Glp-His. A solution of 465 mg (3 mmole) of histidine in 3 ml of 1 N NaOH was treated with 660 mg (3 mmole) of 15-crown-5 and 15 ml of dimethylformamide. The water and 2-3 ml of the dimethylformamide were evaporated off in vacuum. To the residual solution was added a mixture of 387 mg (3 mmole) of pyroglutamic acid, 378 mg (3.3 mmole) of N-hydroxysuccinimide, and 660 mg (3.3 mmole) of dicyclohexylcarbodiimide. The reaction mixture was stirred at room temperature for 24 h and filtered, and the filtrate was evaporated in vacuum at 40-50°C. The residue was neutralized with 3 ml of 1 N HCl solution and, after the water had been evaporated off in vacuum, it was chromatographed on silica gel L 100/250 (0.8 × 75 cm column). Chloroform–methanol (1:1) first eluted 15-crown-5, N-hydroxysuccinimide, and pyroglutamic acid, and then methanol eluted 717 mg (90%) of pyroglutamylhistidine, mp 215-217°C, $[\alpha]_D^{20}$ – 3.26° (c 0.46; CF_3CH_2OH). R_f 0.34 (ethyl acetate–pyridine–acetic acid–water (5:5:1:3) – system 1).

Glp-His-Pro-NH_2. A mixture of 532 mg (2 mmole) of pyroglutamylhistidine, 228 mg (2 mmole) of prolinamide, 253 mg (2.2 mmole) of N-hydroxysuccinimide, and 453 mg (2.2 mmole) of dicyclohexylcarbodiimide in 5 ml of dimethylformamide was stirred for 20 h and was filtered, the precipitate was washed with 2 ml of dimethylformamide, the filtrate was evaporated, and the residue was chromatographed with chloroform–method (1:1). The fraction containing thethyroliberin was treated with 1 ml of acetic acid, the solution was evaporated in vacuum, and the residue was triturated with ethyl acetate. This gave 440 mg (60%) of thyroliberin. Hygroscopic substance, $[\alpha]_D^{20}$ – 58° (c 1; H_2O), R_f 0.74 (system 1).

SUMMARY

1. A two-stage method for the synthesis of thyroliberin has been proposed.

2. The possibility of using complexes of the sodium salts of amino acids with crown ethers for the temporary protection of a carboxylic function has been shown.

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PREPARATION OF ESTERS OF AMINO ACIDS AND OF PEPTIDES UNDER MILD CONDITIONS

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A new method for obtaining esters of N-protected amino acids and peptides from complexes of their sodium salts with 15-crown-5 that are soluble in organic solvents is proposed.

Esters of N-protected amino acids and of peptides are frequently used in peptide synthesis [1]. The most convenient among known methods for their synthesis [2, 3] is their preparation from the cesium salts of the N-protected amino acids or peptides [3]. However, the performance of esterification in a heterogeneous system according to the latest method increases the reaction time and, in a number of cases, lowers the yield.

We have proposed a method for synthesizing esters of N-protected amino acids and peptides (II) in dimethylformamide solution from their sodium salts in the presence of equimolar amounts of 15-crown-5 [1]*

* The nomenclature of the crown ethers is given in accordance with [4].

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TABLE 1. Preparation of Esters of Amino Acids and Peptides

R^1CO_2H	R^2X	$R^1CO_2R^2$	Yield, %	mp, °C	$[\alpha]_D^{25}$ (c, solvent)
Ac-Gly	BzlBr	Ac-Gly-OBzl	93	Oil	—
Z-Glu	BzlBr	Z-Glu (OBzl)-OBzl	86	74—76	—
Z-Asp	BzlBr	Z-Asp (OBzl)-OBzl	88	65—66	—11.8 (1; ethanol)
Z-His	BzlBr	Z-His-OBzl	81	90—92	+9.1 (1; ethanol)
Z-Gly-Gly	BzlBr	Z-Gly-Gly-OBzl	82	Oil	
Z-Gly-Gly	EtI	Z-Gly-Gly-OEt	79	82—83	—
Boc-Gly-Phe	MeI	Boc-Gly-Phe-OMe	79	Oil	—
Z-Gly-Gly	Bu^tCl	Z-Gly-Gly-OBu^t	43	Oil	—

Abbreviations. Ac) Acety l; Z) benzyloxy carbonyl; Boc) tert-butoxycarbonyl; Bzl) benzyl; Me) methyl; Et) ethyl; Bu^t) tert-butyl.

$$R^1CO_2H \;(I) \xrightarrow[2.\ R^2X]{1.\ NaOH,\ 15\text{-}c\text{-}5} R^1CO_2R^2 \;(II)$$

To a solution of a N-protected amino acid (I) in an equimolar amount of an aqueous solution of alkali is added an equimolar amount of crown ether and dimethylformamide, after which the water and 2-3 ml of the dimethylformamide are distilled off in vacuum. After the treatment of the residual solution with an alkyl halide the corresponding ester (II) (Table 1) is obtained. Benzyl esters are formed at room temperature but for the synthesis of methyl, ethyl, and tert-butyl esters heating to 40°C is necessary to decrease the time required.

EXPERIMENTAL

The purity of the compounds was monitored by the TLC method on standard Silufol plates (Kavalier, Czechoslovakia). The substances were detected on the chromatograms with the chlorine:toluidine reagent. The analyses of all the compounds corresponded to the calculated figures. All optically active amino acids were of the L configuration. Reanal (Hungary) N-protected amino acids and peptides were used in the syntheses.

General Procedure for Obtaining Esters of N-Protected Amino Acids and Peptides. A solution of 10 mmole of a N-protected amino acid or peptide in 10 ml of 1 N NaOH solution was treated with 2.2 g (10 mmole) of 15-crown-5 and 15 ml of dimethylformamide. The water and 2-3 ml of dimethylformamide were evaporated off in vacuum. The residual solution was treated with the appropriate amount of alkyl halide (15 mmole of benzyl bromide; 30 mmole of methyl iodide, ethyl iodide, or tert-butyl chloride) and the mixture was kept at 40°C for 2 h (to obtain benzyl esters, esterification can be performed at room temperature). After the elimination of the solvent in vacuum, the residue was treated with ethyl acetate, and the organic solution was washed with water and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated. This gave the ester of the N-protected amino acid or peptide.

SUMMARY

A method is proposed for obtaining methyl, ethyl, tert-butyl, and benzyl esters of N-protected amino acids and peptides.

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COMPARATIVE STUDY OF THE CYTOTOXINS OF THE VENOM OF THE CENTRAL ASIAN COBRA *Naja naja oxiana* BY THE INTRINSIC-FLUORESCENCE METHOD

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The intrinsic fluorescence of cytotoxin V_c1 and V_c5 from the venom of the Central Asian cobra due to the tyrosine residues and differing with respect to quantum yield for these two polypeptides because of the nonidentity of the microenvironments of the tyrosyl residues has been measured and characterized. The dependence of the quantum yield on the pH and the temperature also indicate this nonidentity.

Membrane-active substances – cytotoxins V_c1 and V_c5, which are polypeptides consisting of 60 amino acid residues with molecular weights of about 7000 D – have been isolated from the venom of the Central Asian cobra [1]. Cytotoxin V_c1 contains three tyrosine residues, and V_c5 contains two [2]. The interaction of the cytotoxins with membranes takes place in two stages: the adsorption of the cytotoxin molecules on the membrane surface takes place through electrostatic forces, and then the free hydrophobic parts of the cytotoxin molecules penetrate within the hydrophobic region of the membranes causing structural rearrangements of them [3, 4]. This shows that the spatial organization of the cytotoxin molecules has a definite significance in their functioning. The method of intrinsic fluorescence is an effective one for studying the structure of proteins in solution [5, 6], and we have used it for studying these cytotoxins.

The position of the maximum and the half-width of the fluorescence spectrum of each cytotoxin, on excitation at 280.4 nm, are 305 and 33 nm, respectively. The quantum yields at 25°C and pH 6.0 are 1.67 ±0.04% and 1.27 ±0.04% for cytotoxins V_c1 and V_c5, respectively. The spectral parameters show that the intrinsic fluorescence of the cytotoxins is determined completely by tyrosine residues which is in agreement with the fact that there are no tryptophan residues in the cytotoxin molecules [2]. They therefore belong to fluorescent class A [6]. Since the quantum yields of the two cytotoxins, like those of other proteins of class A, are an order of magnitude lower than the fluorescence yield of free tyrosine, it may be considered that the tyrosine in the cytotoxins is subjected to quenching by the side chains of amino acids and (or) by peptide groups (having hydrogen bonds with the hydroxyl of tyrosine [7]) forming their microenvironment [6]. Apparently, the difference in the quantum yields of V_c1 and V_c5 may be a consequence of the fact that the two tyrosine residues in V_c5 and the three in V_c1 have different microenvironments. Bearing in mind the primary structures of analogous cytotoxins from other courses and their homology with cytotoxins from the venom of the Central Asian cobra, it may be expected that two of the three tyrosyls in V_c1 will have similar quantum yields to the two tyrosyls in V_c5 and their microenvironment may lead to the quenching of the fluorescence to a greater degree than for the third tyrosyl of V_c1. This tyrosyl is Tyr^{11}, as follows from an analysis of the primary sequence of the polypeptide chains of the cytotoxin molecules [2].

Among the factors causing the quenching of fluorescence in proteins, an important role is played by charged residues, and the charge of a cytoxin molecule has functional significance. It therefore appeared of interest to determine the influence of the pH of the

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