[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SIENA HEIGHTS COLLEGE]

The Ultraviolet Absorption Spectra of Cytosine and Isocytosine[1,2]

BY MIRIAM MICHAEL STIMSON AND MARY AGNITA REUTER[3]

The very pronounced changes in the spectrum of guanine with *p*H[4] as well as the modification of the spectrum of isoguanine[5] led us to expect dissimilar spectra of isocytosine and cytosine as well as marked but different response to *p*H. The spectra in aqueous solution of these materials as reported by Heyroth and Loofbourow[4] are substantially identical. We have, therefore, reinvestigated all of these compounds.

Experimental

Materials.—Guanine hydrogen chloride (Eastman Kodak Co.) was purified several times by precipitation from ammoniacal solution with dilute hydrochloric acid. Cytosine was prepared from uracil by the method of Hilbert and Johnson[6] and used as the monohydrate. It darkened about 280° and had completely decomposed at 300° (theoretical; darkens below 300° and decomposes 320–325°). Isocytosine was prepared according to Caldwell and Kime[7] from guanidine and melted at 275°. The sample of 2-aminopyrimidine was kindly furnished by Dr. R. C. Roblin of the American Cyanamid Company. 1,2,4,6-Tetrahydro-4-imino-6-ketopyrimidine-4-acetic acid was prepared by the late Dr. D. E. Worrall.

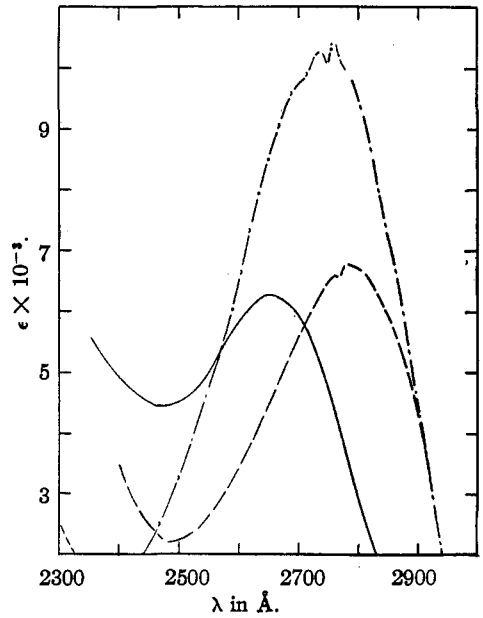


Fig. 1.—Cytosine·H_2O: ———, unbuffered; — — —, acid; — —, base.

Method.—The spectra reported were determined on a Beckman DU spectrophotometer in water, 0.1 *N* hydrochloric acid, and 0.1 *N* sodium hydroxide.

Results and Discussion

The data for cytosine (Fig. 1, Formula 1) agree well with those reported by Heyroth and Loofbourow for the aqueous solution. The pronounced strengthening of the absorption with increasing acidity, however, was unexpected on the basis of previous work[8] with the analogously substituted purine. The change is of the type found to a lesser degree in 2-chloro-6-aminopyrimidine.[9]

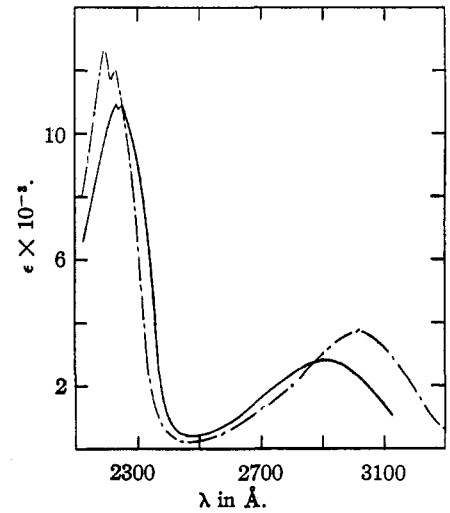


Fig. 2.—2-Aminopyrimidine: ———, unbuffered, base; — — —, acid.

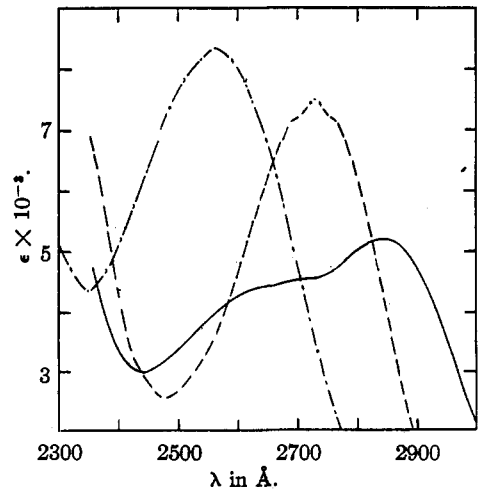


Fig. 3.—Isocytosine: ———, unbuffered; — — —, acid; — —, base.

(1) Part IX. The Ultraviolet Absorption Spectra of Nitrogenous Heterocyclic Compounds.

(2) This work was conducted as part of the research program of Institutum Divi Thomae, with which this Laboratory is affiliated.

(3) Sister Miriam Michael Stimson, O.P., and Sister Mary Agnita Reuter, O.P.

(4) Holiday, *Biochem. J.*, **24**, 619 (1930); Heyroth and Loofbourow, THIS JOURNAL, **56**, 1728 (1934).

(5) Stimson and Reuter, *ibid.*, **64**, 1604 (1942).

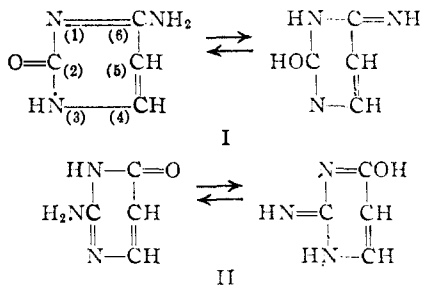
(6) Hilbert and Johnson, *ibid.*, **52**, 1152 (1930).

(7) Caldwell and Kime, *ibid.*, **62**, 2365 (1940).

(8) Stimson and Reuter, *ibid.*, **64**, 1604 (1942).

(9) Stimson and Reuter, *ibid.*, **63**, 1827 (1941).

On the other hand, a similar study of 2-amino-6-chloropyrimidine or of 2-aminopyrimidine (Fig. 2) shows that the two maxima at 2250 and 2900 Å., respectively, remain separated under the three conditions of *p*H investigated, and that in acid reaction the short wave band is somewhat shortened while the long wave maximum moves to slightly longer wave lengths. An examination of the spectra of isocytosine (Fig. 3, Formula II) reveals several points of interest. In none of the media in which it was examined is the spectrum identical to that obtained under corresponding conditions for cytosine. While the spectrum of cytosine has but one sharp maximum in any of the three media the broad band of isocytosine in aqueous solution can be broken down into two bands with centers at 2650 and 2850 Å., respec-



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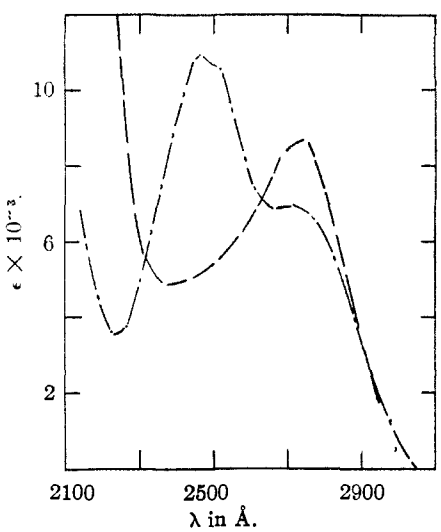

Fig. 4.—Guanine.HCl: ——, base; — — —, acid.

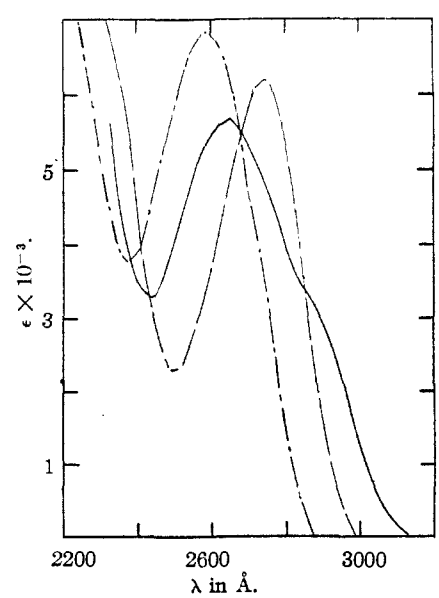

Fig. 5.—Isocytosine-4-acetic acid: ———, unbuffered; — — —, acid; ——, base.

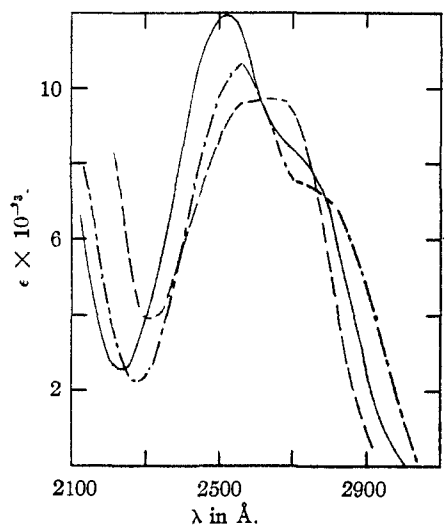

Fig. 6.—Guanosine: ———, unbuffered; — — —, acid; ——, base.

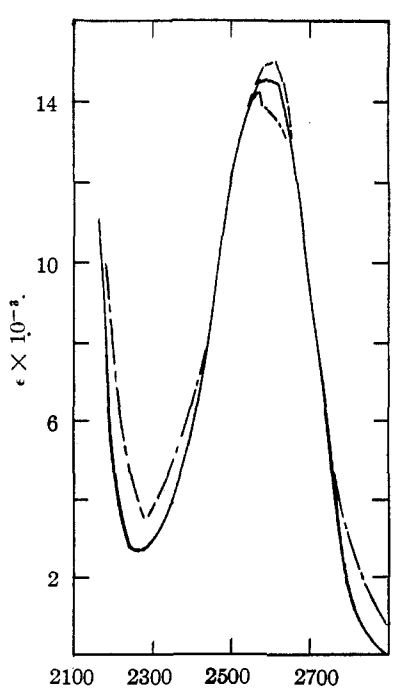

Fig. 7.—Adenosine: ———, unbuffered; — — —, acid; ——, base.

tively. The shorter of these two bands approximates the type of change produced in the short wave band of 2-aminopyrimidine with variations in *p*H, although the effect is much more pronounced by reason of the introduction of the hydroxy group into the molecule. It will be noted from a comparison with Fig. 4 that the wave length separation of the peaks in acid and basic solution is approximately the same in guanine under the same experimental conditions. A further indication that the spectra of isocytosine are as reported in this communication, is seen in the data on isocytosine-4-acetic acid (1,2,4,6-tetrahydro-2-imino-6-ketopyrimidine-4-acetic acid), where, even not considering the influence of the acid, it will be noted (Fig. 5) that in aqueous solution the broad band can be resolved into two bands with centers at 2630 and 2820 Å., respectively, and that the shorter of the two shifts with *p*H as does that of isocytosine. Finally on the assumption that the absorption of these compounds is, to at least a certain extent, additive, the absorption curve of 2-aminopyrimidine and that of 2,4-dimethyl-6-hydroxypyrimidine[10] were combined and gave a curve similar to that of isocytosine. A like addition of 4,6-dimethyl-2-hydroxypyrimidine and 6-aminopyrimidine results in a curve indicative of cytosine in aqueous solution.

(10) Williams, Buchman and Ruehle, THIS JOURNAL, **57**, 1093 (1935).

The discrepancy between these results and those of Heyroth and Loofbourow might be attributed to the possibility of contamination of products when the isomers are prepared simultaneously. Since the isocytosine is the less stable it could very easily be lost. Personal communication with Dr. Heyroth shed no light on this, and unfortunately there is none of the original samples from which comparison might be made under present conditions.

As was pointed out in the work on thymine desoxyriboside[11] the nucleoside does not show up as markedly as does the free base. However, guanosine does present under varying *p*H a much more characteristic picture (Fig. 6) than the former, and in biologic extracts may be differentiated from adenosine (Fig. 7)[12] as well. In conclusion it may be remarked that a general similarity can be traced through the various 2-aminopurines and pyrimidines, which is much more pronounced than a similar comparison involving the 6-amino compounds.

Summary

The ultraviolet absorption spectra of cytosine and isocytosine are not of similar type.

(11) Stimson and Reuter, *ibid.*, **67**, 847 (1945).

(12) Loofbourow, Cook and Stimson, *Nature*, **142**, 573 (1938); Cook, Loofbourow and Stimson, Xth Intern. Cong. Chem., Rome, May, 1938; Cook, Hart and Stimson, *Biochem. J.*, **34**, 1580 (1940).

ADRIAN, MICHIGAN RECEIVED MAY 28, 1945

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Isomerization Accompanying Alkylation. IV.[1] Reaction of Cycloheptanol and Cycloheptene with Benzene. Synthesis of Cycloheptylbenzene

BY HERMAN PINES, ALEXANDER EDELEANU[2] AND V. N. IPATIEFF

In the preceding paper of this series it was shown that a skeletal isomerization of the alkyl group during alkylation of aromatic hydrocarbons may take place; this work has now been extended to include cycloalkylation. The present paper deals with the reaction of cycloheptanol and cycloheptene with benzene using, respectively, aluminum chloride, hydrogen fluoride and sulfuric acid as catalysts.

Sidorova and Tsukervanik[3] reported that cycloheptanol on reaction with benzene in the presence of aluminum chloride yielded cycloheptylbenzene. These authors compared the monoacetamino and monobenzamino derivatives of the obtained hydrocarbon with that of synthetically prepared 1-methyl-2-phenylcyclohexane and phenylcyclohexylmethane. Since the melting point of these derivatives did not compare with each other they concluded that the hydrocarbon they obtained was cycloheptylbenzene. In view of the fact that cycloheptane undergoes isomerization to methylcyclohexane[4] in the presence of aluminum chloride and that the formation of cycloheptylbenzene was not proved conclusively by Sidorova and Tsukervanik it was decided to reinvestigate this reaction.

In order to determine whether cycloheptylbenzene was produced in the reaction studied, this hydrocarbon was prepared synthetically and its physical constants, infrared absorption spectra and solid derivatives compared with those obtained from cycloalkylation.

By repeating the experiment of Sidorova and Tsukervanik it was found that the cycloalkylation of benzene with cycloheptanol in the presence of aluminum chloride was accompanied by isomerization. Contrary to the statement made

(1) For the preceding paper of this series see H. Pines, L. Schmerling and V. N. Ipatieff, THIS JOURNAL, **62**, 2901 (1940).

(2) At present with the United States Army.

(3) N. G. Sidorova and I. P. Tsukervanik, *J. Gen. Chem.* (*U. S. S. R.*), **10**, 2073 (1940).

(4) M. B. Turova-Polak and F. P. Sidelkovskaya, *ibid.*, **11**, 817 (1941).