Radical-Scavenging Activities of New Megastigmane Glucosides from *Macaranga tanarius* (L.) MÜLL.-ARG.

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Four new megastigmane glucosides, named macarangiosides A—D (2—5), together with mallophenol B, lauroside E, methyl brevifolin carboxylate, and hyperin and isoquercitrin as a mixture were isolated from the leaves of *Macaranga tanarius* (L.) MÜLL.-ARG. (Euphorbiaceae). Their structures were elucidated by spectroscopic and chemical analyses. Macarangioside A—C (2—4) and mallophenol B were galloylated on glucose moiety and possessed the potent 2,2-diphenyl-picrylhydrazyl (DPPH) radical-scavenging activity.

Key words *Macaranga tanarius*; Euphorbiaceae; megastigmane glucoside; macarangioside; 2,2-diphenyl-picrylhydrazyl (DPPH)

Megastigmanes and its glycosides are a currently expanding class of compounds. In our continuation of the studies on the sub-tropical plants collected on Okinawa Island, we have phytochemically investigated the leaves of *Macaranga tanarius* (L.) MÜLL.-ARG. (Euphorbiaceae). *M. tanarius* is well known as a pioneer tree and also an ant-plant. It is defended by ants against herbivores by producing the ant-attracting food body.[1)] This plant can be found throughout eastern and southern Asia, especially in southern China, Korea and Japan. In China, root and bark of this plant are used for hemoptysis and dysentery, respectively.[2)] In this paper, we describe the isolation and structure elucidation of four new megastigmane glucosides (**2**—**5**) together with five known compounds (**1**, **6**—**9**), and then discuss about the structural feature on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of these compounds.

Results and Discussion

Air-dried leaves (12.1 kg) of *M. tanarius* were extracted with MeOH (15 l×3) by maceration, and then the combined methanolic extracts were evaporated under reduced pressure. The MeOH extract concentrated to 3 l was defatted with *n*-hexane (3 l), and the remaining MeOH extract was concentrated to viscous gum. The residue was suspended in H_2O (3 l) and partitioned with EtOAc (3 l) and *n*-BuOH (3 l) successively to give EtOAc- (801 g) and *n*-BuOH- (374 g) soluble fractions, respectively. Part of the *n*-BuOH-soluble fraction (181 g) was subjected to various kinds of column chromatography on a highly porous synthetic polymer (Diaion HP-20), silica gel, ODS, droplet counter-current chromatography (DCCC) and HPLC (ODS) to give the above-mentioned nine compounds (**1**—**9**) (Fig. 1).

Compound **1** was obtained as an amorphous powder. Methanolysis of **1** with 0.1 M NaOMe gave **1a**, (6*S*,9*R*)-roseoside.[3)] The presence of D-glucose was also confirmed by acid hydrolysis of **1a** and derivatization with (*S*)-(−)-α-methylbenzylamine.[4)] The ^{13}C- and ^{1}H-NMR spectra indicated the presence of a galloyl moiety (Tables 1, 2). From these results, compound **1** was identified as mallophenol B isolated from *Mallotus furetianus*.[5)] It is noteworthy that a 6′-*O*-galloyl moiety doesn't influence the chemical shift values of aglycone moiety and the cotton effect of CD spectrum by comparison of the physical data of **1** and **1a**.

Macarangioside A (**2**) was obtained as an amorphous powder and its elemental composition was determined to be $C_{26}H_{36}O_{12}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra indicated that **2** was closely related to mallophenol B (**1**) ex-

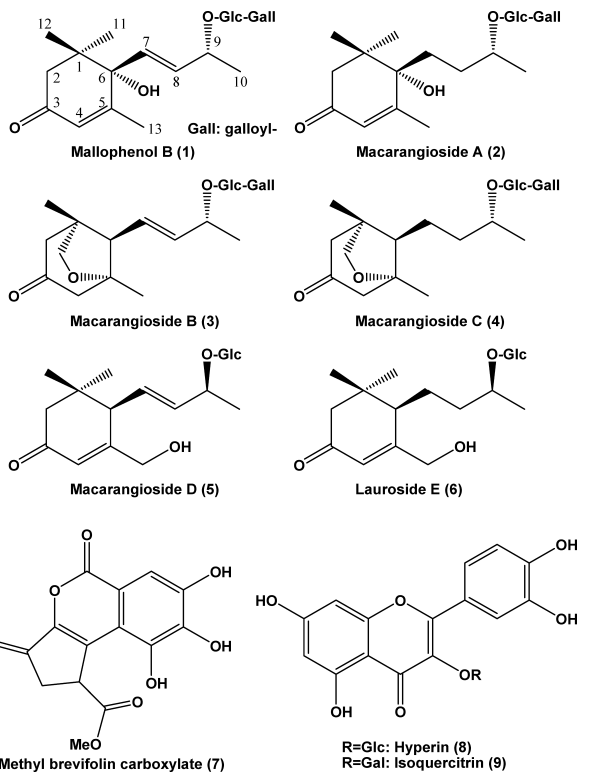

Fig. 1. Structures of Isolated Compounds from *Macaranga tanarius* (L.) MÜLL.-ARG.

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cept for the absence of a double bond between C-7 and 8 (Tables 1, 2). As a result, the plane structure of the megastigmane glucoside moiety was determined to be the same as icariside B5.[6,7] Next, the absolute configuration at C-6 and C-9 was elucidated as 6*S*,9*R* by comparison of the CD spectrum [$\Delta\varepsilon$ (nm) +1.87 (326), −13.8 (253) and +18.1 (221)] and chemical shift value (δ_C 33.5, 76.8 and 20.5 at C-8, 9 and 10, respectively) of **2** with those of icariside B_5 [$\Delta\varepsilon$ (nm) +1.73 (324), −9.33 (250) and +13.0 (218)] and chemical shift (δ_C 33.6, 76.3, 20.1).[6,7] Thus, compound **2** was determined to have the same configuration as icariside B5. The structure of glucose was considered to be the same as **1** because these compounds were isolated from the same plant as also described below. As a result, the structure of compound **2** was established to be (6*S*,9*R*)-megastigman-4-en-3-one-6,9-diol 9-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside.

Macarangioside B (**3**) was obtained as an amorphous powder and its elemental composition was determined to be $C_{26}H_{34}O_{12}$ by HR-FAB-MS. ^{1}H- and ^{13}C-NMR spectra indicated that macarangioside B (**3**) also have a megastigmane skeleton (Tables 1, 2). However, one of the methyl signals was converted to an oxygenated methylene carbon. The index of unsaturation from molecular formula implied an additional ring system. Heteronuclear multiple bond correlation (HMBC) experiment showed the presence of the long-range correlation between H-11 and C-5, which indicated the presence of an oxirane ring system between C-5 and C-11 (Fig. 2). Thus the aglycone moiety of macarangioside B (**3**) was considered to be a 5,11-epoxymegastigmane. The relative configuration of aglycone moiety was confirmed by the observation of NOE correlation between H-6 and pro-*R* H-11

Table 1. ^{13}C-NMR Data for Compounds **1**—**6** (CD_3OD, 100 MHz)

C	1	2	3	4	5	6
1	42.5	43.0	45.2	44.8	37.3	37.3
2	50.7	51.1	49.9	49.6	49.3	48.6
3	201.5	201.2	211.8	212.4	202.0	202.4
4	127.2	126.7	51.0	50.6	122.6	121.5
5	167.2	171.7	84.9	85.6	167.9	172.4
6	80.1	79.3	59.2	54.6	52.5	47.7
7	131.6	35.1	125.4	22.0	131.2	27.0
8	134.9	33.5	141.0	37.6	137.1	37.7
9	77.1	76.8	78.0	76.6	74.9	75.2
10	21.3	20.5	21.5	20.4	22.3	19.9
11	23.4	24.1	20.5	20.8	27.9	27.7
12	24.6	24.6	79.8	79.4	27.5	28.8
13	19.7	21.7	24.3	25.1	64.3	65.1
1′	103.0	102.7	103.0	102.9	101.3	102.1
2′	75.4	75.5	75.6	75.4	75.1	75.1
3′	78.0	78.1	78.1	78.2	78.4	78.1
4′	71.6	71.9	72.0	72.1	71.8	71.8
5′	75.2	75.2	75.3	75.2	78.2	77.9
6′	64.8	65.0	65.0	65.2	63.0	62.9
1″	121.5	121.6	121.6	121.6		
2″, 6″	110.4	110.4	110.4	110.3		
3″, 5″	146.5	146.6	146.7	146.6		
4″	139.9	140.0	140.0	140.0		
C=O	168.5	168.5	168.3	168.3		

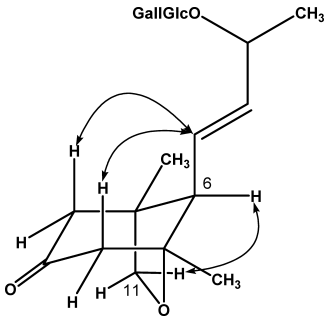

Fig. 2. Important NOE Correlations in Phase Sensitive NOESY Spectrum of **3**

Table 2. ^{1}H-NMR Data for Compounds **1**—**6** (CD_3OD, 400 MHz)

H	Mallophenol B (**1**)	**2**	**3**	**4**	**5**	Lauroside E (**6**)
2	2.11 (1H, d, 17.1)	2.03 (1H, d, 18.1)	2.15 (1H, d, 17.4)	2.05 (1H, d, 16.7)	2.10 (1H, d, 16.8)	2.01 (1H, d, 17.6)
	2.41 (1H, d, 17.1)	2.48 (1H, d, 18.1)	2.47 (1H, d, 17.4)	2.41 (1H, d, 16.7)	2.54 (1H, d, 16.8)	2.56 (1H, d, 17.6)
4	5.83 (1H, s)	5.75 (1H, s)	2.17 (1H, d, 17.0)	2.09 (1H, d, 17.2)	6.15 (1H, s)	6.05 (1H, s)
			2.45 (1H, d, 17.0)	2.40 (1H, d, 17.2)		
6			2.21 (1H, d, 10.8)	1.60 (1H, *t*-like)	2.70 (1H, d, 9.3)	1.98 (1H, m)
7	5.81 (1H, m)[b]	1.68 (1H, m)[a]	5.70 (1H, dd, 15.2, 9.7)	1.96 (2H, m)	5.77 (1H, dd, 15.4, 9.3)	1.51 (2H, m)
		2.04 (1H, m)[a]				
8	5.81 (1H, m)[b]	1.44 (1H, m)[a]	5.89 (1H, dd, 15.2, 6.6)	1.66 (2H, m)	5.57 (1H, dd, 15.4, 7.3)	1.65 (2H, m)
		1.70 (1H, m)[a]				
9	4.39 (1H, m)[a]	3.72 (1H, m)[a]	4.36 (1H, m)[a]	3.84 (1H, *q*-like)	4.45 (1H, qd, 6.6, 7.3)	3.89 (1H, m)
10	1.29 (3H, d, 6.3)	1.17 (3H, d, 5.1)	1.31 (3H, d, 6.4)	1.21 (3H, d, 6.2)	1.28 (3H, d, 6.6)	1.19 (3H, d, 6.2)
11	0.99 (3H, s)[c]	0.96 (3H, s)[c]	3.46 (1H)[a,b]	3.41 (1H)[a,b]	1.03 (3H, s)[c]	1.02 (3H, s)[c]
			3.58 (1H)[a,b]	3.46 (1H)[a,b]		
12	0.95 (3H, s)[c]	0.95 (3H, s)[c]	0.91 (3H, s)	0.96 (3H, s)	1.00 (3H, s)[c]	1.11 (3H, s)[c]
13	1.85 (3H, d, 1.5)	1.96 (3H, s)	1.12 (3H, s)	1.18 (3H, s)	4.13 (1H, dd, 17.8, 1.7)	4.19 (1H, dd, 15.6, 2.0)
					4.23 (1H, dd, 17.8, 1.5)	4.36 (1H, dd, 15.6, 2.0)
1′	4.42 (1H, d, 7.8)	4.33 (1H, d, 7.7)	4.40 (1H, d, 7.7)	4.37 (1H, d, 7.9)	4.28 (1H, d, 7.8)	4.34 (1H, d, 8.0)
2′	3.53 (1H)[a,b]	3.52 (1H)[a,b]	3.51 (1H)[a,b]	3.57 (1H)[a,b]	3.19 (1H)[a,b]	3.15 (1H, dd, 8.0, 8.8)
3′	3.42 (1H)[a,b]	3.39 (1H)[a,b]	3.39 (1H)[a,b]	3.40 (1H)[a,b]	3.31 (1H)[a,b]	3.25—3.35[b]
4′	3.44 (1H)[a,b]	3.42 (1H)[a,b]	3.38 (1H)[a,b]	3.39 (1H)[a,b]	3.24 (1H)[a,b]	3.25—3.35[b]
5′	3.23 (1H)[a,b]	3.17 (1H)[a,b]	3.23 (1H)[a,b]	3.20 (1H)[a,b]	3.16 (1H)[a,b]	3.25—3.35[b]
6′	4.36 (1H)[a,b]	4.37 (1H, dd, 11.7, 5.7)	4.32 (1H)[a,b]	4.35 (1H, dd, 11.9, 6.6)	3.63 (1H, dd, 11.7, 5.9)	3.67 (1H, dd, 12.0, 5.6)
	4.49 (1H, dd, 11.7, 2.0)	4.51 (1H, dd, 11.7, 1.8)	4.56 (1H, dd, 11.9, 2.2)	4.49 (1H, dd, 11.9, 2.2)	3.85 (1H, dd, 11.7, 2.4)	3.88 (1H, dd, 12.0, 2.0)
2″, 6″	7.10 (1H, s)	7.07 (1H, s)	7.08 (1H, s)	7.60 (1H, s)		

a) Determined by HSQC experiments; *b*) overlapped signals; *c*) interchangeable within the same column; *J* values in parentheses are recorded in Hz.

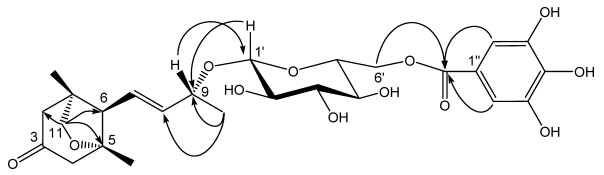

Fig. 3. Important C–H Long-Range Correlations in HMBC Spectrum of **3**
Arrowhead denote carbon atom and arrowtail hydrogen atoms.

as shown in Fig. 3. The remaining carbon signals were very similar to those of **1** and **2**, which indicated the existence of glucose and galloyl moieties in the same manner (Tables 1, 2). This assumption was confirmed by 1D and 2D-NMR analyses. In HMBC spectrum, δ_H 4.36 (H-9) correlated with the anomeric carbon signal at δ_C 103.0 (C-1′) and both δ_H 4.32 (H-6′a) and δ_H 4.56 (H-6′b) correlated with the ester carbonyl carbon signal at δ_C 168.3 (C-7″). Thus, the position of the glucopyranosyl and galloyl moieties was determined to be the same as compound **1** and **2**. ^{13}C chemical shift value of C-9 is diagnostic in assigning the absolute configuration at C-9 in ionol glucosides, *i.e.*, an upfield shift of C-9 is indicative for the (9*S*)-configuration whereas lower field signal for 9*R*. For example, ^{13}C-NMR chemical shifts of C-9 in (9*R*)- and (9*S*)-megastigma-4,7-dien-3-one-9-ol 9-*O*-*β*-D-glucopyranoside are δ_C 77.0 and 74.7, respectively.[8] Therefore, the absolute configuration of **3** at C-9 was determined to be 9*R* by the comparison of chemical shift value (δ_C 78.0 at C-9). On the other hand, the CD spectrum [$\Delta\varepsilon$ (nm) −1.72 (289), +6.22 (243)] of **3** was similar to that of spionoside B [$\Delta\varepsilon$ (nm) −2.3 (296), +2.3 (244)] that was assumed to be 6*S* configuration judging from the structure relationship to (+)-(*S*)-abscisic acid metabolites, phaseic acid, but not with direct comparison of CD data.[9] Thus, compound **3** have the same configuration at C-6 as spionoside B. However, we could not ambiguously determine the configuration at C-6. As a result, the structure of **3** was established to be (7*E*,9*R*)-megastigman-7-en-3-on-5,11-epoxy-9-ol 9-*O*-(6′-galloyl)-*β*-D-glucopyranoside.

Macarangioside C (**4**) was obtained as an amorphous powder and its elemental composition was determined to be $C_{26}H_{36}O_{12}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra indicated that macarangioside C (**4**) exhibited closely similar signals to **3** except for the absence of a double bond between C-7 and 8. This coincided with the fact that the elemental composition of **4** was two mass units larger than **3**. Thus, compound **4** was thought to be a 7,8-dihydro type of **3**. This was also confirmed by HMBC experiment that showed the presence of the long-range correlation between H-11 and C-5. The relative configuration of **4** was also confirmed by the observation of NOE correlation between H-6 and pro-*R* H-11 in the same manner as **3**. Thus, macarangioside C (**4**) was determined to possess 5,11-epoxymegastigmane type skeleton and the plane structure of aglycone moiety was identical to annuionone E isolated from *Helianthus annuus*.[10] The remaining carbon signals were also very similar to that of **1**—**3**, which indicated the existence of glucose and galloyl moieties in the same manner. This was also confirmed by HMBC spectrum, in which δ_H 3.84 (H-9) correlated with the anomeric carbon signal at δ_C 102.9 (C-1′) and both δ_H 4.35 (H-6′a) and δ_H 4.49 (H-6′b) correlated with the ester carbonyl carbon signal at δ_C 168.3 (C=O). Thus, compound **4** was determined to have 9-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranose moiety same as compounds **1**—**3**. The absolute configuration at C-9 was determined to be 9*R* by the comparison of chemical shift value (δ_C 76.7 at C-9) as described in compounds **2** and **3**. On the other hand, the absolute stereochemistry at C-6 could not be ambiguously determined because of the unavailability of appropriate reference data. However, the similar cotton curve of **4** [$\Delta\varepsilon$ (nm) −0.37 (266), +1.73 (221)] indicated that **4** also have the same configuration at C-6 as compound **3**. Thus, the structure of **4** was established to be (9*R*)-megastigman-3-on-5,11-epoxy-9-ol 9-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranoside.

Macarangioside D (**5**) was isolated as a colorless amorphous powder and its elemental composition was determined to be $C_{19}H_{30}O_8$ by HR-FAB-MS. An anomeric proton signal [δ_H 4.28 (1H, d, J=7.8 Hz)] and six carbon signals in the ^{13}C-NMR spectrum indicated the presence of *β*-glucopyranose moiety (Tables 1, 2). The remaining 13 carbon signals suggested that the structure of **5** was a megastigmane glucoside with two hydroxyls and one ketone functional groups. The UV absorption band at 237 nm suggested that the ketone functional group was in a conjugated system, such as between C-3 and C-5. Based on the detailed 2D-NMR spectroscopic analysis, the plane structure of the aglycone moiety was assigned as megastigman-4,7-dien-3-one-9,13-diol. Next, the position of glucosyl moiety was determined to be on the hydroxyl group at C-9 from the HMBC spectrum, in which δ_H 4.45 (H-9) correlated with the anomeric carbon signal at δ_C 101.4 (C-1′). The absolute stereochemistry of C-9 was determined to be 9*S* by comparison of the chemical shift values of the related compounds, which have same planer structure except for 13-OH.[8] As described above, the ^{13}C-NMR chemical shifts of C-9 in (9*R*)- and (9*S*)-megastigma-4,7-dien-3-on-9-ol 9-*O*-*β*-D-glucopyranoside to be δ_C 77.0 and 74.7, respectively.[8] Therefore, the absolute configuration at C-9 (δ_C 74.9) of **5** was assigned as 9*S*. Next, the stereochemistry of C-6 was determined to be 6*R* on the basis of CD spectral data. The observed CD spectrum, $\Delta\varepsilon$ (nm) −0.72 (318) and +17.5 (242), agreed well with that of glochidionionoside C [−1.06 (322) and +20.8 (244)], which has 6*R* configuration.[11] Thus, the aglycone moiety of **5** was determined to be the same as glochidionionol C. The chemical shift values of C-8, 9 and 10 of **5** also agree well with the *β*-D-glucopyranosylation-induced shift trend rule by using the reported value of glochidionionol C as a postulated aglycone of **5**, [($\Delta\delta$ C-8 (−3.1 ppm), C-9 (+6.1) and C-10 (−1.5)].[11] As a result, the structure of **5** was established to be (6*R*,7*E*,9*S*)-megastigma-4,7-dien-3-one-9,13-diol 9-*O*-*β*-D-glucopyranoside.

In this work, the absolute structure of glucose moiety of newly isolated compounds **2**—**5** were determined to be D-glucose same as **1** because of the isolation from the same plant. Actually, acid hydrolysis of major compound **5**, and subsequent derivatization with (*S*)-(−)-*α*-methylbenzylamine[4] also revealed the presence of D-glucose.

Other known compounds were identified as follows. Compound **6** was identified as lauroside E by comparison of the literature data, which had been originally isolated from the roots of *Hordeum vulgare*.[12,13] Recently, we obtained lauroside E from *Bridelia glauca* f. *balansa* and fully determined the absolute stereochemistry (submitted to elsewhere). Com-

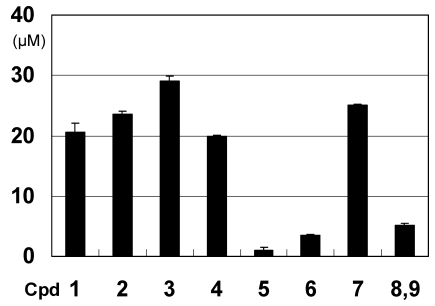

Fig. 4. DPPH Radical Scavenging Activity

The histogram indicated the activity of each compounds (**1**—**9**) as Trolox equivalent (μM). Error bar indicated S.D. (n=3).

pound **7** was identified with methyl brevifolin carboxylate which has previously been found in *Flueggea Microcarpa*.[14] The absolute stereochemistry at C-3′ has never been described previously, probably due to the easy racemization by this labile proton. Actually, compound **7** isolated in this work did not indicate the significant optical rotation (data not shown). Compounds **8** and **9** were identified as a mixture of two quercetin glycosides, hyperin and isoquercitrin, by spectroscopic analysis.[15]

The free radical scavenging activity of compounds **1**—**9** was next evaluated by its ability to quench the stable radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Trolox was used as a reference compound and the Trolox equivalent (μM) was determined for each compounds (Fig. 3). The structural difference between compounds **8** and **9** is the sugar moiety, glucose and galactose, respectively, and these compounds were known to have the same degree of radical scavenging activity.[16] Therefore, we used these compounds as a mixture without isolation. As a result, compounds **1**—**4** had more potent radical-scavenging activity than the well-known antioxidant flavonoids, quercetin glycosides **8** and **9**. On the other hand, compounds **5** and **6** could not eliminate DPPH radical. Considering the structural feature, the double bond between C-7 and C-8, 6-hydroxyl functional group, and 5,11-epoxy ring system were not relevant to the radical scavenging activity. These results clearly indicated that the radical scavenging activity of these galloylated megastigmane glucosides was depending on the galloyl moiety, but not megastigmane glucoside moiety.

Experimental

General Experimental Procedures A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel column chromatogaphy (CC) was performed on silica gel 60 (Merck, Darmstadt, Germany), and reversed-phase [octadecyl silica gel (ODS)] open CC (RPCC) on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=5 cm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 l)→(7 : 3, 1 l), fractions of 10 g being collected]. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 300 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–n-PrOH (9 : 12 : 8 : 2) were used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on ODS (Inertsil; GL Science, Tokyo, Japan; Φ=6 mm, L=250 mm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

Optical rotations were measured on a JASCO P-1030 polarimeter. IR spectra were measured on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/VIS 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 was recorded on a JEOL JMS SX-102 spectrometer. CD spectra were obtained on a JASCO J-720 spectropolarimeter. VERSA max (Molecular Device) was used as a microplate reader.

Plant Material Leaves of *M. tanarius* MÜLL.-ARG (Euphorbiaceae) were collected in Okinawa, Japan, in June, 2003, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima university (03-MT-OKINAWA-0630).

Extraction and Isolation The air-dried leaves of *M. tanarius* (12.1 kg) were extracted with MeOH (15 l) three times. The MeOH extract was concentrated to 3.0 l and then 150 ml of H_2O was added to make a 95% aqueous solution. This solution was defatted with 3.0 l of *n*-hexane and then the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 3.0 l of H_2O, and then extracted with 3.0 l each of EtOAc and *n*-BuOH successively, to afford 801 g and 374 g of EtOAc- and *n*-BuOH-soluble fractions, respectively. The *n*-hexane and the remaining H_2O layer were concentrated to furnish an *n*-hexane- and H_2O-soluble fractions, to afford 70.0 g and 499 g, respectively. A portion of the *n*-BuOH-soluble fraction (181 g) was subjected to highly porous synthetic resin (Diaion HP-20) CC (Mitsubishi Chemical Co., Ltd.; Φ=8 cm, L=40 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), 1 l fractions were collected. The residue (24.9 g in fractions 9—12) of the 40% MeOH eluate obtained on Diaion HP-20 CC was subjected to silica gel (Φ=6 cm, L=50 cm) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 l), and $CHCl_3$–MeOH (49 : 1, 3 l), (19 : 1, 3 l), (47 : 3, 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)], $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 3 l) and MeOH (3 l). 500 ml fractions were collected. The residue (9.1 g in fractions 32—36) of the 15% MeOH eluate obtained on silica gel CC was subsequently subjected to RPCC with gradient mixture of MeOH–H_2O (1 : 9, 1 l; 1 : 1, 1 l, then 1 : 1, 500 ml; 3 : 7, 500 ml). 10 g fractions were collected. The residue (0.95 g in fractions 114—127 of RPCC) was subjected to DCCC. The residues (209 mg in fractions 34—41, 48.2 mg in fractions 46—55 and 30.7 mg in fractions 80—100) were further subjected to HPLC (ODS) to give compounds **1** (46.0 mg) and **2** (3.7 mg) from the first residue, compounds **3** (3.2 mg), **4** (7.6 mg), **5** (11.2 mg) and **6** (2.2 mg) from the second, and compound **7** (18.0 mg) from the third residue. The residue (70.2 mg in fractions 147—156 of RPCC) was subjected to HPLC (ODS) to give the mixture of **8** and **9** (26.2 mg).

Mallophenol B (**1**): Amorphous powder; $[\alpha]_D^{25}$ +76.9° (c=1.36, MeOH); IR ν_{max} (film) cm^{-1}: 3368, 2973, 2939, 1698, 1650, 1448, 1345, 1236, 1035, 768; UV λ_{max} (MeOH) nm (log ε): 223 (4.2), 275 (3.8); ^{13}C-NMR (CD_3OD): Table 1; ^{1}H-NMR (CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): −0.86 (322), +12.6 (237), (c=3.70×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 537.2012 $[M-H]^-$ (Calcd for $C_{26}H_{33}O_{12}$: 537.1972).

Mild Alkaline Methanolysis of 1 A mixture of **1** (20.4 mg) and 0.1 M NaOMe in MeOH (2 ml) was allowed to stand at room temperature for 1.5 h under N_2 atmosphere. The reaction mixture was neutralized with Amberlite IR-120B (Organo) and chromatographed on silica gel CC (8 g, Φ=1 cm, L=17 cm) with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19 : 1, 100 ml, 9 : 1, 100 ml, 17 : 3, 100 ml, 4 : 1, 100 ml and 7 : 3, 100 ml), 10 ml fractions being collected. Degalloylated compound **1a** (7.3 mg) was recovered in fractions 34—40. **1a**: Amorphous powder; $[\alpha]_D^{27}$ +133.3° (c=0.49, MeOH); CD $\Delta\varepsilon$ (nm): −1.00 (321), +19.2 (241), (c=3.83×10^{-5} M, MeOH).

Acid Hydrolysis of 1a A soln of **1a** (3.2 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 1 h. After cooling, the reaction mixture was neutralized by passing through Amberlite IRA-96SB (Organo) and the residue was chromatographed on silica gel CC (6 g, Φ=10 mm, L=12 cm) by gradient mixture of $CHCl_3$–MeOH (20 : 1, 200 ml; 1 : 1, 200 ml) to give a sugar fraction. The sugar fraction was recovered in fractions 26—45 and was suggested to contain glucose by direct TLC comparison with authentic sample. Rf (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1): 0.33 (glucose). The sugar mixture was diluted with H_2O (0.2 ml) and treated with (*S*)-(−)-α-methylbenzylamine (2 mg) and $NaBH_3CN$ (2 mg) in EtOH (0.2 ml) at 40 °C for 4 h. To the reaction mixture was added 50 μl of acetic acid and evaporated. The oily residue was acetylated with Ac_2O–pyridine (1 : 1, 1 ml) at 100 °C for 1 h. H_2O (1 ml) was added, and the mixture was evaporated. The reaction mixture was dissolved in 1 ml of MeCN–H_2O (2 : 3) and passed through a Sep-Pak C18 cartridge (Waters) with MeCN–H_2O (2 : 3) (5 ml) to give a 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative of glucose, which were then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2 : 3); flow rate, 1.6 ml min^{-1}; detection, UV 230 nm. The derivative of sugar sample was detected at t_R (min): 26.0, which were

confirmed by co-chromatography with the derivative of authentic sample (D-glucose).

Macarangioside A (**2**): Amorphous powder; $[\alpha]_D^{27}$ −6.6° (c=0.27, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2968, 2930, 1670, 1649, 1452, 1341, 1228, 1075, 1038, 769; UV λ_{max} (MeOH) nm (log ε): 226 (4.4), 275 (4.2); ^{13}C-NMR (CD_3OD): Table 1; ^{1}H-NMR (CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): +1.87 (326), −13.8 (253), +18.1 (221) (c=3.70×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 539.2111 $[M-H]^-$ (Calcd for $C_{26}H_{35}O_{12}$: 539.2129).

Macarangioside B (**3**): Amorphous powder; $[\alpha]_D^{27}$ +12.1° (c=0.21, MeOH); IR ν_{max} (film) cm^{-1}: 3366, 2971, 2929, 2879, 1710, 1650, 1615, 1452, 1341, 1228, 1038, 769; UV λ_{max} (MeOH) nm (log ε): 226 (4.3), 277 (4.2); ^{13}C-NMR (CD_3OD): Table 1; ^{1}H-NMR (CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): −1.72 (289), +6.22 (243) (c=5.20×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 537.1969 $[M-H]^-$ (Calcd for $C_{26}H_{33}O_{12}$: 537.1972).

Macarangioside C (**4**): Amorphous powder; $[\alpha]_D^{27}$ −0.7° (c=0.45, MeOH); IR ν_{max} (film) cm^{-1}: 3361, 2968, 2932, 2877, 1712, 1701, 1650, 1615, 1455, 1341, 1228, 1079, 1038, 769; UV λ_{max} (MeOH) nm (log ε): 223 (4.1), 277 (3.8); ^{13}C-NMR (CD_3OD): Table 1; ^{1}H-NMR (CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): −0.37 (266), +1.73 (221) (c=5.41×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 539.2153 $[M-H]^-$ (Calcd for $C_{26}H_{35}O_{12}$: 539.2187).

Macarangioside D (**5**): Amorphous powder; $[\alpha]_D^{27}$ +62.0° (c=1.13, MeOH); IR ν_{max} (film) cm^{-1}: 3363, 2964, 2930, 2875, 1651, 1076, 1036; UV λ_{max} (MeOH) nm (log ε): 237 (4.0); ^{13}C-NMR (CD_3OD): Table 1; ^{1}H-NMR (CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): −0.72 (318), +17.5 (242) (c=4.59×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 385.1875 $[M-H]^-$ (Calcd for $C_{19}H_{29}O_8$: 385.1862).

Acid Hydrolysis of 5 Compound **5** (4.7 mg) was subjected to acid hydrolysis and subsequent derivatization as described for **1**. HPLC analysis revealed the presence of D-glucose.

Lauroside E (**6**): Amorphous powder; $[\alpha]_D^{20}$ +27.1° (c=0.70, MeOH); IR ν_{max} (film) cm^{-1}: 3366, 2963, 2928, 1713, 1656, 1374, 1253, 1077, 1035; UV λ_{max} (MeOH) nm (log ε): 235 (4.4); ^{13}C-NMR (CD_3OD): Table 1; ^{1}H-NMR (CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): +0.27 (329), −0.45 (272), +2.11 (222) (c=5.41×10^{-5} M, MeOH); HR-FAB-MS (negative-ion mode) m/z: 387.1998 $[M-H]^-$ (Calcd for $C_{19}H_{35}O_8$: 387.2019).

DPPH Radical Assay The reagents, (*S*)-(−)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Aldrich Chemical Co., and DPPH radical scavenging activities of isolated compounds were tested according to the standard method. In brief, reaction mixtures containing various concentrations of test compounds dissolved in MeOH and 250 mM DPPH solution in a 96-well microtiter plate were incubated at 37 °C for 30 min, and absorbance was measured at 515 nm. The activity is expressed as Trolox equivalents (μM), that is, the data was converted to activity in terms of μM of Trolox using standard curves for the reaction of Trolox with DPPH.

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