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Hippuryl-L-histidyl-L-leucine, a Substrate for Angiotensin Converting Enzyme

ALICE VRIELINK,[a] ANDREAS OBEL-JORGENSEN[b] AND PENELOPE W. CODDING[b]

[a]*Department of Biochemistry, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6, and* [b]*Departments of Chemistry and of Pharmacology and Therapeutics, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada T2N 1N4. E-mail: pcodding@acs.ucalgary.ca*

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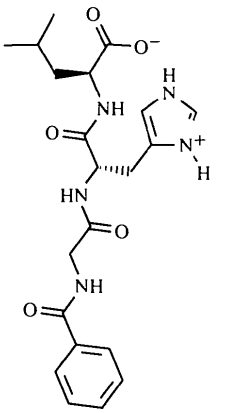
Abstract

The tripeptide crystallizes as a zwitterion with a protonated histidyl ring and the C-terminus ionized and with five water molecules of hydration ($C_{21}H_{27}N_5O_5.5H_2O$). The tripeptide adopts an all *trans* extended conformation with the histidine and phenyl rings parallel to one another. The C-terminus coils into a helical conformation. An intramolecular hydrogen bond between the C-terminus and the Nδ atom of the histidine ring stabilizes the helical conformation. The principal torsion angles are $\varphi_1 = -67.7(8)$, $\psi_1 = 140.8(5)$, $\omega_1 = 171.0(6)$, $\varphi_2 = -156.5(5)$, $\psi_2 = 162.7(5)$, $\omega_2 = 175.0(5)$, $\varphi_3 = -96.4(6)$, $\psi_T{}^1 = 14.5(8)$ and $\psi_T{}^2 = -164.6(6)^\circ$ [IUPAC–IUB Commission on Biochemical Nomenclature (1970). *J. Mol. Biol.* **52**, 1–17]. The tripeptides are linked in infinite chains through a short intermolecular hydrogen bond between the C-terminal carboxylate group and the protonated histidyl Nε atom.

Comment

Angiotensin converting enzyme (ACE) cleaves the neurotransmitter angiotensin(I) to the octapeptide angiotensin(II) and the dipeptide L-histidine-L-leucine. Kinetic studies of the inhibition of ACE are often carried out using hippuryl-L-histidyl-L-leucine (Hip-L-His-L-Leu) as the substrate (Cushman, Cheung, Sabo & Ondetti, 1977; Galardy, Kontoyiannidou-Ostrem & Kortylewicz, 1983; McEvoy, Lai & Albright, 1983; Cheung, Wang, Ondetti, Sabo & Cushman, 1980). Hence the structure of the substrate molecules should provide information regarding the geometric requirements of the ACE active site. In the crystal, the tripeptide terminates in a helical conformation stabilized by an intramolecular hydrogen bond, as has been found in other peptides containing a terminal sequence of L-histidine-L-leucine (Krause, Baures & Eggleston, 1993). The ten-membered ring thus formed, which was also observed in the dipeptide structure (Krause, Baures & Eggleston, 1993), is similar in conformation to a beta turn.



Hip-L-His-L-Leu

The geometric requirements of the ACE active site have been proposed by comparing the observed and preferred conformations of several ACE inhibitors (Vrielink, 1985; Hausin, 1989; Hausin & Codding, 1990, 1991). The crystallographic conformation observed for the ACE substrate is consistent with the postulated requirements for inhibitor binding to the enzyme active site (Hausin & Codding, 1990) and matches the low-energy conformation of a high-affinity inhibitor with a superposition of the atoms that are believed to be involved in binding to the enzyme to within 0.3 Å. The tripeptides are connected by a short, almost symmetrical intermolecular hydrogen bond between the protonated histidyl Nε atom, N2*E*, and the C-terminal O atom, O′, of the $(-\frac{1}{2} + x, -\frac{1}{2} - y, -z)$ symmetry-equivalent position.

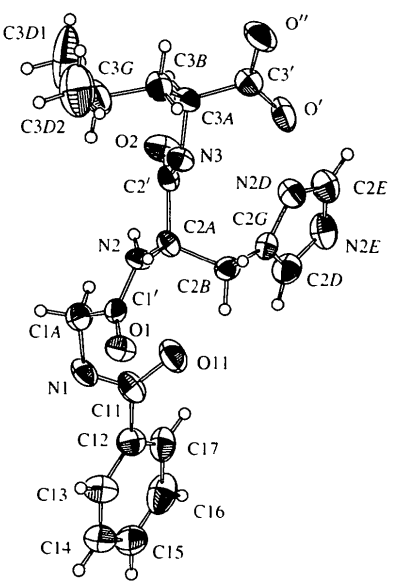


Fig. 1. The tripeptide structure and the atomic labeling scheme. Displacement ellipsoids are drawn at the 50% probability level and the H atoms are assigned arbitrary radii.

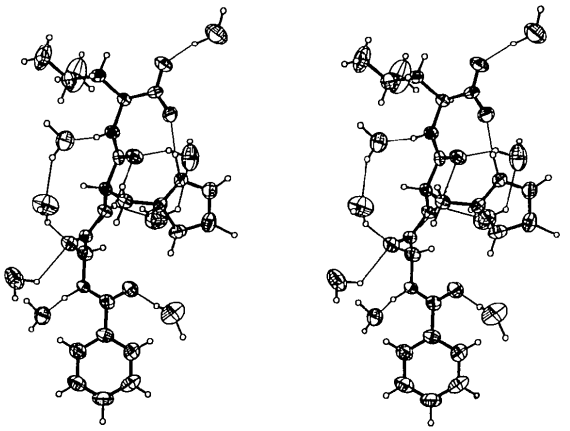


Fig. 2. A stereodrawing illustrating the intramolecular hydrogen bond between the C-terminal carboxylate and the histidyl N atom, as well as the intermolecular interactions with the sheath of water surrounding the tripeptide in the crystal. The structure is drawn as in Fig. 1.

Experimental

Hip-L-His-L-Leu was obtained from Peninsula Laboratories, Belmont, USA, and crystallized by slow evaporation from a methanol–water mixture.

Crystal data

$C_{21}H_{27}N_5O_5.5H_2O$
$M_r = 519.56$
Orthorhombic
$P2_12_12_1$
$a = 10.055\ (2)$ Å
$b = 14.974\ (4)$ Å
$c = 17.825\ (5)$ Å
$V = 2683.8\ (12)$ Å^3
$Z = 4$
$D_x = 1.286$ Mg m^{-3}
D_m not measured

Mo $K\alpha$ radiation
$\lambda = 0.71069$ Å
Cell parameters from 25 reflections
$\theta = 8.7$–$16.4°$
$\mu = 0.102$ mm^{-1}
$T = 293\ (2)$ K
Platelet
$0.4 \times 0.3 \times 0.1$ mm
Colorless

Data collection

Enraf–Nonius CAD-4*F* diffractometer
$\omega/2\theta$ scans
Absorption correction: none
4726 measured reflections
3467 independent reflections
2418 observed reflections $[I > 2\sigma(I)]$

$R_{int} = 0.0359$
$\theta_{max} = 22.43°$
$h = -10 \rightarrow 10$
$k = 0 \rightarrow 16$
$l = 0 \rightarrow 19$
3 standard reflections
frequency: 30 min
intensity decay: 4%

Refinement

Refinement on F^2
$R(F) = 0.0671$
$wR(F^2) = 0.1521$
$S = 0.986$
3463 reflections
325 parameters
H atoms: see below
$w = 1/[\sigma^2(F_o^2) + (0.1000P)^2]$ with $P = (F_o^2 + 2F_c^2)/3$

$(\Delta/\sigma)_{max} = 0.019$
$\Delta\rho_{max} = 0.189$ e Å^{-3}
$\Delta\rho_{min} = -0.202$ e Å^{-3}
Extinction correction: none
Atomic scattering factors from *International Tables for Crystallography* (1992, Vol. C, Tables 4.2.6.8 and 6.1.1.4)

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

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

	x	y	z	U_{eq}
O′	0.2830 (5)	−0.0767 (3)	0.0235 (3)	0.0576 (14)
O″	0.4149 (5)	0.0224 (3)	−0.0272 (3)	0.071 (2)
C3′	0.3148 (6)	0.0015 (5)	0.0086 (4)	0.042 (2)
C3*A*	0.2270 (6)	0.0790 (4)	0.0370 (3)	0.038 (2)
C3*B*	0.3072 (6)	0.1570 (4)	0.0654 (4)	0.044 (2)
C3*G*	0.2305 (8)	0.2393 (5)	0.0893 (5)	0.066 (2)
C3*D*1	0.1635 (12)	0.2823 (7)	0.0238 (7)	0.134 (5)
C3*D*2	0.3228 (10)	0.3059 (6)	0.1268 (6)	0.111 (4)
N3	0.1344 (5)	0.0489 (3)	0.0942 (3)	0.0358 (13)
O2	−0.0436 (4)	0.0267 (3)	0.0188 (2)	0.0531 (13)
C2′	0.0085 (6)	0.0260 (4)	0.0804 (3)	0.0323 (15)
C2*A*	−0.0646 (6)	−0.0113 (4)	0.1501 (3)	0.0344 (15)
C2*B*	−0.0177 (6)	−0.1055 (4)	0.1704 (3)	0.038 (2)
C2*G*	−0.0274 (6)	−0.1731 (4)	0.1096 (3)	0.0356 (15)
C2*D*	−0.1134 (7)	−0.2407 (5)	0.0983 (4)	0.052 (2)
N2*D*	0.0617 (5)	−0.1775 (3)	0.0509 (3)	0.0436 (14)
C2*E*	0.0295 (7)	−0.2451 (5)	0.0077 (4)	0.051 (2)
N2*E*	−0.0776 (6)	−0.2852 (4)	0.0342 (3)	0.052 (2)
N2	−0.2067 (5)	−0.0074 (3)	0.1351 (2)	0.0348 (12)
O1	−0.2633 (4)	−0.0118 (3)	0.2574 (2)	0.0458 (11)
C1′	−0.2949 (6)	−0.0049 (4)	0.1918 (3)	0.0348 (14)
C1*A*	−0.4379 (6)	0.0135 (4)	0.1667 (4)	0.046 (2)
N1	−0.5357 (5)	−0.0386 (4)	0.2080 (3)	0.0429 (14)
O11	−0.4565 (5)	−0.1673 (3)	0.1613 (3)	0.0596 (14)
C11	−0.5409 (6)	−0.1271 (5)	0.1992 (4)	0.045 (2)
C12	−0.6547 (6)	−0.1744 (5)	0.2365 (4)	0.047 (2)
C13	−0.7057 (7)	−0.1470 (5)	0.3051 (4)	0.058 (2)
C14	−0.8145 (8)	−0.1912 (6)	0.3365 (5)	0.069 (2)
C15	−0.8663 (8)	−0.2625 (6)	0.3003 (5)	0.072 (2)
C16	−0.8166 (8)	−0.2912 (5)	0.2322 (5)	0.070 (2)
C17	−0.7086 (7)	−0.2480 (5)	0.2005 (4)	0.054 (2)
*OW*1	−0.3909 (5)	−0.3427 (4)	0.1749 (4)	0.090 (2)
*OW*2	−0.0957 (6)	−0.0522 (4)	−0.1183 (3)	0.088 (2)
*OW*3	0.2120 (5)	0.0616 (4)	0.2493 (3)	0.072 (2)
*OW*4	−0.3265 (6)	−0.0666 (4)	−0.0074 (3)	0.092 (2)
*OW*5	−0.0071 (5)	0.0173 (5)	0.3339 (3)	0.093 (2)

Table 2. *Selected geometric parameters (Å, °)*

The bond lengths in the phenyl ring of the hippuryl residue ranged from 1.352 (11) to 1.395 (10) Å with an average value of 1.381 (14) Å. The internal bond angles in the ring ranged from 119.0 (8) to 121.7 (8)° with an average value of 120.0 (9)°.

O′—C3′	1.242 (7)	C2*B*—C2*G*	1.487 (8)
O″—C3′	1.232 (7)	C2*G*—C2*D*	1.346 (8)
C3′—C3*A*	1.543 (9)	C2*G*—N2*D*	1.380 (7)
C3*A*—N3	1.453 (7)	C2*D*—N2*E*	1.371 (9)
C3*A*—C3*B*	1.508 (8)	N2*D*—C2*E*	1.312 (8)
C3*B*—C3*G*	1.515 (9)	C2*E*—N2*E*	1.321 (9)
C3*G*—C3*D*1	1.494 (12)	N2—C1′	1.345 (7)
C3*G*—C3*D*2	1.518 (11)	O1—C1′	1.217 (7)
N3—C2′	1.335 (7)	C1′—C1*A*	1.531 (8)
O2—C2′	1.217 (7)	C1*A*—N1	1.454 (7)
C2′—C2*A*	1.549 (8)	N1—C11	1.335 (8)
C2*A*—N2	1.454 (7)	O11—C11	1.241 (7)
C2*A*—C2*B*	1.531 (8)	C11—C12	1.502 (9)
O″—C3′—O′	124.1 (6)	C2*G*—C2*B*—C2*A*	115.8 (5)
O″—C3′—C3*A*	116.5 (6)	C2*D*—C2*G*—N2*D*	105.6 (5)
O′—C3′—C3*A*	119.4 (6)	C2*D*—C2*G*—C2*B*	131.5 (6)
N3—C3*A*—C3*B*	110.3 (5)	N2*D*—C2*G*—C2*B*	122.9 (5)
N3—C3*A*—C3′	111.3 (5)	C2*G*—C2*D*—N2*E*	108.8 (6)
C3*B*—C3*A*—C3′	112.7 (5)	C2*E*—N2*D*—C2*G*	108.7 (5)
C3*A*—C3*B*—C3*G*	116.9 (6)	N2*D*—C2*E*—N2*E*	110.0 (6)
C3*D*1—C3*G*—C3*B*	111.1 (7)	C2*E*—N2*E*—C2*D*	106.9 (6)
C3*D*1—C3*G*—C3*D*2	109.7 (8)	C1′—N2—C2*A*	120.7 (5)
C3*B*—C3*G*—C3*D*2	110.3 (7)	O1—C1′—N2	123.1 (5)
C2′—N3—C3*A*	123.9 (5)	O1—C1′—C1*A*	122.8 (5)
O2—C2′—N3	125.0 (6)	N2—C1′—C1*A*	113.9 (5)
O2—C2′—C2*A*	121.5 (5)	N1—C1*A*—C1′	113.0 (5)
N3—C2′—C2*A*	113.2 (5)	C11—N1—C1*A*	120.0 (5)

N2—C2A—C2B	112.5 (5)	O11—C11—N1	121.3 (6)
N2—C2A—C2′	107.7 (5)	O11—C11—C12	122.2 (6)
C2B—C2A—C2′	112.1 (5)	N1—C11—C12	116.5 (6)

Table 3. *Hydrogen-bonding geometry (Å)*

N1···OW3^{i}	3.038 (7)	OW2···O1^{iii}	2.800 (7)
N2···OW4	2.948 (7)	OW2···O2	2.763 (6)
N2D···O′	2.733 (7)	OW3···OW5	2.751 (8)
N2E···O′^{ii}	2.701 (7)	OW3···OW1^{iv}	2.667 (7)
N3···OW3	2.878 (7)	OW4···OW2	3.056 (8)
OW1···O11	2.718 (7)	OW5···O′^{v}	2.710 (7)
OW1···OW2^{ii}	2.782 (8)	OW5···O1	2.947 (7)

Symmetry codes: (i) $x-1, y, z$; (ii) $x-\frac{1}{2}, -\frac{1}{2}-y, -z$; (iii) $-\frac{1}{2}-x, -y, z-\frac{1}{2}$; (iv) $-x, \frac{1}{2}+y, \frac{1}{2}-z$; (v) $\frac{1}{2}-x, -y, \frac{1}{2}+z$.

A single crystal was sealed in a 1.00 mm quartz capillary tube along with mother liquor (McPherson, 1982). The crystal diffracted poorly, undoubtedly due to the inclusion of five water molecules per tripeptide in the structure. Data extended to 1.03 Å resolution; the resulting number of observed reflections was therefore limited, providing insufficient data to refine H-atom parameters and producing large agreement factors. A difference electron density synthesis following the initial structure solution revealed the locations of five water molecules. In order to estimate the validity of parameters introduced in the refinement process, every tenth reflection was set aside for calculation of an R_{free} value (Brunger, 1992). The H atoms involved in intermolecular and intramolecular interactions were placed in observed positions found in electron difference density maps and refined as riding atoms, maintaining the observed non-H-atom–H-atom separation. Inspection of the R_{free} value favored this approach over positions generated by standard geometry. Lifting of the initial restraints in the refinement process resulted in a slight deformation of the phenyl ring. The final difference electron density map showed a uniform distribution of residual electron density with some indication of disorder of the leucine methyl C atoms.

Data collection: *DATCOL* for *CAD*-4 (Enraf–Nonius, 1982). Cell refinement: *TEXSAN* (Molecular Structure Corporation, 1993). Data reduction: *TEXSAN*. Program(s) used to solve structure: *SHELXS*86 (Sheldrick, 1985). Program(s) used to refine structure: *SHELXL*93 (Sheldrick, 1993). Molecular graphics: *ORTEP*II (Johnson, 1971). Software used to prepare material for publication: *SHELXL*93.

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Lists of structure factors, anisotropic displacement parameters, H-atom coordinates and complete geometry have been deposited with the IUCr (Reference: BK1197). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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A Side-Chain Substituted Cholesterol Analog

ZSOLT BÖCSKEI,[a]* ZSUZSA SZENDI[b] AND FREDERICK SWEET[c]

[a]Department of Chemical Research, Chinoin Pharmaceuticals, POB 110, 1325 Budapest, Hungary, [b]Department of Organic Chemistry, József Attila University, Dóm tér 8, H-6720 Szeged, Hungary, and [c]Department of Obstetrics and Gynecology, Washington University, School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, MO 63110, USA. E-mail: bocskei@ludens.elte.hu

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Abstract

The crystal structure of (20*R*,22*RS*)-27-norcholest-5-en-3β,20,22-triol 3,22-diacetate, $C_{30}H_{48}O_5$, is reported.

Comment

A mixture of (20*R*,22*RS*)-27-norcholest-5-en-3β,20,22-triol 3,22-diacetate was fractionally crystallized and provided a single isomer, (1), for the present X-ray analysis. The 20*R*/*S* mixture was obtained by thermal decomposition of the hydrazone by the Wolff–Kishner reduction of (20*R*)-3β,20,26-trihydroxy-27-norcholest-5-en-22-one, which undergoes a base-catalyzed 1,5-