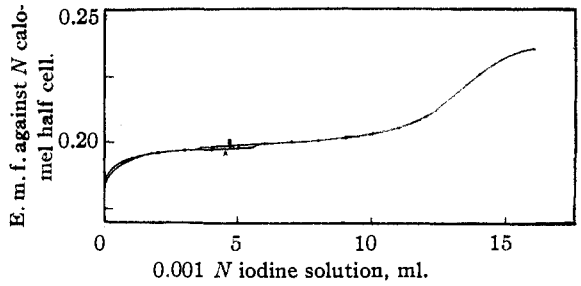


Fig. 6.—Titration of mixtures of corn and potato amyloses: A, 0.0067 g. of potato + 0.0033 g. of corn amylose; B, 0.0033 g. of potato + 0.0067 g. of corn amylose.

as the authors are inclined to do, the results would mean that amyloses from corn and potato have different chain lengths, and the inhomogeneity in the chain length of the amylose from neither source is so great that there is an appreciable overlap in the ranges of chain lengths of the two amyloses.

Preliminary studies on molecular size by viscosity techniques[23] indicate that potato amylose has a greater molecular weight than corn amylose. This result in conjunction with the data in Fig. 1 would mean that the shorter amylose chains require higher iodine potentials for complex formation. This is confirmed by the titration of very short but essentially straight chain amylodextrin (Fig. 7). This material requires a very much larger iodine activity for complex formation than potato amylose. Obviously this point deserves further study.

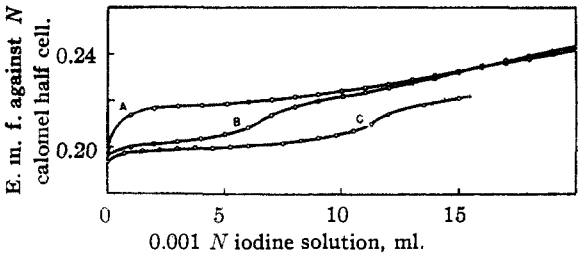


Fig. 7.—Titration of a mixture of amyloses of different chain lengths: A, 0.01 g. of amylose from amylodextrin; B, 0.005 g. of amylose from amylodextrin plus 0.005 g. of potato amylose; C. 0.01 g. of potato amylose.

(23) R. M. Hixon and J. F. Foster, THIS JOURNAL, to be published.

Summary

1. Starch has been shown to possess two components which are quite distinct in their reaction with iodine to form iodine complexes.

2. A potentiometric method has been developed for the rapid quantitative determination of the amylose components of starch.

3. The analytical method has been applied to a number of starches and starch fractions.

4. The amount of iodine bound by the amylose component of starch varies inversely with the iodide concentration.

5. Preliminary results indicate that affinity for iodine varies inversely with the degree of branching of the starch chains.

6. Preliminary results indicate that affinity for iodine varies directly with the length of the starch chain.

7. The amylose component of any one starch appears fairly homogeneous in chain length.

8. The synthetic starch of Hassid, in agreement with his methylation studies, appears to be essentially amylose.

AMES, IOWA RECEIVED SEPTEMBER 21, 1942

[CONTRIBUTION FROM THE LABORATORIES OF INSTITUTUM DIVI THOMAE]

The Ultraviolet Absorption Spectra of Nitrogenous Heterocycles. V. The Blocking Effect of Methyl Groups on the Ultraviolet Absorption Spectra of Some Hydroxypurines and Pyrimidines[1]

BY JOHN R. LOOFBOUROW, SR. MIRIAM MICHAEL STIMSON, O.P., AND SR. MARY JANE HART, O.P.

It has previously been shown that the change in the absorption of uracil may be ascribed to lactim–lactam isomerism.[2] The lactam or hydroxy form was postulated to exist in alkaline solution on the assumption that it would tend to neutralize basicity. On the other hand Fromherz and Hartmann[3] on the basis of absorptiometry of uric acid concluded that its acid nature cannot depend on the complete or partial enolization and dissociation of the hydrogen from oxygen but rather on dissociation of hydrogen from an unsaturated nitrogen. Therefore, uric acid is assumed by these workers to be in the enol form in acid solution and in the keto form in alkaline solution. In order, therefore, to obtain further information as to the *p*H effect on hydroxy com-

(1) Presented at the Buffalo meeting, September, 1942.
(2) Heyroth and Loofbourow, THIS JOURNAL, 53, 3441 (1931).
(3) Fromherz and Hartmann, *Ber.*, 69, 2420 (1936).

pounds, some methyl derivatives have been studied over the *p*H range 3.0–11.0.

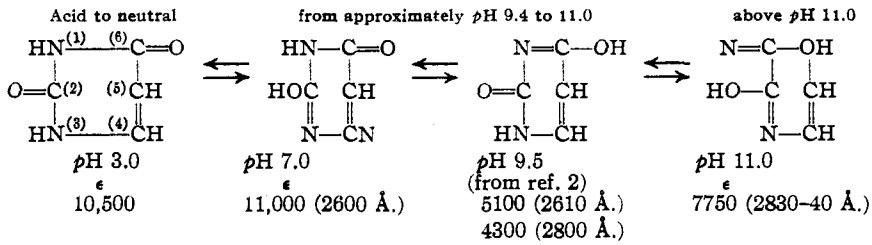
Experimental

Materials.—Uracil was prepared according to the method of Davidson and Baudisch[4] and repurified until constant extinction coefficients were obtained. Dimethyluracil was prepared from uracil by methylation with dimethyl sulfate and was repurified by recrystallization. Xanthine is reported elsewhere by Stimson and Reuter.[5] Caffeine was used as obtained from Eastman Kodak Co. without further purification. Solutions were made in distilled water and buffered with Kolthoff buffer tablets. Equal concentrations of buffers were used in the comparison cells.

Method.—The spectra were obtained on a small Hilger spectrograph in conjunction with a Spekker photometer. Density comparisons were made from photographic enlargements and were checked by visual comparison of the projected image of the plates.

Discussion of Results

Uracil contains two carbonyl groups in the 2 and 6 positions, respectively. According to Lapworth[6] any compound having at least one hydrogen atom situated in the alpha position is formally capable of enolization, therefore, two changes should be possible. However, from titration data, Levene, Bass and Simms[7] concluded that since there is only one dissociation constant the enolization of one position inhibits that of the other, although the two positions have approximately the same tendency to enolize. The value for the enolization of uracil in its natural compounds, nucleoside and nucleic acid, is given by these workers as between 9.17 and 10.2. Unpublished data of Stimson and Reuter for uracil, as a free base, show a change in the slope of the titration curve at *p*H 9.4 and at *p*H 11.2–11.4. An examination of the absorption spectra of this compound for *p*H's 3.0, 7.0, 11.0 indicates that no appreciable change takes place until the high *p*H value is reached. The earlier work of Heyroth and Loofbourow showed the main absorption band still at 2600 Å. at *p*H 11.0 with a secondary absorption band at 2800 Å. Their curve shows the correct trend and is probably a measure of the per cent. change from the keto to the enol form.



However, the *p*H values they reported in the more alkaline range were probably too high by one or more units because of lack of sensitivity of the method at high *p*H. The curve given by them for *p*H 11.0 is more probably that for about *p*H 9.0–9.5 and so represents the transition point. Due to the buffer employed in the present work it was impossible to obtain *p*H values above 11.0 and maintain a constant type of buffer over the *p*H range under investigation, therefore no spectrographic substantiation is at hand for the second change in slope in the titration data of uracil mentioned earlier.

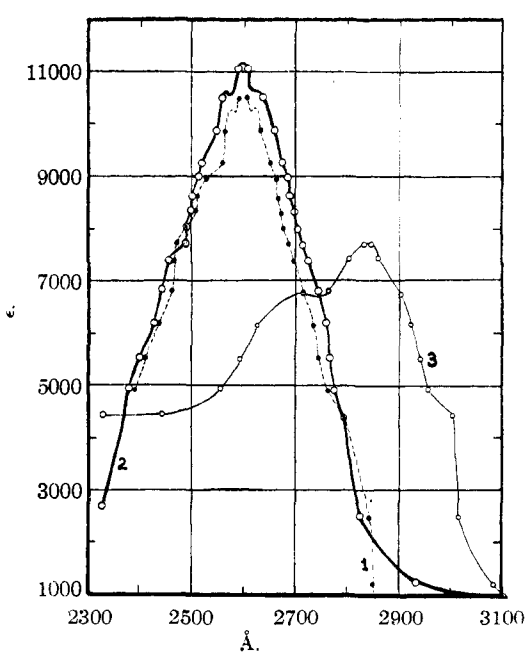


Fig. 1.—Uracil: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0.

Examination of the spectrum of xanthine[8] indicates that it shows a *p*H response analogous

(4) Davidson and Baudisch, THIS JOURNAL, **48**, 2379 (1926).
(5) Stimson and Reuter, *ibid*, **65**, 153 (1943).
(6) Lapworth, *J. Chem. Soc.*, **85**, 30 (1904).
(7) Levene, Bass and Simms, *J. Biol. Chem.*, **70**, 229 (1926).
(8) Stimson and Reuter, THIS JOURNAL, **65**, 153 (1943).

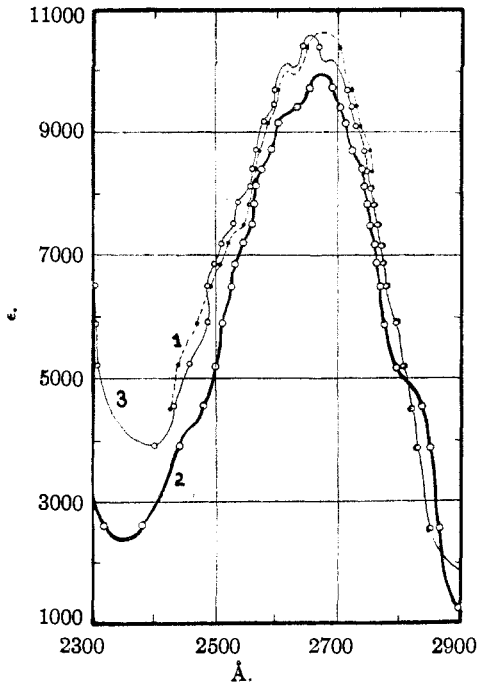


Fig. 2.—1,3-Dimethyluracil: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0.

to that of uracil and since xanthine is the purine analog of uracil its *p*H response is probably associated with the same mechanism.

In order to determine experimentally which form of the 2,6-dihydroxy compounds exists in acid and in alkaline solutions, 1,3-dimethyluracil and caffeine were studied since substitution of the methyl group on the 1,3-positions stabilizes the two oxygen atoms in the carbonyl form. This method of investigation permits comparison with the data of Fromherz and Hartmann[8] and their conclusions that uric acid (a tetrahydroxy compound) exists in the enol form in even acid solution.

Stabilization does result from methylation in that there is practically no *p*H response, however, certain features are to be pointed out. It will be noted that the introduction of the methyl groups causes a shift of the main absorption band 70 Å. toward the red. There is, however, no appreciable change in extinction. At all *p*H's the absorption is of necessity that of the keto form and since the resemblance is between the methylated compound and the acid or neutral parent substance in both cases, it may be concluded that uracil and xanthine are in the keto form until well above the neutral point.

It will also be noted that the difference in fine

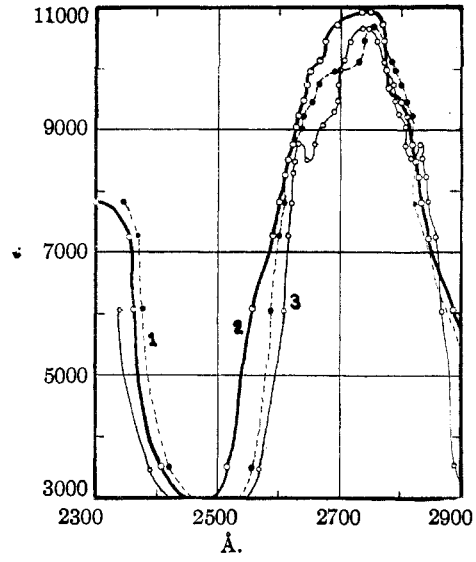
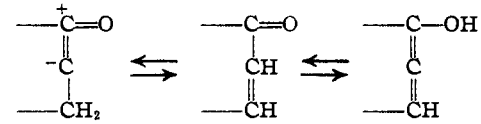
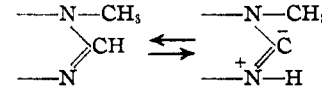


Fig. 3.—Caffeine: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0.

points between the curves for *p*H 3.0 and *p*H 7.0 for both methylated derivatives is greater than for the parent compounds. A possible contributing group in the case of the dimethyl uracil is the combination of an ethylene and carbonyl resonator in conjugated coupling which may resonate as



In the case of caffeine, however, the 4,5 double bond is anchored by the imidazole condensed ring, but there is the possibility of resonance between the 7,8 positions



Monomethylxanthine, as well as caffeine and isocaffeine, were studied spectrographically by Gulland, Holiday and Macrae[9] and potentiometrically by Ogston.[10] The spectroscopic determinations reported, were made at *p*H's 9.5 or 10.0 and at 5.0 or 5.5. These workers found that substitution on the 9 position gave rise to a double absorption band in both acid and alkaline solutions; the 3-substituent gave two bands only in acid solution and the 1- and 7-methylated products showed but one band in either acid or alkaline reaction. It would seem from this that

(9) Gulland, Holiday and Macrae, *J. Chem. Soc.*, 1639 (1934).
(10) Ogston, *ibid.*, 1376 (1935).

the stabilization of unsaturation between the 7,8 positions gives rise to the second band in the cases of the 9-methyl compound and isocaffeine and that when the 6-hydroxy group is blocked in the keto form such unsaturation does exist in alkaline solution. This condition is impossible in the 7-methyl compound by reason of the substitution and in the 3-methyl compound in that the 6-position is free to undergo keto-enol isomerism. It is true that this change is theoretically possible in the 9-methylxanthine, but according to Ogston's values the pK of 3-methylxanthine is 8.5 and of 9-methylxanthine it is 6.3. Therefore, changing the methyl from the 3- to the 9-position gives 2.2 for ΔpK, which indicates that the 9-methyl compound is less acid than the 3-methyl substituent. Since the 9-methylpurine is more basic than xanthine, enolization would probably be beyond pH 9.5, the value used by Gulland and co-workers. Holiday[11] found that the absorption of caffeine is equally intense at pH 5.0 and pH 10.0. The conclusion is that there is no change in the spectrum with pH. Our results show that there is a slight change which, however, would be unnoticed when examining only acid and alkaline solutions.

(11) Holiday, *Biochem. J.*, **24**, 619 (1930).

It may be concluded, therefore, that the 2,6-dihydroxy-purine and -pyrimidine exist in the keto form in acid solution and that enolization does not take place until well above the neutral point. It has likewise been shown that the stabilization of the keto form by methylation permits only slight pH response which may be due to resonance between the low energy forms, the effect and significance of which is obscured in the greater pH response found in the hydroxy compounds employed.

Summary

1. A comparison of uracil with 1,3-dimethyluracil and of xanthine with caffeine shows that uracil and xanthine exist in the ketonic form well into high pH regions.

2. The conclusions of Levene, Bass and Simms as to the enolization of uracil have been confirmed for the pH range 3.0–11.0.

3. The conclusions of Fromherz and Hartmann that uric acid exists in the enol form in acid solution cannot be applied to the 2,6-dihydroxypurine and pyrimidine.

4. There is some pH response by both 1,3-dimethyluracil and caffeine.

CINCINNATI, OHIO RECEIVED SEPTEMBER 10, 1942

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

Ultraviolet Absorption Spectra of Nitrogenous Heterocycles. VI. The Effect of pH on the Spectrum of Uracil-5-carboxylic Acid[1]

BY SR. MIRIAM MICHAEL STIMSON, O.P., AND SR. MARY AGNITA REUTER, O.P.

Representatives of the pyrimidines constitute an important group for both chemical and biological reasons. Uracil-5-carboxylic acid is the first of the pyrimidines to be studied spectroscopically as a pyrimidine derivative. The possible effect of the carboxyl group on the lactam–lactim isomerism of pyrimidine part and the effect of the heterocyclic group on the carboxyl group cannot be studied by the usual chemical methods.

Experimental

Materials.—The uracil-5-carboxylic acid employed was prepared by Doctor T. B. Johnson and Doctor Elizabeth Ballard of Yale and was kindly furnished by them. It was used at a concentration of 0.0075 g./l. and the pH was controlled by Kolthoff buffer tablets. The comparison cells contained in every case an equal concentration of the corresponding buffer; 2-cm. cells were used.

Methods.—The spectra were determined as described in earlier communications.

Results and Discussion

The spectrum of the unbuffered uracil-5-carboxylic acid shows two absorption bands, at 2170 and 2740 Å., respectively. The 2170 Å. band agrees in position with the wave length values found by Ley and Arends[2] and by Ausmuller, Fromherz and Strother[3] for the carboxyl group.

(1) Presented at the Buffalo meeting, September, 1942.

(2) Ley and Arends, *Z. physik. Chem.*, **B17**, 177 (1932); **B4**, 234 (1929).

(3) Ausmuller, Fromherz and Strother, *ibid.*, **B37** (1937).