

The Crystal Structure of Jacobine Bromhydrin at -150°C

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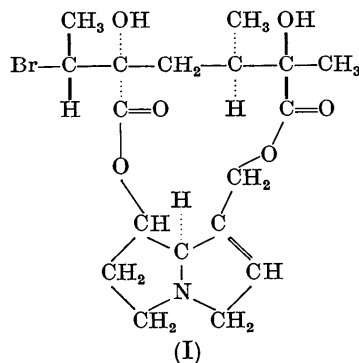
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The structure of jacobine, an important *Senecio* alkaloid, has been determined as its bromhydrin, $\text{C}_{18}\text{H}_{26}\text{NO}_6\text{Br}$. This derivative crystallizes from ethanol in space group $P2_1$ with cell dimensions

$$a = 9.88, b = 15.3, c = 14.634 \text{ \AA}; \beta = 112.7^{\circ} \text{ at } -150^{\circ}\text{C}.$$

Unit-cell contents are $2(2 \times \text{C}_{18}\text{H}_{26}\text{NO}_6\text{Br} + \text{C}_2\text{H}_5\text{OH})$. X-ray analysis was based on 0–10 layers about the b axis (3327 reflexions of 3496 theoretically possible). Coincidence in the y parameters of the two Br atoms of the asymmetric unit resulted in a symmetrical arrangement in both the 'image-seeking' distribution, $F_o^2 \times \varphi_{\text{Br}}$, and the electron-density distribution $F_o(\alpha_{\text{Br}})$ which was phased only with those terms for which the geometrical structure factor, S_{Br} , was greater than 0.45 of its maximum value. From comparison of these two distributions, combined with a cross-check against the projection $\rho(x, z)$, the three-dimensional array of the 55 atoms constituting the skeletons of the two jacobine bromhydrin molecules and the ethanol molecule was derived without further three-dimensional refinement. The first set of structure factors gave an agreement corresponding to $R = 0.27$. The structure of jacobine bromhydrin (I) was placed on an absolute basis by application of Bijvoet's method.



The importance of a high ratio of recorded to possible reflexions which can be gained by a combination of low temperatures and a high-power X-ray generator is stressed to relation to the speed of structure solution.

Introduction

Senecio or pyrrolizidine alkaloids constitute a large group of compounds whose main structure characteristic is that they are mono- or di-ester combinations of one or two acids or of a diacid with a pyrrolizidine diol. Evidence regarding the structures of various members has been reviewed by Warren (1955) and, more recently, Leonard (1960) has brought up to date his earlier summary of the available evidence (Leonard, 1949). Many members have considerable importance as animal poisons causing liver necrosis

(*e.g.* Warren, 1955) and for this reason, physiological studies (*e.g.* Bull, 1961), have been carried out and even extended to attempt correlation with the incidence of liver cancer (Cook, Duffy & Schoental, 1950).

As the main member of a closely related sub-group, jacobine has proved of considerable structural interest and the solution of the structure has given considerable difficulty. A variety of formulae have been proposed at various times, *e.g.* Adams, Gianturco & van Duuren (1956), Bradbury & Willis (1956). Subsequently, reinterpretation of available data by Geissman (1959) and chemical studies by Bradbury & Masamune (1959) resolved the main structural

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problems while chemical studies by Masamune (1960) and X-ray studies by Fridrichsons, Mathieson & Sutor (1960) established the configurational details of the jacobine group.

The work reported here was initiated in 1958 and presented briefly at the 1960 I.U.P.A.C. Symposium on Natural Products, Australia (Mathieson, 1961). The choice of the bromhydrin for X-ray analysis was made because of the ease of introduction of the bromine atom to the molecule. (It is also the bromine analogue of jacoline, which is the chlorhydrin of jacobine.) A parallel investigation of the bromodilactone from jacobine has also been carried out (Mathieson & Taylor, 1961). We are very grateful to Dr C. C. J. Culvenor for his assistance in providing excellent crystals of the derivative.

Experimental

From ethanol, jacobine bromhydrin crystallizes in small diamond-shaped plates with minor faces apical to the short diagonal of the main face. The crystals are monoclinic, the b axis being parallel to the short diagonal and the main face being (001). The cell dimensions measured against a standard (Ag, $a = 4.0862 \text{ \AA}$) are

$$a = 9.889, b = 15.30, c = 14.634 \text{ \AA}; \beta = 112.7^{\circ}$$

at -150°C . The space group is $P2_1$. The presence of ethanol molecules in the crystal structure, indicated by the transformation of the crystals to an opaque state after a few days, was confirmed by the density determination by flotation at room temperature, $D_m = 1.472$ as compared with $D_c = 1.480$ with the low-temperature dimensions for an asymmetric unit $2 \times \text{C}_{18}\text{H}_{26}\text{NO}_6\text{Br} + \text{C}_2\text{H}_5\text{OH}$. For extended exposures, the crystals required to be coated or changed frequently.

Intensity data were collected at -150°C (for details, see Fridrichsons & Mathieson, 1962*a*) for 0–10 layers about the b axis, corresponding to a maximum equi-inclination angle of $\mu = 29.75^{\circ}$. Intensities were determined against a set of standards and

Table 1. *Number of measured and possible terms and R indices for the three sets of three-dimensional structure factors*

hkl	N_m	N_p	N_m/N_p	R_1	R_2	R_3
$h0l$	316	349	0.91	0.206	0.201	0.189
$h1l$	341	347	0.98	0.184	0.170	0.166
$h2l$	333	346	0.96	0.194	0.188	0.183
$h3l$	313	344	0.91	0.220	0.210	0.206
$h4l$	312	331	0.94	0.266	0.261	0.242
$h5l$	303	329	0.92	0.279	0.263	0.261
$h6l$	309	317	0.97	0.305	0.287	0.274
$h7l$	302	303	1.00	0.351	0.342	0.308
$h8l$	285	294	0.97	0.368	0.353	0.350
$h9l$	267	278	0.96	0.398	0.388	0.399
$h10l$	246	258	0.95	0.434	0.409	0.393
hkl	3327	3496	0.95	0.274	0.263	0.253

appropriate corrections applied, the values for the various layers being cross-correlated by reference to $0kl$ and $1kl$ data. The ratio of measured to possible reflexions is shown in Table 1, the 3327 terms representing 0.95.

Two-dimensional structure factors and Fourier syntheses were computed on SILLIAC (Freeman, 1957) while the corresponding three-dimensional calculations were carried out on UTECOM (\equiv DEUCE) using coordinated programs devised by Rollett (1961).

Analysis

The two bromine atoms of the asymmetric unit (final parameters in Table 2) were located from a three-dimensional Patterson distribution for which the $F_o^2(hkl)$ data were sharpened by inverting the temperature factor, derived from a Wilson (1942) plot. The prospect that the two heavy atoms in the asymmetric unit would eliminate the phase ambiguity, inherent in $P2_1$ for one heavy atom, was upset by the coincidence in their y coordinates and the analysis rendered somewhat more problematic in that both molecules required to be resolved from their respective mirror-images.

Earlier experience in the application of image-seeking by calculation (Fridrichsons & Mathieson, 1962*b*) showed limitations inherent in two-dimensional studies and the opportunity was therefore taken to extend tests to three-dimensional operation. With the geometrical structure-factor contributions of the bromine atoms, φ_{Br} , the distribution (1) was obtained.

$$S(x, y, z) = \sum^h \sum^k \sum^l F_{(hkl)}^2 \times \varphi_{\text{Br}}(hkl) \cos\left(\frac{hx}{a} + \frac{ky}{b} + \frac{lz}{c}\right). \quad (1)$$

This operation is equivalent, here, to superposition four times and extracting the sum of the components. The resultant distribution was not wholly convincing but probable atom sites were extracted in an asymmetric array, the sites being selected up to the limit permitted by the cell contents without assumption of chemical significance but in relation to reasonable bond lengths. Structure factors were computed and, after rejection of terms considered inadequately defined, a three-dimensional electron-density distribution was calculated. This distribution was disappointing, returning clearly only those atoms introduced into the structure factor calculation. It was therefore evident that a solution pursued in this manner would involve many cycles of three-dimensional structure factor and Fourier synthesis with only limited progress at each stage.

To avoid this situation, it was decided to revert to an earlier stage of the analysis and to approach the solution to the problem in two (partly complementary) ways — (α) to refine the b -axis projection as far as possible without assumptions as to chemical structure since this projection involves only signs and

(b) to calculate a symmetrical three-dimensional electron-density distribution based only on the Br contributions.

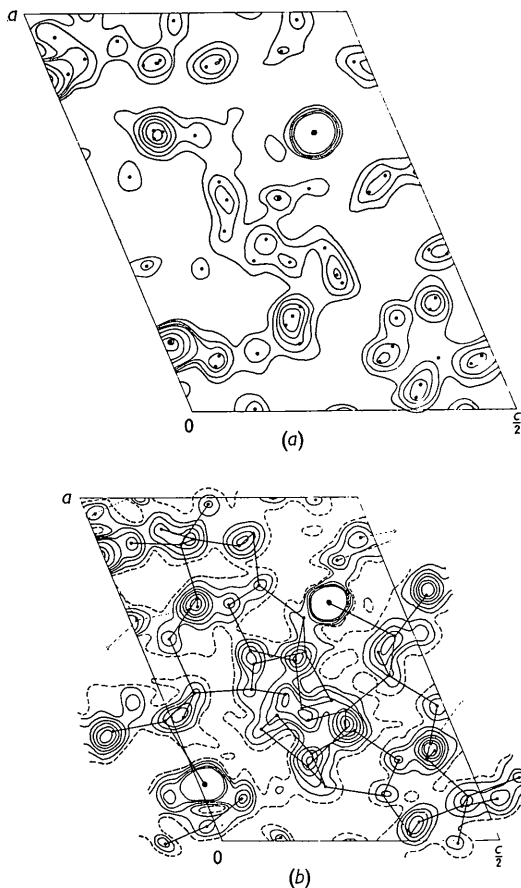


Fig. 1. (a) Partly refined b -axis projection with atom sites (all assumed carbon) allocated solely from interpretation of the $h0l$ data. (b) Final b -axis projection with molecular skeleton.

The refinement of the $h0l$ projection started with Br phases, assuming all light atoms to be carbon, proceeded through several cycles of difference syntheses until the reliability index, R , dropped to 0.21 (including a correction for the asymmetric vibrations of the two Br atoms) additional atom sites being again allocated according to the ρ and $\Delta\rho$ distributions up to the limit set by the cell contents. The distribution of presumed atom centres at this stage is shown in Fig. 1(a). For the three-dimensional electron-density distribution, to make sure that the phase angles defined by the Br contribution should approximate reasonably to the final (correct?) value, a rather high discriminatory level was set, omitting all terms for which the Br contribution was less than 0.45 of its maximum value. This left for the syntheses only 1569 terms of the total 3327. The resultant symmetrical distribution was compared carefully with that of (1) (also symmetrical) and where a definite positive region

occurred in both, it was considered structurally significant. On this basis, guided mainly by the refined projection but not ignoring the possibility that certain regions may contain gross errors, a three-dimensional ball-and-spoke model of the atomic array was gradually constructed. The long-chain (*i.e.* jaconecate) components of both molecules were defined first with reasonable assurance but the location of the two retronecine moieties proved less straightforward. However, on consideration of the structure of pyrrolizidine ring systems (Fridrichsons & Mathieson, 1963), these were also extracted eventually from the available data. Identity of the two molecules proved to be of assistance in selecting true atom sites since occasions arise where one clearly defined in molecule 1 may prove less certain in molecule 2. Although both molecules require to be resolved from their mirror images, two factors assist in this procedure — (a) the relative configurations of the two molecules must be identical and (b) when the orientation of molecule 1 (or 2) is selected (arbitrarily at this stage), packing factors preclude one image of the second molecule. Only near the mirror-planes defined by the Br atoms does the selection of individual atom sites prove more problematic.

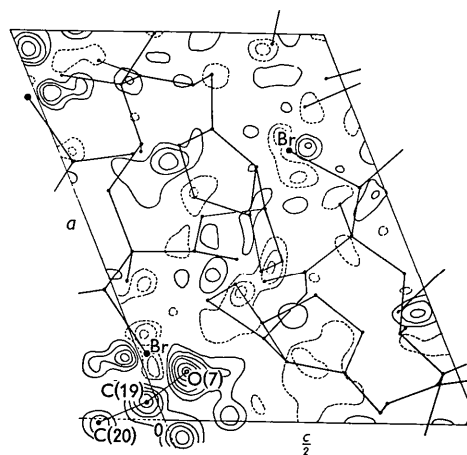


Fig. 2. Difference synthesis for the b -axis projection showing the location of the ethanol molecule.

With the complete set of atomic parameters for the molecules 1 and 2, a difference synthesis, $\Delta\rho(x, z)$, was calculated to locate the atoms of the ethanol molecule (Fig. 2) and their y parameters were then determined from the three-dimensional data. With the complete array of 55 atoms, the oxygen atoms being differentiated both from their peak heights, stereochemical features and from the chemical evidence available, the hkl structure factors yielded a satisfactory agreement (Table 1) the overall value, including unobserved terms, being 0.27. With two more cycles, improvement occurred but was not dramatic. It was clear that refinement of the very

Table 2. *Atomic parameters*

Atom	Molecule A			Molecule B		
	x/a	y/b	z/c	x/a	y/b	z/c
Br	0.165	0.000	0.012	0.700	0.500	0.369
C(1)	0.650	0.503	0.010	0.600	0.480	0.463
C(2)	0.680	0.472	0.115	0.277	0.950	0.445
C(3)	0.437	0.923	0.057	0.487	0.400	0.422
C(4)	0.593	0.923	0.052	0.393	0.383	0.488
C(5)	0.707	0.860	0.133	0.243	0.337	0.433
C(6)	0.683	0.760	0.127	0.267	0.237	0.452
C(7)	0.863	0.890	0.145	0.127	0.358	0.475
C(8)	0.980	0.833	0.222	0.988	0.307	0.423
C(9)	0.447	0.945	0.165	0.380	0.407	0.318
C(10)	0.883	0.990	0.173	0.100	0.462	0.458
C(11)	0.553	0.050	0.288	0.223	0.517	0.210
C(12)	0.412	0.080	0.299	0.317	0.548	0.153
C(13)	0.387	0.167	0.240	0.367	0.642	0.186
C(14)	0.560	0.260	0.189	0.322	0.720	0.317
C(15)	0.697	0.210	0.190	0.233	0.682	0.373
C(16)	0.747	0.145	0.253	0.140	0.622	0.322
C(17)	0.650	0.138	0.313	0.155	0.607	0.225
C(18)	0.892	0.093	0.282	0.020	0.577	0.343
O(1)	0.428	0.888	0.218	0.357	0.367	0.243
O(2)	0.520	0.020	0.190	0.327	0.487	0.304
O(3)	0.363	0.842	0.022	0.555	0.317	0.423
O(4)	0.863	0.007	0.250	0.057	0.487	0.368
O(5)	0.910	0.047	0.123	0.880	0.008	0.472
O(6)	0.873	0.870	0.057	0.817	0.852	0.418
N	0.533	0.207	0.267	0.257	0.677	0.220
C(19)	0.943	0.225	0.017			
C(20)	0.016	0.282	0.110			
O(7)	0.130	0.783	0.064			

} Ethanol molecule

extensive data was not a reasonable proposition with the available computer facilities and it was decided to proceed no further since the main aim, of defining the chemical structure, had been achieved. The final

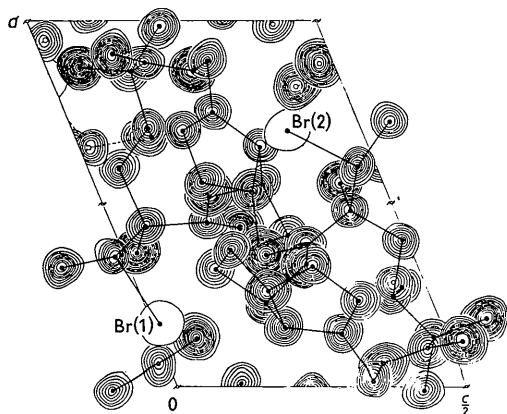


Fig. 3. Three-dimensional distribution shown by sections near atomic centres as viewed down the b -axis.

projection, $\rho_0(x, z)$, based on utilization of all atoms, is shown in Fig. 1(b) while the three-dimensional distribution is depicted in Fig. 3. Atomic parameters are given in Table 2 while comparison of measured and calculated structure factors is recorded in Table 3. The scattering curves were as used by Fridrichsons &

Mathieson (1962a), the overall temperature factor B being 1.90.

Absolute configuration

The absolute configuration has been defined by reference to the anomalous dispersion of $\text{CuK}\alpha$ radiation by the Br atoms. The experimental arrangement was as described previously (Fridrichsons & Mathieson, 1962a), the proper indexing of the film being based on the definitive work of Peerdeman & Bijvoet (1956). In the present case, with two bromine atoms in the asymmetric unit, their overall contribution to the total structure factor tends to be lower than for a single atom, so the most suitable reflexions required careful selection (Table 4). The intensity

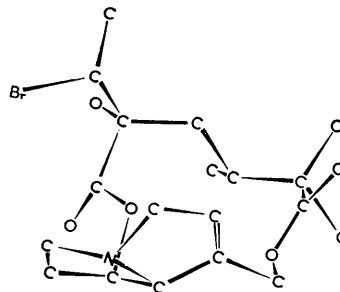


Fig. 4. Structure of the molecule of jacobine bromhydrin indicating the absolute configuration.

inequalities conform to a consistent pattern and the absolute configuration determined in this manner is correctly indicated by the atomic parameters in Table 2 when considered in relation to a right-handed set of axes with symmetry elements grouped as in space group $P2_1$, No. 4 in *International Tables for X-ray Crystallography* (1952). The molecular skeleton in Fig. 4 is also oriented in accord with this conclusion.

Discussion

From the atomic parameters (Table 2) bond lengths and angles were calculated (Table 5). The results for the two independent molecules have been juxtaposed for comparison and also grouped according to their bond-type. From the internal consistency, it is unlikely that any bonds are in error by more than 0.08 \AA at this stage of the analysis. From the agreement for similar bonds (and of details of conformation) it appears permissible to derive mean values for the molecule of jacobine bromhydrin (Fig. 5(a) and (b)).

The two molecules are identical to a first approximation, consisting of a compact retronecine ring system to which is linked, through ester groups, an extended carbon chain with a rather bulky bromethyl group attached to one end of the chain. The whole molecule thus forms a 12-atom ring-system. The overall packing of the molecules in the crystal is determined by this somewhat ungainly shape, parts

Table 3 (cont.)

Table with 15 columns of numerical data, each column labeled with a value and a symbol (e.g., F0, F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15). The table contains multiple rows of data with various numerical values and some sub-totals indicated by underlined numbers.

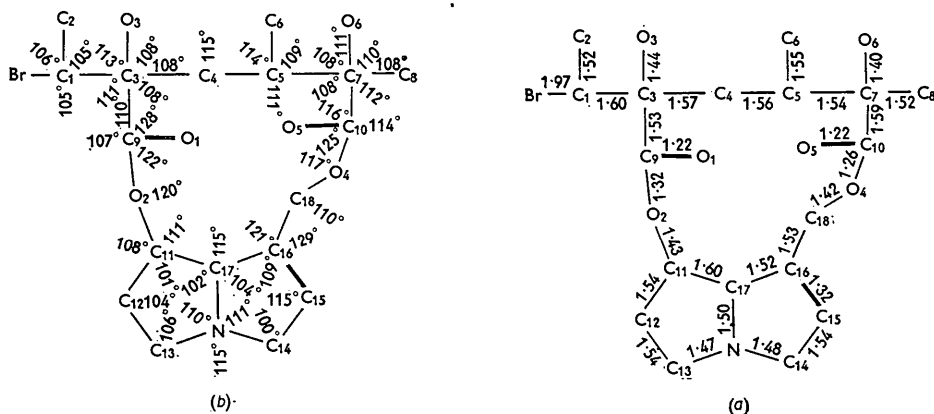


Fig. 5. Molecular diagram giving (a) average bond lengths, (b) average bond angles.

Table 4. Terms used to determine absolute configuration

hkl	F^2_{hko}	$F^2_{\bar{h}\bar{k}o}$	I (obs.)
350	246	202	$350 > \bar{3}50$
760	42	69	$760 < \bar{7}60$
690	294	239	$690 > \bar{6}90$

of one molecule nestling in involuted segments of its neighbour. Certain approach distances may be assigned

Table 5

(a) Bond lengths

Atoms	Bond length		Bond type and mean value
	Mole- cule A	Mole- cule B	
C(1)–C(2)	1.53 Å	1.50 Å	C–C single bond, 1.55 ₁
C(1)–C(3)	1.59	1.61	
C(3)–C(4)	1.55	1.60	
C(4)–C(5)	1.57	1.56	
C(5)–C(6)	1.54	1.56	
C(5)–C(7)	1.56	1.53	
C(7)–C(8)	1.53	1.51	
C(7)–C(10)	1.58	1.61	
C(18)–C(16)	1.55	1.51	
C(16)–C(17)	1.53	1.50	
C(17)–C(11)	1.61	1.59	
C(11)–C(12)	1.54	1.55	
C(12)–C(13)	1.55	1.53	
C(14)–C(15)	1.55	1.53	
C(3)–C(9)	1.58	1.48	
C(15)–C(16)	1.32	1.31	C–C double bond, 1.31 ₅
N–C(13)	1.48	1.46	N–C, 1.48 ₅
N–C(14)	1.50	1.47	
N–C(17)	1.51	1.49	
O(6)–C(7)	1.37	1.44	O–C, hydroxyl bond, 1.42 ₃
O(3)–C(3)	1.44	1.44	
O(4)–C(18)	1.40	1.44	O–C, long ester bond, 1.42 ₅
O(2)–C(11)	1.42	1.44	
O(4)–C(10)	1.24	1.28	O–C, short ester bond, 1.29 ₂
O(2)–C(9)	1.33	1.32	
O(5)–C(10)	1.23	1.20	O–C, ester double bond, 1.21 ₅
O(1)–C(9)	1.23	1.20	
Br–C(1)	1.97	1.97	Br–C, 1.97

Table 5 (cont.)

(b) Bond angles

Atoms	Molecule A	Molecule B
C(2)–C(1)–C(3)	103°	108°
C(1)–C(3)–C(4)	108	112
C(1)–C(3)–C(9)	105	117
C(1)–C(3)–O(3)	112	114
O(3)–C(3)–C(4)	112	103
C(4)–C(3)–C(9)	109	106
O(3)–C(3)–C(9)	112	103
C(3)–C(4)–C(5)	116	115
C(4)–C(5)–C(6)	121	108
C(4)–C(5)–C(7)	108	114
C(6)–C(5)–C(7)	115	103
C(5)–C(7)–O(6)	105	112
C(5)–C(7)–C(8)	111	111
C(5)–C(7)–C(10)	110	105
O(6)–C(7)–C(8)	105	114
O(6)–C(7)–C(10)	115	102
C(8)–C(7)–C(10)	112	112
O(5)–C(10)–O(4)	123	126
C(7)–C(10)–O(5)	112	119
C(7)–C(10)–O(4)	113	115
C(10)–O(4)–C(18)	114	120
O(4)–C(18)–C(16)	110	110
C(18)–C(16)–C(17)	122	120
C(18)–C(16)–C(15)	129	129
C(15)–C(16)–C(17)	108	110
C(16)–C(15)–C(14)	118	112
C(15)–C(14)–N	98	103
C(14)–N–C(13)	118	113
C(14)–N–C(17)	113	109
C(13)–N–C(17)	109	111
C(16)–C(17)–C(11)	112	118
C(16)–C(17)–N	103	105
C(17)–C(11)–C(12)	102	100
C(17)–C(11)–O(2)	113	109
C(12)–C(11)–O(2)	110	106
C(11)–C(12)–C(13)	99	108
C(12)–C(13)–N	106	105
C(11)–O(2)–C(9)	116	123
O(2)–C(9)–O(1)	129	114
O(2)–C(9)–C(3)	106	108
O(1)–C(9)–C(3)	121	135
Br–C(1)–C(2)	109	103
Br–C(1)–C(3)	101	108
C(11)–C(17)–N	101	102
C(20)–C(19)–O(7)	115	

more specific explanation. Thus, the trivalent nitrogen of the retronecine ring system in molecule *A* forms a hydrogen bond through its lone pair of electrons with a hydroxyl group of molecule *B*. However, on account of the shape of the molecule, the crystal structure does not permit the corresponding nitrogen of molecule *B* to hydrogen-bond in a similar manner with a hydroxyl group of molecule *A*. This role is played by an ethanol molecule introduced into the crystal structure to provide a suitably disposed -OH group

Table 6. Shortest approach distances

N-O(3')	2.78 Å	N-C(9')	3.75 Å
N-O(1')	2.98	N'-C(8)	3.81
C(13)-O(1')	3.10	C(14')-C(8)	3.56
C(14)-O(1')	2.90	C(8)-O(6')	3.72
N'-O(7)	2.81	O(4)-O(6')	3.49

for N. Other reasonably short approach distances between hydroxyl and oxo oxygen atoms are listed in Table 6 and shown in Fig. 6. It may be noted in Fig. 6 that the ethanol molecules are situated in a

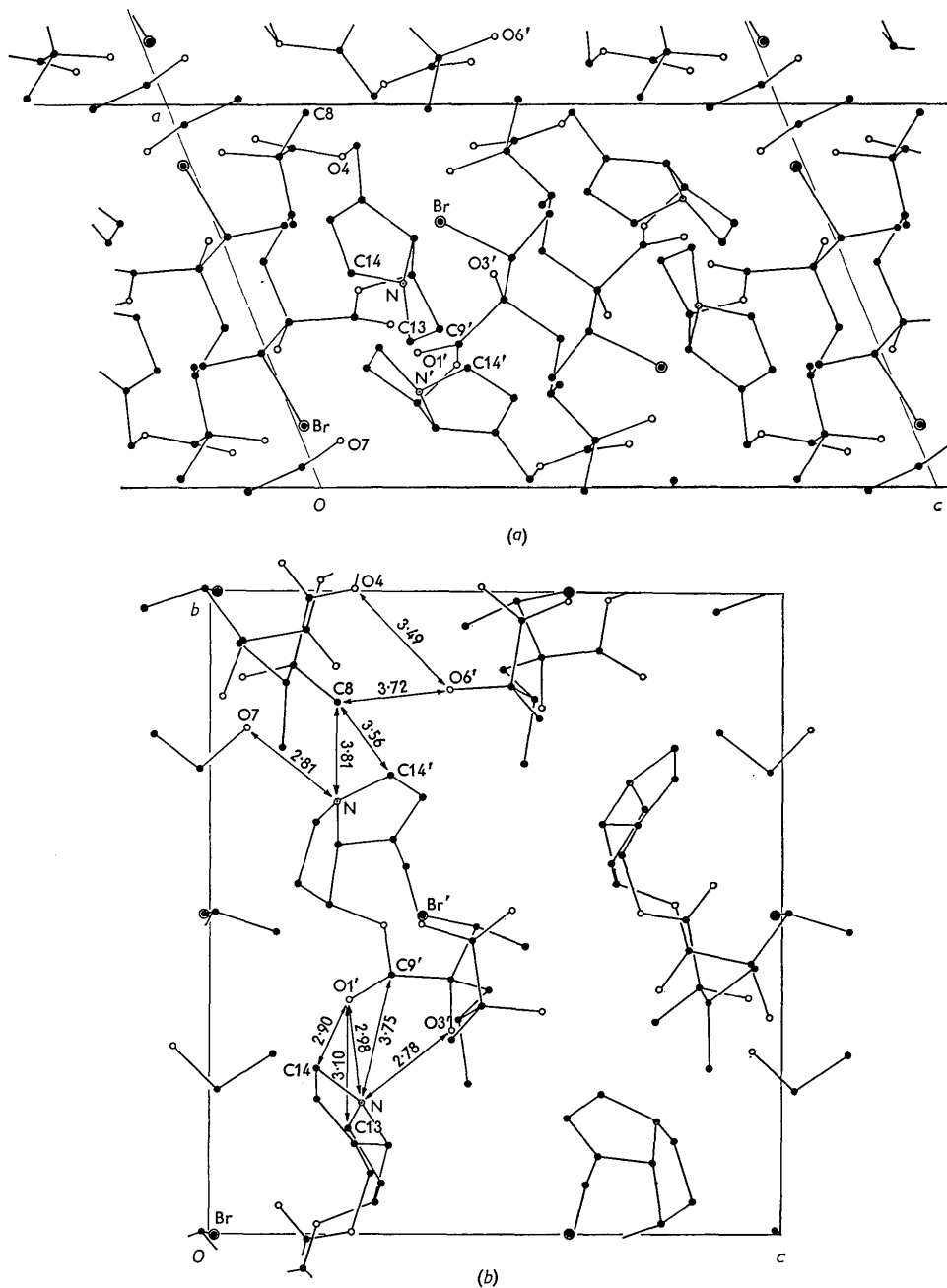


Fig. 6. Molecular packing: (a) viewed down the *b* axis, (b) down the *a* axis.

channel running parallel to b from which they may relatively readily escape (see experimental section).

It would have been possible (*e.g.* Mathieson, 1956), to place the relative configuration of the molecule on an absolute basis by reference to the absolute configuration of one component, retronecine, which has been defined by chemical means (Leonard, 1960) but, as indicated recently for isocitric acid, chemical argument may prove misleading or indecisive (*vide* Patterson, Johnson, van der Helm & Minkin, 1962). It was therefore worth while to provide independent X-ray evidence. The X-ray result (Fig. 4) is in complete accord with the correlation by reference to retronecine and with the deductions, based on chemical studies, of Masamune (1960). Together with the structural and configurational evidence from the dilactone from jacobine (Mathieson & Taylor, 1961), the stereochemistry of jacobine seems to be well established, placing it in an exceptional position in the large group of *Senecio* alkaloids.

A few comments may be offered regarding the crystallographic aspects of the analysis. The three-dimensional image-seeking function (1) was used first to locate the light atoms. In this distribution the Br peaks at $y=0$ and $\frac{1}{2}$ were of height 4000, others on these levels reached 1000 while in all other regions peaks were rarely over 200. This distribution of peak heights largely reflects the influence of the unfortunate relative locations of the Br atoms. As a result, the selection of the light atom sites, even in symmetrical locations, was not regarded as particularly successful and the symmetrical distribution (1) was not considered a suitable test of the capabilities of this type of image-seeking in three dimensions for which it had been intended following earlier tests in two dimensions (Fridrichsons & Mathieson, 1962*b*). More suitable tests with a group providing general phasing are proceeding.

We have noted above that the allocation of atom sites to peak locations in the image-seeking distribution led to an electron-density distribution which yielded back mainly the previously selected atoms and, also, failed to give significant indications as to which could be accounted real and which artefact. This particular situation indicates a very real difficulty which frequently occurs in determining real atom sites and one which has caused difficulties in other analyses dealing with similar types of molecule, *e.g.* epilimonol iodacetate (Arnott, Davie, Robertson, Sim & Watson, 1960), calycanthine (Hamor, Robertson, Shrivastava & Silverton, 1960), ibogaine (Arai, Coppola & Jeffrey, 1959). In these cases, the availability of computing facilities made it feasible to proceed by gradual attrition, introducing atoms progressively (and occasionally discarding previously selected sites), with steady improvement in the definition of phase angles, sometimes utilizing a probability weighting of terms (Sim, 1959). The rate of progress with this procedure may be indicated in a rough manner by the number of three-dimensional

Fourier syntheses involved, which may range up to 10–14. This type of approach would have placed on us a severe restriction due to the limited access to computers. Also it may not be a wholly satisfactory mode of operation if very little is known of the molecule since it may lead to a considerable number of cycles with little positive result. This has been indicated in several cases, *e.g.* for isophotosantonin lactone (Asher & Sim, 1961) attempts were made to solve the structure without reference to known details of the structure but were abandoned after five cycles of calculation. Also it was considered of importance in this type of analysis to maintain as direct a connection with the primary data, $|F_o|$ or F_o^2 , as long as possible and to locate as many of the lighter atoms on this basis before launching into operations involving ill-defined generalized phases. This viewpoint was one of the main reasons for tackling the analysis first through the image-seeking procedure — particularly in its simplest form, summation, since this is equivalent to a simple weighting of the F_o^2 terms by the contribution of the already located and definitely accepted atoms. Since this proved of limited success owing to the special positions of the Br atoms, an alternative approach, still closely allied to the primary data, was to compute an $F_o(\alpha_{\text{Br}})$ electron-density distribution. Unlike the $F_o^2 \times \varphi_{\text{Br}}$ calculation, this process involves selection of an arbitrary cut-off point. To make reasonably certain that the symmetrical component of $|F_o|$ is not used with a sign which is incorrect with respect to its final value, the discrimination must be set at a relatively high level — in this case, 0.45 of the maximum Br contribution. Both distributions, $F_o^2 \cdot \varphi_{\text{Br}}$ and $F_o(\alpha_{\text{Br}})$ were likely to contain significant, as well as adventitious, features and only when coincidence of peaks occurred was this accepted as evidence for a true atom site. By this process, the grouping of atoms on stereochemical grounds yielded thus, in one equivalent step, the atomic array for the two molecules with the agreement shown in Table 1. Despite problems of degeneracy associated with the space group, the location of the heavy atoms and their relative weight $\sum Z_H^2 / \sum Z_L^2 = 1.06$ (Mathieson, 1955) the analysis was held close to the primary data and would suggest that more extensive operations in three dimensions with the primary data (*vide* Mathieson, 1961) is advisable before becoming involved in generalized phase angles by allocation of atoms to sites which may later prove false.* Experience tends to confirm the grave tendency of introduced atoms (real or not) to reproduce convincingly in phased

* We have proposed (Fridrichsons & Mathieson, 1962*b*) that an automatic process of solution could be based upon a combination of product and ratio image-seeking appropriately weighted (2)

$$(1 - n/N)F_o^2 \times \varphi_c + n/N(F_o^2/\varphi_c^*) \quad (2)$$

Here we may note that, for high discrimination, F_o^2/φ_c approximates to $F_o(\alpha_c)$ and that the present combined use of $F_o^2 \times \varphi_c$ and $F_o(\alpha_c)$ corresponds to (2) with approximately equal weighting of terms.

distribution. In relation to the hexacarboxylic acid from vitamin B₁₂, Prosen, Hodgkin, Pickworth, Robertson, Sparks & Trueblood (1959) have suggested that the only basic feature indicating reality of an atom peak in a multi-atom structure is its continued appearance in successive ρ_0 distributions without having been inserted in structure factor calculations.

In suggesting that it is advisable to avoid early involvement with the definition of phase angle by the light atoms, it is necessary to recognize that progress in an analysis is markedly dependent on the condition of the diffraction data. In this respect, we may refer to the recent analysis of ibogaine hydrobromide (Arai, Coppola & Jeffrey, 1960) in which only 48% of the data with Cu radiation was available and this mainly in the low $\sin \theta$ range. Despite knowledge of much of the chemical structure, the authors comment on the considerable difficulty encountered in the solution — atoms being placed and later discarded (see their discussion). In the case of jacobine bromhydrin, although recorded only to $\sin \mu = 0.5$ along the b axis, 95% of the theoretical possible reflexions in this range was achieved and this is reflected in the distributions derived — not merely in terms of parameter accuracy but, of more significance for our purpose, also in terms of the speed of solution and certainty of atom sites.

Where the data ratio (observed/total) is not taken into account, comparison of structures by means of R ($= \sum |F_o - F_c| / \sum |F_o|$) or even R_2 ($= \sum |F_o^2 - F_c^2| / \sum F_o^2$) may give a misleading assessment of their condition. Thus the present value of the index in Table 1 may appear to imply a not wholly satisfactory agreement, but where structures have been established for data of limited range and subsequently extended the increase in R has proved rather dramatic, e.g. penicillin (Pitt, 1952); isoiridomyrmecin (Schoenborn & McConnell, 1962).

Finally, in analyses which involve two crystallographically independent molecules, it appeared that this doubled the number of atoms to be located (while still maintaining the ratio number of reflections/number of atoms) yet it must be recognized that these two molecules must be identical in terms of configurational detail and, from known examples, it is likely that conformational details will be precisely copied so that the molecules will be in all respects identical. From this viewpoint, it is probable that the two molecules will supplement information for one another, i.e. starting from one heavy atom (say) one can work outwards in a parallel sequence. Hence overall, i.e. at the analysis stage and probably at refinement, the presence of two molecules may not be detrimental to a solution — rather the reverse, as we have found in studying α -bromoisotutinone (MacKay & Mathieson, 1963).

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