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Strynuxlines A and B, Alkaloids with an Unprecedented Carbon Skeleton from *Strychnos nux-vomica*

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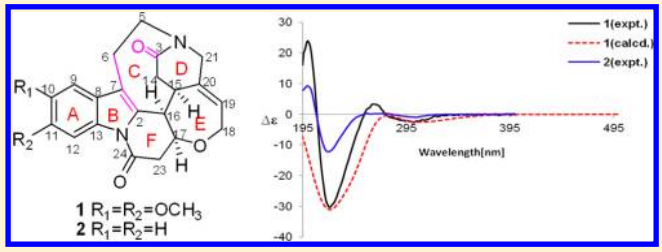
Supporting Information

ABSTRACT: The investigation of the seeds of *Strychnos nux-vomica* led to the isolation of two novel indole alkaloids, strynuxlines A (**1**) and B (**2**), with an unprecedented 6/5/9/6/7/6 hexacyclic ring system. Their structures and absolute configurations were elucidated on the basis of their MS, NMR, and ECD data. A plausible biosynthesis pathway of **1** and **2** is also proposed.



Strychnos nux-vomica is a moderate-sized tree of the family Loganiaceae found in southern Asian countries.[1] The dried ripe seeds of this tree have been applied as a traditional folk medicine in China for the treatment of tumors, rheumatic arthritis, swelling pain, trauma, bone fractures, facial nerve paralysis, myasthenia gravis, and poliomyelitis sequelae.[2] Previous phytochemical analysis has identified strychnine and brucine as the major components,[1] which are also mainly responsible for most of the pharmacological properties, such as the antitumor, cytoprotective, and antitussive activities.[3] Studies aimed at the discovery of trace alkaloids in *S. nux-vomica* led to the isolation of strynuxlines A (**1**) and B (**2**), two novel alkaloids possessing an unprecedented skeleton with a 6/5/9/6/7/6 hexacyclic ring system. This is the first report of the cleavage of the C-3−C-7 bond in strychnan-type alkaloids. In addition to strynuxlines A (**1**) and B (**2**), 16 known alkaloids, strychnine (**3**),[4] α-colubrine (**4**),[5] β-colubrine (**5**),[6] brucine (**6**),[7] strychnine-*N*-oxide (**7**),[8] brucine-*N*-oxide (**8**),[9] pseudostrychnine (**9**),[10] pseudobrucine (**10**),[11] strychnine methiodide (**11**),[12] icajine (**12**),[13] vomicine (**13**),[14] 3-methoxyicajine (**14**),[15] novacine (**15**),[16] isostrychnine (**16**),[17] isobrucine (**17**),[18] and bis-nor-dihydrotoxiferine (**18**),[19] were isolated and identified by comparing the experimental and reported physical data. Herein, we describe the isolation, structure elucidation, plausible biosynthesis pathway, and cytotoxicity of **1** and **2**.

Strynuxline A (**1**) was obtained as a white, amorphous solid with a specific rotation of −254.6 (*c* 0.11, MeOH). Its molecular formula was determined to be $C_{23}H_{24}N_2O_5$, by HREIMS (*m/z* 408.1673 $[M]^+$; calcd 408.1685), with an index of hydrogen deficiency of 13. The UV spectrum exhibited the maximum absorption bands at 263 and 301 nm, both characteristic of a phenyl chromophore substituted with methoxy auxochromes. The IR spectrum showed the presence of aromatic (1485, 1463 cm^{-1}) and amide groups (1669, 1634 cm^{-1}).[20] The ^{13}C NMR and DEPT data revealed the presence of 23 carbon atoms, including 12 sp^2 carbon atoms, six sp^3 methylenes, three sp^3 methines, and two methoxy groups. In addition, the 12 sp^2 carbon atoms were attributable to one indole moiety, one trisubstituted double bond, and two carbonyl groups. In addition, nine indices of hydrogen deficiency were the result of one indole moiety, one trisubstituted double bond, and two carbonyl groups, the remaining four indices of hydrogen deficiency were due to four rings. Accordingly, the key to determining the structure was elucidating the nature of rings C−F, which was described in detail below.

Detailed HSQC, $^1H-^1H$ COSY, and HMBC data revealed that **1** possessed three spin coupling systems: **a** (C-5 to C-6), **b** (C-14 to C-17), and **c** (C-18 to C-19) as shown in Figure 2. In the HMBC spectrum, cross-peaks from H-5β to C-3 (δ_C 174.9) and C-21 (δ_C 52.9) and from H-21 to C-3 and C-5 (δ_C 49.3) indicated that C-3, C-5, and C-21 were interconnected via a nitrogen atom and that there was a unique cleavage of the C-3−

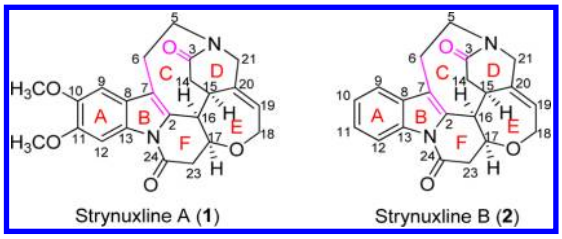


Figure 1. Structures of strynuxlines A (**1**) and B (**2**).

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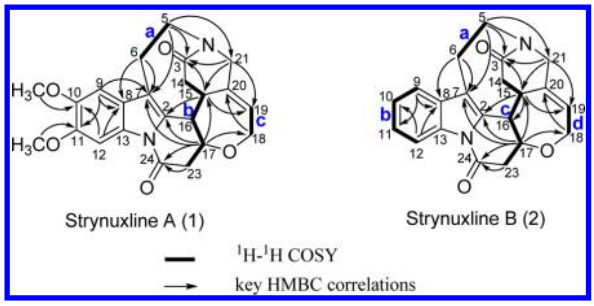

Figure 2. Selected 2D NMR correlations for strynuxlines A (**1**) and B (**2**).

C-7 bond. The HMBC correlations of H-6/C-7 and H-16/C-2 were indicative of the C-6/C-7 and C-16/C-2 linkages, respectively. The above data along with the key HMBC correlations of H-14 to C-3 led to the identification of one nine-membered ring (ring C). Accordingly, the six-membered ring D was established by the cross-peaks from H-21 to C-15 and C-20. In the HMBC spectrum, the correlations of H-17 to C-18 and the chemical shifts at δ_C 83.7 (C-17) and 68.0 (C-18) indicated the linkage of C-17 to C-18 via an oxygen atom. Moreover, the HMBC correlations of H-18 to C-20 and H-15 to C-19 established the connectivity of fragments **b** and **c**, indicating the presence of the seven-membered E-ring. The existence of ring F was illustrated by the key HMBC correlation from H_2-23 to C-24 (δ_C 168.0).

In addition, two aromatic methoxy groups resonating at δ_H 3.86 (3H, s) and 3.86 (3H, s) as well as two sharp aromatic singlets at δ_H 6.78 (1H, s, H-9) and 7.88 (1H, s, H-12) indicated that *O*-methyl groups were located at C-10 and C-11, which was further confirmed by the strong HMBC correlations of 10-OCH_3 to C-10 (δ_C 146.9) and 11-OCH_3 to C-11 (δ_C 148.1). Thus, the gross structure of **1** was determined to be a unique strychnan-type alkaloid possessing a novel 6/5/9/6/7/6 hexacyclic ring system.

The relative configuration of **1** was elucidated by using the ROESY data as shown in the computer-generated 3D drawing (Figure 3). The correlations between H-15, H-16, and H-17 indicated their *cis* relationship.

The absolute configuration of **1** was determined using TD-DFT to calculate its ECD spectrum and then comparing this spectrum with the corresponding experimental spectrum (ECD calculations were performed using Gaussian03 with the TD-DFT-B3LYP/6-31G(d,p) level of theory on B3LYP/6-31G(d)-optimized geometries, and the ECD curves were generated using Spec Dis).[21] The ECD spectrum generated for the (15*R*, 16*R*, 17*S*) diastereomer showed a negative Cotton effect at 221 nm and was in good agreement with the experimental data for **1**. Thus, the absolute configuration is as shown in Figure 3.

Strynuxline B (**2**) was obtained as a white, amorphous solid with a specific rotation of −190.7 (*c* 0.03, MeOH). The UV spectrum exhibited bands at 223 and 278 nm, characteristic of a phenyl chromophore. The IR spectrum revealed the presence of aromatic (1462 and 1436 cm^{-1}) and amide groups (1640 cm^{-1}).[20] The molecular formula was determined as $C_{21}H_{20}N_2O_3$, by HRESIMS (*m/z* 371.1371 $[M + Na]^+$; calcd 371.1372), with a mass 60 mass units lower than that of **1**. The ^{1}H NMR spectrum of **2** was similar to that of **1**, except for peaks indicating the presence of an *ortho*-disubstituted phenyl ring [δ_H 7.16 (1H, m, H-10), 7.18 (1H, m, H-11), 7.37 (1H, d, *J* = 7.5 Hz, H-9), 8.18 (1H, d, *J* = 7.5 Hz, H-12)] and the absence of peaks for two aromatic methoxyl groups in **2**. These differences indicated that **2** was a didemethoxy derivative of **1**. Analysis of the 2D NMR data confirmed that the remainder of the structure of **2** was the same as that of **1**.

The relative configuration of **2** was the same as that of **1**, as demonstrated by comparison of the ROESY spectra. In addition, the absolute configuration of **2** was identical to that of **1**, as revealed by their similar ECD curves (Figure 4) and their similar specific rotation values.

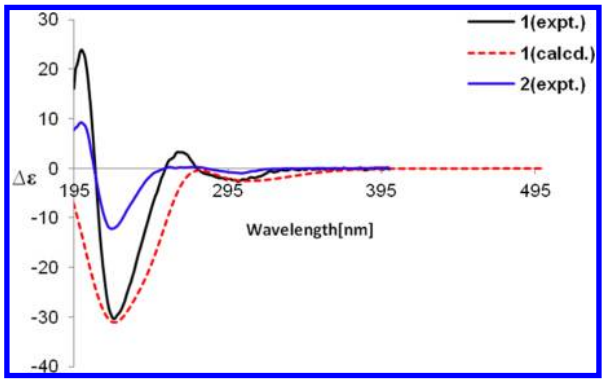

Figure 4. Experimental and calculated ECD spectra of strynuxline A (**1**) and experimental ECD spectrum of strynuxline B (**2**).

Strynuxlines A (**1**) and B (**2**) are the first examples of strychnan-type alkaloids with a 6/5/9/6/7/6 hexacyclic ring system.

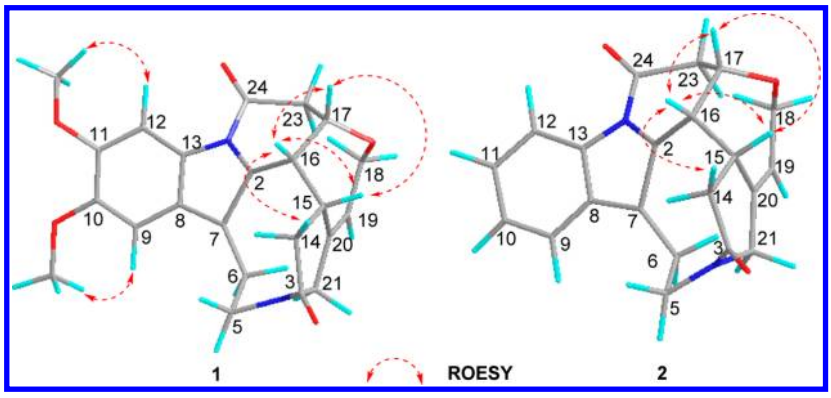

Figure 3. Stereostructure of strynuxlines A (**1**) and B (**2**) with selected ROESY correlations.

A plausible biosynthesis pathway for strynuxlines A (**1**) and B (**2**) is proposed in Scheme 1 (see Supporting Information). The pathway involves the enzymatically catalyzed Pictet–Spengler condensation of tryptamine with secologanin to provide strictosidine in the initial steps. Next, geissoschizine is formed, which is the common intermediate for all monoterpenoid indole alkaloids. Geissoschizine could undergo oxidation, dehydration, and oxidation to yield the key intermediate **i**, with cleavage of the C-2–C-3 bond. After oxidative cyclization, a new bond between the C-16/C-17/C-22 unit and C-2 would be formed.[22] The loss of the methoxycarbonyl group of **ii** would give **iii**, which, upon dehydration and hydroxylation, could lead to the formation of **iv**.[23] To complete the strychnan-type alkaloid backbone, two additional carbons are required. They might be derived from acetate, and the reaction most likely proceeds through **v**, formed by an aldol condensation involving acetyl-CoA.[24]

All 18 alkaloids were assayed for their cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using the MTT method with cisplatin and paclitaxel as positive controls. Strynuxline A (**1**) showed moderate cell growth inhibitory activity against five human cancer cell lines, HL60, SMMC7721, A549, MCF7, and SW480 (IC_{50} values 7.15, 9.40, 17.05, 12.18, and 13.33 μM, respectively), by the MTT method. Strynuxline B (**2**) showed weak cell growth inhibitory activity against the above five human cancer cell lines, (IC_{50} values 16.46, 15.73, 18.05, 11.23, and 18.98 μM for HL60, SMMC7721, A549, MCF7, and SW480, respectively) using the above method. The other alkaloids showed no cytotoxicity in this assay (IC_{50} > 40 μM).

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ECD spectra were recorded on an Applied Photophysics Chirascan spectrometer. UV spectra were recorded on a UV 210A spectrophotometer. IR spectra were taken on a Bio-Rad FTS-135 spectrophotometer (KBr). HRESIMS data were measured on a VG Auto Spec-3000 spectrometer. ESIMS data were obtained on a Finnigan MAT 90 spectrometer. NMR spectra were recorded on Bruker AM-400, DRX-500, and Avance III-600 NMR spectrometers using TMS as an internal standard.

Plant Material. The seeds of *S. nux-vomica* were collected from Mengna County, Yunnan Province, P. R. China, and were identified by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. CUI20090818) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The seeds of *S. nux-vomica* (6 kg) were extracted with 95% EtOH, and the pH of the crude extract was adjusted with saturated tartaric acid to 2. The acidic mixture was defatted with petroleum ether (PE) and then extracted with $CHCl_3$. The aqueous phase was basified to pH ∼ 10 with saturated Na_2CO_3 and then extracted with $CHCl_3$ to obtain crude alkaloids. The crude alkaloids (48 g) were separated on a silica gel column (200–300 mesh; $CHCl_3$/MeOH, 1:0 → 0:1), yielding five major fractions (Fr 1–5). Fraction 1 was chromatographed over a series of silica gel columns ($CHCl_3$/acetone and $CHCl_3$/MeOH) to afford compounds **1** (8 mg) and **2** (25 mg). Fraction 2 (9.2 g) was further chromatographed over a reversed-phase C_{18} silica gel medium-pressure column (MeOH/H_2O, 1:1 → 1:0) to give four fractions (Fr 2A–2D). Fraction 2A (1.6 g) was chromatographed over a series of silica gel columns ($CHCl_3$/acetone and $CHCl_3$/MeOH) to afford compounds **3** (520 mg) and **16** (24 mg). Compound **6** (780 mg) was crystallized in acetone from fraction 2B (1.2 g). Fraction 2C (1.5 g) was separated on a silica gel column (300–400 mesh; PE/acetone, 3:1), yielding three fractions (Fr 2C1–2C3). Fraction 2C1 (600 mg) was purified using a Sephadex LH-20 column eluted with MeOH, followed by semipreparative HPLC using a Waters XBridge C_{18} (4.6 × 250 mm, 5 μm) column with 85% MeOH/H_2O to afford compounds **4** (28 mg), **5** (48 mg), and **17** (33 mg). Compounds **9** (8 mg), **12** (16 mg), **13** (68 mg), and **15** (66 mg) were separated from fraction 2C2 (320 mg) by semipreparative HPLC using a Waters XBridge C_{18} (4.6 × 250 mm, 5 μm) column with 60% MeCN/H_2O. Compounds **10** (25 mg), **11** (36 mg), **14** (13 mg), and **18** (58 mg) were obtained from fraction 2C3 (163 mg) by semipreparative HPLC using an Waters XBridge C_{18} (4.6 × 250 mm, 5 μm) column with 50% MeOH/H_2O. Fraction 3 (7.8 g) was further chromatographed over a reversed-phase C_{18} silica gel medium-pressure column (MeOH/H_2O, 1:1 → 1:0) to give four fractions (Fr 3A–3D). Fraction 3A was separated by semipreparative HPLC using a Waters XBridge C_{18} (4.6 × 250 mm, 5 μm) column with 30% MeOH/H_2O to give compounds **7** (16 mg) and **8** (28 mg).

Strynuxline A (1): white, amorphous solid; $[\alpha]_D^{14}$ −254.6 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3429, 2952, 2924, 1669, 1634, 1463, and 1386 cm^{-1}; UV (MeOH) λ_{max} 263 (ε 14 668), 301 nm (6524); CD (0.0002 M, MeOH) λ_{max} ($\Delta\varepsilon$) 221 (−30.4), 302 (−2.7) nm; EIMS *m/z* 408 $[M]^+$; HREIMS *m/z* 408.1673 (M; calcd for $C_{23}H_{24}N_2O_5$, 408.1685).

Strynuxline B (2): white, amorphous solid; $[\alpha]_D^{14}$ −190.7 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3424, 2955, 2923, 1640, 1462, and 1376 cm^{-1}; UV (MeOH) λ_{max} 223 (ε 15 779), 278 nm (4756); CD (0.0005 M, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−12.2), 304 (−1.0) nm; ESIMS *m/z* 349 $[M + H]^+$, 371 $[M + Na]^+$; HRESIMS *m/z* 371.1371 (M + Na; calcd for $C_{21}H_{20}N_2O_3Na$, 371.1372).

Cytotoxicity Bioassays. The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW480. All cells were

Table 1. ^1H and ^{13}C NMR Data of Strynuxlines A (1) and B (2) in $CDCl_3$

	strynuxline A (1)		strynuxline B (2)	
no.	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[d]
2		130.1 s		131.8 s
3		174.9 s		174.8 s
5α	3.17 overlap	49.3 t	3.19 overlap	49.2 t
5β	4.57 t (13.4)		4.48 t (13.5)	
6α	2.80 m	25.7 t	2.90 overlap	25.5 t
6β	3.14 overlap		3.12 m	
7		117.7 s		117.8 s
8		124.7 s		132.1 s
9	6.78 s	100.4 d	7.37 d (7.5)	118.0 d
10		146.9 s	7.16 m	123.7 d
11		148.1 s	7.18 m	125.3 d
12	7.88 s	99.7 d	8.18 d (7.5)	115.7 d
13		129.5 s		135.3 s
14α	2.48 d (15.1)	39.5 t	2.42 d (15.3)	39.4 t
14β	2.86 dd (15.1, 6.3)		2.88 dd (15.3, 6.4)	
15	3.51 br s	41.5 d	3.52 br s	41.4 d
16	3.29 br s	48.7 d	3.31 br s	48.7 d
17	4.13 overlap	83.7 d	4.16 overlap	83.8 d
18α	4.10 d (13.8)	68.0 t	4.09 d (14.3)	68.0 t
18β	4.26 dd (13.8, 7.0)		4.23 dd (14.3, 7.2)	
19	6.01 br s	123.3 d	6.01 br s	123.3 d
20		145.1 s		145.1 s
21α	3.86 overlap	52.9 t	3.87 overlap	52.8 t
21β	4.19 d (15.9)		4.18 d (16.6)	
23α	2.77 d (18.7)	42.3 t	2.73 d (19.3)	42.3 t
23β	3.15 overlap		3.20 overlap	
24		168.0 s		167.9 s
10-OCH_3	3.86 s	56.3 q		
11-OCH_3	3.86 s	56.3 q		

[a]Measured at 400 MHz. [b]Measured at 100 MHz. [c]Measured at 600 MHz. [d]Measured at 150 MHz.

cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed using the MTT method in 96-well microplates.[25] Briefly, adherent cells (100 μL) were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, and suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the tested compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h. Cisplatin and paclitaxel (Sigma, St. Louis, MO, USA) were used as positive controls. After treatment, cell viability was measured and the cell growth curve was plotted. IC_{50} values were calculated by the Reed and Muench method.[26]

■ ASSOCIATED CONTENT

Supporting Information

This material (biosynthesis pathway proposed for strynuxlines A (**1**) and B (**2**), 1D and 2D NMR, HREIMS, ESIMS, HRESIMS, UV, IR, and ECD spectra of strynuxlines A (**1**) and B (**2**)) is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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