

Research note

Distichamine, a chemotaxonomic marker for the genus *Boophone* Herb. (Amaryllidaceae)

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Abstract

The plant family Amaryllidaceae is known for its ornamental and medicinal value, as well as its unique alkaloid constituents, including several molecules of high biological interest, such as galanthamine, pancratistatin and lycorine. These plants are also used in traditional medicinal practice systems across the globe, and the taxonomy of the family is relatively well-understood. The African genus *Boophone* Herb. comprises two species, *B. disticha* (L.f.) Herb. and *B. haemanthoides* F.M. Leight. Much is known about the ethnobotanical, phytochemical and pharmacological properties of the wide-ranging *B. disticha*. In contrast, *B. haemanthoides* is a threatened and territorially restricted species, and its ethnobotanical usage by Khoi-San of the Northern Cape has only recently been described. During our investigation, *B. haemanthoides* was shown to be a novel source of the known β -crinine alkaloids distichamine, buphanidrine, buphanisine, and crinine. Of note is the presence of distichamine in *B. haemanthoides*, previously identified only in *B. disticha*, and its significance as a distinctive chemotaxonomic marker for the genus *Boophone*. © 2012 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Amaryllidaceae; *Boophone haemanthoides*; Chemotaxonomy; Distichamine

The African Amaryllidaceae constitutes around 300 species in 20 genera, which are divided into three major clades corresponding to the tribes Amaryllideae, Cyrtantheae and Haemantheae (Meerow and Snijman, 1998). The tribe Amaryllideae is primarily South African in distribution, but a few of its genera extend outside this region, of which the pantropical genus *Crinum* is most wide-ranging, with seeds well suited to oceanic dispersal (Meerow and Snijman, 2001). Phylogenetic relationships within the Amaryllideae have been the subject of several indepth studies (Dahlgren et al., 1985; Meerow and Snijman,

1998; Müller-Doblies and Müller-Doblies, 1996; Snijman and Linder, 1996; Traub, 1963). Based on morphological, floral, seed anatomical, and cytological data, the tribe was resolved into two monophyletic subtribes: 1) Crinineae comprising *Boophone* Herb., *Crinum* L., *Ammocharis* Herb., and *Cybistetes* Milne-Redh. & Schweick.; 2) Amaryllidineae comprising *Amaryllis* L., *Nerine* Herb., *Brunsvigia* Heist., *Crossyne* Salisb., *Hessea* Herb., *Strumaria* Jacq., and *Carpolyza* Salisb. (Snijman and Linder, 1996). *Boophone* is a genus of deciduous bulbous geophytes comprising the two species, *B. disticha* (L.f.) Herb. and *B. haemanthoides* F.M. Leight. (Meerow and Snijman, 1998). *B. disticha* is widely distributed in Africa, ranging from Sudan in the north to the Western Cape Province in the south, while *B. haemanthoides* is a rare and threatened species with a restricted territory within the winter rainfall region of South Africa and parts of southern Namibia (Wrinkle, 1984). The use of *B. disticha* for medicinal purposes among the indigenous people of southern

Abbreviations: COSY, correlation spectroscopy; DEPT, distortionless enhancement by polarization transfer; EI, electron impact; HMBC, heteronuclear multiple bond correlation; HRMS, high resolution mass spectrometry; HSQC, heteronuclear spin quantum correlation; LRMS, low resolution mass spectrometry; NMR, nuclear magnetic resonance.

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Africa has been known for several centuries (Watt and Breyer-Brandwijk, 1962). The chemicals likely responsible for many of these effects are alkaloids, structural variants of which occur exclusively within the family Amaryllidaceae (Viladomat et al., 1997). Previous phytochemical investigations of *B. disticha* led to the identification of a total of eleven alkaloids (Cheesman et al., 2012; Hauth and Stauffacher, 1961; Neergaard et al., 2009; Sandager et al., 2005), mainly of the crinane group of compounds. Of these, distichamine **1** (Fig. 1) is structurally unique in possessing both vinylic methoxyl as well as α,β -unsaturated ketone C-ring functionalities, and has currently been isolated only from *B. disticha* (Cheesman et al., 2012; Hauth and Stauffacher, 1961; Neergaard et al., 2009). The ethnobotanical usage of *B. haemanthoides* by Khoi-San of the Northern Cape has recently been described by De Beer and Van Wyk (2011), but information pertaining to its phytochemical make-up is absent from the literature. During our study, the known alkaloids distichamine **1**, buphanidine **2**, buphanisine **3** and crinine **4**, previously shown to occur in *B. disticha* (Hauth and Stauffacher, 1961), were identified in bulbs of *B. haemanthoides*. The presence of the rare alkaloid distichamine in *B. haemanthoides* is significant, and highlights its utility as a distinctive chemotaxonomic marker for the genus *Boophone* Herb.

Bulbs of *B. haemanthoides* were collected during September 2011 in the Saldhana Bay area of the Western Cape and a voucher specimen (Cheesman_02_NU) was authenticated by Dr. Christina Potgieter and retained at the University of KwaZulu-Natal Herbarium. Distichamine **1** (21.9%), buphanidine **2** (46.9%), buphanisine **3** (23.9%) and crinine **4** (7.3%) were isolated consecutively via column chromatography of the crude ethanolic extract and identified by a combination of physical and spectroscopic techniques, according to recently detailed procedures (Cheesman et al., 2012; Neergaard et al., 2009). The above percentage values (expressed as relative contribution to total alkaloids) are contrasted against levels of 5.4%, 19.4%, 16.9% and 7.2% for the four compounds, respectively, originally shown to be present in *B. disticha* (Hauth and Stauffacher, 1961). The remaining seven compounds reported by these authors, including undulatine (18.6%), buphanamine (14.1%), nerbowdine (11.1%), acetylnerbowdine (0.6%), crinamidine (1.2%), lycorine (0.4%) and buphacetine (0.3%) were not detected during the course of our investigation. Thus, of the full complement of eleven alkaloids known in *B. disticha* (Cheesman et al., 2012; Hauth and Stauffacher, 1961; Neergaard et al., 2009; Sandager et al., 2005),

distichamine **1** (Fig. 1) is unique in that it has never been found outside the genus *Boophone*. In relation to spectroscopic data for distichamine, EIMS of the pure compound revealed the $[M]^+$ ion as the base peak at m/z 329, while HRMS analysis gave a mass of 329.1269 g/mol (calculated 329.1263 for $C_{18}H_{19}NO_5$), in agreement with the structure of distichamine. Diagnostic 1H NMR signals were detected downfield at δ 7.62 (1H, s) and 5.85 (2H, 2d, each $J=1.20$ Hz), assignable to H-10 and the methylenedioxy group protons of ring-A, respectively. Also in this region of the spectrum, the trisubstituted ring-C double bond was indicated by a doublet resonance at δ 5.43 ($J=1.28$ Hz) for H-2. Furthermore, the α,β -unsaturated nature of the C-ring keto group was shown by carbon singlet resonances at δ 201.9 (C-1) and 177.1 (C-3), as well as a doublet at δ 103.6 (C-2). Aryl and vinylic methoxy group protons were resonant at δ 3.97 and 3.77 (each 3H, s), respectively, in accordance with C-7 and C-3 substitutions in distichamine. The B-ring heterocycle was characterized by resonance signals for the diastereotropic protons H-6 α (δ 4.14, $J=17.3$ Hz) and H-6 β (δ 3.78, $J=17.3$ Hz), with H-6 α shifted to lower field due to its *syn* proximity to the nitrogen lone pair. Similarly, the bridge protons H-12*exo* (δ 3.42, m) and H-12*endo* (δ 2.91, ddd, $J=13.0, 9.1, 6.04$ Hz) were distinguishable based on their positioning relative to the nitrogen lone pair. All other proton signals were assignable via COSY, HSQC and HMBC correlations, while eighteen carbon atoms were accounted for in the ^{13}C NMR spectrum which resolved to 2 quartets, 5 triplets, 3 doublets and 8 singlets after DEPT refinement, in agreement with published data (Cheesman et al., 2012; Neergaard et al., 2009). In summary, *B. haemanthoides* is identified as a novel source of the β -crinane alkaloids distichamine, buphanidine, buphanisine, and crinine. Furthermore, these findings show that distichamine, previously known only from *B. disticha*, is unique to and characteristic of the genus *Boophone*. To the best of our knowledge, no other phytochemical examples of such generic specificity are recorded for the Amaryllidaceae.

Acknowledgements

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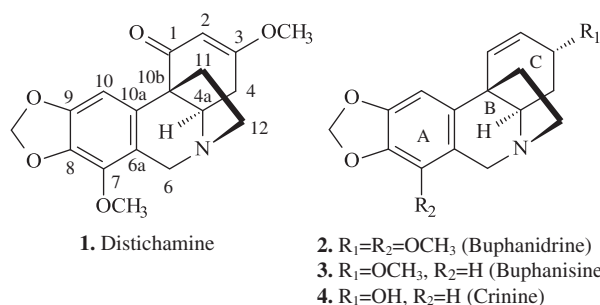


Fig. 1. Structurally-similar β -crinanes isolated from *Boophone haemanthoides*.

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