Stoichiometric Balances and Stability Constants of Amine–Bromthymol Blue Complexes

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Abstract □ The stoichiometric balances and absolute stability constants of some amines (atropine, chlorpheniramine and its maleate salt, dextromethorphan hydrobromide, methapyrilene hydrochloride, and quinine and its hydrochloride salt) were determined, using chloroform as the solvent. In chloroform, bromthymol blue was unable to replace the acid part of the amine salts. Nevertheless, the addition of water appears to help this replacement by separating the charges. The absolute stability constant values (between 2.5×10^4 and 6.0×10^{10}) indicate that the compounds formed by reacting amines with bromthymol blue are complexes in nature rather than the salts.

Keyphrases □ Amine–bromthymol blue complexes—stoichiometric balances, stability constants □ Bromthymol blue–amine complexes—stoichiometric balances, stability constants □ Stability constants—amine–bromthymol blue complexes, in chloroform

Several methods have been published for the quantitative determination of amines and quaternary ammonium ions, in which the acid dyes form color compounds extractable from the aqueous solution with organic solvents. The color in the organic solvent is measured spectrophotometrically. The work in biology was summarized by Axelrod (1), and a number of applications to pharmaceutical products were summarized by Higuchi and Bodin (2).

Gupta and Cadwallader (3) reported the stoichiometric balance of the thiamine–bromthymol blue complex. Schill (4), Gupta *et al.* (5), and Hull and Biles (6) reported the partition coefficients or equilibrium constants of these complexes between the aqueous phase and the organic solvent. In the published literature, there is little information available regarding the stoichiometric balance, the nature of the reaction, and the stability constants of these complexes. The purpose of this research was to investigate these three properties of some amine–bromthymol blue complexes. The amines tested were: atropine, chlorpheniramine and its maleate salt, dextromethorphan hydrobromide, methapyrilene hydrochloride, and quinine and its hydrochloride salt.

EXPERIMENTAL

Reagents—All chemicals and reagents used were either USP, NF, or ACS grade. Bromthymol blue was purchased[1] and used without further purification.

Preparation of Solutions—Solutions of amines and bromthymol blue (2×10^{-4} *M*) in chloroform were prepared using the simple solution method.

Determination of Stoichiometric Balance—Job's method (7), of continuous variation was used to determine the stoichiometric balance of dye to amines. Ten (10.0-), 8.0-, 6.0-, 4.0-, and 2.0-ml. quantities of the dye solution were placed in 25-ml. volumetric flasks. Two (2.0-), 4.0-, 6.0-, 8.0-, and 10.0-ml. quantities of the amine solution were mixed with the dye solution, respectively, and enough chloroform was added to bring it to volume. The absorbance value of each solution was measured at 420 nm. (using a Bausch & Lomb Spectronic 20). In each case, a blank was prepared by substituting pure chloroform for the amine solution. The results are presented in Table I. The stoichiometric balances of amines to dye were determined either by plotting absorbance values against the mole fraction of the dye or intuitively from the maximum absorbance value. For a constant total concentration of dye and amine, the complex is at its greatest concentration (indicated by maximum absorbance value) at a point where the dye and the amine are combined in the ratio in which they occur in the complex. The results are presented in Table II. Samples of curves are presented in Fig. 1.

Effect of Water on Stoichiometric Balances—Separate experiments were carried out on dextromethorphan hydrobromide and methapyrilene hydrochloride solutions to investigate the effect of water on the stoichiometric balances. A 10.0-ml. quantity of the dye solution in chloroform was mixed with 2.0 ml. of the amine salt solution in chloroform, and enough chloroform was added to bring the volume to 25.0 ml. After recording the absorbance value, 4.0 ml. of distilled water was added, and the mixture was shaken for 1 min. in a separator. The chloroform layer was collected and centrifuged at high speed for 5 min.

The absorbance of the clear solution was measured against a blank prepared by substituting pure chloroform for the amine salt solution. The changes in the absorbance values are presented in Table I, and the changes in stoichiometric balances are presented in Table II.

[1] Eastman Organic Chemicals.

Table I—Absorbance Values of Various Amine–Dye Complexes[a]

Dye–Amine Ratio	Absorbance Value of the Amine–Dye Complex When the Amine Is						
	At	Ch	ChM	DeH	MeH	Qu	QuH
5:1	0.289	0.53	0.288	0.016[b]	0.173[c]	0.558	0.283
2:1	0.566	0.942	0.499	0.018	0.289	0.931	0.496
1:1	0.832	0.792	0.597	0.01	0.339	0.877	0.592
2:3	0.579	0.532	0.475	0.012	0.274	0.588	0.458
1:2	0.573	0.532	0.493	0.012	0.323	0.588	0.506
1:5	0.287	0.266	0.282	0.012	0.216	0.305	0.281

[a] At = atropine–dye complex; Ch = chlorpheniramine–dye complex; ChM = chlorpheniramine maleate–dye complex; DeH = dextromethorphan hydrobromide–dye complex; MeH = methapyrilene hydrochloride–dye complex; Qu = quinine–dye complex; and QuH = quinine hydrochloride–dye complex. [b] The absorbance reading was 0.228 after treatment with water. [c] The absorbance reading was 0.385 after treatment with water.

Table II—Stoichiometric Balances of Various Amine–Dye Complexes[a]

Amine–Dye Complex	Stoichiometric Balance (Dye–Amine)
At	1:1
Ch	2:1
ChM	1:1
DeH	No reaction[b]
MeH	1:1[c]
Qu	2:1
QuH	1:1

[a] For the abbreviations used, see Table I, *Footnote a.* [b] The stoichiometric balance was 1:1 after treatment with water since the absorbance value increased from almost zero to 0.228 (Table I). [c] The stoichiometric balance was 2:1 after treatment with water since the absorbance value increased from 0.173 to 0.385 (Table I).

Table III—Stability Constant Values for Various Amine–Dye Complexes[a]

Dye–Amine Ratio	Stability Constant Value When the Complex Is						
	At	Ch	ChM	DeH	MeH	Qu	QuH
5:1	—[b]	5.4×10^{9}	—[b]	—[c]	2.2×10^{4}	2.2×10^{10}	—[b]
2:1	1.6×10^{6}	3.9×10^{10}	1.9×10^{5}	—[c]	2.1×10^{4}	3.0×10^{10}	2.0×10^{5}
1:1	1.4×10^{7}	6.6×10^{10}	1.6×10^{5}	—[c]	2.3×10^{4}	—[d]	1.6×10^{5}
1:2	4.9×10^{6}	6.3×10^{10}	1.8×10^{5}	—[c]	2.8×10^{4}	—[d]	2.5×10^{5}
1:5	—[b]	9.2×10^{10}	—	—[c]	2.4×10^{4}	—[d]	—[b]
2:3	9.8×10^{6}	9.3×10^{10}	2.1×10^{5}	—[c]	2.8×10^{4}	—[d]	2.0×10^{5}
Average values	6.9×10^{6}	6.0×10^{10}	1.9×10^{5}	—	2.5×10^{4}	2.6×10^{10}	2.0×10^{5}

[a] For the abbreviations used, see Table I, *Footnote a.* [b] An average value of these readings was assumed to represent 1.6×10^{-6} *M* concentration of the complex (see text). [c] No reaction. [d] It was not possible to calculate this value, since the free dye concentration turned out to be slightly below zero. This is probably an experimental error due to the assumption made under *Footnote b.*

Determination of Stability Constants—To determine the stability constants, an average of the six absorbance values of the atropine, chlorpheniramine maleate, and quinine hydrochloride complexes (Table I) when the amine–dye ratios were 1:5 and 5:1 was assumed to represent a 1.6×10^{-6} *M* concentration of the complex (the same as that of the amine or the dye, whichever is at a lower concentration). This assumption appears reasonable, since the concentration of either the amine or the dye is five times higher than the other component, and the stoichiometric balance for these complexes is 1:1 (Table II). If the stoichiometric balance was 1:2 (amine–dye), such as in chlorpheniramine and quinine, the same average absorbance value was assumed to represent only 0.8×10^{-6} *M* concentration of the complex, since the dye part is responsible for the absorbance. This assumption facilitated the determination of the concentration of the complex from the absorbance values of all other mixtures. The concentrations of either the free amine or its salt and the dye were calculated by difference. Stability constants were computed using two different formulas, depending upon the stoichiometric balance. For 1:1 complexes:

$$K = \frac{(C_c)}{(C_a)(C_d)} \qquad \text{(Eq. 1)}$$

and for 2:1 (dye–amine) complexes:

$$K = \frac{(C_c)}{(C_a)(C_d)^2} \qquad \text{(Eq. 2)}$$

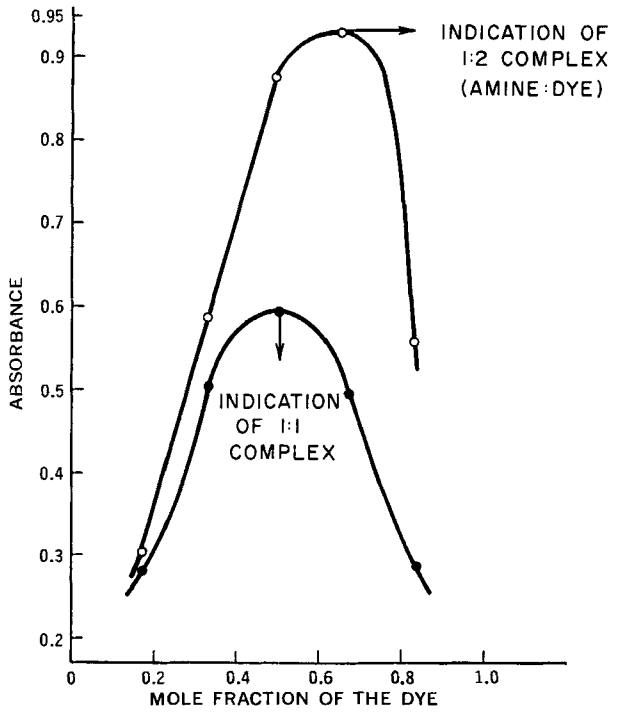

Figure 1—*Plot of absorbance values against mole fraction of the dye. Key:* ○, *quinine–dye complex; and* ●, *quinine hydrochloride–dye complex.*

where (C_c) is the concentration of the complex in moles per liter, (C_a) is the concentration of the amine or its salt in moles per liter, and (C_d) is the concentration of the dye in moles per liter. The results are presented in Table III.

DISCUSSION AND CONCLUSIONS

Stoichiometric Balances and Nature of Reaction—It is obvious that the stoichiometric balance in chloroform varies depending on whether the amine is free or in the form of its salt (Table II). For example, the stoichiometric balance of chlorpheniramine maleate or quinine hydrochloride is 1:1, while it is 2:1 (dye–amine) for free chlorpheniramine base or free quinine base. It appears, therefore, that in the case of chlorpheniramine maleate and quinine hydrochloride, only one nitrogen is occupied by the acidic group and the second nitrogen is free to react with the acidic dye to form a complex. The stoichiometric balance of free atropine base was determined to be 1:1 (Table II), since there is only one nitrogen in the base. It was not possible to conduct studies on easily available atropine salts, such as sulfate and hydrochloride, due to their insolubility in chloroform.

A salt (hydrobromide) of dextromethorphan (one nitrogen) failed to show the formation of any complex in chloroform (Table II), since the only nitrogen available to react with the dye was occupied by a hydrobromic acid group. Nevertheless, treatment with water (see *Experimental*) separated the hydrobromic acid group from dextromethorphan and the dye was able to form a 1:1 complex with the amine, as evidenced by an increase in the absorbance value from almost zero to 0.228. An absorbance value of 0.228 was not high enough to indicate complete extraction of the dextromethorphan–dye complex with chloroform, since a value of 0.285 would represent complete extraction (Table III, *Footnote b*). This was expected since some of the complex stayed in water. The concentration of the complex remaining in the aqueous phase depends upon its pH, as reported earlier (5).

Methapyrilene hydrochloride showed a stoichiometric balance of 1:1 (Table II). By assuming that one nitrogen was occupied by hydrochloric acid and the other two were free, a stoichiometric balance of 2:1 (dye–amine) would have been predicted. It appears, therefore, that one of the free nitrogens in methapyrilene hydrochloride is too weak to react. Even the other one appears to be weakly reactive, since the absorbance value of the complex was lower (Table I) as compared with other amine salts, such as chlorpheniramine maleate and quinine hydrochloride. On treating the methapyrilene hydrochloride–dye complex with water, the dye was able to replace the hydrochloric acid (as explained for dextromethorphan hydrobromide), as evidenced by an increase in the absorbance value from 0.173 to 0.385 (Table I). This increase indicated that the stoichiometric balance changed from 1:1 to 2:1 (dye–amine).

Stability Constants—The absolute stability constant values of various complexes were determined to be within the range of from 2.5×10^{4} to 6.0×10^{10} (Table III). This information may be helpful in deciding the controversial point regarding the nature of the compounds formed when amines react with acidic dyes such as bromthymol blue. Apparently, these compounds are complexes in nature rather than the salts.

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Derivatives of 10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptene and Related Compounds V: Homologous 4-Azaketones and Derivatives

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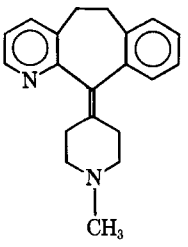
Abstract □ 6,7-Benzo-12*H*-5,6,7,12-tetrahydrocycloocta[1,2*b*]pyridine-12-one (III) and 5,6-benzo-5,6,11,12-tetrahydrocycloocta-[1,2*b*]pyridine-11-one (IV) were prepared. Wolff-Kishner reduction gave the corresponding azahydrocarbons (VII*a* and IX). Alkylation of 6,7-benzo-12*H*-5,6,7,12-tetrahydrocycloocta[1,2*b*]pyridine, using potassium amide and liquid ammonia, gave the corresponding 12-dialkylaminoalkyl derivatives (VII*b* and VII*c*). The dehydration of 12 - hydroxy - 12 - (1 - methyl - 4 - piperidyl) - 6,7 - benzo - 12*H*-5,6,7,12-tetrahydrocycloocta[1,2*b*]pyridine (V), prepared by two methods, gave 4-aza-4*b*-(1-methyl-4-piperidyl)-9,10-dihydroindeno-[1,2*a*]indene (VI). The compounds were without significant biological activity.

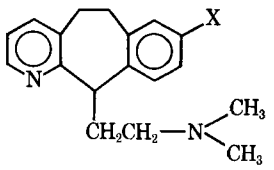
Keyphrases □ 4-Azaketones and derivatives—synthesis, screened for biological activity □ 10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptene and related compounds—synthesis of 4-azaketones and derivatives

Compounds I and II have shown very potent antianaphylactic and antihistaminic activities in laboratory animals[1] and in man (1–4). It was of interest to homologate the seven-membered ring to prepare the corresponding amino derivatives from the benzopyridocyclooctanones (III and IV). This report summarizes attempts in this area.

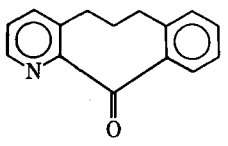
Ketone III was prepared by the intramolecular cyclization of 3-(γ-phenylpropyl)picolinic acid or its corresponding nitrile in a large excess of polyphosphoric acid. Reaction of III with the Grignard reagent, prepared from 1-methyl-4-chloropiperidine, gave the expected tertiary carbinol (V). Reductive alkylation (5), using the disodio derivative of III and 1-methyl-4-chloropiperidine, resulted in a cleaner product in excellent yield.



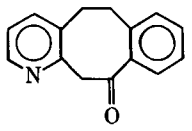
I



II*a*: X = H
II*b*: X = Cl



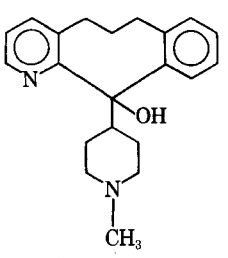
III



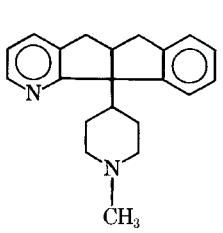
IV

All attempts at the dehydration of V to introduce the exocyclic double bond as in I failed, and the azaindenoindene derivative (VI) was isolated. The structure of VI was established by the usual physical measurements. Of special importance was the weak absorbance in the UV spectrum ($\epsilon_{270\ nm.}$ 6060) compared to that of Compound I ($\epsilon_{239\ nm.}$ 11,000, $\epsilon_{267\ nm.}$ 6100, and $\epsilon_{275\ nm.}$ 5900).

Reduction of III under modified Wolff-Kishner conditions gave the azahydrocarbon VII*a*. Alkylation of VII*a* in the presence of potassium amide gave the



V



VI

[1] For preliminary communication, see F. J. Villani, P. J. Daniels, C. A. Ellis, T. A. Mann, and K. C. Wang, in "Abstracts of the Division of Medicinal Chemistry," 124th meeting of the American Chemical Society, September 1966. A detailed publication from this laboratory is now in preparation.