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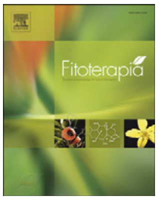

Five new indole alkaloids from the leaves of *Rauvolfia yunnanensis*

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ABSTRACT

Five new indole alkaloids, rauvoloids A–E (**1**–**5**), together with two known ones, raucaffrinoline (**6**) and perakine (**7**) were isolated from the leaves of *Rauvolfia yunnanensis*. Their structures were elucidated by extensive spectroscopic methods. Structurally, rauvoloids A (**1**), B–C (**2**–**3**) and D (**4**) with unusual substitution patterns (no substitution, Cl and (1*E*)-3-oxo-butenyl, respectively) at C-20, are the first examples of perakine-type alkaloids with C_{18} and C_{22} skeletons.

1. Introduction

Rauvolfia yunnanensis belonging to the *Rauvolfia* genus of the family Apocynaceae has long been used for treating hypertension, snake bites and insanity in the Zhuang and Yao minority regions of China. The *Rauvolfia* genus contains about 135 species mainly distributed in America, Africa, Asia and Oceania, of which 9 species, 4 variants and 3 cultivars are present in China [1,2]. *R. yunnanensis* are rich in indole alkaloids, especially reserpine and yohimbine, however, wild resources are reduced rapidly and their use for pharmaceutical purposes are unsatisfactory [3–7]. In 2003, we introduced *R. yunnanensis* from Xichou County into the arid-hot valley region of Nujiang River, Yunnan Province (China) for large scale field cultivation. After transplantation, the contents of total alkaloids, as well as reserpine and yohimbine in the roots of *R. yunnanensis* were obviously increased compared to the indigenous plants [8]. In order to investigate the constitutional variation, our previous study on *R. yunnanensis* resulted in one new macrocyclic diamide and three new indole alkaloids [9–11]. As a continuous investigation, this further study on the leaves of *R. yunnanensis* yielded five new perakine-type indole alkaloids, rauvoloids A–E (**1**–**5**), together with two known ones, raucaffrinoline (**6**) and perakine (**7**). From a biogenetic point of view, perakine-type indole alkaloids with a C_{19} skeleton were derived from raucaffriline (ajmaline-type) by intramolecular rearrangement reaction, and thus, possessed an additional C_1 substitute at C-20 which was derived from the Me-18 in raucaffriline [12–14]. Interestingly, rauvoloids A (**1**), B–C (**2**–**3**) and D (**4**), with no substituent, Cl and (1*E*)-3-oxo-butenyl group at C-20, respectively, were obviously different from perakine (with a formyl group at C-20). To our knowledge, rauvoloids A–C and rauvoloid D are the first examples of perakine-type alkaloids with C_{18} and C_{22} skeletons, respectively. This paper mainly deals with the isolation and structural elucidation of rauvoloids A–E (**1**–**5**) by extensive spectroscopic analyses.

2. Experimental

2.1. General experimental procedures

IR (KBr) spectra were measured on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, California, USA). UV spectra were determined on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotations were recorded on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). 1D and 2D NMR spectra were performed on a Bruker AM-400 NMR and DRX-500 spectrometers with TMS as the internal standard

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(Bruker, Bremerhaven, Germany). MS spectra were determined on a VG Auto Spec-3000 spectrometer (VG, Manchester, UK). Melting points (uncorrected) were measured on an X4 apparatus (Guiguang, Beijing, China). Silica gel (Makall, Qingdao, China), Sephadex LH-20 gel (Pharmacia, Uppsala, Sweden) and Rp-18 gel (Merck, Darmstadt, Germany) were used for column chromatography.

2.2. Plant material

Rauvolfia yunnanensis Tsiang was collected in Nujiang, Yunnan Province, China, in December 2005, which was cultivated in 2003. A voucher specimen (KIB 2005-12-01) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS.

2.3. Extraction and isolation

The air-dried leaves of *R. yunnanensis* (8.0 kg) were powdered and extracted with 95% EtOH at room temperature 3 times (each time 12 h, 20.0 L × 3 times). All the extracts were combined and concentrated under vacuum to give a residue (372.5 g). The residue was suspended in 5.0% HCl–H_2O, and after filtration, the acid solution was basified with 10% ammonia–water to pH 10.0. Then, the basic solution was extracted with $CHCl_3$ (2.0 L × 3 times) to give a total alkaloidal part (57.5 g). This part was performed on an Al_2O_3 column eluted with $CHCl_3$/MeOH (from 100:0 to 0:100) to obtain seven fractions (A–G). Fraction C (15.0 g) was further chromatographed on a silica gel column, eluted with $CHCl_3$/MeOH (from 90:10 to 80:20) to provide three fractions (C1–C3). Fraction C1 (1.0 g) was loaded on the silica gel column, eluted with petroleum ether (PE)/acetone (80:20), and further purified with the Sephadex LH-20 gel column ($CHCl_3$/MeOH, 1:1) to yield compounds **1** (4.6 mg) and **4** (3.2 mg). Separation of fraction C2 (1.2 g) by silica gel column, eluted with PE/acetone gradient (from 80:20 to 60:40), afforded two sub-fractions (C2-1 and C2-2). Sub-fraction C2-1 (100.0 mg) was purified with the Sephadex LH-20 gel column ($CHCl_3$/MeOH, 1:1) to give compound **2** (8.1 mg). Sub-fraction C2-2 (50.0 mg) was chromatographed on a silica gel column with an elution of $CHCl_3$/acetone (80:20) to obtain compound **3** (5.0 mg). Fraction C3 (8.6 g) was loaded on a silica gel column eluted with $CHCl_3$/acetone gradient (from 90:10 to 50:50) to afford three sub-fractions (C3-1–C3-3). Sub-fraction C3-1 (200.0 mg) was further purified with the Rp-18 gel column eluted with MeOH/H_2O (20:80) to yield compound **5** (25.0 mg). Sub-fraction C3-2 (1.0 g) was loaded on an Al_2O_3 column eluted with $CHCl_3$/MeOH (90:10), then purified with the Sephadex LH-20 gel column (MeOH) to give compound **6** (230.5 mg). Sub-fraction C3-3 (3.5 g) was loaded on an Al_2O_3 column eluted with $CHCl_3$/MeOH (85:15), and further purified with the Rp-18 column with an elution of MeOH/H_2O (30:70) to yield compound **7** (2.0 g).

Rauvoloid A (**1**): mp 185–186 °C; $[\alpha]29\ D = +7.5$ (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max}: 2943, 2929, 1737, 1616, 1586, 1469, 1454, 1374, 1354, 1230, 1212, 1029, 1020, 952, 774, 750 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε): 253 (3.52), 205 (3.31) nm; ^1H and ^{13}C NMR data: see Tables 1 and 2; EIMS *m/z* 332 (100, M^+), 279 (45), 263 (32), 235 (43), 196 (83), 168 (51); HRFABMS (+) *m/z* 323.1768 $[M + H]^+$ ($C_{20}H_{23}N_2O_2$, calcd 323.1760).

Table 1
^1H NMR data of compounds **1**–**5** ($CDCl_3$, δ in ppm, *J* in Hz).

No.	**1**	**2**	**3**	**4**	**5**
3	4.17, d, 9.2	4.25, d, 9.4	4.10,d, 8.8	4.11, d, 9.3	4.13, d, 9.4
5	3.61, m	3.62, m	3.80, d, 6.0	3.63, m	3.62, m
6α	1.62, d, 11.8	1.61, m	1.59, m	1.59, d,11.8	1.59, d, 11.8
6β	2.79, dd, 11.8, 5.0	2.78, dd, 12.0, 5.0	2.82, dd, 11.9, 5.0	2.79, m	2.78, m
9	7.46, d, 7.7	7.47, d, 7.6	7.47, d,7.4	7.42, d, 7.8	7.43, d, 7.5
10	7.21, dt, 7.7, 1.2	7.23, t, 7.6	7.23, td, 7.6, 1.2	7.18, dt, 7.8, 1.2	7.19, t, 7.5
11	7.38, dt, 7.7, 1.2	7.41, t, 7.6	7.40, td, 7.6, 1.2	7.35, dt, 7.8, 1.2	7.36, t, 7.5
12	7.61, d, 7.7	7.63, d, 7.6	7.62, d, 7.6	7.53, d, 7.8	7.59, d, 7.5
14α	1.81, m	2.29, m	1.86, m	1.95, m	1.95, m
14β	1.66, m	1.60, m	1.90, m	1.53, m	1.50, m
15	2.40, m	2.62, m	2.80, m	2.38, m	2.44, m
16	2.32, m	2.48, m	2.72, m	2.35, m	2.31, m
17	4.93, s	4.96, s	4.99, s	4.88, s	4.99, s
18	1.24, d, 6.8	1.34, d, 6.8	1.35, d, 6.9	1.18, d, 6.4	1.33, d, 6.5
19	3.03, m	2.94, m	3.34, m	2.74, m	2.73, m
20α	1.86, m	–	4.43, dd, 7.3, 4.6	–	–
20β	1.12, t, 10.0	3.71, d, 7.8	–	2.03, m	1.52, m
21	–	–	–	6.84, dd, 15.9, 7.8	4.53, d, 7.6
22	–	–	–	6.13, d, 15.9	–
23	–	–	–	–	–
24	–	–	–	2.24, s	–
– OAc	2.16, s	2.17, s	2.18, s	2.12, s	2.15, s
– OEt × 2	–	–	–	–	1′a: 3.78, m 1′b: 3.48, m 1″a: 3.60, m 1″b: 3.56, m 2′/2″: 6H, 1.23, m