Monoterpenoid Indole Alkaloids from *Alstonia mairei*

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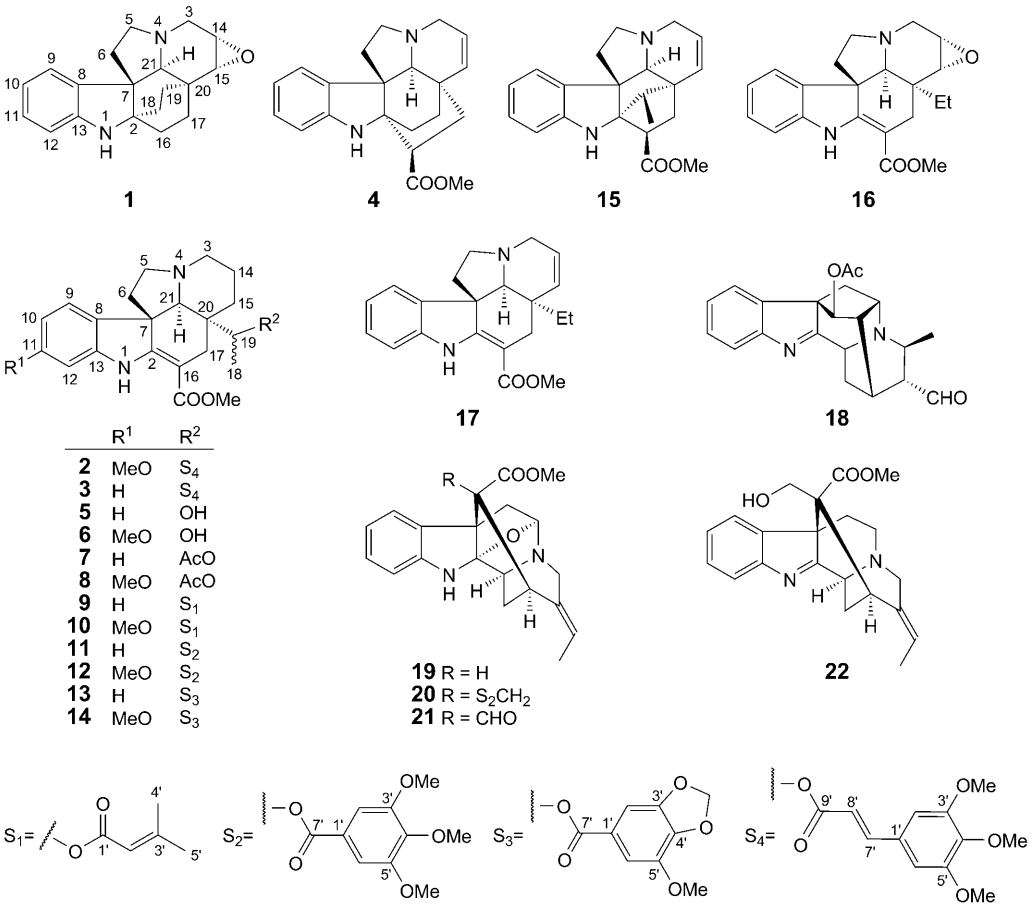
Three new monoterpenoid indole alkaloids, (14α,15α)-14,15-epoxyaspidofractinine (**1**) and maireines A and B (**2** and **3**, resp.), together with 19 known alkaloids, were isolated from the leaves and twigs of *Alstonia mairei*. The structures of the new compounds were elucidated by 1D- and 2D-NMR spectroscopic methods in combination with MS experiments.

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, (14α,15α)-14,15-epoxyaspidofractinine (**1**), and maireines A and B (**2** and **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], (19*S*)-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 (IC_{50} > 40 μM).



	R^1	R^2
2	MeO	S_4
3	H	S_4
5	H	OH
6	MeO	OH
7	H	AcO
8	MeO	AcO
9	H	S_1
10	MeO	S_1
11	H	S_2
12	MeO	S_2
13	H	S_3
14	MeO	S_3

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 $C_{19}H_{23}N_2O$ based on HR-ESI-MS (*m*/*z* 295.1801 ($[M+H]^+$)). 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 cm^{-1}) and aromatic rings (1608, 1479, and 1458 cm^{-1}). In the ^{1}H-NMR spectrum (*Table*), four signals (δ(H) 6.99 (*d*, $J=7.0$, H−C(9)), 6.92 (*t*, $J=7.0$, H−C(11)), 6.63 (*t*, $J=7.0$, H−C(10)), 6.57 (*d*, $J=7.0$, H−C(12))) revealed the presence of an unsubstituted ring *A* in a monoterpenoid indole alkaloid [24]. The ^{13}C-NMR (*Table*) and DEPT spectra of **1** displayed signals for a 2,3-dihydroindole ring (δ(C) 152.0 (*s*, C(13)), 139.8 (*s*, C(8)), 127.5 (*d*, C(11)), 121.9 (*d*, C(9)), 118.9 (*d*, C(10)), 111.1 (*d*, C(12)), 65.2 (*s*, C(2)), 55.7 (*s*, C(7)). Moreover, **1** possesses seven CH_2 (δ(C) 50.0, 49.0, 37.3, 31.0, 28.3, 26.3, 25.2) and three CH C-atoms (δ(C) 62.9, 58.6, 53.4), and another quaternary C-atom (δ(C) 35.9). However, the absence of a Me (C(18)) signal of **1** in its ^{1}H- and ^{13}C-NMR spectra indicated that C(18) might be connected to another

center. Detailed analysis of ^{13}C-NMR and DEPT data revealed that **1** belongs to the aspidosperma-type alkaloids [25]. Comparison with venalstonidine [26] indicated that **1** was similar to this alkaloid with the exception for absence of a COOMe group, which is replaced by an additional CH_2 group (δ(C) 26.3 (*t*)). Its corresponding two H-atom signals at δ(H) 1.75 (*m*, 1 H) and 2.01 (*m*, 1 H) showed HMBCs with C(2) (δ(C) 65.2 (*s*)) and C(7) (δ(C) 55.7 (*s*)), supporting this presumption. In its HMBC spectrum, correlations from δ(H) 2.67 (*d*, $J = 3.2$) to δ(C) 62.9 (*d*, C(21)) and 35.9 (*s*, C(20)), and from δ(H) 3.26 (*m*) to δ(C) 49.0 (*t*, C(3)) and 58.6 (*d*, C(15)) pointed to the presence of a 14,15-epoxy moiety (*Fig. 2*). Absence of a NOE correlation between H–C(14) and H–C(15) with any key H-atoms in the ROESY spectrum suggested α-orientation of the epoxy moiety, which was supported by an upfield shift of C(21) (from δ(C) 68.4 to 62.9) and of the CH_2(19) (from δ(C) 31.1 to 25.2) [3]. Thus, **1** was determined as (14α,15α)-14,15-epoxyaspidofractinine. The negative specific rotation ($[\alpha]_D^{23} = -5$) of **1** compared with those of venalstonidine ($[\alpha]_D^{23} = -96$) [20] and aspidofractinine ($[\alpha]_D^{23} = -20$) [27], suggested that they have same absolute configuration.

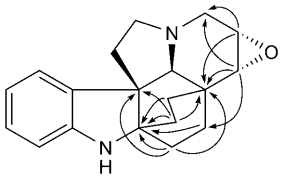


Fig. 2. *Key HMBCs of* **1**

Compound **2** was found to possess the molecular formula of $C_{34}H_{40}O_8N_2$ as evidenced by HR-ESI-MS at *m*/*z* 605.2881 ($[M+H]^+$). Its UV spectrum indicated the characteristic absorption bands of aspidosperma alkaloids with those of an α,β-unsaturated lactone at 232 and 318 nm in agreement with FT-IR bands at 1706 and 1616 cm^{-1}. The ^{1}H-NMR spectra of **2** displayed signals for a mono-substituted indole ring (δ(H) 6.91 (*d*, $J = 7.5$, H–C(9)), 6.32 (br. *s*, H–C(12)), 6.23 (*t*, $J = 7.5$, H–C(10))). Its ^{13}C-NMR and DEPT data showed the presence of 3,4,5-trimethoxycinnamic acid moiety (δ(C) 166.3 (*s*, C(9′)), 153.3 (*s*, C(3′,5′)), 143.9 (*d*, C(7′)), 139.7 (*s*, C(4′)), 129.8 (*s*, C(1′)), 117.0 (*d*, C(8′)), 105.1 (*s*, C(2′,6′)), 60.9 (*q*, *Me*O–C(4′)), 56.1 (*q*, *Me*O–C(3′,5′))) [28]. The remaining ^{13}C-NMR data (see *Table 1*) including signals of eight quarternary C-atoms (δ(C) 168.8, 167.9, 159.8, 144.6, 129.8, 92.0, 54.9, and 43.3), 6 CH_2 (51.2, 49.8, 44.9, 29.2, 26.2, and 20.9), 5 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(9′) which was supported by the HMBC correlation between δ(H) 4.89 (*q*, $J = 6.5$, H–C(19) and δ(C) 166.3 (*s*, C(9′)). The configuration of **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 **3** was found to possess the molecular formula $C_{33}H_{38}O_7N_2$ as evidenced by HR-ESI-MS at *m*/*z* 575.27563 ($[M+H]^+$). It showed UV absorption bands at 232 and 317 nm, and FT-IR bands at 1706 and 1615 cm^{-1} similar to those of **2**. The ^{1}H- and

Table. *[1]H- and [13]C-NMR Data for Compounds* **1**–**3**. δ in ppm, J in Hz.

	δ(C)			δ(H)		
	1	**2**	**3**	**1**	**2**	**3**
H–N(1)					9.04 (br. *s*)	9.31 (br. *s*)
C(2)	65.2 (*s*)	167.9 (*s*)	167.7 (*s*)			
CH_2(3)	49.0 (*t*)	49.8 (*t*)	50.6 (*t*)	3.39 (*dd*, *J* = 12.8, 4.8), 3.08 (*d*, *J* = 12.8)	3.14–3.17 (*m*), 2.54–2.57 (*m*)	3.13–3.18 (*m*), 2.57–2.62 (*m*)
CH_2(5)	50.0 (*t*)	51.2 (*t*)	51.8 (*t*)	2.80 (br. *t*, *J* = 5.0), 2.60–2.63 (*m*)	2.90–2.94 (*m*), 2.51–2.55 (*m*)	2.90–2.94 (*m*), 2.50–2.53 (*m*)
CH_2(6)	37.0 (*t*)	44.9 (*t*)	46.1 (*t*)	2.24–2.28 (*m*), 1.08 (*dd*, *J* = 12.0, 5.0)	2.08–2.11 (*m*), 1.67–1.71 (*m*)	1.97–2.01 (*m*), 1.66–1.70 (*m*)
C(7)	55.7 (*s*)	54.9 (*s*)	56.2 (*s*)			
C(8)	139.8 (*s*)	129.9 (*s*)	138.1 (*s*)			
H–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)
H–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)
H–C(11)	127.5 (*d*)	159.8 (*s*)	128.5 (*d*)	6.92 (*d*, *J* = 8.0)		7.06 (*t*, *J* = 7.5)
H–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*)			
H–C(14) or CH_2(14)	53.4 (*d*)	20.9 (*t*)	22.1 (*t*)	3.26 (br. *t*, *J* = 4.8)	1.83–1.87 (*m*), 1.67–1.70 (*m*)	1.80–1.84 (*m*), 1.66–169 (*m*)
H–C(15) or CH_2(15)	58.6 (*d*)	29.2 (*t*)	28.8 (*t*)	2.67 (*d*, *J* = 3.2)	1.64–1.67 (*m*), 1.58–1.63 (*m*)	1.61–1.66 (*m*), 1.61–1.64 (*m*)
CH_2(16) or C(16)	26.3 (*t*)	92.0 (*s*)	92.7 (*s*)	1.74–1.76 (*m*), 2.00–2.03 (*m*)		
CH_2(17)	28.3 (*t*)	26.2 (*t*)	26.9 (*t*)	1.65–1.68 (*m*), 1.45–1.48 (*m*)	2.61–2.66 (*m*), 2.55–2.59 (*m*)	2.71–2.76 (*m*), 2.50–2.55 (*m*)

Table (cont.)

	δ(C)			δ(H)		
	1	**2**	**3**	**1**	**2**	**3**
CH_2(18) or Me(18)	31.0 (*t*)	15.1 (*q*)	15.0 (*q*)	1.32–1.36 (*m*), 1.60–1.64 (*m*)	1.02 (*d*, *J* = 6.5)	0.96 (*d*, *J* = 6.5)
CH_2(19) or H−C(19)	25.2 (*t*)	71.8 (*d*)	71.1 (*d*)	1.37–1.41 (*m*), 2.16–2.19 (*m*)	4.89 (*q*, *J* = 6.6)	4.79 (*q*, *J* = 6.5)
C(20)	35.9 (*s*)	43.3 (*s*)	43.3 (*s*)			
H−C(21)	62.9 (*d*)	67.7 (*d*)	68.2 (*d*)	2.58 (*s*)	2.66 (*s*)	2.75 (*s*)
COO*Me*		168.8 (*s*)	168.6 (*s*)		3.68 (*s*)	3.55 (*s*)
*Me*O−C(11)		55.0 (*q*)	55.8 (*q*)		3.51 (*s*)	
C(1′)		129.8 (*s*)	130.9 (*s*)			
H−C(2′,6′)		105.1 (*d*)	106.5 (*d*)		6.61 (*s*)	6.92 (*s*)
H−C(3′,5′)		153.3 (*s*)	154.5 (*s*)			
C(4′)		139.7 (*s*)	138.0 (*s*)			
H−C(7′)		143.9 (*d*)	144.8 (*d*)		7.28 (*d*, *J* = 16.5)	7.40 (*d*, *J* = 16.0)
H−C(8′)		117.0 (*d*)	118.2 (*d*)		5.70 (*d*, *J* = 16.5)	6.09 (*d*, *J* = 16.0)
C(9′)		166.3 (*s*)	166.2 (*s*)			
*Me*O−C(3′,5′)		56.1 (*q*)	56.5 (*q*)		3.90 (*s*)	3.88 (*s*)
*Me*O−C(4′)		60.9 (*q*)	60.6 (*q*)		3.87 (*s*)	3.74 (*s*)

^{13}C-NMR spectra of **3** displayed signals for an unsubstituted 2,3-dihydroindole ring *C* (δ(H) 7.25 (*d*, *J* = 7.5, H–C(9)), 7.06 (overlap, H–C(11), H–C(12)), and 6.80 (*t*, *J* = 7.5, H–C(10)); δ(C) 121.7 (*s*, C(9)), 121.2 (*d*, C(10)), 128.5 (*d*, C(11)), and 110.7 (*d*, C(12))). The remaining ^{1}H- and ^{13}C-NMR data were identical to those of **2**, which suggested that the MeO group in the indole ring of **2** was absent in **3**. So, the structure of **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 ($IC_{50} > 40$ μM).

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 (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). TLC: SiO_2 GF_{254} (200–300 mesh, *Qingdao Marine Chemical Factory*, Qingdao, P. R. China); sprayed with *Dragendorff*'s reagent; C_{18} SiO_2 (20–45 μm; *Fuji Chemical Ltd.*, Japan). MPLC: *Büchi* pumps system coupled with glass columns (15 × 230 and 26 × 460 mm, resp., C_{18} SiO_2). HPLC: *Waters 600* pumps coupled with anal. and semi-prep. *Xtera* C_{18} columns (150 × 4.6 and 150 × 7.8 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; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* IR spectrophotometer; KBr pellets; in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *AM-400* and *DRX-500* MHz NMR spectrometer; δ in ppm rel. to Me_4Si 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 EtOH/H_2O 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% aq. HCl and partitioned with AcOEt for three times. The acidic soln. was subsequently basified with $NH_3 \cdot H_2O$ to 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 SiO_2 (45 g) and chromatographed on a prepacked column on SiO_2 (450 g), eluting with a mixture of $CHCl_3$/MeOH (from $CHCl_3$ to $CHCl_3$/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 CC (SiO_2 (30 g); petroleum ether/$CHCl_3$ 1:1–4:1), which gave **17** (5 mg) and **16** (20 mg). *Fr. II* (2.0 g) was subjected to MPLC (RP_{18} SiO_2 (52 g); MeOH/H_2O from 1:1 to 9:1) to afford six subfractions *Frs. II-1*–*II-6*). *Fr. II-5* (0.46 g) was further purified by CC (SiO_2 (35 g); petroleum ether/acetone 9:1–4:1) to give **17** (3.2 mg), **9** (35 mg), **10** (5 mg), *Frs. A* and *B*, and **2** (45 mg). *Frs. A* and *B* were separated by semi-prep. reversed-phase (RP) C_{18} HPLC on *Xterra* column with gradient flow from 50 to 65% aq. MeOH to afford pure compounds **11** (3 mg), **13** (2.5 mg), 1**2** (5.5 mg), **14** (4.5 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 **3** (3 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 mg) and **8** (2.1 mg). *Fr. III* (6.5 g) was subjected to CC (RP_{18} SiO_2; 30–80% aq. MeOH) to give six subfractions, *Frs. III-1–III-6*. *Fr. III-4* (0.8 g) was submitted to CC (SiO_2 (20 g); petroleum ether/acetone from 9:1 to 4:1) to yield **4** (45 mg) and *Fr. C*. *Fr. C* (76.5 mg) was subjected to CC (RP_{18} gel (80 g); 45–60% aq. MeOH) to afford **6** (6 mg). *Fr. III-5* (18.9 mg) were subjected to semi-prep. CC (RP_{18} SiO_2 (150 × 7.8 mm); 40–50% aq. MeOH) to afford **5** (13 mg). *Fr. IV* (7.0 g) was subjected to CC (RP_{18} SiO_2 (160 g); 40–100% MeOH) to afford five subfractions, *Frs. IV-1–IV-5*. *Fr. IV-1* (1.15 g) was purified by CC (SiO_2 (30 g); petroleum ether/acetone 9:1 → 3:1) to give **1** (11 mg) and **18** (64 mg). *Fr. IV-3* (0.9 g) was submitted to CC (RP_{18} SiO_2 (26 g); 50% aq. MeOH) to give **15** (11 mg). Compound **20** (17 mg) separated from *Fr. IV-5*. *Fr. V* (7.0 g) was subjected to CC (RP_{18} SiO_2 (160 g); 40% aq. MeOH) to afford subfraction *V-1*. *Fr. V-1* (0.11 g) was further purified by HPLC semi-prep. column using a gradient flow of 35–45% aq. MeOH to obtain **21** (31 mg) and **22** (12 mg). *Fr. VI* (7.0 g) was subjected to CC (RP_{18} SiO_2 (160 g); 55% aq. MeOH) to afford subfraction *VI-1*. *Fr. VI-1* was purified by CC (SiO_2 (300 g); $CHCl_3$/MeOH 19:1) to give **19** (13 mg).

(14α,15α)-14,15-Epoxyaspidofractinine (= *(1a*R,*8b*R,*11a*R,*12a*S*)-4*H,*12*H-*1b,3a-Ethano-1a,2,3,9,10,12a-hexahydro-11a*H-*oxireno[6,7]indolizino[8,1-*cd*]carbazole*; **1**). White powder. $[\alpha]_D^{23} = -5.0$ ($c = 0.23$, MeOH). UV (MeOH): 205 (4.16), 240 (3.59), 288 (3.27). IR: 3329, 2942, 1608, 1479, 1458. ^{1}H- ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see the *Table*. ESI-MS (pos.): 295 ($[M+H]^+$), 317 ($[M+Na]^+$). HR-ESI-MS: 295.1801 ($[M+H]^+$, $C_{19}H_{23}N_2O^+$; calc. 295.1810).

Maireine A (= *Methyl (5α,12β,19α)-2,3-Didehydro-16-methoxy-20-{[(2*E*)-3-(3,4,5-trimethoxyphenyl)prop-2-enoyl]oxy}aspidospermidine-3-carboxylate*; **2**). White powder. $[\alpha]_D^{23} = -371$ ($c = 0.30$, MeOH). UV (MeOH): 203 (4.23), 232 (4.17), 318 (4.20). IR: 3372, 2940, 1706, 1679, 1616. ^{1}H- ((D_6)acetone, 400 MHz) and ^{13}C-NMR ((D_6)acetone, 100 MHz): see the *Table*. ESI-MS (pos.): 605 ($[M+H]^+$). HR-ESI-MS: 605.2881 ($[M+H]^+$, $C_{34}H_{41}N_2O_8^+$; calc. 605.2862).

Maireine B (= *Methyl (5α,12β,19α)-2,3-Didehydro-20-{[(2*E*)-3-(3,4,5-trimethoxyphenyl)prop-2-enoyl]oxy}aspidospermidine-3-carboxylate*; **3**). White powder. $[\alpha]_D^{23} = -353$ ($c = 0.30$, MeOH). UV (MeOH): 202 (4.21), 231 (4.16), 315 (4.17). IR: 3370, 2941, 1710, 1670, 1614. ^{1}H- ((D_6)acetone, 500 MHz) and ^{13}C-NMR ((D_6)acetone, 100 MHz): see the *Table*. ESI-MS (pos.): 575 ($[M+H]^+$). HR-ESI-MS: 575.2763 ($[M+H]^+$, $C_{33}H_{49}N_2O_7^+$; 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% CO_2 at 37°. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method in 96-well microplates [29]. Briefly, 100 µl 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 1×10^5 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*, USA) as positive control. After compound treatment, cell viability was detected, and cell-growth curve was graphed. The IC_{50} values were calculated by using *Reed* and *Muench*'s method [30].

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