CRYSTAL STRUCTURE OF L-TRYPTOPHAN HYDROBROMIDE*

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1. INTRODUCTION

L-TRYPTOPHAN is an essential amino-acid and is known to be transformed into nicotinamide in biosynthesis. The determination of the crystal structure of L-tryptophan hydrobromide was taken up as part of the major work on the structural studies of amino-acids and peptides in this laboratory. The structure was determined in both the projections down the *b* and *c*-axes and then refined using three-dimensional data by the method of least squares. While the detailed report on the structure was being prepared for publication, it was brought to the notice of the authors that this structure has been determined elsewhere recently.[1] The present determination of the structure has been carried out independently with the hydrobromide and the features of the structure agree well in all essential details with those of Takigawa *et al.*, who have analysed the structure in the hydrochloride form which could therefore be expected to be more accurate. For this reason, a detailed discussion of the hydrobromide structure and the conformational features of the tryptophan molecule will not be given. This paper will therefore deal with only an outline of the method of attack in solving the structure and a comparative study of the structures of the hydrochloride and the hydrobromide.

2. EXPERIMENTAL

The crystallographic data for the hydrobromide are given below :

Cell dimensions : $a = 14{\cdot}57$, $b = 5{\cdot}44$, $c = 7{\cdot}57$ Å and $\beta = 99{\cdot}6°$;
Space group : $P2_1$;
Contents of the unit cell : $2(C_{11}H_{11}N_2O_2.HBr)$;
Calculated density : 1·581 g./c.c. ; Measured density : 1·583 g./c.c. ;
Linear absorption coefficient μ : 51·0 cm.$^{-1}$ (for CuKα).

Three-dimensional intensity data were collected using the multiple film equi-inclination Weissenberg technique. 1220 reflections were recorded with CuKα radiation ($\lambda = 1{\cdot}5418$ Å) for the layers with K = 0, 1, 2, 3 and 4 about the needle axis *b* and the *h k* 0 reflections about the c-axis. All the intensities were estimated visually by comparison with a standard set of spots recorded with the same crystal. These were corrected for the Lorentz and polarisation factors and placed on the absolute scale by layerwise Wilson plots. The *h k* 0 reflections were used for correlating the different layer intensities. Absorption corrections were not applied as the crystal thickness was less than 0·2 mm.

3. DETERMINATION AND REFINEMENT OF THE STRUCTURE

The Lp sharpened Patterson projection down the *b*-axis gave the *x* and *z* co-ordinates of the bromine atom. Using the bromine atom for the known part, a weighted beta general synthesis[2] for this projection was computed (Fig. 1) which gave the structure straight

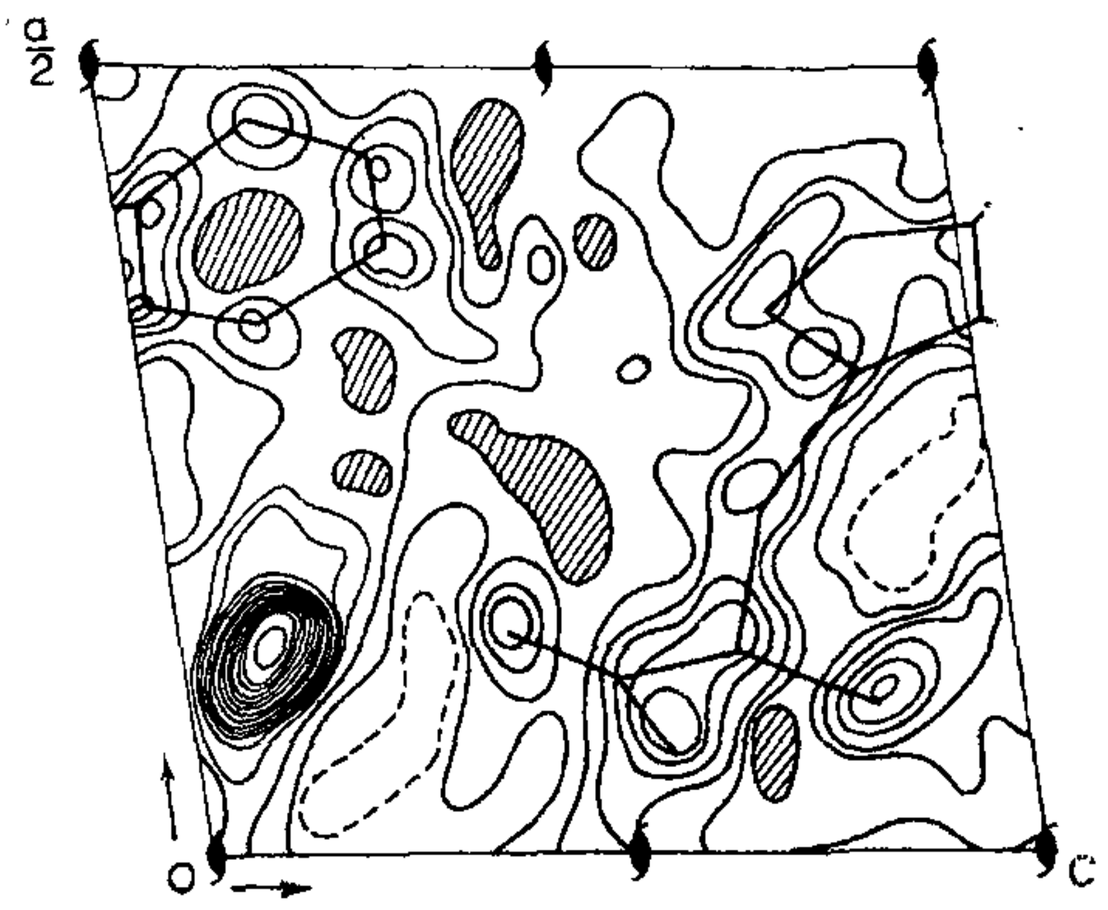

FIG. 1. Weighted β-general synthesis *b*-axis projection. Contours are drawn at intervals of 4e/A^2. Zero contours are indicated by dashed lines.

away. The R-index for the trial structure for the *h* 0 *l* reflections was 0·250 compared with the value of 0·380 given by the bromine alone. The structure was refined in the projection by difference Fourier syntheses to an R-index of 0·200. The difference Fourier maps showed considerable anisotropic thermal vibration for the bromine atom.

For the projection down the c-axis, the *y*-co-ordinate of the bromine atom was arbitrarily chosen to be 0·25. As before, a weighted beta general synthesis was computed.

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Because of the spurious mirror symmetry introduced by the bromine phases, lot of overlapping was observed in the map. However, from stereochemical considerations and with the aid of the map in the *b*-projection, the molecule could be identified and approximate *y*-co-ordinates were assigned to all the atoms. The R-index for the *h k* 0 reflections for this model was 0·290.

This model was used for the three-dimensional refinement of the structure by least squares. In all, eleven cycles of refinement were run which reduced the R-index from 0·310 to 0·110. The first two cycles of refinement were carried out on the Elliot 803 computer using a programme written by Subramanian[3] for the space group $P2_1$. The next seven cycles of refinement were run on the IBM 1620 computer and the last two cycles again on the Elliot 803 computer using the programmes of Mair.[4] The first nine cycles of refinement were carried out with individual isotropic thermal parameters for the atoms. The last two cycles of refinement were anisotropic, block diagonal refinements, in which the three positional and the six thermal parameters were varied together with the layerwise scale factors. Unit weighting scheme was used throughout. Unrecorded reflections were included in the final stages of refinement, by assigning them with half the minimum |Fo| observed in the local sin θ range. The standard deviations were estimated at the end of the refinement from the residuals and the diagonal elements of the inverse matrix of the normal equations. The final R-index was 0·112 for all the reflections, and 0·103 when the unrecorded reflections were omitted.

4. Results and Discussion

The final positional parameters, their standard deviations and the thermal parameters of the atoms of a molecule are given in Table I. The final electron density maps for the *b* and *c*-axes projections are shown in Figs. 2 and 3 respectively. The structure viewed along the *b* and *c*-axes is shown in Figs. 4 and 5 respectively.

TABLE I

Final atomic co-ordinates (fractional) *and their standard deviations in Angstroms and thermal parameters*

Atom	Positional co-ordinates			Standard deviations			Thermal parameters					
	x	*y*	*z*	σ(X)	σ(Y)	σ(Z)	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
	(in Angstroms)						$\times 10^4$					
Br ..	0·1251	0·2500	0·1026	0·0027	0	0·0018	46	293	164	38	52	−121
O_1 ..	0·1487	0·6329	0·4069	0·0180	0·020	0·017	111	321	76	− 80	76	− 26
O_2 ..	0·0757	0·4189	0·5812	0·015	0·019	0·017	58	154	247	−122	56	−176
N_1 ..	0·0961	0·7469	0·8446	0·0 7	0·020	0·015	33	4 7	111	0	38	−113
N_2 ..	0·3963	0·3800	0·8469	0·020	0·022	0·018	48	301	196	− 17	75	67
C_1 ..	0·1130	0·6012	0·5507	0·020	0·018	0·020	28	238	195	76	−23	−155
C_2 ..	0·1342	0·8246	0·6853	0·018	0·022	0·017	34	10	128	24	− 3	12
C_3 ..	0·2367	0·9054	0·7309	0·020	0·024	0·021	9	286	208	18	− 8	14
C_4 ..	0·2983	0·7041	0·8068	0·020	0·022	0·016	39	47	129	− 25	44	37
C_5 ..	0·3393	0·6868	0·9948	0·017	0·018	0·017	31	40	122	5	36	− 3
C_6 ..	0·3285	0·8323	1·1450	0·016	0·019	0·017	29	228	115	− 30	3	− 17
C_7 ..	0·3849	0·7585	1·3128	0·022	0·023	0·022	53	575	159	56	− 9	− 57
C_8 ..	0·4393	0·5501	1·3245	0·020	0·033	0·020	34	390	204	− 27	76	75
C_9 ..	0·4487	0·4026	1·1804	0·021	0·022	0·020	32	276	131	− 22	4	156
C_{10} ..	0·3973	0·4870	1·0200	0·021	0·024	0·018	26	189	193	− 78	20	−158
C_{11} ..	0·3336	0·5092	0·7270	0·018	0·024	0·019	29	287	138	43	25	− 23

Temperature factor = $\exp -(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hk + B_{13}hl + B_{23}kl)$.

The bond lengths and bond angles in the molecule are given in Table II *a* and shown in Fig. 6. Table II *b* gives the standard deviations in bond lengths and angles. Most of the bond lengths and bond angles in the two structures are found to be very nearly equal. Small differences in the bond lengths observed for C^a–N, C_6–C_7 and C_{10}–N_2 in the two structures are not really significant in view of the standard deviation in bond length in the hydrobromide being 0·02 Å. In both the structures, the bond distance C_{10}–N_2 is significantly larger than the value (1·307 Å) found in glycyl-L-tryptophan.[5] A probable reason for this shortening observed in glycyl-L-tryptophan is given by Takigawa *et al.*

In the carboxyl group, the two C–O bond lengths are 1·31 Å and 1·17 Å values close to

single and double bond length in a pure carboxyl group[6]; also, the bond angles observed in this group suggest pure carboxyl group character.

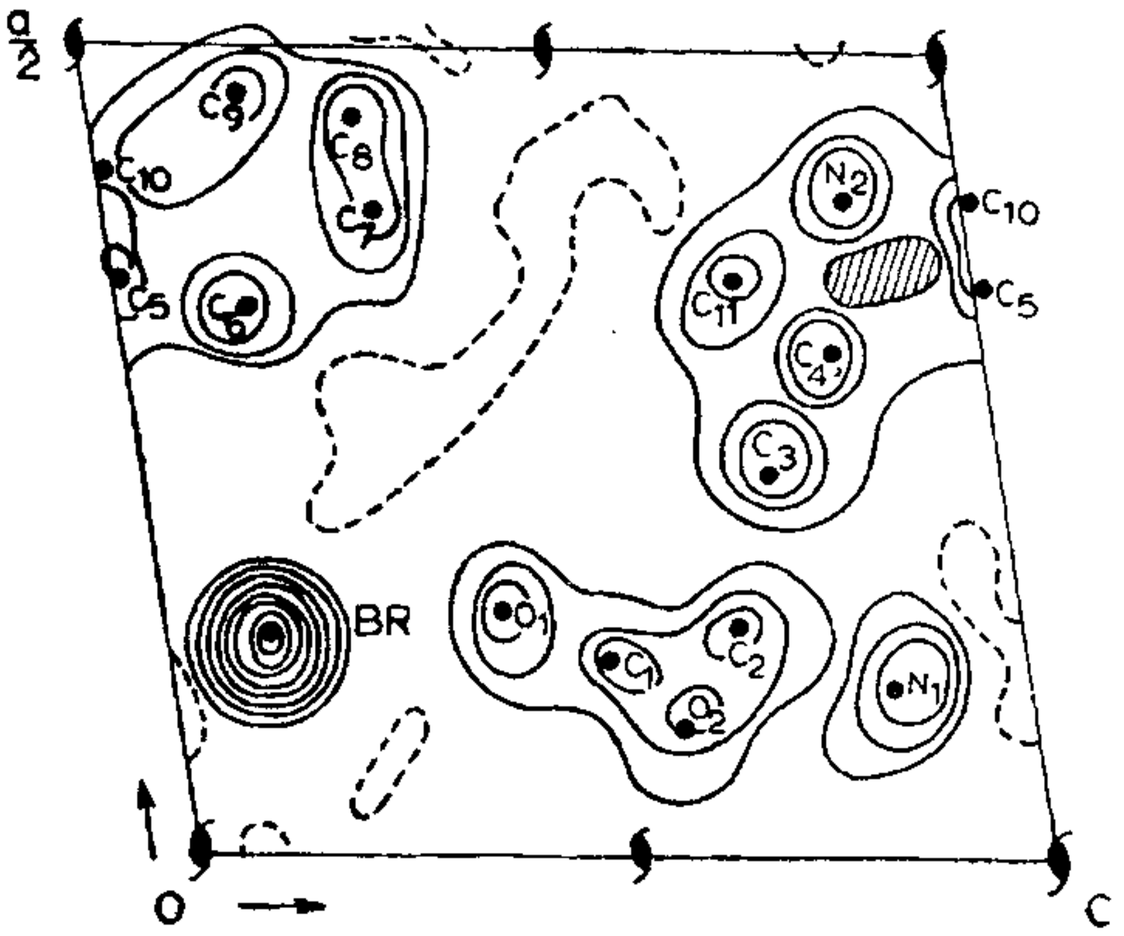

FIG. 2. Final electron density map *b*-axis projection. Contours are drawn at intervals of $2e/A^2$ except near bromine where they are at $5e/A^2$. Zero contours are dashed.

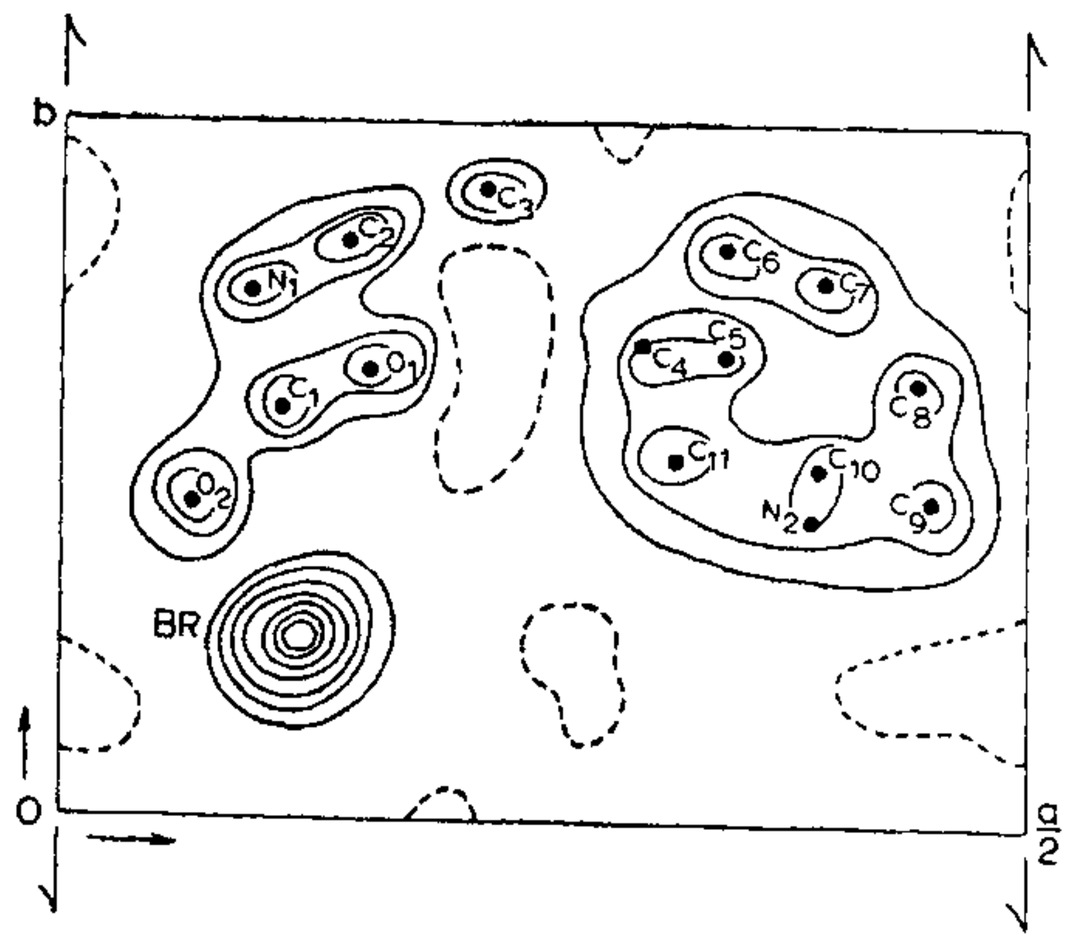

FIG. 3. Final electron density map *c*-axis projection. Contours are drawn as in Fig. 2.

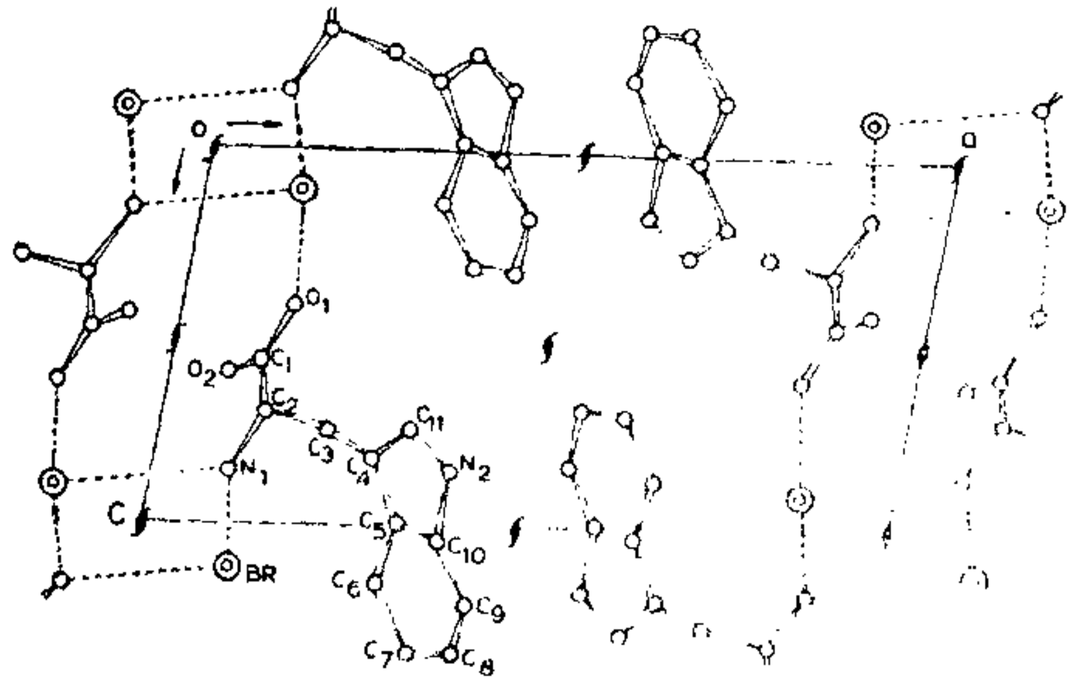

FIG. 4. A view of the structure looked along the *b*-axis.

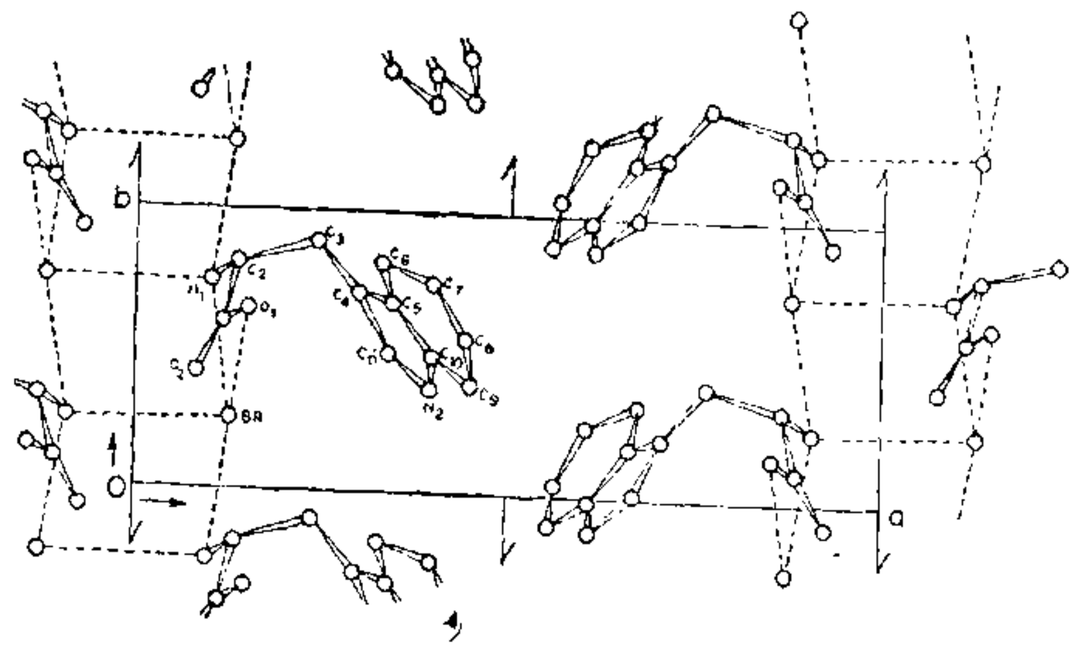

FIG. 5. A view of the structure looked along the *c*-axis

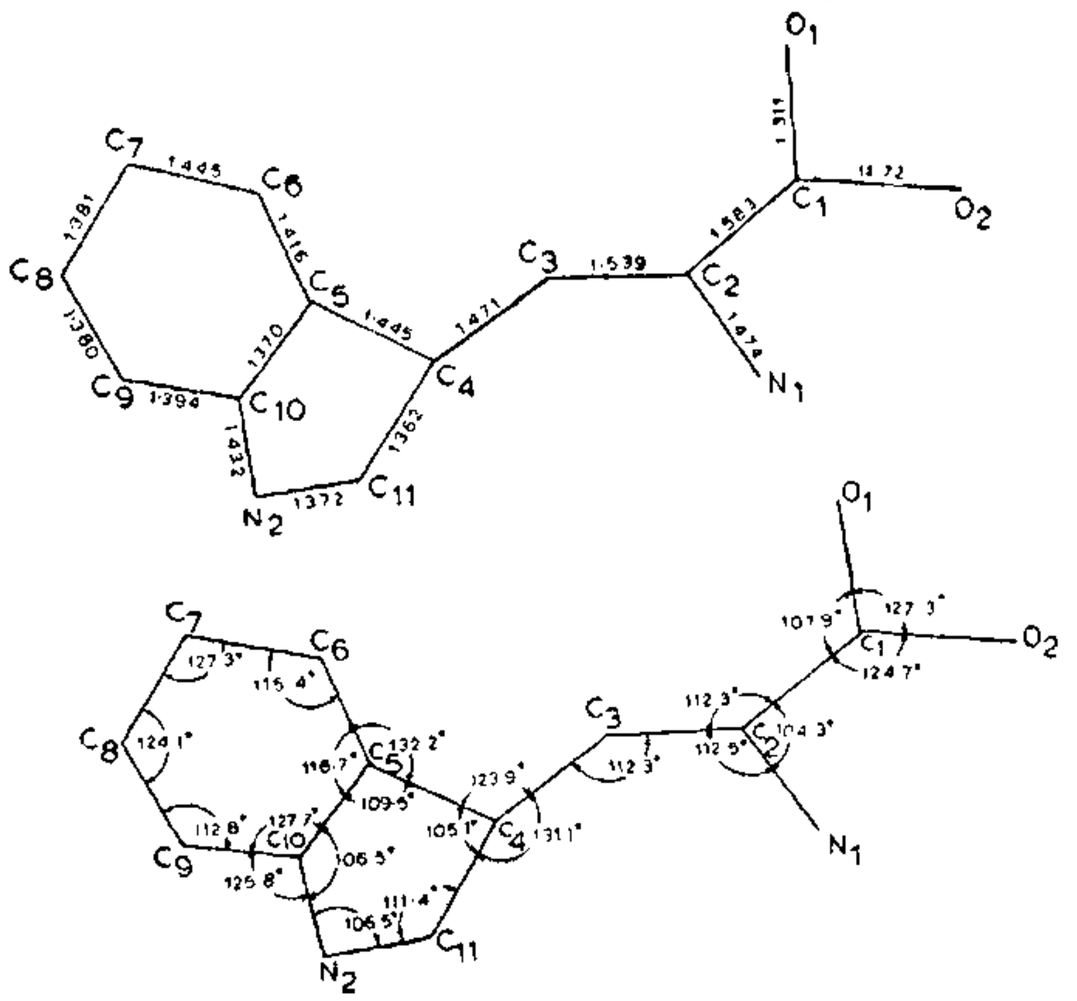

FIG. 6. Bond lengths and bond angles in the molecule.

TABLE II *a*

Bond lengths and bond angles in a molecule

Bond lengths

Bond	Bond length (in Angstroms)	Bond	Bond length (in Angstroms)
C_1–O_1	1·311	C_6 –C_7	1·445
C_1–O_2	1·172	C_7 –C_8	1·381
C_1–C_2	1·583	C_8 –C_9	1·380
C_2–C_3	1·539	C_9 –C_{10}	1·394
C_2–N_1	1·474	C_{10}–N_2	1·432
C_3–C_4	1·471	N_2 –C_{11}	1·372
C_4–C_5	1·445	C_{11}–C_4	1·362
C_5–C_6	1·416	C_{10}–C_5	1·370

Bond angles

Bond angle	Angle in degrees	Bond angle	Angle in degrees
O_1 –C_1–O_2	127·3	C_4 –C_5 –C_6	132·2
O_1 –C_1–C_2	107·9	C_{10}–C_5 –C_6	118·7
O_2 –C_1–C_2	124·7	C_5 –C_6 –C_7	115·4
C_1 –C_2–N_1	104·3	C_6 –C_7 –C_8	121·3
C_1 –C_2–C_3	110·2	C_7 –C_8 –C_9	124·1
N_1 –C_2–C_3	112·4	C_8 –C_9 –C_{10}	112·8
C_2 –C_3–C_4	112·4	C_9 –C_{10}–C_5	127·7
C_3 –C_4–C_5	123·9	C_9 –C_{10}–N_2	125·8
C_3 –C_4–C_{11}	131·1	C_5 –C_{10}–N_2	106·5
C_{11}–C_4–C_5	105·1	C_{10}–N_2 –C_{11}	106·5
C_4 –C_5–C_{10}	109·5	C_4 –C_{11}–N_2	111·4

TABLE II b
Average standard deviations in bond lengths and bond angles

C–C (linear chain)	..	0·020 Å
C–C (aromatic ring)	..	0·023 Å
C–N	..	0·018 Å
C–O	..	0·020 Å
Bond angles	..	1·25°

The indole ring is planar as observed in the hydrochloride. The beta carbon atom also lies nearly in this plane. The amino nitrogen is planar with the C^{α}–COOH group. It is elevated only by 0·003 Å from this plane; the corresponding value in the hydrochloride is 0·15 Å.

All the protons available in a molecule are involved in hydrogen bonding (Table III). The amino nitrogen existing as NH_3^+ forms three hydrogen bonds of the type N–H...Br of lengths 3·32 Å, 3·35 Å and 3·31 A. The carboxyl oxygen atom O_1 forms the fourth hydrogen bond O_1–H...Br = 3·14 Å. The hydrogen bond directions from the amino nitrogen are pointing nearly tetrahedrally towards the three bromide ions with respect to the bond N–C^{α}. A significant feature of the structure is that while the carboxyl oxygen O_1 is involved as a donor in hydrogen bonding, the other oxygen O_2 does not take part in any hydrogen bonding even as an acceptor. This again suggests the existence of pure carboxyl group in the structure.

The scheme of hydrogen bonds may be seen in Figs. 4 and 5. The stability of the structure is maintained by a fine spiral network of hydrogen bonds linking the polar group NH_3^+ and Br⁻ of the molecule and infinitely extending along a screw axis. Two such spiral networks are related by unit translation along the *c* and *a*-axes directions. Around the screw axes that occur midway between these spiral networks, there are only weak van der Waals forces holding the different molecules together. The strong hydrogen bond O_1–H...Br⁻, which is nearly parallel to the *b c* plane, helps to stabilise the structure in this plane by cross-linking the two spiral networks of hydrogen bonds that are translated in the *c*-direction. However, two consecutive spiral networks of hydrogen bonds that are translated in the *a*-direction are separated from each other by two molecules, related by symmetry, which are held together only by van der Waals forces between the atoms of the indole rings.

The non-bonding intermolecular contacts less than 4·0 Å are given in Table IV. There are no unusually short contacts.

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TABLE III
Hydrogen bond lengths and bond angles

Bond	Bond length in Å	Bond angle	Angle in degrees
N_1–H ... Br (A_{001})	3·32	C_2–N_1–Br (A_{001})	133·4
N_1–H ... Br (A_{011})	3·35	C_2–N_1–Br (A_{011})	102·4
N_1–H ... Br (B_{001})	3·31	C_2–N_1–Br (B_{001})	127·2
O_1–H ... Br	3·14	C_1–O_1–Br	116·7
		Br (A_{001}) .. N_1 .. Br (A_{011})	109·3
		Br (A_{001}) .. N_1 .. Br (B_{001})	92·0
		Br (A_{011}) .. N_1 .. Br (B_{001})	92·5

Note: Molecule A is at (x, y, z), B is at $(1 - x, \frac{1}{2} + y, 1 - z)$, A_{001} is at $(x, y\ 1 + z)$, etc.

TABLE IV
Non-bonding inter-molecular contacts less than 4 Å

Atom			Atom		
from	to	distance	from	to	distance
Br (A_{010})	O_1	3·96	O_2(B_{001})	O_2	3·59
Br (A_{001})	O_2	4·00	O_2(B_{001})	N_1	3·85
Br (A_{011})	C_2	3·94	O_2(B_{001})	C_1	3·26
Br (A_{011})	C_3	3·95	O_2(B_{001})	C_2	3·41
Br (A_{011})	C_6	3·71	O_2(B_{011})	O_2	3·59
O_1 (A_{001})	C_6	3·67	N_2(B_{101})	C_6	4·00
O_1 (A_{001})	C_7	3·67	N_2(B_{101})	C_7	3·66
O_2 (A_{010})	C_2	3·40	N_2(B_{101})	C_8	3·42
O_2 (A_{010})	C_3	3·70	N_2(B_{101})	C_9	3·42
N_2 (A_{010})	C_3	3·49	N_2(B_{101})	C_{10}	3·69
N_2 (A_{010})	C_4	3·94	N_2($B_{1\bar{1}1}$)	C_9	3·66
N_2 (A_{010})	C_6	3·96	N_2(B_{102})	C_5	3·98
C_9 (A_{010})	C_6	3·55	C_4($B_{1\bar{1}1}$)	C_9	3·83
C_9 (A_{010})	C_7	3·81	C_5($B_{1\bar{1}1}$)	C_9	3·75
C_{10}(A_{010})	C_6	3·86	C_7($B_{1\bar{1}1}$)	C_8	3·78
C_{11}(A_{010})	C_3	3·58	C_8($B_{1\bar{1}1}$)	C_8	4·00
C_{11}(A_{010})	C_7	3·60	C_8(B_{101})	C_8	4·00
C_{11}(A_{010})	C_8	3·61	C_9(B_{102})	C_{11}	3·79
O_1(B_{001})	O_2	3·59	C_9(B_{101})	C_{10}	3·69

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PLASMA PROTEIN CHANGES IN EXPERIMENTAL CANCER (YOSHIDA ASCITES SARCOMA IN RATS)

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MALIGNANT tumours are known to alter the plasma proteins of the host both qualitatively and quantitatively.[1,2] Many attempts have been made to detect specificity in the pattern of changes to facilitate diagnosis and prognosis of the malignant states. The presence of certain specific proteins have been claimed with certain type of tumours.[3]

The present study relates to the qualitative and quantitative alterations in the serum proteins of rats bearing Yoshida ascites sarcoma. This sarcoma originated in the ascites form in a rat treated with O. amidoazotoluene and Fowler's solution,[4] and has been maintained in ascites form since by serial transplantations in rats. The nature of cancer cell is uncertain. Originally considered to be reticulo-endothelial in nature,[5] recently the tumour is thought to have an epithelial origin.[6]

For our studies the tumour injected rats were obtained from the Indian Cancer Research Centre, Bombay. They have been maintained in highly inbred Wistar strain of rats. This sarcoma—a rapidly developing tumour causes mortality in hundred per cent of the infected animals within ten days of implantation.

Materials and Methods

Male rats weighing 100-120 grams, infected with 100 million cells, were used for the studies.

Heart blood and ascitic fluid were obtained from the transplanted rats on the 8th day when maximal ascites had developed. Separated serum and the supernatant of the ascitic fluid were stored in cold. Normal rat serum served as control.

Total proteins in the sera were estimated by Biuret method[7] after treating with TCA. Analysis of the serum components was carried out by agar-gel electrophoresis[8] at 300 v with constant current of 10–12 mA running for a period of 6 hours. Each stained protein band was estimated by elution method, using cellophane paper technique.[9] The bands were eluted in N/20 NaOH and the color read in Klett-Summerson photoelectric calorimeter using filter 54.

Results are presented in Table I and Figs. 1 and 2.

Table I

Serum proteins in normal and tumour-bearing rats

Sample	% of proteins in grams
Normal rat serum ..	6·2*
Tumour-bearing rat serum ..	4·5*

* Each value represents the average of 3 experiments.

Note : Reduction in protein content of serum from tumour-bearing rats.

Results and Discussion

The results indicate :

(*i*) reduction in the total protein content,
(*ii*) hypoalbuminemia,
(*iii*) slight elevation of α and β-globulins,
(*iv*) complete absence of γ-globulin in the sera of tumour-bearing animals, and
(*v*) a similar pattern of the components with absence of γ-globulin in the ascitic fluid.