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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 azabicylcooctene 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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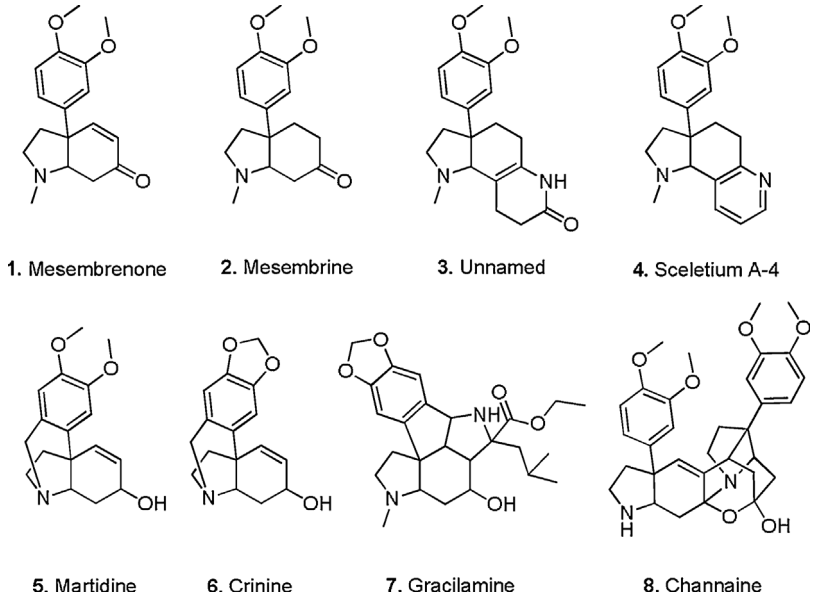

Fig. 1. Several related alkaloids, belonging to different classes, isolated from various species of the genus *Sceletium* or from the Amaryllidaceae family.

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.

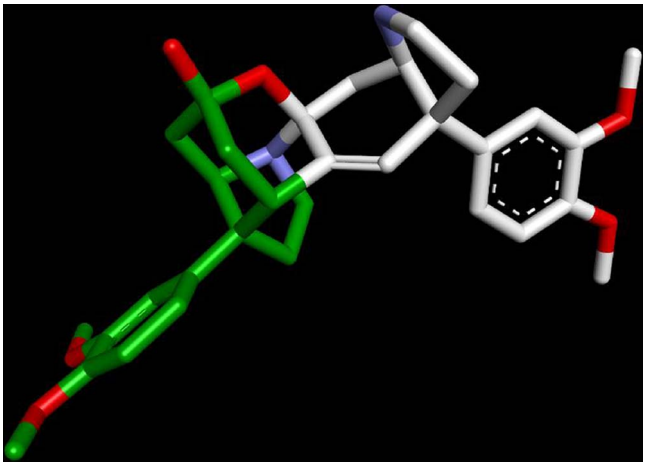

Fig. 2. X-ray crystal structure of channaine (8, CCDC identification number 1124320, Abou-Donia et al., 1978) Highlighted in white and green are the respective arylhydroindole subunits of a putative *N*-demethylmesembrenone, the interface of which forms an unusual cage-like structure. (For interpretation 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