Notes

Chem. Pharm. Bull. 18(7)1475—1477(1970)

UDC 547.963.32.04 : 546.121.64.09 : 546.16.04.09

Hydrogen Fluoride as an Agent in Organic Reactions. I. Degradation of Polynucleotides with Liquid Hydrogen Fluoride[1]

Yuichi Kanaoka, Kazuhiko Itoh, Eisuke Sato, Akihiko Nomura and Yoshihisa Mizuno

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received January 28, 1970)

Hydrogen fluoride (HF) is unique among mineral acids in the area of organic chemistry. It has been used as a synthetic agent.[3,4] Several reviews have extensively dealt with its properties.[5–7] Recently reactions of proteins, carbohydrates and others in HF have also been reviewed.[8] However, liquid hydrogen fluoride (LHF) has been used relatively little in nucleotide chemistry in comparison with other fields.[8] Lipkin, *et al.* recently reported the action of HF on nucleotides, in which remarkably facile fission of phosphorus–oxygen bonds and N-glycosidyl bonds was described.[9]

Although very interesting application of 60% HF to nucleotides has been well established originally by Lipkin, *et al.*, studies of the action of LHF on nucleotides have apparently remained incomplete.[9] As is well known, physical and chemical properties of aqueous HF solutions are highly dependent upon their concentrations.[3,5,6] For example, acidity function values (H_0) for liquid and 60% HF are substantially different (−10 and −6.3, respectively). We therefore sought to explore the application of LHF to degradation of polynucleotides in connection with our studies of LHF as an organic agent[10] as well as studies of nucleotides.[1]

Published data indicate that on treatment with LHF very facile fission of N-glycosidyl bonds of nucleosides and mononucleotides frequently takes place giving corresponding bases.[9] To confirm the general behavior of mononucleotides on exposure to LHF, a solution of nucleoside or nucleotide in anhydrous LHF was stood at room temperature for 2 hr. Recovery of the liberated bases was nearly quantitative as listed in Table I. Thus nucleosides and mononucleotides in general suffer from facile and quantitative fission of their glycosidyl bonds by means of LHF at room temperature for 2 hr. Under these conditions, 10% of cytidine was still recovered uncleaved. After the reaction period of 3 hr, cleavage of cytidine was complete to give cytosine in 97% yield. Treatment of a mixture of these mononucleotides with LHF also resulted in good recovery of the bases.

1) This paper also forms Part III, "Heterocycles related to nucleotides," Part II: E. Sato and Y. Kanaoka, *Tetrahedron Letters*, **1969**, 3547.
2) Location: *Kita–12, Nishi–6, Sapporo.*
3) K. Wiechert, "Newer Methods in Preparative Organic Chemistry," Interscience Publishers, New York, 1948, p. 315.
4) J.H. Simons, "Fluorine Chemistry," Vol. 1, 1950; Vol. 5, 1964, Academic Press, New York.
5) M. Kilpartick and J.G. Jones, "The Chemistry of Non-Aqueous Solvents," Vol. 2, ed. by J.J. Lagowski, Academic Press, New York, 1967, p. 43.
6) H.H. Hyman and J.J. Katz, "Non-Aqueous Solvent Systems," ed. by J.C. Waddington, Academic Press, New York, 1965, p. 47.
7) S. Murahashi and S. Sakakibara, *Yūkigōseikagaku Kyōkaishi*, **25**, 1176 (1967) (in Japanese).
8) J. Lenard, *Chem. Rev.*, **69**, 625 (1969).
9) D. Lipkin, B.E. Phillips and J.W. Abrell, *J. Org. Chem.*, **34**, 1539 (1969), and papers cited therein.
10) Y. Kanaoka and K. Itoh, to be published.

Table I. Action of LHF on Nucleosides and Mononucleotides

Substrate	Base produced	Yield (%)	Substrate	Base produced	Yield (%)
Adenosine	adenine	102	adenosine-5′ phosphate	adenine	100
Guanosine	guanine	100	guanosine-5′ phosphate	guanine	95
Cytidine	cytosine	97[a]	cytidine-5′ phosphate	cytosine	100
Uridine	uracil	100	uridine-5′ phosphate	uracil	95
Thymidine	thymine	97			

a) 3 hr Treatment for 2 hr gave 85%.

It was early noted that most purine and pyrimidine bases including some minor bases can be recovered on exposure to LHF.[11] Control experiments reconfirmed that all major bases were recovered unaltered under these conditions. It should be noted, however, that 5-hydroxymethyluracil, a special pyrimidine base found in some bacteriophage DNA,[12] was altered even on gentle treatment with LHF as checked by paper chromatography. Unusually high reactivity of the hydroxymethyl group[13] may be responsible for this transformation.

DNA and RNA were earlier shown to dissolve in LHF,[14,15] but the products were not characterized. Lipkin, *et al.* described degradation of RNA (and DNA) with 60% HF to give mixtures of purine bases and pyrimidine nucleotides.[9] For comparison, RNA and DNA were now subjected to degradation with LHF. These polynucleotides were degraded by LHF at room temperature in 2 hr to mixtures of the four nucleo-bases. Table II and III present the results of base analysis of RNA and DNA, respectively, by the use of this LHF degradation technique. The results are in moderate agreement with those obtained by conventional hydrolytic method.[16] Side reactions such as deamination of bases[16] were not observed. Sugar moieties gave complex mixtures of polymers, which were not studied further.[17]

The specific feature of this method lies in that only mixtures of bases are formed under mild conditions, since even acid–stable pyrimidine nucleotides[16] are easily cleaved. The degradation method with HF, 60%[9] or anhydrous, may therefore provide a simple and convenient route to structural studies of base components of polynucleotides. After appropriate chemical modification, for example photo-irradiation, polynucleotide would be subjected to this mild degradation possibly to afford uninjured base fractions for further studies, which may have been lost during conventional degradations.

Table II. Base Analysis of RNA[a]

Base produced	HF degradation	1N HCl
Adenine	29.3	26.5
Guanine	27.7	28.1
Cytosine	20.2	20.4[b]
Uracil	22.8	25.0[c]

a) mole % b) cytidine-2′(3′) phosphates c) uridine-2′(3′) phosphates

11) H.A. Rutter, Jr., *J. Franklin Inst.*, **259**, 65 (1955).
12) R.G. Kallen, M. Simons and J. Marmur, *J. Mol. Biol.*, **5**, 248 (1962).
13) D.V. Santi and A.L. Pogolotti, Jr., *Tetrahedron Letters*, **1968**, 6159.
14) J.J. Katz, *Arch. Biochem. Biophys.*, **51**, 293 (1954).
15) J. Bjorksten, F.A. Andrews and H.F. Prahl, *Finska Kemistamfundes Medd.*, **71**, 69 (1962) [*C. A.*, **58**, 12793*f* (1963)].
16) A.M. Michaelson, "Chemistry of Nucleosides and Nucleotides," Academic Press, New York, 1963, p. 339, 369.
17) I.J. Golstein and J.I. Hullar, *Advan. Carbohydrate Chem.*, **21**, 431 (1966).

Table III. Base Analysis of DNA[a)]

Base produced	HF degradation	HCO_2H
Adenine	29.3	30.1
Guanine	26.0	23.4
Cytosine	14.2	16.0
Thymine	30.4	30.6

a) mole%

Conventional degradation methods for the analysis of DNA have been, aside from the enzymatic method, restricted to acid hydrolysis, which requires rather drastic reaction conditions.[16] Whereas the alkaline hydrolysis has been employed for the hydrolysis of RNA, hydrolytic method of DNA in alkaline medium has not been available due to stability of DNA to alkaline treatment. It may be pointed out, therefore, that this mild degradation technique of polynucleotide with LHF could be of considerable potential value particularly for the structural studies of DNA.

Experimental

Materials and Methods——Nucleosides and nucleotides were commercial preparations. Yeast RNA was obtained from Kokoku Jinken Pulp Co., Ltd., lot 1000. Calf thymus DNA was purchased from Sigma Chem. Co., lot D-1501.

All reactions were carried out in a simple vacuum line[18] made from Daifron (poly(monochlorotrifluoroethylene)). This apparatus was purchased from Toho Kasei Co., Ltd., Osaka. The HF is transferred under vacuum from a tank (Daikin Ind. Co.) into a stock vessel, which is cooled with acetone–dry ice. The HF is then distilled into the reaction tube containing the substrate as required.

Toyo Roshi No. 51A filter paper was used in ascending paper chromatography. The solvent systems used were (in volume): methanol–ethanol–conc.HCl–water, 50:25:9:16 (solvent A); methanol–conc.HCl–water, 70:20:10 (solvent B). Paper electrophoresis was performed using a Fuji–Riken Model PA. Buffer system was 2.5% formic acid–7.8% acetic acid, pH 1.8.

Degradation of Nucleosides and Mononucleotides (Table I)——The weighed substrate (0.1 mmole) was transferred to a reaction tube. Into the tube, cooled with acetone–dry ice, LHF (2 ml) was distilled from a stock vessel. The reaction tube was kept at 25—28° for 2 hr. Most of HF was distilled *in vacuo* with a polyethylene aspirator and the residue was treated with excess of 10% NH_3 with cooling. An aliquot from this reaction mixture was subjected to paper chromatography and UV measurement.

Base Analysis of RNA (Table II)——Yeast RNA (3.0 mg) was treated with LHF as above. After evaporation of LHF *in vacuo*, 10% NH_3 (10 ml) was added and a portion (50 μl) of the solution was subjected to paper chromatography (solvent A) or paper electrophoresis. The separated products were extracted with 0.1N HCl (1.2 ml) and determined by UV measurement. Hydrolysis with 1N HCl: A sample (10 mg) of the RNA was hydrolysed with 1N HCl (0.5 ml) at 100° for 1 hr by the described procedure.[19]

Base Analysis of DNA (Table III)——Calf thymus DNA (3.0 mg) was treated with LHF as above and a portion (50 μl) was subjected to paper electrophoresis. The separated products were extracted with 0.1N HCl (4 ml) and determined by UV measurement. Hydrolysis with formic acid: A sample (4.5 mg) of the DNA was hydrolysed with HCO_2H (0.5 ml) in a sealed tube at 175° for 30 min by the described procedure.[20]

Treatment of the Bases with LHF——All major purine and pyrimidine bases were recovered unchanged after standing at room temperature overnight in LHF solution as checked by paper chromatography and UV measurement. This result is in substantial agreement with the literature.[11] 5-Hydroxymethyluracil[21] (HMU) was transformed into a different compound (*Rf*: 0.31, solvent B: HMU, *Rf*: 0.56) after standing for 2 hr in LHF solution.

Acknowledgement We are indebted to Prof. S. Sakakibara, Osaka University, and Prof. O. Yonemitsu, Hokkaido University, for kind advice of the apparatus.

18) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
19) J.D. Smith and R. Markham, *Biochem. J.*, **46**, 509 (1950).
20) G.R. Wyatt and S.S. Cohen, *Biochem. J.*, **55**, 774 (1953).
21) R.M. Fink and R.E. Cline, *J. Am. Chem. Soc.*, **81**, 2521 (1959).