

[CONTRIBUTION FROM RESEARCH LABORATORIES OF INSTITUTUM DIVI THOMAE AND SIENA HEIGHTS COLLEGE]

Ultraviolet Absorption Spectra of Nitrogenous Heterocycles. IV. Effect of pH and Irradiation on the Spectra of Isoguanine and 2-Hydroxy-6,8-diaminopurine

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The introduction of a hydroxy group on the second carbon atom of adenine to give isoguanine and the same relative positions of the hydroxy and amino groups on the pyrimidine ring of 2-hydroxy-6,8-diaminopurine permits a study of the combination effect of tautomerism of the imidine type¹ and of the amide-imidole type.²

Although the spectrum of guanine was found by Holiday³ and by Heyroth and Loofbourow⁴ to be greatly affected by pH change, the position of the amino group on carbon 2 rather than 6 introduces the possibility that some of the variation may be positional⁵ rather than a modification of the spectrum of adenine by introduction of a hydroxy group.

Method.—The details of the techniques employed were given in Part I of this series.

Results and Discussion

Figure 1 shows the spectra of isoguanine at pH 3, 7, 11. The ϵ_{\max} is greatest for pH 7 and is considerably lower for pH 11.0 than for pH 3.0. In 6-aminopyrimidine⁶ and 6-aminopurine¹ ϵ_{\max} for neutral solution is less than that for acid reaction. On the other hand, unpublished findings of Loofbourow and Stimson indicate ϵ_{\max} for cytosine to be greatest at pH 7.0. From this it may be concluded that the downward trend of the amino (on C 6) ϵ_{\max} with change from acid to alkaline conditions is over balanced by the high absorption of the hydroxyl group at pH 7.0.² The effect of the hydroxyl in position 6 is weaker than in 2, however, since the work of Williams, *et al.*,⁶ shows 5-methyl-6-hydroxypyrimidine to absorb less at the long wave maximum in neutral solution than in either acid or alkaline reaction. Unpublished findings of Stimson and Loofbourow for hypoxanthine also show the influence of the hydroxyl on C 6 to be insufficient to raise ϵ_{\max} for neutral solutions above that for acid or alkaline solutions. Although λ_{\max} for 6-aminopyrimidine and 6-aminopurine shifts progressively to longer wave lengths with increase in pH, the reverse condition is true for the compounds with the 2-hydroxy-6-amino arrangement. The introduction of the second amino group in position 8 shows in 2-hydroxy-6,8-diaminopurine, the shift of λ_{\max} to be in the same direction as the other two 2-hydroxy-6-amino compounds although the degree is considerably less (Fig. 2). However ϵ_{\max} here again takes on the characteristics of purely amino substituted compounds, which may be attributed to overbalancing the hydroxyl absorption. The short wave maximum found by Williams, *et al.*, for 6-amino- and 6-hydroxy compounds, by Stimson and Loofbourow for 2-chloro-6-aminopyrimidine and by Uber and Winters⁷ is indicated only at pH 7 for isoguanine and did not appear in the photographic ultraviolet for 2-hydroxy-6,8-di-

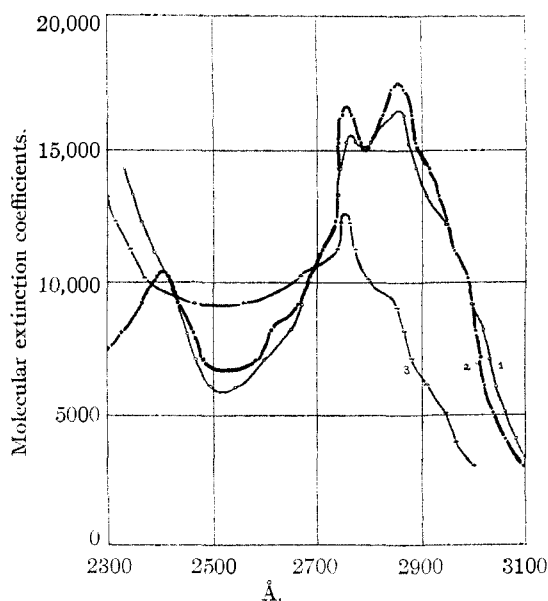


Fig. 1.—Isoguanine: 1, pH 3.0; 2, pH 7.0; 3, pH 11.0.

Experimental

Materials.—The isoguanine and 2-hydroxy-6,8-diaminopurine were kindly furnished by Mr. Joseph R. Spies, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

- (1) Loofbourow and Stimson, *J. Chem. Soc.*, 844 (1940); Stimson and Loofbourow, *THIS JOURNAL*, 63, 1827 (1941).
- (2) Loofbourow and Stimson, *J. Chem. Soc.*, 1275 (1940).
- (3) Holiday, *Biochem. J.*, 24, 619 (1930).
- (4) Heyroth and Loofbourow, *THIS JOURNAL*, 56, 1728 (1934).
- (5) Unpublished laboratory results of Loofbourow and Stimson.

- (6) Williams, Ruehle and Finkelstein, *THIS JOURNAL*, 59, 526 (1937).
- (7) Uber and Winters, *ibid.*, 63, 137 (1941).

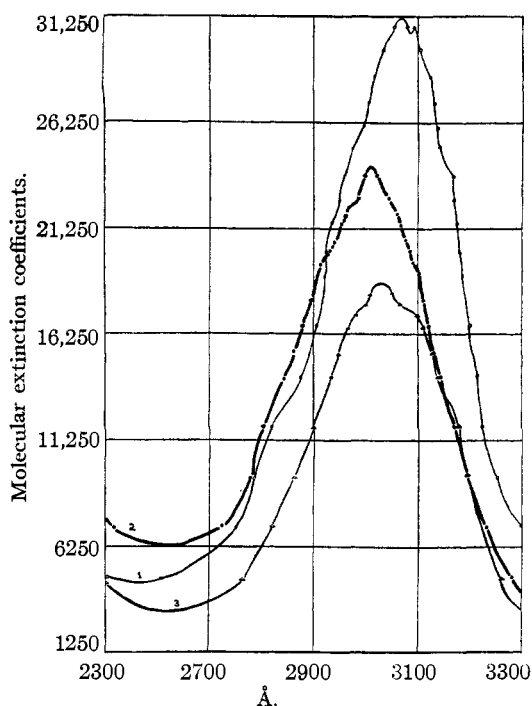


Fig. 2.—2-Hydroxy-6,8-diaminopurine: 1, pH 3.0; 2, pH 7.0; 3, pH 11.0.

aminopurine. In the case of isoguanine the inability to photograph the peak in acid and in alkaline solution may be due to the absorption of the buffers in the short wave end of the spectrum. This, in conjunction with any shift which might occur, could readily prevent it from being recognized under the experimental conditions.⁸

Figure 3 shows the effect of irradiation on isoguanine for as long as four hours. Unlike adenine, which is markedly stable to ultraviolet radiation isoguanine shows response of the type shown by barbituric acid.⁴

Summary

1. A comparison of 2-hydroxy-6-amino-pyrimidine and purine with 6-amino-pyrimidine and

(8) Smakula, *Z. physiol. Chem.*, **230**, 231 (1934).

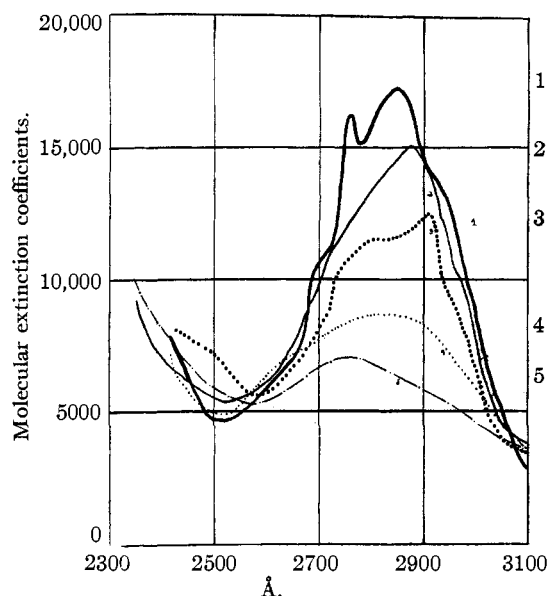


Fig. 3.—Effect of irradiation on isoguanine: 1, 15 min.; 2, 1 hr.; 3, 2 hr.; 4, 3 hr.; 5, 4 hr.

purine shows that the substitution of the hydroxyl group causes λ_{\max} to shift to shorter wave lengths with increase in pH and ϵ_{\max} to rise at pH 7 and then to fall so that the value at pH 11.0 is lower than that at pH 3.

2. Location of the hydroxy group on C 6 in these compounds tend to increase ϵ_{\max} at neutral pH above that in acid or alkaline pH, whereas certain 2-hydroxy purines and pyrimidines have a reduced ϵ_{\max} at pH 7.0.

3. An additional amino group in the case of 2-hydroxy-6,8-diaminopurine shows the same general pH effect on λ_{\max} but to a lesser degree, however ϵ_{\max} follows the amino substitutes.

4. Whereas adenine showed only negligible change on irradiation, isoguanine showed a decided change in extinction.

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