[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

The Synthesis of L-Glutaminyl-L-asparagine, L-Glutamine and L-Isoglutamine from *p*-Toluenesulfonyl-L-glutamic Acid[1]

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L-Glutaminyl-L-asparagine and L-glutamine are readily obtainable by a series of reactions starting from tosyl-L-glutamic acid (I). Phosphorus pentachloride on I gives 1-tosylpyroglutamyl chloride (II) which can be coupled with L-asparagine to form 1-tosylpyroglutamyl-L-asparagine (III). The action of aqueous ammonia on III affords tosyl-L-glutaminyl-L-asparagine and the latter is converted to L-glutaminyl-L-asparagine by removal of the tosyl group with sodium in liquid ammonia. Hydrolysis of II followed by treatment with aqueous ammonia gives tosyl-L-glutamine, from which L-glutamine is obtained in a similar manner. The direct action of concentrated aqueous ammonia on II yields tosyl-L-glutamide. In the course of the work it has been found that sodium can be removed from liquid ammonia solution by Dowex-50 resin and this technique has been utilized in the preparation of L-glutaminyl-L-asparagine, L-glutamine and L-isoglutamine.

In studies directed toward establishing the structure of oxytocin[4] it was shown in this Laboratory that in peptides derived from the hormone the glutamic acid residue is linked to the amino group of aspartic acid and the sequence –glutaminyl-asparaginyl– was postulated. The synthesis of glutaminylasparagine was therefore undertaken as one step toward the synthetic approach to the hormone.[1] In the present communication a new method of synthesis of glutaminyl peptides is described which has led to the desired compound. The key intermediate in this synthesis, 1-*p*-toluenesulfonyl-5-oxo-2-pyrrolidinecarboxyl chloride[5] (1-tosylpyroglutamyl chloride),[6] has also proved useful in the preparation of L-glutamine and L-isoglutamine.

Harington and Moggridge[5] had already shown that heating of tosyl-L-glutamic acid (I) under reflux with acetyl chloride gave the mixed anhydride of 1-tosylpyroglutamic acid and acetic acid, which on hydrolysis in aqueous dioxane gave 1-tosylpyroglutamic acid (V). This compound with phosphorus pentachloride gave 1-tosylpyroglutamyl chloride (II). It was shown that the pyrrolidone ring in these compounds could be opened with aqueous alkali, but was stable to anhydrous ammonia, and proof of structure of the compounds was given.

In the present work 1-tosylpyroglutamyl chloride (II) was obtained directly from tosyl-L-glutamic acid (I) and phosphorus pentachloride. Coupling of II with L-asparagine in the presence of aqueous magnesium oxide as the condensing agent[7] gave 1-tosylpyroglutamyl-L-asparagine (III), which with concentrated *aqueous* ammonia yielded tosyl-L-glutaminyl-L-asparagine (IV), the pyrrolidone ring in III having been opened by addition of the elements of ammonia. The tosyl dipeptide IV was obtained in approximately 80% over-all yield from II. Detosylation of IV by treatment with sodium in liquid ammonia according to the method of du Vigneaud and Behrens[8] gave L-glutaminyl-L-asparagine (IVa) in high yield. The glutaminylasparagine gave a single ninhydrin-positive spot on paper chromatography in each of two solvent systems.

The 1-tosylpyroglutamic acid (V), prepared from II by hydrolysis in magnesium hydroxide or from I by the method of Harington and Moggridge[5] gave tosyl-L-glutamine (VI) when treated with concentrated ammonium hydroxide. The identity of the product was confirmed by synthesis from tosyl chloride and L-glutamine. Removal of the tosyl group with sodium in liquid ammonia gave L-glutamine (VIa). Tests for the presence of isoglutamine and ammonium pyroglutamate were negative. This method of synthesis offers an extremely convenient and economical route to glutamine.

Harington and Moggridge[5] had shown that reaction of II with anhydrous ammonia gives 1-tosylpyroglutamamide (VII), from which tosyl-L-isoglutamine (VIII) can be prepared by the action of sodium hydroxide. They treated VIII with sodium in liquid ammonia and converted the product, without isolation, to carbobenzoxy-L-isoglutamine identical with an authentic sample.[7] By the use of a new technique the L-isoglutamine (VIIIa) has now been isolated from the sodium–liquid ammonia reaction mixture. It can be noted that the action of aqueous alkali (*cf.* VII → VIII) on compounds of type III should provide a route to α-glutamyl peptides.

The reaction of 1-tosylpyroglutamamide (VII) with ammonium hydroxide gave tosyl-L-glutamide (IX), also prepared directly from 1-tosylpyroglutamyl chloride (II) by the action of ammonium hydroxide. The structure of IX was confirmed by treatment with sodium in liquid ammonia followed by carbobenzoxylation of the product to give the known carbobenzoxy-L-glutamide.[9]

In the syntheses reported herein several examples are provided of the cleavage of a tosyl group from

(1) A preliminary report of part of this work was made recently [V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, THIS JOURNAL, **75**, 4879 (1953)].

(2) Fulbright Scholar on Smith Mundt grant-in-aid, on leave from Wool Textile Research Laboratory, C.S.I.R.O., Australia.

(3) Appreciation is expressed to the Lederle Laboratories Division, American Cyanamid Company, for a research grant which has aided greatly in this work.

(4) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953).

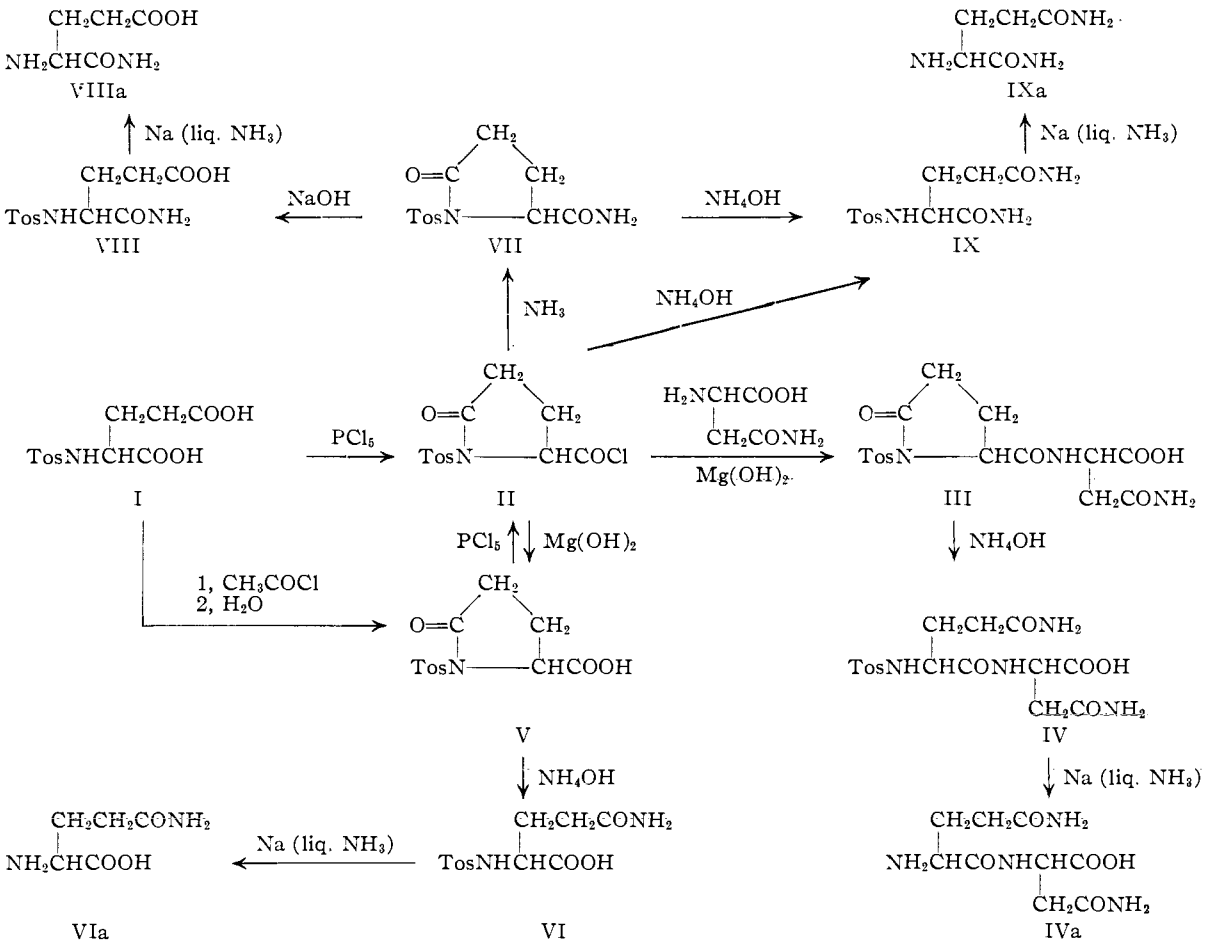
(5) (a) C. R. Harington and R. C. G. Moggridge, *J. Chem. Soc.*, 706 (1940). (b) It has come to our attention since the first report of our work (ref. 1) that Rudinger also has employed 1-tosylpyroglutamic acid and the corresponding chloride as intermediates for the synthesis of glutamine, glutaminylglycine and several γ-glutamyl and α-glutamyl peptides and derivatives [J. Rudinger, *Chem. Listy*, **48**, 235, 244 (1954); J. Rudinger and H. Czurbova, *ibid.*, **48**, 254 (1954)].

(6) *Pyroglutamyl chloride* is synonymous with *5-oxo-2-pyrrolidinecarboxyl chloride; tosyl* is used to designate the *p-toluenesulfonyl* grouping and in structural formulas is abbreviated to Tos.

(7) M. Bergmann and L. Zervas, *Ber.*, **65B**, 1192 (1932).

(8) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).

(9) J. S. Fruton, *ibid.*, **165**, 333 (1946).



a tosyl amide by sodium in liquid ammonia, with subsequent isolation from the reaction mixture of an amino acid or peptide. To accomplish the isolation two new techniques have been developed. In the first of these, glacial acetic acid (rather than, for example, ammonium iodide) was added to the well-cooled liquid ammonia solution and the product was isolated from the aqueous solution of the residue left after evaporation of the ammonia by precipitation with ethanol, in which both sodium and ammonium acetates are soluble. This technique was successful with L-glutaminyl-L-asparagine, but failed with L-isoglutamine. An explanation was found in the fact that L-isoglutamine, although insoluble in ethanol,[7] is very soluble in ethanol saturated with sodium acetate and remains dissolved on considerable dilution. Glutamine and asparagine, on the other hand, are insoluble in this solution. Both L-isoglutamine and L-glutamine were found to be soluble in a saturated solution of sodium iodide in ethanol, the glutamine rather less readily. L-Glutamine could be isolated from the sodium–liquid ammonia reaction mixture after addition of ammonium iodide.

The second technique, which allowed the easy isolation of L-isoglutamine as well as L-glutaminyl-L-asparagine and L-glutamine, was to use a cation-exchange resin to combine with the sodium in the liquid ammonia solution. The resin (Dowex-50) was used in the RSO_3NH_4 form and found to decolorize instantly solutions of sodium in liquid ammonia.[10] The final aqueous solutions from the reaction mixtures were either neutral or only slightly alkaline and addition of ethanol caused the products to crystallize.

Experiments were also carried out on the reaction of 1-tosylpyroglutamic acid (V) and 1-tosylpyroglutamamide (VII) with amines such as benzylamine, morpholine, hydrazine and various amino esters. In all cases the ring was opened and the expected γ-glutamyl and γ-isoglutaminyl derivatives were obtained. This work will be reported in a later communication.

Experimental[11]

1-Tosylpyroglutamyl Chloride (II).—Phosphorus pentachloride (280 g.) was added in one portion with exclusion of moisture to a suspension of 140 g. of tosyl-L-glutamic acid[5] in 1 l. of dry ether at 0°. The mixture was cooled in an ice-bath throughout most of the reaction period. Toward the end of the reaction the mixture was allowed to warm to room temperature and the small excess of phosphorus pentachloride filtered off under suction. The filtrate was diluted with 2–3 l. of petroleum ether and allowed to stand for several hours in the refrigerator. The crystalline product was filtered off, washed with petroleum ether and stored in a vacuum desiccator; yield 125 g. (90%). The chloride, m.p. 71–74° dec., was used in subsequent experiments with-

(10) After this work had been finished, a very interesting study of the behavior of an ion-exchange resin in liquid ammonia was reported by C. W. Keenan and W. J. McDowell [THIS JOURNAL, **75**, 6348 (1953)].

(11) Capillary melting points were determined for all compounds and are corrected.

out further purification and as soon as possible after being dried. In small scale preparations cooling of the mixture was not necessary.

A sample of the chloride dissolved in chloroform saturated with dry ammonia gave 1-tosylpyroglutamamide (VII), melting at 194–196° after recrystallization from 50% ethanol and showing no depression in m.p. on admixture with an authentic sample.[5] Hydrolysis with aqueous magnesium oxide gave 1-tosylpyroglutamic acid (V). When hydrolysis of the chloride was attempted in 50% aqueous acetone containing one equivalent of sodium bicarbonate, a *neutral* substance separated in 30% yield on evaporation of the acetone and this proved to be 1-tosylpyroglutamic acid anhydride, m.p. 205° (after recrystallization from ethyl acetate). The anhydride was soluble in dioxane and ethyl acetate, insoluble in benzene and cold, aqueous sodium carbonate and unaffected by short exposure to boiling water.

Anal. Calcd. for $C_{24}H_{24}O_9N_2S_2$: C, 52.5; H, 4.45; N, 5.11. Found: C, 52.7; H, 4.59; N, 5.04.

When the anhydride was heated with concentrated aqueous ammonia, it slowly dissolved and a second neutral substance was obtained on evaporation. This proved to be tosyl-L-glutamide (IX), m.p. 210–212° dec., identical with the substance obtained directly from 1-tosylpyroglutamyl chloride as described in a subsequent section.

1-Tosylpyroglutamyl-L-asparagine (III).—L-Asparagine monohydrate (60 g.) was dissolved in 300 ml. of water by heating to about 50°, 27 g. of magnesium oxide was added and the mixture cooled rapidly in an ice–salt-bath. 1-Tosylpyroglutamyl chloride (90 g., finely powdered) was then added in portions with extremely vigorous stirring over a period of 15 to 20 minutes. (If the *p*H drops below 8 during this time additional magnesium oxide should be added.) Shortly after the last addition of the chloride the whole mixture set almost solid with precipitation of the insoluble magnesium salt of the product and was allowed to stand at room temperature with intermittent stirring by hand for 1.5 hours. Concentrated hydrochloric acid (90 ml.) was then added, stirring was resumed and the crystalline product filtered off after 30 minutes. The precipitate was washed well with water and dried in air. For highest over-all yield of tosyl-L-glutaminyl-L-asparagine (see next section), it was best to use the crude material directly in the next step. A sample prepared in a similar manner and recrystallized repeatedly from ethanol for analysis melted at 150–151°; $[\alpha]^{21}D$ −42.7° (*c* 5.5, 0.5 *N* $KHCO_3$).

Anal. Calcd. for $C_{16}H_{19}O_7N_3S$: C, 48.4; H, 4.82; N, 10.6. Found: C, 47.9; H, 5.04; N, 10.4.

Tosyl-L-glutaminyl-L-asparagine (IV).—The crude product from the preceding experiment, representing 90 g. of 1-tosylpyroglutamyl chloride, was dissolved in 400 ml. of concentrated ammonium hydroxide and the solution allowed to stand for 30 to 60 minutes. After partial evaporation under reduced pressure to remove most of the ammonia, the solution, from which the ammonium salt sometimes crystallized, was acidified with concentrated hydrochloric acid and cooled for 30 minutes. The product was filtered off, washed with water and dried; wt. 103 g. (83% yield based on 1-tosylpyroglutamyl chloride), m.p. 195–198°. A sample purified for analysis by successive precipitations from sodium bicarbonate solution with acid had m.p. 197–198°, $[\alpha]^{21}D$ −11.4° (*c* 2.1, 0.5 *N* $KHCO_3$).

Anal. Calcd. for $C_{16}H_{22}O_7N_4S$: C, 46.4; H, 5.35; N, 13.5; amide N, 6.8. Found: C, 46.3; H, 5.55; N, 13.2; amide N, 6.7.

L-Glutaminyl-L-asparagine (IVa).—Tosyl-L-glutaminyl-L-asparagine (1 g.) was dissolved in 100 ml. of liquid ammonia and sodium was added in small pieces[12] until the solution remained blue (0.35 g.). Three different procedures were tried for isolation of the product and the results are described for each separately. In the first procedure 3 g. of ammonium iodide was added cautiously and the ammonia evaporated by allowing the flask to stand undisturbed in a hood at room temperature and protected from atmospheric moisture by a tube containing sodium hydroxide. The residue was dissolved in water (3–4 ml.) and the *p*H adjusted to 6 with acetic acid. Ethanol (100 ml.) was then added and the crystalline precipitate filtered off after several hours in the refrigerator. The crude dipeptide weighed 0.5 g. and melted at 197–200° dec. Further crystallization was necessary to remove some inorganic contaminants.

In the second procedure, using 12 g. of the tosyl dipeptide, 750 ml. of liquid ammonia and 4.2 g. of sodium, the flask containing the liquid ammonia solution was cooled in a Dry Ice–acetone bath and, with continual swirling of the contents glacial acetic acid (15 ml.) was run in from a pipet down the side of the flask. After the crude product had been separated in the manner already described for the ammonium iodide procedure, it was digested thoroughly with water (150 ml.) and the dipeptide again precipitated with ethanol. The yield of this recrystallized material was 6.8 g., m.p. 203–205° dec.

For analysis, a sample (1 g.) was dissolved in warm water (40 ml.), diluted with ethanol (120 ml.) to incipient cloudiness and cooled to 0°; wt. 0.9 g., m.p. 210–211° dec., $[\alpha]^{21}D$ +17.1° (*c* 1.5, water), +20.8° (*c* 2.7, 0.5 *N* HCl).

Anal. Calcd. for $C_9H_{16}O_5N_4$: C, 41.5; H, 6.20; N, 21.5. Found: C, 41.2; H, 6.60; N, 21.3.

Chromatography (ascending) on Whatman No. 1 paper at 21° showed that the dipeptide had an R_1 of 0.41 in phenol:water (3:1) and 0.05 in butanol:water:acetic acid (5:4:1).

In the third procedure, using 5 g. of the tosyl dipeptide, 250 ml. of liquid ammonia and 1.75 g. of sodium, Dowex-50 sulfonic acid resin[13] (30 g.) was added to the liquid ammonia reaction mixture, the ammonia evaporated and the flask then thoroughly evacuated. Water (50 ml.) was added and the resin filtered off. The *p*H was adjusted to 6 with a small quantity of acetic acid and the solution was then diluted with 1 l. of ethanol and cooled overnight at 0°. The yield was 3.0 g., m.p. 199–202° dec.

Tosyl-L-glutamine (VI). A. From L-Glutamine.—L-Glutamine (0.5 g.) was dissolved in 6.84 ml. of 1 *N* sodium hydroxide, a solution of 0.8 g. of tosyl chloride in 1 ml. of acetone added and the mixture stirred for 1 hour. The solution was then partly evaporated, the excess tosyl chloride filtered off and the filtrate acidified to give 0.46 g. of product, m.p. 160°. Crystallization and recrystallization from ethyl acetate raised the m.p. to 164–165°; $[\alpha]^{21}D$ +8.7° (*c* 2.4, 0.5 *N* $KHCO_3$). Tosyl-L-glutamine was soluble in cold ethanol, hot water and hot ethyl acetate, insoluble in cold water, ethyl acetate, benzene, chloroform and ether.

Anal. Calcd. for $C_{12}H_{16}O_5N_2S$: C, 48.0; H, 5.37; N, 9.33. Found: C, 48.0; H, 5.50; N, 9.25.

B. From 1-Tosylpyroglutamic Acid or 1-Tosylpyroglutamyl Chloride.—1-Tosylpyroglutamic acid (0.5 g.), prepared either by the method of Harington and Moggridge[5] or from the corresponding acid chloride by hydrolysis with aqueous magnesium oxide, was dissolved in 10 ml. of aqueous ammonia and allowed to stand overnight. The solution was then evaporated under reduced pressure and the residue acidified to give 0.41 g. of product, m.p. 157–159°. Crystallization from ethyl acetate raised the m.p. to 164–165°; $[\alpha]^{21}D$ +8.5° (*c* 2.4, 0.5 *N* $KHCO_3$). Admixture of this product with the compound described in section A caused no depression in m.p.

An alternate procedure, which proved more satisfactory for our purposes, was to prepare tosyl-L-glutamine directly from 1-tosylpyroglutamyl chloride without isolation of the intermediate acid. The acid chloride (33 g.) was stirred vigorously for 1 hour with a mixture of 150 ml. of water, 150 ml. of ether and 12 g. of magnesium oxide. Concentrated aqueous ammonia (400 ml.) was then added and stirring continued for 30 minutes. The mixture was then partly evaporated under reduced pressure to remove most of the ammonia and acidified. The crystalline precipitate was filtered off; wt. 24 g., m.p. 160–162°. This material was sufficiently pure for the preparation of L-glutamine without crystallization.

L-Glutamine (VIa).—The tosyl-L-glutamine was reduced with sodium in liquid ammonia and the L-glutamine was isolated by addition of either ammonium iodide or Dowex-50 resin to the mixture. The two procedures are described separately.

Tosyl-L-glutamine (1.5 g., m.p. 159–162°) was dissolved in 200 ml. of liquid ammonia and sufficient sodium added[12]

(12) All the sodium–liquid ammonia reductions were carried out at the boiling point of the solution.

(13) The Dowex-50 resin used in this and subsequent experiments was a 4% cross-linked, 200–400 mesh sample which was washed successively with large volumes of 1 *N* hydrochloric acid, water, 1 *N* ammonium hydroxide and water and then dried in an oven at 70°.

to give a permanent blue color. During addition of the sodium a precipitate appeared, which dissolved toward the end of the reaction, and the solution became rather yellow. An amount of ammonium iodide equivalent to all the sodium used was added and the ammonia evaporated. The residue was extracted with ether and ethyl acetate, which removed a small amount of yellow impurity, and dissolved in water. The solution was evaporated to dryness *in vacuo* and the residue extracted with 200 ml. of acetone. The insoluble material, which still contained some sodium and ammonium iodide, was triturated with ethanol. This gave 0.65 g. of L-glutamine, which was purified by dissolving it in a small volume of water and adding ethanol; wt. 0.5 g. The product gave a color with Nessler reagent only on standing. This reaction to the test suggested by Archibald[14] indicates the absence of ammonium pyroglutamate in preparations of glutamine. The ninhydrin test was positive and the amino acid showed $[\alpha]^{22}D$ +6.2° (*c* 4.3, water). Fruton[9] gives $[\alpha]^{23}D$ +6.0–6.1° (*c* 3.6, water). For analysis, the material was recrystallized from water–ethanol.

Anal. Calcd. for $C_5H_{10}O_3N_2$: C, 41.0; H, 6.89; N, 19.2. Found: C, 40.9; H, 7.16; N, 18.8.

Paper chromatography showed the glutamine to have an R_f value of 0.54 in phenol:water (3:1) and 0.09 in butanol:water:acetic acid (5:4:1). In both systems the synthetic material gave a single spot and could not be distinguished from an authentic sample of L-glutamine when the two were run either parallel or together. In the butanol:water:acetic acid system, L-glutamine and L-isoglutamine (see subsequent section) had different R_f values.

Tosyl-L-glutamine (24 g.) was dissolved in 700 ml. of liquid ammonia and sodium (7 g.) added[12] to give a permanent blue color. Dowex-50 resin[13] (70 g.) was then added, the ammonia evaporated and the flask thoroughly evacuated. Water (300 ml.) was added and the resin was filtered off and washed with 100 ml. of water. The filtrate was then concentrated to 100 ml. under reduced pressure and diluted with 900 ml. of 95% ethanol. The amino acid was filtered off and washed with ethanol; wt. 11.5 g. A small amount of inorganic material was found to be present and the amino acid was therefore dissolved in 150 ml. of water at 36° and crystallized by the addition of 500 ml. of ethanol; wt. 9 g.

L-Isoglutamine (VIIIa).—The reaction of 1-tosylpyroglutamyl chloride with dry ammonia[5] was carried out as follows. The chloride (14.8 g.) was added in one portion to a saturated solution of ammonia in chloroform (150 ml.). The heat of reaction caused the chloroform to boil. The mixture was then evaporated to dryness, washed with water and filtered. The precipitate of 1-tosylpyroglutamamide weighed 11 g., m.p. 188–190°, and the m.p. was raised to 193–195° by crystallization of the product from 50% ethanol; $[\alpha]^{21}D$ −24.3° (*c* 2.3, acetone). The substance is insoluble in hot chloroform, benzene and ethyl acetate.

Conversion to tosyl-L-isoglutamine and reduction with sodium in liquid ammonia was carried out as described by Harington and Moggridge.[5] When the blue color had been reached, however, Dowex-50 resin[13] (5 g. per g. of tosyl-L-isoglutamine) was added and the L-isoglutamine was isolated according to the procedure described in the preceding section for L-glutamine. The yield of amino acid was 64%; $[\alpha]^{21}D$ +20.5° (*c* 6.1, water). Bergmann and Zervas[7] gave $[\alpha]^{22}D$ +21.1° (*c* 6.5, water). Paper chromatography showed the L-isoglutamine to have an R_f value of 0.52 in phenol:water (3:1) and 0.18 in butanol:water:acetic acid (5:4:1). The isoglutamine melted at 186° dec.

Tosyl-L-glutamide (IX).—This substance was prepared by adding 1-tosylpyroglutamyl chloride to concentrated aqueous ammonia with cooling and then filtering off the product, which was formed in almost quantitative yield.

The diamide could also be prepared in high yield by the action of concentrated ammonium hydroxide on 1-tosylpyroglutamamide (VII) and was obtained from 1-tosylpyroglutamic acid anhydride as mentioned in a preceding section.

Tosyl-L-glutamide melted with decomposition between 210 and 220° depending on the rate of heating, and the m.p. was unaffected by crystallization of the compound from ethanol. The substance is soluble in cold glacial acetic acid, sparingly soluble in ethanol, ethyl acetate, chloroform and acetone; $[\alpha]^{21}D$ +8.3° (*c* 1.7, acetic acid).

Anal. Calcd. for $C_{12}H_{17}O_4N_3S$: C, 48.2; H, 5.72; N, 14.0. Found: C, 48.3; H, 5.89; N, 14.0.

Tosyl-L-glutamide (2 g.) was treated in liquid ammonia (50 ml.) with sodium[12] (0.5 g.) and Dowex-50 resin[13] (5 g.) was added. After evaporation of the ammonia the residue was extracted with hot ethanol and filtered. Evaporation of the filtrate gave a crystalline residue of L-glutamide (IXa) which was characterized by conversion of a small sample to carbobenzoxy-L-glutamide, m.p. 195–196.5°. Fruton[9] reports m.p. 194–196°.

The authors wish to thank Mr. Joseph Albert for carrying out the microanalyses reported herein.

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(14) R. M. Archibald, *Chem. Revs.*, **37**, 161 (1945).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

The Synthesis of *p*-Toluenesulfonyl-L-isoleucyl-L-glutaminyl-L-asparagine and Related Peptides[1]

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The synthesis of tosyl-L-isoleucyl-L-glutamine and tosyl-L-isoleucyl-L-glutaminyl-L-asparagine by the coupling of L-glutamine and L-glutaminyl-L-asparagine with tosyl-L-isoleucyl chloride in the presence of aqueous magnesium oxide is described. Two other protected isoleucyl peptides, namely, tosyl-L-isoleucyl-L-leucine and tosyl-L-isoleucylglycine, have also been prepared.

Studies in our laboratory on peptides from the partial hydrolysis of purified oxytocin preparations revealed the sequence isoleucine·glutamic acid·aspartic acid, and in the postulation of a structure for oxytocin which has guided the synthetic approach to the hormone the sequence –isoleucyl·glutaminyl·asparaginyl– was arrived at.[4] In this synthetic program the preparation of L-isoleucyl-L-glutaminyl-L-asparagine with a protective grouping on the amino group of isoleucine was desired. In view of the earlier synthesis of the dipeptide L-glutaminyl-L-asparagine,[5] it was decided to couple this dipeptide with protected L-isoleucine. For this purpose the N-tosyl-(*p*-toluenesulfonyl) derivative was selected and the synthesis of tosyl-L-iso-

(1) A preliminary report of part of this work was made recently [V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, THIS JOURNAL, **75**, 4879 (1953)].

(2) Fellow of State Scholarships Foundation of Greece.

(3) Appreciation is expressed to the Lederle Laboratories Division, American Cyanamid Company, for a research grant which has aided greatly in this work.

(4) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953).

(5) J. M. Swan and V. du Vigneaud, THIS JOURNAL, **76**, 3110 (1954)