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ALIPHATIC NITRO-COMPOUNDS IN *ASTRAGALUS CANADENSIS*

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Key Word Index—*Astragalus canadensis* var. *mortonii*; Leguminosae; Canada milk-vetch; glucose esters; 3-nitropropanoic acid; [5-oxotetrahydrofuran-3-yl]-acetic acid.

Abstract—A reinvestigation of the alphatic nitro-compounds in *Astragalus canadensis* resulted in the identification of two new esters of glucose with 3-nitropropanoic acid and 5-oxotetrahydrofuran-3-acetic acid, together with six known conjugates of 3-nitropropanoic acid. ^{1}H and ^{13}CNMR data are reported for the new compounds.

INTRODUCTION

Astragalus canadensis L., Canada milk-vetch, is the most widely distributed species of the genus in North America [1, 2]. Although it does not appear in listings of poisonous plants [3, 4], sheep and cattle have been experimentally poisoned with the plant [5, 6], the latter to a much lesser degree, and it is among the 263 species and varieties of *Astragalus* known to contain nitro-compounds [7]. Previous studies have revealed that Canada milk-vetch as well as other species of *Astragalus* contain bound forms forms of 3-nitropropanoic acid (3NPA) [8]. Monogastric mammals are more seriously affected by these conjugates than ruminants [9], and it has been shown that ruminal bacteria can detoxify 3NPA [9] by anaerobic reduction to β-alanine [10]. Six esters of 3NPA with glucose have been reported to be present in two varieties of *A canadensis* four of which were identified as karakin (1,2,6-tri-*O*-[3-nitropropanyl-β-D-glucopyranose), cibarian (1,6-di-*O*-[3-nitropropanoyl]-β-glucopyranose) and a mixture of 6-*O*-[3-nitropropanoyl]-α- and β-D-glucopyranose monoesters [11].

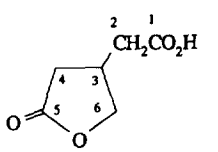
3-(Hydroxymethyl)-glutaric acid γ-lactone (**1**), 'homopilosinic acid', has also been isolated from *A. canadensis* [12].

We now report the results of a reinvestigation of the nitro-compounds of *A. canadensis*. L. var. *mortonii* (Nutt.) Wats. collected in southern British Columbia.

RESULTS AND DISCUSSION

Guided by a colour test for nitro-compounds [13] and extensive chromatographic separations (see Experimental), we isolated eight conjugates from *A. canadensis* L. var. *mortonii*, which were then subjected to sepectrometric analysis. The two major compounds were thus identified as karakin and cibarian, constituting *ca* 1 and 0.6%, respectively, of the plant dry matter. Three others, corynocarpin (1,4,6-tri-*O*-[3-nitropropanoyl]-β-D-glucopyranose), 1,3,6-tri-*O*-(3-nitropropanoyl)-β-D-glucopyranose, a substance which had previously discovered in *Corynocarpus laevigatus* [14], and a new substance (**A**), each accounted for about 0.1% of the dry weight of the plant. The anomeric mixture of 6-*O*-[3-nitropropanoyl]-α- and β-D-glucose, and another new compound (**B**) were present in lesser amounts (< 0.05% of the dry plant).

The 400 MHz ^{1}H NMR spectrum of **A** contained resonances corresponding to two 3-nitropropanyl groups (multiplets at δ_H4.8 and 3.1 ppm, each 4H) and a β-D-glucopyranose unit, esterified at *three* sites: *O*-1, 2 and 6. The connectivities between the ring-protons of the



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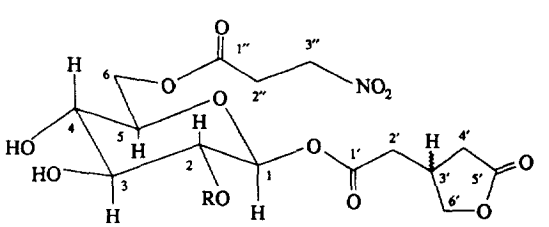

R

2 $CCH_2CH_2NO_2$

3 H

glucopyranose unit were established by a COSY spectrum, while their chemical shifts revealed whether they carried hydroxyl or ester functionalities (see data in Experimental). The identity of the third esterifying component was deduced as follows.

The ^{13}C NMR spectrum of **A** contained 18 signals, with no indication of coincident resonances. Of these, DEPT spectra revealed that four were quaternary (all carboxyl-type carbonyls), six were methines and eight were methylenes. Subtracting the requirements for a glucose diester with 3NPA revealed that the third esterifying component was C_6 unit: constructed from two carbonyls, a methine and three methylene groups.

The chemical shifts of the latter (δ_C 72.8, 37.1 and 34.1 ppm) indicated that one was oxygenated, and the protons of that group were observed in the ^1H NMR spectrum of **A** as the AB portion of an ABX system with chemical shifts consistent with the attachment of an ester (δ_H 4.48, 1H, *dd*, $J = 7.5$ and 9.1 Hz; 4.00, 1H, *dd*, $J = 7.5$ and 9.1 Hz). The X resonance (δ_H 2.96, 1H, *m*) was additionally coupled with both of the other methylenes, in one of which the protons were nearly magnetically equivalent (δ_H 2.65) while in the other they were clearly diastereotopic (δ_H 2.65, 1H *m*; and 2.31, 1H, *dd*, $J = 8.5$ and 17.4 Hz). Adding the two carboxyl groups to this assembly generated **1**, i.e. the third ester component was recognized to be the acid first isolated as a natural product from *A. canadensis* [12].

Additional support for the identification of **A** as a triester of β-D-glucopyranose with two NPA and one **1** was provided by FAB mass spectrometry, which indicated a molecular weight of 508 (M + 1 and M + 23 ions at *m/z* 509 and 531) as required by this formulation. The distribution of the individual esterifying groups was determined by selective INEPT (SELINEPT) NMR measurements [15, 16]. Thus, irradiation of the anomeric proton (δ_H 5.7), using $^3J_{C,H} = 6$ Hz resulted in selective enhancement of one of the carbonyl resonances (δ_C 170.74 ppm). The same carbonyl, as well as another (the γ-lactone one, δ_C 176.76) were enhanced upon irradiating the 3H-multiplet corresponding to H-2'A, H-2'B and H-4'A of **1** (δ_H 2.65). The lactonic ester was, therefore, located at the anomeric centre. Similar irradiation of the α-protons of the two 3NPA units (δ_H 3.09) enhanced two other carbonyl resonances (δ_C 170.67 and 169.87), and irradiation of H-6B (δ_H 4.3 ppm) enhanced one of these (δ_C 170.67), i.e. confirmed that neither of the two 3NPA-units were attached to C-1, but rather were at C-2 and C-6. Thus, **A** has the structure **2**. Similar spectrometric analyses revealed that **B** was the analogous 1,6-diester (**3**) (see Experimental).

One detail of the structures of **A** and **B** eluded us: we were unable to determine the chirality of the lactonic ester unit. The ^1H and ^{13}C NMR data for our isolates contained no indications of the presence of diastereomers, i.e. were consistent with our isolates being single stereomers. Gulati *et al.* reported [12] that the acid (**1**) which they isolated from *A. canadensis* was optically inactive, but in aqueous solution the racemization of the free acid should be facile.

We conclude that *A. canadensis* contains some novel conjugates of 3NPA and **1** (of undecided absolute stereochemistry) with β-D-glucopyranose. Since these should release 3NPA upon hydrolysis, they also contribute to the total toxic burden of the plant.

EXPERIMENTAL

NMR spectra were measured at 400 MHz (^1H) and 100 MHz (^{13}C) of samples dissolved in 99.8 atom % Me_2CO-d_6, using the solvent resonances (δ_H 2.05, δ_C 29.8) as int. reference. ^1H NMR spectra were remeasured after adding 1 drop of 100 atom % D_2O to remove OH couplings. SELINEPT spectra were measured with a Bruker AMX-300 spectrometer. FABMS were determined using a glycerol matrix with a Kratos MS-80 spectrometer fitted with a xenon ion-gun.

Isolation of the nitro-conjugates. Aerial portions of *A. canadensis* var. *mortonii* were collected near Lac de Bois, 12 km from Kamloops, B.C., while the plants were in bloom to pod stages of growth. A voucher specimen has been deposited in the Herbarium of the Royal British Columbia Museum, Victoria, B.C. The freeze-dried powdered plant material (280 g) was extracted with Me_2CO at room temp. The concd extract was suspended in H_2O and extracted with hexane. The aq. phase was then extracted with EtOAC (× 3) and the concd extracts were subjected to CC on silica gel [14]. Individual frs were concd to dryness and assayed for aliphatic nitro-compounds by TLC on silica gel ($CHCl_3$–Me_2CO–HCO_2H, 50:50:1) using diazotized *p*-nitroaniline spray reagent for detection [13]. The triester, 1,3,6-tri-*O*-[3-nitropropanoyl]-β-D-glucopyranose (R_f 0.64) and the diester, cibarian (R_f 0.22) were eluted from the column in pure form. Frs containing karakin (R_f 0.53) and the α,β-anomeric mixt. of the 6-*O*-[3-nitropropanoyl]-D-glucopyranoses were purified by centrifugally accelerated radial TLC (Chromatotron) [14]. Frs containing **A** (R_f 0.41), corynocarpin (R_f 0.44) and karakin were subjected to centrifugal countercurrent chromatography (CCC) [17] using cyclohexane–EtOAC–H_2O (1:3:4) as the two phase system. The upper mobile phase (flow rate 3 ml min^{-1}) yielded karakin and corynocarpin, after which the mobile phase was changed to the upper phase of EtOAC–H_2O (1:1), which yielded pure **A**. Frs containing **B** (R_f 0.35 in Me_2CO–$CHCl_3$–HCO_2H, 50:25:1) were purified by TLC (Chromatotron) and subjected to CCC using EtOAC–CH_3CN–H_2O (2:3:3) as the two phase system. The upper mobile phase yielded cibarian and **B**.

Compound **A**, *1-O-[5-oxotetrahydrofuran-3-yl]acetyl-2,6-di-O-[3-nitropropanoyl]-β-D-glucopyranose* (**2**). This was obtained as a glass. FABMS *m/z* 509 (M + 1) and 531 (M + 23). ^1H NMR δ_H 5.71 (1H, *d*, $J = 8.4$ Hz, H-1), 4.92 (1H, *dd*, $J = 8.4, 9.5$ Hz, H-2), 4.8 (4H, *m*, overlapped *t*, H-3''), 4.48 (1H, *dd*, $J = 7.5, 9.1$ Hz, H-6'A), 4.43 (1H, *dd*, $J = 2.1, 12$ Hz, H-6A), 4.31 (1H, *dd*, $J = 5.7, 12$ Hz, H-6B), 4.00 (1H, *dd*, $J = 7.5, 9.1$ Hz, H-6'B), 3.77 (1H, *t*, $J = 9$ Hz, H-3), 3.75 (1H, *ddd*, $J = 2.2, 5.7, 9.7$ Hz, H-5), 3.53 (1H, *dd*, $J = 9, 9.7$ Hz, H-4), 3.09 (4H, *m*, H-2''), 2.96 (1H, *m*, H-3'), 2.65 (3H, *m*, H-2' and 4'A), 2.31 (1H, *dd*,

J = 8.5, 17.4 Hz, H-4′B). ^{13}C NMR δ_C 176.75 (C-5′), 170.74 (C-1′), 170.66 (C-1″), 169.86 (C-1″), 92.8 (C-1), 75.6 (C-3), 75.0 (C-5), 74.1 (C-2), 72.8 (C-6′), 70.9 and 70.7 (C-3″), 70.7 (C-4), 64.4 (C-6), 37.1 (C-2′), 34.1 (C-4′), 32.4 (C-3′), 31.6 and 31.5 (C-2″). SELINEPT data (see text).

Compound **B**, 1-*O*-[5-*oxotetrahydrofuran*-3-*yl*]*acetyl*-6-*O*-(3-*nitropropanoyl*)-β-D-*glucopyranose* (**B**). This was obtained as a glass. FABMS m/z 408 (M + H) and 430 (M + Na) ^{1}H NMR δ_H 5.53 (1H, *d*, J = 8.1 Hz, H-1), 4.8 (2H, *m*, H-3″), 4.50 (1H, *dd*, J = 7.5, 9.0 Hz, H-6′A), 4.43 (1H, *dd*, J = 2.1, 11.9 Hz, H-6A), 4.28 (1H, *dd*, J = 5.9, 11.9 Hz, H-6B), 4.03 (1H, *dd*, J = 7.3, 9.0 Hz, H-6′B), 3.65 (1H, *ddd*, J = 2.1, 5.9, 9.0 Hz, H-5, 3.51 (1H, *t*, J = 9.0 Hz, H-3), 3.39 (1H, *t*, J = 9.1 Hz, H-4) and 3.36 (1H, *t*, J = 8.1 Hz, H-2), 3.07 (2H, *m*, H-2″), 3.01 (1H, *m*, H-3′), 2.69 (3H, *m*, H-2′ and 4′A), 2.32 (1H, *dd*, J = 8.2, 17.4 Hz, H-4′B); ^{13}C NMR δ_C, 174.1 (C-5′), 171.12 (C-1′), 170.80 (C-1″), 95.5 (C-1), 77.8 (C-3), 75.8 (C-5), 73.8 (C-2), 73.0 (C-6′), 71.0 (C-4), 70.9 (C-3″), 64.9 (C-6), 37.6 (C-2′), 34.3 (C-4′), 32.8 (C-3′), 31.6 (C-2″). SELINEPT experiments resulted in enhancement of δ_C 171.12 when δ_H 5.5 was irradiated, while δ_C 170.8 was similarly enhanced by irradiation at δ_H 3.07 or 4.28 ppm.

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