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GLUCOSE-1-BENZOATE AND PRUNASIN FROM *PRUNUS SEROTINA*

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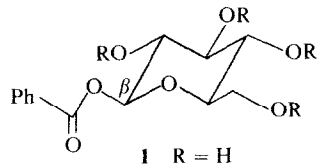
Key Word Index—*Prunus serotina*; Rosaceae; Black cherry; benzoate ester; cyanogenic glycoside; prunasin; allelopathy.

Abstract—β-D-Glucopyranose 1-benzoate and prunasin have been isolated from the leaves of *Prunus serotina*. Both can yield benzoic acid, a potential allelopathic inhibitor of *Acer rubrum*.

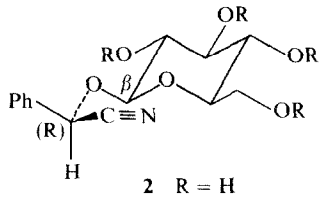
Benzoic acid has been known as a plant growth inhibitor since at least 1914 when Shorey [1] and later Walters [2] isolated it from soils 'fatigued' by citrus. It is known to occur in plants in esterified form [3], and is produced as the oxidation product of benzaldehyde, released on enzymatic hydrolysis of the mandelonitrile glycosides [4, 5]. Benzoic acid has subsequently been shown to inhibit growth of peach seedlings (*Prunus* spp.) [6] and of mycorrhizal fungi [7], as well as the process of ion uptake in isolated barley (*Hordeum vulgare* L.) root tips [8]. Recently, while investigating the allelopathic effects of black cherry (*Prunus serotina* Ehrh.) on red maple (*Acer rubrum* L.), one of us (S.B.H.) found that benzoic acid, released during natural senescence of black cherry leaves, could inhibit the growth of red maple seedlings in concentrations as small as 0.1 mM [9]. We were interested to know the potential sources of benzoic acid in black cherry leaves.

In the present investigation 600 g fr. wt of leaves yielded 22.45 g of crude defatted extract, from which we have isolated two compounds from which benzoic acid could be produced during senescence: β-D-glucopyranose 1-benzoate (**1**) (488 mg) and prunasin (**2**) (337 mg). The former has been reported previously as a synthetic product [10–12], as a secretion of certain insects (*Periplaneta americana* L., *Blatta orientalis* L.) [13], and more recently from the needles of the gymnosperm, *Pinus contorta* Dougl. [14]. However, to our knowledge it has never previously been found in an angiosperm. Prunasin (**2**) was reported 70 years ago [15, 16] to be a constituent of black cherry bark and leaves. We confirm this early report, and provide MS and ^{13}C NMR spectral data to substantiate the structural assignment. Fully assigned ^{13}C NMR spectra of any cyanogenic glycoside have only recently been reported [17]. Both **1** and **2** were isolated by repeated chromatography of an aqueous ethanol extract of black cherry leaves. The IR spectrum of **1** indicated the presence of a monosubstituted aromatic ring, carbonyl, and hydroxyl functions. Although no molecular ion was present in the MS of **1**, prominent ions at *m/e* 105 and 122, corresponding to $C_7H_5O^+$ and $C_7H_6O_2^+$ fragments, respectively, suggested the presence of a benzoate ester. Signals in the ^1H NMR spectrum [δ8.09 (2H, *dd*, $J = 2$ Hz, $J = 8$ Hz) and 7.64 (3H, *m*)] and in the ^{13}C NMR (δ165.09, 134.14, 129.89, 129.48, 129.11) confirmed this part structure. The six remaining signals in the ^{13}C NMR (δ95.35, 78.19, 76.69, 72.88, 69.87, 60.96) suggested the presence of a hexose sugar, but a large HOD peak in the ^1H NMR spectrum prevented a complete analysis of the corresponding proton resonances. Acetylation yielded a chloroform-soluble tetraacetate, **3**, whose ^1H NMR spectrum revealed five methine and two methylene protons between δ3.9 and 5.8, in addition to the signals for the acetyl methyls and benzoate ester. Identification was confirmed by comparison of the physical properties of **1** and **3** with those reported in the literature (mp [13, 18], OR [11], IR [13, 18], ^1H NMR [14]).

Enzymatic hydrolysis of **2** in the presence of picrate paper suggested that it was a cyanogenic glycoside. Although a molecular ion was not observed in the mass



1 R = H
3 R = Ac



2 R = H
4 R = Ac

spectrum of **2**, the base peak at *m/e* 117 ($C_8H_7N^+$) in the EI spectrum was rationalized by H-rearrangement and α-cleavage [19]. Loss of HCN yielded tropylium ion *m/e* 91 ($C_7H_7^+$). The ^{13}C NMR spectrum gave evidence for a nitrile (119.68), a monosubstituted aromatic ring (134.60, 130.57, 129.90, 128.30), a methine carbon (67.54), and a hexose sugar (102.07, 78.15, 77.44, 74.10, 70.82, 62.07). Identification was confirmed by comparison of the physical properties and spectral data of the parent compound and its tetraacetyl derivative, **4**, with those reported in the literature (mp [4, 5, 15], OR [4, 5, 15], IR [4, 5], ^1H NMR [20]). Investigation of other inhibitory constituents of black cherry foliage is continuing.

EXPERIMENTAL

^1H NMR spectra were determined with a Varian EM-390 spectrometer; chemical shifts are reported in δ units (ppm) relative to TMS (δ = 0). ^{13}C NMR spectra were obtained using a Varian CFT-20 Fourier Transform NMR Spectrometer, with DMSO-d_6 as solvent. IR spectra were recorded on a Perkin Elmer 257 Grating Infrared Spectrophotometer. MS were recorded on an AEI MS-902 mass spectrometer operating at 70 eV. Optical rotations were determined on a Perkin Elmer 141 Polarimeter.

Isolation of **1** *and* **2**. Fresh black cherry leaves were extracted in one of two ways: (a) leaves were frozen in liquid N_2, freeze dried, ground to pass a 20-mesh screen, and extracted with cold aq. 80% EtOH or (b) leaves were extracted with boiling aq. 80% EtOH. In either case the extract volume was reduced *in vacuo* leaving a residual which was defatted with Et_2O ether. The aq. phase was reduced to a small vol. and chromatographed on a polyamide column using MeOH–H_2O (4:1) as eluant. Appropriate fractions were combined and chromatographed on a Bio-Gel P-2 column, using H_2O as eluant. Subsequently, appropriate fractions were combined and rechromatographed on a Si gel column, using $CHCl_3$–MeOH (5:1). Compound **2** was monitored in column fractions by release of HCN in the presence of 0.2 mg/ml emulsin at pH 5.6. Samples were incubated overnight at 37°; detection was with neutral Na picrate paper.

β-D-Glucopyranose 1-benzoate (**1**). mp (MeOH–EtOAc) 166.5–168°, (H_2O) 187–188° [lit. [13] (H_2O) 193°; lit. [14] (H_2O) 191–192°]; $[\alpha]_D^{25}$ − 24.5° (H_2O, *c* 0.83) [lit. [11] $[\alpha]_D^{20}$ −27° (H_2O, *c* 0.45)]; IR (KBr): 3400, 1720, 1605, 1590, 1075, 700 cm^{-1}; MS *m/e* (rel. int.): 123 (10), 122 (14), 105 (100), 77 (31), 73 (30); ^1H NMR (D_2O): δ 8.09 (2 H, *dd*, *J* = 2 and 8 Hz) 7.64 (3 H, *m*), 5.80 (1 H, *dd*, *J* = 2 and 5 Hz), 4.67 (HOD) 4.05–3.45 (6 H, *m*); ^{13}C NMR (DMSO): δ 165.09, 134.14, 129.89, 129.48, 129.11, 95.35, 78.19, 76.69, 72.88, 69.87, 60.96.

β-D-Glucopyranose 1-benzoate 2,3,4,6-tetraacetate (**3**). Compound **1** (50 mg) was treated with Ac_2O: pyridine (1:1) at 25° for 16 hr to obtain **3**. mp (benzene, hexanes) 144–145° [lit. [13] (EtOH) 140–141°, lit [14] (MeOH) 140–142°, lit. [18] 145–146°]; $[\alpha]_D^{25}$ −28.6° ($CHCl_3$; *c* 0.73) [lit. [18] $[\alpha]_D^{20}$ −28.3° ($CHCl_3$, *c* 4.0)]; IR (KBr): 1755, 1235, 1090, 745, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.93 (2 H, *dd*, *J* = 2 and 8 Hz), 7.42 (3 H, *m*), 5.83 (1 H, *dd*, *J* = 2 and 5 Hz), 5.20 (3 H, *m*), 4.15 (2 H, *m*), 3.88 (1 H, *m*), 1.98 (12 H, *m*).

Prunasin (**2**). mp (EtOAc) 148.5–149.5° [lit. [4] 148–151°, lit. [15] 145–147°]; $[\alpha]_D^{25}$ −28.5° (H_2O, *c* 0.87) [lit. [4, 5] $[\alpha]_D^{28}$ −30.1° (H_2O, *c* 0.418), lit. [15] $[\alpha]_D$ −29.6°]; IR (KBr): 3400, 2250 (weak), 1625, 1080, 745, 708 cm^{-1}; MS *m/e* (rel. int.): 143 (8), 118 (9), 117 (100), 116 (56), 91 (5), 90 (7), 89 (11), 73 (21), 69 (23), 61 (11), 60 (13), 57 (15), 44 (19), 43 (12); ^1H NMR (D_2O): δ 7.53 (5 H, *s*), 5.90 (1 H, *s*), 4.67 (HOD), 3.9–3.2 (7 H, *m*); ^{13}C NMR (DMSO): δ 134.60, 130.57, 129.90, 128.30, 119.68, 102.07, 78.15, 77.44, 74.10, 70.82, 67.54, 62.07.

Prunasin tetraacetate (**4**). Compound **2** (50 mg) was acetylated as described above to yield **4**. mp (benzene) 139–140° [lit. [4, 5] 136–137°]; $[\alpha]_D^{25}$ −25.25° (EtOH, *c* 0.40) [lit. [15] $[\alpha]_D$ −24°]; ^1H NMR ($CDCl_3$): δ 7.46 (5 H, *s*), 5.50 (1 H, *s*), 5.07 (3 H, *m*), 4.52 (1 H, *dd*, *J* = 1 Hz), 4.17 (2 H, *m*), 3.60 (1 H, *bm*), 2.07 (3 H, *s*), 1.98 (9 H, *s*).

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