



BISCHOFIANIN, A DIMERIC DEHYDROELLAGITANNIN FROM *BISCHOFIA JAVANICA*

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Key Word Index—*Bischofia javanica*; Euphorbiaceae; leaves; tannin; dimeric dehydroellagitannin; bischofianin.

Abstract—A new dimeric ellagitannin, bischofianin, was isolated from the leaves of *Bischofia javanica*, together with five known tannins. The structure was elucidated on the basis of spectroscopic and chemical evidence.

INTRODUCTION

Bischofia javanica is a common subtropical tree and cultivated as an avenue tree. The leaves and bark are known to be rich in tannins. In continuing systematic chemical studies on tannins of euphorbiaceous plants [1-4], we have now examined the leaves of this species and isolated a new dimeric ellagitannin, bischofianin, which consists of galloylglucose and dehydroellagitannin moieties. This paper deals with the isolation and structural elucidation of this tannin.

RESULTS AND DISCUSSION

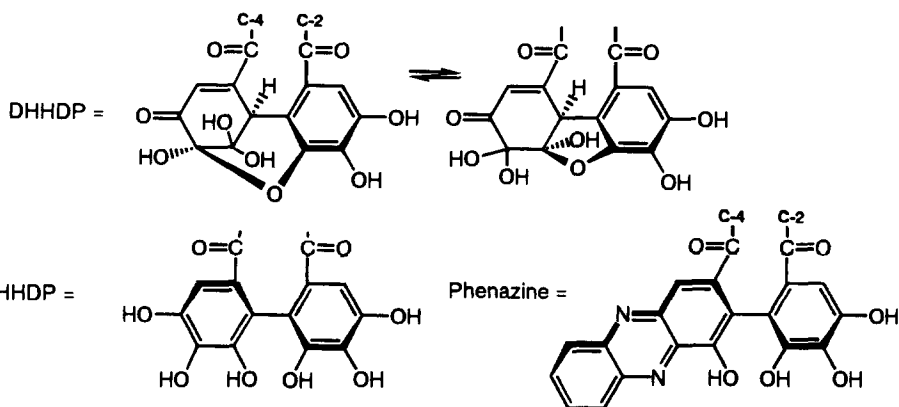
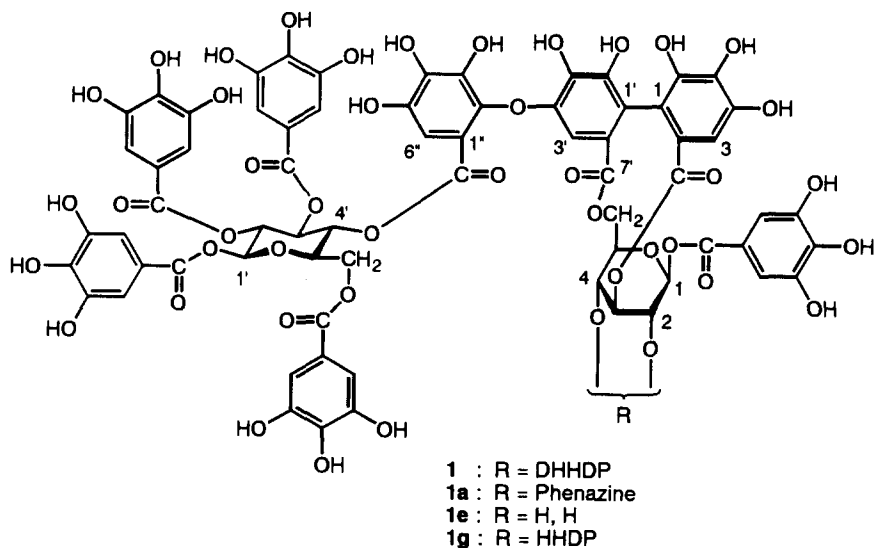
The dried leaves of *B. javanica* were extracted with aqueous acetone and the extract was separated by a combination of chromatography on Sephadex LH-20, cellulose and various reverse-phase gels, such as MCI-gel CHP 20P and Bondapak C₁₈/Porasil B, to yield bischofianin (1), together with five known tannins, geraniin (2) [5], corilagin (3) [6], furosin (4) [7], punicalagin (5) [8] and procyanidin B-1 (6) [9], which were identified by comparison of their physical and spectral data with those described in the literature or by direct comparison with authentic samples.

Bischofianin (1) was characterized as an ellagitannin by its blue coloration with FeCl₃ reagent and reddish-brown coloration with NaNO₂-acetic acid [10]. The ¹³C NMR spectrum showed signals owing to a cyclohexenetrione ring of a dehydrohexahydroxydiphenoyl (DHH DP) group [δ 45.9, 51.9 (CH); 92.1, 93.8, 96.2, 108.9 (hemiketal); 125.4, 128.9, 148.8, 154.1 (olefin); 192.1, 195.0 (ketone)], the chemical shifts of which were closely related to those of geraniin (2). Because of the equilibrium

between five- and six-membered hemiketal ring structures of the DHHDP group, the ¹H and ¹³C NMR spectra were complicated, hence 1 was treated with *o*-phenylenediamine to give the phenazine derivative, 1a. The ¹H NMR spectrum of 1a showed five two-proton aromatic singlets (δ 6.98, 7.08, 7.10, 7.13, 7.16) attributable to five galloyl groups, and two anomeric doublets at δ 5.95 ($J = 8$ Hz) and 6.21 ($J = 6$ Hz) indicating the presence of two sugar residues. The dimeric structure of 1 was further supported by the negative FAB-mass spectra of 1 and 1a which showed [M - H]⁻ peaks at m/z 1871 and 1943, respectively.

Partial hydrolysis of 1a in hot water afforded three major products, phenazine bislactone (1b) [5], 1,2,3,6-tetra-*O*-galloyl- β -D-glucopyranose (1c) [11] and phillyl-aecidin E (1d) [12], suggesting that the valoneyl ester group attached to the C-4 hydroxyl group of 1d links the two sugar moieties together in the molecule of 1. To determine the location of the esters in the other unit, we applied the reaction of dehydroellagitannins with L-cysteine methyl ester [13] for selective hydrolysis of the DHHDP group to yield 1e and f. The ¹H NMR spectrum of 1e showed signals due to two sugar moieties (see Experimental), the chemical shifts and coupling constants of which were almost in line with those of corilagin (3) plus pentagalloylglucose, except for the large upfield shifts of H-5 [δ 3.51 ($d, J = 10$ Hz)] and one of the H-6 [δ 3.67 ($d, J = 12$ Hz)] of the pentagalloylglucose moiety [pentagalloylglucose: δ 4.61 (H-5), 4.50 (2H, H-6)] [14]. These upfield shifts were considered to be caused by the anisotropic effect of the corilagin moiety. The ¹H-¹³C long-range COSY spectrum of 1e showed correlation peaks between the aromatic singlet attributable to the H-3' of the valoneyl group (δ 6.26) [7, 15] and the carboxyl carbon (C-7') signal at δ 168.4, which was also correlated to the signal owing to glucose H-6 (δ 4.70) of the corilagin

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moiety. These results indicated that the structure of **1e**, including the orientation of the valoneyl group, was represented by the formula **1e**.

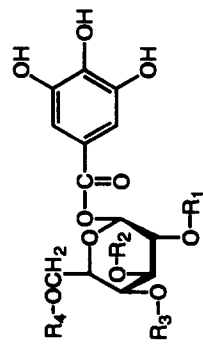
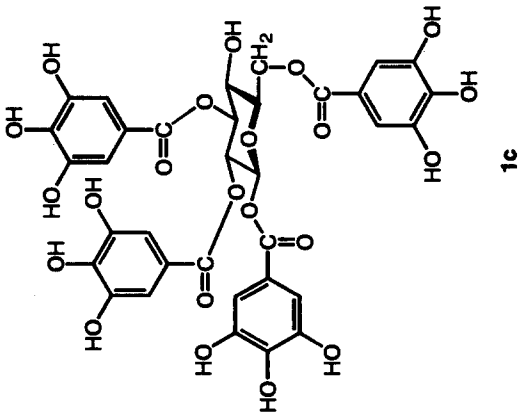
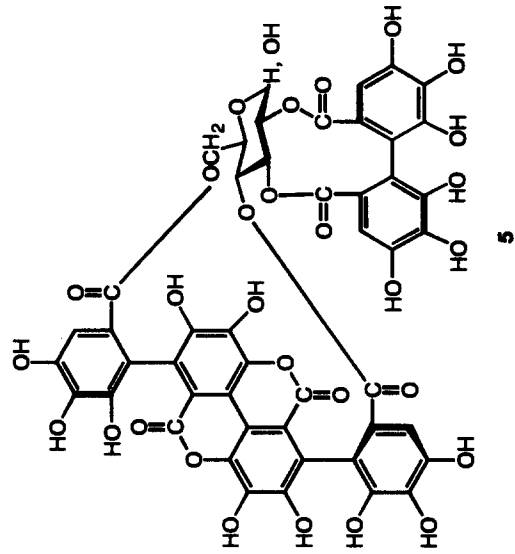
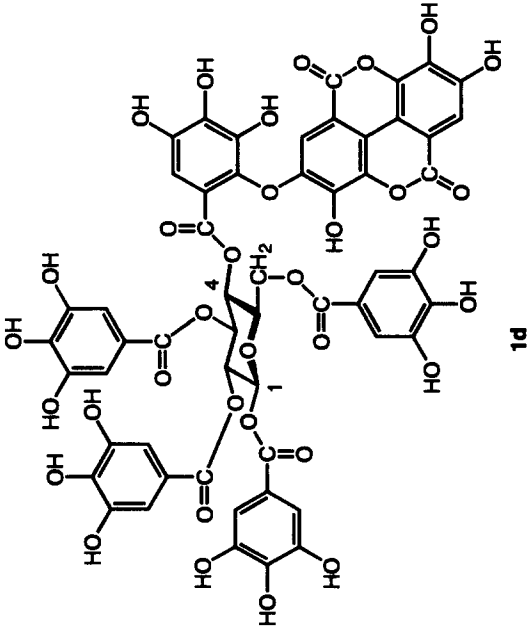
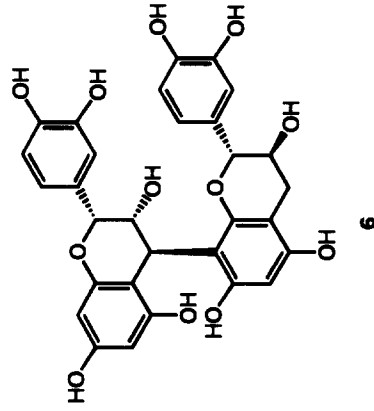
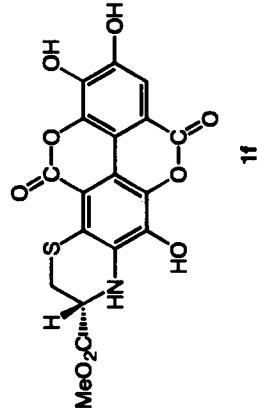
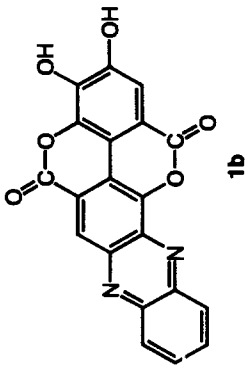
The orientation of the DHHDP group attached to C-2 and C-4 hydroxyl group of the glucose was determined to be the same as that of geraniin (**2**), on the basis of the upfield shift of the anomeric proton signal (δ 6.21) in **1a** as compared with that of **1** (δ 6.64 and 6.60). This phenomenon was similarly observed in the case of **2** and its phenazine derivative [5]. The configuration of the DHHDP and valoneyl groups were determined to be *R* by methylation followed by methanolysis of **1g**, which was prepared by catalytic reduction of **1**, to yield methyl trimethoxybenzoate, dimethyl (*R*)-4,4',5,5',6,6'-hexamethoxydiphenate [5] and trimethyl (*R*)-octamethylvalonate [16]. On the basis of these results, the structure of bischofianin was concluded to be that represented by formula **1**. This tannin is considered to be biosynthesized by oxidative coupling between geraniin (**2**) and pentagalloylglucose. Although **2** is the major constituent of the leaves (1.8% dry weight), pentagalloylglucose was not detected.

EXPERIMENTAL

General. NMR spectra were recorded at 100 and 270 MHz (^1H NMR) and 25 MHz (^{13}C NMR). Chemical shifts are given in δ (ppm) with TMS as int. std. Negative FAB-MS were measured at 1.5 kV (accelerating voltage) with MeOH-glycerol as matrix. CC was carried out on Sephadex LH-20 (25–100 μm , Pharmacia), MCI gel CHP 20P (75–150 μm , Mitsubishi), Bondapak C_{18} /Porasil B (37–75 μm , Waters), Avicel cellulose (Funakoshi), Cosmosil 75C $_{18}$ -OPN (75 μm , Nacalai Tesque) and Kieselgel 60 (70–230 mesh, Merck). TLC was conducted on pre-coated silica gel 60 F $_{254}$ plates (Merck) and pre-coated cellulose F $_{254}$ plates (Merck). Spots were detected under UV and by spraying with ethanolic FeCl_3 .

Plant material. Leaves of *B. javanica* Bl. were collected in the Taiwan Forestry Research Institute, Heng-chun Branch, Taiwan, R.O.C.

Extraction and isolation. Dried leaves (2.8 kg) were extracted with $\text{Me}_2\text{CO}-\text{H}_2\text{O}$ (4:1) at room temp. After removal of Me_2CO *in vacuo*, the aq. soln was filtered and the filtrate subjected to Sephadex LH-20 CC with H_2O



2: R₁-R₃ = DHDP; R₂-R₄ = HHDP
 3: R₁=R₃=H; R₂-R₄ = HHDP
 4: R₁-R₃ = DHDP; R₂-R₄ = H

containing increasing proportions of MeOH, and finally with 50% aq. Me₂CO, to yield four frs, I (50 g), II (19 g), III (57 g) and IV (28 g). Fr. II was sepd by a combination of CC over Sephadex LH-20 (EtOH), MCI-gel CHP 20P (H₂O–MeOH) and Avicel cellulose (2% HOAc) to afford corilagin (3) (2.2 g), furosin (4) (1.1 g) and procyanidin B-1 (6) (41 mg). Repeated CC of fr. III over Sephadex LH-20 (60% MeOH) and MCI-gel CHP 20P (35% MeOH) yielded geraniin (2) (50 g) and punicalagin (5) (72 mg). Fr. IV was applied to a column of Sephadex LH-20 with MeOH–H₂O–Me₂CO (8:1:1) and then Bondapak C₁₈/Porasil B (20% MeOH) to yield bischofianin (1) (2.2 g).

Bischofianin (1). Brown amorphous powder. $[\alpha]_D^{24} - 20.5^\circ$ (MeOH; *c* 2.4). Negative FAB-MS *m/z*: 1889 [M – H][–]. ¹H NMR (Me₂CO-*d*₆ + D₂O): δ 7.27, 7.26 [1H, in total, each *s*, valoneyl(val)-6''], 7.17, 7.16, 7.15, 7.12, 7.10, 7.06 (11H in total, each *s*, DHHDP-3', galloyl-H), 6.64, 6.60 (1H, in total, each *s*, Glc-1), 6.57, 6.56 (1H in total, each *s*, Val-3), 6.21 (1H, *s*, Val-3'), 5.98, 5.96 (1H, in total, each *d*, *J* = 8 Hz, Glc-1'), 5.83 (1H, *t*, *J* = 10 Hz, Glc-3'), 5.69 (1H, *t*, *J* = 10 Hz, Glc-4'), 5.43–5.65 (4H, *m*, Glc-2), 2', 3, 4), 5.22 (2/5H, *s*, DHHDP-1), 4.94 (3/5H, *d*, *J* = 1 Hz, DHHDP-1), 4.79 (2H, *m*, Glc-5, 6) 4.35 (1H, *d*, *J* = 11 Hz, Glc-6'), 3.64–3.85 (Glc-6, 6', overlapped with HOD signal), 3.45 (1H, *br d*, *J* = 10 Hz, Glc-5'). ¹³C NMR (Me₂CO-*d*₆ + D₂O): δ 93.8 (C-1'), 74.1 (C-5'), 73.0 (C-3'), 71.4 (C-2'), 68.4 (C-4'), 61.4 (C-6') (glucose of pentagalloylglucose unit); 92.1, 91.5 (C-1), 73.3, 72.5, 71.0, 70.3, 67.0, 66.0, 64.0, 63.7, 62.8 (C-6) (glucose of geraniin unit); 192.1 (C-4), 154.1 (C-2), 145.0 (C-4'), 143.6 (C-6'), 128.9 (C-3), 115.8 (C-1'), 113.5 (C-3'), 96.2 (C-5), 92.4 (C-6), 45.9 (C-1) (six-membered ring structure of DHHDP); 195.0 (C-4), 148.8 (C-2), 125.4 (C-3), 108.9 (C-6), 92.1 (C-5), 51.9 (C-1) (five-membered ring structure of DHHDP); 103.4 (Val-3'), 106.8 (Val-3), 147.9 (Val-4'); 110.0, 110.2, 110.5 (galloyl-2, 6); 164.6, 165.0, 165.5, 166.0, 166.4, 166.7, 167.0, 168.3 (CO₂). (Found: C, 50.57; H, 3.64. C₈₂H₅₈O₅₃·3H₂O requires: C, 50.62; H, 3.32).

Phenazine derivative (1a). A mixt. of 1 (100 mg) and *o*-phenylenediamine (8 mg) in 20% HOAc–EtOH was left standing for 30 min. The product was purified by Sephadex LH-20 CC with MeOH–H₂O–Me₂CO (14:3:3) to give 1a (42 mg) as a yellow powder. $[\alpha]_D^{25} + 13.3^\circ$ (MeOH; *c* 0.2). Negative FAB-MS *m/z*: 1943 [M – H][–]. ¹H NMR (Me₂CO-*d*₆ + D₂O): δ 8.30–8.37 [2H, *m*, phenazine(phe)-4'', 5''], 8.33 (1H, *s*, phe-3), 8.05 (2H, *m*, phe-3'', 6'') 7.53 (1H, *s*, phe-3'), 7.16, 7.13, 7.10, 7.08, 6.98 (each 2H, *s*, galloyl-H), 7.12 (1H, *s*, Val-3''), 7.07 (1H, *s*, Val-3), 6.26 (1H, *s*, Val-3'), 6.21 (1H, *d*, *J* = 6 Hz, Glc-1), 5.95 (1H, *d*, *J* = 8 Hz, Glc-1'), 5.84 (1H, *t*, *J* = 10 Hz, Glc-3'), 5.74 (1H, *d*, *J* = 6 Hz, Glc-2), 5.73 (1H, *t*, *J* = 10 Hz, Glc-4'), 5.62 (1H, *dd*, *J* = 8, 10 Hz, Glc-2'), 5.57 (1H, *d*, *J* = 5 Hz, Glc-4), 5.42 (1H, *d*, *J* = 4 Hz, Glc-3), 4.97 (1H, *dd*, *J* = 4, 8 Hz, Glc-5), 4.80 (1H, *dd*, *J* = 8, 12 Hz, Glc-6), 4.45 (1H, *d*, *J* = 14 Hz, Glc-6'), 3.88 (1H, *dd*, *J* = 4, 12 Hz, Glc-6), 3.68 (1H, *dd*, *J* = 2, 14 Hz, Glc-6'), 3.47 (1H, *br d*, *J* = 10 Hz, Glc-5'). ¹³C NMR (Me₂CO-*d*₆ + D₂O): δ 91.7 (C-1), 76.8 (C-5), 76.5 (C-2), 68.6 (C-3), 67.7 (C-4), 65.4 (C-6) (glucose of geraniin unit); 93.7, 74.0, 73.0, 71.4, 68.6, 61.7

(glucose of pentagalloylglucose unit); 164.7, 165.2, 166.0, 166.5, 166.8, 167.0, 168.3 (CO₂). (Found: 52.72; H, 3.27; N, 1.33. C₈₈H₆₀O₅₀N₂·3H₂O requires: C, 52.86; H, 3.33; N, 1.40).

Partial hydrolysis of compound 1a. A soln of 1a (30 mg) in H₂O–MeOH (2:1, 6 ml) was heated at 80° for 27 hr. The resulting red ppts 1b were collected by filtration. The filtrate was subjected to CC over Sephadex LH-20 (EtOH) and MCI gel CHP 20P (H₂O–MeOH) to afford 1c (2 mg) and 1d (5 mg). 1,2,3,6-tetra-*O*-galloyl- β -D-glucose (1c). Amorphous powder. $[\alpha]_D^{28} + 38.2^\circ$ (Me₂CO; *c* 0.2). ¹H NMR (Me₂CO-*d*₆ + D₂O): δ 7.17, 7.11, 7.09, 7.03 (each 2H, *s*, galloyl-H), 6.19 (1H, *d*, *J* = 8 Hz, Glc-1), 5.72 (1H, *t*, *J* = 9 Hz, H-3), 5.48 (1H, *t*, *J* = 9 Hz, H-2). Phillylaoeoidin E (1d). Tan amorphous powder. $[\alpha]_D^{28} + 38.1^\circ$ (Me₂CO; *c* 0.5). ¹H NMR (Me₂CO-*d*₆ + D₂O): δ 7.52, 7.13 (each 1H, *s*, Val-H), 7.04 (2H, *s*, Galloyl-H), 7.01 (3H, *s*, Galloyl-H, Val-H), 6.97, 6.72 (each 2H, *s*, Galloyl-H), 6.21 (1H, *d*, *J* = 8 Hz, Glc-1), 5.94 (1H, *t*, *J* = 10 Hz, Glc-3), 5.72 (1H, *t*, *J* = 10 Hz, Glc-4), 5.55 (1H, *dd*, *J* = 8, 10 Hz, Glc-2), 4.47 (1H, *d*, *J* = 13 Hz, Glc-6), 4.41 (1H, *dd*, *J* = 4, 10 Hz, Glc-5), 3.95 (Glc-6, overlapped with HOD signal).

Partial hydrolysate 1e. A mixt. of 1 (200 mg), L-cysteine methyl ester (100 mg) and HCO₂NH₄ (50 mg) in H₂O–MeOH (2:1, 20 ml) was heated at 80° for 5 hr. The resulting yellow ppts (1f), which were identified by direct comparison of the IR data with an authentic sample, were collected by filtration. CC of the filtrate on Cosmosil 75C₁₈-OPN with 30% MeOH gave hydrolysate 1e (85 mg) as a tan amorphous powder. $[\alpha]_D^{25} - 22.0^\circ$ (MeOH; *c* 0.5). ¹H NMR (Me₂CO-*d*₆ + D₂O): δ 7.14, 7.12, 7.11, 7.10, 7.06 (each 2H, *s*, galloyl-H), 7.04 (1H, *s*, Val-6''), 6.88 (1H, *s*, Val-3), 6.26 (each 1H, *s*, Val-3'), 6.37 (1H, *d*, *J* = 3 Hz, Glc-1), 6.02 (1H, *d*, *J* = 8 Hz, Glc-1'), 5.83 (1H, *t*, *J* = 10 Hz, Glc-3'), 5.70 (1H, *t*, *J* = 10 Hz, Glc-4'), 5.62 (1H, *dd*, *J* = 8, 10 Hz, Glc-2'), 4.90 (1H, *br s*, Glc-3), 4.70 (1H, *t*, *J* = 10 Hz, Glc-6), 4.50 (2H, *m*, Glc-4, 5), 4.35 (1H, *d*, *J* = 12 Hz, Glc-6'), 4.15 (2H, *m*, Glc-2, 6), 3.67 (1H, *d*, *J* = 12 Hz, Glc-6'), 3.51 (1H, *d*, *J* = 10 Hz, Glc-5'). ¹³C NMR (Me₂CO-*d*₆ + D₂O): δ 61.3 (Glc-6'), 62.1 (Glc-4), 64.4 (Glc-6), 68.4, 68.8 (Glc-2, 4'), 70.3, 71.1 (Glc-2', 3), 73.1 (Glc-3'), 74.3 (Glc-5'), 75.5 (Glc-5), 93.9 (Glc-1'), 94.3 (Glc-1), 103.3 (Val-3'), 109.2 (Val-3), 110.0, 110.2, 110.6, 110.8 (Val-6'', Galloyl-2, 6), 113.7 (Val-1''), 116.0 (Val-1), 118.6 (Val-1'), 119.5, 120.0, 120.3, 120.6, 121.6 (galloyl-1), 125.3, 125.7 (Val-2, 2'), 135.5 (Val-3''), 136.2 (Val-5'), 136.5 (Val-5), 139.3 (Val-4''), 138.7, 139.1, 139.5, 140.1, 140.3 (Galloyl-4), 140.6 (Val-5''), 143.6 (Val-2''), 145.0 (Val-4), 145.8, 146.0 (Val-6, 6', Galloyl-3, 5), 148.1 (Val-4'), 164.1 (Val-7''), 165.1 (2C), 165.9 (2C), 167.0 (galloyl-7), 167.4 (Val-7), 168.4 (Val-7').

Reduction of compound 1. A soln of 1 (300 mg) in EtOH (10 ml) was hydrogenated for 10 hr at 25° at atmos. pres. in the presence of 8% Pd-C. After filtration, the product was purified by CC on Sephadex LH-20 (EtOH–H₂O–Me₂CO, 27:18:5) to give 1g (46 mg) as a tan amorphous powder. $[\alpha]_D^{28} + 18.8^\circ$ (MeOH; *c* 0.5). ¹H NMR (Me₂CO-*d*₆ + D₂O): δ 7.43 (1H, *s*, Val-H), 7.15 (2H, *s*, Galloyl-H), 7.14 (3H, *s*, Val-H Galloyl-H), 7.13,

7.09, 7.07 (each 2H, s, Galloyl-H), 7.05, 6.99 (each 1H, s, hexahydroxydiphenoyl-H), 6.21 (1H, s, Val-H), 6.16 (1H, d, $J = 6$ Hz, Glc-1), 5.93 (1H, d, $J = 8$ Hz, Glc-1'), 5.82 (1H, t, $J = 10$ Hz, Glc-3'), 5.71 (1H, t, $J = 10$ Hz, Glc-4'), 5.65 (1H, d, $J = 6$ Hz, Glc-2), 5.61 (1H, dd, $J = 8, 10$ Hz, Glc-2'), 5.52 (1H, d, $J = 4$ Hz, Glc-4), 5.17 (1H, d, $J = 4$ Hz, Glc-3), 4.83 (1H, dd, $J = 4, 8$ Hz, Glc-5), 4.74 (1H, dd, $J = 8, 12$ Hz, Glc-6), 4.42 (1H, d, $J = 12$ Hz, Glc-6'), 3.83 (1H, dd, $J = 4, 12$ Hz, Glc-6), 3.66 (1H, d, $J = 12$ Hz, H-6'), 3.43 (1H, d, $J = 10$ Hz, H-5'). (Found: C, 49.56; H, 3.48. $C_{82}H_{58}O_{52} \cdot 6H_2O$ requires: C, 49.66; H, 3.56).

Methylation followed by methanolysis of compound 1g. A mixt. of **1g** (40 mg), Me_2SO_4 (1 ml) and K_2CO_3 (2 g) in dry Me_2CO (20 ml) was heated under reflux for 2 hr with stirring. After removal of inorganic material by filtration, the filtrate was concd and subjected to CC on silica gel (C_6H_6 - Me_2CO , 9:1). The product (26 mg) was hydrolysed with 5% NaOH in H_2O - $MeOH$ (1:1) (90° , 1 hr). After cooling, the mixt. was acidified with HCl, extracted with Et_2O , dried over Na_2SO_4 and methylated with CH_2N_2 . The mixt was sepd by CC on silica gel (C_6H_6 - Me_2CO) to yield methyl 3,4,5-trimethoxybenzoate (10 mg), dimethyl(*R*)-4,4',5,5',6,6'-hexamethoxydiphenate (2.7 mg, $[\alpha]_D^{20} + 24.8^\circ$ ($CHCl_3$; c 0.3)) and trimethyl(*R*)-octamethylvalonate (7.2 mg, $[\alpha]_D^{20} + 15.3^\circ$ ($CHCl_3$; c 0.7)).

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