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7-*O*-GALLOYL-(+)-CATECHIN AND 3-*O*-GALLOYLPROCYANIDIN B-3 FROM *SANGUISORBA OFFICINALIS**

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Key Word Index—*Sanguisorba officinalis*; Rosaceae; tannins; flavan-3-ol gallate; procyanidin gallate; gambiriins; galloylglucoses.

Abstract—7-*O*-Galloyl-(+)-catechin and 3-*O*-galloylprocyanidin B-3, along with gambiriins A-1 and B-3 and four polygalloylglucoses, have been isolated from the root and rhizome of *Sanguisorba officinalis*.

INTRODUCTION

Sanguisorba officinalis is a perennial herb distributed throughout Japan and in the northern parts of China. Its root has been utilized in China and Japan as a haemostatic and an astringent, and is well known as a rich source of tannins. Previously, we have demonstrated the presence of ellagitannins (eugeniin [1], and sanguiins H-1, H-2, H-3 [2] and H-6 [3]) and flavan-3-ol derivatives [(+)-catechin, (±)-gallocatechin and procyanidins B-3 and C-2 [4]] in this plant material. Further chemical studies on phenolic constituents of this material have resulted in the isolation of two new galloyl flavan-3-ol derivatives, together with polygalloylglucoses and gambiriins A-1 and B-3 [5]. This paper describes the isolation and structure elucidation of these compounds.

RESULTS AND DISCUSSION

The ethyl acetate soluble portion of the aqueous acetone extract was fractionated by a combination of Sephadex LH-20 and cellulose chromatography to afford compounds 1 and 2, along with gambiriins A-1 (**6**) and B-3 (**7**) [5], and galloylglucoses (1,2,6-tri- (**8**) [6], 1,2,3,6-tetra- (**9**) [7], 2,3,4,6-tetra- (**10**) [8] and 1,2,3,4,6-penta- (**11**) [9] galloylglucoses)which were identified by comparison with authentic samples.

Compound 1 (**1**), colourless needles (water), mp 165–168°, $C_{22}H_{18}O_{10}\cdot 3/2H_2O$, $[\alpha]_D+38.9°$ (Me_2CO), was strongly positive (a dark blue colour) to the ferric chloride reagent. The ^1H NMR spectrum of **1** was similar to that of (+)-catechin (**1a**) except for the additional signal at δ 7.26 (2H, *s*) due to a galloyl group. On enzymatic hydrolysis with tannase in aqueous solution, **1** gave gallic acid and (+)-catechin. The location of the galloyl group was deduced to be at either C-5 OH or C-7 OH of the catechin moiety from the ^1H NMR spectrum, in which signals due to the C-6 H and C-8 H appeared at lower field than those observed in **1a** (Table 1). This was further supported by the mass spectrum of the hexamethyl ether (**1b**) prepared with dimethyl sulphate and potassium carbonate in dry acetone: it exhibited a characteristic fragment peak at *m/z* 347 formed by a retro-Diels–Alder-type fission of the flavan C-ring. In order to determine unequivocally the position of the galloyl group, hexamethyl ethers of 5- and 7-*O*-galloyl-(+)-catechins (**5** and **1a**) were prepared. Partial methylation of (+)-catechin with dimethyl sulphate and potassium carbonate in dry acetone, followed by chromatography over silica gel, afforded 5,3′,4′- and 7,3′,4′-trimethylcatechins (**3** and **4**) which were identified by comparison of the physical data with those reported previously [10]. These methylates were treated with an equimolar amount of sodium hydroxide and then with trimethoxybenzoyl chloride to give the corresponding galloylcatechin hexamethyl ethers, of which the 7-*O*-galloyl derivative was shown by ^1H NMR comparison to be identical with the methylate derived from the natural source. Consequently, the structure of **1** was concluded to be 7-*O*-galloyl-(+)-catechin.

Compound 2 (**2**), an off-white amorphous powder, $C_{37}H_{30}O_{16}\cdot 3/2H_2O$, $[\alpha]_D-170.1°$ (acetone), gave a dark blue colour with ferric chloride reagent. The ^{13}C NMR spectrum of **2** showed aliphatic carbon signals at δ 36.0 (C-4), 68.1 (C-3), 72.9 (C-3′), 81.0 (C-2′) and 81.9 (C-2) which were closely related to those of procyanidin B-3 (**2a**) [11]. The appearance of signals at δ 109.7, 121.6, 138.0, 145.3 and 165.0 indicated the occurrence of a galloyl group in the molecule. Enzymatic hydrolysis of **2** with tannase yielded gallic acid and **2a**. The location of the galloyl group was established by ^1H NMR measured at 150° in DMSO-d_6. The C-3 proton signal, assigned by means of spin-decoupling techniques, was shifted to a lower field (δ 5.92, *t*, J = 7 Hz) than that of **2a**, indicating that the galloyl group is attached to this position. On the basis of these results, the structure of **2** was confirmed to be 3-*O*-galloylprocyanidin B-3.

Various flavan-3-ol gallates have been reported to occur widely in the plant kingdom, but the location of the galloyl group is limited to the C-3 position, except for the presence of 3,5-digalloyl-(−)-epicatechin and 3,5-digalloyl-(−)-epigallocatechin in green tea leaves [12]. The isolation of 7-*O*-galloyl-(+)-catechin and 3-*O*-

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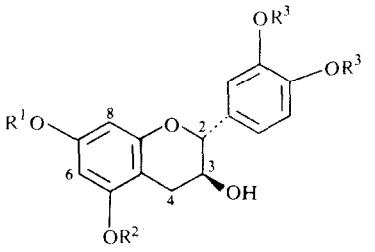
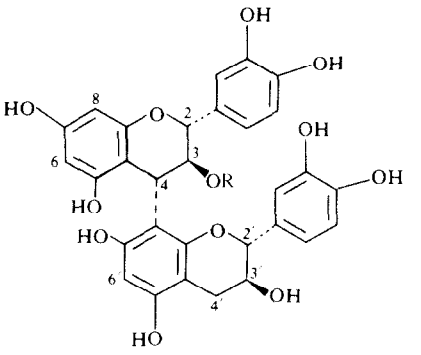
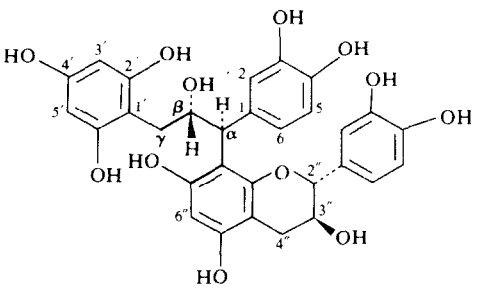


	R^1	R^2	R^3
1	G	H	H
1a	H	H	H
1b	GMe	Me	Me
3	H	Me	Me
4	Me	H	Me
5	Me	GMe	Me



	R
2	G
2a	H



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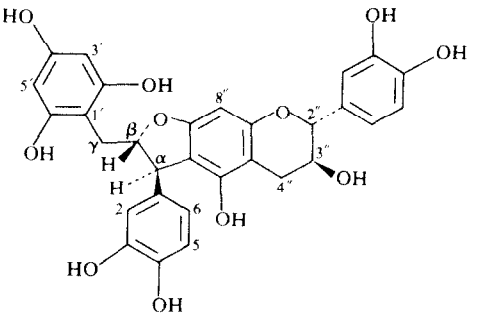

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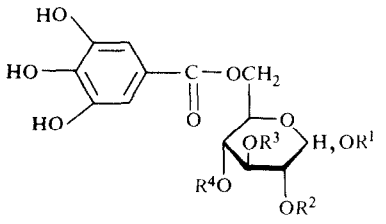
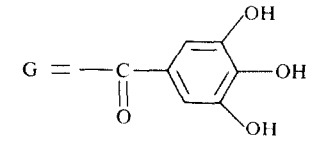
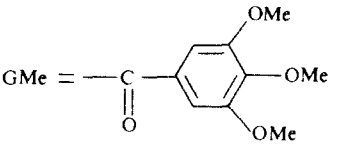


	R^1	R^2	R^3	R^4
8	β–G	G	H	H
9	β–G	G	G	H
10	α,β–H	G	G	G
11	β–G	G	G	G





galloylprocyanidin B-3 from *S. officinalis* implies the random distribution of galloyl groups in higher MW procyanidins of this plant.

EXPERIMENTAL

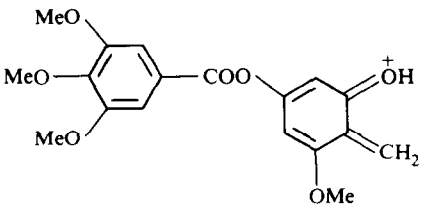
Mps are uncorr. ^{1}H NMR and ^{13}C NMR spectra were recorded at 100 and 25.05 MHz, respectively with TMS as int. standard. MS were measured using a direct inlet system at 75 eV. TLC was conducted on precoated silica gel $60F_{254}$ plates (Merck) and spots were visualized by $FeCl_3$ reagent and by spraying 2% H_2SO_4, followed by heating.

Extraction and isolation. The commercially available roots and rhizomes of *S. officinalis* L. (3 kg) were finely milled and percolated with 80% aq. Me_2CO (20 l.). After evapn of the Me_2CO under red. pres., the aq. soln was partitioned with

Table 1. ^{1}H NMR spectral data for compounds **1**, **1a**, **1b**, **2** and **5** (100 MHz, TMS as internal standard)*

	1†	1a†	1b‡	5‡	2§
H-2	4.65 *d* (8)	4.57 *d* (8)	4.70 *d* (9)	4.71 *d* (8)	4.65 *d* (7)
H-3	4.05 *m*	3.00 *m*	4.00 *m*	4.00 *m*	5.92 *t* (7)
H-4	2.50 *dd* (8, 16)	2.52 *dd* (8, 16)	2.65 *dd* (8, 16)	2.62 *dd* (9, 16)	4.56 *d* (7)
	2.83 *dd* (6, 16)	2.92 *dd* (6, 16)	3.15 *dd* (5, 16)	2.99 *dd* (5, 16)	
H-6	6.30 *d* (2)	5.88 *d* (2)	6.35 *d* (2)	6.42 *d* (2)	5.73 *d* (2)
H-8	6.35 *d* (2)	6.03 *d* (2)	6.45 *d* (2)	6.46 *d* (2)	5.79 *d* (2)
H-2′	—	—	—	—	4.43 *d* (8)
H-3′	—	—	—	—	3.58 *m*
H-4′	—	—	—	—	2.36 *dd* (8, 16)
					2.70 *dd* (5, 16)
H-6′	—	—	—	—	5.90 *s*
Galloyl	7.26 *s*	—	7.41 *s*	7.42 *s*	6.75 *s*
OMe	—	—	3.82, 3.87	3.76, 3.87	—
			3.92	3.92	

*Coupling constants (Hz) in parentheses.
†Me_2CO-d_6.
‡$CDCl_3$.
§DMSO-d_6 at 150°.



m/z 347

EtOAc. The EtOAc-soluble portion (125 g) thus obtained was subjected to CC on Sephadex LH-20, eluting with Me_2CO to give 4 fractions: 1 (30 g), 2 (46 g), 3 (25 g) and 4 (14 g). Fraction 1 was negative to the $FeCl_3$ reagent and was not examined. Fraction 2 was rechromatographed over Sephadex LH-20, eluting with EtOH to furnish gambiriins A-1 (17 mg) and B-3 (12 mg). Rechromatography of fraction 3 on Sephadex LH-20 using EtOH furnished compound 1 (90 mg). Repeated chromatography of fraction 4 over Sephadex LH-20 (EtOH) and cellulose (2% HOAc) yielded compound 2 (600 mg), along with polygalloylglucoses such as 1,2,6-tri-galloyl-β-D-glucose (25 mg), 1,2,3,6-tetragalloyl-β-D-glucose (72 mg), 2,3,4,6-tetragalloyl-D-glucose (450 mg) and 1,2,3,4,6-pentagalloyl-β-D-glucose (2.9 g).

7-O-*Galloyl*-(+)-*catechin* (**1**). Colourless needles (H_2O), mp 165–168°, $[\alpha]_D^{27}$ + 38.9° (Me_2CO; *c* 0.81). ^{1}H NMR: see Table 1. ^{13}C NMR (Me_2CO-d_6): δ 28.4 (*t*, C-4), 67.3 (*d*, C-3), 82.3 (*d*, C-2), 101.0, 103.1 (each *d*, C-6 and C-8), 106.0 (*s*, C-4a), 110.0 (*d*, galloyl C-2 and C-6), 114.9, 115.6 (each *d*, C-2′ and C-5′), 119.8 (*s*, galloyl C-1), 131.0 (*s*, C-1′), 139.5 (*s*, galloyl C-4), 145.3, 145.4 (each *s*, C-3′ and C-4′), 145.9 (*s*, galloyl C-3 and C-5), 151.0 (*s*, C-7), 156.1, 157.1 (each *s*, C-5 and C-8a), 165.0 (*s*, -COO-). (Found: C, 56.74; H, 4.36. $C_{22}H_{18}O_{10} \cdot 3/2H_2O$ requires: C, 56.29; H, 4.51%.)

Enzymatic hydrolysis of **1**. **1** (15 mg) in aq. soln was incubated with tannase at 37°. After 1 hr the soln was evapd to dryness and the residue treated with EtOH. The EtOH-soluble portion was chromatographed over Sephadex LH-20 using EtOH to give gallic acid and (+)-catechin (**1a**) (6.2 mg), colourless needles (H_2O), mp 176–178°, $[\alpha]_D^{18}$ + 10.3° (Me_2CO; *c* 0.29).

Methylation of **1**. **1** (56 mg) was methylated for 3 hr with Me_2SO_4 (0.6 ml) and K_2CO_3 (1 g) in dry Me_2CO (8 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 (9:1) furnished the hexamethyl ether (**1b**), a white amorphous powder, $[\alpha]_D^{18}$ + 10.0° (Me_2CO; *c* 0.48), ^{1}H NMR: see Table 1. MS *m/z* (rel. int.): 526 $[M]^+$ (27), 347 (10), 332 (18), 195 (100), 180 (38). (Found: C, 63. 39; H, 5.77. $C_{28}H_{30}O_{10}$ requires: C, 63.87; H, 5.74%.)

Partial methylation of (+)-*catechin*. (+)-Catechin (20 g) was methylated for 1.5 hr with Me_2SO_4 (20 ml) and K_2CO_3 (40 g) in dry Me_2CO (100 ml). The reaction mixture was worked up as above and the products were separated by CC over silica gel. Elution with C_6H_6–Me_2CO (9:1) furnished the 5,3′,4′-trimethyl ether (**3**) [**10**], colourless needles (MeOH), mp 258–261°, $[\alpha]_D^{18}$ + 49.4° (C_5H_5N: *c* 0.56). ^{1}H NMR (DMSO-d_6): δ 2.38 (1H, *dd*, *J* = 8, 16 Hz, H-4), 2.76 (1H, *dd*, *J* = 6, 16 Hz, H-4), 3.69, 3.74 (× 2) (each *s*, 3 × OMe), 3.93 (1H, *m*, H-3), 4.59 (1H, *d*, *J* = 8 Hz, H-2), 5.87 (1H, *d*, *J* = 2 Hz, H-6), 6.00 (1H, *d*, *J* = 2 Hz, H-8), 6.80–7.00 (3H, *m*, B-ring H). (Found: C, 64.79; H, 6.15. Calc. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07%.) Further elution with C_6H_6–Me_2CO (22:3) gave 7,3′,4′-trimethyl ether (**4**) [10], colourless needles (MeOH), mp 203–204°, $[\alpha]_D^{19}$ + 49.4° (C_5H_5N; *c* 0.55). ^{1}H NMR (DMSO-d_6): δ 2.39 (1H, *dd*, *J* = 8, 16 Hz, H-4), 2.76 (1H, *dd*, *J* = 6, 16 Hz, H-4), 3.60, 3.73 (× 2) (each *s*, 3 × OMe), 3.92 (1H, *m*, H-3), 4.60 (1H, *d*, *J* = 8 Hz, H-2), 5.89 (1H, *d*, *J* = 2 Hz, H-6), 6.00 (1H, *d*, *J* = 2 Hz, H-8), 6.80–7.00 (3H, *m*, B-ring H). (Found: C, 64.70; H, 6.17. Calc. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07%.)

Trimethoxybenzoylation of **3**. A mixture of **3** (100 mg) and NaOH (13 mg) in MeOH was stirred at room temp. for 10 min and the solvent removed by evapn under red. pres. The residue was treated at room temp. with 3,4,5-trimethoxybenzoyl chloride in C_6H_6 for 3 hr and the reaction mixture concd to a syrup which was purified by CC over silica gel. Elution with C_6H_6–Me_2CO (9:1) furnished a hexamethyl ether (95 mg) identical with the sample (**1b**) derived from **1**.

Trimethoxybenzoylation of **4**. **4** (50 mg) was treated in the same way as above to give the hexamethyl ether (**5**) (48 mg), a white, amorphous powder, $[\alpha]_D^{18}$ + 35.0° (Me_2CO; *c* 0.50). ^{1}H NMR: see

Table 1. (Found: C, 63.77; H, 5.84. $C_{28}H_{30}O_6$ requires: C, 63.87; H, 5.74%.)

3-O-*Galloylprocyanidin B-3* (**2**). An off-white, amorphous powder, $[\alpha]_D^{23}$ −170.1° (Me_2CO; *c* 0.72). ^1H NMR: see Table 1. ^{13}C NMR (Me_2CO-d_6): δ 28.3 (*d*, C-4'), 36.0 (*d*, C-4), 68.1 (*d*, C-3'), 72.9 (*d*, C-3), 81.0 (*d*, C-2'), 81.9 (*d*, C-2), 95.7, 96.5, 97.2 (each *d*, C-6, C-6' and C-8), 101.5 (*s*, C-8'), 109.7 (*d*, galloyl C-2 and C-6), 115.5 (*d*, B-ring C-2 and C-5), 121.6 (*s*, galloyl C-1), 130.5, 131.3 (*s*, B-ring C-1), 138.0 (*s*, galloyl C-4), 145.3 (*s*, galloyl C-3 and C-5), 165.0 (*s*, –COO–). (Found: C, 58.64; H, 4.53. $C_{37}H_{30}O_{16}\cdot 3/2H_2O$ requires: C, 58.64; H, 4.39%.)

Enzymatic hydrolysis of **2**. A soln of **2** (100 mg) in H_2O was incubated for 6 hr with tannase at 40°. The mixture was treated similarly as **1** to give gallic acid (20 mg) and procyanidin B-3 (**2a**) [4, 11] (56 mg), a tan, amorphous powder, $[\alpha]_D^{22}$ −217.8° (Me_2CO; *c* 0.93). ^{13}C NMR (Me_2CO-d_6 + D_2O): δ 28.4 (C-4'), 37.7 (C-4), 67.8 (C-3'), 72.9 (C-3), 81.2 (C-2'), 83.1 (C-2). (Found: C, 58.27; H, 5.10. Calc. for $C_{30}H_{26}O_{12}\cdot 2H_2O$: 58.63; H, 4.92%.)

Gambiriin A-1 (**6**) [5]. A tan, amorphous powder, $[\alpha]_D^{23}$ −14.2° (Me_2CO; *c* 0.70). ^1H NMR (Me_2CO-d_6): δ 2.57 (1H, *dd*, *J* = 8, 16 Hz, H-4''), 2.88 (2H, *d*, *J* = 6 Hz, H-γ), 2.95 (1H, *dd*, *J* = 6, 16 Hz, H-4''), 4.00 (1H, *m*, H-3''), 4.52 4.80 (3H, *m*, H-α, H-β and H-2''), 5.92 (2H, *s*, H-3' and H-5'), 6.08 (1H, *s*, H-6''), 6.56–6.85 (6H, *m*, Ar-H). ^{13}C NMR (Me_2CO-d_6): δ 46.0 (*d*, C-α), 68.0 (*d*, C-3''), 76.4 (*d*, C-β), 82.2 (*d*, C-2''), 95.6 (*d*, C-3' and C-5'), 97.2 (*d*, C-6''), 157.3 (*s*, C-2' and C-6').

Gambiriin B-3 (**7**) [5]. Colourless needles (H_2O), mp 271–274° (decomp.), $[\alpha]_D^{25}$ −20.0° (MeOH; *c* 0.75). ^1H NMR (Me_2CO-d_6): δ 2.59 (1H, *dd*, *J* = 9, 16 Hz, H-4''), 2.93 (1H, *dd*, *J* = 6, 16 Hz, H-4''), 3.03 (2H, *d*, *J* = 7 Hz, H-γ), 4.00 (1H, *m*, H-3''), 4.38 (1H, *d*, *J* = 4 Hz, H-α), 4.56 (1H, *d*, *J* = 8 Hz, H-2''), 4.84 (1H, *m*, H-β), 5.87 (1H, *s*, H-8''), 6.01 (2H, *s*, H-3' and H-5'), 6.21–6.91 (6H, *m*, Ar-H). ^{13}C NMR (Me_2CO-d_6): δ 27.6 (*t*, C-4''), 49.9 (*d*, C-α), 67.8 (*d*, C-3''), 82.6 (*d*, C-2''), 92.9 (*d*, C-β), 95.2 (*d*, C-3' and C-5'), 157.7 (*d*, C-2' and C-6').

1,2,6-*Trigalloyl-β-*D-*glucose* (**8**) [6]. Colourless needles (H_2O), mp 229–230°, $[\alpha]_D^{18}$ −88.3° (Me_2CO; *c* 0.63). ^1H NMR (Me_2CO-d_6): δ 3.26–4.08 (2H, *m*, H-3 and H-4), 4.30 4.75 (3H, *m*, H-5 and H-6), 5.24 (1H, *t*, *J* = 8 Hz, H-2), 5.98 (1H, *d*, *J* = 8 Hz, H-1), 7.05, 7.08, 7.12 (each 2H, *s*, galloyl H).

1,2,3,6-*Tetragalloyl-β-*D-*glucose* (**9**) [7]. Colourless needles (H_2O), mp 198–200°, $[\alpha]_D^{20}$ +32.8° (Me_2CO; *c* 0.63). ^1H NMR (Me_2CO-d_6): δ 4.16 (2H, *m*, H-4 and H-5), 4.59 (2H, *m*, H-6), 5.48 (1H, *t*, *J* = 8 Hz, H-2), 5.72 (1H, *t*, *J* = 8 Hz, H-3), 6.19 (1H, *d*, *J* = 8 Hz, H-1), 7.00, 7.08, 7.09, 7.17 (each 2H, *s*, galloyl H).

2,3,4,6-*Tetragalloyl-*D-*glucose* (**10**) [8]. An off-white, amorphous powder, $[\alpha]_D^{20}$ +67.7° (H_2O–Me_2CO, 1:1; *c* 1.18). ^1H NMR (Me_2CO-d_6): δ 4.14–4.70 (*m*, H-5 and H-6), 5.16 (*dd*, *J* = 4, 10 Hz, H-2, α-form), 5.55 (*t*, *J* = 10 Hz, H-4, α-form), 5.62 (*d*, *J* = 4 Hz, H-1, α-form), 5.80 (*t*, *J* = 10 Hz, H-3, β-form), 6.12 (*t*, *J* = 10 Hz, H-3, α-form), 6.95–7.20 (galloyl H). ^{13}C NMR (Me_2CO-d_6): δ 63.1 (C-6, α-form), 68.2 (C-4, α-form), 69.7 (C-2, α-form), 70.9 (C-5, α-form), 72.9 (C-3, α-form), 90.6 (C-1, α-form), 109.8 (galloyl C-2 and C-6), 165.8, 166.1, 166.5 (–COO–).

1,2,3,4,6-*Pentagalloyl-β-*D-*glucose* (**11**) [9]. A tan, amorphous powder, $[\alpha]_D^{20}$ +17.6° (Me_2CO; *c* 2.0). ^1H NMR (Me_2CO-d_6): δ 4.30–4.68 (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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