Detosylation of *N*-Tosyl Amino Acids and Peptides by Electrolytic Reduction[1,2]

Tameo Iwasaki,[3] Kazuo MATSUMOTO,[4] Manabu MATSUOKA, Takeo TAKAHASHI, and Kentaro OKUMURA

Department of Synthetic Chemistry, Research Laboratory of Applied Biochemistry Tanabe Seiyaku Co., Ltd. Kashima-cho, Higashiyodogawa-ku, Osaka

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An electrolytic reductive cleavage of tosyl group in *N*-tosyl amino acids was carried out by the use of 2 mol equivalent of NaCl or KCl as an electrolyte to the tosyl amino acids and of lead as a cathode in methanol–water. The procedure was applicable to the cleavage of *N*-tosyl peptides with preservation of the peptide bond, and with retention of their optical activity. No cleavage of the other protecting groups such as urethane type group and benzyl group was observed. The optimum conditions for reductive cleavage were found to be pH 11, lead being preferable to other cathode metals.

N-*p*-Toluenesulfonyl (tosyl) amino acids are easily prepared from the reaction of *p*-toluenesulfonyl chloride with amino acids in good yields and with retention of their optical activity.

Removal of the *N*-tosyl group with a warm phosphonium iodide–hydroiodic acid mixture was first reported by Fischer[5] in 1915. This drastic method was subsequently applied by Schönheimer[6] to obtain a free peptide from the tosyl peptide.

Since du Vigneaud[7] demonstrated in 1937 that the tosyl group was readily cleaved by reduction in sodium-liquid ammonia, the tosyl group has been employed for peptide synthesis, and is especially useful for the protection of the ω-amino groups in lysine and ornithine peptides.

The *N*-tosyl group was also removed by heating with 48% hydrobromic acid in the presence of phenol[8] and with 30% hydrobromic acid in acetic acid.[9] However, these methods have not been accepted for amino acid and peptide syntheses, since they involve drastic conditions for the cleavage of tosyl amino acids and peptides. Actually they seem to involve a side reaction in the case of hydrolysis of unstable amino acids such as hydroxy amino acid, the concomitant cleavage of peptide bond being often observed. Most of them were not applicable to the detosylation of *N*-tosyl serine because of a partial decompsoition of the resulting serine.

Recently, Horner and Neumann[10] reported a method of electrolytic reductive cleavage of the *N*-tosyl group by the use of tetramethylammonium (TMA) amalgam. The method was new for organic synthesis on a laboratory scale, but the use of TMA and mercury did not make it practicable for carrying out the procedure on a large scale. They stated that cleavage of the tosyl group had not been observed with the use of electrolyte and cathode materials other than TMA and mercury respectively. If a real convenient method with milder conditions could be developed, the tosyl group would serve as a useful protecting group of amino acids and peptides.

We previously reported a method for the cleavage of the tosyl group in various amino acids and peptides by an electrolytic reduction.[11] In the present paper, we wish to report in detail on the electrolytic reduction of tosyl amino acids and peptides.

The reductive cleavage of *N*-tosyl-L-serine under various conditions in particular was investigated. We chose tosyl serine for preparing L-serine practically, the latter being an unstable amino acid under drastic conditions such as heat and strong acid or alkali. Satisfactory results for the preparation of L-serine from tosyl serine readily prepared from *N*-tosyl-L-aspargine[12] have not been obtained.

Results and Discussion

Cathode Material. Experiments on the tosyl serine were carried out with the electrode materials given in Table 1. A lead cathode gave the highest yield of serine under the same conditions, and was used in the investigations.

Electrolyte. Reductive cleavage of the tosyl serine

TABLE 1. EFFECT OF ELECTRODE MATERIAL ON THE ELECTROLYSIS OF TOSYL SERINE AT pH 11 IN 20% MeOH

Electrode	Pb	Zn	Ti	Sn	Cu	C	Porous Zn	amalgamated Pb
Yield(%)	88	7	5	20	0	26	61	42

Cathodic solution: tosyl serine (0.1 mol) and NaCl (0.2 mol) dissolved in 20% MeOH (500 ml); Anodic solution: 20% HCl; Anode: C; Current density: 3.2 A/dm^2; Current concentration: 8 A/L; Temp.: 25°C; Time: 5 hr.

1) Formation of optically active amino acids. I.
2) Presented at the 24th Annual Meeting of the Chemical Society of Japan, Osaka 3rd, April, 1971.
3) Present Address: Institute of Molecular Evolution and Department of Chemistry, University of Miami, 521 Anastasia, Coral Gables, Fla., 33134 U.S.A.
4) To whom inquiries should be addressed.
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11) K. Okumura, T. Iwasaki, M. Matsuoka, and K. Matsumoto, *Chem. Ind.* (London), **1971**, 929.
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Table 2. Effect of electrolyte on the elecrolysis of tosyl serine at pH 11 in 20% MeOH

Electrolyte	Yield(%)
NaCl	88.0
KCl	87.4
LiCl	67.3
NH_4Cl	0
$CH_3NH_2 \cdot HCl$	19.8
$(CH_3)_2NH \cdot HCl$	8.2
$(CH_3)_3N \cdot HCl$	12.0
$(CH_3)_4N \cdot Cl$	62.3

Cathodic solution: tosyl serine (0.1 mol) and NaCl (0.2 mol) dissolved in 20% MeOH (500 ml); Cathode: Pb; Anodic solution: 20% HCl; Anode: Carbon; Current density: 3.2 A/dm²; Current concentration: 8A/L; Temp.: 25°C; Time: 5 hr.

was performed with the electrolytes shown in Table 2. NaCl and KCl gave high yields in methanol–water solvent.

Ammonium chloride, monomethylamine hydrochloride, dimethylamine hydrochloride, and trimethylamine hydrochloride did not give good results, but reduction with tetramethylammonium chloride afforded a relatively good yield.

The electrolytic reduction was carried out with the use of 1.2 mol equivalent of the electrolyte to the tosyl serine. It was found that reaction temperature rose since resistance of the cathodic solution increased. In the case of a large amount of the electrolyte, treatment after the reaction was troublesome.

Reaction was achieved successfully with 2 mol equivalent of the electrolyte such as NaCl or KCl.

Effect of pH in the Cathodic Solution. The electrolytic reduction of various organic compounds such as ketone,[13,14] alkyl halide[15] *etc.* is often observed to a great extent depend on the pH of the cathodic solution. We found this to be strongly so in our electrolytic reduction of the tosyl serine.

The reduction at pH 11 gave the best result (Fig. 1); a considerably lower yield being obtained in either higher or lower pH regions. In a higher pH region the resulting serine was partially decomposed and racemization took place, whereas in a lower one unreacted tosyl serine markedly appeared on the membrane. In curve E, the pH was adjusted to 11.0 before electrolytic reduction, and was not controlled during the course of the reduction. The pH gradually shifted to alkaline region with the lapse of time, and the final concentration of sodium hydroxide in cathodic compartment was about 0.5 N after 5 hr. Hardly any reaction took place during last one hour. The results suggest that cleavage of the tosyl group cannot be performed in a higher pH region than 13.

In the water–methanol system, an adequate amount of methanol was needed to dissolve the tosyl amino acid deposited on a membrane during the course of electrolysis. In the case of tosyl serine, 20% methanol was required.

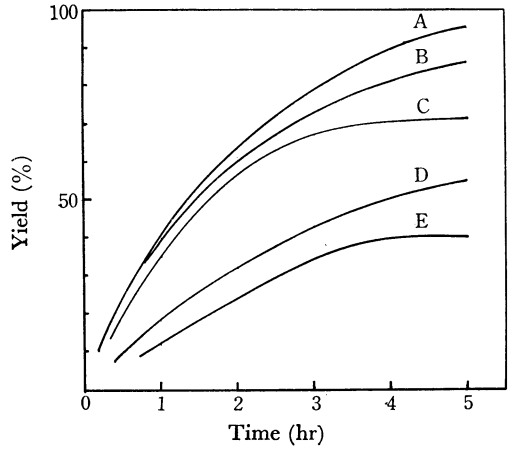

Fig. 1. Effect of pH on the electrolysis of tosyl serine. Cathodic solution: tosyl serine (0.1 mol) and NaCl (0.38 mol) dissolved in 20% MeOH (500 ml); Cathode: Pb; Anodic solution: 20% HCl; Anode: C; Current density: 3.2 A/dm²; Current concentration: 8 A/L; Yield: Analytical value. A: pH 10.8—11.2; B: pH 9.6—10.0; C: pH 12.6—13.0; D: pH 7.0—9.0; E: No controlled pH.

Cathodic Erosion of Lead. The reductive cleavage of 0.1 mol of L-tosyl serine was carried out by the use of 0.2 mol of NaCl and lead cathode in 20% methanol. After 5 hr, L-serine was obtained in 88% yield. However, the cleavage of 0.2 mol of the tosyl serine in the presence of 0.4 mol of NaCl did not proceed sufficiently. The yield of L-serine was only 45%, even when the reaction was allowed to continue for 10 hr. This might be attributed to incorporation of alkali metal into the lead cathode.[16–19] It can also be understood from the fact that use of the lead cathode renewed by washing with 5% nitric acid after 5 hr resulted again in a high yield of the cleavage of the tosyl serine.

In these experiments the electrode potential of the lead cathode showed −2.08 V *vs.* SCE in the initial stage of reaction. After 5 hr, the potential shifted to −1.95 V *vs.* SCE. When the electrode was washed with 5% nitric acid, the potential returned to −2.08 V *vs.* SCE. This result is in line with that of Chernomorskii.[20]

Thus a potential of −2.0 V *vs.* SCE would be required at least in the reductive cleavage of the tosyl serine.

The current density was 3.2 A/dm² at 25°C. A current density greater than 5 A/dm² was not needed, since intensive erosion of lead took place by the incorporation of Na^+ into the cathode and temperature rose markedly in the cathodic solution.

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20) A. I. Chernomorskii, I. G. Kiseleva, and B. N. Kabanov, *Electrokhimiya*, **6**, 429 (1970).

Electrolytic Reductive Cleavage of the Other N-*Tosyl Amino Acids and Peptides.* The above method was applied to various optically active tosyl amino acids and peptides. The results are given in Table 3. All electrolyses were carried out under the same conditions as for tosyl serine.

TABLE 3. ELECTROLYTIC REDUCTIVE CLEAVAGE OF TOSYL-L-AMINO ACIDS AND PEPTIDES

Run	Tos-	Method	Yield[a] (%)	Observed $[\alpha]_D^{25}$deg.	Literature[b] $[\alpha]_D^{25}$deg.
1	Ser–OH	A	88	+14.5	+14.5 (N-HCl
2	Thr–OH	A	91	−28.3	−28.3 (H_2O)
3	Phe–OH	A	66	−34.2	−34.8 (H_2O)
4	Ala–OH	A	84	+13.9	+14.5 (6N-HCl)
5	Val–OH	A	77	+28.6	+28.8 (6N-HCl)
6	Leu–OH	A	84	+15.3	+14.9 (6N-HCl)
7	ILeu–OH	A	85	+41.2	+40.8 (5N-HCl)
8	Glu–OH	A	84	+31.8	+31.6 (2N-HCl)
9	Asp–OH	A	79	+25.5	+25.0 (6N-HCl)
10	Cys(Bzl)–OH	A	78	−26.5	−27.0 (N-HCl)[c]
11	Lys(Z)–OH	B	92	+14.1	+14.4 (2N-HCl)[d]
12	Ala–Gly–OH	A	74	+22.1	+22.6 (0.5N-HCl)[e]
13	Val–Gly–OH	A	83	+102.2	+102.0 (H_2O)[f]
14	*S*–Bzl–glutathione	B	92	−9.7(3N-HCl)	—
15	β-Cl–Ala–OH	A	33[g]	+14.1	+14.5 (6N-HCl)

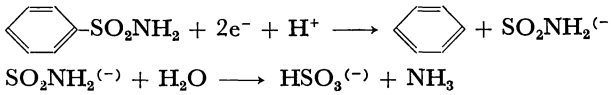
a) Amino acids obtained were paper-chromatographically pure and agreed with authentic specimens.
b) Amino acids (run 1–9, and run 15): "Chemistry of Proteins," Vol. 1, ed. S. Akabori and S. Mizushima, Kyoritsu Shuppan Co. Ltd., (1954) p. 112.
c) L. Zervas, I. Photaki, and N. Ghllis, *J. Amer. Chem. Soc.*, **85**, 1337 (1963).
d), e), f) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley & Son, Inc, (1961) p. 893, p. 1203, p. 1228, respectively.
g) Alanine was obtained.

In the case of tosyl-L-β-chloroalanine,[12] simultaneous fission of sulfur-nitrogen bond and carbon-chlorine bond occurred to give L-alanine.

Urethane type protecting groups such as benzyloxycarbonyl (Z), *t*-amyloxycarbonyl, and *t*-butyloxycarbonyl and *S*-benzyl (S–Bzl) group were not cleaved under the above conditions, but the benzoyl group was attacked.[21] Z and Bzl groups could be cleaved in the electrolytic reduction under basic conditions using other electrode material.[21] Thus, N^{ε}–Z–L-lysine and *S*–Bzl-L-cysteine were obtained by the selective electrolytic reduction of N^{α}-tosyl-N^{ε}-Z-L-lysine and *N*-tosyl-*S*-Bzl-L-cysteine, respectively. *N*-Tosyl-*S*-Bzl-glutathione was treated to give *S*-Bzl-glutathione without cleavage of the *S*-Bzl group.

Reaction Mechanism. In connection with cleavage of the tosyl group in the TMA–mercury–methanol system, Horner and Neumann[22] stated that the radical (stable in the presence of mercury) obtained by reducing TMA cation on the mercury cathode cleaved the tosyl group, and that other electrolytes such as Na or K formed the corresponding amalgam without appreciable cleavage of this group.

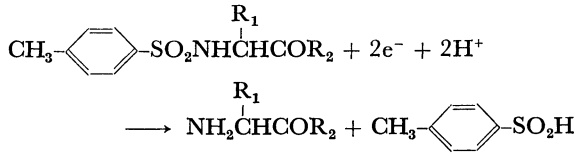
In the polarographic reduction of benzenesulfonylamide, Manousék *et al.*[23] stated that benzene and sulfurous acid monoamide resulting from carbon-sulfur bond cleavage were produced in the initial stage of the reaction, and that the reaction mechanism as represented in the following Scheme was very similar to that of the reduction with sodium-liquid ammonia as reported by Kovacs Ghatak.[24,25]



$$SO_2NH_2^{(-)} + H_2O \longrightarrow HSO_3^{(-)} + NH_3$$

However, in our electrolytic reductive cleavage of tosyl amino acids, *p*-toluenesulfinic acid was isolated from the reaction mixture in a good yield. *p*-Thiocresol was not detected at all even by iodometric titration of the cathodic solution after electrolytic reduction. The electrolytic reduction of *p*-toluenesulfinic acid was not observed under the same conditions as for tosyl amino acids and peptides, although it was reported that *p*-toluenesulfinic acid was reduced to *p*-thiocresol.[26]

The reaction in the electrolytic reduction of tosyl amino acids and peptides under our conditions should proceed *via* two electrons transfer to give sulfur-nitrogen bond cleavage as shown in the following Scheme;



R_1=Amino acid side chains
R_2=OH or amino acid residues

Experimental

Apparatus. A Yanagimoto controlled potential electrolyser was used as a D.C. source. A 600 ml electrolysis cell with a cathode, anode, cellular membrane, thermometer, and a pH autocontroller was used (Fig. 2). Commercial metal plates sufficiently polished before use were used as electrode.

A glass electrode of pH meter was rounded with platinum

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25) J. Kovacs and U. R. Ghatak, *J. Org. Chem.*, **31**, 119 (1966).
26) S. Takagi, T. Suzuki, and J. Date, *Yakugaku Zasshi*, **71**, 126 (1951).

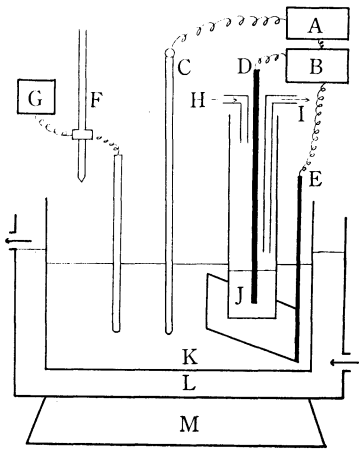

Fig. 2. Apparatus.
A: Recorder, B: DC source, C: Thermometer, D: Carbon anode, E: Lead cathode, F: Automatic buillette, G: pH controller, H: Anodic solution poured, I: Anodic solution drained, J: Anodic compartment, K: Cathodic compartment, L: Cooled water, M: Stirrer.

wire to prevent disturbance on accurate pH value from the electric current for the anode and cathode.

The pH of the cathodic solution was controlled to the desired value within ± 0.1 using a Toa Denpa pH autocontroller equipped with a buret.

Temperature in the cathodic compartment was maintained at 25°C with circulating water cooled by a Yamato cool line model BL-11 around the electrolysis cell. Current, voltage and temperature were recorded on the same chart by a Yokogawa multipoint model ERB, and the pH by a Toa Denpa multipoint pH recorder model HMR-3.

The anodic compartment, in which the anolyte was exchanged at a constant velocity with a fresh solution with a Mitsui Kagaku model SJ-1200, was clamped in position so that the anolyte and catholyte were on the same level. All optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Method. Typical cases *A* and *B* are shown. *A*. Experiments on the effect of pH, electrolyte and cathode material were carried out by the following procedure. After a mixture of 26 g tosyl-L-serine and 12 g of sodium chloride dissolved in 500 ml of 20% methanol was placed in a cathodic compartment, pH of the cathodic solution was adjusted to the desired value with 1N sodium hydroxide solution. On the other hand, 20% hydrochloric acid was put in an anodic compartment with a carbon anode. The anode and cathode compartments were separated by a membrane. An electric current 4A (4—6 V), with density 3.2 A/dm^2, was then passed through the solution at 25°C. The pH of the cathodic solution was maintained at the desired value with 5% hydrochloric acid during the course of electrolytic reduction. The anodic solution was exchanged continuously with 20% hydrochloric acid.

The yield of L-serine was determined by the method of formol titration within an error of 3% in the follwoing way. A 10—20 ml sample taken out from the reaction mixture was adjusted to pH 7.0 with 0.1N hydrochloric acid. To this was added 10 ml of 37% formaldehyde, and pH of the solution was then adjusted to 8.3 with 0.1N sodium hydroxide solution. The amount of L-serine was calculated from that of the consumption of 0.1N sodium hydroxide solution.

For the preparation of L-serine, the cathodic solution was acidified with 20% hydrochloric acid. After removal of the unreacted compound and *p*-toluenesulfinic acid by extraction with ethyl acetate, the aqueous solution was treated with Dowex 50×2 column (H^+ form) and non-amino acid acidic components were eluted with distilled water, then amino acid was eluted with 5% ammonia. The solution was evaporated to dryness *in vacuo* to give a crude serine. The product was recrystallized from water to afford paper-chromatographically and optically pure L-serine as shown in Table 3.

B. A mixture of 55.0 g (0.1 mol) of *N*-tosyl-*S*-Bzl-glutathione, 7.0 g of potassium chloride and 500 ml of 20% methanol was placed in a cathodic compartment with a lead cathode, and potassium hydroxide solution was then added to adjust the pH of the cathodic solution at 11. The anodic solution in an anodic compartment with a carbon anode was exchanged continuously with 20% hydrochloric acid. A membrane was used, and electric current 4A (4—6 V), current density 3.2 A/dm^2, was passed through the solution at 25°C for 5 hr. The pH of the cathodic solution was maintained at 11 with 20% hydrochloric acid during the course of reduction. On completion of electrolysis, the cathodic solution was adjusted to pH 3—4 with hydrochloric acid. The precipitated crystals were collected by filtration and the crude product was washed with methanol. After recrystallization from water, 36 g (92%) of *S*-Bzl-glutathione was obtained, mp 204—205°C (decomp.),[27] $[\alpha]_D^{25}$ −9.7° (*c* 0.70, 3N-HCl). The material showed a single spot when subjected to paper chromatography (Toyo filter paper No. 50), R_f 0.58 (*n*-BuOH–AcOH–H_2O=3:1:1). Found: C, 51.24; H, 5.93; N, 10.87; S, 7.81%. Calcd for $C_{17}H_{23}O_6N_3S$: C, 51.36; H, 5.85; N, 10.57; S, 8.06%.

Isolation of p-*Toluenesulfinic Acid.* The cathodic solution subjected to the above treatment was acidified with hydrochloric acid. After removal of methanol under reduce pressure, the hydrochloric acid solution was extracted with ethyl acetate. The ethyl acetate solution was evaporated to dryness under reduced pressure. The residue was recrystallized from water to obtain *p*-toluenesulfinic acid (80% yield). The crystal showed mp 84—85°C, which was consistent with an authentic specimen.

27) Melting point is uncorrected.