REACTIONS OF PROTEINS, CARBOHYDRATES, AND RELATED SUBSTANCES IN LIQUID HYDROGEN FLUORIDE

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Contents

I. Introduction

Hydrogen fluoride is unique among the mineral acids in the possibilities it offers the organic chemist. Its strongly acidic character ($H_0 = -10.2$) makes it an excellent solvent and protonating agent. It is a liquid from −83 to 19.5°. This enables reactions to be carried out in HF solution over a wide temperature range, and to be terminated readily by removal or neutralization of the HF. Chemically, HF is a nonoxidizing, nonsulfonating medium, and the dehydration reactions common in other strong acids are less often encountered in HF. Thus the acidity of HF can effect specific chemical modifications which may be obscured by side reactions in other acids.

A number of specific reactions of biologically important compounds in HF have been reported in recent years. The results in many cases have been striking and unexpected, but generally represent only beginnings in areas which will repay considerable further effort. This review will draw together the reactions of carbohydrates, proteins, and related substances in HF. Handling techniques and equipment requirements will also be briefly described, since these are simpler than is generally realized for the reactions to be reviewed.

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Recent reviews have dealt comprehensively with HF properties and handling techniques, with methods for the study of dissolved species in HF, and with the chemistry of organic and inorganic solutes in HF.[2,3] The fluorination of organic compounds in HF has also been reviewed.[4,5]

II. HF Handling Equipment and Techniques

Reactions in HF can be safely and conveniently carried out in a simple vacuum line of the kind shown diagrammatically in Figure 1.[6,7] The HF is transferred under vacuum from an aspirator or an oil pump into each of the numbered vessels in turn. The receiving vessel is cooled with liquid nitrogen or acetone–Dry Ice. All the numbered vessels can be individually removed from the line.

In vessel 1 (Figure 1) the HF can be measured and stored. The HF is then distilled into the reaction vessel (2, Figure 1) which already contains the other reactants. As indicated in Figure 1, the line may include any number of these reaction vessels, each with a connection enabling it to be sealed off independently from the line.

The reaction is terminated by distilling the HF into vessel 3. Vessel 2 can then be transferred from the line to a vacuum desiccator containing NaOH pellets to remove the last traces of HF. Disposal of HF from vessel 3 is effected by freezing the HF solidly (liquid nitrogen is required), removing the vessel from the line, and placing it mouth down over a bowl of soda-lime in a hood. If a water aspirator is used, instead of an oil pump, vessel 3 can be omitted and the HF aspirated off directly. The line shown in Figure 1 is available commercially.[8]

Figure 2 is a photograph of a line which was built in 2 days at low cost.[9] The Teflon valves and connectors and the flange clamp fittings are all commercially available. The rest of the

(2) M. Kilpatrick and J. G. Jones in "The Chemistry of Non-Aqueous Solvents," Vol. II, J. J. Lagowski, Ed., Academic Press, New York, N. Y., 1967, p 43.

(3) H. H. Hyman and J. J. Katz in "Non-Aqueous Solvent Systems," T. C. Waddington, Ed., Academic Press, New York, N. Y., 1965, p 47.

(4) M. Hudlicky, "Organic Fluorine Chemistry," Pergamon Press, New York, N. Y., 1962.

(5) J. Burdon and J. C. Tatlow, *Advan. Fluorine Chem.*, 1, 129 (1960).

(6) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jap.*, 40, 2164 (1967).

(7) S. Sakakibara, Y. Shimonishi, M. Okada, and Y. Kishida, "Proceedings of the 8th European Peptide Symposium," H. C. Beyermann, *et al.*, Ed., North Holland Publishing Co., Amsterdam, 1967, p 44.

(8) Toho-kasei Co. Ltd., Kamifukuido 2, Higashi-sumiyoshi, Osaka, Japan.

(9) A. B. Robinson, Ph.D. Thesis, University of California, San Diego, 1967; *Dissertation Abstr.*, 29, 146-B (1967).

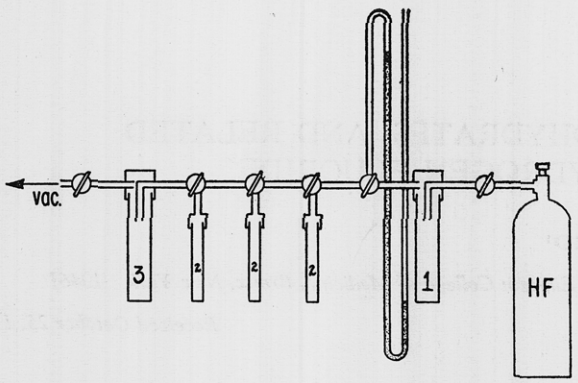


Figure 1. Diagram of a vacuum line for reactions in HF. Reproduced by permission from ref 6.

line, including the reaction vessels, was machined from Kel-F rod.

In addition to Teflon and Kel-F, some other plastics are suitable for the construction of an HF line.[2] In fact, many of the reactions discussed below were carried out by simply condensing HF in a cooled polyethylene flask or test tube. Monel metal has been widely used, but it is expensive, requires silver-soldered connections, and contaminates the HF with small amounts of metal ions (section III.E).

Special cells have been devised for making spectroscopic (uv, visible, ir, Raman, nmr) and electrical measurements in HF solutions.[2,3]

III. Protein Chemistry

A. SOLUBILITY AND STABILITY

The use of hydrogen fluoride in protein chemistry began with the observation that all the common amino acids and many proteins were highly soluble in HF.[10,11] Even tryptophan and cystine, amino acids which are practically insoluble in water, dissolved in HF to give solutions of 3 and 5%, respectively. All of the globular proteins examined dissolved instantaneously at −78° in HF to form solutions of 2% or greater concentration. Fibroin and collagen swelled strongly in HF and dissolved after several hours at room temperature.[10−12] Another collagen preparation was only partially soluble after treatment in HF for 11 days at room temperature.[13]

Proteins are remarkably stable in HF. The hormones insulin and adrenocorticotropic hormone were recovered after 2 hr in HF at 0° with their biological activity still substantially intact.[10,11] The extracellular nuclease from *Staphylococcus aureus* was recovered after 1 hr at 0° with 80% of its original enzymatic activity.[14] Ribonuclease and lysozyme retained about 90% of their activity after 2 hr at −78°.[15] Higher temperature caused a more rapid inactivation and a 15–20% decrease in sedimentation constant.[15] Neither exchange nor reduction of disulfide bonds could be demonstrated in HF using model compounds.[15]

(10) J. J. Katz, *Nature*, **173**, 265 (1954).
(11) J. J. Katz, *Arch. Biochem. Biophys.*, **51**, 293 (1954).
(12) A. Veis and J. J. Katz, *Biochim. Biophys. Acta*, **22**, 96 (1956).
(13) R. E. Dreikorn and V. O'Gorman, *Nature*, **175**, 385 (1955).
(14) C. B. Anfinsen, D. Ontjes, M. Ohno, L. Corley, and A. Eastlake, *Proc. Natl. Acad. Sci. U. S.*, **58**, 1806 (1967).
(15) A. L. Koch, W. A. Lamont, and J. J. Katz, *Arch. Biochem. Biophys.*, **63**, 106 (1956).

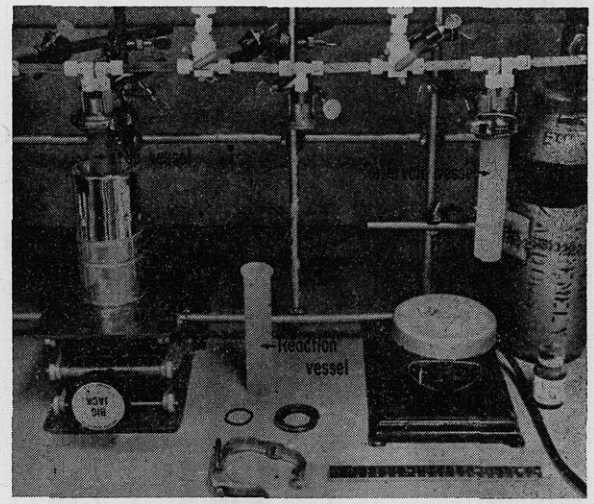


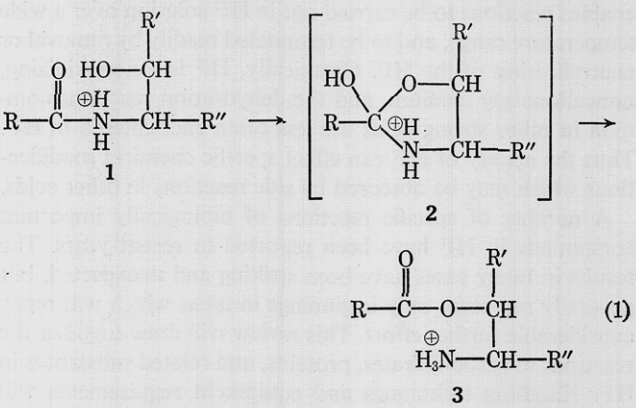
Figure 2. Photo of a vacuum line for reactions in HF. Reproduced by permission from ref 9.

The amino acid analyses of the oxidized A and B chains of insulin and of α-melanocyte-stimulating hormone were not altered after 12 hr in HF at 30° except for about 30% destruction of the tryptophan residue in α-MSH.[16] Electrophoretic examination of oxidized A and B chains of insulin after reversing the N to O acyl shift and hydrolyzing intramolecular ester bonds (see section III.B) showed that nearly all of the material had identical mobility at pH 8.6 with that of the starting material.[16] This showed that cleavage of peptide bonds and side-chain primary amide bonds was not a significant reaction even under these relatively drastic conditions.

The addition of trioxane to proteins in HF led to a typical formaldehyde type of cross-linking, with the protein eventually forming a gel in HF.[10,11] Other cross-linking agents have also rendered proteins insoluble due to reactions occurring in HF.[17]

B. N TO O ACYL SHIFT

The N to O acyl shift has long been known to occur at serine and threonine residues of proteins in strong acids. This reaction, which generates an ester linkage and a free amino group (3) from the peptide bonds of serine and threonine residues (1), is generally considered to proceed through a hydroxyoxazolidine ring intermediate (2) (eq 1). Investigation of this



R, R″ = peptide chain; R′ = H or CH_3

(16) J. Lenard and G. P. Hess, *J. Biol. Chem.*, **239**, 3275 (1964).
(17) J. Bjorksten, F. A. Andrews, and H. F. Prahl, *Finska Kemistamfundets Medd.*, **71**, 69 (1962); *Chem. Abstr.*, **58**, 12793*f* (1963).

reaction in HF was prompted by the stability of proteins in this medium (section III.A), and by the presence of side reactions and variable yields when this reaction was carried out in other acids.[18]

The dipeptides glycylserine, glycylthreonine, and alanylserine were nearly completely converted to the corresponding O-acyl compounds (3) afte r12–15 days in HF at room temperature, without any measurable side reactions.[19,20] All three compounds underwent the N to O shift at the same rate, suggesting that the reaction did not depend upon either the nature of the hydroxyamino acid or the adjacent residue. However, *N*-acetylserine and *N*-benzoylserine did not undergo the N to O shift in HF,[19,20] nor did a larger peptide with *N*-acetylserine at its N terminus.[21]

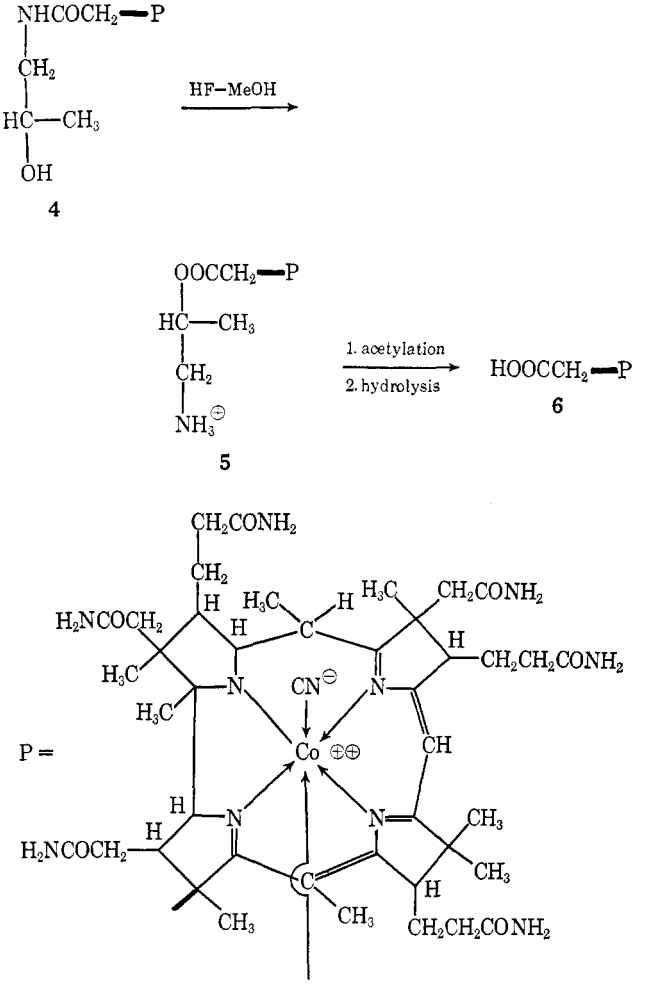
When glycylthreonine underwent the N to O shift in HF, and the shift was subsequently reversed with aqueous bicarbonate, only the starting material was recovered. Glycylallothreonine was not obtained, indicating that inversion at the β-carbon of threonine did not occur in HF.[20]

Preliminary observations of the pentapeptide Leu-Ala-Thr-Leu-Gly showed that it also underwent an N to O acyl shift in HF without apparent side reactions. In this case, however, the reaction was much more rapid than with the dipeptides, and was nearly complete within 2 days.[21]

The N to O acyl shift also proceeded smoothly in larger peptides. Shifts at the serine and threonine residues of oxidized insulin A and B chains and at the internal serine of oxidized α-melanocyte-stimulating hormone were 85–90% complete in each case after 12 hr in 75% HF–25% methanol at 30°.[16] Methanol prevented intramolecular esterification at the serine and threonine hydroxyls, which competed with the N to O acyl shift.[16,22] The N to O shift was quantitated by Van Slyke analysis for amino nitrogen before and after reversing the shift in aqueous bicarbonate.[16,20] It was confirmed for the larger peptides by the isolation and identification of the expected peptides after formylation of the amino groups and cleavage of the ester bonds of the O-acyl products (3) formed in HF. The recovery of specific cleavage products from oxidized α-melanocyte-stimulating hormone exceeded 80%, but was low for the oxidized A chain of insulin.[16]

The N to O acyl shift thus forms the basis for a specific cleavage of protein chains at serine and threonine residues.[18] That this cleavage method has not been widely used is due first to the limitations of variable yield and side reactions when sulfuric and phosphoric acids are used for the N to O acyl shift[18] and second to the lack of a reliable, quantitative method for cleaving the ester linkage (3) without reversing the shift. The findings in HF appear to settle the first problem, and to provide the background for solving the second.[24]

A new synthesis of cobyric acid (6) has been based on this reaction. Treatment of cobinamide (4) or cyanocobalamine (section IV.C) in 25% methanol–75% HF for 3 days at 30° formed 5 *via* an acyl shift.[25] Acetylation of the free amino group of 5 and hydrolysis of the ester bond yielded cobyric acid (6) in about 20% yield.[25] In these compounds, as in insulin chains,[16] the primary amides are stable to treatment with HF.



C. SPECIFIC CLEAVAGE OF METHIONYL PEPTIDE BONDS

When the dipeptide methionylgylcine was treated for 12 hr at room temperature with HF, 67% of the product was recovered as free methionine and glycine.[26] The splitting was complete after 36 hr in HF. It did not occur at all if the dipeptide was first oxidized to the methionine sulfone derivative.[26] The same cleavage occurred at Met-4 of α-melanocyte-stimulating hormone, although in this case the reaction was only about 50% complete after 3 days.[26]

This reaction is altogether different from the cyanogen bromide cleavage of methionyl peptide bonds.[27–30] In this

(18) For a comprehensive review of the N to O acyl shift and its use in the specific cleavage of proteins, see K. Iwai and T. Ando in "Methods in Enzymology," Vol. XI, C. H. W. Hirs, Ed., Academic Press, New York, N. Y., 1967, p 263.

(19) K. H. Shin, S. Sakakibara, W. Schneider, and G. P. Hess, *Biochem. Biophys. Res. Commun.*, **8**, 288 (1962).

(20) S. Sakakibara, K. H. Shin, and G. P. Hess, *J. Am. Chem. Soc.*, **84**, 4921 (1962).

(21) S. Sakakibara and G. P. Hess, unpublished observations.

(22) Esterification was shown to occur at both carboxylic and sulfonic acids in HF.[16] HF may provide a useful medium for the complete esterification of proteins. Almost quantitative conversion of free fatty acids to esters in HF containing alcohol has been reported.[23]

(23) Armour and Co., British Patent 1,055,549; *Chem. Abstr.*, **66**, 104708r (1967).

(24) J. Lenard, Ph.D. Thesis, Cornell University, 1964; *Dissertation Abstr.*, **25**, 1536.

(25) G. Müller and O. Müller, *Z. Naturforsch.*, **21B**, 159 (1966).

(26) J. Lenard, A. V. Schally, and G. P. Hess, *Biochem. Biophys. Res. Commun.*, **14**, 498 (1964).

(27) W. B. Lawson, E. Gross, C. M. Foltz, and B. Witkop, *J. Am. Chem. Soc.*, **83**, 1509 (1961).

(28) E. Gross and B. Witkop, *ibid.*, **83**, 1510 (1961).

(29) W. B. Lawson, E. Gross, C. M. Foltz, and B. Witkop, *ibid.*, **84**, 1715 (1962).

(30) E. Gross and B. Witkop, *J. Biol. Chem.*, **237**, 1856 (1962).

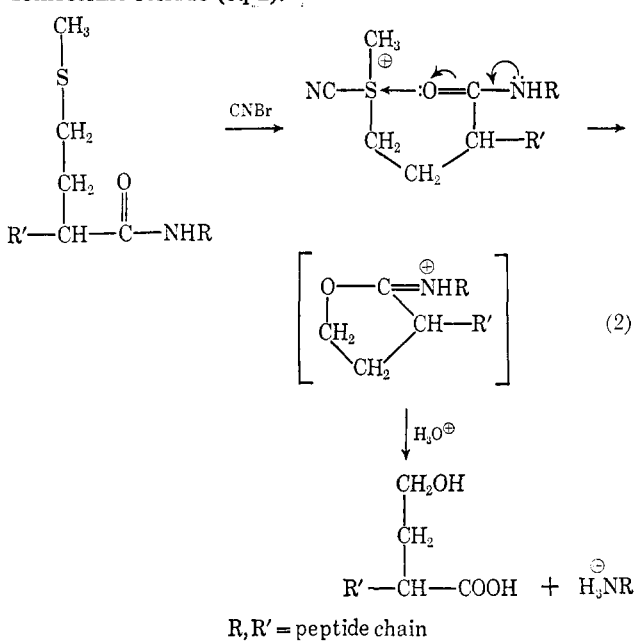
Table I

Conditions for Removal of Various Protecting Groups with HF[6,7]

Protecting group	*Structure*	*Reaction conditions* Temp, °C	Time, min
t-Butyloxy	$(CH_3)_3COCONHR$	0	30
Isoamyloxy	$(CH_3)_2(C_2H_5)COCONHR$	0	30
Isopropyloxy	$(CH_3)_2CHOCONHR$	20	30[a]
Carbobenzoxy	$C_6H_5CH_2OCONHR$	0	30[b]
t-Butyl ester	$RCOOC(CH_3)_3$	0	30
Isopropyl ester	$RCOOCH(CH_3)_2$	20	90[b]
Benzyl ester	$RCOOCH_2C_6H_5$	0	60
Benzyl ether	$ROCH_2C_6H_5$	0	60[b,c]
N-Nitro of arginine	N^G-NO_2	0	30
N-Tosyl of arginine	N^G-$SO_2C_6H_4CH_3$-*p*	0	30[d]
t-Butyl thioether	$RSC(CH_3)_3$	20	60
Benzyl thioether	$RSCH_2C_6H_5$	20	30
p-Methoxybenzyl thioether	$RSCH_2C_6H_4OCH_3$-*p*	0	30
Diphenylmethyl thioether	$RSCH(C_6H_5)_2$	0	30
Diphenylmethylamide	$RCONHCH(C_6H_5)_2$	0	60[b]

[a] Incompletely removed from ε-amino group of lysine. [b] Most vigorous of the reaction conditions listed[6,7] for this protective group. [c] A large excess of anisole is required for deprotection of *O*-benzyltyrosine without side reactions. [d] Reference 36.

widely used reaction, the methionine thioether reacts with cyanogen bromide to form a sulfonium ion, which is then eliminated in a concerted process, generating a C-terminal homoserine residue (eq 2).



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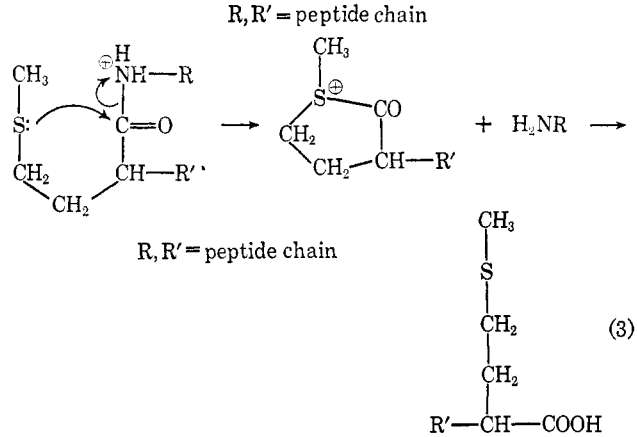


(3)

In contrast, methionine is not converted to homoserine in the HF cleavage.[26] In this case, the sulfur atom apparently acts as the nucleophile, while the protonated peptide nitrogen is the leaving group (eq 3).

D. PEPTIDE SYNTHESIS

Liquid HF is presently most widely used in biologically related research as a reagent in peptide synthesis. Yet the original discovery was reported as recently as 1965, when HF containing excess anisole was found to release a wide variety of protecting groups commonly used in peptide synthesis.[31] Anisole was added to the HF in order to combine with the released benzyl fluoride, thus pulling the reaction to completion and preventing side reactions.[32] This new method was demonstrated by removing the *S*-benzyl, *S*-(*p*-methoxy)benzyl, and *N*-carbobenzoxy groups from protected oxytocin by treatment for 1 hr at room temperature in HF containing anisole.[33] The product thus obtained possessed more than four times the biological activity of the same product deprotected by conventional means.[33]

Systematic studies showed that HF containing excess anisole was effective in removing a wider range of protecting groups from peptides than any other single procedure.[6,7,31] Conditions required to remove various protecting groups from simple model substances are shown in Table I.[6,7,31] Especially noteworthy is the easy removal of the nitro group from nitroarginine,[6,7,31,34,35] since the usual removal of nitro groups by catalytic hydrogenation is hindered by the presence of sulfur-containing amino acids. The following protecting groups are stable in HF under the conditions used: carboethoxy, benzoyl, tosyl (except when the tosyl group is used to protect the guanido group of arginine[36] (see Table I)), formyl, phthalyl, trifluoroacetyl,[14] methyl ester, ethyl ester, *N*-benzyl, *N*-*p*-methoxybenzyl, *S*-methyl, *S*-ethyl, *S*-isopropyl,[6,7] and *S*-acetamidomethyl.[37] The *p*-nitrobenzyl ester is partly cleaved in HF.[6,7]

(31) S. Sakakibara, Y. Shimonishi, and M. Okada, "Proceedings of the 4th Symposium on Peptide Chemistry," Dec 3a nd 4, 1965, Institute for Protein Research, Osaka University, Osaka, Japan, p 39 (in Japanese).
(32) F. Weygand and W. Steglich, *Z. Naturforsch.*, **14B**, 472 (1959).
(33) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Jap.*, **38**, 1412 (1965).
(34) J. Lenard and A. B. Robinson, *J. Am. Chem. Soc.*, **89**, 181 (1967).
(35) J. Lenard, *J. Org. Chem.*, **32**, 250 (1967).
(36) R. H. Mazur and G. Plume, *Experientia*, **24**, 661 (1968).

When a sufficient excess of anisole was used, side reactions were not encountered with any of the common amino acids.[6,7] Simple peptides were prepared in high yield. These contained, among the more reactive amino acids, methionine, tryptophan, aspartic acid, and phenylalanine.[38] This procedure is currently in wide use and has already aided in the synthesis of bradykinin,[34,36,39] arginine vasopressin,[7] monkey β-melanocyte-stimulating hormone,[40] polyglutamine,[41] polyasparagine,[41] and a portion of the sequence of the extracellular nuclease of *Staphylococcus aureus*.[14] No evidence of N to O acyl shift at serine residues was found.[34,39] The conditions required for deprotection of peptides are much milder than for the reactions discussed in sections B and C above.

The usefulness of HF in peptide synthesis in solution suggested its extension to solid-phase peptide synthesis.[42] Treatment with HF–anisole was shown to remove not only the protecting groups from the peptide, but also the peptide from the resin.[34] Thus HF–anisole generates free bradykinin from its fully protected, resin-bound precursor in a single step.[34] This method of removal of completed peptides from the resin has also been used in the synthesis of $(Pro\text{-}Pro\text{-}Gly)_{10}$ and $(Pro\text{-}Pro\text{-}Gly)_{20}$ by the solid-phase method.[43] A number of other peptides containing as many as 15 residues have also been prepared by solid-phase peptide synthesis followed by HF treatment[9] with no evidence of side reactions occurring in HF.

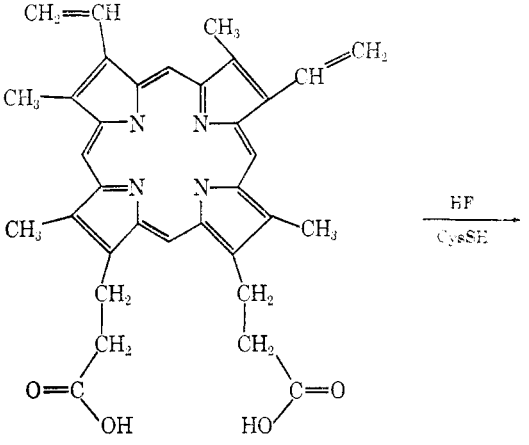
Another potentially useful reaction is the synthesis of nitroarginine in HF. When free arginine was allowed to react with a 50% excess of KNO_3 in HF for 30 min at 0°, nitroarginine was formed in quantitative yield.[35] The optical rotation of this product[35] suggested that it was of substantially greater optical purity than that synthesized by the standard procedure involving fuming nitric and fuming sulfuric acids.[44]

HF has been used as a reaction medium for the synthesis of polypeptides. After several hours at room temperature in HF, leucine *N*-carboxyanhydride was converted to polymers of up to 25–30 units.[45] This reaction is unusual since evidence was presented that the reaction proceeds with formation of the aminoacyl fluoride and its attack upon an unprotonated amino group or upon complexes of the amino group and HF.[45] The significant racemization (up to 40%) which occurs in the course of polymerization[45] has severely limited the usefulness of this reaction. The short chain length of these polymers results from a small amount (*ca.* 5%) of decarbonylation of the leucine fluoride in HF.[46]

E. HEME- AND PORPHYRIN-CONTAINING PEPTIDES AND PROTEINS

The most facile reaction of heme, heme peptides, and cytochrome *c* in HF is the removal of iron from the heme group. The reaction is complete after a few seconds in HF at temperatures well below 0°.[9,47] In the case of cytochrome *c*, the product has been characterized as homogeneous, monomeric porphyrin cytochrome *c* with 96% of its iron removed.[9,47,48] An earlier report that the iron of cytochrome *c* is retained in HF[11] apparently arose from contamination of the HF with metal ions from the monel vacuum line used in the experiments. In contrast, the cobalt ion of cobinamide (**4**) and cobyric acid (**6**) does not seem to be removed by HF.[3,11,25]

HF has recently been used in the synthesis of porphyrin peptides.[9,47] This reaction involves the alkylation of the cysteine sulfur atoms by the vinyl side chains of protoporphyrin IX (**7**) to form the thioether. Thus, when cysteine and **7** were treated in HF at room temperature, porphyrin *c* (**8**) was isolated from the product in 20% yield. The decapeptide Lys-CysSH-Ala-Gln-CysSH-His-Thr-Val-Glu-Arg and other dicysteinyl peptides also combined with **7** in HF to form porphyrin peptides with thioether linkages.[9,47] A high molecular weight polymer containing both starting materials was generally formed as a substantial by-product, which, however, was readily removed by gel filtration. Both mono- and diporphyrin peptides were formed, the relative amounts depending upon the reaction conditions.[9,47]



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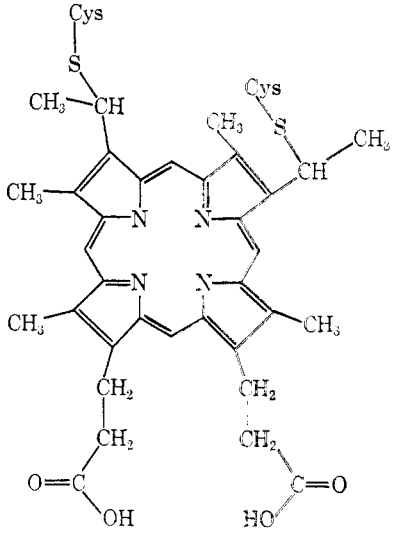

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(37) D. F. Veber, J. D. Milkowski, R. G. Denkenwalker, and R. Hirschmann, *Tetrahedron Letters*, 3057 (1968).
(38) S. Sakakibara, Y. Kishida, R. Nishizawa, and Y. Shimonishi, *Bull. Chem. Soc. Jap.*, **41**, 438 (1968).
(39) S. Sakakibara, N. Nakamizo, Y. Kishida, and S. Yoshimura, *ibid.*, **41**, 1477 (1968).
(40) H. Yajima, Y. Okada, Y. Kinomura, and H. Minami, *J. Am. Chem. Soc.*, **90**, 527 (1968).
(41) M. Fridkin and S. Ariely, *Israel J. Chem.*, **5**, 63p (1967).
(42) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
(43) S. Sakakibara, Y. Kishida, R. Nishizawa, and Y. Shimonishi, *Bull. Chem. Soc. Jap.*, **41**, 1273 (1968).
(44) K. Hofmann, W. D. Peckham, and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).
45) K. D. Kopple and J. J. Katz, *ibid.*, **78**, 6199 (1956).
(46) K. D. Kopple, L. A. Quarterman, and J. J. Katz, *J. Org. Chem.*, **27**, 1062 (1962).
(47) A. B. Robinson and M. D. Kamen in "Structure and Function in Cytochromes," K. Okunuki and M. D. Kamen, Ed., University Park Press, Baltimore, Md., 1968, p 217.
(48) T. Flatmark and A. B. Robinson in ref 47, p 149.

The thioether linkage of the hematoporphyrin group can also be cleaved in HF under suitable conditions. Thus, the porphyrin group of cytochrome *c* has been partially transferred to cysteine and to anisole (see section III.D) by treatment with HF.[47]

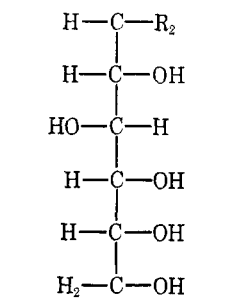
IV. Reactions of Carbohydrates

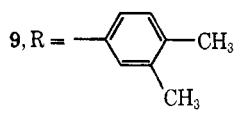
A. FREE MONOSACCHARIDES AND POLYSACCHARIDES

The reactions of cellulose, starch, and D-glucose in HF have recently been reviewed.[49] All these materials dissolve readily and react to form similar products, which are highly dextrorotatory, apparently branched, water-soluble oligosaccharides of 2–14 units. The products have not been precisely characterized, and different workers report somewhat different size distributions of oligosaccharides from the same starting materials. The reaction presumably proceeds by the formation of glycosyl fluorides from the starting materials.

The C_1 of glucose undergoes alkylation in HF. Thus, the reaction of starch or D-glucose with *o*-xylene in HF yields 1-deoxy-1,1-bis(3,4-dimethylphenyl)-D-glucitol (**9**) in 85% yield.[50] Many similar alkylations of the C_1 of saccharides have been carried out in HF, although generally multiple products are obtained. This work has appeared mainly in the patent literature and has recently been comprehensively reviewed.[51]

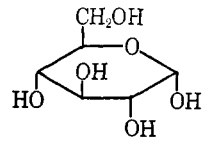
In a similar reaction, D-glucose reacts with *n*-decanethiol in 27% HF–dioxane to yield 1-deoxy-1,1-di-*n*-decyldithioacetal (**10**).[52] The reaction of D-glucose with alkyl dithiols in HF–dioxane at −32° forms polymeric sugar dithioacetals (eq 4).[52]



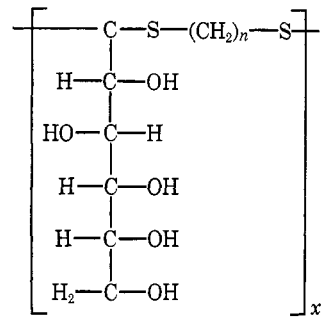
9, R = 

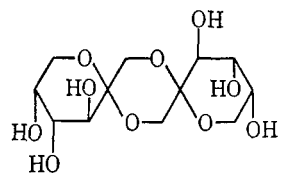
10, R = $-S(CH_2)_9CH_3$

When D-fructose is dissolved in HF, the predominant products are **11** and **12**, the difructose 1,2′:2,1′ dianhydrides (diheterolevulosan I and II).[53] The yield of these products after 4 hr in HF at 0° compares favorably with that from concentrated HCl after 72 hr at −5°.[54]



(4)





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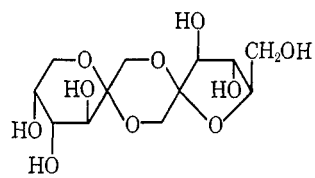


12

The cyclitols, glycitols, and 1,4- and 1,5-anhydroglycitols are all completely stable in HF.[55–58]

B. ACYLATED MONOSACCHARIDES AND RELATED COMPOUNDS

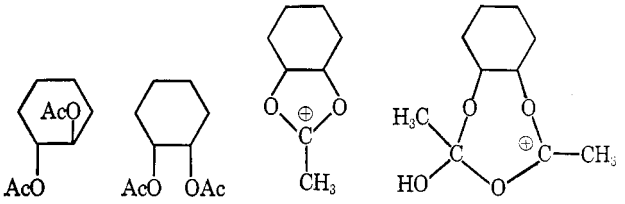
In contrast to the stability in HF of free glycitols and cyclitols, and the limited reactions of free monosaccharides, the reactions of the acylated derivatives of these compounds are far more complex. Glycosyl fluorides are readily obtained by reaction of acylated monosaccharides with HF, but in addition Walden inversions, ring openings and closings, ring contractions, and an allylic shift have all been observed in acylated polyols and monosaccharides. Insights into the mechanisms of these reactions have been speculative until recently, when observations by nmr spectroscopy directly on HF solutions have yielded significant information. Most of the work described below, however, was done by terminating the reaction either by evaporation of the HF in dry air or nitrogen, or by neutralization with aqueous bicarbonate, and identifying the products formed. These two methods of terminating the reaction were shown in certain cases to lead to different products.[59] Knowledge about the species actually formed by HF was limited to inferences from the products isolated.

1. 1,2-Diacetoxycyclohexanes

Nmr observations were first made on the *cis* and *trans* isomers of 1,2-diacetoxycyclohexane[60] which are simple models of

(49) I. J. Goldstein and T. L. Hullar, *Advan. Carbohydrate Chem.*, **21**, 431 (1966).
(50) J. Heerema, G. N. Bollenback, and C. B. Linn, *J. Am. Chem. Soc.*, **80**, 5555 (1958).
(51) A. Wagner in "Friedel-Crafts and Related Reactions," Vol. IV, G. A. Olah, Ed., Interscience Publishers, New York, N. Y., p 235.
(52) E. G. Horvath, S. Gardlund, S. K. Sen, J. W. Berry, and A. J. Deutschman, Jr., *J. Polymer Sci., Part A*, **3**, 1985 (1965).
(53) L. Sattler, F. W. Zerban, G. L. Clark, C.-C. Chu, N. Albon, D. Gross, and H. C. S. DeWhalley, *Ind. Eng. Chem.*, **44**, 1127 (1952).
(54) H. W. Hilton, "Methods in Carbohydrate Chemistry," Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press, New York, N. Y., 1963, p 199.
(55) E. J. Hedgley and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **84**, 3726 (1962).
(56) E. J. Hedgley and H. G. Fletcher, Jr., *ibid.*, **86**, 1583 (1964).
(57) E. J. Hedgley and H. G. Fletcher, Jr., *ibid.*, **85**, 1615 (1963).
(58) E. J. Hedgley and H. G. Fletcher, Jr., **86**, 1576 (1964).
(59) C. Pedersen, *Acta Chem. Scand.*, **17**, 1269 (1963).
(60) C. Pedersen, *Tetrahedron Letters*, **6**, 511 (1967).

acetylated monosaccharides, cyclitols, and glycitols. The nmr spectrum of the *trans* compound **13** remained unchanged for several days in HF, showing that this compound is unreactive.[60] On the other hand, the *cis* isomer **14** underwent a complete change in 6–8 hr in HF. The quantitative formation of the 2-methyl-1,3-dioxolenium ion (**15**) and acetic acid was



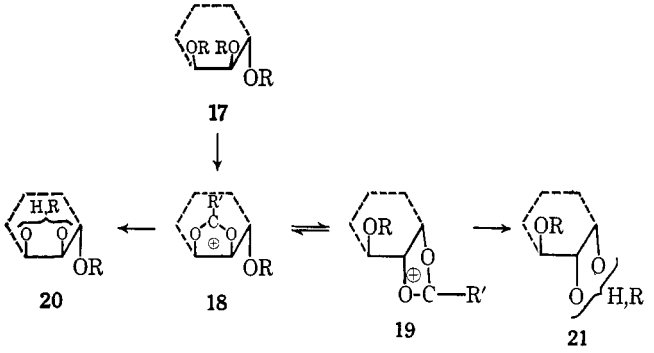
13 **14** **15** **16**

shown by the nmr spectrum.[60] The structure of **15** was confirmed by its isolation from HF in 66% yield as the crystalline tetrafluoroborate,[60] and a comparison of its properties with **15** obtained by a different procedure.[61]

The nmr spectrum of **15** remained unchanged for several days, showing that this is a stable species in HF.[60] This study provided strong support for the contention that a 1,3-dioxolenium cation similar to **15** is the major intermediate in many of the transformations described below. No evidence was obtained in this study for a seven-membered ring (**16**) which had previously been invoked as the first species formed in HF from a *cis*-diacyloxy group.[55]

2. *Cyclitols, 1,5-Anhydroglycitols, and Shikimic Acid*

These compounds undergo Walden inversion in HF at the center carbon of a contiguous *cis–trans* triacyloxy sequence.[55] Inversion always proceeds with deacylation because of formation of the 1,3-dioxolenium cation. The following equilibrium presumably exists in HF. The amount of product which is

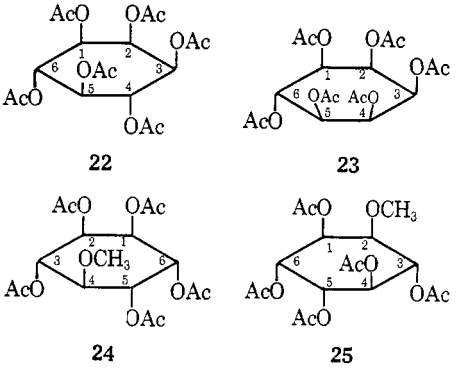


17 **20** **18** **19** **21**

R = CH_3CO or C_6H_5CO
R′ = CH_3 or C_6H_5

inverted at such a sequence varies from 0 to 100% for different compounds and different carbon atoms within a compound. The amount of inversion in the final product will depend both upon the position of the equilibrium **18** ⇆ **19** in HF, and upon the manner in which the equilibrium is disturbed when the hydrolysis products **20** and **21** are formed. In the case of the acetylated cyclitols and 1,5-anhydroglycitols complete deacetylation occurred in HF, yielding stable products. In other compounds, partially acylated products are recovered, and the product distribution may be influenced by the method used to terminate the reaction as well as upon the position of the equilibrium between the various dioxolenium ions in HF, and, possibly upon other mechanisms for deacylation in HF.

Treatment of *myo*-inositol hexaacetate (**22**) in HF for 9 hr at 18° yielded mainly *muco*-inositol, which arose from inversion at both C_1 and C_3 of **22**.[55] C_1 and C_3 are equivalent in **22** and single inversions yielded DL-*chiro*-inositol in small yield. A trace of *myo*-inositol (no inversion) was also found. The same three products arose from the treatment of acylated DL-*chiro*-inositol or *muco*-inositol with HF under similar conditions.[55] This suggests that a common equilibrium between several 1,3-dioxolenium ions arises in HF from all three of these materials.



22 **23** **24** **25**

epi-Inositol hexaacetate (**23**) yields a mixture of *epi*-inositol and *allo*-inositol after HF treatment.[55] The *allo*-inositol arises from inversion at C_1 or C_5 of **23**, these positions being equivalent. 1D-4-*O*-Methyl-*chiro*-inositol pentaacetate (**24**) undergoes inversion at C_2 (1D-3-*O*-methyl-*muco*-inositol, 46%) and at both C_2 and C_6 (1L-4-*O*-methyl-*chiro*-inositol, 11%) but not at C_6 alone.[55] 1L-2-*O*-Methyl-*chiro*-inositol pentaacetate (**25**) inverts at C_6 to give 1L-1-*O*-methyl-*myo*-inositol as the sole product.[55] In this case, *trans*-diaxial attack of the C_1 acyloxy group on C_6 occurs in the preferred conformation, while attack of the C_4 acyloxy group on C_5 requires the less stable conformation. All of these inversions can be understood by assuming the formation of a single or multiple 1,3-dioxolenium cation from *cis*-diacetoxy groupings on each molecule as the first event in HF.

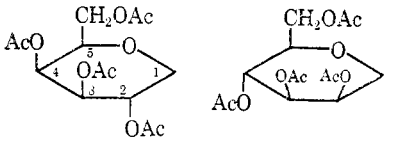
Inversions of cyclitols at the center carbon of a *cis–trans* sequence have also been observed on heating the cyclitols in acetic acid containing *p*-toluenesulfonic acid or other strong acid.[62] The mechanism of this reaction is undoubtedly similar to that of the acylated cyclitols in HF.

The acylated 1,5-anhydroglycitols behave very similarly to the acylated cyclitols. They also undergo Walden inversions in variable yield at the center of a continguous *cis–trans*-triacyloxy sequence. Thus, the tetraacetates of 1,5-anhydro-D-galactitol (**26**) and 1,5-anhydro-D-mannitol (**27**) both underwent partial inversion at C_3 yielding 1,5-anhydro-D-gulitol and 1,5-anhydro-D-altritol, respectively.[57]

1,5-Anhydro-D-glucitol tetraacetate (**28**) has no contiguous *cis–trans* triacetoxy sequence, and no inversion products were found after treatment with HF.[57] Only the arabinitol configuration was recovered from 1,5-anhydro-D-arabinitol triben-

(61) C. B. Anderson, E. C. Friedrich, and S. Winstein, *Tetrahedron Letters*, (1963).

(62) S. J. Angyal, P. A. J. Gorin, and M. E. Pitman, *J. Chem. Soc.*, 1807 (1965).



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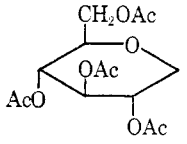

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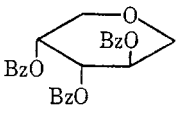
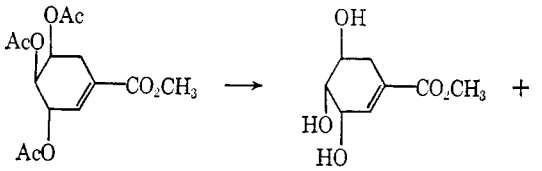


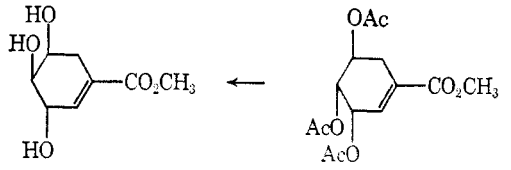
29

zoate (**29**) after HF treatment.[57] Here, inversion at C_3 regenerates the D-arabinitol configuration.

Shikimic acid and 4-epishikimic acid derivatives also undergo Walden inversions,[63] but the mechanism in this case is more complex. Treatment of racemic triacetyl-4-epishikimic acid methyl ester (**30**) with HF for 24 hr at room temperature yielded a mixture of products which, after deacetylation, comprised 80% racemic shikimic acid methyl ester (**31**) and 16% racemic 4-epishikimic acid methyl ester (**32**).[63] Treatment of racemic triacetylshikimic acid methyl ester (33) with HF yielded a mixture of racemic **31** and **32** in the same proportions.[63]

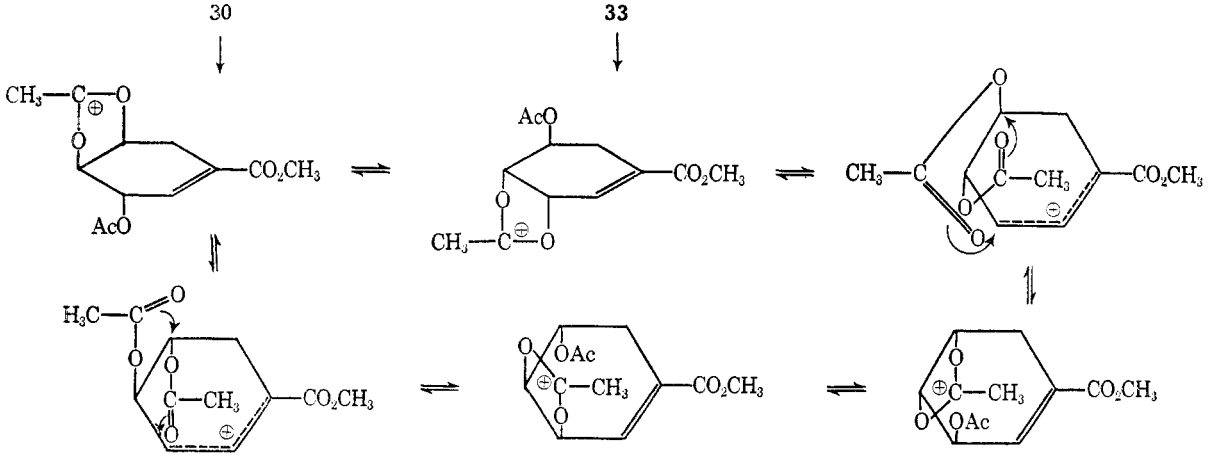


30 31



32 33

formed in each case is therefore not known, and the carbon(s) at which inversion occurs is ambiguous. Table II shows the conversions which acylated galactitol (**34**), D-glucitol (**35**), and D-mannitol (**36**) undergo in HF, and the carbon(s) at which inversion occurs to form each product.[56] The only apparent regularity to the inversions listed in Table II is that in each case only one product arises from a single inversion. The rest require double inversions, or else arise by simple deacylation. DL-Arabinitol pentaacetate (**37**) formed all three pentitols, arabinitol (**38**), ribitol (**39**), and xylitol (**40**) in HF.[56]

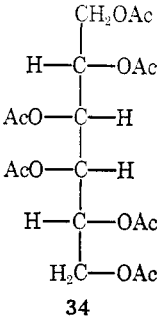


(5)

Quite unexpectedly, treatment of optically active **30** or **33** with HF also gave a mixture of racemic **31** and **32**.[63] This could only occur by inversion of all three acetoxy substituents. Further, no inversion occurred at C_5 alone to yield the *trans–trans* isomer,[63] indicating that inversion at C_3 and C_5 occurred synchronously. It was proposed that the double bond provided resonance stabilization in HF for the equilibrium in eq 5, from which the racemates **31** and **32** would be obtained.[63]

3. *Glycitols*

The flexibility of the acyclic acylated glycitols would be expected to impose no limitation on the formation in HF of 1,3-dioxolenium cations, or on the possibilities for attack by neighboring acyloxy groups. The acylated glycitols in HF do undergo a large number of single and double Walden inversions.[56] The products in this study were identified by paper chromatography and electrophoresis. The optical antipode



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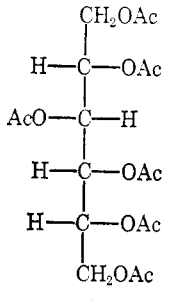

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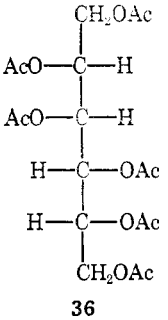


36

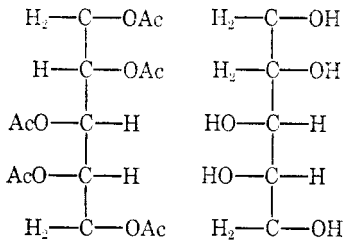
Ring closing to form anhydroglycitols was not observed with any of the fully acylated hexitols or pentitols studied. However, HF treatment of a partially acylated hexitol, 2,3,4,5-tetra-*O*-benzoylgalactitol, generated three different 1,4-anhydroglycitols.[56] This finding is consistent with the suggestion (section IV.B.4)[58] that certain Walden inversions in 1,4-anhydroglycitols occur with ring opening and subsequent reclosing.

(63) R. Grewe and S. Kersten, *Chem. Ber.*, **100**, 2546 (1967).

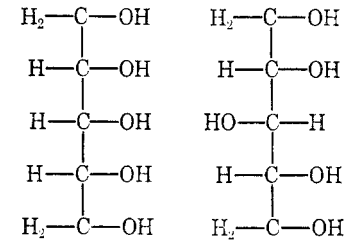
Table II

Transformation of Hexitol Hexaacetates[57]

Starting material, hexaacetate of	*Hexitols detected and inversion(s) required*			
	Galactitol	*Glucitol*	*Iditol*	*Mannitol*
Galactitol (**34**)	+ No inversion	+ C_3 or C_4	+ C_4 and C_5 or C_2 and C_3	+ C_3 and C_5 or C_2 and C_4
D-Glucitol (**35**)	–	+ No inversion or $C_2 + C_5$ or $C_3 + C_4$	+ C_5	–
D-Mannitol (**36**)	+ $C_3 + C_5$ or $C_2 + C_4$	+ C_2 or C_5	+ $C_3 + C_4$ or $C_2 + C_5$	+ No inversion



37 38

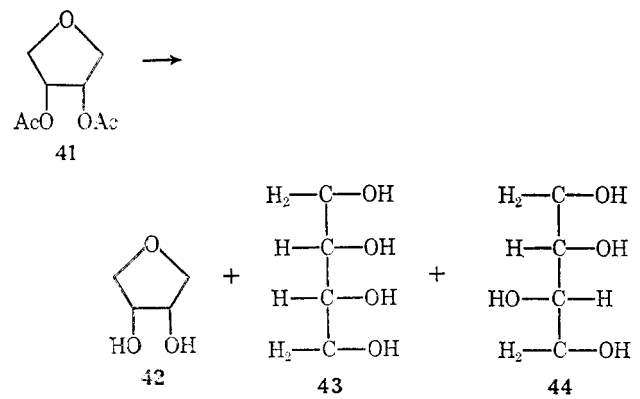


39 40

4. *1,4-Anhydroglycitols*

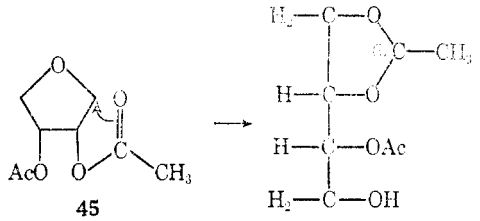
These compounds undergo both Walden inversions and ring opening to form glycitols when treated with HF.[58] As with the compounds already discussed, the reactions are accompanied by partial or complete deacylation.

In the simplest case, 1,4-anhydroerythritol diacetate (**41**) after 24 hr in HF at 18° yielded 1,4-anhydroerythritol (**42**) and a comparable amount of a mixture of erythritol (**43**) and threitol (**44**).[58]

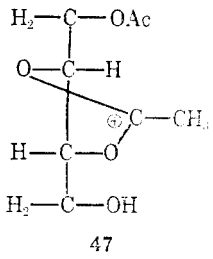


41 42 43 44

To rationalize these products, two competing reactions were postulated.[58] The formation of the 1,3-dioxolenium ion between the *cis*-acetoxy groups would preserve the erythritol configuration, stabilize the ring, and yield **42**. Ring opening and inversion, on the other hand, require attack by an acetoxy group on a methylene carbon (**45**). The resulting 1,3-dioxolenium ion (**46**) could then undergo inversion to form **47**. On hydrolysis **46** and **47** form partially acylated derivatives of erythritol (**43**) and threitol (**44**), respectively.

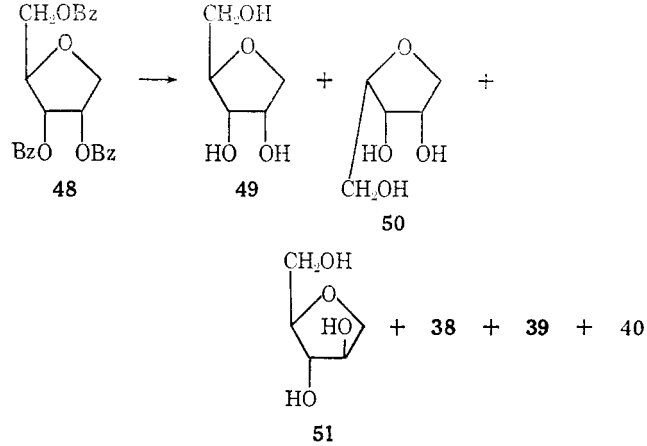


45 46



47

Identical treatment of the next higher analog, 1,4-anhydro-D-ribitol tribenzoate (**48**), yielded a more complex mixture of products.[58] The free pentitols DL-arabinitol (**38**), ribitol (**39**), and xylitol (**40**) accounted for about two-thirds of the product. The remaining product consisted of 1,4-anhydroribitol (**49**), 1,4-anhydrolyxitol (**50**), and a third component tentatively identified as 1,4-anhydroarabinitol (**51**).[58]



48 49 50 51 + 38 + 39 + 40

Table III

Walden Inversion(s) and Ring Contractions of Acylated Saccharides in HF

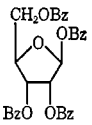
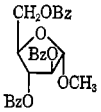
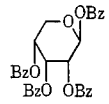
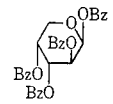
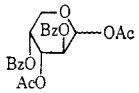
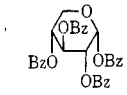
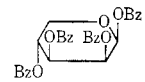
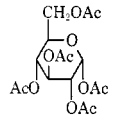
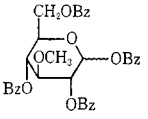
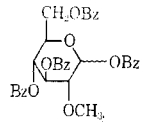
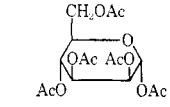
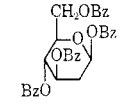
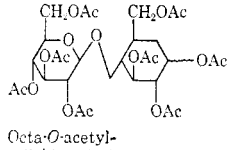
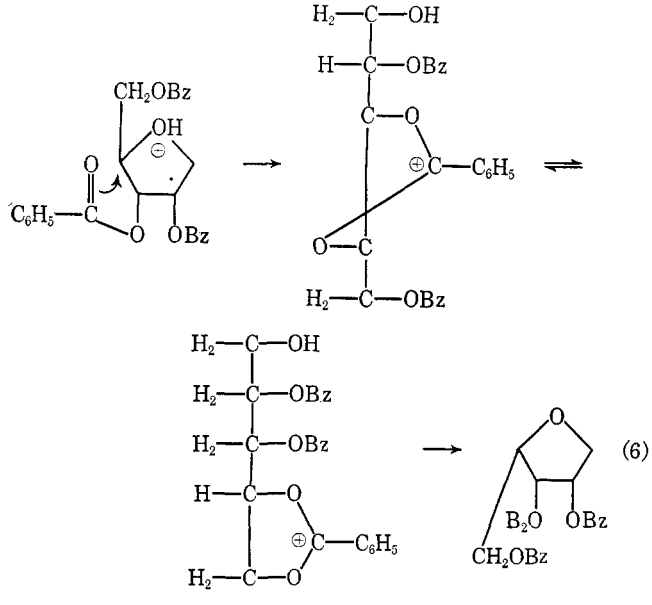
Starting material	*Procedure*[a]	*Inversion(s)*	*Ring contraction*	*Products*	*Ref*
 Tetra-*O*-benzoyl-β-D-ribofuranose (**55**)	6	None	−	Di- and tri-*O*-benzoyl-β-D-ribofuranosyl fluorides (65%)	68
 Tri-*O*-benzoyl-α-methyl-D-ribofuranoside (**56**)	2	C_2	−	Di-*O*-benzoylribofuranosyl fluorides (52%)	68
	6	None	+	Di- and tri-*O*-benzoylribofuranosyl fluorides (52%)	69
 Tetra-*O*-benzoyl-β-D-ribopyranose (**57**)	2	None	+	Di- and tri-*O*-benzoylribofuranosyl fluorides	66, 69
		None	−	Di-*O*-benzoylribopyranosyl fluoride	
	6	C_2	+	Di-*O*-benzoylribofuranosyl fluorides (49%)	69
 Tetra-*O*-benzoyl-β-D-arabinopyranose (**58**)	7	C_2	−	Di-*O*-benzoylribopyranosyl fluorides (60%)	69, *b*
		C_2	+	Di-*O*-benzoylribofuranosyl fluoride (5%)	
		None	−	Tri-*O*-benzoylarabinopyranosyl fluoride (7%)	
58					
 1,3-Di-*O*-acetyl-2,4-di-*O*-benzoyl-D-arabinopyranose (**59**)	8	None	−	2,4-Di-*O*-benzoyl-D-arabinopyranose (30%)	69
 Tetra-*O*-benzoyl-β-D-xylopranose (**60**)	1	$C_2 + C_3$	−	D-Arabinopyranose derivatives (56%)	59
	2	C_2	−	D-Lyxopyranose derivatives (14.5%)	59
		$C_2 + C_3$	−	D-Arabinopyranose derivatives (trace)	
 Tetra-*O*-benzoyl-α-D-lyxopyranose (**61**)	1, 2	C_3	−	2,4-Di-*O*-benzoyl-D-arabinopyranose (~15%)	*c*
		None	−	Tri-*O*-benzoyllyxopyranosyl fluoride (5–15%)	
		None	+	Di-*O*-benzoyllyxofuranosyl fluoride (10%)	
 Penta-*O*-acetyl-β-D-glucopyranose (**62**)	2	C_2	−	Mannopyranose derivatives (28%)	*d*
		$C_2 + C_3$	−	Altropyranose derivatives (11%)	
	5	None	−	Tetra-*O*-acetyl-α-D-glucopyranosyl fluoride (57%)	
 Tetra-*O*-benzoyl-3-*O*-methyl-α (or β-) D-glucopyranose (**63**)	1, 2	None	−	Tri-*O*-benzoyl-3-*O*-methyl-D-glucopyranosyl fluorides (40–45%)	*e*

Table III (Continued)

Starting material	*Procedure*[a]	*Inversion(s)*	*Ring contraction*	*Products*	*Ref*
 Tetra-*O*-benzoyl-2-*O*-methyl-α-(or-β-)D-glucopyranose (64)	1	None	–	Tri-*O*-benzoyl-2-*O*-methyl-D-glucopyranosyl fluorides (42%)	*f*
		None	+	Di-*O*-benzoyl-2-*O*-methyl-D-glucofuranosyl fluorides (23%)	
		None	–	Mannopyranose derivatives (30%)	70
 Penta-*O*-acetyl-α-D-mannopyranose (65)	1, 2	C_3	–	Altropyranose derivatives (~10%)	
		$C_3 + C_4$	–	D-Idopyranose derivatives (1.5%)	
		C_2	–	Glucopyranose derivatives (T)	
 Tetra-*O*-benzoyl-β-D-*arabino*-hexopyranose (66)	4	C_3	–	3,6-Di-*O*-benzoyl-2-deoxy-α-D-*ribo*-hexopyranosyl fluoride (62%)	67
		None	–	Tri-*O*-benzoyl-2-deoxy-α-D-*arabino*-hexopyranosyl fluoride (12%)	
 Octa-*O*-acetyl-cellobiose (67)	3	C_2 of (reducing unit)	–	Hexa-*O*-acetyl-4-gluco-pyranoside-mannopy-ranosyl fluoride (~20%)	*g*

[a] (1) 20 hr in HF, room temp; reaction terminated by pouring into aqueous $NaHCO_3$; (2) 20 hr in HF, room temp; reaction terminated by removing HF with dry air; (3) 5–6 hr in HF, room temp; (4) 1 hr in HF, −17°, terminated by addition of $CHCl_3$, extraction with aqueous $NaHCO_3$; (5) 50% acetic anhydride in HF, 20 hr, room temp; (6) 6–8 hr in HF, room temp; (7) 20 hr in HF, 5°; (8) 36 hr in HF, 0°. T = trace. [b] C. Pedersen and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **82**, 945 (1960). [c] C. Pedersen, *Acta Chem. Scand.*, **18**, 60 (1964). [d] C. Pedersen, *ibid.*, **16**, 1831 (1962). [e] I. Lundt, C. Pedersen, and B. Tronier, *ibid.*, **18**, 1917 (1964). [f] C. Pedersen, *ibid.*, **20**, 963 (1966). [g] D. H. Brauns, *J. Am. Chem. Soc.*, **48**, 2776 (1926).

The formation of the free pentitols **38**, **39**, and **40** and of 1,4-anhydroribitol (**49**) can be rationalized in the same way as suggested for **41**. The formation of **50** requires inversion at C_4, and the sequence of eq 6 was suggested.[58] The finding

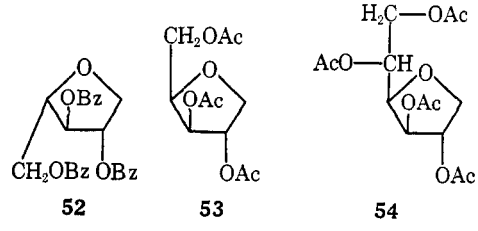


(6)

(section IV.B.3) that 2,3,4,5-tetra-*O*-benzoylgalactitol formed three different 1,4-anhydroglycitols in HF[56] is consistent with this mechanism.

The formation of the tentatively identified 1,4-anhydro-arabinitol (**51**) was visualized[58] as occurring by a transannular attack of the C_5 benzoyloxy group on C_2.

A similar picture was obtained with 1,4-anhydro-L-arabinitol tribenzoate (**52**). Again, after HF treatment all three pentitols, **38**, **39**, and **40**, were obtained, although in much lower yield than from **48**. Most of the product consisted of a mixture of three 1,4-anhydroglycitols arising from deacylation, from inversion at C_2, and from inversion at C_4. Again, these products were rationalized by mechanisms involving ring opening and subsequent reclosing.[58]



52 **53** **54**

In marked contrast, however, was 1,4-anhydro-D-xylitol triacetate (**53**). Treatment with HF under conditions identical with those used above afforded a single product, 1,4-anhydro-

D-ribitol (**49**), which was isolated in 86% yield.[58] In this case reaction in HF is limited to inversion at C_3. The first step was visualized[58] as the formation of an eight-membered ring bridging C_3 and C_5, followed by inversion arising from attack by C_2 on C_3.

Treatment of 1,4-anhydro-D-glucitol tetraacetate (**54**) yielded mainly 1,4-anhydroglucitol, arising by deacylation, 1,4-anhydromannitol, requiring inversion at C_2 or C_5, and a third component which was not identified. Less than 10% of the product was isolated as hexitols. Galactitol, glucitol, iditol, and mannitol were present in the mixture.[58] These are the hexitols found (Table II) after HF treatment of the acetates of galactitol (**34**) and D-mannitol (**36**) but not of D-glucitol (**35**).[56] Also noteworthy is the fact that compounds in which the C_3 and C_4 substituents are *cis* (**53** and **54**) yielded little or no open-chain product. The major path of ring opening thus appears to be attack of the C_3 substituent on C_4, rather than the C_2 substituent on C_1.

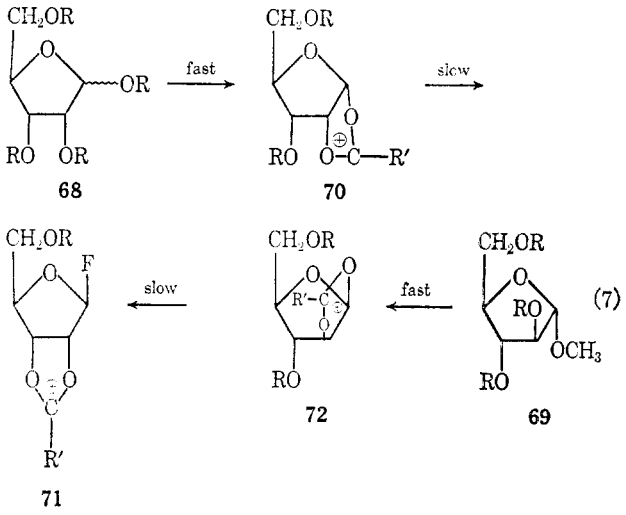
5. *Monosaccharides*

Most acylated mono- and disaccharides yield the corresponding acylated glycosyl fluorides after a few minutes in HF at −15°.[64] Much milder conditions (*e.g.*, 5 min in liquid HF at −70°,[65] or HF solutions in ether[66] or benzene[67]) have been successfully used when Walden inversion occurs very readily or when the desired fluoride is unstable. A review on the preparation and properties of glycosyl fluorides has covered this literature through 1960.[64] In addition, glycosyl fluorides have been prepared from virtually all the reactants and products listed in Table III.

On prolonged treatment in HF, acylated monosaccharides may undergo a variety of single and multiple Walden inversions, and certain glycopyranose rings undergo contraction to the corresponding glycofuranose. These reactions are summarized in Table III. When the glycosyl fluorides obtained from the starting materials were dissolved in HF, they yielded products identical with those shown in Table III.

Very recently, nmr observations have begun to elucidate the considerable diversity of reaction mechanisms which must operate on monosaccharides in HF to generate the products found (Table III). Acylated ribofuranose (**68**) and arabinofuranose, (**69**) underwent in HF the transformation shown in eq 7.[68] Thus, both **68** and **69** dissolve in HF with the very rapid formation of the 1,3-dioxolenium ion generated by attack of the C_2 substituent on C_1. The corresponding glycosyl fluorides were obtained from **70** and **72** on working up the reaction mixture, but were not observed by nmr in HF.[68] These glycosyl fluorides apparently cannot exist in HF, since tri-*O*-benzoyl-β-D-ribofuranosyl fluoride formed the benzoyl analog of **70** when dissolved in HF.[68]

The conversion of the acetyl analog of **68** and **69** to **71** is complete in 24 hr in HF, while the benzoylated analogs of **68** and **69** (**55** and **56**, respectively) require *ca.* 100 hr for this conversion.[68] 1,3,5-Tri-*O*-benzoyl-β-D-ribofuranose and 1,3,5-tri-*O*-benzoyl-β-D-arabinofuranose are both converted to **71** in HF much more rapidly than the corresponding tetra-*O*-benzoyl derivatives.[68] In this case the benzoxonium ions **70** and **72** cannot form, and the glycosyl fluoride is apparently formed in HF.

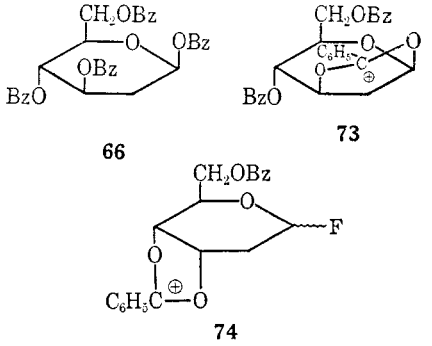


$R' = CH_3$ or C_6H_5

$R = CH_3CO$ or C_6H_5CO

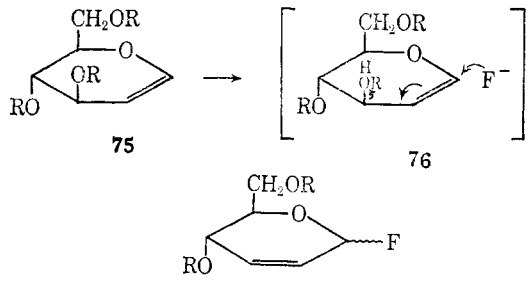
The tetraacetates of ribopyranose and arabinopyranose (the acetyl analogs of **57** and **58**, Table III) dissolve in HF with the rapid formation of the corresponding β-fluorides.[69] On the other hand, the tetrabenzoates of these sugars (**57** and **58**), and also tri-*O*-benzoyl-β-D-ribopyranosyl fluoride, dissolve in HF to form the dioxolenium ion between C_1 and C_2.[69] All four of these sugars eventually form **71** in HF,[69] although the precise sequence of events could not be followed by nmr.

None of the intermediates so far identified in HF adequately accounts for the inversion of **66** (Table III)[67] or for the small amounts of glucose and idose derivatives obtained from **65**.[70] It seems most likely that **66** forms a six-membered dioxolenium ion (**73**) (perhaps preceded by formation of the glycosyl fluoride), inversion then occurring by attack of the C_4 substituent on C_3.[67] Nmr studies of this reaction using the acetyl analog of **66** showed[67] the presence of the acetyl analog of **74** in HF, but precursors of this species could not be identified.



The stability of **59** in HF in contrast to the transformations undergone by the fully benzoylated derivative **58** is noteworthy (Table III).[69] From **59** the C_1–C_2, C_3–C_4 dibenzoxonium ion has been identified in HF by its nmr spectrum. This species

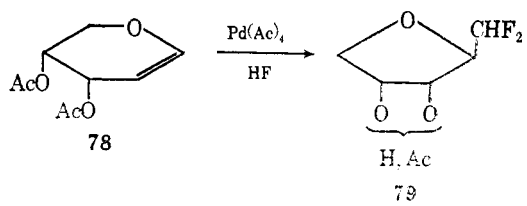
(64) F. Micheel and A. Klemer, *Advan. Carbohydrate Chem.*, **16**, 85 (1961).
(65) L. D. Hall and J. F. Manville, *Can. J. Chem.*, **45**, 1299 (1967).
(66) C. Pedersen and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **82**, 941 (1960).
(67) I. Lundt and C. Petersen, *Acta Chem. Scand.*, **21**, 1239 (1967).
(68) N. Gregersen and C. Pedersen, *ibid.*, **22**, 1307 (1968).
(69) C. Pedersen, *ibid.*, **22**, 1888 (1968).
(70) C. Pedersen, *ibid.*, **17**, 673 (1963).



75 76 77

R = CH_3CO or C_6H_5CO

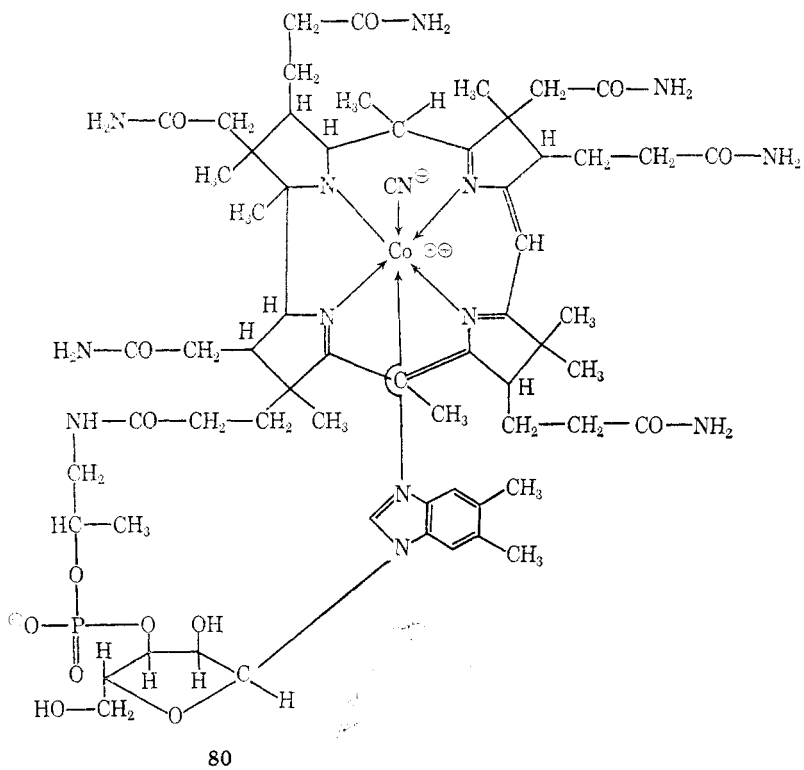
was not found upon comparable treatment of **58** in HF,[69] and provided the first indication that it may be possible to control the nature as well as the rate of transformations of acylated polyols and saccharides in HF by varying the nature of the acyl substituents.



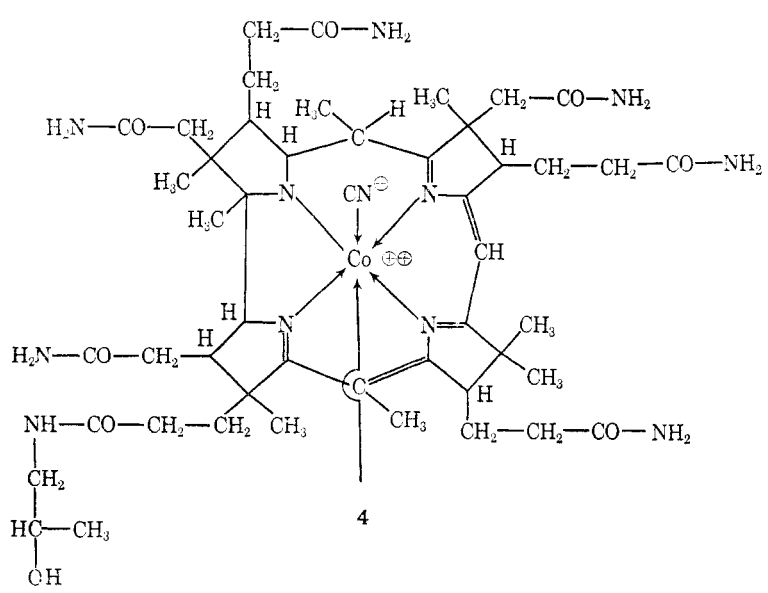
78 79

6. *Glycals*

An unusual reaction occurs when tri-*O*-acetyl- or tri-*O*-benzoyl-D-glucal (**75**) reacts with HF under very mild conditions (0.5 *N* HF in benzene, 0°, 30 min,).[71] Contrary to the



80



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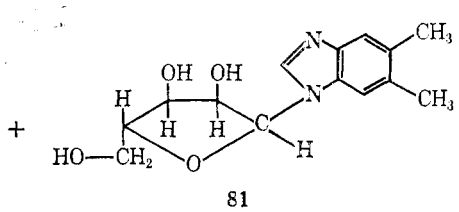

81

expectation, based on reaction of **75** with other hydrogen halides,[72,73] HF does not add to **75** to give an acylated 2-deoxyglucopyranosyl fluoride. Rather, **75** undergoes an allylic shift, presumably through a protonated intermediate **76** to yield a 4,6-di-*O*-acyl-2,3-didehydro-2,3-dideoxy-D-erythrosyl fluoride (**77**), an unstable compound which was identified by its nmr spectrum and by the preparation of several derivatives.[71]

The reaction of arabinal diacetate (**78**) with HF and lead tetraacetate in methylene chloride at −70° (40 min) leads to the formation of the monoacetate of 2,5-anhydro-1-deoxy-1,1-difluoro-D-ribitol (**79**).[74]

C. NUCLEOTIDES, NUCLEOSIDES, AND NUCLEIC ACIDS

Purine and pyrimidine bases are readily soluble in HF. Solutions of up to 12.5% of adenine, guanine, uric acid, thymine, uracil, 2-thiouracil, 2-benzylthiouracil, and 2-benzylmethylthiouracil have been prepared.[75] In each case the base was recovered unaltered. DNA and RNA have been shown to dissolve in HF,[11,17] but the products were not characterized; certainly, extensive cleavage of the molecule occurred (see below).

A study of the behavior of polyphosphoric acid esters in liquid HF showed that the phosphorus atoms are split off in rapid succession from one end of the polyphosphate chain to the other.[76] A fluorine atom becomes attached in each step to the phosphorus released from the chain. Both neutral fluorophosphoric acid estrs, $(RO)_2$–PO–F, and acidic half esters (RO)(HO)–PO–F, have been prepared in this way.[76]

Published studies of the action of liquid HF on mononucleotides are limited. At room temperature, free adenine and ribose are formed after brief exposure of adenosine-3′,5′-phosphoric acid in HF.[77] Treatment of cyanacobalamine (**80**) with 25% methanol in HF results in cleavage at both phosphoester bonds to yield cobinamide (**4**) (which undergoes further reaction, section III.B) and the nucleoside **81** which is stable in methanolic HF.[25] Preliminary experiments showed that cytidylic and uridylic acids were converted to the corresponding nucleosides upon brief treatment in HF at low temperature.[78] The free bases cytosine and uracil were recovered nearly quantitatively after several hours at room temperature. By contrast, brief treatment of thymidylic acid with cold HF led to the formation of thymine, with no measurable formation of thymidine.[78]

The cleavage of both purine and pyrimidine nucleotides to nucleosides has also been observed in 60% hydrofluoric acid.[79] This moderately strong acid ($H_0 = -6.3$) may in some cases have properties in common with liquid HF. In this case, cleavage of nucleotides to nucleosides resulted in the formation of fluorophosphate, which is the species formed in liquid HF, rather than orthophosphate which forms upon hydrolysis. Adenylic, guanylic, cytidylic, and uridylic acids all form the corresponding nucleosides in high yield in 60% hydrofluoric acid. After 1 hr at room temperature the purine bases are quantitatively released from the corresponding nucleosides.[79] The pyrimidine nucleosides are stable to prolonged treatment in this medium. Base analysis of both RNA and DNA have been carried out after degradation in 60% hydrofluoric acid. The results agreed well with those obtained by other methods. Greater convenience and lack of deamination was claimed for the RNA analysis.[79]

Acknowledgments. The author is grateful to Drs. Paul M. Gallop, Christian Pedersen, and Shumpei Sakakibara for helpful criticisms and suggestions. The author is also indebted to Dr. Pedersen for making manuscripts available before publication.

(71) I. Lundt and C. Pedersen, *Acta Chem. Scand.*, **20**, 1369 (1966).
(72) J. Davoll and B. Lythgoe, *J. Chem. Soc.*, 2526 (1949).
(73) J. J. K. Novak and F. Sorm, *Collection Czech. Chem. Commun.*, **27**, 902 (1962).
(74) P. W. Kent and J. E. G. Barnett, *Tetrahedron, Suppl.*, **7**, 69 (1966).
(75) H. A. Rutter, Jr., *J. Franklin Inst.*, **259**, 65 (1955); *Chem. Abstr.*, **49**, 5554*i* (1955).
(76) A. Hood and W. Lange, *J. Am. Chem. Soc.*, **72**, 4956 (1950).
(77) D. Lipkin, W. H. Cook, and R. Markham *ibid.*, **81**, 6198 (1959).
(78) B. E. Phillips, unpublished; quoted in ref 79.
(79) J. W. Abrell, Ph.D. Thesis, Washington University, 1965; *Dissertation Abstr.*, **26**, 4201 (1966).