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Bio-guided isolation of acetylcholinesterase inhibitory alkaloids from the bulbs of *Crinum bulbispermum*

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ABSTRACT

Inhibition of acetylcholinesterase, the key enzyme in the breakdown of acetylcholine, is considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease, senile dementia and myasthenia gravis. A potential source of AChE inhibitors is certainly provided by the abundance of plants belonging to family Amaryllidaceae. In the present work, an *In situ* High Performance Thin Layer Chromatography (HPTLC) autobiographic method was employed for preliminary screening of extracts and fractions of the different organs of *Crinum bulbispermum* Milne-Redhead & Schweick. for compounds with AChE inhibitory effects. Among the tested fractions, the chloroform extract of *Crinum bulbispermum* bulbs demonstrated a significant inhibition of AChE in the assay. Complete phytochemical investigation of this biologically active extract resulted in the isolation of eight crystalline alkaloids (I-VIII) in addition to the well-known Amaryllidaceae alkaloid, lycorine. The structures of the isolated compounds were characterized by spectral evidence and by comparing the results with the reported physical and spectral data as ismine (I), trisphaeridine (II), latifine (III), 1-*O*-Acetyllycorine (IV), crinamine (V), powelline (VI), crinine (VII) and 1-epideacetylbowdensine (VIII). It is worth mentioning that this is the first report for the isolation of alkaloids I-IV and VIII from *Crinum bulbispermum* Milne-Redhead & Schweick.

KEYWORDS

Amaryllidaceae;
Crinum bulbispermum;
HPTLC autobiography;
Acetylcholinesterase inhibition.

INTRODUCTION

Family Amaryllidaceae embraces a large number of plants that are of great importance from the medicinal and economic point of view. The Amaryllidaceae alkaloids comprise a unique group of bases that have been produced only by the members of this family. Amaryllidaceae alkaloids were, and still are, subjected to clinical testing as indicated by recent reports. They aroused great interest among researchers in a wide range of biological areas including, antiviral[14], anti-tumor[2,3], anti-malarial[28], immune-modulatory[22] and AChE inhibitory activities[16]. In particular, galanthamine inhibits cholinesterase activity and has given positive findings for the improvement of memory and intellectual functioning in clinical trials involving patients with mild to

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moderate symptoms of Alzheimer's disease. Several members of the Amaryllidaceae family have been investigated for their potential inhibitory activity against AChE[10,18]. For this purpose, an *In situ* HPTLC autobiographic method was employed for preliminary screening of extracts and fractions of the different organs of *Crinum bulbispermum* Milne-Redhead & Schweick. for compounds with AChE inhibitory effects. Among the tested fractions, the chloroform extract of *Crinum bulbispermum* bulbs demonstrated a significant inhibition of AChE in the assay. It was thus decided to investigate this biologically active extract for the isolation, characterization and structure elucidation of its constituents which eventually resulted in the isolation of nine Amaryllidaceae alkaloids in total, five of them have been isolated for the first time from *Crinum bulbispermum* Milne-Redhead & Schweick.

EXPERIMENTAL

Crinum bulbispermum Milne-Redhead & Schweick was collected in April 2008 during the pre-flowering stage, cultivated in Alexandria, Egypt. The plant was kindly identified by the late Professor Dr. Selim Zedan Heneidy (Professor of Plant Taxonomy, Faculty of Science, University of Alexandria; Alexandria, Egypt). Voucher sample is deposited in the department of Pharmacognosy Herberium, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

Chemicals

- **Buffer:** The following buffer A was used; 50 mM NaH_2PO_4–K_2HPO_4 in H_2O (pH 7.8).
- **Enzyme:** AChE from electric eel (type VI-s, lyophilized powder, 292 U/mg solid, 394 U/mg protein) (Sigma, St.Louis, MO, USA) was dissolved in buffer A to make 1000 U/ml stock solution, and further diluted with buffer A to get 8 U/ml enzyme for HPTLC autobiographic screening.
- **Substrate:** Acetylthiocholine iodide (ATCI) (Sigma, St.Louis, MO, USA) 1 mM of ATCI in buffer A was used for HPTLC autobiographic screening.
- **Ellman's reagent:** 5,5×-Dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma, St.Louis, MO, USA) was used for HPTLC autobiographic screening as 1mM of DTNB in buffer A.

Extraction procedures involved in the HPTLC autobiographic screening method

The different organs of the fresh plant of *Crinum bulbispermum* Milne-Redhead & Schweick. were extracted with ethanol (EtOH) (90%). The different extracts were concentrated under reduced pressure and then defatted with petroleum ether, acidified with 5% tartaric acid to pH 2, filtered and then washed with ether (Et_2O). The acidic aqueous phase was rendered alkaline with ammonium hydroxide (NH_4OH) solution to pH 10, and extracted successively with $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$, EtOAc and *n*-BuOH extracts besides the hydroalcholic extracts of the different organs prior to fractionation were concentrated to a small volume and subjected to HPTLC autobiographic screening for AChE inhibitory activity. In view of the interesting AChE inhibitory activity exhibited by the $CHCl_3$ extract of the bulbs of the studied plant, this extract was considered as a good candidate for further phytochemical investigation.

HPTLC autobiographic screening method

An *in situ* HPTLC autobiographic method was employed using the method outlined by[20] for preliminary screening of the extracts and the fractions of the different organs of the selected plant for compounds with AChE inhibitory effects. The hydroalcholic, $CHCl_3$, EtOAC and n-BuOH extracts of the studied plant were concentrated to dryness. Each extract was dissolved in methanol (MeOH) to a concentration of 10 mg/ml, then 6 *u*L of each sample was spotted on silica gel TLC plate using HPTLC technique. The plate was then developed using solvent system $CHCl_3$: MeOH (8: 2); 0.1 mM physostigmine solution in MeOH was also spotted (2 uL) as a reference. After the development of the TLC plate, the dried plate was sprayed with AChE and then left in moist atmosphere (incubator) for 20 min. at 37 °C. The plate was sprayed with ATCI solution and then allowed to dry in a stream of cold air. The plate was then sprayed with DTNB. The enzyme inhibitory activities of the developed spots were detected as white spots against yellow background according to Ellman's method[7].

Extraction, isolation and purification of compounds (I-VIII)

Fresh chopped bulbs (12 Kg) of *Crinum*

bulbispermum Milne-Redhead & Schweick. collected in the pre-flowering stage were exhaustively extracted with EtOH (70%). The extracts were concentrated under reduced pressure and then defatted with petroleum ether. The defatted extracts were acidified with 5% tartaric acid to pH 2, filtered and then washed with Et_2O. The acidic aqueous phase was rendered alkaline with NH_4OH solution to pH 10, and extracted successively with $CHCl_3$, EtOAC and *n*-BuOH. The biologically active $CHCl_3$ extracts were combined and concentrated to a small volume; at this stage, lycorine (1 g) was precipitated, decanted, washed with MeOH and reserved. The crude alkaloidal residue (13 g) left after removal of the solvent was chromatographed on silica gel column (600 g, 5 cm in diameter). Elution was started using $CHCl_3$ then increasing the polarity with MeOH. Eighty fractions, 250 ml each, were collected, screened by TLC and similar fractions were combined together.

Fractions 19-21

Fractions 19-21 (8% MeOH in $CHCl_3$) were rechromatographed through a silica gel column (40 g, 2 cm in diameter). Elution was started by 2% MeOH in $CHCl_3$. Fractions, 30 ml each, were collected. Fractions 10-18 (4% MeOH in $CHCl_3$) were further purified using preparative TLC (pTLC) on fluorescent silica gel plates using the developing system $CHCl_3$: MeOH (19:1). Two bands were scraped off and eluted with $CHCl_3$: MeOH (3:1). The band of R_f 0.5 afforded 10 mg of I as colourless prisms ($CHCl_3$) and the band of R_f 0.44 afforded 7.8 mg of II as colourless needles ($CHCl_3$). In addition, fractions 27-36 (6% MeOH in $CHCl_3$) were further purified using amino-silica gel open column chromatography (0.3 g, 1cm in diameter). Elution was started by $CHCl_3$ saturated with ammonia, then increasing polarity with MeOH. Fractions, 3ml each, were collected. Fractions 7-8 (1% MeOH in $CHCl_3$) afforded 0.3 mg of III as amorphous white powder ($CHCl_3$).

Fractions 22-29

Fractions 22-29 (10% MeOH in $CHCl_3$) were rechromatographed through a silica gel column (30 g, 1cm in diameter). Elution was started by 1% MeOH in $CHCl_3$. Fractions, 20 ml each, were collected. Fractions 8-11 (4% MeOH in $CHCl_3$) were subjected to pTLC using the developing system $CHCl_3$: MeOH (9:1) and gave after elution with $CHCl_3$: MeOH (3:1) 4.2 mg of IV (R_f 0.54) in the form of colourless needles ($CHCl_3$). Furthermore, Fractions 12-16 (6% MeOH in $CHCl_3$) yielded 15 mg of V as colourless needles ($CHCl_3$) after crystallization from MeOH and $CHCl_3$.

Fractions 43-51

Fractions 43-51 (25% MeOH in $CHCl_3$) were rechromatographed through a silica gel column (30g, 1cm in diameter). Elution was started by 2% MeOH in $CHCl_3$. Fractions, 30 ml each, were collected. Fractions 21-28 (9% MeOH in $CHCl_3$) were subjected to pTLC using the developing system $CHCl_3$: MeOH (9:1) and gave after elution with MeOH: $CHCl_3$ (2:1) 20 mg of VI (R_f 0.42) in the form of colourless crystals (MeOH).

Fractions 52-58

Fractions 52-58 (30% MeOH in $CHCl_3$) were further purified using pTLC on fluorescent silica gel plates using the developing system $CHCl_3$: MeOH (9:1). Two bands were scraped off and eluted with MeOH: $CHCl_3$ (2:1). The band of R_f 0.42 afforded 15 mg of VI as colourless crystals (MeOH) and the band of R_f 0.36 afforded 18 mg of VII as colourless crystals (MeOH).

Fractions 67-78

Fractions 67-78 (40% MeOH in $CHCl_3$) were rechromatographed through basic alumina column (50 g, 2 cm in diameter). Elution was started by 2% MeOH in $CHCl_3$. Fractions, 30 ml each, were collected. Fractions 9-22 (6% MeOH in $CHCl_3$) were further purified using amino-silica gel open column chromatography (8 g, 1cm in diameter). Elution was started by $CHCl_3$. Fractions, 3ml each, were collected. Fractions 5-6 (1% MeOH in $CHCl_3$) afforded 3.2 mg of VIII as amorphous white powder (MeOH).

Ismine (I)

Colourless prisms, m.p. 100-102 °C. $[\alpha]_D^{23}$ -11.2° (MeOH, c, 0.31). UV in MeOH λmax (abs.): 216 (2.03), 240 (1.4), 293 (0.71). EI-MS, m/z (rel. int.): $[M]^+$ 257 (9.2), 239 (4.2), 238 (21.8), 223 (8.5), 87 (10.6), 85 (63.3), 83 (100). ^{1}H-NMR ($CDCl_3$) δ 2.75 (s, 3H, *N*-CH_3), 4.21 (d, *J* =11.96 Hz, 1H, H-6\), 4.27 (d, *J* =11.96 Hz, 1H, H-6), 6.01 (s, 2H, -OCH_2O-), 6.69 (s, 1H, H-10), 6.75 (dd, *J* = 8.04 Hz, *J* = undetectable, 1H, H-4), 6.83 (ddd, *J* =7.32,

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J = 8.32, *J* =1 Hz, 1H, H-2),7 (dd, *J* = 7.56, *J* = 1.2 Hz, 1H, H-1), 7.02 (s, 1H, H-7), 7.25 (ddd, *J* =1.44, *J* = 9, *J* = 7.56 Hz, 1H, H-3). ^{13}C-NMR ($CDCl_3$) δ 30.78 (*N*-CH_3), 63.77 (C-6), 101.26 (-OCH_2O-),109.89 (C-7), 110.25 (C-10), 110.8 (C-4), 118.1 (C-2), 127.3 (C-10b), 129.00 (C-3), 129.9 (C-1), 131.12 (C-10a), 133.9 (C-6a), 146.6 (C-4a), 147.52 (C-9),147.59 (C-8).

Trisphaeridine (II)

Colourless needles, m.p. 139-141°C. $[\alpha]_D^{25}$ Optically inactive. UV in MeOH λmax (abs.): 207.5 (0.69), 252 (1.78), 279 (0.61), 307.5 (0.22), 336(0.08), 352.5 (0.05). EI-MS, m/z (rel. int.): $[M]^+$þ 223 (100), 222 (22.7), 167 (13.0), 166 (8.8), 165 (14.1), 164(18.1), 139 (10.9), 138 (31.2), 137 (12.6), 111 (16.5), 85 (21.1), 83 (32.5), 63 (8.0). ^{1}H-NMR ($CDCl_3$) δ 6.18 (s, 2H, -OCH_2O-),7.34 (s, 1H, H-7), 7.61 (ddd, *J* =1.24, *J* = 6.84, *J* =8.04 Hz, 1H, H-2), 7.67 (ddd, *J* =1.44, *J* = 7.08, *J* = 8.28 Hz, 1H, H-3), 7.92 (s, 1H, H-10), 8.13 (dd, *J* = 1.24, *J* = 8.32 Hz, 1H, H-4), 8.37 (br-d, *J* = 8.05 Hz, 1H, H-1), 9.09 (s, 1H, H-6). ^{13}C-NMR ($CDCl_3$) δ 99.9 (C-10), 101.9 (-OCH_2O-),105.49 (C-7), 121.98 (C-1), 123.08 (C-6a), 124.27 (C-10b), 126.67 (C-2), 127.99 (C-3), 130.04 (C-4), 130.23 (C-10a), 144.1 (C-4a), 148.19 (C-8), 151.46 (C-9), 151.8 (C-6).

Latifine (III)

Amorphous white powder, m.p. 215-217 °C. $[\alpha]_D^{22}$ -27.9° (MeOH, c, 0.32). UV in MeOH λmax (abs.): 203.5 (0.97), 228.5(sh) (0.32), 279.5 (0.1). CD $[\Phi]_{278.6}$ -1.23. EI-MS, m/z (rel. int.): $[M]^+$þ 285 (100), 242 (78), 241 (50.8), 225 (54.7), 211 (63.1), 210 (78), 209 (53.4), 191(37.3), 181 (70.9), 91(55.4), 69 (52.1), 56 (80.6). ^{1}H-NMR (CD_3OD) δ 2.24 (s, 3H, *N*-CH_3), 2.56 (dd, *J* =10, *J* = 5 Hz, 1H, H-3ax), 2.83 (dd, *J* = 10, *J* = 5 Hz, 1H, H-3eq), 3.03 (d, *J* = 14 Hz, 1H, H-1ax), 3.29 (d, *J* =14 Hz, 1H, H-1eq), 3.7 (s, 3H, -OCH_3), 4.16 (t, *J* =5 Hz, 1H, H-4), 6.52 (d, *J* =8.44 Hz, 1H, H-7), 6.53 (dd, *J* = 8.6, *J* = 2.2 Hz, 2H, H-3'and H-5'), 6.74 (d, *J* =8.44 Hz, 1H, H-8), 6.78 (dd, *J* = 8.6, *J* = 2.2 Hz, 2H, H-2'and H-6').

1-*O*-Acetyllycorine (IV)

Colourless needles, m.p. 217-219 °C. $[\alpha]_D^{23}$ -106.0° ($CHCl_3$, c, 0.5). UV in MeOH λmax (abs.): 214 (1.9), 261 (1.1), 288 (1.04). IR, ν_{max} cm^{-1}: 3440 (O-H stretching), 1730 (C=O), 1600 (C=C), 1050 (C-O). EI-MS, m/z (rel. int.): $[M]^+$þ 329 (60.1), 269 (20.4),268 (42.6),252 (13.2),250 (23.8), 227 (91.8), 226 (100), 115 (15.3), 92 (29.5) 91(64.6), 65 (21.3). ^{1}H-NMR ($CDCl_3$) δ 1.95 (s,3H, H-2'), 2.4 (dd, *J* =17.56, *J* = 8.8 Hz,1H, H-5*α*), 2.65 (br-s,2H, H-4), 2.76 (br-d, *J* =10.5 Hz, 1H, H-11c), 2.88 (br-d, *J* =10.7 Hz, 1H, H-11b), 3.37 (ddd, *J* =13.4, *J* =8.76, *J* =4.6 Hz, 1H, H-5*β*), 3.53 (d, *J* =14 Hz,1H, H-7*α*), 4.16 (d, *J* =14 Hz,1H, H-7*β*), 4.22 (s,1H, H-2), 5.56 (br-s,1H, H-3), 5.63 (br-s, 1H, H-1), 5.93 (s,2H, -OCH_2O-), 6.58 (s,1H, H-8), 6.69 (s,1H, H-11). ^{13}C-NMR ($CDCl_3$) δ 21.05 (C-2'), 28.59 (C-4), 39.39 (C-11b), 53.64 (C-5), 56.82 (C-7), 61.48 (C-11c), 69.73 (C-2), 72.64 (C-1), 100.95 (-OCH_2O-),104.89 (C-11), 107.3 (C-8), 117.08 (C-3), 127.03 (C-7a), 129.35 (C-11a), 144.36 (C-3a), 146.23 (C-9), 146.46 (C-10), 170.8 (C-1').

Crinamine (V)

Colourless needles, m.p.190-192°C. $[\alpha]_D^{22}$ +180.0° ($CHCl_3$, c, 0.55). CD $[\Phi]_{248}$ -2.6. UV in MeOH λmax (abs.): 212 (1.74), 240(sh) (0.48), 295 (0.71). EI-MS, m/z (rel. int.): $[M]^+$þ 301(1.1), 270(19.0), 269 (96.9), 268(20.1), 241 (83.4), 240 (25.9),225 (13.5), 224 (14.1), 211(19.9), 196 (44.1), 181 (33.5), 167 (34.7), 154 (100), 149 (71.2), 136 (40.7), 126 (31.0), 124 (31.1), 108 (19.5), 96 (50.9), 79 (20.1), 67 (22.7), 56 (33.6). ^{1}H-NMR ($CDCl_3$) δ 2.04 (ddd, *J* =11.7, *J* = 13.16, *J* = 10 Hz,1H, H-4*α*), 2.13 (ddd, *J* =11.7, *J* = 5, *J* = 6 Hz,1H, H-4*β*), 3.22 (dd, *J* =13.16, *J* = 4.6 Hz, 1H, H-4a), 3.33 (dd, *J* =14, *J* =3.44 Hz, 1H, H-12*β*), 3.4 (dd, *J* =14, *J* =6 Hz, 1H, H-12*α*), 3.41 (s,3H, OCH_3), 3.69 (d, *J* =16.8 Hz,1H, H-6*α*), 3.97 (m,1H, H-3), 4.02 (dd, *J* =6, *J* =4.16 Hz, 1H, H-11), 4.3 (d, *J* =16.8 Hz,1H, H-6*β*), 5.89-5.91 (2d, *J* = 1.44 Hz, 2H, -OCH_2O-), 6.25 (br-s, 1H, H-1), 6.25 (br-s, 1H, H-2), 6.48 (s, 1H, H-7), 6.8 (s,1H, H-10).

Powelline (VI)

Colourless crystals, m.p. 200-202 °C. $[\alpha]_D^{23}$ +3.9° ($CHCl_3$, c, 1.0). CD $[\Phi]_{247}$ +2.5. UV in MeOH λmax (abs.): 221 (2.05), 287 (0.27). EI-MS, m/z (rel. int.): 302 (26.5), $[M]^+$þ 301 (100), 258 (33.1), 246 (27.9), 229 (74.7), 217 (50.8), 115 (55.7), 91 (41.1), 65 (24.8), 58 (75.4), 57 (58.4), 56 (53.2). ^{1}H-NMR

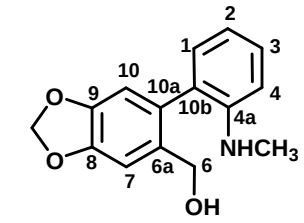
($CDCl_3$) δ 1.72 (ddd, *J* =3.64, *J* =17.32, *J* =13.4 Hz, 1H, H-4*β*), 1.89 (ddd, *J* =17.1, *J* = 5.88, *J* = 11 Hz,1H, H-11*β*), 1.99 (br-d, *J* =11.24 Hz, 1H, H-4*α*), 2.15 (ddd, *J* =12.92, *J* = 9.04, *J* = 4.4 Hz,1H, H-11*α*), 2.85 (ddd, *J* =13.44, *J* = 9.04, *J* = 5.8 Hz,1H, H-12*α*), 3.3 (m,1H, H-12*β*), 3.32 (dd, *J* =13.16, *J* = 4.12 Hz, 1H, H-4a), 3.78 (d, *J* =17.32 Hz,1H, H-6*β*), 3.96 (s,3H, OCH_3), 4.22 (d, *J* =17.32 Hz,1H, H-6*α*), 4.33 (br-s, 1H, H-3), 5.84-5.86 (2d, *J* = 1.44 Hz, 2H, -OCH_2O-), 5.94 (dd, *J* =5.12, *J* =10 Hz, 1H, H-2), 6.54 (d, *J* =10 Hz, 1H, H-1), 6.57 (s, 1H, H-10). ^{13}C-NMR ($CDCl_3$) δ 32.68 (C-4), 44.1 (C-10b), 44.1 (C-11), 53.67 (C-12), 58.6 (C-6), 59.09 (OCH_3), 62.36 (C-4a), 63.95 (C-3), 96.73 (C-10), 100.5 (-OCH_2O-), 117.38 (C-6a), 127.42 (C-2), 132.2 (C-1), 133.34 (C-8), 139.3 (C-10a), 140.93 (C-7), 147.98 (C-9).

Crinine (VII)

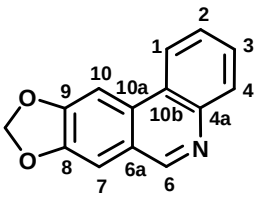
Colourless crystals, m.p. 208-210 °C. $[\alpha]_D^{23}$ -17.5° ($CHCl_3$, c, 1.0). CD $[\Phi]_{248}$ +2.2. UV in MeOH λmax (abs.): 213 (1.8), 237 (0.7), 294 (1.07). EI-MS, m/z (rel. int.): 272 (18.6), $[M]^+$þ 271 (100), 270 (11.2), 228 (22.2), 199 (50.9), 187 (44.8), 173 (15.6), 172 (13), 129 (21.4), 115 (27.9), 57 (32), 56 (10.3). ^{1}H-NMR ($CDCl_3$) δ 1.74 (ddd, *J* =4.2, *J* =17.56, *J* =13.6 Hz, 1H, H-4*β*), 1.92 (ddd, *J* =17.8, *J* = 5.88, *J* = 10.48 Hz,1H, H-11*β*), 1.99 (ddd, *J* =2.68, *J* =4.12, *J* =13.92 Hz, 1H, H-4*α*), 2.18 (ddd, *J* =12.96, *J* = 9.04, *J* = 4.4 Hz,1H, H-11*α*), 2.91 (ddd, *J* =13.2, *J* = 9.04, *J* = 5.88 Hz,1H, H-12*α*), 3.33 (m,1H, H-12*β*), 3.38 (dd, *J* =13.6, *J* = 4.16 Hz, 1H, H-4a), 3.78 (d, *J* =16.84 Hz,1H, H-6*β*), 4.35(m, 1H, H-3), 4.39 (d, *J* =16.84 Hz,1H, H-6*α*), 5.89-5.91 (2d, *J* = 1.44 Hz, 2H, -OCH_2O-), 5.96 (dd, *J* =5.36, *J* =10 Hz, 1H, H-2), 6.48 (s, 1H, H-7), 6.59 (d, *J* =10 Hz, 1H, H-1), 6.85 (s, 1H, H-10).^{13}C-NMR ($CDCl_3$) δ 32.7 (C-4), 44.16 (C-11), 44.2 (C-10b), 53.54 (C-12), 62.28 (C-6), 62.79 (C-4a), 63.96 (C-3), 100.7 (-OCH_2O-),102.8 (C-10), 106.9 (C-7), 126.28 (C-6a), 127.5 (C-2), 132.12 (C-1), 138.28 (C-10a), 145.7 (C-8), 146.1 (C-9).

1-epideacetylbowdensine (VIII)

Amorphous white powder, m.p. 162-164 °C. $[\alpha]_D^{23}$ +22° ($CHCl_3$, c, 0.47). CD$[\Phi]_{248}$ +1.7. UV in MeOH λmax (abs.): 205.5(2.01), 243(0.175), 294 (0.30). EI-MS, m/z (rel. int.): $[M]^+$þ 319 (21.9), 318 (100), 274 (31.6), (13.4), 231 (44.2), 219 (23), 217 (14.7), 202 (16.4), 190 (14.9), 103 (18.5), 91 (22.2), 65 (16.4), 55 (20.5). ^{1}H-NMR ($CDCl_3$) δ 1.54 (dddd, *J* =14.16, *J* =2.6, *J* =3.2 Hz, *J* undetectable, 1H, H-3ax), 1.56 (dddd, *J* =14.6, *J* =4.88, *J* =2.9, *J* =3.2 Hz, 1H, H-4*α*), 1.75 (ddd, *J* =14.6, *J* =3.4, *J* =11.2 Hz, 1H, H-4*β*), 1.96 (ddd, *J* =4.5, *J* = 9, *J* = 12 Hz,1H, H-11*α*), 2.04 (dddd, *J* =14.16, *J* =3.4, *J* =3.2, *J* =3.2 Hz, 1H, H-3eq), 2.73 (ddd, *J* =5.84, *J* = 10, *J* = 11 Hz,1H, H-11*β*), 2.77 (ddd, *J* =6.12, *J* = 9.04, *J* = 14.88 Hz,1H, H-12*α*), 2.89 (dd, *J* =12.72, *J* = 4.88 Hz, 1H, H-4a), 3.36 (ddd, *J* =6.12, *J* = 10.3, *J* = 13.92 Hz,1H, H-12*β*), 3.75 (d, *J* =17.6 Hz,1H, H-6*β*), 3.97 (s,3H, OCH_3), 4.06 (d, *J* =4.4 Hz, 1H, H-1), 4.15 (br-s, 1H, H-2), 4.17 (d, *J* =17.6 Hz,1H, H-6*α*), 5.86 (s,2H, -OCH_2O-), 7.19 (s, 1H, H-10). ^{13}C-NMR ($CDCl_3$) δ 20.47 (C-4), 28.98 (C-3), 36.55 (C-11), 49.41 (C-10b), 51.77 (C-12), 58.31 (C-6), 59.07 (OCH_3), 67.76 (C-4a), 69.92 (C-2), 73.21 (C-1), 99.48 (C-10), 100.45 (-OCH_2O-), 116.76 (C-6a), 133.39 (C-8), 140.08 (C-7), 142.17 (C-10a), 148.08 (C-9).



(I)



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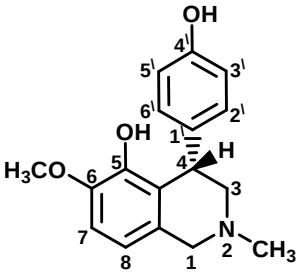

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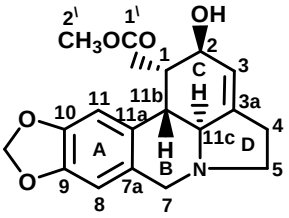
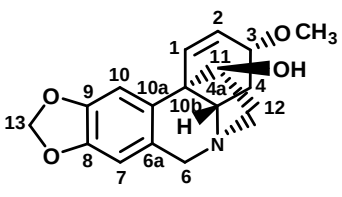
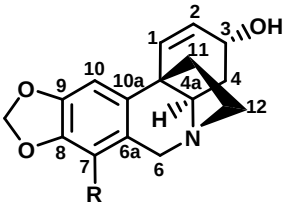


(IV)

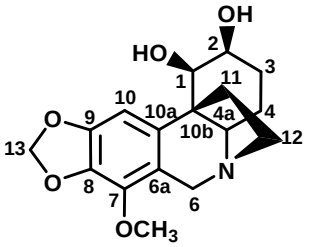


(V)



(VI) R = OCH_3
(VII) R = H

Full Paper



(VIII)

RESULTS AND DISCUSSION

The results for the AChE inhibitory properties of the crude hydroalcholic, $CHCl_3$, EtOAC and *n*-BuOH extracts of the different organs of *Crinum bulbispermum* Milne-Redhead & Schweick., collected at the pre-flowering stage, illustrated that the chloroform extracts of the bulbs, roots and stem discs showed several white spots indicating AChE inhibitory activity while the ethyl acetate and the butanol extracts showed some of the spots already detected in the chloroform extracts but in lesser amount and thus were considered of lower significant AChE inhibitory activity. As a result of bioactivity-directed fractionation of the tested extracts of *Crinum bulbispermum* Milne-Redhead & Schweick. and in view of the interesting AChE inhibitory activity exhibited by the chloroform extract of the bulbs of the studied plant, this extract was considered as a good candidate for further phytochemical investigation which eventually resulted in the isolation of eight crystalline alkaloids (I-VIII) in addition to the well-known Amaryllidaceae alkaloid, lycorine. The structures of compounds I-VIII were characterized by ^{1}H and ^{13}C-NMR spectroscopy combined with EI-mass spectrometry as ismine (I), trisphaeridine (II), latifine (III), 1-*O*-Acetyllycorine (IV), crinamine (V), powelline (VI), crinine (VII) and 1-epideacetylbowdensine (VIII).

The ^{1}H-NMR data of both alkaloids (I and II) showed the presence of six aromatic protons of two aromatic systems, of which two singlets at δ 7.02 and 6.69 for alkaloid (I) and at δ 7.34 and 7.92 for alkaloid (II) indicating the presence of a tetrasubstituted aromatic ring and were assigned for H-7 and H-10, respectively. Moreover, the presence of an ABCD-system in the second disubstituted aromatic ring was deduced from the appearance of two double doublets at δ 7.00 and 6.75 for alkaloid (I) and at δ 8.37 and 8.13 for alkaloid (II), in addition to the presence of two doublets of double doublets at δ 6.83 and 7.30 for alkaloid (I) and at δ 7.61 and 7.67 for alkaloid (II). Furthermore, the ^{1}H-NMR spectra for alkaloids (I and II) exhibited signals due to methylendioxy protons at δ 6.01 and 6.18, respectively. Meanwhile, the ^{1}H-NMR spectrum of alkaloid (II) exhibited characteristic signal for one iminic proton at δ 9.09 (s), which was assigned to H-6. This was further confirmed from the appearance of C-6 signal in the ^{13}C-NMR spectrum at δ 151.8. The observed spectral data for alkaloids (I and II) were in full agreement with the published data for ismine and trisphaeridine, respectively[1,6,23,27].

The EI-MS spectrum of alkaloid (III) exhibited a molecular ion peak at *m/z* 285 corresponding to $C_{17}H_{19}NO_3$, with its fragmentation pattern, similar to that of cherylline- type alkaloid[4], indicating that alkaloid (III) has a 4-phenyltetrahydroisoquinoline skeleton. The ^{1}H-NMR spectrum of alkaloid (III) showed the presence of six aromatic protons of two aromatic systems, of which two doublets at δ 6.52 and 6.74, indicated the presence of a tetrasubstituted aromatic ring and are assigned for H-7 and H-8, respectively. Furthermore, the appearance of two double doublets at δ 6.53 and 6.78, each is integrated for two protons, indicated the presence of a second, *para*-disubstituted, aromatic ring and are assigned for H-3', H-5' and H-2', H-6', respectively. In addition, the ^{1}H-NMR spectrum for alkaloid (III) exhibited signals due to *O*-CH_3 and *N*-CH_3 protons at δ 3.7 and 2.24, respectively, in addition to the presence of AB-type doublets at δ 3.03 and 3.29 assigned for H-1ax and H-1eq, respectively, and the presence of ABX-type signals at δ 2.56, 2.83 and 4.16 assigned for H-3ax, H-3eq and H-4, respectively. All the spectral data of alkaloid (III) were in agreement with those previously reported for latifine[11].

The UV spectrum of alkaloid (IV) referred to Amaryllidaceae alkaloid of the lycorine nucleus with dioxyaryl chromophore[9,21]. The ^{1}H-NMR spectrum revealed the presence of two *para*-oriented aromatic protons at δ 6.58 and 6.69 assigned for H-8 and H-11, respectively, indicating disubstitution at C-9and C-10, while the appearance of signal for one olefinic proton at δ 5.56 assigned for H-3, indicated the presence of double bond at $\Delta^{3,3a}$. The *trans*-linkage of the B and C rings, which is common in lycorine-type alkaloids,

was unambiguously deduced from the large coupling constant between H-11b and H-11c (J=10.5Hz). Moreover, the ^{1}H-NMR spectrum showed the presence of a singlet, integrated for three protons, at δ 1.95 together with its corresponding carbon, in the ^{13}C-NMR spectrum, at δ 21.05 in addition to a characteristic carbonyl signal at δ 170.8, typical for an acetyl group which induces a downfield shift (Δ 1.1 ppm) of both C-1 and H-1, compared with that of lycorine[8]. Thus, it can be inferred that the position of the acetyl group is at C-1. All spectral data of alkaloid (IV) were in agreement with those previously reported for 1-*O*-Acetyllycorine[11,24].

The CD spectrum of alkaloid (V) was similar qualitatively to those of α-5,10b-ethano- phenanthridine alkaloids with a minimum around 250 nm[25]. The EI-MS data showed a very weak molecular ion peak (1%) which excluded the haemanthamine series and suggested the molecular formula $C_{17}H_{19}NO_4$ for alkaloid (V)[26]. The fragmentation pattern of the alkaloid followed the pattern of the crinane compounds possessing aliphatic substituent at C-3 and a substituted ethanobridge[5,13,15]. Alkaloid (V) showed spectral data similar to those reported for crinamine[11,17,19,26].

On the other hand, the CD spectra of alkaloids (VI, VII and VIII) were similar qualitatively to those of β-5,10b-ethanophenanthridine alkaloids with a maximum around 250 nm[25]. The ^{1}H-NMR spectrum of alkaloid (VII) showed the presence of two singlets at δ 6.48 and 6.85 assigned for the two aromatic protons H-7 and H-10, respectively, indicating disubstitution at C-8 and C-9, while the appearance of signals for two olefinic protons, doublet at δ 6.59 assigned for H-1, and double doublet at δ 5.96 assigned for H-2, indicated the presence of double bond $\Delta^{1,2}$ and a substituent at C-3. The small coupling constants between H-2 and H-3 (J=5.12Hz) and between H-3 and H-4β (J=3.6Hz) allowed for the assignment of the *pseudo*-axial disposition for the hydroxyl group at C-3. The large coupling between H-4a and H-4β (J =13.6 Hz) proved their trans/diaxial position characteristic for the crinine series. Each of the protons H-11α, H-11β, H-12α and H-12β was observed as a doublet of double doublet or multiplet, which indicated a non-substituted ethanobridge. The typical AB pattern of the C-6 methylene protons around δ 4.0 confirmed the C-6 unsubstituted member of the crinine series.

Meanwhile, the spectral data of alkaloid (VI) were similar to those of alkaloid (VII) except in the absence of one of the two characteristic singlets in the aromatic region of the ^{1}H-NMR spectrum and the appearance of a singlet, integrated for three protons, at δ 3.96 together with its corresponding carbon at δ 59.09, in the ^{13}C-NMR spectrum, typical for *O*-CH_3 group which induces a pronounced deshielding effect on C-7 and a shielding effect on both C-6a and C-8, with respect to alkaloid (VII). Thus, it can be inferred that the position of the *O*-CH_3 group is at C-7. The EI-MS data of alkaloids (VI and VII) exhibited molecular ion peaks at *m/z* 301 and 271 corresponding to $C_{17}H_{19}NO_4$ and $C_{16}H_{17}NO_3$, respectively, in addition to the typical fragmentation pathway characteristic for crinane alkaloids possessing aliphatic hydroxyl group at C-3 and unsubstituted ethanobridge[5,13,15]. The observed spectral data for alkaloids (VI and VII) were in full agreement with the published data for powelline and crinine, respectively[1,6,23,27].

The ^{1}H-NMR spectrum of alkaloid (VIII) showed the presence of signals for two protons, doublet at δ 4.06 and broad singlet δ 4.15 assigned for H-1 and H-2, respectively, with small coupling constant (J = 4.4 Hz) indicating the absence of both double bond $\Delta^{1,2}$ and a substituent at C-3. This was confirmed from the appearance of C-1, C-2 and C-3 signals in the ^{13}C-NMR spectrum at higher field with respect to alkaloids (VI and VII). Moreover, the ^{1}H-NMR spectrum revealed the appearance of only one singlet in the aromatic region, at δ 7.19, beside the presence of a singlet, integrated for three protons, at δ 3.97 together with its corresponding carbon at δ 59.07, typical for *O*-CH_3 group which induces a pronounced deshielding effect on C-7 and a shielding effect on both C-6a and C-8, with respect to alkaloid (VII). Thus, it can be inferred that the position of the *O*-CH_3 group is at C-7 and that the singlet at δ 7.19 is assigned for H-10. All the spectral data of alkaloid (VIII) were in agreement with those previously reported for 1-epideacetylbowdensine[11,25]. The identification of alkaloids (I-VIII) was further confirmed by direct comparison with reference samples through m.p, m.m.p and co-chromatography.

It is worth mentioning that, in accordance with thor-

ough literature survey, this is the first report for the isolation of alkaloids I-IV and VIII from *Crinum bulbispermum* Milne-Redhead & Schweick.

REFERENCES

[1] A.A.Ali, H.M.El Sayed, O.M.Abdallah, W.Steglich; Phytochemistry, **25**, 2399-2401 **(1986)**.

[2] H.U.Barthelmes, E.Niederberger, T.Roth, K.Schulte, W.C.Tang, F.Boege, H.-H.Fiebig, G.Eisenbrand, D.Marko; British Journal of Cancer, **85**, 1585-1591 **(2001)**.

[3] J.Bohmann, P.Forgo, J.Molnár, K.Wolfard, A.Molnár, T.Thalhammer, I.Máthe, D.Sharples; Planta Medica, **68**, 454-457 **(2002)**.

[4] A.Brossi, G.Grethe, S.Teitel, W.C.Wildman, D.T.Bailey; Journal of Organic Chemistry, **35**, 1100-1104 **(1970)**.

[5] A.M.Duffield, R.T.Aplin, H.Budzikiewicz, C.Djerassi, C.F.Murphy, W.C.Wildman; Journal of the American Chemical Society, **87**, 4902-4912 **(1965)**.

[6] S.A.El-Moghazy; Al-Azhar Journal of Pharmaceutical Sciences, **27**, 249-254 **(2001)**.

[7] G.L.Ellman; Arch.Biochem.Biophys., **74**, 443-450 **(1958)**.

[8] A.Evidente, M.R.Cicala, I.Giudicianni, G.Randazzo, R.Riccio; Phytochemistry, **22**, 581-584 **(1983)**.

[9] Ghosal, Shibnath, Saini, S.Kulwant, Razdan, Sushma; Phytochemistry, **24**, 2141-2156 **(1985)**.

[10] H.M.Hammoda, A.H.Abou-Donia, S.M.Toaima, E.A.Shawky; Alex.J.Pharm.Sci., **25**, 26-28 **(2011)**.

[11] S.Kobayashi, T.Tokumoto, M.Kihara, Y.Imakura, T.Shingu, Z.Taira; Chemical and Pharmaceutical Bulletin, **32**, 3015-3022 **(1984)**.

[12] S.Kobayashi, T.Tokumoto, Z.Taira; Journal of the Chemical Society, Chemical Communications, **15**, 1043-1044 **(1984)**.

[13] M.Kreh, R.Matusch, L.Witte; Phytochemistry, **38**, 773-776 **(1995)**.

[14] M.Leven, D.A.Van den Berghe, F.Mertens, A.J.Vlietnick, E.Lammens; Planta Medica, **36**, 311-321 **(1979)**.

[15] P.Longevialle, D.H.Smith, A.L.Burlingame, H.M.Fales, R.J.Highet; Organic Mass Spectrometry, **7**, 401-415 **(1973)**.

[16] S.Lopez, J.Bastida, F.Viladomat, C.Codina; Life Sciences, **71**, 2521-2529 **(2002)**.

[17] K.G.Oloyede, M.J.Oke, Y.Raji, A.T.Olugbade; World Journal of Chemistry, **5**, 26-31 **(2010)**.

[18] M.P.Pancrasi, P.Metais; Presse Med., **32**, 750 **(2003)**.

[19] J.Refaat, A.Abdel-Lateff, M.S.Kamel, A.A.Ali, M.A.Ramadan, T.Okino, Y.Nogata; Pharmacognosy Research, **1**, 43-52 **(2009)**.

[20] I.K.Rhee, M.Van de Meent, K.Ingkaninan, R.Verpoorte; Journal of Chromatography A, **915**, 217-223 **(2001)**.

[21] A.W.Sangster, K.L.Stuart; Journal of Chemical Review, **65**, 67-130 **(1965)**.

[22] J.J.Sramek, E.J.Frackiewicz, N.R.Cutler; Expert Opinion on Investigational Drugs, **9**, 2393-2402 **(2000)**.

[23] R.Suau, A.I.Gomez, R.Rico; Phytochemistry, **29**, 1710-1712 **(1990)**.

[24] Y.Toriizuka, E.Kinoshita, N.Kogure, M.Kitajima, A.Ishiyama, K.Otoguro, H.Yamada, S.Omura, H.Takayama; Bioorganic and Medicinal Chemistry, **16**, 10182-10189 **(2008)**.

[25] F.Viladomat, G.R.Almanza, C.Codina, J.Bastida, W.E.Campbell, S.Mathee; Phytochemistry, **43**, 1379-1384 **(1996)**.

[26] F.Viladomat, J.Bastida, C.Codina, W.E.Campbell, S.Mathee; Phytochemistry, **35**, 809-812 **(1994)**.

[27] F.Viladomat, M.Selles, C.Codina, J.Bastida; Planta Medica, **63**, 583 **(1997)**.

[28] E.Zvetkova, B.Wirleitner, N.T.Tram, H.Schennach, D.Fuchs; International Immunopharmacology, **1**, 2143-2150 **(2001)**.