299. *Tautomeric Azines. Part I. The Tautomerism of 1-Methyluracil and 5-Bromo-1-methyluracil.*

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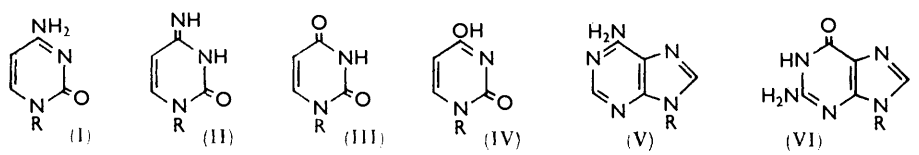
In aqueous solution, the structure 1-methylpyrimidine-2,4-dione is preferred to 4-hydroxy-1-methylpyrimidin-2-one by a considerably greater factor than applies to the corresponding 5-bromo-derivatives. The relation of the present results to theories of mutagenesis is briefly discussed. The tautomerism of 2-ethoxypyrimidin-4-one and that of 4-ethoxypyrimidin-2-one are clarified.

REPLICATION of nucleic acids is considered to occur by building up of a new chain on to an existing molecule. In this process adenine pairs with uracil (or thymine), guanine with cytosine, and *vice versa*. This particular pairing results in favourable hydrogen-bonding relations between the two chains. Spontaneous mutations occur when a " mistake " is made in that an incorrect base is built into the new chain. Watson and Crick [1] have suggested that this might occur when one of the bases reacts in an alternative tautomeric form: thus cytosine (I; R = H) in the imino-form (II; R = H) would simulate uracil (III; R = H), uracil in the hydroxy-form (IV) would simulate cytosine, and similarly for the purine bases. Knowledge of the tautomeric ratios of the relevant forms is



thus of interest for possible correlation with the spontaneous mutation rate. Kenner, Reese, and Todd [2] have shown that for cytosine the amino-form (I; R = H) is preferred

[1] Watson and Crick, *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18**, 123.
[2] Kenner, Reese, and Todd, *J.*, 1955, 855.

to the imino-form (II; R = H) by a factor of $10^{4\cdot7}$. It is known that uracil,[3] adenine,[4] and guanine [4] residues exist predominantly as (III), (V), and (VI) (R = sugar), respectively, in nucleic acids. However, no quantitative information is available on the tautomerism of these compounds. In this paper quantitative data are provided for uracil.

5-Bromouracil is an active mutagenic agent and it has been suggested [5] that this may be due to the bromine atom's causing relatively more of the hydroxy-tautomeric form to be present. We have therefore also investigated this compound.

Preparation.—The compounds were prepared by recorded methods. The gross structures of the isomeric ethoxy-pyrimidinones from the hydrolysis of 2,4-diethoxypyrimidine were elucidated by Shugar and Fox [3] (for tautomeric composition see below). The conversion of 5-bromopyrimidine-2,4-dione successively into 5-bromo-2,4-dichloro- and 5-bromo-2,4-dimethoxy-pyrimidine must be carried out with pure starting material, as separation from the corresponding product of the uracil series is not possible at the later stages. 5-Bromo-2,4-dimethoxypyrimidine was rearranged by methyl iodide to 5-bromo-4-methoxy-1-methylpyrimidin-2-one, the structure of which was proved by hydrolysis to 5-bromo-1-methylpyrimidine-2,4-dione.

Ultraviolet Spectra (Table 1).—Of the many methods available for the determination of tautomeric equilibrium constants, only the basicity method is generally applicable to systems in which one tautomer greatly predominates. If 1-methylpyrimidine-2,4-dione (VIII) and 1-methyl-4-methoxypyrimidin-2-one (X), on protonation, form cations of similar structure (IX; R = H and Me, respectively), the ratio of their K_a values is approximately the ratio between the tautomeric forms of 1-methyluracil. Ultraviolet spectra

TABLE 1.

Ultraviolet spectra.

Substituents at position					Neutral form [1]		Protonated form			pK_a detn.[2]
1	2	3	4	5	$\lambda_{max.}$ (mμ)	$10^{-3}\varepsilon$	$\lambda_{max.}$ (mμ)	$10^{-3}\varepsilon$	H_2SO_4 (N)	mμ
Me	O	Me	O	H	265	8·85	283	8·41	24·6 29·2	265
Me	O	H	O	H	266	9·60	283	9·27	26·4	267
H	O	H	O	H	258	8·16	272 *	6·65	20·6	285 258 260
Me	O	—	OMe	H	272	5·92	290	10·3	20·0	290
H	O	—	OEt	H	268	4·99	284	8·85	10·0 13·8	284 285
—	OEt	H	O	H						
Me	O	Me	O	Br	282	7·88	311	7·15	~36	280
Me	O	H	O	Br	283	8·65	313	7·52	~35·8	285
H	O	H	O	Br	276	7·06	307	4·77	~36·5	275
Me	O	—	OMe	Br	289	4·85	314	6·52	28·5	315

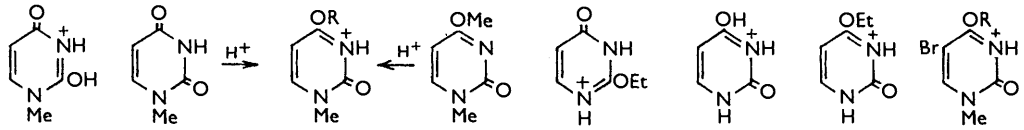
* A constant cationic form was not reached.

[1] Measured at pH 7 in a solution of Burroughs Wellcome & Co. " Soloid " buffer tablets (pH 6·99 at 25°). [2] Wavelength used for determination of pK_a of conjugate acid.

of the cations for (VIII) and (X) are similar (Fig. a), indicating that they possess similar structures (IX; R = H or Me). Considering that an *O*-alkyl group usually has a bathochromic effect on the ultraviolet spectrum, the cation (now shown to be XII) from pyrimidine-2,4-dione resembles the cation (XIII) from 4-ethoxypyrimidin-2-one rather than that (XI) from 2 ethoxypyrimidin-4-one (Fig. b).

Similar considerations (Fig. c) indicated that cations of type (XIV) are found in the bromo-series. The bromine atom exerts a constant bathochromic effect of 17—18 mμ (Table 1) in this series. As expected, the dioxo-form is favoured by the neutral molecules of the tautomeric bromo-compounds (Fig. d).

[3] Shugar and Fox, *Biochim. Biophys. Acta*, 1952, **9**, 199.
[4] Angell, *J.*, 1961, 504.
[5] Crick, personal communication.



(VII) (VIII) (IX) (X) (XI) (XII) (XIII) (XIV)

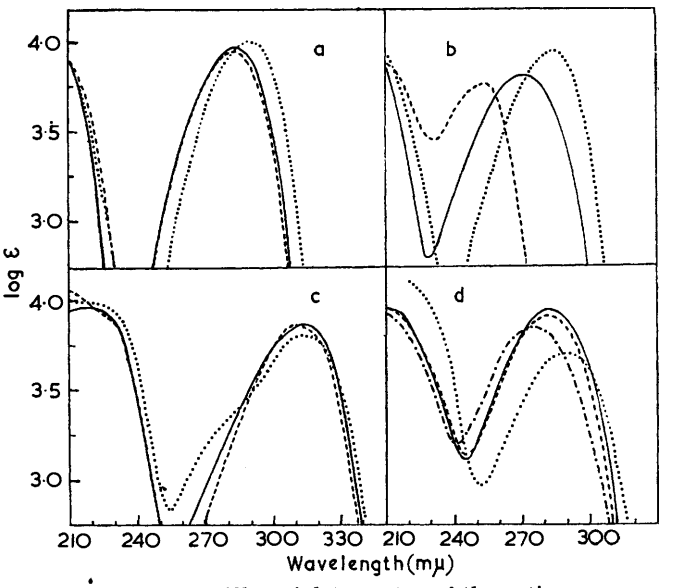
Basicity Measurements.—If we assume that Hammett H_0 values can be used, and that methyl groups have little effect on basicities, the results (Table 2) show that K_T for 1-methyluracil and its 5-bromo-analogue are *ca.* $10^{4\cdot0}$ and $10^{3\cdot3}$, respectively. The Hammett

TABLE 2.

Basicity measurements at 24° ± 1°.

Substituents at positions							
1	2	3	4	5	pK as a base	Gradient [1]	pK_a as an acid [2]
Me	O	Me	O	H	−3·25 ± 0·08	0·57 ± 0·03	—
Me	O	H	O	H	−3·40 ± 0·12	0·50 ± 0·05	9·71 [3]
H	O	H	O	H	−3·38 ± 0·15	0·53 ± 0·03	9·45 [3]
Me	O	—	OMe	H	+0·65 ± 0·05	1·11 ± 0·08	
H	O	—	OEt	H	+1·00 ± 0·01	1·02 ± 0·02	10·7 [4]
Me	O	Me	O	Br	−6·44 ± 0·06	0·84 ± 0·04	
Me	O	H	O	Br	−6·60 ± 0·16	0·69 ± 0·04	7·84 ± 0·03
H	O	H	O	Br	−7·25 ± 0·1	0·49 ± 0·06	7·83 ± 0·02
Me	O	—	OMe	Br	−3·32 ± 0·07	0·34 ± 0·02	

[1] See text. [2] Titrimetric unless otherwise indicated. [3] Levene, Bass, and Simms, *J. Biol. Chem.*, 1926, **70**, 229. [4] Shugar and Fox, *Biochim. Biophys. Acta*, 1952, **9**, 199 (spectrophotometric).



FIGS. a—c. Ultraviolet spectra of the cations.

(a) (——) 1-Methyl- (in 26·4N-H_2SO_4) and (– – –) 1,3-dimethyl-pyrimidine-2,4-dione (in 24·6N- and 29·2N-H_2SO_4); (. . .) 4-methoxy-1-methylpyrimidin-2-one (in 20N-H_2SO_4).

(b) (——) Pyrimidine-2,4-dione (in 20·6N-H_2SO_4); (– – –) 2-ethoxypyrimidin-4-one (in 10N-H_2SO_4); (. . .) 4-ethoxypyrimidin-2-one (in 10·0N- and 13·8N-H_2SO_4).

(c) (——) 5-Bromo-1-methyl- (in *ca.* 36N-H_2SO_4) and (– – –) 5-bromo-1,3-dimethyl-pyrimidine-2,4-dione (in *ca.* 36N-H_2SO_4), and (. . .) 5-bromo-4-methoxy-1-methyl-pyrimidin-2-one (in 28·8N-H_2SO_4).

FIG. (d) Spectra of free bases in phosphate buffer pH 7; as Fig. (c) and 5-bromo-pyrimidine-2,4-dione (— · — · —).

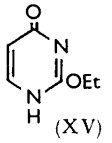
H_0 function is defined so that, for certain weakly basic amines, the concentrations of free base (A) and conjugate acid (HA^+) are given by

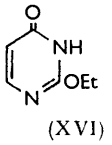
$$\log([A]/[HA^+]) = \text{constant} + H_0.$$

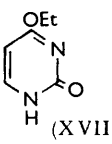
It follows that a plot of log ([A]/[HA^+]) against H_0 should be linear and should have unit gradient. In the present work, the plots were sensibly linear, but the gradients were often much less than unity. It has often been pointed out that bases of different types need not obey the H_0 rule (see, *e.g.*, ref. 6), but there appear to be few available data. However, another method is available for calculating K_T from the basicity data: If an H_0 value is selected midway between the pK values of the two molecules being compared, then by quite short extrapolations the proportion of each of the molecules which is protonated can be calculated. This gives K_T $10^{3\cdot3}$ and $10^{1\cdot7}$ for 1-methyluracil and its 5-bromo-analogue.

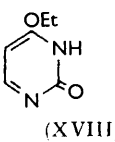
Conclusions.—Although the two methods give different results, it is at least clear that a bromine atom in the 5-position considerably increases the proportion of hydroxy-form present in 1-methyluracil. This could be the cause of the mutagenic activity of the bromo-compound although this may, of course, be due to something quite different: it may be noted that the acidity of 1-methyluracil is also considerably raised by a 5-bromo-substituent (Table 2).

2-Ethoxypyrimidin-4-one and 4-Ethoxypyrimidin-2-one.—These compounds could both exist in a hydroxy-form or in two oxo-forms (XV, XVI and XVII, XVIII). Shugar and Fox [3] discussed the tautomerism of these compounds in connexion with ultraviolet spectra, but did not consider the forms (XVI) and (XVIII). For 4-ethoxypyrimidin-2-one they preferred form (XVII) on the valid ground that the ultraviolet spectrum resembled that of its 1-methyl analogue and not that of 2,4-diethoxypyrimidine; consideration of the relative acidity of the two protons attached to nitrogen in the cation (XIII) makes structure (XVIII) improbable for the neutral molecule. Cations (XIII) and (IX) (R = Me) have similar pK_a values (Table 2), as required by structure (XVII).


(XV)


(XVI)


(XVII)


(XVIII)

Shugar and Fox [3] concluded, however, that 2-ethoxypyrimidin-4-one existed in the hydroxy-form. The available ultraviolet spectra do not decide between the structures, but infrared spectra indicate that the compound exists in an oxo-form: a carbonyl band is found at 1657 (Nujol), 1665 ($CHCl_3$), 1685s, and 1735m cm.$^{-1}$ (CCl_4). Infrared spectra of a dilute solution (*ca.* 3×10^{-3}M, 5 mm. cell) showed an NH bond at 3370 (CCl_4) or 3345 cm.$^{-1}$ ($CHCl_3$). This value for the ν (NH) indicated [7a] that the structure is *o*-quinonoid, *i.e.*, (XVI) rather than (XV); 4-hydroxypyrimidine similarly exists as 3*H*-pyrimidin-4-one.[7]

Experimental

Capital letters refer to the following paper chromatography solvent systems; A, butanol–acetic acid–water (5 : 1 : 4); B, propan-2-ol–conc. HCl–water (170 : 41 : 39); [8] C, butan-1-ol–ammonia–water (50 : 8 : 42); D, saturated $(NH_4)_2SO_4$ soln.–water–propan-2-ol (79 : 19 : 2); [9] E, butanol–acetic acid–water (5 : 3 : 2).

Uracil had m. p. 338° with sublimation and decomposition (lit.,[10] m. p. 335°), and ran as

[6] Hammett and Deyrup, *J. Amer. Chem. Soc.*, 1932, **54**, 2721.
[7] (*a*) Mason, *J.*, 1957, 4874; (*b*) Brown, Hoerger, and Mason, *J.*, 1955, 211.
[8] Wyatt, *Biochem. J.*, 1951, **48**, 584.
[9] Markham and Smith, *Biochem. J.*, 1951, **49**, 401.
[10] Fischer, *Ber.*, 1901, **34**, 3751.

a single spot in A, B, and C. 1-Methyl-4-methoxypyrimidin-2-one had m. p. 147·5—148·5° (lit.,[11] m. p. 149—150°). 1-Methylpyrimidine-2,4-dione had m. p. 238° (lit.,[11] m. p. 237°) and ran as a single spot in B, C, D, and E. 1,3-Dimethylpyrimidine-2,4-dione had m. p. 122—123° (lit.,[12] m. p. 123—124°) and ran as a single spot in B and C.

Ethoxypyrimidinones were prepared by refluxing 2,4-diethoxypyrimidine with aqueous-ethanolic sodium ethoxide.[13] 2-Ethoxypyrimidin-4-one had m. p. 128·5—129·5° (lit.,[13] 127·5—129°); 4-ethoxypyrimidin-2-one had m. p. 169—171° (lit.,[13] 167°); both compounds ran as single, well-separated spots in C.

5-Bromopyrimidine-2,4-dione had m. p. 312° (decomp.) [lit.,[14] m. p. 312° (decomp.)] and after repeated crystallisation from water ran on paper as a single spot in A and B. 5-Bromo-1-methylpyrimidine-2,4-dione had m. p. 267·5—268° [lit.,[15] m. p. 272—274°,[7b] 266° (decomp.), 255—260° [16]] and ran as a single spot on chromatography in B and C.

5-Bromo-1,3-dimethylpyrimidine-2,4-dione had m. p. 181—181·5° (lit., m. p.[15] 182—183°, 184—185° [17]) and ran as a single spot in B and C.

5-Bromo-4-methoxy-1-methylpyrimidin-2-one.—5-Bromo-2,4-dimethoxypyrimidine (2 g.) was shaken with methyl iodide (2 c.c.) until dissolved, and the solution set in the dark at 20° for 48 hr. Solid was filtered off at the pump and washed with dry ether (2 × 3 c.c.). Recrystallisation from light petroleum (b. p. 100—120°) gave the *pyrimidinone* (0·3 g., 15%), as needles, m. p. 148—149·5° [Found: C, 33·1; H, 3·5; N, 12·9. $C_6H_7BrN_2O_2$ requires C, 32·9; H, 3·2; N, 12·8%).

This pyrimidinone (0·033 g.) was heated at 100° with 15N-hydrochloric acid (0·3 c.c.) for 1 hr. and the mixture then evaporated under reduced pressure. Recrystallisation of the residue from water gave 5-bromo-1-methylpyrimidine-2,4-dione (0·021 g., 70%), m. p. 270—273°; the mixed m. p. with an authentic sample was not depressed, and the R_F values were identical in solvents B and C.

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[11] Hilbert and Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 2001.
[12] Davidson and Baudisch, *J. Amer. Chem. Soc.*, 1926, **48**, 2379.
[13] Hilbert and Jansen, *J. Amer. Chem. Soc.*, 1935, **57**, 552.
[14] Hilbert and Jansen, *J. Amer. Chem. Soc.*, 1934, **56**, 134.
[15] Hilbert, *J. Amer. Chem. Soc.*, 1934, **56**, 190.
[16] Johnson and Clapp, *J. Biol. Chem.*, 1908, **5**, 49.
[17] Wang, *Nature*, 1957, **180**, 91.