[7-GLYCINE]DEAMINO-1-CARBA-VASOPRESSIN: SYNTHESIS AND PHARMACOLOGICAL PROPERTIES*

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[8-Arginine]deamino-1-carba-vasopressin and its 7-glycine derivative were prepared by condensation of the amino-terminal, cyclic part of the molecule with carboxy-terminal tripeptide amides. Replacement of proline by glycine in the position 7 results in a substantial decrease in the vasopressin-like activities, the oxytocin-like activities remaining unchanged.

Some time ago we described[1] the synthesis and fundamental pharmacological properties of [8-arginine]deamino-1-carba-vasopressin** (*Ia*). Low yields of some steps and difficulties accompanying the cyclization reaction made a new synthesis of this analogue desirable. Since we planned to prepare other analogues of this type, modified in the carboxy-terminal part of the molecule, we realized condensation of the cyclic part, containing the thioether bridge (*IIb*), with the carboxy-terminal tripeptide-amide.

The synthesis started from S-(γ-methoxycarbonylpropyl)cysteine tert-butyl ester hydrochloride which on reaction with *o*-nitrobenzenesulfenylasparagine 2,4,5-trichlorophenyl ester afforded the dipeptide *III*. After removal of the *o*-nitrobenzenesulfenyl group by treatment with hydrogen chloride[3], the free dipeptide ester was acylated with active ester of *o*-nitrobenzenesulfenylglutamine to give the compound *IV*. In an analogous way, phenylalanine was incorporated into the peptide chain. From the obtained tetrapeptide *Va* the *o*-nitrobenzenesulfenyl group was again removed by hydrogen chloride and the methyl ester was subjected to alkaline hydrolysis. In water the hydrolysis led (after crystallization) to the pure compound *Vb*, whereas in the presence of methanol a mixture was formed which had to be separated by countercurrent distribution[4]. The monoester *Vb* was acylated with N-hydroxysuccinimide ester of *o*-nitrobenzenesulfenyl-O-tert-butyltyrosine and the obtained pentapeptide acid *VI* was transformed into active ester and cyclized in a pyridine solution[5]

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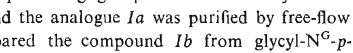
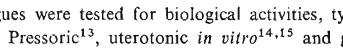
** The amino acids used in this paper are of the L-series. The nomenclature and symbols obey the published recommendations[2].

The cyclopeptide *IIa* was isolated by countercurrent distribution and the protecting groups of the tert-butyl type were cleaved off with trifluoroacetic acid. Since the product *IIb* showed a weakly positive sulfoxide test[6], it was reduced with hydrogen bromide in acetone[7,8] prior further reaction. The cyclopeptide *IIb* was then condensed with prolyl-N^G-*p*-toluenesulfonylarginyl-glycine amide[10] in the presence of dicyclohexylcarbodiimide and N-hydroxybenzotriazole[9] to give the protected peptide *Ic*. The *p*-toluenesulfonyl protecting group was removed by treatment with liquid hydrogen fluoride[11] and the analogue *Ia* was purified by free-flow electrophoresis. Analogously, we prepared the compound *Ib* from glycyl-N^G-*p*-toluenesulfonyl-arginyl-glycine amide[12].

The prepared analogues were tested for biological activities, typical for neurohypophysial hormones. Pressoric[13], uterotonic *in vitro*[14,15] and galactogogic[16,17] activity of *Ia* was identical (within the limits of experimental error) with that described[1] for the compound prepared by stepwise synthesis. Antidiuretic activity, determined by a modified[18] test[19] on conscious rats and based on the half-time of the duration of biological effect, was 1·2–1·4 times higher than the activity of [8-D-arginine]-deamino-vasopressin. The analogue *Ib* showed the following activities: pressoric less than 1 I.U./mg, antidiuretic (determined on anaesthetized rats[20,21]) 47·5 I.U./mg, uterotonic 30 I.U./mg, and galactogogic 50 I.U./mg.



Ia, X = Pro, Y = Arg — *Ib*, X = Gly, Y = Arg
Ic, X = Pro, Y = Arg(Tos) — *Id*, X = Gly, Y = Arg(Tos)



IIa, X = Bu^t, Y = OBu^t — *IIb*, X = H, Y = OH

Nps-Asn-Cys(C_3H_6COOMe)OBu^t

III

Nps-Gln-Asn-Cys(C_3H_6COOMe)OBu^t

IV

X-Phe-Gln-Asn-Cys(C_3H_6COOY)OBu^t

Va, X = Nps, Y = Me — *Vb*, X = H, Y = H

Nps-Tyr(Bu^t)-Phe-Gln-Asn-Cys(C_3H_6COOH)OBu^t

VI

Substitution of proline by glycine in the position 7 thus reduced the vasopressin-like activities whereas the typical oxytocin effects remained unchanged. This phenomenon has been observed already several times in the case of [7-glycine]oxytocin[22–25] and

its derivatives[24-29], however, it was not so pronounced as in the case of compound *Ib*. With analogous vasopressin derivatives[12,30,31] the situation was not clear. It seems therefore probable that a certain conformational change[22], caused by this substitution, is more acceptable for receptors of the oxytocin type than for the kidney or blood vessel receptors. We can thus deduce that replacement of proline by glycine in position 7 in neurohypophysial hormones represents probably a rather universal structural change in designing analogues with desired suppression of vasopressin activities.

We determined also biological activities of protected as well as free carba-analogues without the side chain (*i.e.* compounds *IIa* and *IIb*). Their uterotonic activity was of the same order of magnitude as that of the disulfide compound[32] (deaminopressinoic acid), amounting to 9 mU/mg and 3 mU/mg for the compounds *IIb* and *IIa*, respectively. Pressoric activity was about 2 mU/mg for both compounds and galactogogic activity for the ester *IIa* amounted to 11 mU/mg.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried for at least 24 h at 150 Pa. Thin-layer chromatography was performed on silica gel coated sheets (Silufol, Kavalier, Czechoslovakia) in the following systems: S1 2-butanol–98% formic acid–water (75 : 13·5 : 11·5), S2 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5), S3 1-butanol–acetic acid–water (4 : 1 : 1), S4 pyridine–1-butanol–acetic acid–water (10 : 15 : 3 : 6), S5 n-heptane–tert-butyl alcohol–pyridine (5 : 1 : 1), S6 benzene–ethanol (50 : 1), S7 1-butanol–acetic acid–ethyl acetate–water (1 : 1 : 1 : 1), S8 benzene–methanol (95 : 5). Electrophoresis was carried out on a Whatman paper 3MM (moist chamber, 1 h, 20 V cm^{-1}) in the following buffers: 1M acetic acid (pH 2·4) and pyridine–acetic acid (pH 5·7). The compounds were detected with ninhydrin and/or by the chlorinating 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 (type 6020, Development Workshops, Czechoslovak Academy of Sciences). Reaction mixtures were taken down on a rotatory evaporator under diminished pressure (water pump); dimethylformamide-containing solutions were evaporated using an oil pump (150 Pa). Experiments with liquid hydrogen fluoride were performed in a Toho Kasei Co (Osaka, Japan) instrument. Countercurrent distribution was carried out in an all-glass apparatus (Steady State Distribution Machine, Quickfit & Quartz, Stone, Staffordshire, England) in the system 2-butanol–0·05% aqueous acetic acid. The compounds were located using the Folin–Ciocalteau reaction. Preparative free-flow electrophoresis was performed on a previously described[33,34] apparatus; the peptidic material was detected using the absorption at 280 nm. Optical rotations were measured on a Perkin–Elmer 141 MCA instrument.

S-(γ-Methoxycarbonylpropyl)cysteine Tert-butyl Ester Hydrochloride

Isobutylene (176 ml) was added at —30°C to a suspension of S-(γ-methoxycarbonylpropyl)-cysteine[5] (17·7 g) in tetrahydrofuran (160 ml) and conc. sulfuric acid (32 ml) and the mixture was shaken in a pressure vessel for 10 h at room temperature. After dilution with water (320 ml) the excess isobutylene was removed by extraction with ether, the aqueous solution was adjusted to pH 10 with 4M-NaOH under cooling and the separated product was taken up in ether. The

ethereal solution was dried over magnesium sulfate and the crude hydrochloride of the tert-butyl ester was precipitated with 2M-HCl in ether (40 ml). Crystallization from methanol–ether afforded 12 g (48%) of the product, m.p. 113—115°C, $[\alpha]_D^{20}$ —7·8° (*c* 1, methanol), $[\alpha]_D^{20}$ —3·4° (*c* 0·5, dimethylformamide); R_F 0·52 (S1), 0·62 (S2), 0·51 (S3), 0·70 (S4), 0·50 (S5); $E_{5.7}^{His}$ 0·80, $E_{2.4}^{Gly}$ 1·22. For $C_{12}H_{23}NO_4S$. HCl (313·8) calculated: 45·92% C, 7·71% H, 4·46% N, 10·22% S; found: 45·80% C, 7·56% H, 4·67% N, 10·46% S.

o-Nitrobenzenesulfenylasparaginyl-S-(γ-methoxycarbonylpropyl)cysteine Tert-Butyl Ester (*III*)

A solution of the above-described hydrochloride (15·7 g) in dimethylformamide (30 ml) was cooled and treated with N-ethylpiperidine (7 ml) and 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulfenylasparagine (23·3 g). After standing at room temperature for 24 h, dimethylformamide was evaporated, the residue treated with water and let to solidify at 0°C. The solid portion was dissolved in ethyl acetate and the solution washed with saturated solution of sodium hydrogen carbonate, water, a solution of $KHSO_4/K_2SO_4$ (pH 2) and again water. After drying over magnesium sulfate the solution was taken down and the residue crystallized from aqueous methanol, affording 21·5 g (79%) of the dipeptide *III*, m.p. 111—113°C, $[\alpha]_D^{23}$ —43·4° (*c* 0·5, dimethylformamide), $[\alpha]_D^{23}$ —51·7° (*c* 0·5, methanol); R_F 0·80 (S1), 0·70 (S2), 0·79 (S3), 0·80 (S4), 0·17 (S5). For $C_{22}H_{32}N_4O_8S_2$ (544·6) calculated: 48·52% C, 5·92% H, 10·29% N, 11·77% S; found: 48·72% C, 5·94% H, 9·99% N, 11·49% S.

o-Nitrobenzenesulfenylglutaminyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteine Tert-Butyl Ester (*IV*)

The dipeptide *III* (16·4 g) was dissolved in dimethylformamide (35 ml) and the protecting group was cleaved off by treatment with 7·2M-HCl in ether (8·3 ml). After standing for 4 min at room temperature, the mixture was diluted with ether and the precipitated product collected, ground and dried ($E_{5.7}^{His}$ 0·67, $E_{2.4}^{Gly}$ 1·02). The thus obtained dipeptide hydrochloride was dissolved in dimethylformamide (30 ml) and the solution was adjusted at —5°C to pH 10 (moist indicator paper) with N-ethylpiperidine (4·8 ml). *o*-Nitrobenzenesulfenylglutamine 2,4,5-trichlorophenyl ester (14·4 g) in dimethylformamide (40 ml) was added and the mixture was stirred at room temperature for 40 h. The solvent was evaporated, the residue triturated with ether and washed on the filter with a saturated solution of sodium hydrogen carbonate, water, a solution of $KHSO_4/K_2SO_4$ (pH 2) and again water. Crystallization from methanol afforded 16·1 g (80%) of the product, m.p. 183—185°C, $[\alpha]_D^{21}$ —15·6° (*c* 0·5, dimethylformamide). R_F 0·57 (S1), 0·28 (S2), 0·52 (S3), 0·78 (S4), 0·80 (S6), 0·35 (S7). Amino acid analysis: Glu 1·02, Asp 1·04, Cys($C_4H_7O_2$) 0·94. For $C_{27}H_{40}N_6O_{10}S_2$ (672·8) calculated: 48·20% C, 5·99% H, 12·49% N, 9·53% S; found: 48·00% C, 6·02% H, 12·41% N, 9·37% S.

o-Nitrobenzenesulfenylphenylalanyl-glutaminyl-asparaginyl-S-(γ-methoxycarbonylpropyl)-cysteine Tert-Butyl Ester (*Va*)

A solution of the compound *IV* (13·5 g) in dimethylformamide (35 ml) was treated with ethereal 3·6M-HCl (11 ml) and the mixture was kept at room temperature for 4 h. After trituration with ether, the powdery hydrochloride was collected on filter and dried ($E_{5.7}^{His}$ 0·55, $E_{2.4}^{Gly}$ 0·83). This tripeptide hydrochloride was dissolved in dimethylformamide (40 ml) and the cooled solution was adjusted to pH 10 with N-ethylpiperidine (3·5 ml). After addition of a solution of *o*-nitrobenzenesulfenylphenylalanine 2,4,5-trichlorophenyl ester (10 g) in dimethylformamide (15 ml), the mixture was stirred for 40 h at room temperature. The solvent was evaporated, the residue

triturated with ether and washed on the filter with a saturated solution of sodium hydrogen carbonate, water, a solution of $KHSO_4/K_2SO_4$ (pH 2) and again water. The product *Va* was obtained on crystallization from dimethylformamide–water, m.p. 195—197°C; yield 13 g (79%); $[\alpha]_D^{20}$ +5·1° (*c* 0·6, dimethylformamide). R_F 0·75 (S1), 0·62 (S2), 0·73 (S3), 0·65 (S4), 0·62 (S8). For $C_{36}H_{49}N_7O_{11}S_2 . 0.5\ H_2O$ (829·0) calculated: 52·16% C, 6·08% H, 11·83% N, 7·74% S; found: 52·26% C, 6·30% H, 11·58% N, 8·00% S.

Phenylalanyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)cysteine Tert-Butyl Ester (*Vb*)

The protected tetrapeptide *Va* (8·22 g) was suspended in dimethylformamide (45 ml) and treated with ethereal 5M-HCl (4 ml; 4 min, room temperature) in order to remove the *o*-nitrobenzenesulfenyl group. The tetrapeptide hydrochloride was precipitated with ether, collected and dried ($E_{5.7}^{His}$ 0·45, $E_{2.4}^{Gly}$ 0·73).

A) A suspension of the tetrapeptide hydrochloride (7 g) in methanol (80 ml) was adjusted to pH 11 with 1M-NaOH under stirring and cooling below 5°C and stirred at this temperature for 30 min. After dilution with water (180 ml) the methanol was evaporated at room temperature and the aqueous solution applied on a column of Dowex 50 (H^+; 500 ml). The column was washed until the washings were chloride-negative and then the product was eluted with 20% aqueous pyridine. The effluents were freeze-dried and the residue reprecipitated from 90% aqueous methanol by addition of ether. The precipitate contained, in addition to the desired product, the diacid and the unreacted starting diester. These impurities resisted to the attempted chromatographic separation on Aberlite IR-4B in 50% aqueous dimethylformamide. The mixture (500 mg) was separated by countercurrent distribution (212 transfers of the upper phase and 14 transfers of the lower phase). The desired tert-butyl ester was present in the tubes 46–80 (K = = 0·46). The content of these tubes was concentrated and freeze-dried, affording 250 mg (53%) of material, $[\alpha]_D^{20}$ −48·4° (*c* 0·28, dimethylformamide); R_F 0·30 (S1), 0·23 (S3), 0·61 (S4), 0·62 (S6). For $C_{29}H_{44}N_6O_9S . CH_3COOH . H_2O$ (730·8) calculated: 50·95% C, 6·90% H, 11·50% N, 4·39% S; found: 50·68% C, 6·86% H, 11·77% N, 4·25% S. Amino acid analysis: Asp 0·99, Glu 0·99, Phe 1·01, Cys($C_4H_7O_2$) 1·01.

B) A solution of the tetrapeptide hydrochloride (0·7 g) in water (50 ml) was adjusted to pH 12 with 1M-NaOH under cooling and kept in a cold place for 10 min. The cooled mixture was made neutral with 1M-HCl and applied on a column of Dowex 50. After washing the column with water, the product was eluted with aqueous pyridine, the eluate freeze-dried and the residue reprecipitated from 90% aqueous methanol, affording 400 mg (60%) of material of the same properties as described under *A*).

o-Nitrobenzenesulfenyl-O-tert-butyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)cysteine Tert-Butyl Ester (*VI*)

A solution of the tetrapeptide *Vb* (1 g) in dimethylformamide (25 ml) was adjusted to pH 10 with N-ethylpiperidine (0·35 ml) and treated under stirring with *o*-nitrobenzenesulfenyl-O-tert-butyltyrosine N-hydroxysuccinimide ester (0·74 g). After 24 h a further amount of the active ester (0·37 g in 5 ml of dimethylformamide) was added and the same amount was added again after 24 h. After 24 h the mixture was taken down and the residue triturated with a solution of citric acid, water and ethyl acetate. Crystallization from aqueous methanol afforded 1·1 g (71%) of the peptapeptide tert-butyl ester *VI*, m.p. 188—190°C, $[\alpha]_D^{21}$ +41·8° (*c* 0·5, dimethylformamide); R_F 0·70 (S1), 0·69 (S3), 0·64 (S4); $E_{5.7}^{His}$ 0·34, $E_{2.4}^{Gly}$ 0·70 (the electrophoresis was carried out after removal of the *o*-nitrobenzenesulfenyl group). For $C_{48}H_{64}N_8O_{13}S_2 . H_2O$ (1043) calculated: 55·26% C, 6·37% H, 10·76% N, 6·14% S; found: 55·21% C, 6·30% H, 10·54% N, 6·17% S.

Lactam of O-Tert-butyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)-cysteine Tert-Butyl Ester (*IIa*)

Bis(*p*-nitrophenyl) sulfite (5 g) was added to a solution of the pentapeptide *VI* (1·5 g) in pyridine (100 ml), the mixture was stirred under nitrogen for 9 h and again treated with bis(*p*-nitrophenyl) sulfite (3 g). After 15 h the last portion of bis(*p*-nitrophenyl) sulfite was added and the stirring was continued for 5 h. The solvent was evaporated, the residue triturated with ether and washed on the filter with water and ether, affording 1·1 g of the active ester. This ester (1 g) was dissolved in dimethylformamide (7 ml) and the *o*-nitrobenzenesulfenyl group was removed in the usual manner (1 ml of 3·3M-HCl in ether, 4 min, room temperature). Hydrochloride of the active ester was obtained on addition of ether. A solution of this hydrochloride in dimethylformamide (20 ml) was added under nitrogen at the rate 2·7 ml/h into pyridine (600 ml), containing N-ethylpiperidine (0·14 ml), at 50°C with constant stirring. After stirring for 4 h at 50°C, the mixture was stirred 12 h at room temperature, taken down and the residue triturated with ether. The crude peptide was purified by countercurrent distribution (100 transfers of the upper phase). The desired product was contained in tubes 80—100 ($K = 14$). After pooling, the solution was concentrated and freeze-dried, affording 250 mg (22%) of the pure compound, $[\alpha]_D^{20}$ —87·6° (*c* 0·5, dimethylformamide); R_F 0·79 (S1), 0·73 (S3), 0·85 (S4). For $C_{42}H_{59}N_7O_{10}S \cdot H_2O$ (872·1) calculated: 57·85% C, 7·05% H, 11·24% N; found: 57·77% C, 6·91% H, 11·01% N.

Lactam of Tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-(γ-carboxypropyl)cysteine (*IIb*)

The compound *IIa* (250 mg) was dissolved in trifluoroacetic acid (4 ml) and set aside for 1 h at room temperature. Trifluoroacetic acid was coevaporated with toluene (6 ml), the residue triturated with ether, mixed with acetone (4 ml) and 4M-HBr in acetic acid (8 ml) and allowed to stand for 5 min at room temperature. Acetone was evaporated and acetic acid removed by freeze-drying. Repeated freeze-drying from aqueous acetic acid afforded 200 mg (85%) of material, $[\alpha]_D^{21}$ —16·6° (*c* 0·1, acetic acid), $[\alpha]_D^{20}$ —15·1° (*c* 0·5, dimethylformamide); R_F 0·68 (S1), 0·64 (S3), 0·63 (S4). For $C_{34}H_{43}N_7O_{10}S \cdot C_2H_4O_2 \cdot H_2O$ (819·9) calculated: 52·74% C, 5·90% H, 11·95% N, 3·91% S; found: 52·55% C, 5·95% H, 11·73% N, 3·72% S. Amino acid analysis: Tyr 0·99, Phe 1·06, Glu 0·97, Asp 1·00, Cys($C_4H_7O_2$) 0·97.

[8-N^G-*p*-Toluenesulfonylarginine]deamino-1-carba-vasopressin (*Ic*)

A solution of prolyl-N^G-*p*-toluenesulfonylarginyl-glycine amide[10] (prepared from 93 mg of the benzyloxycarbonyl derivative) in dimethylformamide (1 ml) was added to a solution of the compound *IIb* (50 mg) and N-hydroxybenzotriazole (18 mg) in dimethylformamide (0·5 ml). After cooling to 0°C, dicyclohexylcarbodiimide (15 mg) was added and the reaction mixture was stirred for 1 h at 0°C and for 24 h at room temperature. Dicyclohexylurea was filtered off, the filtrate taken down, the residue triturated with water and washed on the filter successively with 2% HCl, water, saturated solution of sodium hydrogen carbonate, water and ether. Crystallization from methanol–ether afforded 36·6 mg (49%) of the protected peptide, m.p. 175—177°C, $[\alpha]_D^{21}$ —18·3° (*c* 0·4, dimethylformamide); R_F 0·53 (S1), 0·53 (S3), 0·68 (S4). For $C_{54}H_{72}N_{14}O_{14}S_2 \cdot H_2O$ (1223) calculated: 53·02% C, 6·10% H, 16·03% N, 5·24% S; found: 52·86% C, 6·22% H, 15·93% N, 5·04% S. Amino acid analysis: Tyr 0·99, Phe 1·02, Glu 1·02, Asp 1·02, Cys($C_4H_7O_2$) 0·90, Pro 0·97, Arg 0·97, Gly 1·01.

Deamino-1-carba-vasopressin (*Ia*)

Hydrogen fluoride (7 ml) was distilled to the compound *Ic* (30 mg) and anisole (0·07 ml) and the mixture was stirred at 0°C for 30 min. Hydrogen fluoride was evaporated and the residue dissolved in water (20 ml). The aqueous solution was extracted with ether and filtered through a column of Amberlite IR-4B (acetate form). The effluents were freeze-dried and the crude analogue (25 mg) dissolved in 0·5M acetic acid (2·5 ml) and purified by free-flow electrophoresis ($U = 2700$ V, $I = 99$ mA). The product-containing fractions were freeze-dried, affording 10 mg of the product, $[\alpha]_D^{22}$ —52·1° (*c* 0·2, 1M acetic acid); R_F 0·16 (S1), 0·59 (S4), $E_{2,4}^{Gly}$ 0·60, $E_{5,7}^{His}$ 0·35. Amino acid analysis: Tyr 1·04, Phe 1·10, Glu 0·96, Asp 1·00, Cys($C_4H_7O_2$) 0·97, Pro 0·96, Arg 1·06, Gly 0·90. For $C_{47}H_{66}N_{14}O_{12}S \cdot 3\,C_2H_4O_2 \cdot 2\,H_2O$ (1267) calculated: 50·23% C, 6·52% H, 15·47% N; found: 50·15% C, 6·46% H, 15·25% N.

[7-Glycine, 8-N^G-*p*-toluenesulfonylarginine]deamino-1-carba-vasopressin (*Id*)

A solution of glycyl-N^G-*p*-toluenesulfonylarginyl-glycine amide[12] (59 mg) in dimethylformamide (0·5 ml) was added to a solution of the compound *IIb* (50 mg) and N-hydroxybenzotriazole (18 mg) in dimethylformamide (0·5 ml). After cooling to 0°C, dicyclohexylcarbodiimide (14·2 mg) was added and the mixture was stirred for 1 h at 0°C and for 24 h at room temperature. The formed dicyclohexylurea was removed by filtration, dimethylformamide was evaporated and 0·1M-HCl was poured on the residue. The product solidified on standing for 30 min at 0°C. After grinding, it was washed on the filter with water, saturated solution of sodium hydrogen carbonate, again water and ether. Crystallization from aqueous dimethylformamide afforded 36·8 mg (48%) of the product, m.p. 169—171°C, $[\alpha]_D$ —19·8° (*c* 0·5, dimethylformamide); R_F 0·20 (S3), 0·72 (S4), 0·54 (S7). Amino acid analysis: Arg 0·98, Gly 2·17, Pro 0·92, Cys($C_4H_7O_2$) 0·74, Glu 0·95, Asp 0·97, Phe 0·92, Tyr 1·00. For $C_{51}H_{69}N_{14}O_{14}S_2 \cdot H_2O$ (1184) calculated: 51·77% C, 5·96% H, 16·57% N, 5·42% S; found: 51·53% C, 5·97% H, 16·29% N, 5·26% S.

[7-Glycine]deamino-1-carba-vasopressin (*Ib*)

Hydrogen fluoride (7 ml) was distilled to the compound *Id* (36 mg) and anisole (0·6 ml), the mixture was stirred for 30 min at 0°C, hydrogen fluoride was evaporated and the residue dissolved in water (20 ml). The aqueous solution was extracted with ether and passed through a column of Amberlite IR-4B (acetate form). The effluents were freeze-dried and the crude analogue purified by free-flow electrophoresis ($U = 2700$ V, $I = 100$ mA). Fractions, containing the pure compound, were freeze-dried, affording 8 mg of the analogue *Ib*, $[\alpha]_D^{20}$ —57·2° (*c* 0·2, 1M acetic acid); $E_{5,7}^{His}$ 0·35, $E_{2,4}^{Gly}$ 0·62. For $C_{44}H_{52}N_{14}O_{12}S \cdot 2\,C_2H_4O_2 \cdot 3\,H_2O$ (1185) calculated: 48·64% C, 6·46% H, 16·54% N; found: 48·37% C, 6·32% H, 16·28% N. Amino acid analysis: Tyr 0·97, Phe 1·06, Glu 1·00, Asp 1·03, Cys($C_4H_7O_2$) 0·96, Arg 0·90, Gly 1·96.

Pharmacological Methods

Uterotonic activity was determined on rat uterine strips[14,15], placed in a Mg^{2+}-free medium. Galactogogic activity[16,17] was assayed using lactating rats (5—10 days after parturition). The pressor activity[13] was determined on despinalized Wistar-Konárovice strain rats. Antidiuretic activity was estimated either on ethanol anaesthetized rats[20,21] (water load of 6—8% of body weight) or on conscious rats[18,19] in which case it was based on the half-time of duration of the biological effect. The concentration of the solutions used for the biological tests was determined spectroscopically (at 280 nm; N-acetyltyrosine amide taken as standard).

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