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STILBENE GLYCOSIDE GALLATES AND PROANTHOCYANIDINS FROM *POLYGONUM MULTIFLORUM**

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Key Word Index—*Polygonum multiflorum*; Polygonaceae; stilbene glycoside gallates; flavan-3-ols; proanthocyanidins; tannins.

Abstract—Two new stilbene glycoside gallates and proanthocyanidins have been isolated from *Polygonum multiflorum*. The stilbenes were shown to be 2″- and 3″-*O*-monogalloyl esters of 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside.

INTRODUCTION

The genus *Polygonum* (Polygonaceae) produces a wide range of secondary metabolites including phenolcarboxylic acids, flavonoids, anthraquinones and stilbenes [1], and the occurrence of tannins in some of the species has been recognized by their astringency. In the course of a chemical examination of tannins and the related compounds in crude drugs, we have already reported condensed tannins and a gallotannin in commercial rhubarb (the root of *Rheum palmatum* L.) which is closely related to the genus *Polygonum* [2]. We now report the isolation of two new stilbene glycoside gallates (**1**, **2**), together with galloyl procyanidins (**3**, **4**) which occur in rhubarb in large quantities [2], from the root of *Polygonum multiflorum* Thunb., a climbing plant native to China, and also describe the occurrence of gallic acid (**5**), (+)-catechin (**6**), (+)-epicatechin (**7**), 3-*O*-galloyl-(−)-catechin (**8**) and 3-*O*-galloyl-(−)-epicatechin (**9**).

RESULTS AND DISCUSSION

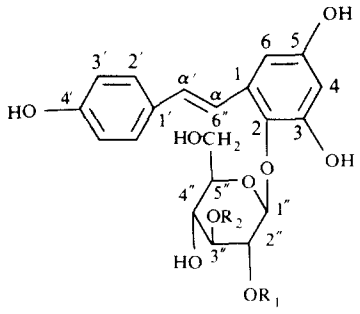
Sephadex LH-20 chromatography of the EtOAc-soluble portion, eluting with H_2O containing increasing amounts of MeOH, and subsequent rechromatography of each fraction using EtOH, gave compounds **1**–**9**. Compounds **5**–**9** were respectively identified as gallic acid, (+)-catechin, (+)-epicatechin and 3-*O*-gallates of (−)-catechin [3] and (−)-epicatechin [2] by direct comparison with authentic samples and/or enzymatic hydrolysis with tannase.

Compound **1**, fluorescent in UV light, showed intense blue colouration with $FeCl_3$ on TLC. The presence of a galloyl group was deduced from a two-proton singlet signal (δ 7.24) and an ester absorption band (1705 cm^{-1}) in the ^1H NMR and IR spectra. Enzymatic hydrolysis of **1** with tannase afforded a hydrolysate (**1a**) and gallic acid (**5**). The ^1H NMR spectrum of **1a** exhibited in the aromatic and olefinic field an A_2B_2-type signal (δ 6.81, 7.47, J = 9 Hz), a pair of *meta*-coupled doublets (δ 6.31, 6.69, J = 3 Hz) and *trans* olefinic proton signals (δ 6.96, 7.78, J = 16 Hz). **1a** formed on acetylation a heptaacetate (**1b**), which revealed three aromatic and four aliphatic acetoxyl signals in the ^1H NMR spectrum. From spectral and chemical evidence, **1a** was suggested to be 2,3,5,4′-tetrahydroxystilbene glycoside, and finally was identical with 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside which had been reported in the root of this plant in large amounts (1.2%) [4]. The position of the galloyl group in **1** was determined by comparison of the ^{13}C NMR spectra of **1** and **1a**. The sugar C-2 signal in **1**, shifted to lower field by 0.6 ppm, while C-3 being shifted upfield by 2.4 ppm, were characteristic, indicating the galloyl group was attached to the C-2 hydroxyl group in glucose moiety. This result was also supported by the ^1H NMR spectrum of **1**, showing a triplet signal (δ 5.32) assignable to the C-2 proton coupled with an anomeric C-1 proton.

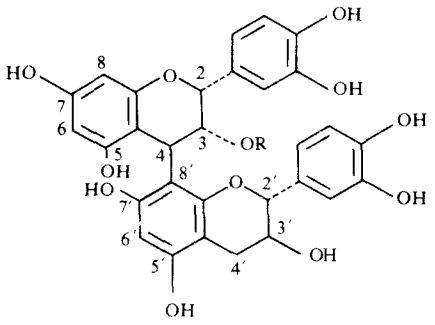
Compound **2** was, as **1**, a stilbene glycoside gallate as revealed by ^1H NMR, ^{13}C NMR and IR spectra, and gave, on enzymatic hydrolysis with tannase, 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside (**1a**) and gallic acid (**5**). The ^1H NMR spectrum of **2** showed a triplet signal (δ 5.29, J = 9 Hz) due to a galloyl-bearing proton which was not coupled with the sugar C-1 proton, suggesting that a galloyl group was attached to the C-3 or C-4 hydroxyl group in glucose moiety. ^{13}C NMR spectral comparison of **2** with **1a** established the location of a galloyl group to be C-3 OH group in glucose moiety, since chemical shift differences in these two spectra were observed only in C-2, C-3 and C-4 atoms (C-3 at low field, C-3 and C-4 at high field, in **2**).

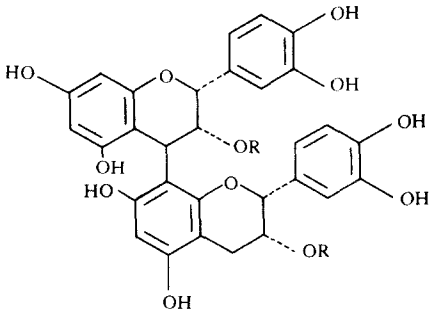
Compound **3**, which formed anthocyan pigments on heating with mineral acids [5], was a proanthocyanidin. The presence of a galloyl group was obvious from a singlet signal (δ 6.96) corresponding to two protons in the ^1H NMR spectrum. Treatment of **3** with tannase yielded procyanidin B-1 (**3a**) [2, 6] and gallic acid (**5**). A C-3 proton signal (δ 5.33) shifted to low field in the ^1H NMR spectrum of **3** suggested that a galloyl group

*Part 2 in the series "Tannins and Related Compounds". For Part 1 see ref. [2].



1 R_1 = Galloyl, R_2 = H
2 R_1 = H, R_2 = Galloyl
1a R_1 = R_2 = H





3 R = Galloyl
3a R = H

4 R = Galloyl
4a R = H

was located at C-3 position. The ^{1}H NMR spectra of **3** and its methylate were consistent with 3-*O*-galloylprocyanidin B-2 reported previously [2].

Compound **4**, a proanthocyanidin, containing two galloyl groups as shown by ^{1}H NMR spectrum (δ 6.99, 7.07), gave on enzymatic hydrolysis with tannase procyanidin B-2 (**4a**) [2, 6] and gallic acid (**5**). The ^{1}H NMR spectrum of **4** implied the position of two galloyl groups to be at C-3 and C-3′ positions. The identification of **4** with 3,3′-di-*O*-galloylprocy-

Table 1. ^{13}C NMR spectra of stilbenes*

		1	2	1a
	C-1	118.8	120.8	121.3
	C-2	134.9	136.8	137.3
	C-3	151.3	151.0	151.5
	C-4	103.2	103.2	103.2
	C-5	155.0	155.2	155.3
	C-6	103.4	107.0	107.3
	C-1′	134.5	129.5	129.2
	C-2′,6′	128.5	128.6	128.6
	C-3′,5′	116.2	116.0	116.0
	C-4′	157.5	157.5	157.6
	C-α,β	129.0, 132.3	129.7, 132.9	130.0, 132.9
	C-1″	102.8	102.2	102.0
	C-2″	75.1	73.1	74.9
	C-3″	75.1	78.5	77.5
	C-4″	70.3	68.4	70.6
	C-5″	77.4	77.3	77.5
	C-6″	61.3	61.3	62.1
Galloyl	C-1	121.1	121.1	—
Galloyl	C-2, 6	110.1	109.9	—
Galloyl	C-3, 5	145.7	145.5	—
Galloyl	C-4	138.9	138.7	—
Galloyl	–COO–	166.7	167.0	—

*Run at 25.05 MHz.

anidin B-2 [2] was confirmed by the ^{1}H NMR spectra of **4** and its methylate.

Polymeric proanthocyanidins (**10**), obtained by Sephadex LH-20 chromatography from the aqueous solution after removal of the EtOAc-soluble portion, were hydrolysed with tannase to give gallic acid (**5**) and polymeric hydrolysates (**10a**). Cleavage reaction of **10** with benzylmercaptan in the presence of acetic acid [6] afforded (+)-catechin (**6**) and 3-*O*-galloyl-(−)-epicatechin (**9**) derived from the lower terminal units of the polymers, and 4-benzylthioethers of 3-*O*-galloyl(−)-epicatechin (**11**) and (−)-epicatechin (**12**) from the upper unit. From these facts coupled with the occurrence of two proanthocyanidin dimers (**3**, **4**), the polymers **10** comprised 3-*O*-galloyl-(−)-epicatechin and (−)-epicatechin units in the upper part and (+)-catechin, and (+)-catechin and 3-*O*-galloyl-(−)-epicatechin units in the lower terminal part. Although these results were consistent with those in rhubarb [2], the polymers in *P. multiflorum* L. were assumed to be less galloylated than those in rhubarb, judging from the low yield of gallic acid on enzymatic hydrolysis.

EXPERIMENTAL

Mps are uncorr. Unless otherwise stated ^{1}H NMR 100 MHz and ^{13}C NMR spectra were obtained in Me_2CO-d_6 + D_2O, and chemical shifts are given in δ (ppm) scale relative to TMS. TLC was conducted on precoated Kieselgel 60 F_{254} plates (Merck) and spots were visualized by $FeCl_3$ reagent.

Extraction and isolation. The dried, milled roots (1.03 kg) of *P. multiflorum* were extracted with 80% aq. Me_2CO, and the aq. soln, after evaporation of Me_2CO under red. pres., was successively extracted with Et_2O and EtOAc. The EtOAc extract (49.7 g) was chromatographed over Sephadex LH-20 eluting with H_2O containing increasing amounts of MeOH to give four fractions (fractions 1–4). Crystallization of fraction 1 (0.7 g) afforded gallic acid (**5**) (0.22 g). Fraction 2 (29.7 g) was rechromatographed over Si gel using C_6H_6–EtOAc (1:1–0:1) to yield compound **1a** (19.1 g) and a mixture of (+)-catechin (**6**) and (+)-epicatechin (**7**). Pure samples of (+)-catechin (**6**) (0.14 g) and (+)-epicatechin (**7**) (0.05 g) were obtained by fractional crystallization from H_2O. Sephadex LH-20 chromatography of fraction 3 eluting with EtOH gave compounds **1** (0.14 g), **2** (0.09 g), **3** (0.10 g) and **9** (0.19 g). Fraction 4 yielded, after separation with Sephadex LH-20 chromatography using EtOH, compounds **4** (0.14 g) and **8** (0.03 g).

Compound **1**. Pale brown needles (H_2O), mp 182–184°, $[\alpha]_D^{23}$ −29.9° (*c* 0.19, Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1705 (ester); ^{1}H NMR (δ): 3.4–4.0 (5H, *m*, sugar H), 5.01 (1H, *d*, *J* = 9 Hz, C-1″), 5.32 (1H, *t*, *J* = 9 Hz, C-2), 6.31, 6.62 (each 1H, *d*, *J* = 3 Hz, C-4, C-6), 6.88, 7.14 (1H, *d*, *J* = 17 Hz, olefinic H), 6.64, 7.18 (each 2H, A_2B_2-type *d*, J = 9 Hz, C-2′, C-3′), 7.24 (2H, *s*, galloyl H). (Found: C, 57.14; H, 4.80. $C_{27}H_{16}O_{13}\cdot 1/2H_2O$ requires: C, 57.30; H, 4.94.)

Enzymatic hydrolysis of **1**. **1** (80 mg) in aq. soln was incubated with tannase at 37°. After 1 hr the soln was evaporated to dryness and the residue was treated with EtOH. EtOH-soluble portion was chromatographed over Sephadex LH-20 using EtOH to give a hydrolysate (**1a**) and gallic acid (**5**). **1a**, a pale yellow powder, $[\alpha]_D^{20}$ +73.0° (*c* 0.63), Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1610 (benzene ring); ^{1}H NMR (δ): 3.44–3.82 (6H, *m*, sugar H), 4.54 (1H, *d*, *J* = 8 Hz, C-1″), 6.31, 6.69 (each 1H, *d*, *J* = 3 Hz, C-4, C-6), 6.81, 7.47 (each 2H, A_2B_2-type *d*, *J* = 9 Hz, C-2′, C-3′), 6.96, 7.78 (each 1H, *d*, *J* = 16 Hz, olefinic H). Hepta-acetate (**1b**), colourless needles (EtOH), mp 167–169°, $[\alpha]_D^{23}$ −25.5° (*c* 0.38, $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ): 3.59 (1H, *m*, C-5″), 3.90 (1H, *dd*, *J* = 2, 12 Hz, C-6″), 4.28 (1H, *dd*, *J* = 4, 12 Hz, C-6″), 4.92 (1H, *d*, *J* = 9 Hz, C-1″), 5.12–5.44 (3H, *m*, C-2″, -3″, -4″), 6.83, 7.26 (each 1H, *d*, *J* = 3 Hz, C-4, C-6), 6.96, 7.33 (each 1H, *d*, *J* = 16 Hz, olefinic H), 7.08, 7.49 (each 2H, A_2B_2-type *d*, *J* = 9 Hz, C-2′, C-3′). (Found: C, 58.29; H, 5.18. Calc. for $C_{34}H_{36}O_{16}$: C, 58.21; H, 5.28.)

Compound **2**. A pale brown amorphous powder, $[\alpha]_D^{23}$ +11.7° (*c* 0.14, Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1700 (ester), 1610 (benzene ring); ^{1}H NMR (δ): 3.4–4.0 (5H, *m*, sugar H), 4.74 (1H, *d*, *J* = 9 Hz, C-1″), 5.29 (1H, *t*, *J* = 9 Hz, C-3″), 6.31, 6.70 (each 1H, *d*, *J* = 3 Hz, C-4, C-6), 7.17 (2H, *s*, galloyl H), 6.80, 7.47 (each 2H, A_2B_2-type *d*, *J* = 9 Hz, C-2′, C-3′), 6.95, 7.28 (each 1H, *d*, *J* = 16 Hz, olefinic H). **2** was, similarly was **1**, hydrolysed with tannase to furnish gallic acid and **1a**.

Compound **3**. An off-white amorphous powder, $[\alpha]_D^{20}$ +1.26° (*c* 0.38, Me_2CO). ^{1}H NMR (δ): 2.56 (1H, *dd*, *J* = 8, 16 Hz, C-4′), 2.89 (1H, *dd*, *J* = 5, 16 Hz, C-4′), 4.06 (1H, *m*, C-3′), 4.46 (1H, *br d*, *J* = *ca* 8 Hz, C-2′), 4.63 (1H, *br s*, C-4), 5.33 (1H, *br s*, C-3), 5.83 (1H, *d*, *J* = 3 Hz, C-6), 5.95 (1H, *d*, *J* = 3 Hz, C-8), 6.10 (1H, *s*, C-6′), 6.96 (2H, *s*, galloyl H). Enzymatic hydrolysis of **3** with tannase, followed by purification with Sephadex LH-20 chromatography, afforded procyanidin B-1 (**3a**) [2, 6] and gallic acid (**5**).

Compound **4**. An off-white amorphous powder, $[\alpha]_D^{25}$ −95.3 (*c* 0.81, Me_2CO). ^{1}H NMR (δ): 2.9–3.1 (2H, *m*, C-4′), 4.79 (1H, *d*, *J* = 3 Hz, C-4), 4.98 (1H, *br s*, C-2′), 5.5–5.6 (2H, *m*, C-3, C-3′), 5.65 (1H, *br s*, C-2), 5.93 (2H, *br s*, C-6, C-8), 6.13 (1H, *s*, C-6′), 6.99, 7.07 (each 2H, *s*, galloyl H). Treatment of **4** with tannase, similarly as **1**, yielded procyanidin B-2 (**4a**) [2, 6] and gallic acid. Methylation of **4** with Me_2SO_4 and K_2CO_3 in dry Me_2CO afforded a tetradecamethylate, a colourless powder, $[\alpha]_D^{20}$ −90.5° (*c* 0.22, $CHCl_3$). ^{1}H NMR (δ): 3.48–3.86 (OMe), 3.08 (2H, *m*, C-4′), 4.83 (1H, *br s*, C-4), 4.94 (1H, *d*, *J* = 11 Hz, C-2′), 5.32 (1H, *m*, C-3′), 5.62 (1H, *br s*, C-3), 5.71 (1H, *br s*, C-2), 5.98 (2H, *s*, C-6, C-8), 6.43 (1H, *s*, C-6′), 7.01, 7.12 (each 2H, *s*, galloyl H).

Compound **8**—3-O-galloyl-(−)-*catechin*. An off-white amorphous powder, $[\alpha]_D^{20}$ −51.5° (*c* 0.20, EtOH). ^{1}H NMR (δ): 2.84 (1H, *dd*, *J* = 6, 10 Hz, C-4), 3.00 (1H, *dd*, *J* = 5, 10 Hz, C-4), 5.08 (1H, *d*, *J* = 7 Hz, C-2), 5.38 (1H, *m*, C-3), 6.00, 6.07 (each 1H, *d*, *J* = 2 Hz, C-6, C-8), 7.03 (2H, *s*, galloyl H). On hydrolysis with tannase **8** afforded gallic acid and (−)-catechin, mp 172–174°, $[\alpha]_D^{21}$ −7.9° (*c* 0.36, Me_2CO).

Compound **9**—3-O-*galloyl*-(−)-*epicatechin*. An off-white amorphous powder, $[\alpha]_D^{23}$ −160.6° (*c* 0.22, Me_2CO). ^{1}H NMR (δ): 2.98 (2H, *m*, C-4), 5.14 (1H, *s*, C-2), 5.56 (1H, *m*, C-3), 6.04, 6.08 (each 1H, *d*, *J* = 3 Hz, C-6, C-8), 6.76 (1H, *d*, *J* = 8 Hz, C-5′), 6.90 (1H, *dd*, *J* = 3, 8 Hz, C-6′), 7.03 (2H, *s*, galloyl H), 7.06 (1H, *d*, *J* = 3 Hz, C-2′).

Isolation of polymeric proanthocyanidins (**10**). The aq. layer, after extraction with EtOAc, was concentrated to a syrup which was chromatographed over Sephadex LH-20 successively eluting with H_2O (1.5 l.), H_2O–EtOH (1:1) (1.0 l.), EtOH (1.0 l.) and H_2O–Me_2CO (1:1) (2.0 l.). The H_2O eluate was further chromatographed over Sephadex LH-20 using EtOH containing increasing amounts of H_2O. The EtOH–H_2O (7:3–1:1) eluate afforded polymeric proanthocyanidins (**10**) (8.5 g).

Enzymatic hydrolysis of **10**. An aq. soln (20 ml) of polymers (**10**) (1.0 g) was incubated with tannase for 4.5 hr at 37°. Ppt. was filtered off and the filtrate was concentrated to

give a brown powder which was subjected to Sephadex LH-20 chromatography. Elution with EtOH gave gallic acid (**5**) (51 mg). Further elution with EtOH–H_2O (7:3–6:4) provided polymeric hydrolysates (0.6 g).

Cleavage reaction of **10**. A mixture of **10** (1.0 g), benzylmercaptan (8 ml), AcOH (5 ml) and EtOH (40 ml) was refluxed for 24 hr under N_2. After evaporation of the solvents, the residue was separated by Sephadex LH-20 chromatography to afford compounds **6**, **9**, **11** and **12**. The procedures for the separation of these compounds and their properties are essentially as reported in [2].

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