

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

The Effect of pH on the Spectra of Thymine and Thymine Desoxyriboside^{1,2}BY MIRIAM MICHAEL STIMSON AND MARY AGNITA REUTER³

Although the spectral data of the purines and pyrimidines are of widest application in the detection of the bases in natural occurring materials, as in the nucleic acids or derivatives, a study of pH influences on the spectrum of the free base may lead to erroneous conclusions if the modifying influence of the sugar group, itself non-absorbing, is not also known. It has been shown⁴ that uracil has, in neutral and acid solutions, a sharp maximum at 2600 Å., which shifts to longer wave lengths in alkaline reaction. As to be expected from its structure, the absorption curves of thymine⁵ closely parallel those of uracil both in shape and pH response (Fig. 1).

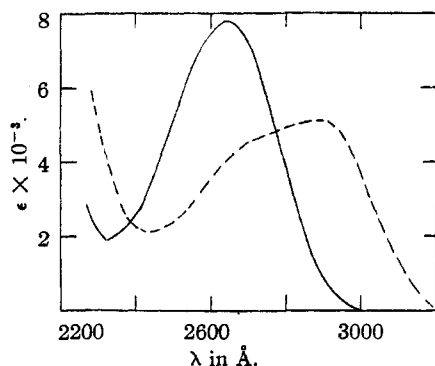
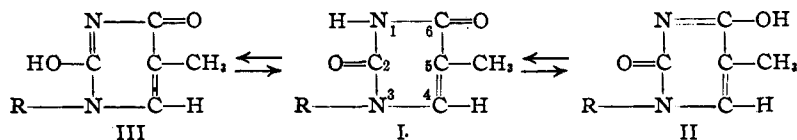


Fig. 1.— ——— in water; 0.1 N HCl; - - - - - in 0.1 N NaOH.

The spectrum of thymine desoxyriboside⁶ differs decidedly from that of the free base in respect to the effect of alkaline reaction (Fig. 2). However, since Levene and Tipson⁷ showed that the pyrimidine-sugar linkage in pyrimidine nucleosides is at position 3, the lactam-lactim tautomerism, involving the 1,6 or 1,2 and the 2,3 positions in uracil and thymine, can operate only at the 1-6 or 1-2 position in the nucleoside. From the following possible forms



(1) Part VIII. Ultraviolet Absorption Spectra of Nitrogenous Heterocycles.

(2) Part of the research program of Institutum Divi Thomae.

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

(4) Stimson and Reuter, *THIS JOURNAL*, **65**, 151 (1943).

(5) The authors wish to acknowledge the kindness of Dr. Marian Van Ess, Golden State Company, for the sample employed.

(6) The sample used was generously supplied by Dr. Thomas G. Brady, University College, Dublin.

(7) Levene and Tipson, *J. Biol. Chem.*, **104**, 385 (1934).

it will be seen that the hydrogen on the 1 position (I) may migrate to either carbonyl, if no other influencing factors are brought to play. However, the $-\text{C}=\text{C}-$ is acid strengthening and resonance inducing⁸ when attached either directly or by conjugation, thus the migration of the labile hydrogen to the 6 position would yield a form with relatively higher acidity than the alternative migration to the 2 would give. In an analogous case Gulland, Holiday and Macrae⁹ working with various mono-methylated xanthenes showed that the 3-methylated derivative does not exhibit any shift of the absorption maximum with pH, whereas the spectrum of the free base has only one maximum in acid and neutral solution and two in alkaline reaction.¹⁰

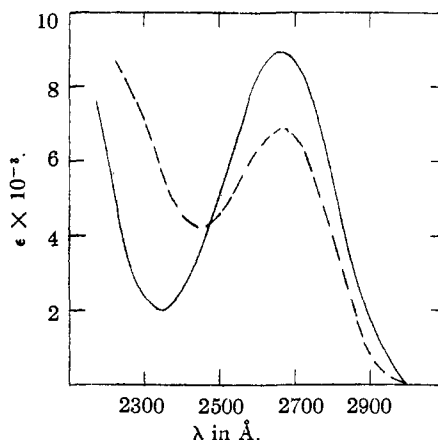


Fig. 2.— ——— in water; 0.1 N HCl; - - - - - in 0.1 N NaOH

The work of Williams, *et al.*,¹¹ shows that pH does not have any marked effect on the absorption maxima of various 6-hydroxypyrimidines, although there is some change in extinction. The pH effect on the spectrum of thymine desoxyriboside is of a similar type and hence leads to the conclusion that if differences in spectral characteristics are to be associated with different molecular forms, lactam-lactim tautomerism involving the 1,6 positions may be effective.

The pyrimidine constituents of the nucleic

(8) A. E. Remick, "Electronic Interpretations of Organic Chemistry," J. Wiley & Sons, Inc., New York, N. Y., 1943; J. R. Johnson in H. Gilman, "Organic Chemistry, Advanced Treatise," J. Wiley & Sons, Inc., New York, N. Y., 1943; G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941.

(9) Gulland, Holiday and Macrae, *J. Chem. Soc.*, 1639 (1934).

(10) Stimson and Reuter, *THIS JOURNAL*, **65**, 153 (1943).

(11) Williams, Ruehle and Finkelstein, *ibid.*, **59**, 526 (1937).

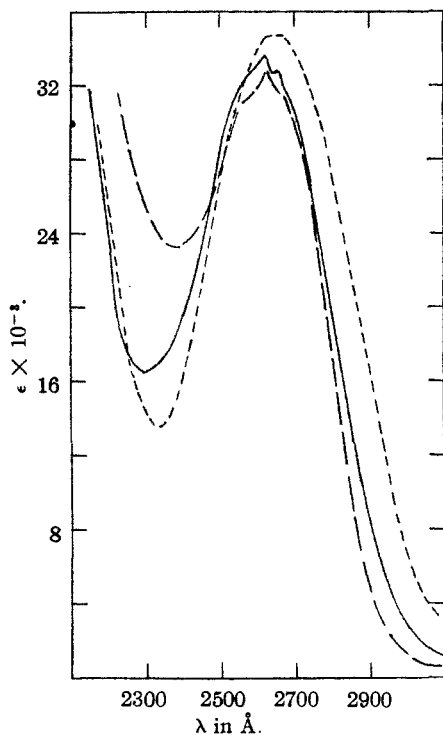


Fig. 3.————— in water; - - - in 0.1 *N* NaOH;
- · - · - in 0.1 *N* HCl.

acids show considerable spectral response to *pH* when they occur as free bases, which is lacking in the parent nucleic acids, giving rise to spectra for yeast and thymus nucleic acids, which show only a slight position and extinction response of the maxima to *pH* (Figs. 3 and 4). It is, therefore, difficult to identify a nucleoside or nucleotide definitely by spectral examination of material at various *pH*'s because of lack of characteristic changes.

Experimental

The spectra were determined on a Hilger spectrograph in conjunction with a "Spekker" photometer and were checked with a Beckman spectrophotometer.

Materials.—Yeast nucleic acid was a commercial preparation (Eastman) purified according to

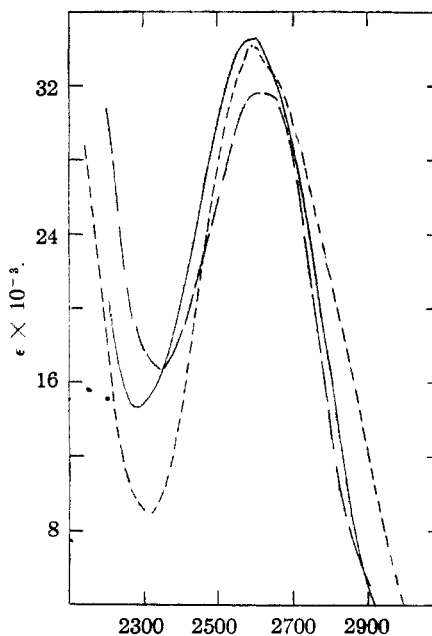


Fig. 4.————— in water; - - - - in 0.1 *N* HCl;
- · - · - in 0.1 *N* NaOH.

Levene¹² to a fine white powder. Thymus nucleic acid was prepared according to Feulgen.¹³ Thymine was prepared by Dr. Marian Van Ess and the nucleoside by Dr. Thomas G. Brady.

In previous work the authors employed Koltzoff buffer tablets, but since these are not available at present, the spectra were run in 0.1 *N* hydrochloric acid, water and 0.1 *N* sodium hydroxide.

Summary

1. The spectra of thymine, thymine desoxyriboside and of yeast and thymus nucleic acids are given.

2. Data presented show that it is not possible to identify pyrimidine-nucleosides, or nucleotides from change in spectra with *pH*.

ADRIAN, MICHIGAN

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(12) P. A. Levene, "Nucleic Acids," The Chemical Catalog Co. New York, N. Y., 1931.

(13) Feulgen, *Z. physiol. Chem.*, **238**, 105 (1936).