SYNTHESIS AND CIRCULAR DICHROISM OF SOME ANALOGUES OF DEAMINO-1-CARBA-OXYTOCIN WITH MODIFICATIONS OF THE AMINO-ACID RESIDUE IN POSITION 2* **

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By a stepwise procedure, and cyclisation by means of active esters in the final stage of the synthesis, 3 analogues of deamino-1-carba-oxytocin were prepared, with substitution of the tyrosine residue by phenylalanine (*Ie*), O-methyltyrosine (*If*) and isoleucine (*Ig*). The circular dichroic spectra of these substances were measured along with those of other oxytocin analogues structurally more similar to the parent molecule (with preservation of the disulphide bridge, the N^{α}-amino group and the tyrosine residue, respectively). The contribution of the aromatic chromophore to separate dichroic bands and the effect of modification of the amino-acid residue in position 2 on the conformation of this residue and neighbouring parts of the molecule are discussed.

The structure–activity relationships of the neurohypophysial hormones oxytocin and vasopressin have been subjected to intense study[2–4]. Naturally, the term structure should comprise not only the constitution of the molecule, but also its spatial arrangement. The latter aspect has also received considerable attention in recent years[5–7]. The basic problem is the question what is the spatial arrangement related to the biological activity. Biological activity starts at that moment when an active molecule in a given conformation interacts with a target tissue. This moment is, however, for the time being unavailable to direct observation. The only possibility is to study the conformation of the biologically active peptides in solution. The relationship between conformations in solution and at the moment of receptor interaction need not be a necessarily obvious one. Nevertheless it would appear to be a useful task to compare data on molecular conformation in solution, without receptor contact, in a series of analogues in order to try to analyse what structural factors influence biological activity. Such a comparison cannot be made at a quantitative level such as that using a linear correlation of free energies (for example, see[8–10]). On the other hand, this empirical approach is not so much taxed with extensive approximations which

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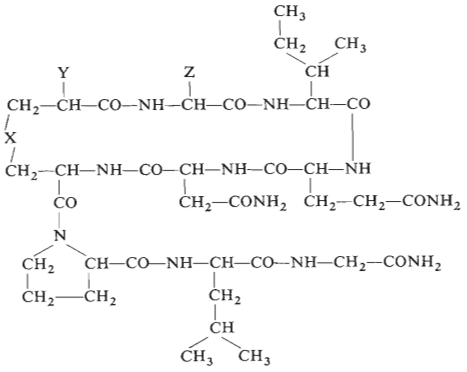
would be necessary in any attempt at quantitative comparison of separate structural factors.

Previous studies have shown that structural changes in the vicinity of amino-acid residue in position 1, changes in the disulphide bridge and the structure of amino acid in position 2 are all important for the activity of analogues of oxytocin. In the present work we have studied the circular dichroism of several analogues of oxytocin with an altered amino acid in position 2.

The synthesis of the hitherto undescribed analogues of deamino-1-carba-oxytocin modified in position 2 (*Ie–Ig*) was derived from common intermediate product, the amide of isoleucyl-glutaminyl-asparaginyl-S-(3-carboxypropyl)cysteinyl-prolyl-



Ia; X = S—S, Y = NH_2, Z = $CH_2C_6H_4OH$
Ib; X = S—S, Y = H, Z = $CH_2C_6H_4OH$
Ic; X = S—S, Y = NH_2, Z = $CH_2C_6H_4OCH_3$
Id; X = CH_2—S, Y = H, Z = $CH_2C_6H_4OH$
Ie; X = CH_2—S, Y = H, Z = $CH_2C_6H_5$
If; X = CH_2—S, Y = H, Z = $CH_2C_6H_4OCH_3$
Ig; X = CH_2—S, Y = H, Z = $CH(CH_3)C_2H_5$
Ih; X = S—S, Y = NH_2, Z = $CH_2C_6F_5$

Ile-Gln-Asn-Cys($C_3H_6CO_2H$)-Pro-Leu-Gly-NH_2
II

X-Ile-Gln-Asn-Cys($C_3H_6CO_2H$)-Pro-Leu-Gly-NH_2

IIIa; X = Nps-Phe
IIIb; X = Boc-Tyr(Me)
IIIc; X = Nps-Ile

TABLE I
Biological Activities (International units/mg)

Compound	Rat uterus[a] isolated	Rat uterus[a] *in situ*	Vasodepressor[b]	Mammary gland[b]	Antidiuretic[a]
Oxytocin	486	450	507	533	2·7
Ie	233	95	71	515	0·6
If	10	40	—	67	0·9
Ig	347	500	171	478	2

[a] Determined by Dr T. Barth of this Institute; [b] determined by Dr I. Krejčí, Research Institute for Pharmacy and Biochemistry, Prague.

leucyl-glycine[12] (*II*),* which was acylated with an active ester of the corresponding acylamino acids, *i.e.* the 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenyl-phenylalanine, the 2,4,5-trichlorophenyl ester of tert-butyloxycarbonyl-O-methyl-tyrosine and the N-hydroxysuccinimide ester of *o*-nitrobenzenesulphenylisoleucine. Active esters were prepared from protected octapeptides *IIIa* to *IIIc* with the use of bis(*p*-nitrophenyl) sulphite[13], and after removal of the protecting groups with HCl in ether (or trifluoroacetic acid in the case of the active ester of substance *IIIb*), cyclisation was carried out in pyridine solution[14]. Analogues *Ie* to *Ig* were isolated and purified using counter-current distribution, gel filtration, and reprecipitation from a mixture of methanol and ether, and were characterised by elemental analysis, amino-acid composition and thin layer chromatography on silica gel. The basic data of the biological activities are presented in Table I.

EXPERIMENTAL

Melting points were determined on a Koffler block and were corrected. Kieselgel G (Merck) plates were used for thin layer chromatography. The solvent systems were: 2-butanol–90% formic acid–water (75 : 13·5 : 13·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2) and 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 3) (S3). Electrophoresis was carried out on Whatman 3MM paper in 1M acetic acid (pH 2·4) and a pyridine–acetic acid buffer (pH 5·7) for 60 minutes at a potential drop of 20 V/cm; where R_F and E values are presented, pure substances were used. Amino-acid analyses were carried out after 20 hours of hydrolysis in 6M-HCl at 105°C on an automatic analyser (type 6020, Development Workshops, Czechoslovak Academy of Sciences). Evaporation of solutions was carried out with a rotary evaporator at a bath temperature of 30—35°C under reduced pressure (water pump, with mixtures containing dimethylformamide an oil vacuum pump).

* All amino acids used in this study are of the L series. The nomenclature and symbols follow suggestions reported elsewhere[11].

Oxytocin[15], deamino-oxytocin[16], [2-O-methyltyrosine]-oxytocin[17] and deamino-1-carba--oxytocin[12] were all prepared by previously published procedures and purified by counter-current distribution and gel filtration. Values of their biological activities and physicochemical constants were in agreement with the literature. The R_F values for deamino-1-carba-oxytocin were 0·30 (S2) and 0·71 (S3).

o-Nitrobenzenesulphenylphenylalanine 2,4,5-Trichlorophenyl Ester

To a solution of *o*-nitrobenzenesulphenylphenylalanine (freed from 2·5 g of the dicyclohexylammonium salt using sulphuric acid) in tetrahydrofurane (30 ml) 2,4,5-trichlorophenol (1·1 g) and after cooling to −15°C dicyclohexylcarbodiimide (1·1 g) were added. The mixture was stirred for 1 h at −10°C, 1 h at 0°C and 1 h at room temperature, the dicyclohexylurea which precipitated was filtered off, the filtrate was evaporated and the remnant was twice crystallized from a mixture of tetrahydrofurane and light petroleum. The yield was 1·97 g (80%) of a product with m.p. 133−135°C; $[\alpha]_D$ − 70·1° (*c* 0.5, dimethylformamide). For $C_{21}H_{15}Cl_3N_2O_4S$ (497·8) calculated: 50·67% C, 3·03% H, 5·63% N; found: 50·89% C, 3·10% H, 5·60% N.

o-Nitrobenzenesulphenylphenylalanyl-isoleucyl-glutaminyl-asparaginyl--S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*IIIa*)

To a solution of heptapeptide[12] *II* (0·50 g) in dimethylformamide (20 ml) and N-ethylpiperidine (0·20 ml) *o*-nitrobenzenesulphenylphenylalanine 2,4,5-trichlorophenyl ester (0·30 g) was added. After 24 h stirring at room temperature a further portion of active ester was added (0·15 g) and after a further 24 h the mixture was evaporated, the residue was ground sequentially with light petroleum and ether, the crystalline portion was filtered and washed with ether, water, 0·2M-H_2SO_4 and again with water. The yield was 0·52 g (78%) of a product with m.p. 218−224°C. This product was used for the next stage. The sample for analysis crystallised from a mixture dimethylformamide−−ether, showed a m.p. of 223−226°C; $[\alpha]_D$ −0·3° (*c* 0·27, dimethylformamide), −2·5° (*c* 0·28, dimethylformamide with 1 equivalent of N-ethylpiperidine). The R_F value was 0·61 in S1, 0·68 in S3; $E_{2.4}^{His}$ 0·57, $E_{5.7}^{Gly}$ 0·12 (electrophoresis was carried out after removal of the protecting group with HCl in methanol). For $C_{50}H_{72}N_{12}O_{14}S_2 \cdot H_2O$ (1147) calculated: 52·35% C, 6·50% H, 14·66% N; found: 51·93% C, 6·26% H, 14·61% N.

The Lactam of Phenylalanyl-isoleucyl-glutaminyl-asparaginyl--S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*Ie*)

Preparation of the active ester and its cyclisation were carried out in a nitrogen atmosphere. To a solution of protected octapeptide *IIIa* (365·3 mg) in a mixture of dimethylformamide(10 ml) and pyridine (10 ml) bis(*p*-nitrophenyl) sulphite (1 g) was added. The mixture was stirred at room temperature for 7 h, diluted with pyridine (5 ml) and a further portion of reagent (1g) was added and the last portion (0·5 g) after 16 h stirring. The mixture was then stirred for 5 h, evaporated, the residue was ground with ether, filtered and washed with ether and water. The active ester of the octapeptide was dissolved in dimethylformamide (4 ml), 2M-HCl in ether (0·3 ml) was added and after 3 min. standing the mixture was diluted with ether. The crystalline portion was filtered off and washed with ether; $E_{2.4}^{Gly}$ 0·69, $E_{5.7}^{His}$ 0·41. The hydrochloride was dissolved in dimethylformamide (10 ml) and this solution was added over 4 h with stirring to a mixture of pyridine (250 ml) and N-ethylpiperidine (36 µl) heated to 50°C. After 12 h standing at room temperature the mixture was evaporated, the residue was ground with ether, filtered off and washed with ether. After drying, the product was dissolved in 25 ml of the upper phase of a solvent system 2-butanol −

-current distribution apparatus (Steady State Distribution Machine, Quickfit & Quartz Ltd., Stone, Staffordshire, England) and 100 transfers of the upper phase were carried out. The peak of the product (localised by Folin – Ciocalteau reagent) with a partition coefficient of 3·0 (tubes 61—93) was isolated by evaporation to a small volume and freeze drying. Thin layer chromatography showed that the product contained two substances which were then separated by filtration on Bio-gel P-4 in 3M acetic acid. The required analogue (73·6 mg) was futher purified by gel filtration on Bio-gel P-2 in 3M acetic acid and Bio-gel P-4 in 1M acetic acid and then precipitated from methanol–ether (27·2 mg; 8·5%); R_F 0·37 (S2), 0·74 (S3); $[\alpha]_D$ −81·7° (*c* 0·19, 1M acetic acid). Amino-acid analysis: Asp 1·03, Cys($C_3H_6CO_2H$) 1·03, Glu 1·00, Gly 0·98, Ile 0·97, Leu 0·99, Phe 0·97, Pro 1·03. For $C_{44}H_{67}N_{11}O_{11}S \cdot 3H_2O$ (1012) calculated: 52·21% C, 7·26% H, 15·22% N; found: 52·41% C, 6·93% H, 15·00% N.

N-Tert-butyloxycarbonyl-O-methyltyrosine Dicyclohexylammonium Salt

To a solution of O-methyltyrosine (5·3 g) in dioxane (30 ml) and water (30 ml) tert-butyloxycarbonyl azide (6 ml) was added with stirring. The pH of the solution was maintained at 10·5 using 4M-NaOH (total added 14 ml). After 6 h mixing a further 2 ml of azide was added. The mixture was stirred for 2 h, extracted with ether, the pH was adjusted with 6M-HCl to 4·5 and the product was extracted into ethyl acetate. The ethyl acetate solution was washed with water, dried with sodium sulphate and the ethyl acetate was evaporated off. The residue was dissolved in ether, to this solution dicyclohexylamine (6·5 ml) was added and the mixture was diluted with light petroleum. After cooling to 0°C the crystals which separated out were filtered off and washed with light petroleum. The yield was 8·65 g (67%) of a product with m.p. 151—152°C. The sample for analysis was crystallised from water without change in m.p.; $[\alpha]_D$ +34·2° (*c* 0·50, dimethylformamide). For $C_{27}H_{44}N_2O_5$ (476·7) calculated: 68·04% C, 9·31% H, 5·88% N; found: 68·17% C, 9·48% H, 5·69% N.

N-Tert-butyloxycarbonyl-O-methyltyrosine 2,4,5-Trichlorophenyl Ester

To a solution of the dicyclohexylammonium salt of N-tert-butyloxycarbonyl-O-methyltyrosine (2·4 g) in 50% ethanol (20 ml) Dowex 50 (H^+-cycle, 20 ml) was added. After 30 min stirring the ion exchanger was filtered off, the filtrate was evaporated and azeotropically dried. The remnant was dissolved in chloroform (20 ml) and to this solution 2,4,5-trichlorophenol (1 g) and after cooling to −20°C dicyclohexylcarbodiimide (1·5 g) was added. The mixture was stirred for 1 h at −10°C and 12 h at room temperature, evaporated, the residue was dissolved in ethyl acetate, the dicyclohexylurea which separated out was filtered off, the filtrate was washed with 0·5M-$NaHCO_3$, water and dried with sodium sulphate. The solution was evaporated and the residue was crystallised from a mixture of ethyl acetate and light petroleum. The yield was 1·2 g (50%) of a product of m.p. 109—110°C. The sample for analysis was recrystallised in the same manner without change in m.p.; $[\alpha]_D$ −31·8° (*c* 0·50, dimethylformamide). For $C_{21}H_{22}Cl_3NO_5$ (474·8) calculated: 53·12% C, 4·67% H, 2·95% N; found: 53·52% C, 4·70% H, 2·89% N.

N-Tert-butyloxycarbonyl-O-methyltyrosyl-isoleucyl-
-glutaminyl-asparaginyl-S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*IIIb*)

To a solution of heptapeptide *II* (0·43 g) in a mixture of dimethylformamide (7·5 ml) and N-ethylpiperidine (0·20 ml) N-tert-butyloxycarbonyl-O-methyltyrosine 2,4,5-trichlorophenyl ester (0·20 g) was added. The mixture was stirred at room temperature for 24 h, a second portion of the active ester (0·10 g) was added, the mixture stirred for 24 h and evaporated. The residue was ground with light petroleum, the crystalline portion was filtered off and washed on the filter with light

petroleum, ether, an aqueous solution of citric acid (pH 3) and water. The yield was 0·50 g (89%) of a product of m.p. 211—213°C; R_F 0·61 (S1), 0·21 (S2), 0·71 (S3). From a sample of this product the protecting group was removed with trifluoroacetic acid: $E_{2.4}^{Gly}$ 0·60, $E_{5.7}^{His}$ 0·13. The sample for analysis was crystallised from dimethylformamide–ether without change in m.p.; $[\alpha]_D$ −36·2° (*c* 0·50, dimethylformamide). For $C_{50}H_{79}N_{11}O_{15}S \cdot 1H_2O$ (1124) calculated: 53·42% C, 7·26% H, 13·70% N; found: 53·42% C, 7·60% H, 13·45% N.

The Lactam of O-Methyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*If*)

To a solution of protected octapeptide *IIIb* (319 mg) in a mixture of dimethylformamide (10 ml) and pyridine (10 ml) bis(*p*-nitrophenyl) sulphite (1 g) was added with stirring and bubbling through of nitrogen. After 8 h the mixture was diluted with pyridine (5 ml), a further portion of reagent (1 g) was added and after 12 h the last portion (0·5 g). After 8 h mixture was evaporated, the residue was ground with ether, filtered off and washed with ether and water. After drying in air the active ester was dissolved in trifluoroacetic acid (10 ml), the solution after 1 h standing at room temperature was diluted with toluene (10 ml) and evaporated. The residue was dissolved in dimethylformamide (10 ml) and this solution was added over 4 h to pyridine (300 ml) with stirring at 50°C and nitrogen bubbling. After 12 h standing at room temperature the mixture was evaporated, the residue was ground with ether, filtered, washed with ether and dissolved in 25 ml of the upper phase of a solvent system for counter-current distribution; 100 transfers of the upper phase were carried out. The peak of the product ($K = 3{\cdot}15$) was concentrated to a small volume and freeze dried. The yield was 130 mg (45%). This product was purified by gel filtration on Bio-gels P-4 and P-2 in 1M acetic acid and precipitated from a methanolic solution with ether. R_F 0·36 (S1), 0·35 (S2), 0·70 (S3); $[\alpha]_D$ −69·0° (*c* 0·25, 1M acetic acid). Amino-acid analysis: Asp 0·94, Cys ($C_3H_6CO_2H$) 1·08, Glu 1·03, Gly 1·00, Ile 0·95, Leu 0·97, Pro 0·94, Tyr+Tyr(Me) 0·98. For $C_{45}H_{69}N_{11}O_{12}S \cdot 2H_2O$ (1024) calculated: 52·78% C, 7·18% H, 15·05% N; found: 52·29% C, 6·90% H, 14·88% N.

o-Nitrobenzenesulphenylisoleucyl-isoleucyl-glutaminyl--asparaginyl-S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*IIIc*)

To a solution of free heptapeptide *II* (0·51 g) in dimethylformamide (20 ml) and N-ethylpiperidine (0·20 ml) we added the N-hydroxysuccinimide ester of *o*-nitrobenzenesulphenylisoleucine[12] (0·15 g). After 24 h stirring at room temperature a further portion of active ester (0·10 g) was added, the mixture stirred for 24 h and evaporated, the residue was ground consecutively with light petroleum and ether, the crystalline portion was filtered off and washed on the filter with ether, water, 0·2M-H_2SO_4 (0°C), and water. The yield was 0·39 g (60%) of a product with m.p. 230—234°C. The sample for analysis was crystallised from dimethylformamide–ether; m.p. 232—234°C; $[\alpha]_D$ −52·4° (*c* 0·5; dimethylformamide). R_F 0·59 (S_1), 0·68 (S_3); $E_{2.4}^{Gly}$ 0·60, $E_{5.7}^{His}$ 0·12 (electrophoresis carried out after removal of the protecting group with HCl in methanol). For $C_{47}H_{74}N_{12}O_{14}S_2 \cdot 0{\cdot}5H_2O$ (1104) calculated: 51·13% C, 6·85% H, 15·23% N; found: 51·01% C, 6·89% H, 15·04% N.

The Lactam of Isoleucyl-isoleucyl-glutaminyl-asparaginyl--S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*Ig*)

Preparation of the active ester and its cyclisation were carried out in a nitrogen atmosphere. To a solution of protected octapeptide *IIIc* (286·8 mg) in a mixture of dimethylformamide (10 ml) —0·05% acetic acid, and the filtered solution was transferred to the second tube of a counter-

and pyridine (10 ml) bis(*p*-nitrophenyl) sulphite (1 g) was added. The mixture was stirred at room temperature for 7 h, diluted with pyridine (5 ml), and a further portion of reagent (1 g) was added. After 15 h stirring 0·5 g of bis(*p*-nitrophenyl) sulphite was added. The mixture was stirred for 5 h and evaporated, the residue was ground with ether, filtered and washed with ether, water and dried in air. The active ester was dissolved in dimethylformamide (4 ml) and to this solution 2·09M-HCl in ether (0·24 ml) was added. After 3 min standing at room temperature the mixture was diluted with ether, the crystalline portion was filtered off and washed with ether; $E_{2.4}^{Gly}$ 0·63, $E_{5.7}^{His}$ 0·48. The hydrochloride was dissolved in dimethylformamide (30 ml) and this solution was added over 10 h to 250 ml pyridine and 28·5 µl N-ethylpiperidine, with stirring at 50°C. After 12 h standing at room temperature the mixture was evaporated, the residue was ground with ether, filtered and washed with ether. After dissolving in 25 ml of the upper phase of the solvent system 2-butanol–0·05% acetic acid, 100 transfers of the upper phase were carried out in the counter-current distribution apparatus. The fraction containing a substance of partition coefficient 2·92 (tubes 58—83) was concentrated to a small volume and freeze dried. The yield was 144 mg (58%) of a product which was purified on columns of Bio-gel P-4 and P-2 in 1M acetic acid and precipitated from methanol–ether; $[\alpha]_D$ −87·2° (*c* 0·24, 1M acetic acid); R_F 0·34 (S2), 0·70 (S3). Amino-acid analysis (6M-HCl, 105°C, 96 h): Asp 1·02, Cys($C_3H_6\overset{\circ}{C}O_2H$) 1·00, Glu 1·00, Gly 0·96, Ile 1·87, Leu 1.02, Pro 1.06. For $C_{41}H_{69}N_{11}O_{11}S \cdot 2\,H_2O$ (960·2) calculated: 51·30% C, 7·66% H, 16·04% N; found; 51·12% C, 7·43% H, 15·63% N.

Spectral Maesurements

Circular dichroic spectra were measured on the Roussel — Jouan Dichrograph CD 185, Model II. Cells with an optical pathway of 0·01 to 1·0 cm were used. The cells were not temperature controlled, and temperature of the solution varied between 22 and 25°C. Concentration of the solutions (about 0·5 mg/ml) was calculated from the weighed amount of dry substance. Spectral data presented in the tables and figures are given in values of molar ellipticity $[\Theta]$ (deg. $cm^2.dmol^{-1}$) and are not corrected for the refractive index of the solvent. As solvents we used for pH 3 0·05M-HCl–KCl buffer, and for pH 7·5 0·01M phosphate buffer. Hexafluoroacetone trihydrate was the commercial product of Hynes Chemical Research Co. and was used without further purification.

RESULTS AND DISCUSSION

From a detailed study of circular dichroic spectra of an extensive series of oxytocin analogues[1,18] it appeared that the characteristic spectral feature of these compounds — a positive band at about 225 nm — is complex in nature and is conditioned by the amino terminal sequence cysteine-tyrosine-isoleucine and its conformation. A particularly important role in the circular dichroism spectra, not only in this region of wavelengths, is played by tyrosine in position 2, the side chain of which interacts with the N^{α}-amino group. In this regard it will be possible to acquire valuable information from studies of circular dichroism of analogues modified in position 2. Only a few such compounds have been studied from this point of view in the past. Beychok and Breslow[19] reported incomplete spectra for [2-isoleucine]-oxytocin, [2-isoleucine]-deamino-oxytocin and [2-glycine]-oxytocin and discussed them from the point of view of the effect of the modification on the character of the positive disulphide band at 250 nm. Urry and coworkers[20] analysed from a similar stand-

point the effect of temperature and dioxane on the spectra of [2-isoleucine]-deamino-oxytocin. The latter investigators measured, but did not present in detail, the spectrum of [2-alanine]-deamino-oxytocin.

The most important spectral data on compounds prepared and studied in this laboratory are presented in Tables II and III along with data of reference, parent compounds oxytocin (*Ia*) and deamino-1-carba-oxytocin (*Id*).[2-Pentafluorophenyl-alanine]-oxytocin (*Ih*), prepared by Kaurov[21] and coworkers, was also included. Observations on these substances have permitted more correct assignment of dichroic spectral bands for the oxytocin analogues. Both phenylalanine derivatives *Ie* (Fig. 1) and *Ih* show a positive band lying in a neutral solution at 222 nm, which is markedly hypsochromically shifted in relation to analogous band of oxytocin. The band is shifted still further to shorter wavelenghts (217 nm; Table II) when the substance is transferred into hexafluoroacetone. This band cannot be assigned to the B_{1u} transition of the phenyl group, because neither position nor intensity correspond. (It is assumed that substitution of the aromatic ring by fluorine does not change significantly the energies of its electronic transitions; UV and rotatory dispersion spectra of phenyl-

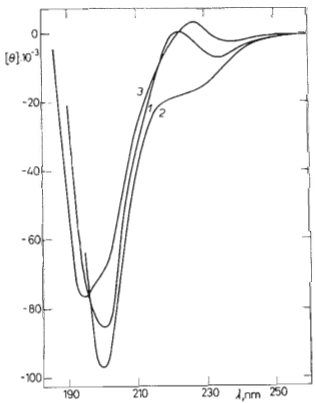


FIG. 1

Circular Dichroism of [2-Phenylalanine]--deamino-1-carba-oxytocin in a Buffer of pH 7·5 (*1*), in Hexafluoroacetone (*2*), and of Deamino-1-carba-oxytocin in a Buffer of pH 7·5 (*3*)

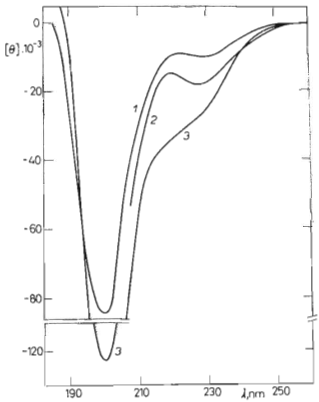


FIG. 2

Circular Dichroism of [2-Isoleucine]-deamino-1-carba-oxytocin

1 In a buffer of pH 7·5; *2* in a mixture of dioxane and water (9 : 1); *3* in hexafluoroacetone.

alanine and 4-fluorophenylalanine are the same in region of the B_{2u} transition in terms of band position[22]). This is clear, *e.g.*, from comparisons with the circular dichroic spectra of rigid cyclodipeptides formed from phenylalanine and a cyclic α-imino acid[23]. In this case one can clearly observe superposition of a sharp, but low intensity, aromatic band at 215 to 218 nm, which does not alter with a change in solvent, on a more intensive n-π^* band of the peptide group. We therefore attribute the major portion of the intensity of the positive band of phenylalanine analogues of oxytocin *Ie* and *Ih* to the peptide n-π^* band. Again, on the basis of comparisons with model substances, with cyclodipeptides of the types cyclo(L-Phe-Gly) and cyclo(L-Phe-L-Leu)[24] and with linear tripeptide Gly-L-Phe-Gly or Gly-L-Phe-L-Leu[25], it is believed that the source of the rotational strength of this n-π^* band is mainly the vicinal effect of a polarisable aromatic group (static coupling) and that the band therefore is connected with the peptide group in the neighbourhood of the phenylalanine side chain. In addition, the isoleucine analogue *Ig* shows a weak positive band at 221 nm (Fig. 2) which can be attributed only to the amide n-π^* transition.

In the example of substance *Ig*, the spectrum of which is not complicated by the presence of aromatic and disulphide chromophores, one can also clear up the nature of the negative extremum between 230 and 240 nm which occurs at room temperature

TABLE II

Circular Dichroic Data of Oxytocin and its Analogues Modified at Position 2

Compound	Solvent	λ^a, nm ($[\theta] \cdot 10^{-3}$, deg . cm^2 . dmol^{-1})			
		disulphide band	complex positive band	amide π-π^* band	aromatic band
Oxytocin (*Ia*)	buffer, pH 7·5	250 (+0·9)	228·5 (+ 6·6)	s 203 (−54)	195 (−78)
	buffer, pH 3·0	250 (+1·9)	226 (+22·3)	*b*	196 (−70)
	hexafluoroacetone	250 (+3·1)	225 (+32·0)	195*c* (−46)	
[2-O-Methyl-tyrosine]--oxytocin (*Ic*)	buffer, pH 7·5	250 (+0·9)	228·5 (+10·0)	s 203 (−57)	194·5 (−100)
	buffer, pH 3·0	250 (+1·6)	226·5 (+22·5)	*b*	195·5 (−62)
	hexafluoroacetone	250 (+2·3)	226 (+32·9)	195*c* (−65)	
[2-Pentafluoro-phenylalanine]--oxytocin (*Ih*)	buffer, pH 7·5	251 (+0·6)	221·5 (+7·8)	199 (−44)	—
	buffer, pH 3·0	250 (+1·1)	221 (+12·3)	*d*	*d*
	hexafluoroacetone	245 (+2·2)	217 (+17·2)	199 (−36)	—

[a] s Denotes shoulder; [b] poorly defined shoulder; [c] only one band can be seen in this region; [d] not measured.

also in analogues *Id* to *If* (Table III) and in further analogues of oxytocin not containing a disulphide bond[1]. The intensity and position of this extremum is markedly dependent upon the solvent. With increasing temperature the negative extremum deepens and shifts hypsochromically, whereas the positive band decreases[18]. The spectrum of compound *Ig* is very similar to that of the tripeptide Gly-L-Leu-Gly[25], which in this spectral region also shows two extrema, a positive at short wavelength and a negative at long wavelength. Their relative intensities and positions change as a function of temperature and solvent. This spectrum has been interpreted[25] as superposition of two n-π^* transitions of opposite sign corresponding to peptide bonds in two different conformations of the tripeptide. In a similar manner, Ziegler and Busch[26] interpreted the spectrum of the cyclic hexapeptide cyclo(Gly_5-L-Leu). Thus, we attribute in oxytocin analogues the negative extremum at 230 to 240 nm to the n-π^* transition of the peptide group. It is probable that the n-π^* band with a positive sign belongs to the peptide group in that part of the chain which is in extended

TABLE III

Circular Dichroic Data of Deamino-1-carba-oxytocin and its Derivatives Modified at Position 2

Compound	Solvent	λ^a, nm ($[\theta] \cdot 10^{-3}$, deg . cm^2 . $dmol^{-1}$)			
		amide n-π^* band	complex positive band	amide π-π^* band	aromatic band
Deamino--carba[1]--oxytocin (*Id*)	buffer, pH 7·5	238 (— 3·7)	227 (+ 3·5)	s 201 (—69)	194·5 (—76)
	dioxan	244 (— 3·1)	231·5 (+ 8·8)	—	—
	hexafluoroacetone	s 223[b] (—15·5)		200 (—80)	—
[2-Phenylalanine] -deamino--1-carba--oxytocin (*Ie*)	buffer, pH 7·5	234 (— 7·4)	225·5 (+ 0·8)	200·5 (—85)	—
	dioxan	236 (— 8·7)	221 (+ 8·0)	—	—
	hexafluoroacetone	s 222[b] (—18·0)		200 (—97)	—
[2-0-Methyl--tyrosine]-deamino-1-carba[1]--oxytocin (*If*)	buffer, pH 7·5	240 (— 2·5)	227·5 (+ 5·5)	s 203 (—60)	195 (—99)
	dioxan	240 (— 5·9)	230·5 (+ 9·6)	—	—
	hexafluoroacetone	s 225[b] (—14·0)		200 (—80)	—
[2-Isoleucine]--deamino--1-carba--oxytocin (*Ig*)	buffer, pH 7·5	228 (—10·3)	221[c] (— 9·2)	200 (—86)	—
	dioxan	228 (—18·0)	219·5[c] (—14·9)	—	—
	hexafluoroacetone	s 219[b] (—32·0)		199·5 (—122)	—

[a] s Denotes shoulder; [b] approximate wavelength of a broad shoulder is given; [c] negative minimum.

conformation[25], *i.e.* the tripeptide Cys-X-Ile. The intensity of the positive band is increased if the side-chain of amino-acid residue X contains a polarisable group. The difference in energy of positive and negative n-π^* bands is estimated, on the basis of comparison of actual and simulated spectra, at 10 nm. Such a difference can be explained, for example, by a different effective dielectric constant in the environment of the carbonyl oxygen of the peptide groups which occur in different conformations.

From the above considerations it would clearly appear that the positive band at 225 nm of substances containing tyrosine or O-methyltyrosine in position 2 is complex. The band arises by superposition of peptide n-π^* and tyrosine B_{1u} transitions, both components being dependent upon the local conformation of the same part of the molecule. The observed relations between parameters – position and intensity – of this band and structure of the analogue can be utilised for considerations on conformation.

All of the oxytocin analogues studied in this laboratory (see also[1]), along with analogues of vasopressin[27], containing tyrosine in position 2 show in aqueous solution a negative maximum at 193–195 nm next to the shoulder at 200–203 nm. This maximum is lacking in substances which have a different amino acid in position 2 (*Ie–Ih*, Tables II and III, Figs 1 and 2) and in deamino derivatives (Table III) also disappears in hexafluoroacetone. The negative maximum, therefore, is in close correlation with the presence and conformation of the side chain of tyrosine. Its origin can be explained in terms of two electronic transitions. In the first place we have the E_{1u} transition of the tyrosine chromophore, which probably lies in this region of wavelengths[28]. The source of rotational strength of aromatic π-π^* transitions of tyrosine in compounds studied in this paper is a coupled oscillator mechanism[29] between transitional dipole moments of the aromatic transitions and π-π^* transition of the peptide group, the result of which is always a pair of bands with positions corresponding to coupled transitions. It cannot therefore be excluded that the discussed negative maximum is due to π-π^* transition of the peptide group, which is in interaction with the aromatic group[26]. In both cases we are dealing with a very intense band and can therefore assume that coupling of transitional dipole moments is extremely effective and the interacting groups are in optimal spatial relation. From this point of view one could take into account most probably the peptide group between cysteine 1 and tyrosine, towards which the aromatic nucleus of tyrosine is oriented. This assumption on the conformation of the tyrosine side-chain is in agreement with conclusions on the basis of other experimental data[1].

Both 2-O-methyltyrosine analogues *Ic* and *If* have qualitatively and quantitatively very similar spectra as the reference, parent compounds. For example, [2-O-methyltyrosine]-oxytocin (*Ic*) shows the same characteristic significant increase in intensity of the positive band as oxytocin itself when transferred from an aqueous medium to hexafluoroacetone (Table II). Qualitatively similar spectral changes in hexafluoro-

acetone are seen with [2-pentafluorophenylalanine]-oxytocin (*Ih*). On the other hand, deamino derivatives *If* and *Ie* react to a change in solvent in the same manner as deamino-1-carba-oxytocin (*Id*) or deamino-oxytocin (*Ib*) (Table III). Conformation of the side chain of the aromatic acid in position 2 and the nature of its interaction with the N^{α}-amino group[18] is obviously the same in modified molecules as in molecules containing an unchanged tyrosine, and does not depend upon the presence of a free or substituted hydroxyl group on the tyrosine. On the basis of this data there is therefore no reason to assume that the oxygen substituent of tyrosine in the isolated molecule of oxytocin plays any role in intramolecular hydrogen bonding on fixation of conformation.

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