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

Ultraviolet Absorption Spectra of Nitrogenous Heterocycles. VII. The Effect of Hydroxy Substitutions on the Ultraviolet Absorption of the Series: Hypoxanthine, Xanthine and Uric Acid[1]

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This investigation was undertaken to study the effect of increasing the number of hydroxyl groups on the ultraviolet absorption of purines.

Experimental

Materials.—Hypoxanthine, xanthine and uric acid, Eastman Kodak Co. products, were purified by recrystallization. The *p*H was controlled by Kolthoff buffer tablets.

Method.—The spectral technique was the same as previously reported.[2]

Results and Discussion

A comparison of the ultraviolet absorption spectra of hypoxanthine, xanthine and uric acid at *p*H 7.0 shows that as the number of hydroxy substitutions increases the position of the main peak is progressively shifted about 200 Å. toward the long wave end of the spectrum and the molecular extinction increases approximately 1000 units for each additional hydroxy group.

The regularity of response at *p*H 7.0 in the purine series to the introduction of a hydroxy group indicates that the hydroxy group itself is the important factor and the position of the introduction is secondary, although as will be seen to be of considerable influence. This influence of the hydroxy group may be the interpretation of the spectral differences between adenine and isoguanine. The introduction of the hydroxy group into adenine to give isoguanine results, at *p*H 7.0, in a shift in the maximum by 240 Å. toward the red, accompanied by increase in the molecular extinction by 5000 units. No data are available at present to determine whether this would be the case on the introduction of the hydroxy into the purine ring as the first active group, but it is definitely not the case in the pyrimidine analogs.

The regular effect of the introduction of hydroxy groups was not found when studied in the pyrimidine series: 6-hydroxy-pyrimidine, uracil and barbituric acid. In this series the maximum is slightly displaced toward shorter wave lengths, although the increase in extinction is even stronger than in the case of the purines, being as great as 20,000 in the change from uracil to barbituric acid. A comparison of the spectrum of 6-hydroxypyrimidine[3] and of pyrimidine[4] shows that although the maximum shifts approximately 200 Å. toward longer wave lengths the extinction is not appreciably affected.

Within a given structure type both the purine and pyrimidine analogs show the same general response to *p*H, the effect being more pronounced in the pyrimidine than in the purine. In the latter the weighting effect of the imidazole ring dampens the influence of any contributing factors. It may be concluded that in hypoxanthine, xanthine and uric acid the introduction of the hydroxy group causes a shift in the maximum toward the red which is accompanied by an increase in the molecular extinction.

Hypoxanthine was previously reported by Holiday[5] for the same *p*H values employed in the present study, and by Bandow[6] in concentrated sulfuric acid. The value for *p*H 7.0 checks with that reported by Holiday when the correction given by Heyroth and Loofbourow[7] is made. For acid and basic solutions, however, the extinctions found by the authors are higher than those given by Holiday. Bandow reported a slightly higher extinction than reported here—12,500 approximately.

In examining the response of hypoxanthine to *p*H it will be noted (Fig. 1.) that the maximum absorption occurs at *p*H 3.0. This falls at *p*H 7.0 with simultaneous shift to slightly shorter wave lengths, but on increasing alkalinity the maximum moves to longer wave lengths. The structure of hypoxanthine shows that it may assume two forms

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

(2) Parts I and V of this series.

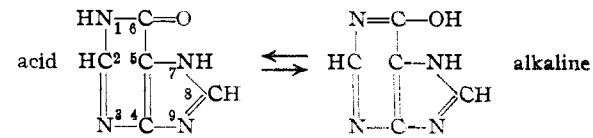
(3) Williams, Ruehle and Finkelstein, THIS JOURNAL, **59**, 526 (1937).

(4) Heyroth and Loofbourow, *ibid.*, **53**, 3441 (1931); Uber and Winters, *ibid.*, **63**, 137 (1941).

(5) Holiday, *Biochem. J.*, **24**, 619 (1930); Gulland and Holiday, *J. Chem. Soc.*, 765 (1936)

(6) Bandow, *Biochem. Z.*, **299**, 199 (1938)

(7) Heyroth and Loofbourow, THIS JOURNAL, **56**, 1728 (1934).



There is also possible a shift of the hydrogen between the 7,8 and the 8,9 positions but the effect would be slight as compared with the change from the lactam to the lactim form and so would probably be obscured.

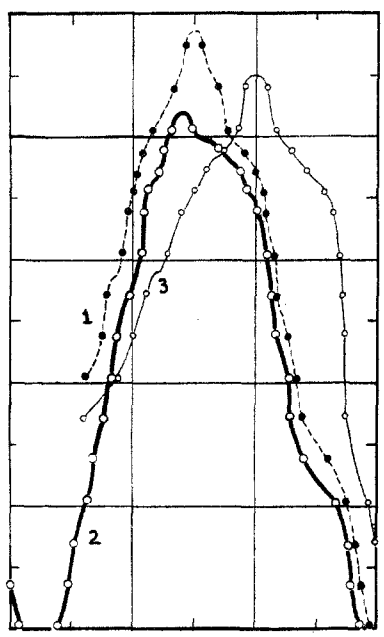


Fig. 1.—Hypoxanthine: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0.

The drop in the extinction for *p*H 7.0 is analogous to that found by Heyroth and Loofbourow[8] for uracil at about *p*H 9.0–9.5, while the band at *p*H 3.0 (2500 Å.; E = 11,700) is due to the keto form. The shift of the band at *p*H 11.0 (2600 Å.; E = 11,000) has been associated with the enol form. It will be noted that at both *p*H's the molecular extinction is almost identical. This may be due to equal hyperchromic values for >C=O and >C=N—.[9]

The spectroscopic values for xanthine in the form of the base have not been reported previously although Gulland, Holiday and Macrae[10] investigated the absorption of the possible monomethyl derivatives. At *p*H 3.0 and *p*H 7.0 the absorption spectrum is practically unchanged, but at *p*H 11.0 two peaks (Fig. 2.) are well developed, each being weaker than the single band for acid or neutral solution.

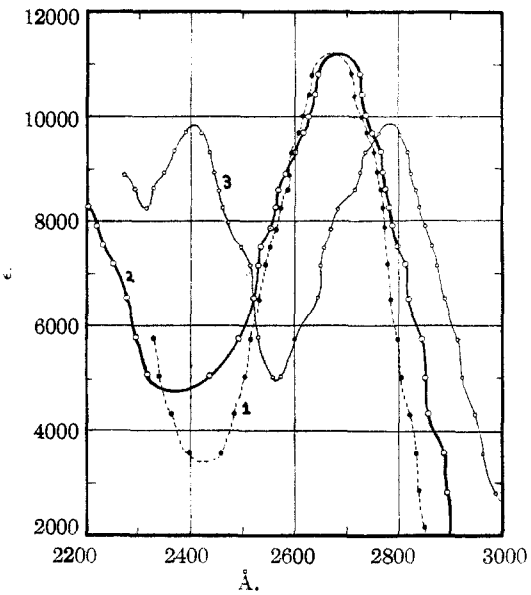
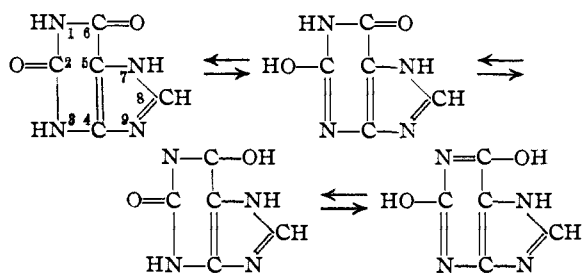


Fig. 2.—Xanthine: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0

Xanthine can, theoretically, exist as a dienolic, a diketonic, or as one of monoketonic forms



Of these the diketonic form predominates in acid and neutral solutions. The spectrum for *p*H 11.0 indicates that partial enolization has taken place. Due to the low solubility of xanthine, potentiometric determinations could not be made, but the titration data of uracil applied to xanthine would permit either of the monoenolic forms to be present at *p*H 11.0, while dienolization would occur at a more alkaline value. On this assumption the double absorption band of xanthine at *p*H 11.0 is associated with the existence of an equilibrium mixture of the two possible monoenolic tautomers.

Uric acid has been investigated by Dhere,[11] Castille and Ruppol,[12] Smith,[13] Holiday,[5] Ban-

(8) Heyroth and Loofbourow, THIS JOURNAL, **53**, 3441 (1931), also Part V of this series.

(9) *Cf.* Loofbourow and Stimson, *J. Chem. Soc.*, 1275 (1940).

(10) Gulland, Holiday and Macrae, *ibid.*, 1639 (1934).

(11) Dhere, "L'absorption des rayons ultra-violets par le albuminoides, les proteides et leurs derives," Fribourg, 1909.

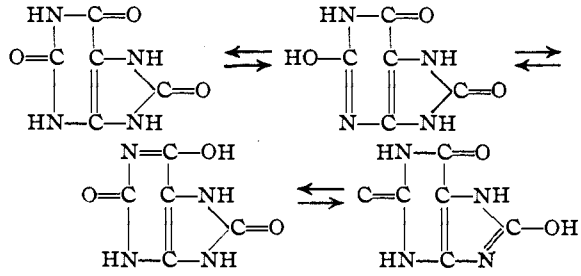
(12) Castille and Ruppol, *Bull. soc. chim. biol.*, **10**, 623 (1928).

(13) Smith, *Biochem. J.*, **22**, 1499 (1928).

dow,[6] and Fromherz and Hartmann.[14] The data presented here are in disagreement with those of Holiday in that the present authors found an increase in the intensity of absorption with rise in *p*H. Smith, also, found such a relation between hydrogen ion concentration and extinction. The trend of the change is likewise similar to that shown in Fig. 2, ref. 5, where dilution of a concentrated sulfuric acid solution results in a slight shift and weakening of the absorption band.

The presence of two absorption bands for uric acid (Fig. 3.) in acid and neutral solution corresponds to the type of absorption displayed by xanthine in basic solution. This seems to substantiate in part the conclusion of Fromherz and Hartmann that uric acid exists in the enol form even in acid solution.

Uric acid has three possible monoenolic forms and three possible dienolic forms besides the trienolic and triketonic arrangements



In view of the work on uracil, hypoxanthine and xanthine it is the authors' conclusion that uric acid exists in the monoenolic form at *p*H 3.0. On the basis of absorption measurements Fromherz and Hartmann concluded that uric acid exists in the enol form in acid solution and in the keto in alkaline. They reported that the salts are of the enol form and that after standing for a period of one week only negligible change was noted. On the other hand Schade and Boden[15] concluded from their investigations that the enol form is the stable form and that uric acid salts in solution go over to it. In attempting to clarify the discrepancies between the work of Fromherz and Hartmann, and Biltz,[16] Ley and Specker[17] showed that by enolization the absorption curve is shifted to the red, although sometimes the individual bands are influenced in different ways.

(14) Fromherz and Hartmann, *Ber.*, **69**, 2420 (1936).
(15) Schade and Boden, *Z. physiol. Chem.*, **83**, 347 (1913).
(16) Biltz, *Ber.*, **69**, 2750 (1936).
(17) Ley and Specker, *ibid.*, **72**, 192 (1939).

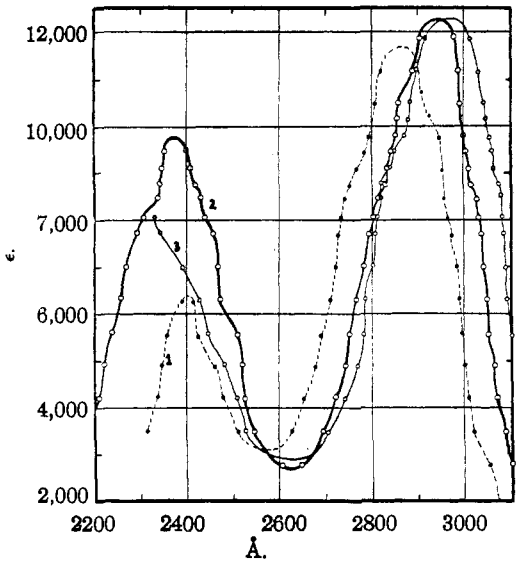


Fig. 3.—Uric acid: 1, at *p*H 3.0; 2, at *p*H 7.0; 3, at *p*H 11.0.

Moreover, it is generally known that the enol form is capable of salt formation as indicated by absorption spectra. On the other hand, certain amidines do show a marked acid character and salt formation, postulated by Fromherz and Hartmann, is theoretically possible. In our interpretation of the spectra of uric acid, potassium urate could be a salt of the lactam (keto) form, since dienolization is probably effective at *p*H 7.0. Trienolization is not clearly indicated for *p*H 11.0.

Conclusions and Summary

1. The spectra of hypoxanthine, xanthine and uric acid have been given for *p*H's 3.0, 7.0, 11.0.

2. At *p*H 7.0 the successive hydroxyl groups cause a shift in the maximum of 200 Å. toward the red with an increase in the molecular extinction of 1000 units.

3. The spectrum of hypoxanthine shows a drop at *p*H 7.0 preliminary to enolization.

4. The spectrum of xanthine, reported for the first time, resembles that of uracil in that no spectroscopic evidence for enolization is found until the curve for *p*H 11.0 is examined.

5. Uric acid, by reason of the similarity of its spectra with the alkaline absorption spectrum of xanthine, is concluded to be in the monoenolic form even in acid solution.

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