion mode) m/z: 223.1338 [M−H]$^-$ (Calcd for $C_{13}H_{19}O_3$: 223.1334). D-Glucose: $[\alpha]_D^{26}$ +43.1° (c=0.53, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MTPA Esters (3b, c, and 4b, c) from 3a and 4a, Respectively Using a similar procedure to that used for the preparation of **2b** and **2c** from **2a**, **3b** (6.3 mg, 77%) and **3c** (5.3 mg, 65%) were prepared from **3a** (2.8 mg each) using the respective amounts of (*R*)- and (*S*)-MTPA (44, 43 mg), DCC (33, 34 mg), and 4-DMAP (16, 16 mg). The developing solvent for preparative TLC was $CHCl_3$–$(CH_3)_2CO$, 19 : 1.

Glochidionionol B 9,13-Di-(*R*)-MTPA Ester (**3b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.917 (3H, s, H_3-12), 0.936 (3H, s, H_3-11), 1.24—1.30 (1H, m, H-7a), 1.33 (3H, d, J=7 Hz, H_3-10), 1.53—1.64 (3H, m, H-7b, 8a, 8b), 1.77 (1H, t, J=5 Hz, H-6), 2.03 (1H, d, J=18 Hz, H-2a), 2.27 (1H, d, J=18 Hz, H-2b), 3.53—3.55 (6H, m, –OCH_3×2), 4.68 (1H, dd, J=16, 2 Hz, H-13a), 4.78 (1H, dd, J=16, 2 Hz, H-13b), 5.08 (1H, m, H-9), 5.85 (1H, br s, H-4), 7.37—7.52 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode) (+NaI) m/z: 681.2228 [M+Na]$^+$ (Calcd for $C_{33}H_{36}O_7F_6Na$:

681.2263).

Glochidionionol B 9,13-Di-(*S*)-MTPA Ester (**3c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.926 (3H, s, H_3-12), 0.993 (3H, s, H_3-11), 1.27 (3H, d, J=6 Hz, H_3-10), 1.42—1.48 (1H, m, H-7a), 1.59—1.74 (3H, m, H-7b, 8a, 8b), 1.84 (1H, t, J=5 Hz, H-6), 2.07 (1H, d, J=18 Hz, H-2a), 2.33 (1H, d, J=18 Hz, H-2b), 3.48—3.54 (6H, m, –OCH_3×2), 4.74 (1H, dd, J=16, 2 Hz, H-13a), 4.88 (1H, dd, J=16, 2 Hz, H-13b), 5.07 (1H, m, H-9), 5.89 (1H, br s, H-4), 7.36—7.51 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode) (+NaI) *m/z*: 681.2224 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_7F_6Na$: 681.2263).

Using a similar procedure to that used for the preparation of **3b** and **3c** from **3a**, **4b** (11.1 mg, 74%) and **4c** (10.2 mg, 68%) were prepared from **4a** (5.3 mg each) using of the respective amounts of (*R*)- and (*S*)-MTPA (46, 49 mg), DCC (32, 34 mg), and 4-DMAP (18, 22 mg). The developing solvent for preparative TLC was $CHCl_3$–$(CH_3)_2CO$, 19 : 1.

Glochidionionol C 9,13-Di-(*R*)-MTPA Ester (**4b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.888 (3H, s, H_3-12), 0.954 (3H, s, H_3-11), 1.42 (3H, d, J=6 Hz, H_3-10), 2.08 (1H, d, J=17 Hz, H-2a), 2.23 (1H, d, J=17 Hz, H-2b), 2.56 (1H, d, J=8 Hz, H-6), 3.51—3.55 (6H, m, –OCH_3×2), 4.67 (1H, dd, J=16, 1 Hz, H-13a), 4.77 (1H, dd, J=16, 1 Hz, H-13b), 5.57 (1H, qui, J=6 Hz, H-9), 5.55 (1H, dd, J=15, 6 Hz, H-8), 5.59 (1H, dd, J=15, 8 Hz, H-7), 5.98 (1H, s, H-4), 7.37—7.52 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode) (+NaI) *m/z*: 679.2111 $[M+Na]^+$ (Calcd for $C_{33}H_{34}O_7F_6Na$: 679.2106).

Glochidionionol C 9,13-Di-(*S*)-MTPA Ester (**4b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.918 (3H, s, H_3-12), 0.961 (3H, s, H_3-11), 1.38 (3H, d, J=6 Hz, H_3-10), 2.11 (1H, d, J=17 Hz, H-2a), 2.28 (1H, d, J=17 Hz, H-2b), 2.59 (1H, d, J=9 Hz, H-6), 3.50—3.53 (6H, m, –OCH_3×2), 4.66 (1H, dd, J=15, 1 Hz, H-13a), 4.83 (1H, dd, J=15, 1 Hz, H-13b), 5.52 (1H, qui, J=6 Hz, H-9), 5.61 (1H, dd, J=15, 6 Hz, H-7), 5.70 (1H, dd, J=15, 9 Hz, H-8), 5.98 (1H, s, H-4), 7.36—7.52 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode) (+NaI) *m/z*: 679.2110 $[M+Na]^+$ (Calcd for $C_{33}H_{34}O_7F_6Na$: 679.2106).

Enzymatic Hydrolysis of Glochidionionoside D (5) Glochidionionoside D (**5**, 23 mg) in 2 ml of H_2O was hydrolyzed with emulsin (20 mg) at 37 °C for 18 h. The reaction mixture was dried and then subjected to silica gel (Φ=15 mm, L=20 cm) CC with $CHCl_3$ (150 ml), and $CHCl_3$–MeOH (9 : 1, 100 ml) and (7 : 3, 300 ml), with 10-ml fractions collected. An aglycone (**5a**) and D-glucose were recovered in fractions 18—21 (9.8 mg) and 31—37 (8.0 mg), respectively.

Aglycone (**5a**): Colorless syrup; $[\alpha]_D^{25}$ −37.9° (c=0.66, MeOH); UV λ_{max} (MeOH) nm (log ε): 229 (4.07); ^{1}H-NMR (CD_3OD) δ: 1.24 (3H, s, H_3-12), 1.27 (3H, s, H_3-11), 1.51 (3H, d, J=1 Hz, H_3-13), 1.50 (1H, dd, J=12, 10 Hz, H-2ax), 1.69 (1H, ddq, J=12, 10, 1 Hz, H-4ax), 1.84 (1H, ddd, J=12, 4, 2 Hz, H-2eq), 2.08 (1H, ddd, J=12, 4, 2 Hz, H-4eq), 2.22 (3H, d, J=0.5 Hz, H_3-10), 3.99 (1H, tt, J=10, 4 Hz, H-3), 5.93 (1H, d, J=0.5 Hz, H-8); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 223.1357 $[M-H]^-$ (Calcd for $C_{13}H_{19}O_3$: 223.1334). D-Glucose: $[\alpha]_D^{24}$ +41.5° (c=0.53, H_2O, 24 h after being dissolved in the solvent).

Synthesis of *p*-Bromobenzoyl Ester (5b) of 5a The aglycone (**5a**, 9 mg) was treated with 18 mg of *p*-bromobenzoyl chloride and 15 μl of pyridine in 1 ml of $CDCl_3$ at 25 °C. Equal amounts of the reagents were added at 12 and 24 h. Six hours after the final addition of the reagents, 2 ml of H_2O and then 1 ml of $CHCl_3$ were added to the reaction mixture. The organic layer was washed successively with 2 N HCl, $NaHCO_3$-saturated H_2O and brine. The residue, taken up in the organic layer, was subjected to preparative TLC (silica gel, 0.25 mm thickness, 18 cm width, developed for 9 cm with C_6H_6–$(CH_3)_2CO$, 4 : 1, and eluted with $CHCl_3$–MeOH, 9 : 1) to give 10 mg of the ester.

p-Bromobenzoyl Ester (**5b**): Colorless plates (EtOH); mp 112—114 °C; $[\alpha]_D^{29}$ −21.0° (c=0.67, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ: 1.31 and 1.34 (each 3H, each s, H_3-11, 12), 1.58 (3H, s, H_3-13), 1.89 (1H, dd, J=14, 5 Hz, H-2ax), 1.98 (1H, ddd, J=14, 4, 1 Hz, H-2eq), 2.05 (1H, dd, J=14, 4 Hz, H-4ax), 2.23 (3H, d, J=0.5 Hz, H_3-10), 2.25 (1H, ddd, J=14, 4, 1 Hz, H-4eq), 5.37 (1H, septet, J=4 Hz, H-3), 6.01 (1H, d, J=0.5 Hz, H-8), 7.89 and 7.58 (each 2H, each d, each J=9 Hz, H-2″, 3″, 5″, 6″); ^{13}C-NMR ($CDCl_3$) δ: 26.6 (C-10), 30.2, 30.6, 31.7 (C-11, 12, 13), 34.8 (C-1), 43.3 (C-2), 44.6 (C-4), 69.1 (C-3), 71.2 (C-5), 102.3 (C-8), 121.3 (C-6), 128.3, 129.2 (C-1″, 4″), 131.1×2, 131.9×2 (C-2″, 3″, 5″, 6″), 165.3 (C-7″), 198.5 (C-7), 209.0 (C-9); HR-FAB-MS (positive-ion mode) (+NaI) *m/z*: 429.0660 and 431.0660 $[M+Na]^+$ (Calcd for $C_{20}H_{23}O_4{}^{79}BrNa$ and $C_{20}H_{23}O_4{}^{81}BrNa$: 429.0677 and 431.0657, respectively).

X-Ray Structure Determination of 5b A suitable crystal (0.50 mm× 0.50 mm×0.10 mm) was used for analysis. All data were obtained with a Rigaku AFC-5S automated four-circle diffractometer with graphite-monochromated Mo$K\alpha$ radiation. Crystal data: $C_{20}H_{23}O_4Br$, M_r=430.30, triclinic, space group P_1, a=7.977(1) Å, b=21.323(2) Å, c=6.406(1) Å, α=91.48(1)°, β=112.56(1)°, γ=82.911(9)°, V=998.3(3) Å^3, Z=2, D_x= 1.352 Mg m^{-3}, $F(000)$=418, and μ(Mo$K\alpha$)=20.842 cm^{-1}. The intensities were measured in the ω-scan mode, 2θ<55°, and measurements were conducted on one component of Bijvoet pairs. Three standard reflections were monitored every 150 measurements. The data were corrected for Lorentz and polarization factors. The absorption correction was applied (transmission factor=0.620—0.998).[15] Of 8867 independent reflections which were collected, 4579 reflections with I>2.00$\sigma(I)$ were used for the structural determination and refinement. The structure was solved by the direct method using the TEXSAN crystallographic software package.[16] This compound has two independent molecules in the asymmetric unit. All nonhydrogen atoms were found in the Fourier map. All H atoms were found in the difference Fourier map and refined isotropically. The refinement of atomic parameters was carried out using the full-matrix least-squares with anisotropic temperature factors for all non-H atoms. The final refinement converged with R=0.043 and R_w=0.048 for 452 parameters. Then 25 Bijvoet pairs with high intensity and high measurement accuracy were selected (Table 2). The absolute configuration of **5b** was determined as shown in Fig. 3 using Bijvoet's anomalous-dispersion method.[13] Atomic scattering factors were taken from the "International Tables for X-ray Crystallography."[17]

The final atom coordinates and a list of the temperature factors and final structure factors have been deposited at the Cambridge Crystallographic Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB12 1EW, U.K.

Acknowledgments The authors are grateful for access to the superconducting NMR instrument at the Analytical Center of Molecular Medicine of Hiroshima University Faculty of Medicine. This work was supported by a grant-in-aid from the Ministry of Education, Science, Sports, Culture and Technology of Japan (No. 13672216). Thanks are also due to the Okinawa Foundation for financial support with an Okinawa Research Promotion Award (H.O.).

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