Acta Cryst. (1974). B30, 2817

An X-ray Study of Ilamycin B_1, a Cyclic Heptapeptide Antibiotic

BY YOICHI IITAKA

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo 113, *Japan*

AND HIKARU NAKAMURA, KUNIO TAKADA AND TOMOHISA TAKITA

Institute of Microbial Chemistry, Kamiohsaki, Shinagawa-ku, Tokyo 141, *Japan*

(*Received* 4 *June* 1974; *accepted* 9 *July* 1974)

The crystal structure of ilamycin B_1 *p*-bromobenzoate (Br-Ila, $C_{61}H_{80}O_{11}N_9Br$) has been determined from the phase angles obtained by the anomalous dispersion method applied to the isomorphous ilamycin B_1 *p*-iodobenzoate (I-Ila) crystal. Br-Ila crystallizes in the monoclinic space group $P2_1$ with lattice constants $a=14{\cdot}32$ (1), $b=19{\cdot}69$ (2), $c=11{\cdot}88$ (1) Å, $\beta=93{\cdot}97$ (5)° and $Z=2$. The final R value was 0·08 for 2539 observed reflexions. The molecule consists of seven L-amino acid residues linked together by peptide linkages. Two of them (residues 3 and 7) involve *N*-methylamide groups; the amide bonds connecting residues 2 and 3 and 6 and 7 are both *cis* and serve to fold the peptide chain to form a cyclic structure. Two transannular intramolecular hydrogen bonds stabilize the conformation.

Introduction

Ilamycin is an antibiotic inhibiting the growth of mycobacteria. It was isolated from the culture filtrate of *Streptomyces islandicus* along with homologous compounds (Takita, Ohi, Okami, Maeda & Umezawa, 1962). Chemical studies have established that ilamycin, ilamycin B_1 and ilamycin B_2 are cyclic heptapeptides consisting solely of L-amino acid residues: L-3-nitrotyrosine, L-alanine, L-*N*-methylleucine (one of the terminal methyl groups of the leucine side chain is replaced by an aldehyde group in ilamycin), L-leucine, L-2-amino-*trans*-4-hexenoic acid, an L-tryptophan derivative [1-(1,1-dimethyl-2,3-epoxypropyl)-L-tryptophan in ilamycin and ilamycin B_2, and 1-(1,1-dimethyl-2,2-propenyl)-L-tryptophan in ilamycin B_1] and L-*N*-methylleucine (Takita, 1963; Takita, Nagasawa, Maeda & Umezawa, 1964). The conformation of the molecule of ilamycin B_1 has been discussed by Cary, Takita & Ohnishi (1971), on the basis of a 300 MHz proton magnetic resonance (p.m.r.) study. The present study by the X-ray diffraction method has been undertaken to determine the structure exactly and to obtain detailed information on the conformation of the molecule. The results will be discussed in comparison with those obtained by the previous p.m.r. study.

Experimental

Two heavy-atom derivatives of ilamycin B_1, Br-Ila and I-Ila, were obtained by esterification of a hydroxyl group of 3-nitrotyrosine residue with either *p*-bromobenzoic acid or *p*-iodobenzoic acid. I-Ila was obtained in two forms when recrystallized from ethanol solution while Br-Ila was obtained only in one form. The crystal data are given in Table 1.

Intensity data for the three kinds of crystals were collected by a Rigaku four-circle X-ray diffractometer using a θ–2θ scan method with a scan speed of $2\theta\ 4°\ \text{min}^{-1}$. Background was measured at both sides of the scan for 10 s. Data for the intensity measurements are summarized in Table 1. In each of the four sets of measurements, intensities of Friedel reflexions ($h\bar{k}l$ *vs.* hkl) were also measured. Intensities were then corrected for Lorentz and polarization factors and were converted to the absolute scale by Wilson's method. No absorption correction was applied.

Determination of the structure

The location of the heavy atom in form I and II crystals was determined by the Patterson method. In view of the fact that the iodine atom in the I-Ila form II crystal lies at a rather special position ($x=0{\cdot}184$, $y=0$, $z=\frac{1}{4}$), giving rise to an ambiguity in phase-angle determination, and that only a limited number of reflexions were observed for this crystal due to large temperature factors, the present structure determination was carried out for the form I crystals of both I-Ila and Br-Ila. The phase-angle determination by the anomalous dispersion method (Hall & Maslen, 1965) was not successful for the bromine derivative even when Mo radiation was used, but was successful for the iodine derivative. The dispersion corrections applied to the scattering factor of the iodine atoms for Cu $K\alpha$ radiation were $\Delta f'=-1{\cdot}1$ and $\Delta f''=7{\cdot}2$. Of the total of 86 atoms (excluding hydrogen) the locations of 81 atoms were found on the first electron-density map with the correct absolute configuration. This map was calculated on the basis of the 1699 structure factors, the phases of 932 of which were determined by the anomalous dispersion method and those of the remaining 767 were assigned merely by the heavy-atom contributions.

Three cycles of least-squares refinement with isotropic, followed by one cycle with anisotropic temper-

ature factors gave an R value of 0·17 for 81 atoms. Further refinement was carried out for Br-Ila (form I). Since the crystals were isomorphous, only the atomic scattering factor of iodine and the 1699 structure factors of I-Ila were replaced by those of Br-Ila. A difference electron-density map calculated after several cycles of least-squares refinement revealed a terminal carbon atom of leucine (residue 3) along with four atoms of ethanol and water which were contained as the solvents of crystallization. Refinement was completed by five cycles of block-diagonal least-squares calculations by the program *HBLS* (Okaya & Ashida, 1967) with anisotropic temperature factors. Unit weight was applied for each reflexion during the refinement. The final difference electron-density map showed no anomalous features and the 20 peaks higher than 0·2 e $Å^{-3}$ could be assigned as hydrogen atoms, but no further attempt has been made to locate them. The final R value was 0·08. Atomic scattering factors of C, N, O and Br were those given in *International Tables for X-ray Crystallography* (1962) in Tables 3.3.1A and Tables 3.3.1B. No anomalous dispersion correction was applied in the refinement of the Br-Ila structure. The final atomic parameters are listed in Table 2.*

Discussion of the structure

Configuration and conformation of the molecule

As has been shown by chemical studies (Takita *et al.*, 1962; Takita, 1963; Takita *et al.*, 1964), ilamycin B_1 is a cyclic peptide consisting of seven L-amino acid residues as shown in Fig. 2(*a*). They are L-3-nitrotyrosine (residue number, $i=1$), L-alanine (2), L-*N*-methylleucine (3), L-leucine (4), L-2-amino-*trans*-4-hexenoic acid (5), L-tryptophan derivative (6) and L-*N*-methylleucine (7), linked together through normal peptide linkages.

The conformation of the molecule is shown in Fig. 1 by an *ORTEP* stereoscopic drawing (Johnson, 1965) which also shows the thermal vibrations of the atoms as ellipsoids of 20% probability. The numbering of the atoms adopted in the present paper is shown in Fig. 2(*b*). Most of the cyclic peptides studied so far contain such residues as glycine, sarcosine and proline, which are considered to be energetically favoured to form the corner of a β-turn. One of the main purposes of the present study was to elucidate the conformation of a cyclic peptide that consists of seven all L-amino acid residues with rather bulky side chains, and to see how the peptide chain is folded to form a stable antiparallel β-structure.

The torsion angles along the peptide chain and those formed by the C^{α}, C^{β}, C^{γ} and C^{δ} atoms are listed in Table 3.

The φ and ψ values are plotted on a φ–ψ chart (Ramachandran, Ramakrishnan & Sasisekharan, 1963) shown in Fig. 3. The planarities of the *N*-methylimino and carbonyl groups are fairly good, as evidenced by the sum of the bond angles subtended at the imino nitrogen or carbonyl carbon atoms, none of which deviate by more than 0·5°.

It can be seen from Figs. 1, 2(*b*) and Table 3 that one of the corners is formed by residues 2 and 3 and the other is occupied by residues 6 and 7. The amide bonds between 2 and 3, and 6 and 7 are *cis*, as seen from the values of ω angles, -11 and 1°, respectively. It is interesting to see that both *cis* configurations occur

* The F_o and F_c table for Br-Ila has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30573 (11 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1. *Crystal data and intensity-measurement data*

I-Ila: Ilamycin B_1 *p*-iodobenzoate.ethanol.monohydrate, F.W. 1306·4*
Br-Ila: Ilamycin B_1 *p*-bromobenzoate.ethanol.monohydrate, F.W. 1259·4*

	I-Ila		Br-Ila	
	Form I Monoclinic	Form II Orthorhombic	Form I Monoclinic	
a (Å)	14·34 (1)	19·71 (2)	14·32 (1)	
b (Å)	19·71 (2)	22·78 (2)	19·69 (2)	
c (Å)	12·00 (1)	15·11 (1)	11·88 (1)	
β (°)	94·08 (3)		93·97 (5)	
U (Å^3)	3382	6782	3341	
Space group	$P2_1$	$P2_12_12_1$	$P2_1$	
Z	2	4	2	
D_x*(g cm^{-3})	1·28	1·23	1·25	
D_m (g cm^{-3})			1·24	
μ (Cu K) (cm^{-1})	46·4	44·6	14·6	
Crystal size (mm)	0·25 × 0·23 × 0·1	0·4 × 0·1 × 0·04	0·3 × 0·2 × 0·1	
Radiation (filter)	Cu(Ni)	Cu(Ni)	Cu(Ni)	Mo(Zr)
$2\theta_{max}$(°)	100	60	120	30
No. obs. refl.	1699	490	2539	558
No. theor. possible refl.	2547	1102	5116	1344

* Calculated assuming $C_{61}H_{80}O_{11}N_9I.C_2H_5OH.H_2O$ or $C_{61}H_{80}O_{11}N_9Br.C_2H_5OH.H_2O$.

at the methyl-substituted amide bonds. The *cis* amide bond is unusual for a peptide unit. However, a few examples have been observed, especially for peptide linkages involving the imino nitrogen atoms of proline, hydroxyproline and other amino acid residues whose imino hydrogen atom is replaced by a methyl group. The ω angles for such amide bonds other than those for proline and hydroxyproline are listed in Table 3. The angles do not deviate greatly from 0°. In the case of the *trans* amide bonds, the ω angles fall in the range $180 \pm 15°$, and the deviations from the ideal value are of the same order of magnitude as in the *cis* bonds.

The conformations of residues 3 and 4 are significantly distorted from the ideal β-conformation and the φ, ψ values of these residues lie in the outer limit of the allowed region of β or even in the forbidden region, especially those of residue 4 which lie just at the borderline between the α and β conformations.

The conformation of the molecule described above is stabilized by the two intramolecular hydrogen bonds, N(1)–H···O(5) of 2·80 Å and N(4)–H···O(1) of 2·98 Å. There is also one shorter transannular close contact, N(5)···O(1) 3·32 Å, for which a weak hydrogen bond might be considered. A 300 MHz p.m.r. study of this molecule suggested the existence of two intramolecular hydrogen bonds, N(1)–H···O(5) and N(5)–H···O(2), on the basis of the temperature dependence of amide proton chemical shifts and of the hydrogen–deuterium exchange rate of amide hydrogen atoms at room temperature (Cary, Takita & Ohnishi, 1971). The latter experiment indicated that the exchanges of the hydrogen atoms at N(1), N(4) and N(5) took several days, whereas those at N(2) and N(6) took only a few hours. As is clear from Fig. 1, only N(1), N(4) and N(5) of the three imino groups turn to the inside of the molecule, which causes a strong interaction with the carbonyl oxygen atoms and the exchange rate of these hydrogen atoms should consequently be reduced.

Table 2. *Final atomic parameters* ($\times 10^4$)

The temperature factors are of the form: $T=\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$. Absolute configuration is represented by the left-hand coordinate system.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br(1*T*)	10813 (2)	0 (0)	10941 (2)	127 (2)	48 (1)	154 (3)	1 (1)	−8 (2)	33 (1)
O(1*H*3)	11084 (11)	4379 (9)	7217 (16)	81 (10)	53 (6)	259 (24)	−13 (7)	−39 (13)	16 (10)
O(1*H*2)	10894 (10)	3464 (10)	8101 (14)	74 (10)	79 (8)	186 (19)	−3 (7)	−7 (11)	35 (10)
O(1*H*1)	9551 (9)	2621 (7)	7443 (10)	104 (10)	39 (5)	63 (11)	19 (5)	8 (8)	−3 (6)
O(1*T*1)	9092 (10)	3071 (7)	9001 (11)	112 (11)	41 (5)	110 (14)	7 (6)	19 (10)	−7 (7)
O(1)	9722 (8)	5375 (6)	3939 (11)	61 (8)	30 (4)	142 (15)	−1 (5)	23 (8)	−13 (6)
O(2)	11107 (9)	3834 (6)	4111 (11)	74 (8)	31 (4)	136 (15)	−10 (5)	−11 (9)	5 (6)
O(3)	12597 (12)	6488 (7)	3757 (13)	148 (13)	26 (4)	169 (17)	−25 (6)	59 (12)	−5 (7)
O(4)	10952 (9)	7312 (8)	6727 (12)	79 (9)	47 (5)	128 (14)	2 (6)	−17 (9)	−26 (7)
O(5)	8677 (7)	6785 (5)	3769 (10)	51 (6)	24 (3)	94 (11)	−1 (4)	1 (7)	−13 (5)
O(6)	6102 (10)	7621 (8)	4623 (12)	85 (10)	52 (6)	137 (15)	−3 (6)	7 (10)	−14 (8)
O(7)	6296 (8)	5157 (6)	3012 (12)	57 (7)	34 (5)	183 (16)	−12 (5)	−18 (9)	26 (7)
N(1)	7759 (8)	5549 (7)	3337 (11)	37 (7)	26 (4)	96 (13)	−2 (5)	−3 (8)	12 (6)
N(1*Z*2)	10636 (12)	3899 (10)	7435 (16)	76 (11)	50 (7)	161 (20)	15 (7)	3 (12)	−3 (10)
N(2)	9435 (10)	4417 (7)	2905 (12)	70 (9)	23 (4)	101 (15)	6 (5)	1 (9)	5 (7)
N(3)	11874 (9)	4794 (6)	3641 (12)	44 (7)	22 (4)	110 (14)	2 (4)	6 (8)	2 (6)
N(4)	11542 (9)	5985 (7)	4769 (12)	42 (7)	32 (5)	96 (14)	3 (5)	22 (8)	−1 (7)
N(5)	10046 (9)	6821 (7)	5349 (11)	35 (7)	35 (5)	89 (14)	−6 (5)	−13 (8)	−2 (7)
N(6)	8039 (9)	7780 (6)	4225 (12)	54 (8)	17 (4)	116 (15)	12 (5)	−20 (9)	−2 (6)
N(6*E*1)	4741 (9)	8250 (9)	1044 (13)	36 (8)	59 (7)	109 (16)	−7 (6)	7 (9)	0 (9)
N(7)	6184 (9)	6705 (7)	3501 (12)	37 (7)	33 (5)	112 (15)	−1 (5)	−11 (9)	13 (7)
C(1*A*)	8150 (9)	4910 (8)	3792 (14)	18 (7)	20 (5)	126 (18)	7 (5)	6 (9)	8 (8)
C(1*B*)	8016 (13)	4876 (9)	5064 (16)	72 (11)	23 (5)	116 (19)	14 (7)	21 (12)	18 (9)
C(1*G*)	8463 (10)	4258 (10)	5588 (15)	23 (8)	50 (8)	103 (18)	16 (6)	22 (10)	−1 (9)
C(1*D*1)	9341 (12)	4337 (9)	6215 (16)	65 (11)	25 (5)	112 (20)	2 (6)	36 (12)	2 (9)
C(1*E*1)	9688 (11)	3787 (9)	6838 (16)	46 (10)	34 (6)	103 (19)	0 (7)	3 (11)	−1 (9)
C(1*Z*1)	9232 (13)	3177 (9)	6821 (16)	78 (12)	25 (5)	102 (18)	19 (7)	1 (12)	14 (8)
C(1*E*2)	8375 (12)	3094 (10)	6191 (16)	55 (10)	33 (6)	111 (19)	7 (6)	0 (11)	11 (9)
C(1*D*2)	8001 (13)	3629 (9)	5594 (17)	75 (13)	22 (5)	127 (21)	10 (7)	−4 (13)	5 (9)
C(1*T*1)	9468 (14)	2609 (10)	8574 (17)	77 (13)	38 (7)	112 (20)	20 (8)	4 (13)	−9 (10)
C(1*T*2)	9791 (12)	1966 (10)	9145 (16)	43 (10)	37 (6)	113 (20)	−3 (7)	−26 (11)	9 (9)
C(1*T*3)	9627 (14)	1918 (11)	10319 (18)	68 (13)	44 (8)	129 (23)	−4 (8)	2 (14)	16 (11)
C(1*T*4)	9904 (16)	1313 (12)	10854 (18)	99 (15)	46 (8)	99 (20)	−11 (9)	11 (14)	−18 (10)
C(1*T*5)	10358 (16)	789 (12)	10222 (18)	102 (16)	48 (9)	122 (23)	−23 (10)	−64 (15)	34 (12)
C(1*T*6)	10535 (13)	876 (9)	9042 (15)	74 (12)	32 (6)	84 (17)	2 (7)	15 (12)	6 (8)
C(1*T*7)	10217 (12)	1442 (9)	8522 (16)	55 (11)	26 (6)	105 (19)	1 (7)	2 (11)	2 (9)
C(1)	9158 (12)	4948 (10)	3582 (13)	79 (12)	26 (5)	70 (15)	−11 (7)	−13 (11)	1 (9)
C(2*A*)	10417 (12)	4403 (9)	2528 (15)	54 (10)	24 (5)	109 (19)	4 (6)	−19 (11)	5 (8)
C(2*B*)	10420 (14)	3793 (11)	1714 (17)	78 (13)	43 (7)	109 (20)	5 (8)	14 (13)	−33 (10)
C(2)	11155 (13)	4368 (10)	3474 (17)	68 (13)	36 (7)	121 (21)	23 (8)	20 (13)	−2 (10)
C(N3)	12649 (13)	4586 (10)	4452 (19)	62 (12)	25 (6)	170 (24)	7 (7)	−18 (14)	19 (10)
C(3*A*)	11976 (13)	5434 (8)	3031 (17)	68 (12)	13 (4)	141 (21)	−3 (6)	−15 (13)	1 (8)
C(3*B*)	12811 (14)	5436 (11)	2271 (18)	76 (13)	35 (7)	146 (23)	−4 (8)	42 (14)	−15 (10)

Table 2 (*cont.*)

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(3*G*)	12747 (14)	4907 (12)	1377 (17)	80 (13)	40 (7)	122 (20)	5 (9)	26 (13)	−13 (11)
C(3*D*2)	12024 (17)	5066 (14)	445 (20)	112 (18)	60 (10)	144 (25)	−22 (12)	27 (17)	10 (14)
C(3*D*1)	13705 (16)	4819 (11)	930 (19)	105 (16)	40 (9)	158 (25)	−12 (9)	37 (16)	−11 (12)
C(3)	12053 (12)	6025 (9)	3923 (16)	56 (11)	23 (5)	130 (21)	−1 (6)	1 (12)	−1 (9)
C(4*A*)	11728 (11)	6456 (9)	5743 (16)	45 (10)	28 (6)	111 (19)	−6 (6)	−4 (11)	−21 (9)
C(4*B*)	12103 (11)	6091 (9)	6810 (13)	40 (9)	41 (7)	63 (15)	17 (6)	−4 (9)	12 (8)
C(4*G*)	13150 (16)	5918 (11)	6697 (18)	99 (16)	41 (7)	129 (22)	14 (9)	−34 (15)	−9 (11)
C(4*D*2)	13734 (17)	6551 (16)	6885 (23)	81 (17)	87 (14)	180 (31)	−30 (12)	−3 (18)	1 (17)
C(4*D*1)	13384 (17)	5351 (13)	7665 (23)	98 (17)	49 (9)	195 (29)	20 (10)	−46 (18)	11 (13)
C(4)	10844 (13)	6864 (9)	5956 (17)	74 (12)	29 (6)	106 (19)	−15 (7)	4 (12)	−2 (9)
C(5*A*)	9334 (12)	7336 (9)	5432 (15)	57 (11)	25 (5)	103 (18)	13 (6)	2 (11)	−8 (8)
C(5*B*)	8749 (14)	7239 (10)	6547 (15)	89 (14)	37 (7)	78 (18)	3 (8)	9 (12)	−15 (9)
C(5*G*)	8221 (14)	6577 (12)	6617 (17)	73 (13)	53 (8)	93 (19)	1 (9)	0 (13)	−30 (10)
C(5*D*)	7376 (18)	6529 (15)	6621 (24)	109 (19)	68 (12)	186 (32)	−25 (12)	40 (20)	−48 (16)
C(5*E*)	6811 (19)	5884 (16)	6889 (25)	105 (20)	82 (14)	206 (34)	−42 (14)	36 (21)	−5 (19)
C(5)	8627 (11)	7279 (9)	4398 (14)	34 (9)	35 (6)	83 (16)	−2 (6)	−8 (9)	−14 (8)
C(6*A*)	7276 (11)	7691 (9)	3344 (16)	41 (9)	25 (5)	135 (20)	0 (6)	1 (11)	6 (9)
C(6*B*)	6944 (12)	8424 (9)	2894 (15)	64 (11)	29 (6)	101 (18)	15 (7)	−40 (12)	−3 (9)
C(6*G*)	6197 (11)	8347 (8)	1967 (15)	54 (10)	24 (5)	95 (17)	16 (6)	−7 (10)	1 (8)
C(6*D*2)	6309 (12)	8179 (8)	798 (15)	67 (11)	17 (5)	111 (19)	−11 (6)	−10 (12)	3 (8)
C(6*E*3)	7149 (14)	8094 (12)	228 (17)	85 (14)	49 (8)	105 (20)	8 (9)	27 (14)	−9 (11)
C(6*Z*3)	6985 (14)	7951 (13)	−883 (21)	56 (12)	57 (9)	196 (30)	−13 (9)	30 (16)	10 (13)
C(6*H*4)	6089 (16)	7896 (13)	−1451 (16)	108 (16)	65 (10)	65 (18)	−15 (11)	18 (14)	−3 (11)
C(6*Z*2)	5282 (14)	7974 (10)	−923 (15)	94 (14)	35 (7)	74 (17)	−6 (8)	19 (12)	16 (9)
C(6*E*2)	5379 (12)	8126 (9)	274 (15)	63 (11)	31 (6)	95 (18)	−9 (7)	6 (11)	18 (8)
C(6*D*1)	5267 (11)	8384 (10)	2052 (15)	41 (9)	45 (7)	103 (18)	−6 (7)	−4 (11)	4 (10)
C(6*Z*1)	3724 (16)	8329 (14)	852 (24)	91 (17)	69 (12)	218 (33)	−41 (12)	−2 (19)	−70 (17)
C(6*H*1)	3341 (26)	7819 (21)	391 (33)	194 (33)	108 (19)	301 (50)	56 (22)	146 (34)	23 (25)
C(6*T*1)	3458 (22)	7253 (16)	374 (30)	154 (27)	64 (12)	296 (45)	−4 (15)	96 (28)	21 (20)
C(6*H*2)	3240 (16)	8478 (18)	1932 (22)	64 (14)	128 (18)	160 (29)	13 (13)	38 (17)	−48 (19)
C(6*H*3)	3427 (19)	8902 (17)	−167 (21)	132 (21)	81 (14)	131 (26)	21 (14)	−16 (19)	−3 (16)
C(6)	6502 (14)	7341 (11)	3847 (15)	79 (13)	45 (7)	76 (18)	17 (8)	−24 (12)	3 (10)
C(N7)	5292 (16)	6421 (14)	3990 (22)	74 (15)	64 (11)	173 (28)	9 (11)	32 (16)	11 (15)
C(7*A*)	6588 (12)	6331 (8)	2579 (14)	70 (11)	19 (5)	76 (16)	1 (6)	−30 (11)	−10 (7)
C(7*B*)	5853 (13)	6300 (10)	1575 (18)	66 (12)	33 (6)	137 (22)	1 (7)	−15 (13)	4 (10)
C(7*G*)	6222 (18)	5877 (13)	587 (21)	109 (18)	55 (9)	149 (25)	−11 (11)	−41 (17)	−1 (13)
C(7*D*2)	5430 (18)	5956 (15)	−438 (20)	124 (21)	74 (12)	129 (25)	−35 (13)	−43 (18)	13 (14)
C(7*D*1)	7149 (17)	6158 (21)	125 (25)	74 (17)	143 (22)	200 (34)	20 (16)	−19 (19)	−38 (23)
C(7)	6862 (11)	5596 (8)	3020 (15)	56 (10)	19 (5)	110 (18)	−18 (6)	−13 (11)	0 (8)
O(*W*)	8051 (11)	3380 (7)	2434 (14)	109 (11)	28 (4)	191 (18)	−13 (6)	−39 (11)	4 (7)
C(ET2)	5244 (25)	4136 (24)	4984 (36)	152 (30)	148 (24)	311 (50)	−6 (23)	107 (32)	16 (30)
C(ET1)	5477 (26)	3585 (24)	4109 (38)	169 (31)	140 (24)	409 (62)	−90 (23)	79 (36)	−99 (33)
O(ET)	6191 (11)	3740 (10)	3289 (16)	99 (12)	66 (7)	236 (23)	−3 (8)	−15 (13)	29 (11)

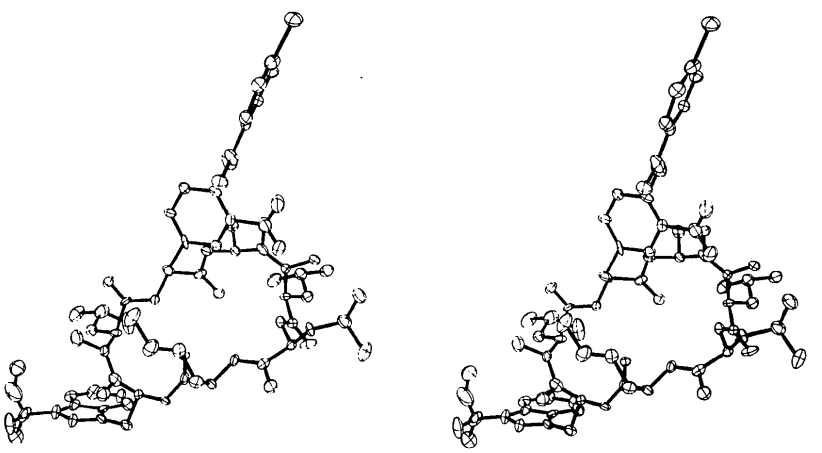
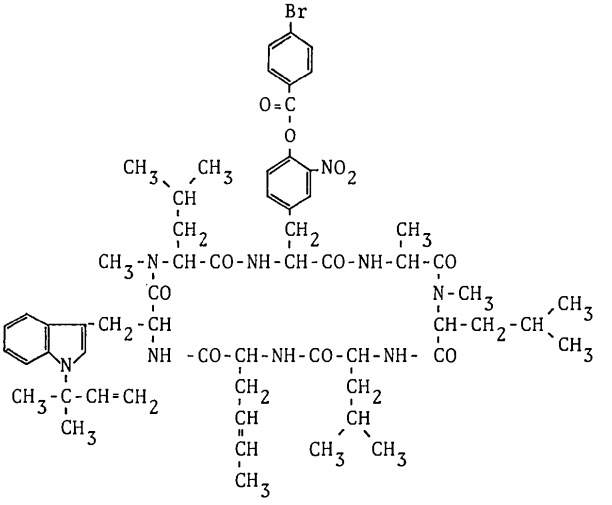
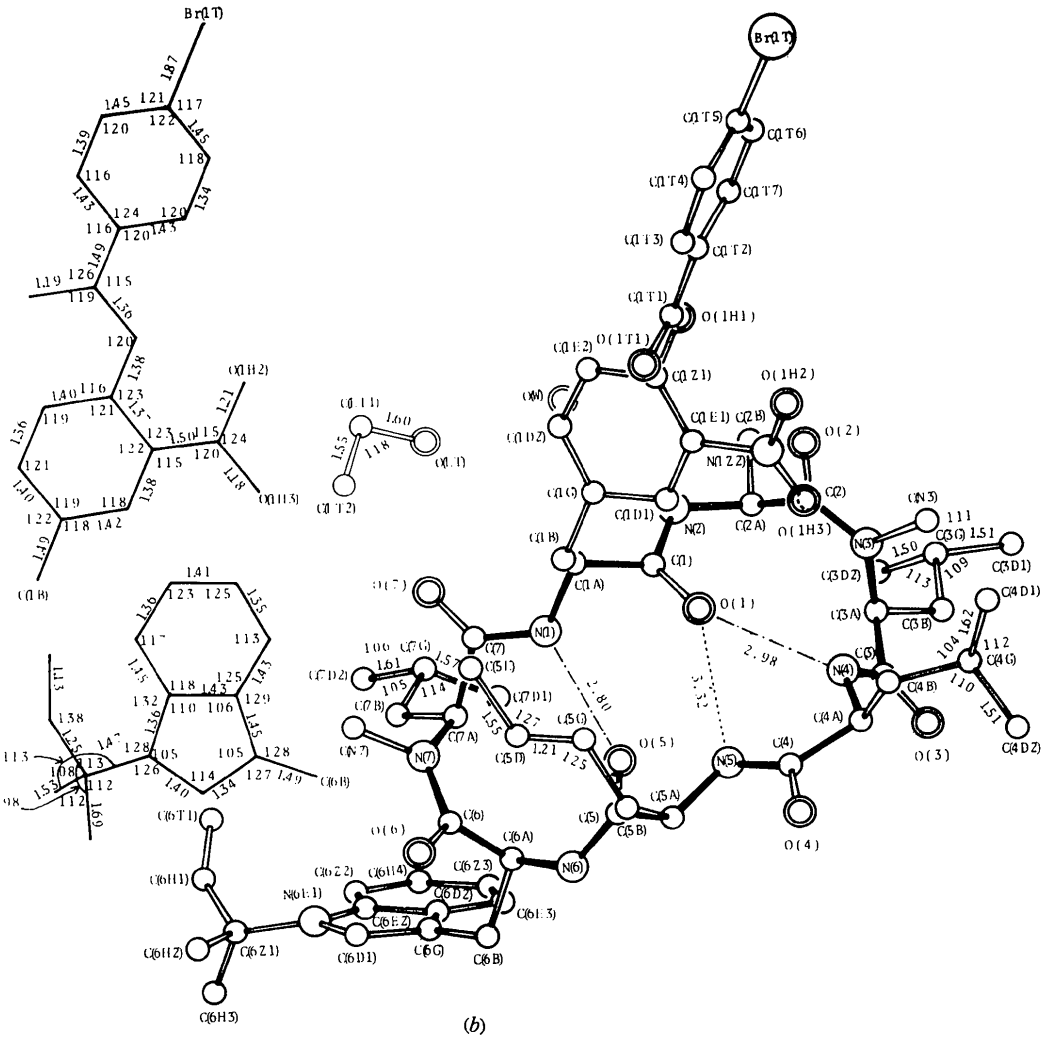


Fig. 1. A stereoscopic view of the molecule of Br-Ila along the *c* axis.



(*a*)



(*b*)

Fig. 2. The structure of Br-Ila. (*a*) Chemical structure. (*b*) Molecular structure. The numbering of the atoms and the bond lengths and angles in the side chains are also shown.

Thus the results of the p.m.r. study could be interpreted more unambiguously, with almost the same conformation as that determined by X-ray analysis. A slight difference is that in a dimethyl sulfoxide solution of intact ilamycin B_1, the hydrogen bond N(5)–H···O(1) may be predominant over N(4)–H···O(1).

Intramolecular interatomic distances and angles

The bond lengths and angles in the skeleton of the molecule are listed in Tables 4 and 5 compared with the values found in other cyclic peptides. The values for the side chains are shown in Fig. 2(*b*). Estimated standard deviations are: 0·025 Å and 1·5° for the bonds between the main-chain atoms, 0·03 Å and 2° for most of the bonds involving side-chain atoms, 0·07 Å and 4° for those involving side-chain terminal atoms, C(4*D*2), C(5*E*), C(6*H*), C(6*T*) and C(7*D*1) and also solvent C(ET). The lengths and angles are compatible with the proposed chemical structure and agree generally with those found in other peptides. However, some significant deviations of angles are observed, mostly for the bonds involved in the *cis* amide groups. The angles C^{α}–C′–N and C′–N–C^{α} (C′ is a carbonyl carbon atom) are significantly larger, while O–C′–N and C′–N–C(*N*-methyl) are smaller than those found in *trans* amide groups. This may be caused by the repulsive forces between the two α-carbon atoms which are *cis* to each other. Indeed, the distances between the two C^{α} atoms across the *cis* amide bonds are found to be C(2*A*)···C(3*A*)=3·05 Å and C(6*A*)···C(7*A*)=2·98 Å, while those between carbonyl oxygen and *N*-methyl carbon atoms are O(2)···C(N3)=2·67 Å and O(6)···C(N7)=2·71 Å. These distances should be compared with the contact distances assumed for deriving the φ–ψ chart, shown in Fig. 3, in which the C···C distances greater than 3·20 Å were assumed for fully allowed regions and at least 3·00 Å for outer-limit regions. For C···O distances the corresponding values were 2·80 and 2·70 Å (Ramachandran, Ramakrishnan & Sasisekharan, 1963). A similar effect is also observed for residue 4, where the two imino nitrogen atoms come to a close contact due to the *cis* disposition of these atoms (ψ_4 is only 2°). In this case the angle C(4*A*)–C(4)–N(5) is widened to 125°.

For bond lengths no such trend is observed but it should be noted that the C′–O bonds are lengthened as the C^{α}–C′–N angles increase. Another point to be noted is that the shorter C(*iA*)–C(*i*) bond and longer amide bond [C(*i*)–N(*i*+1)] are observed for the residues having aromatic side chains. In the present structure, C^{γ} atoms of these residues are all at the *trans* position with respect to their imino nitrogen atoms, and the aromatic rings lie over the amide groups in question. The shortest approaches of atoms between the side chains and the carbonyl groups are C(1*D*1)···O(1)=3·46, C(1*D*1)···C(1)=3·35 and C(6*B*)···C(6)=2·92, C(6*G*)···C(6)=2·99, C(6*G*)···O(6)=3·47 Å; hence C(1*D*1) and C(6*G*) lie at about 3·2∼3·4 Å above the respective carbonyl groups.

The p.m.r. peaks of the isopropylmethyl protons of

Table 3. *Torsion angles* (°)

For abbreviations and residues see Table 4.

	φ_i C(*i*−1) –N(*i*) –C(*iA*) –C(*i*)	ψ_i N(*i*) –C(*iA*) –C(*i*) –N(*i*+1)	ω_i C(*iA*) –C(*i*) –N(*i*+1) –C(*i*+1,*A*)	ω'_i C(*iA*) –C(*i*) –N(*i*+1) –C(N,*i*+1)	φ'_i C(*i*−1) –N(*i*) –C(*iA*) –C(*iB*)	χ^1_i N(*i*) –C(*iA*) –C(*iB*) –C(*iG*)	$\chi^{2,1}_i$ C(*iA*) –C(*iB*) –C(*iG*) –C(*iD*1)	$\chi^{2,2}_i$ C(*iA*) –C(*iB*) –C(*iG*) –C(*iD*2)
Residue No. (*i*)								
Br-Ila								
1	−156	120	−175		85	176	−102	86
2	−61	126	−11	166	174			
3*	−121	38	−168		114	−60	163	−73
4	−123	2	165		112	−76	164	−76
5	−163	168	171		79	61	117	
6	−86	117	1	173	155	−178	−97	79
7*	−128	99	−179		111	177	59	174
CTS								
1	−120·8	65·5	5·4	−173·5				
2*	−93·6	169·5	170·6	−8·1				
CTD								
2*	−102	146	2†			−56	*trans*	−51
4*	−108	121	6†			−67 & *trans*		175
AMD								
4*	80	−169	0†					
	73	179	3†					
5	−123	49	175†					
	−133	53	179†					
PC model‡	−145	142	180					

* The amide bond connecting residues *i* and *i*-1 is *cis*.
† ω_{i-1}.
‡ Antiparallel pleated sheet.

Table 4. *Bond lengths* (Å)

CTS: Cyclotetrasarcosyl (Groth, 1970).
CTD: Cyclotetradepsipeptide, D-HyIv-L-MeIleu-D-HyIv-L-MeLeu (Konnert & Karle, 1969).
AMD: Actinomycin D (in AMD-deoxyguanosine complex: Jain & Sobell, 1972; Sobell & Jain, 1972). The values are for the two cyclic peptide chains obtained by the light-atom analysis.
PC model: Pauling–Corey peptide model (Pauling & Corey, 1953).
Nitro-Tyr: L-3-Nitrotyrosine.
Ala: L-Alanine.
Me-Leu: L-*N*-Methylleucine.
Leu: L-Leucine.
Amino-Hex: L-2-Amino-*trans*-4-hexenoic acid.
Try: L-Tryptophan derivative.
Sar: Sarcosine.
Me-Ileu: L-*N*-Methylisoleucine.
Me-Val: L-*N*-Methylvaline.

Residue No. (*i*)	N(*i*)–C(*iA*)	N(*i*)–C(N*i*)	C(*iA*)–C(*i*)	C(*iA*)–C(*iB*)	C(*iB*)–C(*iG*)	C(*i*)–O(*i*)	C(*i*)–N(*i*+1)
Br-Ila							
1. Nitro-Tyr	1·46		1·48	1·54	1·49	1·22	1·39 (3)
2. Ala	1·50		1·49	1·54		1·30	1·33
3.* Me-Leu	1·47	1·48	1·57	1·55	1·49	1·22	1·29
4. Leu	1·49		1·54	1·52	1·55	1·27	1·31
5. Amino-Hex	1·45		1·55	1·63	1·51	1·22	1·30
6. Try	1·47		1·47	1·60	1·49	1·25	1·39
7.* Me-Leu	1·47	1·54	1·58	1·54	1·56	1·19	1·32
CTS							
1. Sar	1·461	1·471	1·530			1·235	1·352 (4)
2.* Sar	1·454	1·462	1·531			1·215	1·364
CTD							
2.* Me-Leu	1·45	1·52	1·56	1·60	1·48	1·17	1·35 (3)†
4.* Me-Ileu	1·45	1·50	1·52	1·53		1·19	1·29†
AMD							
4.* Sar	1·52	1·45	1·50			1·27	1·36 (3)
	1·45	1·51	1·57			1·23	1·29
5. Me-Val	1·43	1·46	1·52	1·59		1·21	1·35†
	1·49	1·47	1·48	1·59		1·24	1·32†
PC model							
	1·47		1·53			1·24	1·32

* The amide bond connecting residues *i* and *i*−1 is *cis*.
† The amide nitrogen N(*i*+1) is replaced by an ester oxygen atom.

Table 5. *Bond angles* (°)

Residue No. (*i*)	C(*i*−1)–N(*i*)–C(*iA*)	C(*i*−1)–N(*i*)–C(*Ni*)	C(*Ni*)–N(*i*)–C(*iA*)	N(*i*)–C(*iA*)–C(*i*)	N(*i*)–C(*iA*)–C(*iB*)	C(*i*)–C(*iA*)–C(*iB*)	C(*iA*)–C(*iB*)–C(*iG*)	C(*iA*)–C(*i*)–O(*i*)	O(*i*)–C(*i*)–N(*i*+1)	C(*iA*)–C(*i*)–N(*i*+1)
Br-Ila										
1	120			104	109	111	112	127	121	112 (2)
2	120			114	104	114		114	120	126
3*	125	117	118	108	113	112	114	117	125	118
4	119			110	113	111	109	115	120	125
5	121			109	112	107	116	118	125	116
6	117			108	109	110	110	119	117	123
7*	122	119	118	108	108	111	111	121	127	112
CTS										
1	120·1	124·3	115·6	111·2				119·2	121·7	119·1 (3)
2*	123·9	119·8	116·3	112·1				122·6	122·8	114·6
CTD										
2*	125	113	121	106	117	105	113	128	122†	128 (2)†
4*	124	112	124	106	111	111		124	122†	124†
AMD										
4*	124	120	116	110				123	121	116 (1)
	127	118	115	110				121	121	116
5	121	120	118	113	114	109		129	121†	111†
	120	124	116	114	112	111		126	121†	113†
PC model										
	123			111				121	125	114

* The amide bond connecting residues *i* and *i*−1 is *cis*.
† The amide nitrogen N(*i*+1) is replaced by an ester oxygen atom.

a leucine residue appear at very high field, *i.e.* 0·43 and 0·27 p.p.m. below the tetramethylsilane (TMS) signal, whereas those of the other two leucine residues appear at normal regions, *i.e.* around 0·9 p.p.m. below the TMS signal. The reason for such a very high-field shift was explained by the ring current effect of the indole ring of tryptophan or the nitrophenol ring (Cary, Takita & Ohnishi, 1971). As seen in Fig. 1, the isopropyl group of leucine (7) lies just on the indole ring of residue 6 at a short distance and it seems to be reasonable to ascribe the shift to the ring current effect. The perpendicular distances between the atoms of the leucine side chain and the least-squares plane of the indole ring are: C(7*A*) 4·02, C(7*B*) 3·84, C(7*G*) 4·39, C(7*D*1) 3·70 and C(7*D*2) 4·00 Å.

Other short non-bonded interatomic distances to be noted are the following: C(1)···C(2) 3·09 Å (caused by the small angle φ_2), O(3)···C(3*B*) 2·75, N(3)···N(4) 2·76 Å (caused by the small angle of ψ_3), N(5)···O(5) 2·62 Å (caused by the large angle of ψ_5). Furthermore, substitution of a methyl group for imino hydrogen causes very short contacts between the methyl carbon atom and the carbonyl carbon as well as the β-carbon atom within the same residue; C(N3)···C(3*B*) 3·11, C(N3)···C(3) 3·01, C(N7)···C(7) 3·06, C(N7)···C(7*B*) 3·04 Å, which in turn limit the φ_3 and φ_7 angles to about −120°.

Crystal structure

The structure of the crystal is shown in Fig. 4 in a *c*-axis projection in which short intermolecular atomic contacts less than 3·5 Å are also shown. Two imino nitrogen atoms, N(2) and N(6), and carbonyl oxygen atoms O(2), O(3), O(4), O(6) and O(7) turning to the outside of the molecule participate in the strong intermolecular interactions. Most of them form hydrogen bonds which bind the ilamycin molecules either directly to each other or through the solvent molecules. In Table 6, presumed hydrogen bonds are listed. In addition to these hydrogen bonds, there are several rather short contacts of the bromine atom to the atoms of the nitrophenol group in residue 1, the shortest distances being 3·64 Å [Br^1···O(1*T*1)^{ii}] and 3·65 Å [Br^1···N(1*Z*2)^{ii}]. As seen in Fig. 4, the molecules are more closely bound along the dyad screw axis.

Table 6. *Presumed hydrogen bonds*

Symmetry code

i	x,	y, z (shown in Table 2)
ii	$2-x$,	$-\frac{1}{2}+y$, $2-z$
iii	$2-x$,	$\frac{1}{2}+y$, $2-z$

From		To		
Atoms	At	Atoms	At	Distance
N(1)—H	i	O(5)	i	2·80 Å
N(4)—H	i	O(1)	i	2·98
N(6)—H	i	O(2)	iii	3·06
N(2)—H	i	O(*W*)	i	2·87
O(*W*)–H	i	O(4)	ii	2·69
O(*W*)–H	i	O(ET)	i	2·87
O(ET)–H	i	O(7)	i	2·81

The authors wish to express their sincere gratitude to Professor H. Umezawa for his valuable advice.

References

CARY, L. W., TAKITA, T. & OHNISHI, M. (1971). *FEBS Lett.* **17**, 145–148.

GROTH, P. (1970). *Acta Chem. Scand.* **24**, 780–790.

HALL, S. R. & MASLEN, E. N. (1965). *Acta Cryst.* **18**, 265–279.

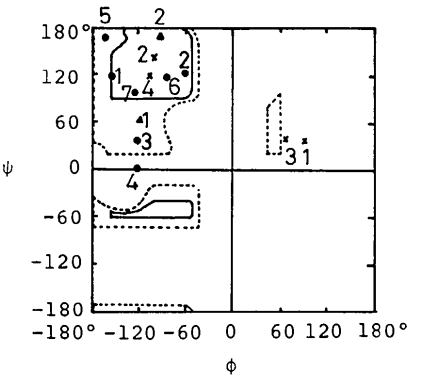


Fig. 3. The φ–ψ chart showing the backbone conformations. The solid line encloses the fully allowed regions and the broken line outer-limit regions. ● Br–Ila, ▲ CTS, × CTD. The numbers correspond to the residue numbers shown in Table 4. Abbreviations of the compound names are also shown in Table 4.

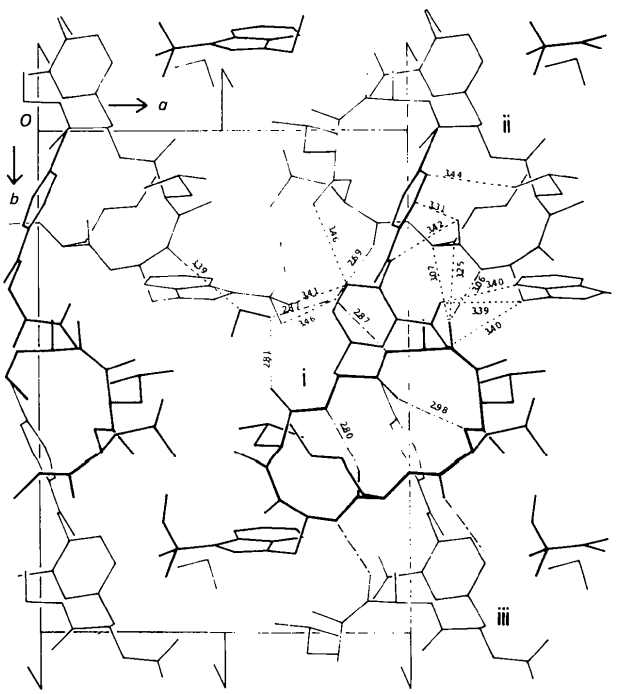


Fig. 4. The crystal structure projected along the *c* axis. Hydrogen bonds are shown by dot-dash lines and intermolecular short contacts by broken lines. Symmetry operations are shown in Table 6.

International Tables for X-ray Crystallography (1962). Vol. III. Birmingham: Kynoch Press.
JAIN, S. C. & SOBELL, H. M. (1972). *J. Mol. Biol.* **68**, 1–20.
JOHNSON, C. K. (1965). *ORTEP*. Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
KONNERT, J. & KARLE, I. L. (1969). *J. Amer. Chem. Soc.* **91**, 4888–4892.
OKAYA, Y. & ASHIDA, T. (1967). *HBLS* IV, *The Universal Crystallographic Computing System* (I), p. 65. Tokyo: The Crystallographic Society of Japan.
PAULING, L. & COREY, R. B. (1953). *Proc. Natl. Acad. Sci. U.S.* **39**, 253–256.
RAMACHANDRAN, G. N., RAMAKRISHNAN, C. & SASISEKHARAN, V. (1963). *J. Mol. Biol.* **7**, 95–99.
SOBELL, H. M. & JAIN, S. C. (1972). *J. Mol. Biol.* **68**, 21–34.
TAKITA, T. (1963). *J. Antibiot. Ser. A*, **16**, 211–212.
TAKITA, T., NAGASAWA, H., MAEDA, K. & UMEZAWA, H. (1964). *J. Antibiot. Ser. A*, **17**, 264–265.
TAKITA, T., OHI, F., OKAMI, Y., MAEDA, K. & UMEZAWA, H. (1962). *J. Antibiot. Ser. A*, **15**, 46–48.

Acta Cryst. (1974). B**30**, 2825

The Structures of the 3-Chloro- and 3-Iodopropyltrimethylammonium Cations in the Form of a Mixed Crystal of the Iodide Salt

BY D. J. H. MALLARD AND D. P. VAUGHAN
Department of Clinical Pharmacology, University of Birmingham Medical School, Birmingham B15 2TJ, England
AND T. A. HAMOR
Chemistry Department, The University, Birmingham B15 2TT, England

(*Received* 17 *July* 1974; *accepted* 19 *July* 1974)

The crystal analysed was orthorhombic, space group *Pnma*, with $a=10{\cdot}905$, $b=7{\cdot}31$, $c=13{\cdot}22$ Å, $Z=4$, and consists of *ca.* 48% of the chloro and 52% of the iodo salt. The cations adopt the extended conformation and possess crystallographic m (C_s) symmetry. The structure was determined by Patterson and Fourier methods from counter data and refined by least-squares and difference syntheses to R 7·1% for 1148 observed structure amplitudes.

Introduction

Muscarine and muscarone are highly specific agonists of acetylcholine at the parasympathetic post-ganglionic (muscarinic) receptor and they possess negligible agonistic activities at nicotinic receptors. Beckett (1967), by considering common structural features in numerous cholinergic agonists, delineated a muscarinic receptor surface. The groups involved in the binding are the onium nitrogen head, the ring oxygen of muscarine (and muscarone) or the ester oxygen atom of acetylcholine, and the 3-hydroxy substituent of muscarine, or the carbonyl oxygen atom of muscarone or acetylcholine. Chothia (1970) and Baker, Chothia, Pauling & Petcher (1971), however, emphasize the importance of the cationic head together with the 2-methyl substituent of muscarine or the acetoxy methyl group of acetylcholine. Bonding of these substituents with the receptor is formalized by the five-atom chain rule (Ing, 1949). According to this, maximal muscarinic activity in compounds of type $R-N^+Me_3$ occurs when R contains a chain of five atoms, exclusive of hydrogens. When the third atom is oxygen a concomitant increase in activity is observed.

Notable exceptions to the five-atom rule are 3-halopropyltrimethylammonium salts, which have potencies of *ca.* 2–10% of that of acetylcholine itself (Friedman, 1967), and it is noteworthy that these compounds do not contain an oxygen atom. We now report the crystal structures of 3-chloro- and 3-iodopropyltrimethylammonium iodide in the form of a mixed crystal consisting of *ca.* 48% of the chloro and 52% of the iodo salt.

Experimental

3-Chloropropyldimethylammonium chloride (Kodak Ltd) was treated with aqueous ammonia at 0°C and the free base in ether solution (dried over anhydrous Na_2SO_4) reacted with methyl iodide. The methiodide was obtained as a white powder and recrystallization from hot ethanol yielded crystals suitable for X-ray analysis. At this stage we were under the impression that the crystals consisted of pure 3-chloropropyltrimethylammonium iodide. This was supported by elemental analysis of the powder: found 48·9% I; calculated 48·15%. Later, in the course of the analysis it became apparent that partial halogen exchange had occurred in the 3-position so that the crystals contained approximately 48% 3-chloro- and 52% 3-iodopropyltrimethylammonium iodide. The density of the crystals was not determined. However, a flotation test using ethyl iodide, density 1·93 g cm^{-3}, indicated that