Nuclear Magnetic Resonance Conformational Studies of the C_α–C_β Fragments of Oxytocin, Oxytocinoic Acid, and Tocinoic Acid in Aqueous Solution

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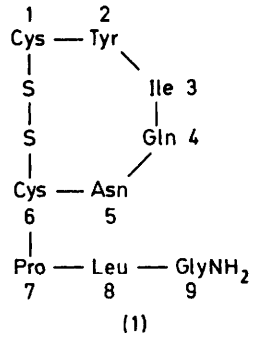
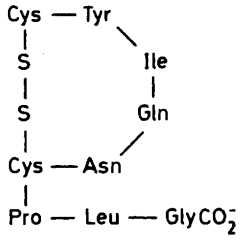
We have recorded the ^1H n.m.r. spectra (300 and 270 MHz) of oxytocin, oxytocinoic acid, and tocinoic acid in aqueous solution in the presence and absence of lanthanide ions. From the analysis of the $C_\alpha HC_\beta H_2$ regions of the spectra, the $J_{\alpha,\beta}$ coupling constants have been derived and found to be similar for corresponding residues in the different molecules indicating these fragments to have similar conformations in the three molecules. The coupling constants for the side-chains of Tyr, Ile, Gln, and Asn have been used to estimate the fractional populations of the three minimum-energy staggered rotamers. The Cys(1)–Cys(6) fragments also have similar conformations and, for tocinoic acid, this conformation has been partially defined by analysing the combined coupling constant and lanthanide induced shift data. The binding of lanthanide ions does not affect the conformation (no change in coupling constants when La^{3+} ions bind) and because tocinoic acid, oxytocin, and oxytocinoic acid have similar conformations for their Cys(1)–Cys(6) fragments, the conformational information derived for tocinoic acid will be valid also for oxytocin and oxytocinoic acid.

OVER the last few years the neurohypophyseal hormone, oxytocin (1), and related peptides have been subjected to many detailed n.m.r. investigations aimed at determining their conformations in solution.[1-7] Most of the information has been deduced from studies of three-bond coupling constants between NH and $C_\alpha H$ protons (related to ϕ dihedral angles) and from the temperature



(1)



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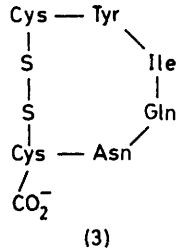


(3)

dependence of the NH proton chemical shifts (to detect intramolecular hydrogen bonding). Urry and Walter [1,2] have proposed a conformational structure for oxytocin in [$^2H_6$]dimethyl sulphoxide which contains two β-turns; one in the ring structure involving the sequence Tyr-Ile-Gln-Asn, with a hydrogen bond between the Asn NH and Tyr C=O and the other in the tail of the molecule, Cys-Pro-Leu-GlyNH_2 with a hydrogen bond between the Gly peptide NH and the Cys(6) C=O. A further hydrogen bond between the Leu NH and the Asn side-chain C=O was also proposed. In aqueous solution oxytocin shows no evidence for intramolecular hydrogen bonding involving NH protons [3-5] and a comparison of the $J_{NH,CH}$ coupling constants in [$^2H_6$]dimethyl sulphoxide and water [6,7] also indicates that its conformation is different from that in dimethyl sulphoxide. Brewster and Hruby [6] have concluded that in aqueous solution oxytocin is a flexible molecule existing as a mixture of several different conformations and the conformational energy calculations of Kotelchuck and his co-workers [8] appear to confirm this view.

Previous studies on oxytocin have neglected any detailed consideration of the $C_\alpha HC_\beta H$ coupling constants probably because of the complexity of the overlapping β-CH_2 multiplets. These coupling constants are of potential value not only because they provide information about the side-chain rotamer populations but, more importantly, because the $J_{\alpha,\beta}$ values for the Cys(1) and Cys(6) residues can provide us with additional knowledge of the ring conformation. In this paper we have obtained this information for oxytocin (1) and the related cyclic peptides oxytocinoic acid (2) and tocinoic acid (3).

Replacement of the *C*-terminal carboxamide of oxytocin by carboxy is accompanied by the loss of almost all oxytocic and avian vasodepressor activity,[9] but has little effect on binding to neurophysin.[10] Further removal of the tripeptide fragment Pro-Leu-Gly to produce tocinoic acid abolishes all avian vasodepressor

[1] D. W. Urry, M. Ohnishi, and R. Walter, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **66**, 111.
[2] D. W. Urry and R. Walter, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 956.
[3] J. Feeney, G. C. K. Roberts, J. H. Rockey, and A. S. V. Burgen, *Nature (New Biol.)*, 1971, **232**, 108.
[4] P. H. von Dreele, A. I. Brewster, J. Dadok, H. A. Scheraga, F. A. Bovey, M. F. Ferger, and V. du Vigneaud, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2169.
[5] J. D. Glickson, D. W. Urry, and R. Walter, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2566.
[6] A. I. Brewster and V. J. Hruby, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 3806.
[7] A. F. Bradbury, A. S. V. Burgen, J. Feeney, G. C. K. Roberts, and D. G. Smyth, *FEBS Letters*, 1974, **42**, 179.
[8] K. Kotelchuck, H. A. Scheraga, and R. Walter, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3629.
[9] B. M. Ferrier and V. du Vigneaud, *J. Medicin. Chem.*, 1966, **9**, 55.
[10] E. Breslow and R. Walter, *Mol. Pharmacology*, 1972, **8**(1), 75.

activity, although some oxytocic activity is still retained.[11]

The aim of this present study is to examine whether there are any conformational differences in these molecules in solution which might influence their very different biological activities. Tocinoic acid is a good model compound to assist in the spectral assignments of the ring residues and to estimate the effects of the oxytocin tail on the ring conformation. Furthermore, the charged carboxylate groups in oxytocinoic and tocinoic acid also afford us the possibility of binding paramagnetic lanthanide ions to assist in the conformational analysis.

EXPERIMENTAL

The ^1H n.m.r. spectra were recorded at 300 and 270 MHz using Varian (SC300) and Bruker (WH270) spectrometers. The peptides were examined in D_2O solutions (5—10mM) and t-butyl alcohol and sodium 4,4-dimethyl-4-silapentanesulphonate (DSS) were used as internal reference materials.

All spectra were recorded in the Fourier transform mode; some of the spectra were recorded using long acquisition times (5 s) and followed by a 32 K transform to ensure that errors in the coupling constant measurements did not arise from inadequate acquisition times or digitisation problems. In some cases convolution difference spectra were obtained which enhanced the resolution and assisted in the spectral assignment of the multiplets. The deuterium in the D_2O solvent was used as an internal field frequency lock. The pH measurements were made at 20 °C using a glass electrode Radiometer model 26 pH meter and the values quoted are meter readings. The experiments in which lanthanide metal ions were added to the peptides were made at constant lanthanide ion concentration by mixing peptide solutions (7mM at pH 4.8 and 5.2) containing the same concentration of La^{3+} and Eu^{3+} (total concentration 200mM).

Materials.—Oxytocinoic acid and tocinoic acid. Boc-*S*-benzyl-*S*-cysteinylbenzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl, and Boc-*S*-benzyl-L-cysteinylbenzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl resin peptides (Boc = benzyloxycarbonyl) were synthesised by the solid phase method of Merrifield [12-14] using a Beckman 990 peptide synthesiser.

In each case the peptide was cleaved from the resin by exposure for 60 min to hydrogen bromide in trifluoroacetic acid, using anisole as scavenger. After evaporation of the trifluoroacetic acid and isolation of the peptide by addition of ether, the cysteinyl-*S*-benzyl groups were removed by exposure to sodium in liquid ammonia according to the method of du Vigneaud,[15] allowing a 15 s end point. The free thiol groups thus produced were oxidised with ferricyanide.[16]

After desalting on Sephadex G15 in 50% acetic acid, purification of the crude peptides was carried out by chromatography on a column of A25 DEAE Sephadex with elution by 0.05M-phosphate at pH 8.0 with a linear sodium chloride gradient to 0.5M. The peptides were eluted at a salt concentration of 0.10M-sodium chloride.

Desalting was carried out on G15 Sephadex according to the procedure of Manning [17] for oxytocin.

Amino-acid analysis gave for oxytocinoic acid, Gly 1.0, Leu 1.0, Pro 0.9, Asp 0.9, Glu 1.0, Ile 0.9, Tyr 0.9, and for tocinoic acid, Asp 1.0, Glu 1.1, Ile 1.1, Tyr 1.1. T.l.c. on Merck 60 F_{254} in chloroform–methanol–acetic acid (90 : 5 : 5) gave for tocinoic acid one spot of R_F 0.36 and for oxytocinoic acid one spot of R_F 0.32, and in butanol–acetic acid–water (4 : 1 : 5) gave for tocinoic acid one spot of R_F 0.27 and for oxytocinoic acid one spot of R_F 0.25.

Oxytocin.—Synthetic oxytocin hydrochloride was kindly supplied by Dr. E. R. Evans, Sandoz Products Ltd.

Lanthanides.—Lanthanide (La^{3+}, Eu^{3+}) chlorides were prepared from the corresponding oxides (Koch–Light) using a small excess (*ca.* 10%) of ^2HCl. After freeze drying the chlorides were made up as 150mM solutions and the pH adjusted with diluted ^2HCl or KO^2H.

RESULTS

Analysis of ^1H N.m.r. Spectra.—Brewster and Hruby [6] have previously made the assignments of the β-CH_2 protons in oxytocin and we have used their results to assist in assigning the spectra of oxytocinoic and tocinoic acid. Confirmation of some of the assignments was obtained from

TABLE 1

The ^1H chemical shifts from DSS reference * for oxytocin (pH 6.6), oxytocinoic acid (pH 5.6), and tocinoic acid (pH 5.9) in aqueous solution †

Amino-acid residues		Oxytocin	Oxytocinoic acid	Tocinoic acid
Cys (1)	α	3.84	4.33	4.29
	β	3.32	3.47	3.48
	β′	3.20	3.32	3.28
Tyr (2)	α	4.84	4.82	4.82
	β	3.23	3.20	3.23
	β′	3.02	3.01	3.02
	o	7.27	7.24	7.24
	m	6.94	6.90	6.90
Ile (3)	α	4.26	4.16	4.13
	β	1.94	1.94	1.97
	γ	1.67	1.67	1.66
	γ-CH_3	0.95	0.94	0.94
	δ-CH_3	0.90	0.91	0.91
Gln (4)	α	4.19	4.15	4.16
	β	2.09	2.0	2.15
	γ	2.43	2.42	2.45
Asn (5)	α	4.73	4.79	4.76
	β	2.88	2.89	2.95
	β′	2.85	2.85	2.90
Cys (6)	α	4.95	4.88	4.50
	β	3.02	3.01	3.10
	β′	3.32	3.28	3.34
Pro (7)	α	4.57	4.54	
	β	2.48	2.3	
	γ	1.96	2.0	
	δ	3.78	3.75	
Leu (8)	α	4.39	4.44	
	β	1.94	1.90	
	δ	0.90	0.91	
Gly (9)	α	3.98	3.82	
	α′	3.90	3.74	

* Measured with respect to Me_3COD (internal) and converted. † Chemical shifts are accurate to ±0.02 p.p.m.

Confirmation of some of the assignments was obtained from

[11] V. J. Hruby, C. N. Smith, D. K. Linn, M. F. Ferger, and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1972, **94**, 5478.
[12] R. B. Merrifield, *J. Amer. Chem. Soc.*, 1963, **85**, 2149.
[13] R. B. Merrifield, *Biochemistry*, 1964, **3**, 1385.
[14] R. B. Merrifield, *Science*, 1965, **150**, 175.
[15] V. du Vigneaud, L. Ressler, J. M. Swan, C. N. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Amer. Chem. Soc.*, 1953, **75**, 4879.
[16] D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, 1962, **237**, 1563.
[17] M. Manning, T. C. Nun, and J. N. M. Baxter, *J. Chromatog.*, 1968, **38**, 396.

ionisation effects on the chemical shifts and from spin decoupling experiments in which α-CH protons were selectively irradiated to indicate which β-CH_2 signals arise from the same residue. From an ABX analysis of the $C_\alpha HC_\beta H_2$ multiplets all the chemical shifts and coupling constants can be obtained; these are summarised in Tables 1 and 2. The coupling constants can be obtained from the analysis of the β-CH_2 multiplets alone which is important for those residues having α-CH signals obscured by the residual HOD signal. For tocinoic acid the β-CH_2 multiplets of Cys(1), Cys(6), Tyr(2), and Asn(5) are well resolved (see Figure 1) and the analysis is straightforward. For oxytocin (Figure 2) and oxytocinoic acid there is accidental overlap of some of the transitions in this region but in both cases the four overlapping AB multiplets could be assigned and the spectra fully analysed; the availability of the tocinoic acid data considerably simplified the analysis of the oxytocin and oxytocinoic acid spectra. Examination of Tables 1 and 2 indicates that for the same residue the observed chemical shifts and coupling constants in the three peptides are remarkably constant.

TABLE 2

The ^1H–^1H $J_{\alpha,\beta}$ coupling constants (H_2) for tocinoic acid, oxytocinoic acid, and oxytocin

Amino-acid residues	Tocinoic acid pH	$J_{\alpha,\beta}$	$J_{\alpha,\beta'}$	Oxytocinoic acid pH	$J_{\alpha,\beta}$	$J_{\alpha,\beta'}$	Oxytocin pH	$J_{\alpha,\beta}$	$J_{\alpha,\beta'}$	Rotamer populations in oxytocin $p_{(I)}$	$p_{(II)}$	$p_{(III)}$
Cys (1)	2.0	5.6	5.6	5.0	5.4	5.4	2.0	5.6	5.6			
	4.1	5.5	5.5	5.6	5.2	5.2	3.8	5.3	5.3			
	5.9	5.3	5.3				4.7	5.5	5.5			
	7.3	5.5	5.5				6.6	5.5	5.5			
							8.7	6.0	6.0			
Tyr (2)	2.0	6.5	8.7	5.0	6.5	9.5	2.0	6.6	9.3	0.70	0.30	0.0
	4.1	6.5	8.7	5.6	6.3	9.0	3.8	6.4	9.2	0.71	0.29	0.0
	5.9	6.3	8.5				4.7	6.3	8.9	0.68	0.29	0.02
	7.3	6.3	8.7				6.6	6.3	9.0	0.68	0.29	0.02
Ile (3) *	2.0	7.0		5.0	6.3		2.0	6.0		0.39	(0.61)	
	4.1	6.8		5.6	6.7		3.8	6.5		0.44	(0.56)	
	5.9	6.4					4.7	6.3		0.42	(0.58)	
	7.3	6.3					6.6	6.7		0.46	(0.54)	
							8.7	6.0		0.39	(0.61)	
Gln (4) †	2.0	7.8		5.0	7.0		2.0	7.5		(1.00)		0.0
	4.1	7.2		5.6	7.0		3.8	7.0		(0.86)		0.14
	5.9	7.0					4.7	7.2		(0.92)		0.08
	7.3	7.1					6.6	7.0.		(0.86)		0.14
							8.7	7.3		(0.95)		0.05
Asn (5) †	2.0	6.8		5.0	7.7		2.0	7.0		(0.86)		0.14
	4.1	6.8		5.6	7.7		3.8	7.7		(1.00)		0
	5.9	5.7	8.3				4.7	7.5		(1.00)		0
	7.3	7.0					6.6	7.2		(0.92)		0.08
							8.7	7.6		(1.00)		0
Cys (6)	2.0	3.5	9.0	5.0	3.7	9.3	2.0	3.9	9.2			
	4.1	3.5	8.9	5.6	3.7	9.1	3.8	3.5	9.2			
	5.9	3.5	9.0				4.7	3.9	9.0			
	7.3	3.5	8.5				6.6	3.5	9.2			
							8.7	3.8	9.0			

* For Ile rotamers H_A is replaced by a methyl group, J_t 12, J_g 2.1 Hz. † For Gln and Asn usually only $\frac{1}{2}(J_{\alpha,\beta} + J_{\alpha,\beta'})$ could be obtained from the deceptively simple spectra.

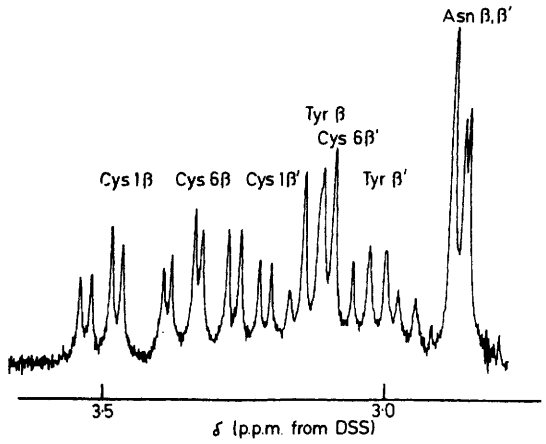


FIGURE 1 Part of the ^1H spectrum at 270 MHz of tocinoic acid in aqueous solution (pH 2.2)

Lanthanide-induced Chemical Shifts.—The addition of Eu^{3+} ions to solutions of tocinoic acid and oxytocinoic acid produces chemical shift changes induced by the paramagnetic ions bound to the carboxylate groups.[18] For

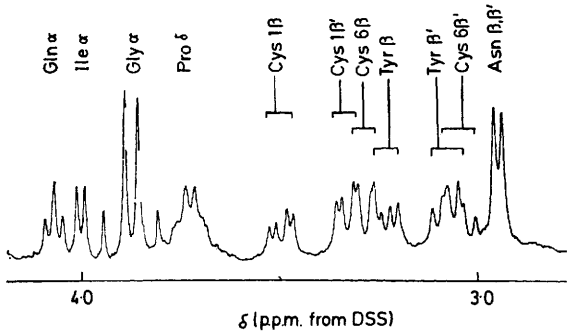


FIGURE 2 Part of the ^1H spectrum at 300 MHz of oxytocin in aqueous solution (pH 3.8). Assignments taken from Brewster and Hruby [6]

[18] B. A. Levine and R. J. P. Williams, *Proc. Roy. Soc.*, 1975, *A*, **345**, 5.

tocinoic acid the $C_\alpha HC_\beta H_2$ protons of the Cys(1) and Cys(6) residues are affected (see Figure 3) while for oxytocinoic acid the only protons appreciably affected are those in the

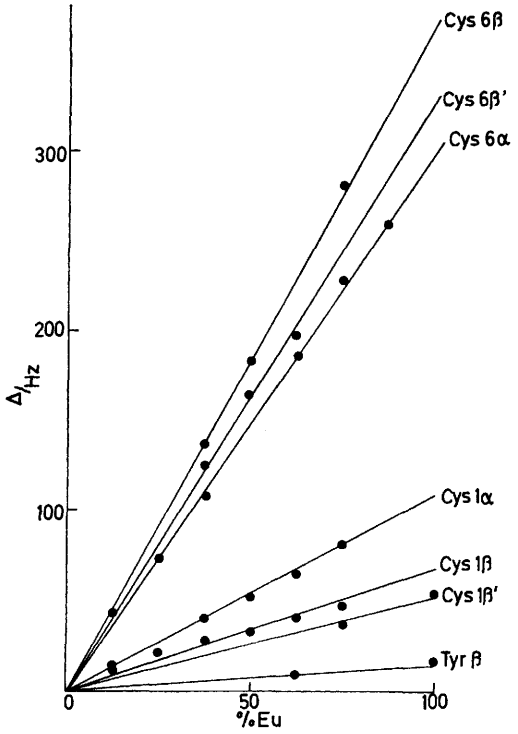


FIGURE 3 The Eu^{3+} induced ^1H chemical shifts (Hz at 270 MHz) for tocinoic acid in aqueous solution (pH 5.2) at 19 °C. Total lanthanide concentration $[La^{3+}] + [Eu^{3+}] = 200$mM

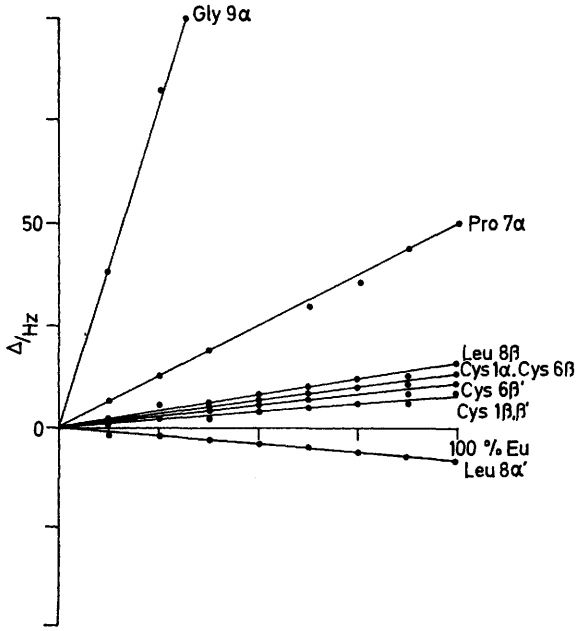
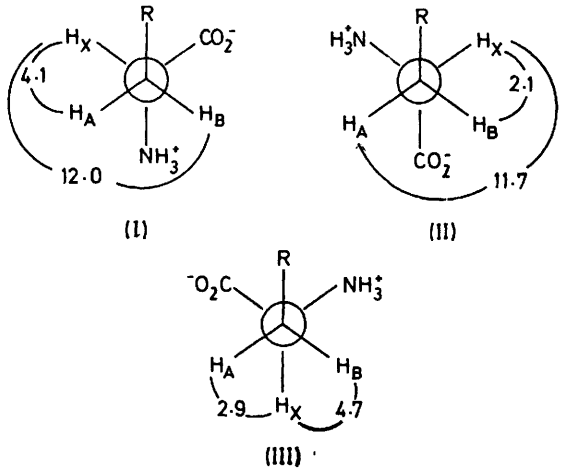


FIGURE 4 The Eu^{3+} induced ^1H chemical shifts (Hz at 270 MHz) for oxytocinoic acid in aqueous solution (pH 4.8) at 19 °C. Total lanthanide concentration $[La^{3+}] + [Eu^{3+}] = 200$mM

$GlyCH_2$ and the $ProC_\alpha H$ groups (see Figure 4). No changes in coupling constants are observed when La^{3+} or Eu^{3+} are added to the peptides.

Data Analysis.—$J_{\alpha,\beta}$ *Coupling constants.* It is usual to treat the side-chain three-bond coupling constants J_{AX} and J_{BX} as averaged values of the component coupling constants in the three minimum energy staggered conformations (I)—(III) weighted according to their fractional



populations $p_{(I)}$, $p_{(II)}$, and $p_{(III)}$. The *gauche-* and *trans-*coupling constants in the three rotamers have been estimated from model compound studies [19] and are shown on rotamers (I)—(III). Thus the averaged coupling constants J_{AX} and J_{BX} can be written as in equations (1) and (2),

$$J_{AX} = 4.1p_{(I)} + 11.7p_{(II)} + 2.9p_{(III)} \quad (1)$$

$$J_{BX} = 12.0p_{(I)} + 2.1p_{(II)} + 4.7p_{(III)} \quad (2)$$

where

$$p_{(I)} + p_{(II)} + p_{(III)} = 1 \quad (3)$$

From the measured values of J_{AX} and J_{BX} we can calculate the fractional populations using these equations. Table 2 gives the fractional populations calculated in this way for the Tyr(2), Ile(3), Gln(4), and Asn(5) side-chains for oxytocin. It is clear that the side-chain conformations are similar for corresponding residues in the different compounds.

For the Cys(1) and Cys(6) $C_\alpha HC_\beta H_2$ fragments the coupling constants are also similar in the three compounds, indicating similar conformations. From an examination of molecular models it is difficult to envisage how an interconverting mixture of rotamers (I)—(III) could exist with the restrictions which cyclisation imposes on the motions of the molecules and one must consider the possibility of conformations other than the staggered conformations (I)—(III). For such cases it is useful to refer to the Karplus type relationship deduced previously [19] for side chain dihedral angles χ in peptides * where the angle χ is defined as zero

$$J_{\alpha,\beta} = 9.9\cos^2\chi + 1.6 \ (\chi = 0—180°) \quad (4)$$

$$J_{\alpha,\beta} = 12.3\cos^2\chi - 0.8 \ (\chi = 180—360°) \quad (5)$$

when the two C_β–H bonds are eclipsed with the C_α–H_X bond and the C_α–N bond respectively; positive angles

* This Karplus curve was deduced for side-chains in which the β-carbon substituent was a carbon rather than a sulphur atom; however, the electronegativity difference between carbon and sulphur is such that this is unlikely to lead to large errors in the estimation of dihedral angles in Cys side-chains.

[19] J. Feeney, *J. Magnetic Resonance*, 1976, **21**, 473.

correspond to a clockwise rotation of the C_α–H_x bond when looking along the C_β–C_α bond. Using these equations it can be shown that the observed coupling constants for tocinoic acid are consistent with the Cys(1) and Cys(6) $C_\alpha HC_\beta H_2$ fragments existing within $\pm 10°$ of one of the following pairs of dihedral angles: Cys(1) χ_5 (53, 127°); (122, 227°); (227, 313°); (313, 53°); Cys(6) χ_1 (65, 144°); (115, 210°); (236, 330°); (304, 36°); (30, 105°); (150, 240°); (205, 305°); (330, 70°). The pairs of dihedral angles for a $C_\alpha HC_\beta H_2$ fragment should differ by 120° and the fact that the deduced values differ by somewhat lower angles (*ca.* 105°) could be due to errors in the Karplus type curve (*ca.* $\pm 10°$) or, more likely, to some averaging over a restricted range of dihedral angles centred on one of these sets of values.

In order to decide which is the correct set of dihedral angles we have considered the lanthanide-induced pseudo-contact shifts for tocinoic acid when Eu^{3+} ions are bound to the carboxylate anion.

Lanthanide-induced shifts (*L.I.S.*). Tocinoic acid. From previous studies of lanthanide ion binding to carboxylate groups in peptides it is known that a 1 : 1 complex is formed and that the induced ^1H shifts are pseudo-contact in origin for all nuclei except the α-CH proton.[15] For a complex with effective axial symmetry the magnitude of the pseudo-contact shift contribution is given by equation (6) where

$$\Delta\nu/\nu_0 = D(3\cos^2\theta_i - 1)/r_i^3 \qquad (6)$$

θ_i is the angle between the principal symmetry axis of the complexed ion and the distance vector r_i between the ion and a specific nucleus. For a carboxylate group binding to a lanthanide ion Levine and Williams [15] have located the lanthanide ion in the plane defined by the O–C–O angle and bound to each oxygen with the principal symmetry axis taken through the lanthanide ion and bisecting the O–C–O angle. We have adopted the same procedure as these workers and have used the BURLESK program [20] (provided by the Oxford Enzyme Group) to search for conformations which give lanthanide-induced shifts (ratios) in agreement with the observed shifts. The fragment of tocinoic acid containing the carboxylate group and the Cys(1) and Cys(6) protons influenced by the bound lanthanide ion can be defined in terms of five torsional angles, χ_i.

Table 3 gives the best solutions within families of BURLESK conformational solutions which agree with the observed shifts within a preset tolerance of the shift ratios and which are acceptable in terms of van der Waal's distance criteria. Table 4 contains the experimental and calculated LIS ratios. The only χ_1 value which gives a good solution for the Cys(6) β-CH_2 proton shifts and is consistent with the large value of the Cys 6β : 6α shift ratio is $\chi_1 = 167°$ (see

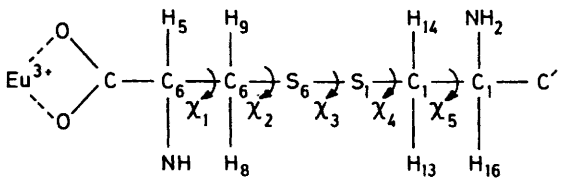


Figure 5). This corresponds to the conformation with the carboxylate group almost *gauche* to the Cys(6) β-CH_2 protons and is consistent with one of the solutions derived from coupling constants (χ_1 150, 240°) if we assume that there is some averaging about this conformation. The conformation of the rest of the Cys(1)–Cys(6) fragment is less certain. The four solutions for χ_3 are near to conformations for a right-handed and left-handed screw (χ_3 90, 270°) and are all reasonable for a disulphide bond. There is a fairly wide variation in the LIS determined values for

TABLE 3

Torsional angles (°) which give solutions for LIS ratio data for tocinoic acid

	I	II	III	IV
χ_1	167	167	167	167
χ_2	250	90	290	125
χ_3	72	110	253	293
χ_4	90	120	177	195
χ_5	200	260	180	160

The conventions for χ_1, χ_5 are defined in the text. χ_2, χ_3, χ_4 are defined as zero where the bonds $C_6\alpha$–$C_6\beta$–S_6–S_1–$C_1\beta$–$C_1\alpha$ are in a synplanar conformation and positive rotation is defined as a clockwise rotation of the far bond looking from the $C_6\beta$, S_6, and S_1 atoms respectively.

TABLE 4

Calculated and experimental LIS ratios for Cys(1) and Cys(6) protons in tocinoic acid

Atom	Experimental LIS ratio	Calculated LIS ratios I	II	III	IV
5-H	81.0				
8-H	100	100	100	100	100
9-H	88.7	88.8	88.8	88.8	88.8
13-H	14.3	14.5	14.4	14.5	14.3
14-H	18.3	17.9	18.1	18.1	18.3
16-H	29.7	29.8	29.7	30.0	29.8

χ_5 (160—260°); the conformation derived from coupling constants which gives the best agreement with the LIS values is χ_5 236, 330° (see Figure 5). It is likely that

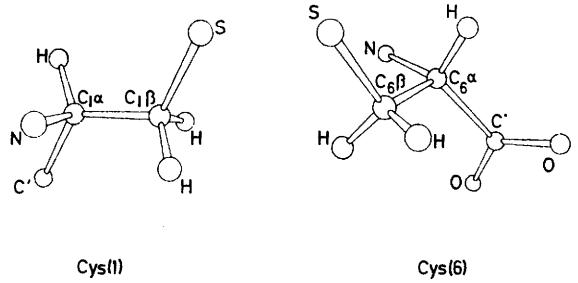


FIGURE 5 Conformation of Cys(1) (χ_5 236°) and Cys(6) (χ_1 167°) fragments in tocinoic acid deduced from LIS and $J_{\alpha,\beta}$ data

there are restricted motions over a range of conformations leading to some averaging of the observed LIS and coupling constant values. This restricted range of conformations for the Cys(1)–Cys(6) fragment can be considered to be centred on the conformations shown in Figure 5.

Oxytocinoic acid. The LIS data for oxytocinoic acid (Figure 4) has not been analysed quantitatively because the small observed shifts are too few in number to define the complex conformational situation. However, it is interesting to note that there are small LIS effects for some of the ring protons [Cys(1), Cys(6), and Ile] in addition to those for the Leu β-CH_2 and the Pro α-CH protons.

[20] C. D. Barry, A. C. T. North, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *Nature*, 1971, **232**, 236.

DISCUSSION

The similarity of the chemical shifts and coupling constants for corresponding residues in the three peptides indicates that the side-chain and the Cys(1) and Cys(6) $C_\alpha HC_\beta H_2$ fragments have similar conformations in the different compounds.

Because the coupling constants do not change by large amounts when the pH is varied there can be no major changes in conformation when the ionisable groups change their ionisation states. The coupling constants also do not change markedly in the presence of lanthanide ions which indicates that the conformational information deduced from LIS ratios for tocinoic acid will be valid also for tocinoic acid in the absence of lanthanide ions.

Tocinoic Acid and Oxytocin.—The conformational information for the Cys(1) and Cys(6) fragments in tocinoic acid obtained from LIS and coupling constant data is probably valid for oxytocin and oxytocic acid. Although reasonably good solutions for single conformations can be obtained for both the coupling constant and LIS data, the agreement between the conformations is only moderate and strongly suggests that there is some conformational averaging taking place. It is likely that most of the contributing conformations are centred on the structures shown in Figure 5 although one cannot exclude the possibility of small contributions from other conformations. Unfortunately the LIS data does not provide unequivocal information about the disulphide bond conformation; all four solutions have C–S–S–C dihedral angles which are somewhat distorted from the value measured in the crystal structure of L-cystine [21] (106 or 286° depending on how the dihedral angle is defined) but they all fall within the range of values measured in cyclic disulphides.[22]

For the side-chain rotamer populations the measured values differ somewhat from those measured for the same amino-acid in simple dipeptides. In particular, rotamer (III) is less populated in the cyclic peptides where there are increased steric interactions between the α-carbon substituents and the side-chain group.

The similar side-chain conformations observed for tocinoic acid and oxytocin imply that the Pro-Leu-Gly tail of oxytocin is not interacting strongly with the side-chain groups of the ring residues.

* The NH protons of oxytocinoic acid in H_2O solution have been measured but not yet assigned: the $J_{\alpha CH-NH}$ coupling constants measured on the unassigned bands are similar to the coupling constants measured for oxytocin and it is likely that the ring conformation is similar in the two compounds.

Oxytocinoic Acid.—The small magnitudes of the lanthanide-induced shifts for protons in the ring of oxytocinoic acid do not suggest a strong interaction between the tail and the ring of oxytocin but clearly the tail must exist for at least some of its time in a conformation over the ring such that these protons can be influenced. LIS Shifts of the observed magnitude could arise from conformations where the lanthanide metal is up to 8 Å from the affected protons but a detailed analysis is not possible from the limited information available.

Oxytocin and oxytocinoic acid have similar conformations which suggest that the charged carboxylate group of oxytocinoic acid is not influencing any of the observed conformational features of the oxytocinoic acid.* Thus the low oxytocic activity of oxytocinoic acid (*ca.* 1/400 of the activity of oxytocin itself when assayed on isolated uteri of rats [9]) cannot be related directly to its conformation in solution. It is possible that the bound conformation of oxytocinoic acid is quite different from that of oxytocin. However, the low oxytocic activity of oxytocinoic acid could equally well result from the absence of an interaction involving the Gly(9) amide group of oxytocin and its receptor.

Conclusions.—It is clear that by using a combination of coupling constant and LIS data it is possible to obtain more conformational information than is available from either method alone. For molecules such as oxytocin, which do not bind strongly to lanthanide ions, their conformations can be inferred from those of related molecules with free carboxylate groups capable of binding to lanthanides if the molecules can be shown to have similar conformations. We intend to extend these studies to other parts of oxytocin by examining the LIS data for oxytocin [Asp (5)] and oxytocin [Glu (4)] and combining this with the $J_{\alpha\text{-CH-NH}}$ coupling constant information already available.

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