Fluorination of Pyrimidines. Part 2.[1] Mechanistic Aspects of the Reaction of Acetyl Hypofluorite with Uracil and Cytosine Derivatives

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The reaction of acetyl hypofluorite (AcOF) with uracil, cytosine, and some N-1-substituted derivatives dissolved in either acetic acid or water has been investigated. Analysis by radio-h.p.l.c., using ^{18}F as a tracer, and by ^1H n.m.r. revealed that a substituent at N-1 of uracil has a remarkable effect on the stability of the intermediate 6-acetoxy-5-fluoro-5,6-dihydrouracils. Substitution at N-1 of cytosine did not really enhance the stability of the intermediate adducts. In addition, it was found that these cytosine adducts rapidly deaminate in water yielding their corresponding uracil analogues.

Recently we reported on the reaction of acetyl hypofluorite (AcOF) with uracil and cytosine.[1] In the fluorination of uracil *cis*- and *trans*-6-acetoxy-5-fluoro adducts were obtained, which were thought to form an acylimine during the removal of the solvent. This interesting acetic-acid elimination would be possible by virtue of the proton at N-1. The instability of the corresponding 6-acetoxy-5-fluoro adducts of cytosine was explained by a fast auto-catalysed acetic-acid elimination by abstraction of 5-H, leading directly to the formation of 5-fluorocytosine. These results imply that a substituent at N-1 should enhance the stability of the uracil adducts by blocking acylimine formation, but should have little effect on the product distribution in the cytosine series.

In this paper the reaction of AcOF with several N-1-substituted pyrimidines and the conversions of the corresponding intermediates are described. In addition, carrying out the fluorination in either acetic acid or water as solvent, provided a means of checking the validity of the recently proposed mechanism for the reaction of AcOF.[1,2] Because of the limited solubility of the uracil derivatives and in order to simplify the detection of the several intermediates the experiments were carried out on a 50-μmol scale using ^{18}F as a tracer.

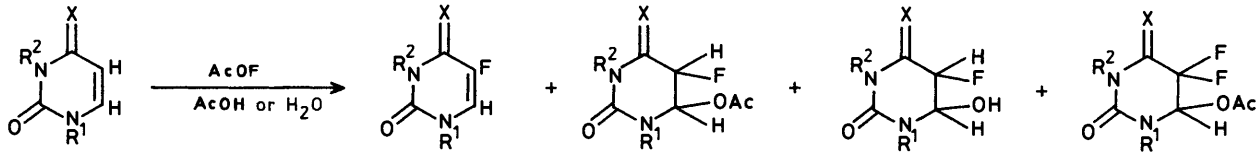
Results and Discussion

The reaction of gaseous AcOF with the uracil derivatives (**1**) was analysed by t.l.c., h.p.l.c., and ^1H n.m.r. (Scheme 1, Tables 1 and 2). In general, the overall yield of each reaction was 85—90%. Reaction of AcOF with (**1**) dissolved in perdeuterioacetic acid gave as the main products the geometric isomers of the 6-acetoxy-5-fluoro-adduct (**3**) with a *cis*:*trans* ratio of *ca.* 10:1. On the basis of earlier arguments[1] the isomers with the smallest $J_{5F,6H}$ coupling constants (2.0—3.8 Hz, Table 2) are assigned to the *cis*-adducts and those with the larger ones (5.9—9.7 Hz) to the *trans* adducts: the relatively small coupling constants again reveal that, due to anomeric effects, all isomers have the preferred conformation with the acetoxy group in the axial position.

Table 1. Chemical yields (%) of the fluorination of (**1**) and (**6**) using gaseous AcOF

Substrate	Solvent	(2)	(3)	(4)	(5)
(**1a, b**)	CD_3CO_2D	5—10	65—75	1—5	1—3
(**1a, b**)	D_2O	5—10	1—5	70—80	
(**1c—e**)	CD_3CO_2D	1—5	70—80	1—5	1—2
(**1c—e**)	D_2O	1—5	30—35	45—50	
		(7)	(8)	(9)	(10)
(**6a, b**)	CD_3CO_2D	55—65			15—25
(**6a, b**)	D_2O	5—10		70—75	

The ^1H n.m.r. spectra showed an interesting difference between the reaction products of (**1c—e**) and (**1a, b**): for (**1c—e**) 80% of the acetoxy adducts carried a non-deuteriated acetoxy group while for both (**1a**) and (**1b**) this was only 60%.



(**1**) **a**; X = O, R^1 = H, R^2 = H
b; X = O, R^1 = H, R^2 = Me
c; X = O, R^1 = Me, R^2 = H
d; X = O, R^1 = Me, R^2 = Me
e; X = O, R^1 = c-C_6H_{11}, R^2 = H

(**2**) X = O (**3**) X = O (**4**) X = O (**5**) X = O

(**6**) **a**; X = NH, R^1 = H, R^2 = H
b; X = NH, R^1 = Me, R^2 = H

(**7**) X = NH (**8**) X = NH (**9**) X = NH (**10**) X = NH

Scheme 1.

Table 2. Physical data of the N-1-substituted pyrimidines (**1**) and (**6**)

			^{1}H-n.m.r. $\delta_H(CD_3CO_2D)$						
Compd.	R_F	R_t (min.)	6-H	5-H	R^1,R^2[a]	OAc	$J_{5F.5H}$	$J_{5H.6H}$	$J_{5F.6H}$
(**1b**)	0.57	13.0	7.47	5.95	3.30			7.7	
(**2b**)	0.66	12.0	7.58		3.32				4.8
cis-(**3b**)	0.77		6.26	5.45	3.16	2.09	45.2	4.3	2.0
trans-(**3b**)	0.77		6.05	5.02	3.19	2.15	45.7	2.9	8.2
cis-(**4b**)	0.60	4.0	5.28	5.26	3.14		46.7	4.0	2.1
trans-(**4b**)	0.60	4.0	5.22	4.93	3.12		46.8	4.1	9.7
(**5b**)	0.80		6.18		3.21	2.12			2.0, 7.2
(**1c**)	0.46	9.5	7.53	5.89	3.39			7.9	
(**2c**)	0.64	8.5	7.68		3.36				5.9
cis-(**3c**)	0.73	27.0	6.44	5.52	3.09	2.14	45.2	4.1	2.7
trans-(**3c**)	0.73	27.0	6.21	4.94	3.13	2.16	45.9	2.9	7.3
cis-(**4c**)	0.58	3.0	5.27	5.32	3.09		46.7	4.0	2.5
trans-(**4c**)	0.58	3.0	5.16	4.89	3.12		46.6	3.7	7.9
(**5c**)	0.76	43.0	6.33		3.13	2.16			2.4, 6.6
(**1d**)	0.52	10.0	7.48	5.94	3.41, 3.32			7.8	
(**2d**)	0.67	7.5	7.66		3.40, 3.34				5.9
cis-(**3d**)	0.74	17.0	6.41	5.47	3.17, 3.12	2.11	45.5	4.3	2.5
trans-(**3d**)	0.74	17.0	6.16	4.99	3.20, 3.14	2.16	45.7	2.9	7.3
cis-(**4d**)	0.63	3.0	5.23	5.28	3.15, 3.11		46.6	4.0	2.4
trans-(**4d**)	0.63	3.0	5.10	4.90	3.14, 3.10		46.8	3.7	7.9
(**5d**)	0.79	26.0	6.31		3.21, 3.15	2.18			2.3, 6.6
(**1e**)	0.27	14.0	7.61	5.94	4.43			8.0	
(**2e**)	0.33	13.5	7.73		4.44				6.6
cis-(**3e**)	0.41	23.5	6.66	5.42	4.11	2.11	45.5	4.0	3.3
trans-(**3e**)	0.41	23.5	6.35	4.86	4.13	2.15	45.9	3.0	7.3
cis-(**4e**)	0.14	6.5	5.40	5.22	4.13		46.5	3.7	3.3
trans-(**4e**)	0.14	6.5	5.27	4.82	4.12		46.8	3.3	5.9
(**5e**)	0.56	39.5	6.49		4.14	2.14			2.9, 5.9
(**6b**)	0.25	6.5	7.73	6.14	3.44			7.6	
(**7b**)	0.38	9.0	7.78		3.41				5.8
(**10b**)	0.69	14.5	6.33		3.09	2.15			2.8, 6.4
			$\delta_H(D_2O)$						
(**9a**)			5.41	5.77			46.9	4.2	2.9
(**9b**)	0.30	2.5	5.38	5.76	3.16		46.9	4.3	3.1
(**4a**)	0.56	2.0	5.47	5.60			45.5	4.1	3.0
(**4c**)	0.58	3.0	5.49	5.63	3.20		45.5	4.2	3.2

[a] For the *c*-C_6H_{11} moiety only the 1-H′ value is given.

Compounds (**2**), (**4**), and (**5**) were formed as by-products. The small amounts of (**4**) are most probably caused by reaction with traces of water, and (**5**) is a consequence of consecutive reactions of preformed (**2**) with AcOF. In the reaction of AcOF with (**1a**) or (**1b**) in D_2O, apart from small amounts of (**2a**, **b**) the *cis*/*trans*-hydroxy adducts (**4a**, **b**) (ratio 10:1; *cis*/*trans* assignment as for (**3**), *vide supra*) were formed as the main products; only a few percent of the acetoxy adducts (**3a**, **b**) could be detected. However, under the same conditions the formation of the hydroxy adducts (**4c**—**e**) was accompanied by the formation of large amounts of their corresponding acetoxy adducts (**3c**—**e**) [ratio (**4**):(**3**) = 1.5, Table 1].

As expected, the adducts (**3b**) showed the same chemical behaviour as those of uracil (**3a**). Upon removal of acetic acid and redissolution of the residue in acetic acid (**3a**) and (**3b**) remained present, while upon redissolution in water both compounds appeared to be converted into the corresponding adducts (**4**).[3,*] Evaporation of the acetic acid in the presence of a small amount of ammonium acetate resulted in the complete conversion of (**3a**) and (**3b**) into the 5-fluorouracils (**2a**) and (**2b**), respectively. Under the same conditions the acetoxy adducts (**3c**—**e**) appeared to be fairly stable. Upon removal of acetic acid and redissolution in water they were still present whereas in water they only underwent a slow conversion, (a few % per day) with retention of configuration leading to a mixture of the analogous 5-fluorouracil derivative (**2**) and the 5-fluoro-6-hydroxy adducts (**4**). Like the hydroxy adducts (**4a**—**e**), compounds (**3c**—**e**) were even stable enough to be isolated by column chromatography, while they were converted into (**2**) only by the addition of a much stronger base such as triethylamine or di-isopropylethylamine. These results clearly confirm our earlier hypothesis that for N-1-unsubstituted 6-acetoxy-5-fluorouracil derivatives acylimine formation is the first step to take place upon removal of the acetic acid.

In order to determine whether the observed differences in the reaction products between (**1a**, **b**) and (**1c**—**e**) were due to a fast exchange reaction of (**3a**, **b**) with the solvent, we studied this possible exchange of (**3a**, **b**) in CD_3CO_2D solution and in CD_3CO_2D solution to which a large amount of D_2O had been added. After 6 h, no detectable exchange of the non-deuteriated acetoxy group with CD_3CO_2D was observed, while only a small amount of (**4a**, **b**) was formed in the CD_3CO_2D–D_2O mixture. This confirmed that the observed differences in the reaction

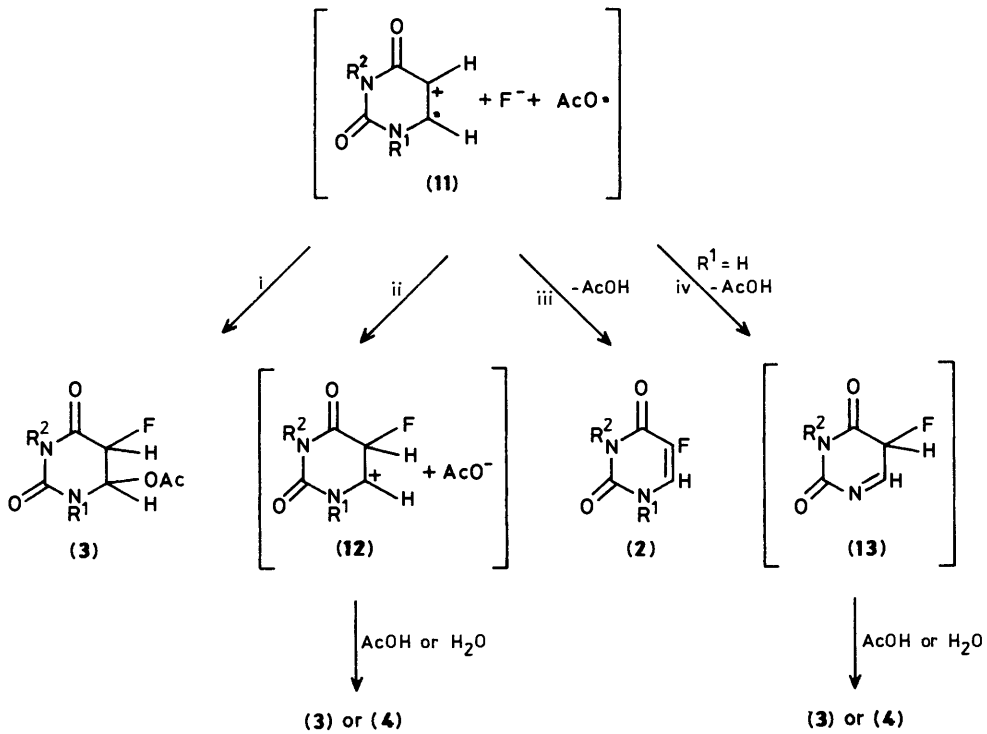
* This facile conversion of (**3**) into (**4**) and ignorance of the formation of hydroxy adducts (**4**), probably accounts for the erroneous assignment of the 6-acetoxy-5-fluoro adducts of uracil made by Diksic *et al.*[3]

products of (**1a**, **b**) and (**1c**—**e**) are really a result of a primary reaction with AcOF.

According to the normal 'electrophilic fluorination' concept,[4,5] (**2**) would be formed by deprotonation of an α-fluorocation, (**4**), by reaction of water with an open α-fluoro cation, while the formation of (**3**) would be the result of a recombination of a tight ion pair. However, in this concept it seems difficult to explain why the reaction of (**1a**, **b**) is less tight-ion-like than that of (**1c**—**e**). Recently, we reported on the mechanistic aspects of the fluorination of cyclohexene[2] and mercury compounds[6] with AcOF in acetic acid. The formation of the variety of products was adequately explained by a single electron transfer (SET) as the first step. For uracil we had already proposed this SET mechanism as an alternative to the electrophilic fluorination reaction.[1] We feel that the results with (**1a**—**e**) favour this SET mechanism and also allow a refinement of our previously proposed mechanism for the reaction of AcOF with enamides (Scheme 2). According to this scheme, AcOF oxidizes the enamide with the formation of a radical cation, a fluoride anion and an acetoxy radical (**11**). Combined with a concerted fluoride incorporation, the acetoxy radical may react in the following ways: (i) recombination of the radicals within (**11**) to give non-deuterioacetoxylated (**3**); (ii) a second single-electron transfer to give the intermediate stabilized carbocation (**12**), which then reacts with acetic acid or water to give (perdeuterioacetoxylated) (**3**) or (**4**); (iii) 5-H-abstraction resulting in the direct formation of (**2**); (iv) 1-H-abstraction leading to the acylimine (**13**), which like (**12**) reacts with the solvent to give (perdeuterioacetoxylated) (**3**) or (**4**). Comparable pathways have been observed in the reaction of AcOF with cyclohexene.[2]

According to this scheme, for the compounds (**1c**—**e**) in CD_3CO_2D, path i is followed for 80% and is still an important one (40%) in water. It was found (*vide supra*) that the acetoxy adducts (**3**) are relatively stable in water and acetic acid. Therefore, the fact that (**3a**) and (**3b**) are only detected in small amounts when the fluorination is performed in water would indicate that when R^1 = H pathway i is not important in this solvent and that pathway ii is mainly followed. However, it is difficult to understand why a substituent at N-1 would have such a drastic effect on the amount reacting through a second single-electron transfer. Therefore, for (**1a**) and (**1b**) we suggest the occurrence of path iv, the abstraction of the hydrogen atom from N-1 by the acetoxy radical to give (**13**) followed by reaction with the solvent, as a more plausible explanation. Consequently, reasonably assuming that compounds (**1a**—**e**) all follow path ii to the same extent, this would imply that (**1a**, **b**) in water follow path iv for nearly 40% and in CD_3CO_2D for some 20%. Finally, path iii explains the direct formation of small amounts of the 5-fluorouracils (**2**) always observed.

For the reaction of the cytosines (**6a**) and (**6b**) with AcOF in acetic acid, it was shown by t.l.c., h.p.l.c. and ^1H n.m.r. analysis,



Scheme 2.

that (**7**) is directly formed under these conditions. The only byproduct found was the corresponding 6-acetoxy-5,5-difluoro adduct (**10**), which is formed by consecutive reactions of (**7**) with AcOF. In acetic acid no difference was observed between (**6a**) and (**6b**) indicating that N-1-substitution does not enhance the stability of the intermediate (**8**). Therefore, the instability of (**8**) must be due to the relatively high acidity of 5-H, presumably caused by the rapid tautomerisation as shown in Figure 1. This conclusion is supported by the fact, that the corresponding 6-acetoxy-5,5-difluoro adducts (**10**) were stable in acetic acid solution (not in water, *vide infra*).

Using D_2O as the solvent in the fluorination of (**6b**), radio-t.l.c. and -h.p.l.c. and ^1H n.m.r. revealed the presence of the hydroxy adduct (**9b**) as the main product. However, when allowed to stand (**9b**) appeared to be converted, with a chemical half life of *ca.* 7 h, into (**7b**) and into a compound with the same

R_F and R_t values and ^{1}H n.m.r. data as (**4c**). This conversion was strongly accelerated by heating of the solution at 50 °C. Therefore, upon dissolution of an evaporated sample of the reaction mixture in CD_3CO_2D, ^{1}H n.m.r. analysis revealed the presence of only the geometric isomers of (**4c**) (c/t = 10), apart from (**7b**). For this reason the n.m.r. data of (**9b**) can only be given for samples dissolved in D_2O; for comparison the data of (**4c**) in D_2O are included (Table 2). Interestingly, upon addition of a small amount of CD_3CO_2D to the reaction mixture in D_2O a rapid 5-H/-D exchange was observed for (**9b**). This latter process was not observed for (**4c**) and again illustrates the high acidity of 5-H *via* the tautomerisation process, which apparently does not occur for (**4c**) (Figure 1).

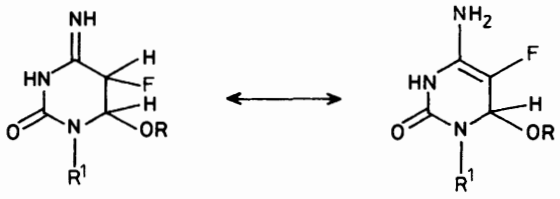


Figure 1.

The same results were obtained with (**6a**) as the substrate. However, in contrast to (**9b**), (**9a**) could not be detected by t.l.c. or h.p.l.c., indicating, as was already suggested earlier,[1] that they are destroyed through interactions with silica. From the results with (**9b**), this difference must be due to the lack of substituent at N-1. Our previous statement that the adducts (**9a**) are stable in CD_3CO_2D solution [1] must therefore be rectified: the observed stable 5-F, 6-OH adducts in the ^{1}H n.m.r. analysis were already the *cis*/*trans* 5-F, 6-OH adducts of uracil (**4a**), formed by a fast deamination reaction during the removal of D_2O at 50 °C. Thus, as with (**9b**) the ^{1}H n.m.r. data of (**9a**) can only be given in D_2O (Table 2). In the ^{1}H n.m.r. spectra the *trans*-isomers of (**9a**) and (**9b**) and those of (**4a**) and (**4c**) could not be detected, due to overlap of either the corresponding *cis*-signals or the HDO peak.

As a consequence of the severe instability of the intermediates, little can be said about the mechanism. However, there seems to be no serious objection to the suggestion that the cytosines react as given in Scheme 2. It then appears that in water (**6b**) mainly follows pathway ii; if (**6b**) reacts analogously to (**1c**) one would expect, *via* the unstable compound (**8b**), a higher amount of (**7b**). This difference is most probably due to a more effective stabilization of the intermediate carbocation *via* a tautomerization similar to that given in Figure 1.

In conclusion, it has been found that a substituent at N-1 of uracil has a marked effect on the stability of the intermediate 5-fluoro-5,6-dihydrouracils. However, irrespective of the presence of a substituent at N-1, the adducts of the cytosines remained unstable; they either rearrange to give the corresponding 5-fluorocytosines or they are converted into uracil derivatives by a fast deamination reaction. The relevance of this deamination under neutral conditions,[7,*] also observed in a series of cytosine nucleosides, will be dealt with in a forthcoming paper.

Experimental

Material and Methods.—The pyrimidines (**1a**), (**1d**), (**1e**), (**6a**), and (**6b**) were purchased from Sigma, (**1b**) and (**1c**) were prepared according to Brown *et al.*[8]

Thin layer chromatography was performed on Merck precoated silica gel F-254 plates (thickness 0.2 mm). The following eluants were used: the organic phases of a 3:2:2 mixture [for (**6a**, **b**)] or a 4:1:2 mixture [for (**1a**, **c**, **d**)] of ethyl acetate, propanol, and water; the organic phase of a 4:1:2 mixture of ethylacetate, butanol, and water for (**1b**), and for (**1e**) a 4:1 mixture of toluene and acetic acid. After development spots were visualized by u.v. light and chromatograms were wrapped in adhesive tape, cut into segments of 0.5 cm and counted in a LKB Compugamma counter.

For reversed-phase h.p.l.c. analysis, all products were dissolved in water prior to injection with the exception of the reaction products of (**1e**); these were dissolved in a 1:1 mixture of MeCN–water. The analysis of the products was performed on a 20-cm Spher C18 column (Chrompack). The following eluants were used: MeOH–water: (30:70 v/v) for (**1d**); MeOH–water (40:60 v/v) for (**1e**); and MeOH–0.1M-$NH_4H_2PO_4$ (2:98 v/v) for (**1a**—**c**) and (**6b**); flow rate 2 ml min^{-1}. Peaks were detected using a radioactivity monitor and u.v. detector [254 nm (**6a**, **b**) or 210 nm (**1a**—**e**)]; fractions of 500 µl were collected and counted for radioactivity. Separation of the ^{18}F-containing products was performed by column chromatography (Lobar Lichroprep. RP-8, 40—63 µm, Merck, eluant: 0.001M-$NH_4H_2PO_4$). Solvent removal was performed under reduced pressure at 50 °C. ^{1}H N.m.r. spectra were measured on a Bruker WM-250 spectrometer; CD_3CO_2D and D_2O were used as solvents. Chemical shifts are reported in δ (p.p.m.) relative to δ(CD_2HCO_2D) = 2.04 and δ(HDO) 4.95.

Fluorination of the Pyrimidines and Related Experiments.—For many of the experiments ^{18}F was used as a tracer, which permitted a simple determination of the yields of fluorinated products as radiochemical yields by using t.l.c. and h.p.l.c. techniques. For the synthesis of the fluorinated adducts, 40—60 µmol of gaseous [^{18}F]-AcOF, (produced by passing [^{18}F]-F_2 through a column of KOAc–HOAc[9]) was bubbled through a solution of the substrate (100 µmol) in CD_3CO_2D (12 ml) or D_2O (12 ml). For (**1a**—**e**) the elimination and exchange experiments were performed as described previously,[1] while the treatment of (**3c**—**e**) with triethylamine or di-isopropylamine was carried out at 70 °C for 30 min. For (**9a**, **b**), and (**10b**) [(**10b**) after removal of CD_3CO_2D and redissolution in D_2O], the solutions were analysed by ^{1}H n.m.r. spectroscopy and h.p.l.c.; immediately following the reaction and then each hour following this; detection at 254 nm for the measurement of the rate of the deamination, detection at 210 nm for the measurement of the formation of analogous uracil counterparts; using ^{18}F as a tracer, both were measured simultaneously during the first 8 h. The stability of (**3c**—**e**) in water was measured at room temperature by daily h.p.l.c. and ^{1}H n.m.r. analysis.

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