mole for KCN and 69 to 76 kcal. for KCNS. The mean value, 72 kcal./mole, is in good agreement with the present calculation, although the temperature dependence of their results creates some uncertainty.

Negative Ions by Resonance Capture. X^-.—Figure 1 shows an ionization efficiency curve for the Br^- ion from cyanogen bromide. The small resonance peak near zero volts may be due to a small HBr impurity. The main peak has an onset of 4.1 ± 0.3 ev. In the case of the iodide no peak was observed near zero volts, the main peak having an onset of 2.6 ± 0.3 ev. On the other hand the chloride had a large peak at or near zero volts and a second peak with an onset of 6.7 ± 0.3 ev. We are not certain as to the origin of the first Cl^- maximum.

If the process is represented by

$$CNX \longrightarrow CN + X^-$$

the appearance potential can be calculated from

$$A(X^-) = \Delta H_f CN + \Delta H_f X^- - \Delta H_f CNX + E_e + E_{k.e.}$$

For $E_{k.e.}$ and E_e zero, the values so obtained are −0.04, −0.43 and −0.43 ev. for the chloride, bromide and iodide respectively. This would indicate that the measured values contain from 2.2 to 6.7 ev. excess energy.

CN^-.—This ion is formed by the process

$$CNX \longrightarrow CN^- + X$$

The onset for this process is nearly zero volts for the bromide and iodide and about 0.4 ev. for the chloride.

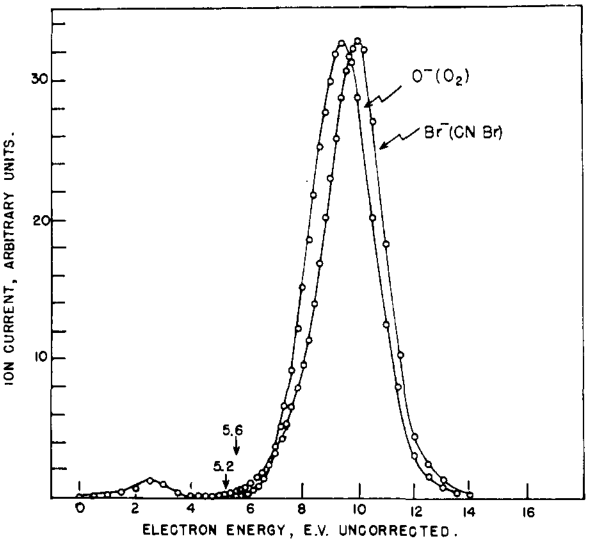

Fig. 1.—Ionization efficiency curve for Br^- from CNBr.

The appearance potential can be calculated from

$$A(CN^-) = \Delta H_f CN^- + \Delta H_f X - \Delta H_f CNX$$

assuming no excess energy is involved in the dissociation. The calculated values are 0.6, 0.0 and −0.4 ev. for the chloride, bromide and iodide, respectively. The experimental and calculated values are sufficiently close to indicate that only relatively small amounts of excess kinetic energy can be involved in the dissociation.

Summary

Appearance potential measurements of both positive and negative ions of the cyanogen halides lead to consistent values for the heat of formation of the CN radical of 89 kcal./mole and of the CN^- ion of 15 kcal./mole. Thus the ionization potential of the CN radical is 337 kcal. (14.6 ev.) and the electron affinity is 74 kcal. (3.21 ev.).

WASHINGTON, D. C.

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, KYUSHU UNIVERSITY]

Effect of Temperature on Ultraviolet Absorption Spectra of Benzoic Acids and its Relation to Hydrogen Bonding

BY MITSUO ITO, HIDEO TSUKIOKA AND SUNAO IMANISHI

RECEIVED AUGUST 18, 1959

The near ultraviolet absorption spectra of benzoic acids in petroleum ether were measured at various temperatures ranging from +100 to −180°. At low temperatures absorption bands due to dimers appear at wave lengths greater than those of monomer bands for all the substances investigated. Effect of hydrogen bonding on the absorption spectra of benzoic acid was also studied at low temperatures for the solution systems containing proton donating or accepting molecules. The results show that the direction of spectral shift due to hydrogen bond formation is determined whether benzoic acid behaves as a proton donor or a proton acceptor in hydrogen bonding. The observed spectral shifts are discussed qualitatively.

Introduction

In a previous paper it was reported that absorption spectra of benzoic acids near 2800 Å. show a remarkable effect of concentration in non-polar hydrocarbon solvents which was interpreted in terms of equilibria between monomer and dimer forms of solute molecules.[1] Hydrogen bonding, through which benzoic acids associate into dimers, is in general a weak binding and its effect on electronic absorption spectra is usually small. To be able to estimate such a small effect, the electronic absorption bands must be sharp enough. Unfortunately, the absorption spectrum of benzoic acid in the region near 2800 Å. is considerably broadened and exhibits only a very vague vibrational structure even with a solution in a non-polar solvent, contrary to the cases of other mono-substituted benzenes which exhibit generally sharp absorption bands.

In order to investigate this apparently small effect of hydrogen bonding on the absorption spectra of benzoic acids, measurements were made at low temperatures by utilizing the fact that

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(1) M. Ito, *J. Mol. Spectroscopy*, in press.

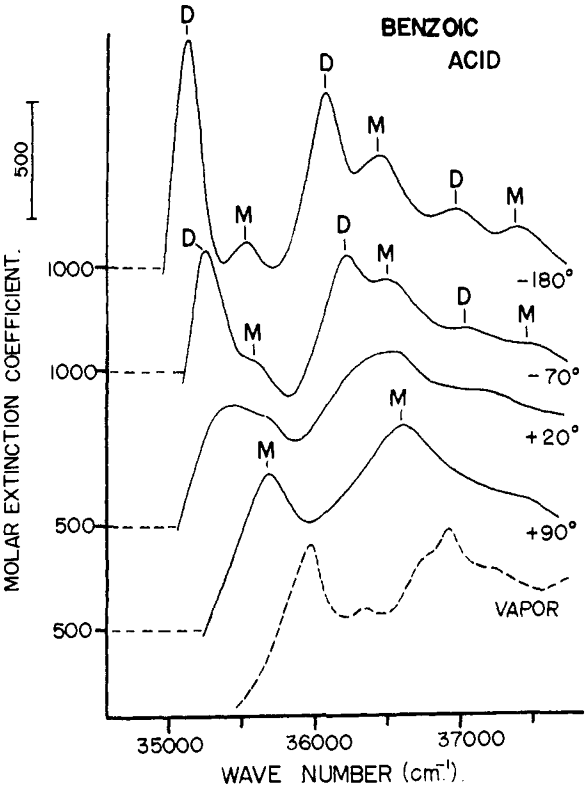

Fig. 1.—Effect of temperature on the absorption spectrum of benzoic acid in petroleum ether: concn. of benzoic acid, 2×10^{-4} mole/l.

absorption bands in general get sharper at low temperatures.[2] An additional advantage of low temperature absorption measurements is that a formation of hydrogen bond is favored at reduced temperatures.

Experimental

Ultraviolet absorption spectra were measured with a Hitachi model EPS automatic recording spectrophotometer. Although this spectrophotometer is constructed for double-beam working, it was used here as a single-beam instrument because the low temperature absorption cells used were originally designed only for single-beam usage. Recording of a spectrum was first made with the solution and then with the pure solvent under nearly the same conditions. Molar extinction coefficient was calculated from the two curves thus obtained. Slit width was kept constant during a run and was controlled below 0.1 mm. for all measurements.

Two absorption cells were used. One was a cell described in detail in a previous paper,[2] which was used for measurements above the Dry Ice temperature. Another cell was one specially designed for the liquid air temperature, of which the principal features are nearly the same as those described by Potts.[3]

Petroleum ether used as a solvent was purified by a method similar to that described for *n*-hexane in a previous paper.[2] It remained liquid above −100°, below this temperature it solidified into rigid glass which was stable even at the liquid air temperature. Best commercially obtainable samples of benzoic acid and its derivatives were used after further purification by recrystallization, sublimation or distillation.

Result and Discussion

1. Dimerization of Benzoic Acids.—The absorption spectrum of benzoic acid was measured in petroleum ether at various temperatures. Concentrations of solutions were kept constant at 2×10^{-4} mole/l. for a series of measurements. The results obtained are shown in Fig. 1, in which the curves for different temperatures are shifted vertically to show the effect clearly. The broken line curve in the figure is for the vapor spectrum. It is noted that the absorption spectrum as a whole tends to shift to the red with decreasing temperature. This type of temperature effect is usually observed and is ascribed to a change in refractive index of the solvent. In addition to this, another significant spectral change is seen. At low temperatures new absorption bands appear at wave lengths greater than the maxima of main bands observed at +90° and the intensity of new bands increases progressively as the temperature is lowered at the expense of the intensity of main bands. In Fig. 1 the original main bands are marked M and the new bands D. On the other hand no such spectral changes were observed in similar experiments for the solution of methyl benzoate, which possesses a methoxyl group replacing the OH group of benzoic acid.

These observations suggest that D bands are to be assigned to benzoic acid dimer linked by hydrogen bonds and M bands to benzoic acid monomer. This assignment is quite in accord with our previous conclusion that the absorption bands of the dimer molecule lie at wave lengths greater than those of monomer bands.[1] Relative intensities of the monomer and dimer bands at a given temperature may be taken as a measure of abundances of the two species at that temperature. Fig. 1 indicates that a considerable amount of monomer exists even at −180°. An explanation for this unexpectedly large intensity of the monomer band at this low temperature may be that the dimerization of benzoic acid does not proceed normally below the temperature at which petroleum ether solvent becomes glassy and the equilibrium concentration is not obtained because of the high viscosity of the solvent at this temperature.

Experiments for the temperature dependence also were made for some derivatives of benzoic acid. The results obtained for *o*-, *m*- and *p*-toluic acids are shown in Figs. 2, 3 and 4, respectively, together with their vapor spectra. Absorption measurement at the liquid air temperature failed for *o*- and *p*-toluic acids owing to the small solubility of these substances in petroleum ether. As seen from the figures, all toluic acid isomers show a temperature effect quite similar to that for benzoic acid, indicating the formation of dimers at low temperatures. On the other hand, no appreciable effect could be found for *o*-chlorobenzoic acid.[4] This probably is due to the special circumstance that in this case an intramolecular hydrogen bond between the Cl atom and the O atom of the carboxylic OH group possibly is formed, hindering intermolecular hydrogen bonding. Positions of 0–0 bands at the longest wave length end of each spectrum of the substances investigated are collected in Table I, where monomer and dimer bands are listed in separate columns. Numbers in the 7th column refer to the tendency for dimerization

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(2) M. Ito, *J. Mol. Spectroscopy*, in press.

(3) W. J. Potts, *J. Chem. Phys.*, **21**, 191 (1953).

(4) Experiments for *m*- and *p*-chlorobenzoic acids failed on account of their small solubility.

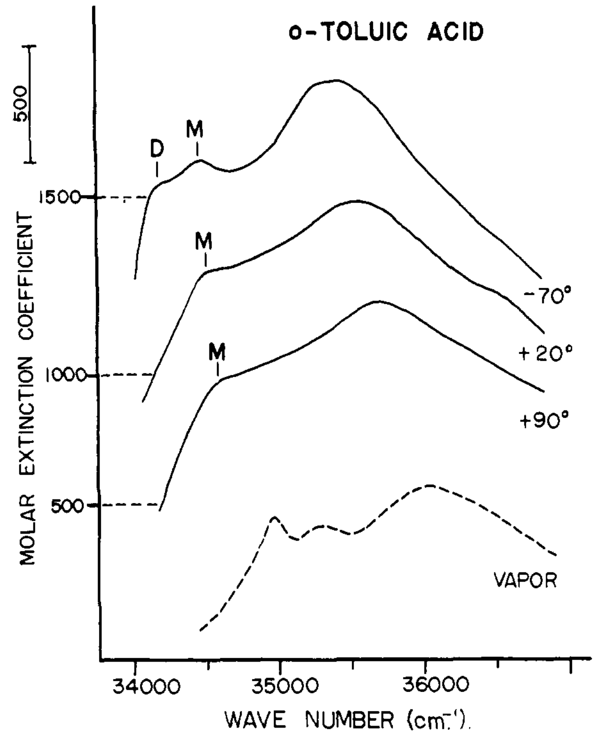

Fig. 2.—Effect of temperature on the absorption spectrum of *o*-toluic acid in petroleum ether: concn. *o*-toluic acid is 2×10^{-4} mole/l.

roughly determined by comparing relative intensities of monomer and dimer bands for each substance under nearly the same conditions of concentration and temperature. It is greatest for benzoic acid and diminishes for *p*-, *m*- and *o*-toluic acid in that order, which coincides with the order of decrease of the vapor state 0–0 band frequency. The 0–0 bands of dimers always appear at wave lengths larger than those of corresponding monomer bands. Wave number differences between the monomer and dimer 0–0 bands are listed in the last two columns of the table. They represent differences in stabilization energy in the electronic ground and excited states by dimer formation. The observed red shifts indicate that stabilization is greater in the electronically excited state than in the ground state. In general, for a system involving hydrogen bonds stabilization energy of the solute molecule mainly comes from hydrogen bonding energy in each electronic state.[5] Then the greater stabilization in electronic excited states means that the hydrogen bond energy is greater in excited states than in ground states. Beside the above contribution of hydrogen bond energy, another important contribution to the frequency shift must be taken into account. It arises from a change of the electronic structure of the solute molecule consequent to hydrogen bonding, which will be discussed in section 3.

2. Hydrogen Bonding Between Benzoic Acid and Proton Accepting or Donating Molecules.—Benzoic acid has two functional groups OH and C=O, the former acting as a proton donating group in forming a hydrogen bond and the latter as a proton accepting group. Which property of these

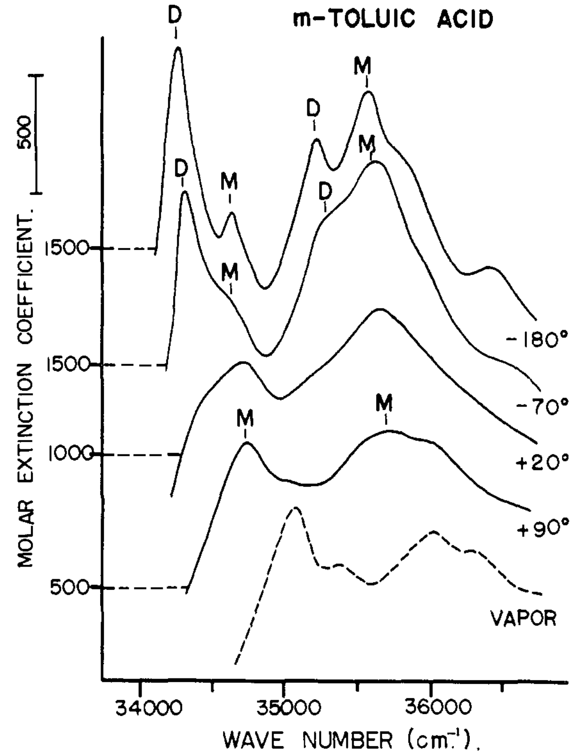

Fig. 3.—Effect of temperature on the absorption spectrum of *m*-toluic acid in petroleum ether: concn. *m*-toluic acid is 2×10^{-4} mole/l.

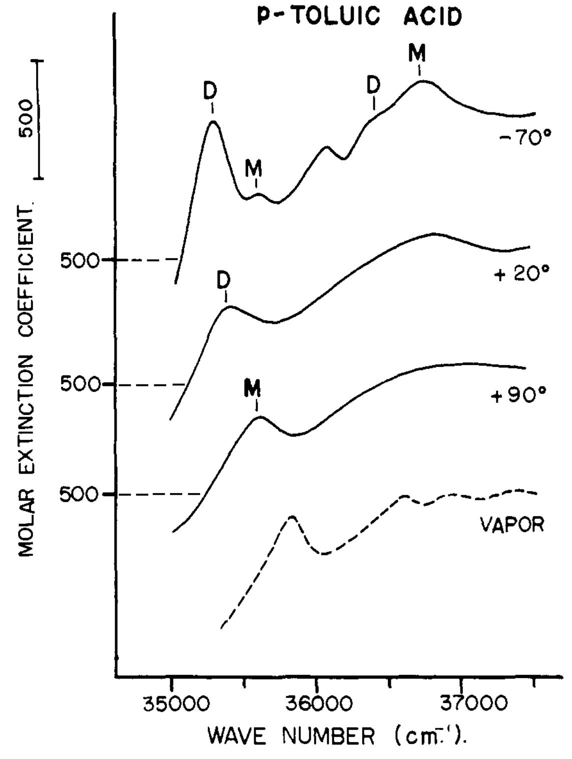

Fig. 4.—Effect of temperature on absorption spectrum of *p*-toluic acid in petroleum ether: concn. *p*-toluic acid is 2×10^{-4} mole/l.

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(5) H. Baba, *Monogr. Res. Inst. Appl. Elec. Hokkaido Univ.*, 61 (1954).

TABLE I

0–0 BANDS OF BENZOIC ACIDS AND FREQUENCY DIFFERENCE BETWEEN 0–0 BANDS OF MONOMER AND DIMER (CM.$^{-1}$)

		0–0 band						
		Monomer		Dimer				
	Vapor	Solution (−70°)	Glass (−180°)	Solution (−70°)	Glass (−180°)	Order of degree of assoc.	ν(dimer) − ν(monomer) at −70°	at −180°
Benzoic acid	35999	35526	35438	35238	35053	1	−290	−390
o-Toluic acid	34955	34628	...	34237	...	4	−390	...
m-Toluic acid	35090	34592	34532	34319	34178	3	−270	−350
p-Toluic acid	35857	35602	...	35288	...	2	−310	...

oppositely functioning groups predominates in actual cases depends on the donating or accepting property of the partner molecule in hydrogen bonding. In this section we will consider first the effect of hydrogen bonding in which benzoic acid acts as a proton donor.

Fig. 5 shows the effect of ethyl ether on the absorption spectrum of benzoic acid at −70°. It is seen that addition of a small amount of ethyl ether weakens monomer bands and at the same time brings out new bands on the blue side whose intensity increases with increasing concentration of ethyl ether, while dimer bands are little affected by the presence of ethyl ether. On the other hand no such spectral changes are observed for the methylbenzoate–ethyl ether system. These observations indicate that the blue-shifted bands are due to the hydrogen bonded species

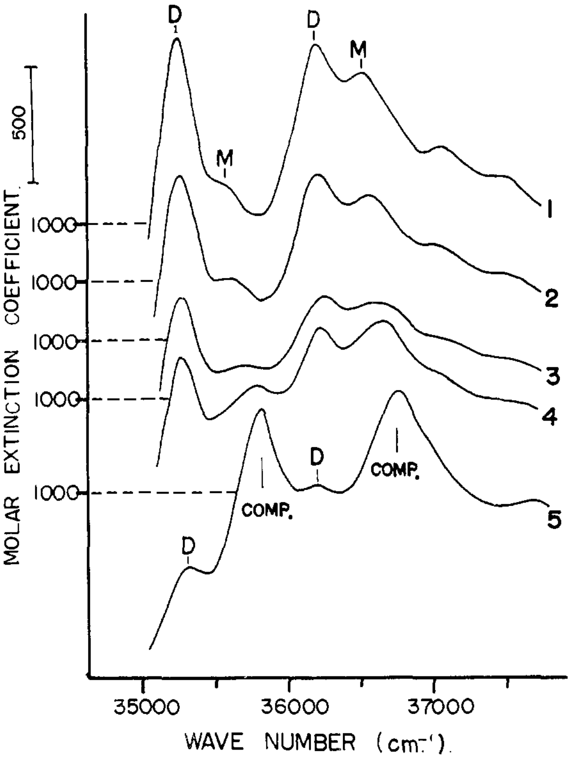
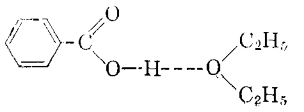


Fig. 5.—Effect of ethyl ether on the absorption spectrum of benzoic acid at −70°: concn. of benzoic acid, 2.2×10^{-4} mole/l.; concn. of ethyl ether (mole/l.): (1) 3×10^{-4}, (2) 6.7×10^{-4}, (3) 1.3×10^{-3}, (4) 2.7×10^{-3}, (5) 1×10^{-2}.



Similar complexes are also formed between benzoic acid and dioxane and between benzoic acid and methyl acetate. Their absorption bands are again blue-shifted with respect to monomer bands. The frequency shifts of these complex bands relative to monomer bands are given in Table II.

TABLE II

FREQUENCY SHIFTS DUE TO HYDROGEN BONDING

Proton donor	Proton acceptor	$\nu_{compl.} - \nu_{free}$ (cm.$^{-1}$)
Benzoic acid	Ethanol	+ 40
Benzoic acid	Methyl acetate	+ 90
Benzoic acid	Dioxane	+140
Benzoic acid	Ethyl ether	+230
Ethanol	Benzoic acid	−100[a]
Trichloroacetic acid	Benzoic acid	−900[a]

[a] The values for methyl benzoate.

As mentioned previously the C═O group of benzoic acid can act as a proton accepting group in hydrogen bonding with a proton donating molecule such as ethanol. In this case many types of hydrogen bonded structures are conceivable, of which some of the more probable ones are shown in Fig. 6.

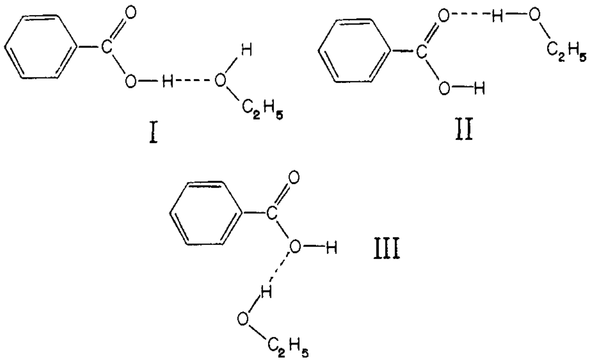


Fig. 6.

We first consider those structures which are more likely to be realized in actual cases. Fig. 7 shows the absorption spectra at low temperatures for the system containing a small amount of ethanol in the solution of benzoic acid. By addition of ethanol the intensity of dimer bands greatly decreases, while that of monomer bands, besides being accompanied by a small blue shift, increases. These spectral changes are essentially similar to those described above for the benzoic acid–ethyl ether system, favoring structure I in Fig. 6 for the hydrogen bonded complex. However, as the magnitude of the blue shift is extremely small, it is better not to assign a definite structure. In view of this ambiguity the benzoic acid–ethanol system is not quite suited for observing the effect of hydrogen

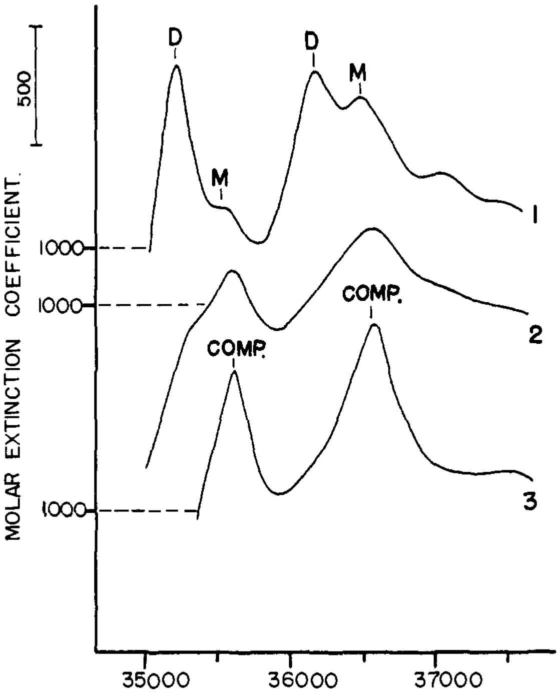
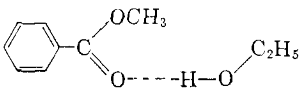
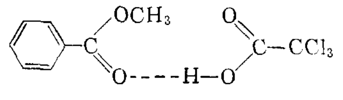


Fig. 7.—Effect of ethanol on the absorption spectrum of benzoic acid at −70°: concn. of benzoic acid, 2 × 10^{-4} mole/l.; concn. of ethanol (mole/l.): (1) 0, (2) 4 × 10^{-3}, (3) 2 × 10^{-2}.

bond attached to the C=O group of benzoic acid. As an alternative we chose the methyl benzoate–ethanol system, in which a complex with a structure analogous to I is excluded; however, in this case again neither large effect nor a definite evidence of complex formation was obtained. However, from an infrared measurement of the C=O vibration frequency of methyl benzoate in solution containing ethanol we could obtain a definite evidence for complex formation. From these findings we may understand the ultraviolet results obtained above in assuming that although methyl benzoate actually forms a hydrogen bonded complex such as



this type of hydrogen bonding can produce no appreciable effect on the π-electronic structure of methyl benzoate since ethanol is a weak proton donor. We next employed trichloroacetic acid as a proton donating molecule which is a more powerful proton donor than ethanol. Fig. 8 shows the effect of trichloroacetic acid on the absorption spectrum of methyl benzoate at room temperature. It is seen that new absorption bands appear at longer wave lengths by the addition of trichloroacetic acid. These bands are assigned to the hydrogen bonded complex.



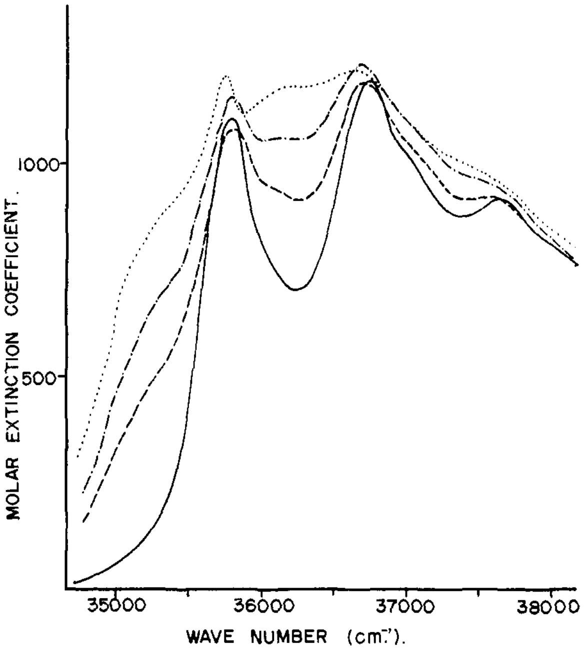


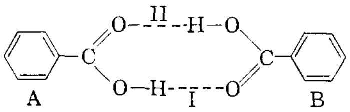
Fig. 8.—Effect of trichloroacetic acid on the absorption spectrum of methyl benzoate in petroleum ether: concn. of methyl benzoate, 7.33 × 10^{-4} mole/l.; concn. of trichloroacetic acid (mole/l.): ······ 2.43 × 10^{-1}, -·-·- 6.08 × 10^{-2}, -------- 2.03 × 10^{-2}, ——— 0.

The difference in frequencies of 0—0 bands of this complex and methyl benzoate is about 900 cm.$^{-1}$ (see Table II).

Thus the hydrogen bond attached to the O atom of the C=O group in the methyl benzoate molecule shifts the absorption spectrum toward the red with quite a large magnitude when the bonding is strong enough. From the similarity of π-electronic structures of methyl benzoate and benzoic acid, a similar spectral effect of hydrogen bonding is expected for benzoic acid also. If a benzoic acid molecule is hydrogen-bonded to a proton donating partner at the position of the O atom of the C=O group of the former, its absorption band will be shifted toward the red and the magnitude of the red shift will be determined by the proton donating power of the partner molecule.

Combining the results obtained above it is concluded that the direction of spectral shift in the absorption of benzoic acid due to hydrogen bond formation is determined by whether benzoic acid behaves as a proton donor or an acceptor in hydrogen bonding. This conclusion is quite in agreement with that obtained in our previous study of the effects of hydrogen bonds on the absorption spectra of phenols[6] and appears to be applicable to all molecules having groups capable of both donating and accepting a proton.

3. Spectral Shift Due to Dimer Formation.—It is well known that the dimer molecule of benzoic acid has the structure



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[6] M. Ito, *J. Mol. Spectroscopy*, in press.

in which two hydrogen bonds I and II link the two molecules A and B. If we confine our attention to only one of the two symmetrically arranged molecules, say A, the effects of the hydrogen bonding on its electronic structure will be the sum of two independent effects contributed by I and II. As mentioned in previous section, the hydrogen bond I causes the absorption spectrum of molecule A to shift toward the blue, while the hydrogen bond II shifts it toward the red. The observed red shift of the absorption of benzoic acid on formation of dimer indicates that perturbing interactions on the π-electronic structure of molecule A due to the bond II predominates over that due to the bond I. When the hydrogen bond is strong enough, as with benzoic acid dimer, the magnitude of the red shift due to the bond II is sufficiently large to overwhelm the blue shift due to the bond I. Since the hydrogen bond energies of I and II must be identical, the above result means that the amount of the spectral shift of absorption bands depends mainly on the type and arrangement of hydrogen bonds rather than the magnitude of their energies.

FUKUOKA, JAPAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Relaxation Spectra of Simple Enzymatic Mechanisms[1,2]

BY GORDON G. HAMMES[3] AND ROBERT A. ALBERTY

RECEIVED JUNE 1, 1959

When a reaction is close to equilibrium, the approach to equilibrium may be characterized by a spectrum of relaxation times which can be related to the rate constants involved in the mechanism.[4] The relaxation spectrum is discussed for the n-intermediate mechanism for a simple enzymatic reaction $S \rightleftarrows P$; such a reaction is characterized by $n + 1$ relaxation times. The inclusion of competitive inhibition in this mechanism is discussed. If the initial substrate concentration is much greater than the initial concentration of enzymatic sites, one relaxation time is characteristic of the steady state and it may be evaluated in terms of the Michaelis constants and maximum velocities. For an n-intermediate mechanism, the steady state relaxation time is

$$\tau_{ss} = [1 + (\bar{s}/K_S)(1 + V_S/V_P)]/(V_S/K_S + V_P/K_P)$$

where $\bar{s}$ is the equilibrium concentration of substrate, K_S and K_P are the Michaelis constants and V_S and V_P are the maximum velocities. If competitive inhibition is included in the mechanism and $i_0 >> e_0$

$$\tau_{ss} = [1 + (\bar{s}/K_S)(1 + V_S/V_P) + i_0/K_1]/(V_S/K_S + V_P/K_P)$$

where i_0 is the total inhibitor concentration and K_I is the competitive inhibition constant. The competitive inhibition constant K_I can be obtained by determining the steady state relaxation time as a function of total substrate concentration and inhibitor concentration. Experimental results for the fumarase reaction at two pH's are presented which show the expected dependence of the long relaxation time on s_0, e_0 and i_0. In addition, one of the Michaelis constants was determined through initial velocity measurements, thus allowing calculation of K_S, V_S/V_P and K_1.

Introduction

Reaction rates usually are measured on systems far from equilibrium. However, Eigen[4] has shown how the study of reactions very close to equilibrium may be used for determining the rate constants of reactions in solution which are too rapid to permit the use of mixing methods. In this manner, Eigen and his co-workers have measured reaction rates with half times as short as 10^{-8} sec. In this method, a reaction mixture is displaced slightly from equilibrium by changing an independent variable such as pressure, electric field strength or temperature. Alternatively, if the reaction rates are sufficiently slow, one may start with a reaction mixture containing concentration ratios slightly different from those of the equilibrium mixture. When close to equilibrium, the return to equilibrium is characterized by a spectrum of relaxation times which are related to the rate constants involved in any particular mechanism. Several discussions of this type of phenomenom are now available.[5-7] This experimental approach is applicable, in principle, to the study of all types of reactions. The purpose of this paper is to describe the relaxation spectrum for an n-intermediate enzymatic reaction; the effect of including inhibition in this mechanism is discussed and detailed equations are given for the case of competitive inhibition. Experimental measurements of the steady state relaxation time for the fumarase reaction are described.

Relaxation Spectrum of an n-Intermediate Enzyme Reaction.—The familiar n-intermediate reaction mechanism to be considered is given in

$$\begin{array}{lcccccccccccc}
 & E & + & S & \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} & X_1 & \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} & \ldots X_i & \underset{k_{-(i+1)}}{\overset{k_{i+1}}{\rightleftarrows}} & \ldots X_n & \underset{k_{-(n+1)}}{\overset{k_{n+1}}{\rightleftarrows}} & E & + & P \quad (1) \\
\text{At equilibrium:} & \bar{e} & & \bar{s} & & \bar{x}_1 & & \bar{x}_i & & \bar{x}_n & & \bar{e} & & \bar{p} \\
\text{At any time } t: & \bar{e} + \Delta e & & \bar{s} + \Delta s & & \bar{x}_1 + \Delta x_1 & & \bar{x}_i + \Delta x_i & & \bar{x}_n + \Delta x_n & & \bar{e} + \Delta e & & \bar{p} + \Delta p \\
\text{At any time } t: & e & & s & & x_1 & & x_i & & x_n & & e & & p
\end{array}$$

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(2) This work was supported by the National Science Foundation and by the Research Committee of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

(3) General Electric Fellow, 1958–1959.

(4) M. Eigen, *Discussions Faraday Soc.*, **17**, 194 (1954).

(5) J. Meixner, *Kolloid Z.*, **134**, 3 (1953).

(6) M. Eigen, *Discussions Faraday Soc.*, **24**, 25 (1957).

(7) G. G. Hammes and L. E. Erickson, *J. Chem. Ed.*, **35**, 611 (1958).