338. *Aryl-2-halogenoalkylamines. Part XVIII.* The Rates of Reduction of Substituted 4-Di-(2-chloroethyl)aminoazobenzenes by Stannous Chloride, Hydrazine, and the Xanthine Oxidase–Xanthine System.*

By W. C. J. Ross and G. P. Warwick.

The rates of reduction of thirty-nine 4-di-(2-chloroethyl)aminoazobenzene derivatives by acid stannous chloride and by hydrazine in neutral ethanolic solution have been measured. The effect of substituents on the reducibility of the azo-linkage is discussed; for reduction in acid solution the effect of 3′- and 4′- substituents can be expressed by the Hammett equation, ρ having the value 1·59. The rates of reduction of some water-soluble azo-derivatives by the xanthine oxidase–xanthine system in pyrophosphate buffer under anærobic conditions have also been measured. The results are consistent with the hypothesis that reduction of the azo-linkage potentiates inhibition of tumour growth by the azo-compounds.

In Part XVI * reference was made to the desirability of finding whether the relative rates of reduction of various 4-di-(2-chloroethyl)aminoazobenzenes could be correlated with their biological activities. Such relative rates have been determined by using polarography, catalytic hydrogenation, chemical reagents, and enzyme systems. It has been difficult to interpret the polarographic results particularly since it has not been agreed that the systems

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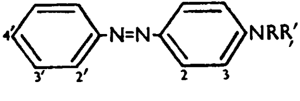
* Parts XVI, XVII, *J.*, 1956, 1364 and preceding paper.

are reversible.[1] It has been stated that "the rate of reaction between an oxidant and a reducing agent is not simply determined by the difference in the oxidation–reduction potential of the two systems. Frequently the chemical nature of the reagent used is of primary importance with regard to the rate of reaction." [2] So we have measured actual rates of reduction. Despite the uncertainties of interpreting the polarographic results and of relating them to biological conditions some tentative conclusions emerge from this earlier work. Shikata and Tachi [3] found the order of reducibility: 4-dimethylamino- $<$ 4-amino-azobenzene $<$ azobenzene. Vladimirstev and Postovski [4] indicate the order: 4-hydroxy- $<$ 4-methoxy- $<$ 4-acetoxy-azobenzene $<$ azobenzene. These results are consistent with Shikata and Tachi's electronegativity rule of reduction potentials,[3] that is, that organic compounds are more easily reduced as more electronegative groups are introduced into the molecule.

The relative rates of catalytic hydrogenation of sulphonamide azo-dyes related to prontosil were measured by Hamon,[5] using a standardised preparation of Raney nickel under controlled conditions of temperature and agitation. There is again considerable difficulty in relating the results to biological systems, for it is known that the relative rate and even the course of hydrogenation can be influenced by a change of catalyst or of solvent. For example, while Hamon [5] found that azobenzene took up two mols. of hydrogen at a steady rate to give aniline under her standard conditions, Skita,[6] using colloidal platinum, found that reduction to hydrazobenzene was rapid and was followed by a further slow reduction. Nevertheless under Hamon's conditions it was shown that in non-basically substituted azobenzenes a 4-methyl substituent hindered reduction whilst a 2-methyl group facilitated it. A 2-carboxy-substituent caused an initial increase in the rate but this decreased before two mols. of hydrogen had been taken up: it was considered that the increased speed of reduction could be attributed to increased electron density at the azo-linkage which resulted from the presence of the 2-substituent.

Tiffeneau [7] showed that, in the reduction of the closely related phenylhydrazine structure by stannous chloride, introduction of one or two methyl groups into the 2- and 4-position of the aromatic ring increased the rate of reaction. Tréfouël and his co-workers [8] measured the rate of reduction of some biologically effective azo-compounds, using cysteine in aqueous solution at 95°, and demonstrated a relation between this rate and activity. Finally, Mecke and Schmähl [9] found that in the enzymic reduction of 4-dimethylaminoazobenzene derivatives by baker's yeast, the introduction of a 2′-hydroxy-substituent reduced the rate of reaction whilst the introduction of a 4′-carboxy-group and especially of a 2′-carboxy-group increased it.

We have used chemical reagents in homogeneous solutions. At first stannous chloride in 0·5N-hydrochloric acid in 75% aqueous ethanol was used, the intensity of the major absorption band (see Part XVII) yielding the concentration of azo-compound. A large excess of stannous chloride was used and, the reaction, being of the first order with respect to azo-compound, exhibited unimolecular kinetics. In order to eliminate errors due to small variations in temperature or in stannous chloride titre, the rate of reduction of the reference compound (I; $R = R' = CH_2{\cdot}CH_2Cl$) was measured simultaneously with that of the compound under test. The unimolecular rate constants are expressed relative to the value for the unsubstituted compound (I; $R = R' = CH_2{\cdot}CH_2Cl$) (Table 1). In all cases the absorption in the region of the *Q*- and *K*-bands fell to an extremely low value at the end of



(I)

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[1] Biilmann and Blom, *J.*, 1924, **125**, 1719; Conant and Pratt, *J. Amer. Chem. Soc.*, 1926, **48**, 2468; Shikata and Tachi, *Mem. Coll. Agr. Kyoto Imp. Univ.*, 1931, **17**, 45; Tachi, *ibid.*, 1937, **40**, 1, 11; 1938, **42**, 1; Hillson and Birnbaum, *Trans. Faraday Soc.*, 1952, **48**, 478; Page, *Quart. Rev.*, 1952, **6**, 298.

[2] Kolthoff and Lingane, "Polarography," Interscience Publ. Inc., New York, 1946, p. 349.

[3] Shikata and Tachi, *J. Chem. Soc. Japan*, 1932, **53**, 834; *Coll. Czech. Chem. Comm.*, 1938, **10**, 368.

[4] Vladimirstev and Postovskii, *Doklady Akad. Nauk, S.S.S.R.*, 1952, **83**, 855.

[5] Hamon, *Ann. Chim. (France)*, 1947, **2**, 233.

[6] Skita, *Ber.*, 1912, **45**, 3312.

[7] Tiffeneau and Orekhoff, II Cons. Chim. Inst. Internat. Chim. Solvay, 1926, p. 247.

[8] Tréfouël, Tréfouël, Nitti, Bovet, and Hamon, *Compt. rend. Soc. Biol.*, 1938, **129**, 950.

[9] Mecke and Schmähl, *Naturwiss.*, 1955, **42**, 153.

the reaction and whenever isolation of products was undertaken only amines were obtained.

Table 1 shows that 3′- and 4′-substituents have the effect on the rate of reduction which would have been predicted from the " Electronegativity rule," electron-attracting groups increasing the rate. A plot of Hammett's σ values [10] against the logarithm of the relative rate constant gives a reasonably straight line which indicates a value of 1·59 for ρ in the equation $\log k - \log k_0 = \sigma\rho$. Considerable divergence from the linear relation occurred only with substituents for which Hammett gave a wide range of values for σ (Fig. 1).

TABLE 1. *Relative rates of reduction of a series of substituted* 4-*di*-(2-*chloroethyl*)*aminoazobenzenes and related compounds using* (i) *stannous chloride in* 0·5N-*hydrochloric acid in* 75% *aqueous ethanol and* (ii) *hydrazine in* 50% *aqueous ethanol at* pH 8.

Subst.	Rel. rate const. (i)	Rel. rate const. (ii)	Biol. activity [a]
(I; R = R′ = $CH_2{\cdot}CH_2Cl$)			
None	100	100	+
2-Me	V. low	359	Low
2′-Me	62	187	Low
3′-Me	94	109	—
4′-Me	36	97	—
2′ : 3′-Benzo	157	204	—
2′-Ph	68	169	—
4′-Ph	101	85	—
2-MeO	152	468	+
3′-MeO	204	122	—
4′-MeO	20	82	—
2′-Cl	113	193	—
3′-Cl	278	107	—
4′-Cl	206	98	—
2′-Br	80	170	—
3′-Br	297	97	—
4′-Br	254	97	—
2′-I	39	189	—
4′-Ac	2100	116	—
2′-CO_2Me	V. high	118	+
2′-CO_2H	V. high	235	+
3′-CO_2H	166	126	—
(I; R = R′ = $CH_2{\cdot}CH_2Cl$)			
4′-CO_2H	1180	136	—
2′-NO_2	>1500 [b]	— [c]	—
3′-NO_2	—	85	—
2′-SO_3H	2470	395	—
4′-SO_3H	374	146	—
2′ : 3′-Cl_2	90	195	—
2′ : 4′-Cl_2	75	149	—
3′ : 4′-Cl_2	417	103	—
2′-CO_2H,2-Me	562	519	+
2′-CO_2H,2-MeO	91	617	+
2′-CO_2H,2 : 4′-Me_2	168	512	+
4′-CO_2H,2-Me	93	366	+
(I; R = Et, R′ = $CH_2{\cdot}CH_2Cl$)			
2′-CO_2H	2330	159	—
(I; R = R′ = Me)			
None	77	94	—
2′-CO_2H	423	61	—
Azobenzene	316	189	
Azobenzene-2-carboxylic acid	1100	100	

[a] As inhibitors of the growth of the transplanted Walker rat carcinoma. [b] One of the end products in this reaction is *o*-nitroaniline (see p. 1728). [c] The end product in this reaction is a triazole oxide (see p. 1728).

In view of the complexity of the reaction which involves the reduction of azo-compounds present in differing proportions of azo- and quinonoid forms (see Table 3, Part XVII) the agreement with the Hammett equation is striking.

The effect of substituents *ortho* to the azo-linkage is, as in other instances, anomalous. A halogen atom in the 2′-position generally hinders reduction, to an extent depending on the size of the atom. For while a 2′-chlorine atom alone has little effect it causes retardation in conjunction with an extra 3′- or 4′-chlorine atom, and a 2′-bromine atom or especially a 2′-iodine atom is rate-reducing. A 2′-phenyl group hinders reduction but a 4′-phenyl group has little influence. 2′-Carboxy- and 2′-methoxycarbonyl groups greatly accelerate the reaction and it is noteworthy that these groups are exceptional in not hindering the addition of a proton to the azo-linkage, an effect observed with most other 2′-substituents (Table 3, Part XVII). It might be considered that this indicates that the quinonoid form of the azo-compound is the more readily reduced but this would not be consistent with the moderate rates of reduction of the 2-methyl-2′- and -4′-acids which are present exclusively in the quinonoid form. The further insertion of a 2-methyl or 2-methoxy-group into the 2′-carboxylic acid reduces the very high rate, the latter substituent being the more

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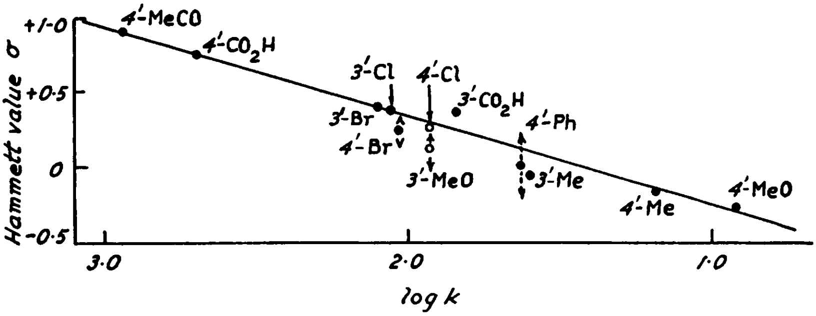
[10] Hammett, " Physical Organic Chemistry," McGraw-Hill Book Co., New York, 1940, p. 188.

effective although the relative effects of these substituents are reversed in the absence of the 2′-carboxyl group.

A 4-dimethylamino-group reduces the rate of reduction more than does a 4-di-(2-chloroethyl)amino-substituent, in accord with the greater electron-releasing potentiality of the former. It is of interest that the introduction of a 2′-carboxy-group into azobenzene and into 4-dimethylaminoazobenzene has not nearly the same accelerating effect that it has in 4-di-(2-chloroethyl)aminoazobenzene.

It was, of course, realised that the relative order of reducibility of the azo-compounds in acid solution did not necessarily bear any relation to the order under physiological conditions, especially as at pH 7·5 all the compounds would be entirely in the azo-form;

FIG. 1. *Relation between* log *k and σ for the stannous chloride reaction.* ←- -→ *indicates the range of values given by Hammett*[10] *in divergent examples.*



so measurements were also made in nearly neutral solutions. Stannous chloride could not be used under these conditions and at first sodium dithionite was examined. However, stock solutions of this reagent deteriorated rapidly, even under nitrogen, owing to a reaction with water which gave thiosulphate and hydrogen sulphite. The development of acidity made it practically impossible to maintain neutrality during reduction runs since adequate concentrations of buffer salts could not be achieved in the alcohol concentration required to dissolve sufficient azo-compound. The results with this reagent were consequently erratic but the general trend indicated that, regardless of their polarity, 3′- and 4′-substituents had little effect on the rate whereas 2′-substituents facilitated reaction.

The use of thiourea dioxide as a reagent for the reduction of azo-compounds has been reported by Gore[11] but it was found to be unsuitable for our studies. Although the azo-compounds were reduced there was a variable time lag before reaction started, which suggested that the dioxide was being converted into some other compound which was the true reducing agent. This was supported by a fairly rapid change in the absorption spectrum of freshly prepared aqueous-ethanolic solutions of thiourea dioxide.

Eventually a partially neutralised solution of hydrazine dihydrochloride in 50% aqueous ethanol was found to be a suitable reagent. The stock solution was stable and acted as its own buffer at pH 8. By using a large excess of hydrazine, unimolecular kinetics were imposed and, as before, reduction runs with the test substance and 4-di-(2-chloroethyl)-aminoazobenzene were carried out simultaneously. The ratios of the unimolecular rate constants were derived by a graphical method and the results are shown in Table 1.

The Table shows that, in striking contrast to their effect on the stannous choride reduction, 3′- and 4′-substituents have here little effect. The outstanding feature is the accelerating effect of all substituents *ortho* to the azo-linkage in 4-di-(2-chloroethyl)aminoazobenzene. Maximum accelerating effects are produced by 2-methoxy, 2-methyl, and 2′-sulphonic acid groups. Insertion of a 2′-halogen atom approximately doubles the rate of reduction irrespective of the size of the halogen atom (cf. p. 1726). The rate-increasing effect of a 2′-carboxylic acid group is reduced on esterification and it is noteworthy that this acid

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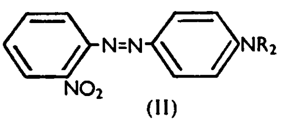
[11] Gore, *Chem. and Ind.*, 1954, 1355.

group hinders reduction when inserted into the azobenzene and 4-dimethylaminoazobenzene structure. There is one feature common to the results of stannous chloride and hydrazine reduction, *viz.*, the rate of reduction of substituted 2′-azobenzenecarboxylic acids is in the order : $4\text{-N}(CH_2\cdot CH_2Cl)_2 > 4\text{-NEt}(CH_2\cdot CH_2Cl) > 4\text{-H} > 4\text{-NMe}_2$.

There is evidence that the initial product obtained in the hydrazine reductions is a hydrazo-compound, for in a number of cases re-oxidation with iodine has resulted in increased absorption in the region of the azo-*K*-band. Eventually this property is lost and it appears that fission of the molecule to give amines occurs. Hydrazobenzene can be isolated when azobenzene is treated with hydrazine under the conditions of the reduction experiments, but 4-dialkylaminohydrazobenzenes seem to be too unstable for ready isolation.[12]

The reduction of the 2′-nitro-derivative (II; $R = CH_2\cdot CH_2Cl$) is potentially complex since either or both the azo-linkage and the nitro-group could be involved. The final steady light absorption of the solution after reduction by acid stannous chloride indicates that only the azo-linkage is affected, with the formation of *o*-nitroaniline. Ultraviolet absorption measurements have now shown that the end product after hydrazine treatment is the triazole oxide (III; $R = CH_2\cdot CH_2Cl$).

There is a discrepancy in the literature regarding the preparation of 2-dimethylaminophenylbenzotriazole oxide (III; R = Me), which was required for comparative absorption measurements; whereas Elbs and his co-workers [13] stated that reduction of 4-dimethylamino-2′-nitroazobenzene (II; R = Me) with ethanolic ammonium sulphide gave the triazole oxide (III; R = Me), Chakrabarty and Dutt [14] claimed to have obtained the triazole (IV; R = Me) when using this reagent. We now confirm the findings of Elbs *et al.* and by a similar reduction of the nitroazo-compound (II; $R = CH_2\cdot CH_2Cl$) we have also prepared 2-[*p*-di-(2-chloroethyl)aminophenyl]benzotriazole 1-oxide (III; $R = CH_2\cdot CH_2Cl$) which exhibits an absorption spectrum similar to that of the dimethylamino-derivative (III; R = Me). Reduction of the triazole oxide (III; R = Me or $CH_2\cdot CH_2Cl$) with stannous chloride gave the corresponding triazole (IV; R = Me or $CH_2\cdot CH_2Cl$).



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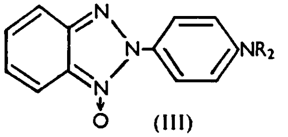

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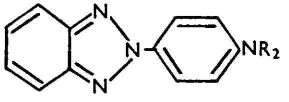

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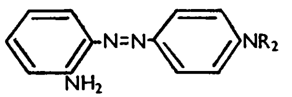

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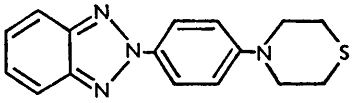

(VI)

Elbs *et al.*[13] also reported that the reduction of the nitroazo-compound (II; R = Me) by ethanolic sodium sulphide gave the aminoazo-derivative (V; R = Me) and this was of interest as an indication of a possible route to the 2′-aminoazobenzene compound (V; $R = CH_2\cdot CH_2Cl$). On repeating the reduction of the compound (II; R = Me) we obtained an apparently homogeneous product of m. p. 133—134° (Elbs *et al.*[13] gave m. p. 133—134° for their so-called aminoazo-compound). However a solution of the product in benzene showed a strong fluorescence similar to that of the triazole (IV; R = Me) and the ultraviolet absorption was unlike that expected for a compound of structure (V; R = Me) since it contained an intense maximum at 365 mμ and an inflection between 410 and 460 mμ (Fig. 2). Chromatographic resolution on alumina showed that the product was composed of 90% of the triazole (IV; R = Me), m. p. 187°, and 10% of the aminoazo-compound

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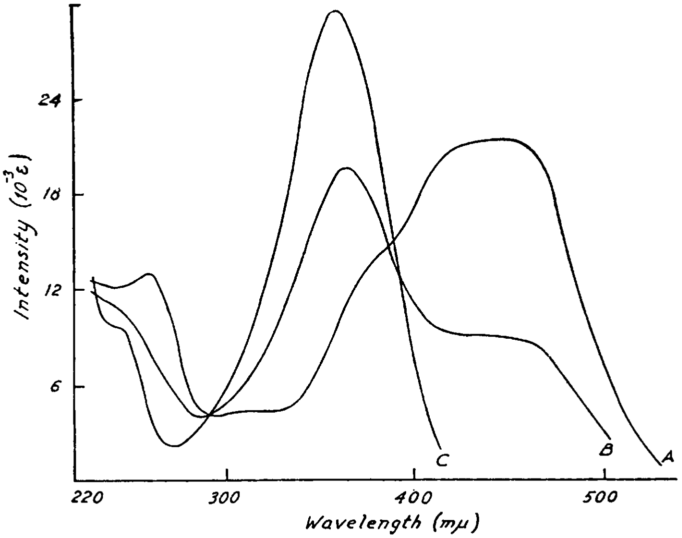
[12] Cf. Miller and Miller, *Adv. Cancer Res.*, 1953, **1**, 387.

[13] Elbs, Hirschel, Wagner, Himmler, Türk, Henrich, and Lehmann, *J. prakt. Chem.*, 1924, **108**, 209.

[14] Chakrabarty and Dutt, *J. Indian Chem. Soc.*, 1928, **5**, 555.

(V; R = Me), m. p. 105—106°. The pure 2′-amino-4-dimethylaminoazobenzene (V; R = Me) exhibited an absorption maximum at 440—450 mμ (Fig. 2). The product of m. p. 133—134° is apparently a molecular complex which resists resolution by crystallisation. Reduction of the nitroazo-compound (II; R = $CH_2{\cdot}CH_2Cl$) by ethanolic sodium sulphide, followed by chromatography, gave the thiazan (VI) as the only identifiable product; the formation of thiazans by the reaction of di-(2-chloroethyl)amines with sodium sulphide has been reported earlier.[15]

FIG. 2. *Absorption spectra in ethanol.*



A, *2′-Amino-4-dimethylaminoazobenzene* (V; R = Me).
B, *Elbs's compound, m. p.* 133—134°.
C, 2-(p-*Dimethylaminophenyl*)*benzotriazole* (IV; R = Me).

It is of interest that the very slow hydrolysis of the 2-chloroethylamine (IV; R = $CH_2{\cdot}CH_2Cl$), less than 1% under standard conditions,[16] confirms the base-weakening effect on arylamines of a *p*-(benzotriazol-2-yl) substituent observed by Elbs *et al.* (ref. 13, p. 223).

It was also desirable to discover whether a naturally occurring enzyme system could effect reduction of the azo-linkage, for if this could be established and if the ease of reduction of a di-(2-chloroethyl)aminoazo-compound by such a system paralleled its biological activity then there would be a strong *a priori* case in favour of the hypothesis that activity

TABLE 2. *Relative rates of reduction of a series of acidic derivatives of* 4-*di*-(2-*chloroethyl*)-*aminoazobenzene by xanthine oxidase–xanthine in pyrophosphate buffer* (pH 8·2) *in* 30 *min. at* 24°.

Subst.	Redn. (%)	Biol. acty.[a]	Subst.	Redn. (%)	Biol. acty.[a]	Subst.	Redn. (%)	Biol. acty.[a]
			(I; R = R′ = $CH_2{\cdot}CH_2Cl$)					
$2\text{-}CO_2H,4'\text{-}NO_2$ ...	*ca.* 85[b]	+	$2'\text{-}CO_2H$	34	+	$2'\text{-}SO_3H$	2	—
$2'\text{-}CO_2H,2\text{-}MeO$ ...	70	+	$2\text{-}CO_2H,2'\text{-}CN$	34	+	$3'\text{-}CO_2H$	2	—
$2:4'\text{-}(CO_2H)_2$	55	+	$4'\text{-}SO_3H$	5	—	(I; R = R′ = Me)		
$2'\text{-}CO_2H,2\text{-}Me$ ...	49	+	$4'\text{-}CO_2H,2\text{-}Me$	4	+			
$2'\text{-}CO_2H,2:4'\text{-}Me_2$	42	+	$4'\text{-}CO_2H$	4	—	$2'\text{-}CO_2H$ (methyl-red)	72	

[a] As inhibitors of the growth of the transplanted Walker rat carcinoma. [b] In 5 min.

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[15] Korshak and Strepikheev, *J. Gen. Chem.* (*U.S.S.R.*), 1944, **14**, 312; Cerkovnikov and Stern, *Arkiv Kem.*, 1946, **18**, 12; Ross, *J.*, 1950, 815.

[16] Ross, *J.*, 1949, 183.

depended on ease of reduction. Since various dyes are known to act as hydrogen acceptors in the xanthine oxidase–xanthine system it seemed logical to examine this enzyme. It was now necessary to work with entirely aqueous solutions, so the rates of reduction of various acidic derivatives, which have adequate water solubility at pH 8·2, were examined at 24° under anærobic conditions. The reaction did not proceed in accordance with any simple kinetic law, so the results are expressed in Table 2 as the percentage reduction in a given time. It had been established that the azo-compounds were unaffected by the enzyme alone or by xanthine alone under the standard conditions. Re-oxidation by iodine led to the re-formation of azo-compounds in the initial stages of the reaction (see above).

The correlation of biological activity with the ease of reduction of the azo-linkage has been discussed elsewhere [17] and it suffices here to indicate that the initial hypothesis has been supported by the results obtained for reduction in neutral solutions in the present work.

EXPERIMENTAL

Rates of Reduction of Azo-compounds in Acid Stannous Chloride.—Stannous chloride solution. A solution of stannous chloride dihydrate (50 g.) in ethanol (2 l.) was heated under reflux for 1 hr. and then distilled, 1½ l. being collected. 500 Ml. of this stabilised ethanol was introduced into the reservoir bottle of an automatic burette, and a stream of oxygen-free nitrogen was passed through the apparatus. When all the air had been displaced stannous chloride dihydrate (4·51 g.) was washed into the bottle with 2N-hydrochloric acid (500 ml.). Unless made up as described a turbid solution was obtained. The burette was subsequently operated by means of pure compressed nitrogen.

Solutions of azo-compounds. The concentration was 10^{-5}M, in redistilled absolute ethanol.

Determination of the relative velocity constant. 10 ml. of a solution of 4-di-(2-chloroethyl)-aminoazobenzene (A) and of a solution of the azo-compound under test (B) were placed in 50-ml. stoppered flasks. Stannous chloride solution (10 ml.) was rapidly run into flask (A), and the stopwatch was started. The thoroughly mixed solution was quickly transferred into a 1-cm. absorption cell and placed in the carrier (adapted for circulation of water at 24°) of a Unicam spectrophotometer. The optical density of the solution was measured after 2 min. against a control solution made by diluting the stannous chloride solution with an equal volume of absolute ethanol. At 3 min. stannous chloride solution was added to flask (B), and the contents were transferred to the Unicam carrier as before. Optical-density readings were taken at the following times: (A) 2, 4, 6, 8, 10, 12, 14 min.; (B) 5, 7, 9, 11, 13, 15, 17 min.

The following result for the 3′-methoxy-derivative of compound (I) is typical:

	Optical density (*D*)			Optical density (*D*)			Optical density (*D*)	
Time (min.)	(A) at 530 mμ *	(B) at 400 mμ *	Time (min.)	(A) at 530 mμ *	(B) at 400 mμ *	Time (min.)	(A) at 530 mμ *	(B) at 400 mμ *
2	0·715	—	8	0·386	—	13	—	0·118
4	0·582	—	9	—	0·269	14	0·209	—
5	—	0·602	10	0·313	—	15	—	0·078
6	0·475	—	11	—	0·181	16	0·173	—
7	—	0·408	12	0·257	—	17	—	0·047

* The wavelengths chosen were those of maximum absorption in 2N-acidic ethanol (see Part XVII)

For very rapidly reduced compounds readings were required more frequently but the rate of reaction of the reference compound was always measured at the same time, usually by returning to solution (A) for measurements after reduction in solution (B) had reached 70—80% completion. By plotting time against the log *D* for the period 4—14 min. in the average run good straight lines were always obtained. This time interval was chosen when it was found that the contents of the cells attained a steady temperature after 3—4 min. From the ratio of of the slopes of the lines the relative unimolecular rate constant was obtained. In the above case 100 × slope B/slope A = 100 × 0·089/0·044 = 202. Values quoted in Table 1 are the result of at least two concordant runs. The pH of the solutions before and after reaction was 0·3, measured by using a glass-electrode.

Rates of Reduction of Azo-compounds in Hydrazine Solutions.—Hydrazine solution. Recrystallised hydrazine dihydrochloride (21 g.) was dissolved in N-sodium hydroxide (300 ml.) and water (200 ml.), and the filtered solution was stored under nitrogen in an automatic burette.

Solutions of azo-compounds were 10^{-5}M, prepared as above.

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[17] Ross and Warwick, *Nature*, 1955, **176**, 298.

Determination of the relative velocity constant. The procedure was similar to that described above except that hydrazine solution (10 ml.) was run in to start the reaction. At the hydrazine concentration chosen the actual rate of reduction of the reference compound was practically the same in the two series of experiments. The relative rate constants were again evaluated graphically. The pH of the solution throughout the reaction was 8.

Rates of Reduction of Azo-compounds by Xanthine Oxidase–Xanthine.— *Solutions of azo-compound.* The acidic azo-compound (15 mg.) and xanthine (30 mg.) were dissolved in warm N-sodium hydroxide (2 ml.), and the cooled solution was diluted to 100 ml. with distilled water.

Buffer solution. Sodium pyrophosphate decahydrate (71·3 g.) and sodium hydrogen pyrophosphate (13·3 g.) were dissolved in water, and the solution made up to 2 l. This solution, to which chloroform (10 ml.) was added as preservative, had pH 8.

Reduction experiment. A solution of the azo-compound (3 ml.), pyrophosphate buffer (12 ml.), water (7 ml.) and N/50-hydrochloric acid (3 ml.) were mixed while oxygen-free nitrogen was passed through the mixture. After 10 min. xanthine oxidase solution (1 ml. of preparation stage M/5, see ref. 18) was added and the solution was rapidly transferred to a stoppered 1 cm. absorption cell. The cell, which was completely filled (no air-space), was used as above for measurement of the optical density at intervals (usually every 5 min.), against a control solution containing everything but the azo-compound. In a number of cases removal of the stopper from the cell to allow access of air increased the absorption in the region of the azo-*K*-band; the following run with methyl-red shows this effect.

Time (min.)	4	8	13	21	36	45	50	Stopper removed	59	68
D (400 mμ)	1·151	1·046	0·860	0·602	0·282	0·178	0·093		0·120	0·134

Reduction of 4-Dialkylamino-2′-nitroazobenzenes with Ethanolic Ammonium Sulphide.—Hydrogen sulphide was passed into a solution of 4-dimethylamino-2′-nitroazobenzene (1 g.) and aqueous ammonia (10 ml.; *d* 0·880) in ethanol (150 ml.). When the solution had become pale yellow it was diluted with water and extracted with ether. The dried ether solution yielded the triazole oxide (III; R = Me) (0·85 g.), m. p. 165—167°, as pale yellow needles from ethanol, as described by Elbs *et al.*[13]

Similar reduction of 4-di-(2-chloroethyl)amino-2′-nitroazobenzene gave an almost quantitative yield of 2-[p-*di-(2-chloroethyl)aminophenyl*]*benzotriazole oxide monohydrate*, yellow plates, m. p. 133—134° (from ethanol) (Found: C, 51·7; H, 4·3; N, 15·1; Cl, 19·2. $C_{16}H_{16}ON_4Cl_2,H_2O$ requires C, 52·0; H, 4·3; N, 15·2; Cl, 19·3%).

Stannous Chloride Reduction of the Triazole Oxide (III; R = $CH_2{\cdot}CH_2Cl$).—To a solution of the triazole oxide (2·0 g.) in ethanol (150 ml.) was added one of stannous chloride dihydrate (25 g.) in concentrated hydrochloric acid (25 ml.) and water (25 ml.). After 2½ hours' refluxing the ethanol was removed and an excess of aqueous sodium hydroxide was added. A dried benzene extract was passed through a column of activated alumina which retained unchanged oxide but allowed the *benzotriazole* (IV; R = $CH_2{\cdot}CH_2Cl$) to pass through. The triazole formed pale yellow plates, m. p. 137—138°, from light petroleum (b. p. 60—80°) (Found: C, 57·0; H, 5·1; N, 16·9. $C_{16}H_{16}N_4Cl_2$ requires C, 57·3; H, 4·8; N, 16·7%).

Reduction of 4-Dialkylamino-2′-nitroazobenzenes with Ethanolic Sodium Sulphide.—A solution of the nitroamine (II; R = Me) (1·0 g.) in ethanol (30 ml.) was heated with a solution of sodium sulphide (3·5 g.) in water (3 ml.) for 1 hr. Addition of water to the cooled solution yielded an orange precipitate which was collected and repeatedly crystallised from ethanol. The product, which appeared to be homogeneous under the microscope, formed orange plates, m. p. 133—134°. When its benzene solution was passed through activated alumina two bands appeared. The fluorescent yellow band which was easily eluted contained the triazole (IV; R = Me), m. p. 183—184°, which was also obtained by reduction of the triazole oxide (III; R = Me) by stannous chloride. A more strongly adsorbed orange band was eluted with ether. It contained *2′-amino-4-dimethylaminoazobenzene*, m. p. 105—106°, which formed orange plates from aqueous ethanol (Found: C, 70·1; H, 6·7; N, 23·1. $C_{14}H_{16}N_4$ requires C, 70·0; H, 6·7; N, 23·3%).

Similar reduction of 4-di-(2-chloroethyl)amino-2′-nitroazobenzene gave a sulphur-containing compound, m. p. 194°, colourless needles from benzene, as the only characterisable product. It is apparently 2-p-(*tetrahydro-4-thiazinyl*)*phenylbenzotriazole* (VI) (Found: C, 64·3; H, 5·3; N, 18·3; S, 10·4. $C_{16}H_{16}N_4S$ requires C, 64·8; H, 5·4; N, 18·9; S, 10·8%).

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[18] Avis, Bergel, and Bray, *J.*, 1955, 1100.

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