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NANDININ: AN ACYLATED FREE CYANOHYDRIN FROM *NANDINA DOMESTICA*

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Key Word Index—*Nandina domestica*; Berberidaceae; cyanohydrin; cyanogenic glycosides; nandinin.

Abstract—The leaves of *Nandina domestica* have yielded a new cyanogenic compound, characterized here as the 3-(3,4-dihydroxyphenyl) -2-propenoic acid 4′-ester of 4-(β-D-glucopyranosyloxy)-α-hydroxybenzeneacetonitrile.

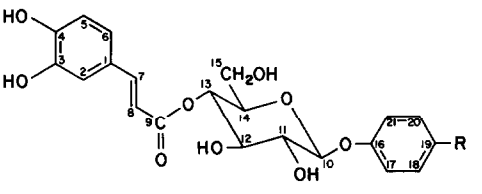
INTRODUCTION

This laboratory has previously reported the isolation and characterization of *p*-glucopyranosyloxymandelonitrile from *Nandina domestica* (Berberidaceae), the garden ornamental hedge commonly called Heavenly Bamboo [1]. We now report the isolation and characterization of nandinin, a new free cyanohydrin and the major cyanogen of young leaves of *N. domestica*. Although many other naturally occurring glycosides have been characterized as acylated products [2], this is only the second instance in which the sugar moiety of a cyanogenic glycoside has been the site of acylation [3] and the first acylated cyanohydrin reported to be found in nature.

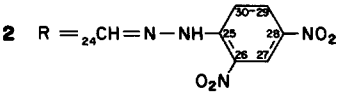
RESULTS AND DISCUSSION

Nandinin (**1**), $C_{23}H_{23}NO_{10}$, was isolated by prep. HPLC as a pale yellow powder. The IR spectrum of **1** exhibits hydroxyl (3300 cm^{-1}) and carbonyl (1700 cm^{-1}) absorption bands. Upon strong acid hydrolysis, it yielded caffeic acid, glucose, *p*-hydroxybenzaldehyde and *p*-hydroxymandelic acid. Very mild alkaline hydrolysis with Tris buffer pH 8.5 released hydrogen cyanide and produced a carbonyl compound which reacted with 2,4-dinintrophenylhydrazine to form the substituted hydrazone **2**. Stronger alkaline hydrolysis released hydrogen cyanide and produced *p*-glucopyranosyloxybenzaldehyde and a strongly oxidized material. A solution of **1** in methanol reacts with ferric chloride to produce a very dark green black color and with lead acetate to form an insolublc yellow precipitate. The ^1H NMR spectrum of **2** showed that there was a one-to-one relationship between all of the peaks in the aromatic portion ($\delta > 6$) with either caffeic acid or with the 2,4-dinitrophenylhydrazone of *p*-glucopyranosyloxybenzaldehyde. The aliphatic portion of the spectrum was similar to that of the 2,4-dinitrophenylhydrazone of *p*-glucopyranosyloxybenzaldehyde except for three downfield peaks: a triplet at δ 4.75, a multiplet at 3.72 and a triplet at 3.61. Proton decoupling experiments indicated that the anomeric proton was not adjacent to the protons causing the three aforementioned peaks. Irradiation of the spectrum at δ 3.39 led to the collapse of both the anomeric doublet and the triplet at δ 3.61 while irradiation of 4.75 caused collapse of the multiplet at 3.72 and the triplet at 3.61. This leads to the conclusion that the caffeic acid is bound to the 4-position of the glucose portion of the molecule. The cleavage of caffeic acid under both acidic and basic conditions, as well as the reactions with ferric chloride and lead acetate, indicate that the caffeic acid is bound to the sugar through an ester linkage at the carboxyl carbon rather than through an ether linkage at either phenolic position. FAB-mass spectrometry of **1** gave an $[M+1]^+$ peak *m/z* 474 and peaks at *m/z* 325, 307 and 289, indicating successive loss of *p*-oxymandelonitrile and two molecules of water. The UV spectrum of **1** was like that of caffeoyl esters while the UV spectrum of the carbonyl compound formed by the loss of hydrogen cyanide from **1** was similar to the additive absorbances of a caffeoyl ester and a *p*-alkoxybenzaldehyde. The ^{13}C NMR spectrum of **1** showed the appropriate absorbances for 21 chemically different carbon atoms in both chemical shifts and multiplicities expected for a caffeoylated *p*-glucopyranosyloxymandelonitrile.

The accumulated evidence led us to assign to nandinin (**1**) the structure as the 3-(3,4-dihydroxyphenyl)-2-propenoic acid 4′-ester of 4-(β-D-plucopyranosyloxy)-α-hydroxybenzeneacetonitrile.



1 R = $_{22}CH_{23}CN$



2 R = $_{24}CH{=}N{-}NH{-}$ … NO_2

EXPERIMENTAL

Plant material. Young leaves of *N. domestica* Thunb. were collected from ornamental hedges on the campus of the University of California at Davis during the month of May, 1980. Only tender foliage was taken; red leaves were avoided during collection.

Extraction and isolation. Leaves (2 kg) were frozen and ground in liquid N_2 and extrated with boiling MeOH. The filtered soln was concd under vacuum and cooled overnight. The tarry ppt was extracted successively with hot Et_2O and hot C_6H_6 and dissolved in EtOH containing 0.1% HOAc. H_2O was added until the soln became cloudy and the mixture was frozen and lyophilized. The residue was a pale yellow solid contaminated by a globule of dark tar which was removed. The solid was extracted again and washed with hot Et_2O and C_6H_6. HPLC was carried out on a system monitored at 325 nm. The eluant, H_2O–MeCN–HOAc (17:3:0.1), was pumped onto a Whatman partisil M9 10/50 ODS 3 column (9.4 mm × 50 cm) at 8 ml/min. Fractions containing **1** were collected, frozen and lyophilized to yield 600 mg, mp 101–104° with loss of HCN, aldehyde decomposition material, mp 180–181°. Treatment of **1** with Tris buffer at pH 8.5 followed by addition of 2,4-dinitrophenylhydrazine hydrochloride provided **2** which was purified by prep. TLC (silica gel, 2500 μm) in C_6H_6–MeOH–HOAc, 14:6:0.1. Sample was applied at 100–200 mg per plate. The orange band was eluted with MeOH. Labile protons were exchanged with a mixture of D_2O and CD_3OD. *p*-Glucosyloxybenzaldehyde was prepared by the method of ref. [4].

Compound **1**. (Found: C, 57.19; H, 4.78; N, 2.75. $C_{23}H_{23}NO_{10}$ requires: C, 58.35; H, 4.87; N, 2.96.) UV λ_{max}^{MeOH} nm: 329. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1690 (C=O), 1605 (C=C), (1200 C–O). ^1H NMR (360 MHz, DMSO-d_6): δ7.83 (2H, *d*, J = 8 Hz, H-18 and H-20), 7.44 (1H, *d*, J = 16 Hz, H-7), 7.18 (2H, *d*, J = 8 Hz, H-17 and H-21), 7.02 (1H, *d*, J = 1 Hz, H-2), 6.96 (1H, *dd*, $J_{5,6}$ = 8 Hz, $J_{5,2}$ = 1 Hz, H-5), 6.72 (1H, *d*, J = 8 Hz, H-6), 6.23 (1H, *d*, J = 16 Hz, H-8), 5.61 (1H, *s*, H-22, pH dependent, H-22), 5.13 (1H, *d*, J = 8 Hz, H-10), 4.72 (1H, *t*, J = 9 Hz, H-13), 3.70 (1H, *m*, $W_{1/2}$ = 8 Hz, H-14), 3.62 (1H, *m*, $W_{1/2}$ = 8 Hz, H-12), H-11 and H-15 unresolved, δ < 3.60. ^{13}C NMR (200 MHz, DMSO-d_6): δ 165.9 (*s*, C-9), 157.6 (*s*, C-16), 148.5 (*s*, C-3), 145.6 (*s*, C-4), 145.5 (*d*, C-7), 130.8 (*s*, C-19), 127.9 (*d*, C-18 and C-20), 125.5 (*s*, C-1), 121.4 (*d*, C-8), 120.7 (*s*, C-23), 116.5 (*d*, C-17 and C-21), 115.8 (*d*, C-6), 114.9 (*d*, C-5), 113.9 (*d*, C-2), 100.0 (*d*, C-10), 74.7 (*d*, C-14), 73.9 (*d*, C-12), 73.3 (*d*, C-11), 70.9 (*d*, C-13), 61.4 (*d*, C-22), 60.5 (*t*, C-15). C-11, 12 and 14 not unequivocally assigned. Mp 101–104° with loss of HCN, aldehyde decomposition material mp 180–181°.

Compound **2**. (Found: C, 51.45; H, 4.44; N, 8.58. $C_{28}H_{26}N_4O_{13}$ requires: C, 51.22; H, 3.96; N, 8.54.) ^1H NMR (360 MHz, DMSO-d_6): δ8.73 (1H, *d*, J = 1 Hz, H-27), 8.54 (1H, *s*, H-24), 8.09 (1H, *dd*, $J_{29,30}$ = 11 Hz, $J_{29,27}$ = 1 Hz, H-29), 7.91 (1H, *d*, J = 11 Hz, H-30), 7.76 (2H, *d*, J = 9 Hz, H-18 and H-20), 7.50 (1H, *d*, J = 16 Hz, H-7), 7.15 (2H, *d*, J = 9 Hz, H-17 and H-21), 7.06 (1H, *d*, J = 2 Hz, H-2), 7.02 (1H, *dd*, $J_{5,6}$ = 8 Hz, $J_{5,2}$ = 2 Hz, H-5), 6.76 (1H, *d*, J = 8 Hz, H-6), 6.27 (1H, *d*, J = 16 Hz, H-8), 5.10 (1H, *d*, J = 8 Hz, H-10), 4.75 (1H, *t*, J = 8 Hz, H-13), 3.75 (1H, *m*, $W_{1/2}$ = 9 Hz, H-14), 3.61 (1H, *t*, J = 8 Hz, H-12), H-11 and H-15 unresolved, δ < 3.60.

p-*Glucopyranosyloxybenzaldehyde: 2,4-dinitrophenylhydrazone.* ^1H NMR (360 MHz, DMSO-d_6): δ8.82 (1H, *d*, J = 1 Hz, H-27), 8.60 (1H, *s*, H-24), 8.31 (1H, *dd*, $J_{29,30}$ = 11 Hz, $J_{29,27}$ = 1 Hz, H-29), 8.04 (1H, *d*, J = 11 Hz, H-30), 7.72 (2H, *d*, J = 9 Hz, H-18 and H-20), 7.11 (2H, *d*, J = 9 Hz, H-17 and H-21), 4.95 (1H, *d*, J = 7 Hz, H-10), unresolved multiplets 3.15–3.71 (6H, *m*, H-11, H-12, H-13, H-14 and H-15).

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