Experimental

Radioactivity determinations were carried out on infinitely thin samples plated on 10 cm.^{2} aluminum planchets with an internal Geiger–Müller flow counter (Radiation Counter Laboratories, Mark 12, model 1, helium–isobutane gas), probable error ±5%, except when otherwise specified. Ultraviolet absorption spectra were determined with the Beckman model DU spectrophotometer with matched 1-cm. quartz cells.

Toxicity Studies.—Male Sherman strain rats (obtained from Rockland Farms, New City, N. Y.) weighing 200 to 300 g. were used in these studies. Animals given intraperitoneal injections of 9-β-D-ribofuranosylpurine (I) at levels of 200, 150 and 100 mg./kg. died after 3, 5 and 20 hours, respectively.

Formation of Phosphorylated Derivatives of 9-β-D-Ribofuranosylpurine.—Eleven male Sherman strain rats, each weighing *ca.* 140 g., were given single intraperitoneal injections of 9-β-D-ribofuranosylpurine-8-C^{14} (I-8-C^{14}) (prepared by diluting I-8-C^{14} [4] with non-radioactive I to give material with a specific activity of 2280 c.p.m./μmole) at the level of 100 mg./kg. After 4 hr. the livers were excised under ether anesthesia and immediately frozen on Dry Ice to give 70 g. of material. All of the subsequent manipulations were done at 4°. The livers were extracted with 3% and then with 2% perchloric acid. The combined extracts were neutralized with 5 *N* potassium hydroxide and clarified by filtration through a Celite pad. The clear filtrate (520 ml.) contained 8840 optical density units[12] and radioactivity totaling 156,000 c.p.m. The material was fractionated by gradient elution chromatography which made use of a 1-liter mixing chamber and a 21.5 × 2 cm. column of Dowex-1 ion exchange resin (formate). After the entire sample of liver perchloric acid-soluble material had been absorbed on the resin, the column was washed with water until the optical density at 260 mμ of the effluent had fallen to 0.06. The water wash contained 1300 optical density units and a total of 9000 c.p.m. The column was then successively eluted with 2.5 *N* formic acid (fractions 1–100, 2 ml. each; fractions 101–520, 4 ml. each), 4.0 *N* formic acid (fractions 521–640, 5 ml. each), 8.0 *N* formic acid (fractions 641–820, 10 ml. each), 8.0 *N* formic acid + 0.4 *M* ammonium formate (fractions 821–900, 10 ml. each) and finally 8.0 *N* formic acid + 1 *M* ammonium formate (fractions 901–960, 10 ml. each). Major peaks of radioactive material were located in tubes 471–500, 760–800 and 881–900. Radioactive fractions were combined and lyophilized in groups consisting of tubes 471–480, 481–489, 490–499, 761–800 and 881–900. Spectral studies indicated that the pooled fractions 471–480 contained the largest amount of a derivative of I uncontaminated by other materials.

The Identification of 9-β-D-Ribofuranosylpurine-5′-phosphate (II).—The R_f values (ascending) of synthetic II, of the major component of fractions 471–480, of 9-β-D-ribofuranosylpurine and of adenosine-5′-phosphate, are, respectively, as follows: (A) 0.50, 0.50, 0.74, 0.35 (1% aqueous ammonium sulfate 33⅓%, isopropyl alcohol 66⅔%)[13]; (B) 0.83, 0.84, 0.72, 0.67 (5% aqueous monobasic sodium phosphate layered with isoamyl alcohol[14]; (C) 0.44, 0.45, 0.58, 0.48 (isopropyl alcohol 75 ml., water 25 ml., trichloroacetic acid 5 g., 28% ammonia 0.3 ml.)[15]; (D) 0.39, 0.39, 0.65, 0.29 (glacial acetic acid 20%, *n*-butyl alcohol 50%, water 30%). The unknown material corresponding to II in each of the above solvent systems gave positive tests for *cis*-glycol groups[9] and for organically bound phosphate[10] (except in solvent B). The spectrum of the material eluted from papers developed using solvent A was identical with that of II, in 0.1 *N* hydrochloric acid, water at *p*H *ca.* 5 and 0.1 *N* sodium hydroxide. The specific activity, calculated with the ε for synthetic II, of the material eluted from papers developed using solvent D was 2100 c.p.m./μmole. Radioautographs prepared from papers developed in solvents B and D showed that the only detectable radioactivity was associated with the material with R_f values corresponding to II, although faint AMP spots were detectable by inspection with ultraviolet light.

Electrophoresis was carried out at *p*H 8.25 as described.[6] The principal component of fractions 471–480, 9-β-D-ribofuranosylpurine-5′-phosphate, and adenosine-5′-phosphate migrated 9 to 10 cm. toward the anode, while 9-β-D-ribofuranosylpurine migrated 1.4 cm. toward the cathode.

The only other component detected in fractions 471–480 during these studies was a small amount of adenosine-5′-phosphate.

The Partial Identification of 9-β-D-Ribofuranosylpurine-5′-diphosphate.—The *p*H of the material in pooled fractions 761–800 was adjusted to 5 with ammonium hydroxide and submitted to electrophoresis. The conditions were the same as those above except that the *p*H of the buffer was 5.25 and the duration of the run was 75 minutes. Seven compounds clearly separated. Guanosine-5′-diphosphate was used as a marker because previous experience[4] indicated that it would be the major contaminant of the metabolite. The guanosine-5′-diphosphate and the major rapidly moving component of the mixture migrated 15 to 16 cm. toward the anode. The latter material gave a positive test for *cis*-glycol groups; its spectrum in 0.1 *N* hydrochloric acid, water and 0.1 *N* sodium hydroxide was very similar to that of 9-β-D-ribofuranosylpurine. The specific activity of the material (assuming an ε at 263 mμ equal to that of the monophosphate) was 1900 ± 200 c.p.m.[16]

Acknowledgments.—The authors wish to thank Mrs. Orsalia Intrieri for competent assistance, and Miss Eva Simmel for the radioautographs.

(12) Optical density units equal the optical density at 260 mμ times the volume in ml.

(13) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).

(14) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

(15) J. P. Ebel, *Bull. soc. chim. France*, 1089 (1953).

(16) A correction factor for the salt present was determined by plating small amounts of 9-β-D-ribofuranosylpyrine-8-C^{14} in the presence of buffer. In view of this correction factor and the use of an uncertain ϵ_{max}, there is no significant difference between this specific activity and those of the II and the administered I.

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[CONTRIBUTION FROM THE CHEMICAL PHYSICS SECTION, DIVISION OF INDUSTRIAL CHEMISTRY, COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION]

Tosyl-α-amino Acids. I. Degradation of the Acid Chlorides and Azides by Aqueous Alkali

BY A. F. BEECHAM

RECEIVED OCTOBER 15, 1956

The decompositions resulting from the treatment of certain tosyl (*p*-toluenesulfonyl)-α-amino acid chlorides and azides with aqueous alkali are described and the mechanisms discussed.

In 1952, Wiley, *et al.*,[1a] observed that α-(benzenesulfonamido)-phenylacetyl chloride undergoes decomposition when treated with aqueous sodium hydroxide forming benzenesulfonamide, benzaldehyde and carbon monoxide. Wiley and Davis[1b] later

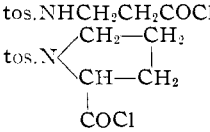
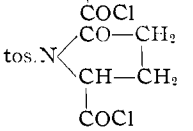
(1) (a) R. H. Wiley, H. L. Davis, D. E. Gensheimer, N. R. Smith, THIS JOURNAL, **74**, 936 (1952); (b) R. H. Wiley and R. P. Davis, *ibid.*, **76**, 3496 (1954),

found that several α-(arylsulfonamido)-propionyl and -phenylacetyl chlorides were similarly degraded. The present author, unaware until recently of this work, already has reported briefly[2] that certain tosyl-α-amino acid chlorides undergo this reaction in the presence of cold aqueous alkali with the formation of *p*-toluenesulfonamide, a carbonyl compound arising from the α-carbon atom with its side chain or chains, carbon monoxide and the chloride ion.

$$CH_3C_6H_4SO_2NHCRR'COCl + OH^- \longrightarrow CH_3C_6H_4SO_2NH_2 + RR'CO + CO + Cl^-$$

Tosylglycyl chloride is only slightly decomposed, being largely hydrolyzed to the parent acid, but with the tosylated chlorides of those α-amino acids in which the α-carbon atom is alkyl substituted, degradation is the main reaction. The amount of carbon monoxide evolved from each of the compounds tested is shown in Table I.

TABLE I

Tosylated acid chloride	R	R′	CO, %
Glycyl	H	H	<2
Alanyl	CH_3	H	86
α-Amino-*n*-butyryl	CH_3CH_2	H	94
α-Amino-isobutyryl	CH_3	CH_3	94
Norleucyl	$CH_3[CH_2]_3$	H	97
Leucyl	$(CH_3)_2CHCH_2$	H	99
Valyl	$(CH_3)_2CH$	H	99
β-Alanyl	tos.$NHCH_2CH_2COCl$		0
Propyl			0
Pyroglutamyl			0

The tosyl-α-amino acid azides undergo a similar reaction. Tosyl-DL-alanyl and -valyl azides both dissolve with effervescence in cold aqueous sodium hydroxide, the production of gas amounting to 11 and 50%, respectively, of the volumes expected from the equation. The tendency to degradation is, therefore, less pronounced than with the corresponding chlorides under like conditions. Also the degradation takes a somewhat different path since, although *p*-toluenesulfonamide and the aldehyde are again produced, the evolved gas is nitrogen containing no trace of carbon monoxide. The residual solution contains the cyanate ion

$$CH_3C_6H_4SO_2NHCHRCON_3 + OH^- \longrightarrow CH_3C_6H_4SO_2NH_2 + RCHO + N_2 + NCO^-$$

Since both the chlorides and the azides appear to be unaffected by suspension in cold water alone and since the chlorides are hydrolyzed normally when such a suspension is heated, it may be assumed that the first step in the alkaline degradation of either class of derivative is formation of the sulfonamide anion. All the susceptible chlorides can ionize in this way, whereas tosyl-L-prolyl chloride, a tosylated secondary amine, cannot and is not degraded.

$$tos.NHCRR'COCl(N_3) + NaOH \longrightarrow tos.\bar{N}CRR'COCl(N_3) + Na^+ + H_2O$$

(2) A. F. Beecham, *Chemistry & Industry*, 1120 (1955).

In the anion formed the tendency to undergo hydrolysis would be reduced by the increased electron density at the carbon atom of the modified carboxyl group resulting from the negative charge on the nitrogen. It would also seem that acquirement of the negative charge imparts a degree of instability to the structure. Apparently with tosylglycyl chloride this is not sufficient for breakdown to predominate over hydrolysis, but in those chlorides in which the α-carbon atom carries an alkyl substituent the disruption becomes the main reaction. Presumably this is due to an added increase in electron density at the carbonyl carbon atom arising from the alkyl group. As may be seen from Table I, in the chlorides tested the extent of degradation increases with increasing electron-releasing power of the alkyl side chain. The same holds for the two azides examined. Of course, steric hindrance to hydrolytic attack would also favor the disruptive reaction.

It was suggested earlier[1a] that, in the chloride degradation, breakdown of the sulfonamide anion might proceed by severance of the ion fragment, $COCl^-$, from which carbon monoxide and the chloride ion might be expected to arise immediately. Similarly, in the azide degradation, ejection of CON_3^- giving rise to nitrogen and the cyanate ion might be postulated. However, if this were so, there seems to be no reason why similar fragments should not be evolved from tosyl α-amino acid derivatives other than the chlorides and azides: esters, for instance, giving $COOR^-$ and amides $CONH_2^-$. But the amides and ethyl esters of tosyl-DL-alanine and -DL-valine were found to be hydrolyzed quantitatively, if sluggishly, by aqueous alkali with regeneration of the tosyl α-amino acids.

Alternatively then, it may be proposed that, in the chloride degradation, the sulfonamido anion breaks down by fission of the carbon–chlorine bond with the chlorine retaining the bonding electrons. The combined electron-releasing effects of the charged nitrogen and the alkyl side chain acting on the already highly polar carbon–chlorine link would be expected to favor this ionization. Such a process would be less likely in the less polar esters and amides. After severance of the chloride ion, internal neutralization of charge in the residual dipolar structure, with elimination of carbon monoxide, would be expected to follow.

$$tos.\bar{N}CRR'COCl \longrightarrow tos.\bar{N}CRR'{-}\overset{+}{C}O + Cl^-$$

$$\downarrow$$

$$tos.NH_2 + RR'CO \xleftarrow{H_2O} tos.N{=}CRR' + CO$$

Wiley[1b] suggests that the chloride degradation may involve displacement at the α-carbon atom of the un-ionized sulfonamido acid chloride in the following way

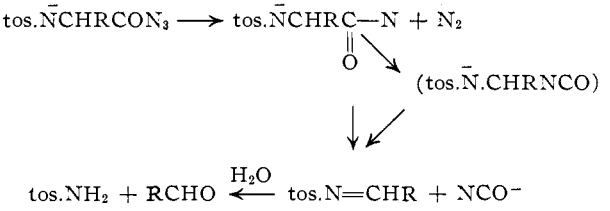
$$ArSO_2NHCHRCOCl + OH^- \longrightarrow ArSO_2NHCHR(OH) + COCl^-$$

$$ArSO_2NH_2 + RCHO \longleftarrow ArSO_2NHCHR(OH)$$

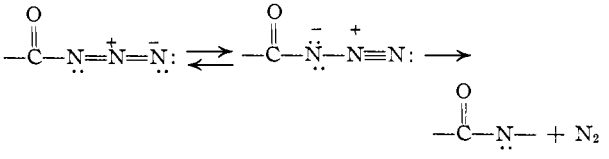
However, this proposal appears to be invalidated by the fact that tosyl-L-prolyl chloride is not de-

graded. The ability to form a sulfonamido anion seems to be essential and, of course, nucleophilic attack by hydroxyl on such an anion is highly unlikely.

The degradation of the azides may be formulated as an example of the Curtius reaction.

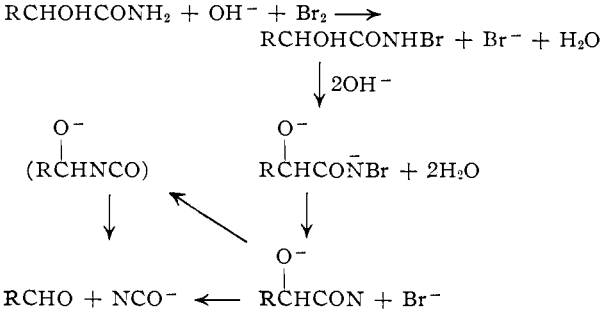


Here the same electron-releasing factors would be operating within the sulfonamido anion as in the chloride reaction and would be expected to promote the evolution of a molecule of nitrogen. Analogously in the Hofmann and Lossen rearrangements the rate-determining release of X^- from the intermediate $RCONX^-$ is favored by electron-releasing substituents at R.[3,4] The nitrogen evolution is akin to an ionization since it leaves an electron-deficient nitrogen atom attached to the organic residue.[3]



The remaining entity may then be visualized either as losing the cyanate ion directly or as rearranging to the organic isocyanate with subsequent loss of the cyanate ion. In view of the negative charge present, perhaps the former alternative is the more likely.

It is interesting to recall that α-hydroxy acid amides when subjected to degradation by alkaline hypohalite yield the next lower aldehyde and the cyanate ion.[5] It may be suggested that this mode of breakdown is favored by electronic factors similar to those operating in the alkaline degradation of the tosyl α-amino acid azides.



If the chloride and azide degradations follow the paths indicated, both give rise to a tosylated imine as the precursor of the carbonyl compound and sulfonamide finally obtained. It is assumed that this imine adds water and disrupts to give the observed products. An attempt was made to isolate the intermediate by dropwise addition of the calculated quantity of aqueous sodium hydroxide to a dioxane solution of tosyl-DL-valyl chloride, followed at once by extraction with ether. Under these conditions, with the chloride dissolved before the base is added, degradation is so rapid that the solution is never more than faintly and transiently alkaline. However, the solid isolated from the ether was *p*-toluenesulfonamide, indicating that the imine, if formed, suffered rapid hydrolysis even in the absence of alkali. The moisture sensitivity of similar structures is mentioned by Lichtenberger, Fleury and Barette.[6]

As shown in Table I, three of the chlorides tested gave no carbon monoxide. If the factors affecting the degradation are those suggested, tosyl-β-alanyl chloride would be expected to be inert, both because the α-carbon atom carries no side chain and because the acid chloride function is insulated from the seat of charge in the sulfonamido anion by the extra methylene group. In tosyl-L-prolyl chloride the nitrogen atom is tertiary so that salt formation is not possible. This also applies to tosyl-L-pyroglutamyl chloride which, however, unlike the prolyl compound, contains an alkali-labile lactam bond. For alkaline degradation to occur, through a sulfonamido anion intermediate, hydrolysis of the lactam system would need to be more rapid than hydrolysis of the acid chloride group. That Swan and du Vigneaud[7] were able to hydrolyze the compound to tosyl-L-pyroglutamic acid using aqueous magnesia indicates that the reverse is true and this is indirectly confirmed by the absence of degradation in the present work.

There are obvious ways in which the range of the degradations described here could be further explored. The tosylated acid chlorides and azides of the more complex α-amino acids might be tested. The reactions are probably general for those compounds capable of forming a sulfonamido anion and bearing an electron-releasing side chain at the α-carbon atom.[1a,b] The arylsulfonyl group might be varied with the expectation that degradation would still occur with derivatives of those amino acids whose tosyl compounds were disrupted.[1a,b] As well as the acid chlorides and azides, other anhydride-type structures might evince instability. Some reference to the effect of bases other than aqueous alkali is made in a subsequent article[8] which deals with the use of tosyl α-amino acid chlorides in the synthesis of peptides.

Experimental[8a]

Tosylamino Acids.—These compounds were prepared by the method used by Harington and Moggridge[9] for tosyl-L-glutamic acid. *p*-Toluenesulfonyl chloride, 0.1 mole, was

(3) M. J. S. Dewar, "The Electronic Theory of Organic Chemistry," Clarendon Press, Oxford, 1949, p. 222.

(4) E. S. Wallis and J. F. Lane, "The Hofmann Reaction" in "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 270.

(5) R. A. Weerman, *Rec. trav. chim.*, **37**, 16 (1917).

(6) J. Lichtenberger, J.-P. Fleury and B. Barette, *Bull. soc. chim. France*, 669 (1955).

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

(8) A. F. Beecham, *ibid.*, **79**, 3262 1957.

(8a) Melting points (capillary) are not corrected. Microanalyses are by the C.S.I.R.O. Microanalytical Service at the University of Melbourne. Mass spectra were determined by Drs. J. D. Morrison and F. H. Dorman of this Section.

(9) C. R. Harington and R. C. G. Moggridge, *J. Chem. Soc.*, 706 (1940).

added to a solution of the amino acid, 0.1 mole, in 100 ml. of water containing NaOH, 0.2 mole (0.3 mole for L-glutamic acid), and the suspension heated at 70–80° with shaking until a clear solution resulted (5–10 minutes). After cooling, acidification caused the product to precipitate except in the case of tosyl-L-glutamic acid which was isolated as described[9] and recrystallized from methyl ethyl ketone–carbon tetrachloride. The products from glycine[10] and DL-alanine[10] were immediately crystalline; those from DL-norleucine,[10] L-leucine,[10] β-alanine[11] and L-proline[12] were oils which crystallized on standing, tosyl-L-proline being hydrated. This last compound was obtained as the hemibenzenate[12] after azeotropic drying.

Tosyl-α-aminoisobutyric Acid.—Acidification gave a clear solution from which the product crystallized during 2 hr. at 0°, yielding 23%, softening 145°, m.p. 148.5–149.5° raised to 149–150° by recrystallization from water (lit.[13] m.p. 147°).

Anal. Calcd. for $C_{11}H_{15}NO_4S$: C, 51.34; H, 5.88; N, 5.44; O, 24.87; S, 12.46. Found: C, 51.71; H, 5.97; N, 5.13; O, 24.4; S, 12.88.

Further crystalline material obtained by concentration of the mother liquor from this preparation consisted largely of a second acidic substance, melting 205–218°, equivalent weight ≃400. This was not further characterized, but may have been the crude ditosyl compound.

Tosyl-DL-α-amino-*n*-butyric Acid.—The compound crystallized rapidly in 62% yield, m.p. 151–152°, after softening at 145°. A sample recrystallized twice from water had m.p. 152–153°.

Anal. Calcd. for $C_{11}H_{15}NO_4S$: C, 51.34; H, 5.88; N, 5.44; O, 24.87; S, 12.46. Found: C, 51.81; H, 5.88; N, 5.16; O, 24.59; S, 12.49.

Tosyl-DL-valine.—The product crystallized rapidly in 71% yield, m.p. 165–166° raised to 166–167° by recrystallization from acetone–water.

Anal. Calcd. for $C_{12}H_{17}NO_4S$: C, 53.12; H, 6.32; N, 5.16; O, 23.59; S, 11.82. Found: C, 53.31; H, 6.18; N, 5.09; O, 23.9; S, 12.26.

Tosylamino Acid Chlorides.—The tosylamino acid, 0.01 mole, and PCl_5, 0.015 mole (0.025 mole for tosyl-L-glutamic acid), were suspended in 20–30 ml. of anhydrous ether (200 ml. for the isobutyryl derivative) and shaken until all the organic material had dissolved, then for a further 30 minutes. Excess PCl_5 was removed by filtration, 100 ml. of anhydrous petroleum ether, b.p. 80–100°, added and the solution set aside at 0° for several hours. The crystalline acid chlorides were then filtered off, washed with petroleum ether and dried in vacuum over solid caustic soda and paraffin wax. The following compounds were prepared in this way.

Tosylglycyl chloride, m.p. 82–84° (lit.[14] m.p. 82–83°).

Tosyl-DL-alanyl chloride, m.p. 93–94°. *Anal.* Calcd. for $C_{10}H_{12}NO_3SCl$: C, 45.89; H, 4.62; Cl, 13.55. Found: C, 46.16; H, 4.69; Cl, 13.37.

Tosyl-DL-α-amino-*n*-butyryl chloride, m.p. 56–57°. *Anal.* Calcd. for $C_{11}H_{14}NO_3SCl$: C, 47.91; H, 5.12; Cl, 12.86. Found: C, 48.21; H, 5.06; Cl, 12.86.

Tosyl-α-amino-isobutyryl chloride, m.p. 115–116°. *Anal.* Calcd. for $C_{11}H_{14}NO_3SCl$: C, 47.91; H, 5.12; Cl, 12.86. Found: C, 47.58; H, 5.09; Cl, 12.76.

Tosyl-DL-valyl chloride, m.p. 76–78°. *Anal.* Calcd. for $C_{12}H_{16}NO_3SCl$: C, 49.74; H, 5.57; Cl, 12.24. Found: C, 49.66; H, 5.59; Cl, 12.13.

Tosyl-DL-norleucyl chloride, m.p. 70–72°. *Anal.* Calcd. for $C_{13}H_{18}NO_3SCl$: C, 51.39; H, 5.97; Cl, 11.67. Found: C, 51.29; H, 6.03; Cl, 11.45.

Tosyl-L-leucyl chloride, m.p. 83–85°. *Anal.* Calcd. for $C_{13}H_{18}NO_3SCl$: C, 51.39; H, 5.97; Cl, 11.67. Found: C, 51.47; H, 6.03; Cl, 11.66.

Tosyl-β-alanyl chloride, m.p. 82–84°. *Anal.* Calcd. for $C_{10}H_{12}NO_3SCl$: C, 45.89; H, 4.62; Cl, 13.55. Found: C, 46.17; H, 4.72; Cl, 13.58.

Tosyl-L-pyroglutamyl chloride, m.p. 103–105° (lit.[12] m.p. 103–105° raised to 106–107° by recrystallization from benzene–ether).

Tosyl-L-prolyl chloride, m.p. 56–57.5° (lit.[12] oil). *Anal.* Calcd. for $C_{12}H_{14}NO_3SCl$: C, 50.08; H, 4.91; Cl, 12.32. Found: C, 50.13; H, 4.91; Cl, 12.24.

Tosyl-DL-Alanyl Azide.—To a solution of tosyl-DL-alanyl chloride, 2.6 g., in 35 ml. of acetone, cooled in ice-water, was added slowly with swirling an ice-cold solution of NaN_3, 0.8 g., in 20 ml. of water. The resulting clear solution was kept for 10 minutes at 0°, then diluted to 200 ml. with ice-water. The crystalline product was filtered off, washed with ice-water and dried in vacuum over silica gel, yield 2.2 g., m.p. 72–73° dec. Reprecipitation with ice-water from acetone solution gave 1.9 g., m.p. 74° dec. The m.p. varied with the rate of heating.

Anal. Calcd. for $C_{10}H_{12}N_4O_3$: C, 44.77; H, 4.51; N, 20.88. Found[15]: C, 45.8; H, 4.7; N, 18.0.

Tosyl-DL-valyl Azide.—Tosyl-DL-valyl chloride, 2.9 g., was converted to the azide by the procedure used for the alanyl derivative, yield 2.8 g., m.p. 65–66° dec. Reprecipitation gave 2.8 g., m.p. 66° dec. The m.p. varied with the rate of heating.

Anal. Calcd. for $C_{12}H_{16}N_4O_3S$: C, 48.63; H, 5.44; N, 18.91. Found[15]: C, 49.7; H, 5.6; N, 15.9.

The Action of Aqueous NaOH on the Tosylamino Acid Chlorides.—When tosyl-DL-valyl chloride, 2.9 g., was added to 20 ml. of cold 1 *N* aqueous NaOH, the crystals dissolved with effervescence leaving a solution smelling strongly of isobutyraldehyde. Acidification with concentrated HNO_3 gave a crystalline precipitate, 1.64 g., m.p. 135–137°, after drying in a vacuum at 70°. The filtrate contained the chloride ion. Recrystallization from water yielded 1.45 g., m.p. 137–137.5°, undepressed on admixture with *p*-toluenesulfonamide. *p*-Toluenesulfonamide was isolated in like fashion after alkali treatment of the tosylated acid chlorides of DL-alanine, DL-α-amino-*n*-butyric acid, α-aminoisobutyric acid, DL-norleucine and L-leucine.

When tosylglycyl chloride, 1.0 g., was added to 10 ml. of cold 1 *N* aqueous NaOH, the compound dissolved with no apparent effervescence. After 10 minutes, concentrated HCl was added causing separation of tosylglycine, 0.8 g., m.p. and mixed m.p. 147–148° after recrystallization from water. Similarly tosyl-β-alanyl chloride or tosyl-L-prolyl chloride dissolved without effervescence in 1 *N* aqueous NaOH, and addition of HCl caused separation of the regenerated tosylamino acid. Tosyl-L-pyroglutamyl chloride dissolved without effervescence in cold 1 *N* aqueous NaOH. Acidification with concentrated HNO_3 caused no separation of sulfonamide or other material. Addition of $AgNO_3$ solution produced a copious precipitate of AgCl.

The Action of Aqueous NaOH on Tosyl-DL-valyl Azide.—The azide, 1.0 g., on treatment with 10 ml. of cold 1 *N* aqueous NaOH dissolved, with effervescence, leaving a solution smelling of isobutyraldehyde. This was filtered to remove a small amount of gummy material and the filtrate acidified with 1 ml. of 10 *N* HCl to give a crystalline precipitate, 0.60 g., m.p. 103–107°, which was added to 10 ml. of 1 *N* aqueous $NaHCO_3$. Part of the solid dissolved in the cold with effervescence, the remainder dissolved on heating. On cooling, crystals separated, 0.22 g. of *p*-toluenesulfonamide, m.p. 136–137°. Acidification of the filtrate gave tosyl-DL-valine, 0.18 g., m.p. 164.5–166°.

Test for the Cyanate Ion.—The presence of the cyanate ion after alkaline degradation of tosyl-DL-valyl azide was detected by its reaction with semicarbazide to form hydrazodicarboxamide.[5] Tosyl-DL-valyl azide, 1.46 g., was shaken with 15 ml. of cold 1 *N* aqueous NaOH until gas evolution ceased. After filtering from a small amount of gum, the solution was added to 0.56 g. of semicarbazide hydrochloride dissolved in 10 ml. of water. The solution was allowed to stand for 5 minutes, then 20 ml. of glacial acetic acid was added. After 10 minutes the resulting solution deposited crystals, 0.12 g., m.p. 251–252° dec. This material did not depress the m.p. of hydrazodicarboxamide prepared from semicarbazide hydrochloride, sodium acetate and potassium cyanate.

The Action of Aqueous NaOH on Tosyl-DL-alanyl Azide.—The azide, 0.51 g., dissolved with effervescence in 10 ml. of cold 1 *N* aqueous NaOH, producing the odor of acetalde-

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hyde. Addition of 1 ml. of 10 *N* HCl gave an oil which quickly crystallized; 0.33 g., m.p. 136–138°. This material dissolved with effervescence in 10 ml. of cold 0.5 *N* aqueous $KHCO_3$. Acidification yielded tosyl-DL-alanine, 0.26 g., m.p. 137–138°.

Identification of Volatile Products from the Degradations.—Crystals of the tosylamino acid chloride or azide were allowed to react in a closed evacuated system with aqueous sodium hydroxide. Volatile material produced was isolated in a liquid-air cooled trap, in which part condensed and part remained gaseous. The mass spectra of the permanent gas and of the condensed material were then determined separately, showing the gas from the acid chlorides to be carbon monoxide and that from the azides, nitrogen. The mass spectra of the less volatile products evolved from the alanyl and α-amino-isobutyryl derivatives showed, as the main components, acetaldehyde and acetone, respectively. Also present were trace amounts of material of higher mass, presumably resulting from self-condensation of the carbonyl compounds. With the remaining tosyl derivatives which underwent degradation, the appropriate aldehyde was the sole volatile product apart from the permanent gas. In each case the mass spectrum of the carbonyl compound evolved was identical with that obtained from an authentic specimen.

Measurement of Gas Evolved in the Degradations.—The tosylated acid chloride or azide was allowed to react with 1 *N* aqueous NaOH in a closed system and the evolved gas collected over water. The results were reproducible to within 1%.

Attempted Isolation of the Tosylated Imine.—Ten ml. of cold 1 *N* aqueous NaOH was run into a solution of tosyl-DL-valyl chloride, 2.9 g., in 10 ml. of dioxane, producing effervescence. Seventy ml. of ether was added at once, the mixture shaken and the aqueous layer separated. This gave a neutral reaction. The ether solution was dried over Na_2SO_4, filtered, diluted with 80 ml. of dry petroleum ether, b.p. 60–80°, and partly evaporated. Crystals formed, 1.4 g. Two recrystallizations from dry ether–petroleum ether gave 0.7 g., m.p. 124.5–125.5°.

Anal. Calcd. for $C_{11}H_{15}NO_2S$ (tosylated imine): C, 58.64; H, 6.71; N, 6.22; S, 14.23. Calcd. for $C_7H_9NO_2S$ (*p*-toluenesulfonamide): C, 49.10; H, 5.30; N, 8.18; S, 18.73. Found: C, 49.43; H, 5.36; N, 8.12; S, 18.80.

Recrystallization from water gave material of m.p. 137–137.5° not depressed on admixture with *p*-toluenesulfonamide. The substance, m.p. 124.5–125.5°, is apparently a dimorph of *p*-toluenesulfonamide.

Hydrolysis of Tosyl-DL-valyl Chloride.—The chloride, 1.0 g., was suspended in cold water. Over 30 minutes there was no effervescence, no odor of aldehyde and little of the solid dissolved. On heating under reflux the solid reverted to oily drops, which did not dissolve at 100°. After 5 minutes at this temperature, crystallization commenced. Dilution to 300 ml. with boiling water gave a clear solution from which, on cooling, tosyl-DL-valine separated; 0.7 g., m.p. 165.5–166.5°.

Samples of tosylglycyl chloride and tosyl-DL-valyl chloride were each suspended in 2% aqueous $AgNO_3$. The suspension of the glycine derivative became cloudy within one minute, that of the valyl compound remained clear for several minutes.

Tosyl-DL-alanine Ethyl Ester.—Tosyl-DL-alanyl chloride, 5.2 g., was dissolved in hot ethanol to form the ester and the product crystallized from benzene–petroleum ether; yield 5.1 g., m.p. 66–67° (lit. m.p. 66–67°,[16] m.p. 68°[17]).

Tosyl-DL-valine Ethyl Ester.—Tosyl-DL-valyl chloride, 2.9 g., was dissolved in hot ethanol. On removal of the solvent under reduced pressure, the ester crystallized; yield 2.7 g., m.p. 86–88° unchanged after recrystallization from petroleum ether, b.p. 60–80°.

Anal. Calcd. for $C_{14}H_{21}NO_4S$: C, 56.16; H, 7.07; N, 4.68; O, 21.38. Found: C, 56.27; H, 6.98; N, 4.54; O, 21.2.

The Action of Aqueous NaOH on the Esters.—Tosyl-DL-valine ethyl ester, 0.29 g., was added to 2 ml. of 1 *N* aqueous NaOH. The solid dissolved slowly to give an odorless solution which was heated at 70° for 8 hr., cooled and treated with 2 ml. of 1 *N* aqueous HCl. A crystalline precipitate formed; 0.25 g., m.p. 165–166°, not depressed when mixed with tosyl-DL-valine. Tosyl-DL-alanine ethyl ester, similarly treated, yielded tosyl-DL-alanine. When either ester was dissolved in 2 equivalents of *N* aqueous NaOH and the solution allowed to stand at room temperature for 8 hr., hydrolysis was not complete. Acidification in each case caused precipitation of material only part of which was soluble in cold aqueous bicarbonate.

Tosyl-DL-alanyl Amide.—Tosyl-DL-alanyl chloride treated with concentrated aqueous ammonia gave the amide, m.p. 195–196°, in 95% yield.

Anal. Calcd. for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56; O, 19.81; S, 13.23. Found: C, 49.64; H, 5.83; N, 11.32; O, 20.05; S, 13.58.

Tosyl-DL-valyl Amide.—Tosyl-DL-valyl chloride on suspension in concentrated aqueous ammonia was converted quantitatively to the amide, m.p. 245–246°.

Anal. Calcd. for $C_{12}H_{18}N_2O_3S$: C, 53.31; H, 6.71; N, 10.36; O, 17.76; S, 11.86. Found: C, 53.67; H, 6.68; N, 9.71; O, 17.9; S, 11.96.

The Action of Aqueous NaOH on the Amides.—Tosyl-DL-alanyl amide, 0.65 g., dissolved in 6 ml. of 1 *N* aqueous NaOH without the production of any aldehyde odor. The solution was heated under reflux for 3 hr., cooled and acidified with 6 ml. of 1 *N* aqueous HCl. An oil separated which crystallized on standing; 0.55 g., m.p. 133–135°, raised to 137–138° by recrystallization, undepressed by admixture with tosyl-DL-alanine. Tosyl-DL-valyl amide, 1.3 g., was dissolved in 10 ml. of 1 *N* aqueous NaOH and heated under reflux for 7.5 hr., during which time the solution became cloudy. Dilution with water to 100 ml. gave a clear solution. After addition of 10 ml. of 1 *N* HCl and cooling, there separated 0.62 g. of unchanged amide, m.p. 245–246°. The filtrate, on evaporation to 10 ml., yielded crystalline material which dissolved in aqueous bicarbonate. Acidification gave tosyl-DL-valine, 0.57 g., m.p. 164–165°.

Solutions of each amide in 2 equivalents of 1 *N* aqueous NaOH, after standing at room temperature for 24 hr., gave, on acidification, unchanged amide, with over 90% recovery.

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