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The Crystal and Molecular Structure of D-α-Benzylpenilloic Acid Monohydrate, $C_{15}H_{20}N_2O_3S.H_2O$*

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D-α-Benzylpenilloic acid [(2*R*,4*S*)-2-phenylacetamidomethyl-5,5-dimethylthiazolidine-4-carboxylic acid] crystallizes in the monoclinic space group $P2_1$ with $a = 10{\cdot}959$ (3), $b = 7{\cdot}287$ (2), $c = 10{\cdot}827$ (3) Å, $\beta = 101{\cdot}5$ (3)°, $Z = 2$. The structure was refined to an R of 0·042 for 1069 counter reflexions. Bond lengths and angles in the thiazolidine ring are comparable with those in phenoxymethylpenicillin and the benzylpenicillin ion. The thiazolidine ring is puckered; four of the five atoms are coplanar and the C atom carrying two methyl groups is out of this plane (−0·711 Å). The dihedral angle is 42·3°. The molecule appears as a zwitterion. The packing is dominated by five intermolecular O—H···O and N—H···O hydrogen bonds. Both O atoms in the carboxyl group are involved in hydrogen bonds: O(*W*)—H···O(8) 2·983 (8), N(12)—H···O(8) 2·841 (8), and N^+(3)—H···O(7) 2·698(8) Å. N(3) is engaged in one more hydrogen bond with O(*W*) of 2·756 (8) Å. The carbonyl O is hydrogen-bonded to a water molecule by O(*W*)—H···O(14), 2·775 (8) Å.

Introduction

The penicillin and cephalosporin antibiotics probably have the same effect on bacterial cell walls. Thus the crystal structures of several biologically active or inactive compounds belonging to the penicillin and cephalosporin groups have been studied (Robertson, 1972); these include the Na, K and Rb salts of penicillin (Crowfoot, Bunn, Rogers-Low & Turner-Jones, 1949). The latter studies were undertaken not only to establish the chemical formula but also to find the relationship between molecular structure and biological activity. Sweet & Dahl (1970) have solved the structures of two cephalosporin antibiotics (cephaloridine.HCl.H_2O and cephaloglycine) and a biologically inactive cephalosporin derivative (phenoxymethyl-Δ^2-deacetoxycephalosporin). Their studies have revealed significant stereochemical information: the pyramidal character of the β-lactam N atom in the penicillin and two active Δ^3-cephalosporin antibiotics, but the nearly planar lactam N atom in the inactive Δ^2-cephalosporin. The ease of base hydrolysis of the lactam amide bond in these antibiotics correlates with biological activity. This behaviour can be explained by the decrease of the amide resonance form in the antibiotic β-lactam relative to that in free β-lactams and in the biologically inactive Δ^2-cephalosporin. This is evident from the C—N and C—O bond-length differences among these compounds.

Besides the antibiotic properties, other pharmaceutical activities of the penicillin derivatives have been observed. Thus D-penicillamine, formerly employed as an antidote in heavy-metal poisoning, has been introduced into medicine as a therapeutic agent in rheumatoid arthritis, chronic aggressive hepatitis and multiple sclerosis (subject of communications presented at the 1st Yugoslav Symposium on D-penicillamine, Zagreb, October 1975). New applications of D-penicillamine have concentrated interest on its economical production. Herak, Kovačević & Gašpert (1977) have described the preparation of D-penicillamine from D-benzylpenilloic acid. These authors studied the epimerization of D-benzylpenilloic acid by PMR spectroscopy. D-Benzylpenilloic acid appears in two stereoisomers described as D-α (monohydrate and anhydrous forms) and D-β (solely anhydrous form) (Clarke, Johnson & Robinson, 1949). Such an assignment was made to differentiate isomers because the configurations at C(2) in the thiazolidine ring were uncertain.

Thus the X-ray crystal structure determination of D-α-benzylpenilloic acid monohydrate was undertaken to establish the configuration at C(2).

The (2*R*,4*S*) configuration is found. The PMR spectrum revealed a mixture of D-α and D-β isomers of benzylpenilloic acid (Herak, Kovačević & Gašpert, 1977). We also observed two kinds of crystals: monoclinic (Table 1) and orthorhombic. The cell parameters of this orthorhombic form are 10·67, 7·30 and 21·96 Å; the space group is $P2_12_12_1$. Attempts to obtain good crystals for structure determination have not yet been successful.

* This structure determination was presented at the Second Yugoslav–Italian Crystallographic Conference, June 1976, Dubrovnik, Yugoslavia.

Table 1. *Crystallographic and physical data*

D-α-Benzylpenilloic acid monohydrate, $C_{15}H_{20}N_2O_3S.H_2O$

FW	326·42	U	847·26 Å^3
Space group	$P2_1$	Z	2
a	10·959 (3) Å*	D_c	1·279 g cm^{-3}
b	7·287 (2)	μ(Mo $K\alpha$)	1·64 cm^{-1}
c	10·827 (3)	Crystal shape	Plate
β	101·5 (3)°	Crystal dimensions	0·707 × 0·234 × 0·035 mm

* Numbers in parentheses here and throughout this paper are the estimated standard deviations in the least significant digit.

Experimental

The space group was determined from Weissenberg photographs recorded with Cu $K\alpha$ radiation. The diffraction symmetry and space-group extinctions indicated $P2_1$ or $P2_1/m$; since the molecule is optically active, the space group is necessarily $P2_1$. Table 1 lists the crystallographic and physical data.

The intensities were collected on a Philips PW 1100 computer-controlled four-circle diffractometer in the ω-scan mode [scan width = 1·4°(θ), scan speed = 0·04°(θ)s^{-1}] with graphite-monochromated Mo $K\alpha$ radiation. 1069 independent reflexions [$I > 2\sigma(I)$] in the range $2 < \theta < 28°$ were observed and only these were used in the calculations. Three standard reflexions were measured every 2h to provide a check on instrument and crystal stability. The data were corrected for background, Lorentz and polarization effects but not for absorption.

Structure determination and refinement

An overall temperature factor (B = 1·30 Å^2) as well as a scale factor were determined (Wilson, 1942) and used to compute normalized structure amplitudes by the routine *NORMAL* included in *MULTAN*. The structure was solved by *MULTAN* (Declercq, Germain, Main & Woolfson, 1973). The solution was based on 250 reflexions with $|E| > 1·1$. The E map corresponding to the solution with the best figure of merit revealed

Table 2. *Final atomic parameters* (×10^4) *for the non-hydrogen atoms*

	x	y	z
S(1)	−2841 (2)	3089	2276 (2)
C(2)	−1163 (5)	3222 (11)	2678 (5)
C(4)	−1707 (5)	2588 (8)	370 (5)
C(5)	−2835 (6)	1738 (10)	843 (5)
C(6)	−1268 (6)	1469 (10)	−657 (6)
C(9)	−2685 (7)	−295 (11)	1141 (7)
C(10)	−4057 (6)	2148 (13)	−88 (6)
C(11)	−733 (6)	5096 (10)	3160 (6)
C(13)	1224 (6)	4570 (10)	4614 (6)
C(15)	2625 (7)	4693 (12)	4774 (6)
C(16)	3144 (6)	2914 (12)	4348 (6)
C(17)	3459 (7)	1448 (12)	5140 (7)
C(18)	3887 (7)	−164 (13)	4727 (8)
C(19)	4007 (7)	−378 (14)	3492 (9)
C(20)	3726 (8)	1071 (15)	2698 (8)
C(21)	·3275 (7)	2731 (13)	3082 (7)
O(7)	−358 (4)	451 (8)	−358 (4)
O(8)	−1903 (4)	1692 (8)	−1730 (4)
O(14)	709 (5)	3927 (8)	5428 (4)
O(W)	602 (5)	9803 (9)	2707 (5)
N(3)	−694 (4)	2743 (8)	1488 (4)
N(12)	597 (5)	5175 (9)	3507 (4)

Table 3. *Positional parameters* (×10^3) *and isotropic thermal parameters* (×10^2) *for the hydrogen atoms*

	x	y	z	U (Å^2)
H(2)	−77	224	333	4·2
H(4)	−188	387	14	3·2
H(9)1	−342	−57	148	5·5
H(9)2	−196	−41	182	5·5
H(9)3	−228	−109	57	5·5
H(11)1	−90	615	254	4·3
H(11)2	−116	564	392	4·3
H(15)1	308	491	575	5·1
H(15)2	277	569	423	5·1
H(17)	319	175	591	6·1
H(18)	415	1118	532	5·4
H(19)	433	−173	331	5·4
H(20)	360	114	167	6·8
H(21)	320	404	261	6·0
H(N3)1	−30	176	160	3·2
H(N3)2	−14	352	129	3·2
H(N12)	105	537	292	4·0
H(OW)1	44	940	339	6·4
H(OW)2	108	927	251	6·4

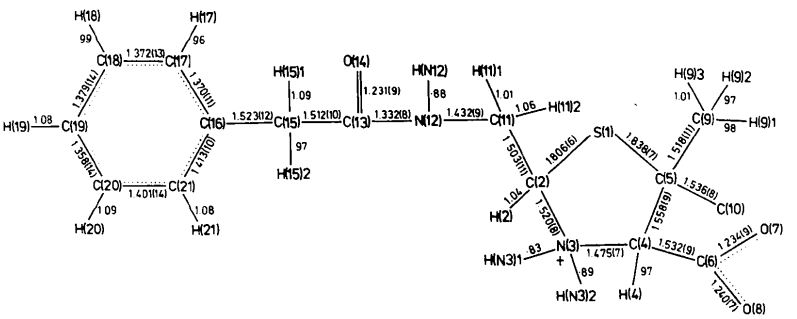

Fig. 1. The structural formula, numbering of the atoms, and bond lengths (Å) [O(W)–H(OW)1 = 0·84, O(W)–H(OW)2 = 0·71 Å].

the positions of 20 non-hydrogen atoms. The remaining two C atoms belonging to the phenyl ring were located from the resulting Fourier synthesis.

Refinement was by full-matrix least squares minimizing $\Sigma w||F_o| - |F_c||^2$ with $w = 1/\sigma^2_{|F_o|}$. Heavy-atom coordinates, isotropic thermal parameters and a scale factor were refined to an R of 0·102. Three cycles of anisotropic refinement (R = 0·062) and a difference synthesis located 19 H atoms. The H atoms from the C(10) methyl group were not determined. In the final refinement a scale factor, heavy-atom coordinates (the y coordinate of S was kept fixed to define the origin in the polar space group) and anisotropic thermal parameters (198 parameters in all) were varied. The H atoms were included in the structure factor calculation only. For the H atoms the isotropic thermal parameters are those of the bonded atom. The final residuals were $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0{\cdot}042$ and $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma|F_o|^2]^{1/2} = 0{\cdot}045$.*

The scattering factors given by Cromer & Mann (1968) and for H by Stewart, Davidson & Simpson (1965) were used. The anomalous-scattering factor for S was from Cromer & Liberman (1970).

The calculations were performed on the Univac 1110 computer at the University Computing Centre in Zagreb with the XRAY 72 System (Stewart, Kruger, Ammon, Dickinson & Hall, 1972).

Atomic positional and (for H) thermal parameters are listed in Tables 2 and 3.*

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33164 (13 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

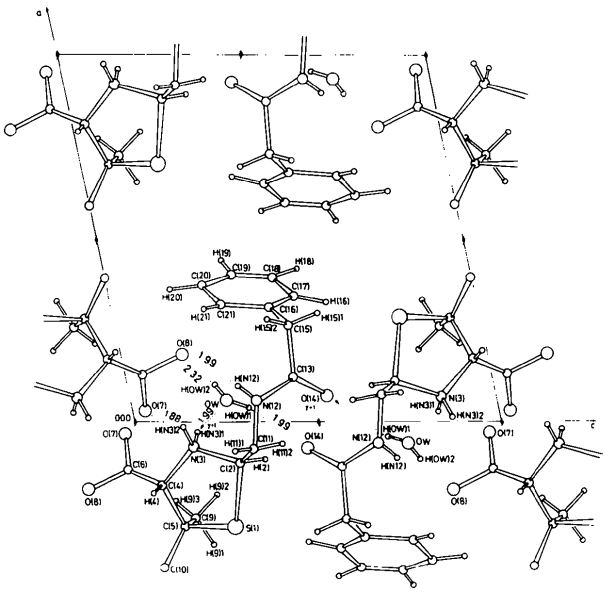

Fig. 2. Molecular packing and hydrogen bonds. The projection is along **b**.

Description and discussion of the structure

The structural formula, numbering of the atoms and bond lengths are given in Fig. 1. Molecular packing and hydrogen bonds viewed along **b** are illustrated in Fig. 2. Torsion angles along bonds showing the conformation of the thiazolidine ring in phenoxymethylpenicillin, the K, Rb and Na salts of penicillin, and D-(α)-benzylpenilloic acid are given in Fig. 3.

Since only the D isomer of penicillamine is of therapeutic value (the racemic form and the L isomer are toxic), D-benzylpenilloic acid was used as the starting material in the reaction with phenylhydrazine (Herak, Kovačević & Gašpert, 1977). An R-factor test on D or L isomers did not show any significant difference. Therefore, preference to the D isomer is given and the compound is assigned as D-(α)-benzylpenilloic acid.

D-(α)-Benzylpenilloic acid is formed from penicillin by opening a β-lactam ring. Thus, a structural comparison with other penicillin derivatives can be made for the thiazolidine moiety only. Bond lengths and angles in the thiazolidine ring (Table 4, Fig. 1) are comparable with the values found in phenoxymethylpenicillin (Abrahamsson, Crowfoot Hodgkin & Maslen, 1963) and the benzylpenicillin ion (Rollett & Vaciago, personal

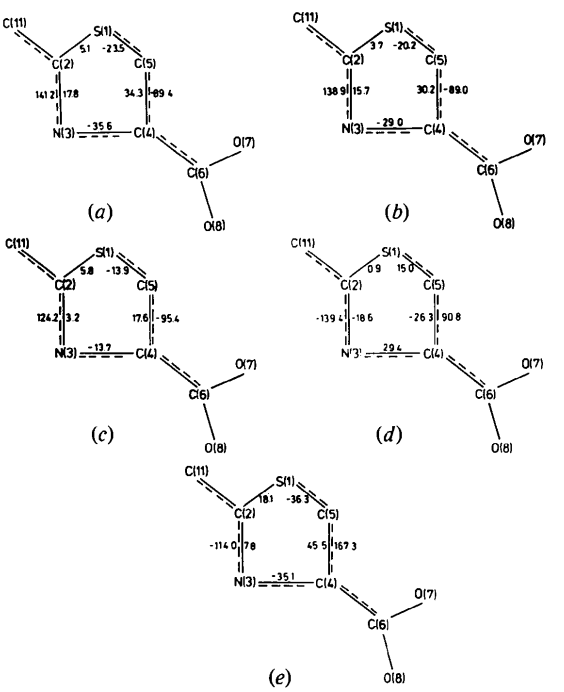

Fig. 3. Torsion angles (°) along bonds in the thiazolidine rings of various penicillin compounds. The calculations were made on the basis of the atomic coordinates given in the references quoted in this paper. (*a*) Phenoxymethylpenicillin (2*S*,4*R*). (*b*) The K salt of penicillin (2*S*,4*R*). (*c*) The Rb salt of penicillin (2*S*,4*R*). (*d*) The Na salt of penicillin (2*R*,4*S*). (*e*) Benzylpenilloic acid (2*R*,4*S*).

Table 4. *Bond angles* (°)

C(2)–S(1)–C(5)	93·3 (3)	C(4)–C(6)–O(7)	118·9 (5)	C(15)–C(16)–C(17)	122·5 (6)
S(1)–C(2)–C(11)	111·5 (5)	C(4)–C(6)–O(8)	114·3 (6)	C(15)–C(16)–C(21)	119·3 (7)
S(1)–C(2)–N(3)	106·5 (3)	O(7)–C(6)–O(8)	126·7 (7)	C(17)–C(16)–C(21)	118·1 (8)
S(1)–C(2)–H(2)	113	C(2)–C(11)–N(3)	111·1 (6)	C(16)–C(17)–C(18)	121·6 (7)
C(11)–C(2)–N(3)	111·5 (6)	C(2)–C(11)–H(11)1	117	C(17)–C(18)–C(19)	121·1 (8)
C(11)–C(2)–H(2)	109	C(2)–C(11)–H(11)2	110	C(18)–C(19)–C(20)	118·2 (9)
N(3)–C(2)–H(2)	105	N(12)–C(11)–H(11)1	101	C(19)–C(20)–C(21)	122·2 (8)
C(5)–C(4)–C(6)	114·3 (5)	N(12)–C(11)–H(11)2	112	C(16)–C(21)–C(20)	118·6 (8)
C(5)–C(4)–N(3)	106·7 (4)	H(11)1–C(11)–H(11)2	106	C(2)–N(3)–C(4)	112·8 (4)
C(5)–C(4)–H(4)	109	C(15)–C(13)–O(14)	122·7 (6)	C(4)–N(3)–H(N3)1	110
C(6)–C(4)–N(3)	110·1 (5)	C(15)–C(13)–N(12)	114·4 (6)	C(4)–N(3)–H(N3)2	107
C(6)–C(4)–H(4)	114	O(14)–C(13)–N(12)	122·8 (6)	C(2)–N(3)–H(N3)1	109
N(3)–C(4)–H(4)	102	C(13)–C(15)–C(16)	110·3 (6)	C(2)–N(3)–H(N3)2	114
S(1)–C(5)–C(4)	101·6 (4)	C(13)–C(15)–H(15)1	112	H(N3)1–N(3)–H(N3)2	103
S(1)–C(5)–C(9)	111·2 (4)	C(13)–C(15)–H(15)2	105	C(11)–N(12)–C(13)	122·6 (6)
S(1)–C(5)–C(10)	107·5 (5)	C(16)–C(15)–H(15)1	107	C(11)–N(12)–H(N12)	120
C(4)–C(5)–C(9)	113·5 (5)	C(16)–C(15)–H(15)2	110	C(13)–N(12)–H(N12)	115
C(4)–C(5)–C(10)	110·6 (5)	H(15)1–C(15)–H(15)2	113	H(O*W*)1–O(*W*)–H(O*W*)2	111
C(9)–C(5)–C(10)	111·9 (6)				

communication to Abrahamsson, Crowfoot Hodgkin & Maslen, 1963). S(1)–C(2), 1·806(6), and S(1)–C(5), 1·838(7) Å, correspond to a C–S single bond. In the thiazolidine ring, the most remarkable bond-length difference between D-α-benzylpenilloic acid and phenoxymethylpenicillin is in the carboxyl group. The difference in C–O bond distances is due to the free-acid form in phenoxymethylpenicillin and the zwitterion form in D-α-benzylpenilloic acid. C(6)–O(7), 1·234(9), and C(6)–O(8), 1·240(7) Å, are equal within experimental error.

Conformation of the thiazolidine ring

The thiazolidine ring is puckered; the angle between planes through C(4), N(3), C(2), S(1) and C(4), C(5), S(1) is 42·3°. C(4), N(3), C(2) and S(1) are in the same plane; C(5) is 0·711 Å below this plane (Table 5). D-α-Benzylpenilloic acid monohydrate possesses the (2*R*,4*S*) configuration.

Table 5. *Deviations of atoms from a least-squares plane*

Atoms included in the least-squares plane calculation are marked by an asterisk.

S(1)*	−0·021 Å	C(6)	−0·561 Å
C(2)*	0·038	C(9)	−2·223
N(3)*	−0·043	C(10)	−0·275
C(4)*	0·026	C(11)	1·315
C(5)	−0·711		

The torsion angles along bonds in the thiazolidine rings of phenoxymethylpenicillin, the K, Rb, Na salts of penicillin, and D-α-benzylpenilloic acid monohydrate are presented in Fig. 3 to show the ring conformation, as well as the configuration at two optically active C atoms, C(2) and C(4). The configuration (2*R*,4*S*) is found in the Na salt of penicillin and D-α-benzylpenilloic acid, but the opposite configuration (2*S*,4*R*) in phenoxymethylpenicillin and the K and Rb salts of penicillin. The thiazolidine rings in phenoxymethylpenicillin (Abrahamsson, Crowfoot Hodgkin & Maslen, 1963), and the Na, K and Rb salts of penicillin (Crowfoot, Bunn, Rogers-Low & Turner-Jones, 1949) are puckered; C(4) is displaced below the least-squares plane [N(3),C(2),S(1),C(5)]. But the carboxylic group with C(6) is situated below the thiazolidine ring in the structures of the Na salt of penicillin and D-α-benzylpenilloic acid monohydrate. In the structure of phenoxymethylpenicillin the carboxyl group is above the thiazolidine ring (Fig. 3). The relative orientation of the β-lactam ring and the carboxyl group in the above penicillin derivatives is *trans*.

Hydrogen bonding and molecular packing

Molecules are connected by five intermolecular O–

Table 6. *Hydrogen bonds*

X–H···*Y*	*X*···*Y*	*X*–H	H···*Y*	∠*X*–H···*Y*	Symmetry operation
O(*W*)–H(O*W*)1···O(14)	2·775 (8) Å	0·84 Å	1·99 Å	153°	$x,y,z;\ \bar{x},\frac{1}{2}+y,\bar{z}+1$
O(*W*)–H(O*W*)2···O(8)	2·983 (8)	0·71	2·32	155	$x,y,z;\ \bar{x},\frac{1}{2}+y,\bar{z}$
N(3)–H(N3)1···O(*W*)	2·756 (8)	0·83	1·99	152	$x,y,z;\ x,y,z$
N(3)–H(N3)2···O(7)	2·698 (8)	0·89	1·88	153	$x,y,z;\ \bar{x},\frac{1}{2}+y,\bar{z}$
N(12)–H(N12)···O(8)	2·841 (8)	0·88	1·99	160	$x,y,z;\ \bar{x},\frac{1}{2}+y,\bar{z}$

H···O and N–H···O hydrogen bonds (Fig. 2, Table 6) forming infinite layers parallel to the *yz* plane at a distance of 10·959 Å.

Both O(7) and O(8) in the carboxyl group are involved in hydrogen bonds: O(*W*)–H···O(8) 2·983(8), N(12)–H···O(8) 2·841(8), and N^+(3)–H···O(7) 2·698 (8) Å. N(3) (carrying a positive charge) in the thiazolidine ring is engaged in one more hydrogen bond of 2·756 (8) Å with a water molecule. The amide O atom is hydrogen-bonded to a water molecule by O(*W*)–H···O(14), 2·775 (8) Å.

The intensities were collected on a Philips PW 1100 diffractometer at the Department of General and Inorganic Chemistry, Faculty of Science, University of Zagreb. The authors thank Magistar Milenko Bruvo for collecting the data, and Drs J. J. Herak, M. Movačević and B. Gašpert for crystals and helpful comments.

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Crystal Structure of L-Tyrosyl-glycyl-glycine Monohydrate, the N-Terminal Tripeptide of the Enkephalins

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The tripeptide L-tyrosyl-glycyl-glycine, the N-terminal portion of endogenous pentapeptides with opiate activity (the enkephalins), crystallizes from acetic acid/isopropanol solutions as the hydrate ($C_{13}H_{17}N_3O_5.H_2O$). Crystals of the tripeptide are orthorhombic, a = 9·549 (2), b = 18·405 (5), c = 8·012 (1) Å, space group $P2_12_12_1$, with Z = 4. Data were collected on a four-circle diffractometer and the structure was solved by direct methods (R = 0·029). The molecule exists in the crystal as a zwitterion with extensive intermolecular hydrogen bonding. Although no intramolecular hydrogen bonds are present, the molecule assumes a conformation ($\varphi_2 = 81°$, $\psi_2 = 12°$) similar to that of a left-handed α-helix ($\varphi = 57°$, $\psi = 48°$). The average C_α^n–C_α^{n+1} is 3·80 (2) Å.

Introduction

The discovery (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) of endogenous materials in porcine brain that mimic opiate activity has generated a great deal of interest. These substances, designated enkephalins, were shown to be two pentapeptides: L-Tyr-Gly-Gly-Phe-Met and L-Tyr-Gly-Gly-Phe-Leu. It was further shown by Simantov, Kuhar, Pasternak & Snyder (1976) that these endogenous ligands were competitors for the opiate receptor sites in brain tissue, implicating a structural similarity between enkephalins

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