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Monoterpenoid indole alkaloids from *Alstonia rupestris* with cytotoxic, antibacterial and antifungal activities

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ABSTRACT

A chemical investigation of the 80% EtOH extract of the aerial plant of *Alstonia rupestris* afforded four new monoterpenoid indole alkaloids, 6,7-epoxy-8-oxo-vincadifformine (1), 11-acetyl-6,7-epoxy-8-oxo-vincadifformine (2), 11-hydroxy-14-chloro-15-hydroxy-vincadifformine (3), and perakine N_1,N_4-dioxide (4), together with two known compounds, 11-hydroxy-6,7-epoxy-8-oxovincadifformine (5) and vinorine N_1,N_4-dioxide (6). Structural elucidation of all the compounds was performed by spectral methods such as 1D- and 2D-NMR, IR, UV, and HRESIMS. Alkaloids 1, 2 and 5 showed significant cytotoxicities against all the tested tumor cell lines of the head and neck squamous cell carcinoma with IC_{50} value less than 20 μM and antimicrobial activities against two fungi (*Alternaria alternata* and *Phytophthora capsici*). Alkaloids 4 and 6 exhibited the activity against bacterium *Staphylococcus aureus*.

1. Introduction

The genus *Alstonia*, which belongs to the family Apocynaceae, comprises about 60 species and is mainly distributed in Asia and South America [1,2]. Among them, 8 species naturally occur in China [3]. Plants of the family Apocynaceae have been proven to be good sources of monoterpenoid indole alkaloids [4–8]. Monoterpenoid indole alkaloids, which originate from the condensation of tryptophan with secologanin to give strictosidine, have attracted the interest of many researchers due to their complicated structures and potent biological activities [9–15]. This type of alkaloids possesses anticancer, antibacterial, antifertility, and anti-tussive activities [16–20]. Galanthamine is a long-acting, selective, reversible and competitive acetylcholine esterase inhibitor that has been approved for use in the European Union and the United States for the treatment of Alzheimer's disease (AD). *Alstonia rupestris* Kerr is usually endemic in the west part of the Guangxi Province of China. In the present paper, chromatographic separation of an EtOH extract of the aerial plant of *A. rupestris* has yielded four new monoterpenoid indole alkaloids, 6,7-epoxy-8-oxo-vincadifformine (1), 11-acetyl-6,7-epoxy-8-oxo-vincadifformine (2), 11-hydroxy-14-chloro-15-hydroxy-vincadifformine (3), and perakine N_1,N_4-dioxide (4), together with two known compounds, 11-hydroxy-6,7-epoxy-8-oxovincadifformine (5) and vinorine N_1,N_4-dioxide (6) (Fig. 1). Their structures were established on the basis of their chromatographic properties, chemical and physicochemical methods. Furthermore, all the triterpenoids were evaluated for their in vitro cytotoxic, antibacterial and antifungal properties.

2. Experimental

2.1. General

Melting points were determined using a Fisher–Johns melting point apparatus (Vernon Hills, Lake, IL, USA). Optical rotations were determined with a JASCO P2000 digital polarimeter (Tokyo, Japan). Ultraviolet (UV) and infrared (IR)

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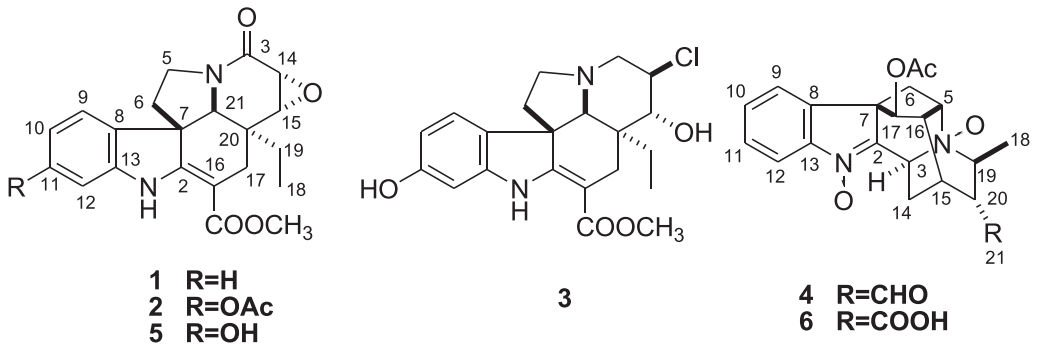

Fig. 1. Structures of compounds 1–6.

spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers, respectively. The NMR spectra were recorded on a Varian Unity INOVA 600 FT-NMR spectrometer (Salt Lake City, UT, USA; 600 MHz for ^{1}H; 125 MHz for ^{13}C, respectively). Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0 ppm) and CD_3OD (δ_H 3.30 and δ_C 49.0 ppm) as internal standards. High resolution ESIMS spectra were obtained on a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA) spectrometer. Silica gel 60 (Merck, Darmstadt, Germany, 230–400 mesh), LiChroprep RP-18 (Merck, 40–63 μm), and Sephadex LH-20 (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) were used for column chromatography (CC). Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F_{254} plates (Merck) were used for analytical thin-layer chromatography analyses.

2.2. Plant material

The aerial parts of *A. rupestris* were collected in the Honghe, Yunnan province, China, in June 2012. A specimen (201206001), identified by one of the authors (Y. Song), was deposited in the Herbarium of Shenyang Medicine College, Shenyang, China.

2.3. Extraction and isolation

The aerial parts of *A. rupestris* (10.0 kg) were cut into small pieces and were extracted with 80% EtOH (20 L × 3) at room temperature for 24 h each time. After removal of EtOH under reduced pressure at 55 °C, the aqueous brownish syrup (1 L) was suspended in H_2O (1 L) and then partitioned with chloroform (1 L × 3) to afford chloroform fraction (87.3 g). The chloroform fraction was further fractionated through a silica gel column (200–300 mesh, 10 × 80 cm, 500 g) using increasing volumes of acetone in petroleum ether (b.p. 60–90 °C) (100:1, 50:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, v/v, each 3 L) as the eluent to give 8 fractions. Fraction 3 (petroleum ether–acetone 15:1, 4.1 g) was applied to an ODS MPLC column (100 g) and eluted with MeOH-H_2O (20:80, 30:70, 40:60, each 500 mL) to yield four subfractions (Fr. 3-1 to Fr. 3-4). Subfraction 3-2 (MeOH-H_2O, 327 mg) was purified by preparative RP–HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (25:75) as mobile phase to obtain 5 (67 mg, 22.42 min). Subfraction 4-2 (MeOH-H_2O, 350 mg) was chromatographed by a Sephadex LH-20 column eluting with MeOH-H_2O (50:50), and purifed by preparative RP–HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (30:70) as mobile phase to yield 1 (68 mg, 23.16 min) and 2 (70 mg, 24.71 min). Subfraction 4-4 (MeOH-H_2O 40:60, 99 mg) was purified by preparative RP–HPLC (ODS column, 250 × 20 mm) eluting with MeOH/H_2O (22:78) to get 4 (57 mg, 24.65 min). Fraction 5 (petroleum ether–acetone 30:1, 1.4 g) was applied to an ODS column eluted with MeOH-H_2O (30:70, 40:60, 50:50) to provide three subfractions (Fr. 5-1 to Fr. 5-3). Subfraction 5-2 (MeOH-H_2O 20:80, 226 mg) was repeatedly chromatographed on silica gel (150 g, 60 × 2.8 cm, chloroform–methanol, 20:1 → 10:1) and then purified on a Sephadex LH-20 column eluted with MeOH-H_2O (50:50) to afford 3 (78 mg, 22.33 min) and 6 (77 mg, 23.90 min).

6,7-Epoxy-8-oxo-vincadifformine (1): Colorless oil. $[\alpha]_D^{23.3} = -97.3$ ($c = 0.14$, MeOH). UV ($CDCl_3$) λ_{max}(log ε): 324 (3.68), 244 (3.79), 228 (3.80), 197 (3.75) nm. IR (KBr) ν_{max} 3385, 1657, 1617, 1442, 1105, 750 cm^{-1}. For NMR data see Tables 1 and 2. EI-MS *m/z*: 366 ($[M]^+$). HR-ESI-MS (pos.) *m/z*: 389.1473 ($[M + Na]^+$, $C_{21}H_{22}N_2O_4Na$, calc. 389.1477).

Table 1
^{13}C NMR data of compounds 1–4 in $CDCl_3$.

No.	1	2	3	4
2	166.5, s	166.1, s	166.2, s	147.2, s
3	165.1, s	164.7, s	54.3, t	68.6, d
5	43.6, t	43.3, t	50.6, t	67.3, d
6	42.0, t	41.7, t	43.9, t	33.3, t
7	56.8, s	56.6, s	54.3, s	58.2, s
8	137.3, s	131.8, s	129.7, s	133.1, s
9	122.9, d	121.0, d	121.5, d	126.3, d
10	120.6, d	112.6, d	104.8, d	132.4, d
11	127.8, d	151.3, s	159.9, s	128.6, d
12	108.7, d	104.3, d	96.6, d	117.5, d
13	143.2, s	143.1, s	144.2, s	149.4, s
14	51.2, d	50.9, d	59.3, d	26.4, t
15	57.1, d	56.9, d	75.6, d	26.2, d
16	89.1, s	88.8, s	92.5, s	50.8, d
17	22.4, t	22.1, t	26.5, t	78.3, d
18	7.3, q	7.1, q	8.0, q	14.4, q
19	26.2, t	26.2, t	22.7, t	66.5, d
20	40.6, s	40.5, s	44.2, s	48.6, d
21	63.3, d	63.2, d	69.5, d	201.5, d
CO_2CH_3	51.2, q	51.2, q	50.8, q	–
CO_2CH_3	168.3, s	168.1, s	168.6, s	–
$COCH_3$	–	20.1, q	–	20.7, q
$COCH_3$	–	169.8, s	–	171.1, s