Acryloylated Glucose 3-Nitropropanoates from *Indigofera kirilowii*

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Two unique glucose esters of 3-nitropropanoic acid with acryloyl moieties, kirilowin A (**1**) and kirilowin B (**2**), were isolated from the roots of *Indigofera kirilowii*. Their structures were elucidated by detailed spectroscopic analysis. The two acrylates constitute new additions to the toxic glucose conjugates of 3-nitropropanoic acid in angiosperms.

The occurrence of glucose esters of 3-nitropropanoic acid (NPA) in many leguminous plants has been frequently reported, notably *Indigofera* spp. since these compounds are known to be toxic to livestock and other animals. Mono-, di-, tri-, and tetrasubstituted glucose esters of NPA have been detected in the aerial parts of over 60 species of *Indigofera*.[1−5] The roots of several *Indigofera* species are used as "ShanDouGen" (*Sophora tonkinensis* Gapnep) in the treatment of inflammatory diseases, especially sore throat, in traditional Chinese medicine. Severe adverse reactions such as headache, vomiting, dystonia, and coma were reported in some patients after taking high dosages.[6] As part of our search for bioactive/toxic principles of the roots of *Indigofera* species used in traditional Chinese medicine, we have investigated the EtOH extract of *Indigofera kirilowii* Maxim. ex Palibin. Fractionation of the crude extract led to the isolation of two new glucose esters of NPA, kirilowin A (**1**) and kirilowin B (**2**), which contained an acryloyl group, which has been rarely observed in natural products research (see Figure 1). Details of the isolation and structural elucidation are presented here.

Specimens of *I. kirilowii* were collected in Ji County of Tianjin, China. The air-dried roots were refluxed with aqueous EtOH, and the extract was evaporated and then partitioned between H_2O and petroleum ether and $CHCl_3$. The $CHCl_3$ extract was concentrated under reduced pressure, and the gummy residue was fractionated via repetitive silica gel chromatography to afford pure kirilowin A (**1**) and kirilowin B (**2**) as white needles.

The molecular formula of **1** was determined as $C_{18}H_{23}N_3O_{16}$ by high-resolution FAB mass spectroscopy (*m/z* 538.1132, calcd for $C_{18}H_{24}N_3O_{16}$ $[M + H]^+$, 538.1151). The ^1H NMR spectrum of **1** in acetone-d_6 contained resonances corresponding to three 3-nitropropanoyl units (multiplets at δ ca. 4.8 and ca. 3.0 ppm, each 6H) and a glucopyranosyl unit. An anomeric proton at δ 5.4, which resonated as a doublet ($J_{1,2} = 3.2$ Hz) upon addition of one drop of D_2O, indicated that the anomeric hydroxyl group of the glucose moiety was not esterified, and the small coupling constant established **1** as the α-anomer. The connectivities between the ring protons of the glucopyranosyl unit were established by a COSY experiment (see Table 1), and the resonances for H-2, 3, 4, and 6 indicated that four sites at *O*-2, 3, 4, and 6 were esterified.[3,7,8]

The ^{13}C NMR spectrum of **1** contained 18 signals (δ 31.5, 3 × C), of which a DEPT spectrum revealed that four were quaternary (all carboxyl-type carbonyls), six were methines, and eight were methylenes. On the basis of the assigned protons, HSQC experiments gave the corresponding carbon assignments of the glucopyranosyl unit and three 3-nitropropanoyl groups, which were confirmed by the HMBC spectrum (see Table 1). Thus, subtracting the requirements for a glucose triester with NPA revealed that the fourth esterifying component was a C_3 unit comprising a carbonyl (δ 165.9), a methine (δ 128.4), and a methylene (δ 132.8) group. The protons of this moiety resonated in the ^1H NMR spectrum as an AMX system similar to those of a vinylic group: three sets of doublets of doublets each representing a single proton. The X proton (δ 6.09) was coupled to the M proton ($J_{XM} = 17.2$ Hz) and to the A proton ($J_{XA} = 10.4$ Hz); the M proton (δ 6.34) was coupled to the X proton and to the A proton ($J_{MA} = 1.2$ Hz); and the A proton (δ 5.92) was coupled to the X proton and to the M proton. Therefore the fourth ester component was recognized to be an acryloyl group.

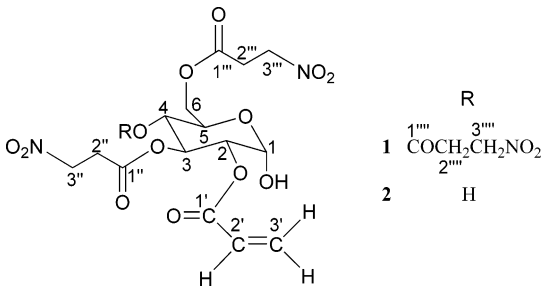

Figure 1. Structures of compounds **1** and **2**.

The positions of the esterifying groups were determined by HMBC experiments: the correlation between H-2 (δ 4.87) and C-1′ (δ 165.9) of the acryloyl group revealed that the acryloyl group was attached to C-2 of the glucose; the correlations between H-3 (δ 5.65), H-4 (δ 5.19), and H-6 (δ 4.22) and three other carbonyl carbons (δ 170.5, 170.3, 170.7), respectively, confirmed that the three 3-nitropropanoyl units were connected to C-3, C-4, and C-6 of the glucose unit. Thus, kirilowin A has the structure **1** (see Figure 1).

Kirilowin B (**2**) possessed a molecular formula of $C_{15}H_{20}N_2O_{13}$ deduced from its high-resolution FABMS (*m/z* 437.1023, calcd for $C_{15}H_{21}N_2O_{13}$ $[M + H]^+$, 437.1038), equivalent to 101 mass units less than **1**, indicating the absence of a 3-nitropropanoyl group. Similar NMR spectrometric analysis revealed that kirilowin B was the analogous 2,3,6-triester (**2**) (see Figure 1 and Table 1).

Thus, the roots of *I. kirilowii* contain some minute but unique conjugates of NPA and acrylic acid with α-D-

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Table 1. NMR Data for Compounds **1** and **2**

	1		**2**	
no.	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H
1	90.4 d	5.41 d (3.2)	90.5 d	5.36 d (3.6)
2	72.4 d	4.87 dd (10.0, 3.2)	72.8 d	ca. 4.73 (overlapped)
3	71.1 d	5.65 dd (10.0, 9.6)	74.0 d	5.51 dd (9.6, 9.6)
4	69.9 d	5.19 dd (10.0, 9.6)	69.1 d	3.68 dd (9.6, 9.6)
5	67.4 d	4.31 m	69.9 d	4.12 ddd (10.0, 5.2, 2.4)
6	63.5 t	4.22 (2H, overlapped)	64.5 t	4.41 dd (12.0, 2.4)
				4.30 dd (12.0, 5.2)
Glc C-2				
1′	165.9 s		166.0 s	
2′	128.4 d	6.09 dd (17.2, 10.4)	128.6 d	6.08 dd (17.2, 10.4)
3′	132.8 t	6.34 dd (17.2, 1.2)	132.2 t	6.33 dd (17.2, 1.6)
		5.92 dd (10.4, 1.2)		5.91 dd (10.4, 1.6)
Glc C-3, 6, 4	170.5 s		170.4 s	
1″	170.7 s		170.8 s	
1‴	170.3 s			
1⁗				
2″	31.5 t	ca. 3.05 (4H, overlapped)	31.6 t	3.04 m (2H)
2‴	(3× C)	ca. 2.96 (2H, overlapped)	31.5 t	3.09 m (2H)
2⁗				
3″	70.7[a] t	ca. 4.76 (6H, overlapped)	70.6 t	4.75 (2H, overlapped)
3‴	70.6[a] t		70.7 t	4.79 (2H, overlapped)
3⁗	70.5[a] t			

[a] Chemical shifts may be interchanged.

glucopyranose. Since these compounds should release NPA upon hydrolysis, they are presumably responsible for the toxicity of the plant.

Experimental Section

General Experimental Procedures. Melting points were measured with an XT4A microscope apparatus and are uncorrected. Optical rotations were determined using a Perkin-Elmer Model 341 polarimeter. IR spectra were recorded on a BIO-RAD 3000 FTIR spectrometer. NMR spectra including DEPT, COSY, HSQC, and HMBC experiments were measured on a Bruker AV400 NMR spectrometer in acetone-d_6 solution, and chemical shifts are expressed in δ (ppm). ^{1}H NMR spectra were remeasured after adding a drop of D_2O to remove OH couplings. High-resolution FABMS were determined with an APEX II FT-ICR MS spectrometer. Silica gel (100−200 mesh and 200−300 mesh, Qingdao Marine Chemical Co.) was used for open column chromatography and silica gel GF_{254} (Qingdao Marine Chemical Co.) for thin-layer chromatography. Solvents were of analytical grade, and phosphomolybdic acid in EtOH was used for visualization.

Plant Material. The roots of *I. kirilowii* were collected from Ji County, Tianjin, People's Republic of China, in September 2004, and a voucher specimen (No. 200409001) has been deposited in the College of Pharmaceutical Sciences and Technology, Tianjin University, Tianjin 300072, People's Republic of China.

Extraction and Isolation. The roots of *I. kirilowii* (14.7 kg) were refluxed in 95% EtOH (3×) and then with 60% EtOH once. The extracts were combined, concentrated, and then suspended in H_2O. The H_2O suspension was extracted in turn with petroleum ether (30.9 g) and $CHCl_3$ (51 g). The $CHCl_3$ extract (45 g) was fractioned via silica gel column chromatography eluting with petroleum ether and acetone (95:5, 9:1, 85:15, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8) to give 130 fractions. Fractions 102 and 103 were further subjected to silica gel column chromatography and eluted with 8% acetone in $CHCl_3$ to afford **1** (3 mg) and **2** (12 mg).

Kirilowin A, 2-*O*-acryloyl-3,4,6-tri-*O*-[3-nitropropanoyl]-α-D-glucopyranose (1): white needles (acetone); mp 162−163 °C; $[\alpha]^{20}_D$ +71.2 (*c* 0.17, acetone); IR (KBr) ν_{max} 3433, 3024, 2925, 1732, 1556, 1381, 1255, 1182, 1049, 980, 870 cm^{-1}; ^{1}H (acetone-d_6 + 1 drop D_2O, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see Table 1; HRFABMS (positive ion mode) *m/z* 538.1132 (calcd for $C_{18}H_{24}N_3O_{16}$ $[M + H]^+$, 538.1151), 520.1035 (calcd for $C_{18}H_{22}N_3O_{15}$ $[M + H - H_2O]^+$, 520.1045).

Kirilowin B, 2-*O*-acryloyl-3,6-di-*O*-[3-nitropropanoyl]-α-D-glucopyranose (2): white needles (acetone); mp 221−222 °C; $[\alpha]^{20}_D$ +60.4 (*c* 0.53, acetone); IR (KBr) ν_{max} 3460, 3029, 2983, 1738, 1715, 1548, 1378, 1298, 1192, 1052, 871 cm^{-1}; ^{1}H (acetone-d_6 + 1 drop D_2O, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see Table 1; HRFABMS (positive ion mode) *m/z* 437.1023 (calcd for $C_{15}H_{21}N_2O_{13}$ $[M + H]^+$, 437.1038), 419.0940 (calcd for $C_{15}H_{19}N_2O_{12}$ $[M + H - H_2O]^+$, 419.0932).

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