and 60 atm., respectively) in a steel container. Under those circumstances, a considerable amount of ethylene appears, and the reaction stops short of completion. Table II gives the results.

Differences in the Boiling Points.—A tabulation of the boiling points makes apparent the regularity of the boiling point depression caused by replacing chlorine by fluorine.

	B. p., °C.	Depression
CCl_3CCl_3	186	
CCl_3CCl_2F	138	48
CCl_2FCCl_2F or CCl_3CClF_2	92 or 91	46 or 47
CCl_2FCClF_2	47	45
or	or	or
CCl_3CF_3	45	46
$CClF_2CClF_2$	3	46
or	or	or
CCl_2FCF_3	− 2	47
$CClF_2CF_3$	−38	41
CF_3CF_3	−78	40
$CCl_2{=}CCl_2$	120	
$CCl_2{=}CClF$	71	49
$CClF{=}CClF$	22	49
or	or	or
$CCl_2{=}CF_2$	15	56
$CClF{=}CF_2$	−23	45
$CF_2{=}CF_2$	−78.4	55
CClFBrCClFBr	139.6	
$CClFBrCF_2Br$	92.8	46.8
CF_2BrCF_2Br	46.4	46.8

Molecular Refractions.—The Lorentz–Lorenz formula has been used to calculate the molecular refractions, and from the experimental results the atomic refraction of fluorine has been computed by subtracting the sum of the atomic refractions of carbon, chlorine, double bonds and bromine ("International Critical Tables" values were used).

	$t°$	Molecular refraction D	Molecular refraction B		F. Atomic refraction D	F. Atomic refraction B	
CCl_2FCCl_2F	25	30.768	30.896	31.242	1.105	1.096	1.097
	35	30.841	30.959	31.316	1.142	1.128	1.134
CCl_2FCClF_2	25	26.065	26.170	26.440	1.147	1.144	1.145
	35	26.142	26.273	26.516	1.173	1.179	1.170
$CClF_2CClF_2$	0	21.35	21.473	....	1.17	1.176	...
CCl_3CF_3	20	....	26.405	....	...	1.223	...
$CClFBrCF_2Br$	20	....	28.533	....	...	1.09	...
$CCl_2{=}CClF$	20	....	25.15	....	...	0.68	...
$CClF{=}CClF$	0	20.36	20.48	20.71	0.99	0.99	0.96
Cis and *trans*	0	20.44	20.60	20.85	1.03	1.05	1.03

The values found for the atomic refractions of fluorine agree well with the values given by Swarts[8] for a large variety of substances; they vary with the type of compound considered.

Summary

All the possible derivatives of ethane and of ethylene (together with their dibromides) which have all their hydrogen atoms replaced by chlorine or fluorine are described.

(8) Swarts, *J. Chim. Phys.*, **20**, 30 (1923).

THE MIDGLEY FOUNDATION COLUMBUS, OHIO RECEIVED MARCH 5, 1934

[CONTRIBUTION FROM THE BASIC SCIENCE RESEARCH LABORATORY, UNIVERSITY OF CINCINNATI]

Correlation of Ultraviolet Absorption and Chemical Constitution in Various Pyrimidines and Purines

BY FRANCIS F. HEYROTH AND JOHN R. LOOFBOUROW

It was shown previously that the ultraviolet irradiation[1] of dilute solutions of uracil and other pyrimidines and purines induces marked changes in their ultraviolet absorption spectra indicative of constitutional changes, and that, with sufficiently long continued irradiation, all selective absorption could be made to disappear. To afford a basis for the interpretation of these changes, information as to the absorption spectra of a series of related compounds was desirable. This paper presents the ultraviolet absorption spectra of eighteen such compounds, and permits comparisons to be made between their spectra and constitutions, so that conclusions may be drawn as to the manner in which various structural modifications are responsible for, or affect, the ultraviolet absorption of the compounds of this series.

Experimental

Method.—The technique used in determining the absorption spectra has been described previously. As there noted,[1,2] the measurements have not been corrected for certain deviations due to failure of the reciprocity law, but with the method employed these deviations are negligible, amounting to less than the experimental error at most wave lengths.

In all cases the solvent was redistilled water. The concentrations were chosen by trial so as to result in the greatest accuracy in the absorption determinations.

Values of P_H were determined by the quinhydrone elec-

(1) Heyroth and Loofbourow, THIS JOURNAL, **53**, 3441 (1931).

(2) Loofbourow, *Bull. Bas. Sci. Res.*, in publication.

trode when less than *P*H 7. More alkaline ones were determined colorimetrically.

Sources and Purification of Materials.—Guanine was prepared by hydrolysis of yeast nucleic acid by the method of Jones.[3] The guanine was employed as the hydrochloride, $C_5H_5N_5O \cdot HCl \cdot H_2O$, in concentrations of 15 and 20 mg. per liter. Adenine was prepared by the method of Levene[4] and was employed as the sulfate $(C_5H_5N_5)_2 \cdot H_2SO_4 \cdot 2H_2O$, in a concentration of 20 mg. per liter. The curves for uracil and dichloromethylpyrimidine are reproduced from our earlier paper.[1]

Thymine was prepared by the Johnson and Harkins[5] modification of the method of Wheeler and Merriam[6] and recrystallized five times. It melted at 321° (capillary tube). Wheeler and Merriam gave 326°, and Johnson and McKenzie[7] 325–335°. The compound was employed in a concentration of 15 mg. per liter. Cytosine was prepared from uracil by the method of Hilbert and Johnson.[8] It melted with decomposition above 311° (320–325°. Wheeler and Merriam[6]) and was employed as the monohydrate, in a concentration of 20 mg. per liter. Isocytosine monohydrate, melting about 280° with decomposition, was obtained as a by-product in this synthesis and photographed in a concentration of 20 mg. per liter. The dichloropyrimidine was prepared from uracil as an intermediate in this synthesis. It melted between 59 and 62° (61°. Hilbert and Johnson[8]). and was used in a concentration of 30 mg. per liter.

Pyrimidine was prepared by the method of Gabriel and Colman.[9] It melted at 18° (20–22°, Gabriel and Colman), and was used in a solution containing 30 mg. per liter. As but a very small quantity was available, this substance could not be recrystallized as many times as might have been desirable, so that its extinction curve may not be regarded as of the same degree of quantitative accuracy as those of the other compounds studied.

The sample of thymine glycol employed was obtained through the kindness of Dr. T. B. Johnson of Yale University. The solution used contained 250 mg. per liter. Barbital was obtained through the courtesy of Dr. Oliver Kamm of Parke, Davis and Co., and recrystallized repeatedly. It melted about 188.5° (191°, Fischer and Dilthey), and was used in a concentration of 5 mg. per liter. Barbituric acid (Eastman Kodak Co.) was recrystallized five times. The anhydrous substance was used in a concentration of 5 mg. per liter.

Isobarbituric acid was prepared by the action of tin and hydrochloric acid on 5-nitrouracil as described by Behrend and Roosen.[10] It was dissolved in potassium hydroxide, reprecipitated by hydrochloric acid, and washed repeatedly with cold water. The anhydrous material was photographed in a concentration of 15 mg. per liter. Isodialuric acid (dihydrate) was obtained from isobarbituric acid by the method of Behrend and Roosen.[10] It was studied in concentrations of 300 and 600 mg. per liter. Dialuric acid was prepared from uric acid by the method of Biltz and Damm.[11] It reddened at 188–189° and melted at 212° (214–215°, Biltz and Damm) and was found to contain one molecule of water.

Alloxan, prepared from uric acid by the method of Biltz and Heyn,[12] was recrystallized three times from water at 35–40°, and kept in a desiccator. At 170° each of the two samples employed lost one molecule of water. The monohydrate was photographed in concentrations of 100, 300 and 500 mg. per liter. Alloxantine was prepared by the oxidation of uric acid by potassium chlorate, followed by reduction by hydrogen sulfide. The recrystallized substance lost 11.8% of water at 170°, which indicates the presence of two molecules of water (theoretical for 2, 11.2%; 3, 15.91%; 3 water of which 2 are lost at 170°, 10.6%). The concentration used was 50 mg. per liter. The adenine methylthiopentose was kindly furnished by Dr. P. A. Levene of the Rockefeller Institute for Medical Research. It was photographed at 20 mg. per liter.

Results and Discussion

It appears from Fig. 1 that ring compounds of the pyrimidine series which lack ethylene linkings within the ring may exhibit end absorption but lack the marked degree of selective absorption

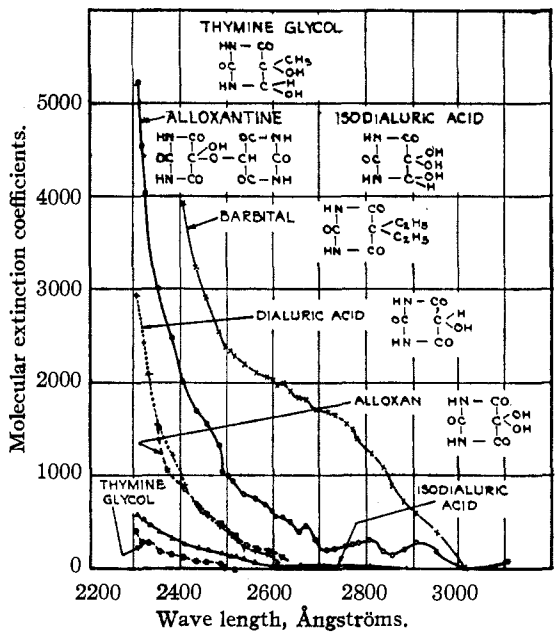


Fig. 1.

possessed by such compounds as uracil and the other pyrimidines derived from nucleic acids (Fig. 2). Since many of the compounds in Fig. 1 possess carbonyl groups in the same or greater number than does uracil, it is evident that the absorption of uracil cannot be attributed to its

(3) Jones, "The Nucleic Acids," 1907.
(4) Levene, "Nucleic Acids," The Chemical Catalog Co., Inc., New York.
(5) Johnson and Harkins, THIS JOURNAL, **51**, 1240 (1929).
(6) Wheeler and Merriam, *Am. Chem. J.*, **29**, 478 (1903).
(7) Johnson and McKenzie, *ibid.*, **42**, 369 (1909).
(8) Hilbert and Johnson, THIS JOURNAL, **52**, 1152 (1930).
(9) Gabriel and Colman, *Ber.*, **32**, 1525 (1899).
(10) Behrend and Roosen, *Ann.*, **251**, 235 (1889).
(11) Biltz and Damm, *Ber.*, **46**, 3662 (1913).
(12) Biltz and Heyn, *Ann.*, **413**, 60 (1917).

carbonyl groups, but is rather due to its single ethylene linkage. The end absorptions of barbital, dialuric acid and alloxan, each of which possesses three carbonyl groups, are greater than

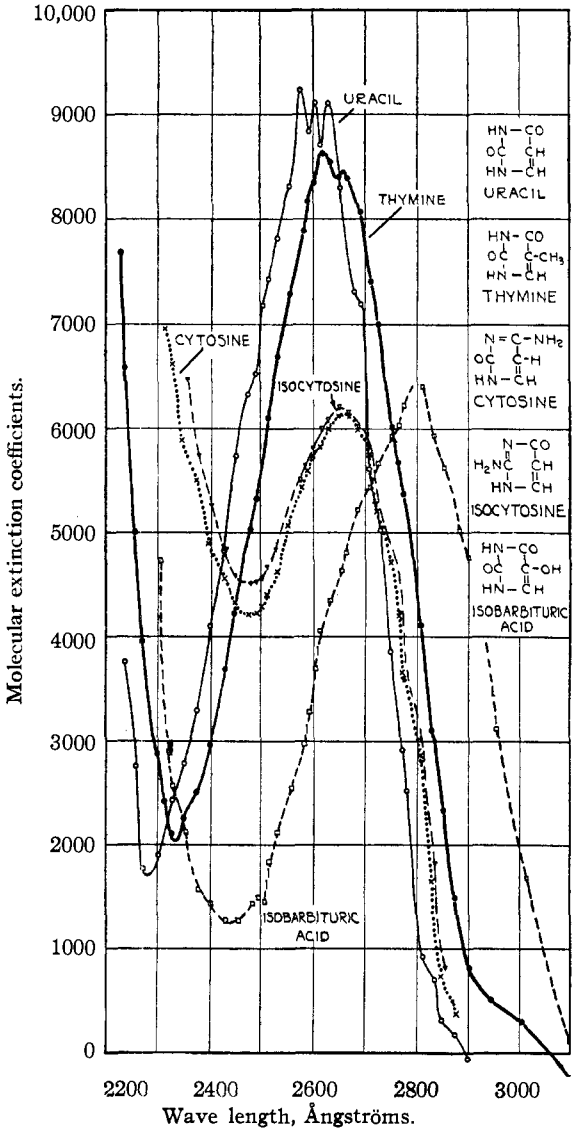


Fig. 2.

those of isodialuric acid or thymine glycol which possess only two carbonyl groups, so that absorption due to the unsaturated valences of this group does play some part, though a small one. Weighting the molecule, either by alkyl groups as in the case of barbital, or by joining together two rings each of which may be considered as reinforcing the absorption of the other as in the case of alloxantine,[13] appears to increase the absorption of the compound, and to shift it toward longer wave lengths. These observations on weighting in the pyrimidine series agree very well with the similar conclusion of Henri and Bielecki[14] in the case of the aliphatic alcohols or fatty acids.

The introduction of a single ethylene linking, as in the case of uracil or thymine (Fig. 2) produces a high degree of selective absorption, with its maximum in the neighborhood of 2600 Å. The case of barbituric acid is of special interest in this connection (Fig. 3). In it, the molecular extinction in this region is of the order of 25,000, while that of uracil is slightly over 9000. Macbeth, Nunan and Traill,[15] who observed deep complex bands in the regions of 2210–2250, 2380–2710 and 2940–3395,[16] attributed this absorption to the

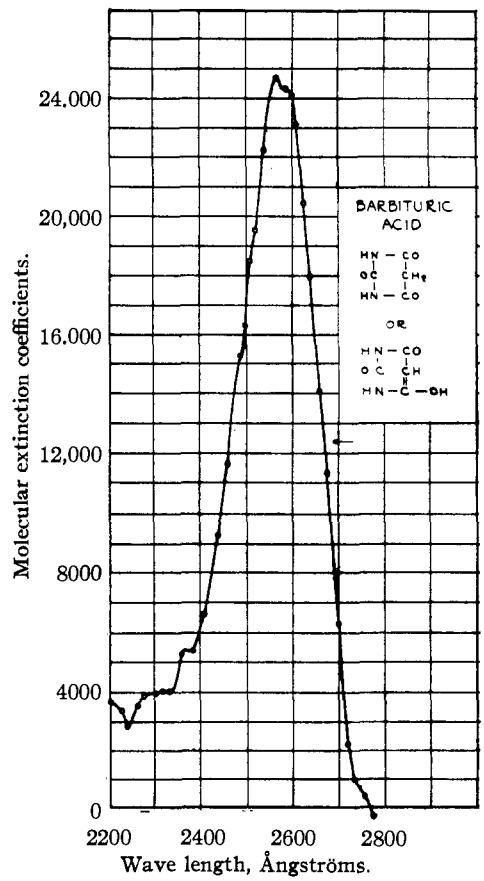


Fig. 3.

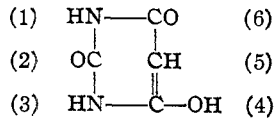
presence of an enolized form in aqueous solutions of the acid. The magnitude of the absorption of barbituric acid is, however, so much greater than

(13) In measuring the absorption of an alloxantine solution, one of course measures the summed absorptions of the components of an equilibrium mixture of dialuric acid, alloxan and alloxantine.

(14) Henri and Bielecki, *Ber.*, **45**, 2819 (1912); **46**, 1304 (1913).

(15) Macbeth, Nunan and Traill, *J. Chem. Soc.*, 1248 (1926).

(16) Neither we nor Marchlewski noted any absorption at 2940–3395 Å. This possibly may be attributed to an impurity in the sample studied by Macbeth, Nunan and Traill.



that of uracil or of thymine as to suggest that there may be a different mechanism responsible for the production of the absorption in these two cases. Macbeth, Nunan and Traill state that barbituric acid loses its absorption to some extent in the presence of hydrochloric acid, by which it is converted largely into the ketonic form. Furthermore, Marchlewski and Wiersuchowska[17] found that Beer's law does not apply to this compound. These facts suggest that in aqueous solutions both enolic and ketonic forms may be present in an equilibrium, the position of which may be shifted by altering the concentration. That such an equilibrium is present suggests the possibility that the high degree of absorption of barbituric acid may be attributed to a tautomeric change from one form to the other, the magnitude of absorption being a measure of the amount of tautomeric change.[18] It is interesting that the absorption thus ascribed to the tautomeric change is situated at practically the same wave length as that due to the 4,5-ethylene linkage in uracil and thymine. It should be noted that this type of tautomerism of barbituric acid is distinct from the lactam—lactim transformation of uracil, the effects of which upon the absorption spectrum have been previously studied by the authors (Fig. 6 of Ref. 1).

The introduction of a second double bond within the ring does not increase the selective absorption about 2600 Å., but rather has the effect of diminishing it (cytosine, Fig. 2). As the unsaturation dissymmetry of the molecule is decreased, the characteristic absorption is also decreased. Cytosine has a molecular extinction coefficient of about 6100 at 2650 Å., while that of uracil is about 9100 at 2600 Å. The effect is seen again in isocytosine (Fig. 2). During the lactam–lactim transformation in uracil, a second double linkage is introduced in the 1,6 position, rendering the molecule analogous to that of cytosine and causing (Fig. 6 of Ref. 1) a similar diminution in absorption at 2600 Å. At the same time there is in both cytosine (and isocytosine) and the lactim form of uracil a shift of the entire absorption band of about 50 Å. toward the longer waves as compared with the band of the lactam form of uracil, which may be correlated with the introduction of the second double linking. (The molecular weight of cytosine and isocytosine is only one less than that of uracil, so that an effect of increased mass need not be considered.)

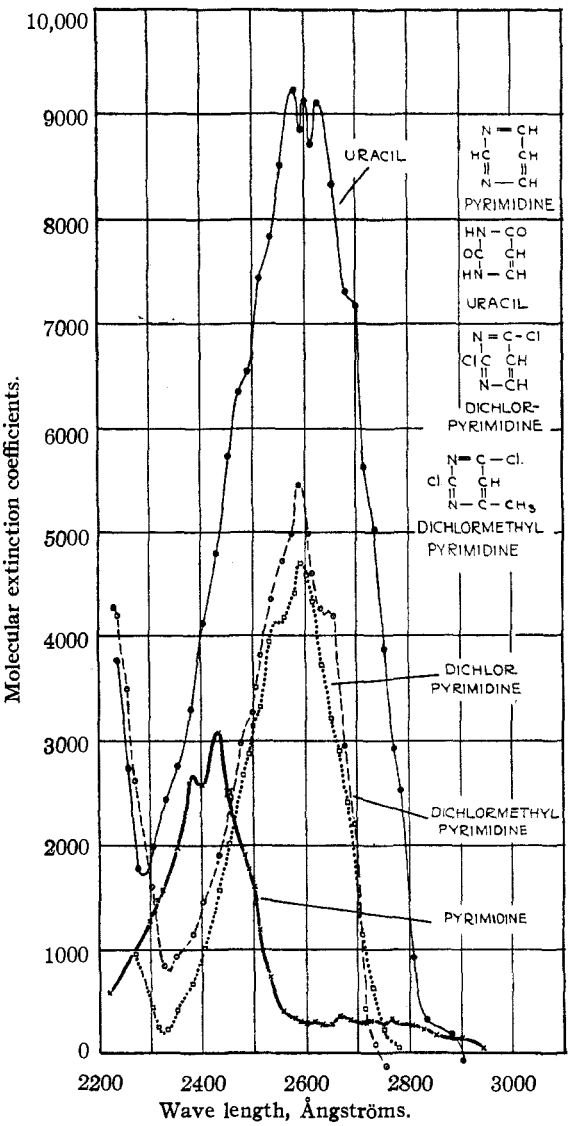


Fig. 4.

Pyrimidine (Fig. 4) has still a third double bond within the ring. With the formation of a symmetrical arrangement of the double linkings, there is still further reduction in the extinction. (The values given do not, however, have the same degree of quantitative significance as most of the

(17) Marchlewski and Wiersuchowska, *Bulletin of Polish Acad.*, 64 (1929).

(18) Compare Baly's theories of the influence of tautomeric changes on the absorption of various organic compounds, *J. Chem. Soc.*, **85**, 1029 (1904); **87**, 766 (1905); **89**, 489 (1906) and several later papers. But see A. Hantzsch, *Ber.*, **43**, 3049 (1910), for a different point of view.

other curves because the compound was of a somewhat lower degree of purity.) There is also in this instance a marked shifting of the absorption band toward the shorter-waved region, the maximum occurring at 2430 Å. There is, then, the following series for the correlation of absorption with the number of double bonds within the ring in this series of compounds.

Number of unsaturated linkings		Approximate E	Wave length of maximum, Å.
0	Only end absorption		
1	(In one tautomer as in barbituric acid)	24,500	2550
1	(Uracil or thymine)	9000	2600
2	(Cytosine or isocytosine)	6100	2650–2660
3	(Pyrimidine)	3000	2430

It is interesting to compare the absorption of these compounds with that of the still more symmetrical benzene, which, in alcohol, has a series of seven or more bands between 2000 and 2600 Å., the maximum molecular extinction being only about 250 at approximately 2580 Å.[19] The magnitude of the extinction is much less than that of pyrimidine. Pyridine,[19] which with its one nitrogen atom is intermediate in symmetry between benzene and pyrimidine, has a molecular extinction coefficient of 1780 at 2550 Å. In these compounds the effect of conjugated double linkings appears to be the unexpected one of lowering the extinction coefficient. Benzene with three conjugated double bonds absorbs the least. The effect of the successive introduction of nitrogen atoms in benzene increases the absorption through that of pyridine to that of pyrimidine. A reduction of the number of conjugated double bonds to that of cytosine increases the extinction, while compounds with no conjugated double linkings within the ring but only a single double bond within the ring, or a conjugated double bond extending without the ring, as in uracil or thymine, have increased absorption. Uracil and thymine and barbituric acid can, however, be written in tautomeric forms, which have three conjugated double linkings. To produce such structures from the structures as usually written, more atoms must shift tautomerically in the case of barbituric acid than in the case of thymine or uracil, and this may be correlated with the greater absorption of barbituric acid.

Effect of Weighting the Ring

In the case of open-chain compounds, the usual effect of weighting a given molecule is to shift its absorption band toward longer wave lengths and to increase the absorption. In discussing the compounds with saturated rings in this series, similar effects were noted as probable. In the unsaturated compounds, the shift in wave length is, in general, clearly marked, although its magnitude appears to vary with the position in the ring upon which the weighting occurs, and possibly also with the nature of the weighting atom, or group of atoms. The introduction of a methyl group (added CH_2 = 14) in position 5 as in thymine (Fig. 2) shifts the band by 50–60 Å. The introduction of any oxygen atom in position 5 of uracil, converting it to isobarbituric acid (Fig. 2), however, exerts a very much greater effect, shifting the band by 200 Å., so that its maximum lies at 2800 Å. In both instances, the expected effect of increasing the absorption is lacking, and, on the contrary, the weighting on position 5 appears to occasion a lessening of the absorption. This is slight (500, or 5.5%, and close to the experimental error) in the case of thymine where the shift is also slight, but greater (about 30%) in the case of isobarbituric acid where the shift is also greater. The value of E for isobarbituric acid is somewhat uncertain because the criteria of purity used are less certain. Elementary analyses were not made. The free acid rather than a salt was used, so that this effect cannot be attributed to the introduction of a second double linking due to a lactam–lactim transformation.

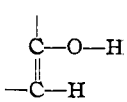
In the compounds with two double linkings studied, cytosine, isocytosine (and the lactim form of uracil), there are no significant differences in the masses of the molecules. As the positions of the maxima agree fairly well, it is evident that the position of the band is independent of the positions, 2 or 6, bearing the weighting group. No compound with two double bonds within the ring weighted in position 5 was examined.

The introduction of two chlorine atoms into pyrimidine in positions 2 and 6 shifts the band by about 200 Å. (Fig. 4) toward the longer wave region, so that its absorption coincides closely with that of uracil. At the same time, the absorption is markedly increased. The further introduction of a methyl group in position 4 (2,6-dichloro-4-methylpyrimidine), while apparently not shifting the band, increases the absorption slightly.

In general, however, it may be stated that, at least in the case of the unsaturated 6-member ring

(19) "International Critical Tables," Vol. V, p. 363.

compounds of this series, weighting exerts the usual effect of shifting the absorption toward the visible. The effect on the magnitude of the absorption is less certain. When the weighting groups are not in position 5, there appears to be the usual expected increase in absorption. When, however, the weighting occurs on position 5, the absorption is decreased, at least in the restricted number of compounds investigated. When OH in position 5 is the weighting group, the decrease in absorption (as well as the shift in wave length) is particularly marked. This may involve some type of saturation of the unsaturated linking by the residual valences on the O so closely adjacent. Such an hypothesis would not, however, account for the slight reduction noted in the case of thymine.



It is also worthy of note that in the case of cytosine and isocytosine, the short-wave absorption minimum occurs at 2480 rather than at 2300, and that at this minimum the absorption is higher than in the case of most of the pyrimidines studied, notwithstanding the fact that absorption in this region has been attributed in the other compounds studied to the carbonyl groups. In these compounds there is, however, only one carbonyl group. This may mean that a single carbonyl group produces a greater absorption than do two or three, just as does a single ethylene linking as contrasted with two or three.

The Purines.—Figure 5 presents the absorption of guanine and of adenine.[20]

In their forms the curves of these purines confirm the observations of Holiday,[21] who found adenine to have one peak at 2630 Å., while guanine had two absorption maxima at 2760 and 2500 Å. Dr. Holiday has advised us in personal communications that the factor 10^5 used in his paper in stating the values for the molecular extinction coefficients of these purines was a misprint for 10^4, that his value for the free base adenine should have been 1.06×10^4, and that all values in that paper, including that just stated for adenine, should be corrected for a sector error by multiplying by 1.16. So corrected, his value for the molecular extinction coefficient of adenine is 1.23×10^4, which is considerably higher than that (1.03×10^4) found by us. On the other hand, his corrected values for guanine at P_H 0.87 are in agreement with those found by us at P_H 1, being 1.26×10^4 at 2500 Å., and 0.73×10^4 at 2760 Å., while we found 1.26×10^4 and 0.79×10^4, respectively. Dr. Holiday has also apprised us that a highly purified specimen of adenine sulfate has given the still higher value of 1.32×10^4 calculated for the free base, and suggests that the discrepancy may be due to the presence in our material of a fluorescent impurity.

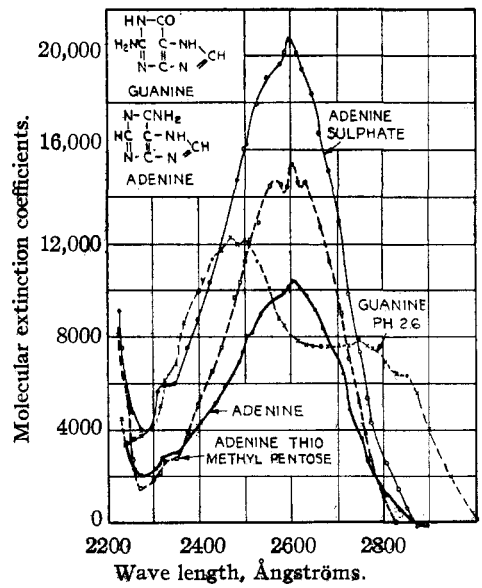


Fig. 5.

We have confirmed the observation by Holiday of the influence of P_H upon the absorption of guanine (Fig. 6), an effect which he noted as absent in the case of adenine and certain other purines. Since in the case of uracil we have shown[1] the reaction greatly to affect the absorption through a lactam–lactim rearrangement, and guanine (but not adenine) is capable of such a change, it might be thought that this type of structural change is responsible for the change in extinction of that compound at 2500 Å. Such a change in structure need not be directly correlated with dissociation, but is merely an indication of a change of HN—C=O to N=C—OH, which, once formed, may dissociate at suitable reactions without materially affecting the absorption.

No explanation can at present be advanced to account for the two types of absorption exhibited by guanine and uric acid, on the one hand, which have two peaks at certain H-ion concentrations,

(20) The molecular extinction values for adenine were erroneously stated in the Fig. 7 of our previous paper (Ref. 1). The experimental values then obtained, when correctly calculated, agree well with our present values. As adenine sulfate contains two molecules of base per molecule of salt, the absorption of free adenine is half as great and is reproduced for comparison.

(21) Holiday, *Biochem. J.*, **24**, 619 (1930).

and adenine, hypoxanthine and other purines on the other, which have but one large band typical of the absorption of the pyrimidine.

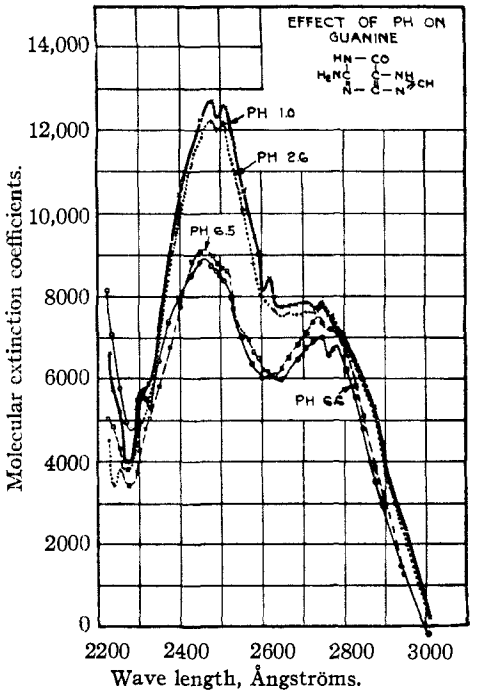


Fig. 6.

Figure 5 also includes the absorption of adenine thiomethylpentose. In this compound the sugar is assumed to be attached to the purine (as in the case of other nucleosides) on position 7. Our value of 15,300 for the molecular extinction coefficient of adenine thiomethylpentose is close to that of 15,080 (*i. e.*, 1.16 times his reported value) found by Holiday for both adenosine and adenine nucleotide. Myrbäck, v. Euler and Hellström,[22] found a somewhat lower value (1.43×10^4) for adenosine. As compared with adenine, these three compounds have increased extinction values (1880 for adenosine), although the shift toward longer wave lengths which usually accompanies weighting by non-absorbing substituents is negligible or absent. In the case of guanine and guanine nucleotide, Holiday found nearly identical values at *P*H 1 at 2480–2500 Å.

A summation of the molar extinctions at 2600 Å. of adenine,[23] guanine, cytosine and thymine (13,200, 7800, 5700 and 8400) gives 35,100 for the extinction coefficient of thymus nucleic acid. Such a calculation, however, neglects the effects of weighting. No observations have been made of this effect in the case of the two pyrimidine nucleosides. In the case of guanine it is negligible, while in the case of adenine it is of the order of 1880, as stated above. With this correction, the molecular extinction coefficient of thymus nucleic acid becomes 36,980, while our highest observed value[1] is 34,900.

(22) Myrbäck, v. Euler and Hellström, *Z. physiol. Chem.*, **212**, 12 (1932).

Summary and Conclusions

By comparing the ultraviolet absorption spectra of eighteen pyrimidines and purines, various features of the effect of molecular constitution are made evident and related to similar phenomena in other groups of organic compounds.

1. Saturation of the ethylene linkages within the ring results in a loss of selective absorption.

2. The introduction of a second or third double linkage into the ring lessens the magnitude of the selective absorption.

3. The effects of substitution depend both upon the nature and position of the substituents. The replacement of a hydrogen atom of position 5 of uracil by the hydroxyl group produces isobarbituric acid, in which the absorption is shifted toward the lesser frequencies. Weighting usually, but not always, shifts the absorption in the same direction. When the weighting group is attached to position 5, it sometimes decreases the absorption. In other situations, it usually increases the absorption.

4. The observations of Holiday upon the influence of *P*H upon the absorption of guanine have been confirmed.

5. The relation of the absorption of the nitrogenous ring compounds of nucleic acid so that of nucleic acid itself has been discussed.

CINCINNATI, OHIO RECEIVED MARCH 21, 1934

(23) Employing Holiday's value.