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New alkaloids from the seeds of *Strychnos nux-vomica*

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ABSTRACT

Eight new trace indolomonoterpenic alkaloids (**1**–**8**) were isolated from the seeds of *Strychnos nux-vomica*. Their structures and absolute configurations were determined by extensive analysis of MS, NMR, CD, and ECD. The skeleton of compound **1** is an unprecedented 6/5/6/5/6/6/7 heptacyclic ring system. Compound **2** possesses a 2-pyridone moiety, and has not yet been found in *strychnos* alkaloids. Compounds **6** and **7** showed neuroprotective activities on PC12 cells with IE 50 values of 0.68–4.81 μM and 0.54–6.50 μM, respectively. The biosynthetic pathway of **1** was also postulated.

1. Introduction

Strychnos nux-vomica L. belongs to Loganiaceae family and is widely distributed in China, India, and Sri Lanka. The seeds of this plant, as a traditional Chinese medicine, are widely used in China for the treatments of rheumatoid arthritis, swelling pain, trauma, bone fracture, facial nerve paralysis, myasthenia gravis, and poliomyelitis sequela.[1] The major components from *S. nux-vomica* were reported to be strychnine and brucine type alkloids.[2,3] However, except for the high content components, the trace alkaloids have not been studied. In present study, the trace bioactive alkaloids with novel skeletons were isolated from this plant. The structures of these new isolates were assigned by spectroscopic data, and their absolute configurations were determined by CD and ECD analysis. The biosynthetic pathway of compound **1** was also proposed to support the structural elucidation. The isolates were tested for their neuroprotective activities on PC12 cells by using MTT methods.

2. Results and discussion

Eight new alkaloids (**1**–**8**) (Fig. 1) were isolated from the $CHCl_3$ partitioned ethanolic extract of the seeds of *S. nux-vomica* L. by using column chromatographic methods.

Stryvomicine (**1**) was obtained as a white amorphous powder. Its molecular formula was established as $C_{22}H_{24}N_2O_4$ by HRESIMS at *m*/*z* 381.1816 $[M+H]^+$, (calcd for 381.1809, $C_{22}H_{25}N_2O_4$), with 12 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 202, 265, and 296 nm, indicating the presence of one phenolic hydroxyl substituted *N*-acyl-dihydroindol chromophore with the carbonyl as part of a lactam function.[4,5] Its IR spectrum showed the absorptions for hydroxyl, carbonyl, and aromatic groups at 3418, 1679, and 1462 cm^{-1}, respectively. The ^1H NMR spectrum of **1** (Table 1) gave three aromatic protons, one olefinic proton, and one phenolic hydroxyl proton in down field region. The ^{13}C NMR and DEPT spectra of **1** showed that it contained 22 carbon signals, including 1 methyl group, 5 methylene groups, 9 methine groups, and 7 quaternary carbons. All of the protons were assigned to corresponding carbons by HSQC experiments. The presence of dihydroindole moiety, trisubstituted double bond, and carbonyl units, was recognized by ^1H and ^{13}C NMR data (Table 1). Above structural units accounted for 7 of the required 12 unsaturation degrees. Therefore, besides dihydroindole nucleus, five additional rings (C–G) containing one nitrogen atom should be assigned to stryvomicine. The elucidation of rings C–G was described in detail as follow.

The ^1H NMR, DEPT, ^1H–^1H COSY, HSQC, and HMBC spectra of **1** revealed the presence of three proton–proton spin systems [a: (H_2-14, H-15, H-16(H-2), H-17, and H_2-24); b: (H_2-18 and H-19) and c: (H_2-5 and H_2-6)]. In the HMBC spectrum, cross-peaks from H-19 to C-15, and H-21 to C-19 established the connections between C-15 and C-20, and C-21 and C-20, respectively. The cross-peaks from

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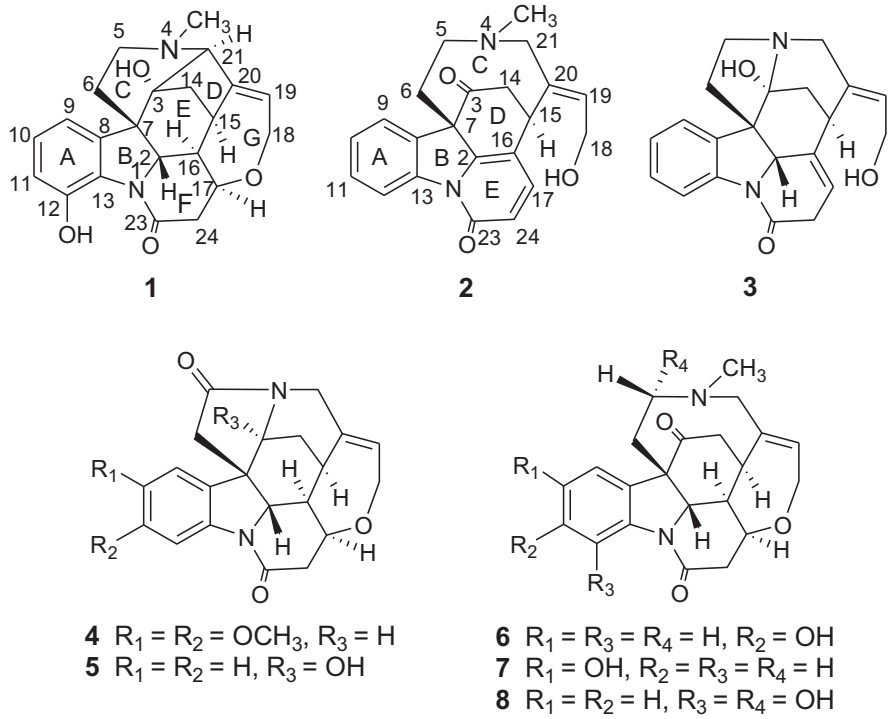

Fig. 1. Structures of new alkaloids.

Table 1
NMR data for **1**–**4**[a]

No.	**1**		**2**		**3**		**4**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2	4.42, d (9.0)	58.9		148.2	4.49, s	66.4	4.07, d (11.0)	61.9
3		78.8		192.4		92.3	4.55, d (4.0)	57.8
5α	2.52, overlap	43.7	1.94, dd (3.5, 13.5)	46.9	3.39, dd (6.5, 9.0)	49.5		176.6
5β	3.37, m		2.49, td (3.0, 13.5)		2.86, m			
6α	2.52, overlap	34.7	2.68, overlap	45.3	2.69, m	42.1	2.86, d (15.5)	50.6
6β	1.11, overlap		1.27, m		1.90, dd (5.0, 12.0)		2.62, d (15.5)	
7		49.9		55.7		57.6		54.6
8		140.2		135.6		133.2		121.2
9	7.99, dd (6.5, 2.0)	119.2	8.08, d (7.5)	123.4	8.18, d (7.5)	129.7	7.18, s	107.9
10	7.17, overlap	127.9	7.29, t (7.5)	125.1	7.16, t (7.5)	123.6		147.5
11	7.15, overlap	118.4	7.38, t (7.5)	127.2	7.35, t (7.5)	129.0		151.1
12		147.4	8.95, d (7.5)	116.1	8.70, d (7.5)	115.0	8.25, s	102.6
13		129.0		139.4		143.5		138.0
14α[b]	1.63, d (12.0)	41.1	2.32, dd (6.0, 17.0)	40.2	1.76, d (13.5)	33.4	1.54, d (14.5)	26.9
14β[b]	2.07, dd (12.0, 6.5)		2.74, d (17.0)		2.28, dd (13.5, 3.5)		2.32, dt (14.5, 4.0)	
15	3.13, m	37.2	4.22, d (6.0)	35.3	3.65, br s	37.8	3.09, br s	31.4
16	1.14, overlap	51.0		116.3		142.1	1.19, m	47.7
17	4.26, m	76.2	7.67, d (9.5)	138.9	5.84, dd (2.0, 5.5)	121.8	4.17, m	78.2
18α	4.17, dd (14.0, 7.0)	64.3	4.71, dd (13.5, 2.0)	58.2	4.62, overlap	59.1	3.92, dd (13.5, 6.0)	64.8
18β	4.06, dd (14.0, 6.5)		4.89, dd (13.5, 2.5)		4.62, overlap		4.01, dd (13.5, 7.0)	
19	6.17, dd (6.5, 7.0)	123.6	5.99, dd (2.5, 2.0)	130.1	5.97, dd (5.5, 5.0)	132.0	5.91, dd (6.0, 7.0)	126.7
20		145.5		136.1		133.5		141.7
21α	3.85, s	73.8	3.41, d (14.5)	62.5	4.13, d (15.5)	52.9	3.61, overlap	44.6
21β			3.22, d (14.5)		3.28, d (15.5)		4.47, d (14.0)	
23		172.2		160.3		168.8		169.7
24α	3.25, dd (10.5, 8.0)	43.7	6.69, d (9.5)	119.5	3.12, overlap	37.6	3.21, dd (16.5, 8.5)	43.0
24β	2.85, overlap				3.12, overlap		2.75, dd (16.5, 4.0)	
N-CH_3	2.86, s	44.3	2.01, s	37.7				
12-OH	12.60, s							
OCH_3-10							3.81, s	56.5
OCH_3-11							3.84, s	57.1

[a] Data were recorded at 500 MHz for proton and at 125 MHz for carbon in pyridine-d_5.
[b] α/β-Orientation of the protons of C-14 was determined according to the ring E of **1**, **3**, and **4**.

H_2-18 to C-17, together with the chemical shifts at δ_C 76.2 (C-17) and 64.3 (C-18), displayed the linkage between C-17 and C-18 through one oxygen atom. The $^1H-^{13}C$ long range correlations and proton–proton spin systems assigned above led to the characterization of one seven-membered ring (ring G). The HMBC correlations from H-21 to C-3, H-15 to C-3, and H-14 to C-21 indicated that C-14 and C-21 were attached to C-3, which was an unique C-3, C-21 linkage pattern and a five-membered ring named ring D. The remaining three rings