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ACKNOWLEDGMENTS AND ADDRESSES

Received March 29, 1974, from the *Pharmacology and Pharmaceutics Sections, School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication June 25, 1974.

Supported by Grant 120759 from the Graduate School, University of Wisconsin.

The authors thank Dr. William E. Dennis and Dr. Donald R. Bennett of the Dow Corning Corp. for supplying the polysiloxane membrane materials and for their helpful discussions and suggestions.

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Charge-Transfer Complexes in Alkaloid Assay

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Abstract □ The intense charge-transfer UV bands in the spectra of molecular complexes of iodine with amines were utilized in a sensitive spectrophotometric assay of alkaloids. Solutions of the alkaloids in chloroform or carbon tetrachloride when mixed with iodine in the same solvent exhibited blue-shifted iodine bands in the 270–305-nm range. Owing to the high molar absorptivities of the complexes, significant increases in sensitivity, accuracy, and precision were observed upon quantitation. The method is directly applicable to the purified alkaloid-containing chloroform fractions obtained in pharmacopeial assays or isolated from plant extracts and drug formulations in the general concentration range of 10^{-5}–10^{-6} M with a relative standard deviation of 0.015. The method is particularly recommended for assaying the tropane alkaloids and other weak UV-absorbing alkaloids such as ephedrine, codeine, and sparteine.

Keyphrases □ Alkaloids—spectrophotometric assay, charge-transfer complexes □ Charge-transfer complexes—alkaloid spectrophotometric assay □ Complexes, charge transfer—alkaloid spectrophotometric assay □ Spectrophotometry—alkaloid assay, charge-transfer complexes

The decolorization of bromine and iodine by many alkaloids is well known (1–4), and colorimetric methods based upon the residual color on adding excess reagent have been developed (4). In some cases, decolorization is due to iodination of the ring, *e.g.*, epinephrine and isoproterenol (4). However, the true nature of this more general reaction as a charge-transfer complex formation between the nitrogen of the alkaloid as the n-donor and the halogen molecule as the σ-acceptor was not recognized by early investigators.

The formation of complexes between electron donors and acceptors is an important phenomenon. Many molecular complexes are colored and give rise to new absorption bands in the electronic spectra. Although a new absorption band in the UV spectra of iodine and benzene solutions was recognized early as characteristic of the molecular complex between benzene and iodine (5), only after Mulliken (6) propounded the charge-transfer theory could the various features of the spectra and other properties of such molecular complexes be understood fully.

Amines are excellent n-donors, and charge-transfer complexes of these compounds with halogens and pseudohalogens have been reported (7–11). However, emphasis was placed on the study of the complexes by various physical methods and the determination of thermodynamic constants rather than on quantitative implications. Furthermore, recent comprehensive monographs on charge-transfer complexes (10, 11) are devoid of explicit reference to alkaloid–halogen complexes.

This work is a preliminary report on charge-transfer complexes of iodine with alkaloids and their utilization in a sensitive assay of many alkaloids, particularly those with very low original absorptivities. The determination of equilibrium constants for some selected alkaloids is also described.

EXPERIMENTAL[1]

Alkaloids—Analytical reagent or pharmaceutical grade alkaloids and alkaloidal salts, which passed compendial limits and possessed the correct physical constants, were used.

[1] A constant-temperature water bath was used in the determination of equilibrium constants. Spectra were made on SP 8000 Pye-Unicam spectrophotometer.

Table I—Peak Position and Intensity for Selected Alkaloids

Alkaloid	Solvent[b]	Position and Intensity of Peak[a]						
		Complex with Iodine		Complex with Solvent		Reported (16)		
		λ, nm	($A^{1\%}_{1cm}$)	λ, nm	($A^{1\%}_{1cm}$)	λ, nm	($A^{1\%}_{1cm}$)	Solvent
Atropine	A	270	(285)	265	(35)	264	(6)	D
		410	(95)	—	—	—	—	—
	B	280	(390)			End absorption		
Scopolamine	B	270	(33)	270	(18)	257	(14)	D
N-Butylscopolammonium bromide	A	280	(1050)	265	(20)	276	(11)	D
Sparteine	C	228	(250)	230	(6)	214	(3.7)	E
		295	(150)					
Ephedrine	B	294	(60)	260	(16)	256	(11.5)	E
Codeine	B	285	(500)	292[c]	(200)	284	(52.5)	E
Quinine	A	280	(180)	285	(140)	278	(133)	E
		340	(200)	340	(165)	332	(163)	
Cinchonidine	B	280	(630)	280	(360)	—	—	
		300	(580)	300	(326)	315	(320)	E
Strychnine	A	258	(429)	258	(400)	255	(315)	D
Brucine	A	270	(440)	270	(350)	267	(302)	E
Emetine	A	293	(220)	290	(210)	285	(208)	D
Papaverine	A	285	(240)	285	(230)	284	(193)	F
		320	(155)	320	(129)	310	(253)	
Reserpine	A	—	—			267	(239)	E
		295	(480)			294	(150)	
		355	(300)			—	—	

[a] Value of A (1%, 1 cm) is the average of five determinations. [b] A = chloroform, B = carbon tetrachloride, C = cyclohexane, D = 0.1 *N* H_2SO_4, E = ethanol, and F = 0.1 *N* HCl. [c] Plateau from 292 to cutoff point (see Fig. 2).

If not available commercially, some alkaloidal free bases were prepared from the pure salts by liberation with appropriate alkali (4) followed by recrystallization, with a purity check by melting-point determination and TLC. Alternatively, an amount of the salt corresponding to the required weight of the free base was treated with alkali and quantitatively extracted with chloroform. The washed and dried (anhydrous sodium sulfate) extract was diluted to the required concentration.

Reagents—Resublimed analytical reagent grade iodine was sublimed again under nitrogen and kept over phosphorus pentoxide. The following spectral grade solvents were utilized: cyclohexane, chloroform (ethanol free), and carbon tetrachloride.

Qualitative Screening for Charge-Transfer Complex Formation—One milliliter of the alkaloid solution in chloroform (1 mg/ml) was mixed at room temperature with an equal volume of iodine solution in chloroform (0.1 mg/ml), and the effect was observed. The mixture was diluted to 50 ml with the same solvent and then warmed to 45–50°, and the effects of dilution and heat upon reversion of color back to violet were noted.

Molecular Ratio of Reactants in Complex—The Job method of continuous variation (12) was employed. With atropine as a model, master solutions of the base [0.145 mg (0.0005 mole)/ml] and iodine [0.127 mg (0.0005 mole)/ml] were prepared in chloroform. A series of 10-ml quantities of mixtures of the master solutions of atropine and iodine was made up comprising complementary proportions of the two solutions (0:10, 1:9, ... 9:1, 10:0) in 10-ml volumetric flasks. The mixtures were allowed to stand in a thermostated water bath at 20 ± 0.5° for 30 min. After this period, the absorbances of the solutions were measured at 270 nm.

A similar experiment was performed for sparteine in cyclohexane using a master solution containing 0.117 mg base/ml and measurement at 295 nm.

Determination of Molar Absorptivity and Association Constant—*Rose–Drago (13) Plot*—A series of alkaloid solutions in chloroform was prepared (1.5, 3.0, 4.5, and 6.0 × 10^{-4} *M*). These solutions and an iodine solution in chloroform (4 × 10^{-5} *M*) were placed in a thermostated water bath at 20 ± 0.5° for 30 min. Five milliliters of each alkaloid solution was mixed with an equal volume of the iodine solution. The mixture was quickly placed in a 1-cm quartz cell and the absorbance was read at λ_{AD}, the wavelength of maximum absorption of the complex, previously determined for the alkaloid (*e.g.*, 270 nm for atropine).

The same experiment was repeated in carbon tetrachloride with measurement at the proper λ_{AD} (280 nm for atropine). In each case, the values computed from the Rose–Drago (13) equation (Eq. 1) for each set of solutions were used to plot a curve of $(K_{AD}{}^c)^{-1}$ *versus* random values of $\epsilon_{AD}{}^\lambda$ (20,000-30,000 for atropine, 40,000–50,000 for brucine, *etc.*):

$$(K_{AD}{}^c)^{-1} = \frac{A}{\epsilon_{AD}{}^\lambda} - a - d + \frac{ad}{A}\epsilon_{AD}{}^\lambda \qquad \text{(Eq. 1)}$$

where:

$(K_{AD}{}^c)^{-1}$ = reciprocal of association constant of charge-transfer complex at 20°
A = absorbance value at concentration (d)
$\epsilon_{AD}{}^\lambda$ = molar absorptivity of charge-transfer complex
a = concentration of acceptor (0.00004 *M*)
d = concentration of donor, moles liter^{-1}

Benesi–Hildebrand (14) Plot—The values of A, a, and d obtained in the previous experiment were utilized to plot $1/d$ values *versus* a/A using the Benesi–Hildebrand (14) equation:

$$(a/A) = [(1/K_{AD}{}^c \cdot \epsilon_{AD}{}^\lambda)\cdot(1/d)] + (1/\epsilon_{AD}{}^\lambda) \qquad \text{(Eq. 2)}$$

The intercept with the ordinate gives $(\epsilon_{AD}{}^\lambda)^{-1}$ and the gradient is equal to $[(\epsilon_{AD}{}^\lambda)(K_{AD}{}^c)]^{-1}$.

Nagakura's (15) Formula—When there was overlapping absorption of the alkaloid with the complex, Nagakura's (15) formula was used to evaluate $K_{AD}{}^c$:

$$K_{AD}{}^c = [d(A_0 - A') + d'(A - A_0)]/[dd'(A' - A)] \qquad \text{(Eq. 3)}$$

where A_0, A, and A' are the absorbances measured at λ_{AD} of solutions whose concentrations of the acceptor are equal and whose donor concentrations are zero, d, and d', respectively.

Quantitative Analysis—The A values (1%, 1 cm) of the alkaloid in the complex are first evaluated from the absorbance values of solutions containing definite amounts of the alkaloid in the selected solvent mixed with iodine solution in the same solvent. The obtained A values (1%, 1 cm) are utilized for computation of the concentration of unknown solutions whose absorbance is determined. Alternatively, standard curves can be constructed by plotting observed absorbance values *versus* the volume taken of equimolar concentrations of the various alkaloids (5 × 10^{-4} *M*) mixed with the iodine solution (4 × 10^{-4} *M*). In all cases, Beer's law held for the system.

Assay of Alkaloids—For assay of alkaloids in crude drugs, galenicals, isolates, principles, and pharmaceutical preparations, the appropriate methods of preparing the sample, liberation of the base, solvent extraction, and purification are first performed fol-

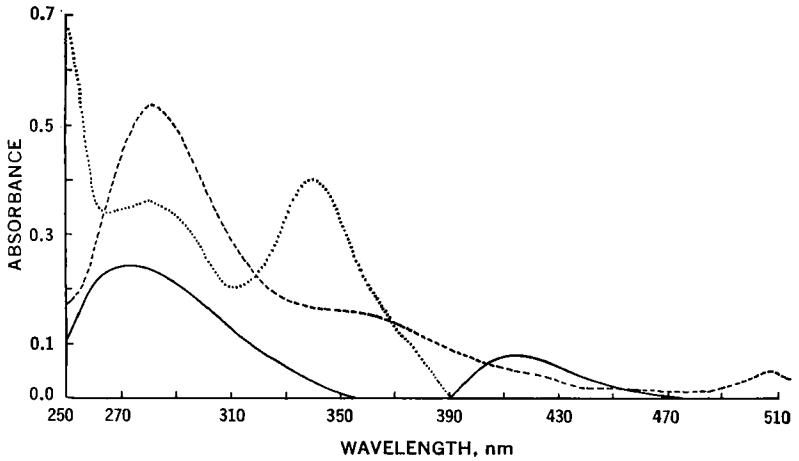

Figure 1—*Absorption spectra of charge-transfer complexes of some alkaloids with iodine in chloroform (iodine concentration = 4 × 10^{-4} M). Key: ———, atropine, 2 × 10^{-4} M; – – – – –, N-butylscopolammonium bromide, 1.2 × 10^{-5} M; and · · · · · ·, quinine, 3.2 × 10^{-4} M.*

lowing standard procedures (4, 16, 17) or compendial directions. The final determination of the alkaloidal content is then made by mixing the properly diluted chloroform solution of the alkaloid (10^{-5}–10^{-4} *M*, corresponding to 2–100 μg/ml depending on the molecular weight) with the iodine solution in chloroform (4 × 10^{-4} *M*) and measuring at the wavelength of maximum absorption of the charge-transfer complex previously determined using the pure alkaloid. The concentration of the alkaloid in the preparation is then calculated from the *A* values (1%, 1 cm) by comparison with a standard or from a calibration curve constructed in the usual manner.

RESULTS AND DISCUSSION

Qualitative Screening for Charge-Transfer Complex Formation—The immediate change of the violet color of iodine in chloroform to lemon yellow or yellowish purple upon reaction with alkaloids was taken as suggestive of charge-transfer complex formation which justified scanning in the UV range for the new band. The complex formation is distinguished from other slow oxidation or substitution reactions of the halogen with alkaloids by being practically instantaneous in analogy to ionic reactions (10).

Further confirmation of the charge-transfer nature of the reaction was obtained on extracting the alkaloid from the complex by shaking with aqueous mineral acid, whereby the violet color of iodine in chloroform was restored. The following compounds were tested and gave positive response: atropine, hyoscyamine, *N*-butylscopolammonium bromide, hyoscyamine hydrobromide, homatropine hydrobromide, ephedrine, codeine, morphine, papaverine, papaverine hydrochloride, quinine, cinchonidine, strychnine, brucine, emetine, and pilocarpine. Negative visual response was obtained with scopolamine, colchicine, and 8-hydroxyquinoline (oxine).

With compounds showing positive response, the degree of stability of the charge-transfer complex varied, as suggested from the extent of reversion of the violet color of iodine upon dilution and warming to 50°. Emetine, strychnine, and, to a lesser extent, quinine and codeine gave the most stable complexes which resisted dissociation on dilution and heating.

Failure of colchicine to give a response confirms that a sufficiently basic nitrogen is necessary to sustain the charge-transfer complex, since the nitrogen of this alkaloid is in amide linkage. Engagement of the lone pair of the nitrogen in hydrogen bonding as in oxine leads to the same effect. In the latter case, however, the negative response may also be due to the steric hindrance.

The apparent negative response of scopolamine was found to be only visual, since instrumental scanning showed a positive but weak response. The weak reactivity of this compound may be explained on the basis of steric hindrance by the epoxide bridge in scopolamine to the approach of the bulky iodine molecule for complex formation with the nitrogen.

The positive response of the hydrohalide salts of some alkaloids is at first sight paradoxical owing to the engagement of the nitrogen lone pair responsible for the charge-transfer complex formation with the proton of the acid. However, the positive response may be readily explained on the basis of interaction of iodine with the halide anion in the salt, leading to a trihalide complex.

Peak Position and Intensity with Various Alkaloids—The results for a number of selected alkaloids are shown in Table I, and some are illustrated in Figs. 1 and 2. The results are to be compared with reported data (16) for the same compound in ethanol or 0.1 *N* acid, as shown in the last column of Table I.

It is clear from Table I that the *A* values (1%, 1 cm) for the iodine-complexed alkaloids are much higher than those for the uncomplexed compounds in ethanol or acid. This result is due to the strong charge-transfer band of the complex which is weak or may be absent in the spectrum of the donor and exists in the visible spectrum of iodine (10). The outcome is an overall increase in the *A* values (1%, 1 cm) from a high of about 100 times for *N*-butylscopolammonium bromide to a low of 1.1 times the uncomplexed value, as in the case of emetine.

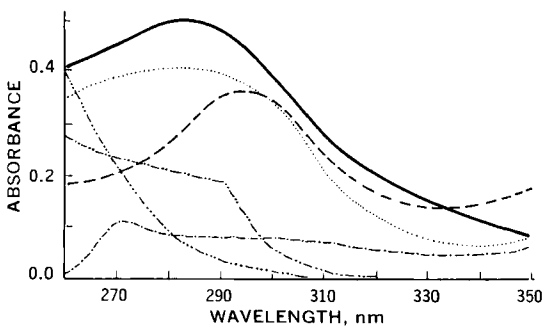

Figure 2—*Absorption spectra of charge-transfer complexes of some alkaloids with iodine in carbon tetrachloride. Key: ———, codeine, 1 × 10^{-4} M; – – – – –, ephedrine, 1.6 × 10^{-3} M; · · · · · ·, atropine, 2.4 × 10^{-4} M; – · – · –, scopolamine, 1.2 × 10^{-3} M; – · · – · · –, codeine, same concentration without iodine; and – · · · – · · · –, atropine, same concentration without iodine.*

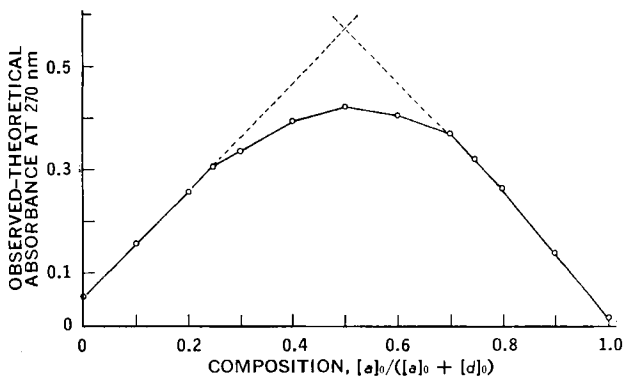

Figure 3—*Continuous variation curve for atropine–iodine complex in chloroform.*

Table II—Electrical Properties of Some Solvents (24)

Solvent	Specific Conductance, $ohm^{-1}\ cm^{-1}$	Dielectric Constant	Dipole Moment, Debye Units, 10^{-18} esu
Chloroform	1×10^{-10}	4.8	1.15
Carbon tetrachloride	4×10^{-18}	2.24	0.0
Cyclohexane	7×10^{-18}	2.02	0.0
Hexane	1×10^{-16}	1.88	0.85
Benzene	4.4×10^{-17}	2.28	0.0
Ether	3×10^{-16}	4.33	1.15
Ethanol	1.35×10^{-9}	24.55	1.66

More interesting is the increased A values (1%, 1 cm) of the alkaloids in the polyhalogenated solvents (without iodine) over the values in ethanol or 0.1 N acid. This finding was taken as suggestive of charge-transfer complex formation between the alkaloidal bases as donors and the polyhalogenated solvents as acceptors. This is in line with reported data (18, 19) concerning the contact charge-transfer pairs of amines with halomethanes, which are characterized by low association constants (20).

On the basis of these findings, it may be suggested that the high solubilities of the relatively polar alkaloidal molecules in these essentially "fat" solvents may be due to charge-transfer interactions. Electron donor–acceptor interaction is recognized as one important intermolecular force contributing to the power of dissolution of many solvents (21). In support of this assumption, the high solubilities of alkaloids in these low conductance, low dielectric constant solvents are to be compared with the lower solubilities of the same compounds in other organic solvents such as hexane, cyclohexane, benzene, and ether (16, 22, 23) which possess comparable electrical properties (Tables II and III). More striking is the high solubilities of alkaloids and some alkaloidal salts in chloroform over their solubility in the essentially more polar ethanol.

Contact pairs with solvent may also explain the fading of the charge-transfer complex color with some alkaloids on dilution with chloroform, because the solvent in massive amounts will compete with iodine as an acceptor in spite of the low stability of the solvent complex.

Alkaloid–Iodine Ratio in Complex—When using atropine as a model, the application of the Job method of continuous variation (12) indicated a 1:1 donor–acceptor ratio (Fig. 3). This finding was anticipated on the basis of the presence of one donating nitrogen in atropine. In the case of sparteine, with two identical nitrogen atoms, a 2:1 donor–acceptor ratio was found.

Molar Absorptivities and Association Constants—These were evaluated at 20° utilizing the well-established Rose–Drago (Eq. 1) (10, 11, 13) and Benesi–Hildebrand (Eq. 2) (10, 14) plots. The graphs for atropine are illustrated in Figs. 4 and 5, respectively.

From these curves the value of $\epsilon_{AD}{}^{\lambda}$ for the atropine–iodine complex at 20° was found to be 24,400 ± 120 in chloroform. Plots

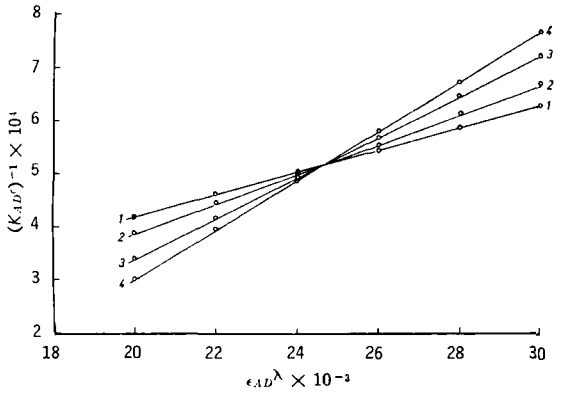

Figure 4—*Graphical solution of Rose–Drago equation (Eq. 1) for a series of atropine–iodine solutions in chloroform (iodine concentration = 4×10^{-5} M). Key (atropine concentration): Set 1, 1.5; Set 2, 3.0; Set 3, 4.5; and Set 4, 6.0×10^{-4} M.*

Table III—Solubilities of Selected Alkaloids in Some Solvents (16)

Alkaloid	Solubility in Chloroform	Ethanol	Ether
Atropine	1:1	1:3	1:60
Quinine	1:1.6	1:17	1:70
Strychnine	1:6	1:250	1:5500
Codeine	1:2	1:2	1:50
Reserpine	1:6	1:2000	Insoluble
Papaverine hydrochloride	1:10	1:120	Insoluble
Hyoscyamine hydrobromide	1:1.7	1:2.5	Very slightly soluble

involving solutions in carbon tetrachloride suggested a higher value of 26,700 ± 160 at 20°.

The association constant, K_{AD^c}, extrapolated from the same plots was also higher in carbon tetrachloride, being 2300 ± 50 liters $mole^{-1}$, and lower in chloroform, 1920 ± 30 liters $mole^{-1}$, at 20° for atropine. These data are in line with the values of association constants of other charge-transfer complexes previously reported (10, 11) in these two solvents which are generally higher in carbon tetrachloride. Similar plots gave a value of $\epsilon_{AD}{}^{\lambda}$ of 45,000 ± 200 and K_{AD^c} of 4430 ± 140 liters $mole^{-1}$ at 20° for *N*-butylscopolammonium bromide in chloroform.

When there is significant contribution at λ_{AD} by the absorption of the alkaloid, as is the case with the strong UV absorbers quinine and brucine, values of K_{AD^c} were evaluated from Nagakura's (15) formula (Eq. 3). Thus, the values for quinine are $\epsilon_{AD}{}^{\lambda}$ of 38,200 ± 200 and K_{AD^c} of 2460 ± 50 liters $mole^{-1}$ at 20° in chloroform, while for brucine $\epsilon_{AD}{}^{\lambda}$ was found to be 48,000 ± 220 and K_{AD^c} 4100 ± 100 liters $mole^{-1}$ at 20° in chloroform.

These relatively high values of association constants compare with the reported values for analogous complexes, *e.g.*, triethylamine–iodine with a K_{AD^c} of 5100 ± 130 at 25° (13) and tri-*n*-butylamine–iodine with a K_{AD^c} of 1600 at 20° (10, 11). The high values are common to complexes between σ-acceptors, such as iodine, and *n*-donors where the intermolecular overlap may be considerable.

Quantitation and Linearity of Beer's Plot—In the determination of the association constant and the molar absorptivity by the Benesi–Hildebrand plot, the equation is valid only when $[d] \gg [a]$. This may lead to anomalies and apparent deviations from Beer's law by the complex species (10). The effect of large donor concentration, expressed in the Benesi–Hildebrand equation, will have its counterpart in solutions where $[d] \ll [a]$. Consequently, the optimum conditions to minimize these deviations will be when $[d] \approx [a]$. It is under such conditions that the anomalies disappear. For this reason, in quantitative work the concentration of iodine was kept in the same order of magnitude as that of the alkaloid. A typical case is the 5×10^{-4} *M* iodine solution and 3×10^{-4} *M* atro-

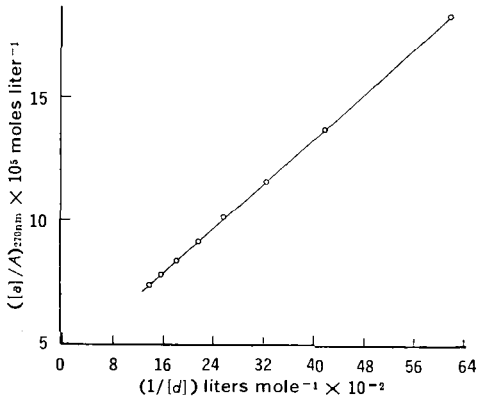

Figure 5—*Benesi–Hildebrand plot for atropine–iodine complex in chloroform at 270 nm. The donor concentration [d] was 1.6, 2.4, 3.2, 4.0, 4.8, 6.0, or 7.2 $\times 10^{-4}$ M. The acceptor concentration [a] was 2×10^{-5} M. The A_{270nm} was the absorbance of the complex at λ_{AD} (270 nm).*

pine solution in chloroform. Under such conditions, perfectly linear Beer's plots were obtained and were quite suitable for quantitation.

Accuracy and Precision—Standard solutions of the alkaloids prepared from the free base, or by quantitative recovery from the salts, when subjected to this procedure afforded results that were accurate to within 1.3% of the true values and were reproducible to ±1.5% at 95% confidence limits.

Assay of Alkaloids—The application of the charge-transfer method to various alkaloidal preparations following preliminary separation by standard and compendial methods afforded data of the same accuracy and precision stipulated. This justifies the adoption of the method to substitute ordinary spectrophotometric assays in routine alkaloidal analysis, since the extent of extra experimental work is negligible while sensitivity is greatly improved. The method is particularly attractive when the alkaloid concerned is an originally weak UV absorber. Thus, significantly improved recoveries were obtained in the assay of preparations containing atropine and other tropane alkaloids, *N*-butylscopolammonium bromide, ephedrine, and codeine.

Limitations—The same limitations of the direct spectrophotometric measurement of alkaloids (4) also apply in this method. Good recoveries are dependent upon the skill in the isolation of the free alkaloid from the preparation. However, an added advantage over the direct method, which balances some of these limitations, is the enhanced sensitivity, accuracy, and precision owing to the high molar absorptivities of the charge-transfer complexes.

SUMMARY

The intense charge-transfer UV bands in the spectra of molecular complexes of iodine with amines were utilized in a sensitive spectrophotometric assay of alkaloids. Solutions of the alkaloids in chloroform or carbon tetrachloride, when mixed with iodine in the same solvent, exhibited blue-shifted iodine bands in the 270–305-nm range.

Using the Job method of continuous variation, the molar ratio of iodine–alkaloid in the complex was found to be 1:1 with monobasic compounds and 1:2 with dibasic compounds.

Molar absorptivities of the charge-transfer complexes, $\epsilon_{AD}{}^{\lambda}$, for selected alkaloids ranged from 24,400 to 48,000, as determined by the Rose–Drago and Benesi–Hildebrand plots. Association constants of the complexes, $K_{AD}{}^{c}$, extrapolated from the same plots or computed from Nagakura's formula, varied from a low of 1920 ± 30 liters mole^{-1} for atropine in chloroform at 20° to a high of 4100 ± 100 liters mole^{-1} for brucine in chloroform at 20° and were solvent dependent, being higher in carbon tetrachloride (2300 ± 50 liters mole^{-1} for atropine at 20°).

The data obtained suggest the formation of similar but weak charge-transfer contact pairs between the alkaloids and the polyhalogen solvents. These weak complexes may account for the presence of moderate absorption maxima in the spectra of alkaloids in chloroform and carbon tetrachloride, which are weak or absent in ethanol, and for the high solubilities of the basic alkaloids in these low conductance solvents as compared to ethanol.

The original *A* values (1%, 1 cm) of the uncomplexed alkaloids showed an increase in the charge-transfer band ranging from a high of 50 times the original value (tropane alkaloids) to a low of 1.2 times (quinine and emetine), with red shift of the alkaloid band in most cases. This increase in absorbance was reflected in proportionate increases in sensitivity, accuracy, and precision upon quantitation.

The method is directly applicable to the purified alkaloid-containing chloroform fractions obtained in pharmacopeial assays or isolated from plant extracts and drug formulations in the general concentration range of 10^{-4}–10^{-5} *M* with a relative standard deviation of 0.015. The method is particularly recommended for assaying the tropane alkaloids and other weak UV-absorbing alkaloids such as ephedrine, codeine, and sparteine.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 25, 1974, from the *Faculty of Pharmacy, University of Assiut, Assiut, Egypt*.

Accepted for publication June 20, 1974.

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