Tannins and Related Polyphenols of Euphorbiaceous Plants. XIV.[1] Euphorbin I, a New Dimeric Hydrolyzable Tannin from *Euphorbia watanabei*

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A new dimeric dehydroellagitannin, euphorbin I (3), was isolated together with twenty-eight known polyphenols including dimeric ellagitannins, euphorbins A (1) and B (2), from the fresh leaves of *Euphorbia watanabei*. The structure of 3 which has a macaranoyl group as a linking unit between the monomers was established based on spectroscopic methods and chemical correlation with 1. The structure of euphorbin B (2c), previously assigned as a dimer having the valoneoyl group as the linking unit, was revised to 2 having a tergalloyl group.

Key words *Euphorbia watanabei*; Euphorbiaceae; hydrolyzable tannin; tannin; euphorbin I; euphorbin B

We previously reported the isolation and structure elucidation of a new class of dimeric hydrolyzable tannins having a geraniin moiety as a monomeric unit, such as euphorbins A-G and antidesmin A, from various euphorbiaceous plants.[2] In our continuing study on the tannins of the euphorbiaceous plants, we have examined the polyphenolic compounds in *Euphorbia watanabei* MAKINO, and isolated a new dimeric tannin, named euphorbin I (**3**), together with twenty-eight known polyphenols including euphorbins A (**1**) and B[2a,e] as major dimeric tannins. This paper describes the isolation and structure determination of the new tannin, and the structural revision of euphorbin B (**2c**), based upon the outcome of chemical transformation of euphorbin I (**3**) into euphorbin A (**1**).

Results and Discussion

The concentrated filtrate from the aqueous acetone homogenate of the fresh leaves of *E. watanabei* was extracted successively with ether, ethyl acetate and *n*-butanol. Repeated chromatography of the ethyl acetate extract over Toyopearl HW-40 and MCI-gel CHP 20P yielded a new tannin, euphorbin I (**3**), together with fifteen known polyphenols, among which seven were flavonol glycosides, identified as quercitrin, isoquercitrin, quercetin 3-*O*-(2″-*O*-galloyl)-β-D-glucopyranoside,[3] quercetin 3-*O*-(2″-*O*-galloyl)-β-D-galactopyranoside,[4] guaijaverin,[5] quercetin 3-*O*-(2″-*O*-galloyl)-α-L-arabinopyranoside,[6] and kaempferol 3-*O*-(2″-*O*-galloyl)-α-L-arabinopyranoside.[7] The other eight were hydrolyzable tannins, which were identified as 1,3,4,6-tetra-*O*-galloyl-β-D-glucose, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose,[8] terchebin,[9] geraniin,[10] galloylgeraniin,[11] didehydrogeraniin,[12] mallotusinic acid[13] and chebulagic acid.[14] Similar chromatographic separation of the *n*-butanol extract afforded an additional crop of euphorbin I (**3**), along with four known hydrolyzable tannin monomers [geraniin, mallotusinin,[15] elaeocarpusin,[16] mallojaponin[15]] and three known dimers [euphorhelin,[17] euphorbins A (**1**) and B]. The water-soluble extract afforded two quinic acid esters [3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid] and five known monomeric hydrolyzable tannins [corilagin,[10] mallotinic acid,[13] hellioscopinin B,[18] putranjivain A[19] and mallotunin[20]]. The major tannin of this plant was geraniin, as found in most other *Euphorbia* species.

Euphorbin I (**3**), $[\alpha]_D$ −20°, showed an ion peak at *m/z* 1913 ascribable to $(M+Na)^+$ in the FAB-MS, and gave a large retention volume on normal-phase HPLC, like euphorbin A (**1**) and other dimers, suggesting it to be a dimeric hydrolyzable tannin. The ^{1}H-NMR spectrum of **3** was not informative because of considerable broadening of most of the signals. The ^{13}C-NMR spectrum of **3**, however, showed characteristic signals of the dehydrohexahydroxydiphenoyl (DHHDP) group [δ 46.2, 154.2, 128.6, 191.8, 96.2, 92.3 (C-1″—6″ of a-form), 51.9, 148.9, 125.0, 194.5, 92.3, 108.9 (C-1″—6″ of b-form)] existing as an equilibrium mixture of six- and five-membered hemiacetal forms, as found in the euphorbin A molecule.[2a,e] The presence of the DHHDP group in **3** was confirmed by formation of its phenazine derivative (**3a**) [FAB-MS *m/z* 1967 $(M+Na)]^+$ upon condensation with *o*-phenylenediamine. The ^{1}H-NMR spectrum of **3a**, which was simplified by the absence of duplication of peaks, clearly disclosed the signals due to five galloyl groups [δ 7.16, 7.04, 7.03, 7.00, 6.98 (each 2H, s)] and three aromatic protons [δ 7.04, 6.95, 6.84 (each 1H, s)] in addition to those of a phenylphenazine moiety [δ 8.28, 7.49 (each 1H, s), 7.98 (2H, m), 8.32, 8.23 (each 1H, br d, $J=9$ Hz)]. The coupling patterns of the sugar proton signals were characteristic of glucopyranoses with $^4C_1$ and skew boat conformations. The chemical shifts of the glucose signals in the ^{1}H- and ^{13}C-NMR spectra were closely similar to those of the euphorbin A-phenazine derivative (**1a**) (see Table 1 and Experimental). The structural similarity between **1** and **3** was confirmed as follows. Euphorbin I (**3**) produced the acetone adduct (**3b**) at the DHHDP moiety upon treatment with acetone in the presence of ammonium formate.[21] Its ^{1}H-NMR spectrum, which was simplified without duplication of each signal in a manner similar to that described for the phenazine derivative (**3a**) mentioned above, showed a close resemblance to that of the corresponding acetone adduct (**1b**) of euphorbin A (Table 1). The conformational change of one of the glucose cores ($^1C_4$→skew boat conformation) was indicated by a comparison of the glucose proton signals between **3b** and **3a**. This phenomenon was

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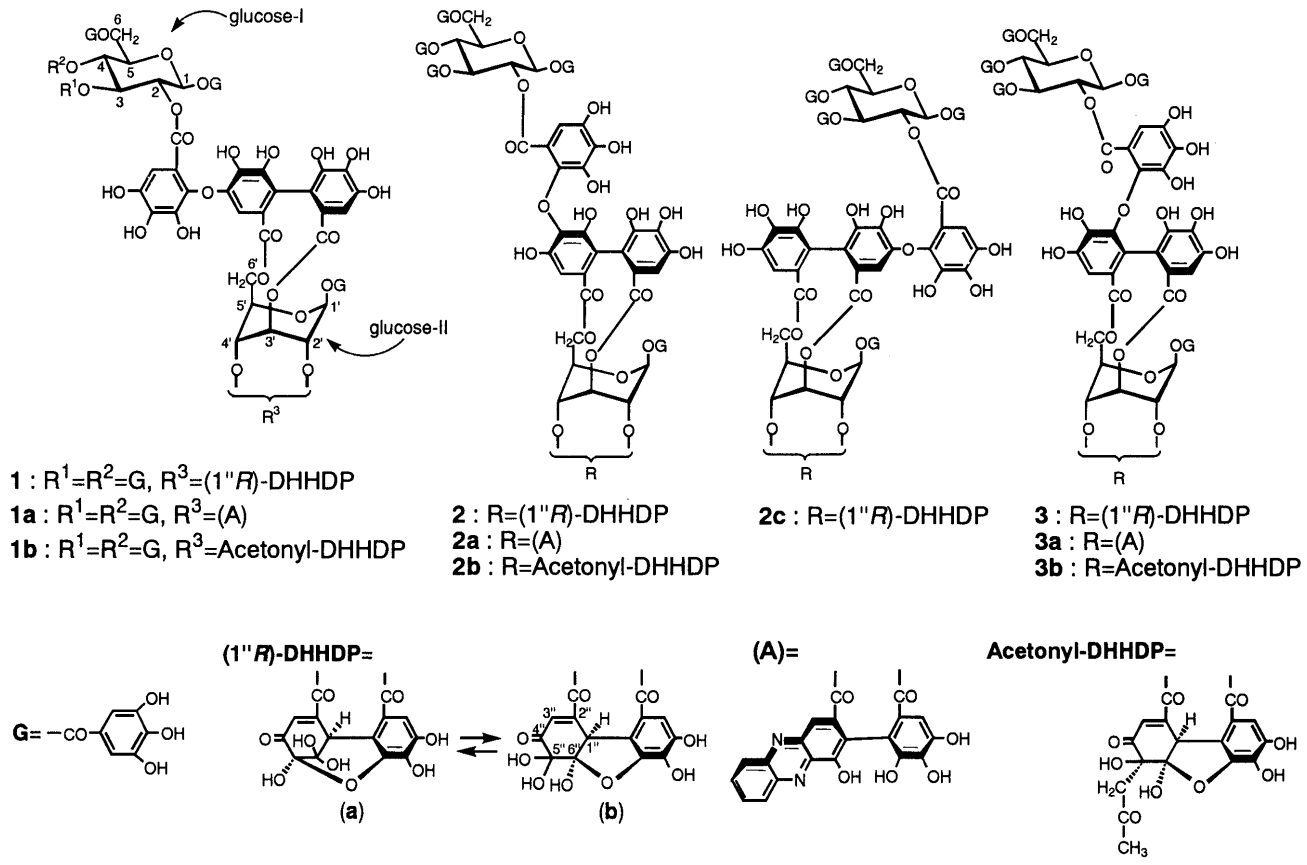

Chart 1

Table 1. ^{1}H-NMR Spectral Data for the Glucose Moieties of **3a**, **3b**, **1a**, **1b**, **2a** and **2b**

Protons	3a	1a	2a	3b	1b	2b
Glucose-I						
H-1	6.24 d (8.5)	6.09 d (8)	6.30 d (8.5)	6.26 d (8)	6.15 d (8)	6.30 d (8)
H-2	5.67 dd (8.5, 10)	5.62 dd (8, 9)	5.72 dd (8.5, 10)	5.67 dd (8, 10)	5.63 dd (8, 10)	5.71 dd (8, 10)
H-3	6.14 t (10)	5.56[b]	6.06 t (10)	6.11 t (10)	5.56[e]	6.05 t (10)
H-4	5.67 t (10)	5.56[b]	5.68 t (10)	5.67 t (10)	5.56[e]	5.68 t (10)
H-5	4.56[a]	4.33 m	4.55[c]	4.55[d]	4.34 m	4.55[f]
H-6	4.56[a]	4.50 dd (1.5, 12.5)	4.55[c]	4.55[d]	4.53 dd (2, 13)	4.55[f]
	4.30 dd (4, 12)	4.32 dd (4.5, 12.5)	4.32 dd (4, 12.5)	4.30 dd (4, 12.5)	4.29 dd (5, 13)	4.33 dd (5.5, 12)
Glucose-II						
H-1′	6.13 d (6)	6.12 d (6)	6.16 d (6)	6.56 br s	6.53 br s	6.56 br s
H-2′	5.72 d (6)	5.65 d (6)	5.67 d (6)	5.59 br s	5.56 br s	5.57 br s
H-3′	5.46 d (4)	5.41 d (4)	5.47 d (4)	5.55 br s	5.52 br s	5.50[g]
H-4′	5.51 d (4)	5.52 d (4)	5.53 d (4)	5.42 br s	5.41 br s	5.50[g]
H-5′	5.03 dd (4, 8)	4.94 dd (4, 8)	5.02 dd (4, 8)	4.83 br t (8)	4.77 br t (8)	4.85[h]
H-6′	4.85 dd (8, 12)	4.67 dd (7.5, 12)	4.72 dd (8, 12)	4.65 br t (11)	4.68 br t (11)	4.55[h]
	3.97 dd (4, 12)	4.05 dd (3.5, 12)	4.07 dd (4, 12)	4.45 dd (8, 11)	4.38 dd (8, 11)	4.31[h]

500 MHz, acetone-d_6 + D_2O. J (Hz) in parenthesis. *a*—*g*) Overlapped each other. *h*) These values may be interchanged.

analogous to that observed upon transformation of euphorbin A (**1**) into its phenazine derivative,[2a,e] indicating that **3** has a geraniin unit in the molecule. Euphorbin I was thus presumed to be a dimer composed of geraniin and pentagalloylglucose, like **1**. Spectral differences between the derivatives of **3** and **1** included a remarkable down-field shift of one of the aromatic 1H-singlets (δ 6.30 in **1a**→δ 6.84 in **3a**; δ 6.21 in **1b**→δ 6.77 in **3b**), which suggested that the linking unit between the monomers in **3** is different from that (valoneoyl group) in **1**.

Methylation of **3a** with diazomethane and subsequent methanolysis yielded methyl tri-*O*-methylgallate (**4**), trimethyl octa-*O*-methylmacaranate (**8**)[22] and the methylated phenylphenazine derivative (**9**),[10] as main products. The linking unit of the monomers in **3** was thus confirmed to be a macaranoyl group. Dimethyl hexamethoxydiphenate (**5**) and trimethyl octa-*O*-methylvaloneate (**6**) were also obtained as minor products in the above reaction. These were regarded as secondary products derived from the macaranoyl moiety in **3** for the following reasons. We recently found that a tergalloyl group in the molecule of hydrolyzable tannins is isomerized to a valoneoyl group

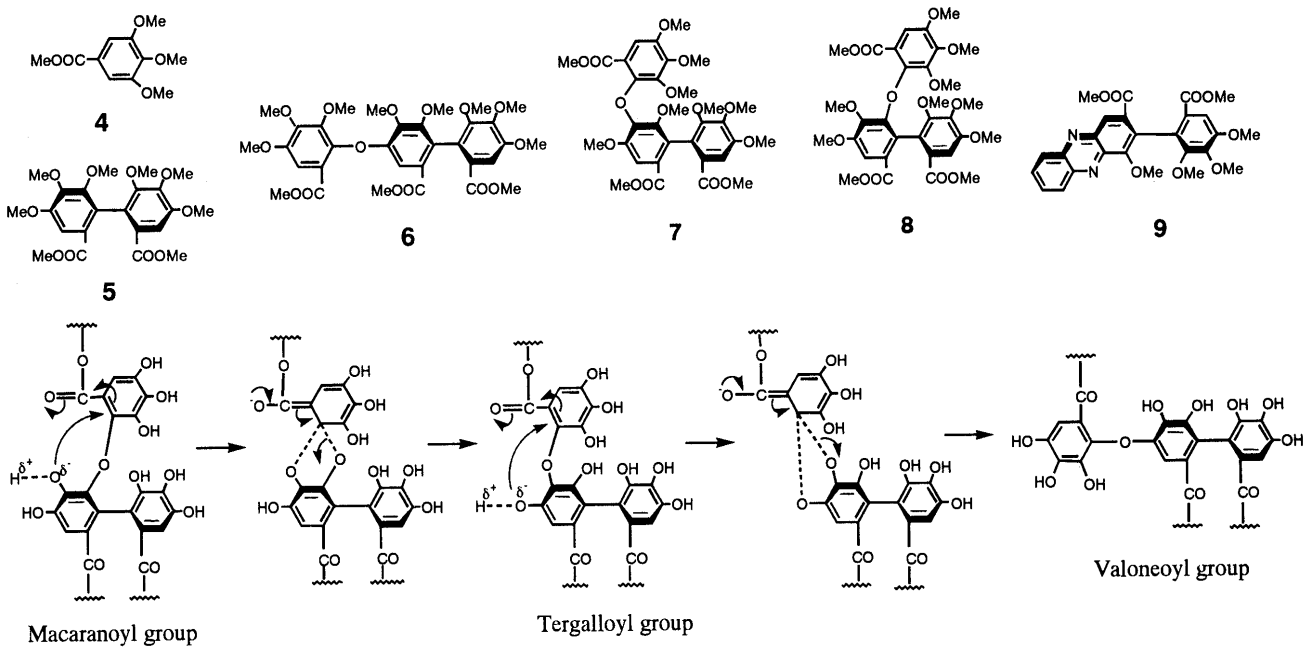

Chart 2

under mild conditions, and this isomerization can be interpreted in terms of Smiles-type rearrangement.[5] Therefore, it seems likely that the macaranoyl group with its crowded substitution mode is isomerized to the valoneoyl group with a sterically less hindered structure, probably *via* the tergalloyl group, by Smiles rearrangement (Chart 2). The formation of **5** is explicable by the ether cleavage of the macaranoyl or valoneoyl group.[23]

The Smiles-type rearrangement has been successfully applied to chemically correlate **3** and **1**. That is, an aqueous solution of **3** containing a small amount of phosphate buffer (pH 7.4) was left standing at room temperature for 3 h to give an isomerized product identical with **1**. Similarly, **3b** was converted into **1b**. Based on these findings, the structure of euphorbin I including the orientation and absolute configuration of the macaranoyl group at O-3/O-6 of glucose was concluded to be represented by the formula **3**.[24]

Upon monitoring the chemical transformation of **3** into **1** by reversed-phase HPLC, we found that the isomerization proceeded *via* an intermediate, whose retention time was identical with that of euphorbin B. This finding, which is inconsistent with the reported structure (**2c**)[2e] of euphorbin B, prompted us to re-investigate its structure. Euphorbin B was previously assigned as the regio-isomer (**2c**) of **1** concerning the binding sites of the valoneoyl group at O-3/O-6 of the $^1C_4$ glucose core, based on spectral data and the hydrolyzates (valoneic acid dilactone and gallic acid) produced by hydrolysis with hot sulfuric acid, which were similar to those of **1**. The reconfirmation of the constituent acyl units of euphorbin B was thus first attempted. A methylated derivative of the euphorbin B phenazine (**2a**), which was prepared by treatment with diazomethane, yielded, upon methanolysis with sodium methoxide, trimethyl octa-*O*-methyltergallate (**7**)[25] in addition to **4** and **9**. On the other hand, methanolysis of the methylated euphorbin B phenazine derivative (**2a**) prepared under weakly alkaline conditions (dimethyl sulfate and potassium carbonate in acetone) afforded trimethyl octa-*O*-methylvaloneate (**6**) and dimethyl hexamethoxydiphenate (**5**) as minor products, besides **7**, **4** and **9**. The former two were regarded as secondary products formed by isomerization and ether cleavage of the tergalloyl group, respectively, leading to the conclusion that euphorbin B has a tergalloyl group, but not a valoneoyl group, as the linking unit between the monomers. The formation of the valoneic acid dilactone in the acid hydrolysis of **2**, which led to the previous erroneous conclusion regarding the constituent unit, was thus regarded as a consequence of isomerization of the tergalloyl group.

In order to confirm the orientation of the tergalloyl group at O-3/O-6 of the glucose-II, Smiles-type rearrangement was again applied to chemical correlation between euphorbins B and A. The isomerized product obtained by treatment of euphorbin B with phosphate buffer was identified as **1**. Similarly, the acetone adduct of euphorbin B (**2b**) was also converted into the corresponding derivative (**1b**) of euphorbin A.[26] Based on these observations, the structure of euphorbin B was revised to the formula **2**.

The possibility that euphorbins A and B might be artefacts formed from euphorbin I is ruled out by the fact that **1** and **2** are the main tannins in the fresh leaves of many Euphorbiaceous plants in which **3** has never been found.

Experimental

General Instruments and chromatographic methods employed in this work were the same as those described in the preceding paper.[1]

Isolation of Tannins The fresh leaves (1.1 kg) of *E. watanabei*, collected in July 1993, were homogenized three times in acetone–H_2O (7 : 3) (7 l × 3) and the homogenate was filtered. The concentrated solution (*ca.* 2 l) was extracted with ether (0.8 l × 3), EtOAc (0.6 l × 10) and *n*-BuOH saturated with H_2O (0.6 l × 10), successively. A part (3.0 g) of the EtOAc extract 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)→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 give quercitrin (20 mg), isoquercitrin (3.4 mg), quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-glucopyranoside (5.6 mg), quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-galactopyranoside (11 mg), guaijaverin (1.2 mg), quercetin 3-*O*-(2″-*O*-galloyl)-α-L-arabinopyranoside (40 mg), 1,3,4,6-tetra-*O*-galloyl-*β*-D-glucose (4.3 mg), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (31 mg), terchebin (10 mg), geraniin (441 mg), galloylgeraniin (17 mg), didehydrogeraniin (50 mg), mallotusinic acid (22 mg), chebulagic acid (5 mg) and euphorbin I (**3**) (24 mg). The *n*-BuOH extract (20 g) was subjected to column chromatography over Dia-ion HP-20 (2.8 cm i.d. × 40 cm) with MeOH–H_2O (5 : 95→3 : 7→4 : 6→5 : 5→7 : 3)→MeOH in a stepwise gradient mode. The eluate with MeOH–H_2O (5 : 5) (5.8 g) was further chromatographed over Toyopearl HW-40 (fine) (2.2 cm i.d. × 23 cm) with MeOH–H_2O (6 : 4→7 : 3)→MeOH–H_2O–acetone (7 : 2 : 1)→acetone–H_2O (7 : 3). 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 give mallotusinin (5 mg), euphorbin A (**1**) (30 mg), euphorbin B (**2**) (23 mg) and euphorbin I (**3**) (26 mg). The eluate with MeOH–H_2O (3 : 7) (1.0 g) in the Dia-ion HP-20 column chromatography was similarly purified by repeated chromatographies on Toyopearl HW-40 (fine), Sephadex LH-20 and MCI-gel CHP-20P to afford mallojaponin (4 mg), elaeocarpusin (20 mg) and euphorhelin (8 mg). The H_2O extract (80 g) was subjected to column chromatography over Dia-ion HP-20 (2.8 cm i.d. × 40 cm) with H_2O→MeOH–H_2O (2 : 8→4 : 6→6 : 4)→MeOH. The eluate with MeOH–H_2O (2 : 8) (5.2 g) was further chromatographed over Toyopearl HW-40 (fine) and MCI-gel CHP-20P to give putranjivain A (42 mg), mallotunin (29 mg), 3-*O*-caffeoylquinic acid (6 mg) and 4-*O*-caffeoylquinic acid (14 mg). The eluate with MeOH–H_2O (4 : 6) (6.2 g) was also rechromatographed over Toyopearl HW-40 (fine) to give hellioscopinin B (2 mg), corilagin (24 mg) and mallotinic acid (42 mg).

Euphorbin I (3) A light brown amorphous powder, $[\alpha]_D$ −20° (c = 1.0, MeOH). FAB-MS m/z: 1913 $(M+Na)^+$. *Anal.* Calcd for $C_{82}H_{58}O_{53}\cdot 12H_2O$: C, 46.73; H, 3.92. Found: C, 46.53; H, 3.63. UV λ_{max}^{MeOH} nm (log ε): 217 (5.17), 275 (4.81). CD (MeOH) [θ] (nm): $+3.0\times10^4$ (202), -1.9×10^5 (230), $+4.5\times10^4$ (236), -3.4×10^4 (292). ^{13}C-NMR (acetone-d_6–D_2O) δ: 46.2, 51.9 (C-1″), 147.6, 154.2 (C-2″), 125.0, 128.6 (C-3″), 191.8, 194.8 (C-4″), 92.3, 96.3 (C-5″), 92.5, 108.9 (C-6″) (DHHDP).

Preparation of the Phenazine Derivatives A solution of *o*-phenylenediamine (8 mg) in 20% AcOH (2 ml) was added to a solution of tannin (20 mg) in MeOH (3 ml), and the reaction mixture was left standing overnight at room temperature. The residue obtained upon evaporation of the solvent was suspended in H_2O, and the insoluble material was collected and washed with H_2O. The dark orange solid was reprecipitated from MeOH–$CHCl_3$ to give the phenazine derivative (15 mg) as an orange amorphous powder.

3a: $[\alpha]_D$ −11° (c = 1.0, MeOH). FAB-MS m/z: 1967 $(M+Na)^+$. *Anal.* Calcd for $C_{88}H_{60}O_{50}N_2\cdot 12H_2O$: C, 48.90; H, 3.92; N, 1.30. Found: C, 49.07; H, 3.90; N, 1.26. UV λ_{max}^{MeOH} nm (log ε): 219 (5.17), 278 (4.92). CD (MeOH) [θ] (nm): -11.0×10^4 (221), $+16.2\times10^4$ (256), -18.3×10^4 (286), $+2.9\times10^4$ (327). ^{1}H-NMR (acetone-d_6–D_2O) δ: 8.32, 8.23 (each 1H, br d, J = 9 Hz), 8.28 (1H, s), 7.98 (2H, m), 7.49 (1H, s) (phenylphenazine), 7.16, 7.043, 7.03, 7.00, 6.96 (each 2H, s, galloyl), 7.044, 6.95, 6.84 (each 1H, s, macaranoyl), glucose protons, see Table 1. ^{13}C-NMR (acetone-d_6–D_2O) δ: 93.5 (C-1), 71.8 (C-2), 73.1 (C-3), 69.4 (C-4), 73.6 (C-5), 62.7 (C-6), 91.6 (C-1′), 76.3 (C-2′), 68.6 (C-3′), 68.4 (C-4′), 76.7 (C-5′), 65.9 (C-6′) (glucose), 168.8, 168.4, 167.7, 166.7, 166.6, 166.5, 165.9, 165.5, 165.3, 164.6 (ester carbonyl).

1a: ^{1}H-NMR (acetone-d_6–D_2O) δ: 8.31, 8.22 (each 1H, dd, J = 1.5, 7.5 Hz), 8.27 (1H, s), 7.98 (2H, m), 7.48 (1H, s) (phenylphenazine), 7.14, 7.13, 7.06, 6.96, 6.90 (each 2H, s, galloyl), 7.01, 7.00, 6.30 (each 1H, s, valoneoyl), glucose protons, see Table 1. ^{13}C-NMR (acetone-d_6–D_2O) δ: 93.5 (C-1), 71.7 (C-2), 73.7 (C-3), 69.1 (C-4), 74.0 (C-5), 62.7 (C-6), 91.7 (C-1′), 76.8 (C-2′), 68.8 (C-3′), 67.9 (C-4′), 77.1 (C-5′), 65.4 (C-6′) (glucose), 169.9, 167.2, 166.6, 166.5, 166.4, 166.2, 165.6, 165.3, 164.8, 164.7 (ester carbonyl).

2a: ^{1}H-NMR (acetone-d_6–D_2O) δ: 8.31, 8.23 (each 1H, dd, J = 1.5, 7.5 Hz), 8.28 (1H, s), 7.98 (2H, m), 7.46 (1H, s) (phenylphenazine), 7.15, 7.11, 7.05, 7.00, 6.96 (each 2H, s, galloyl), 7.01, 6.82, 6.45 (each 1H, s, tergalloyl), glucose protons, see Table 1. ^{13}C-NMR (acetone-d_6–D_2O) δ: 93.2 (C-1), 72.1 (C-2), 73.3 (C-3), 69.4 (C-4), 73.8 (C-5), 62.8 (C-6), 91.5 (C-1′), 76.5 (C-2′), 68.6 (C-3′), 67.8 (C-4′), 76.6 (C-5′), 65.8 (C-6′) (glucose), 168.3, 167.9, 167.6, 166.7, 166.6 (2C), 166.3, 165.9, 165.4, 164.9 (ester carbonyl).

Methylation of 3a Followed by Methanolysis A solution of **3a** (10 mg) in EtOH (1 ml) was treated with an excess of ethereal CH_2N_2 at room temperature overnight. After evaporation of the solvent, 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 to give methyl tri-*O*-methylgallate (**4**) [2.5 mg, EI-MS m/z 226 (M^+)], dimethyl hexamethoxydiphenate (**5**) [1.0 mg, EI-MS m/z 450 (M^+)], methyl 4-methoxy-3-(4,5,6-trimethoxy-2-methoxycarbonylphenyl) phenazine-2-carboxylate (**9**) [1.8 mg, EI-MS m/z 492 (M^+)], trimethyl octa-*O*-methylvaloneate (**6**) [0.5 mg, EI-MS m/z 660 (M^+)] and trimethyl octa-*O*-methylmacaranate (**8**) [1.5 mg, EI-MS m/z 660 (M^+), $[\alpha]_D$ −2° (c = 0.4, acetone). CD (MeOH) [θ] (nm): -3.1×10^4 (231), $+1.6\times10^4$ (262), -8.0×10^3 (320). ^{1}H-NMR (acetone-d_6) δ: 7.38, 7.27, 6.98 (each 1H, s), 3.78 (6H, s) 3.93, 3.87, 3.79, 3.71, 3.65, 3.61, 3.58, 3.56, 3.42 (each 3H, s)], which were identified by direct comparison with authentic samples (TLC, HPLC, MS and ^{1}H-NMR).

Preparation of the Acetone Adducts of 1, 2 and 3 A solution of **3** (30 mg) and ammonium formate (15 mg) in acetone (3 ml) was heated at 50 °C for 4 h, then concentrated and the residue was submitted to column chromatography over MCI-gel CHP-20P with H_2O containing increasing amounts of MeOH. The 40% MeOH eluate gave euphorbin I acetone adduct (**3b**) (5.6 mg), as a white amorphous powder, $[\alpha]_D$ −36° (c = 0.5, MeOH). FAB-MS m/z: 1953 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 218 (5.17), 278 (4.82). ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.18, 7.15, 7.05, 7.03, 7.00 (each 2H, s, galloyl), 7.10, 6.92, 6.77 (each 1H, s, macaranoyl), 7.21 (1H, s), 6.27, 4.88 (each 1H, d, J = 1 Hz), 3.46, 2.96 (each 1H, d, J = 15 Hz), 2.17 (3H, s) (acetonyl-DHHDP), glucose protons, see Table 1.

Euphorbin A (**1**) and euphorbin B (**2**) (each 5 mg) were similarly treated with ammonium formate (2.5 mg) in acetone (1 ml) to give the acetone adducts **1b** and **2b** (each 4 mg), each as a white amorphous powder.

1b: $[\alpha]_D$ −30° (c = 0.5, MeOH). FAB-MS m/z: 1953 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 219 (5.09), 287 (4.73). ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.16, 7.14, 7.09, 7.07, 6.88 (each 2H, s, galloyl), 7.11, 6.98, 6.21 (each 1H, s, valoneoyl), 7.21 (1H, s), 6.25, 4.88 (each 1H, d, J = 1 Hz), 3.43, 2.96 (each 1H, d, J = 15.5 Hz), 2.12 (3H, s) (acetonyl-DHHDP), glucose protons, see Table 1.

2b: $[\alpha]_D$ −27° (c = 0.5, MeOH). FAB-MS m/z: 1953 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 220 (5.20), 284 (4.85). ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.17, 7.14, 7.09, 7.04, 7.00 (each 2H, s, galloyl), 7.11, 6.80, 6.34 (each 1H, s, tergalloyl), 7.20 (1H, s), 6.31, 4.89 (each 1H, d, J = 1.5 Hz), 3.45, 2.96 (each 1H, d, J = 15.5 Hz), 2.18 (3H, s) (acetonyl-DHHDP), glucose protons, see Table 1.

Isomerization of Euphorbin I (3) and Euphorbin B (2) to Euphorbin A (1) A solution of **3** (15 mg) [or **2** (10 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 the reaction was monitored by HPLC. After disappearance of the starting material (8 h), the reaction mixture was acidified with diluted HCl, and applied to a Sep-Pak cartridge (Waters), which was washed with water. The product in the MeOH eluate was further purified by preparative HPLC [YMC-pack ODS A324 (10 × 300 mm); solvent 0.01 M H_3PO_4–0.01 M KH_2PO_4–CH_3CN (41 : 41 : 18)] to give euphorbin A (**1**) (3 mg from **3**; 2 mg from **2**), which was found to be identical with an authentic sample by ^{1}H-NMR spectral comparison.

Isomerization of the Acetone Adducts of Euphorbin I and Euphorbin B to Euphorbin A Acetone Adduct (1b) A solution of **3b** (or **2b**) (1 mg) in 0.02 M phosphate buffer (pH 7.4) (1 ml) was left standing at room temperature for 12 h. The reaction mixture was acidified with diluted HCl, and passed through a Sep-Pak cartridge, which was washed with water. The eluate with 40% MeOH yielded an isomerized product (0.5 mg from **3b**; 0.3 mg from **2b**) that was identical with **1b** by HPLC.

Methylation of 2a Followed by Methanolysis a) A solution of **2a** (10 mg) in EtOH (1 ml) was treated with ethereal CH_2N_2 at room temperature overnight. After removal of the solvent, the methylated product without further purification was subjected to methanolysis with 1% NaOMe in MeOH (1 ml) at room temperature overnight. After acidification with AcOH and evaporation, the residue was purified by preparative TLC to afford methyl tri-*O*-methylgallate (**4**) (2.0 mg), dimethyl hexamethoxydiphenate (**5**) (0.5 mg), methyl 4-methoxy-3-(4,5,6-trimethoxy-2-methoxycarbonylphenyl) phenazine-2-carboxylate (**9**) (1.5

mg) and trimethyl octa-*O*-methyltergallate (**7**) [1.6 mg, EI-MS *m/z* 660 (M^+), $[\alpha]_D$ +8° (*c* = 0.5, acetone). CD (MeOH) [θ] (nm): -1.2×10^4 (222), $+1.5 \times 10^4$ (252), -5.5×10^3 (323). ^{1}H-NMR (acetone-d_6) δ: 7.37, 7.34, 7.18 (each 1H, s), 3.92, 3.87, 3.86, 3.84, 3.76, 3.72, 3.578, 3.576, 3.572, 3.54, 3.39 (each 3H, s)], which were identified by direct comparison with authentic samples (TLC, HPLC, MS and ^{1}H-NMR).

b) A mixture of **2a** (5 mg), K_2CO_3 (100 mg) and $(CH_3)_2SO_4$ (0.01 ml) in acetone (5 ml) was stirred overnight at room temperature, and then refluxed for 2 h. After removal of the inorganic material by centrifugation, the supernatant was evaporated to dryness. The reaction mixture was directly methanolyzed in 1% NaOMe solution, and subjected to preparative TLC to give **4** (0.8 mg), **5** (0.1 mg), trimethyl octa-*O*-methylvaloneate (**6**) (0.1 mg), **7** (0.5 mg) and **9** (0.5 mg), which were identified by direct comparison with authentic samples (TLC, HPLC).

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References and Notes

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