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Alkaloids and triterpenoids from *Ammocharis coranica* (Amaryllidaceae)

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Abstract

The bulbs of *Ammocharis coranica* yielded eight alkaloids: lycorine, acetylcaranine and crinamine, which have been reported previously from *A. coranica*, 1-*O*-acetyllycorine, hippadine, 6α-hydroxypowelline and hamayne, which have been reported from other members of the Amaryllidaceae, 1-*O*-acetyl-9-*O*-demethylpluviine, which has not been described previously, and the known cycloartane compounds: 24-methylenecycloartan-3β-ol, cycloeucalenol, cycloeucalenone and also 24-methylenepollinastanone, which has not been described previously.

Keywords: Amaryllidaceae; *Ammocharis coranica*; Triterpenoids; Alkaloids; 24-Methylenecycloartan-3β-ol; Cycloeucalenol; Cycloeucalenone; 24-Methylene-pollinastanone; Lycorine; 1-*O*-Acetyllycorine; Hippadine; Acetylcaranine; 1-*O*-Acetyl-9-O-demethylpluviine; 6α-Hydroxypowelline; Hamayne; Crinamine

1. Introduction

Ammocharis coranica (Ker Gawl.) Herb., from an African genus of five species (Snijman and Linder, 1996), is one of the most widespread amaryllids of the summer-rainfall region of southern Africa. It is known to the Zulu as *incotho* (Hulme, 1954), a vernacular term also applied to *Boophane disticha* (L.f.) Herb. (Amaryllidaceae) (Gerstner, 1938), a well-documented hallucinogen, arrow poison and homicidal agent of the region (Neuwinger, 1994; Viladomat et al., 1997). A healer from the Nongoma District of Zululand reported that *A. coranica* was used as a substitute for *B. disticha* when the latter was unavailable, for the treatment of mentally ill patients. The treatment of hysteria using *Boophane* has earlier been reported (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). The bulb has further been considered a cure for unspecified afflictions resulting from witchcraft (Hulme, 1954) and (*sensu Ammocharis falcata* Herb.) a useful medicine for cattle (Gerstner, 1938). Across much of its range the outer bulb scales are partially burned in the production of a plastic pitch-like substance which is moulded into traditional headrings for tribal chiefs (Pole-Evans, 1938; Gerstner, 1941) or used as a putty and adhesive (Jacot Guillarmod, 1971; Giess and Snyman, 1972). Extensible cottony threads are recorded (Snijman and Linder, 1996) as a characteristic feature of bulb scales of members of the tribe Amaryllideae to which *Ammocharis* belongs.

Although *A. coranica* apparently harbours psychoactive constituents, these have not been pharmacologically characterised. It is speculated that crinamine may account for the reputed calming or hypnotic effects given that this compound has recently been isolated from *Dioscorea dregeana* (Kunth) Dur. and Schinz (Dioscoreaceae) (Page, 1998), another southern African taxon traditionally employed as a sedative (Gerstner, 1941). Further, crinamine is common to *Brunsvigia radulosa* Herb. (syn. *B. cooperi* Baker) (Dry

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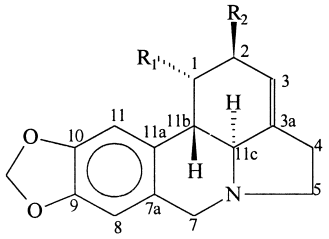
et al., 1958), which is considered to be narcotic (Loubser and Zietsman, 1994). Aerial leaves of *A. coranica* are reputedly grazed by stock (Batten and Bokelmann, 1966; Plowes and Drummond, 1990).

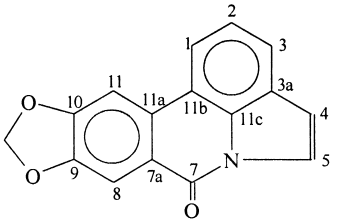
Previous investigations of this species have yielded lycorine, caranine, acetylcaranine, buphanisine, *epi*buphanisine, buphanidrine, ambelline, crinamine, 6-hydroxycrinamine, *epi*vittatine and an uncharacterised alkaloid, coranicine (Mason et al., 1955; Hauth and Stauffacher, 1962). No triterpenoids have been reported previously from this species.

2. Results and discussion

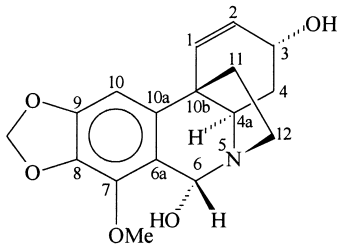
The basic chloroform extract of the bulbs of this species afforded the isoquinoline alkaloids, lycorine (**1**), 1-*O*-acetyllycorine (**2**), hippadine (**3**), 6α-hydroxypowelline (**4**), hamayne (**5**), crinamine (**6**), acetylcaranine (**7**) and 1-*O*-acetyl-9-*O*-demethylpluviine (**8**). Also isolated from the basic chloroform extract were four cycloartane compounds, 24-methylenecycloartan-3β-ol (**9**), cycloeucalenol (**10**), cycloeucalenone (**11**) and 24-methylenepollinastanone (**12**). Structures of the known compounds **1**–**7** and **9**–**11** were confirmed by comparison of spectroscopic and other physical properties against literature values (Kobayashi et al., 1984; Likhitwitayawuid et al., 1993; Evidente, 1986; Pettit et al., 1984; Ghosal et al., 1981; Ali et al., 1981; Lauk et al., 1991; Slabaugh and Wildman, 1971; Viladomat et al., 1996; de Pascual Teresa et al., 1987; Tavares et al., 1995; Khuong-Hu et al., 1975; Akihisa et al., 1986).



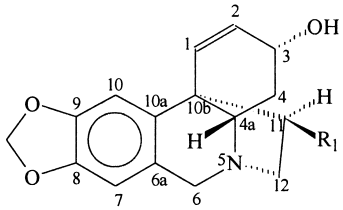
lycorine (**1**) R_1 - OH, R_2 - OH
1-O-acetyllycorine (**2**) R_1 - AcO, R_2 - OH
acetylcaranine (**7**) R_1 - AcO, R_2 - H



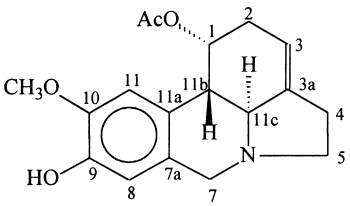
hippadine (**3**)



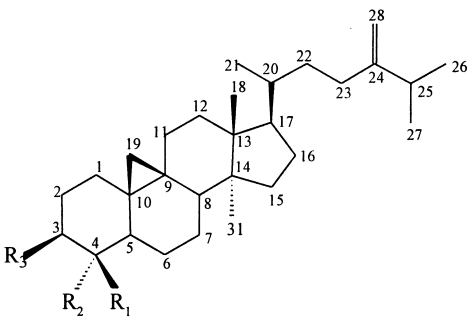
6-α-hydroxy powelline (**4**)



hamayne (**5**) R_1 - OH
crinamine (**6**) R_1 - OCH_3



1-O-acetyl-9-O-demethylpluviine (**8**)



24-methylenecycloartan-3β-ol (**9**) R_1 - CH_3, R_2 - CH_3, R_3 - OH
cycloeucalenol (**10**) R_1 - H, R_2 - CH_3, R_3 - OH
cycloeucalenone (**11**) R_1 - H, R_2 - CH_3, R_3 = O
24-methylenepollinastanone (**12**) R_1 - H, R_2 - H, R_3 = O

The stereochemistry in all cases was confirmed by NOE experiments and the orientation of the 11,12-bridge in compounds **4**–**6** was established by means of CD spectroscopy (Wagner et al., 1996).

The NMR spectra of compound **8** were similar to those of 1-*O*-acetylcaranine (**7**), but instead of the two proton methylenedioxy resonance at δ 5.89, had a methoxy group three proton singlet at δ 3.83. The mass spectrum indicated a molar mass of 315 g/mol for **8**, 18 g/mol higher than for 1-*O*-acetylcaranine. The molecular formula indicated two extra hydrogens and an extra oxygen and this indicated that instead of the methylenedioxy group at C-9 and C-10, a hydroxy and methoxy group occurred at these positions. Irradiation of H-11 gave a positive NOE for the methoxy group protons at δ 3.83 and the H1β proton which occurred at δ 6.06. Thus the methoxy group was placed at C-10 and the hydroxy group at C-9, to give 1-*O*-acetyl-9-*O*-demethylpluviine. This compound has not been reported previously although the isomer, 1-*O*-acetyl-10-*O*-demethylpluviine (Kreh et al., 1995) as well as 9-*O*-demethylpluviine (Uyeo and Yanaihara, 1959) are known. Carbon assignments were made with the aid of DEPT and HETCOR experiments.

Cycloartane triterpenoids have not been reported previously from the Amaryllidaceae family. The ^{1}H- and ^{13}C-NMR spectra of compound **12** showed that it had the same sidechain and 9,10,19-cyclopropane ring as the co-occurring 24-methylenecycloartan-3β-ol (**9**), cycloeucalenol (**10**) and cycloeucalenone (**11**). The presence of a resonance at δ 212.0 in the ^{13}C-NMR spectrum indicated the presence of a 3-ketone as in cycloeucalenone. However, the ^{1}H-NMR spectrum showed that only the 3H-21, 3H-26 and 3H-27 methyl group proton doublets and 3H-18 and 3H-31 methyl group singlets were present. The C-29 methyl group proton doublet present in the ^{1}H-NMR spectrum of cycloeucalenone was absent and thus suggesting the absence of methyl groups at C-4. This was confirmed by an extra downfield methylene carbon resonance at δ 48.5, which was not present in the ^{13}C-NMR spectrum of the other cycloartanoids isolated. This compound has not been reported previously although the related 24-methylenepollinastan-3β-ol is known (Akihisa et al., 1997).

3. Experimental

3.1. General

Fresh bulbs of *A. coranica* were collected at Ashburton, Kwazulu-Natal and a voucher *s*pecimen retained in the Natal Herbarium (*Crouch 766*). The bulbs were cut into small pieces, air dried overnight, and extracted with 95% ethanol on a Labcon shaker for 96 h yielding, after evaporation of solvent, 7.2 g of extract. This was dissolved in water (100 ml) and acidified to pH 4. The acidic extract was then extracted with chloroform (3 × 200 ml) to yield the acidic chloroform extract (2.8 g). The aqueous solution was then made basic to pH 10 and extracted further with chloroform (3 x 200 ml) to yield the basic chloroform extract (3.5 g). The acidic and basic $CHCl_3$ extracts were very similar on TLC and crude ^{1}H NMR spectra. However the acidic $CHCl_3$ extract contained more fats and less of the alkaloids. It was therefore decided to work with the basic $CHCl_3$ extract. The basic chloroform extract was separated by means of column chromatography over silica gel (Merck 9385) and yielded lycorine (**1**)(117 mg), 1-*O*-acetyllycorine (**2**)(23 mg), hippadine (**3**)(14 mg), 6α-hydroxypowelline (**4**)(32 mg), hamayne (**5**)(48 mg), crinamine (**6**)(35 mg), acetylcaranine (**7**)(56 mg), 1-*O*-acetyl-9-*O*-demethylpluviine (**8**)(23 mg), 24-methylenecycloartan-3β-ol (**9**)(29 mg), cycloeucalenol (**10**)(21 mg), cycloeucalenone (**11**)(22 mg) and 24-methylenepollinastanone (**12**)(28 mg). Compounds **1**–**7** and **9**–**11** were identified by comparison of physical data (NMR, IR, UV and CD spectra, MPs and optical rotations) against literature values (Kobayashi et al., 1984; Likhitwitayawuid et al., 1993; Evidente, 1986; Pettit et al., 1984; Ghosal et al., 1981; Ali et al., 1981; Lauk et al., 1991; Slabaugh and Wildman, 1971; Viladomat et al., 1994; de Pascual Teresa et al., 1987; Tavares et al., 1995; Khuong-Hu et al., 1975; Akihisa et al., 1986).

NMR spectra were recorded in CD_3OD on a Varian 300 MHz spectrometer. HRMS and EIMS were recorded at the Cape Technikon on Kratos HRMS 9/50 and Finnigan 1020 GC MS instruments. IR spectra were recorded on a Nicolet Impact 400D instrument. Optical rotations were recorded on an Optical Activity AA-5 polarimeter and CD spectra were recorded on a Jasco J700 spectro-polarimeter.

3.2. 1-O-acetyl-9-O-demethlylpluviine (*8*)

(23 mg) white crystalline, mp 173° HRMS M^+ at *m/z* 315.1466 ($C_{18}H_{21}NO_4$ requires 315.1469), EIMS: *m/z* (rel. int.): 315 (100.00) [M^+], 314 (13.70) [M^+-H], 256 (16.39) [M^+-CH_3COO], 255 (21.97) [M^+-CH_3COOH], 254 (78.08) [M^+-CH_3COOH - H], 229 (38.58) [M^+-$CHOC(O)CH_3$-CH_2], 228 (63.29) [M^+-$CHOC(O)CH_3$-CH_2-H], 43 (9.61)

^{1}H-NMR: δ_H (ppm) (CD_3OD, 300 MHz) 6.86 (1H, *s*, H-11), 6.61 (1H, *s*, H-8), 6.06 (1H, *d*, H-1, J = 3.4 Hz), 5.48 (1H, *d*, H-3, J = 2.4 Hz), 4.14 (1H, *d*, H-7β, J = 14.0 Hz), 3.83 (3H, *s*, OCH_3), 3.56 (1H, *d*, H-7α, J = 14.0 Hz), 3.33 (1H, *m*, H-5b), 2.89 (1H, *bd*, H-11*c*, J = 10.4 Hz), 2.79 (1H, *d*, H-11*b*, J = 10.4 Hz), 2.68 (1H, *m*, H-2b), 2.64 (2H, *m*, H-4), 2.48 (1H, *dd*, H-5*a*, J = 17.7 Hz, 8.8 Hz), 2.40 (1H, *m*, H-2*a*), 1.90 (3H, *s*, $OCOC\underline{H}_3$).

^{13}C-NMR: δ_C (ppm) (CD_3OD, 75 MHz) 172.4 ($O\underline{C}OCH_3$), 147.8 (C-9), 146.2 (C-10), 139.8 (C-3a), 129.3 (C-7a), 126.3 (C-11a), 115.9 (C-3), 114.9 (C-8), 109.5 (C-11), 67.6 (C-1), 62.8 (C-11c), 57.1 (C-7), 56.4 (OCH_3), 54.7 (C-5), 44.1 (C-11b), 34.2 (C-2), 29.2 (C-4), 21.0 ($OCO\underline{C}H_3$).

IR ν_{max} (KBr) (cm^{-1}: 3440 (O–H stretching); 1735 (C═O stretching); 1518 aromatic C═C stretching); 1255, 1235 (C–N stretching); 1235, 1100, 1037 (C–O stretching)$[\alpha]_D^{22} - 106^\circ$ (c = 0.4; MeOH)

3.3. 24-methylenepollinastanone (**12**)

(28 mg) white crystalline, mp 76–77°C HRMS: [M^+] at m/z 410.3559 ($C_{29}H_{46}O$ requires 410.3548), EIMS: m/z (rel. int.): 410 (40.82) [M^+], 395 (22.89) [M^+-CH_3], 367 (20.33), 327 (21.73), 326 (19.66), 286 (20.59), 285 (81.47), 283 (18.79), 243 (16.81), 229 (17.36), 219 (19.29), 217 (20.75), 203 (19.55), 189 (22.95), 175 (41.48), 173 (17.86), 163 (44.45), 161 (32.01), 159 (20.20), 149 (45.67), 148 (21.41), 147 (41.22), 145 (19.39), 137 (23.32), 135 (46.28), 133 (37.81), 123 (44.62), 121 (60.84), 119 (42.61), 109 (64.60), 107 (71.68), 105 (35.80), 97 (30.85), 95 (100.00), 93 (52.14), 91 (27.10), 83 (41.26), 81 (72.38), 69 (78.50), 67 (34.25), 57 (25.34), 55 (84.05), 43 (46.90).

^{1}H-NMR: δ_H (ppm) ($CDCl_3$, 300 MHz) 4.70 (1H, *s*, H-28*b*), 4.65 (1H, *s*, H-28*a*), 1.01 (3H, *d*, H-27, J = 6.8 Hz), 1.00 (3H, *d*, H-26, J = 6.9 Hz), 0.98 (3H, *s*, H-18), 0.90 (3H, *s*, H-31), 0.89 (3H, *d*, H-21, J = 6.6 Hz), 0.60 (1H, *d*, H-19b, J = 3.8 Hz), 0.33 (1H, *d*, H-19*a*, J = 4.2 Hz).

^{13}C-NMR: δ_H (ppm) ($CDCl_3$, 75 MHz) 212.0 (C-3), 156.9 (C-24), 105.9 (C-28), 52.2 (C-17), 48.9 (C-14), 48.5 (C-4), 46.9 (C-5), 45.4 (C-13), 41.2 (C-2), 39.8 (C-8), 36.1 (C-20), 35.3 (C-12), 35.0 (C-22), 33.8 (C-25), 32.8 (C-15), 32.1 (C-1), 31.3 (C-23), 29.2 (C-9), 28.4 (C-19), 28.1 (C-7), 27.2 (C-16), 25.8 (C-6), 24.9 (C-11), 24.5 (C-10), 22.0 (C-27), 21.9 (C-26), 19.1 (C-31), 18.3 (C-21), 17.8 (C-18).

IR ν_{max} (KBr) cm^{-1}: 1715 (C═O stretching), 2960, 2925 (C–H antisymmetric stretching), 2870, 2850 (C–H symmetric stretching), 1468 (C–H antisymmetric bending), 1377 (C–H symmetric bending), 904 (═CH_2 out of plane bending)$[\alpha]_D^{22} + 37^\circ$ (c = 0.4; $CHCl_3$)

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