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THE CONFORMATION OF DEAMINO-OXYTOCIN: X-RAY ANALYSIS OF THE 'DRY' AND 'WET' FORMS

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[Plates 1 and 2]

CONTENTS

Two crystal structures of (1β-mercaptopropionic acid) deamino-oxytocin are reported. The 'dry form' in space group $C2$ has cell dimensions $a = 27.08 \pm 0.03$, $b = 9.06 \pm 0.01$, $c = 22.98 \pm 0.02$ Å, $\beta = 102.06 \pm 0.03$ with one deamino-oxytocin and six water molecules per asymmetric unit. The 'wet form' in space group $P2_1$ has cell dimensions $a = 27.27 \pm 0.02$, $b = 9.04 \pm 0.01$, $c = 23.04 \pm 0.02$ Å, $\beta = 102.24 \pm 0.02$, with two deamino-oxytocin and 13 water molecules per asymmetric

unit. A local twofold parallel to the monoclinic axis gives a pseudo $C2$ packing. Initial phases of the 'dry form' were calculated by the heavy-atom method from the isomorphous and anomalous difference Pattersons and anomalous difference Fourier synthesis. The structure was refined by using restrained least-squares at 1.2 Å resolution to a crystallographic $R = 0.10$. The molecular replacement method yielded the $P2_1$ structure that was refined with geometric restraints to $R < 0.09$, by using all data to 1.09 Å resolution. Deamino-oxytocin consists of a cyclic tocin ring formed by six amino acids, closed by a disulphide bridge, S1—S6, and held by two *trans*-annular hydrogen bonds N2—O5 and N5—O2 with a type II turn at residues 3 and 4. A flexible tripeptide tail has a loosely hydrogen-bonded type I beta-turn between N9 and O6. The sulphur of cysteine at position 1 is disordered in all the molecules leading to alternative hands of disulphide. The conformational flexibility of Ile 3, Asn 5, Pro 7 side chains and the disulphide bridge is consistent with previous models of oxytocin in which flexibility is necessary for biological activity.

1. Introduction

Oxytocin and vasopressin are nonapeptide hormones (figure 1) that are produced in the hypothalamo–neurohypophyseal system of the brain (Acher 1979; Breslow *et al.* 1981). Initially, the hormones and their particular carrier proteins, the neurophysins, which are derived from larger precursors, are synthesized in paraventricular and supraoptic nerve cells of the hypothalamus (Richter 1983; Theodosis 1985). On completion of the synthesis of the inactive hormone precursors, they are transported in secretory granules down axons to the pituitary gland. During this transport, the precursors undergo proteolytic degradation by specific enzymes to produce complexes of neurophysins and their respective hormones.

	S——S								
	1	2	3	4	5	6	7	8	9
oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly-NH_2
mesotocin								Ile	
valitocin								Val	
vasotocin								Arg	
glumitocin				Ser				Gln	
isotocin				Ser				Ile	
arginine vasopressin			Phe	Gln				Arg	
lysosine			Phe					Lys	
aspartocin				Asn				Leu	

Arg at 8 → pressor or antidiuretic (only one property)

Arg at 8 + Phe at 3 pressor + antidiuretic (both properties)

Figure 1. Chemical formulae of oxytocin and analogous hormones.

Oxytocin elicits contraction of the smooth muscle of the uterus for parturition (utertonic effect) and mammary glands for milk ejection (galactobolic effect), and increase in glucose oxidation by adipose cells (insulin-like effect). Vasopressin causes water retention (antidiuretic effect), contraction of blood-vessel muscle to increase systemic blood pressure (vasopressor effect) and breakdown of glycogen in the liver (glycogenolytic effect) (Berde & Boissonnas 1968). Other neurohypophyseal hormones (Acher 1985; Chauvet *et al.* 1983), which belong to the same class of nonapeptides as oxytocin and vasopressin, have a wide range of functions

in the central nervous system, including effects on maternal behaviour, grooming behaviour, lordosis, memory, etc. (Sawyer 1977; Richter & Crabbe 1979; van Nispen *et al.* 1983; Hoffman 1987).

To relate the structure of the hormone to its activity, many analogues have been synthesized (du Vigneaud *et al.* 1960; Ferrier *et al.* 1965). This resulted in the design of highly selective, long acting superagonists and antagonists (Rudinger 1972; Manning *et al.* 1977; Bankowski *et al.* 1980; Hruby 1986; Hruby & Smith 1987). Among these synthetic hormone analogues, deamino-oxytocin (1β-mercaptropropionate oxytocin) was found to be almost twice as potent as the natural hormone (Hope 1962; Ferrier 1965).

To classify these hormone analogues in terms of their structure–activity relations, it is necessary to analyse their three-dimensional structures. As most residues in each sequence of this family of neurohypophyseal hormones are invariant and the presence of a disulphide bridge between residues 1 and 6 is ubiquitous, the overall structures of these peptide hormones are expected to be very similar.

Spectroscopic techniques such as a nuclear magnetic resonance (NMR) and circular dichroism (CD) have defined the general features of the model in various solvents. The first plausible model proposed by Urry & Walter (1971) was based on ^{1}H-NMR of oxytocin in dimethyl sulphoxide (DMSO). The main features of this model are: (i) a relatively planar 20-membered ring; (ii) two β-turns held by intramolecular hydrogen bonds; (iii) the tripeptide tail positioned over the tocin ring.

Although some analyses (Craig *et al.* 1964; Urry *et al.* 1970; Brewster & Hruby 1973; Tu *et al.* 1979; Roy *et al.* 1980*a*, *b*; Krauss & Cowburn 1981) confirmed important aspects of the model, others (Feeney *et al.* 1971; Brewster *et al.* 1973*a*; Ford & Gibbons 1979; Kotelchuck *et al.* 1972*a*, *b*) suggested different conformational features. In fact, NMR studies made on oxytocin in DMSO and in water show that the hormone has different conformations in different solvents. Accordingly, Hruby (1981) suggested that the studies of oxytocin in water could be relevant to its conformation in the plasma while those in hydrophobic DMSO could produce a conformation of the hormone similar to that adopted during receptor binding.

Crystallographic studies were first attempted in 1952 when oxytocin crystals were reported (Pierce & du Vigneaud 1950*a*, *b*, 1952), but these proved unsuccessful in defining the structure. At a later time, crystal data on deamino-oxytocin (Low & Chen 1966) and its 6-seleno-deamino-oxytocin analogue (Chui *et al.* 1969) were reported, but analyses of the structures of these hormone analogues also proved difficult.

Because of the lack of success encountered earlier, we synthesized fresh materials, grew new crystals and recollected X-ray data in our laboratory. A preliminary report of these studies has been described by Wood *et al.* (1986). In this paper, the X-ray analysis and refinement of the 'dry form' of the deamino-oxytocin, which crystallizes in space group $C2$ is described. We then discuss the use of the atomic coordinates of this structure in the solution of the crystal structure of the related 'wet form' of space group $P2_1$. Finally, we describe the conformations of the deamino-oxytocin molecules found in crystals and discuss their possible relations to conformers found in solution.

2. Techniques

(*a*) *Synthesis*

Deamino-oxytocin was synthesized and purified after the method of du Vigneaud *et al.* (1960) by using methods developed in our laboratory (Hruby *et al.* 1977).

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(*b*) *Crystallization*

Deamino-oxytocin and the 6-seleno-analogue were crystallized by cooling from 35–40 °C to room temperature (18 °C) at a peptide concentration of 40 mg ml^{-1}. Deamino-oxytocin crystals grew from 20 % ethanol while the 6-seleno-deamino-oxytocin crystals drew from water that was purged with nitrogen to reduce oxidation of the seleno-compound. Deamino-oxytocin crystallized in space group $P2_1$ with pseudo $C2$ symmetry in which $h+k = 2n+1$ reflections are systematically weak. One crystal that was left to dry in air for several months was found to have undergone a space group change to $C2$. This was termed the 'dry form' and the $P2_1$ type was termed the 'wet form'. Crystal data are shown in table 1.

(*c*) *Data Collection*

Data were collected by using a Hilger–Watts 4-circle diffractometer and by using the variable background–peak–background method of Tickle (1975). All equivalents were measured to a resolution of 1.20 Å for the 'dry form'. Data were corrected for absorption by using the method of North *et al.* (1968), for Lorentz and polarization effects and for radiation damage on the basis of repeated measurements of standard reflections. Statistics for the data collection are shown in table 1.

TABLE 1. X-RAY CRYSTAL STRUCTURE PARAMETERS OF THE 'WET' AND 'DRY' FORMS

	dry form	wet form	6-seleno
		space group	
cell dimensions	$C2$	$P2_1$ (pseudo $C2$)	$C2$
a	27.08±0.03 Å	27.27±0.02 Å	27.26±0.04 Å
b	9.06±0.01 Å	9.04±0.01 Å	9.19±0.05 Å
c	22.98±0.02 Å	23.04±0.02 Å	23.01±0.04 Å
β	102.06±0.03°	102.24±0.02°	102.72°
volume	5513.55 Å^3	5550.73 Å^3	5545Å
ρ_m	1.328±0.005 g/ml[a]	1.328±0.005 g/ml[a]	1.36 g/ml[b]
μ	14.33	14.34	17.02
F_{000}	2352	2372	2504
Z	4	4	4
collecting collimator diameter	3.5 mm	3.5 mm	3.5 mm
goniometer axis	a*	b*	b*
resolution	1.2 Å	1.09 Å	1.98 Å
unique reflections	1840	4681	425
R_{sym}	0.057	0.031	0.052
number of anomalous pairs	1507	—	307
absorption curves	200, 400, 600 & 1000	0–20, 0–60 0–80	0–20
number of water molecules	6	13	6
R value	0.10	0.09	—

[a] Low & Chen 1966. [b] Chiu *et al.* 1969.

3. STRUCTURE DETERMINATION AND REFINEMENT OF THE 'DRY FORM'

Initial phase determination involved the data of the dried form of deamino-oxytocin and the 6-seleno-deamino-oxytocin both having the same space group $C2$ and being isomorphous. First, the position of selenium at residue 6 was obtained from an isomorphous difference Patterson (figure 2*a*) with coefficients $(F_s\text{-}F_{se})^2$ where F_s and F_{se} are the structure factor amplitudes for the deamino-oxytocin and 6-seleno-deamino-oxytocin, respectively. This was confirmed by an anomalous difference Patterson (figure 2*b*) with coefficients $(F_{se}(+)^- F_{se(-)})^2$. Refinement of the selenium atom with centric reflections gave a residual R value of

43 %. The phases due to the single selenium atom were not sufficient to interpret the map. The Harker section of an anomalous difference Patterson (figure 2*c*) for the dried deamino-oxytocin with coefficients $(F_{s(+)}\text{-}F_{s(-)})^2$ showed an extended peak at the same location as a peak in the anomalous difference Patterson of the seleno-analogue. An anomalous difference Fourier with coefficients $(F_{s(+)}\text{-}F_{s(-)})$ (figure 2*d*) phased on the SG6 at 1.2 Å† resolution determined the position of SG_1, although it became apparent later that the rather broad peak showed disorder of this atom.

Phases were calculated by the method of isomorphous replacement combined with the anomalous scattering. This included the isomorphous contribution due to the selenium

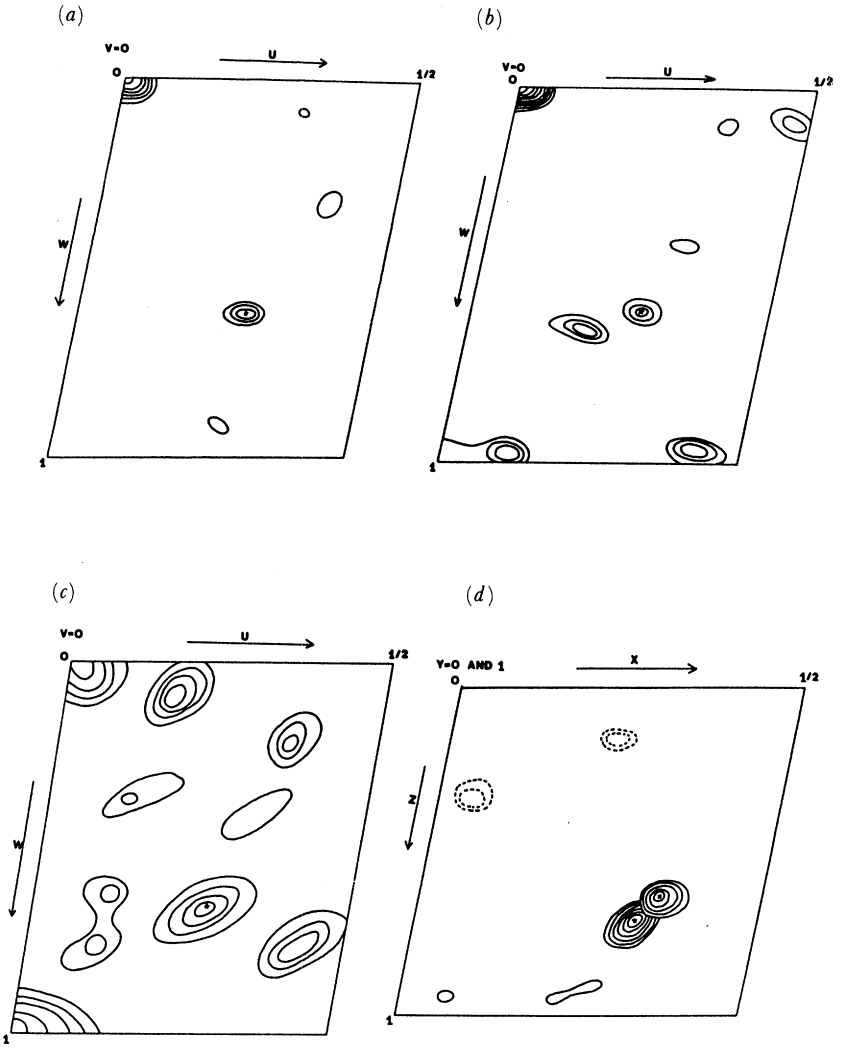

FIGURE 2. Patterson and Fourier maps of the *C*2 crystal form of deamino-oxytocin and its seleno-analogue. (*a*) Harker section of the isomorphous difference Patterson with coefficients $(F_s\text{-}F_{se})^2$. (*b*) Harker section of the anomalous difference Patterson with coefficients $(F_{se(+)}\text{-}F_{se(-)})^2$. (*c*) Harker section of the anomalous differences Patterson with coefficients $(F_{s(+)}\text{-}F_{s(-)})^2$. (*d*) Anomalous difference Fourier map with coefficients $(F_{s(+)}\text{-}F_{s(-)})$ and single isomorphous replacement phases. *, Sulphur 6/Selenium 6 position; +, Sulphur 1 position; (——) = positive density; (----) = negative density.

† 1 Å = 10^{-10} m = 10^{-1} nm.

replacement of sulphur, the anomalous scattering of sulphur and selenium atoms in the 6-seleno derivative and the anomalous scattering due to the sulphur atoms in the deamino-oxytocin. The mean figure of merit was 68% to 2.1 Å resolution. The resultant 'best'-phased map looked more like a 3.5 Å resolution map. By using the most probable conformation of the tocin ring and the 3-residue tail consistent with spectroscopic data, a model of deamino-oxytocin was built on the Evans and Sutherland PS2 computer graphics by using FRODO, which uses standard peptide geometry for model building (Jones 1978). Restrained refinement of the model of deamino-oxytocin in reciprocal space was initiated by using RESTRAIN (Haneef *et al.* 1985) but proved to be unsuccessful. This was because the sulphur atoms, especially those at SG_1 were disordered, as became apparent later. SHELX (Sheldrick 1976) on the NAS 7000, was then used to refine the crystal structure of deamino-oxytocin without any restraint during initial isotropic refinement. The progress of the refinement, shown in figure 3, was monitored at frequent intervals by calculating Sim-weighted (Sim 1959, 1960) difference Fourier (wF_{obs}-F_{calc}) and ($2F_{obs}$-F_{calc}) maps. Although the R value decreased to 0.30, the Fourier maps showed both positive and negative density in the vicinity of the Ile 3 and Gln 4 positions, which had been modelled as a type I β-turn based on Nuclear Overhauser Effect (NOE) data (Ford & Gibbons 1979). Several attempts were made to reposition the ring with retention of the conformation, but these were unsuccessful. Eventually a type II turn was modelled, although the absence of a glycine or asparagine at the second position in the turn (that is, at residue 4) led to the conclusion that this was unlikely. Nevertheless, refinement progressed smoothly and the R value decreased to 0.23. Five water molecules were located in the difference Fourier synthesis and were included in the refinement, the R value converged to 0.19.

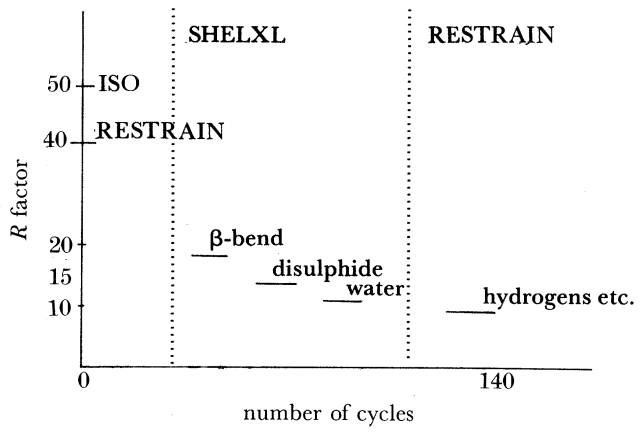

FIGURE 3. Diagram showing the fall in R-factor against number of cycles of refinement.

Throughout the refinement, the electron density map around the disulphide region appeared to be rather noisy, with both sulphur atoms having relatively high thermal parameters. In the later stages of the refinement it became apparent that there are two possible conformations for the disulphide bridge. One conformation resulted in a C—S—S—C torsion angle of +76° with right-handed chirality and the other having left-handed chirality with a corresponding torsion angle of −101°. The two conformations of the disulphide were modelled into the electron density by assigning alternate sites to SG 1, without disturbing the other non-hydrogen atoms, because of the parallel nature of CA1—CB1 and CB6—SG6 bonds.

All non-hydrogen atoms were refined anisotropically ($R = 0.13$) and hydrogens were riding on the carbon and nitrogen atoms. A sixth water molecule was found from the difference map to be on a crystallographic twofold axis. The final R value was 0.10. The final refined coordinates of 'dry form' in the space group $C2$ are listed in table 2*a* and the anisotropic thermal parameters shown in table 3*a*.

TABLE 2*a*. REFINED FRACTIONAL COORDINATES OF MOLECULE C IN $C2$

atom	x	y	z	Occ
Ca 1	0.46354	0.65309	0.59431	1.00
SG 1A	0.36934	0.62528	0.62559	0.55
CB 1A	0.42392	0.53885	0.60704	0.55
SG 1B	0.42200	0.53455	0.57184	0.45
CB 1B	0.41491	0.43389	0.63912	0.45
C 1	0.51750	0.58907	0.62541	1.00
O 1	0.53514	0.47958	0.60534	1.00
N 2	0.53819	0.66888	0.67027	1.00
OH 2	0.60930	0.93764	0.45267	1.00
CD 22	0.64996	0.74890	0.59395	1.00
CE 22	0.64850	0.79183	0.53575	1.00
CZ 2	0.61281	0.89481	0.51043	1.00
CE 12	0.58031	0.96082	0.54291	1.00
CD 12	0.58391	0.91921	0.60196	1.00
CG 2	0.62010	0.81854	0.62715	1.00
CB 2	0.62353	0.77583	0.69278	1.00
CA 2	0.59151	0.64106	0.70004	1.00
C 2	0.59216	0.61744	0.76580	1.00
O 2	0.55588	0.65762	0.78632	1.00
N 3	0.63444	0.56711	0.79935	1.00
CD 13	0.70995	0.21744	0.95536	1.00
CG 13	0.67976	0.35983	0.94335	1.00
CB 3	0.68249	0.43356	0.88563	1.00
CG 23	0.73142	0.50111	0.88061	1.00
CA 3	0.63922	0.53676	0.86299	1.00
C 3	0.64515	0.67771	0.90019	1.00
O 3	0.66745	0.78808	0.88639	1.00
N 4	0.59971	0.68775	0.92737	1.00
NE 24	0.55732	0.81038	1.09362	1.00
OE 14	0.56934	0.56667	1.08018	1.00
CD 4	0.58579	0.70497	1.08049	1.00
CG 4	0.63572	0.73930	1.06180	1.00
CB 4	0.62585	0.85817	1.01316	1.00
CA 4	0.58592	0.81667	0.95852	1.00
C 4	0.57125	0.94735	0.91803	1.00
O 4	0.57102	1.07230	0.93841	1.00
N 5	0.55205	0.91777	0.86098	1.00
ND 25	0.56329	1.26612	0.70480	1.00
OD 15	0.50657	1.31049	0.76625	1.00
CG 5	0.54459	1.23808	0.75223	1.00
CB 5	0.56727	1.11347	0.79330	1.00
CA 5	0.52757	1.03024	0.81911	1.00
C 5	0.49074	0.95552	0.76883	1.00
O 5	0.49777	0.95298	0.71828	1.00
N 6	0.45275	0.88665	0.78618	1.00
CA 6	0.41272	0.81137	0.74449	1.00
SG 6A	0.36436	0.55386	0.70582	0.55
CB 6A	0.41553	0.65950	0.76180	0.55
SG 6B	0.37427	0.55508	0.68695	0.45
CB 6B	0.42121	0.63433	0.74738	0.45
C 6	0.36241	0.87450	0.75423	1.00

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Table 2*a*. (*cont.*)

O 6	0.34342	0.80905	0.79085	1.00
N 7	0.33994	0.98333	0.72059	1.00
CG 7	0.31874	1.16667	0.65033	1.00
CD 7	0.36232	1.06512	0.67663	1.00
CB 7	0.29229	1.19647	0.70195	1.00
CA 7	0.29606	1.05420	0.73719	1.00
C 7	0.24923	0.96005	0.71859	1.00
O 7	0.20929	0.99768	0.73101	1.00
N 8	0.25239	0.84150	0.68562	1.00
CD 28	0.17407	0.80673	0.53268	1.00
CD 18	0.23718	0.61965	0.51706	1.00
CG 8	0.22176	0.72484	0.56236	1.00
CB 8	0.21851	0.63941	0.61776	1.00
CA 8	0.20893	0.74338	0.66706	1.00
C 8	0.20008	0.64526	0.71775	1.00
O 8	0.15729	0.60817	0.72117	1.00
N 9	0.23950	0.59537	0.75561	1.00
CA 9	0.23254	0.51005	0.80736	1.00
C 9	0.28003	0.43521	0.83798	1.00
O 9	0.31988	0.45751	0.82280	1.00
NXT 9	0.27443	0.35022	0.88390	1.00

Table 2*b*. Refined fractional coordinates of molecule A in $P2_1$

atom	x	y	z	Occ	U_{iso}	atom	x	y	z	Occ	U_{iso}
SG 1	0.878	0.619	0.376	0.71		N 5	0.698	0.916	0.151	1.00	
SG 1	0.842	0.456	0.344	0.44		HN 5	0.695	0.804	0.165	1.00	0.048
CB 1	0.826	0.533	0.407	1.00	0.217	CA 5	0.721	1.025	0.194	1.00	
HB 11	0.859	0.593	0.430	1.00	1.154	HA 5	0.740	1.108	0.173	1.00	0.036
HB 21	0.819	0.440	0.434	1.00	1.003	CB 5	0.681	1.100	0.220	1.00	
CA 1	0.784	0.632	0.406	1.00	0.070	HB 15	0.659	1.169	0.186	1.00	0.055
HA 11	0.785	0.668	0.451	1.00	0.485	HB 25	0.658	1.014	0.232	1.00	0.124
HA 21	0.788	0.727	0.379	1.00	0.036	CG 5	0.701	1.196	0.275	1.00	
C 1	0.733	0.563	0.381	1.00		CD 15	0.746	1.253	0.281	1.00	
O 1	0.713	0.464	0.402	1.00		ND 25	0.674	1.220	0.316	1.00	
N 2	0.709	0.641	0.331	1.00		HD 15	0.692	1.296	0.347	1.00	0.157
HN 2	0.731	0.724	0.313	1.00	0.137	HD 25	0.637	1.201	0.321	1.00	0.031
CA 2	0.657	0.616	0.301	1.00		C 5	0.760	0.952	0.242	1.00	
HA 2	0.643	0.519	0.319	1.00	0.073	O 5	0.753	0.914	0.290	1.00	
CB 2	0.624	0.747	0.310	1.00		N 6	0.802	0.917	0.221	1.00	
HB 12	0.585	0.715	0.293	1.00	0.549	HN 6	0.805	0.947	0.176	1.00	0.473
HB 22	0.633	0.839	0.284	1.00	0.044	CA 6	0.842	0.838	0.260	1.00	
CG 2	0.629	0.797	0.373	1.00		HA 6	0.841	0.851	0.306	1.00	0.048
CD 12	0.665	0.900	0.399	1.00		CB 6	0.839	0.673	0.246	1.00	
HD 12	0.691	0.943	0.373	1.00	0.144	HB 16	0.803	0.633	0.251	1.00	0.072
CD 22	0.598	0.741	0.408	1.00		HB 26	0.843	0.655	0.201	1.00	0.620
HD 22	0.571	0.656	0.391	1.00	0.035	SG 6	0.888	0.570	0.297	1.00	
CE 12	0.669	0.949	0.457	1.00		C 6	0.889	0.898	0.244	1.00	
HE 12	0.698	1.027	0.475	1.00	0.095	O 6	0.909	0.854	0.206	1.00	
CE 22	0.601	0.794	0.465	1.00		N 7	0.914	1.010	0.283	1.00	
HE 22	0.575	0.753	0.491	1.00	0.210	CA 7	0.958	1.082	0.269	1.00	
CZ 2	0.636	0.899	0.491	1.00		HA 7	0.955	1.101	0.222	1.00	0.024
OH 2	0.637	0.946	0.546	1.00		CB 7	0.963	1.223	0.304	1.00	
C 2	0.657	0.603	0.235	1.00		HB 17	0.946	1.314	0.277	1.00	0.166
O 2	0.692	0.633	0.213	1.00		HB 27	1.002	1.249	0.322	1.00	0.188
N 3	0.612	0.547	0.202	1.00		CG 7	0.937	1.186	0.352	1.00	
HN 3	0.583	0.512	0.224	1.00	0.390	HG 17	0.962	1.130	0.388	1.00	0.105
CA 3	0.604	0.537	0.138	1.00		HG 27	0.923	1.287	0.369	1.00	0.164
HA 3	0.630	0.461	0.125	1.00	0.112	CD 7	0.892	1.088	0.329	1.00	

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Table 2*b*. (*cont.*)

CB 3	0.550	0.477	0.113	1.00		HD 17	0.859	1.150	0.309	1.00	0.642
HB 13	0.524	0.539	0.133	1.00	0.056	HD 27	0.884	1.013	0.362	1.00	0.582
CG 13	0.546	0.318	0.130	1.00		C 7	1.005	0.987	0.285	1.00	
HG 13	0.565	0.303	0.176	1.00	0.377	O 7	1.044	1.020	0.272	1.00	
HG 23	0.506	0.292	0.126	1.00	0.044	N 8	1.001	0.857	0.318	1.00	
CG 23	0.534	0.503	0.044	1.00		HN 8	0.967	0.829	0.330	1.00	0.000
HG 33	0.538	0.619	0.035	1.00	1.475	CA 8	1.044	0.760	0.334	1.00	
HG 43	0.495	0.471	0.029	1.00	1.272	HA 8	1.076	0.831	0.350	1.00	0.056
HG 53	0.557	0.438	0.022	1.00	0.181	CB 8	1.036	0.656	0.382	1.00	
CD 13	0.568	0.215	0.091	1.00		HB 18	1.003	0.590	0.365	1.00	0.050
HD 13	0.564	0.102	0.105	1.00	1.296	HB 28	1.068	0.585	0.394	1.00	0.069
HD 23	0.607	0.241	0.095	1.00	0.320	CG 8	1.028	0.732	0.439	1.00	
HD 33	0.549	0.229	0.046	1.00	0.069	HG 8	0.998	0.810	0.427	1.00	0.125
C 3	0.613	0.686	0.112	1.00		CD 18	1.015	0.618	0.481	1.00	
O 3	0.586	0.794	0.15	1.00		HD 18	1.009	0.671	0.521	1.00	0.032
N 4	0.650	0.686	0.080	1.00		HD 28	1.045	0.538	0.492	1.00	0.013
HN 4	0.671	0.585	0.077	1.00	0.226	HD 38	0.980	0.562	0.460	1.00	0.099
CA 4	0.664	0.823	0.050	1.00		CD 28	1.074	0.819	0.469	1.00	
HA 4	0.698	0.793	0.035	1.00	0.056	HD 48	1.095	0.846	0.437	1.00	0.136
CB 4	0.624	0.864	−0.004	1.00		HD 58	1.087	0.840	0.514	1.00	0.256
HB 14	0.633	0.969	−0.021	1.00	0.061	HD 68	1.107	0.761	0.486	1.00	0.113
HB 24	0.588	0.874	0.009	1.00	0.154	C 8	1.056	0.674	0.283	1.00	
CG 4	0.613	0.750	−0.053	1.00		O 8	1.099	0.642	0.280	1.00	
HG 14	0.591	0.791	−0.091	1.00	0.042	N 9	1.015	0.618	0.242	1.00	
HG 24	0.604	0.649	−0.038	1.00	0.043	HN 9	0.977	0.642	0.246	1.00	0.208
CD 4	0.666	0.716	−0.073	1.00		CA 9	1.026	0.527	0.195	1.00	
NE 24	0.682	0.578	−0.073	1.00		HA 19	1.041	0.592	0.164	1.00	0.793
HE 14	0.716	0.547	−0.082	1.00	0.120	HA 29	1.052	0.439	0.213	1.00	0.278
HE 24	0.660	0.485	−0.069	1.00	0.105	C 9	0.976	0.463	0.166	1.00	
OE 14	0.694	0.824	−0.084	1.00		O 9	0.935	0.482	0.179	1.00	
C 4	0.679	0.954	0.091	1.00		NXT 9	0.979	0.365	0.119	1.00	
O 4	0.680	1.082	0.075	1.00							

Table 2*c*. Refined fractional coordinates of molecule B in $P2_1$

atom	*x*	*y*	*z*	Occ	U_{iso}	atom	*x*	*y*	*z*	Occ	U_{iso}
SG 1	0.666	0.436	0.646	0.70	—	N 5	0.806	0.922	0.865	1.00	—
SG 1	0.633	0.632	0.621	0.29	—	HN 5	0.808	0.812	0.847	1.00	0.875
CB 1	0.671	0.558	0.581	1.00	—	CA 5	0.781	1.038	0.829	1.00	—
HB 11	0.656	0.557	0.533	1.00	1.029	HA 5	0.761	1.111	0.851	1.00	0.255
HB 21	0.683	0.448	0.596	1.00	0.752	CB 5	0.819	1.134	0.804	1.00	—
CA 1	0.713	0.673	0.599	1.00	—	HB 15	0.840	1.066	0.779	1.00	0.160
HA 11	0.703	0.745	0.633	1.00	0.063	HB 25	0.846	1.182	0.841	1.00	0.016
HA 21	0.716	0.738	0.560	1.00	0.121	CG 5	0.791	1.257	0.765	1.00	—
C 1	0.764	0.608	0.624	1.00	—	OD 15	0.755	1.329	0.783	1.00	—
O 1	0.781	0.496	0.608	1.00	—	ND 25	0.807	1.295	0.716	1.00	—
N 2	0.788	0.691	0.674	1.00	—	HD 15	0.794	1.362	0.679	1.00	0.093
HN 2	0.780	0.784	0.699	1.00	0.528	HD 25	0.837	1.232	0.706	1.00	0.200
CA 2	0.842	0.662	0.702	1.00	—	C 5	0.745	0.962	0.776	1.00	—
HA 2	0.857	0.567	0.683	1.00	0.030	O 5	0.748	0.973	0.723	1.00	—
CB 2	0.873	0.794	0.692	1.00	—	N 6	0.707	0.883	0.794	1.00	—
HB 12	0.912	0.768	0.710	1.00	0.107	HN 6	0.705	0.877	0.840	1.00	0.146
HB 22	0.862	0.888	0.715	1.00	0.201	CA 6	0.669	0.809	0.749	1.00	—
CG 2	0.868	0.833	0.628	1.00	—	HA 6	0.673	0.834	0.704	1.00	0.113
CD 12	0.836	0.945	0.601	1.00	—	CB 6	0.673	0.641	0.757	1.00	—
HD 12	0.816	1.009	0.628	1.00	0.146	HB 16	0.710	0.605	0.753	1.00	0.104
CD 22	0.896	0.757	0.595	1.00	—	HB 26	0.667	0.612	0.801	1.00	0.236
HD 22	0.921	0.669	0.615	1.00	0.115	SG 6	0.626	0.548	0.700	1.00	—

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Table 2*c*. (*cont.*)

CE 12	0.829	0.978	0.541	1.00	—	C 6	0.619	0.861	0.760	1.00	—
HE 12	0.801	1.057	0.520	1.00	0.901	O 6	0.599	0.810	0.798	1.00	—
CE 22	0.892	0.795	0.536	1.00	—	N 7	0.596	0.978	0.725	1.00	—
HE 22	0.915	0.738	0.510	1.00	0.003	CA 7	0.550	1.044	0.738	1.00	—
CZ 2	0.860	0.905	0.509	1.00	—	HA 7	0.553	1.060	0.785	1.00	0.118
OH 2	0.855	0.940	0.450	1.00	—	CB 7	0.545	1.189	0.703	1.00	—
C 2	0.843	0.637	0.767	1.00	—	HB 17	0.565	1.278	0.729	1.00	0.065
O 2	0.808	0.671	0.791	1.00	—	HB 27	0.506	1.219	0.687	1.00	0.069
N 3	0.886	0.573	0.799	1.00	—	CG 7	0.570	1.152	0.651	1.00	—
HN 3	0.917	0.548	0.778	1.00	0.092	HG 17	0.581	1.251	0.631	1.00	0.218
CA 3	0.890	0.539	0.862	1.00	—	HG 27	0.544	1.088	0.617	1.00	0.048
HA 3	0.857	0.482	0.870	1.00	0.017	CD 7	0.615	1.060	0.679	1.00	—
CB 3	0.933	0.431	0.881	1.00	—	HD 17	0.646	1.130	0.699	1.00	0.462
HB 3	0.930	0.340	0.850	1.00	0.046	HD 27	0.626	0.986	0.648	1.00	0.018
CG 13	0.930	0.374	0.943	1.00	—	C 7	0.505	0.947	0.722	1.00	—
HG 13	0.944	0.459	0.976	1.00	0.121	O 7	0.466	0.980	0.737	1.00	—
HG 23	0.891	0.352	0.944	1.00	0.126	N 8	0.509	0.824	0.687	1.00	
CG 23	0.983	0.503	0.882	1.00	—	HN 8	0.544	0.800	0.673	1.00	0.483
HG 33	1.013	0.424	0.896	1.00	0.327	CA 8	0.465	0.726	0.669	1.00	—
HG 43	0.988	0.595	0.913	1.00	0.399	HA 8	0.432	0.794	0.659	1.00	0.121
HG 53	0.984	0.543	0.838	1.00	0.409	CB 8	0.473	0.640	0.615	1.00	—
CD 13	0.961	0.232	0.959	1.00	—	HB 18	0.444	0.557	0.604	1.00	0.282
HD 13	0.958	0.195	1.002	1.00	0.409	HB 28	0.509	0.586	0.626	1.00	0.042
HD 23	1.000	0.254	0.958	1.00	0.716	CG 8	0.472	0.735	0.560	1.00	—
HD 33	0.947	0.147	0.927	1.00	0.277	HG 8	0.499	0.824	0.571	1.00	0.063
C 3	0.889	0.680	0.897	1.00	—	CD 18	0.485	0.636	0.512	1.00	—
O 3	0.919	0.783	0.898	1.00	—	HD 18	0.484	0.700	0.472	1.00	0.182
N 4	0.852	0.685	0.931	1.00	—	HD 28	0.458	0.547	0.502	1.00	0.040
HN 4	0.830	0.586	0.932	1.00	0.082	HD 38	0.522	0.590	0.527	1.00	0.093
CA 4	0.840	0.815	0.965	1.00	—	CD 28	0.421	0.811	0.537	1.00	—
HA 4	0.806	0.791	0.980	1.00	0.016	HD 48	0.413	0.881	0.572	1.00	0.366
CB 4	0.880	0.841	1.020	1.00	—	HD 58	0.392	0.729	0.525	1.00	0.679
HB 14	0.916	0.852	1.088	1.00	0.001	HD 68	0.423	0.878	0.499	1.00	0.669
HB 24	0.872	0.941	1.041	1.00	0.065	C 8	0.456	0.622	0.716	1.00	—
CG 4	0.883	0.714	1.065	1.00	—	O 8	0.415	0.574	0.717	1.00	—
HG 14	0.908	0.745	1.106	1.00	0.058	N 9	0.497	0.569	0.757	1.00	—
HG 24	0.898	0.617	1.046	1.00	0.080	HN 9	0.534	0.610	0.759	1.00	0.113
CD 4	0.833	0.676	1.077	1.00	—	CA 9	0.488	0.457	0.798	1.00	—
NE 24	0.820	0.534	1.077	1.00	—	HA 19	0.461	0.496	0.823	1.00	0.105
HE 14	0.785	0.495	1.080	1.00	0.249	HA 29	0.474	0.357	0.775	1.00	0.272
HE 24	0.842	0.448	1.068	1.00	0.172	C 9	0.537	0.426	0.841	1.00	—
OE 14	0.805	0.788	1.089	1.00	—	O 9	0.578	0.449	0.830	1.00	—
C 4	0.827	0.951	0.926	1.00	—	NXT 9	0.530	0.342	0.889	1.00	—
O 4	0.830	1.076	0.945	1.00	—						

Table 2*d*. Refined fractional coordinates of water molecules in $P2_1$

atom	*x*	*y*	*z*
O A	0.776	0.449	0.903
O B	0.660	0.266	0.810
O C	0.628	0.288	0.973
O D	0.749	0.318	0.510
O E	0.713	0.124	0.595
O F	0.681	0.116	0.912
O G	0.755	0.249	0.992
O H	0.877	0.252	0.053
O I	0.806	0.066	0.101
O J	0.789	0.151	0.407
O K	0.713	0.426	0.084
O L	0.767	0.391	0.195
O M	0.857	0.275	0.186

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Table 3*a*. Refined anisotropic thermal parameters of molecule C in *C*2

atom	*U*11	*U*22	*U*33	*U*12	*U*12	*U*23
CA 1	0.09843	0.08151	0.11654	0.01507	−0.03590	−0.00940
CB 1B	0.09492	0.07389	0.09675	−0.05903	−0.04655	0.00643
SG 1B	0.09431	0.11185	0.10743	0.00256	−0.02476	0.02029
CB 1A	0.08307	0.10648	0.05139	0.00655	0.00162	0.05447
SG 1A	0.09426	0.07104	0.11648	0.01204	0.00318	0.00352
C 1	0.07339	0.10084	0.07724	0.02178	−0.02088	0.00704
O 1	0.11201	0.10959	0.06683	−0.01365	−0.02879	0.01740
N 2	0.07776	0.06146	0.07130	0.00707	0.01249	0.00084
CA 2	0.08766	0.07527	0.06180	0.00114	0.00676	0.00042
CB 2	0.09748	0.10805	0.06701	−0.03400	−0.04796	−0.01803
CG 2	0.07610	0.11643	0.04901	−0.00939	−0.01979	−0.01640
CD 12	0.08876	0.08013	0.06589	−0.00993	−0.01545	0.00126
CD 22	0.10067	0.08966	0.06939	0.01275	−0.00139	−0.04539
CE 12	0.06299	0.06232	0.06308	0.01151	0.00857	0.00333
CE 22	0.09980	0.09559	0.13280	−0.01329	−0.02697	−0.02469
CZ 2	0.09210	0.08483	0.09401	0.01572	0.00013	−0.02833
OH 2	0.14255	0.12146	0.08564	0.00564	0.03172	−0.01175
C 2	0.06540	0.07895	0.09295	−0.01274	−0.01946	−0.01081
O 2	0.06830	0.09894	0.07154	−0.01571	−0.00779	0.00799
N 3	0.09518	0.08624	0.06236	0.01970	−0.00383	0.00361
CA 3	0.06130	0.10408	0.11223	0.01966	−0.03345	0.02337
CB 3	0.11640	0.30853	0.07156	0.07324	0.00093	−0.03666
CG 23	0.17173	0.48981	0.23610	0.00572	0.03170	−0.01692
CG 13	0.29664	0.29970	0.11660	0.07588	−0.07611	0.10901
CD 3	0.30454	0.16686	0.23013	0.01419	0.06402	0.08240
C 3	0.09761	0.06355	0.05857	0.00022	−0.01144	0.00701
O 3	0.09354	0.12189	0.09808	0.00002	−0.01094	−0.03321
N 4	0.07100	0.07335	0.09371	0.00346	−0.00355	−0.01726
CA 4	0.09118	0.07253	0.07371	0.01000	−0.01055	−0.04339
CB 4	0.03195	0.13968	0.17366	0.01833	−0.02428	−0.02493
CG 4	0.12160	0.13107	0.11449	0.04289	0.07003	0.01978
CD 4	0.17618	0.14821	0.06498	0.02827	0.00468	−0.00491
OE 14	0.15582	0.21845	0.17777	0.04652	0.04481	−0.03973
NE 24	0.12343	0.16247	0.10791	0.00088	0.03067	0.04729
C 4	0.10644	0.10033	0.09241	0.02064	0.01403	0.00753
O 4	0.11631	0.09793	0.11050	−0.00447	0.00526	0.00540
N 5	0.06657	0.05638	0.09245	0.01268	−0.02386	−0.00904
CA 5	0.10021	0.10388	0.09689	0.00031	0.00484	−0.00960
CB 5	0.07260	0.05501	0.12900	0.01095	−0.04219	−0.00332
CG 5	0.12343	0.08830	0.20511	−0.05655	−0.03953	0.01997
OD 15	0.15361	0.10469	0.30257	0.07413	−0.07480	−0.03438
ND 25	0.11555	0.13738	0.15078	0.04317	−0.03913	−0.04103
C 5	0.10617	0.07085	0.06653	−0.00302	0.00162	−0.00884
O 5	0.07872	0.09371	0.07690	0.01878	−0.01895	−0.01730
N 6	0.10586	0.06507	0.05781	−0.00091	−0.00723	−0.01128
CA 6	0.07691	0.08076	0.04356	0.00280	−0.00067	0.00121
CB 6B	0.04855	0.03991	0.04627	−0.04529	−0.00260	−0.02084
SG 6B	0.08622	0.07891	0.08761	0.00301	0.00708	−0.02486
CB 6A	0.11785	0.10375	0.05346	−0.03175	−0.00609	0.00131
SG 6A	0.10376	0.11448	0.10656	−0.01852	−0.01305	−0.02531
C 6	0.07306	−0.08135	0.08381	0.03734	−0.02711	−0.02405
O 6	0.09893	0.14158	0.10152	0.07893	−0.01536	−0.01173
N 7	0.06821	0.05530	0.08581	−0.00142	−0.00784	−0.02351
CA 7	0.06178	0.09155	0.08200	0.00818	−0.00946	0.00838
CB 7	0.15330	0.07361	0.11533	0.00526	−0.01026	0.01239
CG 7	0.16871	0.07205	0.11021	0.01466	−0.01614	−0.00661
CD 7	0.12564	0.07613	0.09966	0.03614	−0.03484	−0.03769
C 7	0.04772	0.08327	0.07575	0.00870	0.01279	−0.00283
O 7	0.08253	0.09129	0.11736	−0.01880	0.02641	−0.01526
N 8	0.05489	0.07465	0.07205	0.00415	−0.00876	0.00111

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TABLE 3*a*. (*cont.*)

CA 8	0.07667	0.08689	0.09455	0.03610	0.00239	−0.01372
CB 8	0.08110	0.08331	0.07498	−0.01111	−0.01118	−0.03662
CG 8	0.14565	0.13298	0.09463	−0.01828	0.00773	−0.05663
CD 18	0.15341	0.29111	0.12701	−0.03409	−0.02026	−0.06416
CD 28	0.15986	0.14088	0.15462	0.02026	0.02694	−0.02093
C 8	0.09171	0.10194	0.06311	−0.00180	−0.02235	−0.02826
O 8	0.07661	0.14432	0.08504	0.00354	−0.00315	−0.03676
N 9	0.11593	0.12526	0.09873	0.02874	−0.00701	−0.02392
CA 9	0.08358	0.19566	0.11544	0.04500	−0.02995	−0.01440
C 9	0.14187	0.12995	0.08842	−0.00829	0.01421	0.01253
O 9	0.11029	0.13634	0.13298	−0.00001	−0.00651	−0.00221
NXT 9	0.08645	0.11259	0.14604	−0.01728	−0.00685	0.01723

TABLE 3*b*. REFINED ANISOTROPIC THERMAL PARAMETERS OF MOLECULES A AND B IN $P2_1$

	molecule A							molecule B					
atom	*U*11	*U*22	*U*33	*U*12	*U*13	*U*23	atom	*U*11	*U*22	*U*33	*U*12	*U*13	*U*23
SG 1	0.054	0.083	0.072	−0.008	0.008	−0.004	SG 1	0.052	0.051	0.054	−0.012	0.009	−0.013
SG 1	0.352	0.185	0.217	−0.091	−0.099	−0.060	SG 1	0.106	0.110	0.076	−0.001	−0.007	0.008
C 1	0.046	0.075	0.045	0.015	0.006	−0.006	CB 1	0.070	0.093	0.090	−0.018	−0.004	−0.002
O 1	0.079	0.096	0.052	−0.009	−0.002	0.001	CA 1	0.051	0.111	0.068	−0.009	−0.013	0.007
N 2	0.035	0.055	0.069	0.000	0.002	−0.012	C 1	0.049	0.054	0.045	−0.004	−0.025	0.016
CA 2	0.042	0.064	0.045	0.008	0.010	−0.004	O 1	0.091	0.062	0.068	−0.003	−0.010	0.007
CB 2	0.078	0.064	0.045	0.030	0.013	0.004	N 2	0.027	0.073	0.051	−0.002	0.004	0.009
CG 2	0.036	0.075	0.043	0.018	0.006	−0.001	CA 2	0.049	0.047	0.026	0.007	0.011	0.009
CD 12	0.065	0.091	0.052	0.009	0.012	−0.002	CB 2	0.035	0.079	0.036	−0.014	−0.007	0.020
CD 22	0.040	0.063	0.084	0.008	0.015	0.007	CG 2	0.048	0.072	0.035	−0.013	0.014	−0.007
CE 12	0.053	0.091	0.063	0.001	0.018	−0.013	CD 12	0.062	0.062	0.064	0.008	0.021	0.031
CE 22	0.057	0.099	0.046	0.019	0.026	0.013	CD 22	0.076	0.067	0.030	−0.017	−0.002	0.003
CZ 2	0.072	0.067	0.063	0.015	0.012	0.009	CE 12	0.066	0.084	0.047	−0.019	0.016	0.021
OH 2	0.102	0.100	0.031	0.039	0.028	0.007	CE 22	0.079	0.104	0.054	0.022	0.013	−0.015
C 2	0.038	0.058	0.058	0.015	0.002	−0.027	CZ 2	0.060	0.090	0.050	−0.015	0.020	−0.018
O 2	0.045	0.095	0.050	0.005	0.020	0.005	OH 2	0.109	0.118	0.045	−0.001	0.022	0.003
N 3	0.039	0.065	0.032	0.004	0.009	−0.010	C 2	0.078	0.047	0.037	−0.005	0.026	−0.008
CA 3	0.049	0.047	0.073	−0.015	0.017	−0.022	O 2	0.049	0.077	0.034	0.009	0.021	0.011
CB 3	0.102	0.052	0.040	−0.027	0.010	−0.010	N 3	0.037	0.054	0.046	0.008	0.004	0.012
CG 13	0.105	0.117	0.079	−0.011	−0.003	−0.021	CA 3	0.025	0.056	0.056	0.002	−0.002	0.020
CG 23	0.114	0.217	0.037	−0.060	−0.023	0.016	CB 3	0.060	0.088	0.037	−0.011	0.020	0.015
CD 13	0.163	0.110	0.112	−0.006	0.051	−0.018	CG 13	0.086	0.097	0.116	0.029	0.016	0.032
C 3	0.052	0.043	0.060	−0.001	−0.007	−0.011	CG 23	0.089	0.090	0.103	0.025	−0.001	0.025
O 3	0.060	0.049	0.068	0.008	0.016	0.009	CD 13	0.148	0.130	0.142	0.055	0.030	0.023
N 4	0.036	0.065	0.054	−0.003	0.018	−0.007	C 3	0.055	0.053	0.032	0.011	0.002	−0.001
CA 4	0.042	0.082	0.050	0.010	0.016	0.005	O 3	0.060	0.072	0.068	−0.010	0.020	−0.019
CB 4	0.066	0.052	0.052	0.018	0.032	0.007	N 4	0.050	0.057	0.046	−0.001	0.007	0.007
CG 4	0.064	0.067	0.070	−0.001	0.037	0.013	CA 4	0.083	0.045	0.085	−0.007	0.066	−0.005
CD 4	0.073	0.088	0.061	−0.006	0.007	0.003	CB 4	0.049	0.085	0.040	−0.015	0.002	−0.035
NE 24	0.085	0.098	0.110	−0.015	0.042	−0.010	CG 4	0.096	0.087	0.056	0.014	−0.004	0.019
OE 14	0.075	0.108	0.069	−0.013	0.029	−0.002	CD 4	0.069	0.174	0.043	0.020	0.030	0.009
C 4	0.051	0.073	0.069	0.000	0.024	0.005	NE 24	0.157	0.091	0.249	−0.018	0.109	0.012
O 4	0.124	0.046	0.073	−0.019	0.005	0.017	OE 14	0.107	0.149	0.093	0.021	0.053	0.018
N 5	0.045	0.044	0.071	0.004	−0.002	0.001	C 4	0.039	0.067	0.064	0.009	0.029	0.021
CA 5	0.038	0.034	0.078	−0.009	0.022	−0.002	O 4	0.101	0.057	0.062	0.003	−0.004	−0.010
CB 5	0.068	0.073	0.073	−0.006	−0.012	−0.017	N 5	0.036	0.037	0.060	0.000	0.014	0.000
CG 5	0.042	0.132	0.107	0.025	0.029	0.012	CA 5	0.027	0.069	0.063	−0.012	−0.001	0.032
OD 15	0.059	0.112	0.240	−0.050	0.057	−0.101	CB 5	0.057	0.040	0.086	−0.001	−0.022	0.021
ND 25	0.065	0.080	0.086	0.009	−0.003	−0.030	CG 5	0.070	0.046	0.107	−0.014	0.024	−0.020
C 5	0.043	0.046	0.068	−0.014	0.003	0.003	OD 15	0.069	0.059	0.133	0.004	0.022	0.013
O 5	0.064	0.115	0.053	−0.002	0.011	0.004	ND 25	0.090	0.064	0.072	−0.028	−0.010	0.024
N 6	0.032	0.121	0.068	0.003	0.014	−0.019	C 5	0.061	0.047	0.060	0.017	0.000	0.021
CA 6	0.046	0.087	0.063	0.019	0.004	−0.019	O 5	0.038	0.083	0.048	−0.005	−0.007	0.013
CB 6	0.064	0.118	0.074	0.011	−0.011	−0.012	N 6	0.042	0.036	0.064	0.002	0.012	0.005
SG 6	0.102	0.094	0.113	0.025	0.000	−0.015	CA 6	0.034	0.060	0.035	−0.002	0.007	−0.004
C 6	0.048	0.091	0.048	0.009	−0.002	0.009	CB 6	0.057	0.032	0.083	−0.010	−0.021	−0.013
O 6	0.078	0.169	0.107	−0.003	0.051	−0.073	SG 6	0.079	0.072	0.093	−0.022	0.007	−0.005
N 7	0.058	0.075	0.066	0.014	0.003	0.026	C 6	0.042	0.078	0.060	−0.022	0.013	−0.006
CA 7	0.048	0.085	0.061	0.011	0.014	0.037	O 6	0.056	0.114	0.075	0.013	0.030	0.035
CB 7	0.086	0.081	0.114	0.009	0.032	−0.007	N 7	0.049	0.049	0.043	−0.003	0.024	−0.010
CG 7	0.124	0.090	0.119	0.036	0.054	−0.002	CA 7	0.048	0.045	0.046	−0.014	−0.002	0.001
CD 7	0.081	0.112	0.054	0.009	0.044	−0.027	CB 7	0.051	0.059	0.096	0.015	0.008	−0.014
C 7	0.050	0.083	0.029	0.007	0.003	0.009	CG 7	0.071	0.077	0.080	0.014	−0.022	0.014
O 7	0.056	0.115	0.075	0.005	0.025	0.031	CD 7	0.103	0.045	0.048	0.003	0.040	0.009
N 8	0.019	0.077	0.063	0.015	0.016	0.019	C 7	0.047	0.075	0.063	0.010	0.013	0.046
CA 8	0.028	0.085	0.055	0.003	0.005	0.010	O 7	0.037	0.105	0.096	0.002	0.026	−0.023
CB 8	0.062	0.075	0.059	0.023	0.016	0.001	N 8	0.061	0.049	0.060	−0.001	0.004	−0.009
CG 8	0.086	0.142	0.062	0.046	−0.015	−0.004	CA 8	0.041	0.070	0.063	−0.016	−0.002	−0.009
CD 18	0.109	0.163	0.071	0.030	0.056	0.023	CB 8	0.070	0.089	0.052	−0.061	0.010	−0.021
CD 28	0.207	0.132	0.059	0.102	0.044	−0.024	CG 8	.0137	0.161	0.053	−0.097	0.020	0.019
C 8	0.075	0.080	0.029	0.020	0.036	−0.004	CD 18	0.169	0.244	0.040	−0.113	0.023	−0.037

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TABLE 3*b*. (*cont.*)

O 8	0.048	0.120	0.062	0.040	0.013	−0.022	CD 28	0.189	0.130	0.140	0.005	−0.023	0.062
N 9	0.063	0.125	0.057	0.012	0.011	−0.033	C 8	0.056	0.047	0.056	−0.015	−0.020	−0.009
CA 9	0.074	0.153	0.068	0.002	−0.001	−0.054	O 8	0.038	0.122	0.100	−0.053	−0.004	0.018
C 9	0.111	0.076	0.041	−0.002	0.010	−0.002	N 9	0.061	0.091	0.075	0.007	0.024	0.030
O 9	0.057	0.121	0.064	0.012	0.010	−0.004	CA 9	0.071	0.139	0.069	0.014	0.016	0.046
NXT 9	0.062	0.106	0.202	−0.006	0.020	−0.052	C 9	0.111	0.076	0.041	−0.038	0.008	−0.004
							O 9	0.075	0.090	0.096	−0.010	0.023	0.031
							NXT 9	0.060	0.126	0.098	−0.013	0.025	0.020

TABLE 3*c*. REFINED ANISOTROPIC THERMAL PARAMETERS OF WATER MOLECULES IN $P2_1$

atom	*U*11	*U*22	*U*33	*U*12	*U*13	*U*23
O A	0.082	0.101	0.119	−0.028	0.031	−0.037
O B	0.104	0.107	0.105	0.002	0.013	0.003
O C	0.097	0.112	0.104	0.019	0.001	0.004
O D	0.131	0.095	0.102	0.000	−0.019	−0.008
O E	0.126	0.123	0.112	−0.027	0.039	−0.022
O F	0.167	0.131	0.097	0.007	0.030	0.035
O G	0.117	0.135	0.219	0.025	0.011	−0.009
O H	0.121	0.128	0.163	−0.039	0.036	−0.049
O I	0.156	0.161	0.117	0.036	0.023	0.002
O J	0.140	0.137	0.077	0.045	0.022	0.027
O K	0.124	0.116	0.222	0.064	0.063	−0.002
O L	0.209	0.151	0.175	−0.051	0.039	−0.043
O M	0.240	0.111	0.178	−0.010	−0.034	−0.001

4. CRYSTAL STRUCTURE SOLUTION OF THE 'WET FORM'

The refined model of the 'dry form' of deamino-oxytocin, which has alternate sites for the two cysteine residues 1 and 6 in the *C*2 space group, was used to calculate the initial phases for the 'wet form' ($P2_1$, space group) crystal structure. This was achieved by rotating the *C*2 model twofold to generate a second molecule and translating the two molecules to the $P2_1$ origin by a quarter in the *x*-coordinate.

$$C2 \rightarrow P2_1$$
$$x, y, z \quad \tfrac{1}{4}+x, y, z \quad \text{molecule B}$$
$$\tfrac{1}{4}-x, y, -z \quad \text{molecule A}$$

Molecule A has the position of Cβ1 and SG1 of one set of partial occupancies and molecule B the corresponding atoms of the other set of partial occupancies of the molecule in the *C*2 space group.

Initial structure determination and refinement were carried out by using SHELXL (Sheldrick 1976) on the NAS 7000 at the SERC Daresbury Laboratory. The starting *R* value was 0.35 for all data ($d_{\min} = 1.09$ Å). Bond-length restraints (see table 4) were applied to all main and side-chain peptide bonds and the two molecules were refined isotropically by block matrix least squares refinement (one molecule per block) with $I > 3\sigma(I)$ reflections to decrease the computing time required. The *R* value was 0.33. In this pass of refinement no water molecules were included. Further cycles of refinement lowered the *R* value to 0.26 for all data (4681 reflections). High thermal parameters, were observed for SG1, especially in molecule A, where the occupancy of the sulphur was constrained to unity. Water molecules were included in the refinement and were refined in a third block-matrix. Isoleucine at position 3 in molecule A seemed to be disordered and had to be repositioned from a difference Fourier (F_{obs}-F_{calc}) map

TABLE 4. RESTRAINTS APPLIED TO BOND LENGTHS AND INTERATOMIC DISTANCES IN $P2_1$

bonding atom type	expected bond length (Å)	σ (Å)	observed bond length (Å)	r.m.s.
CB—SG	1.81	0.02	1.84	0.07
SG—SG	2.06	0.02	2.04	0.08
CA—C	1.52	0.02	1.51	0.02
CB—CA	1.53	0.02	1.52	0.03
C—N	1.47	0.02	1.40	0.07
C—O	1.23	0.02	1.22	0.02
N—CA	1.47	0.02	1.46	0.02
CG—CD	1.52	0.02	1.49	0.02
CB—CG	1.53	0.02	1.52	0.02
CG...CD	1.39	0.01	1.39	0.01
CD—NE	1.33	0.01	1.32	0.01
CD—OE	1.33	0.01	1.32	0.01
CG—ND	1.33	0.01	1.32	0.01
CD—OD	1.33	0.01	1.32	0.01
CB—SG—SG	3.85	0.04	3.32	0.54
CB—CG—CD	2.48	0.01	2.49	0.01

from which the side chain was completely omitted in the calculation of phases. An alternative conformation for this side chain was observed and was refined with constrained geometry. Some of the water molecules that had high thermal parameters had to be repositioned from new peaks in the electron density maps. Extensive disorder was seen in residue 1, more in molecule A than in molecule B. Most of the disorder was resolved by assigning alternate sites of SG1 in both molecules A and B and refining these atoms anisotropically, their alternative occupancies restrained to unit. SG6 did not appear disordered and hence had only one site in the model. The S—S bridge was restrained to standard geometry (see table 4) and the two molecules and their waters were refined anisotropically. The final R value was 0.09 for all data (4681 unique reflections) up to 1.09 Å resolution. The quality of the maps is shown in figure 5. Least squares refinement by using only $h+k=2n$ reflections (simulating $C2$ conditions) gave $R=0.07$; while using $h+k=2n+1$ gave $R=0.13$. Tables 2*b–d* and 3*b–c* show the positional coordinates and the anisotropic thermal parameters of the deamino-oxytocin molecules A, B and the water of crystallization.

5. RESULTS AND DISCUSSION

The discussion below refers to the molecules in the asymmetric unit of the 'wet form' in space group $P2_1$ as molecule A and molecule B; and the molecule in the 'dry form' of space group $C2$ is referred to as C. As the molecules A, B and C are similar, and A and B are better refined ($R=0.09$) at 1.09 Å resolution, we will concentrate our discussion on the conformational features of molecules A and B, and make comparisons with C only where specific points of interest arise.

The chemical formula of the neurohypophyseal hormones and their analogues are shown in figure 1. Large temperature factors for residues 3, 5, 7 and the *C*-terminus for molecule C are shown in the Ortep diagram (figure 4*a*). Figure 6 shows the crystal structure of the two independent molecules, A and B, and the distribution of waters of crystallization. Perspective views of the superposition of the molecules A and B in $P2_1$ are shown in figure 9*a* while the

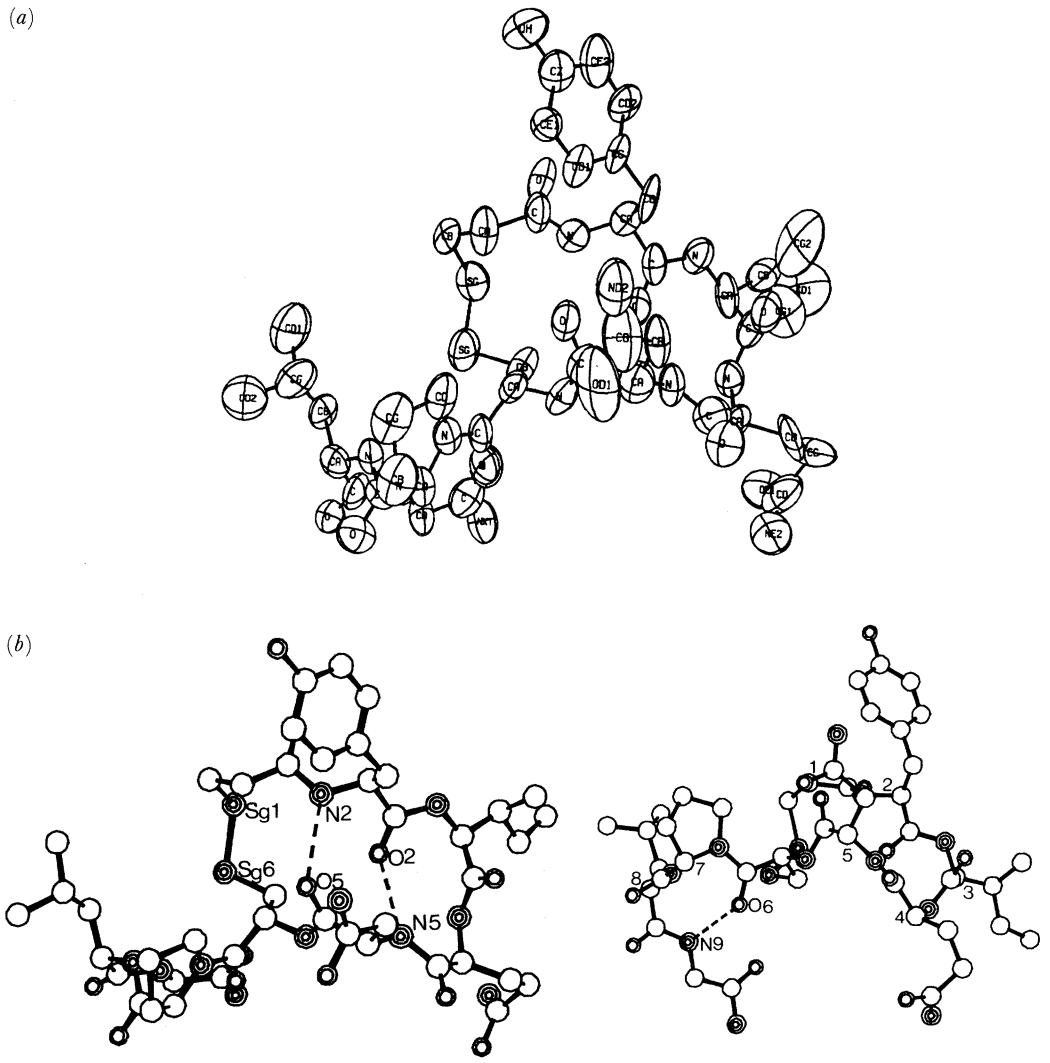

FIGURE 4. (*a*) An ortep plot showing anisotropic thermal vibration of the crystal structure of deamino-oxytocin in the 'dry form' (*C*2). (*b*) Different views of the 'wet form' of the deamino-oxytocin in $P2_1$ molecule (B) showing the three intramolecular H-bonds.

superposition of all three A, B and C are shown in figure 9*b*. Despite the low root mean square (r.m.s.) of the fit (< 0.25 Å) for the Cα atoms calculated by using FITZ (Taylor 1980), major conformational differences are observed at the side chains 2, 3, 5, 7 and the S—S bridge. These apparent differences are better assessed from the torsion angles of molecules given in table 5. Although molecule C is considered to be the average of molecules A and B it is found that conformationally, molecules B and C are more similar than A and C or A and B, especially with respect to Ile 3, Asn 5 and the disulphide conformations.

The deamino-oxytocin molecule has approximate dimensions of $7 \times 9 \times 10$ Å^3. The main chain consisting of 20 atoms (figure 1) bridges at the Cys 1 and Cys 6. There are two short antiparallel β-pleated strands with two *trans*-annular hydrogen-bonds with a β(II) turn at residues Tyr 2, Ile 3, Gln 4 and Asn 5 (figure 4*b*). However, Laser Raman spectroscopy (Tu *et al.* 1978) and Nuclear Overhauser Effect data (Ford & Gibbons 1979) predicted a β(I) turn.

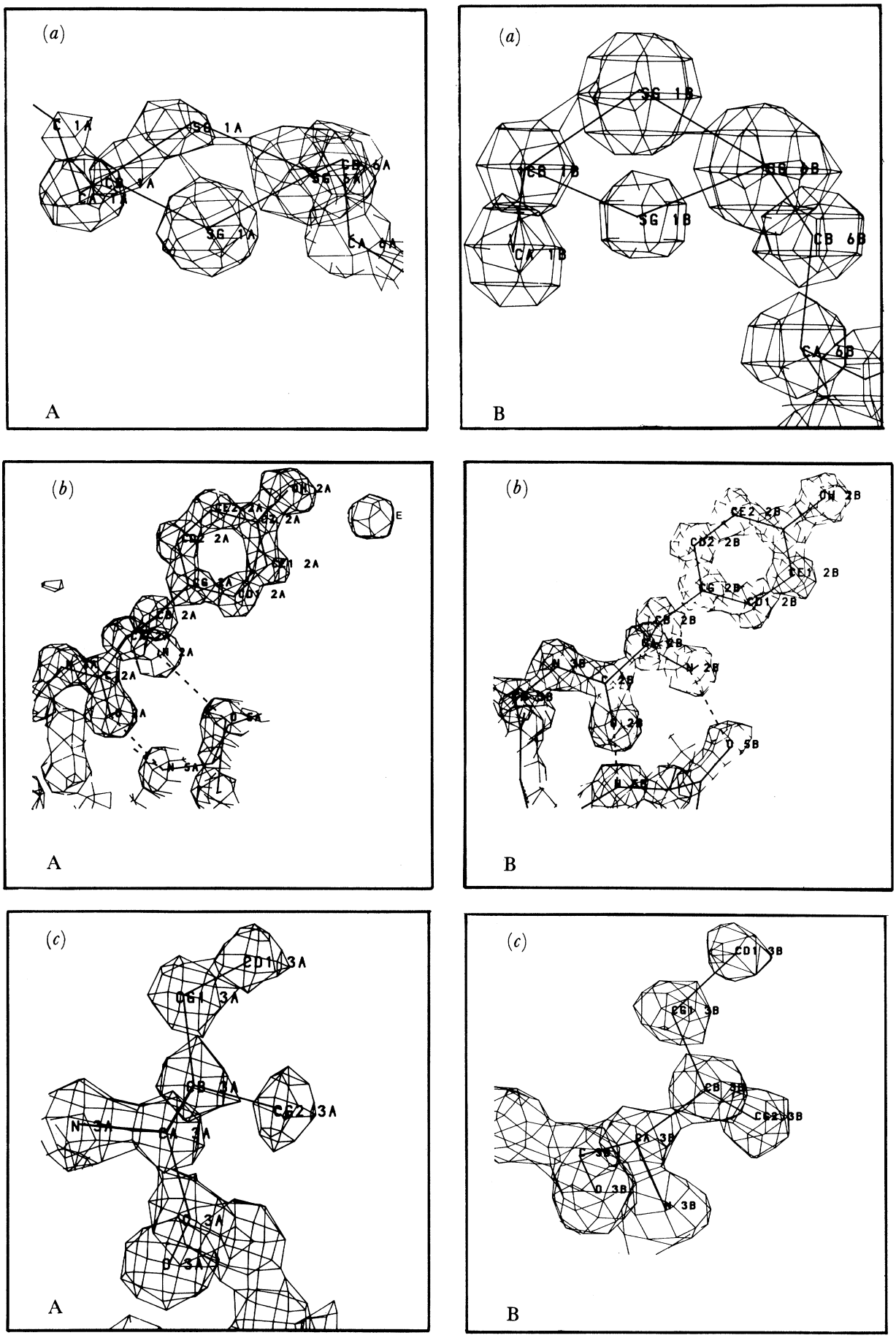

FIGURE 5. For description see page 643.

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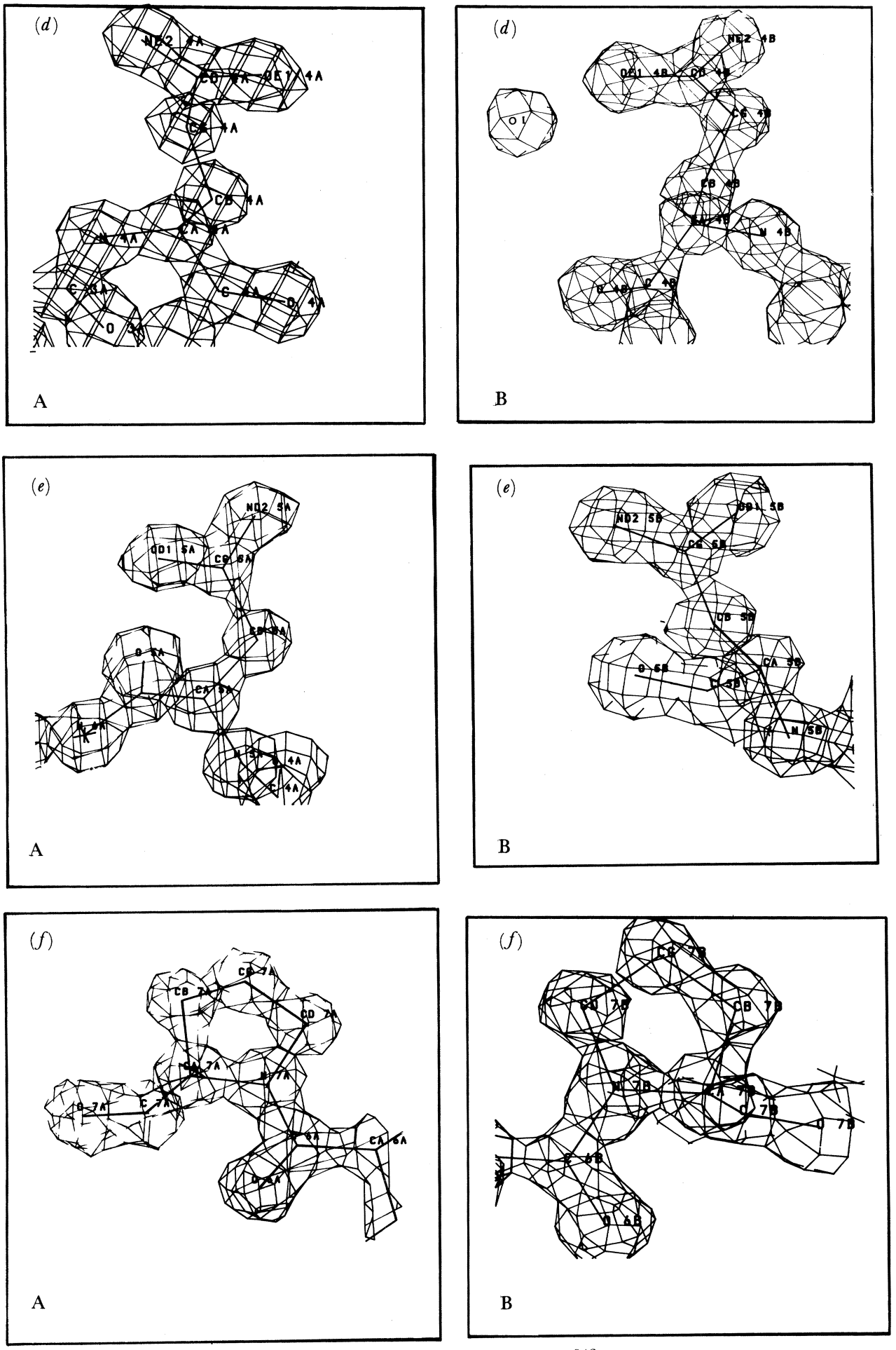

FIGURE 5. For description see page 643.

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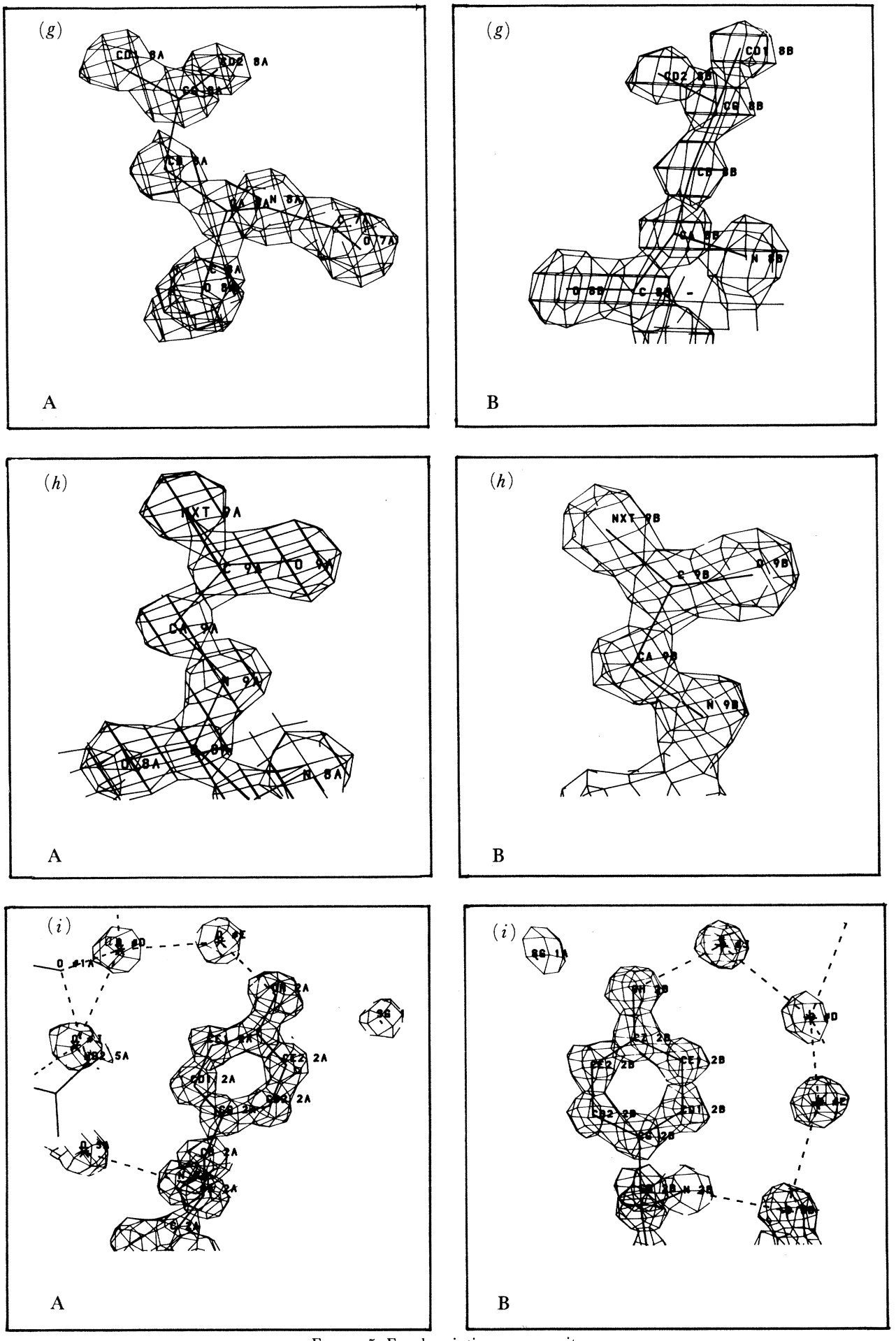

FIGURE 5. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

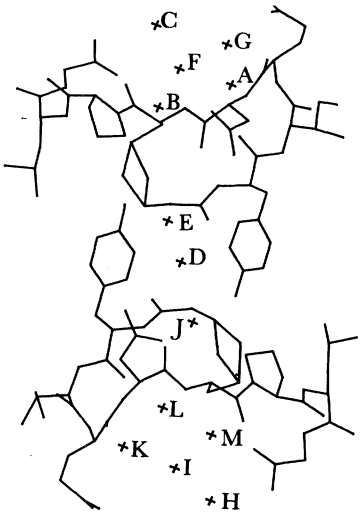

FIGURE 6. Asymmetric unit of the 'wet form' (space group $P2_1$) for crystals of deamino-oxytocin showing molecules A (bottom) and B (top) viewed down the pseudo dyad. Water molecules are shown by crosses.

The tripeptide tail is held by a weak intramolecular hydrogen bond and a β(III) turn is formed by Cys 6, Pro 7, Leu 8 and Gly 9, which confirms the prediction of Tu *et al.* (1978). All molecules of deamino-oxytocin (A, B and C) show β-turns and the three intramolecular hydrogen bonds (table 6). The main chain torsion angles of the tocin ring are such that the ring is curved with Tyr 2 and Asn 5 side chains on the convex side. As a result, the carbonyl oxygen of Cys 1, which is rotated upwards, forms a hydrogen bond with the amide nitrogen of Asn 5 of a neighbouring molecule.

Table 5 shows that there is a marked deviation from planarity of the peptide group between residues Gln 4 and Asn 5, especially in molecule B ($w = 164°$). This suggests that there are both twisting and out of plane bending at the nitrogen of Gln 4 (Winkler & Dunitz 1971). As molecule C was refined by RESTRAIN its peptide geometry was probably restrained rigidly and thus planarity ($w = 177°$) was maintained between residues Gln 4 and Asn 5 to conform to general peptide geometry. The peptide nitrogen of residue Gln 4 in all three molecules A, B and C forms hydrogen bonds with water molecules (see tables 9*a*, *b*, *c*).

(*a*) *S—S Bridges*

The disulphide bridge in both oxytocin molecules appears to be disordered, the extent of disorder in molecule A being greater than that in molecule B. The disorder in molecule A starts at SG1 and is transmitted through Cβ1 to the main chain where it becomes less clearly resolved. This gives rise to the dual conformations of the S—S bridge, right- and left-handed screw, which are characterized by CA1—CB1 bonds perpendicular to the CA6—CB6 bond, and a short CA1 ... CA6 separation distance (Richardson 1981). CA1 ... CA6 distances are 4.45 Å and

FIGURE 5. Final high-resolution ($d_{min} = 1.09$ Å) electron-density map for amino acid residues in molecules A and B of wet form in space group $P2_1$ (*a*) cystine 1–6 showing the disorder, (*b*) tyrosine 2, (*c*) isoleucine 3, (*d*) glutamine 4, (*e*) asparagine 5, (*f*) proline 7, (*g*) leucine 8, (*h*) C-terminus, and (*i*) tyrosine 2 and the water molecules.

Table 5. The torsion angles of the main chain and the side groups of deamino-oxytocin. molecules A, B and C are compared

residue	Φ	Ψ	w	χ^1	χ^2	χ^3	χ^4
				molecule A			
Cys A1	—	120	−171	−109/−178	−102/87	+77/−87	—
Tyr A2	−133	167	176	−50	87	—	—
Ile A3	−54	120	179	−68	−79	—	—
Gln A4	58	24	171	−66	−55	−48	—
Asn A5	−151	75	−177	168	26	—	—
Cys A6	−145	98	−176	−177	57/110	—	—
Pro A7	−78	−8	179	24	−36	31	−15
Leu A8	−73	−40	−176	−59	174	—	—
Gly A9	170	177	—	0	—	—	—
				molecule B			
Cys B1	—	101	−172	+178/−111	80/−93	−94/+83	—
Tyr B2	−126	164	−177	−55	98	—	—
Ile B3	−65	125	−173	−169	163	—	—
Gln B4	56	29	164	−64	−51	−47	—
Asn B5	−158	66	177	−180	−43	—	—
Cys B6	−128	98	−173	−179	113/45	—	—
Pro B7	−73	−12	−179	29	−40	35	−18
Leu B8	−77	−33	−173	−67	175	—	—
Gly B9	−176	168	—	−23	—	—	—
				molecule C			
Cys 1	—	—	−179	—	—	−101/+76	
Tyr 2	−126	169	−179	−57	92	—	
Ile 3	−67	123	−179	−63	158	—	
Gln 4	57	28	177	−62	−57	127	
Asn 5	−155	64	179	−177	−144	—	
Cys 6	−126	95	−177	—	—	—	
Pro 7	−78	−7	−178	23	−36	32	
Leu 8	−76	−37	−180	−65	174	—	
Gly 9	168	176	—	—	—	—	

Table 6. Intramolecular hydrogen-bond distances (Å) and angles (°) of deamino-oxytocin molecules

X	Y	^{r}X–H	^{r}X–Y	$\Theta = \begin{cases} \text{X—H}\ldots\text{Y} \\ \text{X}\ldots\text{H—Y} \end{cases}$
			molecule A	
N A2	O A5	1.93	2.97	161.26
O A2	N A5	1.90	2.95	162.61
O A6	N A9	2.70	3.55	135.97
			molecule B	
N B2	O B5	2.05	3.09	162.75
O B2	N B5	1.82	2.85	156.85
O B6	N B9	2.54	3.49	146.16
			molecule C	
N 2	O 5	2.12	3.09	162.62
O 2	N 5	1.99	2.93	156.31
O 6	N 9	2.60	3.37	134.19

4.09 Å for molecules A and B, respectively. The chirality of the S—S bond is predominantly right-handed in A while that in B is left-handed as suggested by the partial occupanices of the SG1s; 0.7/0.3 and 0.3/0.7 in molecules A and B, respectively. The relevant torsion angles are listed in table 7. The left-handed S–S bridge has *ggg* in χ_1, χ_2 and χ_2', as predicted from laser

TABLE 7. S—S BRIDGE TORSION ANGLE (Å) IN THE 'WET FORM'

H	expected[a]		molecule A		molecule B	
|	left-handed	right-handed	Occ = 0.71	Occ = 0.44	Occ = 0.70	Occ = 0.29
CA1						
| χ_1	−60	−60	−109	−178	+178	−111
| χ_2	−90	+120	−102	+87	+80	−93
SG1						
| χ_3	−90	+90	+77	−87	−94	+83
SG6						
| χ_2'	−90	−50	+57	+110	+113	+45
CB6						
| χ_1'	−60	−60	−177	−177	−179	−179
CB6						
|						
N6			right-handed	left-handed	left-handed	right-handed

[a] Richardson (1981).

Raman spectroscopic studies by Tu *et al.* (1978), while the right-handed one is *ggc* in both the molecules (A and B).

(*b*) *Water structure*

There are 13 water molecules in the asymmetric unit of the $P2_1$ cell (figures 6, 7 & 10) of which two, D and G, lie on the pseudo twofold axis. Deviations from the twofold symmetry of the distribution of water are listed in table 8. The extra water, L, which is absent in the 'dry form' (space group $C2$), is located near the carbonyl oxygen of residue Asn 5 in molecule A. It forms four hydrogen bonds, two to water molecules and two to the deamino-oxytocin molecule A. The hydrogen bonds to and from water molecules are shown in table 10. Figures 10*a* & *b* show the major and minor aqueous zones in the crystal structure and the hydrogen-bond network with the peptide molecules. In the minor aqueous zone three waters E, J and D are involved. D is on the pseudo twofold and hydrogen bonds to the water molecules E, J, as well as the carbonyl oxygen of Cys 1 of both molecules A and B as shown in figures 7 and 10*b*. The minor zone of water molecules is common to the three different crystal structures of deamino-oxytocin. In the major aqueous zone there are nine waters: A, B, C, F, H, I, L and M surrounding G that lies on a pseudo twofold axis.

(*c*) *Inter- and intramolecular hydrogen bonds in oxytocin*

The two *trans*-annular hydrogen-bonds (tables 6*a*, *b*, *c*) found in both the $P2_1$ and the $C2$ structures of deamino-oxytocin are thought to be important for stability of the tocin ring (Urry *et al.* 1970; Urry & Walter 1971; Walter *et al.* 1971; Roy *et al.* 1983). Deuterium NMR studies suggest that the oxytocin molecule undergoes more frequent reorientation about an intramolecular axis than it does as a whole. The conformation of oxytocin observed in water is therefore a time-averaged one (Brewster & Hruby 1973; Glasel *et al.* 1973). The *trans*-annular hydrogen bonds (average 1.925 Å) observed in the $P2_1$ and $C2$ crystal structures of deamino-oxytocin are also time-averaged and correspond closely to the mean O...H distance of the order of 1.85 to 1.95 Å, as quoted in Baker & Hubbard (1984). Thus, if the model proposed by Wood *et al.* (1986), based on the cooperative model of Walter (1971) and the dynamic model of Hruby and co-workers (1981*a*, *b*), is plausible, the H-bond, closer to the S—S bridge and away from the β-II turn, would break during the interconversion of the conformers

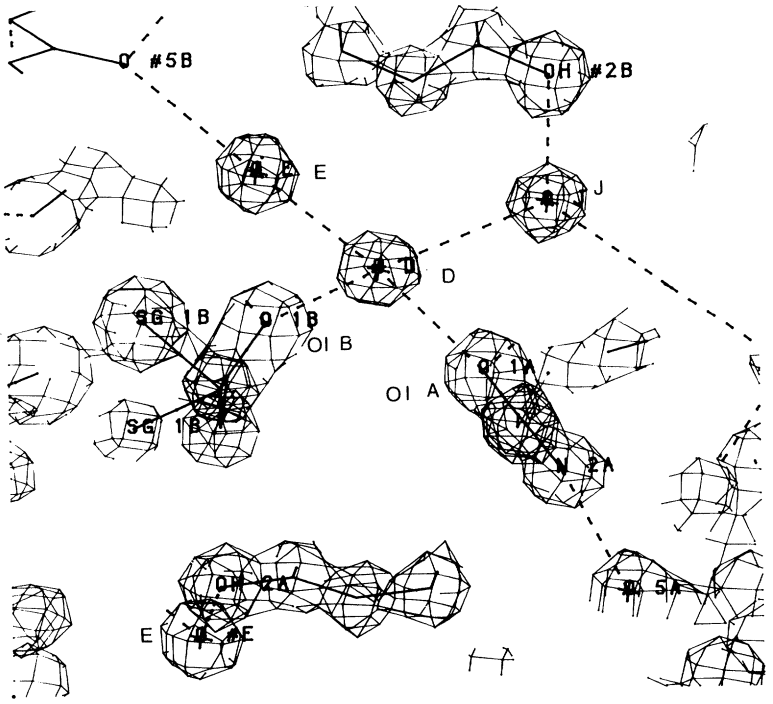

FIGURE 7. Water coordination in the minor aqueous zone in 'wet form' (space group $P2_1$) crystals, showing three water molecules (D, E, J) hydrogen bonded to each other and to residues Cys 1A Cys 1B, Tyr 2B and Asn 5B (see figure 10*b* for schematic drawing).

TABLE 8. DEVIATIONS FROM THE TWOFOLD SYMMETRY OF THE WATER MOLECULES OF THE TWO INDEPENDENT OXYTOCIN MOLECULES IN THE ASYMMETRIC UNIT OF THE 'WET FORM'

water molecules		a pseudo twofold (Å)
OA——	OK	0.46
OB——	OM	0.50
OC——	OH	0.66
OD——	≠OD	0.49
OE——	OJ	0.25
OF——	OI	0.69
OG——	≠OG	0.49
OL	OD15 (B)	1.03
OD15 (A)	OD15 (B)	1.61

of the S—S bridge. Incidentally, this hydrogen bond NH(Tyr 2)—CO(Asn 5) is slightly weaker (reflected in the longer H-bond distance) than the other *trans*-annular H-bond, CO(Tyr 2)—NH(Asn 5), in all the molecules, both in the $P2_1$ and the $C2$ structures. Such a break of the H-bond would lead to the reorientation of the main chain atoms and to a lesser extent of the side-chain atoms about an axis perpendicular to the intact H-bond in the tocin ring. During this very short time required for conformational changes, the tocin ring of the hormone might be adjusting to the changes being induced in the receptor that are necessary for binding.

The tripeptide tail is attached to the tocin ring through hydrogen-bond formed between Gly 9 peptide amide hydrogen and the Cys 6 carbonyl oxygen. A β-III turn is observed, which

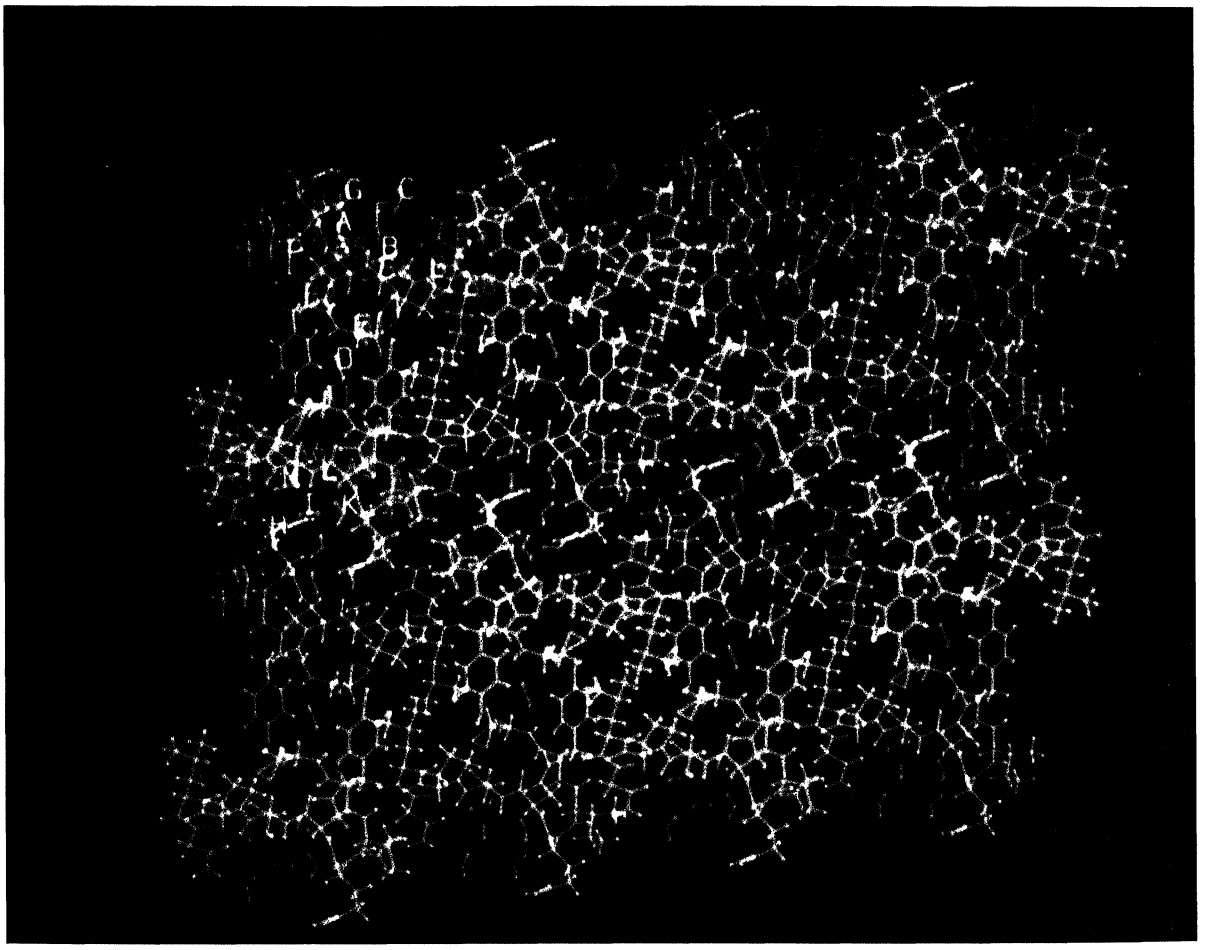

FIGURE 8. The crystal packing of deamino-oxytocin, when viewed down the twofold axis showing hydrophobic and hydrophilic interactions.

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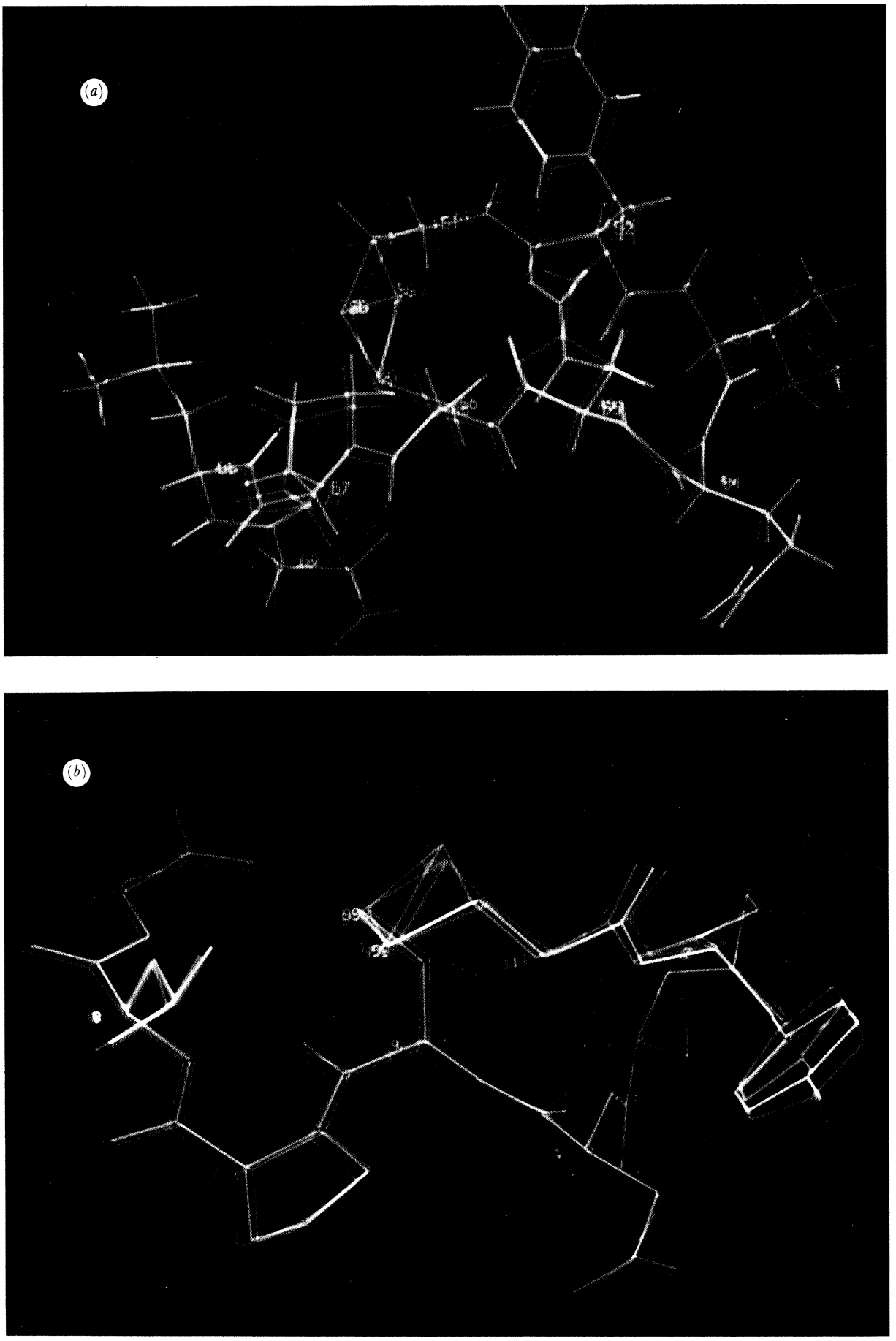

FIGURE 9. Root mean square fitting of Cα atoms of the two independent molecules A and B in $P2_1$ and C in $C2$. (*a*) shows fitting of A (red) and B (green); (*b*) shows the fitting of all three molecules A (blue), B (red) and C (green).

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is made by Cys 6, Pro 7, Leu 8 and Gly 9. Various conformations have been proposed for the tail end of the hormone (Urry & Walter 1971; Brewster *et al.* 1973; Brewster & Hruby 1973; Ford & Gibbons 1979). Recent energy minimization and molecular dynamic studies (Treharne *et al.* 1986) have shown that the tail of the oxytocin molecule is very flexible, as suggested by Wood *et al.* (1986) on the basis of the crystal structure analysis. Ile 3 in the $P2_1$ crystal structure has both the *tt* and g^+g^+ conformations. Molecule A, which has the predominantly right-handed S—S bridge has also a g^+g^+ isoleucine. Here the isoleucine is slightly disordered and its geometry had to be restrained. This was achieved by fixing the distance between Cβ3 and Cδ13 to 2.4 Å with an elasticity of 0.01 Å. A slight increase in the anisotropic thermal parameter was also observed. However, the isoleucine 3 in the $C2$ crystal structure, which has a g^+t conformation similar to that of molecule B in the $P2_1$ has high thermal parameters. Views of the differences and similarities of the $P2_1$ (blue → molecule A; red → molecule B) and $C2$ (green → molecule C) crystal structures of deamino-oxytocin are shown in figures 8 and 9 (plates 1 and 2). Glutamine at position 4 has been investigated by numerous researchers. Substitution of Gln 4 by Ser 4 produces enhanced utertonic and mammary responses with very little depressor activity (Turan *et al.* 1977), whereas Thr 4 produces greatly enhanced oxytocin and depressor potencies (Manning *et al.* 1970). The main chain carbonyl and amide atoms at Gln 4 hydrogen-bond to three water molecules: K, C & I in molecule A and A, G & H in molecule B. Water molecules A and H bridge the independent molecules in the $P2_1$ asymmetric unit. The side-chain carbonyl oxygen Oε14 interacts with the main chain CO and NH of Gln 4 through two water molecules while the amide nitrogen donates a proton to the main chain carbonyl through a water molecule.

The somewhat greater conformational flexibility of the Ile 3, Asn 5, and Pro 7 side-chain

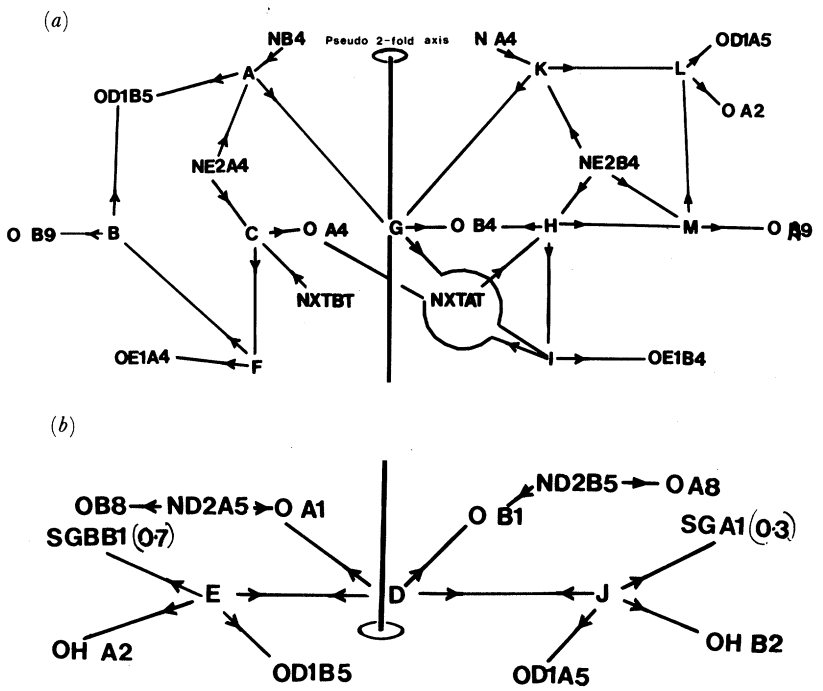

FIGURE 10. Deviations from twofold symmetry of the water environment of the two independent deamino-oxytocin molecules: (*a*) major and (*b*) minor aqueous zone in 'wet form' (space group $P2_1$)

groups and the two different conformations readily accessible to the disulphide bridge in deamino-oxytocin are consistent with the dynamic model for oxytocin agonist activity of Hruby *et al.* (Meraldi *et al.* 1977; Hruby & Mosberg 1981; Hruby 1981), in which it was suggested that oxytocin binds in a stepwise manner to its uterine receptor and this involves interactions of lipophilic amino acid residues including Ile 3 and Pro 7. The side-chain groups of Asn 5 and Tyr 2 were suggested by Walter in his 'cooperative model' (Walter 1977) to be important for agonist biological activity. Furthermore, both models suggested that the Tyr 2 and Asn 5 side-chain groups would be on the same face of the 20-membered ring as indeed is seen in the X-ray structure of deamino-oxytocin reported here. The conformational change of hormone (and the hormone–receptor complex) that presumably occurs during the transduction process may involve the Asn 5 and the disulphide side-chain groups. Obviously structural changes in oxytocin analogues, which would disrupt the necessary dynamic behaviour or topological relations of the side-chain groups such as those of Asn 5, the disulphide bridge or other side-chain groups, could readily provide oxytocin analogues which could still interact with the receptor but which would lead to an oxytocin–receptor conformation state that would be incompatible with transduction. As the analogue can still interact with the receptor, it could have potent antagonist (inhibitory) biological activity. Indeed analysis of oxytocin structure–activity relations (Hruby 1981, 1986, 1987; Hruby & Mosberg 1981*a*, *b*) suggest the agonist and antagonist analogues do indeed use different structural and topographical properties of the hormone for hormone–receptor interaction. Interestingly, consistent with the X-ray results, appropriate steric changes at the cysteine bridge or the Tyr 2 side chain of oxytocin do indeed provide potent antagonists. It would seem that appropriate changes for the Asn 5 side-chain group may lead to similar antagonist properties, though no systematic studies along these lines have appeared as yet. In this context, an alternative approach might be to disrupt the relation of the acyclic tripeptide side chain of oxytocin in relation to the 20-membered ring. In examining the X-ray structure, it occurred to V.J.H. that appropriate rotation about the ring to side-chain junction could place the side-chain groups of the Gln 4 and Leu 8 residues in the same face of the structure in reasonable proximity. Substitution of Gln 4 for Glu 4 and Leu 8 for Lys 8 and subsequent lactam ring formation provided an opportunity to test whether this would 'fix' elements of structure relative to each other that were inappropriate for transduction but which could lead to strong interaction with the receptor (Hill *et al.* 1988). Interestingly, while [β-Mpa1, Glu4, Lys8] oxytocin is a very weak agonist (about $\frac{1}{2000}$ that of the parent deamino-oxytocin), the bicyclic analogue [β-Mpa1, Glu4, Cys6, Lys8] oxytocin is one of the most potent *in vitro* oxytocin antagonists obtained to date with a pA_2 value of 8.2 (Hill *et al.* 1988). Further examination of such topographical structural relations and further exploration of the dynamic properties of oxytocin and its analogues should provide other insights into the relation of oxytocin conformation to biological activity. Some aspects of this have been discussed recently (Hruby 1987), but there is certainly much more to be learned about utilizing the crystal structures reported here and the extensive structure–activity relation that are known (Hruby & Smith 1987).

(*d*) *Disorder and dynamics*

Close contacts to the disordered SG1 have been observed in molecules A and B. Table 10 shows a water molecule and the hydroxyl group of Tyr 2 making contacts of 3.47 and 3.49 Å with the alternative sites of SG1 of molecule A, and 3.42 and 3.39 Å with SG1 in molecule B,

Table 9*a*. H—bond of the residues and the main chain in molecule A (biologically inactive form – probably during transport)

	X	Y	^{r}X–H	^{r}X–Y	Θ^{a}
	O A1	ND2A5	1.98	3.01	172.10
	O A1	OD[a]	1.83	2.81	171.42
	O A1	OJ[a]	2.85	3.48	122.10
	N A2	O A5	1.93	2.97	161.26
accepting	O A2	OL	2.11	3.08	166.04
alternately	O A2	N A5	1.90	2.95	162.61
	OH A2	OE[a]	1.66	2.66	174.22
	N A3	O B7	1.77	2.84	172.56
	O A3	NHTBT	2.16	3.16	161.21
	N A4	OK	1.83	2.90	170.16
	O A4	OC	2.20	3.11	158.70
	O A4	OI	2.37	3.37	177.12
	O A4	OG	2.80	3.43	119.35
	OE1A4	OF	1.72	2.67	163.74
	NE2A4	OC	2.27	3.28	164.72
	NE2A4	OA	1.96	2.97	166.02
	N A5	O A2	1.90	2.95	162.61
	O A5	OJ[a]	3.11	3.47	102.75
	O A5	N A2	1.93	2.97	161.26
	OD1A5	OJ[a]	2.05	3.04	171.67
	OD1B5	OL	1.53	2.51	166.39
	ND2A5	O B8	1.88	2.74	135.03
	ND2A5	O A1	1.98	3.01	172.10
	N A6	OE1B4	2.49	3.28	129.27
		OI	2.04	3.09	163.22
	O A6	N A9	2.70	3.55	135.97
	O A7	N B3	1.75	2.81	165.72
	O A8	ND2B5	1.91	2.90	156.84
	N A9	O A6	2.70	3.55	135.97
	O A9	OM	1.88	2.85	173.28
	NHTAT	OH	2.02	3.05	172.47
	NHTAT	OB	1.99	2.98	156.79

[a] Active water.

Table 9*b*. H—bond of the residues and the main chain in molecule B

X	Y	^{r}X—H	^{r}X—Y	Θ
O B1	ND2B5	2.01	3.04	166.34
O B1	OD[a]	1.79	2.76	171.57
O B1	OE[a]	3.13	3.83	130.30
N B2	O B5	2.05	3.09	162.75
O B2	N B5	1.82	2.85	156.85
OH B2	OJ[a]	1.72	2.68	164.29
N B3	O A7	1.75	2.81	165.72
O B3	NHTAT	1.99	2.98	156.79
N B4	OA	1.93	2.95	154.88
O B4	OG	2.00	2.94	156.19
O B4	OH	2.02	2.99	161.64
O B4	OA	3.02	3.71	135.20
O1 B4	OI	1.58	2.53	174.72
N2 B4	OH	2.07	3.10	169.38
N2 B4	OK	2.09	3.13	176.40
N B5	O B2	1.82	2.85	156.85
O B5	N B2	2.05	3.09	162.75

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TABLE 9*b*. (*cont.*)

O B5	OE[a]	2.29	3.22	153.63
OD1B5	OA	1.96	2.91	163.07
OD1B5	OB	1.94	2.85	159.10
ND2B5	O B1	2.01	3.04	166.34
ND2B5	O A8	1.91	2.90	156.84
N B6	OE1A4	1.90	2.96	166.19
O B6	N B9	2.54	3.49	146.16
O B7	N A3	1.77	2.84	172.56
O B8	ND2A5	1.88	2.74	135.03
N B9	O B6	2.54	3.49	146.16
O B9	OB	1.94	2.89	159.16
NHTBT	O A3	2.16	3.16	161.21
NHTBT	OC	2.28	3.07	125.33

[a] Active water.

TABLE 10. INTERMOLECULAR HYDROGEN H—BOND DISTANCES AND ANGLES BETWEEN THE OXYTOCIN MOLECULES AND THE AQUEOUS ENVIRONMENT

	y	^{r}x—H(A)	^{r}X—Y(A)	$\Theta°$
donor	OD1B5	1.96	2.91	163.07
OA	OG	2.04	2.89	155.96
acceptor	NE2A4	1.96	2.97	166.02
OA	N B4	1.93	2.95	154.88
donor	OD1B5	1.94	2.85	159.10
OB	O B9	1.94	2.89	159.16
acceptor	OF	1.69	2.67	163.47
OB	—	—	—	—
donor	O A4	2.20	3.11	158.70
OC	OF	1.75	2.70	157.42
acceptor	NHTBT	2.28	3.07	125.33
OC	NE2A4	2.27	3.28	164.72
donor	O A1	1.83	2.81	171.42
OD	O B1	1.79	2.76	171.57
acceptor	OE	2.04	2.95	169.86
OD	OJ	2.22	3.21	173.81
donor	OD	2.04	2.95	169.86
OE	O B5	2.29	3.22	153.63
acceptor	OH A2	1.69	2.66	170.03
OE	SGBB1(0.7)	3.22	3.42	171.19
donor	OE1A4	1.72	2.67	163.76
OF	OB	1.69	2.67	163.47
acceptor	OC	1.75	2.70	157.42
OF	—	—	—	—
donor	OI	2.15	3.09	155.24
OG	O B4	2.00	2.94	156.19
acceptor	OA	2.04	2.89	155.96
OG	OK	2.06	3.06	173.78
donor	O B4	2.02	2.99	161.64
OH	OI	2.02	2.96	158.59
acceptor	NE2B4	2.07	3.10	169.38
OH	NHTAT	2.02	3.05	172.47
acceptor	N A6	2.04	3.09	163.22
OI	OH	2.02	2.96	158.59
	OG	2.15	3.03	155.29
donor	O A4	2.37	3.37	164.72
OI	OE1B4	1.58	2.53	174.72
donor	OD	2.23	3.21	171.56

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TABLE 10. (*cont.*)

OJ	OD1A5	2.05	3.04	171.67
acceptor	OH B2	1.70	2.68	173.28
OJ	SGAA1(.5)	—	3.49	—
donor	OG	2.06	3.06	173.78
OK	OL	1.72	2.70	163.49
acceptor	N A4	1.83	2.90	170.16
OK	NE2B4	2.09	3.13	176.40
acceptor	OK	1.72	2.70	163.49
OL	OM	1.78	2.73	171.16
donor	OD1A5	1.53	2.51	166.39
OL	O A2	2.11	3.08	166.04
donor	O A9	1.88	2.85	173.28
OM	OL	1.78	2.73	171.16
acceptor	OH	2.57	3.23	124.53
OM	NE2B4			
	alternative orientation of the waters OE and OJ			
donor	OH A2	1.66	2.66	175.98
OE	O B5	2.24	3.22	176.58
acceptor	OD	1.97	2.95	176.07
OE	SGBB1(0.7)	—	3.42	—
donor	OH B2	1.71	2.67	160.52
OJ	OD1A5	2.10	3.04	157.95
acceptor	OD	2.23	3.21	173.6
OJ	SGAA1(0.7)	—	3.49	—

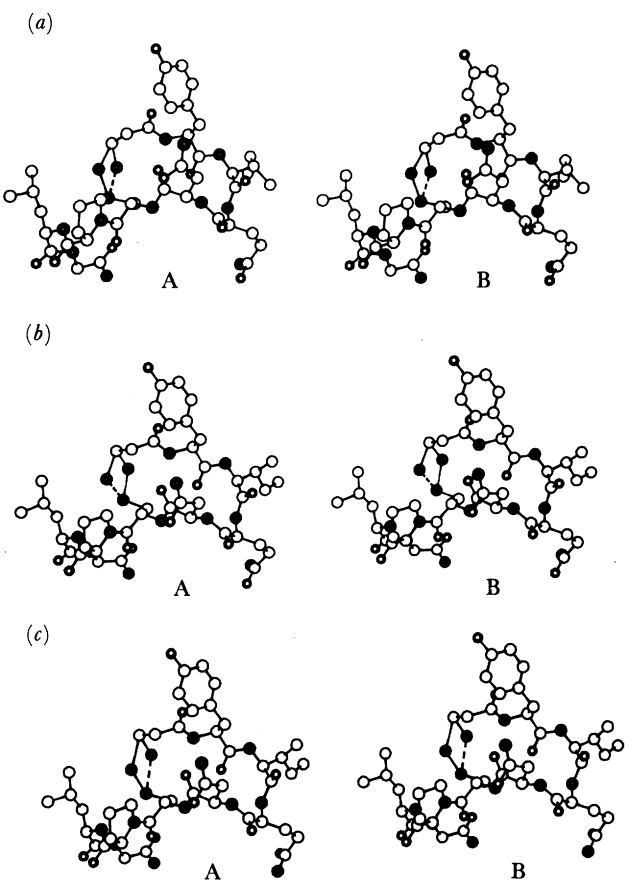

FIGURE 11. Stereo projection of molecules A, B and C are shown in (*a*), (*b*) and (*c*), respectively.

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respectively. The alternative positions of the S—S bond appear to be associated with the alternative van der Waals contacts, and suggest a possible connected motion of the water molecule J near deamino-oxytocin molecule A and the Tyr 2 of the molecule B with the S—S alternative positions. Tables 9 and 10 show how the alternative sites of the SG1 atoms of the disordered cystine residues make close intermolecular contacts with the tyrosyl hydroxyls, either directly or via water molecules. This suggests that the hydroxyl oxygen atom on the tyrosine is able to rotate and hence alternatively donates and accepts from the adjacent water molecules. Energy minimization and molecular dynamics simulation on the isolated deamino-oxytocin molecule A over a period of one nanosecond at room temperature show the molecule to be flexing and contracting, stretching out from residue Tyr 2 down to residue Pro 7; during this time the S—S bridge is alternating from a left-handed conformation to a right-handed one (Treharne *et al.* 1986). This shows that the two conformers may be in thermal equilibrium, even in the crystal lattice. The close contact of a molecule of deamino-oxytocin with a neighbouring tyrosine (table 10) suggests that a similar interaction might occur when the hormone is bound to neurophysin where Tyr 49 is strongly implicated in the intermolecular interactions. If so, the oxytocin molecule might be expected to have a right-handed conformation in the complex. Finally, figure 11 shows stereo views of molecules A, B and C.

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FIGURE 8. The crystal packing of deamino-oxytocin, when viewed down the twofold axis showing hydrophobic and hydrophilic interactions.

FIGURE 9. Root mean square fitting of Cα atoms of the two independent molecules A and B in $P2_1$ and C in $C2$. (*a*) shows fitting of A (red) and B (green); (*b*) shows the fitting of all three molecules A (blue), B (red) and C (green).