Tannins and Related Polyphenols of Euphorbiaceous Plants. VII.¹⁾ Tirucallins A, B and Euphorbin F, Monomeric and Dimeric Ellagitannins from *Euphorbia tirucalli* L.

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A new monomeric hydrolyzable tannin, tirucallin A (7), and two new dimers, tirucallin B (11) and euphorbin F (14), have been isolated, together with six known polyphenols including euphorbin A, from the stems of *Euphorbia tirucalli* L., and their structures were elucidated by spectral and chemical methods. Euphorbin F (14) possesses dehydrohexahydroxydiphenoyl and valoneoyl groups, and tirucallin A (7) has a dilactonized valoneoyl group. The orientation of the valoneoyl group of euphorbins A and B was revised.

Keywords *Euphorbia tirucalli*; Euphorbiaceae; tannin; hydrolyzable tannin; tirucallin A; tirucallin B; euphorbin F; euphorbin A; structure revision

The stems and roots of *Euphorbia tirucalli* L. (Euphorbiaceae) have been used as a poultice applicable to broken bones, ulceration of the nose, swellings, hemorrhoids, and painful parts of the human body, in Indonesia, the Philippines and Malaysia.²⁾ The latex of this plant is known to be poisonous, and more than fifteen phorbol-type diterpenoids³⁾ have been isolated. Triterpenes such as euphorbol⁴⁾ are also known. However, no chemical study on the polyphenolic constituents of this plant has been reported. We have recently reported that *E. hirta* L. produces unique dimeric hydrolyzable tannins, euphorbins A–E,⁵⁾ which possess a dehydrohexahydroxydiphenoyl group in the molecule, whereas some other *Euphorbia* species contain rugosins D, E or G, which are oligomers of a type different from euphorbins.^{1,6)} In our continuing studies on tannins and related polyphenols of Euphorbiaceous plants, we have isolated from the stems of *E. tirucalli* nine polyphenols, including three new hydrolyzable tannins named tirucallins A and B and euphorbin F.

The ethyl acetate- and 1-butanol-soluble fractions of the aqueous acetone extractive of the stems were separately subjected to a combination of Toyopearl HW-40 and MCI-gel CHP 20P column chromatography, developing with aqueous alcohol, to afford nine compounds. Among them, six compounds were identified as quercitrin (1), 5-*O*-caffeoylquinic acid (2),⁷⁾ 3,3',4-tri-*O*-methyl-4'-*O*-rutinosyl-ellagic acid (3),⁸⁾ tellimagrandin II (4),⁹⁾ geraniin (5),¹⁰⁾ and euphorbin A.^{5a)}

Tirucallin A (7) was obtained as a pale yellow amorphous powder, $[\alpha]_D +2.8^\circ$ (acetone), and its molecular formula was indicated to be $C_{55}H_{34}O_{34}$ by the elemental analysis and fast-atom bombardment mass spectrum (FAB-MS), which showed an $(M+Na)^+$ ion peak at m/z 1261. The proton nuclear magnetic resonance (1H -NMR) spectrum of 7 showed the presence of two galloyl groups [δ 6.65 and 6.83 (each 2H, s)] and a hexahydroxydiphenoyl (HHDP) group [δ 6.49 and 6.58 (each 1H, s)]. Three 1H singlets at δ 6.79, 7.11 and 7.61, which are characteristic of a dilactonized valoneoyl group, were also observed. The presence of a 4C_1 glucopyranose core in 7 was indicated by the coupling pattern of a seven-spin system in the aliphatic region (see Experimental). These spectral features, along with the ultraviolet (UV) (218, 266 and 355 nm) and circular dichroism (CD) ($[\theta]_{237} +12.0 \times 10^4$) spectral data, were similar to those of prostratin A (8), which was recently isolated from *Euphorbia prostrata*.¹⁾ A remarkable difference

between 7 and 8 is an upfield shift of the anomeric proton signal of the former (δ 5.69, d, $J=8$ Hz) from that of the latter (δ 6.02, d, $J=8.5$ Hz). This upfield shift of H-1 in 7, relative to the acylated anomeric proton (δ 6.0–6.2) of the hydrolyzable tannins having a galloyl group of O-2 on a 4C_1 glucopyranose core such as 8 and 4, is analogous to that of cornusiins A, B (9) and others,¹¹⁾ which have a valoneoyl group at O-2 of the glucose core. This can be interpreted in terms of an anisotropic effect of the biphenyl moiety of the valoneoyl group.¹¹⁾ Tirucallin A was thus characterized as 1,3-di-*O*-galloyl-2-*O*-dilactonized valoneoyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl- β -D-glucose. This structure was substantiated by enzymatic hydrolysis of 7 to yield cornusin B (9),¹¹⁾ oenotherin C (10)¹¹⁾ and gallic acid.

Tirucallin B (11), $[\alpha]_D -31^\circ$ (acetone), was obtained as a light brown amorphous powder. Acid hydrolysis of 11 yielded gallic acid, ellagic acid, valoneic acid dilactone and glucose. This tannin is regarded as a hydrolyzable tannin dimer consisting of three galloyl groups, an HHDP group and a valoneoyl group, as revealed by three 2H singlets (δ 7.02, 7.05, 7.13), five 1H singlets (δ 6.37, 6.75, 6.79, 6.98, 7.13), and two sugar anomeric proton signals [δ 6.30 (d, $J=3$ Hz), 6.36 (d, $J=2$ Hz)] in the 1H -NMR spectrum. Both glucose cores were shown to adopt 1C_4 conformation by the small $J_{1,2}$ and $J_{3,4}$ values (2–3 Hz) and the absence of coupling between H-2 and H-3, which are similar features to those of geraniin (5).¹⁰⁾ The hydroxyl groups at C-2' and C-4' in one of the glucose cores were shown to be free, by upfield shifts of H-2' [δ 4.01 (brs)] and H-4' [δ 4.36 (brs)], while the other glucose core is fully acylated (see Experimental). The chemical shifts of these glucose signals are in accordance with those of corilagin (12)¹²⁾ and punicafoin (13).¹³⁾ These spectral data, including the FAB-MS data [m/z 1593 ($M+Na$)⁺], are identical with those reported for the dimer 11, which was recently obtained as a degradation product of excoecarianin.¹⁴⁾ The present isolation of 11 is the first report of this compound as a natural product.

Euphorbin F (14), a pale yellow amorphous powder, $[\alpha]_D -28^\circ$ (acetone), gave a positive coloration of ellagitannin with the sodium nitrite reagent¹⁵⁾ in the spot test on paper. It was shown to be a dimer by an $[M+Na]^+$ ion peak at m/z 1911 in the FAB-MS. Acid hydrolysis of 14 yielded the same products (glucose, gallic acid, ellagic acid and valoneic acid dilactone) as those from 11. In the 1H -NMR spectrum

Chart 1

of **14**, the presence of a dehydrohexahydroxydiphenyl (DHHDP) group equilibrating between five- and six-membered hemiacetal forms (**14a** $\rightleftharpoons$ **14b**) was shown by the signal pairs of methine [δ 5.11 (s, **14a**) and 4.89 (d, $J=1.5$ Hz, **14b**)] and vinyl protons [δ 6.46 (s, **14a**) and 6.07 (br s, **14b**)]. The presence of an HHDP group, a valoneoyl group and three galloyl groups was also indicated by five ^1H singlets and three ^2H singlets, each duplicated in a ratio of *ca.* 1:1 (see Experimental). The conformations of the two glucose cores were shown to be $^4\text{C}_1$ (glucose-I) and $^1\text{C}_4$ (glucose-II) by the coupling patterns of their protons. The chemical shifts of the glucose-I signals are analogous to those of tellimagrandin II (**4**), except for the upfield shifts of H-1, H-3 and H-5 in **14**, while the chemical shifts of the glucose-II protons are closely similar to those of geraniin (**5**) (Table I). The glucose carbon resonances in the ^{13}C -NMR spectrum of **14** also showed close similarity to the sum of those of **4** and **5** (Table II). The presence of

the geraniin moiety in **14** was confirmed as follows. Upon treatment with *o*-phenylenediamine in an acidic medium, **14** gave a phenazine derivative (**15**) and a partial hydrolyzate (**16**). The ^1H -NMR spectrum of **15** indicated a conformational change of glucose-II from $^1\text{C}_4$ to the skew-boat form, as revealed by the change of the coupling constants among its protons, H-1'—H-6' (Table I). An upfield shift of H-1' and a downfield shift of H-5' from the corresponding signals of **14** were also observed. These spectral changes were analogous to those observed upon the conversion of **5** into its phenazine derivative (**6**),¹⁰ and support the identity of the position, orientation and absolute stereostructure of the DHHDP group in **14** with those in geraniin (**5**). The location of the DHHDP group at O-2'—O-4' in **14** was further confirmed by the formation of **16**, whose ^1H -NMR spectrum showed upfield shifts of H-2' (δ 4.07, br s) and H-4' (δ 4.38, br s) in the $^1\text{C}_4$ glucose core (II), induced by the loss of the phenylphenazine moiety from **15**. Therefore,

TABLE I. ^1H -NMR Data for the Glucose Moieties of **4**, **5**, **14** and **15** (Acetone- d_6 + D_2O , J in Hz)

	4	5a	5b	14a	14b	15
Glucose-I						
H-1	6.20 (d, $J=8$)			6.14 (d, $J=8$)	6.01	5.95 (d, $J=9$)
H-2	5.58 (dd, $J=8, 9.5$)			5.63 (dd, $J=8, 10$)	5.65	5.60 (t, $J=9$)
H-3	5.83 (t, $J=9.5$)			5.30 (t, $J=10$)		5.31 (t, $J=9$)
H-4	5.20 (t, $J=9.5$)			5.09 (t, $J=10$)		5.09 (t, $J=9$)
H-5	4.54 (dd, $J=6, 9.5$)			4.34 (dd, $J=7, 10$)	4.38	4.31 (dd, $J=6.5, 10$)
H-6	5.36 (dd, $J=6, 3$) 3.87 (d, $J=13$)			5.36 (dd, $J=7, 13$) 3.87 (d, $J=13$)	5.32 3.86	5.24 3.80 (dd, $J=6.5, 14$) (d, $J=14$)
Glucose-II						
H-1'		6.60 (br s)		6.53 (br s)	6.60	6.11 (d, $J=6$)
H-2'		5.60 (br s)		5.56 (br s)	5.48	5.64 (d, $J=6$)
H-3'		5.60 (br s)	5.50	5.60 (br s)	5.44	5.51 (d, $J=4$)
H-4'		5.56 (br s)	5.46	5.60 (br s)		5.47 (br d, $J=4$)
H-5'		4.81 (m)		4.73 (m)		4.92 (dd, $J=4, 8$)
H-6'		4.93 (t, $J=11$) 4.33 (dd, $J=8, 11$)	4.78 (m) 4.45 (dd, $J=6, 9$)	4.79 (t, $J=10$) 4.24 (dd, $J=7, 10$)	4.40 (dd, $J=8, 10$)	4.73 (dd, $J=8, 12$) 4.02 (dd, $J=4, 12$)

TABLE II. ^{13}C -NMR Data for the Glucose Moieties of **4**, **5** and **14** (Acetone- d_6 + D_2O)

		4	5a	5b	14a	14b
Glucose-I	C-1	93.8				93.6
	C-2	71.8				71.6
	C-3	73.3				73.9
	C-4	70.8				70.4
	C-5	73.1				72.7
	C-6	63.1				62.8
Glucose-II	C-1'		90.8	91.8	91.0	91.9
	C-2'		69.9	70.4	69.9	70.8
	C-3'		63.3	62.3	63.3	62.5
	C-4'		65.9	66.8	65.9	66.9
	C-5'		72.6	73.1	72.5	73.4
	C-6'		63.6	63.8	63.8	64.0

euphorbin F was concluded to be a dimer having the tellimagrandin II and geraniin moieties as partial structures.

Conclusive proof of the structure of euphorbin F, including the position of the valoneoyl group in the molecule, was obtained by chemically correlating **14** with euphorbin E (**17**), which has recently been isolated from *E. hirta*.^{5c)} In this experiment, the catalytic hydrogenation of euphorbin E (**17**) yielded two products, one of which was characterized as **18**, which was produced *via* hydrogenolysis of the allylic system in the D-ring of the dehydroeuphorbi-noyl group. This compound was also obtained by similar hydrogenation of **14**.

Since the structures **19** and **20**^{5a)} were previously assigned to euphorbin A and euphorbin B, which are isomers concerning the orientation of the valoneoyl group attached

Chart 3

to O-3'/O-6' of the glucose core-II, euphorbin F (**14**) may be characterized as an oxidative metabolite of euphorbin B. However, euphorbin B was not detected even in a trace amount in the extract of *E. tirucalli*. Therefore, the co-occurrence of euphorbins F and A prompted us to re-examine the previously proposed structures of eu-

phorbins A and B.

The assignment of the orientations of the valoneoyl groups in euphorbins A and B was based on $^1\text{H-NMR}$ spectral comparison, with the inspection of stereomodels.^{5a)} This comparison revealed that the chemical shifts of H-1, H-3 and H-5 of the glucose core-I were noticeably different

Chart 4

Chart 5

between euphorbins A and B. These signals in the former were shifted upfield by *ca.* 0.2–0.5 ppm from the corresponding signals of 1,2,3,4,6-penta-*O*-galloyl- β -D-glucose (**21**), while those in the latter are almost identical with those of **21**. The upfield shifts in the former were attributed to the anisotropic effect of the valoneoyl aromatic ring A, in the conformation arising from the restricted rotation around the ether linkage of the valoneoyl group.^{5a)}

However, as mentioned earlier, H-1, H-3 and H-5 of glucose-I in **14** are shifted upfield from the corresponding signals of tellimagrandin II (**4**). This phenomenon is analogous to that observed in euphorbin A, rather than in euphorbin B, indicating the same orientation of the valoneoyl group in euphorbins A and F (**14**).

The measurement of the ^1H - ^{13}C long-range shift correlation spectrum of the partial hydrolyzate (**22**)^{5a)} from

Fig. 1. ^1H - ^{13}C Long-Range Shift Correlation Spectrum (δ_{C} 165.0–166.8) of **22** in Acetone- d_6 + D_2O

Average J_{CH} was set at 6 Hz.

the condensate of euphorbin A with *o*-phenylenediamine has now been carried out. This spectrum showed a clear cross peak by three-bond coupling between the H-6' signal of the glucose core at δ 4.74 (t, $J=10$ Hz) and the ester carbonyl carbon resonance at δ 168.6, which is correlated with the aromatic proton signal at δ 6.21 (Fig. 1). This

aromatic signal was assigned to H_B of the valoneoyl group, based on its correlation by two-bond coupling with the signal attributed to C-4' (δ 146.7) of the valoneoyl B-ring, and also by three-bond coupling with C-1' (δ 116.8). The connectivity between the valoneoyl B-ring and H-6' of the glucose core-II was thus established, leading to the revised structure **20** for euphorbin A, and consequently structure **19** for euphorbin B.

Experimental

General 1H - (500 MHz) and ^{13}C -NMR (126 MHz) spectra were measured on a Varian VXR 500 instrument and chemical shifts are given in δ (ppm) values relative to acetone- d_6 (2.04 ppm for 1H and 29.8 ppm for ^{13}C). High-performance liquid chromatography (HPLC) was conducted on Superspher Si 60 (4 mm $\times$ 119 mm) and LiChrospher RP-18 (4 mm $\times$ 250 mm) columns, using the following solvent systems: (A) hexane-MeOH-tetrahydrofuran (THF)-HCOOH (60:45:15:1) and oxalic acid (500 mg/1.2 l), (B) 0.05 M phosphate buffer-EtOH-EtOAc (85:10:5), (C) 0.05 M phosphate buffer-CH₃CN (85:15), (D) 0.05 M phosphate buffer-EtOH-EtOAc (87:8:5), (E) 0.05 M phosphate buffer-CH₃CN (87:13), (F) 0.05 M phosphate buffer-EtOH-EtOAc (83:12:5). Column chromatography was carried out on Toyopearl HW-40 (coarse and fine grades, Tosoh) and MCI-gel CHP-20P (Mitsubishi Chemical Industry Co., Ltd.).

Isolation of Tannins and Other Polyphenols The dried stems (900 g) of *E. tirucalli*, collected in a greenhouse at Okayama University, were homogenized in 70% aqueous acetone (13 l), and filtered. The filtrate was concentrated under reduced pressure, and extracted with Et₂O, EtOAc and 1-BuOH (pre-saturated with water), successively. The EtOAc extract (4.8 g) was subjected to column chromatography over Toyopearl HW-40 (coarse) (2.2 cm i.d. $\times$ 50 cm) developing with 50% MeOH $\rightarrow$ 60% MeOH $\rightarrow$ 70% MeOH $\rightarrow$ MeOH-H₂O-acetone (7:2:1). The eluate with 50% MeOH gave quercitrin (**1**) (96 mg). The 70% MeOH eluate yielded tellimagrandin II (**4**) (31 mg) and geraniin (**5**) (267 mg). Euphorbin F (**14**) (366 mg) was obtained from the MeOH-H₂O-acetone (7:2:1) eluate. The fraction (187 mg) eluted prior to **14** was rechromatographed over MCI-gel CHP-20P (30% MeOH $\rightarrow$ 40% MeOH) to give tirucallin A (**7**) (16 mg) and euphorbin A (**20**) (38 mg). The BuOH extract was similarly chromatographed over Toyopearl HW-40, using the same solvents as those for the EtOAc extract mentioned above, to give 5-*O*-caffeoylquinic acid (**2**) (15 mg) and 3,3',4-tri-*O*-methyl-4'-*O*-rutinosyllellagic acid (**3**) (20 mg) from the 60% MeOH eluate. The 70% MeOH eluate was further purified by column chromatography over MCI-gel CHP-20P to give tirucallin B (**11**) (8 mg).

3,3',4-Tri-*O*-methyl-4'-*O*-rutinosyllellagic Acid (3**)** White needles, mp 260–263 °C. FAB-MS m/z : 675 (M+Na)⁺. 1H -NMR (pyridine- d_5) δ : 7.59, 8.29 (each 1H, s, aromatic), 3.63, 3.89, 4.05 (each 3H, s, OMe), 5.61 (d, J = 7 Hz, glucose H-1), 5.25 (s, rhamnose H-1), 3.95–4.16 (8H, m, glucose H-2–H-6, rhamnose H-2–H-5), 1.32 (3H, d, J = 6 Hz, rhamnose Me). ^{13}C -NMR (pyridine- d_5) δ : 18.7, 67.4, 69.8, 71.4, 72.2, 72.8, 74.0, 74.9, 77.8, 78.5, 102.4, 103.3 (rutinosyl), 56.7, 61.6, 62.1 (OMe), 108.2, 113.5, 113.6 (2C), 113.7, 114.5, 142.0, 142.1, 142.2, 143.1, 153.2, 155.1 (aromatic), 159.0, 159.2 (carbonyl).

Hexaacetate. mp 132–135 °C. 1H -NMR (CDCl₃) δ : 7.89 (1H, s), 7.69 (1H, s) (aromatic), 5.0–5.4 (7H, glucose H-1, H-2, H-3, H-4, rhamnose H-2, H-3, H-4), 4.69 (1H, d, J = 1 Hz, rhamnose H-1), 3.6–3.9 (4H, glucose H-5, H-6, rhamnose H-5), 1.17 (3H, d, J = 6 Hz, rhamnose H-6), 4.03, 4.16, 4.20 (each 3H, s, OMe), 1.94, 2.03, 2.05, 2.06, 2.08, 2.11 (each 3H, s, OAc).

Tirucallin A (7**)** A pale yellow amorphous powder, $[\alpha]_D + 2.8^\circ$ (c = 0.5, acetone). UV λ_{max} (MeOH) nm (log ϵ): 218 (4.90), 266 (4.74), 355 (3.78). CD (MeOH) $[\theta]$ (nm): +12.0 $\times 10^4$ (237), –10.0 $\times 10^4$ (265). Anal. Calcd for C₅₅H₃₄O₃₄·10H₂O: C, 46.54; H, 3.81. Found: C, 46.28; H, 3.45. FAB-MS m/z : 1261 (M+Na)⁺. 1H -NMR (acetone- d_6 + D₂O) δ : 5.69 (d, J = 8 Hz, H-1), 5.57 (dd, J = 8, 10 Hz, H-2), 5.63 (t, J = 10 Hz, H-3), 5.08 (t, J = 10 Hz, H-4), 4.31 (dd, J = 6, 10 Hz, H-5), 5.30 (dd, J = 6, 13 Hz, H-6), 3.76 (d, J = 13 Hz, H-6), aromatic protons, see text. ^{13}C -NMR (acetone- d_6 + D₂O) δ : 93.4 (C-1), 71.4 (C-2), 73.6 (C-3), 70.7 (C-4), 72.9 (C-5), 62.9 (C-6) (glucose), 159.6, 160.5, 164.4, 164.8, 166.5, 167.7, 168.1 (ester carbonyl).

Enzymatic Hydrolysis of **7** A mixture of **7** (0.5 mg) and tannase¹⁶⁾ (1 dr.) in H₂O (1 ml) was left standing at 37 °C for 1 h. The concentrated solution was directly analyzed by reversed-phase HPLC (solvent B) to detect gallic acid (t_R 3.10 min), **9** (t_R 4.87, 5.99 min) and **10** (t_R 3.48,

3.83 min). The identities of the products were further confirmed by reversed-phase HPLC using three other solvent systems (C–E), and by normal phase HPLC (solvent A).

Tirucallin B (11**)** A light brown amorphous powder, $[\alpha]_D - 31^\circ$ (c = 0.5, acetone). UV λ_{max} (MeOH) nm (log ϵ): 220 (5.11), 277 (4.76). FAB-MS m/z : 1593 (M+Na)⁺. 1H -NMR (acetone- d_6 + D₂O) δ : 6.36 (d, J = 3 Hz, H-1), 5.28 (br d, J = 3 Hz, H-2), 5.08 (br s, H-3), 5.71 (br d, J = 3 Hz, H-4), 4.56 (t, J = 8 Hz, H-5), 4.68 (dd, J = 8, 11 Hz, H-6), 4.32 (dd, J = 8, 11 Hz, H-6), 6.30 (d, J = 2 Hz, H-1'), 4.01 (br s, H-2'), 4.74 (br s, H-3'), 4.36 (br s, H-4'), 4.32 (dd, J = 8, 11 Hz, H-5'), 4.63 (t, J = 11 Hz, H-6'), 3.83 (dd, J = 8, 11 Hz, H-6'), aromatic protons, see text.

Euphorbin F (14**)** A pale yellow amorphous powder, $[\alpha]_D - 28^\circ$ (c = 0.8, acetone). UV λ_{max} (MeOH) nm (log ϵ): 220 (5.13), 279 (4.76). CD (MeOH) $[\theta]$ (nm): +14.0 $\times 10^4$ (236), –7.0 $\times 10^4$ (288). Anal. Calcd for C₈₂H₅₆O₅₃·6H₂O: C, 49.30; H, 3.41. Found: C, 49.06; H, 3.78. FAB-MS m/z : 1911 (M+Na)⁺. 1H -NMR (acetone- d_6 + D₂O) δ : 6.15, 6.18, 6.62, 6.63, 6.73, 6.78, 6.99, 7.03, 7.09, 7.15, 7.17, 7.23 (each 1/2H, s, HHDP, valoneoyl, H-3'' of DHHDP), 6.46 (1/2H, s), 6.07 (1/2H, br s) (H-3'' of DHHDP), 6.84, 6.87, 7.10, 7.11, 7.16, 7.17 (each 1H, s, galloyl), glucose protons, see text. ^{13}C -NMR (acetone- d_6 + D₂O) δ : 45.9 [DHHDP (a) C-1'], 51.8 [DHHDP (b) C-1'], 154.2 [DHHDP (a) C-2'], 147.6 [DHHDP (b) C-2'], 128.6 [DHHDP (a) C-3'], 124.7 [DHHDP (b) C-3'], 191.8 [DHHDP (a) C-4'], 194.5 [DHHDP (b) C-4'], 96.1 [DHHDP (a) C-5'], 92.2 [DHHDP (b) C-5'], 92.3 [DHHDP (a) C-6'], 164.7 (1/2C), 165.1 (3/2C), 165.2 (3/2C), 165.3 (1/2C), 165.6 (1/2C), 165.7 (1/2C), 166.5 (3/2C), 166.6 (1/2C), 167.6 (1C), 168.1 (1C), 168.2 (1/2C), 168.3 (1/2C) (ester carbonyl); glucose carbons, see text.

Acid Hydrolysis of **14** A solution of **14** (5 mg) in 5% H₂SO₄ (1 ml) was heated on a boiling water-bath for 5 h, and after cooling, extracted with EtOAc. The EtOAc extract was analyzed by HPLC (LiChrospher RP-18, solvent C) to detect gallic acid (t_R 2.95 min), ellagic acid (t_R 10.4 min) and valoneic acid dilactone (t_R 7.12 min). The aqueous layer was neutralized with Amberlite IRA-410 (OH form), and evaporated. The syrupy residue was trimethylsilylated and analyzed by gas-liquid chromatography (GLC) to detect glucose (t_R 3.5 and 5.0 min).

GLC: capillary column, G-250 (1.2 mm $\times$ 40 m); oven temperature, 170 °C; detection, flame ionization detector.

Preparation of the Phenazine Derivative (15**) from Euphorbin F (**14**)** A solution of *o*-phenylenediamine (40 mg) in 15% AcOH (10 ml) was added to a solution of **14** (100 mg) in MeOH (30 ml), and the reaction mixture was left standing at room temperature for 5 h. After removal of the solvent, the residue was suspended in H₂O and the insoluble materials were collected by suction filtration, and washed with H₂O and Et₂O. Reprecipitation of the crude product from MeOH-CHCl₃ gave **15** (31 mg). The mother liquor was chromatographed over MCI-gel CHP 20P developing with 30% MeOH $\rightarrow$ 50% MeOH to yield **15** (5 mg) and **16** (9 mg).

15 An orange amorphous powder, $[\alpha]_D + 26^\circ$ (c = 0.2, acetone). UV λ_{max} (MeOH) nm (log ϵ): 219 (5.22), 281 (4.99). CD (MeOH) $[\theta]$ (nm): –7.5 $\times 10^4$ (215), +19 $\times 10^4$ (236), –11 $\times 10^4$ (280). Anal. Calcd for C₈₈H₅₈N₂O₅₀·12H₂O: C, 48.93; H, 3.80; N, 1.30. Found: C, 48.61; H, 3.15; N, 1.23. 1H -NMR (acetone- d_6 + D₂O) δ : 6.92, 6.96, 7.18 (each 2H, s, galloyl), 6.28, 6.61, 6.69, 6.99, 7.00 (each 1H, s, HHDP and valoneoyl), 7.46 (1H, s), 8.21 (1H, s), 8.3–8.0 (4H, m) (phenylphenazine), glucose protons, see text.

16 A light brown amorphous powder, $[\alpha]_D + 17^\circ$ (c = 1.0, acetone). UV λ_{max} (MeOH) nm (log ϵ): 220 (5.14), 275 (4.79). FAB-MS m/z : 1593 (M+Na)⁺. 1H -NMR (acetone- d_6 + D₂O) δ : 6.82, 7.09, 7.13 (each 2H, s, galloyl), 6.17, 6.63, 6.78, 6.84, 7.06 (each 1H, s, HHDP, valoneoyl), 6.13 (d, J = 8 Hz, H-1), 5.65 (dd, J = 8, 10 Hz, H-2), 5.29 (t, J = 10 Hz, H-3), 5.09 (t, J = 10 Hz, H-4), 4.38 (m, H-5), 5.38 (dd, J = 6, 13 Hz, H-6), 3.88 (d, J = 13 Hz, H-6), 6.32 (d, J = 2 Hz, H-1'), 4.07 (br s, H-2'), 4.80 (dd, J = 2, 3 Hz, H-3'), 4.38 (d, J = 3 Hz, H-4'), 4.43 (t, J = 10 Hz, H-5'), 4.73 (t, J = 10 Hz, H-6'), 4.07 (t, J = 10 Hz, H-6').

Hydrogenation of Euphorbins **17 and **14**** A solution of **17** (100 mg) in EtOAc (10 ml) was hydrogenated over 5% Pd-C for 26 h at room temperature. The catalyst was removed by filtration, the solvent was evaporated off *in vacuo*, and the residue was chromatographed on Toyopearl HW-40 (superfine) (1.1 $\times$ 18 cm) [MeOH $\rightarrow$ MeOH-acetone (9:1)]. The eluate with MeOH-acetone (9:1) was further purified by column chromatography over Sephadex LH-20 (MeOH) to give two fractions, A and B, which showed single peaks at t_R 5.88 min and 9.03 min, respectively, in reversed-phase HPLC (solvent F). Fraction A yielded the reduction product (**18**) (2.6 mg), as an off-white amorphous powder, $[\alpha]_D + 43^\circ$ (c = 0.9, MeOH). UV λ_{max} (MeOH) nm (log ϵ): 217 (5.17), 272 (4.83).

¹H-NMR (acetone-*d*₆ + D₂O) δ : 6.22, 6.60, 6.69, 6.92, 6.97, 7.00, 7.37 (each 1H, s, HHDP and valoneoyl), 6.86, 7.11, 7.12 (each 2H, s, galloyl), 6.04 (d, *J* = 8 Hz, H-1), 5.61 (dd, *J* = 8, 10 Hz, H-2), 5.30 (t, *J* = 10 Hz, H-3), 5.10 (t, *J* = 10 Hz, H-4), 4.33 (dd, *J* = 6.5, 10 Hz, H-5), 5.30 (dd, *J* = 6.5, 14 Hz, H-6), 3.83 (d, *J* = 14 Hz, H-6), 6.12 (d, *J* = 6 Hz, H-1'), 5.56 (d, *J* = 6 Hz, H-2'), 5.15 (br d, *J* = 4 Hz, H-3'), 5.44 (d, *J* = 4 Hz, H-4'), 4.75 (dd, *J* = 4, 8.5 Hz, H-5'), 4.62 (dd, *J* = 8.5, 12 Hz, H-6'), 3.99 (dd, *J* = 4, 12 Hz, H-6').

Euphorbin F (**14**) (5 mg) was hydrogenated in a similar way to that described above to yield the product (4.5 mg), $[\alpha]_D^{25} + 32^\circ$ (*c* = 0.4, acetone), which was identical with **18**. Their identity was confirmed by ¹H-NMR spectral comparison and co-chromatography on HPLC.

¹³C-NMR Spectrum of Partial Hydrolyzate (**22**)^{5a)} δ : 93.4 (C-1), 94.3 (C-1'), 71.4 (C-2), 68.9 (C-2'), 73.3 (C-3), 70.9 (C-3'), 69.4 (C-4), 62.1 (C-4'), 73.8 (C-5), 75.4 (C-5'), 62.8 (C-6), 64.6 (C-6'), 109.9, 110.1, 110.3 (each 2C), 110.5 (4C) (galloyl C-2, 6), 119.2, 119.5, 119.8, 120.5, 120.9 (galloyl C-1), 139.0, 139.40, 139.44 (2C), 139.9 (galloyl C-4), 145.66, 145.7, 145.9 (each 2C), 146.0 (4C) (galloyl C-3, 5), 165.4, 165.5, 165.9, 166.1, 166.6 (galloyl C-7), 116.3 [valoneoyl (Val) C-1], 110.0 (Val C-3), 144.7 (Val C-4), 137.2 (Val C-5), 167.5 (Val C-7), 116.8 (Val C-1'), 105.4 (Val C-3'), 146.7 (Val C-4'), 136.9 (Val C-5'), 168.6 (Val C-7'), 113.3 (Val C-1''), 136.6 (Val C-2''), 140.5 (Val C-3''), 140.6 (Val C-4''), 143.4 (Val C-5''), 110.5 (Val C-6''), 165.0 (Val C-7''), 124.8, 125.7 (Val C-2, 2'), 144.5, 145.6 (Val C-6, 6').

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References and Notes

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