for the values of $\epsilon_{N'}$, K_2, and K_3 (9). With these values (Table II), the microconstants may then be determined. The K_{NZ}, the ratio of the concentration of the neutral species to that of the zwitterionic species, is a constant independent of pH. Since:

$$\epsilon_C[N] + \epsilon_A[Z] = \epsilon_{N'}[N'] \quad \text{(Eq. 14)}$$

and at 495 nm $\epsilon_{N'}$ is equal to 9.10×10^3 cm^{-1} M^{-1}, the following equation can be used to evaluate K_{NZ}:

$$K_{NZ} = \frac{[N]}{[Z]} = \frac{\epsilon_{N'} - \epsilon_A}{\epsilon_C - \epsilon_{N'}} \quad \text{(Eq. 15)}$$

Since [N] = 6.27 [Z], Eq. 2 may be solved for K_{CZ} and K_{CN} as follows:

$$K_2 = \frac{6.27[H^+][Z]}{[C]} + \frac{[H^+][Z]}{[C]} = 7.27\,K_{CZ} \quad \text{(Eq. 16)}$$

Similar substitution into Eq. 3 allows both K_{NA} and K_{ZA} to be calculated.

A similar method for the spectrophotometric evaluation of microconstants involving overlapping equilibria, in which the dissociation of one group affects the absorption spectrum and the other does not, was developed by Edsall *et al.* (10) in studies of the dissociations of tyrosine. This method was applied to the dissociation equilibria of alkanolamines (11). However, the latter approach is somewhat more complicated algebraically than the present one. Moreover, the macroconstants in Edsall's method are calculated from the microconstants. In the present approach, the preliminary evaluation of macroconstants from spectrophotometric data can be checked independently by potentiometry if solubility permits.

The fluorescence spectrum of doxorubicin in the pH region follows the same changes observed in the pH dependence of the absorption spectrum, indicating that protolytic reaction in the excited state is too slow to compete with fluorescence. However, in the Hammett acidity region the fluorescence spectrum of doxorubicin red shifts with increasing acidity in acid solutions more dilute than those in which the corresponding absorption spectral changes occur. This shift is indicative of protonation in the lowest excited singlet state, with the inflection point in the fluorometric titration representing the excited-state dissociation constant (pK*) of −3.1. Doxorubicin becomes more basic in the excited state due to the transfer of charge to a carbonyl group upon excitation.

The method allows the direct calculation of microconstants when overlapping dissociation equilibria are present in a system under spectrometric study. This method is general for any molecule with overlapping equilibria in which dissociation of only one functional group affects the electronic spectral properties of the molecule. The values of the microconstants show that the protonated amino sugar group is slightly more acidic than the phenolic group.

In developing or using any method of analysis for doxorubicin, careful choice of the pH and the analytical wavelength is necessary. The prevalence of only one absorbing species having intense, long wavelength absorption in the pH 4–7 region and one emitting species having intense fluorescence in the pH 1–7 region suggests that the spectrometric analysis of doxorubicin in aqueous solutions be carried out in dilute acidic solutions.

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Determination of Energy Change Associated with Dissolution of a Solid

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Abstract □ The dissolution of a solid immersed in a solvent was considered as a consecutive process, consisting of a primary surface interaction leading to the production of a new surface at the solid–liquid interface, solvation of the solid at the interface, and transfer of the solvated solid into the bulk of the solution. The energy changes involved in each step were studied for the dissolution of *m*-tolylacetamide in hexane and heptane. An energy diagram was constructed according to the proposed dissolution mechanism. The heats of dissolution determined from the energy diagram agreed well with those obtained experimentally.

Keyphrases □ Solids—dissolution in a solvent, energy changes determined □ Dissolution—solid in a solvent, energy changes determined □ Energy changes—determined for dissolution of a solid in a solvent

Several recent investigations concerned the energy changes involved in the dissolution process. Wadke and Reier (1) presented a rate equation, based on previously published results (2–4), involving both the heat of solution of the solid and the activation energy for diffusion. Szinai and coworkers (5, 6), after reviewing several articles on dissolution, proposed a rate equation incorporating an energy term comprised of the energy required to remove a solute molecule from the solid surface, the energy of solvation, and the activation energy for diffusion. A numerical study of the three energy terms was not reported.

The purpose of this study was to quantify the energy associated with initial solvent–solid interaction, the solvation of the solute, and the mass transfer steps of the dissolution process.

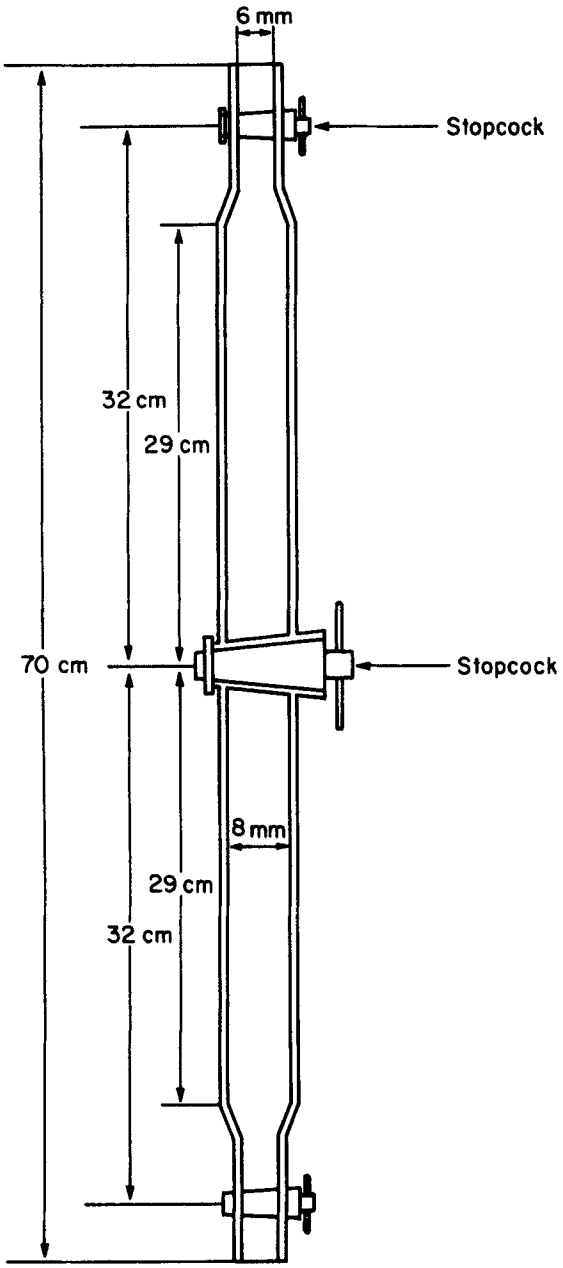


Figure 1—*Diagram of glass dispersion column.*

EXPERIMENTAL

Materials—The solid was *m*-tolylacetamide[1] (I). The solvents used were spectral quality.

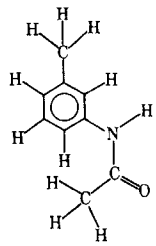
Temperature Dependence of Solubility of I—The solubility of I in hexane and heptane was determined at 14.3–39.9 ± 0.01°. A 300-ml, three-necked, round-bottom flask, containing approximately 20 g of I powder, was put in a constant-temperature water bath at 14.3 ± 0.01°. A stirrer was mounted in the flask, 200 ml of hexane or heptane was added, and the stirring speed was set at 150 rpm.

After 24 hr, a 10-ml sample was taken from the flask and quickly filtered through a 0.45-μm filter. Five milliliters of the filtered solution was diluted in a 250-ml volumetric flask with anhydrous methanol. Duplicate samples were taken each time. The temperature of the water bath was then increased about 5°. After temperature equilibrium was reached in the water bath for 24 hr, another sample was removed, filtered, and diluted as previously described.

The absorbance of each sample was determined at 242 nm with a 1:50 dilution of hexane or heptane in methanol as the reference. A blank was run each time to ensure that the baseline was straight. The absorbance at 242 nm was recorded and converted into concentration through an appropriate calibration curve. The solubility of I in hexane and heptane at the different temperatures was then calculated.

[1] Eastman Kodak Co., Rochester, NY 14605.

Table I—Molecular Structure of *m*-Tolylacetamide



Bond	Bond Length	Group	Geometry	Angle
C=O	1.162	Carbonyl	Planar	120°
C—H	1.09	Methyl	Tetrahedral	109°
C—C	1.54	Alkane	Tetrahedral	109°
N—H	1.038	Amide	Tetrahedral	109°
C=C in benzene	1.39	Benzene	Planar	120°
C—N	1.329	Amide	Tetrahedral	109°

Temperature Dependence of Dissolution Rate of I—The dissolution rate of I in hexane and heptane was determined at 25.0, 29.9, 33.1, and 35.5 ± 0.05° in a constant-temperature chamber (7).

Glass tubes, 45 cm long × 1.65 cm i.d., were flat sealed on one end and employed as dissolution vessels. A stainless steel tablet holder was constructed to fit easily into the dissolution tube without causing bubble formation.

A small amount of I was placed in a casserole, which was then warmed in a water bath at 75°. After the compound was melted, it was poured into a 1.11-cm (7/16-in.) tablet die with a polished, flat-faced punch in place. The cast tablets were removed after solidification, and mottled or chipped tablets were discarded. The punch face was cleaned with anhydrous methanol.

A mixture containing 40% polyethylene glycol 4000 and 60% polyethylene glycol 400 was prepared and carefully applied to the cylindrical surface of the tablet. Care was taken to prevent any of the mixture from contacting the flat surface of the tablet. Solvent, 85 ml, was placed in the dissolution tube and allowed to equilibrate for 8 hr in the controlled temperature chamber. The dissolution tubes were held in a perfect vertical position using a suitable supporting device (8). The tablet was wrapped with aluminum foil and pushed into the tablet holder, which was put through the center of a stopper that sealed the open end of the dissolution tube.

A dissolution run was begun by gently immersing the tablet surface just below the solvent surface. Dissolution was timed for 3 min. The run was stopped by gently removing the tablet holder from the dissolution tube, and the amount dissolved was then determined. Dissolution rate determination was made in triplicate, using a new tablet for each determination. The temperature of the chamber was then increased by 5°, and the dissolution rate was determined at this temperature in the same manner.

Determination of Dispersion Rate—A Pyrex glass column (70 cm long × 8 mm i.d.) was used to measure the dispersion rate of solutions of I in hexane and heptane into the pure solvent (Fig. 1). The column had stopcocks of 2-mm bore at each end and a stopcock with a straight 8-mm bore in the middle.

Dispersion experiments were conducted at 30° in the constant-temperature chamber. The solvent, solution, and column were placed inside the chamber and allowed to equilibrate. The lower stopcock was then closed, and the lower half of the column was filled with solvent to a point above the middle stopcock. Suction was then applied to remove any entrapped air bubbles in the lower column. The middle stopcock was then closed, and the solvent remaining in the upper column was removed. The upper column was then dried with a stream of air.

A series of I solutions in hexane and in heptane were prepared ranging in concentration from 30 to 300 mg/liter. A solution of I was transferred into the upper column while the middle stopcock was still in a closed position. The upper stopcock was then closed, and the entire column was inspected to be certain that no air bubbles were present. Two hours after

Table II—Temperature Dependence of the Solubility of *m*-Tolylacetamide in Hexane and Heptane

Temperature	Solubility in Hexane, mg/liter	Solubility in Heptane, mg/liter
14.3°	294.8	—
19.8°	449.5	—
24.5°	562.4	—
25.7°	—	527.5
29.8°	666.1	—
30.3°	—	688.4
33.8°	758.1	—
35.2°	—	901.0
39.9°	—	1066.9

the column was filled, the middle stopcock was turned to the open position for 3 min and then closed. The upper column was first drained and rinsed with 10 ml of solvent five times. After the upper column was air dried, the middle stopcock was opened to let the liquid inside the stopcock mix with the liquid in the lower column. The solution in the lower column was then removed for assay.

The volumes of the upper and lower (including the stopcock) columns were calibrated with a 50-ml buret and found to be 19.1 ± 0.05 and 20.25 ± 0.05 ml, respectively. From the volume and the final solution concentration in the lower column, the amount of I transferred from the upper column to the lower column and the dispersion rate of I in hexane and heptane were calculated.

Temperature Dependence of Dispersion Rate—The dispersion rates of solutions of I in hexane and heptane into their respective pure solvents were determined at four different temperatures using the same dispersion column and the described method. The concentration of I used in the upper column was that concentration which, during dispersion, transferred to the lower column approximately the same amount of solute as was dissolved from the solid I during the same time period. This concentration was 44.2 mg/liter in hexane and 32.6 mg/liter in heptane. A second concentration, 240 mg/liter in hexane and 265 mg/liter in heptane, was also used to determine if the temperature dependence of the dispersion rate was a function of concentration.

The temperature dependence of the dispersion rate was independent of concentration for the concentrations used. The rates of dispersion, expressed as the amount of solute transferred per minute, were determined by assaying for the amount of I transferred to the lower section of the column.

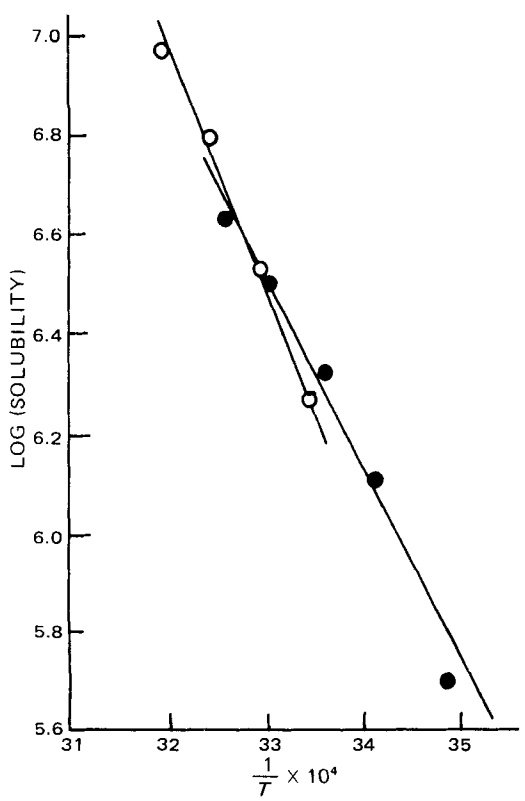


Figure 2—*Temperature dependence of the solubility of I in hexane* (●) *and heptane* (○).

Table III—Temperature Dependence of the Dispersion Rate of *m*-Tolylacetamide in Hexane and Heptane

Temperature	Dispersion Rate in Hexane, μg/min	Dispersion Rate in Heptane, μg/min
27.9°	34.2	23.6
30.0°	36.2	26.4
33.1°	37.9	28.0
35.4°	41.1	29.5

Heat of Fusion—The heat of fusion of I was determined in a differential scanning calorimeter[2] with indium as the standard. Approximately 3 mg of I was weighed to the nearest 0.01 mg on a microbalance[3] into each of five sample holders, which were then sealed. Empty sample holders were used as reference. An indium sample weighing 6.22 mg was used as a standard. The temperature was scanned from 416 to 430 °K for indium and from 316 to 338 °K for I at a rate of 2.5 °C/min. Sharp and symmetric peaks were obtained in each case. The area under the curves was measured with a planimeter.

The calculation of the heat of fusion of I was based on the following equation:

$$\Delta H_1 = \frac{\Delta H_2 A_1 W_2}{A_2 W_1} \qquad \text{(Eq. 1)}$$

where ΔH_1 is the heat of fusion of I, ΔH_2 is the heat of fusion of indium, A_1 is the area under the curve of the sample, A_2 is the area under the curve of indium, W_1 is the weight of the sample, and W_2 is the weight of indium.

RESULTS AND DISCUSSION

Determination of Energy Involved in Each Dissolution Step—Three fundamental processes contribute to the dissolution of a solid immersed in a solvent, regardless of the mechanism by which dissolution is thought to occur (Scheme I). The three processes are: (*a*) the primary surface interaction leading to the continual production of a new surface at the solid–liquid interface, (*b*) solvation of the solid at the solid surface or solid–liquid interface, and (*c*) mass transfer of the dissolved solid into the bulk of the solution. The slowest process determines the dissolution mechanism. The first two steps are interfacial interactions between the solid and the solvent, which are related to interfacial energy and the solid lattice energy. In Step 3, the mass transfer process, solvated solid is brought into the bulk of the solution by diffusion and/or convection.

$$\text{solid} \xrightarrow[\Delta E_1]{\text{surface interaction}} \text{solvent} \cdots \text{solid} \xrightarrow[\Delta E_2]{\text{solvation}} \text{solvated solid at solid surface} \xrightarrow[\Delta E_4]{\text{mass transfer}} \text{solution}$$

Scheme I

The energy required to produce a new surface or to remove a molecule from the surface of a solid, ΔE_1, is equal to the product of the interfacial tension between the solid and the solvent and the surface area of the solid or:

$$\Delta E_1 = \gamma_{SL}\, \Delta A \qquad \text{(Eq. 2)}$$

where ΔA represents the exposed surface area of a mole of solid, and γ_{SL} is the interfacial tension. The literature values of γ_{SL} for I in hexane and heptane are 3.9 and 7.6 dynes/cm, respectively. The value of ΔA for I can be calculated from its molecular structure. The calculation is made possible through a knowledge of the bond lengths and bond angles (Table I), the fact that the benzene ring and acetyl group are planar, and the assumption that the methyl group is spherical. The surface area of I calculated from the molecular structure, based on a two-dimensional planar projection and the assumption that both sides of the molecule interact with the solvent, is 47.08 A^2 or 2.83 × 10^9 cm^2/mole. The ΔE_1 values for I in hexane and heptane calculated from Eq. 2 are 0.26 and 0.51 kcal/mole, respectively.

The heat of solvation consists of two energy terms. The first, ΔE_2, is the energy required to break the solid lattice energy and is equal to the

[2] Model 1B, Perkin-Elmer, Norwalk, Conn.
[3] Cahn R. G. electrobalance, Cahn Instrument Co., St. Paramount, CA 90723.

Table IV—Energy Terms Involved in Each Step of the Dissolution of *m*-Tolylacetamide in Hexane and Heptane

Solvent	ΔE_1 ($\Delta E_{wetting}$), kcal/mole	ΔE_2 (ΔE_{fusion}), kcal/mole	ΔE_3 ($\Delta E_{solvation}$), kcal/mole	ΔE_4 ($\Delta E_{disperson}$), kcal/mole	ΔH_S ($\Delta H_{solution}$), kcal/mole	ΔE_d ($\Delta E_{dissolution}$), kcal/mole (exp)	ΔE_d ($E_{dissolution}$), kcal/mole (calc. from Eq. 7)
Hexane	0.26	4.15	5.61	4.17	10.0	15.9	14.2
Heptane	0.51	4.15	4.63	5.25	9.30	15.3	14.6

heat of fusion of the solid, ΔH_f. The heat of fusion for I determined by differential scanning calorimetry was 4.15 kcal/mole.

The second energy term, ΔE_3, is the energy released or required during solvation. If the interaction energy between the solid and solvent is larger than that between solid molecules, energy is released; if the interaction energy is less than that existing between solid molecules, energy is required. Although ΔE_3 cannot be determined experimentally, it can be calculated from ΔE_1, ΔE_2, and the heat of solution, ΔH_S.

Since the amount of I in the saturated solution in hexane and heptane is relatively small, 602 and 646 mg/liter, respectively, at 30°, the volume change during dissolution may be considered negligible. From the equation:

$$\Delta H_S = \Delta E_S + P\,\Delta V \qquad \text{(Eq. 3)}$$

it can be seen that at constant pressure the heat of solution, ΔH_S, is equal to the energy change in solution, ΔE_S. Then:

$$\Delta H_S = \Delta E_S = \Delta E_1 + \Delta E_2 + \Delta E_3 \qquad \text{(Eq. 4)}$$

The heat of solution, ΔH_S, is the energy difference between two equilibrium states, *i.e.*, the energy of the system before and after the solid is dissolved. This energy term can be obtained from a Van't Hoff plot of the I solubility data (Table II). Figure 2 shows a plot of log (I solubility) as a function of the reciprocal of temperature. From the slope of the line, values of 10.0 and 9.30 kcal/mole were calculated for the heat of solution of I in hexane and heptane, respectively.

From the experimental results of ΔH_S, ΔE_1, and ΔE_2 and Eq. 4, the term ΔE_3 can be obtained such that:

$$\Delta E_3 = \Delta H_S - \Delta E_1 - \Delta E_2 \qquad \text{(Eq. 5)}$$

which yields ΔE_3 = 5.60 kcal/mole for I in hexane and 4.63 kcal/mole for I in heptane. The fact that ΔE_3 has a positive sign shows that energy is required for the solvation step in addition to the heat of fusion.

The activation energy for mass transfer or dispersion is the energy required to transfer the solvated solid from the interfacial region to the bulk of the solution. This energy, ΔE_4, can be determined from the temperature dependence of the liquid–liquid dispersion rate of the I solution into pure solvent (Table III). If it is assumed that the dispersion coefficient is related to temperature in the same manner as the diffusion coefficient, then:

$$D = D_0 e^{-\Delta E_4/RT} \qquad \text{(Eq. 6)}$$

where D is the dispersion coefficient, D_0 is a constant related to the molecular dimensions of the solute, ΔE_4 is the activation energy for dispersion, R is the gas constant, and T is the absolute temperature. Since the dispersion rate is proportional to the dispersion coefficient, an Arrhenius plot may be made of the dispersion rate data as a function of the reciprocal of temperature. Arrhenius-type plots are shown in Fig. 3. From the slope of the lines, values of 4.17 and 5.25 kcal/mole were calculated for ΔE_4 for the systems of I in hexane and heptane, respectively.

The heat of dissolution, ΔE_d, is the energy of activation associated with solid dissolution. This term may also be determined from an Arrhenius plot using the dissolution rate in place of the dispersion rate constant. Figure 4 shows a plot of log (dissolution rate) *versus* the reciprocal of absolute temperature for the dissolution of I in both hexane and heptane. The energies of activation determined from the slope of the lines in Fig. 4, along with the energy terms discussed, are listed in Table IV.

Based on these energy terms, an energy diagram (Fig. 5) can be constructed. From the energy diagram, it can be seen that:

$$\Delta E_d = \Delta E_1 + \Delta E_2 + \Delta E_3 + \Delta E_4 \qquad \text{(Eq. 7)}$$

The activation energy of dissolution, ΔE_d, can then be calculated from Eq. 7 and compared with the value of ΔE_d determined experimentally. This comparison is shown in Table IV.

The comparison between the experimental value of ΔE_d and the value

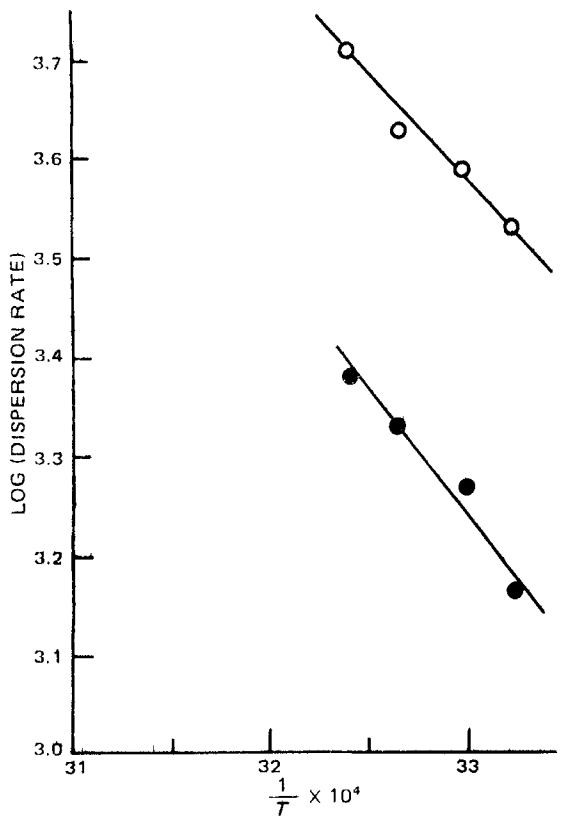


Figure 3—*Temperature dependence of the dispersion rate of I in hexane* (○) *and heptane* (●).

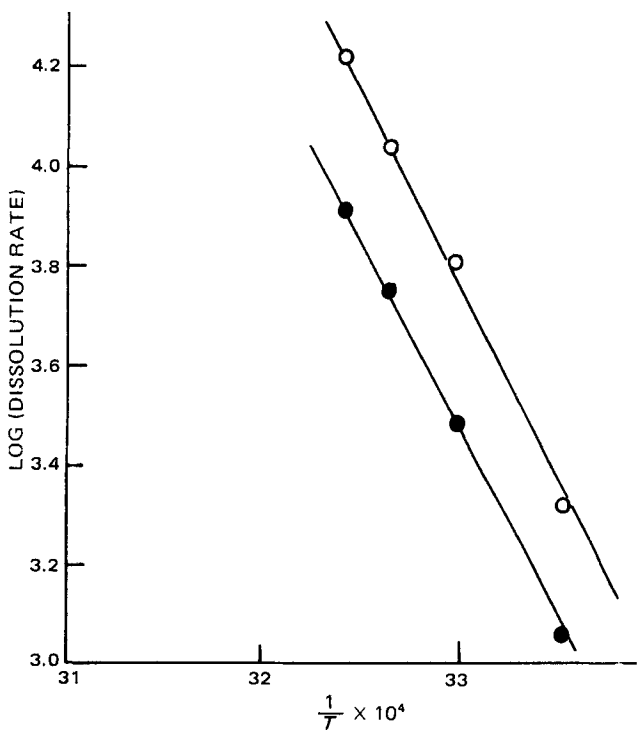


Figure 4—*Temperature dependence of the dissolution rate of I in hexane* (○) *and heptane* (●).

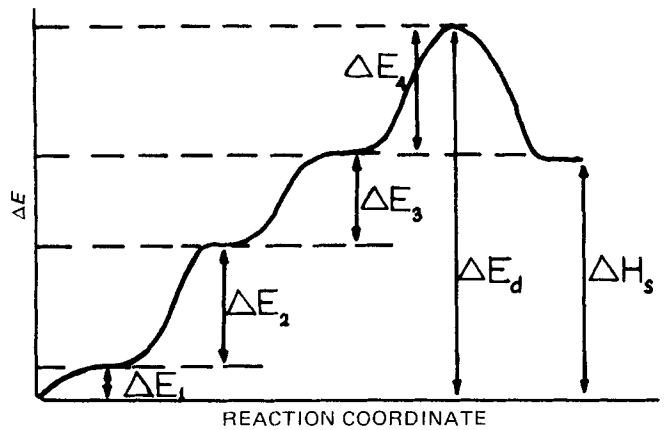


Figure 5—*Energy diagram for the dissolution of I.*

of ΔE_d based on the proposed energy diagram is quite good, considering the errors inherent in the graphical determination of energy values. This finding indicates that the proposed scheme of dissolution should reflect accurately the overall mechanism of the dissolution process. The sum of the energy terms of ΔE_1, ΔE_2, and ΔE_3, all of which are related to the process at the interface between the solid and solvent, is larger than ΔE_4, which is related to the dispersion process. This finding may also indicate that the energy barrier for dissolution of I is the interaction between the solvent and solid at the interface and not the mass transfer of solute into the bulk solution.

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Effect of Laboratory Light on Tetrazolium Reaction and on Stability of Formazans in Various Solvents

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Abstract □ Studies on purified formazans of blue tetrazolium and triphenyltetrazolium showed that ordinary laboratory fluorescent light of about 52-foot-candle intensity provided sufficient energy for phototransformation in certain nonpolar solvents. Slow reoxidation of formazan to tetrazolium was observed in all solvents tested in the presence or absence of light. Water greatly inhibited the oxidation of the red formazan of blue tetrazolium. The blue formazan of blue tetrazolium isomerized to the red formazan in protic solvents in the presence and absence of light. The photochromic phenomena for the blue formazan of blue tetrazolium and the formazan of triphenyltetrazolium in cyclohexane were completely reversible when exposed to alternate periods of intense light and darkness. Under irradiation, the formazans of blue tetrazolium and triphenyltetrazolium in chloroform solution formed their respective photoformazans; darkness reversed the process, and continued exposure of the same solutions for extended times or at a higher light intensity created a solution whose spectrum was no longer reversibly affected by the presence or absence of light. When alcohol USP is used as the solvent for the quantitative determination of corticosteroids, no light precautions need be observed, although reagent blanks have higher absorbances than light-protected solutions. Exact compensation is made by running samples and standards *versus* the reagent blank as recommended by the USP. When other solvents and/or tetrazolium reagents are used, special precautions may be required to keep the reagents, developing solutions, and final formazans protected from light to ensure a quantitative reaction.

Keyphrases □ Tetrazolium reagents—effect of laboratory light, various solvents □ Formazans—of blue tetrazolium and triphenyltetrazolium, stability, effect of laboratory light, various solvents □ Phototransformation—formazans of blue tetrazolium and triphenyltetrazolium, effect of various solvents □ Stability—formazans of blue tetrazolium and triphenyltetrazolium, effect of laboratory light, various solvents

The blue tetrazolium reaction is widely used for the analysis of corticosteroids. USP XIX (1) and NF XIV (2) indicate a slightly modified Mader and Bück procedure (3) for corticosteroid analysis. Blue tetrazolium (I) [3,3′-(3,3′-dimethoxy -4,4′- biphenylylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride)] and triphenyltetrazolium (II) (2,3,5-triphenyl-2*H*-tetrazolium chloride) oxidize the α-keto moiety of the C_{17} side chain in strongly alkaline solution and are reduced quantitatively to highly colored formazans, which are measured spectrophotometrically.

The analytical procedure is subject to several variables such as temperature (4–6), solvent (1–3, 7–13), and concentrations of base (14), water (6, 14), and tetrazolium (6), as well as the steric configuration of the corticosteroid molecule (15). The effect of these variables is minimized by analyzing reagent blanks, standards, and samples concurrently.

Light may or may not influence the tetrazolium reaction. For example, both II (16, 17) and II formazan (10, 12, 16–19) were reported to be light sensitive. Photochemical reoxidation of II formazan to the tetrazolium salt in the presence of intermittent UV irradiation was observed (20). The I formazan was reported (21) to be photosensitive in chloroform solution, and the sensitivity of I to light in 60% chloroform was observed (11) to present problems in the assay. The use of actinic glassware was recommended in