CHROMATOGRAPHIC AND PHARMACOLOGICAL PROPERTIES OF CARBA--ANALOGUES OF NEUROHYPOPHYSEAL HORMONES SULFOXIDES. A GENERAL METHOD OF SULFOXIDE GROUP DETERMINATION IN PEPTIDES

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INTRODUCTION

Conformation of neurohypophyseal hormones in solution is studied relatively intensively, however, conclusions of various authors are not completely identical. Our recent investigations of carba-analogues of these hormones could reveal how certain structural features of the molecule are important for the given type of biological activity, particularly concerning the disulfide bridge region or regions interacting with this bridge. According to the previous CD spectral studies[1] of carba-analogues it seems that the aromatic ring of tyrosine in position 2 interacts with the sulfur atom of cysteine disulfide bridge in position 6. Our investigations on sulfoxides of these carba-analogues confirm this assumption.

RESULTS

Oxidation of deamino-1-carba-oxytocin with sodium periodate affords a mixture of diastereoisomeric sulfoxides which can be separated by reversed-phase high performance liquid chromatography (RPHPLC). Using this method we obtained both diastereoisomers in pure state and compared their biological activity with the already published values[2] (see Table 1). The diastereoisomers can be separated also by countercurrent distribution in the system sec-BuOH:H_2O:AcOH 145o:65o:1 (K= =1,22 and 1,42). On the other hand the sulfoxide obtained by oxidation of deamino-6-carba-oxytocin resisted to attempted separation into the diastereoisomers (if both were formed at all). The same was observed with sulfoxides of larger or smaller ring-containing analogues. 1-Carba-oxytocin (with preserved amino group) as well as 1-carba and 6-carba ana-

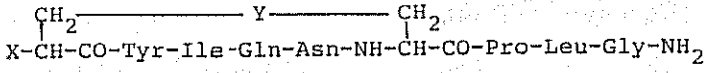
logue of deamino-8-arginine-vasopressin afford a mixture of diastereoisomers, separable with RPHPLC. The pertinent biological activities, together with the k´ values of the studied sulfoxides and sulfides, are given in Table 1.

According to the above-cited work[1], the sulfur atom in position 6 should be to a certain extent protected against action of various reagents, e.g. against oxidation. We followed therefore the kinetics of oxidation of various carba-analogues with sodium periodate. As seen from Fig.1, the oxidation rates for deamino-1-carba-oxytocin and deamino-6-carba-oxytocin differ significantly whereas there is no substantial difference between the carba-analogues of vasopressin (the carba-6 analogue being again oxidized somewhat faster), which are oxidized much faster then deamino-1-carba-oxytocin. For comparison, we followed under the same conditions oxidation of substance P; this compound is oxidized by an order of magnitude faster than any of the carba-analogues mentioned (see Fig.1), In this context also the behaviour of tert-butyl-sulfonium salts is worth notice[3]: in this series the most stable compound is that derived from the substance P; the least stable is the sulfonium salt of deamino-1-carba-oxytocin.

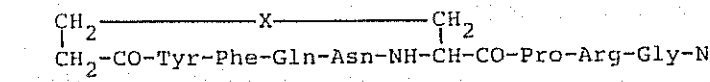
Periodate oxidation leads selectively to sulfoxides and can be employed in combination with RPHPLC for decision whether the given peptide is a sulfide or sulfoxide, or which of the chromatographic peaks can be ascribed to these oxidation states of sulfur. Periodate transforms rapidly sulfides into sulfoxides which are eluted faster as one or two peaks. In ambiguous cases it is advisable to follow the time-dependence of the reaction. We observed for a number of free as well as protected peptides that sulfoxides have lower k´ values[4]; this fact has been described also in the literature (e.g.[5]). In order to check that compound whose chromatographic properties do not change after treatment with periodate is a sulfoxide, it can be reduced with hydrogen bromide and acetone[2,6]. This reaction is very fast, affords no non-volatile side-products and can be easily performed even on micro--scale. Acid-labile protecting groups, if present, must be removed prior the reaction. An example of mentioned procedures is given in Fig.2.

Table 1

Activities (I.U./mg) and k' values of the studied peptides



Compound X	Compound Y	Uterus (rat, *in vitro*)	Mammary gland (rat, *in vivo*)	k' a,b
NH_2	CH_2-S	734	142	1,83
NH_2	CH_2-SO	o,26	o,o2	1,56 and 1,68
H	CH_2-S	1898	6o4	2,93
H	CH_2-SO	o,13	o,67	1,93
H	CH_2-SO	7,2	96	2,22
H	S-CH_2	929	456	2,oo
H	SO-CH_2	455	216	1,55
H	S	9	42[d]	2,54
H	SO	12,6	5,8	1,97
H	CH_2-S-CH_2	6o	136	2,57
H	CH_2-SO-CH_2	1,9	4o,6	1,51



Compound X	Blood pressure (nefrectomized rat)	k' a,c
CH_2-S	55o[e]	3,o2
CH_2-SO	8,1	2,67
CH_2-SO	o,85	2,33
S-CH_2	165 (223[e])	3,6o
SO-CH_2	111	3,o4
SO-CH_2	46	3,52

a) Separon SI C-18, MeOH/buffer (1:1) b) Buffer pH 4
c) Buffer pH 6,4 d) *in vitro* e) Despinalized rat

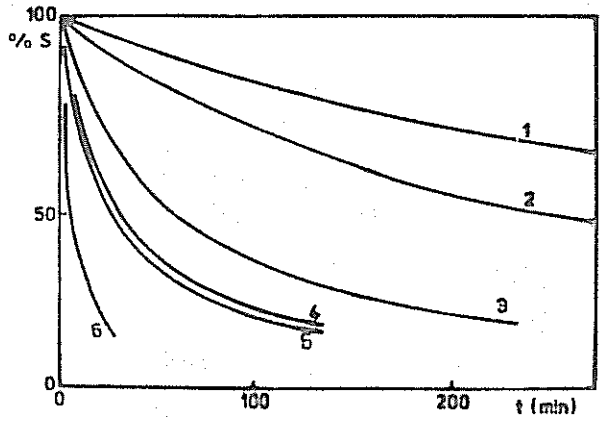


Fig.1. Kinetics of oxidation of some peptides with an equimolecular amount of $NaIO_4$ at 25°C. 1 and 3: dCOT-1, 2: dCOT-6, 4: dCAVP-1, 5: dCAVP-6, 6: substance P; 1 and 2: o,75 mM in $MeOH/H_2O$ (3:1), 3-6: o,65 mM in $MeOH/H_2O$ (1:1).

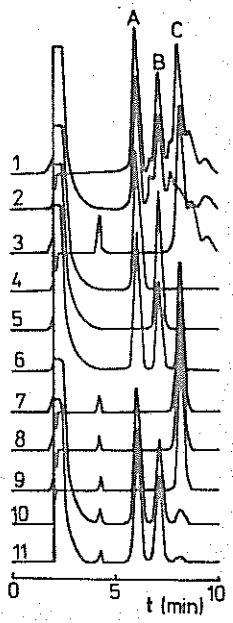


Fig.2. Chromatographic determination of oxidation state of the sulphur atom in peptides in a mixture.

1: crude mixture
2: mixture after oxidation ($NaIO_4$)
3: mixture after reduction (HBr/acetone)
4: peak A after oxidation
5: peak B after oxidation
6: peak C after oxidation
7: peak A after reduction
8: peak B after reduction
9: peak C after reduction
1o: 7 after oxidation
11: 8 after oxidation

DISCUSSION

Carba-analogues in which CD spectra indicate an interaction of the bridge sulfur (in position 6) with the aromatic nucleus on oxidation lose markedly their activity (the only exception being the analogue with the smaller ring which, however, already in the sulfide state exhibits a relatively low activity). On the other hand, for compounds in which such interaction is not assumed the decrease in activity is relatively small (this is true also for analogues derived from deamino-6-carba-oxytocin[7]). Moreover, diastereoisomeric sulfoxides of the deamino-1-carba-oxytocin differ in their activity very markedly. These facts could be explained by assumption that in oxytocin the aromatic ring of tyrosine points to the disulfide bridge and interacts with the sulfur atom in position 6. (The previously assumed[2] effect on the hydrogen bond between Gly-9 and Cys-6 does not seem to be a likely explanation of the lower activity since more recent study[8] shows that this hydrogen bond is of no crucial importance for the oxytocic activity.) If the oxidation affects unfavourably this interaction, the activity is lowered as the result of the unfavourable spatial orientation of the tyrosine side chain which can naturally be influenced differently by different orientation of the oxygen bound to sulfur atom. On the basis of the available facts it is possible to assign absolute configuration R to the sulfoxide with lower k' value and biological activities because the orientation of oxygen corresponding to this configuration should lead to a higher effect on tyrosine than the orientation in sulfoxide of the S--configuration. The same situation exists also in [2-p-fluorophenylalanine]-deamino-1-carba-oxytocin[9] and therefore the tyrosine hydroxyl obviously has no significant effect on the intramolecular interaction. The different activities of diastereoisomeric sulfoxides of deamino-1-carba-8-arginine-vasopressin can be due to similar steric effects; however, for this compound there is as yet no CD-spectral evidence on an interaction between the aromatic ring and sulfur (in so called "biologically active conformation").

A shielding of sulfur atom in position 6 is indicated also by the fact that it is oxidized substantially slower than the

sulfur atom in position 1. The vasopressin analogues do not show this difference, evidently because the aromatic part of tyrosine interacts in an aqueous medium with the phenylalanine moiety[10] and therefore it cannot come close to the disulfide bridge. In the substance P the methionine moiety is unhindered and thus very rapid oxidation occurs. Also behaviour of tert-butylsulfonium salts of these compounds[3] is in a good accord with the assumption of different steric accessibility of their sulfur atom.

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