Three New Indole Alkaloids from the Leaves of *Alstonia scholaris*

by **Hua Zhou**, **Hong-Ping He**, **Xiao-Dong Luo**, **Yue-Hu Wang**, **Xian-Wen Yang**, **Ying-Tong Di**, and **Xiao-Jiang Hao***

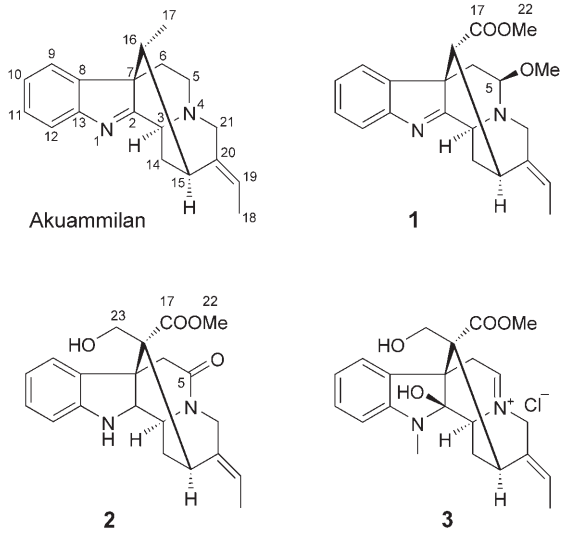
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China
(phone: +86-871-5223263; e-mail: haoxj@mail.kib.ac.cn)

Three new akuamillan-type indole alkaloids, *i.e.*, 5-methoxystrictamine (= methyl (5β,16*R*,19*E*)-5-methoxyakuammilan-17-oate; **1**), methyl (16*R*,19*E*)-1,2-dihydro-16-(hydroxymethyl)-5-oxoakuammilan-17-oate (**2**), and methyl (2β,16*R*,19*E*)-4,5-didehydro-1,2-dihydro-2-hydroxy-16-(hydroxymethyl)akuammilan-4-ium-17-oate chloride (**3**), have been isolated from the leaves of *Alstonia scholaris*, together with ten known compounds. Their structures were determined by spectroscopic means. None of the constituents showed significant cytotoxic activity towards WT cells.

Introduction. – *Alstonia scholaris* is widely distributed in South and Southeast Asia such as Malaysia, India, and China. In China, this plant has been used in traditional medicine for the treatment of cough and fever, as well as for injuries from falls. There are many reports on the isolation of biologically active constituents from *A. scholaris* [1–4], including compounds with antiplasmodial [5] and anticancer [6] properties. However, the alkaloid content of the leaves of *A. scholaris* from the Chinese inland has not been investigated previously.

In this paper, we report the isolation and structure elucidation of three new alkaloids, 5-methoxystrictamine (= methyl (5β,16*R*,19*E*)-5-methoxyakuammilan-17-oate; **1**), methyl (16*R*,19*E*)-1,2-dihydro-16-(hydroxymethyl)-5-oxoakuammilan-17-oate (**2**), and methyl (2β,16*R*,19*E*)-4,5-didehydro-1,2-dihydro-2-hydroxy-16-(hydroxymethyl)akuammilan-4-ium-17-oate chloride (**3**), together with the following ten known compounds: scholaricine [2], 19-epischolaricine [2], vallesamine [2], *N*-methylburnamine [2], methylburnamine [3], picrinine [3], picralinal [3], akuammidine [3], rhazimanine [4], and *N*-demethylechitamine [7].

Results and Discussion. – Compound **1** was shown to have the molecular formula $C_{21}H_{24}N_2O_3$ on the basis of HR-ESI-MS data (*m/z* 353.1857 ($[M+1]^+$, calc. 353.1865), indicating eleven degrees of unsaturation. The ^{13}C-NMR and DEPT spectra (see the *Table* in the *Exper. Part*) displayed signals of three Me, three CH_2, and nine CH groups, together with six quaternary C-atoms. The NMR signals at δ(H) 7.63 (*dd*, $J = 7.7$, 1.0 Hz, H–C(9)), 7.32 (*dt*, $J = 7.7$, 1.0 Hz, H–C(10)), 7.13 (*dt*, $J = 7.7$, 1.0 Hz, H–C(11)), 7.39 (*dd*, $J = 7.7$, 1.0 Hz, H–C(12)), and those at δ(C) 145.4 (C(8)), 120.6 (C(9)), 125.0 (C(10)), 128.0 (C(11)), 123.1 (C(12)), and 155.6 (C(13)) were characteristic for the presence of an indole moiety [1]. In the ^{1}H-NMR spectrum, a Me group appeared at δ(H) 1.54 (*d*, $J = 6.6$ Hz, Me(18)), which was coupled to an olefinic



H-atom (H–C(19)), indicating an ethylidene side chain. The methine signal at δ(C) 89.9 was assigned to C(5) on the basis of an HMBC cross-peak with the H-atoms of Me(24) and CH_2(6) (*Fig. 1*). The HMBC data revealed correlations between δ(C) 53.4 (C(7)) and δ(H) 3.87 (H–C(5)), 1.91 (H–C(16)), 3.43 (H–C(15)), and between δ(C) 89.9 (C(5)) and δ(H) 2.21/3.08 (CH_2(6)), 3.08/4.06 (CH_2(21)), and 3.22 (Me(24)), which indicated the presence of fragment C(16)–C(7)–C(6)–C(5)–N–C(21).

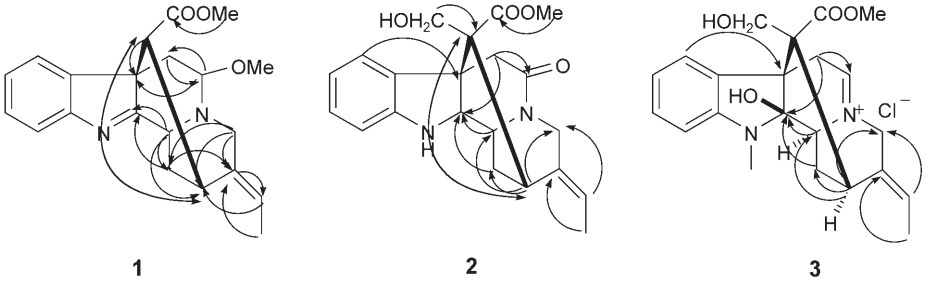


Fig. 1. *Selected HMBC correlations for compounds* **1**–**3**

In the HMBC spectrum of **1** (*Fig. 1*), correlations between δ(C) 190.5 (C(2)) and δ(H) 4.48 (H–C(3)), 1.76/2.68 (CH_2(14)), between δ(C) 32.6 (C(15)) and δ(H) 4.48 (H–C(3)), 1.91 (H–C(16)), 5.53 (H–C(19)), and between δ(C) 51.5 (C(3)) and δ(H) 1.76/2.68 (CH_2(14)) and 3.08/4.06 (CH_2(21)) suggested the fragment C(2)–C(3)–C(14)–C(15)–C(20)–C(21), as further supported by correlations between CH_2(14) and both H–C(3)) and H–C(15)) in ^{1}H,^{1}H-COSY spectrum.

The relative configuration of compound **1** was determined by taking recourse to a 2D ROESY NMR experiment. Some selected ROESY interactions are shown in *Fig. 2*. Cross-peaks between H–C(3) and H_β–C(14), between H–C(15) and H_β–C(14),

between H_α–C(14) and H_β–C(21), between H–C(5) and H_α–C(21), and between H–C(15) and Me(18) were observed. From these data, and by comparison with the literature data of 5,10,11-trimethoxystrictamine [8], the structure of compound **1** could be fully elucidated.

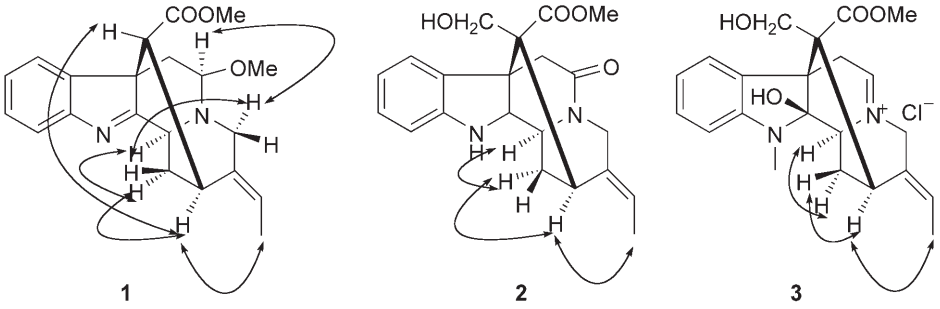


Fig. 2. *Selected $^1H,^1H$-ROESY correlations for compounds* **1**–**3**

Compound **2** had the molecular formula $C_{21}H_{24}N_2O_4$, as determined by HR-ESI-MS (*m/z* 369.1805 ($[M+1]^+$, calc. 369.1814). The ^{13}C-NMR and DEPT spectra displayed signals for two Me, four CH_2, and eight CH groups, as well as seven quaternary C-atoms. The presence of an indole moiety was suggested by characteristic ^{1}H- and ^{13}C-NMR signals at δ(H) 7.36 (*dd*, $J = 7.6$, 1.0 Hz, H–C(9)), 7.02 (*dt*, $J = 7.6$, 1.0 Hz, H–C(10)), 7.18 (*dt*, $J = 7.6$, 1.0 Hz, H–C(11)), 6.82 (*dd*, $J = 7.6$, 1.0 Hz, H–C(12)), and at δ(C) 129.7 (C(8)), 126.2 (C(9)), 122.0 (C(10)), 128.2 (C(11)), 109.2 (C(12)), and 141.4 (C(13)). In the ^{1}H-NMR spectrum, a signal at δ(H) 1.64 (*d*, $J =$ 6.7 Hz) was assigned to Me(18), which showed a vicinal coupling with an olefinic H-atom at δ(H) 5.28 (*d*, $J = 6.7$ Hz, H–C(19)). The signal at δ(C) 183.1 was assigned to C(5) on the basis of HMBC cross-peaks between δ(H) 2.73 (H_α–C(6)) and 1.71 (H_β–C(6)) (see *Fig.* 1). The signals at δ(H) 3.30 (*d*, $J = 12.0$ Hz, H_α–C(23)) and 3.96 (*d*, $J = 12.0$, H_β–C(23)) showed HMBC cross-peaks with C(15), C(16), and C(17), and allowed the identification of the fragment C(23)–C(16)–C(17). In the HMBC spectrum, the correlations between δ(C) 28.0 (C(15)) and δ(H) 2.62 (H_α–C(14)), 1.46 (H_β–C(14)), 3.91 (H_α–C(23)), 3.68 (H_β–C(23)), 1.64 (Me(18)), and 5.28 (H–C(19)), as well as those between δ(C) 60.0 (C(3)) and δ(H) 3.27 (H–C(15)), and between δ(C) 49.0 (C(21)) and δ(H) 3.27 (H–C(15)) and 1.64 (H–C(19)) suggested the fragment C(2)–C(3)–C(14)–C(15)–C(20)–C(21), as further supported by the correlation between H–C(3), H–C(2), and H–C(14) in the ^{1}H,^{1}H-COSY spectrum. The relative configuration of compound **2** was determined from a 2D ROESY NMR experiment. Selected ROESY interactions are shown in *Fig. 2*. ROESY Cross-peaks were observed between H–C(3) and H_α–C(14), between H_α–C(14) and H–C(15), between H_α–C(14) and Me(18), between H–C(15) and Me(18), and between Me(18) and H–C(19). On the basis of the above discussion, the structure of **2** could be fully identified.

Compound **3** had the molecular formula $C_{22}H_{27}N_2O_4Cl$, as determined by HR-ESI-MS (*m/z* 418.1658 (M^-, calc. 418.1659)), and further supported by HR-ESI-MS (*m/z* 382.2013 $[M - HCl]^+$; calc. 382.1892). The ^{13}C-NMR and DEPT spectra showed 22

signals: three Me, four CH_2, eight CH groups, and seven quaternary C-atoms. In the HMBC spectrum, a correlation between δ(C) 165.9 (C(5)) and δ(H) 3.45 (H–C(6)) and 3.27 (H–C(3)) was observed. The quaternary-carbon signal at δ(C) 102.0 was assigned to C(2) on the basis of an HMBC cross-peak between Me(24) and H–C(3) (*Fig. 1*). The HMBC data established the presence of two six-membered rings, C(2)–C(3)–N(4)–C(5)–C(6)–C(7) and C(3)–C(14)–C(15)–C(20)–C(21)–N(4), which pointed to a similar skeleton as for compound **2**. The relative configuration of compound **3** was determined from a 2D-ROESY NMR experiment (*Fig. 2*). ROESY cross-peaks were observed between H–C(3) and H_α–C(14), between H–C(3) and Me(24), between H_β–C(14) and H–C(15), and between H–C(5) and Me(18). On the basis of the above discussion, and by comparison with the literature data of pseudoakuammigine [9], the structure of compound **3** could be fully identified.

All compounds were evaluated for their cytotoxicities towards WT cells. However, none showed a significant effect. Only akuammidine exhibited moderate cytotoxic activity.

This work was financially supported by *The National Foundation for Outstanding Young Scientists* (to *H. X. J.*; No. 39525025) and *The National Nature Science Foundation of Yunnan Province* (No. 2004C0009Z). We thank the analytical group of the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, for recording spectra. Mr. *Quan Chen*, Institute of Zoology (IOZ), Chinese Academy of Sciences, is acknowledged for performing the cytotoxicity tests.

Experimental Part

General. Thin-layer (TLC) and column chromatography (CC) were performed on plates precoated with silica gel F_{254} and *H* (*Qingdao Haiyang Chemical Co., Ltd*, Qingdao, China), respectively. Solvents were distilled before use. Optical rotations were measured on a *Horiba SEAP-300* spectropolarimeter. 1D- and 2D-NMR spectra were recorded on a *Bruker AM-400* spectrometer; δ in ppm, *J* in Hz. EI- and HR-ESI Mass spectra were recorded on a *VG AUTO spec 3000* spectrometer; in *m*/*z* (rel. %).

Plant Material. The leaves of *Alstonia scholaris* were collected in Xishuangbanna (Yunnan Province, P. R. China) in February 2004. The plant was identified by Prof. *Chuan-tao Cai*, Xishuangbanna Botany Garden, Chinese Academy of Science. A specimen of this plant was deposited at the Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The air-dried leaves (20 kg) were ground, and then repeatedly heated at reflux in 95% EtOH (4 × 30 l) for 4, 3, 2 and 1 h, resp. After removal of the solvent by evaporation, the residue was extracted with 1% aq. HCl. The acid-soluble fraction (1.5 kg) was washed with $CHCl_3$, basified to pH 10 with 25% aq. ammonia soln., and extracted with $CHCl_3$ to give a crude alkaloidal fraction (100 g). The latter was purified by initial CC (SiO_2; MeOH/$CHCl_3$ gradient) to afford four fractions: *Fr. 1* mainly contained picrinine, picralinal, vallesamine, and **2** (20 mg). *Fr. 2* was re-subjected to repeated CC (SiO_2; AcOEt/petroleum ether 1:5) to afford rhazimanine, **1** (25 mg), and *N*-demethylechitamine. *Fr. 3* was purified further by CC (SiO_2; AcOEt/MeOH 10:1) to afford akuammidine and burnamine. *Fr. 4* was also purified by CC (SiO_2; AcOEt/MeOH 10:1.5) to afford scholaricine, 19-*epi*-scholaricine, 2-dihydroakuammiline, and **3** (10 mg).

5-Methoxystrictamine (= *Methyl (5β,16*R,*19*E*)-5-methoxyakuammilan-17-oate*; **1**). Colorless powder. M.p. 232–235°. $[\alpha]_D^{13.9} = -119.3$ (*c* = 0.39, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.63 (*dd*, *J* = 7.7, 1.0, H–C(9)); 7.39 (*dd*, *J* = 7.7, 1.0, H–C(12)); 7.32 (*dt*, *J* = 7.7, 1.0, H–C(10)); 7.13 (*dt*, *J* = 7.7, 1.0, H–C(11)); 5.53 (*q*, *J* = 6.6, H–C(19)); 4.48 (*d*, *J* = 5.1, H–C(3)); 4.06 (*d*, *J* = 17.0, H_β–C(21)); 3.80–3.88 (*m*, H–(5)); 3.75 (*dd*, *J* = 4.5, 15.2, H_α–C(6)); 3.70 (*s*, Me(22)); 3.43 (br. *s*, H–C(15)); 3.22 (*s*, Me(24)); 3.08 (*d*, *J* = 17.0, H_α–C(21)); 2.68 (*dd*, *J* = 8.4, 5.1 H_α–C(14)); 2.20 (*d*, *J* = 15.2, H_β–C(6)); 1.91 (*d*, *J* = 4.0, H–C(16)); 1.76 (*dd*, *J* = 8.4, 5.1 H_β–C(14)); 1.54 (*d*, *J* = 6.6, Me(18)). ^{13}C-NMR (50 MHz, $CHCl_3$): see *Table*. FAB-MS: 353 (100, $[M+1]^+$, 337 (20), 321 (30).

5-Oxo-17-deacetyl-1,2-dihydroakuammiline (= *Methyl (16*R,*19*E*)-1,2-dihydro-16-(hydroxymethyl)-5-oxo-akuammilan-17-oate*; **2**). Colorless powder. M.p. 290–292°. $[\alpha]_D^{13.4} = -147.5$ (*c* = 0.35, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.36 (*dd*, *J* = 7.6, 1.0, H–C(9)); 7.18 (*dt*, *J* = 7.6, 1.0, H–C(11)); 7.02 (*dt*, *J* = 7.6, 1.0, H–C(10)); 6.82 (*dd*, *J* = 7.6, 1.0, H–C(12)); 5.28 (*q*, *J* = 6.7, H–C(19)); 3.91 (*d*, *J* = 3.6, H_α–C(23)); 3.80 (*s*,

Table. *^{13}C-NMR Data for Compounds* **1**–**3**. At 100 MHz in $CDCl_3$; δ in ppm.

Position	**1**	**2**	**3**
2	190.5	63.1	102.0
3	51.5	60.0	59.8
5	89.9	183.1	165.9
6	38.4	38.2	52.6
7	53.4	55.7	56.9
8	145.4	129.7	133.0
9	120.6	126.2	126.0
10	125.0	122.0	119.5
11	128.0	128.2	128.9
12	123.1	109.2	110.6
13	155.6	141.4	147.5
14	35.8	26.5	24.4
15	32.6	28.0	32.2
16	56.0	54.8	52.2
17	171.6	174.8	174.5
18	12.9	12.6	13.2
19	120.9	114.9	124.4
20	136.6	138.2	133.2
21	50.2	49.0	56.6
22	51.5	51.9	51.6
23	–	69.3	67.8
1-Me	–	–	44.2
5-MeO	54.6	–	44.2

Me(22)); 3.68 (*d*, $J = 3.6$, H_β–C(23)); 3.60 (*d*, $J = 1.9$, CH_2(21)); 3.27 (br. *s*, H–C(15)); 3.23–3.27 (*m*, H–C(3)); 3.20 (*d*, $J = 5.2$, H–C(2)); 2.73 (*d*, $J = 6.8$, H_α–C(6)); 2.61–2.64 (*m*, H_α–C(14)); 1.71 (*dd*, $J = 6.8$, H_β–C(6)); 1.64 (*d*, $J = 6.7$, Me(18)); 1.45–1.47 (*m*, H_β–C(14)). ^{13}C-NMR: see *Table*. EI-MS: 368 (20, M^+), 265 (100), 158 (35).

*Methyl (2β,16*R*,19*E*)-4,5-Didehydro-1,2-dihydro-2-hydroxy-16-(hydroxymethyl)akuammilan-4-ium-17-oate Chloride* (**3**). Colorless powder. M.p. 229–232°. $[\alpha]_D^{18.2} = -80.1$ ($c = 0.20$, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.56 (br. *s*, H–C(5)); 7.32 (*dd*, $J = 7.6$, 1.0, H–C(9)); 7.08 (*dt*, $J = 7.6$, 1.0, H–C(11)); 6.75 (*dt*, $J = 7.6$, 1.0, H–C(10)); 6.63 (*dd*, $J = 7.6$, 1.0, H–C(12)); 5.46 (*q*, $J = 6.9$, H–C(19)); 3.75 (*s*, Me(22)); 3.46 (*d*, $J = 12.0$, H_α–C(23)); 3.51 (br. *s*, H–C(15)); 3.43–3.46 (*m*, H–C(6)); 3.32 (*d*, $J = 12.0$, H_β–C(23)); 3.26–3.28 (*m*, H–C(3)); 3.18 (*d*, $J = 17.0$, H_α–C(21)); 2.88 (*d*, $J = 17.0$, H_β–C(21)); 2.48 (*s*, MeN); 2.10 (*d*, $J = 14.0$, H_α–C(14)); 1.81–1.84 (*m*, H_β–C(14)); 1.68 (*d*, $J = 6.9$, Me(18)). ^{13}C-NMR: see *Table*. EI-MS: 382 (100, M^+), 356 (10), 282 (5).

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Received March 29, 2005