Isolation of the Product of the Transformation of Phytin from Roasted Rice Middlings. Two 300-g samples of rice middlings were taken. The first was covered with 800 ml of 2% nitric acid and the mixture was carefully stirred and filtered with suction, and the pump was washed with 1000 ml of water.

To the second sample (300 mg) was added the acid extract obtained from the first sample, the mixture was carefully stirred and filtered with suction, and the pulp was washed with the second portion of extract. The mixture was made alkaline with ammonia to pH 8.0, and the phytin was filtered off with suction and washed with water (alkaline mother liquor A).

The yield of technical phytin and phosphate was 19.8 g, which amounts to 3.3% of the weight of the air-dry rice middlings.

On standing, the alkaline mother liquor A deposited small crystals of phosphate. Yield 1.25 g. The phytin transformation product (1.25 g) was dissolved in a small amount of nitric acid, the solution was filtered, the filtrate was made alkaline with ammonia to pH 8.0, and the phosphate that deposited was filtered off with suction. Yield 1.23 g. After two reprecipitations of the technical phytin from the alkaline mother liquor 1.5 g of technical phosphate was obtained.

SUMMARY

1. It has been found that when phytin is heated at 160-200°C for 3 h no phosphate is formed. It is possibly formed at a high temperature and a high pressure by the interaction of phytin with other substances present in the rice middlings.

2. The transformation product of phytin is absent from unroasted rice middlings.

3. It has been established that the fermentation process takes place in the alkaline solution obtained from an acid extract containing formalin; consequently, the use of formalin in the production of phytin is undesirable.

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PREPARATION OF TERT-BUTOXYCARBONYL DERIVATIVES OF AMINO ACIDS USING DI-TERT-BUTYL PYROCARBONATE

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In order to optimize and individualize the process, the influence of conditions of the reactions on the synthesis of Boc derivatives of amino acids using di-tert-butyl pyrocarbonate on the yield of desired product has been studied.

The modern state of peptide synthesis is characterized by the fact that an ever-increasing number of biologically active compounds of peptide nature are becoming products of industrial production. A consequence of this fact is an increase in the demand for the accessibility of auxiliary reagents and, in particular, for the main intermediates of peptide syn-

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thesis — N-protected amino acid derivatives. Few of the amino-protective groupings known at the present time satisfy the requirements of industry. The tert-butoxycarbonyl (Boc) protective group has acquired the greatest value among them in recent times.

The discovery of a new tert-butoxycarbonylating reagent — di-tert-butyl pyrocarbonate (Boc_2O) — has had a fundamental influence on the expansion of the production of Boc-(amino acid)s. tert-Butoxycarbonyl pyrocarbonate is distinguished from reagents known previously by greater accessibility, ease of purification, stability on storage, and high N-acylating capacity, and its reactions form no byproducts interfering with the purification of the Boc derivatives or contaminating the environment. All this has led to a comparatively rapid introduction of the manufacture of Boc_2O both by foreign firms and by the home industry (NPO "Biokhimreaktiv").

Since the time when the use of Boc_2O for obtaining Boc-(amino acid)s was first mentioned [1], several reports have been published showing the high efficacy of the use of this reagent as a donor of a Boc group [2-4]. However, in these publications comparatively little attention has been devoted to the study of the conditions for performing the reactions of Boc_2O with amino acids. At the present time, in view of the use of the method in industry, the task of optimizing the process of the tert-butoxycarbonylation of amino acids with Boc_2O is acquiring particular importance.

In the present paper we give the results of further investigations on the synthesis of Boc derivatives of some amino acids, materials on the synthesis of which with the use of Boc_2O have not been published previously, and also of trifunctional amino acids with protected side functions. In addition, in order to study the possible routes of individualization of the process of introducing a Boc group an attempt has been made to determine the influence of the nature of the salt-forming alkaline reagent and of the organic solvent on the yield of desired product. As a result of investigations in this direction, it has been found that with some change in the conditions of the reaction as compared with those used previously [2] (replacement of sodium hydroxide by potassium carbonate, and of dimethylformamide by isopropanol) and at the same ratio of reagents (20% excess of Boc_2O), the yield of Boc-asparagine rises to 87%. Apparently, the use of the potassium carbonate as salt-forming agent and the performance of reaction in aqueous isopropanol are desirable in the preparation of the Boc derivative of many amino acids [4]. Under these conditions we have obtained high yields of Boc-hydroxypyroline, Boc-isoleucine, and di-Boc-ornithine. However, in a number of cases the use of sodium hydroxide and triethylamine also gives very good results.

In the reaction of cysteine with an equimolar amount of Boc_2O in an alkaline medium, a mixture of N-Boc and S-Boc derivatives is formed and it has not yet been possible to achieve selective N- or S-aceylation. S-Protected cysteine derivatives readily react with Boc_2O with the formation of the corresponding N-Boc derivatives. In order to create an aqueous organic solution we used dimethylformamide, isopropanol, and tert-butanol or dioxane, although no fundamental influence of the nature of the solvent on the yield and rate of the reaction has been detected. The yields of Boc-derivatives of S-substituted cysteines (S-trityl-, S-benzyl-, S-acetamidomethyl-, and S-benzamidomethyl-) are high both in the presence of sodium hydroxide and with triethylamine; as a rule, they are substantially higher and the reaction time is shorter than with the use of other tert-butoxycarbonylating reagents [7-10]. With a 30% excess of Boc_2O and the performance of the reaction at room temperature in the presence of alkali in aqueous isopropanol or with triethylamine in aqueous dioxane, Boc-methionine is obtained with quantitative yield in the form of a chromatographically homogeneous oil.

The product crystallized on cooling and was characterized in the form of the salt with dicyclohexylamine. L-Leucine and L- and D-valines react with Boc_2O in the form of triethylammonium salts very vigorously, and their Boc derivatives are also obtained in high yields. In the preparation of Boc-L-leucine on the one-molar scale, the yield of desired product did not fall. Threonine reacts with Boc_2O better in an alkaline medium. Under these conditions, Boc-threonine is obtained with quantitative yield in crystalline form. N^{ε}-Tosyl-L-lysine reacts identically with Boc_2O in the presence of triethylamine and in sodium hydroxide solution; in both cases the Boc derivative is obtained with yield greater than 90%.

The tert-butoxycarbonylation of the copper complex of lysine with di-tert-butyl pyrocarbonate has been described twice previously [2, 3]. When the reaction was performed in aqueous pyridine in the presence of triethylamine, the copper complex of N^{ε}-Boc-lysine was

obtained with a yield of 60% [2]. Moroder et al. [3] reported a yield of 94%, but the details of the experiment were not given. We found that if the reaction is performed in carbonate solution for 7 h, the yield of the copper complex of N^{ε}-Boc-lysine amounts to 85%.

N^{G}-Nitroarginine reacts with Boc_2O in the presence of potassium carbonate in aqueous dimethylformamide fairly slowly even at an elevated temperature (35-40°C) [4]. When the reaction was performed in the presence of three equivalents of triethylamine at room temperature for 45 min, N^{α}-Boc-N^{G}-nitroarginine was obtained with a yield of 87%. However, the best results (yield 94%) were obtained when the reaction was performed in a mixture of isopropanol, dimethylformamide, and water in the presence of two equivalents of caustic soda.

EXPERIMENTAL

The individuality of the compounds synthesized was checked chromatographically on Silufol plates (Czechoslovakia) in the following solvent systems: 1) benzene—acetone—acetic acid (100:50:2), and 2) chloroform—methanol—acetic acid (85:10:5). The solvent front travelled 6-7 cm. The Boc-derivatives of the amino acids were detected with ninhydrin at 120°C. Extracts in organic solvents were dried with anhydrous Na_2SO_4.

Standard Method of Isolating N-Boc Derivatives of Amino Acids. After the end of the reaction, the organic solvents (alcohols, dioxane) were distilled off from the reaction mixture in vacuum (∿40°C), the residue was diluted with water (1.5-fold), saturated with NaCl, and extracted with petroleum ether, and the aqueous solution was acidified with an excess of citric acid or a small excess of $NaHSO_4$ or with 1 N HCl or H_2SO_4 to pH 3-3.5, and was extracted with ethyl acetate. The extract was washed with a solution of NaCl, dried, and evaporated, and the residue was crystallized from appropriate solvents.

N^{α}-Boc-L-Asparagine. A. A solution of 2.7 g (12 mmole) of Boc_2O in 7 ml of isopropanol was added to a solution of L-asparagine monohydrate in 10 ml of 1 N NaOH, and the mixture was stirred at 35-40°C for 1 h. Then the alcohol was distilled off in vacuum, and the residue was cooled to 2-4°C and treated with a solution of 1.5 g of $NaHSO_4$ in 5 ml of water. The mass was diluted with cooled saturated NaCl solution (∿15 ml), and the precipitate that deposited was filtered off, washed with water and with a mixture of isopropanol and ether, and dried in vacuum over P_2O_5. This gave 2.0 g (86.5%) of N-Boc-L-asparagine with mp 177-178°C, $[\alpha]_D^{20}$ − 8.5° (c 1; DMFA) [4, 5].

B. On the gradual addition of a second equivalent of NaOH during 30 min after the addition of the Boc_2O, followed by keeping the reaction mixture at room temperature (20°C) for two hours, N-Boc-L-asparagine was obtained with a yield of 87%.

N-Boc-L-Hydroxyproline. A solution of 1.3 g of L-hydroxyproline and 1.3 g of $KHCO_3$ in 10 ml of water was treated with 5 ml of isopropanol and the mixture was stirred for 5 min, and then a solution of 2.7 ml of Boc_2O in 5 ml of isopropanol was added. The mixture was stirred at 20°C for 30 min and was worked up by the standard method. This gave chromatographically homogeneous N-Boc-L-hydroxyproline in the form of a viscous oil. The oil was dissolved in ether, the solution was mixed with 2 ml of dicyclohexylamine, and the precipitate was filtered off, washed with ether, and dried in vacuum. This gave 3.9 g (95%) of the dicyclohexylammonium salt of N-Boc-L-hydroxyproline with mp 193-195°C, $[\alpha]_D^{20}$ −23.9° (c1; CH_3OH) [5].

N-Boc-S-Benzyl-L-cysteine. A solution of 1.2 g (5.5 mmole) of Boc_2O in 1.5 ml of tert-butanol was added to a solution of 1.05 g (5 mmole) of S-benzyl-L-cysteine in 5 ml of 1 N NaOH. After being stirred at 20°C for 30 min, the reaction mixture was worked up by the standard method. The oily product was crystallized from a mixture of diethyl ether and petroleum ether. This gave 2.0 g (97%) of N-Boc-S-benzyl-L-cysteine with mp 85-87°C, $[\alpha]_D^{20}$ −45.5° (c 0.13; AcOH) [6].

N-Boc-S-Trityl-L-cysteine. A solution of 0.48 g (2.2 mmole) of Boc_2O in 2 ml of tert-butanol was added to a solution of 0.73 g (2 mmole) of S-trityl-L-cysteine in 4 ml of 1 N NaOH and 4 ml of dioxane, and the mixture was stirred at 25°C for 20 min. After working up by the standard method, 0.82 g (88%) of N-Boc-S-trityl-L-cysteine was obtained with mp 65-70°C. The DCHA salt had mp 210°C, $[\alpha]_D^{20}$ +25.8° (c 0.11; C_2H_5OH) [7].

N-Boc-S-Acetamidomethyl-L-cysteine. A solution of 2.28 g (10 mmole) of the hydrochloride of S-acetamidomethyl-L-cysteine in 20 ml of aqueous dioxane (1:1) and 15 ml of 1 N NaOH was treated with 2.4 g (11 mmole) of Boc_2O and the mixture was stirred for 20 min. Working up by the standard method yielded 2.74 g (94%) of crystalline N-Boc-S-acetamidomethyl-L-cysteine.

After recrystallization from ethyl acetate with ether, mp 110-112°C, $[\alpha]_D^{20}$ −31.7° (c 0.3; H_2O) [8].

N-Boc-S-Benzamidomethyl-L-cysteine. A solution of 14.5 g (50 mmole) of the hydrochloride of S-benzamidomethyl-L-cysteine in 200 ml of dimethylformamide was treated with 7 ml of Et_3N, the mixture was stirred for 15 min, the resulting precipitate was filtered off, and the filtrate was treated with 3.5 ml of Et_3N and 15 ml (65 mmole) of Boc_2O and the resulting mixture was stirred for 1 h. After the standard working up, a viscous oil was obtained which crystallized readily on trituration in hexane. The crystalline product was filtered off and was recrystallized from petroleum ether–ethyl acetate. This gave 16.5 g (96%) of N-Boc-S-benzamidomethyl-L-cysteine with mp 138-139°C, $[\alpha]_D^{20}$ −27.5° (c 0.12; CH_3OH) [9].

N-Boc-L-Methionine. A. A solution of 25 g (0.11 mole) of Boc_2O in 25 ml of dioxane was added to a solution of 15 g (0.1 mole) of L-methionine and 27.5 ml of Et_3N (0.2 mole) in 50 ml of dioxane and 10 ml of dimethylformamide, and the mixture was stirred at 20°C for ∿20 min, after which it was worked up by the standard method to give 24.5 g (98%) of Boc-L-methionine in the form of a viscous oil which crystallized at 0-4°C. The DCHA salt, after recrystallization from ethyl acetate, has mp 136-138°C, $[\alpha]_D^{20}$ +17° (c 1; DMFA) [10].

B. In aqueous isopropanol with the same ratios of reagents and with two equivalents of KOH or NaOH the yield of Boc-L-methionine was quantitative.

N-Boc-L-Threonine. A solution of 2.38 g (20 mmole) of L-threonine in 20 ml of 1 N NaOH and 20 ml of dioxane was treated with 6 g (27 mmole) of Boc_2O, and the mixture was stirred at 40°C for 2 h. The standard working up yielded 4.38 g of a crystalline product. After recrystallization from ethyl acetate with petroleum ether, mp 79-81°C, $[\alpha]_D^{20}$ −9.2° (c 0.2; AcOH) [11].

N-Boc-D-Threonine. A mixture of 1.19 g (10 mmole) of D-threonine, 3.5 ml of Boc_2O in 15 ml of 1 N NaOH, and 15 ml of isopropanol was stirred at 40°C for 1.5 h and was then worked up in the standard way to give 1.75 g (80%) of Boc-D-threonine with mp 77-78°C, $[\alpha]_D^{20}$ +8.9° (c 0.2; AcOH). DCHA salt: mp 139-140°C, $[\alpha]_D^{20}$ −10.2° (c 1; CH_3OH) [12].

N-Boc-L-Leucine. A. A suspension of 1.3 g (10 mmole) of L-leucine in 10 ml of water and 10 ml of isopropanol was treated with 4.2 ml of Et_3N and a solution of 3 ml of Boc_2O in 7 ml of isopronanol. The mixture was stirred at 20°C for 30 min, the alcohol was distilled off in vacuum (40°C), and the residue was diluted with water (25 ml) and extracted with petroleum ether. The aqueous solution was cooled (5°C), acidified with 1 N H_2SO_4 to pH 3, and after 4 h (+4°C) the precipitate was filtered off, washed with water, and dried in air, giving 2.48 g (99%) of the monohydrate of Boc-L-leucine with mp 79-82°C, $[\alpha]^{20}$ −23.4° (c 1; AcOH) [4, 5].

B. Similarly, 131 g (1 mole) of L-leucine in 2 liters of aqueous isopropanol, 280 ml of Et_3N, and 284 g (1.3 mole) of Boc_2O yielded 249 g of the monohydrate of Boc-L-leucine with mp 71-74°C.

C. A solution of 284 g (1.3 mole) of Boc_2O in 300 ml of isopropanol was added to a suspension of 131 g (1 mole) of L-leucine in 1 liter of isopropanol, 50 ml of dimethylformamide, and 1 liter of 1 N NaOH. The mixture was stirred for 10 min and left to stand for 1 h, and then 750 ml of 1 N NaOH was added. After 30 min, the alcohol was distilled off, and the residue was diluted with 1.2 liters of distilled water and worked up as in method B. This gave 242 g (98%) of Boc-L-leucine with mp 78-81°C.

N-Boc-L-Isoleucine. A. A solution of 3 ml of Boc_2O in 5 ml of isopropanol was added to a suspension of 1.3 g of L-isoleucine in 10 ml of 1 M K_2CO_3, and the mixture was stirred at 25°C for 1 h. After the standard working up, an oil was obtained which was crystallized from aqueous ethanol. This furnished 3.3 g (96%) of the hemihydrate of Boc-L-isoleucine with mp 57-58°C, $[\alpha]_D^{20}$ +4.0° (c 1; AcOH) [13].

B. A solution of 27 g (0.12 mole) of Boc_2O in 30 ml of isopropanol was added to a solution of 13 g (0.1 mole) of L-isoleucine in 100 ml of 1 N NaOH and 100 ml of isopropanol, the mixture was stirred for 10 min, and a second equivalent of caustic soda was added over 30 min. The mixture was stirred again at 20°C for 30 min and was worked up by the standard method. This gave an oil which was caused to crystallize by trituration in petroleum ether at 0-5°C. This gave 22.4 g (94%) of N-Boc-L-isoleucine with mp 66-68°C [5].

N-Boc-L-Valine. To 1.17 g (10 mmole) of L-valine in 30 ml of aqueous isopropanol (1:1) were added 4.2 ml of Et_3N and a solution of 3 ml (13 mmole) of Boc_2O in 7 ml of isopropanol.

The mixture was stirred at 25°C for 1 h, and after the standard working up 2.2 g of oily Boc-L-valine was obtained. Crystallization from diethyl ether (10 ml) and petroleum ether (30 ml) in the cold provided 2.1 g (95%) of a crystalline product with mp 70-77°C. After recrystallization, from the same solvents, the yield was 1.95 g and the melting point 77-79°C, $[\alpha]_D^{20}$ −6.1° (c 1; AcOH) [5, 11].

N-Boc-D-Valine. A. Under the conditions for the synthesis of Boc-L-valine, Boc-D-valine was obtained with a yield of 88%. mp 76-78°C, $[\alpha]_D^{20}$ +5.3° (c 1; AcOH).

B. With the same ratio of the reactants but in the presence of 10 ml of 1 N NaOH the yield of Boc-D-valine was 78%, mp 77-78°C.

N^{α}-Boc-N^{ε}-Tosyl-L-lysine. A. A solution of 1.5 ml of Boc_2O in 15 ml of isopropanol was added to a solution of 1.5 g (5 mmole) of N^{ε}-tosyl-L-lysine and 2.1 ml of Et_3N in 20 ml of water and 10 ml of dimethylformamide, and the mixture was stirred at 25°C for 40 min. After the standard working up, N^{α}-Boc-N^{ε}-tosyl-L-lysine was obtained in the form of a viscous oil. The oil was dissolved in 10 ml of ether and a solution of 1.2 ml of dicyclohexylamine in 5 ml of petroleum ether was added, to give 2.7 g (93%) of the DCHA salt with mp 133-135°C, $[\alpha]_D^{20}$ +12.0° (c 2; C_2H_5OH) [5].

B. In aqueous dioxane in the presence of one equivalent of NaOH (1 h, 40°C), N^{α}-Boc-N^{ε}-tosyl-L-lysine was obtained with the same results as in method A.

N^{α},N^{δ}-Di-Boc-L-ornithine. A solution of 0.85 g of L-ornithine hydrochloride and 1.4 g of K_2CO_3 in 15 ml of water was treated with 3 ml of Boc_2O in 10 ml of isopropanol, and the mixture was stirred at 40°C for 1 h. After the standard treatment, 2.2 g of an amorphous product was obtained, and this was converted into the dicyclohexylammonium salt. The yield of the dicyclohexylammonium salt of N^{α},N^{δ}-di-Boc-L-ornithine with mp 156-157°C, $[\alpha]_D^{20}$ +12° (c 1; DMFA) was 2.5 g (97%) [14].

Copper Salt of N^{ε}-Boc-L-Lysine. A solution of 37.4 g (0.2 mole) of the copper salt of lysine was treated with 8 g of NaOH and 16.8 g of $NaHCO_3$ in one liter of aqueous tert-butanol, and the mixture was stirred at 20°C for 7 h. The precipitate was filtered off, washed with water (3 × 200 ml), with ethanol (2 × 100 ml), and with ether (2 × 50 ml), and was dried in vacuum. This gave 48 g (87%) of the copper salt of N^{α}-Boc-L-lysine with decomposition temperature 233-236°C [15].

N^{α}-Boc-N^{G}-Nitro-L-arginine. A. A solution of 1.31 g (6 mmole) of N^{G}-nitro-L-arginine in 10 ml of water and 5 ml of DMFA was treated with 2.3 ml (18 mmole) of Et_3N and 1.5 g (7 mmole) of Boc_2O in 5 ml of dioxane, and the mixture was stirred at 20°C for 45 min. After the standard working up and recrystallization from tetrahydrofuran, 1.66 g (87%) of N^{α}-Boc-N^{G}-nitroarginine was obtained with mp 115-119°C, $[\alpha]_D^{20}$ −5.9° (c 2; DMFA) [4].

B. With stirring, 100 ml of a 1 N solution of NaOH and 32.7 ml (0.15 mole) of Boc_2O in 30 ml of isopropanol were added to a suspension of 21.9 g (0.1 mole) of N^{G}-nitro-L-arginine in 100 ml of isopropanol and 50 ml DMFA. Then 100 ml of 1 N NaOH solution was added over 30 min at 20°C, and after 30 min the mixture was worked up by the standard method. The yield of N^{α}-Boc-N^{G}-nitroarginine with $[\alpha]_D^{20}$ −6.1° (c 2; DMFA) was 30 g (94%).

SUMMARY

In order to optimize and individualize the process, the influence of some conditions of the reaction on the synthesis of Boc derivatives of amino acids using di-tert-butyl pyrocarbonate on the yield of the desired products has been studied.

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ACTION OF TRYPSIN ON A NUCLEOPROTEIN FROM STURGEON GONADS

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A nucleoprotein has been isolated from the gonads of the Caspian sturgeon and its composition has been determined. It has been shown that it contains 55% of DNA, 2% of RNA, 36% of protamines, and about 7% of nonprotamine proteins of nonbasic nature. The nucleoprotein has been hydrolyzed with trypsin, and the amino acid compositions of some hydrolysis products have been studied. On the basis of the results obtained, the hypothesis has been put forward of a possible linkage of the DNA with the basic proteins. It has been shown that protamines react with the DNA through the basic amino acid residues located at various regions of their molecules.

One of the approaches to the study of the structure of the chromatin of the somatic cells is the use of proteolytic enzymes, especially trypsin [1].

Trypsin obviously causes the cleavage of the peptide bond mainly at those carboxyl groups of arginine and lysine that are not included in interaction with DNA. This action disturbs the structure of the nucleoprotein complex, a weakening of the bond of certain sections of the protein molecules with the DNA takes place, and this is accompanied by the migration of individual peptide fragments from the complex. Analysis of these fragments, and also of the peptides remaining bound with the DNA on trypsin hydrolysis enables us to determine which of the proteins present in a given type of chromatin interact more strongly with the DNA.

We have used this method to investigate the chromatin from sturgeon gonads.

We have previously studied the primary structure of the proteins of basic nature — protamines — present in this complex [2, 3]. The general characteristics of other components of it have been obtained in the course of the present investigation.

By using trypsin hydrolysis to study the nucleoproteins from sturgeon gonads we hoped to obtain certain information on the nature of the interaction of the components of chromatin and possible sites of contact of the proteins with the DNA.

The nucleoprotein was isolated from sturgeon gonads by the method of Kardykov et al. [4]. At all stages of the isolation process, to prevent proteolysis we added benzenesulfonyl fluoride. In this method of isolation, the bulk of the somatic chromatin passes into solution [4]. We have noted that the nucleoprotein from sturgeon sperm obtained under these conditions proved to be mainly insoluble. Subsequently, all operations were performed with this insoluble fraction of the DNA.

The amounts of DNA and RNA in the DNP, determined by the methods of Spirin [5] and Davidson [6], amounted to 55% and 2%, respectively.

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