acetate in chloroform shows a band at 11.15 μ which is absent from the spectrum of zygadenine triacetate.

Zygadenine Acetonide.—Zygadenine (285 mg.) was dissolved in boiling methanol (2 ml.) and constant boiling hydriodic acid (5 drops, Merck reagent) was added to *p*H 4. Acetone (2 ml.) was added and, upon rubbing, zygadenine acetonide hydriodide separated in the form of colorless needles (299 mg., m.p. 292–295° dec.).

Anal. Calcd. for $C_{30}H_{47}O_7N\cdot HI$: C, 54.46; H, 7.31. Found: C, 54.16; H, 7.32.

Zygadenine acetonide was liberated from the salt (250 mg.) by the procedure described above for zygacine acetonide. Crystallization of the crude product from acetone–petroleum ether gave zygadenine acetonide containing one mole of acetone of crystallization (130 mg.), m.p. 220–230° after softening from 210°, $[\alpha]^{23}D$ −17° (*c* 1.29, chf.).

Anal. Calcd. for $C_{30}H_{47}O_7N(CH_3COCH_3)$: C, 66.98; H, 9.03. Found: C, 66.79, 67.02; H, 8.69, 8.83.

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, THE HEBREW UNIVERSITY, HADASSAH MEDICAL SCHOOL]

The Relationship between Spectral Shifts and Structural Changes in Uric Acids and Related Compounds

BY FELIX BERGMANN AND SHABTAY DIKSTEIN[1]

RECEIVED JUNE 28, 1954

The absorption maxima of various uric acids have been determined in the non-ionized state and as function of *p*H, and the apparent dissociation constants have been derived. The sequence of ionization is established as 9,3,1. Both alkylation and ionization produce corresponding spectral shifts, but of different magnitude. Alkylation or dissociation at N^9 or N^3 are accompanied by a bathochromic shift, and at N^1 by a hypsochromic effect. An analogous behavior is observed with uracils, but is missing in the xanthine series and in hypoxanthine. The relationship between these spectral changes and the possible resonance forms at various stages of ionization is discussed.

In connection with a study on the metabolism of substituted uric acids a knowledge of the dissociation constants of their ionizable groups was needed. A search in the literature revealed that the required *pK* values are partly unknown and partly still controversial due to the very limited solubility of these acids.[2–4] Since the spectrophotometric determination of *pK* has proved its applicability to related compounds, such as uracils[5] and xanthines,[6] a study of the *p*H dependence of the absorption bands of uric acids was undertaken.

Experimental[7]

Materials.—Commercial uric acid was purified by recrystallization from hot water. 1-Methyluric acid was synthesized according to Fischer.[8] The preparation of 3-methyl-, 1,3-dimethyl- and 1,3-diethyluric acids according to Traube[9] was modified in the following way. Reduction of the 5-nitroso-6-aminouracil was carried out with hot concentrated ammonium sulfide and the yield of diaminouracils was thus considerably improved.[10] 3,7-Diethyl-,[11] 3,7,9-trimethyl-[12] and 1,3,7-trimethyluric[13] acids were synthesized in accordance with the data in the literature.

Method.—Measurements of absorption spectra were carried out with a Beckman ultraviolet spectrophotometer, Model DU, on aqueous solutions, containing 10 μg./ml. A range of *p*H 1–3 was achieved by addition of perchloric acid, *p*H 3–7 by 0.15 *M* acetate buffer, *p*H 7–9 by 0.05 *M* borax, adjustment being made with perchloric acid. *p*H 9–12 by 0.15 *M* borate buffer; *p*H 13 by 0.1 *N* NaOH; *p*H 14 by 1 *N* NaOH. Beyond *p*H 12 the solutions are unstable and have to be prepared immediately before use. Instability was especially noted with caffeine, 3-methyl- and 3,7-dimethyluric acid. The absorption maxima at the higher *p*H values were therefore checked three times with freshly prepared solutions.

The long wave length maximum was first determined approximately by reading at intervals of 50 Å. Then the optical density of a wave length near the expected position of λ_{max} was measured. Going up 5–10 Å. on the wave length scale, the galvanometer was adjusted to zero for the solvent, without changing the transmission, using only sensitivity control and slit width, while the selector switch was on "check." Now the solution was measured with the selector switch on "1." The direction of deviation of the galvanometer needle then determines the change of extinction (movement to the left denoting a decrease.). If extinction is increasing, the new wave length is used to adjust the galvanometer again to zero by turning the transmission knob. The next measurement at a 10 Å. longer wave length is carried out as before. Since the maximum can be approached from both sides, this very sensitive procedure permits localization of the maximum within a short time with an accuracy of ±5 Å.

λ_{max} was finally plotted as function of *p*H and the *pK* values were obtained according to Robinson and Pekrul.[14]

Results

The curves in Fig. 1 represent λ_{max} as function of *p*H. In all derivatives of uric acid with a free NH-group at position 9, the main bathochromic shift occurs in the *p*H range 4–8 and thus permits the evaluation of pK_9 (see Table Ib).[15] When position 3 is unsubstituted, a further bathochromic shift appears at *p*H 9–12 and is thus related to pK_3. On the other hand, a hypsochromic shift is observed above *p*H 12 for 3-methyl- and 3,7-dimethyluric acid and

(1) Part of a Ph.D. Thesis submitted to the Faculty of Science of the Hebrew University, Jerusalem.

(2) F. Gudzent, *Z. physiol. Chem.*, **56**, 150 (1908).

(3) (a) H. Biltz and L. Herrmann, *Ber.*, **54**, 1676 (1921); (b) A. Fromherz and A. Hartmann, *ibid.*, **69**, 2420 (1936).

(4) Sakuji Takagi, *J. Physiol. Soc. (Japan)*, **13**, 129 (1951); *C. A.*, **45**, 8857 (1951).

(5) D. Shugar and J. J. Fox, *Biochim., Biophys. Acta*, **9**, 199 (1952).

(6) L. F. Cavalieri, J. J. Fox, A. Stone and N. Chang, THIS JOURNAL, **76**, 1119 (1954).

(7) Since m.p.'s are not useful for checking the purity of these compounds, recrystallization was repeated until the absorption spectrum remained constant.

(8) E. Fischer, *Ann.*, **215**, 304 (1882); E. Fischer and H. Clemm, *Ber.*, **30**, 3091 (1897).

(9) W. Traube, *Ber.*, **33**, 3035 (1900); J. H. Speer and A. L. Raymond, THIS JOURNAL, **75**, 114 (1953).

(10) The authors wish to thank Prof. B. B. Brodie, National Heart Institute, National Institute of Health, Bethesda, Md., for a sample of 1,3-dimethyluric acid. They are also obliged to Dr. V. Papesch of G. D. Searle and Co., Chicago, Illinois, who generously supplied 3-methylxanthine and several intermediates for the synthesis of substituted uric acids.

(11) H. Biltz and P. Damm, *Ann.* **406**, 35 (1914).

(12) E. Fischer and L. Ach, *Ber.*, **28**, 2484 (1895).

(13) H. Biltz and P. Damm, *Ann.*, **413**, 189 (1917).

(14) E. J. Robinson and L. F. Pekrul, THIS JOURNAL, **67**, 1186 (1945).

(15) Unfortunately, since no 9-substituted uric acid with a free 7-position was available, our experimental data do not yield information as to the magnitude of pK_7.

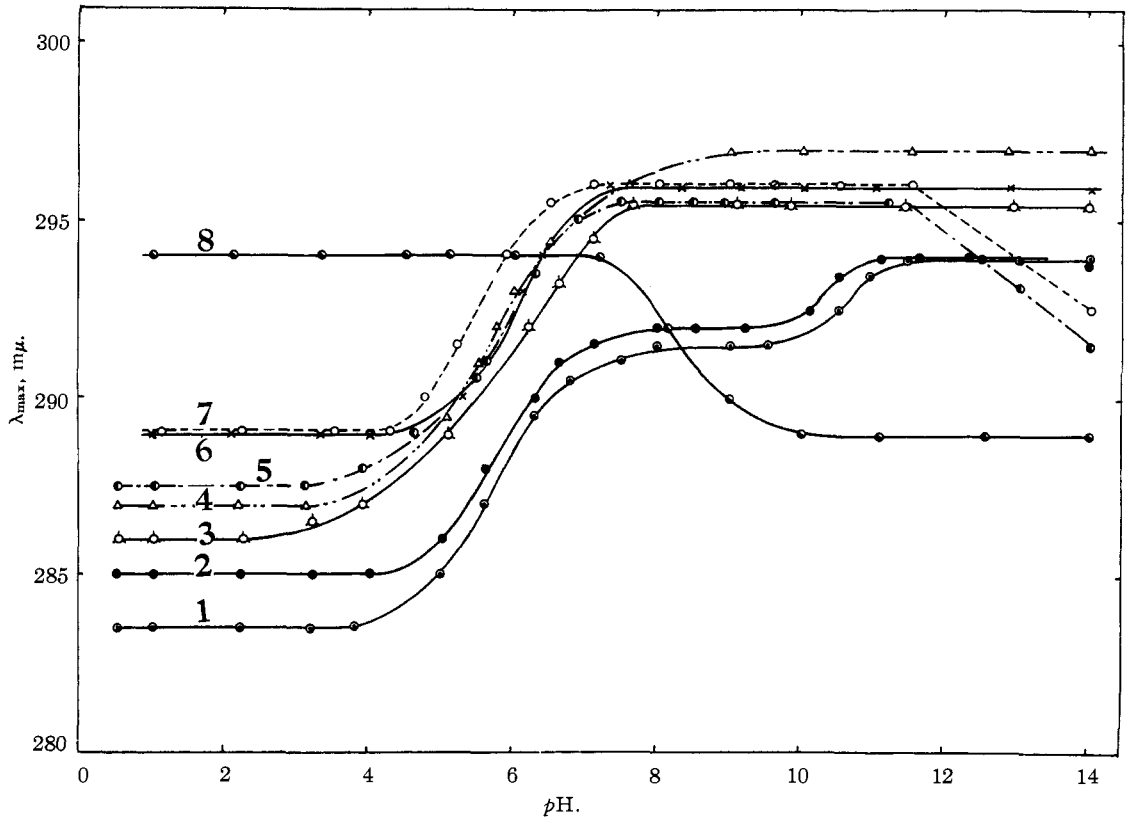


Fig. 1.—Long wave absorption maxima of uric acids as function of *p*H: 1, ⊙—⊙—⊙—⊙, 1-methyluric acid; 2, ●—●—●—●, uric acid; 3, ♀—♀—♁—♁, 1,3-dimethyluric acid; 4, △—- - —△—-, 1,3-diethyluric acid; 5, ◐ - — ◐ - — ◐, 3-methyluric acid; 6, x—x—x—x, 1,3,7-trimethyluric acid; 7, O - - O - - O, 3,7-dimethyluric acid; 8, ◔—◔—◔—◔, 3,7,9-trimethyluric acid. The descending portions of curve 5 and 7 beyond *p*H 12 are not very accurate, due to rapid decomposition of the substances in strongly alkaline solutions.

at *p*H 7–10 for 3,7,9-trimethyluric acid. This phenomenon, which is related to the NH-group in position 1, is however absent in uric acid itself.

The curves in Fig. 1 also reveal a characteristic influence of alkyl substituents on the position of the absorption maximum. In Table II we compare the values of λ_{max} of substituted uric acids at low *p*H, where these compounds exist only in the undissociated form. The N^1-methyl produces a hypsochromic shift, relative to the λ_{max} of uric acid, which parallels the effect of ionization in the same position and contrasts the influence of alkyl groups at N^3, N^7 or N^9. Evidently, both alkyl substitution and ionization influence the spectral properties in the same direction, by changing the excitation energy in the same sense. In order to correlate these phenomena with an over-all increase or decrease in conjugation, two main questions have to be answered. (a) What determines the order of successive steps of ionization? (b) Why is alkylation or ionization at N^1 accompanied by a hypsochromic shift, in contrast to the corresponding process at N^3 or N^9?[16]

The spectral phenomena observed here have to be explained on the basis of the fundamental structure of uric acids. Since the presence and distribution of nitrogen determines the specific behavior of such heterocycles, the participation of nitrogen atoms in resonance phenomena is of utmost importance. Both the imidazole and the pyrimidine rings are characterized by the presence of the triad system N=C—N, which represents one of the strongest resonators in conjugated systems. It has also been shown that a single nitrogen atom, by virtue of its unshared electron pair, extends an adjacent unsaturated system similar to an additional carbon-to-carbon double bond.[17] Thus in semicarbazones the isolated NH-group mediates conjugation between the C=N double bond and the C(=O)–NH group,[18] and the analogous phenomenon is observed in 2,4-dinitrophenylhydrazones, where the aromatic ring becomes conjugated with the C=N— double bond.[19] Therefore while a terminal nitrogen atom acts like an additional double bond, an "isolated" nitrogen atom between two unsaturated groups will produce some kind of cross-conjugation and give only about half the bathochromic shift as in the former case, in analogy to

(16) In this paper only shifts of the maxima at the longest wave lengths are considered and the changes in ϵ_{max} are reserved for future discussion. It is assumed throughout this paper that enolization in the NH-CO- groups is absent. Evidence for the lack of enolization in uric acids will be discussed in a subsequent paper.

(17) K. Bowden, E. A. Braude, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 45 (1946).

(18) L. K. Evans and A. E. Gillam, *ibid.*, 565 (1943).

(19) E. A. Braude and E. R. H. Jones, *ibid.*, 498 (1945).

TABLE I

APPARENT DISSOCIATION CONSTANTS OF VARIOUS PYRIMIDINE DERIVATIVES

(a) Uracils[a]	pK_1	pK_2	
Uracil	9.5	>13	
5-Methyluracil	9.9	>13	
1-Methyluracil	...	9.75	
3-Methyluracil	9.95	...	
2-Ethoxy-4-ketopyrimidine	...	8.2	
4-Ethoxy-2-ketopyrimidine	10.7	...	
(b) Uric acids	pK_3	pK_2	pK_1
Uric acid	5.75	10.3	..
3-Methyluric acid	5.75	...	>12
1-Methyluric acid	5.75	10.6	..
1,3-Dimethyluric acid	5.75	...	..
1,3-Diethyluric acid	5.75	...	..
3,7-Dimethyluric acid	5.5	...	>12
1,3,7-Trimethyluric acid	6.0	...	..
3,7,9-Trimethyluric acid	...	...	8 35
(c) Xanthines	pK_3	$pK_{7(9)}$	pK_1
Xanthine	7.7	10.6[b]	..
3-Methylxanthine	...	8.8[c]	(11.9 ?)[d]
1,3-Dimethylxanthine	...	8.7	..
7-Methylxanthine[e]	8.7	...	10.7
3,7-Dimethylxanthine	...	...	9.9
1,7-Dimethylxanthine[e]	8.8	...	..
(d) Hypoxanthine	$pK_{7(9)}$	pK_1	
	8.8	>12	

[a] Values taken from ref. 5. [b] This figure is 1 unit lower than the value given by Cavalieri and co-workers (ref. 6). [c] Cavalieri and co-workers report 8.3. [d] In our measurements (see Fig. 2) a second ionization step cannot be identified unequivocally. [e] Cavalieri and co-workers report for 7-methylxanthine 8.3 and >12, for 1,7-dimethylxanthine 8.7. Our measurements were carried out with samples supplied by Dr. J. J. Fox, whose generosity is gratefully acknowledged.

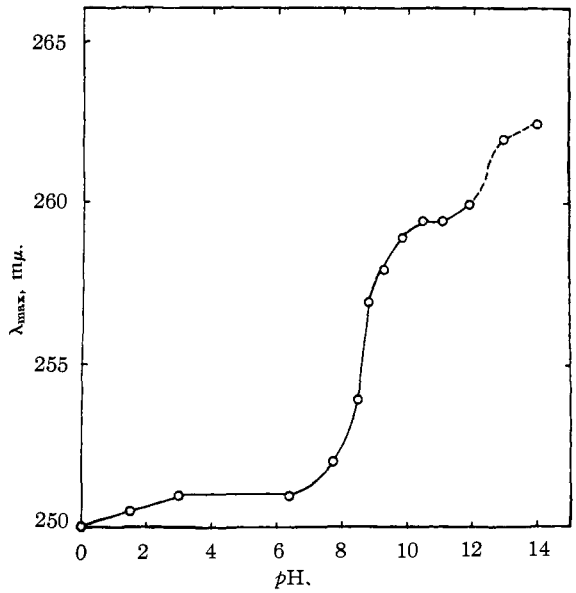
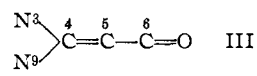


Fig. 2.—Changes of the long wave absorption maximum of hypoxanthine with pH. The values above pH 12 cannot be determined very accurately, due to rapid decomposition of hypoxanthine in strongly alkaline solution.

terminal nitrogen, attached to the right end of the C—C=C— systems, move λ_{max} by about 900 Å.
‖
O
toward the red. This must be ascribed to the mesomeric shift represented in II

$$\text{NH—C=C—C=O} \longleftrightarrow \text{H}^{+}\text{N=C—C=C—O}^{-} \qquad \text{II}$$

which considerably decreases the energy required for electronic transition, as measured by the ultraviolet spectrum. Therefore, we consider the partial structure III of uric acid as the responsible chromophore for the absorption maximum at 2850


III

Å., which is almost identical with λ_{max} 2880 Å. for ethyl β-diethylaminocrotonate.[17] It is also evident that N^7 is much less effective spectroscopically since it behaves not like a linear-, but a cross-conjugated group. Finally, N^1 is not connected directly to the central, conjugated portion of the uric acid

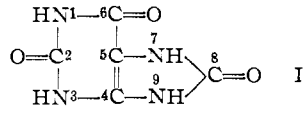
TABLE II

ABSORPTION MAXIMA OF VARIOUS PYRIMIDINE DERIVATIVES IN THE NON-IONIZED STATE

Uric acids		Xanthines		Uracils[b]	
Substance	λ_{max} at pH 2.5, Å.	Substance	λ_{max} at pH 6.0, Å.	Substance	λ_{max} at pH 6.0, Å.
Uric acid	2850	Xanthine	2670	Uracil	2595
1-Methyluric acid	2835	3-Methylxanthine	2715	3-Methyluracil	2585
3-Methyluric acid	2875	7-Methylxanthine[a]	2690	1-Methyluracil	2675
1,3-Dimethyluric acid	2860	1,3-Dimethylxanthine	2720	5-Methyluracil	2645
1,3-Diethyluric acid	2870	3,7-Dimethylxanthine	2730	1,3-Dimethyluracil	2660
3,7-Dimethyluric acid	2890	1,7-Dimethylxanthine[a]	2690	2-Ethoxy-4-keto-pyrimidine	2595
1,3,7-Trimethyluric acid	2890	1,3,7-Trimethylxanthine	2730	4-Ethoxy-2-keto-pyrimidine	2690
3,7,9-Trimethyluric acid	2940				

[a] Values taken from ref. 6. [b] Values taken from ref. 5.

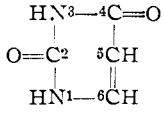
the behavior of terminal or intercalated carbonyl groups. Hence in structure I


I

we have to consider conjugation of the "fixed" double bond in position 4, 5 with three NH-groups in position 3, 7 and 9 and with the 6-keto group. The data of Bowden, *et al.*,[17] show that extension of an ethylenic bond by a (tertiary) nitrogen produces an auxochromic shift of about 200 Å. more than the addition of a carbonyl group and that a

molecule, but through intercalated carbonyl groups (in position 2 and 6).

In order to explain the spectral changes observed for uric acids, we shall first discuss the effect of N-alkylation or of ionization in the simpler structure of uracil (IV),[20] since here only one system of type II, *viz.*, $N^1—C^6=C^5—C^4=O$, and only two ionizable hydrogens are present.



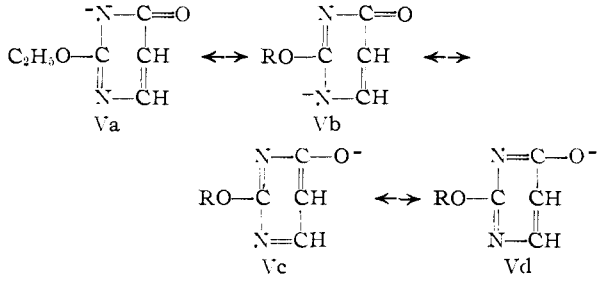
IV

A. Spectral Effects of Alkylation.—From the data of Shugar and Fox,[5] which are included in Table II, it is apparent that, with uracil as reference compound, methylation at N^3 produces a small hypsochromic shift of λ_{max}, whereas 1-methyluracil shows a pronounced bathochromic effect. 1,3-Dimethyluracil represents the algebraic sum of the $\Delta\lambda$-values for the monomethylated derivatives. This relationship can be understood in the light of the above considerations on nitrogen-conjugated systems. Methylation at N^1 enhances the polarization shown in II, thus producing a bathochromic effect. On the other hand, the extension of the ethylenic system by the 4-keto group is impaired by the nitrogen in position 3, which converts it from a terminal to a cross-conjugated carbonyl group. The adverse influence of N^3 becomes more effective by methylation.

Similar rules apply to uric acids (see Table II). Again the influence of a methyl substituent in the non-ionized molecule depends on its position, and the maximum of 1,3-dimethyluric acid in the acid range is determined by the algebraic sum of the $\Delta\lambda$-values of the component monomethyl derivatives, when uric acid serves as standard. 3,7-Dimethyl- and 1,3,7-trimethyluric acid have the same absorption maximum (but the latter possesses a higher extinction). 3,7,9-Trimethyluric acid shows the greatest bathochromic shift, indicating that methylation at N^9 has a stronger effect than in any other position, for reasons given below.

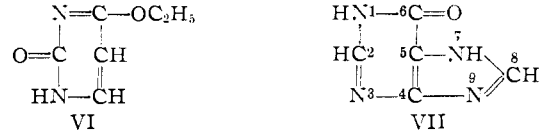
B. Spectral Shifts Produced by Ionization.—The dissociation constants of 1-methyluracil (9.75) and of the 3-methyl isomer (9.95)[5] (see Table Ia) are so close to each other as to prevent an unambiguous assignment of pK values in other uracils to individual ionization steps. However, in the first compound, dissociation is accompanied by a hypsochromic shift of 25 Å. and in the second one by a bathochromic shift of 50 Å. The importance of this fact was not clearly recognized before. It proves that in uracil and thymine ionization at N^1 precedes that at N^3. It is very significant that in uracil and thymine the first ionization step not only greatly delays dissociation at N^3, but also increases the hypsochromic effect considerably (in uracil $\Delta\lambda$ is -75 Å. and in thymine -90 Å.). It can be concluded that conversion of uracil into its mono-anion increases the resonance energy by enhancing the mesomeric shift indicated in II. On the other hand, formation of the bis-anion interferes with this resonating system, since the 4-keto group is now partly occupied for distribution of the negative charge at N^3. Evidently, the stronger the resonance in the partial structure II, the greater the interference from a negative charge at N^3. These effects cannot be evaluated quantitatively at present, since only the number of resonating forms can be determined, but not their relative contribution to the resonance hybrid. Nevertheless, it appears highly significant that for the mono-anion of uracil 6 resonance structures can be written, while for the bis-anion only 4.

In obvious disagreement with the rules which apply to uracil and its N-alkyl derivatives, a bathochromic shift is observed for ionization at N^3 in 2-ethoxy-4-keto-dihydropyrimidine (V).[5] However, O-alkylation introduces into the molecule a N=C-double bond *without electric charge*. Therefore dissociation in V produces—in addition to charge transfer to the 4-keto group (Vc-d)—the new resonating system Va-b, which has no equivalent in uracil or its N-methyl derivatives. It is remarkable that the pK of V, 8.2, is lower even than pK_1 of uracil! Additional support for this explanation is found in the behavior of 4-ethoxy-2-keto-dihydropyrimidine (VI), the isomer of V. In VI charge distribution from N^1 to C^4 is not possible, therefore only a relatively small bathochromic shift accompanies dissociation at N^1 and the pK of 10.7 is appreciably higher than pK_1 of uracil.



Va Vb Vc Vd

These considerations led us to assume that in hypoxanthine (VII) ionization at N^1 (numbering as in uric acid!), by creating a resonating system similar to Va-b and spreading the charge over N^3 and $N^{7(9)}$ must be accompanied by a bathochromic shift of λ_{max}. And indeed, the curve in Fig. 2 reveals 3 dissociation processes, though the measurements in the extreme alkaline region are not too reliable, due to decomposition. In analogy to the xanthines (see below), dissociation at pH 8–10 is ascribed to $N^{7(9)}$ and in the range 11–13 to N^1 [21] (see Table Id). Since all steps produce only bathochromic shifts, our conclusion about the effect of ionization at N^1 holds, independently of the exact value of pK_1.



VI VII

In applying now these rules to uric acids, unambiguous assignment of individual pK-values to N^1

(20) Note that the conventional numbering of uracil (IV) follows the inverse direction of that of uric acid.

(21) The pH dependence of the spectrum of hypoxanthine has been determined before by L. F. Cavalieri, A. Bendich, J. F. Tinker and C. B. Brown, THIS JOURNAL, **70**, 3875 (1948), but their measurements were extended only to pH 9.

becomes possible by virtue of the concomitant hypsochromic effect. Alkylation at different positions modifies the value of pK_9 only very little. These two facts make thus the classification of pK-values in Table Ib more or less reliable. Regarding dissociation, the superiority of N^9 over N^3 in uric acids can be explained as follows. A negative charge at N^9 can be distributed to the oxygen at C^8 and by the principle of vinylogy also to C^6. Although a similar distribution is possible for a negative charge at N^3,

$$\overset{7}{\text{NH}}\text{–}\overset{8}{\text{CO}}\text{–}\overset{9}{\text{NH}}$$
$$\underset{3}{\text{NH}}\text{–}\underset{2}{\text{CO}}\text{–}\underset{1}{\text{NH}}\text{–}\underset{6}{\text{CO}}$$

ionizability is greater in the structure than in NH–CO–NH–CO. In the imidazol ring a double bond at position 8,9 gives a resonating system containing an aromatic-like sextet of π-electrons ($\overset{(5}{\text{C}}=\text{C}-\text{N}=\text{C}-\overset{7)}{\text{N}}$:), whereas in the pyrimidine ring the analogous system is interrupted by the 4-keto group which introduces some kind of cross-conjugation. Therefore the over-all effect is a greater resonance stabilization for the charge at N^9 than at N^3. The second ionization step involves N^3. As before, preceding ionization delays dissociation at N^1, but the hypsochromic effect of the last step is much greater than in the uracil series, due to the much larger degree of previous occupation of the 6-keto group. When previous ionization is lacking, dissociation at N^1 occurs at a much lower pH. The pK of 3,7,9-trimethyluric acid is 8.35! The strong hypsochromic shift of this derivative demonstrates the involvement of the 6-keto group even in the non-ionized molecule in mesomeric shifts of type II and the impairment of the central resonating system of uric acid by ionization at N^1. These effects are analogous to, but more pronounced than, the phenomena observed in uracils. Surprisingly, no change of λ_{max} is observable in uric acid itself between pH 11–14. We explain this effect by lack of dissociation at N^1, when both the oxygen at C^2 and C^6 can simultaneously acquire negative charges from other sources.

The principles underlying the spectral changes of uracils and uric acids can also be applied to explain the changes observed in the xanthine series. As shown in Table II, only bathochromic effects accompany alkylation. 3-Methylxanthine possesses at pH 6 a maximum, which is 45 Å. nearer to the red than λ_{max} of xanthine. This shift is thus greater than in the uric acid series, but smaller than for the corresponding pair of uracils. Hence it is concluded that the inductive effect of an alkyl substituent at N^3 increases when the mesomeric effect, as formulated in II, emanating from N^9 in the imidazol ring, is weakened due to the absence of the 8-keto group. The inductive effect of a methyl group at N^1 now "alternates" more freely with that of N^3. Therefore λ_{max} of 1,3-dimethylxanthine at pH 6 is 5 Å. greater than that of 3-methylxanthine. 3,7-Dimethylxanthine (theobromine) at pH 2–9 has the same λ_{max} as the 1,3,7-trimethyl derivative (caffeine), in analogy to the corresponding pair in the uric acid series.

The effects of ionization in the xanthine series (Fig. 3) can be explained on a similar basis. The

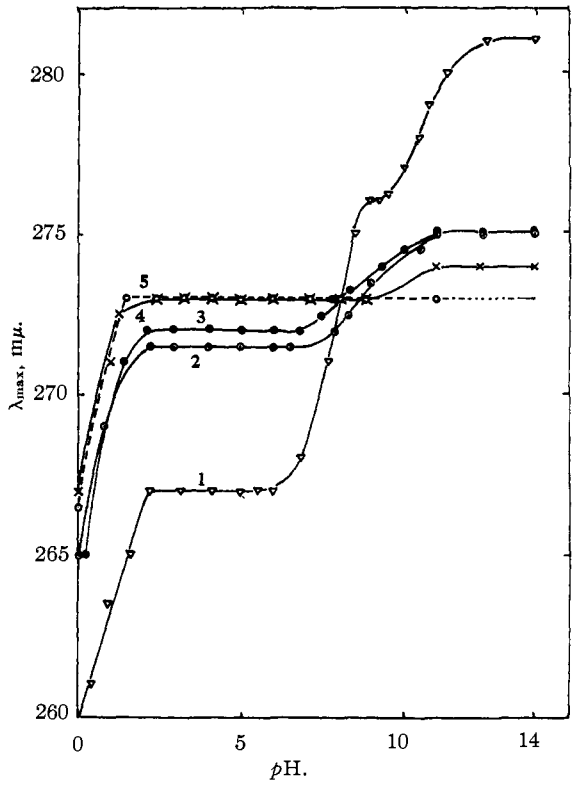


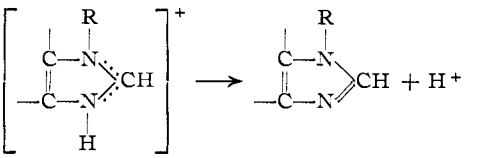
Fig. 3.—The long wave absorption maxima of xanthines as function of pH: 1, ▽—▽—▽—▽, xanthine; 2, ⊙—⊙—⊙—⊙, 3-methylxanthine; 3, ●—●—●—●, 1,3-dimethylxanthine (theophylline); 4, X—X—X—X, 3,7-dimethylxanthine (theobromine); 5, O - - O - - O, 1,3,7-trimethylxanthine (caffeine). The portion of curve 5 beyond pH 11 cannot be determined accurately, due to rapid decomposition of caffeine in strongly alkaline solutions.

NH-group in position 3 probably undergoes dissociation first. This step in xanthine produces a bathochromic shift of 90 Å. between pH 6–9. Similar shifts have been reported for 7-methyl- and 1,7-dimethylxanthine.[6] It is more difficult to identify unequivocally the second dissociation which occurs in xanthine at pH 9–13. Theophylline, in which only position 7 is free, has a pK of 8.7 (Table 1c). Its bathochromic shift is practically identical with that of 3-methylxanthine (pK = 8.8). This makes it probable—but by no means certain—that in both derivatives dissociation takes place at $N^{7(9)}$. Thus ionization at $N^{7(9)}$ appears to be preferred to N^1. On this basis the second dissociation constant of xanthine can be identified as $pK^{7(9)}$. However, the data on uracils, discussed above, show that methyl substitution can shift the pK-value of another nitrogen sufficiently to make the assignment of dissociation constants in the xanthine series less reliable. Assuming analogous relationships in hypoxanthine, we have assigned its ionization at pH 8–10 to $N^{7(9)}$ (see above).

In theobromine dissociation at N^1 is accompanied by a small bathochromic shift, but the pK (9.9) is remarkably low. The latter phenomenon is analogous to our observation on 3,7,9-trimethyluric acid.

Another interesting phenomenon is shown by the

strong bathochromic shift at *p*H 0–2, which is common to all curves in Fig. 3. It can only be associated with the following process



since it appears also in caffeine, but is absent in uric acids. It is remarkable that the pK of this reaction is about 6 units lower than the pK of imidazole itself (≈ 7).[22]

The cation of hypoxanthine is more stable than that of xanthines and the bathochromic shift accompanying conversion to the uncharged form is less pronounced (see *p*H-range 0–3 in Fig. 2). Since in hypoxanthine the proton can be attached to $N^{7(9)}$ or to N^3, the gain in resonance energy upon loss of the proton is less marked than in the xanthine series.

Discussion

Our results show the close relationship of the first dissociation constant of all uric acids with a free 9-position and lead to the conclusion that N^9 is the first to undergo dissociation. The observation that uric acid does not possess more than two dissociation constants, in spite of the presence of 4 ionizable hydrogens, or three potential hydroxyl groups, confirms the old chemical experience that only di-sodium or di-potassium salts of uric acid can be prepared.[23]

The small spread of pK-values for the same group is, however, true only with regard to the first ionization step. In all other positions, pK-values are dependent on preceding ionizations, as demonstrated by the column of pK_1 in Table Ib, and by the corresponding columns in Table Ia and c.

Whereas ionization at N^9 and N^3 in uric acids is accompanied by marked bathochromic shifts, ionization at N^1 gives a hypsochromic effect, which can be explained by the concomitant decrease in resonance energy. This phenomenon is common to uracils and substituted uric acids. (It is absent only in uric acid itself, since ionization at N^1 is made impossible by the two preceding steps.) In the xanthine series such a phenomenon appears to be missing. In xanthine itself, the absence of ionization at N^1 can be ascribed to the preceding dissociation at N^3 and N^7. Our measurements on 3-methylxanthine do not clearly indicate a second ionization, though Cavalieri and co-workers[6] report $pK_2 = 11.9$. In theobromine, however, dissociation at N^1 is accompanied by a very small bathochromic shift.

The hypsochromic effect thus appears only under very specialized conditions. One of them is the absence of resonative charge distribution between N^1 and N^3. When such resonance is possible, as in 2-ethoxy-4-keto-pyrimidine or in hypoxanthine, a bathochromic shift accompanies ionization at N^1 (or at N^3 in uracils). On the other hand, when this effect is precluded as in 3,7,9-trimethyluric acid, ionization at N^1 is accompanied by a considerable lowering of λ_{max}. A second factor is the ability of neighboring oxygen atoms to accept the charge of N^1 (or N^3 in uracils). So for instance, when the 6-keto group is not occupied by previous ionization or by mesomeric effects emanating from N^9, the hypsochromic effect is missing, as in theobromine. It is significant that alkylation of the various nitrogen atoms produces spectral changes similar to ionization. Whereas methylation in general shifts the absorption maximum of the non-ionized molecule toward the red, methylation at N^1 in uric acid (or at N^3 in uracil) gives a hypsochromic effect. Thus the inductive effect of an electron-repelling substituent is similar to a negative charge at nitrogen, but less pronounced. Whereas λ_{max} of 1-methyluric acid at *p*H 2.5 is 15 Å. smaller than λ_{max} of uric acid, ionization at N^1 produces a shift of about 50 Å. toward the ultraviolet. Moreover, the spectral changes produced by alkylation are not only parallel to those caused by ionization, in some cases they even show mutual interference. Thus, for instance, in 1,3,7-trimethyluric acid the value of pK_g is $^1/_2$ unit higher than in uric acid and likewise the pK_1 value in 3-methyluracil is elevated as compared with uracil. Accordingly, the inductive influence, of the methyl group interferes in these cases with the charge distribution required for ionization in another position. It thus follows that both processes, ionization as well as alkylation, produce qualitatively identical electronic changes.

It should, however, be recalled that the above considerations are valid only in the special case of chromophore II representing the central portion of the heterocyclic structure. Different rules apply, when the 6- (or 4-) keto group is missing, as will be discussed in a subsequent paper.

Acknowledgment.—The authors wish to thank Dr. M. Harnik, who synthesized some of the substituted uric acids, and Mr. Eli Breuer for his help with the spectrophotometric measurements. They were assisted by Mrs. Meron in the preparation of the manuscript.

JERUSALEM, ISRAEL

(22) A. H. M. Kirby and A. Neuberger, *Biochem. J.*, **32**, 1146 (1938).
(23) H. Biltz and H. Krzikalla, *Ann.*, **423**, 255 (1921).