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Tosyl-α-amino Acids. II. The Use of the Acid Chlorides for Peptide Synthesis in the Presence of Aqueous Alkali

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Degradation of those tosyl-α-amino acid chlorides in which the side chain is strongly electron-releasing proceeds more rapidly than peptide formation (with glycine) in strong aqueous alkali. To inhibit degradation, the alkalinity must be reduced to a level at which coupling is slow. Hydrolysis of the acid chloride then interferes.

It has been shown recently that tosyl-α-amino acid chlorides,[1a] like other arylsulfonyl-α-amino acid chlorides,[1b] are liable to rapid degradation by cold aqueous alkali in accordance with the scheme

$$\text{tos.NHCRR'COCl} + \text{OH}^- \longrightarrow \text{tos.NH}_2 + \text{RR'CO} + \text{CO} + \text{Cl}^-$$

Evidence was presented[1a] consistent with the postulates that, for such decomposition to occur, the tosylated acid chlorides must be capable of forming a sulfonamido anion, tos.$\bar{\text{N}}$CRR'COCl, and must carry an electron-releasing side chain at the α-carbon atom. It was apparent that this reaction would need to be taken into account if the susceptible compounds were to be used for peptide syntheses in basic aqueous media. Some experiments designed to test the extent of interference with the peptide forming reaction were, therefore, carried out.

It was shown readily that where the tosylamino acid chloride is not degraded, coupling with a second amino acid dissolved in aqueous alkali proceeds smoothly. When tosyl-L-prolyl chloride was caused to react with L-hydroxyproline under these conditions, the tosylated dipeptide was obtained in excellent yield. In tosyl-L-prolyl chloride the nitrogen is tertiary so that sulfonamide salt formation is not possible.[2] Under similar conditions tosylglycyl-L-proline was prepared, with tosylglycyl chloride as the acylating agent. Although this chloride is capable of conversion to the sulfonamido anion, no side chain is present and degradation is slight.[1a]

Tosyl-DL-alanyl chloride is degraded by cold aqueous sodium hydroxide to the extent of 86% as measured by the amount of carbon monoxide evolved.[1a] This compound in the L-form was used by Schönheimer[3] for peptide synthesis, his technique being to shake a benzene solution of the chloride with a solution of the second amino acid in aqueous sodium hydroxide. In the present work, tosyl-DL-alanyl chloride and glycine were coupled by this method. With the reagents in the proportions required by the equation, the peptide was obtained in 80% yield. When solid tosyl-DL-alanyl chloride was added to a solution of glycine in aqueous sodium hydroxide, efferverscence was apparent as the chloride dissolved and the odor of acetaldehyde became evident, but the coupling product was again isolated in 80% yield.

$$\text{tos.NHCHRCOCl} + \text{NH}_2\text{CH}_2\text{COOH} + 2\text{NaOH} \longrightarrow \text{tos.NHCHRCONHCH}_2\text{COONa} + \text{NaCl}$$

Tosyl-L-leucyl chloride is 99% degraded by cold aqueous sodium hydroxide.[1a] When this compound was substituted for tosyl-DL-alanyl chloride under either of the foregoing conditions, a different result was obtained. Effervescence was more pronounced and no coupling product could be isolated. Tosyl-DL-valyl chloride, which is also 99% degraded by cold aqueous sodium hydroxide,[1a] reacted similarly to the leucyl compound. Addition of tosyl-DL-valyl chloride to the solution of glycine in aqueous sodium hydroxide resulted in evolution of 60% of the theoretical quantity of carbon monoxide and *p*-toluenesulfonamide was isolated from the solution.

These results may be interpreted as follows. As already has been shown,[1] when tosyl-α-amino acid chlorides capable of sulfonamido anion formation are treated with aqueous alkali, both hydrolysis and degradation occur. Degradation predominates where the α-carbon atom bears an electron-releasing side chain and predominates increasingly as the inductive power of the side chain increases. When the aqueous alkali contains a second amino acid, a third reaction competes, namely, attack on the acid chloride function by the amino group to form a peptide bond. Under strongly alkaline conditions, the second amino acid is almost wholly in the anionic form and coupling proceeds more rapidly than hydrolysis. With tosyl-DL-alanyl chloride, whose methyl side chain is only weakly electron releasing, coupling is also faster than degradation; but with tosyl-L-leucyl and tosyl-DL-valyl chlorides, which carry the more strongly inductive isobutyl and isopropyl side chains, respectively, degradation is again the main reaction.

A comparison of the proportions of reagents required by the coupling reaction with those recorded by Schönheimer[3] is interesting. To prepare tosyl-L-alanyl-L-leucine he used tosyl-L-alanyl chloride (1 part), L-leucine (1.7 parts) and sodium hydroxide (2.2 parts), but for tosyl-DL-leucylglycine he employed tosyl-DL-leucyl chloride (1 part), glycine (2.5 parts) and sodium hydroxide (0.85 part). Evidently in the second case he found a reduction in alkalinity necessary, although he makes no comment on this.

Reducing the alkalinity seemed the obvious way of modifying the reaction conditions for peptide syntheses involving the more easily degraded chlo-

(1) (a) A. F. Beecham, *Chemistry & Industry*, 1120 (1955); THIS JOURNAL, **79**, 3257 (1957); (b) R. H. Wiley, H. L. Davis, D. F. Gensheimer and N. R. Smith, *ibid.*, **74**, 936 (1952); R. H. Wiley and R. P. Davis, *ibid.*, **76**, 3496 (1954).

(2) This consideration also applies to tosyl-L-pyroglutamyl chloride which has been used successfully for peptide syntheses in the presence of aqueous base; *vide* J. M. Swan and V. du Vigneaud, *ibid.*, **76**, 3110 (1954), and J. Rudinger, *Collection Czechoslov. Chem. Commun.*, **19**, 365 (1954).

(3) R. Schönheimer, *Z. physiol. Chem.*, **154**, 203 (1926).

rides. Tosyl-DL-valyl chloride was chosen for study, since in this compound susceptibility to degradation is high and steric obstruction to coupling is considerable. Reaction with glycine in aqueous media of various alkalinities was attempted, but no conditions were found under which interference was suppressed. At pH <8 hydrolysis was the main reaction and at pH >8 degradation occurred. For glycine, pK'_2 = 9.60,[4] so that at pH 8, although degradation proceeds at a much reduced rate, coupling is also slow, since the concentration of the glycine anion is low. The best yields of tosyl-DL-valylglycine (36–38%) were obtained when a dioxane solution of the chloride was added portionwise to ice-cold aqueous glycine, kept at pH 8–9 with sodium hydroxide or containing excess solid magnesium oxide. In the latter case the initial pH of the aqueous solution was 10.5, but this quickly dropped to lower values as dissolved base was consumed.

Tosyl-L-isoleucyl chloride would be expected to be as readily degraded by aqueous alkali as tosyl-DL-valyl chloride, but Katsoyannis and du Vigneaud[5] have used the compound, in the presence of aqueous magnesia, to prepare tosyl-L-isoleucyl-L-glutamine and tosyl-L-isoleucyl-L-glutaminyl-L-asparagine. The yield of the dipeptide obtained was low but that for the tripeptide was 55–60%. These results are probably accounted for by the different concentrations of the anions of the second component in the two cases; pK'_2 for glutamine is 9.13.[4] The value for glutaminylasparagine is not recorded, but it would be expected to be below 8 (since, *e.g.*, glutaminylglycine has pK'_2 = 7.52[4]).

Experimental[6]

Tosyl-L-prolyl-L-hydroxyproline.—To a solution of L-hydroxyproline, 1.31 g. (0.01 mole) in 1 *N* NaOH, 20 ml., was added tosyl-L-prolyl chloride, 2.875 g. (0.01 mole), and the whole shaken with ice cooling for 20 minutes. Addition of 1 *N* HCl, 10 ml., to the resulting solution caused precipitation of an oil which quickly crystallized to give 3.4 g. of material, m.p. 219–221°. After several recrystallizations, from water or by precipitation with acid from bicarbonate solution, the product had m.p. 224–224.5°, $[\alpha]^{36}D$ −198° (*c* 1 in 0.5 *N* $KHCO_3$).

Anal. Calcd. for $C_{17}H_{22}N_2O_6S$: C, 53.39; H, 5.80; N, 7.33; O, 25.10; S, 8.28. Found: C, 53.19; H, 5.98; N, 6.89; O, 24.9; S, 8.51.

Tosylglycyl-L-proline.—To a solution of L-proline, 5.75 g. (0.05 mole), in a mixture of 1 *N* NaOH, 100 ml., and dioxane, 100 ml., was added tosylglycyl chloride,[7] 12.4 g. (0.05 mole), in portions, with swirling to dissolve after each addition. After standing for 18 hr., the solution was brought to pH 6 by addition of hydrochloric acid, concentrated to 30 ml. and acidified. The oily precipitate crystallized after 3 days at 0°; 13.6 g., m.p. 176–180° after softening at 170°. Three recrystallizations from bicarbonate solution by addition of acid gave 8.2 g. of product, m.p. 183–184°, $[\alpha]^{27}D$ −71° (*c* 1 in 0.5 *N* $KHCO_3$).

Anal. Calcd. for $C_{14}H_{18}N_2O_6S$: C, 51.52; H, 5.57; N, 8.58; O, 24.51; S, 9.82. Found: C, 51.48; H, 5.46; N, 8.37; O, 24.6; S, 9.99.

Tosyl-DL-alanylglycine.—A solution of tosyl-DL-alanyl chloride, 2.61 g. (0.01 mole), in 80 ml. of benzene was shaken with 20 ml. of 1 *N* NaOH containing glycine, 0.75 g. (0.01 mole), for 16 hr. The benzene layer was separated, the aqueous solution warmed to 90°, 10 ml. of 1 *N* HCl added and the solution allowed to cool slowly. Crystals of the tosyl dipeptide separated, 2.35 g., m.p. 149–150° (lit. m.p. 147° cor.,[3] m.p. 150–151° cor.[8]).

When tosyl-DL-alanyl chloride, 2.61 g., was added to a solution of glycine, 0.75 g., in 20 ml. of 1 *N* NaOH, the solid dissolved slowly with effervescence and the production of the odor of acetaldehyde. From the solution, after acidification, was obtained 2.4 g. of the crude tosyl dipeptide, m.p. 147–149°. Recrystallization from 50 ml. of water yielded 1.9 g., m.p. 149–150°.

Tosyl-DL-valylglycine. A.—Tosyl-DL-valyl chloride, 2.895 g. (0.01 mole), was added to a solution of glycine, 0.75 g. (0.01 mole), in 20 ml. of 1 *N* NaOH and the evolved gas collected. The volume of gas produced indicated that 60% of the chloride had suffered degradation. The aqueous liquor contained suspended solid, from which was obtained by recrystallization from aqueous bicarbonate *p*-toluenesulfonamide, 0.7 g., m.p. 136–137.5°.

B.—Addition of the chloride (0.01 mole) to aqueous glycine (0.01 mole) containing triethylamine (0.02 mole) at pH 12 resulted in strong effervescence. The products were not examined.

C.—The chloride, 2.895 g., glycine, 0.75 g., and MgO, 1.0 g., were shaken for 6 hr. in a mixture of 20 ml. of water and 20 ml. of dioxane. After standing overnight the mixture was acidified, the oily precipitate taken into ether, the ether solution dried over Na_2SO_4, evaporated to dryness and the residue dissolved in 400 ml. of hot anhydrous benzene. On cooling crystals separated, 1.3 g., m.p. 165–168° after softening at 160°. This material after three recrystallizations from water yielded tosyl-DL-valylglycine, 0.7 g., m.p. 174–175°.

Anal. Calcd. for $C_{14}H_{20}N_2O_5S$: C, 51.20; H, 6.14; N, 8.53; O, 24.36; S, 9.76. Found: C, 51.25; H, 6.14; N, 8.06; O, 24.2; S, 9.83.

From the benzene filtrate was obtained tosyl-DL-valine, 0.4 g., m.p. 165–166° unchanged when mixed with an authentic sample.[1a]

D.—To a solution of glycine, 0.75 g., in 20 ml. of water containing magnesium oxide, 1.0 g., at pH 10.5 was added, over 20 minutes with shaking and ice cooling, a solution of tosyl-DL-valyl chloride in 20 ml. of dioxane. Shaking with cooling, was continued for 10 minutes, the suspension set aside for 20 minutes and then filtered. The filtrate (pH 8) was acidified and the resulting oily suspension shaken with ether when crystallization commenced. The crystalline material, 1.4 g., m.p. 160–165°, dissolved in aqueous bicarbonate to yield, on acidification, tosyl-DL-valylglycine, 1.06 g., m.p. 173.5–174.5°.

Evaporation of the ether gave further crystalline material from which no pure compound could be obtained.

E.—A solution of glycine, 0.375 g., in 20 ml. of water was maintained at pH 9.6–9.7 by addition of aqueous sodium hydroxide while tosyl-DL-valyl chloride, 1.45 g., was added in portions. Effervescence was noticeable as the chloride dissolved and only *p*-toluenesulfonamide, 0.56 g., was obtained on working up.

F.—From an ice-cooled solution of glycine, 0.375 g., in 10 ml. of water and 10 ml. of dioxane kept at pH 9–10 with sodium hydroxide while tosyl-DL-valyl chloride, 1.45 g., was added, there was obtained *p*-toluenesulfonamide, 0.43 g., and tosyl-DL-valylglycine, 0.52 g.

G.—To a solution of glycine, 0.75 g., in 10 ml. of water were added a solution of tosyl-DL-valyl chloride, 2.895 g., in 20 ml. of dioxane, and 1 *N* sodium hydroxide at such a rate that the pH was kept at 8.5–9. Tosyl-DL-valylglycine, 1.2 g., and *p*-toluenesulfonamide, 0.22 g., were obtained as well as further material which could not be purified.

H.—A solution of glycine, 0.75 g., in water containing sodium bicarbonate had pH 8.2. After addition of tosyl-DL-valyl chloride there was obtained *p*-toluenesulfonamide, 0.77 g., and tosyl-DL-valine, 0.30 g.

I.—Addition of tosyl-DL-valyl chloride, 2.895 g., to a solution of glycine in aqueous pyridine at pH 7.6 resulted in the production of tosyl-DL-valine, 2.0 g.

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(4) D. M. Greenberg, "Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1951, pp. 430–431.

(5) P. G. Katsoyannis and V. du Vigneaud, THIS JOURNAL, **76**, 3113 (1954).

(6) Melting points are not corrected. Microanalyses are by the C.S.I.R.O. Microanalytical Service under the supervision of Dr. K. W. Zimmerman, at the University of Melbourne.

(7) J. M. Swan, *Australian J. Sci. Research*, Ser. A, **5**, 728 (1952).

(8) G. W. Kenner and R. J. Stedman, *J. Chem. Soc.*, 2069 (1952).