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SYNTHESIS OF THE N-TERMINAL TRIPEPTIDE SEQUENCE OF OXYTOCIN WITH VARIOUS PROTECTIVE GROUPS FOR THE CYSTEINE RESIDUE

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UDC 577.175.346:543.42

The influence of the nature of the protective groups at the sulfur and nitrogen atoms in the cysteine molecule on the reactivity of this amino acid in the peptide condensation reaction has been investigated. The synthesis of the N-terminal tripeptide sequence of oxytocin was selected as a model reaction. For identifying the compounds synthesized and checking their purity, in addition to traditional physicochemical methods (TLC, melting points, angles of optical rotation), wide use has been made of the ^{13}C NMR method.

The successful synthesis of oxytocin depends to a considerable degree on the appropriate choice of protective group for the thiol function of cysteine. In spite of the fact that at the present time more than 70 such groups are known, the optimum protection of the mercapto group of cysteine has not been found [1]. This is due to the fact that demands are placed on these protections, on the one hand, for the stable blocking of the mercapto function under the conditions of synthesis and of eliminating the temporary protective grouping (BOC, Z, Trit, OR, etc.) and, on the other hand, for ready elimination under mild conditions excluding the racemization, degradation, or oligomerization of the nonapeptide.

The presence of electron-active groups in the cysteine molecule (—SR', —NR") must have a substantial influence on the properties of the carboxy function of the molecule. It is difficult to predict the combination of the effects of the introduced protections of the thiol function (R') and of the amino function (R") on these properties. It is all the more difficult to predict the reactivities of various cysteine derivatives. And while, as before, great attention is being devoted to the search for new protective groups for the thiol function of cysteine in the literature, there are practically no reports on the comparative characteristics and influence of these groups on the completeness of the creation of the peptide bond [2].

In view of this, it appeared to us to be of interest to elucidate the influence of various readily available protective groups of the thiol function of cysteine on the reactivity of cysteine derivatives, using as an example the synthesis of the N-terminal tripeptide se-

All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormone Preparations, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 684-692, September-October, 1989. Original article submitted December 9, 1988.

 0009-3130/89/2505-0582$12.50

TABLE 1. Properties of the Cysteine Derivatives R"Cys(R')OH Used for the Synthesis

Compound	Protective groups		Angle of opt. rot., $[\alpha]_D^{20}$, deg		mp, °C	Chromatographic mobility, Rf
	R'	R"	DMFA	AcOH		
I	Me	BOC	−35,5	−27,5	71–73	0,56
II	Bzl	BOC	−42,5	−45,5	72–74	0,70
III	Bz	BOC	−58,0	−15,0	107–109	0,67
IV	Bzm	BOC	−36,5	−33,0	133–135	0,50
V	Pym**	BOC	−35,5	−30,5	134–136	0,17
VI	Bzl	Form	−22,0	−9,0	99–102	0,22
VII	Trit	BOC	+26,5	+21,5	76–77	0,69
VIII	Trit	Trit	+79,0	+56,0	98–100	0,92
IX	Trit	Form	+33,5	+40,0	101	0,32
X		Form	−114,0	−175,5*	234 236	0,70

*In HCOOH.
**Pyrrolidonemethyl.

TABLE 2. Physicochemical Properties of the Derivatives Obtained with the Formula R"Cys(R')TyrIleOMe

Compound	Protective groups		Method of preparation	Yield, %	mp, °C	Angle of optical rotation			Chromatographic mobility, Rf
	R'	R"				MeOH	DMFA	AcOH	
XI	Me	BOC	MA	98	59–60	—	−26,5	—	0,90
			DCHC	98	58–60	—	−26,5	—	
XII	Bzl	BOC	MA	92	126–128	−25,0	−24,0	—	0,89
			DCHC	78	145–151	—	−24,0	—	0,88
XIII	Bz	BOC	MA	89	153–155	−23,0	−35,5	−11,0	
XIV	Bzm	BOC	MA	80	59–61	−24,0	−12,5	−14,0	0,83
XV	Pym	BOC	MA	56	63–65	—	−13,5	—	0,64
			DCHC	83	62–64	—	−10,5	—	
XVI	Bzl	Form	DCHC	58	138–140	—	−16,5	—	0,72
XVII	Trit	BOC	DCHC	68	74–77	—	+5,5	−0,5	0,93
XVIII	Trit	Trit	DCHC	60	74–77	—	+35,0	+18,5	0,88
XIX	Trit	Form	DCHC	37	142–144	—	+3,5	—	0,80
XX		Form	DCHC	85	68–70	—	−51,0	−63,5	0,90

quence of oxytocin. In this work we used both commericial cysteine derivatives and derivatives prepared in the laboratory (see Table 1).

Synthesis of the tripeptide was carried out by the 1 + 2 scheme using the mixed-anhydride (MA) and dicyclohexylcarbodiimide (DCHC) methods (see Table 2). It is known [3] that trityl derivatives of amino acids are unsuitable for obtaining peptides by the MA method and therefore the DCHC method was used for the synthesis of the corresponding peptides. In order to compare these methods, derivatives (XI) and (XII) were synthesized by both of them. It was shown that condensation using DCHC at this stage had no advantages. Furthermore, the synthesis of (XII) using DCHC gave a lower yield. Table 2 shows the physicochemical properties of the methyl esters of the compounds obtained. As can be seen from the Table, the yield of BOC derivatives of the tripeptides decreased in the sequence of thioalkyl derivatives Me → Bzl → Bz → Bzm → Pym when condensation was performed by the mixed-anhydride method. When the synthesis was carried out with the use of DCHC, the yield of the BOC derivatives of the tripeptides decreased in the sequence Me → Pym ≈ Bzl → Trit. The yields of S-trityl derivatives of the tripeptides decreased in the sequence of N^{α}-protective groups BOC → Trit → Form.

We used the tripeptide ester derivatives obtained to compare the corresponding hydrazides with the aim of the subsequent synthesis from them of the nonapeptide oxytocin by a 3 + 6 scheme using the azide method.

TABLE 3. Physicochemical Properties of the Derivatives Obtained with the Formula R"cys(R')TyrIleN_2H_3

Compound	Initial compound	Yield, %	mp, °C	Angle of opt. rotation, $[\alpha]_D^{20}$, deg		Chromatographic mobility, R_f
				DMFA	AcOH	
XXI	XI	90*	224—226	—29,5	—26,5	0,72
		92	223—225	—29,0	—	
XXII	XII	72*	227—231	—25,0	—12,0	
		63	225—227	—24,5	—	0,67
XXIII	XIII	0	—	—	—	—
XXIV	XIV	77*	202 - 203	—16,0	—16,5	0,54
XXV	XV	60*	223—225	—20 0	—15,0	
		65	196	—13,5	—	0,47
XXVI**	XVI	<50	223—226	—18,0	—	0.53
XXVII	XII	92	206—207	+7,5	—3 5	0,67
XXIX**	XIX	<50	193 - 195	+8,0	—	0,29
XXX	XX	41	207—208	—53,5	—69,0	0,77

*The initial ester was obtained by the mixed-anhydride method.
**The hydrazide derivatives were not isolated in the pure form.

TABLE 4. Chemical Shifts in the ^{13}C NMR Spectra of the Cysteine Derivatives

Residue	Nucleus	I	II	III	IV	V	VI	VII	VIII	IX
Cys	C_0	172,7	172,6	172,1	172,7	172,5	171,8	172,1	173,9	171,3
	C_2	53,3	53 3	53,2	53,9	53.6	50.4	53,1	54,7	49.9
	C_3	35,1	32,5	30,3	32,4	32,3	32,6	33,0	36,6	33,3
	C = O	155,5	155,4	155,5	155,5	155,4		155.2		
BOC	—C—	78,4	78,3	78.6	78.4	78,3		78,3		
	CH_3	28,3	28,2	28,2	28,2	28,2		28,2		
Form	C = O						161,2			161.0
	—C—								70,9	
Trit (N)	C_1								145 8	
	C_2								128,4	
	C_3								127,8	
	C_4								126,4	

Table 3 gives the physicochemical properties of the hydrazides obtained by the hydrazinolysis of the corresponding derivatives of the tripeptide methyl esters. The procedure for obtaining all the derivatives was fairly simple and their yields, as a rule, were not less than 70%, with the exception of the N-formyl derivatives of the tripeptides [compounds (XXVI), (XXIX), and (XXX)], the yields of which did not exceed 50%.

It is known that protective groups of the thiol function of cysteine of the acyl type possess a high sensitivity to the action of alkaline agents. It is therefore easy to explain the quantitative elimination of a benzoyl protection of the thiol group of cysteine under the conditions of the hydrazinolysis of the methyl ester of tripeptide (XIII) that we observed. The absence of benzoyl protection in the product isolated on the hydrazinolysis of (XIII) was shown by the ^{13}C NMR method. It was impossible to obtain the hydrazide (XXIII) by this method.

It has been reported in the literature that the presence of a thiol group greatly complicates the synthesis of hydrazides from tritylamino acid esters [3]. We found that under the conditions of the hydrazinolysis of the ditrityl derivative, likewise, no quantitative conversion of (XVIII) into the corresponding hydrazide (XXVIII) took place. The presence of one trityl group protecting only the mercapto function of cysteine in the molecule of the tripeptide did not prevent the formation of the corresponding hydrazide [compounds (XXVII) and

TABLE 5. Chemical Shifts in the ^{13}C NMR Spectra of Derivatives of the Tripeptide Methyl Ester*

Residue	Nucleus	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII**	XIX
Cys	C_0	170,4	170,3	169,9	170,6	170,4	169,6	169,5	170,5[d]	168,9
	C_α	54,0[a]	54,1[b]	53,8	54,4	53,8	50,6	53,4[c]	54,9	49,9
	C_β	36,0	33,7	31,0	33,2	32,8	33,5	33,7	***	33,8
Tyr	C_0	171,1	171,0	171,1	171,1	171,2	171,0	170,7	171,4[d]	170,8
	C_α	53,7[a]	53,6[b]	53,8	53,8	58,8	54,0	53,9[c]	53,3	53,8
	C_β	37,0	37,0	37,0	37,0	36,9	36,7	37,0	37,0	36,7
	C_γ	127,4	127,4	127 4	127,4	127,4	127,5	127,1	127,1	127,3
	C_δ	130,3	130,2	130,3	130,2	130,2	130,2	130,1	130,3	130,1
	C_ε	114,9	114,9	114,9	114,9	114 9	114,9	114,8	114,8	114,8
	C_ξ	155,9	155,8	156,0	155,9	155,9	155,8	155,8	155,9	155,8
Ile	C_0	171,7	171,7	171,8	171,7	171,7	171,7	171,6	171,6	171,6
	C_α	56,4	56,4	56,5	56,4	56,4	56,4	56,3	56,4	56,4
	C_β	36,4	36,4	36,4	36,4	36,4	36,3	36,3	36,3	36 4
	C_{γ_1}	24,8	24,8	24,8	24,8	24,8	24,8	24,7	24,8	24,7
	C_{γ_2}	15,4	15,3	15,4	15,3	15,3	15,3	15,3	15,2	15,3
	C_δ	11,1	11,1	11,1	11,0	11,1	11,1	11,0	11,0	11,1
BOC	C=O	155,2	155,2	155,2	155,3	155,3		154,8		
	—C—	78,4	78,5	78,7	78,4	78,4		78,6		
	CH_3	28,2	28,2	28,1	28,1	28,2		28,1		
Form	C=O						160,9			160,8
Me(O)	CH_3	51,7	51,6	51,7	51,6	51,6	51,6	51,5	51,4	51,6

*The opposite assignments are possible for the pairs of signals marked a–a, b–b, c–c, and d–d.

**Chemical shifts for the Trit group at the N-end of the tripeptide (ppm): $-\overset{|}{\underset{|}{C}}-$ −70.9; C_1 − 145.8; C_2 −128.4; C_3 −127.7: C_4 − 126.3.

***The signal overlaps with the signal of the solvent.

(XXIX)], but the partial deblocking of the thiol function of the cysteine residue was possible [compound (XXIX)].

The detection of a relationship between the reactivity of the cysteine derivatives and the structure of the protective groups for the thiol and amino functions showed that of the protective groups for the thiol function of cysteine considered in this work the best (in relation to the reactivity of the cysteine derivative) were the benzyl and benzoyl groups. However, a disadvantage of benzoyl protection is the impossibility of synthesizing the hydrazide of the corresponding derivative. Synthesis using pyrrolidonemethyl protection of the thiol group gave ambiguous results [see (XV), Table 2, and (XXV), Table 3].

The use of the N-formyl protection of cysteine is apparently irrational, since the yields both of the esters and of the hydrazides of the tripeptides using it did not exceed 50%. However, the synthesis of the tripeptides using N-formylthiazolidine took place with high yield.

To confirm the structures and to check the purity of the initial cysteine derivatives and the final tripeptides we used the method of ^{13}C NMR spectroscopy. The chemical shifts for the carbon nuclei of the amino acid residues and the groupings protecting the N^α group of cysteine are given in Tables 4-6 and those for the protective groups of the thiol function of cysteine in Table 7. Analogous results for compounds (X), (XX), and (XXX), containing a thiazolidine ring are given in Table 8.

TABLE 6. Chemical Shifts in the ^{13}C NMR Spectra of the Tripeptide Hydrazide Derivatives*

Residue	Nucleus	XXI	XXII	XXIV	XXV	XXVI	XXVII	XXIX
Cys	C_0	170,4	170,4	170,7[b]	170,4[c]	169,7	169,6	168,9
	C_α	54,0	54,2[a]	54,5	54,5	50,6	53,5[d]	49,9
	C_β	36,0	33,7	33,3	32,8	33,5	33,7	33,8
Tyr	C_0	170,4	170,4	170,5[b]	170,5[c]	170,4	170,1[e]	170,1
	C_α	54,0	53,9[a]	54,2	54,1	54,2	54,0[d]	53,8
	C_β	36,8	36,8	36,8	36,8	**	36,7	**
	C_γ	127,5	127,5	127,5	127,5	127,6	127,2	**
	C_δ	130,2	130,3	130,2	130,2	130,2	130,2	130,1
	C_ε	114,9	114,9	115,0	114,9	114,9	114,8	114,8
	C_ζ	155,8	155,9	155,9	155,8	155,8	155,8	155,8
Ile	C_0	170,0	170,0	170,1	170,0	170,0	170,0[e]	169,9
	C_α	55,5	55,5	55,5	55,5	55,5	55,4	55,4
	C_β	36,8	36,8	36,8	36,8	**	36,7	**
	$C_{\gamma 1}$	24,4	24,5	24,5	24,5	24,5	24,0	24,4
	$C_{\gamma 2}$	15,2	15,2	15,2	15,2	15,2	15,2	15,2
	C_δ	10,9	11,0	10,9	10,9	10,9	10,8	10,8
BOC	C=O	155,2	155,2	155,3	155,3		154,9	
	—C—	78,4	78,5	78,4	78,4		78,6	
	CH_3	28,2	28,2	28,2	28,2		28,1	
Form	C=O							160,8

*The opposite assignments are possible for the pairs of signals marked a–a, b–b, c–c, d–d, and e–e.
**The assignment of these signals was complicated by the presence of the corresponding methyl esters as impurities (see text).

The samples of hydrazides (XXVI) and (XXIX) investigated were not individual tripeptides. Sample (XXVI) consisted of a mixture of the hydrazide and the corresponding methyl ester. Samples (XXIX) contained an impurity of peptide nature, including the initial methyl ester. It can also be assumed from the nature of the ^{13}C NMR spectrum that in this case partial removal of the trityl protection from the thiol function of the cysteine residue had taken place. The presence of impurities made it impossible to identify all the signals in the spectra of these hydrazides, as is mentioned in Table 6.

The assignment of the resonance signals over the whole spectral range was based on literature information [4], on a comparison of the chemical shifts in the series of compounds under consideration, and also on the nature of the splitting of the signals and the values of the SSCCs in the spectra recorded in the gated decoupling regime.

In the identification of the signals in the 160-191 ppm region, a substantial role was played by a comparison of the chemical shifts in the series of methyl esters and hydrazides studied and in the corresponding ester–hydrazide pairs. Results obtained for the intermediates in the synthesis of the N-terminal tripeptide of oxytocin [5] were also brought in. The assignment of the signals in the 160 ppm region to the resonance of the nuclei of the formyl groups was confirmed by the appearance in the gated coupling spectra of a doublet with $^1J_{CH}$ ~190 Hz.

In the analysis of the resonance signals in the 110-160 ppm region, to identify the signals of the carbon nuclei of the benzene ring present in the ortho- and meta-positions to the substituent (in the Bzl, Bz, Bzm, and Trit groups), we used the nature of the splitting of the signals by long-range SSCCs in the gated decoupling spectra. It is known [6] that in the benzene ring the $^3J_{CH}$ constants have values substantially exceeding those of the $^2J_{CH}$ and $^4J_{CH}$ constants (7-10 and 1-3 Hz, respectively). In a monosubstituted ring the carbon nucleus in the ortho-position has two $^3J_{CH}$ SSCCs and the nucleus in the meta-position only one. Thus, the first gives a triplet and the second a doublet with the corresponding SSCCs. In the cases considered by us, the $^3J_{CH}$ values amounted to 6-7 Hz.

TABLE 7. Chemical Shifts in the ^{13}NMR Spectra for Carbon Nuclei Present in the Protective Groups of the Thiol Function of Cysteine

Protective group	Nucleus	II	VI	XII	XVI	XXII	XXVI	Protective group	Nucleus	III	XIII
Bzl	CH_2	35,4	35,5	35,4	35,4	35,5	35,4	Bz	C=O	190,8	190,8
	C_1	138,4	138,3	138,5	138,4	138,5	138,4		C_1	136,4	136,4
	C_2	128,9	129,0	128,9	128,9	128,9	128,9		C_2	127,0	126,6
	C_3	128,4	128,5	128,4	128,4	128,4	128,4		C_3	129,2	129,1
	C_4	126,9	127,0	126,8	126,8	126,9	126,8		C_4	134,1	134,0

Protective group	Nucleus	IV	XIV	XXIV	Protective group	Nucleus	V	XV	XXV
Bzm	CH_2	41,0	41,0	41,5	Pym	(N) CH_2(S)	44,1	43,6	43,6
	C=O	166,4	166,5	166,5		C=O	174,6	175,2	175,2
	C_1	134,1	134,0	134,0		CH_2(N)	45,2	45,2	45,2
	C_2	127,4	127,4	127,4		CH_2(C=O)	30,4	30,4	30,4
	C_3	128,4	128,4	128,4		$-CH_2-$	17,2	17,2	17,2
	C_4	131,5	131,5	131,6					

Protective group	Nucleus	VII	VIII	IX	XVII	XVIII	XIX	XXVII	XXIX
Trit (S)	$-\overset{\vert}{C}-$	66,2	65,6	66,3	66,0	65,6	65,9	66,0	65,9
	C_1	144,4	144,5	144,2	144,4	144,5	144,3	144,4	144,3
	C_2	129,2	129,2	129,1	129,1	129,2	129,1	129,2	129,1
	C_3	128,1	128,0	128,1	128,0	127,9	128,0	128,0	128,0
	C_4	126,8	126,7	126,9	126,7	126,6	126,8	126,8	126,8

Protective group	Nucleus	I	XI	XXI
Me (S)	CH_3	15,2	15,0	15,1

In the assignment of the resonance signals in the 46-80 ppm region the main difficulty consisted in distinguishing the signals of the C_α nuclei of the cysteine and tyrosine residues. In many cases this problem could be solved on the basis of the influence of different protective groups for cysteine on the chemical shifts of the signal of the C_α nucleus obtained in a study of a number of cysteine derivatives (Table 4). The identification of the signals of the CH_2–N and S–CH_2–N nuclei (in the Pym group) was made on the basis of the values of the $^1J_{CH}$ SSCCs: 138 Hz in the first case and 148 Hz in the second (result of the influence of a second electronegative substituent of the sulfur atom).

In the spectra obtained on a spectrometer with a working frequency of 20.115 MHz, the 36-43 ppm region is overlapped by a powerful multiplet of the solvent DMSO-d_6, which interferes with the assignment of the signals of the C_β nuclei of the tyrosine and isoleucine residues from the gated decoupling spectra. This difficulty was eliminated by passed to a spectrometer with a working frequency of 62.9 MHz. In this way it was shown that in the spectra of the methyl esters a signal in the 36.3-36.4 ppm region belonged to the resonance of the C_β nucleus of isoleucine (doublet), while a signal in the 36.7-37.0 ppm belonged to the resonance of the C_β nucleus of tyrosine (triplet). In the spectra of the hydrazides, the signals of the C_β nuclei of Ile and Tyr practically overlapped even at a working frequency of the spectrometer of 62.9 MHz. Exceptions were compounds (XX) and (XXX), which will be considered below.

In the analysis of the 10-36 ppm region of the spectra, the results of gated decoupling spectra were brought in to distinguish the signals of the $C_{\gamma 2}$ nuclei of Ile and of S–CH_3 (methyl protection of the thiol function of cysteine) in the 15.0-15.5 interval, giving a triplet and a quartet, respectively.

The ^{13}C NMR spectrum of formylthiazolidine (X) contained two sets of signals of appreciably differing intensities. On the basis of the gated decoupling spectra it may be assumed that the two sets of signals reflect the existence of two conformers arising thanks to the hindered rotation of the formyl group around the N–C bond. The fact that only one resonance

TABLE 8. Chemical Shifts in the ^{13}NMR Spectra of Compounds Containing a Thiazolidine Ring

Residue	Nucleus	X		XX		XXX	
		1	2	1	2	1	2
Formylthiazolidine (-yl)	C=O	170.8	172.3	168.7	169.4	168.8	169.5
	CH	62,0	65,2	62,9	65,8	63,0	65,9
	CH_2	30,7		30,6		30,7	
	—C—	69,9	70,4	70,2	70,8	70,2	70,9
	CH_3	31,9	29,4	30,6	28,6	30,7	28,7
	CH_3	30,8	27,3	30,6	27,2	30,7	27,2
	HC=O	159,7	160,7	159,9	160,1	159,9	160,2
Tyr	C_0			171,0	171,1	170,4	170,6
	C_α			54,1		54,4	
	C_β			36,4		36,4	
	C_γ			127,3		127,4	
	C_δ			130,2		130,3	
	C_ε			114,9		115,0	
	C_ζ			155,9		155,9	
Ile	C_0			171,7		170,1	
	C_α			56,5		55,7	
	C_β			36,4		37,0	
	$C_{\gamma 1}$			24,8		24,5	
	$C_{\gamma 2}$			15,3		15,3	
	C_δ			11,1		11,0	
Me(O)	CH_3			51,6			

signal corresponds to the nucleus at the CH_2 group does not contradict the hypothesis put forward. The same two sets of signals appeared in the spectra of compounds (XX) and (XXX) with the only difference that the proportion of the predominating conformer had increased. In the spectra of the peptides (XX) and (XXX) splitting of the signal of the C_0 nucleus of Tyr was also observed; it is possible that the same conformational difference appeared here, as well. In Table 8, the predominating conformer is designated as 1 and the second as 2. In the spectrum of the hydroxide (XXX) the presumed assignment of the signals of the C_β nuclei of Tyr and Ile was made on the basis of a law observed in the series of compounds studied. The resonance signal of the C_β nucleus of Ile in the spectrum of a hydrazide shifts downfield by 0.4 ppm in comparison with its position in the spectrum of the corresponding methyl ester.

EXPERIMENTAL

Melting points were determined in open capillaries without correction, and angles of optical rotation on a polarimeter (VNII EKIProdmash [All-Union Scientific-Research Experimental-Design Institute of Food Machinery], Moscow).

The chromatographic purities and mobilities of the peptides obtained were determined by the TLC method on Silufol plates in the ethyl acetate—pyridine—acetic acid—water (15:5:1.5:2.75) system.

Peptides were detected by treating the dried plates in an atmosphere of Cl_2 followed by spraying with a 1 N acetic acid solution containing 0.03% of o-toluidine and 0.1% of KI.

The ^{13}C NMR spectra of solutions of the compounds in DMSO-d_6 (C = 50-100 mg/ml) were recorded on a WP-80DS spectrometer (Bruker) with a working frequency of 20.115 MHz. The recording conditions were similar to those given in [5]. For compounds (III) and (XIII), the spectra of which contained a signal at 190.8 ppm, the volumes of the memory for the accumulation and reproduction of the spectrum were increased to 16 K and 8 K, respectively, with a simultaneous doubling of the spectral range of observation. The chemical shifts were reckoned from the signal of the solvent (39.6 ppm) and were converted to the δ-scale. Commercial DMSO-d_6 was used for the preparation of the solutions, without the preliminary elimination of water.

In a number of cases, spectra were also recorded on a WM-250 spectrometer (Bruker) with a working frequency of 62.9 MHz. Conditions for the recording of spectra with complete suppression of spin—spin interaction with protons: volume of the memory for accumulation

32 K and for reproduction 16 K; machine resolution 0.8 Hz (0.01 ppm), pulse length 8.0 μsec (~25°), interval between pulses 1.3 sec.

For synthesis we used derivatives (II), (IV), and (V) [$(BOCCysOH)_2$, HCys(Bzl)OH, and HCys(Trit)OH] produced by Reakhim and also HCysOH, HCys(Me)OH, and (VIII) produced by Reanal, while compounds (I) and (VII) were obtained by the method of [7], (VI) and (IX) by that of [8], and (X) by that of [9]. Compound (III) was obtained by Photaki's procedure [2] with a yield of 40% thanks to the use of a solution of benzoyl chloride (40% solution in dioxane) in place of neat benzoyl chloride.

Preparation of Esters with Formula R"Cys(R')TyrIleOMe (XI-XX). A. A solution of 6.1 g (26 mmoles) of BOCCys(Me)OH and 8.8 g (28.5 mmoles) of HTyrIleOMe in 60 ml of DMFA was cooled to −5 ± 2°C and a solution of 5.9 g (28.5 mmoles) of DCHC in 20 ml of DMFA cooled to −5 ± 2°C was added. The reaction mixture was stirred at −5 ± 2°C for 24 h and at room temperature for 3 h. The precipitate that deposited was filtered off and was washed with 80 ml of CH_2Cl_2. The combined organic solution was washed with 50 ml of 1 N HCl. The resulting precipitate was again filtered off. The organic layer was washed successively with 30 ml of 30% aqueous MeOH, 30 ml of saturated $NaHCO_3$ solution, 30 ml of 30% aqueous MeOH, and 40 ml of H_2O. The solution was evaporated in vacuum. The residue was treated with 75 ml of hexane and the mixture was left at 0°C for 18 h. The precipitate that had deposited was filtered off and was dried in vacuum to constant weight. The yield of (XI) was 13.3 g (25.3 mmoles). compounds (XII) and (XV-XX) were obtained similarly (see Table 2).

B. The reaction with HTyrIleOMe of the mixed anhydrides of the cysteine derivatives R"Cys(R')OH gave compounds (XI-XV) [5] (see Table 2).

Preparation of Hydrazides with the Formula R"Cys(R')TyrIleN_2H_3 (XXI-XXX). The hydrazides (XXI-XXX) were obtained in a similar way to that described in [5]. The end of the reaction was determined by the TLC method (see Table 3). Hydrazide (XXI) was obtained with the use of anhydrous hydrazine.

SUMMARY

1. The synthesis of the methyl esters and hydrazides of the N-terminal tripeptide of oxytocin with various protective groups for the thiol function of cysteine has been effected by the 1 + 2 scheme.

2. Relationships have been obtained between the yields of methyl esters of tripeptides of the 1-3 sequence of oxytocin and the structures of the protective groups of the N^{α}-function and the mercapto function of cysteine. An advantage of the benzyl grouping used for masking the mercapto group of cysteine has been shown.

3. The signals of the ^{13}C NMR spectra of the compounds obtained have been interpreted.

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