South African Journal of Botany 118 (2018) 188–191

Contents lists available at ScienceDirect

South African Journal of Botany

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

Alkaloids isolated from indigenous South African Amaryllidaceae: *Crinum buphanoides* (Welw. ex Baker), *Crinum graminicola* (I. Verd.), *Cyrtanthus mackenii* (Hook. f) and *Brunsvigia grandiflora* (Lindl)

M. Masi [a], B. Mubaiwa [b], T. Mabank [b], Ç. Karakoyun [c], A. Cimmino [a], W.A.L. Van Otterlo [d], I.R. Green [d,*], A. Evidente [a,*]

[a] Department of Chemical Sciences, University of Naples "Federico II", Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy
[b] School of Pharmacy, University of the Western Cape, Bellville, South Africa
[c] Faculty of Pharmacy, Department of Pharmacognosy, Ege University, Turkey
[d] Department of Chemistry and Polymer Science, University of Stellenbosch, Private Bag X1, Matieland, 7602 Stellenbosch, South Africa

ARTICLE INFO

Article history:
Received 10 May 2018
Received in revised form 13 July 2018
Accepted 18 July 2018
Available online xxxx

Edited by JJ Nair

Keywords:
Natural products
Alkaloids
South African plants
Amaryllidaceae
Lycorine

ABSTRACT

Screening for alkaloids produced in four indigenous South African Amaryllidaceae plants, namely, *Crinum buphanoides* Welw. ex Baker, *Crinum graminicola* I. Verd., *Cyrtanthus mackenii* Hook. f and *Brunsvigia grandiflora* Lindl, leads to the isolation of lycorine as the one metabolite produced by all the species studied. *C. graminicola* produced lycorine in the best yield (2.1 g/kg). Moreover, 1-*O*-acetyl- and 2-*O*-acetyl-lycorine, pratorimine, hippadine and tazettine were isolated from *C. buphanoides*. Haemanthidine, haemanthamine and criwelline were isolated from *C. graminicola*, while tazettine and 11-hydroxyvittatine were produced by *C. mackenii*. Finally, crinamine and 11-hydroxyvittatine were isolated from *B. grandiflora*. This is the very first report on the isolation of these alkaloids from the four South African Amaryllidaceae. Furthermore, some interesting chemosystematic evaluations are reported.

1. Introduction

The Amaryllidaceae are a family of bulbous plants known all over the world for their beautiful flowers and use in folk medicine. This family represents about 1600 species, divided into about 75 genera, which are distributed throughout both tropical and subtropical regions of the world (Christenhusz and Byng, 2016; Cimmino et al., 2017). In particular, they are dominant in the Mediterranean basin, Andean South America and southern Africa, where the majority of the species grow. Amaryllidaceae plants have been shown to produce numerous alkaloids that can be grouped into twelve structural families (Kornienko and Evidente, 2008) with a wide range of bioactivities, including antitumor, antiviral, antibacterial, antifungal, antimalarial, analgesic and cytotoxic activities (Nair et al., 2013; Nair and Van Staden, 2014; He et al., 2015; Cimmino et al., 2017). The structural complexity of a number of these alkaloids has also been the driving force for the interest in these compounds which have in recent years become valuable targets for synthetic organic chemists (Hudlicky et al., 2002; Ghavre et al., 2016; Van Otterlo and Green, 2018). Other alkaloids have demonstrated their importance in medicine. For example, galanthamine, a potent and selective inhibitor of the enzyme acetylcholinesterase, is already present on the market as an Alzheimer's prescription drug (Heinrich and Teoh, 2004; Houghton and Howes, 2005; Cimmino et al., 2017). The important alkaloid, lycorine has shown strong in vitro anticancer activity against different solid tumors with malignant prognosis and several analogues have been synthesized aimed at the development of new anticancer agents (Dasari et al., 2014; Nair, 2014). The search for new alkaloids remains an aspiration of many natural product chemists and thus the isolation and investigation of the alkaloids from indigenous South African Amaryllidaceae provide an opportune source since these have to an extent remained largely unexplored (Nair et al., 2013). Recently, the plant *Nerine sarniensis* was studied and from the acid extract of its bulbs, three new alkaloids, belonging to the mesembrine- and crinine-families, named crinsarnine, sarniensine and sarniensinol, were isolated together with the known bowdensine, hippadine and 1-*O*-acetyl-lycorine. Of particular interest was that sarniensine and crinsarnine showed strong adulticidal activity against *Aedes aegypti*, the vector of yellow and dengue fevers and the Zika virus (Masi et al., 2016, 2017). As part of a South Africa–Italy bilateral project involving the study of bioactive metabolites from South African Amaryllidaceae plants, a further four species, viz., *Crinum buphanoides* (Welw. ex

* Corresponding authors.
E-mail addresses: irg@sun.ac.za (I.R. Green), evidente@unina.it (A. Evidente).

https://doi.org/10.1016/j.sajb.2018.07.021
0254-6299/

Baker), *Crinum graminicola* (I. Verd.), *Cyrtanthus mackenii* (Hook. f) and *Brunsvigia grandiflora* (Lindl) (Germishuizen and Meyer, 2003) were selected and evaluated for their ability to produce alkaloids. It should be noted that to the best of our knowledge, of these four, only *C. graminicola* has been investigated for the ability of its extracts to demonstrate acetylcholinesterase inhibition (Jäger et al., 2004). This manuscript reports the first isolation of lycorine-, haemanthamine- and tazettine-type alkaloids, together with pratorimine and hippadine, from these four species.

2. Materials and methods

2.1. General procedure

Optical rotations were measured in $CHCl_3$ or MeOH on a Jasco P-1010 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded at 400 or 500 and 100 or 125 MHz in $CDCl_3$, MeOD or DMSO on Bruker and Varian spectrometers. ESI MS spectra were recorded on an Agilent 6230 LC MSTOF. Analytical TLC and preparative TLC were performed on silica gel plates (Merck, Kieselgel 60 F254, 0.25 and 0.50, respectively). CC was performed on silica gel (Merck, Kieselgel 60, 0.063–0.200 mm).

2.2. Plant material

The bulbs of *C. buphanoides* (Welw. ex Baker), *C. graminicola* (I. Verd.), *C. mackenii* (Hook. f) and *B. grandiflora* (Lindl) (Germishuizen and Meyer, 2003) were purchased from the South African Bulb Company (Hartbeespoortdam, NW Province, South Africa) and three live specimens of each plant are growing in the greenhouses under the curatorship of Mr. S Kweleta (voucher No BG-2018-01) at the Botanical Gardens of Stellenbosch University, Stellenbosch, South Africa.

2.3. Extraction and isolation of alkaloids

Fresh bulbs (~1.0 kg) of *C. buphanoides* (CB), *C. graminicola* (CG), *C. mackenii* (CM) and *B. grandiflora* (BG) were diced and dried at 40 °C for 48 h and then finely minced. All the resulting materials were extracted using a protocol previously reported (Masi et al., 2016, 2017) and further purified as follows. **CB**: The organic extract (266.6 mg) was fractionated by CC (eluted with $CHCl_3$–EtOAc–MeOH (2:2:1)) to afford seven homogeneous fractions (CBF_{1-7}). The residue (26.7 mg) of CBF_1 was purified by TLC (eluted with *n*-hexane–EtOAc (7:3)) to afford hippadine (**2**, 13.7 mg) and pratorimine (**3**, 2.8 mg) as amorphous solids. The residue (16.7 mg) of CBF_2 was further purified on TLC using as eluent EtOAc–MeOH–H_2O (90:7:3), followed by a second purification with $CHCl_3$–*i*-PrOH (9:1) to afford 1-*O*-acetyl-lycorine (**4**, 3.7 mg) and 2-*O*-acetyl-lycorine (**5**, 3.7 mg) as white solids. The residue (12.6 mg) of CBF_4 was further purified on TLC (eluted with $CHCl_3$–EtOAc–MeOH (2:2:1)) yielding tazettine (**6**, 2.0 mg) as an amorphous solid. The residue (97.7 mg) of CBF_6 was crystallized using hot ethanol to obtain lycorine (**1**, 86.4 mg) as white crystals. **CG**: The organic extract (9.0 g) was fractionated by CC and eluted with a gradient eluent of $CHCl_3$ and *i*-PrOH of increasing polarity which produced nine homogeneous fractions (CGF_{1-9}). The residue (530.2 mg) of the third fraction (CGF_3) was further purified by CC ($CHCl_3$–EtOAc–MeOH, 2:2:1 as eluent) and TLC (EtOAc–MeOH–H_2O, 85:10:5 as eluent) yielding haemanthidine (**7**, 6.4 mg), haemanthamine (**8**, 12.6 mg) and criwelline (**9**, 8.4 mg) as amorphous solids. The residue (2.2 g) of the fifth fraction (CGF_5) was crystallized using hot ethanol to obtain lycorine (**1**, 1.2 g) as white crystals. **CM**: The organic extract (154.6 mg) was further fractionated by CC (eluted with EtOAc–MeOH–Me_2CO–H_2O (65:15:15:5)) affording ten homogeneous fractions (CMF_{1-10}). The residues of the fourth (CMF_4) (24.1 mg) and fifth (CMF_5) (16.4 mg) fractions were further purified by TLC (eluted with $CHCl_3$–EtOAc–MeOH (2:2:1) and EtOAc–MeOH–H_2O (85:10:5), respectively) to afford tazettine (**6**, 3.0 mg) and 11-hydroxyvittatine (**10**, 1.5 mg) as amorphous solids. The residue (35.4 mg) of the sixth fraction (CMF_6) was identified as lycorine (**1**). **BG**: The organic extract (1.3 g) was further fractionated by CC and eluted with EtOAc–MeOH–H_2O (85:10:5) yielding ten (BGF_{1-10}) homogeneous fractions. The residue (118.7 mg) of the fifth fraction (BGF_5) was further purified by CC using as eluent, $CHCl_3$–EtOAc–MeOH, 2:2:1, and TLC, using as eluent EtOAc–MeOH–H_2O, 85:10:5, yielding crinamine (**11**, 32.5 mg) as an amorphous solid. The residues of the sixth (24.0 mg) (BGF_6) and eighth (154.7 mg) (BGF_8) fractions were further purified by TLC (using as eluents EtOAc–MeOH–H_2O (85:10:5) and $CHCl_3$–EtOAc–MeOH (4:4:1), respectively) yielding 11-hydroxyvittatine (**10**, 5.3 mg) and lycorine (**1**, 18.2 mg) as amorphous solids.

3. Results and discussion

3.1. Isolation, purification and structure elucidation of alkaloids

The dried and minced material obtained from ~1.0 kg of fresh bulbs of the four Amaryllidaceae plants, namely *C. buphanoides*, *C. graminicola*, *C. mackenii* and *B. grandiflora*, investigated in this study, were extracted and purified as reported in the Materials and methods section. Lycorine (**1**, Fig. 1) was isolated from the organic extracts of all four species and crystallized when obtained from *C. buphanoides* and *C. graminicola*. Its ESI MS spectrum showed the protonated form $[M + H]^+$ at *m/z* 288 as the base peak, while the proton and carbon spectra were in agreement with the data reported in the literature (Evidente et al., 1983). Furthermore, the optical rotation value was similar to that previously reported (Evidente et al., 1983). Interestingly, the yield of lycorine varied quite significantly for the four different species from the relatively small quantities obtained from the organic extracts of *B. grandiflora*, *C. mackenii* and *C. buphanoides* (18.2, 35.4 and 86.4 mg, respectively) to the relatively high amount obtained from *C. graminicola* (1.2 g).

Furthermore, five known alkaloids were also isolated from the organic extract of *C. buphanoides*, which were identified by comparing their spectroscopic data with those previously reported in literature, namely: hippadine (**2**, Fig. 1) (Ghosal et al., 1981; Masi et al., 2016), pratorimine (**3**, Fig. 1) (Maddry et al., 1985), 1-*O*-acetyl- and 2-*O*-acetyl-lycorine (**4** and **5**, Fig. 1) (Lamoral-Theys et al., 2009; Masi et al., 2016), and tazettine (**6**, Fig. 1) (Ghosal et al., 1984). Furthermore, the optical rotation data obtained for these compounds were similar to those reported in the literature for 1-*O*-acetyl- and 2-*O*-acetyl-lycorine (**4** and **5**) (Lamoral-Theys et al., 2009; Masi et al., 2016) and tazettine (**6**) (Antoun et al., 1993).

Three additional known alkaloids were isolated from the organic extract of *C. graminicola* and identified by comparing their spectroscopic and optical rotational data with those previously reported in literature as haemanthidine (**7**, Fig. 1) (Pabuççuoglu et al., 1989; Antoun et al., 1993), haemanthamine (**8**, Fig. 1) (Pabuççuoglu et al., 1989; Bohno et al., 2007) and criwelline (**9**, Fig. 1) (Boit and Ehmke, 1956; Razafimbelo et al., 1996). These latter two plants have recently been studied by us and the isolation of two non-alkaloid compounds were described. In particular, acetovanillone (also known as apocynin) and 4-hydroxyacetophenone (also named piceol) were isolated from *C. buphanoides*, while only the former was isolated from *C. graminicola* (Masi et al., 2018).

Tazettine (**6**) and 11-hydroxyvittatine (**10**, Fig. 1) were additionally isolated from the organic extract of *C. mackenii*. 11-Hydroxyvittatine **10** was identified by comparing its spectroscopic and optical rotation data with those previously reported in literature (Evidente et al., 2004; Forgo and Hohmann, 2005). The chemical composition of the organic extract of this plant was previously investigated in 1970 by Rao who only reported on the isolation of a crystalline substance identified as lycorine (**1**) (Rao, 1970), while more recently the dichloromethane and methanol extracts of different parts of *C. mackenii* were investigated

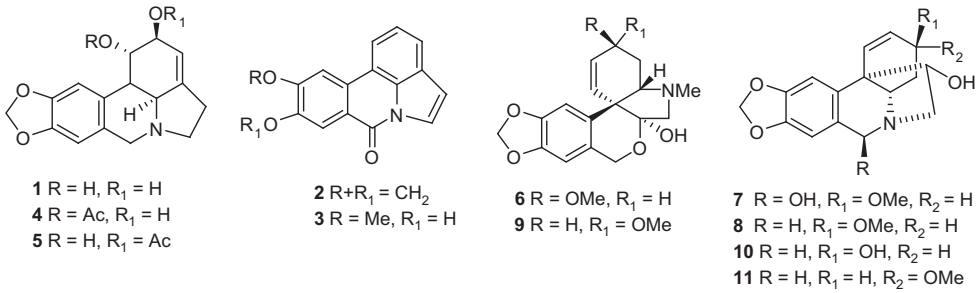

Fig. 1. The structures of lycorine (**1**), hippadine (**2**), pratorimine (**3**), 1-*O*-acetyl-lycorine (**4**), 2-*O*-acetyl-lycorine (**5**), tazettine (**6**), haemanthidine (**7**), haemanthamine (**8**), criwelline (**9**) 11-hydroxyvittatine (**10**) and crinamine (**11**).

for their anti-inflammatory, antibacterial and mutagenic activities by the Van Staden group (Elgorashi and Van Staden, 2004).

Finally, 11-hydroxyvittatine (**10**) and crinamine (**11**, Fig. 1) were also isolated from the organic extract of *B. grandiflora*. Crinamine (**11**) was identified comparing its spectroscopic and optical rotation data with those previously reported in literature (Likhitwitayawuid et al., 1993; Viladomat et al., 1994).

Isolation of the alkaloids **1**–**11** from *C. buphanoides*, *C. graminicola*, *C. mackenii* and *B. grandiflora* is collectively described here for the first time. The alkaloids contained in these indigenous southern African Amaryllidaceae are most interesting from a taxonomic point of view when one considers the phylogenetic relationships of the Amaryllidaceae and the hypothesis that this family evolved in Africa and subsequently spread to other continents. In fact, South Africa is considered the center of primary diversification for these plants, while South America is considered the center of secondary diversification (Ito et al., 1999). The genus *Crinum* comprises several species with wide geographical distribution throughout the tropics and warm temperate regions of the world in Asia, Australia, Africa and America. The organic extracts of these plants have been subjected to extensive chemical and biological investigations that have resulted in the identification of diverse classes of compounds exhibiting a wide range of interesting activities (Tram et al., 2002; Refaat et al., 2013). However, the research activities have been focused predominantly on alkaloids due to their interesting pharmacological activities. The isolation of lycorine (**1**) as the main metabolite and 1-*O*-acetyl- and 2-*O*-acetyl-lycorine (**4** and **5**) from *C. buphanoides* and *C. graminicola* as minor metabolites is not surprising because several alkaloids found in the *Crinum* species belong to the lycorine type and **1** has been isolated from many plants (Tram et al., 2002; Refaat et al., 2012a). Additionally, hippadine (**2**), pratorimine (**3**), tazettine (**6**), haemanthamine (**8**) and criwelline (**9**), were isolated from different *Crinum* species (Tram et al., 2002; Refaat et al., 2012a, 2012b, 2012c) while, to the best of our knowledge, haemanthidine (**7**) was only isolated from *Crinum asiaticum* L. (Refaat et al., 2012c). This latter data is important when taken in the context not only of phylogenetic relationships in the Amaryllidaceae but also for phytogeographic implications considering that *Crinum* is the only cosmopolitan genus while the other Amaryllidaceae genera are reported to be in the same geographic area (Ito et al., 1999).

Cyrtanthus is the largest genus of southern Africa's Amaryllidaceae with well over 90% of its species concentrated in South Africa. Phylogenetic and biogeographic relationships within this genus were recently investigated and its division into three informal infrageneric groups has been proposed. However, future taxonomic studies in the genus are required to better understand the relationships among the species and in this context it is thus important to find distinctive natural products that can be used as important chemotaxonomic markers (Snijman and Meerow, 2010). While tazettine (**6**) has been previously isolated from three different species of *Cyrtanthus*, viz., *Cyrtanthus breviflorus* Harv. (Crouch et al., 2005), *Cyrtanthus falcatus* (Elgorashi et al., 2003) and *Cyrtanthus obliquus* (L.f.) (Brine et al., 2002) this is the first report of the isolation of 11-hydroxyvittatine (**10**).

Brunsvigia has about 20 species in total and is widespread in southern Africa from Tanzania to the Cape Province (Meerow et al., 1999). Different alkaloids have been isolated from these species and in particular, crinamine (**11**) was produced by almost all the species studied, viz., *Brunsvigia radulosa* (Campbell et al., 2000), *Brunsvigia orientalis* (Viladomat et al., 1996), *Brunsvigia josephinae* (Viladomat et al., 1994), *Brunsvigia cooperi* (Dry et al., 1958) and *Brunsvigia rosea* (Mason et al., 1955). To the best of our knowledge, this is the first report of the isolation of 11-hydroxyvittatine (**10**) from this genus.

4. Conclusions

The alkaloids present in the four indigenous South African Amaryllidaceae viz., *C. buphanoides*, *C. graminicola*, *C. mackenii* and *B. grandiflora* were investigated for the first time. Lycorine (**1**) was the main alkaloid present in all the four species in different amounts. Moreover, 1-*O*-acetyl-(**4**) and 2-*O*-acetyl-lycorine (**5**), pratorimine (**3**), hippadine (**2**) and tazettine (**6**) were isolated from *C. buphanoides*. Haemanthidine (**7**), haemanthamine (**8**) and criwelline (**9**) were isolated from *C. graminicola*, while *C. mackenii* produced tazettine (**6**) and 11-hydroxyvittatine (**10**). Finally, *B. grandiflora* produced crinamine (**11**) and 11-hydroxyvittatine (**10**). The isolation of lycorine (**1**) (2.1 g/kg) from *C. graminicola* is noteworthy considering the strong anticancer activity demonstrated by this alkaloid (He et al., 2015). In fact, the good availability of lycorine should advance its further investigation into its mode of action in various preclinical models of human cancers both in vitro and in vivo. Furthermore, haemanthidine (**7**) was isolated for the second time from a *Crinum* species while 11-hydroxyvittatine (**10**) was isolated for the first time from *Cyrtanthus* and *Brunsvigia* which could be taxonomically important. However, further research is clearly required to improve our understanding of relationships among species in the genera of the Amaryllidaceae family. For this purpose, it seems important to find more distinctive compounds with chemotaxonomical significance and if coupled by the study of non-alkaloidal compounds, this could open up new chemical and biological horizons in the future.

Abbreviations

CC	Column chromatography
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
ESI MS	Electrospray Mass Spectrometry
TLC	Thin Layer Chromatography

Acknowledgements

The work was supported in part by grants from a bilateral project between the Italian and South-Africa Ministry of Foreign Affairs Area: South Africa, Medicine and Health Project number: ZA14MO06, titled: Bioactive metabolites from South-African plants with anticancer activity

and in part by the Programme STAR 2017, financially supported by UniNA and Compagnia di San Paolo grant number E62F16001250003. WvO and IRG thank the South African National Research Foundation (NRF) (Incentive and CPRR funding), and Stellenbosch University (Faculty and Departmental support). WvO and IRG acknowledge this work is based in part by support from the National Research Foundation of South Africa (Grant Number 113322). A NEMBA application is being processed. The authors thank Mr. Pierfrancesco Motti and Ms. Nadia Serino for their contributions to this work and thank Dr. Martin Smit (Curator – Stellenbosch University Botanical Garden) for facilitation of live plant specimens.

References

Antoun, M.D., Mendoza, N.T., Ríos, Y.R., Proctor, G.R., Wickramaratne, D.M., Pezzuto, J.M., Kinghorn, A.D., 1993. Cytotoxicity of *Hymenocallis expansa* alkaloids. Journal of Natural Products 56, 1423–1425.

Bohno, M., Sugie, K., Imase, H., Yusof, Y.B., Oishi, T., Chida, N., 2007. Total synthesis of Amaryllidaceae alkaloids, (+)-vittatine and (+)-haemanthamine, starting from D-glucose. Tetrahedron 63, 6977–6989.

Boit, H.G., Ehmke, H., 1956. Uber Amaryllidacea-alkaloide. 12 Alkaloide von *Nerine bowdeni*, *Crinum powelli*, *Amaryllis belladonna* und *Pancratium maritimum*. Chemische Berichte/Recueil 89, 2093–2097.

Brine, N.D., Campbell, W.E., Bastida, J., Herrera, M.R., Viladomat, F., Codina, C., Smith, P.J., 2002. A dinitrogenous alkaloid from *Cyrtanthus obliquus*. Phytochemistry 61, 443–447.

Campbell, W.E., Nair, J.J., Gammon, D.W., Codina, C., Bastida, J., Viladomat, F., Smith, P.J., Albrecht, C.F., 2000. Bioactive alkaloids from *Brunsvigia radulosa*. Phytochemistry 53, 587–591.

Christenhusz, M.J., Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa 261, 201–217.

Cimmino, A., Masi, M., Evidente, M., Superchi, S., Evidente, A., 2017. Amaryllidaceae alkaloids: absolute configuration and biological activity. Chirality 29, 486–499.

Crouch, N.R., Mulholland, D.A., Chetty, J., Van Staden, J., 2005. Lupane triterpenoid and alkaloid isolates from *Cyrtanthus breviflorus* (Amaryllidaceae). South African Journal of Botany 71, 104–106.

Dasari, R., Banuls, L.M.Y., Masi, M., Pelly, S.C., Mathieu, V., Green, I.R., Van Otterlo, W.A.L., Evidente, A., Kiss, R., Kornienko, A., 2014. C1,C2-ether derivatives of the Amaryllidaceae alkaloid lycorine: Retention of activity of highly lipophilic analogues against cancer cells. Bioorganic & Medicinal Chemistry Letters 24, 923–927.

Dry, L.J., Poynton, M., Thompson, M.E., Warren, F.L., 1958. 947. The alkaloids of the amaryllidaceae. Part IV. The alkaloids of *Brunsvigia cooperi* Baker. Journal of the Chemical Society 4701–4704.

Elgorashi, E.E., Van Staden, J., 2004. Pharmacological screening of six Amaryllidaceae species. Journal of Ethnopharmacology 90, 27–32.

Elgorashi, E.E., Van Staden, J., Jäger, A.K., 2003. Alkaloids from *Cyrtanthus falcatus*. South African Journal of Botany 69, 593–594.

Evidente, A., Cicala, M.R., Giudicianni, I., Randazzo, G., Riccio, R., 1983. ^{1}H and ^{13}C NMR analysis of lycorine and α-dihydrolycorine. Phytochemistry 22, 581–584.

Evidente, A., Andolfi, A., Abou-Donia, A.H., Touema, S.M., Hammoda, H.M., Shawky, E., Motta, A., 2004. (−)-Amarbellisine, a lycorine-type alkaloid from *Amaryllis belladonna* L. growing in Egypt. Phytochemistry 65, 2113–2118.

Forgo, P., Hohmann, J., 2005. Leucovernine and acetylleucovernine, alkaloids from *Leucojum vernum*. Journal of Natural Products 68, 1588–1591.

Germishuizen, G., Meyer, N.L., 2003. Plants of southern Africa: an annotated checklist. Strelitzia 14. National Botanical Institute, Pretoria.

Ghavre, M., Froese, J., Pour, M., Hudlicky, T., 2016. Synthesis of Amaryllidaceae constituents and unnatural derivatives. Angewandte Chemie International Edition 55, 5642–5691.

Ghosal, S., Rao, P.H., Jaiswal, D.K., Kumar, Y., Frahm, A.W., 1981. Alkaloids of *Crinum pratense*. Phytochemistry 20, 2003–2007.

Ghosal, S., Kumar, Y., Singh, S., 1984. Glucosyloxy alkaloids from *Pancratium biflorum*. Phytochemistry 23, 1167–1171.

He, M., Qu, C., Gao, O., Hu, X., Hong, X., 2015. Biological and pharmacological activities of amaryllidaceae alkaloids. RSC Advances 5, 16562–16574.

Heinrich, M., Teoh, H.L., 2004. Galanthamine from snowdrop — the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. Journal of Ethnopharmacology 92, 147–162.

Houghton, P.J., Howes, M.J., 2005. Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease. Neurosignals 14, 6–22.

Hudlicky, T., Rinner, U., Gonzalez, D., Akgun, H., Schilling, S., Siengalewicz, P., Martinot, T.A., Pettit, G.R., 2002. Total synthesis and biological evaluation of Amaryllidaceae alkaloids: narciclasine, *ent*-7-deoxypancratistatin, regioisomer of 7-deoxypancratistatin, 10b-*epi*-deoxypancratistatin, and truncated derivatives. The Journal of Organic Chemistry 67, 8726–8743.

Ito, M., Kawamoto, A., Kita, Y., Yukawa, T., Kurita, S., 1999. Phylogenetic relationships of Amaryllidaceae based on matK sequence data. Journal of Plant Research 112, 207–216.

Jäger, A.K., Adsersen, A., Fennell, C.W., 2004. Acetylcholinesterase inhibition of *Crinum* sp. South African Journal of Botany 70, 323–325.

Kornienko, A., Evidente, A., 2008. Chemistry, biology, and medicinal potential of narciclasine and its congeners. Chemical Reviews 108, 1982–2014.

Lamoral-Theys, D., Andolfi, A., Van Goietsenoven, G., Cimmino, A., Le Calvé, B., Wauthoz, N., Mégalizzi, V., Gras, T., Bruyère, C., Dubois, J., Mathieu, V., Kornienko, A., Kiss, R., Evidente, A., 2009. Lycorine, the main phenanthridine Amaryllidaceae alkaloid, exhibits significant antitumor activity in cancer cells that display resistance to proapoptotic stimuli: an investigation of structure–activity relationship and mechanistic insight. Journal of Medicinal Chemistry 52, 6244–6256.

Likhitwitayawuid, K., Angerhofer, C.K., Chai, H., Pezzuto, J.M., Cordell, G.A., Ruangrungsi, N., 1993. Cytotoxic and antimalarial alkaloids from the bulbs of *Crinum amabile*. Journal of Natural Products 56, 1331–1338.

Maddry, J.A., Joshi, B.S., Ali, A.A., Newton, M.G., Pelletier, S.W., 1985. Revised structures of pratorinine and pratorimine. Tetrahedron Letters 26, 4301–4302.

Masi, M., Cala, A., Tabanca, N., Cimmino, A., Green, I.R., Bloomquist, J.R., Van Otterlo, W.A.L., Macias, F.A., Evidente, A., 2016. Alkaloids with activity against the Zika virus vector *Aedes aegypti* (L.) — Crinsarnine and sarniensinol, two new crinine and mesembrine type alkaloids isolated from the South African plant *Nerine sarniensis*. Molecules 21, 1432.

Masi, M., Van der Westhuyzen, A.E., Tabanca, N., Evidente, M., Cimmino, A., Green, I.R., Bernier, U.R., Becnel, J.J., Bloomquist, J.R., Van Otterlo, W.A.L., Evidente, A., 2017. Sarniensine, a mesembrine-type alkaloid isolated from *Nerine sarniensis*, an indigenous South African Amaryllidaceae, with larvicidal and adulticidal activities against *Aedes aegypti*. Fitoterapia 116, 34–38.

Masi, M., Mubaiwa, B., Cimmino, A., Van Otterlo, W.A.L., Green, I.R., Evidente, A., 2018. First isolation of acetovanillone and piceol from *Crinum buphanoides* and *Crinum graminicola* (I. Verd.) Amaryllidaceae. South African Journal of Botany 114, 37–39.

Mason, L.H., Puschett, E.R., Wildman, W.C., 1955. Alkaloids of the Amaryllidaceae. IV. Crystalline alkaloids of *Ammocharis coranica* (Ker-Gawl.) Herb., *Brunsvigia rosea* (Lam.) Hannibal and two *Crinum* species. Journal of the American Chemical Society 77, 1253–1256.

Meerow, A.W., Fay, M.F., Guy, C.L., Li, Q.B., Zaman, F.Q., Chase, M.W., 1999. Systematics of Amaryllidaceae based on cladistic analysis of plastid sequence data. American Journal of Botany 86, 1325–1345.

Nair, J.J., 2014. Cytotoxicity studies of lycorine alkaloids of the Amaryllidaceae. Natural Product Communications 9, 1193–1210.

Nair, J.J., Van Staden, J., 2014. Traditional usage, phytochemistry and pharmacology of the South African medicinal plant *Boophone disticha* (L.f.) herb. (Amaryllidaceae). Journal of Ethnopharmacology 151, 12–26.

Nair, J.J., Bastida, J., Codina, C., Viladomat, F., Van Staden, J., 2013. Alkaloids of the South African Amaryllidaceae: a review. Natural Product Communications 8, 1335–1350 (and literature therein cited).

Van Otterlo, W.A.L., Green, I.R., 2018. A review on recent syntheses of Amaryllidaceae alkaloids and isocarbostyrils (time period mid-2016 to 2017). Natural Product Communications 13, 255–277.

Pabuçcuoglu, V., Richomme, P., Gözler, T., Kivçak, B., Freyer, A.J., Shamma, M., 1989. Four new crinine-type alkaloids from *Sternbergia* species. Journal of Natural Products 52, 785–791.

Rao, R.K., 1970. Chemical examination of *Cyrtanthes mackenii* and *Co-operanthes* hybrid. Current Science 39, 134–135.

Razafimbelo, J., Andriantsiferana, M., Baudouin, G., Tillequin, F., 1996. Alkaloids from *Crinum firmifolium* var. *hygrophilum*. Phytochemistry 41, 323–326.

Refaat, J., Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012a. *Crinum*; an endless source of bioactive principles: a review. Part I; *Crinum* alkaloids: lycorine-type alkaloids. International Journal of Pharmaceutical Sciences and Research 3, 1883.

Refaat, J., Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012b. *Crinum*; an endless source of bioactive principles: a review. Part III; *Crinum* alkaloids: Belladine-, galanthamine-, lycorenine-, tazettine-type alkaloids and other minor types. International Journal of Pharmaceutical Sciences and Research 3, 3630.

Refaat, J., Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012c. *Crinum*; an endless source of bioactive principles: a review, part II; *Crinum* alkaloids: crinine-type alkaloids. International Journal of Pharmaceutical Sciences and Research 3, 3091.

Refaat, J., Kamel, M.S., Ramadan, M.A., Ali, A.A., 2013. *Crinum*; an endless source of bioactive principles: a review. Part V. Biological profile. International Journal of Pharmaceutical Sciences and Research 4, 1239.

Snijman, D.A., Meerow, A.W., 2010. Floral and macroecological evolution within *Cyrtanthus* (Amaryllidaceae): inferences from combined analyses of plastid ndhF and nrDNA ITS sequences. South African Journal of Botany 76, 217–238.

Tram, N.T.N., Titorenkova, T.V., Bankova, V.S., Handjieva, N.V., Popov, S.S., 2002. *Crinum* L. (Amaryllidaceae). Fitoterapia 73, 183–208.

Viladomat, F., Bastida, J., Codina, C., Campbell, W.E., Mathee, S., 1994. Alkaloids from *Brunsvigia josephinae*. Phytochemistry 35, 809–812.

Viladomat, F., Almanza, G.R., Codina, C., Bastida, J., Campbell, W.E., Mathee, S., 1996. Alkaloids from *Brunsvigia orientalis*. Phytochemistry 43, 1379–1384.