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Note

Amino acids and peptides

CLXXVIII*. Correlation between the hydrophobicity of substituents in the phenylalanine moiety of oxytocin carba-analogues and reversed-phase-chromatographic k' values

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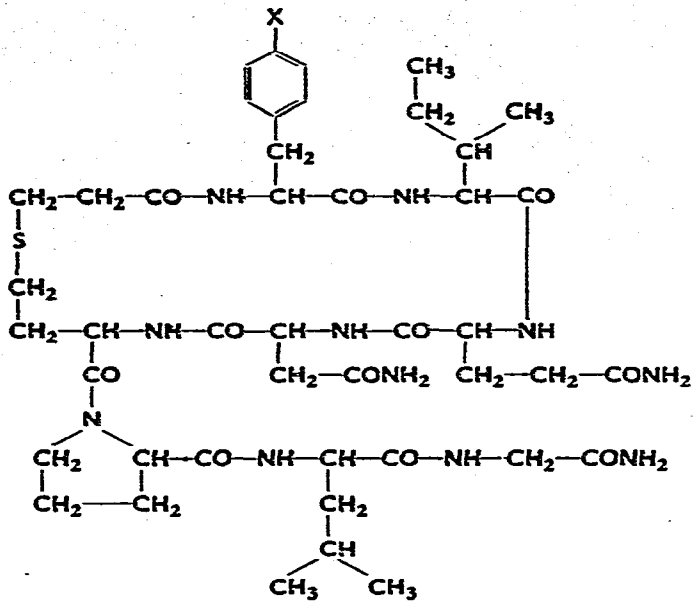
In chromatography on a non-polar reversed-phase, peptides are retained mainly by hydrophobic interactions of their side-chains with the stationary phase[1,2]. The hydrophobicity of a peptide can be approximately evaluated from the partition coefficients between octanol and water for the most hydrophobic amino acid residues[3]. However, the order of elution of peptides actually found[3] often differs from that estimated on the basis of relationship between retention time and hydrophobicity. An approximate prediction of retention time of a peptide of known amino acid composition[4] can be made using empirical parameters derived from the chromatographic behaviour of a series of peptides. Naturally, such an approach is limited to chromatographic conditions that permit the greatest extent of interactions of all of the amino acid side-chains with the stationary phase. By modifying only one side-chain in the whole molecule, we should be able to correlate directly the retention characteristics (capacity factors) of the analogues with the hydrophobicity of the resulting side-chains[5]. On the other hand, a deviation from this correlation could indicate a change in conformation of the analogue being studied.

In this study we tried to correlate the retention characteristics with hydrophobicities (π-values) of various substituents attached to the aromatic ring of the phenylalanine moiety in a series of the oxytocin analogues I–XI.

EXPERIMENTAL

The oxytocin analogues were synthesized in our laboratory[6] and the corresponding sulphoxides were prepared by oxidation with sodium periodate[7]. The chromatography was performed on a 25 × 0.4 cm I.D. column filled with Separon SI-C-18 (Laboratorní Přístroje, Prague, Czechoslovakia), using an SP-8700 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.), equipped with an SP-8400 continuously variable wavelength UV detector and an SP-4100 integrator (both from Spectra-Physics). Mixtures of methanol with water or with a buffer were used as the mobile phase. The following buffers were used: 0.1 % trifluoroacetic acid (pH 2); 0.01

* For Part CLXXVII, see ref. 9.



I. X = NH_2 V. X = H IX. X = Cl
II. X = OH VI. X = $N(CH_3)_2$ X. X = C_2H_5
III. X = NO_2 VII. X = OC_2H_5 XI. X = $NHCOOCH_2C_6H_5$
IV. X = OCH_3 VIII. X = CH_3

M triethylammonium borate (pH 8.1); 0.05 *M* triethylammonium carbonate (pH 5.8–9.2); 0.05 *M* sodium phosphate (pH 4.0–7.5); and 0.1 *M* ammonium acetate (pH 7.0). The regression lines were obtained by a least-squares fit to the experimental points. The π-value for the benzyloxycarbonylamino group was derived on the assumption of additivity of values[8] for methyl, ethyl, benzyl, methyloxycarbonylamino and ethyloxycarbonylamino groups.

RESULTS AND DISCUSSION

The series of deamino-6-carbaoxytocin analogues I–XI was attractive for the study of correlation of lipophilicities with k' values, as the analogues differed only in the *para*-substituents on the phenylalanine moiety in position 2. We studied their reversed-phase chromatographic behaviour at various pH values. The plots obtained of k' values against the π-values[8] of substituents attached to the aromatic nucleus (at pH 8.1) are depicted in Fig. 1. The correlation is good (correlation coefficient r = 0.972), the only substantial deviations being for the hydroxy and dimethylamino groups. At pH 2, the dimethylamino and amino groups are protonated and the corresponding π-values (−2.5 and −4.19, respectively) cannot be used in the correlation because the charge obviously changes the character of the analogue. The deviation of the k' value from the correlation straight line for the dimethylamino compound does not change within the pH range 5.8–9.2; also, no substantial change in the deviation of the k' value was found for the hydroxy compound in the pH range

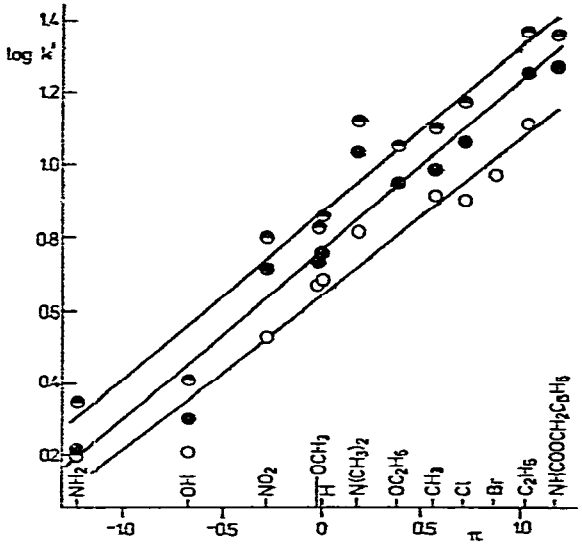


Fig. 1. Plot of log k' of analogues of deamino-6-carbaoxytocin (◑), their sulphoxides (●) and substituted benzenes (○) against π-values of the corresponding substituents. Mobile phase: methanol–borate buffer (pH 8.1) (55:45, or 65:35 for substituted benzenes).

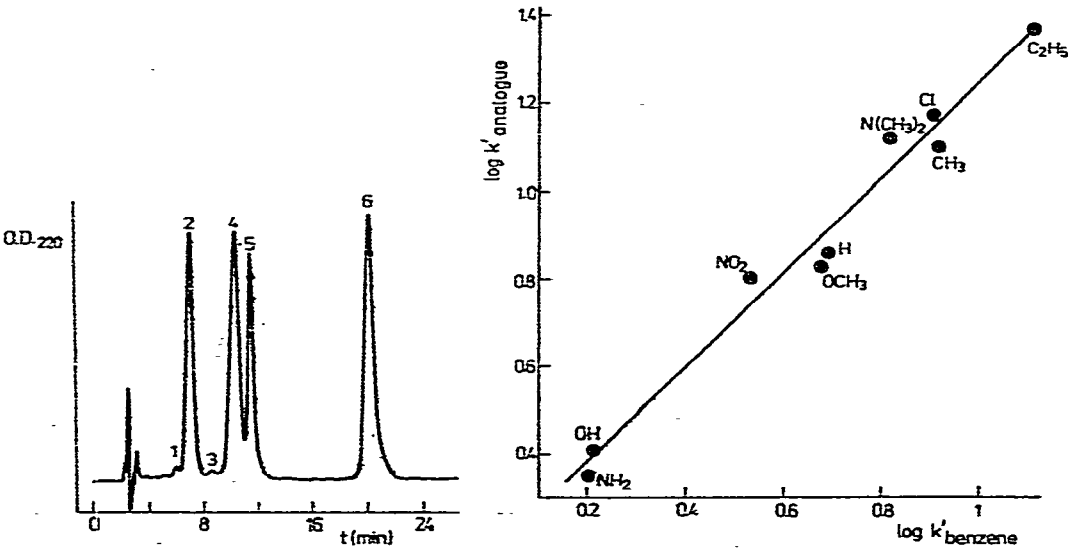


Fig. 2. Chromatogram of a mixture of (2-phenylalanine)deamino-6-carbaoxytocin (2), its sulphoxide (1), (2-*p*-methylphenylalanine)deamino-6-carbaoxytocin (4), its sulphoxide (3), benzene (5) and toluene (6). Mobile phase: methanol–acetate buffer (pH 7.0) (62:38). Flow-rate: 1 ml/min.

Fig. 3. Plot of log k' of deamino-6-carbaoxytocin analogues against log k' of the correspondingly substituted benzenes. Conditions as in Fig. 1.

2.0–8.1. The same behaviour was also observed for the retention characteristics of the corresponding sulphoxides ($r = 0.968$).

In order to check whether it is really possible to correlate the k' values with the π-values of substituents on an aromatic ring (obtained from measurements of the distribution of model compounds between octanol and water[8]) we plotted similarly (Fig. 1) the retention characteristics of monosubstituted benzenes determined under analogous conditions (it was necessary to increase the methanol content in the mobile phase by 10%). A good correlation was obtained ($r = 0.973$), the points for the hydroxy and dimethylamino groups deviating in the same direction as found for the oxytocin analogues.

The chromatographic behaviour of oxytocin analogues, containing unsubstituted phenylalanine and *p*-methylphenylalanine, was compared with that of benzene and toluene. As can be seen from Fig. 2, the aromatic hydrocarbons are eluted substantially later and the column efficiency for these compounds is about four times higher.

The elution order of the chloro and methyl derivatives of benzene is the opposite of that of the oxytocin analogues. This could be explained in the following way: in chlorobenzene the bulky halogen atom negatively influences the interaction with the stationary phase because it hinders the optimal parallel orientation of the aromatic nucleus with the aliphatic chains, whereas in the *p*-chlorophenylalanine oxytocin analogue the peptide conformation may not necessarily allow an optimal orientation of the aromatic nucleus relative to the octadecyl chains, and thus steric effects of the substituents are not so pronounced.

Fig. 3 shows the plot of the retention characteristics of oxytocin analogues, substituted in the aromatic nucleus, against those of the corresponding substituted benzenes; the correlation straight line has a correlation coefficient $r = 0.988$.

It follows from the above dependences that it is possible to correlate retention characteristics even of compounds of higher molecular weight with π-values of the corresponding substituent. However, because of the different behaviour of substituted benzenes in distribution between octanol and water and in chromatography on a reversed phase, it is advisable to use corrected π-values of the substituents, *i.e.*, retention data obtained by the chromatography of substituted benzenes on the given phase in the given system.

As all of the compounds of the series studied obey the correlation between the π-values of substituents on the aromatic nucleus and the capacity factor of the analogue, we can assume that the conformations of all of the analogues (or their sulphoxides) are probably very similar.

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