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# Tannins and Related Polyphenols of Euphorbiaceous Plants. IV.[1] Euphorbins A and B, Novel Dimeric Dehydroellagitannins from *Euphorbia hirta* L.

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Two new dimeric dehydroellagitannins, named euphorbin A (**6**) and euphorbin B (**7**), were isolated from the aerial parts of *Euphorbia hirta*, and their structures, containing $^4C_1$ and $^1C_4$ glucopyranose residues and a dehydrohexahydroxydiphenoyl group, were elucidated on the basis of chemical and spectral studies. Five monomeric hydrolyzable tannins, *i.e.*, 2,4,6-tri-*O*-galloyl-D-glucose, 1,3,4,6-tetra-*O*-galloyl-β-D-glucose, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, geraniin and terchebin, as well as two quinic acid esters, *i.e.*, 5-*O*-caffeoylquinic acid and 3,4-di-*O*-galloylquinic acid, and three flavonol glycosides were also isolated.

**Keywords**——*Euphorbia hirta*; Euphorbiaceae; tannin; dimeric hydrolyzable tannin; euphorbin A; euphorbin B; dehydrohexahydroxydiphenoyl group; dehydroellagitannin

*Euphorbia hirta* L. (Chinese name: fēi yang cǎo) (Euphorbiaceae) is a pantropic herbal plant, and its decoction has been traditionally used as an antidiarrheic, an antidiuretic and an expectorant, and also as a remedy for bronchitis, asthma, intestinal ailments of children and various skin diseases in China and Indonesia.[2] Some phytochemical studies of this plant have revealed the presence of diterpenoids,[3] triterpenoids,[3,4] and flavonoids[4,5] in the bark and leaves. However, the tannins which should be responsible for the main activities of the herb have not yet been investigated. We have now isolated two dimeric hydrolyzable tannins of a new class, referred to as euphorbin A and euphorbin B,[6] together with twelve known polyphenols, from the aerial parts of this plant and elucidated the structures of the new tannins.

An aqueous acetone homogenate of the dried leaves of *E. hirta* L. was extracted successively with ether, ethyl acetate and *n*-butanol. The ethyl acetate extract was chromatographed on Toyopearl HW-40 and Sephadex LH-20 using aqueous alcohol as an eluant to yield euphorbin A and euphorbin B, in addition to three galloylglucoses [2,4,6-tri-*O*-galloyl-D-glucose (**1**),[7] 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**2**)[8] and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**3**)[9]], and a dehydroellagitannin [terchebin (**4**)[10]]. The identity of the latter four tannins was established by proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy and cochromatography (high-performance liquid chromatography, HPLC). Two additional compounds isolated were identified as flavonol glycosides [quercitrin and myricitrin]. Chromatographic separation of the *n*-butanol extract afforded geraniin (**5**)[11] and two quinic acid esters [5-*O*-caffeoylquinic acid[12] and 3,4-di-*O*-galloylquinic acid[13]].

Dried stems of the plant were similarly treated to yield gallic acid, ellagic acid and isoquercitrin as well as quercitrin.

It is noteworthy that terchebin (**4**), a rare tannin which had been isolated only from

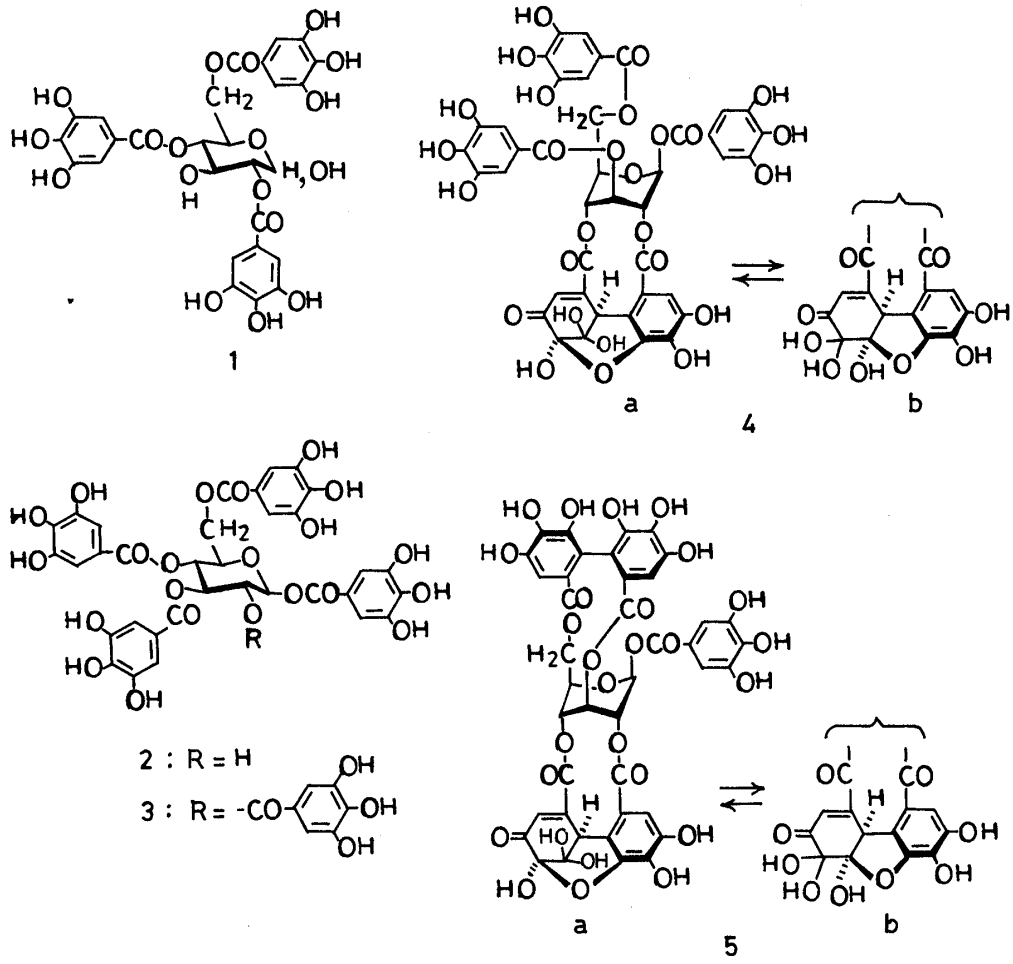


Chart 1

*Terminalia chebula* (Combretaceae),[10] has now been found in *Euphorbia* species.

The new tannins, euphorbins A and B were assigned the structures **6** and **7**, respectively, on the basis of the following evidence.

Euphorbin A, $[\alpha]_D$ −43° (MeOH), $C_{82}H_{58}O_{53}\cdot 5H_2O$, was isolated as a light yellow amorphous powder. Acid hydrolysis of **6** afforded gallic acid, valoneic acid dilactone and ellagic acid, which were characterized as the corresponding methyl derivatives. Glucose was also identified by gas liquid chromatography (GLC) after trimethylsilylation. The $^1$H-NMR spectrum of **6** exhibited methine proton signals at $\delta$ 5.13 (s) (**6a**, H-1″) and 4.94 (d, $J$=2 Hz) (**6b**, H-1″), and vinyl proton signals at $\delta$ 6.52 (s) (**6a**, H-3″) and 6.19 (d, $J$=2 Hz) (**6b**, H-3″), which are characteristic of a dehydrohexahydroxydiphenoyl (DHHDP) group equilibrating between six- and five-membered hemiacetal forms (**6a**⇄**6b**), as found for **4** and **5** in aqueous solution.[10,11] The presence of a valoneoyl group, five galloyl groups and two glucose residues was also indicated, although individual protons appear as duplicate signals induced by the equilibration of the DHHDP group. The glucose protons showed a resonance patterns typical of the $^4C_1$ and $^1C_4$ glucopyranose, and the chemical shifts of protons on the latter (glucose core II) are well compatible with those of geraniin (**5**) (Table I). Confirmation of the presence of the DHHDP group in **6** was obtained by the production of a phenazine derivative (**8**), $[\alpha]_D$ +49° (MeOH), upon condensation of **6** with *o*-phenylenediamine in an acidic medium. The $^1$H-NMR spectrum of **8** was more informative than that of **6** as regards the structural units because of the absence of peak duplication for each proton. In the aromatic proton region, sharp singlets at $\delta$ 8.30 and 7.51, and multiplets at $\delta$ 8.33, 8.21 and 7.99, which are assignable

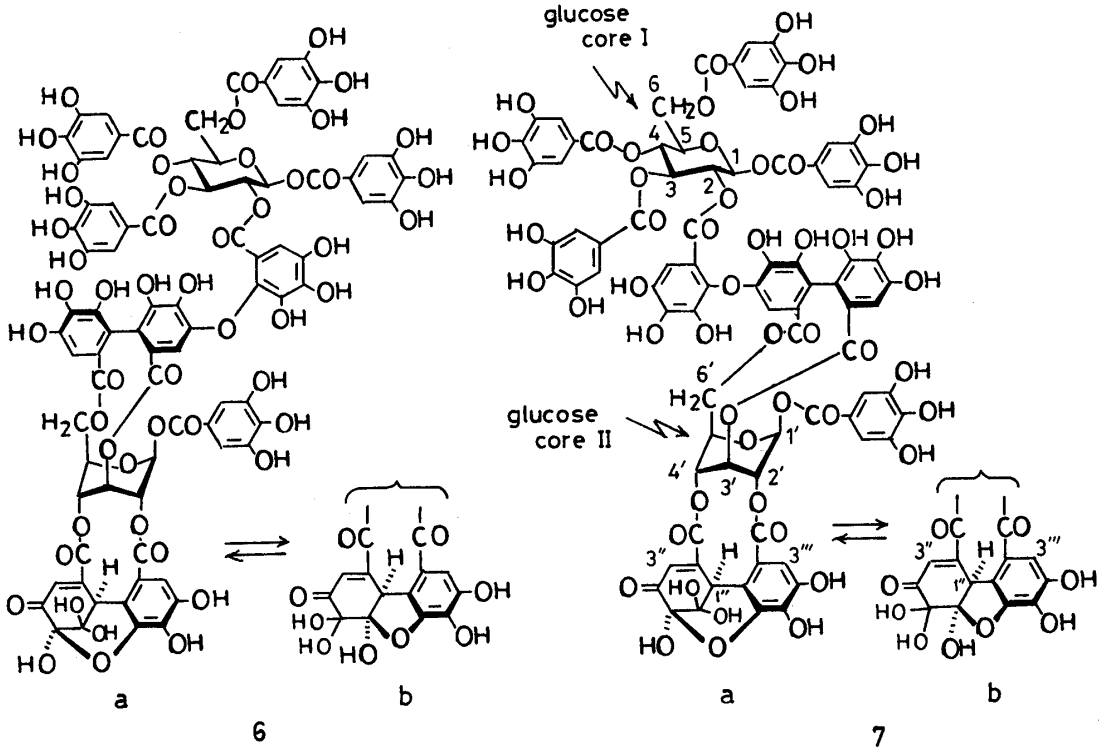


Chart 2

to the phenylphenazine moiety, were observed. Two-proton singlets at $\delta$ 7.16, 7.15, 7.07, 6.98 and 6.90, due to five galloyl groups, and the one-proton singlets at $\delta$ 7.02, 7.00 and 6.34, attributable to a valoneoyl group, were also exhibited. Upon comparison of the sugar proton signals of **8** with those of **6**, a significant difference was observed in the coupling pattern of the protons in the glucose core II (Table II), which is indicative of a conformational change from $^1C_4$ to skew boat form. A marked upfield shift for H-1′ ($\delta$ 6.51→6.15) and downfield shift for H-5′ ($\delta$ 4.69→4.96) in the glucose core II were also observed. These distinguishing spectral features between **8** and **6** are analogous to those observed upon transformation of geraniin (**5**) to its phenazine derivative (**9**),[11] and thus the shifts of H-1′ and H-5′ are reasonably explained by the anisotropy effect of the phenylphenazine moiety in the conformation illustrated by the stereostructure **8**. In addition, the proton signals for the glucose core II in **8** are again in good agreement with those of **9**[14] (Table II). These observations led to the assignment of the geraniin structure including stereostructure, as one of the constituent monomer units of **8**, and consequently of **6**. Another monomeric unit in **6** was suggested to be pentagalloylglucose (**3**) from the carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum of **8**. In this spectrum, the glucose carbon signals showed chemical shifts virtually identical with those of **3** and **9** (Table III). Accordingly, euphorbin A is regarded as a dimer produced biogenetically by intermolecular C–O oxidative coupling between the galloyl group in pentagalloylglucose (**3**) and the hexahydroxydiphenoyl group at O-3′—O-6′ of geraniin (**5**). This assignment, based on the spectral data, was substantiated by the following chemical evidence.

Upon partial hydrolysis in boiling water for 1 h, **8** gave a hydrolysate (**10**), $[\alpha]_D$ −9.4° (MeOH), and phenylphenazine dilactone (**11**).[15] The $^1$H-NMR spectrum of **10** showed the retention of the five galloyl groups and the valoneoyl group. One of the two glucose cores in **10** again adopts $^1C_4$ conformation as revealed by the coupling pattern (see Experimental), and remarkable upfield shifts for H-2′ ($\delta$ 5.68→4.09) and H-4′ ($\delta$ 5.56→4.39), from those of **8**, were observed, indicating the location of the phenylphenazine group at O-2′ and O-4′ in **8**. Methanolysis of **8** in a mixture of acetate buffer (pH 6.0) and methanol at 37 °C for 20 h, gave,

TABLE I. $^1$H-NMR Chemical Shifts of the Glucose Moieties of **3**, **5**, **6** and **7** (500 MHz, Acetone-$d_6$) ($J$ in Hz)

| | | **3**[a] | **5**[a] | | **6** | | **7** | |
|---|---|---|---|---|---|---|---|---|
| | | | **a** | **b** | **a** | **b** | **a** | **b** |
| Glucose core I | H-1 | 6.39 (d, $J$=9.5) | | | 6.22 (d, $J$=8) | 6.14 | 6.30 (d, $J$=9.5) | |
| | H-2 | 5.66 (t, $J$=9.5) | | | 5.65 (t, $J$=8) | 5.62 | 5.71 (dd, $J$=8, 10) | |
| | H-3 | 6.06 (t, $J$=9.5) | | | 5.51 (t, $J$=8) | 5.52 | 6.04 (t, $J$=10) | |
| | H-4 | 5.70 (t, $J$=9.5) | | | 5.55 (dd, $J$=8, 8.5) | | 5.68 (t, $J$=10) | 5.67 |
| | H-5 | 4.60 (m) | | | 4.34 (m) | | 4.55 (m) | |
| | H-6 | 4.60 (m) 4.45 (dd, $J$=3, 12) | | | 4.51 (dd, $J$=12, 1.6) 4.37 (d, $J$=12) | 4.49 | 4.55 (m) 4.33 (dd, $J$=5, 12) | |
| Glucose core II | H-1′ | | 6.60 (br s) | | 6.51 (br s) | | 6.56 (br s) | |
| | H-2′ | | 5.60 (br s) | | 5.60 (br s) | | 5.60 (br s) | |
| | H-3′ | | 5.50 (br s) | 5.60 | 5.46 (br s) | 5.40 | 5.56 (br s) | 5.48 |
| | H-4′ | | 5.56 (br s) | 5.46 | 5.57 (br s) | 5.61 | 5.59 (br s) | 5.48 |
| | H-5′ | | 4.81 (m) | | 4.69 (br dd, $J$=7, 10) | | 4.81 (br dd, $J$=7, 10) | |
| | H-6′ | | 4.93 (t, $J$=11) 4.33 (dd, $J$=8, 11) | 4.78 (m) 4.45 (dd, $J$=6, 9) | 4.86 (t, $J$=10) 4.24 (dd, $J$=7, 10) | | 4.89 (t, $J$=10) 4.32 (dd, $J$=7, 10) | 4.76 4.41 |

a) 400 MHz.

in addition to methyl gallate and **11**, three partial hydrolysates among which two were characterized as 1,3,4,6-tetra-*O*-galloyl-*β*-D-glucose (**2**) and 3,4,6-tri-*O*-galloyl-D-glucose (**12**), based on their $^1$H-NMR spectral data (see Experimental). The third hydrolysate was identified as corilagin (**13**)[16] which could be produced by cleavage of the ether bond of the valoneoyl group. Production of ellagic acid upon the afore-mentioned acid hydrolysis of **6** is thus explainable by hydrolysis of **13** and also partly by disproportionation of the DHHDP group.[17] From these results, the location of the valoneoyl group in **8**, and consequently in **6**, was established to be at O-3′—O-6′ of glucose core II and at O-2 of glucose core I. In order to obtain further proof of the absolute configurations of the valoneoyl group and C-1″ of the DHHDP group in **6**, methanolysis of the permethylated derivative (**14**), $[\alpha]_D$ +11° (acetone), was carried out, and trimethyl octa-*O*-methylvaloneate (**15**) and dimethyl ester (**16**) were obtained along with methyl tri-*O*-methylgallate. The specific rotations of **15** [+13° (acetone)] and **16** [+33° (acetone)] indicated their chiralities to be in the *R*-series.[1b,11] As the chirality of **16** arises from the configuration at the methine carbon of the precursor (DHHDP group),[11] the absolute configuration at C-1″ of the DHHDP group in **6** was established to be *R*, which is the same as that of **5**.[11] The orientation of the valoneoyl group in the proposed structure **6** will be discussed later.

Euphorbin B (**7**), $C_{82}H_{58}O_{53}\cdot 7H_2O$, $[\alpha]_D$ −26° (MeOH), was obtained as a light yellow

TABLE II. $^1$H-NMR Chemical Shifts of the Glucose Moieties of **3**, **8**, **9** and **17** (400 MHz, Acetone-$d_6$)

| | | **3** | **9** | **8** | **17**[a] |
|---|---|---|---|---|---|
| Glucose core I | H-1 | 6.39 (d, $J$=9.5) | | 6.01 (d, $J$=8) | 6.36 (d, $J$=8.5) |
| | H-2 | 5.66 (t, $J$=9.5) | | 5.61 (dd, $J$=8, 9) | 5.73 (dd, $J$=8.5, 10) |
| | H-3 | 6.06 (t, $J$=9.5) | | 5.56 [b] | 6.07 (t, $J$=10) |
| | H-4 | 5.70 (t, $J$=9.5) | | 5.56 [b] | 5.67 (t, $J$=10) |
| | H-5 | 4.60 (m) | | 4.30 (m) | 4.57 (ddd, $J$=1.5, 4, 10) |
| | H-6 | 4.60 (m) | | 4.48 (br d, $J$=11) | 4.53 (dd, $J$=1.5, 13) |
| | | 4.45 (dd, $J$=3, 12) | | 4.32 (br d, $J$=11) | 4.41 (dd, $J$=4, 13) |
| Glucose core II | H-1′ | | 6.18 (d, $J$=6) | 6.15 (d, $J$=6) | 6.21 (d, $J$=5.5) |
| | H-2′ | | 5.69 (d, $J$=6) | 5.68 (dd, $J$=6, 1) | 5.69 (dd, $J$=5.5, 1) |
| | H-3′ | | 5.45 (d, $J$=3.5) | 5.44 (dd, $J$=4, 1) | 5.52 (br d, $J$=4) |
| | H-4′ | | 5.55 (d, $J$=3.5) | 5.56 (br d, $J$=4) | 5.56 (dd, $J$=4, 1) |
| | H-5′ | | 4.99 (dd, $J$=4, 8) | 4.96 (dd, $J$=4, 8) | 5.05 (dd, $J$=4.5, 8) |
| | H-6′ | | 4.72 (dd, $J$=8, 11) | 4.67 (dd, $J$=6, 12.5) | 4.75 (dd, $J$=8, 12) |
| | | | 4.03 (dd, $J$=4, 11) | 4.12 (dd, $J$=4, 12.5) | 4.09 (dd, $J$=4.5, 12) |

*a*) 500 MHz. *b*) Overlapped with each other.

TABLE III. $^{13}$C-NMR Chemical Shifts of the Glucose Moieties of **3**, **8**, **9** and **17** (126 MHz, Acetone-$d_6$)

| | | **3** | **9**[a,b] | **8**[b] | **17** |
|---|---|---|---|---|---|
| Glucose core I | C-1 | 93.3 | | 93.5 | 93.2 |
| | C-2 | 71.7 | | 71.7 | 72.1 |
| | C-3 | 73.2 | | 73.7 | 73.3 |
| | C-4 | 69.3 | | 69.1 | 69.4 |
| | C-5 | 73.9 | | 74.0 | 73.8 |
| | C-6 | 62.8 | | 62.7 | 62.8 |
| Glucose core II | C-1′ | | 91.6 | 91.7 | 91.5 |
| | C-2′ | | 76.6 | 76.8 | 76.5 |
| | C-3′ | | 68.7 | 68.8 | 68.6 |
| | C-4′ | | 67.6 | 67.9 | 67.8 |
| | C-5′ | | 76.8 | 77.1 | 76.6 |
| | C-6′ | | 65.2 | 65.4 | 65.8 |

*a*) From reference 14. *b*) 100 MHz.

amorphous powder, and afforded upon acid hydrolysis, the same products (gallic acid, valoneic acid dilactone, ellagic acid and glucose) as those from acid hydrolysis of euphorbin A (**6**). The $^1$H-NMR spectrum of **7** exhibited the signals due to five galloyl groups and a

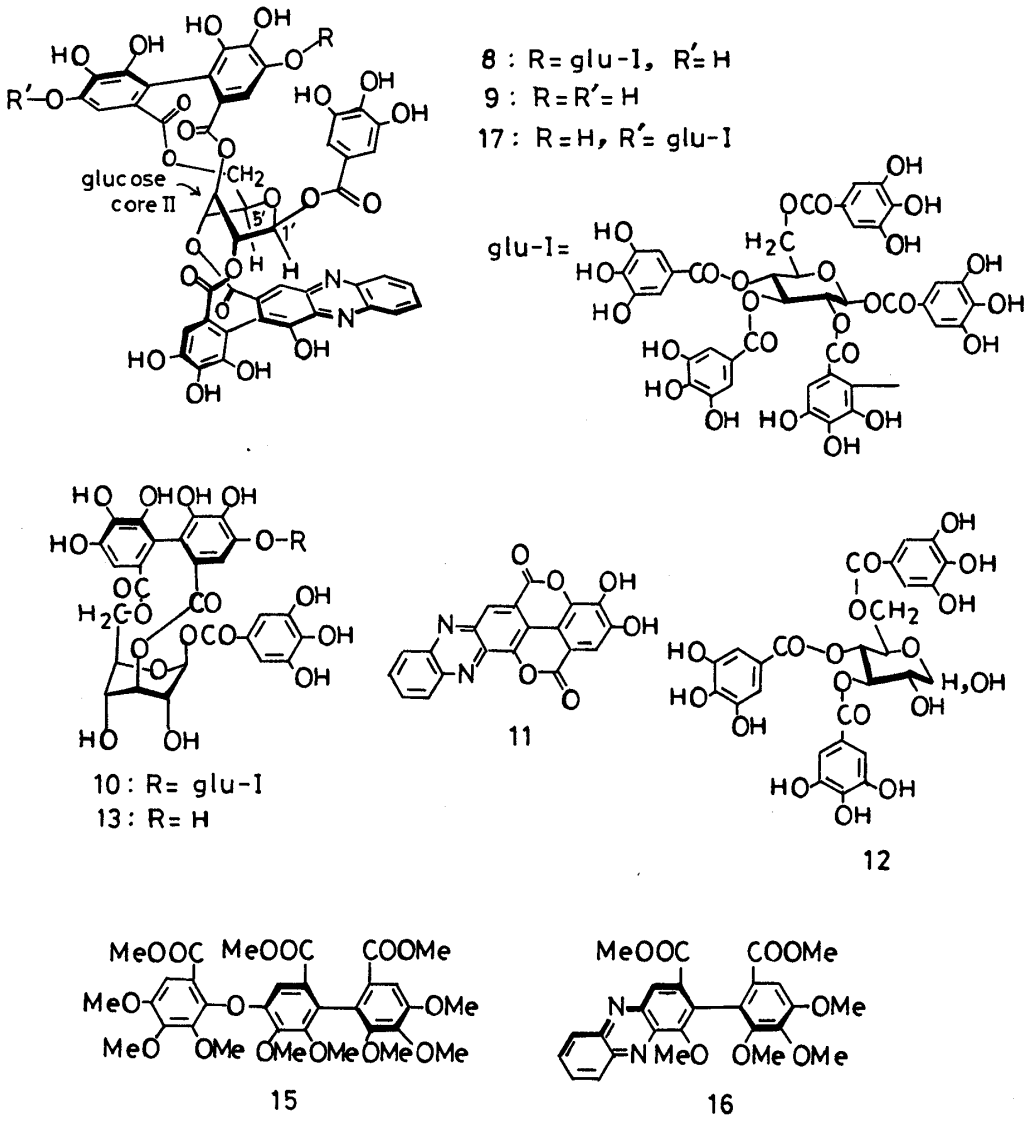


Chart 3

valoneoyl group, which show dual peaks for each proton owing to the presence of an equilibrated DHHDP group. This spectrum also showed the presence of $^4C_1$ and $^1C_4$ glucopyranose residues, and their proton signals coincide with those of **3** and **5**, as shown in Table I. These observations indicate that euphorbin B is a dimeric hydrolyzable tannin closely related to euphorbin A (**6**). A phenazine derivative (**17**), $[\alpha]_D$ +6° (MeOH) was obtained by the treatment of **7** with *o*-phenylenediamine in 15% acetic acid, and its $^1$H-NMR signals (Table II) were analogous to those of euphorbin A-phenazine (**8**) except for the chemical shifts of H-1, H-3 and H-5 which will be discussed later. The $^{13}$C-NMR spectrum also showed close similarity to that of **8** (Table III). Further evidence for the structural resemblance between **8** and **17** was obtained by the partial hydrolysis of **17** in boiling water, which afforded the same products (**2**, **11**, **12** and **13**) as those obtained by methanolysis of **8**. The circular dichroism (CD) spectrum of **17** exhibited a strong positive Cotton effect at 250 nm ($[\theta] = +9.83 \times 10^4$) and a negative one at 283 nm ($[\theta] = -13.6 \times 10^4$), which are almost superimposable on those of **8** and **9** (Fig. 1). These CD spectral data indicate that the configurations of the valoneoyl group and also the phenylphenazine moiety in **17**, are the same as those of **8**. The absolute configuration at C-1'' of the DHHDP group in **7** is also *R*, as in **6**.

These observations indicate that euphorbin B is an isomer of euphorbin A concerning the

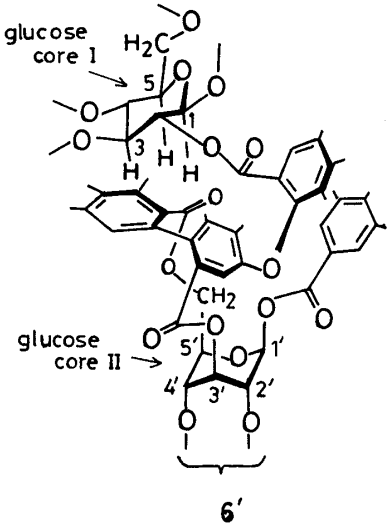


Chart 4

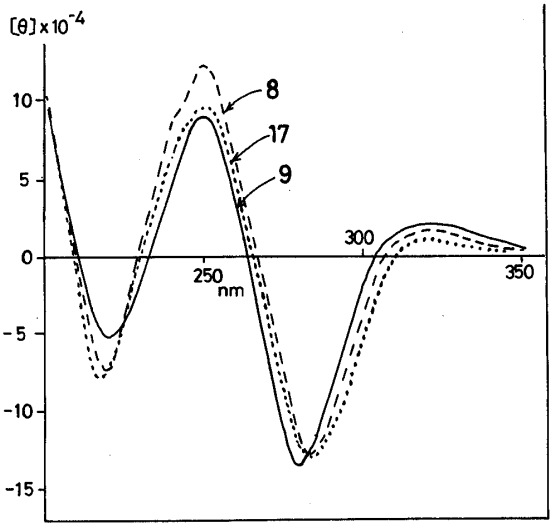


Fig. 1. CD Spectra of Phenazine Derivatives, **8**, **9** and **17** in MeOH

binding sites of the valoneoyl group on O-3′—O-6′ of the glucose core II. The orientations of the valoneoyl group as shown in the formulas **6** and **7** were deduced by $^1$H-NMR spectral analysis. Upon comparison of the $^1$H-NMR spectra between **8** and **17**, noticeable differences in the chemical shifts of the protons at C-1, C-3 and C-5 of the glucose core I were observed as mentioned earlier. These signals of **17** are observed at $\delta$ 6.36 (d, $J$=8.5 Hz, H-1), 6.07 (t, $J$=10 Hz, H-3) and 4.57 (ddd, $J$=1.5, 4, 10 Hz, H-5), respectively, and coincide well with the corresponding signals of 1,2,3,4,6-penta-*O*-galloyl-$\beta$-D-glucose (**3**), whereas these signals in **8** are shifted upfield to $\delta$ 6.01 (d, $J$=8 Hz, H-1), 5.56 (H-3) and 4.30 (m, H-5), respectively. The other proton signals of the glucose core I of **8** and **17** are comparable to those of **3**, as indicated in Table II. Marked upfield shifts of H-1, H-3 and H-5 in the spectra of **6** and the hydrolysate (**10**), relative to those of **3**, were also observed. Therefore, these upfield shifts could be interpreted in terms of the shielding effect by the aromatic ring(s) of the valoneoyl group. Inspection of the Dreiding model shows that the glucose core I of **6** should be fixed in such a way as to force H-1, H-3 and H-5 into an orientation where they are just above the plane of the aromatic ring(s) of the valoneoyl group, as illustrated by the structure **6′**. This conformation could be induced by strictly limited rotation around the ether bond of the valoneoyl group, due to severe steric hindrance between the galloyl group at O-1′ and the bulky monomeric unit (glucose core I) attached to the galloyl part of the valoneoyl group. On the other hand, structure **7** (euphorbin B) apparently permits free rotation around the ether bond, and hence the protons of glucose core I would be unaffected by the neighboring aromatic ring(s), and should show chemical shifts similar to those of the monomer **3**. Consequently the most plausible structures for euphorbin A and B are **6** and **7**, respectively.

These tannins represent a new class of dimeric hydrolyzable tannins which are composed of $^4$C$_1$ and $^1$C$_4$ glucopyranose cores and contain a dehydroellagitannin[18] monomer in the molecule, and are present in equilibrium states induced by the equilibration in the DHHDP group.

## Experimental

Optical rotations were measured on a JASCO DIP-4 polarimeter. NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for $^1$H and 126 MHz for $^{13}$C) at the SC-NMR Laboratory of Okayama University, and on a Bruker AM-400 (400 MHz for $^1$H and 100 MHz for $^{13}$C) spectrometer; the chemical shifts are given in $\delta$-values (ppm) downfield from tetramethylsilane as internal standard. Mass spectrum (MS) were taken on a Shimadzu

LKB-9000 GC-MS spectrometer. Normal phase HPLC was performed on a column of Nomura Develosil 60-5 (4 × 150 mm) with hexane–MeOH–tetrahydrofuran–formic acid (55 : 33 : 11 : 1, v/v) containing oxalic acid (450 mg/l) as an eluant. Reversed-phase HPLC was performed on a column of YMC A312 (ODS) (5 × 150 mm) with 0.01 M $H_3PO_4$–0.01 M $KH_2PO_4$–EtOH–EtOAc (40 : 40 : 15 : 5, v/v). Kieselgel $PF_{254}$ (Merck) was used for analytical and preparative thin layer chromatography (TLC), and the spots were visualized by ultraviolet (UV) irradiation (254 nm). Sephadex LH-20 (100 $\mu$m) (Pharmacia Fine Chemicals) and Toyopearl HW-40 (coarse and fine grades) (Toyo Soda Mfg.) were used for column chromatography. Solvents were removed by evaporation under reduced pressure below 40 °C.

**Isolation of Tannins**——The dried leaves (2.4 kg) of *E. hirta*, collected at Fuzhou, Fujian, China in September, were homogenized in a mixture of acetone–$H_2O$ (7 : 3) and the homogenate was filtered. After removal of acetone, the aqueous solution was extracted with ether, EtOAc and then *n*-BuOH saturated with $H_2O$. The yields of these extracts from the dried leaves were as follows: ether extract, 2.53 g; EtOAc extract, 117 g; *n*-BuOH extract, 133 g. A part of the EtOAc extract (2.4 g) was chromatographed over Sephadex LH-20 (2.2 × 34 cm) developing with 50% EtOH (1.0 l) and then with 70% EtOH (1.5 l). The following five fractions were obtained: fractions I and II eluted with 50% EtOH; fractions III—V eluted with 70% EtOH. Fraction I (540 mg) was rechromatographed over Sephadex LH-20 with EtOH to yield quercitrin (221 mg), myricitrin (81 mg) and 3,4-digalloylquinic acid (37 mg). Fraction II (139 mg) was further chromatographed on Sephadex LH-20 with EtOH to afford 2,4,6-tri-*O*-galloyl-D-glucose (**1**) (15 mg). After rechromatography over Toyopearl HW-40 (fine), fractions III and IV gave 1,3,4,6-tetra-*O*-galloyl-$\beta$-D-glucose (**2**) (31 mg) and 1,2,3,4,6-penta-*O*-galloyl-$\beta$-D-glucose (**3**) (14 mg), respectively. Fraction V gave euphorbin A (**6**) (325 mg). In a separate experiment on the EtOAc extract (20 g) by Toyopearl HW-40 (course) (5 × 25 cm) column chromatography with stepwise elution using aqueous MeOH (60% MeOH→70% MeOH→80% MeOH→MeOH), the fraction eluted with 70% MeOH yielded terchebin (**4**) (39 mg). Euphorbin A (**6**) (3.6 g) was obtained from 80% MeOH eluate. The fraction eluted after **6** was further chromatographed over Sephadex LH-20 using MeOH as the eluant to give euphorbin B (**7**) (181 mg). The BuOH extract (42.6 g) was fractionated by column chromatography over Diaion HP-20 using a mixture of $H_2O$–MeOH. A part (295 mg) of the 30% MeOH eluate (4.1 g) was chromatographed over Toyopearl HW-40 (fine) to yield 5-*O*-caffeoylquinic acid (58 mg) and 3,4-di-*O*-galloylquinic acid (11 mg). A part (9.5 g) of the 50% MeOH eluate (20 g) was further purified by column chromatography over Toyopearl HW-40 (coarse) (70% EtOH) and Sephadex LH-20 (80% EtOH) to give geraniin (**5**) (89 mg).

The dried stems of *E. hirta* (290 g) were treated similarly. The EtOAc extract (410 mg) was chromatographed on a column of Toyopearl HW-40 (coarse) using 50% MeOH as the eluant to give gallic acid (7 mg), isoquercitrin (6 mg), quercitrin (31 mg) and ellagic acid (7 mg).

**Terchebin (4)**——This compound was isolated as a light yellow amorphous powder, $[\alpha]_D$ −40° ($c$ = 1.0, EtOH). $^1$H-NMR (500 MHz, acetone-$d_6$) $\delta$: 7.29, 7.26 (each s, 2H in total), 7.23, 7.17 (each s, 2H in total), 7.05, 7.04 (each s, 2H in total) (galloyl), 7.28, 7.27 (each s, 1H in total, H-3″), 6.57 (s), 6.254 (d, $J$ = 1 Hz) (H-3′), 6.57 (d, $J$ = 2.5 Hz), 6.55 (br s) (H-1), 6.04, 5.80 (each diffused s, H-3), 5.54, 5.52 (each diffused s, H-4), 5.40, 5.30 (each diffused s, H-2), 5.26 (s), 4.96 (d, $J$ = 1 Hz) (H-1′), 4.70 (br dd, $J$ = 7, 8 Hz, H-5), 4.93 (dd, $J$ = 8, 11.5 Hz, H-6), 4.83 (dd, $J$ = 7, 11 Hz, H-6), 4.86 (dd, $J$ = 6, 11.5 Hz, H-6′), 4.72 (dd, $J$ = 6, 11 Hz, H-6′).

**Euphorbin A (6)**——This compound was isolated as a light yellow amorphous powder, $[\alpha]_D$ −43° ($c$ = 1.0, MeOH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 221 (5.19), 280 (4.87). *Anal.* Calcd for $C_{82}H_{58}O_{53} \cdot 5H_2O$: C, 49.71; H, 3.46. Found: C, 49.53; H, 3.53. $^1$H-NMR (500 MHz, acetone-$d_6$), **6a** $\delta$: 7.21, 7.16, 7.114, 7.10, 6.83 (6/5H each, s, galloyl), 7.22, 7.10, 7.02, 6.20, 6.52 (3/5H each, s, valoneoyl and DHHDP), 5.13 (3/5H, s, H-1″); **6b** $\delta$: 7.20, 7.16, 7.11, 7.08, 6.86 (4/5H each, s, galloyl), 7.27, 7.16, 6.99, 6.26 (2/5H each, s), 6.19 (2/5H, d, $J$ = 2 Hz) (valoneoyl and DHHDP), 4.94 (2/5H, d, $J$ = 2 Hz, H-1″).

**Euphorbin B (7)**——This compound was isolated as a light yellow amorphous powder, $[\alpha]_D$ −26° ($c$ = 0.9, MeOH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (5.34), 277 (4.93). *Anal.* Calcd for $C_{82}H_{58}O_{53} \cdot 7H_2O$: C, 48.80; H, 3.60. Found: C, 48.65; H, 3.46. $^1$H-NMR (500 MHz, acetone-$d_6$), **7a** $\delta$: 7.18, 7.15, 7.10, 7.04, 7.004 (10/7H each, s, galloyl), 7.17, 7.11, 6.83, 6.53, 6.42 (5/7H each, s, valoneoyl and DHHDP), 5.15 (5/7H, s, H-1″); **7b** $\delta$: 7.19, 7.14, 7.09, 7.04, 7.002 (4/7H each, s, galloyl), 7.20, 7.13, 6.84, 6.40 (2/7H, each, s), 6.259 (2/7H, d, $J$ = 2 Hz) (valoneoyl and DHHDP), 4.92 (2/7H, d, $J$ = 2 Hz, H-1″).

**Acid Hydrolysis of Euphorbin A (6) and Euphorbin B (7)**——A solution of **6** (5 mg) in 5% $H_2SO_4$ (2 ml) was heated in boiling water bath for 6 h. After cooling, the reaction mixture was extracted with EtOAc. Methylation of the EtOAc soluble portion with $CH_2N_2$, followed by preparative TLC gave methyl tri-*O*-methylgallate [1.6 mg, MS $m/z$: 226 ($M^+$)], tetra-*O*-methylellagic acid [0.2 mg, MS $m/z$: 358 ($M^+$)] and methyl hexa-*O*-methylvaloneate dilactone [0.3 mg, MS $m/z$: 568 ($M^+$)]. The aqueous layer was neutralized with Amberlite IRA-410 (OH form) and filtered. After evaporation, followed by trimethylsilylation, the sugar was identified as glucose by GLC (column, 3% OV-1, column temperature, 170 °C). Euphorbin B (**7**) (5 mg) was similarly hydrolyzed to give the same products as described above.

**Euphorbin A-Phenazine (8)**——A mixture of **6** (100 mg) and *o*-phenylenediamine (20 mg) in MeOH (2 ml) and 15% AcOH (6 ml) was left at room temperature overnight, and then evaporated. The residue was suspended in water and the insoluble material was collected and washed with water and ether. Reprecipitation from MeOH–$CHCl_3$ gave

euphorbin A-phenazine (**8**) (105 mg) as an orange solid, $[\alpha]_D$ +49° ($c$=0.2, MeOH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 221 (5.19), 280 (5.02). *Anal.* Calcd for $C_{88}H_{59}N_2O_{49}\cdot 11H_2O$: C, 49.24; H, 3.80; N, 1.32. Found: C, 49.19; H, 3.50; N, 1.68. $^1$H-NMR (400 MHz, acetone-$d_6$) $\delta$: 7.16, 7.15, 7.07, 6.98, 6.90 (2H each, s, galloyl), 7.02, 7.00, 6.34 (1H each, s, valoneoyl), 7.51 (1H, s, H-3‴), 8.30 (1H, s, H-3″), 8.33, 8.21, 7.99 (each, m), glucose protons, see Table II. $^{13}$C-NMR (100 MHz, acetone-$d_6$) $\delta$: 169.9, 167.2, 166.6, 166.5, 166.4, 166.2, 165.6, 165.3, 164.7, 164.8 (ester carbonyl), glucose carbons, see Table III. CD ($c$=0.01, MeOH): $[\theta]_{218}-7.8\times10^4$, $[\theta]_{250}+12.36\times10^4$, $[\theta]_{283}-13.1\times10^4$, $[\theta]_{320}+1.4\times10^4$.

**Hydrolysis of Euphorbin A-Phenazine (8)**——A solution of **8** (60 mg) in acetone (1 ml) and water (35 ml) was refluxed for 1 h. After concentration, the crystalline materials were collected and recrystallized from tetrahydrofuran to furnish dark red needles (7 mg): this product was identical with phenylphenazine dilactone (**11**) obtained from geraniin-phenazine (**9**), based on TLC and infrared (IR) spectral comparisons. The filtrate gave, after chromatography on Sephadex LH-20 using 70% MeOH as the eluant, the hydrolysate (**10**) (10 mg), $[\alpha]_D$ −9.4° ($c$=0.5, MeOH). $^1$H-NMR (500 MHz, acetone-$d_6$) $\delta$: 7.15, 7.11, 7.10, 7.09, 6.83 (2H each, s, galloyl), 6.23, 6.86, 7.04 (1H each, s, valoneoyl), [glucose core I, $\delta$: 6.22 (d, $J$=8 Hz, H-1), 5.67 (dd, $J$=8, 10 Hz, H-2), 5.55 (2H, triplet like, H-3 and H-4), 4.37 (ddd, $J$=1.5, 5, 9.5 Hz, H-5), 4.52 (dd, $J$=1.5, 12 Hz, H-6), 4.32 (dd, $J$=5, 12 Hz, H-6′)], [glucose core II, $\delta$: 6.34 (d, $J$=2 Hz, H-1), 4.09 (br s, H-2), 4.83 (br s, H-3), 4.39 (br s, H-4), 4.43 (dd, $J$=8, 10 Hz, H-5), 4.79 (t, $J$=10 Hz, H-6), 4.08 (dd, $J$=8, 10 Hz, H-6′)].

**Methanolysis of Euphorbin A-Phenazine (8)**——A solution of **8** (180 mg) in a mixture of acetate buffer (pH 6.0) (2 ml) and MeOH (18 ml) was kept at 37 °C for 72 h. The reaction mixture, after evaporation, was suspended in dilute HCl and the insoluble material was collected and crystallized from tetrahydrofuran to give (**11**) (21 mg). The filtrate was extracted with EtOAc and the organic layer was evaporated. The residue was chromatographed over Toyopearl HW-40 (fine) by stepwise elution with MeOH–$H_2O$ (1 : 1), MeOH–$H_2O$ (6 : 4), MeOH–$H_2O$ (7 : 3) and MeOH. From the MeOH–$H_2O$ (1 : 1) fraction, three hydrolysates were obtained and identified as methyl gallate (2 mg), corilagin (**13**) (6 mg) [$^1$H-NMR (acetone-$d_6$) $\delta$: 7.12 (2H, s, galloyl), 6.84, 6.70 (1H each, s, HHDP), 6.36 (1H, br s, H-1)] and 3,4,6-tri-*O*-galloyl-D-glucose (**12**) (6 mg) [$^1$H-NMR (acetone-$d_6$) $\delta$: 7.16, 7.15 (each s, 2H in total), 7.04 (2H, s), 7.03, 7.02 (each, s, 2H in total), α-glucose, $\delta$: 5.33 (d, $J$=3.5 Hz, H-1), 3.84 (br d, H-2), 5.72 (t, $J$=10 Hz, H-3), 5.36 (t, $J$=10 Hz, H-4), 4.51 (ddd, $J$=2, 5, 10 Hz, H-5), 4.41 (dd, $J$=2, 12 Hz, H-6), 4.26 (dd, $J$=5, 12 Hz, H-6′); β-glucose, $\delta$: 4.87 (d, $J$=7.5 Hz, H-1), 3.62 (dd, $J$=7.5, 9.5 Hz, H-2), 5.51 (t, $J$=9.5 Hz, H-3), 5.34 (t, $J$=9.5 Hz, H-4), 4.12 (ddd, $J$=2, 5, 9.5 Hz, H-5), 4.40 (dd, $J$=2, 12 Hz, H-6), 4.25 (dd, $J$=5, 12 Hz, H-6′)]. The fraction eluted with MeOH–$H_2O$ (7 : 3) gave 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**2**) (11 mg).

**Methylation of Euphorbin A-Phenazine (8)**——Compound **8** (100 mg) in EtOH (2 ml) was methylated with ethereal $CH_2N_2$ for 1.5 h at room temperature. After removal of the solvent, the residue was purified by preparative TLC [ligroin–$CHCl_3$–acetone (2 : 3 : 1, v/v)] to furnish the permethylated derivative (**14**) (16 mg) as an orange amorphous powder, $[\alpha]_D$ +11° ($c$=1.1, acetone). $^1$H-NMR (400 MHz, acetone-$d_6$) $\delta$: 7.40 (2H, s), 7.07 (4H, s), 7.20 (2H, s), 7.199 (2H, s) (galloyl), 8.48 (1H, s), 7.48 (1H, s), 7.09 (1H, s), 6.67 (2H, s), 8.30 (2H, m), 8.03 (2H, m) (phenylphenazine and valoneoyl), [glucose core I, $\delta$: 6.16 (d, $J$=8 Hz, H-1), 5.89 (dd, $J$=8, 10 Hz, H-2), 5.83 (t, $J$=10 Hz, H-3), 5.95 (t, $J$=10 Hz, H-4), 4.59 (m, H-5), 4.81 (dd, $J$=3, 12 Hz, H-6), 4.35 (d, $J$=4.5, 12 Hz, H-6′)], [glucose core II, $\delta$: 6.33 (d, $J$=3 Hz, H-1), 5.61 (dt, $J$=1, 3 Hz, H-2), 5.57 (m, H-3), 5.50 (br d, $J$=4 Hz, H-4), 4.59 (m, H-5), 3.96 (dd, $J$=8, 9 Hz, H-6), 4.53 (dd, $J$=3, 8 Hz, H-6′)].

**Methanolysis of 14**——A mixture of **14** (11 mg) and 1% NaOMe (0.1 ml) in MeOH (1 ml) was kept at room temperature for 3 h. After neutralization with AcOH and evaporation, the residue was purified by preparative TLC [benzene–acetone (15 : 1, v/v)] to give methyl tri-*O*-methylgallate [2 mg, MS $m/z$: 226 ($M^+$)], trimethyl octa-*O*-methylvaloneate (**15**) [1.4 mg, $[\alpha]_D$ +13° ($c$=0.2, acetone), MS $m/z$: 660 ($M^+$)], and dimethyl ester (**16**) [1.4 mg, $[\alpha]_D$ +33° ($c$=0.2, acetone), MS $m/z$: 492 ($M^+$)], which were identical with authentic samples obtained from mallotusinic acid[1b] as judged from $^1$H-NMR and MS spectral comparisons.

**Euphorbin B-Phenazine (17)**——Euphorbin B (**7**) (100 mg) was treated with *o*-phenylenediamine in a way similar to that described above for **6**, to yield the phenazine derivative (**17**) (71 mg) as an orange amorphous powder, $[\alpha]_D$ +6° ($c$=0.6, MeOH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (5.20), 281 (5.11). *Anal.* Calcd for $C_{88}H_{59}N_2O_{49}\cdot 9H_2O$: C, 50.55; H, 3.68; N, 1.34. Found: C, 50.23; H, 3.41; N, 1.66. $^1$H-NMR (500 MHz, acetone-$d_6$) $\delta$: 7.17, 7.11, 7.05, 6.98, 6.97 (2H each, s, galloyl), 7.03, 6.84, 6.54 (1H each, s, valoneoyl), 8.31 (1H, s, H-3″), 7.50 (1H, s, H-3‴), 8.21, 8.01, 8.33 (each m), glucose protons, see Table II. $^{13}$C-NMR (126 MHz, acetone-$d_6$) $\delta$: 168.3, 167.9, 167.6, 166.7, 166.6 (2C), 166.3, 165.9, 165.4, 164.9 (ester carbonyl), glucose carbons, see Table III. CD ($c$=0.01, MeOH): $[\theta]_{217}-7.2\times10^4$, $[\theta]_{250}+9.83\times10^4$, $[\theta]_{283}-13.6\times10^4$, $[\theta]_{320}+0.76\times10^4$.

**Partial Hydrolysis of 17**——A solution of **17** (63 mg) in acetone (1 ml) and $H_2O$ (25 ml) was refluxed for 10 h. After concentration, the reddish brown precipitate was collected and recrystallized from tetrahydrofuran to give phenylphenazine dilactone (**11**) (8 mg), identical with that obtained from **8**. The filtrate was chromatographed over Toyopearl HW-40 (fine) using 50% MeOH as the eluant to afford gallic acid (15 mg), corilagin (**13**) (2 mg), 3,4,6-tri-*O*-galloyl-D-glucose (**12**) (4 mg) and 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**2**) (4 mg).

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## References and Notes


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