to be in harmony with magnetic measurements on cytochrome and its derivatives, with spectrophotometric and titrimetric determinations on cytochrome, as well as with the investigations of Pauling and his co-workers on the constitution of hemoglobin. We see here a striking analogy with hemoglobin, which is also supposed to contain 2 histidine-imidazole groups in the vicinity of each iron atom (Conant,[11] Wyman[12]). One main difference between hemoglobin and cytochrome c, as regards the incorporation of the iron atom in the molecule, is that in hemoglobin one imidazole group is in a favorable position for coördination with the iron atom, whereas the other is not. Thus one of the six octahedral valences of the iron atom is left free for addition of oxygen, carbon monoxide, etc. In cytochrome c, on the other hand, both imidazole groups are in such a favorable position for coördination with the iron atom that a very firm compound is formed. Only in strongly alkaline or strongly acid solutions are one or both of the imidazole groups freed from the iron atom, after which compounds with carbon monoxide (to Fe^{++}) or CN^- and F^- (to Fe^{+++}) can be formed.

The heme of the cytochrome is thus built into the protein component in a manifold way: by means of thioether bindings from the side chains of the porphyrin to the protein, and by means of two histidine-imidazole groups strongly bound to Fe on each side of the flat heme disc. Thus the heme group appears to be built into a crevice in the protein molecule. This explains why cytochrome c is not autoxidizable, since oxygen can never approach the iron atom, and why no CO-compounds or cyanide compounds are formed at physiological *p*H values.

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RECEIVED OCTOBER 1, 1940

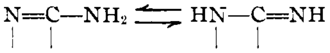
(12) J. Wyman, *J. Biol. Chem.*, **127**, 1 (1939).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SIENA HEIGHTS COLLEGE, AND FROM THE RESEARCH LABORATORIES OF THE INSTITUTUM DIVI THOMAE]

Ultraviolet Absorption Spectra of Nitrogenous Heterocyclic Compounds. III. Effect of *p*H and Irradiation on the Spectrum of 2-Chloro-6-aminopyrimidine

BY SISTER MIRIAM MICHAEL STIMSON, O.P., AND JOHN R. LOOFBOUROW[1]

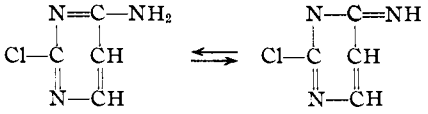
The change in the absorption of adenine with *p*H[1a] was accounted for by tautomerism of the amidine type



$$N{=}C{-}NH_2 \rightleftharpoons HN{-}C{=}NH$$

In adenine there are four nitrogen atoms which have a potentially labile hydrogen. With the exceptions of the 1,6 and 6 positions the possible tautomers may be expected to be all equal in absorption characteristics since in every case the —N=C< chromophore is within the ring and hence is approximately equal. In the shift involving the 1,6 and 6 positions the resonator is the free >C=N— in which the weight of the attached hydrogen is negligible. Brode[2] points out that the effect of a weight on a vibrating body is to decrease the frequency of oscillation and consequently of the absorption. Therefore a change in the position of a selected resonator from the 1,6 to 6 atoms should be detectable. We, therefore, investigated 2-chloro-6-aminopyramidine (I), since in this compound the introduction of a chlorine in the 2 position permits tautomerism involving only the 1,6 and 6 positions.

(I)



(II)

Experimental

Materials.—2-Chloro-6-aminopyrimidine was prepared according to the method of Johnson and Hilbert,[3] and separated from its isomer by steam distillation and repeated recrystallization from glass distilled water. Spectra were obtained with concentrations of 1.25 and 1.29 mg./liter; 4 and 2 cm. cells were employed. Kolthoff buffer tablets were used to adjust the *p*H and the comparison cell contained the corresponding buffer at the same concentration.

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

Results and Discussion

2-Chloro-6-aminopyrimidine shows two bands between 2200–2900 Å. as do also 6-aminopyrimi-

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[1] Present address: Massachusetts Institute of Technology.

[1a] Loofbourow and Stimson, Part I, *J. Chem. Soc.*, 844 (1940).

[2] Brode, "Chemical Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1939, p. 124.

[3] Johnson and Hilbert, THIS JOURNAL, **52**, 1152 (1930).

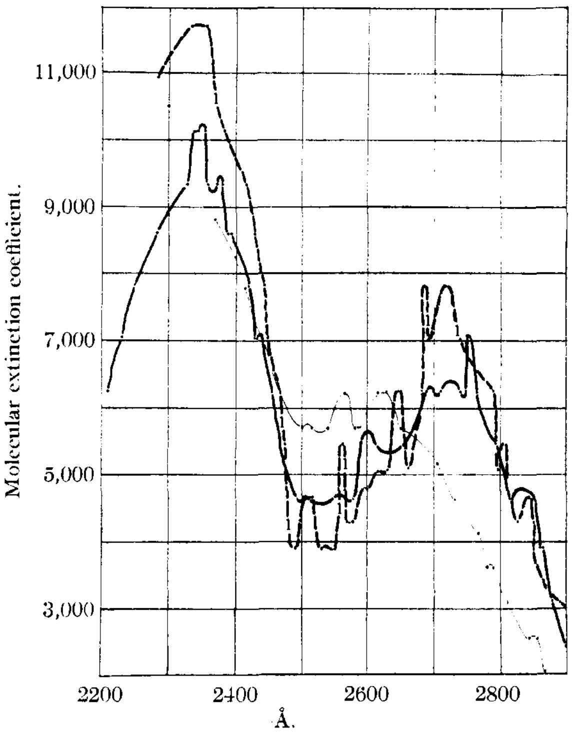

Fig. 1.—Absorption of 2-chloro-6-aminopyrimidine: heavy line at *p*H 7.0; broken line at *p*H 11.0; light line at *p*H 3.0.

dine and 6-hydroxypyrimidine.[4] This cannot be due solely to weighting of the vibrating group, for in the spectra of the chlorination products from pyrimidine to tetrachloropyrimidine.[5] Uber and Winters found that the second absorption peak at about 2300 Å. was not noted until the last member of the series, in spite of the fact that the chlorine atom in the 6 position contributes about twice the weight of the —NH_2 group.

Table I

Dependence of Two Bands on Weighting and Structure

Compound	Short wave band Å.	E	Long wave band Å.	E
6-Oxypyrimidine[4]	2280	6,500	2610	3,000
6-Aminopyrimidine[4]	2320	10,000	2660	3,500
2-Chloro-6-aminopyrimidine	2350	10,000	2750	7,000
Guanine[6]	2450	8,500	2750	7,000
Adenine[1]	..	....	2610	13,200
Pyrimidine[5]	..	....	2405	2,335
2,6-Dichloropyrimidine[5]	..	....	2575	4,100
2,4,6-Trichloropyrimidine[5]	..	....	2620	4,900
Tetrachloropyrimidine[5]	2307	8,262	2790	5,200

On the other hand, in the compound under consideration, since the weighting is less than in the case of dichloropyrimidine, the second peak is possibly due to the existence of dissimilar resonators and is more a function of electron forces than of mass. This would account for the occurrence of two bands also in 6-hydroxypyrimidine, 6-aminopyrimidine and guanine (Table I). Adenine, the purine analog of 6-aminopyrimidine, has but one peak at 2600, which has an extinction approximately twice that of 2-chloro-6-aminopyrimidine. It has six possible tautomers but they all involve the amidine type.

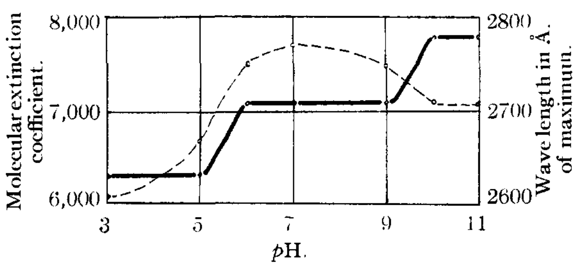

Fig. 2.—Broken line, relation between *p*H and wave length maximum; continuous line, relation between *p*H and maximum extinction for >C=N absorption.

Effect of *p*H.—The short wave band at 2350 Å. shows no appreciable change in position with increased *p*H. On the other hand, there is noted (Figs. 1, 2) change both in the extinction and the position of the >C=N— absorption at 2600 Å. The extinction increases up to *p*H 10.0. The change in absorption with *p*H must be due to tautomerism of the 1,6 and 6 positions and thus substantiates the interpretation of our results with adenine. Under the conditions of irradiation, for as long as four hours, there was practically no change in absorption, as was also the case with adenine, in contrast to the effect on uracil[7] and barbituric acid,[8] thus indicating under the conditions employed breakdown parallels the number of >C=O groups present. Further discussion of the relation of chemical constitution and *p*H with absorption spectrum will appear in subsequent papers.

Summary

1. The presence of two bands in the region 2200–2900 may be due to the presence of dissimilar chromophores.
2. The >C=N— absorption shifts to longer

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[4] Williams, Ruehle and Finkelstein, This Journal, **59**, 526 (1937).

[5] Uber and Winters, *ibid.*, **63**, 137 (1941).

[6] Holiday, *Biochem. J.*, **24**, 619 (1930); Heyroth and Loofbourow, This Journal, **56**, 1728 (1934).

[7] Heyroth and Loofbourow, *ibid.*, **53**, 3441 (1931).

[8] Loofbourow and Stimson, *J. Chem. Soc.*, 1275 (1940).

wave lengths with increase in *p*H from 3 to 7 and then slowly back.

3. The extinction of $>C=N-$ absorption increases from *p*H 3 to 10.

4. There is no apparent breakdown after ultraviolet irradiation for as long as four hours.

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RECEIVED MARCH 31, 1941

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

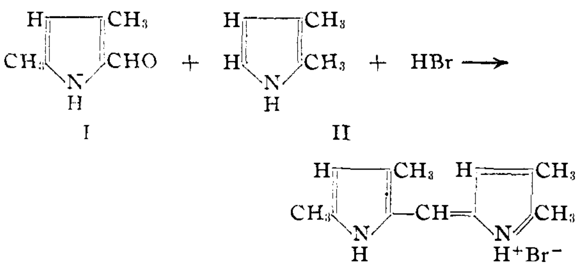
A Reinvestigation of the Configuration of Hemin[1]

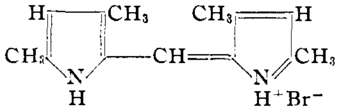
BY ALSOPH H. CORWIN AND ROBERT H. KRIEBLE[2]

The preceding papers of this series[3] have developed a study of the anomalies which occur in the aldehyde synthesis of dipyrrylmethenes. They have shown that in an attempted synthesis of an unsymmetrical methene by this method, the possibility must be taken into account that either of two symmetrical methenes may result in addition to the normal product. An anomalous course of this type would be discovered readily by analytical methods in cases in which the three methenes differed in composition. More subtle methods would be required, however, in those cases in which the possible products were isomeric. In the proof of the structure of hemin two of the key porphyrins, meso-porphyrin and deutero-porphyrin, were prepared from intermediates which fall into this indeterminate class. Since deutero-porphyrin was used in the synthesis of hemin, its configuration has been reinvestigated with the possibility of this anomaly in mind.

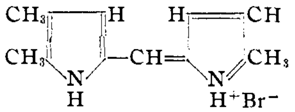
The critical reaction in the deutero-porphyrin synthesis is the preparation of compound III, Chart I:

CHART I





IV



V

The products which might be formed in addition to compound III are represented by formulas IV and V.[3] All three of these substances have been prepared previously[4] but no attempt to compare them was made by the investigators who first prepared them. The methene salts themselves are not suitable for melting point comparison and a description of the properties of the free base of V was not available for comparison at the time that the synthesis of hemin was completed. The recorded melting points of the free bases of III and IV are 115° and 118–119°, respectively.

Compounds III, IV and V have now been prepared for the purpose of direct comparison of properties. Under mild condensing conditions in the presence of hydrogen chloride instead of hydrobromic acid and in an anhydrous medium, the yield of III (hydrochloride) was raised to 95%. The free base for identification was prepared in a yield of 83% making an over-all yield of over 78% from the pyrroles. This yield is structurally significant since neither of the symmetrical methenes can be formed from the unsymmetrical aldehyde synthesis in a yield greater than 50%.

Compounds IV and V were prepared by the reactions shown in Chart II.

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[1] Studies in the Pyrrole Series. V. This paper is from the doctoral dissertation of Robert H. Krieble, The Johns Hopkins University, 1939, and was presented at the Baltimore Meeting of the American Chemical Society, April, 1939.

[2] Mary Carroll Garvan Fellow; present address, Socony-Vacuum Oil Co., Paulsboro, N. J.

[3] THIS JOURNAL, **58**, 1081 (1936); **58**, 1086 (1936); **59**, 1973 (1937); **62**, 418 (1940).

[4] III, Fischer and Kirstahler, *Ann.*, **466**, 183 (1928); IV, Piloty, Krannich and Will, *Ber.*, **47**, 2544 (1914); V, Fischer and Nüssler, *Ann.*, **491**, 170 (1931).