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Prolyl Endopeptidase Inhibitors. I. A Peptidyl α-Keto Ester Derivative

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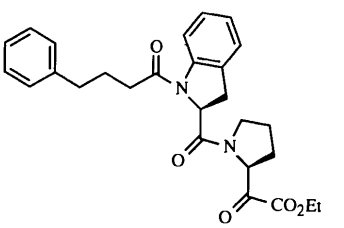
Abstract

In the crystal structure of the synthetic prolyl endopeptidase (PEP) inhibitor ethyl α-oxo-1-{1-(4-phenylbutanoyl)-2(*S*)-indolinoyl}-2(*S*)-pyrrolidineacetate, $C_{27}H_{30}N_2O_5$, the N-terminal amide bond is *cis*. The proline amide bond is *trans*. The dipeptide has a polyproline II conformation. The O atoms of both the ketone and the ester carbonyl groups point in the same direction.

Comment

Prolyl endopeptidase (PEP) (E.C. 3.4.21.26) (Welches, Brosnihan & Ferrario, 1993) is a serine protease that cleaves proline-containing peptides such as substance P, vasopressin and bradykinin. It is thought that PEP inhibitors may improve learning and memory by prolonging the half-life of neuropeptides (Angelucci *et al.*, 1993). A series of α-keto esters have been successfully incorporated into peptidyl protease inhibitors (Angelastro, Mehdi, Burkhart, Peet & Bey, 1990). In order to investigate the inhibition mechanism, it is important to determine the molecular structure of the α-keto ester inhibitor, (I), which is the subject of this study.



(I)

The title dipeptide, with φ_1 and ψ_1, and φ_2 and ψ_2 of $-59(1)$ and $154(9)$, and $-70(1)$ and $159(9)°$, respectively (IUPAC–IUB Commission on Biochemical Nomenclature, 1971), has a polyproline II conformation. The N-terminal amide bond is *cis* [C19—C18—N1—C4 $-8(1)°$]. The C-terminal proline amide bond is *trans*

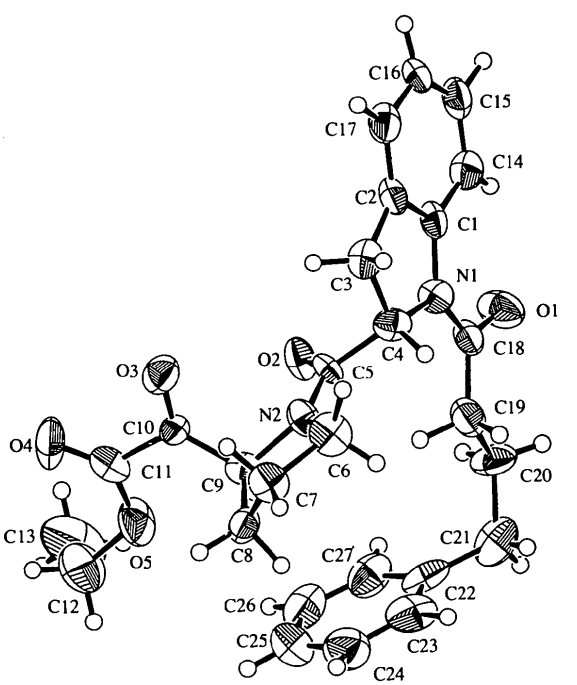


Fig. 1. The molecular structure of title compound with the crystallographic numbering scheme (*ORTEP*II; Johnson, 1976). Displacement ellipsoids are shown at the 50% probability level and H atoms are drawn as spheres of arbitrary size.

[C4—C5—N2—C9 −177 (9)°]. PEP reportedly has an absolute requirement for a *trans* peptide bond at the position preceding the peptide bond that is cleaved (Lin & Brandts, 1983). Therefore, the enzyme serine hydroxy group may react with the ketone carbonyl group at the C-terminus to form the tetrahedral intermediate. The carbonyl O atom (O3) of the ketone points in the same direction as the ester carbonyl O atom (O4). Knowledge of the conformation of the molecule might aid the design of α-keto ester bioisostere inhibitors.

Experimental

The title compound was synthesized according to the method described by Henning, Urbach & Hock (1991). Single crystals were grown from ethyl acetate solution.

Crystal data

$C_{27}H_{30}N_2O_5$	Cu $K\alpha$ radiation
M_r = 462.54	λ = 1.5418 Å
Orthorhombic	Cell parameters from 25 reflections
$P2_12_12_1$	θ = 24.89–42.53°
a = 15.209 (3) Å	μ = 0.674 mm^{-1}
b = 25.479 (4) Å	T = 23.0 K
c = 6.260 (2) Å	Needle
V = 2425.7 (9) Å^3	0.3 × 0.09 × 0.05 mm
Z = 4	Colorless
D_x = 1.266 Mg m^{-3}	

Data collection

AFC-5*R* diffractometer	θ_{max} = 60°
ω with profile analysis scans	$h = 0 \rightarrow 17$
Absorption correction: none	$k = 0 \rightarrow 28$
2126 measured reflections	$l = 0 \rightarrow 7$
2126 independent reflections	3 standard reflections monitored every 150 reflections
2098 observed reflections [$I > 0$]	intensity decay: 2.59%

Refinement

Refinement on F	$\Delta\rho_{max}$ = 0.57 e Å^{-3}
R = 0.229	$\Delta\rho_{min}$ = −0.66 e Å^{-3}
wR = 0.079	Extinction correction: type 2 Gaussian isotropic (Zachariasen, 1967)
S = 1.24	Extinction coefficient: 3.3 × 10^{-7}
2098 reflections	Atomic scattering factors from *International Tables for X-ray Crystallography* (1974, Vol. IV)
307 parameters	
H-atom parameters not refined	
Weighting scheme based on measured e.s.d.'s	
$(\Delta/\sigma)_{max}$ = 0.94	

Table 1. *Fractional atomic coordinates and equivalent isotropic displacement parameters* (Å^2)

$U_{eq} = (1/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	U_{eq}
O1	1.2366 (5)	0.7831 (3)	0.858 (1)	0.086 (3)
O2	1.0059 (4)	0.8615 (2)	0.797 (1)	0.061 (2)
O3	0.7949 (4)	0.8840 (3)	0.692 (1)	0.070 (3)
O4	0.7625 (6)	0.9881 (3)	0.761 (2)	0.120 (3)
O5	0.8894 (5)	1.0025 (3)	0.580 (2)	0.090 (3)
N1	1.0963 (5)	0.7657 (3)	0.772 (2)	0.051 (3)
N2	0.9403 (5)	0.8465 (3)	0.480 (2)	0.053 (4)
C1	1.0766 (6)	0.7314 (4)	0.943 (2)	0.050 (4)
C2	0.9911 (7)	0.7162 (4)	0.936 (2)	0.052 (4)
C3	0.9454 (6)	0.7397 (4)	0.745 (2)	0.058 (3)
C4	1.0155 (7)	0.7738 (4)	0.642 (2)	0.054 (4)
C5	0.9866 (7)	0.8314 (4)	0.646 (2)	0.046 (4)
C6	0.9124 (7)	0.8148 (4)	0.296 (2)	0.069 (4)
C7	0.8415 (7)	0.8501 (4)	0.190 (2)	0.063 (4)
C8	0.8776 (6)	0.9043 (4)	0.229 (2)	0.057 (4)
C9	0.9111 (6)	0.9017 (4)	0.456 (2)	0.050 (4)
C10	0.8437 (6)	0.9153 (4)	0.612 (2)	0.044 (4)
C11	0.826 (1)	0.9748 (5)	0.670 (2)	0.083 (5)
C12	0.878 (1)	1.0608 (5)	0.591 (3)	0.107 (6)
C13	0.919 (1)	1.0799 (5)	0.778 (3)	0.147 (7)
C14	1.1324 (7)	0.7133 (4)	1.104 (2)	0.064 (4)
C15	1.0965 (7)	0.6789 (4)	1.248 (2)	0.067 (4)
C16	1.0135 (8)	0.6646 (4)	1.243 (2)	0.069 (4)
C17	0.9589 (7)	0.6826 (4)	1.085 (2)	0.067 (4)
C18	1.1737 (7)	0.7903 (4)	0.737 (2)	0.053 (4)
C19	1.1812 (7)	0.8221 (4)	0.538 (3)	0.066 (4)
C20	1.2643 (7)	0.8554 (4)	0.534 (2)	0.074 (4)
C21	1.2744 (7)	0.8864 (5)	0.330 (2)	0.095 (5)
C22	1.2073 (8)	0.9269 (5)	0.292 (2)	0.071 (5)
C23	1.154 (1)	0.9257 (5)	0.117 (2)	0.086 (5)
C24	1.092 (1)	0.9639 (6)	0.071 (2)	0.094 (6)
C25	1.080 (1)	1.0040 (6)	0.216 (3)	0.097 (6)
C26	1.132 (1)	1.0057 (6)	0.397 (3)	0.088 (6)
C27	1.1921 (9)	0.9678 (6)	0.427 (2)	0.081 (5)

The intensities were extremely weak due to the very small size of the crystal and hence the value of R is high. Refinement using reflections with $I > 3\sigma(I)$ led to a better value of R, but with an unacceptably low observation-to-parameter ratio. This refinement, however, yielded geometrical parameters very close to those of the determination reported here, but with greater e.s.d.'s. H atoms were placed in calculated positions and were not refined.

Data collection: *MSC/AFC Diffractometer Control Software* (Molecular Structure Corporation, 1988). Cell refinement: *MSC/AFC Diffractometer Control Software*. Data reduction: *TEXSAN PROCESS* (Molecular Structure Corporation, 1989). Program(s) used to solve structure: *MITHRIL* (Gilmore, 1984). Program(s) used to refine structure: *TEXSAN LS*. Molecular graphics: *ORTEP*II (Johnson, 1976). Software used to prepare material for publication: *TEXSAN FINISH*.

Lists of structure factors, anisotropic displacement parameters, H-atom coordinates and complete geometry have been deposited with the IUCr (Reference: VJ1012). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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Prolyl Endopeptidase Inhibitors. II. A Peptidyl α-Keto Thiazole Derivative

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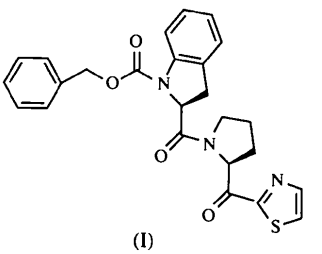
(*Received* 18 *April* 1994; *accepted* 16 *March* 1995)

Abstract

In benzyl 2-[2-(2-thiazoloyl)pyrrolidinoyl]indoline-1-carboxylate, $C_{25}H_{23}N_3O_4S$, the N-terminal urethane bond is *cis* and the proline amide bond is *trans*. The dipeptide adopts a polyproline II conformation and shows coplanarity of the ketone carbonyl group and the thiazole ring, with the carbonyl O atom *cis* with respect to the ring S atom.

Comment

The rational design and synthesis of protease inhibitors is an attractive field in medicinal and bioorganic chemistry (Rich, 1990). A general approach has been the replacement of the scissile amide unit by an electron-deficient carbonyl group (Wiley & Rich, 1993). Prolyl endopeptidase (PEP) (E.C. 3.4.21.26) is a serine protease that cleaves proline-containing peptides such as substance P, vasopressin and bradykinin (Welches, Brosnihan & Ferrario, 1993). It is thought that PEP inhibitors may improve learning and memory by prolonging the half-life of neuropeptides (Angelucci *et al.*, 1993). The peptidyl α-keto thiazole compound was found to be more potent as an inhibitor than both the α-keto ester and aldehyde derivatives (Tsutsumi *et al.*, 1994). The α-keto heterocyclic functional group may be an effective bioisostere of the α-keto ester moiety. The structure determination of the new α-keto thiazole inhibitor, (I), was undertaken as a step towards elucidating the inhibition mechanism.



(I)

The title dipeptide, with $\varphi_1 = -67(1)$, $\psi_1 = 143(9)$, $\varphi_2 = -74(1)$ and $\psi_2 = 150(1)°$ (IUPAC–IUB Commission on Biochemical Nomenclature, 1971), has a polyproline II conformation. Recent structure determinations of Src homology 3 (SH3) domains from PI3K, fyn and Grb2 complexed with proline-rich ligands indicate that SH3 domains recognize the polyproline II conformation (Lim, Richards & Fox, 1994; Feng, Chen, Yu, Simon & Schreiber, 1994). The present study may aid the design of small compounds interacting with SH3 domains. The N-terminal urethane bond is *cis* [O2—C9—N1—C8 −2(1)°]. Conformationally constrained amino acids like indolecarboxylic acid permit such a *cis* conformation (Magaard, Sanchez, Bean & Moore, 1993; Tsutsumi, Okonogi, Takeuchi & Kodama, 1995). The proline amide bond is *trans* [C8—C19—N2—C20 179(8)°]. The ketone carbonyl group and the thiazole ring are coplanar [O4—C21—C22—S1 −2(2) and N3—C22—C21—O4 173(1)°]. The carbonyl O atom of the ketone moiety and the S atom of the thiazole ring are *cis* with respect to each other.

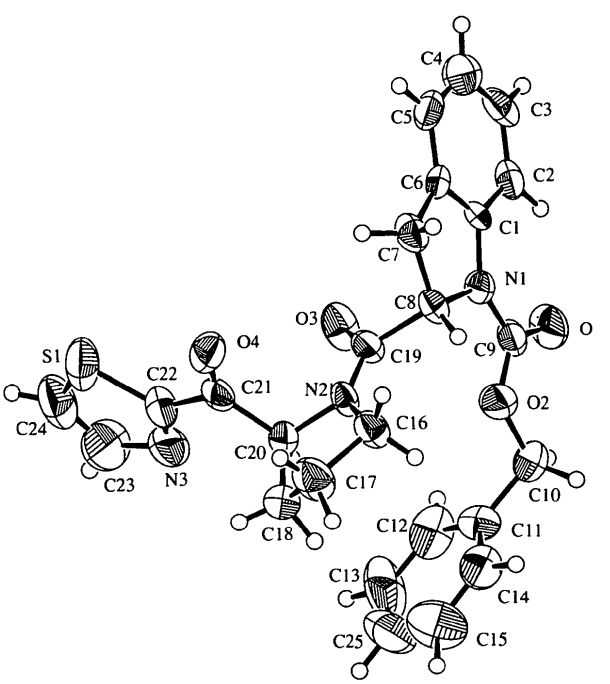


Fig. 1. The molecular structure of the title compound with the crystallographic numbering scheme (*ORTEPII*; Johnson, 1976). Displacement ellipsoids are shown at the 50% probability level and H atoms are drawn as spheres of arbitrary size.