AMINO ACIDS AND PEPTIDES. CII.*

LARGE-SCALE PREPARATION OF ORNITHINE FROM GLUTAMIC ACID**

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A previously described 8-stage conversion of glutamic acid to optically active ornithine through the N-tosyl derivatives has been simplified to require the isolation of only 4 intermediates, and improved to give an overall yield of 40%. Raney nickel in acetic anhydride is used for the hydrogenation of a side-chain nitrile to an acetamido group. The procedure has been used to prepare D-ornithine hydrochloride on a kilogram scale.

L-Ornithine has been prepared directly, without optical resolution, from L-arginine (*cf.*[2]) and from L-glutamic acid[3,4]. Faced with a requirement for rather large amounts of D-ornithine we chose the readily available D-glutamic acid as starting material and reinvestigated the synthesis earlier described from this laboratory[4] (Scheme 1) to simplify the procedure and improve the yields. As a result we have been able to prepare D-ornithine in kilogram amounts from D-glutamic acid with an overall yield of about 40%.

Tosyl-D-glutamic acid (D-*I*), prepared as described in the Experimental part by a slight modification of an earlier procedure[5], was converted to tosyl-D-glutamine isopropyl ester (D-*IVb*), without isolation of the intermediates *II* and *IIIb*, in 82% overall yield; the isopropyl ester was chosen because, unlike the methyl and ethyl esters, it is resistant to ammonia under the conditions required for the ammonolysis of the tosyl-lactam ring[4]. The dehydration of the amide D-*IVb* to the nitrile D-*Vb* was carried out essentially as described earlier except that benzenesulphonyl chloride rather than toluene-*p*-sulphonyl chloride was used since the liquid reagent permitted better regulation of the course of this exothermic reaction.

The hydrogenation of the nitrile group of the acid *VI* had previously been ef-

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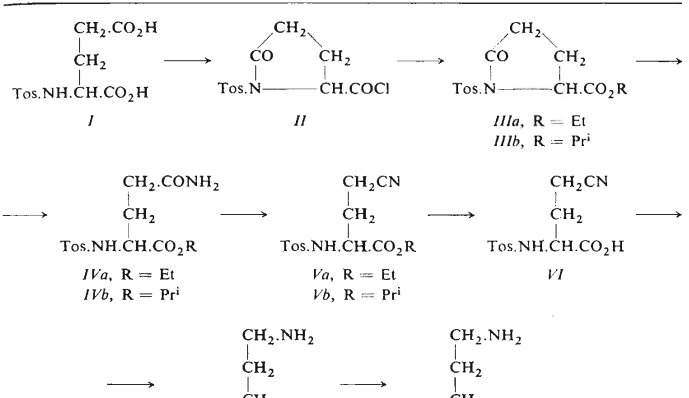
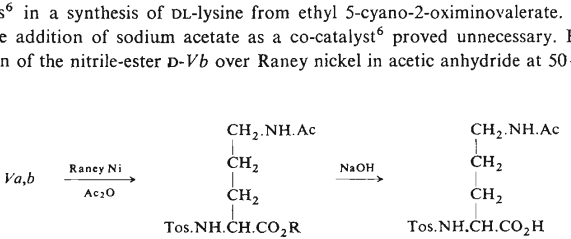
** Taken in part from the Thesis of V. G.; *cf.*[1].



SCHEME 1

fected over Adams' catalyst in acid solution or over Raney nickel in aqueous–methanolic ammonia[4]. For work on a larger scale we adopted the reductive acetylation with Raney nickel and acetic anhydride which had been used by Ferris and coworkers[6] in a synthesis of DL-lysine from ethyl 5-cyano-2-oximinovalerate. In our case the addition of sodium acetate as a co-catalyst[6] proved unnecessary. Hydrogenation of the nitrile-ester D-*Vb* over Raney nickel in acetic anhydride at 50–60°C



SCHEME 2

and 50 atm afforded N^{δ}-acetyl-N^{α}-tosyl-D-ornithine isopropyl ester (D-*IXb*; Scheme 2) in almost 90% yield. The L-enantiomer (L-*IXb*) and the ethyl ester of the L-series (L-*IXa*) were prepared by the same method.

All the protecting groups were removed simultaneously with aqueous hydrogen bromide in the presence of phenol. The D-ornithine (D-*VIII*) was isolated as the monohydrobromide which was converted to the hydrochloride by ion exchange. Recrystallisation gave D-ornithine monohydrochloride of high chemical and optical purity.

It should be noted that potentially useful partially protected derivatives of ornithine can also be obtained from the fully protected *IX*. Thus the ester and acetyl groups could be selectively hydrolysed by boiling 4M-HCl to give N^{α}-tosyl-D-ornithine (D-*VII*) and alkaline hydrolysis of D-*IXa* afforded N^{δ}-acetyl-N^{α}-tosyl-L-ornithine (D-*X*) which was characterised as the crystalline dicyclohexylamine salt.

EXPERIMENTAL

Samples for analysis were dried at 60—80°C/0.5 Torr over phosphorus pentoxide for 8 hours unless otherwise stated. Melting points were determined on a Kofler block. Optical rotations were measured with a photoelectric polarimeter at 25°C. Electrophoresis was carried out in a moist-chamber apparatus on Whatman No 3MM paper in 1M acetic acid, pH 2·4 (E_1) or in pyridine–acetic acid buffer, pH 5·7 (E_2) at about 20 V/cm for 30—60 min.

Tosyl-D-glutamic Acid (D-*I*)

D-Glutamic acid (3·00 kg) in water (10 l) containing sodium hydroxide (1·6 kg) was tosylated essentially as described[5] but using purified toluene-*p*-sulphonyl chloride (4·10 kg) with added toluene-*p*-sulphonic acid* (75—100 g) in ether (13 l). After completion of the reaction the alkaline aqueous phase was washed with ether (7 l), heated to 80—85°C and acidified, at 65°C, with concentrated hydrochloric acid (about 4 l) to pH 1—2. The solution was allowed to cool with stirring; at 50—55°C it turned milky and later the product crystallised. Stirring was continued for 10—12 h while the mixture was cooled, eventually to 0°C. The product was collected and washed with ice-water (1 l). Yield 5·38 kg (90%), m.p. 143—146°C, $[\alpha]_D$ + 14,6° (*c* 0·5, water) (literature[7] m.p. 143—146°C).

Tosyl-D-glutamine Isopropyl Ester (D-*IVb*)

Tosyl-D-glutamic acid (5·15 kg) was refluxed with thionyl chloride (10 l) with stirring until all had dissolved (45 min) and for 20 min more, and the thionyl chloride was distilled off under reduced pressure. The viscous oily residue was treated with benzene (5 l), the benzene was distilled off and this procedure was repeated. The oily residue was immediately** treated with 2-propanol

* It has been noted[5] that the reaction sets in with considerable delay if purified toluene-*p*-sulphonyl chloride is used but rapidly with the crude reagent. This we believe to be due to the emulsifying properties of the sodium toluene-*p*-sulphonate formed from the free acid present in the crude reagent. The use of the purified sulphonyl chloride with added sulphonic acid avoids the stoichiometric uncertainties involved in using the crude chloride.

** The acid chloride should not be allowed to crystallise.

(10 l), the mixture was refluxed for 20 min, cooled to 40—50°C and cautiously treated with concentrated aqueous ammonia (10 l). The mixture was refluxed for 20 min, diluted with hot water (10 l) and allowed to cool to 0°C overnight. The crystalline product was collected by centrifugation and washed with 10% aqueous 2-propanol (6 l). Yield 4·80 kg (82%) of material melting at 118—120°C, resolidifying and remelting at 131—133°C. This material was used in the next step. A sample recrystallised from 2-propanol–light petroleum had the same double m.p., 118 to 120 and 132—134°C; $[\alpha]_D$ +16·6° (*c* 0·5, dimethylformamide). For $C_{15}H_{22}N_2O_5S$ (342·4) calculated: 52·61% C, 6·48% H, 8·18% N; found: 52·85% C, 6·53% H, 8·16% N. The literature[4] gives m.p. 134°C (capillary).

Isopropyl 4-Cyano-D-1-tosylaminobutyrate (D-*Vb*)

To tosyl-D-glutamine isopropyl ester (4·8 kg) in pyridine (5 l) benzenesulphonyl chloride (2·15 l) was added, with stirring, at such a rate as to keep the temperature of the reaction mixture between 50 and 60°C. The mixture was kept at 55—60°C for 25 min more, cooled to room temperature, mixed with crushed ice (13 kg) and acidified with concentrated hydrochloric acid (3 l) to pH 1—2. The oily product crystallised on stirring during 2 h. The suspension was diluted with water (25 l), set aside overnight at room temperature, the product was collected and washed with water (about 120 l) until the filtrate was chloride-free. Yield 4·35 kg (96%), m.p. 68—69°C; this material was used in the next stage. A sample was recrystallised from 2-propanol–light petroleum and dried at 20°C/0·5 Torr over phosphorus pentoxide for 24 h; m.p. 69—70°C, $[\alpha]_D$ + 20·6° (*c* 0·5, dimethylformamide). For $C_{15}H_{20}N_2O_4S$ (324·4) calculated: 55·53% C, 6·21% H, 8·64% N; found: 55·39% C, 6·28% H, 8·79% N. The literature[4] gives m.p. 70—72°C for the L-enantiomer.

N^{δ}-Acetyl-N^{α}-tosyl-D- and -L-ornithine Isopropyl Ester (D- and L-*IXb*)

The nitrile-ester D-*Vb* (500 g) in acetic anhydride (2·4 l) was stirred with Raney nickel (15 g) at room temperature for 30 min, filtered through a mixture of active charcoal and kieselguhr (Hyflo Supercel) and the filter was washed with acetic anhydride (100 ml). The combined filtrates were hydrogenated in a 5-l rotating autoclave over Raney nickel (15 g) at an initial pressure of about 60 atm and 50—60°C for 2—3 h. The warm solution was filtered through a mixture of charcoal and kieselguhr, the filter was washed with warm (50—60°C) acetic anhydride (twice 100 ml) and the combined filtrates were kept overnight at —10°C. The crystalline product was collected and washed with ether (1 l); yield 435 g, m.p. 159—161°C. The acetic anhydride filtrate was evaporated under reduced pressure to about 1/5 of its bulk, the partly crystalline residue was diluted with ether (250 ml) and after standing overnight at 0°C the product was collected and washed with ether (500 ml); yield 71 g, m.p. 157—159°C. The two crops (total yield 88%) were combined for the preparation of D-ornithine. A sample for analysis was recrystallised from 2-propanol; m.p. 159—161°C, $[\alpha]_D$ +13·9° (*c* 0·5, dimethylformamide). For $C_{17}H_{26}N_2O_5S$ (370·5) calculated: 55·11% C, 7·07% H, 7·56% N; found: 55·41% C, 7·15% H, 7·65% N.

The L-ornithine derivative L-*IXb* was prepared essentially by the same procedure on a smaller scale in 92% yield; m.p. 158—160°C (2-propanol), $[\alpha]_D$ — 15·5° (*c* 0·5, dimethylformamide). Found: 55·19% C, 7·12% H, 7·31% N.

N^{δ}-Acetyl-N^{α}-tosyl-L-ornithine Ethyl Ester (L-*IXa*)

Ethyl 4-cyano-L-1-tosylaminobutyrate[4] (31·0 g) in acetic anhydride (250 ml) was hydrogenated over Raney nickel (3 g) in a steel autoclave at 50—60°C and an initial pressure of 25 atm for 2 h. The filtered reaction mixture was evaporated to dryness under reduced pressure, the crystal-

line residue was triturated with ether (100 ml), collected, and washed with more ether (150 ml). Yield 32·3 g (91%), m.p. 131—132°C, unchanged by crystallisation from ethanol–ether. For $C_{16}H_{24}N_2O_5S$ (356·4) calculated: 53·92% C, 6·79% H, 7·86% N; found: 53·77% C, 6·82% H, 7·68% N.

D-Ornithine Hydrobromide (D-*VIII* . HBr)

N^{δ}-Acetyl-N^{α}-tosyl-D-ornithine isopropyl ester (D-*IXb*) (4·50 kg) was refluxed with 42% aqueous hydrobromic acid (22 l) and with phenol (5 kg) for 18 h (after 16 h, electrophoresis of a sample in E_1 still showed the presence of a small amount of tosylornithine, which had disappeared 2 h later). After cooling the aqueous layer was separated, the phenol layer washed with water (3 l) and the combined aqueous solutions were evaporated down to about 4 l under reduced pressure. Water (about 8 l) was added and the solution again evaporated down to 4 l. This procedure was repeated 3 times more: before the last evaporation the solution was filtered through a layer of active charcoal and kieselguhr. The solution was brought to a pH of about 5 with pyridine, with cooling to 20°C, and gradually diluted with acetone (15 l). After standing at 0°C overnight the hydrobromide was collected, resuspended in acetone (5 l) and again filtered off; this procedure was repeated twice. Finally the product was washed with ether (3 l) and dried. This crude product (2·40 kg; m.p. 231—234°C, decomp.) was used in the next stage; analysis for bromine indicated that it was approximately a monohydrobromide. A sample for analysis was recrystallised twice from aqueous acetone; m.p. 232—234°C (decomp.), $[\alpha]_D$ —1·0° (*c* 0·5, water). For $C_5H_{13}BrN_2O_2$ (213·1) calculated: 28·17% C, 6·15% H, 37·50% Br, 13·14% N; found: 28·39%C, 6·16% H, 37·22% Br, 12·86% N.

D-Ornithine Hydrochloride (D-*VIII* . HCl)

The crude hydrobromide prepared as above (2·40 kg) in water (6 l) was filtered through a layer of charcoal and kieselguhr and then passed through a column (125 × 15 cm) of a sulphonic acid ion exchange resin (Katex S, Spolek pro chemickou a hutní výrobu, Ústí nad Labem, Czechoslovakia). The column was washed with water (about 60 l) until the effluent was free of bromide, and the ornithine was eluted with 6M-HCl (65 l). The ninhydrin-positive fractions of the eluate were pooled and evaporated under reduced pressure to about 4 l. The solution was diluted with water (about 8 l), evaporated once more to 4 l, and this procedure was repeated 3 times more, with a filtration (charcoal-kieselguhr) before the last evaporation. The stirred and cooled solution was brought to a pH of about 5 with pyridine (about 1 l) and the resulting suspension was gradually diluted with ethanol (20 l). After standing overnight at 0°C the product was collected, resuspended in ethanol (4 l), once more collected and this procedure was repeated once more. Finally the product was washed with ether; yield 1·51 kg (74% on tosyl derivative), m.p. 237—238·5°C (decomp.). This material was suspended and partly dissolved in boiling water (1·25 l) and the suspension was diluted with hot ethanol (2 l). After standing overnight at 0°C the product was collected and washed with ethanol (2 l), acetone (1 l), and ether (1 l); yield 1·33 kg (65% on tosyl derivative), m.p. 240—241·5°C (decomp.), $[\alpha]_D$ —24·5° (*c* 0·5, 6M-HCl). The product appeared homogeneous by the criterion of paper electrophoresis (E_1 and E_2; 0·1% or less of ninhydrin-positive impurity) and gave a single symmetrical peak on the automatic amino-acid analyser[8]. For $C_5H_{13}ClN_2O_2$ (168·6) calculated: 35·62% C, 7·76% H, 21·02% Cl, 16·61% N; found: 35·73% C, 7·67% H, 20·76% Cl, 16·47% N. The literature records $[\alpha]_D^{23-27}$ —22·1° (*c* 2, 5M-HCl) (ref.[9]) (recalculated for the monohydrochloride) and m.p. 236·5—237·5°C, $[\alpha]_D^{20}$ —22·5° (*c* 5·5, 6M-HCl) (ref.[4]).

N^{α}-Tosyl-D-ornithine (D-*VII*)

N^{δ}-Acetyl-N^{α}-tosyl-D-ornithine isopropyl ester (D-*IXb*) (35·2 g) was refluxed with 4M-HCl (560 ml) for 4 h,* the solution was evaporated to dryness, the residue was taken up in water and the solution was once more evaporated. The residue crystallised from a small volume of water; it was collected and the mother liquors were progressively evaporated to give three more crops of the hydrochloride. The combined crystals (29·2 g) in water (about 200 ml) were brought to pH 6·5 with pyridine. The product which separated was collected and recrystallised from water to afford 22·3 g (88%) of tosyl-D-ornithine hemihydrate, m.p. 190–194°C, $[\alpha]_D$ +5·6° (*c* 0·4 in 5M-HCl). For $C_{12}H_{18}N_2O_4S.\frac{1}{2}H_2O$ (295·3) calculated: 48·80% C, 6·48% H, 9·49% N; found: 48·67% C, 6·61% H, 9·32% N. The literature gives m.p. 194–195°C (Kofler block)[4] or 212–213°C (capillary)[11] for the L-enantiomer and $[\alpha]_D$ +12·6° (*c* 5, 50% acetic acid)[4] for its hydrochloride.

N^{δ}-Acetyl-N^{α}-tosyl-L-ornithine Dicyclohexylamine Salt (L-*X* . $C_{12}H_{23}N$)

The ethyl ester L-*IXa* (1·76 g) dissolved in 4M-NaOH (2·5 ml) was set aside at room temperature for 2 h. The oil which separated on acidification was converted by trituration to an amorphous solid which was washed with water, dried (1·19 g; m.p. about 70°C, unsharp) and treated with dicyclohexylamine (0·2 ml) in ethanolic solution. The dicyclohexylamine salt crystallised on trituration under ether; yield 55%, m.p. 180–183°C. A sample for analysis was recrystallised twice from ethanol with the addition of a small amount of dicyclohexylamine: m.p. 182–183°C. For $C_{26}H_{43}N_3O_5S$ (509·7) calculated: 61·26% C, 8·50% H, 8·24% N; found: 61·26% C, 8·49% H, 8·37% N.

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* After 2 h, electrophoresis (E_2; detection according to Reindel and Hoppe[10]) still revealed the presence of some N^{δ}-acetyl-N^{α}-tosylornithine.