# **441.** *Aromatic Azo-compounds. Part II.* Oxidation of Substituted Azobenzenes.*

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The rates of oxidation of various substituted azobenzenes with perbenzoic acid have been determined at three temperatures, and the energies of activation calculated. All *meta*- and *para*-substituted compounds are found to have the same entropy of activation. Electron-releasing substituents decrease, and electron-attracting substituents increase, the heat of activation and free energy of activation. The effects of two substituents appear to be accurately additive, and there is a linear relationship between the rate constants and the Hammett " sigma " constants for the various substituents.

MANY derivatives of azobenzene produce cancer of the liver when administered to rats and mice (for review, see Badger and Lewis, *Brit. J. Cancer,* 1952, in the press). Most of the active compounds are 4-amino-, 4-methylamino-, or 4-dimethylamino-derivatives, but additional substituents have a profound effect on the relative potency of the resulting compound. For example, 4-dimethylamino-4′-methylazobenzene is much less active than 4-dimethylaminoazobenzene, but 4-dimethylamino-4′-fluoroazobenzene is rather more active than the parent base. More than thirty substituted 4-dimethylaminoazobenzenes have been tested for carcinogenic activity; some are more active than the parent base, some less active, and several entirely inactive. The relationship between chemical constitution and carcinogenic activity has not been extensively studied, but Pullman (*Compt. rend.*, 1946, **222**, 1501) has suggested that the electron density at the azo-linkage is an important factor.

It has not been possible to examine the rate of oxidation of 4-dimethylaminoazobenzene with perbenzoic acid as reaction occurs far too rapidly. However, the effect of many other substituents on the electron density around the azo-nitrogen atoms has been examined by determining the rates of oxidation of various substituted azobenzenes.

The oxidations were carried out, at three temperatures, by the method given in the preceding paper, and the results are summarised in the Table. For every compound, the logarithms of the three rate constants fell very nearly on a straight line when plotted against the reciprocal of the absolute temperature, and the heat of activation ($\Delta H^{\ddagger}$) has been calculated in each case. The difference in the heat of activation relative to azobenzene itself ($\Delta H^{\ddagger} - \Delta H_0^{\ddagger}$) has also been evaluated. The difference of the free energy of activation relative to azobenzene ($\Delta F^{\ddagger} - \Delta F_0^{\ddagger}$) has been calculated from the rate constants at 25° for the compound in question and that for azobenzene. In addition, the difference in the entropy of activation relative to azobenzene ($\Delta S^{\ddagger} - \Delta S_0^{\ddagger}$) has been evaluated from the *PZ* factors at 25° and multiplied by the absolute temperature.

The probable error for $\Delta F^{\ddagger} - \Delta F_0^{\ddagger}$ must be very small, but that for $\Delta H^{\ddagger} - \Delta H_0^{\ddagger}$ and for $T(\Delta S^{\ddagger} - \Delta S_0^{\ddagger})$ is in the region of $\pm 0.5$ kcal. Within experimental error, therefore,

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* Part I, preceding paper.

and with the exception of 2 : 2′-dimethylazobenzene (the only *ortho*-substituted derivative examined) it is found that the entropy of activation remains constant for all the substituted azobenzenes examined. This is indicated by the fact that $T(\Delta S^{\ddagger} - \Delta S_0^{\ddagger}) \approx 0$ in each case, and that there is a linear relationship between the heat of activation and the logarithm of the rate constant at 25° (Fig. 1). All the points fall about the line of gradient $-2{\cdot}303\boldsymbol{R}T$ which is drawn through the point for azobenzene. The point for 2 : 2′-dimethylazobenzene shows the greatest displacement from the line.

*Rate constants and activation energies for reaction between substituted azo-compounds and perbenzoic acid.**

| No. | Substituted azobenzene | $10^2k_2$ (mole$^{-1}$ min.$^{-1}$ l.) 15° | 25° | 30° | $\Delta H^{\ddagger}$ (kcal.) | $X$ | $Y$ | $Z$ |
|---|---|---|---|---|---|---|---|---|
| 1. | 4 : 4′-Dimethoxy- | 80·1 ± 2·9 | 179 ± 7 | 255 ± 6 | 13·4 ± 0·7 | −1·4 | +0·1 | −1·5 |
| 2. | 4-Methoxy- | 25·4 ± 0·4 | 58·2 ± 1·8 | 83·3 ± 2·0 | 13·7 ± 0·5 | −1·1 | −0·2 | −0·8 |
| 3. | 4 : 4′-Dimethyl- | 21·4 ± 0·4 | 49·1 ± 1·6 | 71·6 ± 2·1 | 14·0 ± 0·6 | −0·8 | −0·1 | −0·7 |
| 4. | 4-Methyl- | 12·0 ± 0·2 | 28·2 ± 0·8 | 41·7 ± 1·1 | 14·4 ± 0·5 | −0·4 | 0 | −0·4 |
| 5. | 3 : 3′-Dimethyl- | 10·1 ± 0·2 | 23·9 ± 0·6 | 34·7 ± 0·7 | 14·3 ± 0·5 | −0·5 | −0·2 | −0·3 |
| 6. | 3-Methyl- | 7·20 ± 0·09 | 17·1 ± 0·4 | 25·3 ± 0·4 | 14·5 ± 0·3 | −0·3 | −0·2 | −0·1 |
| 7. | 3-Methoxy- | 5·81 ± 0·08 | 14·4 ± 0·4 | 21·6 ± 0·4 | 15·2 ± 0·4 | +0·4 | +0·4 | 0 |
| 8. | Parent substance | 5·80 ± 0·07 | 13·9 ± 0·3 | 20·9 ± 0·3 | 14·8 ± 0·3 | 0 | 0 | 0 |
| 9. | 4-Fluoro- | 3·99 ± 0·08 | 9·73 ± 0·17 | 14·6 ± 0·4 | 15·0 ± 0·6 | +0·2 | 0 | +0·2 |
| 10. | 4-Chloro- | 3·07 ± 0·07 | 7·54 ± 0·12 | 11·5 ± 0·2 | 15·3 ± 0·5 | +0·5 | +0·1 | +0·4 |
| 11. | 4-Bromo- | 2·92 ± 0·05 | 7·09 ± 0·15 | 11·0 ± 0·2 | 15·3 ± 0·4 | +0·5 | +0·1 | +0·4 |
| 12. | 3-Carbethoxy- | 2·29 ± 0·05 | 5·65 ± 0·18 | 8·61 ± 0·17 | 15·3 ± 0·5 | +0·5 | 0 | +0·5 |
| 13. | 4-Carbethoxy- | 2·03 ± 0·04 | 5·03 ± 0·14 | 7·54 ± 0·13 | 15·2 ± 0·4 | +0·4 | −0·2 | +0·6 |
| 14. | 3-Bromo- | 1·73 ± 0·04 | 4·51 ± 0·12 | 6·82 ± 0·13 | 15·9 ± 0·5 | +1·1 | +0·4 | +0·7 |
| 15. | 4 : 4′-Dichloro- | 1·71 ± 0·03 | 4·38 ± 0·14 | 6·76 ± 0·11 | 15·9 ± 0·4 | +1·1 | +0·4 | +0·7 |
| 16. | 2 : 2′-Dimethyl- | 1·70 ± 0·03 | 4·36 ± 0·13 | 6·88 ± 0·14 | 16·2 ± 0·5 | +1·4 | +0·7 | +0·7 |
| 17. | 3-Chloro- | 1·69 ± 0·03 | 4·33 ± 0·10 | 6·58 ± 0·11 | 15·7 ± 0·4 | +0·9 | +0·2 | +0·7 |
| 18. | 4 : 4′-Dibromo- | 1·64 ± 0·03 | 4·10 ± 0·15 | 6·49 ± 0·12 | 15·9 ± 0·4 | +1·1 | +0·4 | +0·7 |
| 19. | 3-Fluoro- | 1·66 ± 0·03 | 4·06 ± 0·12 | 6·34 ± 0·12 | 15·5 ± 0·4 | +0·7 | 0 | +0·7 |
| 20. | 3-Nitro- | 0·767 ± 0·015 | 2·05 ± 0·09 | 3·13 ± 0·09 | 16·3 ± 0·6 | +1·5 | +0·3 | +1·1 |
| 21. | 3 : 3′-Dichloro- | 0·559 ± 0·014 | 1·45 ± 0·06 | 2·24 ± 0·07 | 16·0 ± 0·7 | +1·2 | −0·1 | +1·3 |
| 22. | 4-Nitro- | 0·158 ± 0·005 | 0·416 ± 0·021 | 0·681 ± 0·022 | 16·9 ± 0·7 | +2·1 | 0 | +2·1 |

* $X = \Delta H^{\ddagger} - \Delta H_0^{\ddagger}$; $Y = T(\Delta S^{\ddagger} - \Delta S_0^{\ddagger})$; $Z = \Delta F^{\ddagger} - \Delta F_0^{\ddagger}$; all in kcal.

As the entropy of activation is essentially constant for all *meta*- and *para*-substituted azobenzenes, then $\Delta H^{\ddagger} - \Delta H_0^{\ddagger} = \Delta F^{\ddagger} - \Delta F_0^{\ddagger}$, and either $\Delta H^{\ddagger}$ or $\Delta F^{\ddagger}$ may be used as an index of relative electron density or electron availability at the reacting centre (cf. Hammett, " Physical Organic Chemistry," McGraw-Hill, 1940, pp. 118 *et seq.*). In the Table, the various compounds have been arranged in order of decreasing electron density around the azo-nitrogen atoms; but clearly, it is impossible accurately to distinguish between compounds showing only small differences in rate constants.

Examination of the Table shows that the various substituents affect the heat of activation and the free energy of activation (and hence the electron density at the azo-nitrogen atoms) more or less as expected. Electron-releasing substituents decrease the energy of activation and increase the rate of reaction, and electron-attracting substituents have the reverse effects. The results for 3-methyl- and 3 : 3′-dimethyl-azobenzenes, for example, indicate that a *meta*-methyl group does act slightly as an electron-donor to the azo-nitrogen atoms. Similarly, the results for 4-methyl- and 4 : 4′-dimethyl-azobenzenes indicate that a *para*-methyl group produces a much greater increase in electron density at the reacting centre. On the other hand, the halogens produce a decrease in the electron density. The effect was found to be relatively small when the halogen substituent was in the *para*-position, for in these circumstances the $-I$ effects of the substituents are largely counterbalanced by the $+M$ effects. When the halogen substituent was present in the *meta*-position, its effect was much greater. Incidentally, it was not possible to examine iodoazobenzenes in this reaction, as the perbenzoic acid also attacked the iodo-substituent.

Further examination of the effects of the various substituents shows that there is a linear relationship between the logarithm of the rate constant (at 25°) and Hammett's " sigma " constant for the substituent (Hammett, *op. cit.*, pp. 184 *et seq.*). This is shown in Fig. 2, and as some of the sigma constants are not known very accurately, the relationship must be considered very satisfactory. In some cases the probable error in the sigma constant is of the order of 0·1, and this no doubt accounts for one or two apparent anomalies. For example, the free energy of activation for 3-methoxyazobenzene (see Table) was found to be the same as that for azobenzene, in spite of the fact that Hammett gives a 3-methoxy-group a sigma constant of +0·115. This value would suggest that $\Delta F^{\ddagger} - \Delta F_0^{\ddagger}$ should be about +0·3 kcal., and that the reaction rate for 3-methoxyazobenzene should be between those for the 4-fluoro- and the 4-chloro-derivative. However, the probable error in the sigma constant for this substituent is 0·102, and it may well be that the true value for a *m*-methoxy-substituent is close to zero. Hammett gives two values for a *p*-nitro-group and it is difficult to choose which is the more satisfactory for the present comparison. Both values have therefore been included in Fig. 2.

The effects of two substituents in the *meta*- and *para*-positions appear to be accurately additive. This is shown by the fact that the $\Delta F^{\ddagger} - \Delta F_0^{\ddagger}$ values for disubstituted compounds are almost identical with the sum of the $\Delta F^{\ddagger} - \Delta F_0^{\ddagger}$ values for the corresponding mono-substituted compounds. It is also confirmed by the fact that the rate constants for the disubstituted compounds fall accurately on the curve (Fig. 2) when plotted against the sum of the relevant sigma constants.

FIG. 1.

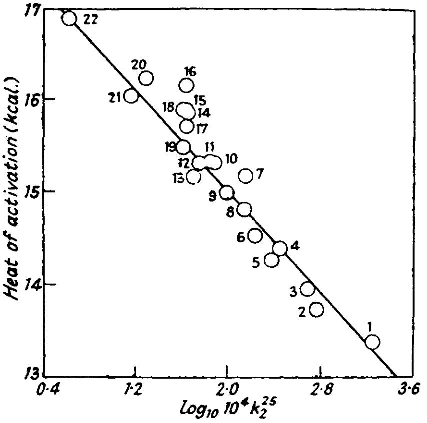

FIG. 2.

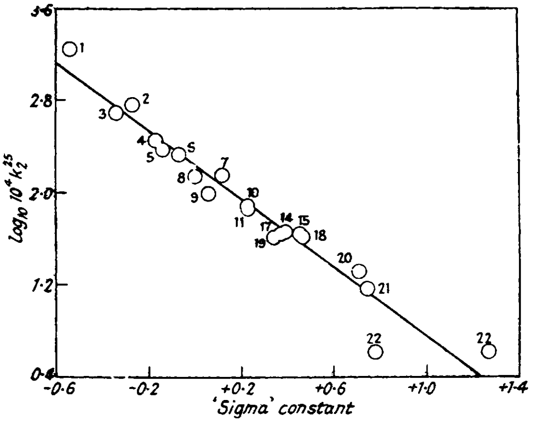

FIG. 1. *Relation between heat of activation and the logarithm of the rate constant (at 25°) for substituted azobenzenes. The line is drawn through the point for azobenzene at a slope of* −2·303 **R***T. The key to the numbers is given in the Table.*

FIG. 2. *Relation between the logarithm of the rate constant (at 25°) and the Hammett " sigma " constant for the particular substituent. The key to the numbers is given in the Table.*

2 : 2′-Dimethylazobenzene reacted more slowly than azobenzene and much more slowly than 4 : 4′-dimethylazobenzene. Steric hindrance is evidently a factor here, for the $T(\Delta S^{\ddagger} - \Delta S_0^{\ddagger})$ value is somewhat greater than that for the *meta*- and *para*-substituted derivatives. Moreover, the absorption spectra of 2 : 2′-dimethylazobenzene and of its azoxy-derivative (Part IV, *J.*, **1953**, 2156) indicate that the latter compound is sterically hindered.

Most of the substituted azo-compounds used in this investigation were prepared by standard methods, but some improvements in experimental procedure have been effected. 3- and 4-Fluoroazobenzene were prepared by condensing nitrosobenzene with the appropriate fluoroaniline. 4-Thiocyanatoazobenzene was prepared from *p*-thiocyanatoaniline and nitrosobenzene, but it was unsuitable for examination as perbenzoic acid oxidised the substituent. 4-Thiocyanatoazobenzene was also found to be converted by passage through a column of alumina into a sparingly soluble product which is probably the disulphide, $NPh{:}N{\cdot}C_6H_4{\cdot}S{\cdot}S{\cdot}C_6H_4{\cdot}N{:}NPh$. A similar conversion of a thiocyanatoderivative into a disulphide has been reported by Wood and Fieser (*J. Amer. Chem. Soc.*, **1941**, 63, 2323).

*Application to Problem of Carcinogenesis.*—The observed linear relationship between the logarithm of the rate constant and the Hammett sigma constant is of some importance. It implies that the electron density around the azo-nitrogen atoms in substituted azobenzenes is directly related to the sigma constants of the substituents, and this is of value in determining the relative electron densities at this region in the carcinogenic compounds. For example, the relative electron densities in a series of substituted 4-dimethylaminoazobenzenes can be evaluated in terms of the sigma constants for the " additional " substituents. Unfortunately, relatively few 3'- and 4'-substituted 4-dimethylaminoazobenzenes have so far been tested for carcinogenic activity, and no firm conclusions can be reached. The available data (Badger and Lewis, *loc. cit.*) are, however, not inconsistent with the view that carcinogenic activity is related to an optimum electron density around the azo-nitrogen atoms.

## EXPERIMENTAL

*Mono-substituted Azobenzenes.*—Most of the mono-substituted azobenzenes were prepared by condensing nitrosobenzene with the appropriate substituted aniline, the temperature being kept below 30° (cf. Hickinbottom, " Reactions of Organic Compounds," p. 326). Except 3-methylazobenzene, all the products separated from the reaction mixture as solids. The crude material was purified by chromatography on alumina from light petroleum, elution being effected with solutions of 10%, 20%, and then 30% benzene in light petroleum. After evaporation of the solvent the azo-compound was recrystallised from alcohol. 3-Methylazobenzene was first obtained as an oil, but after purification in the above manner, it had m. p. 20° (lit. 18°). 3-*Fluoroazobenzene*, prepared from *m*-fluoroaniline, was obtained as orange plates, m. p. 44° (Found: C, 72·1; H, 4·6. $C_{12}H_9N_2F$ requires C, 72·0; H, 4·5%). 4-*Fluoroazobenzene*, prepared from *p*-fluoroaniline, was obtained as orange-yellow needles, m. p. 83° (Found: C, 72·3; H, 4·7%). The crude product obtained by condensing *p*-thiocyanatoaniline with nitrosobenzene was purified by recrystallisation from alcohol. The 4-*thiocyanatoazobenzene* thus obtained formed pale orange plates, m. p. 72° (Found: C, 65·2; H, 3·8. $C_{13}H_9N_3S$ requires C, 65·3; H, 3·8%). Attempted purification of this compound by chromatography on alumina was unsuccessful. A sparingly soluble substance separated out on the column and this could only be removed from the alumina with boiling chloroform. Evaporation of the solvent and recrystallisation from alcohol gave the *disulphide* as orange plates, m. p. 166° (Found: C, 67·6; H, 4·2. $C_{24}H_{18}N_4S_2$ requires C, 67·6; H, 4·2%).

4-Methoxyazobenzene was obtained by methylation of 4-hydroxyazobenzene with methyl sulphate. 4-Chloroazobenzene was obtained from 4-aminoazobenzene by the Sandmeyer reaction; and 3-methoxyazobenzene was obtained from nitrobenzene, *m*-anisidine, and sodium hydroxide at 170—180° (Martynoff, *Bull. Soc. chim.*, 1951, 214), the product being purified by chromatography.

4-Nitro- and 4 : 4'-dinitro-azobenzene were prepared by direct nitration of azobenzene (Werner and Stiasny, *Ber.*, 1889, **22**, 3268). The crude product was extracted with light petroleum (Soxhlet) for 24 hr. Evaporation of the solvent and repeated recrystallisation from glacial acetic acid gave 4-nitroazobenzene, m. p. 145°. The fraction insoluble in light petroleum was repeatedly recrystallised from glacial acetic acid and gave 4 : 4'-dinitroazobenzene, m. p. 223°.

*Di-substituted Azobenzenes.*—All the di-substituted azobenzenes were prepared from the appropriate nitrobenzene as follows: A mixture of the substituted nitrobenzene (1 mole), and sodium hydroxide (150 g.) in water (150 c.c.) and alcohol (600 c.c.) was treated with zinc dust (350 g.) in small portions at a rate sufficient to maintain vigorous boiling. After all the zinc dust had been added, the reaction mixture was heated for another 2 hr. on the water-bath. The mixture was then filtered, and the zinc residues washed with boiling alcohol (500 c.c.). Air was then drawn through the solution for 6 hr. (to oxidise any hydrazo-compound), and the crude azo-compound collected. Purification was effected by recrystallisation, but in each case the purity of the product was checked by a trial chromatographic analysis. No bands due to impurites were observed.

*Reaction.*—Rates of reaction with perbenzoic acid were determined by the method outlined in Part I for *trans*-azobenzene.

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