Four new hydrolyzable tannins and an acylated flavonol glycoside from *Euphorbia maculata*

Yoshiaki Amakura, Keita Kawada, Tsutomu Hatano, Isao Agata, Takeshi Sugaya, Sansei Nishibe, Takuo Okuda, and Takashi Yoshida

Abstract: The structure of a new acylated flavonol glycoside from the leaf extract of *Euphorbia maculata* was characterized as quercetin 3-*O*-(2″,3″-di-*O*-galloyl)-β-D-glucopyranoside. Four new hydrolyzable tannins together with twelve known tannins were also isolated and their structures were established by spectral and chemical means as 1,2,6-tri-*O*-galloyl-α-D-glucose, 1-*O*-galloyl-2,4;3,6-di-*O*-chebuloyl-β-D-glucose (eumaculin E), and dimers (eumaculins B and D) having a tergalloyl and a macaranoyl group as the linking unit of monomers, respectively.

Key words: *Euphorbia maculata*, Euphorbiaceae, tannin, eumaculin B, eumaculin E.

Résumé : On a déterminé la structure d'un nouveau glycoside acylé du flavonol extrait de la feuille d'*Euphorbia maculata*; il s'agit du 3-*O*-(2″,3″-di-*O*-galloyl)-β-D-glucopyranoside de quercétine. On a aussi isolé quatre nouveaux tannins hydrolysables ainsi que douze tannins connus et on a déterminé leurs structures à l'aide de méthodes tant spectrales que chimiques; il s'agit du 1,2,6-tri-*O*-galloyl-α-D-glucose, du 1-*O*-galloyl-2,4;3,6-di-*O*-chébuloyl-β-D-glucose (eumaculine E) et de dimères (eumaculines B et D) comportant respectivement un groupe tergalloyle et un groupe macaranoyle pour relier les unités de monomère.

Mots clés : *Euphorbia maculata*, Euphorbiaceae, tannin, eumaculine B, eumaculine E.

[Traduit par la rédaction]

Introduction

Many plants of the family Euphorbiaceae that grow in warm and temperate climates have been widely used as folk medicines in various areas of the world including East Africa, Southeast Asia, China, and Japan. The plants in this family, particularly *Euphorbia*, *Phyllanthus*, and *Macaranga* species, have been recognized to produce the ellagitannins with a vast structural diversity as represented by the geraniin metabolites (1–5). Amongst them, euphorbin A (**1**) and its analogous dimers possessing the geraniin unit as a constructing component were characterized as the abundant constituent of chemotaxonomical significance in many euphorbiaceous plants (5).

We previously examined the tannins of *Euphorbia maculata* L. (Euphorbiaceae), which has been used as a folk medicine for wound treatment in Mexico and China (6), and as an antidiarrheal in China (7), and isolated a new dimeric hydrolyzable tannin, eumaculin A (**11**), which contains the $^1C_4$ and $^4C_1$ glucopyranose cores linked through a valoneoyl group (8). Further investigation on the polyphenolic constituents in the leaf extract of the plant has revealed the presence of euphorbin A (**1**) and its analogs, euphorbins B (1) and D (9). In addition to these dimers together with eight known tannins, a new digalloylated flavonol glycoside (**2**) and four new hydrolyzable tannins (**3**, **4**, **9**, **10**) have also been isolated. This paper describes the structural elucidation of the new compounds.

Results and discussion

The aqueous acetone homogenate of dried leaves of *E. maculata* was extracted successively with ether, EtOAc, and *n*-BuOH. The EtOAc extract was chromatographed over Toyopearl HW-40 and MCI-gel CHP-20P to yield 17 polyphenolics including new compounds **2**–**4**, **9**, and **10**. Among them, 12 were identified as 1,3,6-tri-*O*-galloyl-β-D-glucose, 3,4,6-tri-*O*-galloyl-D-glucose, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (10), euphormisin M_3 (2), chebulanin (11), chebulagic acid (**8**) (12), tercatain (13), granatin B (14), excoecarianin (15), and euphorbins A (**1**) (16, 17), B (1, 16, 17), and D (9), by direct comparisons with authentic specimens or by comparisons of their physical data with those reported in the literature.

Compound **2** showed UV absorption bands characteristic of a flavonol derivative. The ^{1}H NMR spectrum of **2** indicated the presence of a quercetin moiety as revealed by 1,3,4-trisubstituted benzene proton signals at δ 7.78 (d, J = 2.5 Hz), 7.59 (d, J = 2.5, 8.5 Hz), and 6.94 (d, J = 8.5 Hz) and *meta*-coupled signals at δ 6.45 (d, J = 2 Hz) and 6.22 (d, J = 2 Hz). Besides these signals, two singlets attributable to two galloyl

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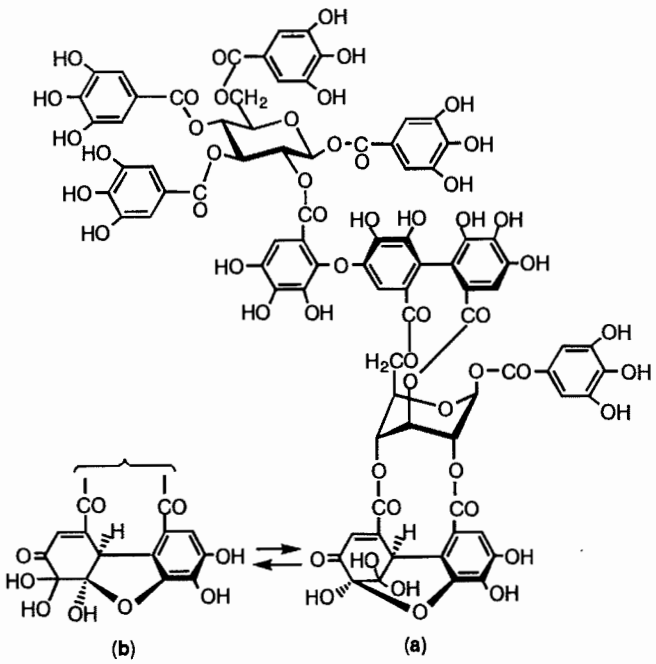
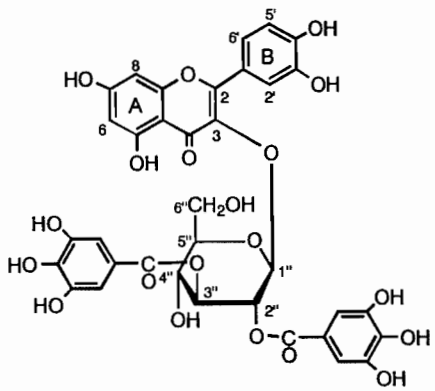
groups (δ 7.04, 7.10, each 2H) were observed in the aromatic region. The coupling patterns of the aliphatic proton signals, which were assigned with the aid of ^{1}H–^{1}H shift correlation spectroscopy (COSY), were consistent with those of $^4C_1$ glucopyranose. The ^{13}C NMR resonances of the aglycone moiety were also in agreement with those of isoquercitrin (18). These data coupled with the FABMS data (*m/z* 791 $(M+Na)^+$) indicated that **2** is a digallate of isoquercitrin. The galloyl groups in **2** were located at O-2 and O-3 of the glucose residue based on the remarkable downfield shifts of the H-2″ (δ 5.39) and H-3″ (δ 5.51) signals. Enzymatic hydrolysis of **2** with tannase yielded gallic acid, isoquercitrin, and quercetin 3-*O*-(2″-*O*-galloyl)-β-D-glucopyranoside (19). The compound **2** was thus characterized as quercetin 3-*O*-(2″,3″-di-*O*-galloyl)-β-D-glucopyranoside (**2**). Although various acylated flavonol glycosides are found widely in the plant kingdom (20), digallates such as **2** are quite rare, and to our knowledge compound **2** is the first example of a natural flavonol glycoside galloylated at O-3 of the glucose residue.

Compound **3** gave an $(M+Na)^+$ ion peak at *m/z* 659 corresponding to the molecular formula $C_{27}H_{24}O_{18}$ in the FABMS. Its ^{1}H NMR spectrum showed signals attributable to three galloyl groups (δ 7.16, 7.12, 7.06, each 2H singlet) and seven aliphatic protons characteristic of a $^4C_1$ glucopyranose residue. The glucose H-3 and H-4 signals were observed at higher field (δ 4.26, 3.83) than the other proton signals, indicating that the hydroxyl groups at C-3 and C-4 are not acylated, while the others are all acylated. The α-configuration of the galloyloxy group at the anomeric center was apparent from the small coupling constant (J = 3.5 Hz) of the H-1 signal. Compound **3** was thus characterized as 1,2,6-tri-*O*-galloyl-α-D-glucose[2] (**3**).

A new compound (**4**), which was designated as eumaculin E, showed an ion peak $(M+H)^+$ at *m/z* 973 in the FABMS. The ^{1}H NMR spectrum of **4** displayed a two-proton singlet at δ 7.22 due to a galloyl group, and signals assignable to two chebuloyl groups (12) (δ 2.18 (2H, m), 2.26 (dd, J = 3.5, 17 Hz), 2.18 (dd, J = 12, 17 Hz) (H-5); 3.87 (ddd, J = 1.5, 5, 11 Hz), 3.80 (ddd, J = 2, 3.5, 12 Hz) (H-4); 5.10 (dd, J = 1.5, 7 Hz), 4.94 (dd, J = 2,7 Hz), (H-3); 4.87 (d, J = 7 Hz), 4.82 (d, J = 7 Hz) (H-2); 7.52 (s) and 7.48 (s) (H-3′)). The presence of the chebuloyl groups in **4** was also substantiated by the ^{13}C NMR spectrum (δ 66.4 (2C, C-2), 39.9, 41.1 (C-3), 39.3, 39.6 (C-4), 30.5, 30.7 (C-5) and eight carbonyl carbon resonances), and by the formation of trimethyl tri-*O*-methylchebulate (**6**) (23) and methyl tri-*O*-methylgallate (**5**) on methanolysis of the methylated derivative of **4**. The coupling patterns of the sugar proton signals were characteristic of glucopyranose adopting a skew-boat conformation (24). The positions of the acyl groups in **4** were determined by HMBC measurement ($^{2,3}J_{C-H}$ = 8 Hz). The presence of the galloyl group at O-1 was indicated by correlation of the galloyl proton signal through three-bond coupling with an ester carbonyl carbon signal at δ 165.1 that also showed a cross peak with the anomeric proton. The signals at δ 7.52 and δ 7.48 (chebuloyl H-3′) showed correlations with the H-6 and H-2 signals through common ester carbonyl carbon signals at δ 167.5 and 165.6, respectively (Fig. 1), establishing the orientation of the chebuloyl groups at O-2/O-4 and O-3/O-6 of the glucose core. Although the stereochemistry of the chebuloyl moieties is apparent from the CD spectrum of **6**, which is identical with that of authentic specimens (23),



1



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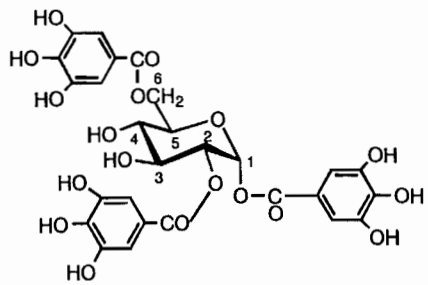
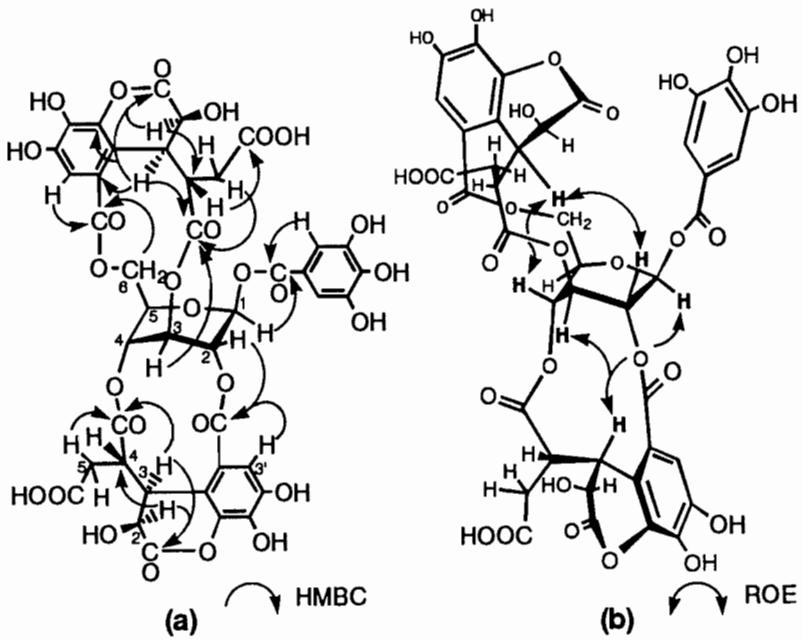


3

[2] Although the structure **3** was once assigned to a constituent of *Nuphar japonicum* (21), it was later revised to 1,2,4-tri-*O*-galloyl-α-D-glucose (22), and thus **3** is a new compound.

Fig. 1. (*a*) HMBC and (*b*) ROE correlations of **4**.



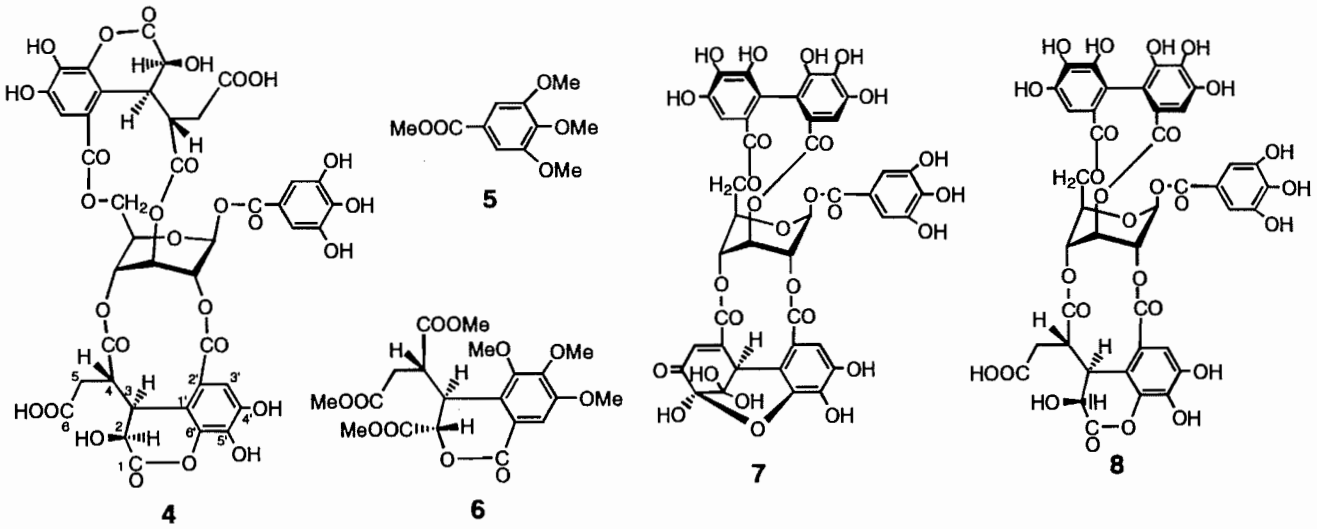
further evidence for the absolute configuration at C-3 of each chebuloyl group in **4** was provided by ROE's between the chebuloyl H-3 signals (δ 5.10, 4.94) and the glucose proton signals (H-1, H-3 and H-2, H-4, respectively), in rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY). On the basis of these results, the structure of eumaculin E was established as 1-*O*-galloyl-2,4;3,6-di-*O*-chebuloyl-β-D-glucopyranose (**4**). Tannin **4** might be regarded as a metabolite which can be produced via chebulagic acid (**8**) by structural modifications of geraniin (**7**) .

Compounds **9** and **10** were shown to be new dimers analogous to eumaculin A (**11**) as described below, and thus named eumaculins B and D, respectively. The molecular formula of eumaculin B (**9**), $C_{68}H_{52}O_{44}$, was deduced from an $(M+Na)^+$ ion peak at *m/z* 1595 in the FABMS. The ^{1}H NMR spectrum of **9** disclosed signals due to five galloyl groups (δ 7.17, 7.13, 7.11, 7.05, 7.02 (each 2H, s)), three aromatic protons (δ 6.98, 6.84, 6.48 (each 1H, s)), and two glucose cores (see Table 1). The coupling patterns of the glucose protons are typical for those of $^4C_1$ and $^1C_4$ glucopyranoses. The hydroxyl groups on the $^4C_1$ glucopyranose core are fully acylated as revealed by the chemical shifts of the H-1–H-4 and H-6 signals, whereas the hydroxyl groups at C-2′ and C-4′ in the $^1C_4$ glucose core were shown to be unacylated by the appearance of H-2′ (δ 4.12) and H-4′ (δ 4.60) in the high-field region. These NMR features of **9** were similar to those of eumaculin A (8), indicating that **9** is a dimer composed of corilagin and pentagalloylglucose units like **11**. A spectral difference between **9** and **11** includes a remarkable downfield shift of one of the aromatic 1H-singlets (δ 6.19 in **11** → δ 6.48 in **9**), which suggested that the linking unit of the monomers in eumaculin B (**9**) differs from that (valoneoyl group) of eumaculin A (**11**). Methylation of **9** with dimethyl sulphate and potassium carbonate in acetone and subsequent methanolysis yielded methyl tri-*O*-

Table 1. ^{1}H NMR spectral data for the glucose moieties of compounds **11**, **9**, and **10**[a] (500 MHz in acetone-d_6 + D_2O, *J* in Hz).

Proton	**11**	**9**	**10**
Glucose-I			
H-1	6.27 d	6.28 d	5.89 d
	(*J* = 8)	(*J* = 8.5)	(*J* = 8.5)
H-2	5.69 dd	5.73 dd	5.69 dd
	(*J* = 8, 9.5)	(*J* = 8.5, 10)	(*J* = 8.5, 9)
H-3	5.56 m[b]	6.05 t	5.93 t
		(*J* = 10)	(*J* = 9)
H-4	5.56 m[b]	5.67 t	5.53 t,
		(*J* = 10)	(*J* = 9)
H-5	4.40 m	4.55 m[c]	4.68 m
H-6	4.54 dd	4.55 m[c]	4.51 dd
	(*J* = 2, 12.5)		(*J* = 1.5, 12.5)
	4.27 dd	4.37 dd	4.36 dd
	(*J* = 5, 12.5)	(*J* = 4.5, 13)	(*J* = 3, 12.5)
Glucose-II			
H-1′	6.33 d	6.39 br s	6.42 br s
	(*J* = 2)		
H-2′	4.07 br s	4.12 br s	4.32 br s
H-3′	4.81 br s	4.84 br s	5.09 br s
H-4′	4.38 br s	4.60 br s	4.85 br s
H-5′	4.43 t-like	4.55 m[c]	4.41 m
	(*J* = 9.5)		
H-6′	4.72 t	4.92 t	4.90 t
	(*J* = 11)	(*J* = 11)	(*J* = 10)
	4.07 dd	4.15 dd	4.24 dd
	(*J* = 8, 11)	(*J* = 8.5, 11)	(*J* = 8, 10)

[a]Measured in CD_3OD.
[b,c]Overlapped with each other.

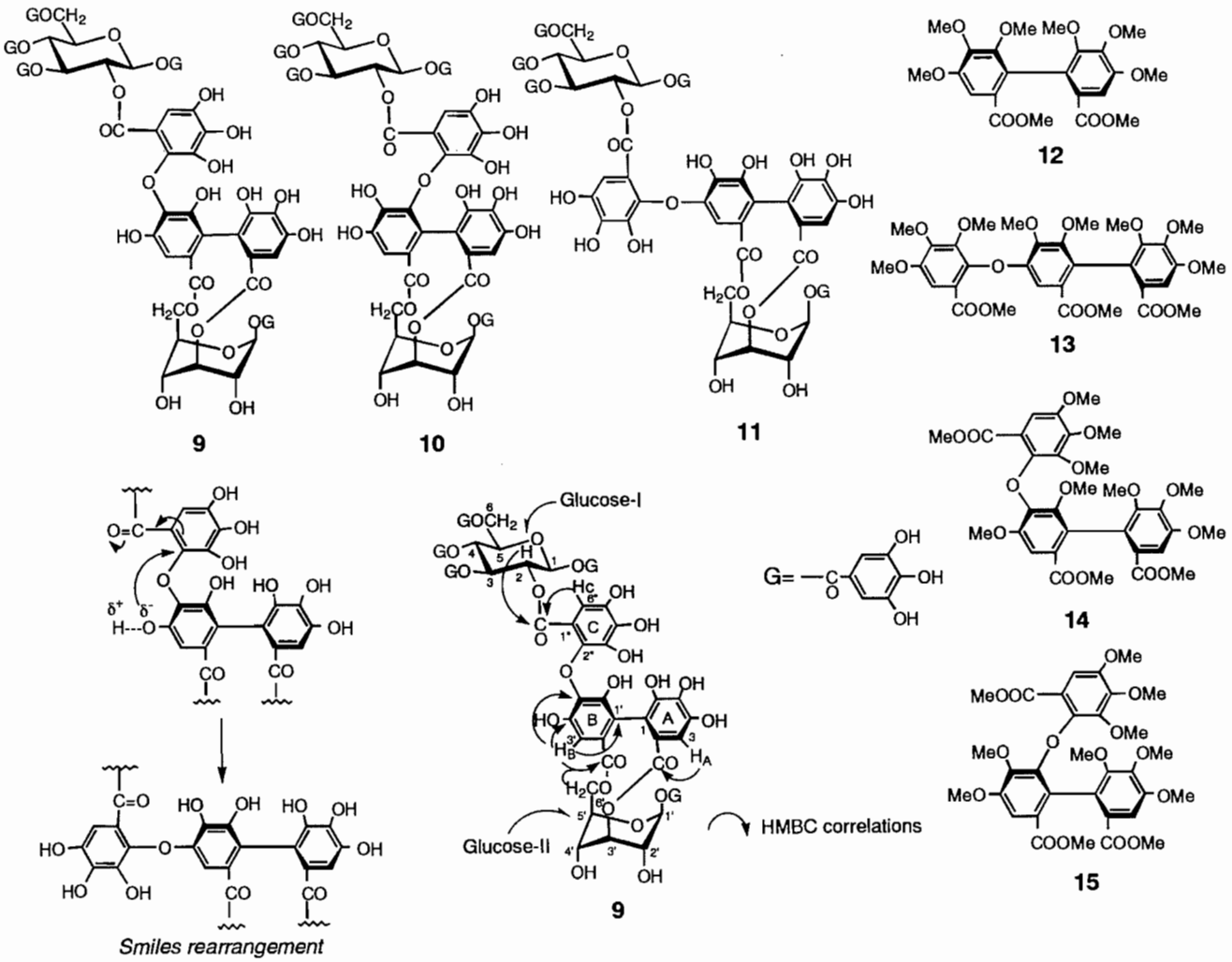


methylgallate (**5**) and trimethyl octa-*O*-methyltergallate (**14**) (1, 25), as major products. The linking unit of the monomers in eumaculin B (**9**) was thus confirmed to be a tergalloyl group. Dimethyl hexamethoxydiphenate (**12**) and trimethyl octa-*O*-methylvaloneate (**13**) were also obtained as minor products in the above reaction. These two products were regarded as secondary products derived from the tergalloyl moiety in **9** by cleavage of the biphenyl ether bond and Smiles-type rearrangement, respectively (1). The HMBC ($^{2,3}J_{C-H}$ = 8 Hz) of **9** showed a cross peak by three-bond coupling between the glucose H-6′ signal (δ 4.92 (t, J = 11 Hz)) and the ester carbonyl carbon resonance at δ 168.3, which, in turn, correlated with the aromatic proton signal at δ 6.48. This aromatic proton signal was assigned to H_B of the tergalloyl group based on its correlation through two-bond coupling with the signal attributed to C-4′ (δ 149.1) of the tergalloyl B-ring (4), and also through three-bond coupling with C-1′ (δ 115.3). The connectivity between the tergalloyl B-ring and C-6′ of the glucose core-II was thus established. The structure **9** thus proposed for eumaculin B was substantiated by chemical transformation of eumaculin B (**9**) into eumaculin A (**11**) through Smiles-type rearrangement (1, 25). Namely, an aqueous solution of **9** containing a small amount of phosphate buffer (pH 7.4) was left at room temperature for 5 h to give an isomerized product identical with **11**. Based on these findings, the structure of eumaculin B including the orientation and absolute configuration of the tergalloyl group at O-3/O-6 of glucose was represented by the formula **9**.

Eumaculin D (**10**) showed an ion peak at *m/z* 1595 ascribable to $(M+Na)^+$ in the FABMS, and gave a large retention volume on normal-phase HPLC, like **11** and **9**, suggesting it to be a dimeric hydrolyzable tannin (5). The ^{1}H NMR spectrum of **10** measured in acetone-d_6 and D_2O at ambient temperature showed duplicate signals for each proton, probably due to the presence of rotamers resulting from restricted rotation about the ether linkage of a macaranoyl group. The spectrum of **10** measured in MeOH-d_4, which was simplified by enhanced ratio of one rotamer, was similar to those of **11** and **9**, particularly in the chemical shifts and coupling patterns of the sugar moieties. A significant difference in the ^{1}H NMR spectra among **10**, **9**, and **11** was a downfield shift of one of the aromatic 1H singlets in **10** (δ 6.19 in **11**; δ 6.48 in **9** → δ 6.67 in **10**), which suggested that the linking unit of two monomers in **10** is different from that in both **11** and **9**. Methylation of eumaculin D (**10**) with dimethyl sulphate and potassium carbonate in acetone, followed by methanolysis with sodium methoxide, yielded methyl tri-*O*-methylgallate (**5**) and trimethyl octa-*O*-methylmacaranate (**15**) (1), as major products, accompanied by the minor products **12**, **13**, and **14**. The latter three were regarded as secondary products formed from the macaranoyl group in a similar manner to that from the tergalloyl group in **9**. The position and orientation of the macaranoyl group were based on the HMBC measurement ($^{2,3}J_{C-H}$ = 8 Hz) of **10**, which revealed the connectivity between glucose H-6 and the macaranoyl H_B signal through three-bond couplings with an ester carbonyl carbon signal at δ 170.1. The assignment of the macaranoyl H_B signal was based on two- and three-bond couplings with the macaranoyl C-4′ and C-1′ signals. Eumaculin D (**10**) was thus concluded to be an isomer of **11** and **9** differing only in the structure of the linking unit of the monomeric constituents. The structure **10** for eumaculin D was finally substantiated by chemical correlation between **10** and **11**. The isomerized product obtained by the Smiles-type rearrangement upon treatment of **10** with phosphate buffer (pH 7.4) was identical in all respects with eumaculin A (**11**).

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 instrument at 500 MHz and 126 MHz, respectively, and chemical shifts are given in δ values (ppm), based on the ^{1}H and ^{13}C signals of solvents (acetone-d_6 (δ_H 2.04; δ_C 29.8) or CD_3OD (δ_H 3.30; δ_C 49.0)) on a TMS scale. EI and FAB mass spectra were measured on a JEOL GMS HX-100 or a VG 70-SE mass spectrometer. ESI mass spectra were carried out with a Micromass AutoSpec OA-Tof mass spectrometer (solvent: MeOH-H_2O (1:1) + 0.1% $AcONH_4$, flow rate: 20 μL/min). Optical rotations were obtained on a JASCO DIP-1000 digital polarimeter, CD spectra on a JASCO J-720W spectropolarimeter, and UV spectra on a Shimadzu UV-180 spectrophotometer. Normal-phase HPLC was carried out with a Shimadzu LC-6A using a YMC-Pack SILA-003 column (4.6 × 250 mm) devel-



oped with *n*-hexane–MeOH–THF – formic acid (60:45:15:1, v/v) containing oxalic acid (500 mg/1.2 L) (flow rate, 1.5 mL/min; detection 280 nm) at room temperature. Reversed-phase HPLC was performed on a YMC-Pack A312 (ODS) column (4.6 × 150 mm) developed with 0.01 M H_3PO_4 – 0.01 M KH_2PO_4 – CH_3CN (41:41:18, v/v) (flow rate, 1.0 mL/min; detection 280 nm) at 40°C. TLC was conducted on Kieselgel 60 PF_{254} (Merck) plates with *n*-hexane–$CHCl_3$–acetone (4:6:1). Column chromatography was performed on Toyopearl HW-40 (coarse and fine grades, Tosoh), Sephadex LH-20 (Pharmacia Fine Chemicals), Dia-ion HP-20, and MCI-gel CHP-20P (Mitsubishi Kasei Industry).

Plant material

The leaves of *E. maculata* L. were collected at the campus of Okayama University in summer 1992 and a voucher specimen has been deposited at the Herbal Garden of the Faculty of Pharmaceutical Sciences, Okayama University.

Extraction and isolation

The dried leaves (2.0 kg) were homogenized (× 3) in acetone–H_2O (7: 3) (12 L × 3) and the homogenate was filtered. The filtrate was concentrated and further extracted with Et_2O (3 L × 3), EtOAc (3 L × 6), and *n*-BuOH saturated with H_2O (3 L × 6). A part (6.0 g) of the EtOAc extract (124.5 g) was chromatographed over Toyopearl HW-40 (fine) (2.2 cm i.d. × 35 cm) with MeOH–H_2O (5:5 → 6:4 → 7:3) → MeOH–H_2O–acetone (7:2:1 → 6:2:2) → acetone–H_2O (7:3) in a stepwise gradient mode. The fractions showing similar HPLC patterns were combined and further purified by rechromatography over Sephadex LH-20 with EtOH and (or) MCI-gel CHP-20P with aqueous MeOH to afford 3,4,6-tri-*O*-galloyl-D-glucose (1.7 mg), 1,3,6-tri-*O*-galloyl-β-D-glucose, euphormisin M_3 (1.3 mg), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (8 mg), chebulanin (16 mg), chebulagic acid (**8**) (3 mg), tercatain (5 mg), granatin B (4 mg), excoecarianin (14 mg), euphorbin A (**1**) (53 mg), euphorbin B (5 mg), euphorbin D (99 mg), quercetin 3-*O*-(2″,3″-di-*O*-galloyl)-β-D-glucopyranoside (**2**) (7.6 mg), 1,2,6-tri-*O*-gallyl-α-D-glucose (**3**) (2 mg), eumaculin E (**4**) (4.8 mg), eumaculin B (**9**) (30 mg), and eumaculin D (**10**) (45 mg). To obtain additional crops of the new tannins, the other part (10 g) of the EtOAc extract was similarly fractionated by repeated column chromatographies over Toyopearl HW-40 (fine), Sephadex LH-20, and MCI-gel CHP-20P to give eumaculin E (**4**) (25 mg), eumaculin B (**9**) (50 mg), and eumaculin D (**10**) (55 mg).

Quercetin 3-O-(2″,3″-di-O-galloyl)-β-D-glucopyranoside (**2**):

yellow amorphous powder; $[\alpha]_D$ −18 (*c* 1.0, MeOH); UV λ_{max} MeOH nm (log ε): 215 (5.75), 254sh (5.31), 265 (5.43), 292sh (5.39), 357 (5.39); ^{1}H NMR (500 MHz, acetone-d_6 + D_2O) δ: 7.10, 7.04 (each 2H, s, galloyl), 7.78 (1H, d, *J* = 2.5, B-ring H-2′), 7.59 (1H, dd, *J* = 2.5, 8.5 Hz, B-ring H-6′), 6.94 (1H, d, *J* = 8.5 Hz, B-ring H-5′), 6.45 (1H, d, *J* = 2 Hz, A-ring H-8), 6.22 (1H, d, *J* = 2 Hz, A-ring H-6), 6.04 (1H, d, *J* = 8 Hz, Glc H-1″), 5.39 (1H, dd, *J* = 8, 10 Hz, Glc H-2″), 5.51 (1H, t, *J* = 10 Hz, Glc H-3″), 3.90 (1H, t, *J* = 10 Hz, Glc H-4″), 3.60 (1H, ddd, *J* = 2.5, 5, 10 Hz, Glc H-5″), 3.78 (1H, dd, *J* = 2.5, 12 Hz, Glc H-6″), 3.70 (1H, dd, *J* = 5, 12 Hz, Glc H-6″); ^{13}C NMR (126 MHz, acetone-d_6 + D_2O) δ: 157.7 (C-2), 134.2 (C-3), 178.4 (C-4), 162.6 (C-5), 99.4 (C-6), 166.7 (C-7), 94.4 (C-8), 157.8 (C-9), 105.4 (C-10), 122.8 (C-1′), 115.8 (C-2′), 145.4 (C-3′), 149.1 (C-4′), 117.1 (C-5′), 122.7 (C-6′) (quercetin moiety), 121.2, 121.0 (galloyl C-1), 110.2, 110.0 (each 2C, galloyl C-2,6), 145.8, 145.9 (each 2C, galloyl C-3,5), 138.9, 139.0 (galloyl C-4), 165.1, 166.3 (galloyl C-7), 99.8 (Glc C-1″), 73.3 (Glc C-2″), 76.4 (Glc C-3″), 69.2 (Glc C-4″), 78.0 (Glc C-5″), 61.7 (Glc C-6″). FABMS: *m/z* 791 $(M+Na)^+$; HRESIMS: *m/z* 769.1223 (calcd. for $C_{35}H_{29}O_{20}$: 769.1252 $(M+H)^+$).

Enzymatic hydrolysis of ***2***

An aqueous solution of **2** (5 mg/ 3 mL) was incubated at 37°C with tannase that was prepared from *Aspergillus niger* (26), and the progress of the reaction was monitored by HPLC. The formation of isoquercitrin and quercetin 3-*O*-(2″-*O*-galloyl)-β-D-glucopyranoside, together with gallic acid, was observed by the accompanying disappearance of the starting material, and these products were identified by co-chromatography on HPLC with authentic samples.

1,2,6-Tri-O-*galloyl*-α-D-glucose (**3**): off-white amorphous powder; $[\alpha]_D$ +31 (*c* 0.5, MeOH); UV λ_{max} MeOH nm (log ε): 218 (4.79), 277 (4.42); ^{1}H NMR (500 MHz, acetone-d_6 + D_2O) δ: 7.16, 7.12, 7.06 (each 2H, s, galloyl), 6.50 (1H, d, *J* = 3.5 Hz, Glc H-1), 5.02 (1H, dd, *J* = 3.5, 10 Hz, Glc H-2), 4.26 (1H, t, *J* = 10 Hz, Glc H-3), 3.83 (1H, t, *J* = 10 Hz, Glc H-4), 4.16 (1H, m, Glc H-5), 4.52 (1H, dd, *J* = 2, 12 Hz, Glc H-6), 4.12 (1H, dd, *J* = 4, 12 Hz, Glc H-6). FABMS: *m/z* 659 $(M+Na)^+$.

Eumaculin E ***(4)***: light-brown amorphous powder; $[\alpha]_D$ +5 (*c* 1.0, MeOH); UV λ_{max} MeOH nm (log ε): 220 (4.83), 278 (4.48); CD: $[\theta]_{216}$ +186 000, $[\theta]_{242}$ −62 300, $[\theta]_{267}$ +38 200, $[\theta]_{294}$ −66 700 (MeOH); ^{1}H NMR (500 MHz, acetone-d_6 + D_2O) δ: 7.22 (2H, s, galloyl), 7.52, 7.48 (each 1H, s, chebuloyl H-3′), 5.10 (1H, dd, *J* = 1.5, 7 Hz), 4.94 (1H, dd, *J* = 2, 7 Hz) (chebuloyl H-3), 4.87 (1H, d, *J* = 7 Hz), 4.82 (1H, d, *J* = 7 Hz) (chebuloyl H-2), 3.87 (1H, ddd, *J* = 1.5, 5, 11 Hz), 3.80 (1H, ddd, *J* = 2, 3.5, 12 Hz) (chebuloyl H-4), 2.18 (2H, m), 2.26 (1H, dd, *J* = 3.5, 17 Hz), 2.18 (1H, dd, *J* = 12, 17 Hz) (chebuloyl H-5), 6.38 (1H, d, *J* = 5 Hz, Glc H-1), 5.45 (1H, dd, *J* = 1.5, 5 Hz, Glc H-2), 5.65 (1H, dd, *J* = 1.5, 3.5 Hz, Glc H-3), 5.30 (1H, brd, *J* = 3.5 Hz, Glc H-4), 4.73 (1H, brt, *J* = 3.5 Hz, Glc H-5), 5.42 (1H, dd, *J* = 3.5, 13.5 Hz, Glc H-6), 4.21 (1H, brd, *J* = 13.5 Hz, Glc H-6); ^{13}C NMR (126 MHz, acetone-d_6 + D_2O) δ: 119.6 (galloyl C-1), 110.2 (2C, galloyl C-2, 6), 146.2 (2C, galloyl C-3, 5), 139.9 (galloyl C-4), 66.4 (2C, chebuloyl C-2), 39.9, 41.1 (chebuloyl C-3), 39.3, 39.6 (chebuloyl C-4), 30.5, 30.7 (chebuloyl C-5), 115.3, 115.8 (chebuloyl C-1′), 116.9, 117.1 (chebuloyl C-2′), 110.2 (2C, chebuloyl C-3′), 146.4, 146.5 (chebuloyl C-4′), 139.0, 139.7 (chebuloyl C-5′), 140.7, 141.1 (chebuloyl C-6′), 94.2 (Glc C-1), 74.7 (Glc C-2), 66.6 (Glc C-3), 69.7 (Glc C-4), 78.6 (Glc C-5), 65.7 (Glc C-6), 173.6, 173.1, 172.8, 172.7, 169.9, 169.6, 167.5, 165.6, 165.1 (ester carbonyl). Anal. calcd. for $C_{41}H_{32}O_{28}\cdot 6H_2O$: C 45.56, H 4.10; found: C 45.87, H, 4.19. FABMS: *m/z* 973 $(M+H)^+$.

Methylation of ***4*** *followed by methanolysis*

A mixture of **4** (8 mg), K_2CO_3 (150 mg), and $(CH_3)_2SO_4$ (0.02 mL) in acetone (2.5 mL) was stirred overnight at room temperature, and then heated at reflux for 2 h. After removal of the inorganic material by centrifugation, the resulting supernatant was evaporated to dryness. The residue was directly methanolyzed with 1% NaOMe in MeOH (1 mL), and subjected to preparative TLC (Kieselgel PF_{254}, toluene–acetone, 4:1) to give methyl tri-*O*-methylgallate (**5**) (2 mg; EIMS: *m/z* 226 $(M)^+$) and trimethyl tri-*O*-methylchebulate (**6**) (2.5 mg; CD (MeOH): $[\theta]_{210}$ −34 000, $[\theta]_{227}$ +31 000, $[\theta]_{244}$ −5000, $[\theta]_{271}$ +28 000 (MeOH); ^{1}H NMR (500 MHz, acetone-d_6) δ: 7.32 (1H, s), 5.35 (1H, d, *J* = 1 Hz), 3.85 (1H, dd *J* = 1, 9.5 Hz), 3.22 (1H, dt, *J* = 5.5, 9.5 Hz), 2.75 (1H, dd, *J* = 9.5, 17 Hz), 2.49 (1H, dd, *J* = 5.5, 17 Hz), 3.93, 3.91, 3.87, 3.68, 3.62, 3.50 (each 3H, s); EIMS: *m/z* 440 $(M)^+$), which were identical with those of authentic samples in all respects.

Eumaculin B ***(9)***: light-brown amorphous powder; $[\alpha]_D$ −50 (*c* 1.0, MeOH); UV λ_{max} MeOH nm (log ε): 220 (5.28), 278 (4.92); CD (MeOH): $[\theta]_{204}$ +99 000, $[\theta]_{217}$ −26 000, $[\theta]_{236}$ −64 000, $[\theta]_{263}$ +36 000, $[\theta]_{293}$ −56 000; ^{1}H NMR (500 MHz, acetone-d_6 + D_2O) δ: 7.17, 7.13, 7.11, 7.05, 7.02 (each 2H, s, galloyl), 6.92, 6.84, 6.48 (each 1H, s, tergalloyl), glucose protons see Table 1; ^{13}C NMR (126 MHz, acetone-d_6 + D_2O) δ: 93.2 (Glc C-1), 94.3 (Glc C-1′), 72.3 (Glc C-2), 69.0 (Glc C-2′) , 73.3 (Glc C-3), 71.1 (Glc C-3′), 69.3 (Glc C-4), 62.0 (Glc C-4′), 73.7 (Glc C-5), 75.3 (Glc C-5′), 62.7 (Glc C-6), 64.6 (Glc C-6′), 110.1, 110.3, 110.5 (each 2C), 109.8 (4C) (galloyl C-2,6), 119.5, 119.96, 120.02, 120.05, 121.0 (galloyl C-1), 139.0, 139.2, 139.37, 139.42 (2C) (galloyl C-4), 145.80, 145.82 (each 2C), 145.87, 145.9 (each 4C) (galloyl C-3,5), 165.2, 165.3, 165.74, 166.42, 166.45 (galloyl C-7), 108.3, 108.5, 110.1, 113.1, 115.3, 117.1, 124.7, 130.8, 136.6, 137.0, 140.0, 140.6, 140.7, 142.1, 144.7 (2C), 149.1, 149.4, 167.3, 167.8, 168.3 (tergalloyl-C). Anal. calcd. for $C_{68}H_{52}O_{44}\cdot 8H_2O$: C 47.56, H 3.99; found: C 47.65, H 4.12. FABMS: *m/z* 1595 $(M+Na)^+$.

Methylation of ***9*** *followed by methanolysis*

A mixture of **9** (5 mg), K_2CO_3 (100 mg), and $(CH_3)_2SO_4$ (0.01 mL) in acetone (2 mL) was stirred overnight at room temperature and then heated at reflux for 2.5 h. After removal of K_2CO_3 by centrifugation followed by evaporation of the solvent, the residue was directly subjected to methanolysis with 1% NaOMe in MeOH (1 mL) at room temperature overnight. After acidification with AcOH and evaporation of the solvent, the residue was submitted to preparative TLC (Kieselgel PF_{254}, *n*-hexane–$CHCl_3$–acetone, 3:7:1) to give **5** (0.9 mg), trimethyl octa-*O*-methyltergallate (**14**) (0.6 mg, EIMS *m/z* 660 $(M)^+$), dimethyl hexamethoxydiphenate (**12**) (0.2 mg), and trimethyl octa-*O*-methylvaloneate (**13**) (0.1 mg).

Eumaculin D ***(10)***: off-white amorphous powder; $[\alpha]_D$ −6 (*c* 1.2, MeOH); UV λ_{max} MeOH nm (log ε): 218 (5.20),

278 (4.85); CD (MeOH): $[\theta]_{216}$ −110 000, $[\theta]_{241}$ −22 000, $[\theta]_{263}$ +89 000, $[\theta]_{290}$ −92 000, $[\theta]_{320}$ +19 000; ^{1}H NMR (500 MHz, MeOH-d_4) δ: 7.10, 7.07, 7.05, 6.94, 6.81 (each 2H, s, galloyl), 7.06, 7.01, 6.67 (each 1H, s, macaranoyl), glucose protons see Table 1; (500 MHz, acetone-d_6 + D_2O) δ: 5.90, 6.29 (each d, J = 8 Hz, Glc H-1), 5.73, 5.69 (each dd, J = 8, 9.5 Hz, Glc H-2), 6.01, 6.12 (each t, J = 9.5 Hz, Glc H-3), 5.57, 5.68 (each t, J = 9.5 Hz, Glc H-4), 4.55–4.64 (Glc H-5, 6), 4.27, 4.30 (each dd, J = 5, 13 Hz, Glc H-6), 6.43, 6.37 (each br s, Glc H-1′), 4.38, 4.13 (each br s, Glc H-2′), 5.16, 4.85 (each br s, Glc H-3′), 4.87, 4.44 (each br s, Glc H-4′), 4.51, 4.57 (each t, J = 11 Hz, Glc H-5′), 4.80, 4.82 (each t, J = 11 Hz, Glc H-6′), 4.22, 4.15 (each dd, J = 8.5, 11 Hz, Glc H-6′); ^{13}C NMR (126 MHz, MeOH-d_4) δ: 93.8 (Glc C-1), 95.1 (Glc C-1′), 72.0 (Glc C-2), 69.3 (Glc C-2′) , 74.5 (Glc C-3), 71.9 (Glc C-3′), 70.4 (Glc C-4), 62.1 (Glc C-4′), 73.0 (Glc C-5), 76.3 (Glc C-5′), 63.3 (Glc C-6), 65.2 (Glc C-6′), 110.4, 110.9, 111.0 (each 2C), 110.3 (4C) (galloyl C-2,6), 120.6 (2C), 120.7, 121.0, 121.1 (galloyl C-1), 139.0, 139.5, 139.9, 140.0, 140.3 (galloyl C-4), 146.0, 146.2 (each 2C), 146.31, 146.32 (each 4C) (galloyl C-3,5), 166.0, 166.7, 166.8, 166.9, 167.3 (galloyl C-7), 108.2, 110.0, 111.0, 113.4, 118.7, 119.1, 124.6, 125.6, 138.4, 138.6, 140.3, 140.4, 141.3, 142.1, 142.3, 143.2, 143.7, 146.8, 168.0, 168.4, 170.6 (macaranoyl-C). Anal. calcd. for $C_{68}H_{52}O_{44}\cdot 6H_2O$: C 48.58, H 3.83; found: C 48.60, H, 4.16. FABMS: *m/z* 1595 $(M+Na)^+$; HRESIMS: *m/z* 1590.2370 (calcd. for $C_{68}H_{56}NO_{44}$: 1590.2175 $(M+NH_4)^+$).

*Methylation of **10** followed by methanolysis*

A solution of **10** (10 mg), K_2CO_3 (200 mg), and $(CH_3)_2SO_4$ (0.02 mL) in acetone (3 mL) was stirred overnight at room temperature, and then heated at reflux for 4 h. After removal of the inorganic material by centrifugation, the supernatant was evaporated to dryness. The residue was directly methanolyzed with 1% NaOMe in MeOH (1 mL) at room temperature overnight. After acidification with AcOH and evaporation, the residue was subjected to preparative TLC (Kieselgel PF_{254}, *n*-hexane–$CHCl_3$–acetone, 3:7:1) to give **5** (2.2 mg), trimethyl octa-*O*-methylmacaranate (**15**) (1.0 mg, EIMS *m/z* 660 $(M)^+$), **12** (0.2 mg), **13** (0.5 mg), and **14** (0.3 mg), which were identical in all respects by direct comparison with authentic samples.

*Isomerization of **9** and **10** to **11***

A solution of **10** (3 mg) (or **9** (3 mg)) in 0.02 M phosphate buffer (KH_2PO_4–Na_2PO_4, pH 7.4) (2 mL) was left standing at room temperature and the progress of reaction was monitored by HPLC. After disappearance of the starting material (3 h) (or 5 h for eumaculin B), the reaction mixture was acidified with dilute HCl, and applied to a Sep-Pak cartridge (Waters). After washing with H_2O, the products were eluted with MeOH to yield **11** (2.8 mg from **10**; 2.5 mg from **9**), which was identical with an authentic sample by ^{1}H NMR spectral comparison.

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