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Circular dichroism studies of some arginine-vasopressin analogues

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CD spectra of arginine-vasopressin (AVP) and of its analogues substituted in position 1 and/or 7 were measured in aqueous solution at different pH values. The shapes of the CD spectra of AVP analogues substituted in position 1 are strongly influenced by the type of group attached to the β-carbon of residue 1. The substitution of the proline residues in position 7 by *N*-methylalanine also leads to a change in conformation of the peptide. The differences in the CD spectra are interpreted in terms of conformational changes, which are due to the interaction of the tyrosine side chain with neighbouring residues (for 1-substituted analogues of AVP), or to that between the hexapeptide ring and acyclic tripeptide chain (for 7-substituted analogues).

1. Introduction

Arginine-vasopressin (AVP):

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2
1 2 3 4 5 6 7 8 9

is a hormone exerting a wide variety of actions, in both the periphery and the central nervous system. Certain modifications of the structure of AVP affect its characteristic spectrum of activities and are exemplified by the specific agonist of AVP, [1-deamino,8-D-arginine]-vasopressin [1], which is a long-acting, potent and very selective antidiuretic peptide used as the drug of choice for treatment of diabetes insipidus. On the other hand, since the discovery by Du Vigneaud and coworkers [2–6] that the antagonists of neurohypophyseal hormones oxytocin and vasopressin could be obtained by substitution of a β,β-dialkyl-β-mercaptopropionic acid for the cysteine at position 1, many highly potent and selective antagonists of AVP have been described [7–10]. It is now widely accepted that there are different structural and conformational requirements for AVP agonists and antagonists interacting with different hormone receptors.

In an effort to determine the conformation of some selective agonists and antagonists of AVP synthesized in our laboratory, we have investigated the circular dichroism (CD) of these peptides [11–15]. Even if the CD spectra of AVP and its analogues prove to be too complex due to the presence of numerous chromophores (i.e., aromatic, disulfide and peptide groups), they are very useful for analysis of conformational changes of peptides influenced by steric and electronic factors [16,17].

In this paper, we have focused our attention on the CD spectra of AVP and its analogues with modifications in position 1 and/or 7. The structures of the peptides presented here are as follows:

1, arginine-vasopressin, AVP;
2, [7-glycine]-arginine-vasopressin, GAVP [15];
3, [7-sarcosine]-arginine-vasopressin, SAVP [11];
4, [7-*N*-methylalanine]-arginine-vasopressin, MAAVP [11];

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5, [des-7-proline]-arginine-vasopressin, [des-Pro^7]AVP [13];
6, [1-β-mercaptopropionic acid,7-sarcosine]-arginine-vasopressin, dSAVP [11];
7, [1-deaminopenicillamine,7-sarcosine]-arginine-vasopressin, dPSAVP [12];
8, [1-(β-mercapto-β,β-cyclopentamethylene)propionic acid]-arginine-vasopressin, [Mca^1]AVP;
9, [1-(β-mercapto-β,β-cyclopentamethylene)propionic acid,7-sarcosine]-arginine-vasopressin, [Mca^1]SAVP [12];
10, [1-β-mercaptopropionic acid,7-*N*-methylalanine]-arginine-vasopressin, dMAAVP [11];
11, [1-deaminopenicillamine,7-*N*-methylalanine]-arginine-vasopressin, dPMAAVP [12];
12, [1-(β-mercapto-β,β-cyclopentamethylene)propionic acid, 7-*N*-methylalanine]-arginine-vasopressin, [Mca^1]MAAVP [12];
13, [1-thiosalicylic acid]-arginine-vasopressin, [Ths^1]AVP [14];
14, [1-thiosalicylic acid,2-D-phenylalanine]-arginine-vasopressin, [Ths^1,D-Phe^2]AVP [14];
15, [1-thiosalicylic acid,2-D-phenylalanine,4-isoleucine]-arginine-vasopressin, [Ths^1,D-Phe^2, Ile^4]AVP [14].

The last two peptides (**14** and **15**), in addition

Table 1

CD data on arginine-vasopressin and analogues in aqueous solution at pH 6.9

Peptide	λ_{max} (nm)	θ_{max} (degree cm^2 dmol^{-1})	Peptide	λ_{max} (nm)	θ_{max} (degree cm^2 dmol^{-1})
AVP, **1**	286	−305	[Mca^1]SAVP, **9**	268	240
	225	13900		231sh	−2220
	205	−20500 [a]		205	−29690 [a]
GAVP, **2**	280	−210	dMAAVP,**10**	274	−200
	224	8040		235	−3680
	207	−15230 [a]		205	−34340 [a]
SAVP, **3**	295	−130	dPMAAVP, **11**	256	700
	223	19470		210	−20840 [a]
	205	−17570 [a]			
			[Mca^1]MAAVP, **12**	270	520
				205	−31060 [a]
MAAVP, **4**	287	−160			
	249	750	[Ths^1]AVP, **13**	290	−160
	225	7080		222sh	−6960
	205	−18530 [a]		205	−55190 [a]
[des-Pro^7]AVP, **5**	280	−240	[Ths^1, D-Phe^2]AVP, **14**	288	460
	223	−10270		254	−450
	207	−16600 [a]		232	−1360
				220	2170
dSAVP, **6**	270	−445		210	−11290 [a]
	226	−8010			
	205	−35470 [a]	[Ths^1,D-Phe^2,Ile^4]AVP, **15**	278	360
dPSAVP, **7**	252	640		210	−17140 [a]
	231	2230			
	205	−29990 [a]			
[Mca^1]AVP, **8**	267	−805			
	238	−460			
	210	−26420 [a]			

[a] Values of the ellipticity at arbitrarily chosen wavelength.

to the thiosalicylic acid residue in position 1, have modifications in positions 2, and 2 and 4, respectively.

2. Materials and methods

Peptides were synthesized by solid-phase peptide synthesis [11–15]. CD spectra were measured on a Jasco J-20 spectropolarimeter, using cells with path lengths of 0.1–1 cm, at a temperature of 20–22° C. Aqueous solutions of concentration approx. 0.5 mg/dm^3 were prepared by weighing lyophilized peptides and dissolving them in double distilled water or in KH_2PO_4-Na_2HPO_4 buffer (pH 6.88). The solutions of pH 3 and 11 were adjusted by adding concentrated HCl or NaOH (4 N) solutions of spectroscopic grade, respectively, and were measured with a Mera Elmat N 5122 pH-meter. The intensity and wavelength of the CD bands were correlated with the spectra of (+)-10-camphorsulphonic acid.

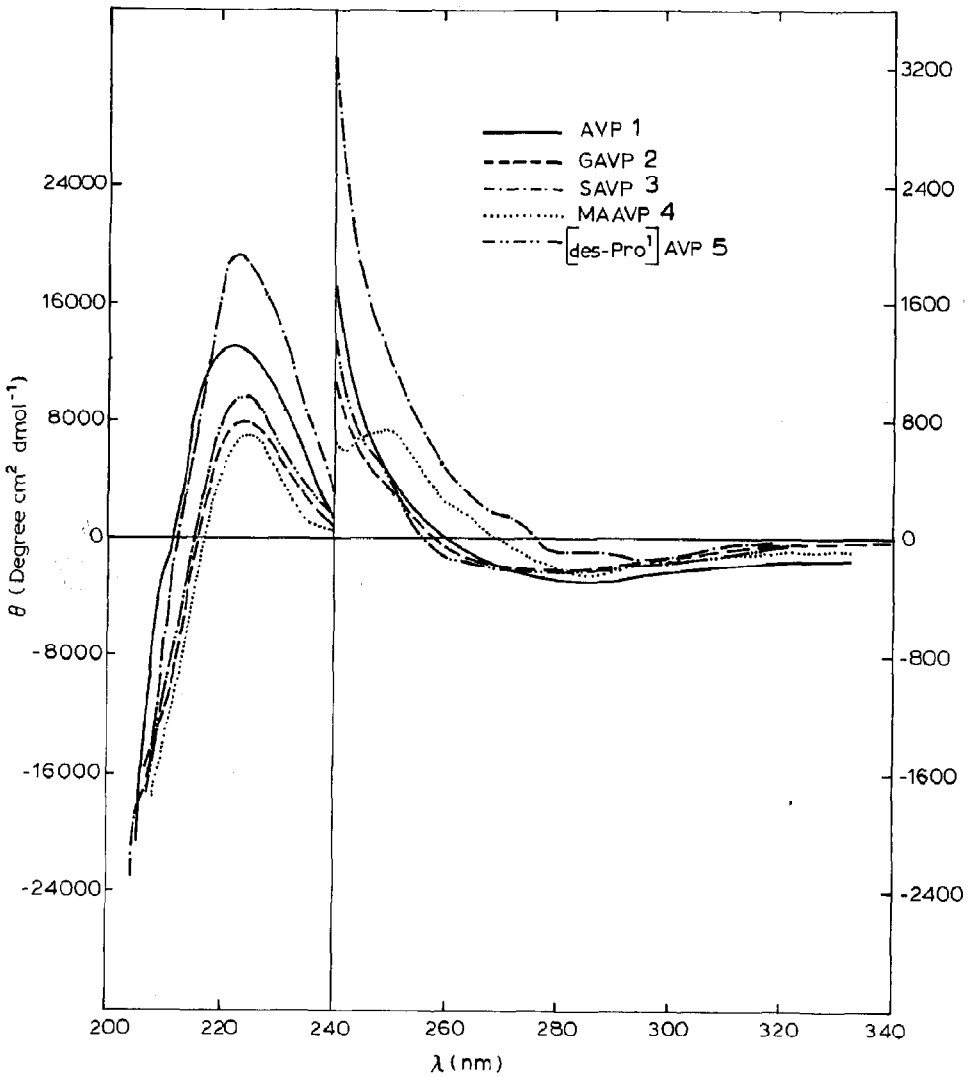


Fig. 1. CD spectra of arginine-vasopressin (AVP) and analogues in aqueous solution at pH 6.9.

3. Results and discussion

The CD spectra of AVP were identical with those previously published [17–19]. In fig. 1, the spectrum of AVP taken at pH 6.9 is juxtaposed with the appropriate spectra of GAVP (**2**), SAVP (**3**), MAAVP (**4**) and [des-Pro^7]AVP (**5**). The shapes of the CD spectra of the AVP analogues shown in fig. 1 closely resemble that of the parent hormone. The greatest difference, particularly over the range 240–270 nm, is observed in the spectrum of MAAVP. Here, a second positive CD band (λ_{max} 249 nm) is evident. The CD spectra of GAVP, SAVP and [des-Pro^7]AVP differ from that of AVP in the intensity of the positive CD band at 220–225 nm (a combination of the aromatic B_{1u} transition and of the amide n-π^* transition). In comparison to AVP, the spectrum of SAVP shows increased ellipticity of the positive CD band (220–225 nm), whereas for GAVP, MAAVP and [des-Pro^7]AVP the intensity of this band is decreased.

The differences in the CD spectrum of MAAVP can be interpreted in terms of conformational changes arising from the change in the torsional angle ϕ (fig. 2) [20]. The presence of two methyl groups attached to neighbouring N and C atoms in the *N*-methylalanine residue should change the angle in relation to AVP, GAVP and SAVP. Therefore, the interaction of an acyclic tripeptide with the N-terminal hexapeptide ring in MAAVP should differ from that of AVP and the other analogues studied. It is interesting that removal of the proline residue from the AVP structure does not significantly influence the CD spectrum.

An increase in intensity of the positive CD band (220–225 nm) of AVP, SAVP and MAAVP, in acidic solution (fig. 3) can be observed, which is due to the protonation of basic groups. The greatest increase in ellipticity is seen for the parent hormone. In the spectrum of MAAVP recorded at pH 3, a second positive CD band can be observed as a shoulder at 250 nm.

Dissociation of the phenolic group of the tyrosine residue causes a shift of the aromatic B_{1u} band to a longer wavelength [17] (fig. 4). Consequently, the usually unresolved combination band of amide and aromatic transitions of AVP and SAVP manifests itself as two separate positive CD bands at about 220 and 245 nm. In the case of MAAVP there is only one positive band at about 250 nm. This observation lends further support to the explanation of the different conformational behaviour of MAAVP in comparison with other 7-substituted analogues. Fig. 4 also shows that the long-wavelength aromatic B_{2u} band in the CD spectra of AVP, SAVP and MAAVP at pH 11 has a positive value, in contrast to the spectra recorded at pH 3 and 6.9.

Removal of the amino group from either SAVP or MAAVP results in different shapes of the CD spectra (figs. 5 and 6). A change in intensity, or the presence of a 225 nm CD band in the spectra

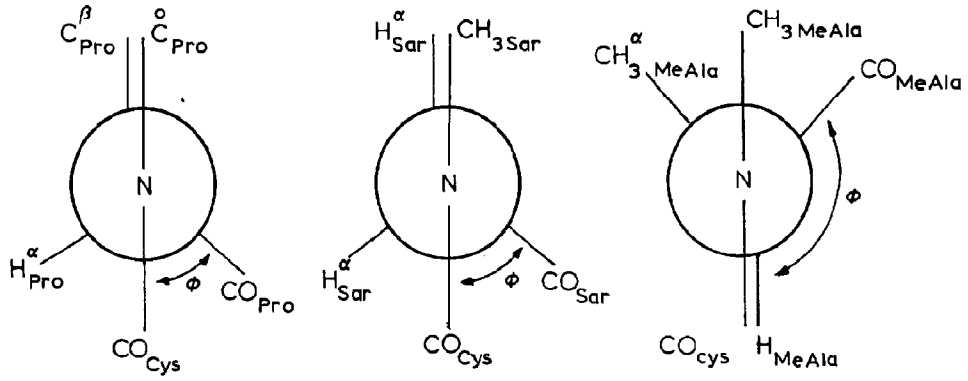


Fig. 2. Torsional angle ϕ in AVP, SAVP and MAAVP with respect to the amino acid residues in position 7.

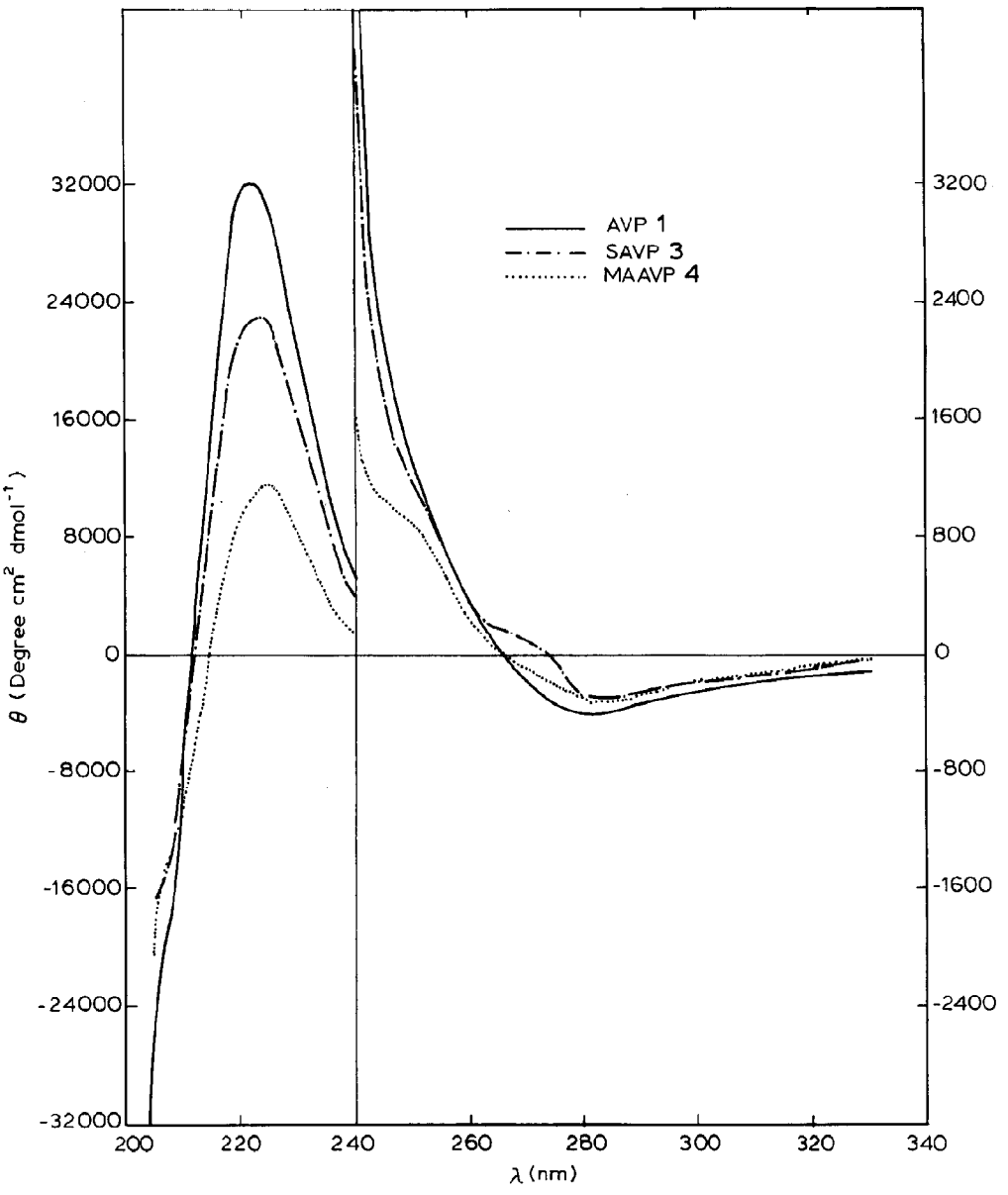


Fig. 3. CD spectra of AVP, SAVP and MAAVP in aqueous solution at pH 3.

of the respective analogues substituted in position 1 by the β-mercaptopropionic acid residue (**17**) indicates clearly the existence of an interaction between the aromatic nucleus of tyrosine with the amino group of the residue in position 1 in AVP, SAVP and MAAVP. On the other hand, examination of the CD curves of dSAVP and dMAAVP again confirms the strong influence of the *N*-

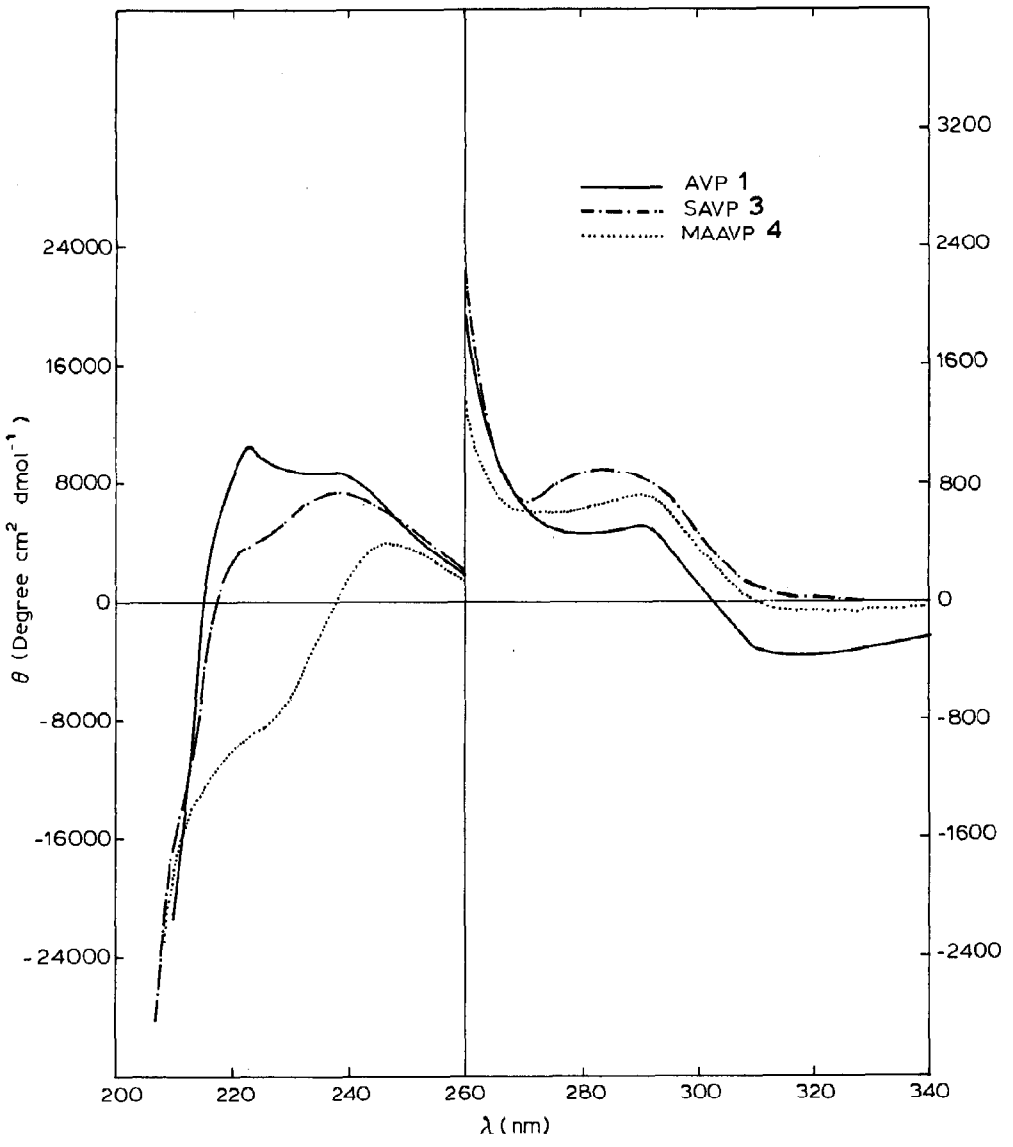
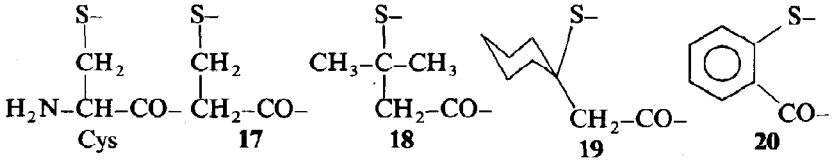


Fig. 4. CD spectra of AVP, SAVP and MAAVP in aqueous solution at pH 11.

methylalanine residue in position 7 on the conformation of vasopressin.

Therefore, in our opinion, the low agonistic activity of MAAVP and dMAAVP [11] as compared to the great potency of other 7-substituted analogues of AVP, i.e., GAVP [15], SAVP and dSAVP [11], can be explained by differences in conformation. In particular, the presence of a



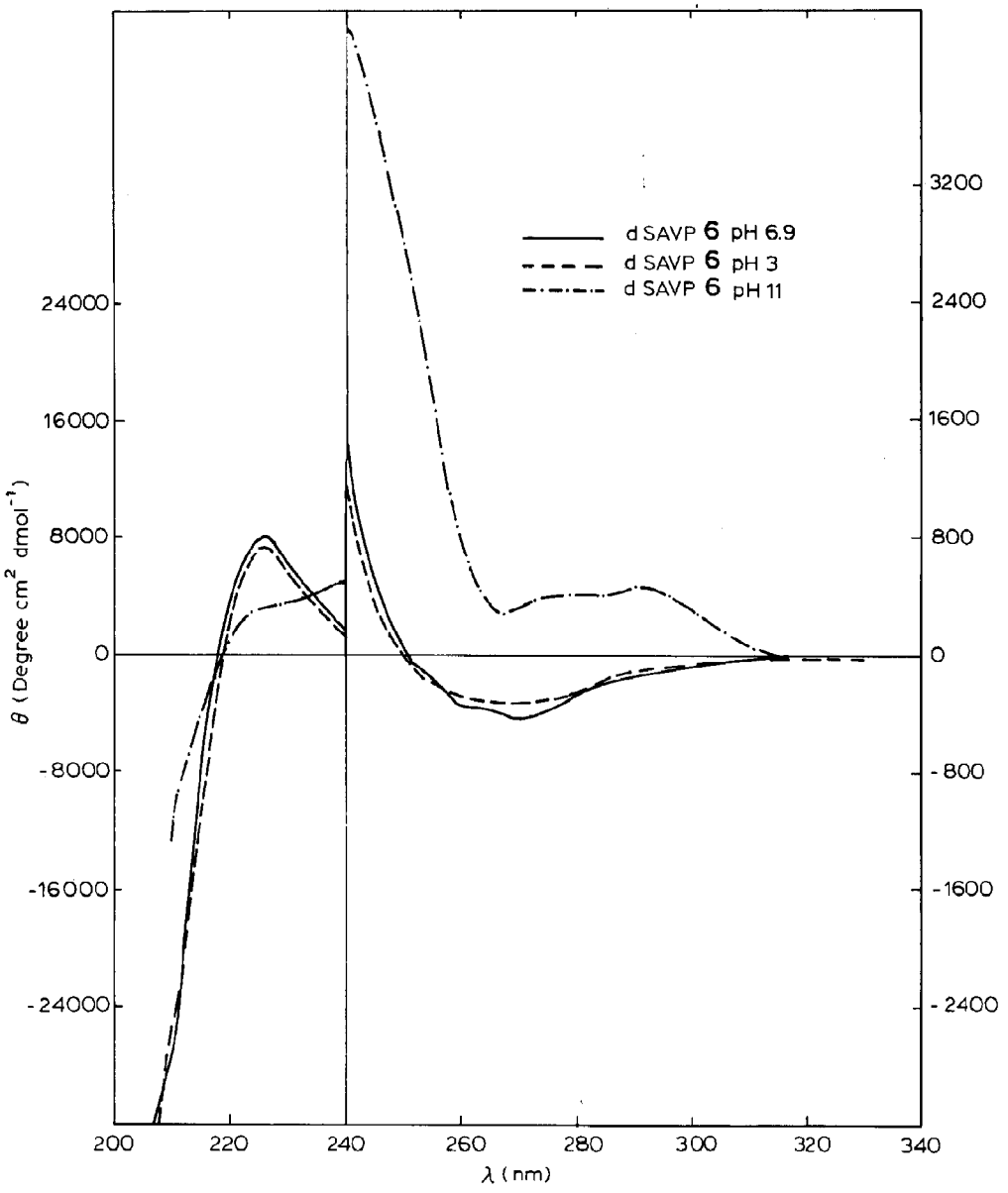


Fig. 5. CD spectra of dSAVP in aqueous solution at three different pH values.

C-terminal tripeptide is essential for the agonistic activity of AVP analogues [9,10].

Besides the interaction between amino, phenolic and amide chromophores, the disulphide group can also influence the shape of the CD curves discussed [16,17]. However, because of the lack of appropriate carba analogues and because of the low intensity of the disulphide dichroic bands which overlap with aromatic bands, no definite conclusion on the contribution of the disulphide

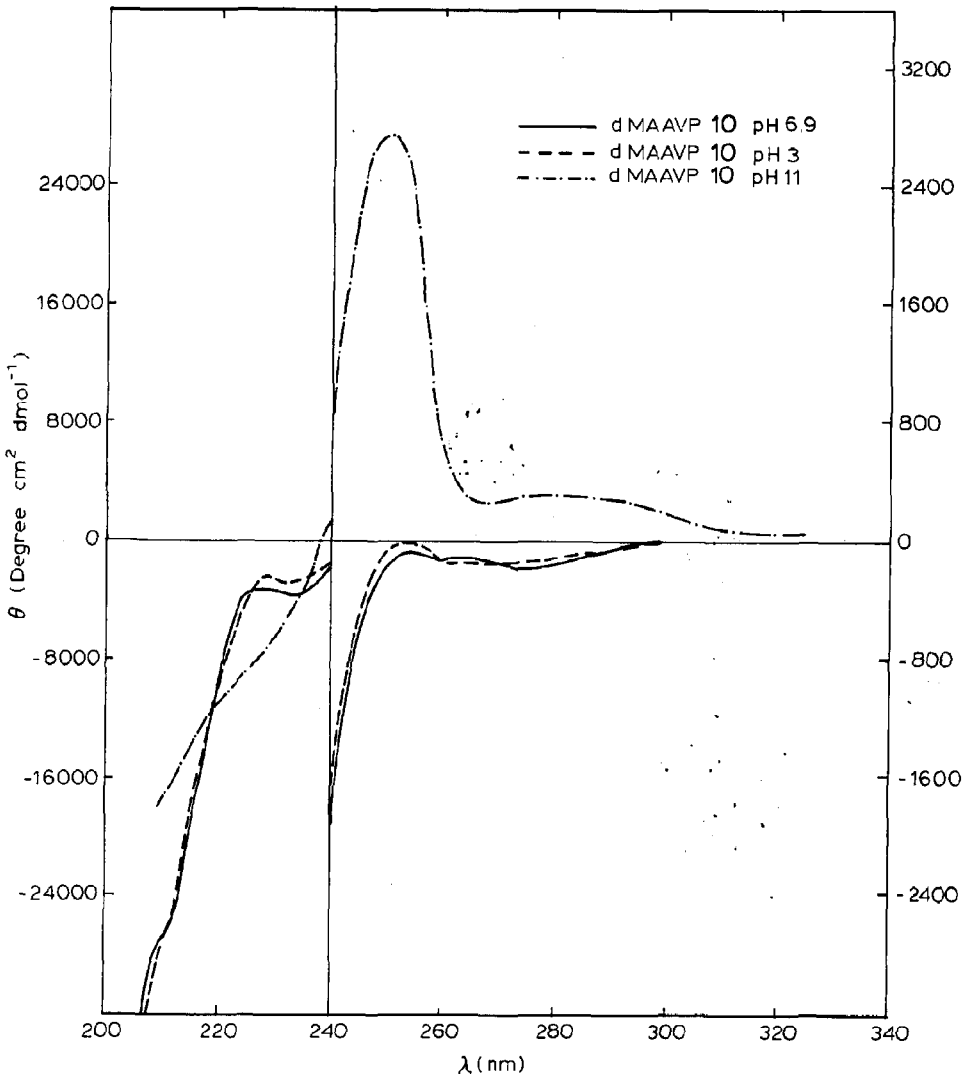


Fig. 6. CD spectra of dMAAVP in aqueous solution at three different pH values.

group to the observed changes of the CD curves can be drawn.

It is well known from the work of Du Vigneaud and co-workers [2–6] that the substitution of hydrogen atoms on the β-carbon atom of cysteine or β-mercaptopropionic acid residues in position 1 of oxytocin and vasopressin by alkyl groups brings about a sharp decrease in agonistic activities, and leads to antagonism of neurohypophyseal hormones.

Introduction of two methyl groups into the β-mercaptopropionic acid residue in SAVP and MAAVP gives rise to further pronounced changes in the CD spectra, not solely in the short-wavelength region (figs. 7 and 8). This is due to the enhanced rigidity of the AVP molecule as a result of the presence of the deaminopenicillamine residue (**18**) in position 1 [21,22]. In the case of dPSAVP and dPMAAVP, the ellipticity over 250 nm has a positive value, independent of the pH.

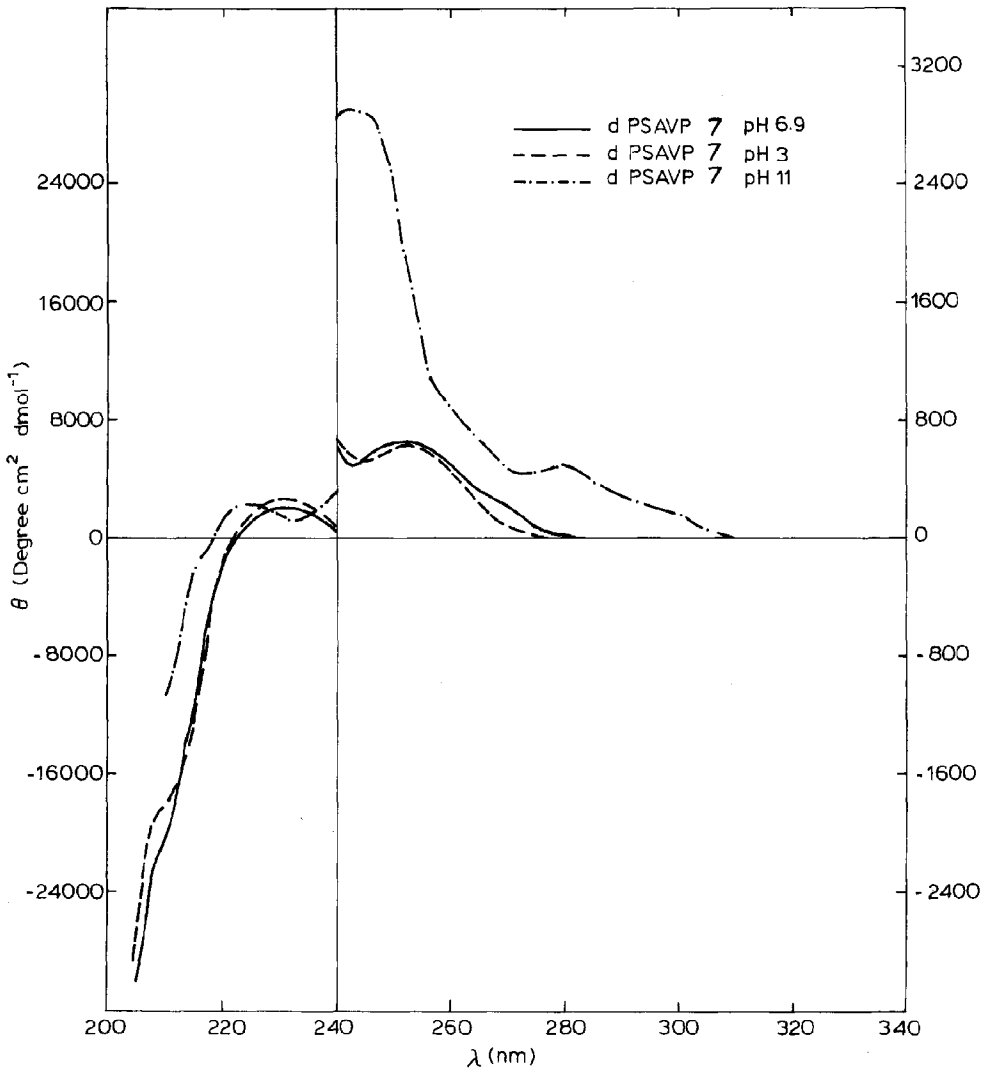


Fig. 7. CD spectra of dPSAVP in aqueous solution at three different pH values.

Still greater changes in the CD spectra are observed for analogues with the β-mercapto-β,β-cyclopentamethylenepropionic acid residue (**19**) in position 1 (figs 9 and 10). The CD spectrum of [Mca[1]]AVP at pH 6.9, as well as those of [Mca[1]]SAVP and [Mca[1]]MAAVP at pH 3, 6.9 and 11, are characterized by a negative ellipticity in the 210–245 nm region, and by the positive bands over 250 nm. It can be concluded that differences between these spectra and the others shown in the present article indicate the high degree of rigidity of the analogues containing an Mca residue in position 1.

Recently, in our continuing efforts to design new antagonists of AVP, we have synthesized some analogues with thiosalicylic acid (Ths) (**20**) in position 1 [14]. Contrary to our expectations, which were based on the hydrophobic properties of this residue and on the size of the hexapeptide ring, these analogues lack any antagonistic effect. Fig. 11 compares the spectrum of [Mca[1]]AVP, in which the cyclohexyl ring is situated perpendicular

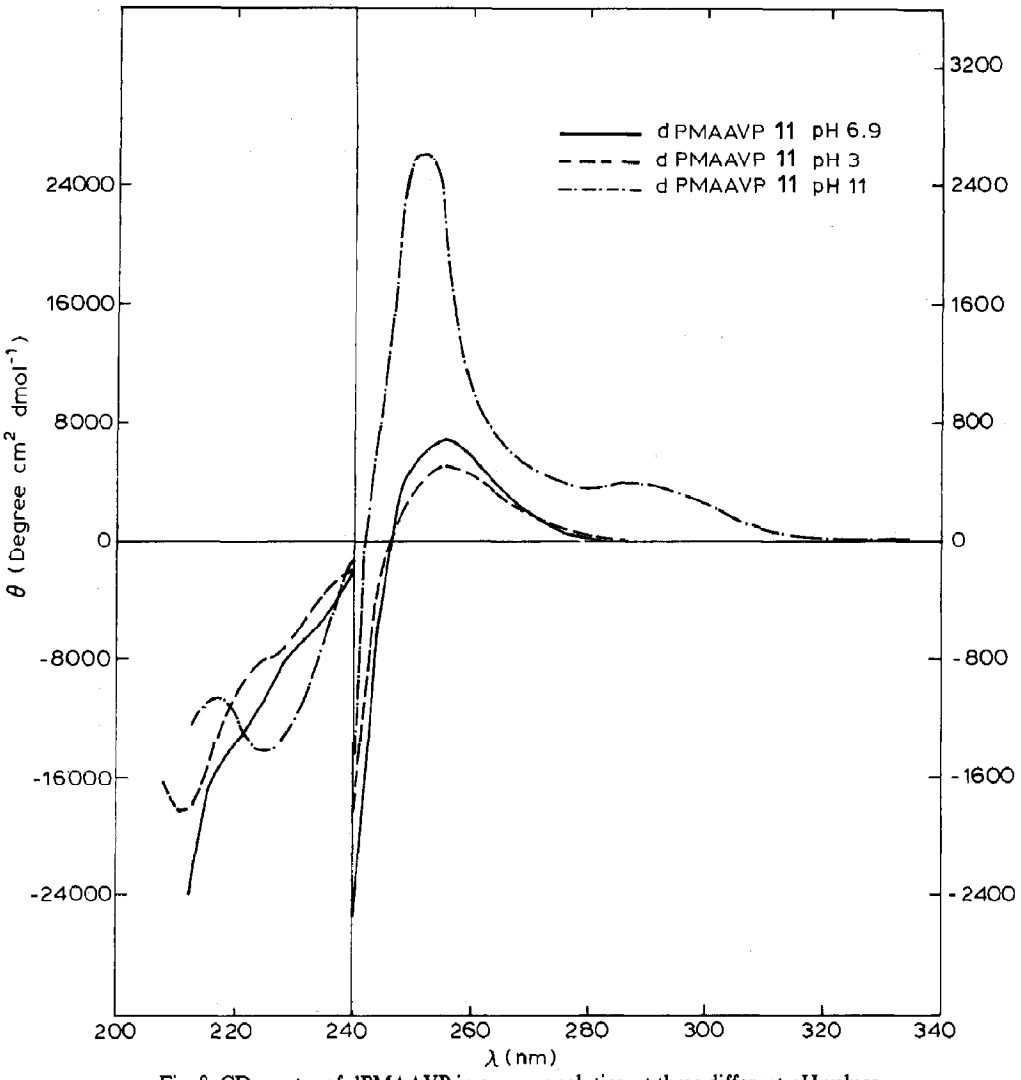


Fig. 8. CD spectra of dPMAAVP in aqueous solution at three different pH values.

to the hexapeptide unit, with that of Ths[1]-containing analogues, which have a parallel orientation of the aromatic ring. As can be seen in fig. 11, the spectra of [Mca[1]]AVP and [Ths[1]]AVP are characterized by very different shapes for their respective CD curves, thus indicating different conformations for the two peptides. In the spectra of two other analogues containing the Ths residue, further changes resulting from substitutions of tyrosine in position 2 by D-phenylalanine and of glutamine in position 4 by isoleucine can be observed.

Molecular mechanics calculations (energy minimization) have been carried out for [Mca[1]]AVP and [Ths[1]]AVP [14]. The calculated dihedral angles show that both peptides possess different conformations, especially in the hexapeptide unit, thus supporting the CD results.

4. Conclusions

Previous CD investigations of the conformation of arginine-vasopressin and its analogues have indicated that these peptides, due to the flexibility of the peptide backbone, possess an unordered conformation in solution [17]. Our present data also suggest that AVP and its analogues exist in aqueous solutions as a mixture of conformers.

However, we were able to obtain some evidence from the CD spectra of local conformational changes arising from substitutions in position 1 and/or 7. Comparison of the CD spectra of AVP, GAVP, SAVP, MAAVP and [des-Pro^7]AVP, as well as of analogues with additional substitutions in position 1, indicates that the *N*-methylalanine residue in position 7, in contrast to other amino acid residues, alters the conformation of the C-terminal tripeptide in relation to the cyclic N-terminal hexapeptide. This is due to non-bonding interactions of the two methyl groups of the *N*-methylalanine residue.

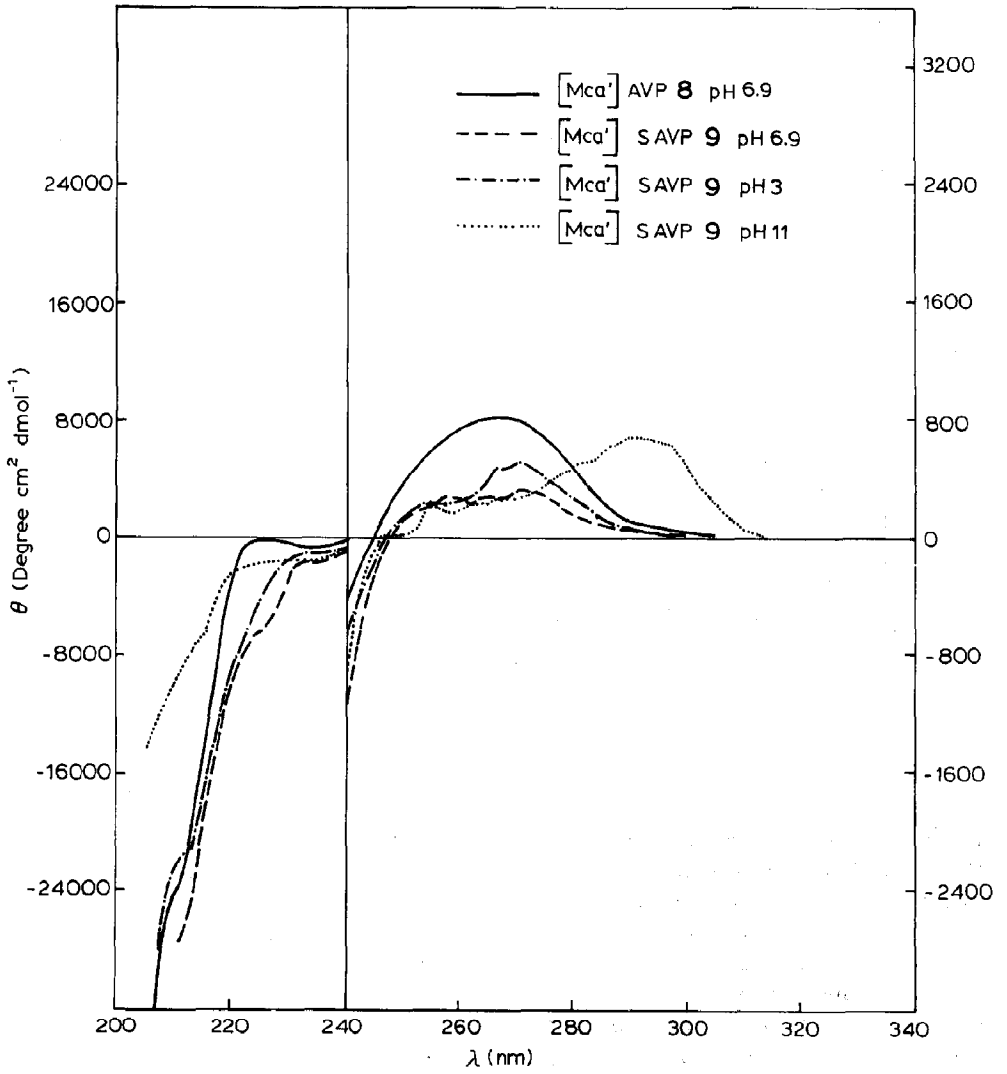


Fig. 9. CD spectra of [Mca^1]AVP and [Mca^1]SAVP in aqueous solution at four different pH values.

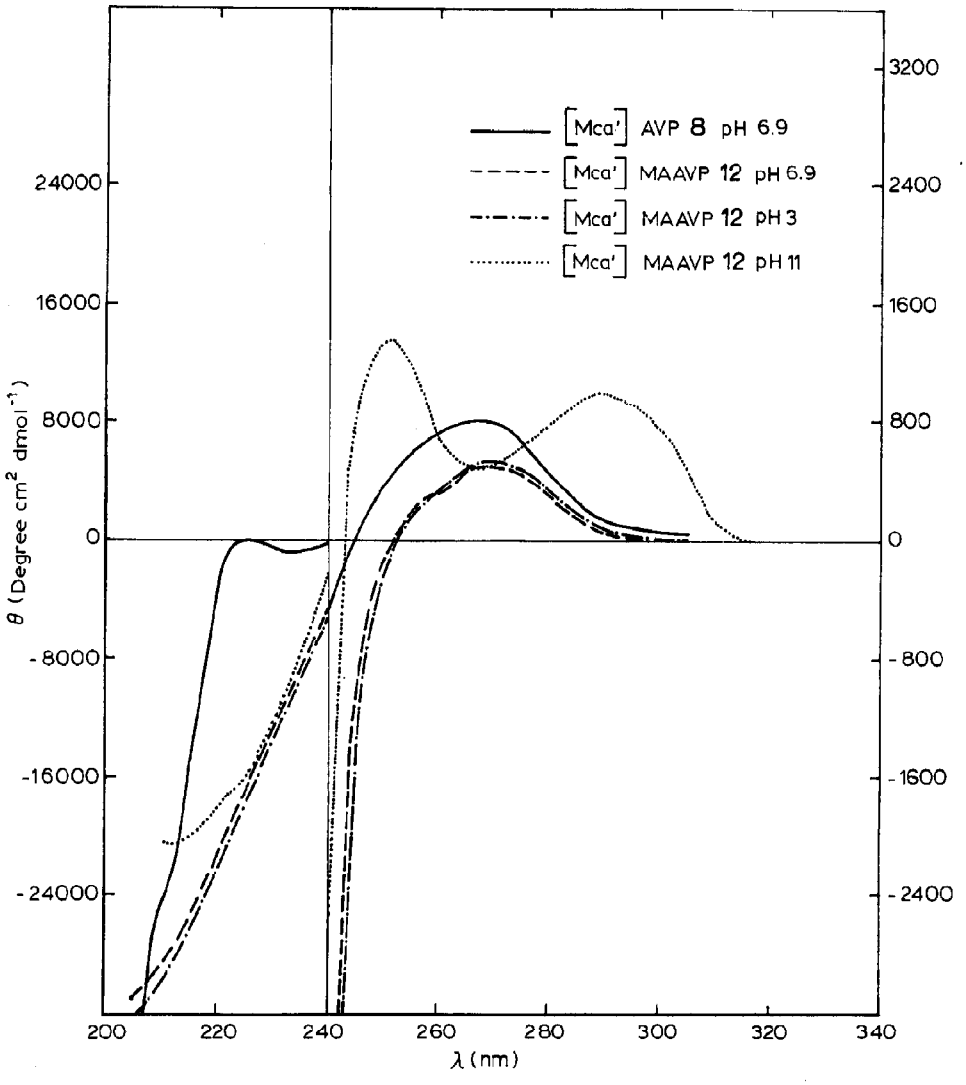


Fig. 10. CD spectra of [Mca[1]]AVP and [Mca[1]]MAAVP in aqueous solution at four different pH values.

Substitution of the cysteine in position 1 by β-mercaptopropionic acid and its β,β-dialkyl derivatives or by thiosalicylic acid also gives rise to profound changes in the CD spectra. The differences in local conformation around the N-terminal fragment are a consequence of the removal of the amino group, which makes the interaction of the aromatic phenolic group of tyrosine in position 2 with the amino group of cysteine in position 1 impossible. On the other hand, the CD spectra provide evidence of the high degree of rigidity of the AVP analogues containing the β,β-dialkyl-substituted β-mercaptopropionic acid residue in position 1. Introduction of the thiosalicylic acid residue into position 1, in addition to its stiffening influence, causes significant changes in torsional angles for the backbone of the hexapeptide fragment. The different conformation of the

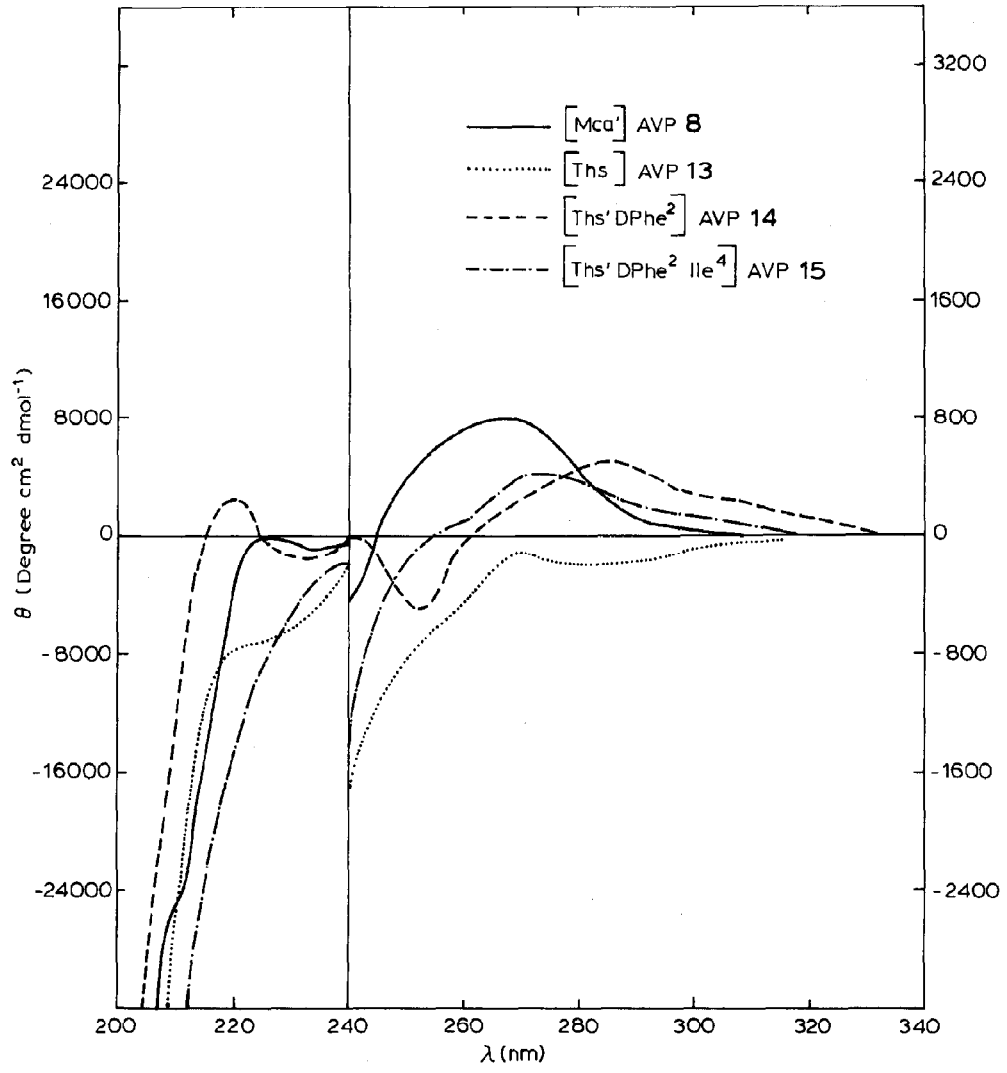


Fig. 11. CD spectra of [Mca¹]AVP, [Ths¹]AVP, [Ths¹,D-Phe²]AVP and [Ths¹,D-Phe²,Ile⁴]AVP in aqueous solution at four different pH values.

Ths-substituted analogues, demonstrated by their CD spectra, could explain the very low activity of these peptides.

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References

1 M. Zaoral, J. Kolc and F. Šorm, Coll. Czech. Chem. Commun. 32 (1967) 1250.
2 H. Schulz and V. du Vigneaud, J. Med. Chem. 9 (1966) 647.
3 R.J. Vavrek, M.F. Ferger, G.A. Allen, D.H. Rich, A.T. Blomquist and V. du Vigneaud, J. Med. Chem. 15 (1972) 123.
4 W.Y. Chan, J.J. Nester, Jr, M.F. Ferger and V. du Vigneaud, Proc. Soc. Exp. Biol. Med. 146 (1974) 346.

5 D.F. Dyckes, J.J. Nester, Jr, M.F. Ferger and V. du Vigneaud, J. Med. Chem. 17 (1974) 250.
6 J.J. Nester, Jr, M.F. Ferger and V. du Vigneaud, J. Med. Chem. 18 (1975) 284.
7 W.H. Sawyer, Z. Grzonka and M. Manning, Mol. Cell. Endocrinol. 22 (1981) 117.
8 W.H. Sawyer and M. Manning, in: Oxytocin: clinical and laboratory studies, eds. J.A. Amico and A.G. Robinson (Elsevier, Amsterdam, 1985) p. 423.
9 M. Manning and W.H. Sawyer, in: Vasopressin, ed. R.W. Schrier (Raven Press, New York, 1985) p. 121.
10 K. Jošt, M. Lebl and F. Brtnik, Handbook of neurohypophyseal hormone analogs (CRC Press, Boca Raton, 1987).
11 Z. Grzonka, B. Lammek, F. Kasprzykowski, D. Gazis and I.L. Schwartz, J. Med. Chem. 26 (1985) 555.
12 D. Gazis, I.L. Schwartz, B. Lammek and Z. Grzonka, Int. J. Peptide Protein Res. 23 (1984) 78.
13 M. Manning, J. Przybylski, B. Lammek, Z. Grzonka, A. Olma, K. Bańkowski, A. Misicka, M. Kruszyński and W.H. Sawyer, in: Peptides 1986, ed. D. Theodoropoulos (De Gruyter, Berlin, 1987) p. 459.
14 Z. Grzonka, L. Łankiewicz, F. Kasprzykowski and A. Liwo, in: Peptides 1986, ed. D. Theodoropoulos (De Gruyter, Berlin, 1987) p. 493.
15 F. Kasprzykowski, Z. Grzonka and P. Melin, Pol. J. Chem. 61 (1987) 641.
16 R.W. Woody, in: The peptides, vol. 7, ed. V.J. Hruby (Academic Press, Orlando, 1985) p. 15.
17 I. Frič, in: Handbook of Neurophypophyseal Hormone Analogs, eds. K. Jošt, M. Lebl and F. Brtnik (CRC Press, Boca Raton, 1987) p. 159.
18 I. Frič, M. Kodicek, M. Flegel and M. Zaoral, Eur. J. Biochem. 56 (1975) 493.
19 A.T. Tu, J. Lee, K.K. Deb and V.J. Hruby, J. Biol. Chem. 254 (1979) 3272.
20 E. Gwizdała, B. Lammek Z. Grzonka, Pol. J. Chem. 59 (1985) 1153.
21 V.J. Hruby, K.K. Deb, J. Fox, J. Bjarnason and A.T. Tu, J. Biol. Chem. 253 (1978) 6060.
22 V.J. Hruby, H.I. Mosberg, J.W. Fox and A.T. Tu, J. Biol. Chem. 257 (1982) 4916.