APPLICATION OF OXO REACTION TO TWO CARBOHYDRATE DERIVATIVES.
NUCLEOSIDE SYNTHESIS.

by

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ABSTRACT

Hydroformylation of 5,6-anhydro-1,2-0-isopropylidene-\(\alpha\)-D-glucofuranose (XXIV) with carbon monoxide (70 atm.) and hydrogen (70 atm.) at a temperature of 100-105° C. gave 6-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (XXV) in 78% yield. Minor quantities of the rearrangement product, 6-deoxy-5-keto-1,2-0-isopropylidene-\(\alpha\)-D-glucofuranose (XXVI), and the hydro-hydroxymethylation product, 6-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-hepto-1,4-furanose (XXVII), were isolated in 9% and 4% yields, respectively. Acetylation of crude (XXV) afforded two anomeric derivatives (XXIX, XXVII).

Under identical experimental conditions, 5,6-dideoxy-1,2-0-isopropylidene-\(\alpha\)-D-xylo-hex-5-enofuranose (XXXIII) gave 5,6-dideoxy-\(\alpha\)-D-xylo-heptodialdo-1,4-furanose-3,7-pyranose (XXXIV) in 51% yield. A minor amount of 5,6-dideoxy-\(\alpha\)-D-xylohepto-1,4-furanose (XXXV) in about 5% yield was also detected by thin layer chromatography (T.L.C.)

Fusion of 5,7-di-0-acetyl-6-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (XXVIII) with 5,6-dimethylbenzimidazole using chloroacetic acid as a catalyst at 170-175° C. gave two anomeric nucleosides 1-(3'-0-acetyl-2'-deoxy-6',7'-0-isopropylidene-
heptodialdo-4',7'-pyranose-\(\alpha(\text{and } \beta-)\)-L-gulopyranosyl)-5,6-dimethyl-benzimidazole (XXXVII, XXXVIII) in 42% yield. These nucleosides were separated by multiple ascending development on preparative T.L.C. plates of silica gel G. impregnated with 2% G.E.Phosphor. Assignment of structures of the nucleosides was based on an analysis of their N.M.R. spectra.
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GENERAL INTRODUCTION

(1) Historical Background of the Oxo Reaction.

Excellent reviews on the application of the oxo reaction to unsaturated compounds and carbohydrates have appeared from time to time (1,2,3,4,5). In general, the term oxo reaction describes the reaction of olefins with carbon monoxide and hydrogen under high temperature (100-200°C.) and pressure (200-400 atm.) in the presence of a cobalt catalyst. When the product is an aldehyde the process is often referred to as "hydroformylation" whereas, the term "hydrohydroxymethylation" is reserved to describe the process when an alcohol instead of an aldehyde is obtained:

\[ \text{R.CH=CH.R} + \text{H}_2 + \text{CO} \rightarrow \text{R.CH}_2\text{CHR.CH}_2\text{CHO} \quad \text{(Hydroformylation)} \]
\[ \text{R.CH=CH.R} + \text{H}_2 + \text{CO} \rightarrow \text{R.CH}_2\text{CHR.CH}_2\text{OH} \quad \text{(Hydrohydroxymethylation)} \]

Very often when temperatures between 75° and 200°C. and pressures of synthesis gas from 100-300 atmospheres are employed, alcohols rather than aldehydes are the resulting products.

The first work on the application of the oxo reaction to the field of carbohydrates was done by Rosenthal and Read (6). From 3,4,6-tri-O-acetyl-D-galactal (I) new seven-carbon sugar alcohols were obtained which were tentatively assigned branched-chain structures. These anhydrodeoxyheptitols (II, III) were later shown to be linear and not branched-chain as shown below. The reaction has since been extended to other glycals and shown to be a general reaction (7,8,9,10,11,12).
Up to 1960, very little was known about the reaction of epoxy derivatives with carbon monoxide. Hamada (13) treated ethylene oxide with carbon monoxide and hydrogen in toluene using dicobalt octacarbonyl as a catalyst but obtained a mixture of hydrogenated products only. Lenel (14) reported that, under oxo reaction conditions, epoxides gave rise to mixtures of isomers which were difficult to separate. Seon and Lenel (15) claimed the preparation of monoethylene glycol hydracrylate from ethylene oxide, carbon monoxide and water, and of mixtures of hydracrylates by replacing water with alcohol in the reaction medium. Eisenmann (16) treated propylene oxide in methanol with carbon monoxide in the presence of preformed dicobalt octacarbonyl to give predominately methyl β-hydroxybutyrate. Under such conditions minor amounts of 1-methoxy-2-propanol and 2-methoxy-1-propanol were also formed together with very small amount of unidentified hydroformylation products, one of which was believed to be methyl crotonate. Two years later (17), however, the major by-product of this reaction was identified as acetone. It was therefore concluded that dicobalt octacarbonyl caused isomerization as well as carbonylation of propylene oxide. Meanwhile, the reaction
of cobalt hydrotetracarbonyl with olefins was studied by Heck and Breslow (18). They demonstrated that cobalt hydrotetracarbonyl added to olefins to form alkylcobalt tetracarbonyls in the first step in the hydroformylation process. They isolated these products as their mono-triphenyl phosphine adducts. In the subsequent year it was found that cobalt hydrotetracarbonyl reacted with epoxides and carbon monoxide at 0°C. to give high yields of β-hydroxyacylcobalt tetracarbonyls (19). Thus, the possibility of the hydroformylation of olefins oxide was evidenced, but no definite characteristics of this reaction were yet known. Simultaneously, Japanese workers demonstrated that propylene oxide underwent hydroformylation reaction at a relatively low temperature (80°C.) to give β-hydroxy-n-butyraldehyde as the major product (20), whereas Orchin and co-workers showed that cyclohexene oxide underwent hydroformylation reaction to yield a dimeric hexahydrosalicylaldehyde (21).

In this thesis the investigation of the hydroformylation reaction on carbohydrate derivatives with a terminal epoxy ring, namely, 5,6-anhydro-1,2-0-isopropylidene-α-D-glucofuranose (XXIV), and a terminal double bond, namely, 5,6-dideoxy-1,2-0-isopropylidene-α-D-xylo-hex-5-enofuranose (XXXIII) will be discussed. The extended synthesis of one hydroformylation product to two nucleosides will also be described.

(11) Sugar Epoxides.

The interest in sugar epoxides, as distinct from other anhydro derivatives, stemmed from the fact that anhydro sugars played an important part in the study of Walden inversion in carbohydrate chemistry, in the syntheses of deoxy sugar, in the determination of structures, and in the
syntheses of O-alkyl, halogeno- and amino- sugars. The oxide ring was opened by nucleophilic reagents to give, usually, two products with the trans configuration and a substituent group which was derived from the reagent. The reaction was easily carried out, but separation and characterization of the products often needed prolonged study.

One of the earliest reactions in which a sugar epoxide was formed occurred in the attempt to synthesize glucosamine from glucal (22). 3,4,6-Tri-O-acetyl-D-glucal-1,2-dibromide was first converted into methyl 3,4,6-tri-O-acetyl-2-bromo-2-deoxy-\(\beta\)-D-glucoside (III). When this compound was treated with ammonia the product was 3-amino-3-deoxy-\(\beta\)-D-altrose (IV) and not the expected 2-amino-2-deoxy-\(\beta\)-D-glucose or mannose. Fischer suspected that a 2,3-anhydro ring might have been formed as an intermediate. It can of course be recognised now that the intermediate was methyl 2,3-anhydro-\(\beta\)-D-mannoside, but it was not until 13 years later that the existence of 2,3-anhydrohexoside was fully established.

\[
\begin{align*}
\text{CH}_2\text{OAc} & \quad \text{CH}_2\text{OH} \\
\text{AcO} & \quad \text{OH} \\
\text{OAc} & \quad \text{OH} \\
\text{Br} & \quad \text{OMe} \\
\text{(III)} & \quad \text{NH}_2 \\
\text{Epoxide} & \quad \text{(IV)}
\end{align*}
\]

In fact, the first sugar epoxide appeared in literature as early as 1921. Brigl (23) found that when tetra-O-acetyl-\(\beta\)-D-glucose was treated with phosphorous pentachloride, 3,4,6-tri-O-acetyl-2-0-trichloroacetyl-\(\beta\)-D-glucosyl chloride (V) was formed. Careful treatment
of this compound with ammonia removed only the trichloroacetyl group, forming 3,4,6-tri-O-acetyl-\(\beta\)-D-glucosyl chloride (VI) which on further mild treatment with ammonia was converted into 3,4,6-tri-O-acetyl-1,2-anhydro-\(\alpha\)-D-glucose (VII). This anhydro derivative was called Brigl's anhydride. Subsequently, methyl 3,4-anhydro-\(\beta\)-D-galactoside and methyl 2,3-anhydro-\(\beta\)-D-hexoside (25) were soon reported.

\[
\begin{align*}
(V) & \quad \begin{array}{c}
\text{CH}_2\text{OAc} \\
\text{OAc} \\
\text{AcO} \\
\text{OCOCCl}
\end{array} \\
(VI) & \quad \begin{array}{c}
\text{CH}_2\text{OAc} \\
\text{OAc} \\
\text{AcO} \\
\text{OH}
\end{array} \\
(VII) & \quad \begin{array}{c}
\text{CH}_2\text{OAc} \\
\text{OAc} \\
\text{AcO} \\
\text{O}
\end{array}
\end{align*}
\]

It may be appreciated that much impetus has been given to the chemistry of sugar epoxides by the desire to synthesize naturally occurring sugars, for example, the synthesis of glucosamine (2-amino-2-deoxy-D-glucose) in 1939 (26) and chondrosamine (2-amino-2-deoxy-D-galactose) in 1946 (27). In the years following 1946, much attention was also given to sugar epoxides as intermediates in the preparation of rare sugars.

The standard method for the preparation of anhydro sugars of the ethylene oxide type is achieved through the hydrolysis of sugar derivative containing a halogen or sulfonic acid group situated in suitable juxtaposition to a hydroxy group. In all these reactions the formation of an anhydro ring has been accompanied by inversion of configuration at that carbon atom to which the sulfonyloxy group is attached.
alkaline medium the anion of the vicinal trans-hydroxyl group can displace the p-toluene-sulfonyloxy anion to form an epoxide, and this can be represented as the intramolecular $S_N^2$ process:

\[
\begin{align*}
\text{OTs} & \quad \text{HOMe} \\
\text{OMe} & \quad \text{HOMe}
\end{align*}
\]

If the vicinal group is cis, as in methyl 2,3,6-tri-O-acetyl-4-O-methanesulfonyl-$\beta$-D-galactose, then only deacetylation occurs and the methanesulfonyloxy group is not displaced (28). The reason is that, in the base-catalysed formation of an epoxide from a trans-1,2-diol mono-O-p-toluenesulfonate, the intramolecular $S_N^2$ process in a six-membered ring requires the two groups to be in the diaxial position. The entering and the departing anion, and the carbon atoms to which they are attached, are then co-planar and this permits maximum participation. This condition is found in 1,6-anhydro-2-O-methanesulfonyl-$\beta$-D-galactose (VIII), and it is easily converted into the 2,3-talo-epoxide (IX) by mild alkali. On the other hand, the majority of p-toluenesulphonates vicinal to a trans hydroxyl group are in the diequatorial position. The ease with some of these compounds that react suggests some structural modification before the epoxide formation, as
In contrast, the conversion of 6-bromo-6-deoxy-1,2-0-isopropylidene-α-D-glucofuranose (29) and 1,2-0-isopropylidene-6-0-tosyl-α-D-glucofuranose (30) into 5,6-anhydro-1,2-0-isopropylidene-α-D-glucofuranose (XXIV) is not complicated by Walden inversion.

Epoxides may also be prepared by alkaline hydrolysis of di-0-tosyl compounds. A well known example of this is the formation of methyl 2,3-anhydro-4,6-0-benzylidene-α-D-alloside from methyl 4,6-0-benzylidene-2,3-di-0-tosyl-α-D-glucoside (31,32). In this reaction one of the ester groups must undergo O-S cleavage. Angyal and Gilham (33) consider that the first tosyl group, which will be the more accessible one, will be facilitated
by the inductive effect of the other tosyl group. However, the alkaline

$$\text{TsO} + \text{MeO}^- \rightarrow \text{MeOTs} + \text{TsO}^-$$

hydrolysis of 1,2-0-isopropylidene-5,6-di-0-\(\alpha\)-D-glucofuranose follows a
different pathway. Instead of 5,6-anhydro-1,2-0-isopropylidene-\(\alpha\)-D-
glucofuranose (XXIV), another compound, namely, 3,6-anhydro-1,2-0-
isopropylidene-5-0-tosyl-\(\alpha\)-D-glucofuranose, was formed \((34)\). It is not
immediately obvious why the reaction should follow this course and not
yield the 5,6-epoxide. Perhaps a bicyclic furanoid structure is more
thermodynamically stable. On the other hand, when 1,2-0-isopropylidene-
5,6-di-0-tosyl-\(\alpha\)-D-glucofuranose is refluxed with sodium iodide in dry
methyl ethyl ketone an unsaturated carbohydrate, namely, 5,6-dideoxy-
1,2-0-isopropylidene-\(\alpha\)-D-xylo-hex-5-enofuranose (XXXIII), is formed
\((35,36)\).

5,6-Anhydro-\(\alpha\)-D-glucofuranose (XXIV) is extremely reactive due
to its epoxide ring being situated at the terminal position. For instance,
6-0-acyl derivatives were obtained by the action of carboxylic acid; 6-thio-
6-deoxy derivatives by the action of hydrogen sulphide in the presence
of barium hydroxide \((37)\); C-6-N derivatives \((38,39,40)\) by the reaction
with amines and amino acids; 6-alkyl ethers \((41)\), 6-aryl ether \((42)\) by
the actions of alkoxides and phenol, respectively; and the reaction with
potassium hydrogen phosphate (43) or with salts of dibenzylphosphoric acid (44) led to derivatives of the Robison ester.

(III) Unsaturated Sugars.

Unsaturated monosaccharide derivatives are of great importance from synthetic point of view. In unsaturated aldohexoses, those with a double bond located in position C-5,6 may be named 5,6-glycoseens or glyco-5,6-enoses, but the first name is more commonly used.

Jones (35) and Hall (36) prepared an unsaturated compound (XXXIII) from 1,2-0-isoporpypylidene-5,6-di-0-tosyl-α-D-glucofuranose by a modification of Oldham and Rutherford's method (45). By refluxing this di-0-tosyl compound with sodium iodide in anhydrous methyl ethyl ketone, a high yield of 5,6-dideoxy-1,2-0-isopropylidene-α-D-xylo-hex-5-enofuranose (XXXIII) was obtained. Recently Turner (46) reported that (XXXIII) was prepared from 1,2-0-isopropylidene-α-D-glucofuranose via the 5,6-thionocarbonate using the alkene synthesis of Corey and Winter (47).

5,6-Glycoseens are stable only in the form of their derivatives. Attempts to prepare free 5,6-glycoseens by saponification of the respective 5,6-glycoseen derivatives in acid solution have always resulted in hydrolysis at the site of the double bond, so that instead of the expected unsaturated compound, the 6-deoxy-5-keto derivative of the initial aldohexoses have been formed (48).

5,6-Dideoxy-1,2-0-isopropylidene-α-D-xylo-5-enofuranose (XXXIII) has been reported to undergo hydrogenation readily with palladium charcoal (36), and to react with thiolacetic acid under illumination from a tungsten lamp (49) to yield 5,6-dideoxy-1,2-0-isopropylidene-α-D-glucofuranose
and 6-S-acetyl-5-deoxy-1,2-0-isopropylidene-6-thio-\(\alpha\)-D-xylo-hexofuranose, respectively.

(IV) **Nucleosides.**

The second part of the research was devoted to nucleoside syntheses. The reasons for so doing were due to their importance in nature as well as their intrinsic chemical interest.

(a) **Introduction.** Work on nucleic acids, the polymeric materials isolated from living cell nuclei, was initiated in the nineteenth century by Miescher, Altmann and Kossel, and important contributions in the early part of this century were made by Levene and others (50). The structure of the major bases and of the sugars was then established by these workers.

After the second War, there were two major developments in the field of nucleic acid chemistry, due to the advent of new chromatographic techniques. Improved methods of isolation and careful analytical work by Chargaff (51) and others established that nucleic acids were polymers of high molecular weight, and that in deoxyribose nucleic acid (DNA), the ratio of the base content of adenine to thymine, and of guanine to cytosine, was equal to one.

At about the same time, chemical investigations, mainly by Todd and coworkers, led to the synthesis of nucleosides and the determination of the structures of nucleotides obtained by chemical and enzymatic hydrolysis of nucleic acids. This made it possible to formulate nucleic acids as 3', 5'-linked linear polynucleotides. The relationship between the various derivatives is indicated by the following hydrolytic sequence:
Nucleic acids ——— Nucleotides
Nucleotide ——— Nucleoside + Orthophosphate
Nucleoside ——— Base (Purine or Pyrimidine)
+ Sugar (Ribose or 2-deoxyribose).

With this work as a basis, Watson and Crick (52) were led to their world renowned interpretation of the X-ray crystallographic studies of polymeric DNA by Wilkins and others, and to propose the specifically hydrogen-bonded double stranded helical structure for DNA.

(b) **Definition.** The term nucleoside was originally restricted to the purine and pyrimidine N-glycosides of ribose and 2-deoxyribose derived from nucleic acids of living cells, but it is now applied to many other heterocyclic glycosides, including the 5,6-dimethylbenzimidazolriboside from vitamin B_{12}; psuedopuridine, a C-glycoside; the antibiotic puromycin, in which an amino sugar is attached to a purine; and the arabinosides found in certain sponges.

(c) **Methods of Preparation.** There are chiefly six methods for the syntheses of nucleosides.

(i) **Fischer-Helferich or Metal Salt Method.** Fischer and Helferich (53) first condensed silver 2,8-dichloroadenine (X) with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (XI) and obtained 2,8-dichloro-9-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-adenine (XII). Deacetylation of this derivative afforded one of the first synthetic nucleosides ever reported. Davoll and Lowy (54) found that chloromercuriederivatives of purines, rather than silver purines, gave better results. Since then, the syntheses of nucleosides of purines, pyrimidines and other hetero-
cyclics using mercury salts have become practical methods.

(ii). Hilbert-Johnson Method. Pyrimidine nucleoside synthesis was achieved successfully by Hilbert and Johnson (55) who prepared $\beta-\text{D-glucopyranosyl uracil (XVI)}$ and $\beta-\text{D-glucopyranosyl cytidine (XVII)}$ by the reaction of 2,4-diethoxypyrimidine (XIII) with 2,3,4,6-tetra-$\text{O-acetyl-\text{D-glucopyranosyl bromide (XIV)} followed by hydrolysis. The first synthesis of cytidine and uridine by Todd and co-workers (56) was based on this method.
(iii) Glycosylamine Method. Shaw (57) observed that 2-cyano-3-ethoxy-N-(ethoxycarbonyl)-acrylamide \((XVIII)\) reacted with amines to yield 1-substituted 5-cyanouracil \((XV)\). This reaction proceeded by way of the acyclic intermediate which upon acidification with acetic acid, afforded the pyrimidine. Other workers (58) applied this reaction to the synthesis, in good yields, of the 5-cyano-1-(D-glycopyranosyl)-uracil derivatives containing D-glucose, D-galactose, D-xylose, and D-ribose residues, by the use of the appropriate glycosyl amines. This
method is interesting from the chemical point of view but is not very commonly adopted in nucleoside syntheses.

(iv) **Nucleoside Interconversions.** By oxidation (59, 60, 61, 62) anhydro nucleosides formation (63) and thiation of pyrimidine nucleosides (64, 65) various nucleosides have been synthesized.

(V) **Trimethylsilyl Method.** A new method for the synthesis of pyrimidine and purine nucleosides, based on Birkofer's original discovery (66, 67) of the activation of heterocyclic ring nitrogens by silylation, was described by Nishimura and co-workers (68, 69, 70, 71). Trimethylsilyl ethers of uracil (XX), fused at 180-190°C with 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl chloride (XXI), produced 1-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl)-uracil (XXIII) upon hydrolysis.
(vi) **Fusion Method.** Shimadate and co-workers (72,73,74) initiated a method of nucleoside synthesis, which involved heating 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose with various purines and a catalyst, p-toluene-sulfonic acid, to yield various nucleosides. Subsequently, zinc chloride, concentrated sulphuric acid (75), sulphamic acid (76), chloroacetic acid (77,78,79), bis-(p-nitrophenyl)-hydrogen phosphate and its methyl derivative (80) have also been used by other workers as effective catalysts for the fusion method.

Non-catalytic fusion (81) of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose with 6-chloro-, 6-iodo-, 6-cyano-, 2-chloro-6-iodo-,...
6-chloro-2-iodo- and 2,6,8-trichloro- purines gave the corresponding ribonucleosides in 10-67% yields.

Deoxyribosides were in general much more difficult to synthesize than ribosides, partly because 2-deoxyribose itself was not readily available, and partly because of the labile nature of deoxyribofuranosyl halides. Therefore, in our present research, failure and difficulties were anticipated.
DISCUSSION

Previous work in our laboratory (6,7,8,9,10,11) demonstrated that the oxo reaction is applicable to glycols to yield anhydrodeoxyalditols. Under very careful controlled experimental conditions glycols were also successfully hydroformylated (12). Propylene oxide and cyclohexene oxide were also reported to undergo hydroformylation reaction (20,21). These findings, therefore, led us to investigate the application of the oxo reaction to a carbohydrate epoxide and to an unsaturated carbohydrate.

EXPERIMENT A. Hydroformylation of 5,6-anhydro-1,2-0-isopropylidene-\(\alpha\)-D-glucofuranose (XXIV).

(a) Reaction Conditions. In this work a relatively lower temperature (100-105° C.) and pressure (140-150 atm.), similar to the hydroformylation conditions employed by Yokohawa and co-workers (19) and Orchins (21), were used as it was thought that temperatures above 130° C. would favor decomposition of the carbohydrate under investigation and reduction of the product aldehyde(s) to alcohol(s). A high partial pressure of hydrogen was also undesirable as hydrogenation of both the reactant (anhydro sugar) and products (aldehydes) might be effected.

The time (2 hours) for the hydroformylation reaction was chosen arbitrarily although it was observed that the pressure of the reaction
vessel began to decrease after 45 minutes, thus indicating that the course of reaction had reached its peak. After the reaction the vessel was let stand at room temperature for 18 hours so as to allow sufficient time for the products to crystallize (in case they did).
Characterization of Fraction X: 6-Deoxy-1,2-D-isopropylidene-\(\alpha-D\)-gluco-heptodialdo-1,4-furanose-3,7-pyranose.

The crystalline portion (2.16 g.; 78%) of the hydroformylation product was designated as Fraction X, which was shown to be homogeneous, \(R_f 0.71\). After being recrystallized from ethanol-ethyl acetate to constant m.p. (159-160°C), this compound showed no carbonyl peak in its I.R. spectrum; its mass spectrum revealed a mol. wt. 232; the base in the spectrum was \(M^+ - 15\), due to loss of \(\text{CH}_3\); and microanalysis gave an elemental composition of \(\text{C}_{10}\text{H}_{16}\text{O}_6\). Its N.M.R. spectrum showed a multiplet at 7.7\(\tau\) equal to two methylenic hydrogens and multiplets at 4.25\(\tau\) and 4.82\(\tau\) equal to one anomeric C-7-H which were in agreement with the assigned structure (XXV). Crude (XXV) had an initial \(\alpha [D]_{22} +39^\circ\) which mutarotated to \(+60^\circ\) after 3 hours. Therefore, (XXV) was 6-deoxy-1,2-D-isopropylidene-\(\alpha-D\)-gluco-heptodialdo-1,4-furanose-3,7-pyranose (82). Presumably, (XXV) was formed from the aldehyde intermediate (XXVa).

Further confirmation of the structure of (XXV) was obtained by N.M.R. and elemental analysis (all satisfactory) of the following

1. Data refer to silica gel G. activated at 100°C with methyl ethyl ketone-water azeotrope as the developing solvent. Homogeneity of each substance was checked by T.L.C. by at least four different tests using different solvent system.

2. Data refer to a solution of (XXV) in \(D_2O\) at 100 Mc/s.
derivatives:

1. 6-Deoxy-1,2-O-isopropylidene-α-D-gluco-heptodialdo-1,4-furanose semicarbazone monohydrate, m.p. 190-191°C, \([\alpha]_D^{20} -9^\circ (c 1, \text{ water})\).

2. 6-Deoxy-1,2-O-isopropylidene-α-D-gluco-heptodialdo-1,4-furanose phenylhydrazone, m.p. 134-135°C, \([\alpha]_D^{22} -33^\circ\) (c 2, chloroform).

3. 5,7-Di-O-acetyl-6-deoxy-1,2-O-isopropylidene-α-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose; two fractions: crystalline portion, m.p. 176-177°C, \([\alpha]_D^{22} +42^\circ (c 2, \text{ chloroform})\); mother liquor, \([\alpha]_D^{24} +209^\circ (c 1.75, \text{ chloroform})\).

(c) N.M.R. Determination of the Structures of Acetate Derivatives.

Acetylation with acetic anhydride and dry pyridine converted (XXV) into two derivatives: the α- and the β-anomers (XXVIII, XXIX). The N.M.R. spectrum of the crystalline fraction, have a m.p. 176-177°C and \([\alpha]_D^{22} +42^\circ\), was first studied with the aim of determining its structure as well as its ring conformation. In this respect, the two anomers of 2-deoxy glucose were useful as a starting point. From a model of its β-anomer, it can be seen readily that its equatorial hydroxyl group on the C-1 carbon atom is comparatively free from strain between the C-1 and C-2 position; hence the molecule readily assumes the lowest energy conformation with the C-1 hydroxyl group bisecting the two C-2
protons and giving dihedral angles of 61° and 181° for the two
C1H1a - C2H2 bonds. In the case of the α-anomer, the axial hydroxyl
group is probably subject to considerable steric strain in a chair
conformation, caused by the axial proton at the C-3 and C-5 position
and, as a result, the anomic hydroxyl group is believed to have
been repelled by the other ring substituents and forced into a more
equatorial position. From measurements of coupling constant, it has
been reported that this repulsion caused some 12° deviations of the
C1H1e - C2H2 dihedral angles (83).

Attention was then turned to the α and β anomers of 1,3,4,6-
tetra-O-acetyl-2-deoxy-D-glucopyranose. The chemical shifts and coupling
constants of various protons may be found in the literature (84):

<table>
<thead>
<tr>
<th></th>
<th>H1</th>
<th>H2x</th>
<th>H5a</th>
<th>H3</th>
<th>H4</th>
<th>H5</th>
<th>H6</th>
<th>H61</th>
<th>H62</th>
<th>OAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td>7.73</td>
<td>8.07</td>
<td>4.68</td>
<td>4.91</td>
<td>5.95</td>
<td>5.68</td>
<td>5.97</td>
<td>7.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.99</td>
<td>7.95</td>
<td>7.95</td>
<td>7.95</td>
<td>8.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.13</td>
<td>7.67</td>
<td>8.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.87</td>
<td>5.02</td>
<td>6.22</td>
<td>5.68</td>
<td>5.95</td>
<td>7.92</td>
<td>7.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.99</td>
<td>8.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The N.M.R. parameters for 1,3,4,6-tetra-O-acetyl-2-deoxy-α-
(and β)-D-glucopyranose.

The information in Table 1. assisted in analyzing the observed
spectrum (Fig. 1) To assign correctly all chemical shifts of various
protons and to account for their coupling constants was difficult, since such assignments always involved some uncertainty. For this reason the technique of double-resonance (85) was used.

It may be pointed out here that various esters of 1,2-D-isopropylidene-\(\alpha\)-D-glucofuranose have been studied (86) in some detail and that the furanoid ring was found to adopt a twist conformation, i.e., C-2 was below and C-3 was above the plane defined by C-4, O, and C-1. Thus C-1 proton and C-2 proton would have the same coupling constant, \(J=4.2\) cps.

![Diagram 1. The twist conformation for an ester of 1,2-D-isopropylidene-\(\alpha\)-D-glucofuranose.](image)

The C-1 proton of (XXVIII) was now subjected to irradiation, i.e., by applying a strong, stationary radiofrequency field at the resonance frequency of this proton, and the C-2 proton signal (5.25\(\pi\)) was found to collapse from a doublet to a singlet. The C-3 proton
Fig. 1. N.M.R. Spectrum of 5,7-Di-Q-acetyl-6-deoxy-1,2-Q-isopropyldene-
α-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (XXVIII) in CDCl₃ at 100 Mc/s.
Fig. 1a. N.M.R. Spectrum of 5,7-Di-O-acetyl-6-deoxy-1,2-O-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (XXVIII) in CDC\(_3\) at 100 Mc/s, showing splitting patterns of various protons.
Fig. 2. Double-resonance at 100 Mc/s: (A) decoupling of $H_5, H_7$ from $H_6$; (B) decoupling of $H_2$ from $H_1$. 
could not be irradiated in like manner in order to study the C-4 proton (5.70\( \tau \)) since their chemical shifts were too close to each other. However, the coupling constant of C-3 proton, \( J_{3-4} = 1.2 \) cps, assisted in its assignment in the spectrum. By irradiating the C-4 proton, the C-5 proton signal (4.89\( \tau \)) was simplified. Thus from the \( J \)'s (coupling constants) of C-1, C-2, C-3 protons, and in particular from the near zero value of \( J_{2-3} \), the furanoid part of the molecule of (XXVIII) was noted to exist in the same twist conformation as described above for an ester of 1,2-\( \text{D} \)-isopropylidene-\( \alpha \)-D-glucofuranose.

The C-5 proton (4.89\( \tau \)) and the C-7 proton (4.40\( \tau \)) of (XXVIII) both gave very complex splitting patterns. By irradiation of the C-6 deoxy protons (8.00\( \tau \)) the multiplicities of both the C-5 and C-7 protons resonances were reduced; however, these resonances were still too complex for the direct determination of the required coupling constants. Unfortunately the C-6 protons resonances appeared at the same region as the acetate methyl resonances (7.95\( \tau \)) so that these resonances could not be analysed either. Nevertheless it was possible to use the N.M.R. spectrum to assign the configuration C-7. Since the C-7-H resonance was chemically shifted from the C-6-H(2) resonances, and all three were mutually coupled, these resonances might be termed as an \( A(H_{61})B(H_{62})X(H_7) \) system. On a first-order basis the \( X(H_7) \) gave six lines which meant that the first-order approximation did not hold here; hence it was only possible to obtain from the \( X(H_7) \) resonance the sum of \( |J_{61-7} + J_{62-7}| \) which was 12 cps. Insofar that this sum was exactly equal to the sums of \( |J_{1a-2e} + J_{1a-2a}| \) for
1,3,4,6-tetra-O-acetyl-2-deoxy-\(\alpha\)-D-glucopyranose (B) it seemed reasonable to suggest that the geometry of the C\(_6\)-C\(_7\) portion of (XXVII) was closely similar to that of (B). This geometry of (XXVII) could exist in two ways: the chair and the boat form, which could not be distinguished by the N.M.R. data available. The four possible \(*\)stereochemistries for (XXVII) are shown in C, D, E, and F. D and E can be immediately eliminated because

\* Care must be taken to interpret the configuration at C-5, since ambiguity may exist between the Fischer projection and Haworth projection.
they have an equatorial proton at C-7; the total width of the C-7-H resonance would be about 5 cps, which was significantly different from the observed value (12 cps). D can also be eliminated because a boat conformation is less thermodynamically stable. Therefore, the geometry for (XXVIII) is equal to C.

(d) Characterization of the mother liquor of the acetate derivatives of (XXV).

The mother liquor of the acetate derivative of (XXV) was purified by passing through a column of silica gel and eluting with methyl ethyl ketone. Evaporation of the eluant yielded a syrup which had an identical R as the crystalline fraction but a different optical rotation. The N.M.R. spectrum showed a multiplet at 3.70^ equal to the C-7-H; the resonance width here was equal to about 4.8cps. Therefore this compound was the α-anomer (XXIX).

(e) Separation and Identification of Fraction Y: The benzene fraction of the hydroformylation product.

The combined benzene filtrate and petroleum ether washings afforded a colorless syrup upon purification and evaporation to dryness. The syrup (0.412 g.) was designated as Fraction Y.

Fraction Y (0.203 g.) was resolved into five sub-fractions by ascending development on preparative T.L.C. plates of silica gel G., using methyl ethyl ketone-water azeotrope as the developing solvent:
<table>
<thead>
<tr>
<th>Zone</th>
<th>Wt.(g.)</th>
<th>R</th>
<th>M.P.(°C)</th>
<th>$[\alpha]^{22}_D$</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.091</td>
<td>0.86</td>
<td>99-100°</td>
<td>-47°</td>
<td>XXVI</td>
</tr>
<tr>
<td>B</td>
<td>0.004</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.042</td>
<td>0.73</td>
<td>159-160°</td>
<td>+60°</td>
<td>XXV</td>
</tr>
<tr>
<td>D</td>
<td>0.049</td>
<td>0.53</td>
<td>syrup</td>
<td>+8.9°</td>
<td>XXVII</td>
</tr>
<tr>
<td>E</td>
<td>0.005</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The compound obtained from Zone A was characterized as 6-deoxy-5-keto-1,2-O-isopropylidene-\(\alpha\)-D-glucofuranose (XXVI). Its I.R. spectrum showed a strong peak at 1720 cm\(^{-1}\) indicating the presence of a carbonyl group, while its N.M.R. spectrum (Fig.3) gave a sharp singlet at 7.75 ppm attributed to an isolated methyl group. Its mass spectrum gave a mol. wt. 202, and microanalysis gave an elemental composition of C\(_9\)H\(_{14}\)O\(_5\), which agreed with calculated values. The melting point, but not the optical rotation agreed with the literature value (87). However, the optical rotation did agree with that of an authentic sample prepared by another method in our laboratory (88).

Compound (XXVI) (9%) might thus be regarded as a rearrangement product of the starting material. Its presence was not surprising since it had been reported that epoxides rearranged to ketones in an alcoholic solution of dicobalt octacarbonyl (17).

The substance derived from Zone B was suspected of being a branched-chain sugar, since in all probability the additions of the
Fig. 3. N.M.R. Spectrum of 6-Deoxy-5-keto-1,2-0-isopropylidene-
α-D-glucofuranose (XXVI) in CDCl₃ at 100 Mc/s.
formyl group might have occurred at the C-5 of the initial anhydro sugar. This substance did not occur in sufficient quantity for characterization. It had a higher $R_f$ value than compound (XXV) which might suggest that it was less polar.

The substance from Zone D (4%) showed an identical N.M.R. spectrum to a sodium borohydride reduction of (XXV); therefore, this compound (XXVII) was 6-deoxy-1,2-0-isopropylidene-α-D-gluco-hepto-1,4-pyranose. It may be emphasized here that, under the experimental conditions employed in the present research, hydroformylation was the chief course of the reaction and that any alcohol production was due to the further reduction of the aldehyde already formed (12).

Zone E was believed to be the hydrolysed product of (XXV). It was suspected that cobalt hydrotetracarbonyl generated from dicobalt octacarbonyl was sufficiently acidic to hydrolyse the isoporpylidene group. This was only an assumption, since Zone E did not afford sufficient material for characterization.

**EXPERIMENT B.** Hydroformylation of 5,6-dideoxy-1,2-0-isopropylidene α-D-xylo-hex-5-enofuranose (XXXIII).

(a) **Background of hydroformylation of a similar unsaturated sugar.**

Earlier investigation (89) on the oxo reaction of 3-0-acetyl-
5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hex-5-enofuranose (XXX) afforded an inseparable mixture of polyols, the major components of which, as revealed by N.M.R. data, were suspected of being seven-carbon sugar alcohols of the type shown below:

\[
\begin{align*}
\text{CH}_2\text{OH} & \\
\text{OAc} & \\
\text{CH}_3 & \\
\text{CH}_3 & \\
\text{CH}_2\text{OH} & \\
\end{align*}
\]

The reason for the occurrence of such a large number of products (more than seven) are these:

1. High temperatures cause decomposition of reactants and products.
2. High partial pressure of hydrogen causes reduction of both reactants and products.
3. At high temperatures hydrolysis of isopropylidene groups by cobalt hydrotetracarbonyl is facilitated.

On account of the above and in view of the success of the hydroformylation of (XXIV) it was therefore decided to investigate
the reaction of 5,6-dideoxy-1,2-0-isopropylidene-\(\alpha\)-D-xylo-hex-5-enofuranose (XXXIII) as it had a free C-3-OH which would be capable of cyclization with a formyl group. A dialdehyd compound similar to (XXV) was the expected product.

\[
\text{CH}_2 \quad \text{C} \quad \text{H} \quad \text{OH} \quad \text{O} \quad \text{O} \quad \text{CH}_3
\]

\[
\text{OH} / \text{CH}_2 \quad \text{O} \quad \text{CH}_3
\]

\[
\text{NaBH}_4
\]

(b) Work-up of hydroformylation products. The experimental procedure and conditions were identical to those described in Experiment A. After letting the reaction product stand at room temperature for 18 hours the high pressure reaction vessel was opened; there was no crystalline carbohydrate portion but a dark greenish
brown solution. The contents of the vessel were transferred to a flask and refluxed at 60° for two hours in order to decompose the cobalt catalyst. Filtration of the dark solution through a norit-celite column yielded a colorless syrup upon evaporation to dryness. This syrup proved to be very difficult to crystallize although many solvents were tried. However, after 60 days, a few grains of crystal was discovered in the thick syrup. After removing these seed crystals and seeding an ethyl ether-petroleum ether solution of the mixture a pure crystalline compound was obtained. This crystalline fraction was designated as Fraction Z.

Filtration of the crystalline portion afforded a mother liquor which was shown to consist of at least four components by T.L.C. These components were extremely difficult to separate. No further attempts were made to characterize this mother liquor.

(c) **Characterization of Fraction Z:** The crystalline portion of the hydroformylation product of (XXXIII).

Several recrystallization of the crystalline component from ethyl ether-petroleum ether gave a pure compound having a sharp m.p. 101-102° C; R 0.83; and an elemental composition of C_{10}H_{16}0_5. Its mass spectrum revealed a mol. wt. of 216 as was expected. There was no carbonyl peak in its I.R. spectrum; in its N.M.R. spectrum (Fig.4) there appeared multiplets centered at 4.82τ and 5.32τ equal to one proton, the anomeric C-7-H. These resonances thus revealed a mixture of α- and β- anomers. The crystalline substance had an initial optical
Fig. 4. N.M.R. Spectrum of 5,6-Dideoxy-1,2-0-isopropylidene-\(\alpha\)-\(\beta\)-D-xylo-heptodialdo-1,4-furanose-3,7-pyranose (XXXIV) in CDCl\(_3\) at 100 Mc/s.
rotation, \([\alpha]_D^{24} = +18^\circ\) which mutarotated to +46° after 2 hours. On the basis of these evidences, this substance was assigned as (XXXIV). Additional confirmation of (XXXIV) was obtained by elemental and N.M.R. analysis of the following derivatives:

1. 5,6-Dideoxy-\(\beta\)-isopropylidene-\(\alpha\)-D-xylo-heptodialdo-1,4-furanose phenylhydrazone, m.p. 146-149° C; \([\alpha]_D^{24} = -32.3^\circ\) (c 0.65, chloroform).

2. 7-\(\alpha\)-Acetyl-5,6-dideoxy-1,2-\(\beta\)-isopropylidene-\(\alpha\)-D-xylo-heptodialdo-1,4-furanose-3,7-pyranose; colorless syrup, N.M.R. gave multiplets at 3.95T and 4.45T equal to one anomeric C-7-H; a doublet at 4.15T equal to anomeric C-1-H; a singlet at 5.88T equal to C-3-H; a quartet at 5.50T equal to C-4-H; a doublet at 5.30T equal to C-2-H; and multiplets centered at 8.20T equal to four C-5,6-H's; \([\alpha]_D^{24} = +54^\circ\), which represented the optical rotation of a mixture of \(\alpha\)- and \(\beta\)-anomer. Attempt to separate the mixture by T.L.C. plates was not successful.

Sodium borohydride reduction of (XXXIV) afforded a seven-carbon sugar alcohol (XXXV). Since one of the components in the mother liquor (described in section (c)) had an identical \(R_f\) (in various developing solvents) as this sugar alcohol, it might be reasonably to suggest that some hydrohydroxymethylation did take place. From T.L.C. data, (XXXV) occurred to the extend of about 5%.
EXPERIMENT C. NUCLEOSIDE SYNTHESIS

(a) History of Benzimidazole Nucleosides.

Recent work (90,91) has revealed that the biological effect of certain purine nucleosides might be due to the contaminating mercuric ions which were present in concentrations as low as $10^{-8}$ M, introduced during the preparation of the nucleosides via the mercury salt method. In view of this problem, numerous synthetic procedures have been devised to avoid the use of mercury salts of purine and pyrimidine. The most successful method thus far was the fusion method.

The biochemical interest in 2'-deoxy-D-ribofuranosylbenzimidazoles was reported as early as 1956 (92), yet there were no reports of their chemical syntheses until the work of Robins (77,78,79). Such nucleosides, e.g., 5,6-dimethyl-1-(O-D-ribofuranosyl)-benzimidazole, were found to incorporate into vitamin $B_{12}$ in various microbiological systems, without cleavage of the nucleoside linkage (93,94). The importance of vitamin $B_{12}$ as a cofactor in many biochemical reactions was well established (95,96,97). In addition, vitamin $B_{12}$ was found to play a significant role in the conversion of ribonucleotides without glycosidic cleavage in microorganism L. leichmannii (98,99,100). Therefore, it was suggested that these compounds were simulating purine nucleoside analogs since the inhibition of
influenza A virus by 5,6-dichloro-1-\((\beta-D\text{-ribofuranosyl})\)-benzimidazole (DRB) was reversed by adenosine (101) and that DRB interfered with preliminary synthesis of ribonucleic acid (102). Recent work (103) has confirmed this suggestion and has shown that DRB exhibits specific inhibition of chromosomal RNA synthesis. Furthermore, a benzimidazole nucleoside has been isolated as a component of an enzyme system in wheat embryos (104). The desirability of obtaining benzimidazole 2'-deoxynucleosides for biological evaluation thus cannot be denied. In this thesis the syntheses of benzimidazole deoxynucleosides is discussed. Compound (XXVIII) was chosen since it resembled a 2-deoxy-hexopyranose.

![Chemical structures](image.png)
(b) Methods and results.

The procedure used was similar to that described by Robins (79). The results are summarized in the following Table 2.
<table>
<thead>
<tr>
<th>Attempt No.</th>
<th>Carbohydrate</th>
<th>Base</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Press. (mm)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Compound (XXVIII)</td>
<td>B</td>
<td>160°</td>
<td>15</td>
<td>Water-pump</td>
<td>Tan melt; 3 faint spots (2 nucleosides + base) shown in U.V.</td>
</tr>
<tr>
<td>2</td>
<td>Compound (XXVIII)</td>
<td>B</td>
<td>165°</td>
<td>15</td>
<td>10⁻¹</td>
<td>Chiefly sublimation of (XXVIII)</td>
</tr>
<tr>
<td>3</td>
<td>Compound (XXVIII)</td>
<td>B</td>
<td>180-185°</td>
<td>15</td>
<td>10⁻¹</td>
<td>Decomposition of (XXVIII)</td>
</tr>
<tr>
<td>4</td>
<td>Compound (D)</td>
<td>B</td>
<td>170-175°</td>
<td>15</td>
<td>10⁻¹</td>
<td>Chiefly 3 spots (2 nucleosides + base) shown in U.V.</td>
</tr>
<tr>
<td>5</td>
<td>Compound (XXVIII)</td>
<td>B</td>
<td>170-175°</td>
<td>25</td>
<td>10⁻¹</td>
<td>Chiefly 3 spots (2 nucleosides + base) shown in U.V.</td>
</tr>
</tbody>
</table>

Table 2. Fusion of compound (XXVIII) with 5,6-dimethylbenzimidazole (B). Compound (D) was 2-deoxy-1,3,4,6-tetra-O-acetyl-α( and β-)D-glucopyranose. In all experiments chloroacetic acid was used as a catalyst.

4. Data refer to silica gel G, activated at 130° C with 2% G.E. Phosphor impregnated; the developing solvent was methanol:benzene (1:9).
In the first attempt a negligible amount of nucleosides was formed as evidenced by U.V. viewing of T.L.C. plates. One of the spots appeared on spraying with sulfuric acid but did not show up in U.V. By comparing the $R_f$ of this dark spot with that of the starting material, (XXVIII), equal values were obtained, which evidenced that most of the starting material had not reacted. It was interesting to note that nucleosides in general took a little while to show up on T.L.C. sprayed with concentrated sulfuric acid whereas, carbohydrates showed up quite readily once sprayed with sulfuric acid and then heated in an oven. The base (5,6-dimethylbenzimidazole) itself did not show up at all under such conditions, but would show up in U.V. This valuable experience played a vital role in the separation of the fusion mixture in the later stage.

Attempt 2 resulted in much sublimation of the starting (XXVIII). This was due to incomplete mixing of the reactants and that high vacuum was applied too soon to allow a complete melt of all the reactants.

Attempt 3 showed that high temperatures only resulted in decomposition of the carbohydrates. T.L.C. revealed that the melt contained many components as evidenced by spraying with concentrated sulfuric acid.

After these three trials which met with little success, the validity of the fusion method was doubted. Attention was therefore turned to the conventional Davoll-Lowy's mercury salt method. Again the results were only disappointing; the failure of this method will
be the subject of discussion later on.

Thus, it was realized that a study of the fusion method by using a model compound was necessary. It was not difficult to understand after the above three failures that (a) a higher temperature than was required should be avoided; (b) high vacuum should only be applied after a clear melt was obtained since sublimation of the reactant might thus be eliminated; and (c) a homogeneous mixing of the reactants was necessary before fusion since the catalyst was present in such a small amount. Hence it was decided to use 2-deoxy-1,3,4,6-tetra-O-acetyl-D-glucopyranose as a model compound.

The model compound, the base (5,6-dimethylbenzimidazole) together with the catalyst (chloroacetic acid) were first dissolved in absolute methanol. Upon evaporation of the solvent a homogeneous mixture of the reactants was thus secured. A temperature of 170-175°C was regarded suitable from past experience. A high vacuum was applied only one minute after a clear melt was obtained; this was necessary in order to remove the acetic acid produced during the fusion. This procedure showed promise since on T.L.C. two major spots appeared under U.V. in addition to the spot produced by the unreacted base. Although no attempts to separate this melt were made it was suspected that the two intense spots were but nucleosides. This was Attempt 4.

Attempt 5 was achieved in a similar manner as described for Attempt 4. The mixing of the reactants and the removal of the methanol
had to be brief, since chloroacetic acid might be acidic enough to hydrolyse the isopropylidene group of (XXVIII). Separation of the melt was achieved by multiple ascending development on preparative T.L.C. plates (30 plates: 20cm X 20cm X 0.8mm thick) using G.E. Phosphor impregnated silica gel G. From 0.506 g. of the melt the following were obtained:

<table>
<thead>
<tr>
<th>Zone</th>
<th>$R_f$</th>
<th>Wt. (g.)</th>
<th>M.P. (°C.)</th>
<th>$[\alpha]^24_D$</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.44</td>
<td>0.049</td>
<td>168-170°</td>
<td>+120°</td>
<td>(XXXVIII)</td>
</tr>
<tr>
<td>B</td>
<td>0.37</td>
<td>0.045</td>
<td>108-109°</td>
<td>+70°</td>
<td>(XXXVII)</td>
</tr>
<tr>
<td>C</td>
<td>0.28</td>
<td>0.113</td>
<td></td>
<td></td>
<td>Decomposed (XXVIII)</td>
</tr>
<tr>
<td>D</td>
<td>0.19</td>
<td>0.096</td>
<td>201-202°</td>
<td></td>
<td>Unreacted base</td>
</tr>
<tr>
<td>E</td>
<td>0.95</td>
<td>0.095</td>
<td>175-176°</td>
<td>+42°</td>
<td>Unreacted (XXVIII)</td>
</tr>
</tbody>
</table>

Compound (XXXVII) derived from Zone A was characterized by N.M.R. (Fig. 5) as 1-(3-O-acetyl-2-deoxy-6,7-O-isopropylidene-heptodialdo-4,7-furanose-α-L-gulopyranosyl)-5,6-dimethylbenzimidazole. The 5,6-dimethyl protons, which were formerly magnetically equivalent,
now became slightly non-equivalent. This was revealed by the small
difference in their chemical shifts. The quartet for δ-4-H, which
formerly occurred at 5.70τ (see Fig. 1), had now been shifted down-
field to 5.50τ thus overlapping with the doublet for C-2-H. In
addition, the H-4 and H-7 of the dimethylbenzimidazole which were
also formerly equivalent, now became non-equivalent and appeared as
two separate peaks (2.45τ and 2.85τ). A multiplet (4.55τ) with
resonance width of about 9.9 cps indicated that C′-7-H was axial, on
the same basis of argument already given for (XXVIII). Therefore, the
nucleoside was assigned the α-configuration (XXXVII).

Fig. 6 shows the N.M.R. spectrum of (XXXVIII). The multiplet
at 4.10τ with a resonance width of about 4.0 cps indicated that C′-7-H
was equatorial, again on the same basis of argument already give for
(XXIX). The nucleoside had the β-configuration.

One additional interesting aspect of this work has been the
finding that the α-anomer of the nucleoside (XXXVIII) was the more
dextro-rotatory. This was in agreement with Hudon's isorotation
rules (105). However, it should be emphasized here that assignment
of configuration to N-glycosides on the basis of rotation data is
by no means reliable: exceptions to these rules have been found (106).

Substances recovered from Zone C, D, and E were revealed by
N.M.R. as decomposed product of (XXVIII), unreacted 5,6-dimethyl-
benzimidazole, and unreacted (XXVIII), respectively. Since about
65-75% of all the substances were recovered from T.L.C. plates, the
yields of nucleosides were calculated to be about 42%.
Fig. 5. N.M.R. Spectrum of 1-(3'-0-acetyl-2'-deoxy-6',7'-0-isopropylidene-heptodialdo-4',7'-furanose-\(\alpha\)-L-gulopyranosyl)-5,6-dimethylbenzimidazole (XXXVII) in CDCl\(_3\) at 100 Mc/s.
Fig. 6. N.M.R. Spectrum of 1-\((3'-O\text{-acetyl-2'}-deoxy-6',7'-O-iso-
propylidene-heptodialdo-4',7'-furanose-\(\beta\)-L-gulopyranosyl)\)-5,6-dimethyl-
benzimidazole (XXXVIII) in CDCl₃ at 100 MHz.
(c) Mechanism of Reaction of Fusion.

Since an approximately equal amount of the two anomers (XXXVII, XXXVIII) of the nucleoside was obtained from the fusion of one single anomer (XVIII), this finding supported the proposed reaction mechanism (79) which involved alkylation of the benzimidazole nitrogen (tertiary nitrogen with the lone electron pair) by a carbonium ion intermediate. The reaction may thus be regarded to have proceeded by an $S_N^1$ mechanism.
(d) **Conclusion.**

Deoxyribosides are more difficult to synthesize than ribosides because of the labile nature of deoxyribofuranosyl halides which readily lose a molecule of hydrogen halide. At attempt to synthesize 5-O-acetyl-7-chloro-6-deoxy-1,2-O-isopropylidene-α-D-glucopyranose using thionyl chloride in dry pyridine (107) was unsuccessful. The chloro derivative hydrolysed rapidly during the sodium bicarbonate washing. The method utilizing ethereal hydrogen chloride (108) did not work either since the chloro derivative decomposed readily at room temperature after both the ether and hydrogen chloride had been evaporated.

It was thought that perhaps condensation of the titanium chloride complex of compound (XXVIII) with dithyminyl mercury using the procedure devised by Baker and co-workers (109) might give thyminyl nucleosides. But a preliminary attempt using this method involving 2-deoxy-1,3,4,6-tetra-O-acetyl-α( and β-) -D-glucopyranose gave a large number of products. Consequently, it was suspected that (XXVIII) would not work either. Attempt to use benzoates, p-chlorobenzoates, and p-methylbenzoates (106) as blocking groups instead of acetates on compound (XXV) and to couple these derivatives with the more reactive monomercuri pyrimidine⁵ was now considered.

Within the last two decades, the understanding of the important role of nucleic acids in the metabolism of living matters stimulated
the syntheses of many analogs of nucleic acid derivatives as antimi-
metabolites for the chemotherapy of cancers. Success in chemotherapy
has been limited but the investigation might one day prove rewarding.

5. Unlike thymine, N-acetylcytosine forms a mercuri derivative
containing mercury and pyrimidine in a ratio of 1:1. With
an intermediate of this type, two moles of poly-O-acyl-
glycosyl halide are required in order to effect the conden-
sation reaction, a fact which as Fox (110) pointed out,
emphasized the necessity of knowing precisely the type of
mercuri pyrimidine being employed.


EXPERIMENTAL

(I) General Consideration.

High pressure reactions were carried out using an Aminco 2\frac{3}{16}\text{ inches o.d. Micro Series reaction vessel of manganese steel (Americal Instrument Co. Inc., Silver Spring, Md.). Infrared (I.R.) spectra were measured on Perkin-Elmer Model 21 and Model 137 (Sodium Chloride) spectrophotometers. Nuclear Magnetic Resonance (N.M.R.) spectra were recorded at 60 Mc/s and 100 Mc/s on Varian A60 and A100 Spectrometers; Japanese N.M.R. spectrometer (Japan Electronic Optic Lab., Tokyo, Japan) was also used; resonance positions were recorded in ppm from tetramethylsilane as reference, set at zero (external with D₂O, internal with other organic solvents). Double resonance spectra were measured on a Varian D.P. spectrometer, using a Heteronuclear Decoupler (Nuclear Magnetic Resonance Specialties). Thin layer chromatography (T.L.C.) was performed on plates of silica gel G. (acc. to Stahl) with or without 2\% G.E. Phosphor (General Electric Co. Ltd., U.S.A.); zones were located by ultraviolet viewing (U.V.) or by spraying with reagent concentrated sulfuric acid containing 5\% fuming nitric acid and heating at 100°-130°C. Melting points were determined on a Kofler block and were uncorrected. Mass spectroscopy was done with A.E.I.-M.S. 9 spectrometer. Elemental analyses were performed by the Microanalytical Laboratory, University of British Columbia.
Preparations of:

(i) 1,2:5,6-Di-\(\alpha\)-isopropylidene-\(\alpha\)-D-glucofuranose.

Anhydrous reagent grade \(\alpha\)-glucose (300 g.), powdered in a Waring blender, was placed in a dry winchester containing anhydrous acetone (2 liters) placed in an ice bath. Molten zinc chloride (240 g.) was then poured in slowly, followed by 85% phosphoric acid (15 g.). The winchester was fastened onto a shaker. After 3 days at room temperature the undissolved glucose (140 g.) was collected and washed with a little acetone. The filtrate and washings were cooled and made slightly alkaline with sodium hydroxide solution (170 g. of sodium hydroxide in 170 ml. of water). The insoluble inorganic material was removed by filtration and washed with acetone. The almost colorless filtrate and washings were concentrated under reduced pressure, and the residue was diluted with 300 ml. of water and extracted with chloroform (4 x 300 ml.). The combined chloroform extracts were washed with a little water and concentrated to give a white crystalline residue of crude 1,2:5,6-di-\(\alpha\)-isopropylidene-\(\alpha\)-D-glucose; yield: 196 g. (70%, based on the \(\alpha\)-glucose consumed). Recrystallization from ligroin (b.p. 60°-120°C) gave a m.p. 110°-112°C, and \([\alpha]_D^{22} = -13.2°\) (c 1, chloroform) which were in agreement with literature values (111).

(ii) 1,2-\(\alpha\)-Isopropylidene-\(\alpha\)-D-glucofuranose.

1,2:5,6-Di-\(\alpha\)-isopropylidene-\(\alpha\)-D-glucofuranose (100 g.) was dissolved in hot water (60-70°C, 500 ml.). This homogeneous solution
was poured into a large beaker containing Amberlite IR-120 (H⁺) (120 g.) and was well stirred. After 10-15 minutes at 60°, the Amberlite was filtered off by suction and washed with a little water. The combined washing and filtrate was evaporated to dryness under reduced pressure to a solid white cake (80 g.); 95% yield. Recrystallization from ethyl acetate gave a m.p. 159-162° C, [α]$_D^{22}$ -11° (c 1, water) which were in agreement with literature values (112).

(iii) 1,2-O-Isopropylidene-6-O-tosyl-α-D-glucofuranose.

1,2-O-Isopropylidene-α-D-glucofuranose (60 g.) was dissolved in dry pyridine (200 ml), and a solution of p-toluene-sulfonyl chloride (52.5 g.) in dry chloroform (120 ml.) was added at 0° C. The yellow solution was kept at room temperature for 48 hours after which time it was cooled to 0° C and water was added dropwise, followed 30 minutes later by 2N-hydrochloric acid (200 ml). The aqueous extracts were washed successively with 2N-hydrochloric acid (2 x 100 ml.), 1N-sodium hydrogen carbonate (2 x 100 ml.), and water (2 x 100 ml.) and dried over sodium sulphate (anhydrous). Concentration produced a pale yellow syrup which crystallized from ethyl acetate (42.8 g.; 42% yield), m.p. 106-107° C, [α]$_D^{22}$ -8.8° (c 1, chloroform), which agreed with literature values (113).
(iv) 5,6-Anhydro-1,2-0-isopropylidene-α-D-glucofuranose.

1,2-0-Isopropylidene-6-0-p-tosyl-α-D-glucofuranose (20 g.) was dissolved with cooling in dry chloroform (50 ml), and to the cooled solution was added dry methanol (10 ml.) containing sodium (1.5 g.) dissolved in it. The gelatinous mixture was stirred vigorously; water (50 ml) was added after 5-7 minutes to dissolve the sodium p-toluenesulfonate which separated. The chloroform layer was separated, and the aqueous layer was extracted with more chloroform. The extracts were combined and dried over magnesium sulfate (anhydrous). Evaporation of the solution gave a solid which was recrystallized from ethyl acetate, 4.1 g. 38% yield; m.p. 130 -132°C, [α]22D -25.4° (c, 1 water). Literature values (114) were in agreement.

(v) 1,2-0-Isopropylidene-5,6-di-0-tosyl-α-D-glucofuranose.

1,2-0-Isopropylidene-α-D-glucofuranose (30 g.) was dissolved in pyridine (50 ml.) and a solution of toluene-p-sulfonyl chloride in chloroform (50 ml.) was added at 0°C. The yellow mixture was kept at room temperature for 48 hours, then cooled to 0°C; water was added dropwise, followed by the addition of 2N hydrochloric acid (200 ml.). The aqueous layer was extracted with chloroform (3 x100 ml.), the combined extracts were washed successively with 2N-hydrochloric acid, 1N sodium hydrogen carbonate, and water; and dried over sodium sulphate. Concentration produced a pale yellow syrup, which crystallized from ethanol as colorless needles. Further recrystallization from ethanol gave pure di-0-tosyl compound
(26 g., 35% yield), m.p. 159 -160°C, $[\alpha]_D^{22} = -6.5^\circ$ (C 1, chloroform). Literature values (36) were in agreement.

(vi) 5,6-Dideoxy-1,2-0-isopropylidene-$\alpha$-D-xylo-hex-5-enofuranose.

1,2-0-Isopropylidene-5,6-di-O-tosyl-$\alpha$-D-glucofuranose (20 g.) and sodium iodide (35 g.) were heated under reflux for 6 hours in dry methyl ethyl ketone. The precipitated sodium p-toluene-sulfonate was filtered off, washed with methyl ethyl ketone, dried and weighed. Concentration of the combined filtrates produced a brown residue which was fractionated between aqueous sodium thiosulfate and chloroform. The aqueous layer was then extracted with chloroform and the combined chloroform extracts were washed with water, and dried over anhydrous sodium sulphate. Concentration under reduced pressure produced a yellow syrup, a methanolic solution of which was decolorized with norit and concentrated to a solid. Sublimation at 40°-60°C at 10^{-3} mm afforded white needles of 5,6-dideoxy-1,2-0-isopropylidene-$\alpha$-D-xylo-hex-5-enofuranose, 3.2 g., 51 %, m.p. 54-56°C., $[\alpha]_D^{20} = -51.5^\circ$, which were in agreement with Lit. value (36).

(III) Hydroformylation of 5,6-anhydro-1,2-0-isopropylidene-$\alpha$-D-glucofuranose.

To a solution of 5,6-anhydro-1,2-0-isopropylidene-$\alpha$-D-glucofuranose (2.4 g.) in anhydrous benzene (50 ml), contained in the glass lining of a high pressure reaction vessel, was added dicobalt octacarbonyl (0.3 g.) (These operations were performed in
a dry box). The stoppered lining was then inserted into the reaction vessel, which was flushed with carbon monoxide. Reagent carbon monoxide was then added to a pressure of 70 atm. followed by hydrogen to a total pressure of 140 atm. and the reactants were heated, with rocking, at 100-105° C for about 1½ hours. After cooling to room temperature for 18 hours, the unreacted synthesis gas was released and the contents of the vessel and glass lining were transferred to a flask. The crystalline portion was removed by filtration, then washed with petroleum ether (dried over aluminium oxide, b.p. 30-60° C) and kept in a desiccator. This was designated as Fraction X, 2.16 g., 78% yield. The combined filtration and washings was refluxed at 60° C for two hours to decompose the cobalt catalyst, then filtered through a norit-celite column. Evaporation of the solution to dryness yielded a colorless syrup which was designated as Fraction Y (0.412 g.)

Fraction X was recrystallized several times from ethanol-ethyl acetate to yield a pure product. Fraction Y (0.203 g.) was chromatographed into five sub-fractions by preparative T.L.C. using silica gel G (acc. to Stahl): 6 plates (20cm X 80cm), and methyl ethyl ketone-water azeotrope as developing solvent. Detection of zones was done by spraying the outer edges of each plate with concentrated sulphuric acid while covering the middle section of it. As a result only 60-70% of the substances could be recovered.

After their locations the zones were carefully scraped off the plates and extracted with chloroform followed by ethanol-methanol.
Upon evaporation to dryness, these extracts yielded the following: Zone A (0.091 g.), m.p. 99-100° C., [α]$_D^{22}$ - 47°; Zone B (0.004); Zone C (0.042 g.) m.p. 159-160° C.; [α]$_D^{22}$ + 60°; Zone D (0.049 g.), [α]$_D^{22}$ + 8.9°; Zone E (0.005 g.).

(vii) 6-Deoxy-1,2-0-isopropylidene-α-0-glucop-heptodialdo-1,4-furanose Semicarbazone Monohydrate.

Semicarbazone hydrochloride (0.040 g.) was dissolved in 3 drops of distilled water containing sodium acetate (0.040 g.). To this solution was added 6-deoxy-1,2-0-isopropylidene-α-0-glucop-heptodialdo-1,4-furanose-3,7-pyranose which had been dissolved in one drop of water. The combined solution was heated in steam bath for 5 minutes, after which time the solution still remained clear. Upon cooling, the solution was extracted with chloroform. Needle shaped crystals appeared in the aqueous fraction after 24 hr., 0.126 g., 93% yield; m.p. 190-191° C.; [α]$_D^{20}$ - 9° (c 1, water) calcd. for C$_{11}$H$_{16}$N$_3$O$_5$.H$_2$O: C, 43.00%; H, 6.84%; N, 13.68%; found: C, 43.00%; H, 6.66%; N, 14.00%.

(viii) 6-Deoxy-1,2-0-isopropylidene-α-0-glucop-heptodialdo-1,4-furanose Phenylhydrazone.

Phenylhydrazine hydrochloride (0.100 g.) was first dissolved in distilled water (2 ml.); sodium acetate (0.150 g.) was then added. The solution was filtered. To a solution of 6-deoxy-1,2-0-isopropylidene-α-0-glucop-heptodialdo-1,4-furanose-3,7-pyranose (0.030 g.) in one drop of water was then added four drops of the above prepared phenylhydrazine solution. The solution was mixed well by stirring. This resulting
solution was warmed on a steam bath for 1 minute, and crystals
appeared upon cooling in ice bath; 0.032 g., 78%; m.p. 134 -135° C,
\[ \alpha \]_D^{22} = -33° (c 2, chloroform); calcd. for C\textsubscript{16}H\textsubscript{22}N\textsubscript{2}O\textsubscript{5}: C, 59.62%;
H, 6.83%; N, 8.69%; found: C, 59.52%; H, 6.97%; N, 8.83%.

(ix) 5,7-Di-O-acetyl-6-deoxy-1,2-0-isopropylidene-\( \alpha \)-D-gluco-
heptodialdo-1,4-furanose-3,7-pyranose.

To a solution of 6-deoxy-1,2-0-isopropylidene-\( \alpha \)-D-gluco-
heptodialdo-1,4-furanose-3,7-pyranose (1.223 g.) in dry pyridine
(10 ml.) was added acetic anhydride (5 ml.) at 0° C under anhydrous
conditions. After 48 hours at room temperature the solution was
evaporated to dryness in a flash evaporator, then pumped under
high vacuum. A clear glass (1.79 g; 105%) was obtained which
yielded needle shape crystals when a minimal amount of ethyl
acetate was added. The crystals were re-crystallized several times
in ethyl acetate; 1.31 g. (76.5% yield); m.p. 176 -177° C; \( R_f \) 0.73
(81); \[ \alpha \]_D^{22} +42° (c 2, chloroform); calcd. for C\textsubscript{14}H\textsubscript{20}O\textsubscript{8}: C, 53.20%;
H, 6.20%; found: C, 53.12%; H, 6.11%; N.M.R. gave C-7-H signal at
4.36\( \tau \) (J 1,2,2,=12 cps.): C-7-0Ac was \( \beta \).

The mother liquor was passed through a column of silica
gel (2 X 40 cm) and eluted with methyl ethyl ketone. Evaporation
of the eluant yielded a syrup, 0.35 g. (20.6%), \[ \alpha \]_D^{24} +209°;
N.M.R. gave C-7-H signal at 3.70\( \tau \)(J=4.8 cps.): C-7-0Ac was \( \alpha \).
(x) Sodium Borohydride Reduction of 6-Deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose.

To a solution of 6-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (0.030 g.) in methanol (20 ml.) was added sodium borohydride (0.30 g.) at 0°C. After 24 hours at room temperature, acetic acid (1 ml.) was added followed by 10 ml. of water. When effervescence stopped the solution was passed through a column of Amberlite IR-120 (H\(^+\)), then a column of Dowex A-4. The aqueous eluant was evaporated to dryness and azeotroped with methanol then ethanol-benzene, to a syrup (0.027 g., 90% yield), \(R_f\) 0.53 (81), \([\alpha]_D^{24} +16.0^\circ\) (c 1.6, ethanol).

(xi) 5,7-Di-0-Benzoyl-6-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose.

To a solution of 6-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (0.101 g.) in dry pyridine (2 ml.) was added freshly distilled benzoyl chloride (0.5 ml.) at 0°C under anhydrous conditions. After 48 hours at room temperature the solution was evaporated to dryness in a flash evaporator, then passed through a column of silica gel and eluted with ethyl ether. Evaporation of the eluant afforded a syrup which crystallized upon addition of ligroin, 0.106 g., 69% yield. The crystalline portion had a m.p. of 113-121°C; \([\alpha]_D^{24} +6.6^\circ\) (c 0.9, chloroform); the mother liquor had an optical rotation, \([\alpha]_D^{24} +25.4^\circ\) (c 1.1, chloroform).
(xii) 6-Deoxy-5-keto-1,2-0-isopropylidene-α-D-glucofuranose Phenyldrazone.

Phenyldrazine hydrochloride (0.100 g.) was first dissolved in distilled water (2 ml.); then sodium acetate (0.150 g.) was added. The solution was filtered. To a solution of 6-deoxy-5-keto-1,2-0-isopropylidene-α-D-glucofuranose (0.080 g.) in one drop of methanol was then added 2 drops of the above prepared phenyldrazine solution. The resulting orange colored solution was warmed on a steam bath for 1 minute, and the methanol was let to evaporate. Crystals appeared upon cooling of the solution in an ice bath; 0.032 g., 32% yield; m.p. 200-204°C; \([\alpha]_D^{24} \approx -12^\circ (c 0.48,\) chloroform); calcd. for C_{15}H_{20}O_N: C, 61.60%; H, 6.85%; found C, 61.54%; H, 6.78%.

(IV) Hydroformylation of 5,6-Dideoxy-1,2-0-isopropylidene-α-D-xylo-hex-5-enofuranose.

To a solution of 5,6-dideoxy-1,2-0-isopropylidene-α-D-xylo-hex-5-enofuranose (1.40 g.) in anhydrous benzene (50 ml.), contained in the glass lining of a high pressure reaction vessel, was added dicobalt octacarbonyl (0.30 g.). The stoppered lining was then inserted into the reaction vessel which was flushed with carbon monoxide. Reagent carbon monoxide was then added to a pressure of 70 atm., followed by hydrogen to a total pressure of
140 atm., and the reactants were heated, with rocking, at 100-105°C.
for about 1½ hours. After cooling to room temperature for 18 hours,
the unreacted synthesis gas was released and the contents of the vessel
were transferred to a flask. The solution was then refluxed at 60°C.
for 2 hours to decompose the cobalt catalyst; then filtered through
a norit-celite column. Evaporation of the filtrate gave a colorless
syrup which did not crystallize until after 60 days. Seeding an ethyl
ether-petroleum ether solution of this syrup afforded a major crystalline
portion which was designated as Fraction Z (0.820 g.; 50.5%). The
mother liquor (0.804 g.) was not characterized due to the difficulties
of separating the components (more than four).

The crystalline portion of the hydroformylation product was
recrystallized several times from ethyl ether-petroleum ether, m.p.
103-104°C; homogeneous, $R_F$ 0.83 (81); mol. wt. 216 (the base peak in
the mass spectrum was at $M^+ - 15$ (loss of CH$_3$)) ; calc'd. for C, H, O : $^{10}$ $^{16}$ $^{5}$
C, 55.60%; H, 7.40%; found: C, 55.55%; H, 7.10%; the crude crystalline
fraction had an initial $[\alpha]_D^{24}$ +18° which mutarotated to +36.4° after
1½ hours; and gave a positive Schiff test but did not show a carbonyl
peak in its I.R. spectrum. Its N.M.R. spectrum gave multiplets at
4.82$\tau$ and 5.32$\tau$ equal to one anomeric C-7 H, and a multiplet at 8.20$\tau$
equal to four C-5 and C-6 protons.
(xiii) 5,6-Dideoxy-1,2-0-isopropylidene-α-D-xylo-heptodialdo-1,4-1,4-furanose Phenylhydrazone.

Phenylhydrazone hydrochloride (0.200 g.) was first dissolved in 3 ml. of distilled water; sodium acetate (0.300 g.) was then added. The solution was filtered. To a solution of 5,6-dIDEOXY-1,2-0-isopropylidene-α-D-xylo-heptodialdo-1,4-furanose-3,7-pyranose (0.090 g.) in one drop of water was then added 4 drops of the above prepared solution. The resulting orange colored solution was warmed on a steam bath for 1 minute, and crystals appeared upon cooling in an ice bath; 0.056 g., 49%; m.p. 146-149° C., \([\alpha]_D^{24} = -32.3^\circ\) (c 0.65, chloroform); cald. for C_{16}H_{22}N_{2}O_4: C, 62.74%; H, 7.18%; N, 9.10%; found:C, 62.82%; N, 8.92%.

(xiv) 7-0-Acetyl-5,6-dideoxy-1,2-0-isopropylidene-α-D-xylo- heptodialdo-1,4-furanose-3,7-pyranose.

To a solution of 5,6-Dideoxy-1,2-0-isopropylidene-α-D-xylo-heptodialdo-1,4-furanose-3,7-pyranose (0.040 g.) in dry pyridine (4 ml.) was added acetic anhydride (1 ml.) at 0° C under anhydrous conditions. After 48 hours at room temperature the solution was evaporated to dryness; then passed through a column of silica gel and eluted with ethyl ether. A colorless syrup (0.032 g.; 60%) was obtained; \([\alpha]_D^{24} = +54^\circ\) (which represented the optical rotation of both α and β anomer.) (c 2.65, chloroform); T.L.C. revealed 2 spots (by more than 3 developing solvents).
(xv) Sodium Borohydride Reduction of 5,6-Dideoxy-1,2-0-isopropylidene-\(\alpha-D-xylo\)-heptodialdo-1,4-furanose-3,7-pyranose.

To a solution of 5,6-dideoxy-1,2-0-isopropylidene-\(\alpha-D-xylo\)-heptodialdo-1,4-furanose-3,7-pyranose (0.045 g.) in methanol (20 ml.) was added sodium borohydride (0.45 g.) at 0° C. After 24 hours at room temperature, acetic acid (2 ml.) was added followed by water (10 ml.) When effervescence stopped the solution was passed through a column of Amberlite IR-120 (\(H^+\)), then through a column of Dowex A-4. The aqueous eluant was evaporated to dryness and azeotroped with methanol, then with ethanol-benzene, to a syrup (0.035 g., 75%), \(R_f\) 0.46, \([\alpha]_D^{24} +1.6^\circ\) (c 2.4, ethanol). Its N.M.R. spectrum gave a multiplet at \(\delta 8.10^\circ\) equal to four C-5,6 protons, a triplet at \(6.25^\circ\) equal to two C-7 protons, which were in agreement with the assigned structure for compound (XXXV).

(V) Nucleoside Synthesis: Fusion of 5,7-Di-0-acetyl-6-deoxy-1,2-0-isopropylidene-\(\alpha-D-gluco\)-heptodialdo-1,4-furanose-3,7-pyranose (XXVIII) with 5,6-Dimethylbenzimidazole, Using Chloroacetic Acid as a Catalyst.

Attempt 1. Compound (XXVIII) (0.051 g.), 5,6-dimethylbenzimidazole (XXXVI) (0.023 g.), and a catalytic amount of chloroacetic acid (0.001 g.) were mixed and heated at 160° C. for 15 minutes under reduced pressure (water-pump). The resulting melt showed 3 faint spots on T.L.C. viewed under U.V., while a dark spot showed up only when the plate was sprayed with concentrated sulfuric acid.
Attempt 2. Compound (XXVIII) (0.048 g.), 5,6-dimethylbenzimidazole (XXXVI) (0.022 g.) and chloroacetic acid (0.001 g.) were mixed and heated at 165° C. for 15 minutes under vacuum (10⁻³ mm.). A large amount of (XXVIII) sublimed on the upper part of the container; a small quantity of fusion melt was obtained which gave 3 faint spots on T.L.C. viewed under U.V.

Attempt 3. Compound (XXVIII) (0.048 g.) and (XXXVI) (0.022 g.) and chloroacetic acid (0.001 g.) were mixed and heated at 185° C. for 3 minutes; then high vacuum was applied to the system. Heating was continued for another 12 minutes. A dark melt was obtained which showed more than 7 spots in acid spray.

Attempt 4. See Experimental Section (VI).

Attempt 5. Compound (XXVIII) (1.223 g.) and 5,6-dimethylbenzimidazole (0.970 g.) and chloroacetic acid (0.026 g.) were first intimately mixed by dissolving in methanol, then evaporating all solvent in high vacuum to yield a homogeneous mixture. This mixture was fused at 170-175° C. After 1½ minutes when a clear melt was obtained, high vacuum was applied. Heating was continued for another 24 minutes. Separation of the melt was achieved by multiple ascending development on preparative T.L.C. plates (30 plates: 20cm X 20cm X 0.8mm) of 2% G.E.Phosphor (General Electric Co. Ltd, U.S.A.) impregnated silica gel G., using methanol-benzene (1:9) as the developing solvent. Location of zones was done by viewing the plates under U.V. From 0.516 g. of the melt, 5 zones were obtained: Zone A (0.049 g.), m.p. 168-170° C, [α] D24 +120°; calcd. for C₂₁H₂₆N₂O₆: C, 62.68% ;
H, 6.46%; N, 6.96; found: C, 54.60%; H, 6.80%; N, 6.11%; Zone B
(0.045 g.), m.p. 108-109° C, $\alpha_{D}^{24} +70$ ; calcd. for $C_{21}H_{26}N_{2}O_{6}$:
C, 62.68%; H, 6.46%; N, 6.96%; found: C, 56.10%; H, 6.79%;
N, 6.85%. Zone C (0.113 g.), decomposed product of compound (XXVIII).
Zone D (0.096 g.), m.p. 201-202° C, unreacted base. Zone E (0.095 g.),
m.p. 175-176° C, unreacted (XXVIII).
Microanalyses of compounds from zones A and B did not give satisfactory
results due to impurities present (possibly silica gel) since some
ash was discovered in the boat during microanalysis.

(VI) Fusion of 2-Deoxy-1,3,4,6-tetra-O-acetyl-$\alpha$(and $\beta$)-D-
glucopyranose with 5,6-Dimethylbenzimidazole, Using Chloro-
acetic Acid as Catalyst.

2-Deoxy-1,3,4,6-tetra-O-acetyl-$\alpha$(and $\beta$)-D-glucopyranose
(0.300 g.) and 5,6-dimethylbenzimidazole (0.164 g.) and chloroacetic
acid (9.525 g.) were first dissolved in methanol; then evaporated
to dryness in high vacuum. The resulting mixture was fused at
170-175° C for 25 minutes in high vacuum. On T.L.C. plates, this
melt revealed 3 intense spots under U.V. No attempts were made to
isolate these components.
(xvi) 2-Deoxy-1,3,4,6-tetra-O-acetyl-αβ-D-glucopyranose.

2-Deoxy-D-glucose (2.5 g.) was dissolved in dry pyridine (10 ml.). Acetic anhydride (7.5 ml.) was added at 0° C under anhydrous conditions, and the mixture was allowed to stand overnight. It was then poured into water (100 ml.), and stirred for 1 hour, and extracted three times with chloroform (50 ml.). The extract was washed with 25% hydrochloric acid, water, and finally with saturated sodium bicarbonate solution, then dried over anhydrous sodium sulfate, filtered, and stripped of the solvent in a flash evaporator; 5.80 g. (109% yield) of a clear, crude, syrupy product was obtained. The syrup was passed through a column of silica gel (2 x 60 cm) and eluted with ethyl acetate to remove any impurities. Evaporation of the eluant yielded a syrup which crystallized after 10 days; \( [\alpha]_D^{24} +32^\circ \). The optical rotation corresponded to a mixture containing both α- and β-anomer and was in agreement with literature value (115). No attempt was made to solate these isomers before use in the fusion experiment.

(xvii) N-Acetylcytosine.

Under anhydrous conditions cytosine (0.800 g.) was refluxed for 4 hours in acetic anhydride (20 ml.) The pale brown mixture was allowed to stand at 0° C overnight. The colorless crystalline product was collected on a filter and washed well with cold ethanol and water; yield, 0.75 g. (70%), m.p. 325° C (d) which agreed with literature value (116).
(xviii) **N-Acetylcystosine Mercury.**

To a 1-liter, wide-mouthed Erlenmeyer flask equipped with a mechanical stirrer was added water (370 ml.) and N-acetylcystosine (0.700 g.). While stirring the suspension vigorously 1N sodium hydroxide (5 ml.) was added. Solution was complete within a few minutes. Addition of mercuric chloride (1.35 g.) in ethanol (15 ml.) was done immediately. The pH of the turbid mixture was about 5. While stirring, the mixture was warmed to 70° C, then cooled to about 40° C. It was brought to pH 7 by the addition of sodium hydroxide (6 ml.; 1.0 N). After again warming to 70° C, the suspension was cooled several hours at 0-5° C. The colorless solid was collected on a filter paper and washed well with water until free of chloride ion (tested by a solution of silver nitrate). It was then washed with ethanol and dried at 100-110° C; yield of colorless powder was essentially quantitative. The product was kept in the dark as it turned dark when exposed to light.

(xix) **Dithyminelmercury.**

Thymine (1.26 g., 0.01 mole) was dissolved in hot water (40 ml.) containing sodium hydroxide (0.40 g.). To the clear solution, 10 ml of an alcoholic solution containing 1.35 g. (0.005 mole) of mercuric chloride was added. A fine, white precipitate formed immediately. The resulting pH of the solution was approximately 5. After cooling several hours at 5-10° C, the amorphous precipitate was collected on a filter paper and washed repeatedly with water until the filtrate was free from chloride ion. The
white solid was washed successively with ethanol and ether and dried at 110°C; yield, 2.2 g., 98%; indefinite m.p. above 320°C which was in agreement with literature value (117).

Attempted Preparation of 5-O-Acetyl-7-chloro-6-deoxy-1,2-0-isopropylidene-$\alpha$-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose, by Using Ethereal Hydrogen Chloride.

5,7-Di-O-acetyl-6-deoxy-1,2-0-isopropylidene-$\alpha$-D-gluco-heptodialdo-1,4-furanose-3,7-pyranose (0.045 g.) was dissolved in 30 ml. of absolute ether in a 50-ml Erlenmeyer flask fitted with an injector septum (from a serum bottle). A hypodermic needle connected to a hydrogen chloride source was inserted through the septum into the ether solution. Another hypodermic needle connected to a drying tube was inserted likewise but above the ether layer. The system was cooled in an ice bath, and the ether solution was thus bubbled with hydrogen chloride gas for 5 minutes. The needles were then removed and the clear solution was kept in a cold room (-10°C). After 7 days the solution which had now turned slightly yellow and turbid was evaporated to dryness first with a flash evaporator then under high vacuum at room temperature. During evaporation the syrup changed rapidly to a dark gummy layer and T.L.C. showed many components. No further attempt was made to identify this decomposed mixture.

*Hydrogen chloride gas from gas cylinder was passed through a concentrated sulfuric acid medium.
(xxi) Attempted Preparation of $\beta$-Acetyl-7-chloro-6-deoxy-1,2-$\alpha$-isopropylidene-$\alpha$-gluco-heptodialdo-1,4-furanose-3,7-pyranose, by Using Thionyl Chloride in Pyridine.

Compound (V) (0.030 g.) was dissolved in dry pyridine and thionyl chloride (5 drops) was added under anhydrous conditions. After 48 hours at room temperature the solution was poured into 10 g. of ice, followed 30 minutes later by 1N-hydrochloric acid (10 ml). The solution was extracted with chloroform (2 X 10 ml.) and the extracts were washed with saturated sodium bicarbonate solution (2 X 10 ml.) Evaporation of the chloroform layer did not yield any carbohydrate which presumably had been hydrolysed by the sodium bicarbonate solution and remained in the aqueous extract. No attempt was made to recover the sugar.

(xxii) Attempted Synthesis of 1-(2'-Deoxy-3',4',6'-tetra-$\alpha$-glucopyranosyl)-thymine Using Titanium Tetrachloride.

To a solution of 0.090 of 2-deoxy-1,3,4,6-tetra-$\alpha$-$\beta$-D-glucopyranosyl in 10 ml. ethylene dichloride was added a solution of 0.030 ml. of titanium tetrachloride in 0.40 ml. of ethylene dichloride. A yellow solution was obtained after refluxing for 30 minutes. The solution was then added to a stirred suspension of dithyminylmercury (0.08 g.) in 10 ml of ethylene dichloride. The solution rapidly turned dark and a black precipitate separated. T.L.C. of the dark solution revealed a large number of components. No further attempt was made to work up the mixture as described in literature (109).
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