Values of K calculated from data at five wave lengths are presented in Table I. By means of standard formulas the probable error was calculated for each of the five slopes and intercepts. From an average value of the slopes, intercepts, and probable errors in those values, the probable error for the average value of K was found to be 0.249.

For each of the five solutions used in this investigation, the concentration of $CuBr^+$, x, was calculated using the average value of K. From the approximation mentioned above

$$D_1 = D_{01} - D_0$$

then

$$\epsilon_1 = \frac{D_1}{x} = \frac{D_{01} - D_0}{x}$$

Values of the molar extinction coefficient of $CuBr^+$, ϵ_1, were calculated for each of the five solutions, and the average value of ϵ_1 was found for each of the nine wave lengths. Figure 1 shows the curve obtained by plotting $\log_{10} \epsilon_1$ against wave length.

Experimentally, the work of Näsänen[3] differed from this investigation in one major respect: his solutions never contained an excess of cupric copper, and his assumption that higher complexes were negligible is less valid than is the same assumption for the present study. The results of the two investigations agree reasonably well. For solutions of similar ionic strength Näsänen reports an equilibrium constant of 3 to 4 as compared with 2.1 found in this work. In more dilute solutions he found the constant to be about 1.8.

Acknowledgment.—The author wishes to thank Professors N. R. Davidson and E. H. Swift for their helpful advice.

Pasadena, California Received August 27, 1951

[Contribution from the Fundação A. e V. Matarazzo, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brasil]

Molecular Compounds of Aminoazo Dyes and Bile Acids. II[1]

By G. Cilento

The investigation of the coördinating ability of carcinogenic aminoazo dyes for desoxycholic and cholic acids has been extended to *m'*-methyl-*p*-dimethylaminoazobenzene (I), *o*-aminoazotoluene (II) and *p*-diethylaminoazobenzene (III). The choleic acids were prepared and found to be tetracholeic acids. Phase equilibrium diagrams indicate that almost certainly (I) and (III) form an unstable 1:2 molecular compound with cholic acid. Like *p*-dimethylaminoazobenzene, (I) and (III) show quite different spectra in sodium desoxycholate and in sodium cholate solutions. The possible significance of the observed results is recorded.

In previous papers,[2,3] coördination compounds of *p*-aminoazobenzene and *p*-dimethylaminoazobenzene ("butter yellow") with desoxycholic acid were described. It was also reported[3] that none of these azo dyes forms an addition compound with cholesterol, whereas only the methylated azo dye forms a molecular compound, although a very unstable one, with cholic acid. The investigation has been extended to *m'*-methyl-*p*-dimethylaminoazobenzene, *m,o'*-dimethyl-*p*-aminoazobenzene ("*o*-aminoazotoluene") and *p*-diethylaminoazobenzene. Of these azo dyes, the first is known to be even more active than *p*-dimethylaminoazobenzene in inducing hepatomas in rats, the next only slightly active for the rat and the last devoid of any activity. Phase equilibrium diagrams of cholesterol and these aminoazo dyes were not investigated since the first systems involving this sterol showed no indication of molecular addition.[3,4]

Choleic Acids.—The choleic acids were prepared by the current procedure. No phase diagrams were worked out.

Like *p*-dimethylaminoazobenzene choleic acid, the choleic acids of the three azo dyes showed an acholic content between that expected for a tetracholeic and a hexacholeic acid. It has been again found that recrystallization from ethyl alcohol raised the percentage of the acholic component. Initially, it was thought that they also[3] may be a mixture of tetracholeic and hexacholeic acids, or that they are perhaps formed in the unusual 1:5 molar ratio. However, another explanation can be advanced, namely, that they are tetracholeic acids contaminated with the solvent, choleic acid. This explanation is supported by the striking rise in melting point following recrystallization observed for some of these choleic acids. It should be noticed that all these choleic acids were analyzed directly for their acholic content, for which reason possible contaminations would not have been masked. On these grounds, a rough estimation for the melting point of pure *m'*-methyl-*p*-dimethylaminoazobenzene- and *o*-aminoazotoluenetetracholeic acids would lead to figures still higher by 2 or 3°. The following sequence would then result: *m'*-methyl-*p*-dimethylaminoazobenzenecholeic acid (m.p. 210°), *p*-dimethylaminoazobenzenecholeic acid (m.p. 207°), *o*-aminoazotoluenecholeic acid (m.p. 200°), *p*-diethylaminoazobenzenecholeic acid (m.p. 194°) and *p*-aminoazobenzenecholeic acid (m.p. 183°). It may be further mentioned that during the preparation of these choleic acids the first two apparently crystallized faster than the others. These facts seem to suggest an increased stability in the choleic acids of carcinogenic aminoazo dyes.

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(1) From a paper presented at the 3rd Annual Meeting of the Sociedade Brasileira para o Progresso da Ciência, Belo Horizonte, November, 1951.

(2) G. Cilento, This Journal, **72**, 4272 (1950).

(3) G. Cilento, *ibid.*, **73**, 1355 (1951).

(4) A eutectic and very regular curves are depicted in the binary systems of cholesterol with anthracene, 1,2-benzanthracene or 9,10-dimethyl-1,2-benzanthracene (G. Cilento, 2nd Annual Meeting of the Sociedade Brasileira para o Progresso da Ciência, Curitiba, 1950).

Systems Involving Cholic Acid.[5]—Like *p*-dimethylaminoazobenzene, *m*′-methyl-*p*-dimethylaminoazobenzene forms a 1:2 molecular compound of incongruent melting point with cholic acids (Fig. 1). Possibly in this case the coördination compound is somewhat less unstable. The diagram for *o*-aminoazotoluene is illustrated in Fig. 2. Evidently no molecular addition takes place between this azo dye and cholic acid. However, the data depicted in Fig. 3 for the system involving *p*-diethylaminoazobenzene indicate the existence of an 1:2 compound.

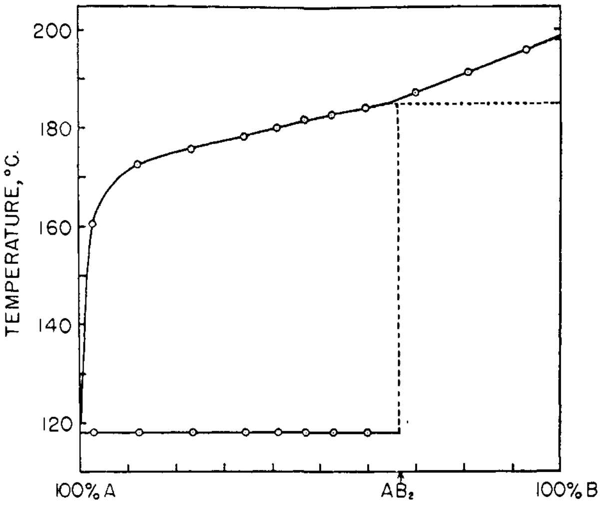

Fig. 1.—Binary system of *m*′-methyl-*p*-dimethylaminoazobenzene (A) and cholic acid (B).

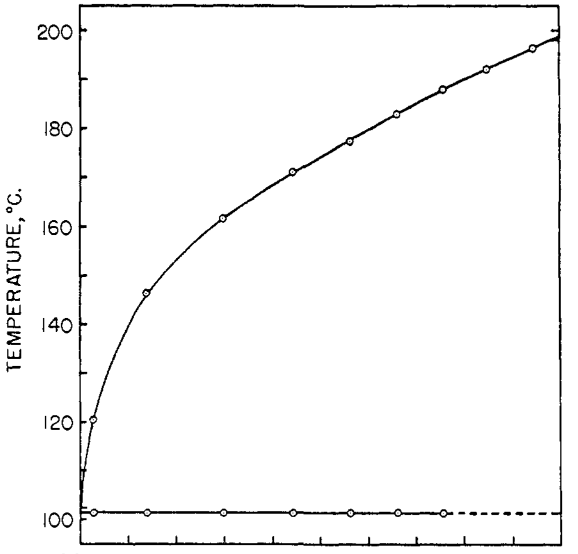

Fig. 2.—Binary system of *o*-aminoazotoluene (A) and cholic acid (B).

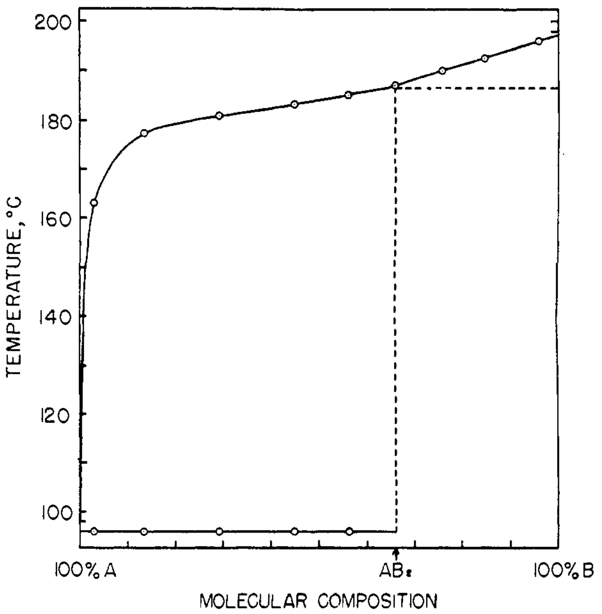

Fig. 3.—Binary system of *p*-diethylaminoazobenzene (A) and cholic acid (B).

Absorption Spectra.—The spectrum of the three azo dyes in absolute ethyl alcohol, sodium desoxycholate and sodium cholate solutions was also investigated. Considerable changes in the relative intensities, rather than in position, of the bands appear in passing from alcoholic to sodium desoxycholate solutions. One might have anticipated similar spectra in sodium cholate and sodium desoxycholate solutions. However, like *p*-dimethylaminoazobenzene, *m*′-methyl-*p*-dimethylaminoazobenzene and *p*-diethylaminoazobenzene showed different spectra in these solutions.[6] As an example the spectrum of *m*′-methyl-*p*-dimethylaminoazobenzene in these solutions is shown in Fig. 4. It is interesting to note that these N-alkylated aminoazo compounds are also the ones

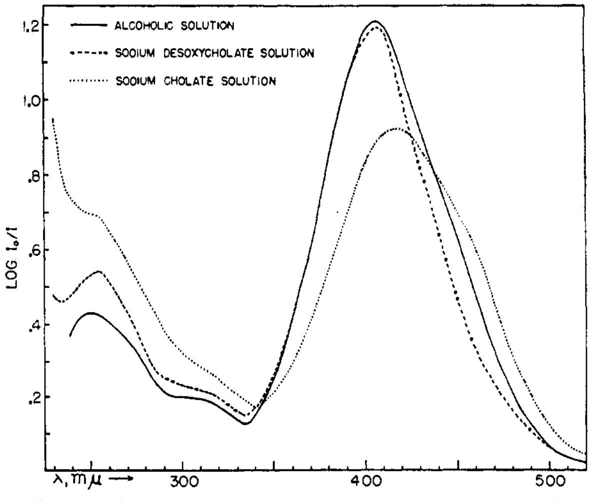

Fig. 4.—Absorption spectrum of *m*′-methyl-*p*-dimethylaminoazobenzene in different solutions (unknown concentrations of the azo dye).

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[5] Due possibly to the fact that mixtures for these systems were prepared by grinding without previous melting (*cf.* G. Cilento, reference 3), in all cases thawing points for all mixtures were coincident with the melting point of the pure azo compound. Anyway, the very fact that in some systems an inflection is present in the liquid curve whereas in others it is not, disposes of the possibility of ascribing the inflection to an enantiotropic transition of cholic acid and leaves little, if any, doubt as to the formation of a molecular compound.

[6] Evidence has been found that this result cannot be ascribed to departures from Beer's law.

TABLE I

ANALYSES OF CHOLEIC ACIDS BY LIGHT ABSORPTION

		Choleic acid soln.			Pure acholic soln.			Acholic content, %		
Choleic acid from	M.p., °C.	Concn., mg./l.	$\log_{10} I_0/I$	λ, mμ	Concn., mg./l.	$\log_{10} I_0/I$	Calcd.	Found 1:4	1:5	1:6
m'-Methyl-*p*-dimethylaminoazobenzene	200–204[a]	49.2	0.637	405	4.80	0.572	10.8			
	200–204[a]	52.4	.691	410	5.02	.611	10.8	13.2	10.8	9.2
	203–207.5[b]	30.5	.438	405	4.80	.572	12.0			
o-Aminoazotoluene	190.5–194[a]	51.0	.631	390	4.72	.523	11.1			
	190.5–194[a]	58.6	.722	390	5.88	.658	11.0	12.5	10.3	8.7
	196–198[b]	36.8	.484	390	4.72	.523	11.8			
p-Diethylaminoazobenzene	192–193.5[a]	44.9	.676	415	4.82	.602	12.0			
	192–193.5[b]	51.3	.767	415	5.02	.622	12.0	13.8	11.4	9.7
	193–194[b]	37.7	.585	415	4.82	.602	12.4			

[a] Unrecrystallized. [b] Recrystallized.

which form a molecular compound with cholic acid. It may be that these facts are indirectly related. Apart from any possible explanation, it is of considerable interest that the same compound may show a different behavior when dissolved in very similar solubilizing agents of biological origin. It may be further mentioned that a change in light absorption following irradiation has been observed for the N-alkylated aminoazo dyes in sodium desoxycholate solutions. These changes may perhaps have been produced by a photochemical *trans* ⇄ *cis* isomerization.[7]

Discussion

Any observed parallelism between carcinogenic activity and some other property in a class of carcinogenic compounds will, in general, be subject to limitations, since the cancer inducing power should be the result of several, rather than of a single, factors. Further limitation comes from our ignorance regarding possible chemical alterations in the administered compound. In the investigation of the coördinating ability of aminoazo dyes for bile acids, it has been observed that in solid–liquid phase diagrams the two azo dyes with carcinogenic activity give evidence of chemical interaction with cholic acid whereas the inactive *p*-aminoazobenzene and *o*-aminoazotoluene[8] give no indication of such a behavior at least in the region of the temperatures investigated. Furthermore, unexpected spectra were observed for the carcinogenic azo dyes in sodium cholate solution. However in both phase diagrams and light absorption investigation the inactive *p*-diethylaminoazobenzene showed a behavior similar to that of the carcinogenic derivatives. In the light of the above exposed arguments it may be pertinent to note that this molecule differs from the others in showing enlarged distortions from planarity. Thus, in marked contrast with the others, it cannot assume a completely planar configuration in the electronically excited states, where a double bond between the nitrogen atom and the ring is required.

Experimental

o-Aminoazotoluene, Eastman Kodak Co., was twice recrystallized from ethyl alcohol and once from benzene. *p*-Diethylaminoazobenzene and *m'*-methyl-*p*-dimethylaminoazobenzene were prepared according to Miller and Miller[9]; the former was recrystallized from a mixture of benzene and petroleum ether and then from ethyl alcohol whereas the latter was twice recrystallized from ethyl alcohol. The three azo dyes were dried in an Abderhalden drying apparatus and showed (in the order mentioned above) m.p. 101.4–102.6°, 96.0–97.8° and 118.0–120.0°. Desoxycholic acid (m.p. 171–173°) and cholic acid (m.p. 198–200°) were described in previous papers.[2,3]

Choleic acids were prepared by pouring a hot alcoholic solution of the azo dye (0.08 g. in 2 ml.) into a hot alcoholic solution of desoxycholic acid (1.1 g. in 4 ml.). After cooling, the choleic acids were filtered and then dried in a vacuum desiccator over calcium chloride. A sample of these choleic acids was recrystallized from absolute ethyl alcohol and dried as before. Melting points are recorded in Table I. Analyses were carried out by the method previously described[2] and are summarized in Table I. Figures in this table refer to alcoholic solutions in 1.002-cm. silica cells. In all cases the choleic acid showed practically the same spectrum of the pure azo dye. Irradiation did not show any effect upon the absorption of these solutions.

Binary solid–liquid diagrams were investigated according to Rheinboldt.[10] Incongruent melting points of the molecular compound of *m'*-methyl-*p*-dimethylaminoazobenzene and *p*-diethylaminoazobenzene with cholic acid are 185 and 186.5°, respectively.

Sodium desoxycholate and sodium cholate solutions were prepared by dissolving 1 g. of the acid in 100 ml. of 0.1 *N* sodium hydroxide. Some milliliters of each of these solutions were shaken with the azo dye in a dim room. After filtration, the spectrum was determined. When a change in absorption was observed on exposure, the spectrum was investigated after no further change was noticed on irradiation.

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[7] G. S. Hartley, *J. Chem. Soc.*, 633 (1938).

[8] *o*-Aminoazotoluene is inactive in the rat at concentrations where *p*-dimethylaminoazobenzene and its *m'*-methyl derivative are active. (J. A. Miller, R. W. Sapp and E. C. Miller, *Cancer Research*, **9**, 652 (1949)).

[9] J. A. Miller and E. C. Miller, *J. Exptl. Med.*, **87**, 139 (1948).

[10] H. Rheinboldt, *et al.*, *J. prakt. Chem.*, [2] **111**, 242 (1925); **112**, 187, 199 (1926); **113**, 348 (1926); *Ber.*, **74**, 756 (1941).