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Unique Monoterpenoid Indole Alkaloids from *Alstonia scholaris*

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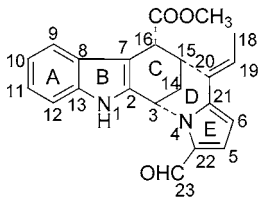
ABSTRACT



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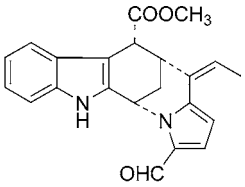

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A pair of geometrically isomeric monoterpenoid indole alkaloids with a skeleton rearrangement and two additional carbons, named (19,20) *E*-alstoscholarine (1) and (19,20) *Z*-alstoscholarine (2), were obtained from the leaf extract of *Alstonia scholaris*. Their structures were elucidated on the basis of spectroscopic methods and then confirmed by X-ray crystal diffraction. The biogenesis of these compounds was also proposed.

The genus *Alstonia* found in Africa and Asia comprises about 60 species, 8 of which are distributed in China. Four species of this genus have been found in the Yunnan province.[1] The phytochemical constituents of the *Alstonia* sp. have been investigated intensively. Until now, more than 300 compounds have been reported from this genus. Most of them are monoterpenoid indole alkaloids, which originate from the condensation of tryptophan with secologanin to give strictosidine and then elaborate to give an impressive array of structural variants.[2] This type of *Alstonia* alkaloid possesses 19 (or 18) carbon atoms in the skeleton and reportedly has anticancer, antibacterial, antifertility, and antitussive activity.[3] *Alstonia scholaris* is widely distributed in South and Southeast Asia, where it is used as a medicinal plant by local ethnic people. For example, the bark is used in traditional medicines throughout the region to treat dysentery and malaria.[4] The leaves are used to treat chronic respiratory disease in "dai" ethnopharmacy historically in the Yunnan province, P. R. of China.[5] On the basis of traditional usage, the leaf extract was also developed to be a commercial *traditional Chinese medicine* in China, which has been both prescribed in hospital clinics and sold in drug stores over the counter. Previous phytochemical studies on the leaves of *A. scholaris* collected in India, Pakistan, Thailand, the Philippines, Malaysia, and Indonesia showed diverse monoterpenoid indole alkaloidal patterns.[6] However, the chemical constituents from leaves of *A. scholaris* cultivated in Yunnan, the raw medicine material of the above-mentioned over the counter drugs, have not been reported. As part of a continuing effort to discover novel secondary metabolites from Yunnan local medicinal plants, we undertook phytochemical research on this plant.[7] In this paper, we describe the isolation and structural elucidation of two novel monoterpenoid indole

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alkaloids with a skeleton rearrangement and two additional carbons (Figure 1).

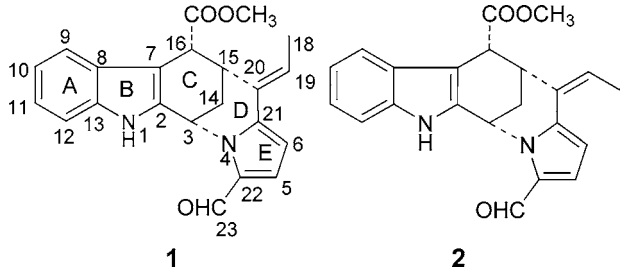

Figure 1. Structure of compounds **1** and **2**.

The dried and powdered leaves of *Alstonia scholaris* (50 kg) were extracted with EtOH (150 L × 3) under reflux, and the solvent was evaporated in vacuo. The residue was dissolved in 1% HCl, and the acidic solution was adjusted to pH 9−10 with ammonia. The basic solution was partitioned with EtOAc, to afford basic solution, EtOAc, and emulsion fractions. The emulsion fractions (250 g) were dissolved in MeOH and subjected to column chromatography on silica gel eluting with $CHCl_3$−Me_2CO [from $CHCl_3$ to $CHCl_3$−Me_2CO (1:1)] to afford five fractions (I−V). Fraction II (21 g) was chromatographed once again using petroleum ether−Me_2CO (4:1) to give compounds *E*-alstoscholarine (**1**) (22 mg) and *Z*-alstoscholarine (**2**) (12 mg).

Compound **1**[8] was found to possess a molecular formula of $C_{22}H_{20}N_2O_3$ as evidenced by HRESIMS at *m*/*z* [361.1550] in combination with ^{1}H NMR, ^{13}C NMR, and DEPT spectra. Its UV spectrum showed the existence of conjugated groups based on the maximum absorption at 223, 291, and 300 nm. The IR spectra exhibited absorption bands for −NH (3418 cm^{-1}), C═O (1746 cm^{-1}), and benzene rings (1623 cm^{-1}). The ^{1}H NMR, ^{13}C NMR, and DEPT spectra displayed signals for a substituted indole ring [δ_C 135.4 (s, C-2), 105.4 (s, C-7), 128.5 (s, C-8), 118.7 (d, C-9), 119.8 (d, C-10), 122.4 (d, C-11), 111.7 (d, C-12), 135.7 (s, C-13); δ_H 7.20 (1H, d, J = 7.5 Hz, H-9), 7.03 (1H, t, J = 7.5 Hz, H-10), 7.14 (1H, t, J = 7.5 Hz, H-11), 7.32 (1H, d, J = 7.5 Hz, H-12)].[9] Besides indole ring signals, the alkaloid possessed six olefinic carbons [δ_C 140.1 (s), 129.8 (s), 128.5 (s), 127.9 (d), 125.4 (d), 106.1 (d)], an aldehyde group ([δ_C 179.2 (d) and δ_H 9.43 (s)], a methyl ester group [δ_C 173.3 (s), 51.7 (q)], three methines, a methene, and a methyl group in the ^{13}C NMR spectrum.

In the HMBC spectrum of **1**, cross-peaks of δ_H 4.47 (1H, d, J = 7.5 Hz, H-16) with C-7 (δ_C 105.4, s) and C-8 (δ_C 128.5, s) suggested that CH-16 is directly connected to C-7 (Figure 2). δ_H 4.00 (1H, m) was assigned to be H-15 because

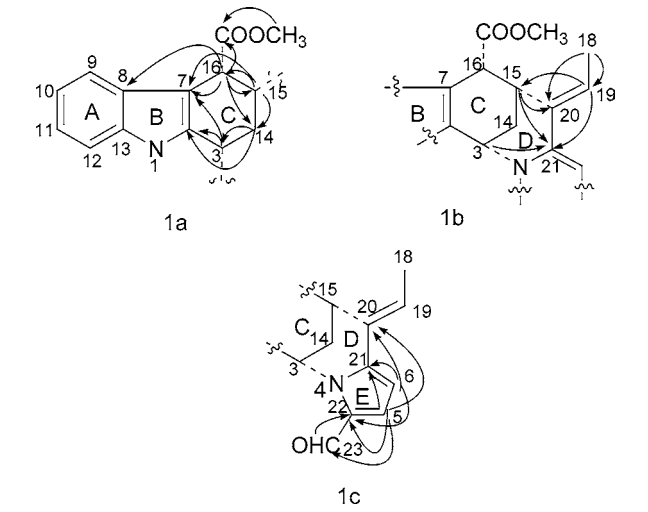

Figure 2. Key HMBC correlations of structural fragments of **1**.

of its correlations with C-7 (δ_C 105.4, s) and C-16 (δ_C 45.7, d), which was also supported by the ^{1}H−^{1}H COSY spectral data. Likewise, correlations between δ_H 6.27 (1H, t, J = 2.7 Hz, H-3) with C-7 and C-2 in the HMBC spectrum assigned another −CH adjacent to the indole ring. In the ^{1}H−^{1}H COSY spectrum of **1**, cross signals between δ_H 2.47 (2H, m, H-14) with δ_H 6.27 (H-3) and δ_H 4.00 (H-15) indicated the six-membered ring C. The carboxylic methyl ester group was positioned at C-16, which was supported by correlations between H-16 with δ_C 173.3 and 51.7 (−$COOCH_3$). In the ROESY spectrum of **1**, NOE correlations among H-16, H-15, and H-3 placed three protons at the same side. Ultimately, these three protons were assumed to be in β orientation because the H-16 of other monoterpenoid indole alkaloids from this genus was in the β location (Figure 3).[10] The above

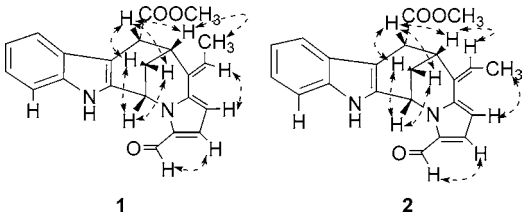

Figure 3. Key ROESY correlations of **1** and **2**.

data established a partial structure of **1a**.

The downfield chemical shift of H-3 (δ_H 6.27, t) required another nitrogen atom connected to C-3. In the HMBC

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(8) Compound **1**: green prism crystal; mp 168−170 °C; $[\alpha]_D^{20} = -53°$ (*c*, 0.065); UV ($CHCl_3$) λ max 223, 291, 300 nm; IR (KBr) ν_{max} 3418, 1746, 1623 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; postive FABMS *m*/*z* $[M]^+$ 360 (100); HRESIMS *m*/*z* 361.1550 (cacld for $C_{22}H_{20}N_2O_3$, 361.1552).

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spectrum, the correlation between H-3 and δ_C 128.5 (s, C-21) suggested that C-3 and an olefinic carbon are joined through a nitrogen atom. Additionally, H-15 was also correlated with this olefinic carbon and another one at δ_C 140.1 (s, C-21), which gave another six-membered ring D. Also, in the HMBC, correlations of δ_H 6.41 (q, J = 7.0 Hz, H-19) with δ_C 29.7 (C-15, d) and δ_C 128.5 (s, C-21), as well as of δ_H 1.90 (d, J = 7.0 Hz, H-18) with δ_C 140.1 (s, C-21), further supported the existence of a six-membered ring and corroborated an exocyclic substituted propylene group of C-19, 20, and 18 at the D ring (**1b**, Figure 2). The double bond of C-19/20 was determined to be *E* on the basis of NOE correlations of H-15/18 and H-6/H-19, in its ROESY spectrum (Figure 3).

Considering the 14 degrees of unsaturation of compound **1**, one more ring was required for the structure. The E-ring was proposed to be a substituted pyrrole by the HMBC spectrum with cross-peaks of two coupled protons at δ_H 6.36 (d, J = 4.5 Hz) and δ_H 6.81 (d, J = 4.5 Hz) with δ_C 140.1 (C-20, s), δ_C 128.5 (C-21, s), and δ_C 129.8 (s, C-22). The above data also placed the aldehyde at C-22 together with a correlation between δ_H 6.81 and δ_C 179.2 (−CHO, d). On the basis of the above evidence, we propose structure **1** for this novel alkaloid, named *E*-alstoscholarine. Finally, the structure of **1** was confirmed by X-ray diffraction (Figure 4).[11] The compound numbering system corresponded in biogenetic origin to the monoterpenoid indole alkaloids, in which two additional carbons were labeled as C-22 and C-23.

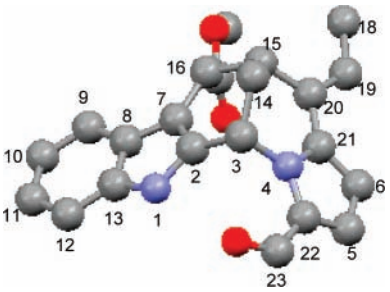

Figure 4. X-ray crystal structure of **1**.

Its full assignments of ^{1}H and ^{13}C spectra were determined by HMBC, HSQC, and ROESY spectra (Table 1).

Table 1. ^{1}H and ^{13}C NMR Assignments of **1** and **2**[a]

entry	**1** δ_C	**1** δ_H (J in Hz)	**2** δ_C	**2** δ_H (J in Hz)
1		9.03 (1H, s)		8.97 (1H, s)
2	135.4 s		135.4 s	
3	45.2 d	6.27 (1H, t, 2.7)	45.6 d	6.32 (1H, t, 2.7)
5	125.4 d	6.81 (1H, d, 4.5)	125.1 d	6.89 (1H, d, 4.5)
6	106.1 d	6.36 (1H, d, 4.5)	112.1 d	6.43 (1H, d, 4.5)
7	105.4 s		105.2 s	
8	128.5 s		128.5 s	
9	118.7 d	7.20 (1H, d, 7.5)	119.3 d	7.23 (1H, d, 7.5)
10	119.8 d	7.03 (1H, t, 7.5)	119.8 d	7.02 (1H, t, 7.5)
11	122.4 d	7.14 (1H, t, 7.5)	119.3 d	7.12 (1H, t, 7.5)
12	111.7 d	7.32 (1H, d, 7.5)	111.7 d	7.30 (1H, d, 7.5)
13	135.7 s		135.7 s	
14	31.7 t	2.47 (2H, m)	31.4 t	2.50 (1H, m)
				2.42 (1H, m)
15	29.7 d	4.00 (1H, m)	37.9 d	3.59 (1H, m)
16	45.7 d	4.47 (1H, d, 7.5)	47.2 d	4.36 (1H, d, 6.5)
18	14.1 q	1.90 (1H, d, 7.0)	16.0 q	1.92 (1H, d, 7.5)
19	127.9 d	6.41 (1H, q, 7.0)	129.4 d	5.90 (1H, q, 7.5)
20	140.1 s		137.1 s	
21	128.5 s		129.5 s	
22	129.8 s		129.8 s	
23	179.2 d	9.43 (1H, s)	180.1 d	9.51 (1H, s)
−*C*O−	173.3 s		172.9 s	
OMe	51.7 q	3.64 (3H, s)	51.4 q	3.63 (3H, s)

[a] Data were recorded in $CDCl_3$ on Brucker DRX-500 MHz spectrometers (^{1}H, ^{13}C, HSQC, HMBC, ROESY). Chemical shifts (δ) are given in parts per million with reference to the most downfield signal of $CDCl_3$ (δ 7.26 ppm) for ^{1}H and to the center peak of the downfield signal of $CDCl_3$ (δ 77.0 ppm) for ^{13}C.

Compound **2**[14] also possessed the molecular formula $C_{22}H_{20}N_2O_3$ by HRESIMS, which was identical to that of **1**. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except for an olefinic proton at δ_H 5.90 (1H, q, J = 7.0 Hz, H-19) in **2** instead of at δ_H 6.41 (1H, q, J = 7.0 Hz, H-19) in **1**. The NOE correlations [H-6 (δ_H 6.43, 1H, d, J = 4.5 Hz)/H-18 (δ_H 1.92, 3H, d, J = 7.5 Hz), H-15 (δ_H 3.59, 1H, m)/H-19 (δ_H 5.90, 1H, q, J = 7.5 Hz)] in the ROESY spectrum of **2** (Figure 3) supported a *Z*-type of double bond C-19/20 for **2**. The stereochemistry at the other chiral centers in **2** was identical to that of compound **1**, as supported by its ^{1}H NMR, ^{13}C NMR, HMBC, HMQC, ^{1}H−^{1}H COSY, and ROESY spectra.

Compounds **1** and **2** were inactive toward both *Penicillium avellaneum* UC-4376 and *Candida albicans* HSP90 in antibacterial biotest experiments.

By comparison with known monoterpenoid indole alkaloids, we believe that compounds **1** and **2** are rearranged monoterpenoid indole alkaloids with two additional carbons in the skeleton. We considered the possible biogenetic origin.

(11) X-ray crystal data of **1**: $C_{22}H_{20}N_2O_3$, MW = 360.40; monoclinic, space group $P2_1$; a = 10.483(2) Å, b = 11.115(2) Å, c = 15.144(3) Å, V = 1764.5(6) Å^3, Z = 4, D_{calcd} = 1.357 g/cm^3, Mo Kα (λ = 0.71073 Å). The data were collected on a MAC DIP-2030K diffractometer, with a graphite monochromator. Mo Kα radiation was found using a colorless crystal of dimensions of 0.52 × 0.52 × 0.40 mm^3 and a maximum 2θ value of 16.0°. Independent reflections: 3990. Observed number of reflections: 3653 [$|F|^2 \geq 8\sigma(|F|^2)$]. The structure was solved by the direct method SHELX-86[12] and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[13] and full-matrix, least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were R = 0.0346 and R_w = 0.0756. The CCDC deposit number is 631977. Copies of these data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.html (or Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K., fax +44 1223 336033, e-mail deposit@ccdc.cam.ac.uk).

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(14) Compound **2**: white power; $[\alpha]_D^{25}$ = −140° (*c*, 0.05); UV ($CHCl_3$) λ_{max} 220, 286, 293 nm; IR (KBr) ν_{max} 3418, 1745, 1634 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; postive FABMS *m/z* [M]$^+$ 360 (100); HRESIMS *m/z* 361.1547 (cacld for $C_{22}H_{20}N_2O_3$, 361.1552).

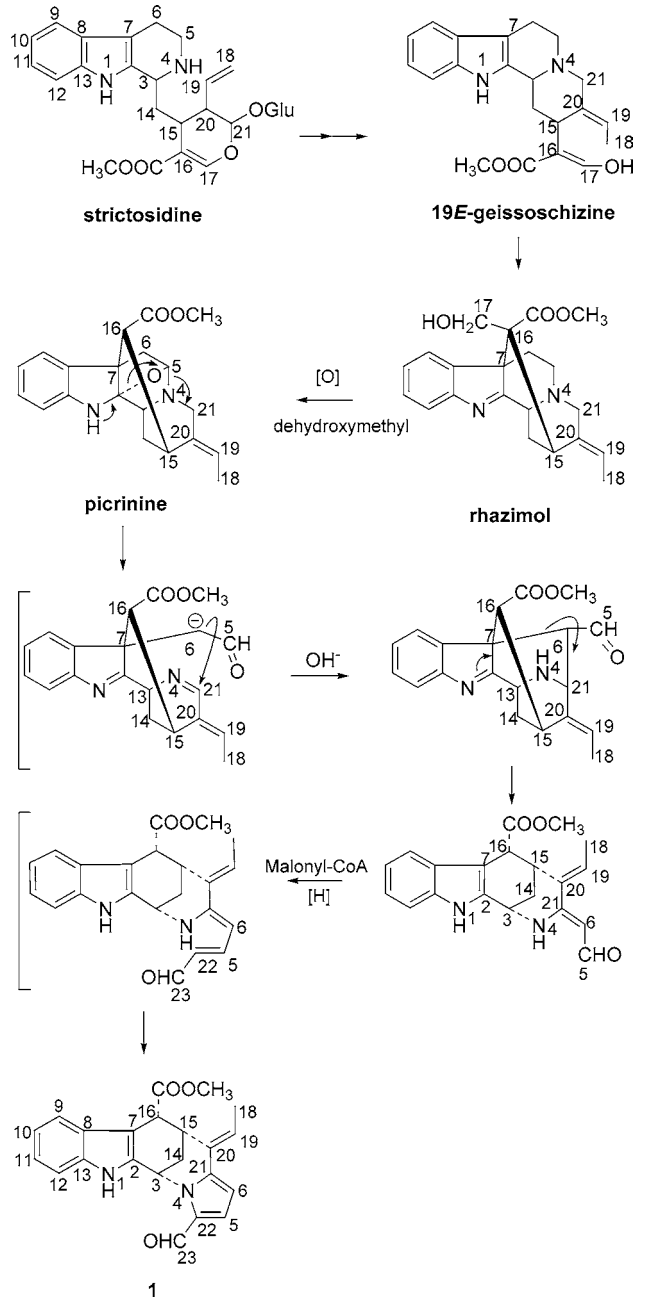

Figure 5. Proposed bioway of **1** and **2**.

As the monoterpenoid indole alkaloids precursor, the strictosidine would be further elaborated to give 19-*E*/*Z*-geissoschizine (Figure 5).[15]

Rhazimol could be formed by bond formation between C-16 and C-7 of 19-*E*-geissoschizine, followed by oxygenation and dehydroxymethylation of rhazimol to afford picrinine. The chemical bonds of C_2-O and C_5-N of picrinine could be cleaved to form an aldehyde carbon of C-5 and a double bond between N-4 and C-21. Subsequently, nucleophilic attack of C-6 on C-21 led to cleavage of C-6/7 and formed another double bond between C-6 and C-21. Finally, the formation of a new ring E with two more carbons might pass through the intermediate adding a malonyl-coenzyme A.[16]

The difference between compounds **2** and **1** was the configuration of the double bond C-19/20. The concurrence of those geometry isomers might be involved in an elimination reaction in the biosynthesis of geissoschizine to form a doule bond, which affords both 19-*E* and 19-*Z* types. Nevertheless, the 19-*E* type is dominant in all monoterpenoid indole alkaloids because of its stability. Therefore, **2** should be derived from an analogic pathway from *Z*-type geissoschizine, although it has been isolated now. To our knowledge, there are four more pairs of stereoisomeric monoterpenoid indole alkaloids with an ethylidene group (*E* or *Z*) at the 19-position, that is, 19-*E*/*Z*-16-epiisositsirikine,[17] 19-*E*/*Z*-isositsirikine,[17,18] 19-*E*/*Z*-vallesamine,[19] and 19-*E*/*Z*-picralinal.[20]

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Supporting Information Available: 1D, 2D, and X-ray data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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