SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF OXYTOCIN ANALOGUES MODIFIED SIMULTANEOUSLY IN POSITION 2 AND IN THE DISULFIDE BRIDGE*

Michal LEBL, Tomislav BARTH and Karel JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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Analogues of deamino-6-carba-oxytocin, containing isoleucine, O-methyltyrosine or methionine in position 2, either in the sulfide or sulfoxide form, were prepared and studied pharmacologically. These compounds had considerably lower biological activities than the analogous compounds of the 1-carba series. Furthermore, analogues of [1,6-homolanthionine]deamino-oxytocin were prepared with O-methyltyrosine or isoleucine in position 2; these compounds had a certain degree of oxytocic activity.

The synthesis and pharmacological properties of analogues of deamino-1-carba-oxytocin (*Ia*), the tyrosine residue of which had been substituted by O-methyltyrosine,** phenylalanine or isoleucine, have already been described[1]. As compared with effectors containing a disulfide bridge[3-5], all the analogues with a 1-carba bridge had significantly higher activities. Moreover, [2-O-methyltyrosine]deamino-1-carba-oxytocin (*Ib*), like other oxytocin analogues containing O-methyltyrosine in position 2, had protracted activity in the uterotonic and galactogogic assays *in vivo*[6,7]. Earlier experiments also suggested[6] that 6-carba analogues might have higher uterotonic activity *in vivo*; we therefore decided to prepare analogues derived from deamino-6-carba-oxytocin and modified in position 2. The increase of activity should have been most pronounced in the case of [2-O-methyltyrosine]deamino-6-carba-oxytocin (*Ic*) and we also expected it to have hormonogen properties.

Several analogues of deamino-oxytocin containing an alifatic amino acid in position 2 have already been prepared. The isoleucine derivative[4] (*Id*) had a rather high degree of biological activities, whereas analogues containing leucine[9] or valine[10] had very low activities. An analogue with methionine has not been synthetized so far though the electronic properties of the side chain of methionine would place it somewhere

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** The nomenclature and symbols used are based on published suggestions[2]. The abbreviation —OTcp denotes 2,4,5-trichlorophenyl ester. The amino acids used in this work were of L-configuration.

between an aromatic and alifatic residue. We therefore decided to prepare analogues of deamino-6-carba-oxytocin with isoleucine or methionine in position 2. We assumed that if the effects of the substitution of sulfur by a methylene group and the modification in position 2 on the biological activity were of an additive nature, the uterotonic activity of [2-isoleucine]deamino-6-carba-oxytocin (*Ie*) would be remarkably high.

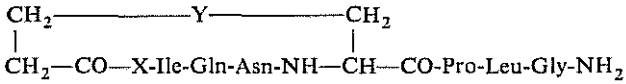
The oxidation of sulfur in the thioether bridge of carba analogues decreased their activities[11]; this was most pronounced in the case of 1-carba compounds. The oxidation of 6-carba analogues did not influence their activity to such a degree[11]. If we take into account the fact that rapid oxidation of thioether sulfur may occur in the blood stream, *i.e.* under conditions of assays *in vivo*, as proved in the case of an ACTH fragment[12], it follows that 6-carba-analogues should be more active in assays *in vivo* than 1-carba-analogues.

The oxytocin analogues, the cyclic part of which was enlarged and contained a disulfide bond, either were not active at all or had minimum activity[13]. However, the compound containing a CH_2—S—CH_2 group instead of the disulfide bond was found to be relatively highly potent[13]. We were therefore interested in investigating how further modifications of its molecule might influence its activity. Again we performed the modifications in position 2, which enabled us to compare the activities of the resulting analogue with those of the 1-carba and 6-carba series.

When synthetizing analogues of deamino-6-carba-oxytocin, we started with heptapeptide[14] *IIa* which we acylated with the corresponding active ester, thus obtaining octapeptides *IIb*–*IId*. We performed cyclization after the removal of the protecting groups by the active ester method. In every case, the cyclization product was subjected to countercurrent distribution. Regarding the isoleucine (*Ie*) and O-methyltyrosine derivatives (*Ic*), one of the fractions obtained by this purification procedure contained a compound that, judging by results of thin-layer chromatography, was the main component of the crude reaction mixture after cyclization. The compounds obtained after countercurrent distribution were characterized as sulfoxides[11,15] (*If*, *Ig*) and were transformed into sulfides by reduction with hydrogen bromide and acetone[11,16]. (It had been established previously that the methyl group is not split off from O-methyltyrosine during reduction under these conditions.) In the case of the methionine analogue (*Ih*), countercurrent distribution was performed under mild conditions and the resulting product contained only very small traces of substances with a sulfoxide-positive reaction. On the one hand, these substances could be easily removed by gel filtration, on the other, reduction by hydrogen bromide and acetone transformed them into the required product. By contrast, oxidation with sodium periodate yielded a bis-sulfoxide (*Ii*), the chromatographic properties of which were identical with those of one of the products of oxidation contaminating the final product after countercurrent distribution.

We detected sulfoxides by means of Thompson's test[15] and infrared spectroscopy. There is practically only one method for the quantitative determination of sulfoxides, namely X-ray photoelectron spectroscopy[17], which, however, is not currently available. Approximate results can be obtained in the case of amino acid sulfoxides by means of an amino acid analyzer, either from the decreased values for amino acids containing sulfur (after hydrolysis with hydrochloric acid[11,18]), or by determining sulfoxide itself after hydrolysis which does not destroy the sulfoxide grouping (alkaline hydrolysis or hydrolysis with toluenesulfonic acid[18,19]), or otherwise by indirect methods[20].

When preparing analogues with a larger ring, we started with the heptapeptide[13] *IIIa* which we acylated with active esters of O-methyltyrosine or isoleucine, thus obtaining octapeptides *IIIb* and *IIIc*. Cyclization was performed as mentioned above and the isoleucine analogue *Ij* was purified by countercurrent distribution and gel filtration. No sulfoxide formation was observed in this case. The analogue containing O-methyltyrosine (*Ik*) was purified only by gel filtration.



Ia, X = Tyr, Y = CH_2—S
Ib, X = Tyr(Me), Y = CH_2—S
Ic, X = Tyr(Me), Y = S—CH_2
Id, X = Ile, Y = S—S
Ie, X = Ile, Y = S—CH_2
If, X = Ile, Y = SO—CH_2
Ig, X = Tyr(Me), Y = SO—CH_2
Ih, X = Met, Y = S—CH_2
Ii X = Met(O), Y = SO—CH_2
Ij X = Ile, Y = CH_2—S—CH_2
Ik, X = Tyr(Me), Y = CH_2—S—CH_2
Il, X = Ile, Y = CH_2—S

R-Ile-Gln-Asn-Hcy(C_2H_4COOH)-Pro-Leu-Gly-NH_2

IIa, R = H
IIb, R = Boc-Tyr(Me)
IIc, R = Nps-Ile
IId, R = Boc-Met

R-Ile-Gln-Asn-Hcy(C_3H_6COOH)-Pro-Leu-Gly-NH_2

IIIa, R = H
IIIb, R = Boc-Tyr(Me)
IIIc, R = Nps-Ile

The formation of sulfoxides during the different procedures apparently depended to a great extent on the structure of the individual compounds. We noticed that the analogues belonging to 6-carba series were more susceptible to sulfur oxidation. Analogous derivatives of the 1-carba series (2-phenylalanine, 2-isoleucine), described earlier[1], did not contain sulfoxide, although they were subjected to the same procedures as compound *Ie* in the course of their synthesis and purification. The amino acid in position 2 was also of some importance; in the presence of O-methyltyrosine,

sulfur oxidation occurred to a greater extent than when isoleucine was situated in this position. We also observed a strong tendency to sulfoxide formation in the case of 1-carba analogues in which the glutamine residue in position 4 was substituted by an alifatic amino acid[21]. All these findings show that changes of the primary structure cause alterations of the conformation of the molecule (at least in the region of the disulfide bridge[22]), thus exposing one or the other sulfur atom to oxidation.

The products obtained were subjected to the uterotonic assay *in vitro* and to the galactogogic assay *in vivo*. As can be seen in Table I, the oxidation of thioether sulfur does not significantly influence the biological activity of this type of compound[11], but the influence on the uterotonic activity is quite the reverse of that on the galactogogic activity. We must stress that the increase of the uterotonic activities of sulfoxides *If* and *Ig* is rather surprising; it demonstrates that when the conformation of the molecule has already been altered, the subsequent oxidation of sulfur can have an unexpected effect on the activity of the analogue. Oxidation resulting in bis-sulfoxide *Ii* led to an almost complete loss of activity; this could be explained by a tendency of both the sulfoxide groups to form a stable polar arrangement which profoundly disturbed the conformation of the peptide necessary for evoking a biological response.

The expected increase of activities of the analogues prepared, as compared with

TABLE I

Biological Activities (I.U./mg) of Some Carba-Analogues of Oxytocin

Compound	Uterus *in vitro*	Mammary gland *in vivo*
Ia	1 899[a]	604[a]
Ib	17·1[b]	35[b]
Ic	3·14	18·0
Id	22[c]	80[c,d]
Ie	3·1	90·0
If	8·44	88·1
Ig	5·25	10·1
Ih	4·74	7·3
Ii	0·13	<0·05
Ij	2·36	6·54
Ik	0·24	0·74
Il	347[b]	327[b]

[a] Ref.[8]; [b] ref.[1]; [c] ref.[4]; [d] value obtained in experiments with rabbits *in vivo*.

analogous compounds from the 1-carba series, was not observed. It can be seen that the effects of the substitution of sulfur by a methylene group in the disulfide bridge and the substitution in position 2 are not additive and, furthermore, that there is a great difference between the two sulfur atoms with respect to their importance for eliciting certain biological responses, which is in agreement with a number of published results[11]. In the case of isoleucine analogues of the 1-carba (*Il*) and 6-carba series (*Ie*), quite the opposite shift of uterotonic activities was observed as compared with [2-isoleucine]deamino-oxytocin (*Id*), depending on the position of the methylene group. (It was not possible to compare the galactogogic activities due to the different methods used for the assay.) The methionine analogue *Ih* had the highest uterotonic and at the same time the lowest galactogogic activity of all the thioether 6-carba analogues described in this paper. The activities of the O-methyltyrosine analogue *Ic* in assays *in vivo* are presented in another paper[7]. Table I shows that the character of the substitution in position 2 is not as important for uterotonic activity as for galactogogic activity.

The analogues with increased ring size (*Ij* and *Ik*) had a certain degree of typical oxytocin activities; the uterotonic activity of the isoleucine derivative *Ij* was comparable with the activity of the analogue *Ie* of the 6-carba series. The activities of the O-methyltyrosine analogue *Ik* were lower by approximately one order of ten. Nevertheless, the specificity of the galactogogic effect of these two compounds was practically identical, by contrast with the analogues of the 1-carba or 6-carba series.

EXPERIMENTAL

Samples for elemental analysis were dried at room temperature at 150 Pa. Thin-layer chromatography 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% aqueous ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), and pyridine–butanol–acetic acid–water (10 : 15 : 3 : 6) (S4). Electrophoresis was performed in a moist chamber on paper Whatman 3 MM with the use of 1M acetic acid (pH 2·4) and pyridine–acetic acid (pH 5·7) as buffers at 20 V/cm for 60 min. Detection was by means of ninhydrin or by the chlorination method; sulfoxide were detected according to ref.[15]. Samples for amino-acid analysis were hydrolysed for 20 or 200 h at 105°C in 6M-HCl (in ampoules sealed at 150 Pa). Analyses were carried out on an automatic analyzer (Development Workshops, Czechoslovak Academy of Sciences, type 6020). Reaction mixtures were taken down under diminished pressure on a rotatory evaporator (150 Pa, bath temperature, 40°C). Melting points were determined on a Kofler block and are uncorrected. The all-glass fully automatic Steady State Distribution Machine (Quickfit & Quartz, Stone, Staffordshire, England) with possibilities of transfers of both upper and lower phases, was used for countercurrent distribution in the solvent system 2-butanol–0·05% aqueous acetic acid. The peptide material was localized by the Folin–Ciocalteau reaction. For gel filtration we used columns (100 × 1 cm) of Bio-Gel P—4 (Bio-Rad Laboratories); localization of peptide material was by means of UV absorption. The IR spectra were taken on a UR-10 (Carl Zeiss, Jena) apparatus.

Water or acetic acid in elemental analyses were calculated to optimally fit the values determined and were not estimated analytically.

General Method for Preparing Octapeptides *IIb—IId*, *IIIb*, and *IIIc*

To the solution of heptapeptide *IIa* or *IIIa* (0·5 mM) in dimethylformamide (20 ml), N-ethylpiperidine (pH 8—9; wet pH paper) and the appropriate active ester (0·7 mM) were added. The mixture was stirred for 20 h at room temperature, a further part of active ester (0·3—0·7 mM) and base (pH 8—9) were added and the mixture was stirred till all the compound with a free amino group disappeared (reaction with ninhydrin). The total reaction time is given in Table II. The reaction mixture was evaporated, the residue triturated with light petroleum, the product was collected and washed with ether, ethyl acetate, water, 3% solution of citric acid, and again with water and ether. Table II gives the analytical values of the products. Samples for elemental analysis were precipitated from dimethylformamide with water. Electrophoretic mobilities were determined after the removal of the amino-protecting group. Amino-acid analyses are given in Table III.

Sulfoxide of [2-O-Methyltyrosine]deamino-6-carba-oxytocin (*Ig*)

The preparation was carried out under nitrogen. To a solution of protected octapeptide *IIb* (250 mg) in a mixture of dimethylformamide (8 ml) and pyridine (8 ml), bis-(*p*-nitrophenyl) sulfite (0·8 g) was added and the solution was stirred at room temperature for 7 h. A further part of sulfite (0·8 g) was added and the last part (0·4 g) after 16 h. 6 h later the mixture was evaporated and triturated with ether, water, and again with ether. The compound obtained (210 mg) was dissolved in trifluoroacetic acid (8 ml) and after 70 min the mixture was diluted with toluene (8 ml) and evaporated. The evaporation was repeated after the addition of more toluene (5 ml). The compound was triturated with ether, dried and dissolved in dimethylformamide (8 ml). The solution was added in the course of 4 h to a mixture of pyridine (200 ml) and N-ethylpiperidine (40 µl) under stirring, heating to 50°C, and bubbling through with nitrogen. After a further 4 h at 50°C and 14 h at room temperature the mixture was evaporated and triturated with ether. The countercurrent distribution (100 transfers of the upper phase and 25 transfers of the lower) gave two compounds with $K = 0{\cdot}79$ and 2·3. The compound from the former peak was ninhydrin-positive and the latter sulfoxide-positive. The compound with $K = 2{\cdot}3$ was dissolved in methanol (0·3 ml) and 3M acetic acid (3 ml) and purified on a Bio-Gel P—4 column. Lyofilization afforded 29 mg of the compound with R_F 0·13 (S1), 0·08 (S2), 0·15 (S3), 0·60 (S4); $[\alpha]_D$ —64·8° (*c* 0·1, 1M acetic acid). IR spectrum: ν (SO) 1015 cm^{-1} (w). For $C_{45}H_{69}N_{11}O_{13}S.2\,C_2H_4O_2.2\,H_2O$ (1160) calculated: 50·72% C, 7·04% H, 13·28% N; found: 50·82% C, 6·79% H, 13·36% N. For amino-acid analysis see Table III.

[2-O-Methyltyrosine]deamino-6-carba-oxytocin (*Ic*)

A solution of HBr in acetic acid (35%; 0·5 ml) was added to a suspension of sulfoxide *Ig* (10 mg) in acetone (1 ml) and after 5 min at room temperature the solution was evaporated. The residue was suspended in acetone and the mixture evaporated again. The remnant was dissolved in methanol (0·4 ml) and 3M acetic acid (2 ml) and the solution was transferred onto a Bio-Gel column. Lyofilization gave 7·1 mg of the compound with negative reaction for sulfoxides; R_F 0·17 (S1), 0·12 (S2), 0·20 (S3), 0·62 (S4). $[\alpha]_D$ —78·3° (*c* 0·1, 1M acetic acid). For $C_{45}H_{69}N_{11}O_{12}S.2\,C_2H_4O_2.H_2O$ (1126) calculated: 52·25% C, 7·07% H, 13·68% N; found: 51·88% C, 6·76% H, 13·42% N. For amino-acid analysis see Table III.

Sulfoxide of [2-Isoleucine]deamino-6-carba-oxytocin (*If*)

The octapeptide *IIc* (250 mg) was transformed into the active ester in the same way as described for the compound *IIb*. The ester was dissolved in dimethylformamide (8 ml), 2·9M-HCl in ether

TABLE II

Some Characteristics of the Octapeptides Prepared

Compound	Active ester reaction time/yield	R_F S1 S3	R_F S2 S4	$E^{His}_{5,7}$ $E^{Gly}_{2,4}$	M.p., °C $[\alpha]_D$[a]	Formula (m.w.)	Calculated/Found % C	% H	% N
IIb	Boc-Tyr(Me)-OTcp	0·66	0·33	0·17	224—230	$C_{50}H_{79}N_{11}O_{15}S.3\,H_2O$	51·77	7·38	13·28
	44 h/95%	0·62	0·65	0·66	—23·2°	(1 160)	51·94	7·01	13·07
IIc	Nps-Ile-ONSu	0·50	0·15	0·23	238—242	$C_{47}H_{74}N_{12}O_{14}S_2.3\,H_2O$	49·12	7·02	14·62
	70 h/80%	0·58	0·64	0·61	—42·7°	(1 149)	48·78	6·67	14·45
IId	Boc-Met-OTcp	0·47	0·13	0·15	220—225	$C_{49}H_{84}N_{10}O_{15}S_2.2\,H_2O$	51·03	7·69	12·14
	48 h/82%	0·34	0·64	0·56	—26·6°	(1 153)	51·31	7·32	12·40
IIIb	Boc-Tyr(Me)-OTcp	0·68	0·43	0·16	229—233	$C_{51}H_{61}N_{11}O_{15}S.3\,H_2O$	52·19	7·46	13·12
	95 h/50%	0·71	0·75	0·56	—26·4°	(1 173)	51·92	7·24	12·84
IIIc	Nps-Ile-ONSu	0·56	0·10	0·18	234—239	$C_{48}H_{76}N_{12}O_{14}S_2.2\,H_2O$	50·34	7·05	14·69
	90 h/64%	0·69	0·74	0·57	—45·7°	(1 145)	50·32	7·22	14·35

[a] *c* 0·2, dimethylformamide.

(0·38 ml) was added and after 7 min standing at room temperature the hydrochloride was precipitated by the addition of ether. Cyclization was carried out in the same way as in the case of the compound *Ig*. Countercurrent distribution (109 transfers of the upper and 101 of the lower phase) again gave two compounds: a ninhydrin-positive one ($K = 0{\cdot}14$) and a product with $K = 1{\cdot}9$. This was, after dissolving it in methanol (0·4 ml) and 3M acetic acid (3 ml), purified by Bio-Gel filtration. Yield, 25·3 mg of sulfoxide-positive compound with R_F 0·22 (S1), 0·14 (S2), 0·12 (S3), 0·62 (S4); $[\alpha]_D$ —72·5° (*c* 0·1, 1M acetic acid). IR spectrum: ν(SO) 1020 cm^{-1} (w). For $C_{41}H_{69}N_{11}O_{12}S.2\,C_2H_4O_2.2\,H_2O$ (1096) calculated: 49·30% C, 7·45% H, 14·05% N; found: 48·98% C, 7·13% H, 14·02% N. For amino-acid analysis see Table III.

[2-Isoleucine]deamino-6-carba-oxytocin (*Ie*)

Sulfoxide *If* (10 mg) was reduced and purified as was described for the compound *Ig*. The yield of sulfoxide-negative product was 7·6 mg; R_F 0·26 (S1), 0·17 (S2), 0·16 (S3), 0·63 (S4); $[\alpha]_D$ —90·1° (*c* 0·1, 1M acetic acid). For $C_{41}H_{69}N_{11}O_{11}S.2\,C_2H_4O_2.H_2O$ (1054) calculated: 48·99% C, 7·55% H, 14·61% N; found: 48·72% C, 7·24% H, 14·78% N. For amino-acid analysis see Table III.

TABLE III

Amino-Acid Analyses of the Peptides Prepared

Compound	Asp	Glu	Pro	Gly	Ile	Leu	Tyr	X	Y	Conditions of hydrolysis[a]
IIb	0·94	1·00	1·04	1·03	0·93	1·05	0·60	1·02[b]	0·32[c]	A
IIc	1·01	0·99	1·00	1·00	1·86	1·05		0·74[b]		B
IId	1·02	0·98	1·02	1·02	0·98	1·24		0·74[b]	0·26[d]	C
IId	1·04	1·00	1·03	1·03	1·03	1·05		0·90[b]	0·92[d]	D
IId	1·06	1·01	1·03	1·06	1·08	1·12		0·76[b]	0·26[d]	E
IIIb	0·99	0·95	1·03	0·94	1·03	1·06	0·65	1·03[e]	0·34[c]	A
IIIc	1·00	0·97	1·02	0·98	1·99	1·05		1·03[e]		B
Ic	1·03	1·01	0·98	1·02	1·02	1·05	0·62	0·94[b]	0·35[c]	A
Ie	1·04	0·98	1·00	1·00	1·96	1·01		0·98[b]		B
Ih	1·02	0·99	1·02	1·00	1·00	1·04		0·97[b]	0·96[d]	A
Ig	1·08	1·01	0·96	1·03	1·00	1·07	0·58	0·56[b]	0·35[e]	A
If	1·00	0·99	1·01	1·01	1·95	1·04		0·49[b]		B
Ii	1·06	0·96	0·97	1·02	0·99	1·03		0·54[b]	0·90[d]	A
Ik	0·99	0·97	1·03	0·99	0·99	1·06	0·60	1·02[e]	0·35[c]	A
Ij	0·99	0·97	1·03	1·00	1·91	1·04		1·08[e]		B
Ii	1·95[g]	0·79[h]	0·97	1·06	0·58[h]	1·02				F

[a] A: 20 h, phenol, B: 200 h, phenol, C: 20 h, without phenol, D: as A, after removing the Boc group E: 20 h, mercaptoethanol, F: toluenesulfonic acid, ref.[19]; [b] Hcy($C_2H_4CO_2H$); [c] Tyr(Me); [d] Met [e] Hcy($C_3H_6CO_2H$); [f] Hcy(O)($C_2H_4CO_2H$) not determined; [g] sum of Asp + Met(O); [h] lower values are probably caused by incomplete hydrolysis.

[2-Methionine]deamino-6-carba-oxytocin (*Ih*)

The preparation of the active ester from octapeptide *IId* (250 mg) and the cyclization was carried out in the same way as was described for the compound *IIb*. Countercurrent distribution (44 transfers of the upper phase) afforded a compound with $K = 1{\cdot}44$; from this peak 92 mg was obtained, containing according to TLC about 20% of a by-product with lower R_F. Repeated gel-filtration gave 34·6 mg of sulfoxide-negative compound with R_F 0·18 (S1), 0·13 (S2), 0·14 (S3), 0·64 (S4); $[\alpha]_D$ —95·5° (*c* 0·1, 1M acetic acid). For $C_{40}H_{67}N_{11}O_{11}S.1{\cdot}5\ C_2H_4O_2.H_2O$ (1029) calculated: 49·01% C, 7·24% H, 14·97% N; found: 49·15% C, 6·96% H, 14·75% N. For amino-acid analysis see Table III.

Bis-Sulfoxide of [2-Methionine]deamino-6-carba-oxytocin (*Ii*)

To the solution of compound *Ih* (10 mg) in methanol (40 µl) and water (0·5 ml), sodium periodate (10 mg) was added and the mixture was set aside at room temperature for 2 h. 3M Acetic acid (2 ml) was then added and the compound was purified by gel-filtration; yield, 8·8 mg of the title compound, with R_F 0·02 (S1), 0·00 (S2), 0·02 (S3), 0·37 (S4), which gave a strongly positive reaction for sulfoxides. $[\alpha]_D$ —68·7° (*c* 0·1, 1M acetic acid). IR spectrum: ν(SO) 1 025 cm^{-1} (broad band). For $C_{40}H_{67}N_{11}O_{13}S.C_2H_4O_2.H_2O$ (1052) calculated: 47·94% C, 6·99% H, 14·64% N; found: 48·16% C, 7·08% H, 14·36% N. For amino-acid analysis see Table III.

[2-O-Methyltyrosine, 1,6-homolanthionine]deamino-oxytocin (*Ik*)

The transformation of octapeptide *IIb* (80 mg) into the active ester and its cyclization was carried out as described for the compound *IIb*. Purification by gel-filtration afforded 6·8 mg of a sulfoxide-negative compound with R_F 0·51 (S1), 0·37 (S2), 0·51 (S3), 0·66 (S4), containing a very small amount of a ninhydrin-positive compound with lower R_F values. $[\alpha]_D$ —86·6° (*c* 0·1, 1M acetic acid). For $C_{45}H_{69}N_{11}O_{12}.C_2H_4O_2S.H_2O$ (1066) calculated: 52·94% C, 7·09% H, 14·15% N; found: 52·66% C, 6·81% H, 14·64% N. For amino-acid analysis see Table III.

[2-Isoleucine, 1,6-homolanthionine]deamino-oxytocin (*Ij*)

The preparation of the active ester from octapeptide *IIIc* (350 mg) and its cyclization was performed as described in the case of peptide *IIc*. The purification was by means of countercurrent distribution (100 transfers of the upper phase, $K = 2{\cdot}9$); yield 120 mg. Half of the amount of this product was further purified by gel-filtration; 24 mg of the title compound, giving negative reaction for the presence of sulfoxides, was obtained. R_F 0·21 (S1), 0·23 (S2), 0·34 (S3), 0·74 (S4); $[\alpha]_D$ —68·8° (*c* 0·1, 3M acetic acid). For $C_{43}H_{73}N_{11}O_{11}S.3{\cdot}5\ H_2O$ (1015) calculated: 50·87% C, 7·94% H, 15·17% N; found: 50·74% C, 7·58% H, 15·04% N. For amino-acid analysis see Table III.

Pharmacological Methods

The uterotonic assay was carried out on uterine strips of adult Wistar strain rats, under the influence of oestrogens. The strips were placed into medium[23] which was bubbled through with 95% O_2 — 5% CO_2 at 30°C. Oxytocin was used as a standard. 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.[24].

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