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Indole alkaloids from cultivated *Vinca major*

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ABSTRACT

Nine new indole alkaloids, vinmajines A–I (**1**–**9**), and 43 known indole alkaloids were isolated from cultivated *Vinca major* in Kunming. The new structures were elucidated by extensive spectroscopic and quantum theory analysis. In addition, the results also supported that types of indole alkaloids from *V. major* might be influenced significantly by the ecological environment.

1. Introduction

Plants of the Apocynaceae family are rich sources of structurally diversified indole alkaloids, which originate from the condensation of tryptophan with secologanin.[1] Many of them, such as yohimbine,[2] reserpine,[3] and camptothecin[4] are well known for their pharmacological significance. We focused on antitumor indole alkaloids from the *Alstonia*, *Melodinus*, and *Tabernaemontana* genera of Apocynaceae, and reported several novel and bioactive indole and bisindole alkaloids recently.[5] Particularly, the reported (19,20)-*E*/*Z*-alstoscholarine,[6] scholarisine A,[7] melohenine B,[8] and melotenine A[9] have been total synthesized by other chemists, due to their complex molecular architectures. The genus *Vinca* (Apocynaceae) is distributed natively to Europe, Northwest Africa, and South-west Asia. Pharmacological investigation on *Vinca* alkaloids and their derivatives are mainly focused on anticancer activity, such as vinblastine, vincristine, vindesine, and vinorelbine.[10] *Vinca major* is a perennial evergreen herb with medicinal and ornamental values, and have also been cultivated in China widely as an outside ornament. Previous phytochemical investigation on this specie collected from Turkey,[11] Yugoslavia,[12] Canada,[13] Iran,[14] India,[15] and Japan,[16] reported diverse alkaloidal patterns such as yohimbine, sarpagine, ajmaline, akuammine, and oxindole types. In our investigation, we found that the type of plant secondary metabolites would be influenced significantly by the ecological environment,[17] which encouraged us to search for structurally unique and biologically active indole alkaloids from *V. major* in different habitats. As a result, nine new indole alkaloids (Fig. 1) including four *ajmaline*-type alkaloids, vinmajines A–D (**1**–**4**), two *sarpagine*-type alkaloids, vinmajines E and F (**5**–**6**), one *oxindole*-type alkaloid, vinmajine G (**7**), two pyridino-indolo-quinolizidinone alkaloids, vinmajines H and I (**8**–**9**) together with 43 known alkaloids were isolated from *V. major* cultivated in Kunming botanical garden. The new alkaloids were elucidated by means of spectroscopic methods. To the best of our knowledge, the pyridino-indolo-quinolizidinone alkaloids, vinmajines F/G, 19-*O*-acetylangustoline,[18] angustoline,[18] 19-*O*-methylangustoline,[19] and angustidine[20] were first reported from plants of the genus *Vinca*. All compounds were evaluated for cytotoxicity against five human cancer cell lines. Reported herein are the isolation, structural elucidation, and cytotoxic activities of these compounds.

2. Results and discussion

Vinmajine A (**1**) was obtained as colorless oil and gave a positive reaction with Dragendorff's reagent, characteristic for an alkaloid. Its molecular formula was established as $C_{25}H_{31}N_3O_5$ by the molecular ion at *m*/*z* 453.2269 $[M]^+$ in the HREIMS, indicating twelve

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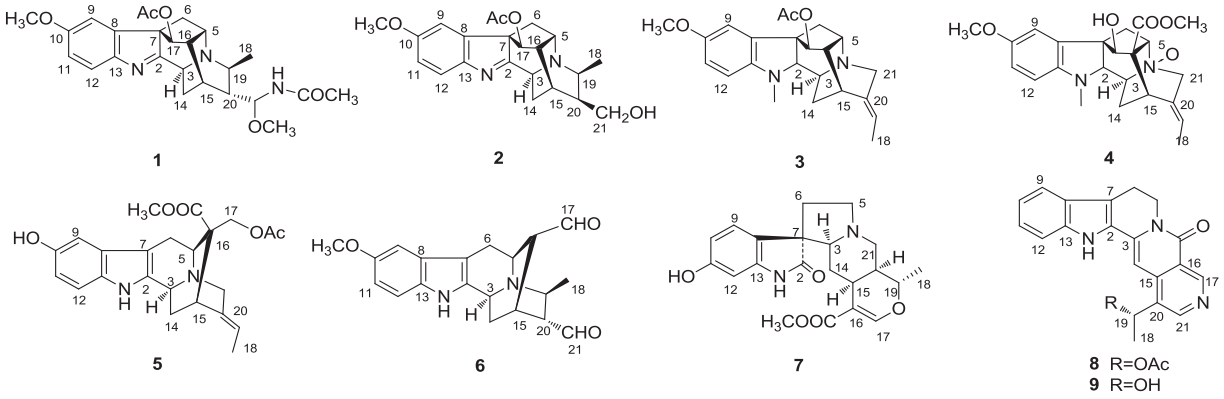

Fig. 1. Structures of vinmajines A–I (**1**–**9**).

degrees of unsaturation. The UV spectrum showed absorption maxima characteristic of an indolenine chromophore (279, 222, 201 nm), which was supported by the characteristic chemical shift of C-2 at δ_C 181.3 in the ^{13}C NMR spectrum. The IR absorption at 1743 cm^{-1} showed the presence of ester carbonyl group. In the ^1H NMR spectrum, three aromatic protons at δ_H 7.48, 6.99, and 6.87 form an ABX system with J_{AB}=8.5 Hz and J_{Bx}=2.5 Hz revealed the presence of a mono-substituted ring in a monoterpenoid indole alkaloid.[21] Besides substituent groups (two methoxyls and two acetyls), compound **1** possessed 19 skeleton carbon resonances, ascribable to one methyls, two sp^3 methylenes, eight sp^3 methines, three sp^2 methines, and four sp^2 quaternary and one sp^3 quaternary carbons in the ^{13}C NMR and DEPT spectra (Table 3). Comparison of its NMR spectral data (Tables 1 and 3) and molecular formula with those of 10-methoxyperakine[11] suggested that **1** was an *Ajmaline*-type indole alkaloid bearing an additional methoxyl group (δ_H 3.29, δ_C 55.9) and an acetamido group (δ_H 2.06, δ_C 170.3, 23.5). Signal of aldehyde group in 10-methoxyperakine[11] was disappeared, a downfield methine at δ_C 81.1 (d, C-21) was appeared as instead in the ^{13}C NMR spectrum of **1**, which was supported by the HMBC correlations of δ_H 2.57 (1H, t, J=5.0 Hz, H-15) to δ_C 81.1 (d, C-21). Furthermore, the HMBC correlations from the methine proton at δ_H 5.21 (1H, dd, J=9.6, 7.8 Hz, H-21) to δ_C 55.9 (−O*C*H$_3$) and 170.3 (−NCOCH$_3$), and from δ_H 3.29 (−OC*H*$_3$) and 2.06 (−NCOC*H*$_3$) to δ_C 81.1 suggested that both the methoxyl (δ_H 3.29; δ_C 55.9) and the acetamido group (δ_H 2.06; δ_C 170.3 and 23.5) were connected at C-21 (Fig. 2).

Table 1
^1H NMR data of **1**–**3** in $CDCl_3$ (δ in ppm and J in Hz)

No.	**1**[a]	**2**[b]	**3**[a]
2			3.10, overlap
3	4.13, d (9.2)	4.10, d (8.6)	3.57, dd (9.5, 5.0)
5	3.59, m	3.58, m	3.21, d (6.3, 5.0)
6a	2.71, dd (11.8, 4.9)	2.72, dd (11.9, 4.9)	2.41, m
6b	1.66, d (11.8)	1.61, d (11.9)	1.79, d (11.7)
9	6.99, d (2.5)	6.98, d (2.5)	6.73, d (2.6)
10			
11	6.87, dd (8.5, 2.5)	6.87, dd (8.5, 2.5)	6.70, dd (8.4, 2.6)
12	7.48, d (8.5)	7.47, d (8.5)	6.55, d (8.4)
14a	2.14, overlap	1.82, dd (13.6, 4.8)	2.46, dd (13.1, 3.9)
14b	1.51, dd (14.8, 4.8)	1.76, m	1.47, dd (13.7, 10.0)
15	2.57, t (5.0)	2.56, d (4.4)	3.11, overlap
16	2.31, t (6.0)	2.38, t (6.0)	2.13, t (5.8)
17	4.95, s	5.01, s	5.17, s
18	1.28, d (6.5)	1.19, d (7.4)	1.63, d (6.5)
19	2.82, m	3.21, dd (9.2, 7.4)	5.24, q (6.5)
20	1.36, t (8.3)	1.99, dt (13.5, 9.2)	
21a	5.21, dd (9.6, 7.8)	3.83, dd (10.9, 6.4)	3.47, br s
21b		3.70, t (10.2)	
OCO*CH*$_3$	2.14, s	2.13, s	1.94, s
NCO*CH*$_3$	2.06, s		
10-O*CH*$_3$	3.79, s	3.81, s	3.74, s
21-O*CH*$_3$	3.29, s		
−N*CH*$_3$			2.58, s

[a] Recorded at 400 MHz.
[b] Recorded at 500 MHz.

In ROESY spectrum of **1**, NOE correlations of δ_H 4.13 (H-3) with 2.14 (H-14a) and 2.82 (H-19), and of δ_H 1.28 (H$_3$-18) with 3.59 (H-5) and 1.36 (H-20) placed H-3, H-14a, and H-19 at the same side (α-orientation), while H-20 at another side, because *Ajmaline*-type alkaloids took α-orientation of H-3 from their biosynthetic consideration. Furthermore, other chiral centers were identical to those of 10-methoxyperakine supported by ROESY spectrum of **1** (Fig. 2). A quantum chemical calculation was applied to assign the configuration of C-21 in **1**. All DFT and TDDFT calculations were carried out at 298.15 K in the gas phase with Gaussian 09. The electronic circular dichroism (ECD) was investigated at the B3LYP/6-311++G(d,p) level.[22] The CD spectrum generated was in agreement with the experimental data of **1**. The recorded and the computed CD curves are illustrated in Fig. 3. All of the evidence from NMR and ECD suggested that **1** has an absolute configuration of 3*S*, 5*S*, 7*R*, 15*R*, 16*S*, 17*R*, 19*S*, 20*R*, 21*S*.

The molecular formula of **2** was determined to $C_{22}H_{26}N_2O_4$ by the positive HRESIMS ($[M+H]^+$ at m/z 383.1964), and was the same as that of 10-methoxyraucaffrinoline[23] They have similar physical data in the UV and IR spectra, assuming the existence of the same functional groups. The ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 3) were similar to those of 10-methoxyraucaffrinoline except for chemical shift of δ_H 1.99 (1H, dt, J=13.5, 9.2 Hz, H-20) and δ_C 41.6 (d, C-20). The difference was that the configuration of H-20 in **2** was α-oriented, as supported by NOE correlations of δ_H 3.21 (H-19) with 4.10 (H-3) and 1.99 (H-20) in ROESY spectrum. Other parts of **2** were identical to those of 10-methoxyraucaffrinoline, which was supported by detailed analysis of 2D NMR spectra of **2**. Thus, compound **2** was assigned as shown, and named vinmajine B.

Vinmajine C (**3**) gave the molecular formula $C_{23}H_{28}N_2O_3$ on the basis of HREIMS. The ^{13}C NMR and DEPT spectra of compound **3** displayed 23 carbon resonances ascribable to four methyls, three methylenes, ten methines, and six quaternary carbons (Table 3). These data were closely related to those of vincamedine,[24] suggesting that **3** was also an *ajmaline*-type alkaloid. The most notable difference between the two alkaloids was the loss of −COOCH$_3$ group at C-16 in **3**, as supported by the HMBC correlations of δ_H 2.13 (1H, d, J=5.8 Hz, H-16) with δ_C 21.6 (t, C-14), 55.2 (s, C-7), and 77.5 (d, C-17). The *E* configuration of double bond at side chain was indicated by the NOE correlations between δ_H 1.63 (CH$_3$-18) and 3.11 (H-15). Furthermore, the NOE correlations of H-17 (δ_H 5.17) with H-14a (δ_H 2.46), and H-15 (δ_H 3.11) suggested H-17 to be β-oriented, identical to the configuration of vincamedine. Thus, the structure of vinmajine C (**3**) was assigned as shown.

Table 2
^{1}H NMR data of **4**–**9**[a] (δ in ppm and J in Hz)

No.	**4**[b]	**5**[c]	**6**[c]	**7**[c]	**8**[c]	**9**[c]
N_1–H		10.49, s		10.26, s	11.0, s	11.90, s
3	3.64, d (8.6)	4.21, br s	4.21, s	2.26, overlap		
5a	3.61, m	3.05, overlap	3.91, m	3.16, m	4.35, tdd (13.5, 10.4, 6.2)	4.42, qt (13.4, 6.5)
5b	3.83, d (4.0)			2.21, m		
6a	2.56, d (13.0)	3.10, d (15.0)	3.08, dd (15.5, 4.9)	2.13, dd (12.3, 8.2)	3.07, m	3.14, m
6b	2.44, m	2.68, d (12.1)	2.63, t (12.2)	1.77, m		
9	7.13, d (7.2)	6.65, d (2.1)	6.88, d (1.8)	6.93, d (7.7)	7.53, d (8.0)	7.65, d (7.9)
10	6.73, t (7.2)			6.34, d (7.7)	6.99, t (8.0)	7.12, t (7.9)
11	7.10, t (7.8)	6.52, dd (8.5, 2.1)	6.78, dd (8.4, 1.8)		7.13, t (8.0)	7.29, t (7.9)
12	6.59, d (7.8)	7.05, d (8.5)	7.21, d (8.4)	6.29, s	7.31, d (8.0)	7.50, d (7.9)
14a	2.48, m	2.54, m	1.91, m	2.35, m	7.04, s	7.25, s
14b	1.71, dd (13.4, 9.7)	1.80, t (10.3)	1.53, m	0.75, dd (23.5, 11.6).		
15	3.48, d (4.9)	3.05, overlap	2.78, dd (22.0, 7.1)	1.33, m		
16			2.55, t (6.2)	2.55, t (6.2)		
17a	4.05, s	4.13, d (10.5)	9.79, s	7.42, s	9.21, s	9.26, s
17b		4.05, d (10.5)				
18	1.54, d (6.6)	1.50, d (6.8)	1.33, d (6.7)	1.35, d (6.1)	1.51, d (6.7)	1.50, d (6.5)
19	5.17, q (6.6)	5.36, q (6.8)	3.28, dq (13.6, 6.7)	4.25, br s	6.17, q (6.7)	5.35, q (6.5)
20			2.06, m	1.57, m		
21a	3.64, m	3.56, m	9.77, s	3.26, m	8.58, s	8.78, s
21b	3.44, d (15.4)			2.29, m		
NCH_3	2.54, s					
10-OCH_3			3.84, s	3.54, s		
–*OH*		8.58, s		9.45, s		5.61 d (4.1)
OCOCH_3		1.93, s			1.99, s	
COOCH_3	3.61s	2.92, s				

[a] Compounds **4** and **6** were measured in $CDCl_3$, **5**, **7**, and **9** in DMSO-d_6, **8** in acetone-d_6.
[b] Recorded at 400 MHz.
[c] Recorded at 600 MHz.

Table 3
^{13}C NMR spectral data of **1**–**9**[a] (δ in ppm)

No.	**1**[b]	**2**[c]	**3**[b]	**4**[b]	**5**[d]	**6**[d]	**7**[d]	**8**[d]	**9**[d]
2	181.3C	181.4C	75.9CH	69.5CH	138.1C	137.8C	180.7C	128.7C	127.7C
3	56.9CH	56.8CH	53.7CH	69.2CH	49.7CH	52.1CH	70.8CH	138.1C	136.4C
5	51.2CH	51.1CH	59.1CH	75.5CH	57.0CH	44.4CH	53.1CH_2	41.2CH_2	40.2CH_2
6	37.4CH_2	38.1CH_2	36.7CH_2	30.3CH_2	23.7CH_2	27.5CH_2	34.5CH_2	20.2CH_2	19.1CH_2
7	64.7C	64.8C	55.2C	55.8C	103.0C	104.6C	55.7C	116.4C	114.5C
8	137.8C	137.9C	131.2C	128.9C	126.9C	127.9C	123.5C	126.7C	125.3C
9	110.9CH	110.8CH	111.3CH	124.9CH	101.8CH	100.5CH	124.7CH	120.5CH	119.6CH
10	157.8C	157.8C	153.3C	119.3CH	150.2C	154.3C	108.2CH	121.0CH	119.8CH
11	112.7CH	112.7CH	112.4CH	128.4CH	110.4CH	111.7CH	157.3C	125.5CH	124.4CH
12	121.1CH	120.9CH	109.3CH	109.4CH	111.3CH	111.9CH	97.5CH	112.7CH	111.9CH
13	150.0C	149.8C	148.4C	154.0C	130.8C	131.5C	142.4C	139.7C	138.4C
14	22.2CH_2	27.8CH_2	21.6CH_2	21.6CH_2	28.4CH_2	29.0CH_2	30.1CH_2	93.8CH	93.7CH
15	26.6CH	26.0CH	25.9CH	29.1CH	29.0CH	24.9CH	30.0CH	139.6CH	138.3C
16	49.1CH	43.4CH	47.2CH	60.7C	48.6C	53.2CH	109.6C	120.4C	118.8C
17	77.9CH	78.4CH	77.5CH	73.4CH	69.0CH_2	202.2CH	154.6CH	151.2CH	149.1CH
18	19.5CH_3	14.0CH_3	12.9CH_3	12.5CH_3	12.5CH_3	19.1CH_3	18.4CH_3	22.3CH_3	25.2CH_3
19	52.2CH	51.7CH	114.7CH	119.7CH	116.1CH	51.9CH	72.1CH	68.5CH	63.8CH
20	48.2CH	41.6CH	139.3C	129.5C	136.8C	51.7CH	37.2CH	131.1C	134.8C
21	81.1CH	61.3CH_2	55.7CH_2	69.9CH_2	54.4CH_2	202.1CH	53.7CH_2	148.9CH	147.5CH
22								162.1C	161.1C
OCOCH$_3$	169.9C	169.9C	170.0C		170.1C			170.4C	
OCOCH_3	21.1CH_3	21.0CH_3	21.2CH_3					21.1CH_3	
*C*OOCH$_3$				170.7C	171.6C		166.7C		
COOCH_3				52.0CH_3	51.1CH_3		51.0CH_3		
NCOCH_3	23.5CH_3								
N*C*OCH$_3$	170.3C								
10-OCH_3	55.6CH_3	55.5CH_3	55.8CH_3			56.1CH_3			
19-OCH_3	55.9CH_3								
NCH_3			34.8CH_3	34.4CH_3					

[a] Compounds **1**–**4** and **6** were measured in $CDCl_3$, **5**, **7**, and **9** in DMSO-d_6, **8** in acetone-d_6.
[b] Recorded at 400 MHz.
[c] Recorded at 500 MHz.
[d] Recorded at 600 MHz.

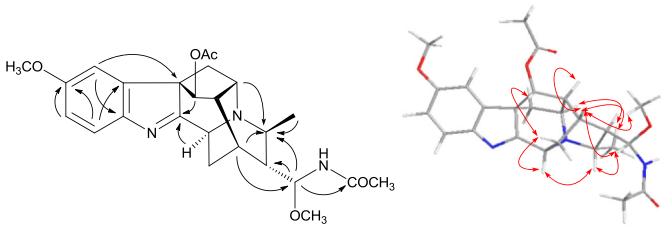

Fig. 2. Key HMBC (⌒) and ROESY (⌒) correlations of **1**.

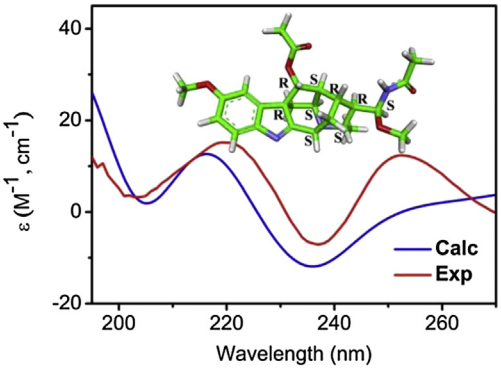

Fig. 3. Calculated and experimental ECD spectra of **1**.

Vinmajine D (**4**) was isolated as a white amorphous powder and had a molecular ion peak $[M]^+$ at *m*/*z* 382.1895 in its HREIMS, identified as $C_{22}H_{26}N_2O_4$, 16 Da higher than that of vincamajine.[25] Compound **4** was readily assumed as vincamajine-*N*(4)-oxide from its ^1H and ^{13}C NMR spectral data, in particular the characteristic downfield shifts of the carbon resonances at δ_C 69.2, 69.9, and 75.5 for C-3, C-21, and C-5, respectively, with respect to those of vincamajine. Other parts of **4** were identical to those of vincamajine supported by its HSQC, HMBC, and ROESY spectra.

The molecular formula $C_{23}H_{26}N_2O_5$ of vinmajine E (**5**) was given by HREIMS (*m*/*z* 410.1841 $[M]^+$). Its ^1H and ^{13}C NMR spectral data showed that **5** had a structure similar to that of acetylakuammidine,[26] except for one more hydroxyl group (δ_H 8.58) in **5**. The hydroxyl was placed at C-10, which was supported by correlations of δ_H 6.65 (1H, d, *J*=2.1 Hz, H-9) with δ_C 150.2 (s, C-10), and 130.8 (s, C-13); and of δ_H 7.05 (1H, d, *J*=8.5 Hz, H-12) with δ_C 126.9 (s, C-8), and 150.2 (s, C-10) in the HMBC spectrum of **5**. Complete analysis of 2D NMR spectral data of **5** indicated that the other parts were the same to those of acetylakuammidine.

Vinmajine F (**6**) had the molecular formula $C_{22}H_{22}N_2O_3$ as determined by the positive HREIMS at *m*/*z* 338.1628 $[M]^+$. Its ^{13}C NMR spectrum showed close similarities to those of 19,20-dihydroperaksine-17,21-al,[27] with the presence of an additional methoxyl group (δ_H 3.84; δ_C 56.1) in the aromatic region of **6** (Tables 2 and 3). The HMBC correlations of δ_H 3.84 (3H, s) with δ_C 154.3 (s, C-10); and of δ_H 7.21 (1H, d, *J*=8.4, H-12) with δ_C 127.9 (s, C-8) and 154.3 (s, C-10) allowed placement of the methoxyl group at C-10. Thus, the structure of compound **6** was established as shown, and all the signals were assigned on the basis of its 2D NMR spectra.

The HREIMS of vinmajine G (**7**) displayed its molecular ion peak $[M]^+$ at *m*/*z* 384.1690 ($C_{21}H_{24}N_2O_5$), identical to that of rumberine.[28] In addition, its NMR spectral data also suggested the similarity of two compounds. Detailed comparison of ^1H and ^{13}C NMR spectral data of the two compounds suggested that the hydroxyl group was assigned at C-11 in **7**, rather than at C-10, which was further supported by the HMBC correlations of δ_H 9.45 (s, −OH) with δ_C 157.3 (s, C-11), of δ_H 6.93 (1H, d, *J*=7.4 Hz, H-9) with δ_C 55.7 (s, C-7), 142.4 (s, C-13), and 157.3 (s, C-11). Moreover, ROESY correlations indicated that the relative configuration of **7** was the same as that of rumberine.

The mixture of vinmajine H (**8**) and 19-*O*-acetylangustoline was obtained as yellow amorphous powder with the molecular formula $C_{22}H_{19}N_3O_3$, as established by HRESIMS based on the $[M+H]^+$ peak (*m*/*z* 374.1497), indicating fifteen degrees of unsaturation. The IR spectrum displayed absorption bands due to −NH (3445 cm^{-1}), ester (1720 cm^{-1}), and conjugated amide (1654 cm^{-1}) groups. The ^{13}C NMR spectrum displayed indole ring signals [δ_C 128.7 (s, C-2), 116.4 (s, C-7), 126.7 (s, C-8), 120.5 (d, C-9), 121.0 (d, C-10), 125.5 (d, C-11), 112.7 (d, C-12), 139.7 (s, C-13)], and signals for three sp^2 methines (δ_C 93.8, 148.9, and 151.2), one sp^3 methine (δ_C 68.5), two sp^3 methylenes (δ_C 41.2 and 20.2), two methyls (δ_C 21.1 and 22.3), and six quaternary carbons (δ_C 120.4, 131.1, 138.1, 139.6, 162.1, and 170.4). These data indicated that it was a pyridino-indolo-quinolizidinone alkaloid, and identical to those of 19-*O*-acetylangustoline.[18] However, the optical rotation value indicated a racemate for it. Then, the racemate was subsequently resolved by HPLC column on a chiral stationary phase to afford the individual enantiomers, (+)-**8**, and (−)-19-*O*-acetylangustoline, showing opposite Cotton effects in the CD spectra and opposite optical rotations. The absolute configuration of **8** was also assigned using the quantum method.[22] The optical rotation was computed at the B3LYP/6-311++g(2d,2p)//B3LYP/6-311++g(d,p) level. The calculated OR value for the absolute configuration of 19*S* was +55.9, which is close to the experimental value of +51.3. In addition, the ECDs calculated at the B3LYP/6-311++G(d,p) level, and showed agreement with the experimented spectra with the experimental data of **8** (Fig. 4). Thus, the structure of vinmajine H (**8**) was determined as 19*S*-*O*-acetylangustoline.

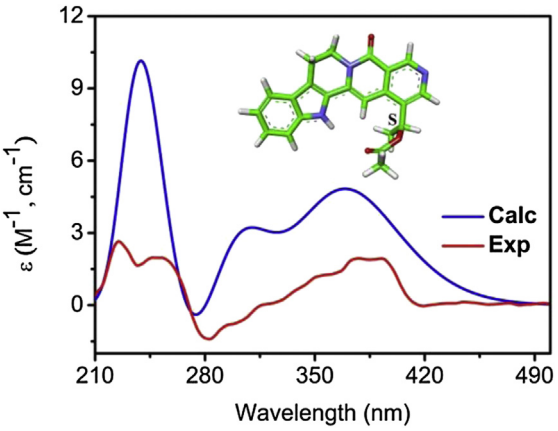

Fig. 4. Calculated and experimental ECD spectra of **8**.

The similar TLC behavior, UV, and IR spectral data of vinmajine I (**9**) and **8** suggested both compounds have the same skeleton. Vinmajine I (**9**) and angustoline were also separated in the form of an enantiomeric mixture by HPLC on a Chiralpak IC column. The HREIMS gave the molecular formula $C_{20}H_{18}N_3O_2$. However, the optical rotation value of **9** [+31.7 (*c* 0.10, MeOH)] was similar magnitude, but of opposite sign to that of angustoline [−34 ($CDCl_3$)].[18] Hence, compound **9** was elucidated to be 19*S*-angustoline.

In addition, other 43 known indole alkaloids were also isolated from *V. major* cultivated in Kunming, PR China. The known compounds were identified as 19-*O*-acetylangustoline,[18] angustoline,[18] 19-*O*-methyl-angustoline,[19] angustidine,[20] perakine,[29] 10-methoxy perakine,[11] 10-methoxyraucaffrinoine,[23] vincamedine[24] vincamajine,[25] vincamajoreine,[30] majoridine,[30] 10-methoxyvinorine,[11]

vinorine,[31] 10-methoxyvellosimine,[32] 10-hydroxystrictamine,[33] cathafoline,[34] 17-hydroxy-pseudoakuammigine,[35] pseudoakuammigine,[35] lanceomigine,[35] 17-methoxypseudoakuammigine,[36] akuammine,[37] strictosamide,[38] ajmalicine,[39] reserpinine,[40] vallesiachotamine,[30] isovallesiachotamine,[41] vallesiachotamine lactone,[42] majdine,[43] isomajdine,[43] vinerine,[44] vineridine,[44] herboxine,[45] reserpinine oxindole,[46] carboxine B,[28] tetraphylline pseudoindoxyl,[47] vincadiff ormine,[48] 3-oxo-tabersonine,[49] lochnericine,[50] 3-oxo-lochnericine,[50] vincamine,[51] 14,15-dehydrovincamine,[52] akuammicine *N*(4)-oxide,[53] and quebrachamine[54] by comparison of their spectroscopic data with those reported in the literature.

All alkaloids were evaluated for their cytotoxicity against five human cancer cell lines using MTT method as reported previously.[55] Compound **6** displayed stronger inhibitory effects against A-549 with lower IC_{50} values than those of cisplatin. The compounds 3-oxo-lochnericine, and 14,15-dehydrovincamine displayed moderate cytotoxicity against some of the cell lines, while perakine, isomajdine, 10-methoxyvellosimine, and vincadifformine exhibited weak cytotoxicity (Table S1). Other compounds were inactive (IC_{50} values >40 μM).

3. Conclusion

Monoterpenoid indole alkaloids (MIAs), including more than 2000 compounds, play a very important role in natural medicinal history. Vinca alkaloids, one of the most notable examples of terpenoid alkaloids from strictosidine condensed by tryptamine and secologanin, are characteristic of the genus *Vinca* and comprise five main groups: eburna alkaloids, sarpagine alkaloids, ajmaline alkaloids, akuammine alkaloids, and oxindole alkaloids. In this paper, nine new indole alkaloids with 43 known alkaloids were isolated from cultivated *V. major*. To the best of our knowledge, the alkaloids with pyridino-indolo-quinolizidinone skeleton, including vinmajines F/G, 19-*O*-acetylangustoline, 19-*O*-methylangustoline, angustoline, and angustidine, were first reported from plants of the genus *Vinca*, which have been isolated from genera of *mitragyna*, *nauclea*, *uncaria*, and *strychnos*. Furthermore, three *akuammine* alkaloids, lanceomigine, 17-hydroxypseudoakuammigine, and 17-methoxypseudoakuammigine, two *ajmalicine* alkaloids, vallesiachotamine lactone, and tetraphylline pseudoindoxyl, two *aspidospermine* alkaloids, lochnericine, and 3-oxo-lochnericine, one *ajmaline* alkaloids, perakine, one *oxindole* alkaloids. reserpinine oxindole, one *sarpagine* alkaloid, cathafoline, and one strychnos alkaoid, akuammicine *N*(4)-oxide, as well as 14,15-dehydrovincamine were also obtained firstly from the genus *Vinca*. In addition, twelve alkaloids, 10-methoxy-raucaffrinoline, vinorine, 10-methoxyvinorine, vinerinine, vineridine, herboxine, 10-hydroxystrictamine, vallesiachotamine, isovallesiachotamine, 3-oxo-tabersonine, vincadifformine, and quebrachamine were first reported in *V. major*, which have been isolated from other species of Vinca.

4. Experimental section

4.1. General information

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were obtained by a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D spectra were run on an AVANCE III-600 MHz, a Bruker DRX-500 MHz spectrometer, or an AV-400 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in parts per million with reference to solvent signals. HREIMS was recorded on a Waters Auto Premier P776 spectrometer. HRESIMS was recorded on an API QSTAR Pulsar 1 spectrometer. CD spectra were obtained on a JASCO 810 spectrometer. Column chromatography (CC) was performed on Silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd. Qingdao), and spots were visualized by Dragendorff's reagent. Medium pressure liquid chromatography (MPLC) was employed using a Buchi pump system coupled with C_{18}-silica gel-packed glass column (15×230 and 26×460 mm). High performance liquid chromatography (HPLC) was performed using an Agilent 1260 pump coupled with Agilent semi-preparative and preparative C_{18} columns (150×9.4 and 250×21.2 mm, respectively). Chiral separation was performed by HPLC on a Daicel Chiralpak IC column (250×10.0 mm).

4.2. Plant material

V. major was collected from Kunming Botanical Garden, Yunnan province, PR China, and identified by Prof. Wei-Bang Sun, Kunming institute of Botany. A voucher specimen (No. Sun20110820) has been deposited at deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

An air-dried and powdered sample (20 kg) was extracted with MeOH (3×50 L) at room temperature and the solvent removed in vacuo. The residue was dissolved in 0.3% aqueous hydrochloric acid (v/v), and the solution was subsequently basified to pH 9–10, using ammonia, then partitioned with EtOAc (3×10 L) to give an alkaloidal extract. The extract was subjected to a silica gel column ($CHCl_3$/MeOH, 1:0–0:1) to afford fractions (I–VII). Fraction I (4.6 g) was further applied to a silica gel column using a petroleum ether/acetone gradient eluent (12:1–8:1) to yield alkaloids ajmalicine (217 mg), vallesiachotamine lactone (47 mg), and vincadifformine (64 mg). Fraction II (15.8 g) was separated by silica gel CC (petroleum ether/Me_2CO, 8:1–2:1), then by RP-18 CC, eluted with MeOH/H_2O (5:5–10:0) to afford reserpinine (6.21 g), vallesiachotamine (2.25 g), and a mixture (1.33 g). The mixture was chromatographed on a silica gel column (petroleum ether/EtOAc, 6:1–2:1), then purified on a preparative C_{18} HPLC column with a gradient of MeOH/H_2O (65:35–70:30) to yield lochnericine (47 mg), 3-oxo-tabersonine (97 mg), and 3-oxo-lochnericine (43 mg). Fraction III (28.6 g) was purified on a preparative C_{18} HPLC column with a gradient of MeOH/H_2O (40:60–70:30) to yield six subfractions III-1–6. Subfraction III-1 (6.5 g) was further subjected to silica gel CC using a petroleum ether/Me_2CO gradient eluent (10:1–5:1) to yield majdine (3.65 g), isomajdine (145 mg), and quebrachamine (43 mg). Subfraction III-2 (130 mg) was separated on a preparative C_{18} HPLC column with a gradient of MeOH/H_2O (55:45–70:30) to yield isovallesiachotamine (42 mg) and tetraphylline pseudoindoxyl (66 mg). Subfraction III-3 (210 mg) was further purified on a C_{18} HPLC column with a gradient of MeOH/H_2O (50:50–60:40) to afford perakine (108 mg), herboxine (35 mg), and reserpinine oxindole (21 mg). Subfraction III-4 (67 mg) was further purified on a C_{18} HPLC column with a gradient MeOH/H_2O (55:45–70:30) to afford vincamine (17 mg) and 14,15-dehydro-16-epivincamine (11 mg). Subfraction III-5 (1.9 g) was chromatographed on a silica gel column (petroleum ether/Me_2CO, 3:1–3:2), then purified on a preparative C_{18} HPLC column with a gradient of MeOH/H_2O (55:45–65:45) to yield isovinerine (97 mg), and carboxine B (16 mg). Fraction III-6 (5.3 g) was subjected to MPLC with RP-18 CC (MeOH/H_2O, 6:4–8:2), then followed by silica gel CC (petroleum ether/Me_2CO, 8:1–3:1) to yield **5** (15 mg), 10-methoxyperakine (1.95 g), vincamajine (1.39 g), majoridine (121 mg), 10-

methoxyvinorine (48 mg), and vinorine (36 mg). Fr. IV (8.2 g) was purified by C_{18} MPLC with a MeOH/H_2O gradient (40:60–80:20) to yield subfractions IV-1–3. 10-Hydroxystrctamine (3.2 g) was crystallized from subfraction IV-1. The mother liquid of this subfraction (121 mg) was further separated on a preparative C_{18} HPLC column with a gradient MeOH/H_2O (55:45–70:30) to yield **3** (42 mg), **4** (20 mg), **6** (4 mg), and vincamedine (26 mg). Subfraction IV-2 was further separated on the same column with MeOH/H_2O (60:40) to yield **7** (29 mg), cathafoline (11 mg), and akuammicine *N*(4)-oxide (7 mg). Fraction V (91 g) was separated by silica gel CC ($CHCl_3$/MeOH, 15:1–10:1) to yield 10-methoxyvellosimine (14 mg), 17-methoxypseudoakuammigine (780 mg), strictosamide (6.8 g), and a mixture. Further separation of the mixture by a preparative C_{18} column gives subfractions V-1–5. Subfraction V-1 (1.2 g) was separated on a preparative C_{18} column with a gradient MeOH/H_2O (30:70–80:20), then separated on a semipreparative C_{18} HPLC column (MeOH/H_2O, 75%) to produce pseudoakuammigine (11 mg), 17-hydroxypseudoakuammigine (6 mg), and akuammine (6 mg). Subfraction V-2 (6.3 g) was purified by CC over silica gel with $CHCl_3$/Me_2CO (9:1–4:1) to yield V-2-1 (11 mg), V-2-2 (10 mg), and 19-*O*-methylangustoline (22 mg), HPLC separation of V-2-1 and V-2-2 with a Chiralpak column (CH_2Cl_2/MeOH, 5:95) led to two pairs of enantiomers **8** (4 mg) and 19-*O*-acetylangustoline (4 mg), as well as **9** (2 mg) and angustoline (2 mg). Subfraction V-3 (2.1 g) was subjected to silica gel CC using $CHCl_3$/Me_2CO eluent (3:1) to yield V-3-1–3. V-3-1 (130 mg) was further separated on a preparative C_{18} HPLC column with a gradient MeOH/H_2O (50:50–70:30) to give a mixture, then chiral separated by HPLC on a Chiralpak IC column to afford 2 (32 mg) and 10-methoxyraucaffrinoline (46 mg). V-3-2 (86 mg) was further separated on a preparative C_{18} column with a gradient MeOH/H_2O (40:65–70:30) to afford **1** (7 mg) and vinerine (6 mg). V-3-3 (163 mg) was further separated on the same column with a gradient MeOH/H_2O (55:45–65:35) to vincamajoreine (56 mg).

4.4. Spectroscopic data of the isolated compounds

*4.4.1. Vinmajine A (**1**).* Colorless oil; $[\alpha]_D^{22}$ −23.2 (*c* 0.118, MeOH); UV (MeOH) λ_{max} (log ε): 279 (3.97), 222 (4.25), 201 (4.28); IR (KBr) ν_{max} 3439, 2935, 1743, 1658, 1640, 1593, 1530, 1469, 1374, 1033 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Tables 1 and 3; HREIMS *m*/*z* 453.2269 (calcd for $C_{25}H_{31}N_3O_5$ $[M]^+$, 453.2264).

*4.4.2. Vinmajine B (**2**).* Colorless oil; $[\alpha]_D^{22}$ +45.5 (*c* 0.123, MeOH); UV (MeOH) λ_{max} (log ε): 279 (3.44), 223 (4.14), 202 (4.08); IR (KBr) ν_{max} 3429, 2938, 1742, 1622, 1592, 1469, 1220, 1029, 822 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data ($CDCl_3$), see Tables 1 and 3; positive ion HRESIMS *m*/*z* 383.1964 (calcd for $C_{22}H_{27}N_2O_4$ $[M+H]^+$, 383.1971).

*4.4.3. Vinmajine C (**3**).* White amorphous powder; $[\alpha]_D^{22}$ −36.7 (*c* 0.112, MeOH); UV (MeOH) λ_{max} (log ε): 310 (3.60), 246 (4.01), 204 (4.47); IR (KBr) ν_{max} 2947, 1737, 1481, 1373, 1250, 1213, 1157, 1119, 1033, 817, 714 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Tables 1 and 3; HREIMS *m*/*z* 380.2100 (calcd for $C_{23}H_{28}N_2O_3$ $[M]^+$, 380.2100).

*4.4.4. Vinmajine D (**4**).* White amorphous powder; $[\alpha]_D^{21}$ −15.2 (*c* 0.089, MeOH); UV (MeOH) λ_{max} (log ε): 291 (3.44), 247 (3.86), 204 (4.47); IR (KBr) ν_{max} 3417, 2950, 1735, 1611, 1465, 1293, 1245, 1139, 1079, 996, 759, 746 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Tables 2 and 3; HREIMS *m*/*z* 382.1895 (calcd for $C_{22}H_{26}N_2O_4$ $[M]^+$, 382.1893).

*4.4.5. Vinmajine E (**5**).* White amorphous powder; $[\alpha]_D^{23}$ +41.1 (*c* 0.106, MeOH); UV (MeOH) λ_{max} (log ε): 279 (3.99), 225 (4.44), 207 (4.44); IR (KBr) ν_{max} 3434, 2928, 1714, 1458, 1381, 1233, 1109, 1035, 985 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (DMSO-d_6), see Tables 2 and 3; HREIMS *m*/*z* 410.1841 (calcd for $C_{23}H_{26}N_2O_5$ $[M]^+$,410.1842).

*4.4.6. Vinmajine F (**6**).* Colorless oil; $[\alpha]_D^{23}$ +55.9 (*c* 0.101, MeOH); UV (MeOH) λ_{max} (log ε): 277 (3.85), 224 (4.34), 209 (4.34); IR (KBr) ν_{max} 3397, 2929, 1715, 1596, 1281, 1217, 1151, 1032 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data ($CDCl_3$), see Tables 2 and 3; HREIMS *m*/*z* 338.1628 (calcd for $C_{20}H_{22}N_2O_3$ $[M]^+$, 338.1630).

*4.4.7. Vinmajine G (**7**).* White amorphous powder; $[\alpha]_D^{22}$ −92.2 (*c* 0.098, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.39), 194 (4.14); IR (KBr) ν_{max} 3370, 3289, 2947, 1704, 1629, 1440, 1314, 1211, 1151, 1086 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (DMSO-d_6), see Tables 2 and 3; HREIMS *m*/*z* 384.1690 (calcd for $C_{21}H_{24}N_2O_5$ $[M]^+$,384.1685).

*4.4.8. Vinmajine H (**8**).* Yellow amorphous powder; $[\alpha]_D^{22}$ +51.3 (*c* 0.230, MeOH); UV (MeOH) λ_{max} (log ε): 395 (3.59), 376 (3.59), 220 (3.54); IR (KBr) ν_{max} 345, 1720, 1654, 1606, 1534, 1374, 1253, 1029, 740 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (acetone-d_6), see Tables 2 and 3; positive ion HRESIMS *m*/*z* 374.1497 (calcd for $C_{22}H_{20}N_3O_3$ $[M+H]^+$, 374.1505).

*4.4.9. Vinmajine I (**9**).* Yellow amorphous powder; $[\alpha]_D^{22}$ +31.7 (*c* 0.101, MeOH); UV (MeOH) λ_{max} (log ε): 395 (4.03), 378 (4.14), 222 (4.14); IR (KBr) ν_{max} 3440, 1605, 1501, 1453, 1384, 1325, 1117, 746 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (DMSO-d_6), see Tables 2 and 3; HREIMS *m*/*z* 332.1392 (calcd for $C_{20}H_{18}N_3O_2$ $[M]^+$, 332.1399).

4.5. Computational methods

Conformational analysis is initially performed using Discovery Studio 3.5 Client. The step provides a large number of MD minimized geometries and an energy cutoff of 20 kcal/mol was applied to reduce the number of energy minima to be subsequently refined.[56] The geometries and energies of the stationary points on the potential energy surface were estimated using the DFT (B3LYP) method in conjunction with the 6-31G(d). Vibrational analysis at the B3LYP/6-311++G(d,p) level of theory resulted in no imaginary frequencies, confirming the considered conformers as real minima. Free energy values were used to obtain the Boltzmann population of conformers at 298.15 K. The theoretical calculation of ECD was performed using time-dependent density-functional theory (TDDFT) at B3LYP/6-311++G(d,p) level methanol with PCM model or in the gas phase. The specific rotation of a chiral molecule in dilute solution calculated at B3LYP/6-311++g(2d,2p)//B3LYP/6-311++g(d,p) with PCM model. All above calculations were carried out with the Gaussian09 package.[22] The calculated ECD spectra were obtained by weighing the Boltzmann distribution rate of lowest-energy conformers.[57] The ECD Cotton effect is approximated by the Gaussian distribution.[58]

4.6. Cytotoxicity assay

Five human cancer cell lines: human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7 and colon cancer SW480 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates.[55] Briefly, 100 μL adherent cells were seeded into each well of 96-

well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug adding with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed. IC_{50} value was calculated by Reed and Muench's method.[59]

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Supplementary data

1D, 2D NMR (HSQC, HMBC, ROESY), and MS spectra of *vinmajines* A–I (**1**–**9**), CD curves of *vinmajines* A and I, and the cytotoxic activities of the alkaloids are available. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.09.026. These data include MOL files and InChiKeys of the most important compounds described in this article.

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