Thus cinnamal chloride undergoes solvolysis very rapidly.[9]

Of the halides in question only methylvinylcarbinyl chloride, 1,3-dichloro-1-butene and crotyl chloride display reasonably high solvolysis rates. On the other hand only the 1,3-dichloropropenes, allyl chloride and crotyl chloride show marked disposition to undergo bimolecular replacement reactions. These results lend support to the previously reported evidence[2] that the solvolytic reactions of crotyl chloride are very likely to a large degree bimolecular reactions with the solvent.

Experimental

The α- and β-1,3-Dichloropropenes.—A mixture of the two isomers kindly supplied by the Shell Chemical Corporation was separated by fractionation through a four-foot silvered, vacuum-jacketed column packed with glass helices as previously described. Properties of the isomers were as follows: α-, b. p. 103.3–103.5° (762 mm.), $n^{20}D$ 1.4690; β-, b. p. 112.0–112.3° (762 mm.), $n^{20}D$ 1.4740.

3,3-Dichloropropene.[10]—A mixture of 3,3-dichloropropene and 1,3-dichloropropene was prepared from phosphorus pentachloride and acrolein in a manner similar to that used in preparing 1,3-dichloro-1-butene.[5] The dried crude product was carefully fractionated to isolate the lower-boiling isomer. A sample of b. p. 83.0°, $n^{20}D$ 1.4510, d^{20}_4 1.175 was collected for use in this work.

Allyl Chloride.—A sample from Paragon Testing Laboratories was fractionated. A cut of b. p. 44.9–45.0, $n^{19}D$ 1.4158, was taken for use in this work.

Kinetic Studies.—The reaction rates were followed by determining the rate of disappearance of base or of production of acid as previously described.[2,5] Commercial absolute ethanol was further dried by distillation from magnesium ethoxide for use in the rate runs.

Summary

The reaction kinetics at 25° for the ethanolysis and hydrolysis of the *cis* and *trans* isomers of 1,3-dichloropropene have been studied. The reactivities of the isomers with respect to S_N2 replacement or solvolysis are closely similar in these particular reactions. The relative reactivities of certain allylic chlorides as influenced by differences in substituents attached at various positions to the allyl radical are also considered.

DAVIS, CALIFORNIA RECEIVED DECEMBER 17, 1947

(9) (a) Andrews and Linden, THIS JOURNAL, **69**, 2091 (1947); (b) Andrews, *ibid.*, **69**, 3062 (1947).

(10) Hübner and Geuther, *Ann.*, **114**, 36 (1860).

[CONTRIBUTION FROM THE MCARDLE MEMORIAL LABORATORY, MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN]

The Absorption Spectra of Certain Carcinogenic Aminoazo Dyes and the Protein-bound Derivatives Formed from These Dyes *in Vivo*[1]

BY J. A. MILLER, R. W. SAPP AND E. C. MILLER

As a group, the various C-monomethyl derivatives of 4-dimethylaminoazobenzene cover a wide range of carcinogenic activity toward the liver of the rat.[2,3] The potency of one of these compounds, 3′-methyl-4-dimethylaminoazobenzene, exceeds that of the parent dye.[2,4,5] The relative carcinogenicities[3,6] of these dyes and of several closely related compounds are as follows:

Azobenzene	0
4-Aminoazobenzene	0
4-Monomethylaminoazobenzene	6
4-Dimethylaminoazobenzene	6 (reference compound)
4′-Methyl-4-dimethylaminoazobenzene	<1
3′-Methyl-4-dimethylaminoazobenzene	10–12
2′-Methyl-4-dimethyaminoazobenzene	2–3
2-Methyl-4-dimethylaminoazobenzene	0–<1
3-Methyl-4-dimethylaminoazobenzene	0

Recently the formation of liver tumors in rats fed 4-dimethylaminoazobenzene has been found to be preceeded by and probably casually related to the formation of firmly bound compounds between azo dye metabolites of the parent dye and the liver proteins.[7] Protein-bound dyes have also been found in the livers of rats fed various C-monomethyl derivatives of this dye. Degradation of the proteins by tryptic or alkaline hydrolysis frees the bound dyes and it has been found that the major fraction of the released bound dyes can be extracted from the protein digests only by ethyl ether–ethanol. This polar property distinguishes this fraction from the dye fed and the free dyes found in the liver; hence it has been designated as the "polar bound dye."[7] The occurrence of these dyes in only microgram quantities in the liver has prevented for the time being not only their isolation and characterization but also any exact correlation of the level of bound dye produced by feeding each dye with its carcinogenic potency. Hence as an aid in further analysis the absorption spectra of 4-dimethylaminoazobenzene and its C-monomethyl derivatives in ethanol and aqueous hydrochloric acid–ethanol solution have been determined in the 215–1200 mμ region; some of these spectra have not been recorded previously. The absorption spectra of the corresponding polar bound dyes in the 400–600 mμ region are also presented. The principal absorption maxima of the acid forms of the parent and bound dyes occur

(1) This investigation was aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the National Cancer Institute, and the Wisconsin Section of the American Cancer Society.

(2) Miller and Baumann, *Cancer Research*, **5**, 227 (1945).

(3) Miller and Miller, *J. Exp. Med.*, **87**, 139 (1948).

(4) Giese, Miller and Baumann, *Cancer Research*, **5**, 337 (1945).

(5) Giese, Clayton, Miller and Baumann, *ibid.*, **6**, 679 (1946).

(6) Hartwell, "Survey of Compounds which have been Tested for Carcinogenic Activity," Federal Security Agency, United States Public Health Service, 1941.

(7) Miller and Miller, *Cancer Research*, **7**, 468 (1947).

near 520 mμ and are of considerable value in analysis.[7,8]

Experimental[9]

Preparation of Compounds.—Azobenzene,[10] m.p. 68–68.5°, was prepared from hydrazobenzene by oxidation with air. 4-Dimethylaminoazobenzene and its various C-monomethyl derivatives with the exception of the 3-methyl compound were prepared by coupling the appropriate diazotized amine with the desired tertiary amine.[2] These dyes included 4-dimethylaminoazobenzene,[11] m.p. 117–118°; 4′-methyl-4-dimethylaminoazobenzene,[11] m.p. 170.5–171°; 3′-methyl-4-dimethylaminoazobenzene,[11] m.p. 119.5–120.5°; 2′-methyl-4-dimethylaminoazobenzene,[11] m.p. 73.5–74°; and 2-methyl-4-dimethylaminoazobenzene,[12] m.p. 68–68.5°. 3-Methyl-4-dimethylaminoazobenzene[2] was prepared by the methylation of 3-methyl-4-monomethylaminoazobenzene[3] with methyl sulfate in benzene. Unlike the properties of the dye briefly reported by Hantzsch[13] our preparation was liquid at room temperature and all attempts to crystallize it have been unsuccessful. Hence the spectra of this compound are presented apart from those of the crystalline dyes. The preparations of 4-monomethylaminoazobenzene, m.p. 88–88.5°, its 3-methyl derivative, m.p. 89–90°, and 3′-methyl derivative, m.p. 109–110°, have been described recently.[3]

Solvents and Molarities of Dye Solutions.—Redistillation of commercial grain 95% ethanol yielded a sample suitable for spectroscopic work. The aqueous acid-ethanol solution (henceforth referred to as acid-alcohol) was prepared from ethanol and 7*N* hydrochloric acid in the volume ratio of 4 to 5, respectively; a liter of mixture was found to result from mixing 457.0 ml. of ethanol and 571.2 ml. of acid. This particular combination was used to correspond with previous work[7] with the bound dyes. The concentrations of the dyes in either solvent needed to obtain optical densities of 0.5 to 1.0 varied from $5 \times 10^{-4} M$ to $2 \times 10^{-5} M$. The solutions of the dyes in acid-alcohol were made just prior to use because of their instability on standing more than one or two days.

Preparation of Polar Bound Dyes.—Acid-alcohol solutions of the polar bound dyes were prepared from the liver proteins of rats fed each dye at a molar level equivalent to 0.054% of 4-dimethylaminoazobenzene in a semi-synthetic diet.[7] The rats were sacrificed after one month, the livers were freed of blood by perfusion with saline *in situ* and then homogenized in water in a Waring blendor. The particulate components (nuclei, mitochondria and microsomes) of the liver were then precipitated with calcium chloride following the method of Schneider.[14] Centrifugation at about 1600 × *g* yielded a supernatant fluid containing soluble liver proteins. The removal of the particulate matter is useful since it contains hemin-like pigments which give interfering colors in acid-alcohol. The soluble proteins were precipitated by trichloroacetic acid at a final concentration of 10%. They were then washed, extracted exhaustively with hot ethanol, and dried as described previously.[7]

The dyes bound to the proteins were released by hydrolysis in alcoholic potassium hydroxide.[7] The small quantities of non-polar bound dyes present were removed from the hydrolysates by extraction with petroleum ether and the polar bound dyes were then extracted with ethyl ether–ethanol. The solvent was removed *in vacuo* and the residue of polar bound dye was then taken up in acid-alcohol as previously described.[7] The optical densities reported here represent the dye obtained from 40 mg. of liver protein. Similar extracts from the livers of normal rats fed the diet without dye (basal extracts) were prepared and their spectra determined. The spectra of the bound dyes were corrected for the absorption given by these extracts.

Absorption Spectra Measurements.—The spectra were measured with a Beckman quartz spectrophotometer. Readings were taken up to 1200 mμ, the limit[15] of the instrument; however, all readings from 700–1200 mμ were essentially zero. The maxima near 410 and 520 mμ were determined by readings taken 2 mμ apart; the maxima in the ultraviolet region were generally determined within 1 mμ. At other points readings were taken every 5 to 10 mμ. A gradually increasing slit width from 0.02 mm. in the visible to 2 mm. at the lowest wave length was employed; thus the band widths used were generally not above 2 mμ. One cm. matched cells were used throughout; silica cells were used up to 360 mμ and corex cells at the longer wave lengths since 360 mμ was the best match point for the two kinds of cells. Readings from day to day with new solutions made from the same standards were generally reproducible to within 0.005 unit of optical den-

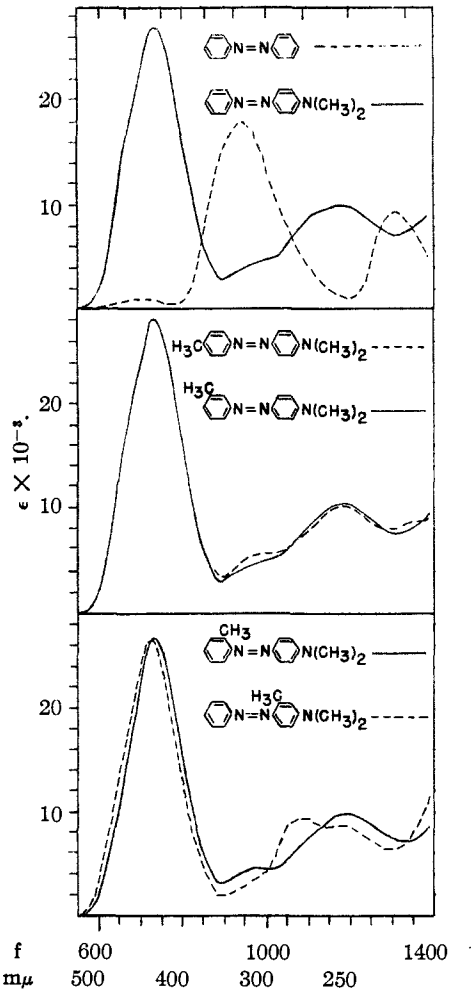

Fig. 1.—The absorption spectra of 4-dimethylaminoazobenzene and certain of its C-monomethyl derivatives in ethanol.

(8) Miller and Baumann, *ibid.*, **5**, 157, 162 (1945).

(9) All melting points corrected for stem exposure.

(10) Adkins, McElvain and Klein, "Practice of Organic Chemistry," McGraw-Hill, New York, N. Y., 1940, p. 175.

(11) Mechel and Stauffer, *Helv. Chim. Acta*, **24**, 151E (1941). These and previous authors obtained m. p.'s from 65–68° for the 2′-methyl derivative; we have also on occasion obtained this m. p. but repeated recrystallization yields a product melting within the higher range recorded here.

(12) Samelson, *Ber.*, **33**, 3479 (1900).

(13) Hantzsch, *ibid.*, **48**, 167 (1915).

(14) Schneider, *J. Biol. Chem.*, **166**, 595 (1946).

(15) Gibson and Balcom, *J. Research Nat. Bur. Standards*, **38**, 601 (1947).

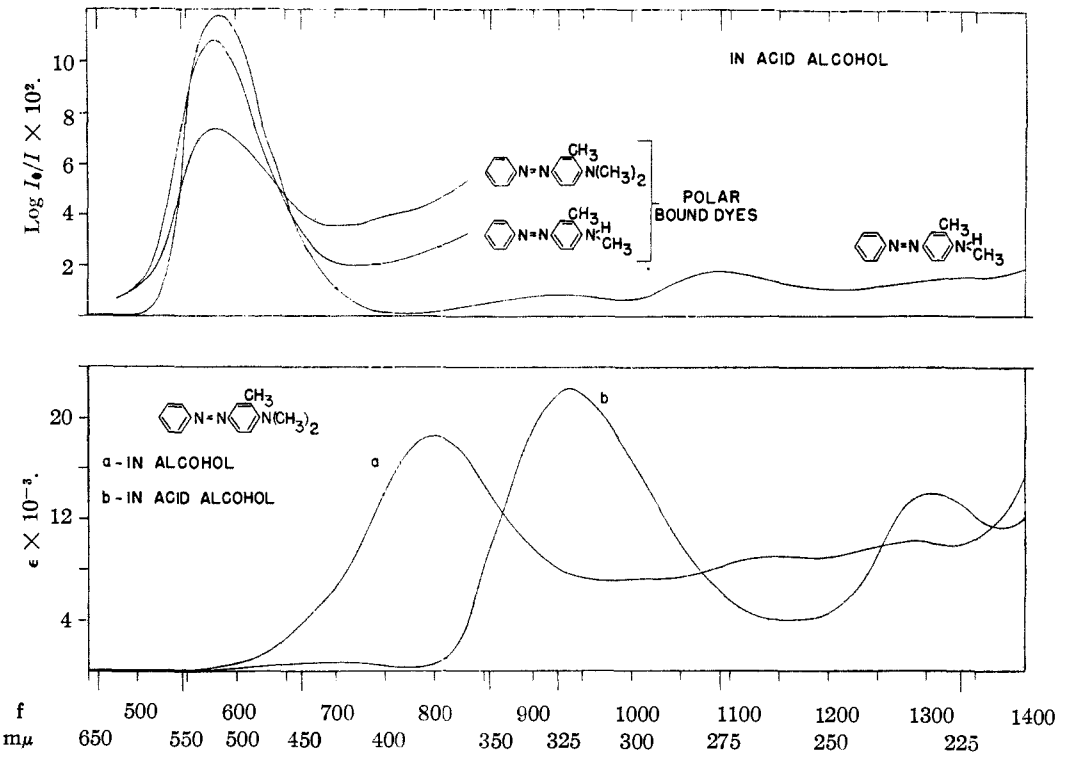

Fig. 2.—The absorption spectra of 3-methyl-4-dimethylaminoazobenzene, its N-monomethyl derivative and the protein-bound dyes formed from these dyes *in vivo*.

sity and often within 0.003 unit. The wave length scale of the instrument agreed with the 656.3 mμ H line and the reported absorption of potassium chromate in 0.05 *M* potassium hydroxide.[16] Following Brode[17] the spectra were plotted on a linear frequency scale in fresnels. The molar extinction coefficient was calculated from the equation

$$\epsilon = \frac{1}{cd} \log \frac{I_0}{I}$$

where c is the concentration in moles per liter.

Results and Discussion

Absorption Spectra of the Azo Dyes in Ethanol Solution.—Figure 1 presents the spectra of the crystalline azo dyes in ethanol. Apparently the presence of a methyl group in either the 4′; 3′ or 2′ positions in 4-dimethylaminoazobenzene does not alter the spectrum of this dye in ethanol to a significant extent. However, the 2-methyl substitution shifts the spectrum toward slightly longer wave lengths and gives rise to a small extra peak at 277.5 mμ. Among the six dimethylaminoazo dyes 3-methyl-4-dimethylaminoazobenzene possesses the most atypical spectrum in ethanol solution (Fig. 2). The dye appears to have a spectrum in this solvent intermediate between the spectra of its structural isomers and the spectrum of azobenzene.

Absorption Spectra of the Azo Dyes in Acid–Alcohol Solution.—The great change effected by adding an acid to the ethanol solutions of the crystalline aminoazo dyes is evident from a comparison of Figs. 1 and 3. In contrast the spectra of azobenzene in ethanol and acid–alcohol are very similar except for a slight increase in absorption in the latter solvent. Unlike the situation in ethanol solution the spectra of the dimethylaminoazo dyes in acid–alcohol resemble the spectrum of azobenzene in two of the three bands, although wide quantitative differences are apparent. In acid–alcohol as in ethanol solution 3-methyl-4-dimethylaminoazobenzene again has the most atypical spectrum (Fig. 2). A comparison of Figs. 2 and 3 shows that the spectra of the 3-methyl derivative and azobenzene in acid–alcohol solution resemble each other strongly. In each case symmetrical bands of approximately the same intensity occur at the same wave lengths.

The great differences between the spectra of the aminoazo dyes in ethanol and in acid–alcohol indicates that acid effects a large change in structure. Hartley[18] and Lewis and Calvin[19] have suggested that in acid solution the aminoazo dyes have in part a quinoid structure. Resonance between the quinoid form with a positive charge on the nitrogen farthest from the amino group and the azo form with the charge on the amino group is thought to account for most of the light absorption. It is interesting to examine the cases of the 2′-methyl and 3-methyl derivatives of 4-di-

(16) Hogness, Zscheile and Sidwell, *J. Phys. Chem.*, **41**, 379 1937).

(17) Brode, "Chemical Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1943, p. 193.

(18) Hartley, *J. Chem. Soc.*, 633 (1938).

(19) Lewis and Calvin, *Chem. Rev.*, **25**, 273 (1939).

methylaminoazobenzene from this standpoint. The exceptionally low absorption of the 2'-methyl derivative at 520 mμ (Fig. 3) may indicate that the proximity of the 2'-methyl group to the charged N of the azo group tends to force the system out of a plane and thereby reduces the amount of resonance.[20] The increased ε values exhibited by the 2'-derivative at 226 and 326 mμ must be due to an increase in other absorbing forms. Some of these forms are probably similar to azobenzene since in acid–alcohol each of the dimethylamino dyes attains a small maximum at the wave lengths where azobenzene absorbs in either solvent. Similarly, Fig. 2 indicates that a methyl group ortho to the dimethylamino group as in the 3-methyl derivative effectively prevents resonance in the quinoid form. A comparison of Figs. 1 and 2 also shows that the 3-methyl group almost entirely inhibits the auxochromic effect of the 4-$N(CH_3)_2$ group; hence the spectrum is nearly that of azobenzene itself.

There does not appear to be any obvious correlation relating the absorption spectra of the aminoazo dyes in either solvent with their carcinogenicity toward the rat liver. However, it is of interest that the two strongest carcinogens, 4-dimethylaminoazobenzene and 3'-methyl-4-dimethylaminoazobenzene, show the greatest over-all similarity in their absorption spectra.

Absorption Spectra of the Polar Bound Dyes.—In general it was not possible to determine the spectra of the polar bound dyes in ethanol since the extracts contained large amounts of tissue substances which absorbed strongly at the lower wave lengths. However, the strong carcinogens 4-dimethylaminoazobenzene, 3'-methyl-4-dimethylaminoazobenzene, and their N-monomethyl derivatives form sufficiently high levels of bound dyes that the spectra exhibit maxima even after subtraction of the relatively large absorption (often up to 50% of the total) of the basal extracts. The position of the maxima of these dyes, their corresponding monomethyl derivatives and of the polar bound dyes formed from either dye in each pair are listed in Table I. The maxima of the anomalous 3-methyl derivatives in acid–alcohol (Fig. 2) are also given for comparison. For each pair of dyes the spectra of the polar bound dyes in ethanol correspond most closely to the spectra of the *N-monomethyl* dye; this is also true for the spectra of the bound dyes formed from the 3-methyl dyes although here the solvent is acid–alcohol. However, the spectra of most of the polar bound dyes in acid–alcohol solution (Fig. 4) correspond more closely with the spectra of the *N-dimethyl* dyes. For the present it is considered that the atypical behavior of the 3-methyl derivatives and the bound dyes formed from these dyes points to the former correspondence as the more likely one; *i.e.*, the bound dyes may be more closely related in structure to a monomethylaminoazo dye than to a dimethylaminoazo dye. The correspondence of the spectra of most of the bound dyes and the free dimethylaminoazo dyes in acid–alcohol solution could be largely accidental and due to a shift of the absorption maxima in acid by conjugation of bonds in the unidentified polar groups with bonds in the aminoazo dye portion of the bound dyes.

(20) We have also noticed a similar situation at 500 mμ in the spectrum of 2',3-dimethyl-4-aminoazobenzene ("*o*-aminoazotoluene," a weak carcinogen) as compared to the spectrum of 4-aminoazobenzene.

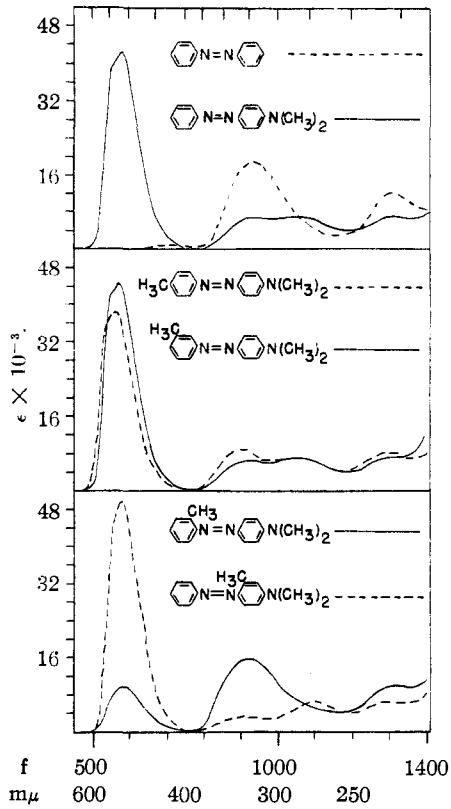

Fig. 3.—The absorption spectra of 4-dimethylaminoazobenzene and certain of its C-monomethyl derivatives in acid–alcohol.

TABLE I

THE ABSORPTION MAXIMA (IN mμ) OF SEVERAL PAIRS OF N,N-DIMETHYL AND N-MONOMETHYL AMINOAZO DYES AND THE POLAR BOUND DYES FORMED FROM THESE DYES *in Vivo*

	Solvent			
	Ethanol		Acid–alcohol	
Dye	Free dye	Bound dye	Free dye	Bound dye
DAB[a]	408	402	518	523
MAB[b]	402	402	511	523
3'-Methyl-DAB	408	400	524	525
3'-Methyl-MAB	401	400	513	524
3-Methyl-DAB	375	...	320	517
3-Methyl-MAB	404	...	514	518

[a] DAB = 4-dimethylaminoazobenzene. [b] MAB = 4-monomethylaminoazobenzene.

It is of interest that a *rough* correlation (Table II) may be made between the carcinogenicities of

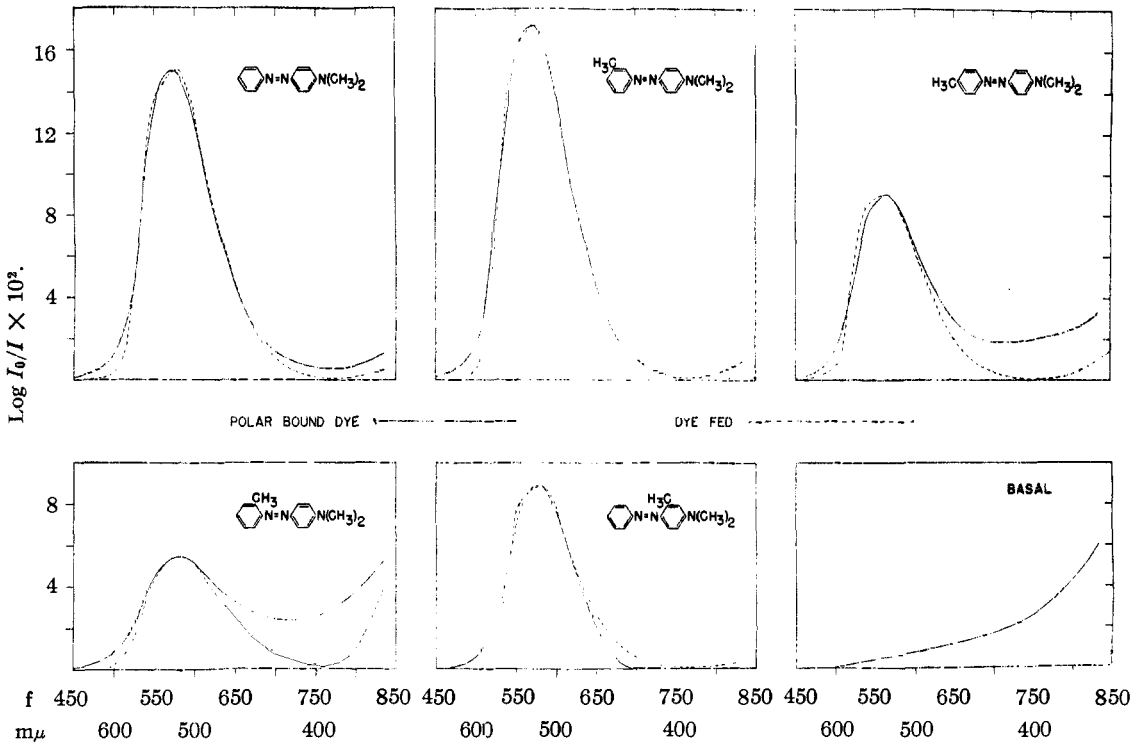

Fig. 4.—The absorption spectra in acid-alcohol of several dimethylaminoazo dyes and the protein-bound polar dyes formed from these dyes *in vivo*. The curves of the dyes fed are drawn to coincide with the curves of the bound dyes at the maxima of the latter.

the dyes fed (see introduction) and the concentrations of the resulting bound dyes in the soluble liver proteins if it is assumed that the extinction coefficients of the bound dyes bear approximately the same ratio to each other as do the parent dyes to one another. Only 2′-methyl-4-dimethylaminoazobenzene, a moderately strong carcinogen, seems to be seriously out of line, since on this basis the amount of bound dye formed by this dye would appear to exceed that of any other dye. However, the anomalous spectrum of this dye in acid indicates that the assumption made in making these comparisons may be least likely to hold for the bound dye formed from this derivative. The need for a more direct method of determining the concentration of the bound dyes in the liver proteins is obvious.

TABLE II

COMPARISON OF THE CARCINOGENICITY OF SEVERAL AMINOAZO DYES AND THE CALCULATED LEVELS OF POLAR BOUND DYE IN THE SOLUBLE PROTEINS OF THE LIVERS OF RATS FED THE DYES

Dye	Polar bound dye liberated from 40 mg. of liver protein: Optical density	"μgm."[b]	Relative carcinogenicity[a]
3′-methyl-DAB[a]	0.172	4.16	10–12
DAB	.150	3.55	6, ref. cpd.
2′-methyl-DAB	.055	5.88	2–3
4′-methyl-DAB	.090	2.50	<1
2-methyl-DAB	.090	1.92	0–<1
3-methyl-DAB	.075	1.46	0

[a] DAB = 4-dimethylaminoazobenzene. [b] The concentrations of the bound dyes are calculated on the assumption that their molar extinction coefficients are equal to those of the parent compounds. In the case of 3-methyl-DAB the comparison had to be made with the extinction coefficient of the corresponding N-monomethyl dye (see Fig. 2).

Summary

1. The absorption spectra in 95% ethanol and in 7*N* hydrochloric acid: 95% ethanol (5:4 by volume) were determined for azobenzene, 4-dimethylaminoazobenzene, 4′-methyl-4-dimethylaminoazobenzene, 3′-methyl-4-dimethylaminoazobenzene, 2′-methyl-4-dimethylaminoazobenzene, 2-methyl-4-dimethylaminoazobenzene, 3-methyl-4-dimethylaminoazobenzene and 3-methyl-4-monomethylaminoazobenzene from 215 to 1200 mμ. The spectra of the last five compounds have not been previously recorded.

2. The absorption spectra of the polar bound dyes liberated by alkaline hydrolysis from the liver proteins of rats fed any one of the aminoazo dyes were determined in acid–ethanol solution in the range 400–600 mμ. In the case of 4-dimethylaminoazobenzene, its 3′-methyl derivative, and the N-monomethyl derivatives of these dyes it was also possible to obtain the spectra of the polar bound dyes in ethanol solution.

3. The effect of a C-methyl group on the spectrum of 4-dimethylaminoazobenzene and the possible structural relationship between the parent dyes and the polar bound dyes formed *in vivo* are discussed.

RECEIVED FEBRUARY 9, 1948

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA]

Raman Spectra of Germanium Tetrachloride and Lead Tetrachloride

BY JOHN T. NEU AND WILLIAM D. GWINN

The molecules of the type XY_4 have been the subject of considerable investigation and the Raman spectra of a large number of these compounds have been measured and tabulated.[1] Professor Hildebrand[2] has for some time been especially interested in the tetrahalide molecules and has recorded that the distance from the central atom to the halogen atom plotted against the Raman frequencies ν_1 and ν_2 gives generally smooth curves as shown in Fig. 1. The ν_3 frequency for germanium tetrachloride on such a plot, however, shows a considerable deviation from what might be expected. Haun and Harkins[3] have noted a possible alternate value for ν_3, which would give a point closer to the one predicted by the graph. It was, therefore, considered worth while to repeat the determination of the Raman spectrum for germanium tetrachloride and to check the value of ν_3.

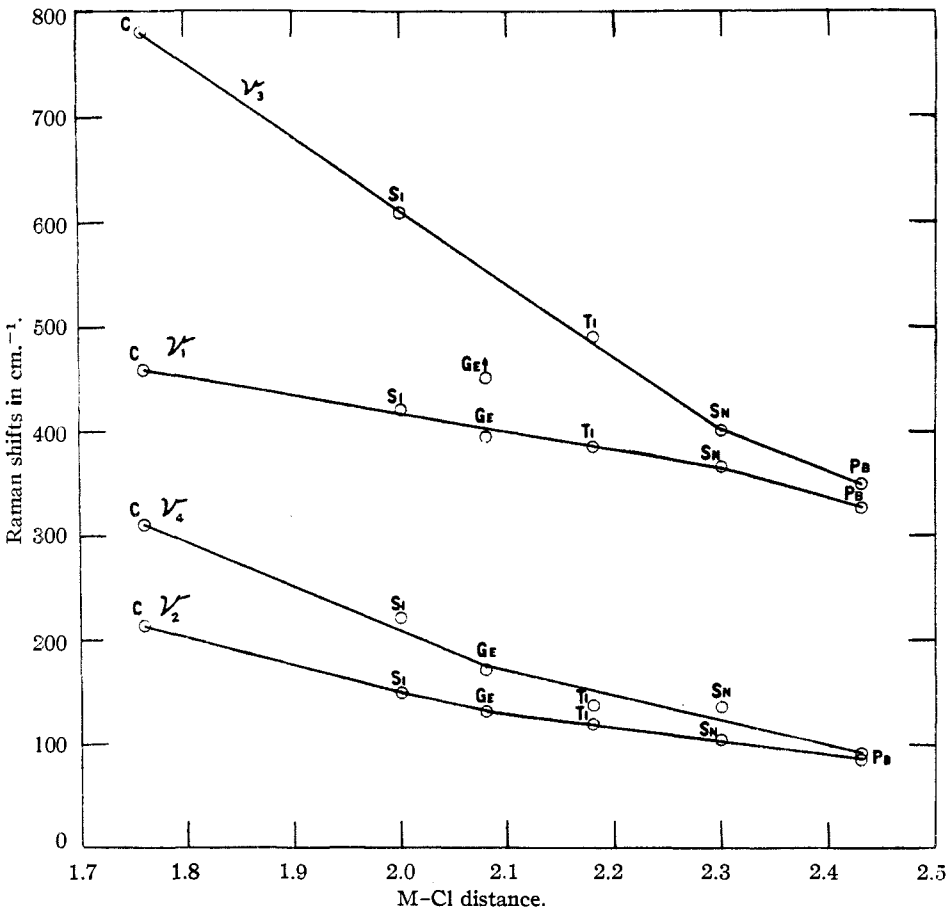

Fig. 1.—Raman frequencies plotted against central atom–chlorine distance in Å.

It also seemed possible that the above-mentioned graph might be used to predict Raman frequencies of tetrahalides where the central atom–halide distance was known. Lead tetrachloride appeared to be a suitable compound with which to verify such a prediction, and at Professor Hildebrand's suggestion, the determination was made

(1) G. Herzberg, "Infrared and Raman Spectra," D. Van Nostrand, New York, N. Y., 1945.

(2) J. H. Hildebrand, *J. Chem. Phys.*, **15**, 727 (1947).

(3) Haun and Harkins, THIS JOURNAL, **54**, 3971 (1932).