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Iridoid glucosides from *Strychnos nux-vomica*

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Abstract

From seeds of *Strychnos nux-vomica* three iridoids, 6′-*O*-acetylloganic acid, 4′-*O*-acetylloganic acid and 3′-*O*-acetylloganic acid were isolated together with two known iridoid glucosides, loganic acid and 7-*O*-acetylloganic acid. The structures of the compounds were established by ESI-MS and by 1D and 2D NMR spectroscopic methods.

Keywords: *Strychnos nux-vomica*; Loganiaceae; Iridoids; Loganic acid derivates

1. Introduction

Strychnos nux-vomica L. (Loganiaceae) is an evergreen tree native to southeast Asia, especially India and Sri Lanka. Its dried seeds are used for herbal remedies in traditional Chinese Medicine, for treatment of nervous diseases, arthritis, and vomiting. The fruits are a rich source of iridoid glucosides (Bisset and Chouhury, 1974).

In continuation of the investigation of its chemical constituents, we now report the isolation and structural elucidation of new iridoid glucosides (**1**–**3**) from the seeds of *Strychnos nux-vomica* together with two known compounds **4** and **5**.

2. Results and discussion

The molecular weight of **1** was indicated by the peak at *m*/*z* $[M-H]^-$ 417 in the electrospray mass spectra (ESI-MS). The IR spectrum of **1** showed a hydroxyl absorption at 3422 cm^{-1}, an α, β-unsaturated carboxyl absorption at 1685, 1636 cm^{-1}, and another carbonyl absorption at 1705 cm^{-1}. ^{1}H NMR signals (Table 1) revealed a typical iridoid skeleton and a β-glucose moiety (Mitsunaga et al., 1991). The ^{13}C NMR spectrum of **1** (Table 2) confirmed the presence of 18 carbons. These spectral features were very close to those observed for 7-*O*-acetylloganic acid **5** (Nakamoto et al., 1988; Garcia and Chulia, 1986). The ^{1}H and ^{13}CNMR spectra of compound **1** showed the absence of a carbomethoxy function (δ 3.6 *s*; 51.3) as in loganin, a common iridoid in many plants (Jensen et al., 2002; Calis et al., 1984). These data, as well as IR spectra at 1685 cm^{-1}, revealed that compound **1** is an acid derivative. The main differences between the NMR spectra of **1** and loganic acid (**4**) were seen for the signals of the 6′-CH_2 group which in **1** had downfield shifts of ca 0.5 ppm and ca 2.5 ppm in the ^{1}H and ^{13}C spectra, respectively. In addition, the 6′-protons showed correlations to the CO-signal (δ 176.8) of the acetyl group in the HMBC spectrum. Compound **1** was therefore 6′-*O*-acetylloganic acid (Scheme 1). Since the spectra of the aglucone parts of **1** and **4** were almost identical, the β-configuration at C-1 could also be assumed.

The UV absorption maxima and molecular ions obtained for **2** were identical to those observed for **1**. Besides the molecular ion peak, the main information obtained from the ESI-MS spectrum of **2** was given by the intensive fragment at *m*/*z* 213 corresponding to the loss of an acetyl glucose moiety, which also was observed in the MS spectrum of compound **1**. According to the analysis of ^{1}H NMR and ^{13}C NMR spectra data (Tables 1 and 2) compound **2** was identified as an iridoid glucoside whose skeleton must be the same as that of known loganic acid **4**. Comparison of the ^{13}C NMR spectra data of **2** with those for **1** and loganic acid **4** indicated the same iridoidal system for **2** with

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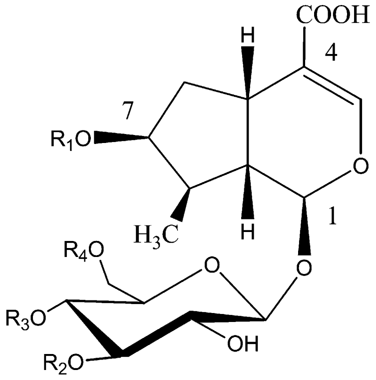
Table 1
^{1}H NMR data for compounds 1, 2, 3, 4 and 5 in D$_2$O (δ in ppm, *J* in Hz)

H	**1**	**2**	**3**	**4**	**5**
1	5.33 (*d*, 3.6)	5.41 (*d*, 3.6)	5.42 (*d*, 3.6)	5.41 (*d*, 3.6)	5.40 (*d*, 3.6)
3	7.45 (*s*)	7.40 (*s*)	7.40 (*s*)	7.41 (*s*)	7.47 (*s*)
5	3.06 (*dd*, 7.6, 8.0)	3.04 (*dd*, 7.6, 8.0)	3.04 (*dd*, 7.2, 8.4)	3.05 (*dd*, 7.6,8.0)	3.06 (*dd*, 7.6, 8.0)
6a	2.19 (*m*)	2.21 (*m*)	2.20 (*m*)	2.18 (m)	2.29 (*m*)
6b	1.76 (*m*)	1.77 (*m*)	1.77 (*m*)	1.77 (*m*)	1.83 (*m*)
7	4.15 (*m*)	4.15 (*m*)	4.15 (*m*)	4.15 (*m*)	5.13 (*m*)
8	1.90 (*m*)	1.92 (*m*)	1.92 (*m*)	1.91 (*m*)	2.09 (*m*)
9	2.10 (*m*)	2.12 (*m*)	2.12 (*m*)	2.11 (*m*)	2.19 (*m*)
10	1.06 (*d*, 7.2)	1.07 (*d*, 7.2)	1.07 (*d*, 7.2)	1.06 (*d*, 7.2)	1.05 (*d*, 7.2)
1′	4.81 (*d*, 8.0)	4.85 (*d*, 8.4)	4.90 (*d*, 8.4)	4.79 (*d*, 8.4)	4.81 (*d*, 8.4)
2′	3.29 (*t*, 8.0, 9.2)	3.19 (*t*, 8.4, 9.2)	3.29 (*t*, 8.4, 9.2,)	3.28 (*t*, 8.4, 9.2)	3.29 (*t*, 8.4, 9.2)
3′	3.51 (*t*, 9.2)	3.71 (*m*)	4.99 (*t*, 9.2)	3.50 (*t*, 9.2)	3.51 (*t*, 8.0, 9.2′)
4′	3.47 (*t*, 9.2, 9.6)	4.81 (*t*, 9.2)	3.60 (*t*, 9.2)	3.40 (*t*, 9.2, 9.6)	3.40 (*t*, 9.2, 9.6)
5′	3.68 (*m*)	3.69 (*m*)	3.59 (*m*)	3.48 (*m*)	3.51 (*m*)
6′a	4.42 (*dd*, 2.4, 12.4)	3.73 (*dd*, 1.6, 12.4)	3.92 (*dd*, 1.6, 12.4)	3.91 (*dd*, 1.6, 12.4)	3.94 (*dd*, 1.6, 12.4)
6′b	4.33 (*dd*, 6.0, 12.4)	3.39 (*dd*, 4.8, 12.4)	3.75 (*dd*, 6.0, 12.4)	3.72 (*dd*, 6.0, 12.4)	3.72 (*dd*, 6.0, 12.4)
CH$_3$CO	2.12 (*s*)	2.17 (*s*)	2.17 (*s*)	–	2.17 (*s*)



	R$_1$	R$_2$	R$_3$	R$_4$
1	H	H	H	CH$_3$CO
2	H	H	CH$_3$CO	H
3	H	CH$_3$CO	H	H
4	H	H	H	H
5	CH$_3$CO	H	H	H

Scheme 1. Structures of compounds **1**, **2**, **3**, **4** and **5** discussed in the text.

differences in the position of an acetyl group. To elucidate the substitution pattern of **2**, ^{1}H–^{1}H COSY data correlated spectroscopy COSY was used to assign all resonance signals in the ^{1}H NMR spectra. The main differences between the NMR spectra of **1** and loganic acid (**4**) were seen for the signals of the 4′-H which in **2** had downfield shifts of ca 1.4 ppm and ca 1.3 ppm in the ^{1}H and ^{13}C spectra, respectively. The ^{1}H–^{1}H COSY relationships permitted the assignment of the proton chemical shifts and also supported the substitution pattern. The acetyl function was ultimately determined to be linked to C-4′, and **2** was thus identified as 4′-*O*-acetylloganic acid.

Compound **3** revealed an $[M-H]^-$ ion peak at m/z 417 in the ESI-MS spectrum, consistent with the molecular weight of **1**, **2** and 7-*O*-acetylloganic acid **5**. The data of ^{1}H and ^{13}C NMR spectra were identical to those of compound **1** and **2** for the aglycone unit. Comparison between the ^{1}H NMR spectra of **3** and **4** showed a large downfield shift for H-3′ (1.5 ppm) in the former and proved that this was the position of the acetyl group. The expected shifts in the ^{13}C spectrum were also observed (Table 2). These changes suggested that the acetyl group was attached to the glucose at C-3′. It was thus concluded that the structure of compound **3** was 3′-*O*-acetylloganic acid.

3. Experimental

3.1. General

Optical activities were measured on a Jasco P1010 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity spectrometer at 400 and 100.57 MHz, respectively with DSS as an internal standard. IR spectra were obtained on a Perkin-Elmer GSII spectrometer. ESI-MS was conducted using a Finnigan TSQ 700 mass spectrometer. ESI-TOF/MS spectra were recorded on Applied Biosystems Mariner Mass spectrometer. Purification of samples was performed on Waters Delta 4000 preparative HPLC.

3.2. Isolation of authentic compounds

The seeds of *Strychnos nux-vomica* L. were supplied by Dalian Company of Chinese Herbal Drugs (Dalian, China) in 2001. The voucher specimens were identified by Prof. Cheng Daixian and deposited in Dalian Insti-

Table 2
^{13}C NMR spectroscopic data for compounds **1**, **2**, **3**, **4** and **5** in D_2O (δ in ppm)

C	**1**	**2**	**3**	**4**	**5**
1	99.5	99.4	99.3	99.2	99.4
3	154.0	153.4	153.4	153.5	154.6
4	115.6	116.2	116.2	116.1	115.4
5	32.5	32.4	32.5	32.5	33.0
6	43.0	42.9	42.9	42.9	41.2
7	77.1	77.1	77.1	77.1	81.7
8	42.8	42.7	42.6	42.7	41.9
9	47.6	47.6	47.6	47.7	48.3
10	14.7	14.7	14.6	14.7	15.0
11	174.0	174.3	174.3	174.3	174.1
1′	101.5	101.2	101.1	101.2	101.7
2′	75.3	75.2	73.7	75.3	75.7
3′	78.1	76.2	79.8	78.3	79.4
4′	72.1	73.5	70.5	72.2	72.6
5′	76.4	76.7	78.7	79.0	78.7
6′	65.7	62.8	63.1	63.3	63.7
$\underline{C}H_3CO$	22.9	23.1	23.0		23.6
$CH_3\underline{C}O$	176.8	176.4	175.9		177.6

tute of Chemical Physics, Chinese Academy of Sciences. Dried seeds (5 kg) were extracted with 15 L 95% EtOH (60 °C) for 3 h. The extract was concentrated to dryness and then dissolved with water. The water-soluble fraction was applied directly to a Dianion HP-20 column and eluted using a step gradient of ethanol–water 0:100, 10:90, 30:70, and 70:30, respectively. One subfraction (10% ethanol, 20 g) was separated by MPLC on a silica gel column (200–300 Mesh), eluting with EtOAc–EtOH (7:3). The eluants were collected and evaporated, individually. The iridoid fraction (500 mg) was then separated by HPLC on a Superiorex ODS column (5 μm, 250×20mm i.d.,) eluted with MeOH–0.2%HAc 20:80 to afford compound **1** (61 mg), **2** (15 mg), **3** (9 mg), **4** (150 mg) and **5** (43 mg) in pure form.

3.3. *6′-O-acetylloganic acid* ***1***

White amorphous solid. $[\alpha]_D^{20}$ −85.1 (MeOH; *c* = 0.07); UV λ_{max}^{MeOH} nm: 235; IR ν_{max} KBr cm^{-1}: 3422, 1705, 1685, 1636, 1376, 1074; ESI-MS *m*/*z*: 417 $[M-H]^-$, ESI-MS-MS *m*/*z*: 375, 357, 213, 169; API-TOF/MS *m*/*z*: 417.1380 $[M-H]^-$ (calculated for $C_{18}H_{25}O_{11,}$ 417.1397); ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

3.4. *4′-O-acetylloganic acid* ***2***

White amorphous solid. $[\alpha]_D^{20}$ −67.6(MeOH; *c* = 0.07); UV λ_{max}^{MeOH} nm: 235; IR ν_{max} KBr cm^{-1}: 3423, 1707, 1685, 1637, 1376, 1079; ESI-MS *m*/*z*: 417 $[M-H]^-$, ESI-MS-MS *m*/*z*: 375, 357, 213, 169; API-TOF/MS *m*/*z*: 417.1374 $[M-H]^-$ (calculated for $C_{18}H_{25}O_{11}$, 417.1397); ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

3.5. *3′-O-acetylloganic acid* ***3***

White amorphous solid. $[\alpha]_D^{20}$ −70.1 (MeOH; *c* = 0.06); UV λ_{max}^{MeOH} nm: 235; IR ν_{max} KBr cm^{-1}: 3421, 1706, 1685, 1636, 1376, 1077; ESI-MS *m*/*z*: 417 $[M-H]^-$, ESI-MS-MS *m*/*z*: 375, 357, 213, 169; API-TOF/MS *m*/*z*: 417.1427 $[M\text{-}H]^-$(calculated for $C_{18}H_{25}O_{11,}$ 417.1397); ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

3.6. *Loganic acid* ***4***

White amorphous solid. $[\alpha]_D^{20}$ −75.8 (MeOH; *c* = 1.3); UV λ_{max}^{MeOH} nm: 235; IR ν_{max} KBr cm^{-1}: 3348, 1685, 1637, 1076; ESI-MS *m*/*z*: 375 $[M-H]^-$, ESI-MS-MS *m*/*z*: 213,169; ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

3.7. *7-O-acetylloganic acid* ***5***

White amorphous solid. $[\alpha]_D^{20}$ −67.2 (MeOH; *c* = 0.07); UV λ_{max}^{MeOH} nm: 235; IR ν_{max} KBr cm^{-1}: 3423, 1707, 1685, 1636, 1376, 1074; ESI-MS *m*/*z*: 417 $[M-H]^-$, ESI-MS-MS *m*/*z*: 375, 357, 255, 211, 195; ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

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