337. *Aryl-2-halogenoalkylamines. Part XVII.* The Ultraviolet Absorption Spectra of Some 4-Di-(2-chloroethyl)aminoazobenzenes in Neutral and Acidic Ethanol Solutions.

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The absorption spectra of some substituted 4-di-(2-chloroethyl)amino-azobenzenes and related compounds in neutral and in acidic ethanol solutions have been studied. The absorption spectra of certain 2'-carboxy-derivatives which present unusual features have also been examined in chloroform and in alkaline ethanol solution. Neutral solutions of these acids are considered to contain appreciable proportions of a hydrogen-bonded hybrid (IIa ⬌ c ⬌ d).

DURING studies of cytotoxic chloroethylamino-derivatives of azobenzene an attempt has been made to correlate various chemical and physical properties with biological activities. The measurement of the ultraviolet absorption spectra of a number of derivatives became of interest, first, because of a possible relation between the effects of substituents on the position of absorption maxima and on the biological activity and, secondly, because methods for assessing the reducibility of the azo-linkage in the compounds demanded a knowledge of their absorption characteristics in neutral and acidic solutions.[1]

Absorption Spectra in Neutral Ethanol Solution.—Azobenzenes exhibit weak absorption bands in the regions 210—290 and 440—470 mμ (*R*-band), due respectively to the aromatic ring system and to the –N:N– linkage, and a strong band at about 320—370 mμ (*K*-band) ascribed to the conjugation between the azo-group and the aromatic ring system.[2] Our compounds exhibit the low-intensity band at about 270 mμ and a broad band of high

TABLE 1. *Absorption spectra of some substituted 4-di-(2-chloroethyl)aminoazobenzenes and related compounds in 95% aqueous ethanol.*

Subst.	$\lambda_{max.}$ (mμ)	ε	Subst.	$\lambda_{max.}$ (mμ)	ε
(I; R = R' = $CH_2 \cdot CH_2Cl$)			(I; R = R' = $CH_2 \cdot CH_2Cl$)		
None	397	25,700	4'-SO_3H	405	33,300
2'-Me	395	27,750	2 : 2'-(MeO)$_2$	405—415	25,600
3'-Me	395	26,900	2'-CO_2H, 2-MeO	450—460	33,100
4'-Me	395	29,300	2'-CO_2H, 2-Me	430—450	14,500
2'-Ph	400	26,500		485	17,590
4'-Ph	400—410	40,500	2'-CO_2H, 2 : 4'-Me$_2$	480—490	28,000
2-MeO	405	29,000	4'-CO_2H, 2-Me	420	28,100
3'-Cl	406	31,400	4'-CO_2H, 3-MeO	420	22,100
4'-Cl	405	31,600	2'-OH, 5'-NO_2	485—490	34,560
4'-Br	405	33,000	2-CO_2H, 4'-NO_2	444	29,000
2'-I	410—415	31,850			
2'-CO_2H	410—430	19,150	(I; R = R' = Me)		
	470	20,300	None	410	27,800
3'-CO_2H	402	30,550	2'-CO_2H	498	32,200
4'-CO_2H	412	32,300	2'-NH_2	440—460	21,480
4'-Ac	435	34,100	(I; R = Me, R' = $CH_2 \cdot CH_2Cl$)		
2'-NO_2	420	27,900			
3'-NO_2	416	30,800	2'-CO_2H	415—445	20,650
4'-NO_2	446	21,300		485	24,500
2'-SO_3H	400	27,400	2-Carboxyazobenzene	315	12,100

intensity in the region 390—430 mμ; the latter is apparently a composite of the *R*-band and of the *K*-band which has undergone a bathochromic shift due to the *p*-alkylamino-substituent. Interest has centred on this intense band and the data obtained are shown in Table 1.

The position of the absorption maximum is strongly displaced bathochromically by 2'-, 3'-, and 4'-nitro-groups and by a 4'-acetyl group; the effect from the 4'-position is

* Part XVI, *J.*, 1956, 1364.

[1] Ross and Warwick, following paper.

[2] Burawoy, *J.*, 1935, 1865; Morton, "Practical Aspects of Absorption Spectrophotometry," *Inst. Chem. Monograph*, 1938, p. 33; Badger and Buttery, *J.*, 1953, 2156.

especially marked. A 4'-phenyl group, a 2-methoxy-group, 2'-, 3'-, and 4'-halogeno-groups, 2'-, 3', and 4'-carboxy-groups, and 2'- and 4'-sulphonic groups all cause small bathochromic shifts, whilst 2'-, 3'-, and 4'-methyl groups are without significant effect. Substitution of methyl groups in butter yellow (I; $R = R' = Me$) by 2-chloroethyl groups causes a hypsochromic displacement.

Introduction of a 2'-carboxy-substituent into the structure (I; $R = R' = CH_2 \cdot CH_2Cl$) considerably modified the absorption spectrum and this was of especial interest in view of
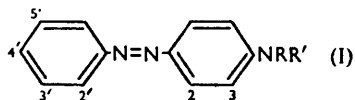
the enhanced biological activity of the compounds. In 95% aqueous ethanol the 2'-carboxy-derivative shows the usual single low-intensity maximum at 270 mμ but at longer wavelengths a broad band having two maxima of approximately equal intensity at 410—430 and 470—480 mμ. In absolute ethanol two maxima were exhibited but the peak at about 470 mμ was relatively more intense than in 95% aqueous ethanol. In chloroform and in N/250-hydrochloric acid in 95% aqueous ethanol a single maximum at 465 mμ was shown while in N/250-sodium hydroxide in 95% aqueous ethanol a single maximum at 395 mμ appeared.
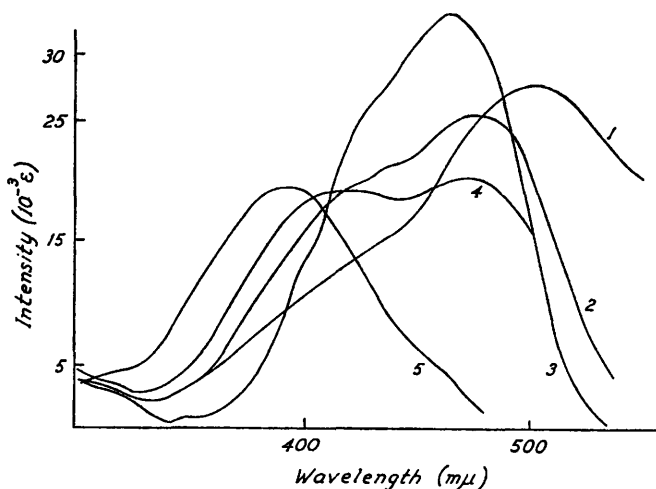
Absorption spectra of 4-di-(2-chloroethyl)aminoazobenzene-2'-carboxylic acid in various solvents.

1, In N/2-ethanolic HCl.
2, In N/250-ethanolic HCl.
3, In pure CHCl₃.
4, In 95% aq. EtOH.
5, In N/250-ethanolic NaOH.

The spectrum in absolute ethanol was practically identical with curve 2.

A single maximum was shown at 505 mμ in N/2-hydrochloric acid in 75% aqueous ethanol. These results which were briefly recorded elsewhere [3] are shown in the Figure.

The 3'- and the 4'-carboxy-derivative and the methyl ester of the 2'-carboxy-compound do not show this anomalous absorption which appears to require the presence of an ionisable 2'-carboxyl group. Dr. S. F. D. Orr of this Institute has examined the infrared spectra of several derivatives and has drawn the following conclusions. Carboxylic acids are known to form stable dimers, which are only partially dissociated even in dilute solutions or in the vapour phase at low temperatures. Thus benzoic acid in carbon tetrachloride solution (0·05%) absorbs at 1743 and 3536 cm.⁻¹ owing to the CO and OH groups of the monomeric forms and also at 1696 and around 2600 cm.⁻¹ owing to hydrogen-bonded CO and OH groups of the dimers; in the solid state, there are only associated forms and absorption is at 1685 and 2500—2700 cm.⁻¹. The 3'-carboxy-derivative of the present series behaves in an analogous manner; absorption is found in CCl₄ solution at 1746 and 3537 cm.⁻¹, and at 1696 and 2500—2700 cm.⁻¹, owing to the monomeric and the dimeric form respectively, while the latter gives rise to absorption in the solid state at 1688 and around 2600 cm.⁻¹. With the 2'-carboxy-compound, however, in dilute CCl₄ solution, bands at 1746 and 2785 cm.⁻¹ are observed, and in the solid state at 1730 and about 2750 cm.⁻¹; these results mean that the carbonyl group is free but the hydroxyl group is associated under all conditions; this is possible only if an intramolecular hydrogen bond is formed between the hydroxyl group and the azo-nitrogen atom as postulated below. Methyl-red behaves in the same manner as

³ Ross and Warwick, *Chem. and Ind.*, 1955, 745; see also Warwick, *Thesis*, London, 1955.

this 2′-carboxy-compound; the 4′-compound could only be measured in the solid state and was similar to the 3′-compound.

It is considered that solutions of 4-di-(2-chloroethyl)aminoazobenzene-2′-carboxylic acid (IIa; R = R′ = CH₂·CH₂Cl) contain the species indicated in the annexed scheme. In neutral solution an equilibrium exists between the dissociated form (IIb) and the undissociated form which is regarded as a resonance hybrid (IIa ◄──► c ◄──► d) of a
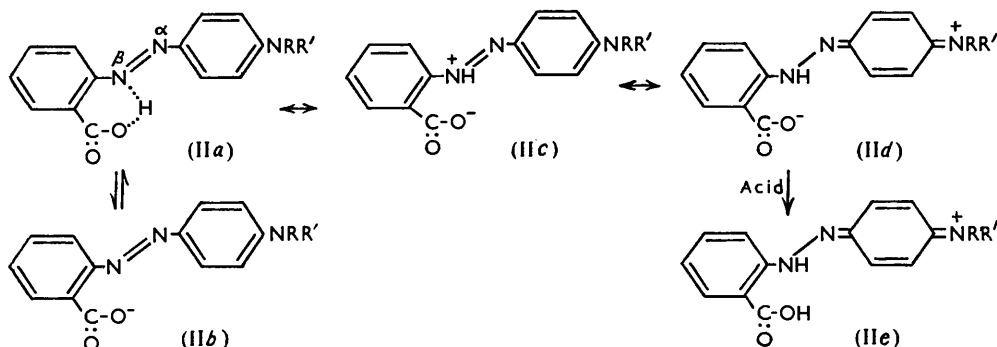
hydrogen-bonded structure. It seems probable that the quinonoid form (IId) is present in the highest proportion since the maximum at 470—480 mμ, now considered to be associated with the hybrid, approaches that of the cationic quinonoid form (IIe) which is present in strongly acid solutions.

This scheme appears to account for the various changes in spectra which have been observed. Thus in chloroform solution and in weakly acidic solution there will be practically no dissociation and the spectrum is that of the resonance hybrid; in alkaline solution the anionic form (IIb) predominates; both forms are present in absolute ethanol and in 95% aqueous ethanol and, as would be expected, rather more of the dissociated form is present in the latter solution. A hydrogen-bonded structure for 4-di-(2-chloro-ethyl)aminoazobenzene-2′-carboxylic acid (IIa; R = R′ = CH₂·CH₂Cl) and methyl-red [5] (IIa; R = R′ = Me) is also supported by the greater solubility in chloroform and carbon tetrachloride of the 2′-carboxy-derivative compared with the 3′- and the 4′-isomer. The powerful chelating action of 2′-carboxy-azo-compounds [6] also suggests such bonding in the parent acids.

If hydrogen bonding occurs between the carboxyl group and the β-nitrogen atom of the azo-linkage then modifications to the molecule which would increase the basicity of this nitrogen atom should increase the proportion of the bonded hybrid structure (IIa ◄──► c ◄──► d) at the expense of the anionic form (IIb). This would be due partly to the decreased acidity of the carboxyl group and partly to the increased probability of hydrogen bonding. It now appears to be established that increasing electron-release from the 4-position increases the basicity of the β-nitrogen atom [7] and it has now been shown (Table 1) that the proportion of hydrogen-bonded form in neutral alcoholic solution increases in the order (IIa; R = R′ = CH₂·CH₂Cl) < (IIa; R = Et, R′ = CH₂·CH₂Cl) < (IIa; R = R′ = Me), *i.e.*, in the order of increasing basicity of the β-nitrogen atom. The last-mentioned compound, methyl-red, represents the extreme case in which only the hydrogen-bonded form is present in 95% aqueous ethanol, the position of the absorption maximum in this solvent being identical with that in chloroform (Tables 1 and 2). The introduction of a 2-methyl substituent into (IIa; R = R′ = CH₂·CH₂Cl) also increases the basicity of the β-nitrogen atom (cf. ref. 7c) and the intensity of the maximum at 485 mμ is increased at the expense of the maximum at 430—450 mμ. Further substitution in the

[4] Davies and Sutherland, *J. Chem. Phys.*, 1938, **6**, 755; Flitt, *J.*, 1951, 962.
[5] Cf. Mecke and Schmähl, *Naturwiss.*, 1955, **42**, 153.
[6] Drew and Landquist, *J.*, 1938, 292.
[7] (a) Rogers, Campbell, and Maatman, *J. Amer. Chem. Soc.*, 1951, **73**, 5122; (b) Badger, Buttery, and Lewis, *J.*, 1954, 1888; (c) Sawicki and Ray, *J. Org. Chem.*, 1954, **19**, 1686.

4'-position gives the 2 : 4'-dimethyl-2'-carboxylic acid which resembles methyl-red in showing a single maximum at the longer wavelength.

It is of interest that adding appreciable amounts of water to alcoholic solutions of azo-compounds causes the appearance of a new band bathochromically displaced some 50 mμ from the original intense band : this is ascribed to hydrogen bonding between water molecules and the more basic nitrogen atom of the azo-linkage.[8] The displacement is in the same direction and of the same order of magnitude as that now observed in the case

TABLE 2. *Absorption spectra of some substituted 4-di-(2-chloroethyl)aminoazobenzenes and related compounds in chloroform and in* N/250-*sodium hydroxide in aqueous ethanol solution.*

Subst.	In CHCl₃ $\lambda_{max.}$ (mμ)	ε	In N/250-NaOH $\lambda_{max.}$ (mμ)	ε	Subst.	In CHCl₃ $\lambda_{max.}$ (mμ)	ε	In N/250-NaOH $\lambda_{max.}$ (mμ)	ε
(I; R = R' = Me)					(I; R = R' = CH₂·CH₂Cl)				
2'-CO₂H ...	495	39,730	405	21,550	2'-CO₂H, 2-Me ...	475	25,900	400	11,900
(I; R = Et, R' = CH₂·CH₂Cl)					2'-CO₂H, 2 : 4'-Me₂	475	32,100	400	35,770
2'-CO₂H ...	480—485	45,800	405	22,200	2'-SO₃H	555	31,150	400	20,320
(I; R = R' = CH₂·CH₂Cl)						530—540	29,880		
2'-CO₂H ...	465	34,000	395	19,600	2'-OH, 5'-NO₂	475—490	—	505—520	—
					None	395	32,220	385	17,700

of the 2'-carboxy-derivative when compared with the corresponding 3'- and 4'-isomers. The high activity of the 2'-carboxylic acid derivatives as tumour-growth inhibitors may be connected with an ability to form chelate compounds with traces of metals or with the increased lipoid-solubility resulting from a hydrogen-bonded structure, but it seems more likely to be related to the relative ease of reduction of the azo-linkage which is discussed in the following paper.

The 2'-sulphonic acid is a sufficiently strong acid ($pK_a < 3$ in 50% aqueous acetone) for it to exist entirely in the anionic form in 95% aqueous ethanol, a maximum being shown at 400 mμ at the same position as in alkaline solution (Tables 1 and 2). In absolute ethanol a second peak appears at 530 mμ, and in chloroform, in which a hydrogen-bonded structure is presumed to exist, the maximum at 400 mμ is absent.

Absorption Spectra in Acidic Ethanol Solution.—According to Badger, Buttery, and Lewis [7b] it seems probable that the species (III), (IV) are present in moderately acidic solutions of *p*-alkylaminoazobenzenes. Of these (IV*a* and *b*) are extreme forms of a
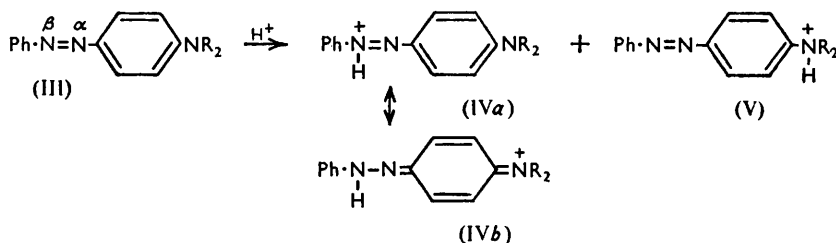
resonance hybrid which is considered to give rise to a band in the region of 530 mμ (called the Q-band), while the tautomeric form (V) is considered responsible for a band which appears at about 320 mμ (called the K'-band). The acid concentration (0·5N-hydrochloric acid in 75% aqueous ethanol) used in our measurements was dictated by the requirements of the stannous chloride reduction experiments—the conditions chosen gave conveniently measured rates of reaction and all the compounds which it was desired to examine were then in solution. At this acid concentration the base-strength-reducing effect of the 2-chloroethyl substituents on the amino-nitrogen atom, due to the electron-attracting capacity of the chlorine atoms [9] possibly coupled with steric hindrance, results

[8] Brode, Seldin, Spoerri, and Wyman, *J. Amer. Chem. Soc.*, 1955, **77**, 2762.
[9] Ross, *J.*, 1949, 183.

in proton addition exclusively at the azo-linkage in all but one example (see below) and no K'-bands were detected. Most of the compounds examined were present partly in the uncharged form (giving a K-band) and partly as the cation (giving a Q-band); the results are shown in Table 3.

As would be expected from their effect on the basicity of the azo-linkage, electron-releasing substituents tended to increase the proportion in the cationic form whilst electron-attracting substituents decreased the proportion. 2'-Substituents, independently of their polarity, greatly reduced the tendency for proton addition to the azo-linkage, only K-bands being observed under the conditions employed. This effect, which is well established,[10] supports the view that the proton is added to the β-nitrogen atom, for a 2'-substituent would hinder such addition. The effect of a 2'-carboxy-group and of a 2'-methoxycarbonyl

TABLE 3. *Absorption spectra of some substituted 4-di-(2-chloroethyl)aminoazobenzenes and related compounds in* N/2-*hydrochloric acid in* 75% *aqueous ethanol.*

| Subst.	Q-Band $\lambda_{max.}$ (mμ)	ε	K-Band $\lambda_{max.}$ (mμ)	ε	K'-Band λ_{max} (mμ)	ε
	(I; R = R' = CH₂·CH₂Cl)					
None	530	22,660	400	19,770	—	—
2-Me	520	46,390	—	—	—	—
2'-Me	—	—	400	27,100	—	—
3'-Me	530	23,340	400	19,130	—	—
4'-Me	530	23,750	400	20,130	—	—
2'-Ph	—	—	400	28,860	—	—
4'-Ph	555	25,220	410—415	24,610	—	—
2-MeO	(480—490	55,310) ᵃ	—	—	—	—
3'-MeO	530	21,270	400	22,080	—	—
4'-MeO	565	19,830	410	19,950	—	—
2'-Cl	—	—	410	29,610	—	—
3'-Cl	520	9,280	410	29,400	—	—
4'-Cl	530	11,070	400	26,940	—	—
2'-Br	—	—	415	37,360	—	—
3'-Br	515	7,300	410—415	27,930	—	—
4'-Br	530	10,780	410	28,470	—	—
2'-I	—	—	415	29,430	—	—
2'-CO₂H	505	30,200	—	—	—	—
3'-CO₂H	530	9,300	405	26,810	—	—
4'-CO₂H	530	Infl.	425	22,480	—	—
2'-CO₂Me	530	17,510	405	18,930	—	—
4'-Ac	—	—	435	27,760	—	—
2'-SO₃H	550	33,410	405—410	7,200	—	—
	535	34,610				
4'-SO₃H	525	14,940	420	24,850	—	—
2'-CO₂H, 2-Me	530	45,300	—	—	—	—
2'-CO₂H, 2-MeO	500—510	54,800	—	—	—	—
2'-CO₂H, 2 : 4'-Me₂	530—540	46,070	—	—	—	—
4'-CO₂H, 2-Me	530	40,940	—	—	—	—
4'-CO₂H, 3-MeO	515—520	8,980	425—430	14,400	310—315	9,770
Azobenzene	—	—	320	18,400	—	—
	(I; R = R' = Me)					
None	525	34,700	—	—	320	10,940
2'-CO₂H	515	39,900	—	—	325—330	3,980
	(I; R = Et, R' = CH₂·CH₂Cl)					
2'-CO₂H	525	36,000	—	—	—	—

(a) For a discussion of the position of this maximum see p. 1724.

group is exceptional. It has already been postulated that the 2'-carboxylic acid exists in neutral solutions in the forms (IIa—d). It would be expected that proton addition could occur quite readily to the unhindered carboxylate ion in forms (IIc and d). Similarly the methyl ester probably exists in a number of forms, including (VI), to which proton addition would proceed without difficulty.

The 2-methoxy-derivative shows a maximum at 480—490 mμ, which is at a considerably

[10] (a) Miller, Sapp, and Miller, *J. Amer. Chem. Soc.*, 1948, **70**, 3458; (b) Badger and Lewis, *J.*, 1953, 2147; (c) Badger and Buttery, *J.*, 1953, 2156.

shorter wavelength than that associated with other quinonoid forms. Since the corresponding 2-methyl derivative shows a normal maximum at 530 mμ it appears that the presence of the oxygen atom is responsible for the hypsochromic displacement of the Q-band. It is possible that the form (VII) is stabilised by hydrogen bonding as shown, thus increasing the azo-character of the absorption.

(VI) (VII)
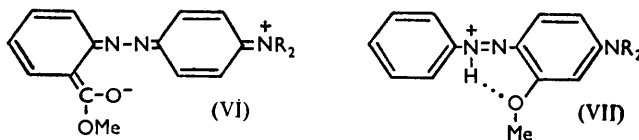
K'-Bands corresponding to form (V) have been shown in our series only by methyl-red and by 4-di-(2-chloroethyl)amino-3-methoxyazobenzene-2'-carboxylic acid. The presence of this form indicates increased basicity of the amino-group. In the first case this is due to the replacement of the chloroethyl groups by methyl groups and in the second to the steric hindrance to coplanarity of the di-(2-chloroethyl)amino-group with the conjugated system—a similar effect due to a 3-methyl substituent has been recorded.[10a]

Experimental.—The preparation of the substituted *p*-di-(2-chloroethyl)aminoazobenzenes was described in Part XVI.

Ultraviolet absorption spectra were measured at 24° by means of a Unicam Model SP 500 quartz spectrophotometer which had been adapted to have thermostatic control of the cell housing. The concentration of the azo-compound was 10^{-5}M in all cases. N/250-Ethanolic sodium hydroxide (Table 2) was prepared by diluting 4 ml. of 0·1N-aqueous sodium hydroxide to 100 ml. with ethanol, and the N/2-ethanolic hydrochloric acid (Table 3) by diluting 25 ml. of 2N-aqueous acid to 100 ml. with ethanol.

Infrared spectra were measured by using a Perkin-Elmer Model 112 spectrometer, with a rock-salt prism below 2000 cm.$^{-1}$, and one of lithium fluoride above this.