Phytochemistry, Vol. 28, No. 9, pp. 2369–2371, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

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

MICHAEL H. BENN and WALTER MAJAK*†

Chemistry Department, The University, Calgary, Alberta, Canada T2N 1N4; *Research Station Agriculture Canada, 3015 Ord Road, Kamloops, B.C., Canada V2B 8A9

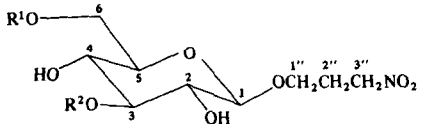
(*Received* 1 *December* 1988)

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

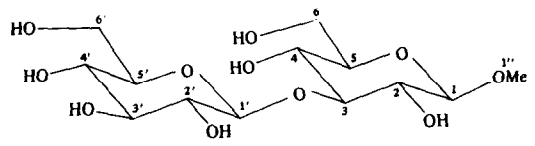
Abstract—A new glycoside of 3-nitropropanol, 3-nitro-1-propyl-β-D-laminaribioside, has been isolated from the aerial portions of *Astragalus miser* var. *serotinus*. Its structure was established by spectroscopic studies which compared closely related glucosides of 3-nitropropanol.

INTRODUCTION

The genus *Astragalus* is known to produce glucose esters of 3-nitropropionic acid and the β-D-glucoside of 3-nitropropanol, miserotoxin (**1**) [1]. In recent communications we reported on the isolation of gentitoxin, 3-nitro-1-propyl-β-D-gentiobioside (**2**) [2], and 3-nitro-1-propyl-β-D-allolactoside (**3**) [3] from *A. miser* Dougl. var. *serotinus* Gray (Barneby) also known as timber milkvetch. These disaccharides were minor constituents of timber milkvetch occurring as <0.1% of the herbage dry matter in contrast to miserotoxin, the major nitropropyl glycoside which can account for >5% of the dry matter [4]. In this communication we report the isolation and structural determination of a fourth glycoside of 3-nitropropanol, 3-nitro-1-propyl-β-D-laminaribioside (**4**), which we have isolated from the same source. The susceptibility of these glycosides to enzymatic hydrolysis is also discussed.



1 R^1 = H, R^2 = H
2 R^1 = β-D-Glucose, R^2 = H
3 R^1 = β-D-Galactose, R^2 = H
4 R^1 = H, R^2 = β D-Glucose



5

†Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Chromatographic fractionation of an ethanol extract of *A. miser* var. *serotinus* resulted in the isolation of the new substance as an amorphous solid, homogeneous on TLC and HPLC analysis, which gave the typical colour reactions of *C*-nitro alkyl compounds [5]. As with the other nitropropyl disaccharides (**2**, **3**), **4** was also isolated as a minor component of *A. miser* and we estimate that it constitutes <0.1% of the herbage dry matter. In a behaviour similar to **1** and **2**, **4** was readily hydrolysed in the presence of almond emulsin (EC 3.2.1.21, Sigma No. G-4511), a β-glucopyranosidase, to yield glucose and 3-nitropropanol, which were identified by specific TLC systems [5, 6].

Based on its chromatographic behaviour we surmised that the new substance was a disaccharide, i.e. a glucosyl-miserotoxin and for further structural information we resorted to NMR spectroscopy. The ^{1}H NMR spectra of the glycoside at 200 or 400 MHz, including ^{1}H-COSY spectra, were not particularly helpful, although resonances for the 3-nitropropyl fragment could be observed, and the magnitudes of the vicinal spin–spin couplings seen in two low-field (anomeric) proton signals were consistent with the presence of two sugars, both being β-D-glucopyranosides (Table 1). However, 50.3 MHz ^{13}C NMR spectra of our compound were very informative, and enabled us to deduce its structure. Thus in broad-band spectra, 13 signals were observed, two of which corresponded in intensity to coincident resonances, i.e. 15 carbons were present in accord with our deduction that the substance was a monoglucopyranosylated miserotoxin. DEPT spectra established the numbers of attached protons per carbon, and comparison of the data with that reported for miserotoxin (**1**) enabled us to identify the resonances due to the aglycone and glycosidic units (Table 2). As both the enzymatic cleavage and the ^{1}H NMR evidence had indicated that the linkage between the sugars was β, and we had previously isolated as a different compound the 1,6-linked gentiobioside isomer (**2**) [2], we knew that the new glycoside had to have 1,2-, 1,3- or 1,4- linkages, i.e. be a sophoroside, laminaribioside, or cellobioside. Comparison of the ^{13}C NMR spec-

Table 1. ^{1}H NMR data for laminaritoxin (4)

H	δ
1	4.54 (8)
2	3.53 (8, 9)
3	*ca* 3.78
4	*ca* 3.58
5	*ca* 3.58
6A	3.96 (12, 2)
6B	*ca* 3.78
1′	4.81 (8)
2′	3.39 (8, 9.5)
3′	*ca* 3.58
4′	*ca* 3.58
5′	*ca* 3.58
6′A	3.96 (12, 2)
6′B	*ca* 3.78
1″A	4.07 (10.5, 6.5)
1″B	3.84 (10.5, 6.5)
2″	2.34 (6.5)
3″	4.74 (6.5)

At 400 MHz. Solvent D_2O. Shifts are in ppm from internal DSS, coupling constants are in parentheses, in Hz.

Table 2. Comparison of ^{13}C NMR spectra for miserotoxin (1), laminaritoxin (4) and methyl β-D-laminaribioside (5)

C	1*	4*	5†
1	105.0	104.8[a]	105.3[a]
2	75.7	76.2	76.0
3	78.7[a]	87.2	87.4
4	72.3	70.9	70.8
5	77.4[a]	78.8	78.4[b]
6	63.5	63.5	63.4
1′		105.6[a]	105.5[a]
2′		75.6	75.3
3′		78.3	78.1
4′		72.4	72.2
5′		78.3	78.1[b]
6′		63.5	63.4
1″	69.4	69.6	59.8
2″	29.6	29.6	
3″	75.3	75.3	

The solvent was D_2O. Shifts are relative to internal TSP-d_4.

*At 50.3 MHz.

†At 22.6 MHz.

[a,b]Assignments may be interchanged.

tra reported for β-D-sophorose, β-D-laminaribiose and β-D-cellobiose [7] with that of our glycoside suggested that it was the laminaribioside (in particular the downfield resonance of the glucopyranosylated carbon at δ86.7 was similar to that reported for β-D-laminaribiose, but neither of the other possibilities). Further comparison with the ^{13}C NMR spectrum of methyl β-D-laminaribiose (5) (kindly provided by Professor K. Takeo) revealed excellent agreement in the signals for the resonances due to the disaccharide components (Table 2). Thus we identified the new glycoside as 3-nitropropyl-β-D-laminaribioside (4) and have named it laminaritoxin.

The discovery of this compound extends the list of laminaribiosides isolated from plants, recent examples of which include some saponins [8, 9]. As it has been established that transglucosylation of glucosides can produce laminaribiosides, as for example from cycasin [10] we considered the possibility that 4 is a product of such a 'reverse emulsin' type reaction. However we have not detected β-glucosidase activity in *A. miser* var. *serotinus*. Tests for the enzyme proved to be negative when (i) frozen plant material was autolysed at room temperature and an increase in the level of the aglycone was not detected; (ii) buffered extracts of *A. miser* were incubated with amygdalin and the formation of hydrogen cyanide was not detected and (iii) buffered extracts were incubated with exogenous miserotoxin and there was no detectable increase in the amount of nitropropanol which is usually present in trace amounts. Thus we conclude that the new glycoside is a natural constituent and not an artefact of the extraction procedure.

As the aglycone, 3-nitropropanol, has been detected in hydrolysates from *ca* 50 additional species of *Astragalus* [11] it is possible that these species may also contain the nitropropyl disaccharides, possibly at higher concentrations. In contrast to 1, 2 and 4, compound 3 resisted hydrolysis by β-glucosidase but it was readily hydrolysed in the presence of β-galactosidase (EC 3.2.1.23, Sigma No. G-6512) [3]. Using the synthetic substrates *o*-nitrophenyl β-D-galactopyranoside (Sigma No. N-1127) and *p*-nitrophenyl-β-D-glucopyranoside (Sigma No. 7006), we have shown that rumen microorganisms of cattle contain much higher levels of β-glucosidase, than of β-galactosidase (Majak, W., unpublished results). For example, when the animal diet consisted of orchard-grass (*Dactylis glomerata*) hay (a feed which is devoid of β-glucosidase activity), the substrate for β-glucosidase (2 mM) was hydrolysed at 16.6 μg/ml/min but the substrate for β-galactosidase was hydrolysed at a much slower rate, 0.54 μg/ml/min. Similarly, when the bovine rumen fluid was obtained from an animal fed fresh alfalfa (*Medicago sativa*) the substrate for β-glucosidase hydrolysed at 53.0 μg/ml/min but the β-galactosidase substrate was hydrolysed at 2.78 μg/ml/min. As the release of the aglycone is essential for toxicity to occur [12], these results indicate that 1, 2 and 4 would be much more toxic to cattle than 3.

EXPERIMENTAL

For general methods, except for those described below, see previous papers [2, 3]. The EtOH-extract [2] of fresh-frozen plant material was further fractionated on a coconut charcoal column and the fraction eluted with 50–60% EtOH (20 l) was subjected to centrifugally accelerated TLC (Chromatotron) as described previously [2]. Fractions (100 ml) were eluted with

EtOH in $CHCl_3$ (1:4–3:2) and those containing 35–45% EtOH yielded the new glycoside as shown by Avicel TLC ($R_f = 0.37$ as compared to $R_f = 0.46$ for miserotoxin, *n*-BuOH–EtOH–H_2O, 4:1:5 [5]). Final purification of the glycoside was achieved after two sequential developments on prep. HPLC utilizing 3% aq. MeOH as the isocratic mobile phase [2]. The retention times for miserotoxin and the new glycoside were 16 and 32 min respectively. Evaporation of the final HPLC fraction *in vacuo* yielded the glycoside as a colourless glass.

^1H and ^{13}C NMR: experimental conditions are given in Tables 1 and 2.

Acknowledgements—Thanks are due to Ruth McDiarmid for excellent technical assistance. We are also indebted to Professor Ken'ichi Takeo (Department of Agricultural Chemistry, Kyoto Prefectural University) for a copy of the ^{13}C NMR spectrum of methyl β-D-laminaribioside.

REFERENCES

1. Stermitz, F. R. and Yost, G. S. (1978) in *Effects of Poisonous Plants on Livestock* (Keeler, R. F., van Kampen, K. R. and James, L. F., eds) p. 371. Academic Press, New York.
2. Majak, W. and Benn, M. H. (1988) *Phytochemistry* **27**, 1089.
3. Majak, W., Benn, M. H. and Huang, Y. Y. (1988) *J. Nat. Prod.* **51**, 985.
4. Majak, W., Quinton, D. A., Douwes, H. E., Hall, J. W. and Muir, A. D. (1988) *J. Range Manage.* **41**, 26.
5. Majak, W. and Bose, R. J. (1974) *Phytochemistry* **13**, 1005.
6. Kuroki, G., Lizotte, P. A. and Poulton, J. E. (1984) *Z. Naturforsch.* **39c**, 232.
7. Usui, T., Yamaoka, N., Matsuda, K., Tuzimura, K., Sugiyama, H. and Seto, S. (1973) *J. Chem. Soc. Perkin Trans. I* 2425.
8. Jain, D. C. (1987) *Phytochemistry* **26**, 1789.
9. Ishii, H., Tori, K., Tozyo, T. and Yoshimura, Y. (1981). *J. Chem. Soc. Perkin Trans. I*. 1928.
10. Yagi, F., Tadera, K. and Kobayashi, A. (1985). *Agric. Biol. Chem.* **10**, 2985.
11. Williams, M. C. and Gómez-Sosa, E. (1986) *J. Range Manage.* **39**, 341.
12. Majak, W., Pass, M. A. and Madryga, F. J. (1983) *Toxicol. Letters* **19**, 171.