SYNTHESIS AND SOME PHARMACOLOGICAL PROPERTIES OF [8-L-ARGININE]DEAMINO-1-CARBA-VASOPRESSIN*

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By means of stepwise synthesis of peptides in solution and cyclisation of the linear octapeptide using active esters, an analogue of deamino-vasopressin, [8-L-arginine]deamino-1-carba-vasopressin was prepared, in which the disulphide group has been substituted by a CH_2—S group. Some basic pharmacological activities were determined; pressor and particularly antidiuretic potencies were very high.

In the case of oxytocin it has already been shown[1-4] that replacement of the disulphide bond by S—CH_2 or CH_2—S results in almost all cases in a cyclic nonapeptide with increased and prolonged biological activity. When the disulphide bridge is replaced by CH_2—CH_2, biological activity which is prolonged also remains, but for most of these activities potency is far less than that of the parent substance. A similar situation has been reported for vasopressin (*Ia*): replacement of S—S by S—CH_2 resulted in analogues with high levels of biological activities, reflecting other structural features[5]. Again, complete substitution by CH_2—CH_2 resulted[6] in analogues with prolonged activities but loss of potency, with the single exception of antidiuresis[7] where activity was both prolonged and high. Since in the case of oxytocin there was some degree of dissociation of biological activities between the 1- and 6-monocarba forms, it was considered to be useful to complete the series for vasopressin as well. The present communication describes the synthesis and some pharmacological properties of deamino-arginine-vasopressin with the S—S bridge replaced by CH_2—S (*i.e.* a 1-monocarba analogue, *Ib*).**

Synthesis of compound *Ib* followed procedures reported elsewhere for analogues of this type[3-7]. The peptide chain was prolonged in stepwise fashion[9] using active esters and amino-protecting groups easily removed in acid medium. Cyclisation of the linear octapeptide was again by means of active esters[10]. The *p*-toluenesulphonyl

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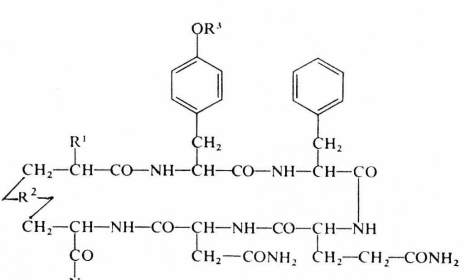
** Amino acids used in this work were all in the L-configuration. Nomenclature and symbols are according to published suggestions[8].

protecting group on the guanidine group of arginine was removed in the last step by means of liquid hydrogen fluoride[11].

The amide of prolyl-N^G-*p*-toluenesulphonylarginyl-glycine[12] was condensed with the 5-chloro-8-quinolyl ester of N-benzyloxycarbonyl-S-γ-methoxycarbonylpropyl-cysteine[3]. The benzyloxycarbonyl group was split from the resulting tetrapeptide *II* by means of hydrogen bromide in acetic acid and the peptide chain was prolonged by residues of asparagine, glutamine and phenylalanine by consecutive acylation of the 2,4,5-trichlorophenyl esters of the corresponding *o*-nitrobenzenesulphenylamino acids. This gave rise consecutively to substances *III*, *IV* and *Va*, from which the *o*-nitrobenzenesulphenyl protecting groups were removed with hydrogen chloride; the hydrochloride of the ester of the heptapeptide amide was subject to alkaline hydrolysis and substance *Vb* was purified by countercurrent distribution. By reaction with the N-hydroxysuccinimide ester of N-tert-butyloxycarbonyl-O-tert-butyltyrosine we achieved protected octapeptide acid *VIa*, which was converted to the active ester by the action of bis-(*p*-nitrophenyl) sulphite, both tert-butyl protecting groups were removed by trifluoroacetic acid and a dimethylformamide solution of the salt of the active ester of the peptide was slowly added to pyridine. After evaporation the mixture was purified by means of countercurrent distribution: a ninhydrin-positive product of non-cyclic nature with a partition coefficient $K = 0{\cdot}92$ was obtained in 96% yield.

Therefore the heptapeptide *Vb* was acylated by the N-hydroxysuccinimide ester of *o*-nitrobenzenesulphenyl-O-tert-butyltyrosine, the octapeptide *VIb* was converted to an active ester, the *o*-nitrobenzenesulphenyl protecting group was removed with hydrogen chloride in ether and cyclisation was carried out by slow addition of the active ester in solution to pyridine. Product *Ic* was again isolated by countercurrent distribution (a substantially higher yield was obtained using the 2,4,5-trichlorophenyl ester than with the *p*-nitrophenyl ester), the O-tert-butyl and *p*-toluenesulphonyl protecting groups were removed by liquid hydrogen fluoride and [8-arginine]-deamino-1-carba-vasopressin (*Ib*) was isolated and purified by continuous free flow electrophoresis and gel filtration.

Biological activities typical for neurohypophysial hormones were determined for analogue *Ib*. Pressor activity determined on despinalised rats showed a mean value of 550 I.U./mg. This means that in comparison with [8-L-arginine]deamino-6-carba-vasopressin[5] (*Id*) there was a 100% increment in potency. This level is higher than in the corresponding disulphide prototypes (substance *Ia* and the deamino variants of same). In this assay we did not observe prolongation of action either for *Ib* (index of persistence[13] 0·9) or *Id* (index of persistence 1·16). In measurements of regional haemodynamics[14,15] analogue *Ib* behaved in a manner similar to *Id* in terms of effects on blood flows to separate organs, qualitatively and quantitatively[15], *i.e.*, it produced a decrease in blood flow to the gastrointestinal tract and uterus with minimal cardiac and systemic effects. These actions, analogously as for *Id*, showed a prolonged time course.



Ia, $R^1 = NH_2$, $R^2 = S—S$, $R^3 = H$, $R^4 = H$
Ib, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = H$, $R^4 = H$
Ic, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = t\text{-Bu}$, $R^4 = Tos$
Id, $R^1 = H$, $R^2 = S—CH_2$, $R^3 = H$, $R^4 = H$

Z-Cys($C_3H_6CO_2Me$)-Pro-Arg(Tos)-Gly-NH_2

II

Nps-Asn-Cys($C_3H_6CO_2Me$)-Pro-Arg(Tos)-Gly-NH_2

III

Nps-Gln-Asn-Cys($C_3H_6CO_2Me$)-Pro-Arg(Tos)-Gly-NH_2

IV

R^1-Phe-Gln-Asn-Cys($C_3H_6CO_2R^2$)-Pro-Arg(Tos)-Gly-NH_2

Va, $R^1 = Nps$, $R^2 = Me$
Vb, $R^1 = H$, $R^2 = H$

R-Tyr(Bu^t)-Phe-Gln-Asn-Cys($C_3H_6CO_2H$)-Pro-Arg(Tos)-Gly-NH_2

VIa, R = Boc, *VIb*, R = Nps

In anaesthetised rats, analogue *Ib* was antidiuretically active at dosages 1 to 1·5 orders of ten lower than analogue *Id*. Antidiuretic activity calculated from threshold doses[16] indicates that analogue *Ib* is one of the most potent substances known. Since *Ib* does not have D-amino acid residue at sequence positions 8, this high activity must

be attributed only to absence of α-amino group and suitable modification of the disulphide bridge. These changes must involve not only increased metabolic stability of the analogue due to the structural alterations, but also a very high affinity of *Ib* for renal tubular receptors, similarly as it was observed for *Id* and [8-L-ornithine]-deamino-6-carba-vasopressin[17,18]. Both analogues *Ib* and *Id* show markedly prolonged antidiuretic actions.

The uterotonic activity of *Ib* determined on isolated rat uterus strips was 34 I.U./mg (about half of that of *Id*) and the galactogogic activity was 65 I.U./mg (twice that of *Id*). The action on the rat uterus *in situ* was protracted and amounted to 90 I.U./mg.

EXPERIMENTAL

Melting points were determined on a Kofler block and were corrected. Samples for elemental analysis were dried over phosphorus pentoxide at 1 Torr and room temperature for 12 h or more. Thin-layer chromatography was carried out on silica gel plates (Kieselgel G, Merck) in the solvent systems 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S_1), 2-butanol–90% aqueous formic acid–water (75 : 13·5 : 11·5) (S_2) and 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6 (S3). Electrophoresis was carried out on Whatman paper No 3 MM for 1 h at a potential drop of 20 V cm^{-1} in buffer solutions 1M acetic acid (pH 2·4) and pyridinium acetate (pH 5·7). Spots were detected by ninhydrin or by chlorination. Samples for amino-acid analysis were hydrolysed for 20 h at 105°C in 6M-HCl (in ampoules sealed at 1 Torr) and analyses were carried out on an automatic analyser (Developmental Workshops, Czechoslovak Academy of Sciences, type 6020). Solutions were evaporated on a rotatory evaporator at a bath temperature of 30—35°C under reduced pressure (water pump; with mixtures containing dimethylformamide an oil vacuum pump).

The all-glass, fully automatic Steady State Distribution Machine produced by Quickfit & Quartz, Ltd., Stafordshire, England, with possibilities of transfers of both upper and lower phases, was used for countercurrent distribution. The solvent system was 2-butanol–0·05% acetic acid (1 : 1) and the peptide material was located by the Folin–Ciocalteau reaction (samples removed every other tube). Continuous free-flow electrophoresis was carried out on an instrument described elsewhere[19,20] (conditions: 0·5M acetic acid, 4°C, 2·6 kV across the electrodes). The peptide material was located by measurement of optical density at 280 nm. For gel filtration we used Bio-gel P-4 (Bio-Rad Laboratories, Richmond, California, USA) in 1M acetic acid; the peptide material was detected by absorption at 280 nm.

N-Benzyloxycarbonyl-S-γ-methoxycarbonylpropylcysteinyl-prolyl-N^G-*p*-toluenesulphonyl-arginyl-glycine Amide (*II*)

To a solution of prolyl-N^G-*p*-toluenesulphonylarginyl-glycine amide[2] (1·45 g) in dimethylformamide (15 ml) we added the 5-chloro-8-quinolyl ester of benzyloxycarbonyl-S-γ-methoxycarbonylpropylcysteine[3] (1·6 g). After 2 days of stirring the reaction mixture was evaporated and the residue dissolved in chloroform (50 ml). The latter solution was sequentially extracted with 0·2M-H_2SO_4, water, 0·5M-$NaHCO_3$ and water and the emulsion was separated by centrifugation. After drying over sodium sulphate the solution was evaporated to dryness, dissolved in 2-propanol, cooled to −40°C (the product must separate out as a suspension; if an oil forms the mixture must be heated to redissolve and solvent volume must be increased) and to this sus-

pension ether was added. The product was filtered off and washed on the filter with ether and then dried. The yield was 1·26 g (51%) of a product with m.p. 87—89°C; $[\alpha]_D^{25}$ —38·3° (*c* 0·5; dimethylformamide). For $C_{36}H_{50}N_8O_{10}S_2.H_2O$ (837·0) calculated: 51·70% C, 6·27% H, 13·40% N; found: 51·65% C, 6·21% H, 13·35% N.

o-Nitrobenzenesulphenylasparaginyl-S-γ-methoxycarbonylpropylcysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*III*)

To a solution of protected tetrapeptide *II* (3·07 g) in acetic acid (15 ml) we added 35% HBr in acetic acid (15 ml). After 15 min at room temperature the mixture was diluted with ether, the hydrobromide which separated out was triturated with ether, filtered off, washed with ether and dried. $E_{2.4}^{Gly}$ 0·74, $E_{5.7}^{His}$ 0·47. To the solution of this product in dimethylformamide (25 ml), neutralised with N-ethylpiperidine (moist pH paper; pH 8—9) we added 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenylasparagine (1·8 g). After 24 h stirring at room temperature a further portion of active ester was added (0·8 g) and after 24 h the mixture was evaporated, the residue was triturated with light petroleum and ether, the solid product was filtered out and washed with ether. After precipitation from methanol with ether in the same way as in the case of the compound *II*, 2·2 g (16%) of a product with m.p. 110—115°C (softening from 105°C) was obtained. Sample for analysis was precipitated from methanol with ether without a change in the melting point, $[\alpha]_D^{25}$ —48·2° (*c* 0·5; dimethylformamide). For $C_{38}H_{53}N_{11}O_{12}S_3.1{\cdot}5\ H_2O$ (979·1) calculated: 46·60% C, 5·78% H, 15·73% N; found: 46·60% C, 5·74% H, 15·64% N.

o-Nitrobenzenesulphenylglutaminyl-asparaginyl-S-γ-methoxycarbonylpropylcysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*IV*)

To a solution of protected pentapeptide *III* (2·13 g) in dimethylformamide (10 ml) we added 2M-HCl in ether (3·5 ml). After 5 min at room temperature the reaction mixture was diluted with ether, the oil which separated out was triturated with ether, the solid portion filtered off, washed on the filter with ether and dried. $E_{2.4}^{Gly}$ 0·68; $E_{5.7}^{His}$ 0·41. The pentapeptide hydrochloride was dissolved in dimethylformamide (30 ml) and to this solution we added N-ethylpiperidine (pH 8·0—9·0; moist indicator paper) and the 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenylglutamine (1·1 g). After 24 h stirring at room temperature we added a further portion of the active ester (0·55 g) and after a further 24 h the reaction mixture was evaporated to dryness, the remnant was triturated with light petroleum and ether and then precipitated twice from methanol and ether in the same manner as described for product *II*. The yield was 1·95 g (81%) of a product of m.p. 115—120°C (with softening from 110°C); $[\alpha]_D^{25}$ —37·7° (*c* 0·2; dimethylformamide). For $C_{43}H_{61}N_{13}O_{14}S_3.1{\cdot}5\ H_2O$ (1 107) calculated: 46·60% C, 5·82% H, 16·45% N; found: 46·44% C, 5·67% H, 16·27% N.

o-Nitrobenzenesulphenylphenylalanyl-glutaminyl-asparaginyl-S-γ-methoxycarbonylpropyl-cysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*Va*)

From protected hexapeptide *IV* (1·82 g) we split off the *o*-nitrobenzenesulphenyl protecting group in the usual manner (6 ml dimethylformamide; 2·7 ml 2M-HCl in ether; $E_{2.4}^{Gly}$ 0·63; $E_{5.7}^{His}$ 0·38). The hexapeptide hydrochloride was dissolved in dimethylformamide (20 ml) and to this solution we added N-ethylpiperidine (pH 8·0—9·0) and the 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenylphenylalanine (0·9 g). After 24 h stirring at room temperature a further portion of active ester (0·4 g) was added to the reaction mixture and after a further 24 h the mixture was evaporated to dryness, the remainder triturated sequentially with light petroleum and ether, the crystalline

portion filtered off and washed with ether on the filter. The product was recrystallised from methanol (the same volume of ether was added to the crystalline product), filtered and washed with ether. The yield was 1·57 g (76%) of a product of m.p. 162—167°C; $[\alpha]_D^{25}$ −18·5° (*c* 0·2; dimethylformamide). For $C_{52}H_{70}N_{14}O_{15}S_3.2\,H_2O$ (1263) calculated: 49·45% C, 5·91% H, 15·53% N; found: 49·44% C, 5·75% H, 15·44% N.

Phenylalanyl-glutaminyl-asparaginyl-S-γ-carboxypropylcysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*Vb*)

The *o*-nitrobenzenesulphenyl protecting group was split off from protected heptapeptide *Va* (1·09 g) in the usual manner (10 ml dimethylformamide; 1·5 ml 2M-HCl in ether; $E_{2.4}^{Gly}$ 0·54; $E_{5.7}^{His}$ 0·27). The heptapeptide hydrochloride was dissolved in a mixture of methanol (10 ml) and 1M-NaOH (4 ml). After 1 h at room temperature the methanol was evaporated off and the aqueous solution was filtered through a column of Dowex 50 (25 ml; H^+-cycle). The column was washed through with water and the product eluted with 10% pyridine. The pH of the latter was lowered to 6·0 with acetic acid, concentrated and freeze-dried. The powder was recrystallised from a mixture of methanol and ether. The product yield was 0·55 g (58%), m.p. 143—145°C.

The product was purified by countercurrent distribution (445 upper phase transfers and 79 lower). The contents of tubes 15—50 ($K = 0{\cdot}27$) were pooled, concentrated to a small volume and freeze-dried. After recrystallisation from methanol and ether the yield was 0·245 g (26%) of a product of m.p. 145—152°C; $[\alpha]_D^{25}$ +37·1° (*c* 0·2; dimethylformamide); $E_{2.4}^{Gly}$ 0·66; $E_{5.7}^{His}$ 0·12. Amino-acid analysis: Arg 1·00, Asp 1·01, Glu 1·03, Pro 0·98, Gly 1·01, Cys ($C_3H_6CO_2H$) 1·03, Phe 0·97. For $C_{45}H_{65}N_{13}O_{13}S_2.3\,H_2O$ (1114) calculated: 48·50% C, 6·42% H, 16·32% N; found: 48·38% C, 6·21% H, 16·02% N.

Tert-butyloxycarbonyl-O-tert-butyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-γ-carboxypropylcysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*VIa*)

To a solution of free heptapeptide amide *Vb* (0·22 g) in dimethylformamide (3 ml) and N-ethylpiperidine (0·1 ml) we added the N-hydroxysuccinimide ester of tert-butyloxycarbonyl-O-tert-butyltyrosine (0·1 g). After 24 h stirring at room temperature we added a further portion of active ester (0·04 g) and after a further 24 h the reaction mixture was diluted with ether, the product was triturated and filtered off. The product was sequentially washed on the filter with ether, water, 0·5M-H_2SO_4 and water. The yield was 0·25 g (87%) of a product with m.p. 173—176°C; $[\alpha]_D^{25}$ −29·6° (*c* 0·34; dimethylformamide); $E_{2.4}^{Gly}$ 0·55; $E_{5.7}^{His}$ 0·18 (after removal of the tert-butyloxycarbonyl and O-tert-butyl groups with trifluoroacetic acid). For $C_{63}H_{90}N_{14}O_{17}S_2.3\,H_2O$ (1433) calculated: 52·75% C, 6·75% H, 13·67% N; found: 52·73% C, 6·32% H, 13·58% N.

o-Nitrobenzenesulphenyl-O-tert-butyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-γ-carboxypropylcysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*VIb*)

To a solution of free heptapeptide-amide *Vb* (0·4 g) in dimethylformamide (5 ml) and N-ethylpiperidine (0·2 ml) we added the N-hydroxysuccinimide ester of *o*-nitrobenzenesulphenyl-O-tert-butyltyrosine[6] (0·2 g). After 24 h stirring at room temperature we added a further portion of active ester (0·08 g) and after a further 24 h the reaction mixture was evaporated to dryness. The remainder was triturated sequentially with light petroleum, ether and 0·5M-H_2SO_4. The product was filtered off and washed with water and dried. The yield was 0·513 g (94%) of a product with m.p. 170—173°C; $[\alpha]_D^{25}$ +7·7° (*c* 0·2; dimethylformamide); $E_{2.4}^{Gly}$ 0·55; $E_{5.7}^{His}$ 0·08 (after

removal of the *o*-nitrobenzenesulphenyl group). For $C_{64}H_{85}N_{15}O_{17}S_3.0{\cdot}5\ H_2O$ (1442) calculated: 53·30% C, 6·01% H, 14·57% N; found: 53·44% C, 6·08% H, 14·12% N.

Bis(2,4,5-trichlorophenyl) Sulphite

This compound was prepared in a manner similar to that for bis(*p*-nitrophenyl) sulphite[21]. M.p. 98—100°C; R_F 0·76 (benzene). For $C_{12}H_4O_3Cl_6S$ (440·9) calculated 32·70% C, 0·91% H, 48·25% Cl, 7·27% S; found: 32·73% C, 1·23% H, 48·11% Cl, 7·32% S.

Lactam of O-Tert-butyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-γ-carboxypropyl-cysteinyl-prolyl-N^G-*p*-toluenesulphonylarginyl-glycine Amide (*Ic*)

Preparation and cyclisation of the active ester were carried out in a nitrogen atmosphere. To a solution of protected octapeptide *VIb* (0·12 g) in a mixture of dimethylformamide (10 ml) and pyridine (10 ml) we added bis (2,4,5-trichlorophenyl) sulphite (1 g). The reaction mixture was stirred 6 h at room temperature, diluted with pyridine (5 ml) and a further portion (1 g) of reagent added; after 16 h stirring we added a further portion of bis(2,4,5-trichlorophenyl) sulphite (0·5 g) and after a further 5 h the mixture was evaporated to dryness. The oily remainder was sequentiatially triturated with ether and water, the crystalline portion filtered off and washed with water and ether and then dried. The yield was 0·132 g (98%).

The product was dissolved in dimethylformamide (4 ml) and we added 2M-HCl in ether (0·1 ml). After 5 min at room temperature the reaction mixture was diluted with ether and the hydrochloride which separated out was triturated, filtered off, washed with ether and dried over NaOH, then dissolved in dimethylformamide (10 ml). This solution was added over 4 h to 10 ml pyridine and 50 μl N-ethylpiperidine with stirring, nitrogen bubbling and heating to 50°C. After 24 h stirring at room temperature under nitrogen the reaction mixture was evaporated to dryness, the remainder was triturated with ether, filtered and washed with ether. The dried product was purified by countercurrent distribution (100 upper phase transfers). The peak with a partition coefficient of 6·1 (tubes 75—97) was isolated and freeze-dried. After recrystallisation from methanol and ether the yield was 0·065 g (62%), m.p. 175—178°C; R_F 0·02 (S_1), 0·29 (S_2) 0·80 (S_3). On paper electrophoresis (pH 2·4 and 5·7) there was no mobility. For $C_{58}H_{80}N_{14}O_{14}S_2.5\ H_2O$ (1352) calculated: 51·55% C, 6·71% H, 14·51% N; found: 51·79% C, 6·11% H, 14·45% N.

If we used bis(*p*-nitrophenyl) sulphite to prepare the active ester, the yield of *Ic* after countercurrent distribution and recrystallisation from methanol and ether was only 8%.

Lactam of Tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-γ-carboxypropylcysteinyl-prolyl--arginyl-glycine Amide (*Ib*)

Protected cyclic octapeptide *Ic* (68·4 mg) was reduced in liquid HF (5 ml) in the Toho Kasei Co. (Osaka) apparatus for non-aqueous HF at 0°C for 30 min. Hydrogen fluoride was evaporated off *in vacuo* (water pump) and the remainder was dried 2 h *in vacuo* (oil pump). After solution in 1M-acetic acid (20 ml) and several extractions with ethyl acetate, the product was freeze-dried. The powder was dissolved in water (3 ml) and filtered through a column of Amberlite IR-4B (2·5 ml; acetate cycle, pH 2·5). The eluate, after freeze-drying, was purified by continuous free-flow electrophoresis and gel filtration (P-4 Biogel, 1M-acetic acid). The yield was 23·4 mg (41%); $E^{Gly}_{2.4}$ 0·63; $E^{His}_{5.5}$ 0·35; R_F 0·00 (S_1), 0·15 (S_2), 0·57 (S_3); $[\alpha]_D^{25}$ −51·5° (*c* 0·2; 1M-acetic acid). Amino acid analysis: Asp 1·05, Glu 1·05, Pro 1·03, Gly 1·02, Cys($C_3H_6CO_2H$) 0·95, Tyr 0·89, Phe 0·99, Arg 1·02. For $C_{47}H_{66}N_{14}O_{12}S.2\ CH_3COOH.7\ H_2O$ (1297) calculated: 47·22% C, 6·83% H, 15·16% N; found: 47·48% C, 6·07% H, 14·85% N.

Pharmacological Methods

The pressor activity[22] was determined on despinalised Wistar-Konárovice strain rats. The activity was calculated from threshold doses; lysine-vasopressin was used as the standard. Antidiuretic activity was estimated on ethanol anaesthetised rats (maintained water load of 6—8% of body weight), with urine flow rate measured as drop counts recorded automatically, and continuous measurement of urine conductivity[16]. Milk-ejecting activity was determined on lactating rats (5—10 days after parturition) according to[23]. Isolated rat uterus assay was carried out on uterine strips of adult virgin Wistar rats, under the influence of oestrogens. The strips were placed into medium[24] and bubbled through with 95% O_2 — 5% CO_2 at 30°C. Isometric contractions were recorded using a magneto-electric transducer[25]. Uterotonic activity *in vivo* was determined[26] on the rats in ethanol anaesthesis under the influence of oestrogens.

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