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HIPTAGIN AND OTHER ALIPHATIC NITRO ESTERS IN *LOTUS PEDUNCULATUS*

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Key Word Index—*Lotus pedunculatus*; Leguminosae; aliphatic nitro compounds; *Costelytra zealandica*; feeding deterrent; hiptagin; 3-nitropropanoyl glucopyranose.

Abstract—1,2,4,6-Tetra-(3-nitropropanoyl)-β-D-glucopyranose (hiptagin) was isolated from foliage and roots of *Lotus pedunculatus* together with 1,4,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (karakin), 2,6-di-(3-nitropropanoyl)-β-D-glucopyranose (coronarian) and 1,6-di-(3-nitropropanoyl)-β-D-glucopyranose (cibarian). Structural assignments for hiptagin were made on the basis of 400 MHz ^{1}H NMR; the compound significantly reduced feeding by third instar larvae of *Costelytra zealandica*.

INTRODUCTION

Recent studies of the chemical basis of resistance in the pasture legume *Lotus pedunculatus* Cav. to the grass grub, *Costelytra zealandica* (White) (Coleoptera: Melolonthinae) led to the isolation of a group of aliphatic nitro compounds from roots of the plant. Three were identified as the triester karakin and the diesters coronarian and cibarian [1, 2]. These compounds, together with several other related esters of 3-nitropropionic acid, have previously been found in other legume species [3, 4]. Williams [5] reported the presence of unidentified aliphatic nitro compounds in the foliage of several species of *Lotus* including *L. pedunculatus*.

Karakin, coronarian, cibarian and 3-nitropropionic acid all deter feeding by third instar *C. zealandica* larvae, and are toxic to the grubs [1, 2]. Toxicity of coronillin and cibarian to *Trichoplusia ni* has also been reported [6] and the toxicity of these and other nitro compounds to vertebrates is well known [3–5, 7].

We report here the presence of the tetraester, hiptagin [1,2,4,6-tetra-(3-nitropropanoyl)-β-D-glucopyranose] in extracts of *L. pedunculatus* root and foliage and the identity of the nitro esters present in the foliage.

RESULTS AND DISCUSSION

Extracts of the foliage and root of *L. pedunculatus* were separately chromatographed on silica gel columns. When the columns of each extract were eluted with increasing concentrations of ethyl acetate in chloroform, four fractions each yielding a white crystalline compound were obtained. On TLC all gave the characteristic red colour of primary aliphatic nitro compounds when sprayed with the diazotized sulphanilic acid reagent [8]. They migrated with R_f values which corresponded to those of karakin, coronarian and cibarian. The identity of each was confirmed by comparison of the melting points and IR spectrum with those of the corresponding authentic compound. While these three nitro esters of glucose were known to be present in the roots of *L. pedunculatus* [2], this is the first report of their isolation and identification in the foliage of the plant.

The fourth crystalline compound which eluted from the silica gel column prior to karakin had a melting point of 109–110°. The 400 MHz ^{1}H NMR indicated a glucose moiety with the 1,2,4 and 6 positions esterified, and the presence of a characteristic low field doublet at δ5.8 ($J_{1,2}$ = 8.4 Hz) [9] indicated that it was a β-anomeric hydroxyl group that was esterified. Two sets of multiplets were also observed. One set centred at δ3.1 (3.08, 3.09, 3.11, 3.14) was assigned to a $-CH_2-CO-$ group and the other set, centred at δ4.83 (4.82, 4.83(2), 4.85), to a $-CH_2-NO_2$ group. The ^{1}H NMR data suggested that the compound was the 1,2,4,6-tetraester of β-glucopyranose and the 400 MHz ^{1}H NMR spectra of authentic 1,2,4,6-tetra-(3-nitropropanoyl)-β-D-glucopyranose (hiptagin) was identical to that of our unknown compound. The IR spectra of the two were superimposable confirming the identity. Hiptagin has previously been isolated from *Hiptage madablota* [10], *Indigofera endocaphylla* [11], *Astragalus* [12] and *Heteropteris* [13].

Although karakin was the major nitro ester present in the foliage of *L. pedunculatus*, hiptagin levels varied between 3% and 48% of all the identified nitro esters in 3- to 12-month old plants. The total concentration of the combined nitro esters was estimated as 1–2% dry weight. Further studies on the variability of the levels of nitro esters during growth are presently being undertaken.

Ingestion by third instar *C. zealandica* larvae was significantly reduced when the insects were offered test discs containing 0.1 and 0.2% hiptagin, levels which are similar to those at which the compound was found in the plant. This feeding deterrent activity is closely similar to that of the triester karakin, the diester cibarian and 3-nitropropionic acid [1].

Lotus pedunculatus has been developed as a legume suitable for pastoral agriculture on poorer soils in New Zealand and elsewhere. The plant is rejected as a food plant by foliar-feeding insect pests such as black field cricket (*Teleogryllus commodus*) [14] and grass caterpillar (*Wiseana cervinata*) [15]; nitro compounds in the plant

tissue may well be responsible for this phenomenon. The implications of the presence of these toxins in the foliage for the pastoral use of the plant are discussed elsewhere [2].

EXPERIMENTAL

Insect feeding deterrent assays. These were performed as described previously [16, 17]. Field collected 3rd instar larvae of *Costelytra zealandica* which had been starved for 24 hr were enclosed individually in petri dishes each with a 1.5 cm diam. disc cut from an artificial 4% agar–4% cellulose powder medium containing a standard feeding stimulant (0.1 M sucrose + 0.01 M ascorbic acid) plus the test material. Hiptagin was dissolved in Me_2CO and appropriate vols of the soln were added to the cellulose powder. The solvent was evaporated prior to the preparation of the agar-based medium. Media containing hiptagin at 0.01, 0.05, 0.1 and 0.2% were tested. Feeding deterrent activity was assessed by comparing counts of faecal pellets after 24 hr, from larvae offered discs containing the test material (T) with those from larvae offered standard discs containing feeding stimulants only (C) and from a third group offered blank discs prepared with H_2O. Twenty larvae were tested with each medium. Wilcoxon's Rank Sum test was used to determine whether T was significantly less than C ($P < 0.05$).

Plant material. Lotus pedunculatus plants ranging from 3 to 12 months were harvested and separated into foliage and roots. The roots were washed and towel-dried. The plant material was frozen until extracted.

Large scale isolation. The frozen plant material was directly extracted with Me_2CO in a Waring blender. The filtered extract was concd under red. pres. at a temp. not exceeding 30°. The concentrate was then partitioned and chromatographed on silica gel columns according to Moyer *et al.* [9]. Nitro compounds in each of the fractions were identified by comparison of their R_f values with those of authentic compounds. Pre-coated silica gel F-254 TLC plates (Merck) were used and were developed in (a) EtOH–$CHCl_3$ (1:4) or (b) hexane–EtOAc–HCO_2H (60:40:1) and sprayed with diazotized sulphanilic acid–NaOH [8]. The four crystalline compounds were recrystallized using 10% EtOAc in $CHCl_3$.

Analysis. 1,2,4,6-Tetra-(3-nitropropanoyl)-β-D-glucopyranose: mp 109–110°; IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1735 (ester group) and 1560 (NO_2); ^1H NMR (400 MHz, Me_2CO-d_6): δ5.83 (*d*, H-1, $J_{1',2}$ = 8.4 Hz), 5.04 (*dd*, H-2, $J_{2,3}$ = 9.5 Hz), 4.08 (*dd*, H-3, $J_{3,4}$ = 9.5 Hz), 5.07 (*dd*, H-4, $J_{4,5}$ = 10.0), 4.06 (*ddd*, H-5, $J_{5,6A}$ = 5.2 Hz, $J_{5,6B}$ = 2.2 Hz), 4.31 (*dd*, H-6A, $J_{6A,6B}$ = 2.2 Hz), 4.2 (*dd*, H-6B).

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REFERENCES

1. Sutherland, O. R. W., Hutchins, R. F. N., Russell, G. B., Lane, G. A. and Biggs, D. R. (1981) in *Proc. 3rd Australasian Conf. Grassl. Invert. Ecol.* (Lee, K. E., ed.) p. 245. S. A. Govt. Printer, Adelaide, Australia.
2. Hutchins, R. F. N., Sutherland, O. R. W., Gnanasunderam, C., Greenfield, W. J., Williams, E. M. and Wright, H. J. (1984) *J. Chem. Ecol.* **10**, 81.
3. Gustine, D. L. (1979) *Crop Sci.* **19**, 197.
4. 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.
5. Williams, M. C. (1983) *Agron. J.* **75**, 520.
6. Byers, R. A., Gustine, D. L. and Moyer, B. G. (1977) *Environ. Entomol.* **6**, 229.
7. Williams, M. C. and James, L. F. (1978) in *Effects of Poisonous Plants on Livestock* (Keeler, R. F., Van Kampen, K. R. and James, L. F., eds) p. 391. Academic Press, New York.
8. Majak, W. and Bose, R. J. (1974) *Phytochemistry* **13**, 1005.
9. Moyer, B. G., Pfeffer, P. E., Valentine, K. M. and Gustine, D. L. (1979) *Phytochemistry* **18**, 113.
10. Finnegan, R. A. and Stephani, R. A. (1968) *J. Pharm. Sci.* **57**, 353.
11. Finnegan, R. A. and Mueller, W. H. (1965) *J. Pharm. Sci.* **54**, 1136.
12. Harlow, M. C., Stermitz, F. R. and Thomas, R. D. (1975) *Phytochemistry* **14**, 1421.
13. Stermitz, F., Hnatyszyn, O., Bandoni, A. L., Rondina, R. V. D. and Coussio, J. D. (1975) *Phytochemistry* **14**, 1341.
14. Blank, R. H., Olson, M. H., Cox, N. R. and Bell, D. S. (1980) *N.Z. J. Agric. Res.* **23**, 409.
15. Farrell, J. A. K., Sweney, W. J. and Jones, A. E. (1974) *N.Z. J. Agric. Res.* **17**, 373.
16. Sutherland, O. R. W. and Hillier, J. R. (1974) *N.Z. J. Zool.* **1**, 211.
17. Sutherland, O. R. W., Russell, G. B., Biggs, D. R. and Lane, G. A. (1980) *Biochem. Syst. Ecol.* **8**, 73.