Monoterpenoid Indole Alkaloids from *Alstonia yunnanensis*

Tao Feng,[†,‡] Yan Li,[†] Xiang-Hai Cai,[†] Xun Gong,[†] Ya-Ping Liu,[†,‡] Rong-Ting Zhang,[†,‡] Xiang-Yun Zhang,[†,‡] Qin-Gang Tan,[†,‡] and Xiao-Dong Luo*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, and Graduate School of the Chinese Academy of Sciences, Beijing 100039, People's Republic of China

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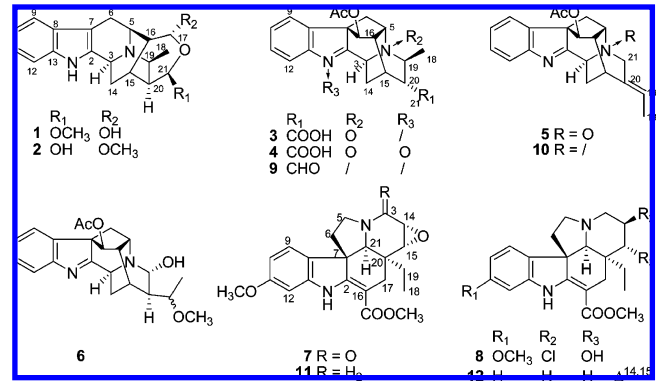
Eight new monoterpenoid indole alkaloids, alstoyunines A−H (**1**−**8**), along with 17 known analogues, were isolated from *Alstonia yunnanensis*. The structures of the new alkaloids were established by means of extensive spectroscopic methods. Alstoyunines C (**3**), E (**5**), and F (**6**) showed selective inhibition of Cox-2 (>75%). Alstoyunine F (**6**) showed weak cytotoxicity against the human myeloid leukemia HL-60 (IC_{50} = 3.89 μM) and hepatocellular carcinoma SMMC-7721 (IC_{50} = 21.73 μM) cell lines.

Monoterpenoid indole alkaloids, which originate from the condensation of tryptophan with secologanin to give strictosidine and then elaborate to give an impressive array of structural variants,[1] occur abundantly in the family Apocynaceae. Many of them, such as reserpine,[2] vincristine,[3] and yohimbine,[4] are well known for their pharmacological significance. In our previous study of the monoterpenoid indole alkaloids from the family Apocynaceae, a series of alkaloids, including scholarisines A−G, (19,20)-*E*-alstoscholarine, and (19,20)-*Z*-alstoscholarine, have been isolated from *Alstonia scholaris*.[5−7] As a continuation of our search for biologically active alkaloids, *Alstonia yunnanensis* Diels, a medicinal plant used for the treatment of fever, headache, and inflammation in the southwest of China,[8] has been investigated. Eight new monoterpenoid indole alkaloids, alstoyunines A−H (**1**−**8**), as well as 17 known ones, perakine (**9**),[9,10] vinorine (**10**),[11] lochnerinine (**11**),[12,13] tabersonine (**12**),[14,15] raucaffrinoline,[16,17] 11-methoxytabersonine,[18] 19-acetoxy-11-methoxytabersonine,[19] (−)-echitoveniline,[20] echitoserpidine,[21] vellosimine,[22] vellosiminol,[23] picrinine,[23] picraline,[24] 19(*Z*)-burnamine-17-*O*-3′,4′,5′-trimethoxybenzoate,[25] compactinervine,[26,27] 19-*epi*-ajmalicine,[28,29] and alloyohimbine,[30,31] were isolated from the whole plant extract of *A. yunnanensis*. The new compounds were evaluated for their anti-inflammatory activity against Cox-1, Cox-2, and 5-Lox, and compounds **3**, **5**, and **6** showed selective inhibitory effects on Cox-2, with inhibitory rates of 75−95% at 100 μM. In addition, the new compounds were tested for antitumor activity against five human tumor cell lines. Compound **6** displayed weak cytotoxicity against the human myeloid leukemia HL-60 (IC_{50} = 3.89 μM) and hepatocellular carcinoma SMMC-7721 (IC_{50} = 21.73 μM) cell lines.

Results and Discussion

An alkaloidal extract of the whole plant of *A. yunnanensis* was repeatedly chromatographed on silica gel, RP-18, and Sephadex LH-20 columns to yield a total of 25 monoterpenoid indole alkaloids, including eight new ones, which were elucidated as alstoyunines A−H (**1**−**8**).

Compound **1** was isolated as colorless needles. The molecular formula was established as $C_{20}H_{24}N_2O_3$ by the quasimolecular ion peak at *m/z* 341.1862 $[M + H]^+$ in the positive HRESIMS, indicating 10 degrees of unsaturation. The UV absorption bands at λ 289, 279, and 228 nm indicated an indole chromophore,[32] while the IR absorption bands at 3398 and 3220 cm^{-1} showed the existence of OH and NH groups, respectively. The ^1H NMR, ^{13}C NMR, and DEPT spectra of compound **1** displayed a substituted indole ring [δ_C 104.4 (s, C-7), 111.9 (d, C-12), 118.6 (d, C-9), 119.7 (d, C-10), 121.9 (d, C-11), 128.7 (s, C-8), 138.2 (s, C-13), 139.2 (s, C-2); δ_H 6.94 (t, *J* = 8.0 Hz, H-10), 7.01 (t, *J* = 8.0 Hz, H-11), 7.25 (d, *J* = 8.0 Hz, H-12), 7.35 (d, *J* = 8.0 Hz, H-9)]. Besides the signals of the indole ring, the ^{13}C NMR spectrum displayed 12 additional carbon signals, which were classified by the chemical shifts and HSQC spectrum as eight methine carbons (δ_C 25.8, 38.0, 42.5, 44.6, 52.7, 55.2, 93.1, 101.0), two methylene carbons (δ_C 29.4, 33.2), one methyl (δ_C 12.4), and one *O*-methyl group (δ_C 55.4). The methine carbons at δ_C 93.1 and 101.0 were ascribed to two hemiacetal groups located at C-17 and C-21, respectively, according to the HMBC correlations of δ_H 1.53 (1H, d, *J* = 5.0 Hz, H-16) and 3.81 (1H, dd, *J* = 5.5, 5.2 Hz, H-5) with δ_C 93.1 (C-17) and of δ_H 1.75 (1H, dd, *J* = 10.0, 3.5 Hz, H-20) and 3.25 (1H, m, H-19) with δ_C 101.0 (C-21) (Figure 1). The $^1H-^1H$ COSY correlations established the linkages of C-9/C-12, C-5/C-16, C-15/C-20/C-21, and C-18/C-20, as shown in Figure 1, which suggested that **1** possessed a similar carbon skeleton to that of (19*S*,20*R*)-dihydroperaksine.[32] However, the key difference between the two compounds was that compound **1** possessed one more ring. In the HMBC spectrum, the cross-peak of H-17 with C-21 indicated the linkage between C-17 and C-21 through an oxygen atom (Figure 1), which established the additional ring. In addition, the HMBC correlation between the *O*-methyl hydrogens at δ_H 3.40 (3H, s) and C-21 indicated that the methoxy group should be connected to C-21. The relative configuration of **1** was assigned on the basis of a ROESY experiment (Figure 1). The ROESY correlations of H-3 and H-20 with H-19 and of H-18 with H-5 indicated that H-3, H-5, H-19, and H-20 were α-oriented. The ROESY correlations of H-15 and H-16 with H-17 and of H-21 with H-18 indicated the *R**



* To whom correspondence should be addressed. Tel: 86-871-5223177. Fax: 86-87-5150227. E-mail: xdluo@mail.kib.ac.cn.
† Kunming Institute of Botany, Chinese Academy of Sciences.
‡ Graduate School of the Chinese Academy of Sciences.

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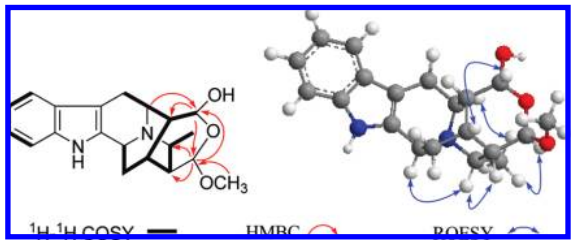

Figure 1. Key 2D NMR correlations of alstoyunine A (**1**).

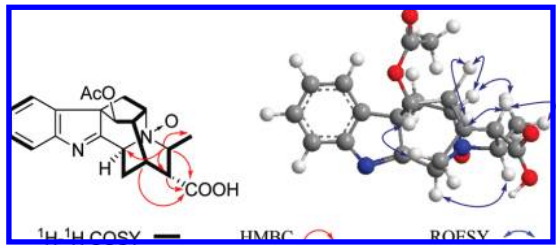

Figure 2. Key 2D NMR correlations of alstoyunine C (**3**).

configuration of C-15, C-16, C-17, and C-21. Thus, the structure of alstoyunine A was elucidated as **1**.

Compound **2** was isolated as a white, amorphous powder. The molecular formula $C_{20}H_{24}N_2O_3$ was determined by the positive HRESIMS ($[M + H]^+$ at *m/z* 341.1857), suggesting that **2** was an isomer of **1**. The 1D and 2D NMR data were closely related to those of **1**. The only difference was the exchange of the hydroxy and methoxy groups. The HMBC correlation between the *O*-methyl hydrogens at δ_H 3.48 (s) and δ_C 99.1 (C-17) suggested that the *O*-methyl group should be placed at C-17 rather than at C-21 as in **1**. The ROESY spectrum showed correlations of H-19/H-3, H-19/H-20, H-18/H-5, H-15/H-16, H-15/H-17, H-17/H-16, and H-18/H-21. These were in good agreement with those of compound **1**, which showed the same relative configurations at C-3, C-5, C-15, C-16, C-17, C-19, C-20, and C-21. Thus, the structure of alstoyunine B was established as **2**.

Compound **3** was isolated as white needles with a molecular formula of $C_{21}H_{22}N_2O_5$ assigned by the positive ion HRESIMS ($[M + H]^+$ at *m/z* 383.1617). The UV absorption bands at λ 264 and 219 nm indicated the presence of an indolenine chromophore,[33] which was cofirmed by the characteristic chemical shift of C-2 at δ_C 179.6 in the ^{13}C NMR spectrum. According to the other characteristic signals for the normal monoterpenoid indolenine alkaloids at δ_C 65.4 (s, C-7), 156.6 (s, C-2), 171.4 (s, $CH_3\underline{C}O$), 20.7 (q, $\underline{C}H_3CO$), 28.8 (d, C-15), 50.8 (d, C-16), 78.0 (d, C-17), and 14.5 (q, C-18) in the ^{13}C NMR spectrum, compound **3** could be derived from **9**.[9,10] The key difference was a carboxylic group at C-21 in compound **3** instead of an aldehyde group in **9**. This could be inferred from the HMBC correlations of δ_H 2.88 (1H, m, H-20), 4.08 (1H, m, H-19), and 2.83 (1H, m, H-15) with δ_C 173.8 (C-21) (Figure 2). In addition, compound **3** possessed an N_4-oxide group, deduced from the downfield chemical shifts at δ_C 74.6 (C-3), 66.8 (C-5), and 69.0 (C-19) compared to δ_C 56.8 (C-3), 56.2 (C-5), and 48.6 (C-19) in **9**.[10] The ROESY spectrum established the relative configuration of **3** (Figure 2). The correlations of H-5/H-18, H-3/H-19, and H-18/H-20 indicated the α-orientation of H-3, H-5, and H-19 and the β-orientation of H-20. In addition, according to the ROESY correlations of H-5/H-16, H-15/H-16, H-15/H-20, and H-17/H-14b, the relative configuration of the C-15, C-16, and C-17 stereogenic center was deduced as *R**, *S**, and *R**, respectively. Thus, the structure of alstoyunine C was elucidated as **3**.

Compound **4** ($C_{21}H_{22}N_2O_6$), amorphous powder, displayed a quasimolecular ion peak at *m/z* 399.1550 $[M + H]^+$ in the positive ion HRESIMS. The 1D and 2D NMR (HSQC, HMBC, ROESY) data were closely related to those of **3**. However, compound **4** contains one more oxygen atom compared to **3**. The upfield chemical shift of C-2 at δ_C 147.3, combined with the HMBC correlations of δ_H 5.16 (1H, d, J = 9.2 Hz, H-3), 2.64 (1H, dd, J = 14.4, 9.2 Hz, H-14a), and 2.14 (1H, dd, J = 14.4, 4.0 Hz, H-14b) with δ_C 147.3 (C-2), indicated that **4** was an N_1-oxide derivative of **3**, which was confirmed by the *m*-CPBA oxidation of **3** to yield **4**. This is the first report of an N_1-oxide indolenine alkaloid. The ROESY correlations of H-5/H-18, H-3/H-19, H-18/H-20, H-5/H-16, H-15/H-16, H-15/H-20, and H-17/H-14b established that the relative configuration of **4** was the same as that of **3**. Thus, the structure of alstoyunine D was elucidated as **4**.

Compound **5** was obtained as a colorless oil. The molecular formula of $C_{21}H_{22}N_2O_3$ was established by a quasimolecular ion peak at *m/z* 351.1707 $[M + H]^+$ in the positive HRESIMS. The ^{13}C NMR spectrum was very similar to that of **10**,[11] also isolated during this investigation, except for three highly downfield signals of C-3 (δ_C 72.6), C-5 (δ_C 73.1), and C-21 (δ_C 69.6) due to the N_4-oxide functionality. The *E* configuration of the C-19−C-20 double bond was determined by the ROESY correlations of H-18 at δ_H 1.69 (3H, d, J = 6.6 Hz) with H-15 at δ_H 3.96 (1H, dd, J = 6.0, 5.0 Hz) and of H-19 at δ_H 5.43 (1H, q, J = 6.6 Hz) with H-21a at δ_H 4.42 (1H, d, J = 15.0 Hz) and H-21b at δ_H 4.02 (1H, d, J = 15.0 Hz). The ROESY analysis additionally showed that the relative configuration of the C-3, C-5, C-15, C-16, and C-17 stereogenic center was in good agreement with those of compounds **3** and **4**. Detailed analysis of the 2D NMR spectra (HSQC, HMBC, ROESY) finally established the structure of **5** as the N_4-oxide of **10**. Thus, the structure of alstoyunine E was elucidated as **5**.

Compound **6** was obtained as a colorless oil. The molecular formula $C_{22}H_{26}N_2O_4$ was determined by a quasimolecular ion peak at *m/z* 383.1967 $[M + H]^+$ in the positive ion HRESIMS. The IR spectrum displayed absorption bands at 3423 (OH) and 1743 (C=O) cm^{-1}. The UV absorption bands at λ 258 and 219 nm indicated the presence of an indolenine chromophore, which was confirmed by the characteristic quaternary carbon at δ_C 182.5 (C-2) in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed similar patterns to those of 19,20-dihydrovomilenine,[14,15] the difference being an additional *O*-methyl group (δ_H 3.32, s; δ_C 56.1) in **6**. The connection between OCH_3 and C-19 was established by the HMBC correlation of the protons of the *O*-methyl group with C-19 (δ_C 76.8). Detailed analysis of the 2D NMR spectra (HSQC, HMBC) finally established the planar structure of **6** as 19-methoxy-19,20-dihydrovomilenine. The ROESY data analysis showed that the relative configuration of the C-3, C-5, C-15, C-16, and C-17 stereogenic center was the same as that of compounds **3**, **4**, and **5**. In addition, the ROESY correlation between H-5 and H-21 suggested the β-orientation of H-21, and the correlation of H-15 with H-20 established the α-orientation of H-20. However, the relative configuration of C-19 could not be determined, and attempts to grow a single crystal have so far not been successful. Therefore, the structure of alstoyunine F (**6**) was established as 19-methoxy-19,20-dihydrovomilenine.

Compound **7** was isolated as a colorless oil. The molecular formula $C_{22}H_{24}N_2O_5$ was established by a positive ion HRESIMS quasimolecular ion peak at *m/z* 397.1774 $[M + H]^+$. The IR absorption bands at 3373, 1658, and 1618 cm^{-1} indicated the presence of a β-anilinoacrylate chromophore,[34] corresponding to the characteristic carbon signals for an acrylate double bond at δ_C 166.3 (C-2) and 88.9 (C-16). The ^1H and ^{13}C NMR spectra, combined with MS analysis, inferred the existence of an *O*-methyl group, an acylamide group, and an oxirane ring. In addition, three armonatic signals at δ_H 7.10 (d, J = 8.0 Hz, H-9), 6.43 (dd, J = 8.0, 2.1 Hz, H-10), and 6.48 (d, J = 2.1 Hz, H-12) and one *O*-methyl group at δ_H 3.79 (3H, s) suggested the *O*-methyl group was located at C-11 (δ_C 160.7). A key HMBC correlation between δ_H 3.79 and C-11 was also observed. The 14,15-epoxy group was established by the HMBC correlations of δ_H 3.61 (1H, d, J = 3.8

Table 1. ^{1}H NMR Data of Compounds **1**−**4** (methanol-d_4, δ in ppm and J in Hz)

no.	1	2	3	4
3	4.07, d (9.0)	4.68, d (9.5)	4.52, d (9.8)	5.16, d (9.2)
5	3.81, dd (5.5, 5.2)	4.20, dd (5.5, 5.2)	4.30, dd (6.0, 5.0)	4.27, dd (6.0, 5.0)
6a	2.97, dd (15.5, 5.5)	3.18, dd (16.5, 5.5)	2.91, m	2.90, dd (13.0, 4.3)
6b	2.62, d (15.5)	2.80, d (16.5)	2.46, d (13.0)	2.60, d (13.0)
9	7.35, d (8.0)	7.43, d (8.0)	7.62, d (7.5)	7.74, d (7.8)
10	6.94, t (8.0)	7.03, t (8.0)	7.31, t (7.5)	7.58, t (7.8)
11	7.01, t (8.0)	7.13, t (8.0)	7.43, t (7.5)	7.62, t (7.8)
12	7.25, d (8.0)	7.32, d (8.0)	7.61, d (7.5)	7.79, d (7.8)
14a	1.99, dd (13.8, 9.0)	2.34, dd (14.0, 9.5)	2.60, dd (14.5, 9.8)	2.64, dd (14.4, 9.2)
14b	1.59, dd (13.8. 4.2)	1.88, m	2.07, dd (14.5, 5.0)	2.14, dd (14.4, 4.0)
15	2.11, br s	2.49, br s	2.83, m	2.85, m
16	1.53, d (5.0)	1.93, br s	3.08, m	3.07, m
17	5.05, d (1.1)	4.98, d (1.3)	4.99, s	5.11, d (1.0)
18	1.37, d (7.2)	1.57, d (7.1)	1.54, d (6.5)	1.39, d (6.3)
19	3.25, m	3.85, m	4.08, m	4.15, m
20	1.75, dd (10.0, 3.5)	2.08, m	2.88, m	2.79, d (9.5)
21	4.81, br s	5.39, br s		
$OCOCH_3$			2.19, s	2.19, s
OCH_3	3.40, s	3.48, s		

Table 2. ^{1}H NMR Data of Compounds **5**−**8** ($CDCl_3$, δ in ppm, J in Hz)

no.	5	6	7	8
N_1-H			8.95, s	8.94, s
3a	4.61, d (9.8)	4.41, d (9.2)		3.32, dd (12.0, 5.0)
3b				3.25, dd (12.0, 4.2)
5a	3.96, dd (6.0, 5.0)	3.49, m	4.43, dd (12.0, 6.8)	2.97, m
5b			3.20, m	2.68, m
6a	2.88, d (13.0)	2.79, dd (12.0, 4.7)	1.73, m	2.09, m
6b	2.70, dd (13.0, 4.0)	1.68, d (12.0)	1.68, m	1.70, dd (11.3, 4.4)
9	7.63, d (7.7)	7.43, d (7.5)	7.10, d (8.0)	7.05, d (8.0)
10	7.24, t (7.7)	7.20, t (7.5)	6.43, dd (8.0, 2.1)	6.39, dd (8.0, 2.0)
11	7.40, t (7.7)	7.38, t (7.5)		
12	7.46, d (7.7)	7.63, d (7.5)	6.48, d (2.1)	6.42, d (2.0)
14a	2.42, dd (15.0, 9.8)	1.88, m	3.61, d (3.8)	4.18, m
14b	2.09, dd (15.0, 5.0)	1.78, dd (14.0, 5.5)		
15	3.36, t (5.0)	2.70, m	3.47, d (3.8)	3.91, d (6.0)
16	2.75, m	2.37, m		
17a	4.85, br s	5.03, br s	2.66, d (16.0)	2.84, d (14.8)
17b			1.85, d (16.0)	2.71, d (14.8)
18	1.69, d (6.6)	1.11, d (6.0)	0.78, t (7.2)	0.70, t (7.4)
19a	5.43, q (6.6)	3.27, m	1.28, q (7.2)	1.18, q (7.4)
19b			1.09, q (7.2)	0.90, q (7.4)
20		1.58, d (8.0)		
21a	4.42, d (15.0)	4.46, br s	3.62, s	2.78, s
21b	4.02, d (15.0)			
$OCOCH_3$	2.18, s	2.18, s		
CO_2CH_3			3.81, s	3.79, s
OCH_3		3.32, s	3.79, s	3.79, s

Hz, H-14) with δ_C 164.8 (s, C-3) and of δ_H 3.47 (1H, d, J = 3.8 Hz, H-15) with δ_C 40.6 (s, C-20) and 63.3 (d, C-21). This information suggested that the structure of **7** was closely related to **11**.[12,13] The distinct difference was that the methylene at C-3 in **11** was replaced by an acylamide group (δ_C 164.8, s) in **7**, which was supported by the HMBC correlations of H-14 and H-15 with C-3. According to ROESY data, the α-orientation of the 14,15-epoxy group was determined by the correlation between H-15 and H-17a, which is possible only if the orientation of H-15 is β.[34] Finally, the structure of alstoyunine G (**7**) was established as 3-oxo-11-methoxytabersonine-14,15-α-epoxide by the detailed analysis of 2D NMR data (HSQC, HMBC, ^{1}H−^{1}H COSY, ROESY).

Compound **8** was isolated as a colorless oil. The positive HRESIMS displayed a quasimolecular ion peak at m/z 419.1727 $[M + H]^+$, consistent with a molecular formula $C_{22}H_{27}N_2O_4Cl$. The chlorine atom was identified by the appropriate ^{13}C NMR chemical shift at δ_C 59.5 (d, C-14) and EIMS analysis. The EIMS showed an isotope peak at m/z 420 and the fragment ion peak at m/z 382 for the loss of HCl.[35] The ^{1}H and ^{13}C NMR data were similar to those of **11**,[12,13] except for a hydroxy group at C-15 (δ_C 75.8) and a chlorine atom at C-14 (δ_C 59.5) in **8** instead of the 14,15-epoxy group in **11**, as evidenced by the MS analysis and the ^{1}H−^{1}H COSY correlations of H-14 at δ_H 4.18 (1H, m) with H-14a at δ_H 3.32 (1H, dd, J = 12.0, 5.0 Hz) and H-3b at δ_H 3.25 (1H, dd, J = 12.0, 4.2 Hz) and of H-14 with H-15 at δ_H 3.91 (1H, d, J = 6.0 Hz). The α-orientation of H-14 and the β-orientation of H-15 were deduced from the ROESY correlations of H-14/H-21 and H-15/H-17a, respectively. Thus, the structure of alstoyunine H was established as **8**. However, alstoyunine H is likely an artfact of **11** because HCl was used during the extraction process.

The NMR data of compounds **1**−**8** as shown in Tables 1, 2, and 3 were assigned on the basis of 2D NMR spectra (HSQC, HMBC, ^{1}H−^{1}H COSY, ROESY).

Generally, alstoyunines A−H represent three kinds of carbon skeletons derived from peraksine, perakine (**9**), and tabersonine (**12**). Since the absolute configuration of peraksine,[36] perakine (**9**),[9,10] and tabersonine (**12**)[14,15] was elucidated previously, biogenetically, the absolute configuration of alstoyunines A−H could be assumed as (3*S*,5*S*,15*R*,16*R*,17*R*,19*S*,20*S*,21*R*) for alstoyunine A (**1**), (3*S*,5*S*,15*R*,16*R*,17*R*,19*S*,20*S*,21*R*) for alstoyunine B (**2**), (3*S*,5*S*,15*R*,16*S*,17*R*,19*S*,20*R*) for alstoyunine C (**3**), (3*S*,5*S*,15*R*,16*S*,17*R*,19*S*,20*R*) for alstoyunine D (**4**), (3*S*,5*S*,15*R*,16*S*,17*R*,19*E*) for

Table 3. ^{13}C NMR Data of Compounds **1**−**8**[a]

no.	1	2	3	4	5	6	7	8
2	139.2 s	134.3 s	179.6 s	147.3 s	178.0 s	182.5 s	166.3 s	166.4 s
3	52.7 d	52.6 d	74.6 d	68.7 d	72.6 d	50.0 d	164.8 s	54.5 t
5	44.6 d	47.5 d	66.8 d	67.5 d	73.1 d	55.5 d	43.4 t	50.8 t
6	29.4 t	27.6 t	33.8 t	33.5 t	32.4 t	37.3 t	41.9 t	44.2 t
7	104.4 s	103.4 s	65.4 s	58.3 s	63.5 s	65.0 s	56.7 s	54.4 s
8	128.7 s	127.8 s	136.3 s	132.8 s	134.7 s	136.3 s	127.5 s	129.9 s
9	118.6 d	119.0 d	125.5 d	126.1 d	123.8 d	123.6 d	121.8 d	121.7 d
10	119.7 d	120.5 d	127.8 d	130.9 d	126.2 d	125.5 d	105.5 d	105.0 d
11	121.9 d	123.3 d	130.3 d	131.0 d	129.1 d	128.7 d	160.7 s	160.1 s
12	111.9 d	112.3 d	122.1 d	116.1 d	121.6 d	121.1 d	97.2 d	96.7 d
13	138.2 s	138.5 s	156.6 s	149.2 s	155.9 s	156.4 s	143.9 s	144.3 s
14	33.2 t	31.7 t	26.6 t	26.4 t	28.4 t	27.7 t	51.1 d	59.5 d
15	25.8 d	24.2 d	28.8 d	28.9 d	26.7 d	25.3 d	57.1 d	75.8 d
16	42.5 d	41.4 d	50.8 d	50.8 d	49.0 d	42.7 d	88.9 s	92.7 s
17	93.1 d	99.1 d	78.0 d	78.4 d	76.5 d	78.2 d	22.2 t	26.7 t
18	12.4 q	12.3 q	14.5 q	14.4 q	12.6 q	16.3 q	7.2 q	8.1 q
19	55.2 d	58.0 d	69.0 d	69.2 d	119.8 d	76.8 d	26.2 t	22.8 t
20	38.0 d	37.9 d	51.1 d	51.3 d	130.9 s	51.9 d	40.6 s	44.4 s
21	101.0 d	92.6 d	173.8 s	174.0 s	69.6 t	84.5 d	63.3 d	69.6 d
$\underline{C}O_2CH_3$							168.2 s	168.8 s
$CO_2\underline{C}H_3$							51.2 q	51.0 q
OCH_3	55.4 q	56.6 q				56.1 q	55.5 q	53.4 q
$O\underline{C}OCH_3$			171.4 s	171.2 s	169.8 s	169.6 s		
$OCO\underline{C}H_3$			20.7 q	20.8 q	20.8 q	21.1 q		

[a] Compounds **1**−**4** were recorded in methanol-d_4, **5**−**8** in $CDCl_3$, δ in ppm.

Table 4. Evaluation of Anti-Inflammatory Activity of Compounds **1**−**8**[a]

compound	COX-1	COX-2	5-LOX
1	27.76	32.14	38.36
2	26.84	33.91	33.54
3	45.03	94.84	<0
4	39.33	42.21	<0
5	41.76	93.94	77.22
6	39.82	77.18	61.82
7	37.24	<0	<0
8	<0	<0	0.84
SC-560	61.30		
NS-398		97.09	
zileuton			83.05

[a] Percent inhibition (all compounds and reference drugs concentration: 100 μM).

alstoyunine E (**5**), (3*S*,5*S*,15*R*,16*S*,17*R*,20*R*,21*R*) for alstoyunine F (**6**), (7*R*,14*R*,15*R*,20*S*,21*R*) for alstoyunine G (**7**), and (7*R*,14*R*,-15*R*,20*S*,21*R*) for alstoyunine H (**8**), based on their relative configurations.

The new compounds **1**−**8** were tested for their anti-inflammatory activity and cytotoxicity. The results of the anti-inflammatory assay are shown in Table 4. Alstoyunines C (**3**), E (**5**), and F (**6**) showed selective inhibition of Cox-2 (>75%). The cytotoxic assay showed that alstoyunine F (**6**) displayed weak activity against the human myeloid leukemia HL-60 (IC_{50} = 3.89 μM) and hepatocellular carcinoma SMMC-7721 (IC_{50} = 21.73 μM) cell lines, while other compounds were noncytotoxic (IC_{50} > 40 μM).

Experimental Section

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained by a Tenor 27 spectrophotometer using KBr pellets. 1D and 2D spectra were run on Bruker DRX-500 and AV-400 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Autospec-3000 spectrometer or an API QSTAR Pulsar 1 spectrometer. Column chromatography 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 or by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The whole plants of *A. yunnanensis* were collected in Kunming, Yunnan Province, People's Republic of China, in September 2007. The sample was identified by Dr. Chun-Xia Zeng. A voucher specimen (Luo 070908) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. An MeOH extract of the dried whole plants of *A. yunnanensis* (12 kg) was concentrated to dryness, then dissolved in 7% HCl (2 L) and filtered. The filtration was basified using 10% ammonia−water to pH 9−10. The basic solution was partitioned with EtOAc to give a total alkaloidal extract (27 g). The latter was subjected to a silica gel column, eluted with $CHCl_3$−Me_2CO (from 1:0 to 0:1), to obtain eight fractions (1−8). Fraction 2 (2.8 g) yielded **7** (12 mg), **8** (8 mg), **10** (13 mg), **12** (60 mg), and 11-methoxytabersonine (30 mg) after repeated silica gel column chromatography eluted with petroleum ether−Me_2CO (4:1). Separation of fraction 3 (4.2 g) by silica gel column chromatography, eluted with petroleum ether−Me_2CO (from 8:1 to 1:1), afforded six subfractions (3a−3f). Both fractions 3c (80 mg) and 3e (100 mg) were subjected to a Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1) to yield (−)-echitoveniline (12 mg) and echitoserpidine (10 mg), respectively. Fraction 3a (260 mg) was subjected to silica gel column chromatography (petroleum ether−Me_2CO, 2:1) to afford raucaffrinoline (90 mg). Fraction 3d (110 mg) was subjected to silica gel column chromatography eluted with petroleum ether−Me_2CO (from 5:1 to 1:1) to give 19-acetoxy-11-methoxytabersonine (40 mg) and picrinine (7 mg). 19-*epi*-Ajmalicine (13 mg) was recrystallized from fraction 3b (70 mg) in MeOH, and 19(*Z*)-burnamine-17-*O*-3′,4′,5′-trimethoxybenzoate (7 mg) from fraction 3f (20 mg) in MeOH. Fraction 4 (1.8 g) was subjected to an RP-18 column (MeOH−H_2O, 6:4), followed by silica gel column chromatography eluting with $CHCl_3$−MeOH (13:1) and $CHCl_3$−MeOH (15:1) to yield **2** (16 mg) and **1** (5 mg), respectively. Fraction 5 (2.0 g) was purified by RP-18 (MeOH−H_2O, 5:5) column chromatography to afford **9** (680 mg) and fraction 5c (800 mg) as a mixture. The latter was chromatographed on a silica gel column ($CHCl_3$−MeOH, 10:1) to afford picraline (18 mg) and compactinervine (33 mg). Fraction 6 (3.8 g) was separated by silica gel ($CHCl_3$−MeOH, from 10:1 to 1:1) column chromatography to afford five subfractions (6a−6e). Fraction 6b (140 mg) was further purified by Sephadex LH-20 column chromatography to yield **11** (45 mg) and vellosiminol (37 mg). Vellosimine (1.1 g) precipitated from fraction 6e (1.8 g). Fraction 7

(3.2 g) was subjected to RP-18 (MeOH−H_2O, from 2:8 to 6:4) and Sephadex LH-20 (MeOH) column chromatography to yield **5** (23 mg), **6** (18 mg), and alloyohimbine (120 mg). Fraction 8 (1.8 g) was subjected to Sephadex LH-20 (MeOH−H_2O, 7:3) column chromatography to afford four subfractions (8a−8d). Fractions 8c (120 mg) and 8d (60 mg) were further purified by RP-18 column chromatography (eluting with MeOH−H_2O, 2.5:7.5 and 2:8, respectively) to afford **3** (13 mg) and **4** (2 mg), respectively.

Alstoyunine A (1): colorless needles (MeOH); mp 147−148 °C; $[\alpha]_D^{20}$ −74.3 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 289 (3.82), 279 (3.89), 228 (3.87), 193 (3.84) nm; IR (KBr) ν_{max} 3398, 2925, 1453, 1032, 744 cm^{-1}; ^1H and ^{13}C NMR data (methanol-d_4), see Tables 1 and 3, respectively; positive ion HRESIMS *m/z* 341.1862 (calcd for $C_{20}H_{25}N_2O_3$ $[M + H]^+$, 341.1865).

Alstoyunine B (2): white, amorphous powder; $[\alpha]_D^{20}$ −69.0 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 289 (3.79), 278 (3.83), 222 (3.83), 194 (3.81) nm; IR (KBr) ν_{max} 3406, 2936, 1455, 1029, 747 cm^{-1}; ^1H and ^{13}C NMR data (methanol-d_4), see Tables 1 and 3, respectively; positive ion HRESIMS *m/z* 341.1857 (calcd for $C_{20}H_{25}N_2O_3$ $[M + H]^+$, 341.1865).

Alstoyunine C (3): white needles (MeOH); mp 293−295 °C; $[\alpha]_D^{20}$ −111.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 264 (3.77), 219 (3.80) nm; IR (KBr) ν_{max} 3433, 2952, 1739, 1232, 1040, 766 cm^{-1}; ^1H and ^{13}C NMR data (methanol-d_4), see Tables 1 and 3, respectively; positive ion HRESIMS *m/z* 383.1617 (calcd for $C_{21}H_{23}N_2O_5$ $[M + H]^+$, 383.1607).

Alstoyunine D (4): white, amorphous powder; $[\alpha]_D^{20}$ −27.8 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 278 (3.88), 227 (3.84), 207 (3.79), 197 (3.85) nm; IR (KBr) ν_{max} 3440, 2962, 1743, 1228, 1051, 761 cm^{-1}; ^1H and ^{13}C NMR data (methanol-d_4), see Tables 1 and 3, respectively; positive ion HRESIMS *m/z* 399.1550 (calcd for $C_{21}H_{23}N_2O_6$ $[M + H]^+$, 399.1556).

Alstoyunine E (5): colorless oil; $[\alpha]_D^{20}$ −73.7 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 264 (3.84), 220 (3.82), 194 (3.83) nm; IR (KBr) ν_{max} 3422, 2965, 1742, 1593, 1228, 1037, 752 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Tables 2 and 3, respectively; positive ion HRESIMS *m/z* 351.1707 (calcd for $C_{21}H_{23}N_2O_3$ $[M + H]^+$, 351.1709).

Alstoyunine F (6): colorless oil; $[\alpha]_D^{20}$ −27.7 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 258 (3.87), 219 (3.84) nm; IR (KBr) ν_{max} 3423, 2940, 1743, 1592, 1234, 1032, 750 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Tables 2 and 3, respectively; positive ion HRESIMS *m/z* 383.1967 (calcd for $C_{22}H_{27}N_2O_4$ $[M + H]^+$, 383.1971).

Alstoyunine G (7): colorless oil; $[\alpha]_D^{20}$ −137.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 324 (3.80), 244 (3.84), 228 (3.82), 197 (3.79) nm; IR (KBr) ν_{max} 3392, 1658, 1618, 1439, 1106, 753 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Tables 2 and 3, respectively; positive ion HRESIMS *m/z* 397.1774 (calcd for $C_{22}H_{25}N_2O_5$ $[M + H]^+$, 397.1763).

Alstoyunine H (8): colorless oil; $[\alpha]_D^{20}$ −337.3 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 326 (3.83), 245 (3.75), 228 (3.50), 223 (3.80), 205 (3.45), 198 (3.44) nm; IR (KBr) ν_{max} 3424, 1677, 1617, 1263, 1112, 758 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Tables 2 and 3, respectively; positive ion HRESIMS *m/z* 419.1727 (calcd for $C_{22}H_{28}N_2O_4Cl$ $[M + H]^+$, 419.1738).

In Vitro Anti-Inflammatory Assay. The anti-inflammatory activity was performed according to the literature with minor modifications.[37] Briefly, the reaction system was incubated for 5 min at 25 °C, by sequential addition of the buffer, heme, test compounds, and Cox-1 or Cox-2 into the system followed by mixing with TMPD and arachidonic acid, and soft agitation for several seconds. The absorbance value was recorded at a wavelength of 590 nm after another 15 min of incubation at 25 °C. The performance of the assay was checked using SC-560 and NS-398 as positive controls, which gave the inhibition of Cox-1 (61.3%) and Cox-2 (97.1%), respectively (Table 4). Different from the method mentioned above, the reaction system was added to the assay buffer with 5-Lox in the presence of the colorimetric substrate and test compounds and then incubated for a period of 5 min at 25 °C. After the completion of the reaction, the chromogen was added, and the plate was shaken softly for a few seconds. A further period of 5 min incubation was performed at 25 °C. The inhibitory effect against 5-Lox was determined by measuring the absorbance at a wavelength of 500 nm. The performance of the assay was checked using zileuton as a positive control, which led to the inhibition of 83.05% (Table 4).

Cytotoxicity Assay. The following human tumor cell lines were used: breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) 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.[38] 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 addition with initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, St. Louis, MO) as positive control, which gave IC_{50} values of 1.11 μM (HL-60), 16.53 μM (SMMC-7721), 23.15 μM (A-549), 29.96 μM (SK-BK-3), and 89.40 μM (PANC-1). After compound treatment, cell viability was detected and the cell growth curve was graphed. The IC_{50} value was calculated by the Reed and Muench method.[39]

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Supporting Information Available: 1D and 2D NMR and MS spectra of alstoyunines A−H (**1**−**8**). These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hutchinson, C. R. *Tetrahedron* **1981**, *37*, 1047–1065.
(2) Mueller, J. M.; Schlittler, E.; Bein, H. J. *Experientia* **1952**, *8*, 338.
(3) Moncrief, J. W.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1965**, *87*, 4963–4964.
(4) Bader, F. F.; Dichel, D. F.; Schlittler, E. *J. Am. Chem. Soc.* **1954**, *76*, 1695–1696.
(5) Cai, X. H.; Du, Z. Z.; Luo, X. D. *Org. Lett.* **2007**, *9*, 1817–1820.
(6) Cai, X. H.; Tan, Q. G.; Liu, Y. P.; Feng, T.; Li, W. Q.; Luo, X. D. *Org. Lett.* **2008**, *10*, 577–580.
(7) Feng, T.; Cai, X. H.; Zhao, P. G.; Du, Z. Z.; Li, W. Q.; Luo, X. D. *Planta Med.*, in press.
(8) Tsiang, Y.; Li, P. Y. *Flora of China*; Science Press: Beijing, 1977; Vol. 63, p 92.
(9) Ulshafer, P. R.; Bartlett, M. F.; Dorfman, L.; Gillen, M. A.; Schlittler, E.; Wenkert, E. *Tetrahedron Lett.* **1961**, 363–367.
(10) Khan, M. A.; Horn, H.; Voelter, W. *Z. Naturforsch., B: Chem. Sci.* **1982**, *37B*, 494–498.
(11) Meisel, H.; Doepke, W.; Gruendemann, E. *Tetrahedron Lett.* **1971**, 1291–1293.
(12) Moza, B. K.; Trojanek, J.; Bose, K. A.; Das, K. G.; Funke, P. *Tetrahedron Lett.* **1964**, 2561–2566.
(13) Moza, B. K.; Trojanek, J.; Bose, K. A.; Das, K. G.; Funke, P. *Lloydia* **1964**, *27*, 416–427.
(14) Plat, M.; Men, J. L.; Janot, M. M.; Wilson, J. M.; Budzikiewicz, H.; Durham, L. J.; Nakagawa, Y.; Djerassi, C. *Tetrahedron Lett.* **1962**, 271–276.
(15) Ziegler, F. E.; Bennett, G. B. *J. Am. Chem. Soc.* **1973**, *95*, 7458–7464.
(16) Khan, M. A.; Siddiqui, S. *Experientia* **1972**, *28*, 127–128.
(17) Libot, F.; Kunesch, N.; Posson, J. *Phytochemistry* **1980**, *19*, 989–991.
(18) Baassou, S.; Mehri, H.; Plat, M. *Phytochemistry* **1978**, *17*, 1449–1450.
(19) Kutney, J. P.; Choi, L. S. L.; Kolodziejczyk, P.; Sleigh, S. K.; Stuart, K. L.; Worth, B. R.; Kurz, W. G. W.; Chatson, K. b.; Constabel, F. *Phytochemistry* **1980**, *19*, 2589–2595.
(20) Majumder, P. L.; Joardar, S.; Chanda, T. K.; Dinda, B. N.; Banerjee, M.; Ray, A. B.; Chatterjee, A.; Varenne, P.; Das, B. C. *Tetrahedron* **1979**, *35*, 1151–1157.
(21) Majumder, P. L.; Joardar, S.; Dinda, B. N.; Bandyopadhyay, D.; Joardar, S.; Basu, A. *Tetrahedron* **1981**, *37*, 1243–1248.
(22) Rapoport, H.; Moore, R. E. *J. Org. Chem.* **1962**, *27*, 2981–2985.
(23) Grossmann, E.; Sefcovic, P.; Szasz, K. *Phytochemistry* **1973**, *12*, 2058.
(24) Britten, A. Z.; Smith, G. F. *J. Chem. Soc.* **1963**, 3850–3854.
(25) Abe, F.; Yamauchi, T.; Shibuya, H.; Kitagawa, I.; Yamashita, M. *Chem. Pharm. Bull.* **1998**, *46*, 1235–1238.
(26) Djerassi, C.; Nakagawa, Y.; Wilson, J. M.; Budziliewicz, H.; Gilbert, D.; Antonaccio, L. D. *Experientia* **1963**, *19*, 467–469.
(27) Verpoorte, R.; Kos-Kuyck, E.; Tsoi, T. A.; Ruigrok, C. L. M.; Jong, G.; Svendsen, A. B. *Planta Med.* **1983**, *48*, 283–289.
(28) Melchio, J.; Bouquet, A.; Pais, M.; Goutarel, R. *Tetrahedron Lett.* **1977**, 315–316.
(29) Uusvuori, R.; Lounasmaa, M. *Planta Med.* **1981**, *41*, 406–407.

(30) Wenkert, E.; Chang, C. J.; Chawla, H. P. S.; Cochran, D. W.; Hagaman, E. W.; King, J. C.; Orito, K. *J. Am. Chem. Soc.* **1976**, *98*, 3645–3655.
(31) Toke, L.; Honty, K.; Szabo, L.; Blasko, G.; Szantay, C. *J. Org. Chem.* **1973**, *38*, 2496–2500.
(32) Sheludko, Y.; Gerasimenko, I.; Kolshorn, H.; Stockigt, J. *J. Nat. Prod.* **2002**, *65*, 1006–1010.
(33) Kan, T. S.; Nyeoh, K. T.; Sim, K. M.; Yoganathan, K. *Phytochemistry* **1997**, *45*, 1303–1305.
(34) Lim, K. H.; Hiraku, O.; Komiyama, K.; Kam, T. S. *J. Nat. Prod.* **2008**, *71*, 1591–1594.
(35) Luo, X. D.; Wu, S. H.; Ma, Y. B.; Wu, D. G. *Heterocycles* **2000**, *53*, 2795–2768.
(36) Kiang, A. K.; Loh, S. K.; Demanczyk, M.; Gemenden, C. W.; Papariello, G. J.; Taylor, W. I. *Tetrahedron* **1966**, *22*, 3293–3300.
(37) Duan, W. G.; Zhang, L. Y. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2006**, *74*, 317–321.
(38) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(39) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493–497.

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