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THE MOLECULAR STRUCTURE OF CYCLOCHLOROTINE, A TOXIC CHLORINE-CONTAINING CYCLIC PENTAPEPTIDE

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The molecular structure of the title oligopeptide has been determined by X-ray diffraction methods. The dihydrate crystals contain two independent molecules in the unit cell, and they have a quite similar conformation. The configuration of the two chlorine atoms around the C^β-C^γ bond of the pyrrolidine ring is nearly *gauche*. Each molecule contains a β-loop of α-*U*-folding type.

Cyclochlorotine(CCT)[1] is one of the toxic metabolites of *Penicillium islandicum* Sopp, the mold of islandia yellow rice, and has been isolated by one of the authors(T. T.) et al.[2] This substance is a chlorine containing cyclic pentapeptide, and the amino acid sequence has been determined to be as follows:[3]

$$\begin{tikzcd}
\text{L-Ser} \arrow[rr] & & \text{L-DichloroPro} \arrow[d] \\
\text{L-}\beta\text{-Phe} \arrow[u, "(1)"'] & \underset{(2)}{\text{L-Ser}} \arrow[l] & \text{L-Abu} \arrow[l]
\end{tikzcd}$$

(L-Ser: L-Serine, L-DichloroPro: L-Dichloroproline, L-Abu: L-α-Amino-n-butylic Acid, L-β-Phe: L-β-Amino-β-phenylpropionic Acid)

However, the location and the configuration of the two chlorine atoms bonded to the pyrrolidine rings have not been established yet with certainty. Since the L-DichloroPro residue seemed to play an important role in exhibiting the mortal toxicity of CCT, there was much need to be studied in detail. On the other hand, it would be of much interest to examine the conformational structure of the annular skeleton, especially that of a hydrogen-bonded chelate ring, called "β-loop"[4] or "β-turn structure", which is expected to be present in the skeleton.

Single crystals, grown from a water-methanol solution, are colorless tabular, and contain two moles of water of crystallization. The crystal data are:

Cyclochlorotine dihydrate, $C_{24}H_{31}N_5O_7Cl_2 \cdot 2H_2O$, crystal system; triclinic, space group; $\underline{P}1$, cell dimensions; $\underline{a}$ = 13.704 ± 0.003, $\underline{b}$ = 13.807 ± 0.002, $\underline{c}$ = 9.907 ± 0.001 Å, $\underline{\alpha}$ = 99.45 ± 0.01, $\underline{\beta}$ = 107.30 ± 0.01, $\underline{\gamma}$ = 106.97 ± 0.01°, $\underline{V}$ = 1645.9 Å^3, $\underline{D}_{\underline{x}}$ = 1.228 g cm^{-3}, $\underline{Z}$ = 2.

Intensity data were collected on a four circle automatic diffractometer. The independent reflections within the range of 2θ less than 50° were measured by the ω-2θ scan technique using Mo $\underline{K}\alpha$ radiation. A total of 6071 reflections was measured, of which 3666 had intensities greater than three times their standard deviations. These reflections were submitted to the analysis.

The structure was solved by the reiteration of Fourier syntheses combined with least-squares calculation, which was initiated with the chlorine atoms deduced from a Patterson map. Several cycles were needed to yield all the non-hydrogen atoms. Least-squares refinement with anisotropic thermal parameters for all but the water oxygen atoms, to which isotropic parameters are assumed, reduced the conventional R value to 9.0 %. A difference map revealed many of the hydrogen atoms, but they are not included in the refinement. The standard deviations of the positional parameters were about 0.007 and 0.02 Å for the chlorine and most of the other atoms, respectively.

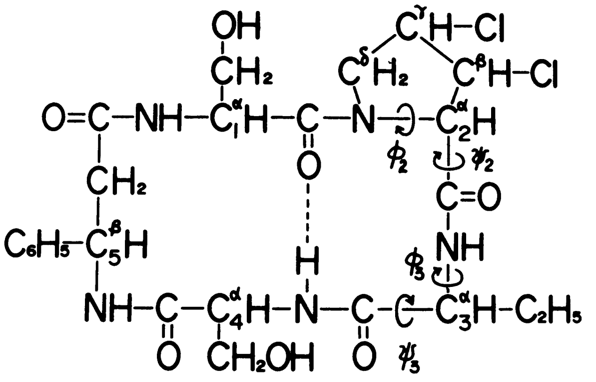

Fig. 1. The chemical structure of CCT, with naming of some atoms and internal rotation angles.

The stereochemistry thus established confirms the proposed chemical structure, given in Fig. 1. Fig. 2 shows the structures of both molecules(Molecule I and II), which are quite similar to each other and differ in only a few detail. One of the chlorine atoms is attached to C^{β} and the other to C^{γ}. The configuration of the chlorine atoms around the C^{β}-C^{γ} bond, as shown in Fig. 3, is gauche type, the torsion angles θ(Cl-C^{β}-C^{γ}-Cl) being 46° and 50° in I and II, respectively. In each pyrrolidine ring, N, C^{α}, C^{β} and C^{δ} are nearly coplanar, and C^{γ} deviates from the plane by about 0.60 Å, which is greater than those so far reported. Thus it seems that the conformation of the pyrrolidine ring is much influenced by the chlorine atoms.

A predominant characteristic of the peptide ring is the presence of the β-loop, or the β-turn structure, the constituents of which are L-DichloroPro, L-Abu and the transannular hydrogen bond, N-H[Ser(2)]...O=C[Ser(1)]. Similar β-loops have been found in several cyclic and linear oligopeptides, and their conformations in the crystalline state have been collectively examined by Ueki et al.[5] According to the internal rotation angles[6] of (ϕ_2, ψ_2) and (ϕ_3 , ψ_3)(see Fig. 1) Ueki et al. classified the β-turn conformations into the two groups, and called them "α- and β-U-folding". In the present molecules the average values of these angles, (118°, 158°) and (79°, 184°), are fairy close to those of α-U-folding conformation. The distances of the transannular hydrogen bonds are 2.85 and 2.83 Å in Molecule I and II, respectively. These distances are somewhat shorter than any of those so far observed in the β-loops. In Table I are listed the internal rotation angles and the distances relevant to the conformation.

It seems to be interesting to compare the β-loop conformation with that found in tuberactinomycin-O(TUM-O), the structure of which has been determined by two of the authors(H. Y. and K. N.) et al.[7] This oligopeptide also contains a cyclic skeleton composed of one β- and four α-amino acid residues. The internal rotation angles in the β-loop are similar to those of the α-U-folding but the sense of

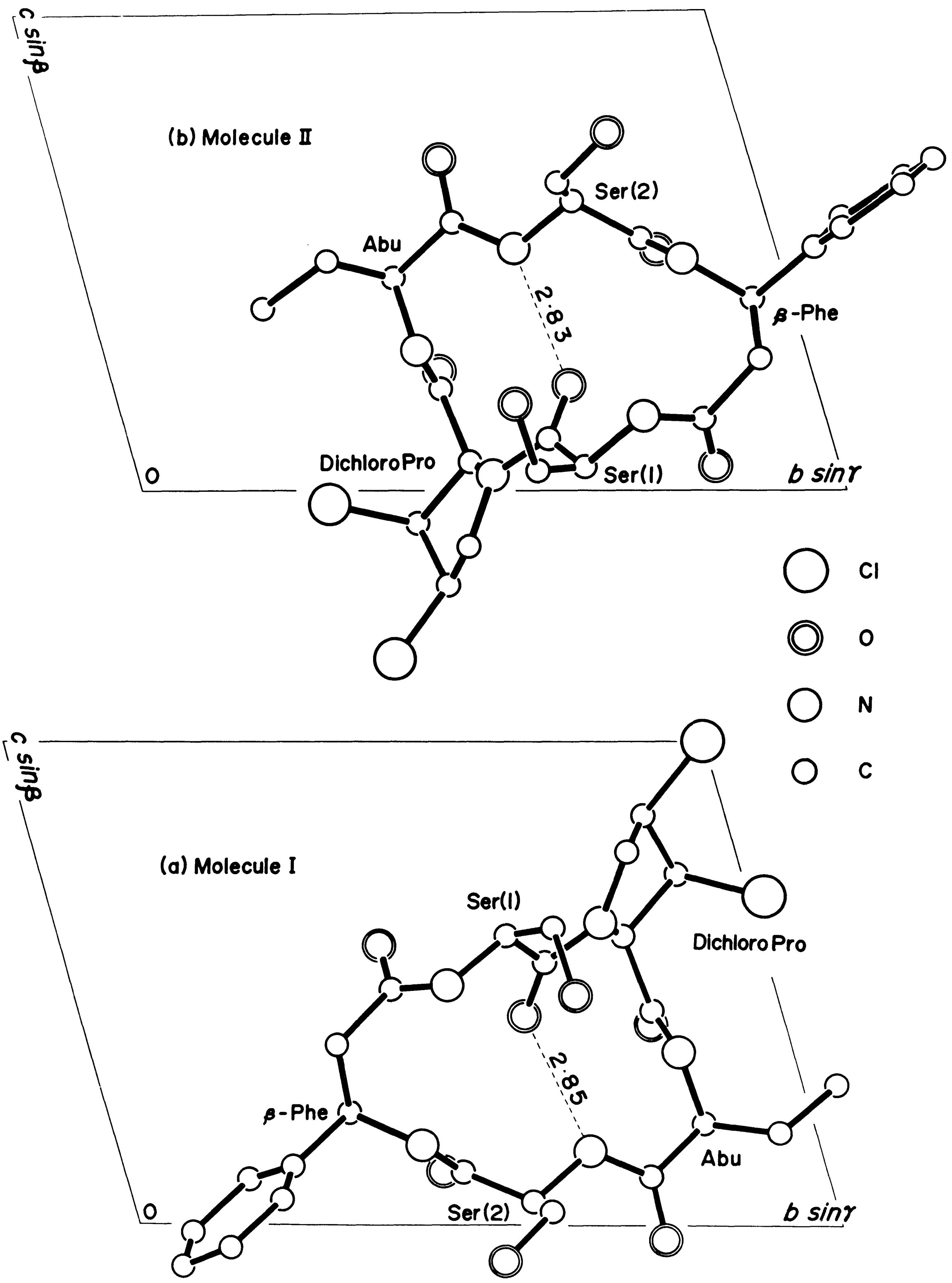

Fig. 2. The crystal structure of CCT viewed along the a axis. The whole crystal structure is obtained by stacking the figure (a) on top of (b). The water molecules are not shown.

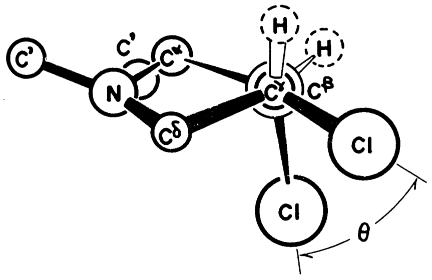

Fig. 3. Configuration of L-DichloroPro as viewed down the C -C bond. The positions of the hydrogen atoms are assumed. θ = 46 and 50°, and, Cl...Cl = 3.16 and 3.24 Å, in Molecule I and II, respectively.

Table I. Conformation of the β-loop.

	ϕ_2	ψ_2	ϕ_3	ψ_3	N-H...O	$C_1^{\alpha}...C_4^{\alpha}$
CCT I	120°	157°	75°	185°	2.85 Å	5.20 Å
CCT II	116	159	83	182	2.83	5.25
Average	118	158	79	184	2.84	5.23
α-U-folding[a)]	113	152	82	188	3.02	(5.04)[b)]
TUM-O	-121	-145	-93	-186	2.95	5.39

a) Averaged over the values found in the β-loops having α-U-folding[5)].

b) Average value of *p*-bromocarbobenzoxy-Gly-L-Pro-L-Leu-Gly(OH) and *o*-bromobenzoxy-Gly-L-Pro-L-Leu-Gly-L-Pro(OH)[5)].

rotation is opposite(see Table I) in spite of that all the constituents have the L-configuration.

A remarkable feature of the crystal structure is that Molecule I and II are related by a local, approximate 2_1 axis parallel to the *a* axis. When Fig. 3(a) is stacked on top of (b) in order for the unit cell edges to coincide, the 2_1 axis is seen clearly through the point (*y* = 0.647, *z* = 0.326).

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