

Tannins and Related Polyphenols of Euphorbiaceous Plants. XI.¹⁾ Three New Hydrolyzable Tannins and a Polyphenol Glucoside from *Euphorbia humifusa*

Takashi YOSHIDA,^{*,a} Yoshiaki AMAKURA,^a Yan-Ze LIU,^b and Takuo OKUDA^a

Faculty of Pharmaceutical Sciences, Okayama University,^a Tsushima, Okayama 700, Japan and Henan College of Traditional Chinese Medicine,^b Zhengzhou 450003, China. Received March 15, 1994; accepted May 9, 1994

Three new hydrolyzable tannins, euphormisins M₁, M₂ and M₃, were isolated from *Euphorbia humifusa* WILLD., and respectively characterized as 1,3,6-tri-*O*-galloyl-4-*O*-brevifolincarboxyl- β -D-glucose (19), an oxidative metabolite (23) of geraniin, and 1,3,6-tri-*O*-galloyl- α -D-glucose (18), by spectroscopic and chemical methods. A new ellagic acid glucoside (16) and fifteen known tannins, including geraniin (8) and four dimers [euphorbins A (13), B (14), excoecarianin (15) and eumaculin A (12)], were also isolated.

Keywords *Euphorbia humifusa*; Euphorbiaceae; tannin; euphormisin M₁; euphormisin M₂; euphormisin M₃

In the course of chemical studies on tannins of euphorbiaceous medicinal plants in Japan and China, we have reported the isolation of euphorbins A—E²⁾ and antidesmin A,¹⁾ unique dimeric hydrolyzable tannins having a geraniin unit as one of the constituent monomers, and we demonstrated the specific distribution of the dimers of this type in Euphorbiaceae.³⁾ Further examination of the tannins in this family may allow the use of these dimers as chemotaxonomical markers. In the present study on the tannins of *Euphorbia humifusa* WILLD., we have confirmed the occurrence of euphorbins A (13) and B (14). In addition to these dimers, three new tannins, named euphormisins M₁ (19), M₂ (23) and M₃ (18), and a new polyphenolic glucoside (16) together with thirteen known tannins, were also isolated. This paper describes the isolation and characterization of these tannins.

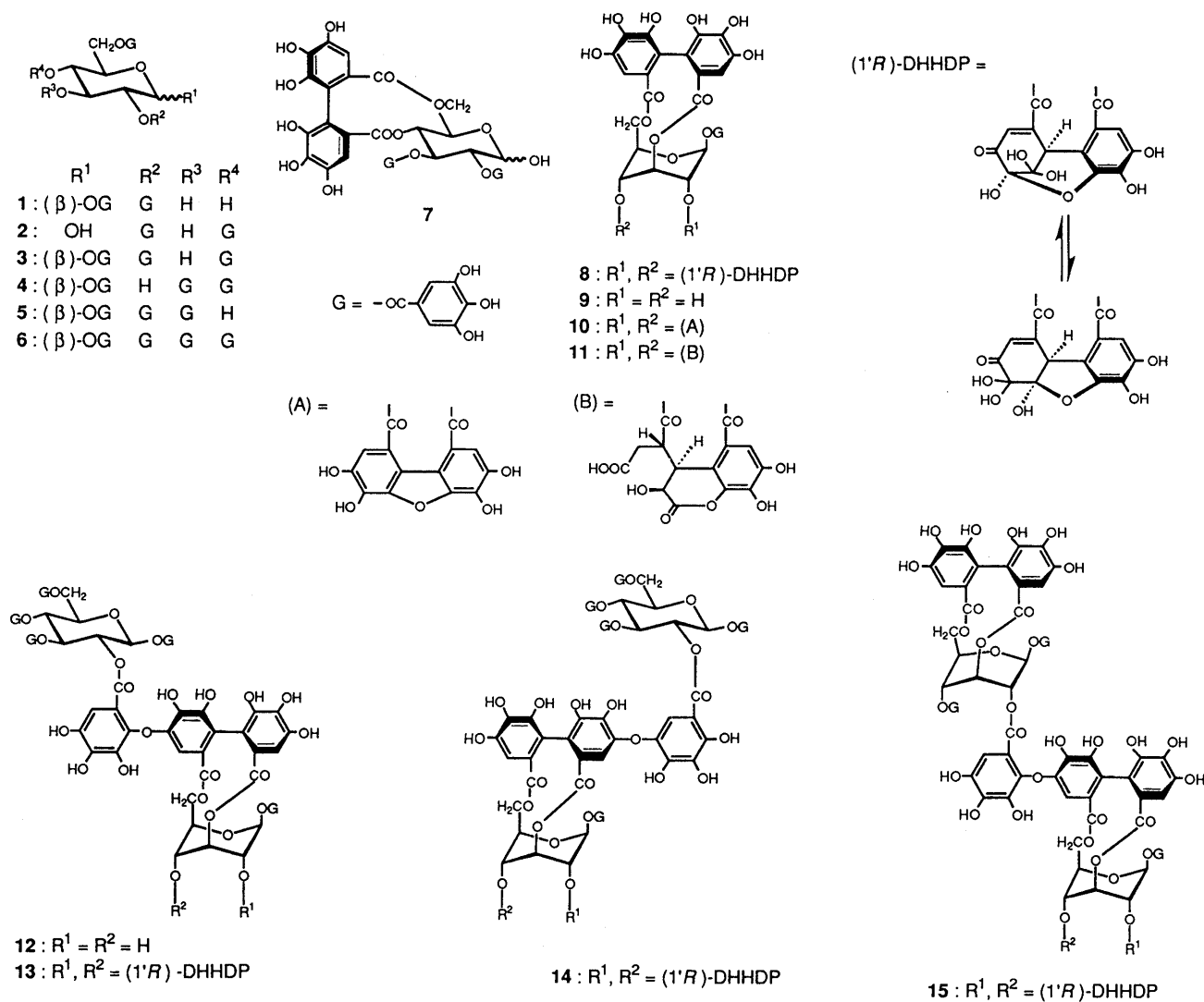
Dried aerial parts of *E. humifusa* collected in Henan, China were homogenized in aqueous acetone and filtered. The concentrated filtrate was extracted with ether, ethyl acetate and *n*-butanol, successively. The ethyl acetate extract was fractionated and purified by repeated column chromatography over Toyopearl HW-40 and MCI-gel CHP-20P to yield euphormisins M₁ (19), M₂ (23) and M₃ (18), and eleven known tannins which were identified as 1,2,6-tri-*O*-galloyl- β -D-glucose (1), 2,4,6-tri-*O*-galloyl-D-glucoses (2), 1,2,4,6- (3), 1,3,4,6-tetra-*O*-galloyl- β -D-glucoses (4), 1,2,3,4,6-penta-*O*-galloyl- β -D-glucose (6),⁴⁾ tellimagrandin I (7),⁵⁾ geraniin (8),⁶⁾ mallotusin (10),⁷⁾ eumaculin A (12),⁸⁾ and euphorbins A (13)^{2a,b)} and B (14).^{2a,b)} The *n*-butanol extract was similarly subjected to a combination of chromatographies to give a new polyphenolic glucoside (16) and three known hydrolyzable tannins, corilagin (9), chebulagic acid (11)⁹⁾ and excoecarianin (15).¹⁰⁾ Among them, the major tannin was geraniin (8).

The new polyphenolic glucoside (16) was obtained as a pale brown amorphous powder. Its UV spectrum showed absorption maxima at 220, 348 and 362 nm, which are characteristic of ellagic acid.¹¹⁾ In the ¹H-NMR spectrum of 16, a doublet (δ 5.12, J =6.6 Hz) and multiplets (6H, δ 3.45—4.05) ascribable to sugar protons were observed besides two aromatic singlets at δ 7.81 and 7.60 (each 1H), indicating that 16 is an ellagic acid glucoside. Methylation

of 16 followed by acid hydrolysis gave glucose and 3,3',4'-tri-*O*-methylellagic acid (17).^{11,12)} The glycosidic linkage is β , as shown by the coupling constant of the anomeric proton signal (δ 5.12). This glycoside was thus characterized as ellagic acid 4-*O*- β -D-glucopyranoside (16).

Euphormisin M₃ (18) was obtained as an off-white amorphous powder. Its ¹H-NMR spectrum showed the signals attributable to three galloyl groups (δ 7.11, 7.12 and 7.15, each two-proton singlet) and the aliphatic proton signals characteristic of a ⁴C₁ glucopyranose residue. The positions of the galloyl groups in 18 were straightforwardly assigned to O-1, O-4 and O-6 based on the chemical shifts of the H-1 (δ 6.12), H-4 (δ 5.50) and H-6 (δ 4.5 and 4.7) signals. The glycosidic linkage was shown to be α by the small coupling constant (J =3.5 Hz) of the H-1 signal. Thus, euphormisin M₃ was characterized as 1,4,6-tri-*O*-galloyl- α -D-glucose (18). Galloylglucoses, which are distributed widely in the plant kingdom, mostly have a β -glycosidic linkage, and those having an α -glucosidic linkage are quite rare.¹³⁾

Euphormisin M₁ (19) was obtained as a light brown amorphous powder, and showed the pseudomolecular ion peak (M+Na)⁺ at m/z 933 in the FAB-MS. The ¹H-NMR spectrum displayed three two-proton singlets at δ 7.11, 7.15 and 7.17 ascribable to three galloyl groups. The coupling pattern of the sugar proton signals which were assigned on the basis of the ¹H—¹H shift correlation spectroscopy (COSY) spectrum was consistent with that of ⁴C₁ glucopyranose. The H-2 signal resonated at higher field (δ 4.01) than the other glucose proton signals, indicating that the hydroxyl group at C-2 is not acylated, while the others are all acylated. Besides the glucose proton signals, ABX-type signals were observed at δ 4.60 (dd, J =2, 8 Hz), 2.84 (dd, J =8, 18.5 Hz), 2.29 (dd, J =2, 18.5 Hz). These signals along with an aromatic proton singlet at δ 7.38 suggested the presence of a brevifolin carboxyl group¹⁴⁾ in the molecule. The existence of this acyl group was also consistent with the ¹³C-NMR spectrum which shows the signals due to two aliphatic carbons (δ 37.8 and 41.5), three carbonyl carbons (δ 193.1, 172.5 and 161.0) and eight *sp*² carbons (δ 107.0, 114.1, 116.0, 139.0, 139.2, 144.5, 147.0, 150.0).¹⁴⁾ The structures of the acyl groups in euphormisin M₁ were chem-



that of **5**. Since an analogous upfield shift (0.22 ppm) was observed at the H-4 signal of **19** upon comparison between **19** and 1,3,4,6-tetra-*O*-galloyl- β -D-glucose (**4**)⁴⁾ (Table I), the brevifolincarboxyl group in **19** should be at O-4. Euphormisin M₁ was thus characterized as 1,3,6-tri-*O*-galloyl-4-*O*-brevifolincarboxyl- β -D-glucose (**19**).¹⁶⁾

Euphormisin M₂ (**23**) was obtained as a light brown amorphous powder, and gave the ion peak at m/z 947 attributable to the (M+Na)⁺ ion, which suggested its molecular formula to be C₄₀H₃₄O₂₉. The ¹H-NMR

spectrum of **23** showed a two-proton singlet (δ 7.13) and two one-proton singlets (δ 7.02 and 6.65) attributable to galloyl and HHDP groups, and an additional one-proton singlet at δ 7.29 in the aromatic region. The sugar proton signals were similar to those of geraniin, in both the chemical shifts and coupling patterns, indicating the presence of the fully acylated glucopyranose ring with ¹C₄ conformation. ABXY-type signals (a methylene and two methine protons) were also observed at δ 2.12 (dd, $J=6.5$, 12 Hz), 1.91 (dd, $J=3$, 12 Hz), 4.95 (d, $J=3$ Hz) and 3.62 (m). These spectral features are analogous to those of phyllanthusiins¹⁴⁾ which are oxidative metabolites of geraniin (**8**). Euphormisin M₂ gave corilagin (**9**)⁶⁾ in hot water, confirming the positions of the galloyl and HHDP groups at O-1 and O-3/O-6 of the glucose core.

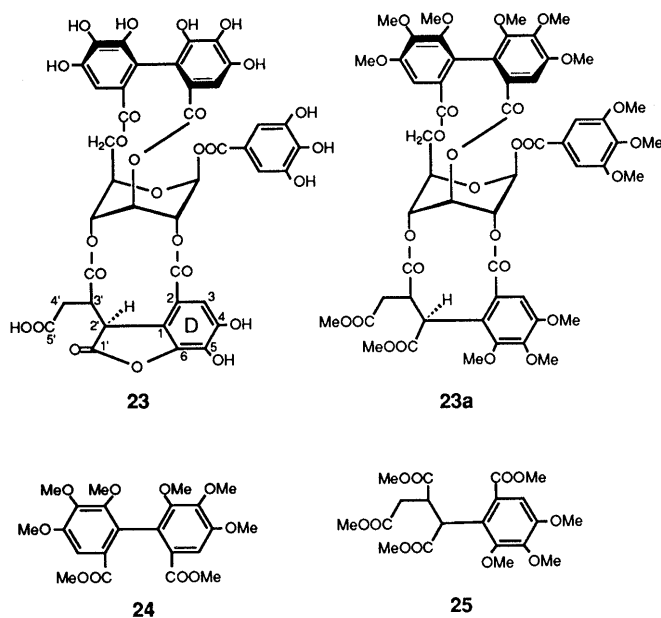


Chart 4

TABLE I. ¹H-NMR Data for the Glucose Moieties of **4**, **5**, **19**, and **20** (500 MHz, acetone-*d*₆ + D₂O, J in Hz)

	5	20 ^{a)}	4	19
H-1	6.14 d ($J=8$)	6.10 d ($J=8$)	5.98 d ($J=8$)	5.98 d ($J=8$)
H-2	5.45 dd ($J=8, 10$)	5.24 t ($J=8$)	4.03 dd ($J=8, 10$)	4.00 dd ($J=8, 9.5$)
H-3	5.66 t ($J=10$)	5.56 t ($J=8$)	5.65 t ($J=10$)	5.61 t ($J=9.5$)
H-4	4.08 t ($J=10$)	4.05 br d ($J=8$)	5.46 t ($J=10$)	5.24 t ($J=9.5$)
H-5	4.14 dd ($J=4.5, 10$)	^{b)}	4.35 ddd ($J=2, 5, 10$)	4.32 m
H-6	4.61 dd ($J=4.5, 12.5$)	^{b)}	4.51 dd ($J=2, 12.5$)	4.77 d ($J=11$)
	4.52 d ($J=12.5$)		4.03 dd ($J=5, 12.5$)	4.32 m

a) Measured at 100 MHz. b) Not described in the ref. 15.

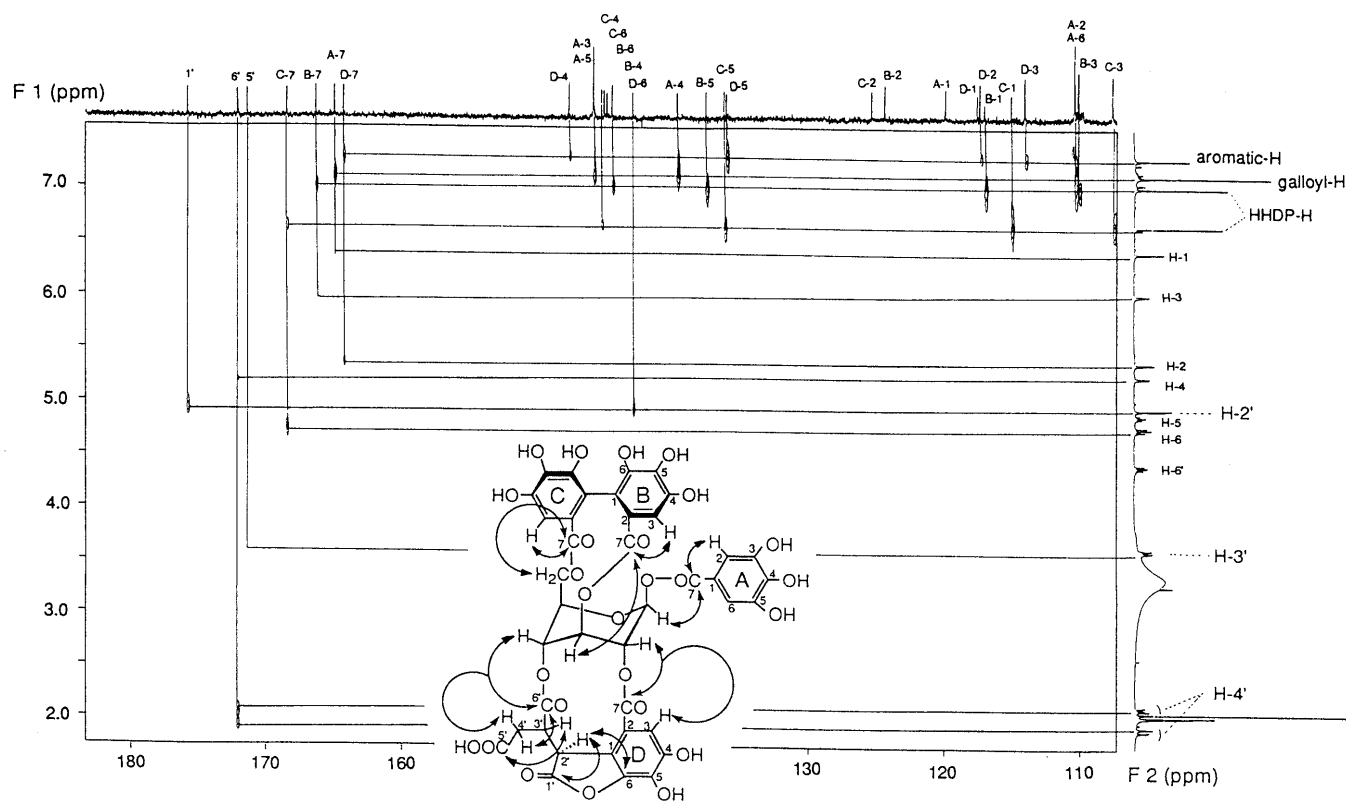


Fig. 1

The ABXY-type aliphatic protons and an aromatic proton (δ 7.29) are thus due to an acyl group at O-2/O-4. The carbon framework of this 2,4-acyl group was shown to consist of three aliphatic, six aromatic and four carboxyl carbons (δ 30.5, 45.0, 47.7, 114.2, 117.2, 117.7, 136.1, 143.0, 147.7, 164.3, 171.4, 172.1, 175.8) by subtracting the signals of the corilagin moiety from those in the ^{13}C -NMR spectrum of **23**. The presence of the D-ring in the 2,4-acyl group was demonstrated in the ^1H - ^{13}C long-range COSY spectrum of **23**, by the cross peaks of $\text{H}_{\text{D}-3}$ (δ 7.29) with the aromatic carbon resonances [δ 114.2 ($\text{C}_{\text{D}-3}$), 117.2 ($\text{C}_{\text{D}-2}$), 117.7 ($\text{C}_{\text{D}-1}$), 136.1 ($\text{C}_{\text{D}-5}$), 147.7 ($\text{C}_{\text{D}-4}$)] through two- and three-bond couplings. The connectivity of $\text{H}_{\text{D}-3}$ with H-2 of the glucose core was also shown by their three-bond couplings with the ester carbonyl carbon at δ 164.3 ($\text{C}_{\text{D}-7}$). The methine proton signal at δ 4.95 exhibited a cross peak with $\text{C}_{\text{D}-6}$ (δ 143.0), allowing its assignment to H-2'. Taking the presence of the ABXY (H-2'—H-4') system and the other long-range couplings shown in Fig. 1 into consideration, the structure of the acyl group at O-2/O-4 in euphormisin M_2 was deduced to be as shown in the formula **23**. The presence of the γ -lactone ring was supported by the IR absorption at 1800 cm^{-1} , characteristic of γ -lactone fused with a benzene ring.¹⁷⁾ The absolute configuration at C-2' was determined as *S* by rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY) which showed a clear nuclear Overhauser effect between H-2' and the anomeric proton signal of the glucose core.

Methylation of **23** with dimethyl sulfate in acetone gave a tetradecamethyl derivative (**23a**) [m/z 1139 ($\text{M} + \text{H}$)⁺], accompanied with cleavage of the lactone ring in the acyl group at O-2/O-4 during the reaction. Methanolysis of **23a** yielded methyl tri-*O*-methylgallate (**21**), dimethyl hexamethoxydiphenate (**24**), and a heptamethyl derivative (**25**). The mass and ^1H -NMR spectral data of the methylated new polyphenolic acid (**25**) (see Experimental) were consistent with structure **25**. The structure of euphormisin M_2 was thus represented by **23**, although the stereochemistry at C-3' is not yet determined.

Experimental

UV spectra were taken on a Hitachi 200-10, and optical rotations on a JASCO DIP-4 polarimeter. ^1H - and ^{13}C -NMR spectra were measured in acetone- d_6 - D_2O unless otherwise stated, on a Varian VXR-500 instrument (500 MHz for ^1H -NMR and 127 MHz for ^{13}C -NMR). Chemical shifts are given in δ values (ppm) relative to that of solvent [acetone- d_6 (δ_{H} 2.04; δ_{C} 29.8)] on a TMS scale. FAB-MS were recorded on a VG 70-SE mass spectrometer using 3-nitrobenzylalcohol as the matrix agent. Normal-phase HPLC was carried out on a Superspher SI60 (Merck) column (4 $\times$ 125 mm) developed with *n*-hexane–MeOH–THF–formic acid (55:33:11:1) containing oxalic acid (450 mg/l) (flow rate, 1.5 ml/min; detection 280 nm) at room temperature. Reversed-phase HPLC was performed on a LiChrospher RP-18 column (5 μm ; 4 $\times$ 250 mm) developed with 10 mM H_3PO_4 –10 mM KH_2PO_4 –MeCN (9:9:2) (flow rate, 1 ml/min; detection 280 nm) at 40 $^\circ\text{C}$. Analytical TLC and preparative TLC were conducted on Kieselgel PF₆₀ with toluene–acetone (4:1). Solvents were removed by evaporation under reduced pressure below 40 $^\circ\text{C}$.

Isolation of Tannins The dried leaves (2.8 kg) of *E. humifusa*, collected in Zhengzhou, China, were extracted with 70% aqueous acetone (151 $\times$ 3), and the concentrated solution (2 l) was extracted with ether (11 $\times$ 5), EtOAc (11 $\times$ 10) and *n*-BuOH saturated with H_2O (11 $\times$ 10), successively. A part (5 g) of the EtOAc extract (78 g) was chromatographed over Toyopearl HW-40 (coarse) (CC-1) (2.2 cm i.d. $\times$ 60 cm)

with aqueous MeOH (60% $\rightarrow$ 70% MeOH) $\rightarrow$ MeOH– H_2O –acetone (8:1:1 $\rightarrow$ 7:1:2) (CC-1). The fractions showing similar HPLC patterns were combined, and purified further by rechromatography over Toyopearl HW-40 (fine) and/or MCI-gel CHP-20P with aqueous MeOH to give 1,2,6-tri-*O*-galloyl- β -D-glucose (**1**) (5.4 mg), 2,4,6-tri-*O*-galloyl-D-glucose (**2**) (51 mg), 1,2,4,6-tetra-*O*-galloyl- β -D-glucose (**3**) (2.2 mg), tellimagrandin I (**7**) (3.4 mg), geraniin (**8**) (218 mg), eumaculin A (**12**) (2.2 mg), mallotusin (**10**) (2 mg), euphorbin A (**13**) (17 mg) and euphorbin B (**14**) (16 mg). The fractions containing new compounds (**19** and **23**) were combined and subjected to preparative HPLC as described later. The other part (40 g) of the EtOAc extract was similarly fractionated by column chromatography over Dia-ion HP-20 with H_2O – H_2O –MeOH (10% $\rightarrow$ 20% MeOH $\rightarrow$ 30% $\rightarrow$ 40% $\rightarrow$ 50% MeOH) in a stepwise gradient mode (CC-2). The 50% MeOH eluate was rechromatographed over Toyopearl HW-40 (coarse) with MeOH– H_2O (6:4 $\rightarrow$ 7:3) MeOH–acetone– H_2O (8:1:1 $\rightarrow$ 6:2:2) to give 1,3,4,6-tetra-*O*-galloyl- β -D-glucose (**4**) (14 mg), 1,2,3,4,6-penta-*O*-galloyl- β -D-glucose (**6**) (57 mg), and euphormisin M_1 (**19**) (1 mg) and M_2 (**23**) (12 mg). The 40% MeOH eluate from CC-2 was purified further by rechromatography over MCI-gel CHP-20P with aqueous MeOH to afford euphormisin M_3 (**18**) (15 mg). The fractions (214 mg) containing **19** and **23** were combined, and finally purified by preparative HPLC [YMC A312 (10 $\times$ 300 mm); solvent, 10 mM H_3PO_4 –10 mM KH_2PO_4 – CH_3CN (4:4:2)] to give euphormisins M_1 (**19**) (8.6 mg) and M_2 (**23**) (20 mg).

A part (30 g) of the *n*-BuOH extract (86 g) was similarly fractionated and purified by a combination of column chromatographies over Dia-ion HP-20 and MCI-gel CHP-20P to give ellagic acid 4-*O*- β -D-glucoside (**16**) (17 mg), corilagin (**9**) (13 mg), chebulagic acid (**11**) (42 mg) and excoecarianin (**15**) (17 mg).

Ellagic Acid 4-*O*- β -D-Glucopyranoside (16**)** A pale brown amorphous solid, $[\alpha]_{\text{D}} -5^\circ$ ($c=1.0$, acetone). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 220 (4.37), 256 (4.39), 290sh (4.08), 348 (3.75), 362 (3.76). ^1H -NMR δ : 7.81, 7.60 (each 1H, s, H-5, 5'), 5.12 [1H, d, $J=6.6$ Hz, glucose (Glc) H-1], 3.45–4.05 [Glc H-2–H-6].

Methylation of **16 Followed by Acid Hydrolysis** A solution of **16** (2 mg) in EtOH (1 ml) was methylated with an excess of CH_2N_2 – Et_2O at room temperature for 4 h. The residue obtained after evaporation was suspended in 5% H_2SO_4 (1 ml) and heated in a boiling-water bath for 2 h. The insoluble crystalline material was collected by centrifugation, washed with H_2O and identified as 3,3',4'-tri-*O*-methylgallate (**17**) by co-chromatography with an authentic sample on TLC. After neutralization of the aqueous supernatant followed by evaporation, the syrupy residue was trimethylsilylated and analyzed by GLC to detect glucose.

Euphormisin M_3 (18**)** A pale brown amorphous powder, $[\alpha]_{\text{D}} +2^\circ$ ($c=0.5$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 200 (4.72), 258 (4.39). FAB-MS m/z : 659 ($\text{M} + \text{Na}$)⁺. ^1H -NMR δ : 6.34 (1H, d, $J=3.5$ Hz, H-1), 3.86 (1H, dd, $J=3.5$, 10 Hz, H-2), 4.24 (1H, t, $J=10$ Hz, H-3), 5.34 (1H, t, $J=10$ Hz, H-4), 4.33 (1H, m, H-5), 4.46 (1H, dd, $J=2$, 12.5 Hz, H-6), 4.13 (1H, dd, $J=4$, 12.5 Hz, H-6), aromatic protons, see text. ^{13}C -NMR δ : 62.9 (Glc C-6), 71.2 (Glc C-5), 71.5 (Glc C-4), 72.2 (Glc C-2), 72.5 (Glc C-3), 92.9 (Glc C-1), 109.8, 110.1, 110.2 [each 2C, galloyl (Gal) C-2, 6], 120.9, 121.0, 121.3 (Gal C-1), 138.8, 139.0, 139.2 (Gal C-4), 145.9, 146.0, 146.1 (each 2C, Gal C-3, 5), 165.6, 166.2, 166.5 (Gal C-7).

Euphormisin M_1 (19**)** A pale brown amorphous powder, $[\alpha]_{\text{D}} -20^\circ$ ($c=1.0$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 215 (4.69), 278 (4.42). FAB-MS m/z : 933 ($\text{M} + \text{Na}$)⁺. ^1H -NMR δ : 7.38 [1H, s, brevifolinicboxyl (Brev) H-3'], 7.17, 7.15, 7.11 (each 2H, s, Gal), 4.60 (1H, dd, $J=2.0$, 8.0 Hz, Brev H-4), 2.84 (1H, dd, $J=8.0$, 18.5 Hz, Brev H-5a), 2.29 (1H, dd, $J=2.0$, 18.5 Hz, Brev H-5b), glucose-H, see Table I. ^{13}C -NMR δ : 95.2 (Glc C-1), 73.7 (Glc C-2), 75.7 (Glc C-3), 70.5 (Glc C-4), 71.9 (Glc C-5), 63.5 (Glc C-6), 120.2–121.0 (Gal C-1), 110.0–110.2 (Gal C-2, C-6), 145.3–145.8 (Gal C-3, C-5), 142.5–145.8 (Gal C-4), 165.6–167.5 (Gal C-7), 193.1 (Brev C-1), 150.0 (Brev C-2), 147.1 (Brev C-3), 41.5 (Brev C-4), 37.8 (Brev C-5), 172.5 (Brev C-6), 114.2 (Brev C-1'), 116.0 (Brev C-2'), 107.0 (Brev C-3'), 139.2 (Brev C-4'), 139.0 (Brev C-5'), 144.5 (Brev C-6'), 161.0 (Brev C-7').

Euphormisin M_2 (23**)** A pale brown amorphous powder, $[\alpha]_{\text{D}} -82^\circ$ ($c=1.3$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 222 (4.70), 277 (4.34). IR ν (KBr) cm^{-1} : 1800, 1710 (sh), 1705, 1605. CD (MeOH) $[\theta]$ (nm): -1.82×10^4 (217), -0.73×10^4 (240), $+1.45 \times 10^4$ (256), -3.8×10^4 (284). FAB-MS m/z : 947 ($\text{M} + \text{Na}$)⁺. Anal. Calcd for $\text{C}_{40}\text{H}_{34}\text{O}_{29} \cdot 3\text{H}_2\text{O}$: C, 49.09; H, 3.50. Found: C, 48.86; H, 3.60. ^1H -NMR δ : 7.13 (2H, s,

Gal), 7.02, 6.65 (1H each s, HHDP), 7.29 [1H, s, ring D (D) H-3], 4.95 (1H, d, $J=3.0$ Hz, H-2'), 3.62 (1H, m, H-3'), 2.12 (1H, dd, $J=6.5$, 12 Hz, H-4'), 1.91 (1H, dd, $J=3$, 12 Hz, H-4'), 6.41 (1H, brs, Glc H-1), 5.38 (1H, brs, Glc H-2), 6.02 (1H, brs, Glc H-3), 5.25 (1H, brs, Glc H-4), 4.88 (1H, brt, Glc H-5), 4.76 (1H, t, $J=10$ Hz, Glc H-6), 4.41 (1H, dd, $J=8.5$, 10 Hz, Glc H-6). ^{13}C -NMR δ : 30.5 (C-4'), 45.0 (C-3'), 47.7 (C-2'), 61.2 (Glc C-3), 63.9 (Glc C-6), 67.1 (Glc C-4), 70.2 (Glc C-2), 73.5 (Glc C-5), 91.7 (Glc C-1), 107.7 [ring C (C) C-3], 110.1 [ring B (B) C-3], 110.5 [ring A (A) C-2, 6], 114.2 [ring D (D) C-3], 115.2, 117.2, 117.5, 117.7, 120.1, 124.5, 125.4, 136.1, 136.3, 137.6, 139.8, 143.0, 144.6, 144.9, 145.1, 145.2, 145.9, 147.7, 164.3, 165.0, 166.4, 168.5, 171.4, 172.1, 175.8.

Partial Hydrolysis of Euphormisin M_2 (23) An aqueous solution of **23** (1 mg/1 ml) was heated in a water-bath at 70 °C for 5 h. After removal of the solvent, the residue was analyzed by HPLC (normal and reversed phases) to detect corilagin (**9**).

Methylation of Euphormisin M_2 (23) A mixture of **23** (5 mg), dimethyl sulfate (100 μl) and potassium carbonate (100 mg) in acetone (3 ml) was stirred overnight at room temperature and then refluxed for 3 h. The inorganic material was removed by centrifugation and the supernatant was concentrated. The residue was purified by preparative TLC to give the tetradecamethyl derivative (**23a**) (2 mg), $[\alpha]_{\text{D}} -80^\circ$ ($c=0.5$, acetone). FAB-MS m/z : 1139 ($\text{M}+\text{H}$) $^+$. ^1H -NMR δ : 7.28 (2H, s, Gal), 7.06, 6.90 (1H each s, HHDP), 7.45 (1H, s, ring D H-3), 6.65 (1H, brs, Glc H-1), 5.56 (1H, brs, Glc H-2), 5.85 (1H, brs, Glc H-3), 5.30 (1H, brs, Glc H-4), 4.93 (1H, m, Glc H-5), 5.10 (1H, t, $J=5$ Hz, Glc H-6), 4.50 (1H, dd, $J=1.5$, 5 Hz, Glc H-6), 5.53 (1H, brs, H-2'), 4.15 (1H, m, H-3'), the methylene protons H-4', were hidden by OMe signals, 3.93, 3.92, 3.90, 3.89, 3.86, 3.85, 3.84, 3.75, 3.67, 3.65, 3.53, 3.27 (each 3H, s, OMe $\times 12$), 3.68 (6H, s, OMe $\times 2$).

Methanolysis of 23a A solution of **23a** (5 mg) in MeOH (0.2 ml) was treated with 1% NaOMe (0.1 ml), and the reaction mixture was left standing overnight at room temperature. The solvent was evaporated after acidification with a few drops of AcOH to give a syrupy residue, which, upon purification by preparative TLC, afforded methyl tri-*O*-methylgallate (**21**) (1 mg), dimethyl hexamethoxydiphenate (**24**) [0.4 mg; EI-MS m/z : 450 (M) $^+$] and the heptamethyl derivative (**25**) [0.4 mg; EI-MS m/z : 442 (M) $^+$. ^1H -NMR δ : 7.32 (1H, s, aromatic-H), 3.90, 3.87, 3.86, 3.83, 3.67, 3.56, 3.40 (each 3H, s, OMe $\times 7$).

References and Notes

- 1) Part X. T. Yoshida, O. Namba, C.-F. Lu, L.-L. Yang, K.-Y. Yen, T. Okuda, *Chem. Pharm. Bull.*, **40**, 338 (1992).
- 2) a) T. Yoshida, L. Chen, T. Shingu, T. Okuda, *Chem. Pharm. Bull.*, **36**, 2940 (1988); b) T. Yoshida, K. Yokoyama, O. Namba, T. Okuda, *ibid.*, **39**, 1137 (1991); c) T. Yoshida, O. Namba, L. Chen, T. Okuda, *ibid.*, **38**, 86 (1990); d) *Idem*, *ibid.*, **38**, 1113 (1990); e) T. Yoshida, O. Namba, K. Yokoyama, T. Okuda, Symposium Papers of the 31st Symposium on the Chemistry of Natural Products, Nagoya, 1989, p. 601.
- 3) T. Okuda, T. Yoshida, T. Hatano, *Phytochemistry*, **32**, 507 (1993).
- 4) E. A. Haddock, R. K. Gupta, S. M. K. Al-Shafi, E. Haslam, D. Magnolato, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2515.
- 5) a) T. Okuda, T. Yoshida, M. Ashida, K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1765; b) C. K. Wilkins, B. A. Bohm, *Phytochemistry*, **15**, 211 (1976).
- 6) T. Okuda, T. Yoshida, T. Hatano, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 9.
- 7) R. Saijo, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.*, **37**, 2063 (1989).
- 8) I. Agata, T. Hatano, Y. Nakaya, T. Sugaya, S. Nishibe, T. Yoshida, T. Okuda, *Chem. Pharm. Bull.*, **39**, 881 (1991).
- 9) T. Yoshida, T. Okuda, T. Koga, N. Toh, *Chem. Pharm. Bull.*, **30**, 2655 (1982).
- 10) J.-H. Lin, T. Tanaka, G. Nonaka, I. Nishioka, I.-S. Chen, *Chem. Pharm. Bull.*, **38**, 2162 (1990).
- 11) W. E. Hillis, Y. Yazaki, *Phytochemistry*, **12**, 2963 (1973).
- 12) T. Okuda, T. Yoshida, N. Toh, *Phytochemistry*, **14**, 2513 (1975).
- 13) a) G. Nonaka, M. Ishimatsu, T. Tanaka, I. Nishioka, M. Nishizawa, T. Yamagishi, *Chem. Pharm. Bull.*, **35**, 3127 (1987); b) J. H. Lin, G. Nonaka, I. Nishioka, *ibid.*, **38**, 1218 (1990).
- 14) T. Yoshida, H. Itoh, H. Matsunaga, R. Tanaka, T. Okuda, *Chem. Pharm. Bull.*, **40**, 53 (1992).
- 15) S.-H. Lee, T. Tanaka, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.*, **39**, 630 (1991).
- 16) Although the methyl ester (**22**) was optically inactive, the brevifolincarboxyl moiety in the molecule of **19** may be optically active. Its stereochemistry, if it is chiroptical, should be examined in the future.
- 17) T. Tanaka, H. Fujisaki, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.*, **40**, 2937 (1992).