Spectrophotometric Studies of Nucleic Acid Derivatives and Related Compounds. VI. On the Structure of Certain 5- and 6-Halogenouracils and -cytosines[1]

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It is shown by ultraviolet absorption spectral comparisons in aqueous solutions that the neutral species of 6-chloro- and 6-fluorocytosine are best represented by the 4-amino-2-oxo structure (VI) in which the dissociable proton is affixed to N-3. The neutral species of 6-fluoro- and 6-chlorouracils exist in the dicarbonyl form. The monoanions of these 6-halogeno derivatives are formed by loss of a proton from N-1 to give an anion of type II (R = F or Cl; R' = H). The monoanionic forms of 5-halogenouracils are best represented as *mixtures* of monoanions I and II. Analysis of the spectral data in aqueous solution of 5-bromouracil and its 1- and 3-methylated derivatives shows that the relative proportion of I and II in the 5-bromouracil monoanionic mixture is in the ratio of *ca.* 1:2. The preponderance of II probably applies also to the 5-chloro- and the 5-iodo-uracil monoanions. With the biologically important 5-fluorouracil, however, the monoanionic mixture (I and II, R = H, R' = F) contains a greater proportion of I.

Previous publications have described the synthesis of 6-fluorocytosine[2] and 6-fluorouracil.[3] It was noted that the over-all ultraviolet absorption spectral pattern exhibited by these 6-fluoropyrimidines was similar to that of their 6-chloro analogs but dissimilar to that exhibited by cytosine and uracil and their 5-halogeno derivatives. However, the spectral patterns of the 6-halogenouracils and -cytosines do bear a strong resemblance to those of the 3-alkylated uracil and cytosine, respectively (see Fig. 1). Therefore, a more detailed analysis of the available spectral data was undertaken.

It is apparent from examination of the spectra of the 5-halogenouracils[4] that the 5-halogen substituents exert a bathochromic effect relative to the spectrum of uracil in the order of F < Cl < Br < I (see Table I.). This same order of bathochromic progression has also been noted in the spectra of the 2- and 3-halogenopyridines relative to pyridine.[5] In contrast, the presence of a fluorine in the 6-position of uracil exerts a general hypsochromic shift of 12–19 mμ (see Table I) in the absorption maxima relative to that of uracil; a 6-chloro substituent, on the other hand, has practically the same maxima as uracil itself. This hypsochromic effect of a 6-fluoro substituent is also found in a comparison of the absorption maxima of 2,4,6-trifluoropyrimidine (232 mμ) with unsubstituted pyrimidine (240 mμ).[6] 2,4,6-Trichloropyrimidine (261 mμ)[7] does show a bathochromic effect relative to unsubstituted pyrimidine.

As can be seen in Fig. 1, the curves representing the neutral species (pH 1) of 6-chloro- and 6-fluorouracil are similar (although the positions of the maxima differ *ca.* 14 mμ) and they are also similar to the corresponding curve (pH 7.2) for 3-methyluracil. Likewise, the direction and magnitude of the spectral shift of the maxima exhibited by the 6-fluoro-, 6-chloro-, and 3-methyluracil in passing from neutral to monoanionic species (pH 6.5, 9.5, or 14, respectively) are also similar. There-

fore, since it has been proved[8,9] that the neutral species of 3-methyluracil exists in the dicarbonyl form, it follows that the 6-fluoro- and 6-chlorouracils in aqueous solution also exist in the lactam form.

On the basis of a comparison of the spectral curves for the neutral and anionic species of uracil with the corresponding curves for 1-methyl- and 3-methyluracil, Nakanishi, *et al.*,[10] concluded that the monoanionic curve for uracil (pH 12) represents a 1:1 mixture of monoanionic forms I and II (see Fig. 2). The curves for the monoanionic species of 6-fluorouracil (pH 6.5) and 6-chlorouracil (pH 9.5) do not resemble those for the uracil or the 1-methyluracil monoanions (pH 12), but do resemble that for the anionic form of 3-methyluracil (pH 12–14), Fig. 1. With 3-methyluracil, anion formation must involve loss of a proton from N-1. This dissociation of 3-methyluracil is manifested spectrally by a bathochromic shift of the maximum of 24 mμ (Table I) accompanied by an appreciable rise in the extinction coefficient.[8] A similar pattern of behavior is exhibited by the first dissociation (neutral to monoanionic forms, Δmμ ∼ 20) of the 6-halogenouracils. It is reasonable, therefore, to attribute the formation of the 6-halogenouracil monoanions to the loss of a proton from N-1 rather than from N-3. It follows, then, that the monoanion of 6-fluoro- and 6-chlorouracil in aqueous solution is best represented by structure II (R = F or Cl, R' = H). The proportion of I, if present at all, must be very small. This conclusion is derived from the fact that the monoanionic curve for the 6-chloro- and 6-fluorouracils show the same degree of symmetry as the monoanionic curve of 3-methyl-6-chlorouracil[11] which must exist as structure II. A pattern of monoanionic dissociation similar to that shown by the 6-halogenouracils is exhibited by the methyl ester of orotic acid,[12] a uracil derivative which also bears an electronegative substituent in position 6.

Dianion formation of the 6-halogenouracils involves a hypsochromic shift (8–9 mμ) of the maximum (see Table I) similar to that shown by the uracil dianion.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-07).

(2) I. Wempen and J. J. Fox, *J. Med. Chem.*, **6**, 688 (1963).

(3) I. Wempen and J. J. Fox, *ibid.*, **7**, 207 (1964).

(4) K. Berens and D. Shugar, *Acta Biochim. Polon.*, **10**, 25 (1963).

(5) H. C. Brown and D. H. McDaniel, *J. Am. Chem. Soc.*, **77**, 3752 (1955).

(6) S. F. Mason, *J. Chem. Soc.*, 1247 (1959).

(7) The relatively large difference in the position of the maxima between 2,4,6-trifluoro- and 2,4,6-trichloropyrimidine was utilized to monitor spectrally the completeness of conversion of the chloro to the fluoro derivative.[3]

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

(9) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).

(10) K. Nakanishi, N. Suzuki, and F. Yamazaki, *Bull. Chem. Soc. Japan*, **34**, 53 (1961).

(11) This compound was prepared by the method of G. Nübel and W. Pfleiderer, *Chem. Ber.*, **95**, 1605 (1962). The authors thank Dr. Pfleiderer of the Institut für Organische Chemie und Org.-Chem. Technologie, Stuttgart, West Germany, for an authentic sample of the compound.

(12) J. J. Fox, N. Yung, and I. Wempen, *Biochim. Biophys. Acta*, **23**, 295 (1957).

TABLE I

ULTRAVIOLET ABSORPTION DATA FOR SOME URACIL DERIVATIVES

Compound	Neutral (pH) mμ	Monoanion (pH) mμ	Dianion (pH) mμ	Δmμ, neutral to monoanion	Δmμ, monoanion to dianion	pKa₁[a]
Uracil[b]	(7.0) 259.5	(12) 284	(14)[c] 276.5	+24.5	−7.5	9.5
3-Methyl-[b]	(7.0) 258.5	(14) 282.5		+24		9.95
1-Methyl-[b]	(7.0) 267.5	(14) 265		−2.5		9.75
5-Fluoro-[d]	(5.0) 268	(11) 270	(14) 286	+2	+16	7.98[e]
5-Chloro-[d]	(5.0) 275	(11) 300	(14) 289	+25	−11	7.95
5-Bromo-[d]	(5.0) 277.5	(11) 302.5	(14) 291	+25	−11.5	8.05
5-Iodo-[d]	(5.0) 285	(11) 305	(14) 292.5	+20	−13	8.25
6-Fluoro-[f]	(1.0) 248	(6.5–9) 267	( )[g] 258	+19	−9	4.03
6-Chloro-[f]	(1.0) 262[h]	(9.0–11.5) 283[h]	(14) 275	+21	−8	5.67
6-Chloro-3-methyl-[i]	(1.0) 261	(9–14) 281		+20		5.84
6-Carbomethoxy-[j]	(1.0) 285	(9.75) 317.5	[k]	+32.5		

[a] The pKa₂ values of these compounds are all ca. 13. [b] Ref. 8. [c] 1 N NaOH is taken as pH 14. [d] Ref. 4. [e] Ref. 23. A value of 8.0 is reported in ref. 4. [f] Ref. 3. [g] 3 N NaOH. [h] H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, J. Org. Chem., 26, 792 (1961), report maxima at 260 (pH 1) and 280 mμ (pH 11). [i] Ref. 11. [j] Ref. 12. [k] The compound hydrolyzes in basic solution giving the same value as for orotic acid.
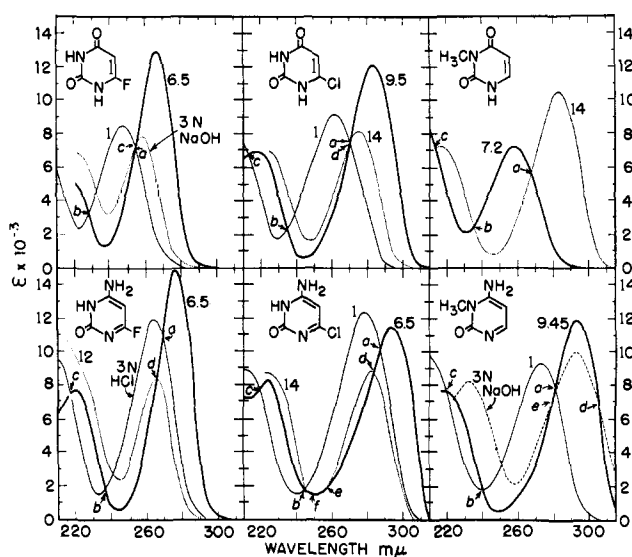

Fig. 1.—The spectra of the 6-halogenouracils and -cytosines were determined with a Cary Model 15 recording spectrophotometer. The spectrum of 3-methyluracil was taken from Shugar and Fox[8] and that for 3-methylcytosine from Ueda and Fox.[18] The curves shown represent neutral or pure ionic species at the pH values given; the italicized letters refer to isosbestic points. 1 N NaOH is taken as pH 14; 0.1 N HCl is taken as pH 1.
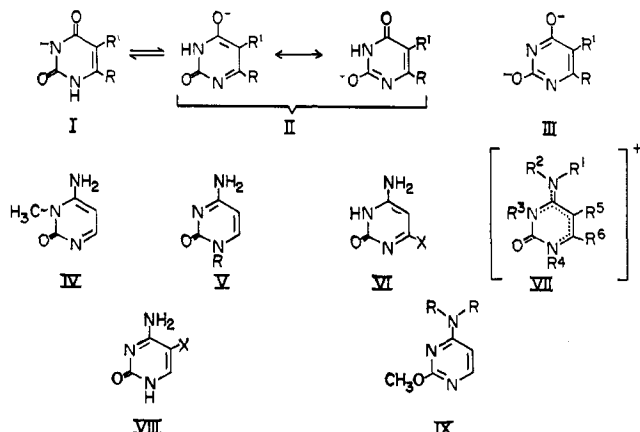

Figure 2.

Since the spectrum of the 6-halouracil dianion resembles that for the uracil dianion, it is likely that the structures of these dianions are similar. Though the structure of the dianion of uracil[8] and the 5-halogenouracils[4] has
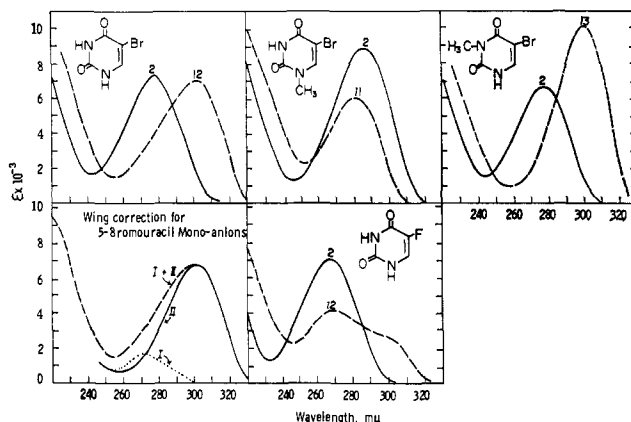

Fig. 3.—All spectra, with the exception of that showing the wing correction, were taken from Berens and Shugar.[4] In these four spectra, the solid-line curve (pH 2) represents the neutral species, while the dotted line (- - - - -) represents the pure anionic species. For calculation of the wing correction for the 5-bromouracil monoanions, see the text.

been presented as III, conclusive evidence supporting this likely structure is lacking.[13]

From the foregoing discussion, a reinterpretation of the spectral data of the 5-halogenouracil monoanions recently reported by Berens and Shugar[4] is warranted. Since 5-bromouracil and its 1- and 3-methylated derivatives (see Fig. 3) exhibit spectra similar to uracil and its N-methylated derivatives, Berens and Shugar[4] concluded that "it is likewise the 2-carbonyl which dissociates first." This reasoning was applied equally to the 5-chloro and 5-iodo analogs. With 5-fluorouracil, they did note ambiguity in the spectrum of the monoanionic species when compared to those for the other 5-halogenouracils. However, on the basis of the spectrum of 5-fluorouridine (an analog of 1-methyl-5-fluorouracil) they suggested that, with 5-fluorouracil also, monoanion formation was attributable to dissociation at the "2-carbonyl."[4] As will be shown in the following discussion, this order of dissociation cannot now be accepted in this strict sense.

(13) The absorption maximum of 2,4-diethoxypyrimidine[8] is hypsochromically displaced by 17.5 mμ from that of the uracil dianion (pH 14, 276.5 mμ).[8] This same behavior is found also in comparison of the spectra of the dianion of 6-fluorouracil (258 mμ) and of 6-chlorouracil (275 mμ) with the respective 2,4-dimethoxy-6-fluoropyrimidine (246 mμ) and 2,4-dimethoxy-6-chloropyrimidine (259 mμ). The maxima of the absorption spectra of the latter compounds lie 12–16 mμ hypsochromically to the curves for the corresponding 6-halouracil dianons.

The $\Delta m\mu$ values (neutral to monoanion) in Table I attest to the fact that the 5-chloro-, 5-bromo-, and 5-iodouracils are similar to each other and to the corresponding $\Delta m\mu$ values for uracil and 3-methyluracil, all showing a bathochromic shift in the maximum of 20–25 $m\mu$. The spectral curve of the 5-bromouracil monoanion (pH 12, see Fig. 3) shows a certain degree of skewness, not as pronounced, however, as in the curve of the uracil monoanion. This dissymmetry, as in the case of the uracil monoanion, suggests the presence of a mixture of monoanionic structures I and II (R = H, R′ = Br). The availability of the spectra of the 1- and 3-methylated 5-bromouracils (see Fig. 3) offers an opportunity to apply a procedure[10] analogous to that used for calculating the relative proportions of I and II for the uracil monoanion. The curves for the 1- and 3-methyl-5-bromouracil monoanions, of necessity, must be analogous to the curves for pure I and pure II, respectively.[14] (In this case, each of the dissociable protons of pure I and pure II is replaced by a methyl group.) Therefore, if wing correction[15] is carried out on the 5-bromouracil monoanion (see Fig. 3), a peak is revealed at ca. 273 $m\mu$ (A 1650)[16] which is due to the amount of monoanion I present in the mixture. It is apparent, therefore, that the mixture of monoanions of 5-bromouracil is more heavily weighted in favor of structure II than in the case of the uracil monoanion. Calculation of the relative proportions[17] of the ionic structures I and II in the 5-bromouracil monoanion by a method analogous to that carried out for the uracil monoanion[10] reveals 64% of II and thus, 36% of I, or a ratio of II–I of 1.8:1.0 as the composition of the monoanionic mixture of 5-bromouracil. A similar proportion probably also holds for the 5-chloro- and 5-iodouracil monoanions due to the similarity of their curves to those of 5-bromouracil.

The ambiguity noted by Berens and Shugar[4] for the spectrum of the 5-fluorouracil monoanion now finds ready explanation. Whereas with the other 5-halo-genouracils the monoanionic mixture is heavily weighted in favor of II, with 5-fluorouracil structure I predominates. This conclusion is warranted since the monoanionic curve for 5-fluorouracil exhibits only a slight bathochromic shift (2 $m\mu$) and a loss of extinction relative to the curve for the neutral species (see Fig. 3). By analogy with the 1-methyluracil anion,[10] such spectral behavior is due to the loss of a proton from N-3 resulting in a monoanion similar in structure to I (R = H, R′ = F). In addition, the monoanionic curve for 5-fluorouracil has only a pronounced shoulder in the 300–310 $m\mu$ region where the other 5-halogeno-uracil monoanions have their maxima, thus indicating the presence of some of structure II monoanion in the former. The relative proportions of I and II in the monoanionic mixture can be calculated only when the 1- and 3-methyl-5-fluorouracils are available.

The predominance of I in the mixture of 5-fluorouracil monoanions may be of importance in attempts to correlate the potent biological activity of this pyrimidine with structure at physiological pH values where, as has been noted,[4] a significant amount of 5-fluorouracil is ionized.

The close similarity of the over-all spectral patterns of the 6-halogenouracils to 3-methyluracil has been discussed above. Likewise, examination of the over-all spectra[2] of 6-fluoro- and 6-chlorocytosine (see Fig. 1) shows a strong resemblance to that of 3-methyl-cytosine[18] rather than to that for cytosine[8] and 1-methylcytosine. In Fig. 1, a comparison of the curves representing the neutral species of the 6-halogenocyto-sines (pH 6.5) to the corresponding curve (pH 9.45) for 3-methylcytosine shows a close similarity. Therefore, since it has recently been rigorously established[18] that in aqueous solution the neutral species of 3-methyl-cytosine exists predominantly in the 4-amino-2-oxo structure (IV), it follows that in the neutral species of the 6-halogenocytosines the proton resides on N-3 (and not on N-1 as in the case of cytosine V, R = H). Therefore, the structure of 6-fluoro and 6-chlorocyto-sine in aqueous solution (pH 5–8) is represented as VI (X = F or Cl).

It can also be seen in Fig. 1 that the curves representing the pure cationic species of 6-fluorocytosine (3 N HCl) and 6-chlorocytosine (pH 1.0) are similar to each other and to the corresponding curve for 3-methylcyto-sine (pH 1.0). Since it has been established that 3-methylcytosine protonates on N-1,[18,19] it is logical to assume that protonation of the 6-halogenocytosines must likewise occur on N-1 and not on N-3 as in the case of cytosine[8] and 1-methylcytosine.[20] The curves for the pure cationic species of the 6-halogeno- and 3-methylcytosines in Fig. 1 are likewise similar to those of cytosine, 1-methylcytosine, and indeed all of the other cytosine derivatives listed in Table II. This similarity of the cationic species may be explained in terms of the same resonant cation (VII, Fig. 2)[18] resulting from protonation of the neutral species of any of these cytosines.

It can be seen from the $\Delta m\mu$ values (neutral to cation) in Table II that those compounds which are known to protonate on N-3 (cytosine, 1-methylcytosine, and

(14) The position of the maxima of II (R ~ H, R′ = Br) and that of 3-methyl-5-bromouracil anion would be the same except for the slight spectral shift due to the methylation at N-3. Likewise, the maxima of 1 and that of 1-methyl-5-bromouracil anion are similar, allowance being made for the effect of methylation at N-1.[10]

(15) The wing correction[10] is obtained here by substituting the proper values at any given wave length in the following equations.

$$A_{II} (m\mu) = E \text{ (3-methyl-5-bromouracil anion)} \times$$
$$E_{max} \text{ (5-bromouracil anion)}/E_{max} \text{ (3-methyl-5-bromouracil anion)}$$
$$A_I (m\mu) = E \text{ (5-bromouracil anion)} - E_{II}$$

For example A_{II} (280 $m\mu$) = 4650 × 6900/10,020 = 3210
A_I (280 $m\mu$) = 4500 − 3210 = 1290

(16) The A value is not the molecular extinction coefficient of pure 1 but represents the actual amount of 1 in the mixture of anions 1 and 11. The molecular extinction coefficient of 1 is calculated[17] to be ca. 4800. These figures are, of necessity, approximate, since the values used in the calculation were read directly from the published spectral plots.[4]

(17) This calculation is carried out as follows: $E_{II} = E_N \times E_{3A}/E_{3N}$ where E_N, E_{3A}, and E_{3N} are the extinction coefficients of the maxima of 5-bromo-uracil (neutral species), 3-methyl-5-bromouracil (anion), and 3-methyl-5-bromouracil (neutral species). The percentage of structure 11 present in the bromouracil anion mixture is

% II = E_{max} (5-bromouracil anion)/E_{II} × 100

thus E_{II} = 7140 × 10,020/6600 = 10,840

% II = 6900/10,840 × 100 = 64%

and, therefore, the amount of structure 1 is 36%. Similarly, E_I can be calculated using the data for 1-methyl-5-bromouracil instead of the values for the 3-methyl derivative in the above formula. The percentage of 1 is then estimated from the new peak at 273 $m\mu$ determined in the wing correction (see Fig. 3)

E_I = 7140 × 6030/8900 = 4840

% I = 1650/4840 × 100 = 34%

This value is in good agreement with the value of 36% as determined above.

(18) Part V of this series: T. Ueda and J. J. Fox, J. Am. Chem. Soc., 85, 4024 (1963).

(19) P. Brookes and P. D. Lawley, J. Chem. Soc., 1348 (1962).

(20) J. J. Fox and D. Shugar, Biochim. Biophys. Acta, 9, 369 (1952).

<div align="center">TABLE II</div>

<div align="center">ULTRAVIOLET ABSORPTION DATA FOR SOME AMINOPYRIMIDINES[a]</div>

Compound	Cationic (pH) mμ	Neutral (pH) mμ	Anionic (pH) mμ	Δmμ, neutral to cation	Δmμ, neutral to anion	pKa₁[b]
Cytosine[c]	(1.0) 276	(7–10) 267	(14)[d] 282	+9	+15	4.61[e]
1-Methyl-[f]	(1.0) 283	(7.2–14) 274		+9		4.55
3-Methyl-[g]	(4.0) 274	(12) 294	( )[h] 293	−20	−1	7.4
N,N-Dimethyl-[e]	(1.0) 282	(7.1) 274	(14) 289	+8	+15	4.25
5-Fluoro-[i]	(1.0) 281	(7.1) 275	(14) 292	+6	+17	2.83
5-Chloro-[j]	(1.0) 293	(7.6) 282	(14) 296	+11	+14	2.94
5-Bromo-[k]	(1.0) 297	(7.44) 282.5	(14) 296	+14.5	+13.5	3.04
6-Fluoro-[j]	( )[l] 264	(6.5) 277	(14) 265	−13	−12	1.52
6-Chloro-[j]	(1.0) 279	(6.5) 293	(14) 282	−14	−11	3.26
Cytidine[f]	(1.0) 280	(7.2–12) 271		+9		4.1
N,N-Dimethyl-[e]	(1.0) 287	(6–12) 278		+9		3.62
5-Fluoro-[e]	(1.0) 290	(5–12) 281		+9		2.26
Pyrimidine						
4-Amino-2-methoxy-[c]	(1.0) 260.5	(7.2) 270.5		−10		5.3
4-Dimethylamino-2-methoxy-[m]	(1.0) 269	(10) 284		−15		6.17

[a] Only those cytosine derivatives which have at least one unsubstituted heteroatom are considered in this table. Some of the compounds listed also have a shoulder or second maximum in the short wave length region; these data have been omitted for sake of clarity. [b] The pKa₂ values have been omitted for brevity. [c] Ref. 8. [d] N NaOH is taken as pH 14. [e] See ref. 23. [f] Ref. 20. [g] Ref. 19. [h] Value determined in 3 N NaOH (see discussion of this dissociation in the text). [i] Sample furnished by Dr. R. Duschinsky of Hoffmann LaRoche, Inc., Nutley, N. J. [j] Ref. 2. [k] Prepared according to G. E. Hilbert and E. F. Sansen, J. Am. Chem. Soc., 56, 134 (1934). [l] 3 N HCl. [m] Ref. 21.

cytidine)[18,19,21,22] show a *bathochromic* spectral shift of the position of the maxima of 9 mμ, whereas protonation of 3-methylcytosine which occurs on N-1[18,19] is manifested spectrally by a *hypsochromic* shift of the maxima of 20 mμ. With these data, it becomes possible to predict the most probable site of protonation of the other cytosine derivatives listed in Table II. Those cytosine derivatives in Table II which exhibit a *hypsochromic* shift (neutral to cation) with a corresponding Δ mμ value averaging *ca.* 15 mμ, protonate on N-1. Those cytosine derivatives which show a *bathochromic* spectral shift of the position of the maxima, with a Δmμ value (neutral to cation) of *ca.* 9 mμ, protonate on N-3. This assignment of the site of protonation as N-3 for the N,N-dimethyl- and the 5-fluorocytosines is further supported by the similar Δmμ values of their 1-β-D-ribofuranosyl derivatives[23] (see Table II) in which the N-1 position is already substituted. Since the other 5-halogenocytosines listed show a shift similar to that of 5-fluorocytosine and cytosine, it is reasonable to assume that, in these derivatives also, cation formation occurs by protonation on N-3, and their neutral species is to be represented by structure VIII.

Also listed in Table II are two compounds, 4-amino- and 4-dimethylamino-2-methoxypyrimidine (IX, R = H or CH₃, respectively), which in the neutral species have no proton on the heteroatoms and may be considered to be analogous to the anion of cytosine. The Δmμ values (neutral to cation) of these two methoxypyrimidines show a hypsochromic shift of the maxima of 10 and 15 mμ, respectively, in the same direction and of the same order of magnitude as that shown by 3-methylcytosine. These data suggest that, as with 3-

methylcytosine, protonation occurs on N-1 and not N-3. A similar conclusion has been suggested[18] on the basis of methylation studies of 4-amino-2-methoxy-pyrimidine in which the 1-methiodide, rather than the N-3 derivative, is obtained.

With the acceptance of V (R = H) and VI (Fig. 2) as the true structural representations of the neutral species of cytosine and the 6-halogenocytosines, respectively, then it is obvious that anion formation must involve removal of the proton from a different heteroatom, *i.e.*, from N-1 in cytosine and from N-3 in the 6-halogenocytosines. As can be seen in the Δmμ values (neutral to anion) in Table II, proton removal in the case of cytosine (V, R = H) is reflected spectrally by a bathochromic shift of the maxima of 15 mμ. The corresponding Δmμ values resulting from anion formation in the case of the 6-halogenocytosines (VI, X = F or Cl) show a hypsochromic shift of the maxima of *ca.* 12 mμ. Thus, the direction and magnitude of the Δmμ values (neutral to anion) appears to be a direct spectral reflection of the site of proton removal from the neutral species. For example, the dissociation (neutral to anionic species) of the 5-halogenocytosines is accompanied by a bathochromic shift of the maxima averaging 15 mμ (similar to that shown by cytosine). Therefore, proton removal occurred from N-1, and further corroboration is provided that, in the neutral species of the 5-halogenocytosines, the proton resides on N-1 (VIII).

It has been demonstrated[18] recently that 3-methylcytosine (Fig. 1) possesses an acidic dissociation (above pH 9.5) attributed to proton removal from the *exocyclic* amino group. Unlike the acidic dissociations of the other cytosines (Table II, Δmμ neutral to anion) proton removal from 3-methylcytosine shows no appreciable spectral shift.

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(23) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *ibid.*, 83, 4755 (1961).