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Alistonitrine A, a Caged Monoterpene Indole Alkaloid from *Alstonia scholaris*

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Supporting Information

ABSTRACT: Alistonitrine A, a new monoterpene indole alkaloid incorporating a third nitrogen atom, was isolated from the leaves of *Alstonia scholaris* and found to possess an unprecedented caged skeleton with a unique 6/5/6/5/5/6 ring system. Its structure and absolute configuration were established by extensive spectroscopic analyses and electron circular dichroism calculations. A plausible biogenetic pathway has been proposed for the biosynthesis of alistonitrine A from picrinine.

Monoterpene indole alkaloids (MIAs) have consequently received considerable attention from natural products and synthetic chemists and pharmacologists in terms of their structural intricacies and interesting biological properties.[1] It has been proposed that MIAs originate from the coupling of tryptophan with secologanin by a series of biosynthetic processes and are characterized by their unique structures with two nitrogen atoms.[2] However, several MIAs incorporating a third nitrogen atom have been isolated from the plants of *Alstonia*,[3] *Kopsia*,[4] and *Neolamarckia*.[5] The incorporation of an additional nitrogen atom would result in the formation of an unprecedented MIA skeleton, such as those found in arboflorine[4] and aminocadambines,[5] and this possibility encouraged us to search for more natural MIAs containing three nitrogen atoms.

Alstonia scholaris R. Br. (Apocynaceae) is widely distributed in the South of China as well as several other countries in South and Southeast Asia. The bark and leaves of *A. scholaris* have been used as traditional medicines by local people to treat a number of ailments, including dysentery, malaria, and chronic respiratory disease.[6] A series of phytochemical investigations showed that MIAs are the major components of this plant.[7] Luo et al.[8] reported the isolation of three novel MIAs with unprecedented skeletons from the leaves of *A. scholaris*. Furthermore, the extracts and alkaloids of this plant have been shown to possess a diverse range of pharmacological activities, including anticancer, antibacterial, anti-inflammatory, antiasthmatic, expectorant, analgesic, and antitussive activities.[9] To date, however, there have been no reports concerning the isolation of MIAs containing three nitrogen atoms from *A. scholaris*. Our preliminary study of this species identified several minor compounds containing three nitrogen atoms by UHPLC-TOF-MS from the total alkaloids of *A. scholaris*. Subsequent phytochemical investigation of this plant allowed us to successfully isolate a new MIA incorporating a third nitrogen atom that we named alistonitrine A. In this paper, we reported the isolation and structural elucidation of this novel caged alkaloid (Figure 1).

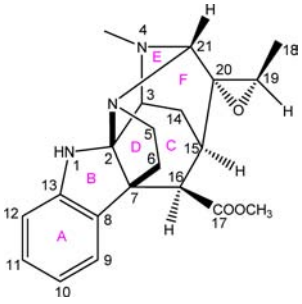

Figure 1. Structure of alistonitrine A.

Air-dried leaves[10] of *A. scholaris* (15 kg) were extracted with 80% EtOH (2 × 150 L) under reflux conditions, and the combined extracts were concentrated under reduced pressure. The resulting residue was dissolved in 1% HCl and the solution was then centrifuged. The supernatant was collected and basified using ammonia hydroxide to pH 9−10. The solution was then partitioned with EtOAc to afford an EtOAc-soluble fraction (136 g), which was subjected to column chromatography over silica gel eluting with a $CHCl_3$/MeOH gradient (from 1:0 to 2:1) to give 51 fractions (F.1−F.51). LC-MS analysis identified F.16−F.18 (showed an ion peak at m/z 368) as being of particular interest, and these fractions were chromatographed on a reversed-phase C-18 column eluting with MeOH/0.05% diethylamine in H_2O (55:45→100:0) and further purified by semipreparative HPLC eluting with MeOH/0.05% diethylamine in H_2O (65:35) and MeCN/0.05% diethylamine in H_2O (35:65) to yield alistonitrine A (5.0 mg). Alistonitrine A was also identified by UHPLC-TOF-MS from the total alkaloids of *A. scholaris* prepared by an ammonia-free method (Figure S1), suggesting that it is a genuine natural product.

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Alistonitrine A[11] was obtained as a pale yellow powder. The molecular formula $C_{21}H_{25}N_3O_3$, requiring 11 degrees of unsaturation, was established by HRMS. Its FTIR spectrum showed absorption bands consistent with a carbonyl group (1737 cm^{-1}) and an aromatic ring (1608, 1464 cm^{-1}). The ^{13}C NMR (Table 1) spectrum displayed 21 carbon resonances that

Table 1. NMR Data of Alistonitrine A in $CDCl_3$[a]

no.	δ_C mult		δ_H mult (J in Hz)	HMBC ($^1H-^{13}C$)
2	101.7	C		
3	69.6	CH	3.03, m	2, 14, 15, 21, NCH_3
5a	44.7	CH_2	3.15, brt (9.9)	2, 6, 7, 21
5b			2.86, ddd (6.9, 10.8, 12.0)	6, 21
6a	43.6	CH_2	2.50, dt (9.2, 12.7)	5, 8, 16
6b			2.06, dd (6.8, 13.6)	2, 16
7	53.5	C		
8	135.3	C		
9	122.5	CH	7.38, dd (0.9, 7.5)	7, 11, 13
10	118.9	CH	6.77, dt (0.9, 7.5)	8, 12
11	127.9	CH	7.06, dt (1.0, 7.5)	9, 13
12	109.5	CH	6.62, brd (7.5)	8, 10
13	146.4	C		
14a	35.5	CH_2	2.42, ddd (1.1, 5.1, 12.8)	2, 3, 15, 16,
14b			1.38, dd (4.8, 12.8)	3, 15, 16, 20
15	40.4	CH	1.78, brt (3.8)	3, 14, 16, 20, 21
16	51.4	CH	3.32, d (3.5)	2, 6, 7, 8, 14, 15, 17, 20
17	172.9	C		
18	13.5	CH_3	1.41, d (5.7)	19, 20
19	61.7	CH	3.63, q (5.7)	15, 18, 20
20	61.3	C		
21	81.5	CH	3.51, brs	2, 3, 15, 20, NCH_3
OCH_3	51.6	CH_3	3.80, s	17
NCH_3	42.5	CH_3	2.76, s	3, 21
NH			4.11, brs	

[a]600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR. Data were assigned based on the DEPT, HSQC, HMBC, $^1H-^1H$ COSY, and NOESY spectroscopic data.

were classified by DEPT and HSQC experiments as three methyls, three methylenes, nine methines, and six quaternary carbons. The integration of the 1D NMR and HMBC data (Table 1) allowed for the signals corresponding to a substituted dihydroindole ring [i.e., δ_C 146.4 (s, C-13), 135.3 (s, C-8), 127.9 (d, C-11), 122.5 (d, C-9), 118.9 (d, C-10), 109.5 (d, C-12), 101.7 (s, C-2), 53.5 (s, C-7); δ_H 7.38 (1H, dd, J = 0.9, 7.5 Hz, H-9), 7.06 (1H, dt, J = 1.0, 7.5 Hz, H-11), 6.77 (1H, dt, J = 0.9, 7.5 Hz, H-10), 6.62 (1H, brd, J = 7.5 Hz, H-12] to be readily assigned. The HMBC spectrum showed correlations between δ_H 3.32 (1H, d, J = 3.5 Hz, H-16) and the carbons of the dihydroindole skeleton (C-8, C-7, and C-2) that allowed for the assignment of the methine group (C-16) adjacent to the dihydroindole ring at C-7 (Figure 2, **1a**). The $^1H-^1H$ COSY cross signals of H-16/H-15, H-15/H-14, and H-14/H-3 suggested the presence of a $CHCHCH_2CH$ fragment corresponding to the $C_{16}-C_{15}-C_{14}-C_3$ unit. HMBC correlations from H-14 and H-3 to C-2 indicated that C-3 was linked to C-2 and that the $C_{16}-C_{15}-C_{14}-C_3$ unit was fused with the dihydroindole skeleton to form the C-ring (Figure 2, **1a**). Two downfield carbon shifts at δ_C 69.6 (C-3) and 81.5 (C-21) were

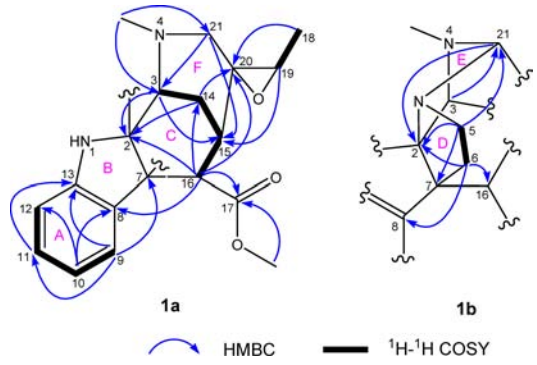

Figure 2. Key HMBC and $^1H-^1H$ COSY correlations of alistonitrine A.

attributed to the carbons connected to N-4, which was supported by HMBC correlations from the *N*-methyl protons (δ_H 2.76, 3H, s) to C-3 and C-21. By combining the observed HMBC correlations from H-21 to C-15 and C-20, and from H-14 and H-16 to C-20, we were able to deduce the structure of the F-ring (Figure 2, **1a**). The methyl ester group (δ_C 172.9, 51.6; δ_H 3.80) was assigned at C-16, based on an HMBC correlation between H-16 and C-17. The C-linkage of the $C_{18}-C_{19}-C_{20}$ unit was supported by HMBC correlations from H-18 to C-19 and C-20 and from H-19 to C-15 and C-20, as well as a COSY correlation between H-18 and H-19. The chemical shifts of H-19 at δ_H 3.63, C-19 at δ_C 61.7, and C-20 at δ_C 61.3 were similar to those of MIAs possessing an epoxy ring at C-19 and C-20[12] and, therefore, suggested that the remaining oxygen atom was connected to C-19 and C-20 to form an epoxy ring in alistonitrine A. Based on the above evidence, a partial structure **1a** was established as shown (Figure 2).

Besides signals corresponding to partial structure **1a**, a nitrogen atom and two methylenes were left to be assigned. First, $^1H-^1H$ COSY correlations between the remaining methylenes (H-5 at δ_H 3.15, 2.86 and H-6 at δ_H 2.5, 2.06) indicated the presence of a CH_2CH_2 fragment. Considering that alistonitrine A contained 11 degrees of unsaturation, two more rings were required in the structure and it was envisaged that the third nitrogen atom could be acting as a bridging atom between these two rings. Analysis of the ^1H and ^{13}C NMR data (Table 1) indicated that the third nitrogen atom was connected to C-2, C-5, and C-21, which was supported by the observed HMBC correlations from H-5 to C-2, C-7, and C-21 and from H-21 to C-2. Furthermore, HMBC correlations from H-6 to C-2, C-8, and C-16 indicated that C-6 was linked to C-7. These connections formed two five-membered rings (rings D and E) as shown in Figure 2, **1b**. These data suggested that alistonitrine A was an unusual MIA incorporating a third nitrogen atom with the unique 6/5/6/5/5/6 ring system (Figure 1).

The relative configuration of alistonitrine A was determined by a NOESY experiment (Figure 3A). Like echitamine,[1c,13] an MIA containing the same rings A,B,C,D as alistonitrine A, the C_6-C_7 bond and D-ring were assigned to be in a β-orientation, which is above the dihydroindole ring. The NOE correlation between the methyl ester group at C-17 and H-6b indicated that C-17 is in the same orientation (β) as C-6; thus, H-16 is α-oriented. Based on a Dreiding model, it was found that the rigid ring system of alistonitrine A only allows a β-orientation of the $C_{15}-C_{20}$ bond like the D-ring. Therefore, the H-15 should be α-oriented, which was supported by the coupling constant (J =

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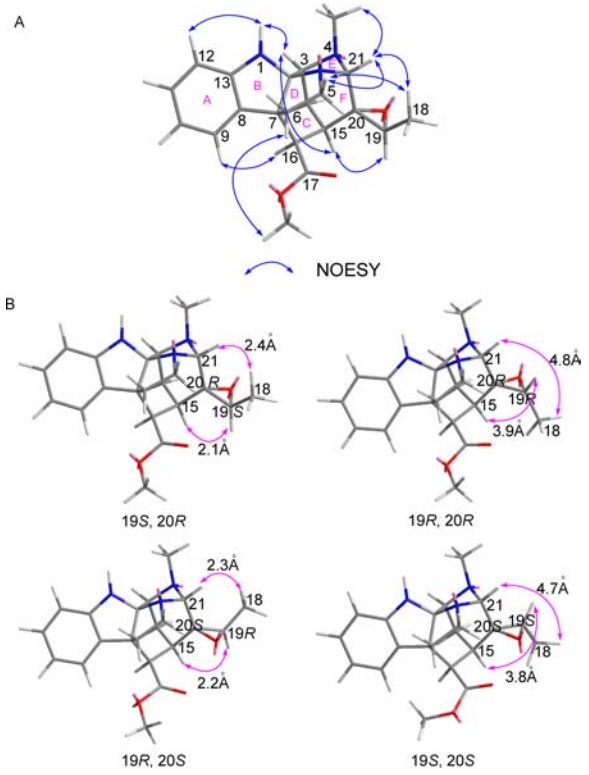


Figure 3. (A) Key NOESY correlations of alistonitrine A. (B) The atom distances for the candidate stereoisomers calculated by Chem3D.

3.5 Hz) of H-16 and H-15. NOE correlations of H-3/H-1, H-15/H-3 (weak), H-16/H-14b, and H-16/H-9 further confirmed that H-3, H-15, and H-16 are all α-oriented which are opposite to the D-ring. H-21 was assigned to be in a β-orientation by the NOE correlation between H-21 and H-5b. The α- or β-orientation of the epoxy ring at C-19/20 could not be determined by NOE correlations because of similarities in the spatial distances between H-15 and H-19 and between H-21 and H-18 (Figure 3B). To determine the absolute configuration of alistonitrine A, ECD curves for the four possible stereostructures (19*S*/20*R*; 19*S*/20*S*; 19*R*/20*S*; 19*R*/20*R*, Figure 3B) were calculated using the TD-DFT theory method.[14] As shown in Figure 4, the calculated curves of 19*S*/20*R* (blue) and 19*R*/20*R* (green) were in good agreement with the experimental CD spectrum (pink). Furthermore, NOE correlations of H-15/H-19, H-21/H-18, and H-18/H-5b supported the 19*S*/20*R*

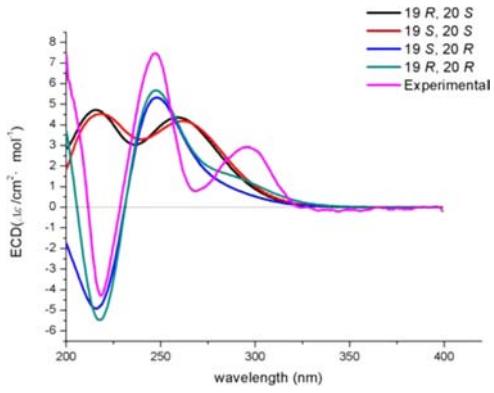
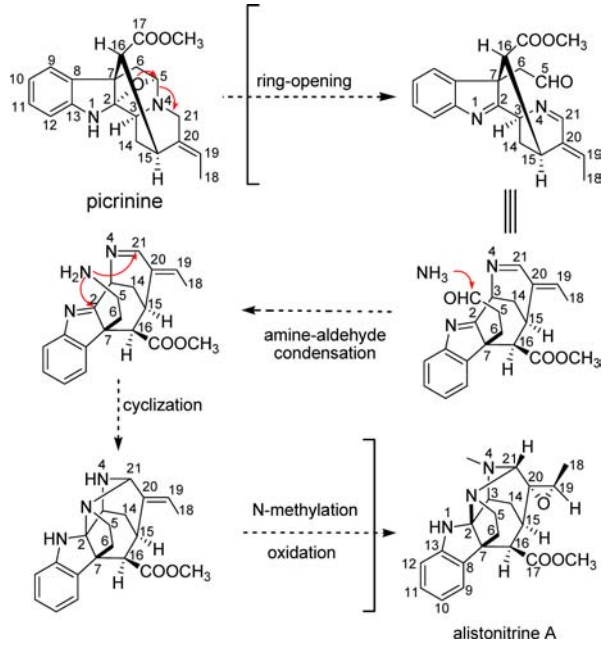


Figure 4. Experimental and calculated CD spectra of alistonitrine A and the candidate stereoisomers.

configuration (Figure 3). Thus, the absolute configurations of chiral carbons of alistonitrine A were established as 2*S*, 3*S*, 7*R*, 15*R*, 16*R*, 21*S*, 19*S*, 20*R*, in which C-7 keeps the same configuration with that of scholarisine A,[8] an MIA with a similar structure isolated from the same plant *A. scholaris*.

In contrast to the known MIAs, the skeleton of alistonitrine A incorporated a third nitrogen atom to form a unique pyrroloimidazole ring in its nonindole moiety. Biosynthetically, alistonitrine A could plausibly be derived from picrinine, which is a major component of *A. scholaris*.[7b] As shown in Scheme 1,

Scheme 1. Proposed Biogenetic Pathway for Alistonitrine A



the hexacyclic ring attached to the indole of picrinine could be opened via cleavage of the oxygen bridge and the C_5–N_4 bond. An aldehyde group at C-5 and two double bonds at N_1/C_2 and N_4/C_{21} could also be formed simultaneously. Ammonia could then react with the aldehyde to form a primary amine at C-5. This amine could then be linked at C-2 and C-21 to form a pyrroloimidazole ring though a Michael addition. Finally, N-4 and the C-19/20 double bond could be methylated and oxidized, respectively, to form alistonitrine A (Scheme 1).

We evaluated the effects of alistonitrine A on NF-κB and HIF-α activities and found that this compound was inactive in both of these models (Figures S2 and S3).

■ ASSOCIATED CONTENT

Supporting Information

Experimental details; UV, CD, IR, HRMS, 1D and 2D NMR spectra of alistonitrine A. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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(10) The leaves of *Alstonia scholaris* R. Br. were collected from Shenzhen of Guangdong Province, People's Republic of China, in August 2013. The species was identified by the author, Dr. Zhu G-Y. A voucher specimen (AS-201308) was deposited at the State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology.

(11) Pale yellow powder; $[\alpha]^{25}_{D}$ −15.2 (c = 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 245 (2.98), 294 (2.57) nm; IR (KBr) ν_{max} (cm^{-1}) 3430, 2953, 1737, 1608, 1464, 1382, 1348, 1261, 1167, 1134, 1029, 963, 900, 751, 614, 534; CD (0.0004 M, MeOH) λ_{max} ($\Delta\varepsilon$) 248 (−4.3), 247 (+7.4), 296 (+2.9) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS m/z 368.1970 $[M + H]^+$ (calcd for $C_{21}H_{26}N_3O_3$, 368.1969).

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