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CHEMICAL STUDIES ON TUBERACTINOMYCIN. II.
THE STRUCTURE OF TUBERACTINOMYCIN O

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A group of new peptide antibiotics called tuberactinomycin (TUM) effective against tubercular bacilli was found in the culture broth of Streptomyces griseoverticillatus var. tuberacticus.[1,2] TUM-A[3] and B were first isolated from the broth filtrate by column chromatography on Amberlite IRC-50, latter of which was identical with the known antibiotic viomycin.[4] A mutant prepared by treatment of the original microorganism with nitrosoguanidine produced two similar antibiotics termed TUM-N and O.[4]

Amino acid components of these four antibiotics are listed in Table 1.

Table 1. Amino Acid Components of Tuberactinomycins

TUM	Molecular Formula	Ser	Dpr	3-Ureido -DeAla	Tbd	Cpd	γ-Hy-β-Lys	β-Lys
A	$C_{25}H_{43}N_{13}O_{11}$	2	1	1	1	—	1	—
B	$C_{25}H_{43}N_{13}O_{10}$	2	1	1	1	—	—	1
N	$C_{25}H_{43}N_{13}O_{10}$	2	1	1	—	1	1	—
O	$C_{25}H_{43}N_{13}O_{9}$	2	1	1	—	1	—	1

Ser:L-serine, Dpr:L-α,β-diaminopropionic acid, Tbd:L-tuberactidine[5], Cpd:L-capreomycidine[6], γ-Hy-β-Lys:erythro-γ-hydroxy-L-β-lysine, β-Lys:L-β-lysine, 3-UreidoDeAla:3-ureidodehydroalanine[8]

Among these peptides, only TUM-O was crystallized either as hydrochloride or as hydrobromide. Crystals suitable to X-ray analysis were obtained as $C_{25}H_{43}N_{13}O_9 \cdot 2HBr \cdot HCl \cdot 3H_2O$ from water-dimethyl formamide. They belong to the monoclinic space group C2, with four formula units in a cell of dimensions: a=25.29, b=10.50, c=20.27 Å, β=130.30°. The independent reflections with 2θ less than 50° were measured by the ω-2θ scanning technique using Mo Kα radiation. Thus a total of 3852 reflections were obtained, of which 2758 had intensities greater than three times their standard deviations.[9]

The structure was solved by the heavy atom method, and refined by the successive Fourier syntheses and the least-squares method. In the course of the analysis the species of halogens and the amount of water of crystallization were established. When isotropic thermal vibrations were assumed to all of the non-hydrogen atoms, the conventional R factor was 0.14.

The electron density distribution calculated at this stage of the refinement is given in Fig.1. The distinction between O(31) and N(32), and the locations of hydrogen atoms are not definite yet. However, the molecular skeleton is clearly revealed and it is quite consistent with the one so far obtained with various chemical studies. Fig.1 represents also the absolute configuration of the molecule which is assigned by reference to the configurations of L-amino acids involved.

In the molecule of TUM-O a sixteen membered peptide ring is present, in which an intramolecular hydrogen bond is formed between N(5) and O(36), the distance being 2.95 Å. The conformation of the hydrogen bonded chelate ring, which contains the atoms from N(5) to O(36) via C(7), is very similar to those found in several oligopeptides.[10]

When TUM-A and N were partially hydrolyzed with conc. HCl, γ-hydroxy-β-lysine was splitted out easily in both cases, giving a ring peptide in which α-amino group of α,β-diaminopropionic acid was made free newly. Furthermore each one mole of serine was obtained from partial hydrolysate after bromine oxidation of TUM-A, B and N. This common fact could be elucidated by similar situation that the serine was liberated from the carboxyl terminal formed by oxidative cleavage followed by acid treatment of seryl-3-ureidodehydroalanine unit present

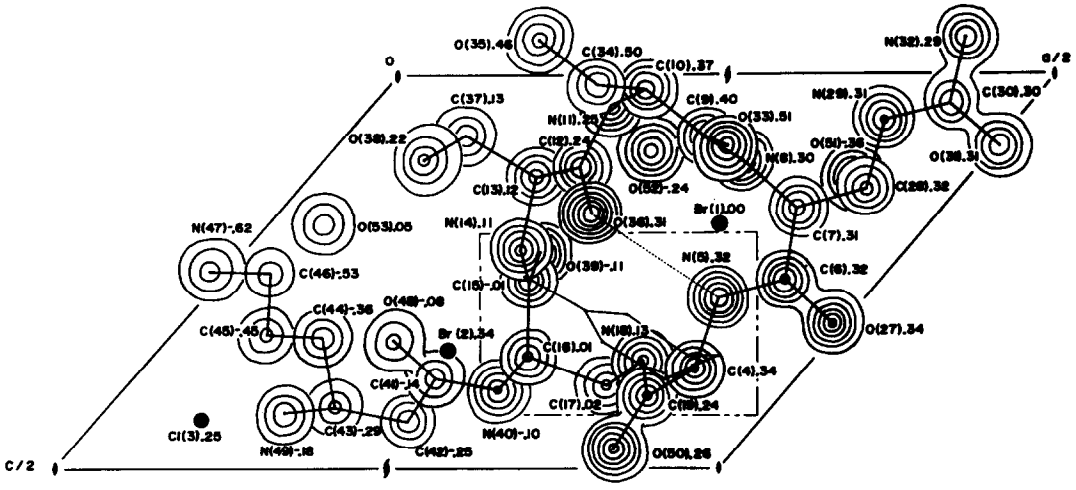
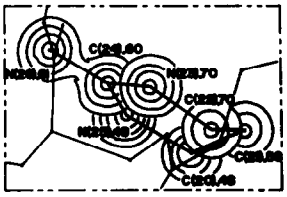
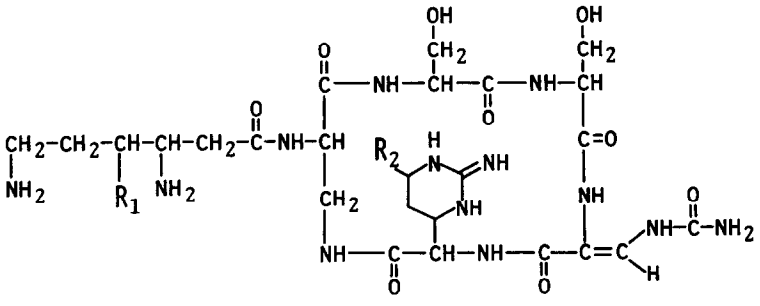


Fig.1 Composite electron density distribution viewed along the b-axis. Contours are drawn at intervals of 2 e.Å^{-3} beginning at 2 e.Å^{-3} ; those for the bromide and chloride ions are not drawn. A part of the map is shown separately. The fractional y-coordinate is given after the name of the atom.





TUM	A	B	N	O
R_1	OH	H	OH	H
R_2	OH	OH	H	H

Fig.2 Chemical Structure of Tuberactinomycins

in the ring part through N,O-rearrangement.

From the results described above together with other chemical and physical properties, it is suspected that all of the four compounds may have the same peptide sequence, differing only in possessing or not of hydroxyl group at R_1 and R_2 as shown in Fig.2. Although many presumed structures for viomycin have been presented,[8,11] the correct structure could be now represented as one corresponding to TUM-B mentioned in Fig.2 where R_1=H and R_2=OH.

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