# Monoterpenoid Indole Alkaloids from Alstonia mairei 

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[^0]Introduction. - Monoterpenoid indole alkaloids play a most important role in natural medicinal history; for example, vincristine and quinine showed excellent anticancer and antimalaria activity, respectively [1][2]. So far, no other anticancer monoterpenoid indole alkaloids than vincristine analoges have been employed as drugs. Recently, it was reported that anticancer monomers of this type are as potent as the corresponding dimers (vincristine) [3]. The genus Alstonia of Apocynaceae is rich in monoterpenoid indole alkaloids. Four species, including two endemic plants of the genus Alstonia, are distributed in Yunnan Province [4]. The phytochemical constituents of Alstonia sp. have been investigated intensively, with anticancer, antibacterial, antifertility, and antitussive activities having been reported [5]. In our continuing study of the Yunnan endemic resources, we have reported new alkaloids from $A$. scholaris and A. yunnanensis [6-8]. Another species, A. mairei, is also rich in monoterpenoid indole alkaloids [9]. In the current study, separation of the alkaloid extract led to 22 monoterpenoid indole alkaloids. In this article, we describe the isolation and structure elucidation of three new alkaloids, $(14 \alpha, 15 \alpha)$-14,15-epoxyaspidofractinine (1), and maireines A and B ( $\mathbf{2}$ and $\mathbf{3}$, resp.), together with 19 known isolates, venalstonine (4) [10], (-)-minovincinine (5) [11], (-)-11-methoxyminovincinine (6) [12], (-)-echitovenine (7) [13], echitovenaldine (8) [14], echitovenidine (9) [15], 11-methoxyechitovenidine (10) [15], echitoveniline (11) [13], 11-methoxyechitoveniline (12) [13], echitoserpidine (13) [16], 11-methoxyechitoserpidine (14) [17], (19S)-vindolinine (15) [10], lochnericine (16) [18], tabersonine (17) [18], perakine (18) [19], picrinine (19) [20], deacetylpicraline 3,4,5-trimethoxybenzoate (20) [21], picralinal (21) [22], and rhazimol (22) [23] (Fig. 1). In addition, all compounds were tested for their cytotoxicity against five human cancer cell lines, but no significant activity was found ( $I C_{50}>$ $40 \mu \mathrm{M}$ ).


1


4


15


16


|  | $\mathrm{R}^{1}$ | $\mathrm{R}^{2}$ |
| ---: | :--- | :--- |
| $\mathbf{2}$ | MeO | $\mathrm{S}_{4}$ |
| $\mathbf{3}$ | H | $\mathrm{S}_{4}$ |
| $\mathbf{5}$ | H | OH |
| $\mathbf{6}$ | MeO | OH |
| $\mathbf{7}$ | H | AcO |
| $\mathbf{8}$ | MeO | AcO |
| $\mathbf{9}$ | H | $\mathrm{S}_{1}$ |
| $\mathbf{1 0}$ | MeO | $\mathrm{S}_{1}$ |
| $\mathbf{1 1}$ | H | $\mathrm{S}_{2}$ |
| $\mathbf{1 2}$ | MeO | $\mathrm{S}_{2}$ |
| 13 | H | $\mathrm{S}_{3}$ |
| $\mathbf{1 4}$ | MeO | $\mathrm{S}_{3}$ |



17

$19 \mathrm{R}=\mathrm{H}$
$20 \mathrm{R}=\mathrm{S}_{2} \mathrm{CH}_{2}$
$21 \mathrm{R}=\mathrm{CHO}$


18


22




Fig. 1. Alkaloids from A. mairei

Results and Discussion. - Compound 1 gave a positive reaction with Dragendorff's reagent and had a molecular formula of $\mathrm{C}_{19} \mathrm{H}_{23} \mathrm{~N}_{2} \mathrm{O}$ based on HR-ESI-MS ( $\mathrm{m} / \mathrm{z}$ $\left.295.1801\left([M+\mathrm{H}]^{+}\right)\right)$. Its UV spectrum showed the characteristic absorption bands of indole alkaloids at 240 and 288 nm [20]. The FT-IR spectra exhibited absorption bands for NH ( $3329 \mathrm{~cm}^{-1}$ ) and aromatic rings ( 1608,1479 , and $1458 \mathrm{~cm}^{-1}$ ). In the ${ }^{1} \mathrm{H}-\mathrm{NMR}$ spectrum (Table), four signals $(\delta(\mathrm{H}) 6.99(d, J=7.0, \mathrm{H}-\mathrm{C}(9)), 6.92(t, J=7.0$, $\mathrm{H}-\mathrm{C}(11)), 6.63(t, J=7.0, \mathrm{H}-\mathrm{C}(10)), 6.57(d, J=7.0, \mathrm{H}-\mathrm{C}(12)))$ revealed the presence of an unsubstituted ring $A$ in a monoterpenoid indole alkaloid [24]. The ${ }^{13} \mathrm{C}$-NMR (Table) and DEPT spectra of $\mathbf{1}$ displayed signals for a 2,3-dihydroindole ring ( $\delta(\mathrm{C}) 152.0(s, \mathrm{C}(13)), 139.8(s, \mathrm{C}(8)), 127.5(d, \mathrm{C}(11)), 121.9(d, \mathrm{C}(9)), 118.9(d, \mathrm{C}(10))$, $111.1(d, \mathrm{C}(12))$, $65.2(s, \mathrm{C}(2))$, $55.7(s, \mathrm{C}(7))$. Moreover, $\mathbf{1}$ possesses seven $\mathrm{CH}_{2}(\delta(\mathrm{C})$ $50.0,49.0,37.3,31.0,28.3,26.3,25.2)$ and three CH C-atoms ( $\delta(\mathrm{C}) 62.9,58.6,53.4$ ), and another quaternary C -atom $(\delta(\mathrm{C}) 35.9)$. However, the absence of a $\mathrm{Me}(\mathrm{C}(18))$ signal of $\mathbf{1}$ in its ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}$-NMR spectra indicated that $\mathrm{C}(18)$ might be connected to another
center. Detailed analysis of ${ }^{13} \mathrm{C}-\mathrm{NMR}$ and DEPT data revealed that $\mathbf{1}$ belongs to the aspidosperma-type alkaloids [25]. Comparison with venalstonidine [26] indicated that $\mathbf{1}$ was similar to this alkaloid with the exception for absence of a COOMe group, which is replaced by an additional $\mathrm{CH}_{2}$ group $(\delta(\mathrm{C}) 26.3(t))$. Its corresponding two H -atom signals at $\delta(\mathrm{H}) 1.75(m, 1 \mathrm{H})$ and $2.01(m, 1 \mathrm{H})$ showed HMBCs with $\mathrm{C}(2)(\delta(\mathrm{C}) 65.2$ $(s))$ and $\mathrm{C}(7)(\delta(\mathrm{C}) 55.7(s))$, supporting this presumption. In its HMBC spectrum, correlations from $\delta(\mathrm{H}) 2.67(d, J=3.2)$ to $\delta(\mathrm{C}) 62.9(d, \mathrm{C}(21))$ and $35.9(s, \mathrm{C}(20))$, and from $\delta(\mathrm{H}) 3.26(m)$ to $\delta(\mathrm{C}) 49.0(t, \mathrm{C}(3))$ and $58.6(d, \mathrm{C}(15))$ pointed to the presence of a 14,15-epoxy moiety (Fig. 2). Absence of a NOE correlation between H-C(14) and $\mathrm{H}-\mathrm{C}(15)$ with any key H -atoms in the ROESY spectrum suggested $\alpha$-orientation of the epoxy moiety, which was supported by an upfield shift of $\mathrm{C}(21)$ (from $\delta(\mathrm{C}) 68.4$ to 62.9) and of the $\mathrm{CH}_{2}(19)$ (from $\delta(\mathrm{C}) 31.1$ to 25.2) [3]. Thus, $\mathbf{1}$ was determined as $(14 \alpha, 15 \alpha)$-14,15-epoxyaspidofractinine. The negative specific rotation $\left([\alpha]_{\mathrm{D}}^{23}=-5\right)$ of 1 compared with those of venalstonidine $\left([\alpha]_{\mathrm{D}}^{23}=-96\right)$ [20] and aspidofractinine $\left([\alpha]_{\mathrm{D}}^{23}=-20\right)[27]$, suggested that they have same absolute configuration.


Fig. 2. Key HMBCs of $\mathbf{1}$

Compound 2 was found to possess the molecular formula of $\mathrm{C}_{34} \mathrm{H}_{40} \mathrm{O}_{8} \mathrm{~N}_{2}$ as evidenced by HR-ESI-MS at $m / z 605.2881\left([M+\mathrm{H}]^{+}\right)$. Its UV spectrum indicated the characteristic absorption bands of aspidosperma alkaloids with those of an $\alpha, \beta$ unsaturated lactone at 232 and 318 nm in agreement with FT-IR bands at 1706 and $1616 \mathrm{~cm}^{-1}$. The ${ }^{1} \mathrm{H}$-NMR spectra of 2 displayed signals for a mono-substituted indole ring $(\delta(\mathrm{H}) 6.91(d, J=7.5, \mathrm{H}-\mathrm{C}(9)), 6.32$ (br. $s, \mathrm{H}-\mathrm{C}(12)), 6.23(t, J=7.5, \mathrm{H}-\mathrm{C}(10)))$. Its ${ }^{13} \mathrm{C}-\mathrm{NMR}$ and DEPT data showed the presence of 3,4,5-trimethoxycinnamic acid moiety ( $\delta(\mathrm{C}) 166.3\left(s, \mathrm{C}\left(9^{\prime}\right)\right), 153.3\left(s, \mathrm{C}\left(3^{\prime}, 5^{\prime}\right)\right), 143.9\left(d, \mathrm{C}\left(7^{\prime}\right)\right), 139.7\left(s, \mathrm{C}\left(4^{\prime}\right)\right), 129.8$ $\left(s, \mathrm{C}\left(1^{\prime}\right)\right), 117.0\left(d, \mathrm{C}\left(8^{\prime}\right)\right), 105.1\left(s, \mathrm{C}\left(2^{\prime}, 6^{\prime}\right)\right), 60.9\left(q, M e \mathrm{O}-\mathrm{C}\left(4^{\prime}\right)\right)$, 56.1 ( $q$, $\left.M e \mathrm{O}-\mathrm{C}\left(3^{\prime}, 5^{\prime}\right)\right)$ ) [28]. The remaining ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data (see Table 1 ) including signals of eight quarternary C -atoms ( $\delta(\mathrm{C}) 168.8,167.9,159.8,144.6,129.8,92.0,54.9$, and $43.3), 6 \mathrm{CH}_{2}(51.2,49.8,44.9,29.2,26.2$, and 20.9$), 5 \mathrm{CH}(121.4,104.4,97.0,71.8$, and 67.7) , and 2 Me ( 55.0 and 15.1) were identical to those of 11-methoxyminovincinine (6) [12]. Two moieties were connected at $C(19)$ and $C\left(9^{\prime}\right)$ which was supported by the HMBC correlation between $\delta(\mathrm{H}) 4.89\left(q, J=6.5, \mathrm{H}-\mathrm{C}(19)\right.$ and $\delta(\mathrm{C}) 166.3\left(s, \mathrm{C}\left(9^{\prime}\right)\right)$. The configuration of $\mathbf{2}$ was identical to that of 11-methoxyminovincinine, as supported by its negative specific rotation and ROESY spectra. Thus, 2 was structurally determined as shown and named maireine $A$.

Compound $\mathbf{3}$ was found to possess the molecular formula $\mathrm{C}_{33} \mathrm{H}_{38} \mathrm{O}_{7} \mathrm{~N}_{2}$ as evidenced by HR-ESI-MS at $m / z 575.27563\left([M+\mathrm{H}]^{+}\right)$. It showed UV absorption bands at 232 and 317 nm , and FT-IR bands at 1706 and $1615 \mathrm{~cm}^{-1}$ similar to those of $\mathbf{2}$. The ${ }^{1} \mathrm{H}$ - and
Table. ${ }^{1} H$ - and ${ }^{13} C$-NMR Data for Compounds $\mathbf{1}-\mathbf{3} . \delta$ in ppm, $J$ in Hz .

|  | $\delta(\mathrm{C})$ |  |  | $\delta(\mathrm{H})$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 1 | 2 | 3 |
| $\mathrm{H}-\mathrm{N}(1)$ |  |  |  |  | 9.04 (br. $s$ ) | 9.31 (br. $s$ ) |
| C(2) | 65.2 (s) | 167.9 (s) | 167.7 (s) |  |  |  |
| $\mathrm{CH}_{2}(3)$ | 49.0 ( $t$ ) | 49.8 (t) | 50.6 (t) | $\begin{aligned} & 3.39(d d, J=12.8,4.8), \\ & 3.08(d, J=12.8) \end{aligned}$ | $\begin{aligned} & 3.14-3.17(\mathrm{~m}), \\ & 2.54-2.57(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 3.13-3.18(m), \\ & 2.57-2.62(m) \end{aligned}$ |
| $\mathrm{CH}_{2}(5)$ | 50.0 (t) | 51.2 (t) | 51.8 (t) | $\begin{aligned} & 2.80 \text { (br. } t, J=5.0 \text { ), } \\ & 2.60-2.63(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 2.90-2.94(m), \\ & 2.51-2.55(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 2.90-2.94(\mathrm{~m}), \\ & 2.50-2.53(\mathrm{~m}) \end{aligned}$ |
| $\mathrm{CH}_{2}(6)$ | 37.0 (t) | 44.9 (t) | 46.1 ( $t$ ) | $\begin{aligned} & 2.24-2.28(\mathrm{~m}), \\ & 1.08(d d, J=12.0,5.0) \end{aligned}$ | $\begin{aligned} & 2.08-2.11(\mathrm{~m}), \\ & 1.67-1.71(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 1.97-2.01(\mathrm{~m}), \\ & 1.66-1.70(\mathrm{~m}) \end{aligned}$ |
| C(7) | 55.7 (s) | 54.9 (s) | 56.2 (s) |  |  |  |
| C(8) | 139.8 (s) | 129.9 (s) | 138.1 (s) |  |  |  |
| $\mathrm{H}-\mathrm{C}(9)$ | 121.9 (d) | 121.4 (d) | 121.7 (d) | 6.99 ( $d, J=8.0)$ | $6.91(d, J=7.5)$ | $7.25(d, J=7.5)$ |
| $\mathrm{H}-\mathrm{C}(10)$ | 118.9 (d) | 104.4 (d) | 121.2 (d) | 6.63 ( $d, J=8.0)$ | $6.23(t, J=7.5)$ | $6.80(t, J=7.5)$ |
| $\mathrm{H}-\mathrm{C}(11)$ | 127.5 (d) | 159.8 (s) | 128.5 (d) | $6.92(d, J=8.0)$ |  | $7.06(t, J=7.5)$ |
| $\mathrm{H}-\mathrm{C}(12)$ | 111.1 (d) | 97.0 (d) | 110.7 (d) | $6.57(d, J=8.0)$ | $6.32(d, J=7.5)$ | 7.06 ( $d, J=7.5$ ) |
| C (13) | 152.0 (s) | 144.6 (s) | 144.5 (s) |  |  |  |
| $\begin{aligned} & \mathrm{H}-\mathrm{C}(14) \\ & \text { or } \mathrm{CH}_{2}(14) \end{aligned}$ | 53.4 (d) | 20.9 (t) | 22.1 (t) | 3.26 (br. $t, J=4.8$ ) | $\begin{aligned} & 1.83-1.87(\mathrm{~m}), \\ & 1.67-1.70(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 1.80-1.84(\mathrm{~m}), \\ & 1.66-169(\mathrm{~m}) \end{aligned}$ |
| $\begin{aligned} & \mathrm{H}-\mathrm{C}(15) \\ & \text { or } \mathrm{CH}_{2}(15) \end{aligned}$ | 58.6 (d) | 29.2 (t) | 28.8 (t) | $2.67(d, J=3.2)$ | $\begin{aligned} & 1.64-1.67(\mathrm{~m}), \\ & 1.58-1.63(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 1.61-1.66(\mathrm{~m}) \\ & 1.61-1.64(\mathrm{~m}) \end{aligned}$ |
| $\begin{aligned} & \mathrm{CH}_{2}(16) \\ & \text { or } \mathrm{C}(16) \end{aligned}$ | 26.3 (t) | 92.0 (s) | 92.7 (s) | $\begin{aligned} & 1.74-1.76(\mathrm{~m}), \\ & 2.00-2.03(\mathrm{~m}) \end{aligned}$ |  |  |
| $\mathrm{CH}_{2}(17)$ | 28.3 (t) | 26.2 (t) | 26.9 (t) | $\begin{aligned} & 1.65-1.68(\mathrm{~m}), \\ & 1.45-1.48(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 2.61-2.66(\mathrm{~m}), \\ & 2.55-2.59(\mathrm{~m}) \end{aligned}$ | $\begin{aligned} & 2.71-2.76(\mathrm{~m}), \\ & 2.50-2.55(\mathrm{~m}) \end{aligned}$ |

Table (cont.)

|  | $\delta(\mathrm{C})$ |  |  | $\delta(\mathrm{H})$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 1 | 2 | 3 |
| $\begin{aligned} & \hline \mathrm{CH}_{2}(18) \\ & \text { or } \mathrm{Me}(18) \end{aligned}$ | 31.0 (t) | 15.1 (q) | 15.0 (q) | $\begin{aligned} & \hline 1.32-1.36(\mathrm{~m}), \\ & 1.60-1.64(\mathrm{~m}) \end{aligned}$ | $1.02(d, J=6.5)$ | 0.96 ( $d, J=6.5$ ) |
| $\begin{aligned} & \mathrm{CH}_{2}(19) \\ & \text { or } \mathrm{H}-\mathrm{C}(19) \end{aligned}$ | 25.2 (t) | 71.8 (d) | 71.1 (d) | $\begin{aligned} & 1.37-1.41(\mathrm{~m}), \\ & 2.16-2.19(\mathrm{~m}) \end{aligned}$ | $4.89(q, J=6.6)$ | 4.79 ( $q, J=6.5)$ |
| C (20) | 35.9 (s) | 43.3 (s) | 43.3 (s) |  |  |  |
| $\mathrm{H}-\mathrm{C}(21)$ | 62.9 (d) | 67.7 (d) | 68.2 (d) | 2.58 (s) | 2.66 (s) | 2.75 (s) |
| COOMe |  | 168.8 (s) | 168.6 (s) |  | 3.68 (s) | 3.55 (s) |
| $\mathrm{Me} \mathrm{O}-\mathrm{C}(11)$ |  | 55.0 (q) | 55.8 (q) |  | 3.51 (s) |  |
| $\mathrm{C}\left(1^{\prime}\right)$ |  | 129.8 (s) | 130.9 (s) |  |  |  |
| $\mathrm{H}-\mathrm{C}\left(2^{\prime}, 6^{\prime}\right)$ |  | 105.1 (d) | 106.5 (d) |  | 6.61 (s) | 6.92 (s) |
| $\mathrm{H}-\mathrm{C}\left(3^{\prime}, 5^{\prime}\right)$ |  | 153.3 (s) | 154.5 (s) |  |  |  |
| $\mathrm{C}\left(4^{\prime}\right)$ |  | 139.7 (s) | 138.0 (s) |  |  |  |
| $\mathrm{H}-\mathrm{C}\left(7^{\prime}\right)$ |  | 143.9 (d) | 144.8 (d) |  | $7.28(d, J=16.5)$ | 7.40 ( $d, J=16.0)$ |
| $\mathrm{H}-\mathrm{C}\left(8^{\prime}\right)$ |  | 117.0 (d) | 118.2 (d) |  | 5.70 ( $d, J=16.5$ ) | $6.09(d, J=16.0)$ |
| C( $9^{\prime}$ ) |  | 166.3 (s) | 166.2 (s) |  |  |  |
| $\mathrm{Me} \mathrm{O}-\mathrm{C}\left(3^{\prime}, 5^{\prime}\right)$ |  | 56.1 (q) | 56.5 (q) |  | 3.90 (s) | 3.88 (s) |
| $\mathrm{Me} \mathrm{O}-\mathrm{C}\left(4^{\prime}\right)$ |  | 60.9 (q) | 60.6 (q) |  | 3.87 (s) | 3.74 (s) |

${ }^{13} \mathrm{C}$-NMR spectra of $\mathbf{3}$ displayed signals for an unsubstituted 2,3-dihydroindole ring $C$ $(\delta(\mathrm{H}) 7.25(d, J=7.5, \mathrm{H}-\mathrm{C}(9)), 7.06$ (overlap, $\mathrm{H}-\mathrm{C}(11), \mathrm{H}-\mathrm{C}(12)$ ), and $6.80(t, J=$ $7.5, \mathrm{H}-\mathrm{C}(10)) ; \delta(\mathrm{C}) 121.7$ ( $s, \mathrm{C}(9)), 121.2$ ( $d, \mathrm{C}(10))$, 128.5 ( $d, \mathrm{C}(11))$, and 110.7 (d, $\mathrm{C}(12))$ ). The remaining ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data were identical to those of 2 , which suggested that the MeO group in the indole ring of 2 was absent in $\mathbf{3}$. So, the structure of $\mathbf{3}$ was determined as shown, and the compound was named maireine B.

All alkaloids 1-22 were tested for their ability to prevent the cytopathic effects of cancer in breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells, and their cytotoxicities were determined using cisplatin as the positive control. Unfortunately, none of them showed a significant activity ( $I C_{50}>40 \mu \mathrm{~m}$ ).

Herewith, our group has terminated the phytochemical research on $A$. scholaris, $A$. yunnanensis, and A. mairei. Most of alkaloids from the former were of picrinine type, together with its derivatives; and those from A. yunnanensis were both of picrinine and aspidospermine types [8]. Most of constituents from the title plant belong to the aspidospermine type.

## Experimental Part

General. Column chromatography (CC): silica gel ( $\mathrm{SiO}_{2} ; 200-300$ mesh; Qingdao Marine Chemical Factory, Qingdao, P. R. China). TLC: $\mathrm{SiO}_{2} G F_{254}(200-300$ mesh, Qingdao Marine Chemical Factory, Qingdao, P. R. China); sprayed with Dragendorff's reagent; $C_{18} \mathrm{SiO}_{2}(20-45 \mu \mathrm{~m}$; Fuji Chemical Ltd., Japan). MPLC: Büchi pumps system coupled with glass columns $\left(15 \times 230\right.$ and $26 \times 460 \mathrm{~mm}$, resp., $C_{18}$ $\mathrm{SiO}_{2}$ ). HPLC: Waters 600 pumps coupled with anal. and semi-prep. Xtera $C_{18}$ columns ( $150 \times 4.6$ and $150 \times 7.8 \mathrm{~mm}$, resp.). The HPLC system employed a Waters 2996 photodiode array detector and a Waters fraction collector II. Optical rotations: Horiba SEAP-300 spectropolarimeter. UV Spectra: Shimadzu double-beam 210A spectrophotometer; $\lambda_{\max }(\log \varepsilon)$ in nm. IR Spectra: Bio-Rad FTS-135 IR spectrophotometer; KBr pellets; in $\mathrm{cm}^{-1} .{ }^{1} \mathrm{H}$-, ${ }^{13} \mathrm{C}-$, and $2 \mathrm{D}-\mathrm{NMR}$ spectra: $A M-400$ and $D R X$ 500 MHz NMR spectrometer; $\delta$ in ppm rel. to $\mathrm{Me}_{4} \mathrm{Si}$ as internal standard, $J$ in Hz. MS: API Qstar Pulsar I and Finnigan LCQ Advantage spectrometer; in $m / z$ (rel. \%).

Plant Material. A. mairei was identified by Dr. Ende Liu in November 2008 in Yunnan Province, P. R. China, and the specimen (cai-20081101) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried leaves and twigs ( 13.0 kg ) of $A$. mairei were crushed and extracted with $\mathrm{EtOH} / \mathrm{H}_{2} \mathrm{O} 9: 1$ under reflux for three times (3,2, and 1 h ) to yield an EtOH extract. After removal of EtOH under reduced pressure, the residue was dissolved in $1 \% \mathrm{aq} . \mathrm{HCl}$ and partitioned with AcOEt for three times. The acidic soln. was subsequently basified with $\mathrm{NH}_{3} \cdot \mathrm{H}_{2} \mathrm{O}$ to $\mathrm{pH} 9-10$, and partitioned with AcOEt for three times, to afford a two-phase mixture including the aq. phase and AcOEt/org. phase (total alkaloids). Total alkaloids ( 34 g ) was absorbed on $\mathrm{SiO}_{2}(45 \mathrm{~g})$ and chromatographed on a prepacked column on $\mathrm{SiO}_{2}(450 \mathrm{~g})$, eluting with a mixture of $\mathrm{CHCl}_{3} / \mathrm{MeOH}$ (from $\mathrm{CHCl}_{3}$ to $\mathrm{CHCl}_{3} / \mathrm{MeOH} 9: 1$ ), to give seven fractions, Fr. I-VII, according to differences in composition monitored by TLC plate after spraying with Dragendorff's reagent. Fr. I ( 2.6 g ) was further purified by $\mathrm{CC}\left(\mathrm{SiO}_{2}(30 \mathrm{~g})\right.$; petroleum ether/ $\left.\mathrm{CHCl}_{3} 1: 1-4: 1\right)$, which gave $\mathbf{1 7}(5 \mathrm{mg})$ and $\mathbf{1 6}(20 \mathrm{mg})$. Fr. II ( 2.0 g ) was subjected to MPLC $\left(R P_{18} \mathrm{SiO}_{2}(52 \mathrm{~g}) ; \mathrm{MeOH} / \mathrm{H}_{2} \mathrm{O}\right.$ from 1:1 to $\left.9: 1\right)$ to afford six subfractions Frs. $I I-1-I I-6)$. Fr. II-5 $(0.46 \mathrm{~g})$ was further purified by $\mathrm{CC}\left(\mathrm{SiO}_{2}(35 \mathrm{~g})\right.$; petroleum ether/acetone $\left.9: 1-4: 1\right)$ to give $\mathbf{1 7}(3.2 \mathrm{mg}), \mathbf{9}(35 \mathrm{mg}), \mathbf{1 0}(5 \mathrm{mg}), F r s . A$ and $B$, and $\mathbf{2}(45 \mathrm{mg})$. Frs. $A$ and $B$ were separated by semiprep. reversed-phase (RP) $C_{18}$ HPLC on Xterra column with gradient flow from 50 to $65 \%$ aq. MeOH to afford pure compounds $\mathbf{1 1}(3 \mathrm{mg}), \mathbf{1 3}(2.5 \mathrm{mg}), 12(5.5 \mathrm{mg}), \mathbf{1 4}(4.5 \mathrm{mg})$, resp. Fr. II-6 ( 0.11 g ) was further purified on a semi-prep. column with gradient flow from 50 to $65 \%$ aq. MeOH to give $\mathbf{3}(3 \mathrm{mg})$. The same semi-prep. column with gradient flow from 30 to $60 \%$ aq. MeOH was used to separate the
mixture of Fr. II-3 and Fr. II-4 ( 0.15 g ). This technique afforded $7(5 \mathrm{mg})$ and $\mathbf{8}(2.1 \mathrm{mg})$. Fr. III ( 6.5 g ) was subjected to $\mathrm{CC}\left(R P_{18} \mathrm{SiO}_{2} ; 30-80 \%\right.$ aq. MeOH$)$ to give six subfractions, Frs. III-1 - III-6. Fr. III-4 $(0.8 \mathrm{~g})$ was submitted to $\mathrm{CC}\left(\mathrm{SiO}_{2}(20 \mathrm{~g})\right.$; petroleum ether/acetone from $9: 1$ to $\left.4: 1\right)$ to yield $4(45 \mathrm{mg})$ and $\operatorname{Fr}$. C. $\mathrm{Fr} . C(76.5 \mathrm{mg})$ was subjected to $\mathrm{CC}\left(R P_{18}\right.$ gel $(80 \mathrm{~g}) ; 45-60 \%$ aq. MeOH$)$ to afford $\mathbf{6}(6 \mathrm{mg})$. Fr. III-5 (18.9 mg) were subjected to semi-prep. $\mathrm{CC}\left(R P_{18} \mathrm{SiO}_{2}(150 \times 7.8 \mathrm{~mm}) ; 40-50 \%\right.$ aq. MeOH$)$ to afford $5(13 \mathrm{mg})$. Fr. $I V(7.0 \mathrm{~g})$ was subjected to $\mathrm{CC}\left(R P_{18} \mathrm{SiO}_{2}(160 \mathrm{~g}) ; 40-100 \% \mathrm{MeOH}\right)$ to afford five subfractions, Frs. $I V-1-I V-5$. Fr. IV-1 $(1.15 \mathrm{~g})$ was purified by $\mathrm{CC}\left(\mathrm{SiO}_{2}(30 \mathrm{~g})\right.$; petroleum ether/acetone $9: 1 \rightarrow 3: 1)$ to give $\mathbf{1}(11 \mathrm{mg})$ and $\mathbf{1 8}(64 \mathrm{mg})$. Fr. $I V-3(0.9 \mathrm{~g})$ was submitted to $\mathrm{CC}\left(R P_{18} \mathrm{SiO}_{2}(26 \mathrm{~g}) ; 50 \%\right.$ aq. MeOH ) to give $15(11 \mathrm{mg})$. Compound $20(17 \mathrm{mg})$ separated from $\operatorname{Fr} . I V-5 . \operatorname{Fr} . V(7.0 \mathrm{~g})$ was subjected to $\mathrm{CC}\left(R P_{18} \mathrm{SiO}_{2}(160 \mathrm{~g}) ; 40 \%\right.$ aq. MeOH$)$ to afford subfraction $V-1 . \operatorname{Fr} . V-1(0.11 \mathrm{~g})$ was further purified by HPLC semi-prep. column using a gradient flow of $35-45 \%$ aq. MeOH to obtain 21 $(31 \mathrm{mg})$ and $22(12 \mathrm{mg}) . F r . V I(7.0 \mathrm{~g})$ was subjected to $\mathrm{CC}\left(R P_{18} \mathrm{SiO}_{2}(160 \mathrm{~g}) ; 55 \%\right.$ aq. MeOH$)$ to afford subfraction VI-1. Fr. VI-1 was purified by CC $\left(\mathrm{SiO}_{2}(300 \mathrm{~g}) ; \mathrm{CHCl}_{3} / \mathrm{MeOH} 19: 1\right)$ to give $19(13 \mathrm{mg})$.
(14 $\alpha, 15 \alpha)-14,15$-Epoxyaspidofractinine $(=(1 a \mathrm{R}, 8 b \mathrm{R}, 11 a \mathrm{R}, 12 a \mathrm{~S})-4 \mathrm{H}, 12 \mathrm{H}-1 b, 3 a$-Ethano-1a, 2,3 , 9,10,12a-hexahydro-11aH-oxireno[6,7]indolizino[8,1-cd]carbazole; 1). White powder. $[\alpha]_{\mathrm{D}}^{23}=-5.0$ $(c=0.23, \mathrm{MeOH}) . \mathrm{UV}(\mathrm{MeOH}): 205$ (4.16), 240 (3.59), 288 (3.27). IR: 3329, 2942, 1608, 1479, 1458. ${ }^{1} \mathrm{H}-\left(\mathrm{CDCl}_{3}, 400 \mathrm{MHz}\right)$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\mathrm{CDCl}_{3}, 100 \mathrm{MHz}\right)$ : see the Table. ESI-MS (pos.): $295\left([M+\mathrm{H}]^{+}\right)$, $317\left([M+\mathrm{Na}]^{+}\right)$. HR-ESI-MS: $295.1801\left([M+\mathrm{H}]^{+}, \mathrm{C}_{19} \mathrm{H}_{23} \mathrm{~N}_{2} \mathrm{O}^{+}\right.$; calc. 295.1810).

Maireine $A(=$ Methyl $(5 \alpha, 12 \beta, 19 \alpha)-2,3-D i d e h y d r o-16-m e t h o x y-20-\{[(2 \mathrm{E})-3-(3,4,5-$ trimethoxyphe-nyl)prop-2-enoyl]oxy\}aspidospermidine-3-carboxylate; 2). White powder. $[\alpha]_{\mathrm{D}}^{23}=-371 \quad(c=0.30$, $\mathrm{MeOH})$. UV (MeOH): 203 (4.23), 232 (4.17), 318 (4.20). IR: 3372, 2940, 1706, 1679, 1616. ${ }^{1} \mathrm{H}-$ $\left(\left(\mathrm{D}_{6}\right)\right.$ acetone, 400 MHz$)$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\left(\mathrm{D}_{6}\right)\right.$ acetone, 100 MHz$)$ : see the Table. ESI-MS (pos.): 605 $\left([M+\mathrm{H}]^{+}\right)$. HR-ESI-MS: $605.2881\left([M+\mathrm{H}]^{+}, \mathrm{C}_{34} \mathrm{H}_{41} \mathrm{~N}_{2} \mathrm{O}_{8}^{+}\right.$; calc. 605.2862).

Maireine $B(=$ Methyl (5 $2,12 \beta, 19 \alpha)-2,3-D i d e h y d r o-20-\{[(2 \mathrm{E})-3-(3,4,5-$ trimethoxyphenyl)prop-2-enoyl]oxy]aspidospermidine-3-carboxylate; 3). White powder. $[\alpha]_{\mathrm{D}}^{23}=-353(c=0.30, \mathrm{MeOH})$. UV (MeOH): 202 (4.21), 231 (4.16), 315 (4.17). IR: 3370, 2941, 1710, 1670, 1614. ${ }^{1} \mathrm{H}-\left(\left(\mathrm{D}_{6}\right)\right.$ acetone, $500 \mathrm{MHz})$ and ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(\left(\mathrm{D}_{6}\right)\right.$ acetone, 100 MHz$)$ : see the Table. ESI-MS (pos.): $575\left([\mathrm{M}+\mathrm{H}]^{+}\right)$. HR-ESI-MS: $575.2763\left([M+\mathrm{H}]^{+}, \mathrm{C}_{33} \mathrm{H}_{49} \mathrm{~N}_{2} \mathrm{O}_{7}^{+}\right.$; calc. 575.2758) .

Cytotoxicity Assay. Five human cancer cell lines, breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 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 $5 \% \mathrm{CO}_{2}$ at $37^{\circ}$. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) method in 96 -well microplates [29]. Briefly, $100 \mu \mathrm{l}$ of 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 $1 \times 10^{5}$ cells $/ \mathrm{ml}$. Each tumor cell line was exposed to the test compound at concentrations of $0.0625,0.32,1.6,8$, and $40 \mu \mathrm{~m}$ in triplicates for 48 h , with cisplatin (Sigma, USA) as positive control. After compound treatment, cell viability was detected, and cell-growth curve was graphed. The $I C_{50}$ values were calculated by using Reed and Muench's method [30].

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[^0]:    Three new monoterpenoid indole alkaloids, $(14 \alpha, 15 \alpha)$-14,15-epoxyaspidofractinine (1) and maireines A and B ( $\mathbf{2}$ and $\mathbf{3}$, resp.), together with 19 known alkaloids, were isolated from the leaves and twigs of Alstonia mairei. The structures of the new compounds were elucidated by 1D- and 2D-NMR spectroscopic methods in combination with MS experiments.

