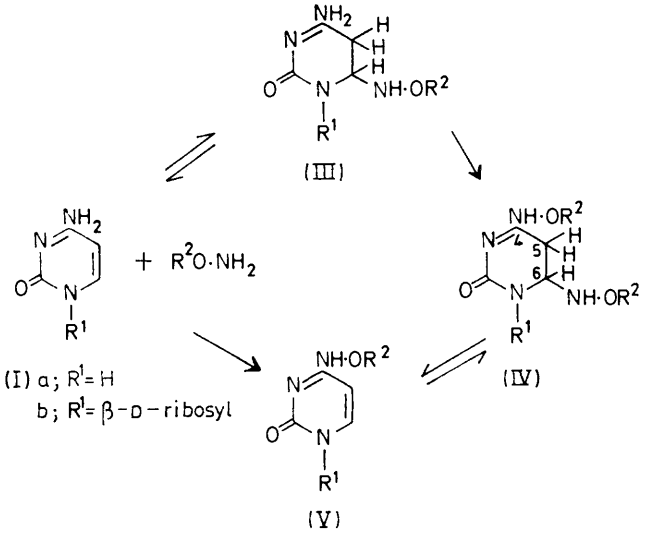
Reactions of Cytosine and Cytidine with *O*-Substituted Hydroxylamines

By **Peter M. Schalke** and **C. Dennis Hall,*** Department of Chemistry, King's College, Strand, London WC2R 2LS

Reactions of cytosine (Ia) and cytidine (Ib) with a wide variety of *O*-alkylhydroxylamines (II) are reported. At pH 4 in methanol–water and in the presence of an excess of hydroxylamine, the major product in each case is the *N*(4)-alkoxy-6-alkoxyamino-5,6-dihydro-derivative (IVa or b). Compounds containing primary and secondary alkyl or arylalkyl and tertiary alkyl groups in the hydroxylamine function are described and characterised by analysis and u.v., i.r., and ^{1}H and ^{13}C n.m.r. spectra. Treatment of the addition–substitution products (IVa) with acid gives *N*(4)-alkoxycytosines (Va) by elimination of *O*-alkylhydroxylamine. The reaction of cytosine with bis-1,6-amino-oxyhexane is also reported.

THE mutagenic activity of hydroxylamines towards DNA and bacteriophages is now well established [1-4] and appears to depend upon the alteration of a cytosine residue so that, during replication, the modified base directs the incorporation of adenine rather than guanine in the newly synthesised DNA. In consequence the *in vitro* reactions of hydroxylamines with cytosine (Ia) and cytidine (Ib) have attracted considerable attention.[5-11]



SCHEME 1 Reaction of cytosine (or cytidine) with *O*-alkylhydroxylamines

The two major products which may be isolated from the reactions are an addition–substitution product (IV) and a substitution product (V) (Scheme 1). Mechanistic studies [7-9] with hydroxylamine itself have established the interconvertibility of (IV; $R^2 = H$) and (V) and suggest that the principal pathway to (IV) involves the undetected addition intermediate (III). The product (V) may arise either by direct substitution of R^2ONH– for NH_2– or *via* elimination of R^2ONH_2 from (IV); the evidence suggests that under the appropriate conditions, (V) is obtained by both routes. If the pH is maintained between 4 and 6 then (IV) is the major product, whereas more acidic media give increasing amounts of (V). There is still some debate, however, as to whether the addition step (I) ⟶ (III) is reversible,[8,9,12] and little is known about the effects of substituents in the hydroxylamine on the rates of the various reactions leading to (IV) and (V).

The present work represents the first stage of a systematic study of the reactions between *O*-alkylhydroxylamines and cytosine (or cytidine) with the objective of establishing the influence of steric and electronic factors within the *O*-alkyl group of (II) on the products and rates of the reactions. This paper reports the isolation and characterisation of a wide variety of addition–substitution products (IVa and b) of cytosine and cytidine and several substitution products (Va) of cytosine, the latter either obtained directly from cytosine or derived from the addition–substitution products by treatment with acid.

RESULTS AND DISCUSSION

Addition–substitution products (IVa and b) were prepared in methanol–water solution by using an excess (*ca.* 5 : 1 molar) of *O*-alkylhydroxylamine. There is a rate maximum at pH 4—5 which is close to the pK_a values of the hydroxylamines employed and also close to the pK_a values of cytosine (4.45) and cytidine (4.11).[13] This is a typical mechanistic feature of nucleophilic addition–elimination reactions.[14] In most cases the extent of the reaction was easily followed by observing the disappearance of the cytosine or cytidine absorption at 280 nm and monitoring the appearance of a new band due to (IVa or b) at 225—230 nm. The cytidine products were more difficult to crystallise and hence to purify and characterise.

Apart from the definitive u.v. spectra, the products were identified by analysis and mass, i.r., and ^{1}H and ^{13}C n.m.r. spectra. The yields, m.p.s, and analytical

[1] D. R. Kreig, *Progr. Nucleic Acid Res.*, 1963, **2**, 125.
[2] E. Freese in 'Molecular Genetics,' ed. J. H. Taylor, Academic Press, New York, 1963, pt. I, p. 207.
[3] L. E. Orgel, *Adv. Enzymol.*, 1965, **27**, 289.
[4] J. H. Phillips and D. M. Brown, *Progr. Nucleic Acid Res.*, 1967, **7**, 349.
[5] D. M. Brown and P. Schell, *J. Mol. Biol.*, 1961, **3**, 709.
[6] N. K. Kochetkov, E. I. Bodowsky, and R. P. Shibaeva, *Biochim. Biophys. Acta*, 1963, **68**, 496.
[7] D. M. Brown and P. Schell, *J. Chem. Soc.*, 1965, 208.
[8] N. K. Kochetkov, E. I. Budowsky, E. D. Sverdlov, R. P. Shibaeva, V. N. Shibaev, and G. S. Monastinobaya, *Tetrahedron Letters*, 1967, 3253.
[9] D. M. Brown and M. J. E. Hewlins, *J. Chem. Soc.*, 1968, 1922.
[10] D. M. Brown and P. F. Coe, *Chem. Comm.*, 1970, 568.
[11] D. M. Brown, P. F. Coe, and D. P. L. Green, *J. Chem. Soc.* (*C*), 1971, 867.
[12] N. K. Kochetkov and E. I. Bodowsky, *Progr. Nucleic Acid Res.*, 1969, **9**, 403.
[13] D. D. Perrin, 'Dissociation Constants of Organic Bases in Aqueous Solution,' Butterworths, London, 1965.
[14] E. S. Gould, 'Mechanism and Structure in Organic Chemistry,' Holt, Rinehart, and Winston, New York, 1959.

TABLE 1

Yields, m.p.s, and analytical data for *N*(4)-alkoxy-6-alkoxyamino-cytosines (IVa) and -cytidines (IVb)

R²	Yield (%) [a]	M.p. (°C) [b]	Required (%) C	H	N	Found (%) C	H	N
(a) (IVa)								
Bu^n	90	85	52.95	8.8	20.6	53.45	8.75	20.45
Bu^s	85	118	52.95	8.8	20.6	52.75	8.85	20.6
Bu^i	80	132	52.95	8.8	20.6	52.7	8.6	20.85
Bu^t	50	165	52.95	8.8	20.6	52.95	8.9	20.8
PhCHMe	90	142	65.2	6.5	15.2	64.95	6.5	15.55
$PhCH_2$	90	140	63.5	5.9	16.45	63.1	5.9	16.55
$PhCH_2{\cdot}CH_2$	80	126	65.2	6.5	15.2	64.85	6.55	15.4
o-$ClC_6H_4{\cdot}CH_2$	80	138	52.95	4.15	13.7	52.65	4.1	13.55 [c]
p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$	95	157	50.25	4.2	19.55	50.0	4.3	19.8
p-$MeO{\cdot}C_6H_4{\cdot}CH_2$	80	144	60.0	6.0	14.0	60.15	6.1	14.25
Ph_2CH	80	137	73.15	5.7	11.4	72.9	5.75	11.3
(b) (IVb)								
$PhCH_2$	60	172	58.45	5.95	11.85	58.65	6.1	12.0
p-$MeO{\cdot}C_6H_4{\cdot}CH_2$	70	189	56.4	6.0	10.5	56.05	6.05	10.3
p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$	85	194	49.1	4.65	14.95	49.15	4.7	15.05
o-$ClC_6H_4{\cdot}CH_2$	25	130	51.0	4.8	10.35	50.9	4.95	10.55 [d]

[a] Based on starting cytosine or cytidine. [b] Small amounts of impurity change the m.p.s considerably. [c] Found: Cl, 17.65. Required: Cl, 17.4%. [d] Found: Cl, 12.8. Required: Cl, 13.1%.

data are collected in Table 1 and the n.m.r. data appear in Tables 2 (^{1}H) and 3 (^{13}C). Signals due to the two protons at position 5 of the dihydropyrimidine ring are easily assigned since they appear as the eight signals of the AB part of an ABX system at *ca.* δ 2.6; the H_X signal appears as a multiplet in the δ 4.4 region. Likewise the $-NH{\cdot}O{\cdot}CH<$ signals are easily distinguished from those of other aliphatic or aromatic protons in R². Assignment of the signals due to the three NH groups in the cytosine derivatives is not so straightforward, however. One can be fairly certain that the signal between δ 6.15 and 7.6 (depending upon the solvent and the nature of R²) belongs to the proton on N-1 since it is not apparent in the spectra of the cytidine derivatives. The NH of the 6-alkoxyamino-group is assigned to the resonance occurring between δ 4.8 and 6.9; this is reinforced by the observation that the signal is frequently a doublet (coupling with H_X). In fact, in the case of the s-butyl derivative, irradiation at the H_X frequency caused the NH doublet at δ 5.3 to collapse to a singlet. Further support for this assignment is provided by the observation that the NH signal of *NO*-dimethylhydroxylamine appears at δ 5.43. In view of the potential conjugation with the ring carbonyl, it seems reasonable to expect the NH of the 4-alkoxyamino-group to be in a similar chemical environment to the proton on N-1; accordingly, the signal between δ 7.7 and 8.8 is assigned to this proton.

TABLE 2

^{1}H N.m.r. spectra (δ values)

R²	Solvent	5-H_AH_B	6-H_X	4-NH	N(1)H	6-NH	4-NH·O·CH_n	6-NH·O·CH_n	Aromatic	Aliphatic
(a) Compounds (IVa)										
Bu^n	$CDCl_3$	2.64 (oct)	4.50 (m)	7.75	6.58	5.5 *	4.00 (2 H, t)	3.68 (2 H, t)		0.9 (6 H, t) 1.1—1.8 (8 H, m)
Bu^i	$CDCl_3$	2.65 (oct)	4.50 (m)	7.70	6.35	5.50	3.78 (2 H, d)	3.45 (2 H, d)		1.88 (2 H) 0.67—1.0 (12 H)
Bu^s	$CDCl_3$	2.64 (oct)	4.50 (m)	7.70	6.22	5.28 (d)	4.02 (1 H, m)	3.65 (1 H, m)		0.7—1.9 (16 H)
Bu^t	$CDCl_3$	2.64 (oct)	4.43 (m)	7.67	6.15	4.80				1.26 (9 H), 1.16 (9 H)
$PhCH_2{\cdot}CH_2$	$CDCl_3$	2.60 (oct)	4.40 (m)	7.72	6.80	5.95	4.20 (2 H, t)	3.90 (2 H, t)	7.22 (10 H)	2.7—3.05 (4 H, 2 × t)
$PhCH_2$	$CDCl_3$–$(CD_3)_2SO$	2.52 (m)	4.38 (m)	8.12	7.3 *	6.35 (d)	4.94 (2 H, s)	4.58 (2 H, s)	7.25 (10 H)	
p-$MeO{\cdot}C_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	2.50 (m)	4.30 (m)	8.15	7.2 *	6.50	4.85 (2 H, s)	4.48 (2 H, s)	6.7—7.5 (8 H)	3.78 (6 H)
p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	2.50 (m)	4.35 (m)	8.80	7.6 *	6.80	5.05 (2 H, s)	4.75 (2 H, s)	7.4—8.2 (8 H)	
o-$ClC_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	2.55 (m)	4.35 (m)	8.60	7.4 *	6.80	5.00 (2 H, s)	4.70 (2 H, s)	7.1—7.7 (8 H)	
PhCHMe	$CDCl_3$–$(CD_3)_2SO$	2.50 (m)	4.30 (m)	7.85	6.75	5.0 *	5.10 (1 H, q)	4.68 (1 H, q)	7.25 (10 H)	1.2—1.6 (6 H 2 × d)
(b) Compound (VI)										
	$CDCl_3$	2.65	5.20	7.2†	5.9		4.93 (2 H, s)	4.90 (2 H, s)	7.25—7.9 (14 H)	2.32 (3 H s)
(c) Compounds (IVb)‡										
$PhCH_2$	$CDCl_3$–$(CD_3)_2SO$	2.81 (m)	4.66 (m)	8.80		6.82	4.98 (2 H, s)	4.51 (2 H, s)	7.17—7.45 (10 H)	
p-$MeO{\cdot}C_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	2.71 (m)	4.53 (m)	8.49		6.50	4.90 (2 H, s)	4.39 (2 H, s)	6.67—7.34 (8 H)	3.62 (6 H)
p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	2.77 (m)	4.6 (m) *	8.60		6.85	5.11 (2 H, s)	4.66 (2 H, s)	7.28—8.30 (8 H)	
o-$ClC_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	2.85 (m)	4.6 (m) *	7.99		6.60 (d)	5.08 (2 H, s)	4.72 (2 H, s)	7.05—7.48 (8 H)	
(d) Compounds (Va)		5-H	6-H	4-NH	N(1)H	6-NH	4-NH·O·CH_n	6-NH·O·CH_n	Aromatic	Aliphatic
$PhCH_2$	$CDCl_3$	5.50 (d)	6.48 (q)	8.2 *	9.10		5.00 (2 H, s)		7.32 (5 H)	
$PhCH_2{\cdot}CH_2$	$CDCl_3$	5.55 (d)	6.52 (q)	8.35	9.30		4.30 (2 H, t)		7.25 (5 H)	2.98 (2 H, t)
Bu^n	$CDCl_3$	5.52 (d)	6.58 (d)	8.8 *	8.8 *		3.98 (2 H, t)			0.7—1.8 (7 H)
$Me_2C{:}N{\cdot}O{\cdot}[CH_2]_6$	$CDCl_3$	5.62 (d)	6.60 (q)	8.4	9.30 (d)		4.05 (4 H, t)			1.2—2.0 (8 H) 1.9 (6 H, s)

* Very broad; exact chemical shift difficult to estimate. † Exact chemical shift difficult to estimate owing to signals in same region. ‡ Signals due to the sugar appear at 5.5—5.8 (1 H, d), 3.2—4.5, and *ca.* 5.1. Cytidine shows δ [$(CD_3)_2SO$] 5.72 (1 H, d), 3.2—4.1, and 4.8—5.3.

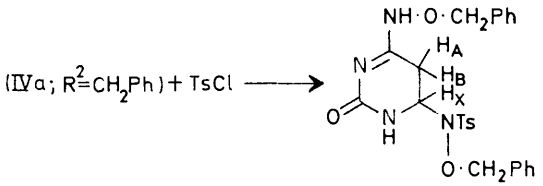
TABLE 3

^{13}C N.m.r. data (δ values) [a, b]

R²	Solvent	C-2	C-4	C-5	C-6	4-NH·O·*C*	6-NH·O·*C*
(a) Compounds (IVa)							
Bu^n	$CDCl_3$	151.6	142.5	27.2	64.6	75.2	73.9
Bu^s	$CDCl_3$	152.5	142.2	27.3	64.7	80.9	80.3
$PhCH_2{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	151.3	144.0	26.5	63.5	75.0	73.4
$PhCH_2$	$CDCl_3$–$(CD_3)_2SO$	151.4	144.1	26.7	63.7	76.5	74.6
p-$MeO{\cdot}C_6H_4{\cdot}CH_2$	$CDCl_3$–$(CD_3)_2SO$	151.4	143.9	26.9	63.7	76.3	74.6
p-$NO_2{\cdot}C_6H_4CH_2$	$CDCl_3$–$(CD_3)_2SO$	151.5	144.9	26.7	63.6	75.1	73.3
(b) Compounds (Va) [c]							
Bu^n	$CDCl_3$	153.1	145.1	97.8	131.3	75.2	
$PhCH_2$	$CDCl_3$–$(CD_3)_2SO$	149.4	148.5	93.9	135.9	76.2, 75.9 [d]	

[a] Signals for carbon atoms in the alkyl or aryl groups of R², other than those α to oxygen, are not quoted. [b] For comparison purposes: cyclohexane has δ 27.6; $PhCH_2{\cdot}CH_2{\cdot}ONH_2$ has δ 76.4; p-$NO_2{\cdot}C_6H_4{\cdot}CH_2{\cdot}ONH_2$ has δ 76.4. [c] For comparison purposes, cytidine has δ (D_2O) 165.6 (C-2), 155.7 (C-4), 94.1 (C-5), and 141.5 (C-6). [d] The two OCH_2 signals are probably due to the presence, in $CDCl_3$–$(CD_3)_2SO$ solution, of both the alkoxyamino-tautomer [*cf.* (V)] and the alkoxyimino-form [*cf.* (XII)].

The tosyl derivative (VI) of the *O*-benzylhydroxylamine derivative of cytosine has NH signals at δ 5.9



(VI)

and 7.2. This suggests that at least one of the remaining NH signals has moved upfield under the influence of the tosyl group; the origin of this effect is unknown. Both $O{\cdot}CH_2$ groups now resonate at δ *ca.* 4.9, which pinpoints the $O{\cdot}CH_2$ resonance at 4.58 in the spectrum of (IVa; $R^2 = CH_2Ph$) as that due to the 6-benzyloxyamino-group. In view of the anticipated conjugation between the ring and the N atom of the 4-benzyloxyamino-group, it is not surprising that the $O{\cdot}CH_2$ of this group should be deshielded relative to the $-OCH_2-$ of the 6-benzyloxyamino-group. As expected, H_X adjacent to the tosylated hydroxylamine function in (VI) is also deshielded and its signal moves downfield (to δ 5.2) relative to H_X in (IVa; $R^2 = CH_2Ph$) (δ 4.38).

Analysis of the AB parts of the spectra for the n-butyl, s-butyl, t-butyl, and isobutyl derivatives allowed evaluation of J_{AB}, J_{AX}, J_{BX}, ν_A, and ν_B (Table 4). In one case ($R^2 = Bu^n$), exchange of the NH proton of the 6-butoxyamino-group with deuterium removed coupling between H_X and NH and revealed the H_X signal as a triplet with $|J_{AX} + J_{BX}|$ 8.5 Hz as required by the analysis of Table 4.

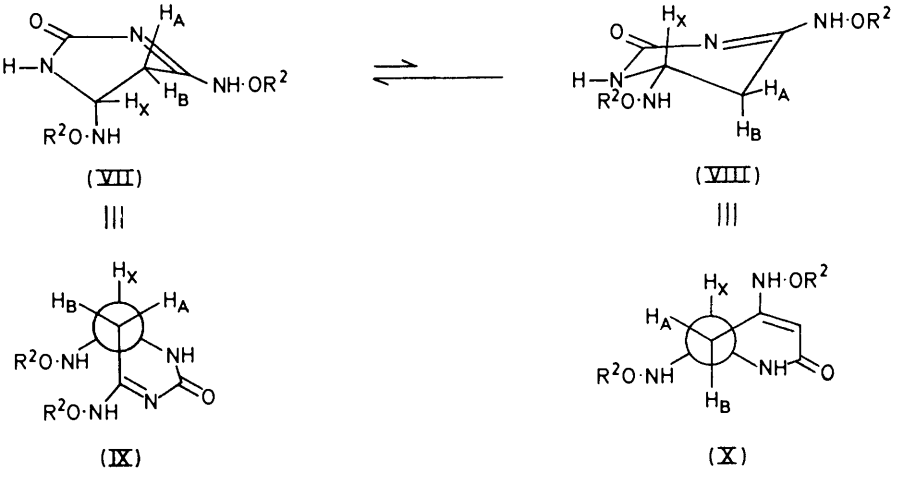
Two conformers [(VII) and (VIII), also pictured as their Newman projections (IX) and (X), respectively] of the addition–substitution products are, in theory, possible. Assuming that N-1 is planar, or at least capable of rapid inversion, the opposite configuration at C-6 simply provides enantiomers of (VII) and (VIII) (which in themselves are conformational diastereoisomers). The data of Table 4 reveal that, at least in chloroform as solvent, (VII) is the predominant conformer. This follows from the observation that J_{AX} and J_{BX} are almost equal (*ca.* 4—5 Hz), which implies torsion angles of *ca.* 60° between H_A or H_B and H_X. In view of the size of the alkoxyamino-groups in position 6, predominance of (VII) [(IX)] is at first sight surprising. However, molecular model studies suggest a more efficient intramolecular hydrogen bonding in this conformer between N(1)–H and the oxygen of the 6-alkoxyamino-group.

TABLE 4

Analysis of the AB part of the ABX spectra of compounds (IVa)

R^2	J_{AB}/Hz	J_{AX}/Hz	J_{BX}/Hz	$(\nu_A - \nu_B)$/Hz
Bu^n	−15.5	+4.4	+4.2	15.0
Bu^n *	−15.8	+4.3	+4.3	16.3
Bu^i	−15.4	+5.2	+4.2	16.6
Bu^s	−15.5	+5.1	+4.4	17.3
Bu^t	−14.8	+4.7	+4.4	18.4
$PhCH_2$	−16.9	+5.8	+3.3	13.0

* In D_2O: deuterium exchange simplifies the H_X signal to a triplet with $J_{AX} + J_{BX}$ 8.5 ± 0.5 Hz, which confirms the analysis of the AB part of the spectrum and eliminates the second possible set of coupling constants for which $J_{AX} \simeq -12$ and $J_{BX} \simeq +22$ Hz.



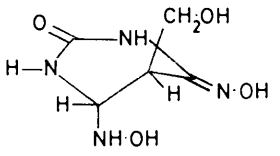
(VII) (VIII)

(IX) (X)

In the ^{13}C n.m.r. spectra the carbonyl carbon signal appears at lowest field (*ca.* 152 p.p.m. from Me_4Si) and the C(4)–NH signal occurs between δ_C 142 and 145 as expected. The $O{\cdot}CH_2$ or O·CH signals of the alkoxy-groups are separated by 1—2 p.p.m., the low field signal being assigned to the alkoxyamino-group at C-4 by analogy with the proton spectra. The 5- and 6-carbon atoms are plainly tetrahedral; cyclohexane shows δ_C 27.6 and the tertiary carbon atom in *N*-methyl-*N*-1-*p*-nitrophenylethyl-*O*-methylhydroxylamine resonates at δ_C 60.0.[15] Thus the ^1H and ^{13}C n.m.r. data support a structure similar to that proposed by Brown for the bis-

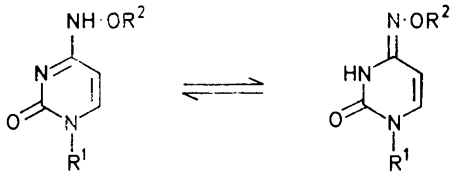
[15] T. Posner and C. D. Hall, unpublished results.

hydroxylamine derivative of 5-hydroxymethylcytosine (XI).[9,*]



(XI)

On treatment with acid the addition–substitution products of cytosine (IVa) decomposed to yield *N*(4)-alkoxycytosines (Va). These compounds were also obtained directly from cytosine when a lower pH (<3) was used for the reaction of cytosine with hydroxylamines. The compounds were purified by preparative t.l.c. and had R_F values slightly lower than those of the addition–substitution products. Yields, m.p.s, and analyses are detailed in Table 5; ^{1}H and ^{13}C n.m.r. data appear in Tables 2 and 3, respectively. Again the NH signals appear at low field (δ *ca.* 8.2 and 9.2) and the ^{13}C spectra are notable for the olefinic carbon signals at δ_C *ca.* 98 (C-5) and 131 (C-6). On the basis of u.v., i.r., and dissociation constant data, Brown and Hewlins [16] proposed a tautomeric equilibrium, (V) ⇌ (XII) (for R^1 = H or Me and R^2 = H or Me) with the 4-imino-structure (XII) predominating in aqueous solution. Similar u.v. data (Table 6) suggest analogous equilibria for the compounds reported here.



(V) R^1 = H or Me, R^2 = H or Me (XII)

Formation of (V) may be monitored by the appearance of the u.v. absorption at *ca.* 270 nm. Qualitative rate data indicate a rate maximum at low pH for this reaction since in concentrated hydrochloric acid the u.v. spectrum of (IV; R^2 = CH_2Ph) did not change over several days. Presumably the elimination of *O*-alkylhydroxylamine is catalysed by acid [(IV) ⟶ (XIII) ⟶ (V), Scheme 2]

TABLE 5

Yields, m.p.s, and analytical data for *N*(4)-alkoxycytosines (Va)

	Yield (%) [a]			Required (%)			Found (%)		
R^2	Method A	Method B	M.p. (°C)	C	H	N	C	H	N
$PhCH_2$	70	60	174	60.85	5.05	19.35	60.5	5.0	19.1
$PhCH_2{\cdot}CH_2$	60	50	126	62.35	5.65	18.2	61.9	5.85	18.45
Bu^n	50		86	52.45	7.1	22.95	52.5	7.15	23.15

[a] Based on starting (IVa) or cytosine.

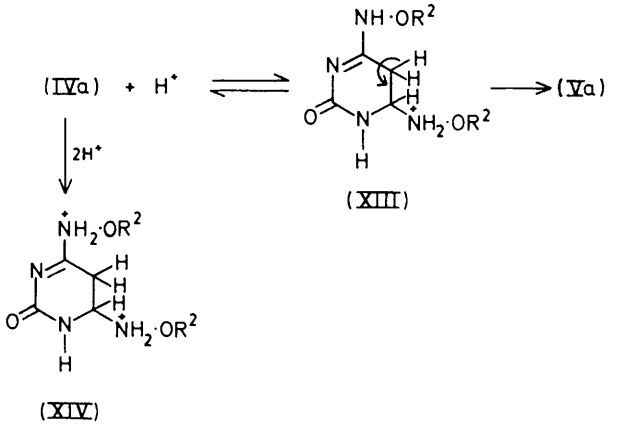
TABLE 6

U.v. spectra of *N*(4)-alkoxycytosines (Va) in (a) 95% EtOH, (b) 0.1N-HCl, and (c) 0.1N-NaOH

	R^2	$\lambda_{max.}$/nm (ε)	$\lambda_{min.}$/nm (ε)
(a)	Bu^n	281 (6 500) 235 (11 700)	263 (5 300)
	$PhCH_2$	279 (6 400) 236 (10 400)	262 (5 500)
	H *	283 (5 800) 231 (11 300)	260 (4 000)
(b)	Bu^n	285 (11 800) 216 (13 300)	249 (3 200)
	$PhCH_2$	285 (10 900) 217 (9 600)	248 (2 700)
	H *	276 (11 800) 216 (8 700)	240 (2 800)
(c)	Bu^n	300 (6 400) 242 (10 100) 220 (9 800)	275 (4 700) 228 (9 200)
	$PhCH_2$	302 (6 300) 247 (9 100) 224 (14 600)	278 (4 700) 238 (8 800)

Isosbestic points: Bu^n 264 (5 300) and 223 (10 000)
$PhCH_2$: 266 (6 100) and 222 (8 800)

* From ref. 16.



SCHEME 2 Elimination of *O*-alkylhydroxylamine from addition–substitution products

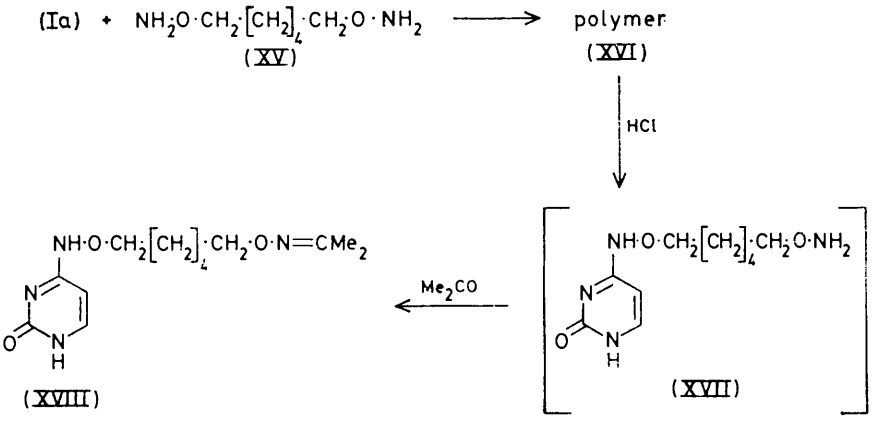
but in highly acidic media a double protonation to give (XIV) may inhibit the elimination. This aspect of the elimination will be investigated further and the results reported at a later date, but if the above interpretation is correct it implies a degree of neighbouring group participation by the *N*(4)-alkoxyamino-group in removing a proton.

* Brown [9] draws the hydroxyimino-structure (XI) for this derivative of 5-hydroxymethylcytosine and for the addition–substitution compound from cytosine and hydroxylamine (IVa; R^2 = H). In solution, both tautomers may be present in equilibrium [*n.b.* (IVa; R^2 = H) gives a positive hydroxamic acid test with ferric ion [7]] but our spectral data do not allow a clear distinction between a hydroxyimino-form of (IV) [analogous to (XI)] and the alkoxyamino-tautomer as drawn for (IV) in this paper. But see also D. M. Brown and M. J. E. Hewlins, *J. Chem. Soc.* (*C*), 1968, 2050.

[16] D. M. Brown, M. J. E. Hewlins, and P. Schell, *J. Chem. Soc.*, (*c*), 1968, 1925.

After treatment of the addition–substitution derivatives of cytidine (IVb) with acid, *N*(4)-alkoxyaminocytidines could not be isolated.* The reason for the failure of the elimination reaction in these cases is not yet established but it is possible that, at low pH, decomposition of the sugar occurs faster than the elimination reaction.

Reaction of a bifunctional hydroxylamine (XV) with cytosine (molar ratio 1 : 2) gave a polymeric product (XVI) of undetermined stoicheiometry and unknown structure. Treatment of the polymer with acid however, gave an unstable monomer (XVII), which was



isolated as an oxime (XVIII) after treatment with acetone. When the monomer (XVII) was generated *in situ*, attempts to cause it to react with more cytosine produced only new polymeric mixtures. Presumably, the 5,6-double bond of (XVII) is particularly susceptible to nucleophilic addition; further investigation of this system is also in hand.

EXPERIMENTAL

U.v. spectra were obtained with a Pye-Unicam 1700 spectrophotometer; i.r. spectra were recorded with a Perkin-Elmer 257 grating spectrometer for KBr discs; ^{1}H n.m.r. spectra were recorded on either a Perkin-Elmer R12 60 MHz spectrometer or a Brüker HFX 90 instrument with $CDCl_3$ or $CDCl_3$–$(CD_3)_2SO$ as solvent and Me_4Si as internal standard; ^{13}C n.m.r. spectra were obtained by the Fourier transform technique with the Brüker HFX 90 instrument [$CDCl_3$ or $CDCl_3$–$(CD_3)_2SO$ solvent], with a deuterium lock and Me_4Si as internal standard. Mass spectra were obtained with an A.E.I. MS9 spectrometer. Merck silica gel GF_{254} was used for preparation of t.l.c. plates.

O-Alkyl- (or -Arylalkyl-)hydroxylamines.[17]—These were synthesised by *O*-alkylation of *N*-hydroxyphthalimide with alkyl halides or alkyl acetates, and treatment of the products with ammonia (in methanol).

N(4)-*Alkoxy*-6-*alkoxyamino*-5,6-*dihydrocytosines* (IVa).—The following general procedure was employed. A mixture of *O*-alkylhydroxylamine (*ca.* 2 g) and cytosine (500 mg, 4.5 mmol) was dissolved or suspended in water (10 ml) and methanol (20 ml). The pH of each mixture was adjusted to *ca.* 4 by dropwise addition of concentrated hydrochloric acid. The solutions (or suspensions) were stirred at room temperature and the reactions were monitored by observing the disappearance of absorbance at 280 nm and the appearance of absorbance at *ca.* 225 nm. The appearance of gas bubbles afforded a sign that reaction was proceeding. On completion (*ca.* 7 days), the mixture was evaporated to dryness, the residue treated with dilute sodium hydroxide (50 ml), and the suspension extracted with chloroform (5 × 40 ml). The extracts were combined, dried (Na_2SO_4), and evaporated. The resultant solid was recrystallised from chloroform–methanol ether to give a colourless, crystalline *compound* (Table 1). The i.r. spectra showed $\nu_{max.}$ 3 400—3 200 (doublet, NH str.), 1 700—1 600s, br (C=O and C=N), *ca.* 1 300m (unassigned), and 1 050m cm^{-1} (C–O str.); bands associated with the R^2 groups (*e.g.* NO_2 str. in the *p*-nitrobenzyl group) were also observed. The u.v. spectra showed $\lambda_{max.}$ (95% EtOH) 225—230 nm (ε 11 000—16 000); R_F values on silica gel [$CHCl_3$–MeOH (10 : 1 v/v)] were 0.4—0.5.

N(4)-*Alkoxy*-6-*alkoxyamino*-5,6-*dihydrocytidines* (IVb).—The procedure was essentially the same as for (IVa) except that evaporation of the chloroform extracts gave, in each case, a residual gum. Crystallisation was achieved by boiling under reflux with methanol (20 ml) for 2 h then adding chloroform (20 ml) followed by ether until the solution became cloudy. After several days at 0 °C the *products* were obtained crystalline (see Tables 1 and 2). The i.r. spectra were essentially the same as those of the analogous cytosine derivatives except that the strong, broad OH stretching vibrations between 3 500 and 3 300 cm^{-1} obscured the weaker NH stretching vibrations.

N(4)-*Benzoyloxy*-6-(N-*benzyloxy*-p-*tolylsulphonylamino*)-*cytosine* (VI).—The adduct (IVa; R^2 = CH_2Ph) (300 mg) and tosyl chloride (1.5 g) were dissolved in dry chloroform (50 ml), the mixture was cooled to −10 °C and cold pyridine (0.5 g) was added. The mixture was set aside overnight at 0 °C, dilute hydrochloric acid (50 ml) was added, and the mixture was extracted with chloroform (3 × 40 ml). The combined extracts were treated with solid, anhydrous sodium carbonate, filtered, and concentrated and the concentrate was applied to preparative t.l.c. plates (silica gel) [$CHCl_3$–MeOH (10 : 1 v/v) as eluant]. The product (R_F 0.75) was extracted with methanol and the crude

* Heating the addition–substitution derivatives also failed to promote elimination but caused extensive decomposition.

[17] Houben–Weyl, 'Methoden der Organischen Chemie,' 1971, 10(1), p. 1181.

material was recrystallised from chloroform–ether to give 0.24 g (50%) of the tosyl derivative (VI) (0.24 g, 50%), m.p. 90° (see Tables 1 and 2), $\nu_{max.}$ 1 170 cm^{-1} (SO_2 str.) (Found: C, 60.65; H, 5.5; N, 11.15; S, 6.4 $C_{25}H_{26}N_4SO_5$ requires C, 60.75; H, 5.25; N, 11.35; S, 6.5%).

N(4)-*Alkoxycytosines* (Va).—*Method A*. Samples of the cytosines (IVa; R^2 = Bu^n, CH_2Ph or $CH_2{\cdot}CH_2Ph$) (0.2 g in each case) were dissolved in methanol (50 ml) and concentrated hydrochloric acid (5 ml) was added with stirring. After 2 days at room temperature, u.v. spectra indicated that reaction was complete and each mixture was neutralised with aqueous potassium hydroxide. The solutions were evaporated and the residues, dissolved in chloroform (20 ml), were subjected to preparative t.l.c. on silica gel [$CHCl_3$–MeOH (5 : 1 v/v)]. The *products* (R_F *ca.* 0.4) were extracted with methanol and crystallised from chloroform–ether (see Tables 2, 3, and 5). I.r. bands were observed at $\nu_{max.}$ 3 200—3 400m (NH str), 1 740s and 1 630s (C=N and C=O str. respectively), 1 230—1 300m (unassigned), and 1 050m cm^{-1} (C–O str.).

Method B. A mixture of cytosine (0.2 g) and *O*-alkylhydroxylamine (*ca.* 0.5 g) in aqueous methanol (10 ml) was adjusted to pH *ca.* 3 (conc. HCl) and kept at room temperature for 2—3 weeks, until t.l.c. revealed the formation of the required product. The solution was then neutralised and evaporated and the residue subjected to the work-up procedure described in Method A (see Table 5 for yields).

Reaction of Cytosine with Bis-1,6-amino-oxyhexane (XV).—A mixture of cytosine (0.2 g) and bis-1,6-amino-oxyhexane dihydrobromide (0.5 g) in aqueous methanol (20 ml) was adjusted to pH 4. After 48 h, concentrated hydrochloric acid (10 ml) was added, followed by acetone (50 ml). After 24 h at room temperature the solvent was evaporated off and the residue was dissolved in chloroform (20 ml). T.l.c. on silica [chloroform–methanol (10 : 1 v/v)] gave a major product with R_F 0.6; extraction with methanol and evaporation afforded N(4)-[6-(*isopropylideneamino-oxy*)*hexyloxy*]-*cytosine* (0.18 g, 60%), m.p. 89° (see Table 2 for ^1H n.m.r. data) (Found: M^+, 282.1697 $C_{13}H_{12}N_4O_3$ requires *M*, 282.1692).

We thank the Deutsche Akademischer Austauschdienst for a grant (to P. M. S.), Dr. J. M. Briggs (King's College) for 90 MHz n.m.r. spectra, and University College, London, for use of the mass spectrometer.

[5/883 *Received*, 9*th May*, 1975]