isomers were available, curves of this form were actually obtained. A similar behavior was first observed and discussed by Weitkamp.[7]

Experimental[8]

2-Methylpentanoic Acid.—Diethyl methyl-*n*-propylmalonate was prepared by alkylation, in the usual way,[9] of diethyl methylmalonate (prepared from ethyl oxalpropionate). The average yield was 83%, b.p. 108–112° (16 mm.), n^{22}_{D} 1.4210. The diester was saponified with alcoholic potassium hydroxide, alcohol was distilled, and the acidified residue was extracted with ether. The crude malonic acid (m.p. 99–103°) was decarboxylated at 175°, to give 90% yield of 2-methylpentanoic acid, b.p. 95–96° (14 mm.), n^{24}_{D} 1.4128. Hommelen[10] has reported b.p. 192–193.6°, n^{20}_{D} 1.4136.

2-Ethylbutanoic acid, b.p. 113.4–113.6° (40 mm.), was obtained in a pure state by fractionation of the commercial acid through a three-foot packed column.

2-Bromopentane.—(A) The silver salt and bromine reaction was carried out as described for preparation of tridecyl bromide[11] except that acid was removed as the sodium salt rather than the barium salt. The carbon tetrachloride solvent was flash-distilled through a three-foot packed column, keeping the mole fraction of bromide in the pot below 0.2 until all solution had been added. After an intermediate fraction, the 2-bromopentane was collected in 55–65% yield at 117.0–117.8°, n^{24}_{D} 1.4389. Stodola and Lauer[3] reported b.p. 116.4–117.4°, n^{20}_{D} 1.4414.

This reaction was also carried out in methylene dichloride, in order to avoid the tedious separation from carbon tetrachloride, but this method proved even more troublesome on account of formation of small amounts of bromodichloromethane. From a 1-mole run there was obtained 2.4 g. of material of b.p. 90.4–92°, which after redistillation had n^{20}_{D} 1.4911 (reported[12] for bromodichloromethane, b.p. 90.2°, n^{20}_{D} 1.4985).

(B) Synthetic 2-pentanol[13] was converted to the bromide by the method of Hsueh and Marvel[5] except that the final heating period was omitted; yield 82%, b.p. 117–117.4°, n^{20}_{D} 1.4413, n^{24}_{D} 1.4392.

3-Bromopentane, prepared from silver 2-ethylbutanoate by the method described for the 2-isomer, was obtained in 76% yield, b.p. 118.1–118.9°, n^{20}_{D} 1.4452. Lauer and Stodola[3] reported b.p. 117.4–118.4°, n^{20}_{D} 1.4443.

Anilides from the bromo**pentanes** were prepared by the method of Lauer and Stodola[3] except that the Grignard reaction with freshly distilled phenyl isocyanate was carried out in a three-necked test-tube fitted with mercury sealed stirrer, dropping funnel and reflux condenser. Crystallization of the anilides from aqueous methanol was as previously described except that the final addition of water was omitted.

The anilide from 2-bromopentane from the silver salt reaction, after a single crystallization, melted at 95.9–96.2°, and the best sample obtained (three crystallizations) melted at 96.0–96.4°.

The anilide from 2-bromopentane prepared from 2-pentanol, after a single crystallization, melted at 92.5–93.0°, and after three crystallizations, the m.p. was 94.7–95.6°. The best value previously reported[3] was 94.3°.

The anilide from 3-bromopentane, after a single crystallization, melted at 127.2–127.7° (best previous value[3] 125°). This anilide is polymorphic, for on removing the capillary from the m.p.-bath until crystallization sets in then immediately returning it to the bath, the substance rapidly remelts completely at temperatures down to 123.8°. Below this temperature, reversion to the original form occurred, with re-m.p. 127.4°. With the larger sample used for the binary melting point diagram, no difficulty with the lower-melting polymorph was encountered.

The binary melting point diagram was determined by the method described in detail by Cason and Winans.[6]

DEPT. OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIF.

RECEIVED JULY 5, 1950

---

[7] Weitkamp, THIS JOURNAL, **67**, 447 (1945).

[8] All melting points are corrected; all boiling points are uncorrected. All distillations, unless otherwise specified, were through a half-meter Vigreux type column with heated jacket and partial-reflux head.

[9] Adams and Kamm, "Organic Syntheses," Coll. Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 250.

[10] Hommelen, *Bull. soc. chim. Belg.*, **42**, 243 (1933).

[11] Cason and Winans, *J. Org. Chem.*, **15**, 142 (1950).

[12] Timmermans and Martin, *J. chim. phys.*, **23**, 766 (1926).

[13] Cason and Coad, THIS JOURNAL, **72**, 4695 (1950).

Molecular Compounds of Aminoazo Dyes and Bile Acids I[1]

BY G. CILENTO

This paper describes the first results of an investigation of the coördinating affinity of aminoazo dyes for biologically important compounds. Related to this research was a recent note[2] in which the preparation of *p*-aminoazobenzenecholeic acid and of its N-dimethyl derivative was reported. Interest in this subject was induced by the present views on chemical carcinogenesis.

It is a well founded hypothesis that a common feature to carcinogenic molecules is the presence of at least one region which has a high electron density and an increased ability for addition reactions.[3] Actually there is an increasing amount of evidence which suggests that the carcinogen forms, in a first step—as it may be expected on the basis of its peculiar properties—an addition complex with some tissue constituent.[3] The results of the investigation, herein reported, of the coördinating ability of *p*-aminoazobenzene and *p*-dimethylaminoazobenzene, "butter yellow," for cholesterol, desoxycholic and cholic acids are in agreement with the views mentioned above.

p-Aminoazobenzene is a border line molecule with slight, if any, carcinogenic power, whereas the dimethyl derivative is very active when orally administered in inducing hepatomas in rats. Desoxycholic acid is known easily to form molecular compounds, the so-called choleic acids, with organic derivatives. This property, however, is rarely found in cholesterol or cholic acid.[4]

The research consisted of working out the solid-liquid equilibrium diagram between the azo dye and the sterid. Application was also made of ultraviolet and visible light spectrophotometry.

It has been found that neither of the two azo dyes form mixed crystals or molecular compounds with cholesterol. The eutectic point lies at 48% (by weight) *p*-aminoazobenzene, 108.5° in one case and at 60% (by weight) *p*-dimethylaminoazobenzene, 105.5° in the other case.

Figures 1 and 2 illustrate the phase diagrams involving desoxycholic acid. As it was expected, in both cases a maximum occurs in the liquid curve which indicates molecular addition. However, due to the flat shape of these maxima, it was impossible to decide if four or six molecules of the bile acid were associated with one molecule of the azo dye, nor could additional information be obtained from the

---

[1] From a paper presented at the Second Meeting of the Sociedade Brasileira Para o Progresso da Ciência, Curitiba, November, 1950.

[2] G. Cilento, THIS JOURNAL, **72**, 4272 (1950).

[3] For a survey see P. Daudel and R. Daudel, *Biol. méd.* (*Paris*), **39**, 201 (1950).

[4] Harry Sobotka, "The Chemistry of the Sterids," The William and Wilkins Company, Baltimore, Md., 1938, p. 122.

solid curve.[5] A pentacoördination was ruled out on symmetry grounds.[6]

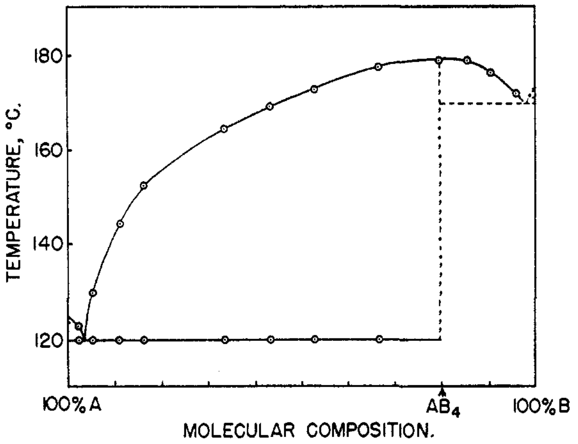

Fig. 1.—Binary system of *p*-aminoazobenzene (A) and desoxycholic acid (B).

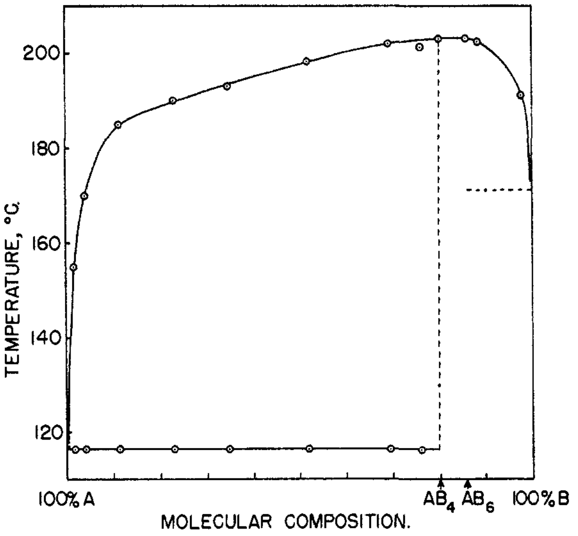

Fig. 2.—Binary system of *p*-dimethylaminoazobenzene (A) and desoxycholic acid (B).

Therefore the choleic acids were prepared and analyzed for their acholic content. It was found[2] that *p*-aminoazobenzene was associated with four molecules of the biliary acid. Unexpected results were obtained with its N-dimethyl derivative. Different samples showed a different content of the azo dye, which was always located between those expected for a tetra- and an hexacholeic, though in one case the expected percentage for an hexacholeic was obtained. It was also observed that recrystallization from ethyl alcohol raised the content of the acholic component. Therefore it was assumed that this azo dye can form both a tetra- and an hexacholeic.

Choleic acids of nitrogenous substances seem not to have been previously reported. It is interesting to note that while *p*-aminoazobenzene and its N-dimethyl derivative melt at 124.5° and 118°, respectively, their choleic acids melt at 183° and 204°. Among all the choleic acids hitherto reported only that of 1,2,5,6-dibenzanthracene melts higher (m.p. 223-224°)[7] than butter yellow choleic acid.

p-Aminoazobenzene choleic acid and its N-dimethyl derivative show, in alcoholic solution, the same spectrum as the pure azo dye. In alkaline solution the former showed a 10–12 mμ shift of the main bands toward shorter wave length making the shoulder representative of the N=N absorption more prominent. The faint shoulder at around 310 mμ disappeared. In the case of the N-dimethyl derivative no substantial shift nor disappearance of the 310 mμ shoulder was observed.

p-Aminoazobenzene and cholic acid display no ability for mutual addition at the temperatures investigated. The diagram shows a simple eutectic point (at 4.5 mole per cent. azo dye, 120°) and a very regular liquidus curve. However the observed data for the system involving the dimethyl derivative, shown in Fig. 3, indicate the existence of a molecular compound between one molecule of *p*-dimethylaminoazobenzene and two molecules of cholic acid.

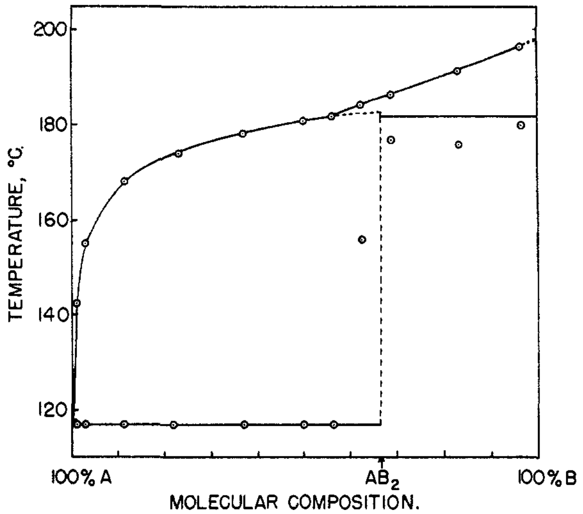

Fig. 3.—Binary system of *p*-dimethylaminoazobenzene (A) and cholic acid (B).

Owing to the existence of different polymorphic modifications of cholic acid, mixtures for the diagrams were prepared by close grinding of the components, without previous melting. Indeed, it was observed that if cholic acid (m.p. 200°) is previously melted and resolidified, it melts at around 135°, provided the sample is heated from low temperatures, otherwise it appears already melted even below 135°.

Some difficulties were found on ascertaining the solidus curve of the diagram depicted in Fig. 3. It was initially observed that all mixtures showed a thaw point coincident with the melting point of pure *p*-dimethylaminoazobenzene. Therefore the mixtures were partially melted between glass slides

---

(5) Mixtures rich in desoxycholic acid started melting at relatively low temperature and apparently resolidified again, yet, it was impossible to determine their thaw point.

(6) H. Rheinboldt, *Ann.*, **451**, 256 (1927).

(7) L. F. Fieser and M. S. Newman, THIS JOURNAL, **57**, 1602 (1935).

and after resolidification, the slides rubbed against each other. It was observed that this procedure lowered the proportion of liquid phase to that of solid phase for mixtures containing less than 60 mole per cent. cholic acid and raised by some 40–60° the thaw point of mixtures richer in cholic acid. Thaw points for the latter mixtures shown in the diagram are averages of several determinations and are of rather poor accuracy.

p-Aminoazobenzene showed in sodium cholate solution practically the same spectrum as in sodium desoxycholate solution. With the N-dimethyl derivative in sodium cholate solution considerable changes, such as a shift toward longer wave length, were observed.[8]

Experimental

p-Dimethylaminoazobenzene, cholesterol and cholic acid were several times recrystallized from ethyl alcohol. The former two were dried in vacuum and showed m.p. 117–118° and m.p. 147.5–148.5°, respectively. Cholic acid was dried in Abderhalden drying apparatus at 94° until constant weight; m.p. 198–200°; desoxycholic acid (m.p. 171–173°). *p*-Aminoazobenzene (m.p. 123.5–124.5°) and its choleic acid (m.p. 180–183°) were described in a previous note.[2] Solid–liquid equilibrium diagrams were carried out according to Rheinboldt.[9] Thaw points were determined in a microscope equipped with a heating stage. *p*-Dimethylaminoazobenzene choleic acid was repeatedly prepared by the current method[2]; m.p. 201–204°. Analyses were carried out by the method previously described.[2] Percentages were computed from the optical densities D measured in 1.002 cm. silica cells at 410 mμ, which was, approximately, the wave length of the absorption maximum of *p*-dimethylaminoazobenzene. Alcoholic solutions of this azo compound showed, in average, D/c = 0.1304, c being the concentration in mg. per liter. Analyses of various samples are summarized in Table I. Calculated percentages of azo dye for a tetra-, penta- and hexacholeic acid were 12.5, 10.3 and 8.7, respectively.

TABLE I

ANALYSES OF VARIOUS SAMPLES OF *p*-DIMETHYLAMINOAZOBENZENE CHOLEIC ACID

	Choleic acid solution		
Sample	Mg./liter	D_{410}	Acholic in choleic, %
A[a]	103.1	1.219	9.0
B[a]	97.2	1.261	9.9
C[a,b]	50.9	0.722	10.8
	51.2	0.724	10.8
C[c]	49.8	0.728	11.2
	49.2	0.722	11.2
D[c]	68.4	0.951	10.6
	102.8	1.459	10.8
E[c]	118.5	1.620	10.5
	96.6	1.364	10.8

[a] Unrecrystallized. [b] Prepared from alcoholic solutions of butter yellow and desoxycholic acid in such concentrations that even if no choleic were formed on mixing them, there would not be precipitation of any reactant upon cooling. [c] Recrystallized.

FUNDAÇÃO ANDRÉA E VIRGINIA MATARAZZO
FACULDADE DE MEDICINA
UNIVERSIDADE DE SÃO PAULO
SÃO PAULO, BRASIL

RECEIVED OCTOBER 20, 1950

---

(8) All solutions were prepared in 0.1 N sodium hydroxide and had arbitrary concentrations of azo dye and bile acid.

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

Cleavage of Dimercaptols of Acetonylacetone by Mercuric Chloride

BY R. A. DINERSTEIN

When sugar mercaptals are treated with mercuric chloride in alcohol, the sulfur precipitates as the alkylmercapto mercuric chloride, RSHgCl, and the aldehyde is regenerated.[1] That this cleavage is general for mercaptals and mercaptols has been recognized in this Laboratory. To demonstrate the effect of treating dimercaptols with mercuric chloride, the dimercaptols of acetonylacetone were prepared from three different mercaptans according to the method of Rietz, Chapman and Fernandez[2] and then treated with mercuric chloride. The precipitates were analyzed for sulfur and compared by mixing melting point with the RSHgCl compound prepared directly from the mercaptan and mercuric chloride.[3] Mixed melting points (Table I) and sulfur analyses (Table II) demonstrated that the products are identical. Thus, cleavage takes place in the reaction of dimercaptols and mercuric chloride.

TABLE I

MELTING POINTS OF RSHgCl COMPOUNDS

R	From RSH and $HgCl_2$	From dimercaptol and $HgCl_2$	Mixed	Reported by Rietz[4] for "addition product"
n-Amyl	183–184	181–182	182–183	184–185
n-Decyl	155–156	154–155	154–155	155–156
n-Dodecyl	153–154	152–153	152–153	153–154

TABLE II

SULFUR CONTENTS (% S) OF RSHgCl COMPOUNDS

R	Obsd. from RSH and $HgCl_2$	Obsd. from dimercaptol and $HgCl_2$	Calcd. for RSHgCl	Calcd. for "addition product"
n-Amyl	9.4	9.7	9.5	6.9
n-Decyl	7.7	7.6	7.8	6.9
n-Dodecyl	7.1	7.2	7.3	6.5

This conclusion differs from that of Rietz, *et al.*,[4] regarding the same reaction. In a study of the dimercaptols of acetonylacetone prepared from mercaptans containing one to twelve carbons, these authors described the "mercuric chloride addition products" derived from each member of the series. Carbon and hydrogen analyses led the authors to assume that four to eight mercuric chloride molecules were attached to each dimercaptol molecule, the exact number being a function of chain length; two cases in which this number was 4.5 were considered "anomalous."

Identification of the reaction products as the RSHgCl compounds permits a simpler explanation. Confirmation is provided by the sulfur analyses (Table II). Furthermore, the calculated carbon and hydrogen contents of the RSHgCl compounds

---

(1) H. Gilman, "Organic Chemistry," second edition, John Wiley and Sons, Inc., New York, N. Y., 1943, Vol. II, pp. 1562, 1575.

(2) E. G. Rietz, R. D. Chapman and J. B. Fernandez, THIS JOURNAL, **70**, 3486 (1948).

(3) S. Blackburn and F. Challenger, *J. Chem. Soc.*, 1872 (1938).

(4) E. G. Rietz, J. B. Fernandez, L. T. Snider and T. K. Todsen, THIS JOURNAL, **71**, 3433 (1949).