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# THE ULTRAVIOLET SPECTRA OF SOME MONOSULPHONATED DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE

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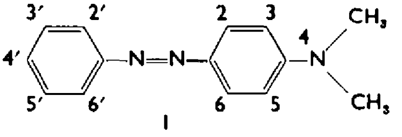
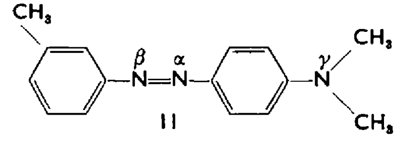
**Abstract**—The spectra of *o*-, *m*- and *p*-methyl orange, the 2'-, 3'- and 4'-sulphonated derivatives of 2-methyl-4-dimethylaminoazobenzene, the 4'-sulpho derivative of 2'- and 3'-methyl-, 2,2'-, 2,3'- and 2',6'-dimethyl-, and 2,2',6'-trimethyl-4'-sulpho-4-dimethylaminoazobenzene, together with 2,2',6'-trimethyl-4-dimethylaminoazobenzene have been examined in neutral and acidic solution. The effects observed in both cases can be accounted for by the electron attracting inductive effect of the sulphonate group. The relative amounts of protonated tautomers in acidic solution have been calculated. The amount of azonium form (III) of the protonated dyes was found to be dependent upon the position of the sulphonate group, diminishing in the order 2', 3', 4'. The presence of methyl groups in the same ring as the sulphonate group tended to increase the amount of azonium tautomer in comparison to the unsulphonated dye.

## INTRODUCTION

The aminoazobenzenes are a class of compounds which have aroused much interest over recent years because of the specific induction of tumours in rat liver by a large number of them,[1] in particular 4-dimethylaminoazobenzene (I) and 3'-methyl-4-dimethylaminoazobenzene (II). In attempting to correlate structure and biological activity considerable chemical[2] and physicochemical[3] work has been recorded in the literature and much attention has been paid to the absorption spectra of these compounds in neutral and acidic solution.[4]



I



II

In neutral solution these dyes display more or less similar spectra. The main feature is a strong band at 410–440 m$\mu$. Azobenzene has an absorption maximum at

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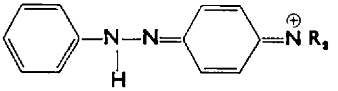
[1] J. A. Miller and E. C. Miller, *Adv. Cancer Res.* **1**, 339 (1953).
[2] G. M. Badger and G. E. Lewis, *Brit. J. Cancer* **6**, 270 (1952).
[3] J. A. Miller, E. C. Miller and G. C. Finger, *Cancer Res.* **13**, 93 (1953), **17**, 389 (1957); A. Heller and B. Pullman, *Ibid.* **19**, 618 (1959).
[4] G. E. Lewis, *Tetrahedron* **10**, 129 (1960).

320 mμ and 4-aminoazobenzene at 380 mμ. The bathochromic shift in the N-substituted compounds is due to the increased conjugation of the amino group with the azo linkage.

In acidic solution profound changes occur in the spectra. In general two main absorption bands arise, one at about 320 mμ and the other in the region of 500–540 mμ. Lewis[4] designates these as the K′ and Q bands respectively. They are due to the tautomeric mixture formed by the protonation of aminoazobenzenes, since these can carry a proton either on the β-nitrogen of the azo group or on the amino group. Protonation of the amino group causes the absorption band at 320 mμ to appear, since the amino group can no longer resonate with the remainder of the molecule. The long wavelength Q band is caused by the highly resonating azonium ion (III) formed by the protonation of the β-azo nitrogen.[4]



III

The relative intensities of these bands depend upon the structure of the dye[5] and are especially affected by substituents *ortho* to the azo link. A 2-methyl group enhances the basicity of the β-azo nitrogen by electron repulsion. A similar effect should operate in the case of a 2′-methyl group, but here steric hindrance opposes the protonation of the azo group. These effects are reflected in the low intensity K′ band and high intensity Q band of the 2-methylated dyes, a situation which is reversed with a 2′-methyl group. A 3-methyl group sterically inhibits the resonance of a 4-dimethylamino group with the azo group and such dyes do not possess a Q band in their spectra in acid.

The influence of sulphonic acid groups in the spectra of dyes has received little systematic examination. Brode[6] measured the spectra of a number of phenylazonaphthylamines and noted the shifts in maxima for various positions of the sulphonic acid group, but did not attempt to account for them.

During a project on the excretion and metabolism of water soluble azo dyes in this laboratory a number of water soluble dimethylaminoazobenzenes have been prepared substituted with methyl and sulphonate groups. The present report is concerned with the effects of these substituents on the spectra in neutral and acidic solution of these compounds.

## RESULTS AND DISCUSSION

The details of the spectra are recorded in Table 1. The spectra of some unsulphonated aminoazobenzenes are summarized in Table 2.

(i) *Neutral spectra.* The spectra of the monosulphonated aminoazobenzenes examined in neutral solution were all broadly similar, but a range of long wavelength maxima from 420–480 mμ was observed. Generally these maxima occurred at longer wavelengths than the unsulphonated compounds. This bathochromic shift increased as the sulphonate group was shifted from the 2′ to 3′ to 4′ positions in the phenyl ring. This is exemplified by the three methyl oranges. *o*-Methyl orange has a maximum at 446 mμ, the *meta* compound at 467 mμ and the *para* at 472 mμ. The corresponding

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[5] J. A. Miller, R. W. Sapp and E. C. Miller, *J. Amer. Chem. Soc.* **70**, 3458 (1948).

[6] W. R. Brode and D. R. Eberhardt, *J. Org. Chem.* **5**, 157 (1940).

TABLE 1. ABSORPTION MAXIMA OF SULPHONATED 4-DIMETHYLAMINOAZOBENZENES

| Position of substituents | | Neutral solution | | Acidic solution | | | |
|---|---|---|---|---|---|---|---|
| | | | | Q Band | | K′ Band | |
| Sulphonic acid | Methyl | $\lambda_{max}$ | $\varepsilon_{max} \times 10^{-3}$ | $\lambda_{max}$ | $\varepsilon_{max} \times 10^{-3}$ | $\lambda_{max}$ | $\varepsilon_{max} \times 10^{-3}$ |
| 2′ | — | 446 | 21 | 518 | 53 | 321 | 4.4 |
| 3′ | — | 467 | 20 | 513 | 40 | 318 | 8·0 |
| 4′ | — | 472 | 22 | 500 | 30 | 321 | 13 |
| 2′ | 2 | 446 | 21 | 513 | 50 | 333 | 2·9 |
| 3′ | 2 | 467 | 21 | 513 | 47 | 321 | 2·9 |
| 4′ | 2 | 481 | 25 | 516 | 38 | 329 | 8·3 |
| 4′ | 2′ | 455 | 29 | 513 | 23 | 316 | 17·3 |
| 4′ | 3′ | 470 | 25 | 521 | 48·3 | 325 | 7·4 |
| 4′ | 2,3′ | 476 | 22·4 | 521 | 51 | 333 | 3·4 |
| 4′ | 2′,2 | 455 | 22·2 | 513 | 40 | 333 | 7·4 |
| 4′ | 2′,6′ | 420 | 22 | 472 | 16 | 305 | 7·2 |
| 4′ | 2,2′,6′ | 424 | 19 | 470 | 31 | 316 | 4·4 |

TABLE 2. SPECTRA OF 4-DIMETHYLAMINOAZOBENZENES

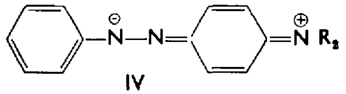
| Position of methyls | Neutral solution | | Acid solution | | | |
|---|---|---|---|---|---|---|
| | | | Q Band | | K′ Band | |
| | $\lambda_{max}$ | $\varepsilon_{max} \times 10^{-3}$ | $\lambda_{max}$ | $\varepsilon_{max} \times 10^{-3}$ | $\lambda_{max}$ | $\varepsilon_{max} \times 10^{-3}$ |
| — | 420 | 24 | 520 | 34 | 320 | 10 |
| 2 | 420 | 27 | 520 | 50 | 326 | 4 |
| 2′ | 410 | 27 | 520 | 10 | 326 | 16 |
| 3′ | 410 | 26 | 520 | 38 | 320 | 9 |
| 2,2′ | — | — | 510 | 15 | 332 | 14 |
| 2,2′,6′ | 385 | 21 | 470 | 20 | 320 | 8 |

2-methyl derivatives have maxima at 446, 467 and 481 mμ. These may be compared with the value of 420 mμ for the unsulphonated dyes (Table 2).[4]

The remainder of the sulphonated dyes examined all have this substituent in the 4′-position. These dyes show a large bathochromic shift compared with their unsulphonated parent. An interesting example is the highly hindered 2,2′,6′-trimethyl-4-dimethylaminoazobenzene and its 4′-sulphonated derivative. These have maxima at 385 and 424 mμ respectively. The former compound was obtained as an uncrystallizable oil, but its spectrum was typical of aminoazobenzenes.

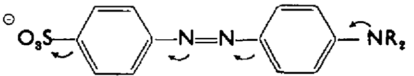
It is interesting to note that the ε values for the sulphonated and unsulphonated dyes are much the same. The presence of a sulphonate group apparently does not alter the transition probability very much even though the transition energy is lowered.

The effects noted here may all be ascribed to the electron attracting inductive effect of the sulphonate group. Among the contributing resonance structures of the aminoazobenzenes (IV) is very important.



IV

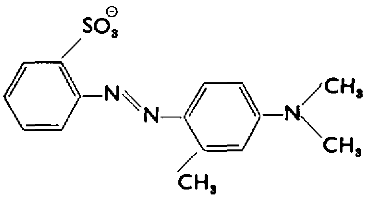
A strongly electron attracting group would stabilize this form by the general electron drift towards it. This effect is clearly shown in the 3′- and 4′-sulphonated compounds. The slightly greater absorption maximum for the 4′-sulphonated dyes may be due to resonance involving the sulphonate group as in (V). This resonance form is possible



V

since the outer octet of sulphur can easily expand to a decet.[7] This, however, can only make a small contribution to the resonance energy of the dyes. Sulphonated aromatic compounds have spectra only slightly differing from the parent hydrocarbon.[8] Zollinger[9] has reported p*K* measurements on sulphanilic and metanilic acids compared with the anilinium ion. Both sulphonated compounds are stronger acids than anilinium, with sulphanilic acid slightly stronger. These facts are explicable by an inductive base weakening effect of the sulphonate group with a slight mesomeric effect operating in sulphanilic acid. Similar effects operating in the case of the dyes under discussion provide an explanation of the observed spectra.

The 2′-sulphonated dyes show the smallest bathochromic shifts in neutral solution. This is most likely due to the proximity of the bulky, negatively charged sulphonate group to the partially negative *β*-azo nitrogen atom. Simple electrostatic repulsion would produce some destabilization of IV. This is clearly insufficient to block the operation of the inductive effect, but it does seriously weaken it. It is most unlikely that the sulphonate group is exerting any steric effects since 2-methyl-2′-sulpho-4-dimethylaminoazobenzene has a spectrum almost identical with that of *o*-methyl orange, with its long wavelength maximum at 446 m$\mu$. A 2-methyl group enhances the electron density about the *β*-azo nitrogen. Models show that this compound almost certainly has the conformation (VI) in which the inductive effect of the sulphonate



VI

group should be enhanced by the electron repulsion of the methyl group. That this is not so can only be accounted for by an electrostatic repulsion due to the sulphonate group.

(ii) *Acid spectra.* The spectra of these compounds in acid are similar to those of the unsulphonated dyes. Two main maxima are observed, the K′ band at 310–330 m$\mu$ and a Q band at about 500–520 m$\mu$. The highly hindered 2′,6′-di- and 2,2′,6′-trimethyl-4′-sulpho-4-dimethylaminoazobenzene have Q bands at about 470 m$\mu$.

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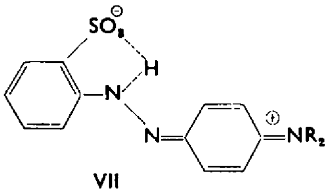
[7] G. Cilento, *Chem. Rev.* **60**, 147 (1960).

[8] E. A. Braude, *Ann. Rpts. Chem. Soc.* **42**, 105 (1945).

[9] Hch. Zollinger, W. Buchler and C. Williver, *Helv. Chim. Acta* **36**, 1711 (1953).

In acidic solution the highest $\varepsilon$ values for the Q band were found for the 2′-sulphonated compounds. This is due to the strong hydrogen bond between the proton and the $\beta$-azo nitrogen and the sulphonate group (VII). Similar strong absorptions have been noted in other cases where a 2′-substituent can form a hydrogen bond with the azonium proton.[10] This is in contrast to the normal behaviour of 2′-substituents which sterically inhibit the protonation of the $\beta$-azo nitrogen.[5,10] The result of such a hydrogen bond is to shift the azonium–ammonium equilibrium almost completely in favour of the azonium form. This is reflected in the spectra by the weak K′ band absorption (ammonium form) and the very intense Q band absorption.



VII

The spectra of the 3′- and 4′-sulphonated aminoazobenzenes examined showed a small hypsochromic shift of the Q band in acid solution compared to the spectra of the parent dyes. 4-Dimethylaminoazobenzene (I) and its 2-methyl derivative have $\lambda_{max}$ 520 m$\mu$ and $\varepsilon_{max}$ 34,000 and 50,000 respectively. *m*-Methyl orange and *p*-methyl orange have $\lambda_{max}$ 513 and 500 m$\mu$ with $\varepsilon_{max}$ 40,000 and 30,000 respectively. This pattern is repeated in the 2-methyl derivatives, although the $\varepsilon$ values are higher as expected. In this series the 3′- and 4′-sulpho-compound have $\lambda_{max}$ at 513 m$\mu$ and $\varepsilon_{max}$ 47,000 and 38,000 respectively. These results indicate that sulphonation produces a slight increase of the electron transition energies and lowers the transition probability.

Such a result is to be expected if the arguments in the section on the neutral spectra are correct. UV and visible light absorption depend mainly upon the energy transitions of the valency electrons.[11] Any group which tends to localize these would produce both hypsochromic and hypochromic effects. In the case of the spectra of the compounds under discussion, these effects are complicated by the tautomeric equilibrium between the protonated forms and the relative amounts of each.

The relative amounts of each tautomer can be readily estimated from the $\varepsilon$ values of the K′ and Q bands. Plotting the value of $\varepsilon_{K'}$ against the $\varepsilon_Q$ value for each dye and drawing the line of best fit leads to a value of 58,000 and 27,000 for the limiting values of the Q and K′ bands respectively.

The ratios

$$\frac{\varepsilon_{Q'}}{\lim \varepsilon_{Q'}} \times 100$$

and

$$\frac{\varepsilon_{K'}}{\lim \varepsilon_{K'}} \times 100$$

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[10] W. C. J. Ross and G. P. Warwick, *J. Chem. Soc.* 1719 (1956).

[11] S. F. Mason, *Quart. Rev. Chem. Soc., Lond.* **15**, 287 (1961).

then give the percentage of the azonium and ammonium forms of the protonated dyes.

A similar procedure has been carried out for the unsulphonated dyes.[12] The limiting $\varepsilon$ values are different from those obtained for the sulphonated compounds; lim $\varepsilon_Q$ was found to be 62,000 and the lim $\varepsilon_{K'}$ 23,000.[12]

For the hindered dyes 4′-sulpho-2′,6′-dimethyl- and 4′-sulpho-2,2′,6′-trimethyl-4-dimethylaminoazobenzene the $\varepsilon_{K'}$ values were very low. Plotting these values against the $\varepsilon_Q$ values produced points well off the line of best fit. However, a line drawn through these points passed through the same lim $\varepsilon_Q$ value as the other dyes and gave a lim $\varepsilon_{K'}$ value of 10,000. For calculating the relative amounts of azonium forms of these dyes this figure gave results (Table 3) in good agreement with the other compounds.

From the results set out in Table 3 the relative amounts of the two protonated forms of the dye can be seen. These figures are not corrected for overlapping of the K′ and Q bands. Examination of the spectra showed this to be insignificant for the Q band. However, other shorter wavelength bands did overlap with the K′ band. This was assumed to be negligible. As expected, the 2′-sulphonated compounds exist to a large extent as the azonium form for reasons already outlined. The relative amounts of azonium and ammonium forms found for the 3′- and 4′-sulphonated dyes support the ideas already elaborated of the effects of a sulphonate group on the spectra of these compounds. A comparison of the amounts of azonium ion for 4-dimethylaminoazobenzene and its 3′- and 4′-sulpho derivatives in acid solution shows that the 3′-sulpho group increases the amount of azonium ion considerably, while a 4′-sulpho group lowers it slightly. This is in agreement with an inductive effect operating in the former case and an inductive and mesomeric effect in the latter as has already been discussed. A similar picture is obtained from the 2-methyl derivatives which contain a sulphonate group. The results obtained from the dyes which contain one or more methyl groups in the same ring as the sulphonate group are not so clear cut. All of the compounds examined possessed a 4′-sulpho group and in acid solution more of the azonium form was found than was expected. This is probably due to a balance of the electron repulsion of the methyl groups and the electron attraction of the sulphonate group, which lead to the observed localization of electrons on the $\beta$-azo nitrogen. The reality of this effect is evidenced by comparing the relative amounts of the azonium and ammonium forms of the unsulphonated parent compounds in acid solution (Table 3). In particular, the difference between the hindered dyes with a 2′-methyl and 2,2′,6′-trimethyl groups in comparison to their 4′-sulphonated derivatives are quite illuminating.

Other authors have reported work on the tautomeric equilibrium of the conjugate acids of aminoazobenzenes. Sawicki[13] has used the ratio $\varepsilon_A/\varepsilon_B$, where $\varepsilon_A$ is the extinction coefficient of the azonium band and $\varepsilon_B$ the extinction coefficient of the ammonium band as a rough measure of the tautomeric equilibrium. However, since these quantities are inversely proportional[12] the ratio between them gives an estimate weighted in favour of the azonium. Hence this ratio leads to an overestimate of the relative amount of the azonium form and underestimates the ammonium form of the tautomeric mixture.

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[12] G. Cilento, E. C. Miller and J. A. Miller, *J. Amer. Chem. Soc.* **78**, 1718 (1956).

[13] E. Sawicki, *J. Org. Chem.* **21**, 605 (1956).

TABLE 3. RELATIVE AMOUNTS OF PROTONATED TAUTOMERS OF SULPHONATED-4-DIMETHYLAMINOAZOBENZENES

| Position of sulphonate | Position of methyls | Percentage azonium | Percentage ammonium |
|---|---|---|---|
| 2′ | — | 92 | 16 |
| 3′ | — | 70 | 30 |
| 4′ | — | 52 | 48 |
| 2′ | 2 | 88 | 11 |
| 3′ | 2 | 83 | 11 |
| 4′ | 2 | 67 | 31 |
| 4′ | 2′ | 40 | 64 |
| 4′ | 3′ | 83 | 27 |
| 4′ | 2,3′ | 88 | 13 |
| 4′ | 2,2′ | 69 | 28 |
| 4′ | 2′,6′ | 28 | 72 |
| 4′ | 2,2′,6′ | 56 | 44 |
| — | — | 57 | 46 |
| — | 2 | 82 | 19 |
| — | 2′ | 16 | 81 |
| — | 3′ | 62 | 45 |
| — | 2,2′ | 25 | 67 |
| — | 2,2′,6′ | 35 | 65 |

Granacher *et al.*[14] have estimated the amount of ammonium form of the fluorborates of several dimethylaminoazobenzenes in anhydrous acetonitrile by NMR methods. Because of rapid exchange of protons no N–H signals could be found and these authors used the average chemical shift of the N-methyl protons and the 2,6, 3,5 protons to calculate their results. While the results obtained are essentially in agreement with the present work, the method is not quite as satisfactory as using the signals from the particular tautomers as can be done with keto-enol tautomers.[15] Katritzky and Waring[16] have pointed out some of the dangers inherent in interpreting NMR spectra where rapid proton exchange takes place. In the case of the dyes under consideration it is possible that resonance effects as in III affecting the amount of positive charge of the $\gamma$-nitrogen could lead to errors in the estimation of the relative amounts of each tautomer.

## EXPERIMENTAL

UV spectra were measured on a Unicam SP 700 recording spectrophotometer. The compounds examined were dissolved in distilled water or aqueous ethanol. Spectra in acid solution were measured by diluting 1 ml aliquots of stock dye solutions to 100 ml with 1N HCl aq. In the case of 2′-sulphonated dyes it was necessary to use ethanolic 1N HCl.

*Preparation of dyes.* The azo dyes were prepared by dissolving the appropriate sulphonated amine (0·01 mole) in water (25 ml) containing $Na_2CO_3$ (0·05 mole). Sodium nitrite (0·011 mole) was added and the solution cooled to 0° and slowly added to a mixture of conc. HCl (3·0 ml) and crushed ice (10 g) with stirring.

After the diazotization was complete a solution of the appropriate dimethylaniline (0·01 mole) in glacial acetic acid (2 ml) was added to the diazonium salt suspension. The mixture was allowed to stand at 0° for 10 min, a solution of NaOH (1·4 g) in water (10 ml) was added with stirring, and

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[14] J. Granacher, H. Suhr, A. Zenhausern and Hch. Zollinger, *Helv. Chim. Acta* **44**, 313 (1961).

[15] L. W. Reeves, *Canad. J. Chem.* **35**, 1351 (1957).

[16] A. R. Katritzky and A. J. Waring, *Chem. & Ind.* 695 (1962).

the resulting precipitate coagulated on the water bath. The crystalline precipitate was recrystallized from hot water.

Where the sodium salt failed to precipitate even after the addition of NaCl, the solution was evaporated to about half bulk. If no dye crystallized out the pH was adjusted to about 6 and the theoretical quantity of S-benzylisothiouronium chloride was added. The resultant precipitate was collected and recrystallized from aqueous ethanol.

*p-Methyl orange* was a commercial sample purified by recrystallization of the sodium salt from hot water.

*o-Methyl orange* was recrystallized from hot water as the sodium salt. (Found: C, 50·2; H, 4·7; N, 12·5. $C_{14}H_{14}N_3O_3SNa\cdot\frac{1}{2}H_2O$ requires: C, 50·1; H, 4·5; N, 12·5%).

*m-Methyl orange* was recrystallized from hot water as the sodium salt. (Found: C, 48·6; H. 4·4; N, 12·4. $C_{14}H_{14}N_3O_3SNa\cdot H_2O$ requires: C, 48·8; H, 4·7; N, 12·2%).

2-*Methyl-4′-sulpho*-DAB* was recrystallized from hot water as the sodium salt. (Found: C, 51·8; H, 5·4; N, 11·4. $C_{15}H_{16}N_3O_3SNa\cdot\frac{1}{2}H_2O$ requires: C, 51·4; H, 4·9; N, 12·0%).

2-*Methyl-3′-sulpho*-DAB was recrystallized from aqueous ethanol as the S-benzylisothiouronium salt, m.p. 167°. (Found: C, 56·85; H, 5·7; N, 14·0. $C_{23}H_{27}N_5O_3S_2$ required: C, 56·8; H, 5·6; N, 14·4%).

2-*Methyl-2′-sulpho*-DAB was recrystallized from hot water as the sodium salt. (Found: C, 51·1; H, 5·2; N, 11·8. $C_{15}H_{16}N_3O_3SNa\cdot\frac{1}{2}H_2O$ requires: C, 51·4; H, 5·5; N, 12·0%).

2′-*Methyl-4′-sulpho*-DAB was recrystallized from water–dioxan as the sodium salt–dioxan solvate. (Found: C, 52·6; H, 5·8; N, 9·7. $C_{15}H_{16}N_3O_3SNa\cdot C_4H_8O_2$ requires: C, 53·2; H, 5·6; N, 9·8%).

3′-*Methyl-4′-sulpho*-DAB was recrystallized from hot water as the sodium salt. (Found: C, 50·0; H, 5·2; N, 11·8. $C_{15}H_{16}N_3O_3SNa\cdot H_2O$ requires: C, 50·1; H, 5·1; N, 11·7%).

2,2′-*Dimethyl-4′-sulpho*-DAB was recrystallized from hot water as the sodium salt. (Found: C, 52·9; H, 5·4; N, 11·2. $C_{16}H_{18}N_3O_3SNa\cdot\frac{1}{2}H_2O$ requires: C, 52·7; H, 5·3; N, 11·5%).

2,3′-*Dimethyl-4′-sulpho*-DAB was recrystallized from hot water as the sodium salt. (Found: C, 52·4; H, 5·4; N, 11·8. $C_{16}H_{18}N_3O_3SNa\cdot\frac{1}{2}H_2O$ requires: C, 52·7; H, 5·3; N, 11·5%).

2′,6′-*Dimethyl-4′-sulpho*-DAB was recrystallized from aqueous ethanol as the S-benzylisothiouronium salt, m.p. 192°. (Found: C, 57·7; H, 6·00; N, 14·00. $C_{24}H_{29}N_5O_3S_2$ requires: C, 57·7; H, 5·9; N, 14·0%).

2,2′,6′-*Trimethyl-4′-sulpho*-DAB was recrystallized from aqueous ethanol as the S-benzylisothiouronium salt, m.p. 200°. (Found: C, 57·9; H, 6·00; N, 13·5. $C_{25}H_{31}N_5O_3S_2$ requires: C, 58·4; H, 6·1; N, 13·6%).

2,2′,6′-*Trimethyl-4-Dimethylaminoazobenzene.* 2,6-Dimethylaniline (1·0 g) was mixed with conc. HCl (2·5 ml) and diluted with water (3·0 ml). The solution was cooled to 0° and $NaNO_2$ (0.75 g) in water (9 ml) slowly added. At the end of the diazotization N,N-dimethyl-*m*-toluidine (1·2 g) and sodium acetate (2·4 g) dissolved in ethanol–water mixture was added all at once. The reaction mixture was stored for 1 hr at 0° and diluted with water and extracted into benzene and the benzene solution washed with water, 5% NaOH and water. The residue from the benzene was gummy. Excess amines were removed by steam distillation and the gummy dye taken up into benzene and chromatographed on alumina with benzene–petrol (1:2) as the eluent. Only one red band was seen on the column. A clear red gum was obtained on elution and removal of the solvent. The UV spectrum was that expected for an amino–azo dye.

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* DAB is an abbreviation for 4-dimethylaminoazobenzene.