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A dinitrogenous alkaloid from *Cyrtanthus obliquus*☆

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Abstract

The ethanolic extract of bulbs of *Cyrtanthus obliquus* (L.f.) Ait yielded the new dinitrogenous alkaloid obliquine (**1**), 3*S*, 4a*S*, 11*S*, 10b*S*-3,4,4a,13,11,5,6-heptahydro-5[2-(4-hydroxyphenyl)ethyl]-3-methoxy-13-methyl-[1,3-dioxolo[4,5-g]indolo[3,3a-c]-isoquinolin-12-one, together with the five known structures 11α-hydroxygalanthamine, 3-epimacronine, narcissidine, tazettine and trisphaeridine. All structures were established using 1D and 2D NMR techniques and HREIMS. The alkaloids were tested for cytotoxicity against two mammalian cell lines and did not show activity at concentrations up to 100 μg/ml.

Keywords: *Cyrtanthus obliquus*; Amaryllidaceae; Alkaloids; Dinitrogenous alkaloids; 11α-Hydroxygalanthamine; 3-Epimacronine; Narcissidine; Obliquine; Tazettine; Trisphaeridine

1. Introduction

As part of our ongoing phytochemical and cytotoxicity studies on South African Amaryllidaceae, we investigated *Cyrtanthus obliquus* (L.f.) Ait, a species indigenous to the Western Cape, Eastern Cape and KwaZulu Natal Provinces of South Africa (DuPlessis and Duncan, 1989). A decoction of *Cyrtanthus obliquus* bulbs is taken as a traditional medicine by the Zulu people to treat chronic coughs and scrofula, whilst the dried bulb scales are used as a snuff for the relief of headaches (Watt and Breyer-Brandwijk, 1962). In this study we describe the isolation and characterisation of the novel dinitrogenous alkaloid obliquine (**1**), 3*S*, 4a*S*, 11*S*, 10b*S*-3,4,4a,13,11,5,6 - heptahydro - 5[2 - (4 - hydroxyphenyl)ethyl] - 3 - methoxy - 13 - methyl - [1,3 - dioxolo[4,5 - g]indolo[3,3a - c] - isoquinolin-12-one, together with the known structures, tazettine (**2**) 11α-hydroxygalanthamine, 3-epimacronine, narcissidine, and trisphaeridine. Obliquine represents the third member of a new subgroup of the Amaryllidaceae alkaloids, where a nitrogen atom replaces the oxygen atom in position 5 of a tazettine type of molecule, and that nitrogen atom is substituted by a pendant 6-hydroxyphenethyl moiety, and follows the isolation of (+)-plicamine (**3**) and (−)-secoplicamine (**4**) (Ünver et al., 1999) (Fig. 1). The structure and stereochemistry of **1** was determined by detailed 1D and 2D NMR experiments and HREIMS.

2. Results and discussion

A HREIMS provided *m*/*z* 448.19980 [M^+], which is in accordance with the calculated molecular weight of 448.19982 for the formula $C_{26}H_{28}N_2O_5$. Initial examination of the ^{1}H NMR spectrum of **1** showed the characteristic singlets associated with the *para*-orientated aromatic protons H-7 and H-10 at δ 6.46 and 6.75, respectively, which together with the methylenedioxy singlet at δ 5.88, established the substitution pattern of the aromatic A ring. The presence of an additional two doublets in the aromatic region (δ 7.01, δ 6.73), integrating for two hydrogens each, suggested the presence of a *para*-disubstituted benzene ring, which was determined to be the pendant phenethyl moiety. These doublets were assigned to the two pairs of *ortho*-coupled protons at 4′ and 8′, and at 5′ and 7′, respectively. The

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four aliphatic protons of this phenethyl moiety were distinguished as a *dt* at δ 3.29, a further *dt* at δ 3.23, and a separate triplet at δ 2.75. The two double triplets were assigned to the geminal protons on C-1′, whilst the triplet was assigned to the geminal protons on C-2′ of the phenethyl moiety. The ROESY experiment supported this assignment by providing spatial correlations for H-1′a and b, and H-2′a and b with the AB system of benzylic protons H-6α and H-6β, which resonated as two doublets at δ 3.67 and δ 3.92, respectively. H-6β was more deshielded due to its coplanarity with the nitrogen lone pair.

The C ring was characterised by a *d* at δ 5.89 and a *dd* at δ 6.05, which were assigned to the methine protons H-1 and H-2 respectively. A multiplet at δ 3.89 linked to a carbon resonating at δ 71.2 suggested that the methoxyl group was positioned at C-3, confirmation coming from a $^3J_{CH}$ HMBC contour with C-3 and ROESY correlations with H-2 and H-3. H-3 in turn, coupled in the COSY spectrum with the geminal protons H-4α

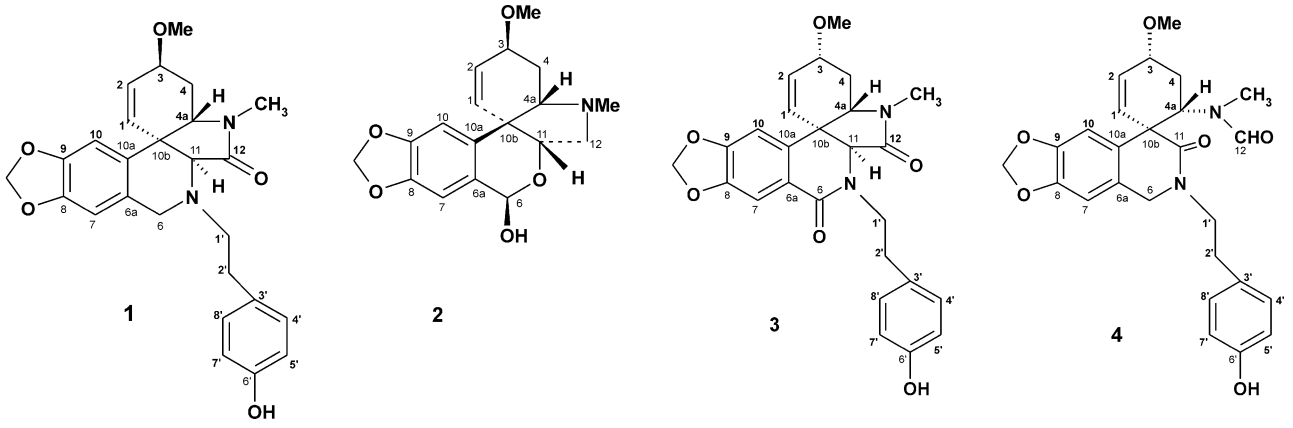

Fig. 1. Structures of obliquine (**1**), tazettine (**2**), plicamine (**3**) and secoplicamine (**4**).

Table 1
^{1}H NMR, HSQC and HMBC data for **1** in $CDCl_3$. Carbon multiplicities were established by DEPT data

Position	δH (*J* in Hz)	HSQC	HMBC
1	5.89 *d* (9.5)	136.5 *d*	C-2, C-3, C-4a, C-10a, C-10b, C-11
2	6.05 *dd* (9.5, 5.3)	124.3 *d*	C-1, C-3, C-4, C-10b
3	3.89 *m*	71.2 *d*	C-1, C-2, C-4a, OMe
4	α 1.49 *ddd* (13.5, 11.5, 3.0)	30.4 *t*	
	β 2.36 *dt* (13.5, 4.0)		C-2, C-3, C-4a, C-10b
4a	3.75 *dd* (11.5, 4.5)	61.6 *d*	C-10a, C-10b, C-11, C-12
6	α 3.67 *d* (15.3)	50.5 *t*	C-6a, C-7, C-10a, C-11, C-1′
	β 3.92 *d* (15.3)		C-6a, C-7, C-10a, C-11, C-1′
6a		129.1 *s*	
7	6.46 *s*	106.0 *d*	C-6, C-9, C-10a
8		146.8 *s*	
9		146.9 *s*	
10	6.75 *s*	108.4 *d*	C-6a, C-8, C-10a, C-10b
10a		130.5 *s*	
10b		44.4 *s*	
11	3.64 *s*	66.0 *d*	C-1, C-6, C-10a, C-10b, C-12, C-1′
12		172.3 *s*	
1′a	3.29 *dt* (12.5, 8.0)	56.8 *t*	C-6, C-11, C-2′, C-3′
1′b	3.23 *dt* (12.5, 8.0)		C-6, C-11, C-2′, C-3′
2′a, 2′b	2.75 *t* (8.0) 2H	33.8 *t*	C-1′, C-3′, C-4′, C-8′
3′		132.0 *s*	
4′, 8′	7.01 *d* (8.7) 2H	129.8 *d*	C-2′
5′, 7′	6.73 *d* (8.7) 2H	115.2 *d*	C-3′, C-6′, C-4′, C-8′
6′		154.3 *s*	
NMe	2.71 *s*	27.7 *q*	C-4a, C-12
OMe	3.45 *s*	56.5 *q*	C-3
OCH_2O	5.88 *s*	100.9 *t*	C-8, C-9

Table 2
Scalar and spatial correlation of the protons of **1**

Proton	COSY	ROESY
1	H-2	H-2, H-11
2	H-1, H-3	H-1, H-3, OMe
3	H-2, H-4α, H-4β	H-2, H-4α, H-4β, OMe
4	α H-3, H-4β, H-4a	H-3, H-4β, H-4a, H-11, NMe
	β H-3, H-4α, H-4a	H-3, H-4α, H-4a, NMe
4a	H-4α, H-4β	H-4α, H-4β, H-10, NMe
6	α H-6β	H-6β, H-7, H-11, H-1′a, H-1′b, H-2′a, H-2′b
	β H-6α	H-6α, H-7, H-1′a, H-1′b, H-2′a, H-2′b
7		H-6α, H-6β
10		H-4a
11		H-1, H-4α, H-6α, H-1′a, H-1′b, H-2′a, H-2′b, NMe
1′a	H-1′b, H-2′a, H-2′b	H-6α, H-6β, H-11, H-2′a, H-2′b, H-4′, H-8′
1′b	H-1′a, H-2′a, H-2′b	H-6α, H-6β, H-11, H-2′a, H-2′b, H-4′, H-8′
2′a	H-1′a, H-1′b, H-4′, H-8′	H-6α, H-6β, H-11, H-1′a, H-1′b, H-4′, H-8′
2′b	H-1′a, H-1′b, H-4′, H-8′	H-6α, H-6β, H-11, H-1′a, H-1′b, H-4′, H-8′
4′, 8′	H-2a′, H-2′b, H-5′, H-7′	H-1′a, H-1′b, H-2′a, H-2′b, H-5′, H-7′
5′, 7′	H-4′, H-8′	H-4′, H-8′
NMe		H-4α, H-4β, H-4a, H-11
OMe		H-2, H-3
OCH_2O		

(δ 1.49) and H-4β (δ 2.36). Ünver et al. (1999), assigned an alpha orientation to the OMe group in both plicamine (**3**) and secoplicamine (**4**), which is surprising since their CD curves closely resembled that of tazettine (**2**) (Fig. 1). We have established that in the obliquine structure the configuration is beta. For H-3α the dihedral angles between H-4α and H-3α and H-4α and H-4a are approximately 45° and 180° respectively. This is consistent with the calculated coupling constants for the H-4α *ddd* of 13.5, 11.5 and 3.0 Hz. An H-3β would result in two trans diaxial couplings. Assignments in this ring were completed by a *dd* at δ 3.75 attributed to H-4a.

The identification of ring D as a γ-lactam structure was confirmed by $^3J_{CH}$ HMBC correlations between the deshielded N–Me protons (δ 2.71) to both C-4a (δ 61.6) and the carbonyl group at C-12 (δ 172.3) and a $^2J_{CH}$ contour from H-11 to C-12. Key ROESY contours in this region of the structure were H-4a to H-10 and N–Me and H-11 to H-4α, H-1′a and 1′b and H-2′a and 2′b, confirming the beta orientation for H-4a and the alpha orientation for H-11.

All protonated carbon resonances could be assigned from the HSQC spectrum, and the multiplicities were determined from the DEPT spectra. The ^{13}C NMR and DEPT spectra confirmed the 26 carbon resonances, made up of two methyl, five methylene, eleven methine and eight quaternary carbon resonances. The HMBC correlations which identified all the quaternary carbons were from H-1 to C-10a and C-10b, H-4β to C-10b, H-4a to C-10a, C-10b and C-12, H-6α to C-6a and C-10a, H-6β to C-6a and C-10a, H-7 to C-9 and C-10a, H-10 to C-6a, C-8 and C-10b, H-11 to C-10a, C-10b and C-12, H-1′a and H-1′b to C-3′, H-2′a and H-2′b to C-3′, H-5′, 7′ to C-3′ and C-6′, NMe to C-12, and OCH_2O to C-8 and C-9 (Table 1). Further key ROESY correlations were from H-1 to H-11, and H-6α and H-6β to H-1′a, H-1′b, H-2′a and H-2′b (Table 2).

The alkaloids 3-epimacronine, narcissidine, tazettine and trisphaeridine were identified by direct comparison of their melting points and their spectroscopic properties (MS, IR, OR, ^1H and ^{13}C NMR) with those given in the literature (Bastida et al., 1998; Viladomat et al., 1997). The structure of 11α-hydroxygalanthamine was confirmed by comparison of its spectroscopic data with an authentic sample previously isolated in our laboratories (Brine et al., 2002).

3. Experimental

3.1. General

Mps are uncorr. Optical rotations were taken on a Perkin-Elmer 241 Polarimeter. CD: Jasco J-700 Spectropolarimeter. HREIMS were run on a Varian VG70-SEQ instrument and EIMS were run on a Hewlett Packard 5989A Mass Spectrometer at 70 eV. ^1H, ^{13}C NMR, DEPT, COSY, HSQC, HMBC (60 and 110 ms) and ROESY (300 ms) spectra were recorded on Varian VXR 500, 300 or 200 instruments in $CDCl_3$ with TMS as internal standard. Chemical shifts are reported in

units of δ (ppm) and coupling constants (J) are expressed in Hz. Analytical TLC (0.25 mm) was carried out on Silica gel 60 plates F_{254} (Merck). Viewing the chromatogram spots under UV light (254/365 nm) and spraying with Dragendorff's reagent confirmed the presence of alkaloids. Prep. TLC was performed on Silica gel 60 F_{254} (Merck) glass plates (0.25 mm, 20×20 cm). After development, the alkaloids were recovered from the silica in methanol.

3.2. Plant material

C. obliquus (L.f.) Ait bulbs were supplied by Welland Cowley of Cape Flora Nurseries in Port Elizabeth. A voucher specimen is housed in the Selmar Schonland Herbarium, Grahamstown, South Africa.

3.3. Extraction and isolation of alkaloids

The fresh bulbs were finely chopped and exhaustively extracted by vigorous shaking with cold MeOH (5 × 2 l) for 24 h, followed by Soxhlet extraction (2 l) for 12 h. The hot and cold crude extracts were pooled and evaporated under reduced pressure. The residue was then dissolved in distilled water with sonication, and acidified with 10% hydrochloric acid to pH 3. The neutral material was removed by partitioning with diethyl ether (6 × 50 ml). The remaining solution was then basified with 10% NH_4OH solution to pH 8–9 and repartitioned with ethyl acetate (15 × 50 ml) and finally, with ethyl acetate: methanol (9:1) (3 × 50 ml). The basic extracts were combined and dried in vacuo to yield brown gummy residues (1.56 g). The acidic and basic extracts were analysed by TLC, eluting with 10% methanol: ethyl acetate in an ammonia atmosphere. The basic extract was chromatographed by VLC (10 × 5 cm) on Silica gel eluting with hexane, increasing the polarity with EtOAc (up to 100% EtOAc) and later with EtOAc: MeOH (up to 100% MeOH), at 200 ml per fraction. Fractions were combined to give 6 main fractions containing alkaloids, on the basis of similar TLC profiles.

Fraction Alk1 (18 mg) was purified by prep. TLC, eluting with hexane–EtOAc (8:2) to yield trisphaeridine (3 mg). Fraction Alk2 (76 mg) was further fractionated by prep. TLC with hexane–EtOAc (1:9) to give two further fractions. Fraction Alk2.1 yielded the novel alkaloid obliquine (**1**, 9 mg). Fraction Alk2.2 was purified by column chromatography on Sephadex LH 20, and yielded 3-epimacronine (6 mg). Fraction Alk3 (34 mg) was subjected to prep. TLC, eluting with 100% EtOAc in NH_3 atmosphere, and yielded brown crystals of tazettine (**5**, 8 mg). Fraction Alk4 (238 mg) was subjected to another round of acid-base extraction to yield a basic alkaloidal extract, which was purified by prep. TLC, in EtOAc–MeOH (1:1), resulting in two main alkaloid regions. Fraction Alk4.1 was purified by further prep. TLC in 100% EtOAc in NH_3 atmosphere, yielding narcissidine (23 mg). Fraction Alk4.2 was identified as 11α-hydroxygalanthamine (4 mg). Fraction Alk5 was further purified by a second acid-base extraction, yielding a basic alkaloidal extract, which was then fractionated by prep. TLC hexane–EtOAc (1:1), and yielded more trisphaeridine (4 mg) and more tazettine (**2**, 5 mg). Fraction Alk6 was subjected to further acid-base extraction, and then purified by prep. TLC with 100% EtOAc in NH_3 atmosphere, resulting in the isolation of more tazettine (**2**, 3 mg).

3.3.1. Obliquine (*1*)

Amorphous solid, mp 136–139 °C. $[\alpha]_D^{20}$ = -20.5° (MeOH; *c* 0.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3310 (OH), 3013, 2927, 2855, 2362, 1672 (lactam CO), 1513, 1482, 1238, 1037, 1039, 934, 833, 759 136–139. HREIMS *m*/z 448.19980 $[M]^+$ ($C_{26}H_{28}N_2O_5$ requires: 448.19982).

3.4. Cytotoxicity assay

The cytotoxicity of the alkaloids against Chinese Hamster Ovarian (CHO) cells and Human Heptoma (HepG2) cells was evaluated in 96 multiwell plates, plated at 1 × 10^4 cells/well in 200 μl of $HAMS_{F\text{-}12}$: DMEM (1:1) for the CHO cells and DMEM + Glutamax-1 for the HepG2 cells, supplemented with 10% heat-inactivated FCS (all purchased from Highveld Biological, Johannesburg, South Africa). The cells were incubated with the alkaloids for 48 h and the cell viability was then determined using MTT tetrazolium salt colorimetric assay, first described by Mosmann (1983).

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