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A NEW GLYCOSIDE OF 3-NITROPROPANOL FROM *ASTRAGALUS MISER* VAR. *SEROTINUS*

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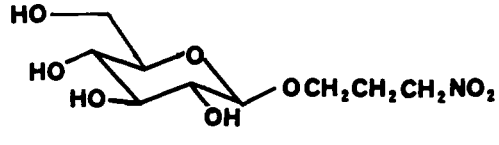
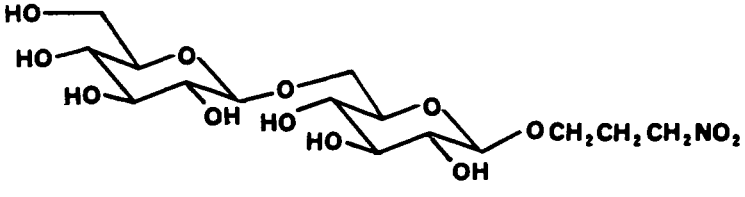
Astragalus miser Dougl. var. *serotinus* (Gray) Barneby (Leguminosae) or timber milkvetch, a perennial forb widely distributed on rangelands in the interior of British Columbia, is well-known for its toxicity to cattle and sheep. Miserotoxin (3-nitro-1-propyl-β-D-glucopyranoside) [**1**] is the poisonous principle in timber milkvetch, and it is also found in ten other species of *Astragalus* in the Western U.S.A. (1). About 50 additional species of *Astragalus*, primarily from the temperate regions of North and South America, have also yielded the aglycone 3-nitro-1-propanol after acid hydrolysis (2), and presumably these species contain miserotoxin or other bound forms of 3-nitropropanol yet to be identified. The aglycone is much more toxic to rats than the glycoside **1** (3). In our earlier communication we reported the isolation and characterization of gentitoxin (3-nitro-1-propyl-β-D-gentiobioside) [**2**] as a minor constituent of *A. miser* var. *serotinus* (4). Miserotoxin and gentitoxin are rapidly hydrolyzed when treated with β-glucosidase (EC 3.2.1.21) yielding glucose and 3-nitropropanol as the hydrolysis products. We now report the isolation of another nitropropyl glycoside from the same source, which glycoside we have identified as the allolactose (6-*O*-β-D-galactopyranosyl-D-glucose) **3** derivative of 3-nitropropanol on the basis of the following evidence.

The new glycoside, which like **2** was present in the plant only as a minor component comprising <0.1% of the dry matter, was hydrolyzed by dilute HCl to yield **1**, 3-nitropropanol, galactose, and glucose. The glycoside was also hydrolyzed by β-galactosidase (EC 3.2.1.23) at C-1′ and only slowly hydrolyzed by β-glucosidase at C-1. These results suggest that **3** is less toxic to ruminants than **1** or **2** because the aglycone of **3** would be released at a slower rate by microbial enzymes of the rumen.

The 200-MHz ^{1}H-nmr spectrum of the glycoside revealed signals attributable to the presence of a nitropropyl moiety [δ 4.61 (2H, t, $J=7$ Hz, CH_2NO_2), 2.30 (2H, p, $J=7$ Hz,



1



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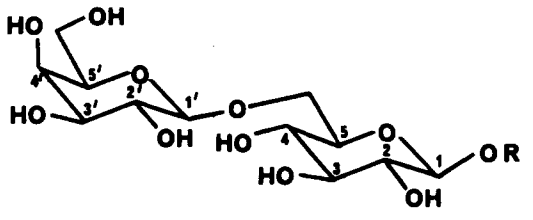

1″ 2″ 3″

3 R=$CH_2CH_2CH_2NO_2$

4 R=Me

-O-$CH_2CH_2CH_2NO_2$)]. The appearance of two low-field doublets [δ 4.31 (1H, J=7.9 Hz), 4.28 (1H, J=7.5 Hz)] was consistent, with the expectation for protons at the anomeric centers being *anti*-periplanar with protons on the adjacent carbon, i.e., both glycopyranosidic linkages being β (8). However, the remainder of the spectrum, which involved considerable overlap of signals in the region δ 3.0–4.0, was complex, and we were unable to decide the connectivities between the galactosyl and glucosyl units. We therefore turned to an examination of the ^{13}C-nmr spectrum of the glycoside. This revealed the presence of 15 absorptions (Table 1)

TABLE 1. Comparison of the ^{13}C-nmr Spectra of 3-Nitropropyl-β-D-allolactoside [**3**] and Methyl β-D-allolactoside [**4**].[a]

Carbon	Compound	
	3	**4**
1	103.2	104.2
2	73.9	73.9
3	76.5	76.6
4	70.3	70.3
5	75.9	75.8
6	69.3	69.3
1′	104.3	104.2
2′	71.6	71.7
3′	73.5	73.6
4′	69.5	69.5
5′	76.0	75.9
6′	61.8	61.8
1″	67.7	58.2
2″	27.7	
3″	73.4	

[a]In D_2O, the chemical shifts are in ppm from internal acetonitrile (CH_3 1.70 relative to dioxane 67.40 ppm, from TMS = 0).

as required for the nitropropyl disaccharide. The absence of any of these in the range 79–88 ppm suggested that the glucopyranosyl unit was not glycosylated at O-2, 3, or 4 (4,9); by elimination of these possibilities the site of attachment was deduced to be O-6. This resulted in the identification of the glycoside as 3-nitropropyl-β-D-allolactoside [**3**].

As we were unable to locate the ^{13}C-nmr spectrum of methyl β-D-allolactoside [**4**] or any other suitable model substance in the literature, we synthesized **4** by conventional procedures (10, 11) and compared its ^{1}H- and ^{13}C-nmr spectra with those of the nitropropyl glycoside. The 200-MHz ^{1}H-nmr spectrum of **4** showed anticipated similarities to that of **3**, in particular the resonances from the anomeric protons [δ 4.32 (1H, d, J=7.5 Hz), 4.27 (1H, d, J=7.9 Hz)], but most striking was the concordance of the 12 absorptions seen for the disaccharide unit in the ^{13}C nmr with those for the corresponding part of the nitropropyl glycoside (Table 1).

Thus, we conclude that the new glycoside is, indeed, 3-nitropropyl-β-D-allolactoside. Inasmuch as we are unaware of any other plant-derived naturally occurring allolactoside, this result surprised us. Presumably the glycoside could be formed by a reversion reaction involving the β-galactosidase-catalyzed galatosylation of miserotoxin, but as we were unable to demonstrate that this occurred during the processing of our plant extracts, it seems that the new glycoside is a genuine natural product and not an artifact of the isolation procedure.

EXPERIMENTAL

PLANT MATERIAL.—The aerial parts of *A. miser* var. *serotinus* were collected from the Lac du Bois area near Kamloops, B.C. Voucher specimens have been deposited in the herbarium at the Agriculture Canada Research Station in Kamloops.

SPECTROSCOPY.—Measurements of ^{1}H- and ^{13}C-nmr spectra were made using Varian XL-200 and Bruker AC-200 and AM-400 spectrometers.

ISOLATION AND IDENTIFICATION.—An EtOH extract of fresh-frozen plant material was fractionated on a coconut charcoal column, and the fraction eluted with 40% EtOH was subjected to centrifugally accelerated tlc (Chromatotron) as described previously (4). Fractions (100 ml) from the Chromatotron, eluted with 45–50% EtOH in $CHCl_3$, yielded the new glycoside of 3-nitropropanol. Final purification was achieved with preparative hplc utilizing 2% aqueous MeOH as the isocratic mobile phase (4). The retention times for miserotoxin and the new glycoside were 36 and 48 min, respectively. The retention times for miserotoxin and gentitoxin were previously reported as 36 and 65 min, respectively (4). The R_fs for miserotoxin, gentitoxin, and the new glycoside were 0.49, 0.27, and 0.25, respectively, on tlc [Avicel, *n*-BuOH-EtOH-H_2O (4:1:5)]. The compounds were readily detected with the *p*-nitroaniline reagent specific for aliphatic nitrocompounds (5).

HYDROLYSIS.—When the new glycoside was treated with 1N HCl for 2 h at 90°, tlc of the hydrolysate revealed misertoxin and 3-nitropropanol as hydrolysis products. When the acid hydrolysate was chromatographed in tlc systems for simple sugars (6,7), glucose and galactose were detected as hydrolysis products by conventional procedures (5,6). Treatment of the glycoside with almond emulsin (Sigma No. G-4511) yielded 3-nitropropanol but only trace quantities of miserotoxin. In the presence of emulsin, the hydrolysis of **3** appeared to progress at a much slower rate than the hydrolysis of **1**. Miserotoxin (100 μg), for example, was completely hydrolyzed within 1 h at room temperature in phosphate buffer (pH 7.2), but the hydrolysis of **3** at C-1 required >20 h for completion as evidenced by hplc. Hydrolysis of the glycoside in the presence of β-galactosidase (Sigma No. G-6512) yielded miserotoxin but not the aglycone. The reaction went to completion within 2 h at room temperature as evidenced by tlc. On the basis of this evidence we concluded that the substance was an *O*-β-D-galactopyranosyl derivative of miserotoxin.

NEW GLYCOSIDE.—A colorless glass: ^{1}H nmr (D_2O, 200 MHz) δ 4.61 (2H, t, J = 7 Hz), 4.31 (1H, d, J = 7.9 Hz), 4.28 (1H, d, J = 7.5 Hz), 4.1–3.0 (ca. 14H, complex), 2.30 ppm (2H, p, J = 7 Hz). Irradiation at δ 2.30 ppm collapsed the signal at δ 4.61 to a singlet, while two doublets emerged at δ 3.9 and 3.68 ppm (both J = 12 Hz). Irradiation at δ 4.61 reduced the signal at δ 2.30 to a triplet (J = 7 Hz). Irradiation at δ 3.25 ppm collapsed the signal at δ 4.31 ppm to a singlet, while irradiation at δ 3.5 ppm similarly collapsed the doublet at δ 4.28 ppm. ^{13}C nmr (D_2O, 50.3 MHz) see Table 1.

METHYL-β-D-ALLOLACTOSIDE.—Koenigs-Knorr coupling of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl bromide (Sigma Chemical Co.) and methyl β-D-2,3,4-tri-*O*-acetylglucopyranoside (10) was performed as previously described for the synthesis of octa-*O*-acetylallolactose (11), except that an ultrasonic bath (Branson Co.) was used to maintain continuous vigorous agitation of the heterogeneous reaction mixture. Centrifugal tlc (Chromatotron) of the reaction products using toluene-EtOAc (2:1↦1:1) as eluent on Si gel Merck 60 F254 gave methyl hepta-*O*-acetyl-β-D-allolactoside (48%) homogeneous on tlc and crystalline, mp 146–148° [lit. (12) mp 145°]. Deacetylation of this material was carried out in anhydrous MeOH in the presence of catalytic amounts of NaOMe (8 h room temperature) followed by careful neutralization of the base with 5% H_2SO_4 in MeOH. The reaction mixture was then centrifuged and the supernatant evaporated to dryness (bath 40°) in vacuo to provide a nearly quantitative yield of methyl β-D-allolactoside as a colorless gum, homogeneous by tlc and nmr analysis: ^{1}H nmr (D_2O, 200 MHz) δ 4.34 (1H, d, J = 7.5 Hz), 4.26 (1H, d, J = 7.9 Hz), 4.2–3.0 (ca. 12H, complex), 3.44 (3H, s, OMe); ^{13}C nmr (D_2O, 50.3 MHz) see Table 1.

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