SUMMARY

1. It has been established that the nature of the action of pectin on the solubility of gossypulin and its derivatives with different degrees of chemical modification and of denaturation is the same at a weight ratio of protein to pectin of 4:1.

2. It is assumed that when protein solutions are stabilized in the presence of pectin, ionic, hydrogen, and/or hydrophobic interactions take place between the proteins and the pectins. When protein is precipitated from solution by acid titration in the presence of pectin, complexes between the protein and the pectin with pronounced ionic interactions are formed.

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SYNTHESIS OF A HEPTAPEPTIDE WITH SEQUENCE 17-23 OF HUMAN CALCITONIN

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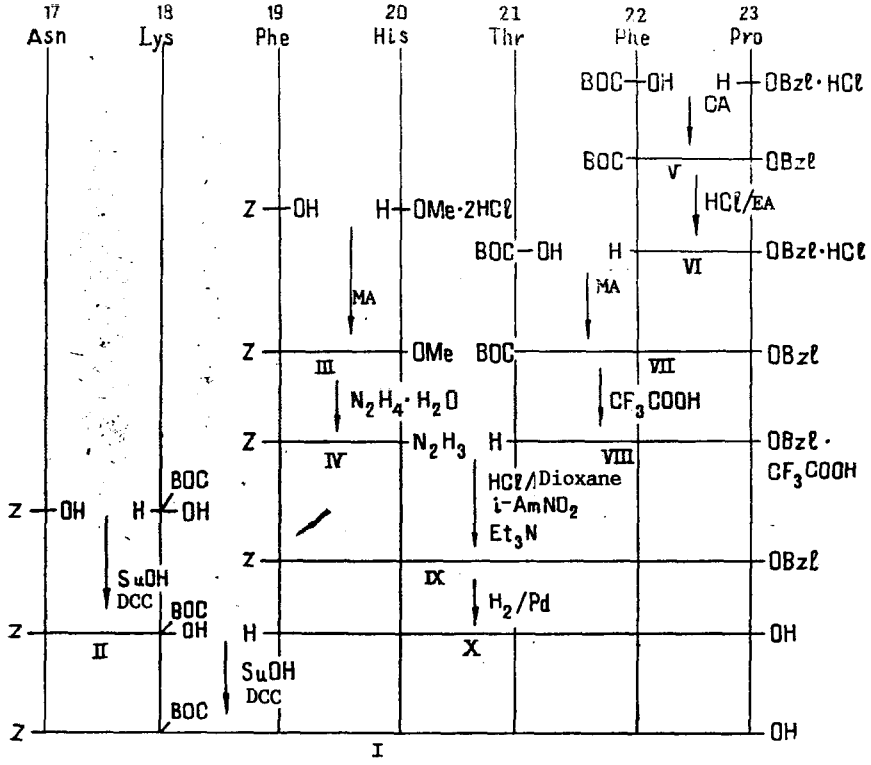
UDC 547.962.5

Two schemes for the synthesis of a peptide with sequence 17-23 of human calcitonin with the minimum protection of the lateral functions of the amino acids are proposed.

The heptapeptide ZAsnLys(Boc)PheHisThrPheProOH (I) with the natural sequence of human calcitonin [1] is a fragment in the synthesis of the complete calcitonin molecule with the minimum protection of the lateral functional groups of the peptide which permits simpler amino acid derivatives to be used and the yield of the desired product at the end of the synthesis to be raised. In an attempt to synthesize (I) by analogy with [2] without the protection of the hydroxy groups of the threonine residue, a substance was formed that contained two impurities close in chromatographic mobility and other properties to the heptapeptide which could not be separated from the heptapeptide by the usual methods (extraction, recrystallization).

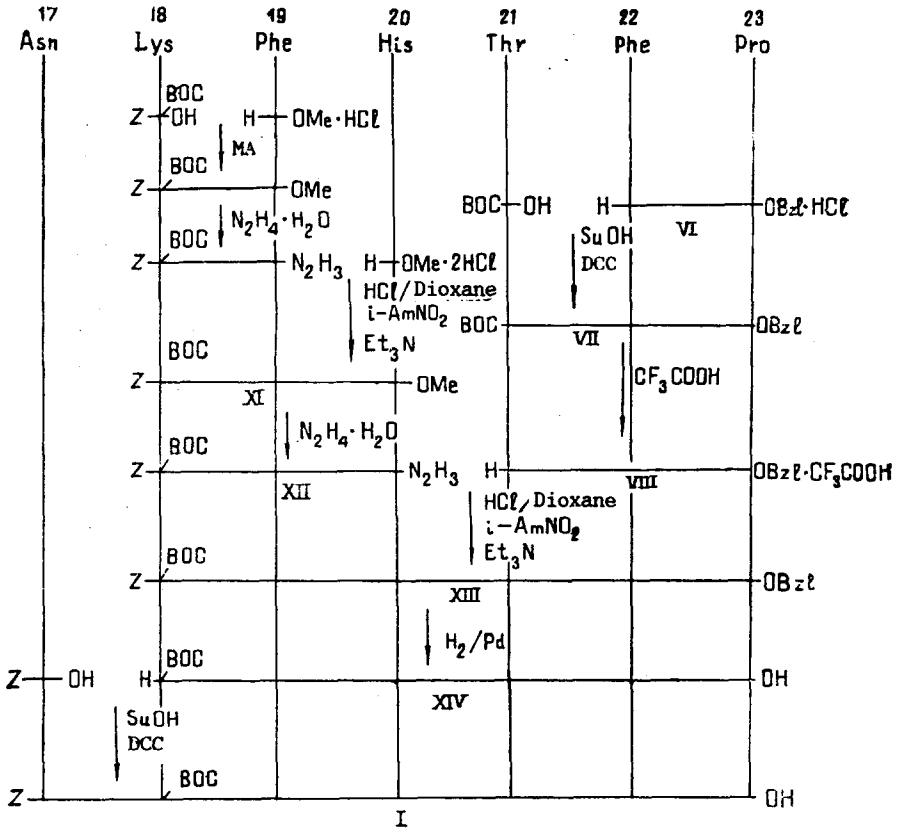
All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormonal Preparations, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 480-488, July-August, 1986. Original article submitted March 19, 1986.



Scheme 1

Two schemes for the synthesis of (I) have been used in the present work. According to scheme 1, the heptapeptide (I) was obtained by the addition of the dipeptide ZAsnLys(Boc)OH (II) was added to the pentapeptide HPheHisThrPheProOH (C) by the activated ester (AE) method; under these conditions, the (I) formed contained the initial dipeptide (II) as an impurity the removal of which was associated with considerable losses of the desired product, and the yield at this stage fell to 30%. Scheme 2 was therefore selected, which consists in the condensation of the tripeptides ZLys(Boc)PheHisN_2H_3 (XII) and HThrPheProOBzl·CF_3COOH (VIII) by the azide method followed by the addition of ZAcnOH to the hexapeptide HLys(Boc)PheHisThrPheProOH (XIV) by the AE method: the ZAsnOH that had not reacted was separated from the (I) by washing the reaction mixture with water, and the ZAsnOSu by reprecipitation with ether from $CHCl_3$. According to the literature [2, 3], the addition of ZAsnOH to peptides containing N-terminal lysine by the AE method takes place with a yield of up to 50%. Schemes 1 and 2 make use of the tripeptide HThrPheProOBzl·CF_3COOH (VIII), which is obtained by the successive growth of the polypeptide chain from the C-end totally by the mixed-anhydride (MA) method (Scheme 1) or by the addition of BocThrOSu to the dipeptide (V) (Scheme 2). When using the MA method, the tripeptide BocThrPheProOBzl (VI) that was formed contained as a slight impurity a by-product with R_f 0.9 in system 4 from which it could be separated by eliminating the Boc protective group. The yield on the addition of the tripeptide ZLys(Boc)PheHisN_2H_3 (XII) to the tripeptide (VIII) was lower than on the addition of the dipeptide ZPheHisN_2H_3 (IV) to the tripeptide (VIII) because considerable amounts of the hexapeptide were lost on its purification from the ZLys(Boc)PheHisN_2H_3 (XII) that had not reacted, which dissolves considerably less well in 0.1 N HCl than (IV). However, the purification of the heptapeptide (I) obtained by Scheme 2 was simpler and more effective than that obtained by Scheme 1.

To protect the N^{α}-amino groups of phenylalanine (No. 19), lysine, and asparagine the benzyloxycarbonyl group was used; for the N^{ε}-amino group of lysine and for the N^{α}-amino groups of phenylalanine (No. 22) and of threonine, the tert-butoxy carbonyl group; for the α-carboxy groups of histidine and proline, methyl and benzyl ester groups, respectively; and for the α-carboxy group of lysine, the trimethylsilyl ester group. In both schemes, the MA method was used to obtain the di- and tripeptides and the azide and AE methods for linking the fragments. The peptides obtained were chromatographically homogeneous according to TLC on Silufol and were characterized by their melting points and angles of optical rotation (Table 1). To confirm the structures and check the purity of the compounds obtained we used the ^{13}C NMR method. Table 2 gives the chemical shifts of peptides (II, IV, V, VII-IX, and XIII).



Scheme 2

TABLE 1. Properties of the Peptides Obtained

Peptide	Method of preparation	Yield (%)	mp, °C	$[\alpha]_D^{20}$, deg (c=1) (solvent)	R_f (system_)
II	BSA	65	164—177	+4 (MeOH)	0,6 (2)
	AE				0,13 (4)
II	Me_3SiCl	30	167—170	+3 (MeOH)	0,58 (2)
	AE				0,12 (4)
III	MA	50,3	107—108	−13,5 (MeOH)	0,58 (3)
					0,23 (4)
IV	—	97,2	175—176	−21,0 (MeOH)	0,39 (2)
					0,26 (3)
					0,15 (4)
V	MA	92	97,5—98	−47,0 (MeOH)	0,83 (4)
VI	—	97	147,5—149	−10,5 (DMFA)	0,5 (2)
				−26 (MeOH)	0,1 (4)
VII	MA	91,7	Oil	−52 (MeOH)	0,68 (4)
VII	AE	93	Oil	−58 (MeOH)	0,81 (3)
VIII	—	70	170—173	−18,5 (DMFA)	0,15 (4)
IX	Azide	74,6	96—98	−50 (MeOH)	0,85 (2)
					0,2 (4)
X	—	92,6	166—169	−6 (DMFA)	0,4 (1)
					0,08 (2)
XI	Azide	78	139—141	−26 (MeOH)	0,6 (2)
					0,3 (4)
XII	—	95,3	115—117	−21,5 (DMFA)	0,37 (2)
XIII	Azide	45,5	102—106	−30 (DMFA)	0,9 (2)
					0,25 (4)
XIV	—	90	—	−19 (DMFA)	0,19 (2)
I·HCl	Scheme 1	30	146,5—148,5	−34,5 (DMFA)	0,31 (2)
I	Scheme 2	39,2	139,5—140,5	−36 (DMFA)	0,29 (2)

For (XII), $[\alpha]_D^{20}$ −15° (c = 1; DMFA), mp 136-137°C [10].

TABLE 2. Chemical Shifts in the ^{13}C NMR Spectra of Solutions of Peptides (II, IV, V, VII-IX, and XIII) in DMSO-d^6 Relative to TMS*

Amino acid residue	Nucleus	II	IV	V	VII	VIII	IX	XIII
Asn	C_0	171,41						
	C_α	5 ,93 [a]						
	C_β	37,51						
	C_γ	171.41						
Lys	C_0	173,46						171,91
	C_α	51,62 [a]						53,66
	C_β	30,77						31,63
	C_γ	22,58						22,76
	C_δ	29,09						29,22
	C_ε	40,30						†
Phe	C_0		171,18				171.44	170,91 [c]
	C_α		56,30				56,35	54,94
	C_β		37,51					
	C_1		138,14				138 23	137,68
	C_2		128,13				128,08	128,08
	C_3		129,22				129,22	129,31
	C_4		126,30				126,30	126,40
His	C_0		170,23				170,91	170,73 [c]
	C_α		51,66				52,89 [b]	52,94 [d]
	C_β		30,00				29,54	29,22
	C_γ		134,63				134,86	134,82
	C_{δ_2}		117,02				116,70	116,93
	C_{ε_1}		133,27				133,54	132,86
Thr	C_0				170,14	167,00	169,86	169,86
	C_α				60,40	57,85	58,08	58,08
	C_β				66,82	65,95	66,41	66 50
	C_γ				19,80	19,66	19,48	19,53
Phe	C_0			170,46	169 68	169,32	169,59	169,59
	C_α			53,71	51,75	52,48	52,21 [b]	52,16 [d]
	C_β			36,50	37,10	36,70	†	†
	C_1			137,87	137,32	137,09	137,46	137,46
	C_2			128,04	128,08	128,36	128,08	128,08
	C_3			129,26	129,35	129,22	129,22	129,31
	C_4			126,26	126,35	126,62	126,30	126,40
Pro	C_0			171,64	171,55	171,46	171,55	171,55
	C_α			58,76	58,81	58,85	58,81	58,85
	C_β			28,49	28,63	28,63	28,58	28,58
	C_γ			24,67	24,58	24,63	24,53	24,58
	C_δ			46,38	46,56	46,61	46,47	46,52
Z	C=O	155,75	155,94				155,94	155,94
	CH_2	65,50	65,36				65,32	65,50
	C_1	137,00	137,05				137,05	137,00
	C_2	127,76	127,44				127,40	127,81
	C_3	128,35	128,35				128,31	128,35
	C_4	127,76	127,72				127,67	127,81
BOC	C=O	155,62		155,25	155,30			155,62
	$-C-$	77,42		78,06	78,33			77 42
	CH_3	28,31		28,08	28,13			28 31
Bzl	CH_2			65,82	65,91	65,95	65,82	65,86
	C_1			136,05	136,09	136,05	136,04	136,04
	C_2			127,76	127,81	127,86	127,76	127,81
	C_3			128,40	128,49	128,49	128,44	128,44
	C_4			128,04	128,08	128,13	128,03	128,08

*The assignment of the signals marked a–a, b–b, c–c, and d–d may be reversed.

†Signal not observed becuase of overlapping with a signal from the solvent.

The assignment of the signals of the C_α atoms and the C atoms of the side chains was made on the baiss of literature information [4-6] and by comparing the chemical shifts in the series of compounds under consideration. To assign the signals of the C atoms of the carbonyl groups we also used the spectra of mixtures of deuterated and nondeuterated peptides (VII) and (IX), which permitted the signals of the C=O groups of threonine and of the phenylalanine residue adjacent to it, No. 22, to be distinguished.

The complete assignment of the signals in the spectrum of the final heptapeptide (I) proved to be impossible in view of the absence of shorter model peptides with a protected NH_2 group and a free COOH group of a proline residue. However, analysis of the spectrum (its general form, the number of signals observed, their chemical shifts and interval intensities, and the presence of characteristic signals of each amino acids residue) and a comparison of it with the spectra of the pentapeptide (IX) and the hexapeptide (XIII) convincingly confirmed the structure of the heptapeptide (I) and its purity.

The following signals were observed in the ^{13}C NMR spectrum of the heptapeptide (I): 173.14 ppm, C=O of Pro; 171.94, 171.59, 171.59, 171.05, 170.27 ppm, C=O of Asn, Lys, Phe No. 19, and His, and C_γ of Asn; 169.68 and 169.32 ppm, C=O of Phe No. 22 and Thr; 155.57 ppm, C=O of the Boc group; 137.87, 137.36, 136.91 ppm, C_1 of the Z group, of Phe No. 19, and of Phe No. 22; 134.13 and 131.18 ppm, C_γ and C_{ε_1} of His; 129.40-126.35 ppm, a group of signals relating to C_{Ar}; 117.02 ppm, C_{δ_2} of His; 77.42 ppm, quaternary C of the Boc group; 66.59 ppm, C_β of Thr; 65.59 ppm, CH_2 of the Z group; 58.81 and 58.31 ppm, C_α of Pro and Thr; 54.21, 53.25, 52.16, 52.16, and 51.80 ppm, C_α of Asn, Lys, Phe No. 19, Phe No. 22, and His; 46.43 ppm, C_δ of Pro; 31.36 ppm, C_β of Lys; 29.22, 29.22 ppm, C_β of His and C_δ of Lys; 28.68 ppm, C_β of Pro; 28.27 ppm, CH_3 of the Boc group; 24.53 ppm, C_γ of Pro; 22.35 ppm, C_γ of Lys; 19.48 ppm, C_γ of Thr. The signals of C_ε of Lys, and C_β of Phe No. 19 and Phe No. 22 fell under a signal from the solvnet.

EXPERIMENTAL

The melting points of the peptides were determined in open capillaries without corrections, and the angles of optical rotation on a polarimeter. The chromatographic purity and mobility of the peptides obtained were determined by the TLC method on Silufol plates in the following systems (ratio by volume): 1) EtOAc–pyridine–AcOH–H_2O (3:2:0.6:1.1); 2) EtOAc–pyridine–AcOH–H_2O (3:1:0.3:0.55); 3) EtOAc–pyridine–AcOH–H_2O (3:0.5:0.15:0.275); 4) EtOAc–pyridine–AcOH–H_2O (3:0.25:0.075:0.1375).

The ^{13}C spectra of solutions of the peptides (c = 100 mg/ml) in DMSO-d_6 were recorded on a WP-80DS spectrometer (Bruker) with a working frequency of 20.115 (MHz). Conditions of recording the spectra with broad-band suppression of proton couplings: volume of the memory for the accumulation of the spectrum 8 K and for reproduction 8 K; machine resolution 0.45 Hz (0.023 ppm); length of a pulse 3 μsec (25°C); time interval between the scanning pulses 1.1 sec; number of accumulations 3000-70,000. The chemical shifts were reckoned from the signal of the solvent and were converted to the δ scale relative to tetramethylsilane (TMS) from the formula $\delta_{TMS} = \delta_{DMSO\text{-}d_6} + 39.60$ ppm. The substitution of the mobile hydrogen atoms in the peptides (VII) and (IX) was carried out as described in [4]. For the preparation of the solutions, commercial DMSO-d_6 was used without the preliminary elimination of water. In the investigation of the deuterated peptides, DMFA-d_7 with a negligibly small water content was used as solvent.

The following amino acid derivatives were used in the work: ZAsnOH, HHisOMe·2HCl, BocThrOH·DCHA, HLys(Boc)OH, and ZPheOH from Reanal; ZLys(Boc)OH, BocPheOH, and HPheOMe·HCl from Reakhim; and the condensing agent DCC from Ferak, BuOCOCl – ch ["pure"], Me_3SiCl – ch, and CF_3COOH –ch. HProOBZl·HZl was obtained as described in [7], N-bis(trimethylsilyl)acetamide (BSA) as in [8], and isoamyl nitrite as in [9]. N-Hydroxysuccinimide (SuOH) from Reakhim was recrystallized from EtOAc. ZLys(Boc)PheHisOMe (XI) was obtained in accordance with [10].

Preparation of ZAsnLys(Boc)OH (II). With stirring at 0°C, 2.7 g ($1.2 \cdot 10^{-2}$ mole) of DCC was added in portions to a solution of 2.7 g ($1 \cdot 10^{-2}$ mole) of ZAsnOH and 1.4 g ($1.2 \cdot 10^{-2}$ mole) of SuOH in 10 ml of DMFA, and stirring was continued at room temperature for 2 h; the urea that deposited was filtered off, the precipitate was washed with CH_2Cl_2 (2 × 15 ml), and the filtrate was evaporated at 15 mm Hg. To a solution of ZAsnOSu in DMFA was

added a solution obtained from 2.2 g ($9.1 \cdot 10^{-3}$ mole) of HLys(Boc)OH and 4.9 ml ($2 \cdot 10^{-2}$ mole) of BSA in DMFA at 40°C for 1 h or from 2.2 g ($9.1 \cdot 10^{-3}$ mole) of HLys(Boc)OH, 2.8 ml ($2.2 \cdot 10^{-2}$ mole) of Me_3SiCl, and 3.2 ml of Et_3N in 25 ml of $CHCl_3$ at room temperature for 12 h. The reaction mixture was stirred at room temperature for 24 h. Then 200 ml of a mixture of H_2O and ether (1:2) was added; the precipitate that deposited was filtered off, washed with ether, and dried at 40°C/0.1 mm Hg. This gave 2.9 g ($5.9 \cdot 10^{-3}$ mole) of compound (II) (Tables 1 and 2).

Preparation of ZPheHisOMe (III). To a solution of 5 g ($1.67 \cdot 10^{-2}$ mole) of ZPheOH in 20 ml of CH_2Cl_2 were added 2.6 ml of Et_3N and, at −25°C, 2.3 ml of BuOCOCl. The reaction mixture was stirred at −20°C for 20 min and then a solution obtained from 4.45 g ($1.84 \cdot 10^{-3}$ mole) of HHisOMe·2HCl and 5.2 ml of Et_3N in 25 ml of $CHCl_3$ was added. The reaction mixture was stirred at −5 to 0°C for 2 h and was kept at this temperature for 14 h. Then 100 ml of $CHCl_3$ was added and the product was washed successively with a mixture of 0.1 N HCl and a saturated solution of NaCl (1:1) (2 × 30 ml), with saturated NaCl solution (2 × 30 ml), with saturated $NaHCO_3$ solution (3 × 50 ml), and with saturated NaCl solution again (2 × 30 ml). The organic solution was dried with anhydrous Na_2SO_4, the solvent was evaporated off at 15 mm Hg, and the residue was treated with 200 ml of ether. The resulting precipitate was filtered off and was washed with ether (2 × 30 ml). The compound (III) was dried at 40°C/0.1 mm Hg, giving 3.8 g ($8.4 \cdot 10^{-3}$ mole) of compound (III) (Table 1).

Preparation of ZPheHisN$_2$H$_3$ (IV). A solution of 3.6 g ($8 \cdot 10^{-3}$ mole) of (III) in 5 ml of MeOH was treated with 3.9 ml of hydrazine hydrate. The reaction mixture was stirred at room temperature for 10 min, and the precipitate was filtered off, washed with ether (2 × 30 ml), and dried at 40°C/0.1 mm Hg. This gave 3.5 g ($7.77 \cdot 10^{-3}$ mole) of (IV) (Tables 1 and 2).

Preparation of BocPheProOBzl (V). To a solution of 15 g ($5.65 \cdot 10^{-2}$ mole) of BocPheOH in 35 ml of EtOAc were added 8.7 ml of Et_3N and at −25°C, 7.9 ml of BuOCOCl. The reaction mixture was stirred at −20°C for 10 min, and then $6.2 \cdot 10^{-2}$ mole of HProOBzl, obtained from 25 g ($6.2 \cdot 10^{-2}$ mole) of HProOBzl·HCl and 8.7 ml of Et_3N in 40 ml of $CHCl_3$, was added. The reaction mixture was stirred at −5 to 0°C for 2 h and was kept at this temperature for 14 h. Then 200 ml of $CHCl_3$ was added and it was washed successively with H_2O (2 × 30 ml), 0.1 N HCl (3 × 30 ml), H_2O (2 × 30 ml), saturated $NaHCO_3$ solution (2 × 50 ml), and H_2O (2 × 30 ml). The organic solution was dried with anhydrous Na_2SO_4 and the solvent was evaporated off at 15 mm Hg. The residue was treated with 100 ml of hexane. The precipitate was filtered off, washed with hexane, and dried at 40°C/0.1 mm Hg. This gave 23.5 g ($5.2 \cdot 10^{-2}$ mole) of (V) (Tables 1 and 2).

Preparation of HCl·HPheProOBzl (VI). A solution of 15.6 g ($3.45 \cdot 10^{-2}$ mole) of (V) in 20 ml of EtOAc was treated with 34.5 ml of a 4 N solution of HCl in dioxane and the mixture was stirred at room temperature for 2 h. Then 200 ml of ether was added and the resulting precipitate was filtered off and washed with ether (2 × 20 ml) and was dried at 40°C/0.1 mm Hg. This gave 13.0 g ($3.34 \cdot 10^{-2}$ mole) of (VI) (Table 1).

Preparation of BocThrPheProOBzl (VII). AE Method. A solution of 8.6 g ($2.15 \cdot 10^{-2}$ mole) of the BocThrOH·DCHA salt in 100 ml of EtOAc was washed successively with 1 N H_2SO_4 (2 × 30 ml) and H_2O (2 × 30 ml) to pH 6-7; the EtOAc solution was dried with anhydrous Na_2SO_4, the solvent was evaporated off at 15 mm Hg, and the residue was dissolved in 30 ml of dioxane, and to this solution were added 2.72 g ($2.36 \cdot 10^{-2}$ mole) of SuOH and, at 5°C, in portions, 5.3 g ($2.58 \cdot 10^{-2}$ mole) of DCC. The reaction mixture was stirred at room temperature for 20 h, the urea that had deposited was filtered off and was washed with dioxane (2 × 10 ml), and the filtrate was evaporated at 15 mm Hg. To the residue was added 7.6 g ($1.95 \cdot 10^{-2}$ mole) of (VI), the mixture was dissolved in 10 ml of DMFA, 2.7 ml of Et_3N was added, and the resulting reaction mixture was stirred at room temperature for 24 h; then 200 ml of $CHCl_3$ was added and it was wahsed successively with 0.1 N HCl (3 × 20 ml) and H_2O (3 × 25 ml). The organic solution was dried with anhydrous Na_2SO_4 and was evaporated at 15 mm Hg. This gave 10.04 g ($1.8 \cdot 10^{-2}$ mole) of (VII) (Tables 1 and 2).

MA Method. To the mixed anhydride obtained from $2.18 \cdot 10^{-2}$ mole of BocThrOH, 3.36 ml of Et_3N, and 3.24 ml of BuOCOCl in 30 ml of EtOAc at −20°C over 30 min was added a solution obtained from 9.3 g ($2.4 \cdot 10^{-2}$ mole) of (VI) and 3.36 ml of Et_3N in 35 ml of $CHCl_3$ at −25°C. The reaction mixture was stirred at −5°C for 2 h and was left at 0°C for 20 h. Then 150 ml of $CHCl_3$ was added and it was washed with 0.1 N HCl (3 × 30 ml) and with H_2O (3 × 30

ml). The organic solution was dried with anhydrous Na_2SO_4 and evaporated at 15 mm Hg. The residue was dried at 40°C/0.1 mm Hg, giving 11.1 g ($2 \cdot 10^{-2}$ mole) of (VII) contaminated with a substance having R_f 0.9 (4) (Tables 1 and 2).

Preparation of HThrPheProOBzl·CF_3COOH (VIII). At −20°C, 28 ml of CH_3COOH was added to 10 mg ($1.8 \cdot 10^{-2}$ mole) of (VII), and the mixture was kept at room temperature for 1.5 h. Then the CF_3COOH was evaporated off and 15 mm Hg, 0.5 liter of ether was added to the residue, and the mixture was kept at 0°C for 12 h. The precipitate was filtered off, washed with ether (2 × 30 ml), and dried at 40°C/0.1 mm Hg. This gave 7.15 g ($1.26 \cdot 10^{-3}$ mole) of (VIII) (Tables 1 and 2).

Preparation of ZPheHisThrPheProOBzl (IX). At −25°C, 2.7 ml of 4 N HCl in dioxane and 2.9 ml of isoamyl nitrite were added to a solution of 1.95 g ($4.32 \cdot 10^{-3}$ mole) of (IV) in 15 ml of DMFA, and the mixture was stirred at −25°C for 25 min, after which 1.5 ml of Et_3N, 2.7 g ($4.76 \cdot 10^{-3}$ mole) of (VIII), and 0.67 ml of Et_3N were added. The reaction mixture was stirred at 0°C for 1 h, and then 0.6 ml of Et_3N was added and, after being kept at the same temperature for 20 h, it was poured into 200 ml of H_2O. The precipitate was filtered off and dissolved in 150 ml of $CHCl_3$, and the solution was washed with H_2O (2 × 30 ml). The organic solution was dried with anhydrous Na_2SO_4, the solvent was evaporated off at 15 mm Hg, and 150 ml of ether was added to the residue. After the decantation of the ether, the product was dried at 40°C/0.1 mm Hg, giving 2.8 g ($3.22 \cdot 10^{-3}$ mole) of (IX) (Tables 1 and 2).

Preparation of HPheHisThrPheProOH (X). A solution of 2.6 g ($3 \cdot 10^{-3}$ mole) of (IX) in 30 ml of MeOH was treated with 0.2 g of palladium and was subjected to hydrogenation at 40°C for 8 h. The catalyst was filtered off, the filtrate was evaporated, the residue was dissolved in 30 ml of H_2O, and the solution was washed with $CHCl_3$ (2 × 10 ml). The aqueous solution was evaporated at 5 mm Hg and the compound (X) was dried at 40°C/0.1 mm Hg. The yield of (X) was 1.8 g ($2.78 \cdot 10^{-3}$ mole) (Tables 1 and 2).

Preparation of ZLys(Boc)PheHisN_2H_3 (XII). A solution of 2.1 g ($3.09 \cdot 10^{-3}$ mole) of (XI) in 20 ml of MeOH was treated with 1.5 ml of hydrazine hydrate. The reaction mixture was stirred at room temperature for 24 h, and then 250 ml of ether was added and the precipitate was filtered off and washed with ether (2 × 30 ml). The (XII) was dried at 40°C/0.1 mm Hg, the yield being 2 g ($2.95 \cdot 10^{-3}$) (Table 1).

Preparation of ZLys(Boc)PheHisThrPheProOBzl (XIII). Compound (XIII) was obtained by the azide method from (XII) and (XVII) in a similar manner to the peptide (IX) (Tables 1 and 2).

Preparation of HLys(Boc)PheHisThrPheProOH (XIV). Compound (XIV) was obtained by the catalytic hydrogenation of (XIII) in a similar manner to the preparation of peptide (X) (Tables 1 and 2).

Preparation of ZAsnLys(Boc)PheHisThrPheProOH (I). Method 1 (Scheme 1). With stirring, 0.73 g ($3.56 \cdot 10^{-3}$ mole) of DCC was added to a solution of 1.6 g ($3.24 \cdot 10^{-3}$ mole) of (II) and 0.41 g ($3.56 \cdot 10^{-3}$ mole) of SuOH in 10 ml of DMFA at −10°C, and stirring was continued at room temperature for 12 h; the urea that had precipitated was filtered off, the precipitate was washed with CH_2Cl_2 (2 × 15 ml), and the filtrate was evaporated at 15 mm Hg. To the ZAsnLys(Boc)OSu obtained, in 10 ml of DMFA, was added 1.5 g ($2.3 \cdot 10^{-3}$ mole) of (X) and 0.4 ml of Et_3N, and the mixture was kept at room temperature for 24 h. Then 200 ml of ether was added, the precipitate was filtered off and was dissolved in 100 ml of $CHCl_3$, and the solution was washed with 0.1 N HCl (3 × 15 ml), with saturated NaCl solution (2 × 15 ml), with a 1:1 mixture of 10% Na_2CO_3 and saturated NaCl solution (3 × 15 ml), with saturated NaCl solution (2 × 15 ml), with 0.1 N HCl (2 × 15 ml), and again with saturated NaCl solution (2 × 15 ml). The organic solution was dried with anhydrous Na_2SO_4, the solvent was evaporated off at 15 mm Hg, and 100 ml of ether was added to the residue. The resulting precipitate was filtered off, and it was washed with ether (2 × 20 ml) and with EtOAc (2 × 15 ml) and was dried at 40°C/0.1 mm Hg. This gave 0.8 g ($6.9 \cdot 10^{-4}$ mole) of (I)·HCl (Table 1).

Method 2 (Scheme 2). A solution of 270 mg ($1.02 \cdot 10^{-3}$ mole) of ZAsnOH and 135 mg ($1.17 \cdot 10^{-3}$ mole) of SuOH in 8 ml of DMFA at 0°C was treated with 1.17 ml of a 1 N solution of DCC in CH_2Cl_2 and the mixture was stirred at room temperature for 2 h, after which the urea that had precipitated was filtered off and was washed with CH_2Cl_2 (2 × 15 ml), and the fil-

trate was evaporated at 15 mm Hg. To the ZAsnOSu obtained in 8 ml of DMFA, was added 450 mg ($0.51 \cdot 10^{-3}$ mole) of (XIV), and 0.1 ml of Et_3N, and the mixture was kept at room temperature for 50 h. Then 100 ml of $CHCl_3$ was added and it was washed with H_2O (3 × 20 ml), after which the organic solution was dried with anhydrous Na_2SO_4 and the $CHCl_3$ was evaporated off at 15 mm Hg; the residue was treated with 150 ml of ether and the resulting precipitate was filtered off, washed with ether (2 × 25 ml) and dried at 40°C/0.1 mm Hg. This gave 220 mg ($2.0 \cdot 10^{-4}$ mole) of (I) (Table 1).

SUMMARY

Two schemes for the synthesis of a heptapeptide having sequence 17-23 of human calcitonin with the minimum protection of the lateral functions of the amino acids have been proposed.

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A STUDY OF THE WATER-SOLUBLE PROTEINS OF THE SEEDS OF THE COTTON PLANTS Gossypium hirsutum AND G. Barbadense

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UDC 547.965:633.511

It has been shown by electrophoresis in polyacrylamide gel that different enzymes with similar electrophoretic mobilities may be localized in one and the same zone of water-soluble cottonseed proteins. Some zones not stained by the usual protein dyes also possess enzymatic activity. It has been established that the majority of the electrophoretic fractions consist of a series of polypeptides with different molecular weights, mainly of low-molecular-weight nature. The results are given of an investigation of the peroxidase, α-amylase, lipase, NADH-DCPIP oxidoreductase, and NADPH-DCPIP oxidoreductase activities of the water soluble fraction of the proteins of the seeds of the species mentioned.

The water soluble proteins of cotton seeds are separated on electrophoresis in polyacrylamide gel (PAAG) in the alkaline pH range into several main and a number of minor components. One of the main components of the proteins from the seeds of Gossypium hirsutum L. and G. barbadense L. is distinguished by its electrophoretic mobility and is inherited as a monofactorial trait [1]. In a study of the water-soluble proteins of cotton seeds by electrophoresis in PAAG in the presence of sodium dodecyl sulfate (NaDDS) more than 20 components were obtained and no differences were detected between the seeds of G. hirsutum and G. barbadense [2].

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Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 488-491, July-August, 1986. Original article submitted March 2, 1986.

0009-3130/86/2204-0456$12.50