[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF SIENA HEIGHTS COLLEGE AND OF THE INSTITUTUM DIVI THOMAE]

The Infrared and Ultraviolet Absorption Spectra of Cytosine and Isocytosine in the Solid State[1,2]

BY MIRIAM MICHAEL STIMSON[3] AND MARIE JOANNES O'DONNELL[3]

A method has been developed whereby the usual nujol mull employed for the study of solid organic compounds in the infrared region of the spectrum may be obviated. This method has been applied to cytosine and its isomer, isocytosine. A comparison of the absorption of these two compounds is made in the infrared and the ultraviolet regions employing a disk of solid potassium bromide, and additional comparison is made with the known solution spectra of these compounds; thus permitting the correlation of both regions of the spectrum with solution spectra.

This work was undertaken to develop a method of preparing specimens of various pyrimidines and purines for study on the same physical sample in both the ultraviolet and infrared regions of the spectrum in order: (1) that direct correlation of the absorbing entities in these regions would be possible; (2) to obviate the use of a mull, with its attendant reflection losses, particularly in the ultraviolet and short wave length end of the infrared regions of the spectrum.

It has been found possible, by making dilutions of cytosine and isocytosine in solid potassium bromide and with subsequent pressing, to prepare disks of these materials which are visually clear and of known concentration and weight. Furthermore, a comparison of the ultraviolet absorption in solution and in the solid potassium bromide, together with the infrared absorption of the solid, permits us to conclude that cytosine occurs, probably, in the enol form even in the solid state, while isocytosine occurs in the keto form, which undergoes enolization or hydrogen bonding on solution. This is indicated in the latter case by the shift in the maximum on solution as compared with the position found for the solid.

Experimental

Materials.—The potassium bromide used in making the plates is of C.P. grade, is ground to 200 mesh or better, and is then dried in an oven for several days at 110°. High temperatures must be avoided to prevent either the melting of the salt or discoloration due to partial oxidation of bromine. While this latter may not be apparent in the powder due to general reflection, it becomes readily noticeable, even visually, in the formed disk. The samples of cytosine and isocytosine were those used for solution spectra reported earlier.[4] Mixing was done in a mullite mortar with successive mixing and grinding for five minutes to ensure uniformity of distribution. That this can be achieved within the limits of sensitivity of the method is shown in Table I. The disks 53–55 were prepared from random samples of one mixing process to test the efficiency of the method of distribution.

TABLE I

Disk	Concentration, %	Weight, g.	Optical density/ % × wt.
53	0.06	0.1785	2150
54	.06	.1463	2170
55	.06	.1610	2180

In Fig. 1 is shown the transmittancy of the pressed potassium bromide as compared with that of air for the same slit width. It will be noted that the salt does not introduce any selective absorption. Most of the energy loss shown is due to the loss by reflection at the surfaces as can be seen in a comparison of disks of varying thickness of potassium bromide.

It has been found that pyrimidine concentrations ranging from 0.01 to 0.1% can be employed in disks weighing from 100 to 400 mg. Larger concentration results in uniform cloudiness with attendant energy losses. Too low a concentration reduces the accuracy with which the optical density can be measured.

The intimate mixture of pyrimidine and potassium bromide is placed in a cylindrical steel die which is then enclosed in a larger cylinder connected to a vacuum pump. The dies are made of AISI-52-100, a ball bearing material, and thoroughly hardened to 64 Rockwell "C" hardness. The pressing surfaces of the plunger and anvil are optically flat and are as square as possible with the axis. Evacuation for about five minutes at approximately 40 microns is generally sufficient for the removal of occluded air, which otherwise causes crevasses in the pressed disk after release from the die. After evacuation for about five minutes, and while the system is still being evacuated, the die is subjected to a pressure of 15 tons (dial reading) for three to five minutes. Air is then admitted to the system. Upon removal from the die, the weight of the experimental plate in terms of pure potassium bromide is determined, and a plate of pure potassium bromide is made of the same weight to the nearest mg. for use as a comparison plate.

Absorption Measurements.—All ultraviolet measurements were made using a Beckman DU spectrophotometer with a Victoreen Hi-Meg resistor in the phototube compartment. Infrared measurements were obtained by manual operation of a Beckman IR-2 spectrophotometer. In both cases the measurements were comparison readings of transmittances of the experimental disks and the potassium bromide disks of corresponding weight made at the same slit setting.

Discussion

A comparison of the ultraviolet absorption of cytosine in the solid state (Fig. 2) with that of the solution in a *p*H range from 0.1 *N* HCl to 0.1 *N* NaOH[5] shows that the long wave maximum is substantially in the same position for both states. However, a similar comparison for isocytosine (Fig. 3) shows a shift of the maximum from 286 mμ in solution to 298 mμ in a solid potassium bromide disk. It was concluded that this does not represent the influence of the potassium bromide since a disk of isocytosine dissolved in 10 ml. of distilled water has a maximum at 296 mμ. That this shifted spectrum for isocytosine is not dependent on the potassium bromide is further shown from a comparison of the data of Sinsheimer, Scott and Loofbourow[6] in which samples prepared by sublimation were studied. The fine structure shown by isocytosine in Fig. 3 represented reproducible data and was not, as far as visible appearance showed,

(1) Presented before the Division of Biological Chemistry, Chicago Meeting of the American Chemical Society, Sept., 1950.

(2) This work was aided by a grant from the Research Corporation.

(3) Sister Miriam Michael Stimson, O.P., and Sister Marie Joannes O'Donnell, O.P.

(4) M. M. Stimson and M. A. Reuter, THIS JOURNAL, **67**, 2191 (1945).

(5) M. M. Stimson, *ibid.*, **71**, 1470 (1949).

(6) R. L. Sinsheimer, J. F. Scott and J. R. Loofbourow, *J. Biol. Chem.*, **187**, 313 (1950).

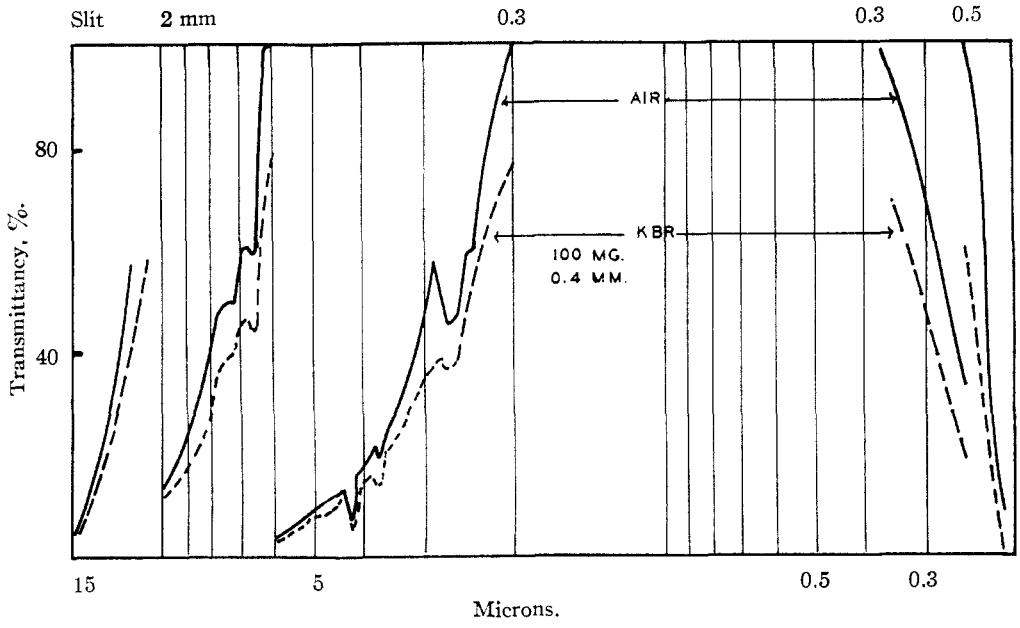

Fig. 1.

the result of the formation of a film of water on the surface.[7]

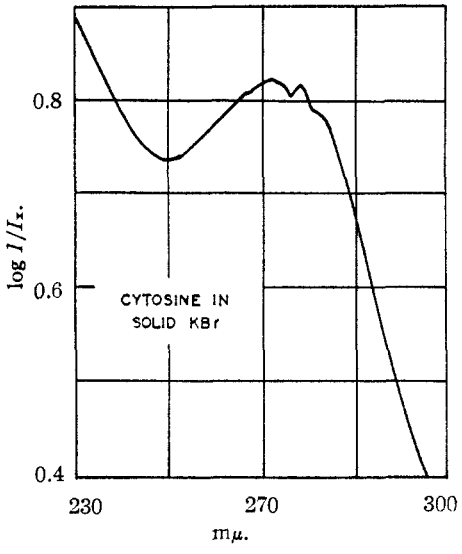

Fig. 2.

The infrared absorption of isocytosine (Fig. 4) and of cytosine (Fig. 5) were obtained by measurement of the same disks with which the ultraviolet absorption spectra were obtained. The data shown here for cytosine agree very well with those of Blout and Fields[8] with the single exception that the doublet they report at 6.7 microns was not resolved. This shows remarkably slight sample variation.[9] In a footnote these workers have suggested that their samples, prepared by sublimation, might show molecular orientation, and thus conceivably might have some frequency of enhanced and some of diminished or zero intensity. Such seems not to be the case, since samples prepared as were ours have, more probably, a random arrangement and yet show substantially the same spectrum.

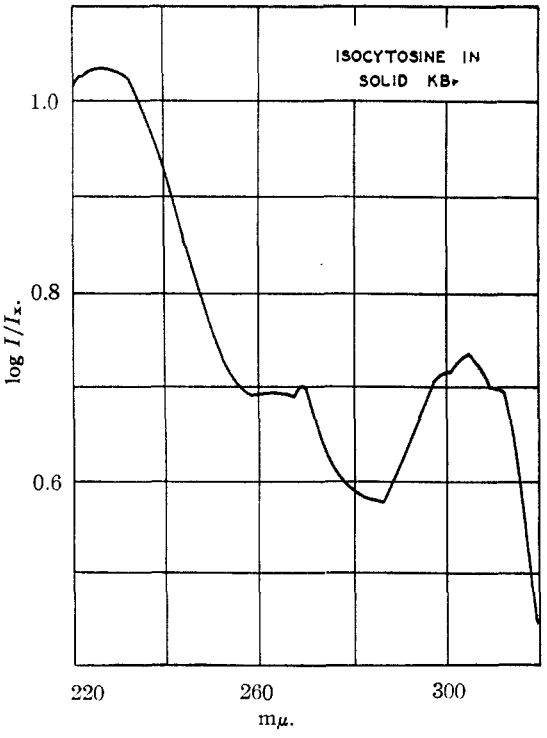

Fig. 3.

Blout and Fields suggest that the oxygen of cytosine in the solid state exists completely in association with hydrogen, either by enolization or by intermolecular hydrogen bonding. This conclusion is based on the fact that cytosine shows three intense bands in the 3-micron region, but a

(7) R. L. Sinsheimer, J. F. Scott and J. R. Loofbourow, *Nature*, **164**, 796 (1949).

(8) E. R. Blout and M. Fields, THIS JOURNAL, **72**, 479 (1950).

(9) C. C. Clark reported on three preparations of cytosine, all studied spectroscopically under the same conditions, and found differences greater than those reported here between our data and those of Blout and Fields; C. C. Clark, University Microfilms No. 18381.

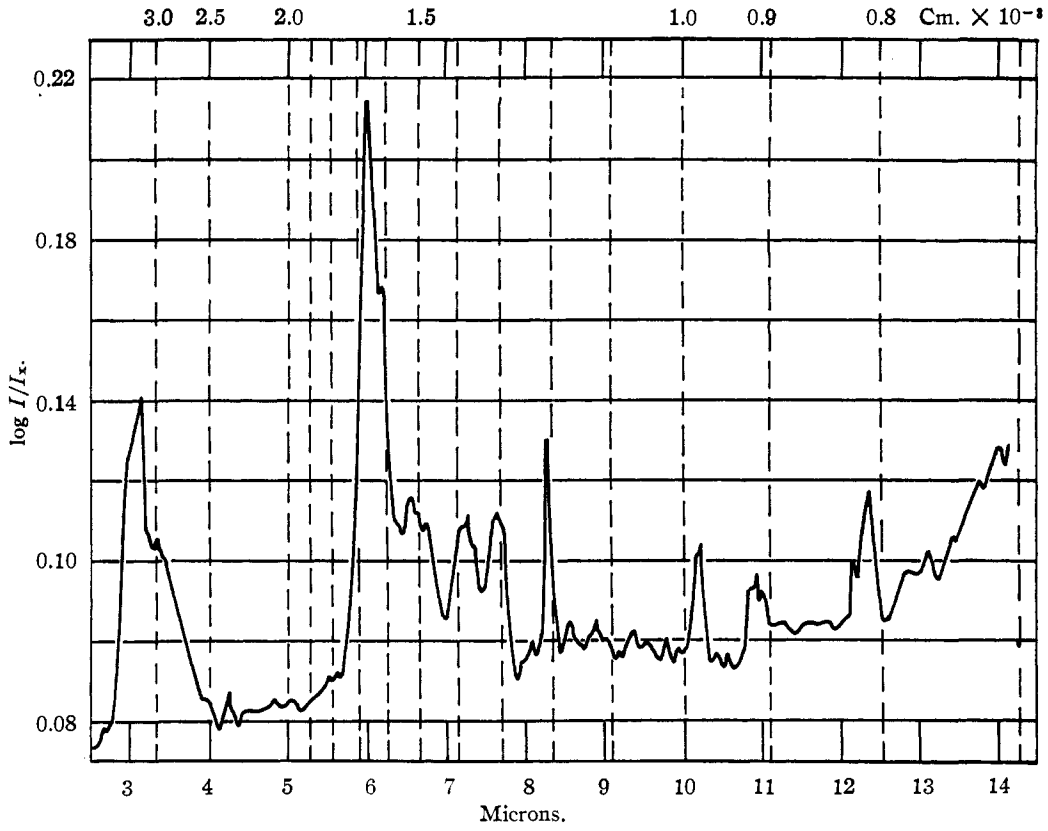

Fig. 4.—Isocytosine.

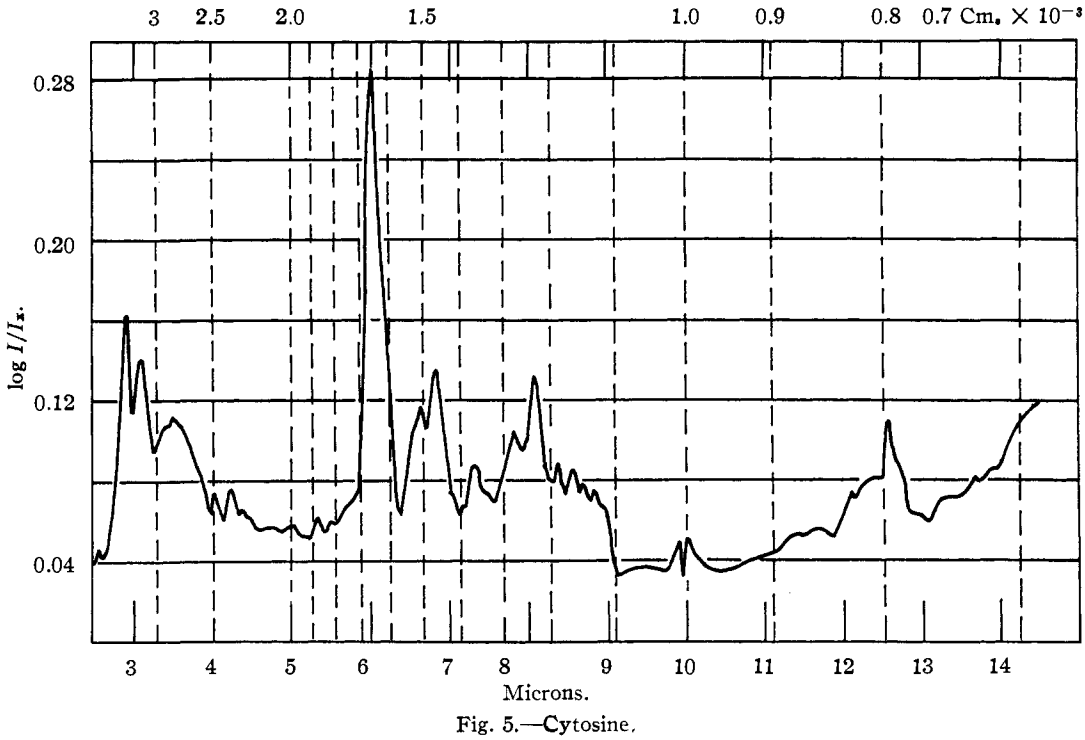

Fig. 5.—Cytosine.

singlet around 6μ. That this indicates hydrogen bonding is in accordance with the solution data for the ultraviolet which showed no change in the position of maximum over the position of λ_{max} for the solid. On the other hand, in the case of isocytosine, a singlet is found in the neighborhood of 3μ and the band at 6μ shows the amide absorption[10]; this, together with the shift found on solution in the ultraviolet region, leads to the conclusion that, in the solid state, isocytosine is in the keto

(10) The assignments of infrared frequencies are taken chiefly from H. W. Thompson, *J. Chem. Soc.*, 328 (1948).

form and undergoes hydrogen bonding on solution, thus giving rise to a different absorbing entity in solid than in solution. It will be further noted that no band shorter than 3.2 occurs for isocytosine, which would indicate the presence of a free NH rather than a free NH_2 group. This is substantiated by the failure of isocytosine to react with nitrous acid.

It is hoped that by extending the compounds investigated in solid potassium bromide, to arrive at some empirical values which may be used in analysis for concentration in mixtures and crude preparations of extracts.

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The Influence of Nitro Compounds on the Catalytic Exchange Reaction Between Deuterium Gas and Acetic Acid. Evidence for the Mechanism of the Catalytic Hydrogenation of Nitro Groups

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Studies of the kinetics of exchange between deuterium and glacial acetic acid in the presence of Adams platinum catalyst indicate that the reaction is first order with respect to the deuterium pressure and essentially zero order with respect to acetic acid concentration (in *n*-heptane over range 10–100%) and has an activation energy of about 13,600 calories. The exchange reaction is completely suppressed by the addition of a small amount of nitrobenzene, but is unaffected by the addition of nitroethane. In either case, the nitro group is deuterated to form the amine. The results are discussed in the light of the mechanism of catalytic exchange and catalytic hydrogenation reactions.

Previous studies of the hydrogenation of nitro groups have shown that nitrobenzene undergoes hydrogenation on Adams platinum catalyst in the presence of glacial acetic acid at a rate which is zero order with respect to the concentration of acceptor, but proportional to the hydrogen pressure. For nitroethane, and nitroalkanes in general, the kinetic behavior is reversed.[3] The rate of this reaction is essentially independent of the hydrogen pressure, but directly proportional to the concentration of the nitroethane in the acetic acid solvent. For both compounds the nitro group is reduced to form the amine.

The catalytic exchange between deuterium gas and glacial acetic acid containing these two acceptors has been studied with a view to obtaining further information regarding the kinetics of these two hydrogenation reactions.

Experimental

Materials.—du Pont C.P. glacial acetic acid, nitrobenzene and nitroethane were purified as in previous work.[3] Adams platinum catalyst was prepared from C.P. platinic chloride by the usual method.[4] That which passed a 325-mesh screen was used for the exchange experiments. Research grade *n*-heptane (99.87 mole per cent.) was purchased from the Phillips Petroleum Company.

Preliminary experiments with this material indicated an impurity containing exchangeable hydrogen which was no longer detected after prolonged shaking with hydrogen gas and platinum catalyst. Accordingly, the solvent was pretreated in this manner before use.

Hydrogen gas was obtained from the National Cylinder Gas Company and deuterium gas (minimum purity 99.5 per cent.) from the Stuart Oxygen Company. Both were used without further purification. Pure hydrogen for use in standardizing the analytical apparatus was obtained by liberation with clean J. T. Baker C.P. metallic sodium from glacial acetic acid.

Exchange Apparatus and Procedure.—The apparatus used for the exchange studies consisted of a 50-ml. erlenmeyer flask, the opening of which was extended by sealing on a 12-cm. length of 10-mm. tubing. One side of a two-way, straight-bore stopcock (size 6) was sealed to this unit at a point 3 cm. below the barrel. On the opposite side there was attached a socket from a standard ball and socket joint assembly. The volume of the reaction flask to the stopcock was about 67 ml.

Exchange studies were made by introducing the requisite liquids and platinum oxide through a funnel which extended through the bore of the stopcock, and evacuating the flask while it was cooled in a Dry Ice–acetone-bath. The flask was then warmed to room temperature, shaken for a few minutes, and recooled in the Dry Ice–acetone mixture. It was again evacuated, warmed to the requisite temperature, and filled with deuterium. The second evacuation was necessary in order to remove dissolved gases from the liquid. The reaction flask and contents were then placed in a jacket attached to a cradle and shaken for a definite period of time, usually three minutes. The temperature of the circulating water in the jacket was held to ±0.2°. The shaker was usually operated at about 350 cycles per minute; this was sufficient to ensure equilibrium conditions. Finally the flask was removed from the cradle and attached to the analytical apparatus by means of the ball and socket joint.

Preliminary experiments at 30° with 7 mg. of prereduced catalyst showed no exchange over a ten-minute period of time in the absence of shaking; hence some delays could be tolerated at this temperature in the handling of the mixtures before and after shaking. At 43°, however, some 16% exchange did occur on 7 mg. of prereduced catalyst over the same time interval. In the study of the temperature-dependence of the exchange, therefore, the contents were warmed to the requisite temperature before adding deuterium and placed in the jacket and shaken without delay. Within about 30 seconds the circulating water in the jacket came to the required temperature. At the end of the shak-

(1) A.E.C. Predoctoral Fellow. Present address: E. I. duPont de Nemours & Co., Oak Ridge National Laboratory, Oak Ridge Tennessee.

(2) Work supported by the Atomic Energy Commission.

(3) H. A. Smith and W. C. Bedoit, *J. Phys. Colloid Chem.*, **55**, 1085 (1951).

(4) R. Adams, V. Voorhees and R. L. Shriner, *Org. Syntheses*, **8**, 92 (1928).