REACTIONS OF METHYL 2,3-ANHYDRO-RIBOSIDES WITH HYDROGEN FLUORIDE

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By

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Moissan, who discovered elemental fluorine in 1886, also investigated its reactions with many organic compounds and was the first to test the pharmacological activity of a fluoro compound. He found that fluoroethane, in contrast to ethyl chloride, ethyl bromide, and ethane, is very toxic. (1) In low concentrations in air it produces acceleration of breathing rate in guinea pigs. At increasingly higher concentrations the animal becomes excited; the breath comes spasmodically; there are convulsive jerks; the rear extremities become lame; and, at a concentration of six to seven percent, death results.

The value of fluoride ion in the construction of tooth enamel has elicited much controversy in the past decade and is part of the increasing interest and awareness of the anomalous behavior of fluorine, fluorides, and fluoro-compounds. The chemistry of this element has enjoyed a logarithmic exploitation as notable as any other.

In the field of chemotherapy, interest in pseudometabolites has brought attention to fluoro-compounds, among others. Closely related to the object of this thesis is the pseudometabolite 2-fluoroadenosine, prepared by the Schiemann reaction (2) by Montgomery and Hewson. (3) 2-Fluoroadenosine in a concentration of 10^{-8} g. per ml. inhibited human epidermoid carcinoma; azaserine and 6-diazo-5-oxo-L-norleucine inhibit this tissue at 10^{-7} g. per ml.

6-Methyl purine has shown marked effects on neoplastic growth. (4,5) In prevention of mitosis in both sarcoma 180 and normal skin cells in tissue culture, it is effective at a concentration of 0.05 umole per milliliter, but 6-trifluoromethyl purine shows no toxicity at five hundred times this concentration. (6)

The antitumor activity of purine itself and of 6-chloropurine (11,6) aroused interest in 6-fluoropurine, but attempts to prepare this compound by several routes were without success. This was interpreted as due to the instability of the compound because similar difficulty was encountered by Roe and Hawkins in the unsuccessful Schiemann synthesis of 4-fluoropyridine, although 2- and 3-fluoropyridine were preparable. (12)

Many other purine and pyrimidine derivatives have been prepared and tested for antitumor activity. Among those that have had some success are 6-mercaptopurine, 5-fluorouracil, 5-bis(2-chloroethyl)-aminouracil, and 8-azaguanine.

6-Mercaptopurine is thought to be a competitive inhibitor between the conversion of inosinic acid to adenine or guanine polynucleotides. (7) It has given temporary relief from the symptoms of leukemia. (8)

5-Fluorouracil is a potent uracil competitor which is not greatly differentially toxic to tumor and normal tissue.

8-Azaguanine has been shown to enter the ribonucleic acid of cells. Among the azapurine analogues, it has been

more interesting than the adenine, hypoxanthine, and xanthine analogues because of its inhibition of a variety of living systems. R. E. F. Matthews gives a broad review of the literature. (9) Incorporation seems to be into shorter polynucleotides according to Matthews' work and that of others in the discussion following the paper. An interesting aspect is the work of Creaser (13) which showed that the enzyme adaptation of Staphylococcus aureus toward lactose, which necessitates the production of β-galactosidase, was prevented in whole growing cells at relatively low concentrations of 8-azaguanine.

5-Bis(2-chloroethyl)-aminouracil is a nitrogen mustard derivative prepared by Lyttle and Petering. (14) It is not a uracil competitor; the uracil moiety serves as a carrier for the nitrogen mustard which acts as an alkylating agent. The nitrogen mustards have been used for some time in leukemia therapy with temporary remission of symptoms. The new compound has produced complete repression of experimental growing tumors of several types in rats and is being tested in human patients at present. The ratio of its effective dose to toxicity is favorable.

The compound 9(β-D-xylofuranosyl)adenine gives further hope for the rationale behind this thesis. This compound has been shown to be highly toxic by Biesele at Sloan-Kettering Institute. In as much as it has been strongly indicated that the nucleic acids are polymers of ribose or

of 2-deoxyribose linked by phosphate ester bridges between the 3- and 5-hydroxyls, (16,17) the l-configuration on the 3-carbon of xylose in xylofuranosyladenine may mean that a stable termination of a nucleic acid is produced which, because of the orientation of the 3-hydroxyl, is incapable of further extension and completion of the normal nucleic acid. Arabinofuranosyladenine is not toxic. In this case the 3-hydroxyl has the same configuration as in the natural ribosyl- or deoxyribosyladenine.

Work with puromycin, 6-dimethylamino-9-(3'-p-methoxy-L-phenylanylamino-3'-deoxy-D-ribofuranosyl)purine, a compound produced by Streptomyces alboniger, has shown it to have a broad-spectrum activity against bacteria in vitro (18) and against a number of protozoa in vivo. (10) Puromycin also displays activity against higher animals and against mammary adenocarcinoma in the C_3H strain of mice. Although it has undesirable side effects, these are remitted on discontinuance of administration and are not sufficiently severe to prevent its use in combating Trypanosoma gambiense (19) and Endamoeba hystolytica (20) in man.

A number of structural modifications of puromycin have been made. Hutchings (10) states that the structural requirements for activity vary with the organisms studied. "The intact molecule is required for the antibacterial activity in vitro, antiamoebic activity in vivo, and anthelmintic activity. The aminonucleoside is more effective on

a molar basis than the parent compound when tested against trypanosomes in vivo and the mammary adenocarcinoma of the C_3H mouse." Because in several studies (21,22) purines have reversed the action of puromycin, it is suspected that it may interfere with purine metabolism. With respect to substituents on the purine ring, a 6-dipropylaminonucleoside was eight times more effective than the nucleoside and thirty-two times more effective than puromycin against Trypanosoma equiperdum in the mouse. (10a)

Again the 3' position of the nucleoside is indicated as a significant point in the molecule. B. R. Baker and his group at Stanford Research Institute are concentrating on syntheses of pentofuranoses of potential interest deriving from his work with puromycin. The methyl 3-deoxy-3-amino-xylo-furanoside has been synthesized (23) by ammonolysis of methyl 2,3-anhydro-D-ribofuranoside. The 3'-deoxy-3'-mercaptoribonucleosides are now holding their attention. (24)

One might interject comments on the surprising activity of various fluorosteroids at this point. Adrenal cortical hormones, e.g. cortisone, have found use in the treatment of allergies, the prevention of scarring, treatment of rheumatoid arthritis, and the treatment of shock. Synthetic methods for the preparation of cortisone provided routes and material for various derivatives of the parent compound. 9-α-Fluorohydrocortisone has elicited considerable interest since its glucorticoid activity, i.e., the extent of deposition

of liver glycogen from metabolized protein, is about ten times that of hydrocortisone, the lithium aluminum hydride reduction product of cortisone which has the other two keto groups protected. 9-α-Fluorohydrocortisone was prepared (25) by opening a 9β, 11β- epoxide with hydrogen fluoride. It was found that the activity of the compound was inversely proportional to the size of the substituted halogen. 9-α-Fluorohydrocortisone had undesirable side effects which included the production of edema. Introduction of a 16α- or -β-methyl has overcome some of the side effects of the parent fluorosteroid. 16α-Methyl-9α-fluoroprednisolone 21-acetate, prepared by the Shering group, has enhanced glucocorticoid activity and anti-inflammatory activity, with complete avoidance of salt and water retaining characteristics found in the parent 9α-fluoroprednisolone. (26)

Ringold and Bowers (27) have used Henbest and Wrigley's synthesis (28) to open a 5,6-epoxide by reaction with boron trifluoride-ether complex in a benzene medium to produce 6-fluorosteroids. They report that 6α-fluorocortisone acetate, 6α-fluorohydrocortisone acetate, 6α-fluoroprednisolone acetate, and 6α-fluoroprednisone acetate had respective anti-inflammatory and thymolytic activities of (10, 6), (10, 8), (20, 62), and (20, 23) times that of hydrocortisone acetate. In addition the latter three promoted sodium excretion in non-salt-loaded adrenalectomized rats.

Pattison and his students at the University of Western Ontario have written many papers in their series on toxic fluoro-compounds. The papers mentioned here give reference to their work.

ω-Fluoroaklyl nitrogen compounds have been prepared to lead to new barbiturates, in this case, 5-ethyl-5-(4'-fluorobutyl)barbiturate and 5-ethyl-5(5'-fluoroamyl)barbiturate. (29) Their activity as central nervous system depressants was lower than that of neonal and amytal, and their toxicity was low as, according to similar data from Bruce and Huber, (30) could be expected.

ω-Fluoro fatty acids ahd ketones show higher toxicity for members having an even number of carbons such as ethyl 8-fluoro-3-oxo-octanoate and ethyl 12-fluoro-3-oxo-dodecanoate than for members having an odd number of carbons such as ethyl 9-fluoro-3-oxo-nonoate. (31) This is in accord with the beta-oxidation theory of fatty acid metabolism, which would yield fluoroacetic acid as the end product of the acids with an even number of carbons.

Even chain 1-fluoroalkanes, -alkenes, and -alkynes are very toxic to mice. Ethyl 3-fluoro-lactate, discussed in the same paper, was non toxic. (32)

18-Fluoro-10-methyloctadecanoic acid, 18-fluoro-tuberculostearic acid, was prepared to test it as an anti-tubercular compound. It showed complete inhibition of Mycobacterium tuberculosis var. hominis at a concentration of

1.25 gamma per ml. *in vitro* in the absence of bovine serum, but was inactive in the presence of ten percent bovine serum at a concentration of 20 gamma per ml. In mice it had no effect on tuberculosis at a diet concentration of 25 mg. per kg. per day. Intraperitoneally, the lethal dose for fifty percent of mice was 2.7 mg. per kg. (33)

The activity of fluoro-compounds in biological systems and the possible strong effect of a 2- or 3-substituted pentose on nucleic acid metabolism prompted this thesis.

CHEMICAL BACKGROUND

2-Deoxy-2-fluoro-D,L-glyceraldehyde has been prepared by Taylor and Kent. (34). Diethyl fluoro-oxalacetate was reduced to 2-deoxy-2-fluorotetritols with potassium borohydride and lithium aluminum hydride, then oxidized with sodium periodate to the fluoroglyceraldehyde. The fluoro atom is removed by reaction with dinitrophenylhydrazine in 2 N hydrochloric acid.

Taylor and Kent have also prepared 6-deoxy-6-fluoro-D-galactose and 5-deoxy-5-fluoro-D-ribose by reaction of protected 6- or 5-mesyl derivatives with anhydrous potassium fluoride in ethylene glycol at reflux. (35)

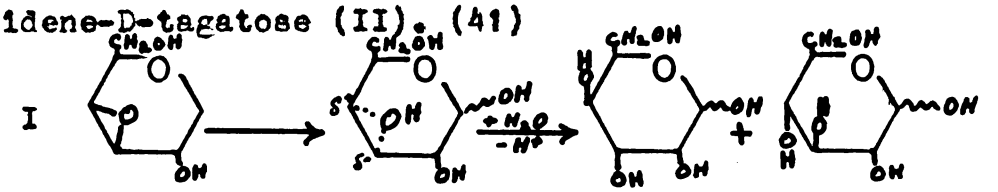
6-Fluoro-6-deoxy-D-glucose has been prepared by Helferich and Gnüchtel (36) and the compound has been used by Blakeley and Boyer (37) in a study of its effect on yeast fermentation and on hexokinase. Its competitive inhibition of glucose and fructose was greater in intact cells than in cell free extracts and in its competitive effect on hexokinase. Its toxicity toward rats was moderate and it did not affect virus synthesis or behavior in tissue culture. Its effects on glycolysis were not as marked as found by McDonald's co-workers, Cramer and Woodward, (38) in their studies of the effects of 2-deoxy-D-glucose on the rate of yeast glycolysis.

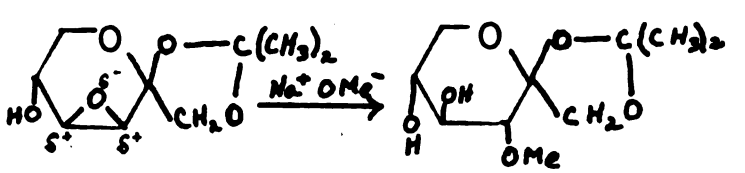
Micheel and his students at the University of Münster have synthesized a number of fluorosugars with the fluoro

substituent on the glycosidic carbon or on the primary carbon. Michael states that "of the halosugars with free hydroxyl groups which carry the halogen on the glycosidic carbon atom, only those of fluorine are stable." (39)

Other than the 2-fluoro-D,L-glycerose of Taylor and Kent (34) no other sugars are recorded in the literature which have fluorine on a secondary carbon.

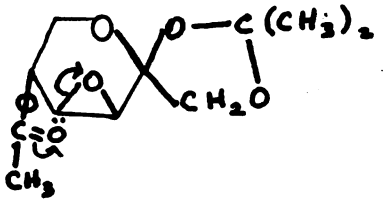
The route to the synthesis of a 2- or 3-substituted fluorosugar was selected to use methyl 2,3-anhydro-D-ribofuranoside as the terminal intermediate. This intermediate should allow the preparation of methyl 3-deoxy-3-fluoro-D-xylo-furanoside or methyl 2-deoxy-2-fluoro-D-arabino-furanoside. The production of these as the result of scission of the 2,3-epoxide ring is illustrated by the acid fission of methyl 3,4-anhydro- -D-galactoside (I) (40) and the alkaline fission of 3,4-anhydro-1,2-O-isopropylidene-D-tagatose (II). (41)





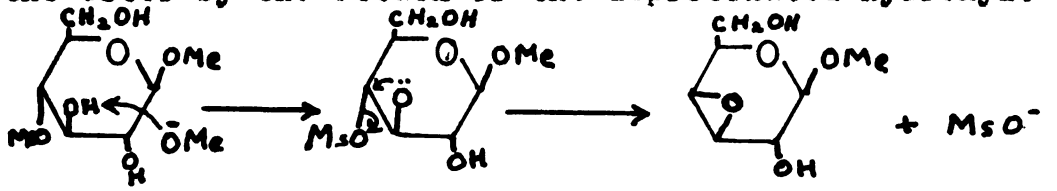
Interestingly the compound II reacts with base to give principally the sorbose derivative, but with acetic acid, acetic anhydride, and a little pyridine the product is 3,4,5-triacetyl-1,2-O-isopropylidene-D-fructose. This might

result from an orthoester type of interaction between an acetylated C-5 and C-4. (42,43)



Peat (44) has given a thorough review of the chemistry of the anhydro sugars through 1946. Goodman, Benitez, and Baker have studied epoxypentene as a model compound for syntheses involving reactions compatible with 2',3'-anhydro-ribo-nucleosides. (45)

The formation of anhydro sugars results from the attack of a base on the proton of a hydroxyl situated trans to a cationogen such as a sulfonic ester. This is displaced in a cission between the carbon of the sugar and the oxygen of the ester by the attack of the deprotonated hydroxyl. (46)



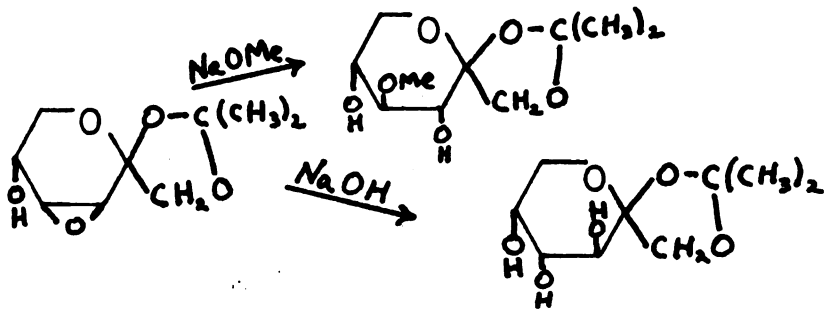
(47)

Anhydro sugars of several types can be formed: the ethylene oxide type illustrated above, the propylene oxide, hydrofuranol, and five member rings containing two oxygens. (44)

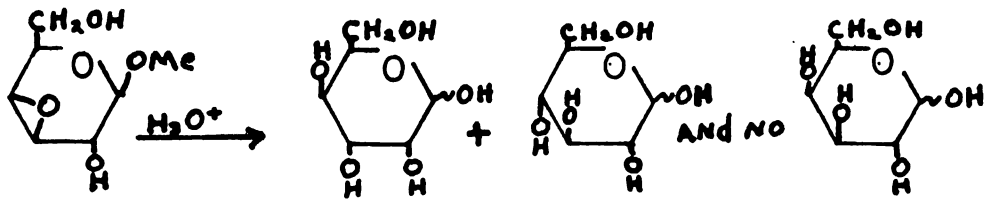
Anhydro rings can be cleaved by either acids or bases,

including those of the Lewis definition. Four cases will be illustrated.

With sodium methoxide, 1,2-O-isopropylidene-3,4-anhydro-D-psicose is converted principally to 1,2-O-isopropylidene-4-O-methyl-D-sorbose, whereas with sodium hydroxide 1,2-O-isopropylidene-D-fructose was detected in the mixture of products. (48) Stereochemical consideration of the pyranose ring would predict the reaction with a large base to result in inversion on C-4, since an entering group of any size could be alternately equatorial with the other substituents in a sorbose configuration.

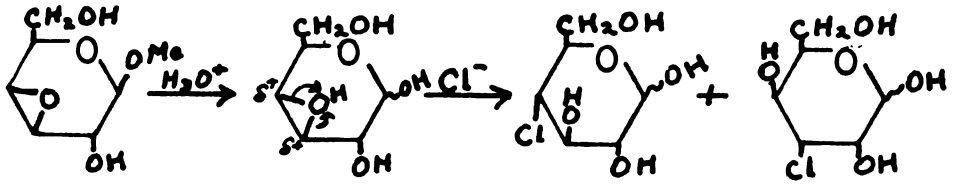


In aqueous solution methyl 3,4-anhydro-β-D-galactoside gave on hydrolysis with sulfuric acid a mixture of sugars in which D-glucose and D-gulose were identified. No galactose is formed. (40)



In an earlier publication on the reactions of this same methyl 3,4-anhydro-β-D-galactoside, Müller reported the introduction of chlorine into the sugar on reaction with

normal aqueous hydrogen chloride at steam bath temperature. (49) This would indicate that the hydronium ion and chloride ion were responsible for the epoxide opening in this case.



In as much as Müller did not prove the configuration of the compounds he obtained, the work of Robertson and Dunlop (50) and of Peat and Wiggins (46) should be mentioned. In the former paper it was stated that two methyl chlorohexosides were produced when methyl benzylidine-2,3-anhydro-D-alloside was treated with a very dilute solution of hydrogen chloride in acetone. These produced the same methyl 2,3-anhydro-D-alloside when treated with silver oxide. The latter paper concerned work done on methyl 2,3-anhydro-dimethyl-D-alloside and methyl 3,4-anhydro-dimethyl-D-alloside which each opened to give two chlorodimethylhexoses when treated with hydrochloric acid, all of which were unique.

Another mechanism for opening anhydrorings is evidenced in the amination with ammonia in dry alcohol or ether, or, as in a recent paper (23), with concentrated ammonium hydroxide. In none of these three cases can the attack of a base other than ammonia result in the observed product, and an acid catalysed opening is improbable in view of the magnitude of the equilibrium $NH_2^- + H^+ \rightleftharpoons NH_3$.

A Lewis acid opening of epoxide rings has been described by Henbest and Wrigley (28) in the preparation of 6,5-fluorohydrins of sterols. This is a very rapid reaction with boron trifluoride-ether complex in benzene which has been followed polarimetrically and terminated by shaking with aqueous sodium bicarbonate. No mechanism for the donation of fluorine from the very stable boron trifluoride is given although discussions of the effect of configurational and electrical factors in relation to the 5β, 6β-epoxide is treated.

MATERIALS AND METHODS

All reagents used in this research were of reagent grade except those used in extractions, which were of USP grade.

Pyridine was dried over calcium hydride and distilled.

p-Toluenesulfonyl chloride was purified by the method of Pelletier. (57)

Derivatives which were purified by distillation were distilled using the distilling head designed by Dr. John C. Speck, Jr.

Solvent removal *in vacuo* was accomplished in most cases with the flash evaporator obtainable from Arthur S. Lapine Company.

Melting points were determined in capillary tubes in a stirred oil bath.

Intermediates were identified and experimental results were interpreted with a Perkin-Elmer Model 21 Infrared Spectrophotometer.

EXPERIMENTAL

The first attempts at a preparation of a 2-(or 3-) fluoro-2-(or 3-)deoxy-pentose were made using the 2,3-anhydro-β-methyl-ribopyranoside of Honeyman. (51)

Methyl β-L-arabinopyranoside.--Beginning with 54 g. of L-arabinose in one liter of 1% methanolic hydrogen chloride the sugar was converted to methyl β-L-arabinopyranoside by refluxing for seven hours. The product (32 g.) crystallized from the solution on cooling.

Methyl 3,4-O-isopropylidene-β-L-arabinopyranoside was made by Levene and Raymond's method (52) for preparing diacetone xylose because Honeyman's preparation using phosphorus pentoxide as the catalyst failed to work. Twenty-five g. of methyl β-L-arabinopyranoside was stirred with 1 ml. of sulfuric acid (sp. g. 1.84) and 40 g. of anhydrous cupric sulfate in 1/2 l. of dry acetone for 65 hours. At this time the mixture began to darken considerably. The acid was neutralized by the addition of 25 g. of sodium carbonate with continued stirring for 24 hours. The solution was then separated from the inorganic residue by drawing it through a sintered glass filterstick, of medium porosity, into an evaporating flask where it was concentrated to a syrup. The inorganic residue was thoroughly rinsed with acetone a number of times. Considerable β-methyl arabinoside crystallized out in the evaporator. The syrup was separated from these crystals and transferred

to another flask for distillation. The product distilled close to the value recorded by Honeyman (82°/0.1 mm.) at 74°/0.03 mm., 80°/0.05 mm., and 85°/0.05 mm. to give a yield of 16 g., or 51%.

Methyl 2-O-Tosyl-3,4-O-isopropylidene-β-L-arabinopyranoside.--The whole amount of the distillate was treated with 33 g. of p-toluenesulfonyl chloride in 100 ml. of dry pyridine. After standing at room temperature for five days, the mixture was poured onto 400 g. of ice (cubes of about 1 g. each) and allowed to stand for two hours until the ice melted. The crude 2-O-tosyl-3,4-O-isopropylidene-β-methyl-L-arabinopyranoside weighed 21.7 g. after drying over phosphorus pentoxide *in vacuo* and represented a 76% yield.

Methyl 2-O-Tosyl-β-L-arabinopyranoside.--A 5.7-g. sample of the compound prepared in the previous step was dissolved in 275 ml. of methanol to which 5.7 ml. of 0.5 N hydrochloric acid had been added and the solution was refluxed for 9 hours according to the directions of Honeyman. Following treatment with 7 g. of silver carbonate, filtration, and evaporation, the product was obtained as a glass which was exhaustively pumped with the water aspirator and dried for 4 hours over phosphorus pentoxide at 15 mm.

Methyl 3,4-Di-O-acetyl-2-O-tosyl-β-L-arabinopyranoside.--The compound obtained above was treated *in situ* with 6 ml.

of acetic anhydride in 50 ml. of pyridine and allowed to stand at room temperature for three days. The acetylated compound was then obtained by pouring the reaction mixture onto 1/2 l. of ice and allowing the ice to melt. The methyl 3,4-di-O-acetyl-2-O-tosyl-arabinoside was collected on a sintered glass filter in 92% yield from the acetone derivative. Its melting point of 114° compared with Honeyman's 116°.

Methyl 2,3-Anhydro-β-L-arabinopyranoside.--The above compound (5.9 g.) was dissolved in 75 ml. of methanol and 6 g. of sodium methoxide was added with cooling. The mixture was allowed to stand at room temperature for two days. It was then carefully brought to neutrality with acetic acid using an external indicator and diluted with water to 500 ml. Extraction with three 50-ml. portions of chloroform failed to give on evaporation more than a few drops of 2,3-anhydro-β-methyl-riboside. A fact which was discovered too late to be of any use and a mistake which was repeated in following Baker's preparation of methyl 2,3-anhydroribofuranoside is the inordinately unfavorable distribution coefficient of the methyl anhydroglycosides between water and organic solvents. For example, in a paper by Mathers and Robertson (53) washing of a 0.15 g. sample of methyl 4,6-di-O-methyl-2,3-anhydro-α-hexoside with water almost quantitatively eluted the compound into the water phase, which had optical activity corresponding to

0.13 g. of starting material. The benzene solution had very little optical activity.

The water phase of the extraction above was evaporated to dryness and extracted with chloroform to give about two grams of an oil which had the same infrared spectrum as that obtained from the chloroform extract of the water solution and presented an epoxide $-CH_2-$ peak at 3.92 μ and epoxide -O- peaks at 11.6 and 12.1 μ in films between salt plates.

This material was used in several experiments in treatment with hydrogen fluoride in reagent grade chloroform which had been allowed to stand in contact with anhydrous calcium chloride for a week to remove the preservative amounts of ethanol and had then been purified by filtration and distillation.

One-half g. was dissolved in 89 g. of chloroform in a polyethylene bottle and weighed. Hydrogen fluoride gas was added from a cylinder with protection from moisture in the air and frequent checks of weight until the equivalent weight (70 mg.) of hydrogen fluoride had been added. The mixture was allowed to stand for four hours at room temperature and evaporated to a syrup in a glass beaker within a filtering bell. Because silicon tetrafluoride would not interfere with infrared examination of the product, contamination from this source was not of any consequence.

The spectrum was identical with that of the reactant.

More rigorous treatment was given 500 mg. of the epoxide in chloroform with hydrogen fluoride in equimolar concentration at the temperature of boiling acetone overnight plus two days at room temperature. A syrup which still retained the epoxide peaks at 11.7 and 12.15 μ was obtained on evaporation.

A like quantity of the anhydro compound was treated with an equimolar amount of potassium acid fluoride (KHF_2) in 10 ml. of glacial acetic acid at room temperature for 12 hours. Removal of the acetic acid in vacuo left a paste which retained the epoxide peaks.

The chloroform-hydrogen fluoride treatment was repeated once more with the same result.

Anderson, Goodman, and Baker's preparation (23) of a methyl anhydro-riboside was followed for subsequent experiments because it is prepared unequivocally in higher yield than is Honeyman's and produces methyl 2,3-anhydro-α and β-D-ribofuranosides which have widely separated boiling points. They may be resolved by vacuum distillation through a short Vigreaux column to give the pure anomers, which are in the correct (furan) ring for subsequent reactions terminating in nucleosides.

1,2:3,5-Di-O-isopropylidene-D-xylofuranose.--To 1 l. of acetone, which had been dried with magnesium sulfate and

distilled, were added 150 g. of D-xylose and 300 g. of anhydrous cupric sulfate. Ten ml. of sulfuric acid (sp. gr. 1.84) were added and the mixture was stirred for 25 hours. Twenty-five g. of anhydrous sodium carbonate was added at that time with continued stirring overnight. The solution was then filtered off and the residue washed with acetone. The solution was evaporated to a syrup which distilled at 135-138° at 12 mm. to yield 173 g. of light-brown diacetone xylose.

1,2-O-Isopropylidene-D-xylofuranose.--The contents of the flask were washed into 1100 ml. of 0.2% hydrochloric acid (6 ml. of 37.6% HCl diluted to 1100 ml.) and shaken vigorously for 25 minutes. The hydrogen ion concentration was then reduced to that corresponding to a pH of 7-8 with 6.5 g. of sodium bicarbonate and the water was removed overnight at less than 40° C. in vacuo. The residual syrup was then taken up in 500 ml. of chloroform, treated with a little "Darco" and filtered on to 20 g. of sodium sulfate to dry.

The chloroform solution was filtered into the distilling apparatus and pumped to a syrup with heating to 80° at the end of the concentration process. Following pumping for 4 hours at 80° and 12 mm., the pressure was lowered to 0.2 to 0.3 mm. The pot was heated at about 100-110° until the thermometer in the distilling head began to rise. Slow distillation at 82° proceeded until liquid stopped coming

over. About 10 g. distilled under these conditions, which are characteristic of diacetone xylose. The head temperature then rose to 132° and a few drops were distilled to flush the side arm of the distilling head. The receiver was changed and distillation was resumed. At a pot temperature of 170° and a pressure of 0.3 mm., the product distilled at 132-134°. Most of the material was distilled and the receiver again was changed. Approximately 10 g. was distilled into the third receiver. The product in both the second and third flasks crystallized. The net weight of the second flask was 97.5 g. This weight plus about 10 g. in the third receiver and 10 g. of recovered reactant allows calculation of a yield of 80% for the conversion and purification of monoacetone xylose from diacetone xylose.

1,2-O-Isopropylidene-5-O-methoxycarbonyl-D-xylofuranose.--The 97.5 g. of monoacetone xylose was washed out of the flask with 200 g. of chloroform into a three-necked flask fitted with a dropping funnel. Two hundred g. of pyridine was added and the whole mixture was cooled to -5° C. in an ice-salt bath. Fifty-five g. of methyl chloroformate were added dropwise over a period of 1.5 hours with stirring and maintenance of temperature within the range -5° to 0°. The mixture was then stored in the refrigerator for three days. The contents of the flask were then rinsed into 1300 ml. of water in a separatory funnel and extracted with 150 ml. of chloroform in four extractions until 600 ml. was used. This

extract was washed twice with 400 ml. of water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. It was then taken up in 300 ml. of benzene warmed to boiling and 300 ml. of petroleum ether was added. The collected crystals weighed 101 g. and represented a yield of 80%.

1,2-O-Isopropylidene-3-O-mesyl-5-O-methoxycarbonyl-D-xylofuranose.--The material obtained above was dissolved in 300 ml. of dry pyridine and cooled to 0° in an ice bath. Ninety-five g. of methanesulfonyl chloride were added. After allowing it to stand for 30 hours in a closed flask, the mixture was poured into 1 l. of ice water and extracted with 1 l. of chloroform in 250-ml. portions. The combined chloroform extracts were washed with four 250-ml. portions of 1% sodium bicarbonate solution and twice with 250-ml. portions of water. The extract was then dried with magnesium sulfate and evaporated to a residue (46.5 g.) which was used in the next step.

It had been found that solvolysis of the isopropylidene group in methanolic hydrogen chloride resulted in formation of the xylopyranoside derivative as well as the desired furanoside. Intermediate solvolysis to a 1,2-di-O-acetyl derivative followed by methanolic solvolysis circumvented this undesirable consequence.

1,2-Di-O-acetyl-3-O-mesyl-5-O-methoxycarbonyl-D-xylofuranose.--The mesylated compound obtained above (46.5 g.)

was dissolved in 640 ml. of glacial acetic acid which contained 71 ml. of acetic anhydride. A 39-ml. volume of concentrated sulfuric acid was then added in small portions, with mechanical stirring and ice cooling, to maintain the temperature below 20°. The flask was covered and allowed to stand at room temperature for 20 hours. The contents were then poured into 3 l. of ice-water mixture and extracted with two 500-ml. portions of chloroform. The combined extracts were washed quickly with a liter of 1% sodium bicarbonate solution in 250-ml. portions followed by one wash with 250 ml. of water. The chloroform solution was then dried with magnesium sulfate and evaporated to an amber gum (53.5 g.) which was used in the next step.

Methyl 3-O-Mesyl-5-O-methoxycarbonyl-α(and β)-D-xylofuranoside.--The gum was solvolysed to methyl 3-O-mesyl-5-O-methoxycarbonyl-D-xylofuranoside by dissolving it in 1 l. of 1% methanolic hydrogen chloride and allowing the mixture to stand at room temperature for 20 hours. This mixture was then neutralized by the addition of 32 g. of sodium bicarbonate. After filtering, the methanol was removed from this mixture *in vacuo*. The residue was extracted with two 200-ml. and one 100-ml. portions of boiling methylene chloride. It was then filtered with the aid of Celite and evaporated to dryness *in vacuo* to give a residue weighing 35.5 g.

Methyl 2,3-Anhydro-α(and β)-D-ribofuranoside.--The 35.5 g. of the methyl glycoside was converted to the anhydro derivative according to the literature directions by dissolution in 75 ml. of dry methanol and treatment at ice temperature with a solution of seven grams of sodium methoxide in 65 ml. of methanol. The flask was stored in the refrigerator for four days at which time four grams of Celite was added and the mixture was filtered, neutralized with 3 ml. of acetic acid, and evaporated to dryness *in vacuo*. The residue was dissolved in 500 ml. of water (the literature did not specify the amount) and extracted with seven 80-ml. portions of chloroform. The chloroform extract was dried over magnesium sulfate and evaporated to a residue *in vacuo*.

The α- and β-methyl 2,3-anhydro-D-ribofuranoside mixture was distilled through a short Vigreaux column to obtain a small yield of methyl 2,3-anhydro-β-D-ribofuranoside at 5 μ and 50° and of methyl 2,3-anhydro-α-D-ribofuranoside at 3 μ and 90°. The original authors obtained clear white syrups, the more volatile of which was shown by its optical rotation and previous deductions concerning its physical characteristics to be the named compound. The α-methyl glycoside would be expected to be less fluid because of hydrogen bonding between molecules and the β-anomer more fluid because of intramolecular hydrogen bonding between the 5-hydroxyl and the glycosidic oxygen.

Numerous experiments were conducted with the methyl anhydroribofuranosides as follows:

A 3.35-g. sample was dissolved in 100 ml. of a standardized solution of hydrogen fluoride in chloroform which contained 0.2524 millimole of hydrogen fluoride per g. Samples of the solution were titrated from time to time to determine whether the epoxide reacted with the hydrogen fluoride. The solution was contained in a polyethylene wash bottle. The amount of hydrogen fluoride was determined by weighing a sample into an excess of 0.2099 N aqueous sodium hydroxide. The flask was shaken vigorously and the excess of base was titrated with 0.1198 N hydrochloric acid to the faintest discernable pink of the indicator phenolphthalein. It was thought that overrunning this end point with acid required a larger than equivalent amount of base to return to the pink basic form of phenolphthalein. This effect was, however, too close to the limit of experimental error. The discrepancy was thought to be due to an acid catalysed hydrolysis of a glycosidic fluoro substituent. (54)

One hundred mg. of the anhydro sugar was treated with the same weight of potassium acid fluoride in 25 ml. of dry methanol, in which the salt is slightly soluble. Evaporation, after 2 days at room temperature, gave a syrup which indicated no diminution of epoxide absorption at 11.6 μ.

The experiment was repeated at the boiling point of

methanol at 60° ± 5° for 36 hours with the same result.

Hydrogen fluoride in methanol at a concentration of 655 mg. in 50 ml. of methanol which contained 250 mg. of the anhydro sugar failed to show altered absorption in the infrared after 2 days at room temperature.

Since Müller (49) opened a sugar epoxide with hydrogen chloride in aqueous solution under conditions in which it would have high concentration and ionic character, an analogous experiment with hydrogen fluoride was tried. One and one-tenth g. of the anhydro sugar was dissolved in 5 g. of 9.1% hydrofluoric acid and heated in the steam bath for 3 hours. It was then neutralized with calcium carbonate, filtered with the aid of a little Celite, evaporated and dried over phosphorus pentoxide at less than 15 mm. overnight. This was also unsuccessful by the infrared criterion. The syrup was strongly reducing to Benedict's reagent, however, and hence had lost its glycosidic methoxyl.

Success appeared imminent when the anhydro sugar was treated with a slight excess of hydrogen fluoride and a catalytic trace of boron trifluoride.

A 1.55-g. sample of the anhydro sugar was dissolved in 100 ml. of anhydrous methanol. The polyethylene tubing which led from the valve to the reaction vessel was inserted through a cork which allowed for some volume change. Hydrogen fluoride was added from the cylinder, the cylinder valve was turned off, the needle valve was turned off, then removed

from the cylinder without disconnecting the receiving bottle. The exposed end of the needle valve was then cleaned and sucked repeatedly with the aspirator to remove condensed hydrogen fluoride and water. It was then screwed into the boron trifluoride cylinder and enough boron trifluoride was added to make a fog about one-quarter of an inch deep above the surface of the liquid. The cylinder valve and needle valve were closed and the bottle was sealed with a polythene cap and left at room temperature for two days. For lack of any other means of removing solvent and acid, phosphorus pentoxide was used in a vacuum desiccator. The solution was reduced to a viscous syrup much different from the mobile fluid of the reactant. A small sample had been removed earlier and dried to a hard glass which was free of epoxide peaks in the infrared. On further drying in the desiccator, the main portion of the product was converted to a glass which charred badly. This material was taken up in about 25 ml. of methanol, treated with a little "Darco", and filtered. It was insoluble in chloroform and hydrocarbons and crystallized to a very slight extent in the cold from methanol, in which it was very soluble. Dissolution in a minimal amount of n-propanol produced white crystals on cooling which grew on standing. These were filtered out with the aid of a small Hirsch funnel and washed with a small amount of methylene chloride, whereupon they became a sticky gum. They were taken up again in propanol, but were not recovered.

A 1.95-g. sample of anhydro sugar was dissolved in 80 g. of anhydrous methanol and 1.73 g. of hydrogen fluoride including a trace of boron trifluoride was added as described above. Subsequent removal of solvent over phosphorus pentoxide after two days again resulted in charring. The charcoal treatment was repeated and on nearing fruition in the crystallization step the compound was lost through accident.

A 3.5-g. sample of the anhydro sugar was dissolved in 100 g. of methanol and treated with 1.000 g. of hydrogen fluoride including a trace of boron trifluoride. After 2 days, 5 g. of anhydrous sodium carbonate was added to this mixture, carbon dioxide was removed under reduced pressure, and the solution was filtered and evaporated in glass on the flash evaporator to obtain a thin syrup which resembled the reactant and was shown to have its composition by infrared examination.

A 1.75-g. sample of the α-anomer of the anhydro sugar was dissolved in 40 g. of methanol and treated with 0.85 g. of hydrogen fluoride, including a trace of boron trifluoride. This was left for two days at room temperature, split into two portions, the first of which was treated with 1/2 equivalent (1 g.) of calcium carbonate, evacuated, filtered with Celite, and evaporated to a syrup which began turning dark immediately. The other portion was treated with about 100 mg. of sodium fluoride and evaporated to dryness over phosphorus pentoxide at first and finally over crushed

magnesium turnings which appeared to remove both hydrogen fluoride and methanol. The residue was a white crystalline mass. This was extracted with 25 ml. of hot propanol. Filtration through a small Hirsch funnel was attempted but evaporation of the solvent precluded this. By adding a little methanol, the hot solution was made filterable, but on partial evaporation the carbohydrate did not crystallize and on evaporation to dryness over phosphorus pentoxide, the material again charred very badly.

Eleven g. of partially purified methyl 2,3-anhydro-α-D-ribofuranoside was dissolved in about 100 ml of anhydrous methanol and treated with 6 g. of hydrogen fluoride and a trace of boron trifluoride. After two days at room temperature the solution was treated with 30 g. of calcium carbonate and evacuated. The effect of the calcium carbonate was more noticable on this scale and it was found that the solution was still acid after overnight contact. Twenty-five g. of sodium bicarbonate was then added. The bottle was placed in the vacuum desiccator and evacuated, whereupon carbon dioxide bubbled off vigorously. The solution was then filtered through Celite and evaporated to a thin syrup which was not the compound sought. (Infrared examination showed prominent epoxide peaks.)

DISCUSSION

It seems feasible to prepare a fluorosugar using an anhydro sugar intermediate which is subjected to ionic ring-opening conditions in methanol. Boron trifluoride in trace amounts was used to enhance the ionic character of hydrogen fluoride. Presumably the ring-opening agent was fluoboric acid, which is much more ionic than hydrogen fluoride.

The compound which was obtained was readily decomposed by residual boron trifluoride and easily converted to the anhydro precursor by basic materials in methanol, even such mild bases as sodium bicarbonate. Its value as an antimetabolite thus is open to question since its existence in solutions buffered near a pH of 7 might be ephemeral.

If an ionic mechanism of epoxide opening is necessary, the failure of short term exposures in aqueous and methanolic solutions to hydrogen fluoride alone is readily understandable, since Deussen's data (55) quoted in Mellor (56) gives the molor electrical conductivity of aqueous hydrogen fluoride at a concentration of 0.25 M at 25° as 29.6 compared to 366, 377, and 376 for HCl, HBr, and HI respectively.

That the initial step in opening the epoxide ring may be the attack of a proton on the epoxide oxygen would explain the failure of potassium acid fluoride to bring about the desired reaction. This reagent was used in the hope that an electron-sharing mechanism such as is found with opening by ammonia would be operative.

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