Isolation of a pentacyclic triterpenoid from *Sceletium tortuosum*

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Abstract

Traditionally, extracts from Sceletium tortuosum have been used, both as a medicine as well as for social and spiritual purposes. The genus is distributed in the south-western parts of South Africa. Methanolic extracts of S. tortuosum were prepared and fractionated using column chromatography. Indole alkaloids have been reported from S. tortuosum with mesembrine as the most abundant alkaloid and mesembrenone, 4'-odemethylmesembrenol and tortuosamine also present. A triterpenoid, novel to this genus was isolated and reported for the first time. NMR and HPLC-MS/MS analyses were used to confirm the structure of the compound.

Key words: Sceletium tortuosum, alkaloids, triterpenoid, obtusalin.

Introduction

The genus Sceletium is classified under the family Mesembryanthemaceae (Aizoaceae) and belongs to the sub-family Mesembryanthemoideae (Patnala and Kanfer, 2009; Smith et al., 1996). The genus name is derived from the word 'sceletus' which means 'skeleton', referring to the prominent vein-like lines, which are easily visible in the old, dry and withered leaves (Figure 1) (Gericke and Viljoen, 2008). The word Mesembryanthemum originates from the name 'midday flower', which refers to the opening of the flowers around noon (as shown in Figure 2). Plants that belong to the Mesembryanthemaceae family are known by most South Africans as vygies (Chesselet et al., 2002)

Figure 1: Characteristic skeletonised appearance of old leaves (Gericke and Viljoen, 2008).

Figure 2: Represents that decumbent habit and succulent leaves of *Sceletium* spp. (Gericke and Viljoen, 2008).

The general application of Sceletium (Aizoaceae, subfamily Mesembryanthemoideae) had been revised by various authors since the genus was established in 1925 by N.E. Brown (Gericke and Viljoen, 2008). This group of plants is characterised by the skeletonised leaf venation pattern visible in dried leaves. In 1986, Bittrich argued for a broader application of Phyllobolus which included Sceletium as one of five subgenera (Gericke and Viljoen, 2008). Since Gerbaulet (1996) was unable to find a synapomorphy (a unique derived character) for Bittrich's broad concept of Phyllobolus, she reinstated Sceletium as a genus (Gericke and Viljoen, 2008). Species of this genus are distinguished on the basis of vegetative, flower, fruit and seed characteristics. Some species are reduced to synonymy including S. joubertii L. Bol., and S. namaquense L. Bol. now considered part of S. tortuosum. Sceletium exhibits a climbing or decumbent habit and has

characteristic succulent leaves with “bladder cells” or idioblasts.

The genus Sceletium is distributed in the south-western parts of South Africa and has an affinity for arid environments. It is amongst taxa that have been extensively researched in the past few decades. Traditionally, plants of this genus have been used to relieve thirst and hunger, to combat fatigue, as medicines and for social and spiritual purposes by San hunter-gatherers and Khoi pastoralists (Gericke and Viljoen, 2008). South Africa can benefit from the scientific evaluation of this indigenous plant and its knowledge base. However; the chemistry and pharmacology of many medicinal plants, such as Sceletium tortuosum, have not yet been thoroughly investigated (Harvey, 2000). It is believed that the phytochemical exploration of the genus Sceletium commenced in 1898 when Meiring isolated a crude alkaloid mixture from S. tortuosum. This was followed by the work of Zwicky in 1914 that isolated several alkaloids including mesembrine and mesembrenine (Gericke and Viljoen, 2008). The number of Sceletium species within the Aizoaceae family that have been examined for the presence of alkaloids has been restricted by their geographical inaccessibility. Within the Sceletium genus, a number of alkaloids are produced which mainly belong to the crinane class of compounds (Jeffs et al., 1982), but no compound belonging to the triterpene class has been reported to have been isolated from Sceletium.

2. Material and Methods

2.1 Plant collection

Nineteen kg of Sceletium whole plant material was harvested on 11 – 12 June 2009 under sunny and dry conditions at Kamieskroon in the Northern Cape of South Africa. The plant material was supplied to the Council for Scientific and Industrial Research (CSIR) for this study by Enterprise Creation for Development (ECD). Plant material was collected by an independent contractor who practiced standard operating procedures during harvesting/handling, storage and transportation. This study required harvesting of whole plant material by the supplier. The plant material was positively identified and confirmed to be Sceletium tortuosum N.E. Br. by the South African National Biodiversity Institute (SANBI), batch number: 10025 (Genspec No. ECDMP-100 22).

2.2 Plant preparation and extraction

About 6.3 kg of wet plant material was placed in a 60 oC oven to dry. The dry plant material was not ground to a fine powder, but was broken and crushed into smaller pieces. From that, 3.15 kg was transferred to a 1000 ml glass beaker. Two large magnetic stirrers and sufficient methanol (MeOH) solvent, enough to cover the plant material was added. The beaker was covered tightly with a piece of aluminium foil before being placed on a stirring heating plate and heated at 40 oC for 2 days (48 hrs). A mercury thermometer was used to monitor the temperature throughout the extraction process. After extraction, the contents were mixed thoroughly and then filtered through filter paper into a 1000 ml conical flask connected to a vacuum pump. The extract was filtered twice to remove any debris remaining in the suspension. The MeOH was evaporated at 45 oC using a GeneVac® Personal evaporator. The crude extract was then stored in a dark, cold room at 4 oC.

2.3 Compound isolation and purification

Obtusalin was isolated from the methanol extract of S. tortuosum using Preparative Layer Chromatography (PLC) with a solvent system containing hexane, ethyl acetate and triethylamine [Hex: EtOAc: Et3N (6:4:1)] as eluant. The plate was run twice to improve separation between compounds. A very thick band was observed under UV light and used for further purified using PLC with Hex: EtAc: Et3N (6:4:1) as solvent system. Ultimately, 2 mg of obtusalin was isolated. NMR (600 MHz, Varian,) was used to confirm the structure, using 1H, 13C, COSY, DEPT, HSQCAD and HMBCAD protocols to generate chemical shift data for subsequent comparison with that found in the literature.

3 Results and discussion

The methanol crude extract of S. tortuosum was fractionated and several compounds were isolated, purified and identified. These compounds were confirmed to be alkaloids that have commonly been reported in the extraction of S. tortuosum; namely, mesembrine, Δ4 mesembrenone, Δ7 mesembrenone (mesembranol) and epimesmbrine. An unexpected finding which later became apparent, was that these compounds degrade over a period of time, verified by the degradation of mesembrine. Consequently; the compounds had to be re-isolated, with more attention being paid to chemical instability problems. A separate High Performance Liquid Chromatography (HPLC) study was undertaken to evaluate the stability and

determine if there were gradual changes occurring within the extracts while in storage. Mesembrine and Δ7 mesembrenone were re-isolated, with some measure of stability, but the other two compounds had degraded to such an extent within the extract that they could not be re-isolated. In addition to these compounds, pinitol and sucrose were also isolated from the plant (Setshedi, 2014).

A pentacyclic triterpenoid confirmed as obtusalin (Figure 3) was isolated for the first time from the methanol extract of S. tortuosum. Triterpenoids are generally very stable and, unlike the alkaloids of this plant material, do not decompose, but were found in low concentrations. Triterpenoids are commonly found in most plants and are produced by arrangements of squalen epoxide in a chair-chair-chair-boat arrangement subsequently followed by condensation (Patočka, 2003). These compounds are isopentenoids composed of thirty carbon atoms and may possess acyclic, mono, di-, tri-, tetra- or pentacyclic carbon skeletons. Pentacyclic triterpenoids are dominant constituents of this class and have been widely investigated (Mahato and Kundu, 1994). Obtusalin is a pentacyclic triterpene belonging to the lupane class of compounds (Zheng et al., 2010; Siddiqui et al., 1989) that was first isolated by Siddiqui and colleagues in 1989 from the leaves of Plumeria obtusa. Obtusalin forms part of is the few naturally occurring pentacyclic triterpenoids possessing a C-27-hydroxyl group in conjunction with a double bond at C-12 in the lupine chain of triterpenoids (Begum et al., 1994 and Siddiqui et al., 1989). Pentacyclic triterpenes are reported to possess a wide spectrum of biological activities, where some may be used as medicines (Patočka, 2003).

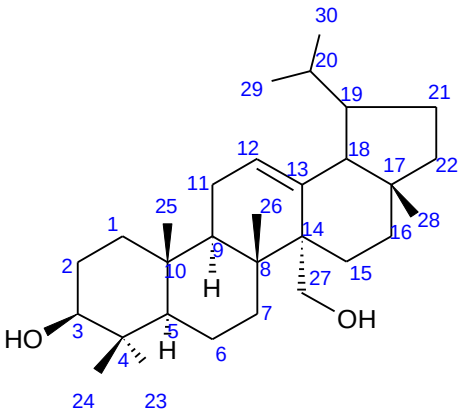

Figure 3: Structure of Compound 1

Table 1: ^{1}H NMR spectral data of compound 1 ($CDCl_3$) in comparison to that found in literature ($CDCl_3$) (Siddiqui et al., 1989)

Compound 1		
Proton Number	**δH (*J* in Hz)(isolated)**	**δ^{13}H(*J* in Hz) (Literature)**
3α	3.16 (br*d*, *J* = 12.69)	3.21 (*dd*, *J* = 10.8; 4.9)
5α	0.68 (br*t*, *J* = 12.1)	0.72 (*dd*, *J* = 11.6; 1.5)
9	1.54 (m)	1.54 (*dd*, *J* = 10.0, 3.4)
11α	1.85 (br*dd*, *J* = 8.8, 6.4)	1.84 (*ddd*, *J* = 13.2, 3.6, 3.4)
11β	1.63 (m)	1.61 (*ddd*, *J* = 13.2, 10.0, 3.6)
12	5.07 (*brs*)	5.13 (*t*, 3.6)
23	0.94 (*s*)	1.01 (*s*)

24	0.93 (s)	0.98 (*s*)
25	0.72 (s)	0.78 (*s*)
26	0.88 (s)	0.94 (*s*)
27a	3.46 (brd, *J* = 11.2)	3.52 (*d*, *J* = 10.9)
27b	3.14 (*brd*, *11.2*)	3.18 (*d*,10.9)
28	1.03 (*s*)	1.10 (*s*)
29/30	0.87 (*brd*, *J* = 5.2)	0.93 (*d*, *J* = 5.8)
30/29	0.75 (*brd*, *J* = 7.0)	0.80 (*d*, *J* = 5.9)

Table 2: ^{13}C NMR spectral data of compound 1 ($CDCl_3$) in comparison to that found in literature ($CDCl_3$) (Siddiqui *et al*., 1989 and Begum *et al*., 1994)

Compound 1

Carbon Number	**δ^{13}C (Isolated)**	**δ^{13}C (Literature)**
1	38.8	38.8
2	27.2	27.3
3	79.0	79.1
4	38.0	38.0
5	55.1	55.2
6	18.3	18.4
7	32.8	32.9
8	40.0	40.1
9	47.6	47.7
10	36.9	36.9
11	23.4	23..4
12	125.0	125.1
13	138.7	138.8
14	42.0	42.1
15	23.4	23.4
16	26.0	26.0
17	38.8	38.8
18	54.0	54.0
19	39.4	39.5

20	39.3	39.4
21	30.6	30.7
22	35.2	35.2
23	28.1	28.2
24	16.8	16.8
25	15.6	15.6
26	15.7	15.7
27	69.9	69.9
28	23.3	23.3
29	21.3	21.3
30	17.3	17.3

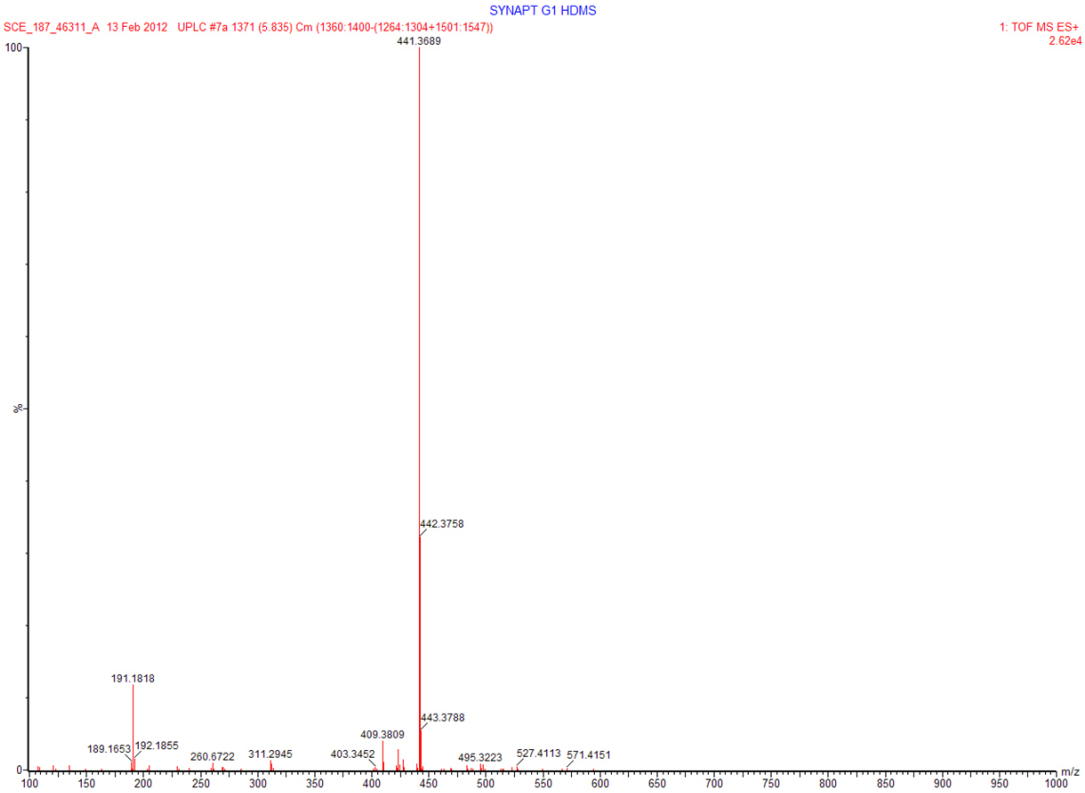

Figure 4.: HRTOFMS (ESI$^+$) chromatogram of compound 1

The HRTOFMS (ESI+) spectrum (Figure 4) of compound 1 showed a pseudo-molecular ion signal [M]+ at m/z 441.3689 which corresponds to the molecular formula C30H50O2. There are significant fragments as indicated in Figure 5. This data strongly indicates that the compound is of a lup-12-ene type. 1H NMR, 13C NMR and MS spectral data of compound 1 are in agreement with those reported in literature for obtusalin (Siddiqui et al., 1989 and Begum et al., 1994). One olefinic proton was observed in the proton NMR spectrum (Table 1) and resonated at δH 5.07, while two secondary methyl groups resonated at δH 0.87 (J= 5.2 Hz) and δH 0.75 (J= 7.0 Hz), five three-proton singlets indicating tertiary methyls that resonated at δ 0.94, 0.93, 0.72, 0.88 and 1.03. The 13C NMR spectrum (Table 2) showed the presence of 30 carbon signals: olefinic carbons at δC13 125.0 and 138.7; oxygenated carbons at δ

79.0 and 69.9 and seven methyls at δC13 15.6, 15.7, 16.8, 17.3, 21.3, 23.3 and 28.1. Accordingly, the structure of compound 1 was assigned as obstusalin.

4 Conclusion

Sceletium species have been shown to contain indole alkaloids with mesembrine reported as the most abundant alkaloid in S. tortuosum. A pentacyclic triterpenoid, novel to this species as well as the genus was isolated and reported for the first time. NMR and HPLC-MS/MS analyses were used to confirm the structure of the compound. Pentacyclic triterpenes are reported to possess a wide spectrum of biological activities, with some used as medicines.

5 Acknowledgments

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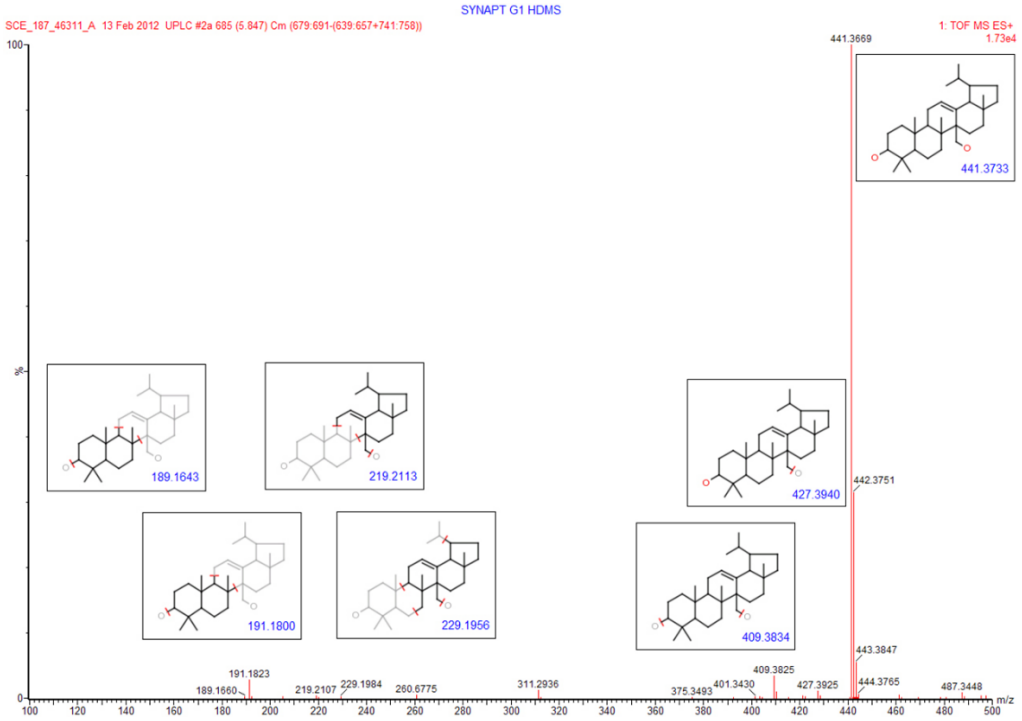

Figure 5: HRTPFMS (ESI$^+$) fragmentation of compound 1. Showing peak fragmentation correlating to those significant fragments which strongly suggests that the compound is of a lup-12-ene type.

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