mine content, depending on sample position within the bale (Table V). This finding demonstrates the difficulty in obtaining a representative sample from a consignment.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 14, 1974, from the *Department of Pharmacy, University of Queensland, St. Lucia, Brisbane, Queensland, Australia.*

Accepted for publication March 4, 1975.

The authors acknowledge the gift of a commercial bale of *Duboisia* leaves by G. R. Davis, Hornsby, N. S. W., and assistance of their colleague W. R. Owen in furnishing computer programs for the calculation of regression equations (LREG3) and the prediction of x values and tolerance limits from known regression lines (LRIP).

[x] To whom inquiries should be directed.

Solubilization and Stabilization of the Cytotoxic Agent Coralyne

M. J. CHO [*§], A. J. REPTA [*x], C. C. CHENG [‡], K. Y. ZEE-CHENG [‡], T. HIGUCHI [*], and I. H. PITMAN [*]

Abstract □ Kinetic studies were carried out on the ring opening of the quaternary nitrogen cation, coralynium ion (I), to yield 6′-acetylpapaverine (III), on the cyclization of III to yield I, and on a photochemical reaction undergone by I in aqueous solutions exposed to visible light. From the results, it was concluded that: (*a*) I and III are in facile equilibrium in aqueous solution but appreciable amounts of III do not exist in dilute solutions with pH values below 10; (*b*) the photochemical reaction of I in water (presumably a photohydration) can be reversed by lyophilization, by heating, and by increasing the pH of solutions to values greater than 12; (*c*) the photochemical reaction of I can be inhibited by protecting the aqueous solutions from visible light, and the rate in the presence of light can be reduced by increasing the concentration of I in the solution; and (*d*) although the chloride and sulfoacetate salts of I react identically and have similar solubilities in water, it is possible to prepare more concentrated and, hence, more stable solutions of the sulfoacetate salt by including sodium hydroxide in the solvent. The solubility of coralyne chloride remains about the same in dilute sodium hydroxide as in water.

Keyphrases □ Coralyne salts—photohydration, ring cleavage, pH dependence of solubility, stabilization, formation of 6′-acetylpapaverine □ Cytotoxic agents—coralyne salts, solubilization and stabilization, photohydration, pH dependence, formation of 6′-acetylpapaverine □ 6′-Acetylpapaverine—kinetics of formation from coralynium ion, cyclization □ Solubility—coralyne chloride and sulfoacetate, pH dependence

Coralyne chloride (I*a*) is a berbinium salt possessing antileukemic activity against both the P-388 and L-1210 strains (1, 2). However, the clinical evaluation of I*a* has been hampered by the relatively high expected dose of 1-2 g[1] together with the rather low aqueous solubility of about 5 mg/ml and a report[2] that solutions of the drug stored under different conditions or for varying times exhibited altered spectral characteristics relative to freshly prepared solutions.

This study was undertaken to determine the stability of coralynium ion (I) in aqueous solution with respect to chemical transformation. Previous reports led to the expectation that the most likely degradation pathways would be a ring opening to yield 6′-acetylpapaverine (III) (3) and a covalent hydration reaction (4). Although these reactions were postulated, no quantitative information about reaction rates was reported.

An investigation into the chemical stability and solubility of coralyne sulfoacetate (I*b*), isolated as the initial product in a general synthesis of I (1), suggests that I*b* may be a better coralynium salt to use in liquid dosage forms than I*a*.

EXPERIMENTAL

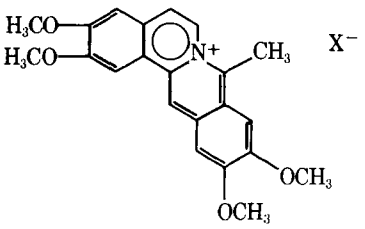
Materials—Coralyne chloride[3] and coralyne sulfoacetate[4] were provided by the National Cancer Institute and used without further purification. The IR spectrum of coralyne chloride used was identical to the reported spectrum[2]. Coralyne sulfoacetate has an extra absorption band at 1720 cm^{-1} due to the carbonyl group in the anionic part. 6′-Acetylpapaverine was prepared, following the

[1] J. P. Davignon, National Cancer Institute, personal communication.

[2] P. Lim and S. Stone, Stanford Research Institute Report 9180 to National Cancer Institute, June 28, 1972.

[3] NSC 96349, lot pH 9-29-1, mol. wt. 399.9, mp 246–250° dec. [lit. (1) 248–250° dec.].

[4] NSC 154890, lot pH 5-113-1, mol. wt. 503.5, mp 268–274° dec. [lit. (1) 278–280° dec.].



I: coralynium ion
Ia: X = Cl
Ib: X = $HO_2C—CH_2—SO_3$

procedure given by Schneider and Schroeter (3), from coralyne chloride.

Water was double distilled, with the second distillation being from acid–permanganate solution in an all-glass still. Indoor light was from an ordinary fluorescent bulb[5], and the photohydration was carried out about 1.5 m (5 ft) from such lights. Deoxygenated water was obtained by bubbling double-distilled water with nitrogen gas passed through a pyrogallol solution in concentrated sodium hydroxide. Commercially available borosilicate glass containers were used except where otherwise specified.

Kinetic Experiments—Formation of 6′-acetylpapaverine from coralyne was followed by the absorbance change at 297 nm on a UV spectrophotometer[6]. The reaction was carried out in such a way that, after rapid mixing with sodium hydroxide solution in an optical cell, a coralyne chloride concentration of 1.64×10^{-5} *M* and a coralyne sulfoacetate concentration of 1.82×10^{-5} *M* were obtained. Whenever the half-life of the reaction was shorter than 10 sec, a stopped-flow spectrophotometer[7] was used. In this case, a 1:1 volume ratio of coralyne solution to sodium hydroxide solution was adopted. A temperature of 25.0 ± 0.5° was maintained during the reaction. Ionic strength was not controlled, since no significant primary salt effect was observed when the final ionic strength of the solution, which was 0.5 *M* in sodium hydroxide, was adjusted to 1.0 *M* with sodium sulfate.

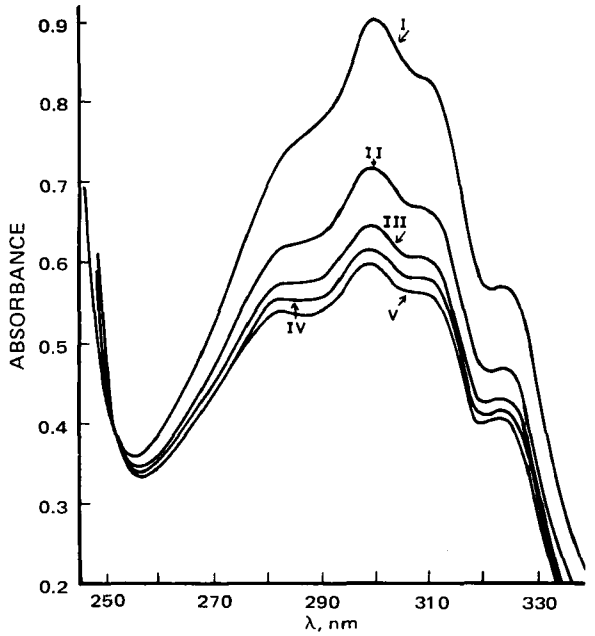
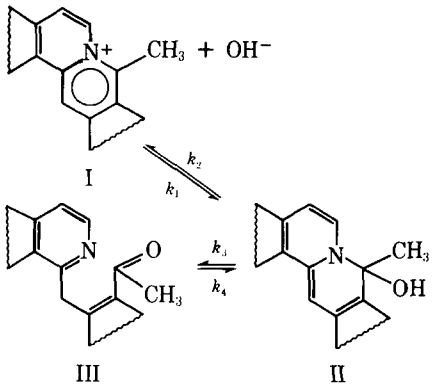


Figure 1—*Spectral changes observed at various times for a solution of* Ia *(~2 × 10⁻⁵ M) in aqueous 0.1 M NaOH at 25°. (Identical spectral changes were observed for* Ib *under the same conditions.) The spectra were recorded at various times after preparation of the solution. Key: I, 38 sec; II, 218 sec; III, 218 sec; IV, 308 sec; and V, 647 sec.*

[5] F40CW, Lifeline, Sylvania.
[6] Cary model 16, Varian Instruments.
[7] Durrum-Gibson.



Scheme I

The aromatization reaction of 6′-acetylpapaverine was followed in a similar manner. A concentrated solution, freshly prepared in chloroform, was used as a stock solution (~6 mg/ml). After about 3.5 μl of this stock solution was directly transferred into an optical cell through a microsyringe, the solvent was evaporated by nitrogen gas, the residue was rapidly dissolved in the appropriate buffer solutions, and the absorbance change at 297 nm was followed on a spectrophotometer. Since the reaction was extremely slow in 0.1 *N* HCl (Table I), sometimes a concentrated stock solution (~6 mg/ml) prepared in 0.1 *N* HCl was used in the kinetic experiments. Temperature and the final ionic strength were maintained at 25.0 ± 0.1° and 0.1 *M*, respectively.

Solubility Studies—The solubility of Ia and Ib in aqueous media was determined as follows. About 100 mg of either Ia or Ib was placed into separate 4-dram screw-capped vials. To each vial was added about 5 ml of distilled water. The tightly closed vials were wrapped in aluminum foil and attached to a rotating shaft in a water bath thermostated at 25 ± 0.1°. The samples were allowed to equilibrate for 7 days.

The solubility of Ib in various concentrations of sodium hydroxide was determined similarly. To a series of vials were added successively increasing amounts of Ib and 5 ml of increasingly concentrated aqueous sodium hydroxide solution. In a typical series of vials, the composition of the contents of each vial relative to the added amounts of Ib and the concentration of the sodium hydroxide solution, respectively, were as follows: vial I, ~125 mg, 0.02 *M*; vial II, ~200 mg, 0.04 *M*; vial III, 275 mg, 0.06 *M*; and vial IV, 350 mg, 0.08 *M*.

The vials were tightly closed and equilibrated as already described. Equilibration was complete after about 30 hr. Following equilibration, samples of the various solutions were withdrawn using a filter[8] and the filtrate was analyzed.

Analysis of Coralyne Chloride and Coralyne Sulfoacetate in Solution—The determination of the concentration of the coralyne salts in solution was carried out spectrophotometrically. The UV spectra of both compounds were qualitatively identical in solutions of anhydrous methanol and in 1% water–methanol. Beer's law plots were constructed for each salt in anhydrous methanol at λ_{max} = 312 nm. The molar absorptivity of the two compounds differed slightly. The experimental values determined from Beer's law plots were $\epsilon_{312} = 5.3 \times 10^4$ liters/mole cm for Ia and $\epsilon_{312} = 5.85 \times 10^{-4}$ liter/mole cm for Ib. This difference in absorptivities may be due to the presence of greater amounts of water in Ia (up to 10%)[9] as compared to Ib (0–2% water)[10].

Filtered aliquots of the aqueous coralyne solutions were suitably diluted (100-fold or more) with anhydrous methanol. The absorbance was then measured at 312 nm in a 1-cm cell, and the concentration was calculated utilizing the molar absorptivity value obtained for the particular salt being analyzed.

Methanol was used as the solvent for the analysis to prevent photohydration, which would have occurred rapidly in dilute aqueous solutions.

[8] Metricell, Scientific Products Co., Kansas City.
[9] P. Lim and S. Stone, Stanford Research Institute Report 1274 to National Cancer Institute, July 27, 1972.
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RESULTS AND DISCUSSION

Stability of Ia and Ib in Solutions Protected from Light—The UV spectra of aqueous solutions of *Ia* and *Ib* that had pH values below 10 and were completely protected from light (*e.g.*, stored in stoppered flasks wrapped in aluminum foil) did not change more than ±2% at any wavelength during 48 hr. The spectra of these two solutions were identical and were similar to the spectrum of *Ia* in methanol.

When the pH values of solutions of *Ia* and *Ib* were above 12, spectral changes occurred in a manner suggesting that coralynium ion was being converted into one or more products. An isosbestic point was maintained at 253 nm during these changes. Figure 1 displays the spectra measured at several times after the mixing of 0.6 ml of a 1×10^{-4} *M* solution of *Ia* in acetonitrile (a solvent in which *Ia* appeared to be stable for several days) with 3.0 ml of 1×10^{-1} *M* NaOH.

It was reported previously (3) that coralynium ion (I) is converted into 6′-acetylpapaverine (III) in alkaline solutions. Hence, it is believed that the spectral changes observed in solutions of *Ia* and *Ib* at pH > 12 were caused by the conversion of I to III. The results of studies (5) on the ring opening of other ring systems containing a quaternary nitrogen atom led to the conclusion that the reactions shown in Scheme I are probably involved in the conversion of I to III.

Compound III, formed by the reactions of *Ia* and *Ib* in solutions at pH ≥ 12, reverted to coralynium ion when the pH value of the solutions was reduced to values below 11. The spectral changes observed during the reaction for the conversion of III to I were precisely the reverse of those shown in Fig. 1, and the isosbestic point maintained during the closure reaction was at the same wavelength (253 nm) as that observed during the conversion of I to III in solutions with pH > 12. These spectral data represent evidence that the reactions observed at pH < 11 were the reverse of the reactions of *Ia* in more alkaline solutions.

Furthermore, a precipitate obtained 24 hr after III (5 mg/ml) was dissolved in 0.1 *N* HCl had an identical IR spectrum (measured in a KBr disk) and NMR spectrum (when dissolved in trifluoroethanol) to those of an authentic sample of *Ia*. It has recently been observed (2) that the pharmacological activities of *Ia* and III are similar. This observation is consistent with the postulates that I and III are rapidly interconvertable in solution and that III would be rapidly converted to I at physiological pH.

When the initial concentration of sodium hydroxide, $[NaOH]_0$, was at least five times greater than the initial concentration of *Ia* or *Ib*, the conversion of I to III was a first-order process. Pseudo-first-order rate constants, k^0_{obs}, for this reaction sequence were calculated from the slopes of linear plots of log $(A_t - A_\infty)$ against time. Values of k^0_{obs} are shown plotted against $[NaOH]_0$ in Fig. 2.

The conversion of III to I in aqueous buffers was also a first-order process, and values of a pseudo-first-order rate constant, k^c_{obs}, were calculated from the slopes of linear plots of log $(A_t - A_\infty)$ against time. Values of k^c_{obs} at several different pH values, together with the buffer systems, are listed in Table I. No attempt

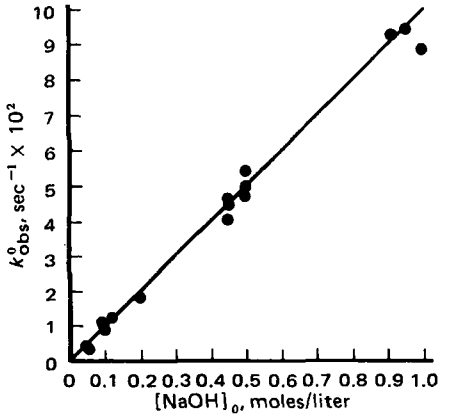

Figure 2—*Plot of the observed pseudo-first-order rate constants for the conversion of I to III as a function of the sodium hydroxide concentration in aqueous solution at 25°.*

Table I—Observed First-Order Rate Constant for the Conversion of III to I as a Function of pH[a]

pH (Buffer System)	$k^c_{obs} \times 10^4$ sec^{-1}
3.07 (monochloroacetate)	1.20
4.81 (acetate)	9.81
6.05 (succinate)	27.7
7.05 (phosphate)	67.3
8.41 (tromethamine)	19.4
9.87 (carbonate)	8.32
10.57 (carbonate)	3.54

[a] Ionic strength and temperature were kept at 0.1 *M* and 25°, respectively.

was made to evaluate buffer-independent rate constants or general-acid and general-base catalysis constants.

The facts that pure first-order kinetics were observed during both the conversion of I to III and the reverse reactions and that the same isosbestic point was maintained during the forward and reverse reactions strongly suggest that no significant concentrations of an intermediate (*i.e.*, the carbinolamine, II) accumulated during either the forward or the reverse reactions. Therefore, an identity relating k^0_{obs} and k^c_{obs} values to the microscopic rate constants in Scheme I can be arrived at by using a steady-state treatment. An identity that should apply at any pH value where species III exists primarily as a neutral molecule is:

$$k^0_{obs} = k^c_{obs} = \frac{k_1 k_3}{(k_2 + k_3)}[OH^-] + \frac{k_2 k_4}{(k_2 + k_3)} \qquad \text{(Eq. 1)}$$

Values of the slope and intercept of the plot of k^0_{obs} against $[NaOH]_0$ indicate that $k_1k_3/(k_2 + k_3) = 0.1\ M^{-1}$ sec^{-1} and $k_2k_4/(k_2 + k_3) \ll 10^{-2}$ sec^{-1}. Values of k^c_{obs} calculated from experiments in which the pH values were varied between 6 and 11 (*i.e.*, where $[H^+]$ and $[OH^-]$ varied by 10^5) only differed from one another by a factor of about 20. Hence, it appears that under these conditions the term $k_1k_3[OH^-]/(k_2 + k_3) \ll k_2k_4/(k_2 + k_3)$.

The relatively small change observed in k^c_{obs} values was perhaps caused by the different general acids and general bases in each reaction system. If this conclusion is correct, the value of the buffer-independent term, $k_2k_4/(k_2 + k_3)$, is likely to be no greater than $\sim 3.5 \times 10^{-4}$ unit, which is the smallest value of k^c_{obs} observed throughout this pH range (Table I). The decrease in the value of k^c_{obs} that occurred when the pH value was reduced from 6 to 3 was most likely caused by protonation of the nitrogen atom of 6′-acetylpapaverine. The conjugate acid of III would be expected to have a pKa value similar to that of isoquinoline, 5.4 (6). Protonation of III would be expected to reduce its rate of cyclization to yield II and would thus reduce the apparent value of k_4.

It can be concluded that $k_1k_3/k_2k_4 = [III]/[I][OH^-] \simeq 10^{-1}/3 \times 10^{-4} \simeq 3 \times 10^2\ M^{-1}$. Hence, at pH < 11, [III]/[I] < 0.3; at pH ~ 6, [III]/[I] would be of the order of 10^{-5}. Thus, the interconversion of I and III occurs relatively rapidly in solution and only an insignificant fraction of the total alkaloid persists as III in solutions with pH values ≤10.

Photochemical Reactions of Ia and Ib—It has been suggested (4) that I becomes covalently hydrated in aqueous solution. This conclusion was based on similarities between the UV spectra of freshly prepared aqueous coralyne solutions and solutions of coralyne salts in anhydrous solvents, such as acetonitrile and methanol, and the differences between these spectra and that of an aged aqueous solution. The reversibility of the hydration reaction was established from the fact that lyophilization of aged solutions of *Ia* or *Ib* yielded residues which, on redissolution in methanol, produced solutions with UV and NMR spectra identical to those obtained from freshly prepared solutions of the authentic salts.

In the present study, the reactions that occur in aqueous solutions of *Ia* and *Ib* at pH values where no appreciable amounts of III are formed were investigated and the following additional information is reported.

1. The reaction of I that has been referred to as a covalent hydration reaction is a photochemical reaction and appeared to occur as a result of exposure of solutions of I to visible light. Hence, whereas the UV spectra of solutions of *Ia* and *Ib* in water do not change appreciably during 24 hr if the solutions are completely

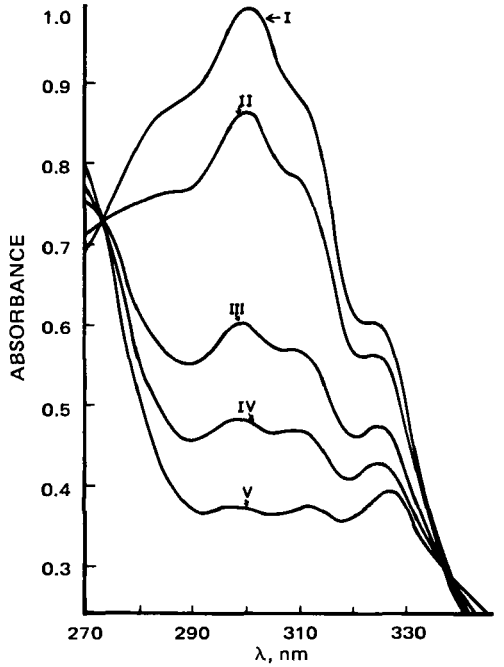

Figure 3—*Spectral changes for an aqueous solution of Ia (~2 × 10^{-5} M) exposed to fluorescent laboratory light for various periods. Key: I, 0 min (freshly prepared solution); II, 15 min; III, 60 min; IV, 180 min; and V, 2 days (no further spectral changes occurred).*

protected from light, they do change rapidly upon exposure to sunlight or normal laboratory illumination in either open or closed glass vessels.

Figure 3 displays the spectrum of a freshly prepared solution of I*a* in water and the spectra measured after various periods of exposure of the solutions to normal laboratory illumination. If a solution that had been exposed to illumination for any length of time was then protected from light (*e.g.*, by storing it in an amber glass container or in a container painted black or wrapped with aluminum foil), its spectrum underwent no further changes during the protection period.

The rate and nature of spectral changes upon illumination were the same in solutions of I*a* and I*b* stored in either borosilicate glass or quartz containers. Because the materials of the former type of container absorb UV light whereas those of the latter type transmit UV light, it seems highly probable that the visible range of radiant energy is involved in the photochemical reaction. Thus, it is

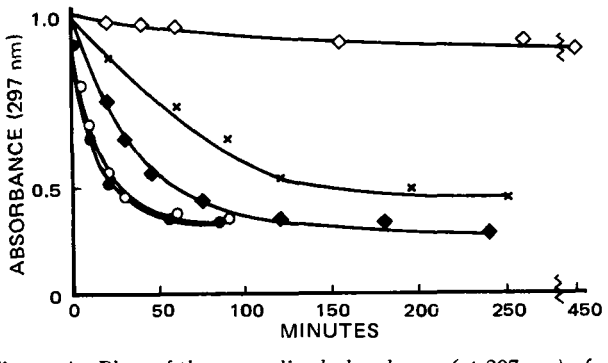

Figure 4—*Plots of the normalized absorbance (at 297 nm) of solutions of I*b versus *the time of exposure to normal laboratory light. The studies were conducted at room temperature (about 19–22°). The initial concentrations of I*b *in the solutions were: ◇, 2.15 × 10^{-3} M; ×, 2.15 × 10^{-4} M; ♦, 7.54 × 10^{-5} M; ○, 1.08 × 10^{-5} M; and ●, 7.10 × 10^{-6} M. Normalization was achieved by multiplying measured absorbances at 297 nm by a factor that took into account differences in the initial concentration and the path length of the cell. The initial absorbance of a 2.15 × 10^{-5} M solution of I*b *in a 1-cm cell was 0.984.*

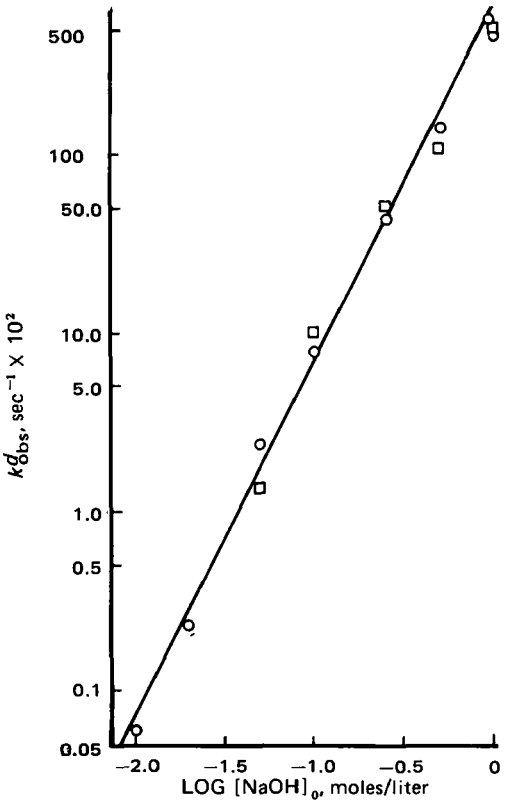

Figure 5—*Logarithmic plot of observed first-order rate constants for the conversion of the postulated photohydrate to I as a function of sodium hydroxide concentration. T = 25°. Identical results were obtained with I*a *(○) and I*b *(□).*

necessary to exclude light to prevent the reaction from occurring.

2. The rate of spectral changes during illumination was not affected by the presence of dissolved oxygen in the solutions. This fact was established by comparing the rates of spectral changes in illuminated solutions of I*a* in water that had been saturated with either oxygen or nitrogen. It can be concluded from this result that oxygen is not involved in the photochemical reaction. Furthermore, oxygen is a quencher of many photochemical reactions involving triplet-state excited molecules (T^1). Hence, the fact that the rate of the photochemical reaction of I is insensitive to oxygen concentrations suggests that it involves single-state (S^1) rather than triplet-state (T^1) excited molecules. A similar conclusion was drawn (7) about the photohydration of uracil, because the rate of this reaction was also insensitive to oxygen concentrations.

3. The rate of photohydration reaction was the same in 0.1 *N* HCl as it was in water. This result suggests that the rate-determining step involves a reaction between an excited form of the coralynium ion (presumable S^1 species) and undissociated water molecules rather than hydroxide ions or hydrogen ions.

4. The apparent rate of the photohydration reaction decreased as the initial concentration of I in the solution was increased. This result can be seen in Fig. 4, where plots of the absorbance (at 297 nm) of different solutions of I*b* against time of exposure to laboratory illumination are displayed.

In a typical experiment, 10–12 identical volumetric flasks (25 ml), wrapped with aluminum foil, were filled with a solution of I*b* in water. This operation was performed in a photographic darkroom where no appreciable photochemical reaction occurred. The flasks were then placed on an illuminated laboratory bench and the aluminum foil was removed. At various time intervals, a flask was selected at random and the UV absorbance of its solution at 297 nm was measured in a 0.2-, 1.0-, or 5.0-cm cell or an appropriate dilution was made in a darkroom and the absorbance then was measured. The absorbance data were normalized so that they could be conveniently presented on a single graph.

No simple rate law could be derived to account for the absorbance changes as a function of time. This observation suggests that the distribution of light energy is not uniform throughout the reac-

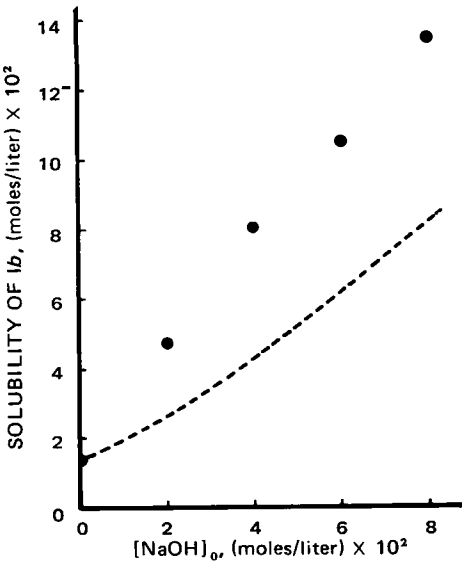

Figure 6—*Plot of aqueous solubility of Ib as a function of added sodium hydroxide, [NaOH]₀. The points represent the experimental results. The dashed line represents the curve calculated using Eq. 6 and a value of* $K_{sp}^{Ib} = 1.72 \times 10^{-4}$ M²; T = 25°.

tion solutions, a phenomenon that is commonly encountered in photochemical reactions where the excited-state molecules have only a very short lifetime (8). However, the results clearly indicate that the apparent rate of the photochemical reaction decreases as the concentration of I increases. Further confirmation of this fact comes from the observation that rapid dilution with water of a saturated solution of Ia, which had been stored in a flask on a laboratory bench for 2 months, yielded a solution that had a UV spectrum identical to that of a freshly prepared solution of Ia.

It was reported (4) that the presumed hydration of I can be reversed by lyophilization of solutions or by dilution of solutions of the hydrate with methanol. Two other methods of causing a reversal of the hydration reaction have become apparent as a result of this work. In the first case, when a 10^{-4} *M* solution of Ia, which had been illuminated with laboratory light for several weeks, was refluxed for 1 hr and then cooled in the dark, its spectrum was almost the same as that of a freshly prepared solution of Ia.

In the second case, if a solution of Ia in water was exposed to light for several weeks and was then made alkaline to pH > 12, its spectrum rapidly changed from that of the hydrate to one that was very similar to the spectrum of Ia. This spectrum then changed in the manner that has been attributed to the conversion of I to III. Since an isosbestic point is maintained at 253 nm during the conversion of I to III and the absorbance of I and its hydrate at 253 nm differ significantly, the kinetics of conversion of the hydrate to I could be studied by measuring absorbance changes at this wavelength. The reaction appeared to be first order in the concentration of the hydrate, and pseudo-first-order rate constants, k_{obs}^{d}, were calculated from the slopes of linear plots of log $(A_t - A_\infty)$ against time.

As can be seen from the logarithmic plot of k_{obs}^{d} against $[NaOH]_0$ in Fig. 5, the rate of this reaction had a second-order dependence on hydroxide-ion concentrations. An explanation of this result cannot be offered at this time. Dehydration reactions of photohydrates of molecules such as uracil have been reported (9) to be first order with respect to hydroxide-ion concentration, but we are unaware of any dehydration reactions that have been shown to be second order with respect to this reactant. This result may cast some doubt on whether the product of the photochemical reaction is indeed a photohydrate.

This study did not yield additional evidence as to the structure of the product; its characterization rests on the observations that it only forms in water and not in anhydrous methanol or acetonitrile and that it is readily converted into I by lyophilization, by heating, or by making the solution alkaline.

Irrespective of the exact nature of the product of the photochemical reaction of I, the reaction can be prevented by completely protecting the aqueous solutions from visible light and the rate in the presence of light can be greatly reduced by increasing the concentration of I in the solution. These facts, together with the ability to reverse the photochemical reaction through lyophilization, are extremely important in the ultimate development of formulations for parenteral solutions of I.

Solubility of Ia and Ib—It was desirable to identify a method for obtaining more concentrated solutions of I to facilitate the intravenous administration of the cytotoxic agent, especially in view of the data in Fig. 4 indicating the apparent increase in chemical stability achieved through increased concentration.

The solubility of Ia and Ib in water at 25° was determined to be about 4.5 and 6.5 mg/ml, respectively. It had been shown[1] that the solubility of Ia was no greater in weakly alkaline aqueous media than in distilled water, as expected for such a compound. The solubility of Ib as a function of added sodium hydroxide is shown in Fig. 6. It is obvious that the apparent solubility of Ib is greatly increased by the presence of added sodium hydroxide. Qualitatively, this solubility behavior of Ib was expected, since the sulfoacetate anion (in contrast to Cl^-) is an acid and would be ionized by the added hydroxide ion. This behavior can be expressed mathematically as follows.

The apparent solubility product (K_{sp}) for Ib is given by:

$$K_{sp}^{Ib} = [I][(HO_2C—CH_2—SO_3)^-] \quad \text{(Eq. 2)}$$

Because $(HO_2C—CH_2—SO_3)^-$ is a weak acid, it would react with each added hydroxide ion according to Scheme II:

$$(HO_2C—CH_2—SO_3)^- + (OH)^- \rightarrow (O_2C—CH_2—SO_3)^{-2} + H_2O$$

Scheme II

and:

$$[NaOH]_0 \simeq [(O_2C—CH_2—SO_3)^{-2}] \quad \text{(Eq. 3)}$$

As long as the only solid phase in the system is Ib, the following identities must be obeyed:

$$[I] = [(HO_2—C—CH_2—SO_3)^-] + [(O_2C—CH_2—SO_3)^{-2}] \quad \text{(Eq. 4)}$$

$$[I] = [(HO_2C—CH_2—SO_3)^-] + [NaOH]_0 \quad \text{(Eq. 5)}$$

Substituting Eq. 5 into Eq. 2 and solving for [I] lead to:

$$[I] = \{[NaOH]_0 + ([NaOH]_0^2 + 4K_{sp}^{Ib})^{1/2}\}/2 \quad \text{(Eq. 6)}$$

The dashed line in Fig. 6 was calculated using Eq. 6 and a K_{sp}^{Ib} value that was calculated from the identity:

$$K_{sp}^{Ib} = [I]_{sat}^2 \quad \text{(Eq. 7)}$$

where $[I]_{sat}$ ($= 1.31 \times 10^{-2}$ *M*) is the solubility of Ib in water at 25°.

There are at least two possible explanations for the fact that the actual solubility of Ib was higher in aqueous sodium hydroxide than was predicted using Eq. 6. First, no allowance was made for the fact that the K_{sp}^{Ib} value would change as the ionic strength of the solution increased. The solubility of Ib and the concentration of the dianionic species $(O_2C—CH_2—SO_3)^{-2}$ obviously increased as the initial concentration of sodium hydroxide was increased. Therefore, the ionic strength of the solution increased, and such an increase probably would be expected to increase the value of K_{sp}^{Ib} (10).

Second, there is a strong possibility that I dimerizes or forms higher order complexes in solution. The occurrence of this phenomenon might also be expected to lead to an increase in the apparent solubility of the salt. Although this latter phenomenon was not investigated further, related quaternary nitrogen-containing ring systems such as acridines do form dimers and higher order complexes in aqueous solution (11).

The important result of this solubility study is that much more concentrated solutions of I can be formed by dissolving Ib (or, presumably, other salts of I and the anions of polyprotic acids) *in* aqueous alkaline solutions than can be prepared using Ia. Moreover, the salt Ib is the initial product obtained by a general synthesis of I (1).

Application of Results—These results have been useful in the development of a formulation of I which obviates the problems that had previously limited the clinical evaluation of the cytotoxic

coralyne salts. This formulation is presently being utilized and is prepared by dissolving Ib in sufficient aqueous sodium hydroxide to yield a final concentration of 25 mg/ml (calculated on the basis of Ib) at pH ~ 6.5. The solution obtained is sterile filtered, frozen, and lyophilized. The lyophilized product is stable, and reconstitution as a solution is easily accomplished upon the addition of water. Such a formulation affords good solubility and stability with respect to I and appears to be completely suitable for intravenous use.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 22, 1974, from the *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66044, and the ‡Midwest Research Institute, Kansas City, MO 64110

Accepted for publication March 11, 1975.

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Annual Meeting, New Orleans, La., November 1974.

Supported in part by the National Institutes of Health, Department of Health, Education, and Welfare, through Research Grant RO1-GM-18348, Research Career Development Award GM-70100 (to I. H. Pitman), and Contracts NO1-CM-23217 and NO1-CM-33743 from the Division of Cancer Treatment, National Cancer Institute.

§ Present address: The Upjohn Co., Kalamazoo, Mich.

ˣ To whom inquiries should be directed.

Micelle Formation and Its Relationship to Solubility Behavior of 2-Butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl Ketone Hydrochloride

LOUIS J. RAVIN ˣ, ELIE G. SHAMI ‡, and ELISABETH S. RATTIE

Abstract □ Micelle formation by 2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride was studied by conductance measurements. The CMC was approximately 0.05% and was independent of temperature between 20 and 50°. The heat of formation for the micelle was calculated to be 6.9 kcal/mole. The unusual solubility behavior of the compound was attributed to its ability to form micelles. Ultracentrifuge studies indicate the molecular weight of the micelle to be approximately 100,000. Anions such as chloride, sulfate, acetate, tartrate, and citrate significantly affect the equilibrium solubility of the compound. NMR spectroscopic data indicate that the solubility behavior, in part, is related to an effect on the CMC of the compound by the anionic environment.

Keyphrases □ 2-Butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride—micelle formation, relationship to solubility □ Solubility—2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride, micelle formation □ Micelles—2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride, relationship to solubility

It was reported previously (1, 2) that 2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride[1] (I) was capable of existing in the micellar state and that surfactants such as polysorbate 80, sodium lauryl sulfate, and cetyldimethylbenzylammonium chloride had a pronounced effect on its equilibrium solubility. This paper reports the results of studies on the micellar behavior of I, its relationship to the solubility characteristics, and the effect of various anions on its equilibrium solubility.

EXPERIMENTAL

Materials—Ultrapure water[2] was used. All other chemicals were of analytical reagent grade quality.

Equipment—A recording spectrophotometer[3] was used for UV absorption measurements. A conductivity bridge[4] and a conductivity cell[5] were used in the conductance experiments.

Equilibrium Solubility Studies—*Effect of Temperature*—Approximately 200 mg of I was added to screw-capped vials containing 15 ml of water. The vials were rotated for 24 hr at 20, 25, 30, 35, 40, 45, and 60°. The contents of the vials were filtered through syringes fitted with Swinney filter adapters containing a

[1] SK&F 33134-A, marketed as Cordarone by Labaz Laboratories in several European countries.

[2] Harleco, Philadelphia, Pa.

[3] Cary model 15.

[4] Surfass model RCM 15B1, Arthur Thomas.

[5] K-1.00/CM, Beckman Instruments.