SYNTHESIS, REDUCTION, AND PHARMACOLOGICAL PROPERTIES OF THE SULFOXIDES OF SOME CARBA-ANALOGUES OF OXYTOCIN*

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Received July 26th, 1977

Direct selective oxidation afforded the sulfoxides of deamino-1-carba-oxytocin (*Ia*), deamino--6-carba-oxytocin (*Ic*), and 1-carba-oxytocin (*Id*). The sulfoxide *Ia* was also prepared by a stepwise synthesis of the peptide chain. For purposes of comparison, the sulfone of deamino-1-carba--oxytocin (*Ib*) was prepared. The pharmacological properties of compounds *Ia*—*Id* were examined. Of a special interest is the difference in activities of sulfoxides of the 1-carba and 6-carba series and dissociation of effects on uterus and lactation. With the use of model compounds, the reduction of sulfoxides was examined and the most advantageous procedure (hydrogen bromide/acetone) was applied to the sulfoxide *Ia* which afforded the sulfide *Ie* without formation of by-products.

The sulfides are known to undergo oxidation more readily than the disulfides[1]. It may be thus expected that the so-called carba-analogues of neurohypophysial hormones will be more exposed to the danger of oxidation reactions than the naturally occurring disulfide hormones.** Since the structural requirements and influence of the structure in the region of the disulfide bridge on the biological activity have not been so far examined except for the substitution of sulfur by the methylene group[3-5] or selenium[6], it is hardly possible to predict any biological properties of the oxidized carba-analogue. According to a hypothesis, the sulfoxide of deamino-1-carba-oxytocin could exhibit a low activity[3] and its formation could cause the decreased activity of older preparations[7], but this idea has been neither confirmed nor controverted. By the formation of a sulfoxide, there is explained the inactivation or decreased activity of ACTH (ref.[8,9]) and its fragments[10], MSH (ref.[11]), calcitonin[12], parathormone[13], eledoisin[14] or carboxyterminal tetrapeptide gastrin[15] when these substances are treated with aqueous hydrogen peroxide. In this connection, some of these hormones containing the sulfoxide of methionine have been synthesized[12,14,15].

* Part CIL in the series of Amino Acids and Peptides; Part CXLVIII: This Journal *43*, 1300 (1978).

** The nomenclature and symbols obey the earlier published recommendations[2]. The amino acids appearing in this paper are of the L-series. The abbreviations Hcy and Picr designate homocystein and picric acid, resp.

The sulfide can be converted to the sulfoxide by several procedures[16] from which, however, only a few are suitable for the present field of substances. The procedure of choice must afford selectively the sulfoxide, the reaction conditions must be mild, and the reaction must be as fast as possible. Analogous requirements apply to reduction of the sulfoxide.

As a model substance in investigations on the oxidation of sulfides and reduction of the corresponding sulfoxides, S-(γ-methoxycarbonylpropyl)cysteine[3] (*IVa*) was used, *i.e.*, the parent amino acid of the so-called 1-carba-analogues of neurohypophyseal hormones. Oxidation with hydrogen peroxide in acid and neutral media was examined. The sulfone *VIa* is quantitatively formed in acid media whereas a slow oxidation to the sulfoxide *Va* occurs in neutral media. Twenty four hours at room temperature are required for a complete conversion. When compared with cystathionine[17] and the thialysine derivatives[18], the present reaction is more specific. In the case of cystathionine, the formation of the sulfoxide was observed[19] even in acid media under vigorous conditions. A similar behaviour was also reported for the case of methionine[20] which affords a pure sulfoxide by the action of excess hydrogen peroxide in 1M-HCl. When the oxidation is performed with performic acid, the results are analogous to those obtained in the case of S-methylcysteine[21] (*II*). At room temperature, the sulfone *VIa* is exclusively obtained whereas at −15°C, a mixture of the sulfone *VIa* and the sulfoxide *Va* is formed. Like the German authors[18] working on thialysine derivatives, we did not succeed in preparing a pure sulfoxide by the action of bromine. Best results were obtained with the use of the periodate as oxidant[16] (this reagent has not been as yet used in the oxidation of amino acids). The reaction is fast enough and the first order reaction constant equals to $1{\cdot}1 \cdot 10^{-3}$. s^{-1} at 22°C. The use of excess reagent and prolonged reaction time does not result in the formation of the sulfone. The conversion of the sulfide is quantitative after 2 h; after additional 200 h, no sulfone was detected by chromatography. The advantage of potassium periodate consists in its low solubility in water and the great solubility of the iodate as the reaction product (in spite of excess reagent, the reaction takes place in an excess of the oxidized component). Sodium periodate is much more soluble in water and its reduction product, sodium iodate, can be precipitated with methanol after completion of the oxidation.

Interesting results may be observed in the acid hydrolysis (6M-HCl, 105°C under diminished pressure) of the sulfoxide *Va*. Irrespective of the reaction time, the hydrolysis of the sulfoxide (either free or as component of a peptide chain) affords S-(γ-carboxypropyl)cysteine (*Vb*) in 50% yield. A similar behaviour may be observed in the case of S-(β-methoxycarbonylethyl)homocysteine sulfoxide (*VIII*) and cystathionine sulfoxide as components of the peptide. Such a behaviour was also reported in the case of S-methylcysteine sulfoxide[21] (*III*) but was not quantitatively evaluated. Under analogous conditions, methionine sulfoxide affords methionine in high yield[22] (*cf.* ref.[9]) whereas S-carboxymethylcysteine sulfoxide gives a mixture of pro-

ducts in which cysteine predominates[21]. Contrary to S-carboxymethylcysteine sulfone[21], the sulfones *VIa* and *IX* and S-methylcysteine sulfone do not decompose (only the methyl ester is hydrolysed); we may thus exclude explanation of the 50% recovery of the sulfide from the sulfoxide *Va* by means of a disproportionation to the sulfide *IV* and the sulfone *VI* followed by decomposition of the sulfone with the formation of ninhydrin-negative substances. It was not possible to exclude *a priori* another mechanism consisting in the decomposition of the sulfoxide *Va* to a sulfenic acid derivative[23] (and dehydroalanine which is converted to pyruvic acid); the sulfenic acid derivative would make possible reduction of the other portion of the sulfoxide *Va* to the corresponding sulfide. However, in the case of the sulfoxide *VIII* such an explanation based on a partial reduction is not as evident since the yield of S-(β-carboxyethyl)homocysteine *VIIb* in the hydrolysis of the sulfoxide *VIII* was 20% in the case of the free amino acid and 54% in the case of the peptide *Ic*. By the formation of a sulfoxide and its subsequent 50% destruction it is possible to explain the low values of S-(γ-carboxypropyl)cysteine (*IVb*) in the amino-acid analysis and to determine in this manner the content of the sulfoxide *V* in the peptide. The hydrolysis of the substance is performed in a properly evacuated (10 min) and sealed ampoule since in the case of an incomplete evacuation or when the evacuation is omitted, the yield of the amino acid is as low as 70%. When the hydrolysis is performed in the presence of an oxidant ($NaIO_4$), the recovery of the sulfide *IVb* is not recorded and the hydrolysate contains the original sulfoxide *Vb* (in the form of a diacid); such a result does not correspond to the above proposed mechanism.

From circular dichroism curves of the present sulfoxides, the absolute configuration on sulfur of the predominant diastereoisomer was determined as *R* (ref.[24]). In the

TABLE I

R_F and *E* Values of Some Amino Acids and Their Oxidation Products

Compound	R_F					$E^{Gly}_{2,4}$
	S1	S2	S3	S4	S5[a]	
IVa	0·39	0·17	0·29	0·46	0·58	0·41
Va	0·15	0·09	0·10	0·26	0·45 and 0·34	0·30
VIa	0·22	0·13	0·20	0·40	0·36	0·24
VIIa	0·35	0·12	0·17	0·26	0·65	0·61
VIII	0·10	0·03	0·07	0·12	0·50	0·50
IX	0·18	0·04	0·12	0·23	0·00—0·65	0·45

[a] Paper chromatography.

periodate oxidation of S-(γ-methoxycarbonylpropyl)cysteine (*IVa*), the ratio of the resulting diastereoisomers is 54 : 46; in the oxidation with hydrogen peroxide, the ratio is 57 : 43. These diastereoisomeric sulfoxides may be separated by paper chromatography and ion exchange chromatography under conditions of an analysis in amino-acid analyser, see Table I and II. The separation by ion exchange chromatography is also successful in the case of diastereoisomeric sulfoxides *VIII* but fails with diastereoisomers of S-methylcysteine sulfoxide (*III*).

TABLE II

Elution Times of Some Amino Acid Derivatives in Amino-acid Analyzer

Compound	Time, min	Compound	Time, min
IVa	117	*II*	72
Va	46 and 48	*III*	43
VIa	47·5	*VIII*	50 and 51·5
IVb	93	*IX*	63
Vb	43 and 45	Asp	48
VIb	43	Gly	85
VIIa	86	Ile	123
VIIb	116	Phe	141

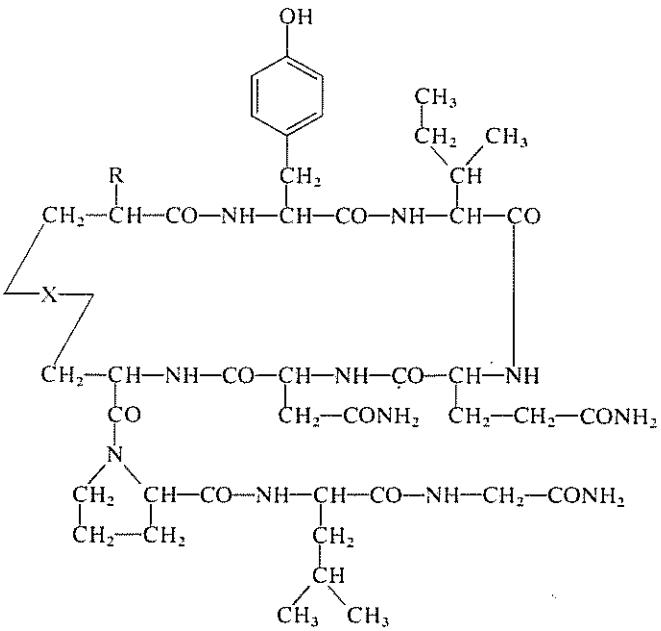
TABLE III

Chromatographical Data (R_F) of Some Oxytocin Analogues

Compound	S1	S2	S3	S4	S5[a]
Ia	0·16	0·12	0·23	0·62	0·48
Ib	0·19	0·11	0·26	0·62	—
Ic	0·13	0·11	0·02	0·45	—
Id	0·02	0·00	0·00	0·07	—
Ie	0·22	0·17	0·21	0·62	0·57
If	0·18	0·14	0·03	0·52	—
Ig	0·03	0·01	0·00	0·06	—

[a] Paper chromatography.

In the preparation of sulfoxides of carba-analogues of oxytocin, the sodium periodate oxidation was used as the procedure of choice. After two hours, the excess oxidant and the corresponding iodate were removed by gel filtration and the product was freeze-dried. This procedure was used in the preparation of sulfoxides *Ia*, *Ic*, and *Id* derived from deamino-1-carba-oxytocin, deamino-6-carba-oxytocin, and 1-carba-oxytocin, resp. In order to demonstrate that no sulfone is formed from the sulfide in this case, the authentic sulfone *Ib* of deamino-1-carba-oxytocin was prepared by performic acid oxidation and its chromatographic data were examined. The chromatographic behaviour (Table III) of the sulfone *Ib* was different from that of the sulfoxide *Ia* and the product of the periodate oxidation did not contain even a trace amount of the sulfone *Ib*. When a solution of the sulfide *Ie* in methanol was kept at room temperature for half a year, formation of the sulfoxide *Ia* was detected by chromatography.



Ia, X = CH_2—SO, R = H
Ib, X = CH_2—SO_2, R = H
Ic, X = SO—CH_2, R = H
Id, X = CH_2—SO, R = NH_2

Ie, X = CH_2—S, R = H
If, X = S—CH_2, R = H
Ig, X = CH_2—S, R = NH_2
Ih, X = CH_2—CH_2, R = H

The amino acid analysis may be successfully applied to prove sulfoxide and sulfone structures. Sulfoxide *Ia* gives 50% of parent amino acid in the form of sulfide *IVb*, whereas sulfone of S-(γ-carboxypropyl)cysteine(*VIb*) remained unchanged. Further-

more, the vibrations of —SO— and —SO_2— groups can be detected by IR spectroscopy. The spectrum of a model mixture containing 90% of deamino-dicarba-oxytocin (*Ih*) and 10% of sulfoxide *III* contains a band at 1006 cm^{-1} of medium intensity, whereas the spectrum of compound *Ia* has two weak bands in the expected area with frequencies shifted to the greater values. This finding should indicate the intramolecular interaction of sulfoxides with some polar group of the peptide molecule. Such an interaction is demonstrated by splitting the band into a doublet with lower intensity and therefore it is not possible to use IR spectroscopy to detect small amounts of sulfoxides in the analogue synthesized.

Polarography seemed to be promising for detection of sulfoxides[25]. S-Methylcysteine sulfoxide (*III*) and sulfoxide *Va* were used as model compound. In the first case no wave attributable to the reduction of sulfoxide was observed and in the other case only a poorly developed wave was detected just before the area of the final current increase. This wave may be attributed to the catalytic wave of hydrogen and it is not therefore possible to use it for the detection of sulfoxides in the molecules with molecular weights similar to those of neurohypophysial hormones. The Thompson test[26], which is still the most specific assay, although not quantitative, was used for verifying the newly synthesized sulfoxides.

For completely unambiguous confirmation of the identity of the synthesized analogues the stepwise building up of deamino-1-carba-oxytocin sulfoxide (*Ia*) was performed. This analogue was selected as a representative of the sulfoxide series because of synthetic procedure reasons and a dissociation of biological activities accompanied by a decrease of biological potencies. Sulfoxide *Va* was converted by means of Bunte salt[27] to N-benzyloxycarbonyl-S-(γ-methoxycarbonylpropyl)cysteine sulfoxide, which was obtained in the form of dicyclohexylammonium salt (*X*). The extremely low yield of this reaction obviously resulted from the intramolecular interaction of the amino group with the sulfoxide that served as protection against acylation. Carbobenzoxylation using benzyloxycarbonyl chloride at higher pH (8·5) gives the desired product *X* in a good yield. Owing to the fact that partial reduction of sulfoxide of S-benzylcysteine was described[28] when the benzyloxycarbonyl group from the cysteine derivative was split off by hydrogen bromide in acetic acid, we verified whether similar reduction did not occur also in the case of sulfoxide *Va*. By TLC we found that approximately 5% of sulfide was regenerated and for this reason the procedure was avoided.

Tetrapeptide amide *XIb* obtained by decarbobenzoxylation of peptide *XIa* (ref.[3]) was oxidized with sodium periodate. Sulfoxide *XII* in contrast to peptide *XIb* was obtained in a crystalline form, in accordance with the expected better crystallisation properties of sulfoxides[20]. Peptide *XII* had clearly different chromatographic properties when compared with compound *XIb* and it was possible to prove that no traces of the original sulfide were present in the sulfoxide prepared. Because of our intention to use amino acid derivatives protected with the 2-nitrobenzenesulfenyl group

Cys(Me)

II

Cys(O) (Me)

III

Cys(C_3H_6COOR)

IVa, R = Me
IVb, R = H

Cys(O) (C_3H_6COOR)

Va, R = Me
Vb, R = H

Cys(O_2) (C_3H_6COOR)

VIa, R = Me
VIb, R = H

Hcy(C_2H_4COOR)

VIIa, R = Me
VIIb, R = H

Hcy(O) (C_2H_4COOMe)

VIII

Hcy(O_2) (C_2H_4COOMe)

IX

Z—Cys(O) (C_3H_6COOMe).(C_6H_{11})$_2$NH

X

X—Cys(C_3H_6COOMe)—Pro—Leu—Gly—NH_2

XIa, X = Z
XIb, X = H

Cys(O) (C_3H_6COOMe)—Pro—Leu—Gly—NH_2

XII

Nps—Asn—Cys(O) (C_3H_6COOMe)—Pro—Leu—Gly—NH_2

XIII

X—Gln—Asn—Cys(O) (C_3H_6COOMe)—Pro—Leu—Gly—NH_2

XIVa, X = Nps; *XIVb*, X = H

X—Ile—Gln—Asn—Cys(O) (C_3H_6COOR)—Pro—Leu—Gly—NH_2

XVa, X = Nps, R = Me
XVb, X = H, R = H

Nps—Tyr(Bu^t)—Ile—Gln—Asn—Cys(O) (C_3H_6COOH)—Pro—Leu—Gly—NH_2

XVI

for the further building up of the peptide chain it was necessary to examine whether the sulfoxide group of the molecule would not be influenced when the protecting group was split off. The stability of sulfoxide was proved by splitting off this group

from 2-nitrobenzenesulfenylasparagine in mixture with sulfoxide *Va*. Peptide *XII* was prolonged by the 2,4,5-trichlorophenyl ester of 2-nitrobenzenesulfenylasparagine; this resulted in appearance of water soluble pentapeptide *XIII*. When the protecting group was split off hexapeptide *XIVa* was prepared using the same active ester of 2-nitrobenzenesulfenylglutamine. This peptide was also soluble in water and part of it was filtered through Amberlite IR-4B. The protecting group was split off by HCl and the hydrochloride generated was transformed into the free peptide *XIVb* by filtration through Amberlite IR-4B. Alternatively the oxidation-hydrolytic method[24] was applied at this stage for the removal of the 2-nitrobenzenesulfenyl protecting group. This method based on the simultaneous action of sodium periodate and water gave the free peptide in a high yield. The resulting peptide *XIVb* acylated by the N-hydroxysuccinimide ester of 2-nitrobenzenesulfenylisoleucine gave a poorly water soluble peptide *XVa*. The amino-protecting group was split off and methyl-ester was hydrolyzed by aqueous sodium hydroxide. Peptide *XVb* was then acylated by N-hydroxysuccinimide ester of 2-nitrobenzenesulfenyl-O-tert-butyltyrosine. The octapeptide *XVI* obtained was converted into the active ester by treatment with bis(*p*-nitrophenyl) sulfite and after the amino-protecting group was split off cyclization was performed in the usual manner[3]. Then, the protecting group on tyrosine was removed by means of trifluoroacetic acid and the analogue *Ia* thus prepared was purified by counter-current distribution and gel filtration. In the counter-current distribution in the system 2-butanol–0·01% aqueous acetic acid, sulfoxide *Ia* had a different distribution coefficient than peptide *Ie* (K = 1·30 and 2·15, resp.) and it was therefore possible to exclude the contamination of analogue *Ie* by the product of sulfur oxidation if the final hormone analogue was purified by counter-current distribution. The chromatographic properties of sulfoxide *Ia* (synthesized or obtained by a direct oxidation) differ from those of sulfide *Ie* into which sulfoxide *Ia* may be converted by reduction.

A series of methods for sulfoxide reduction was published[29–31] but only a part of them may be used for our purposes[32–36]. We tested the reduction of model compounds (*III*, *Va*) by means of 2-chloro-1,3,2-benzodioxaphosphole[37] (giving a mixture of products) and 2-mercaptoethanol[20], which turned to be utilisable but due to reaction conditions (50°C, 24 h) not the best one. Considering the reaction conditions the reduction by hydrogen bromide and acetone[20] (a procedure described some time ago but rarely used) proved to be best. The mild reaction conditions (20°C, 5 min) are comparable only with dichloroborane reduction[38] (0°C, several min). It was verified that under the mentioned conditions the tyrosine residue was not damaged and no cleavage of the molecule occurred even at reaction time longer by the magnitude of ten. The yields of the reduction were nearly theoretical when ionex chromatography was used for the product isolation. By the above-mentioned method sulfoxide *Ia* was reduced giving sulfide *Ie* with full biological activity; in this case the product was isolated by gel filtration.

The biological activities of oxidized monocarba-analogues together with the activities of some reference compounds are presented in Table IV. Compound *Ia* had both the activities tested, *i.e.* uterotonic *in vitro*[39] and milk-ejecting[40], lowered and clearly dissociated. The biological activities of a sample prepared by the oxidation of *Ie* were approximately same as those found for the sample prepared by the stepwise synthesis. Significantly higher potencies were found for sulfoxide *Ic*, they represented one half of the values estimated for the corresponding sulfide and this decrease was proportional for both the activities. The oxidation of carba-analogue with maintained amino group led to a decrease of potencies by several magnitudes of ten, just as it was found for the oxidation of deamino-1-carba-oxytocin to the sulfone stage. No inhibitory properties of the compound *Ib* were observed in the uterotonic assay (ratio *Ib*/oxytocin, 400 : 1).

It has been mentioned in many publications that the sulfur atoms of the oxytocin molecule are not equivalent. Differences in biological potencies were found for deamino-1-carba- and deamino-6-carba-oxytocins[41,42] just as in the case of monoseleno analogues of deamino-oxytocin[6]. Also the chiroptical properties of monocarba-analogues were different[43]. The great difference between the activities of *Ia* and *Ic* showed that non-equivalency of sulfur atoms was more evident when they were oxidized instead of having been replaced by methylene or by a selenium atom. Concerning the biological activities of sulfoxide the general tendency was similar to seleno-analogues where compounds of the 1-seleno series were more active as compared with 6-seleno-analogues; in our case the analogues possessing sulfoxide group in position 1 (*i.e.* analogues of the 6-carba series) had higher activities. Though we did not verify it experimentally, we did not consider it provable that the difference in the activities of compounds *Ia* and *Ic* was caused by a different metabolic stability

TABLE IV
Biological Activities of Oxytocin Analogues[a]

Analogue	Isolated rat uterus	Milk-ejection (rat)
Ia	13·1	91·0
Ib	0·05	0·03
Ic	455·4	216·2
Id	0·26	0·02
Ie	1 899[b]	604·3[c]
If	929[b]	456·5[c]
Ig	734[d]	142[c]

[a] Activities given in I.U./mg; [b] ref.[41]; [c] ref.[42]; [d] ref.[48].

of the analogues during their interaction with receptors or different elimination rates from the receptor compartment. The available information on the oxytocin receptor (uterus) is not sufficient to support fully the correctness of the hypothesis according to which the agent intrudes "the receptor pocket" by a part of disulfide bond belonging to the sulfur atom in position 6 and that sulfoxide derived from this sulfur (*i.e.* sulfoxide of the 1-carba series) cannot attain such a similar "receptor fit" due to spatial reasons. It is more plausible to consider that the spatial position of the tripeptide side chain is influenced by a strongly polar group in position 6. From the proposed secondary structure[44] of oxytocin as well as from the measurements of diffusion rates through membranes[45] follows that the tripeptide side chain is oriented above the cyclic part of molecule. *A priori* one cannot exclude the interaction of the sulfoxide in position 6 with this chain, mainly the effect on the hydrogen bond formed by the amino group of glycine and the carbonyl group of cysteine in position 6 which concenquently forces the adoption of a less suitable conformation of the tripeptide side chain in its interaction with the receptor. Taking in account the model of oxytocin proposed by American authors[44] for the conformation of hormone in water, it is evident that the oxidized sulfur in position 6 influenced dramatically the above mentioned hydrogen bond whereas the oxidized sulfur in position 1 was without effect. Sulfoxide in position 1 could affect the hydrogen bond between the amino-group of cysteine in position 1 and the carbonyl group of glycine which, however, is excluded in the case of deamino-analogue *Ic*. Therefore, the oxidation of this sulfur did not have a pronounced effect on the activity of deamino-6-carba--oxytocin. The significantly lower activity of sulfone *Ib* could be explained by higher steric requirements of the —SO_2— group or by higher probability of effecting a hydrogen bond between the side-chain and the cyclic part of the molecule, in comparison with sulfoxide *Ia* in which only one of the diastereoisomers could disturb this hydrogen bond (*cf.*[46]). We cannot be sure that sulfoxide *Ia* is exclusively of one configuration although measurements of dichroic spectra of model sulfoxides[24] show that the configuration of the sulfoxide sulfur changes quite simply and becomes thermodynamically more favorable.

The explanation of the extremely lowered activity of sulfoxide *Id* may be based on the preferential formation of a hydrogen bond between the sulfoxide group and the amino group of cystein in position 1 (which in oxytocin is engaged in a hydrogen bond with the glycine carbonyl) as a result of which not only the conformation of the cyclic part of the molecule is altered but also that of the tripeptide side chain.

EXPERIMENTAL

Samples for elemental analysis were dried for 24 h at room temperature and 1 Torr. Thin-layer chromatography (TLC) was carried out on silica gel plates (Silufol, Kavalier) in the solvent systems: 2-butanol–98% formic acid–water 75 : 13·5 : 11·5 (S1), 2-butanol– 25% ammonia–water

85 : 7·5 : 7·5 (S2), 1-butanol–acetic acid–water 4 : 1 : 1 (S3) and pyridine–1-butanol–acetic acid–water 10 : 15 : 3 : 6 (S4). Paper chromatography was carried out on Whatmann 3MM paper in methanol–water–pyridine 85 : 15 : 4 (S5). Electrophoresis was performed in a wet chamber apparatus in 1M acetic acid (pH 2·4) and in pyridinium acetate buffer (pH 5·7) at a potential drop of 20 V/cm for 60 min. Detection was by means of ninhydrin or by chlorination in the case of ninhydrin-negative compounds. Samples for amino-acid analysis were hydrolyzed for 20 h at 105°C in 6M-HCl (in ampoules sealed at 1 Torr). The analyses were carried out on an automatic analyser (Development Workshops, Czechoslovak Academy of Sciences, Prague, type 6020). For evaporation we used rotatory evaporators (water pump, bath temperature 30—40°C). Mixtures containing dimethylformamide were evaporated at 1 Torr. Melting points were determined on a Kofler block and values are uncorrected. For counter-current distribution the all-glass Steady State Distribution Machine from Quickfit & Quartz, Ltd, Stone, Staffordshire, England, with independent shifting of both upper and lower phases, was used. In all cases the solvent system was 2-butanol–0·05% aqueous acetic acid and the peptide material was located by the Folin-Ciocalteau reaction. For gel filtration we used the columns (100 × 1 cm) of Bio-gel (Bio-Rad Laboratories) P—2 and P—4.

S-(γ-Methoxycarbonylpropyl)cysteine Sulfoxide (*Va*)

a) To the solution of S-(γ-methoxycarbonylpropyl)cysteine[3] (*IVa*) (3·15 g) in water (180 ml) sodium periodate (4 g) was added and the mixture was stirred 2 h at room temperature. After filtration, the mixture was cooled to 0°C and placed on a column of Dowex-50 (H^+-cycle, 100 ml). The ion exchanger was washed with water (0°C, negative test for iodate ions) and the product was eluted with 10% pyridine (0°C). The eluate was evaporated and the residue was crystallized from water and acetone; the yield was 3·05 g (90%) with the m.p. 139—143°C. The sample for analysis was recrystallized in the same manner, m.p. 144—145°C; $[\alpha]_D^{25}$ −10·9° (*c* 0·2, water). For $C_8H_{15}NO_5S$ (237·3) calculated: 40·50% C, 6·37% H, 5·90% N; found: 40·67% C, 6·57% H, 5·91% N.

b) To the solution of *IVa* (22 mg) in water (3·5 ml) potassium periodate (25 mg) was added at 22°C. The samples were withdrawn after 5, 10, 30 and 60 min and the amount of sulfoxide (*Va*) in the reaction mixture was determined by means of paper electrophoresis. The conversion is a first-order reaction with a reaction constant equal to $1{\cdot}1 \cdot 10^{-3}\ s^{-1}$.

c) The suspension of *IVa* (220 mg) in a mixture of water (4·5 ml), methanol (2·5 ml) and 30% H_2O_2 (0·3 ml) was agitated 1 h at room temperature. The resulting solution contained 45% of sulfoxide *Va* and 55% of unreacted sulphide *IVa*. After further 24 h of standing at room temperature the product was precipitated by the addition of acetone; the yield was 190 mg of a compound, m.p. 131—133°C, containing about 1% of sulfone *VIa*.

d) To the solution of *IVa* (220 mg) in water (25 ml) 100 µl of Br_2 was added. After mixing, the colour of the mixture immediately disappeared and pH value became lower. The solution was freeze-dried and the oily product which was obtained, contained the same amount of sulfide *IVa* and sulfoxide *Va* (TLC).

e) Compound *IVa* (220 mg) was suspended in chloroform (25 ml) and the solution obtained after addition of 100 µl of Br_2 was evaporated 5 min later, the residue was dissolved in water and freeze dried. The same mixture of compounds was obtained as in the previous attempt.

Hydrochloride of S-(γ-Methoxycarbonylpropyl)cysteine Sulfoxide (*Va*. HCl)

To the solution of sulfoxide *Va* (237 mg) in methanol (6 ml) 2M-HCl in ether (0·5 ml) was added and the product was precipitated by the addition of ether (40 ml). Crystallisation from methanol and ether yielded 220 mg (81%) of the product with m.p. 126—128°C; $[\alpha]_D^{25}$ 0° (*c* 0·2, water). For $C_8H_{16}ClNO_5S$ (273·7) calculated: 35·10% C, 5·89% H, 5·12% N; found: 34·89% C, 6·01% H 5·15% N.

S-(γ-Carboxypropyl)cysteine Sulfoxide (*VI*

To the solution of methyl ester *Va* (237 mg) in water (2 ml) 1M-NaOH (2 ml) was added. After 1 h at room temperature the solution was cooled down to 0°C and filtered through the column of Dowex 50 (H^+-form, 10 ml). The column was washed with water (0°C) and the product was eluted with 10% pyridine. The eluate was taken to dryness, yield 200 mg (90%), m.p. 163 to 165°C, R_F 0·10 (S1), 0·00 (S2), 0·13 (S3), 0·14 (S4); $E_{5,7}^{Pier}$ 0·26. The sample for analysis was crystallized from methanol without change in the melting point; $[\alpha]_D^{25}$ −4·5° (*c* 0·2, water). For C_7H_{13}. NO_5S (223·2) calculated: 37·65% C, 5·87% H, 6·28% N; found: 37·41% C, 5·76% H, 6·12% N.

S-(γ-Methoxycarbonylpropyl)cysteine Sulfone (*VIa*)

a) Compound *IVa* (220 mg) was oxidized 1 h at room temperature with performic acid, prepared from formic acid (4 ml) and 30% H_2O_2 (0·3 ml). Lyofilisation afforded 250 mg (99%) of the product *VIa*, m.p. 158—159°C (decomp.). The sample for analysis was crystallized from water and acetone without change in m.p.; $[\alpha]_D^{25}$ −6·0° (*c* 0·2, water). For $C_8H_{15}NO_6S$ (253·3) calculated: 37·94% C, 5·97% H, 5·53% N; found: 37·63% C, 5·71% H, 5·34% N.

b) When the reaction as described under *a*) above was carried out at −15°C a mixture of 25% sulfoxide *Va* and 75% sulfone *VIa* was obtained.

c) To the solution of *IVa* (220 mg) in a mixture of water (1·5 ml), concentrated hydrochloric acid (0·2 ml) and methanol (2·5 ml), 10% H_2O_2 (0·8 ml) was added. After 1 h at room temperature methanol was evaporated and the mixture was freeze-dried. The yield was 240 mg of hydrochloride *VIa*, m.p. 146—148°C, chromatographically identical with the compound prepared as under *a*) above.

S-(β-Methoxycarbonylethyl)homocysteine Sulfoxide (*VIII*)

Analogously to the preparation of the compound *Va* (oxidation with $NaIO_4$) sulfoxide *VIII* was prepared from *VIIa* (0·5 g) in a 88% yield, m.p. 180—182°C, $[\alpha]_D^{25}$ +13·0° (*c* 0·2, water). For $C_8H_{15}NO_5S$ (237·3) calculated: 40·50% C, 6·37% H, 5·90% N; found: 40·71% C, 6·41% H, 5·95% N. R_F values are given in Table I.

S-(β-Methoxycarbonylethyl)homocysteine Sulfone (*IX*)

Oxidation of *VIIa* (0·5 g) with performic acid afforded sulfone *IX*. The yield was 0·49 g (90%), m.p. 224—225°C (decomp.), $[\alpha]_D^{25}$ +11·4° (*c* 0·2, water). For $C_8H_{15}NO_6S$ (253·3) calculated: 37·94% C, 5·97% H, 5·53% H; found: 38·25% C, 6·20% H, 5·47% N. R_F values are given in Table I.

Reduction of Sulfoxides *III* and *Va*

a) To the suspension of *Va* (340 mg) in acetone (5 ml) 35% hydrogen bromide in acetic acid (2 ml) was added and after 5 min at room temperature the resultant solution was evaporated. After briefly drying (1 Torr) the residue was suspended in acetone (5 ml), the solvent was evaporated and this operation was repeated once more. The remnant was dissolved in water (10 ml) and the solution was passed through Amberlit IR-4B (OH^--form, 25 ml) column. Evaporation of the eluent afforded 292 mg (92%) of the product, m.p. 200—207°C (ref.[3] gives m.p. 209 to 212°C). Amino-acid analysis revealed 3% of *IVb* but no sulfoxide *Va*.

b) Reduction of sulfoxides *III* and *Va* with 2-chloro-1,3,2-benzo-dioxaphosphole was carried out according to ref.[37]; a mixture of unidentified products (TLC) was obtained.

c) Reduction of sulfoxides *III* and *Va* with 2-mercaptoethanol (according to ref.[20]) gives chromatographically pure (TLC) sulfides *II* resp. *IVa*.

Dicyclohexylammonium Salt of N-Benzyloxycarbonyl-S-(γ-methoxycarbonylpropyl)cysteine Sulfoxide (*X*)

a) To the solution of sulfoxide *Va* (1·5 g) in water (40 ml) sodium benzyloxycarbonyl thiosulphate (2·5 g) was added and the pH was maintained at 7 by the addition of 2M-$NaHCO_3$ (21 ml) After 2 h no more $NaHCO_3$ is consumpted and the pH raises spontaneously. The mixture was acidified with 1M-HCl to pH 3, extracted with ether (4 × 20 ml), ethereal layers were combined, washed with water and dried. After evaporation, the remnant was dissolved in benzene, dicyclohexylamine (1·6 ml) was added, the solution was diluted by the addition of light petroleum, cooled to 0°C and crystals which separated were collected, dried and crystallized from ethyl acetate and light petroleum. The yield was 0·34 g (10%) of a product with m.p. 101—107°C. The sample for analysis was recrystallized in the same manner, m.p. 111—115°C, $[\alpha]_D^{25}$ −20·2° (*c* 0·2, dimethylformamide). For $C_{28}H_{44}N_2O_7S$ (552·7) calculated: 60·84% C, 8·02% H, 5·06% N; found: 60·56% C, 7·74% H, 5·39% N. The TLC comparison of the product with dicyclohexylammonium salt of N-benzyloxycarbonyl-S-(γ-methoxycarbonylpropyl)cysteine[3] (the values in parenthesis): R_F: 0·65 and 0·48 (0·76 and 0·48) (S1), 0·16 and 0·63 (0·26 and 0·63) (S2), 0·58 and 0·35 (0·74 and 0·35) (S3).

b) To the solution of *Va* (385 mg) in water (1 ml) 4M-NaOH (pH 8·5, 0·4 ml) was added and, under stirring and maintaining the pH at 8·5 (total 1·5 ml of 4M-NaOH), benzyloxycarbonyl chloride (0·5 ml) was added. After 2 h the consumption of NaOH ceased. The mixture was diluted by water (2 ml), extracted with ether, the aqueous layer was acidified to pH 3 with concentrated hydrochloric acid (0·5 ml) and the product was taken up in ethyl acetate. The organic layer was washed with water, dried and evaporated; the yield was 487 mg (81%) of an oil with R_F 0·65 (S1), 0·16 (S2), 0·58 (S3), 0·65 (S4); $[\alpha]_D^{25}$ −30·3° (*c* 0·2, dimethylformamide). From a part of this oily product the dicyclohexylammonium salt was prepared, identical with the one prepared as under *a*) above.

The Splitting of the Protecting Groups in the Presence of Sulfoxide

a) Amino acid was liberated from its dicyclohexylammonium salt (*X*) (100 mg) by means of 0·025M-H_2SO_4. The oil, obtained by usual working up of the mixture, was dissolved in acetic acid (0·1 ml) and 35% HBr in acetic acid (0·2 ml) was added. After 15 min at room temperature the product was precipitated by the addition of ether. TLC analysis revealed the presence of 5% of sulfide.

b) To the solution of 2-nitrobenzenesulfenylasparagine (143 mg) and sulfoxide *Va* (120 mg) in methanol (20 ml) 2M-HCl in ether (0·80 ml) was added. The mixture was evaporated to small bulk and during trituration with ether it turned to solid state. Paper electrophoresis as well as quantitative amino-acid analysis revealed only asparagine and sulfoxide *Va* in the reaction mixture.

Sulfoxide of S-(γ-Methoxycarbonylpropyl)cysteinyl-prolyl-leucyl-glycine Amide (*XII*)

To the solution of the protected peptide *XIa* (4·5 g) in acetic acid (22·5 ml) 35% HBr in acetic acid (45 ml) was added. After 10 min at room temperature the mixture was diluted with ether (450 ml), the precipitate formed was triturated with ether, collected, dried and dissolved in water (100 ml). The aqueous solutions was passed through a column of Amberlite IR-4B (acetate form, 60 ml) and eluted with water. A part of the eluate was withdrawn for comparison with the product of the oxidation reaction. To the remaining eluate (270 ml) sodium periodate (2 g) was added and the mixture was stirred at room temperature for 2 h. The cooled (0°C) solution was transferred to a column of Dowex 50 (H^+-cycle, 60 ml), the column was washed with water (0°C; negative reaction for iodate ions) and the product was eluted with 10% pyridine (0°C). The solution was taken to dryness, dissolved in a small amount of methanol, diluted with benzene and evaporated; this operation was repeated once more. After the last evaporation, the residue was dissolved in methanol, diluted with ether, and the oil which was obtained crystallized on standing (contrary to the sample of sulfide where an attempt at crystallization failed). The yield was 3·3 g (90%) of a product with m.p. 75—79°C; $[\alpha]_D^{25}$ —46·5° (*c* 0·2, dimethylformamide). For $C_{21}H_{37}N_5O_7S.2\,H_2O$ (539·7) calculated: 46·74% C, 7·66% H, 12·97% N; found: 46·61% C, 7·35% H, 12·99% N. TLC comparison with unoxidized sample *XIb* (the values are given in parenthesis: R_F 0·06 (0·12) (S1), 0·03 (0·07) (S2), 0·06 (0·07) (S3), 0·29 (0·41) (S4)) proved that the product did not contain sulfide *XIb*.

Sulfoxide of 2-Nitrobenzenesulfenylasparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl-prolyl--leucyl-glycine Amide (*XIII*)

To the solution of the tetrapeptide *XII* (3·2 g) in dimethylformamide (45 ml), N-ethylpiperidine (0·4 ml) and 2,4,5-trichlorophenyl ester of 2-nitrobenzenesulfenylasparagine (3·6 g) were added. After standing at room temperature for 18 h a further base (0·6 ml) and active ester (1·8 g) were added. After 72 h, the mixture was evaporated, the residue was triturated with ether and crystals which formed were collected and washed with ether. The product is water soluble and therefore water washings must be avoided. Precipitation from a mixture of methanol, dimethylformamide and ether yielded 3·2 g (63%) of a chromatographically pure product with m.p. 176—179°C; R_F 0·27 (S1), 0·17 (S2), 0·24 (S3), 0·55 (S4) and 0·41 ($CHCl_3$-methanol; 4 : 1). The sample for analysis was reprecipitated in the same way, m.p. 183—186°C; $[\alpha]_D^{25}$ —82·9° (*c* 0·2, dimethylformamide). For $C_{31}H_{46}N_8O_{11}S_2.H_2O$ (788·9) calculated: 47·20% C, 6·13% H, 14·20% N; found: 47·39% C, 6·53% H, 14·29% N. Amino-acid analysis: Asp 1·01, Pro 1·00, Gly 1·02, Leu 0·99, Cys(C_3H_6COOH) 0·50.

Sulfoxide of 2-Nitrobenzenesulfenylglutaminyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl-prolyl-leucyl-glycine Amide (*XIVa*)

To the solution of protected pentapeptide *XIII* (3·1 g) in dimethylformamide (40 ml) 2M-HCl in ether (2 ml) was added. After 4 min at room temperature the solution was diluted with ether

(200 ml), the precipitate was collected, washed with ether and dried. It was then dissolved in dimethylformamide (40 ml), the solution was alkalinized with N-ethylpiperidine (1·1 ml) and 2,4,5-trichlorophenyl ester of 2-nitrobenzenesulfenylglutamine (2·5 g) was added. After 12 h at room temperature a second portion of N-ethylpiperidine (0·4 ml) was added and 32 h later the mixture was taken to dryness and the residue was triturated with ether. Precipitation from methanol and ether provided 4·1 g of a mixture of hexapeptide *XIVa* and N-ethylpiperidine hydrochloride. A part of this mixture (0·5 g) was dissolved in water (20 ml) and filtrated through a column of Amberlite IR-4B (acetate form; 10 ml). The eluate was evaporated, dissolved in benzene, taken to dryness and precipitated from a mixture of methanol, dimethylformamide and ether. The yield of chromatographically and electrophoretically pure product was 0·44 g (88%), m.p. 111—114°C. R_F 0·24 (S1), 0·21 (S2), 0·09 (S3), 0·55 (S4). $E_{5.7}^{His}$ 0·32, $E_{2.4}^{Gly}$ 0·60 (after splitting off the protecting group). The total yield equals 100%. The sample for analysis was reprecipitated from dimethylformamide and ether, m.p. 122—125°C; $[\alpha]_D^{25}$ −63·9° (*c* 0·2, dimethylformamide). For $C_{36}H_{54}N_{10}O_{13}S_2.H_2O$ (917·0) calculated: 47·15% C, 6·16% H, 15·27% N; found: 47·22% C, 6·19% H, 14·98% N.

Sulfoxide of Glutaminyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl-prolyl-leucyl--glycine Amide (*XIVb*)

a) To the solution of hexapeptide *XIVa* (3·5 g of a preparation of 88% purity, *i.e.* 3·08 g of pure compound) 2M-HCl in ether (1·95 ml) was added. After 4 min at room temperature the solution was diluted with ether (300 ml), the precipitate obtained was triturated with ether and reprecipitated from methanol with ether. After dissolving in water (100 ml) it was passed through a column of Amberlite IR-4B (acetate form; 60 ml), the product was eluted with water, evaporated and twice recrystallized from methanol and ether. The yield was 2·56 g (96%), m.p. 131—138°C; $E_{2.4}^{His}$ 0·47, $E_{5.7}^{His}$ 0·35, R_F 0·48 (S4). The sample for analysis was recrystallized in the same manner, m.p. 135—142°C; $[\alpha]_D^{25}$ −55·2° (*c* 0·2, dimethylformamide). For $C_{30}H_{51}N_9O_{11}S.H_2O$ (763·9) calculated: 47·17% C, 6·99% H, 16·50% N; found: 46·87% C, 6·71% H, 16·24% N.

b) To the solution of hexapeptide *XIVa* (180 mg) in water (25 ml) $NaIO_4$ (90 mg) was added. After 4 h at room temperature (the colour of the mixture disappeared) the solution was passed through a column of Dowex-50 (H^+-cycle; 4 ml), the ion exchanger was washed with water and the product was eluted with 10% pyridine. The eluate was evaporated and the residue was precipitated from methanol and ether. The yield was 130 mg (87%) of a product of the same properties as had the compound prepared as under *a*) above.

Sulfoxide of 2-Nitrobenzenesulfenylisoleucyl-glutaminyl-asparaginyl-8-(γ-methoxycarbonylpropyl)cysteinyl-prolyl-leucyl-glycine Amide (*XVa*)

To the solution of *XIVb* (2·5 g) in dimethylformamide (35 ml) N-hydroxysuccinimide ester of 2-nitrobenzenesulfenylisoleucine (2·3 g) and N-ethylpiperidine (250 µl and after 2 h 100 µl) was added. After 70 h stirring at room temperature the mixture was evaporated, the residue was triturated with ether, the crystalline portion was collected and washed with ether, ethyl acetate, water, ethyl acetate and ether. The crystallization from dimethylformamide, methanol and ether yielded 2·67 g (82%) of the product with m.p. 210—214°C; R_F 0·24 (S1), 0·20 (S2), 0·12 (S3), 0·63 (S4). The sample for analysis was recrystallized from dimethylformamide and ether, m.p. 217—219°C; $[\alpha]_D^{25}$ −39·7° (*c* 0·4, dimethylformamide). For $C_{42}H_{65}N_{11}O_{14}S_2.2\,H_2O$ (1042) calculated: 48·13% C, 6·63% H, 14·70% N; found: 48·32% C, 6·34% H, 14·42% N.

Sulfoxide of Isoleucyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycine Amide (*XVb*)

To the solution of heptapeptide *XVa* (2·0 g) in dimethylformamide (60 ml) 2M-HCl in ether (2·4 ml) was added. After 5 min at room temperature the mixture was diluted with ether (300 ml), the precipitate was collected, washed with ether and dried; R_F 0·05 (S1), 0·07 (S2), 0·08 (S3), 0·41 (S4), $E_{5.7}^{His}$ 0·42. The compound was dissolved in water (50 ml), the solution was alkalinized with 1M-NaOH (6 ml, pH 12) and after 25 min the pH was brought to the value of 7 (5·7 ml of 1M-HCl). The solution was cooled (0°C), transferred to a column of Dowex-50 (H^+-cycle, 60 ml), the column was washed with water (0°C) and the product was eluted with 10% pyridine. The eluate was evaporated, dissolved in a mixture of methanol and benzene and evaporated. The precipitation from dimethylformamide, methanol and ether yielded 1·3 g (77%) of the product with m.p. 181—185°C; R_F 0·05 (S1), 0·01 (S2), 0·08 (S3), 0·28 (S4), $E_{5.7}^{His}$ 0·12, $E_{2.4}^{His}$ 0·43. The sample for analysis was reprecipitated from dimethylformamide and ether, m.p. 186 to 189°C, $[\alpha]_D^{25}$ −52·4° (*c* 0·2, dimethylformamide). For $C_{35}H_{60}N_{10}O_{12}S.2{\cdot}5\,H_2O$ (890·0) calculated: 47·23% C, 7·36% H, 15·73% N; found: 47·25% C, 6·98% H, 15·45% N. Amino-acid analysis: Asp 0·99, Glu 0·97, Pro 1·02, Gly 1·01, Ile 0·99, Leu 1·01, Cys(C_3H_6COOH) 0·56.

Sulfoxide of 2-Nitrobenzenesulfenyl-O-tert-butyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycine Amide (*XVI*)

To the solution of free heptapeptide *XVb* (1 g) in dimethylformamide (50 ml), N-ethylpiperidine (0·25 ml) and N-hydroxysuccinimide ester of 2-nitrobenzenesulfenyl-O-tert-butyltyrosine (1·3 g) was added. After 10 h more base (0·3 ml) was added and after additional 28 h the solution was evaporated and the residue was triturated with light petroleum and ether. The crystalline portion was collected and washed with ethyl acetate, water, ethyl acetate and ether. The yield was 1·3 g (91%), m.p. 202—204°C, R_F 0·47 (S1), 0·13 (S2), 0·73 (S3), 0·70 (S4). The sample for analysis was crystallized from dimethylformamide and ether without a change in the melting point; $[\alpha]_D^{25}$ +1·0° (*c* 0·2, dimethylformamide). For $C_{45}H_{80}N_{12}O_{16}S_2.2\,H_2O$ (1253) calculated: 51·74% C, 6·75% H, 13·41% N; found: 51·52% C, 6·55% H, 13·40% N. Amino-acid analysis: Asp 0·99, Glu 0·97, Pro 1·00, Gly 1·01, Ile 0·98, Leu 1·01, Tyr 0·98, Cys(C_3H_6COOH) 0·53.

Sulfoxide of Tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycine Amide Lactam (*Ia*)

a) To the solution of protected octapeptide *XVI* (500 mg) in dimethylformamide (15 ml) and pyridine (15 ml) was, under nitrogen, bis(*p*-nitrophenyl)sulfite (1 g) added and after 6 h at room temperature a further sulfite (1 g). After 12 h another 0·5 g portion of sulfite was added and 5 h later the mixture was evaporated, the residue was triturated with ether, collected, dried and dissolved in dimethylformamide (9 ml). To the solution 2M-HCl in ether (1 ml) was added and after 6 min at room temperature the mixture was diluted with ether (300 ml), the precipitate was collected, washed with ether and dissolved in dimethylformamide (20 ml). This solution was added, at a rate of 5 ml/h, to a mixture of pyridine (320 ml) and N-ethylpiperidine (150 µl) under heating (50°C) and nitrogen bubbling. The mixture was stirred 2 h at 50°C, then 14 h at room temperature, taken to dryness and the residue was triturated with ether. The oily product was dissolved in trifluoroacetic acid (15 ml) and after 50 min at room temperature the mixture was diluted with toluene (15 ml) and evaporated. The residue was triturated with ether and the solid compound was dissolved in 25 ml of the upper phase of the solvent system 2-butanol–0·05% aqueous acetic acid, the filtered solution was placed in the second tube of the counter-current distribution machine and 150 transfers of the upper phase and 60 transfers of the lower phase were carried

out. Three peaks with $K = 0{\cdot}14$, $0{\cdot}60$ and $1{\cdot}30$ were found. The first peak contained a ninhydrin-positive compound, the second one contained only a very small amount of peptide material and was not analysed. The third peak with $K = 1{\cdot}30$ (tubes No 49—73) was concentrated to a small volume and lyofilized. The yield was 130 mg (31%). A part of this product (60 mg) was dissolved in 3M acetic acid (2 ml) and transferred to a column of Bio-gel P-4. Lyofilisation of a peak of biologically active material (localised by OD_{280}) yielded 46·2 mg of the product identical in all properties with the product obtained by oxidation. The sample for analysis was precipitated from methanol and ether, $[\alpha]_D^{25}$ $-50{\cdot}0°$ (*c* 0·12, water). For $C_{44}H_{67}N_{11}O_{13}S.3\,H_2O.C_2H_4O_2$ (1104) calculated: 50·03% C, 7·02% H, 13·95% N; found: 49·83% C, 6·70% H, 13·82% N. Amino-acid analysis: Asp 1·00, Glu 0·99, Pro 1·02, Gly 1·00, Ile 0·98, Leu 1·02, Tyr 0·97, Cys(C_3H_6COOH) 0·47.

b) To the solution of deamino-1-carba-oxytocin[3] (*Ie*) (15 mg) in water (0·5 ml) sodium periodate (6·4 mg) was added. After 2 h at room temperature the mixture was diluted with 3M acetic acid (1 ml) and transferred to a column of Bio-gel P—2 (100 × 1 cm) in 3M acetic acid. The product was localized by OD_{280} and lyofilisation of the peak afforded 9·5 mg of the product. R_F value are given in Table III.

c) The solution of deamino-1-carba-oxytocin[3] (*Ie*) (15 mg) in methanol (0·2 ml) was left aside for 6 months at room temperature. TLC analysis revealed 30% of sulfoxide *Ia*.

Sulfone of Deamino-1-carba-oxytocin (*Ib*)

Deamino-1-carba-oxytocin[3] (*Ie*) (15 mg) was dissolved in a mixture (0·15 ml) of formic acid (4 ml) and 30% H_2O_2 (0·3 ml), 30 min after the preparation of this mixture. After standing for 1 h at room temperature, the solution was freeze-dried, the lyofilisate was dissolved in water (0·5 ml) and again freeze-dried. After dissolving in 3M acetic acid the solution was transferred to a column of Bio-gel P-2; the yield was 11 mg. The sample for analysis was precipitated from methanol and ether, $[\alpha]_D^{25}$ $-65{\cdot}5°$ (*c* 0·1, water). For $C_{44}H_{67}N_{11}O_{14}S.2\,H_2O.C_2H_4O_2$ (1102) calculated: 50·13% C, 6·86% H, 13·98% N; found: 50·23% C, 6·57% H, 14·12% N. Amino-acid analysis: Asp 1·02, Glu 1·00, Pro 1·03, Gly 1·01, Ile 0·98, Leu 1·02, Tyr 0·97, Cys(O_2)(C_3H_6.COOH) 0·95 (for calculation the constant for Cys(C_3H_6COOH) was used); no trace of Cys.(C_3H_6COOH) was found.

Sulfoxide of Deamino-6-carba-oxytocin (*Ic*)

To the solution of deamino-6-carba-oxytocin[4] (*If*) (3·4 mg) in a mixture of water (200 µl) and methanol (20 µl) a solution of sodium periodate (1·5 mg) in water (10 µl) was added. After 2 h standing at room temperature the mixture was worked up as described above; yield was 2·1 mg. TLC analysis proved that the product contained no sulfide *If*, *cf.* Table III. Amino-acid analysis: Asp 1·06, Glu 1·00, Pro 1·00, Gly 1·00, Ile 0·97, Leu 1·04, Tyr 0·95, Hcy(C_2H_4COOH) 0·51.

Sulfoxide of 1-Carba-oxytocin (*Id*)

To the solution of 1-carba-oxytocin[4,8] (*Ig*) (5 mg) in a mixture of water (300 µl) and methanol (20 µl) a solution of sodium periodate (2·4 mg) in water (10 µl) was added. After 2 h standing at room temperature the mixture was worked up as described for *Ia* above. The yield was 2·5 mg, R_F values are given in Table III. Amino-acid analysis: Asp 1·00, Glu 0·98, Pro 1·02, Gly 1·02, Ile 0·98, Leu 1·00, Tyr 0·98, Cyth 0·54.

Deamino-1-carba-oxytocin (*Ie*)

To the suspension of sulfoxide *Ia* (2 mg) in acetone (200 µl) 35% HBr in acetic acid (100 µl) was added. The obtained solution was after 12 min standing at room temperature evaporated, the residue was suspended in acetone (2 ml) and evaporated again. The remnant was triturated with ether, dried, dissolved in 3M acetic acid and transferred to a column of Bio-gel P-2 in 3M acetic acid. The procedure yielded 1·2 mg of a compound chromatographically identical with deamino-1-carba-oxytocin, having 1790 I.U./mg when tested on the isolated rat uterus.

Pharmacological Methods

The assay on the isolated rat uterus was carried out on strips of uterus of adult Wistar strain rats, under the influence of oestrogens. The strips were placed into the medium[39] which was bubbled through with 95% O_2 — 5% CO_2 at 30°C. Isometric contractions were recorded using a magnetoelectric transducer[47]; oxytocin was used as a standard. The inhibitory effect of analogue *Ib* was measured by the shift of the dose–response curve. For the determination of the milk-ejecting activity, lactating female rats (5—14 days after delivery) of the same strain were used. The assay was carried out as described in ref.[40].

Our thanks are due to Dr J. Smolíková for the interpretation of IR spectra, to Dr J. Krupička for the polarographic determination and to Dr I. Frič for measuring and interpretation of CD-*spectra. We would like to thank Mrs H. Kovářová and Mrs J. Kellerová for carrying out the pharmacological tests, to Mrs H. Farkašová for amino-acid analyses and to Mrs Z. Ledvinová for measuring the optical rotation. The elemental analyses were carried out in the analytical department of this Institute (head Dr J. Horáček).*

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