Discussion. The *N*-methyl amide bond takes a *trans* form and its plane is almost vertical to the dichlorobenzene ring (Fig. 1). The cyclohexyl ring which takes a 'chair' form is separated from the aromatic ring by the amide bond. The pyrrolidine N(20) atom is in a protonated state and forms an ion pair with the methanesulfonate O(2)′ atom: N(20)—O(2)′ = 2·768 (3) Å. The S(1)′—O(2)′ bond of the methanesulfonate molecule is meaningfully longer than other S—O bonds (Table 2), and this would indicate the localization of a negative charge. The methanesulfonate molecule is further linked to a methanol solvent by a hydrogen bond: O(3)′—O(2)″ = 2·813 (6) Å. The methanesulfonate and methanol molecules compose a layer expanding along the *bc* plane, which is alternately arranged with the hydrophobic layer of U-50488 as shown in Fig. 2.

The U-50488 molecule assumes an 'opened' conformation to avoid intermolecular short contacts among the three bulky rings. Such a conformation would be expected in the absence of the organic acid (non-ionized state), and consequently the relative distance between the aromatic ring and the nitrogen N(20), which is very important to the analgesic activity of morphine, would be invariably kept. Tifluadom, a related κ-agonist which also has a protonated nitrogen and bulky rings like U-50488, was crystallized with a similar conformation (Petcher, Winder, Maetzel & Zeugner, 1985). Thus, such an 'opened' conformation would be closely related with the emergence of κ-agonist activity.

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Structure of the Tetrahydrate of the N-Terminal Tetrapeptide from Angiotensin II

BY SCOTT H. FELDMAN AND DRAKE S. EGGLESTON

Smith Kline & French Laboratories, Department of Physical and Structural Chemistry, L-950, PO Box 1539, King of Prussia, PA 19406, USA

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Abstract. L-Aspartyl-L-arginyl-L-valyl-L-tyrosine tetrahydrate, $C_{24}H_{37}N_7O_8.4H_2O$, $M_r = 623{\cdot}67$, triclinic, $P1$, $a = 4{\cdot}796\ (2)$, $b = 11{\cdot}791\ (5)$, $c = 13{\cdot}681\ (6)$ Å, $\alpha = 91{\cdot}91\ (2)$, $\beta = 92{\cdot}75\ (2)$, $\gamma = 90{\cdot}94\ (2)°$, $V = 772{\cdot}2\ (10)$ Å^3, $Z = 1$, $D_x = 1{\cdot}341$ Mg m^{-3}, λ(Cu $K\bar{\alpha}$) = 1·54184 Å, $\mu = 8{\cdot}714$ mm^{-1}, $F(000) = 334$, $T = 196$ K, $R = 0{\cdot}041$ for 2740 observations. The tetrapeptide comprises the first four residues of human angiotensin II. Crystals were grown *via* hanging-drop vapor diffusion against various high molarity salt solutions. The tetrapeptide is a double zwitterion in the crystal and adopts an extended conformation. Principal backbone torsion angles are $\psi_1 = 153{\cdot}2\ (2)$, $\omega_2 = 162{\cdot}0\ (2)$, $\varphi_2 = -106{\cdot}5\ (3)$, $\psi_2 = 120{\cdot}8\ (3)$, $\omega_3 = -168{\cdot}2\ (2)$, $\varphi_3 = -129{\cdot}6\ (3)$, $\psi_3 = 120{\cdot}1\ (3)$, $\omega_4 = -176{\cdot}0\ (2)$, $\varphi_4 = -107{\cdot}6\ (3)°$. The aspartyl side chain [$\chi_1^1 = -65{\cdot}4\ (3)°$] is hydrogen bonded intramolecularly to the N terminus. The tyrosyl ring sits over the preceeding peptide bond; the dihedral angle between phenolic and peptide planes is 38·9 (3)°. An extensive hydrogen bonding network exists in the crystals. The peptide backbone amides are hydrogen bonded in a parallel β-sheet motif. The guanidinum group of arginine participates in both a type *B* and a type *C* interaction.

Introduction. The renin–angiotensin system plays a key role in cardiovascular homeostasis with inappropriate activity implicated in the development of essential hypertension, renal disease and congestive heart failure (Capponi, Aquilera, Fakunding & Catt, 1981). Angiotensin II, an octapeptide first isolated in the 1950's (Peach, 1981) and an element of this system, is a potent endogenous vasoconstrictor with direct arterial action.

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This peptide continues to be the subject of considerable biological and physicochemical interest. An understanding of its structure will be useful in the design of angiotensin II antagonists for treatment of disease states consequential to its inappropriate activity. No crystal structure of the octapeptide, or a large fragment of it, has yet been reported. Solution studies by NMR have provided information, especially regarding the C-terminal residues (Weinkam & Jorgensen, 1971; Smeby & Fermandjian, 1978); however there is disagreement regarding key structural features of this region (Moore, 1985). Little study has been focused on the N-terminal residues despite the known importance of arginine in position 2 for both optimal agonist and antagonist activities (Khosla, Munoz-Ramirez, Hall, Khairallah & Bumpus, 1977) and the observation that modification of Asp[1] to sarcosine leads to potent antagonist activity (Hota, Ogihara, Mikami, Nakamura, Maruyama, Mandai & Kumahara, 1978).

We have undertaken studies to crystallize this important peptide and/or fragments thereof. The structure of the N-terminal tetrapeptide fragment, L-aspartyl-L-arginyl-L-valyl-L-tyrosine, represents the first contribution. This structure is also illustrative of the application of hanging-drop vapor diffusion techniques to growth of peptide crystals suitable for X-ray diffraction studies at atomic resolution.

Experimental. The peptide was purchased from Peninsula Laboratories, Belmont, CA 94002. Traditional slow evaporation experiments in a variety of solvent systems failed to yield crystals suitable for diffraction studies. Using hanging-drop vapor diffusion experiments, the general utility of which we have been investigating for peptide crystallization (Eggleston & Feldman, 1990), conditions which produced single crystals were identified, consuming in the process less than 2 mg of peptide. The crystal used for data collection grew in one day from a 10 mg ml^{-1} solution of peptide. The hanging drops were of 4 μL total volume, containing a 50:50 mixture of the peptide and precipitant solutions and were diffused against 1 ml wells containing 2 *M* sodium acetate, pH 6·8 at room temperature. Similarly, crystals can be obtained *via* diffusion against various concentrations of Li_2SO_4 or against mixtures of sodium acetate with 2-methyl-2,4-pentanediol or polyethylene glycols over a pH range of 6·8–7·6. We have not observed any evidence for crystal polymorphism in these systems.

The specimen used for data collection was approximately 0·10 × 0·20 × 0·65 mm on edge, had a lath shape and was mounted with epoxy on a glass fiber. Cell constants were determined from a least-squares analysis of 25 reflections [$60 \le 2\theta(\mathrm{Cu}) \le 70°$] measured on an Enraf–Nonius CAD-4 diffractometer equipped with a graphite monochromator and an Enraf–Nonius FR558 low-temperature system. Data were also collected on the diffractometer using variable speed (2·5 to 6·7 ° min^{-1}) ω–2θ scans. There were 3217 measured intensities, $2\theta \le 132°$; $0 \le h \le 5$, $-14 \le k \le 14$, $-16 \le l \le 16$. Data were corrected for Lorentz and polarization effects. A theta dependent correction for absorption was applied using the method of Walker & Stuart (1983). The correction factors from the *DIFABS* program were 0·729 (min.) and 1·255 (max.). No systematic fluctuations in the intensities of reflections 084, $\bar{2}63$, $\bar{1}6\bar{5}$, monitored at the beginning, end and each 3 h during data collection (15 times) were observed; mean values of F_o 324·9 (9), 418·1 (10), 310·2 (9), respectively. A unique set of 2846 intensities was obtained by averaging the $0kl/0\bar{k}\bar{l}$ and $0k\bar{l}/0\bar{k}l$ reflections; $R_{\mathrm{int}} = 0·030$. The structure was determined by direct methods. A 12 atom fragment obtained from *MULTAN*80 (Main, Fiske, Hull, Lessinger, Germain, Declercq & Woolfson, 1980) after removal of reflection 0,0,12 from consideration in the 'starting set' was expanded *via* least-squares refinement and difference Fourier syntheses. Eventually, non-H positions were refined with U_{ij}'s by full-matrix least squares (on *F*) minimizing the function $\sum w(|F_o| - |F_c|)^2$. The position of atom O1 was fixed to define the origin.

Positions of hydrogens attached to carbon were assigned from geometrical considerations assuming a C—H length of 1 Å. All H atoms attached to heteroatoms were located from difference maps and included in the refinement. Convergence, indicated by max. $(\Delta/\sigma) = 0·08$, led to final $R = 0·041$, $wR = 0·056$, $S = 2·084$ for 2740 observations with $I \ge 3\sigma(I)$, 385 variables. Weights were defined as $4F_o^2/s^2(I)$ where $s(I) = [\sigma^2(I_c) + 0·04(F_o)^2]^{1/2}$. Maximum final $|\Delta\rho|$ excursions were less than 0·276 e Å^{-3}. Refinement using all 2819 observations with $I \ge 0·01\sigma(I)$ gave $R = 0·042$. All programs used were from the locally modified CAD-4 *SDP* (Frenz, 1987); neutral atom scattering factors from *International Tables for X-ray Crystallography* (1974), for H from Stewart, Davidson & Simpson (1965).

Discussion. The molecular structure is displayed in Fig. 1; positional parameters are listed in Table 1 along with their standard deviations as estimated from the inverse least-squares matrix.* The tetrapeptide crystallizes as a double zwitterion with the amino terminus and guanidinium group protonated and the C-terminal and aspartyl carboxyl groups

* Lists of structure factors, H-atom positions and anisotropic librational parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52423 (21 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

ionized as indicated by nearly equivalent bond lengths (see Table 2) such as C1*G*—O1*D*1 = 1·251 (3), C1*G*—O1*D*2 = 1·247 (3), C2*Z*—N2*H*1 = 1·336 (3), C2*Z*—N2*H*2 = 1·340 (3) Å. The crystals also contain four molecules of water per asymmetric unit.

The overall peptide conformation is highly extended in a parallel β-sheet form which is maintained by hydrogen bonding interactions (see below) typical of such a structure. The principal backbone torsion angles are $\psi_1 = 153{\cdot}2$ (2), $\omega_2 = 162{\cdot}0$ (2), $\varphi_2 = -106{\cdot}5$ (3), $\psi_2 = 120{\cdot}8$ (3), $\omega_3 = -168{\cdot}2$ (2), $\varphi_3 = -129{\cdot}6$ (3), $\psi_3 = 120{\cdot}1$ (3), $\omega_4 = -176{\cdot}0$ (2), $\varphi_4 = -107{\cdot}6$ (3)°.

Amongst both cyclic and linear peptides, observations of non-planar peptide bonds, such as ω_2 and ω_3 in this structure, increase steadily. Out-of-plane amide bond deformations of the order of 12–18° require expenditure of less than 8 kJ mol^{-1} of energy (Winkler & Dunitz, 1971; Dunitz & Winkler, 1975). In this structure, such distortions may arise in response to requirements for formation of the parallel β-sheet conformation and are facilitated by formation of accompanying hydrogen bonds (see below).

The side-chain torsion angles are all typical for these residues. In the aspartyl side chain $\chi_1^1 = -65{\cdot}4$ (3), $\chi_1^{2,1} = 7{\cdot}9$ (4) and $\chi_1^{2,2} = 173{\cdot}0$ (2)°. The arginyl side chain is highly extended with $\chi_2^1 = -66{\cdot}9$ (3), $\chi_2^2 = 173{\cdot}0$ (2), $\chi_2^3 = 173{\cdot}0$ (2), $\chi_2^4 = 104{\cdot}4$ (3), $\chi_2^{5,1} = -7{\cdot}2$ (3), $\chi_2^{5,2} = 172{\cdot}8$ (3)°. Based on previous observations (Yokomori & Hodgson, 1988), this conformation presents the seventeenth variant documented for this very flexible side chain, in peptide crystal structures reported to date.

The valyl side chain adopts a conformation with $\chi_3^{1,1} = -52{\cdot}6$ (4) and $\chi_3^{1,2} = -175{\cdot}8$ (3)°, the conformation most commonly observed for valine in peptide structures (Ashida, Tsunogae, Tanaka & Yamane, 1987). The tyrosyl conformation is described by $\chi_4^1 = -54{\cdot}2$ (3), $\chi_4^{2,1} = 84{\cdot}7$ (4), $\chi_4^{2,2} = -94{\cdot}3$ (4)°. In this orientation the tyrosyl ring sits over the preceding peptide bond. The dihedral angle between the phenolic and peptide planes is 38·9 (3)° and the distances from the phenolic ring centroid to peptide bond atoms (N3, C2′, O2) range from 4·6 to 4·9 Å. Other examples of a tyrosine ring folding against an extended backbone are provided by conformers *A* and *D* in one of the enkephalin structures (Karle, Karle, Mastropaolo, Camerman & Camerman, 1983). In these latter instances, with Tyr at the peptidal N terminus, it is the succeeding peptide bond over which the phenolic ring folds. Such a recurring motif may indicate a preferred conformation for this side chain.

There is an extensive network of hydrogen bonding in the crystal, as detailed metrically in Table 3 and illustrated in part by Fig. 2. The protonated amino terminus donates intramolecularly through H3*N*1, to the ionized aspartyl carboxylate oxygen,

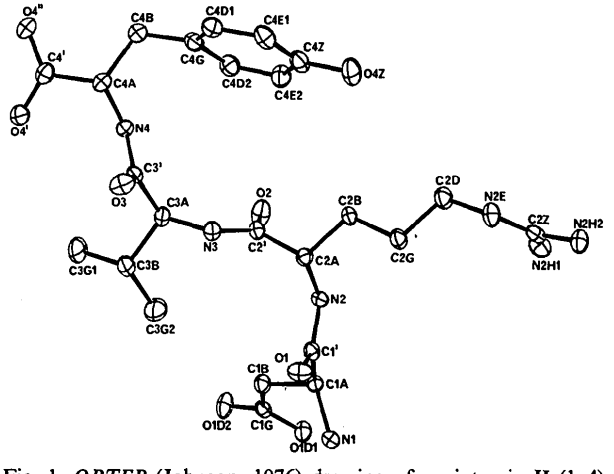

Fig. 1. *ORTEP* (Johnson, 1976) drawing of angiotensin II (1–4) with non-H atoms depicted at the 50% probability level, H atoms as spheres of arbitrary size.

Table 1. *Positional parameters and their estimated standard deviations*

	x	*y*	*z*	B_{eq}(Å^2)*
O1	0·834	0·776	0·923	2·16 (4)
O1*W*	0·0702 (5)	1·0307 (2)	0·7778 (2)	3·17 (5)
O2	0·1938 (4)	0·8793 (2)	1·1504 (2)	2·37 (4)
O2*W*	0·2247 (6)	1·4028 (2)	0·5459 (2)	3·34 (5)
O3	0·9467 (4)	0·8568 (2)	1·3959 (2)	2·34 (4)
O3*W*	0·1130 (6)	0·6310 (2)	1·5745 (3)	3·86 (6)
O4*W*	0·0662 (6)	0·3314 (3)	1·1172 (2)	4·26 (6)
O4′	0·6191 (6)	0·7625 (2)	1·6170 (2)	2·74 (5)
O4″	0·5470 (5)	0·9133 (2)	1·7132 (2)	2·45 (4)
O4*Z*	0·5535 (5)	1·3198 (2)	1·2156 (2)	2·72 (5)
O1*D*1	0·0429 (5)	0·5202 (2)	0·7998 (2)	1·87 (4)
O1*D*2	0·0196 (5)	0·4390 (2)	0·9435 (2)	2·43 (4)
N1	0·5360 (5)	0·6459 (2)	0·7786 (2)	1·54 (4)
N2	0·4355 (5)	0·8602 (2)	0·9693 (2)	1·38 (4)
N2*E*	0·6025 (5)	1·2889 (2)	0·8548 (2)	1·96 (4)
N3	0·6368 (5)	0·8530 (2)	1·2099 (2)	1·50 (4)
N4	0·5174 (5)	0·8806 (2)	1·4528 (2)	1·50 (4)
N2*H*1	0·2580 (5)	1·2535 (2)	0·7321 (2)	2·16 (5)
N2*H*2	0·6246 (6)	1·3751 (2)	0·7082 (2)	2·00 (5)
C1′	0·5796 (6)	0·7823 (2)	0·9203 (2)	1·42 (5)
C1*A*	0·3943 (6)	0·6938 (2)	0·8645 (2)	1·40 (5)
C1*G*	0·1110 (6)	0·5117 (2)	0·8886 (2)	1·45 (5)
C1*B*	0·3254 (6)	0·5985 (2)	0·9339 (2)	1·59 (5)
C2*G*	0·5936 (6)	1·1098 (2)	0·9470 (2)	1·71 (5)
C2′	0·4455 (6)	0·8872 (2)	1·1427 (2)	1·42 (5)
C2*A*	0·5587 (6)	0·9334 (2)	1·0490 (2)	1·38 (5)
C2*Z*	0·4955 (6)	1·3043 (2)	0·7666 (2)	1·78 (5)
C2*B*	0·4722 (6)	1·0567 (2)	1·0364 (2)	1·52 (5)
C2*D*	0·4711 (7)	1·2265 (3)	0·9319 (2)	2·04 (5)
C3′	0·6936 (6)	0·8454 (2)	1·3866 (2)	1·44 (5)
C3*A*	0·5585 (6)	0·7882 (2)	1·2931 (2)	1·55 (5)
C3*B*	0·6537 (7)	0·6645 (3)	1·2840 (2)	2·19 (6)
C4*A*	0·6078 (6)	0·9425 (3)	1·5422 (2)	1·68 (5)
C4′	0·5858 (6)	0·8682 (3)	1·6319 (2)	1·85 (5)
C4*B*	0·4514 (7)	1·0550 (3)	1·5519 (2)	2·03 (5)
C4*G*	0·4741 (6)	1·1272 (3)	1·4639 (2)	1·93 (5)
C4*Z*	0·5209 (6)	1·2564 (3)	1·2965 (2)	2·17 (6)
C3*G*1	0·548 (1)	0·5975 (3)	1·3690 (3)	3·32 (8)
C3*G*2	0·553 (1)	0·6109 (3)	1·1857 (3)	3·72 (8)
C4*D*1	0·2914 (7)	1·1098 (3)	1·3819 (3)	2·14 (6)
C4*E*1	0·3164 (7)	1·1720 (3)	1·2986 (2)	2·25 (6)
C4*E*2	0·7025 (7)	1·2755 (3)	1·3775 (3)	2·40 (6)
C4*D*2	0·6777 (7)	1·2117 (3)	1·4603 (2)	2·21 (6)

*$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

Table 2. *Principal bond distances* (Å) *and angles* (°)

O1—C1′	1·222 (3)	C1′—C1*A*	1·521 (3)
O2—C2′	1·219 (3)	C1*A*—C1*B*	1·538 (4)
O3—C3′	1·219 (3)	C1*G*—C1*B*	1·533 (3)
O4′—C4′	1·270 (3)	C2*G*—C2*B*	1·529 (3)
O4″—C4′	1·241 (3)	C2*G*—C2*D*	1·521 (3)
O4*Z*—C4*Z*	1·370 (3)	C2′—C2*A*	1·530 (3)
O1*D*1—C1*G*	1·251 (3)	C2*A*—C2*B*	1·531 (3)
O1*D*2—C1*G*	1·247 (3)	C3′—C3*A*	1·535 (3)
N1—C1*A*	1·485 (3)	C3*A*—C3*B*	1·538 (3)
N2—C1′	1·340 (3)	C3*B*—C3*G*1	1·529 (3)
N2—C2*A*	1·463 (3)	C3*B*—C3*G*2	1·521 (3)
N2*E*—C2*Z*	1·307 (3)	C4*A*—C4′	1·538 (3)
N2*E*—C2*D*	1·467 (3)	C4*A*—C4*B*	1·539 (3)
N3—C2′	1·343 (3)	C4*B*—C4*G*	1·505 (3)
N3—C3*A*	1·453 (3)	C4*G*—C4*D*1	1·397 (3)
N4—C3′	1·329 (3)	C4*G*—C4*D*2	1·388 (3)
N4—C4*A*	1·448 (3)	C4*Z*—C4*E*1	1·388 (3)
N2*H*1—C2*Z*	1·336 (3)	C4*Z*—C4*E*2	1·386 (3)
N2*H*2—C2*Z*	1·340 (3)	C4*D*1—C4*E*1	1·385 (3)
		C4*E*2—C4*D*2	1·388 (3)

C1′—N2—C2*A*	123·5 (2)	O3—C3′—N4	124·3 (2)
C2*Z*—N2*E*—C2*D*	126·3 (2)	O3—C3′—C3*A*	120·1 (2)
C2′—N3—C3*A*	121·7 (2)	N4—C3′—C3*A*	115·6 (2)
C3′—N4—C4*A*	122·9 (2)	N3—C3*A*—C3′	108·4 (2)
O1—C1′—N2	125·4 (2)	N3—C3*A*—C3*B*	111·6 (2)
O1—C1′—C1*A*	121·2 (2)	C3′—C3*A*—C3*B*	109·7 (2)
N2—C1′—C1*A*	113·3 (2)	C3*A*—C3*B*—C3*G*1	109·8 (2)
N1—C1*A*—C1′	110·9 (2)	C3*A*—C3*B*—C3*G*2	110·6 (2)
N1—C1*A*—C1*B*	109·8 (2)	C3*G*1—C3*B*—C3*G*2	111·3 (2)
C1′—C1*A*—C1*B*	108·8 (2)	N4—C4*A*—C4′	111·3 (2)
O1*D*1—C1*G*—O1*D*2	125·7 (2)	N4—C4*A*—C4*B*	110·7 (2)
O1*D*1—C1*G*—C1*B*	117·1 (2)	C4′—C4*A*—C4*B*	113·2 (2)
O1*D*2—C1*G*—C1*B*	117·1 (1)	O4′—C4′—O4″	124·2 (2)
C1*A*—C1*B*—C1*G*	113·0 (2)	O4′—C4′—C4*A*	116·2 (2)
C2*B*—C2*G*—C2*D*	110·0 (2)	O4″—C4′—C4*A*	119·6 (2)
O2—C2′—N3	124·5 (2)	C4*A*—C4*B*—C4*G*	112·9 (2)
O2—C2′—C2*A*	119·3 (2)	C4*B*—C4*G*—C4*D*1	120·7 (2)
N3—C2′—C2*A*	116·2 (2)	C4*B*—C4*G*—C4*D*2	121·7 (2)
N2—C2*A*—C2′	105·5 (2)	C4*D*1—C4*G*—C4*D*2	117·6 (2)
N2—C2*A*—C2*B*	110·7 (2)	O4*Z*—C4*Z*—C4*E*1	121·9 (2)
C2′—C2*A*—C2*B*	110·5 (2)	O4*Z*—C4*Z*—C4*E*2	118·8 (2)
N2*E*—C2*Z*—N2*H*1	122·7 (2)	C4*E*1—C4*Z*—C4*E*2	119·3 (2)
N2*E*—C2*Z*—N2*H*2	119·3 (2)	C4*G*—C4*D*1—C4*E*1	121·6 (2)
N2*H*1—C2*Z*—N2*H*2	117·9 (2)	C4*Z*—C4*E*1—C4*D*1	119·9 (2)
C2*G*—C2*B*—C2*A*	113·0 (2)	C4*Z*—C4*E*2—C4*D*2	120·3 (2)
N2*E*—C2*D*—C2*G*	113·3 (2)	C4*G*—C4*D*2—C4*E*2	121·3 (2)

Table 3. *Hydrogen bonding interactions* (Å *and* °)

				Sym. Op.*
N1···O1*D*1	2·876 (3)	N1—H1*N*1···O1*D*1	172 (1)	1,100
H1*N*1···O1*D*1	1·94 (2)			
N1···O4′	2·684 (4)	N1—H2*N*1···O4′	167 (1)	1,00$\bar{1}$
H2*N*1···O4′	1·77 (2)			
N1···O1*D*1	2·802 (3)	N1—H3*N*1···O1*D*1	128 (1)	1,000
H3*N*1···O1*D*1	2·07 (2)			
N2···O1	3·068 (2)	N2—H*N*2···O1	154 (1)	1,$\bar{1}$00
H*N*2···O1	2·23 (2)			
N3···O2	2·845 (3)	N3—H*N*3···O2	159 (1)	1,100
H*N*3···O2	1·95 (2)			
N4···O3	2·816 (3)	N4—H*N*4···O3	165 (1)	1,$\bar{1}$00
H*N*4···O3	1·91 (2)			
N2*E*···O1*D*2	2·850 (3)	N2*E*—H*N*2*E*···O1*D*2	167 (1)	1,110
H*N*2*E*···O1*D*2	1·97 (2)			
N2*H*1···O2*W*	3·143 (4)	N2*H*1—N1*N*2*H*1···O2*W*	151 (1)	1,000
H1*N*2*H*1···O2*W*	2·25 (2)			
N2*H*1···O1*W*	2·862 (4)	N2*H*1—H2*N*2*H*1···O1*W*	147 (1)	1,000
H2*N*2*H*1···O1*W*	1·97 (2)			
N2*H*2···O2*W*	2·894 (3)	N2*H*2—H1*N*2*H*2···O2*W*	140 (1)	1,000
H1*N*2*H*2···O2*W*	2·14			
N2*H*2···O1*D*1	2·833 (3)	N2*H*2—H2*N*2···O1*D*1	161 (1)	1,110
H2*N*2*H*2···O1*D*1	1·92			
O4*Z*···O4*W*	2·648 (4)	O4*Z*—H*O*4*Z*···O4*W*	157 (1)	1,010
H*O*4*Z*···O4*W*	1·77			
O1*W*···O4″	2·930 (3)	O1*W*—H1*O*1*W*···O4″	167 (1)	1,$\bar{1}$0$\bar{1}$
H1*O*1*W*···O4″	2·08			
O1*W*···O4″	2·853 (4)	O1*W*—H2*O*1*W*···O4″	165 (1)	1,00$\bar{1}$
H2*O*1*W*···O4″	1·76			
O2*W*···O3*W*	2·771 (4)	O2*W*—H1*O*2*W*···O3*W*	168 (1)	1,01$\bar{1}$
H1*O*2*W*···O3*W*	1·99			
O3*W*···O4′	2·923 (4)	O3*W*—H1*O*3*W*···O4′	170 (1)	1,$\bar{1}$00
H1*O*3*W*···O4′	2·21			
O3*W*···O4′	2·883 (4)	O3*W*—H2*O*3*W*···O4′	155 (1)	1,000
H2*O*3*W*···O4′	1·98			
O4*W*···O1*D*2	2·732 (4)	O4*W*—H1*O*4*W*···O1*D*2	158 (1)	1,000
H1*O*4*W*···O1*D*2	1·75			
O4*W*···O4*Z*	2·862 (4)	O4*W*—H2*O*4*W*···O4*Z*	153 (1)	1,$\bar{1}$$\bar{1}$0
H2*O*4*W*···O4*Z*	1·97			

* Translations are along *x*, *y* and *z* directions, respectively.

O1*D*1. Such intramolecular interactions involving the Asp side chain have often been inferred in protein crystallographic results but are rarely observed in structures of peptides containing this residue (Eggleston, 1989). The arginyl guanidinum group participates in one type *B* interaction (Salunke & Vijayan, 1981) involving, intermolecularly, a convergent, coplanar pair of hydrogen bonds from N2*E* and N2*H*2 to the aspartyl side chain. Type *B* interactions have been suggested from theoretical calculations (Sapse & Russell, 1984) to be more favorable than those of type *A* (defined as a convergent, coplanar pair of hydrogen bonds from N2*H*1 and N2*H*2 to both oxygens of a carboxylate group). Statistically, however, the latter have been observed more frequently in peptide structures containing arginine. There also is a type *C* interaction involving water oxygen O2*W* and convergent hydrogen bonds from N2*H*1 and N2*H*2. The extended parallel β-sheet-like structure is maintained by three hydrogen bonds between backbone peptide bond atoms parallel to the short *a* axis, as illustrated in Fig. 3. Additional hydrogen bonds involve the water molecules, all four of which participate in trigonal interactions. One water, O3*W*, bridges between the C terminus of molecules translated along the *a* axis. The 'head-to-tail' packing of translation related molecules along *c* is characterized by only one hydrogen bond between the protonated amino terminus and ionized carboxyl.

Most models of angiotensin II–receptor binding (for reviews see Marshall, Bosshard, Vine, Glickson & Needleman, 1974; Smeby & Fermandjian, 1978; Capponi *et al.*, 1981) focus on aspects of the C-terminal pentapeptide. Potent antagonists of A-II have been developed, however, by replacement of Asp[1] with the *N*-methylamino acid sarcosine (Case, Wallace & Laragh, 1979). A Sar[1] substitution may block formation of an intramolecular hydrogen bond between the N terminus and the aspartyl side chain such as the one observed in this structure. The importance of Arg[2] for both agonist and antagonist activity (Bumpus & Khosla, 1977; Samanen *et al.*, 1988) may arise from its ability to form convergent parallel planar hydrogen bonds with receptor residues such as the type *B* and *C* interactions we observe. Furthermore, the overall highly extended conformations of the side chains and their many interactions with waters of crystallization are observations which agree with free accessibility to solvent suggested from NMR studies (Smeby & Fermandjian, 1978). Similarly, the tetrapeptide itself

has been studied in solution (Smeby & Fermandjian, 1978). Chemical shift and temperature dependent NMR studies suggest that, in zwitterionic form, the Arg[2] and Val[3] amide protons are protected from solvent or are hydrogen bonded while that of Tyr is undetermined. The crystal structure is fully in agreement with NMR results on these points. In the crystal, the observed hydrogen bonds are of a parallel β-strand form; an observation also compatible with some models of the larger structure derived from solution studies (Smeby & Fermandjian, 1978; Marshall *et al.*, 1974).

It would seem, therefore, that our crystal structure results are highly relevant to observations in solution and to possible models for angiotensin–receptor interactions. Certainly the plethora of intermolecular interactions we observe in this structure gives some hint as to plausible roles for backbone and side-chain functional groups in a receptor environment.

Based on the results of many physicochemical studies it may be concluded that angiotensin II displays well defined conformations in solution. Preliminary observations in our hanging-drop vapor diffusion experiments (Eggleston & Feldman, 1990) give us optimism for defining proper conditions to select out one (or more) of these conformations in a crystalline environment.

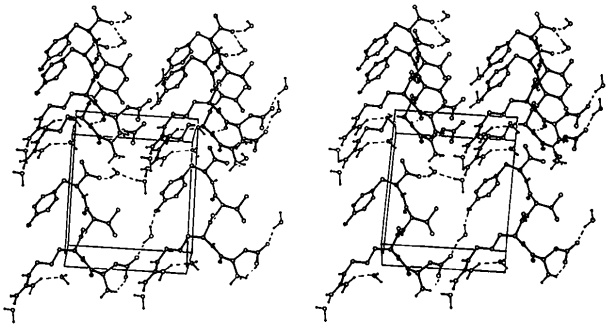

Fig. 2. Stereodiagram of the unit cell illustrating hydrogen-bonding interactions of angiotensin II (1–4). Only hydrogens attached to heteroatoms have been included. The *c* and *b* axes parallel the horizontal and vertical directions, respectively.

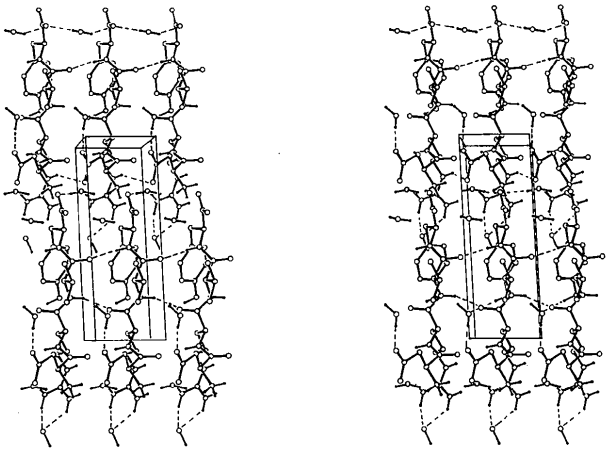

Fig. 3. Stereodiagram of the unit cell illustrating hydrogen-bonding interactions forming the parallel β-sheet structure as well as linkages through water between C termini. The view has the *a* axis horizontal and the *c* axis vertical.

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