Phytochemistry Letters 31 (2019) 78–84

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Sceletorines A and B, two minor novel dimeric alkaloids of *Mesembryanthemum tortuosum* (synonym *Sceletium tortuosum*)

Hongquan Yin[a,b], Zulfiqar Ali[a,*], Yuanqing Ding[a], Yan-Hong Wang[a], Michael. J. Cunningham[a], Mohamed A. Ibrahim[a], Amar G. Chittiboyina[a], Wei Wang[d], Alvaro M. Viljoen[e], Ikhlas A. Khan[a,c,**]

[a] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS, 38677, USA
[b] Department of Applied Chemistry and Pharmacy, School of Chemical Engineering and Environment, Beijing Institute of Technology, Beijing, 100081, PR China
[c] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, MS, 38677, USA
[d] TCM and Ethnomedicine Innovation, Development Laboratory, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, Hunan 410208, PR China
[e] Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa

ARTICLE INFO

Keywords:
Mesembryanthemum tortuosum
Sceletium tortuosum
Sceletorine
Alkaloids
Absolute configuration
Structure elucidation
NMR

ABSTRACT

Sceletorines A and B, two unique alkaloids, were isolated for the first time from the aerial parts of *Mesembryanthemum tortuosum*. Their structures were determined by the extensive spectroscopic methods and their absolute configuration was established with ECD calculations. Plausible biosynthetic pathways for **1** and **2** are proposed and a relationship to the previously assumed artifact, channaine is also addressed.

1. Introduction

Mesembryanthemum tortuosum L. (synonym *Sceletium tortuosum* (L.) N.E.Br.), a succulent subshrub indigenous to South Africa, belongs to the family Mesembryanthemaceae (Smith et al., 1996). It is mainly distributed in the Karroid areas of the Cape region of South Africa and is locally referred to as “kanna” or “canna” (Khoi) and “kougoed” (Afrikaans) (Shikanga et al., 2012b). *M. tortuosum* is traditionally used to treat central nervous system (CNS) conditions, such as depression, stress, anxiety, and as a masticatory drug for the relief of thirst and hunger (Shikanga et al., 2012a,b). Recently, a ^{1}H NMR and UPLC–MS based metabolomic study, exploring chemotypic variation in *S. tortuosum* from two provinces in South Africa, was reported (Zhao et al., 2018). Alkaloids, commonly referred to as “mesembrine type alkaloids” (mesembrine, mesembrenone, mesembrenol and mesembranol), are recognized as the major chemical and bioactive constituents responsible for the psychoactive activities of the plant (Carpenter et al., 2016; Loria et al., 2014; Shikanga et al., 2012a, 2011, 2012b; Smith et al., 1996). As few alkaloids have been reported from this psychoactive plant species so far, we decided to investigate it further for its undiscovered phytoconstituents. The current phytochemical investigation of *M. tortuosum* resulted in the isolation and characterization of two novel alkaloids, sceletorine A (**1**) and sceletorine B (**2**), which are shown to have unusual carbon architecture (Fig. 1). Their structures and absolute configurations (ACs) were determined by the analysis of extensive spectroscopic data and electronic circular dichroism (ECD) calculations. Interestingly, compound **2** appears to be a precursor of channaine, which was reported as an artifact originating from the condensation of pair of *N*-desmethylmesembrenone molecules in 1978 by Jeffs et al. from *S. strictum* (Abou-Donia et al., 1978) and recently isolated and characterized on the basis of NMR from *M. tortuosum* (Veale et al., 2018). The possibility of compounds **1** and **2** to be the artifacts formed during acid-base extraction and/or isolation procedures was ruled out by observing their presence in the freshly prepared methanol extract of the plant by HPLC (see Supplementary file). Herein, the isolation, structure elucidation, stereochemical assignment, and plausible biosynthetic pathways of these alkaloids are presented.

* Corresponding author.
** Corresponding author at: National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS, 38677, USA.
E-mail addresses: zulfiqar@olemiss.edu (Z. Ali), ikhan@olemiss.edu (I.A. Khan).

https://doi.org/10.1016/j.phytol.2019.03.013
Received 12 September 2018; Received in revised form 14 February 2019; Accepted 14 March 2019
Available online 27 March 2019

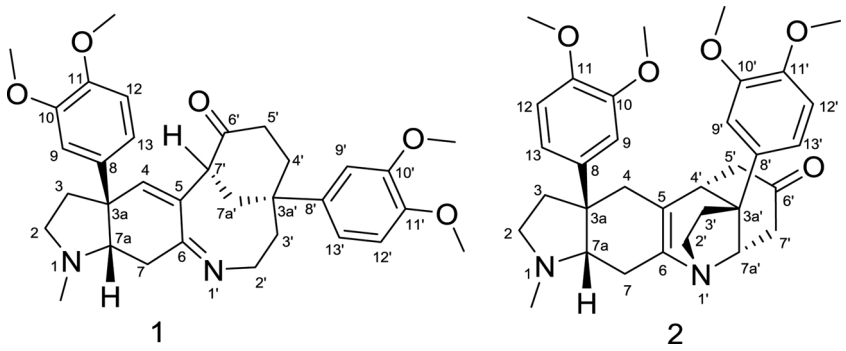


Fig. 1. Structures of compounds **1** and **2**.

2. Results and discussion

Sceletorine A (**1**) was isolated as colorless solid. The molecular formula of **1** was determined to be $C_{33}H_{40}N_2O_5$ by its ^{13}C NMR data and positive HRESIMS, which showed a $[M+H]^+$ ion peak at *m/z* 545.3026 (calcd for $C_{33}H_{41}N_2O_5$, 545.3015). The ^{13}C NMR and HSQC spectra analysis revealed that **1** possessed 33 carbons, including five methyls, eight methylenes, nine methines, and 11 nonprotonated carbons, out of which 15 were olefinic/aromatic carbons. The resonance at δ_C 211.3 was due to a carbonyl group. The NMR resonances (Table 1) at δ_H 2.32 (3H, s), and δ_C 39.8 were ascribed to a methyl group on the nitrogen atom. The resonances at δ_H 7.11 (1 H), 7.04 (1 H), 6.94 (1 H), 6.86 (3 H), 3.89 (s, 3 H), 3.93 (s, 9 H) and at δ_C 149.6, 148.9, 147.9, 147.7, 139.0, 135.8, 118.8, 118.7, 111.5, 111.1, 110.3, 110.0, 56.2, 56.0, 56.0, 55.9 in ^1H or ^{13}C NMR spectra suggested the presence of two 3, 4-dimethoxyphenyl groups, the typical aryl moiety for mesembrine type alkaloids (Bastida et al., 1989; Chavan et al., 2004), which was further supported by the detailed HMBC correlations (Fig. 2). The aforementioned resonances implied that **1** could be a dimeric alkaloid, which was confirmed by the HMBC correlations of H-7′ (δ_H 3.86) with C-4, C-5, C-6; H-7a′ (δ_H 2.76, dd, J = 6.9, 15.8 Hz) with C-5; H-2′ (δ_H 3.82 and 3.68, brd, J = 18.6 Hz) with C-6; and H-4 (δ_H 5.95, s) to C-7′. Determination of the relative configuration at each stereogenic center of **1** was achieved with NOESY experiment in benzene-d_6. The correlations

Table 1
$^1H^*$ and ^{13}C NMR spectroscopic data of **1** and **2** (δ in ppm).

No.	**1**			**2**	
	$\delta_C{}^a$, type	$\delta_H{}^a$ mult. (*J* in Hz)	$\delta_H{}^b$ mult. (*J* in Hz)	$\delta_C{}^c$	$\delta_H{}^c$ mult. (*J* in Hz)
N-Me	39.8, CH_3	2.32 s	2.25 s	40.0, CH_3	2.28 s
2	56.5, CH_2	2.51, 3.23 brt (8.0)	2.30 m, 3.05 brt (8.0)	53.5, CH_2	2.30 m, 3.16 td (4.8, 9.0)
3	40.1, CH_2	2.45, 2.08 ddd (8.0, 10.2, 13.9)	2.04, 2.20	38.6, CH_2	2.01
3a	51.0, C			48.2, C	
4	142.1, CH	5.95 s	6.23 s	38.0, CH_2	2.09, 2.58 d (15.0)
5	134.5, C			122.3, C	
6	164.8, C			138.9, C	
7	36.2, CH_2	2.51	2.62 brd (13.5)	29.9, CH_2	2.16
			2.79 dd (3.0, 13.5)		2.23 dd (6.3, 18.6)
7a	73.3, CH	2.52	2.53	68.4, CH	2.66 d (6.3)
8	139.0, C			139.6, C	
9	110.3, CH	6.86	6.96 d (2.3)	111.4, CH	6.99 brs
10	148.9, C			149.8, C	
10-OMe	56.0, CH_3	3.93	3.64 s	55.1, CH_3	3.86 s
11	147.7, C			148.1, C	
11-OMe	55.9, CH_3	3.89 s	3.49 s	55.3, CH_3	3.79 s
12	111.1, CH	6.86	6.66 d (8.5)	112.0, CH	6.91
13	118.8, CH	6.86	6.93	118.7, CH	6.91
2′	47.9, CH_2	3.68 brd (18.6)	3.74 m	54.0, CH_2	2.86
		3.82	3.97 dd (6.4, 17.8)		2.89
3′	45.5, CH_2	1.84	1.64 dd (6.4, 15.0)	40.9, CH_2	1.75 ddd (2.5, 8.3, 12.6)
			1.88 dd (10.0, 15.0)		1.93
3a′	43.3, C			46.2, C	
4′	38.3, CH_2	2.15, 2.28 brd (13.2)	2.02, 2.20	48.0, CH	2.46 brs
5′	31.8, CH_2	2.16, 2.41	2.09	40.8, CH_2	1.95, 2.15
6′	211.3, C			207.3, C	
7′	42.2, CH	3.86	3.55	39.9, CH_2	2.53 d (18.9)
					2.72 dd (8.3, 18.9)
7a′	42.6, CH_2	2.49, 2.76 dd (6.9, 15.8)	2.47	62.4, CH	3.84 dd (2.4, 8.3)
8′	135.8, C			137.4, C	
9′	110.0, CH	7.04 brs	6.78 d (2.3)	111.3, CH	6.92 d (2.4)
10′	149.6, C			149.1, C	
10′-OMe	56.2, CH_3	3.93	3.41 s	55.2, CH_3	3.80 s
11′	147.9, C			147.8, C	
11′-OMe	56.0, CH_3	3.93	3.47 s	55.5, CH_3	3.79 s
12′	111.5, CH	6.94 d (8.4)	6.63 d (8.4)	110.5, CH	6.91
13′	118.7, CH	7.11 brd (8.4)	6.70 dd (2.3, 8.0)	117.9, CH	6.85 dd (2.4, 8.4)

*Multiplicity not clear for some signals due to overlapping. Data were recorded in $^a CDCl_3$, $^b C_6D_6$, and $^c C_3D_6O$.

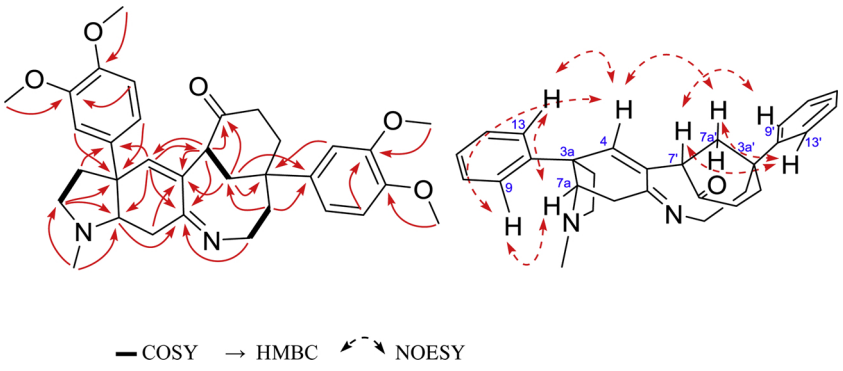


Fig. 2. Key COSY, HMBC, and NOESY correlations of **1**.

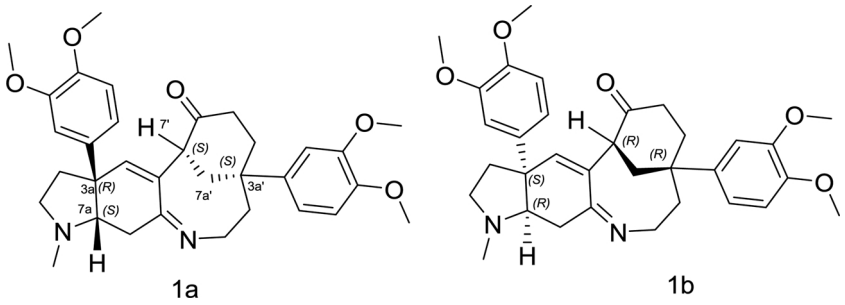


Fig. 3. Possible configurations of **1**.

between H-7a with H-9/H-13 are in agreement with mesembrine alkaloid type *cis* ring fusion. Whereas, NOESY correlations of H-7′ with H-9′/H-13′ and H-4 (olefinic) with H-7a′ and H-9/H-13 indicated that both aryl groups, H-7a, and H-7′ placed on same side of the plane, implying that the absolute configuration (AC) of compound **1** might be either 3a*R*,7a*S*,3a′*S*,7′*S*- or 3a*S*,7a*R*,3a′*R*,7′*R*- (Fig. 3).

Electronic circular dichroism (ECD) spectra were calculated to assign the exact AC of compound **1** (Ding et al., 2007, 2010; Li et al., 2015; Yang et al., 2014). Since the ECDs of enantiomers are completely opposite, only configuration **1a** was employed for the conformational random search with an energy window of 130 kJ/mol by using the OPLS_2005 force field in MacroModel software package of Schrodinger suite, yielding 320 conformers. Thirty-two conformers within an energy cut-off of 20 kJ/mol were submitted to the geometry optimization and harmonic vibrational frequency computation at the B3LYP/6-311 + + G** level in the gas phase. Twenty-one conformers were relocated and conformational analysis was performed using the electronic and zero-point energies obtained above (Table S1 and Fig. 4). The ECDs of individual conformers were calculated using time-dependent density functional theory (TDDFT) at the B3LYP-SCRF(COSMO)/6-311 + + G**//B3LYP/6-311 + + G** level in MeOH. The weighted ECD of **1a** convincingly matches the experimental curve of **1** (Fig. 5). Exampled by the predominant conformer **1a7** which possesses 35.5%, the experimentally observed positive Cotton Effect (CE) at 288 nm is mainly contributed by the electronic transition at 286 nm, whereas the negative CE at 241 nm is attributed to the transition states at 264, 257, and 234 nm, respectively, and that at 211 corresponds to the excitations at 217, 207, 204, 203 and 201 nm, respectively. Therefore, the AC of compound **1** was unambiguously assigned as 3a*R*,7a*S*,3a′*S*,7′*S* (**1a** in Fig. 3).

Sceletorine B (**2**) was obtained as a colorless solid. Its molecular formula, $C_{33}H_{40}N_2O_5$, was established by the positive HR-ESI-MS (*m/z* 545.3073 $[M+H]^+$, calcd. for $C_{33}H_{41}N_2O_5$, 545.3015) and ^{13}C NMR data. Analysis of ^1H NMR, ^{13}C NMR and HSQC spectra revealed that the 33 carbons in this compound are comprised of five methyls, eight methylenes, nine methines (six olefinic/aromatic and three aliphatic) and 11 nonprotonated carbons (one non-conjugated carbonyl, eight olefinic/aromatic and two aliphatic). Analysis of the $^1H/^{13}C$ NMR data (Table 1) demonstrated that **2** contained a non-conjugated carbonyl group (δ_C 207.3), *N*-methyl [δ_H/δ_C 2.28 (3H, s)/40.0], four methoxy groups [δ_H/δ_C 3.79 (6H, s)/55.3, 55.5, 3.80 (3H, s)/55.2, and 3.86 (3H, s)/55.1], and two 1,3,4-trisubstituted phenyl groups [δ_H/δ_C 6.99/111.4, 6.91/112.0, 6.91/118.7, 6.92/111.3, 6.91/110.5, and 6.85/117.9 and δ_C 139.6, 149.8, 148.1. 137.4, 149.1, 147.84]. The aforesaid data suggested that **2** is also a dimeric alkaloid, which was further supported by the HMBC correlations of H-2′ with C-6, H-4′ with C-5/C-6, H-5′ with C-5, and H-7a′ with C-6, as well as supported by the ^1H–^1H COSY correlations of H-2/H-3, H-7/H-7a, H-2′/H-3′, H-4′/H-5′, and H-7′/H-7a′ (Fig. 6). Thus, the planar structure of **2** was determined to be that shown in Fig. 1.

The relative configuration at the stereogenic centers in **2** was assigned by analysis of the NOESY correlation (Fig. 6). The correlations of H-7a with H-9/H-13 in the NOESY spectrum revealed that H-7a and phenyl group at 3a are on the same side, similar to mesembrine type alkaloids. The presence of similar correlations of H-4′ with H-9′/H-13′, and H-7a′ with H-9′/H-13′ and no correlation between H-4′ and H-7a′ verified that these two protons occupy equatorial-equatorial positions flanked by a cyclopentane and dimethoxy aryl group as shown in Fig. 6. Similar to compound **1**, ECD spectra were calculated to facilitate the AC assignment of compound **2**. Since configurations **2a** and **2c** are enantiomeric to **2b** and **2d**, respectively, only the ECDs of **2a** and **2c** were calculated using the previous protocols. Fifty-two of 242 conformers of **2a** and 48 of 264 conformers of **2c** within an energy cut-off of 10 kJ/mol were included in the ECD computations. Fifty-two conformers of **2a** and 42 conformers of **2c** were relocated at the B3LYP/6-311 + + G** level in the gas phase, both with very broad distribution (Tables S2 and S3). The weighted ECD spectrum of configuration **2a** overall matches the experimental ECD curve, whereas that of **2c** does not, even though noticeable redshift was found in the simulated ECD spectrum of **2a** for the experimental CEs at 235 and 253 nm (Fig. 7). The experimentally negative CEs at 205 and 235 nm were reproduced at 202 and 254 nm, respectively, whereas those positive CEs at 217 and 253 nm correspond to the simulated ones at 227 and 279 nm, respectively. Therefore, the AC of compound **2** was assigned as 3a*S*,7a*S*,3a′*R*,4′*R*,7a'*S* (**2a**) (Fig. 8).

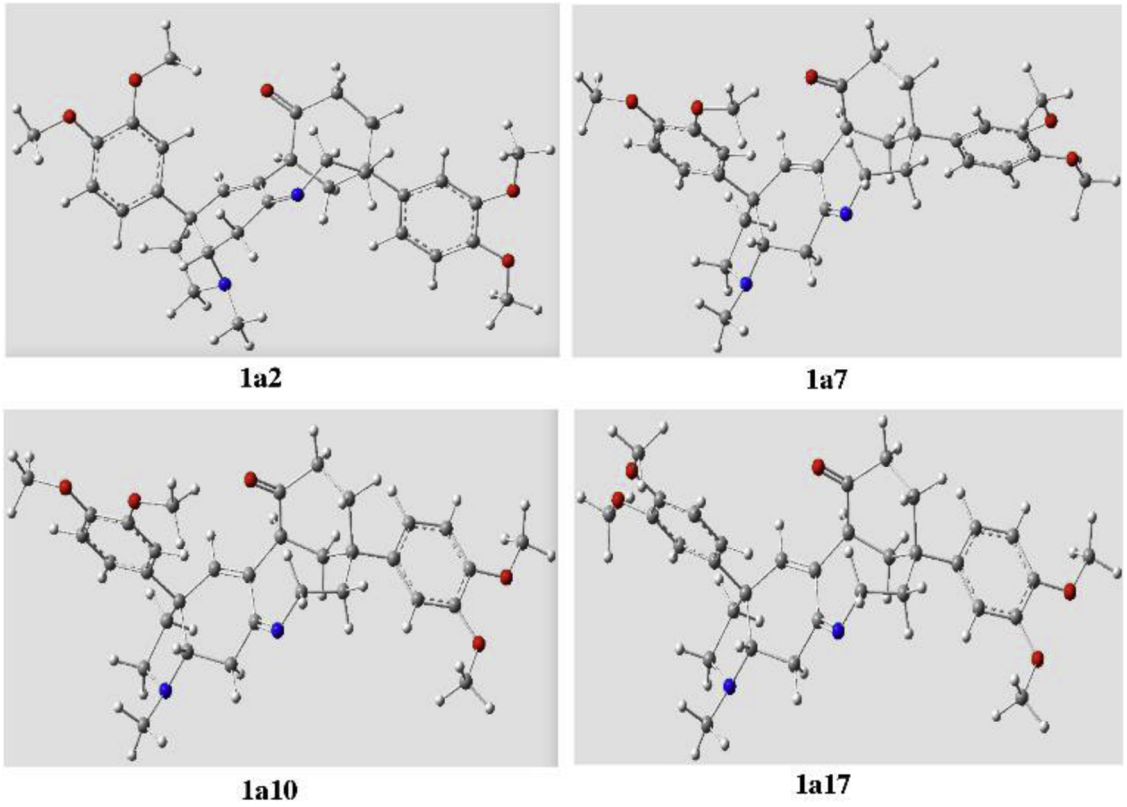


Fig. 4. Optimized geometries of predominant conformers of 3a*R*,7a*S*,3a′*S*,7′*S*- configuration of compound **1** at the B3LYP/6-311 + + G** level in the gas phase.

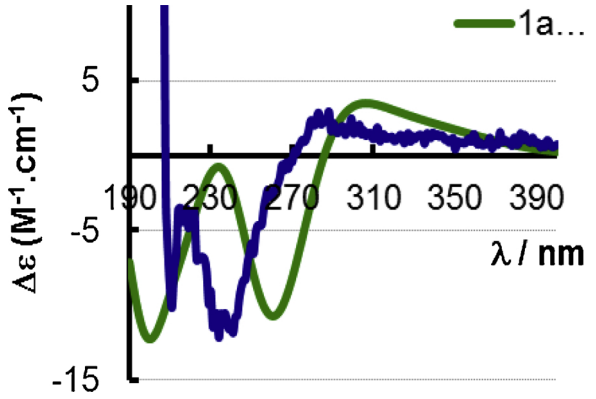


Fig. 5. Experimental (recorded in methanol) and weighted ECD spectra of **1**.

A plausible biogenetic route towards the unique dimer (**1**) formation may be initiated by oxidative keto-enamine coupling of mesembrine with a *N*-desmethylmesembrine unit(Guo et al., 2012) to yield a tetracyclic intermediate. A proton rearrangement followed by an allylic oxidation and a subsequent elimination of a water molecule results in compound **1** (Scheme 1). Similar to compound **1**, the pentacyclic compound **2** could be envisioned from intramolecular Michael addition of (desmethyl)mesembrine enolate to the *N*-demethylmesembrenone (Guo et al., 2012), *via* enamine/iminium intermediate. Further, possible P450 mediated allylic oxidation (when R=H), followed by a rearrangement of the hydroxyl group could yield amino-enone, aminol or hemiacetal, channaine (Scheme 1). Based on the results along with the proposed biogenesis for these dimeric alkaloids, it was speculated that the possible intermolecular condensation of monomeric mesembrine alkaloids as well as cytochrome P450 mediated secondary oxidations are plausible in *M. tortuosum*. Moreover, the optical rotation of these two dimeric alkaloids, conserved a stereopreference at the bicyclic bridge and their presence in freshly prepared extract indicate that these compounds are not artifacts of extraction/isolation procedures. Indeed, they are natural secondary metabolites of mesembrine-type mono alkaloids present in *M. tortuosum*. Nonetheless, the current findings highlight the complex chemistry of *M. tortuosum* wherein the major mono alkaloids are used as building blocks for the formation of novel, unique scaffolds.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured on an autopol IV polarimeter. CD spectra were recorded on an Olis DSM20 instrument. UV spectra were obtained on Varian Cary 50 Bio UV–vis spectrophotometer. IR spectra were detected on an Agilent Cary 630 FTIR spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker AVIII-400, AVIII-500 and AVIII-600 MHz spectrometers. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS spectra were determined on API QSTAR time-of-flight spectrometer and on Waters Autospec Premier P776 mass spectrometer. Semi-preparative HPLC analysis was conducted using an Agilent 1100 HPLC system equipped with a degasser, quaternary pump, auto sampler, column oven, and UV-Diode detector controlled by Chemstation software using RP-C18 column (250 × 10 mm; particle size 10 µm; Luna). HPLC grade acetonitrile and water were used as eluting solvents. Preparative TLC was performed on Sigma-Aldrich and Analtech uniplate silica gel plates (20 cm × 20 cm, 500 µm), and column chromatography was carried out using silica gel (230–400 mesh, Silicycle Co., Ltd., Canada). Fractions were monitored by TLC (aluminum-backed cards, pre-coated with silica gel F254 (200 µm, 60 Å, Merck)) and spots were visualized by Dragendorff's and vanillin staining solutions.

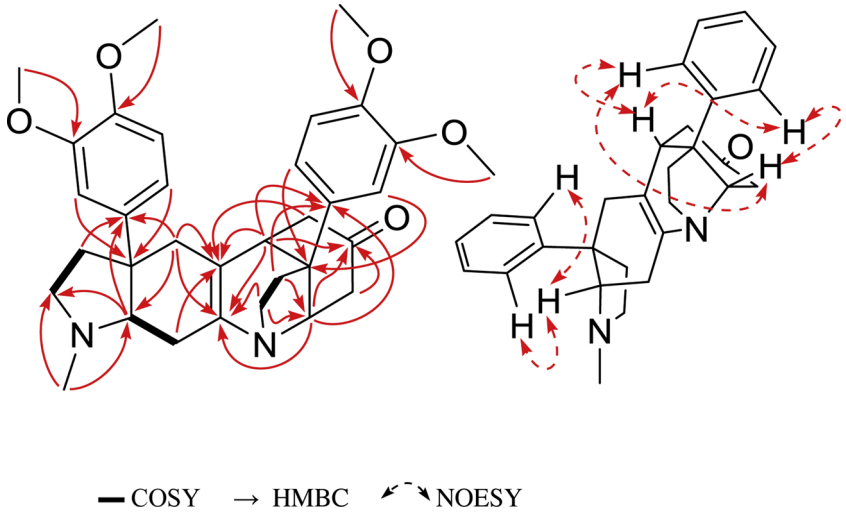


Fig. 6. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of **2**.

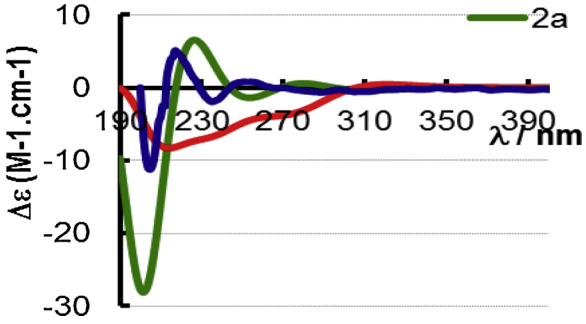


Fig. 7. Experimental (recorded in methanol) and weighted ECD spectra of **2**.

3.2. Plant material

The aerial parts of *M. tortuosum* were collected (2012) from cultivated material, Farm Vredelus, Uhlenhorst (Namibia). Retention samples are retained in the Department of Pharmaceutical Sciences, Tshwane University of Technology (Pretoria, South Africa). The sample (#13120) was also deposited at the National Center for Natural Products Research, University of Mississippi, USA.

3.3. Extraction and isolation

Powdered aerial parts of *M. tortuosum* (1.5 kg) were extracted with methanol (4 L × 24 h × 4). The combined extracts were concentrated under reduced pressure to afford methanol extracts (256.1 g). The major part of extract (225.5 g) was suspended in 5% HCl in water (1.3 L) and extracted with EtOAc (1.3 L × 3). The water soluble part was then basified (pH = 9–10) with NH_4OH solution and again extracted with EtOAc (1.7 L × 5) to afford alkaloid extract (40.8 g). The major part (32.2 g) of the alkaloid extract was chromatographed on a silica gel column, eluted with $CHCl_3$ – CH_3OH–NH_4OH (from 100:0:0 to 0:100:0.3), to yield 62 fractions. Fraction 28–32 (3.1 g) was separated over a silica gel column, eluted with $CHCl_3$, $CHCl_3$ – CH_3OH–NH_4OH (300:7.5:1) and (2000:100:7), to afford 37 subfractions. Compound **1** (1.7 mg) was obtained from subfractions 12–15 by preparative TLC.

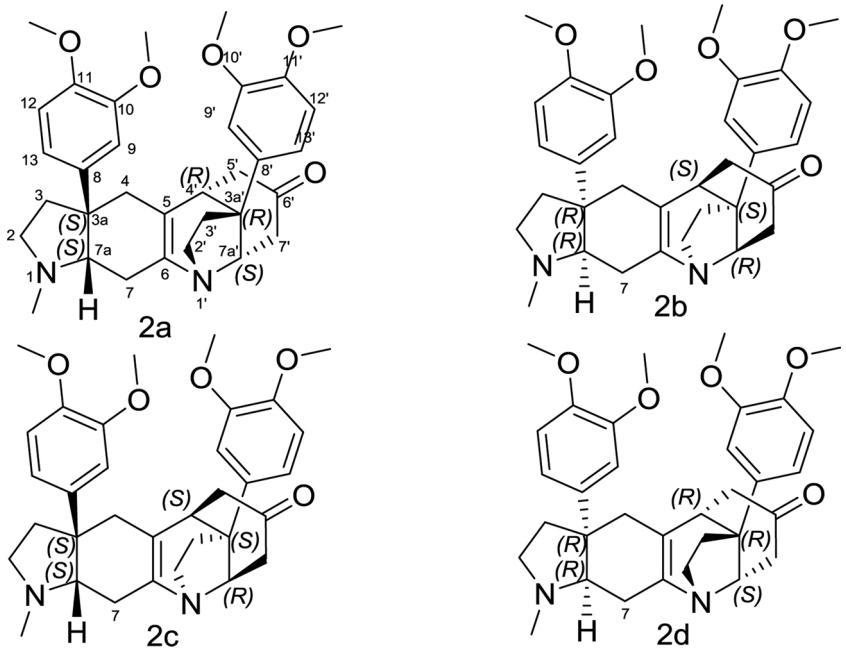
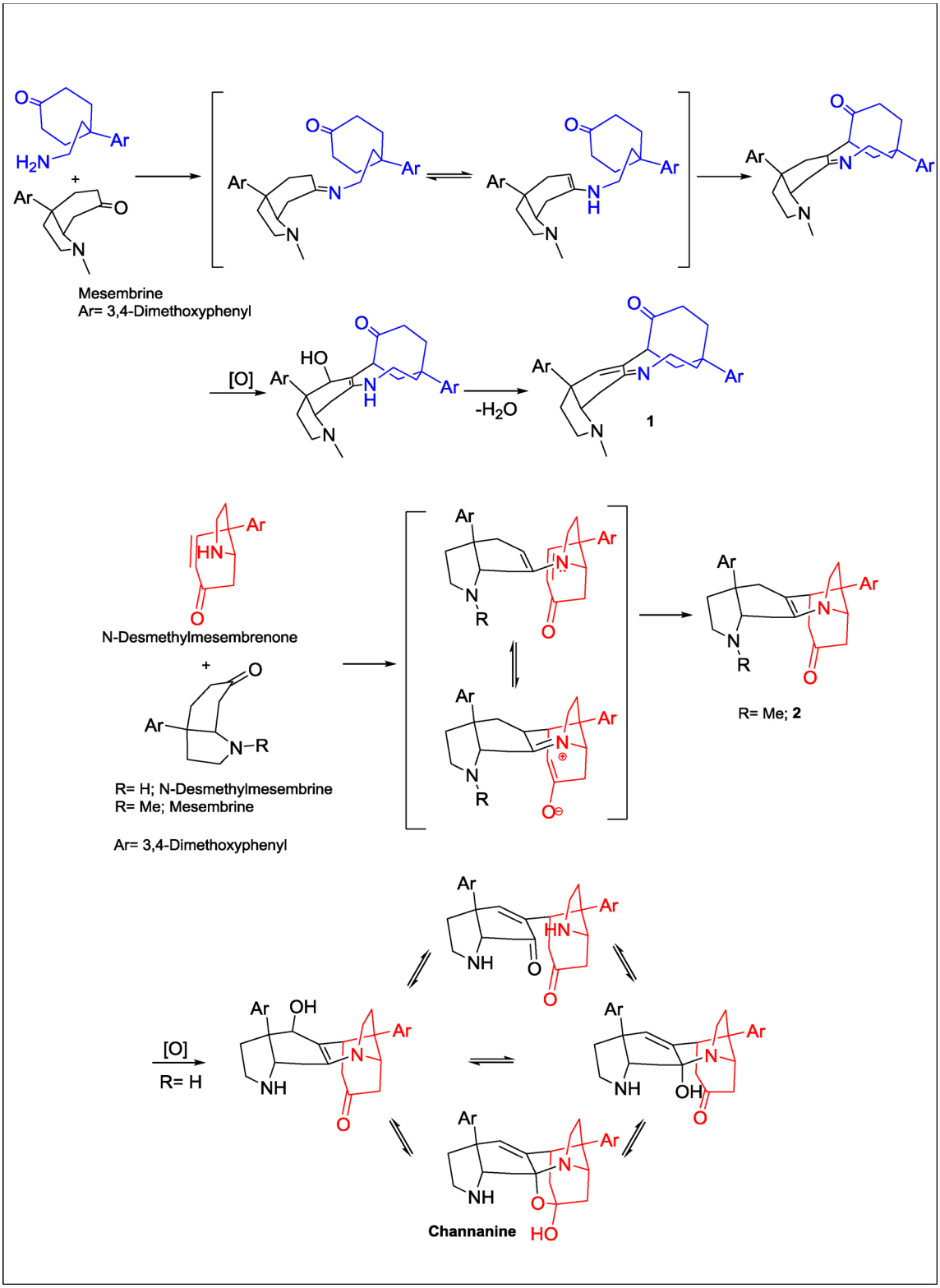


Fig. 8. Possible configurations of compound **2**.



Scheme 1. Plausible biogenetic pathway for **1**, channaine and its precursor **2**.

Compound **2** (3.5 mg) was isolated from subfraction 21 by semi preparative reversed phase HPLC. The HPLC analysis was carried out on RP-C18 column (250 × 10 mm; particle size 10 μm) with column oven temperature set at 25 °C and using gradients of eluent A (water with 0.1% acetic acid) and B (acetonitrile with 0.1% acetic acid). The gradient condition was as follows: 0–2 min (5% B), 2–32 min (5% B → 40% B), 32–33 min (40% B → 100% B), 33–37 min (100% B). The flow rate of the solvent was 4.0 mL/min and the injection volume was 100 μL. All the analysis was carried out at wavelengths of 257 nm.

3.3.1. Sceletorine A (1)

$C_{33}H_{40}N_2O_5$; Colorless solid; $[\alpha]_D^{26}$ +320 (*c* 0.05, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 279.0 (3.59) nm; IR (neat) υ_{max} 2940, 1740, 1694, 1631, 1595, 1522, 1466, 13.72, 1260, 1154, 1059, 1031, 919,

858, 808, 774 cm^{-1}; HRESIMS *m/z* 545.3026 $[M+H]^+$ (calcd for $C_{33}H_{41}N_2O_5$ 545.3015); ^1H NMR and ^{13}C NMR data: see Table 1.

3.3.2. Sceletorine B (2)

$C_{33}H_{40}N_2O_5$; colorless solid; $[\alpha]_D^{26}$ +60 (*c* 0.02, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 279.1 (3.30) nm; IR (neat) υ_{max} 2951, 1706, 1695, 1634, 1522, 1455, 1422, 1388, 1260, 1154, 1031, 830, 769 cm^{-1}; HRESIMS: *m/z* 545.3073 $[M+H]^+$ (calcd for $C_{33}H_{41}N_2O_5$, 545.3015); ^1H NMR and ^{13}C NMR data: see Table 1.

Acknowledgments

This work was supported by the Food and Drug Administration "Science Based Authentication of Dietary Supplements" [grant number 2U01FD004246-06]. FDA Disclaimer: The findings and conclusions in this report are those of the authors only and do not represent FDA positions or policies. Thanks to China Scholarship Council for financial support to Dr. Yin. The authors are thankful to Dr. Raman for keeping record of the plant material and Dr. Avula for providing the mass spectral data.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.03.013.

References

Abou-Donia, A., Jeffs, P.W., McPhail, A.T., Miller, R.W., 1978. X-ray crystal and molecular structure of channaine, an unusual alkaloid, probably an artefact from *Sceletium strictum*. J. Chem. Soc. Chem. Commun. 1078–1079.

Bastida, J., Viladomat, F., Llabres, J.M., Ramirez, G., Codina, C., Rubiralta, M., 1989. Narcissus alkaloids. VIII. Mesembrenone: an unexpected alkaloid from *Narcissus pallidulus*. J. Nat. Prod. 52, 478–480.

Carpenter, J.M., Jourdan, M.K., Fountain, E.M., Ali, Z., Abe, N., Khan, I.A., Sufka, K.J., 2016. The effects of *Sceletium tortuosum*(L.) NE Br. extract fraction in the chick anxiety-depression model. J. Ethnopharmacol. 193, 329–332.

Chavan, S.P., Khobragade, D.A., Pathak, A.B., Kalkote, U.R., 2004. A simple and efficient synthesis of (±)-mesembrine. Tetrahedron Lett. 45, 5263–5265.

Ding, Y., Li, X.-C., Ferreira, D., 2007. Theoretical calculation of electronic circular dichroism of the rotationally restricted 3,8″-biflavonoid morelloflavone. J. Org. Chem. 72, 9010–9017.

Ding, Y., Li, X., Ferreira, D., 2010. 4-Arylflavan-3-ols as proanthocyanidin models: absolute configuration via density functional calculation of electronic circular dichroism. J. Nat. Prod. 73, 435–440.

Guo, F., Clift, M.D., Thomson, R.J., 2012. Oxidative coupling of enolates, enol silanes, and enamines: Methods and natural product synthesis. Eur. J. Org. Chem. 2012, 4881–4896.

Li, C.-S., Ding, Y., Yang, B.-J., Miklossy, G., Yin, H.-Q., Walker, L.A., Turkson, J., Cao, S., 2015. A new metabolite with a unique 4-pyranone-γ-lactam-1,4-triazine moiety from a Hawaiian plant-associated fungus. Org. Lett. 17, 3556–3559.

Loria, M.J., Ali, Z., Abe, N., Khan, I.A., Sufka, K.J., 2014. Effects of *Sceletium tortuosum* in rats. J. Ethnopharmacol. 155, 731–735.

Shikanga, E.A., Viljoen, A., Combrinck, S., Marston, A., 2011. Isolation of *Sceletium* alkaloids by high-speed countercurrent chromatography. Phytochem. Lett. 4, 190–193.

Shikanga, E.A., Kamatou, G.P.P., Chen, W., Combrinck, S., Viljoen, A.M., 2012a. Validated RP-UHPLC PDA and GC–MS methods for the analysis of psychoactive alkaloids in *Sceletium tortuosum*. S. Afr. J. Bot. 82, 99–107.

Shikanga, E.A., Viljoen, A.M., Combrinck, S., Marston, A., Gericke, N., 2012b. The chemotypic variation of *Sceletium tortuosum* alkaloids and commercial product formulations. Biochem. Syst. Ecol. 44, 364–373.

Smith, M.T., Crouch, N.R., Gericke, N., Hirst, M., 1996. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: a review. J. Ethnopharmacol. 50, 119–130.

Veale, C.G., Chen, W., Chaudhary, S., Kituyi, S.N., Isaacs, M., Hoppe, H., Edkins, A.L., Combrinck, S., Mehari, B., Viljoen, A., 2018. NMR structural elucidation of channaine, an unusual alkaloid from *Sceletium tortuosum*. Phytochem. Lett. 23, 189–193.

Yang, X.-W., Ding, Y., Zhang, J.-J., Liu, X., Yang, L.-X., Li, X.-N., Ferreira, D., Walker, L.A., Xu, G., 2014. New acylphloroglucinol derivatives with diverse architectures from *Hypericum henryi*. Org. Lett. 16, 2434–2437.

Zhao, J., Khan, I.A., Combrinck, S., Sandasi, M., Chen, W., Viljoen, A.M., 2018. ^1H-NMR and UPLC–MS metabolomics: functional tools for exploring chemotypic variation in *Sceletium tortuosum* from two provinces in South Africa. Phytochemistry 152, 191–203.