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REVISED STRUCTURE OF THE ALKALOID CLIVORINE

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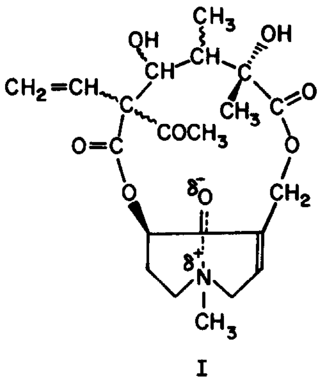
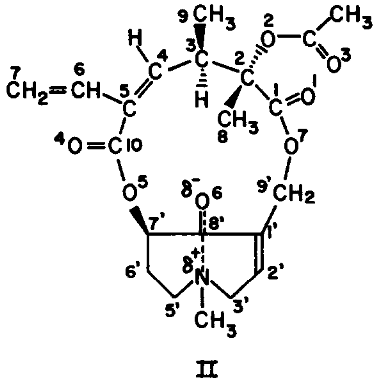
On the basis of chemical evidence and PMR spectral data the structure I (empirical formula $C_{21}H_{29}NO_8$) was ascribed to clivorine[1], an alkaloid isolated[2] from *Ligularia clivorum* Maxim. In order to determine the configuration at C(3), C(4) and C(5) it was decided to carry out an X-ray analysis. Its results showed that the structure is II and that a water molecule of crystallization is present, corresponding to the empirical formula $C_{21}H_{27}NO_7 \cdot H_2O$. After the determination of the structure by X-ray diffraction a sample of clivorine, dried for 5 hrs at 100°C and 0.04 mm Hg, was analysed. The results agreed with those calculated for $C_{21}H_{27}NO_7$.

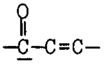


I



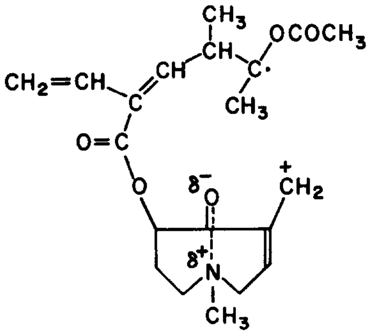
II

Further spectroscopic studies proceeded concurrently and we now present a reinterpretation of our results. A positive Cotton effect at ca. 260-280 nm (ethanol, tail) or 285 nm (methyl cyclohexane, distinct band) is indeed characteristic for all othonecine diesters[3], a positive CD-band at 244 nm ($\Delta\varepsilon$ = +4.29) and another negative one at 219 nm (Δε = -8.70) indicates, however, the presence of a conjugated α,β-unsaturated ester system in the acidic moiety[3]. This finding necessitated a revision of the structure of clivorine.

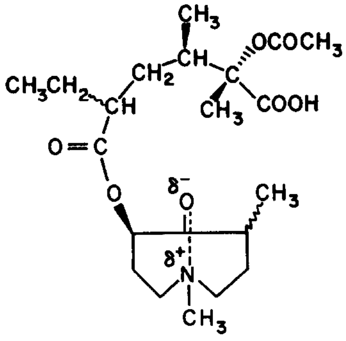
The ^{13}C NMR spectrum[4] shows peaks assignable to all 21 carbons. There are six peaks in the 100-150 ppm region resulting from three carbon-carbon double bonds; two of them belong to the carbon atoms which bear no protons (based on the off-resonance CW decoupling). Moreover, the chemical shift of one of the four carbonyl carbon resonances indicates a  $-\overset{O}{\overset{\|}{\underline{C}}}-C=C-$ grouping. The correct structure (II) has therefore a double bond between C(4) and C(5) and an O-acetyl group at C(2). The PMR spectrum is compatible with this structure, only the nature of H(C4) and of the two exchangeable protons must be changed.

Consequently, the empirical formula of clivorine is $C_{21}H_{27}NO_7$. It is consistent with the mass spectrum which exhibits a molecular peak at M^+ 405. As in the case of analogous alkaloids[5], fragmentation of a molecular ion gives rise to the fragment III at m/e 361 which then loses an acetyl to give an ion at m/e 318 and, moreover, an ion at m/e 346 ascribable to loss of an acetoxy group. The highest peak in that part of the spectrum which shows the fragmentation of the acidic moiety of the molecule is exhibited at m/e 302 and corresponds to the simultaneous loss of CO_2 and CH_3COO from the molecular ion. The occurrence of ions at m/e 168, 151, 150, 149, 123, 110, 96 and 94 is characteristic[5] for the diesters of othonecine.

In view of these findings, octahydroclivorine[1] will have to be allotted the revised structure IV whereas the validity of the structures assigned to clivonecinol[1] and to its transformation products remains firm. The structure of the so-called acetylclivorine[1] will have to be studied further.



III



IV

Clivorine crystallizes from acetone in the form of well-developed, colourless bipyramids which belong to the orthorhombic space group $P2_12_12_1$. The cell dimensions at -160°C are a = 12.765, b = 14.772 and c = 11.145Å. The data were collected at low temperature (-160°C) on a manual four-circle diffractometer using the $\theta/2\theta$ scan technique and Cu*K*α radiation. Of the 2628 reflections with $2\theta \leq 163°$ 2515 were considered observed. In view of the absence of heavy atoms the structure had to be solved by direct methods[6]. In the initial attempts symbols were assigned to a few reflections and, using the Σ_2 formula, their phases as well as those of 50-80 other reflections were determined. Additional phases were then determined by the application of the tangent formula. This procedure did not lead to a solution of the structure. A successful solution was reached by using the following reflections as a starting set:

h	k	ℓ	$\|E\|$	ϕ
0	11	7	4.04	$\pi/2$
11	3	0	3.20	$\pi/2$
4	5	0	2.39	0
0	2	5	2.03	0
6	2	8	3.12	
2	6	9	2.75	
4	7	8	2.70	

The first three reflections define the origin and the fourth defines the enantiomorph. The last three were assigned the phases $\pm \pi/4$ and $\pm 3\pi/4$. All possible 64 combinations were tried and the phases of 235 reflections ($|E| \geq 1.6$) were then determined by the tangent formula. Two sets resulted in much lower *R*(Karle)[6] values, 18.7 and 17.9%, as compared to 26.5-35.2% for the others. The set with the lowest *R*(Karle) value was extended to a total of 360 reflections ($E \geq 1.45$) and these were used in the calculation of an *E*-map. From this map all the non-hydrogen atoms were located on the 30 highest peaks. Their parameters were refined by the least-squares method. The positions of the hydrogen atoms, except one in the water molecule, were determined from a low-angle difference Fourier map and they were included in the refinement. At present the reliability factor *R* is 4.4%. The refinement is being continued.

A perspective view of the molecule can be seen in Figure 1. There is a transannular interaction between the nitrogen atom and C(8'): the N----C(8') distance is 1.993(3)Å, approximately 1Å shorter than the sum of van der Waals radii, and the C(8')$\overset{-}{=}$O distance 1.258(3)Å is significantly longer than a normal C=O bond length (1.215Å). This interaction implies a re-hybridization of C(8') to a state intermediate between sp^2 and sp^3. This is manifested by the fact that C(8') is displaced by 0.213(3)Å from the plane through the three atoms to which it is bonded. As expected, the substituents of the nitrogen atom are tetrahedrally arranged. The water oxygen atom makes two close contacts, each to a different molecule of clivorine. One of them, O(W)····O(6) 2.762(3)Å, can be ascribed to a hydrogen bond. The other one, O(W)····O(4) 3.125(3)Å, cannot be interpreted with certainty since this distance is longer than a normal hydrogen bond and the position of the relevant hydrogen atom was not determined.

The conformation of the eight-membered ring can be described as follows:

C(8'), C(1'), C(2'), C(3') and N are coplanar; C(7'), C(8'), N and C(5') lie approximately in one plane while C(6') is displaced by 0.648Å from it. The dihedral angle between the two planes is 59.6°. The conformation of the twelve-membered ring can be seen in Figure 2.

Complete results of the X-ray analysis will be published elsewhere (by K.B.B.).

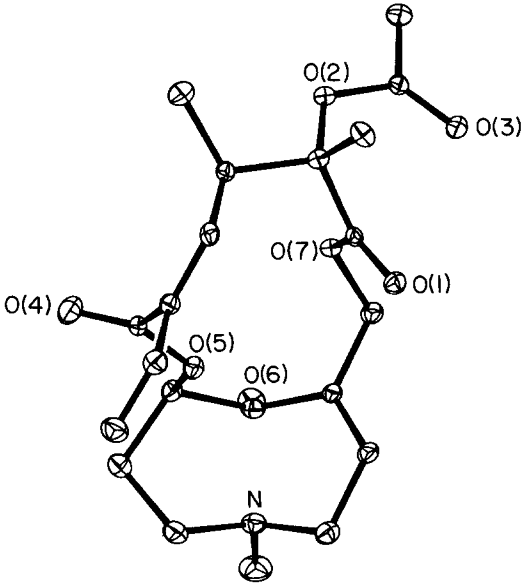


Fig. 1 A perspective view of clivorine.

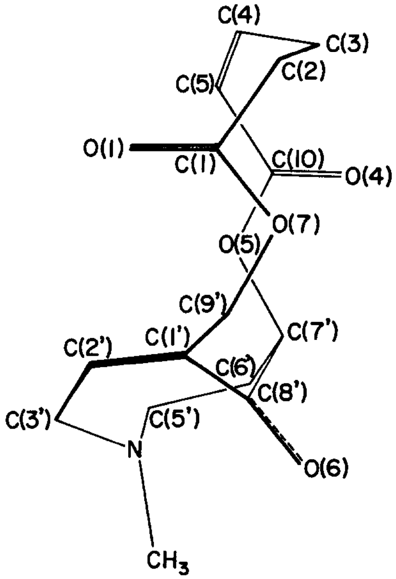


Fig. 2 Partial structure of clivorine viewed along the b-axis.

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References

1. A. Klásek, P. Sedmera and F. Šantavý, *Coll.Czech.Chem.Comm.* 35, 956 (1970).
2. A. Klásek, P. Vrublovský and F. Šantavý, *Coll.Czech.Chem.Comm.* 32, 2512 (1967).
3. J. Hrbek, Jr., L. Hruban, A. Klásek, N.K. Kochetkov, A.M. Likhosherstov, F. Šantavý and G. Snatzke, *Tetrahedron*, to be submitted.
4. ^{13}C NMR (25.15 MHz, dioxane, δ_{TMS} = 0): 194.8 (−$\overset{\overset{O}{\|}}{\underline{C}}$−C=C−); 171.3, 169.7, 161.5 (3x $\underline{C}$=O); 137.0, 134.8 (2x −$\overset{|}{\underline{C}}$=); 136.6, 135.1, 132.8, (3x H$\overset{|}{\underline{C}}$=); 115.9 (=$\underline{C}H_2$); 82.6, 77.8, 66.2 (3x −$\underline{C}$−O); 58.7, 53.4 ($\underline{C}$−N); 41.4 ($\underline{C}H_3$−N); 40.3 (−$\underline{C}HCH_3$); 36.7 (CH$\underline{C}H_2CH_2$); 20.5 ($\underline{C}H_3COO$); 15.0, 14.4 (2x $\underline{C}H_3C$).
5. M.P. Cava, K.V. Rao, J.A. Weisbach, R.F. Raffauf and B. Douglas, *J. Org. Chem.* 33, 3570 (1968).
6. J. Karle and I.L. Karle, *Acta Cryst.* 21, 849 (1966).