SYNTHESIS OF ISOGLUTAMINE, ISOASPARAGINE, AND DERIVATIVES*

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Condensation of N^{α}-benzyloxycarbonyl and N^{α}-tosyl derivatives of glutamic acid and aspartic acid with paraformaldehyde in the presence of benzenesulfonic acid and the *in situ* ammonolysis of resulting oxazolidinones affords the corresponding N^{α}-acylisoglutamines and N^{α}-acylisoasparagines which can be either converted into isoglutamine and isoasparagine or directly used in peptide synthesis.

For investigations on isoglutamine** and isoasparagine peptides, an unambiguous, simple, and quick synthesis of the starting N^{α}-acyl derivatives of isoglutamine and isoasparagine was required. The recommended methods[1-10], though accessible, did not meet our demands.

Some 19 years ago, a method of peptide synthesis based on oxazolidinones of N^{α}-acylamino acids has been reported by two Laboratories[11,12]. The obvious preparative value of this method has been for example demonstrated on the preparation of α-amides of glutamic acid and aspartic acid by Itoh[13]. However, the reported procedure is laborious and affords low yields. In order to check the oxazolidinone approach in detail, some experiments with N^{α}-benzyloxycarbonyl and N^{α}-tosyl derivatives of glutamic acid and aspartic acid have been now carried out. From the two reported methods[11,12] for the preparation of oxazolidinones of acylamino acids, the condensation of acylamino acids with paraformaldehyde in benzene in the presence of a catalytic amount of benzenesulfonic acid was selected. The removal of water from the reaction mixture has been earlier[13] accomplished by an azeotropic distillation; the reaction time for the formation of oxazolidinones has been now considerably shortened by additions of fresh dry benzene during the distillation. Furthermore, in the ammonolysis of oxazolidinones, methanolic ammonia has been replaced by aqueous ammonia. With the use of these modifications, the synthesis of benzyloxycarbonyl-D-isoglutamine, benzyloxycarbonylisoasparagine, and tosyl-

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** Unless stated otherwise, all the optically active amino acids are of the L series.

isoglutamine was surprisingly simple and satisfactory with respect to the yields. As useful intermediates in the synthesis of peptides, the two benzyloxycarbonyl derivatives become readily accessible by a two-step synthesis from glutamic acid or aspartic acid. The protecting group of benzyloxycarbonyl-D-isoglutamine and benzyloxycarbonylisoasparagine was removed by the action of hydrogen bromide in acetic acid[14] with the formation of pure hydrobromides of D-isoglutamine and isoasparagine. The two hydrobromides did not contain even a trace of ω-amides. The attempted removal of the tosyl residue from tosylisoglutamine by means of hydrogen bromide in acetic acid afforded the hydrobromide of aminoglutarimide as the only product. On the other hand, the tosyl residue may be readily removed with sodium in liquid ammonia[15]. When compared with other methods, the present oxazolidinone method represents the most advantageous approach to isoglutamine, isoasparagine, and their derivatives.

EXPERIMENTAL

Melting points (uncorrected) were taken on a heated microscope stage (Kofler block). Optical rotations were measured on a Perkin-Elmer Type 141 polarimeter. The purity of products was checked by paper electrophoresis in a veronal buffer solution (ref.[6]) and on an amino-acid analyser Type 6020 (Developmental Workshops, Czechoslovak Academy of Sciences, Prague) according to ref[16]. Analytical samples were dried at 100°C/0·1 Torr for 10 h.

Benzyloxycarbonyl-D-isoglutamine

A mixture of benzyloxycarbonyl-D-glutamic acid (11·2 g; 40 mmol), benzenesulfonic acid (0·5 g), paraformaldehyde (2·0 g), and benzene (300 ml) was subjected to a slow distillation (1—3 drops of the distillate per second) with the use of a Liebig condenser equipped with a dropping funnel. The benzene in the distillate was substituted by occasional additions of fresh dry benzene into the reaction mixture through the dropping funnel. When the water was removed (after about 30 min), the mixture was evaporated under diminished pressure. Aqueous ammonia (27%, 40 ml) was added to the residual oil, the mixture kept at room temperature for 1 h, diluted with an equal volume of water, filtered with active charcoal, and the filtrate adjusted to pH 2 with hydrochloric acid. The precipitate solidified to crystals in the course of several minutes. The mixture was kept at 0°C for 1 h, the crystals collected with suction, washed on the filter with water, and dried. Yield, 9·3 g (83%); m.p. 173—175°C. This material was recrystallised from water to afford 8·4 g (75%) of benzyloxycarbonyl-D-isoglutamine, m.p. 175—176°C, $[\alpha]_D^{20}$ +5·9° (*c* 2·0; methanol). Reported[17], m.p. 175—176°C, $[\alpha]_D^{27}$ +6·1° (*c* 0·8; methanol).

Benzyloxycarbonylisoasparagine

A mixture of benzyloxycarbonylaspartic acid (3·34 g; 12·5 mmol), paraformaldehyde (0·5 g), benzenesulfonic acid (0·15 g), and benzene (100 ml) was processed analogously to the preceding paragraph. Yield, 3·10 g (93%) of benzyloxycarbonylisoasparagine, m.p. 165—166°C. Optical rotations were taken with the use of a product recrystallised from water (the m.p. value did not change): $[\alpha]_D^{25}$ +4·5° (*c* 0·7; CH_3COOH); $[\alpha]_D^{25}$ −25·6° (*c* 0·95; dimethylformamide). Reported[1,5,9], m.p. 164°C (corr.), $[\alpha]_D^{18}$ +6·9° (*c* 1·66; CH_3COOH); m.p. 169·5—172°C, $[\alpha]_D^{21.5}$ +4·8°

(*c* 1·66; CH_3COOH), $[\alpha]_D^{23}$ −27·2° (*c* 1·0; dimethylformamide); m.p. 164°C, $[\alpha]_D^{25}$ −25·5° (*c* 1·0; dimethylformamide).

Tosylisoglutamine

A mixture of tosylglutamic acid (6·05 g; 20 mmol), paraformaldehyde (1·0 g), benzenesulfonic acid (0·30 g), and benzene (200 ml) was processed analogously to preceding paragraphs. Yield, 5·55 g (92%) of the crude (m.p. 145—155°C) and 3·9 g (65%) of the pure tosylisoglutamine melting at 157—158°C, solidifying, and remelting at 167°C (reported[10], m.p. 158—170°C). Optical rotation: $[\alpha]_D^{25}$ +25·2° (*c* 1·5; dimethylformamide).

D-Isoglutamine

Benzyloxycarbonyl-D-isoglutamine (7·0 g; 25 mmol) in acetic acid (15 ml) was treated with a solution of hydrogen bromide in acetic acid (15 ml, about 35%), the mixture heated at 60°C for 5 min, cooled down, and precipitated with excess ether. The precipitate was collected with suction, washed on the filter with ether, dried (5·64 g; 100%), and recrystallised from water to afford 5·1 g (89%) of D-isoglutamine hydrobromide, m.p. 215—216°C, $[\alpha]_D^{25}$ −18·0° (*c* 5·98; water). For the L-compound reported[8], m.p. 216—217°C, $[\alpha]_D^{24}$ +17·3° (*c* 6·2; water). The product was homogeneous on paper electrophoresis[6] and on the amino-acid analyser[16]. A sample of the hydrobromide was dissolved in a small volume of water, pH adjusted to 7 with aqueous ammonia, the precipitate collected with suction, and recrystallised from water–acetone; $[\alpha]_D^{25}$ −20·6° (*c* 0·96; water); reported[17], $[\alpha]_D^{24}$ −21·0° (*c* 2·1; water).

Isoasparagine

The preparation was analogous to that of D-isoglutamine. Benzyloxycarbonylisoasparagine (1·3 g; 5 mmol) afforded (after recrystallisation from methanol–ether) 0·83 g (78%) of isoasparagine hydrobromide, m.p. 146—148°C, $[\alpha]_D^{25}$ +9·0° (*c* 0·56; 5M-HCl). Analogously to the preparation of D-isoglutamine, the hydrobromide was converted to the isoasparagine monohydrate, $[\alpha]_D^{25}$ +15·0° (*c* 1·55; 0·1M-HCl); reported[1], $[\alpha]_D^{18}$ +15·5° (*c* 1·55; 0·1M-HCl).

Removal of the Protecting Group from Tosylisoglutamine

A. A mixture of tosylisoglutamine (2·0 g; 6·7 mmol), hydrogen bromide in acetic acid (about 35% solution; 15 ml), and phenol (4·0 g) was heated at 70°C for 5 h, cooled down, and precipitated with excess ether. The precipitate was collected with suction, dried (0·5 g), and recrystallised from aqueous ethanol–ether. Yield, 0·3 g of aminoglutarimide hydrobromide, subl. 240°, decomp. 289°C. On paper electrophoresis in pyridine–acetate buffer solution (pH 5·7), the product behaved as a homogeneous (basic) substance. IR spectrum: —CONHCO— 1705 cm^{-1}. For $C_5H_9BrN_2O_2$ (209·0) calculated: 28·73% C, 4·34% H, 13·40% N, 38·22% Br; found: 28·49% C, 4·25% H, 13·32% N, 38·58% Br.

B. The tosyl residue was removed with sodium in liquid ammonia and the product isolated according to ref.[10]. Tosylisoglutamine (0·5 g; 1·67 mmol) afforded 230 mg (49%) of benzyloxycarbonylisoglutamine, m.p. 174—175°C.

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