tion with the ylide and a product containing 70% d_4, 20% d_3, 5% d_2, 3% d_1, and 2% d_0 was obtained.

$\Delta^{4(8)}$-Menthene-9,9,9,10,10,10-d_6.—This product was prepared using the same procedure described for the nondeuterated analog except that appropriately labeled triphenylisopropylphosphonium bromide was employed. The product contained 88% d_6 and 12% d_5.

Camphor-3,3-d_2.—A solution of camphor (0.01 mole), trifluorodeuterioacetic acid (0.10 mole) and deuterium oxide (0.495 mole) was heated at 130° in a sealed tube for 9 days. The reaction mixture was basified with anhydrous potassium carbonate and extracted with pentane. The extract was dried and filtered, and the solvent was removed. The residue was recrystallized from hexane and sublimed: m.p. 179.5–180.5° (unlabeled camphor, m.p. 179.5–180.5°). The difficulty in introducing more than one atom of deuterium experience by Thomas and Willhalm[18] was avoided under these conditions, and a product containing 95% d_2 and 5% d_1 was obtained after one exchange.

(18) A. F. Thomas and B. Willhalm, *Tetrahedron Letters*, 1309 (1965).

Investigation on the Sodium–Liquid Ammonia Cleavage of a Tosyl Protecting Group of Tosylamino Acids and Peptides[1]

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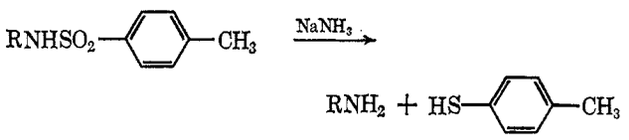
The major reaction product of the sodium–liquid ammonia cleavage of tosyl protecting groups of tosylamino acids and peptides is not thiocresol, as it is presented in the literature, but sulfite and toluene. The reaction can be best represented as two parallel reactions: (a) the major course of reaction involves carbon–sulfur cleavage giving sulfite and toluene, in addition to the amino acid or peptide; and (b) the minor path is sulfur–nitrogen cleavage giving sulfinic acid which again undergoes two types of reaction leading primarily to *p*-thiocresol through reduction and to sulfite and toluene by carbon–sulfur cleavage.

The removal of a tosyl protecting group of tosylamino acids was reported first by Fischer in 1915 using a warm phosphonium iodide–hydriodic acid mixture.[2] This quite drastic procedure was applied by Schönheimer[3] in 1926 to generate the free peptide from the tosyl derivatives. In 1937 du Vigneaud[4] demonstrated that a tosyl protecting group can be cleaved under very mild conditions using sodium–liquid ammonia. Since then the tosyl protecting group has become an important tool in peptide chemistry.

However, not much information was available regarding the fate of the *p*-toluenesulfonyl group in such reactions. The over-all reaction has been represented by Birch[5] in his reveiw on metal–amine reduction as follows. In connection with the synthesis of



some peptides using a tosyl protecting group we found a considerable quantity of sulfite instead of *p*-thiocresol during sodium–ammonia cleavage, which led us to investigate the details of this reaction.

The sodium–liquid ammonia cleavage of *p*-toluenesulfonamide, which was selected as the simplest model compound, gave 70 to 81% of sulfite along with 10 to 14% of *p*-thiocresol when 3.5 g.-atoms of sodium was used. The results are compiled in Table I. When a lesser amount of sodium was used, *i.e.*, 2.5 g.-atoms/mole, again a considerable amount of sulfite (58 to 65%) formed, along with traces of *p*-thiocresol.

For further investigations simple tosylamino acids were selected and the results are shown in Table I (expt. 3–7). The major part of the cleavage was sulfite again. The hydrocarbon part of the cleavage reaction, that is toluene, was not obtained in a quantitative yield since its major part evaporated with the ammonia. In the case of tosyl-L-glutamic acid and tosyl-DL-alanine using modified experimental techniques, it was possible to isolate about 24–25% toluene using vapor phase chromatography. This value was corrected to about 60%, counting the approximate loss observed during isolation of toluene in a control experiment.

Results of cleavage reactions of tosyl peptides are similar to that of tosylamino acids and are summarized in Table I (expt. 8–12).

p-Toluenesulfinic acid was considered first as an intermediate in this reaction, which undergoes further cleavage to sulfur dioxide and toluene or is reduced to thiocresol. Sodium–liquid ammonia reaction of *p*-toulenesulfinic acid as shown in Table I (expt. 13) yielded 49–50% of thiocresol and 21–23% of sulfite, *i.e.*, the major part of the reaction is reduction, along with some carbon–sulfur bond cleavage. The ratio of cleavage to reduction is about 1:2. Similar results were observed with benzenesulfinic acid (expt. 14, Table I).

These results show that *p*-toluenesulfinic acid cannot be the major intermediate in cleavage reactions; if this were the case, then *p*-thiocresol rather than sulfite would be the major cleavage product of tosylamino acids and peptides.

This led to the conclusion that the cleavage reaction can be best represented as shown in Scheme I.

Path a represents the major course of reaction involving carbon–sulfur cleavage leading to sulfur dioxide and toluene. Path b is the minor course of reaction which indicates sulfur–nitrogen cleavage giving sulfinic acid. Sulfinic acid again undergoes two types of reaction leading primarily to thiocresol through

(1) This investigation was presented at the Metropolitan Regional Meeting of the New York and New Jersey Sections of the American Chemical Society, 1963; a part of the results was communicated in *Chem. Ind.* (London), 913 (1963).

(2) E. Fischer, *Ber.*, **48**, 93 (1915).

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

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

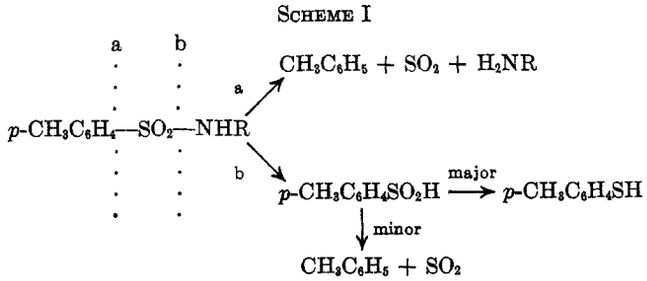
(5) A. J. Birch and H. Smith, *Quart. Rev.* (London), **12**, 17 (1958).

TABLE I

Expt.	Compound	G.-atoms of sodium/mole	% sulfite: Iodometric	% sulfite: Gravimetric	% thiocresol	Remarks
1	*p*-Toluenesulfonamide	3.5	70, 81	74	14, 10	
2	*p*-Toluenesulfonamide	2.5	59, 65	58	Trace	Some starting material recovd.
3	Tosyl-L-glutamic acid[a]	5.5 (3.5 + 2 for 2COOH)	74, 75	67	14, 15	63% toluene (corrected for 60% loss during isolation)
4	Tosyl-L-glutamic acid[a]	4 (2 + 2 for 2COOH)	50		Trace	
5	Tosyl-L-glutamic acid[a]	3.26 (1.26 + 2 for 2COOH)	52	...	...	16% sulfinic acid isolated
6	Tosyl-DL-alanine[b]	3.5 (2.5 + 1 for 1COOH)	73		16	61% toluene (corrected for 60% loss during isolation)
7	Tosyl-L-aspartic acid[c]	4.5 (2.5 + 2 for 2COOH)	60		15.7	
8	Tosyl-L-alanyl-L-leucine[d]	4.5 (3.5 + 1 for 1COOH)	19	41	6	
9	Tosyl-DL-valyl-L-leucine[d]	4.5 (3.5 + 1 for 1COOH)	21	35	10	9% *p*-toluenesulfinic acid isolated
10	Tosyl-DL-valyl-L-leucine[d]	4 (3 + 1 for 1COOH)	34	34	12	
11	Tosyl-L-glutamylglycine[e]	5.5 (3.3 + 2 for 2COOH)	Trace, 5	44, 40	19	
12	Tosyl-L-glutamyl-L-tyrosine[e]	6.4 (3.4 + 3 for 2COOH and phenolic hydroxyl)	13	57	10	
13	*p*-Toluenesulfinic acid[f]	3	21, 23	23, ...	49, 50	
14	Benzenesulfinic acid[f]	3	39	34.6	57 (thiophenol)	

[a] C. R. Harrington and R. C. G. Moggridge, *J. Chem. Soc.*, 706 (1940). [b] C. S. Gibson and J. L. Simonsen, *ibid.*, **107**, 798 (1915). [c] K. Freudenberg and A. Noe, *Ber.*, **58**, 2399 (1925). [d] See Experimental Section. [e] J. Rudinger, *Collection Czech. Chem. Commun.*, **19**, 375 (1954). [f] A. I. Vogel, "A Textbook of Practical Organic Chemistry," Longmans, Green and Co., London, 1961, pp. 607, 626, 1078.

SCHEME I



reduction, and through carbon–sulfur cleavage to some sulfite and toluene.

The presence of sulfinic acid could be detected by a color test in nearly every case of those reactions listed in Table I. More convincing results were obtained when the sodium–liquid ammonia cleavage of tosylglutamic acid was carried out with 1.26 g.-atoms of sodium instead of 3.5 (expt. 5, Table I); here 16% sulfinic acid was isolated as ferric salt along with 52% sulfite. Furthermore, this experiment clearly indicates that path a is a faster reaction than path b or the reduction of sulfinic acid to thiophenol.

The above results indicate that a longer reaction time and excess of sodium is desirable for complete elimination of sulfinic acid from the reaction mixture to simplify the purification procedure of the free peptide.

Experimental Section[6]

A typical experiment with *p*-toluenesulfonyl-L-glutamic acid is described below. All reactions were carried out under identical conditions. Slight variations were needed in certain cases, which will be mentioned separately.

Reductive Cleavage of *p*-Toluenesulfonyl-L-glutamic Acid with Sodium in Liquid Ammonia.—*p*-Toluenesulfonyl-L-glutamic acid (3.01 g., 0.01 mole) was dissolved in 100 ml. of anhydrous liquid ammonia in a three-necked flask, fitted with a stirrer, a Dry Ice–acetone condenser protected with a sodium hydroxide tube, and an inlet for dry nitrogen. The reaction vessel was flushed with a slow stream of nitrogen and the nitrogen stream was continued during the reaction. With rapid stirring, sodium metal was added to the solution in thin slices of about 0.1 g. each or using du Vigneaud's method as follows. Sodium was melted under toluene and then the end of a graduated glass tube (broken pipet) was filled with sodium. This sodium-filled tube was inserted into the liquid ammonia solution and the sodium used was easily measured. During the addition of this first 0.04 g.-atom of sodium, the blue color rapidly disappeared. On addition of more sodium the blue color reappeared, then slowly turned yellow to dark orange in color, and finally a green color developed which persisted for about 5–15 min. The consumption of sodium to this point was 1.26 g. (0.055 g.-atom). Solid ammonium acetate was added until the green color disappeared and the ammonia was allowed to evaporate, while a rapid stream of nitrogen was maintained. The dry white residue, practically free from ammonia, was taken up in water (50 ml.) and the reaction flask was thoroughly washed with several portions of water. The combined turbid aqueous solution (*ca.* 80 ml.) was extracted with ether and the ether (solution A) was washed once with water which was combined with the aqueous solution. The aqueous solution was cooled with ice, acidified with 6 *N* hydrochloric acid to pH 5, and extracted with ether to remove the thiocresol (solution B). The aqueous phase was filled up to 100 ml. This aqueous phase gave a white precipitate with barium chloride solution which almost completely dissolved in concentrated hydrochloric acid with the liberation of sulfur dioxide, and was used for the estimation of sulfite by iodometric titration, and gravimetrically after oxidation by iodine. The presence of sulfinic acid would not raise considerably the value of the iodometric titration since it is known that benzenesulfinic acid reacts with iodine solution only slowly in the cold.[7] The differences between the results obtained through iodometric titration and gravimetric method can be explained mainly by partial oxidation of sulfite to sulfate during the work-up procedures. This was indicated by the fact that the precipitate obtained with barium chloride always left some insoluble barium sulfate when dissolved in hydrochloric acid.

The ether layer (solution A) was washed with 2 *N* sodium hydroxide solution until no thiophenol was detectable on acidification of the alkaline extract with acetic acid and addition of lead acetate solution. The ether layer was dried over sodium hydroxide pellets; after evaporation of most of the ether through

(6) All analyses were performed by Weiler and Strauss, Oxford, England. All melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected.

(7) M. Bazlen, *Ber.*, **60**, 1479 (1927).

a fractionating column, the remaining solution was used for vapor phase chromatographic analysis of toluene.

The alkaline layer from the ether extraction (solution C) was acidified with 6 *N* hydrochloric acid to pH 4 and extracted with ether (solution D). The ether extracts containing thiophenol were combined (solutions B and D) and washed with water, dried over sodium sulfate, and evaporated to an oily residue. This was dissolved in ethanol, and the lead salt of thiophenol was precipitated with aqueous lead acetate solution (0.25 *M*). The lead salt was filtered, washed with water, dried *in vacuo* over phosphorous pentoxide, and used for estimation of thiocresol.

Experiments for the Isolation of Toluene.—Experiments were carried out with tosyl derivatives as described above except that no nitrogen stream was used during the reaction and evaporation of ammonia. Ammonia was allowed to evaporate through a condenser at room temperature. The residue was taken up in water and extracted with ether. The ether layer was washed thoroughly with 2 *N* sodium hydroxide solution and then with water. The ether extract was dried over solid potassium hydroxide and evaporated through a fractionating column. The residual liquid was examined by vapor phase chromatography by comparison with the retention time of sample of toluene using a 2-m. polypropylene glycol UCON LB-550-X column at 84° in a Perkin Elmer 154 B vapor fractometer with thermal detector. The liquid residue proved to be toluene with small contamination of ether. In the case of tosylglutamic acid, the yield of toluene was 26% (584 mg.) from 7.8 g. (0.025 mole) using 4 g.-atoms of sodium (2.3 g.) in 200 ml. of ammonia. Reduction of tosylalanine (4.86 g., 0.02 mole) in 150 ml. of ammonia with 1.6 g. of sodium (0.035 g.-atom) gave 448 mg. (25%) of toluene.

In order to determine the loss of toluene during the isolation procedure, 170 ml. of liquid ammonia containing 2.3 g. of toluene was allowed to evaporate under conditions identical with those described above. The residue was treated with 150 ml. of water, acidified with concentrated hydrochloric acid, and extracted twice with ether (50 ml.). The ether phase was washed with water and dried over sodium sulfate, and the solvent was evaporated through a fractionating column. Recovery of toluene was 915 mg., 40%.

Isolation of Sulfinic Acid. A.—Reaction was carried out with tosyl-L-glutamic acid (0.01 mole), and sodium (0.0326 g.-atom) in liquid ammonia (100 ml.), under the general conditions described above. After decomposition of the reaction mixture with ammonium acetate and water, *p*-thiophenol and toluene were removed by extraction at pH 4 with ether. An aliquot of the aqueous phase (total volume 50 ml.) was used for the estimation of sulfite by iodometric titration. Sulfinic acid was isolated from another part of the aqueous phase by precipitation with saturated ferric chloride at pH 2 and 0°. Ferric salt of sulfinic acid (17% yield) was filtered and dried and finally decomposed with 5% sodium hydroxide solution. After removal of the ferric hydroxide precipitate, the aqueous solution was acidified with dilute hydrochloric acid at 0°. The white precipitate of *p*-toluenesulfinic acid was filtered. After careful drying it melted at 85° and was identical with an authentic sample.

B.—In case of tosyl-DL-valyl-L-leucine, 1.04 g. (0.0033 mole) was reduced with 340 mg. (0.0148 g.-atom) of sodium, and after the usual procedure, aliquots of the water phase (100 ml.) were used for the determination of thiocresol, sulfite, and sulfinic acid. The sulfinic acid was extracted with ether from a 30-ml. portion after acidification (pH 1) with 6 *N* hydrochloric acid. The ether layer was washed with water, dried over sodium sulfate, and evaporated under reduced pressure. The residue was dissolved in water (20 ml.) and treated with an acidic saturated solution of ferric chloride. The orange-red precipitate was filtered and washed with 10 ml. of water and dried: yield 15.5 mg. (9.2%). The sulfinic acid was regenerated as described above, m.p. 84–85°.

Tosyl-L-alanyl-L-leucine.—Tosyl-L-alanine (6.1 g.), m.p. 134–135°, was coupled with 3.3 g. of L-leucine ethyl ester hydrochloride, m.p. 134°, in 30 ml. of methylene chloride according to Sheehan's method,[8] using dicyclohexylcarbodiimide (5.3 g., 0.0257 mole). The oily dipeptide was crystallized from ether–petroleum ether (b.p. 40–60°), m.p. 94–96°. The crude tosyl-L-alanyl-L-leucine ethyl ester (7 g.) was saponified with 20 ml. of 2 *N* sodium hydroxide at room temperature for 2 hr. The reaction mixture was diluted with 30 ml. of saturated sodium chloride solution and extracted with ethyl acetate. The ice-cold aqueous layer was acidified with hydrochloric acid to pH 2 when an oil separated, which solidified after a few hours. It was filtered, washed with water, and dissolved in ethyl acetate. The ethyl acetate solution was washed with water, dried over sodium sulfate, and evaporated *in vacuo*. The white solid residue was recrystallized from aqueous methanol, m.p. 190–191°, yield 3.5 g. It was identical with tosyl-L-alanyl-L-leucine, m.p. 186°, prepared by Schönheimer through a different method.

Tosyl-DL-valyl-L-leucine.—Tosyl-DL-valine (13.6 g.) was coupled with 6.6 g. of L-leucine ethyl ester hydrochloride using Sheehan's dicyclohexylcarbodiimide method.[8] The glassy reaction product was crystallized from ethyl acetate–petroleum ether giving 13.2 g. (95%) of white needles, m.p. 111–113°.

Saponification of the ethyl ester to tosyl-DL-valyl-L-leucine was carried out with 10 g. in 25 ml. of dioxane by 25 ml. of 2 *N* sodium hydroxide at room temperature for 2 hr. The oily crude reaction product (9.3 g.) obtained on acidification was crystallized from ethyl acetate–petroleum ether, m.p. 100–103°, with resolidification and melting again at 145–150°. Recrystallization from methanol–ether gave prisms, m.p. 107–109°, resolidification and remelting at 154–155°.

Anal. Calcd. for $C_{18}H_{28}N_2O_5S$: C, 56.22; H, 7.34. Found: C, 55.78; H, 7.36.

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(8) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).