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FOUR 6-HYDROXYLATED ALKALOIDS IN THE CRININE SERIES FROM *CRINUM AUGUSTUM**

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Key Word Index—*Crinum augustum*; Amaryllidaceae; alkaloids; crinamine; 6-α- and 6-β-hydroxybuphanisine; 6-α- and 6-β-hydroxycrinine.

Abstract—The structures of five alkaloids present in *Crinum augustum* were elucidated by spectral arguments. Four of them were shown to be new and constitute two pairs of epimers: 6-α- and 6-β-hydroxybuphanisine and 6-α- and 6-β-hydroxycrinine. The fifth alkaloid was identified as crinamine.

INTRODUCTION

In our previous paper [1], we reported the isolation of nine alkaloids from *Crinum augustum* Rox. cultivated in Assiut, Egypt. Two of these alkaloids were identified as lycorine and buphanisine. In addition, the new alkaloid augustine was characterized. Physical and spectral data of a fourth alkaloid of unusual type were given. As a continuation of our studies on this plant, we now report on the remaining five alkaloids.

RESULTS AND DISCUSSION

By solvent extractions, column chromatography and crystallization, the five alkaloids **5**–**9** were obtained and numbered according to ref. [1]. In the course of the structural elucidation we started with alkaloid **7** because it could be related to a known Amaryllidaceae alkaloid: crinamine ($C_{17}H_{19}NO_4$) by its mp [2], UV, IR, high resolution MS [3–6], ^{1}H NMR [7] and microchemical reactions [8] which are identical to those reported for crinamine. Mmp with authentic crinamine showed no depression.

Compounds **5** and **6** were detected on TLC as two overlapping spots [1]. The mixture was not susceptible to separation by conventional chromatographic methods including HPLC. They crystallize together from both MeOH and Et_2O and have a sharp mp. UV and IR data revealed the presence of olefinic unsaturation, an aromatic methylenedioxy chromophore, OMe and OH groups but the absence of carbonyl groups. The OH group is alcoholic based on insolubility in dil. aqueous NaOH and a negative $FeCl_3$ test.

The ^{1}H NMR spectra provided additional proof of these findings. The $CDCl_3$ spectrum exhibits signals for two olefinic protons at δ 6.05 (*dd*, J = 10 and 5 Hz) and 6.70 (*d*, J = 10 Hz) as an AB pattern partially overlapped by a methylenedioxy signal. The former olefinic signal is further split by a third proton. Singlet signals for aromatic protons are observed at δ 6.90, 6.95 and 7.10. The second signal integrates for one proton equal to the sum integration of the first and third signals. Two singlets with the sum integration of one proton equivalent appear at 5.18 and 5.87. The spectrum exhibits two singlets at 3.33 and 3.37 assignable to two OMe groups with a sum integration of only three proton equivalents. A broad signal for one proton was noticed in the range δ 7.00–8.80 and disappeared after the treatment with D_2O. Additional information about the nature of this signal was obtained from the spectrum of the alkaloid mixture in DMSO-d_6. It showed two exchangeable doublets of, together, one proton equivalent at δ 6.00 and 6.36. In the range from δ 1 to 4 the $CDCl_3$ spectrum exhibits a complicated, unresolved pattern with an integration for eight protons other than the signals assignable to the OMe groups.

In the olefinic part, the spectrum shows a close resemblance with that of buphanisine [1], buphanidrine and related alkaloids but differs completely from that of crinamine, haemanthamine and closely related alkaloids hydroxylated at C-11 [7]. The shifts of the other signals, on the other hand, are similar to those of the 6-hydroxyalkaloids of the crinine series, 6-hydroxycrinamine and 6-hydroxyhaemanthamine, with a non-oxygenated C-7 position [9, 10]. Accordingly, the signal at δ 6.95 is assigned to H-10 whereas the signals at 6.90 and 7.10, with the sum integration of one proton equivalent, are correlated both to H-7. This indicates the presence of two epimeric species which is proved by the two singlets at δ 5.18 and 5.87 due to the benzylic H-6 proton for the two C-6 epimers, again with the sum integration of one proton equivalent. As a confirmation of the C-6 hydroxylation, the spectrum does not exhibit the typical AB pattern for the C-6 methylene protons around δ 4.00 which is observed in all of the C-6 unsubstituted members of the crinine series.

The singlet signals at δ 5.18 and 5.87 observed in the $CDCl_3$ spectrum are split into two doublets at 4.83 and

* Part 2 in the series "Alkaloids of *Crinum augustum*". For Part 1 see ref. [1].

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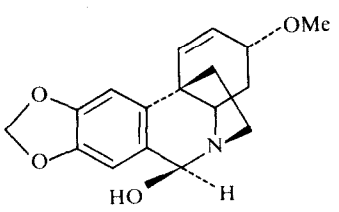

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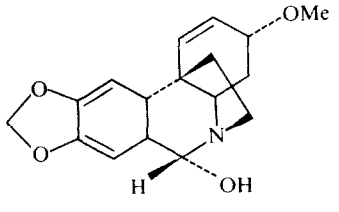

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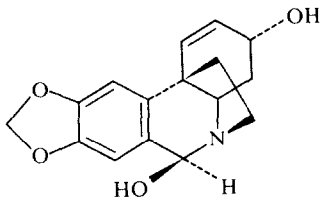

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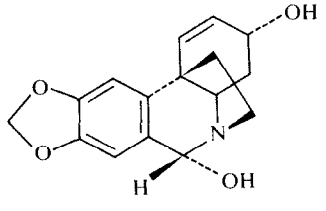

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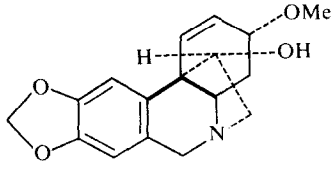

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5.48 in the DMSO-d_6 spectrum, which in turn collapse to two singlets on addition of D_2O giving evidence of CH groups in a secondary alcohol fragment. The relative lowfield shift of these signals is only explained by a benzylic structure indicating that the OH groups are located at C-6. Hydroxylation at either C-3 or C-11, which commonly occurs in the crinine series, is thus ruled out. From the size of the coupling constants $J_{6-OH,\,6-H}$ of the doublets at δ_1 4.83 ($J = 3$ Hz) and δ_2 5.48 ($J = 4.5$ Hz), it is apparent that the first signal belongs to an equatorial proton coupled with an axial OH group (α) and the second signal to an axial proton coupled with an equatorial OH group (β). The OH doublets in the DMSO-d_6 spectrum are discriminated as well at δ 6.00 (α-OH) and 6.36 (β-OH) and disappear on deuteration.

The location of the OMe group, found in the $CDCl_3$ spectrum in the aliphatic part of the molecule, is derived from the splitting pattern of the H-2 signal group. Only in the case of the C-3 methoxylation, which is of common occurrence in the crinine series, can the observed doublet from doublet structure thus be explained. H-2 couples, additional to H-1, only with one more proton at C-3. The latter is in the equatorial position, which is explained by the size of the coupling constant $J_{2,3} = 5$ Hz. In follow up, the OMe group must be axial. Thus the two OMe signals found close together in the $CDCl_3$ spectrum cannot be attributed to two epimeric C-3 species, which would have required two H-2 signals as well, but to the 6-α- and 6-β-hydroxy-epimers. The axial 3-OMe position is also found in buphanisine.

This stereoisomerism at C-6 is restricted to those C-6 hydroxyalkaloids which are not substituted at C-7 and is due to their lability [9, 10]. The epimerization is observed even in $CDCl_3$. The failure in separating **5** from **6** is in favour of this deduction. The partial fractionation of **5** and **6** by HPLC was followed by a time-dependent equilibration to the original composition of the mixture.

The epimers **5** and **6** have a MW of 301.1326 consistent with a molecular formula of $C_{17}H_{19}NO_4$. The base peak at m/z 246 arose from the expulsion of the C_3H_5N fragment from M^+, the loss of which is characteristic for the alkaloids in the crinine series, with a double bond between C-1 and C-2 and no hydroxy substituent at C-11 [5]. The high mass region displayed almost all of the peaks which have been reported in this area for buphanisine [4–6] with the difference that all peaks are shifted by an additional 16 mass units gained due to the fourth oxygen atom present in the molecular formula of the two epimers. This finding confirms the close structural relation of the two epimers to buphanisine which is strengthened by the presence of identical peaks at m/z 187 ($C_{12}H_{11}O_2$) and 115 (C_9H_7), with almost similar intensities, in the low mass regions.

The mass spectrum also confirmed the earlier findings concerning the existence of both an aliphatic OMe and OH group in the epimers. The peak with a composition $M^+ - OMe$ appeared at m/z 270, that with the composition $M^+ - CH_5O_2$ at m/z 252 must be formed by the elimination of both OMe and OH groups in addition to H from M^+. Both the resemblance of the fragmentation

pattern of the two epimers with that of buphanisine and the ready expulsion of the OH group [6] are compatible with the location of the OMe group at C-3 and the OH group at the benzylic C-6 which has already been deduced from the ^{1}H NMR spectrum.

Thus, the spectral data show that the two epimers are 6-α-hydroxybuphanisine (**5**) and 6-β-hydroxybuphanisine (**6**) respectively. They represent the sixth and seventh C-6 hydroxyalkaloids in the crinine series [3, 9, 10]. They are unique among them because they contain neither an OH group at C-11 nor an oxygen function at C-7.

The chromatographic behaviour of the mixture of **8** and **9** on column, TLC and HPLC is similar to that of **5** and **6**, indicating that they are probably epimers also. The UV and IR spectra showed the presence of a methylenedioxy phenyl system, of olefinic unsaturation and an OH group. The high resolution mass spectrum revealed a molecular formula of $C_{16}H_{17}NO_4$. The fragmentation pattern also indicated that **8** and **9** belong to the C-1,2 unsaturated alkaloids of the crinine type, without an OH group at C-11. The high mass region exhibits almost all of the peaks which have been reported in the same zone of the MS of crinine ($C_{16}H_{17}NO_3$) [4–6] with the shift of additional 16 mass units due to the fourth oxygen atom in the empirical formula. The presence of two OH groups is indicated by the appearance of a peak at *m/z* 252 with the composition $M^+ - H_3O_2$. The strong structural relation between **8/9** and crinine was further supported by the identity in composition of the peaks appearing in both spectra at *m/z* 199, 198, 187, 173, 172, 115 after the expulsion of the two and one OH groups respectively.

The ^{1}H NMR spectrum recorded in DMSO-d_6 confirms the presence of the methylenedioxy chromophore and the two olefinic protons at C-1 with identical, and at C-2 with very close chemical shifts to those of **5** and **6**. It also revealed the presence of the dual chemical shifts of both the benzylic H-6 and the aromatic H-7 protons, similar to those of **5** and **6**, indicating the presence of an OH group at the benzylic C-6. Deuteration caused the doublet at δ 4.71, with one proton equivalent, and the two doublets at 6.00 and 6.27 with, together, one proton equivalent to disappear. The latter two signals are assigned to the 6-α- and 6-β-hydroxy groups respectively. The coupling constant $J_{6\beta\text{-OH},6\alpha}$ (4.7 Hz) as expected is around 1 Hz larger than $J_{6\alpha\text{-OH},6\beta}$ (3.6 Hz). The H-6α and H-6β doublets both collapse to singlets. The H-3 proton appears as a multiplet centred at δ 4.11. The high-field OH doublet at δ 4.71 is attributed to a hydroxy group located at C-3 in an axial position. The C-3 location is derived as well from the splitting pattern of the vicinal H-2 proton, as already described for **5** and **6**, and from the half height bandwidth of the H-3 signal with 14 Hz only for the four couplings to H-2, H-4α, H-4β and 3-OH, which is only explained if H-3 is in an equatorial position. A support for the axial 3-OH group configuration is found in the size of both coupling constants $J_{2,3} = 5$ Hz and $J_{3\text{-OH},3} = 4.8$ Hz. This axial location of the 3-OH group is in harmony with that in crinine. Based on the foregoing findings, compounds **8** and **9** are elucidated as 6α-hydroxycrinine and 6β-hydroxycrinine respectively.

EXPERIMENTAL

General directions have been reported previously [1]. Additionally, the ^{1}H NMR spectrum of the epimer mixture (**8/9**) was recorded on a Bruker WH-90 MHz spectrometer.

6α/6β-Hydroxybuphaninisine (**5/6**). Colourless prisms (MeOH), mp 126–28°, $[\alpha]_D^{25}$ + 40.04° (*c* = 0.5, EtOH). UV $\lambda_{max}^{95\%\,EtOH}$ nm: 216 (ε 24 923), 239 (1896) and 294 (4063). IR (KBr) cm^{-1}: 1615, 1480, 1040, 940 (aromatic methylenedioxy), 3450, (OH), 3032 (olefinic C=C), 2950, 1070 (OMe). ^{1}H NMR ($CDCl_3$): δ 3.33 (3 H, *s*, 3-OMe (in **5**), 3.37 (3 H, *s*, 3-OMe (in **6**)), 5.18 (1H, *s*, H-6β (in **5**)), 5.87 (1 H, *s*, H-6α (in **6**)), 6.00 (1 H, *s*, OCH_2O), 6.05 (1 H, *ddd*, $J_{1,2} = 10$, $J_{2,3} = 5$ Hz, H-2), 6.70 (1 H, *d*, $J_{1,2} = 10$ Hz, H-1), 6.90 (1 H, *s*, H-7 (in **5**)), 6.95 (1 H, *s*, H-10), 7.10 (1 H, *s*, H-7 (in **6**)). ^{1}H NMR (DMSO-d_6): δ 3.23 (3 H, *s*, 3-OMe (in **5**)), 3.31 (3 H, *s*, 3-OMe (in **6**)), 4.83 (1 H, *d*, $J_{6\alpha\text{-OH},6\beta\text{-H}}$ = 3 Hz, H-6β (in **5**)), 5.48 (1 H, *d*, $J_{6\beta\text{-OH},6\alpha\text{-H}}$ = 4.5 Hz, H-6α (in **6**)), 6.00 (1 H, *d*, 6α-OH), 6.36 (1 H, *d*, 6β-OH), 6.69 (1 H, *s*, H-7 (in **5**)), 6.81 (1 H, *s*, H-7 (in **6**)). MS: MW: 301.1330 ($C_{17}H_{19}NO_4$, 78%). Other significant peaks appeared at *m/z* 286 (M^+ − Me, 7), 270 (M^+ − MeO, 14), 269 ($M^+ - CH_4O$, 9), 258 ($M^+ - C_2H_5N$, 10), 257 ($M^+ - C_2H_6N$, 20), 252 ($M^+ - CH_5O_2$, 11), 246 ($M^+ - C_3H_5N$, base peak), 231 ($M^+ - C_4H_8NO$, 41), 203 ($C_{12}H_{11}O_3$, 10), 187 ($C_{12}H_{11}O_2$, 17), 185 $C_{12}H_9O_2$, 10), 115 (C_9H_7, 11).

6α/6β-Hydroxycrinine (**8/9**). Colourless prisms (MeOH), mp 268–70°, $[\alpha]_D^{25}$ + 13.58° (*c* = 0.5, EtOH). UV $\lambda_{max}^{95\%\,EtOH}$ nm: 215 (30 032), 240 (2875) and 295 (3833). IR (KBr) cm^{-1}: 1615, 1480, 1040, 940 (aryl methylenedioxy), 3420 (OH), 3032 (olefinic C=C). ^{1}H NMR (DMSO-d_6): δ 4.11 (1 H, *m*, $J_{3\text{-OH},3}$ = 4.8, $J_{2,3}$ = 5 Hz, H-3), 4.71 (1 H, *d*, $J_{3\text{-OH},3}$ = 4.8 Hz, 3-OH), 4.82 (1 H, *d*, H-6β (in **8**)), 5.50 (1 H, *d*, H-6α (in **9**)), 5.80 (1 H, *ddd*, *J* = 10 and 5 Hz, H-2), 5.93 (2 H, *s*, OCH_2O), 6.00 (1 H, *d*, $J_{6\alpha\text{-OH},6\beta}$ = 3.6 Hz, 6α-OH), 6.27 (1 H, *d*, $J_{6\beta\text{-OH},6\alpha}$ = 4.7 Hz, 6β-OH), 6.70 (1 H, *d*, *J* = 10 Hz, H-1), 6.77 (1 H, *s*, H-7 (in **8**)), 6.87 (1 H, *s*, H-7 (in (**9**)), 7.05 (1 H, *s*, H-10). MS: MW: 287.1165 ($C_{16}H_{17}NO_4$, base peak), other significant peaks appeared at *m/z* 270 (M^+ − OH, 17%), 269 ($M^+ - H_2O$, 32.7), 252 ($M^+ - H_3O_2$, 11.6), 244 ($M^+ - C_2H_5N$, 19.4), 243 ($M^+ - C_2H_6N$, 45.9), 232 ($M^+ - C_3H_5N$, 9.4), 215 ($M^+ - C_3H_6NO$, 24), 214 ($M^+ - C_3H_7NO$, 51.6), 203 ($C_{12}H_{11}O_3$, 41.2), 199 ($C_{13}H_{11}O_2$, 9.4), 187 ($C_{12}H_{11}O_2$, 9.8), 185 ($C_{12}H_9O_2$, 26.5), 173 ($C_{11}H_9O_2$, 11.7), 172 ($C_{11}H_8O_2$, 5.7), 157 ($C_{11}H_9O$, 7.6), 115 (C_9H_7, 16.9).

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