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TWO NON-HYDROXYLATED ALKALOIDS IN *CRINUM AUGUSTUM**

A. A. ALI,†‡ H. KATING,‡ A. W. FRAHM,§ A. M. EL-MOGHAZI‖ and M. A. RAMADAN‖

‡Institut für Pharmazeutische Biologie der Universität Bonn, West Germany; § Pharmazeutisch-Chemisches Institut der Universität Bonn, West Germany; ‖ Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt

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Key Word Index—*Crinum augustum*; Amaryllidaceae; lycorine; buphanisine; alkaloid in the crinine series; augustine.

Abstract—Several alkaloids were isolated from *Crinum augustum* grown in Assiut, Egypt. Two were identified as lycorine and buphanisine. In addition two new non-hydroxylated alkaloids of identical molecular formula $C_{17}H_{19}NO_4$ were investigated by spectroscopic methods. The gross structure of the first, termed augustine, belongs to the 1,2-epoxy-5,10-b-ethanophenanthridine-type. In contrast the second could not be related to any of the known alkaloid types of the Amaryllidaceae.

INTRODUCTION

The genus *Crinum* comprises many species which have been reported to elaborate a variety of alkaloids [1]. This paper represents the first phytochemical study of *Crinum augustum* Rox. It is restricted to the active constituents of the alkaloidal type, isolated from plants cultivated in Assiut, Egypt.

RESULTS AND DISCUSSION

The crude alkaloidal residue was obtained as described in the Experimental. Its treatment with MeOH affected the separation of lycorine as a yellowish powder. It was purified by recrystallization from EtOH and fully characterised (mmp, IR, TLC [2], MS [3] and ^{1}H NMR [4] and ^{13}C NMR spectra.)

Preliminary fractionation of the above mentioned mother liquor into two main fractions was performed by chromatography over Al_2O_3 column. The first fraction comprised the three alkaloids **2–4** eluted in a mixture with C_6H_6–AcOEt (1:1). The second fraction constituted the subsequent five alkaloids, eluted in a mixture with AcOEt–MeOH (4:1). The final separation of the alkaloidal components of both fractions was performed by chromatography on Si gel column. The alkaloids are numbered according to their R_f values on TLC under standard conditions (see Experimental). They were purified by recrystallization from appropriate solvents.

In this paper, besides lycorine, only the compounds **2–4** are considered. Investigation of the five other alkaloids will be reported later. The second alkaloid that could be correlated with one of the known Amaryllidaceae alkaloids was **4**. Its physical, UV, IR and high-resolution MS data are in full agreement with those reported for buphanisine ($C_{17}H_{19}NO_3$) [5–8].

The compounds **2** and **3** are represented by one molecular formula $C_{17}H_{19}NO_4$, which differs from that of buphanisine by one additional oxygen atom, and they appear to be new alkaloids. Compounds **2–4** were subjected to comparative spectral studies. They exhibit very similar UV spectra corresponding to the aromatic methylenedioxy chromophore, the presence of which was also indicated by IR bands at 1615, 1480 and 940 cm^{-1}. Their IR spectra showed neither hydroxyl nor carbonyl bands.

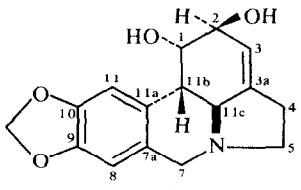
Compound **3** shows a close structural relation to buphanisine. Their close R_f values reflect a similar degree of basicity. Each of the high-resolution MS exhibits peaks arising from the elimination of Me and OMe from M^+. In the low mass region they show peaks at 187, 185, 172, 157, 115 with identical composition. Both findings are indicative of partial structural similarity between the two alkaloids and, according to [6–8], **3** should be a member in the crinine series.

The two spectra differ widely in the middle region. That of compound **3** does not reveal the expulsion of the fragment C_3H_5N from M^+ which is typical for members with both unsaturated C-1,2 and non-hydroxylated at C-11 [7, 8] of several fragments, constituting two oxygen atoms which are completely absent in the same region of the buphanisine spectrum. In view of the already stated lack of carbonyl and OH groups, **3** must contain the additional oxygen in a cyclic ether environment. Based on the elimination of a CHO fragment from M^+, the presence of an oxiran ring is suggested.

The coincidence of the relevant signals from the ^{1}H NMR spectra of both compounds led to the gross structural elucidation of **3**. Each of the two 60 MHz spectra exhibits three singlets in the range from δ 5–7 assignable to two *para*-aromatic protons H-7 and H-10, and to two protons of the methylenedioxy group. Additionally two doublets around δ 4 corresponding to the two benzylic methylene protons and a singlet for three protons due to OMe group at C-3 are observed. The major observable differences in the two spectra are

* Part I in the projected series "The Alkaloids of *Crinum augustum*".

† Permanent address: Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.



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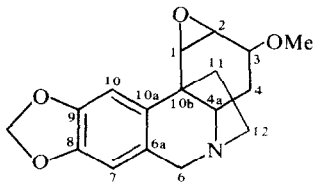

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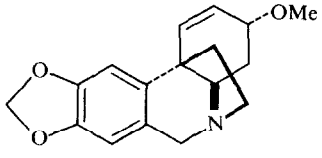

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associated with the signals resulting from C-1 and C-2 protons. The spectrum of buphanisine exhibits signals assignable to the olefinic H-1, as a doublet at δ 6.70 ($J_{1,2}$ = 10 Hz), and to the second olefinic proton H-2 as a doublet from doublet at δ 6.21 with further long-range coupling ($J_{1,2}$ = 10 Hz, $J_{2,3}$ = 5 Hz). The 60 MHz spectrum of **3** does not show such olefinic signals indicating the saturation of its C-1,2 double bond. This is confirmed by its IR which does not exhibit any olefinic band but absorption peaks at 1280 and 840 cm^{-1} assignable to an oxiran system. The epoxy group thus constitutes the fourth oxygen atom required by the empirical formula. In the ^1H NMR spectrum only one of the two oxiran protons could be identified as a doublet at δ 3.83 assignable to H-1. The signal due to the second proton H-2 was not resolved.

Thus compound **3** has the structure shown. It represents the ninth member in the series of the 1,2-epoxyalkaloids of the 5,10-b-ethanophenathridine type, after crinamidine [9], undulatine [10], flexinine [11], nerbowdine [12], crinalbine [13], flexamine [14], tubispacine [15] and cavinine [16]. **3** is considered new in the sense that it is the only alkaloid in this series which contains neither OH nor aromatic OMe. Its stereochemistry as well as the details of its MS fragmentation will be presented later.

From calculation based on its molecular formula $C_{17}H_{19}NO_4$, **2** must contain 9 double bond equivalents. Five of them are due to the methylenedioxy phenyl system, the presence of which was confirmed by the ^1H NMR spectrum. It showed the two singlets due to the *para*-aromatic protons and the expected two-proton singlet for the methylenedioxy group. It did not exhibit any doublet or multiplets in the area from δ 5–10, indicating that **2** is free from olefinic unsaturation. Its IR showed that carbonyl or C=N groups are absent. In conclusion, **2** must possess four rings in addition to the methylenedioxy phenyl system. The ^1H NMR spectrum also revealed a singlet for three protons of a NMe group. Signals assignable to OMe or exchangeable protons were not observed. In the high-resolution spectrum, the M^+ peak appeared at *m/e* 301.1298 and the base peak at 300. The other significant peaks were observed at *m/e* 244 ($M^+ - C_3H_5O$), 215 ($M^+ - C_4H_8NO$), 201 ($M^+ - C_5H_{10}NO$), 185 ($M^+ - C_5H_{10}NO_2$).

From these findings, it was not possible to relate **2** to any of the known types of the Amaryllidaceae alkaloids. It must constitute a new skeletal structure which could not be derived from our present available spectral data. Intensive investigation comprising high-field ^1H NMR, ^{13}C NMR and X-ray crystallography is now in progress.

EXPERIMENTAL

Mps are uncorr. UV spectra are measured in MeOH, IR spectra in KBr, ^1H NMR spectra in $CDCl_3$ unless otherwise stated with TMS as an internal standard; chemical shifts are given on a δ (ppm) scale and the coupling constants in Hz. MS were recorded at 70 eV using a direct inlet system with high resolution. Column chromatography was performed on alkaline Al_2O_3 (E. Merck) or Si gel (E. Merck 6,3–20 u); the latter was moistened with 0.5% aq. NaOH and then activated. TLC was performed on Si gel (E. Merck) activated layers, previously slurried with 0.5% aq. NaOH. Spots were detected by treatment with I_2 vapour or spraying with modified Dragendorff (Monier) reagent.

Plant material. Whole plants of *Crinum augustum* Rox. cultivated in Assiut University campus were collected in May 1977. The plants were previously propagated by offsets from a plant identified by the late Prof. Vivi Tackholm of Cairo University Herbarium, Giza, Egypt.

Extraction. Air-dried chipped leaves, bulbs and roots (10 kg) were extracted with alcohol (95%) by firstly maceration and then percolation. The solvent was evapd under red. pres. The residue was partitioned between AcOEt and 5% aq. HCl. The acid layer was basified with NH_4OH solution and extracted with AcOEt. This organic layer was evapd to dryness leaving the dark brown residue constituting the crude alkaloids under investigation.

Fractionation of the alkaloids. The residue was treated with MeOH (200 ml) where most of lycorine (**1**) separated as a creamy powder, removed by filtration and purified by recrystallization from EtOH as colourless prisms of mp 270–2°. The mother liquor, left after removal of lycorine, was evapd then chromatographed over Al_2O_3 column at first with C_6H_6–AcOEt (1:1) till complete exhaustion (fraction A), then with AcOEt–MeOH (4:1) till complete elution of any Dragendorff-positive substance (fraction B). TLC of fraction A by system 1 [alkaline Si gel layers and the solvent system $CHCl_3$–MeOH (19:1)] revealed three major spots of R_f 0.81 (**2**), 0.59 (**3**) and 0.48 (**4**). The fraction A was rechromatographed over alkaline Si gel column. Elution was affected by $CHCl_3$–MeOH gradients. The eluate fractions were monitored by TLC. The fractions eluted with $CHCl_3$–MeOH (99:1) afforded upon evapn 1.1 g **2** which was recrystallized from acetone as colourless prisms of mp 173–5°.

The fractions eluted with $CHCl_3$–MeOH (49:1) yielded upon evapn 2.3 g **3**. Recrystallization from C_6H_6 afforded colourless needles of mp 174–6°. The fractions eluted with $CHCl_3$–MeOH (97:3) yielded upon evapn 2.5 g **4**. Recrystallization from C_6H_6 afforded colourless prisms mp 122–4°. Fraction B, from the foregoing Al_2O_3 column chromatography was evapd. The residue was treated with MeOH (50 ml). The solution was filtered off from the undissolved lycorine, which escaped from the previous precipitation. Examination of the filtrate by system 2 [alkaline Si gel–$CHCl_3$–MeOH (85:15)] revealed four major spots of R_f 0.56 (**5** and **6** in two overlapping spots), 0.50 (**7**) and 0.31 (**8** and **9** in two overlapping spots). The residue from the filtrate was rechromatographed over Si gel column and eluted with $CHCl_3$–MeOH gradient. The fractions eluted with $CHCl_3$–MeOH (19:1) yielded upon evapn 3.4 g mixture of **5** and **6**. Recrystallization from MeOH afforded large colourless prisms of mp 126–8°. The fractions eluted with $CHCl_3$–MeOH (93:7) yielded upon evapn 2.6 g **7**. Recrystallization from acetone afforded colourless small prisms mp 197–9°. The fractions eluted with $CHCl_3$–MeOH (17:3) yielded upon evapn 56 mg **8** and **9**. Recrystallization from acetone afforded colourless prisms mp 266–8°.

Lycorine (**1**). Colourless prisms (EtOH), mp 270–2°(lit. values range from 250–83°. $[\alpha]_D^{21} = 85.42$ ($c = 0.2$, EtOH). IR: 3350 (H-bonded), 3032 (olefinic double bond), 1615, 1480 and 940 cm^{-1} (aromatic methylenedioxy). ^1H NMR (in d_6-DMSO): δ 6.80 (1 H, *s*, H-11), 6.60 (1 H, *s*, H-8) line broadening ($W_{1/2} = 1.0$ Hz) because of long range coupling between the two aromatic protons, 5.90 (2 H, *s*, $-OCH_2O-$), 4.80 (1 H, *d*, $J = 6$ Hz, OH – 1), 4.70 (1 H, *d*, $J = 4$ Hz, OH – 2). MS: MW *m/e* 287 ($C_{16}H_{17}NO_4$, 39.2%), other significant peaks appeared at 286, 270, 268, 227 ($C_{14}H_{13}NO_2$) and 226 (base peak). ^{13}C NMR (in d_6-DMSO): δ 145.7 (*s*, C-9), 145.3 (*s*, C-10), 141.8 (*s*, C-3a), 129.8; 129.7 (*s*; *s*, C-7a/C-11a), 118.5 (*d*, C-3), 107.1 (*d*, C-8), 105. 1 (*d*, C-11), 100.6 (*t*, CH_2O_2), 71.8 (*d*, C-1), 70.3 (*d*, C-2), 60.8 (*d*, C-11c), 56.7 (*t*, C-7), 53.4 (*t*, C-5), 40.3 (*d*, C-11b), 28.2 (*t*, C-4).

2 (*New base*). Colourless prisms (acetone) mp 173–5°. UV: λ_{max} 214 nm ($\varepsilon = 83{,}437$), 241 (45511), 292 (46304). IR: 2950 (Me), 1620, 1480 and 940 cm^{-1} (aromatic methylenedioxy). ^1H NMR: δ 6.78 (1 H, *s*, aromatic proton), 6.65 (1 H, *s*, aromatic proton), 5.95 ($-O-CH_2-O-$), 2.31 (3 H, *s*, $-N-Me$). MS: MW *m/e* 301.1282 ($C_{17}H_{19}NO_4$, 74.4%), 300 ($C_{17}H_{18}NO_4$, base peak), 245 ($C_{14}H_{14}NO_3$, 65.3), 215 ($C_{13}H_{11}O_3$, 15.3), 201 ($C_{12}H_9O_3$, 11.1), 185 ($C_{12}H_9O_2$, 5.3).

Augustine **3**. Colourless prisms (benzene) mp 174–6°, $[\alpha]_D^{21} - 46.25$ ($c = 0.8$, EtOH). UV: λ_{max} 216 nm ($\varepsilon = 22{,}274$), 238 (2167), 294 (4816). IR: 2950 and 1070 (OMe), 1615, 1480 and 940 (aromatic methylenedioxy), 1280 and 840 cm^{-1} (epoxy). ^1H NMR: δ 6.97 (1 H, *s*, H-10), 6.53 (1 H, *s*, H-7) line broadening ($W_{1/2} = 2$ Hz) because of long-range coupling between the two aromatic protons; 5.93 (2 H, *s*, $-O-CH_2-O-$), 4.40 (1 H, *d*, $J = 17$ Hz, β H-6), 4.03 (1 H, *m*, H-3), 3.83 (1 H, *d*, $J = 3$ Hz, H-1), 3.70 (1 H, *d*, $J = 17.0$ Hz, α H-6), 3.47 (3 H, *s*, aliphatic OMe). MS: MW *m/e* 301.1332 ($C_{17}H_{19}NO_4$, base peak), 286 ($C_{16}H_{16}NO_4$, 9.2%), 272 ($C_{16}H_{18}NO_3$, 7.9), 270 ($C_{16}H_{16}NO_3$, 256 ($C_{15}H_{14}NO_3$, 10.9), 230 ($C_{13}H_{12}NO_3$, 12.3), 228 ($C_{14}H_{14}NO_2$, 30.3), 215 ($C_{13}H_{11}O_3$, 7.2), 202 ($C_{12}H_{12}NO_2$, 5.7), 202 ($C_{12}H_{10}O_3$, 7.2), 200 ($C_{12}H_{10}NO_2$, 6.8), 189 ($C_{11}H_9O_3$, 8), 187 ($C_{12}H_{11}O_2$, 18), 185 ($C_{12}H_9O_2$, 7.8), 175 ($C_{11}H_{11}O_2$, 77.4), 173 ($C_{11}H_9O_2$, 15.2), 172 ($C_{11}H_8O_2$, 7.9), 161 ($C_{10}H_9O_2$, 5.1), 159 ($C_{10}H_7O_2$, 23.7), 157 ($C_{11}H_9O$, 9.7), 145 ($C_9H_5O_2$, 14.8), 115 (C_9H_7, 18.3).

Buphanisine (**4**). Colourless prisms (C_6H_6) mp 122–4° $[\alpha]_D^{20} - 26.00$ ($c = 0.7$, EtOH). UV: λ_{max} 216 nm ($\varepsilon = 29\,070$), 239 (2052) and 296 (5472). IR: 3032 and 1640 ($-C=C-$), 2950 and 1070 ($-OMe$), 1615, 1480 and 940 cm^{-1} (aromatic methylenedioxy). ^1H NMR: δ 6.92 (1 H, *s*, H-10), 6.70 (1 H, *d*, $J_{1,2} = 10$ Hz, H-1), 6.59 (1 H, *s*, H-7), 6.21 (1 H, *ddd*, $J_{1,2} = 10$ Hz, $J_{2,3} = 5.0$ Hz, H-2) 5.97 (2 H, *s*, $-O-CH_2-O-$), 4.50 (1 H, *d*, $J = 17$ Hz, β H-6), 3.90 (1 H, *d*, $J = 17$ Hz, αH-6), 3.87 (1 H, *m*, H-3), 3.42 (3 H, *s*, $-OMe$). MS: MW *m/e* 285 ($C_{17}H_{19}NO_3$, base peak), 270 ($C_{16}H_{16}NO_3$, 22.31%), 254 ($C_{16}H_{16}NO_2$, 23.6), 230 ($C_{14}H_{14}O_3$, 29.7), 215 ($C_{13}H_{12}O_3$, 57.4), 187 ($C_{12}H_{11}O_2$, 10.3), 185 ($C_{12}H_9O_2$, 14.2), 172 ($C_{11}H_8O_2$, 13.0), 157 ($C_{11}H_9O$, 16.5) and 115 (C_9H_7, 15.8). ^{13}C NMR: δ 145.9; 145.5 (*s*; *s*, C-8, C-9), 138.4 (*s*, C-10a), 132.9 (*d*, C-2), 126.4 (*s*, C-6a), 125.2 (*d*, C-1), 106.7 (*d*, C-7), 102.7 (*d*, C-10), 100.5 (*t*, CH_2O_2), 72.6 (*d*, C-3), 62.9 (*d*, C-4a), 62.4 (*t*, C-6), 56.3 (*q*, C-3-OMe), 53.5 (*t*, C-12), 44.2 (*s*, C-10b), 44.2 (*t*, C-11), 28.8 (*t*, C-4).

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