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The Structure of Akuammidine

By Stuart Silvers and A. Tulinsky

Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut, U.S.A.

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The structure of akuammidine methiodide has been solved employing three-dimensional X-ray crystallographic techniques and it has been shown to be identical with that of the methiodide of an alkaloid recently isolated and called 'rhazine'. The crystals are orthorhombic; $P2_12_12_1$,

$$a = 9{\cdot}86,\ b = 12{\cdot}34,\ c = 18{\cdot}75\ \text{Å},$$

4 molecules/unit cell. Intensity data were collected to 1 Å resolution employing the stationary crystal–stationary counter technique. The structure was solved by the heavy atom method and refined through successive electron and difference density considerations. The ninth and final structure factor computation, which included 24 hydrogen atoms, gave $R = 0{\cdot}095$. The previously proposed skeletal structure of akuammidine has been confirmed and uncertainties about side chain stereochemistry have been removed. A methyl ester group (–CO_2Me) of the alkaloid is disordered between two positions approximately 180° apart. Adjacent alkaloid molecules are linked together in the *a* direction through a hydrogen-bonding water molecule.

1. Introduction

In a preliminary account of this work (Silvers & Tulinsky, 1962), the structure of akuammidine ($C_{21}H_{24}N_2O_3$), isolated from seeds of *Picralima klaineana* (Henry, 1932), is identical with an alkaloid recently isolated from the shrub *Rhazya stricta* Decaisne and called 'rhazine' (Chatterjee *et al.*, 1961). The unit cells, the space groups and the single-crystal X-ray diffraction patterns of the two are the same to a high degree, as are their infra-red absorption spectra.

$C_{21}H_{26}N_2O_3$, tertiary base
–OMe –CO_2Me
2 active H's –OH –NH
–CMe (50% theoretical), probably 2 NMe's, u.v.
spectrum typical of indole:

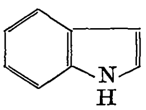
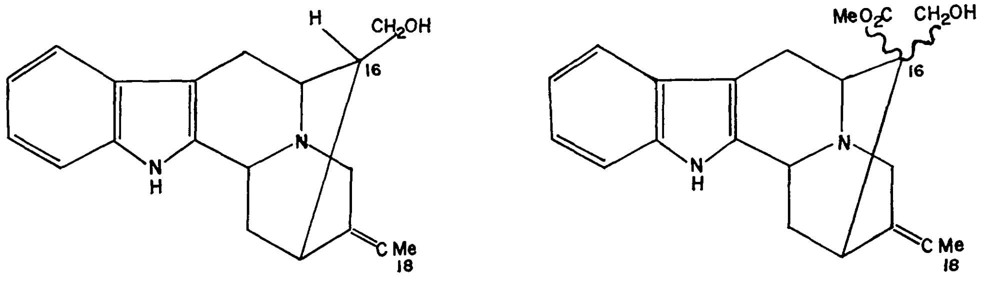


Fig. 1. The structural information known about 'rhazine' at the onset of the X-ray structure determination.

This X-ray structure determination was begun on 'rhazine'. The structural information then known about 'rhazine', hereinafter called akuammidine, is summarized in Fig. 1 (Chatterjee *et al.*, 1961). A later communication (Chatterjee *et al.*, 1962) changed the empirical formula to $C_{21}H_{24}N_2O_3$, established the presence of the indole with certainty, and said that the alkaloid contains ethylidine ($-CH=CHMe$) and primary hydroxyl groups but no $-NMe$ groups. By this time, however, the structure of akuammidine had already been related by degradative methods to that of normacusine-B, (I) of Fig. 2, and it had been established to be (II) of Fig. 2, with the stereochemistry at C_{16} and C_{18} uncertain (Levy, LeMen & Janot, 1961).

2. Experimental

The structure determination was carried out on akuammidine methiodide.* Single crystals, displaying pseudo-octahedral morphology, were grown by slow



(I) (II)

Fig. 2. (I) Normacusine-B; (II) Akuammidine, structural uncertainties indicated.

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* We wish to thank Dr A. Chatterjee for supplying us with 10 milligrams of this derivative.

evaporation of an aqueous solution. The crystal selected for intensity measurements was a fragment in the form of a triangular plate with dimensions of about 0·5 mm on each edge and 0·25 mm in thickness.

All the X-ray work (Cu $K\alpha$) was carried out with a General Electric Single Crystal Orienter. Preliminary measurements indicated that the crystal was orthorhombic with $a=9{\cdot}86$, $b=12{\cdot}34$, $c=18{\cdot}75$ Å. Systematic absences fixed the space group as $P2_12_12_1$ and there are 4 molecules/unit cell.

Since the atomic arrangement, rather than particularly accurate bond distances and bond angles, was of interest in this investigation, intensity data collection was confined to 1 Å resolution. Intensities were measured by the stationary crystal–stationary counter technique (Furnas, 1957). Mosaic spreads of a group of selected reflections were found to be exceptionally symmetric and sharp (0·25–0·30° from background to background). With the aid of these spreads, a 'best quadrant' was chosen for data collection (quadrant rather than octant, since but little over a hemisphere is accessible with the Orienter). The positions of diffraction maxima were located with the aid of angular coordinates precomputed from lattice dimensions. These were often checked to assure that maximum intensity was being observed. The intensity of a reflection was taken to be the difference between the two measurements with balanced Ni–Co (CoO embedded in plastic) filters. Of the 1396 reflections possible, 1261 (90·3%) were taken to be observed.

When intensity data collection was initiated, the intensities of five convenient reflections were monitored and plotted as a function of the time the crystal was exposed to X-rays. A direct beam shutter wired in parallel with a time meter permitted such observations to be made. Although there was appreciable scatter in these data and individual plots differed slightly, all five decreased continuously and perceptibly (by about 8%) during the first 8–10 hours of exposure and then remained approximately constant for the last eight hours. Since a comparison of the relative intensity distribution along the c axis before and after data collection showed it to be essentially unchanged, an average curve, obtained from all the monitor data was used to correct intensities for decay due to crystal exposure time.

The intensities were also corrected for absorption. Intensities of the ($h00$) reflections ($\chi=90°$) were measured as a function of azimuthal orientation, φ. The ratio between maximum and minimum varied between 1·37 in the low orders to 1·18 in the high orders. This observed variation of intensity with φ of a particular ($h00$) reflection was assumed to be due only to a corresponding change in absorption with φ. Such functions can strictly be used to correct for absorption only the intensities of those reflections with the same 2θ values as the ($h00$) reflections and, also, with $\chi=90°$. However, as an approximation, the several ($h00$) reflections were used to obtain an average curve (maximum–minimum ratio of 1·28) with which all the intensities were then corrected for absorption as a function of their φ value.

Finally, Lorentz and polarization factors were applied to the corrected intensities to obtain a set of relative $|F|^2$'s and $|F|$'s. An absolute scale was then approximated by Wilson's method.

3. Structure analysis

Since the iodide ion was expected to dominate the diffraction pattern, it was considered unnecessary to use three-dimensional data in order to determine its positions; thus only the three principal Patterson projections were examined. The Patterson coefficients were sharpened with $(z_{\rm I}/f_{\rm I})^2$, where $z_{\rm I}$ and $f_{\rm I}$ are, respectively, iodine's atomic number and scattering form factor.* Such a sharpening transforms the iodine–iodine vectors into ones due to point atoms with thermal vibration. To be sure, the light atom vectors are also sharpened by this procedure, but not to point atom vectors, because their scattering powers fall off much more rapidly with scattering angle than does that of iodine (at 1 Å resolution, $z_{\rm I}/f_{\rm I}=1{\cdot}96$, whereas $z_{\rm N}/f_{\rm N}=3{\cdot}89$, where N refers to nitrogen). Thus, such a sharpening insures a definite and preferential enhancement of the heavy atom vectors. Shown in Fig. 3 are the three principal projections from which iodine coordinates of $x=0{\cdot}085$, $y=0{\cdot}178$, $z=0{\cdot}111$ were obtained.

Phases based on iodine were assigned to all the observed structure amplitudes and a three-dimensional

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* The iodine form factor was used throughout this analysis; therefore, iodine and iodide are hereafter used interchangeably.

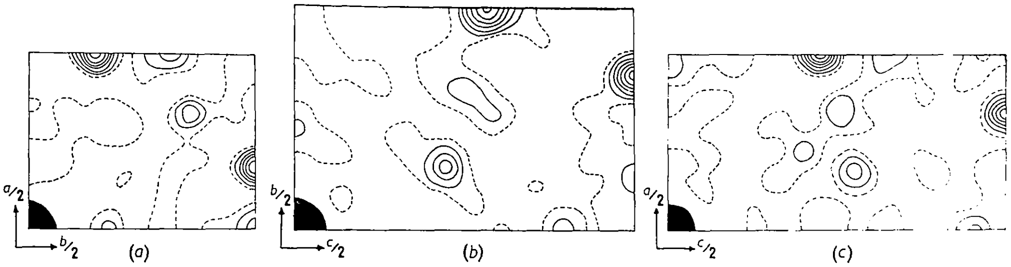

Fig. 3. The three principal sharpened Patterson projections; contours at arbitrary but equal increments; zero contour broken, negative ones not shown; origin peak darkened.

electron density (ϱ_1) was computed. The electron density obtained with these phases contained 35 peaks greater than 2·2 e.Å^{-3} of which 3 were immediately eliminated as improbable by virtue of their close contact with iodine. From the remaining peaks, the skeletal structure of akuammidine (Fig. 2) was easily recognized. Moreover, so were the ethylidine and carboxylic acid functional groups and, also, the quaternary nitrogen's methyl group. The only uncertainties as to the complete structure at this stage were: (1) the location of the methyl ester's methyl group (2) a –CH_2OH or a crystallographically equivalent group with a large C–C distance to the ring system and (3) a peak of 3·1 e.Å^{-3}, possibly a water molecule within hydrogen bonding distance of the indole nitrogen of one molecule and the forementioned carbinol group of a neighboring molecule.

Two structure factor computations were then carried out. One was based on iodine plus 24 atoms: 20 carbons, 2 nitrogens and 2 oxygens (omitting the above mentioned uncertainties) and the other on iodine plus 11 atoms: 10 carbons and one nitrogen (the reasonably certain indole and the two atoms connected to it). The latter structure factor computation was made to verify the atoms included in the former. The R factor for the first was 0·18 while that of the second was 0·27. A detailed comparison of the electron densities, ϱ_2 and ϱ_3 respectively, which were determined by these two sets of phases confirmed the 24 atoms included in the larger computation. Furthermore, it established the presence of the –CH_2OH group and the water molecule. Although not suspected at the time, the only remaining uncertainty was the ester's methyl group. In ϱ_1 there was a 1·6 e.Å^{-3} peak within bonding distance of a carboxyl oxygen. In ϱ_2 it increased to 2·2 e.Å^{-3} while in ϱ_3 it remained the same, appearing at 1·7 e.Å^{-3}. Therefore, this peak was assumed to be the methyl of the ester group. However, in what follows, it will be shown that this is not completely correct. The three other peaks of ϱ_1 not included in the larger structure factor computation were taken to be spurious because they decreased in height by more than a factor of two, practically disappearing in both ϱ_2 and ϱ_3. The atoms omitted from the structure factor computation for ϱ_3, but included in that for ϱ_2, increased or maintained their peak heights in ϱ_3 relative to their heights in ϱ_1. In both ϱ_2 and ϱ_3 the included atoms returned to near their final peak heights.

The next structure factor computation was based on iodine plus 28 atoms. These included a hydrogen bonded water molecule, a –CH_2OH group put in as two carbon atoms in an attempt to distinguish between the carbon and oxygen atoms, and the ester's methyl group. The R factor dropped to 0·15. The electron density (ϱ_4) based on these phases confirmed the water molecule and the –CH_2OH group (carbon with peak height of 5·4 e.Å^{-3}, oxygen of 7·3 e.Å^{-3}). However, it raised serious doubts about the ester's methyl group. Although it was included in the structure factor computation, it increased in ϱ_4 to less than twice its original height (only to 4·0 e.Å^{-3}). Moreover, a small peak (1·7 e.Å^{-3}) appeared within bonding distance of the other carboxyl oxygen. Reexamination of this region in ϱ_1, ϱ_2 and ϱ_3 showed that this peak was persisting (0·7, 1·4 and 0·8 e.Å^{-3}, respectively). Therefore, it was concluded that the peak originally included as methyl (Me_1 hereafter) was wrong, and the new peak (Me_2) was assumed to be the correct methyl group.

The next structure factor computation included Me_2 rather than Me_1 and coordinate shifts from ϱ_4. In addition, individual isotopic thermal parameters were assigned on the basis of the relative peak heights of the atoms. The R factor decreased to 0·144.

The phases of this computation were then employed to compute electron and difference densities (ϱ_5 and D_1). The most interesting feature of these densities was again the ester's methyl group, this time Me_2. It returned to a peak height of 3·7 e.Å^{-3} in ϱ_5 and was $-1·5$ e.Å^{-3} in the difference density. On the other hand, Me_1 appeared, respectively, as 2·0 e.Å^{-3} and $+1·6$ e.Å^{-3}. It was then evident that the carboxyl group is disordered between two positions approximately 180° apart. Moreover, the behavior of the peak heights of Me_1 and Me_2 in ϱ_4, ϱ_5 and D_1 suggested that the two positions are about equally occupied.

In order to establish conclusively that the carboxyl group is disordered, the next structure factor computation included Me_1 as carbon but not Me_2. In addition, more quantitative atomic parameter shifts were obtained from a comparison of the observed and calculated electron densities. The R factor decreased to 0·124.

Electron and difference densities (ϱ_6 and D_2) were computed. In ϱ_6, Me_1 appeared at 3·9 e.Å^{-3} but was $-1·5$ e.Å^{-3} in D_2 while Me_2 was $+1·6$ e.Å^{-3} in both densities. Accordingly, the next structure factor computation included both Me_1 and Me_2 as half-carbon atoms and the R factor decreased to 0·107. In ϱ_7, Me_1 was 3·0 e.Å^{-3} while Me_2 was 2·7 e.Å^{-3}. In D_3, Me_1 was $+0·2$ e.Å^{-3} while Me_2 was zero. The behavior of these two peaks throughout the analysis is summarized in Table 1.

Table 1. *Peak heights of* Me_1 *and* Me_2 *in* e.Å^{-3}

	ϱ_1	ϱ_2	ϱ_3	ϱ_4	ϱ_5, D_1	ϱ_6, D_2	ϱ_7, D_3
Me_1	1·6	2·2	1·7	4·0*	2·0, +1·6	3·9, −1·6*	3·0, +0·2*
Me_2	0·7	1·4	0·8	1·7	3·7, −1·5*	1·6, +1·6	2·7, 0·0†

* Included in the structure factor computation as C.
† Included in the structure factor computation as ½ C.

A detailed examination of D_3 revealed an interesting development. Positive regions of density were found at or near the expected positions for hydrogen atoms on the ring system, the quaternary nitrogen's methyl, the ethylidene, the carbinol, and the water molecule.

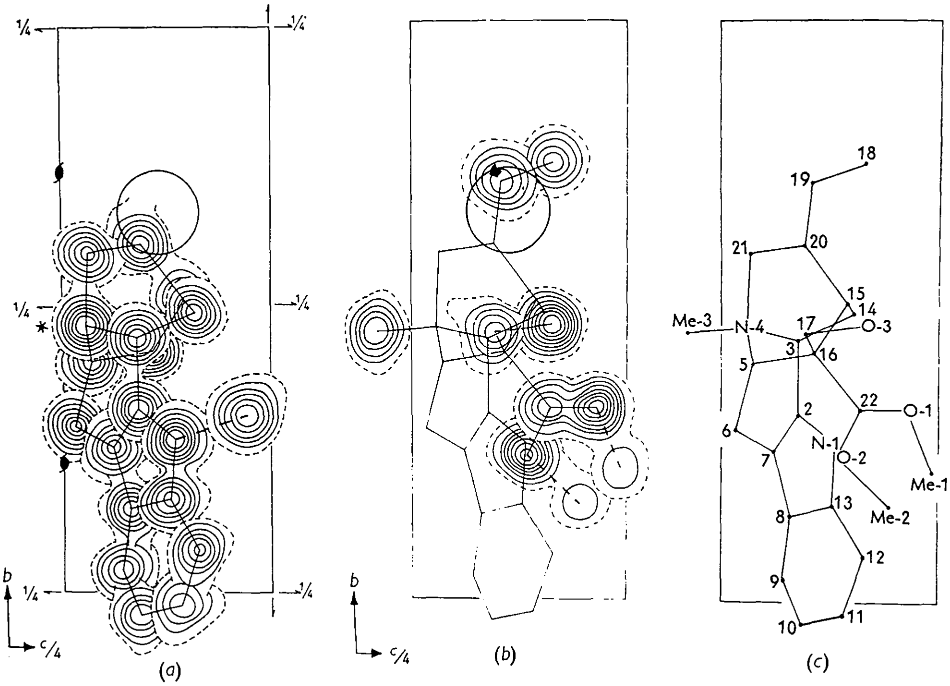

Fig. 4. Akuammidine methiodide. (*a*) Composite electron density of the basic ring structure viewed along *a*; contours at 1 e.Å^{-3}, 1 e.Å^{-3} broken; water hydrogen bonding to indole indicated by broken line, quaternary nitrogen by asterisk, iodide ion by open circle. (*b*) As in (*a*) except that only side chains are shown; the ethylidene is below the iodide ion; bonds to the disordered methyl groups are broken. (*c*) The numbering system employed. Screw axes indicated in (*a*).

Although the heights of these regions varied over a wide range and some regions were quite distorted, it is indeed significant that they all appeared. A structure factor computation was then carried out based on adjusted atomic parameters and the R factor decreased to 0·099; when 24 hydrogen atoms were included at the above mentioned positive peak positions, this value dropped to 0·095. No attempt was made to refine these positive regions and the structure analysis was terminated at this stage.

4. Results*

The final electron density (ϱ_7) is shown in Fig. 4(*a*), (*b*); positive peaks of D_3 at or near expected hydrogen atom positions are shown in Fig. 5. The final atomic parameters are listed in Table 2. The interatomic and hydrogen bond distances are given in Fig. 6, while the interbond angles are given in Fig. 7.

The accuracy of the structure has been assessed employing Cruickshank's method. The estimated standard deviation of the electron density is 0·43 e.Å^{-3}; the standard errors of the first differential of the electron density with respect to x, y, z are 1·12, 0·94 and 1·07 e.Å^{-4}, respectively. In general, the curvatures of the atoms varied in different directions and, also, from atom to atom, ranging from about 40 to 70 e.Å^{-5}. This led to a root mean square error in atomic position of about 0·03–0·04 Å and a root mean square error in bond distance of approximately

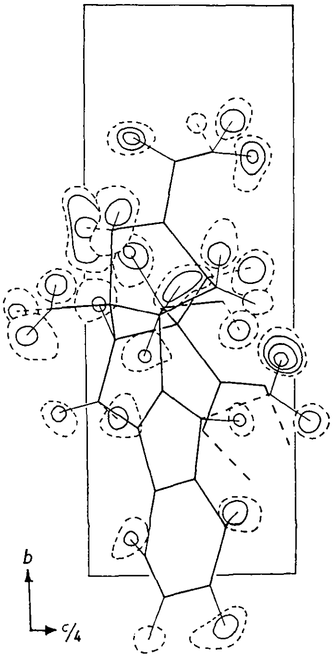

Fig. 5. Positive regions in D_3 at or near expected hydrogen atom positions; contours at 0·25 e.Å^{-3}, 0·5 e.Å^{-3} contour broken; everything else as in Fig. 4; 0·25 e.Å^{-3} contour not shown.

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* Copies of the observed intensity data are available from the authors.

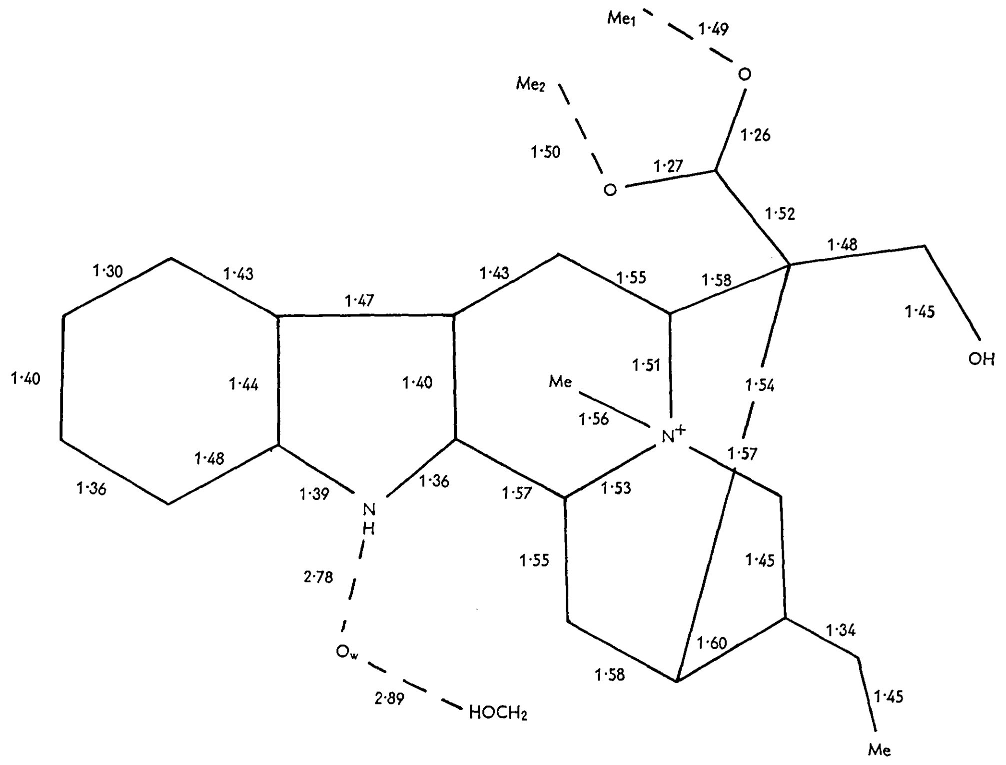

Fig. 6. Interatomic and hydrogen bond distances.

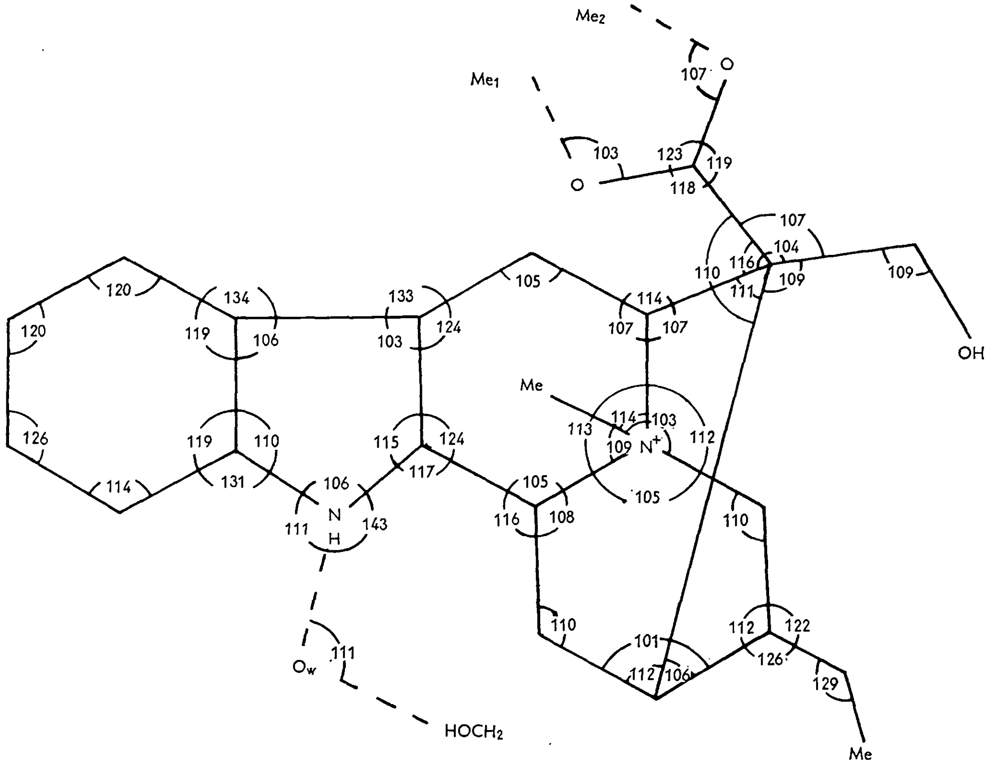

Fig. 7. Interbond angles including that of the hydrogen bond.

0·04–0·06 Å. The estimated standard error in bond angles is of the order of 1·5–2·0 degrees.

Table 2. *Final atomic parameters*

Atom	x	y	z	B (Å^2)	$\varrho_0(0)$ (e.Å^{-3})
I^-	0·0856	0·1772	0·1114	3·68	72·9
C(2)	0·3383	0·8270	0·0925	2·85	6·7
C(3)	0·3480	0·9542	0·0888	2·50	6·7
C(5)	0·4250	0·4130	0·4690	3·15	6·6
C(6)	0·4615	0·2935	0·4860	3·50	7·1
C(7)	0·4330	0·7572	0·0605	3·70	6·0
C(8)	0·3845	0·6493	0·0820	3·55	6·5
C(9)	0·4335	0·5415	0·0715	4·25	5·5
C(10)	0·3650	0·4593	0·0960	4·20	6·0
C(11)	0·2570	0·4755	0·1430	4·55	4·6
C(12)	0·2040	0·5730	0·1625	4·15	6·0
C(13)	0·2760	0·6680	0·1318	3·40	6·7
C(14)	0·4110	0·0010	0·1580	3·35	6·1
C(15)	0·4330	0·5260	0·3535	2·95	6·6
C(16)	0·3610	0·4330	0·3930	2·90	7·7
C(17)	0·2205	0·4660	0·4090	3·60	5·7
C(18)	0·2970	0·7580	0·3393	4·20	5·1
C(19)	0·3810	0·7228	0·3985	4·10	5·7
C(20)	0·4380	0·6255	0·4083	3·45	6·0
C(21)	0·4735	0·1030	0·0317	2·55	7·1
C(22)	0·3520	0·3340	0·3452	3·80	5·5
N(1)	0·2465	0·7780	0·1355	3·05	7·7
N(4)	0·4450	0·9782	0·0275	3·20	7·9
O(1)	0·4145	0·3340	0·2865	4·50	8·2
O(2)	0·2805	0·2545	0·3660	4·45	8·1
O(3)	0·1480	0·4815	0·3425	3·95	8·8
O_W	0·0200	0·8150	0·2200	5·75	6·5
Me(1)	0·3815	0·2300	0·2500	3·80	3·0
Me(2)	0·2940	0·1750	0·3056	4·25	2·7
Me(3)	0·1340	0·0345	0·4560	3·35	5·8

Where $\varrho_0(0)$ is the peak height at the atomic position.

5. Discussion

In addition to confirming that part of the structure of akuammidine known through chemical methods, this determination removes the uncertainties about akuammidine's side chain stereochemistry. The methyl group of the ethylidene and the methyl ester function are directed away from the quaternary nitrogen atom. Thus, the structure is identical in all respects with that of macusine-*A* (McPhail *et al.*, 1961) except that the positions of the ester and carbinol groups are interchanged. The absolute stereochemistry is also known since akuammidine has been related to normacusine-*B*, of known absolute configuration (Bartlett *et al.*, 1962).

The disorder of the methyl ester, shown rather conclusively by X-ray crystallographic techniques, has been independently confirmed with infra-red absorption evidence. When the structure determination was in its final stages there was good reason to suspect that 'rhazine' is, in fact, the alkaloid akuammidine.* That this is so has been established through a comparison of the single crystal X-ray diffraction patterns of their corresponding methiodides.† Their infra-red absorption spectra were also measured and shown to be identical. However, the most interesting feature of both spectra was in the carbonyl absorption region; the carbonyl peak was split into two components appearing at 5·73 and 5·82 microns. This is readily accounted for by the disordered methyl ester function, the two orientations presenting different environments for the carbonyl.

The water molecule is of interest in two respects. First, it hydrogen bonds simultaneously with the indole hydrogen of one molecule and the carbinol hydrogen of another. Furthermore, the angle between the hydrogen bonding groups ($\angle$ N–O_W–O) is nearly tetrahedral (111·0°) as in ice. The other notable feature of the water molecule is that it makes the closest approach to the iodide ion (3·74 Å), with its hydrogen atoms (from D_3) apparently directed toward the ion. The iodide ion is seemingly indifferent to which one of two quaternary nitrogen atoms, or positive charges, it is associated. There are thus two relatively large and similar N^+–I^- contacts (4·6 and 5·1 Å).

Finally, the unexpected positive regions of difference density at or near hydrogen atom positions suggest that hydrogen atoms can probably be located by the X-ray method in structures of greater complexity than those that have customarily been considered.

All the computations during this analysis were carried out on an IBM Type 709 Data Processing System using programs written by Busing & Levy (ORXLS) and Sly & Shoemaker (MIFR-1). We are grateful to the foregoing for making these programs available to us.

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* Dr W. I. Taylor, private communication.

† We should like to thank Dr J. Le Men for kindly supplying us with an authentic sample of akuammidine methiodide via Dr W. I. Taylor.