[CONTRIBUTION FROM THE CANCER RESEARCH LABORATORY, UNIVERSITY OF FLORIDA]

THE RELATION OF PHYSICAL PROPERTIES OF CARCINOGENS TO THEIR ACTIVITY. I. THE BASICITY OF ALKYLATED 4-AMINOAZOBENZENE DYES¹

EUGENE SAWICKI AND FRANCIS E. RAY

Received June 3, 1954

Although a large number of 4-dimethylaminoazobenzene (DAB) derivatives have been found to be hepatocarcinogenic in the rat (1), in our opinion no satisfactory theory is available to correlate the activity of these dyes with (a) their chemical and physical properties and (b) the biochemical effects of the dye and its metabolites on the animal. In this respect a worthwhile theory would have to explain the variable effect of substitution on the cancer producing activity of 4-aminoazobenzene derivatives (1). In this paper the first phase of this project is undertaken, namely, the investigation of the basicity of 4-aminoazobenzene dyes.

The azo group has two nitrogen atoms in close proximity connected by a double bond. This type of grouping usually shows in its absorption spectra a well developed low energy n band of moderate intensity. This type of band is due to $n \rightarrow pi$ electronic transitions (2). The spectra of azomethane (3), cinnoline (4), 1-benzeneazo-1-phenylcyclohexane (5), and 1-azo-bis-1-arylalkanes (6), in which the azo group is present, show low energy n bands of moderate intensity also. In the presence of an amino group this band may be hidden. This is shown in Figure 1 where the low intensity azobenzene n band at 441 m μ is displaced to 429 m μ in 4-methylformylaminoazobenzene and is hidden in DAB. On the other hand, the intense phenylazo pi band of azobenzene at 317 m μ is displaced to 338 m μ by substitution of a 4-methylformylamino group and to 408 m μ by substitution of a 4-dimethylamino group.

In the 4-aminoazobenzenes the resonance effect causes the *beta* nitrogen to acquire a high electron density. Some evidence has been presented that the *beta*



nitrogen actually is the most basic nitrogen (7). In the reaction with a proton it is evident that if the proton attached itself to the amino nitrogen, the consequent salt would be spectrally similar to azobenzene. This is not so, for the spectral curves of azobenzene and DAB hydrochloride are dissimilar, Figure 1. Consequently a proton or a Lewis acid should be attracted preferably to the *beta* nitrogen. This has been stated to occur with sulfur dioxide (8) and palladium dicyanide (9). On the other hand a 2'-alkyl group should (as in o-toluidine) decrease the basicity of the ortho nitrogen, Table II, thus causing a relative increase in the basicity of the amino nitrogen while in 4-diethylaminoazobenzene

¹ This investigation was supported by research grant C-1308 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

Compound	m.p., °C. (ref.)	pK_a in 50% alcohol
3-Methyl DAB ^a	oil (10)	3.48
4-Diethylaminoazobenzene	97-98 (11)	3.08
2-Methyl DAB	68-69 (12)	3.08
2,3'-Dimethyl-4-aminoazobenzene	81-82 (13)	2.92
2,4'-Dimethyl-4-aminoazobenzene	127 - 128 (14)	2.92
4'-Ethyl-4-methylethylaminoazobenzene ^b	80-81	2.72
2,2'-Dimethyl DAB ^c	82-83	2.64
4-Methylethylaminoazobenzene	72–73 (11)	2.58
4-Ethylaminoazobenzene	87-88 (11)	2.58
3'-Methyl-4-methylaminoazobenzene	109-110 (11)	2.44
3,4'-Dimethyl-4-aminoazobenzene	130-131 (14)	2.39
4-Methylaminoazobenzene	89-90 (11)	2.37
4'-Methyl DAB	170-171 (15)	2.36
4-Aminoazobenzene	124 - 125 (16)	2.35
3'-Methyl DAB	120-121 (11)	2.33
4'-Ethyl DAB ^d	131-132	2.30
4-Dimethylaminoazobenzene (DAB)	117-118 (15)	2.28
2'-Methyl DAB	73-74 (10)	2.04
2',5'-Dimethyl DAB	157-158 (11)	2.0
2'-Ethyl DAB	82-83	1.85
4-Benzylmethylaminoazobenzene	99-100 (11)	1.6

TABLE I

BASICITY OF ALKYLATED 4-AMINOAZOBENZENE DYES

^a Anal. Cale'd for C₁₅H₁₇N₃: N, 17.6. Found: N, 17.5.

^b Anal. Cale'd for C₁₇H₂₁N₃: C, 76.4; H, 7.9; N, 15.7. Found: C, 76.3; H, 7.8; N, 15.4.

^c Anal. Calc'd for C₁₆H₁₉N₃: N, 16.6. Found: N, 16.3.

^d Anal. Calc'd for C₁₆H₁₉N₃: C, 75.9; H, 7.5; N, 16.6. Found: C, 75.6; H, 7.6; N, 16.5.

• Anal. Cale'd for C16H19N3: C, 75.9; H, 7.5; N, 16.6. Found: C, 75.8; H, 7.4; N, 16.4.

the basicity of the amino nitrogen would be strengthened by the ethyl groups thus causing a relative increase of the basicity of the amino group as compared to the β -nitrogen. In the same table the basicities of DAB derivatives are compared with analogous aniline compounds.

Although the azo nitrogens are usually weakly basic, the *beta* nitrogen of the 4-aminoazobenzenes has a moderate basic strength. The base strengthening oscillation of the positive charge in the salt is the probable explanation of this



anomaly. Many more examples of this base strengthening effect have been given by Albert, et al. (20).

A comparison of the ionization constants of 4-aminoazobenzene derivatives, substituted in the 2-position by a methyl group, with DAB is enlightening, Table I. 2-Methyl DAB, 2,4'-dimethyl DAB, 2,4'-dimethyl-4-aminoazobenzene, and 2,3'-dimethyl-4-aminoazobenzene have pK_a 's of 3.08, 3.14, 2.92, and 2.92, respectively, as compared to a pK_a of only 2.28 for DAB. From this data it is apparent that a methyl group in the 2-position of the 4-aminoazobenzenes



Fig. 1. Ultraviolet and Visible Absorption Spectra: 1. Azobenzene in 95% Ethanol; 2. 4-Methylformylaminoazobenzene in 95% Ethanol; 3. DAB in 95% Ethanol; 4. DAB in 10% Ethanolic 0.1 N Hydrochloric Acid.

has a base strengthening effect. This increase in basicity may be due to a slight increase in resonance caused by some such contribution as



X X 3_2 NH2		$\begin{array}{c c} & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\$			
x	ref.	¢Ka in H₂O	x	ref.	¢K _a in 50% ETOH
4-OMe	(17)	5.29	4'-OMe	(7)	2.35
4-Me	(17)	5.07	4'-Me		2.360
3-Me	(17)	4.69	3'-Me		2.33
Н	(17)	4.58	Н		2.28 ^b
2-Me	(17)	4.39	2'-Me		2.04
4-Cl	(17)	4.00	4'-Cl	(7)	2.00
$4-NO_2$	(18)	1.0	4'-NO2	(7)	1.81
2,4,6-Br3	(19)	abt-0.8	2',4',6'-Br ₃		1.0

TABLE	II
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pK_a Comparisons of Aniline and Azobenzene Derivatives

^a Rogers, et al. (7) obtained 2.31.

^b Rogers, et al. obtained 2.27.

The pK_a 's of 2'-methyl DAB and 2',5'-dimethyl DAB are 2.04 and 2.0, respectively, as compared to DAB with a pK_a of 2.28. This base weakening effect of a methyl group in the 2'-position is probably caused by the weak steric strain of the 2'-methyl group on the *beta* nitrogen of the salt. The decrease in basicity of *o*-toluidine, as compared to aniline, has been explained on this basis



(21). In line with this is the decreased basicity of 2'-ethyl DAB, pK_a 1.85, as compared to DAB, pK_a 2.28 and 2'-methyl DAB, pK_a 2.04. The pK_a of 2,2'-dimethyl DAB is 2.64. In this molecule both the base weakening effect of a 2'-methyl group and the base strengthening effect of a 2-methyl group are present. As the effect of a 2-methyl group has a more powerful effect on the basicity of the *beta* nitrogen, it is consistent with the facts that 2,2'-dimethyl DAB is intermediate in basicity between 2-methyl DAB and 2'-methyl DAB, but is more basic than DAB.

The base strengthening effect of an N-ethyl group is well known. For example dimethylaniline has a pK_a in 50% alcohol of 4.21 as compared to a pK_a of 5.85 for diethylaniline (22). In the 4-aminoazobenzene dyes increasing the basicity of the amino nitrogen must strengthen the resonance effect, thus causing an increase in basicity of the *beta* nitrogen. In this respect 4-diethylaminoazobenzene has a pK_a of 3.08 as compared to a pK_a of 2.28 for DAB.

The base weakening effect of a benzyl group is shown in 4-methylbenzylamino-

azobenzene, pK_a 1.6. This is evidently due to the electron withdrawal effect of the benzene ring in the benzyl group on the amino nitrogen.

A methyl group in the 3'- or 4'-position of a 4-aminoazobenzene has only a slight effect on the basicity. On the other hand, in DAB, a 3-methyl group would cause definite steric hindrance and consequently would have a large base strengthening effect on the dimethylamino nitrogen and a base weakening effect on the *beta* nitrogen. The latter effect would be a direct result of the decreased resonance effect caused by a crowding of the dimethylamino group out of the plane of the molecule. Consequently the amino nitrogen in 3-methyl DAB could be expected to be the most basic part of the molecule. Miller, *et al.* (10) have shown that the absorption spectrum of their 3-methyl DAB in acid solution is similar to that of azobenzene. This we have confirmed. The salt of 3-methyl DAB must then have the following structure. Of all the monomethyl DAB's 3-methyl DAB is



the most basic with a pK_a of 3.48. On the basis of these facts the amino nitrogen is the strongest proton acceptor and so is the most basic nitrogen in the com pound.



FIG. 2. THE ABSORPTION SPECTRA OF 4-DIETHYLAMINOAZOBENZENE IN 50% ETHANOL

EXPERIMENTAL²

Preparation of compounds. The compounds were prepared by procedures given in the literature. Each dye was crystallized from two to four different solvents to a constant melting point. The melting point and reference of preparation are given in Table I.

4'-Ethyl DAB, 2'-ethyl DAB, and 4'-ethyl-4-methylethylaminoazobenzene were prepared by combining the ethyl benzene diazonium chloride with the appropriate dialkylaniline by standard procedure.

Determination of the dissociation constant. All absorption spectra were measured with a Beckman Model DU spectrophotometer and 1-cm. Corex cells at $26 \pm 1^{\circ}$. The absorption spectra of unbuffered solutions were measured in 50% aqueous ethanol (100 ml. of the solution contained 50 ml. of water) for various pH values. The concentrations of all dyes were 5×10^{-5} M. For most of the compounds two isosbestic points were observed in the range of 320 to 600 m μ . The type of curves obtained are shown in Figure 2 for 4-diethylaminoazobenzene.

The dissociation constants were calculated (23) by the equation $pK_{a} = pH_{m} + \log p$

 $E_B - E_m$ where E_B , E_{BH+} are the optical densities of base and salt and E_m is the optical $E_m - E_{BH+}$

density of a mixture of base and salt at an intermediate pH_m fairly close to the value of the pK_a of the compound. The average value of pK_a so obtained is shown in Table I. All basicities were determined at several different pH values over a 60 m μ wave length range within the two isosbestic points at 4 m μ intervals. The average deviation of all compounds was within $\pm 0.05 \ pH$ units.

SUMMARY

The basicity of 22 alkylated 4-aminoazobenzene dyes were determined spectrophotometrically in 50% alcohol.

The spectra and the basicity of these dyes were discussed. The change in the basicity of 4-aminoazobenzene caused by substitution of various alkyl groups in the molecule was explained on the basis of electronic theory.

GAINESVILLE, FLORIDA

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² Melting points are uncorrected. Analyses are by Peninsular ChemResearch, Inc., Gainesville, Fla.

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