SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF [2-*p*-FLUOROPHENYLALANINE]DEAMINO-1-CARBA-OXYTOCIN AND ITS DIASTEREOISOMERIC SULFOXIDES*

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Systematic build-up of the peptide chain in solution led to [2-*p*-fluorophenylalanine]deamino--1-carba-oxytocin which after purification by countercurrent distribution was obtained in the form of diastereoisomeric sulfoxides. The sulfoxides were separated by chromatography and reduced with hydrogen bromide and acetone to the corresponding sulfide. Uterotonic and galactogogic activity of all compounds was determined; the diastereoisomeric sulfoxides differed markedly in these activities.

Oxytocin analogues, containing *p*-fluorophenylalanine** in the position 2, are of interest for the investigation of structure-activity relationship because they contain instead of the tyrosine hydroxyl a substituent which cannot participate as a proton donor in an intramolecular hydrogen bond. At the same time, this substituent bears certain polarity without changing the steric demands in the *para*-position of the tyrosine nucleus which is a region of critical importance for evoking biological responses. First attempt to synthesize such modified oxytocin analogue was unsuccessful because the removal of benzyl protecting group from the cysteine sulfur atom by action of sodium in liquid ammonia was accompanied by removal of the halogen atom[3]. This problem can be solved by using one of the following two approaches. One consists in using other sulfhydryl-protecting groups or other conditions for removal of the benzyl group (*e.g.* liquid hydrogen fluoride), the second is synthesis of an analogue which does not contain any disulfide bridge. The first path was successfully realized[4] and led to considerably active oxytocin analogues of which the deamino derivative *Ic* exhibited a lower uterotonic activity than the α-amino analogue *Ib*.

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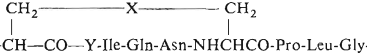
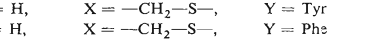
** The amino acids in this work are of L-configuration. The nomenclature and symbols of the amino acids and peptides obey the published recommendations[1]: Phe(*p*-F) denotes the *p*-fluorophenylalanine moiety.

Several years ago we published[5] the synthesis and some pharmacological properties of several deamino-1-carba-oxytocin (*Id*) analogues, containing phenylalanine (*Ie*), O-methyltyrosine or isoleucine in the position 2 and it appeared to be of interest to extend this series by preparation of the *p*-fluorophenylalanine analogue (*If*). Biological properties of this compound should lie somewhere between those of the tyrosine-containing and phenylalanine-containing analogues.

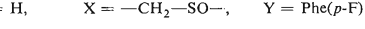
Our synthesis started from the heptapeptide[6] *III* which was acylated either by tert-butyloxycarbonyl-*p*-fluorophenylalanine N-hydroxysuccinimide ester or by *o*-nitrobenzenesulfenyl-*p*-fluorophenylalanine 2,4,5-trichlorophenyl (or better pentachlorophenyl) ester to give the respective octapeptides *IVa* and *IVb*. These were transformed into the active ester by treatment with bis(*p*-nitrophenyl) sulfite[7] and, after removal of the amine-protecting group, cyclized under high dilution conditions in pyridine. Purification by countercurrent distribution afforded a mixture of compounds differing in their chromatographic behaviour on thin-layers of silica gel. Gel filtration gave a fraction the elution volume of which roughly corresponded to the molecular weight of the monomer but which still was a mixture of products. Chromatography on a silica gel column in system 2-butanol–water (9 : 1) separated this mixture into two individual compounds of the same amino acid and elemental composition



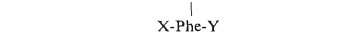
Ia: R = NH_2, X = —S—S—, Y = Tyr
Ib: R = NH_2, X = —S—S—, Y = Phe(*p*-F)
Ic: R = H X = —S—S—, Y = Phe(*p*-F)
Id: R = H, X = —CH_2—S—, Y = Tyr
Ie: R = H, X = —CH_2—S—, Y = Phe
If: R = H, X = —CH_2—S—, Y = Phe(*p*-F)
Ig: R = H, X = —CH_2—SO—, Y = Phe(*p*-F) (*k'* 2·0)
Ih: R = H, X = —CH_2—SO—, Y = Phe(*p*-F) (*k'* 2·55)



IIa: X = Nps, Y = OH *IId*: X = Boc, Y = OH
IIb: X = Nps, Y = OTcp *IIe*: X = Boc, Y = ONSu
IIc: X = Nps, Y = OPcp *IIf*: X = Boc, Y = ONp



III



IVa: X = Nps
IVb: X = Boc

which differed only in their melting points and chromatographic properties (thin-layer and high performance liquid chromatography). Both the compounds exhibited positive sulfoxide reaction[8], their infrared spectrum displayed characteristic sulfoxide vibrations and their chromatographic properties did not change on oxidation with periodate[9]. Both compounds were reduced with hydrogen bromide and acetone[10] to give identical compounds which on oxidation with periodate afforded in both cases a mixture of two compounds of the same capacity factor values as had both the fractions before the reduction. These experiments proved that the compounds are diastereoisomeric sulfoxides of the compound *If*. The corresponding sulfide *If* was prepared by reduction of the sulfoxides *Ig* and *Ih* with hydrogen bromide and acetone. Small amount of *If* was obtained also by chromatographic separation on Separon SI-C-18 from the mixture after countercurrent distribution. Sensitivity toward oxidation in analogues, containing a thioether grouping, depends strongly on structure because in syntheses of the mentioned analogues modified in position 2 no oxidation products have been observed[5] although these compounds were subjected to the same purification procedures (including countercurrent distribution). On the other hand, sulfoxides were formed from 6-carba-analogues, modified in position 2 (ref.[11]), as well as from 1-carba-analogues, modified in position 4 (ref.[12]).

The results of biological tests performed on the synthesized compounds are given in Table I. The analogue *If* exhibits a significant uterotonic activity, comparable with that of oxytocin (*Ia*) whereas its galactogogic activity is somewhat lower. Such behaviour of an oxytocin analogue is relatively exceptional, a reverse dissociation of activity being more common. The sulfoxide *Ig* shows no marked selectivity

TABLE I
Biological Activities (I.U./mg) and Values of Capacity Factor (k')

Compound	Rat uterus (*in vitro*)	Mammary gland (rat *in vivo*)	k' [a]	Ref.
Ia	450	450	—	—
Ib	97	—	—	4
Ic	57	—	—	4
Id	1 899	604	—	22, 26
Ie	233	221	—	22, 26
If	400	319	3·04	[b]
Ig	0·11	0·15	2·00	[b]
Ih	6·9	34·7	2·55	[b]

[a] Phosphate buffer (0·02M, pH 4·4)–methanol (7 : 13); [b] this paper.

whereas the other diastereoisomer *Ih* (exhibiting greater values of both the activities) exhibits a five times greater galactogogic than uterotonic activity. Oxidation of the sulfur atom in deamino-1-carba analogues is known to shift the specificity in favour of the galactogogic activity[13]; this effect can be observed also in the case of both our diastereoisomeric sulfoxides.

CD spectra of oxytocin carba-analogues indicate[14] an interaction of the aromatic ring with sulfur atom in position 6 of the disulfide bridge. This finding agrees well with the results of biological tests showing that oxidation of the sulfur atom in position 1 has only a negligible effect on the activities in question. Oxidation of sulfur in position 6 in 1-carba-analogues can disturb this interaction (which probably is important for adopting a biologically active conformation) which, on the other hand, is not affected by oxidation of sulfur atom in position 1. Activities of the diastereoisomeric sulfoxides *Ig* and *Ih* differ by order of magnitude, particularly as concerns the galactogogic activity. This fact might be explained by a different effect on interaction of the aromatic moiety with the sulfur atom, caused by a different orientation of the sulfoxide oxygen. If one considers the modified[15] model of American authors[16], the sulfoxide group of the absolute configuration (*S*) (assumed in compound *Ig*) somewhat "pushes away" the aromatic moiety whose exact position is important for the galactogogic activity whereas the effect of the (*R*)-sulfoxide (*Ih*) is much smaller. For the uterotonic activity, a direct interaction with receptor in the region of the sulfur atom in the position 6 of the disulfide bridge can be important; this interaction, however, is perturbed already by the oxidation itself and does not depend so strongly on the sulfoxide configuration or on a changed orientation of the aromatic moiety.

As seen from the values of k' (Table I), chromatographic properties of the less active diastereoisomer *Ig* differ from those of the sulfoxide *If* more than do those of *Ih* which indicates that conformation of the former sulfoxide differs from that of *If* more than the conformation of the other diastereoisomer, particularly in the region, responsible for interaction with the stationary phase (which might well be also the aromatic moiety).

These considerations about the spatial arrangement of analogues, described in this work, somewhat contradict the results obtained with the analogues *Ib* and *Ic*. On the basis of ^{19}F-NMR spectra measurements it was concluded[4] that the *p*-fluorobenzyl side chain in compounds *Ib* and *Ic*, as well as in their S-protected acyclic precursors, is freely exposed to the solvent influence and thus does not interact with the amino-terminal part of the molecule. Possible difference in the spatial arrangement of compounds *Ib* and *Ic* on the one hand and compound *If* on the other hand could thus also contribute to the difference in their biological activities.

The sulfoxides *Ig* and *Ih* exhibit low pressor activity (<0·5 I.U./mg) whereas the sulfide *If* shows an activity of 6·5 I.U./mg in the same test. The high antidiuretic activity of the compound *If* (75 I.U./mg) is not very surprising for a carba-analogue[17].

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried for 24 h at room temperature and 150 Pa. Thin-layer chromatography was performed on silica gel-coated plates (Kieselgel G — Merck) or Silufol sheets (Kavalier) in the following solvent systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4), n-heptane–pyridine–tert-butyl alcohol (5 : 1 : 1) (S5), and 2% ethanol in benzene (S6). Paper electrophoresis was carried out in 1M-acetic acid (pH 2·4) and in a pyridine–acetate buffer (pH 5·7) on a Whatman 3MM paper (moist chamber, 1 h, 20 V cm^{-1}). The compounds were detected with ninhydrin or using the chlorination method. Amino acid analyses were performed after hydrolysis of the samples for 20 h in 6M-HCl at 105°C in evacuated (150 Pa) ampoules, using an automatic analyzer (Development Workshops, Czechoslovak Academy of Sciences, Prague, type 6020). Reaction mixtures were taken down on a rotatory evaporator under diminished pressure (water pump); dimethylformamide-containing solutions were evaporated at 150 Pa.

Isolation and Purification Methods

Countercurrent distribution was carried out in an all-glass apparatus (Steady State Distribution Machine, Quickfit & Quartz, Stone, Staffordshire, England) with ability of transfer of the upper as well as the lower phases. Gel filtration was performed on 1 × 100 cm columns filled with Bio-Gel P—2 or P—4 (Bio-Rad Laboratories, Richmond, Ca., USA) in 3M acetic acid with flow rates 5—7 ml/h. High performance liquid chromatography was carried out using a Milton Roy Instrument MiniPump model 396—74 (Laboratory Data Control, Staffordshire, England) and a Separon SI C—18 column (Laboratorní přístroje, Prague, Czechoslovakia) (15 × 0·6 cm), containing spherical particles (6 μm diameter) of silica gel with chemically bonded octadecyl chains. For analytical purposes the sample was applied by means of the septum injector LCI-02 (Laboratorní přístroje, Prague), modified for a stopped-flow performance. In preparative runs the sample was applied by means of an injection valve of 2 ml capacity (250 × 0·1 cm). The flow rate of the mobile phase was 1·2 ml/min at 25—30 MPa. In countercurrent distribution and gel filtration the peptide material was located by the Folin–Ciocalteau reaction. Column chromatography on silica gel and high performance liquid chromatography were followed using a single beam UV spectrophotometer at 225 nm and 230 nm, respectively.

o-Nitrobenzenesulfenyl-*p*-fluorophenylalanine (*IIa*)

o-Nitrobenzenesulfenyl chloride (1·0 g) and 2M-NaOH (3 ml) were alternately added to a solution of *p*-fluorophenylalanine[18] (0·92 g) in 2M-NaOH (3 ml) and dioxane (5 ml). After stirring for 15 min the mixture was diluted with ice-cold water (40 ml), made acid with 0·5M-H_2SO_4 and the product was taken into ethyl acetate. The organic extract was washed with water, dried over sodium sulfate and taken down. The residue was dissolved in ethyl acetate (5 ml) and light petroleum was added until the solution became turbid. After standing overnight in a refrigerator the crystallized product was collected on filter and washed with light petroleum, affording 0·89 g (53%) of the product, m.p. 112—118°C. An analytical sample was obtained by crystallization from a mixture of ethyl acetate and light petroleum without change in the melting point. $[\alpha]_D$ —36·0° (*c* 0·5, dimethylformamide); R_F 0·39 (S2), 0·74 (S4), 0·07 (S6). For $C_{15}H_{13}FN_2O_4S$ .1/2 H_2O (345·3) calculated: 52·15% C, 4·09% H, 8·11% N; found: 52·16% C, 3·76% H, 8·16% N.

Dicyclohexylammonium salt: crystallized from methanol, m.p. 177—180°C; $[\alpha]_D$ —10·3° (*c* 0·5; dimethylformamide), $[\alpha]_D$ +20·4° (*c* 0·5; methanol). For $C_{27}H_{36}FN_3O_4$ (517·6) calculated: 62·70% C, 7·02% H, 8·13% N; found: 62·87% C, 7·08% H, 8·18% N.

o-Nitrobenzenesulfenyl-*p*-fluorophenylalanine 2,4,5-Trichlorophenyl Ester (*IIb*)

A suspension of dicyclohexylammonium salt of *IIa* (0·52 g) in ethyl acetate (10 ml) was shaken with 0·1M-H_2SO_4. The organic layer was washed with water, dried over sodium sulfate, taken down and dissolved in tetrahydrofuran (10 ml). 2,4,5-Trichlorophenol (0·22 g) was added, followed after cooling to —15°C by dicyclohexylcarbodiimide (0·23 g). The mixture was stirred for 1 h at —10°C, kept for 1 h in a refrigerator and stirred for another hour at room temperature. The separated dicyclohexylurea was filtered off, the filtrate taken down, dissolved in a small amount of ether and a further portion of dicyclohexylurea was filtered. The filtrate was taken down and the residue crystallized from 2-propanol and light petroleum. After one week (or even longer time) the product was collected; m.p. 93—97°C, yield 0·30 g (58%). The analytical sample was crystallized twice from the same solvent mixture, m.p. 98—99°C; $[\alpha]_D$ —73·7° (*c* 0·25, dimethylformamide). R_F 0·73 (S1), 0·75 (benzene). For $C_{21}H_{14}Cl_3FN_2O_4S$ (515·8) calculated: 48·65% C, 2·74% H, 5·43% N; found: 48·90% C, 2·76% H, 5·27% N.

o-Nitrobenzenesulfenyl-*p*-fluorophenylalanine Pentachlorophenyl Ester (*IIc*)

The active ester *IIc* was prepared from pentachlorophenol (0·28 g) in the same manner as the derivative *IIb*. Crystallization from tetrahydrofuran and light petroleum afforded 0·47 g (81%) of the product, m.p. 150—152°C. An analytical sample was crystallized from the same solvent without change in the melting point; $[\alpha]_D$ —63·0° (*c* 0·5, dimethylformamide); R_F 0·73 (S2), 0·85 (benzene). For $C_{21}H_{12}Cl_5FN_2O_4S$ (584·7) calculated: 43·15% C, 2·05% H, 4·79% N; found: 43·17% C, 2·04% H, 4·52% N.

Tert-Butyloxycarbonyl-*p*-fluorophenylalanine N-Hydroxysuccinimide Ester (*IIe*)

Dowex 50W (H^+-form; 5 ml) was added to a solution of dicyclohexylammonium salt of *IId* (ref.[4]; 0·47 g) in ethanol (25 ml) and water (10 ml). After stirring for 30 min the ion exchanger was filtered off and washed with ethanol. The filtrates were taken down, dried by azeotropic distillation with benzene and the residue was dissolved in ethyl acetate (10 ml). N-Hydroxysuccinimide (0·12 g) was added, followed after cooling to —10°C by dicyclohexylcarbodiimide (0·23 g). The mixture was stirred at —10° for 1 h, kept in a refrigerator overnight and at room temperature for 1 h. The separated dicyclohexylurea was filtered and the filtrates taken down. The crystalline residue was triturated with light petroleum, filtered, washed with light petroleum and after drying crystallized from ethanol, affording 0·33 g (87%) of the product, m.p. 168—170°C; $[\alpha]_D$ —48·9° (*c* 0·5, dimethylformamide); R_F 0·12 (benzene), 0·35 (S6). For $C_{18}H_{21}FN_2O_6$ (380·4) calculated: 56·85% C, 5·57% H, 7·37% N; found: 56·81% C, 5·57% H, 7·29% N.

Tert-Butyloxycarbonyl-*p*-fluorophenylalanine *p*-Nitrophenyl Ester (*IIf*)

The active ester *IIf* was prepared from *p*-nitrophenol (0·15 g) in the same manner as the derivative *IIe* and crystallized from ether and light petroleum; the analytical sample was recrystallized from ethyl acetate and light petroleum. Yield 0·29 g (72%) of the product, m.p. 109—111°C; R_F 0·36 (S6). For $C_{20}H_{21}FN_2O_6$ (404·4) calculated: 59·40% C, 5·23% H, 6·93% N; found: 59·28% C, 5·28% H, 7·08% N.

o-Nitrobenzenesulfenyl-*p*-fluorophenylalanyl-isoleucyl-glutaminyl-asparaginyl-
-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycine Amide (*IVa*)

A solution of the heptapeptide *III* (ref.[6]; 0·40 g), the pentachlorophenyl ester *IIc* (0·30 g) and N-ethylpiperidine (0·2 ml) in dimethylformamide (20 ml) was stirred for 24 h at room temperature. Another portion of the active ester (0·12 g) was added and after 24 h the solvent was evaporated. The residue was brought to crystallization by trituration successively with light petroleum, ether and 0·1M-H_2SO_4, filtered and washed on the filter with 0·1M-H_2SO_4 and water. Yield 0·48 g (89%) of the product, m.p. 197—201°C. The analytical sample was crystallized from dimethylformamide and ether; m.p. 201—204°C; $[\alpha]_D$ —28·8° (*c* 0·25, dimethylformamide); R_F 0·61 (S1), 0·67 (S4). $E^{Gly}_{2,4}$ 0·57, $E^{His}_{5,7}$ 0·12 (after removal of the *o*-nitrobenzenesulfenyl protecting group with HCl in ether and dimethylformamide). For $C_{50}H_{71}FN_{12}O_{14}S_2 \cdot 1{\cdot}5\ H_2O$ (1174) calculated: 51·20% C, 6·35% H, 14·32% N; found: 51·11% C, 6·12% H, 13·98% N. When the preparation was carried out with the active ester *IIb*, lower yields were achieved.

Tert-Butyloxycarbonyl-*p*-fluorophenylalanyl-isoleucyl-glutaminyl-asparaginyl-
-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycine Amide (*IVb*)

A solution of the heptapeptide *III* (0·60 g), N-hydroxysuccinimide ester *IIe* (0·28 g) and N-ethylpiperidine (0·3 ml) in dimethylformamide (5 ml) was stirred at room temperature for 24 h. Another portion of the active ester (0·14 g) was added and after 24 h the mixture was diluted with ether. The separated product was filtered, washed with ether, water, 0·25M-H_2SO_4, again with water, and dried, yielding 0·54 g (70%) of *IVb*, m.p. 213—215°C; $[\alpha]_D$ —41·7° (*c* 0·23, dimethylformamide); $E^{Gly}_{2,4}$ 0·60, $E^{His}_{5,7}$ 0·20 (after removal of the tert-butyloxycarbonyl protecting group with trifluoroacetic acid). For $C_{49}H_{76}FN_{11}O_{14}S.3\ H_2O$ (1148) calculated: 51·25% C, 7·20% H, 13·42% N; calculated: 51·15% C, 6·90% H, 13·19% N.

[2-*p*-Fluorophenylalanine]deamino-1-carba-oxytocin (*If*) and the Corresponding Sulfoxides (*Ig* and *Ih*)

a) Bis(*p*-nitrophenyl)sulfite[7] (1·5 g) was added to a solution of the protected octapeptide *IVa* (390 mg) in dimethylformamide (15 ml) and pyridine (15 ml) into which nitrogen was introduced (the preparation of the active ester as well as the cyclization were performed under nitrogen). After stirring for 6 h at room temperature, another portion of the reagent (1·5 g) and pyridine (7 ml) was added, followed after 16 h by further portion of the reagent (0·7 g). After 5 h the solvents were evaporated and the residue triturated successively with ether and water, filtered and washed with water, affording 377 mg (88%) of the ester, m.p. 196—198°C. This was dissolved in dimethylformamide (10 ml) and the solution was treated with 2M-HCl in ether (0·35 ml). After 5 min the mixture was diluted with ether, the precipitate filtered and after washing with ether dried in a desiccator over sodium hydroxide; $E^{Gly}_{2,4}$ 0·51, $E^{His}_{5,7}$ 0·35. This hydrochloride of the octapeptide active ester was dissolved in dimethylformamide (15 ml) and the solution was added in the course of 6 h to a mixture of pyridine (300 ml) and N-ethylpiperidine (37 μl) at 50°C. The reaction mixture was set aside overnight at room temperature, taken down, the residue triturated with ether, filtered and washed on the filter with ether. The product was dissolved in the upper phase (25 ml) of solvent system 2-butanol–0·05% aqueous acetic acid and subjected to countercurrent distribution (148 transfers of the upper phase and 354 transfers of the lower phase) which afforded a peak of k 3·65 (tubes 25—60). The material obtained after concentration and freeze-drying was purified on a column of Bio-gel P—4, the fraction 49 to 59 ml being collected. Freeze-drying of this fraction afforded 33 mg of material which still was

a mixture of several compounds and required purification by high performance liquid chromatography. A part of the material (20 mg) was dissolved in a methanol–water mixture (2 : 3, 2 ml), applied to a column of Separon SI C—18 and eluted with a methanol–water mixture (46 : 54). The single obtained fractions were concentrated *in vacuo* and freeze-dried. Their purity was checked by chromatography on the same column using a mixture of methanol and a phosphate buffer pH 4·4 (13 : 7) as the mobile phase. This procedure gave 4·8 mg of compound, identical with the portion A in the procedure *b*) ($k' = 2·0$), 3·1 mg of compound identical with the portion B in the procedure *b*) ($k' = 2·55$), 2·6 mg of compound of $k' = 3·04$ (contaminated with a compound of $k' = 3·33$; in an unbuffered system this pair of compounds was separated with great difficulty), and two 1·5 mg portions of compounds of $k' = 2·88$ and $k' = 3·33$ which were not further studied. Samples of the products (0·1 mg) of $k' = 2·0$, 2·55 and 3·04 were dissolved separately in methanol (0·1 ml), treated with a solution of sodium periodate (10 μl, 50 μg/ml) and the reaction mixtures were analyzed in the above-mentioned system. Chromatographic behaviour of compounds of $k' = 2·0$ and 2·55 did not change whereas the compound of $k' = 3·04$ was transformed into a mixture of compounds of $k' = 2·0$ and 2·55 in the ratio 5 : 3. Other samples (0·1 mg) of the products of $k' = 2·0$, 2·55 and 3·04 were separately suspended in acetone (30 μl) and mixed with a solution of hydrogen bromide (35%) in acetic acid (30 μl). The mixture was freeze-dried and evaporated twice with acetone (100 μl). The residue was dissolved in methanol and analyzed by chromatography. The compounds of $k' = 2·0$ and 2·55 were converted into the product of $k' = 3·04$ whereas the compound of $k' = 3·04$ did not change its chromatographic properties and was indistinguishable from the product arising by reduction of compounds of $k' = 2·0$ and 2·55. The identity of the reduction products was proved also by thin-layer chromatography in four systems. Methanolic solutions of the products were again treated with a solution of sodium periodate (as described above) and the reaction mixture was again analyzed. In all cases the reaction gave a mixture of the compounds of $k' = 2·0$ and 2·55 in the ratio approximately 5 : 3.

A mixture of the compounds of $k' = 2·0$ and 2·55 (7 mg) was suspended in acetone (0·2 ml), treated with a solution of hydrogen bromide in acetic acid and worked up as described above. Precipitation from methanol and ether afforded 4·3 mg of the compound *If*, m.p. 161—163°C, $k' = 3·04$. R_F 0·25 (S1), 0·14 (S2), 0·21 (S3), 0·62 (S4). For $C_{44}H_{66}FN_{11}O_{11}S.2·5\ H_2O$ (976·1) calculated: 51·75% C, 7·01% H, 15·09% N; found: 51·60% C, 6·75% H, 14·88% N. Amino acid analysis: Gly 1·03, Leu 1·01, Pro 1·00, Cys($C_3H_6CO_2H$) 1·01, Asp 1·01, Glu 1·01, Ile 0·90, Phe(*p*-F) 0·98.

b) The protected octapeptide *IVb* (330 mg) was transformed into the active ester in the same manner as described under *a*); m.p. 219—222°C, yield 300 mg (83%). A solution of the active ester in trifluoroacetic acid (10 ml) was kept at room temperature for 1 h, treated with toluene (10 ml) and taken down. The residue was dissolved in dimethylformamide (10 ml), added during 4 h to pyridine (200 ml) and warmed to 50°C with stirring under nitrogen. The mixture was set aside overnight at room temperature, taken down, the residue was triturated with ether, filtered and washed with ether. The product was dissolved in the upper phase (25 ml) of the solvent system 2-butanol–0·05% acetic acid. After 100 transfers of the upper phase a peak of the distribution coefficient 4·00 was isolated (tubes 68—94). Concentration and freeze-drying afforded 94 mg of the product which was purified by chromatography on columns of Bio-gel P—4 (fraction 43—57 ml) and of Bio-gel P—2 (fraction 54—66 ml) and finally precipitated from methanol and ether. This procedure gave 50 mg of a mixture of two diastereoisomeric sulfoxides; R_F 0·29 and 0·23 (S1), 0·29 and 0·16 (S2), 0·68 (S4), 0·45 and 0·39 (S3) (all values refer to thin-layer chromatography on silica gel).

The mixture was separated by chromatography on a column of silica gel (1 × 100 cm, particle size 30—60 μ, eluant 90% 2-butanol–10% water) which afforded fraction A (190 to 247 ml)

and fraction B (290 ml — 390 ml). Precipitation from methanol and ether yielded 13·2 mg of fraction A (*Ig*), m.p. 230—233°C, R_F 0·29 (S1), 0·29 (S2), and 9·7 mg of fraction B (*Ih*), m.p. 172 to 174°C, R_F 0·23 (S1), 0·16 (S2). *Ig*: For $C_{44}H_{66}FN_{11}O_{12}S.4\ H_2O$ (1064) calculated: 49·66% C, 7·01% H, 14·48% N; found: 49·87% C, 6·57% H, 14·36% N. Amino acid analysis: Asp 1·00, Glu 0·99, Pro 1·00, Gly 1·04, Ile 1·00, Leu 1·02, Phe (*p*-F) 1·02, Cys (C_3H_6COOH) 0·69. IR spectrum: 1019 cm^{-1}, 1025 cm^{-1} (doublet), sulfoxide group. *Ih*: For $C_{44}H_{66}FN_{11}O_{12}S.2{\cdot}5\ H_2O$ (1039) calculated: 50·89% C, 6·91% H, 14·84% N; found: 50·82% C, 6·57% H, 14·86% N. Amino acid analysis: Asp 0·99, Glu 1·00, Pro 0·97, Gly 1·05, Cys (C_3H_6COOH) 0·73, Ile 1·00, Leu 1·02, Phe (*p*-F) 1·03. IR spectrum: 1018 cm^{-1}, 1031 cm^{-1} (doublet), sulfoxide group.

Pharmacological Methods

The uterotonic activity was assayed on the isolated rat uterus[19,20], galactogogic activity[21,22] on anaesthetized lactating rats (9—15 days of lactation). Antidiuretic activity was determined using anaesthetized male rats under 6—8% water load[23,24]. The pressor activity was assayed on pithed male rats[25]. The biological tests were performed exclusively with freshly prepared solutions; the stock solutions were used for maximum one day.

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