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A New Crystalline Form of the Thyroliberin Analogue Thi^2–TRH. Structure and Molecular Arrangements

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L-Pyroglutamyl-β-(2-thienyl)-L-alanyl-L-prolinamide, Thi^2-TRH ($C_{17}H_{22}N_4O_4S$), a biologically active thienyl analogue of the thyrotropin-releasing peptidhormone (TRH), is polymorphic. Crystals with $P2_1$ and $C2$ space group symmetries have been obtained. The present investigation concerns the $C2$ modification (Z=8, a=15.358(4), b=10.830(4), c=23.155(2) Å and β=108.29(1)°), for which a suitable structural model could be deduced from results of an earlier determination of the $P2_1$ structure by assuming closely related molecular packings in the two structures. The structural parameters were refined isotropically *versus* the 1173 most significant observable reflections (d_{min}=1.0 Å) to a conventional R-value of 0.115. Thus, the structure is composed of hydrogen bonded dimers in which the molecules are built up in antiparallel β-pleated sheet arrangements. The conformation properties of the $C2$ molecules deviate slightly in the main chain backbone of the second residue but adopt approximately the same conformation as the $P2_1$ molecules. The rotational disorder of the thiophene rings as well as the conformational freedom of the pyrrolidine rings found in $P2_1$ are also present in the $C2$ structure.

The biologically highly active thienyl analogue, Thi^2-TRH ($C_{17}H_{22}N_4O_4S$) L-pyroglutamyl-β-(2-thienyl)-L-alanyl-L-prolinamide[2] (Fig. 1) of the thyrotropin-releasing hormone (TRH), thyroliberin, has been crystallized from both ethanol and water, yielding two different monoclinic crystal forms. The prismatic shaped material obtained from water solutions shows $P2_1$ space group symmetry [Z=4, a=9.340(1) Å, b=21.961(3) Å, c=9.449(1) Å, β=109.58(1)° and V=1826.0(4) Å^3] and has previously[1] been studied by X-ray diffraction techniques. Even after determining the $P2_1$ structure, a study of the molecular conformations in the alternative $C2$ form remains interesting due to the possible influence on the conformations by the different symmetry requirements of the molecular arrangements. The analysis of different crystal forms can provide information concerning the flexibility of the observed conformation and/or the possible existence of conformational isomers. In the $P2_1$ structure, the two molecules of the fundamental unit form hydrogenbonded dimers, related via an approximate twofold axis, while the twofold screw axis only influences the packing of these dimers. Careful analysis of crystallographically independendent molecules have in most cases shown dimeric molecules to be conformationally distinct with a finite barrier to interconversions. It is resonable to believe that in the $C2$ structure the molecules of the dimers are related by a true twofold axis, while the twofold

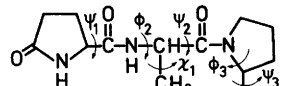

Fig. 1. The Thi^2-TRH (∠Glu–Thi–Pro–NH_2) molecule with the notation of torsional angles.

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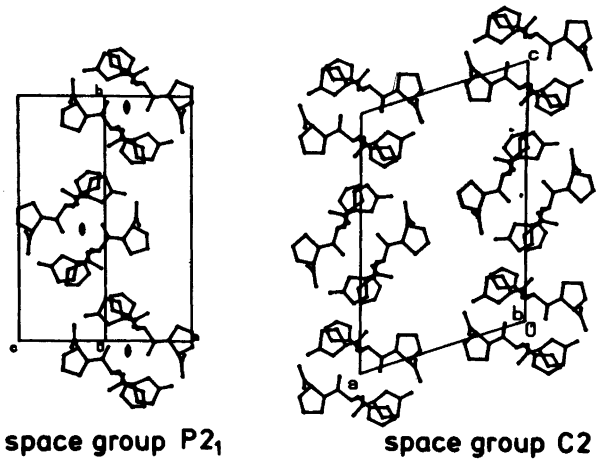

Fig. 2. Projections of parts of the two Thi[2]-TRH structures. a. The $P2_1$ structure viewed along ⟨101⟩. The positions of the pseudo twofold axis and are indicated. b. The $C2$ structure viewed along ⟨010⟩.

screw becomes a pseudo symmetry. The derivation of a suitable $C2$ model from the $P2_1$ structure, which is composed of molecules related by pseudo twofold symmetry, is described in some detail below.

DERIVATION OF THE STRUCTURAL MODEL

In Fig. 2, a selected part of the $P2_1$ crystal structure in a suitable orientation is shown. The pseudo twofold axis is roughly parallel to the ⟨101⟩ direction in the $P2_1$ structure and passes through the point $(x_0,y_0,z_0)\approx(0,0.46,0.26)$. Due to the twofold axis the following relations are valid

$$\begin{cases}(x_1,\ y_1-y_0,\ z_1-z_0)\approx(z_2-z_0,\ \bar{y}_2-\bar{y}_0,\ x_2)\\ (\bar{x}_1,\ \frac{1}{2}+y_1-y_0,\ \bar{z}_1-z_0)\approx(\bar{x}_2-z_0,\ \frac{1}{2}+\bar{y}_2-\bar{y}_0,\ \bar{x}_2)\end{cases}$$

where (x_1, y_1, z_1) and (x_2, y_2, z_2) refer to the $P2_1$ atomic coordinates of the two crystallographically independent molecules. From these relations the values of y_0 and z_0 can be obtained by averaging over all pairs of pseudo symmetry related atoms as

$$y_0=\tfrac{1}{2}\langle y_1+y_2\rangle;z_0=\tfrac{1}{2}\langle x_1+x_2+z_1+z_2\rangle$$

The y_0 and z_0 values obtained, were 0.4589(20) and 0.2645(47), respectively. The small esd of the position of the pseudo twofold axis indicates that the pseudo symmetry is remarkably well satisfied. After determining the y_0 and z_0 idealized coordinates (x_1',y_1',z_1') and (x_2',y_2',z_2'), yielding two molecules in $P2_1$ exactly related by a pseudo twofold axis, can be estimated by averaging as

$$\begin{cases}(x_1',y_1',z_1')=\frac{1}{2}(x_1+z_2-z_0,y_1-y_2+2y_0,x_2+z_1+z_0)\\ (x_2',y_2',z_2')=\frac{1}{2}(z',x_1'+z_0)\end{cases}$$

y_1

The coordinates to be transformed to $C2$ should be those describing two molecules related by the twofold screw axis in $P2_1$ shifted by $(0,\bar{y}_0,\bar{z}_0)$, *e.g.* $(x_1',y_1'-y_0,z_1'-z_0)$ and $(\bar{x}_1',\frac{1}{2}+y_1'-y_0,\bar{z}_1'-z_0)$.

By comparing the cell dimensions of the $P2_1$ and $C2$ structures of Thi[2]-TRH and in accordance with the pseudo symmetry described above, the following two sets of relations (upper and lower signs respectively) are derived,

$$\begin{pmatrix}A\\B\\C\end{pmatrix}=\begin{pmatrix}\mp1 & 0 & \pm1\\ \pm1 & 0 & \pm1\\ \pm\frac{1}{2} & 0 & \mp\frac{1}{2}\end{pmatrix}\begin{pmatrix}a\\b\\c\end{pmatrix}$$

where the lower case letters refer to the $P2_1$ unit translations and the upper case ones to the $C2$ translations. The predicted $C2$ unit cell parameters become 15.35, 10,83, 23.26 Å and 109.3°, in close agreement with those observed (15.358, 10.830, 23.155 Å and 108.29°, respectively). The associated coordinate transformation becomes

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \begin{pmatrix} \mp\frac{1}{2} & \frac{1}{2} & \pm\frac{1}{2} \\ \pm\frac{1}{2} & 0 & \pm\frac{1}{2} \\ 0 & 1 & 0 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$

where the lower case letters refer to the idealized and shifted $P2_1$ coordinates and the upper case ones to the $C2$ coordinates.

STRUCTURE REFINEMENT

The two different structural models, derived as above, yielded linear R-values of 0.44 and 0.35 for the 1173 most significant reflections (uncorrected for the minor absorption effects). An inspection of the calculated and observed structure factor amplitudes, revealed that the second alternative is the more correct one. By treating each molecule as a rigid group with individual isotropic temperature factors in a least-squares refinement, the R-value for the selected alternative dropped to 0.15.

During the final stages of the refinements of the $C2$ structural model it became apparent that the thienyl groups of the molecules in resemblance with the $P2_1$ structure were disordered,

Table 1. Crystal data for L-pyroglutamyl-β-(2-thienyl)-L-alanyl-L-prolinamide.

$C_{17}H_{22}N_4O_4S$; F.W. 378.45	
Space group $C2$	
a=15.358(4) Å	V=3657(2) Å^3
b=10.830(4)	Z=8
c=23.155(2)	D_x=1.375(1) g·cm^{-1}
β=108.29(1)°	μ(Cu$K\alpha$)=18.0 cm^{-1}

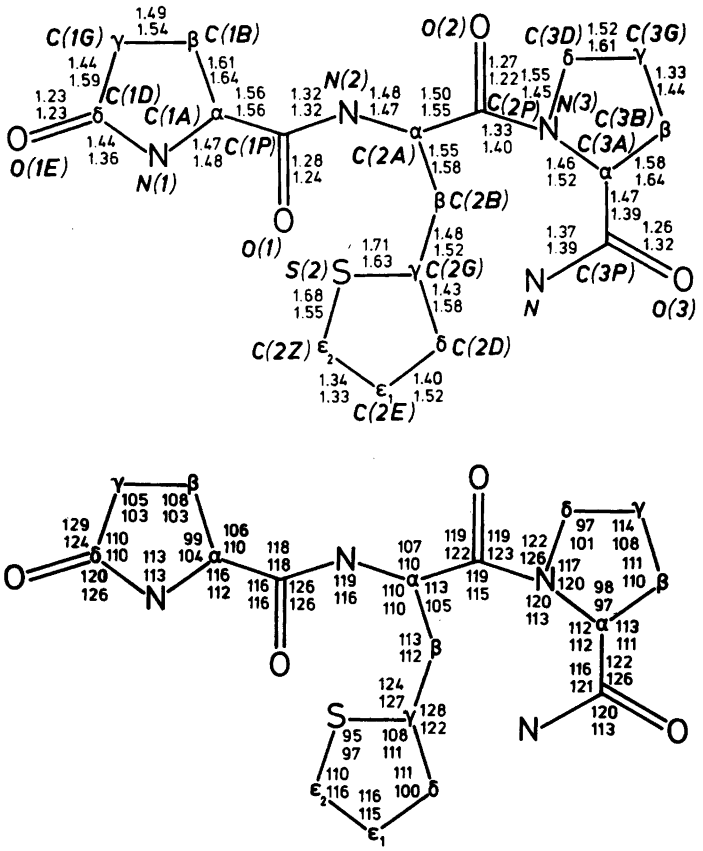

Fig. 3. a. Interatomic bond distances (Å). b. Bond angles (°) for the $C2$ molecules of Thi²-TRH. The esd's of the bond lengths range between 0.023 and 0.046 Å with the average value of 0.033 Å and the bond angles vary between 1.3 and 3.6° with the average of 2.3°.

Table 2. Fractional atomic coordinates and thermal parameters multiplied by 10.[4] The atomic labels (IUPAC recommendations) for the two independent molecules (A and B) are unprimed and primed, respectively. The *y*-parameter of the S(2′) was kept fixed to define the origin along the *b*-axis.

Atom	*x*	*y*	*z*	*U*
S(2)	−47(9)	1404(16)	6687(6)	1167(57)
N(1)	−651(15)	5033(25)	6134(10)	584(68)
C(1A)	319(23)	5381(35)	6380(15)	780(99)
C(1P)	1027(21)	4375(33)	6352(14)	640(94)
O(1)	1866(13)	4635(22)	6631(8)	625(57)
C(1B)	395(21)	5518(34)	7086(15)	750(99)
C(1G)	−368(19)	4792(31)	7191(13)	580(84)
C(1D)	−1037(22)	4663(34)	6599(14)	721(96)
O(1E)	−1841(14)	4331(23)	6464(9)	782(67)
N(2)	731(13)	3441(23)	5979(9)	433(59)
C(2A)	1405(15)	2512(27)	5917(10)	309(63)
C(2P)	1669(17)	2857(29)	5368(11)	412(69)
O(2)	1070(13)	2771(23)	4845(9)	612(55)
C(2B)	979(16)	1204(29)	5871(11)	446(72)
C(2G)	800(17)	810(29)	6434(11)	405(72)
C(2D)	1280(13)	−108(23)	6861(9)	482(95)
C(2E)	841(24)	−324(37)	7298(15)	883(99)
C(2Z)	103(22)	379(35)	7252(15)	757(99)
N(3)	2552(15)	2874(25)	5409(10)	545(62)
C(3A)	2825(18)	3027(31)	4862(13)	549(80)
C(3P)	2844(24)	1843(42)	4559(17)	941(99)
O(3)	3202(15)	889(28)	4853(11)	990(79)
N	2680(15)	1895(27)	3944(10)	584(66)
C(3B)	3808(23)	3583(36)	5181(17)	835(99)
C(3G)	4086(24)	3361(38)	5778(17)	960(99)
C(3D)	3315(19)	3004(32)	6029(13)	637(88)
S(2′)	1694(8)	6215	1658(5)	747(49)
N(1′)	1615(15)	2538(25)	1047(10)	561(68)
C(1A′)	887(20)	2113(31)	1297(13)	643(91)
C(1P′)	170(16)	3147(27)	1276(11)	379(69)
O(1′)	−380(12)	2934(22)	1555(8)	582(54)
C(1B′)	1441(21)	1753(36)	2004(14)	755(98)
C(1G′)	2323(21)	2536(32)	2140(14)	679(92)
C(1D′)	2432(19)	2735(32)	1485(12)	542(78)
O(1E′)	3148(13)	3062(22)	1403(9)	675(59)
N(2′)	185(13)	4149(23)	953(9)	429(60)
C(2A′)	−514(17)	5090(29)	933(12)	465(74)
C(2P′)	−1336(17)	4930(30)	346(12)	485(77)
O(2′)	−1247(13)	4964(23)	−157(9)	600(56)
C(2B′)	−121(16)	6415(29)	879(11)	473(76)
C(2G′)	657(14)	6766(25)	1443(9)	237(59)
C(2D′)	533(10)	7782(20)	1903(7)	551(64)
C(2E′)	1511(24)	7878(39)	2325(15)	891(99)
C(2Z′)	2099(20)	7105(33)	2198(14)	686(95)
N(3′)	−2181(13)	4724(22)	443(9)	366(54)
C(3A′)	−2953(19)	4493(32)	−145(13)	620(88)
C(3P′)	−3317(20)	5590(35)	−429(14)	615(87)
O(3′)	−3390(14)	6631(27)	−153(10)	963(76)
N(′)	−3653(16)	5679(27)	−1058(11)	653(72)
C(3B′)	−3657(22)	3817(35)	161(16)	759(99)
C(3G′)	−3385(27)	4075(43)	805(19)	1188(99)
C(3D′)	−2323(18)	4495(30)	1024(12)	543(83)

yielding unreasonable high and low values of the temperature factors of the sulfur atom S(2) and the carbon atom C(2D) respectively (*cf.* atom labelling in Fig. 3). Due to the limitations in the diffraction data, it was considered relevant to treat this disorder in the same way as in the $P2_1$ case. Consequently, it was assumed that the thiophene ring occupied two alternative positions corresponding to a rotation around the C(2B)–C(2G) bond by 0 and 180° degrees, respectively. This would imply a considerable overlap between *e.g.* the sulfur S(2) and the carbon C(2D) atoms from rings of different orientations, and could explain the anomalous values of their temperature factors. Accordingly, a combination of sulfur and carbon atom form factors was used for these positions and the atomic ratio was estimated by refining occupancy factors. The S(2) position in the first molecule has the occupancies 89(3)% S and 11(3)% C (*e.g.* the C(2D) position has 11 % S and 89 % C), while the position S(2′) in the second independent molecule has the occupancies 68(3)% S and 32(3)% C. Due to this disorder the relevance of the geometry of the thienyl group, especially as regards bond distances and angles involving S(2) and C(2D), is limited.

In the final least squares refinements of scale, positional, isotropic thermal and two independent occupancy parameters (210 parameters, 1173 observations) the *R*-value became 0.115. The weights used were estimated as inversely proportional to $\sigma(F_{obs})^2+0.001\,|F_{obs}|^2$. The crystallographic calculations were performed by using a local version of the program system SHELX-76[3] and an interactive program system XTAPL.[4]

Atomic coordinates and thermal parameters are listed in Table 2. The obtained bond distances and bond angles are shown together with the atomic labelling scheme in Fig. 3. Lists of structure factors are available from one of the authors (B.S.) on request.

DISCUSSION

As is evident from the close agreement between the molecular arrangements in the *C*2 and $P2_1$ structures of Thi²-TRH, the intermolecular contacts and gross molecular conformations are almost identical in the two structures (Fig. 3). In the $P2_1$ structure, where the two independent molecules designated A and B are associated in an antiparallel β-pleated sheet arrangement, while the *C*2 structure consists of alternative A–A and B–B molecules along the *c*-axis. The three-dimensional molecular packing scheme, constructed from infinite predominantly hydrophobic layers separated by ordinary van der Waals distances, is in the $P2_1$ structure consisting of "mixed" A–B molecules perpendicular to the *b*-axis, while in *C*2 every second layer parallel to the *a*-axis contains only A or B molecules.

A detailed discussion of the structural features as well as some biochemical implications of the molecular structure can be found in the paper by Stensland & Castensson.[1] The present paper will only consider some supplementary points.

In Table 3 a selected set of torsional angles (*cf.* Fig. 1) labelled according to the IUPAC-IUB recommendations[5] are given for the *C*2 and $P2_1$ structures. Since the esd's of the torsion angles, range from 2.7° to 4.3° with the average value of 3.3° for the *C*2 structure, most of the conformational differences between the two independent molecules are insignificant. There is only one minor deviation found in the ψ_2 angle, which influences the direction of the main chain back-

Table 3. Torsion angles in the two polymorphic crystal forms of Thi²-TRH. The average esd is 3.3° in the *C*2 structure and 0.7° in $P2_1$, respectively.

∠Glu–Thi–Pro–NH_2	Residue 1		Residue 2			Residue 3	
	ψ_1	ϕ_2	ψ_2	χ_1	χ_2	ϕ_3	ψ_3
Space group *C*2							
Molecule A	18	−96	133	−66	71	−86	152
Molecule B	12	−97	122	−66	72	−83	147
Space group $P2_1$							
Molecule A	8	−92	121	−63	70	−78	144
Molecule B	10	−97	124	−67	71	−84	142

bone. Also in the $P2_1$ structure where the average esd of the torsion angles is 0.7°, the geometries of the two independent molecules deviate substantially. The observed conformation in $P2_1$ agrees well with the second (primed labels) of the two molecules in $C2$.

As mentioned above the molecular disorder in the thiophene- and in the pyrrolidine rings observed in the $P2_1$ structure is also present in $C2$. The rotation disorder around the C(2B)–C(2G) bond of the two independent thienyl groups is associated with different magnitudes of the sulfur occupancies. The main sulfur position in the A and B molecules of $C2$ are 89(3) and 68(3)% compared with 66(1) and 80(1)% in the two $P2_1$ molecules.

In view of the influence of the limited data quality and the crude way of handling the rotation disorder problem the accuracy of the bond lengths and angles are affected (Fig. 2). As was found in the $P2_1$ structure, the C(3B)–C(3G) distance in the pyrrolidine ring of the A molecule is very short (1.33(4) Å). Although this shortening of the C–C bond, can partly be due to the large, possibly uncoupled, thermal vibrations at this residue, it is likely that this distance could be considered as an anomalous one.[6] Substantial conformational freedom in the pyrrolidine ring among TRH analogues seems not to be uncommon[7] and in the Phe^2–TRH · H_2O analogue even two different C(3G)conformers have been observed.[8]

The conformations of the three different five membered rings of each molecule were analyzed by calculating puckering coordinates.[9] These coordinates indicate that all of the rings adopt slightly distorted envelope conformations with apex atoms C(1B), C(1B′), S(2), S(2′), C(3B) and C(3B′), respectively. Least squares planes calculated through the four approximately coplanar atoms of each ring, show that the apex atoms above deviate from these planes by 0.15, 0.29, 0.25, 0.43, 0.35 and 0.10 Å, respectively. The root mean square deviations of the four atoms defining each of the planes were negligible (less than 0.02 Å) in comparison with the esd's of the atomic positions (about 0.03 Å). Although, the thiophene rings are more planar than the pyrrolidone- and the pyrrolidine rings, all three rings are significantly nonplanar.

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