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RELATIVE CONFIGURATION OF THE ALKALOID AUGUSTINE*

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Key Word Index—*Crinum augustum*; Amaryllidaceae; alkaloid; augustine; relative configuration; ^{1}H NMR; ^{13}C NMR; high resolution MS.

Abstract—Structural confirmation and the relative configuration of the alkaloid augustine were achieved by NMR spectral analysis. The high resolution MS fragmentation pattern of the alkaloid is also discussed.

INTRODUCTION

The isolation and purification of the new alkaloid augustine from *Crinum augustum* Rox. has been reported in [1]. Its gross structure as a 1,2-epoxy alkaloid in the crinine series has been derived from UV, IR, MS and 60 MHz ^{1}H NMR data. The presence of an oxiran ring in the alkaloid has been inferred from its composition and the absence of both olefinic unsaturation and of any additional functionality necessary to accommodate the fourth oxygen atom in the formula in comparison with buphanisine.

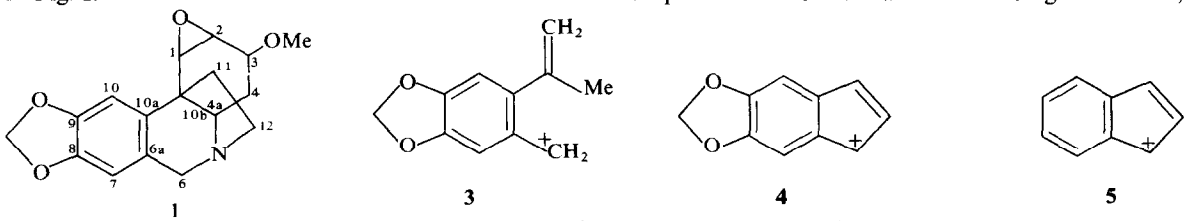
In the present study we wish to report the results of intensive NMR spectroscopic studies which led to the definite establishment of the previously proposed structure for augustine and to its relative configuration. A study of its MS fragmentation under high resolution conditions is also included.

RESULTS AND DISCUSSION

The 90 MHz ^{1}H NMR spectrum of augustine exhibits distinct signals assignable to the methylenedioxy, OMe group, two aryl, two benzylic and the H-1 and H-3 protons. Furthermore it affords a good separation and some clarification of the signals due to the remaining 9 protons in the region δ 1.4–3.4 which were previously observed in the 60 MHz spectrum as a series of unresolved peaks [1]. The planar formula of the alkaloid is depicted in Fig. 1.

The signals have been assigned to specific protons on the basis of their shifts, their multiplicities and the size of the coupling constants which were partly evaluated directly from the normal spectrum, by subspectral analysis, and partly from selective decoupling experiments. The complete set of data is shown in Table 1. Accordingly, the signal at δ 3.83 assignable to H-1 is observed as doublet, coupling with the signal appearing as a multiplet (*ddd*) at δ 3.35 attributable to the second oxiran ring proton H-2. Irradiation of the latter signal confirms this assignment in causing the collapse of the H-1 doublet to a singlet which is overlapping with one line of the doublet assignable to H-6α. This finding lends strong support to the presence of a C-(1)–C-(2) oxiran ring. The irradiation experiment also induces simplification of both the 'quartet' (*ddd*) at δ 4.03 due to the H-3 to a 'triplet' (*dd*) and the multiplet centred at δ 1.70 which loses the long-range splitting of *ca* 1 Hz and is consequently correlated to one of the H-4 protons. This long-range coupling in a *W*-mechanism between H-2 and one H-4 proton in an α-position is best explained with their almost equatorial positions in the half-chair conformation of ring C caused by the oxiran ring in position 1 and 2. The coupling constant $J_{1,2}$ = 3.5 Hz is at the lower margin of the known *cis*-1,2-epoxycyclohexane derivatives coupling [2].

Saturation of the resonance frequency of H-3 at δ 4.05 causes the expected collapse of the H-2 multiplet to a doublet of doublets and simplification of the H-4α multiplet. This is observed as well for the signal at δ 1.47,



1 3 4 5

Fig. 1.

* Part 3 in the series "Alkaloids of *Crinum augustum*". For Part 2 see Ali, A. A., Kating, H. and Frahm, A. W. (1981) *Phytochemistry* **20**, 1731.

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Table 1. 90 MHz ^{1}H NMR spectrum of augustine*

Proton	δ (ppm)	Multiplicity	Coupling protons	J (Hz)
H-10	6.95	*s*	—	—
H-7	6.55	*s* broad	7,6β	0.5
OCH_2O–	5.93	*s*	—	—
H-6β	4.42	*d* broad	6β,6α	−17.0
			6β,7	0.5
H-3	4.03	*ddd*	3,2	2.5
			3,4β	2.8
			3,4α	2.4
H-1	3.83	*d*	1,2	3.5
H-6α	3.70	*d*	6α,6β	−17.0
3-OMe	3.47	*s*	—	—
H-2	3.35	*ddd*	2,1	3.5
			2,3	2.5
			2,4α	0.9
H-12	3.20	*ddd*	12,12′	−12.5
			12,11	10.5
			12,11′	4.8
H-4a	3.15	*dd*	4a,4α	4.0
			4a,4β	12.5
H-12′	2.80	*ddd*	12′,12	−12.5
			12′,11′	9.0
			12′,11	5.4
H-11	2.42	*ddd*	11,11′	−12.0
			11,12	10.5
			11,12′	5.4
H-11′	2.05	*ddd*	11′,11	−12.0
			11′,12	9.0
			11′,12	4.8
H-4α	1.70	*dddd*	4α,4β	−13.5
			4a,4a	4.0
			4α,3	2.4
			4α,2	0.9
H-4β	1.42	*ddd*	4β,4α	−13.5
			4β,4a	12.5
			4β,3	2.8

*Solvent $CDCl_3$.

then appearing as a doublet of doublets which was accordingly assigned to H-4β.

The H-3 signal was found with a narrow bandwidth of *ca* 7.5 Hz. This value excludes the accommodation of H-3 in an axial orientation which requires at least double that bandwidth. From this finding, the 1β,2β oxiran configuration is also derived. The opposite configuration would have required a broader bandwidth for H-3 because of the enlarged *J* value for the coupling between H-2 and H-3 in a nearly ecliptic position (Dreiding model).

The equatorial H-3 proton couples with H-2, H-4α and H-4β in form of a so-called 'quartet', the three coupling constants of which are accidentally *ca* equal and amount to *ca* 2.6 Hz. Therefore the 3-OMe group must have the axial position.

Decoupling experiments with both H-4α and H-4β enable the assignment of the doublet of doublets signal at δ 3.15 to the nitrogen-adjacent H-4a proton. The size of $J_{4\alpha,4\beta} = 12.5$ Hz proves the *trans*/axial positions of H-4a and H-4β protons. H-4β couples in addition to H-4a to the *gem* H-4α and the *vic* H-3 as H-4α does with H-4α, H-4β, H-3 and long range with H-2 confirming its quasi equatorial location. From the size of the coupling constant of H-4α, H-4β and H-3, it can also be deduced that ring B and C are linked together in a *trans* juncture. In the case of a *cis* B–C linkage the H-4a would still be kept in an axial position and would show similar coupling constants. But in particular the coupling constant $J_{2,3}$ should then increase strongly and differ from $J_{3,4\alpha} \approx J_{3,4\beta}$ as can be deduced from the Dreiding model. Also, the long range coupling between H-2 and H-4α should not occur. This evidence finds a support in the ^{13}C NMR shift data (see Table 2). The ^{1}H spectrum shows additionally an interaction between the aromatic H-7 and the equatorial H-6β proton resulting in a line-broadening.

The protons H-12, H-12′, H-11 and H-11′ show an ABXY pattern which is consistent with the 5,10b-ethano bridge in a pyrrolidine structure type. The two signals at δ 3.20 and 2.80 comparatively lowfield shifted are ascribable to the nitrogen-adjacent H-12 and H-12′ protons respectively. The correlation of these two signals was done in accordance with [3] on the basis that H-12 is coplanar to the nitrogen lone pair electrons and acquires a downfield shift of *ca* 0.4 ppm in comparison with H-12′ which is directed towards the aromatic ring A. The signals attributable to H-11 and H-11′ protons are found at δ 2.42 and 2.05 respectively. Each of the four signals appear as a multiplet (*ddd*) with *gem*, *cis* and *trans* coupling constants. H-11 and H-12 as well as H-11′ and H-12′ each are located in the *cis* position with $J_{cis} > J_{trans}$ because of the eliptic conformation in the rigid 5β,10bβ-ethano bridge.

The inspection of the 90 MHz ^{1}H NMR spectra led to the stereo formula **2** of augustine with a *trans* B–C ring juncture, a 5β,10bβ ethano bridge, a 1β,2β oxiran ring and a 3-α OMe group.

Figure 2 represents its relative configuration, which matches with those of the buphanisine type.

The ^{13}C NMR spectrum of augustine gave a complete confirmation of its proposed structure and its elucidated stereochemistry. It was possible to make virtually complete assignments of all signals on the basis of general shift rules, the observed multiplicities in the off-resonance decoupled and coupled spectra, selective proton decoupling and comparison with data from certain other alkaloids in the crinine series (unpublished results) including undulatine [4]. The results are summarized in Table 2.

The spectra reveal signals for five nonprotonated (S), six methine (D), five methylene (T) and one Me (Q) carbons. The signals in the lowfield range above δ 95 are

Table 2. 20 MHz ^{13}C NMR spectrum of augustine*

Carbon	δ, Multiplicity	Carbon	δ, Multiplicity
C-9	146.0 *s*†	C-4a	61.5 *d*
C-8	145.6 *s*†	C-3 OMe	57.4 *q*
C-10a	137.8 *s*	C-2	54.9 *d*
C-6a	126.8 *s*	C-1	53.7 *d*
C-7	107.0 *d*	C-12	52.3 *t*
C-10	102.3 *d*	C-10b	41.5 *s*
OCH_2O–	100.7 *t*	C-11	39.2 *t*
C-3	74.8 *d*	C-4	25.3 *t*
C-6	62.4 *t*		

*Solvent $CDCl_3$.
†Interconvertible signals.

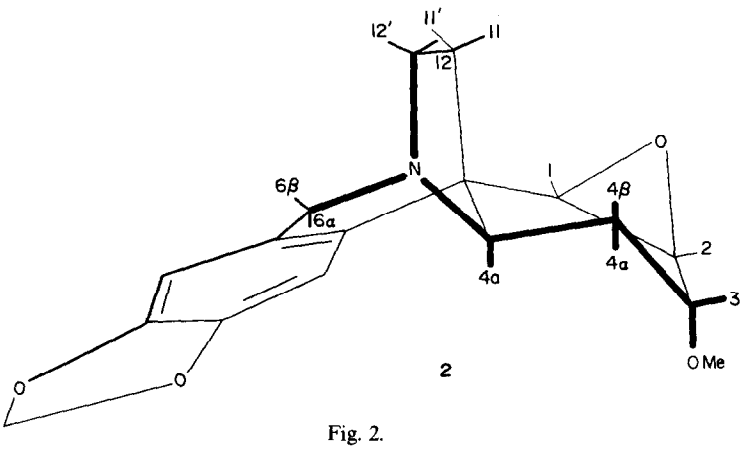

Fig. 2.

restricted to four singlets, two doublets and one triplet. The latter is observed at *ca* δ 100 which is characteristic for a methylene carbon in an aromatic methylendioxy fragment. The four quaternary singlets indicate a fourfold substituted aromatic ring. The two aromatic methine doublets do not show any further splitting in the fully coupled spectrum thus clarifying the 1,2,4,5-benzene ring substitution. Comparison with the ^{13}CNMR data of buphanisine [1] clearly shows matching chemical shifts for the corresponding seven signals of C-6a, 7, 8, 9, 10, 10a and OCH_2O. The singlet due to the fifth quaternary carbon at δ 41.5 arises from C-10b which is observed upfield shifted in comparison with the C-10b singlet in most of the investigated alkaloids in the crinine series (unpublished results; [1,4]). Contrary to the lowfield shifted doublets of C-1 and C-2 in the C-1, 2 unsaturated alkaloids (unpublished results; [1,4]) these two doublets in the spectrum of augustine are found at δ 53.0 and 54.95 respectively, which is in accordance with the range of oxiran carbon resonances [4, 5]. The doublet signal at δ 74.83 clearly indicates methoxylation at the C-3 atom. The remaining doublet in the highfield range is correlated to C-4a, provided that ring B and C are linked in a *trans* juncture and that the C-3 substituent is in the axial position.

The triplet at highest field at δ 25.3 is assigned to C-4. The highfield shift of this signal with respect to the corresponding signal in the C-1, 2 unsaturated alkaloids in this series which absorbs at a lower field between δ 28 and 32.5, is an indicator for the 1,2-oxiran ring in augustine. The shift of the C-11 signal is influenced as well by this structural feature. It is found at δ 39.2, normally observed at *ca* δ 44 in the case of C-11-nonhydroxylation. The triplet at δ 52.3 is attributed to C-12 adjacent to the nitrogen. The left triplet signal at δ 62.4 originates from C-6 with a strong-line stability in the investigated alkaloids in this series when C-7 is not substituted (unpublished results; [1,4]). The quartet at δ 57.7 belongs to the 3-OMe carbon.

The only ^{13}C NMR spectrum for the group of 1,2 epoxy alkaloids so far reported is that of undulatine [4]. Comparison revealed that the signals in the high-field range are almost matching with those of augustine except for the C-6 signal which exhibits an upfield shift of *ca* 5 ppm due to the 7-OMe group. In the lowfield range all of the signal shifts differ because of the C-7 substitution.

The MS of augustine [1] provides some information which could facilitate the systematic structure elucidation of new epoxy alkaloids in the crinine series. The spectrum exhibits M^+ as base peak in harmony with the findings for all reported alkaloids in this series except both crinamine [6, 7] and the 1,2-epoxy alkaloid cavinine [8] which differs from augustine by a C-11 OH and a C-7 OMe group. The different composition of the base peak in both augustine and cavinine spectra indicates that the oxiran ring is not a discriminating feature in the formation of the base peak in this group of alkaloids. The different origin of the base peak from $M^+ - MeOH$ in case of crinamine may be attributed to the unusual configuration of crinamine with the equatorial α-3-OMe group together with an equatorial β-C-10b–C-10a linkage. The base peak of cavinine was reported to arise from $M^+ - CHO$ and has been claimed to originate entirely through the expulsion of a CHO fragment from the oxiran ring. In contradiction, the spectrum of augustine exhibits the peak $M^+ - CHO$ in a comparatively low relative intensity (8%). So the base peak in the cavinine spectrum is only to be understood if it is established by elimination of the CHO fragment from both the oxiran ring and mainly from the hydroxylated C-11 which has been reported to be a source for this fragment in the MS of the C-11 oxygenated alkaloids in this series [6].

The second significant peak in the high mass region of augustine is observed at *m/z* 228 and is consistent with $M^+ - C_3H_5O_2$. The coincidence of the corresponding peak in the MS of cavinine [8] with almost the same relative abundance (32%) is in favour of their formation by one mechanism involving the elimination of the C-2,3 element with its substituents, in agreement with what has been reported from the MS of alkaloids in this series which have a saturated ring C [9]. This $M^+ - C_3H_5O_2$ ion is unlikely to originate from the combined removal of CHO and the C-11,12 ethano-bridge fragments as has been described in case of cavinine [8].

The third abundant peak appears in the middle mass region at *m/z* 175 with the composition $C_{11}H_{11}O_2$ (77%). No corresponding peak has been recorded in the spectra of either cavinine or any other alkaloids in this series. Its likely formation from M^+ is supported by a small metastable peak at *m/z* 102 with the proposed structure **3** (Fig. 1).

The next significant peak was noticed at *m/z* 159 (23%) with the composition $C_{10}H_7O_2$. The corresponding peak for cavinine at *m/z* 205 has a composition of $C_{11}H_9O_4$ in a higher abundance (50%). This composition indicates that the substituents OH and OMe at C-11 and C-7 respectively are still retained in this ion. This means that

C-11 exists in both of the two corresponding ions at *m/z* 159 and *m/z* 205. Then the ion at *m/z* 159 should likely possess structure **4** (Fig. 1).

The only significant peak in the low mass region is observed at *m/z* 115 and is composed of C_9H_7. It is characteristic of all of these alkaloids. Its postulated structure is **5** (Fig. 1).

The high mass region of augustine exhibits peaks of relatively low intensities at *m/z* 286 and 270 due to expulsion of Me and OMe fragments respectively from M^+. No peak corresponding to the elimination of MeOH is observed, in agreement with the MS of cavinine [8].

The middle mass region shows peaks of low abundance at *m/z* 215, 187, 185, and 157 with compositions identical to those of buphanisine. The peak at *m/z* 215 was described in the MS of buphanisine as a parent ion in this series from which the three other fragments originate in succession [6]. In the case of augustine the identical fragmentation of the *m/z* 215 key ion is concluded. This ion is expected to arise from the expulsion of both the 3-OMe group and the nitrogen-containing fragment C_3H_5N which on rearrangement could adapt the structure of the corresponding ion in buphanisine [6].

The elucidated relative configuration obtained from the resonance techniques is not contradicted by the MS fragmentation pattern. Support is found in the base peak M^+, and a generally parallel fragmentation line with both buphanisine and cavinine.

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