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COROLLIN, CORONILLIN AND CORONARIAN: THREE NEW 3-NITROPROPANOYL-D-GLUCOPYRANOSES FROM *CORONILLA VARIA*

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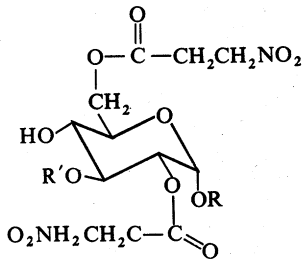
Key Word Index—*Coronilla varia*; Leguminosae; crownvetch; aliphatic nitro compounds; 3-nitropropanoyl-D-glucopyranoses.

Abstract—Three new 3-nitropropanoyl-D-glucopyranoses, 2,3,6-tri(3-nitropropanoyl)-α-D-glucopyranose (corollin), 1,2,6-tri(3-nitropropanoyl)-α-D-glucopyranose (coronillin) and 2,6-di(3-nitropropanoyl)-α-D-glucopyranose (coronarian) were isolated from the aerial parts of *Coronilla varia*. Structural assignments were made on the basis of 220 MHz NMR spectra.

INTRODUCTION

The toxicity of crownvetch, *Coronilla varia*, to non-ruminants such as chicks, pigs and meadow voles is due to the presence of 3-nitropropionic acid (NPA) [1, 2]. In addition to NPA, we previously reported the presence of a series of compounds thought to be glucose esters of NPA in crownvetch [1]. Three of these have been identified by Majak and Bose [3] as 1,2,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (karakin), 1,6-di-(3-nitopropanoyl)-β-D-glucopyranose (cibarian) and 6-mono-(3-nitropropanoyl)-D-glucopyranose. This study describes the characterization of three additional glucose esters of NPA, corollin (**1**), coronillin (**3**) and coronarian (**4**).



(1) R=H, R′=—C(=O)—$CH_2CH_2NO_2$
(3) R=—C(=O)—$CH_2CH_2NO_2$, R′=H
(4) R=R′=H

RESULTS AND DISCUSSION

The procedure described by Stermitz [4] for the isolation of cibarian from *Astragalus cibarius* was used for the extraction and purification of five glucose esters of NPA from crownvetch. Eluates from Si gel column chromatography were characterized by TLC. Isolated compounds were purified by crystallization and PLC. Compounds eluted from the Si gel column with 5% EtOH in $CHCl_3$ were: 2,3,6-tri-(3-nitropropanoyl)-α-D-glucopyranose (**1**, corollin), 1,2,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (**2**, karakin) and 1,2,6-tri-(3-nitropropanoyl)-α-D-glucopyranose (**3**, coronillin). Compounds eluted with 10% EtOH in $CHCl_3$ were: 2,6-di-(3-nitropropanoyl)-α-D-glucopyranose (**4**, coronarian) and 1,6-di-(3-nitropropanoyl)-β-D-glucopyranose (**5**, cibarian). Compounds **1** and **3** are reported for the first time: compound **4** was reported in *Indigofera* [4], but without supporting data for the structure. The NMR spectra of compounds **2** and **5** were identical to those reported for karakin [5] and cibarian [4], respectively. The structures of compounds **1**, **3** and **4** were assigned primarily on the basis of 220 MHz NMR spectra, which are summarized in Table 1.

Table 1. NMR data* for glucose protons of corollin (**1**), coronillin (**3**) and coronarian (**4**)

Esterification pattern	H-1	H-2	H-3	H-4	H-5	H-6
2,3,6-α (**1**)	5.34 $J_{1,2}=3$	*Ca* 4.82†	5.52 $J_{2,3}=J_{3,4}=10$	3.70 $J=10$	4.15	4.39 $J_{AB}=12, J_{AX}=5, J_{BX}=1.5$
1,2,6-α (**3**)	6.25 $J_{1,2}=4$	*Ca* 4.85†	3.91	3.55	3.91	4.35 $J_{AB}=12, J_{AX}=5, J_{BX}=2$
2,6-α (**4**)	5.30 $J_{1,2}=3.7$	4.65 $J_{1,2}=3.4, J_{2,3}=9.5$	3.95	3.45 $J_{3,4}=9.5, J_{4,5}=10$	3.95	4.35 $J_{AB}=12, J_{AX}=5.5, J_{BX}=2$

* Expressed as ppm (δ) from TMS in Me_2CO-D_6. † Found under the signals for $OOCCH_2C\underline{H}_2NO_2$.

Corollin (**1**) exhibited an anomeric proton signal at $\delta 5.3$, which became a doublet ($J_{1,2} = 3$ Hz) upon addition of D_2O, indicating that the C-1 position is not esterified; the small coupling constant indicates that the proton is equatorial. Substitution at C-6 was verified by the low field position ($\delta 4.4$) of the C-6 methylene proton signals. Areas of 6 + 1 and 6 protons corresponding to $C\underline{H}_2$–NO_2 ($\delta 4.82$) and $C\underline{H}_2$–COO ($\delta 3.16$), respectively, per anomeric proton, established the compound to be a triester. The C-2 proton signal must be superimposed on the low field $C\underline{H}_2$–NO_2 signals since integration indicated a total of 7 protons. Significantly, there were no observable signals with a coupling constant of 3 Hz other than those from the anomeric proton. The low field position indicated that the C-2 hydroxyl group is esterified.

Since our NMR data indicate esterification of the C-2 and C-6 hydroxyl groups, the low field triplet at $\delta 5.52$, which is farther down field than the anomeric proton signal, must arise from either the C-3 or C-4 proton. That the C-3 hydroxyl group is esterified may be deduced as follows: Birkofer *et al.* [6] found, that in relation to the C-1 proton signal, C-3 proton signals of *p*-coumaroyl and hydroxybenzoyl C-3 glucose esters are downfield, C-4 proton signals of C-4 esters are upfield or are the same and the C-2 proton signal of C-2 esters are shifted upfield. Both the C-4 and C-2 protons of corollin are deshielded. The C-4 proton signal is found at $\delta 3.7$, as compared to $\delta 3.55$ and $\delta 3.45$ for **3** and **4**, respectively. Such a shift downfield by about 0.2 ppm is due to deshielding by an adjacent ester carbonyl, as shown by Lemieux and Stevens [7]. The C-2 proton of **1** is also deshielded since the C-2 proton signals of **1** and **3** are at $\delta 4.82$ and $\delta 4.65$, respectively. Only the 2, 3, 6-substitution pattern can account for both the C-2 and C-4 proton signals being shifted downfield. This assignment is consistent with the NMR spectra of glucose having the C-2 and C-3 hydroxyl groups esterified [8]. Addition of D_2O sharpened the C-4 broad multiplet signal at $\delta 3.7$ to a triplet ($J = 10$ Hz), also indicating that the C-4 hydroxyl group is not esterified.

The low field anomeric proton signal of coronillin (**3**) at $\delta 6.25$ denoted esterification, while the small coupling constant ($J = 4$ Hz) established **3** to be an α-anomer. Substitution at C-6 was verified by the low field position ($\delta 4.35$) of the C-6 methylene proton signals. Areas of 6 + 1 and 6 protons corresponding to $C\underline{H}_2$-NO_2 ($\delta 4.85$) and $C\underline{H}_2$–COO (δ 3.05, 3.18), respectively, established that **3** is a triester. The C-2 proton signal was found superimposed on the low field $C\underline{H}_2$–NO_2 signals since integration indicated a total of 7 protons. As with **1** there were no observable signals with a coupling constant of $J = 4$ Hz other than those from the anomeric proton. The low field position indicated esterification of the C-2 hydroxyl group. Further evidence for esterification was the lack of migration of the NPA residue from C-1 to C-2 upon heating; such migrations were observed by Pfeffer [9] with 1-α-fatty acid esters of glucose and have been documented as a facile rearrangement [10].

Upon addition of D_2O, the anomeric proton signal of coronarian (**4**) appeared at $\delta 5.3$ as a doublet ($J_{1,2} = 3.4$ Hz). The anomeric proton therefore must be equatorial with the C-1 hydroxyl group unesterified. Substitution at C-6 is indicated by the low field position ($\delta 4.35$) of the methylene proton signals. Two areas of 4 protons corresponding to $C\underline{H}_2$–NO_2 ($\delta 4.85$) and $C\underline{H}_2$–COO ($\delta 3.07$, 3.17) per anomeric proton established this compound to be a diester. The low field position of the C-2 proton signal at $\delta 4.65$ ($J_{1,2} = 3.4$ Hz) indicated that the C-2 hydroxyl group is esterified. The position of the C-2 proton signal is upfield compared to **1** because of the absence of deshielding by adjacent ester carbonyl groups [7].

Additional experimental results confirmed the structural assignments of **1**, **3** and **4**. Acid hydrolysis yielded NPA (identified by TLC) and glucose (identified by GLC of TMSi esters). Elemental analyses were consistent with the calculated molecular weights. Colorimetric determinations of NO_2 [11] indicated 2.93 mol NO_2/mole of compound **1**, 3.02 mol NO_2/mole of compound **3**, and 2.95 mol NO_2/mole of compound **4**, values which were consistent with assigned structures.

Glucose esters of NPA have now been found in six dicot genera distributed among three families. The occurrence of these compounds is shown in Table 2. For each of the three nonleguminous genera, a single compound predominates: karakin in *Corynocarpus* (Anacardiaceae) and hiptagin in *Heteropteris* and *Hiptage* (Malphigiaceae). *Coronilla* and *Indigofera* have a wide range of glucose esters of NPA. Only cibarian, karakin and the tetraester, hiptagin, have been reported in *Astragalus* spp., although the complete range of esters in many of the species has not been fully investigated. A wider range of glucose esters of NPA may yet be found in most *Astragalus* spp. containing these compounds.

All known glucose esters of NPA, including the monoester, are substituted at C-6 (Table 2), which suggests that

Table 2. Distribution of 3-nitropropanoyl-D-glucopyranoses

Compound	Common name	Structural data	Genera
6-α, β	—	MS [3], NMR [3]	*Coronilla* [3]
1,6-β (**5**)	Cibarian	MS [4], NMR [4]	*Astragalus* [4], *Coronilla* [3]
2,6-α (**4**)	Coronarian	NMR*	*Coronilla*,* *Indigofera* [4]
4,6-α	—	Synthesis [12]	*Indigofera* [4]
4,6-β	—	—	*Indigofera* [4]
1,2,6-β (**2**)	Karakin	MS [5], NMR [5], synthesis [12]	*Astragalus* [5], *Corynocarpus* [12], *Coronilla* [3], *Indigofera* [12]
1,2,6-α (**3**)	Coronillin	NMR*	*Coronilla**
2,3,6-α (**1**)	Corollin	NMR*	*Coronilla**
1,2,4,6-β	Hiptagin	NMR [13], synthesis [13]	*Astragalus* [5], *Heteropteris* [14], *Hiptage* [13], *Indigofera* [13]

* Data presented in this paper.

the first step in the biosynthesis of these compounds is esterification of the hydroxyl group at that position. Of the compounds that are esterified at C-1, β-anomers are both more widely distributed and make up a greater proportion of total esters in a species than α-anomers.

We found the esters present in roots, stems, leaves and flowers of *C. varia* (TLC analysis). The concentration of NPA in flowers, both free and in the form of glucose esters, was as high as 5% of the dry wt, as determined colorimetrically [11]. The concentration in leaves was about 3%, while stems and roots had 1% or less. Two other species, *C. scorpoides* and *C. globosa*, have no NPA [15]. We also detected trace amounts (TLC analysis) of several other NPA esters in crownvetch.

EXPERIMENTAL

Isolation. Corinilla varia L. cv Penngift was collected at mid-bloom July 9, 1975, dried at 60° overnight and ground in a Wiley mill to pass through a 1 mm screen. Ground plant material (250 g) was extracted with a total of 15 l. Me_2CO over a 3-day period. Tar obtained (38.6 g) after evaporation of Me_2CO was mixed with 30 g Si gel and chromatographed on a 3.2 × 33 cm Si gel column (130 g). The column was eluted with 2.5 l 2% EtOH in $CHCl_3$, 1.5 l. 5% EtOH in $CHCl_3$ and 2.5 l. 10% EtOH in $CHCl_3$. Fractions were characterized using Si gel TLC: EtOAc–MeCOEt–HCO_2H–H_2O (50:48:1:1), solvent system A: R_f values, 0.86 (**1**), 0.77 (**2**, **3**), 0.71 (**4**), 0.58 (**5**); $CHCl_3$–EtOAc–HCO_2H (10:89:1), solvent system B: R_f values, 0.39 (**1**), 0.25 (**2**), 0.21 (**3**), 0.16 (**4**), 0.14 (**5**). Compounds containing NPA were detected with diazotized sulfanilic acid [1]. Compounds **1**, **2** and **3** were eluted with 5% EtOH in $CHCl_3$; compounds **4** and **5** were eluted with 10% EtOH in $CHCl_3$. Yields were 3.3 g of **5**, 0.34 g of **4**, 1.65 g of a mixture of **5** and **4**, and 1.5 g of a mixture of **1**, **2** and **3**. Compounds **2** (karakin), **4** (coronarian) and **5** (cibarian) were crystallized in nearly pure form from their respective mixtures from Me_2CO–CCl_4. Compounds **1** (corollin) and **3** (coronillin) were isolated in mg amounts by PLC (solvent system B on 1-mm Si gel plates, followed by elution with EtOAc and crystallization).

Analysis. Corollin (**1**); mp 158.5–160° (Me_2CO–CCl_4). Found: C, 36.84; H, 4.32; N, 8.68. $C_{15}H_{21}N_3O_{15}$ requires: C, 37.27; H, 4.34; N, 8.70%. Karakin (**2**); mp 122–123° (Me_2CO–CCl_4); NMR data identical to those reported [5]. Found: C, 37.25; H, 4.42; N, 8.68. $C_{15}H_{21}N_3O_{15}$ requires: C, 37.27; H, 4.34; N, 8.70%. Coronillin (**3**); mp 112.5–114° (Me_2CO–CCl_4). Found: C, 37.84; H, 4.42; N, 8.60. $C_{15}H_{21}N_3O_{15}$ requires: C, 37.27; H, 4.34; N, 8.70%. Coronarian (**4**); mp 151–153° (Me_2CO–CCl_4). Found: C, 37.74; H, 4.76; N, 7.53. $C_{12}H_{18}N_2O_{12}$ requires: C, 37.70; H, 4.71; N, 7.34%. Cibarian (**5**); mp 123.5–124° (Me_2CO–CCl_4); NMR data in D_2O identical to those reported [4]. Found: C, 37.69; H, 4.62; N, 7.46; $C_{12}H_{18}N_2O_{12}$ requires: 37.70; H, 4.71; N, 7.34%. All NMR spectra data are reported as δ values (ppm from TMS in Me_2CO-D_6) and were recorded at the Middle Atlantic Regional NMR facility (supported by NIH grant RR 542, The University of Pennsylvania).

REFERENCES

1. Gustine, D. L., Shenk, J. S., Moyer, B. G. and Barnes, R. F. (1974) *Agron. J.* **66**, 636.
2. Shenk, J. S., Wangsness, P. J., Leach, R. M., Gustine, D. L., Gobble, J. L. and Barnes, R. F. (1976) *J. Anim. Sci.* **42**, 616.
3. Majak, W. and Bose, R. J. (1976) *Phytochemistry* **15**, 415.
4. Stermitz, F. R., Lowry, W. T., Ubben, E. and Sharifi, I. (1972) *Phytochemistry* **11**, 3527.
5. Harlow, M. C., Stermitz, F. R. and Thomas, R. D. (1975) *Phytochemistry* **14**, 1421.
6. Birkofer, L., Kaiser, C., Hilges, B. and Becker, F. (1969) *Lieb. Ann. Chem.* **725**, 196.
7. Lemieux, R. U. and Stevens, J. D. (1965) *Can. J. Chem.* **43**, 2059.
8. Agahigian, H., Vickers, G. D., von Saltza, M. H., Reid, J., Cohen, A. I. and Gauthier, H. (1965) *J. Org. Chem.* **30**, 1085.
9. Pfeffer, P. E., Moore, G. G. and Hoagland, P. A. Personal communication.
10. Pederson, C., Fletcher, H. G., Jr. (1960). *J. Am. Chem. Soc.* **81**, 3215.
11. Matsumoto, H., Unrau, A. M., Hylin, J. W. and Temple, B. (1961) *Anal. Chem.* **33**, 1442.
12. Finnegan, R. A. and Stephani, R. A. (1968) *Lloydia* **33**, 441.
13. Finnegan, R. A. and Stephani, R. A. (1968) *J. Pharm. Sci.* **57**, 353.
14. Stermitz, F., Hnatyszyn, O., Bandoni, A. L., Rondina, R. V. D. and Coussio, J. D. (1975) *Phytochemistry* **14**, 1341.
15. Gustine, D. L., Moyer, B. G. and Risius, M. L. Personal communication.