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1-*O*-galloyl-α-L-rhamnose from *Acer rubrum*

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Abstract

Leaves of *Acer rubrum* L. afforded the novel 1-*O*-galloyl-α-L-rhamnose as well as 1-*O*-galloyl-β-D-glucose; gallic acid; methyl gallate; ethyl gallate; *m*-digallate and ethyl digallate. Their structures were established on the basis of spectral and chemical evidence.

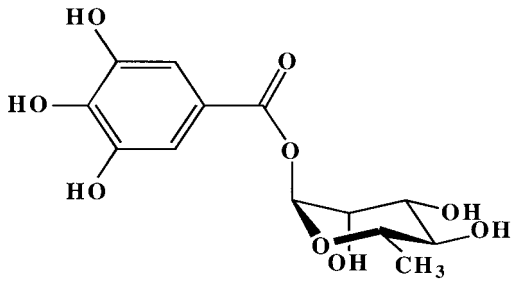
Keywords: *Acer*; *Acer rubrum* L; Aceraceae; Red maple; Phenolics; Hydrolysable tannins; 1-*O*-galloyl-α-L-rhamnose; 1-*O*-galloyl-β-D-glucose; *m*-digallate; Gallic acid

1. Introduction

Acer rubrum L. (red maple) (Aceraceae) is a prominent species in the hardwood forests of eastern Canada (Farrar, 1995). It is horticulturally important and widely planted for the brilliant autumn colours of its leaves. We are attempting to isolate and identify the constituents of red maple leaves that make it resistant to the forest tent caterpillar (Nicol, Arnason, Helson & Abou-Zaid, 1997). Galloyl-rhamnose, reported here for the first time and one of many gallates in the leaf, may be such a resistance factor.

2. Results and discussion

Compound **1** was found to possess a characteristic UV spectral maximum (275 nm) in methanol which suggested that it has galloyl ester-like characteristics. FAB-MS analysis (negative ion mode) established that **1** was a galloylrhamnose ($[M\text{-}H]^-$, m/z 315) with a M_r of 316. On acid hydrolysis (2N HCl) **1** yielded gallic acid (HPLC, UV, MS, ^1H and ^{13}C NMR spectral analyses) together with rhamnose (CoPC, TLC). The ^1H NMR spectrum of **1** was assigned on the basis of coupling constants. The proton resonances due to the rhamnose moiety appeared in the region δ 3.18–5.30 ppm. The anomeric proton of the rhamnose moiety was observed as a singlet at δ 5.30 ppm. The vicinal coupling constants in the ^1H NMR spectrum ($J_{4,5}=9.5$) of the rhamnose moiety showed it to have the α-L-rhamnopyranose configuration. Analysis of the ^1H–^1H COSY spectrum of the sugar moiety, the upfield shift of H-1 of rhamnose (δ 5.30 ppm), indicated that gallic acid was attached to OH-1. The resonance of the galloyl moiety of **1** appeared as a



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singlet, integrated to two protons at δ 6.96 ppm. From the data it is evident that **1** is a monogalloylrhamnose, whose anomeric hydroxyl group is galloylated.

The ^{13}C NMR data of **1** is also in accordance with this structure. The anomeric carbon was recognised from the resonance at δ 94.4 ppm, being shifted upfield in comparison with the resonance of the corresponding carbon in the spectrum of unsubstituted L-rhamnose. This shift is due to galloylation of the anomeric hydroxyl group. Consequently, **1** is identified as 1-*O*-galloyl-α-L-rhamnopyranose, which is a novel natural product.

Gallic acid is commonly found in plants in the esterified form with D-glucose as 1-*O*-galloyl-β-D-glucose, first isolated from the roots of *Rheum officinale* in 1903 (Gilson, 1903) and is also esterified to itself in depside form (Haslam, 1982). Several galloyl-D-glucose derivatives have been described but there are few reports of ester formation with sugars other than glucose; Haslam (1989) names only fructose and hamamelose. Chevalley, Marston and Hostettmann (1999) describe a gallic acid fructose ester from *Saxifraga stellaris* L. A preliminary report (Gvazava, 1998), unsupported by detailed chemical and physical analyses, of the isolation of 2,4-di-*O*-galloyl-α-L-rhamnopyranose from *Euphorbia glareosa* Pall. ex Bieb. together with the present study, adds rhamnose to this short list.

Other gallate compounds isolated from red maple leaves in the present study and identified by physical analyses and comparison to standards where available were: 1-*O*-galloyl-β-D-glucose; gallic acid; methyl gallate; ethyl gallate; *m*-digallate and ethyl digallate. Results obtained with UV spectroscopy; ^{1}H-NMR; ^{13}C-NMR and (positive and negative) FAB-mass spectroscopy were identical to published data (Okuda, Yoshida & Hanata, 1989; Self, et al., 1986; Yoshida, et al., 1997).

3. Experimental

3.1. Plant material

Red maple leaves were collected in June 1992 from 10 mature trees in Sault Ste. Marie, Ontario, Canada (46.34N, 84.17W). Pressed voucher specimens are deposited in the Canadian Forest Service-Sault Ste. Marie herbarium as *Acer rubrum* L. (CFS-SSM # s 1001-1010), family Aceraceae.

3.2. Extraction

Fresh red maple leaf material (2 kg) was extracted at room temperature in two steps: first, by steeping for 24 h in 100% EtOH (4 L), followed by chopping in a commercial Waring blender and decanting the solvent; second, by steeping the chopped residue for an additional 24 h with 4 L of EtOH:H_2O (1:1). The combined ethanolic extracts were evaporated under reduced pressure until most of the EtOH had been removed. The residue was freeze-dried to obtain 242 g of crude extract.

3.3. Fractionation

The ethanolic freeze-dried extracts were adsorbed onto polyvinylpolypyrrolidone (PVPP) powder (Sigma) packed in a Buchner funnel (2 L). Elution was carried out at a slow rate initially with water followed by aliquots of increasing concentrations (0, 20, 50, 70 and 100%) of ethanol. The ethanol–water (20–80) fraction was further fractionated on a PVPP column with the following solvent system: CH_2Cl_2 – EtOH – MeCOEt – Me_2CO (1:1:1:1) and yielded 1-*O*-galloyl-α-L-rhamnose as well as 1-*O*-galloyl-β-D-glucose in fraction number 4. Separation of these two compounds was achieved with the aid of a low pressure liquid chromatograph (Chemco low-prep pump, model 9 1-M-8R, with 6-port valve, max. 80 ml/min) using a methanol–water gradient. Final cleanup of the compounds was achieved on a Sephadex LH-20 column (1 × 50 cm), using methanol as the eluting solvent, a step essential to obtaining good spectra of purified compounds.

3.4. Identification of purified compounds

Structural elucidation was achieved by physical analyses: UV spectroscopy; ^{1}H-NMR; ^{13}C-NMR and (positive and negative) FAB-mass spectroscopy.

3.5. 1-O-galloyl-α-L-rhamnose

UV λ MeOH: 275 nm; FAB-MS (neg. ion) m/z (rel. int.): 315.1 $[M-1]^-$, 169.1 $[M-147]^-$; galloyl moiety: ^{1}H-NMR of δ 6.96 (2H, s, H-2 and H-6). ^{13}C-NMR of δ 118.4 (C-1), 108.5 (C-2 and C-6), 145.4 (C-3 and C-5), 138.4 (C-4), 165.5 (C═O); rhamnosyl moiety: ^{1}H-NMR of δ 5.30 (s, J = 9.5 Hz, H-1), 3.70 (s, J = 9.5 Hz, H-2), 3.54 (s, J = 9.5 Hz, H-3), 3.18 (t, J = 9.5 Hz, H-4), 3.48 (dd, J = 9.5, 5.9 Hz, H-5); ^{13}C-NMR of δ 94.4 (C-1), 70.4 (C-2), 70.8 (C-3), 72.1 (C-4), 69.9 (C-5), 16.3 (C-6).

Acknowledgements

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