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# GALLOYLARBUTIN AND OTHER POLYPHENOLS FROM *BERGENIA PURPURASCENS**

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**Key Word Index**—*Bergenia purpurascens*; Saxifragaceae; polyphenols; 4,6-di-*O*-galloylarbutin; 6-*O*-galloylarbutin; galloylbergenins; 2,4,6-tri-*O*-galloyl-D-glucose; procyanidins.

**Abstract**—Two new polyphenols, 4,6-di-*O*-galloylarbutin and 2,4,6-tri-*O*-galloyl-D-glucose, were isolated from the root of *Bergenia purpurascens*, which also contains 6-*O*-galloylarbutin, bergenin and its gallates, and five other phenolic compounds. The structures of the new compounds were determined by chemical and spectroscopic methods.

## INTRODUCTION

*Bergenia purpurascens* Engl. (Saxifragaceae) is a tannin-rich plant grown on mountains over 2000 m in southwest China. Although its roots have been used as tonics, haemostatics and antitussives in China [1] no chemical constituents have been identified except for bergenin [1]. We now report the isolation and characterization of a new galloylarbutin and a trigalloylglucose, along with nine known polyphenols from the root of this plant.

## RESULTS AND DISCUSSION

The ethyl acetate soluble portion of the aqueous acetone homogenate of the roots of *B. purpurascens* was separated into seven fractions by CC on Toyopearl HW-40. Each fraction was further purified by a combination of chromatography on Toyopearl HW-40 (fine), Sephadex LH-20 and cellulose to afford the new compounds (**1**) and (**4**), and the known polyphenols: 6-*O*-galloylarbutin (**2**) [2], 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**5**) [3], bergenin (**6**) [4], 11-*O*-galloylbergenin (**7**) [5], 4-*O*-galloylbergenin (**8**) [5], (+)-catechin (**9**), 7-*O*-galloyl-(+)-catechin (**10**) [6], procyanidin B-3 (**11**) [7] and 3-*O*-galloylprocyanidin B-1 (**12**) [8], which were identified by comparison of their spectral and physical data with those of authentic samples and with literature values.

Compound **1**, one of the major components, gave a dark blue colour with ferric chloride reagent on TLC. The $^1$H NMR spectrum showed $A_2B_2$-type signals at δ6.98 and 6.72 ($J$ = 8.6 Hz), a 2H-singlet at δ7.19, and a doublet ascribable to an anomeric proton of glucose at δ4.93 ($J$ = 7.4 Hz), which are closely related to those of 6-*O*-galloylarbutin (**2**). A singlet due to an additional galloyl

*Part 1 in the series "Tannins and related polyphenols of the Saxifragaceae".

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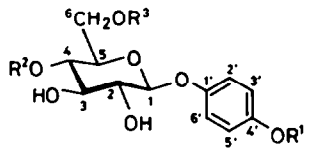


**1**: $R^1$=H, $R^2$=$R^3$=G

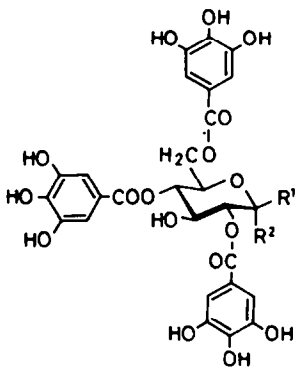
**2**: $R^1$=$R^2$=H, $R^3$=G

**3**: $R^1$=$R^2$=$R^3$=H

**1a**: $R^1$=Me, $R^2$=$R^3$=GMe

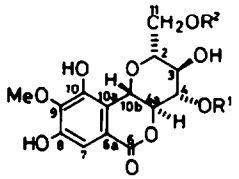
**2a**: $R^1$=Me, $R^2$=H, $R^3$=GMe

**3a**: $R^1$=Me, $R^2$=$R^3$=H



**4**: $R^1$, $R^2$ = H, OH

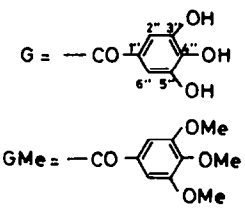
**5**: $R^1$=OG, $R^2$=H



**6**: $R^1$=$R^2$=H

**7**: $R^1$=H, $R^2$=G

**8**: $R^1$=G, $R^2$=H



group was also present at $\delta$7.17. Methylation of **1** with diazomethane furnished a heptamethyl derivative (**1a**), which upon methanolysis yielded methyl tri-*O*-methylgallate and arbutin monomethyl ether (**3a**), proving that **1** is a digalloylarbutin. The location of the galloyl groups in **1** was established to be at O-4 and O-6 of the glucose moiety by $^1$H NMR and $^{13}$C NMR analysis. The signals due to H-4 in **1** [$\delta$5.15 (*t*, $J$ = 8.6 Hz)] shifted to a field lower than that of **2**, whereas the other signals were almost identical with those of **2**. The $^{13}$C resonances of **2** were assigned by comparison with those of arbutin (**3**) as shown in Table 1. When the $^{13}$C NMR signals of **1** were compared with those of **2**, the C-4 signal in **1** was found to have shifted downfield by 0.72 ppm and the C-3 and C-5 signals had shifted upfield compared with **2**. These data are consistent with the presence of galloyl groups at O-4 and O-6, and hence **1** was characterized as 4,6-di-*O*-galloylarbutin.

Compound **4** gave a positive ferric chloride reaction and hydrolysis with 5% sulphuric acid yielded gallic acid and glucose. The $^1$H NMR spectrum, in which each proton signal formed dual peaks, exhibited two doublets due to an anomeric proton at $\delta$5.48 ($J$ = 3.6 Hz) and 5.01 ($J$ = 8 Hz) and six singlets assignable to three galloyl groups at $\delta$7.16–7.20. These data, coupled with the presence of two anomeric carbon signals at $\delta$97.03 and 91.70 in the $^{13}$C NMR spectrum, indicated that **4** is an equilibrium mixture of $\alpha$- and $\beta$-anomers (*ca* 3:1) of trigalloylglucose. The location of the galloyl groups in **4** was determined to be at O-2, O-4 and O-6 by $^1$H NMR spectral analysis, i.e. the C-3 proton signal, assigned by spin–spin decoupling experiments, appeared at a higher field [$\delta$4.45 ($\alpha$-anomer) and 4.17 ($\beta$-anomer)] than the C-2 proton [$\delta$4.93 ($\alpha$) and 5.07 ($\beta$)] and C-4 proton [$\delta$5.34 ($\alpha$) and 5.30 ($\beta$)], and this pattern was in accord with that of 1,2,4,6-tetra-*O*-galloyl-$\beta$-D-glucose (**5**) (see Experimental) except for the C-1 proton. The proposed structure (**4**) is also consistent with the $^{13}$C NMR spectrum, and was confirmed by selective degalloylation of **5** with tannase to furnish **4**. Consequently, the structure of **4** is 2,4,6-tri-*O*-galloyl-D-glucose (**4**).

Although bergenin is a well known phenolic component in *Bergenia* species, the results of the present study, along with the isolation of galloylbergenin from *Saxifraga stolonifera* [9] and of monogalloylarbutin from *Bergenia crassifolia* and *B. cordifolia*, which was once proposed as the biogenetic precursor of bergenin [2], indicate that gallates of bergenin and arbutin could also be widespread phenolic components in the Saxifragaceae.

Table 1. $^{13}$C NMR data of **1**–**3**

| | **1** | **2** | **3*** |
|---|---|---|---|
| C-1 | 103.66 | 103.75 | 103.26 |
| C-2 | 75.39 | 75.39 | 74.60 |
| C-3 | 75.78 | 78.18 | 77.46 |
| C-4 | 72.56 | 71.84 | 71.03 |
| C-5 | 73.51 | 75.13 | 77.46 |
| C-6 | 64.18 | 65.02 | 62.26 |
| C-1′ | 152.79 | 152.92 | 152.36 |
| C-2′,6′ | 117.31 | 117.30 | 117.30 |
| C-3′,5′ | 119.74 | 119.71 | 118.83 |
| C-4′ | 154.32 | 154.28 | 153.86 |
| C-1″ | 122.25, 122.02 | 122.44 | |
| C-2″,6″ | 111.04, 110.91 | 110.84 | |
| C-3″,5″ | 146.81 | 146.94 | |
| C-4″ | 139.89, 139.73 | 139.79 | |
| Ester CO | 167.58, 167.35 | 167.90 | |

*Measured in $Me_2CO$-$d_6$–$D_2O$.

## EXPERIMENTAL

*General.* $^1$H NMR and $^{13}$C NMR spectra were recorded at 270 MHz and 67.8 MHz, respectively, in $Me_2CO$-$d_6$ with TMS as int. standard. EIMS were measured on a Shimadzu LKB-9000 GC-MS spectrometer. TLC was conducted on cellulose (Avicel, Funakoshi) using 7% HOAc and on a Kieselgel $PF_{254}$ plate (Merck) using the solvent systems: (A) $C_6H_6$–$Me_2CO$ (3:1); (B) EtOAc–MeOH (99:1). Avicel microcrystalline cellulose (Funakoshi), Toyopearl HW-40 (fine and coarse grades) (Toyo Soda) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for CC.

*Extraction and isolation.* Dried roots (166 g) of *B. purpurascens*, collected in Sichuan, China, were homogenized in 70% aq. $Me_2CO$ (2.3 l.) and the concd soln extracted with $Et_2O$ and EtOAc. The EtOAc extract (13.4 g) was fractionated into seven fractions (I–VII) by CC on Toyopearl HW-40 (coarse), eluted with 50% MeOH and 70% MeOH. Fraction I, after removal of the solvent gave bergenin (**6**) (2.57 g) as white crystals. Fraction II (0.665 g) was further purified by CC on Sephadex LH-20 using 30% EtOH to provide 6-*O*-galloylarbutin (**2**) (298 mg). Fraction III (1.67 g) was crystallized from 20% EtOH to give 11-*O*-galloylbergenin (**7**) (180 mg), and the mother liquor was chromatographed on Sephadex LH-20 and then Toyopearl HW-40 (fine) eluted with 40% MeOH to furnish (+)-catechin (**9**) (222 mg), 4-*O*-galloylbergenin (**8**) (28 mg) and more **7** (99 mg). Fraction IV (285 mg) was rechromatographed on Toyopearl HW-40 (fine) using 30% MeOH to give procyanidin B-3 (**11**) (159 mg). Fraction V (545 mg) was subjected to CC on Sephadex LH-20 developed with 40% EtOH to yield 4,6-di-*O*-galloylarbutin (**1**) (294 mg) and crude 2,4,6,-tri-*O*-galloyl-D-glucose (**4**). Rechromatography of fraction VI (423 mg) on cellulose using $H_2O$ gave crude **4**, 7-*O*-galloyl-(+)-catechin (**10**) (31 mg) and crude 3-*O*-galloylprocyanidin B-1 (**12**) (81 mg) which was finally purified by CC on Toyopearl HW-40 (fine) (50% MeOH). Crude 2,4,6-tri-*O*-galloyl-D-glucose obtained from fractions V and VI was further subjected to CC on Toyopearl HW-40 (fine) to give pure **4** (133 mg). Fraction VII was chromatographed on cellulose eluted with $H_2O$ and 10% MeOH to yield 1,2,4,6-tetra-*O*-galloyl-$\beta$-D-glucose (**5**) 54 mg).

4,6-*Di*-O-*galloylarbutin* (**1**). An off-white amorphous powder, $[\alpha]_D$ −15.5° ($Me_2CO$; *c* 1.0). TLC (cellulose) $R_f$ 0.34, UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.74), 280 (4.35). $^1$H NMR: $\delta$7.19, 7.17 (each 2H, *s*, galloyl), 6.98 (2H, *d*, $J$ = 8.6 Hz, H-2′,6′), 6.72 (2H, *d*, $J$ = 8.6 Hz, H-3′,5′), 4.93 (1H, *d*, $J$ = 7.4 Hz, H-1), 3.64 (1H, *dd*, $J$ = 8.6, 7.4 Hz, H-2), 3.93 (1H, *t*, $J$ = 8.6 Hz, H-3), 5. 15 (1H, *t*, $J$ = 8.6 Hz, H-4), 4.14 (1H, *dt*, $J$ = 8.6, 6.7, 2.2 Hz, H-5), 4.50 (1H, *dd*, $J$ = 11.6, 2.2 Hz, H-6), 4.26 (1H, *dd*, $J$ = 11.6, 6.7 Hz, H-6′). $^{13}$C NMR, see Table 1. (Found: C, 52.55; H, 4.50. $C_{26}H_{24}O_{15}\cdot H_2O$ requires: C, 52.53; H, 4.41%.)

*Methylation of* **1**. A mixture of **1** (22 mg) and ethereal $CH_2N_2$ in $Me_2CO$ (1 ml) was kept at room temp. for 6 hr, and at 4° for 8 hr. After conc. the residue was purified by prep. TLC (silica gel, solvent A) to give the heptamethyl ether (**1a**) (9 mg), colourless needles, mp 198–200°, $[\alpha]_D$ −18.5° ($CHCl_3$; *c* = 0.83), TLC (silica gel, solvent A) $R_f$ 0.21, $^1$H NMR ($CDCl_3$): $\delta$7.30, 7.27 (2H each, *s*, galloyl), 7.00 (2H, *d*, $J$ = 9 Hz, H-2′,6′), 6.72 (2H, *d*, $J$ = 9 Hz, H-3′,5′), 4.88 (1H, *d*, $J$ = 8 Hz, H-1), 3.91 (12H, *s*, 4 × OMe),

3.85 (6H, *s*, 2 × OMe), 3.73 (3H, *s*, OMe); MS *m/z*: 674 $[M]^+$ 550, 480, 356, 318, 212, 195, 124.

*Methanolysis of* **1a**. To a soln of **1a** (8.3 mg) in MeOH (0.5 ml), 1% NaOMe (0.1 ml) was added, and the reaction mixture kept at room temp. for 10 hr. After neutralization and conc. the residue was purified by prep. TLC (silica gel, solvent B) to afford methyl tri-*O*-methylgallate (3.8 mg) and methylarbutin (**3a**) (3.8 mg), MS *m/z*: 286 $[M]^+$, 162, 124, $^1$H NMR: $\delta$7.05 (2H, *d*, *J* = 9 Hz, H-2',6'), 6.84 (2H, *d*, *J* = 9 Hz, H-3',5'), 4.83 (1H, *d*, *J* = 8 Hz, H-1), 3.75 (3H, *s*, OMe), which was identical with the methyl ether prepared from authentic arbutin.

6-O-*Galloylarbutin* (**2**). Colourless needles, mp 250–251°, TLC (cellulose), $R_f$ 0.63, $[\alpha]_D$ −33.6° ($Me_2CO$–$H_2O$ 43:1; *c* 1.1), UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.08), 282 (4.17); $^1$H NMR: $\delta$7.20 (2H, *s*, galloyl), 6.95 (2H, *d*, *J* = 9 Hz, H-2', 6'), 6.70 (2H, *d*, *J* = 9 Hz, H-3',5'), 4.81 (1H, *d*, *J* = 7 Hz, H-1), 4.62 (1H, *dd*, *J* = 12, 2.7 Hz, H-6), 4.39 (1H, *dd*, *J* = 12, 6 Hz, H-6'), 3.79 (1H, *dt*, *J* = 9, 6, 2.7 Hz, H-5), 3.44–3.59 (3H, *m*, H-2, 3, 4). $^{13}$C NMR, see Table 1. (Found: C, 50.54; H, 4.94. Calcd. for $C_{19}H_{20}O_{11} \cdot 3/2H_2O$: C, 50.55; H, 5.14%.) Tetramethyl ether (**2a**) was prepared with $CH_2N_2$ as above. White needles, mp 178–180°, $[\alpha]_D$ −23.4° ($Me_2CO$; *c* = 0.5), TLC (silica gel, $C_6H_6$–$Me_2CO$, 2:1), $R_f$ 0.23, MS *m/z*: 480 $[M]^+$, 356, 338, 212, 195, 124. $^1$H NMR: $\delta$7.35 (2H, *s*, galloyl), 7.02 (2H, *d*, *J* = 9 Hz, H-2',6'), 6.72 (2H, *d*, *J* = 9 Hz, H-3',5'), 4.78 (1H, *d*, *J* = 7 Hz, H-1), 3.87 (6H, *s*, 2 × OMe), 3.83 (3H, *s*, OMe), 3.69 (3H, *s*, OMe).

2,4,6-*Tri*-O-*galloyl*-D-*glucose* (**4**). A light brown amorphous powder, TLC (cellulose), $R_f$ 0.25, $[\alpha]_D$ +53° (MeOH; *c* 0.72), UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 217 (4.57), 277 (4.18), $^1$H NMR: $\delta$7.16–7.20 (6H in total, galloyl), 5.48 (1H, *d*, *J* = 3.6 Hz, $\alpha$-anomer H-1), 5.01 (1H, *d*, *J* = 8 Hz, $\beta$-anomer H-1), 4.93 (1H, *dd*, *J* = 9, 3.6 Hz, $\alpha$-anomer H-2), 5.07 (1H, *dd*, *J* = 8.4, 8 Hz, $\beta$-anomer H-2), 4.45 (1H, *t*, *J* = 9 Hz, $\alpha$-anomer H-3), 4.17 (1H, *t*, *J* = 8.4 Hz, $\beta$-anomer H-3), 5.34 (1H, *t*, *J* = 9, $\alpha$-anomer H-4), 5.30 (1H, *t*, *J* = 8.4 Hz, $\beta$-anomer H-4), 4.45–4.55 (3H, *m*, $\alpha$-anomer H-5, 6), 4.07 (1H, *ddd*, *J* = 2, 6, 8.4 Hz, $\beta$-anomer H-5), 4.20 (1H, *ddd*, *J* = 2, 6, 13 Hz, $\beta$-anomer H-6). $^{13}$C NMR: $\delta$97.03, 91.70, 77.16, 75.62, 73.44, 68.87, 73.12, 73.05, 72.95, 70.29, 64.25, 64.15 (glucose), 111.13, 110.55, 122.44, 122.34, 122.15, 139.92, 146.88, 167.80, 167.28. (Found: C, 48.49; H, 4.48. $C_{27}H_{24}HO_{18} \cdot 2H_2O$ requires: C, 48.22; H, 4.20%.)

*Hydrolysis of* **4**. **4** (3 mg) in 5% $H_2SO_4$ (0.5 ml) was heated at 100° for 4 hr, and extracted with $Et_2O$. The $Et_2O$ layer gave gallic acid (TLC and HPLC). The aq. layer neutralized with Amberlite IRA-410 resin and evapd to gave glucose (GC after trimethylsilylation).

1,2,4,6-*Tetra*-O-*galloyl*-$\beta$-D-*glucose* (**5**). A light brown amorphous powder, TLC (cellulose), $R_f$ 0.10, $[\alpha]_D$ −5.4° (MeOH; *c* = 0.7). $^1$H NMR (400 MHz): $\delta$7.19, 7.17, 7.13, 7.10 (2H each, *s*, galloyl), 6.09 (1H, *d*, *J* = 8.5 Hz, H-1), 5.42 (1H, *dd*, *J* = 8.5, 9.5 Hz, H-2), 4.40 (1H, *t*, *J* = 9.5 Hz, H-3), 5.44 (1H, *t*, *J* = 9.5 Hz, H-4), 4.33 (1H, *ddd*, *J* = 2, 5.5, 9.5 Hz, H-5), 4.57 (1H, *dd*, *J* = 13, 2 Hz, H-6), 4.24 (1H, *dd*, *J* = 13, 5.5 Hz, H-6').

*Degalloylation of* **5**. A soln of **5** (2 mg) in $H_2O$ (0.5 ml) was treated with tannase at 37°. The reaction was monitored by the HPLC of an aliquot. After 20 min, the peak ($R_t$ 18.19 min) due to **5** was completely displaced by two peaks at $R_t$ 5.32 and 8.16 min ($\alpha$- and $\beta$-anomers), which were identical with those of **4**. HPLC: column, YMC-Pack A312(ODS) (6 × 150 mm), solvent, 0.05 M $KH_2PO_4$–0.05 M $H_3PO_4$–EtOH–EtOAc (17:17:4:2), detection, 280 nm.

11-O-*Galloylbergenin* (**7**) [5]. Colourless fine needles, mp 188–190°, TLC (cellulose) $R_f$ 0.17, $[\alpha]_D$ +39.3° (EtOH; *c* = 0.9). $^1$H NMR: $\delta$7.17 (2H, *s*, galloyl), 7.07 (1H, *s*, H-7), 5.13 (1H, *d J* = 10 Hz, H-10b), 4.8–5.03 (2H, H-11), 3.89 (3H, s, OMe).

4-O-*Galloylbergenin* (**8**) [5]. A light brown amorphous powder, TLC (cellulose) $R_f$ 0.38, $[\alpha]_D$ −41.3° (MeOH; *c* = 0.8). $^1$H NMR: $\delta$7.19 (2H, *s*, galloyl), 7.08 (1H, *s*, H-7), 5.25 (1H, *d*, *J* = 10 Hz, H-10b), 5.62 (1H, *t*, *J* = 9 Hz, H-4), 3.91 (3H, *s*, OMe).

7-O-*Galloyl*-(+)-*catechin* (**10**) [6]. Colourless needles, mp 183–186°, TLC (cellulose), $R_f$ 0.31, $[\alpha]_D$ +34° ($Me_2CO$; *c* = 0.52), UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 215 (4.85), 282 (4.11). $^1$H NMR: $\delta$7.27 (2H, *s*, galloyl), 6.29 (1H, *d*, *J* = 2 Hz, H-6), 6.36 (1H, *d*, *J* = 2 Hz, H-8) 4.65 (1H, *d*, *J* = 8 Hz, H-2), 4.05 (1H, *m*, H-3), 2.52 (1H, *dd*, *J* = 16, 6 Hz, H-4), 2.85 (1H, *dd*, *J* = 16, 8 Hz, H-4').

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