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Note

Amino acids and peptides

CLXXXI*. Separation of diastereoisomers of oxytocin analogues

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A number of examples of reversed-phase chromatographic separations of diastereoisomeric peptides have been reported[1–5]. The separation of all oxytocin isomers formed by a change in configuration of any one amino acid may be achieved[5].

In this study, the separation of diastereoisomers was investigated in the synthesis of analogues containing an unnatural amino acid in position 2, starting with the racemic derivatives of phenylalanine, and it was examined whether diastereoisomers of oxytocin analogues modified in other ways (in position 1 and in the disulphide bridge) may also be separated.

EXPERIMENTAL

The oxytocin analogues were synthetized as described previously[6,7]. Chromatography was carried out on a 25 × 0.4 cm I.D. column packed with Separon SI-C-18 (Laboratory Apparatus, Prague, Czechoslovakia), using an SP-8700 liquid chromatograph equipped with an SP-8400 continuously variable wavelength UV detector and an SP-4100 integrator (all from Spectra-Physics, Santa Clara, CA, U.S.A.). Mixtures of methanol or tetrahydrofuran with aqueous buffers were used as mobile phases.

RESULTS AND DISCUSSION

A number of oxytocin analogues, I–XX (Table I) containing an amino acid of L or D configuration in position 2, were prepared. The synthesis of some compounds I–X employed optically inactive aromatic amino acids as starting materials, and in order to obtain pure compounds reversed-phase liquid column chromatography was used. For the determination of which enantiomer of phenylalanine derivative is present in an analogue, a comparison was made with a substance prepared from optically pure amino acid, and enzymatic incubation of the hydrolysate with L-amino acid oxidase was also carried out.

* For Part CLXXX see ref. 6.

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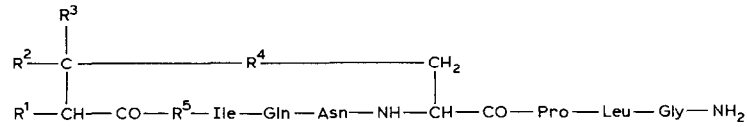
TABLE I

STRUCTURES OF OXYTOCIN ANALOGUES



Et = Ethyl; Me = methyl.

Compound	R^1	R^2	R^3	R^4	R^5
I	NH_2	H	H	S–S	L-Phe(Et)
II	NH_2	H	H	S–S	D-Phe(Et)
III	H	H	H	S–S	L-Phe(Et)
IV	H	H	H	S–S	D-Phe(Et)
V	NH_2	CH_3	CH_3	S–S	L-Phe(Et)
VI	NH_2	CH_3	CH_3	S–S	D-Phe(Et)
VII	H	H	H	S–CH_2	L-Phe(Et)
VIII	H	H	H	S–CH_2	D-Phe(Et)
IX	H	H	H	S–CH_2	L-Phe(Me)
X	H	H	H	S–CH_2	D-Phe(Me)
XI	H	H	H	S–CH_2	L-Phe
XII	H	H	H	S–CH_2	D-Phe
XIII	H	H	H	S–CH_2	L-Phe(Cl)
XIV	H	H	H	S–CH_2	D-Phe(Cl)
XV	H	H	H	S–CH_2	L-Tyr(Et)
XVI	H	H	H	S–CH_2	D-Tyr(Et)
XVII	H	H	H	S–CH_2	L-Tyr
XVIII	H	H	H	S–CH_2	D-Tyr
XIX	H	H	H	CH_2–S	L-Tyr
XX	H	H	H	CH_2–S	D-Tyr

As is evident from Table II, the diastereoisomers were separated in all cases. Almost in all instances the substance containing a D-amino acid was eluted later, indicating its higher hydrophobic interaction with the stationary phase. The analogues containing tyrosine in position 2 are the sole exception. In this case the analogue with a D-tyrosine was eluted earlier when methanol was used as the organic component of the mobile phase, but with respect to the L-tyrosine-containing compound its separation coefficient is very low and only little affected by the composition and the pH value of the buffer used. (For XVII and XVIII the value of α is between 0.93 and 0.96 for buffers of pH 2–8.1.) However, if tetrahydrofuran is used instead of methanol the separation factor is 1.2, while the D-tyrosine-containing compound XVIII is eluted later, in agreement with the chromatographic behaviour[5] of (2-D-tyrosine)oxytocin. Tetrahydrofuran also increased the selectivity of the separation in other cases; for example, compounds IX and X have $\alpha = 1.59$ (21% tetrahydrofuran in the mobile phase) in contrast to $\alpha = 1.39$ obtained when methanol was used.

In the case of analogues VII–XX we also investigated the chromatographic behaviour of the corresponding sulphoxides. The separation factor of the sulphoxides of analogues containing a D- and L-amino acid in position 2 is in most instances not much different from the corresponding value for the pair of analogues in sulphide form, and the order of elution of the D and L diastereoisomers is also unchanged (see

TABLE II

RETENTION DATA OF DIASTEREOISOMERIC PEPTIDES

Compound	*Amino acid in position 2 (R^5)*	*k'*	*α**	*k'_{so}***	*α_{so}***	*Mobile phase****	
I	L-Phe(Et)	2.09	1.74			A	7:3
II	D-Phe(Et)	3.65					
III	L-Phe(Et)	9.25	1.61			B	13:7
IV	D-Phe(Et)	14.87					
V	L-Phe(Et)	3.05	1.96			A	7:3
VI	D-Phe(Et)	5.89					
VII	L-Phe(Et)	5.18	1.44	4.32	1.40	B	7:3
VIII	D-Phe(Et)	7.48		6.04			
IX	L-Phe(Me)	4.75	1.39	3.84	1.35	B	3:2
X	D-Phe(Me)	6.60		5.18			
XI	L-Phe	3.90	1.41	3.12	1.38	B	3:2
XII	D-Phe	5.51		4.30			
XIII	L-Phe(Cl)	4.45	1.39	3.67	1.42	B	13:7
XIV	D-Phe(Cl)	6.18		5.22			
XV	L-Tyr(Et)	7.03	1.30	5.48	1.15	B	11:9
XVI	D-Tyr(Et)	9.14		6.28			
XVII	L-Tyr	10.44	0.95	7.56	0.63	B	2:3
XVIII	D-Tyr	9.92		4.77			
XIX	L-Tyr	17.8	0.98	11.4	1.02	B	2:3
XX	D-Tyr	17.4		11.6 and 8.25	or 0.73		

* Ratio of k' for D-amino acid-containing analogue/k' for L-amino acid-containing analogue.
** Value for the corresponding sulphoxide.
*** A, Methanol–0.1 *M* ammonium acetate pH 7; B, methanol–0.05% trifluoroacetic acid.

Table II). However, in compounds XVII and XVIII containing tyrosine the value of the separation factor changed distinctly ($\alpha = 0.63$) on oxidation, while the analogue containing D-tyrosine is eluted earlier. Use was made of this fact in the preparation of analogue XVIII from which even a trace of contamination with compound XVII had to be excluded. The sulphoxide was prepared by oxidation with sodium periodate and purified by liquid chromatography. After reduction with hydrogen bromide and acetone[8,9] and rechromatography, pure analogue XVIII was obtained. The entire reaction sequence, including lyophilization of the product, could be carried out in 1 day. For compounds XIX and XX a similar procedure was employed, even though the situation is complicated here by the fact that the sulphoxide of XX is eluted in two peaks (*R* and *S* configuration of the sulphur atom of the sulphoxide) even when an acidic buffer is used (in contrast to other sulphoxides of the analogues of the carba-1 series*), and that only the more rapidly eluted peak ($\alpha = 0.73$) is utilizable, which decreases the yield of the whole procedure.

* Carba-substitution means replacement of the sulphur atom in the disulfide group by a methylene group. Here, we distinguish carba-1 of carba-6 series: carba-1, if cysteine in sequence position 1 was changed for methylene group (analogues XIX and XX); carba-6 if this replacement was done in position 6 (analogues VI–XVIII).

On the basis of the oxidation rate of individual carba-analogues, attempts had previously been made to infer a better or poorer accessibility of the oxidant to the sulphur atom of the carba-bridge[10,11]. In the case of carba-6 analogues, no interaction of the aromatic residue with the sulphur atom should take place on the basis of earlier findings, and so the rate of oxidation of analogues with a different phenylalanine configuration should be the same. This assumption was checked by following the course of the oxidation of analogues VII and VIII with sodium periodate, and the oxidation rate in each case was found to be the same within the experimental errors.

The possibilities of preparing oxytocin analogues by starting with a racemate of the amino acid in position 2, containing various modifications of the molecule (deamination in position 1, introduction of penicillamine into this position, carba-1 and carba-6 modification of the bridge) have been examined. It was shown than some difficult separations of the carba-analogues can be circumvented by preparing easily reducible sulphoxides.

REFERENCES

1 B. Larsen, V. Viswanatha, S. Y. Chang and V. J. Hruby, *J. Chromatogr. Sci.*, 16 (1978) 207.
2 G. Lindeberg, *J. Chromatogr.*, 193 (1980) 427.
3 T. Takaya, Y. Kishida and S. Sakakibara, *J. Chromatogr.*, 215 (1981) 279.
4 D. J. Pietrzyk, W. J. Cahill and J. D. Stodola, *J. Liquid Chromatogr.*, 5 (1982) 443.
5 B. Larsen, B. L. Fox, M. F. Burke and V. J. Hruby, *Int. J. Pept. Protein Res.*, 13 (1979) 12.
6 M. Lebl, P. Hrbas, J. Škopková, J. Slaninová, A. Machová, T. Barth and K. Jošt, *Collect. Czech. Chem. Commun.*, 47 (1982) 2540.
7 M. Lebl, T. Barth, L. Servítová, J. Slaninová and K. Jošt, in K. Bláha and P. Maloň (Editors), *Proc. 17th Eur. Pept. Symp., Prague, 1982*, Walter de Gruyter, Berlin, in press.
8 B. Iselin, *Helv. Chim. Acta*, 44 (1961) 61.
9 M. Lebl, T. Barth and K. Jošt, *Collect. Czech. Chem. Commun.*, 43 (1978) 1538.
10 M. Lebl, T. Barth and K. Jošt, in K. Brunfeldt (Editor), *Proc. 16th Eur. Pept. Symp., Helsingør, 1980*, Scriptor, Copenhagen, 1981, p. 719.
11 M. Lebl, J. Pospíšek, J. Hlaváček, T. Barth, P. Maloň, L. Servítová, K. Hauzer and K. Jošt, *Collect. Czech. Chem. Commun.*, 47 (1982) 689.