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NMR structural elucidation of channaine, an unusual alkaloid from *Sceletium tortuosum*

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ABSTRACT

Chemical interrogation of the *Sceletium* genus and Amaryllidaceae family of plants has yielded a diverse array of aryl-hydroindole containing alkaloids. Included in this class is channaine, which was tentatively identified, without comprehensive structural elucidation from *Sceletium tortuosum* in 1957. Following its isolation from *S. strictum*, the structure of channaine was eventually resolved by X-ray crystallographic analysis, which revealed an unusual cage-like ring structure at the interface of two aryl-hydroindole subunits. However, since this report in 1978, channaine has not re-appeared in the literature. In this letter, the full NMR characterisation of channaine, isolated from *S. tortuosum* collected from St Helena in the Western Cape Province of South Africa, is reported for the first time.

1. Introduction

The genus *Sceletium*, which is endemic to South Africa, has garnered significant interest as a plant for ethnopharmacological enquiry (Gericke and Viljoen, 2008; Harvey et al., 2011). Numerous related alkaloids have been isolated and characterised from the genus *Sceletium*, and from the Amaryllidaceae family, which is richly represented in South Africa (Fig. 1). These alkaloids have complex core structures and their diverse biological activities have resulted in innumerable studies into their chemical synthesis and biosynthesis (Das et al., 2015; Denmark and Marcin, 1997; Jeffs, 1981; Jin, 2016, 2013, 2009, 2007, 2005). The most prominent alkaloids of this class, including mesembrenone (**1**) and mesembrine (**2**) feature a distinct 3 ring aryl-hydroindole scaffold. However, several others, including the unnamed alkaloid (**3**) and sceletium alkaloid A-4 (**4**), feature an additional fourth ring attached to the parent scaffold. Furthermore, more diverse alkaloids of this class such as maritidine (**5**) and crinine (**6**) feature a fused azabicyclooctene ring, formed through a bond between the hydroindole nitrogen and position 6 of the respective aryl rings. Further diversity in the ring systems is observed in the unusual gracilamine (**7**), which features an esterified methyl leucine residue at the 6 position of the aryl ring, which in turn, forms covalent bonds with 2 positions on the hydroindole to form a complex 6-ring system.

In 1957, Bodendorf and Krieger (1957) reported the isolation of a new alkaloid from *Sceletium tortuosum*, with an empirical formula of $C_{16}H_{19/21}NO_3$, of which the infrared spectrum contained both NH and OH functional groups, in the absence of corresponding carbonyl bands. This compound was assigned the trivial name channaine (**8**). This was followed by a review article by Popelak and Lettenbauer (1967), who had determined that channaine contained two veratrole rings, and was likely a dimer of two $C_{16}H_{19}NO_3$ subunits. They also reported that channaine was racemic in nature.

Acknowledging that a dimer of this nature would likely result in the characterisation of a new ring system for this class, Jeffs and McPhail were interested in identifying channaine in their thorough exploration of *S. namaquense* (Capps et al., 1977; Jeffs et al., 1971). Although initially unsuccessful, Jeffs and McPhail did manage to isolate an alkaloid from *S. strictum* with spectral data and physical properties that matched

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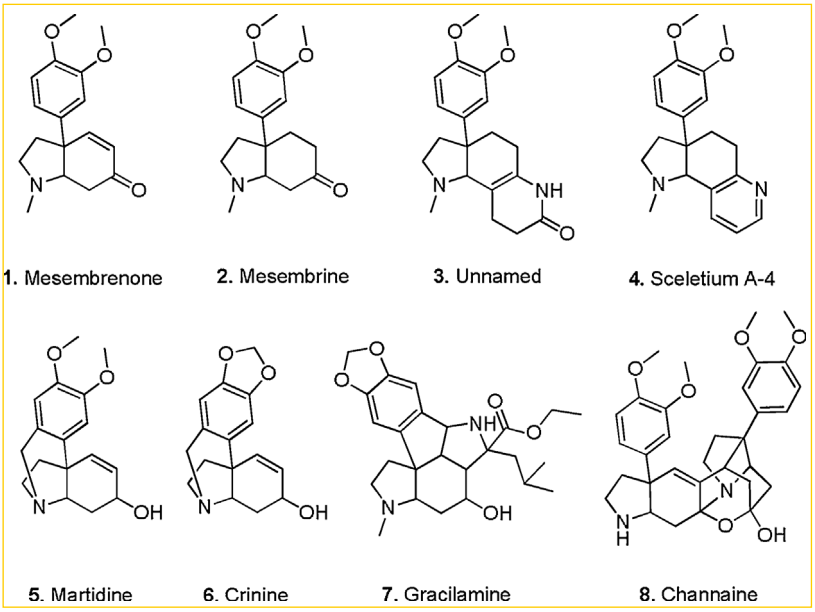

Fig. 1. Several related alkaloids, belonging to different classes, isolated from various species of the genus *Sceletium* or from the Amaryllidaceae family.

closely with those previously reported for channaine (Abou-Donia et al., 1978). However, owing to the lack of optical purity, they initially proposed that channaine is an artefact, resulting from the condensation of two putative *N*-demethylmesembrenone subunits (which is yet to be isolated from a natural source), under either the acidic or basic conditions provided during the extraction process. However, following the synthesis of *N*-demethylmesembrenone, Jeffs et al. (1983), were unsuccessful in attempts to induce dimerization under either acidic or basic conditions, leading them to tentatively conclude that channaine is a natural product (Jeffs et al., 1983). Furthermore, mesembrenone, mesembranol and sceletium alkaloid A-4 have all been isolated as racemic mixtures (Snyckeres et al., 1971).

While in their initial report, the authors (Abou-Donia et al., 1978) confidently assigned the structure of channaine through X-ray crystallography (Fig. 2), their NMR analysis only permitted the assignment of the proton NMR signals to the veratrole subunits, as well as the olefinic methine. Since then, no further reports regarding channaine appeared in the literature. Accordingly, in this letter we report the first full NMR characterisation of channaine.

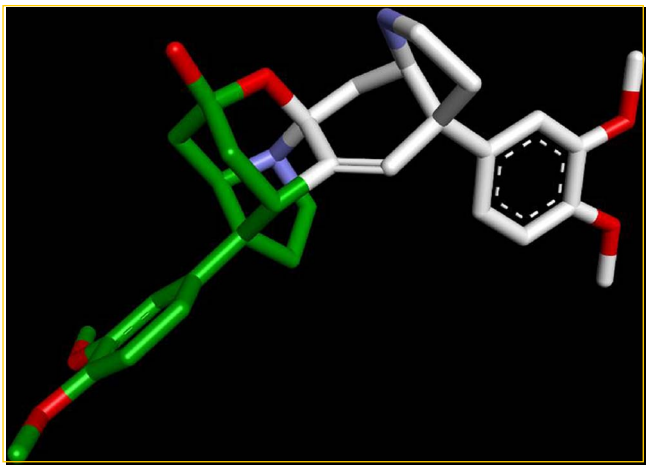

Fig. 2. X-ray crystal structure of channaine (8, CCDC identification number 1124320, Abou-Donia et al., 1978) Highlighted in white and green are the respective arylhydroindole subunits of a putative *N*-demethylmesembrenone, the interface of which forms an unusual cage-like structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Results and discussion

2.1. Structural elucidation

Following an acid-base extraction of *S. tortuosum*, UPLC–MS analysis identified a molecular ion at *m/z* 547.2813 $[M+H]^+$ which correlated, with a deconvoluted molecular formula, to that of channaine ($C_{32}H_{38}N_2O_6$). Following purification, initial analysis of the ^{13}C NMR spectrum, in conjunction with the ^1H and HSQC NMR spectra, revealed the presence of 11 quaternary carbons, seven methylene carbons (CH_2, each bound to diastereotopic protons), 10 methine signals (CH) and four methoxy signals, while the proton spectrum ($CDCl_3$) displayed four aromatic multiplets, (δ_H 6.94–6.92, 2H; 6.87–6.85, 2H; 6.84–6.82, 1H; 6.80–6.79, 1H) integrating for a total of six aromatic protons. Furthermore, four proton signals in the methoxy region of the spectrum (δ_H 3.89, 3.88, 3.86, 3.84) each integrating for three protons, were present, thus suggesting the presence of two possible veratrole rings.

Corresponding HMBC correlations between the proton resonances at δ_H 6.80–6.79 and δ_H 3.88 with a quaternary carbon at δ_C 147.6 allowed us to assign a specific relationship between these three positions. Similarly, the same aromatic proton resonance (δ_H 6.80–6.79) and the methoxy proton resonance at δ_H 3.89, both correlated to a quaternary carbon at δ_C 149.2, which allowed us to establish that those specific methoxy groups were attached to the same ring system, which were subsequently designated as the aromatic B-ring (Table 1; Fig. 3). Similarly, the multiplet at δ_H 6.84–6.82 and the methoxy at δ_H 3.84 correlated to a quaternary carbon at δ_C 149.1, while the aromatic proton resonances at δ_H 6.84–6.82 and δ_H 6.94–6.92, as well as the methoxy residue at δ_H 3.86 correlated with the quaternary carbon at δ_C 148.2. This allowed us to correlate all these signals to the same ring, which we designated as ring A. Finally, HMBC correlations between the A-ring protons, and an aromatic quaternary carbon (δ_C 133.6), allowed us to assign the final position on the ring, while an HMBC correlation between the B-ring proton resonance (δ_H 6.80–6.79) as well as the outstanding proton resonance at δ_H 6.87–6.85, with an aromatic quaternary carbon at δ_C 134.5, allowed the outstanding aromatic residues of the B-ring to be assigned. NMR data was also collected in CD_3OD, and showed the same correlations. However, the methoxy and aromatic regions were more condensed and we were unable to unambiguously assign some of these positions (Table 1). The points of attachment of the A- and B-rings were assigned as δ_C 50.3 (C-4) and δ_C 48.9 (C-4′), respectively, due to HMBC correlations between the correlating aromatic

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for Channaine (1).

	$CDCl_3$				CD_3OD			
C/H.	δ_H [ppm]	mult	*J* (Hz)	δ_C [ppm]	δ_H [ppm]	mult	*J* (Hz)	δ_C [ppm]
1								
2	3.45	t	9.9	44.5	3.39–3.34	m		45.5
2	3.29–3.23	m			3.27	dt	11.2, 7.3	
3	2.52	dd	11.8, 10.9	41.2	2.55	dd	11.2, 10.1	42.0
	2.15–2.09	m			2.24	dd	6.8, 6.1	
4				50.3				51.3
5	5.68	s		123.4	5.92	s		125.7
6				142.9				142.9
7				86.3				87.5
8	2.47	d	15.5	29.8	2.08	dd	15.8, 2.0	31.3
	1.78	dd	15.5, 2.2		1.87–1.82	m		
9	3.84[a]	m		63.4	3.85 – 3.83	m		64.5
1′								
2′	2.89	td	11.7, 5.6	47.2	2.91	td	11.1, 5.6	48.1
	2.62	qd	8.6, 4.4		2.62	qd	8.8, 4.1	
3′	2.24–2.19	m		41.9	2.36–2.31	m		42.4
	1.85	td	11.5, 2.9		1.89	dd	11.5, 4.0	
4′				48.9				50.2
5′	3.77–3.74	m		65.2	3.89–3.86	m		66.02
6′	2.38	dt	12.9, 3.3	33.8	2.36–2.31	m		34.7
	1.99	dd	12.9, 2.4		2.04–1.99	m		
7′				95.1				95.9
8′	2.15–2.09	m		41.0	2.04–1.99	m		41.3
	1.96–1.92	m			1.87–1.82	m		
9′	3.29–3.23	m		47.3	3.46–3.43	m		47.9
1”				133.6				135.0
2”	6.84–6.82	m		111.3[d]	7.08–7.03	m		113.1[l]
3”				149.1[e]				150.7[m]
4”				148.2[e]				149.9[m]
5”	6.94–6.92	m		118.9[f]	7.00–6.98	m		120.4[l]
6”	6.94–6.92	m		109.7[f]	7.08–7.03	m		111.7[l]
OMe	3.86[b]	s		56.0[g]	3.87[j]	s		56.7[n]
OMe	3.84[b]	s		55.9[g]	3.84[j]	s		56.4[n]
1‴				134.5				135.9
2‴	6.80–6.79	m		108.9	7.00–6.98	m		111.2[l]
3‴				149.2[i]				150.9[p]
4‴				147.6[i]				149.3[p]
5‴	6.87–6.85	m		111.2 [d,e]	7.00–6.98	m		113.1[l]
6‴	6.87–6.85	m		117.9[e]	7.03–7.01	m		119.4[l]
OMe	3.89[c]	s		55.9[h]	3.84[k]	s		56.6[o]
OMe	3.88[c]	s		55.9[h]	3.83[k]	s		56.4[o]

[a] Assigned by HSQC.
[b–p] Positions interchangeable.

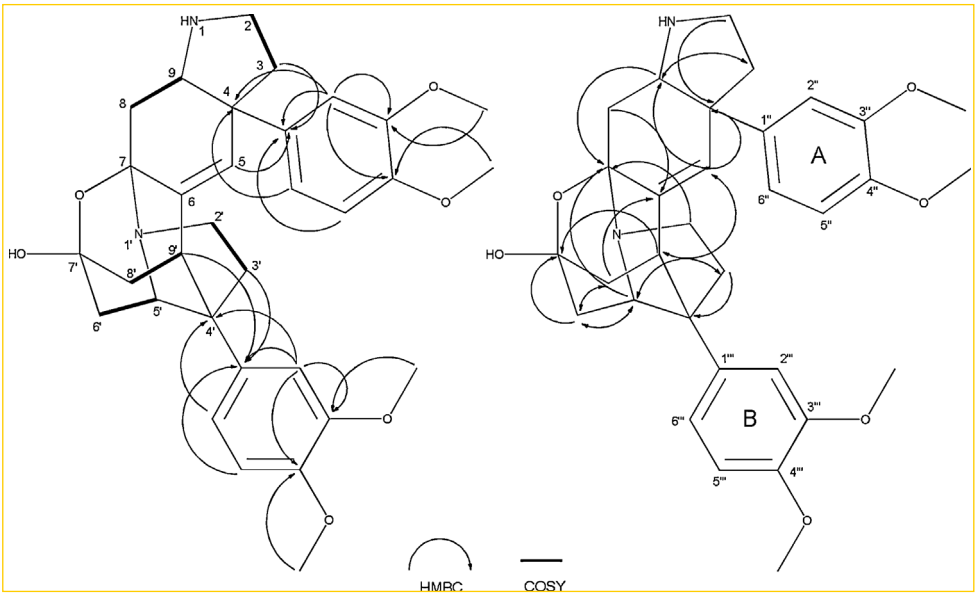

Fig. 3. Left: Key correlations pertaining to the veratrole rings A and B and their respective points of connectivity. Right: Key correlations pertaining to the hydroindole dimer portion of channaine.

protons and these positions.

The point of attachment for the A-ring was further elucidated through the presence of a vinyl singlet (δ_H 5.68, H-5) and two diastrophic methylene signals (δ_H 2.52, 2.15–2.09, H-3; 3.45, 3.29–3.23,H-2), which all correlated through HMBC to C-4 (Fig. 3 **Right**). Furthermore, the proton resonance for H-3 and H-5 correlated with the aromatic carbon C-1″ through HMBC. Finally, H-2 and H-3 were found to correlate through COSY. The H-3 and H-5 protons both correlated to a methine carbon signal (δ_C 63.4) which was assigned as the position 9 ring junction. Position 8 resonances were assigned by a COSY correlation between the H-8 (δ_H 2.47, 1.78) and H-9 (δ_H 3.84) protons. The H-5 vinyl proton was found to correlate through HMBC to a downfield quaternary carbon (δ_C 142.9), a methine carbon (δ_C 47.3) and a methylene carbon (δ_C 41.0). The corresponding proton signals for the latter two carbons also correlated through COSY, which allowed these to be assigned as positions 9′ and 8′ respectively. In turn, both the H-8′ and H-9′ proton resonances, in addition to the H-8 resonance, correlated through HMBC with the carbon signal at δ_C 142.9 which was assigned confidently to the vinylic C-6.

HMBC correlations between the H-5, 9 and 9′ protons to a quaternary carbon signal (δ_C 86.3) allowed us to assign this as position 7 and completing the first putative hydroindole scaffold. The H-9′ proton resonance, in addition to a diasterotopic methylene resonance (δ_H 2.24–2.19, 1.85, H-3′) and a methine signal (δ_H 3.77–3.74, H-5′), correlated through HMBC to a quaternary carbon signal at δ_C 48.9, which had already been identified as the position of attachment to ring B. This hypothesis was confirmed by corresponding HMBC correlations between H-9′ and H3′ and the C-1‴ carbon signal. While positions 3′ and 5′ correlated with each other through corresponding HMBC correlations, COSY correlations between the protons at 3′ and 2′, as well as between 5′ and 6′, confirmed our positional assignments for these signals. In addition, the protons assigned to 2′ and 5′ both correlated to C-7 through HMBC. Corresponding HMBC correlations between the nuclei at positions 5′ and 8′ with 6′ confirmed this assignment. Finally, the carbon signal at δ_C 95.1was assigned to the hemi-acetal C-7′ through HMBC correlations from protons at positions 5′, 6′, 8′ and 9′.

2.2. Biological evaluation

As part of our wider interest in the identification of biologically active compounds, we were interested in the potential medicinal properties of channaine. The related mesembrenone (**1**) has been shown to be cytotoxic (Weniger et al., 1995), while the hydrochloride salt of compound **2**, which shares structural homology with rolipram (**9**) (Fig. 4), has been found to inhibit phosphodiesterase-4 (PDE-4, Harvey et al., 2011). Notably, inhibition of PDE has been investigated as an anti-cancer target (Hirsh et al., 2004; Savai et al., 2010), Furthermore, the catalytic domain of PDE enzymes is highly conserved between humans and protozoans, meaning that inhibition of PDEs has been exploited to inhibit several protozoan parasites, including malaria and trypanasoma. (De Koning et al., 2012; Díaz-Benjumea et al., 2006; Gould et al., 2013; Gould and De Koning, 2011; Howard et al., 2015; Kunz et al., 2006; Oberholzer et al., 2007; Orrling et al., 2012; Seebeck et al., 2011; Wang et al., 2012a,b; Zoraghi and Seebeck, 2002) Furthermore, inhibition of protozoan PDEs has been achieved with known human PDE inhibitors sildenafil (**10**) and tadalafil (**11**, Ochianaa et al., 2012; C. Wang et al., 2012a,b).

Accordingly, channaine was assessed for anti-protozoal biological activity against *Plasmodium falciparum* (3D7) and *Trypanosoma brucei brucei*, as well as for cytotoxic biological activity against a HeLa (cervical carcinoma) cell line and HCC70 (triple negative breast carcinoma) cell line, using previously published methodology (Adeyemi et al., 2017; De La Mare et al., 2012; Svogie et al., 2016). However, in this battery of assays, channaine showed no significant biological activity.

3. Methods and materials

3.1. Plant material preparation, extraction and purification

Samples of *S. tortuosum* were collected from St Helena on the West Coast of South Africa. Voucher specimens were prepared, taxonomically identified by Mr. Eugene Pienaar and have been retained in the Department of Pharmaceutical Sciences, Tshwane University of Technology. The collected plant material was air-dried prior to extraction. The ground material was extracted using a typical alkaloid acid/base extraction procedure (Shikanga et al., 2011). The dried extract was re-dissolved in HPLC grade methanol and filtered through a 0.2 µm syringe filter. Ultra-performance liquid chromatography analysis of the solvent extracts was performed using a Waters Acquity UPLC system (Waters, Milford, MA, USA) in tandem with a quadrupole time-of-flight (Waters Xevo® G2QToF) mass spectrometer.

Isolation of channaine was achieved through use of a Waters preparative high-performance liquid chromatography-mass spectrometry (prep HPLC–MS) system fitted with a Waters photodiode array (PDA) (Model 2998) and interfaced with a QDa MS detector (Waters, Milford, MA, USA). An injection volume of 100 µL was introduced. Separation was achieved on an XBridge Prep C18 column (19 × 250 mm, i.d., 5 µm particle size, Waters) maintained at 40 °C. The chromatographic conditions were optimised to obtain the best resolution of the target compounds. The mobile phase consisted of 0.1% ammonium hydroxide in water (Solvent A) and acetonitrile (Solvent B) at a flow rate of 20 mL/min. Gradient elution was applied as follows: The initial ratio was 95% A:5% B, held for 1 min, changed to 60% A:40% B within 3.0 min, changed to 38%: A:62% B within 4.0 min, changed to 15%: A:85% B in 1.0 min, and maintained for 1.0 min before returning to the initial ratio in 0.5 min. Data were collected by the chromatographic software MassLynx 4.1 (Waters, USA). The electrospray ionisation was carried out in positive mode. The probe temperature was maintained at 600 °C, while the source temperature was 120 °C. The capillary and cone voltages were set to 800 and 10 V, respectively. Data were collected between *m/z* 100 and 750.

The eluent was fractionated into 220 drops/tube (about 2 mL) using a fraction collector. The target compound was collected in various fractions, subsequently combined and concentrated to yield a residue, which were analyzed by UPLC–MS to establish its purity.

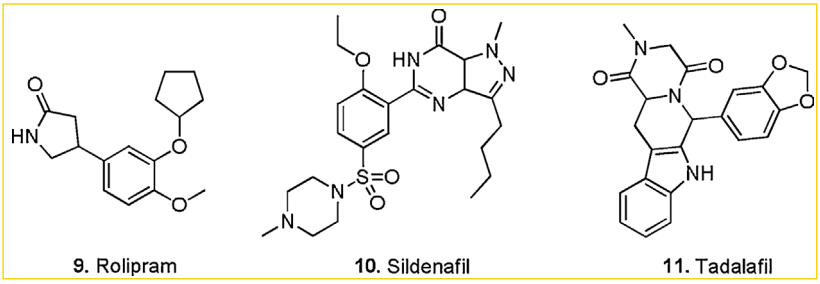

Fig. 4. Known inhibitors of phosphodiesterase.

3.2. NMR spectroscopy

NMR spectra were acquired on a Bruker 600 MHz Avance II spectrometer. Chemical shifts are reported in ppm, referenced to residual solvent resonances ($CDCl_3$ δ_H 7.25, δ_C 77.0; CD_3OD δ_H 3.31, δ_C 49.0). Chemical structures were resolved using a combination of ^1H, ^{13}C, COSY, HSQC and HMBC NMR spectroscopy

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.phytol.2017.11.018.

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