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3-NITRO-1-PROPYL-β-D-GENTIOBIOSIDE FROM *ASTRAGALUS MISER* VAR. *SEROTINUS*

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Key Word Index—*Astragalus miser*, Leguminosae, timber milkvetch, aliphatic nitrocompounds, toxic glycoside.

Abstract—A new glycoside of 3-nitropropanol, 3-nitro-1-propyl-β-D-gentiobioside, has been isolated from the aerial portions of *Astragalus miser* var. *serotinus* and characterized by spectral studies.

INTRODUCTION

Timber milkvetch, *Astragalus miser* Dougl. var. *serotinus* (Gray) Barneby, and at least 10 other species of *Astragalus* synthesize the glycoside miserotoxin (3-nitro-1-propyl-β-D-glucopyranoside) [1]. The aglycone, 3-nitro-1-propanol has been detected in 49 additional species of *Astragalus* that primarily occur on rangelands in the temperate regions of the New World [2, 3] Presumably, these species also contain miserotoxin or other bound forms of 3-nitropropanol yet to be identified In cattle and sheep, miserotoxin is readily hydrolysed by microbial enzymes of the rumen and the aglycone is rapidly absorbed and oxidized to 3-nitropropionic acid [4, 5] 3-Nitropropionic acid is a potent inhibitor of mitochondrial enzymes essential to respiration [6, 7]

A number of *Astragalus* species can also synthesize glucose esters of 3-nitropropionic acid but with the exception of *A palenae* [3], conjugates of this acid and 3-nitropropanol do not occur together in the same plant [1] Ten substitution patterns have been described for 3-nitropropionic acid glucose esters [8, 9] but to our knowledge, miserotoxin is the only known conjugate of 3-nitropropanol in nature This report now describes the isolation and identification of a new glycoside of 3-nitropropanol from timber milkvetch

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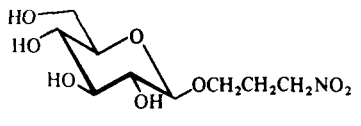
RESULTS AND DISCUSSION

The new glycoside of 3-nitropropanol was isolated as a minor component of *A miser* var *serotinus*. Whereas miserotoxin can occur as >5% of the dry matter of timber milkvetch [10], it is estimated that the new glycoside accounts for <0 1% of its dry matter

Its structure was established as **2** on the basis of the following evidence The mobility of the substance in TLC and HPLC systems, relative to miserotoxin (**1**), suggested that it was a disaccharide When treated with almond emulsin (EC 3 2 1 21) it was cleaved to yield only 3-nitropropanol, glucose and miserotoxin The progress of this hydrolysis was followed by TLC [11, 12] and HPLC. In the latter case the appearance of the nitro-containing products was monitored by following their absorption at 210 nm After incubation with the enzyme for one hr, the absorptions for 3-nitropropanol and **1** were of equal intensity, after which that of 3-nitropropanol increased while there was a corresponding decrease for **1**. These observations suggested that our toxin was a β-D-glucoside of miserotoxin, and to locate the position of attachment of the 'extra' glucose we turned to an examination of the ^{13}C NMR spectrum.

Glycosylation of sugars is known to result in a downfield shift of the resonance of the oxygenated carbon [13] In the case of 2-, 3- or 4-*O*-β-D-glucopyranosylation of glucopyranosides (formation of sophorosides, laminaribiosides or cellobiosides) this absorption appears below 79 ppm (usually in the range 79–88 ppm) [13] No such



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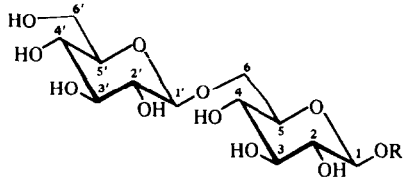

2 R = $CH_2^{1''}CH_2^{2''}CH_2^{3''}NO_2$
3 R = Me
4 R = H

signal appeared in the ^{13}C NMR spectrum of our glycoside The remaining possibility is attachment of the β-D-glucopyranosyl unit to 0–6 of miserotoxin, to form 3-nitropropyl β-D-gentiobioside (**2**). As revealed by the data in Table 1, the 12 carbon resonances observed for our glycoside in addition to the three attributable to the nitropropyl unit were in excellent agreement with those expected for this structure, showing a very close fit to those of methyl β-D-gentiobioside (**3**) [14] The results of 400 MHz ^1H NMR measurements (including RCT COSY 2D-spectra) were also in full accord with our toxin being the β-D-gentiobioside **2** (see data in Table 2), and we have named it gentitoxin

Table 1 Comparison of the ^{13}C NMR spectra* of gentitoxin (**2**) and methyl β-D-gentiobioside (**3**) [14]

C	2	3
1	103 1	104 2
2	73 9	73 9
3	76 5	76 7
4	70 2	70 6
5	75 8	75 8
6	69 4	69 5
1′	103 7	103 6
2′	73 9	74 0
3′	76 5	76 7
4′	70 5	70 7
5′	76 8	76 8
6′	61 6	61 8
1″	67 7	58 1
2″	27 7	-
3″	73 3	

*The values given are of the chemical shifts in ppm relative to internal 1,4-dioxane (δ67 4 ppm) The solvent was D_2O

EXPERIMENTAL

Isolation Aerial parts of the plant material were collected from the Lac du Bois area near Kamloops, B C The fresh plant material (7 5 kg) was thoroughly extracted with hot 80% EtOH and the concentrated extract was treated with Celite and polyamide as described previously [11] The polyamide eluate was fractionated on a coconut charcoal column (8 × 83 cm) [15] The column was developed with H_2O (17 l), aq 10% EtOH (pH 4, 45 l), 20% EtOH (20 l), 30% EtOH (10 l) and 40% EtOH (10 l) Most of the glycoside was eluted in the last 20 l which were combined and concd to a brown gum (8 g) under red pressure Portions of the gum (1 g) were fractionated by centrifugally accelerated TLC (Chromatotron, 4 mm silica gel 60, Merck No 7749) as follows the sample to be applied was initially suspended in 90% EtOH (25 ml), clarified by centrifugation and the supernatant was applied to the prep layer as a 2 cm band at the origin The band was thoroughly dried in a stream of air and the plate was developed with a stepwise gradient of 95% EtOH in $CHCl_3$ (HCl-free) 1 4 (100 ml), 1 3 (150 ml), 3 7 (200 ml), 3 5 6 (200 ml), 4 6 (200 ml), 4 5 5 5 (300 ml) Fractions (× 100 ml) eluted with 25-30% EtOH yielded the major glycoside, miserotoxin, as shown by avicel TLC (R_f 0 47, *n*-BuOH–EtOH–H_2O, 4 1 5 [11]) Those eluted with 45% EtOH yielded a new, minor glycoside (R_f 0 27) detected with the *p*-nitroaniline reagent specific for aliphatic nitrocompounds [11] Final purification of the glycoside was achieved with prep HPLC utilizing a Varian Micropak MCH-10 (10 μm octadecylsilane [monomeric, bonded to LiChrosorb]) column (50 cm × 8 mm) The isocratic mobile phase consisted of 2% aq MeOH and the RR_ts were 36 and 65 min respectively for miserotoxin and the new glycoside, which were detected with a UV detector set at 210 nm A flow rate of 2 ml/min gave a pressure of 38–40 atmos The column was cleaned with a MeOH gradient (2–20%) between injections (0 4 ml) of the concd chromatotron fractions which were redissolved in H_2O For analytical HPLC a similar, but smaller column was employed (Micropak MCH-5, 30 cm

Table 2 A comparison of the ^1H NMR spectra* of gentitoxin (**2**) and β-D-gentiobiose (**4**) [16]

H	2	4
1	4 47 $J_{1,2}$=8 Hz	4 66 $J_{1,2}$=7 8 Hz
2	3 27 $J_{2,3}$=9 Hz	3 26 $J_{2,3}$=9 Hz
3	(3 49 $J_{3,4}$=9 Hz)	(3 50) too strongly coupled for analysis
4	(3 46 $J_{4,5}$=9 Hz)	(3 50)
5	3 62 $J_{5,6A}$=2 Hz, $J_{5,6B}$=6 Hz	3 63 $J_{5,6A}$=1 9 Hz, $J_{5,6B}$=5 5 Hz
6	4 21(A) and 3 87(B) $J_{6A,6B}$=12 Hz	4 21(A) and 3 85(B) $J_{6A,6B}$=−12 Hz
1′	4 52 $J_{1,2}$=8 Hz	4 51 $J_{1,2}$=7 8 Hz
2′	3 32 $J_{2,3}$=9 Hz	3 33 $J_{2,3}$=9 Hz
3′	3 50 $J_{3,4'}$=9 Hz	3 51 $J_{3,4}$=9 Hz
4′	3 40 $J_{4',5}$=9 Hz	3 39 $J_{4,5}$=10 Hz
5′	3 47 $J_{5',6A}$=2 5 Hz, $J_{5,6B}$=6 Hz	(3 47) $J_{5,6A}$=2 Hz, $J_{5,6B}$=5 5 Hz
6′	3 95(A) and 3 76(B) $J_{6A,6B}$=12 Hz	3 93(A) and 3 73(B) $J_{6A,6B}$=−11 4 Hz
1″	4 03 and 3 80 $J_{1'A,1B}$=10 5 Hz	
2″	2 34 $J_{1'A,2}$=$J_{1B,2}$=$J_{2,3}$=7 Hz	
3″	4 68	

*In D_2O, **2** at 400 MHz, **4** at 300 MHz Chemical shifts are in ppm from TSS internal standard The complexity of the region 3 45–3 50 makes a first-order analysis questionable, and the values given are tentative

× 4 mm) Gentitoxin (approximately 15 mg/g gum) was obtained as a white amorphous powder after drying

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