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3-NITROPROPANOYL-D-GLUCOPYRANOSES OF *CORYNOCARPUS LAEVIGATUS*

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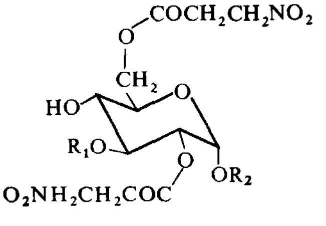
Key Word Index—*Corynocarpus laevigatus*; Corynocarpaceae; aliphatic nitro compounds; 3-nitropropanoyl-D-glucopyranoses; structural dtermination.

Abstract—A new nitropropanoyl glucopyranose, 1,4,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (corynocarpin), and three known compounds, 1,6-di-(3-nitropropanoyl)-β-D-glucopyranose (cibarian), 2,6-di-(3-nitropropanoyl)-α-D-glucopyranose (coronarian) and 2,3,6-tri-(3-nitropropanoyl)-α-D-glucopyranose (corollin), were isolated from seeds and plants of *Corynocarpus laevigatus*. Structural assignments were made on the basis of 220 MHz PMR spectra.

INTRODUCTION

Karakin (**2**) was first isolated from seeds of *Corynocarpus laevigatus* [1], a tree native to New Zealand. Subsequently, karakin and other 3-nitropropanoyl glucopyranose (NPG) compounds were reported in *Astragalus* spp. [2, 3], *Coronilla varia* [4, 5] and *Indigofera spicata* [6]. Although several nitro-containing compounds other than karakin were detected in *C. laevigatus* seeds [7], these were not identified. This paper describes the isolation of 4 additional NPG compounds from *C. laevigatus* plants and seeds: corollin (**1**), corynocarpin (**3**), coronarian (**4**) and cibarian (**5**). Of these, **1**, **4** and **5** were previously isolated from other plant sources. This is the first unambiguous report of **3** as a naturally occurring compound.



(**1**) $R_1 = -COCH_2CH_2NO_2$, $R_2 = H$
(**4**) $R_1 = R_2 = H$

RESULTS AND DISCUSSION

C. laevigatus seeds, leaves, stems and roots were extracted with acetone. At least 11 compounds containing NO_2 and reacting with diazotized sulfanilic acid were present in all extracts; because the composition of the 3 extracts from plant parts differed little (TLC), they were combined. Five compounds were isolated from both seed and plant extracts, either by direct crystallization or PLC of Si gel column eluates. These, in order of elution from columns, were: 2,3,6-tri-(3-nitropropanoyl)-α-D-glucopyranose (**1**, corollin), 1,2,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (**2**, karakin), 1,4,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (**3**, cornynocarpin), 2,6-di-(3-nitropropanoyl)-α-D-glucopyranose (**4**, coronarian) and 1,6-di-(3-nitropropanoyl)-β-D-glucopyranose (**5**, cibarian).

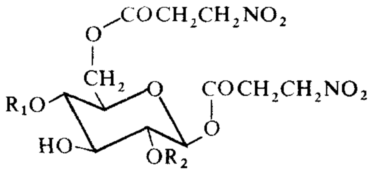
The PMR spectra of compounds **1**, **2**, **4** and **5** were consistent with published data for corollin [5], karakin [3], coronarian [5] and cibarian [2], respectively. The structure proposed for **3** (corynocarpin) was based on PMR spectral data which are summarized in Table 1.

Esterification of the β-anomeric hydroxyl group of **3** was indicated by the characteristic low field doublet at δ 5.6 ($J_{1,2}$ = 8 Hz, area = 1 proton). Triplets (area 2 protons) at δ 3.48 ($J_{2,3\,\mathrm{avg.}}$ = 8.5 Hz) and δ 3.76 ($J_{3,4\,\mathrm{avg.}}$ = 9.1 Hz) were ascribed to the protons at the unsubstituted positions C-2 and C-3, respectively. A triplet at δ 4.9 ($J_{3,4\,\mathrm{avg.}}$ = 9.5 Hz, area = 1 proton) was ascribed

Table 1. PMR data for 1,4,6-tri-(3-nitropropanoyl)-β-D-glucopyranose (3)

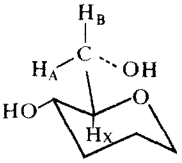
H_1	H_2	H_3	H_4	H_5	$H_{6,6'}$
Chemical shifts (ppm) of glucose protons					
5.6	3.48	3.76	4.9	3.9	4.17
J (Hz)					
$J_{1,2} = 8$	$J_{1,2} = 8$ $J_{2,3} = 9.2$*	$J_{2,3} = 9.2$* $J_{3,4} = 9$*	$J_{3,4} = 9$* $J_{4,5} = 10$	$J_{4,5} = 10$ $J_{5,6} = 5.4$ $J_{5,6'} = 1.9$	$J_{6,6'} = 12$ $J_{5,6} = 5.4$ $J_{5,6'} = 1.9$

* Unaveraged $J_{2,3}$ and $J_{3,4}$ obtained by decoupling experiments.



(2) $R_1 = H$, $R_2 = -COCH_2CH_2NO_2$
(3) $R_1 = -COCH_2CH_2NO_2$, $R_2 = H$
(5) $R_1 = R_2 = H$

to the C-4 proton, since esterification of the hydroxyl group was indicated by the low field position. An 8-line multiplet centered at δ 3.9 ($J_{4,5} = 10$ Hz, $J_{5,6} = 5.4$ Hz, $J_{5,6'} = 1.9$) was assigned to the C-5 proton. The C-6 proton signals were observed at δ 4.09 and 4.24 as two doublets of doublets ($J_{6,6'} = 12$ Hz, $J_{5,6} = 5.4$ Hz, and $J_{5,6'} = 1.9$ Hz, area = 2 protons). The $—C\underline{H}_2NO_2$ resonances were seen as overlapping triplets centered at δ 4.76 (area = 6 protons); overlapping triplets at δ 3.1 and δ 3.9 (areas = 4 and 2 protons, respectively) were assigned to $—C\underline{H}_2—CO—$.



(6)

Shift assignments for 3 were verified by proton homonuclear decoupling at 60 MHz. Irradiation of the triplet resonance of C-2 H centered at δ 3.48 collapsed the C-1 H resonance at δ 5.6 to a sharp singlet. Irradiation of the δ 4.9 triplet collapsed the C-3 H triplet at δ 3.76 to a doublet.

Lemieux and Brewer [8], in their 220 MHz PMR studies of hexopyranose conformation, demonstrated that rotamers having the C-6 methylene protons oriented as depicted (6) predominate over the other two possible rotational configurations. Since the H_A bond makes a 60° dihedral angle with respect to the H_X bond, $J_{A,X}$ is smaller than $J_{B,X}$. Relative to the H_B proton signal, the H_A proton signal was consistently observed downfield due to deshielding by the C-4 hydroxyl oxygen.

Table 2. Shielding effects on C-6 methylene protons due to esterification of C-4 hydroxyl group

Esterification pattern	Chemical shift (ppm)		Coupling constants (Hz)		
	H_A	H_B	J_{AX}	J_{BX}	J_{AB}
Cibarian, 1,6 β (5)*	4.42	4.29	2.5	6.0	12
Karakin, 1,2,6 β (2)*	4.39	4.24	2.0	6.0	12
Corynocarpin, 1,4,6 β (3)	4.00	4.24	1.9	5.4	12

* Data taken from Moyer *et al.* [5].

As shown in Table 2, H_A resonances of 2 and 5 are downfield from H_B resonances; however, the H_A resonance of 3 shows a decided upfield shift. This is presumably due to the predominant shielding effect of the carbonyl group of the ester function at C-4.

Karakin was first assigned structure 3 by Carter [9], but Finnegan and Stephani found that synthetic 3 had properties distinguishing it from karakin that they isolated from *C. laevigatus* and thus subsequently assigned structure 2 to karakin [10]. Harlow *et al.* [3] confirmed 2 (1,2,6-tri-3-nitropropanoyl)-D-β-glucopyranose) as the structure of karakin and showed that a compound they isolated from *Astragalus* spp. was identical to karakin isolated from *C. laevigatus* by Carter. Although 3 was synthesized by Finnegan and Stephani [10], it has not been rigorously characterized or unambiguously isolated from a natural source.

Of plants known to contain NPG compounds, a series of such compounds is produced by all but *Hiptage benghalensis*, from which only hiptagin has been isolated [11]. An examination of this species is likely to show the presence of other NPG compounds.

We found that the concentration of NPG compounds in one-year-old *C. laevigatus* plants is higher in leaves than in roots. The total amounts of NPG compounds found in plants exceed those in seeds. Since translocation of NPG compounds from seeds during germination does not account for amounts found in plants, *de novo* synthesis presumably occurs in the plant.

The 6 unidentified nitro-containing compounds in *C. laevigatus* presumably are also NPG compounds; elucidation of their structures awaits further studies.

EXPERIMENTAL

PMR spectra data are reported as δ values (ppm from internal TMS in Me_2CO-d_6) and were run at 220 MHz (60 MHz for decoupling expts).

Plant materials. Seeds of *Corynocarpus laevigatus* (J. R. & G. Forst.) were obtained from Park Seed Co., Inc., Greenwood, S.C. Plants were grown from seed in equal parts sand, peat moss and loam.

Extraction and isolation. Seeds (150 g) were ground and extracted with Me_2CO. Fresh leaves, roots and stems from 15 one-year-old plants (1.5 kg) were separately extracted. Extracts from plant parts were combined as explained in the text. The solvent was removed and the resulting aq. phase washed with equal vols of hexane (× 3) and EtOAc (× 3). EtOAc washes were concd and the resulting tar was combined with Si gel and chromatographed on Si gel (100 mesh) columns (3.2 × 33 cm). NO_2-containing compounds were eluted using 40–60% EtOAc and 1% HCO_2H in $CHCl_3$. Eluates were characterized by TLC [5]. The order of elution was Unknown$_1$ (U_1), **1**, U_2, **2**, **3**, U_3, **5**, U_4, U_5, and U_6. Compounds **2**, **3**, **4**, and **5** were crystallized from eluates; **1** was isolated from eluates containing **1** and **2** using PLC [5].

Identification. Acid hydrolysis of 3 yielded glucose (GLC of TMS esters) and 3-nitropropanoic acid, (TLC in 2 solvent systems [5]. Transesterification with MeOH, EtOH and PrOH produced compounds co-chromatographing on Si gel TLC (hexane–EtOAc–HCO_2H, 60:40:1) with authentic Me, Et and Pr 3-nitropropanoate, respectively.

Greiss-Ilosvay colorimetric determination of NO_2 [12] indicated 3 mol NO_2/mol of 3.

Analysis. Mps are uncorr. The PMR spectra of compounds **1**, **2**, **4** and **5** were consistent with published data for corollin [5], karakin [3], coronarian [5] and cibarian [2], respectively. Corollin (**1**); mp 158.5–160° (Me_2CO–CCl_4). (Found: C, 37.32; H, 4.18; N, 8.73. $C_{15}H_{21}N_3O_{15}$ requires: C, 37.27; H, 4.34; N, 8.70%). Karakin (**2**); mp 122–123° (Me_2CO–CCl_4). (Found: C, 37.13; H, 4.36; N, 8.79. $C_{15}H_{21}N_3O_{15}$ requires: C, 37.27; H, 4.34; N, 8.70%). Corynocarpin (**3**); mp 114.5–115° (EtOAc–CCl_4). (Found: C, 37.56; H, 4.50; N, 8.50. $C_{15}H_{21}N_3O_{15}$ requires: C, 37.27; H, 4.34; N, 8.70%). Coronarian (**4**); mp 147.5–148° (Me_2CO–CCl_4). (Found: C, 38.26; H, 4.42; N, 7.83. $C_{12}H_{18}N_2O_{12}$ requires: C, 37.70; H, 4.71; N, 7.34%). Cibarian (**5**); mp 123.5–124° (Me_2CO–CCl_4). (Found: C, 37.79; H, 4.60; N, 7.47. $C_{12}H_{18}N_2O_{12}$ requires: C, 37.70; H, 4.71; N, 7.34%).

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