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Tannins and Related Polyphenols of Euphorbiaceous Plants. V.[1] Euphorbin C, an Equilibrated Dimeric Dehydroellagitannin Having a New Tetrameric Galloyl Group

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Euphorbin C (5), a member of a new class of dimeric hydrolyzable tannins, has been isolated from *Euphorbia hirta* L. In its molecule, a monomeric hydrolyzable tannin unit having a $^1C_4$ glucopyranose core which is esterified with an equilibrated dehydrohexahydroxydiphenoyl group, and another monomer unit having a $^4C_1$ glucose core are bonded through ester linkages of a euphorbinoyl group, which is a new galloyl tetramer.

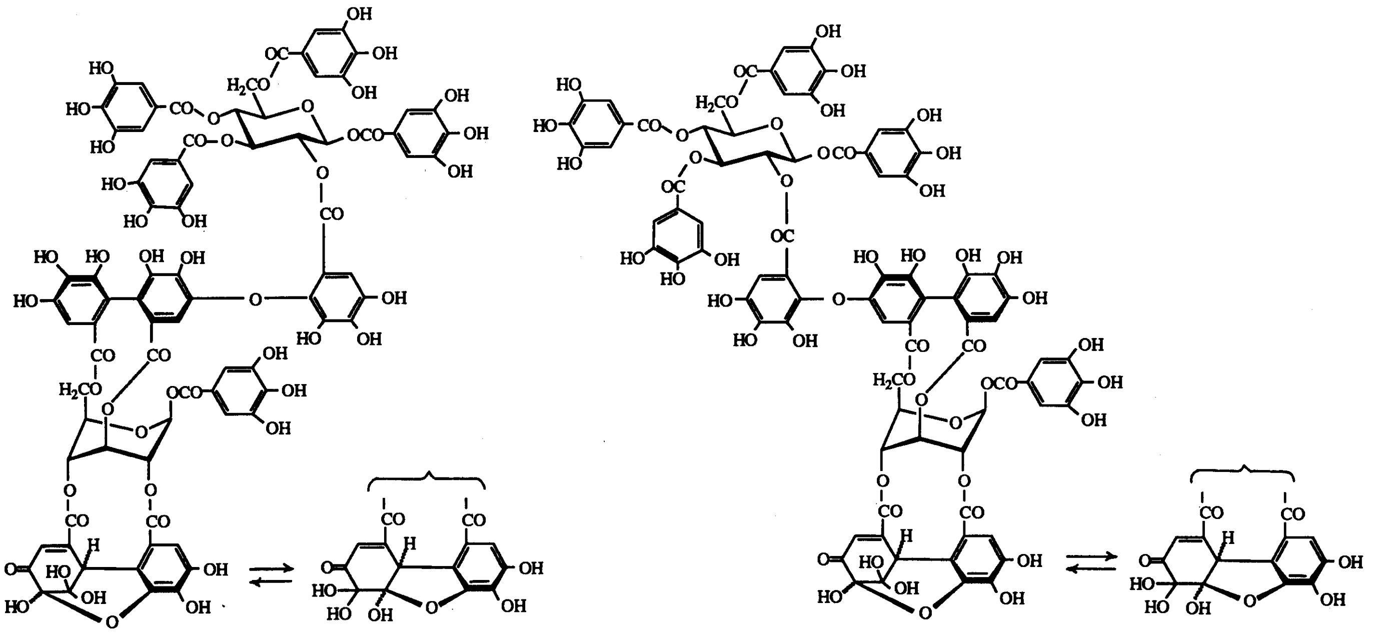
Keywords *Euphorbia hirta*; Euphorbiaceae; hydrolyzable tannin dimer; tannin; euphorbin C; euphorbinic acid; ^1H–^{13}C long-range 2D NMR

Recent advances in methods of analyzing tannins have led to the isolation and characterization of many hydrolyzable tannins, particularly oligomers, from a variety of medicinal plants.[2] The polyphenolic groups in the oligomeric hydrolyzable tannins hitherto isolated are galloyl, hexahydroxydiphenoyl (HHDP), dehydrodigalloyl, valoneoyl and sanguisorboyl groups. The latter three acyl groups can couple two monomer units as in agrimoniin,[3] rugosins[4] and sanguiins,[5] and are regarded as the linking units for dimer formation in plants.[2] We have recently isolated from *Euphorbia hirta* L. (Euphorbiaceae), euphorbins A (**1**) and B (**2**),[1] which are the first examples of dimeric hydrolyzable tannins possessing a $^1C_4$ glucopyranose core and a $^4C_1$ glucopyranose core bonded *via* a valoneoyl group, and an equilibrated dehydrohexahydroxydiphenoyl (DHHDP) group[6] in each molecule. Upon further investigation on the polyphenolics of this plant, a dimeric tannin belonging to a new class has been isolated. This tannin, designated euphorbin C (**5**), has an unusual linking unit (euphorbinoyl group), which is a galloyl tetramer, between the two monomers. We report herein the isolation and structure determination of this tannin.

Euphorbin C (**5**), $C_{82}H_{54}O_{53} \cdot 6H_2O$, was isolated from the ethyl acetate-soluble portion of the aqueous acetone extract of the leaves, by column chromatography over Toyopearl HW-40. It showed ellagitannin coloration with the sodium nitrite and acetic acid reagent[7] on a thin-layer chromatography (TLC) plate. The retention time in normal-phase high-performance liquid chromatography (HPLC) was similar to those of the dimers, **1** and **2**.

Euphorbin C (**5**) has been shown to consist of two galloyl groups, an HHDP group, a DHHDP group, a euphorbinoyl group and two glucose residues, by the following experiments.

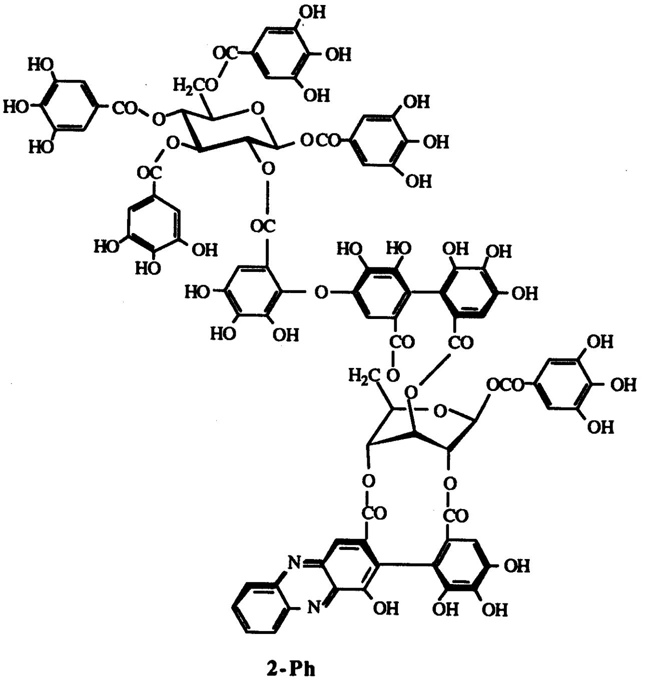
The proton nuclear magnetic resonance (^1H-NMR) spectrum (500 MHz, acetone-d_6) of **5** was complicated by dual-peak formation for each proton, due to the equilibration between six- and five-membered hemiacetal forms (**5a**$\rightleftarrows$**5b**) of the DHHDP group [δ 5.20 (s) and 6.54 (s) (H-1″ and H-3″ of **5a**), and 4.95 and 6.21 (each d, $J=2$ Hz) (H-1″ and H-3″ of **5b**)]. The signals attributable to two galloyl groups [δ 7.24, 7.23 (each s, 2H in total), and 6.93, 6.94



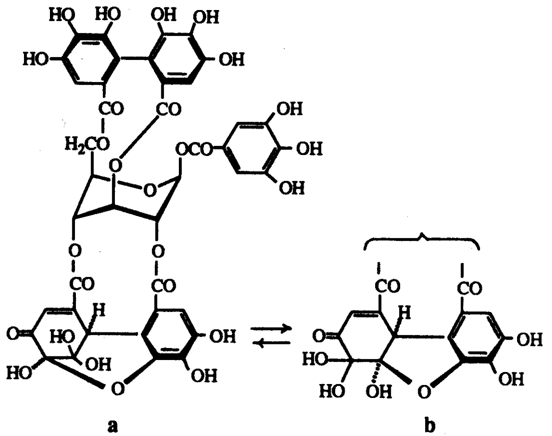
1

2

Chart 1



2-Ph



a 3 b

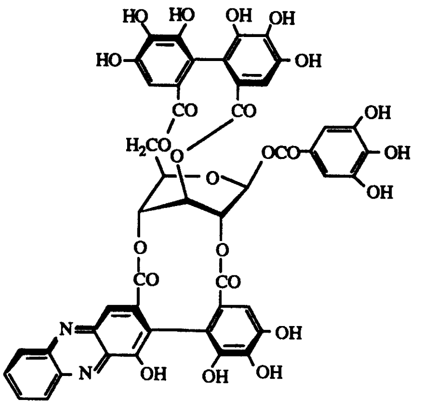


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Chart 2

(each, s, 2H in total)], and seven pairs of aromatic one-proton singlets were also exhibited (see Experimental). The sugar protons were those of the $^4C_1$ and $^1C_4$ glucopyranose residues, among which the proton signals of the latter residue showed chemical shifts consistent with those of geraniin (**3**) in aqueous solution.[8] The presence of a DHHDP group in **5** was confirmed by production of a phenazine derivative (**6**) upon condensation with *o*-phenylenediamine. The ^{1}H-NMR spectrum of **6** exhibited the conformational change of the $^1C_4$ glucopyranose (glucose core-II) to skew boat form as observed upon the transformation of **3** into its phenazine derivative (**4**).[6] The chemical shifts of the signals ascribable to protons and carbons of the glucose core-II in **6** also coincided well with those of **4** and also of the phenazine derivative (**2-Ph**)[1] of euphorbin B (**2**), as shown in Tables I and II. However, differences in the chemical shifts of the $^4C_1$ glucose protons of **5** and **6** from those of **2** and **2-Ph** (Table I) suggest that the glucose core-I in **5** has a substitution pattern different from that of **2**.

Methylation of **6** with diazomethane afforded a hexacosamethyl derivative (**7**). Methanolysis of **7** with sodium methoxide gave glucose, a methylated phenylphenazine derivative (**8**),[6,9] methyl tri-*O*-methylgallate, and dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenate (**9**),[10] which were identified by comparison with authentic specimens. The sign of specific optical rotation of **8**, $[\alpha]_D$ +34° (EtOH) and **9**, $[\alpha]_D$ −25° (EtOH), showed the atropisomerism to be *R* and *S*, respectively. The fourth methanolysate (**11**), $C_{42}H_{46}O_{20}$, *m*/*z* 870 $[(M)^+]$, showed in the ^{1}H- and ^{13}C-NMR spectra four aromatic one-proton singlets at δ 6.90, 7.12, 7.19 and 7.35, in addition to fourteen methoxyl signals, and four ester carbonyl carbon resonances (δ 165.9, 166.2, 166.8 and 167.3). Its parent tetracarboxylic acid, named euphorbinic acid, has been found to have the structure **10** in the following way. Acid hydrolysis of **5** afforded euphorbinic acid dilactone (**13**), gallic acid, ellagic acid, and a small amount of valoneic acid dilactone (**15**),[11] which were characterized as methyl derivatives. The dilactone **15** was also obtained when **13** was treated further with 5% sulfuric acid. Euphorbinic acid dilactone, therefore, should have the structure in which gallic acid associates

Table I. ^{1}H-NMR Chemical Shifts for the Glucose Moieties of **2**, **2**-Ph, **3**, **4**, **5** and **6** [500 MHz, in Acetone-d_6–D_2O; J Values in Hz]

H-atom	**2**		**3**		**5**		**2-Ph**	**4**	**6**
	a	b	a	b	a	b			
Glucose-I									
1	6.30				4.72	4.55	6.36		4.60
	(d, J=8)				(d, J=8.5)		(d, J=8.5)		(d, J=8)
2	5.71				5.35		5.73		5.32
	(dd, J=8, 10)				(dd, J=8.5, 10)		(dd, J=8.5, 10)		(dd, J=8, 9.5)
3	6.04				5.15	5.18	6.07		5.25
	(t, J=10)				(t, J=10)		(t, J=10)		(t, J=9.5)
4	5.68	5.67			5.16	5.07	5.67		5.08
	(t, J=10)				(t, J=10)		(t, J=10)		(t, J=9.5)
5	4.55				4.05		4.57		4.03
	(m)				(dd, J=6, 10)		(ddd, J=1.5, 4, 13)		(dd, J=6, 9.5)
6	4.55				5.31	5.32	4.53		5.28
	(m)				(dd, J=6, 13.5)		(dd, J=1.5, 13)		(dd, J=6, 13.5)
	4.33				3.93		4.41		3.93
	(dd, J=5, 12)				(d, J=13.5)		(dd, J=4, 13)		(d, J=13.5)
Glucose-II									
1′	6.56		6.60		6.57	6.58	6.21	6.14	6.19
	(br s)		(br s)		(br s)		(d, J=5.5)	(d, J=6)	(d, J=6)
2′	5.60		5.60		5.67	5.61	5.69	5.63	5.75
	(br s)		(br s)		(br s)		(dd, J=1, 5.5)	(d, J=6)	(dd, J=1, 6)
3′	5.56	5.48	5.50	5.60	5.67	5.54	5.52	5.45	5.44
	(br s)		(br s)		(br s)		(br d, J=4)	(d, J=3.5)	(dd, J=1, 4)
4′	5.59	5.48	5.56	5.46	5.53	5.45	5.56	5.55	5.60
	(br s)		(br s)		(br s)		(dd, J=1, 4)	(d, J=3.5)	(d, J=4)
5′	4.81		4.81		4.70		5.05	4.99	5.01
	(br dd, J=7, 10)		(m)		(m)		(dd, J=4.5, 8)	(dd, J=4, 8)	(dd, J=4.5, 8)
6′	4.89	4.76	4.93	4.78	4.75		4.75	4.72	4.72
	(t, J=10)		(t, J=11)	(m)	(m)		(dd, J=8, 12)	(dd, J=8, 11)	(dd, J=8, 11)
	4.32	4.41	4.33	4.45	4.43		4.09	4.03	4.02
	(dd, J=7, 10)		(dd, J=8, 11)	(dd, J=6, 9)	(m)		(dd, J=4.5, 12)	(dd, J=4, 11)	(dd, J=4.5, 11)

Table II. ^{13}C-NMR Chemical Shifts of the Glucose Carbons of **2**-Ph, **4**, **6**, **21** and **22**

C-atom	**2**-Ph$^{a)}$	**4**$^{a)}$	**6**$^{a)}$	**21**$^{a)}$	**22**
Glucose-I					
1	93.2		95.5	94.1	
2	72.1		72.1	73.1	
3	73.3		73.2	74.3	
4	69.4		70.4	71.1	
5	73.8		73.7	73.1	
6	62.8		63.0	63.5	
Glucose-II					
1′	91.5	91.6	91.5	93.5	93.5
2′	76.5	76.6	76.4	76.1	76.4
3′	68.6	68.7	68.5	67.8	67.4
4′	67.8	67.6	68.0	61.3$^{b)}$	61.3
5′	76.6	76.8	76.3	75.5	76.0
6′	65.8	65.2	66.2	64.7	64.4

a) Assignments were confirmed by ^{1}H–^{13}C COSY. *b)* Overlapped with methoxy carbon signal.

through an ether linkage with one of the phenolic hydroxyl groups of the valoneic acid dilactone core. In the differential nuclear Overhauser effect (NOE) spectrum of the decamethyl derivative (**12**) which was obtained by alkaline hydrolysis of **11**, three aromatic signals at δ 7.21, 7.28 and 7.42 (each 1H, s) showed appreciable NOE (*ca.* 19%) with methoxyl signals. However, the one-proton singlet at δ 7.09 exhibited no NOE with any methoxyl signal, permitting its assignment to a proton (H_B) of ring B in **12**. The ^{1}H–^{13}C long-range shift correlation spectrum of **11** indicated that, among the signals ascribable to the oxygen-bearing carbons, those at δ 150.2 and 144.7 are due to C-4 and C-5 of ring B, by their correlations with H_B (δ 6.90) through two- and three-bond couplings. These carbons did not show long-range coupling with any methoxyl methyl protons, thus indicating that the two aroyl ether linkages are at the relevant positions of ring B. The other sp^2 carbon resonances of **11** were fully assigned with the aid of the ^{1}H–^{13}C long-range correlation spectroscopy (COSY) and also by comparison with those of the related compounds, **9**, trimethyl octa-*O*-methylvaloneate (**17**)[12] and trimethyl octa-*O*-methyltergallate (**18**),[13] as summarized in Table III. Euphorbinic acid was thus assigned structure **10**.

Partial hydrolyses of the phenazine derivative (**6**) were then attempted to clarify the locations of the acyl groups on the glucose cores in euphorbin C. Mild hydrolysis of **6** in boiling water gave a phenylphenazine derivative (**19**)[9] and a partial hydrolysate (**20**). The ^{1}H-NMR spectrum of **20** showed elimination of the phenylphenazine moiety, accompanied by the upfield shifts of the 2-H and 4-H signals of the glucose core-II by 1.55 and 1.10 ppm from the relevant signals of **6**. These results, and the close similarity of the chemical shifts of the $^1C_4$ glucopyranose protons of **5** and **6** to those of **3** and **4** mentioned earlier in this paper, strongly indicate that the glucose core-II in **5** has a substitution system similar to that of **3**.

The hydrolysis of **5** with hot 5% sulfuric acid for 2 h, followed by column chromatography over Sephadex LH-20, afforded two other partial hydrolysis products (**23** and **25**), together with gallic acid, ellagic acid and **13**. The hydrolysate (**23**), *m*/*z* 951 [(M − H)$^-$], was shown to exist as a mixture of α- and β-anomer by the presence of two doublets attributable to anomeric protons [δ 5.91 (J=

3.5 Hz) and 4.89 ($J=8$ Hz)] in the ^{1}H-NMR spectrum, and also by the duplication of each proton signal. The ^{1}H-NMR spectrum of **23** also showed the presence of a galloyl group [δ 6.63, 6.98 (2H in total)] and a euphorbinoyl group [δ 7.06, 6.99 (1H in total), 7.16, 7.11 (1H, in total), 7.18, 7.13 (1H, in total), and 7.55, 7.54 (1H in total)]. These groups should be located at O-2 and O-3 of the $^4C_1$ glucopyranose, as indicated by the low-field shifts of the signals of 2-H and 3-H [δ 5.06 (dd, $J=8$, 9.5 Hz), 4.93 (dd, $J=3.5$, 9.5 Hz), and 5.69 (t, $J=9.5$ Hz) and 5.21 (t, $J=9.5$ Hz)]. Selective hydrolysis of **23** with tannase gave a degalloylated derivative (**24**), m/z 799 [$(M-H)^-$]. The ^{1}H-NMR spectrum of **24** showed the absence of the galloyl signal and an upfield shift of 3-H of glucose residue to δ 3.88 and 3.50 (each t, $J=9.5$ Hz, 1H in total), as well as the retention of the euphorbinoyl dilactone group, which has been thus confirmed to be at O-2. The structure of **25** was readily deduced from an ion peak at m/z 1253 [$(M-H)^-$], and the ^{1}H-NMR spectrum. Its ^{1}H-NMR spectrum was similar to that of **23** except for the extra aromatic signals assignable to an HHDP group, and low field shifts of 4-H and 6-H [δ 5.00, 4.96 (each t, $J=10$ Hz), and 3.80 (br d, $J=13$ Hz), 3.75 (dd, $J=1.5$, 13 Hz), 5.35 overlapped by the signals of 1-H and 2-H of the β-anomer)].

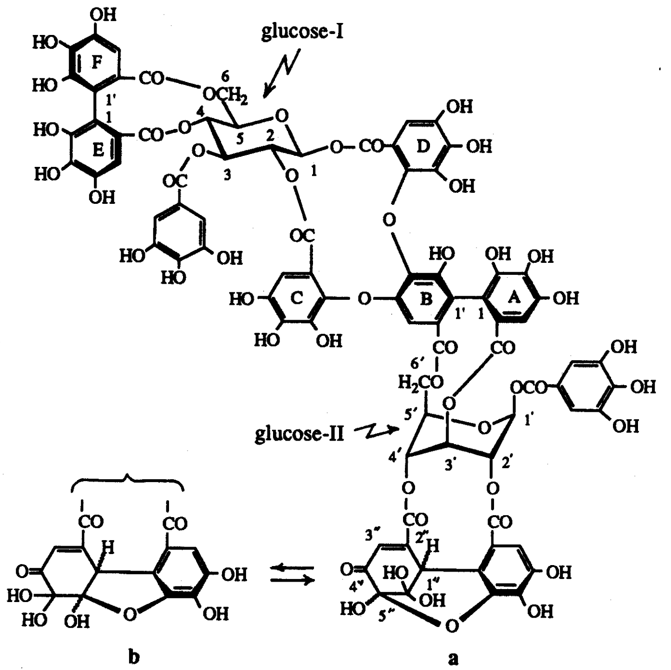
TABLE III. ^{13}C-NMR Chemical Shifts of **9**, **11**, **17** and **18** (126 MHz, $CDCl_3$)

		9	**11**		**17**	**18**
Ring A	1	126.6	126.2	127.3$^{a)}$	126.3	126.4
	2	125.0	125.2	126.3$^{a)}$	125.2	125.1
	3	108.9	108.9	109.6$^{a)}$	108.9	108.8
	4	152.0	152.1	153.2$^{a)}$	152.1	152.1
	5	145.4	145.3	146.3$^{a)}$	145.4	145.3
	6	151.2	151.2	152.3$^{a)}$	151.5	151.3
	7	166.9	166.9	167.3$^{a)}$	167.1	166.8
Ring B	1	126.6	127.7	128.6$^{a)}$	127.3	127.1
	2	125.0	124.0	124.9$^{a)}$	124.9	123.9
	3	108.9	111.9	112.5$^{a)}$	111.6	109.6
	4	152.0	149.4	150.2$^{a)}$	151.6	149.7
	5	145.4	143.9	144.7$^{a)}$	145.4	143.9
	6	151.2	148.9	149.9$^{a)}$	151.2	148.5
	7	166.9	166.5	166.8$^{a)}$	166.6	166.1
Ring C	1		119.4	120.6$^{a)}$	119.3	
	2		142.2	142.6$^{a)}$	142.5	
	3		147.1	148.0$^{a)}$	147.0	
	4		147.0	147.9$^{a)}$	147.2	
	5		150.1	151.4$^{a)}$	150.2	
	6		108.8	109.5$^{a)}$	108.8	
	7		165.6	165.9$^{a)}$	165.4	
Ring D	1		117.6	118.9$^{a)}$		117.0
	2		145.7	146.51$^{a)}$		145.5
	3		146.1	146.53$^{a)}$		146.1
	4		146.6	147.5$^{a)}$		146.5
	5		148.5	149.7$^{a)}$		148.5
	6		108.1	109.1$^{a)}$		108.2
	7		165.8	166.2$^{a)}$		166.1

a) Measured in acetone-d_6.

The binding sites of the euphorbinoyl group to the glucose cores in euphorbin C were established to be as in the formula **5** by extensive NMR spectral analyses of a tricosamethyl derivative (**21**), which was prepared by methylation of **20** with dimethyl sulfate and potassium carbonate. In the ^{1}H- and ^{13}C-NMR spectra of **21**, the signals of glucose core-II showed close similarity to those of decamethylcorilagin (**22**)[14] (Table II), indicating that methylation occurred at O-2′ of glucose core-II as well as the phenolic hydroxyl groups.

Among six aromatic one-proton singlets (δ 6.65, 6.79, 6.81, 6.96, 6.51 and 6.99), four at δ 6.65, 6.79, 6.81 and 6.96 were assigned to the protons on the rings A, B, E and F, based on the three-bond long-range couplings with the signals at δ 120—125 ppm, which were ascribed to C-1 and C-1′ of each biphenyl moiety, in the ^{1}H–^{13}C long-range shift correlation spectrum of **21**. Three (δ 6.79, 6.81 and 6.96) of them also showed long-range couplings with the



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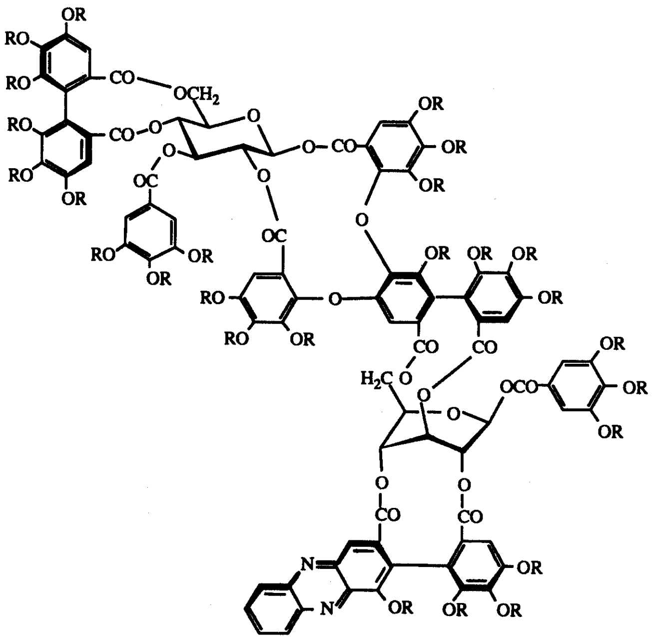
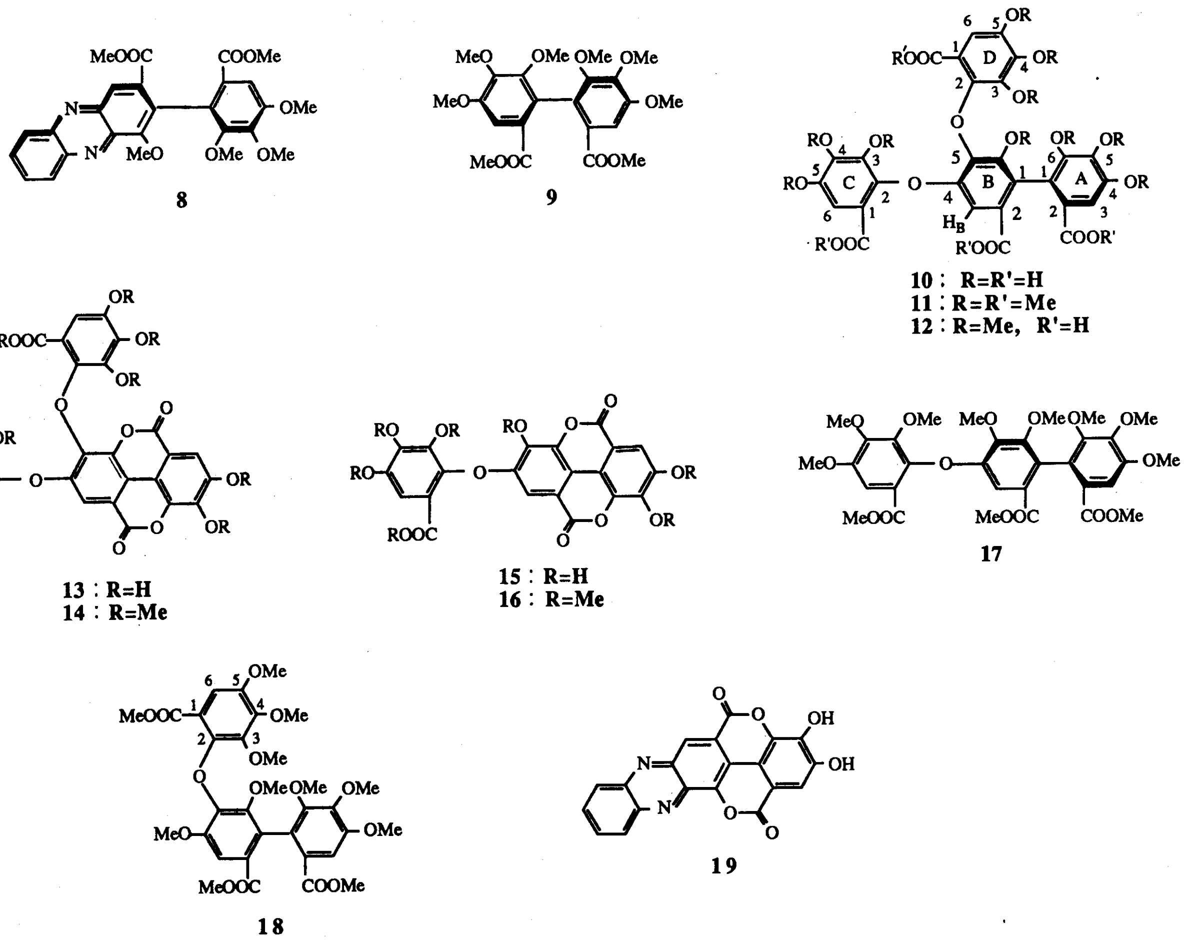


6 : R=H
7 : R=Me

Chart 3



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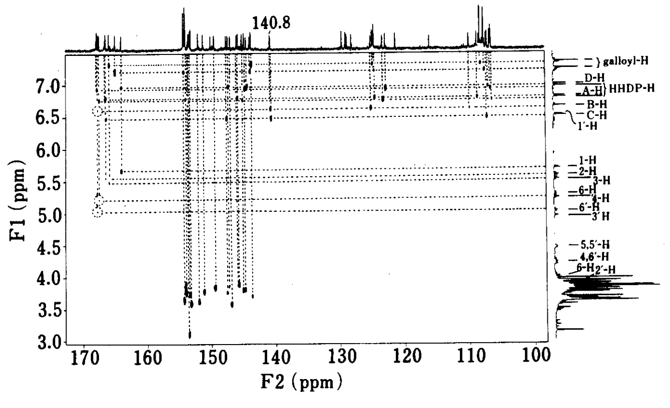
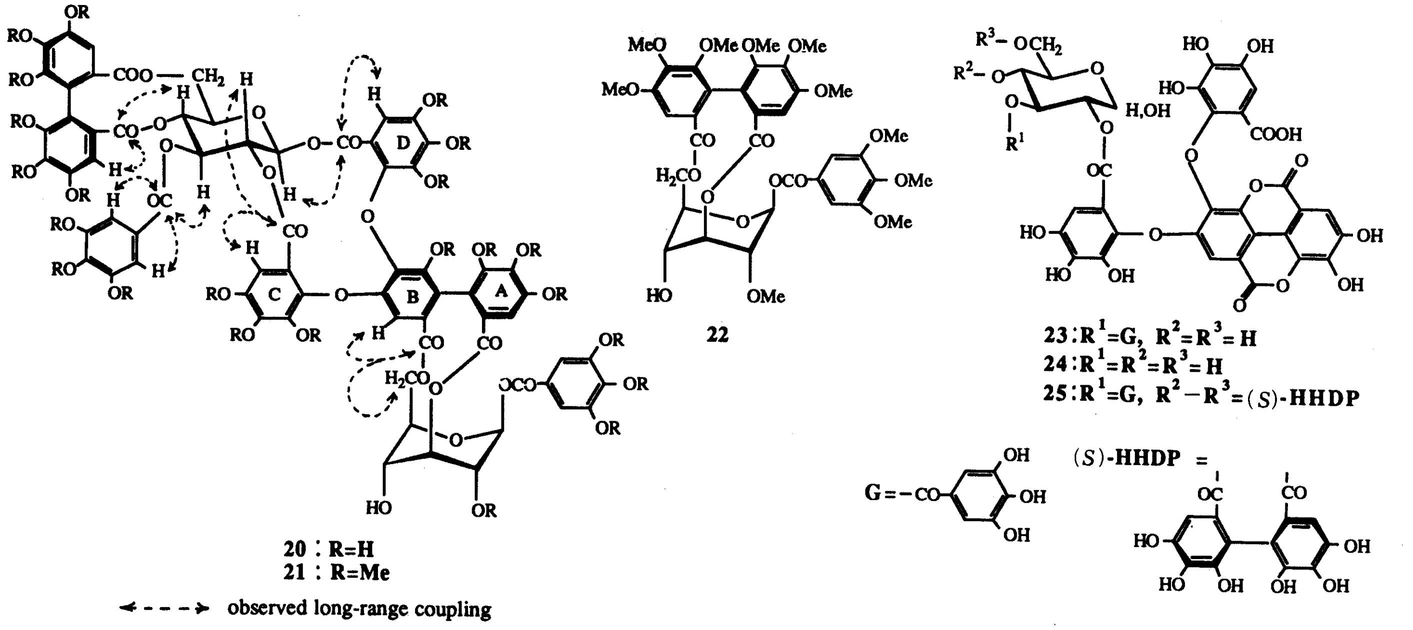


Fig. 1. ^{1}H–^{13}C Long-Range Shift Correlation Spectrum (δ 100—170 ppm) of **21** ($J_{CH}=7$ Hz) in Acetone-d_6

oxygen-bearing carbon resonances at δ 140—145, which gave cross peaks with methoxyl protons. However, the carbon resonance (δ 140.8) which showed a long-range coupling with the signal at δ 6.65 displayed no cross peak with any methoxyl proton signal. The signal at δ 6.65 is therefore that of the ring-**B** proton. The remaining signals at δ 6.51 and 6.99 were assigned to the ring-C and ring-D protons, respectively. These assignments were based on connectivities between each proton and two carbons (methoxy-bearing carbon and phenylether carbon) at δ 140—148 through three-bond couplings, and also on a comparison with the assigned signals for tetramethyl deca-*O*-methyleuphorbinate (**11**) (Table III). The ring-C and ring-D protons thus assigned showed long-range correlation with the ester carbonyl carbons (δ 166.1 and 163.7), which have cross peaks with 2-H and 1-H of the glucose core-I of **21**. Similarly, connectivity between the ring B proton (δ 6.65) and 6′-H (δ 5.03) of glucose core-II was revealed by long-range correlation through the same ester carbonyl carbon (δ 167.4). The locations of the galloyl group at C-3 and the HHDP group at C-4 (and C-6) on the glucose core-I were also confirmed by the long-range ^{1}H–^{13}C connectivity as shown in Fig. 1.

The β-configuration at C-1 of the glucose core-I in euphorbin C (**5**) was assigned based on the large coupling constant ($J=8.5$ Hz) of the anomeric proton [δ 4.72 (a-form) and 4.55 (b-form)]. A significant upfield shift of H-1 might be interpreted as a shielding effect of the B-ring of the euphorbinoyl group as illustrated in formula **5′**.

The (*R*)-configuration at the biphenyl moiety of the euphorbinoyl group in euphorbin C (**5**) was deduced by the identity of the sign of optical rotation of **11** {$[\alpha]_D$ +17° (EtOH)} with that of trimethyl (*R*)-octa-*O*-methylvaloneate (**17**) {$[\alpha]_D$ +13° (acetone)}.[12] Additional support for this assignment, and for the absolute configurations of the HHDP group and of C-1″ of the DHHDP group, was obtained from the circular dichroism (CD) spectrum of **6** (Fig. 2). It exhibited Cotton effects similar to those of the phenazine derivative[1] of euphorbin A, with some differences of the pattern and amplitude of each Cotton effect caused by the presence of the (*S*)-HHDP group.



20 : R=H
21 : R=Me

22

23 : R^1=G, R^2=R^3= H
24 : R^1=R^2=R^3= H
25 : R^1=G, R^2−R^3=(S)-HHDP

Chart 5

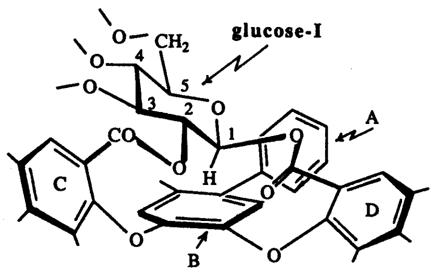


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Chart 6

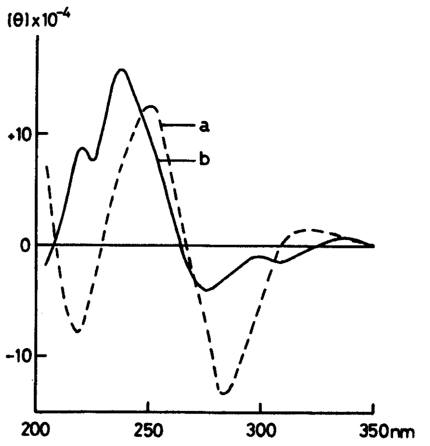

Fig. 2. CD Spectra of Phenazine Derivatives of Euphrobin A (a) and Euphrobin C (b) in MeOH

On the basis of the above findings, the structure of euphorbin C was determined to be **5**.

According to the hypothesis proposed for the biogenesis of dimeric hydrolyzable tannins,[3,15)] euphorbin C can be regarded as a dimer biogenetically formed by intramolecular C–C and C–O oxidative couplings between two galloyl groups at C-4 and C-6 of euphorbin B, and also between a galloyl group at C-1 and a valoneoyl group at C-2.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR 500 instrument at 500 MHz and 126 MHz, respectively, and chemical shifts are given in δ (ppm) values from tetramethylsilane. Electron-impact (EI) and fast atom bombardment (FAB) mass spectra (MS) were measured on a JEOL GMS HX-100 or a VG 70-SE mass spectrometer. Optical rotations were obtained on a JASCO DIP-4-digital polarimeter and CD spectra on a JASCO J500A. Normal-phase HPLC was carried out with Waters M-4 machine using a Zorbax Sil column (4.6 × 150 mm) with a flow rate of 2.5 ml/min and a solvent composition of hexane–MeOH–tetrahydrofuran (THF)–HCOOH (55 : 33 : 11 : 1, v/v) containing oxalic acid (450 mg/l), and reversed-phase HPLC with a Shimadzu LC-6A using a YMC-Pack A312 (ODS) (6.1 × 150 mm) with a flow rate of 1.3 ml/min and a solvent system of 0.05 M H_3PO_4–0.05 M KH_2PO_4–CH_3CN (42.5 : 42.5 : 15, v/v). Si gel [Kieselgel PF_{254} (Merck)] in the following solvent systems: A, benzene–acetone (15 : 1, v/v), B, benzene–acetone (25 : 1, v/v); C, light petroleum–benzene–acetone (2 : 3 : 1, v/v); D, light petroleum–benzene–acetone (1 : 1 : 1, v/v). Column chromatography was performed on Toyopearl HW-40 (coarse, fine grade, Tosoh), Sephadex LH-20 (Pharmacia Fine Chemicals), Diaion HP-20 (Mitsubishi Chemical Industry).

Isolation of Euphorbin C A part (20 g) of the ethyl acetate extract (118 g) which was obtained from an aqueous acetone homogenate of dried leaves (2.4 kg) of *E. hirta*,[1)] was chromatographed over Toyopearl HW-40 (coarse) using MeOH–H_2O (6 : 4)→MeOH–H_2O (7 : 3)→MeOH–H_2O (8 : 2)→MeOH–acetone–H_2O (7 : 1 : 2) as the eluant. The eluate with MeOH–acetone–H_2O (7 : 1 : 2) gave euphorbin C (**5**) (2.07 g).

Euphorbin C (5) A pale yellow amorphous powder, $[\alpha]_D$ +60° (c = 0.7, MeOH). *Anal.* Calcd for $C_{82}H_{54}O_{53} \cdot 6H_2O$: C, 49.36; H, 3.33. Found: C, 49.27; H, 3.52. UV λ_{max}^{MeOH} nm (log ε): 220 (5.12), 275 (4.78). ^{1}H-NMR (acetone-d_6–D_2O) showed **5a** : **5b** ratio of *ca.* 3 : 1. δ: 7.24, 7.23 (each s, 2H in total), 6.93, 6.94 (each s, 2H in total) (galloyl), 7.35, 7.30 (each s, 1H in total), 6.56, 6.55 (each s, 1H in total), 6.36, 6.40 (each s, 1H in total), 6.62 (1H, s), 6.35, 6.38 (each s, 1H in total), 6.34, 6.35 (each s, 1H in total), 7.23, 7.26 (each s, 1H in total) (aromatic), 6.54 (s, 3''-H of DHHDP of **5a**), 6.21 (d, J = 2 Hz, 3''-H of DHHDP in **5b**), 5.20 (s, 1''-H of DHHDP in **5a**), 4.95 (d, J = 2 Hz, 1''-H of DHHDP in **5b**), glucose protons, see Table I.

Formation of the Phenazine Derivative (6) from Euphorbin C (5) A solution of *o*-phenylenediamine (40 mg) in 15% AcOH (12 ml) was added to a solution of **5** (200 mg) in MeOH (4 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 filtered. The dark orange precipitate was reprecipitated from MeOH–$CHCl_3$ and washed with $CHCl_3$ to give a phenazine derivative (**6**) (192 mg) as a yellow amorphous powder, $[\alpha]_D$ +97° (c = 0.2, MeOH). *Anal.* Calcd for

$C_{88}H_{56}N_2O_{50}\cdot 6H_2O$: C, 51.57; H, 3.34; N, 1.37. Found: C, 51.57; H, 3.73; N, 1.24. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 221 (5.17), 280 (4.99). CD (MeOH): $[\theta]_{220}$ $+8.8\times 10^4$, $[\theta]_{225}$ $+7.7\times 10^4$, $[\theta]_{238}$ $+16\times 10^4$, $[\theta]_{275}$ -4.0×10^4, $[\theta]_{297}$ -1.0×10^4, $[\theta]_{306}$ -1.5×10^4, $[\theta]_{337}$ $+0.6\times 10^4$. ^{1}H-NMR (acetone-d_6) δ: 7.53, 8.30 (each 1H, s), 8.29 (1H, br d, $J=8$ Hz), 8.21 (1H, br d, $J=8$ Hz), 7.95 (2H, m) (phenylphenazine), 6.98, 6.97 (each 2H, s, galloyl), 7.14, 6.62, 6.55, 6.47, 6.46, 6.38 (each 1H, s, HHDP and euphorbinoyl), glucose protons, see Table I. ^{13}C-NMR (acetone-d_6) δ: 164.8, 166.0, 166.1, 166.5, 166.7, 167.3, 167.5, 167.6, 167.7, 168.2 (ester CO), glucose carbons, see Table II.

Methylation of 6 Methylation of **6** (200 mg) in EtOH (10 ml), with an excess of CH_2N_2 followed by preparative TLC (solvent C) gave a hexacosamethyl derivative (**7**) (13 mg) as a pale yellow amorphous powder, $[\alpha]_D$ $+19°$ ($c=1.6$, acetone). *Anal.* Calcd for $C_{114}H_{108}N_2O_{50}\cdot 3H_2O$: C, 58.02; H, 4.87; N, 1.19. Found: C, 57.80; C, 4.87; N, 1.32. TLC (solvent C) *Rf* 0.36. ^{1}H-NMR (acetone-d_6) δ: 8.37, 7.47 (each 1H, s), 8.28, 8.02 (each 2H, m) (phenylphenazine), 7.35, 7.19 (each 2H, s, galloyl), 7.16, 6.99, 6.96, 6.80, 6.73, 6.53 (each 1H, s, HHDP and euphorbinoyl), 5.65 (1H, d, $J=8$ Hz, 1-H), 5.58 (1H, t, $J=8$ Hz, 2-H), 5.51 (1H, dd, $J=8$, 10 Hz, 3-H), 5.28 (1H, dd, $J=7$, 13 Hz, 6-H), 5.23 (1H, t, $J=10$ Hz; 4-H), 4.44 (1H, dd, $J=7$, 10 Hz, 5-H), 3.99 (1H, d, $J=13$ Hz, 6-H) (glucose core-I), 6.33 (1H, d, $J=3$ Hz, 1′-H), 5.58 (1H, 2′-H, overlapped by 2-H), 5.50 (1H, 4′-H, overlapped by 3-H), 5.37 (1H, d, $J=4$ Hz, 3′-H), 4.91 (1H, t, $J=8$ Hz, 5′-H), 4.57, 4.52 (each 1H, dd, $J=8$, 11 Hz, 6′-H) (glucose core-II), 4.05—3.57 (26 × OMe).

Methanolysis of 7 A mixture of **7** (110 mg) and 1% NaOMe in MeOH (6 ml) was kept at room temperature overnight. After neutralization and evaporation, the residue was submitted to preparative TLC (solvent A) to give methyl tri-*O*-methylgallate [4.3 mg, MS *m/z*: 226 (M^+)], methyl (*R*)-4-methoxy-3-(4,5,6-trimethoxy-2-methoxycarbonylphenyl)-phenazine-2-carboxylate (**8**) [10 mg, *m/z* 492 (M^+), $[\alpha]_D$ $+34°$ ($c=1.0$, EtOH)], dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate (**9**) [9 mg, *m/z* 450 (M^+), $[\alpha]_D$ $-25°$ ($c=0.6$, EtOH)], and tetramethyl deca-*O*-methyleuphorbinate (**11**) (20 mg). The identities of the former four were confirmed by comparison with authentic samples TLC (solvent A), $[\alpha]_D$ and ^{1}H-NMR.

Tetramethyl Deca-*O*-methyleuphorbinate (11) This compound was obtained as a pale yellow oil, $[\alpha]_D$ $+17°$ ($c=1.0$, EtOH). *Anal.* Calcd for $C_{42}H_{46}O_{20}$: C, 57.93; H, 5.32. Found: C, 57.51; H, 5.30. MS *m/z*: 870 (M^+). CD (MeOH): $[\theta]_{215}$ -6.3×10^4, $[\theta]_{247}$ $+3.5\times 10^4$, $[\theta]_{315}$ -0.4×10^4. ^{1}H-NMR (acetone-d_6) δ: 7.35, 7.19, 7.12, 6.90 (each 1H, s, aromatic), 3.93, 3.90, 3.89, 3.83, 3.78, 3.74, 3.69, 3.60, 3.59, 3.58, 3.53, 3.41 (each 3H, s), 3.87 (6H, s) (OMe). ^{13}C-NMR, see Table III.

Deca-*O*-methyleuphorbinic Acid (12) A solution of **11** (20 mg) in MeOH (4 ml) was treated with 5% NaOH (1 ml) and the reaction mixture was kept at 37 °C for 5 h. The precipitate obtained upon addition of dilute HCl was collected and washed with H_2O to give **12** (14 mg) as a white amorphous powder. MS *m/z*: 813 $(M-H)^-$. ^{1}H-NMR (acetone-d_6) δ: 7.42, 7.28, 7.21, 7.09 (each 1H, s, aromatic), 3.92, 3.90, 3.88, 3.87, 3.85, 3.84, 3.65, 3.59, 3.57, 3.51 (each 3H, s, OMe).

Acid Hydrolysis of Euphorbin C (5) A solution of **5** (5 mg) in 5% H_2SO_4 (1 ml) was heated on a boiling-water bath for 6 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was neutralized with ion exchange resin, Amberlite IRA-410 (OH form), filtered and evaporated. The sugar component was identified as glucose by gas liquid chromatography (2.5% OV-1, 170 °C) after trimethylsilylation. The EtOAc extract was methylated with CH_2N_2 and the product was purified by preparative TLC (solvent A) to afford methyl tri-*O*-methylgallate (1.1 mg), tetra-*O*-methylellagic acid [0.4 mg, *m/z* 358 (M^+)], methyl hexa-*O*-methylvaloneate dilactone (**16**) [0.4 mg, *m/z* 568 (M^+)] and dimethyl octa-*O*-methyleuphorbinate dilactone (**14**) [0.5 mg, *m/z* 778 (M^+)]. ^{1}H-NMR (acetone-d_6) δ: 7.70, 7.28, 7.24, 7.15 (each 1H, s, aromatic), 4.12, 4.10, 3.93, 3.91, 3.77, 3.72, 3.71, 3.61 (each 3H, s), 3.84 (6H, s) (OMe).

Degradation of Euphorbinic Acid Dilactone (13) A suspension of **13** (18 mg) in 5% H_2SO_4 (7 ml) was heated on a boiling-water bath for 8 h. Reversed-phase HPLC of the reaction mixture showed peaks of valoneic acid dilactone (**15**) and the starting material. These compounds were further characterized as their methyl derivatives **16** and **14**, prepared with CH_2N_2, and purified by preparative TLC (solvent B) after extraction with EtOAc.

Partial Hydrolysis of 6 A solution of **6** (100 mg) in acetone (1 ml) and H_2O (50 ml) was refluxed for 1 h. The dark reddish precipitates that deposited upon concentration of the reaction mixture were collected and washed with MeOH. Recrystallization from THF afforded phenylphenazine dilactone (**19**) (10 mg) as dark reddish brown needles. This product was identical with the specimen obtained from the phenazine derivative (**4**) of geraniin, by comparison of infrared (IR) spectra. The filtrate and washing were combined and evaporated. The residue (70 mg) was submitted to column chromatography on Sephadex LH-20 with EtOH–H_2O (7 : 3) to give a partial hydrolysate (**20**) (14 mg) as a pale yellow amorphous powder, $[\alpha]_D$ $+56°$ ($c=0.6$, MeOH). *Anal.* Calcd for $C_{68}H_{48}O_{44}\cdot 3H_2O$: C, 50.32; H, 3.35. Found: C, 50.09; H, 3.81. FAB-MS *m/z*: 1569 $(M-H)^-$. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 217 (5.13), 273 (4.78). ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.13, 6.96 (each 2H, s, galloyl), 7.00, 6.64, 6.56, 6.41, 6.39, 6.36 [each 1H, s, HHDP and euphorbinoyl], 5.35 (1H, dd, $J=8.5$, 10 Hz, 2-H), 5.30 (1H, dd, $J=6.5$, 13 Hz, 6-H), 5.17 (1H, t, $J=10$ Hz, 3-H), 5.08 (1H, t, $J=10$ Hz, 4-H), 4.88 (1H, d, $J=8.5$ Hz, 1-H), 4.13 (1H, dd, $J=6.5$, 10 Hz, 5-H), 3.91 (1H, d, $J=13$ Hz, 6-H) (glucose core-I), 6.39 (1H, br s, 1′-H), 4.92 (1H, br s, 3′-H), 4.71 (1H, t, $J=10.5$ Hz, 6′-H), 4.50 (1H, br s, 4′-H), 4.47 (1H, br dd, $J=8.5$, 10.5 Hz, 5′-H), 4.21 (1H, dd, $J=8.5$, 10.5 Hz, 6′-H), 4.20 (1H, br s, 2′-H) (glucose-II). ^{13}C-NMR (acetone-d_6–D_2O) δ: 61.9, 63.0, 64.7, 69.0, 70.4, 71.1, 72.0, 73.0, 73.4, 75.1, 94.4, 94.9 [glucose (I) and (II)]; 104.9, 106.8, 107.6, 107.8, 108.1, 110.0 (2C), 110.4 (2C), 110.6, 111.0, 115.6, 115.7, 115.9, 116.1, 118.1, 120.1, 120.5, 124.0, 125.3, 126.0, 130.1, 132.5, 134.0, 136.3, 136.4, 137.2, 137.8, 138.48, 138.51, 138.8, 139.2 (2C), 139.5, 141.9, 144.0, 144.3 (2C), 144.34, 145.0, 145.1, 145.2, 145.7 (2C), 145.76, 145.8, 148.7, 149.4 (2C) (aromatic); 165.6, 166.1, 166.4, 166.8, 167.3, 167.6, 167.7, 168.2 (ester CO).

Partial Acid Hydrolysis of Euphorbin C (5) A suspension of **5** (300 mg) in 5% H_2SO_4 (35 ml) was refluxed for 2 h. After cooling, the reaction mixture was passed through a column of Diaion HP-20 and eluted with H_2O and MeOH. The MeOH eluate was evaporated and then chromatographed over Sephadex LH-20, developing with EtOH and EtOH–H_2O–acetone (7 : 2 : 1) to give two partial hydrolysates, **23** (27 mg) and **25** (5 mg), together with gallic acid (22 mg), ellagic acid (8 mg) and euphorbinic acid dilactone (**13**) (3 mg). Euphorbinic acid dilactone (**13**) was obtained as a pale yellow amorphous powder, UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 219 (4.60), 256 (4.67), 355 sh (3.95), 365 (3.99). FAB-MS *m/z*: 637 $(M-H)^-$. ^{1}H-NMR (acetone-d_6) δ: 7.53, 7.22, 7.19, 7.09 (each 1H, s).

A hydrolysate (**23**) was obtained as a pale yellow amorphous powder, $[\alpha]_D$ $+65°$ ($c=0.4$, MeOH). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 219 (4.80), 257 (4.75), 355 sh (4.01), 367 (4.04). FAB-MS *m/z*: 951 $(M-H)^-$. ^{1}H-NMR (acetone-d_6–D_2) δ: 7.55, 7.54 (each s, 1H in total), 7.18, 7.13 (each s, 1H in total), 7.16, 7.11 (each s, 1H in total), 7.06, 6.99 (each s, 1H in total), 6.98, 6.63 (each br s, 2H in total) (aromatic), 5.41 (d, $J=3.5$ Hz, 1-H), 4.93 (dd, $J=3.5$, 10 Hz, 2-H), 5.69 (dd, $J=9.5$, 10 Hz, 3-H), 4.10—3.40 (4-H—6-H) (α-anomer), 4.89 (d, $J=8$ Hz, 1-H), 5.06 (dd, $J=8$, 9.5 Hz, 2-H), 5.21 (t, $J=9.5$ Hz, 3-H), 4.10—3.40 (4-H—6-H) (β-anomer).

A hydrolysate (**25**) was obtained as a pale yellow amorphous powder, $[\alpha]_D$ $+73°$ ($c=0.3$, MeOH). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 221 (4.98), 257 (4.78), 355 sh (3.96), 367 (3.98). FAB-MS *m/z*: 1253 $(M-H)^-$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.12, 6.61 (each 2H, s), 7.53, 7.52 (2H in total), 7.22, 7.16 (1H in total), 7.14, 7.01 (1H in total) (aromatic), 5.50 (d, $J=4$ Hz, 1-H), 5.16 (dd, $J=4$, 10 Hz, 2-H), 5.79 (t, $J=10$ Hz, 3-H), 5.00 (t, $J=10$ Hz, 4-H), 4.62 (br dd, $J=6.5$, 10 Hz, 5-H), 3.75 (dd, $J=1.5$, 13 Hz, 6-H), another H-6 was overlapped with 1-H—2H and 6-H of β-anomer) (α-anomer), 5.28—5.22 (m, 1-, 2-, 6-H), 5.42 (t, $J=10$ Hz, 3-H), 4.96 (t, $J=10$ Hz, 4-H), 4.23 (m, 5-H), 3.80 (br d, $J=12$ Hz, 6′-H) (β-anomer).

Hydrolysis of 23 with Tannase A solution of **23** (60 mg) in H_2O (30 ml) was incubated at 37 °C with tannase which was prepared from *Aspergillus niger*,[16] and the reaction process was monitored by HPLC. The reaction mixture was evaporated and the residue was dissolved in EtOH. The insoluble material was removed by centrifugation, and the supernatant was chromatographed on Sephadex LH-20 using EtOH as the eluant to give a degalloyl derivative (**24**) (8 mg) as a white amorphous powder, $[\alpha]_D$ $+19°$ ($c=0.86$, MeOH). *Anal.* Calcd for $C_{34}H_{24}O_{23}\cdot 3H_2O$: C, 47.99; H, 3.54. Found: C, 47.85; H, 3.50. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 210 (4.65), 255 (4.78), 355 sh (4.06), 366 (4.09). FAB-MS *m/z*: 799 $(M-H)^-$. ^{1}H-NMR (acetone-d_6–D_2O) δ: 7.50, 7.49 (1H in total), 7.23, 7.20 (1H in total), 7.19, 7.15 (1H in total), 7.09, 7.07 (1H in total) (aromatic), 5.20 (d, $J=3.5$ Hz, 1-H of α-anomer), 4.50 (br d, $J=8$ Hz, 1-H of β-anomer), 4.60 (dd, $J=3.5$, 10 Hz, 2-H of α-anomer), 4.70 (dd, $J=8$, 9.5 Hz, 2-H of β-anomer), 3.88 (dd, $J=9$, 10 Hz, 3-H of α-anomer), 3.50 (br t, $J=9.5$ Hz, 3-H of β-anomer), 3.75—3.30 (4-H—6-H).

Methylation of 20 A mixture of **20** (90 mg), dimethyl sulfate (0.5 ml) and anhydrous potassium carbonate (500 mg) in dry acetone (10 ml) was stirred overnight at room temperature and refluxed for 3 h. After removal of potassium carbonate by filtration and evaporation of the solvent, the residue was purified by preparative TLC (solvent D) to give a tricosamethyl derivative (**21**) (36 mg) as a white amorphous powder, $[\alpha]_D$

$+11°$ ($c=1.5$, acetone). *Anal.* Calcd for $C_{91}H_{94}O_{44}\cdot 3H_2O$: C, 56.17; H, 5.18. Found: C, 56.27; H, 4.92. ^{1}H-NMR (acetone-d_6) δ: 7.34, 7.23 (each 2H, s, galloyl), 6.99, 6.96, 6.81, 6.79, 6.65, 6.51 (each 1H, s, HHDP and euphorbinoyl), 5.70 (1H, d, $J=8.5$ Hz, 1-H), 5.58 (1H, dd, $J=8.5$, 9.5 Hz, 2-H), 5.52 (1H, t, $J=9.5$ Hz, 3-H), 5.23 (1H, t, $J=9.5$ Hz, 4-H), 4.46 (1H, dd, $J=6.5$, 9.5 Hz, 5-H), 5.29 (1H, dd, $J=6.5$, 13.5 Hz, 6-H), 3.97 (1H, d, $J=13.5$ Hz, 6-H) (glucose core-I); 6.52 (1H, br s, 1′-H), 3.93 (2′-H), overlapped with methoxy protons), 4.94 (1H, br s, 3′-H), 4.20 (1H, br s, 4′-H), 4.45 (5′-H, overlapped with 5-H), 5.03 (1H, t, $J=11$ Hz, 6′-H), 4.23 (1H, dd, $J=11$, 8 Hz, 6′-H) (glucose core-II). ^{13}C-NMR (acetone-d_6) δ: 167.5, 167.4, 167.1, 166.2, 166.1, 165.6, 164.7, 163.7 (ester CO).

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