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A Cage-Monoterpene Indole Alkaloid from *Alstonia scholaris*

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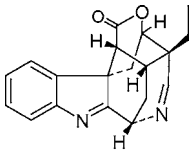
ABSTRACT



scholarisine A

An unprecedented cage-like alkaloid, scholarisine A was isolated from the leaves of *Alstonia scholaris* and its structure determined on the basis of 1D and 2D NMR, FTIR, UV, and high-resolution mass spectroscopic data. This alkaloid might be derived from picrinine via oxygenation, rearrangement, and lactonization.

The genus *Alstonia* is widely distributed in the tropical regions of Africa and Asia.[1] The phytochemical constituents of *Alstonia* sp. have been investigated extensively, and nearly 400 compounds have been isolated and characterized.[2] Most of the compounds identified so far belong to monoterpene indole and quinoline alkaloids and are thought to originate from the condensation of tryptophan with secologanin.[3] Previous investigations have indicated that the existence of monoterpene indole alkaloids is related to plant inhabitability. For example, picrinine-type indole alkaloids are generally found in *Alstonia* plants from continental countries such as India, Pakistan, and Thailand, while alkaloids bearing the angustilobine skeleton exist predominantly in those in Indonesia and the Philippines.[4] Potent anticancer, antibacterial, anti-inflammatory, and antitussive activities for several of these natural compounds were reported in the literature.[5]

Four species of the *Alstonia* genus have been found in the Yunnan province of the People's Republic of China.[6] The leaves of *A. scholaris* (L.) R. Br. have been historically used in "dai" ethnopharmacy to treat chronic respiratory diseases.[7] The leaf extract, developed as a commercially available traditional chinese medicine, has also been prescribed in hospitals and sold over the counter in drug stores.[8] As part of a continuing effort to discover novel secondary metabolites from Yunnan local medicinal plants, we undertook phytochemical research on *A. scholaris.* As part of this endeavor, a pair of rearranged alkaloids were previously isolated and reported.[9] In this paper, we describe the isolation and structure elucidation of a novel cage monoterpenoid indole alkaloid (Figure 1).

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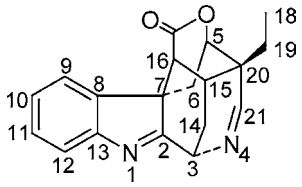

Figure 1. Structure of scholarisine A.

The dried and powdered leaves[10] of *A. scholaris* (50 kg) were extracted with EtOH (150 L × 3) under reflux conditions, and the solvent was evaporated in vacuo. The residue was dissolved in 1% HCl, and the solution was subsequently basified using ammonia water to pH 9−10. The basic solution was partitioned with EtOAc, affording a three-phase mixture including the aqueous phase, EtOAc/organic phase, and the emulsion phase. The emulsion fraction (250 g) was collected and then dissolved in MeOH, and the resulting solution was subjected to column chromatography over 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 IV (19 g) was further chromatographed using petroleum ether−Me_2CO (7:3) as eluent to give scholarisine A (15 mg).

This newly isolated alkaloid[11] was found to possess a molecular formula of $C_{19}H_{18}N_2O_2$ as evidenced by HRESIMS at *m*/*z* 307.1440 $[M + H]^+$. Its UV spectrum showed the characteristic absorption bands of indolenine alkaloids at 220, 268 nm.[12] The FTIR spectra exhibited absorption bands for lactone groups (1766 cm^{-1}) and aromatic rings (1641, 1575 cm^{-1}). The ^1H, ^{13}C NMR and DEPT spectra displayed signals for a substituted indole ring [δ_C 153.2 (s, C-13), 142.0 (s, C-8), 129.0 (s, C-11), 126.9 (d, C-10), 122.1 (d, C-9), 121.3 (d, C-12), 180.1 (s, C-2), 53.1 (s, C-7); δ_H 7.65 (1H, d, J = 7.5 Hz, H-12), 7.40 (1H, t, J = 7.5 Hz, H-11), 7.28 (1H, d, J = 7.5, H-9), 7.26 (1H, t, J = 7.5 Hz, H-10].[13,14] Besides the indole ring signals, the alkaloid possessed three methylenes (δ_C 34.7, 33.2, 30.1), five methine carbons (δ_C 169.8, 78.5, 58.2, 50.0, 32.2), one methyl group (δ_C 8.7), and one ester group [δ_C 171.4 (s)]. In its HMBC spectrum, correlations between δ_H 5.45 (1H, s, H-3) and the indole skeleton carbons (C-7 and C-2) allowed the assignment of a -CH adjacent to C-2, and correlations between one of the signals overlapping at δ_H 2.30 (H, brs, H-16) and C-8, as well as C-7, suggested that a −CH is connected to the indole ring at C-7 (Figure 2, **1a**). In the $^1H-^1H$ COSY spectrum, cross

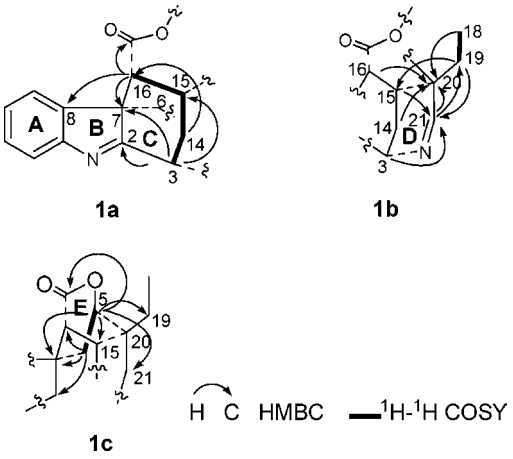

Figure 2. Key HMBC and $^1H-^1H$ COSY correlations of structural fractions of scholarisine A.

signals between δ_H 5.45 (H-3) and δ_H 1.77, 2.02 (1H, brd, J = 12.0 Hz, H-14) suggested the linkage between C-3 and a methylene (δ_C 33.2, C-14), while cross signals between δ_H 1.77, 2.02 (1H, brd, J = 12.0 Hz, H-14) and a signal at δ_H 2.30 (1H, brs) allowed the connection of a methine to C-14. δ_H 2.30 (2H, brs) was attributed to two methine (δ_C 50.0, 32.2) protons according to its HSQC. In addition to the assignment of a methine, C-16 (δ_C 50.0) adjacent to C-7, δ_C 32.2 was assigned to the methine between δ_C 50.0 (C-16) and δ_C 33.2 (C-14), which was supported by correlations between H-3, 14, and 16 with δ_C 32.2 (C-15) in its HMBC. The carboxylic ester group was positioned at C-16, an assignment supported by the correlations between H-16 and the signal of δ_C 171.4 (s). Like other monoterpenoid indole alkaloids from this genus, C-16 was assigned to be beta to the indole ring[14,15] NOE correlations among H-16, H-15, and H-3 in its ROESY spectrum (see Figure 3), showed that the

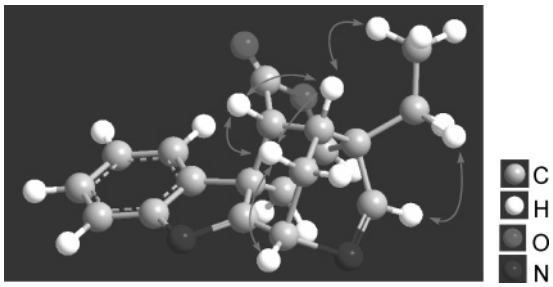

Figure 3. Key ROESY correlations of scholarisine A.

three protons are the same side of the indole ring. Based on the above data analysis, partial structure **1a** was established and shown in Figure 2.

The downfield signal of H-3 (δ_H 5.45, s) suggested the attachment of another nitrogen atom to C-3. In the HMBC

(10) The leaves of *A. scholaris* (L.) R. Br. were collected in April 2006 in Simao of Yunnan Province, People's Republic of China, and identified by Dr. Chun-Xia Zeng, Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (Luo20060407) has been deposited in the herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN).

(11) Scholarisine A: white powder; $[\alpha]^{20}_D = +188$ (*c*, 0.55); UV (CH_3-OH) λ_{max} 220 (ϵ 2370), 268 (ϵ 604) nm; IR (KBr) ν_{max} 1766, 1641, 1575 cm^{-1}; ^1H and ^{13}C NMR, data see Table 1; positive ESIMS *m*/*z* $[M + H]^+$ 307 (100); HRESIMS *m*/*z* 307.1440 (calcd for $C_{22}H_{29}N_2O_3$, 307.1446).

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experiment, correlations of H-3 and H-15 with the downfield signal of δ_C 169.5 (d, C-21) gave a possible −CH═NC$_3$H− structural unit. In addition, correlations between H-14, H-15, and δ_H 7.85 (1H, s, H-21) with δ_C 47.5 (s, C-20) suggested a six-membered ring in *D* (see Figure 2). Signals at δ_H 1.99 (2H, q, J = 8.0 Hz, H-19) and 1.04 (3H, t, J = 8.0 Hz, H-18) pointed to a connection between an ethyl and a quaternary carbon (δ_C 47.5, s, C-20), which was further supported by correlations between δ_H 1.99 with δ_C 32.2 (C-15), 47.5 (C-20), and 169.5 (C-21) in the HMBC spectrum. Based on the above spectral data, partial structure **1b** was drawn. The proton at δ_H 4.66 (1H, brs, H-5), assigned to an oxymethine, showed a correlation to the carbonyl carbon (δ_C 171.4, s) and C-20 in the HMBC spectrum, and thus suggested a six-memberd lactone ring. Considering that there are 12 degrees of unsaturation in this alkaloid, one more ring was required in the structure. In the ^{1}H−^{1}H COSY spectrum, cross signals between δ_H 4.66 (1H, brs, H-5) and δ_H 2.09, 2.11 (each 1H, brd, J = 17.0 Hz, H-6) suggested that the last methylene is neighboring to C-5. In the HMBC spectrum, correlations between δ_H 2.09 and 2.11 with the indole carbon signals at δ_C 180.1, 53.1, and 142.0 (C-2, 7, 8), indicated a spiral ring (*E*-ring) with indole derivative sharing C-7 (see Figure 2, **1c)**. On the basis of the above evidence, the structure of scholarisine A was constructed, in combination with a molecular model. The numbering system corresponded to the biogenetic origin of the monoterpene indole alkaloids.[15] All of the ^{1}H and ^{13}C spectral data were assigned by the ^{1}H−^{1}H COSY, HMBC, HSQC, and ROESY spectra (Table 1).

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

entry	δ_H (J in Hz)	δ_C	HMBC (^{1}H−^{13}C)
2		180.1 s	
3	5.45 (1H, brs)	58.2 d	2, 7, 14, 15, 21
5	4.66 (1H, brs)	78.5 d	7, 15, 19, 20, CO
6	2.11 (1H, brd, 17)	34.7 t	2, 5, 7, 8, 16, 20
	2.09 (1H, brd, 17)		
7		53.1 s	
8		142.0 s	
9	7.26 (1H, d, 7.5,)	122.1 d	7, 8, 10, 11, 12, 13
10	7.26 (1H, t, 7.5)	126.9 d	8, 9, 11, 12, 13
11	7.40 (1H, t, 7.5)	129.0 d	8, 9, 10, 12, 13
12	7.65 (1H, d, 7.5)	121.3 d	8, 9, 10, 11, 13
13		153.2 s	
14	1.77 (1H, brd, 12.0)	33.2 t	2, 3, 15, 16, 20
	2.02 (1H, brd, 12.0)		
15	2.30 (1H, brs, overlap)	32.2 d	3, 5, 7, 15, 19, CO
16	2.30 (1H, brs, overlap)	50.0 d	3, 6, 7, 8, 20, CO
18	1.04 (3H, t, 8.0)	8.7 q	19, 20,
19	1.99 (2H, q, 8.0)	30.1 t	18, 15, 20, 5, 21
20		47.5 s	
21	7.85 (1H, s)	169.8 d	15, 19, 20, 3, 2
-CO-		171.4 s	

[a] Data were recorded in $CDCl_3$ on Bruker AMD-400 (^{1}H, ^{13}C) and DRX-500 MHz spectrometers (^{1}H−^{1}H COSY, HSQC, HMBC, ROESY); chemical shifts (δ) are given in parts per million with references to the most downfield signal of $CDCl_3$ (δ 7.23 ppm) for ^{1}H and to the center peak of the downfield signal of $CDCl_3$ (δ 77.0 ppm) for ^{13}C.

A molecular model indicated that the cage skeleton was rigid. Dihedral angles between the bridgehead protons (H-3, 5, 15, and 16) and their neighborhood protons in structure were nearly 90°, suggesting that the vicinal coupling constants between the protons should be very small. That is what was observed in the ^{1}H NMR spectrum where all bridgehead protons appeared as broad singlets. Unfortunately, attempts to prepare a single crystal of this molecule have so far not been successful.

As the monoterpenoid indole alkaloid precursor, strictosidine would be elaborated to give picrinine. Similar to *E/Z*-alstoscholarine,[9] biogenesis of scholarisine A might also be derived from picrinine. The oxygen bridge between C-2 and C-5 and the C_5−N_4 bond could be cleaved and formed an aldehyde carbon at C-5. Subsequently, an enamine among N-4, C-21, and 20 might be formed by double bond migration from C-19/20 to C-20/21 of the intermediate, and spontaneous nucleophilic addition[16] to form a new C−C bond between C20/5 and an imine at N_4/C_{21}. Finally, the formation of a lactone bridge ring might be enabled through an intramolecular condensation reaction (Figure 4). Since C-16 of

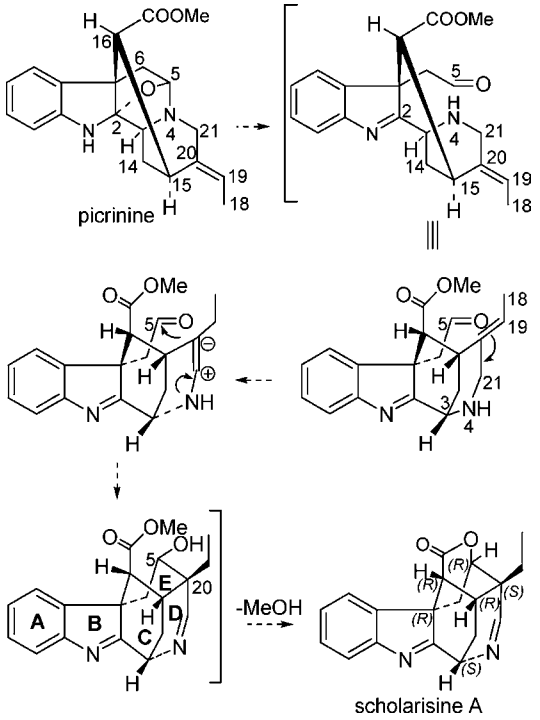

Figure 4. Proposed biosynthesis of scholarisine A.

picrinine was established to be *R*,[17] the absolute configuration at chiral carbons of scholarisine A could be determined to

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be 3*S*,5*R*,7*R*,15*R*,16*R*,20*S*, based on their relative configuration.

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Supporting Information Available: 1D and 2D data of scholarisine A. This material is available free of charge via the Internet at http://pubs.acs.org.

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