

1,6-DI-3-NITROPROPANOYL- β -D-GLUCOPYRANOSIDE FROM *ASTRAGALUS CIBARIUS**

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Key Word Index—*Astragalus cibarius*; Leguminosae; 1,6-di-3-nitropropanoyl- β -D-glucoside; aliphatic nitro compounds.

Abstract—Extraction of dried, above-ground plant parts of *Astragalus cibarius* Sheld. with acetone and purification by silica gel chromatography of the residue from the acetone extract yielded a colorless, crystalline compound, m.p. 122°. This compound has been shown to be 1,6-di-3-nitropropanoyl- β -D-glucopyranoside.

INTRODUCTION

WE PREVIOUSLY showed¹ that, from the extraction of *Astragalus cibarius* Sheld. (Leguminosae) with hot ethanol, could be isolated 3-nitropropanoic acid, along with a trace of an oil which was tentatively suggested to be a diester of 3-nitropropanoic acid with D-glucose. It was pointed out that extraction with acetone rather than ethanol yielded much more of the diester. This paper reports on improved methods of isolation and purification of the diester and proof of its structure.

TABLE 1. INTERPRETATION OF THE CHEMICAL IONIZATION MS OF I

<i>m/e</i>	Relative intensity	Fragment(s) lost from QM+
318	18	$\text{HNO}_2 + \text{H}_2\text{O}$
246	35	$\text{HOOCCH}_2\text{CH}_2\text{NO}_2 + \text{H}_2\text{O}$
264	45	$\text{HOOCCH}_2\text{CH}_2\text{NO}_2$
217	100	$\text{HOOCCH}_2\text{CH}_2\text{NO}_2 + \text{HNO}_2$
199	44	$\text{HOOCCH}_2\text{CH}_2\text{NO}_2 + \text{HNO}_2 + \text{H}_2\text{O}$
145	63	2 $\text{HOOCCH}_2\text{CH}_2\text{NO}_2$
127	42	2 $\text{HOOCCH}_2\text{CH}_2\text{NO}_2 + \text{H}_2\text{O}$

RESULTS

The methods of isolation and purification described in the Experimental yielded colorless needles of the diester (I), m.p. 122° $[\alpha]_D^{25} -14^\circ$ (*c* 0.14, H_2O). Combustion analysis was consistent with the formula $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_{12}$. The chemical ionization MS² employing either methane or isobutane as reactant gases did not show a molecular ion plus H (QM+) corresponding to the above formula. However, the fragmentation pattern was consistent with the diester structure. Chemical ionization MS typically show fragment ion peaks correspond-

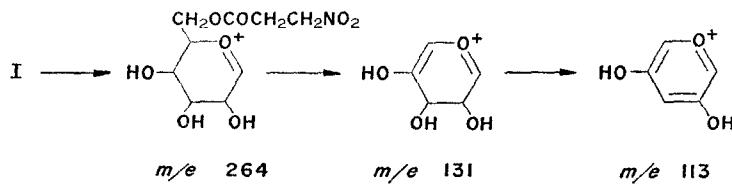
* This work was conducted in cooperation with the Colorado Agricultural Experiment Station (for which this report is Scientific Journal Series No. 1759).

¹ F. R. STERMITZ, W. T. LOWRY, F. A. NORRIS, F. A. BUCKERIDGE and M. C. WILLIAMS, *Phytochem.* **11**, 1117 (1972).

² This MS was obtained at Battelle's Columbus Laboratories Mass Spectrometry Center supported by the National Institutes of Health, Contract No. NIH-71-2483.

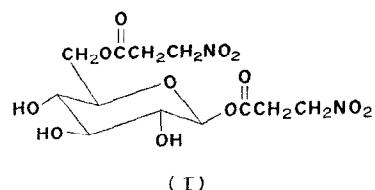
ing to the loss of neutral molecules from the $Q\bar{M}^+$. Table 1 shows the pertinent results of the chemical ionization MS.

A molecular ion was, of course, also not observed in the normal MS of I. However, the three most intense peaks above m/e 73 were at m/e 264, 131 and 113. The interpretation of Scheme 1 is consistent with typical substituted glucopyranose MS fragmentations.



The 60 MHz NMR spectrum in D_2O was not interpretable. However, if I was dissolved in D_2O to which 20% DCl in D_2O was added and the solution allowed to stand, the complex spectrum of I changed to a simple one showing two triplets at $3\cdot18\delta$ ($J = 6$ Hz) and $4\cdot97\delta$ ($J = 6$ Hz) and a multiplet at $3\cdot1\text{--}4\cdot2\delta$. This spectrum was superimposable on a combination of the spectrum of glucose (1 part) and 3-nitropropanoic acid (2 parts) combined in D_2O -DCl. Previously¹ we had isolated these individual components from the hydrolysis of I.

Final confirmation of the structure of I was obtained by the 220 MHz NMR. Two triplets at $3\cdot05$ and $3\cdot144\delta$ and two triplets at $4\cdot75$ and $4\cdot79\delta$ (the latter pair clearly visible on the expanded spectrum in spite of the nearly overlapping HDO peak) could be readily assigned to the methylenes of the two side chain 3-nitropropanoyl groups (the upfield triplets being next to the carbonyl and the downfield triplets being next to the nitro function). A one-proton doublet at $5\cdot54\delta$ was typical for an α -oriented anomeric proton on an acylated glucopyranose. That the second nitropropanoyl group was at the C-6 oxygen was clear from the position ($4\cdot36\delta$) of the two proton set of four doublets representing the 6,6' hydrogens. The C-5 proton appeared as two sets of four peaks each at $3\cdot72\delta$. Analysis of these peaks was completely first order, is given in the Experimental and corresponded closely to the analysis³ of the same protons on another C-6 acylated glucopyranose-type compound. The C-2, C-3 and C-4 protons fell together in a multiplet centering at $3\cdot45\delta$ and this chemical shift again indicated that none of the hydroxyl groups at these positions were acylated. Thus, I is clearly established as the structure for this diester.



DISCUSSION

Finnegan and Stephani^{4a} have isolated nine 3-nitropropanoate esters of glucose from *Indigofera endecaphylla* and have given these the general name 'endecaphyllins'. These

³ C. V. HOLLAND, D. HORTON, M. J. MILLER and N. S. BHACCA, *J. Org. Chem.* **32**, 3077 (1967).

^{4a} R. A. FINNEGAN and W. H. MUELLER, *J. Pharm. Sci.* **54**, 1136 (1965); R. A. FINNEGAN and R. A. STEPHANI, *J. Pharm. Sci.* **57**, 353 (1968).

include three diesters which have been assigned^{4b} the 4,6- α , 4,6- β and 2,6- α structures. Thus, the diester here described from *A. cibarius* is a new variation of these heretofore rarely observed compounds. We could find no evidence for any other nitro compounds in *A. cibarius* except for the occasional occurrence in trace amounts of 3-nitropropanoic acid. Even these traces may have been artifacts arising from hydrolysis of the diester. Both *Astragalus* and *Indigofera* are genera of the Leguminosae and one may perhaps expect the presence of similar compounds in other legumes. It seems likely from our work that 220 MHz NMR will provide the most rapid method for characterization of these esters.

EXPERIMENTAL

Data on the collection of *Astragalus cibarius* Sheld. have been given previously.¹ Isolation and characterization of the diester was as follows.

Air-dried, ground *A. cibarius* (900 g) was wetted with distilled acetone, until the acetone was 7.5 cm above the wet plant material, and left to stand for 24 hr with occasional stirring. The acetone was decanted and fresh acetone was again added to cover the plant material. After an additional 24 hr, the mixture was filtered and washed well with acetone. All the acetone solutions were then combined and evaporated at reduced pressure to yield 28 g of gummy brown residue. For a typical chromatographic separation, 14 g of residue was mixed well with 50 ml of 60–200 mesh silica gel (Baker No. 3405) and this gray-brown powder was added to the top of a 3 × 37 cm column of the same silica gel set up in CHCl₃. The column was then successively eluted with 500 ml each of the following mixtures of CHCl₃ and 95% EtOH: 100:0, 97:3, 19:1, 9:1, 17:3 and 1:1. The fractions eluted with 5–15% EtOH in CHCl₃ tested positive using the Griess-Ilosay test for nitro compounds. These fractions were combined and evaporated to yield 3 g of a greenish gummy residue of crude diester. The NMR of this crude material did not change significantly upon final purification. This was accomplished simply by rechromatographing the crude diester as above, but continuing to elute at the 10% EtOH–CHCl₃ concentration until fractions were negative in the nitro compound test. Fractions eluted with 10% EtOH were evaporated to 5 ml *in vacuo* and then allowed to evaporate in air. Needles formed as the solvent evaporated and residual solvent was decanted and the crystals washed with CHCl₃ to yield needles, m.p. 121–122°.

Anal. Calcd. for C₁₂H₁₈N₂O₁₂: C, 37.70; H, 4.75; N, 7.33. Found: C, 37.59; H, 4.83; N, 7.21. MS and optical rotation (Perkin-Elmer Model 141 polarimeter) are described above. The IR spectrum (KBr) showed absorptions at 3500 (hydroxyl) 1735 (ester) and 1550 cm⁻¹ (nitro group).

The 220 MHz NMR (Morgan-Schaffer Corp., D₂O) was analyzed as follows (chemical shift values are in ppm from TMS): 3.05 δ (*t*, 2H, *J* = 5.8, -OOCCH₂CH₂NO₂); 3.14 δ (*t*, 2H, *J* = 5.8, -OOCCH₂CH₂NO₂); 3.35–3.60 δ (*m*, 3H, protons at C-2, C-3, C-4); 3.72 δ (*m* of eight equal peaks, 1H, proton at C-5 coupled to C-4, C-6 and C-6', *J*_{4,5} = 10, *J*_{5,6} = 5.5, *J*_{5,6'} = 2.4); 4.36 δ (*m* of eight peaks, 2H, protons at C-6 and C-6', *J*_{6,6'} = 12.5, *J*_{5,6} = 5.5, *J*_{5,6'} = 2.4); 4.72 δ (*s*, HDO); 4.75 δ (*t*, 2H, *J* = 5.8, -OOCCH₂CH₂NO₂); 4.79 δ (*t*, 2H, *J* = 5.8, OOCCH₂CH₂NO₂); 5.54 δ (*d*, 1H, *J* = 8.0, anomeric proton C-1).

^{4b} R. A. FINNEGAN, private communication.