161. *Ultra-violet Absorption Spectra of Nitrogenous Heterocyclic Compounds. Part I. Effect of* p_H *and Irradiation on the Spectrum of Adenine.*

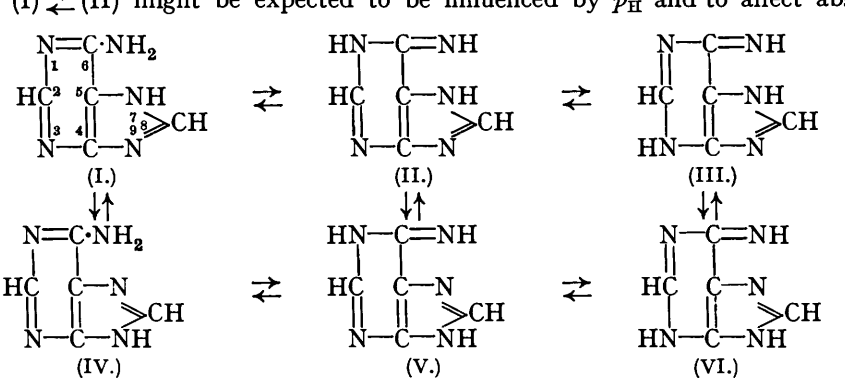
By JOHN R. LOOFBOUROW and MIRIAM M. STIMSON.

Examination of the ultra-violet absorption spectrum of adenine over a wide range of p_H showed, contrary to previous reports in the literature, that the spectrum of this compound in aqueous solution is appreciably influenced by p_H. The possible relationship of tautomerism to this effect is considered. Ultra-violet irradiation of adenine

resulted in little change of absorption as compared with that observed in other purines and pyrimidines.

IN this series it is proposed to consider the relationship between tautomerism and the effect of p_H on the ultra-violet absorption spectra of purines and pyrimidines, and the lability of these compounds to ultra-violet irradiation. The reasons for undertaking these investigations were twofold: (1) further data regarding the ultra-violet absorption spectra of such compounds were required for use in identifying certain cellular products affecting cellular metabolism (Loofbourow, Dwyer, and Lane, *Biochem. J.*, 1940, in the press, etc.), and (2) data regarding the changes in absorption accompanying tautomeric rearrangements were needed for developing quantitative relationships between ultra-violet absorption and chemical constitution in accordance with considerations to be presented in a subsequent paper.

The spectrum of adenine was reported by Holiday (*ibid.*, 1930, **24**, 619) to be unaffected in extinction and but slightly affected in wave-length by change in p_H, whereas his data for guanine showed that p_H markedly influenced its spectrum. Heyroth and Loofbourow (*J. Amer. Chem. Soc.*, 1934, **56**, 1728) confirmed Holiday's results for guanine and found that the absorption of uracil (*ibid.*, 1931, **53**, 3441) was also markedly influenced by p_H. They attributed the effect in uracil to tautomerism of the amide–imidol type (H—N—C=O ⇄ N=C—O—H), and since guanine can undergo this type of tautomerism but adenine cannot (lacking, as it does, C=O groups), they suggested that this might account for the lack of p_H effect reported by Holiday for adenine. On undertaking the present investigation, however, we noted that adenine can undergo tautomerism of the amidine type (N=C—NH_2 ⇄ HN—C=NH) as shown in (I)—(VI), and that of these changes (I) ⇄ (II) might be expected to be influenced by p_H and to affect absorption



materially (because of the markedly different weighting of the —N=C< group in the 1 : 6 position and >C=N— in the 6 position). We therefore deemed it advisable to reinvestigate the absorption of this compound over a wide range of p_H.

The results show that adenine absorption does change considerably in both extinction and wave-length with p_H change, contrary to Holiday's statement (*loc. cit.*) and to Gulland and Holiday's spectra for adenine at two different p_H values (J., 1936, 765). They are in close agreement, however, with data for two p_H's presented by Warburg, Christian, and Giese (*Biochem. Z.*, 1935, **282**, 157; 1936, **287**, 291). The matter is of particular importance now that spectra are being used extensively in attempts to identify various biologically active materials related to nucleic acids and their derivatives, since the influence of p_H must be taken into account in deducing from spectra the constituents present in crude preparations.

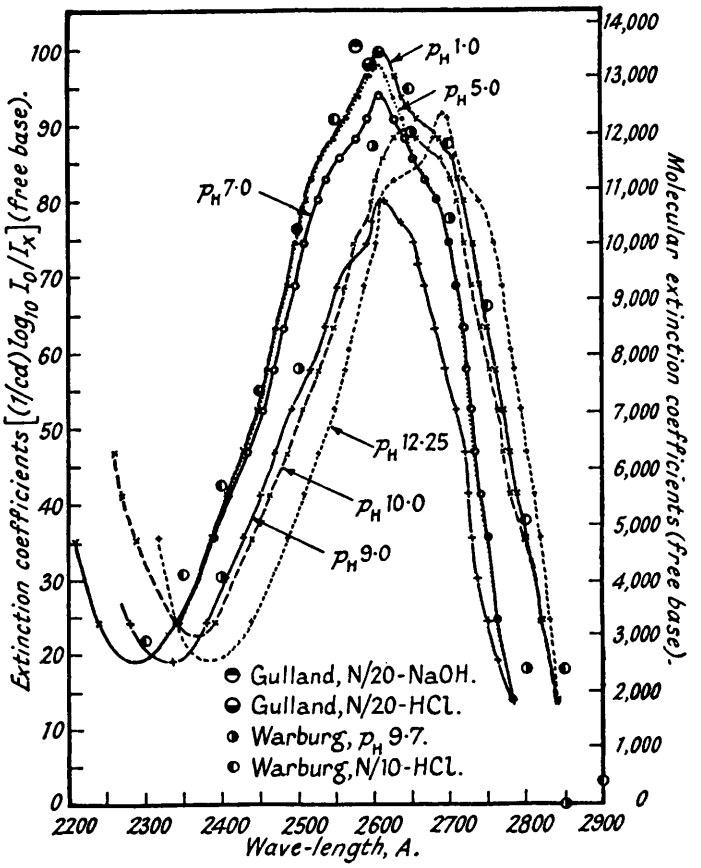
EXPERIMENTAL.

The spectra were determined with a Hilger quartz spectrograph and Spekker photometer, a tungsten-spark source and Cramer contrast plates being used. The plates were enlarged on contrast bromide to facilitate matching, or were matched photoelectrically (Loofbourow, *J. Opt. Soc. Amer.*, 1939, **29**, 535).

All solutions were made in glass-distilled water, usually at a concentration of 16 mg. per l. Kolthoff buffers were used for adjusting the p_H except in the case of the most extreme acid and alkaline solutions, for which hydrochloric acid and sodium hydroxide, respectively, were employed. The solvent cell was filled with a corresponding solution of the buffer alone in each instance, in order to compensate for absorption by the buffer and solvent. The glass electrode was used for determining p_H. Adenine was employed as the sulphate, $(C_5H_5N_5)_2,H_2SO_4,2H_2O$, after repeated recrystallisation from glass-distilled water.

Water-filtered ultra-violet radiation from a Burdick A.C. quartz–mercury arc, operated at 75 volts across the arc, was used for determining the effects of ultra-violet radiation on the spectrum of adenine. Unbuffered solutions were irradiated at 25 cm. from the arc in quartz-covered containers, a liquid thickness of 5 cm. being used. Samples were withdrawn at 0, 5, 15, and 30 mins., 1, 2, 3, and 4 hours. Throughout the period of irradiation, the solution was stirred repeatedly.

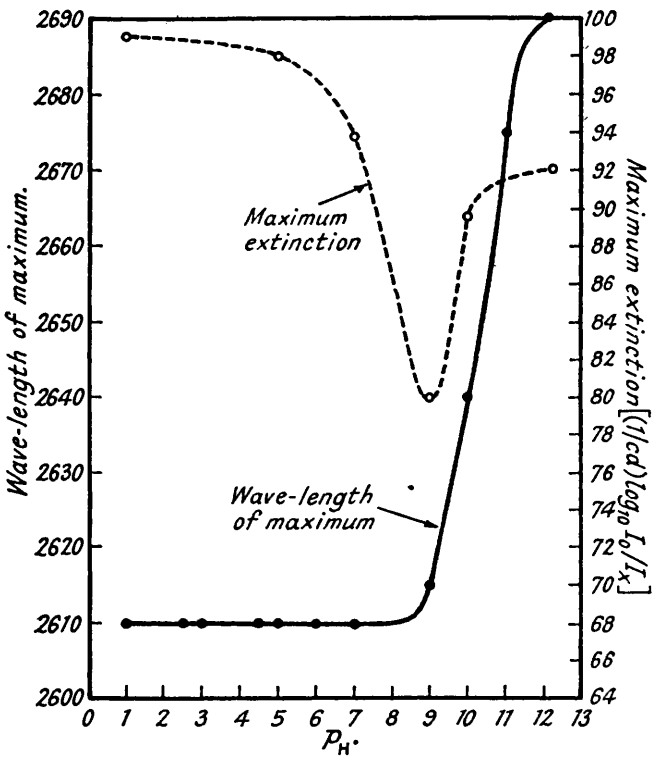
FIG. 1.



Results and Conclusions.—The effect of p_H on the spectrum of adenine is shown in Figs. 1 and 2. In the interests of clarity, several curves for intermediate p_H values (1·62, 3·0, 4·0, 6·0, and 11·0) have been omitted from Fig. 1. The p_H 5·0 solution (the p_H of unbuffered solutions of equivalent concentration was about 5·3) had a maximum at 2610 A. and a molecular extinction (calculated for the free base) of 13,200. This is higher than the value of 10,300 reported by Heyroth and Loofbourow (*loc. cit.*, 1934) and that of 12,300, after correction for sector error (*idem, ibid.*), obtained by Holiday (1930), unbuffered solutions having been used in both cases. Our highest extinction, 13,400, occurred at p_H 1·0. This value agrees precisely with that of Warburg *et al.* (*loc. cit.*, 1935) for solutions in 0·1N-hydrochloric acid and is slightly higher than Gulland and Holiday's value (13,200) for solutions in 0·05N-acid (*loc. cit.*).

Starting with the most acid solution (p_H 1·0), we found a progressive diminution in the extinction at 2610 A. up to p_H 9·0 (Figs. 1 and 2). Further increase in alkalinity resulted in a shift of the maximum toward longer wave-lengths, and ultimately to an increase in extinction with maximum at 2690 A. The values of Warburg *et al.* (*loc. cit.*, 1936) for adenine at p_H 9·7 (Fig. 1 and Table) show a diminution in extinction and shift of the maximum toward longer wave-lengths, as in our results. Holiday's values (*loc. cit.*) for p_H 3 and p_H 10 show no change in extinction but a shift to longer wave-lengths on change to alkaline solution, the latter agreeing with Warburg's results and ours. On the other hand, Gulland and Holiday report an *increase* in extinction and shift toward *shorter* wave-lengths for solutions in 0·05N-sodium hydroxide as compared with 0·05N-hydrochloric acid. We are at a loss to account for their results (inconsistent as they are with Holiday's earlier values, as well as with Warburg's and our own) unless the method used by them did not compensate adequately for the absorption of the alkali and acid.

FIG. 2.



Absorption Maximum of Adenine.

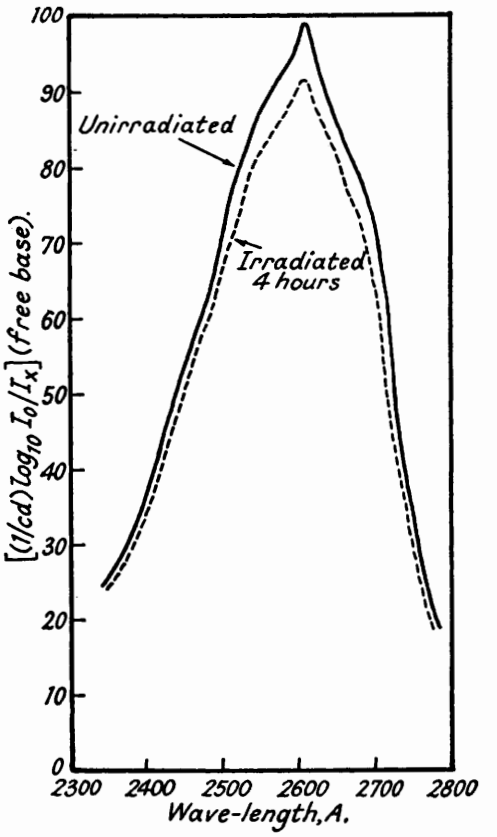
Reference.	Wave-length of maximum. Acidic.	Alkaline.	Molecular extinction at maximum. Acidic.	Alkaline.
Holiday (1930)	p_H 3 2630 A.	p_H 10 2660 A.	p_H 3 140,000 (12,300)*	p_H 10 140,000 (12,300)*
Gulland and Holiday (1936)	N/20-HCl 2600 A.	N/20-NaOH 2580 A.	N/20-HCl 13,200	N/20-NaOH 13,600
Warburg *et al.* (1935, 1936)	N/10-HCl 2600 A.	p_H 9·7 2650 A.	N/10-HCl 13,400	p_H 9·7 12,300
	p_H 1 2610 A.	p_H 10·0 2645 A.	p_H 1 13,400	p_H 10 12,000

* Values in parentheses are obtained from the reported values by using Heyroth and Loofbourow's method of correction (*loc. cit.*, 1934).

We believe the changes in absorption observed to be attributable to a tautomeric rearrangement of the type shown in (I) $\rightleftarrows$ (II). This, however, becomes more clearly evident on comparing the p_H effects for adenine with those for other purines and pyrimidines, and we therefore defer discussion of this point to a subsequent paper.

Fig. 3 shows the changes in spectra obtained on irradiating adenine. The most striking

FIG. 3.



feature is the marked stability of this compound toward ultra-violet radiation. Irradiation for as long as 4 hours resulted in inappreciable decrease in absorption. This is in contrast to the effects of ultra-violet radiation on uracil, etc. (Heyroth and Loofbourow, *loc. cit.*), and should serve as a helpful means of identifying this compound. In general, the lability of purines and pyrimidines to ultra-violet radiation appears to parallel the number of C=O groups they contain, as will appear from the subsequent reports.

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