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 *p*H 9.5, the value used by Gulland and co-workers. Holiday[11] found that the absorption of caffeine is equally intense at *p*H 5.0 and *p*H 10.0. The conclusion is that there is no change in the spectrum with *p*H. Our results show that there is a slight change which, however, would be unnoticed when examining only acid and alkaline solutions.

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 *p*H response which may be due to resonance between the low energy forms, the effect and significance of which is obscured in the greater *p*H 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 *p*H regions.
2. The conclusions of Levene, Bass and Simms as to the enolization of uracil have been confirmed for the *p*H 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 *p*H response by both 1,3-dimethyluracil and caffeine.

CINCINNATI, OHIO RECEIVED SEPTEMBER 10, 1942

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

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

Ultraviolet Absorption Spectra of Nitrogenous Heterocycles. VI. The Effect of *p*H 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 *p*H 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.

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[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).

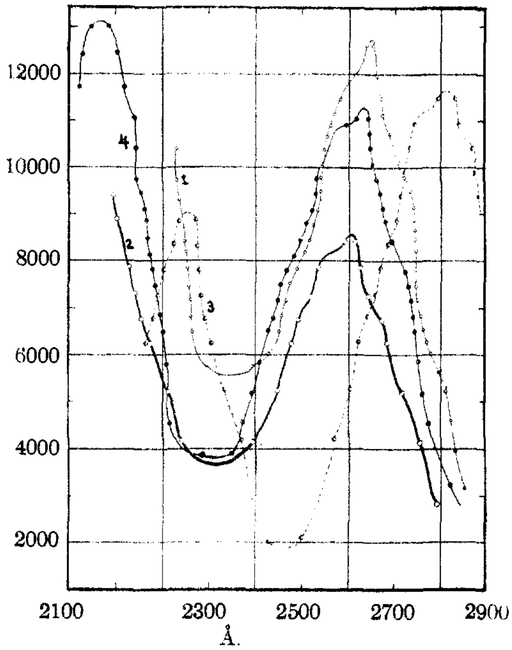
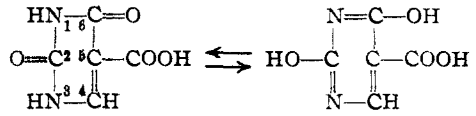


Fig. 1.—Uracil-5-carboxylic acid: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0; 4, unbuffered.

Due to the natural absorption of the buffers beyond 2200 Å. the response of the short wave band to change in *p*H could not be measured under the conditions of the experiment. However, certain trends are indicated: in acid solution this maximum is closer to the visible than in neutral solution, while in alkaline solution the peak is well defined. It is located at 2360 Å., which is a shift of approximately 200 Å. from the position of the band in the unbuffered solution. The shift is accompanied by a drop in the molecular extinction coefficient of 4000 units.

The long wave band, which is the uracil absorption, shows considerable difference with the absorption spectrum of the parent compound as reported by Loofbourow, Stimson and Hart.[4] A preliminary comparison shows that the marked shift in position of the uracil maximum to longer wave lengths at *p*H 11.0 is repeated in the carboxylic acid. In this instance the extinction is higher in alkaline than in neutral or weakly acid solution, whereas the band weakened considerably for the same *p*H in the case of uracil. This difference is probably due to the influence of the carboxyl group which affects the resonance of the pyrimidine residue. The carboxyl radical is conjugated with the ethylene group and when in the keto form the 6-carbonyl is also conjugated with the ethylene group. It is noteworthy that although the individual resonators all possess strong absorption the combination does not intensify the uracil absorption band over that displayed by the unsubstituted compound.



The low absorption of the long wave band at *p*H 7.0 may indicate that, due to the carboxyl influence, enolization takes place at a lower *p*H than is the case for uracil. This is suggested by a comparison of the spectra of uracil-5-carboxylic acid and uracil [4,5] from which it is seen that the absorption band drops as the end-point for enolization is approached. In uracil this drop takes place beyond the neutral point but the enol-absorption does not appear until approximately *p*H 9.5. In uracil-5-carboxylic acid even at *p*H 7.0 the absorption of the uracil band is considerably weakened, although from the spectra there are no indications of the enol shift. The next *p*H at which the spectrum was determined is 11.0 and at this value it is possible that both carbonyl groups have enolized. This would account for the intensity of the band. The absorption spectrum of uracil-5-carboxylic acid comparable to uracil (at *p*H 11.0) on this assumption would be between *p*H 7.0 and *p*H 11.0. The possibility of enolization of both carbonyl groups is being studied potentiometrically.

Summary

1. The ultraviolet absorption spectrum of uracil-5-carboxylic acid has been reported for *p*H's 3.0, 7.0, 11.0.

2. Uracil absorption is so modified that the band (2700–2900 Å.) shows its weakest absorption at *p*H 7.0 rather than at *p*H 11.0.

3. The absorption at 2170 Å. for the unbuffered solution agrees with the position assigned by Ley and Arends, and by Ausmuller, Fromherz and Strother for the absorption of the carboxylic group.

ADRIAN, MICHIGAN RECEIVED SEPTEMBER 10, 1942

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(4) Loofbourow, Stimson and Hart, THIS JOURNAL, **65**, 148 (1943).

(5) Heyroth and Loofbourow, *ibid.*, **53**, 3441 (1931).