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GALLOYL GLUCOSE DERIVATIVES FROM *HEUCHERA CYLINDRICA*

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Key Word Index—*Heuchera cylindrica*; Saxifragaceae; gallotannins; hydrolysable tannins; polyphenols.

Abstract—Two major polyphenolic compounds from *Heuchera cylindrica* var. *gabella* have been shown to be 1,2,6-trigalloyl-β-D-glucopyranose and 3,4,6-trigalloyl-D-glucopyranose.

INTRODUCTION

In our chemotaxonomic studies of the Saxifragaceae, Bohm and I reported on the presence of gallate esters in *Tellima grandiflora* (gallo-ellagitannins) [1] and *Heuchera micrantha* Dougl. var. *diversifolia* (flavanol gallate) [2]. We also reported on the flavonoid derivatives of *H. cyclindrica* var. *glabella* [3]. I now report structures for two major polyphenolic compounds from this plant.

RESULTS AND DISCUSSION

Concentrated 80% acetone extracts of the plant were first chromatographed on a Sephadex LH-20 column using gradient elution (from water to methanol to acetone) [1, 2]. Fractions of similar composition were combined after monitoring by polyamide and cellulose TLC. The last Sephadex LH-20 column fractions contained flavonoid aglycones, small amounts of flavonoid monoglucosides and flavonoid monoglucoside gallates. TLC analysis also revealed large quantities of material which absorbed UV radiation (360 nm) and gave blue fluorescent spots on polyamide layers after spraying with 2-aminoethyl diphenyl borinate. The same materials on cellulose layers gave grey spots after treatment with 2,6-dichloro-*p*-benoquinone-4-chloroimine and ammonia vapour. This behaviour is typical for gallate esters. Chromatography on cellulose partition columns yielded fractions containing nearly pure tannins. Additional purification by column chromatography with cellulose adsorption or Sephadex LH-20 yielded tannins A, B and C which were astringent tasting. Hydrolysis of all three tannins yielded gallic acid and glucose as well as intermediate products.

NMR data are shown in Table 1. Tannin A showed three gallate groups, the signals of two of which were split into double signals. An apparent β-anomeric proton and two sets of signals for glucose H-2 integrated for 0.6, 0.6 and 0.4 protons respectively. The double signals indicated an anomeric pair mixture which was confirmed by decoupling experiments in which βH-1 and βH-2 and α-H-2 and αH-3 were observed. Thus the structure is clearly α and β-3,4,6-trigallolyl-D-glucopyranose. The NMR data is similar to that from Gemin D, 3-galloyl-4,6-hexahydroxydiphenoyl-D-glucopyranose (Table 1).

Tannin B crystallized from dilute methanol solutions on cooling and showed an identical NMR spectrum to that reported for 1,2,6-trigalloyl-β-D-glucopyranose. Complete analysis of all coupling constants could be obtained from 500 MHz NMR spectrum .

Tannin C was shown by HPLC to be a mixture which was not resolved by Sephadex LH-20 column chromatography. Methanolysis yielded methyl gallate and primarily 1,2,6-trigalloyl-β-D-glucopyranose. The presence of signals at δ7.56 and 7.46 (*d*, 2 Hz) in the ^{1}H NMR and a shift for C-6 of 0.3–0.4 ppm in the ^{13}C NMR suggested that the main constituents are 1,2-diagalloyl-6-(*m* and *p*) digalloyl-β-D-glucopyranose by analogy to data from Turkish gallotannin [6] and *Spirogyra* tannin [7]. Preparative HPLC separations to obtain pure samples for definitive assignments are in progress.

EXPERIMENTAL

Chromatographic procedures were described earlier [1, 2]. Microcrystalline cellulose (Merck 2331) was used for partition columns. Solvents were Merck reagent grade unless noted. TLC: DC-Plastik-folien Cellulose (Merck 5577) with 15% HOAc–2 BuOH–HOAc–H_2O (14:1:5) or H_2O–2BuOH–HOAc (18:1:1) and MN-Polyamide-DC 6.6 (0.3 mm, Machery–Nagel & Co. D-5160 Düren) with MeOH–butanone–HOAc (5:5:1). HPLC: LiChrosorb RP-18 (5 nm 250 × 4.6 mm). Solvent A: 600 ml MeOH containing 1 ml HCO_2H dild to 1000 ml with distilled deionized H_2O and 100 ml EtOAc added. Solvent B: H_2O. All samples were run with A/B (2:3)

Tannin A. Partition column fractions rich in this material were combined and purified with a cellulose adsorption column eluted with H_2O. Off-white amorphous solid, mp softens 161°, 164–166°. Found: C, 49 50; H, 4.25. $C_{27}H_{24}O_{18}\cdot H_2O$ requires: c 49.5 H 4.0. C D; $(\theta)_{294} - 15{,}000$, $(\theta)_{266}$ 18,000 in EtOH. Approximate yield 0.12% of fr. wt.

Tannin B. Partition column fractions containing tannins B and C were combined and purified further by chromatography on Sephadex LH-20 columns eluted with MeOH–H_2O (1:1) chaging to (7:3) and finally Me_2CO–H_2O (7:3) Tannin B. Crystalline grey solid, mp 206–208° (lit. [5] 208.5–210.5°). Approximate yield 0.14%. fr. wt. Tannin C. Off-white amorphous solid. Approximate yield, 0.43%. fr. wt.

Methanolysis of tannin C was carried out according to ref. [6]. The tannin (3 mg) was dissolved in 2 ml MeOH and 1 ml

Table 1. NMR data for *Henchera* glucose gallates (deuteroacetone, TMS as int. stand.)

	H-1	H-2	H-3	H-4	H-5	H-6	Gallates
Tannin A (90 MHz)	α *ca* 5.3* β 4.90 (7.8) *d*	3.87 (9.8, 3.4) *dd* 3.65 (*ca* 8)	5.74 (9.8) *t* *ca* 5.4*	5.14–5.30*	4.00–4.65*	4.00–4.65*	7.02, 7.04, 2H 7.05 *s*, 2H 7.14, 7.16, 2H
Gemin D (200 MHz) [4]	α 5.28 (3.5) *d* β 4.75 (8.0) *d*	3.84 (10, 3.5) *dd* 3.58 (10, 8) *br t*	5.51 (10) *t* 5.33 (10) *t*	4.95 (10) *t* 5.98 (10) *t*	4.58 (10, 7) *dd* 4.13 (10, 6) *dd*	5.26 (13, 7) *dd* 3.74 (13) *d* 5.24 (13, 6) *dd* 3.82 (13) *d*	7.01, 7.02, 2H 6.63, 6.641H‡ 6.43 *s*, 1H‡
Tannin B (90 MHz)	5.97 (9.4) *d*	5.25 (9.4) *br t*	3.57–4.15*	3.57–4.15*	4.38–4.73*	4.38–4.73*	7.06 *s*, 2H 7.09 *s*, 2H 7.16 *s*, 2H
β-1,2,6 Trigalloyl-β-D-glucose (100 MHz) [5]	5.98 (8) *d*	5.28 (9) *t*	†	†	†	4.41 (12, 4) *dd* 4.67 (12) *br d*	7.08 *s*, 2H 7.10 s, 2H 7.16 *s*, 2H
Tannin B (500 MHz)	6.02 (8.4) *d*	5.31 (9.5, 8.4) *dd*	4.05 (9.5, 9.0) *dd*	3.82 (9.8, 9.0) *dd*	4.00 (9.8, 4.9, 2.0) *ddd*	4.52 (12.2, 4.9) *dd* 4.65 (12.2, 2.0) *dd*	7.12 *s*, 2H 7.15 *s*, 2H 7.20 *s*, 2H

*Not resolved.
†Not reported.
‡Hexanydroxydiphenate group.

0.5 M Ac buffer, pH 6.0. HPLC analysis was carried out after 20 hr at room temp. Total hydrolysis of tannins was carried out as described earlier [1].

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