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234. Reduction of Tosylamino Acids and Related Compounds with Sodium in Liquid Ammonia; Stoichiometry and Products[1])

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This paper is dedicated to Professor *František Šorm,* in respect and gratitude, on the occasion of his 60th birthday.

(29. V. 73)

Summary. Using an extraction procedure which permits determination of end-points, the reduction of a number of tosylamino acids, their derivatives, and model compounds with sodium in liquid ammonia has been investigated. When the molecule contains a single acidic group in addition to the tosylamino group (as in the case of the simple mono-tosylamino monocarboxylic acids) reduction proceeds with the consumption of 2 g-at. Na per tosyl group and the formation of toluene-4-sulphinic acid. If the number of acidic groups in the products is insufficient to accommodate the number of electrons consumed in the reduction, the reduction of ionisable tosylamino groups is incomplete unless an acid is added. If the number of acidic groups is higher, or excess acid is added, the sulphur-containing products include thiocresol and sulphite. This last reaction course is also observed whenever calcium is used in place of sodium. A partial interpretation of the results is offered.

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[1]) Much of the work described in this paper was carried out at the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, in 1962–1966 and formed part of a thesis submitted by the second author (*cf.* [1]). Some of the results were presented at the XIXth International Congress of Pure and Applied Chemistry, London 1963, and at the 6th European Peptide Symposium, Athens 1963 (*cf.* [2–3]).

The tosyl protecting group has found extensive application in amino-acid and peptide chemistry (see [4]). The most widely used method for removing the tosyl group from peptides is the reduction with sodium in liquid ammonia, introduced by *du Vigneaud & Behrens* [5]. However, a number of side reactions and other difficulties have been encountered in the use of this reagent (*cf.* [4] [6]). In some instances the removal of tosyl groups has been incomplete [7–8] or excessively slow [9] under the standard reaction conditions. Cleavage of peptide chains was first observed by *Hofmann & Yajima* [10] and identified by *Guttmann* [11] as being due to the reductive fission of peptide bonds involving the imino group of proline (for further references see [4] [6]). S-substituted cysteine may be desulphurated by sodium in ammonia [12]; other reactions which have been observed include damage to threonine [13], serine [14], arginine [11], and tryptophan [15], demethylation of methionine [16–17], cleavage of the N–C_α bond in proline [18], attack on *t*-butyl ester groups [1] [18–19], and racemisation [17] [20]. In peptides of 2,4-diaminobutyric acid, $N_{(2)} \rightleftharpoons N_{(4)}$ acyl migration and intramolecular aminolysis of peptide bonds may occur though these side reactions can be avoided by a suitable choice of procedures [3] [21].

It seemed not unlikely that a better knowledge of the course and mechanism of the sodium–ammonia reduction of sulphonamides might indicate reaction conditions which would eliminate or reduce some of these difficulties and dangers. In spite of the extensive use of this procedure its course and even its stoichiometry were quite unclear at the time when this work was initiated. It was widely assumed that the sulphur-containing coproduct formed by the reduction of toluene-4-sulphonamides is thiocresol (*e.g.* [22]). Some years ago we noted the presence of sulphite and sulphate ions in the reaction mixtures after metal–ammonia reductions of tosylamino acids and tosylpeptides [23] and we have also reported the isolation of toluene-4-sulphinic acid salts from such reaction mixtures [24]. These findings have been confirmed by other authors [25–26] but the relation between these products, and the conditions for their formation, remained unclear.

The extraction technique developed by *Nesvadba* [27] for reductions with sodium in liquid ammonia, in which the metal is introduced into the reaction mixture as a solution in the refluxing solvent, offers particular advantages for studying the course of the reaction. With this technique [7] excess of sodium can be avoided, the rate of sodium consumption can be approximately estimated, and the end-point can be determined more accurately than with the conventional procedures[2]). We have made use of this method to determine the stoichiometry of the reduction of a series of tosylamino acids and some model compounds and its dependence on certain reaction conditions. The results give an insight into the reaction course and some factors affecting it.

Experimental Part

General Remarks. – Melting-points were determined on a *Kofler* block. Thin-layer chromatograms (tlc.) were run on silica gel (Kieselgel G, *Merck*) in the solvent systems (composition by volume): A) *n*-BuOH/H_2O/AcOH 4:1:1; B) *n*-BuOH/pyridine/H_2O/AcOH 15:10:6:3; C) *i*-PrOH/26% aq. NH_3 7:1; D) H_2O-satd. PhOH/26% aq. NH_3 9:1. Electrophoresis was carried out on

[2]) More recently, *Katsoyannis et al.* [28] have described what is essentially a burette for the addition of sodium in liquid ammonia.

Whatman No. 3MM paper in a moist-chamber apparatus with 6% AcOH (pH 2.4) as the electrolyte, at about 20 V/cm for 1 h.

Materials. The tosylamino acids and tosylglycylglycine were prepared by standard procedures (*cf.* [29]). Tosylproline[3]) was used as the solvate with 1/2 molecule of benzene [31a]. The ditosyl-, $N_{(2)}$-tosyl-, and $N_{(4)}$-tosyl derivatives of 2,4-diaminobutyric acid were available from earlier work [31b]. Monosodium tosylglutamate sesquihydrate [31c] was converted to the anhydrous salt by drying at 100°/0.1 Torr for 48 h; m.p. 222–230° (dec.). The disodium salt of tosylglycine [31d] was freshly dried before use. Na toluene-4-sulphinate [32] and the Ca salt [33] were obtained as the hydrates and were dried at 0.1 Torr and 50° and 100°, respectively. Toluene-4-sulphinic acid was prepared from the Na salt by careful acidification with HCl in concentrated solution, collected, washed with ice-cold water and dried over KOH *in vacuo*; m.p. 83–86°. N-Tosylpiperidine [34] and S-4-tolyl toluene-4-thiosulphonate [35] were prepared essentially as described.

Tosylglycyl piperidide. Tosylglycine chloride (3.2 g) in $CHCl_3$ (20 ml) was treated with piperidine (2.3 g) at room temperature overnight and the mixture was evaporated to dryness under reduced pressure. The residue was taken up in ethyl acetate, the solution was filtered, washed with water, 5% $NaHCO_3$, dil. HCl, and water, dried (Na_2SO_4), and evaporated to dryness; yield 1.5 g; m.p. 129–130°, unchanged by recrystallisation from ethyl acetate/light petroleum.

$C_{14}H_{20}N_2O_3S$ (296.4) Calc. C 56.73 H 6.80 N 9.45% Found C 56.94 H 6.24 N 9.27%

Standard Reduction Procedure. The apparatus, comprising the reduction flask containing a stirring-bar, the extractor proper [7] and a dry-ice reflux condenser was oven-dried, assembled, and allowed to stand overnight with a flask containing P_2O_5 attached to the inlet arm. Ammonia (about 120 ml) was distilled into the reaction flask, using the dry-ice condenser, from a solution containing excess Na. The inlet arm was detached and the inlet closed with a stopper. The compound to be reduced (5 mmol unless otherwise stated) was introduced through the lower side-arm and dissolved with stirring. The ammonia was allowed to come to boiling-point and the required amount of freshly cut Na was rapidly weighed in air to an accuracy of about 10 mg (the approximate stoichiometry was determined in preliminary experiments). The Na was placed on the sintered-glass disc of the extractor through the upper side-arm and the NH_3 was caused to reflux vigorously, with continued stirring, by immersing the flask in an alcohol bath at 20–40°. The rate of reflux aimed at was such that the sodium was introduced as rapidly as it was consumed, keeping the solution a faint blue. When the solution acquired a deep-blue colour the reaction flask was immersed in an ethanol-dry ice bath to stop reflux and, if the colour proved to be stable for 2 min or more, the solution was treated with sufficient NH_4Cl to discharge the colour, the reaction flask was detached and the NH_3 was evaporated from the frozen state. Evaporation was continued for 2 h after the residue was apparently dry.

The residue was taken up in ice-water (100 ml) and most of the Na^+ was removed [23] by stirring the solution with the carboxylate ion exchange resin Amberlite IRC-50 (NH_4^+ salt) (3 g) for 30 min. The resin was filtered off, washed with water, dried in air and extracted with ethanol and with ethyl acetate; the extracts were examined for water-insoluble products. The combined aqueous filtrate and washings were passed through a column of the sulphonic acid ion exchange resin Zerolite 225 (H^+ form) (15 ml) and the column was washed with water and then with 1% aq. NH_3 until the eluate was ninhydrin-negative. The aqueous eluates, in which SO_2 and thiocresol, if present, were detected by the odour, were evaporated down to about 3–5 ml on a rotary evaporator and the toluene-4-sulphinic acid which crystallised was collected, washed with ice-cold water (3 ml) and dried in a desiccator over NaOH. The combined mother liquor and washings were treated with aq. $FeCl_3$ until precipitation was complete. The precipitate (essentially iron (III) toluene-4-sulphinate [36]) was dried and weighed. In some cases, the distillate collected during evaporation of the aqueous eluate from the Zerolite column was extracted with ether, the extract was dried and evaporated, and the residue of thiocresol (m.p.'s 40° to 43°) was weighed.

The ammoniacal eluates from the Zerolite column were evaporated to dryness under reduced pressure and the residue was examined by tlc. (systems A und B) and by paper electrophoresis. The amino acid or peptide was transferred to a filter with ethanol (in the case of proline, with 2-propanol), dried, and weighed.

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[3]) All amino acids (except glycine, sarcosine, and α-aminoisobutyric acid) are of the L-configuration. Standard abbreviations are used for amino acids and their derivatives [30].

In particular experiments, the standard procedure was varied as follows:

Reductions with Ca and Li. Ca shavings and Li freshly cut from blocks kept under petroleum were used in place of Na. The oxide or carbonate film on the Ca was retained on the sintered-glass disc when the metal dissolved.

Tosylglutamic acid. After reduction of tosylglutamic acid and its monosodium salt, the residue obtained by evaporation of the ammoniacal eluate from the Zerolite column was evaporated several times with concentrated HCl, the hydrochloride was transferred to the filter with a small volume of conc. HCl, washed with the acid and dried *in vacuo* over P_2O_5 and KOH.

Ditosyl-2,4-diaminobutyric acid. The reduction of the ditosyl derivative (2.5 mmol) was carried out by the standard procedure. The ammoniacal eluates from the Zerolite 225 column were evaporated almost to dryness, the solution was treated with picric acid (1.15 g) in ethanol and the mixture was further evaporated to crystallisation. The product (0.54 g) was identical, by m.p. and mixed m.p. (183–185°), with the dipicrate of 2,4-diaminobutyric acid [37]. The mother liquors after separation of the picrate were analysed by paper electrophoresis; 2,4-diaminobutyric acid and its $N_{(2)}$- and $N_{(4)}$-tosyl derivatives were run for reference.

Tosylglycine piperidide. No clear end-point was reached after 2 g-at. Na/mol had been added. In one experiment, the reaction was stopped at this stage and the reaction mixture was worked up by the standard procedure. The ammoniacal eluates from the Zerolite 225 column were evaporated to a small volume and analysed by paper electrophoresis. Three ninhydrin-positive products were detected, identical in mobility with reference samples of glycine, glycine piperidide[4]), and 2-aminoethanol. A similar mixture of products was obtained with Ca (consumption 2 g-at./mol) instead of Na.

Disodium salt of tosylglycine. A suspension of the salt (5 mmol) in NH_3 turned blue after 2–3 drops of Na solution had been added. After addition of NH_4Cl (5 mmol) more Na was consumed; the reaction slowed down distinctly after 1 g-at./mol had been added. After the consumption of 2 g-at./mol the reaction was stopped. Tosylglycine (0.28 g; 24%) was recovered (identified by tlc. in system C and by m.p. and mixed m.p. after recrystallisation from ethanol). Glycine (0.210 g; 56%) was isolated in the usual way. No sulphinic acid was found.

S-4-Tolyl toluene-4-thiosulphonate. The thiosulphonate (5 mmol) was reduced by the standard procedure; it gradually dissolved during the addition of Na. A clear end-point was reached after 10 mg-at. of Na had been added. The colour was discharged with NH_4Cl, the solution was dried from the frozen state, the residue was taken up in water, the solution was filtered and brought to pH 5. The precipitated thiocresol (0.400 g; m.p. and mixed m.p. 42–43°) was collected, the solution was extracted with ether (the extract contained more thiocresol), evaporated to small bulk and acidified with HCl. Yield 0.613 g of toluene-4-sulphinic acid, m.p. 84–85°, undepressed on admixture of an authentic sample.

Treatment of toluene-4-sulphinic acid with liquid NH_3. Toluene-4-sulphinic acid (0.156 g; 1 mmol) was refluxed in liquid NH_3 for 10 min, the solution was evaporated from the frozen state, the residue was taken up in water and the solution was passed through a column of Zerolite 225 (H^+). The sulphinic acid in the eluate was determined manganometrically [39]. Recovery was quantitative. The same result was obtained when NH_4Cl (1 mmol) was added to the solution in liquid NH_3. In another experiment, tosylglycine (5 mmol) was reduced with Na by the standard procedure. After the end-point of the reduction had been reached, toluene-4-sulphinic acid (5 mmol) was added, the solution was allowed to reflux for 10 min and worked up by the standard procedure. The yield and recovery of sulphinic acid (0.972 g) and its Fe^{3+} salt (0.391 g) corresponded to 8.5 mmol.

Results. – The amounts of sodium consumed in the reduction of a number of tosylamino acids and a tosylpeptide are given in Table 1 together with the yields of products isolated. It will be seen that the tosyl derivatives of amino acids and imino acids (proline and sarcosine) not containing additional acidic functional groups are

[4]) A reference sample of glycine piperidide was obtained from the tosyl derivative by treatment with 35% HBr in acetic acid at 70° for 4 h (*cf.* [38]).

cleanly reduced by two atoms of sodium, with the formation of the amino acid and toluene-4-sulphinic acid in high yields. The reduction of tosylglycylglycine takes an analogous course.

In the reduction of ditosyl-2,4-diaminobutyric acid a clear end-point was reached after 3 g-at. Na per mole of the ditosyl derivative had been added. Electrophoretic analysis of the amino-acid products showed incomplete reduction, the 2,4-diaminobutyric acid (isolated in 40% yield as the picrate) being accompanied by the $N_{(4)}$-

Table 1. *Reduction of tosylamino acids and a tosylpeptide with sodium in liquid ammonia*

Starting material[a])	Na consumed g-at./mol	Yield %	
		sulphinic acid[b])	amino acid
Tos-Gly	2	87	99
Tos-Gly[c])	4	18	88
Tos-Leu	2	74	74
Tos-Pro	2	72	66
Tos-Sar	2	70	81
Tos-Aib	2	81	87
Tos-Gly-$N(CH_2)_5$	>2[d])	32	d
Tos-Ser	3.5	49	66[e])
Tos-Glu	3.5	33	70[f])
Tos-Glu-ONa	2	56	46[f])
Tos-Dab(Tos)	3	59	40[g])
Tos-Gly-Gly	2	70	98

[a]) For symbols and conventions see [30]; Aib denotes 2-aminoisobutyric acid.
[b]) Including material isolated as Fe^{3+} salt.
[c]) EtOH (2 mol/mol) added after first end-point; thiocresol detected.
[d]) Reaction stopped after consumption of 2 g-at. Na/mol; glycine piperidide, glycine, and 2-aminoethanol identified electrophoretically, thiocresol and SO_2 detected.
[e]) A trace of glycine was detected by tlc.
[f]) Isolated as hydrochloride.
[g]) As picrate; Dab(Tos) and a trace of Tos-Dab identified by electrophoresis.

Table 2. *Reduction of some model compounds with sodium in liquid ammonia*

Starting material (R = 4-tolyl)	Na consumed g-at./mol	Yield or recovery %			
		Starting material	$R \cdot SO_2H$	$R \cdot SH$	SO_2
$R \cdot SO_2 \cdot NH_2$	1	22	38	–	–
$R \cdot SO_2 \cdot NH_2 + NH_4Cl$[a])	2	7	54	+	+
$R \cdot SO_2 \cdot NH_2 + 2\ AcNH_2$[b])	2	0	16	+	+
$R \cdot SO_2 \cdot NHCH_3$	1	35	47	–	–
$R \cdot SO_2 \cdot N(CH_2)_5$	2	trace	68	–	–
$R \cdot SO_2H$	2	37		23	11[c])
$R \cdot SO_3Na$	0	64		–	–
$R \cdot SO_2 \cdot S \cdot R$	2	0	79	>65	+

[a]) Added after first end-point.
[b]) Added before reduction.
[c]) Determined gravimetrically as $BaSO_4$ in an aliquot after oxidation.

tosyl derivative and traces of the $N_{(2)}$-tosyl derivative. Incomplete reduction was also observed with the model compounds, toluene-4-sulphonamide and its N-methyl derivative (Table 2). Both these compounds consumed only 1 g-at. Na/mol and considerable amounts of the starting materials were recovered from the water-insoluble fraction of the products. On the other hand, the reduction of N-tosylpiperidine proceeded to completion, 2 g-at. Na/mol being consumed. When, in the reduction of toluene-4-sulphonamide, 1 mol of ammonium chloride was added after the apparent end-point had been reached, or 1 mol of acetamide or urea at the beginning of the experiment, a total of 2 g-at. Na/mol was again consumed and the sulphinic acid was formed in high yield. The disodium salt of tosylglycine was resistant to sodium reduction but after addition of ammonium chloride (1 mol/mol) to the liquid ammonia solution, sodium was consumed. After 2 g-at. Na/mol had been taken up some tosylglycine was recovered on work-up but no toluene-4-sulphinic acid was isolated. In all other cases of incomplete reduction, the sulphinic acid was the only sulphur-containing reduction product detected.

In the reduction of tosylserine and tosylglutamic acid, the sodium consumption generally slowed down distinctly after 2 g-at. Na/mol had been added but a stable blue colour did not appear until about 3.5 g-at. Na/mol had been taken up. In these cases the yields of toluenesulphinic acid were low (see Table 1) and the presence of thiocresol and sulphur dioxide was evident from the odour. Anhydrous monosodium tosylglutamate consumed only 2 g-at. Na/mol and the yield of toluenesulphinic acid was higher. In model experiments (Table 2) it was found that whereas sodium toluene-4-sulphinate dissolved in liquid ammonia consumed no sodium, a solution of the free acid took up about 2 g-at. Na/mol; from the reaction products some of the sulphinic acid was recovered but thiocresol and sulphur dioxide were also found.

The consumption of more than 2 g-at. Na/mol was also observed in the reduction of tosylglycine piperidide; even when the reaction was stopped after addition of 2 g-at. Na/mol a complex mixture of products was formed, including at least three ninhydrin-positive materials which were electrophoretically identical with glycine, glycine piperidide, and 2-aminoethanol.

The results of some reductions with lithium and calcium are given in Table 3. The course of the reaction with lithium was the same as with sodium but with calcium the end-point was not reached until 2 g-at. (4 g-eq.) Ca had been consumed per mol of

Table 3. *Reductions with lithium and calcium in liquid ammonia*

Starting material[a])	Metal	Consumed g-eq./mol	Yield or recovery %		
			$R \cdot SO_2H$	$R \cdot SH$	Amino acid
Tos-Gly	Li	2	80	–	80
Tos-Gly	Ca	4	0	present	80
Tos-Leu	Ca	4	0	5	66
Tos-Gly-$N(CH_2)_5$	Ca	4	present	present	[b])
$R \cdot SO_2Ca_{0.5}$	Ca	5	0	50	

[a]) R = 4-tolyl.

[b]) Glycine piperidide, glycine, and 2-aminoethanol identified by electrophoresis.

tosylamino acid. Reduction of tosylglycine piperidide with 2 g-at. Ca/mol again gave a mixture of products.

A solution of calcium toluene-4-sulphinate in liquid ammonia took up approximately 2.5 g-at. Ca/mol; no toluene-4-sulphinic acid was recovered but thiocresol was isolated in 50% yield.

Discussion. – The results given in Table 1 clearly demonstrate that, at least under the conditions prevailing when the extraction procedure [7] is used, the fundamental reaction course involves reduction of the tosylamino group by two electrons with fission of the S–N bond and formation of the amine and sulphinic acid [2–3][5]. This reaction course has also been deduced for the particular case of S-(2-tosylaminoethyl)cysteine by *Hope* & *Horncastle* [26]. With tosylamino acids containing no additional groups the reaction stops at this stage (or slows down greatly) and high yields of both the amino acid and toluene-4-sulphinic acid can be isolated. It is of interest to note that the electrolytic cleavage of sulphonamides in methanolic tetramethylammonium chloride also consumes two electrons and affords the sulphinic acids [41].

At first sight it is surprising that the reduction of the simpler model compounds, toluene-4-sulphonamide and its N-methyl derivative, with sodium in ammonia comes to a stop when only one-half of the sulphonamide has been reduced. However, consideration of the fate of the two electrons consumed during the reduction shows why this is so. During the reduction of the amino-acid derivatives, these electrons are evidently accommodated by formation of the sulphinate and carboxylate anions (eq. 1a). In tosylamides not containing an acid functional group one negative charge is lodged on the conjugate base of the most acidic species present – the tosylamide group, so that for every mol of amide reduced one mol of amide is ionised (eq. 1b). On the reasonable assumption that the tosylamide anion is resistant to reduction, the stoichiometry of the reaction becomes entirely plausible. The failure of the disodium salt of tosylglycine (in which the tosylamino group as well as the carboxyl group is present as the anion) to react with sodium in ammonia lends experimental support to this assumption. The behaviour of the fully N-substituted sulphonamide, N-tosylpiperidine, which is completely reduced with the consumption of 2 g-at. Na/mol, is also in accord with this explanation; the second electron here evidently goes to form NH_2^- (eq. 1c). The complete reduction of toluene-4-sulphonamide in the presence of urea or acetamide can also be rationalised: Evidently these amides are sufficiently strong acids in the ammonia system to compete with the sulphonamide for the negative charge and make uncharged sulphonamide available for reduction.

$$C_7H_7 \cdot SO_2 \cdot NH \cdot CH_2 \cdot COOH + 2e = C_7H_7 \cdot SO_2^- + NH_2 \cdot CH_2 \cdot COO^- \quad (1a)$$

$$2\ C_7H_7 \cdot SO_2 \cdot NHCH_3 + 2e = C_7H_7 \cdot SO_2^- + C_7H_7 \cdot SO_2 \cdot NCH_3^- + NH_2CH_3 \quad (1b)$$

$$C_7H_7 \cdot SO_2 \cdot N(CH_2)_5 + 2e + NH_3 = C_7H_7 \cdot SO_2^- + NH_2^- + HN(CH_2)_5 \quad (1c)$$

The same explanation without doubt applies to other, more complex cases of incomplete reduction. Thus ditosyl-2,4-diaminobutyric acid contains two reducible

[5] The possibility that the formation of sulphinic acid might be due to a base-catalysed elimination (see [40]) is excluded [2–3] by the stoichiometry, the formation of optically active reduction products, the normal behaviour of 2-aminoisobutyric acid, the stability of tosylleucine to sodium amide, etc.

groups which should consume four electrons, but only three acid groups (two sulphinic, one carboxylic) are available to accommodate these electrons so that some tosylamide groups are ionised and thereby protected against reduction. The tosylamino grouping in position 4 is evidently the more acidic since more of the $N_{(4)}$- than of the $N_{(2)}$-tosyl derivative survives.

Nesvadba & Koleff [8] have noted that tosyl-S-benzylcysteine and its derivatives are incompletely reduced by sodium in liquid ammonia even after a clear end-point (blue coloration) has been reached: Tosylcysteine and tosylcystine were identified among the reaction products. Here again, tosyl-S-benzylcysteine contains an excess of reducible over (potential) acidic groups. The benzyl anion, which formally is the primary product of reduction of the benzyl thioether grouping, would be expected to be a stronger base than sulphonamide anion and the tosylamino group should again be ionised. The most conspicuous case of sluggish reduction recorded in the literature [9] involved a benzyloxycarbonyl-tetratosyl-pentapeptide, *Z*-Lys(Tos)-Lys(Tos)-Arg(Tos)-Arg(Tos)-Pro-OH, once more a compound with a large excess of reducible over acidic groups.

It should be noted that this explanation can be applied to peptides only if it is assumed that the peptide NH group, unlike urea or acetamide in the reduction of toluene-4-sulphonamide discussed above, is not capable of competing with the tosylamino group for the electron to the necessary extent.

In several other reductions recorded in Table 1 no clear end-point was reached after the consumption of 2 g-at. Na/mol although the rate of reduction distinctly decreased at this point. A stable blue coloration usually developed after 3.5–4 g-at. Na/mol had been added, and the reaction products included thiocresol and sulphur dioxide. Two types of compounds showed this behaviour. The first, represented by tosylserine[6]) and tosylglutamic acid, characteristically contain an acidic group in addition to the α-carboxyl. That this structural feature is responsible for the altered reaction course was shown by two experiments: When one acidic group was blocked in advance by salt formation (monosodium tosylglutamate) the 'normal' stoichiometry (2 g-at. Na/mol) was restored; when, on the other hand, ethanol was added during the reduction of tosylglycine, consumption of sodium continued beyond 2 g-at./mol and thiocresol and sulphur dioxide were formed.

The obvious conclusion is that sodium toluene-4-sulphinate is stable to sodium in liquid ammonia whereas the free acid, formed to some extent in the presence of additional acids of comparable strength, undergoes further reaction with the consumption of sodium. This conclusion was borne out by the appropriate experiments (Table 2): A solution of sodium toluene-4-sulphinate in liquid ammonia turned blue with the first drops of sodium but a solution of the free acid consumed sodium, to give thiocresol and sulphur dioxide.

As to the course of this further reaction, the simplest explanation would be that free toluenesulphinic acid directly undergoes further reduction. However, this does not account for the formation of sulphur dioxide since this product represents the same oxidation state of sulphur as the sulphinic acid and cannot therefore arise from

[6]) It has been noted that the reduction of tosylserine [14] and tosylthreonine [13] with sodium in liquid ammonia gives rise to ninhydrin-positive by-products; under our conditions only a trace of glycine could be detected in the serine formed.

the latter by a reductive process. The conclusion of *Kovacs & Ghatak* [25] that the sulphite arises by a reductive attack on the C–S bond in the sulphonamide, as a parallel reaction path to the reduction of the S–N bond, cannot stand in view of our demonstration that toluene-4-sulphinic acid is practically the exclusive primary product of sulphonamide reduction, and that sulphur dioxide is formed from toluene-4-sulphinic acid itself. On the other hand, sulphite is known [42] to result when sodium benzenesulphonate is reduced with sodium in liquid ammonia (eq. 2). Also,

$$C_6H_5 \cdot SO_3^- + 2e + NH_3 = C_6H_6 + SO_3^{2-} + NH_2^- \quad (2)$$

sulphinic acids are known [43] to undergo a characteristic disproportionation reaction under a variety of conditions which gives rise to sulphonic acid and thiosulphonate (eq. 3). Since thiosulphonate would be expected to be reduced in turn to thiol and sulphinate (eq. 4) a reaction scheme in which toluene-4-sulphonic acid (but not its

$$3\ R \cdot SO_2H = R \cdot SO_3H + R \cdot SO_2 \cdot S \cdot R + H_2O \quad (3)$$

$$R \cdot SO_2 \cdot S \cdot R + 2e = R \cdot SO_2^- + R \cdot S^- \quad (4)$$

sodium salt) undergoes such disproportionation under the conditions of the sodium–ammonia reduction would account at least qualitatively for the course of the further reaction. We have, indeed, found that S-4-tolyl toluene-4-sulphinate is reduced by sodium (2 g-at./mol) with the formation of toluene-4-sulphinic acid and thiocresol. However, no evidence could be found for the disproportionation of toluene-4-sulphinic acid kept in liquid ammonia for up to 30 min. No traces of the tolyl toluenethiosulphonate were found, and permanganate titration indicated quantitative recovery of sulphinic acid. Similar results were obtained when the sulphinic acid was treated with ammonium chloride in liquid ammonia, or when it was added to a reaction mixture resulting from the reduction of tosylglycine with sodium under the standard conditions. It is still possible that dissolution in liquid ammonia itself is not fully equivalent to the conditions of the sodium–ammonia reduction, or that the first stages of the disproportionation, which are thought to be equilibrium reactions [43b], do occur and that reductive removal of an intermediate causes the disproportionation to continue.

These considerations are also relevant to the course of reduction of tosylamino acids with calcium in ammonia, in which four equivalents of the metal are consumed (Table 3). Unlike sodium toluene-4-sulphinate, the calcium salt in liquid ammonia (though it is only partially soluble) consumes calcium, or sodium, with the formation of thiocresol. Evidently the calcium salt either itself undergoes whatever reaction is responsible for the behaviour of the free acid, or it gives rise to the free acid by solvolysis, favoured presumably by the well-known tendency of ammonia to solvate calcium ions.

Tosylglycine piperidide, which also consumes more than 2 g-at. Na/mol in liquid ammonia, obviously does not belong to the same structural category as tosylserine or tosylglutamic acid. The higher sodium consumption here is associated with reductive cleavage of the amide bond as indicated by the formation of 2-aminoethanol. This is the same type of reaction as the reductive fission of peptide bonds involving the imino groups of proline or sarcosine. Although there is some dispute on the matter [44] it seems likely [11] [45–46] that this cleavage is a metal/acid reduction, with methanol [45], water, tosylamino groups [11] or adventitious impurities as the proton

source. Our experiment in which attack at the amide bond was observed after only two equivalents of sodium had been added shows that this reaction must be concomitant with reductive fission of the sulphonamide bond, a conclusion which agrees with the results obtained by *Guttmann* [11] using the conventional reduction conditions.

In summary, our results show that the course of reductions with sodium in liquid ammonia is greatly affected by the presence of acids or bases in the reaction medium. Preponderance of base[7]) may lead to incomplete reduction of tosylamino groups and probably also favours racemisation; preparatively, this situation can be corrected by adding a relatively weak acid (in the ammonia system) such as acetamide [47] or urea [48]. Preponderance of acid groups leads to a more complex course of reduction of the sulphur-containing moiety (a consideration which is immaterial to the primary preparative purpose) and favours attack on certain peptide bonds. Neutralisation of excess acid groups can to some extent restore the 'normal' course of reaction, but the finding that attack at the dialkylamide bond appears to proceed concomitantly with reduction of the tosylamide group, with both sodium and calcium as the reducing agent, makes it unlikely that this side-reaction can be completely suppressed.

The conclusions derived from this study do not necessarily apply to reactions carried out by the conventional procedure. The high metal concentration and consequent local excess created by the dissolving sodium and the presence of a metal-ammonia interface might well cause some reactions which are consecutive under our conditions to occur simultaneously (giving rise to a different stoichiometry) and even lead to reactions which do not occur when the extraction procedure is used.

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