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PUNICAFOLIN, AN ELLAGITANNIN FROM THE LEAVES OF *PUNICA GRANATUM**

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Key Word Index—*Punica granatum*; Punicaceae; pomegranate; punicafolin; ellagitannin; gallotannin.

Abstract—A new ellagitannin, punicafolin has been isolated from the leaves of *Punica granatum* and characterized by physicochemical data and spectral evidence as 1,2,4-tri-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-β-D-glucose. The occurrence in the leaves of the known tannins, granatins A and B, corilagin, strictinin, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose has also been demonstrated.

INTRODUCTION

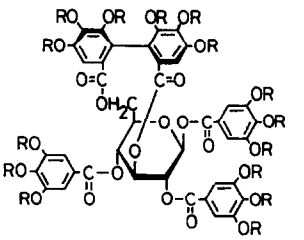
Punica granatum, native to the Mediterranean regions and south west Asia, is cultivated throughout the world, and has been used since ancient times as an anthelmintic and an astringent drug. From the pericarp of the plant, four yellow-coloured ellagitannins, granatins A and B [1], punicalagin and punicalin [2] have been isolated. The former two tannins contain a dehydrohexahydroxydiphenoyl and a hexahydroxydiphenoyl ester group attached to a D-glucose moiety, while the latter are unique in that they contain a gallagyl (tetraphenyl) ester group in the molecule.

As a part of chemical studies on tannins and related compounds in crude drugs, we have examined tannins of the leaf; this has resulted in the isolation of a new ellagitannin, punicafolin, which is presumed to be a biosynthetic intermediate of granatin B [3]. In addition, four ellagitannins and two gallotannins have been isolated, and they were characterized as granatins A and B, corilagin, strictinin, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose.

RESULTS AND DISCUSSION

Repeated chromatography of an aqueous acetone extract of the fresh leaves over high-porosity polystyrene gel (Diaion HP-20 Ag, MCI-gel) [4–6] and Sephadex LH-20 afforded tannins **1**–**7**, of which compounds **2**–**7** were identified as granatins A (**2**) and B (**3**) [1], corilagin (**4**) [7], strictinin (**5**) [8, 9], 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**6**) [10] and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**7**) [11] by comparison of the physical and spectral data with those of authentic samples. Although it was reported that punicalagin and punicalin were the major constituents in the pericarp [2], these tannins could not be isolated from the leaves. The major components of the leaves were granatins A (**2**) and B (**3**) which comprised *ca* 1.5% of the fr. wt of the leaves.

The new tannin, punicafolin (**1**), was strongly positive (a dark blue colour) to the ferric chloride reagent. The ^{1}H NMR spectrum exhibited three two-proton singlets at δ7.18, 7.19 and 7.27 and two one-proton singlets at δ6.79 and 7.01, suggesting the occurrence of three galloyl and one 4,4′,5,5′,6,6′-hexahydroxydiphenoyl ester groups in the molecule. Glucose, gallic acid and ellagic acid were



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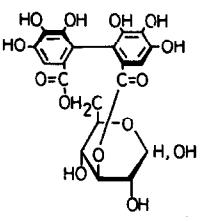
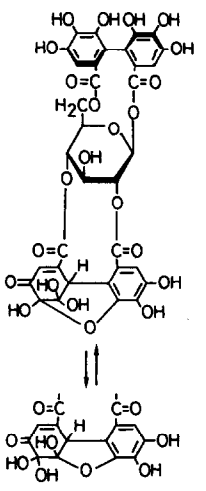
1b R = Me



1a



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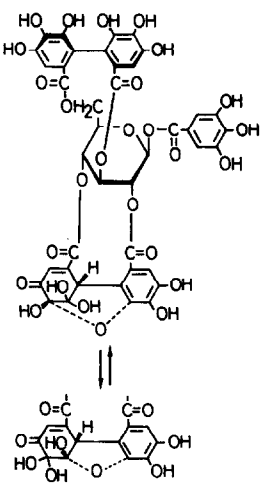

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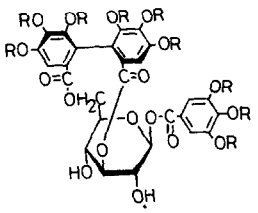
*Part 30 in the series "Tannins and Related Compounds". For Part 29 see Morimoto, S., Nonaka, G. and Nishioka, I., *Chem. Pharm. Bull. Tokyo* (in press).



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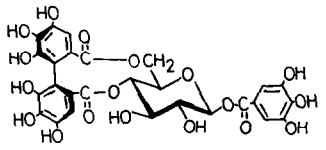
4a R = Me



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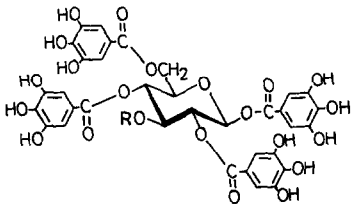
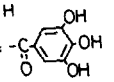
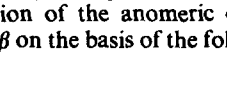


6 R = H
7 R = 

obtained upon hydrolysis of **1** in boiling water. The lowfield shifts (δ6.54, *d*, J = 5 Hz, H-1; δ5.87, *d*, J = 4 Hz, H-4; δ5.55, *d*, J = 5 Hz, H-2; δ5.11, *d*, J = 4 Hz, H-3; δ4.42–4.82, *m*, H-5 and H-6) of all of the glucose ring protons indicated that the glucose hydroxyl groups are exhaustively esterified. Furthermore, the small coupling constants of these signals implied that the conformation of the glucopyranose ring is $^1C_4$ or an intermediate skew boat, and not $^4C_1$ [3]. Since it was reported that when the hexahydroxydiphenoyl ester group is located at the 2,3- and/or 4,6-positions of the glucopyranose moiety, the glucose ring adopts the most stable $^4C_1$ conformation [12], these observations suggested that the hexahydroxydiphenoyl group in **1** is attached to neither the 2,3- nor the 4,6-positions of the glucopyranose core.

Enzymatic hydrolysis of **1** with tannase afforded gallic acid and a hydrolysate (**1a**), whose ^{1}H NMR spectrum showed the presence of a hexahydroxydiphenoyl group and no galloyl peak. The appearance of duplicated sugar proton signals indicated the occurrence of α- and β-anomers. From these spectral data coupled with the above consideration, **1a** was assumed to be either 3,6- or 2,4-hexahydroxydiphenoylglucose. Consequently, by comparison of the ^{1}H NMR spectrum, **1a** was identified as 3,6-hexahydroxydiphenoyl-D-glucose which was obtained by similar tannase hydrolysis of corilagin (**4**). The chirality of the hexahydroxydiphenoyl group was ascertained to be *R* by comparison of the specific optical rotation [+24.7° ($CHCl_3$)] of dimethyl hexamethoxydiphenoate (**8**) obtained by alkaline methanolysis of the pentadeca-*O*-methyl ether (**1b**) of **1**. Accordingly, punicafolin was characterized as 1,2,4-tri-*O*-galloyl-3,6-*O*-(*R*)-hexahydroxydiphenoyl-β-D-glucose.

The configuration of the anomeric centre in **1** was determined to be β on the basis of the following chemical



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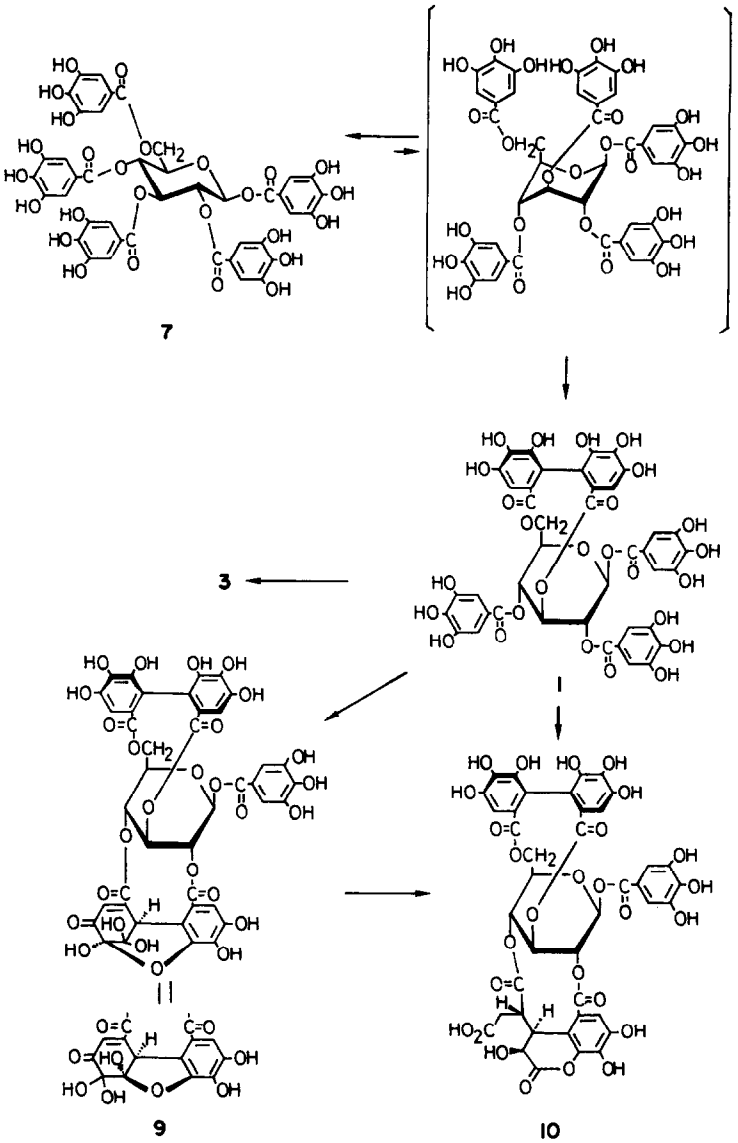
examinations. Corilagin (**4**), which is known to have a β-linkage at the anomeric centre of the glucose residue [13], was methylated with diazomethane to yield the nona-*O*-methyl derivative (**4a**). This methylate was treated with trimethoxybenzoyl chloride in pyridine to give a methyl ether which was shown to be identical with the methyl ether (**1b**) by comparison of their ^{1}H NMR spectra and specific optical rotations.

Previously, Haslam and his collaborators suggested that 1,2,4-tri-*O*-galloyl-3,6-hexahydroxydiphenoylglucose, although they did not isolate this compound, is derived by oxidative coupling of two 3,6-positioned galloyl groups in 1,2,3,4,6-penta-*O*-galloylglucose, and is a key intermediate in the biosynthesis of granatin B and other related ellagitannins, viz. geraniin (**9**) and chebulagic acid (**10**) [3]. Therefore, the isolation of punicafolin is of interest from the viewpoint of biosynthesis of such ellagitannins.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 100 and 25.05 MHz, respectively, and chemical shifts are given in δ (ppm) scale with TMS as int. standard. TLC was conducted on precoated silica gel 60 F_{254} plates (Merck) with C_6H_6–HCO_2Et–HCO_2H (1:7:1) (for phenolics) and C_6H_6–Me_2CO (4:1) (for Me ethers), and precoated cellulose F_{254} plates (Merck) with 2% HOAc (for phenolics) and *n*-BuOH–pyridine–H_2O (6:4:3) (for sugars). Spots were visualized by spraying $FeCl_3$ (for phenolics), 5% H_2SO_4 (for phenolics and Me ethers) and aniline-hydrogen-phthalate (for sugars) reagents. The plant material was collected in October 1982 in the campus of Kyushu University, Fukuoka, Japan.

Extraction and isolation. Fresh leaves of *P. granatum* L. (950 g) were extracted × 3 at room temp with 80% aq. Me_2CO. After evapn of Me_2CO *in vacuo*, the resulting aq. soln was filtered to remove chlorophylls. The filtrate was concd and subjected to CC over high-porosity polystyrene gel (MCI-gel CHP-2OP). Elution first with H_2O and then H_2O with an increasing amount of MeOH yielded three fractions. The last fraction contained mainly flavonoids and was not examined. The first fraction was chromatographed on MCI-gel using H_2O with increasing amounts of MeOH to give mannitol (5.2 g), granatin A (12.4 g) (**2**), corilagin (450 mg) (**4**) and strictinin (60 mg) (**5**). The second



Scheme 1.

fraction was repeatedly chromatographed on Sephadex LH-20 with aq. MeOH and aq. EtOH (60–80%) to yield granatin B (16.3 g) (3), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (100 mg) (6), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (220 mg) (7) and punicafolin (130 mg) (1).

Punicafolin (1). White powder (H_2O), mp 235–237° (dec.), $[\alpha]_D^{20}$ −59.5° (MeOH; *c* 0.4). ^{1}H NMR (Me_2CO-d_6): δ4.42–4.82 (3H, *m*, H-5 and H-6), 5.11 (1H, *d*, *J* = 4 Hz, H-3), 5.55 (1H, *d*, *J* = 5 Hz, H-2), 5.87 (1H, *d*, *J* = 4 Hz, H-4), 6.54 (1H, *d*, *J* = 5 Hz, H-1), 6.79, 7.01 (each 1H, *s*, HHDP*-H), 7.18, 7.19, 7.27 (each 2H, *s*, galloyl H). ^{13}C NMR (Me_2CO-d_6): δ64.7 (C-4, C-6), 71.1, 72.2, 75.8 (C-2, C-3, C-5), 91.9 (C-1), 108.6, 109.8, 115.7, 116.7, 124.9, 125.0, 136.7, 137.2, 145.0, 145.4 (HHDP-C), 110.5, 119.8, 120.3, 139.9, 146.0 (galloyl C), 165.7 (× 2), 166.4, 166.8, 168.7 (ester C). (Found: C, 50.7; H, 3.5. $C_{41}H_{30}O_{26}\cdot 2H_2O$ requires: C, 50.5; H, 3.5%.)

*HHDP = Hexahydroxydiphenoyl.

Hydrolysis of 1. A soln of 1 (3 mg) in H_2O (3 ml) was refluxed for 15 hr and extracted with EtOAc. The EtOAc-soluble portion was concd and analysed by silica gel TLC. Gallic acid (R_f 0.77) and ellagic acid (R_f 0.68) were identified by co-chromatography with authentic samples. The aq. layer was concd and analysed by cellulose TLC which showed a spot (R_f 0.40) coinciding with that of glucose.

Enzymatic hydrolysis of 1. A soln of 1 (40 mg) in H_2O (10 ml) was incubated with tannase for 30 min at 35°. After evapn of solvent *in vacuo*, the residue was treated with EtOH. The EtOH-soluble portion was chromatographed over Sephadex LH-20 using EtOH to give gallic acid and 3,6-(+)-hexahydroxydiphenoyl-D-glucose (12 mg), an off-white amorphous powder, $[\alpha]_D^{22}$ −21.3° (EtOH; *c* 0.3). ^{1}H NMR (Me_2CO-d_6–D_2O): δ6.76, 6.77, 6.78, 6.79 (each *s*, HHDP-H). This compound was identified by ^{1}H NMR comparison with an authentic sample obtained by tannase hydrolysis of corilagin (4).

Methylation of 1. Compound 1 (60 mg) was methylated for 2.5 hr with Me_2SO_4 (1 ml) and K_2CO_3 (1.2 g) in dry Me_2CO

(30 ml). After filtration of the inorganic ppt, the soln was concd to a syrup which was purified by CC over silica gel. Elution with C_6H_6–Me_2CO (5:1) furnished the pentadeca-*O*-Me ether (**1b**) (32 mg), a white amorphous powder, $[\alpha]_D^{18}$ −69.6° ($CHCl_3$; *c* 0.8). ^1H NMR ($CDCl_3$): δ3.57–4.00 (OMe), 4.36 (1H, *dd*, *J* = 7, 11 Hz, H-6), 4.92 (1H, *dd*, *J* = 7, 11 Hz, H-5), 5.21 (1H, *t*, *J* = 11 Hz, H-6), 5.38 (1H, *d*, *J* = 2 Hz, H-3), 5.47 (1H, *br s*, H-2), 5.82 (1H, *d*, *J* = 2 Hz, H-4), 6.75 (1H, *br s*, H-1), 6.86, 6.94 (each 1H, *s*, HMDP*-H), 7.19 (4H), 7.31 (2H) (each *s*, galloyl H).

Methanolysis of **1b**. A soln of **1b** (20 mg) in 2% NaOMe–MeOH (10 ml) was stirred for 4 hr. The soln was neutralized with Amberlite 120 B and filtered. The filtrate was concd to dryness and subjected to CC over silica gel with C_6H_6–Me_2CO (24:1) to afford Me trimethoxybenzoate (2 mg), mp 81–82°, and (*R*)-diMe hexamethoxydiphenoate (**8**) (3.5 mg), colourless syrup, $[\alpha]_D^{20}$ +24.7° ($CHCl_3$; *c* 0.35).

Methylation of **4**. A soln of **4** (110 mg) in MeOH (5 ml) was treated with CH_2N_2–Et_2O for 30 min. The crude product was purified by CC over silica gel. Elution with C_6H_6–Me_2CO (9:1) afforded the nona-*O*-Me ether (**4a**) (67 mg), colourless needles (MeOH), mp 229–231°, $[\alpha]_D^{20}$ −146.0° (Me_2CO; *c* 0.9). ^1H NMR ($CDCl_3$): δ6.52 (1H, *br s*, H-1), 6.56, 6.75 (each 1H, *s*, HMDP-H), 7.19 (2H, *s*, galloyl H).

Trimethoxybenzoylation of **4a**. A mixture of **4a** (41 mg) and 3,4,5-trimethoxybenzoyl chloride (80 mg) in dry pyridine (5 ml) was stirred at room temp for 20 hr and then heated at 70° for 1 hr. The reaction mixture, after concn *in vacuo*, was extracted with Et_2O and the Et_2O soln washed with 3% HCl (× 2) and H_2O (× 2), dried over Na_2SO_4 and evapd. The residue was subjected to CC over silica gel with C_6H_6–Me_2CO (9:1) to afford a Me ether (49 mg), $[\alpha]_D^{22}$ −70.2° ($CHCl_3$; *c* 1.4). This compound was shown to be identical with the pentadeca-*O*-Me ether (**1b**) by comparison of their physical and spectral data.

Granatin A (**2**). Yellow amorphous powder, $[\alpha]_D^{21}$ +43.7° (EtOH; *c* 1.6). ^1H NMR (Me_2CO-d_6): δ4.10 (1H, *m*, H-5), 4.50–4.75 (2H, *m*, H-3 and H-6), 5.00 (2H, *m*, H-2 and H-4), 6.10 (1H, *br s*, H-1).

Granatin B (**3**). Yellow powder (H_2O), $[\alpha]_D^{22}$ −122.4° → 134.6° (Me_2CO–H_2O, 9:1, 13 hr; *c* 0.6). ^1H NMR (Me_2CO-d_6): δ4.22 (1H, *dd*, *J* = 8, 10 Hz, H-6), 4.84 (1H, *t*, *J* = 10 Hz, H-5), 5.08 (1H, *s*, H-1'), 5.17 (1H, *t*, *J* = 10 Hz, H-2), 5.28 (1H, *t*, *J* = 10 Hz, H-6), 5.50 (1H, *br s*, H-3), 5.95 (1H, *d*, *J* = 3 Hz, H-4), 6.57 (1H, *br s*, H-1), 6.59 (1H, *s*, H-3'), 6.78, 6.98 (each 1H, *s*, HHDP-H), 7.15 (2H, *s*, galloyl H), 7.20 (1H, *s*, H-3''). ^{13}C NMR (Me_2CO-d_6): δ46.2 (C-1'), 62.0, 63.8, 64.8, 69.2, 72.3, 90.9 (glucosyl C), 92.6 (C-6'), 96.1 (C-5'), 108.1, 110.5, 115.3, 115.6, 124.4, 125.5, 136.7, 137.8 (HHDP-C), 111.2, 119.9, 139.6, 145.7 (galloyl C), 113.7 (C-3''), 116.9 (C-1''), 119.9 (C-2''), 129.3 (C-3'), 138.8 (C-5''), 143.2 (C-6''), 144.8 (C-4''), 153.9 (C-2'), 164.8, 165.6 (× 2), 166.7, 168.8 (ester C), 191.7 (C-4'). (Found: C, 47.1; H, 3.5. Calc. for $C_{41}H_{28}O_{27} \cdot 5H_2O$: C, 47.2; H, 3.7%.)

Hydrolysis of **3**. A soln of **3** (500 mg) in H_2O (80 ml) was refluxed for 2 hr. After concn *in vacuo*, the residue was subjected to CC over Sephadex LH-20 with EtOH to afford corilagin (**4**) (41 mg).

Preparation of phenazine derivative of **3**. A mixture of **3** (300 mg), *O*-phenylenediamine (40 mg) and HOAc (4 ml) in MeCN (40 ml) was stirred at room temp for 19 hr. The resulting yellow ppt was collected by filtration and crystallized from $CHCl_3$–MeOH to yield a phenazine derivative as a yellow powder (173 mg), mp > 290°. ^1H NMR (Me_2CO-d_6): δ5.09 (1H, *br s*, H-2), 5.44 (1H, *br s*, H-3), 5.60 (1H, *br d*, *J* = 3 Hz, H-4), 6.51 (1H, *br s*, H-1), 6.72, 7.01, 7.03 (each 1H, *s*, aromatic H), 7.15 (2H, *s*, galloyl H).

Corilagin (**4**). Off-white amorphous powder, $[\alpha]_D^{21}$ −202.2° (EtOH; *c* 0.7), ^1H NMR (Me_2CO-d_6): δ4.01–4.20 (2H, *m*, H-2 and H-6), 4.43–4.64 (2H, *m*, H-4 and H-5), 4.83–5.08 (2H, *m*, H-3 and H-6), 6.38 (1H, *br s*, H-1), 6.69, 6.84 (each 1H, *s*, HHDP-H), 7.12 (2H, *s*, galloyl H).

Strictinin (**5**). Off-white amorphous powder, $[\alpha]_D^{21}$ −9.1° (MeOH; *c* 0.5). ^1H NMR (Me_2CO-d_6): δ4.91 (1H, *t*, *J* = 9 Hz, H-4), 5.22 (1H, *dd*, *J* = 6, 14 Hz, H-6), 5.76 (1H, *d*, *J* = 8 Hz, H-1), 6.61, 6.76 (each 1H, *s*, HHDP-H), 7.20 (2H, *s*, galloyl H).

1,2,4,6-*Tetra*-*O*-*galloyl*-β-D-*glucose* (**6**). Colourless needles (H_2O), mp 211–213°, $[\alpha]_D^{22}$ −4.8° (Me_2CO; *c* 0.67). ^1H NMR (Me_2CO-d_6): δ4.20–4.64 (4H, *m*, H-3, H-5 and H-6), 5.43 (2H, *t*, *J* = 9 Hz, H-2 and H-4), 6.14 (1H, *d*, *J* = 8 Hz, H-1), 7.10, 7.12, 7.18 (8H in total, each *s*, galloyl H). ^{13}C NMR (Me_2CO-d_6): δ63.2 (C-6), 71.7 (C-4), 73.3 (C-2), 74.0 (C-3 and C-5), 93.5 (C-1), 110.3, 120.0, 121.2, 121.5, 138.9, 139.1, 139.6, 146.0 (galloyl C), 165.2, 166.0 (× 2), 166.6 (ester C). (Found: C, 48.9; H, 3.8. Calc. for $C_{34}H_{28}O_{22} \cdot 5/2\ H_2O$: C, 49.0; H, 4.0%.)

1,2,3,4,6-*Penta*-*O*-*galloyl*-β-D-*glucose* (**7**). Off-white amorphous powder, $[\alpha]_D^{20}$ +16.9° (Me_2CO; *c* 0.76), ^1H NMR (Me_2CO-d_6): δ4.30–4.68 (3H, *m*, H-5 and H-6), 5.61 (1H, *dd*, *J* = 8, 9 Hz, H-2), 5.66 (1H, *t*, *J* = 9 Hz, H-4), 6.03 (1H, *t*, *J* = 9 Hz, H-3), 6.34 (1H, *d*, *J* = 8 Hz, H-1), 6.96, 7.00, 7.05, 7.11, 7.16 (each 2H, *s*, galloyl H).

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*HMDP = Hexamethoxydiphenoyl.

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