Table: Migrated triethyl citrate contents (% w/w) of cores as a function of different relative humidity conditions of the storage medium after 14 days of storage

Relative humidity	Initial triethyl citrate concentration of the coating		
	10% w/w	5% w/w	1% w/w
75%	1.13 ± 0.11	0.56 ± 0.05	0.14 ± 0.01
65%	1.09 ± 0.10	0.47 ± 0.05	0.14 ± 0.01
35%	1.00 ± 0.09	0.44 ± 0.03	0.08 ± 0.01

Experimental

Direct compression was applied to obtain placebo tablets based on Ludipress of 6 mm diameter. The average weight of the tablets was 0.10 ± 0.01 g and the breaking force was 90 N (Erweka). The inner phase of the tablets contained Ludipress and the applied lubricant was 2%w/w Macrogol 4000. 20%w/w Acryl-EZE (Aqueous Acrylic Enteric System, Colorcon, UK) dispersion containing triethyl citrate (Ph.Eur.4) of 1, 5 and 10%w/w concentrations were used for the coating procedure. After the determination of the surface of one tablet, the amount of coating dispersions containing triethyl citrate of different concentrations was calculated to obtain 5.5 g/1000 cm^2 polymer on the core surface of each tablet charge. Fluidized bed coating was achieved with a Aeromatic Strea-1 (Aeromatic AG, Switzerland) laboratory-scale fluidization equipment. The process parameters were the following: Quantity of each charge: 250 g tablets; amount of Acryl-Eze dispersions: 69.2 g; inlet air temperature: 35 °C; drying temperature: 35 °C; atomizing pressure: 2 bar. The coated tablets were transferred into separated desiccators kept at 35%, 65% and 75% RH and room temperature. For storage periods 3, 6, 9 and 14 days were chosen at each RH value. After clearing the coating from the tablet core away with a microtome, the triethyl citrate contents of the tablet cores were quantified by GC/MS. Each sample was dissolved in methanol. A model GCQ mass spectrometer system (Finnigan Corp., Austin, TX) was used with manual split injection (split: 1/50) and 30QC2/BPX5 (SGE) bonded and cross-linked (5% phenyl) methylpolysiloxane capillary column (30 m × 0.25 mm). The temperature of the injection port was 220 °C; the initial temperature of the column was 100 °C and the heating rate was 9 °C/min and the final temperature of the column was 200 °C. The transfer line temperature was constant (200 °C) and the source temperature was 180 °C. Detection was started 4 min after injection. The selective ion method was applied to determine the diagnostic fragments of triethyl citrate (103 m/z) and that of the internal standard (163 m/z). Dimethylphtalate was employed as internal standard and triethyl citrate as external standard. Calibration curves were constructed by plotting the area ratio of triethyl citrate (103 m/z) and internal dimethylphtalate standard (163 m/z) against the amount injected.

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Alkaloids from the bulbs of *Crinum bulbispermum*

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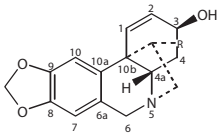
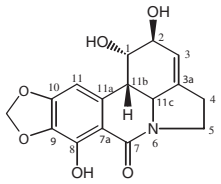
Two new alkaloids, namely 8-hydroxylycorin-7-one and 2-deoxylycorine were isolated from *Crinum bulbispermum* along with the known compounds vittatine, 11-hydroxyvittatine and hippamine.

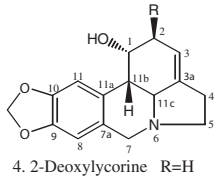
The genus *Crinum* (Amaryllidaceae) is a rich source of alkaloids that are derived mainly from three fundamental types; norbelladine, lycorine and vittatine/crinine-type (Gosal et al. 1985). Many *Crinum* species have been widely used in traditional and modern medicine (Fennell and Van Stader 2001). Recent studies showed that some species of the genus have anti-allergic (Ckpo and Adeyemi 2002a), antianaphylactic (Ckpo and Adeyemi 2002b), analgesic (Ckpo et al. 2001) and anti-inflammatory activities (Samud et al. 1999). Earlier investigations of *C. bulbispermum* grown in Egypt led to the isolation of crinamine, hippadine, powelline and lycorine (El-Moghazi and Ali 1976), crinine and the new alkaloid bulbispermine (Ali et al. 1984). The present study deals with the isolation and structure elucidation of two new alkaloids namely 8-hydroxylycorin-7-one and 2-deoxylycorine, in addition to the known alkaloids vittatine, 11-hydroxyvittatine and hippamine. Structural elucidation of the isolated alkaloids could be achieved using EIMS, 1D- and 2D-NMR spectra.



1. Vittatine R=H
2. Hydroxyvittatine R=OH



3. 8-Hydroxylycorin-7-one



4. 2-Deoxylycorine R=H
5. Hippamine R=OCH_3

The phenolic nature of alkaloid **3** was indicated by a positive $FeCl_3$ test. NMR spectral data showed basic similarities to those of lycorine, with two main differences. The first is the absence of the aromatic signal characteristic for H-8 and the appearance of a D_2O-exchangeable low-field resonance at δ 11.6 attributable to an OH group *peri* to a carbonyl group. The second is the absence of the characteristic signals of CH_2-7 in lycorine, and the appearance of a quaternary carbon signal at δ 168, of a carbonyl group in **3**. NMR spectra showed the presence of a singlet signal at δ 6.99 attributed to H-11, with its corresponding carbon at δ 105.33 as observed from HMQC spectrum. HMBC correlated H-11 to carbons at δ 146.80, 131.21, 124.93 and 35.83. A broad signal was also observed at δ 5.70, attributed to H-3 along with its carbon detected at δ 122.17. HMBC correlated H-3 to carbons at δ 69.01, 135.75 and 30.25. Accordingly the chemical structure was suggested to be; 8-hydroxylycorin-7-one. This suggestion was confirmed by EI-MS, which showed a molecular ion peak at m/z 317 corroborating to a molecular formula to be $C_{16}H_{15}O_6N$. In addition, MS showed fragments characteristic and diagnostic to the lycorine-type at m/z 257 and 256, due to loss of $C_2H_4O_2$ and $C_2H_5O_2$ from the parent molecular ion, respectively (Ibuka et al. 1966). 8-hydroxylycorin-7-one appears to be a new alkaloid.

The ^1H NMR spectrum of alkaloid **4** indicated the presence of two *p*-oriented aromatic protons at δ 6.59 (H-8) and 6.88 (H-11) and an olefinic proton at δ 5.62 (H-3). HMQC indicated their corresponding carbons to be at δ 105.00, 107.42 and 118.37, respectively. It showed a simple AB system at δ 3.65 and 4.17 with a large coupling constant (10.0 Hz) typical for the two geminal protons of C-7. The two coupled pairs of methylene groups at C-5 and C-4 showed a more complex spin system. However, the spectrum showed the absence of N-methyl group, indicating a lycorine-type skeleton (Evidente et al. 1983). Chemical shifts observed at δ 4.50 in ^1H NMR and at δ 70.83 in ^{13}C NMR spectra, were typical for hydroxymethine group at position 1. The *trans*-linkage of the B and C rings was unambiguously deduced from the large coupling constant (9.8 Hz) between H-11b and H-11c (Quirion et al. 1991). All data of this alkaloid are similar to those of lycorine, except for the absence of characteristic signals of the β-hydroxymethine group at position 2 and the appearance of a two-proton multiplet at δ 2.58. 2-D NMR spectra correlated the latter signal to carbons at δ 31.76 (HMQC), 118.37, 141.67, 70.83 and 39.58 (HMBC) indicating a non-substituted C-2. MS confirmed the suggested molecular formula, as it revealed the molecular ion peak at m/z 271, i.e.: 16 mass units less than that of lycorine. In conclusion, alkaloid **4** was identified as 2-deoxylycorine. This is the first report for the isolation of this alkaloid from a natural source.

Spectral data of alkaloids **1** and **2** were found to be of the crinine-type. They were identified as vittatine (Kihara et al. 1991) and 11-hydroxyvittatine (Mugge et al. 1994), respectively, by comparison of the data observed with those published and by co-chromatography with reference samples.

Furthermore, the spectral data of alkaloid **5** were in agreement with those reported for hippamine (Evidente et al. 1984). This is the first report for the isolation of hippamine, vittatine and 11-hydroxyvittatine from the genus *Crinum*.

Brine shrimp bioassay was conducted as a simple, rapid, reliable and very convenient method of biological screening. In this method, the observed toxicity can be understood as an indication of biological activities at lower doses. Values of LD_{50} (μg/ml) of the examined samples were determined. The most effective extracts were the butanol fraction of the acidic extract of the non-flowering bulbs (LD_{50} = 63.1 μg/ml) followed by the ether fraction of the alkaline extract of the flowering bulbs (LD_{50} = 73.0 μg/ml). The other samples showed variable degrees of activity but of lower potencies than the previously mentioned ones.

Experimental

1. General

Melting points were determined using a Stuart SMPI melting point apparatus and were uncorrected. UV spectra were determined using a Perkin Elmer Lambda EZ 201 UV-VIS spectrophotometer. NMR spectra were recorded at 399.95 MHz for ^1H- and 100.58 MHz for ^{13}C NMR, using standard Varian pulse sequence programs. MS were obtained on a VG 7070 E-HF. Silica gel (70–230 mesh, Merck), neutral alumina (BDH) were used for CC and silica gel G (Merck) was used for TLC.

2. Plant material

The flowering and non-flowering ornamental plant *C. bulbispermum* were obtained from the garden of El-Shallalat, Alexandria, Egypt. The identity of the plant was established by Prof. Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Egypt. A voucher sample (MAHA) deposited at Pharmacognosy Department, Faculty of Pharmacy, University of Alexandria, Egypt.

3. Extraction, isolation and purification of compounds

The flowering (1 kg) and non-flowering (500 g) bulbs were macerated in alcohol. The hydroalcoholic extracts of both of them were defatted using light petroleum, then acidified using citric acid (pH 2). The acidified extracts were further fractionated using ether, $CHCl_3$, ethyl acetate and butanol, respectively. The acidic aqueous extracts were then rendered alkaline (pH 10) by ammonia solution and subjected to further fractionation using the previously mentioned sequence of solvents. The obtained extracts were screened by TLC and subjected to the brine shrimp bioassay. The results of the bioassay indicated that, both ether of the alkaline extract of the flowering bulbs and butanol of the acidic extract of the non-flowering bulbs were the most active. Accordingly, the ether fraction of the alkaline extract of the flowering bulbs (3 g) was subjected to CC neutral alumina. Elution was performed with $CHCl_3$ and MeOH with increasing polarity. Fractions eluted with 12% MeOH in $CHCl_3$ were subjected to pTLC on silica gel plates (MeOH : $CHCl_3$; 1 : 9). Two zones were scrapped off and eluted with $CHCl_3$–MeOH, 2 : 1. The zone of R_f 0.44 afforded 100 mg of alkaloid **1** after crystallization from $CHCl_3$ and petroleum ether, while the zone of R_f 0.32 afforded 50 mg of alkaloid **2**. The butanol fraction (300 mg) of the acidic extract of the non-flowering bulbs was subjected to CC silica gel. Fractions eluted with 60% EtOH in EtOAc, gave 10 mg of alkaloid **3** in the form of amorphous powder (MeOH), R_f 0.4 (EtOAc : EtOH : H_2O, 2 : 1 : 2). Ethyl acetate fraction of the alkaline extract of the non-flowering bulbs (100 mg) was subjected to pTLC ($CHCl_3$: MeOH; 1 : 1) and afforded 5 mg of alkaloid **4** in the form of white powder (MeOH); R_f 0.7, and 10 mg of alkaloid **5**, R_f 0.5.

3.1. 8-Hydrocylycorin-7-one (**3**)

Amorphous powder. MS, m/z (rel. int.): $[M]^+$ 317 (40), 257 (100), 256 (70). ^1H NMR ($CDCl_3$ + few drops of CD_3OD) δ 4.44 (br.s, 1 H, H-1), 4.12 (br.s, 1 H, H-2), 5.70 (br. s, 1 H, H-3), 2.69 (m, 2 H, 2 H-4), 2.50 (t, J = 1.6 Hz, 1 H, H-5α, H-5β), 6.99 (s, 1 H, H-11), 2.91 (d, J = 11 Hz, 1 H, H-11b), 3.79 (d, J = 11 Hz, 1 H, H-11c), 6.03 (s, 2 H, $-OC\underline{H}_2O-$), 11.6 (s, 1 H, OH-8). ^{13}C NMR ($CDCl_3$) δ 67.74 (C-1), 69.01 (C-2), 122.17 (C-3), 135.75 (C-3a), 30.25 (C-4), 53.56 (C-5), 168.00 (C-7), 124.93 (C-7a), 145.53 (C-8), 147.80 (C-9), 146.80 (C-10), 105.33 (C-11), 131.21 (C-11a), 35.83 (C-11b), 58.61 (C-11c), 101.11 ($-O\underline{C}H_2O-$).

3.2. 2-Deoxylycorine (**4**)

White powder. UV in MeOH λ_{max} (abs.): 217.5(3.69), 284(2.57). MS, m/z (rel. int.): $[M]^+$ 271 (25), 227 (50), 226 (100), 203 (90), 121 (80). ^1H NMR ($CDCl_3$) δ 4.50 (br.s, 1 H, H-1), 2.58 (m, 2 H, 2 H-2), 5.62 (br.s, 1 H, H-3), 2.63 (m, 2 H, 2 H-4), 2.25 (m, 1 H, H-5α) 3.35 (m, 1 H, H-5β), 3.65 (d, J = 10 Hz, 1 H, H-7α), 4.17 (d, J = 10 Hz, 1 H, H-7β), 6.59 (s, 1 H, H-8), 6.88 (s, 1 H, H-11), 2.71 (d, J = 9.8 Hz, 1 H, H-11b), 2.49 (d, J = 9.8 Hz, 1 H, H-11c), 5.94 (s, 2 H, $-OC\underline{H}_2O-$). ^{13}C NMR ($CDCl_3$) δ 70.83 (C-1), 31.76 (C-2), 118.37 (C-3), 141.67 (C-3a), 28.30 (C-4), 53.78 (C-5), 56.71 (C-7), 128.05 (C-7a), 105.00 (C-8), 146.96 (C-9), 146.43 (C-10), 107.42 (C-11), 129.00 (C-11a), 39.58 (C-11b), 61.29 (C-11c), 101.12 ($-O\underline{C}H_2O-$).

4. The brine shrimp bioassay **(Meyer et al. 1982)**

Different extracts of the plant were prepared and the shrimp eggs were hatched. Ten shrimps were transferred into vials containing the examined samples using a pipette and artificial sea water was added to attain 5 ml. Nauplii can be counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension (3 mg in 5 ml artificial sea water) was added as nutrient to each vial. Surviving nauplii were counted after 24 h for each dose alongside with control.

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