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The Crystal Structure of a Pepsin Substrate: *N*-Acetyl-L-phenylalanyl-L-tyrosine

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An X-ray crystallographic study of *N*-acetyl-L-phenylalanyl-L-tyrosine is reported. This pepsin substrate crystallizes in space group $P2_1$ with $a=11{\cdot}530$ (5), $b=8{\cdot}589$ (3), $c=10{\cdot}635$ (3) Å, $\beta=114{\cdot}52$ (2)°, $Z=2$. The structure was determined by direct methods, and most of the 22 hydrogen and 27 non-hydrogen atoms were refined by ΔF syntheses and least-squares calculations based on 2967 three-dimensional data. The final R is 0·047. The molecule is in an extended form in the crystal with the phenyl and tyrosyl rings separated by 10 Å. The peptide group is not planar; there is a dihedral angle of 17·7° about the peptide bond.

Introduction

N-Acetyl-L-phenylalanyl-L-tyrosine (APTPL) (Fig. 1) was first synthesized in the early 1950's as a model substrate for pepsin (Baker, 1951). Since that time, the kinetics of hydrolysis of the compound and of its diiodotyrosine, dibromotyrosine, ethyl, and methyl ester derivatives have been extensively studied (Zeffren & Kaiser, 1966; Clement, Snyder, Price & Cartmell, 1968; Jackson, Schlamowitz & Shaw, 1965). It has been found that the rate of hydrolysis of the peptide bond is most rapid for the diiodo compound and slowest for the nonhalogenated molecule. A competitive inhibition model has been advanced as an explanation of this behavior, but it would be useful to know the structures of the three molecules to determine any noticeable structural differences which could account for the different reaction rates. Attempts to crystallize the diiodo compound have not produced suitable crystals for X-ray work, but crystals of the parent compound were easily obtained. Attempts to obtain suitable crystals of the halogenated derivatives are continuing.

Experimental

APTPL was obtained from Mann Research Laboratories and crystallized by dissolving in hot 95% ethanol, adding hot water, and cooling slowly. This method gives fairly large, clear crystals. All the X-ray work was done with one crystal, approximately 0·1 × 0·1 × 0·3 mm in size. After the initial photographic work to determine the space group and approximate unit-cell parameters, intensities were collected with a four-circle diffractometer equipped with magnetic tape output. Table 1 contains the unit-cell parameters, space group, and information concerning data collection. The space group, $P2_1$, was assumed from the systematic absences ($0k0$ for k odd) and the fact that the molecule is presumably present as only one enantiomer.

A plot of the intensities of the monitor reflections (collected after every 200 reflections) as a function of reflection count showed the crystal had suffered no significant deterioration during the course of data collection. Several of the larger reflections were remeasured at reduced current, and standard coincidence corrections were applied. No absorption corrections were deemed necessary since Mo radiation was used, $\mu=1{\cdot}00\ cm^{-1}$, and the maximum dimension of the crystal was 0·3 mm.

During the course of data collection the shutter was found to be malfunctioning and a number of reflections which were suspect were remeasured. Inadvertently, three reflections ($\bar{9}$,3,14; $\bar{9}$,8,7 and $\bar{3}$,6,11) were not recollected. They appear in the structure factor table,

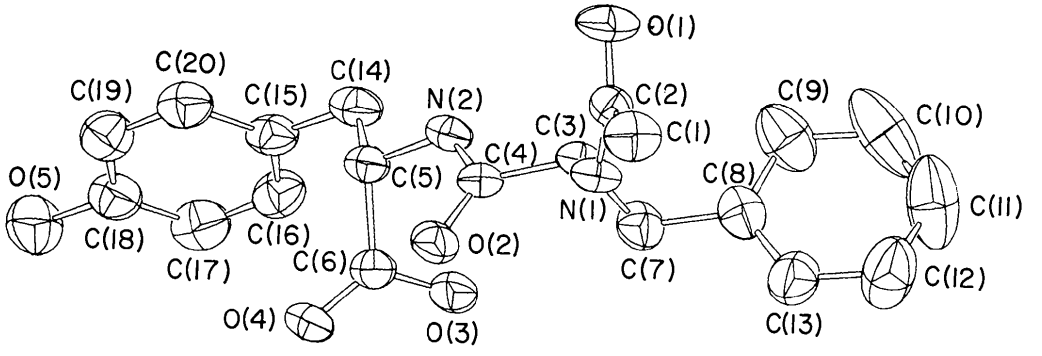


Fig. 1. Molecule with thermal ellipsoids. View along **b**.

the first two with zero weight, but the third was processed as a normal reflection.

Most of the computer programs used in the structure determination and refinement are parts of the X-RAY system of J. M. Stewart. Figs. 1 and 2 were made with the new version of the *ORTEP* program of C. Johnson which eliminates overlapping lines.

Determination of the structure

Several methods were tried to solve the structure. Initial attempts by Patterson methods failed to lead to a satisfactory phasing model. It was possible to find the patterns of 19 vectors characteristic of six-member aromatic rings in the Harker section of the Patterson map. The structure failed to appear in ΔF and F_o syntheses based on phases calculated for the rings corresponding to these patterns, and tangent-formula refinement of the calculated phases also failed to yield the structure. Use of the direct methods programs of the X-RAY system in attempts to expand several sets of origin and enantiomorph defining phases did not produce the structure but did make apparent the fact that the data set of 238 E's above 1·6 consisted of

Table 1. *Crystal data*

Space group $P2_1$	Mo $K\alpha$ radiation	$C_{20}H_{22}N_2O_5$
a = 11·530 (5) Å	Unmonochromatized	M.W. 370·4
b = 8·589 (3)	2967 reflections	$F(000)=392$
c = 10·635 (3)	$242<2\sigma(F)$	Linear absorption coefficients = 1·00 cm^{-1}.
$\beta=114{\cdot}52\ (2)^\circ$	Maximum $\sin\theta/\lambda=0{\cdot}715$	Data collected by ω–2θ scan
$V=958{\cdot}28$ Å^3	Crystal size 0·1 × 0·1 × 0·3 mm	Scan rate 2° min^{-1}
$Z=2$	Mounted along **b**	Scan width 1·3°
		20 s backgrounds on each side of reflection

Table 2. *Results from MULTAN*

h	k	l	E	Code*	α	Possible phases	Starting phases that gave the solution
6	0	4	2·35	0		0	0
9	3	0	2·60	1		0	0
4	6	5	2·55	1		45,135	45
2	1	−2	2·37	2		45	45
2	2	−2	2·33	3	0·0	±45,±135	135
8	0	−14	3·78	3	0·0	0,180	0
9	1	−1	2·24	3	0·0	±45,±135	45
3	0	2	2·63	3	1·8	0,180	0

* 0 = from $\sum_1$ relations, 1 = origin definition, 2 = origin and enantiomorph definition, 3 = α's of zero or low value.

Table 3. *Course of refinement*

Procedure	R*	R_w*	Goodness of fit*	Average shift/error	Comments
E map	0·333†				Found 23 atoms
Four cycles of ΔF refinement	0·227‡	0·225‡			Found additional 4 atoms
One cycle of *CRYLSQ* least-squares refinement	0·166‡	0·176‡	8·24‡	2·69	Isotropic thermal parameters on carbon, nitrogen and oxygen atoms. Refinement with random third of data.
One cycle of *CRYLSQ*	0·134§	0·132§	7·90§	2·62	Same as directly above but with full data set
One cycle of *CRYLSQ*	0·089♀	0·083♀	5·21♀	1·85	Anisotropic thermal parameters on nonhydrogen atoms. Block diagonal refinement with random half of data.
Four cycles of *CRYLSQ*	0·059§	0·052§	2·48§	2·14	Added 22 hydrogens. Anisotropic thermal parameters on nonhydrogen atoms. Isotropic hydrogen atoms. Block-diagonal refinement with full data set. Beginning $R=0{\cdot}083$.
Two cycles of *CRYLSQ*	0·047§	0·037§	2·10§	0·717	Anisotropic nonhydrogens. Isotropic hydrogens. Fixed 5 hydrogens. Block-diagonal refinement with full data set.

* $R=\sum||F_o|-|F_c||/\sum|F_o|$, $R_w=\sqrt{[\sum w(||F_o|-|F_c||)^2/\sum w(|F_o|)^2]}$, goodness of fit $=\sqrt{[\sum w(||F_o|-|F_c||)^2/(\text{number reflections}-\text{number variables})]}$.

† R on full data set including reflections less than $2\sigma(F)$.

‡ R on random third of data set omitting reflections less than $2\sigma(F)$.

§ R on full data set omitting reflections less than $2\sigma(F)$.

♀ R on random half of data set omitting reflections less than $2\sigma(F)$.

Table 4. *Structure factors*

Each set of four columns contains respectively, h, $10\times F_o$, $10\times F_c$, $10\times \sigma(F_o)$.

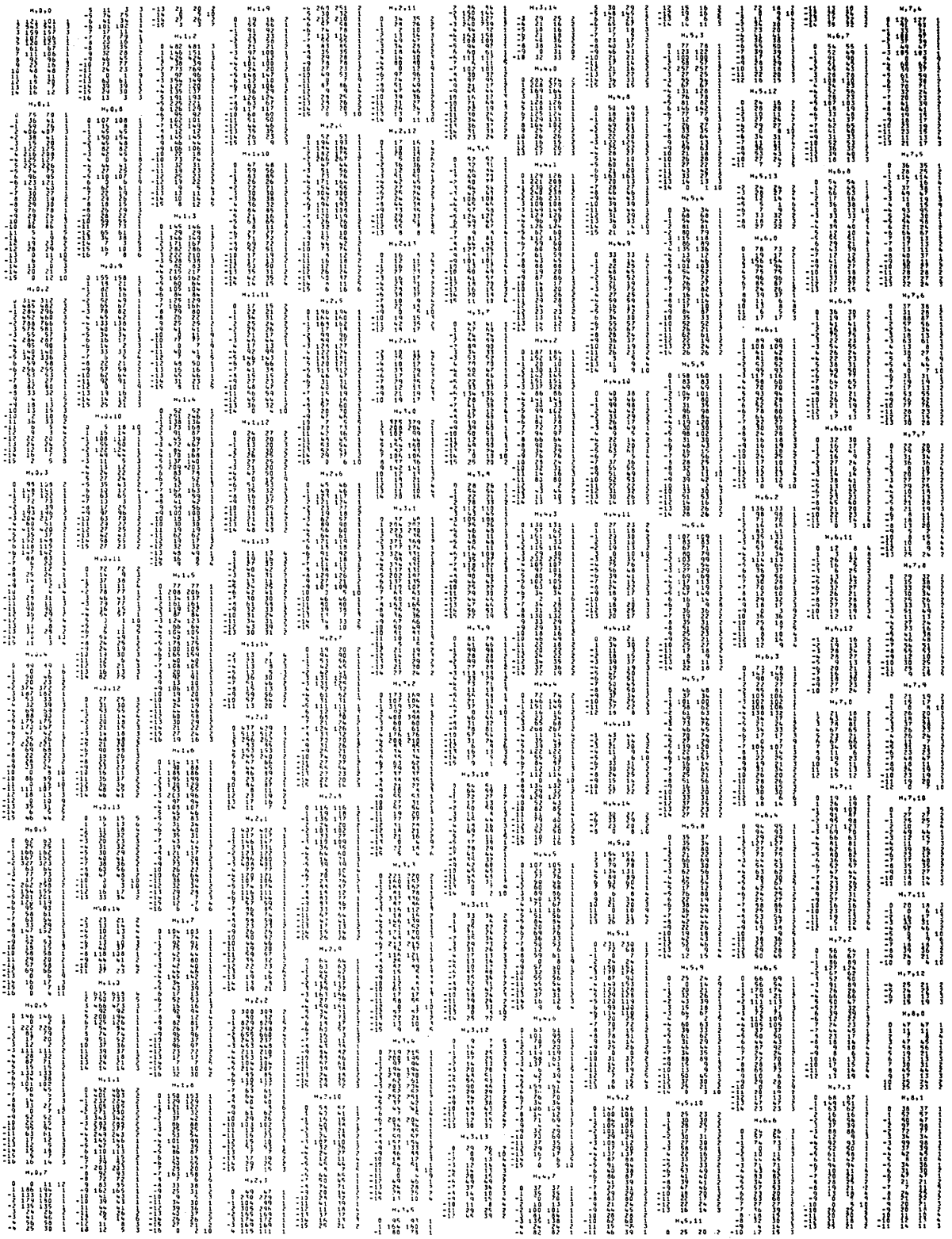
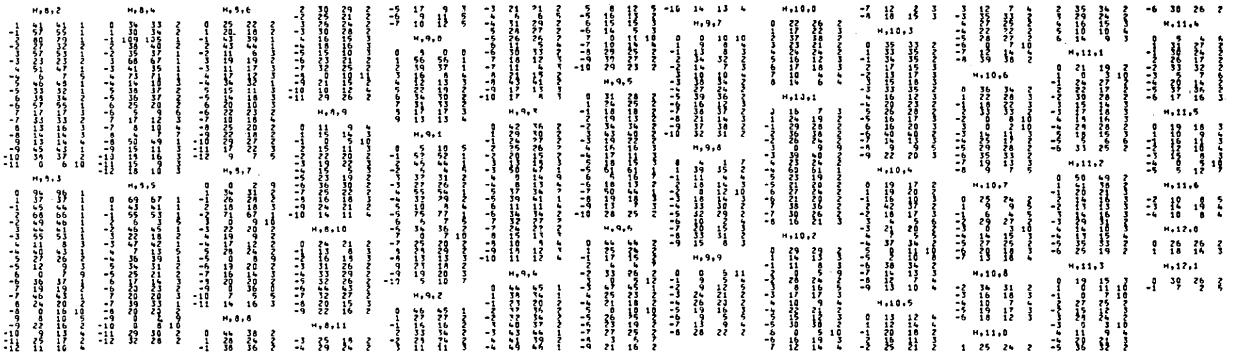


Table 4 (*cont.*)



domains of reflections weakly connected through reflections with low E values.

The structure was solved by use of the direct-methods program, *MULTAN* (Main, Woolfson & Germain, 1971). This program calculates a number for each reflection, α, which is related to how strongly the phase of that reflection appears to be determined. The reflection with the lowest α is eliminated from the data set, and α is recalculated for each reflection based on the remaining reflections. This process is repeated until the program converges on an origin and enantiomorph defining set. In the convergence listing some reflections may have α's of zero, and these reflections can not be determined by the phases of the remaining reflections. Therefore, if the phase determination is to proceed from the origin definition, these reflections must be assigned phases.

Table 2 gives the origin and enantiomorph reflections chosen by *MULTAN*, the reflections which had low α's, their E values, and the phases which were assigned to these reflections and permuted to ensure that the starting phases were within 45° of their true values. The presence of three reflections with α's of zero again suggests that this set of data has separated domains of reflections which are not strongly connected.

Initially, 65 of the possible 128 starting sets were ex-

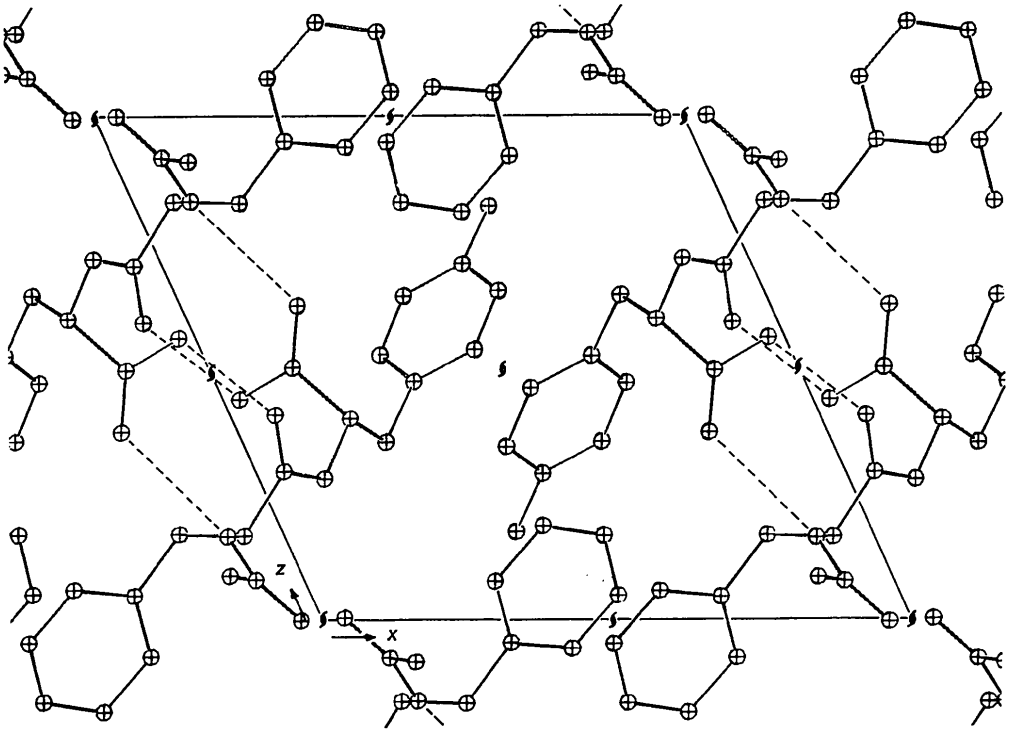


Fig. 2. Unit-cell contents. View along **b**.

panded and refined by the tangent formula. Of these, 61 sets gave a weighted Karle R

$$R_K = \frac{\sum |w|E_h| - K\langle E_{h'} E_{h-h'}\rangle_{h'}|}{\sum |w|E_h||}$$

where K and w are the scale factor and weight defined in the *MULTAN* instructions (Main *et al.*, 1971) greater than 0·27, while four of them gave residuals of 0·2208 (set 1), 0·2200 (set 2), 0·2237 (set 3) and 0·2206 (set 4). While all four sets of phases may contain the solution to the structure, the phases in sets 3 and 4 were not obviously related to those of sets 1 or 2. A comparison of sets 1 and 2 showed that while the starting sets varied in the phase assigned to the $2,2,\bar{2}$ reflection [in set 1, $\varphi(2,2,\bar{2})=45°$, in set 2, $\varphi(2,2,\bar{2})=135°$], the tangent refinement gave nearly the same set of phases. The average difference in the 266 noncentrosymmetric phases in the two sets is 22°. In set 1, $\varphi(2,2,\bar{2})$ refined to 85°, and in set 2, it refined to 92°.

Twenty-three of the 27 nonhydrogen atoms were found in the E map calculated from the phases from set 2 with 312 E's above 1·5. Four atoms in the phenylalanine ring did not appear as prominent peaks in the map, but if it had been contoured at a lower level, these atoms and some noise peaks would have appeared. A structure-factor calculation based on 23 atoms gave a conventional $R = \sum||F_o|-|F_c||/\sum|F_o|$ of 0·33. The other four atoms were found in a ΔF map. A second structure-factor calculation based on all 27 nonhydrogen atoms gave an R of 0·258.

Refinement of the structure

Table 3 shows the results of the various steps in the refinement of the structure. The refinement to an R of 0·089 by use of ΔF syntheses and least-squares methods was routine. All hydrogen atoms were found in a ΔF map except H(C12). It was placed in a reasonable position, and the refinement was continued.

Two more cycles of least squares decreased R to 0·056, but the hydrogen atoms of the acetyl group had high thermal parameters. A disorder model was postulated, but it failed to fit the electron density satisfactorily. Further investigation showed that two hydrogens H3(C1) and H(C12) had refined to positions about 2 Å from the carbon atoms to which they are bonded. They were repositioned closer to the carbons and two more cycles of least squares were computed. Again, they began to diverge, and several other hydrogen atoms had unreasonable thermal parameters. It was

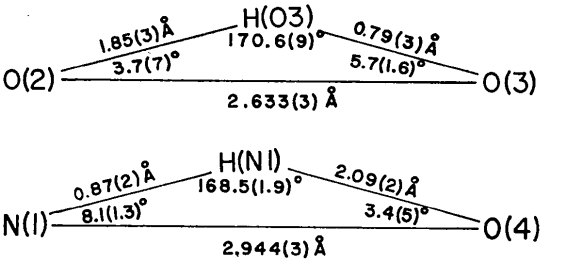


Fig. 3. The hydrogen bonds (not to scale).

Table 5. *Positional and thermal parameters*

(*a*) Atomic parameters for carbon, nitrogen and oxygen atoms. Positional parameters are $\times 10^5$. Thermal parameters are defined by the expression: $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)\times 10^{-4}]$.

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	12552 (22)	−14127 (28)	91310 (23)	709 (14)	419 (12)	638 (13)	77 (11)	347 (12)	76 (11)
C(2)	8358 (16)	2324 (25)	92104 (18)	430 (10)	424 (11)	374 (9)	15 (9)	174 (8)	57 (9)
O(1)	3818 (14)	6104 (20)	100290 (14)	861 (11)	548 (9)	562 (8)	116 (9)	516 (8)	153 (8)
N(1)	9808 (16)	12507 (21)	83376 (15)	573 (10)	381 (9)	363 (8)	45 (8)	297 (8)	31 (7)
C(3)	6940 (18)	28861 (25)	83270 (18)	513 (11)	374 (10)	344 (9)	43 (9)	249 (8)	25 (8)
C(4)	−4872 (17)	33389 (24)	70389 (18)	441 (10)	418 (11)	365 (9)	10 (9)	243 (8)	−19 (8)
O(2)	−7731 (12)	27010 (19)	59109 (13)	529 (8)	556 (9)	382 (7)	−78 (7)	207 (6)	83 (7)
N(2)	−11227 (15)	45770 (21)	71822 (15)	505 (10)	414 (10)	317 (8)	−65 (8)	227 (7)	−16 (7)
C(5)	−20243 (19)	54040 (25)	59823 (18)	453 (11)	423 (11)	355 (9)	−35 (9)	219 (9)	−31 (9)
C(6)	−14309 (17)	58050 (24)	49750 (18)	445 (10)	432 (11)	397 (10)	−50 (9)	220 (8)	−35 (9)
O(3)	−2829 (14)	64141 (22)	56046 (14)	512 (9)	780 (11)	390 (7)	128 (8)	222 (7)	−78 (8)
O(4)	−19914 (12)	56475 (21)	37440 (12)	577 (8)	711 (10)	317 (7)	56 (8)	224 (6)	2 (7)
C(7)	17923 (21)	38899 (29)	82937 (22)	520 (12)	477 (13)	454 (11)	−34 (10)	169 (10)	109 (10)
C(8)	30431 (20)	37035 (26)	95269 (20)	561 (13)	380 (11)	485 (11)	−82 (10)	146 (10)	72 (10)
C(9)	32433 (32)	43942 (35)	107706 (26)	1042 (22)	610 (17)	593 (16)	104 (17)	146 (16)	−60 (14)
C(10)	44896 (51)	42923 (43)	118840 (34)	1770 (43)	578 (21)	560 (19)	−128 (24)	−21 (23)	−73 (16)
C(11)	54449 (34)	35241 (46)	116896 (44)	859 (22)	648 (21)	1105 (28)	215 (18)	−164 (20)	−221 (21)
C(12)	52244 (26)	27950 (43)	104912 (35)	597 (17)	951 (24)	1074 (24)	91 (18)	223 (17)	−315 (22)
C(13)	40311 (22)	28863 (35)	94108 (27)	534 (13)	764 (18)	673 (15)	12 (14)	260 (12)	218 (15)
C(14)	−24507 (23)	69190 (30)	64526 (22)	634 (14)	542 (14)	453 (11)	−127 (12)	327 (11)	−40 (11)
C(15)	−33916 (19)	78298 (27)	52565 (20)	577 (13)	486 (12)	492 (11)	−153 (11)	318 (10)	−37 (10)
C(16)	−30135 (23)	91131 (29)	47208 (24)	541 (13)	514 (14)	649 (14)	−89 (12)	308 (11)	−53 (11)
C(17)	−38721 (26)	98851 (32)	35605 (27)	713 (17)	522 (14)	714 (16)	−127 (13)	390 (14)	−162 (12)
C(18)	−51164 (22)	94005 (30)	29256 (22)	626 (14)	561 (14)	534 (12)	−204 (12)	290 (11)	−70 (12)
O(5)	−60151 (17)	101015 (24)	17643 (18)	741 (11)	731 (13)	673 (11)	223 (10)	246 (9)	189 (10)
C(19)	−55214 (24)	81588 (29)	34578 (24)	604 (14)	559 (15)	606 (14)	109 (12)	281 (12)	−0 (12)
C(20)	−46641 (22)	73844 (30)	46198 (24)	645 (15)	526 (15)	599 (13)	−74 (12)	348 (12)	−73 (11)

Table 5 (*cont.*)

(*b*) Atomic parameters for hydrogen atoms. Positional parameters are $\times 10^4$. The thermal parameter is defined by the expression $\exp[-8\pi^2U^2(\sin^2\theta/\lambda^2)]$.

	x	*y*	*z*	$U^2(\times 10^3)$
H1(C1)*	1315	−1560	8255	121
H2(C1)*	2181	−1609	9980	121
H3(C1)*	559	−2216	9210	121
H(N1)	1261 (17)	935 (23)	7741 (18)	45 (5)
H(C3)	551 (15)	3140 (22)	9162 (18)	31 (5)
H(N2)	−899 (19)	5021 (28)	8011 (21)	61 (6)
H(C5)	−2807 (19)	4728 (28)	5497 (20)	54 (6)
H(O3)	−35 (24)	6816 (31)	5093 (27)	57 (7)
H1(C7)	1949 (19)	3617 (27)	7462 (22)	64 (7)
H2(C7)	1524 (19)	4969 (30)	8115 (20)	56 (6)
H(C9)	2562 (19)	4837 (28)	10924 (21)	59 (7)
H(C10)	4467 (31)	4709 (44)	12585 (32)	104 (14)
H(C11)*	6399	3539	12514	169
H(C12)*	5983	2132	10369	154
H(C13)	3774 (25)	2495 (39)	8436 (30)	113 (10)
H1(C14)	−1662 (20)	7556 (28)	7007 (21)	64 (7)
H2(C14)	−2813 (20)	6586 (28)	7033 (22)	55 (7)
H(C16)	−2131 (23)	9346 (34)	5228 (23)	59 (8)
H(C17)	−3609 (22)	10744 (34)	3262 (24)	80 (8)
H(O5)	−5610 (34)	10961 (48)	1543 (37)	139 (14)
H(C19)	−6420 (22)	7800 (32)	2968 (22)	68 (7)
H(C20)	−4980 (29)	6586 (37)	5010 (32)	83 (9)

* Denotes atoms with calculated parameters.

decided, therefore, to calculate the positions of H1(C1), H2(C1), H3(C1), H(C11) and H(C12), assign them *B*'s five units greater than the *B*'s of the atoms to which they are bonded, and to fix their parameters in the remaining refinement. After two more refinement cycles *R* converged to 0·047 with a final average shift/error of 0·72 and a goodness of fit of 2·10. Table 4 is a listing of the observed and calculated structure factors, and Table 5 lists the final atomic parameters.

Table 6. *Bond lengths and bond angles*

C(1)—C(2)	1·507 (3) Å	C(1)—C(2)—O(1)	121·83 (21)°
C(2)—O(1)	1·231 (3)	C(1)—C(2)—N(1)	116·07 (20)
C(2)—N(1)	1·335 (3)	O(1)—C(2)—N(1)	122·10 (20)
N(1)—C(3)	1·442 (3)	C(2)—N(1)—C(3)	123·29 (19)
C(3)—C(7)	1·545 (3)	N(1)—C(3)—C(4)	111·79 (15)
C(3)—C(4)	1·528 (2)	N(1)—C(3)—C(7)	110·87 (19)
C(4)—O(2)	1·233 (2)	C(7)—C(3)—C(4)	105·76 (17)
C(4)—N(2)	1·336 (3)	C(3)—C(4)—O(2)	122·57 (19)
N(2)—C(5)	1·454 (2)	C(3)—C(4)—N(2)	115·31 (16)
C(5)—C(14)	1·546 (4)	O(2)—C(4)—N(2)	121·81 (15)
C(5)—C(6)	1·531 (3)	C(4)—N(2)—C(5)	121·02 (17)
C(6)—O(3)	1·319 (2)	N(2)—C(5)—C(6)	111·09 (18)
C(6)—O(4)	1·203 (2)	N(2)—C(5)—C(14)	109·57 (16)
C(7)—C(8)	1·502 (3)	C(6)—C(5)—C(14)	109·37 (19)
C(8)—C(9)	1·379 (4)	C(5)—C(6)—O(3)	112·33 (16)
C(9)—C(10)	1·436 (5)	C(5)—C(6)—O(4)	122·82 (18)
C(10)–C(11)	1·371 (7)	O(3)—C(6)—O(4)	124·74 (22)
C(11)–C(12)	1·346 (6)	C(3)—C(7)—C(8)	114·83 (19)
C(12)–C(13)	1·381 (3)	C(7)—C(8)—C(9)	120·64 (24)
C(13)–C(8)	1·386 (4)	C(7)—C(8)—C(13)	120·19 (21)
C(14)–C(15)	1·504 (3)	C(9)—C(8)—C(13)	119·11 (21)
C(15)–C(16)	1·391 (4)	C(8)—C(9)—C(10)	118·47 (34)
C(16)–C(17)	1·389 (3)	C(9)—C(10)–C(11)	119·77 (34)
C(17)–C(18)	1·373 (4)	C(10)–C(11)–C(12)	121·24 (30)
C(18)–O(5)	1·378 (3)	C(11)–C(12)–C(13)	119·51 (33)
C(18)–C(19)	1·377 (4)	C(12)–C(13)–C(8)	121·76 (28)
C(19)–C(20)	1·390 (3)	C(5)—C(14)–C(15)	112·26 (18)
C(20)–C(15)	1·390 (3)	C(14)–C(15)–C(16)	121·32 (20)
		C(14)–C(15)–C(20)	120·92 (22)
		C(16)–C(15)–C(20)	117·72 (19)
		C(15)–C(16)–C(17)	120·87 (22)
		C(16)–C(17)–C(18)	120·44 (27)
		C(17)–C(18)–O(5)	123·52 (25)
		C(17)–C(18)–C(19)	119·75 (21)
		O(5)—C(18)–C(19)	116·72 (21)
		C(18)–C(19)–C(20)	119·92 (23)
		C(19)–C(20)–C(15)	121·24 (25)

The average tetrahedral C–H bond is 0·986 (23) Å.
The average aromatic C–H bond is 0·942 (29) Å.
The N(2)–H(N2) distance is 0·894 (22) Å.
The O(5)–H(O5) distance is 0·954 (43) Å.

Discussion

Table 6 contains the bond lengths and bond angles uncorrected for the effects of thermal motion. The values are consistent with the expected values except for the bond lengths and angles in the phenylalanine ring, atoms C(8) through C(13). These atoms have rather larger thermal parameters and, as can be seen in Fig. 1, the whole ring appears to be vibrating about the C(8)–C(13) bond. This large thermal motion gives rise to unreliable bond lengths and angles in the aromatic ring. It may also account for the fact that four of the ring carbons were not seen in the initial *E* map, for the non-appearance of H(C12) in the ΔF map where the other hydrogen atoms were found, and for the problems in refining H(C11) and H(C12).

The molecule exists in a conformation with the peptides and other hydrogen bond-forming groups situated in the middle of the molecule with the aromatic rings far removed from one another, the distance between the centers of the rings being about 10 Å. Each molecule participates in four hydrogen bonds, and it can be seen in Fig. 2 that this leads to a structure with chains of hydrogen bonds running diagonally through the crystal lacing the molecules together. Between these chains are regions of aromatic rings stacked in an arrangement where the tyrosine ring is roughly parallel to **b** with the phenylalanine ring parallel to the *xz* plane. The angle between the least-squares planes through the two rings is 84·5°. The over-all packing arrangement is an example of 'like likes like'. The polar parts of the molecules are close to one another while the aromatic nonpolar parts are packed together.

The hydrogen bonds formed in the crystal are shown in Fig. 3. The atoms involved in the hydrogen bonds are: O(2) of the molecule at x,y,z is bonded to O(3) of the molecule at $-x,-\frac{1}{2}+y,1-z$; and N(1) of the molecule at x,y,z is bonded to O(4) of the molecule at $-x,-\frac{1}{2}+y,1-z$. The hydrogen bonds are fairly linear, and the distances between the donor and acceptor atoms are typical of moderately strong hydrogen bonds.

Not all of the possible hydrogen bond acceptors and donors in the molecule are involved in the hydrogen-bond scheme. The hydroxyl on the tyrosine ring is not close enough to any other group to take part in any

hydrogen bonding interaction. Table 5 shows that the phenylalanyl ring has large thermal parameters while the tyrosine ring does not. The tyrosine is not fixed by hydrogen bonding, so the reason for the difference must be the packing of the groups around the rings. The motion of the phenylalanine ring is relatively unrestricted in the direction of the thermal ellipsoids. On the other hand, the tyrosine ring is held in place by its own symmetry-related partner, the phenylalanine ring and the carboxyl group.

In most of the molecules containing peptide-type linkages that have been studied in the solid state by X-ray crystallography, the atoms surrounding the peptide bonds have been found to be coplanar. The nonplanar peptides which have been found to this date have been in small cyclic compounds or in peptides containing proline (Winkler & Dunitz, 1971; Ramachandran, 1968). Although the peptide group in this molecule is in a *trans* configuration, the dihedral angle about the peptide bond is 17·7° [$\omega = 162{\cdot}3\ (4)°$]. Table 7 shows the results of fitting a least-squares plane to the atoms comprising the peptide group. In this molecule, the twist in the peptide plane must be caused by the crystal-packing forces. The molecule is not forced into its configuration by internal bonding as in cyclic compounds. It seems likely that if such forces can produce a nonplanar peptide in a small polypeptide, the various interactions in a protein structure could also produce nonplanar peptides.

Table 7. *Deviations from the least-squares plane through* C(3), C(4), O(2), N(2), C(5)

C(3)	0·090Å
C(4)	−0·062
O(2)	−0·007
N(2)	−0·129
C(5)	0·108

As in all crystallographic studies that are undertaken to study the chemistry of the molecules, questions must be raised concerning the structures of the molecules when not in the crystal. Because of the crystal-packing forces, it is possible that the structure of the molecule in the crystal is not the same as that in biological systems or in solution. This could obviate any chances of explaining the kinetics of the pepsin catalyzed hydrolysis on the basis of the solid-state results. Nevertheless, the structure found is useful for comparative purposes and as a point of departure for other methods. Other techniques such as n.m.r. spectroscopy or energy minimization programs would be useful in determining the structure of the molecule in solution.

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References

BAKER, L. E. (1951). *J. Biol. Chem.* **193**, 809–819.
CLEMENT, G. E., SNYDER, S. L., PRICE, H. & CARTMELL, R. (1968). *J. Amer. Chem. Soc.* **90**, 5603–5610.
JACKSON, W. T., SCHLAMOWITZ, M. & SHAW, A. (1965). *Biochemistry*, **4**, 1537–1543.
MAIN, P., WOOLFSON, M. M. & GERMAIN, G. (1971). *MULTAN – A Computer Programme for the Automatic Solution of Crystal Structures.* Univ. of York Printing Unit, York, England.
RAMACHANDRAN, G. N. (1968). *Biopolymers*, **6**, 1494–1496.
WINKLER, F. K. & DUNITZ, J. D. (1971). *J. Mol. Biol.* **59**, 169–182.
ZEFFREN, E. & KAISER, E. T. (1966). *J. Amer. Chem. Soc.* **88**, 3129–3131.

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The Crystal and Molecular Structure of *cis*-1,3-Diethyl-2,4-diphenyl-2,4-dithiocyclodiphosphazane

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Crystals of the title compound, $[NEtP(S)Ph]_2$, are monoclinic, $a = 7{\cdot}17$, $b = 16{\cdot}27$, $c = 16{\cdot}59$ Å, $\beta = 96{\cdot}0°$, space group $C2/c$, $Z = 4$. The atomic positions have been determined by least-squares refinement from X-ray diffractometer intensities, the final R being 0·075 for 1574 reflexions. The molecules possess exact symmetry C_2. The phosphazane ring is non-planar with the bond angles N–P–N 84·5° and P–N–P 95·0°, the departure from planarity being attributed to steric overcrowding in the molecule. The bond lengths: P–N 1·695, P–S 1·922, P–C 1·797, and N–C 1·46 Å, are not significantly different from the corresponding lengths in the *trans* isomer.

Introduction

The possibility of *cis–trans* isomerism in the cyclodiphosphazanes (I) was first suggested by Trippett (1962) but until recently pairs of isomers had not been separated. Flint, Ibrahim, Shaw, Smith & Thakur (1971) were able to isolate two isomers of 1,3-diethyl-2,4-diphenyl-2,4-dithiocyclodiphosphazane (I: R = Et,