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OXOCRININE AND OTHER ALKALOIDS FROM *CRINUM AMERICANUM*

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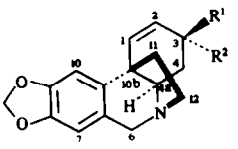
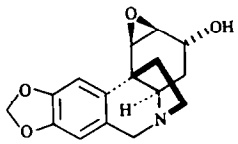
Key Word Index—*Crinum americanum*; Amaryllidaceae; flowering bulbs; alkaloids; oxocrinine.

Abstract—From bulbs of *Crinum americanum* oxocrinine, an intermediate in the biosynthesis of Amaryllidaceae alkaloids, was isolated for the first time. In addition, the plant contains crinine, flexinine, *O*-acetylcrinine, lycorine, hippadine, pratorinine, pratorimine, pratosine, ungeremine and trisphaeridine. The alkaloids were identified by spectroscopic evidence, chemical transformations and partial syntheses.

INTRODUCTION

Crinum americanum L. is an ornamental plant of temperate zones. A botanical study of this plant as well as tentative evidence for the presence of lycorine and crinine have already been presented [1]. The present study of its constituents is part of an integrated programme on members of the Amaryllidaceae cultivated at the Campus of Assiut University. In this contribution we report on the isolation and characterization of its alkaloidal constituents.





1 R^1, R^2 = —O—
2 R^1 = H, R^2 = OH
3 R^1 = H, R^2 = OAc

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RESULTS AND DISCUSSION

The air-dried powdered bulbs (40 kg) were extracted with 95% ethanol. Subsequent extensive chromatography of the evaporated extract afforded the alkaloids in amounts adequate for their identification by spectral studies.

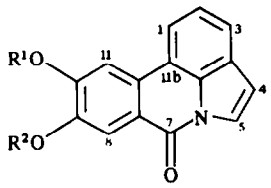
Crinine (**2**) [2] and flexinine (**4**) [3, 4] occur as major alkaloids and were identified by their spectral data. As minor components of the same type *O*-acetylcrinine (**3**) [5] and oxocrinine (**1**) could be identified. Compound **1** is an intermediate in the biosynthesis of crinine and related alkaloids [6] and has not yet been isolated from a natural source. However, the compound has been prepared by oxidation of crinine with manganese dioxide [7]. Its structure was elucidated by spectral data including the ^{1}H-coupled ^{13}C NMR spectrum (Table 1) and was finally confirmed by comparison with a sample prepared from crinine.

From the basic fraction lycorine (**5**) was isolated as an additional major alkaloid. It is accompanied by minor amounts of ungeremine (**10**) [8] and trisphaeridine (**11**) [9]. Ungeremine was identical to a sample obtained by oxidation of **5** with *N*-bromosuccinimide in acetic acid [10]. From the non-basic fraction the indole derivatives hippadine (**6**) [11, 12], pratorinine [13, 14], pratorimine [14] and pratosine (**9**) [14] could be obtained.

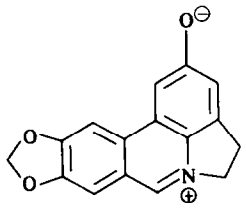
There is considerable confusion regarding the structures of pratorinine and pratorimine. Pratorinine, mp 265–267°, has been assigned formula **7** on the basis of the ^{1}H NMR upfield shift of H-8 on addition of NaOD–D_2O [13]. According to Professor Frahm [personal communication] this compound is identical with 'Alkaloid B' from *Crinum bulbispermum* [15], for which structure **7** has been recently rigorously established by an X-ray structural analysis [15]. Unfortunately the reference IR spectra of pratorinine and pratorimine submitted to Maddry *et al.* [15] had been wrongly labelled [Frahm, A. W., personal communication], which causes a reversal of the naming of the alkaloids in ref. [15]. Pratorimine ('Alkaloid A' [15], mp 224–225°) therefore has structure **8** and the mp 263–265° given in ref. [14] is erroneous.

To ascertain the assignments of the NMR signals selective ^{1}H decouplings in the ^{1}H-coupled ^{13}C NMR spectrum of pratorimine were performed. It could be shown that the slightly sharper singlet at δ7.82 (in DMSO–$CDCl_3$) is due to H-8 because it exhibits a ^3J-coupling of 4 Hz with the carbonyl carbon at δ157.3. Irradiation at the frequency of H-8 also causes the simplification of the C-10 signal (δ152.3, *dd*, J = 7.5 and 3.5 Hz) into a 3.5 Hz doublet. Simplification of the same signal to a 7.5 Hz doublet was observed on irradiation at the frequency of H-11 (δ7.73). C-9 appears as a multiplet, due to ^3J-coupling with the methoxy protons and shows the expected decouplings on irradiation at H-8 or H-11. These results lead to formula **8** for pratorimine, in accord with ref. [15].

The minor alkaloids of the lycorine group appear to be products of oxidative degradation of the parent compound. Ungeremine (**10**) has been isolated only once before from *Ungernia minor* [8], whereas trisphaeridine (**11**) occurs in *U. trisphaera*, *U. spiralis*, *Galanthus plicatus* [9] and *Pancratium trianthum* [16]. The indole type



6 R^1, R^2 = —CH_2—
7 R^1 = Me, R^2 = H
8 R^1 = H, R^2 = Me
9 $R^1 = R^2$ = Me



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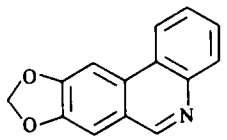

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Table 1. ^{13}C NMR data of oxocrinine (**1**), *O*-acetylcrinine (**3**) and flexinine (**4**) in $CDCl_3$*

Carbon no.	1†	3	4
1	149.3	123.7	53.6
2	128.7	134.5	56.5
3	197.9	66.6	64.5
4	44.7	29.9	29.0
4a	68.8	63.3	61.8
6	61.8	62.4	61.9
6a	126.2	126.4	125.1
7	107.3	107.0	107.2
8	146.3‡	146.2‡	146.3‡
9	146.5‡	145.9‡	149.3‡
10	102.4	102.0	102.8
10a	135.9	138.2	137.3
10b	44.8	44.3	42.0
11	40.1	44.1	38.8
12	54.0	53.6	52.2
OCH_2O	101.0	100.0	101.2
Ac	—	170.0/21.2	—

*Compound **4** in $CDCl_3$–DMSO-d_6.

†Assignments were proven by ^{1}H, ^{13}C shift correlation and the observed $^3J_{H,C}$ couplings.

‡Values may be interchanged.

alkaloids **6**–**9** have been obtained before from several *Crinum* species [11–15]. It is possible that these minor alkaloids have been overlooked in other cases where only small amounts of plants have been investigated.

EXPERIMENTAL

General. Mps are uncorr. ^{1}H NMR: 90 and 400 MHz, $CDCl_3$ if not otherwise stated, δ-values, TMS as int. standard. ^{13}C NMR: 22.6 and 100.4 MHz. MS, 70 eV. CC: silica gel 60 (63–200 μm) and alumina (neutral, grade I), E. Merck. TLC on silica gel plates 60 F_{254} (E. Merck), eluents EtOAc (S-I) and $CHCl_3$–MeOH (4:1) (S-II), spots were visualized by spraying with Dragendorff's reagent.

Plant material. The bulbs of *Crinum americanum* L. were collected from the cultivated plants at Assiut University Campus in July 1981 during flowering. The identity was confirmed by Prof. Dr. N. El-Hadidy (Cairo University). A voucher sample is kept in the Faculty of Pharmacy, Assiut University.

Extraction and isolation. The air-dried powdered bulbs (40 kg) were exhaustively extracted by percolation with 95% EtOH, yielding 6 kg residue upon evaporation. The residue was acidified with 5% aq. HCl and defatted by shaking with petrol (3 × 3 l.) and then with $CHCl_3$ (5 × 2.5 l.). The combined $CHCl_3$ extracts yielded after evaporation fraction A (500 g). The aq. phase was basified with conc. NH_3 and extracted with $CHCl_3$ (4 × 2 l.). Evaporation of the combined $CHCl_3$ extracts gave fraction B (250 g).

Chromatographic fractionation of fraction A. Fraction A was separated on an alumina column (150 × 6 cm) by elution with petrol, followed by petrol–EtOAc (1:1), thereafter gradually increasing the polarity. Fractions (100 ml) were monitored by TLC using S-I. The fractions were combined according to similarity in contents. All the isolated compounds were recrystallized from MeOH. The start of the elution afforded compound **6** (R_f 0.84, S-I; 1 g), followed by fractions containing compounds **8** (R_f 0.68, 8 mg), **7** (R_f 0.62, 100 mg) and **9** (R_f 0.56, 10 mg).

Chromatographic fractionation of fraction B. Fraction B was digested in MeOH (250 ml), whereby **5** separates as a creamy powder. The filtrate was concd and passed through a silica gel column (150 × 6 cm). Elution started with $CHCl_3$, then $CHCl_3$–MeOH (4:1), and eluates were monitored by S-II. The compounds appeared in the following order: **11** (R_f 0.72, 10 mg), **3** (R_f 0.63, 200 mg), **1** (R_f 0.60, 100 mg), **4** (R_f 0.56, 800 mg), **2** (R_f 0.52, 1 g), **10** (R_f 0.15, yellow, 80 mg).

Oxocrinine (**1**). Colourless needles (MeOH), mp 185–186° (lit. [7] mp 184–186°); $[\alpha]_D = -24.6°$ ($CHCl_3$; *c* 1.0); UV λ_{max} nm (log ε): 223 (4.25), 292.5 (3.64); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3020–2900, 1695 (CO), 1490, 940 (OCH_2O); ^{1}H NMR (400 MHz): δ2.18 (*ddd*, *J* = 12, 10, 6.5 Hz, H-11), 2.38 (*ddd*, *J* = 12.5, 10, 4.5 Hz, H-11'), 2.50 (*dd*, *J* = 16.5, 12.5 Hz, H-4β), 2.70 (*dd*, *J* = 16.5, 5 Hz, H-4α), 3.04 (*ddd*, *J* = 12.5, 9, 6.5 Hz, H-12), 3.55 (*ddd*, *J* = 12, 9, 4.5, H-12'), 3.66 (*dd*, *J* = 12.5, 5 Hz, H-4a), 3.82 (*d*, *J* = 17 Hz, H-6α), 4.42 (*d*, *J* = 17 Hz, H-6β), 5.93, 5.94 (AB*q*, *J* = 1.3 Hz, OCH_2O), 6.10 (*d*, *J* = 10.5 Hz, H-2), 6.53 (*br s*, H-7), 6.93 (*s*, H-10), 7.62 (*d*, *J* = 10.5 Hz, H-1). The ^{1}H, ^{1}H-couplings were proven by a 2D COSY experiment; ^{13}C NMR: see Table 1. MS *m/z* (rel. int.):

269.1054 $[M]^+$ (100) ($C_{16}H_{15}NO_3$ requires 269.1052), 268 $[M-H]^+$ (8), 241 $[M-H-CO]^+$ (12), 240 $[C_{15}H_{14}NO_2]$ (14), 226 $[C_{14}H_{12}NO_2]$ (7), 214 (7), 213 (9), 212 $[C_{13}H_{10}NO_2]$ (19), 201 (8), 200 $[C_{12}H_9NO_2]$ (10), 197 $[C_{13}H_9O_2]$ (6), 186 $[C_{12}H_{10}O_2]$ (63), 185 $[C_{12}H_9O_2]$ (22), 156 $[C_{11}H_8O]$ (21), 128 $[C_{10}H_8]$ (34), 127 (13), 115 $[C_9H_7]$ (10).

Crinine (**2**). Colourless needles (MeOH), mp and mmp 209° (lit. [2] 208–210°).

O-*Acetylcrinine* (**3**). Colourless needles (MeOH), mp 141° (lit. [4] mp 142–143°); $[\alpha]_D$ + 59.3° ($CHCl_3$; *c* 1); UV λ_{max} nm (log ε): 203 (4.35), 240 (3.30), 293 (3.86); IR ν_{max}^{KBr} cm^{-1}: 1730 (CO), 1490, 928 (OCH_2O); ^1H NMR (90 MHz, selection): δ2.00 (*s*, Ac), 3.87 (*d*, *J* = 17 Hz, H-6α), 4.44 (*d*, *J* = 17 Hz, H-6β), 5.35 (*m*, H-3), 5.91 (ABq, *J* = 1.2 Hz, OCH_2O), 6.00 (*ddd*, *J* = 10, 5.3, 0.8 Hz, H-2), 6.65 (*d*, *J* = 10 Hz, H-1), 6.50 (*s*, H-7), 6.90 (*s*, H-10); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 313.1308 $[M]^+$ (20) (calc. for $C_{18}H_{19}NO_4$ 313.1314). The compound was identical to a sample prepared by acetylation of crinine with pyridine–Ac_2O (no depression in mmp).

Flexinine (**4**). Colourless needles (Me_2CO), mp 222° (lit. [3] mp 222°); $[\alpha]_D$ − 15.8 ($CHCl_3$; *c* 1); UV λ_{max} nm (log ε): 203 (4.66), 242 (3.89), 295 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3180 (*br*, OH), 2900, 1515, 1490, 1250, 1235, 1035, 928 (OCH_2O), 842; ^1H NMR (400 MHz): δ1.56, 1.57 (*m*, H-4α,H-4β), 1.83 (*br s*, OH), 2.02 (*ddd*, *J* = 12.4, 9.0, 4.8 Hz, H-11), 2.39 (*ddd*, *J* = 12.4, 10.5, 5.7 Hz, H-11'), 2.79 (*ddd*, *J* = 12.4, 9.0, 5.7 Hz, H-12), 3.17 (*ddd*, *J* = 12.4, 10.5, 4.8 Hz, H-12'), 3.18 (*m*, H-4a), 3.28 (*dd*, *J* = 3.7, 2.5 Hz, H-2), 3.65 (*d*, *J* = 16.5 Hz, H-6α), 3.78 (*d*, *J* = 3.7 Hz, H-1), 4.29 (*d*, *J* = 16.5 Hz, H-6β), 4.47 (*ddd*, *J* = 2.5, 2.5, 2.5 Hz, H-3), 5.90, 5.91 (ABq, *J* = 1.2 Hz, OCH_2O), 6.43 (*s*, H-7), 6.90 (*s*, H-10); ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 287.1161 $[M]^+$ (61) (calc. for $C_{16}H_{17}NO_4$ 287.11157), 258 $[C_{15}H_{16}NO_3]$ (100), 229 $[C_{14}H_{15}NO_2]$ (16), 228 $[C_{14}H_{14}NO_2]$ (17), 214 $[C_{13}H_{12}NO_2]$ (16), 200 $[C_{12}H_{10}NO_2]$ (11), 187 $[C_{12}H_{11}O_2]$ (28), 186 (12), 185 (11), 175 $[C_{11}H_{10}O_2]$ (16), 173 (22), 159 $[C_{10}H_7O_2]$ (17), 145 $[C_{10}H_9O]$ (13), 143 $[C_{10}H_7O]$ (52), 115 $[C_9H_7]$ (26).

Lycorine (**5**). Colourless needles (EtOH), mp 252–254°; mp remained undepressed on admixture with an authentic sample.

Hippadine (**6**). Colourless flakes, mp 207–209°, undepressed on admixture with an authentic sample (lit. [11] mp 213–215°).

Pratorinine (**7**). Colourless needles (MeOH), mp 264° (lit. [13,14] mp 265–267°, [15] mp 265–267° = 'Alkaloid B' [Frahm, A. W., personal communication]; UV λ_{max} nm: 226, 235, 248, 255, 285, 295, 337, 350, 364; ^1H NMR (90 MHz): δ4.09 (*s*, OMe), 6.91 (*d*, *J* = 3.6 Hz, H-4), 7.49 (*t*, *J* = 7.7 Hz, H-2), 7.78 (*dd*, *J* = 7.7, 1 Hz, H-3), 7.80 (*s*, H-11), 7.96 (*br dd*, *J* = 7.7, 1 Hz, H-1), 8.04 (*s*, H-8), 8.07 (*d*, *J* = 3.6 Hz, H-5); MS *m/z* (rel. int.): 265.0744 $[M]^+$ (100) (calc. for $C_{16}H_{11}NO_3$ 265.0739), 250 $[C_{15}H_8NO_3]$ (39), 222 $[C_{14}H_8NO_3]$ (45), 194 $[C_{13}H_8NO]$ (8).

Pratorimine (**8**). Needles (MeOH), mp 224° (lit. [15] mp 224–225° = 'Alkaloid A' [Frahm, A. W., personal communication]); the mp 263–265° of ref. [14] is incorrect [Frahm, A. W., personal communication]; the UV and IR spectra are in agreement with ref. [14]; ^1H NMR (DMSO-d_6–$CDCl_3$): δ7.73 (*s*, H-11), 7.82 (*s*, H-8), other signals are in agreement with refs [13, 14]; ^{13}C NMR (DMSO-d_6): δ55.7 (*Q*, *J* = 147 Hz, OMe), 108.6 (*D*, *J* = 160 Hz, C-8), 110.0 (*D*, *J* = 161 Hz, C-11), 110.6 (*Ddd*, *J* = 176, 7, 2 Hz, C-4), 116.1 (*dm*, *J* = 8 Hz, C-11b), 118.4 (*Dd*, *J* = 161 and 7 Hz, C-1 or C-3), 118.6 (*d*, *J* = 6.5 Hz, C-7a), 124.0 (*D*, *J* = 161 Hz, C-2), 122.2 (*Dd*, *J* = 164, 8 Hz, C-1 or C-3), 123.4 (*Dd*, *J* = 192, 10 Hz, C-5), 127.8 (*m*, C-3a), 129.1 (*dd*, *J* = 7, 3.5 Hz, C-11a), 130.4 (*m*, C-11c), 148.6 (*ddq*, *J* = 4, 5, 2 Hz, C-9), 152.3 (*dd*, *J* = 7.5, 3.5 Hz, C-10), 157.3 (*d*, *J* = 4 Hz, C-7); MS *m/z* (rel. int.): 265.0736 $[M]^+$ (100) (calc. for $C_{16}H_{11}NO_3$ 265.0739, 250 $[C_{15}H_8NO_3]$ (66), 222 $[C_{14}H_8NO_2]$ (51).

Pratosine (**9**). Colourless needles, mp 232° (lit. [14] mp 232–233°); the UV, IR and ^1H NMR data coincide with those of ref. [14]; MS *m/z* (rel. int.): 279.0898 $[M]^+$ (100) (calc. for $C_{17}H_{13}NO_3$ 279.0895).

Ungeremine (**10**). Yellow crystals, mp 265° (lit. [8] mp 270–272°); UV λ_{max} nm: 208, 262, 285 (sh), 293 (sh), 325, 365, 435; in 0.1 N HCl 250, 270, 360; in 0.1 N NaOH 260, 315, 420; IR ν_{max}^{KBr} cm^{-1}: 3400–2600 (OH), 1620, 1510, 1480, 1390, 1275, 1045, 932 (OCH_2O), 890, 858; ^1H NMR (MeOD, 400 MHz): δ3.72 (*m*, H-4), 5.19 (*t*, *J* = 7 Hz, H-5), 6.37 (*s*, OCH_2O), 7.25 (*td*, *J* = 1.08, 1.8 Hz, H-3), 7.44 (*d*, *J* = 1.8 Hz, H-1); 7.63 (*s*, H-8), 8.01 (*s*, H-11), 9.13 (*s*, H-7); MS *m/z* (rel. int.): 265.0723 $[M]^+$ (57) (calc. for $C_{16}H_{11}NO_3$ 265.0739), 264 $[M-1]$ (100), 206 $[C_{14}H_8NO]$ (14). This alkaloid was prepared by oxidation of lycorine according to ref. [10] and proved to be identical in all aspects.

Trisphaeridine (**11**). Colourless needles (MeOH), mp 138° (lit. [9] mp 140–141°); UV λ_{max} nm (log ε): 252 (4.58), 265 (sh), 278 (4.13), 308 (3.77), 335 (3.51), 350 (3.45); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3080, 2960, 1610, 1500, 1460, 1380, 1080, 1035, 910; ^1H NMR (400 MHz): δ6.17 (*s*, OCH_2O), 7.33 (*s*, H-7), 7.61, 7.68 (each *td*, *J* = 7.5, 1.5 Hz, H-2 and H-3), 7.90 (*s*, H-10), 8.12 (*d*, H-4), 8.37 (*d*, H-1), 9.08 (*s*, H-6); MS *m/z* (rel. int.): 223.0637 $[M]^+$ (100) (calc. for $C_{14}H_9NO_2$ 223.0634), 222 $[C_{14}H_{18}NO_2]$ (37), 167 $[C_{12}H_9N]$ (8), 165 $[C_{12}H_7N]$ (11), 164 $[C_{12}H_6N]$ (12), 147 $[C_9H_9NO]$ (10), 138 $[C_{11}H_6]$ (20). The structure was further confirmed by a synthesis according to ref. [17]. The product agreed with all spectral data, and in mmp no depression was observed.

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