

Article

Monoterpene Indole Alkaloids with Ca_v3.1 T-Type Calcium Channel Inhibitory Activity from *Catharanthus roseus*

Zhen-Tao Deng^{1,2,3,†}, Wen-Yan Li^{2,†}, Lei Wang², Zhi-Ping Zhou^{1,2}, Xing-De Wu^{2,4,*}, Zhong-Tao Ding^{1,*} and Qin-Shi Zhao^{2,*}

- ¹ Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education and Yunnan Province, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China; dengzhen tao@mail.kib.ac.cn (Z.-T.D.); zhouzhiping@mail.kib.ac.cn (Z.-P.Z.)
- ² State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; liwenyan@mail.kib.ac.cn (W.-Y.L.); laralei@163.com (L.W.)
- ³ University of Chinese Academy of Sciences, Beijing 100049, China
- ⁴ Key Laboratory of Ethnic Medicine Resource Chemistry, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University, Kunming 650500, China
- * Correspondence: wuxingde@mail.kib.ac.cn (X.-D.W.); ztding@ynu.edu.cn (Z.-T.D.); qinshizhao@mail.kib.ac.cn (Q.-S.Z.); Tel.: +86-871-65223058 (Q.-S.Z.)
- † These authors contributed equally to this work.

Abstract: *Catharanthus roseus* is a well-known traditional herbal medicine for the treatment of cancer, hypertension, scald, and sore in China. Phytochemical investigation on the twigs and leaves of this species led to the isolation of two new monoterpene indole alkaloids, catharanosines A (**1**) and B (**2**), and six known analogues (**3–8**). Structures of **1** and **2** were established by ¹H-, ¹³C- and 2D-NMR, and HREIMS data. The absolute configuration of **1** was confirmed by single-crystal X-ray diffraction analysis. Compound **2** represented an unprecedented aspidosperma-type alkaloid with a 2-piperidinyl moiety at C-10. Compounds **6–8** exhibited remarkable Ca_v3.1 low voltage-gated calcium channel (LVGCC) inhibitory activity with IC₅₀ values of 11.83 ± 1.02, 14.3 ± 1.20, and 14.54 ± 0.99 μM, respectively.

Keywords: *Catharanthus roseus*; monoterpene indole alkaloid; catharanosine A; Ca_v3.1 low voltage-gated calcium channel (LVGCC)



Citation: Deng, Z.-T.; Li, W.-Y.; Wang, L.; Zhou, Z.-P.; Wu, X.-D.; Ding, Z.-T.; Zhao, Q.-S. Monoterpene Indole Alkaloids with Ca_v3.1 T-Type Calcium Channel Inhibitory Activity from *Catharanthus roseus*. *Molecules* **2021**, *26*, 6516. <https://doi.org/10.3390/molecules26216516>

Academic Editor:
Giovanni Palmisano

Received: 30 August 2021
Accepted: 25 October 2021
Published: 28 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Monoterpene indole alkaloids (MIAs) are one of the largest natural product families constructed from indole and monoterpene moieties, and commonly found in Apocynaceae, Rubiaceae, and Loganiaceae families [1]. To date, more than 3000 MIAs have been reported, many of which have been found to exhibit important pharmaceutical effects [2,3]. Representative MIAs such as reserpine, vinblastine/vincristine, and quinine are used clinically for the treatment of hypertension, cancer, and malaria, respectively [4]. In light of their diverse and complex structures and high druggability, MIAs have attracted great interest from chemical and pharmacological communities and have been a potent resource for new drug discovery.

Catharanthus roseus (L.) G. Don (Apocynaceae), a tropical perennial subshrub, is a well-known traditional herbal medicine for treating cancer, hypertension, scald, and sore in China [5]. Early phytochemical studies on this plant have led to the isolation of an array of MIAs, including the well-known anticancer drugs vinblastine and vincristine [6]. The discovery of these two drugs has been regarded as one of the most important developments in both natural product chemistry and the clinical treatment of cancer during the 1960s to 1980s [7–10]. In recent years, some new and bioactive MIAs were still reported from this

plant [11–17]. In our continuing search for structurally unique and pharmaceutically interesting MIAs from medicinal plants [18–22], a phytochemical study of the twigs and leaves of *C. roseus* was undertaken and led to the identification of two new MIAs, catharanosines A (1) and B (2), and six known analogues (Figure 1). Compound 2 was found to represent an unprecedented aspidosperma-type alkaloid with a piperidine moiety at C-10. Due to the limited amount of 1 and 2, only compounds 3–8 were screened for their inhibitory activity on Ca_v3.1 low voltage-gated calcium channel (LVGCC), an important therapeutic target for cardiovascular disease [23]. Herein, the isolation, structure determination, and bioactivities of compounds 1–8 are described.

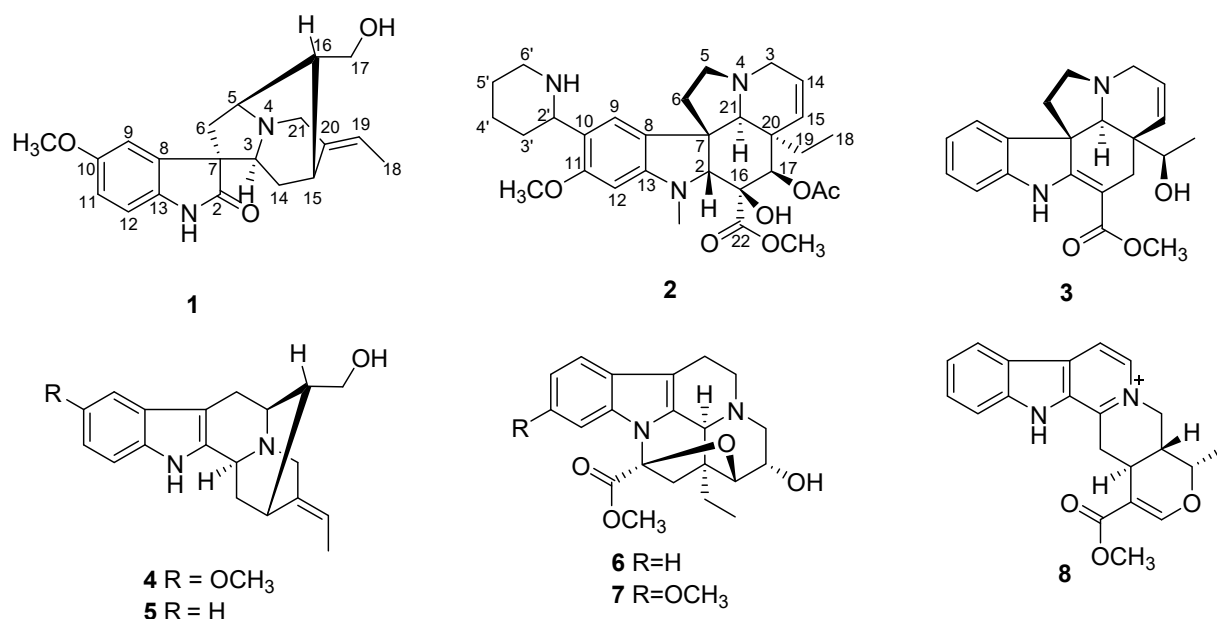


Figure 1. Structures of compounds 1–8.

2. Results

2.1. Structure Elucidation

The total crude alkaloid fraction was subjected to MCI gel, silica gel, and Sephadex LH-20 to afford two new MIAs, catharanosines A (1) and B (2), and six known analogues. Compared with literature data, the known compounds were identified as (*R*)-19-hydroxytabersonine (3) [24], lochnerine (4) [25], normacusine B (5) [26], vincapusine (6) [27], vincarodine (7) [28], and serpentine (8) [29], by comparing their spectroscopic data with those reported in the literature.

Compound 1, colorless crystals, had a molecular formula of C₂₀H₂₄N₂O₃ according to the ¹³C NMR data and the HR-EI-MS ion at *m/z* 340.1782 [M]⁺ (calcd. 340.1787), which corresponded to 20 indices of hydrogen deficiency. The IR spectrum (Figure S9) displayed absorption bands resulting from hydroxy (3439 cm⁻¹), carbonyl (1712 cm⁻¹), and aromatic (1630 and 1465 cm⁻¹) functionalities. The presence of an oxindole chromophore was revealed by the UV absorptions (Figure S8) at 205, 248, 298 nm [30]. The ¹H NMR spectrum displayed resonances (Table 1 and Figure S1) for a 1,2,4-trisubstituted benzene ring (δ_{H} 6.59 dd, *J* = 8.4, 2.4 Hz, H-11; 6.65 d, *J* = 8.4 Hz, H-12; and 6.76 d, *J* = 2.4 Hz, H-9), an olefinic proton (δ_{H} 5.09 q, *J* = 6.7 Hz, H-19), an oxymethylene (δ_{H} 3.34, m, H-17), a methyl group (δ_{H} 1.40 d, *J* = 6.7 Hz, H₃-18), and a methoxy group [δ_{H} 3.63 (s)]. The ¹³C NMR spectrum (Figure S2), with the aid of the HSQC data (Figure S3), showed 20 carbon resonances (Table 1) attributable to two methyls (one methoxy at δ_{C} 55.7), four methylenes (one oxygenated at δ_{C} 65.0), eight methines (three aromatic at δ_{C} 109.7, 111.8, and 114.9 and one olefinic at δ_{C} 114.6), and six nonprotonated carbons (three aromatic at δ_{C} 132.0,

135.3, and 154.9, one olefinic at δ_C 135.6, and one lactam carbonyl at δ_C 184.4). Comparison of the ^1H and ^{13}C NMR data (Table 1) of **1** with those of rauvomitorine V, an affinisine oxindole type alkaloid from *Rauwolfia vomitoria* [31], revealed their structural similarity, except the absence of one methoxy group and that one aromatic methine in **1** replaced one aromatic nonprotonated carbon in the latter. The only methoxy group was located at C-10 through HMBC correlations (Figure 2) of H-9 (δ_H 6.76, d, $J = 2.4$ Hz) with C-7 (δ_C 57.4), C-8 (δ_C 132.0), C-10 (δ_C 154.9), and C-13 (δ_C 135.3), as well as cross-peaks of H-9 and H-11 with the methoxy group in ROESY spectrum (Figure 2).

Table 1. ^1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) spectroscopic data of compounds **1** and **2** in CDCl_3 (δ in ppm, J in Hz).

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
2		184.4	3.71, s	83.3
3	3.11, d (8.8)	62.8	3.48, d (13.2) 2.90, d (13.2)	50.8
5	2.87, dd (6.0, 2.9)	59.3	3.38, m 2.69, m	51.6
6	2.50, dd (12.8, 6.0) 1.74, d (12.8)	44.3	2.41, m 2.17, m	43.6
7		57.4		52.8
8		132.0		124.2
9	6.76, d (2.4)	114.9	7.36, s	122.1
10		154.9		114.4
11	6.59, dd (8.4, 2.4)	111.8		158.0
12	6.65, d (8.4)	109.7	5.97, s	92.3
13		135.3		153.8
14	1.98, m 1.33, m	28.4	5.82, ddd (10.2, 4.9, 1.3)	124.4
15	2.65, s	26.1	5.18, d (10.2)	130.0
16	1.78, m	47.8		79.4
17	3.34, m	65.0	5.34, s	76.2
18	1.40, d (6.7)	12.0	0.43, t (7.4)	7.5
19	5.09, q (6.7)	114.6	1.56, m 0.97, m	30.9
20		135.6		42.7
21	3.38, s	48.3	2.76, s	66.5
22				171.8
2'			4.29, br s	54.6
3'			2.10, m 1.80, m	28.7
4'			1.92, m 1.53, m	23.4
5'			1.86, m 1.66, m	22.0
6'			3.10, d (11.8) 2.83, m	45.7
N-Me			2.65, s	37.9
10-OMe	3.63, s	55.7		
11-OMe			3.80, s	55.7
22-OMe			3.76, s	52.3
OAc				170.8
			2.03, s	21.0

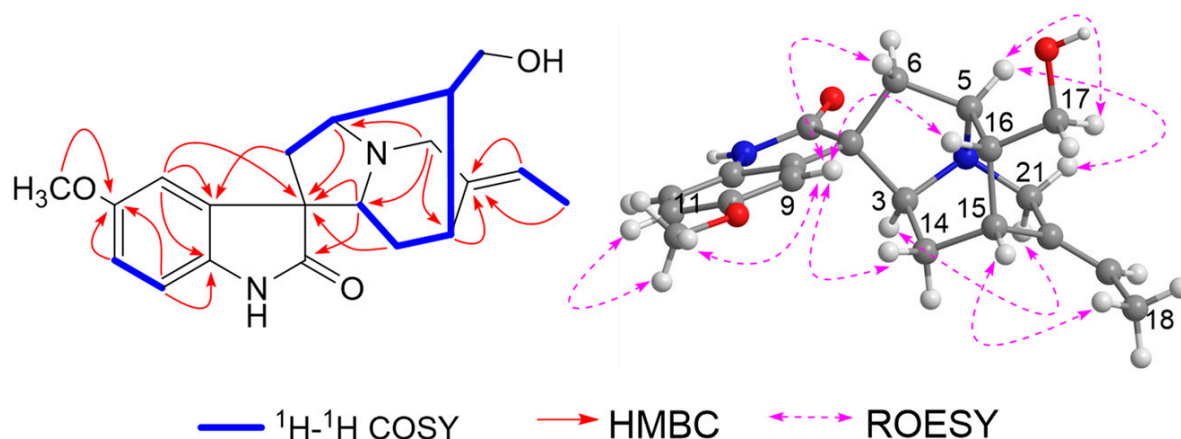


Figure 2. Key ^1H - ^1H COSY, HMBC and ROESY correlations of **1**.

The relative configuration of **1** was assigned via the ROESY (Figure S6) and ^{13}C NMR data (Table 1). The configuration of the spirocyclic C-7 was assigned as *S* by the ROESY correlations (Figure 2) of H-9 with H-6 β , H-14 β , and H-16, coupled with the diagnostic chemical shifts of C-2 (δ_{C} 184.4), C-3 (δ_{C} 62.8), and C-8 (δ_{C} 132.0) [32,33]. The ROESY correlations of H-3 and H-5 with H₂-21, of H-5 with H₂-17, and of H-9 with H-16 indicated the α -orientations of H-3, H-5, and H₂-17. The rigid structure of the bridge ring system required H-15 to be α -oriented. Moreover, the *E*-geometry for the double bond between C-19 and C-20 was determined by the ROESY cross-peak of H-15 and H₃-18. Finally, single-crystal X-ray diffraction analysis of **1** using Cu-K α radiation unambiguously assigned the absolute configuration as (3*S*, 5*S*, 7*S*, 15*R*, 16*R*) based on a Flack parameter of 0.11(8) (Figure 3). Accordingly, the structure of **1** was deduced and named as catharansine A. Many sarpagine type alkaloids have been reported from natural resources [34–38], however, only a few of the corresponding oxindoles were discovered, including affinisine, talpinine, and chitosenine types oxindole alkaloids [31,39–42]. To date, no general scaffold name for this small group of oxindole alkaloid are reported, so we tentatively name them as spiroindoxyl sarpagane alkaloid.

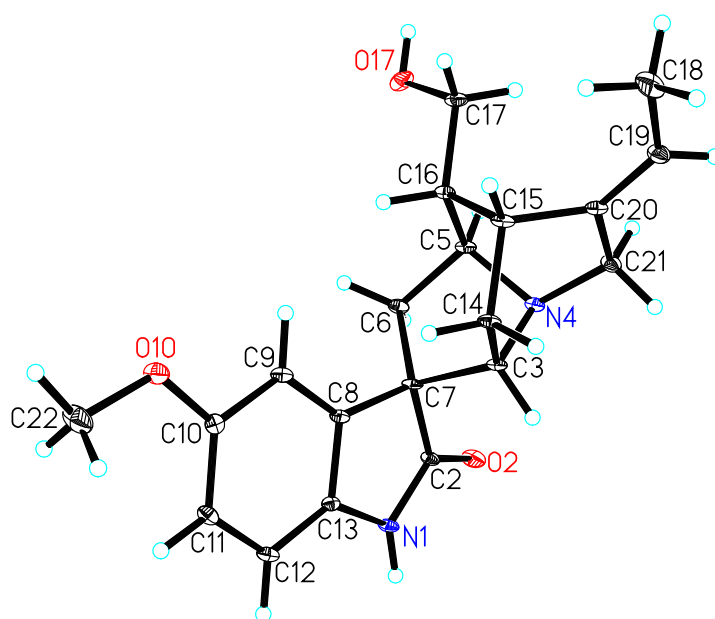


Figure 3. X-ray ORTEP drawing of **1**.

The HREIMS spectrum of **2** showed a molecular ion peak at m/z 539.3000 $[M]^+$ (calcd. 539.2995), consistent with a molecular formula of $C_{30}H_{41}N_3O_6$, suggesting 24 indices of hydrogen deficiency. The IR spectrum (Figure S18) exhibited absorptions attributable to hydroxy (3432 cm^{-1}) and carbonyl (1741 cm^{-1}) groups, and a benzene ring (1621 , 1506 , and 1453 cm^{-1}). The characteristic UV absorptions (Figure S17) at 213, 261, 309 nm indicated a dihydroindole chromophore [43]. In ^1H NMR spectrum (Figure S10 and Table 1), two aromatic singlets at δ_{H} 7.36 (s, H-9) and 5.97 (s, H-12) were observed for the 10,11-disubstituted dihydroindole moiety. The ^{13}C NMR spectrum (Figure S11 and Table 1) showed the presence of five methyls (one N-methyl, one acetyl methyl, and two methoxy groups), four methylenes, seven methines (one oxygenated at δ_{C} 76.2, two aromatic at δ_{C} 92.3 and 122.1, and two olefinic at δ_{C} 124.4 and 130.0), and nine nonprotonated carbons (one oxygenated at δ_{C} 79.4, two ester carbonyl at δ_{C} 170.8 and 171.8 and four aromatic at δ_{C} 114.4, 124.2, 153.8, and 158.0). In addition, the carbon resonances (Table 1) at δ_{C} 54.6, 28.7, 23.4, 22.0, and 45.7, along with the ^1H - ^1H COSY correlations (Figure 4) of H-2'/H₂-3'/H₂-4'/H₂-5'/H₂-6', indicated the presence of 2-piperidinyl unit in **2**. The ^1H and ^{13}C NMR spectroscopic data (Table 1) of **2** closely resembled those of vindoline [44], with the exception of the aforementioned 2-piperidinyl moiety. The HMBC correlations (Figure 4) from H-9 (δ_{H} 7.36) to C-7 (δ_{C} 52.8), C-8 (δ_{C} 124.2), C-10 (δ_{C} 114.4), and C-13 (δ_{C} 153.8), from H-2' (δ_{H} 4.29, br s) to C-9 (δ_{C} 122.1), C-10, and C-11 (δ_{C} 158.0), as well as the cross-peak of H-12 with N-methyl and 11-methoxy groups, revealed the linkage of vindoline with piperidine moiety through C-10-C-2' bond.

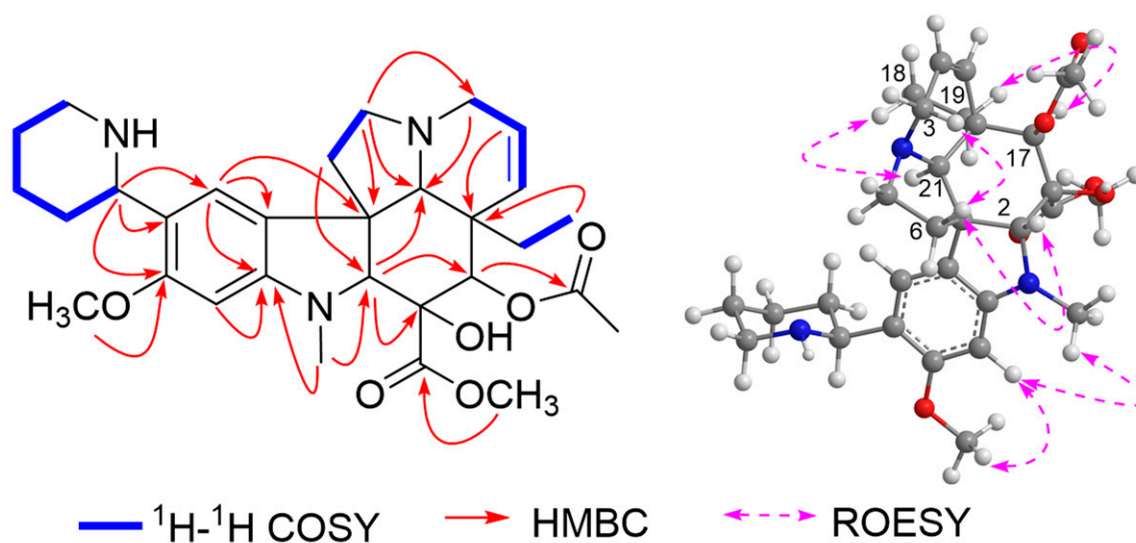
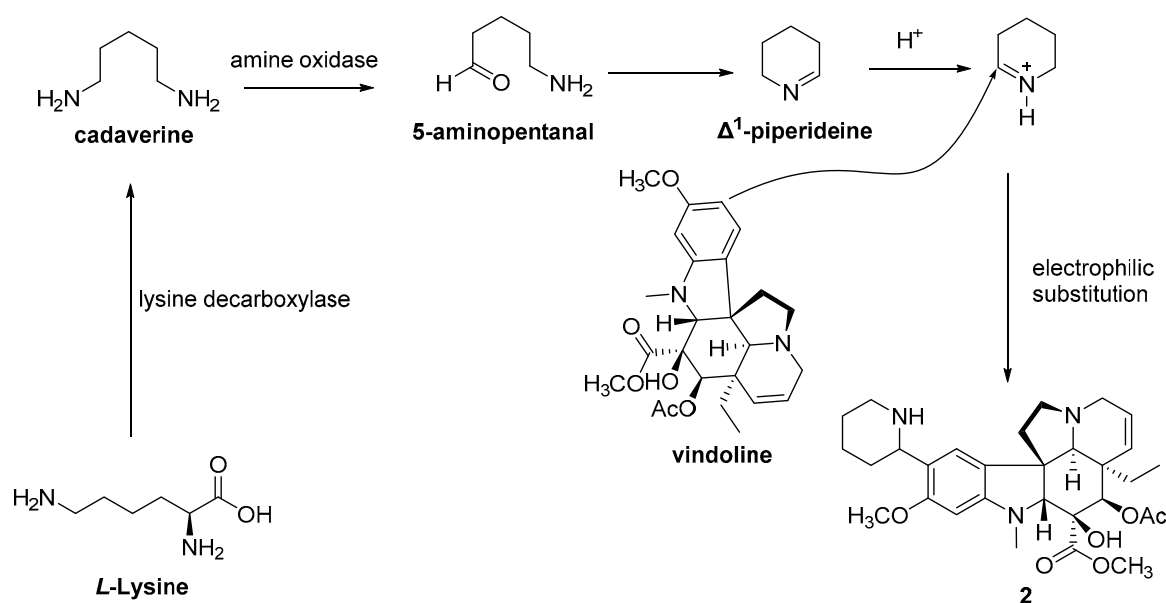


Figure 4. Key ^1H - ^1H COSY, HMBC and ROESY correlations of **2**.

The ROESY correlations (Figure 4) of H-2/H-6 β and H-6 β /H-3 β suggested the β -orientation of H-2 and the R^* configuration for C-7. H-17, H-21, and the ethyl groups were determined as α -orientated by the ROESY cross-peaks of H-21/H₃-18 and H-17/H₂-19. The β -orientation of 16-OH was established by similar carbon resonances at C-2 (δ_{C} 83.3; $\Delta\delta_{\text{C}}$ +0.1), C-16 (δ_{C} 79.4; $\Delta\delta_{\text{C}}$ -0.1), and C-17 (δ_{C} 76.2; $\Delta\delta_{\text{C}}$ +0.0) with those of vindoline [44]. However, other ROESY correlations for **2** were insufficient to determine the configuration at C-2'. Consequently, the structure of compound **2** was identified and named as catharansine B. To our knowledge, compound **2** represented the first aspidosperma-type alkaloid with a 2-piperidinyl moiety at C-10. Biogenetically, compound **2** was likely to be formed through electrophilic substitution at C-10 of vindoline by Δ^1 -piperidinium cation derived from *L*-lysine via decarboxylation and oxidation under the catalysis of lysine decarboxylase and amine oxidase, and subsequently cyclization and protonation (Scheme 1) [45,46].



Scheme 1. Plausible biosynthesis pathway of 2.

2.2. Biological Activity

Due to the traditional use of *C. roseus* for treating hypertension in China, compounds 3–8 were evaluated for the effects on $Ca_v3.1$ low-voltage-gated calcium channel, which plays an important role in the regulation of cardiovascular disease. At a concentration of $50 \mu\text{M}$, compounds 6–8 showed strong inhibitions on $Ca_v3.1$ (Figure 5), while compounds 3–5 exhibited weak activity with inhibition rate of less than 50%. Then, compounds 6–8 were further evaluated for their dose-dependent relationships on $Ca_v3.1$ at a concentration range from 1.6 to $50.0 \mu\text{M}$. The results showed that compounds 6–8 dose-dependently inhibited on $Ca_v3.1$ with IC_{50} values of 11.83 ± 1.02 , 14.30 ± 1.20 , and $14.54 \pm 0.99 \mu\text{M}$, as compared to mibefradil, an inhibitor of T-type VGCC, with IC_{50} value of $3.09 \pm 0.41 \mu\text{M}$ (Figure 6). These results indicated that compounds 6–8 were important antihypertensive active components of *C. roseus*.

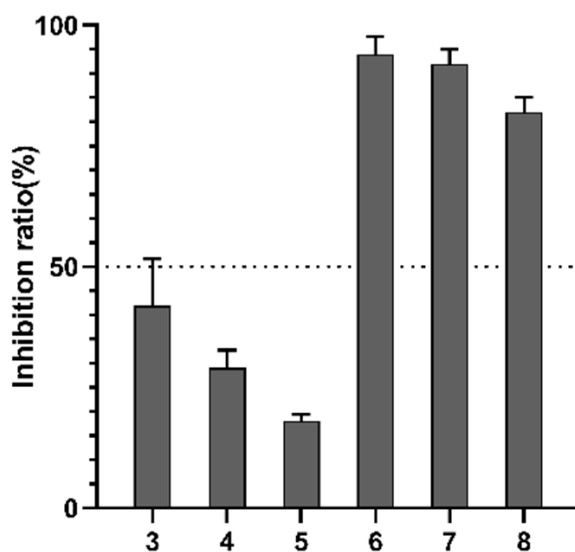


Figure 5. Effects of compounds 3–8 on current $Ca_v3.1$ low-voltage-gated calcium channel at $50 \mu\text{M}$. All the data were represented as mean \pm SD ($n = 3$).

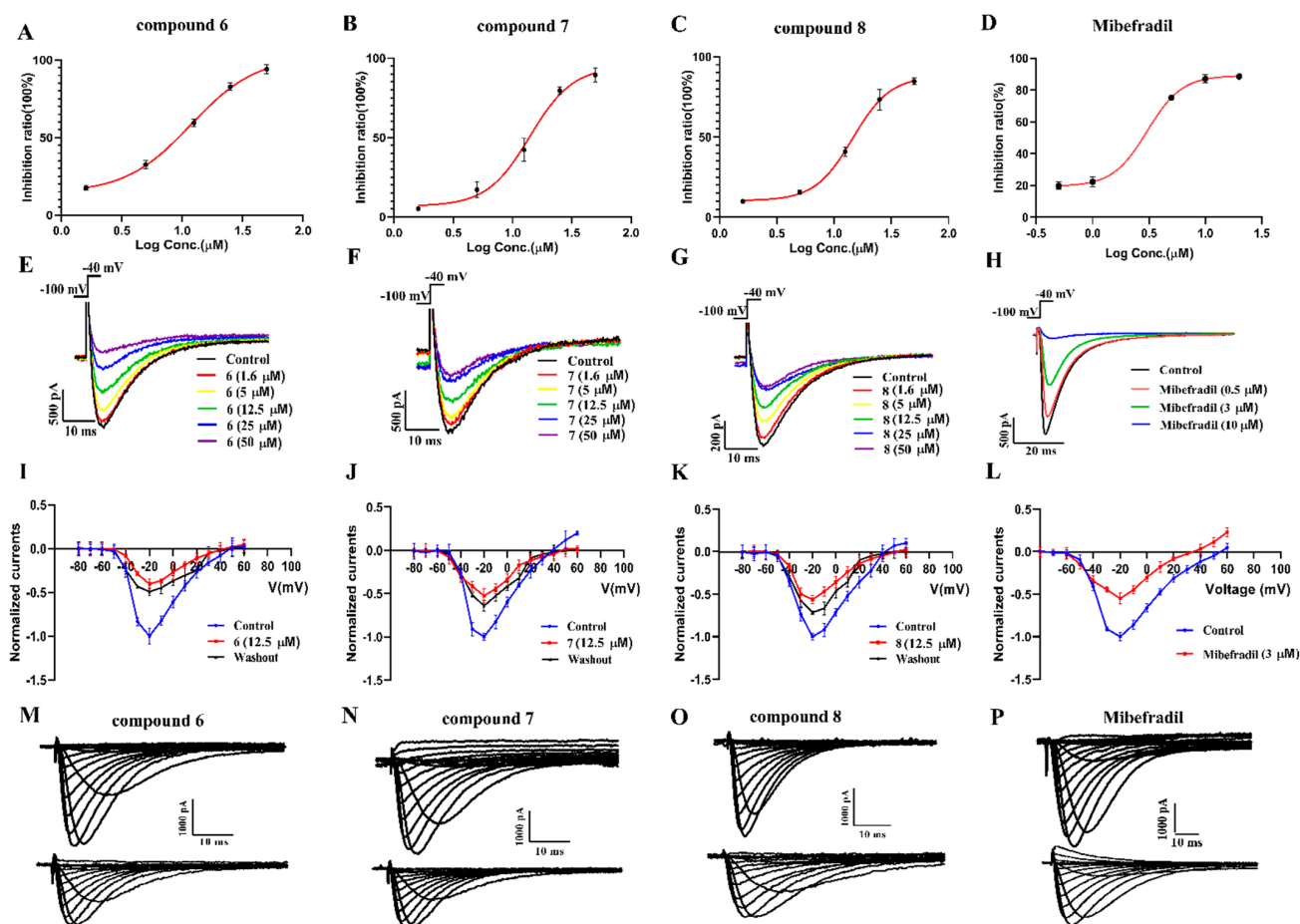


Figure 6. Effects of compounds 6–8 and the positive control (mibefradil) on current of $Ca_v3.1$. (A–D) Dose-responsive curves of 6–8 and mibefradil on peak current of $Ca_v3.1$. Data points represent mean \pm SD of three or four repetition measurements. Solid curve represents fit to the Hill equation. (E–H) Representative $Ca_v3.1$ peak current traces in the absence and presence of different concentrations of 6–8 and mibefradil. (I–L) Normalized I–V curves of $Ca_v3.1$ control (blue), $Ca_v3.1$ with 12.5 μ M 6–8 (red), 3 μ M mibefradil, and washout (black). All the data were represented as mean \pm SD ($n = 3$). (M–P) Current traces obtained with $Ca_v3.1$ at various membrane potentials (from -80 to $+60$ mV in 10 mV increment at 4 s intervals) from a holding potential of -100 mV (upper panel). Current traces obtained with $Ca_v3.1$ with 6–8 (12.5 μ M) and mibefradil (3 μ M) using same stimulating voltages (lower panel).

3. Materials and Methods

3.1. General

Optical rotations were measured with a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). Melting point was recorded on an X-4 micro melting point apparatus (Beijing Second Optical Instrument Factory, Beijing, China). IR spectra were obtained by a Tensor 27 spectrophotometer (Bruker, Karlsruhe, Germany) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). 1D and 2D spectra were run on a Bruker AM-400 or an Avance III 600 spectrometer (Bruker, Karlsruhe, Germany) with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. EIMS were recorded on a Waters Autospec Premier P776 spectrometer (Waters Corporation, Milford, MA, USA). Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China) and MCI gel (75–150 mm; Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents were distilled prior to use.

3.2. Plant Material

The whole plants of *C. roseus* were purchased from the Herb Material Market of Juhuaacun, Kunming, Yunnan Province, P. R. China, in June 2011, and identified by Prof. Xiao Cheng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (20110620C) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and Isolation

The air-dried whole plants of *C. roseus* (60 kg) were powdered and extracted with methanol (4 × 200 L) under reflux. The methanol was evaporated under reduced pressure to produce a residue, which was dissolved in hot water and adjusted to pH 2 with 0.5% HCl and then extracted with ethyl acetate (50 L × 3). The water-soluble portion was adjusted to pH 9.0 with sat. Na₂CO₃ and partitioned with CHCl₃ to yield the total crude alkaloids (200 g), which were chromatographed over silica gel column using a step-gradient eluting with CHCl₃-Me₂CO (1:0-0:1) to obtain five fractions A–E. Fraction B was applied to MCI gel column eluted with MeOH-H₂O (40%-100%) to give subfractions B1–B4. Fraction B1 was subjected to silica gel CC (CHCl₃-MeOH, 10:1) to obtain compounds **6** (15 mg), **7** (15 mg), and **8** (12 mg). Fraction B2 was subjected to silica gel CC (CHCl₃-MeOH, 10:1) to obtain compounds **3** (13 mg) and **4** (11 mg). Fraction C was subjected to silica gel CC (CHCl₃-MeOH, 9:1) to obtain compound **5** (5 mg). Fraction D was applied to MCI gel column eluted with MeOH-H₂O (30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0, each 3 L) to give subfractions D1–D3. Fraction D1 was subjected to silica gel CC (CHCl₃-MeOH, 20:1) and then purified by Sephadex LH-20 (CHCl₃-MeOH, 1:1) to obtain compound **1** (2 mg). Compound **2** (1.5 mg) was obtained by Sephadex LH-20 (MeOH) from Subfraction D3.

3.4. Spectroscopic Data of Compounds 1 and 2

Catharanosine A (**1**): Colorless crystals, mp 104–108 °C; $[\alpha]_D^{23}$ -21.80 (*c* 0.12, MeOH); UV (MeOH): λ_{\max} (log ϵ) 298 (2.61), 248 (3.04), 205 (3.64) nm; IR (KBr) ν_{\max} : 3439, 2932, 1712, 1630, 1465, 1206 cm⁻¹. ¹H and ¹³C NMR spectral data, see Table 1; HR-EI-MS *m/z* 340.1782 (calcd. for C₂₀H₂₄N₂O₃, 340.1787).

Catharanosine B (**2**): Yellow oil; $[\alpha]_D^{23}$ -98.25 (*c* 0.14, MeOH); UV (MeOH): λ_{\max} (log ϵ) 309 (3.29), 261 (3.52), 213 (3.99) nm; IR (KBr) ν_{\max} : 3432, 2937, 1741, 1621, 1506, 1453, 1433, 1383, 1231, 1041 cm⁻¹. ¹H and ¹³C NMR spectral data, see Table 1; HR-EI-MS *m/z* 539.3000[M + H]⁺ (calcd. for C₃₀H₄₁N₃O₆, 539.2995).

3.5. X-ray Crystal Data of 1

C₂₀H₂₄N₂O₃·H₂O, *M* = 358.43, *a* = 12.2146(2) Å, *b* = 12.2146(2) Å, *c* = 24.9041(6) Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, *V* = 3715.60(15) Å³, *T* = 100(2) K, space group *P*41212, *Z* = 8, μ (CuK α) = 0.727 mm⁻¹, 17875 reflections measured, 3394 independent reflections (*R*_{int} = 0.1146). The final *R*₁ values were 0.0651 (*I* > 2 σ (*I*)). The final *wR*(*F*²) values were 0.1599 (*I* > 2 σ (*I*)). The final *R*₁ values were 0.0654 (all data). The final *wR*(*F*²) values were 0.1603 (all data). The goodness of fit on *F*² was 1.125. Flack parameter = 0.11(8). Crystallographic data for compound **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers: CCDC 2106217). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk.

3.6. Ca_v3.1 T-Type Calcium Channel Inhibitory Activity Assay

HEK293T cells purchased from ATCC were cultured at 37 °C with 5% CO₂ in Dulbecco's modified Eagle medium with glucose, L-glutamine, pyruvate, 10% FBS, and 1% Pen-Strep. Cells were seeded at low density onto 24-well plates 24 h before transfection. Adherent cells were transfected using Lipofectamine 2000 reagent (Invitrogen) with 300 ng Ca_v3.1 cDNA and recorded after 48 h. Whole-cell voltage-clamp recordings were performed at room temperature (24 °C). The peak currents of Ca_v3.1 were elicited by 150 ms depolarization from a holding potential of -100 mV to -40 mV at 4 s intervals. Borosil-

icate glass micropipettes were pulled to produce a resistance of 4–6 M Ω and filled with intracellular recording solution containing 130 mM CsCl, 2 mM MgCl₂, 10 mM EGTA, 5 mM Na-ATP, 10 mM HEPES (pH 7.2 with CsOH). The extracellular recording solution was composed of 145 mM CsCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM glucose, 10 mM HEPES (pH 7.4 with CsOH). The current trace of Ca_v3.1 in different states was analyzed by the Clampfit 10.6. Data were processed using the software Graphpad Prism 8.0.

4. Conclusions

In summary, two new MIAs, catharanosine A (**1**) and catharanosine B (**2**), together with six known compounds (**3–8**) were isolated from the twigs and leaves of *Catharanthus roseus*. The absolute configuration of compound **1** was confirmed by X-ray crystal diffraction analysis. Compound **2** represented the first aspidosperma-type alkaloid with a 2-piperidinyl moiety at C-10. Compounds **6–8** dose-dependently inhibited on Ca_v3.1 with IC₅₀ values of 11.83 ± 1.02, 14.30 ± 1.20, and 14.54 ± 0.99 μ M.

Supplementary Materials: Supplementary Materials are available online. Figures S1–S9: 1D and 2D NMR, HREIMS, UV, and IR spectra of compound **1**. Figures S10–S18: 1D and 2D NMR, HREIMS, UV and IR spectra of compound **2**.

Author Contributions: Z.-T.D. (Zhen-Tao Deng) isolated the compounds and elucidated the structures, and wrote the manuscript; W.-Y.L. tested the bioactivity assay; L.W. and Z.-P.Z. performed the experiments and analyzed the data; X.-D.W. offered guidance to the experiment and revised the manuscript; Z.-T.D. (Zhong-Tao Ding) and Q.-S.Z. designed the research project and supervised this work. All authors have read and agreed to the published version of the manuscript.

Funding: Please add: This work was supported by the project of the Natural Science Foundation of China (Nos. 81773611 and 21837003), the Science and Technology Program of Yunnan province (No. 2019FA037), the NSFC-Joint Foundation of Yunnan Province (No. U1502223), the Top Young Talent of the Ten Thousand Talents Program of Yunnan Province (X.-D. Wu), and the Youth Innovation Promotion Association CAS (X.-D. Wu).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Supplementary materials.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Zhang, Y.; Yuan, Y.X.; Goto, M.; Guo, L.L.; Lee, K.H.; Hao, X.J. Tabernaemines A–I, cytotoxic vobasiny-iboga-type bisindole alkaloids from *Tabernaemontana corymbosa*. *J. Nat. Prod.* **2018**, *81*, 562–571. [[CrossRef](#)]
2. Facchini, P.J.; De Luca, V. Opium poppy and Madagascar periwinkle: Model non-model systems to investigate alkaloid biosynthesis in plants. *Plant J.* **2008**, *54*, 763–784. [[CrossRef](#)] [[PubMed](#)]
3. De Luca, V.; Salim, V.; Atsumi, S.M.; Yu, F. Mining the biodiversity of plants: A revolution in the making. *Science* **2012**, *336*, 1658–1661. [[CrossRef](#)] [[PubMed](#)]
4. Ban, Y.; Murakami, Y.; Iwasawa, Y.; Tsuchiya, M.; Takano, N. Indole alkaloids in medicine. *Med. Res. Rev.* **1988**, *8*, 231–308. [[CrossRef](#)]
5. Nanjing University of Traditional Chinese Medicine. *Dictionary of Traditional Chinese Medicine*; Shanghai Science and Technology Press: Shanghai, China, 2006; pp. 628–630.
6. Zhou, X.J.; Rabmani, R. Preclinical and clinical pharmacology of *Vinca* alkaloids. *Drugs* **1992**, *44*, 1–16. [[CrossRef](#)]
7. Himes, R.H. Interaction of the *Catharanthus* (*Vinca*) alkaloids with tubulin and microtubules. *Pharmacol. Ther.* **1991**, *51*, 257–267. [[CrossRef](#)]
8. Jordan, M.A.; Thrower, D.; Wilson, L. Mechanism of inhibition of cell proliferation by *Vinca* alkaloids. *Cancer Res.* **1991**, *51*, 2212–2222. [[PubMed](#)]
9. Ngan, V.K.; Bellman, K.; Hill, B.T.; Wilson, L.; Jordan, M.A. Mechanism of mitotic block and inhibition of cell proliferation by the semi-synthetic *Vinca* alkaloids vinorelbine and its newer derivative vinflunine. *Mol. Pharmacol.* **2001**, *60*, 225–232. [[CrossRef](#)]
10. Okouneva, T.; Hill, B.T.; Wilson, L.; Jordan, M.A. The effects of vinflunine, vinorelbine, and vinblastine on centromere dynamics. *Mol. Cancer Ther.* **2003**, *2*, 427–436. [[PubMed](#)]

11. Toki, K.; Saito, N.; Irie, Y.; Tatsuzawa, F.; Shigihara, A.; Honda, T. 7-O-Methylated anthocyanidin glycosides from *Catharanthus roseus*. *Phytochemistry* **2008**, *69*, 1215–1219. [[CrossRef](#)]
12. Tiong, S.M.; Looi, C.Y.; Ary, A.; Wong, W.F.; Hazni, H.; Mustafa, M.R.; Awang, K. Vindogentianine, a hypoglycemic alkaloid from *Catharanthus roseus* (L.) G. Don (Apocynaceae). *Fitoterapia* **2015**, *102*, 182–188. [[CrossRef](#)]
13. Wang, C.H.; Zhang, Y.; Jiang, M.M. Indole alkaloids from the roots of *Catharanthus roseus*. *Chem. Nat. Comp.* **2014**, *49*, 1177–1178. [[CrossRef](#)]
14. Zhang, W.K.; Xu, J.K.; Tian, H.Y.; Wang, L.; Zhang, X.Q.; Xiao, X.Z.; Li, P.; Ye, W.C. Two new vinblastine-type N-oxide alkaloids from *Catharanthus roseus*. *Nat. Prod. Res.* **2013**, *27*, 1911–1916. [[CrossRef](#)]
15. Wang, C.H.; Wang, G.C.; Wang, Y.; Zhang, X.Q.; Huang, X.J.; Ye, W.C. Three new monomeric indole alkaloids from the roots of *Catharanthus roseus*. *J. Asian Nat. Prod. Res.* **2012**, *14*, 249–255. [[CrossRef](#)] [[PubMed](#)]
16. Wang, L.; He, H.P.; Di, Y.T.; Zhang, Y.; Hao, X.J. Catharoseumine, a new monoterpenoid indole alkaloid possessing a peroxy bridge from *Catharanthus roseus*. *Tetrahedron Lett.* **2012**, *53*, 1576–1578. [[CrossRef](#)]
17. Wang, B.; Liu, L.; Chen, Y.Y.; Li, Q.; Li, D.; Liu, Y.P.; Luo, X.D. Monoterpenoid indole alkaloids from *Catharanthus roseus* cultivated in Yunnan. *Nat. Prod. Comm.* **2015**, *10*, 2085–2086. [[CrossRef](#)]
18. Wang, K.; Zhou, X.Y.; Wang, Y.Y.; Li, M.M.; Li, Y.S.; Peng, L.Y.; Cheng, X.; Li, Y.; Wang, Y.P.; Zhao, Q.S. Macrophyllionium and macrophyllines A and B, oxindole alkaloids from *Uncaria macrophylla*. *J. Nat. Prod.* **2011**, *74*, 12–15. [[CrossRef](#)]
19. Jiang, W.W.; Su, J.; Wu, X.D.; He, J.; Peng, L.Y.; Cheng, X.; Zhao, Q.S. Geissoschizine methyl ether N-oxide, a new alkaloid with antiacetylcholinesterase activity from *Uncaria rhynchophylla*. *Nat. Prod. Res.* **2015**, *29*, 842–847. [[CrossRef](#)] [[PubMed](#)]
20. Zhang, Z.J.; Du, R.N.; He, J.; Wu, X.D.; Li, Y.; Li, R.T.; Zhao, Q.S. Vinmajorines C-E, monoterpenoid indole alkaloids from *Vinca major*. *Helv. Chim. Acta.* **2016**, *99*, 157–160. [[CrossRef](#)]
21. Zhang, Z.J.; Du, R.N.; He, J.; Wu, X.D.; Li, Y.; Li, R.T.; Zhao, Q.S. Three new monoterpenoid indole alkaloids from *Vinca major*. *J. Asian Nat. Prod. Res.* **2016**, *18*, 328–333. [[CrossRef](#)]
22. Zhang, Z.J.; Yang, J.; He, J.; Wu, X.D.; Shao, L.D.; Li, Y.; Huang, S.X.; Li, R.T.; Zhao, Q.S. Vincamajorines A and B, monoterpenoid indole alkaloids with new carbon skeletons from *Vinca major*. *Tetrahedron Lett.* **2014**, *55*, 6490–6494. [[CrossRef](#)]
23. Liu, Y.; Wang, P.; Ma, F.F.; Zheng, M.Q.; Liu, G.; Kume, S.; Kurokawa, T.; Ono, K. Asparagine-linked glycosylation modifies voltage-dependent gating properties of Cav3.1-T-type Ca²⁺ channel. *J. Physiol. Sci.* **2019**, *69*, 335–343. [[CrossRef](#)] [[PubMed](#)]
24. Giddings, L.A.; Liscombe, D.K.; Hamilton, J.P.; Childs, K.L.; DellaPenna, D.; Buell, C.R.; O'Connor, S.E. A stereoselective hydroxylation step of alkaloid biosynthesis by a unique chinaytochrome P450 in *Catharanthus roseus*. *J. Biol. Chem.* **2011**, *286*, 16751–16757. [[CrossRef](#)] [[PubMed](#)]
25. Abaul, J.; Philogène, E.; Bourgeois, P.; Mérault, G. Alkaloids indoliques de *Rauwolfia biauriculata*. *J. Nat. Prod.* **1986**, *49*, 829–832. [[CrossRef](#)]
26. Clivio, P.; Richard, B.; Deverre, J.R.; Sevenet, T.; Zeches, M.; Men-Oliver, L.L. Alkaloids from leaves and root bark of *Ervatamia hirta*. *Phytochemistry* **1991**, *30*, 3785–3792. [[CrossRef](#)]
27. Mitra, A.K.; Patra, A.; Mukhopadhyay, A.K. Vincapusine, a minor indole alkaloid of *Vinca pusilla*. *Phytochemistry* **1981**, *20*, 865–866. [[CrossRef](#)]
28. Neuss, N.; Boaz, H.E.; Occolowitz, J.L.; Wenkert, E.; Schell, F.M.; Potier, P.; Kan, C.; Plat, M.M.; Plat, M. The Structure of Vincarodine, *Helv. Chim. Acta.* **1973**, *56*, 2660–2666. [[CrossRef](#)] [[PubMed](#)]
29. Wachsmuth, O.; Matusch, R. Anhydronium bases from *Rauwolfia serpentina*. *Phytochemistry* **2002**, *61*, 705–709. [[CrossRef](#)]
30. Jin, P.F.; Zhan, G.Q.; Zheng, G.J.; Liu, J.J.; Peng, X.; Huang, L.; Gao, B.; Yuan, X.H.; Yao, G.M. Gelstriamine A, a triamino monoterpenoid indole alkaloid with a caged 6/5/7/6/6/5 scaffold and analgesic alkaloids from *Gelsemium elegans* Stems. *J. Nat. Prod.* **2021**, *84*, 1326–1334. [[CrossRef](#)]
31. Zhan, G.Q.; Miao, R.K.; Zhang, F.X.; Chang, G.; Zhang, L.; Zhang, X.X.; Zhang, H.; Guo, Z.J. Monoterpene indole alkaloids with acetylcholinesterase inhibitory activity from the leaves of *Rauwolfia vomitoria*. *Bioorg. Chem.* **2020**, *102*, 104136.
32. Tan, S.J.; Lim, J.L.; Low, Y.Y.; Sim, K.S.; Lim, S.H.; Kam, T.S. Oxidized derivatives of macroline, sarpagine, and pleiocarpamine alkaloids from *Alstonia angustifolia*. *J. Nat. Prod.* **2014**, *77*, 2068–2080. [[CrossRef](#)]
33. Yeap, J.S.Y.; Navanesan, S.; Sim, K.S.; Yong, K.T.; Gurusamy, S.; Lim, S.H.; Low, Y.Y.; Kam, T.S. Ajmaline, oxindole, and cytotoxic macroline–akuammiline bisindole alkaloids from *Alstonia penangiana*. *J. Nat. Prod.* **2018**, *81*, 1266–1277. [[CrossRef](#)]
34. Lim, S.H.; Low, Y.Y.; Sinniah, S.K.; Yong, K.T.; Sim, K.S.; Kam, T.S. Macroline, akuammiline, sarpagine, and ajmaline alkaloids from *Alstonia macrophylla*. *Phytochemistry* **2014**, *98*, 204–215. [[CrossRef](#)]
35. Zhan, G.Q.; Miao, R.K.; Zhang, F.X.; Hao, X.C.; Zheng, X.; Zhang, H.; Zhang, X.X.; Guo, Z.J. Monoterpene indole alkaloids with diverse skeletons from the stems of *Rauwolfia vomitoria* and their acetylcholinesterase inhibitory activities. *Phytochemistry* **2020**, *177*, 112450. [[CrossRef](#)] [[PubMed](#)]
36. Kogure, N.; Nishiya, C.; Kitajima, M.; Takayama, H. Six new indole alkaloids from *Gelsemium sempervirens* Ait. f. *Tetrahedron Lett.* **2005**, *46*, 5857–5861. [[CrossRef](#)]
37. Sheludko, Y.; Gerasimenko, I.; Kolshorn, H.; Stöckigt, J. New alkaloids of the sarpagine group from *Rauwolfia serpentina* hairy root culture. *J. Nat. Prod.* **2002**, *65*, 1006–1010. [[CrossRef](#)] [[PubMed](#)]
38. Gao, Y.; Yu, A.L.; Li, G.T.; Hai, P.; Li, Y.; Liu, J.K.; Wang, F. Hexacyclic monoterpenoid indole alkaloids from *Rauwolfia verticillate*. *Fitoterapia* **2015**, *107*, 44–48. [[CrossRef](#)] [[PubMed](#)]

39. Yeap, J.S.Y.; Tan, C.H.; Yong, K.T.; Lim, K.H.; Lim, S.H.; Low, Y.Y.; Kam, T.S. Macroline, talpinine, and sarpagine alkaloids from *Alstonia penangiana*. An NMR-based method for differentiating between *A. penangiana* and *A. Macrophylla*. *Phytochemistry* **2020**, *176*, 112391. [[CrossRef](#)]
40. Kam, T.S.; Choo, Y.M. Alkaloids from *Alstonia angustifolia*. *Phytochemistry* **2004**, *65*, 603–608. [[CrossRef](#)]
41. Sakai, S.; Aimi, N.; Yamaguchi, K.; Ohhira, H.; Hori, K.; Haginiwa, J. Gardneria alkaloids -IX structures of chitosenine and three other minor bases from *gardneria multiflora makino*. *Tetrahedron Lett.* **1975**, *10*, 715–718. [[CrossRef](#)]
42. Braekman, J.C.; Lampe, T.M.; Pecher, J. Indole alkaloids. XX isolation and structural elucidation of four minor alkaloids from *Voacanga chalitiana pierre ex stapf*. *Bull. Soc. Chim. Belges.* **1969**, *78*, 523–538.
43. Zeng, T.; Wu, X.Y.; Yang, S.X.; Lai, W.C.; Shi, S.D.; Zou, Q.; Liu, Y.; Li, L.M. Monoterpenoid indole alkaloids from *Kopsia officinalis* and the immunosuppressive activity of rhazinilam. *J. Nat. Prod.* **2017**, *80*, 864–871. [[CrossRef](#)] [[PubMed](#)]
44. Wenkert, E.; Hagaman, E.W.; Wang, N.; Gutowski, G.E.; Miller, J.C. The C (16') configuration of vincalukoblastine-like natural and synthetic substances. *Heterocycles* **1981**, *15*, 255–258. [[CrossRef](#)]
45. Bunsupa, S.; Komastu, K.; Nakabayashi, R.; Saito, K.; Yamazaki, M. Revisiting anabasine biosynthesis in tobacco hairy roots expressing plant lysine decarboxylase gene by using ¹⁵N-labeled lysine. *Plant Biotechnol.* **2014**, *31*, 511–518. [[CrossRef](#)]
46. Yeap, J.S.Y.; Lim, K.H.; Yong, K.T.; Lim, S.H.; Kam, T.S.; Low, Y.Y. *Lycopodium* alkaloids: Lycoplaryne A, an unusual lycodine–piperidine adduct from *Lycopodium platyrhizoma* and the absolute configurations of lycoplanine D and lycogladine H. *J. Nat. Prod.* **2019**, *82*, 324–329. [[CrossRef](#)]