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Alstoscholarisines F and G, two unusual monoterpenoid indole alkaloids from the leaves of *Alstonia scholaris*

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ABSTRACT

Alstoscholarisine F (**1**), a monoterpenoid indole alkaloid pigment with unprecedented carbon skeleton, and alstoscholarisine G (**2**) incorporated with a third nitrogen atom were isolated from the long-term stored leaves of *Alstonia scholaris*. Their structures were established by extensive MS and NMR spectroscopic analysis and the absolute configuration of **1** was defined by comparison of experimental and calculated ECDs. Compounds **1** and **2** were subjected to hippocampal neuronal stem cells (NSCs) proliferation evaluation, but they did not show significant effect.

Plants of *Alstonia* (Apocynaceae) have been reported to be a rich source of monoterpenoid indole alkaloids (MIAs),[1] which originated from the condensation of tryptophan with secologanin.[2] MIAs are a group of structurally fascinating and synthetically challenging natural products and collectively exhibit a remarkably broad range of bioactivities such as tumor inhibitive, anti-asthmatic, and antidepressant.[3] The leaves of *A. scholaris* have been historically used in 'dai' ethnopharmacy to treat chronic respiratory diseases in Yunnan province, China.[4] A defined mixture of alkaloids from *A. scholaris* leaf,[5] registered as investigational new botanical drug (No. 2011L01436), has been approved for clinical trials (phase I and II) by China Food and Drug Administration (CFDA). Our previous chemical studies on this plant led to the isolation of a series of MIAs,[6] including alstoscholarisines A-E, five unprecedented MIAs with 6/5/6/6/6 fused-bridge rings activating neuronal stem cells (NSCs) proliferation significantly.[6e] In our continuous search for novel and bioactive MIAs from *A. scholaris*, alstoscholarisines F and G (**1** and **2**, Fig. 1), two new MIAs were isolated from the long-term stored leaves.[7] Compound **1** possessed an unprecedented carbon skeleton and may be biogenetically derived from isovallesiachotamine[8] via intricate transformation. It appeared yellow-green under visible light due to extended conjugation system in the molecule; hence, it may be considered as a MIA pigment. Compound **2**, incorporated with a third nitrogen atom, was the second example of MIAs with three nitrogen atoms from the same plant.[9] In this Letter, we report isolation, structural elucidation, and bioactivity in regulating hippocampal NSCs proliferation of the two compounds, though they showed subtle activity.

Alstoscholarisine F (**1**)[10] was obtained as a yellow-green gum. Its molecular formula $C_{18}H_{16}N_2O_2$ was established by ^{13}C NMR and HR-EIMS (*m/z* 292.1210, $[M]^+$) data. The UV spectrum showed maximal absorptions at 288, and 420 nm, indicating an extended conjugation system in the molecule, while the FTIR spectrum exhibited absorption bands due to conjugated carbonyls (1699 and 1655 cm^{-1}) and the aromatic ring (1594 and 1456 cm^{-1}).

The ^{13}C and DEPT NMR spectra of **1** presented 18 carbon signals of six quaternary carbons (conjugated carbonyls for a ketone at δ_C 201.1, an acylamino at δ_C 159.4), three methines (two olefinic carbons at δ_C 101.0 and 143.5), three methyls (N-Me at δ_C 38.5), and other six signals assignable to an *ortho*-substituted benzene moiety (δ_C 143.5, s, C-8; 158.1, s, C-13; 122.9, d, C-9; 127.0, d, C-10; 129.4, d, C-11; 122.8, d, C-12), which were in agreement with δ_H 7.54 (d, H-9), 7.34 (t, H-10), 7.44 (t, H-11), and 7.76 (d, H-12) in its ^1H NMR spectrum (Table 1).

In the HMBC spectrum, the correlation from δ_H 7.54 (H-9) to δ_C 60.8 (C-7), and from a singlet methyl at δ_H 1.28 (Me-6) to C-7, C-8, and a deshielded quaternary carbon at δ_C 175.8 (C-2) attributable to its imine form proposed a methylindole moiety (partial structure A, Fig. 2) in the molecule, which was unlike other intact MIAs.[6]

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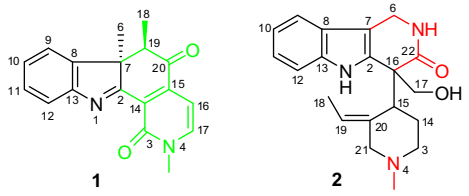


Figure 1. Structures of compounds **1** and **2**.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data (δ in ppm) of **1** and **2**

	1[a]		**2**[b]	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	175.8		135.3	
3	159.4		52.3	2.53, m
				2.12, m
6	24.3	1.28, s	40.2	4.90, d (15.0)
				4.80, d (15.0)
7	60.8		107.2	
8	143.5		125.8	
9	122.9	7.54, d (7.5)	118.1	7.63, d (7.5)
10	127.0	7.34, t (7.5)	119.5	7.23, t (7.5)
11	129.4	7.44, t (7.5)	121.9	7.26, t (7.5)
12	122.8	7.76, d (7.5)	112.0	7.57, d (7.5)
13	158.1		138.2	
14	122.2		28.4	2.40, br d (13.9)
				2.04, m
15	140.6		39.0	3.80, br d (6.4)
16	101.0	6.68, d (6.8)	54.4	
17	143.5	8.00, d (6.8)	66.1	4.98, d (10.5)
				4.65, d (10.5)
18	14.0	0.48, d (7.2)	13.8	1.46, d (6.8)
19	52.9	3.36, q (7.2)	126.2	5.50, q (6.8)
20	201.1		135.0	
21			62.5	3.10, d (12.0)
				2.91, d (12.0)
22			174.7	
N-CH_3	38.5	3.64, s	45.6	1.96, s
N_1-H				12.10, s
CON*H*				9.28, br s

[a] Recorded in acetone-d_6.
[b] Recorded in pyridine-d_5.

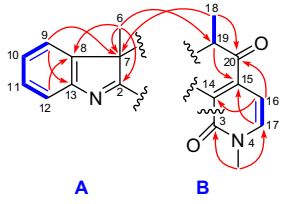


Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

The correlation of a methyl doublet at δ_H 0.48 (Me-18) with δ_H 3.36 (H-19) in the ^{1}H–^{1}H COSY spectrum, as well as the correlations of Me-18 with a carbonyl at δ_C 201.1 (C-20) in the HMBC spectrum suggested a fragment C-18/C-19/C-20. Furthermore, correlations of a methyl singlet at δ_H 3.64 (attributable to N4-Me with corresponding carbon at δ_C 38.5) with an acylamino carbon at δ_C 159.4 (C-3) and an olefinic carbon at δ_C 143.5 (C-17) in the HMBC spectrum, together with two mutual coupling protons at δ_H 8.00 (d, J = 6.8 Hz, H-17) and δ_H 6.68 (d, J = 6.8 Hz, H-16), established another fragment C-3/N-4/C-17/C-16. The remaining two signals at δ_C 122.2 (C-14) and 140.6 (C-15) were assigned to a pair of olefinic quaternary carbons, which combined the two fragments mentioned above indicated by the correlations from both H-19 and H-17 to C-15, and from H-16 to C-14 and C-20 in the HMBC spectrum. Therefore, a second partial structure B was elucidated as shown (Fig. 2).

The two partial structures A and B were linked by C-7/C-19 based on the key correlations of Me-6 with C-19, and of Me-18 with C-7 in the HMBC spectrum. Then, the remaining unique linkage of C-14/C-2/C-3 was finally established by the indices of hydrogen deficiency, which met the extended conjugation system observed in the UV spectrum. Hence, the planer structure of **1** was established to possess an unprecedented carbon skeleton. The relative configuration of **1** was defined by the obvious correlation of Me-6/H-19 observed in the ROESY spectrum, indicating the *trans*-substitutions of Me-6 and Me-18.

The absolute configuration of **1** was determined by the comparison of experimental and theoretical electronic circular dichroism (ECD) spectra.[11] Conformational analysis using molecular mechanics (MM) calculations was performed in the Discovery Studio 3.5 Client with MM force field of 10 kcal/mol upper energy limit. Using the Gaussian 09 software package, the selected conformers were optimized at the B3LYP/6-31G(d,p) level of theory. The theoretical calculation of ECD was performed using time-dependent density functional theory (TDDFT) at B3LYP/6-31G (d, p) level in MeOH with PCM model. As shown in Figure 3, the calculated ECD for 7*R*,19*R* of **1** matched with the experimental curve and determined its absolute configuration.

The plausible biosynthetic pathway of **1** from isovallesiachotamine[8] is also proposed in Scheme 1, which involves the key oxidation, Hoffmann degradation, oxidative cleavage of C-2/C-3, nucleophilic attack of C-7 to C-9, N-methylation, nucleophilic attack of C-14 to C-2, and dehydrogenation.

Alstoscholarisine G (**2**)[12] was assigned the molecular formula $C_{20}H_{25}N_3O_2$ on the basis of its ^{13}C NMR and HR-TOFMS (m/z 340.2022, $[M+H]^+$) data, containing three nitrogen atoms in the molecule. The UV spectrum exhibited maximal absorption of an indole chromophore (232 and 286 nm).[13] The ^{1}H and ^{13}C NMR spectral data of **2** (Table 1) were similar to those of 19*E*-vallesamine,[13] except that the signals for a methoxy (δ_C 53.0 and δ_H 3.66) in 19*E*-vallesamine were replaced by a *N*-methyl (δ_C 45.6 and δ_H 1.96) and an extra active hydrogen (δ_H 9.28, br s) in 2. In the HMBC spectrum of 2, the correlations of the *N*-methyl (δ_H 1.96) with δ_C 52.3 (C-3) and 62.5 (C-21) suggested that compound **2** was a derivative of 19*E*-vallesamine by methylation of N-4 and cleavage of C-6/N-4 bond. Furthermore, the correlations from the active hydrogen at δ_H 9.28 to δ_C 40.2 (C-6), 107.2 (C-7), 54.4 (C-16), and a carbonyl at δ_C 174.7 (C-22), combined with the existence of one extra nitrogen, proposed the presence of a lactam moiety in **2** (Fig. 4). Moreover, HMBC correlations of δ_H 4.90 and

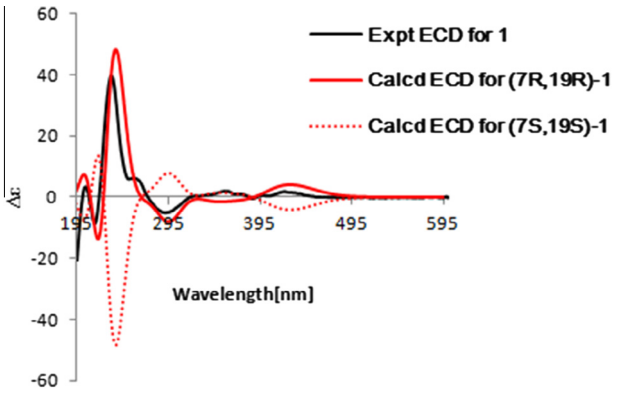
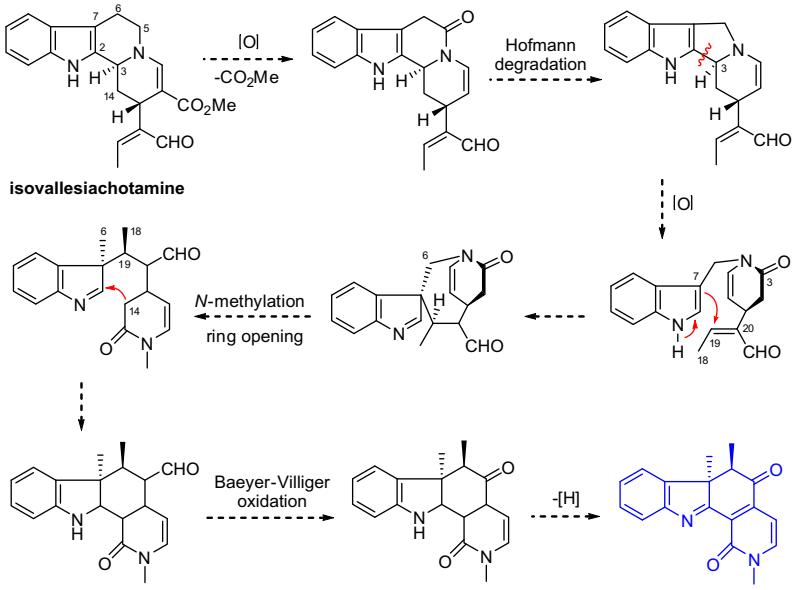


Figure 3. Calculated and experimental ECD spectra of **1**.



Scheme 1. Putative biosynthesis of **1** from isovallesiachotamine.

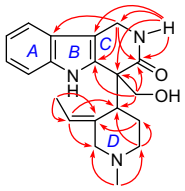
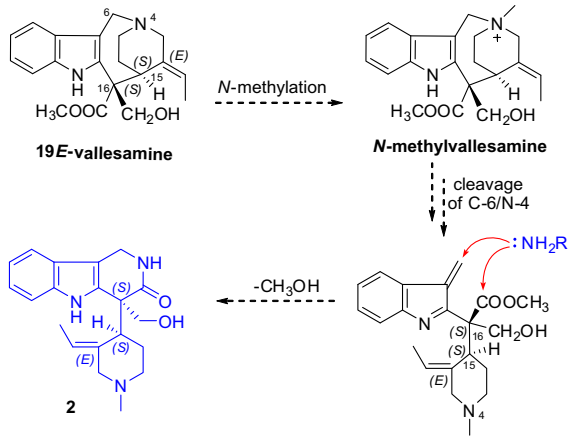


Figure 4. Key HMBC correlations of **2**.



Scheme 2. Putative biosynthesis of **2** from 19*E*-vallesamine.

4.80 (2H, H-6) with δ_C 135.3 (C-2), 125.8 (C-8), and of δ_H 4.98, 4.65 (2H, H-17) with C-2, C-16, and C-22 further supported the assumption and constructed an unusual six-membered lactam (ring-C). As shown in Scheme 2, compound **2** might be biogenetically derived from 19*E*-vallesamine, and then the chiral centers of C-15 and C-16 kept. The rest part of the structure of **2** was determined to be the same as that of 19*E*-vallesamine by the detailed analysis of 2D NMR spectroscopic data of **2** (Fig. 4). Thus, the structure of **2** was elucidated as shown in Figure 1.

The isolated alkaloids were evaluated for their bioactivities of regulating hippocampal NSC proliferation in vitro.[14,15] However, they did not enhance NSC proliferation significantly.

Acknowledgments

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Supplementary data

Supplementary data (computational details of **1** and original MS, 1D and 2D NMR spectra for **1** and **2**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.10.051.

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7. The leaves of *A. scholaris* (18 kg) were collected in Xishuangbanna, Yunnan Province, PR China, and were extracted with EtOH (40 L × 3) under reflux conditions, and the solvent was evaporated in vacuum. The residue was

dissolved in 0.37% HCl, and the solution was subsequently basified using ammonia water to pH 9–10. The basic solution was partitioned with EtOAc, affording a two-phase mixture including the aqueous phase and the EtOAc/organic phase. The organic fraction (total alkaloids, 180 g) was dissolved in MeOH, and the resulting solution was subjected to column chromatography over silica gel eluting with $CHCl_3$–MeOH (from 1:0 to 2:8) to afford six fractions (Fr. A–F). Fr. A (25 g) was further chromatographed using $CHCl_3$–Me_2CO (from 9:1 to 7:3) as eluent to give five fractions (Fr. A1–A5). Fr. A3 (1.6 g) was further purified by preparative HPLC (CH_3CN–H_2O, 2:1) to afford **1** (4 mg). Fr. E (6.3 g) was further chromatographed over silica gel and preparative HPLC (CH_3CN–H_2O, 1:4) to obtain **2** (6 mg).

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10. *Alstoscholarisine F* (**1**): yellow-green gum; $[\alpha]_D^{23}$ +454 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) = 288 (3.86), 420 (4.05) nm; IR (KBr) ν_{max} 2964, 2926, 1699, 1655, 1594, 1534, 1456, 1384, 1326, 1301, 1088, 746 cm^{-1}; CD (0.00034 M, MeOH) λ_{max} ($\Delta\varepsilon$) 210 (+3.1), 235 (+40.1), 260 (+5.6), 341 (−4.8) nm; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 315 $[M+Na]^+$; HREIMS *m*/*z* 292.1210 $[M]^+$ (calcd for $C_{18}H_{16}N_2O_2$, 292.1212).
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12. *Alstoscholarisine G* (**2**): colorless gum; $[\alpha]_D^{23}$ +11 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) = 204 (4.30), 232 (4.48), 286 (3.84) nm; IR (KBr) ν_{max} 3437, 2931, 1683, 1578, 1456, 1338, 1215, 1048, 741 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 340 $[M+H]^+$; HRTOFMS *m*/*z* 340.2022 $[M+H]^+$ (calcd for $C_{20}H_{26}N_3O_2$, 340.2025).
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14. *NSCs proliferation assay:* 1.8×10^4 adult mouse dentate gyrus neural stem cells (early passage cells between passage 4 and 10) were cultured in serum-free growth medium containing 20 ng/mL epidermal growth factor (EGF), 20 ng/mL basic fibroblast growth factor (bFGF), 1% penicillin/streptavidin, 1% N_2 supplement (Gibco), 1× B27 (Gibco) and 10 mg/mL heparin as previously described. Then the cells were trypsinized to single cells and seeded into each well of 96 well plates. After being grown for 24 h, different compounds in the same concentration of 10 μM were added to the cells of different wells. The plates were incubated at 37 °C for 48 h in 5% CO_2 incubator. NSCs proliferation rate was measured by SRB assay according to the standard protocol.
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