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Deoxy Sugars

A symposium co-sponsored by the Division of Carbohydrate Chemistry and the Division of Microbial Chemistry and Technology at the 152nd Meeting of the American Chemical Society, New York, N. Y., Sept. 13-14, 1966.

Stephen Hanessian

Symposium Chairman

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FOREWORD

ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented, their papers distributed among several journals or not published at all. Papers are refereed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications. Papers published in ADVANCES IN CHEMISTRY SERIES are original contributions not published elsewhere in whole or major part and include reports of research as well as reviews since symposia may embrace both types of presentation.

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PREFACE

The last five years have witnessed a reawakening interest in modern carbohydrate chemistry, a feature which is reflected in the number of symposia held by the Division of Carbohydrate Chemistry during national meetings of the American Chemical Society. The scheduling of a symposium on Deoxy Sugars for the fall meeting of 1966 seemed very opportune if not somewhat overdue.

Efforts toward the organization of this symposium were started early in 1965, and we were quite fortunate to have among the scheduled speakers several European scientists who have pioneered in the area of deoxy sugars. Regrettably some of those could not eventually participate, but it is still evident from the roster of participants that representation from foreign countries almost equalled that from the United States. Since reviews on the subject were somewhat scarce it seemed worthwhile to compile under one cover expanded versions of the individual topics that were discussed during the day-and-a-half symposium. The topics were selected so as to encompass four general aspects of deoxy sugars. One phase of the symposium was devoted to presentations on the synthesis and selected properties of various biologically-derived deoxy sugars or suitable model compounds. The first chapter in this monograph is thus concerned with details of the utilization of stable crystalline glycosyl halide derivatives in the synthesis of biologically important glycosides and nucleosides of deoxy sugars (Zorbach and co-workers). The total structure of the antibiotic Kasugamycin containing an unusual polydeoxy diaminosugar is presented in detail by Maeda and co-workers. The synthesis and properties of certain methylated deoxy sugars which are constituents of antibiotics and cardiac glycosides are the subject of the chapter by Stacey and co-workers.

The deoxy cyclitols, long-known naturally occurring cyclohexane derivatives are discussed by McCasland, with special reference to configurational effects and detailed NMR spectral data. L. Szabó reviews the synthesis and reactions of phosphorylated deoxy sugars, with particular emphasis on the selection of proper O-protecting groups. The methods available for the quantitative analysis of deoxy sugars, especially those based on the formation of malondialdehyde are discussed in detail by Patricia Szabó.

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A second phase in the symposium was involved with topics dealing with synthetic and mechanistic aspects of deoxy sugars. The synthesis of various unsaturated sugar derivatives of the vinyl ether type which can be considered as precursors to deoxy sugars, is thus described in detail by Hough and co-workers. Overend reviews the synthetic work aimed at devising new syntheses of deoxy sugars with particular emphasis on the rearrangement of 2-hydroxyglycals and ring expansion reactions. Some approaches to the synthesis of halodeoxy sugars with special reference to the utilization of new reagents is the subject of the chapter by Hanessian.

Finally, a third phase of the program was devoted to two presentations dealing with the utility of physical methods, particularly NMR spectroscopy and mass spectrometry, in the study of deoxy sugars and their derivatives. In this monograph, the potential application of nuclear magnetic double resonance to halodeoxy sugars and some unsaturated sugars is discussed by Hall and Manville with reference to stereospecific dependencies and coupling constants. The electron-impact-induced fragmentation of glycosides and O-isopropylidene ketals of several deoxy sugars is discussed by DeJongh, Hribar, and Hanessian. The mass spectra of isomeric deoxy sugars are interpreted with the aid of metastable peaks and deuterium exchange studies.

A presentation by Webber and co-workers dealing with the synthesis of fluorodeoxy sugars regrettably had to be left out of this monograph because of unexpected difficulties in the preparation of the manuscript. The fourth aspect was represented in the symposium by two presentations involving important aspects of deoxy and dideoxy sugars by Osborn and Heath respectively which unfortunately were not incorporated in this monograph.

Finally I would like to express my sincere thanks to all the participants in this first symposium on Deoxy Sugars, and acknowledge the cooperation to those who were able to contribute to this monograph.

Ann Arbor, Michigan
March 29, 1968

STEPHEN HANESSIAN

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The Direct Synthesis of Deoxyglycosides Employing Crystalline O-Acyldeoxyglycosyl Halides

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The preparation of a stable, crystalline O-*acylglycosyl halide of 2-deoxy-*D-arabino-*hexofuranose and* O-p-*nitrobenzoylglycopyranosyl halides of four 2-deoxy sugars is discussed; their utility in the direct synthesis of biologically important 2-deoxyglycosides is demonstrated by successful couplings with cardiac aglycons or with dialkoxypyrimidines. Tri-*O-*benzoyl-*α*-*D-*rhamnosyl bromide couples with two cardiac aglycons to give two cardenolides having the "unnatural", α-*D-*configuration. 4-*O-*Benzoyl-2,3-*O-*carbonyl-6-deoxy-*α*-*D-*mannosyl bromide also couples with cardiac aglycons, resulting in two 1,2-*cis *cardenolides, each having the β-*D *configuration. Exploration of some routes to a halide of 2-deoxy-*D-ribo-*hexofuranose is delineated.*

In 1958, we reported (*19*) on the preparation of two crystalline, stable acylglycosyl halides of digitoxose (2,6-dideoxy-D-*ribo*- hexose), which occurs as the carbohydrate component of cardiac glycosides obtained from *Digitalis* spp. Prior to this work, the only report of a crystalline *O*-acylglycosyl halide of a 2-deoxy sugar was given by Bergmann and co-workers (*1*) who prepared 3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-*arabino*-hexosyl bromide. The bromide is unstable and cannot be stored; consequently, it was added to methanol containing silver carbonate, giving methyl 2,3,4-tri-*O*-benzoyl-2-deoxy-β-D-*arabino*-hexoside. Failure to secure crystalline, relatively stable halides of 2-deoxy sugars (therefore, having utility in the direct synthesis of 2-deoxyglycosides) was undoubtedly a major barrier to the synthesis of 2-deoxyglycosides, as evidenced by the scarcity of published material on this subject prior to 1958.

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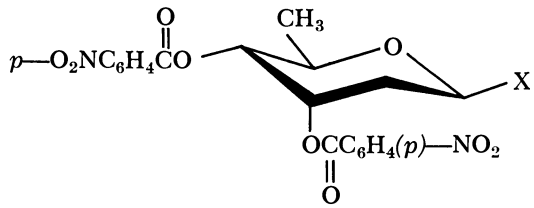
Preliminary investigations (*19*) with digitoxose, employing, alternatively, its triacetate or tribenzoate, failed to yield a crystalline halide. However, when the hexose was *p*-nitrobenzoylated, a high-melting tris-*p*-nitrobenzoate was obtained (*19*). The latter, when treated with a little more than one equivalent of hydrogen bromide in dichloromethane, yielded crystalline 2,6-dideoxy-3,4-di-*O*-*p*-nitrobenzoyl-D-*ribo*-hexosyl bromide (**1a**) (*19*). The choice of dichloromethane was fortuitous; owing to its low solubility in this solvent, the liberated *p*-nitrobenzoic acid separated in almost quantitative yield. Filtration left a solution containing the desired bromide (**1a**), which crystallized readily from ether-dichloromethane. Crystalline **1a** was extremely reactive; consequently, the less reactive chloride (**1b**), likewise crystalline, was prepared in an analogous manner (*19*).



1a, X=Br ([M] = + 70800)

1b, X=Cl ([M] = + 86500)

Assuming the chair form shown is the correct one, the halides **1a** and **1b** most likely have the β-D anomeric configuration, in which the halogen atom is an equatorial substituent. Inasmuch as the two halides lack an oxygenated substituent at C–6, they should behave like 2-deoxypentopyranosyl halides, in that the substituent at C–3, rather than C–5, should govern the configuration about C–1. Were the halogen atom an α (and, therefore, axial) substituent, a severe 1,3-interaction with the C–3 *p*-nitrobenzoyloxyl group would result. Support for the tentative assignment (*20*) of the β-D-configuration to the halides (**1a** and **1b**) is provided by the fact that the molecular rotation of the chloride (**1b**) is greater than that of the bromide (**1a**), a reversal of the relationship that obtains with α-D-aldopyranosyl halides.

Preliminary experiments on the coupling of the chloride (**1b**) with digitoxigenin (3β,14β-dihydroxy-5β-card-20(22)-enolide) in the presence of silver carbonate, led to gross decomposition of the halide, and it was suspected that, under the conditions of the experiments, the silver carbonate was causing elimination of hydrogen chloride. When, however, digitoxigenin was treated with an excess of **1b** in a small volume of

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dichloromethane, there was formed, in 45% yield, digitoxigenin α-digitoxoside (3β-*O*-(2,6-dideoxy-α-D-*ribo*-hexopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide) (*20*). The preparation of the latter was a significant development in the chemistry of 2-deoxyglycosides, in that it constituted, simultaneously, the first synthesis of a 2-deoxycardenolide and the first recorded instance in which a biologically important 2-deoxyglycoside was synthesized using a crystalline *O*-acyl-2-deoxyglycosyl halide. The α-digitoxoside and the naturally occurring evatromonoside (*13*) (3β-*O*-(2,6-dideoxy-β-D-*ribo*-hexopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide) are the first known anomeric pair of cardenolides; when assayed intravenously in cats, the α-D anomer showed a potency only two-fifths that of evatromonoside.

The possibility of synthesizing evatromonoside by a direct method has been investigated (*17*). Digitoxigenin and digitoxose were dissolved in a relatively small volume of *p*-dioxane, and the solution was treated with a small proportion of hydrogen chloride in dichloromethane. The acid was neutralized, and the crude, solid product was chromatographed, giving an approximately 1:1 mixture of the anomeric digitoxosides in a combined yield of 10% based on digitoxigenin.

Having demonstrated the utility of 2,6-dideoxy-3,4-di-*O*-*p*-nitrobenzoyl-β-D-*ribo*-hexosyl chloride (**1b**) in a cardenolide synthesis, it was of interest to determine whether the halide could be successfully employed in the synthesis of nucleosides, thereby broadening the scope of this direct method of synthesis of 2-deoxyglycosides. Attempts to couple **1b** with dithyminyl mercury resulted only in elimination of hydrogen halide from the molecule, as evidenced by the isolation from the reaction mixture of a carbohydrate product that had a composition agreeing with that for 1,2,6-trideoxy-3,4-di-*O*-*p*-nitrobenzoyl-D-*ribo*-hex-1-enopyranose (*15*). However, **1b** reacted smoothly with 2,4-diethoxy-5-methylpyrimidine to give, after de-ethylation and saponification, 1-(2,6-dideoxy-β-D-*ribo*-hexopyranosyl)thymine (*15*). It also reacted with chloromercuri-*N*-benzoyladenine to give, after debenzoylation, 9-(2,6-dideoxy-β-D-*ribo*-hexopyranosyl)adenine (*14*).

In extending this direct method of synthesis, we next investigated the possibility of preparing similarly constituted halides from 2-deoxy-D-*arabino*-hexose (2-deoxy-D-glucose) (*21*). The hexose was subjected to a partial anomerization procedure described by Bergmann and co-workers (*1*). The solid material obtained by this procedure is a mixture of the anomeric forms of 2-deoxy-D-*arabino*-hexose; low temperature *p*-nitrobenzoylation of the latter in pyridine resulted in a mixture of crystalline, anomeric tetrakis-*p*-nitrobenzoates in a ratio of approximately 1:1. They were readily separable by fractional recrystallization, and treatment of either with an excess of hydrogen bromide in dichloromethane, or with

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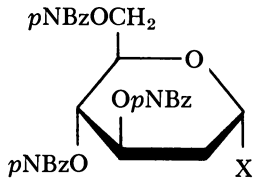
hydrogen chloride in dichloromethane, resulted in 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexosyl bromide (**2a**) and the corresponding chloride (**2b**), respectively (*21*). Both halides are crystalline and high melting, and have a stability which appears to exceed that of *O*-acetyl-glycosyl halides of normal hexoses.



2a, X = Br

2b, X = Cl where *p*NBz is *p*-nitrobenzoyl

Taking precedent from the reaction conditions employed in the synthesis of the α-digitoxoside of digitoxigenin, attempts were made to couple the chloride (**2b**) with digitoxigenin in the absence of an acid acceptor. Even on prolonged standing, there was no evidence of even partial coupling having taken place, and the starting materials were recovered unchanged (*21*). When the more reactive bromide (**2a**) was employed, glycoside formation took place; however, the product was shown to be 3β-*O*-(2-deoxy-α-D-*arabino*-hexopyranosyl)-5β-carda-8(14), 20(22)-dienolide, in which removal of the C–14 hydroxyl group from the aglycon moiety was brought about by the liberated hydrogen bromide. A second coupling was performed in the conventional manner, using the less reactive chloride (**2b**). Treatment of digitoxigenin with a 1,2-dichloroethane solution of **2b** in the presence of silver carbonate gave, after saponification of the reaction mixture, 35% of an approximately 1:1 mixture of both anomers of the 2-deoxy-D-*arabino*-hexopyranosides of digitoxigenin (*21*).

The bromide (**2a**) reacted smoothly with 2,4-diethoxy-5-methylpyrimidine to give, after de-ethylation and deacylation, 1-(2-deoxy-β-D-*arabino*-hexopyranosyl)thymine (**3**) (*15*). The new nucleoside (**3**) is the first truly competitive inhibitor of a pyrimidine phosphorylase (*7*), that is, it inhibits the phosphorylase, yet is not a substrate for the enzyme. It was recently shown that **3** enhances the incorporation of 2′-deoxy-5-iodouridine *in vivo* in cats (*8*).

The bromide (**2a**) reacted also with chloromercuri-*N*-benzoyladenine to give, after debenzoylation, 9-(2-deoxy-β-D-*arabino*-hexopyranosyl)-adenine (*15*). A later paper reported (*18*) on the coupling of **2a** with

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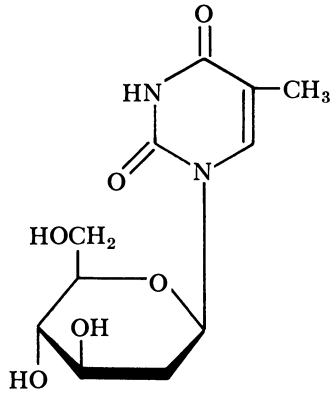
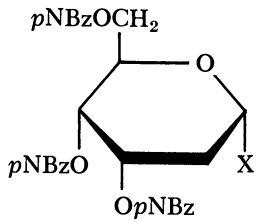
2,4-diethoxypyrimidine to give 1-(2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-*arabino*-hexosyl)-4-ethoxy-2(1*H*)-pyrimidinone. De-ethylation and de-acylation of the latter gave 1-(2-deoxy-β-D-*arabino*-hexopyranosyl)uracil, whereas ammonolysis of the protected intermediate led to 1-(2-deoxy-β-D-*arabino*-hexopyranosyl)cytosine.



3

2-Deoxy-D-*ribo*-hexose has not been reported to occur naturally, but it may be synthesized in five steps, starting with methyl α-D-glucopyranoside (*10, 11, 19*). Low temperature *p*-nitrobenzoylation of 2-deoxy-D-*ribo*-hexose in pyridine gave an anomerically pure tetrakis-*p*-nitrobenzoate, presumably having the β-D configuration (*14*). Treatment of the nitrobenzoate in dichloromethane with hydrogen bromide or with hydrogen chloride gave crystalline 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*ribo*-hexosyl bromide (**4a**) and the corresponding, crystalline chloride (**4b**), respectively (*14*). Both halides are high melting and extremely stable.



4a, X = Br

4b, X = Cl

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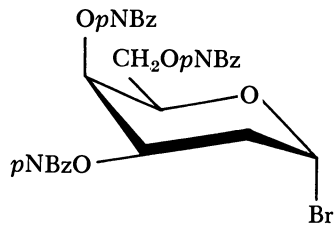
Coupling of the bromide (**4a**) with digitoxigenin, using freshly prepared silver carbonate, gave, after saponification of the reaction products, 46% of 3β-*O*-(2-deoxy-β-D-*ribo*-hexopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (*14*). The bromide (**4a**) also coupled with chloromercuri-*N*-benzoyladenine or with 2,4-diethoxy-5-methylpyrimidine to give, after removal of the protecting groups in each case, 9-(2-deoxy-β-D-*ribo*-hexopyranosyl)adenine and 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)thymine, respectively (*22*). In later work, **4a** (*18*) was coupled with 2,4-diethoxypyrimidine to give 1-(2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-*ribo*-hexosyl)-4-ethoxy-2(1*H*)-pyrimidinone, which underwent de-ethylation and deacylation to yield 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)uracil. Ammonolysis of the protected intermediate led directly to 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)cytosine.

With a view to preparing "fraudulent" nucleosides containing a "2-deoxy-D-galactose" residue, the preparation of a stable halide of 2-deoxy-β-D-*lyxo*-hexose (2-deoxy-D-galactose) was investigated. *p*-Nitrobenzoylation of 2-deoxy-β-D-*lyxo*-hexose was performed in the usual manner, to give an anomerically pure tetrakis-*p*-nitrobenzoate, presumably having the same configuration as the starting material. Treatment of the latter in dicholoromethane with hydrogen bromide gave 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide (**5**) as crystalline, high-melting



5

material. It reacted readily with methanol in the presence of silver carbonate, by inversion, to give methyl 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-*lyxo*-hexoside, and methoxide-catalyzed saponification of the latter gave crystalline methyl 2-deoxy-β-D-*lyxo*-hexopyranoside.

The bromide (**5**) failed to couple with dialkoxypyrimidines, even at elevated temperatures; the failure is probably because of steric conditions imposed by the axially oriented C–4 *p*-nitrobenzoyloxyl group (*see* structure **5**); this same group in the other halides (**1a, 1b, 2a, 2b, 4a,** and **4b**) discussed in the foregoing is an equatorial substituent. Accordingly, the preparation of a differently constituted halide of 2-deoxy-D-*lyxo*-hexose is being investigated for the synthesis of pyrimidine nucleosides.

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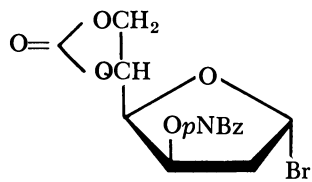
The discovery that "2-deoxy-D-glucosylthymine" is a pyrimidine phosphorylase inhibitor prompted the preparation of pyrimidine nucleosides containing, as the sugar residue, 2-deoxy-D-*arabino*-hexofuranose. We considered means, therefore, for securing a properly constituted halide for preparing the corresponding furanosyl nucleosides. For obvious reasons, the preparation of such a halide would be considerably more complicated than the relatively routine procedures for preparing *O*-acylglycopyranosyl halides.

Treatment of "2-deoxy-D-glucose" in methanol, for 15 minutes, led (*2*) to an anomerically pure methyl 2-deoxy-D-*arabino*-hexofuranoside, as well as preponderant amounts of an approximately 1:1 mixture of the anomeric 2-deoxy-D-*arabino*-hexopyranosides, and unconsumed "2-deoxy-D-glucose." *p*-Nitrobenzoylation of the sirupy, quadripartite mixture gave a mixture of *p*-nitrobenzoic esters from which, by fractional recrystallization, there was obtained in pure form a methyl 2-deoxy-3,5,6-tri-*O*-*p*-nitrobenzoyl-D-*arabino*-hexoside. Attempts to replace directly the severely hindered methoxyl group at C–1 of the latter by hydrogen halide failed; therefore, the nitrobenzoylated furanoside was deacylated, giving a crystalline methyl 2-deoxy-D-*arabino*-hexofuranoside. On conformational grounds, and because of its strongly positive specific rotation, the α-D anomeric configuration is provisionally assigned to the furanoside. Treatment of the latter with carbonyl chloride gave methyl 5,6-*O*-carbonyl-2-deoxy-α-D-*arabino*-hexofuranoside, and the unsubstituted hydroxyl group at C–3 was protected by *p*-nitrobenzoylation. Replacement of the methoxyl group of the completely protected intermediate was facile, yielding crystalline 5,6-*O*-carbonyl-2-deoxy-3-*O*-*p*-nitrobenzoyl-D-*arabino*-hexosyl bromide (**6**) (*2*). The anomeric configuration of the bromide (**6**) has not yet been determined, but it is presumed to be α-D, in which the bromine atom occupies a position *trans* to the substituted side chain at C–4 of the furanoid ring. To the best of our knowledge, this constitutes



6

the first report of a stable, crystalline *O*-acylglycofuranosyl halide of a 2-deoxyaldohexose.

The bromide (**6**) coupled readily with 2,4-diethoxypyrimidine, or with 2,4-diethoxy-5-methylpyrimidine, to give 1-(5,6-*O*-carbonyl-2-deoxy-3-*O*-*p*-nitrobenzoyl-D-*arabino*-hexosyl)-4-ethoxy-2(1*H*)-pyrimidinone (*2*) and 1-(5,6-*O*-carbonyl-2-deoxy-3-*O*-*p*-nitrobenzoyl-D-*arabino*-hexosyl)-4-ethoxy-5-methyl-2(1*H*)-pyrimidinone, respectively. The former underwent ammonolysis to yield 1-(2-deoxy-D-*arabino*-hexofuranosyl)cytosine, and, when de-ethylated, gave 1-(5,6-*O*-carbonyl-2-deoxy-3-*O*-*p*-nitrobenzoyl-D-*arabino*-hexosyl)uracil. The bromide (**6**) also reacted with silver *p*-nitrobenzoate to give 5,6-*O*-carbonyl-2-deoxy-1,3-di-*O*-*p*-nitrobenzoyl-D-*arabino*-hexose (*2*) the anomeric configuration of which has not yet been determined.

Owing to an interest in preparing nucleosides containing a 2-deoxy-D-*ribo*-hexofuranosyl residue, an effort was made to secure an *O*-acylglycofuranosyl halide of 2-deoxy-D-*ribo*-hexose by a procedure analogous to that for preparing the bromide (**6**) described in the foregoing. Whereas the methyl glycosidation of 2-deoxy-D-*arabino*-hexose (C–3–OH, *e*) gave methyl 2-deoxy-α-D-*arabino*-hexofuranoside in 35% yield, similar treatment of 2-deoxy-D-*ribo*-hexose (C–3–OH, *a*) failed to yield even traces of furanoside, as disclosed by paper chromatography. In fact, it was discovered that, in less than one minute, all of the sugar was consumed and converted, presumably, into a mixture of the anomeric pyranosides. The reaction was followed polarimetrically and, after 24 hours, the observed rotation did not change. Column partition-chromatography of the sirupy mixture gave crystalline methyl 2-deoxy-β-D-*ribo*-hexopyranoside. The alternative component, present in a very small proportion, is presumed to be the α-D anomer, which was not further investigated.

An alternative route to a furanosyl halide of 2-deoxy-D-*ribo*-hexose was envisaged, involving the lithium aluminum hydride reduction of ethyl 2,3-anhydro-β-D-allofuranoside which could, presumably, lead to ethyl 2-deoxy-β-D-*ribo*-hexofuranoside and, thence, to an *O*-acylglycofuranosyl halide. The known ethyl 5,6-*O*-carbonyl-β-D-glucofuranoside (*5*) was treated with *p*-toluenesulfonyl chloride in pyridine for 10 days, giving ethyl 5,6-*O*-carbonyl-2,3-di-*O*-(*p*-tolylsulfonyl)-β-D-glucoside, and also ethyl 5,6-*O*-carbonyl-2(or 3)-*O*-(*p*-tolylsulfonyl)-β-D-glucofuranoside, the two of which were separable by fractional recrystallization. Treatment of the ditosylate with sodium methoxide gave a complex mixture from which no material identifiable as the desired 2,3-anhydroalloside could be obtained. Consequently, this route was abandoned.

A third, and more promising, route is currently under investigation. Bromine oxidation of an aqueous solution of 2-deoxy-D-*ribo*-hexose gave the hitherto unreported 2-deoxy-D-*ribo*-hexono-1,4-lactone, which was benzoylated to yield 3,5,6-tri-*O*-benzoyl-2-deoxy-D-*ribo*-hexono-1,4-lactone. Studies are in progress to effect a reduction of the benzoylated

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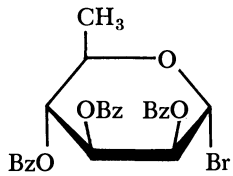
lactone, using disiamylborane, in an effort to secure 3,5,6-tri-*O*-benzoyl-2-deoxy-D-*ribo*-hexose, which should react with hydrogen halide to yield a 3,5,6-tri-*O*-benzoyl-2-deoxy-D-*ribo*-hexosyl halide, having utility in coupling reactions with dialkoxypyrimidines.

Halides of 6-Deoxy Sugars

Klyne (*6*) has shown that natural cardenolides containing D-sugars are β-D anomers, whereas those that contain L-sugars have the same absolute, or α-L-configuration, and it is to be noted also that they are 1,2-*trans* isomers. Moreover, it has been adequately demonstrated by Reichstein and co-workers (*9*) that conventional methods for the partial synthesis of cardenolides always lead to 1,2-*trans* glycosides, and this behavior conforms to Tipson's *trans* rule (*12*). Accordingly, it is not possible, by the methods usually employed, to synthesize, for example, cardenolides containing either α-L-glucose or β-D-mannose.

Because of this situation, the prospect of synthesizing D-rhamnosyl cardenolides containing the "unnatural," α-D-linkage was investigated. D-Rhamnose has not been reported to occur naturally, but it has been synthesized by Hudson and co-workers (*4*) in six steps, starting with methyl α-D-mannopyranoside. An intermediate in the synthesis is methyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannoside, and a consideration of its structure suggested a relatively direct route to a halide for coupling reactions. Prolonged treatment of the benzoylated rhamnoside with hydrogen bromide in acetic acid resulted in good yields of crystalline 2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannosyl bromide (**7**) (*24*). The bromide



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(**7**) coupled with digitoxigenin in the presence of silver carbonate to give, after saponification of the reaction products, 3β-*O*-(6-deoxy-α-D-mannopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (*24*), having the "unnatural," α-D configuration. At the same time, it is a 1,2-*trans* glycoside, and, when assayed intravenously in cats, the new cardenolide showed a very low order of cardiotonic activity. A similar coupling of the bromide (**7**) with strophanthidin (3β,5β,14β-trihydroxy-19-oxocard-20(22)-enolide) gave, after saponification, 3β-*O*-(6-deoxy-α-D-mannopyranosyl)-5β,14β-dihydroxy-19-oxocard-20(22)-enolide (*23*). The new cardenolide has the

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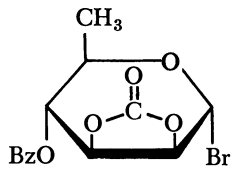
"unnatural," α-D configuration, and has a low order of cardiotonic activity.

Recently, we again attacked the knotty problem of synthesizing 1,2-*cis* cardenolides. Taking precedent from work by Gorin and Perlin (*3*) who were successful in preparing 1,2-*cis* mannopyranosides through the use of the "non-participating" 2,3-*O*-carbonyl group, we set out to prepare a D-rhamnosyl halide containing the latter grouping (*16*).

Methyl α-D-mannopyranoside was treated in succession with *p*-toluenesulfonyl chloride, carbonyl chloride, and benzoyl chloride, and, without isolating the intermediates, there was obtained in 37% yield methyl 4-*O*-benzoyl-2,3-*O*-carbonyl-6-*O*-(*p*-tolylsulfonyl)α-D-mannoside. The tosyloxyl group of the latter was replaced by iodine, and hydrogenation of the 6-iodo derivative in the presence of a "nickel boride" catalyst gave methyl 4-*O*-benzoyl-2,3-*O*-carbonyl-6-deoxy-α-D-mannoside. Treatment of the latter with hydrogen bromide in acetic acid gave *crystalline* 4-*O*-benzoyl-2,3-*O*-carbonyl-6-deoxy-α-D-mannosyl bromide (**8**) (*16*). The



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halide is very reactive and decomposes rapidly in moist air, but it can be stored for short periods in a desiccator, away from light at −78°C. The bromide (**8**) readily underwent methanolysis in the presence of silver carbonate to give, by inversion, methyl 4-*O*-benzoyl-2,3-*O*-carbonyl-6-deoxy-β-D-mannoside (*16*), and these results are consistent with those reported by Gorin and Perlin (*3*).

Coupling of digitoxigenin with the bromide (**8**) in 1,1,2-trichloroethane in the presence of silver carbonate, followed by saponification of the reaction mixture, gave 48% of the previously described α-D-rhamnoside (*24*) but only 13% of the desired 3β-*O*-(6-deoxy-β-D-mannopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide (*16*). Coupling of **8** with strophanthidin under essentially the same conditions, gave 8% of the known α-D-rhamnoside (*23*) and 6% of 3β-*O*-(6-deoxy-β-D-mannopyranosyl)-5β,14β-dihydroxy-19-oxocard-20(22)-enolide (*16*). In both cases, failure to yield inverted products exclusively is most probably because of steric factors in which the bulky steroid aglycon attacks the initially formed carbonium ion from below the plane of the ring to give the α-D anomer as the major product.

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Assay results with the two new 1,2-*cis* (β-D) cardenolides show enhanced activity as compared with the two "unnatural," α-D-rhamnosides. They have potencies that fall well within the range for those of the naturally occurring cardenolides. These results support the postulate that the α-D-glycosidic linkage in cardenolides containing D-sugars is unfavorable for cardiotonic activity.

Table I. Physical Properties of *O*-Acyldeoxyglycosyl Halides Reported Herein

Systematic Name	*Formula Number*	*Melting Point, °C.*	$[\alpha]_D$, *degrees* [a]	*References*
arabino-*Hexosyl bromide*, α-D-, *2-deoxy-3,4,6-tri*-O-p-*nitrobenzoyl*-	**2a**	145–148 (dec.)	+88.1, *A*	*20*
arabino-*Hexosyl chloride*, α-D-, *2-deoxy-3,4,6-tri*-O-p-*nitrobenzoyl*-	**2b**	151–153 (dec.)	+69.5, *A*	*20*
arabino-*Hexosyl bromide*, α-D-, *5,6-O-carbonyl-2-deoxy-3*-O-p-*nitrobenzoyl*-	**6**	125–153 (dec.)	−35.1, *B*	*32*
lyxo-*Hexosyl bromide*, α-D-, *2-deoxy-3,4,6-tri*-O-p-*nitrobenzoyl*-	**5**	160–162 (dec.)	+122, *A*	
Mannosyl bromide, α-D-, *4-O-benzoyl-2,3-O-carbonyl-6-deoxy*-	**8**	148–156 (dec.)	+82.9, *A*	*16*
Mannosyl bromide, α-D-, *2,3,4-tri*-O-*benzoyl-6-deoxy*-	**7**	163–164	−67.4, *C*	*24*
ribo-*Hexosyl bromide*, α-D-, *2-deoxy-3,4,6-tri*-O-p-*nitrobenzoyl*-	**4a**	119 (dec.)	+198, *A*	*14*
ribo-*Hexosyl chloride*, α-D-, *2-deoxy-3,4,6-tri*-O-p-*nitrobenzoyl*-	**4b**	122 (dec.)	+192, *A*	*14*
ribo-*Hexosyl bromide-β-D-*, *2,6-dideoxy-3,4-di*-O-p-*nitrobenzoyl*-	**1a**	96–103 (dec.)	+139.1, *C*	*19*
ribo-*Hexosyl chloride*, β-D-, *2,6-dideoxy-3,4-di*-O-p-*nitrobenzoyl*-	**1b**	91–101 (dec.)	+185.9, *C*	*19*

[a] Key: *A*, dichloromethane; *B*, acetone; *C*, chloroform.

Experimental

2-Deoxy-1,3,4,6-tetra-O-p-*nitrobenzoyl*-D-lyxo-*hexose.* To a stirred suspension of 4.64 grams (25 mmoles) of *p*-nitrobenzoyl chloride in 50 ml. of anhydrous pyridine at 0°C. is added, in small portions over a period of 15 minutes, 0.82 grams (5 mmoles) of 2-deoxy-D-*lyxo*-hexose purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. Stirring is maintained at 0°C. for 2 hours and the mixture is kept in a refrigerator

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for 2 days. It is then stirred at room temperature for 2 hours and 20 ml. of saturated aqueous sodium hydrogen carbonate is cautiously added. The resulting mixture is poured, with vigorous stirring, onto 500 grams of crushed ice. Stirring is continued until the ice melts, and the separated product is filtered off by suction and washed several times with water. The solid is dried in the open and then in a desiccator containing phosphorus pentaoxide for 24 hours. Four recrystallizations from ether–dichloromethane gives 2.5 grams (66%) of solvated tetrakis-*p*-nitrobenzoate, melting at 163°–165° C. and having $[\alpha]_D$ + 39.6° in dichloromethane. The product is desolvated by heating it in an oil bath at 170°C.; it melts, becoming a brown mass, and heating is continued until some decomposition takes place, as evidenced by the appearance of *p*-nitrobenzoic acid which collects on the walls of the flask. Heating is discontinued and the residue is allowed to cool. It is dissolved in dry dichloromethane, the solution is decolorized with Darco G–60 decolorizing carbon, and filtered. On the addition of ether, the pure product crystallizes and has m.p. 244°–245°C. and $[\alpha]_D$ + 40.5° in dichloromethane. The yields vary from about 45–55%.

Anal. Calcd. for $C_{34}H_{24}N_4O_{17}$: C, 53.60; H, 3.16; N, 7.36. Found: C, 53.77; H, 3.14; N, 7.62.

2-Deoxy-3,4,6-tri-O-p-*nitrobenzoyl-α*-D-lyxo-*hexosyl bromide (5)* To a solution of 105 mg. of 2-deoxy-1,3,4,6-tetra-*O*-*p*-nitrobenzoyl-D-*lyxo*-hexose (m.p. 244°–245°C.) in 2 ml. of dry dichloromethane is added 2 ml. of a saturated solution of hydrogen bromide in dichloromethane. The mixture is stirred magnetically under exclusion of moisture for 20 minutes, and the separated *p*-nitrobenzoic acid is filtered by suction, using a sintered-glass funnel, and washed with 2 ml. of dry dichloromethane. The filtrate is diminished in volume by one half by evaporation at room temperature, and 4 ml. of ether is added. Crystallization begins in 10 minutes and the flask containing the mixture is kept in a refrigerator for 2 hours. The crystals are filtered off, and washed with dry ether, and have m.p. 155°–157°C. (dec.). Recrystallization from ether-dichloromethane gives 85 mg. (91%) of the pure bromide (**5**), m.p. 160°–162°C. (dec.) and $[\alpha]_D$ + 122° in dichloromethane.

Methyl 2-deoxy-3,4,6-tri-O-p-*nitrobenzoyl-β*-D-lyxo-*hexoside.* A solution of 370 mg. of 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-*α*-D-*lyxo*-hexosyl bromide (**5**) in 15 ml. of dry dichloromethane is added to a stirred suspension of 500 mg. of silver carbonate in 100 ml. of absolute methanol. The mixture is stirred for 20 hours and filtered; the silver salts are washed several times with warm dichloromethane. The combined filtrate and washings are evaporated to dryness under diminished pressure, and the residue is recrystallized five times from ether–dichloromethane, giving 61% of pure product, having m.p. 173°–174°C. and $[\alpha]_D$ + 36° in chloroform.

Anal. Calcd. for $C_{28}H_{23}N_3O_{14}$: C, 53,72; H, 3.71; N, 6.72. Found: C, 53.82; H, 3.56; N, 6.80.

Methyl 2-deoxy-β-D-lyxo-*hexopyranoside.* A suspension of 170 mg. of the nitrobenzoylated hexoside (obtained in the preceding preparation) in 50 ml. of 0.01*M* methanolic sodium methoxide is stirred for 4 hours at room temperature. The solvent is removed at 40°C. under diminished pressure, and the residue is suspended in 15 ml. of water. The suspension

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is extracted five times with 15-ml. portions of ether, the aqueous layer is stirred for 10 minutes with 2 grams of Rexyn 300 (H^+, OH^-) ion-exchange resin, and filtered. The filtrate is evaporated to dryness at 45°C. under diminished pressure, the residue is dissolved in 10 ml. of methanol, and the solution is decolorized with Darco G–60 decolorizing carbon, filtered, and evaporated to dryness. The sirupy residue is dissolved in 5 ml. of ethyl acetate, and ether is added to incipient turbidity. The solution is kept in a refrigerator overnight, and the separated crystals are recrystallized from ether–ethyl acetate, giving 40 mg. (82%) of pure product, having m.p. 123°–124°C. and $[\alpha]_D$ + 46.8° in methanol.

Anal. Calcd. for $C_7H_{14}O_5$; C, 47.19; H, 7.92. Found: C, 47.47; H, 7.86.

*Methyl 2-deoxy-β-*D-ribo-*hexopyranoside.* To 20 ml. of 0.2% hydrogen chloride–methanol is added 175 mg. of 2-deoxy-D-*ribo*-hexose and the solution is kept at room temperature for 24 hours. The hydrogen chloride is neutralized with an excess of silver carbonate, decolorizing carbon is added, and the mixture is filtered. Evaporation of the filtrate gives a sirup, which is chromatographed on a column (3 x 30 cm.) of cellulose powder by eluting with butyl alcohol–toluene (9:1) saturated with water. The β-D-pyranoside is recovered as a sirup which solidifies on storage in a vacuum desiccator containing phosphorus pentaoxide. The solid is dissolved in tetrahydrofuran, and pentane is added to incipient turbidity, giving 91 mg. of amorphous product having m.p. 115°–120°C. and $[\alpha]_D$ − 45° in methanol.

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 47.47; H, 8.15.

*Ethyl 5,6-*O*-carbonyl-2(or 3)-*O*-(*p*-tolylsulfonyl)-β-*D*-glucofuranoside and ethyl 5,6-*O*-carbonyl-2,3-di-*O*-(*p*-tolylsulfonyl)-β-*D*-glucoside.* To 690 mg. of ethyl 5,6-*O*-carbonyl-β-D-glucofuranoside (5) in 10 ml. of dry pyridine is added 4.4 grams of *p*-toluenesulfonyl chloride. The solution is kept at room temperature for 10 days, and is then poured, with stirring, onto 200 grams of crushed ice. When the ice has melted, the mixture is extracted with four 100-ml. portions of dichloromethane. A generous amount of ice is added to the combined extracts, which are then extracted successively with two 20-ml. portions of 6*N* sulfuric acid, several small volumes of water, saturated aqueous sodium hydrogen carbonate, and water. The extract is dried with calcium chloride, decolorized, and filtered, and the filtrate is evaporated under diminished pressure to a sirupy mixture of the mono- and di-*p*-toluenesulfonates, which co-crystallize on addition of absolute ethanol. Fractional recrystallization from pentane–chloroform gives 300 mg. of the mono-ester, m.p. 172°–174°C. and $[\alpha]_D$ − 42.3° (in chloroform). Processing of the mother liquors yields 360 mg. (after recrystallizing from pentane–tetrahydrofuran) of the di-ester, m.p. 122°–124°C. $[\alpha]_D$ − 52.0° (in chloroform).

Anal. Calcd. for monotosylate: $C_{16}H_{20}O_9S$: C, 49.48; H, 5.19; S, 8.26. Found: C, 49.69; H, 5.21; S, 8.18. *Anal.* Calcd. for ditosylate: $C_{23}H_{26}O_{11}S_2$: C, 50.91; H, 4.84; S, 11.82. Found: C, 50.96; H, 5.05; S, 11.74.

*2-Deoxy-*D-ribo-*hexono-1,4-lactone.* To 286 mg. of 2-deoxy-D-*ribo*-hexose in 4 ml. of water is added 0.3 ml. of bromine. The solution is kept overnight at 37°C. and is then aerated to remove the excess bromine. Silver carbonate (1.5 grams) is added and the mixture is filtered. The clear filtrate is stirred with 5.4 grams of Dowex-50W X8 (H^+) ion-exchange resin, decolorized, and filtered, and the filtrate is evaporated

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under diminished pressure. The resulting sirup crystallizes on storage in a vacuum desiccator containing phosphorus pentaoxide. Recrystallization from ether–ethanol gives hygroscopic, crystalline product, having m.p. 71°–73°C. and $[\alpha]_D$ −42° in absolute ethanol.

Anal. Calcd. for $C_6H_{10}O_5$: C, 44.45; H, 6.22. Found: C, 44.64; H, 6.34.

*3,5,6-Tri-*O*-benzoyl-*D*-ribo-hexono-1,4-lactone.* To 1.55 grams of 2-deoxy-D-*ribo*-hexono-1,4-lactone in 10 ml. of dry pyridine is added 7 ml. of benzoyl chloride. The mixture is stirred for 1 hour at room temperature and then kept in a refrigerator for 3 days. The excess benzoyl chloride is decomposed by the careful addition of an excess of saturated aqueous sodium hydrogen carbonate, and the mixture is poured, with efficient stirring, into 600 ml. of ice-water. The separated, gummy solid is filtered off and dissolved in chloroform. The solution is decolorized with Darco G–60 decolorizing carbon and filtered, and the filtrate is evaporated under diminished pressure. The resulting, clear sirup is dissolved in a small volume of chloroform, and pentane is added to incipient turbidity, giving 2.5 grams (55%) of product, melting at 104–105°C. and having $[\alpha]_D$ + 5.5° in chloroform.

Anal. Calcd. for $C_{27}H_{22}O_8$: C, 68.34; H, 4.67. Found: C, 68.22; H, 4.88.

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Kasugamycin

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Kasugamycin (**1**) *is chemically degraded into four main fragments;*—i.e., d-*inositol*, *2,4-diamino-2,3,4,6-tetradeoxy-α-*arabino-*hexopyranoside of* d-*inositol* (**4**), *methyl 2,4-diamino-2,3,4,6-tetradeoxy-β-*arabino-*hexopyranoside* (**5**), *and 2,4-diamino-N⁴-(carboxyformyl)-2,3,4,6-tetradeoxy-α-*arabino-*hexopyranoside* (**9a**). *The presence of an amidine structure at* C_4 *of the amino sugar moiety has been verified by synthetic transformation of* **4** *to kasugamycin itself. Since the linking position of* d-*inositol has been decided by x-ray analysis, the whole structure of* **1** *has been assigned to structure* **14,** *2,4-diamino-N⁴-(carboxyformidoyl)-2,3,4,6-tetradeoxy-α-*D-arabino-*hexopyranoside of* d-*inositol.*

Kasugamycin was discovered in the screening study of antibiotics useful for prevention of rice blast (*26*). In this study, each culture filtrate was sprayed on a young rice plant infected with *Piricularia oryzae*, and the preventive effect was examined. This test is called a "pot test" and a culture filtrate of *Streptomyces kasugaensis* showed a strong preventive effect. However, on a yeast extract medium usually applied to the test of the inhibitory effect against this fungus *in vitro*, this culture filtrate showed no inhibition of this fungus. An important step of the study of this antibiotic was to find a method of testing the activity *in vitro*. On a medium consisting of rice plant juice adjusted to pH 5, the active agent was found to exhibit a strong inhibition of the fungus. Thus, as a result of the testing method established, the active agent was successfully extracted and purified.

On the rice plant juice medium adjusted to pH 5, the growth of *P. oryzae* is inhibited at 0.39 μg./ml. of kasugamycin, but on the same medium adjusted to pH 7, 100 μg./ml. is necessary for the inhibition

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

as shown in Table I. The environment where this pathogenic fungus grows in leaves of rice plants is acidic and the growth of the fungus in this acidic environment is inhibited by kasugamycin. Kasugamycin has low toxicity and inhibits growth of *Pseudomonas* (*8*) and a therapeutic effect on *Pseudomonas* infection (*25*) in humans was also confirmed. Kasugamycin is an important antibiotic useful not only for prevention of rice blast (*12*) but also for the treatment of *Pseudomonas* infection in humans.

Table I. Inhibitory Effect of Kasugamycin on Growth of *Piricularia oryzae* at pH 5.0 and pH 7.0 in Rice Plant Juice Medium

	Kasugamycin μg/ml										
	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0
pH 5.0	−	−	−	−	−	−	−	−	−	+	+
pH 7.0	+	+	+	+	+	+	+	+	+	+	+

− : no growth; + : positive growth. Observation 72 hours at 27°C.

Table II. Properties of Kasugamycin (1)

	Hydrochloride		*Free base*	
m.p. 236° – 239°C. (dec.)			214° – 216°C. (dec.)	
$[\alpha]_D^{25}$ +125°C. (c=1.6, H_2O)			+115°C. (c=1, H_2O)	
Anal. $C_{14}H_{25}O_9N_3 \cdot HCl \cdot H_2O$			$C_{14}H_{25}O_9N_3 \cdot H_2O$	
	Calcd.	*Found:*	*Calcd.*	*Found:*
C	38.67	38.59	42.31	42.13
H	6.72	6.71	6.85	7.14
O	36.79	36.90	40.26	39.78
N	9.66	9.68	10.58	10.49
Cl	8.15	8.34		

pKa′ <2, 7.1, 10.6, NH_2: 1, C–CH_3: 1

Color reaction: Positive Ninhydrin, Nitroprusside-reagent, Br_2–H_2O, Lemieux, and Negative Tollens, Molish, Sakaguchi, Elson-Morgan, Ferric chloride.
Ultraviolet: End absorption.

Structural Studies

Kasugamycin (**1**) was first isolated in a state of a crystalline hydrochloride and afterwards as a crystalline free base. The properties of kasugamycin are summarized in Table II. Kasugamycin has three pK′a values: 10.6, 7.1, and below 2.0. The presence of a carboxyl group which should show the lowest pK′a value was expected and definitely confirmed later by a partial synthesis of kasugamycin. Kasugamycin has one primary amino group and one carbon methyl group. The polyhydroxylic nature of kasugamycin is shown by absorption bands at 3500–3000 cm^{-1}

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and strong bands at 1100–1000 cm^{-1}. Formation of borate complex and a high oxygen value of the elemental analysis were additional evidence for polyhydroxyl groups in kasugamycin. Thus, kasugamycin was considered to be an amino-glycosidic compound.

Acid hydrolysis of kasugamycin hydrochloride yielded a crystalline product (**2**). This product has a molecular formula of $C_6H_{12}O_6$ and shows no absorption in carbonyl region of its infrared spectrum, but shows strong bands in hydroxyl group region. Furthermore, the product gives negative Benedict and positive Scherer's reaction (*18*) characteristic of inositol. It was shown that the specific rotation of the kasugamycin hydrolysis product (**2**) was identical with that of *d*-inositol. The identity with *d*-inositol was confirmed by no depression of the mixed melting point and the same infrared spectrum with an authentic sample of *d*-inositol.

The NMR spectrum of *d*-inositol has a signal at 3.83 p.p.m. for four axial protons, and a signal at 4.10 p.p.m. for two equatorial protons. The NMR spectrum of kasugamycin contains similar signals of *d*-inositol.

The treatment of kasugamycin hydrochloride with saturated methanolic hydrogen chloride gave a C_9–amine (**3**) and *d*-inositol. The C_9–amine (**3**) has a molecular formula of $C_9H_{17}O_4N_3$.

$$\underset{\mathbf{1}}{C_{14}H_{25}O_9N_3 \cdot HCl} \xrightarrow[MeOH]{HCl} \underset{\mathbf{3}}{C_9H_{17}O_4N_3} + \underset{\mathbf{2}}{d\text{-inositol}}$$

The functional groups of kasugamycin and the C_9–amine are shown in Table III. Both have similar properties except that the C_9–amine has one methoxyl group which is not found in kasugamycin.

Table III. Functional Groups of Kasugamycin (1) and C_9-Amine (3)

	Kasugamycin (**1**)	*C_9-Amine* (**3**)
$-C-CH_3$	1	1
$-NH_2$	1	1
$-OCH_3$	0	1
$-NCH_3$	0	0
pKa′	<2.0, 7.1, 10.6	<2.0, 7.2, 10.8

The NMR spectra of kasugamycin hydrochloride and the C_9–amine hydrochloride are shown in Figures 1 and 2. The spectrum of the C_9–amine has no signal for the tertiary hydrogens of *d*-inositol, but has a sharp singlet at 3.44 p.p.m. for three protons of methoxyl group. Therefore, this evidence indicates that the *d*-inositol moiety of kasugamycin is replaced by a methoxyl group during the methanolysis.

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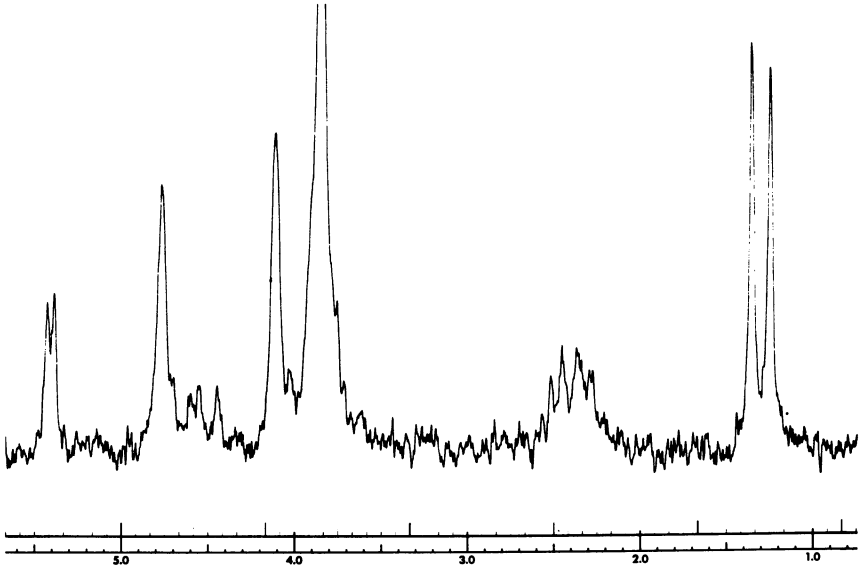

Figure 1. NMR spectrum of kasugamycin hydrochloride in deuterium oxide at 60 Mc.

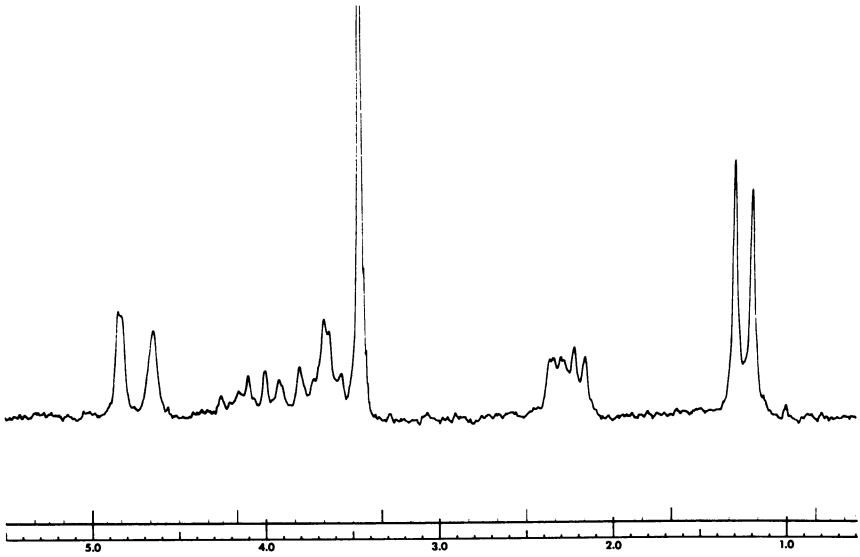

Figure 2. NMR spectrum of C_9-amine hydrochloride in deuterium oxide at 60 Mc.

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The treatment of kasugamycin hydrochloride with saturated barium hydroxide solution at 100°C. for 10 hours gave a mole each of oxalic acid, ammonia, and a diamine named kasuganobiosamine. Kasuganobiosamine (**4**) crystallized as dihydrochloride has pK'a 6.7 and 8.5. The hydrolysis of C_9–amine (**3**) under the similar condition yielded another diamine named methylkasugaminide (**5**), oxalic acid, and ammonia. Methylkasugaminide (**5**) has one carbon methyl group, one methoxyl group, and two primary amino groups. By the methanolysis, kasuganobiosamine (**4**) is cleaved into methylkasugaminide (**5**) and *d*-inositol. These results indicate that kasuganobiosamine (**4**) consists of *d*-inositol and kasugamine (**6**). Since kasuganobiosamine has no ester band in its infrared spectrum, kasugamine must be connected to *d*-inositol through the glycosidic linkage to form kasuganobiosamine (**4**).

Acetylation of kasuganobiosamine with acetic anhydride in methanol gave *N,N'*-diacetylkasuganobiosamine which contained no titrable group and reduced 6.6 moles of sodium periodate, affording 4.7 moles of formic acid, 0.5 mole of carbon dioxide, and a reducing substance;—*i.e.*, *N,N'*-diacetylkasugamine.

The oxidation of *N,N'*-diacetylkasugamine with bromine-water (*11*) yielded a δ-lactone (**7**), showing an absorption at 1752 cm.$^{-1}$ (KBr) in the infrared spectrum. It suggested the pyranose structure of kasugamine (**6**).

The molecular formula of methylkasugaminide (**5**) was established as $C_7H_{16}N_2O_2$ by the parent peak $m/e = 160$ in the mass spectrum. The presence of one carbon methyl group, one methoxyl group, and two primary amino groups in methylkasugaminide was confirmed by chemical and spectroscopic methods. Then, the next question was how to assign one methyl and two amino groups to the pyranose structure. The answer was obtained by the NMR spectroscopy, especially by the application of the spin decoupling technique. The NMR spectra of 10% methylkasugaminide solution in deuterium oxide were taken by A-60, and HR-100 Varian spectrometers. The chemical shift is expressed in p.p.m. taking 0.2% sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal reference.

The NMR spectrum of methyl kasugaminide in deuterium oxide at 100 Mc is shown in Figure 3. The anomeric proton at C–1 linking with methoxyl group is shown as a doublet at 4.57 p.p.m. indicating one proton at C–2. The weak coupling, 1.6 c.p.s., is possible between protons in *cis* relation (*28*) or in equatorial-equatorial relation (*6*) at C–1 and C–2 of the six-membered ring.

The next low field signal of one proton at 3.73 p.p.m. can be assigned to a proton at C–5 connected to the ether linkage and the splitting pattern of the signal can be explained by the coupling with three

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protons of a methyl group and one proton at C–4. Therefore, the location of the methyl group must be at C–5. The large coupling constant, 9.6 c.p.s., of the proton at C–5 indicates the axial-axial relation of the protons at C–5 and C–4 in the chair form of the six membered ring. Three protons of the methoxyl group appear at 3.40 p.p.m. as a sharp singlet.

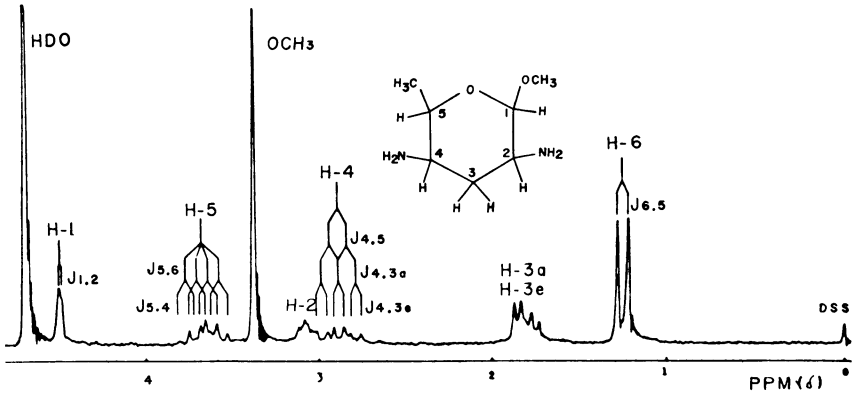

Figure 3. NMR spectrum of methylkasugaminide in deuterium oxide at 100 Mc.

A doublet signal of three protons at 1.27 p.p.m., $J = 6.5$ c.p.s., must be the signal of a methyl group indicating one proton at C–5.

The positions of methoxyl and methyl groups were thus determined. Therefore, C–2 and C–4 must contain one hydrogen and one amino group each. The relative location of two amino groups conforms with the evidence that methylkasugaminide is strongly resistant to periodate oxidation. Then, the signals of two protons at 1.82 p.p.m. can be assigned to methylene protons at C–3. The signals at 3.15 and 2.92 p.p.m. must be a result of protons at C–2 and C–4. The large coupling constant of the proton at 2.92 p.p.m. confirms the axial-axial relation between protons at C–5 and C–4, and this signal is the result of the proton at C–4. Then, the signal at 3.15 p.p.m. can be assigned to one proton at C–2.

From the evidence discussed above, the framework of methylkasugaminide is determined to be methyl 2,4-diamino-2,3,4,6-tetradeoxyhexopyranoside in which hydrogens at C–4 and C–5 are axial-axial and hydrogens at C–1 and C–2 are not in axial-axial relation. The structure was definitely proved by the application of the spin decoupling technique and, moreover, the relative relations of all hydrogens were confirmed.

The irradiation of C–5 proton at 3.73 p.p.m. shows a singlet resonance at 1.27 p.p.m. for three protons of the methyl group and reverse irradiation shows a doublet at 3.73 p.p.m. for one proton at C–5 with coupling constant, 9.6 c.p.s., confirming the neighboring relationship of the methyl

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group and C–5 proton and that protons at C–5 and C–4 are axial (A and B in Figure 4).

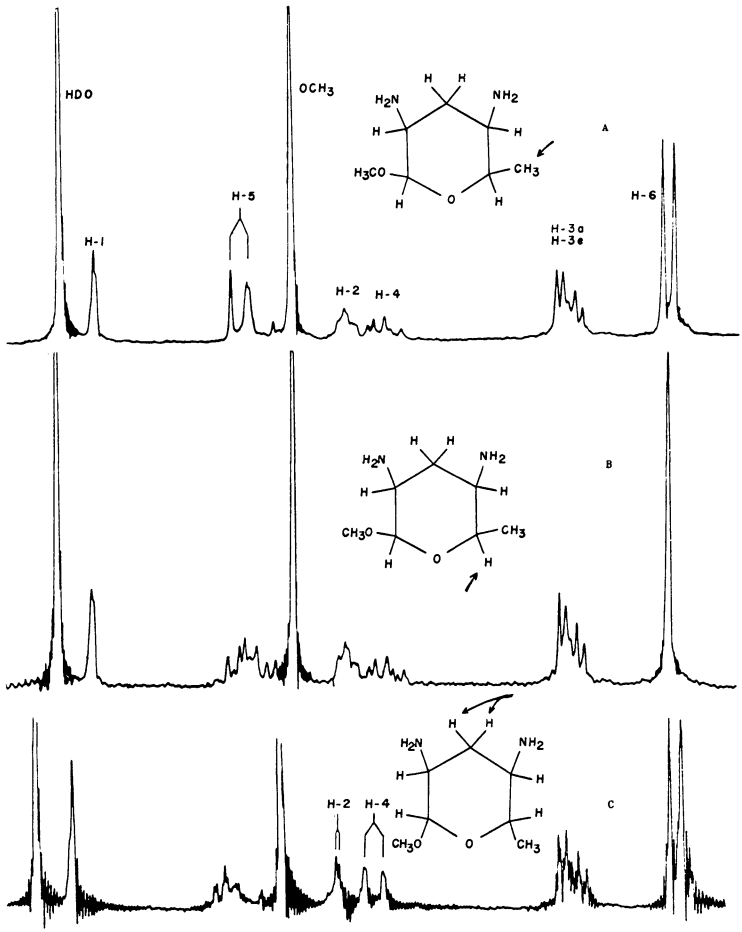

Figure 4. NMR spectra of methylkasugaminide in deuterium oxide at 100 Mc.

(A) C_6 protons irradiated
(B) C_5 proton irradiated
(C) C_3 protons irradiated

When methylene protons at 1.82 p.p.m. are irradiated, each signal at 3.15 and 2.92 p.p.m. splits into a doublet with a coupling constant, 1.6 c.p.s., and a doublet with 9.6 c.p.s., respectively. Therefore, the assignment of the signals at 3.15 and 2.92 p.p.m. to C–2 and C–4 protons, is clearly established (C in Figure 4). The coupling constants again confirmed the axial- equatorial or equatorial-equatorial relation between C–1 and C–2 protons and axial-axial relation between C–4 and C–5 protons.

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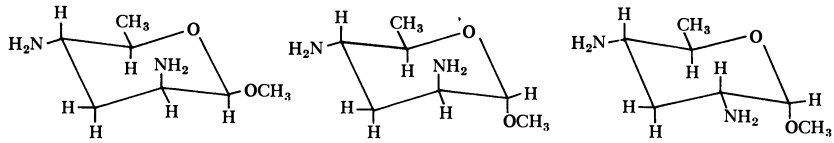
From the preceding spectral evidence, the stereochemical formula of methyl kasugaminide is limited to three possible structure **8a, 8b,** or **8c.**



8a **8b** **8c**

The following experiments exclude the structure having an axial proton at C–2 (**8c**). The two patterns of methylene protons are different, when C–2 proton at 3.15 p.p.m. and C–4 proton at 2.92 p.p.m. are irradiated separately (*A* and *B* in Figure 5). This evidence excludes the structure

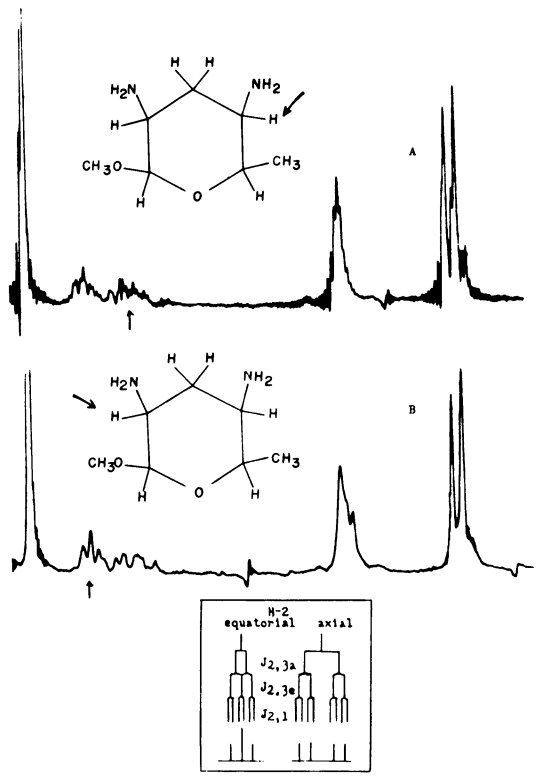


Figure 5. NMR spectra of methylkasugaminide in deuterium oxide at 100 Mc.

(A) C_4 proton irradiated
(B) C_2 proton irradiated

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8c, since it should give essentially the same pattern by the same treatment. Furthermore, the C–2 proton signals at 3.15 p.p.m. have a total width of 9–10 c.p.s. and appear as a triplet character. This width is practically equal to the sum of three coupling constants ($J_{2,3a}$, $J_{2,3e}$, $J_{2,1}$) with which the equatorial C–2 hydrogen is coupled to the methylene protons (H_3 axial and H_3 equatorial) and to the anomeric proton, while the structure **8c** should give proton signals of quartet character with a width of 12–15 c.p.s.

According to van deer Veen (*27*) and Rao and Foster (*17*), the anomeric proton line positions for α-D-glycopyranosides (H_1 equatorial) appear in the region of 4.8 to 5.5 p.p.m., while for β-D-glycopyranosides (H_1 axial), the peaks appear at 4.4 to 4.6 p.p.m. The chemical shift of the anomeric proton of methylkasugaminide (**5**) is located at 4.57 p.p.m. and thus the proton must be axial, excluding structure **8b** and **8c** in which the anomeric proton is equatorial. Structure **8a** is thus completely in agreement with the NMR spectra.

On the other hand, the NMR spectrum (Figure 6) of kasuganobiosamine (**4**) shows that the anomeric proton signal is located at 5.02 p.p.m., therefore the configuration of the proton is assigned to the equatorial configuration or the same ground (*17*,*27*).

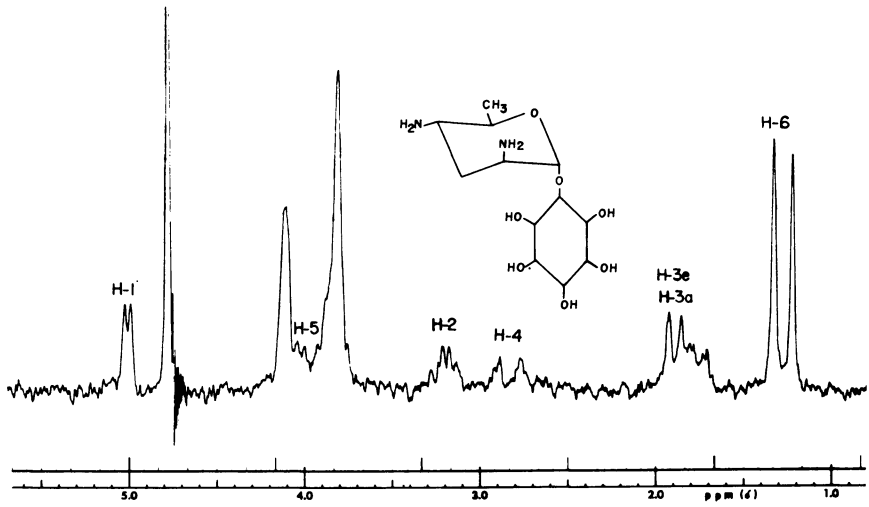

Figure 6. NMR spectrum of kasuganobiosamine in deuterium oxide at 60 Mc.

Consequently, the glycosidic linkage in kasuganobiosamine must be α-configuration, showing conversion of the anomeric proton during methanolysis.

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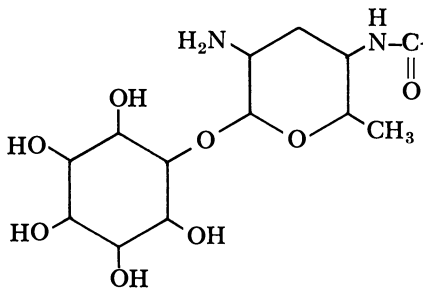
The next step of our study was to determine which amino group of kasuganobiosamine binds with the residual moiety.

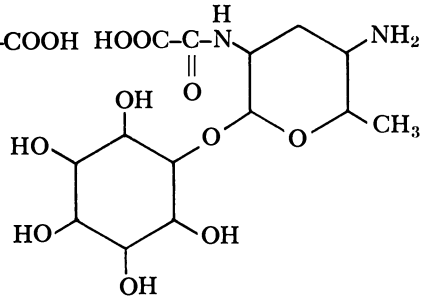
Alkaline hydrolysis of kasugamycin (**1**) with saturated equeous barium hydroxide gave an amphoteric compound named kasugamycinic acid along with kasuganobiosamine (**4**), oxalic acid, and ammonia. The crystalline kasugamycinic acid contains one acidic group and one primary amino group, showing pK'a 1.8 and 7.8.

Kasugamycinic acid gave kasuganobiosamine (**4**) and oxalic acid by alkaline hydrolysis, and yielded *d*-inositol by acid hydrolysis.

The abnormaly high infrared absorption frequencies and low pK'a value of the carboxyl group in kasugamycinic acid are consistent with those in oxamic acid, which shows an infrared absorption at 1740 cm^{-1} (KBr) and pK'a 1.9. Consequently, these data are consistent with the structure of kasugamycinic acid in which one carboxyl group of oxalic acid is free and the second carboxyl group forms an amide linkage with one amino group of kasuganobiosamine (**4**). The foregoing results suggests two possible structures for kasugamycinic acid (**9a** or **9b**).



9a



9b

Two kinds of *N*-monoacetylkasuganobiosamine were prepared to determine the structure of kasugamycinic acid. Acetylation of kasuganobiosamine (**4**) with one mole of acetic anhydride in methanol gave two monoacetylated compounds (**10a** and **10b**) having pK'a 7.8 and 8.2 along with *N,N'*-diacetyl derivative. On the other hand, when kasugamycinic acid was converted into *N*-monoacetylkasuganobiosamine by acetylation followed by alkaline hydrolysis, the product was identical with the compound having pK'a 8.2 (**10b**).

The method of predicting pK'a values by Clarke and Perrin (*1*) was tentatively applied to determine the structures of the two monoacetylated compounds, and the results indicate that kasugamycinic acid is 4-*N*-carboxyformyl-kasuganobiosamine (**9a**).

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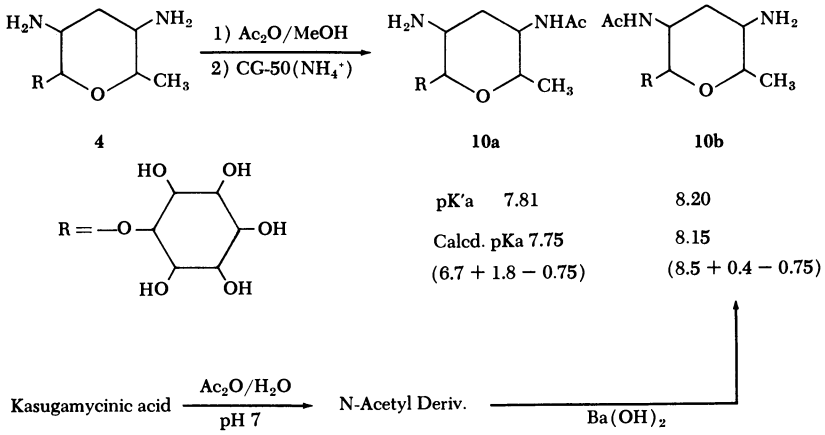
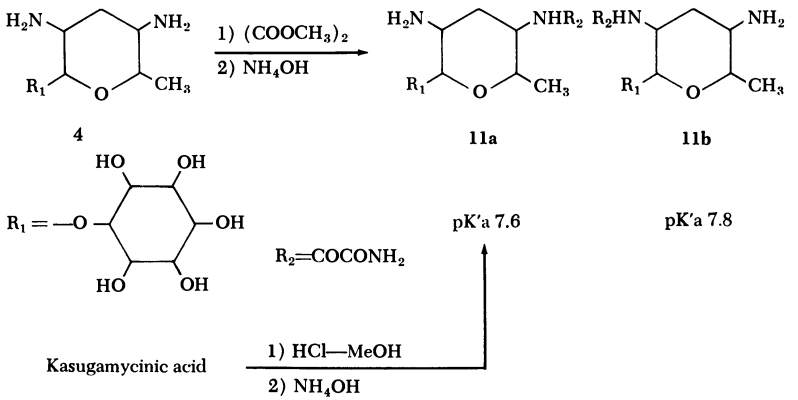


A clear determination of the position of substituted amino group was obtained by comparison of NMR spectra of kasuganobiosamine with those of its derivatives (**11a** and **11b**). It is known that the hydrogens on the carbon with acetylated amino groups shifts to lower field by 0.5 to 0.7 p.p.m. (*15*). Since characteristic patterns of the tertiary hydrogens are well analyzed as described before, such method was applied to kasuganobiosamine *N*-monoderivatives and the results are shown in Figure 7 and Table IV. The 4-oxamide derivative of kasuganobiosamine (**11a**) was identical with the amide of kasugamycinic acid. Consequently, the side chain of kasugamycin must be located at C–4 of the amino sugar moiety.

When we reached the structure of kasugamycinic acid by chemical methods, x-ray group (*10*) suggested that kasugamycin could be the amide of kasugamycinic acid. Therefore, the amide of kasugamycinic acid was first prepared from kasuganobiosamine by the procedure shown below.



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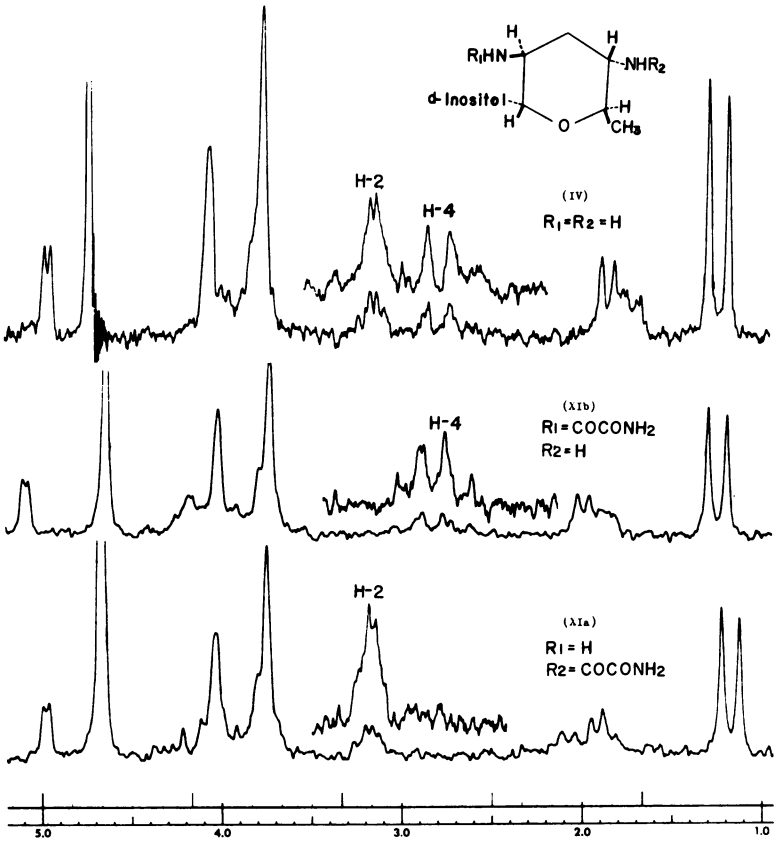

Figure 7. NMR spectra of kasuganobiosamine derivatives in deuterium oxide at 60 MC.

The treatment of kasuganobiosamine with dimethyl ester of oxalic acid followed by concentrated aqueous ammonia gave two kinds of amides. The amide (**11a**) with pK′a 7.6 was identical with the amide of kasugamycinic acid, which was prepared from kasugamycinic acid by treatment with anhydrous hydrogen chloride-methanol followed by concentrated aqueous ammonia. The other isomer (**11b**) with pK′a 7.8, was named amide of isokasugamycinic acid.

It was found that the spectroscopic and biological data on the amide of kasugamycinic acid were different from those of kasugamycin.

The next step of our study, therefore, was to determine the structure of the side chain attached to the amino sugar moiety.

Although three possible structures (**11a, 12,** and **13**) of kasugamycin were considered on the basis of the stoichiometrical data presented previously, the amide structure (**11a**) was excluded as already described.

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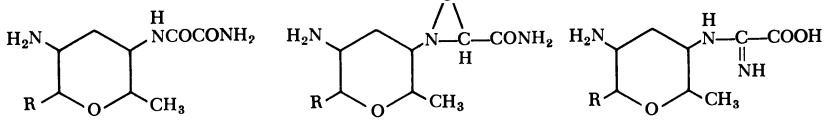
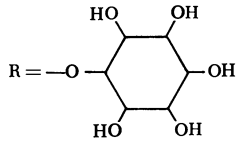


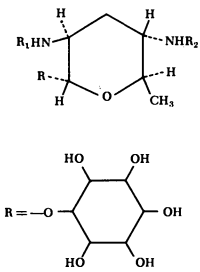
11a 12 13



The oxazirane structure (**12**) for kasugamycin was also eliminated by negative reactions characteristic of the oxazirane group (*4*) and by the observation of no proton corresponding to an oxazirane group in the NMR spectrum of kasugamycin.

During these structural studies by chemical methods, we found the following interesting reactions, which were substantially useful for the presence of an unique amidine group.

Table IV. Chemical Shifts of the Tertiary Hydrogens at C–2 and C–4 of Kasuganobiosamine N-Monoderivatives



			NMR(δ)	
	R_1	R_2	*H–2*	*H–4*
4	H	H	3.20	2.83
10a	H	Ac	3.15	3.5∼4.0[a]
10b	Ac	H	3.5∼4.0[a]	2.77
11a	H	$COCONH_2$	3.20	3.5∼4.0[a]
11b	$COCONH_2$	H	3.5∼4.0[a]	2.83
1	Kasugamycin	(Free Base)	3.22	3.5∼4.0[a]

[a] Overlap with inositol hydrogens.

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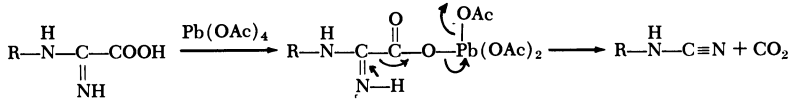
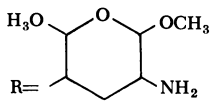
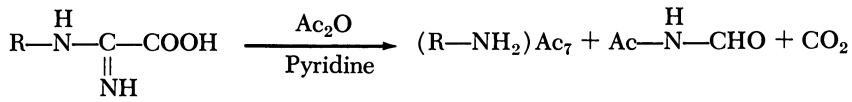
The C_9–amine, originally obtained by the methanolysis of kasugamycin, on treatment with lead tetraacetate or sodium periodate afforded a nitrile amine, with evolution of carbon dioxide, showing a maximum at 2200 cm.$^{-1}$. This reaction is explained only by the structure (**13**). The –N–C≡N group of the product can be formed by oxidative decarboxylation and can be easily rationalized by the present understanding of such reagents (*2*, *13*) as shown below. On the other hand, the treatment



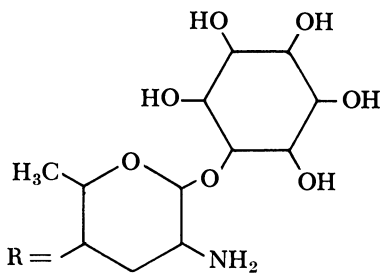
3



of kasugamycin with acetic anhydride-pyridine gave heptaacetylated kasuganobiosamine, carbon dioxide, and acetylformamide, which has been synthesized by Pinner from ethyl formimidate and acetic anhydride (*16*).



1



Furthermore we found that kasugamycin forms a chelate compound with basic cupric carbonate (*7*), which is stable to acid and unstable to heat and base. This evidence together with the results obtained above strongly supports the amidine structure (**13**) for kasugamycin. Finally the amidine compound was successfully prepared by the reaction of kasuganobiosamine with the diethyl ester of oxalimidic acid (*14*) and

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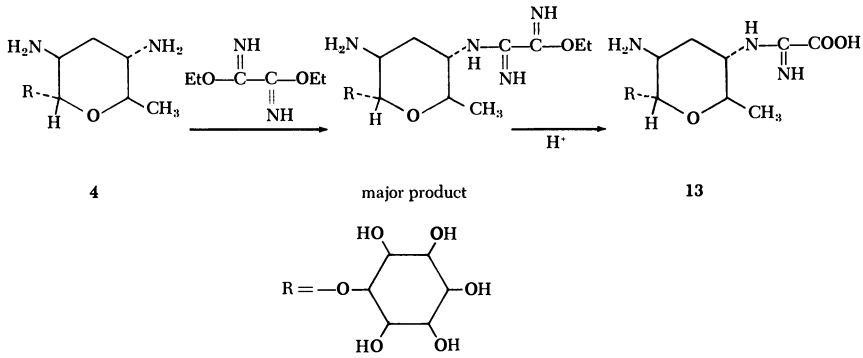
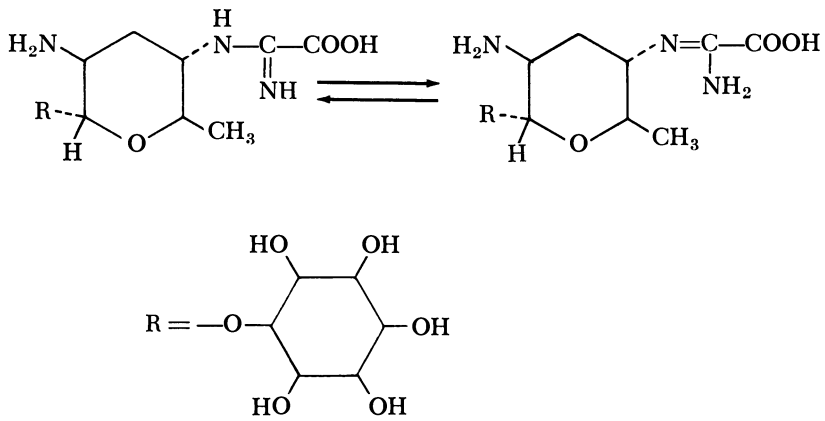
subsequent mild hydrolysis with hydrochloric acid. The hydrochloride of this synthetic material is completely identical with natural kasugamycin hydrochloride in all respect such as mixed m.p., infrared and NMR spectra and bio-assay. In this case, no isomeric amidine compound was obtained, and the selectivity of the reaction may be rationalized from the stereo-chemical stability at the transition state.



4 major product 13

From a large amount of knowledge on amidine compounds it is known that monosubstituted amidines having various groups on the nitrogen atoms exhibit tautomerism (*21*). The existence of tautomerism in case of kasugamycin is also shown by the fact that the hydrolysis with barium hydroxide produces a mixture of kasuganobiosamine and kasugamycinic acid. Therefore, the intrinsic structure of kasugamycin in solution is considered as shown below.



It should be mentioned also that the pK'a of kasugamycin shows 7.1 as the amino group at C–2, 10.6 as amidine, and below 2.0 as carboxylic

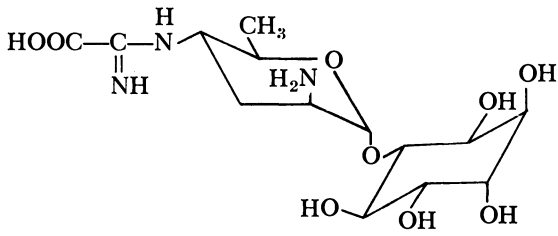
Table V. Incorporation of Various

Substrate	Isotope Added μc	Incorporation into KSM Activity Rel. Act. [a] %	c.p.s.
Maltose–U–C^{14}	25.6	1.98	311
D–Glucose–U–C^{14}	25.0	8.46	2,040
Myoinositol–U–C^{14}	5.0	53.70	1,969
Glycerol–1–C^{14}	10.0	1.24	45.9
Glycine–1–C^{14}	25.0	17.09	2,724
Glycine–2–C^{14}	10.0	19.61	1,214

Medium: 1.5% maltose, 1.5% soybean meal, 0.1%

[a] Specific activity of KSM/μ mol of C.
[b] Specific activity of fragments/μ mol of C/specific activity

acid, and the infrared spectrum displays absorption at 1670 $cm.^{-1}$ for the carboxyl group attached to the amidine.



14

X-ray analysis of kasugamycin hydrobromide gave the stereochemical structure of kasugamycin completely. The result of x-ray analysis has disclosed the linking position of *d*-inositol with kasugamine and D-configuration of kasugamine. Therefore, the structure **14**, 2,4-diamino-N^4-(carboxyformidoyl)-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranoside of *d*-inositol has been assigned to kasugamycin.

This is the first case to observe 2,4-diamino-2,3,4,6-tetradeoxy-D-*arabino*-hexopyranose in natural products and the first isolation of *d*-inositol from microbial products. Kasugamycin is the first compound possessing a unique group of an amidine carboxylic acid.

Biosynthesis

The route of kasugamycin's biosynthesis, especially of the amidine carboxylic acid moiety, is interesting. Either C–1 or C–2 labeled glycine

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Compounds into KSM and Its Fragments

Relative Incorporation [b] *into KSM Fragments*		
d-*Inositol*	*Kasugamine*	–C–COOH ‖ NH
0.48	1.74	0.33
0.54	1.75	0.13
2.22	0.11	0.03
1.10	1.04	0.57
0.005	0.007	6.96
0.006	0.006	6.97

K_2HPO_4, 0.1% $MgSO_4$.$7H_20$, 0.3% NaCl, (pH 6.0).

of KSM/μ mol of C.

was incorporated into kasugamycin (**1**) in high rate such as 17% or 19% of the glycine added. Most of the radioactivity was found in the amidine carboxylic acid moiety. Thus, both carbons of glycine are used for synthesis of the amidine carboxylic acid moiety.

Table V indicates the incorporation and distribution of labeled compounds into kasugamycin (**1**), when they are added during the production of this antibiotic. Glucose is incorporated into kasugamine and *d*-inositol. *Myo*-inositol is mainly incorporated into the *d*-inositol moiety, suggesting the synthesis of *d*-inositol moiety through *myo*-inositol or its derivative from glucose or other carbon sources.

It is interesting to note the same relationship between the activity and the functional groups of kasugamycin derivatives. The amidine carboxylic acid moiety plays an important role to the activity. Kasugamycinic acid (**9a**) and kasuganobiosamine (**4**) have no biological activity. The amide of kasugamycinic acid (**11a**) shows about 10% inhibitory activity of kasugamycin (**1**) against *Pseudomonas* but shows no activity against *Piricularia oryzae*. However, C_9-amine (**3**) has substantially no activity and therefore, the *d*-inositol moiety may also contribute to the activity of kasugamycin.

Experimental

(Note: All the melting points are uncorrected.)

Kasugamycin (**1**) *and its Hydrochloride.* The hydrochloride (*26*) was used after recrystallization from aqueous ethyl alcohol, and the free

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base was prepared from the hydrochloride by chromatography using IRA-400, and recrystallized from aqueous ethyl alcohol. Their properties are shown in Table II.

ACID HYDROLYSIS. A solution of kasugamycin hydrochloride (1.5 grams, 3.46 mmoles) dissolved in 15 ml. of 6*N* hydrochloric acid was heated at 105°C. for five hours in a sealed tube. The solution was condensed to 5 ml. under a reduced pressure and the addition of 50 ml. of ethyl alcohol afforded a crude solid overnight. It was recrystallized from aqueous ethyl alcohol, showing m.p. 246°-247°C. (dec.). It showed no depression in the mixed-melting point and completely identical infrared spectrum with *d*-inositol which was supplied by L. Anderson of the University of Wisconsin. The yield was 81% (503 mg., 2.79 mmoles). *Anal.* Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.71; O, 53.29; mol. wt., 180.16. Found: C, 40.11; H, 6.67; O, 53.33; mol. wt., 180 (vapor pressure osmometer).

*C_9-Amine (**3**) by Acid Methanolysis.* Dry hydrogen chloride gas was saturated in a suspended solution of kasugamycin hydrochloride (5 grams, 11.52 mmoles) and 300 ml. of methanol to afford a colorless transparent solution. It was refluxed for 10 hours under bubbling of dry hydrogen chloride gas. The reaction mixture was completely dried under a reduced pressure and the residue was dissolved in 200 ml. of water. It was neutralized with Dowex-3 (free form) to pH 7.0 and filtered. The filtrate (250 ml.) was placed on a column (3 x 70 cm.) of Amberlite CG-50 (ammonium form), allowed to pass (0.5 ml./minute) and then developed with water (1 ml./minute). Each fraction (15 ml.) was separately collected. All of the fractions were subjected to Lemieux and ninhydrin tests. Fractions from No. 20 to 35 positive to Lemieux reaction afforded *d*-inositol (320 mg.). Fractions from No. 66 and 94 positive to ninhydrin test were combined and condensed under a reduced pressure. The residue was adjusted to pH 4.0 with dilute hydrochloric acid and lyophilized, affording a pale yellow powder (825 mg.). It was first crystallized from ethyl alcohol-ether and then recrystallized from ethyl alcohol showing m.p. 210°-213°C. (dec.) and $[\alpha]_D^{15}$ +105° (c=1.4, H_2O). *Anal.* Calcd. for $C_9H_{17}O_4N_3 \cdot HCl \cdot 1/2H_2O$: C, 39.06; H, 6.92; N, 15.19; Cl, 12.81; C-CH_3, 5.43; O-CH_3, 11.2; Van Slyke N, 5.06. Found: C, 38.86; H, 7.09; N, 14.99; Cl, 12.98; C-CH_3, 3.97; O-CH_3, 9.76; Van Slyke N, 4.77. The functional groups of **1** and **3** are shown in Table III.

*Kasuganobiosamine (**4**) by Hot Alkaline Hydrolysis.* An aqueous solution (150 ml.) saturated with barium hydroxide was added to a solution of kasugamycin hydrochloride (5.3 grams, 12.22 mmoles) dissolved in 50 ml. of water free from carbon dioxide. The solution was refluxed on a steam bath for 10 hours. By the similar treatment of the reaction mixture as described in the case of cold alkaline hydrolysis, ammonia (11.10 mmoles), barium oxalate (3.037 grams, 12.48 mmoles) and kasuganobiosamine (3.638 grams, 11.80 mmoles) were obtained.

*Methylkasugaminide (**5**) from C_9-Amine (**3**).* An aqueous solution (30 ml.) saturated with barium hydroxide was added to a solution of C_9-amine hydrochloride (250 mg., 0.93 mmole) dissolved in water (3 ml.) free from carbon dioxide. The solution was refluxed for 10 hours on a steam bath. By the similar treatment of the reaction mixture as described in the case of cold alkaline hydrolysis, ammonia (0.91 mmole), barium

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oxalate (223.9 mg., 0.92 mmole) and methylkasugaminide (**5**) (118 mg., 0.74 mmole) were obtained.

*Methylkasugaminide (**5**) from Kasuganobiosamine (**4**).* A solution of kasuganobiosamine (617 mg., 2 mmoles) dissolved in 100 ml. of methanol saturated with hydrogen chloride was refluxed for 24 hours. After removal of the solvent under a reduced pressure, the residue was dissolved in 40 ml. of water and neutralized with Dowex-3 (free form) to pH 7.0. The resin was removed by filtration and washed with water. The combined filtrate (60 ml.) was placed and passed (0.5 ml./minute) on a column (1.5 x 30 cm.) of Amberlite CG-50 (ammonium form), and development of the chromatogram with water (200 ml.) gave fractions (54 mg.) positive to Lemieux reaction from which d-inositol was obtained. Development of the chromatogram with 0.1 *N* ammonia afforded fractions positive to ninhydrin test. The fractions were lyophilized to afford a pale yellow powder. The powder was sublimed to give a colorless material (19 mg.), which was identified to be methylkasugaminide (**5**) (1 mm., 110°C.).

N,N′-Diacetylkasuganobiosamine. To a suspension of kasuganobiosamine (500 mg., 1.6 mmoles) in 10 ml. of methanol was added 2.5 ml. of acetic anhydride. The mixture became a colorless transparent solution after allowing to stand at room temperature for 5 hours. After adding 20 ml. of water, the reaction mixture was allowed to stand at room temperature for 2 hours. An oily material was obtained after removal of the solvent, and dissolved in 50 ml. of water to adjust to pH 5.0 with dilute sodium hydroxide. The solution was placed and passed on a column (1.5 x 20 cm.) of Amberlite CG-50 (H form). Development of the chromatogram with water afforded fractions positive to Lemieux reaction. A colorless powder (620 mg.) was obtained after removal of the solvent. The powder was dissolved in methanol (15 ml.) to remove insoluble material. To the filtrate was added ether until no precipitate was produced. This procedure was repeated twice, affording a colorless material, m.p. 143°-150°C., $[\alpha]_D^{20}$ +113° (c=1.1, H_2O) which was identified to be *N,N′*-diacetyl derivative of **4**. The yield was 313 mg. (0.79 mmole). Anal. Calcd. for $C_{16}H_{28}O_9N_2$: C, 48.97; H, 7.19; O, 36.70; N, 7.14. Found: C, 48.93; H, 7.46; O, 37.10; N, 6.92.

*Periodate Oxidation of N,N′-Diacetylkasuganobiosamine. N,N′-*Diacetylkasuganobiosamine (1.2 grams, 3.06 mmoles) and sodium periodate (5.35 grams, 24.48 mmoles) were dissolved in 200 ml. of water and the solution was allowed to stand at room temperature for 3 days in a dark place. The amount of the consumed periodate was determined by the general method (*3*). About seven mmoles (6.6 mmoles) equivalent of the periodate to the substrate was consumed, and carbon dioxide (1.53 mmoles, 0.5 mole/substrate) and formic acid (14.4 mmoles, 4.7 moles/substrate) were obtained. After the reaction, ethylene glycol (310 mg., 5 mmoles) was added and allowed to stand at room temperature for one hour and the reaction mixture was neutralized with dilute barium hydroxide. Precipitates were removed by filtration and the filtrate was condensed by lyophilization to afford a colorless powder. The powder was treated with 200 ml. of acetone to remove insoluble material. The organic layer was subjected to dryness by evaporation of the solvent to afford a glassy solid (715 mg.). The solid was placed on a column of silica gel (3 x 43 cm.) and washed with 500 ml. of acetone. Development

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of the chromatogram with acetone-ethanol (1:1) afforded fractions positive to Benedict reaction. The combined fractions afforded a colorless powder after removal of the solvent. The powder was treated with 20 ml. of acetone at 60°C. to remove insoluble material. After filtration and cooling, a crystalline solid precipitated. It melted at 123°–125°C., and proved to be *N,N'*-diacetylkasugamine. *Anal.* Calcd. for $C_{10}H_{18}O_4N_2$: C, 52.16; H, 7.88; O, 27.79; N, 12.17. Found: C, 52.09; H, 8.02; O, 27.28; N, 11.81.

Oxidation of N,N'-Diacetylkasugamine with Bromine. *N,N'*-Diacetylkasugamine (230 mg., 1 mmole) and barium benzoate (600 mg., 1.4 mmoles) were dissolved in 20 ml. of water, and bromide (187 mg., 1.17 mmoles) was added. The reaction mixture was stirred at 10°C. for 42 hours in a dark place. After the reaction, a solid deposit was removed by filtration and the filtrate was treated with 2.5 ml. of 0.515*N* sulfuric acid followed by the treatment with silver carbonate, which was freshly prepared from 500 mg. of silver nitrate and 200 mg. of sodium bicarbonate. After stirring for 30 minutes, precipitates were removed by filtration and the filtrate thus obtained was extracted with ether. The aqueous layer was passed through a column of Amberlite CG-50 (H form, 1 x 15 cm.), and condensed by lyophilization to afford 288 mg. of a colorless powder. The powder was treated with 20 ml. of acetone to remove insoluble material. After filtration and removal of the solvent, the residue was recrystallized from ether, affording a crystalline material (**7**) (23.6 mg.), m.p. 168°–172°C. (dec.). Its infrared spectrum showed strong absorption at 1750 cm^{-1} (KBr) characteristic of δ-lactone. *Anal.* Calcd. for $C_{10}H_{16}O_4N_2$: C, 52.62; H, 7.07; O, 28.04; N, 12.27. Found: C, 52.46; H, 7.20; O, 27.97; N, 11.91.

*Kasuganobiosamine (**4**) and Kasugamycinic Acid (**9a**) by cold Alkaline Hydrolysis.* Kasugamycin hydrochloride (622 mg., 1.43 mmoles) was dissolved in 5 ml. of water free from carbon dioxide and 50 ml. of water saturated with barium hydroxide was added. The solution was allowed to stand at room temperature for 36 hours. Ammonia (0.30 mmole) was produced and barium oxalate (199 mg., 0.80 mmole) was obtained. After removal of barium oxalate by filtering, the filtrate was neutralized with dry ice. After removal of barium carbonate by filtering, the filtrate was adjusted to pH 7.0 and placed on a column of Amberlite CG-50 (ammonium form, 1.5 x 22 cm.), allowed to pass with a rate of 0.5 ml/minute, and washed with water. The fractions positive to ninhydrin test were collected and condensed to 1 ml. under a reduced pressure. Addition of ethyl alcohol to the residue afforded a crystalline material. It was recrystallized from aqueous ethyl alcohol to afford a colorless material (201 mg.), m.p. 218°–221°C. (dec.), $[\alpha]_D^{15}$ + 119° (c=1, H_2O), pK'a 1.8 and 7.8 (H_2O). It proved to be kasugamycinic acid (**9a**). *Anal.* Calcd. for $C_{14}H_{24}O_{10}N_2 \cdot H_2O$: C, 42.21; H, 6.58; O, 44.18; N, 7.03; C–CH_3, 3.77; Van Slyke N, 3.52. Found: C, 42.31; H, 6.67; O, 43.86; N, 7.12; C–CH_3, 2.95; Van Slyke N, 3.61.

Further development of the chromatogram with 0.1*N* ammonia afforded fractions positive to ninhydrin test. From the fractions, 249 mg. of a colorless material was obtained. It was dissolved in 19 ml. of water and the solution was adjusted to pH 4.0 with dilute hydrochloric acid. A colorless material, after condensing under a reduced pressure and lyophilization, was recrystallized from aqueous methanol with a small amount

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of ether to afford needle crystals (201 mg.), m.p. 222.5°–225° (dec.), $[\alpha]_D^{15}$ +101° (c=1.8, H_2O), pK'a 6.7 and 8.5 (H_2O). It proved to be kasuganobiosamine dihydrochloride. *Anal.* Calcd. for $C_{12}H_{24}O_7N_2$ 2HCl: C, 37.80; H, 6.87; O, 29.37; N, 7.35; Cl, 18.60; Van Slyke N, 9.10. Found: C, 37.76; H, 7.02; O, 29.61; N, 7.33; Cl, 17.56; Van Slyke N, 8.35.

*Alkaline Hydrolysis of Kasugamycinic Acid (**9a**) with Barium Hydroxide.* Kasugamycinic acid (250 mg., 0.63 mmole) was hydrolyzed with barium hydroxide-saturated-water at 100°C. for 10 hours, and the reaction mixture was treated in the similar manner as described in the case of alkaline hydrolysis of kasugamycin, affording kasuganobiosamine (**4**) (179 mg., 0.68 mmole) and barium oxalate (151 mg., 0.62 mmole).

*Acid Hydrolysis of Kasugamycinic Acid (**9a**) with 6N Hydrochloric Acid. d*-Inositol (103 mg., 0.57 mmole) was obtained from the treatment of kasugamycinic acid (515 mg., 1.29 mmoles) with 6*N* hydrochloric acid followed by the similar procedure as described in the case of acid hydrolysis of kasugamycin.

*Acid Hydrolysis of Kasuganobiosamine (**4**) with 6N Hydrochloric Acid. d*-Inositol (117 mg., 0.65 mmole) was obtained from the treatment of kasuganobiosamine (507 mg., 1.64 mmoles) with 6*N* hydrochloric acid followed by the similar procedure as mentioned above.

Mono-N-acetylation of Kasuganobiosamine (**4**). Acetic anhydride (0.4 ml.) was added to a suspension of kasuganobiosamine (1.243 grams, 4.03 mmoles) in 20 ml. of methanol and the reaction mixture was allowed to stand at room temperature for 6 hours. After removal of the solvent, the residue was dissolved in 75 ml. of water and placed on a column of Amberlite CG-50 (ammonium form, 2 x 36 cm.). Development with water afforded two major fractions, both positive to ninhydrin test. The first fraction afforded 299 mg. of a colorless solid after complete dryness by lyophilization. It was recrystallized from methanol-acetone to afford a crystalline material (105 mg., 0.30 mmole), m.p. 140°–143°C. (dec.), $[\alpha]_D^{20}$ +116° (c=1.3, H_2O), pK'a 7.8. It proved to be 4*N*-acetyl derivative (**10a**). *Anal.* Calcd. for $C_{14}H_{26}O_8N_2$: C, 47.99; H, 7.48; O, 36.53; N, 8.00; Van Slyke N, 4.00. Found: C, 47.85; H, 7.51; O, 36.21; N, 8.15; Van Slyke N, 3.79.

The second fraction afforded 255 mg. of a colorless solid after the similar treatment as described above. It was rechromatographed on Amberlite CG-50 column to afford 175 mg. (0.50 mmole) of a colorless solid, m.p. 138°–143°C., $[\alpha]_D^{20}$ +90° (c=1.0, H_20), pK'a 8.2. It proved to be the isomer of the first fraction, 2-*N*-acetyl derivative (**10b**). *Anal.* Found: C, 47.43; H, 7.38; O, 36.35; N, 7.87; Van Slyke N, 3.85.

The structures of these two isomers of mono-*N*-acetyl derivative of **4** were decided on the basis of their NMR spectra as discussed in the part of structural studies.

N-Acetylation of Kasugamycinic Acid (**9a**). A solution of kasugamycinic acid (225 mg.) dissolved in 10 ml. of water was treated with acetic anhydride (0.3 ml.) under cooling; sodium bicarbonate was used to keep the pH 7.2 and stirring continued for 30 minutes. The reaction product was passed through Dowex 50W-X2 (H form) and the column was washed with water. The combined filtrate was subjected to lyophilization to afford 234 mg. of a crude *N*-acetyl derivative. Its infrared spectrum showed strong absorptions at 1740 cm^{-1} characteristic of oxamic acid group. The *N*-acetyl derivative (178 mg.) was treated with 40 ml.

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of water saturated with barium hydroxide at 100°C. for 1 hour. After the reaction, the solution was neutralized with dry ice. Precipitates were removed by filtration and the filtrate was condensed to 10 ml. and placed on a column of Amberlite CG-50 (ammonium form, 2 x 15 cm.) and the chromatogram was developed with water to afford fractions positive to ninhydrin test. The fractions afforded 48 mg. of a colorless solid which was found to be completely identical with 2-*N*-acetylkasuganobiosamine (**10b**) synthesized from kasuganobiosamine.

*Oxamide Derivatives. (**11a**) and (**11b**), from Kasuganobiosamine (**4**).* Kasuganobiosamine (3 grams, 9.7 mmoles) and methyloxalate (1.77 grams, 14.9 mmoles) were dissolved in 100 ml. of methanol, and the solution was allowed to stand at room temperature for 50 minutes. After removal of the solvent, the residue was treated with 12 ml. of 28% ammonia at room temperature for 3 hours with stirring. After ammonia was removed under a reduced pressure, the residue was dissolved in 200 ml. of water and adjusted to pH 7.0. The solution was placed on a column of Amberlite CG-50 (ammonium form, 3.5 x 17 cm.) and the chromatogram was developed with water. Fifteen ml. of each fraction were separately collected. First ninhydrin-positive fractions (No. 4 to 18) were condensed to 1 ml. and adding 40 ml. of ethyl alcohol deposited a crystalline material (124 mg.) which was found to be identical with **9a**. Second ninhydrin-positive fractions (No. 77 to 100) were condensed and lyophilized to afford 453 mg. of a colorless powder. It was recrystallized from water to give a crystalline material (363 mg., 0.97 mmole), m.p. 174°–177°C., which proved to be identical with the amide (**11a**) obtained from a reaction of methyl kasugamycinate and ammonia. Third ninhydrin-positive fractions (No. 106 to 170) were condensed and lyophilized to afford 706 mg. of a colorless powder. It was purified by rechromatography on a column of Amberlite CG-50, affording a colorless powder (534 mg.), m.p. 150°–158°C., $[\alpha]_D^{26}$ +83° (c=1, H_2O), pK'a 7.8. It proved to be **11b** on the basis of its elemental analysis and spectral evidence. *Anal.* Calcd. for $C_{14}H_{25}O_9N_3$: C, 44.32; H, 6.64; O, 37.96; N, 11.07; Van Slyke N, 3.69; mol. wt., 379.4. Found: C, 43.97; H, 6.75; O, 38.39; N, 10.88; Van Slyke N, 3.72; mol. wt., 389 (titration).

*Methyl Kasugamycinate and its Amide (**11a**).* Kasugamycinic acid (1.1 grams) was dissolved in 10 ml. of methanol containing *N* hydrochloric acid and the solution was allowed to stand at room temperature for 10 minutes. After removal of the solvent, ether was added to the residue. Crude methyl ester of **9a** (1.19 grams) was separated by trituration. Its infrared spectrum showed strong absorption at 1750 $cm.^{-1}$. The methyl ester (1 gram) was treated with 5 ml. of 28% ammonia and the solution was allowed to stand at room temperature for 3 hours. After removal of the solvent, the residue was dissolved in 50 ml. of water and the solution was placed on a column of Amberlite CG-50 (ammonium form, 1.5 x 50 cm.). The chromatogram was developed with water. Fifteen ml. of each fraction were separately collected. Ninhydrin-positive fractions (No. 95 to 153) were combined, condensed and dried by lyophilization to afford 523 mg. of a colorless powder. It was recrystallized from water to give a crystalline material (195 mg.), m.p. 174°–177°C. (dec.), $[\alpha]_D^{26}$ +111° (c=1, H_2O), pK'a 7.6. It proved to be an amide (**11a**) of methyl kasugamycinate on the basis of its elemental

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analysis, molecular weight, and spectral evidence. *Anal.* Calcd. for $C_{14}H_{25}O_9N_3$: C, 44.32; H, 6.64; O, 37.96; N, 11.07; Van Slyke N, 3.69; mol. wt., 379.4. Found: C, 44.17; H, 6.80; O, 37.76; N, 11.02; Van Slyke N, 3.58; mol. wt., 390 (titration).

*Periodate Oxidation of C_9-Amine (**3**).* C_9-Amine hydrochloride (268 mg., 1 mmole) was treated with a solution of sodium periodate (235 mg., 1.1 mmoles) dissolved in 20 ml. of water at room temperature for two days. During the reaction, carbon dioxide generated was introduced to 1*N* barium hydroxide solution with nitrogen. Thus, barium carbonate (139.2 mg., 0.7 mmole) was obtained. After the reaction, ethylene glycol (31 mg., 0.5 mmole) was added, and the solution was allowed to stand at room temperature for 30 minutes. A pale yellow powder was obtained after dryness by lyophilization. It was treated with 50 ml. of acetone to remove insoluable material. It was filtered and the filtrate was then dried under reduced pressure. The residue was dissolved in 20 ml. of water and the solution was placed on a column of Amberlite CG-50 (ammonium form, 2 x 10 cm.). Development of the chromatogram with water afforded fractions positive to both ninhydrin and nitroprusside reactions (9). The fractions were condensed and dried by lyophilization to give a colorless powder. It was treated with 20 ml. of acetone and the insoluble material was removed by filtration. Removal of the solvent from the filtrate deposited a crystalline material (105 mg., 0.62 mmole), m.p. 123°–126°C. by allowing to stand. Its infrared spectrum showed a sharp absorption at 2200 cm^{-1} (–N–C≡N). *Anal.* Calcd. for $C_8H_{15}O_2N_3$: C, 51.87; H, 8.16; N, 22.69. Found: C, 51.63; H, 8.03; N, 22.37.

*Oxidation of C_9-Amine (**3**) with Lead Tetraacetate.* C_9-Amine hydrochloride (53 mg., 0.17 mmole) was dissolved in 0.1 ml. of water and 5 ml. of acetic acid and to the solution was added lead tetraacetate (190 mg., 0.3 mmole) at 16°–18°C. in 10 minutes. Insoluble material was removed by filtration, and the filtrate was dried by lyophilization. The residue was dissolved in 5 ml. of water and the solution was placed on a column of Amberlite CG-50 (ammonium form, 1 x 10 cm.) followed by a similar treatment as described in the case of periodate oxidation. *N*-Cyano derivative (5.1 mg.) was obtained and proved to be identical with the *N*-cyano derivative obtained from the periodate oxidation.

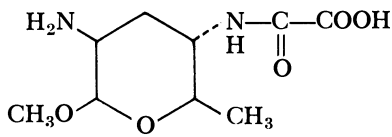
*Treatment of Kasugamycin (**1**) with Acetic Anhydride.* Kasugamycin hydrochloride (1.0 grams) was suspended in 20 ml. of acetic anhydride under cooling with ice-water and 2 ml. of pyridine was added. The reaction mixture was allowed to stand at room temperature for 15 hours and further stirred at 28°C. for 72 hours. The solution became yellow and transparent. After the ice-water was added, the mixture was allowed to stand overnight. It was extracted with chloroform twice. The organic layer was dried over sodium sulfate. After removal of the solvent, the residue was dissolved in 3 ml. of ethyl alcohol and allowed to stand. A colorless crystalline material deposited. It was found to be heptaacetylkasuganobiosamine (825 mg.). By extracting the filtrate with ether, 688 mg. of a residue were obtained. The ethereal solution deposited a needle crystalline material (124 mg.) which was recrystallized from chloroform-ether, showing m.p. 64°–66°C. It proved to be acetylformamide on the basis of its elemental analysis and spectral evidence. The yield was 70 mg. *Anal.* Calcd. for $C_3H_5O_2N$: C, 41.38; H, 5.79; O, 36.75; N, 16.09. Found: C, 42.10; H, 5.85; O, 36.44; N, 15.51.

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

It was observed that carbon dioxide was generated during the reaction by trapping as barium carbonate.

*Partial Synthesis of Kasugamycin (**1**) from Kasuganobiosamine (**4**).* Kasuganobiosamine (3.08 grams, 10 mmoles) and diethylester of oxalimidic acid (1.60 grams, 11 mmoles) was dissolved in 50 ml. of water and the solution was allowed to stand at room temperature for 24 hours. Five ml. of 35% hydrochloric acid was added to the reaction mixture, and the solution was heated at 95°–98°C. for 3 hours. After the reaction, 450 ml. of water was added and the solution was neutralized with Dowex-3 (free form) to pH 6.0 and condensed to 200 ml. The condensed solution was placed on a column of Amberlite CG-50 (ammonium form, 2.5 x 40 cm.) and developed with water. Fifteen ml. of each fraction was separately collected. Each fraction was subjected to biological activity using a disc-plate method (*26*) against *Piricularia oryzae*. Active fractions (No. 277 to 366) were collected and adjusted to pH 5.0 with dilute hydrochloric acid. After condensing and dryness by lyophilization, a pale yellow powder (442 mg.) was obtained. The powder was recrystallized from aqueous ethanol to afford a crystalline material, m.p. 236°–239°C. (dec.), which was found to be identical with natural kasugamycin hydrochloride in all respects including m.p., infrared, NMR, $[\alpha]_D$, and biological activity.

*Methylkasugaminide (**5**) by Cold Alkaline Hydrolysis of C_9-Amine (**3**) with Barium Hydroxide.* A solution of C_9-amine (519 mg., 2.24 mmoles) dissolved in 5 ml. of water free from carbon dioxide was treated with 50 ml. of barium hydroxide saturated solution at room temperature for 48 hours. Generation of ammonia was observed and barium oxalate (241 mg., 0.99 mmole) was obtained. After removal of barium oxalate, barium carbonate produced by neutralization with dry ice was also removed by filtration. The filtrate thus obtained was placed on a column of Amberlite CG-50 (ammonium form, 1.5 x 25 cm.) and developed with water. After the similar treatment as described in the cold hydrolysis of kasugamycin, ninhydrin-positive fractions afforded a colorless crystalline material (154 mg., 0.62 mmole), m.p. 223°–225°C. (dec.), $[\alpha]_D^{20}$ +110° (c=1.7, H_2O), pK′a 1.8 and 7.9, which was identified to be C_9-acid (**15**). *Anal.* Calcd.



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for $C_9H_{16}O_5N_2$ H_2O: C, 43.19; H, 7.25; O, 38.36; N, 11.20; mol. wt., 250.26. Found: C, 42.95; H, 7.23; O, 38.42; N, 10.96; mol. wt., 245 (titration).

In the next, the chromatogram was developed with 0.1*N* ammonia to afford ninhydrin-positive fractions, from which methylkasugaminide (**5**) (106 mg.) was obtained. It was sublimed at 110°C. under a reduced pressure (1 mm.), showing m.p. 111.5°–116°C., $[\alpha]_D^{15}$ +70° (c=1, H_2O), pK′a 6.9 and 8.7. *Anal.* Calcd. for $C_7H_{16}O_2N_2$: C, 52.47; H, 10.07;

O, 19.97; N, 17.47; C–CH_3, 9.37; O–CH_3, 19.35; Van Slyke N, 17.50; mol. wt., 160.221. Found: C, 51.86; H, 10.03; O, 20.14; N, 17.35; C–CH_3, 9.20; O–CH_3, 16.97; Van Slyke N, 15.7; mol. wt., 160 (*m/e*, parent peak).

*Acetylation of Kasuganobiosamine (**4**).* Acetic anhydride (2 ml.) and pyridine (4 ml.) were added to kasuganobiosamine (212 mg., 0.688 mmole) and the solution was allowed to stand at room temperature for 24 hours. The reaction mixture was treated with ice-water and extracted three times with chloroform. The organic layer was dried over sodium sulfate. After removal of the solvent, an oily material was treated with 30 ml. of ether to deposit a crystalline material. It was further recrystallized from ether-ethyl alcohol to afford a needle crystalline material (409 mg., 0.679 mmole), m.p. 242°–243°C., and identified to be heptaacetate of **4**. *Anal.* Calcd. for $C_{26}H_{38}O_{14}N_2$: C, 51.58; H, 6.44; O, 36.69; N, 4.41. Found: C, 51.27; H, 6.44; O, 36.42; N, 4.53.

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3

Deoxycyclitols: Stereochemical and NMR Studies

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The deoxyinositols (quercitols, cyclohexanepentols) are useful model compounds which display many of the physical and chemical properties of true deoxy sugars. Although (+)-proto-*quercitol, the best known isomer, was isolated from nature 118 years ago, no synthesis has been reported up until now. The synthesis here described is actually that of the (−)-enantiomer, starting with (−)-inositol; however, identical procedures applied to the readily available (+) or* DL-*inositol would give (+) or* DL-proto-*quercitol, respectively. The natural occurence of (—)-*proto-*quercitol has also been reported recently. Configurational interpretation of the NMR spectra of quercitols was at first difficult or impossible. Such interpretations can now easily be accomplished by recording the proton spectra at 220 MHz. (51.7 kilogauss), using a superconducting solenoid. The synthesis of ring-deoxy sugars (pseudo-hexoses) is also discussed.*

Hydroxylated cyclohexanes, especially those with five hydroxyl groups, are valuable model compounds which exhibit much of the stereochemical and physical behavior of true sugars and deoxy sugars, without complications attributed to ring-opening and closing, or anomerization. They may be important, also, because of their relationship to *myo*-inositol (**2**) which is present in every living cell of every plant or animal, so far as is known, and is one of the very small group of organic compounds essential for growth of isolated human cells in cultures (*21,37*).

The cyclohexanediols, -triols, and -tetrols each have three structures (*e.g.*,. 1,2; 1,3; and 1,4 for the diols), but the cyclohexanepentols and -hexols and cyclohexanol itself each have only one structure. For these twelve structures a total of fifty diastereomeric forms (28 meso, 22 racemic) is possible. Cyclohexanol and the -diols have long been known,

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

and the last of the -triols (*29,30*), -pentols (*23,32*), and -hexols (*4*) have recently been reported. All but two of the numerous -tetrol diastereomers have been reported, and work on these two remaining isomers is in progress (*27*). The large number of meso diastereomers (28 out of 50) is an interesting feature of these cyclitols; it results from the symmetry of the cyclohexane ring, which is not present in the hexopyranose ring.

Although the best alicyclic analogs of deoxyhexoses might be the deoxyinososes—*e.g.* **1**, very little is yet known about these compounds. The present chapter will therefore be devoted mainly to the deoxyinositols (quercitols, cyclohexanepentols), which strictly speaking are analogs of deoxyhexitols. A section is also included on the new ring-deoxy or "pseudo" sugars—*e.g.* **34**, in which the pyranose ring-oxygen atom is replaced by methylene. These compounds have the primary alcohol side-chain typical of hexopyranoses.

Monodeoxyinositols or Quercitols

Since earlier reviews (*20,21,37*) are available, only very recent developments will be considered here.

Synthesis of Deoxyinosamines from vibo-*Quercitol.* As yet, the quercitols have rarely been used as synthetic starting materials. Recently, however, Suami and Yabe (*42*) have reported clever syntheses of two (acetylated) deoxyinosamines (**8** and **9**, Y= NHAc) and one (acetylated) deoxyinosadiamine (**10**, Y = NHAc) from racemic *vibo*-quercitol (*4*). The syntheses were effected by selective mesylation of one or two hydroxyl groups and displacement of each mesyloxy group by an azido group, which was reduced to amino. Although attempted S_N2 displacement of cyclohexane substituents is often unsuccessful, the powerfully nucleophilic azide ion is usually able to displace an alkylsulfonoxy group, and this route has been exploited in several recent cyclitol syntheses.

The *vibo* or DL (124/35) quercitol (**4**) needed for the synthesis was prepared from *myo*-inositol (**2**) *via* the bromoquercitol (**3**) according to the method of McCasland and Horswill, (*28*). By acetonation, acetylation, deacetonation, and equatorial mesylation the mesyloxy derivative (**5**) was obtained. This was converted to the azidotetrol derivative (**8**) (Y = N_3); the anchimeric effect of the position 3 acetoxy group (formula **5**) resulted in inversions of configuration at positions **2** and **3**. The *proto* or DL (134/25) configuration was retained in the final product, **8** (Y = NHAc), a derivative of 3-amino-1,2,4,5-cyclohexanetetrol (*42*).

The 1-mesyloxy intermediate (**6**) was similarly prepared *via* the equatorial monobenzoate, and it reacted with azide ion by a single S_N2 displacement, since no anchimeric effect was possible here. The *scyllo*

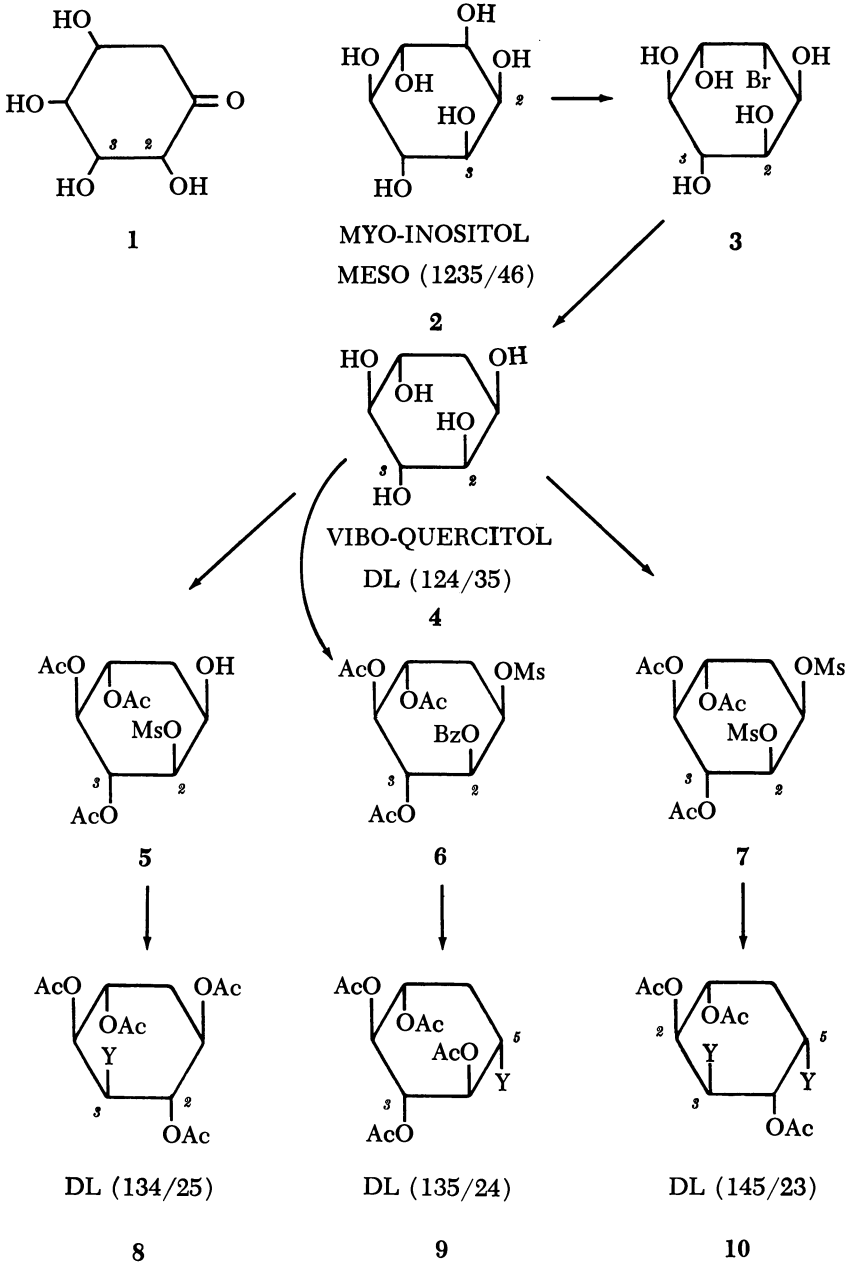

CHART A

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or DL (135/24) configuration was retained in the final product, **9** (Y = NHAc), a derivative of 5-amino-1,2,3,4-cyclohexanetetrol (*42*).

Finally the dimesyl derivative (**7**) was prepared in a similar manner. When it reacted with azide ion, configurational inversions were observed at positions 1, 2, and 3 (formula **7**), owing in part to the anchimeric effect of the position 3 acetoxy group. The *vibo* or DL (145/23) configuration (same as in the starting material) was retained in the final product **10** (Y = NHAc), a derivative of 3,5-diamino-1,2,4-cyclohexanetriol (*42*).

All of the intermediates and products were characterized by NMR (*42*).

Natural Occurrence of (−)-proto-*Quercitol.* Although the dextrorotatory form (**12**) of *proto*-quercitol was discovered in acorns more than a century ago by Braconnot (*5*), who at first thought that it was lactose, the levorotatory form (**13**) remained unknown until 1961. In that year, Plouvier isolated it from leaves of the tree *Eucalyptus populnea;* the yield was 0.55% (*36*). The optical rotation of the new compound was equal and opposite to that of the dextro enantiomer, and it was identical to the latter in its crystal form, melting point, solubilities, molecular formula and infrared spectrum.

Plouvier then prepared the previously unknown racemic form of *proto*-quercitol by mixing equal weights of the two enantiomers. The melting point (237°C.) of the mixture was not depressed, and its (presumably solid state) infrared spectrum reportedly (*36*) was identical with that of either active form. It thus appears that DL-*proto*-quercitol exists as a solid solution, not a racemic compound or conglomerate.

The discoverer of levorotatory *proto*-quercitol unfortunately described it (36) as "L-quercitol." The capital letter "L" should of course be understood to designate configuration, not rotation. And according to one widely accepted convention (*18,19*), the quercitol stereosiomer which has the configuration **13** would be designated "D", not "L". (*See* formulas **12** and **13**.) The name *quercitol* is now used in a generic sense (cyclohexanepentol), so that there are actually six diastereomers to which the name "L-quercitol" might apply.

According to the modified Maquenne system (*18,19,31*) used in this chapter, the diastereomeric configuration of any cyclitol is expressed by a fraction, and position-numbering, if otherwise equivocal, is so assigned that the numerator will have the lowest possible numbers. For example, *proto*-quercitol (**12** or **13**) is designated (134/25,), not (14/235) or (25/134).

To specify enantiomeric configuration, the pre-numbered perspective formula is so oriented in three dimensions that its numbering will proceed from right to left around the front of the ring, as customary for

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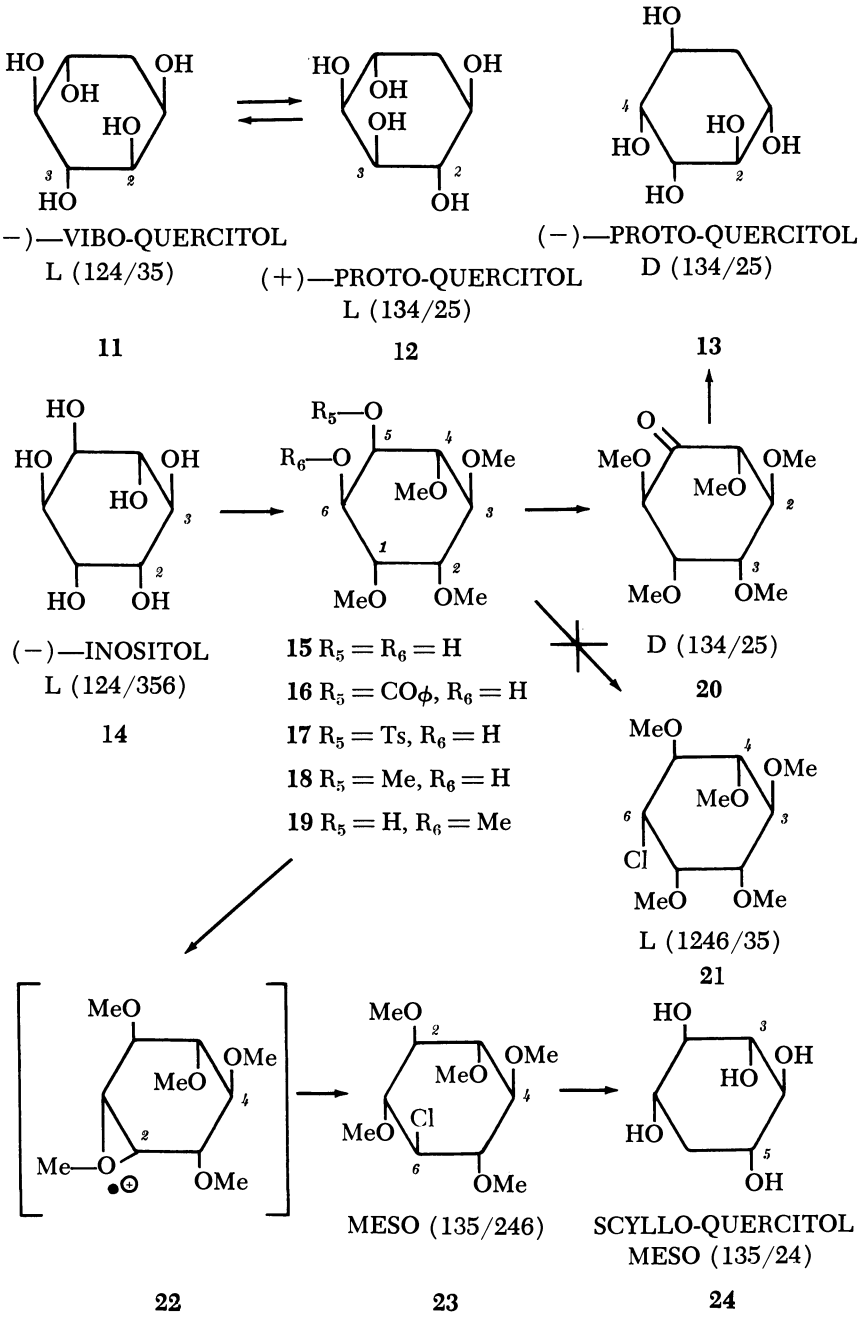

CHART B

monosaccarides. If the lowest-numbered group is then down, the enantiomer is designated "D"; if up, it is designated "L". For example, dextrorotatory *proto*-quercitol **12** is called L (134/25).

Until some uniform configurational nomenclature for cyclitols has been generally accepted, it would appear safer for authors in this field to specify the nomenclature used in every article, or to indicate configurations by means of formulas.

*The Synthesis of (−)-*proto*-Quercitol.* Although *proto*-quercitol (dextro) was discovered in 1849 (*5*), its cyclohexanepentol structure was not established until 1885 (*13*), and its configuration not until 1932. (*38*). The synthesis of this well-known cyclitol has been a difficult problem, since it appears that nearly every synthetic reaction commonly employed for other cyclitols would lead stereospecifically to the "wrong" product.

It was not until 1966 that the synthesis of *proto*-quercitol (levo) was finally accomplished (*30*) by indirect removal of the position 2 hydroxyl group in (−)-inositol (**14**).

The tetramethyl ether (**15**) of (−)-inositol was first prepared by acetonation, methylation, and deacetonation, and selectively esterified to the equatorial monotosylate **17**, whose remaining free hydroxyl group was then methylated. By reductive detosylation, the pentamethyl ether (**19**) was obtained and oxidized to (−)-*proto*-inosose pentamethyl ether (**20**) by means of the very useful new reagent (*35*) ruthenium dioxide/sodium periodate. Efforts to prepare the free inosose by ether cleavage were unsuccessful. The carbonyl group could not be hydrogenated to methylene by Posternak's well-known procedure (*38,39*); it now appears that this procedure works only when the carbonyl group has one or more neighboring free hydroxyl or acyloxyl groups (*29*).

Success finally resulted when the inosose ether (**20**) was converted to its ethylene mercaptal. The crude mercaptal was reduced with nickel, and the crude pentol pentamethyl ether cleaved in the usual manner.

The pure, crystalline (−)-*proto*-quercitol (**13**) which was isolated had an infrared spectrum identical with that of authentic (+)-*proto*-quercitol, and its optical rotation was equal and opposite. Further characterization and preparation of the racemic form, by mixing the enantiomers, is described elsewhere (*30*).

The identical synthetic procedures applied to (+)-inositol or DL-inositol should lead to (+)-*proto*-quercitol and DL-*proto*-quercitol, respectively. Since the total synthesis of DL-inositol had previously been reported (*33*), the new syntheses of the various forms of *proto*-quercitol are "total," with the possible exception of the step for resolution of DL-inositol, which so far has been accomplished only by a microbiological method (*43*).

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In 1965, Angyal, Gorin, and Pitman (*2,3*) obtained an equilibrium mixture containing 54% of (acetylated) (−)-*vibo*-quercitol, (**11**) by prolonged heating of (+)-*proto*-quercitol (**12**) with 95% acetic acid containing a little strong acid. Similar treatment of (−)-*vibo*-quercitol gave the same equilibrium mixture. The mixture was shown by vapor phase chromatography to contain (acetylated) (+)-*proto*-quercitol, but none of the latter product was actually isolated.

The tetramethyl ether (**15**) mentioned above was also converted to its equatorial monobenzoate (**16**), and the latter methylated and deesterified to give a hexol pentamethyl ether. As benzoyl migration occurred during methylation, this pentamethyl ether had the configuration **18**, and was not the diastereomer (**19**) mentioned above (*27*).

In early experiments, the pentamethyl ether (**18**) was treated with phosphorus pentachloride in the hope of obtaining a chloropentol reducible to (−)-*proto*-quercitol. (Allowing for the benzoyl migration, the expected product would have been (−)-*vibo*-quercitol (**11**).) Surprisingly, the quercitol actually obtained after demethylation and dehalogenation was neither of these but still another previously known isomer, meso-*scyllo*-quercitol (**24**) (*27*).

This result is attributed to participation by the neighboring methoxy group at position 1 (formula **18**) in the phosphorus pentachloride displacement reaction. A cyclic methoxonium ion (**22**) is postulated as the intermediate. The overall result is methoxyl migration from 1 to 6, with inversion of configuration at both positions (*27*).

NMR Studies on the Deoxyinositols. The successful application of proton magnetic resonance to configurational and conformational studies on cyclohexanol and the cyclohexanediols (*11*), -triols (*29*), -tetrols (*24,25*) and -hexols (*14,15*) has been reported by various authors. The cyclohexanepentols (quercitols) were found to be considerably more difficult and at first presented a severe challenge to the NMR method. This was because of complicated spin-coupling and overlapping of signals at 60 and even 100 MHz. In most isomers lack of the time-averaging and symmetry effects, which have often simplified the interpretations for diols, tetrols and hexols, added to the difficulties.

It now appears that such difficulties can largely be overcome by the application of proton-proton spin-decoupling and especially by means of the very high resolution now available in spectrometers of the superconducting solenoid type (*see* below).

Early in 1961, the first successful use of NMR for the assignment (*22*) of configuration to a quercitol (**26**) was accomplished indirectly, by analysis of the spectrum of its 6-bromo derivative. The spectrum of this bromoquercitol (**25** or **28**) fortunately contained a well-resolved *AB*

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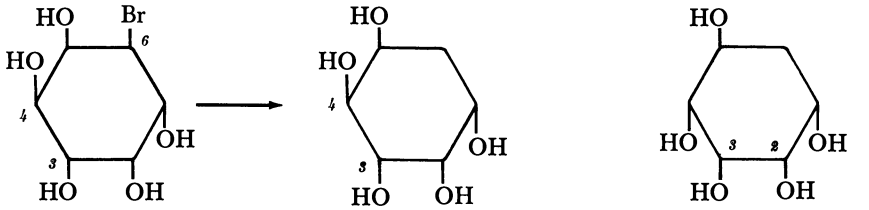
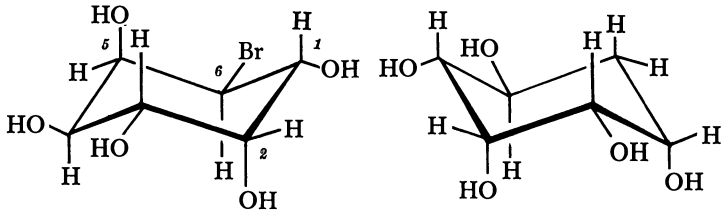
pattern assignable to the axial protons H–1 and H–6, formula **28**. The observed splittings of the *AB* pattern components revealed that the neighboring protons, H–2 and H–5, were equatorial. When chemical evidence was also considered, the (levorotatory) bromoquercitol could have only the D (123/456) configuration **25**, and the favored conformation **28**. The related (dextrorotatory) quercitol then necessarily had the D (123/45) or *"talo"* configuration **26**.



D (123/456) — **25**

D (123/45) (+)—TALO-QUERCITOL — **26**

DL (1234/5) ALLO-QUERCITOL — **27**



28 **29**

CHART C

Later in the year 1961, the first direct interpretations for a quercitol spectrum was reported (*23*) for the *"allo"* diastereomer (**27**). The methylene pattern (16-peak *AB* pattern) for this isomer was unusually well resolved, even at 60 MHz. From the coupling constants of this four-component *AB* pattern, it was apparent that each component was further split by two protons neighboring the methylene group, one axial, the other equatorial. This limited the possible configurations to four. Three of these were excluded by comparing the NMR spectra of authentic samples of these compounds with that obtained from the compound under investigation. The one remaining possibility was the DL (1234/5) or "allo" configuration **27**, which exists in the favored conformation **29**.

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Cyclitol Spectra at 220 MHz with the Superconducting Solenoid. In 1964, Nelson and Weaver (*34*) at Varian Associates constructed a superconducting solenoid with which proton spectra can be observed at 51.7 kilogauss (220 MHz.) or even higher fields. Other nuclei have been observed at suitable field/frequency combinations.

The niobium-zirconium wire used remains superconducting at 4° K. even in the strong field of the solenoid itself. The unique feature of the new apparatus is the very high field homogeneity in the sample region (2 cm. diameter sphere) kept at room temperature (*34*).

The power of the new spectrometer to reveal configurations of difficult cyclitols or sugars was first tested with *myo*-inositol (**2**), using deuterium oxide as solvent. At 60 or 100 MHz. the one equatorial and five axial protons appear to have different chemical shifts as shown by Lemieux in 1956 with a 40 MHz. instrument (*14,15*). However, since the five-proton axial signal could not be resolved, one could probably not have assigned the configuration **2** (which was already known from laborious chemical correlations extending over many years.)

At 220 MHz. (Figure 1), the spectrum was beautifully spread out and complete interpretation was easily accomplished. The axial proton H–5 appears as a triplet (3.26 p.p.m.) with coupling constants indicating that H–4 and H–6 are also axial. The smaller constants of the H–2 triplet (4.04 p.p.m.) reveal that H–2 is equatorial (since H–1 and H–3 are axial —*see* below). The equivalent protons H–1 and H–3 appear as a perturbed pair of doublets (3.51 p.p.m.); it is apparent that each proton is axial and has one axial and one equatorial neighbor. The equivalent axial protons H–4 and H–6 appear as a perturbed triplet (3.61 p.p.m.), from which it appears that each of these protons is coupled to two axial neighbors. The spectrum is consistent only with the *meso* (1235/46) configuration (**2**). If the configuration of *myo*-inositol had been unknown, it probably could have been assigned within a few hours after the spectrum was recorded.

The technique was next applied to (+)-*proto*-quercitol (**12**) with equally successful results. The axial methylene proton H–6_a (1.81 p.p.m.) shows splittings (large, large, small) owing to coupling with three protons (geminal, axial, and equatorial). (*See* formula in Figure 2.) The equatorial proton H–6_e (1.99 p.p.m.) similarly shows coupling (large, small, small) with the geminal and neighboring protons. The triplet of the axial proton H–2 (3.56 p.p.m.) reveals that its neighbors H–1 and H–3 are axial. The narrow patterns of H–4 and H–5 reveals that each is equatorial. It is clear that (+)-*proto*-quercitol has the diastereomeric configuration (134/25), formula **12**, and the favored conformation shown in Figure 2. The absolute configuration L (134/25) could be deduced from optical rotation calculations (*6,7,8,44*). The results confirm previous assignments based on years of laborious chemical correlations.

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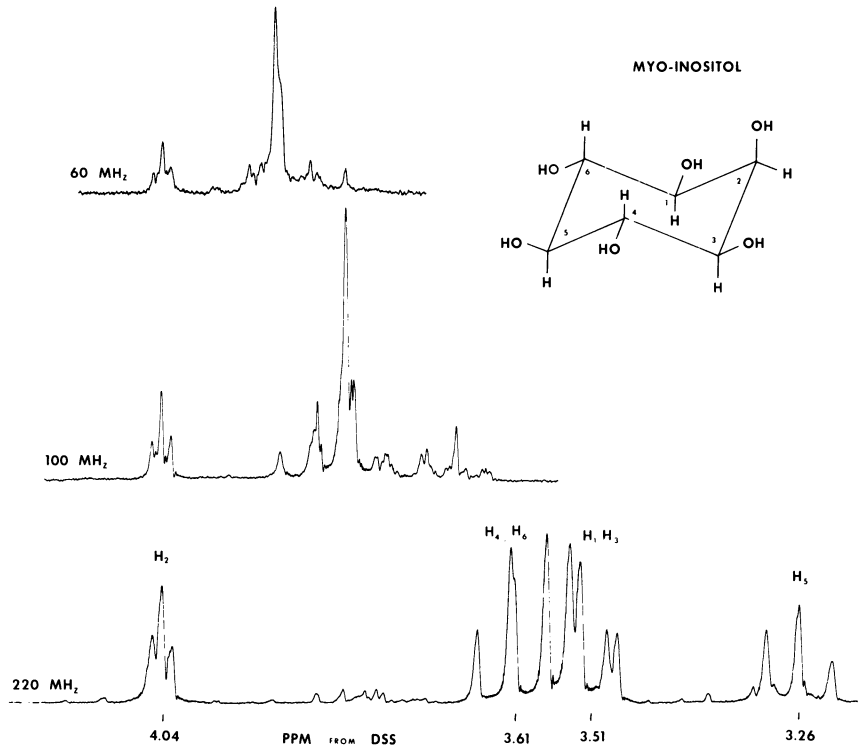

Figure 1. Proton magnetic resonance spectra at 14.1, 23.5, and 51.7 kilogauss (60, 100 and 220 MHz.) of myo*-inositol in deuterium oxide.*

In Figure 2, the H–1 signal (3.75 p.p.m.) appears to consist of an 8-line pattern (J = 11.5, 9.0 and 5.0 Hz) partially superposed on the H–3 signal (3.71 p.p.m.), a pair of doublets (J = 9.0 and 3.0 Hz.)

The 220 MHz spectra shown in the figures were recorded at Varian Associates (*see* Acknowledgement) on their Model HRSC-IX or -220 Spectrometer.

With the new very high resolution NMR spectra, it should be a simple matter to assign configurations to other "difficult" cyclitols or carbohydrates, for example, the numerous still undiscovered isomers of 6-bromo, 6-chloro, and 6-iodoquercitol (20 diastereomers predicted for each).

Periodate Titrations of Deoxyinositols

In the past, periodate titrations have been of limited value for establishing the structure of quercitols or cyclohexanetetrols. The former show "overoxidation," because of the fact that malonaldehyde is formed, and this compound undergoes further oxidation. Some isomers of the tetrols

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show the expected uptake of one mole of periodate/mole for each vicinal glycol grouping present, while others show "overoxidation" which may or may not be attributed to the formation of malonaldehyde, depending on the nature of the tetrol.

It is now reported by P. Szabó that the quercitols can be titrated with completely normal results, by using suitable experimental conditions, and especially low temperature and low pH, for the titrations (*40,41*). This method might also be applicable to the estimation of the tetrols.

Synthesis of Ring-Deoxy Sugars (Pseudo-Hexoses)

There has been much recent work on replacement of the ring-oxygen atom of pyranose or furanose sugars by other atoms, such as sulfur or nitrogen (NH). If the oxygen atom is replaced by carbon (CH_2), the product is called a pseudo-sugar (*26, 27*), for example, pseudo-α-DL-*talo*-pyranose (**34**). These products may have biological importance, since a pseudo-sugar should be acceptable in place of the corresponding true sugar to some, but not all, enzymes present in (normal or malignant)

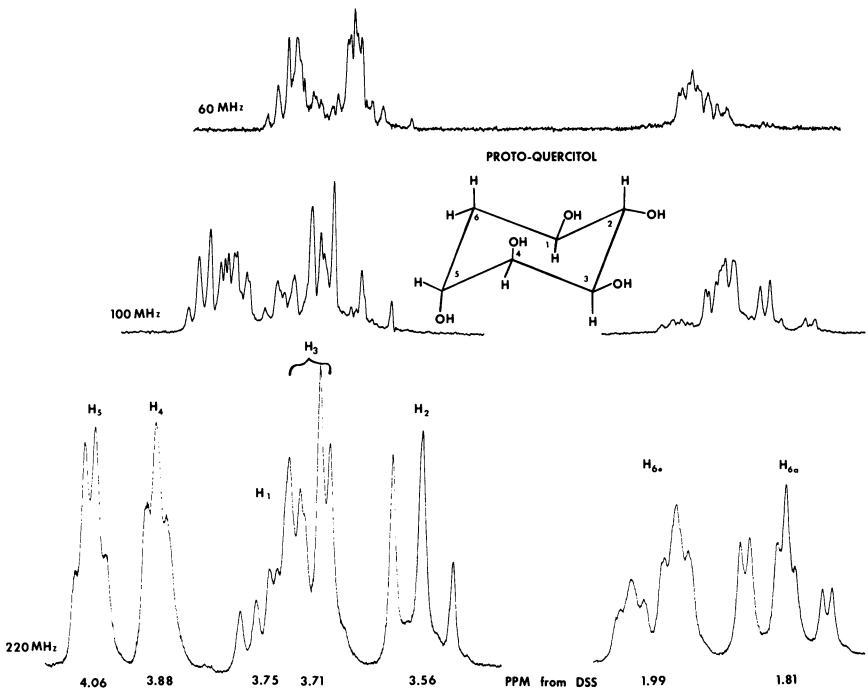

Figure 2. Proton magnetic resonance spectra at 14.1, 23.5, and 51.7 kilogauss (60, 100 and 220 MHz.) of (+)-proto-quercitol in deuterium oxide.

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human cells or tissues, or in pathogenic microorganisms. The pseudo-sugar thus might be able to inhibit harmful biological processes (*26*, *27*).

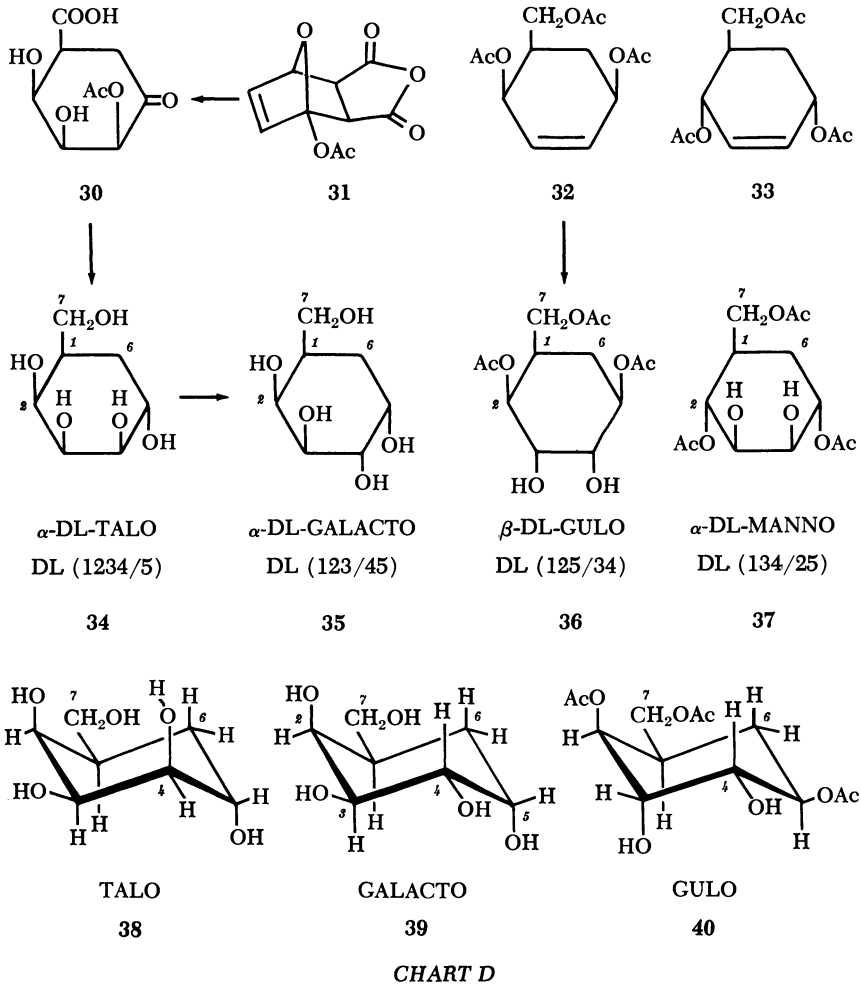

CHART D

The first known pseudo-hexose, pseudo-α-DL-*talo*pyranose (**34**) was prepared by reduction of the keto-acid monoacetate (**30**) (*26*, *27*). This intermediate, which had been used by Daniels, Doshi, and Smissman (*9*, *10*) for a synthesis of shikimic acid, is prepared from the Diels-Alder adduct (**31**) of 2-acetoxyfuran and maleic anhydride, by a remarkable series of transformations.

By the use of nuclear magnetic double (and triple) resonance, the configuration (**34**) of pseudo-talose was established, confirming chemical evidence, and the sidechain-equatorial conformation (**38**) was also

established, since the tertiary proton H–1 was found to be axial (note that sugar numbering is not used for these compounds). The three equatorial (AcO–C*H*) protons produced a signal at higher field, and the one axial proton a signal at lower field, contrary to the rules originally postulated by Lemieux and co-workers (*14, 15, 26, 27*). These results can be explained by more refined rules recently proposed by Lemieux and Stevens (*16, 17*) which allow for the changes in shielding and deshielding effects caused by inversions of configuration at other positions in the six-membered ring.

Two additional pseudo-hexoses have recently been prepared. Pseudo-α-DL-*galacto*pyranose (**35**) was obtained by heating pseudo-talose (**34**) with the Angyal epimerization reagent (*2, 3*) (*see* above). This reagent tends to invert an acetoxy group which has one neighboring *trans* and one neighboring *cis* acetoxy group. The product was isolated as the penta-acetate and converted to the free pentol. The anticipated configuration (**35**) and favored conformation (**39**) were confirmed by NMR. The protons H–2 and H–5, and H–3 and H–4, appeared to be equivalent or nearly so, because of the near-symmetry of the molecule **39**, even though the latter has no true symmetry element (*27*).

Pseudo-β-DL-*gulo*pyranose triacetate (**36**) was prepared by hydroxylation of the enetriol triacetate (**32**) and converted to the corresponding pentol and pentaacetate. The intermediate **32** was obtained by Diels-Alder reaction (200°C., two days) of *trans/trans*-1,4-diacetoxy-1,3-butadiene with allyl acetate. The double bond was surprisingly inert to the usual additive reagents and not detectable by infrared spectroscopy because of near-symmetry, but it did react with *tert*-butyl hydroxperoxide to give **36** in about 30% yield (*27*).

The NMR spectrum at first appeared to indicate the α-DL-*manno*-pyranose configuration (**37**) corresponding to a configuration (**33**) for the Diels-Alder adduct. However, by a systematic spin-decoupling study, involving all seven of the ring-protons, the β-DL-*gulo*pyranose configuration (**36**) and the favored conformation **40** were finally established for the pentol triacetate, and thus indirectly for the pentol and its penta-acetate. The Diels-Alder adduct then must have the all-*cis* configuration **32** (*27*).

The biological properties of the pseudo-hexoses are being examined.

Acknowledgment

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4

Syntheses of 6-Deoxy-2 and 3-*O*-Methyl-D-allose and Some 6-Deoxyhexopyranoside Phenylboronates

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*New reagents that are of value in the oxidation of partially protected sugars are reviewed briefly. 6-Deoxy-3-O-methyl-*D*-allose, a component sugar of the sarcostin glycosides of* Ascelepias lilacina *Weimarck and* A. swynnertonii *S. Moore, has been synthesized by way of methyl 4,6-O-benzylidene-2-O-toluene-*p*-sulfonyl-α-*D*-ribo-hexopyranosid-3-ulose. The same compound has also been used in two syntheses of javose (6-deoxy-2-O-methyl-*D*-allose), a constituent of two cardenolides found in the seeds of* Antiaris toxicaria *Lesch. Condensation of methyl 6-deoxy-β-*D*-allo- and gluco-pyranoside with triphenylboroxole afforded a cyclic 2,4-phenylboronate, in each case. In an analogous reaction with methyl α-*L*-rhamnopyranoside and methyl α-*L*-fucopyranoside, 2,3- and 3,4-cyclic esters, respectively, were formed. Preliminary results of the oxidation of some glycoside phenylboronates with methyl sulfoxide and acetic anhydride are reported.*

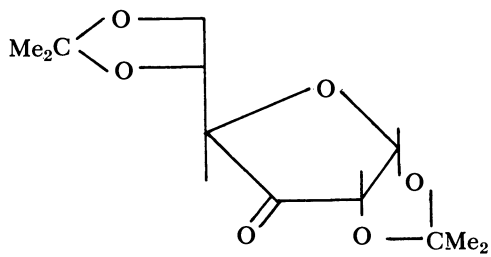
The replacement of a secondary hydroxyl group by a carbonyl group in pyranoid and furanoid rings provides a versatile functionality for further synthesis (*9*, *45*). Details of the preparation and chemistry of carbohydrates containing a carbonyl group, in addition to that at C(1), have appeared (*31*, *53*) relatively recently but several important developments have since taken place in this area. These stem from the introduction of new oxidation procedures—*e.g.*, ruthenium textroxide (*6*, *7*), acid anhydride-methyl sulfoxide (*1*,*2*), dicyclohexylcarbodiimide-methyl sulfoxide-pyridinium phosphate (*47*)—which promise to make keto-sugars more readily available for synthetic work. The scope of these new

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

oxidation reagents will be reviewed briefly, together with their application to the synthesis of two naturally-occurring methyl ethers of 6-deoxy-D-allose. In addition, preliminary investigations on the oxidation of some carbohydrate phenylboronates, with acetic anhydride-methyl sulfoxide (*1*), will be described.

In 1964, Beynon, Collins, and Overend (*6*) reported that ruthenium tetroxide is an extremely potent oxidant for preparing the partially protected glycopyranosiduloses. This reagent offered distinct advantages over the chromium trioxide-pyridine complex, the reagent usually employed (*45*, *53*), and affected neither glycosidic, ester, acetal, nor ketal substituents in the oxidized molecule (*6*). Oxidation is achieved either at room temperature with a slight excess of ruthenium tetroxide (prepared from the dioxide with sodium metaperiodate) in carbon tetrachloride, or, less satisfactorily, with a catalytic quantity of the tetroxide in the presence of sodium metaperiodate. The latter procedure, however, was developed by Parikh and Jones (*46*) into a single-stage oxidation which gave essentially quantitative yields of oxidized products. In passing, this reagent fulfills the long-standing need (*5*) for an oxidant which functions under neutral conditions at ambient temperature.

Of particular value, was the reagent's ability to oxidize a hydroxyl group attached directly to a furanoid ring (*6*, *7*, *46*), as is illustrated by the conversion of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose into 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (**1**). The follow-



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ing keto-sugars are among those which have been prepared, in good to quantitative yield, using the ruthenium textroxide procedures (*6*, *7*, *46*): methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose, 6-deoxy-1,3:2,5-di-*O*-methylene-L-*lyxo*-hex-4-ulose, methyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosidulose, methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro* and *threo*-hexopyranosid-3-ulose, methyl 3,4,6-tri-*O*-benzoyl-α-D-*arabino*-hexopyranosidulose, 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D-*xylo*-hex-3-ulose, and methyl 6-deoxy-3,4-*O*-isopropylidene-α-L-*lyxo*-hexopyranosidulose. Another significant development in the oxidation

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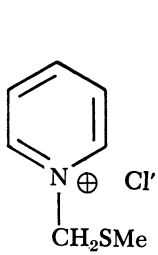
of partially protected carbohydrates arose through the introduction of methyl sulfoxide-carbodiimide-pyridinium phosphate (*47*) and related oxidants (*1*, *2*). The former combination of reagents was discovered by Pfitzner and Moffatt (*47*, *48*) when attempting to polymerize thymidine 5'-phosphate using dicyclohexylcarbodiimide in anhydrous methyl sulfoxide. With these reagents, the nucleotide was degraded completely to thymine and a second product (**2**) was also formed if an excess of pyridine was added. It was shown (*48*) subsequently that only nucleotides containing an unsubstituted 3'-hydroxyl group underwent rapid and complete cleavage of the *N*-glycosidic and ester bonds. Degradation was attributed to oxidation of the 3'-hydroxyl group to a ketone



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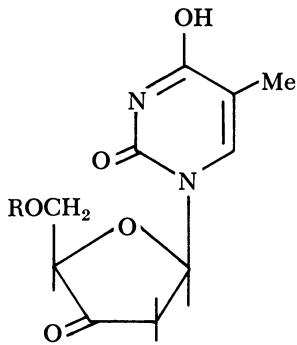

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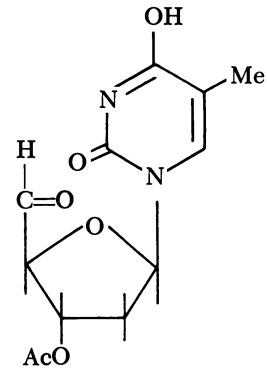

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(**3**), which underwent spontaneous β-elimination of the base and phosphate group. This argument is enhanced by the recent isolation (*19*) of 2'- and 3'- ketouridine following similar oxidation and detritylation of 3',5'- and 2',5'-di-*O*-trityluridine, respectively. It is also pertinent to record that oxidation of 3'-*O*-acetylthymidine yielded the aldehydo-compound (**4**) without concomitant formation of carboxylic acid derivatives.

The facile and selective oxidation of both primary and secondary hydroxy groups with certain nucleotides led Pfitzner and Moffatt (*48*) to explore the scope of the carbodiimide-methyl sulfoxide reagent with steroid and alkaloid alcohols. Relatively minor differences were apparent in the rate of oxidation of epimeric pairs of 3- and 17- hydroxy steroids whereas the equatorial 11α-hydroxyl group in several steroids was readily oxidized under conditions where the axial epimer was unreactive [*cf.* chromic oxide oxidation (*51*)].

The mechanism of the oxidation reaction, resulting from treatment of an alcohol with dicyclohexylcarbodiimide and methyl sulfoxide in the presence of a proton source, was elucidated by isotope experiments (*24*). These confirmed that the reaction proceeded by formation of a

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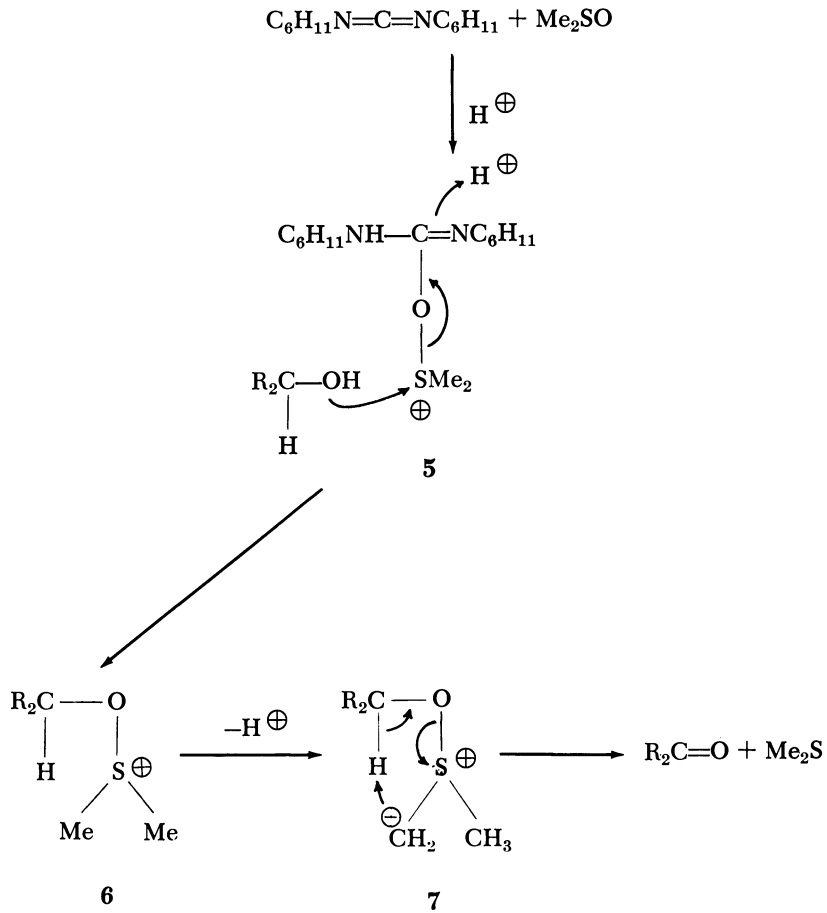
sulfoxide-carbodiimide adduct (**5**) which was attacked by the alcohol to give an alkoxysulfonium salt (**6**). This undergoes abstraction of a proton from the α-carbon atom and concerted collapse of the resulting intermediate **7** with formation of the carbonyl compound and methyl sulfide. The intermediacy of alkoxysulfonium salts relates this reaction to the oxidation of various reactive alkyl halides, alkyl sulfonates, and α-halogeno esters on heating with methyl sulfoxide in the presence of a proton source (*3*, *34*, *37*, *38*, *39*, *43*). Oxysulfonium salts are also intermediates in the reactions of methyl sulfoxide with alkyl chloroformates (*5*) and with alcohols in the presence of acid anhydrides (*see* below).



Following closely on the foregoing oxidation procedure, Albright and Goldman (*1,2*) described a novel method for the oxidation of alcohols which uses methyl sulfoxide and certain acid anhydrides—*e.g.*, acetic

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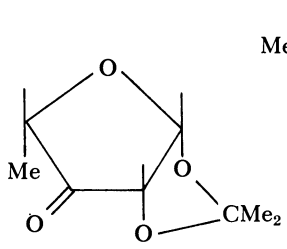
anhydride, benzoic anhydride, or phosphorus pentoxide. The method appears to be generally applicable and is claimed to be of particular value for the oxidation of sterically hindered hydroxyl groups. The mechanism of the oxidation of alcohols with methyl sulfoxide and acid anhydrides has been discussed recently by Albright and Goldman (*2*). Although acyloxysulfonium salts are undoubtedly intermediates in the oxidation (*c.f.*, Reference *49*) the way in which these break down to give the carbonyl compound and methyl sulfide is by no means established.

A review of oxidations using methyl sulfoxide is now available (*23*). These new oxidation procedures were quickly applied in the carbohydrate field with remarkable success. Oxidation of 5-deoxy-1,2-*O*-isopropylidene-β-L-arabinofuranose with the Pfitzner-Moffatt reagent (*48*) gave the keto-sugar **8**, which was ultimately transformed into L-streptose (5-deoxy-3-*C*-formyl-L-lyxose) (*22*), thus providing the first constitutional synthesis of this elusive antibiotic sugar. A more extensive appraisal of the Pfitzner-Moffatt reagent was conducted by Baker and Buss (*4*) who prepared the keto-sugars **9** and **10**, in high yield, from



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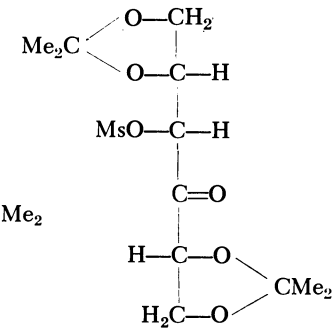

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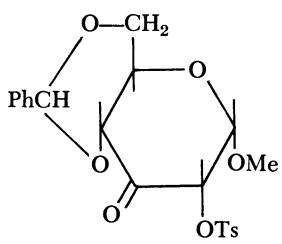

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1,2:5,6-di-*O*-isopropylidene-3-*O*-methanesulfonyl-D-mannitol and methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulfonyl-α-D-glucopyranoside, respectively. In some instances, oxidation of a carbohydrate secondary hydroxyl group—*e.g.*, in 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose—was not achieved, and it would appear that the reagent has certain limitations.

Fortunately, the oxidation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose to 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (**1**) can be accomplished using either phosphorus pentoxide (*10*, *44*) or acetic anhydride (*10*, *52*) in methyl sulfoxide; although this oxidation is effected with ruthenium tetroxide (*6*, *7*, *46*), it is exceeding difficult with other oxidizing agents (*53*). Keto-sugar **1** is reduced stereospecifically

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(*54*) with sodium borohydride to 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose and so provides a convenient and cheap route to D-allose. Oxidation of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranoside and 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose with methyl sulfoxide-acetic anhydride gave high yields of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose and 2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone (*33*), respectively. The use of methyl sulfoxide-acetic anhydride offers advantages in that the oxidized product can be isolated simply by lyophilization of the excess of reagents and crystallization.

Rearrangement of methyl 4,6-*O*-benzylidene-3-deoxy-3-phenylazo-aldohexopyranosides with alkali provides a new route to hexosid-3-uloses (*18*).

As part of a program in this laboratory on the reactions of D-allose derivatives, we have been concerned with the synthesis of some naturally-occurring methyl ethers of 6-deoxy-D-allose. Mycinose [6-deoxy-2,3-di-*O*-methyl-D-allose (*21*)] is a constituent of the macrolide antibiotic chalcomycin and its synthesis has been reported (*15*, *16*) already. 6-Deoxy-3-*O*-methyl-D-allose is now known to be a component of the sarcostin glycosides of *Ascelepias lilacina* Weimarck (*50*) and *Ascelepias swynnertonii* S. Moore (*36*). A facile entry into the D-allopyranoside series can be achieved by stereospecific reduction, with sodium borohydride, of methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulfonyl-α-D-*ribo*-hexopyranosid-3-ulose (**10**) to yield (*4*) methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulfonyl-α-D-allopyranoside (**11**). Compound **11** was then converted (*14*) into 6-deoxy-3-*O*-methyl-D-allose (**12**) by a series of well-established reactions (Figure 1). Alternatively, this sugar has been prepared (*40*) by partial benzoylation of methyl 6-deoxy-β-D-allopyranoside to give the 2,4-di-*O*-benzoate followed by methylation at C(3) and removal of the protecting groups.

An isomeric sugar, D-javose, is a constituent of two cardenolide glycosides (strophanthojavoside and antiarojavoside) found (*42*) in the seeds of *Antiaris toxicaria* Lesch. Degradative studies indicated (*42*) that javose had the structure 6-deoxy-2-*O*-methyl-D-allose (**17**) and this assignment was confirmed by two stereospecific syntheses.

In the first approach (*13*) methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulfonyl-α-D-allopyranoside (**11**) was transformed by successive benzylation and alkali treatment into methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-allopyranoside (**13**) (*see* Figure 2). Methylation of the C(2)-hydroxyl group and mild acid treatment then gave methyl 3-*O*-benzyl-2-*O*-methyl-α-D-allopyranoside (**14**). Introduction of the 6-deoxy group was achieved by toulene-*p*-sulfonylation of the primary hydroxyl group of compound **14** and desulfonyloxylation using lithium aluminum hydride. The resulting 6-deoxyglycoside derivative **15** was catalytically debenzylated and

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on further treatment with hot acid gave crystalline 6-deoxy-2-*O*-methyl-D-allose (**17**).

In a second approach (*see* Figure 3) the alloside derivative **11** was converted, by mild acid hydrolysis, into methyl 2-*O*-toluene-*p*-sulfonyl α-D-allopyranoside (**18**). Acid catalyzed acetonation of compound **18**

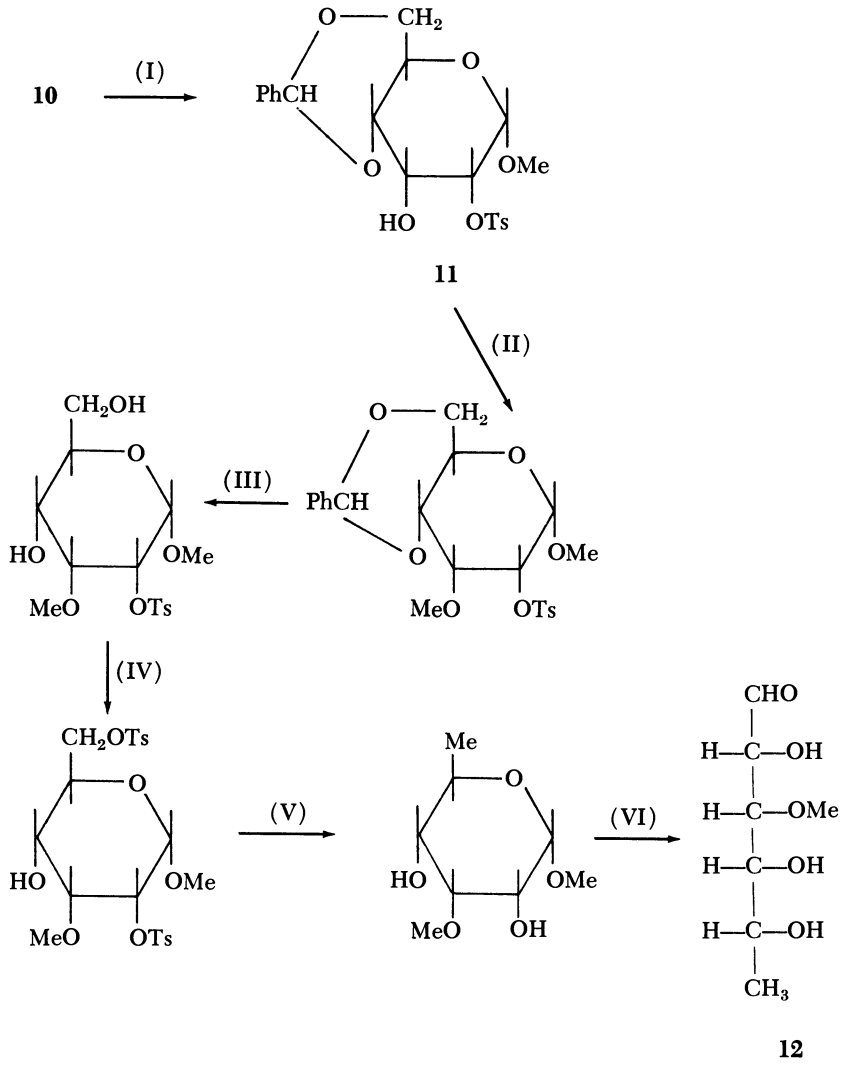

Figure 1. Synthesis of 6-deoxy-3-O-methyl-D-allose.

(I) $NaBH_4$
(II) MeI/Ag_2O
(III) H_2/Pd–C
(IV) $MeC_6H_4SO_2Cl$
(V) $LiAlH_4$
(VI) N–H_2SO_4

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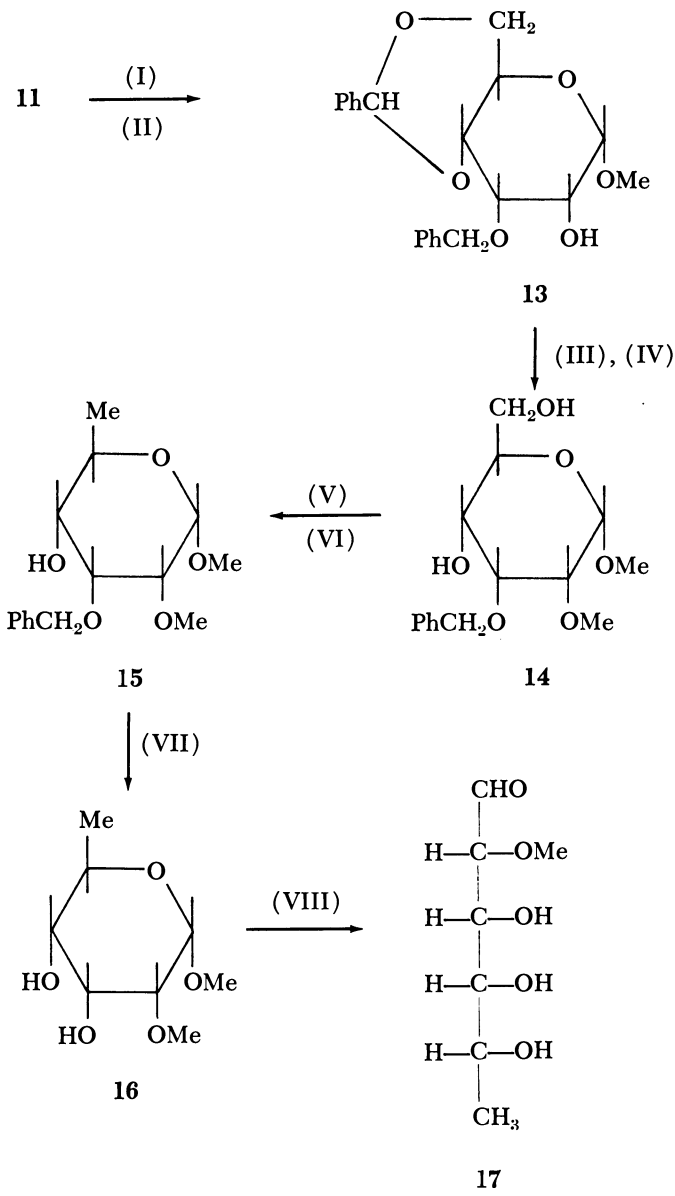

Figure 2. Synthesis of 6-deoxy-2-O-methyl-D-allose (javose).

(I)	*$PhCH_2Br/NaH/DMF$*	*(V)*	*$MeC_6H_4SO_2Cl$*
(II)	*NaOEt*	*(VI)*	*$LiAlH_4$*
(III)	*MeI/NaH/DMF*	*(VII)*	*H_2/Pd–C*
(IV)	*H_3O^+*	*(VIII)*	*N–H_2SO_4*

followed the expected pattern to yield methyl 3,4-*O*-isopropylidene-2-*O*-toluene-*p*-sulfonyl-α-D-allopyranoside (**19**). Treatment with toluene-*p*-sulfonychloride then formed the disulfonate (**20**), which gave methyl 6-deoxy-3,4-*O*-isopropylidene-α-D-allopyranoside (**21**, R-H) on treatment with lithium aluminum hydride. Finally, methylation of the C(2)-hydroxyl group and removal of the protecting ketal and glycosidic substituents afforded crystalline 6-deoxy-2-*O*-methyl-D-allose (**17**). The synthetic compounds proved to be identical with javose, which was isolated originally (*42*) in amorphous form but has since been crystallized. Yet a third synthesis of javose has been reported by Reichstein *et al.* (*32*).

Our interest in the oxidation of carbohydrate phenylboronates arose through an unsuccessful attempt (*14*) to prepare 6-deoxy-3-*O*-methyl-D-allose (**12**) by methylation of methyl 6-deoxy-β-D-allopyranoside 2,4-phenylboronate (**23**). Although methods for preparing the partially protected aldopyranosiduloses have increased, little additional work seems to have been reported on the preparation of their unsubstituted analogs. It will be recalled that unsubstituted keto-glycosides have been prepared, in low yield, by oxidation of certain glycopyranoside derivatives with chromium trioxide-acetone (*53*); this procedure usually yields complex product mixtures which require extensive chromatography. By oxidizing glycosides having all but one of the hydroxyl groups protected, the importance of competing side-reactions is greatly diminished, but, on the other hand, losses and rearrangements occur on removal of the protecting groups. Catalytic oxidation of certain pentopyranosides (*31*) and 6-deoxyhexopyranosides (*11*) affords moderate yields of unsubstituted keto-glycosides.

The revived interest (*26*, *28*, *30*) in phenylboronic acid as a protecting group in carbohydrate chemistry suggested an alternative approach to preparing the unsubstituted keto-glycosides, since cyclic boronate esters are formed and removed under mild, neutral conditions. Oxidation of glycoside phenylboronates should be possible under the anhydrous conditions employed with the methyl sulfoxide-acetic anhydride reagent (*1*, *2*) while removal of the phenylboronic acid residue should occur on addition of water. Other methods for removing the boronate ester are available. Brown and Zweifel (*17*) removed butylboronic acid from cyclic boronate esters using ethylene glycol and separated ethylene glycol butylboronate by distillation. In the carbohydrate field, propane-1,3-diol seems to be preferred for this purpose (*26*); six-membered borate (*20*, *35*) and boronate (*8*, *29*) cyclic esters are more stable than the analogs formed from 1,2-diols.

A number of pentopyranoside phenylboronates are known already through the work of Ferrier and Prasad (*26*). The mobility of methyl

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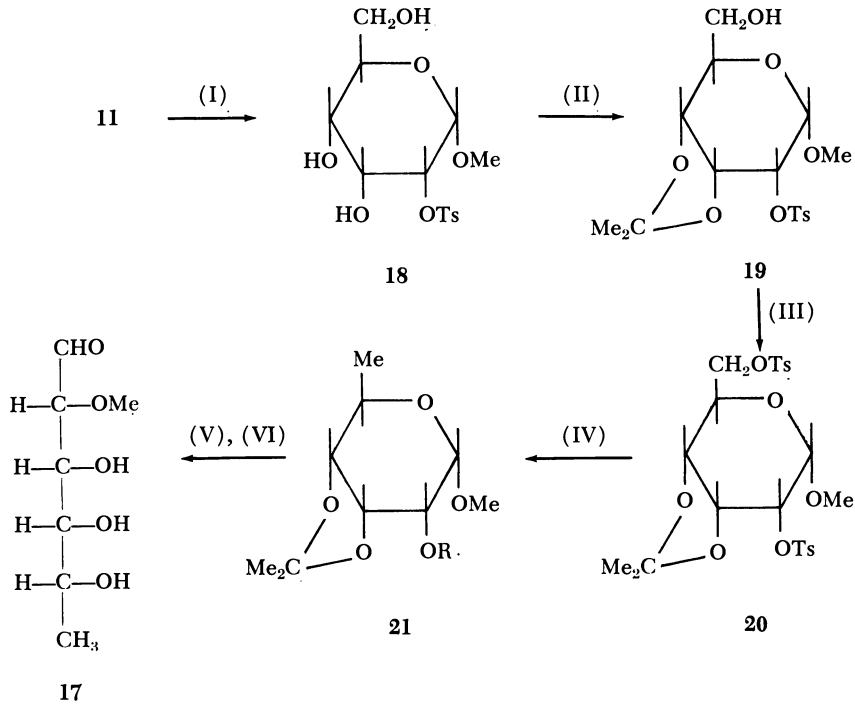

Figure 3. Alternative synthesis of javose.

(I)	H_3O^+	*(IV)*	$LiAlH_4$
(II)	Me_2CO/H_3O^+	*(V)*	*MeI/NaH/DMF*
(III)	$MeC_6H_4SO_2Cl$	*(VI)*	N–H_2SO_4

β-D-ribopyranoside on paper chromatograms is greatly enhanced (*25*) in the presence of phenylboronic acid and the crystalline 2,4-phenylboronate ester (**22**) is obtained (*26*) when the glycoside is heated with triphenylboroxole under dehydrating conditions. On similar treatment, methyl α- and β-D-xylopyranoside yield 2,4-cyclic esters (*28*); solubility differences between the xylopyranoside phenylboronates in benzene provides a convenient means for their separation (*27*). Methyl β-L-arabinopyranoside and methyl α-D-lyxopyranoside also condense smoothly with triphenylboroxole to give (*26*) crystalline cyclic esters which possess 3,4- and 2,3- cyclic structures, respectively.

Our initial studies were directed towards the synthesis and characterization of phenylboronate esters derived from methyl 6-deoxy-β-D-allopyranoside, methyl α-L-rhamnopyranoside, methyl α-L-fucopyranoside, and methyl 6-deoxy-β-D-glucopyranoside. Previous work (*14*) in this laboratory indicated that the reaction of triphenylboroxole and methyl

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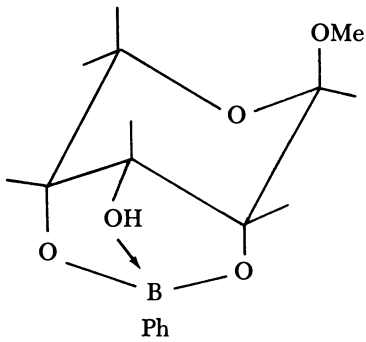
6-deoxy-β-D-allopyranoside yields a single, cyclic ester although attempts to characterize the ester by methylation of the unsubstituted hydroxyl group were unsuccessful. However, treatment of the alloside boronate with phenyl isocyanate gave a crystalline carbamate. Cleavage of the phenylboronate group, using propane-1,3-diol in acetone, yielded a product which did not reduce sodium metaperiodate and is, therefore, methyl 6-deoxy-β-D-allopyranoside 3-*N*-phenylcarbamate. This condensation parallels the reaction of methyl β-D-ribopyranoside (*26*) in that, although *cis*-vicinal diols are present at the 2,3- and 3,4- positions, the 2,4-positions are preferentially esterified. These findings are in agreement with the observations (*8*, *29*) that six-membered boronate rings are more stable than five-membered rings but the main stabilizing influence is likely to result from co-ordination of the hydroxylic oxygen to boron (*see* **22** and **23**).



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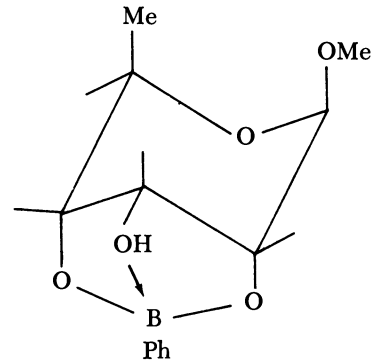
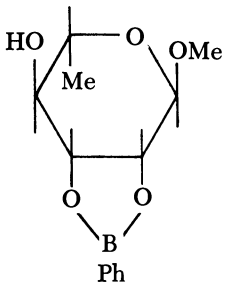


23

Reaction of methyl α-L-rhamnopyranoside with triphenylboroxole gave a syrupy boronate ester which was characterized as a crystalline phenylcarbamate. Removal of the phenylboronic acid residue gave a product identified as methyl α-L-rhamnopyranoside 4-*N*-phenylcarbamate, since it was identical with that resulting from removal of the ketal group from methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside 4-*N*-phenylcarbamate (*12*). This establishes the structure of the original ester as methyl α-L-rhamnopyranoside 2,3-phenylboronate (**24**).

The crystalline phenylboronate derived by similar treatment of methyl α-L-fucopyranoside was shown to possess the 3,4-cyclic structure (**25**). This assignment is based on oxidation of compound **25** with methyl sulfoxide-acetic anhydride and the chromatographic identification of

6-deoxy-L-talose and L-fucose following reduction of the resulting keto-glycoside and acidic hydrolysis. Methyl 6-deoxy-β-D-glucopyranoside also gave a crystalline phenylboronate; the structure of this ester was not definitively established but, by analogy with the corresponding reactions of methyl α- and β-D-xylopyranosides (*28*), is likely to be methyl 6-deoxy-β-D-glucopyranoside 2,4-phenylboronate (**26**).



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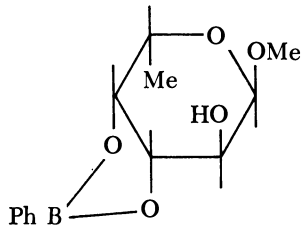

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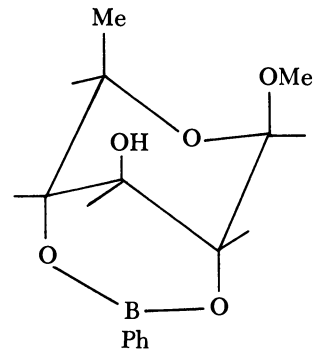

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Shortly before completing the preparation of the foregoing phenylboronates, Lindberg and Slessor (*41*) reported that both methyl α- and β-D-xylopyranoside 2,4-phenylboronates were smoothly oxidized, in 60 to 80% yield, using methyl sulfoxide-acetic anhydride. The phenylboronate group was cleaved and largely removed by partitioning the reaction mixture between chloroform-water and the keto-glycosides, subsequently identified as methyl D-*erythro*-pentopyranosid-3-uloses, were recovered by absorption on to Dowex-IX (HSO_3^- form) and elution with aqueous acetone. These results clearly substantiated our original reasonings and demonstrated the feasibility of this approach.

An essentially similar procedure was used in our preliminary experiments although longer reaction times were generally necessary. Oxidation of methyl β-L-arabinopyranoside 3,4-phenylboronate (*26*) afforded methyl β-L-*erythro*-pentopyranosidulose, in *ca* 20% yield. Methyl α-L-fucopyranoside 3,4-phenylboronate and methyl α-L-rhamnopyranoside 2,3-phenylboronate, however, gave lower yields (<15%) of the carbonyl compounds **27** and **28**, respectively. It is possible that considerably longer reactions times may be required to improve the yield of these compounds and to render the method of preparative significance. These investigations are continuing.

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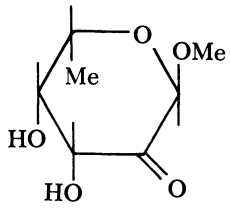

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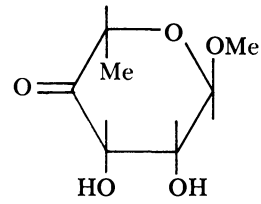

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Acknowledgments

One of us (F.H.) thanks the Libyan Government for financial support.

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5

The Synthesis and Reactions of Phosphorylated Deoxy Sugars

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Following a critical review of methods used for the synthesis of phosphorylated deoxy sugars, the stability of these esters in various reaction conditions is discussed. The use of cyclic phosphodiesters of sugars for the synthesis of phosphorylated deoxy sugar acids is described and the occurrence of phosphate group migration in phosphorylated deoxy sugars, which are unable to form a pyranoid ring, is pointed out.

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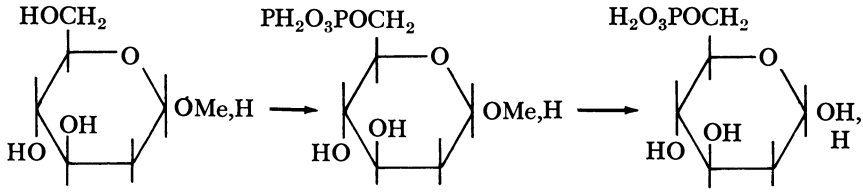
Deoxy sugar phosphates can be prepared by all of the general methods used for the syntheses of sugar phosphates and, if we except the 2-deoxy glycosyl phosphates and certain phosphates having a furanose ring, the difficulty of obtaining them lies essentially in preparing the appropriately protected deoxy sugar and not in introducing and maintaining the phosphoryl group in the sugar.

As in the case of normal sugars (*56*) and amino sugars (*36*), the primary alcohol group of the otherwise unprotected hexopyranosides can be phosphorylated with appropriate phosphorylating agents. Thus, for example, 2-deoxy D-glucose 6-phosphate (2-deoxy D-*arabino*-hexose 6-(dihydrogen phosphate)), (**3**) has been obtained from both the α- (*42*) and the β-methyl glycoside (**1**) (*61*) by direct phosphorylation with diphenyl phosphorochloridate; the 6-diphenyl phosphate (**2**) is formed. From this, the protecting phenyl groups can be removed by hydrogenolysis and the aglycone by mild acid treatment (the compound's own acidity can be used (*61*)) to yield the free, phosphorylated sugar (**3**).

(The pyranose or furanose structures shown for the free sugars are arbitrary assignments.)

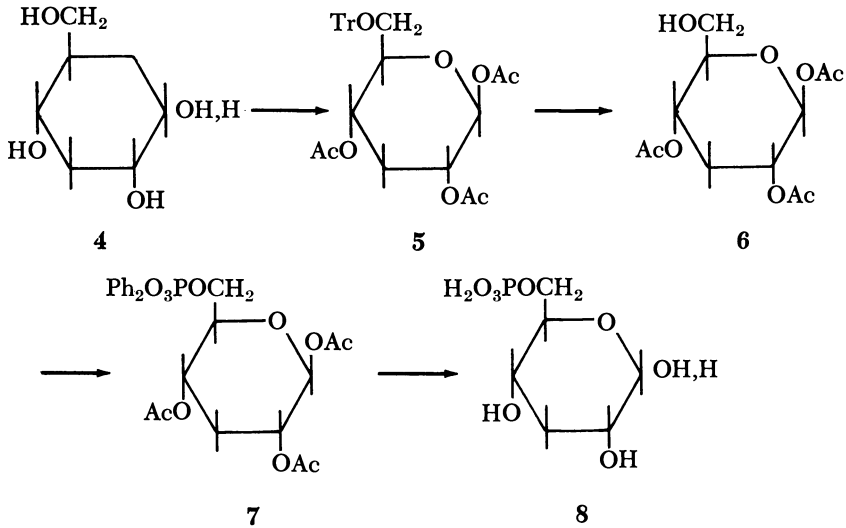
Apparently no phosphate migration was noticed by either group of authors, in spite of the acid conditions employed for the cleavage of the glycosidic bond.

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.



1 2 3

Starting with the corresponding free deoxy sugar (**4**), Dahlgard and Kaufmann (*13*) used a classical reaction sequence for the synthesis of 3-deoxy D-glucose 6-phosphate (3-deoxy D-*ribo*-hexose 6-(dihydrogen phosphate)), (**8**) : tritylation, followed by acetylation, gave the fully protected sugar (**5**); after selective removal of the trityl group, the free primary alcohol of the triacetate (**6**) was phosphorylated with diphenyl phosphochloridate; from the phosphotriester (**7**), the protecting phenyl and acetyl groups were removed by hydrogenolysis and mild alkaline hydrolysis, in that order, to yield the phosphorylated deoxy sugar (**8**). As the free phosphomonoester was not exposed to acid treatment, the assignment of the phosphate group to the terminal hydroxyl function is, in this case, unambiguous.



4 5 6 7 8

Phosphate esters of 2-deoxy D-galactose (2-deoxy-D-*lyxo*-hexose) have also been obtained (*15*). The 6-phosphate (**12**) was synthesized from the methyl glycoside (**9**) by condensing the latter with acetone; the 3,4-

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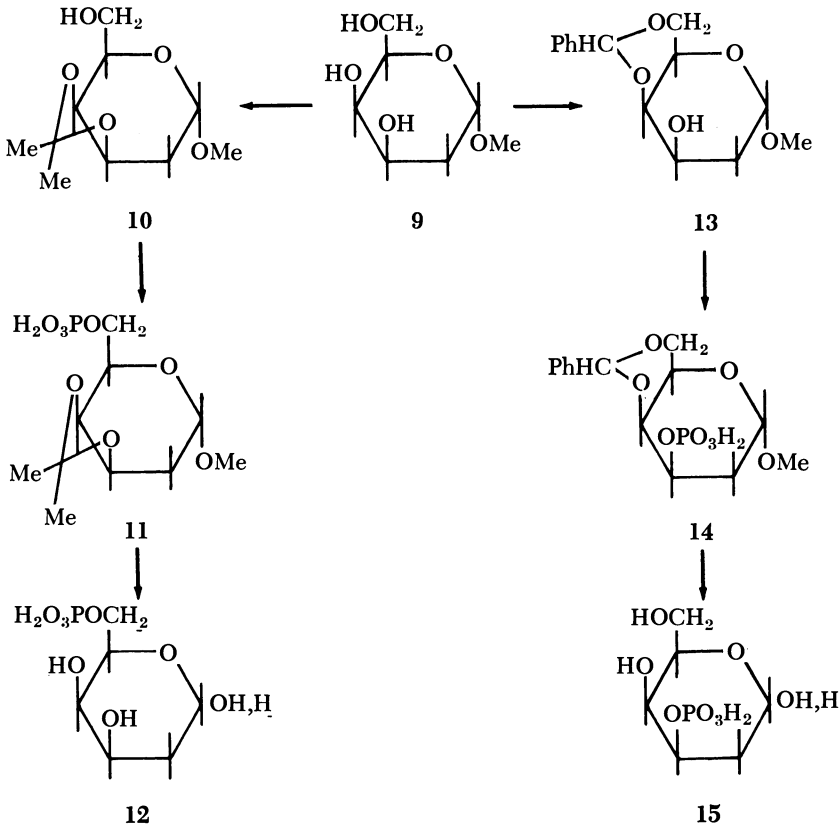
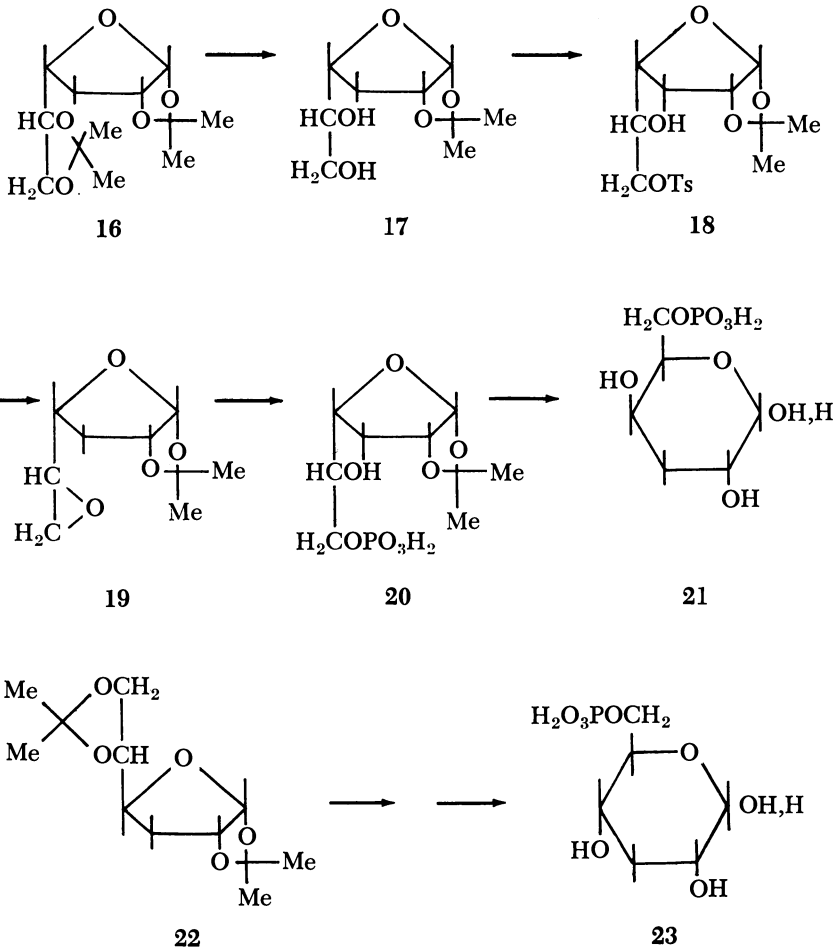
O-isopropylidene derivative (**10**) was then phosphorylated with phosphorous oxychloride to form the phosphate ester (**11**) from which the protecting groups were removed by mild acid hydrolysis. The 3-phosphate (**15**) was obtained by phosphorylating the 4,6-benzylidene derivative (**13**) of the same glycoside with phosphorus oxychloride, followed by hydrolytic removal of the protecting groups, from the ester (**14**) thus obtained.



3-Deoxy-D-*xylo* hexose 6-(dihydrogen phosphate) (**21**) has also been synthesized (*2*); the reaction sequence makes use of 3-deoxy 1:2,5:6-di-*O*-isopropylidene D-galactofuranose (**16**), a compound that can be easily prepared from D-glucose (*2, 60*). The mono-isopropylidene derivative (**17**) formed by partial hydrolysis of the di-ketal is converted into the 6-tosylate (**18**) by reaction with one molar equivalent of *p*-toluenesulfonyl chloride. From this the epoxide (**19**) is formed by reaction with sodium methoxide. Treatment of the anhydro sugar with an aqueous solution of disodium hydrogen phosphate (*26*) leads to the 6-phosphate (**20**)

from which the phosphorylated free sugar (**21**) is easily obtained by very mild acid hydrolysis. An analogous reaction sequence (*52*) starting with 3-deoxy 1,2:5,6-di-*O*-isopropylidene D-glucofuranose (**22**) [also prepared from D-glucose (*9*, *10*)] leads to 3-deoxy D-glucose 6-phosphate (**23**). It may be noted, that partial hydrolysis of the di-isopropylidene derivative of 3-deoxy glucose (*21*) is more easily accomplished with good yields than is that of the deoxy galactose derivative. In neither synthesis does the final acid hydrolysis affect the position of the phosphate group, which remains attached to the primary alcohol.



A more complicated reaction sequence has been used by Ukita and Nagasawa (*59*) in their synthesis of 2-deoxy D-ribose 5-phosphate (2-deoxy D-*erythro*-pentose 5-(dihydrogen phosphate)), (**29**). They phosphorylated a mixture of the anomeric methyl deoxyribofuranosides (**24**)

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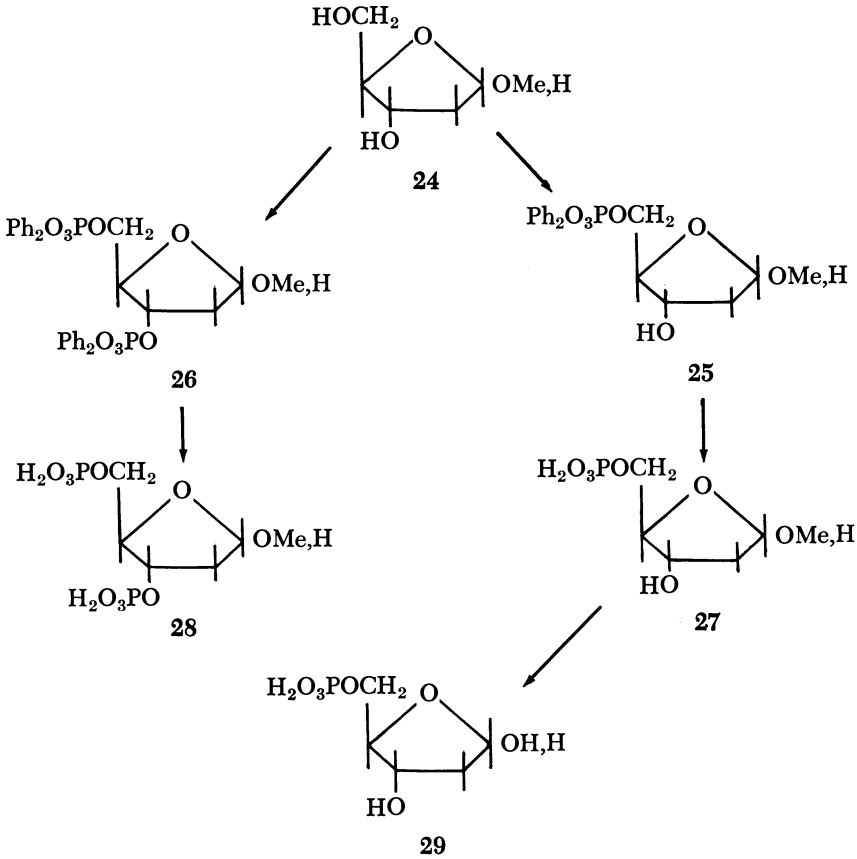
with diphenyl phosphorochloridate and obtained a mixture of the 5-diphenyl phosphate (**25**) and of the 3,5-bis-diphenyl phosphate (**26**). Apparently no 3-diphenyl phosphate was formed. Hydrogenation of the above esters yielded the monophosphate (**27**) and the diphosphate (**28**), also as a mixture; these esters could be separated by fractional precipitation of their barium salts followed by chromatography on a cellulose column. Mild acid hydrolysis of the monophosphate (**27**) then produced the free phosphorylated deoxy sugar (**29**).



As with normal sugars (*3,25,44,45*) cyclic phosphates of deoxy sugars can be synthesized if phenyl phosphorodichloridate is used as the phosphorylating agent. Thus the 4,6-phenyl phosphate of 2-deoxy methyl glucoside (methyl 2-deoxy β-D-*arabino*-hexopyranoside 4,6-(phenyl phosphate)) (**30**) is obtained (*61*), if the above phosphorylating agent is allowed to react with the unprotected glycoside (**1**). The same triester is formed (*61*) by weak alkaline treatment of the 6-diphenyl phosphate

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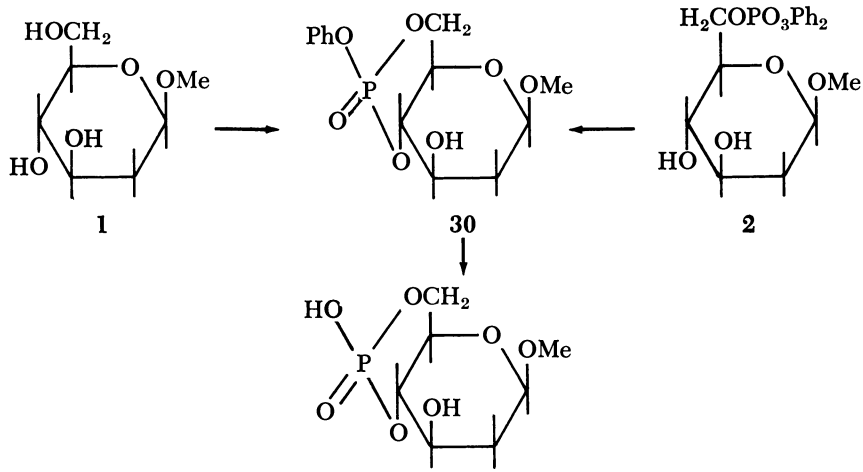
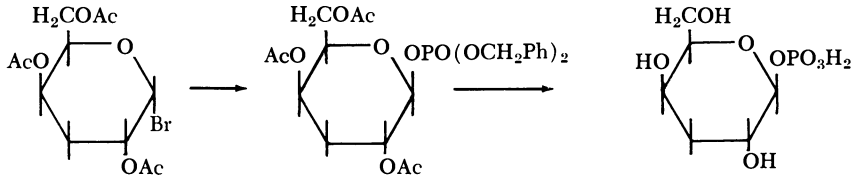
(**2**). The phenyl group can be removed by catalytic hydrogenation to yield the cyclic phosphodiester (**31**).



1 **30** **2**

31

If we except the 2-deoxy aldosyl phosphates, it seems, from the few cases described, that the deoxy group does not render difficult the preparation of glycosyl phosphates. Thus, no difficulty was encountered (*1*) during the synthesis of the β-1-phosphate of 3-deoxy D-galactopyranose (3-deoxy-D-*xylo*-hexose) (**34**) which was accomplished by allowing the silver salt of dibenzylphosphoric acid to react with the acetobromosugar (**32**) and then removing the protecting benzyl and acetyl groups from the phosphorylated sugar (**33**) by hydrogenolysis and alkaline hydrolysis, respectively. On the other hand, the purity of the corresponding α-1-phosphate which is said to be formed (*1*), when the silver dibenzyl phosphate is replaced by silver diphenyl phosphate in the above reaction sequence, is very uncertain.



32 **33** **34**

The vicinal diol groups of deoxy sugar phosphates can be cleaved by periodate in the usual conditions without affecting the stability of the phosphate group, so that this reaction can be used for preparative purposes in certain cases. Thus, if 3-deoxy glucose 6-phosphate (**23**) is

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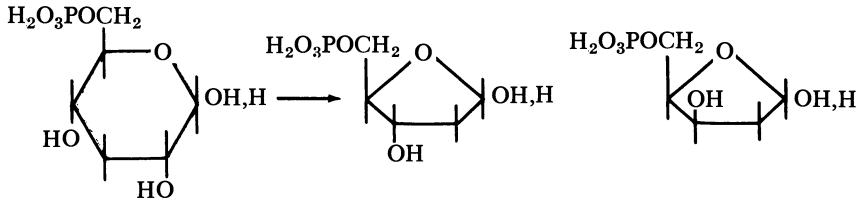
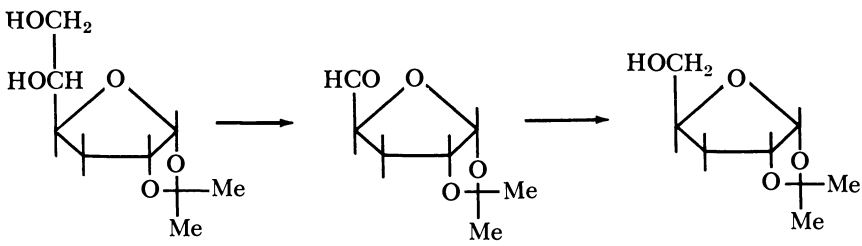
treated with one molar equivalent of sodium periodate in unbuffered solution, 2-deoxy ribose 5-phosphate (**35**) can be isolated in excellent yield (*52*). Similarly, from 3-deoxy galactose 6-phosphate (**21**) 2-deoxy D-*threo*-pentose 5-(dihydrogen phosphate) (**36**) is obtained (*2*). The reaction sequences just outlined are probably the most convenient ones actually available for the synthesis of sizeable amounts of these 2-deoxy pentose phosphates. The method has the special advantage that inorganic phosphate is used as phosphorylating agent and that the phosphate is introduced at a late stage of the synthesis; it is therefore easy to obtain these sugar phosphates marked with the radioactive isotope ^{32}P and possessing high specific activity.

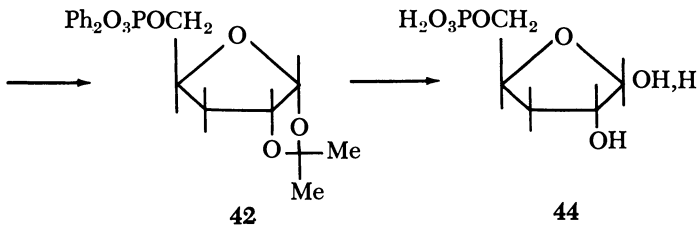


23 35 36

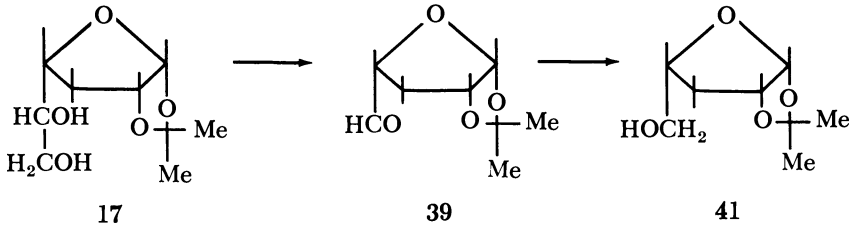
The 3-deoxy 1,2-*O*-isopropylidene D-gluco- (**37**) and D-galacto-furanoses (**17**) have been used (*53*, *58*) in yet another way to prepare deoxy sugars phosphorylated in the terminal position. If either of these compounds is treated with one molar equivalent of periodate, carbon 6



37 38 40



42 44

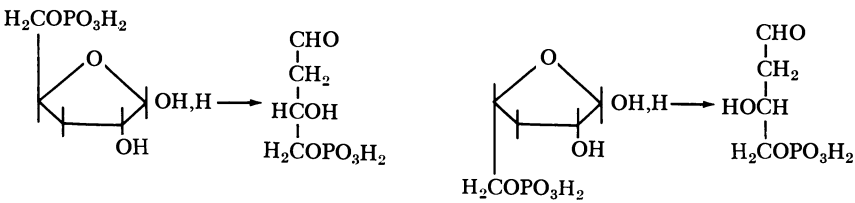


17 39 41

43 45

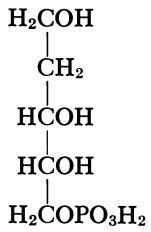
is removed as formaldehyde. The newly formed aldehyde group of the acetals (**38** and **39**) can be easily reduced with Raney nickel to primary alcohols. Thus 3-deoxy 1,2-*O*-isopropylidene D-*erythro*-pentofuranose (**40**) and 3-deoxy 1,2-*O*-isopropylidene L-*threo*-pentofuranose (**41**) are obtained from the 3-deoxy glucose- and 3-deoxy galactose derivatives, respectively. As in both of these compounds the only free hydroxyl group is that of the primary alcohol, excellent yields of the phosphorylated derivatives (**42** and **43**) are obtained upon phosphorylation with diphenyl phosphorochloridate. Catalytic hydrogenolysis, followed by very mild acid hydrolysis, then yields 3-deoxy-D-*erythro*-pentose 5-(dihydrogen phosphate) (**44**) (*53*) and 3-deoxy-L-*threo*-pentose-5-(dihydrogen phosphate) (**45**) (*58*). The former compound is of special interest, as 3-deoxy D-ribose is the sugar moiety of the antibiotic cordycepine.

The 3-deoxy pentose phosphates (**44** and **45**) can be further degraded to phosphorylated deoxy sugars (*58*) : treatment of either of them with periodate will cleave the carbon–carbon bond between C_1 and C_2 to yield 2-deoxy-D- (**46**) and -L-*glycero*-tetrose-4-phosphates (**47**).



44 46 45 47

Generally speaking, the phosphorylated deoxysugars undergo the usual reactions of carbohydrates without complication. For instance, both 2-deoxy D-ribose 5-phosphate (*52*, *59*) and 2-deoxy D-xylose 5-phosphate (*2*) can be reduced to the corresponding 2-deoxy D-*erythro*- (**48**) and 2-deoxy D-*threo*-pentitol 5-phosphates (**49**). 2-deoxy ribose 5-phosphate has also been oxidized (*52*) to the corresponding phosphorylated acid (**50**).



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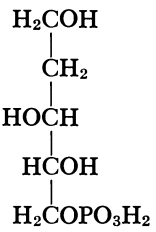

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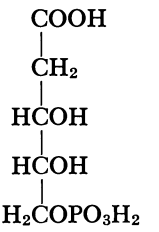

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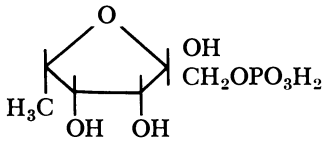
Oxidations can be carried out in the usual conditions. It seems, however, that there is some advantage in using the hypoiodide method, which gave very good yields in the preparation of 2-deoxy-D-gluconic acid 6-phosphate (*61*) and 3-deoxy D-gluconic acid 6-phosphate (*13*), whereas low yields were obtained when 3-deoxy D-galactose 6-phosphate was oxidized with bromine (*24*) to the phosphorylated deoxy galactonic acid. The yields obtained in this reaction are low probably, not because of the presence of a phosphate group in the molecule, for it has been shown (*62*) that oxidation of the non-phosphorylated 3-deoxy D-galactose also proceeds in low yields, while electrolytic oxidation gives excellent yields of the desired product.



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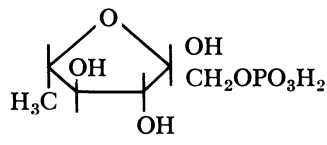

52

It is well known that the 1-phosphates of the ketoses, L-fuculose (**51**) and L-rhamnulose (**52**) have considerable biochemical interest. Their chemical synthesis has not been described as far as is known to the writer, but the rate of acid hydrolysis of L-fuculose 1-phosphate, obtained by enzymatic synthesis, has been determined by Heath and Ghalambor (*20*) and that of L-rhamnulose 1-phosphate by H. Sawada (*48*) and by Chiu and Feingold (*11*). They found that the rate of

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hydrolysis of the respective 6-deoxy-hexose 1-phosphates in 1*N* HCl at 100°C. was of the same order of magnitude as that of the 1-phosphate of D-fructose under the same conditions (*54*). This observation is rather intriguing in view of the fact that in acid, the 3- and 6-phosphates of 2-deoxy D-galactose are hydrolyzed considerably more rapidly than are the corresponding phosphates of D-galactose (*16*). 2-Deoxy ribose 5-phosphate is also hydrolyzed more rapidly than is ribose 5-phosphate (*40*). The difference that may exist between the hydrolysis rates of the ketose 1-phosphates is perhaps obscured by the relatively vigorous conditions (1*N* acid at 100°C.) in which the ketose phosphates were hydrolyzed.

As one would expect from the general behavior of 2-deoxy sugars, the lability of the phosphate group is enhanced in 2-deoxy glycosyl phosphates and particularly so if the sugar is in the furanose form. The only example of successful synthesis (*34*) of such an ester is that of the biochemically very important 2-deoxy α-D-*erythro*-pentofuranosyl phosphate (**56**). The compound has been previously obtained by Friedkin *et al.* (*17*, *18*, *19*) and also by Tarr (*55*) by enzymic methods and isolated as the crystalline cyclohexylammominum salt. In both laboratories the extreme lability of the phosphate was noticed: Friedkin and his colleagues (*17*, *18*, *19*) found that the ester had a half-life of 10 to 15 minutes at pH 4 and 23°C., Tarr (*55*) observed 98% hydrolysis in 100 minutes in a 0.06*M* acetate buffer of the same pH. The starting point for the chemical synthesis (*34*) is the crystalline 2-deoxy 3, 5-di-*O*-*p*-toluyl α-D-ribofuranosyl chloride (**53**) (*22*, *23*) which is condensed with disilver phosphate: a mixture of the anomeric phosphates (**54** and **55**), in which the α-anomer (**54**) predominates, is formed. Next the protecting groups are removed by mild alkaline treatment and the mixture of the free ribofuranosyl phosphates crystallized as the dicyclohexylammonium salts (**56** and **57**). By a very elaborate purification procedure, the α-phosphate was then enriched to somewhat more than 80%, but further purification was considered impracticable in view of the extreme acid-lability of the compound which is reflected during paper chromatography in ammonia containing solvents: mere drying of the paper at room temperature is sufficient to hydrolyze, at least partially, the ester, because of loss of ammonia during the process and concomitant, slight acidification of the spot. The synthesis, as it stands, therefore, represents a remarkable achievement. It has been found in several instances that in the type of phosphorylation just described, the ratio of the anomeric esters formed varies considerably with the cation of the phosphorylating agent. This is so in this case too: if the tri-*n*-pentylammonium phosphate is used instead of the silver phosphate, the β-anomer (**57**) predominates. A

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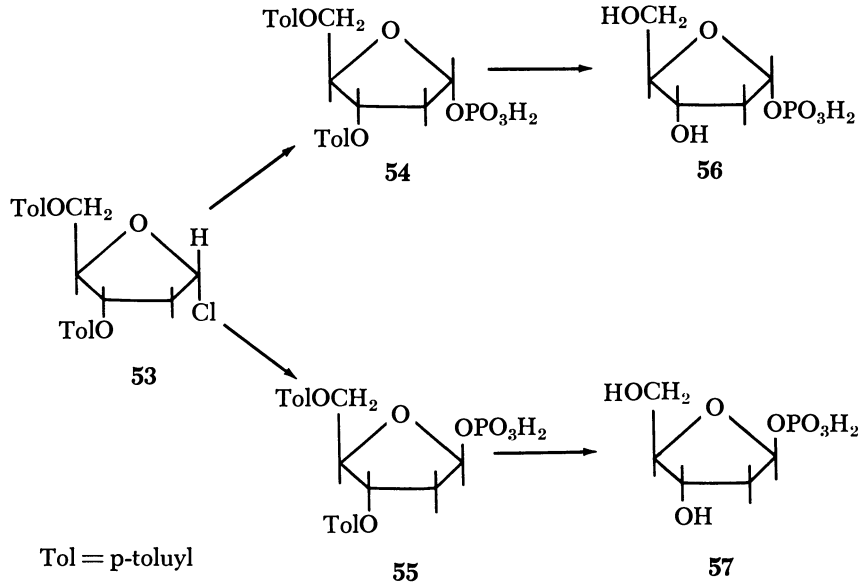

preparation enriched to about 65% in this isomer has been obtained by the same authors (*34*).

Complications other than unusually high acid lability can also occur with deoxy sugar phosphates. Thus a synthesis of 2-deoxy D-ribose 5-phosphate (**35**) has been elaborated by MacDonald and Fletcher (*33*), following the discovery, by the latter author, that 2-deoxy D-ribose can be easily prepared from D-glucose (*14*). The sequence of steps in the synthesis of 2-deoxy ribose 5-phosphate follows closely that elaborated (*5*) for obtaining D-erythrose 4-phosphate: the diisopropyl mercaptal of 2-deoxy ribose (**58**) was tritylated and then benzoylated; the fully protected mercaptal (**59**) was then transformed by the mercury chloride/mercuric oxide method (*8*) into the dimethyl acetal (**60**) from which the trityl group can be removed by hydrogenolysis. The dibenzoate (**61**) was then phosphorylated with diphenyl phosphorochloridate. The phenyl groups of the diphenyl phosphate (**62**) were next cleaved by hydrogenolysis, the benzoate groups removed by alkaline treatment and the phosphorylated dimethyl acetal (**63**) isolated as the crystalline dicyclohexylammonium salt. It was expected that exposure to weak acid would be sufficient to hydrolyze the acetal group and yield the phosphorylated deoxy sugar (**35**). However, when the hydrolysis was carried out by treating an aqueous solution of the cyclohexylammonium salt with a strong acid ion exchange resin at room temperature, it was observed that the reducing 2-deoxy D-ribose 5-phosphate was not the major product of the hydrolysis; a non-reducing phosphate ester was

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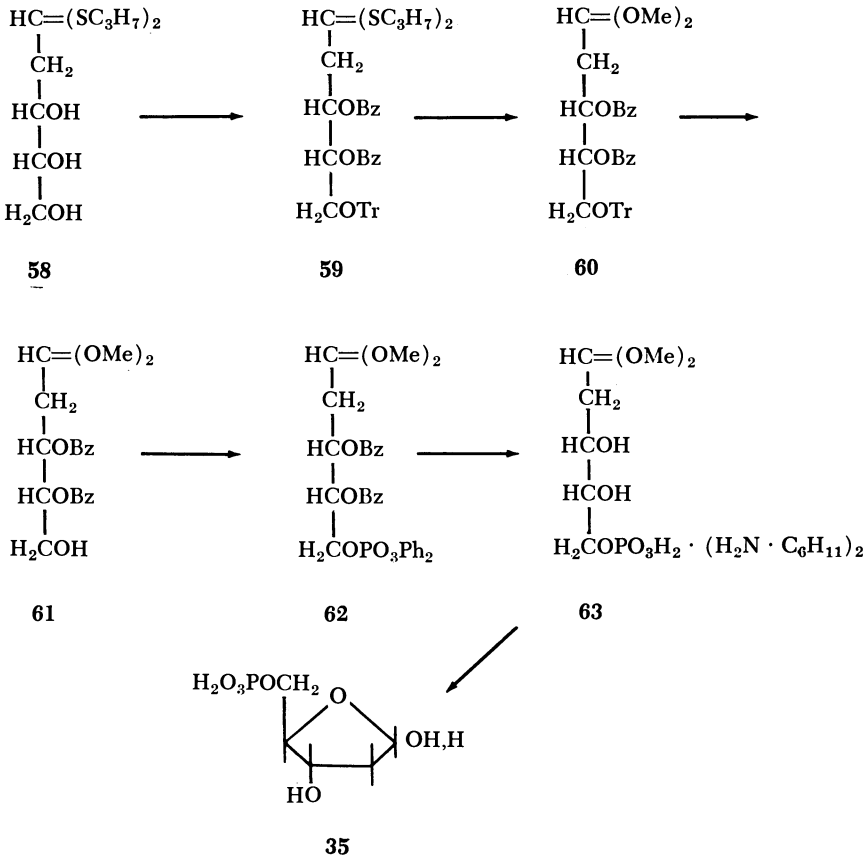
formed instead and this was transformed only very slowly into the expected sugar phosphate (**35**), the complete transformation requiring several days. The authors tentatively suggested that the nonreducing, periodate resistant phosphate ester formed in the first place might be the methyl glycoside, as addition of methanol to the hydrolysis mixture appeared to increase the amount of the unknown phosphate ester. This suggestion is, however, not easily reconciled with the observation that the methyl glycosides of 2-deoxy D-ribose 5-phosphate are hydrolyzed at pH 1.25 (own acidity) at 37°C. within one hour (59). Whatever the



nature of this nonreducing compound may be, its appearance indicates that 2-deoxy ribose 5-phosphate is different in its behavior from the other phosphate esters whose chemistry was discussed above.

At about the same time that MacDonald and Fletcher worked on their synthesis of 2-deoxy ribose 5-phosphate, we ourselves tried to work out a synthesis for the same compound, using a quite different approach.

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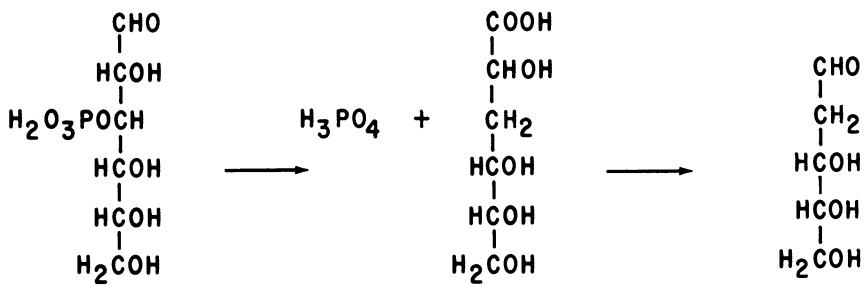
Trying to avoid the use of the then virtually inaccessible 2-deoxy ribose (the starting point for both the American (*33*) and the Japanese (*59*) chemists), we elaborated a synthesis starting with D-glucose and making use of cyclic phosphate esters. It had been shown previously that, on mild alkaline treatment, glucose 3-phosphate (**64**) yielded (*7*), besides inorganic phosphate, glucometasaccharinic acid (**65**) and that this acid could be degraded (*43*) to 2-deoxy D-ribose (**66**). It was, therefore, to be expected that D-glucose, 3, 6-(hydrogen phosphate) (**70**) would form the 6-phosphate of glucometasaccharinic acid (**71**), whose degradation, according to Ruff's method, should lead to 2-deoxy ribose 5-phosphate (**35**). It was found (*51*) that the required cyclic phosphate of glucose (**70**) could be easily obtained, if 1,2-*O*-isopropylidene-D-glucose 3-phosphate (**67**) were treated with dicyclohexylcarbodiimide in pyridine solution: isopropylidene glucose 3, 5-(hydrogen phosphate) (**68**) was formed first, and then underwent a transesterification reaction to form isopropylidene glucose 3, 6-(hydrogen phosphate) (**69**). Treatment of this phosphorylated acetal with weak acid yielded the required glucose 3, 6-(hydrogen phosphate) (**70**). As expected, treatment of this cyclic phosphodiester with baryta transformed (*29*) it into glucometasaccharinic acid 6-phosphate (**71**). The structure of the compound was confirmed (*29*) by an independent synthesis, based on the alkaline degradation of 3-*O*-methyl glucose 6-phosphate (**72**) which gave the same phosphorylated acid. When this compound was submitted to the Ruff degradation and the resulting mixture separated by column chromatography in a sulfate system (*28*), the deoxyribose phosphate was easily separated from other material. In spite of correct analyses (elementary and functional groups), and appropriate checks on purity, all indicating homogeneity of the substance, the product was, in fact, a mixture: according to enzymic analysis—based on the cleavage of the 5-phosphate by a specific aldolase to triose phosphate and acetaldehyde



64 **65** **66**

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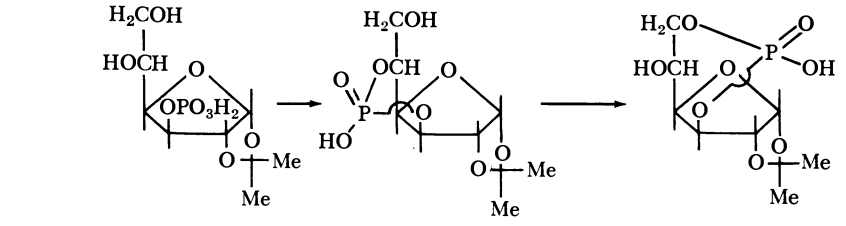

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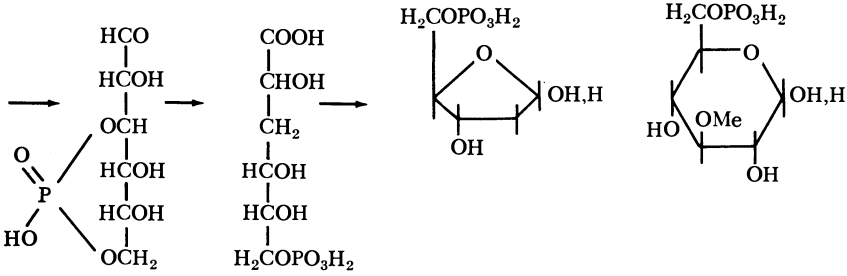

70 71 35 72

(*39*, *40*, *46*)—it contained not more than about 25% of the expected 5-phosphate. From the above mentioned analytical results it was obvious that some sort of isomerization must have occurred during the preparation and, accordingly, attempts were made to identify the components of the mixture. By treating the isomerized mixture with acid phosphatase and estimating the amount of 2-deoxy ribose liberated, it was shown (*58*) that the deoxyribose content of the substance was that theoretical for a monophosphate of 2-deoxy ribose; as an equimolar amount of inorganic phosphate was formed at the same time, the substance evidently consisted of a mixture of positional isomers of 2-deoxy ribose phosphate, resulting from phosphate migration either during the degradation or the succeeding chromatographic separation.

In theory, periodate oxidation could have given a clear-cut answer as to the composition of the isomeric mixture of deoxy ribose phosphates. The 4-phosphate (**73**), devoid of vicinal diol groups, should be resistant to periodate; the 3-phosphate (**74**) should reduce one and only one molar equivalent of the oxidant and yield one molar equivalent of both formaldehyde and the phosphorylated dialdehyde (**75**), whereas the 5-phosphate (**76**) could be expected to reduce one molar equivalent of periodate relatively rapidly, followed by a slower "overoxidation" reaction owing to the oxidation of malonaldehyde, formed as a result of the glycol cleavage.

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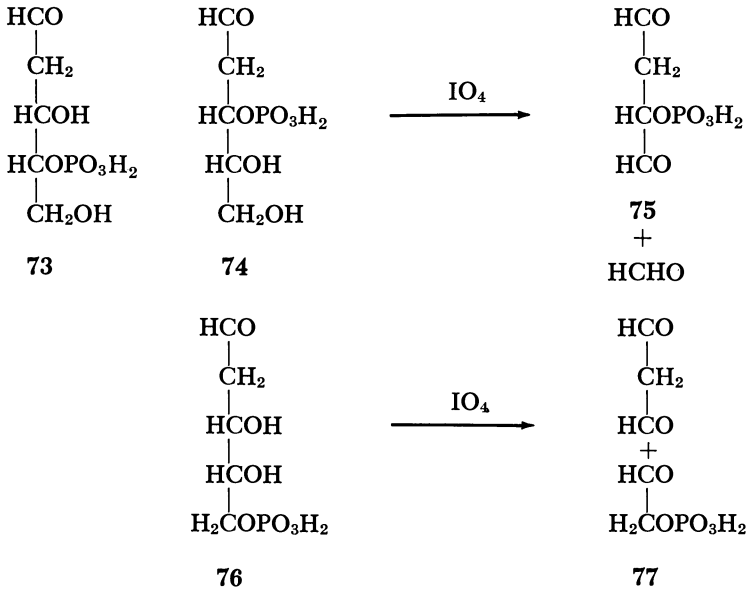

In fact, it has been found (52) that in unbuffered solution, at room temperature, authentic 2-deoxy ribose 5-phosphate reduces more than 4 molar equivalents of periodate, but that there is no noticeable slowing down of the reaction rate after the reduction of the first molar equivalent. This may be owing to the fact that only the aldehydo form (**76**) of 2-deoxy ribose 5-phosphate has a free vicinal diol group; as the acyclic 2-deoxy ribitol 5-phosphate reduces one molar equivalent of periodate quite fast (58), it is probable that the time-curve of periodate uptake by the phosphorylated sugar reflects the rate of formation of the aldehyde form from the furanose form.

Attempts to separate the isomeric deoxy ribose phosphates by ion exchange techniques met with no success; this may be because of the fact that eluents having a pH lower than 5 were not used, as migration of phosphomonoesters is known to be acid catalysed, and, because of the free sugar involved, alkaline eluents could not be considered.

That the postulated phosphate group migration did in fact, occur, was eventually proved (58) in the following way. During the many experiments done to find conditions in which the phosphate group migration could be demonstrated by periodate oxidation, the conclusion was reached that one of the difficulties in the interpretation of the results was because of the fact that the reaction between the phosphorylated

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deoxy ribose and periodate is rather slow and provides ample opportunity for secondary reactions to occur as, for example, the self-condensation of glycol aldehyde phosphate (**77**). It is well known that this compound gives erratic results during paper chromatography (*32*, *38*, *47*) and it can be reasonably expected that the instability of the phosphorylated dialdehyde (**75**), which would be formed from deoxy-ribose 3-phosphate if present in the mixture, would be even greater. On the other hand it is known (*58*) that 2-deoxy ribitol 5-phosphate is oxidized rapidly by periodate. It was therefore decided to treat the deoxyribose phosphate mixture with borohydride and then cleave the vicinal glycol groups with periodate; the aldehydes formed were reduced to alcohols by a second treatment with borohydride; secondary reactions of the phosphorylated aldehydes were thus avoided. The reduction products of the oxidizing agent were then removed as free iodine and the solution submitted to paper electrophoresis. The reliability of the method was tested by submitting authentic 2-deoxy D-ribose 5-phosphate to the reaction sequence indicated: besides a trace of inorganic phosphate, the only phosphate ester found was glycol phosphate. When the deoxyribose phosphate mixture, obtained through the Ruff degradation of glucometasaccharinic acid 6-phosphate, was treated in a similar way, paper electrophoresis revealed two phosphate esters: the faster moving, minor compound had a mobility identical to that of glycol phosphate, thereby confirming the presence of 2-deoxy ribose 5-phosphate in the mixture, the slower moving, major compound had a mobility equal to that of 2-deoxy ribitol-5-phosphate. The presence of a phosphate ester, other than glycol phosphate, in the final reaction mixture represents an unequivocal proof for the postulated phosphate migration.

The unknown phosphate ester had the same electrophoretic mobility as 2-deoxy ribitol 5-phosphate and it seemed reasonable to expect that in the conditions used (0.1*M* pyridinium acetate buffer of pH 5) 2-deoxy ribitol-4- and -5-phosphates would behave similarly: therefore it was considered probable that the unknown phosphate ester is 2-deoxy ribitol 4-phosphate, resulting from the reduction of the periodate resistant 2-deoxy ribose 4-phosphate. However, the possibility that both 2-deoxy ribitol 4-phosphate and 2-deoxy erythritol 3-phosphate (formed from 2-deoxy ribose 3-phosphate, if present in the isomerized mixture) were present, but insufficiently separated to be distinguished, had to be examined. Accordingly 2-deoxy D-*glycero*-tetritol 4-phosphate, which could be expected to behave similarly on electrophoresis to the 2-deoxy erythritol 3-phosphate, was synthesized (*58*). It was clearly separated from glycol phosphate and migrated slightly faster than both 2-deoxy ribitol 5-phosphate and the unknown phosphorylated deoxy sugar alcohol. The mixture of isomeric 2-deoxy ribrose phosphates was next treated with periodate

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until no more periodate was reduced and then the mixture submitted to paper electrophoresis in conditions in which the "glycolaldehyde spot" and the "2-deoxyribose 4-phosphate spot" are separated. The latter, eluted from the paper, was treated with acid phosphatase and the ratio of deoxyribose to inorganic phosphate determined and found to be unity: this established that no phosphate ester other than 2-deoxy ribose 4-phosphate was present in that spot. The "glycolaldehyde phosphate spot" was also eluted, reduced with borohydride and then submitted to paper electrophoresis in conditions in which glycol phosphate is well separated from the 2-deoxy tetritol and pentitol phosphates: it was found that only one phosphate ester, corresponding in mobility to glycol phosphate, was present and this definitely proves that in the mixture of the isomeric deoxyribose phosphates, obtained by the Ruff degradation of glucometasaccharinic acid 6-phosphate, only two esters are present, namely the 4- (**73**) and the 5- (**35**) phosphates of 2-deoxy D-ribose.

The ease of the phosphate group migration during the attempted synthesis of 2-deoxy ribose 5-phosphate from glucometasaccharinic acid 6-phosphate is quite surprising. The Ruff degradation is carried out at neutral pH values, and we have seen that deoxy sugar phosphates may be exposed to acid conditions more severe than those used during the ion exchange chromatography without any phosphate migration being noticeable. The reasons for the occurrence of this unexpected reaction are now under investigation.

2-Deoxy ribose 5-phosphate is not the only sugar phosphate which undergoes this easy phosphate migration. 2-Keto 3-deoxy D-gluconic acid 6-phosphate (3-deoxy D-*erythro*-hexulosonic acid 6-phosphate) (**78**) and 2-keto 3-deoxy D-galactonic acid 6-phosphate (3-deoxy D-*threo*-hexulosonic acid 6-phosphate) (**80**) are also subject to such instability. These two phosphorylated sugar acids can be obtained (*24*) by oxidation of glucometasaccharinic acid 6-phosphate (**71**) and 3-deoxy galactonic acid 6-phosphate (**79**) respectively, with chlorate, in the presence of a vanadium pentoxide catalyst, followed by ion exchange chromatography in a monochloracetate system of pH 4. The method (*41*) has been used to oxidize 3-deoxy D-*gluco*-heptonic acid 7-phosphate (**81**) to the corresponding 3-deoxy D-*arabino*-heptulosonic acid 7-phosphate (**82**) without any apparent anomaly as far as the stability of the phosphate group is concerned (*50*). The phosphorylated hexulusonic acids (**78** and **80**) are both cleaved by specific aldolases to yield, by the fission of the C_3–C_4 bond, equimolar amounts pyruvate and triose phosphate (*30*, *35*). When tested by this method it was found that preparations giving correct figures for elementary and functional group analyses, yielded only 60% to 70% of the expected cleavage products. For both hexulosonic acids the percentage of cleavage products varied from one

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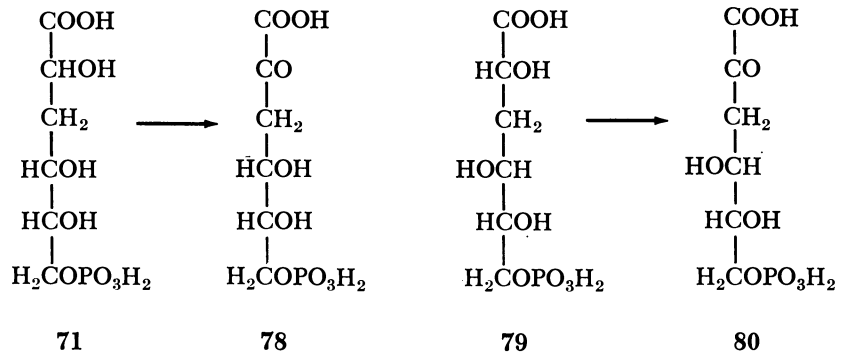
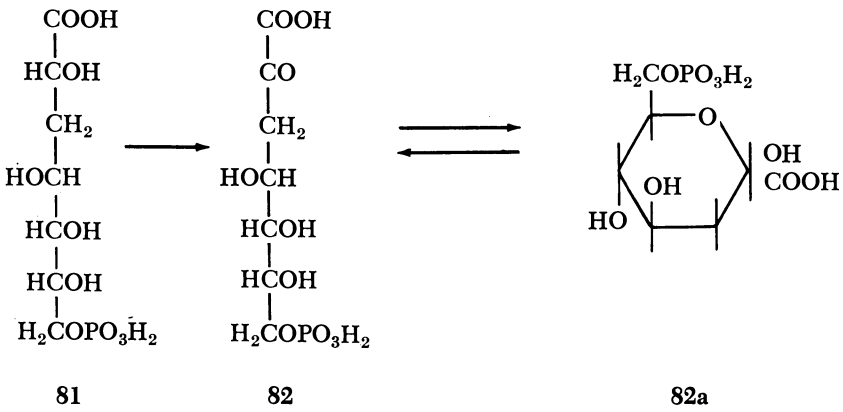
preparation to another. In view of the fact that all preparations had a 100% α-keto acid content as measured by both the semicarbazide (*30*, *35*) and the *o*-phenylenediamine method (*27*), we suspected that in this





case too, phosphate migration had occurred. Indeed it was found that in very carefully controlled conditions it is possible to resolve, at least partially, the seemingly homogeneous 3-deoxy D-*erythro*-hexulosonic acid phosphate by ion exchange chromatography (Figure 1) into two fractions. This proof is, however, not unequivocal on account of the possibility of phosphate migration occurring during the analytical ion-exchange chromatography itself.

The features common to 2-deoxy ribose 5-phosphate and the two 3-deoxy hexulosonic acid 6-phosphates are that they all have a deoxy group next to the carbonyl group and that they can form only the furanoid ring (**35, 78a, 80a**) as long as the phosphate group is attached to the terminal, primary alcohol. However, they can all assume the pyranoid ring

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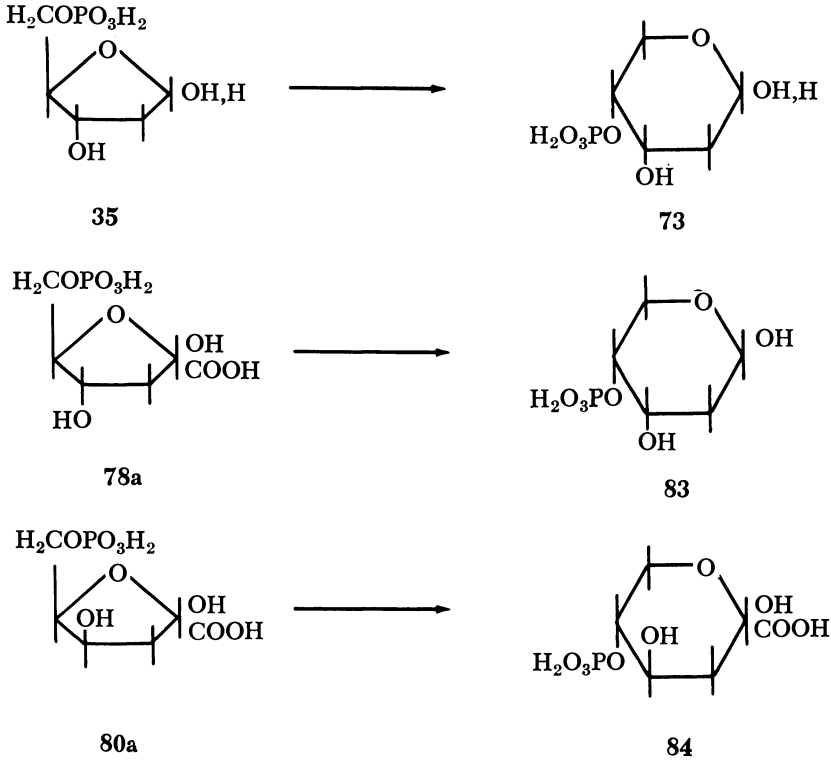
form (**73a, 83, 84**) if the primary alcohol group is free. It is known that, in solution, most sugars exist preferentially in the pyranose form and we believe that for 2-deoxy ribose 5-phosphate and the 3-deoxy hexulosonic acid 6-phosphates the driving force for the phosphate migration is the furanose to pyranose transformation. This structural particularity, and hence the driving force, is absent from the 3-deoxy heptulosonic acid (**82, 82a**) and from the other deoxy sugar phosphates, whose syntheses have been described, and, as a corollary, the phosphate group in these compounds is not subject to easy phosphate migration. It will, however, be necessary to determine the exact conditions in which the phosphate migration occurs, before a fully satisfactory explanation can be given for this interesting phenomenon.



The alkaline degradation of various cyclic sugar phosphates is a general reaction and it can be used for preparing a number of phosphorylated deoxy sugar acids. Thus, if glucose 3, 5-phosphate (**85**) is treated with baryta (*29*), glucometasaccharinic acid 5-phosphate (**86**) is obtained. In the same conditions, glucose 4, 6-phosphate (**87**) (*3, 25*) is transformed into isosaccharinic acid 6-phosphate (**88**) (*25*), while the

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easily accessible (37) xylose 3, 5-phosphate (**89**) yields "xylometasaccharinic acid" 5-phosphate (**90**) (25). However, it was found that the reaction was only partially successful when the synthesis and alkaline degradation of D-arabinose 3, 5-phosphate (**92**) were attempted (25), To obtain the cyclic phosphate of the pentose, glucose 4, 6-phosphate

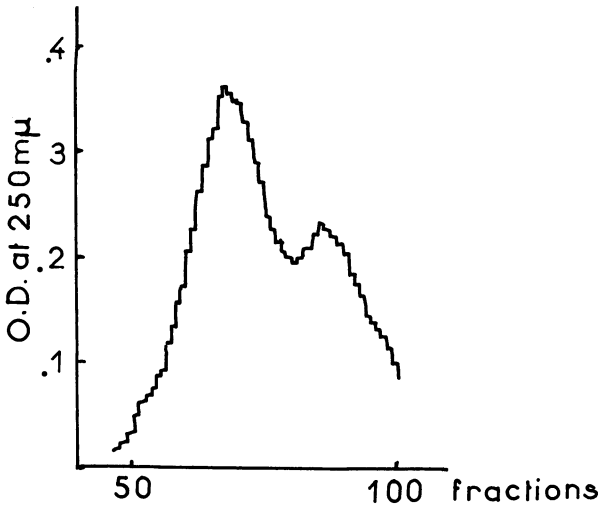
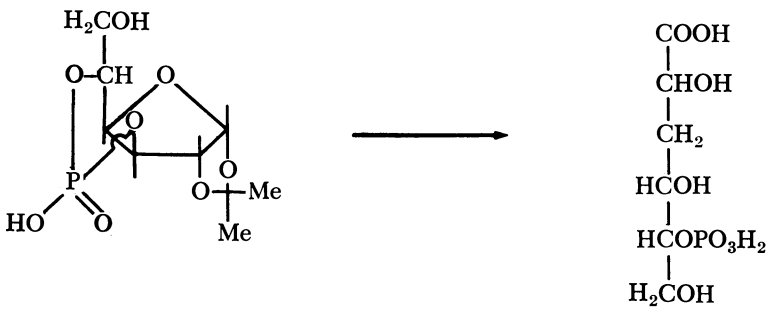
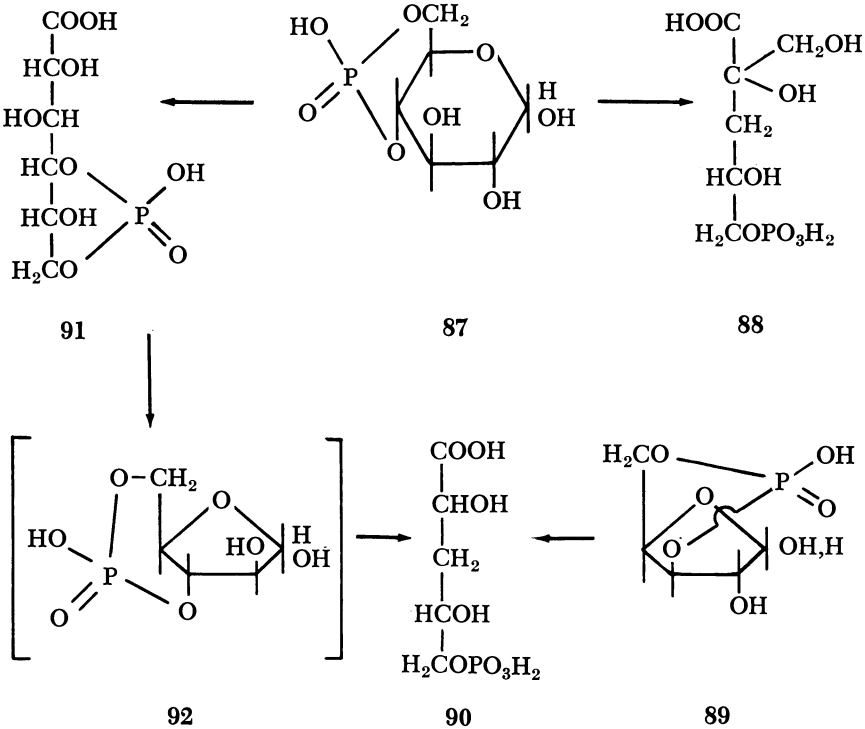


Figure 1. Separation of isomeric 3-deoxy D-erythro-*hexulosonic acid phosphates.*

11.5 mg. of the mixed, isomeric Ba-salts were adsorbed onto a column of Dowex 2 x 10 resin (200–400 mesh, 11.5 x 320 mm., monochloracetate form) and eluted with a buffer of pH 3.95 (0.05 molar with respect to monochloracetic acid and 0.120 molar with respect to sodium monochloracetate); four 5 ml. fractions were collected per hour. The elution was followed by the semicarbazide test (36).



85 **86**



91 87 88

92 90 89

was oxidized to gluconic acid 4, 6-phosphate (**91**) and the latter subjected to a Ruff degradation. However, in spite of considerable effort, we failed to find conditions for the isolation of the cyclic phosphate of the pentose, whose formation could be, nevertheless, demonstrated by chromatographic techniques. Its presence in the reaction mixture was also substantiated by the fact that if, after the completion of the Ruff degradation, the mixture was treated with baryta, a small yield of the same phosphorylated 5-carbon metasaccharinic acid, as that formed in good yield from D-xylose 3, 5-phosphate was obtained.

The discrepancy between the behavior of arabinose- and xylose-3, 5-(hydrogen phosphates) probably reflects the difference which exists in the steric arrangement of these two cyclic phosphodiesters. In the xylofuranose derivative (**89**), the hydroxyl group on C–3 and the primary hydroxyl group are *cis:* the cyclic phosphate is easily formed and perfectly strainless. In arabinofuranose these same groups are in position *trans* and, although an apparently strainless molecule can be constructed from models, it is probable that in this case the main ring of the compound will be that formed by the phosphodiester, with a concomitant tendency for the compound to assume the aldehydo form. Objection to

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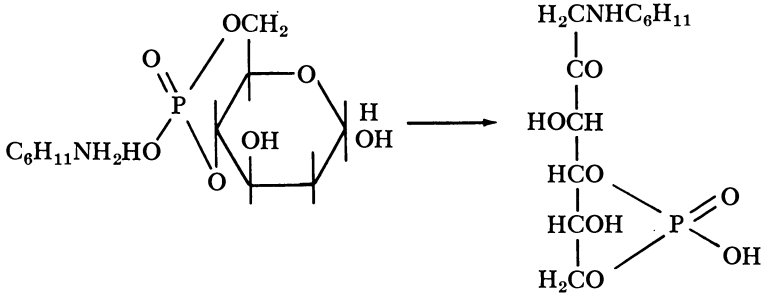
this argument would be that in the ribonucleoside and deoxyribonucleoside 3′, 5′-cyclic phosphates the same situation exists, nevertheless the furanoid ring and the cyclic phosphate coexist in the molecule. However, the nucleoside cyclic phosphates are formed (*12*, *31*, *49*, *57*) in conditions in which the glycosidic bond, which maintains the sugar in the furanose form, is not broken; that the formation of 3′, 5′-cyclic phosphate does, even so, affect the state of the glycosidic carbon atom is shown by the fact that, while the acyclic thymidine 5′-phosphate is not attacked by 0.1*N* hydrochloric acid at 100°C. in five minutes, the cyclic thymidine 3′, 5′-phosphate is completely hydrolyzed in the same conditions (*49*, *57*). Another observation also supports the above hypothesis. It has been reported (*3*) that the cyclohexylammonium salt of glucose 4, 6-phosphate (**93**), when recrystallized from hot ethyl alcohol, undergoes an Amadori rearrangement and forms 1-cyclohexylamino 1-deoxy D-fructose 4, 6-phosphate (**94**). This compound has the same steric configuration as arabinose 3, 5-phosphate (**92**) and although it could form a furanose ring, as both the reducing group and the hydroxyl group on C–5 are free, in solution it is nevertheless in the acyclic, keto-form, as shown by its ultraviolet absorption at 280 mμ, a band also observed with other keto-fructose derivatives of known constitution (*6*). We think therefore, that arabinose 3, 5-phosphate is also preponderantly in the aldehydo form; it is not unexpected that the aldehydo compound should undergo considerable destruction in the conditions of the Ruff degradation: this would explain the difficulties encountered during the attempted isolation of the cyclic sugar phosphate and also the low yields of the phosphorylated deoxy sugar acid when prepared by the method described.



93 94

Acknowledgments.

The author wishes to thank M. Doudoroff for carrying out the analyses in which the phosphorylated hexulusonic acids (**78** and **79**)

were both cleaved by specific aldolases to yield, by the fission of the C_3–C_4 bond, equimolar amounts of pyruvate and triose phosphate.

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6

Quantitative Estimation of Deoxy Sugars and Related Compounds with Special Reference to Periodate Oxidation.

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Known methods of quantitative analysis of deoxy sugars are reviewed, including methods which rely on the formation of malonaldehyde during periodate oxidation of certain deoxy sugars. The behavior of malonaldehyde, and that of its postulated oxidation product, triose reductone ("hydroxy malonaldehyde") towards periodate is treated in detail. Under certain conditions malonaldehyde is produced in stoichiometric amounts from appropriate deoxy sugars, sugar alcohols, and cyclitols. It is also stable with periodate and can be estimated quantitatively, without the use of standards, by known colorimetric methods. Simultaneously, the number of vicinal diol groups present in the molecule can also be determined quantitatively. The method can be applied to the estimation of deoxy sugars in biological material.

In view of the biological importance of deoxy sugars, many specific methods have been elaborated for their identification and quantitative estimation. Obviously, many of the methods generally employed for the analysis of monosaccharides can also be used for the estimation of deoxy sugars; for example, the Willstätter-Schudel hypoiodite titration, methoxy and ethoxy group determinations, quantitative estimation of the terminal methyl group in such compounds as 6-deoxy hexoses, and so on. However, one of the most useful analytical methods in the carbohydrate field, namely periodate oxidation, has, until now, had only limited application for the analysis of deoxy sugars, owing to the fact that many of these compounds yield malonaldehyde and related substances upon oxidation. Such intermediates, in the usual oxidation conditions, are subject to non-Malapradian or "over-oxidation" reactions and thus interfere with the

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

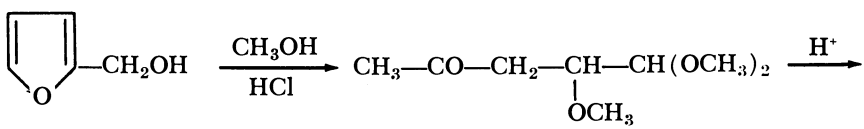
selective estimation of the vicinal diol groups. The present review will deal mainly with this aspect of the estimation of deoxy sugars, as in recent years we have studied these "over-oxidation" reactions in the hope of extending the use of periodate oxidation to the analysis of all deoxy sugars.

In as far as other analytical methods are concerned, many specific reactions have been elaborated for the quantitative determination of 2-deoxy aldoses. 2-Deoxy-D-ribose (2-deoxy-D-*erythro*-pentose), a compound which was recognized early as playing an important role in biological systems, has been of particular interest. Overend and Stacey (*43*) have given a critical review of the methods available until 1952 for the estimation of 2-deoxy pentoses. A recent summary of specific methods for the identification and quantitative estimation of the different classes of deoxy sugars has been prepared by Dische (*13*).

In general, the methods involve the transforming of the sugar to some highly reactive compound, which then forms a colored product with such reagents as thiols, aromatic amines, or phenols. In many cases, the sugar forms a chromogen upon treatment with strong acid; however, in other cases, the chromogen is malonaldehyde or a related compound and is formed upon periodate oxidation of the sugar.

To obtain reliable, accurate, and reproducible methods for quantitative estimation of deoxy sugars, certain conditions must be fulfilled. Thus, it is necessary that the chromogen be formed quantitatively from the sugar. The chromogen must then react quantitatively with the compound used for color formation, and lastly, the dye, once formed, should be stable and have a well defined molar extinction coefficient. In methods in which all of these conditions are not or cannot be fulfilled, recourse must be had to simultaneous determinations with suitable standard substances, a requirement not always easy to fulfil.

In some cases, these problems, or one or more of them, have been solved. For example, Birkofer and Dutz (*4*) have shown that splitting of furfuryl alcohol (**1**) with methanolic hydrochloride yielded the dimethyl



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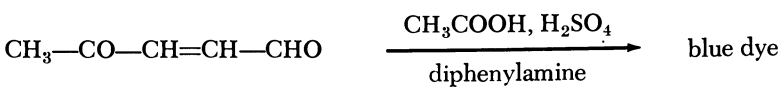

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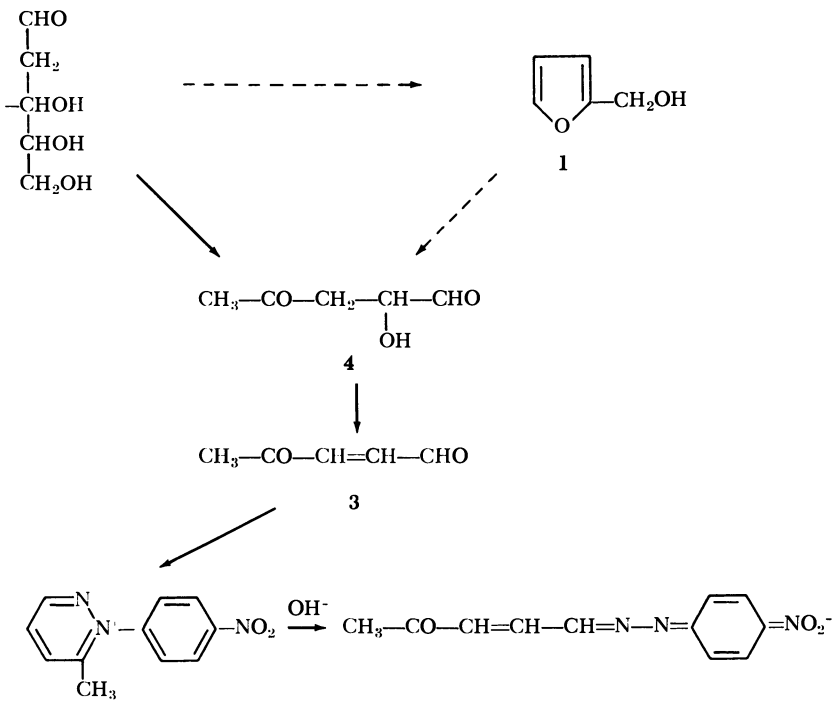
acetal of α-methoxy levulinic aldehyde (**2**). This acetal, when further treated with acid, could be transformed into β-acetyl acrolein (**3**) which gave a strongly positive reaction in Dische's diphenylamine test (*12*). It is therefore highly probable that a similar reaction sequence, involving the free hydroxyaldehyde (**4**), formed by acid treatment of 2-deoxy pentoses, takes place in the Dische test. This well-known test is specific for 2-deoxy pentoses and permits their quantitative estimation; when the sugars are heated in a strongly acid solution containing diphenylamine, a blue dye, having an absorption maximum at 600 mμ, is formed.

In the Webb and Levy test (*60*) for 2-deoxy pentoses, the same hydroxyaldehyde intermediate (**4**) (*30*), formed by treating the sugars with trichloracetic acid, reacts with *p*-nitrophenylhydrazine to yield the pyridazinium salt (**5**) from which a quinonoid dye (**6**) absorbing at 560 mμ, is formed in alkaline medium.



Although these results establish with reasonable certainty the reaction sequences leading from the sugar to the dye, no stoichiometric relationships have been shown to exist between the amounts of sugar employed and the quantities of dyes formed. Both tests are, therefore, still empirical,

and for quantitative estimations, it is necessary to perform simultaneous determinations using suitable standards.

In connection with the Webb and Levy test, it should be mentioned that this test can also be applied to the estimation of all 2-deoxy aldoses, including those containing a terminal deoxy group, provided they have at least five carbon atoms in a straight chain; 2-deoxy tetroses do not react (*62*). It has also been used for the estimation of 3-deoxy and 3, 6-di-deoxy hexoses, after their conversion to the corresponding 2-deoxy pentoses by removal of carbon 1 with periodate (*24,25*).

As has already been mentioned, some methods of estimating deoxy sugars rely on the fact that chromogens, such as malonaldehyde, are formed when these compounds are treated with periodate. Thus, Waravdekar and Saslaw (*58,59*) have shown that 2-deoxy aldoses such as 2-deoxy-D-ribose, 2-deoxy-D-xylose (2-deoxy-D-*threo*-pentose), 2-deoxy-D-glucose (2-deoxy-D-*arabino*-hexose) and digitoxose (2, 6-di-deoxy-D-*ribo*-hexose), when oxidized with periodate at pH 2 and room temperature, all yielded approximately one molar equivalent of malonaldehyde. This intermediate reacts with 2-thiobarbituric acid to form a red dye which can be estimated by colorimetry. It was shown that a linear relationship exists between the concentration of malonaldehyde and the optical density of the dye formed. Malonaldehyde bis-bisulfite, sodium salt, was used as standard. However, other compounds, such as 3-deoxy-D-glucose (3-deoxy-D-*ribo*-hexose), yielded, in the same conditions, only a small fraction of the expected amount of malonaldehyde. Shortly afterwards, Cynkin and Ashwell (*11*) found that yields of malonaldehyde from 3-deoxy and 3, 6-di-deoxy sugars could be greatly increased if the oxidations were carried out at 55°C.

As has already been indicated, the periodate oxidation of those deoxy sugars which yield malonaldehyde involves two reactions; the primary reaction of glycol cleavage and the secondary reaction in which the malonaldehyde thus formed is further oxidized. In compounds such as 2-deoxy aldoses, which can be estimated by the method of Waravdekar and Saslaw, the rate of the primary reaction is very fast, and thus, at room temperature, during the time required for the quantitative release of malonaldehyde, the chromogen is not destroyed to any measurable extent. However, in cases where the glycol cleavage is slow, as for example in 3-deoxy-D-glucose, already at room temperature a considerable quantity of malonaldehyde is destroyed before glycol cleavage is complete and therefore the method fails (*54*). If the rate of glycol cleavage is increased by performing the oxidations at more elevated temperatures, the rate of oxidation of malonaldehyde is also increased and thus equimolar quantities of malonaldehyde are not obtained in this case either. For example, in the method of Cynkin and Ashwell, the

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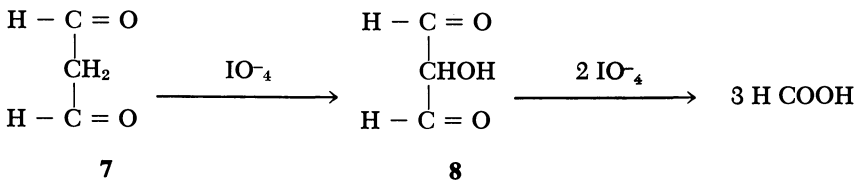
maximum yields of malonaldehyde from the 3-deoxy and 3, 6-di-deoxy sugars attained only 88% of the theoretical value. In addition to the fact that quantitative yields of malonaldehyde are not obtained, another inconvenience of the method is that the heating time required for the formation of the maximum quantities of malonaldehyde is different for each sugar.

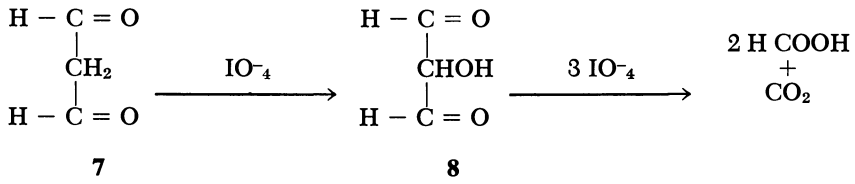
Despite the above-mentioned short-comings, this approach to the estimation of those deoxy sugars which yield malonaldehyde when oxidized with periodate, seemed promising, since, as has been seen (*58,59*), the dye is formed·quantitatively in the reaction of malonaldehyde with 2-thiobarbituric acid; also, more recently, its constitution (*49,57*) and molar extinction coefficient (*36*) have been established. Thus, if conditions could be found in which malonaldehyde, while being formed quantitatively from the deoxy sugars, would be stable, an ideal method, independent of standard compounds, would be available for the quantitative determination of all of these sugars.

Two different pathways have been proposed to explain the "over-oxidation" reaction of malonaldehyde. Huebner and his collaborators (*32*) based their conclusion on the observed behavior of digitoxose and suggested that malonaldehyde (**7**) was oxidized by three molar equivalents of periodate with the concomitant formation of three molar equivalents of formic acid:



7 **8**

while Fleury *et al.* (*22*) oxidized malonaldehyde, obtained by hydrolysis of 1, 1, 3, 3-tetra-ethoxypropane, and found an uptake of four molar equivalents of the oxidant and the formation of two molar equivalents of formic acid and of one molar equivalent of carbon dioxide:



7 **8**

In neither case were the final products obtained in stoichiometric amounts. The behavior of crystalline malonaldehyde (*33*) has not been investigated, probably because it is extremely unstable.

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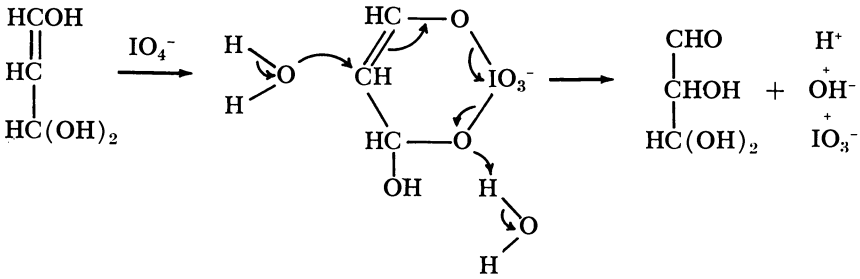
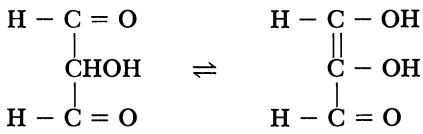
The oxidation of active methylene groups by periodate both in acyclic (*18,19,32*) and in cyclic (*63*) compounds, is thought to proceed by an initial hydroxylation stage in which one of the "active" hydrogen atoms is replaced by a hydroxyl group, and both of the above-mentioned authors (*22,32*) also suggest that the first step in the oxidation of malonaldehyde is the "hydroxylation" of the molecule, to give "hydroxymalonaldehyde" (**8**). According to Foster and his colleagues (*5*), the hydroxylation could result from the breakdown of a six-membered cyclic complex (**10**) formed from the hydrated form of malonaldehyde (**9**), and periodate, with the participation of two molecules of water:



9 **10**

The hydroxylated compound thus formed would be "hydroxymalonaldehyde"—*i.e.* tartronic dialdehyde (**8**). This compound has never been obtained or studied, but its enol form, which is the so-called triose reductone (**11**) (*14*), is well known, and it is generally agreed that, in solution, the equilibrium



8 **9**

is entirely in favor of triose reductone. Thus, one might expect that most, if not all, of the tartronic dialdehyde formed in the first step, will be oxidized as triose reductone.

On the basis of experiments with *myo*inositol (*50*), it has been suggested that triose reductone should be oxidized by periodate to yield two molar equivalents of formic acid and one molar equivalent of carbon dioxide. However, it has been reported by two groups (*1,29*) that crystalline triose reductone is oxidized by two moles of periodic acid to give formic acid and glyoxylic acid, free iodine being liberated during the

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oxidation. In one case (*29*), glyoxylic acid was isolated in high yield. At first this seemed surprising, as it is well known (*17,31,32,51*) that glyoxylic acid is oxidized by periodate to formic acid and carbon dioxide.

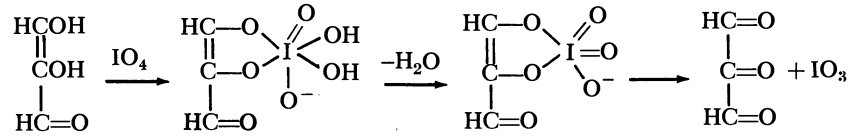
The appearance of free iodine during the periodate oxidation of compounds having an active hydrogen atom (*27*) or an ene-diol structure (*1,39*) has frequently been observed, and this implies that further reduction of iodate, formed from periodate during the main reaction, takes place. It has, in fact, been shown that, in acid solution, iodate is fairly readily reduced by such compounds as triose reductone (*27*), dihydroxyfumaric (*39*), and tartronic (*32*) acids.

We examined the reaction of triose reductone with both periodate and iodate (*55,56*), and found that, whereas iodine was invariably set free from both sodium periodate and sodium iodate if the concentration of the reductone were greater than $10^{-3}M$, no iodine was liberated at lower concentrations (*e.g.* 6 x $10^{-4}M$) of substrate, even in the presence of relatively large amounts of the oxidants.

The reaction of iodate with triose reductone is not only a function of the concentration of the reagents, it is also dependent on the pH of the solution. In solutions of triose reductone more dilute than $10^{-3}M$, iodine is set free from iodate, if the pH of the solution is lower than about 3 (*55*). Dihydroxyfumaric and L-ascorbic acids (*26*), which also have free ene-diol structures, behave similarly.

Hesse and Mix (*29*) oxidized a relatively concentrated solution of triose reductone using limited amounts of free periodic acid. In these conditions, the iodic acid formed by the initial reduction of periodic acid could be further reduced and the reduction product could then, in turn, react with the remaining periodic acid and liberate iodine. Thus glyoxylic acid could be isolated from the oxidation mixture, as no periodate was available for its oxidation.

We therefore carried out periodate oxidation of triose reductone in dilute solutions using sodium metaperiodate as the oxidizing agent (*55,56*), Triose reductone could react with periodate according to the following reaction sequence:

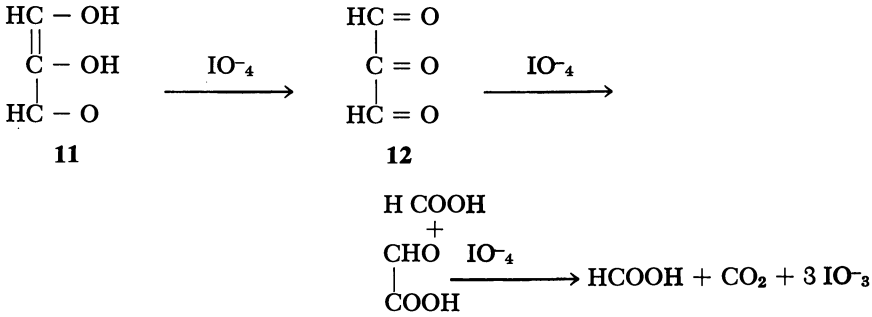


11 **12**

to give mesoxalic dialdehyde (**12**) in a reaction analogous to glycol cleavage (*6,7,8*). Triose reductone is an acid which can be titrated with a strong base (*10*). It is also an α-β unsaturated dicarbonyl compound

and absorbs strongly between 270 and 290 mμ (*15*). On the other hand, mesoxalic dialdehyde is a neutral substance and has a very low extinction coefficient. Accordingly, when one molar equivalent of periodate was added to a solution of triose reductone, a neutral solution of low absorbancy was obtained.

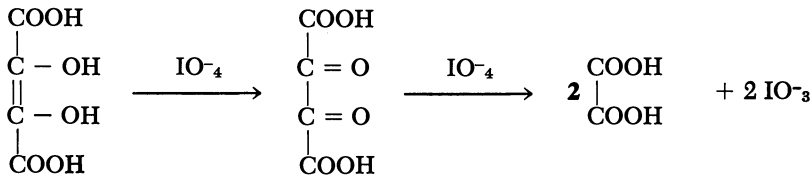
Mesoxalic dialdehyde can be reasonably expected (*16,28,50*) to undergo normal glycol cleavage and give one mole of formic acid and one mole of glyoxylic acid; in fact, when a second molar equivalent of periodate was added to the above solution, two molar equivalents of titratable acid were formed. If an excess of periodate is now added, two molar equivalents of titratable acid remain, but in addition, one molar equivalent of carbon dioxide can be expelled from the solution. Thus, in the overall reaction, one mole of triose reductone is oxidized by three moles of periodate to give two moles of formic acid and one mole of carbon dioxide:



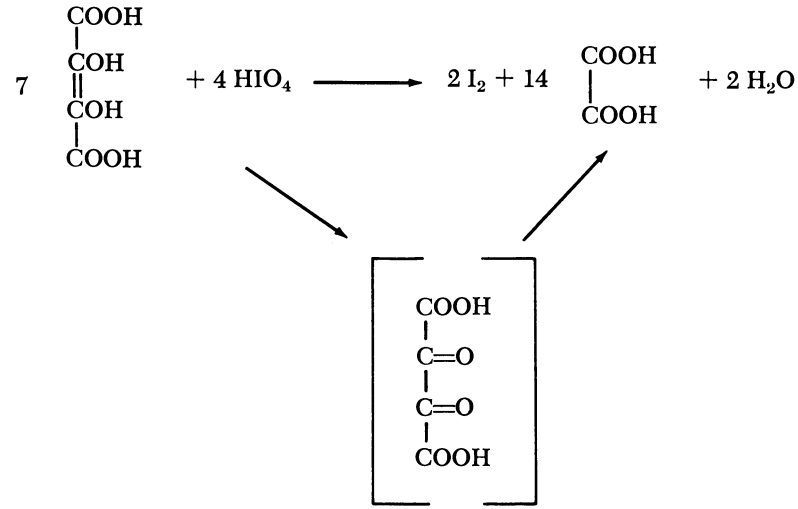
It is significant that the uptake of the third mole of periodate is appreciably slower than that of the first two moles, indicating that another type of reaction is taking place (*56*).

It must be stressed again, that the reaction sequence given here proceeds quantitatively, without formation of free iodine, only if the concentration of triose reductone is less than $10^{-3}M$ and if the pH is above 3.

This reaction sequence is a general one for ene-diols. For example, in the same conditions, dihydroxyfumaric acid is oxidized by two moles of periodate, first to a diketone and then to two moles of oxalic acid (*25,56*).



Previously (*39*), the oxidation of this compound was reported to proceed by the following sequence:

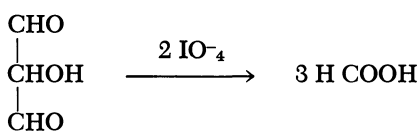


Obviously, simultaneous reduction of both periodate and iodate occurs.

Thus, if triose reductone is, in fact, the first intermediate in the periodate oxidation of malonaldehyde, the total consumption of periodate per mole of malonaldehyde should be four molar equivalents; two moles of formic acid and one mole of carbon dioxide should be formed, in accordance with the sequence proposed by Fleury and his collaborators (*22*). As in the case of the periodate oxidation of malonic acid (*32*) the rate determining step should be the "hydroxylation" step.

However, when we oxidized malonaldehyde (*56*) in the conditions just described for triose reductone, although formic acid and carbon dioxide were produced in high yields, the periodate consumption was erratic. Similar results were obtained with deoxy sugars. This discrepancy may be caused by the incomplete enolization of the first intermediate, "hydroxy malonaldehyde"—*i.e.* tartronic dialdehyde (*5,22,32*), to triose reductone, or may concern the "hydroxylation" step itself.

As to the first point, tartronic dialdehyde (**8**) could, as has already been suggested (*32*), be oxidized by classical glycol cleavage to give three molar equivalents of formic acid (and no carbon dioxide) with the concomitant reduction of two (instead of three for the enol form) molar equivalents of periodate:



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Crystalline triose reductone has been shown (*56*) by titration with strong base and with iodine, to exist in solution, for practical purposes, entirely as the enol form. In addition, the fact that it reduces exactly three molar equivalents of periodate to give quantitative yields of formic acid and of carbon dioxide indicates that it is also oxidized entirely in this form. However, nothing is known of the rate of enolization of tartronic dialdehyde and the possibility therefore remains that part of it may be oxidized in the dialdehydo form. If this were the case, the results of periodate oxidations would be dependent on the ratio of the rate of enolization of tartronic dialdehyde to the rate of its oxidation by periodate, since the oxidation of triose reductone is, again, for practical purposes, instantaneous.

It is, however, more likely that the discrepancies observed in the periodate oxidation of malonaldehyde concern mainly the hydroxylation step. In the mechanism proposed (*5*) for this reaction, it is the enol form of malonaldehyde which is hydroxylated. However, titrations of a solution of malonaldehyde, prepared by hydrolysis of an aqueous solution (*33*) of carefully distilled 1, 3, 3-tri-ethoxypropene (*46*, *47*), both with strong base and with iodine, indicate that only about 80% of the enol form is present in the equilibrium solution. On the other hand, the thiobarbituric acid test (*58*, *59*) gave consistently higher values for the malonaldehyde content of the solution. The fact that only about 80% of the enol form is present in the equilibrium solution is all the more important as it can be shown (*56*) by titration with strong base that the enolization is slow, and moreover does not seem to go to completion.

It is interesting to note that Fleury and his collaborators (*22*) found that the malonaldehyde solution used for their oxidation experiments, which was prepared by acid hydrolysis of 1, 1, 3, 3-tetra-ethoxypropane, also reacted with about 80% of the theoretical amounts of strong base and of iodine. These authors interpreted their results as being caused by some unidentified reaction of the starting material or to the decomposition of the free malonaldehyde; accordingly, they calculated the periodate consumption, acid production, and carbon dioxide evolution on the basis of the quantity of malonaldehyde determined by titration. In this way, they obtained analytical figures in fair agreement with the equation:

$$OHC - CH_2 - CHO \xrightarrow{4\ IO^-_4} 2\ HCOOH + CO_2$$

However, in view of the above observations, it seems that malonaldehyde solutions, obtained by the hydrolysis of the appropriate acetals, contain two different forms of malonaldehyde; one, which can be titrated with base and with iodine and which reacts with periodate according

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to the equation proposed by Fleury *et al.* (*22*), is certainly the enol form; the other, which is nonacidic and which does not follow the above equation, could be the dialdehydo form.

Attempts were made to estimate the amount of the second form by studying the weak absorption band of malonaldehyde solutions at 350 mμ. This band has been attributed to nonconjugated carbonyl absorption—*i.e.* to the dialdehydo form of malonaldehyde (*40*, *48*).

When 1, 3, 3-triethoxypropene was hydrolyzed with 1*N* sulfuric acid, a solution of malonaldehyde whose optical density was perfectly stable at 350 mμ for at least one week was obtained. If the solution was made alkaline, the optical density at the same wavelength increased by a small value and then remained virtually constant for at least one week (*56*). It was also observed that in these solutions the extinction coefficient at 350 mμ was very low (observed: 8.3, 61.5 and 69, for solutions of pH 0.4, 7.15 and 9.4 respectively) compared with previously reported values which varied from 200 (*40*) to 1000 (*48*). On the other hand, the absorption of solutions having a pH of 3 to 5, increased considerably with time (at pH 4.75, the extinction coefficient of malonaldehyde at 350 mμ was initially about 40; after four weeks a value of about 930 was recorded and the optical density of the solution was still increasing). This increase in absorption was accompanied by a marked decrease in the malonaldehyde content of the solution, as measured by the thiobarbituric acid method. As a corollary, it was found that aqueous solutions of malonaldehyde, prepared by autocatalyzed hydrolysis (*33*) of the same acetal and which had a pH of about 3.5, showed, at the completion of the hydrolysis, considerably higher extinction coefficient values at 350 mμ than did those malonaldehyde solutions which were prepared by hydrolysis with 1*N* acid and subsequently adjusted to pH 4. It appears, therefore, that at pH values at which most of the periodate oxidations are carried out, malonaldehyde is unstable and undergoes a chemical reaction, the nature of which is not, as yet, known.

These observations provide at least one explanation for the fact that variable results are obtained when malonaldehyde is oxidized with periodate. They also explain why widely differing values for the extinction coefficient of malonaldehyde at 350 mμ have been reported and make it unlikely that the absorption band at this wavelength is caused by the dialdehydo form of malonaldehyde.

In view of these results, periodate titrations of malonaldehyde were carried out at several pH values other than 4. However, in no instances were stoichiometric amounts of periodate reduced; the deoxy sugars gave similar results.

It has been noted previously (*9*, *18*, *19*, *32*) that the rate of oxidation of malonic acid is markedly affected by pH; it is at its maximum at pH 4

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and decreases with decreasing pH. Similarly, malonaldehyde has been shown (*53*) to be oxidized more slowly at pH 2.5 that at higher pH values. In view of these results, the oxidation of 2-deoxy-D-glucose was carried out at low pH, namely in 0.1*N* sulfuric acid solution (pH 1.5). As can be seen in Figure 1, the rate of "over-oxidation" is much slower in these conditions than in unbuffered solutions and it is possible to distinguish the reaction of glycol cleavage from that of "over-oxidation." Similar results were obtained with other deoxy sugars, such as 2-deoxy-D-ribose and 3-deoxy-D-galactitol (3-deoxy-D-*xylo*-hexitol), which undergo rapid glycol cleavage. However, malonaldehyde is still oxidized to a marked extent, so that in less favorable cases, that is when the glycol cleavage is slow, as for example in 3-deoxy-D-glucose, the breakage point between glycol cleavage and "over-oxidation" reactions is not well defined (*54*).

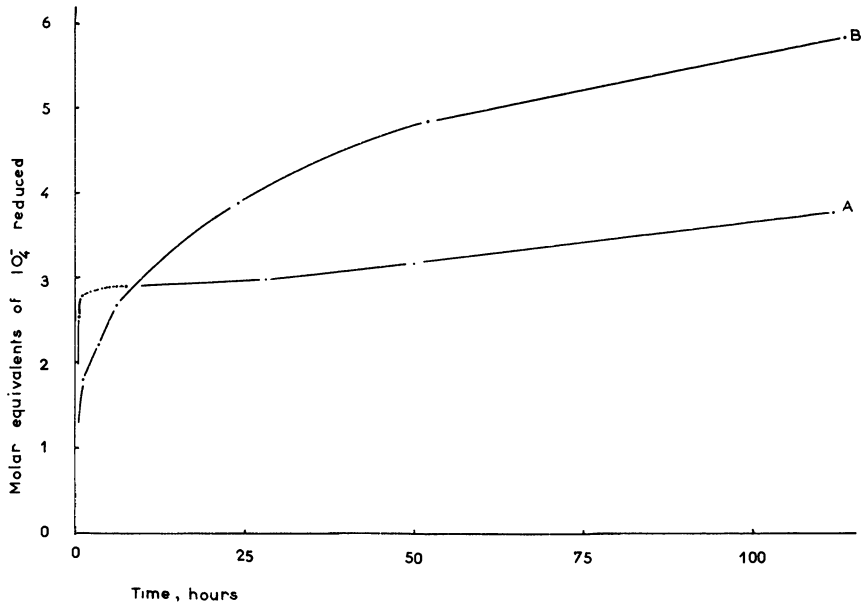

Figure 1. Oxidation of 2-deoxy-D-glucose (6 • 10⁻⁴M) with sodium metaperiodate (6.6 • 10⁻³M) in 0.1N H_2SO_4 (curve A) and in unbuffered solution (curve B) at room temperature.

As it is known (*9*, *20*, *21*, *34*, *35*, *45*) that the temperature coefficient of "over-oxidation" reactions is quite large, and greater than that of glycol cleavage (*9*), similar oxidations were carried out at +4°C. In these conditions, deoxy sugars undergo only normal glycol cleavage, and no "over-oxidation" occurs: the malonaldehyde formed is not oxidized and

is stable for at least 200 hours. It is possible to estimate all deoxy sugars, deoxy sugar alcohols, and cyclohexanepentols (*see* below) which yield malonaldehyde upon periodate oxidation (Table I), including those which react slowly with periodate as, for example, do 3-deoxy and 3, 6-di-deoxy hexoses.

Table I. Oxidation of Deoxy Sugars and Related Compounds ($6 \cdot 10^{-4}$M) in 0.1N H_2SO_4 at +4°C. with Sodium Metaperiodate ($6.6 \cdot 10^{-3}$M).

Substrate	Moles IO^-_4 reduced per mole of substrate		Moles of malonaldehyde formed per mole of substrate	Approx. Time for complete glycol cleavage
	Calc.	Found	Calc. 1	
2-Deoxy-D-*erythro*-pentose (2-Deoxy-D-ribose)[a]	2	1.9	0.91	6
2-Deoxy-D-*arabino*-hexose (2-Deoxy-D-glucose)[a]	3	2.9	0.96	20
3-Deoxy-D-*ribo*-hexose (3-Deoxy-D-glucose)[a]	3	3.1	0.99	75
3-Deoxy-D-*arabino*-hexose (3-Deoxy-D-mannose)[a]	3	3.0	1.0	85
2,6-Di-deoxy-D-*ribo*-hexose (Digitoxose)[a]	2	2.0	1.0	2
3-Deoxy-D-*erythro*-pentitol (3-Deoxy-D-ribitol)[a]	2	2.1	0.97	6
3-Deoxy-D-*xylo*-hexitol (3-Deoxy-D-galactitol)[a]	3	2.9	1.0	20
3,6-Di-deoxy-D-*ribo*-hexitol [a]	2	2.0	0.90	50
1,3,5/2,4-Cyclohexanepentol (*scyllo*-Quercitol)	4	3.94	0.95	130
(1 L)-1,2,4/3,5-Cyclohexanepentol [(−)-*vibo*-Quercitol]	4	3.94	0.99	90
DL-1,2,3,4/5-Cyclohexanepentol (DL-*allo*-Quercitol)	4	4.0	—	75
(+)-Quercitol [(+)-*proto*-Quercitol]	4	3.94	0.94	35
(1 D)-1,2,5/3,4-Cyclohexanepentol [(−)-*gala*-Quercitol]	4	4.0	0.95	35

[a] Reference (*56*).

The malonaldehyde thus formed can be estimated quantitatively by the thiobarbituric acid method (*58*, *59*). As a control of the method's reliability, we used, as primary standard, 1, 3, 3-tri-ethoxypropene (*46*, *47*) purified by gas-liquid chromatography (*56*) and hydrolyzed to malonaldehyde at room temperature with 1*N* sulfuric acid. The molar

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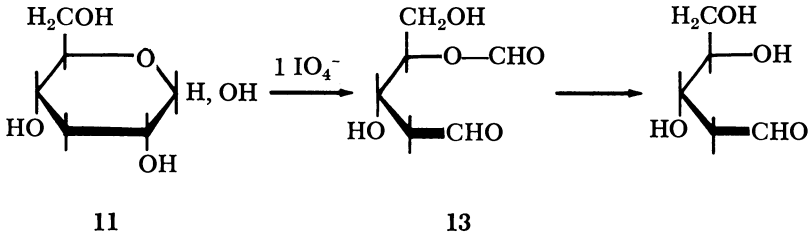
extinction coefficient for the dye formed from this malonaldehyde solution and thiobarbituric acid was found to be 1.45×10^5 at 352 mμ; the molar extinction coefficient obtained from the purest samples of deoxy sugars was found to be 1.53×10^5. The latter value was used to calculate the yields of malonaldehyde in all experiments. Kuhn and Lutz (*36*) have reported molar extinction coefficients of 1.35×10^5 at 352 mμ in water and 1.53×10^5 at 534 mμ in cyclohexanone for the isolated pigment. It has been shown (*58*, *59*) that the absorption of the dye is maximum at acid pH values and decreases above pH 2. This observation would explain the fact that the extinction coefficient of the dye in aqueous solution is lower than the value given above.

The advantages of the method indicated are that the number of vicinal diol groups present in deoxy sugars can be determined; one mole of malonaldehyde is formed per mole of deoxy sugar, even if the glycol cleavage is slow, and the absolute quantity of malonaldehyde formed can be calculated (as has been done for the values given in Table I) directly from the optical density observed, using the known molar extinction coefficient of the malonaldehyde-thiobarbituric acid dye.

The method can also be used for the determination of deoxy sugars in complex biological material. Thus abequose (3, 6-di-deoxy-D-*xylo*-hexose) has been estimated quantitatively in a heteropolysaccharide isolated from cell wall lipopolysaccharides of *Salmonella typhimurium*. As the heteropolysaccharide also contains 3-deoxy octulosonic acid (KDO) whose carbonyl group is free (*42*) and which is therefore oxidized by periodate to give β-formyl pyruvic acid (*37*, *38*), a compound known to react with thiobarbituric acid to form a dye absorbing at 545-550 mμ (*61*), the unhydrolyzed material was first treated with borohydride until the periodate-thiobarbituric acid test (*42*, *61*) was negative, then hydrolyzed to set free the glycosidically bound (*52*), and hence initially periodate resistant, di-deoxy sugar, and finally oxidized with periodate; the malonaldehyde formed was estimated as above.

An interesting observation resulted from this work. Professor Angyal (*3*) has presented NMR spectral data indicating that, while in aqueous solution 2-deoxy D-glucose is entirely in the pyranose form, solutions of 3-deoxy D-glucose contain about 30% of the furanose form. When 3-deoxy D-glucose (Figure 2) is oxidized in the cold, it rapidly reduces 1.4 molar equivalents of periodate. The time curve shows a neat break at this point; the periodate uptake then continues more slowly. If one considers the reactions of both the pyranose and the furanose forms of this sugar, it will be seen that the pyranose form (**11**) should consume one molar equivalent of periodate rapidly (by oxidation between carbons 1 and 2) and then the rate of periodate uptake would be slowed down by the relatively lower rate of hydrolysis of the formyl ester (**13**). On

the other hand, the furanose form (**12**) should be oxidized rapidly by two moles of periodate (between carbons 1 and 2, and 5 and 6) and



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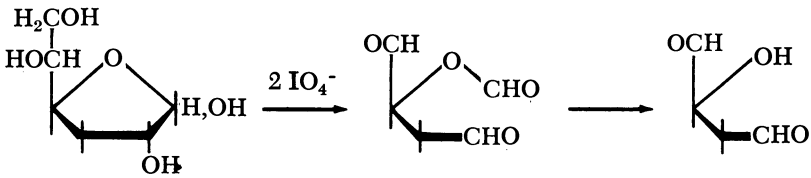

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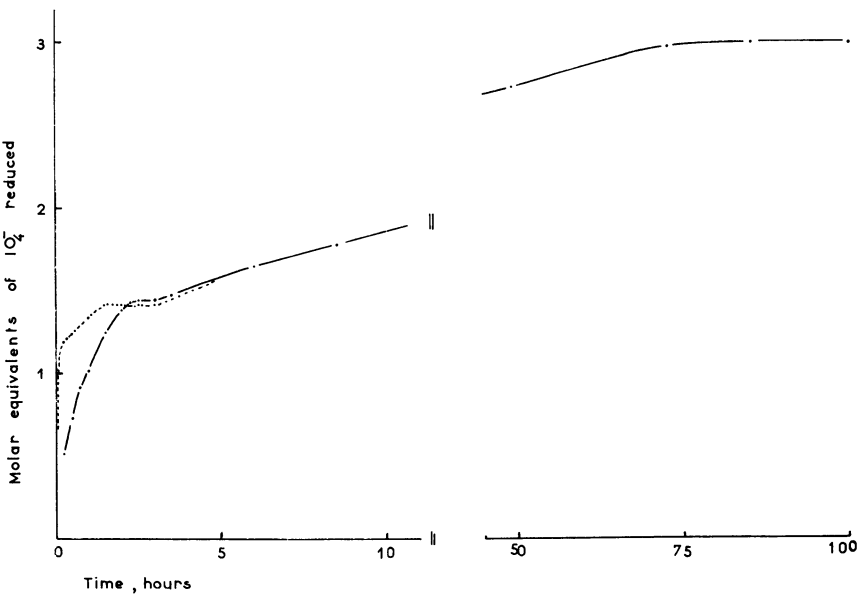

Figure 2. Oxidation of 3-*deoxy*-D-*glucose* (——•——) *and of* 3-*deoxy*-D-*mannose* (- - - • - - -) *with sodium metaperiodate* (c.f. *Table 1).*

then the reduction of the third mole would be controlled by the hydrolysis rate of the formyl ester (**14**). Thus the break seen in the time curve when 1.4 molar equivalents of periodate have been consumed, seems to indicate that, in this medium, about 40% of the sugar is in the furanose form. It is interesting to note that 3-deoxy D-mannose (3-deoxy-D-*arabino*-hexose) gives a similar titration curve, the only difference being that the initial reduction of periodate is more rapid.

Unfortunately, whereas the method was so successful for the estimation of deoxy and di-deoxy sugars, it does not appear to be applicable to deoxy sugar phosphates. For example, 2-deoxy-D-ribose 5-phosphate, which should reduce one molar equivalent of periodate, is oxidized very slowly and irregularly and, even after 120 hours, only about 0.2 molar equivalents of periodate are reduced and approximately the same amount of malonaldehyde is formed. Curiously enough, 2-deoxy-D-xylose 5-phosphate reduces more periodate (about 0.6 molar equivalents after 120 hours and forms more malonaldehyde than does 2-deoxy ribose 5-phosphate; but even so, the reaction does not go to completion. Similarly, 3-deoxy D-glucose 6-phosphate is oxidized by about one molar equivalent of periodate, presumably to a formyl ester of 2-deoxy ribose 5-phosphate, and thereafter the oxidation is very slow and irregular and similar to that of 2-deoxy ribose 5-phosphate itself. Since the oxidation of the vicinal diol groups in glucose 6-phosphate proceeds normally and quantitatively (*54*), it would appear that the presence of a phosphate group in the terminal position of a deoxy sugar, or at least of a 2-deoxy pentose, has a marked influence on its behavior towards periodate in acid medium. This phenomenon is being further investigated. In unbuffered medium, the phosphates behave similarly to the nonphosphorylated deoxy sugars: they undergo typical "over-oxidation" reactions and yield products in comparable amounts.

On the other hand, the method gives excellent results when applied to the cyclohexanepentols (mono-deoxy inositols). The reaction of this class of compound with periodate has not been extensively studied. To our knowledge, the only cyclohexanepentol whose overall oxidation has been examined is (+)-quercitol: Fleury *et al.* (*23*) found that approximately eight molar equivalents of periodate were reduced, with the formation of approximately five molar equivalents of formic acid and of one molar equivalent of carbon dioxide. Malonaldehyde was shown to be an intermediate in the reaction. Cleavage of the vicinal diol groups of (+)-quercitol would require four molar equivalents of periodate; formally, this cleavage would yield three molar equivalents of formic acid and one molar equivalent of malonaldehyde. The latter, if oxidized according to the sequence previously proposed by Fleury *et al.* (*22*), would reduce a further four molar equivalents of periodate; two molar

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equivalents of formic acid and one molar equivalent of carbon dioxide would be formed. However, as in the case of the oxidation of malonaldehyde itself, the analytical results were not entirely satisfactory. It is thus obvious that in the conditions usually employed, cyclohexanepentols undergo "over-oxidation" reactions similar in nature to those of the deoxy sugars. They also behave similarly to the deoxy sugars in the "cold acid" method described above. All of the cyclohexanepentols tested reduced four molar equivalents of periodate and yielded one molar equivalent of malonaldehyde (*54*) (cf. Table I).

It is generally admitted that the first step in the oxidation of cyclitols and of cyclohexanepentols is the ring opening (*2*) and that the rate of this step is dependent on the *cis*-or *trans*-relationships of the hydroxyl groups. As in the case of the sugars, vicinal *cis*-hydroxyl groups are oxidized, as a general rule, more rapidly than vicinal *trans*-hydroxyl groups. The ring opening leads to the formation of a hexodialdose which could exist in the straight chain or in a cycle form and it has been stated (*2*, *44*) that further oxidation is then independent of the configuration of the original compound. [Periodate oxidations of cyclitols have been discussed in detail by Posternak (*44*)].

Angyal and McHugh (*2*) have compared the initial rates of periodate oxidation of several cyclohexanepentols. From the results obtained, it appears that a cyclohexanepentol having a single pair of vicinal *cis*-hydroxyl groups distant from the deoxy group [*e.g.* (+)-quercitol] reacts more rapidly with periodate than does a cyclohexanepentol having a single pair of vicinal *cis*-hydroxyl groups adjacent to the deoxy group [*e.g.*, (1 L)-1,2,4/3, 5-cyclohexanepentol]. This relationship holds true when the same two cyclohexanepentols are oxidized with periodate in cold acid (Figure 3).

In an attempt to elucidate the reaction sequence by which the cyclohexanepentols are oxidized, we recorded simultaneously the time curves of periodate reduction and of malonaldehyde production during the oxidations. By this procedure, it is, in fact, possible to propose a reaction sequence for (1 D)-1, 2, 5/3, 4-cyclohexanepentol.

(1 D)-1, 2, 5/3, 4-Cyclohexanepentol (**15**) rapidly reduces somewhat more than three molar equivalents of periodate (about 3.3) and at the same time about 0.3 molar equivalents of malonaldehyde are formed (Figure 4). Both time curves show a break at this point. A slower reduction of periodate then takes place and at the same time, the remainder of the malonaldehyde is set free. In view of the results obtained with (+)-quercitol and (1 L)-1, 2, 4/3, 5-cyclohexanepentol, the main attack of periodate will probably take place on the pair of vicinal *cis*-hydroxyl groups distant from the deoxy group (the hydroxyl groups on carbons 3 and 4). In this case, ring opening would lead to the formation of the

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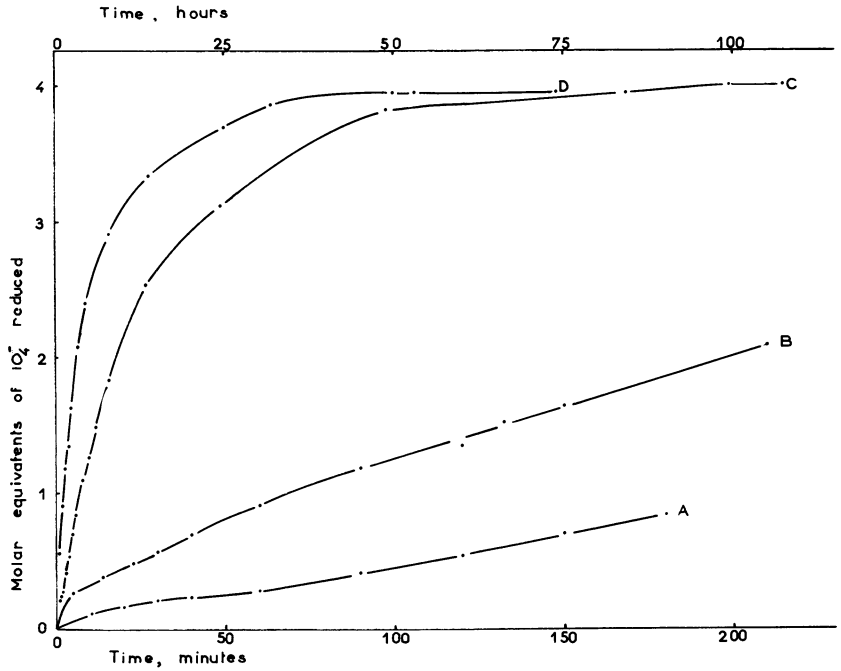
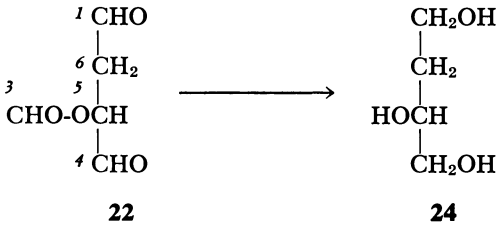
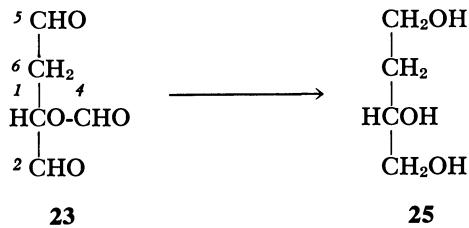


Figure 3. Oxidation of (1 L)-1,2,4/3,5-cyclohexanepentol (curve A: minutes; Curve C: hours) and of (+)-quercitol (curve B: minutes; curve D: hours) with sodium metaperiodate (c.f. *Table I).*

hexodialdose (**16**). Cyclization of this dialdehyde could yield two pyranose (**17**) and (**18**) and one furanose (**19**) structure. The first pyranose (**17**) would be cleaved rapidly between carbons 4 and 5 by a second molar equivalent of periodate to give a formyl ester (**20**) who hydrolysis would be the rate limiting step for the uptake of the third and fourth molar equivalents of periodate and for the liberation of malonaldehyde. Since, experimentally, three molar equivalents of periodate are reduced rapidly, there is little likelihood that this pyranose structure is involved in the periodate oxidation of (1 D)-1, 2, 5/3, 4-cyclohexanepentol. The second pyranose (**18**) would be cleaved rapidly between carbons 3 and 2 and the dialdehyde (**21**) thus formed would reduce rapidly the third molar equivalent of periodate to yield the formyl ester (**22**). The hydrolysis of this ester would be the rate limiting step for the uptake of the fourth molar equivalent of periodate and for the coincident formation of malonaldehyde. This sequence would, therefore, be in agreement with the experimental data. So would be the reaction sequence starting with the furanose (**19**). This intermediate would be cleaved rapidly (between carbons 4 and 5 and carbons 2 and 3) by the second and third

molar equivalents of periodate to yield the formyl ester (**23**), which would then behave similarly to the formyl ester (**22**). Therefore, on the basis of the data available, it is not possible to distinguish between these two pathways. It should, in principle, be possible to determine which reaction sequence is actually involved, by stopping the oxidation reaction at the stage at which three molar equivalents of periodate have been consumed, reducing the intermediate dialdehyde with, say, sodium borohydride, and determining the optical rotation of the 2-deoxy tetritol formed after removal of the formyl group. If the pyranose (**18**) is the cyclic compound involved in the oxidation sequence, 2-deoxy-L-*glycero*-tetritol (**24**) should be formed. The furanose (**19**) should, on the other hand, yield 2-deoxy-D-*glycero*-tetritol (**25**).





The initial rapid uptake of more than three molar equivalents of periodate (3.3 molar equivalents) and the coincident production of 0.3 molar equivalents of malonaldehyde could result from simultaneous attack of two moles of periodate on hydroxyl groups 1, 2 and 3, 4 of the cyclohexanepentol, which would give one two-carbon fragment and one four-carbon fragment, both of which would then be rapidly oxidized; it could also be attributed to the direct oxidation of a small proportion of the straight chain dialdehyde (**16**).

In the cases of the slowly reacting cyclohexanepentols, more complicated results are obtained. This is probably because of the fact that, as has been pointed out by Angyal and McHugh (2), the rate of oxidation of the product of ring fission is likely to be more rapid than the rate of fission itself.

Thus, as far as (+)-quercitol is concerned (Figure 5), the time curves show that a small quantity of malonaldehyde is liberated at the beginning

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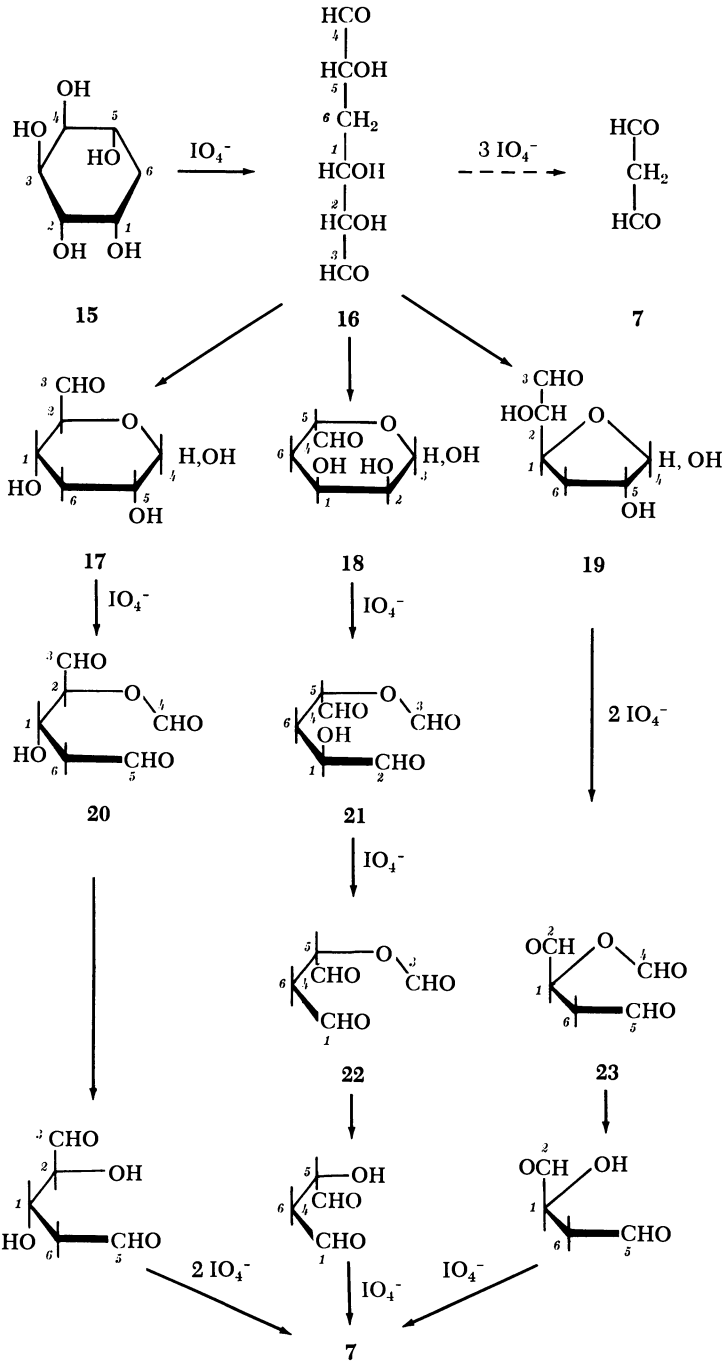


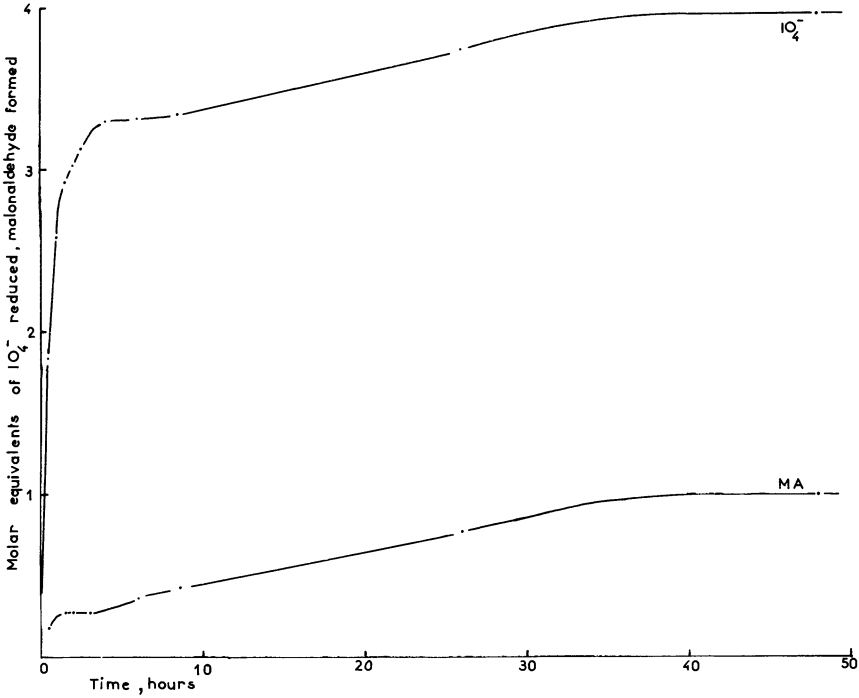
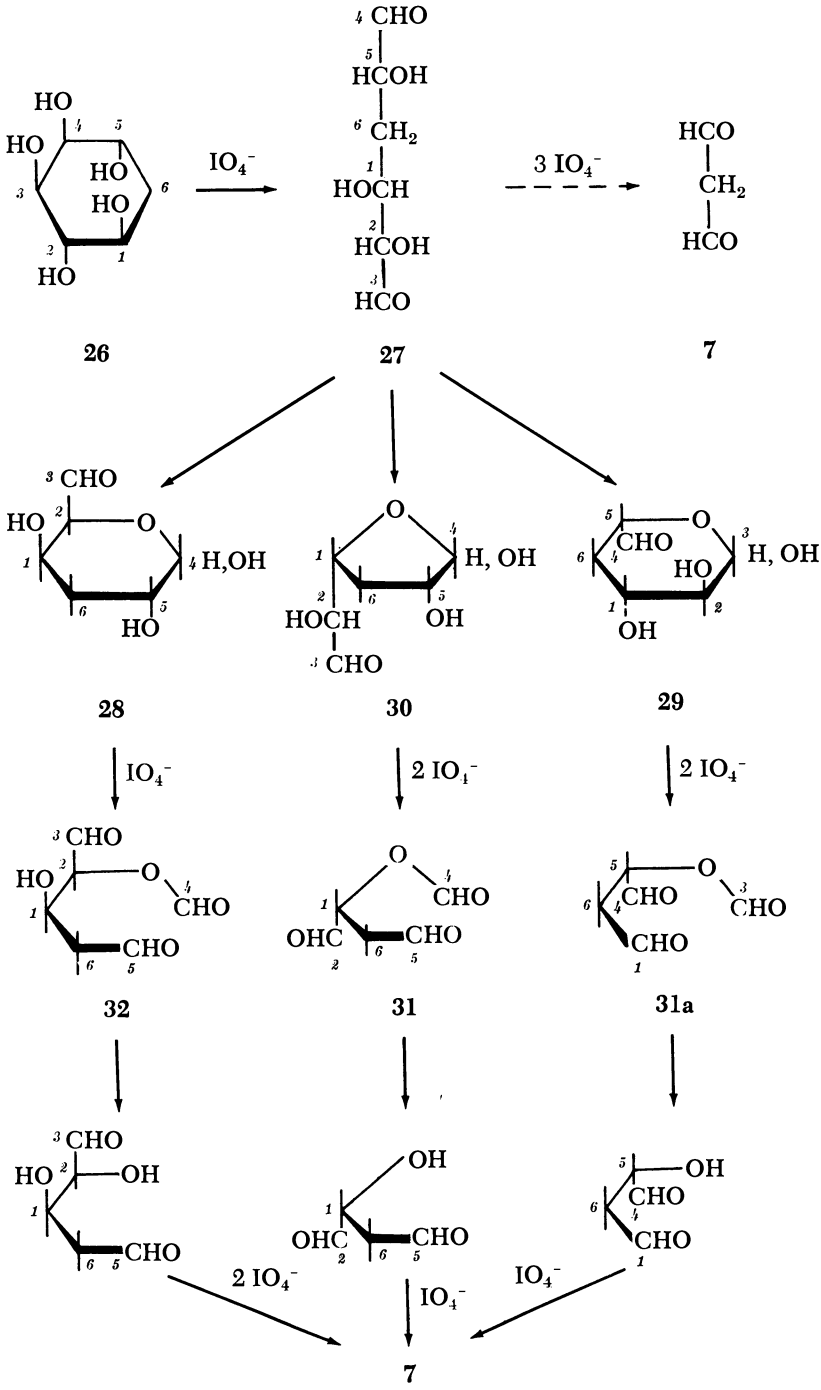


Figure 4. Periodate uptake (10_4^-) and malonaldehyde formation (MA) during the oxidation of (1 D)-1,2,5/3,4-cyclohexanepentol with sodium metaperiodate (c.f. *Table I*).

of the oxidation, as in the case of (1 D)-1, 2, 5/3, 4-cyclohexanepentol, and that the major part of the malonaldehyde is liberated during the reduction of both the third and fourth molar equivalents of periodate. As (+)-quercitol (**26**) possesses only one pair of *cis*-vicinal hydroxyl groups, the major attack of periodate will probably occur at this point. Ring scission would thus yield the hexodialdose (**27**) which could then cyclize to give two pyranoid (**28** and **29**) and one furanoid (**30**) structures. The furanose (**30**) and the pyranose (**29**) would rapidly reduce a second and a third molar equivalent of periodate to give similar formyl esters (**31** and **31a**), and the rate of hydrolysis of these esters would control both the uptake of the fourth molar equivalent of periodate and the formation of malonaldehyde. Although these reaction sequences cannot be completely ruled out, the sequence involving the pyranose structure (**28**) would be more in accord with the experimental results. This structure would be oxidized rapidly by the second molar equivalent of periodate to give the formyl ester (**32**), whose hydrolysis would control both the uptake of the third and fourth molar equivalents of periodate



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as well as the production of malonaldehyde. As in the case of (1 D)-1, 2, 5/3, 4-cyclohexanepentol, the initial formation of a small amount of malonaldehyde could be caused by either simultaneous attack of more than one mole of periodate on the cyclohexanepentol, or by the direct oxidation of a small proportion of the straight chain form of the hexodialdose (**27**) formed by ring cleavage.

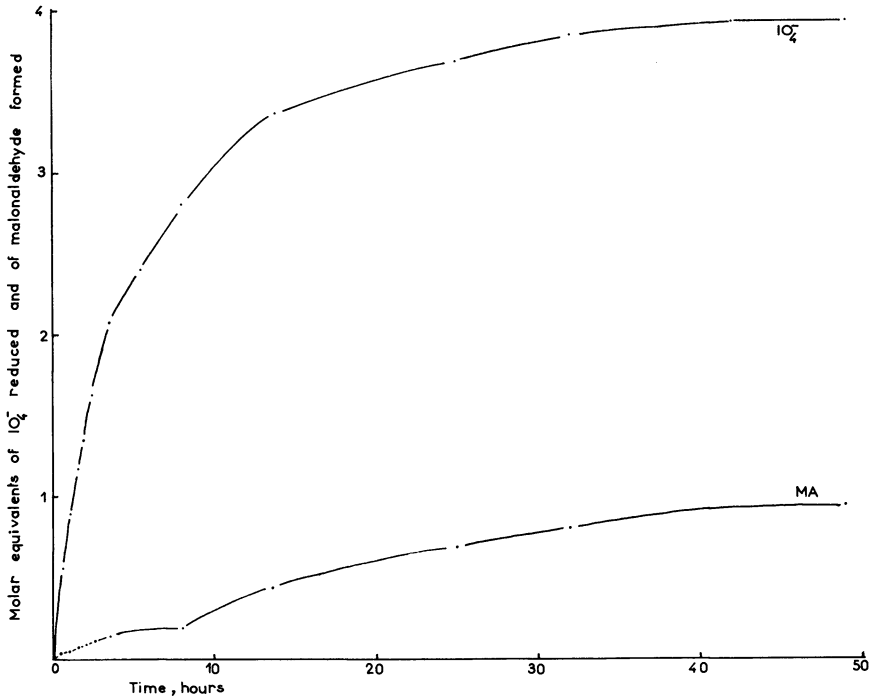

Figure 5. Periodate uptake (10_4^-) and malonaldehyde formation (MA) during the oxidation of (+)-quercitol with sodium metaperiodate (c.f. *Table I).*

No hypotheses can be advanced for the sequences involved in the oxidation of cyclohexanepentols which react even more slowly with periodate than does (+)-quercitol. For instance, when the all-trans 1 3, 5/2, 4-cyclohexanepentol (**33**) is oxidized (Figure 6), production of malonaldehyde starts very early and the time curve of periodate reduction indicates a very complex reaction. Nor is it possible to analyze satisfactorily the curves obtained with (1 L)-1, 2, 4/3, 5-cyclohexanepentol (**34**) or with DL-1, 2, 3, 4/5-cyclohexanepentol (**35**) [this is the configuration predicted for the (1 D)-entantiomorph (*41*)].

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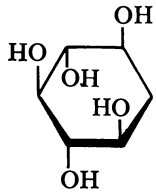

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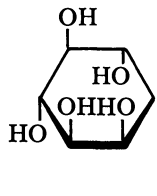

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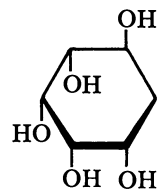

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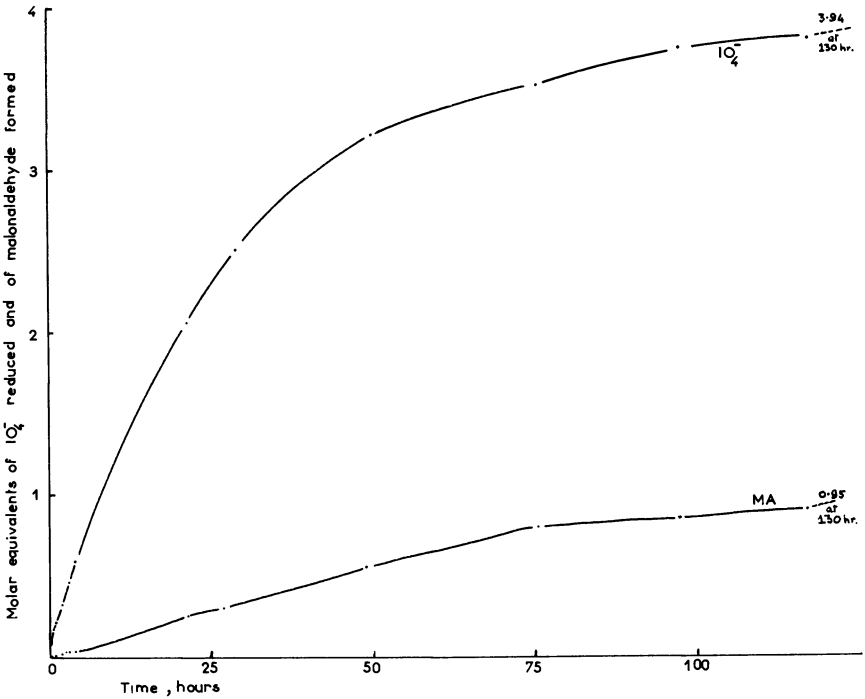

Figure 6. Periodate uptake (10_4^-) and malonaldehyde formation (MA) during the oxidation of 1,3,5/2,4-cyclohexanepentol with sodium metaperiodate (c.f. *Table I).*

It is, in fact, interesting to note that the latter cyclohexanepentol, which has four adjacent *cis*-hydroxyl groups, reacts slowly with periodate. Perhaps further light will be thrown on this problem when the results of periodate oxidations of DL-1, 2, 3, 5/4-cyclohexanepentol (**36**), 1, 2, 4, 5/3-cyclohexanepentol (**37**), and (1 D)-1, 2, 3/4, 5-cyclohexanepentol (**38**) will be available.

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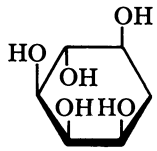

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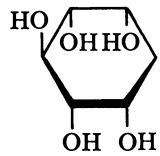

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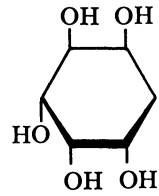

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(1 L)-enantiomorph

Acknowledgments

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7

Synthesis and Reactions of Unsaturated Sugars: 6-Deoxyhex-5-enose and 5-Deoxypent-4-enose Derivatives

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*Various 6-deoxy-hex-5-enose and 5-deoxy-pent-4-enose derivatives, which are vinyl ethers, have been prepared by the elimination of hydrogen halide from 6-deoxy-6-iodo-hexopyranoid, 6-deoxy-6-iodo-hexulofuranoid, and 5-deoxy-5-iodo-pentofuranoid systems by treatment with silver fluoride in pyridine. In the hexulofuranoid and pentofuranoid systems, unless all the hydroxy groups are substituted, oxetans can be formed under certain circumstances in preference to the vinyl ethers. The chemistry and biological significance of these unsaturated sugars is reviewed. (The chemistry of the pyranoid derivatives has formed part of a recent review (6), hence only new developments are discussed herein.) Catalytic hydrogenations of the vinyl ethers are stereoselective. Thus, with palladium catalyst, hydrogenation of 6-deoxy-2,3-O-isopropylidene-β-*D-threo-*hexulo-5-enofuranose occurs from the least hindered side of the molecule to give 6-deoxy-2,3-O-isopropylidene-β-*D-arabino-*hexulofuranose only.*

The potential importance of unsaturated carbohydrates as synthetic and biological intermediates has stimulated considerable interest. Added interest has resulted from the detection of these derivatives in the naturally occurring nucleosides cytosinine (**1**), a component of the antibiotic blasticidin S, (*7*, *45*) and angustmycin A (decoyinine, **2**) (*15*). The exocyclic, vinyl ethers of pyranoid derivatives, analogous to the methyl pyranoside (**3**) first described in 1928 by Helferich and Himmen

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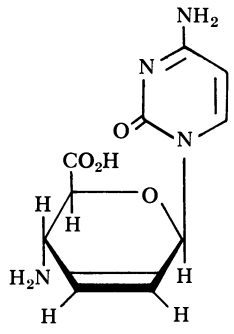

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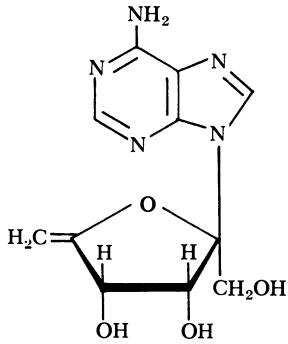

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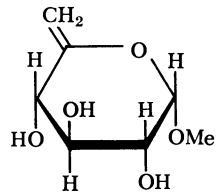

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(*11*), are now thought to be intermediates in the biosynthesis of 6-deoxy-L-hexoses. Since these vinyl ether derivatives lack asymmetry at C-5, they are potential intermediates in the chemical conversion of readily available D-sugars into less common L-sugars. Furthermore, hydrolysis affords, by subsequent rearrangement, the appropriate deoxy dicarbonyl sugar. The usual method for the synthesis of these vinyl ethers involves treatment of a suitably protected 6-bromo-6-deoxy- or 6-deoxy-6-iodo-D-hexopyranose with anhydrous silver fluoride in pyridine (*11*). Alternatively, compounds bearing alkali-stable protecting groups may be dehydrohalogenated by sodium methoxide in methanol (*8*, *25*). Although the silver fluoride reaction has been known for many years, extension to the pentofuranoid and hexulofuranoid series was reported only recently (*18*). Previously, the reaction had been applied successfully only to derivatives of aldohexoses and hexitols where C-5 forms part of a pyranose (*6*) or 3,5-*O*-isopropylidene ring (*14*). Thus compounds such as 3,5-di-*O*-acetyl-6-deoxy-6-iodo-1,2-*O*-isopropylidene-α-D-glucofuranose (*13*), and tetra-*O*-benzoyl-1,6-dideoxy-1,6-diiodo-D-mannitol (*34*) do not afford unsaturated products when treated with silver fluoride. A variety of pyranoid and furanoid vinyl ethers have now been prepared and some aspects of their chemistry, with particular reference to the synthesis of deoxy sugars, forms the subject of this paper.

Nucleotide derivatives of 6-deoxy-α-D-*xylo*-hex-5-eno-pyranose (**4**) are plausible intermediates in several biosynthetic reactions. For example, Blumsom and Baddiley (*2*) postulated that the thymidine diphosphate (TDP) derivative (**5**) was an intermediate in the biosynthesis of TDP-β-L-rhamnose (**10**) from TDP-α-D-glucose in *Streptomyces griseus*, the vinyl ether (**5**) being formed by dehydration of TDP-α-D-glucose and then rearranged *via* the enol (**6**) to TDP-6-deoxy-β-L-*arabino*-hexopyranosid-4-ulose (**7**). The latter (**7**) was thought to rearrange further *via*

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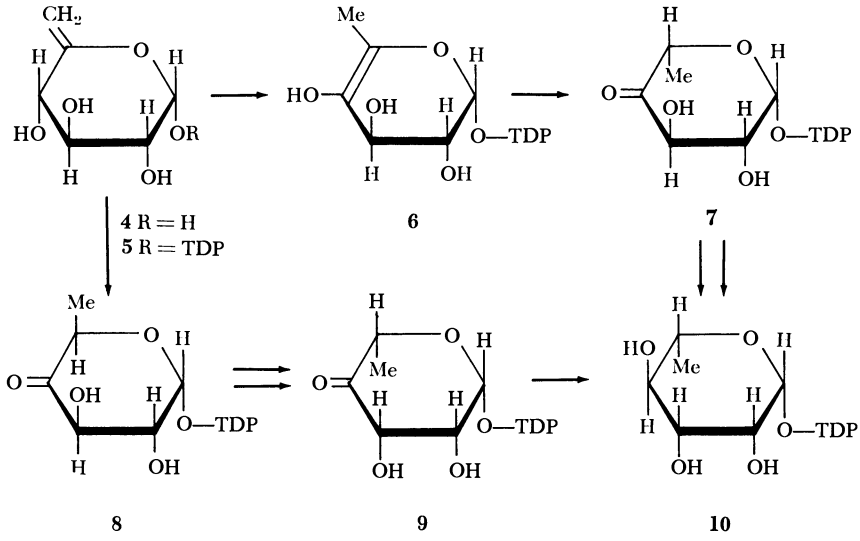
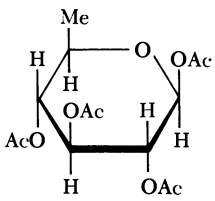
an enediol intermediate to TDP-6-deoxy-β-L-*arabino*-hexopyranosid-3-ulose and then undergo stereospecific reduction to TDP-β-L-rhamnose



(**10**). In a similar scheme, Okazaki and co-workers (35) proposed that the vinyl ether (**5**) rearranged to TDP-6-deoxy-α-D-*xylo* hexopyranosid-4-ulose (**8**) and this is supported by the isolation of the latter (**8**) from a mutant strain of *Escherichia coli*. Incubation of this TDP-derivative (**8**) with extracts of another strain of *E. coli* in the presence of NADPH afforded TDP-β-L rhamnose (**10**) and it was suggested that the conversion proceeded in several steps *via* the L-*lyxo* intermediate (**9**). Enzymic conversion of the guanosine diphosphate (GDP) derivative of α-D-mannose into GDP-β-L-fucose occurs *via* the intermediate, GDP-6-deoxy-α-D-*lyxo*-hexopyranosid-4-ulose (*9*), which probably arises from a 5-ene by rearrangement. However, unsaturated compounds of this type (**5**) have not so far been detected in natural systems. If formed, they would probably occupy an unfavorable equilibrium position with respect to the 6-deoxy-4-keto compounds and would, therefore, be present in very small amounts. No example of the conversion of exocyclic vinyl ethers such as **3** to 6-deoxy-4-keto sugars by chemical means has been reported. The 4-keto-pyranoside (**7**) is of further interest as an intermediate in the biosynthesis of 5-deoxy-3-*C*-formyl-L-lyxose, (streptose). The formyl group at C–3 of streptose is known to be derived from C–3 of D-glucose (*3*). Baddiley and co-workers (*2*, *3*, *4*) have suggested that TDP-streptose is formed by a concerted enzymic process involving cleavage of the C–2, C–3 bond of the 4-keto-derivative (**7**) and attack of C–2 on C–4. Since the configuration at C–3 of the intermediate is not important,

presumably the L-*lyxo* derivative (**9**) could also be considered as a precursor of streptose.

Hydrogenation of exocyclic, pyranoid vinyl ethers could afford a mixture of both possible 6-deoxy-D and L-hexoses. Our observations show that the proportion of each isomer is dependent upon the catalyst and the substituents on the vinyl ether. Thus, treatment of a methanol solution of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-β-D-xylo-hex-5-eno-pyranose (**12**) with hydrogen in the presence of a palladium catalyst afforded a mixture which was shown by gas chromatography to contain 96% of the 6-deoxy-D-*gluco* isomer (**11**) and 4% of the 6-deoxy-L-*ido* isomer (**13**). In this



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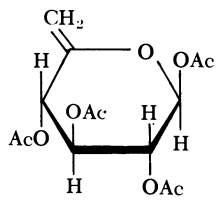

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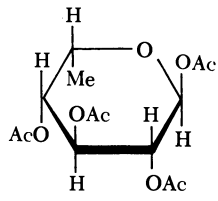

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case reduction occurs predominantly by axial attack at C–5. However, when the reaction was catalyzed by platinum oxide, the D-*gluco* and L-*ido* isomers were formed in the ratio of 70:30. In ether solution very similar results were obtained and the solvent used does not appear to affect the course of the reduction. On a preparative scale, using platinum oxide catalyst, the D-*gluco* and L-*ido* isomers were isolated as their tetra-acetates in 56% and 20% yield respectively.

A mixture of D- and L- hexoses also results from the hydroboration of these 5-enes. Hydroboration results in anti-Markownikoff, *cis*-hydration of the double bond and the amount of each hexose formed varies according to the nature of the substituent groups. For example, hydroboration (23) of methyl 6-deoxy-α-D-*xylo*-hex-5-enopyranose (**3**) affords methyl α-D-glucopyranoside and methyl β-L-idopyranoside in the ratio of 1:2.5 respectively whereas hydroboration of the *tris*-trimethylsilyl ether of **3** afforded them in the ratio 1:0.6 respectively. The hydroboration method can be used to achieve specific labelling of hexoses with tritium; methyl-β-L-idopyranoside[5–H^3] and methyl α-D-glucopyranoside [5–H^3] were thus prepared (23). Similarly, hydroboration of the D-*lyxo*-hex-5-eno derivative (**14**) with diborane-H^3 followed by removal of the isopropylidene group, afforded methyl α-D-mannopyranoside [5–H^3] and methyl β-L-gulopyranoside [5–H^3] in the ratio of 1:2 respectively (23).

Derivatives of 6-deoxy-hex-5-enopyranosides are important intermediates in the synthesis of dicarbonyl sugars, namely hexos-5-uloses. For

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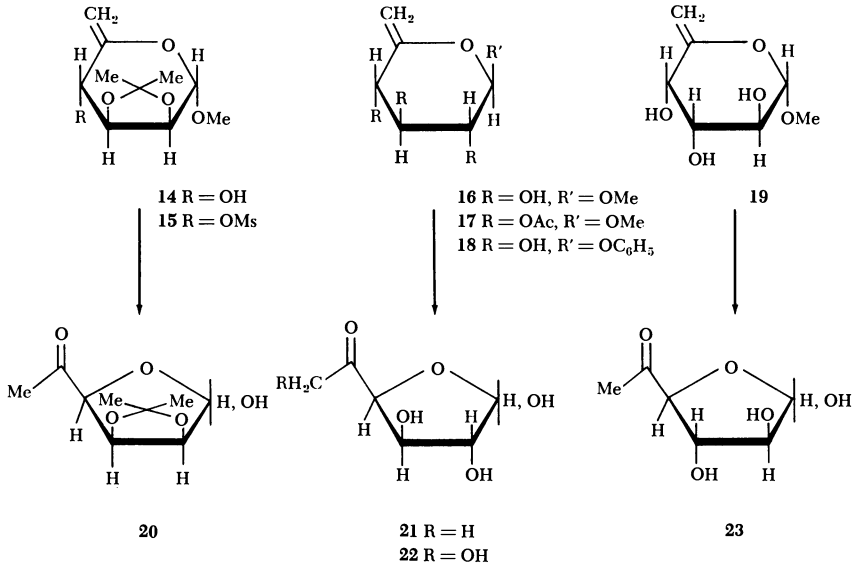

example, the D-*xylo*-hex-5-enoside (**16**) is acid labile and undergoes hydrolysis and subsequent rearrangement (*12*) to the isomeric 6-deoxy-D-*xylo*-hexos-5-ulose (**21**). The D-*lyxo*-hex-5-enoside (**14**) is particularly labile and and readily rearranges to 6-deoxy-2,3-*O*-isopropylidene-D-*lyxo*-hexofuranos-5-ulose (**20**) (25). Reduction of **20** with sodium borohydride and removal of the isopropylidene group afforded 6-deoxy-L-gulitol (1-deoxy-D-glucitol) in 73% yield (*25*). Only a trace of the isomeric 1-deoxy-D-mannitol was formed. Mild hydrolysis of the D-*arabino*-hex-5-enoside (**19**) afforded (*41*) 6-deoxy-D-*arabino*-hexafuranos-5-ulose (**23**), previously shown to be the carbohydrate moiety of the antibiotic Hygromycin A (*31*). The structure of **23** was confirmed by the NMR spectrum of its 1,2-*O*-isopropylidene derivative and by the conversion of the latter compound to 6-deoxy-L-galactose diethyl dithioacetal. Acetoxylation of the D-*xylo*-hex-5-enoside (**17**) with lead tetraacetate afforded a crystalline 2,3,4,5,6-penta acetyl derivative which was hydrolyzed to D-xylo-hexos-5-ulose (**22**) (*10*). Evidence of the ring structures of the hexos-5-uloses (**20–23**) is lacking but these compounds probably exist in the furanose form.

It was of interest to determine whether glycosides of 6-deoxy-D-*xylo*-hex-5-enopyranose were susceptible to enzyme hydrolysis by β-glucosidase. Since aromatic glucosides are hydrolyzed by this enzyme at a much faster rate than aliphatic glycosides, phenyl 6-deoxy-β-D-*xylo*-hex-5-enopyranoside (**18**) was prepared (*20*). Phenyl β-D-glucopyranoside was converted to the 6-tosylate by selective esterification and then, by conventional procedures, transformed to phenyl 2,3,4-tri-*O*-acetyl-6-deoxy-

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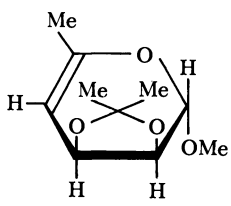
6-iodo-β-D-glucopyranoside. Treatment of this 6-iodo derivative with silver fluoride in pyridine and deacetylation of the resulting 5-enoside afforded the required product. In aqueous, unbuffered solutions, the phenyl hex-5-enopyranoside (**18**) was slowly hydrolyzed by β-glucosidase to give the 6-deoxy-dicarbonyl sugar (**21**). The degree of hydrolysis, however, was only some 10–20% in four days whereas under these conditions, phenyl β-D-glucopyranoside was hydrolyzed completely within 4 hours. With a tenfold increase in enzyme concentration, hydrolysis of the 5-ene proceeded to the extent of 60–70% in 45 hours. This slow rate of hydrolysis presumably reflects the different geometry at C-5 of the 5-ene owing to sp^2 hybridization, as compared with phenyl β-D-glucopyranoside.

Migration of the double bond from an exocyclic 5-position to an endocyclic 4-position occurred on treatment of the 4-mesylate of the 5-enoside (**15**) with lithium aluminum hydride in ether (*22*). The structure of



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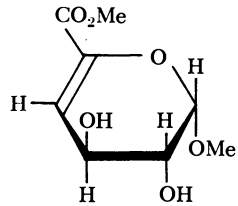

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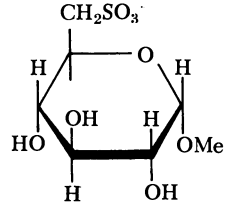

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the sirupy product (**24**) was confirmed by NMR spectroscopy. This 4-enoside, which may be regarded as a glycal, should be a useful synthetic intermediate. Hydrogenation of the related methyl uronate (**25**), (*5, 39*) followed by reduction with lithium aluminum hydride, afforded a mixture of methyl 4-deoxy-α-D-*xylo*-hexopyranoside and methyl 4-deoxy-β-L-*arabino*-hexopyranoside.

Zill and Cheniae (*46*) have suggested that 6-deoxy-6-sulfo-D-glucose, a component of plant sulfolipids, is formed by sulfonyl group transfer from phosphoadenyl-sulfate to a nucleotide derivative of 6-deoxy-D-*xylo*-hex-5-enopyranose (**4**). As a model for this reaction, Lehmann and Benson (*25*) examined the reaction of sodium bisulfite with methyl 6-deoxy-α-D-*xylo*-hex-5-enopyranoside (**3**) and with the corresponding D-*lyxo* isomer. In each case addition of bisulfite to the double bond occurred rapidly in aqueous solution at pH 6.4-7.0 and the corresponding 6-deoxy-6-sulfo-D-hexopyranosides (**26** and the corresponding mannopyranoside) were isolated in good yield as the sodium or cyclohexylammonium salts. The alternative 5-sulfonated products, expected from nucleophilic attack of bisulfite ion, were not formed and it was concluded that the addition took place by a free-radical mechanism. This was supported by an observed inhibition of the reaction by hydroquinone. The

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free radical reaction is stereospecific for the formation of the D-configuration since the other possible product from **3** namely methyl 6-deoxy-6-sulfo-β-L-idopyranoside, was not detected. Similarly, adding the free radical of benzylthiol to the hex-5-enopyranoside (**3**) afforded only methyl 6-*S*-benzyl-6-thio-α-D-glucopyranoside (*24*). The product was formed in quantitative yield and was characterized by conversion to methyl 6-deoxy-α-D-glucopyranoside.

A crystalline 5,5′-diene, or bis (vinyl ether), derivative of sucrose has been prepared from 6,6′-dideoxy- 6,6′-diiodo-sucrose hexa-acetate, derived from the 6,6′-ditosylate, by treatment with silver fluoride in pyridine (*26*).

For preliminary study of the synthesis of exocyclic, vinyl ethers of pentofuranoid derivatives, the readily available 5-deoxy-5-iodo-1,2-*O*-isopropylidene-α-D-xylofuranose (**30**) (*28*) was selected. Treatment of the latter (**30**) with anhydrous silver fluoride in pyridine, however, did not afford an unsaturated product. Instead there was isolated in 85% yield the oxetan, namely 3,5-anhydro-1,2-*O*-isopropylidene-α-D-xylofuranose (**28**). This compound, first prepared by Levene and Raymond (*29*) by reaction of the 5-tosylate (**27**) with sodium methoxide in methanol, also resulted from the interaction of the 5-tosylate (**27**) with silver fluoride. Acetylation of the 5-iodo derivative (**30**) afforded the crystalline 3-acetate (**31**) which reacted with silver fluoride-pyridine to give an 83% yield of a crystalline, unsaturated sugar. The NMR spectrum, which will be discussed later, afforded conclusive evidence that the product was the required 3-*O*-acetyl-5-deoxy-1,2-*O*-isopropylidene-β-L-*threo*-pent-4-enofuranose (**34**). Catalytic deacetylation of this product (**34**) with sodium methoxide in methanol gave a quantitative yield of 5-deoxy-1,2-*O*-isopropylidene-β-L-*threo*-pent-4-enofuranose (**33**) which was further characterized as the crystalline 3-benzoate (**35**) and the sirupy 3-tosylate (**32**). Since these vinyl ethers lack asymmetry at C-4, they could also be prepared from precursors having the L-*arabino*-configuration. Thus, 5-deoxy-5-iodo-1,2-*O*-isopropylidene-β-L-arabinofuranose (**37**), where the stereochemistry prohibits anhydro-ring formation, and its 3-acetate (**38**) afforded the vinyl ethers **33** and **34** respectively. Similarly, both 1,2-*O*-isopropylidene-3,5-di-*O*-tosyl-β-L-arabinofuranose (**36**) and the corresponding D-*xylo* isomer (**29**) afforded the same unsaturated product (**32**) when treated with silver fluoride in pyridine. This elimination of the elements of toluene-*p*-sulfonic acid by silver fluoride, although novel, was slow and in each case the product was isolated in only 40% yield. A further elimination was observed (*20*) when *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-6-*O*-tosyl-β-D-glucopyranoside was treated with silver fluoride.

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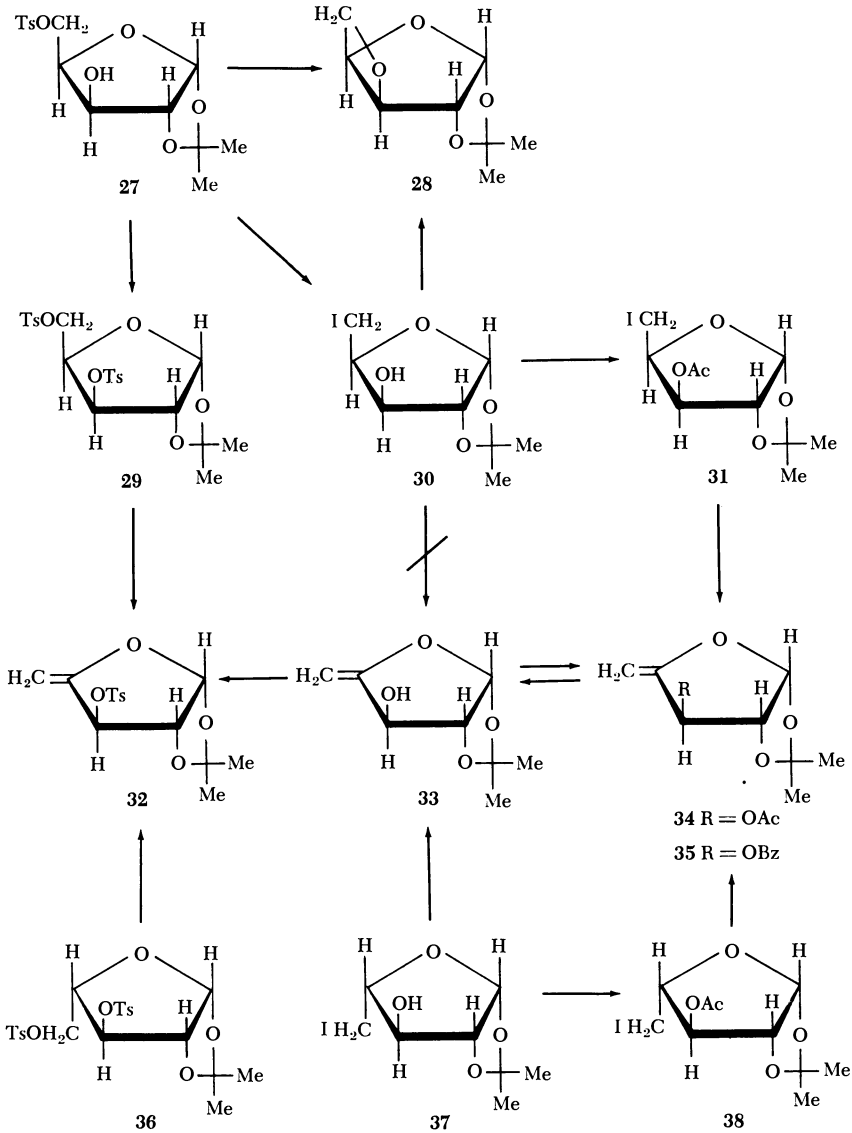

The structures assigned to the foregoing vinyl ethers are supported by physical data. Each compound showed intense absorption in the infrared near 1660 cm.$^{-1}$ This absorption is characteristic of vinyl compounds and is attributed to C=C stretching. Analysis of the NMR spectra was straightforward although only the spectrum of the 3-tosylate (**32**) (Figure 1) showed completely resolved signals. The spectrum showed unambiguously that the 3-tosylate (**32**) has the structure assigned and

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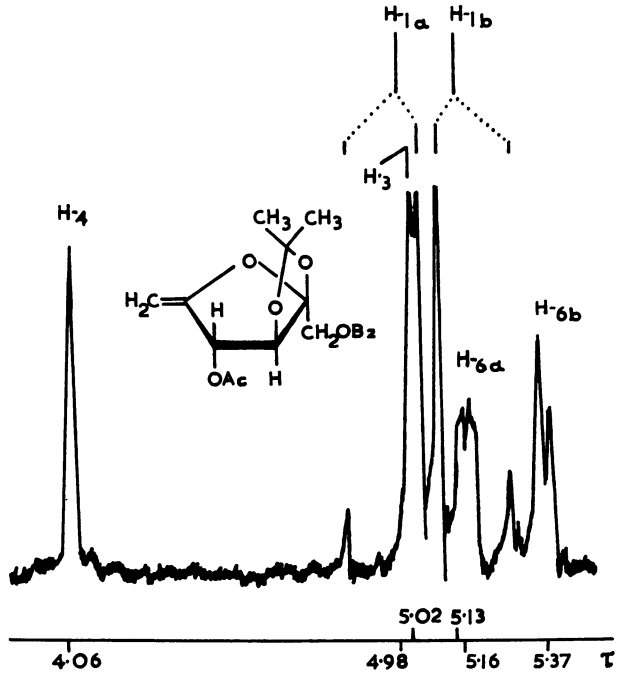

*Figure 1. Part of NMR spectrum of 5-deoxy-1,2-O-isopropylidene-3-O-tosyl-β-*L*-*threo*-pent-4-enofuranose* (**32**) *at 60 Mc.p.s. in deuterochloroform.*

excluded the alternative structure having an endocyclic double bond. The signals of the vinyl protons appeared as narrow doublets centered at τ 5.50 and 5.96. The H–1 and H–2 signals appeared as doublets centered at τ 4.02 and 5.39 respectively; the remaining singlet at τ 4.91 was assigned to H–3. The H–3 signal would be absent if **27** were a 3-enose and the C–5 protons would be shifted to about τ 8.0. The values for the coupling constants and chemical shifts observed for the vinyl ethers (**32–35**) are recorded in Table I. The values for $J_{1,2}$ and $J_{2,3}$ are similar to those reported by Abraham and co-workers(*1*) for derivatives of 1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose. The latter derivatives exist in solution in a twist (T^3_2) conformation and it is likely that the vinyl ethers (**32–35**) adopt a similar conformation. The signals for the vinyl protons (H_{5a},H_{5b}) and the allylic protons (H_3) showed additional splittings when recorded at a sweep width of 100 c.p.s. The magnitude of these splittings was of the order 0.5-0.7 c.p.s. and they are presumably caused by allylic couplings between H–3 and and the protons at C–5.

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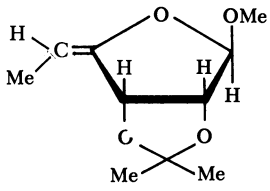
Of the four possible 5-deoxy-pent-4-enofuranoses, the D-*erythro*-isomer was of interest as a potential source of derivatives of L-lyxofuranose. For this purpose, a vinyl ether having the D-*erythro*-configuration has been prepared from derivatives of D-ribose. Condensation of D-ribose with acetone in the presence of methanol, cupric sulfate and sulfuric acid at 30°C., as described by Levene and Stiller(*30*) afforded a sirupy product consisting mainly of methyl 2,3-*O*-isopropylidene-D-ribofuranose (**40**). Treatment of a pyridine solution of the sirup with tosyl chloride



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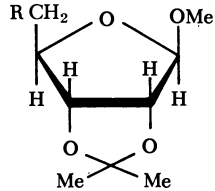
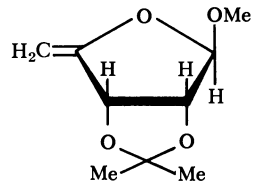


40 R = OH

41 R = OTs

42 R = I



43

afforded the crystalline 5-tosylate (**41**). The NMR signal of the anomeric proton of the latter (**41**) was a sharp singlet thus indicating a projected valency angle between H–1 and H–2 of about 90°. This requirement is met only by the β-anomer. The 5-tosylate (**41**) was then converted to the 5-iodo derivative (**42**) which on treatment with silver fluoride-pyridine gave the required methyl 5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranoside (**43**). The NMR spectrum of the latter (**43**) measured in deuterochloroform at 100 Mc.p.s. confirmed the assigned structure (*see* Table 1). Hydrogenation of the pent-4-enofuranosides (**43**) and (**33**), which affords a convenient route to 5-deoxy pentoses, is under investigation and will be reported elsewhere.

Stereospecificity was observed in the hydrogenation and hydroboration of the alkene (**39**) (38). Attack from below the furanose ring of both D-erythro-4-enosides (**39** and **43**) is hindered by the 2,3-O-isopropylidene ring and the products resulting from topside attack therefore predominate.

The furanoid vinyl ethers described above are of interest in view of the structure of the nucleoside antibiotic angustmycin A (**2**) (*15*) which also contains an exocyclic double bond. As a first step towards the synthesis of analogs of angustmycin A (**2**), preparation of exocyclic vinyl ethers from hexulofuranoid derivatives was studied. Benzoylation of 2,3;4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**44**) followed by selective acid hydrolysis of the 4,6-*O*-isopropylidene group afforded 1-*O*-benzoyl-2,3-*O*-isopropylidene-α-L-*xylo*hexulofuranose (**45**) in 72%

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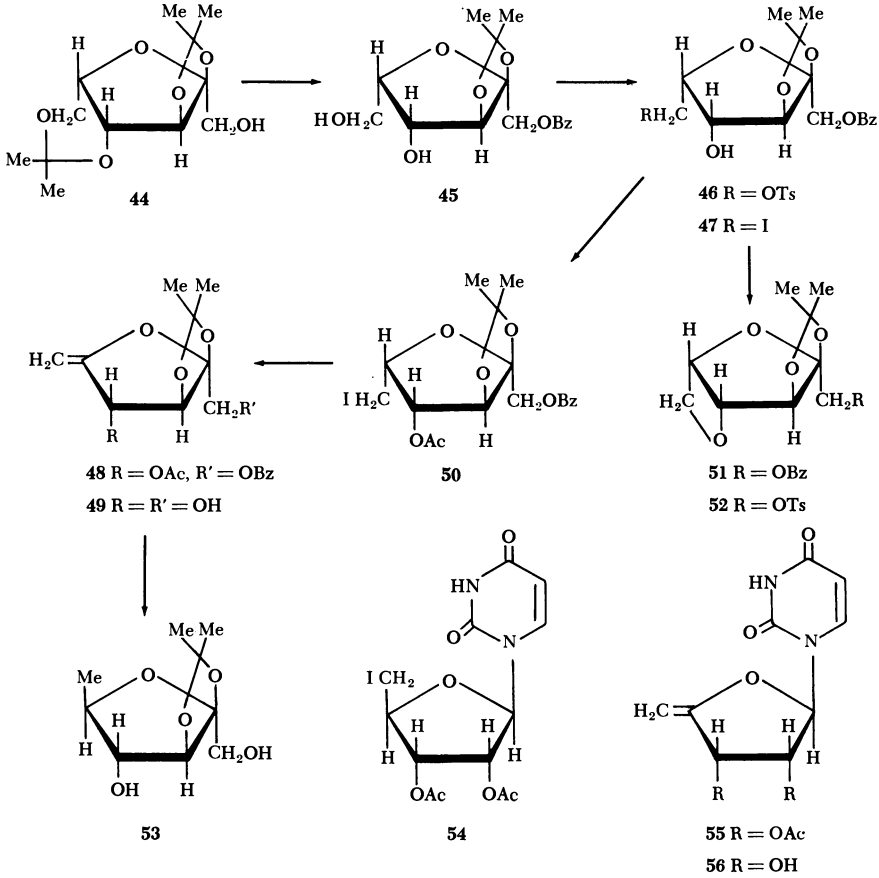

yield. Selective tosylation of this 1-benzoate (**45**) in pyridine gave a 90% yield of the 6-tosylate (**46**) which on treatment with sodium iodide in refluxing butanone afforded the 6-iodo derivative (**47**), subsequently converted to the 4-acetate (**50**). Both the 6-iodo (**47**) and the 6-tosylate (**46**) derivatives, with free hydroxyl groups at C–4, reacted with silver fluoride in pyridine to give the 4,6-oxetan (**51**) which was converted by debenzoylation and tosylation to the 1-tosylate (**52**). This product (**52**) was identical with a compound erroneously reported (*42*) to be 1,4-anhydro-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*hexulofuranose. The proof of structure of the oxetans **51** and **52** has been described elsewhere (*19*). The reaction of the 4-acetate (**50**) with silver fluoride afforded the required 4-*O*-acetyl-1-*O*-benzoyl-6-deoxy-2,3-*O*-isopropylidene-β-D-*threo*-hexulo-5-enofuranose (**48**). Catalytic de-esterification of this 5-enose (**48**) gave the crystalline 6-deoxy-2,3-*O*-isopropylidene-β-D-*threo*-hexulo-5-enofuranose (**49**). The NMR spectra of the latter 5-

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enoses (**48** and **49**), measured in pyridine solution at 60 Mc.p.s., showed completely resolved signals. The signals of the two vinyl protons of **48** (Figure 2) appeared as doublets centered at τ 5.16 and 5.37. The C–1 protons appeared as an AB quartet at τ 4.98 and 5.13. The H–4 signal at τ 4.06 was distinguished from the H–3 signal at τ 5.02 by small splittings owing to allylic coupling of H–4 to the C–5 protons. The spectrum of compound **49** showed the expected diamagnetic shifts of the signals

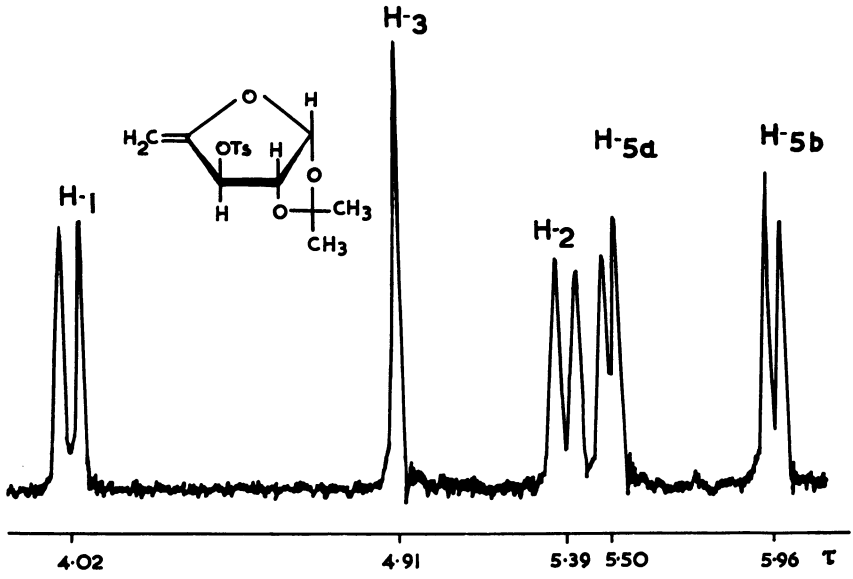

*Figure 2. Part of NMR spectrum of 4-O-acetyl-1-O-benzoyl-6-deoxy-2,3-O-isopropylidene-β-D-threo*hexulo-*5-enofuranose* (**48**) *at 60 Mc.p.s. in pyridine.*

because of H–4 and H–1. In this case the two protons at C–1 were equivalent and appeared as a two-proton singlet at τ 5.71. The spectra are consistent only with the assigned structures (**48** and **49**).

Hydrogenation of the vinyl ether (**49**) in ether solution in the presence of palladium-on-carbon catalyst afforded 6-deoxy-2,3-*O*-isopropylidene-β-D-*arabino*-hexulofuranose (**53**) (17) as the only product. As with the vinyl ethers (**39**) and (**43**), reduction of the double bond occurred from the least hindered side of the molecule, namely opposite to the isopropylidene ring.

The use of the silver fluoride reaction in the preparation of a nucleoside containing a 4′,5′ double bond was recently reported (*44*). Thus treat-

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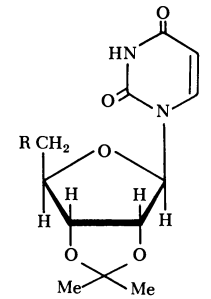
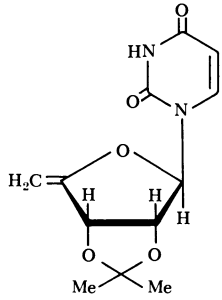


57 R = I
58 R = F
59 R = OTs



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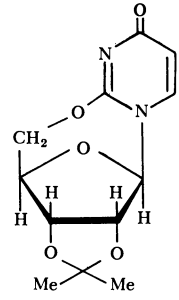

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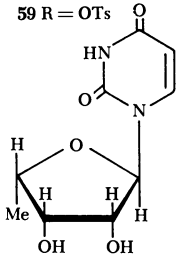

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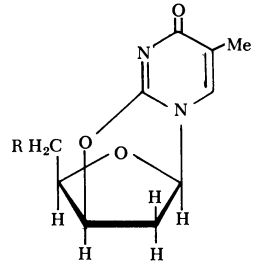
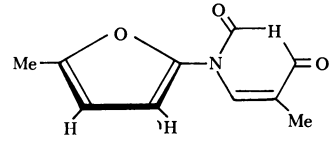


63 R = I
64 R = OMS



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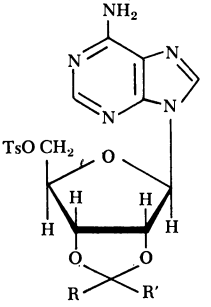
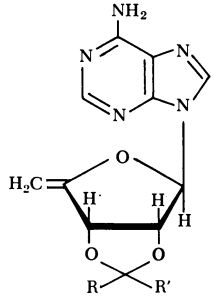
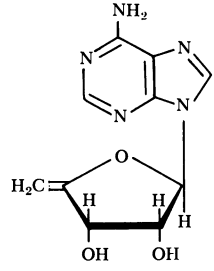


66 R = R′ = Me
67 R = H, R′ = OEt



68 R = R′ = Me
69 R = H, R′ = OEt



70

ment of 2′,3′-di-O-acetyl-5′-deoxy-5′-iodo uridine (**54**) with silver fluoride in pyridine afforded the amorphous 4′-ene (**55**) in 84% yield. Removal of the protecting groups with ammonia in methanol gave crystalline 1-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl) uracil (**56**). On the other hand when 2′,3′-*O*-isopropylidene-5′-deoxy-5′-iodo-uridine (**57**) was treated (*36*) with silver fluoride in pyridine, the product was the 5′-fluoride (**58**) and not the 4′-ene (**60**). Under similar conditions, the 5′-fluoride (**58**) was formed in good yield from the 2,5′-anhydride (**61**) and this compound is presumably an intermediate in the formation of **58** from the 5′-iodo derivative (**57**). In contrast to the 2′,3′-di-acetate (**54**), the 2′,3′-

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O-isopropylidene derivative (**57**) must exist in pyridine solution in a conformation which favors anhydro-ring formation rather than elimination. Considerable degradation occurred when the 5-iodo derivative (**63**) was treated with silver fluoride in pyridine (*36*). The products, which were isolated in small yield, were identified as thymine and 1-[2-(5-methylfuryl)]-thymine (**65**). This same compound (**65**) was formed in high yield when the 5′-mesylate **64** was treated with potassium *tert*-butylate in dimethyl sulfoxide (*16*). The formation of **65** from **63** or **64** clearly involves the rearrangement of an intermediate 2′,4′-diene. In a different approach to the problem of introducing terminal unsaturation into pentofuranoid nucleosides, Robins and co-workers (*32,37*) have employed mild base catalyzed E_2 elimination reactions. Thus, treatment of the 5′-tosylate (**59**) with potassium *tert*-butylate in *tert*-butyl alcohol afforded a high yield of the 4′-ene (**60**) (*37*). This reaction may proceed *via* the 2,5′

Table I. Nuclear Magnetic Resonance Data [a]

	proton chemical shifts (τ)							*proton coupling constants (c.p.s.)*		
Compound	*H-1*	*H-2*	*H-3*	*H-5a*	*H-5b*	*C(Me)₂*		$J_{1,2}$	$J_{2,3}$	$J_{5a,5b}$
32	4.02[d]	5.39[d]	4.91	5.50[d]	5.96[d]	8.60	8.65	3.3	<0.5	2.3
33	3.86[d]	[e]	[e]	[e]	5.70[d]	8.54	8.60	3.4	<0.5	2.3
34	3.90[d]	[e]	4.48	[e]	5.61[d]	8.53	8.61	3.4	<0.5	1.8
35	3.88[d]	5.32[d]	4.29	5.38[d]	5.56[d]	8.50	8.59	3.5	<0.5	1.9
43[c]	4.98	5.59[d]	5.03[d]	5.48[d]	5.71[d]	8.60	8.72	<0.5	6.0	2.0
	H–1a,1b	H–3	H–4	H–6a	H–6b	$C(Me)_2$		$J_{1a,1b}$	$J_{3,4}$	$J_{6a,6b}$
48[b]	4.98[q] 5.13	5.02	4.06	5.16[d]	5.37[d]	8.44	8.57	12.0	<0.5	1.8
49[b]	5.71	4.97	5.07	5.36[m]	5.60[d]	8.39	8.50	—	<0.5	1.8

[a] Except for compounds **48** and **49,** spectra were determined in deuterochloroform solution (15% w.v.) containing 1% tetramethylsilane as internal reference, with a Varian A-60 spectrometer. Values were obtained by first-order analysis. Signals are singlets unless unless otherwise designated.

[b] Determined in pyridine solution. [c] Determined at 100 Mc.p.s. [d] doublet. [e] Not fully resolved. [q] AB quartet—second order analysis. [m] multiplet.

anhydride (**61**) and indeed, when **61** was treated with potassium *tert*-butylate in dimethyl sulfoxide, the 4′-ene (**60**) and isopropylidene uridine were formed. Hydrogenation of the 4′-ene (**60**) over a palladium-on-carbon catalyst proceeded in a stereospecific manner to give a high yield of 1-(5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl) uracil which was subsequently converted to 1-(5-deoxy-α-L-lyxofuranosyl) uracil (**62**). Treatment of the 2′,3′-*O*-isopropylidene and 2′3′-*O*-ethoxymethylidene derivatives of 5′-*O*-tosyl adenosine (**66** and **67**) with potassium *tert*-butylate in

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tert-butyl alcohol afforded the 4′-enes (**68** and **69**) in 26% and 31% yields respectively (*32*). Attempts to remove the isopropylidene group of **68** by means of mild acid hydrolysis resulted in cleavage of the glycosyl bond but similar treatment of the 2′,3′-*O*-ethoxymethylidene derivative (**69**) afforded a 32% yield of 6-amino-9-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl) purine (**70**). This compound, which differs from the naturally occurring angustmycin A (**2**) only in the lack of a hydroxymethyl substituent at C–1′, is reported (**32**) to posses higher activity against *Streptococcus faecalis* than does angustmycin A.

Experimental Memoranda

Concentrations were carried out under reduced pressure. Melting points were determined on a Koffler microstage apparatus. Thin layer chromatography (t.l.c.) was performed on Silica gel G (Merck) with the indicated solvent system. The separated materials were detected by spraying with 5% ethanolic sulfuric acid followed by heating at 110°-115°C. for about 10 minutes. Optical rotations were measured on a Zeiss Polarimeter in 10 cm. tubes of 1 or 10 ml. capacity: Chloroform solutions were used unless otherwise stated. Light petroleum (b.p. 60°-80°C.) was used throughout. Pyridine was dried by several distillations from phosphoric oxide and stored over potassium hydroxide. Methanol was dried by the magnesium-iodine method and butanone was purified by distillation from sodium iodide.

Experimental

3-O-Acetyl-5-deoxy-5-iodo-1,2-O-isopropylidene-α-D-xylofuranose (**31**). Acetic anhydride (0.66 ml., 6.95 mmole) was added dropwise to a stirred solution of 1.4 grams, (4.67 mmole) of 5-deoxy-5-iodo-1,2-*O*-isopropylidene-α-D-xylofuranose (*28*) (**30**) in pyridine (10 ml.). The reaction mixture was left at room temperature for 3 hours and then processed in the usual way. The product (1.5 grams, 94%) crystallized from methanol, m.p. 67°–68°C. $[\alpha]_D^{2.75}$ — 47.5° (c, 1.55). *Anal.* Calcd. for $C_{10}H_{15}IO_5$: C, 35.1; H, 4.4. Found: C, 35.3; H, 4.4.

3-O-Acetyl-5-deoxy-5-iodo-1,2-O-isopropylidene-β-L-arabinofuranose (**38**). Acetylation of 5-deoxy-5-iodo-1,2-*O*-isopropylidene-β-L-arabinofuranose (**37**) (*27*) in pyridine as described above for the D-*xylo*-isomer afforded the 3-acetate (**38**) as a colorless sirup which did not crystallize. $[\alpha]_D^{24}$ + 13.7° (c, 3.5). *Anal.* Calcd. for $C_{10}H_{15}IO_5$: C, 35.1; H, 4.4. Found: C, 35.0; H, 4.5.

1-O-Benzoyl-2,3-O-isopropylidene-α-L-xylohexulofuranose (**45**). The following procedure was modified from that described by Sullivan (*40*). Benzoyl chloride (25.0 ml., 0.22 mole) was added slowly to a stirred solution of 46.4 grams (0.18 mole) of 2,3;4,6-di-*O*-isopropylidene-α-L-*xylo*hexulofuranose (**44**) (*43*) in pyridine (100 ml.), and the reaction mixture stood at room temperature for 1 hour. Water (200 ml.) was added and the sirupy product extracted into chloroform (2 × 100 ml). The combined chloroform solutions were washed sequentially with 2*N*

hydrochloric acid until the washings were acidic, with saturated sodium bicarbonate solution until the washings were neutral, and finally with water. Concentration of the chloroform solution afforded sirupy 1-*O*-benzoyl-2,3;4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulofuranose. This sirup was heated at 100° with 50% aqueous acetic acid (350 ml.). Dissolution of the sirup was complete within 15 minutes and after 1 hour chromatography (benzene-methanol 4:1 v/v) showed that none of di-isopropylidene acetal remained. The solution was cooled and concentrated to a thin sirup which was then dissolved in ethyl acetate (200 ml.). The remaining acetic acid was removed by washing the solution with saturated sodium bicarbonate solution and then with water. The solution was dried over potassium carbonate and evaporated to a semi-crystalline mass. Recrystallization from ethyl acetate-petroleum gave fine needles of **45** (45 grams, 72%), m.p. 86°–90°C. Recrystallization from the same solvent pair afforded pure material, m.p. 91°–92°C., $[\alpha]_D^{24}$ + 6.8° (c, 2.8). Sullivan (*40*) reported m.p. 92°C. and $[\alpha]_D^{25}$ + 7.2°C.

1-O-*Benzoyl-2,3*-O-*isopropylidene-6*-O-*tosyl*-α-L-xylo*hexulofuranose* (**46**). A solution of toluene-*p*-sulfonyl chloride (13.0 grams, 68 mmole) in pyridine (50 ml.) was added over a period of two hours to a stirred solution of 20 grams (62 mmole) of **45** in dry pyridine (100 ml.) at −5°C. After the addition, the reaction mixture remained at room temperature for 16 hours. Water (1 ml.) was added, and after 30 minutes the solution was poured into ice-water (700 ml.). The crystalline solid which separated was collected, washed with water, and recrystallized from hot methanol. The yield of pure product, m.p. 150°–152°C. (decomp.), $[\alpha]_D^{24}$ − 5.0° (c, 5.6) was 26.7 grams, (90%). *Anal.* Calcd. for $C_{23}H_{26}O_9S$: C, 57.7; H, 5.5; S, 6.7. Found: C, 57.9; H, 5.5; S, 6.5.

1-O-*Benzoyl-6-deoxy-6-iodo-2,3*-O-*isopropylidene*-α-L-xylo*hexulofuranose* (**47**). A solution of 15 grams (31.3 mmole) of **46** in butanone (200 ml.) containing dry sodium iodide (9.3 grams, 62 mmole) was refluxed for 24 hours. The reaction mixture was cooled, the sodium tosylate removed by filtration, and the filtrate concentrated to dryness. The residue was partitioned between water (50 ml.) and chloroform (2 × 50 ml.); the combined chloroform solutions were washed with water, dried over sodium sulfate and concentrated to a colorless sirup which spontaneously crystallized. Recrystallization from aqueous methanol afforded pure material in two crops (11.6 grams, 85%), m.p. 115°–117°C., $[\alpha]_D^{24}$ + 36.0° (c, 5.3). *Anal.* Calcd. for $C_{16}H_{19}O_6$: C, 44.2; H, 4.4; I, 29.3. Found: C, 44.3; H, 4.5; I, 29.4.

4-O-*Acetyl-1*-O-*benzoyl-6-deoxy-6-iodo-2,3*-O-*isopropylidene*-α-L-xylo *hexulofuranose* (**50**). Acetylating **47** with acetic anhydride in pyridine and isolating the product in the usual way afforded the 4-acetate (**50**) in 93% yield. m.p. 83°–84°C., $[\alpha]_D^{24}$ + 40.3° (c, 3.0). *Anal.* Calcd. for $C_{18}H_{21}IO_7$: C, 45.4; H, 4.4; I, 27.6. Found: C, 45.2; H, 4.4; I, 27.2.

3-O-*Acetyl-5-deoxy-1,2*-O-*isopropylidene*-β-L-threo-*pent*-*4*-*enofuranose* (**34**). (1) FROM 3-*O*-ACETYL-5-DEOXY-5-IODO-1,2-*O*-ISOPROPYLIDENE-α-D-XYLOFURANOSE (**31**). Anhydrous silver fluoride (7.5 grams) was added to a solution of 7.2 grams **26** in dry pyridine (50 ml.), and the mixture shaken at room temperature for 24 hours. The black reaction mixture was diluted with ether (50 ml.), and the supernatant liquid was decanted from the dark, inorganic residue. The residue was further extracted with ether (3 × 50 ml.) and the pyridine-ether solution partially decolorized

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by passage through a column of silica gel (1.5 × 20 cm.). The pale yellow effluent and column washings were concentrated to a mobile sirup which was shown by chromatography to consist of a major unsaturated component together with traces of two other components. The sirup was placed on a column of silica gel (2.5 × 80 cm.), and the column was eluted with benzene-ether, 2:1 v/v. Fractions containing the major component were combined and concentrated to a pale yellow sirup (3.75 grams, 83%) which was chromatographically pure (t.l.c. and g.l.c.). The product was distilled at 90°C. (bath) at 3×10^{-4}mm. The clear distillate crystallized on cooling to 0°C.; m.p. 31°–33°C., $[\alpha]_D^{24} - 6.3°$ (c, 4 in acetone). *Anal.* Calcd. for $C_{10}H_{14}O_5$: C, 56.1; H, 6.6. Found: C, 56.3; H, 6.7.

(2) FROM 3-*O*-ACETYL-5-DEOXY-5-IODO-1,2-*O*-ISOPROPYLIDENE-β-L-ARABINOFURANOSE (**38**). Treating 100 mg. of (**38**) in pyridine (1 ml.), with anhydrous silver fluoride (200 mg.) for 4 hours and isolating the product as described above afforded crystalline **34** in 87% yield. Pure material had m.p. 31°–33°C. and $[\alpha]_D^{24} - 6.5°$ (c, 4 in acetone). Both samples had identical infrared spectra and had the same g.l.c. retention time at 100°C.

(3) From 5-DEOXY-5-IODO-1,2-*O*-ISOPROPYLIDENE-β-L-ARABINOFURANOSE (**37**). Anhydrous silver fluoride (600 mg.) was added to a solution of 300 mg. of **37** in pyridine (4.0 ml.), and the mixture was shaken at room temperature for 24 hours. Ether (4 ml.) was added, and the mixture was passed through a column of silica gel (1.5 × 12 cm.). The column was washed with ether/pyridine, 1:1 v/v. (10 ml.), and the effluent, which contained 5-deoxy-1,2-*O*-isopropylidene-β-L-*threo*-pent-4-enofuranose (**33**), was concentrated to 4 ml. Acetic anhydride (0.2 ml.) was added, and the reaction mixture was kept at room temperature for 16 hours. Concentration afforded a sirup from which the last traces of solvent were removed by storage in high vacuum at 20°C. The sirup was distilled at 90°C. (bath) at 2.5×10^{-4}mm. The distillate (110 mg., 51%), which crystallized on standing, had physical constants which were identical to material prepared as above.

*5-Deoxy-1,2-O-isopropylidene-β-L-*threo-*pent-4-enofuranose* (**33**). A solution of sodium methoxide (0.05 mmole) in methanol was added to a solution of 540 mg. (2.52 mmole) of **34** in dry methanol (8.0 ml). The solution was kept at room temperature for 18 hours and then concentrated to dryness. The sirupy residue was dissolved in acetone (10 ml.), the solution filtered, and the filtrate evaporated to a colorless sirup (400 mg., 83%) which was chromatographically pure. $[\alpha]_D^{24} - 73.0°$ (c, 4 in acetone). *Anal.* Calcd. for $C_8H_{12}O_4$: C, 55.8; H, 7.0. Found: C, 55.6; H, 7.1.

*3-O-Benzoyl-5-deoxy-1,2-O-isopropylidene-β-L-*threo*-pent-4-enofuranose* (**35**). Treating 220 mg. (1.3 mmole) of **33** in pyridine (5 ml.) with benzoyl chloride (0.165 ml., 1.4 mmole) at room temperature and isolating the product in the usual way afforded the 3-benzoate as a yellow sirup (234 mg., 70%). Distilling at 120°C. (bath) and 0.5 mm. afforded a colorless sirup which crystallized on standing, mp. 52–54°C., $[\alpha]_D^{21} -$ 28.5° (c, 1.1 in acetone). *Anal.* Calcd. for $C_{15}H_{16}O_5$: C, 65.2; H, 5.8. Found: C, 65.1; H, 5.8.

*5-Deoxy-1,2-O-isopropylidene-3-O-tosyl-β-L-*threo*-pent-4-enofuranose* (**32**). (1) FROM 5-DEOXY-1,2-*O*-ISOPROPYLIDENE-β-L-THREO-PENT-4-ENO-

FURANOSE (**33**). Toluene-*p*-sulfonyl chloride (500 mg. 2.6 mmole) was added to a cooled (0°C.) solution of 380 mg. (2.2 mmole) **33** in pyridine (10 ml.). The reaction mixture was kept at 0°C. for 24 hours and then poured into cold water. The dark oil, which separated, was extracted into chloroform (2 x 10 ml.), the chloroform solution washed with water (10 ml.) and dried over sodium sulfate. Concentration afforded a brown sirup which decomposed on attempted distillation. Chromatography on silica gel with benzene-ether, 2:1 v/v, afforded the 3-tosylate as a colorless sirup (480 mg. 65%) $[\alpha]_D^{25}$ — 15.0° (c, 2.0 in acetone). *Anal.* Calcd. for $C_{15}H_{18}O_6S$: C, 55.3; H, 5.5. Found: C, 55.1, H, 5.4.

(2) FROM 1,2-*O*-ISOPROPYLIDENE-3,5-di-*O*-TOSYL-β-L-ARABINOFURANOSE (*33*) (**36**). Anhydrous silver fluoride (600 mg.) was added to a solution of 260 mg. **36** in pyridine (5 ml.), and the mixture shaken at room temperature for 42 hours. Isolation of the product was carried out as described for the preparation of **34** except that final purification was achieved by means of preparative thin-layer chromatography. The product (77 mg., 45%) had an infrared spectrum identical with that of **33** prepared as above. $[\alpha]_D^{25}$ — 15.0° (c, 1.5 in acetone).

(2) FROM 1,2-*O*-ISOPROPYLIDENE-3,5-DI-*O*-TOSYL-β-D-XYLOFURANOSE (*21*) (**29**). Treating **29** with silver fluoride in pyridine and isolating as described above for the L-*arabino* isomer gave a 40% yield of **32** after a reaction time of 48 hours. The product had $[\alpha]_D^{25}$ — 14.9° and had an infrared spectrum identical with material prepared as above.

*3,5-Anhydro-1,2-*O*-isopropylidene-α-*D*-xylofuranose* (**28**). (1) FROM 1,2-*O*-ISOPROPYLIDENE-5-*O*-TOSYL-α-D-XYLOFURANOSE (**27**). Silver fluoride (1.0 gram) was shaken with a solution of 1.0 gram of **27** in pyridine (8.0 ml.) at room temperature for 49 hours. The product was isolated as described previously except that recourse to column chromatography was not necessary. The crude product (425 mg., 85%) was distilled at 40°C. (bath) at 5.7 × 10^{-4}mm. to give pure material with m.p. *ca* 18°C.$[\alpha]_D^{22}$ + 11.9° (c, 3.0). Lit. values (*29*); m.p. *ca* 17°C.; $[\alpha]_D$ + 11.4° ($CHCl_3$). The product had an infrared spectrum identical with authentic **27** prepared by the method of Levene and Raymond (*29*).

(2) FROM 5-DEOXY-5-IODO-1,2-*O*-ISOPROPYLIDENE-α-D-XYLOFURANOSE (**30**). A solution of 1.14 grams of **30** in pyridine (8.0 ml.) was shaken at room temperature with silver fluoride (2.0 grams). The reaction was slower than with the corresponding 5-tosylate (**22**) and was complete after 72 hours. The reaction mixture was processed as described above to give a pale yellow sirup which contained, in addition to **28**, three minor components. Distillation afforded pure material (0.4 grams, 75%) identical with material prepared as above.

*4-O-Acetyl-1-O-benzoyl-2,3-O-isopropylidene-β-*D*-threo-hexulo-5-eno-furanose* (**48**). Anhydrous silver fluoride (6.0 grams) was shaken with a solution of 5.25 grams of **50** in pyridine (50 ml.) for 4 hours at room temperature. The reaction mixture was processed as described in the preparation of **34** to give a yellow sirup (3.6 grams) which contained a major unsaturated compound and a small amount of another compound. Chromatography on silica gel with petroleum-ether, 1:1 v/v, afforded **48** (3.0 grams, 71%) as a chromatographically pure sirup. $[\alpha]_D^{24}$ — 25° (c, 4.0). *Anal.* Calcd. for $C_{18}H_{20}O_7$: C, 62.1; H, 5.8. Found: C, 61.7; H, 5.9.

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*6-Deoxy-2,3-O-isopropylidene-β-*D-threo-*hexulo-5-enofuranose* (**49**). A solution of 860 mg. (2.5 mmole) of **48** in dry methanol (10 ml.) was treated with a solution of sodium methoxide (0.05 mmole) in methanol. The reaction mixture was stored at room temperature for 16 hours and then evaporated to dryness. The solid residue was dissolved in acetone, the solution filtered, and the filtrate evaporated to a small volume. Petroleum was added until the solution was faintly turbid; the solution was then cooled. The resulting crystals (400 mg., 80%) were further purified by sublimation at 115°C. and 0.1 mm. m.p. 118°–120°C., $[\alpha]_D^{24}$ + 91.0° (c, 2.0). *Anal.* Calcd. for $C_9H_{14}O_5$; C, 53.5; H, 7.0. Found: C, 53.2; H, 6.9.

*6-Deoxy-2,3-O-isopropylidene-β-*D-arabino*hexulofuranose* (**53**). Palladium (10%) on carbon (*ca* 40 mg.) was added to a solution of 300 mg. of **45** in ether (40 ml.), and the suspension was shaken in an atmosphere of hydrogen for 20 hours. The catalyst was filtered off, washed well with ether, and the filtrate concentrated to a sirup. The product crystallized from ether-petroleum. Yield 250 mg. (71.5%), m.p. 110°C., $[\alpha]_D^{23}$ + 6.5° (c, 2 in ethanol). Hough and Jones (*17*) reported m.p. 112°C. and $[\alpha]_D$ + 8°. *Anal.* Calcd. for $C_9H_{16}O_5$: C, 52.9; H, 7.8. Found: C, 52.8; H, 8.05.

*Methyl 5-deoxy-2,3-O-isopropylidene-β-*D-erythro-*pent-4-enofuranoside* (**43**). A solution of methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-ribofuranoside (**42**) (*30*) (1.0 grams) in pyridine (10 ml.) was shaken for 7 hours at room temperature with anhydrous silver fluoride (1.5 grams). Isolation of the product as described previously afforded the 4-ene (**43**) as a sirup (414 mg., 70%) which distilled at 40°C. (bath) at 0.1 mm. G.l.c. showed the presence of a major component (95%) together with 5% of another component which was not investigated. $[\alpha]_D^{21}$ + 26.3° (c, 2.3). *Anal.* Calcd. for $C_9H_{14}O_4$: C, 58.05; H, 7.6. Found: C, 57.9; H, 7.7.

*Hydrogenation of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-β-*D-xylo-*hex-5-enopyranose* (**11**). A solution of the 5-enose (**12**) (*11*) (2 grams) in ether (400 ml.) was shaken in the presence of platinum oxide (0.5 grams) for 12 hours in an atmosphere of hydrogen at room temperature. The catalyst was filtered off, and the filtrate was concentrated to a sirup which crystallized. T.l.c. showed the presence of traces of unchanged starting material and two other slower moving components [solvent: ether/petrol 4:1 v/v]. Recrystallization from ethanol gave 6-deoxy-β-D-glucopyranose tetra-acetate (**11**) (1.03 grams) with m.p. 150°–151°C., $[\alpha]_D^{22}$ + 20° (c, 1.0). The mother liquors were evaporated and the residual sirup separated by preparative t.l.c. to give 6-deoxy-β-D-glucopyranose tetra-acetate (**11**) (90 mg., total yield 56%) and the slower moving 6-deoxy-α-L-idopyranose tetraacetate (**13**) (0.4 grams, 20%) which crystallized from ethanol-petrol, m.p. 124°–126°C., $[\alpha]_D^{19}$ − 65.2° (c, 1.0). *Anal.* Calcd. for $C_{14}H_{20}O_9$: C, 50.6; H, 6.0. Found: C, 50.9; H, 6.0.

Gas liquid chromatography of similar hydrogenation reaction mixtures on a 6 foot column, packed with 3.1% S.E. 30 supported on Diatoport S, at 150°C., showed that the proportion of the D-*gluco* to L-*ido* isomers formed was in the ratio of approximately 70:30 whereas in methanol and using palladium as catalyst the ratio was 96:4.

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8

Novel Syntheses of Deoxy Sugars

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Recent developments in the synthesis of deoxy sugars from derivatives of aldosuloses and from peracylated 2-hydroxyglycals are reviewed. The value of ruthenium tetroxide as an oxidant for converting partially protected sugars into derivatives of aldosuloses has been established. A new type of branched chain deoxy sugar has been prepared by the ring expansion of derivatives of aldosuloses. Thus treatment of 5-O-benzoyl-1,2-O-isopropylidene-α-D-erythro-pentofuranos-3-ulose with excess of diazomethane, and then lithium aluminum hydride afforded 4-deoxy-1,2-O-isopropylidene-3-C-methyl-α-D-xylo-hexopyranose together with 1,2-O-isopropylidene-3-C-methyl-α-D-xylo-pentofuranose. Similarly some methyl 5,7-O-benzylidene-2,3-dideoxy-4-C-methyl-α-D-heptoside was obtained from methyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose. The preparation of 3-deoxyaldoses from peracylated 2-hydroxyglycals by sequential rearrangement, reduction, and deacylation is discussed by reference to examples based on 2-hydroxyglycal esters derivable from D-glucose, D-galactose, 6-deoxy-D-glucose, 6-deoxy-D-galactose, and D-xylose.

This paper reviews some recent work, mainly carried out in the laboratories of Birkbeck College, on the conversion of normal sugars into deoxy sugars *via* glycopyranosiduloses or unsaturated sugars as intermediates.

Deoxy Sugars from Aldosuloses and Glycopyranosiduloses

There had long been a need in carbohydrate chemistry for an oxidant which would enable the conversion of partially protected sugars, and particularly partially protected glycosides, into mono-oxo derivatives in good yield and under mild conditions. It is not surprising that in recent years this problem has been studied fairly extensively and several

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reasonable methods have been reported (*1,2,28,44,45,47,48,52*). Limitations of space preclude a general account of the oxidants that have been used and in this article only oxidations achieved with ruthenium tetroxide will be discussed. This is an oxidant which we have found to be an extremely useful reagent in carbohydrate chemistry (*2,3,4,13*) and in our opinion the one of choice when a good yield of clean product is required quickly.

We have shown (*2*) that with partially protected methyl glycosides the glycosidic linkage is unaffected by the reagent and that acetate, benzoate, benzylidene, isopropylidene, trityl, benzyl, and tosyl groups can be used successfully for protection. Two procedures are adopted generally: oxidation either at room temperature for reasonable periods with a slight excess of ruthenium tetroxide in carbon tetrachloride or, less favored, with a catalytic quantity of the tetroxide in the presence of sodium metaperiodate (*46*). Generally good yields are obtained. Equatorial and axial hydroxyl groups on a pyranoid ring seem to be oxidized with equal ease (*3*) as are *exo* and *endo* hydroxyl groups in 1,4:3,6-dianhydrohexitols (*13*). The reagent can be used to oxidize a hydroxyl group in a partially protected *N*–acylated amino sugar.

At one stage in our project we were surprised to learn that some workers had found difficulties in preparing the tetroxide from the dioxide, until we experienced the same trouble. This problem has now been resolved (*3*). Ruthenium dioxide is available commercially in both anhydrous and hydrated forms, the former being obtained by direct oxidation of ruthenium metal and the latter by a precipitation process. Only the hydrated form is oxidizable under the mild conditions (*2,3*) that we use and this form must be specified when purchasing the dioxide. It is noteworthy that the dioxide recovered from carbohydrate oxidations is always easily re-oxidized to the tetroxide. The stoichiometry has been determined of both the oxidation of the dioxide by periodate and reduction of the tetroxide which results on oxidation of an alcohol.

As an oxidant for secondary hydroxyl groups in protected monosaccharides, ruthenium tetroxide is equally as versatile as the more generally employed methyl sulfoxide and has several advantages over chromium trioxide-pyridine (*9,10,14,15,32*) which is another oxidant which we have used extensively. Thus, although oxidation of a hydroxyl group attached directly to a furanoid ring has been achieved, the examples reported are limited. Ruthenium tetroxide can be used effectively for this purpose. More relevant, in the context of this symposium, is that it can be used as a general oxidant for methyl 4,6-*O*-benzylidene-2-deoxyglycopyranosides. Although it was shown (*25*) that methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*arabino*-hexopyranoside can be oxidized by

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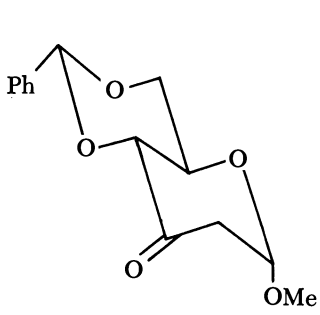
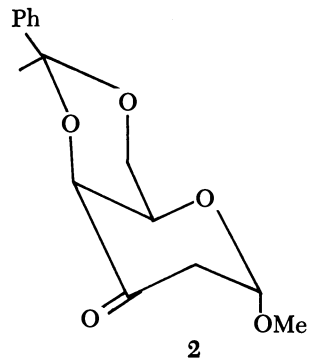
chromium trioxide-pyridine to give methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (**1**), this oxidant did not convert methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*lyxo*-hexopyranoside into methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*threo*-hexopyranosid-3-ulose (**2**) in isolable amount. Instead, a pyranodioxin (**3**) was produced by elimination of methanol in addition to oxidation (*2*).



1



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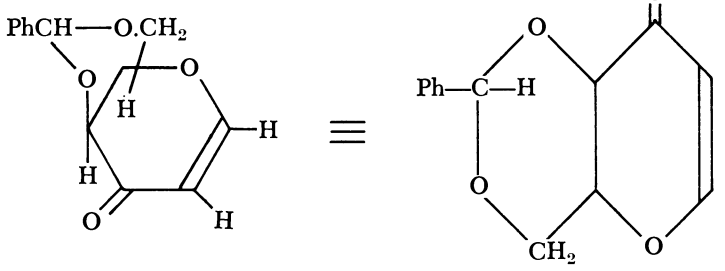

3

By monitoring the intensity of the carbonyl absorption it was observed that oxidation of methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*lyxo*-hexopyranoside with chromium trioxide-pyridine at room temperature gave initially the hexopyranosid-3-ulose (**2**) in low concentration, but attempts to increase this yield resulted in elimination of methanol to give compound **3**. However, when methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*lyxo*-hexopyranoside is oxidized by ruthenium tetroxide in either carbon tetrachloride or methylene dichloride it affords compound **2** without concomitant elimination. When compound **2** was heated for 30 minutes in pyridine which was 0.1*M* in either perchloric acid or hydrochloric acid it afforded compound **3**, but in pyridine alone it was recoverable unchanged (*2*). Another example of this type of elimination, leading to the introduction of unsaturation into a glycopyranoid ring, was observed

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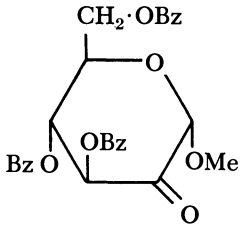
when the oxidation of methyl 3,4,6-tri-*O*-benzoyl-α-D-glucoside was studied (*2*). The product from its oxidation with ruthenium tetroxide was shown to be methyl 3,4,6-tri-*O*-benzoyl-α-D-*arabino*-hexopyranosidulose (**4**). From an attempt to purify this hexopyranosidulose on a silica gel column, another crystalline compound was obtained which is considered to have structure **5** and to be formed from compound **4** by an elimination reaction.



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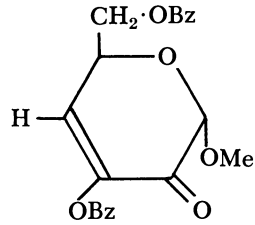

5

Bz=C_6H_5CO—

With methods available for the preparation of glycopyranosiduloses and other derivatives of aldosuloses, attention was turned to the conversion of the carbonyl group in these compounds into a methylene group. In our experience the direct conversion of a carbonyl group in a glycopyranosidulose into a methylene group is unsatisfactory, although Lindberg and Theander (*37*) have reported a direct conversion of methyl β-D-*ribo*-hexopyranosid-3-ulose into methyl 3-deoxy-β-D-*ribo*-hexopyranoside.

Three routes for the conversion, each involving more than one stage, are as follows:

$$\rangle C{=}O \longrightarrow \rangle C{=}N \cdot NH \cdot SO_2 \cdot C_6H_4{-}CH_3 \longrightarrow \rangle CH_2 \qquad (1)$$

The tosylhydrazone is prepared from the carbonyl compound and then reduced with lithium aluminium hydride, sodium borohydride or potassium borohydride. In this way D-glucose tosylhydrazone was converted into crystalline 1-deoxyglucitol by reduction with potassium borohydride (*16*).

$$\rangle C{=}O \longrightarrow \rangle C{=}N \cdot NH_2 \longrightarrow \rangle CH \cdot NHNH_2 \longrightarrow \rangle CHI \longrightarrow \rangle CH_2 \qquad (2)$$

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Treatment of hydrazino-compounds with iodine in chloroform can afford iodides which are convertible into methylene derivatives (*7,8*). An extension of this approach currently being investigated involves reduction of the hydrazones obtainable from glycopyranosiduloses and treatment of the products with iodine.

$$\rangle C{=}O \longrightarrow \rangle C(SEt)_2 \longrightarrow \rangle CH_2 \qquad (3)$$

Rennie (49), working in my laboratory, succeeded in converting D-glucose into 4-deoxy-D-*xylo*-hexose, albeit with considerable difficulty, by the following sequence of reactions which incorporate mercaptalation of the carbonyl group and reductive desulfurization of the dithiol:

D-glucose $\xrightarrow{1}$ methyl 2,3-di-*O*-benzyl-6-*O*-trityl-α-D-glucopyranoside $\xrightarrow{2}$ methyl 2,3-di-*O*-benzyl-6-*O*-trityl-α-D-*xylo*-hexapyranosid-4-ulose $\xrightarrow{3}$ methyl 2,3-di-*O*-benzyl-α-D-*xylo*-hexopyranosid-4-ulose $\xrightarrow{4}$ dibenzyl mercaptal of methyl 2,3-di-*O*-benzyl-α-D-*xylo*-hexopyranosid-4-ulose $\xrightarrow{5}$ methyl 2,3-di-*O*-benzyl-4-deoxy-α-D-*xylo*-hexoside $\xrightarrow{6}$ 4-deoxy-D-*xylo*-hexose. Stages 1 and 3 were effected by standard procedures. The oxidation (Stage 2) was difficult and after four treatments with chromium trioxide-pyridine reaction was not complete but the procedure was discontinued to avoid losses and the mixture was detritylated. Effective separation of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (from incomplete oxidation) and its '4-oxo' derivative was troublesome but treatment of the mixture with benzaldehyde and zinc chloride gave a 4,6-*O*-benzylidene derivative of the unoxidized material, which was chromatographically separable from the '4-oxo' derivative. The remaining Stages (4–6) were straightforward, bearing in mind that a mixture of benzyl mercaptan-acetic acid-boron trifluoride diethyl etherate was most effective for the mercaptalation.

Derivatives of aldosuloses can be used in other ways than those described so far to obtain deoxy sugars. For example, a glycopyranosidulose obtained by oxidation with ruthenium tetroxide of a partially protected glycoside prepared from a common deoxy sugar, when reduced may afford an isomeric deoxyglycoside of rarer configuration. The value of this sequence of reactions is illustrated by the synthesis of 6-deoxy-L-talose (*15*), a sugar interesting because of its isolation some years ago from several natural products (*11,30,38,39,40,51*). It was prepared either from L-fucose or L-rhamnose as initial material: both are sugars which are readily available.

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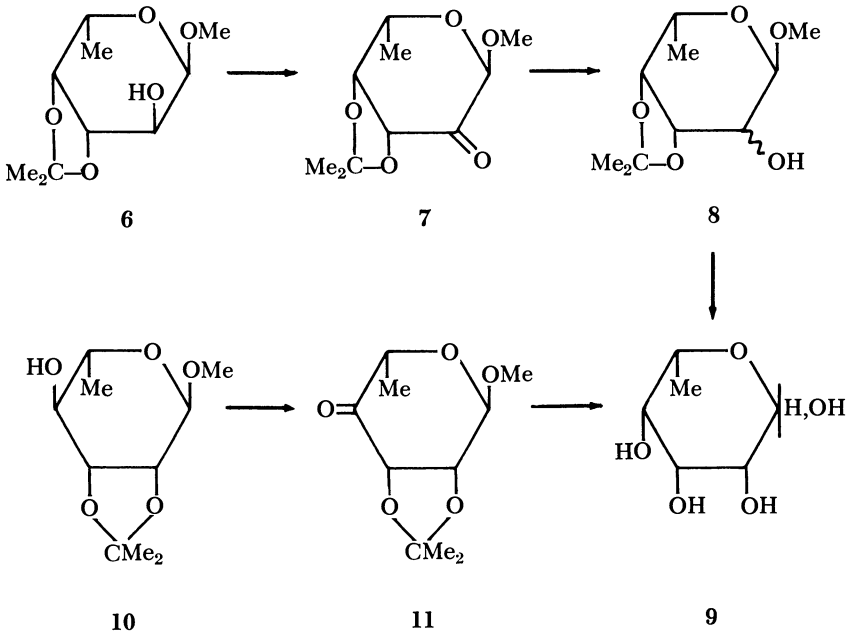
Oxidation of methyl 3,4-*O*-isopropylidene-α-L-fucoside (**6**) gave methyl 6-deoxy-3,4-*O*-isopropylidene-α-L-*lyxo*-hexopyranosidulose (**7**) which on catalytic hydrogenation yielded a mixture of methyl 6-deoxy-3,4-*O*-isopropylidene-α-L-hexosides (**8**) which was readily deacetonated by mild acidic hydrolysis. From the mixture a crystalline 2,3,4-triacetate was obtained which on complete hydrolysis gave 6-deoxy-L-talose (**9**). The configuration of the sugar follows from the fact that the same compound can be obtained from methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (**10**) by sequential oxidation to give compound **11** from which 6-deoxy-L-talose (**9**) was produced by reduction and removal of protecting groups.



The well-known reaction leading to ring expansion of cyclic ketones has been applied to derivatives of 'oxo sugars' in an attempt to develop a new route to novel deoxy sugars. By treatment with diazomethane both a five-membered (*42*) and a six-membered (*26*) sugar ring have been expanded by insertion of a methylene group.

From the reaction of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-3-ulose (prepared in 80% yield by oxidation of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose (*35,36*) with ruthenium tetroxide) with an excess of diazomethane in methanol-ether, two main products (m.p. 44°–45°C. and 76°–77°C.), both epoxides, could be isolated by chromatography of the product on a silica column. An

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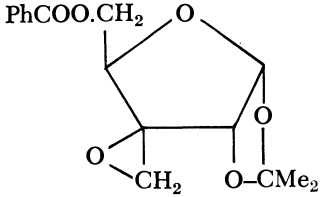
unidentified minor product and some unchanged starting material were also detected. Elemental analysis indicated that the epoxides differed by a methylene group, the higher melting compound having the higher molecular weight. The amount of each epoxide produced is dependent to some extent on the relative proportions of methanol and ether used as solvent. If methanol is in excess the higher melting compound predominates, whereas in excess of ether the other epoxide is the main product. These epoxides have been shown to have structures **12** and **13**.



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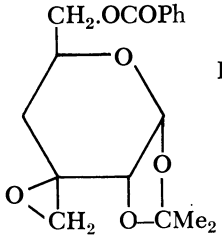

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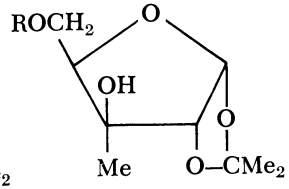

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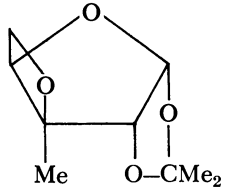

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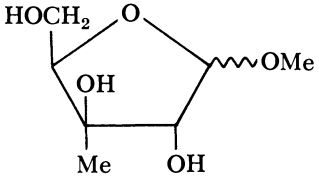

16

Treatment of the lower melting epoxide **12** with excess of lithium aluminum hydride in ether resulted both in cleavage of the epoxide and debenzoylation and afforded a 1,2-*O*-isopropylidene-3-*C*-methyl-α-D-pentofuranose (**14**, R = H) considered to have the D-*xylo* configuration, together with a trace of another product which is probably an isomer. Other work (*21,26*) in our laboratory has shown that spiro-epoxides of this type are cleaved by lithium aluminium hydride to give a *C*-methyl and not a *C*-hydroxymethyl substituent. Mono-*O*-tosylation of compound **14** (R = H) at the primary hydroxyl group and subsequent treatment of the tosyl ester **14** (R = Ts) with sodium methoxide in methanol gave a 3,5-anhydride (**15**) (*cf.* Levene and Raymond (*35,36*) who described a similar compound but lacking a methyl group

In Deoxy Sugars: Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

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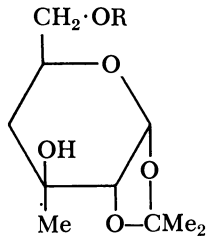
at C–3). Deacetonation and mild glycosidation of compound **14** (R = H) afforded a syrupy methyl glycoside which consumed 1 mole of periodate as expected for a glycofuranoside **16**. The uptake of oxidant was slow (42 hours at 35°C.), thereby suggesting that the hydroxyl substituents at C–2 and C–3 were trans disposed.

When the higher melting epoxide **13** was treated with lithium aluminium hydride, best in boiling tetrahydrofuran, cleavage of the epoxide and debenzoylation occurred to give a substance which has been assigned structure **17** (R = H). In methanol in the presence of the aci form of an ion-exchange resin this substance gave a syrupy methyl 3-*C*-methyl-glycoside which was found to consume slowly (42 hours at 35°C). 1.04 mole of periodate per mole of glycoside without production of formaldehyde, thereby indicating that the methylene group had been inserted between C–3 and C–4 in the original pentofuranos-3-ulose. Mono-*O*-tosylation of compound **17** (R = H) and treatment of the tosyl ester **17** (R = Ts) with sodium methoxide in methanol gave a 3,6-anhydride (**18**).



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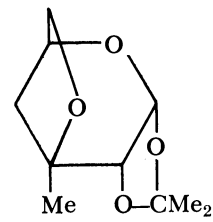

18

This procedure can be used to prepare derivatives of dideoxy sugars, as exemplified by the reaction of methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (**19**) (*25*) with an excess of diazomethane in methanol-ether at room temperature. Two crystalline epoxides **20** (m.p. 116.5°–117°C.) and **21** (m.p. 154°–154.5°C.) were isolated. The assignment of structure to epoxide **20** presented no difficulty because with lithium aluminium hydride it gave a product isomeric with that obtained either from the reaction of the glycopyranosidulose (**19**) with methylmagnesium iodide or from reductive cleavage with lithium aluminium hydride of the spiro-epoxide produced by treatment of compound **19** with dimethylsulfoxonium methylide (*31*). The *C*-methyl-glycoside produced by the latter two routes has been shown previously to have the *ribo* configuration (*25*) and so the new branched-chain glycoside **22** has the *arabino* configuration. This configurational assignment was supported by

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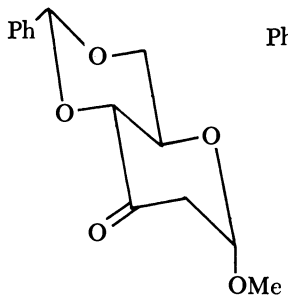
the NMR spectrum of the epoxide **20**, by high-resolution infrared spectral analysis (*20*) of the hydroxyl stretching region of compound **22**, and by periodate oxidation and the ionophoretic behavior of compound **23** obtained from the debenzylidenation of compound **22**.



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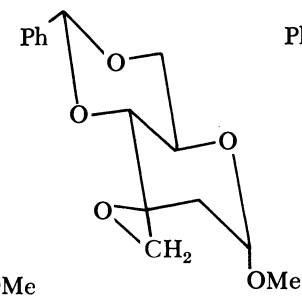

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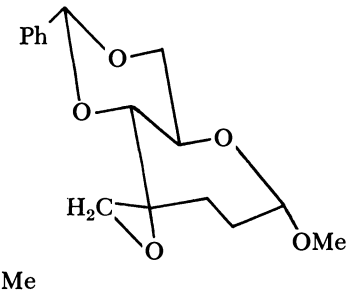

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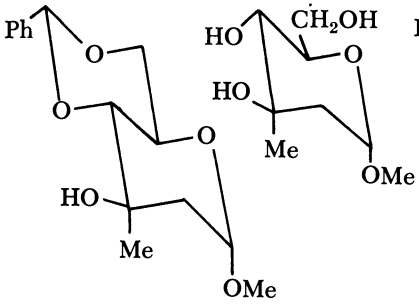
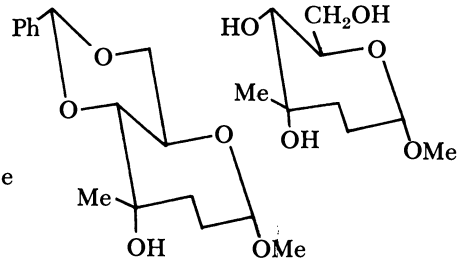


22 **23**



24 **25**

The other epoxide **21** gave a positive test for a 2-deoxy sugar and liberated benzaldehyde on acidification. Elemental analysis and molecular weight determinations indicate that epoxides **20** and **21** were not isomeric but differed by a methylene group. The NMR spectrum of this higher melting epoxide was fully consistent with structure **21** and precludes all structures containing a methyl substituent or a ring of the trimethylene oxide type. An unambiguous proton count of 20 was obtained and signal assignments were as follows: five protons of the phenyl group at τ 2.60; the singlets at τ 4.50 and 6.63 (3 protons) were owing respectively to the acetal proton and the methoxyl group at C–1; the 2 protons in the two sharp doublets τ 7.45 and 6.80 formed a typical AB system and were assigned to the protons of the epoxide ring; the

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anomeric proton gave rise to a quartet at τ 5.30 with $J_{1,2}$ = 8.0 c./sec. (whereas in epoxide **20** the quartet caused by the anomeric proton at τ 5.12 has $J_{1,2}$ = 4.0 c./sec.). A model of the epoxide shows that the anomeric proton is quasi-axial which accounts for the large splitting observed; the multiplet in the region τ 5.60–6.40 (4 protons) was no more complex than that observed in the region τ 5.60-6.30 (4 protons) of the spectrum of epoxide 20 and was assigned to H–5, H–6, H–7_{ax}, and H–7_{eq}; in the high field region the multiplet at τ 8.00-8.80 (4 protons) arises from the protons of the methylene groups at C–2 and C–3.

Reduction of epoxide **21** with lithium aluminium hydride gave a crystalline branched-chain methyl heptoside derivative **24**. The NMR spectra of compounds **21** and **24** were very similar. In the spectrum of compound **24** the disappearance of the two sharp doublets at τ 6.80 and 7.45 (2 protons) and the appearance of a singlet at τ 8.65 (3 protons) is consistent with the reductive cleavage of epoxide **21** to give a substance **24** with a methyl substituent. The multiplet at τ 7.40-8.50 (5 protons) was assigned to the four protons of the two methylene groups and the hydroxylic proton.

Debenzylidenation of compound **24** yielded a crystalline glycoside **25** and the extent and rate of its periodate oxidation supports the structure and configuration shown.

Further work on this approach to deoxy sugars is in progress, particularly to determine the directive influences which operate in the ring expansion.

Deoxy Sugars from 2-Hydroxyglycal Esters

The reaction undergone by esterified glycals when heated in water, namely, the allylic displacement of the C–3 ester grouping and formation of 2,3-unsaturated compounds (pseudoglycals) is well known, (*18,27*) but the general nature of this type of rearrangement has become apparent only recently, as a result of work in our and other laboratories (*6,12,17,19,43*). An analogous rearrangement in the 2-hydroxyglycal series has now been studied and the products of the rearrangement have been shown to be valuable intermediates in the preparation of deoxy sugars.

Initially it was necessary to devise an improved method for the preparation of 2-hydroxyglycal esters, because the standard procedure (treatment of an acylglycosyl bromide with diethylamine in benzene or chloroform solution) was inconveniently lengthy in time and frequently afforded only a moderate yield of product (5). As a result of their recent thorough investigation of the kinetic features of the dehydrobromination of tetra-*O*-acetyl-α-D-glucopyranosyl bromide Lemieux and Lineback

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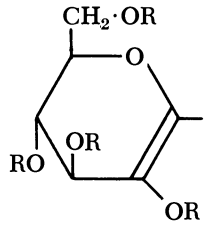
(*33*) developed a simple and efficient synthesis of tetra-*O*-acetyl-2-hydroxy-D-glucal, using acetonitrile as solvent and diethylamine and tetra-*n*-butylammonium bromide as catalysts. When my colleagues attempted to apply this method for the preparation of other esterified hydroxyglycals they found it was not successful (*23*) and they prefer to convert the bromides into iodides by brief treatment with sodium iodide in acetone solution and then to add diethylamine directly when rapid elimination takes place and is complete in 10 minutes. This reaction time compares favorably with those reported to be necessary for other methods. Fair to good yields of crystalline products are obtainable after a simple extraction procedure. In Table I the yields obtained in representative reactions are listed.

Table I. Preparation of Hydroxyglycal Esters [a]

Compound	*Yield (%)* *Iodide Method*	*Other Methods*
2-Hydroxyglucal acetate	60	51,[b] 80 [c]
2-Hydroxyglucal benzoate	70	57-68 [b]
2-Hydroxyxylal acetate	24	28 [b]
2-Hydroxyxylal benzoate	54	23 [b]
2-Hydroxygalactal acetate	32	12.5 [b]
6-Deoxy-2-hydroxyglucal acetate	55	—
2-Hydroxylactal acetate	77	44 [b]

[a] Ref. 23.
[b] Treatment of acylglycosyl bromide with diethylamine in benzene.
[c] Method of Lemieux and Lineback (*33*).

When 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal (**26**, R = Ac) was heated in boiling acetic acid, two isomeric products **27** (54%, $[\alpha]_D$ + 52°) and **28** (10%, $[\alpha]_D$ + 156°) were obtained after fractional crystallization (*22*). Elemental and NMR spectral analysis revealed that they were also isomeric with the initial hydroxyglycal acetate. They were shown to be the α- and β-anomers of 1,2,4,6-tetra-*O*-acetyl-2,3-didehydro-3-deoxy-D-*erythro*-hexose. Neither NMR spectroscopy nor polarimetry afforded reliable means of assigning anomeric configurations to the unsaturated acetates **27** and **28** but both methods, when applied to the dihydro derivatives of these compounds, revealed clearly that the major product was the α anomer, despite the fact that it is the less dextrorotatory form. Thus these unsaturated compounds constitute an exception to Hudson's rule (*29*). Optical rotatory dispersion studies demonstrated that the effect is not caused by a fortuitous crossing of the O.R.D. curves since the same relationship as pertains at 589 mμ holds over the range 230 to 400 mμ (*22*). Subsequent work in our laboratory has revealed other anomalies in unsaturated sugar derivatives (*50*).



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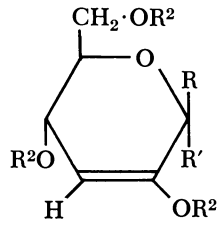

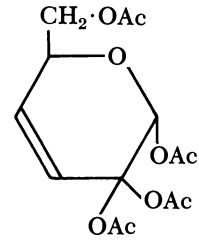

29

27, R = H, R′ = OAc, R^2 = Ac

28, R = OAc, R′ = H, R^2 = Ac

30, R = H, R′ = OAc, R^2 = Bz

31, R = OAc, R′ = H, R^2 = Bz

Clearly compound **26** (R = Ac) had undergone a reaction analogous to the glycal rearrangement. It has been demonstrated that the rearrangement of this compound also occurs at room temperature in acetic anyhydride in the presence of zinc chloride (*34*). Under these conditions, however, a further slower isomerization takes place and a third product, assigned the acetylated enone-hydrate structure **29**, was isolated. As noted later this structure has been shown to be incorrect.

Several features of the rearrangement have been elucidated. Although in the treatment of the ester **26** with acetic acid the products were isolated in only 64% yield, evidence was obtained (*22*) that finally no 1,2-unsaturated compounds remained, since the noncrystalline portion on hydrogenation and deacetylation afforded only 3-deoxy-D-*ribo* and -D-*arabino*-hexose and no 1,5-anhydrohexitols. That the components of the final mixture were in equilibrium was indicated by the observation that the main component **27** underwent reaction in boiling acetic acid to give a solution with the same optical activity as that of the original reaction mixture. Thus the 2,3-unsaturated compounds are more stable than the hydroxyglycal derivatives and the α isomer **27** is more stable than its anomer **28**.

In the presence of strong acids the reactions of glycals leading to 2,3-unsaturated compounds are superseded by additions which give rise to 2-deoxyglycosyl derivatives. With tetra-*O*-acetyl-2-hydroxy-D-glucal in acetic acid solution, however, methanesulfonic acid (in a concentration which would cause addition of acetic acid to the double bond of tri-*O*-acetyl-D-glucal) merely accelerated the rearrangement and the unsaturated acetates **27** and **28** were obtained just as from the noncatalysed

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reaction. The presence of the C–2 acetoxyl group, which can oppose the mesomeric effect of the ring oxygen atom, therefore apparently inhibited the protonation at this site which would be the first step in an addition process, and the added acid facilitated removal of the C–3 acetoxyl group. By heating tetra-*O*-benzoyl-2-hydroxy-D-glucal in acetic acid two 1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-2,3-didehydro-3-deoxy-D-*erythro*-hexoses [**30**, 63%, $[\alpha]_D$ + 52°; **31**, 13%, $[\alpha]_D$ + 91°] were obtained (*22*). NMR spectral measurements revealed that these compounds were present in 70%, and 30% respectively at equilibrium. It was shown (*22*) that this pair of compounds also represents an exception to the isorotation rules.

On hydrogenation (Pd catalyst) both compound **27** and **28** gave mixtures of 1,2,4,6-tetra-*O*-acetyl-3-deoxy-D-*ribo*- and -*arabino*-hexoses (-3-deoxy-D-glucose and -D-mannose). Deacetylation of these mixtures was effected with sodium methoxide in methanol solution. Although such treatment of aldose peracetates, in general, is complicated by the sensitivity of the sugars themselves to the reagent, with 3-deoxyaldoses the de-esterification proceeds without degradation because an elimination β- to the reducing function cannot occur. Since no 3-deoxy-D-*arabino*-hexose was produced during the methoxide treatment of tetra-*O*-acetyl-3-deoxy-β-D-*ribo*-hexose the de-esterification also occurs without epimerization.

The 3-deoxyhexoses were obtained crystalline after separation on a column of cellulose, a rather tedious operation: recently separation of these sugars by fractional crystallization has been reported (*41*). From the major unsaturated ester **27**, 3-deoxy-D-*ribo*- and -*arabino*-hexose were shown to have been produced respectively in the ratio 66:34, and from ester **28** in the ratio 24:76. Saturation of the enol grouping of the esters **27** and **28** gives, therefore, the 1,2-*cis* products preferentially. However, some hydrogenolysis of the anomeric acetate group accompanies simple saturation of the double bond and poses another separation problem.

Hydrogenation of 1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-2,3-didehydro-3-deoxy-α-D-*erythro*-hexose (**30**) gave a crystalline 1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-3-deoxyhexose in 60% yield, which on de-esterification with methoxide in methanol afforded 3-deoxy-α-D-*ribo*-hexose (*22*). It was not possible to hydrogenate the β isomer under the conditions used for the α form, presumably because the large *trans*-related C–1 and C–4 ester groupings prevent the necessary contact with the catalyst.

Subsequently this route to 3-deoxy sugars was developed by further work in our laboratory by Ferrier and Sankey (*23*). They demonstrated that although tetra-*O*-acetyl-2-hydroxy-D-galactal undergoes rearrangement in boiling acetic acid, and that reaction is speeded up and goes to completion if catalytic amounts of methanesulfonic acid are added, the

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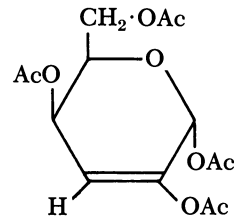
reaction characteristics differ markedly from those found with tetra-*O*-acetyl-2-hydroxy-D-glucal (**26**, R = Ac). In the hydroxy-galactal series reaction is slower (for completion four hours at 96°C. in the presence of 0.004*M* methanesulfonic acid are required, whereas the glucal analog **26** reacts in 30 minutes and in 0.002*M* methanesulfonic acid at this temperature) and is complicated by a competitive addition reaction. These differences may be attributed to anchimeric assistance provided by the C–4 acetoxyl group which facilitates the allylic rearrangement in the glucal series specifically. In addition only the α anomer of the rearranged unsaturated ester is formed and an explanation of this in terms of stereochemical and stereoelectronic factors has been given by Ferrier and Sankey (*24*).

From the rearrangement of tetra-*O*-acetyl-2-hydroxy-D-galactal in boiling acetic acid it was possible to isolate 1,2,4,6-tetra-*O*-acetyl-2,3-dide-hydro-3-deoxy-α-D-*threo*-hexose (**32**) (58%) and a small amount of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose. In the reaction mixture the presence of some α-pentaacetate was demonstrated chromatographically but NMR spectroscopy indicated no resonances corresponding to the β anomer of compound **32**. These spectral measurements indicate that compound **32** constituted 80% of the mixture of products.



32

It was noted (*23*) that the NMR spectrum of compound **32** was identical with that published (*34*) for the third product (assigned structure **29**) isolated from the reaction of tetra-*O*-acetyl-2-hydroxy-D-glucal with acetic anhydride and zinc chloride. The identity of the compounds was fully established and a revised structure proposed for this third product. In the presence of zinc chloride, therefore, epimerization can occur at an allylic site and the *quasi*-equatorial C–4 acetoxy group in the *erythro* isomers **27** and **28** can assume the favored *quasi*-axial orientation (*24*).

Hydrogenation of the α-unsaturated ester **32** was accompanied by extensive hydrogenolysis and NMR spectroscopy revealed that only 45% of a 3-deoxyhexose tetraacetate was present in the products and only one

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resonance in the anomeric region of the spectrum was observed. Nevertheless, crystalline 1,2,4,6-tetra-*O*-acetyl-3-deoxy-α-D-*lyxo*-hexose (32%) was isolated which on deacetylation afforded syrupy 3-deoxy-D-*lyxo*-hexose which, although chromatographically distinguishable from 3-deoxy-D-*xylo*-hexose, was found to give the same osazones as this sugar. Although hydrogenation of the *erythro* isomers **27** and **28** had afforded predominantly products with *cis*-related 1,2-groups, in the case of the *threo* compound **32** only the saturated α-*lyxo* product—*i.e.*, that with the *trans* configuration at C–1—C–2—was detected by chromatography and NMR spectroscopy. Besides hydrogenation, however, appreciable hydrogenolysis of the ester **32** occurred. That it was the C–1 acetoxyl group that undergoes cleavage was established by deacetylating the product of a hydrogenation and separating the products on a cellulose column into two components, namely 3-deoxy-D-*lyxo*-hexose and a more mobile, nonreducing, substance which gave a crystalline tribenzoate. The nonreducing material is considered to be 1,5-anhydro-3-deoxy-D-*lyxo*-hexitol (*23*), so the hydrogenolysis of the acetoxyl group at C–1 does not affect the course of the addition reaction at the unsaturated linkage between C–2 and C–3.

Likwise the 2,3,4-tri-*O*-acetyl and 2,3,4-tri-*O*-benzoyl derivatives of 2-hydroxy-D-xylal were isomerized in boiling acetic acid to give equilibriated mixtures of respectively anomeric 1,2,4-tri-*O*-acetyl-2,3-didehydro-3-deoxy-D-*glycero*-pentoses and 1-*O*-acetyl-2,4-di-*O*-benzoyl-2,3-didehydro-3-deoxy-D-*glycero*-pentoses. These rearrangements differ from those found for the hydroxyglycals derived from hexoses because the β forms predominate and the α anomers are present only in small amount. The rearrangements of both the acetate and benzoate of 2-hydroxy-D-xylal were accompanied by additions to the 1,2-double bond to give minor amounts of saturated products. Hydrogenolysis interfered with the hydrogenation of the 2,3-unsaturated esters but after deacylation it was possible to obtain 3-deoxy-D-*erythro*-pentose from both 1,2,4-tri-*O*-acetyl-2,3-didehydro-3-deoxy-β-D-*glycero*-pentose and 1-*O*-acetyl-2,4-di-*O*-benzoyl-2,3-didehydro-3-deoxy-β-D-*glycero*-pentose.

In similar manner 3,6-dideoxyhexoses have been prepared from esterified 6-deoxy-2-hydroxyglycals. 2,3,4-Tri-*O*-acetyl-6-deoxy-2-hydroxy-D-glucal was converted into the α and β forms of 1,2,4-tri-*O*-acetyl-2,3-didehydro-3,6-dideoxy-D-*erythro*-hexose. The α anomer was the main product (77%, 55% isolated crystalline) and, in addition to the β anomer (19%), a small amount (4%) of saturated products was obtained. On hydrogenation, the major product also suffered some hydrogenolysis but afforded two tri-*O*-acetyl-3,6-dideoxyhexoses which were shown by NMR spectroscopy to be present in the ratio 12:13 and to have the α configuration. Deacetylation of the reduction products gave

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a mixture from which the epimeric free sugars (3,6-dideoxy-D-*ribo*- and -*arabino*-hexose) were obtained.

Just as inversion at the allylic C–4 position occurs on treatment of 1,2,4,6-tetra-*O*-acetyl-2-3-didehydro-3-deoxy-α-D-*erythro*-hexose with acetic anhydride and zinc chloride, so was it found that 1,2,4-tri-*O*-acetyl-2,3-didehydro-3,6-dideoxy-α-*erythro*-hexose isomerizes partially to the D-*threo* isomer under these conditions. The same compound could be obtained alternatively from 2,3,4-tri-*O*-acetyl-6-deoxy-2-hydroxy-D-galactal by its almost specific rearrangement to 1,2,4-tri-*O*-acetyl-2,3-didehydro-3,6-dideoxy-α-D-*threo*-hexose. In the rearrangement no detectable amount of β anomer and only a very small amount (3%) of saturated products were produced. As with tetra-*O*-acetyl-2-hydroxy-D-galactal the rearrangement was slower than with the analogous glucal derivative. On hydrogenation of tri-*O*-acetyl-2,3-didehydro-3,6-dideoxy-α-D-*threo*-hexose there was a competitive hydrogenolysis but it was possible to isolate 1,2,4-tri-*O*-acetyl-3,6-dideoxy-α-D-*lyxo*-hexose from the reaction products and deacetylation of this compound afforded 3,6-dideoxy-D-*lyxo*-hexose (*23*). Hydrogenation had occurred, therefore, in a similar direction to that found for 1,2,4,6-tetra-*O*-acetyl-2,3-didehydro-3-deoxy-α-D-*threo*-hexose.

In addition to rearrangements induced by boiling acetic acid, the efficacy of substituted acetic acids—*e.g.*, monochloroacetic, trichloroacetic, and trifluoroacetic acids—in suitable inert solvents has been examined (*50*). For example, from the mixture obtained when tetra-*O*-benzoyl-2-hydroxy-D-glucal was heated with trichloroacetic acid in boiling benzene it was possible to isolate 2,4,6-tri-*O*-benzoyl-2,3-didehydro-3-deoxy-1-*O*-trichloroacetyl-α-D-*erythro*-hexose which in ethanol-benzene underwent reaction to give ethyl 2,4,6-tri-*O*-benzoyl-2,3-didehydro-3-deoxy-β-D-*erythro*-hexoside in good yield. It was anticipated that this sequence of reactions would be useful to obtain intermediates for preparing 3-deoxyaldosides, but unfortunately hydrogenation of the unsaturated glycoside was accompanied by hydrogenolysis and deoxyglycosides were not obtained.

Acknowledgement

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9

Some Approaches to the Synthesis of Halodeoxy Sugars

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Selected methods of introducing halogen atoms in sugar molecules (exclusive of the anomeric carbon atom) are outlined with special emphasis on the use of new reagents. Recent developments in the preparation of halodeoxy sugars from sulfonate esters are summarized with particular reference to the reactivity of sulfonate esters at C–4 in sugar derivatives. Several procedures which were discovered many years ago but have received little attention are brought to light and compared with the presently available routes to identical or similar compounds. The utility of some halodeoxy sugars as intermediates in the synthesis of deoxy sugars of biological significance is illustrated by some examples from the literature.

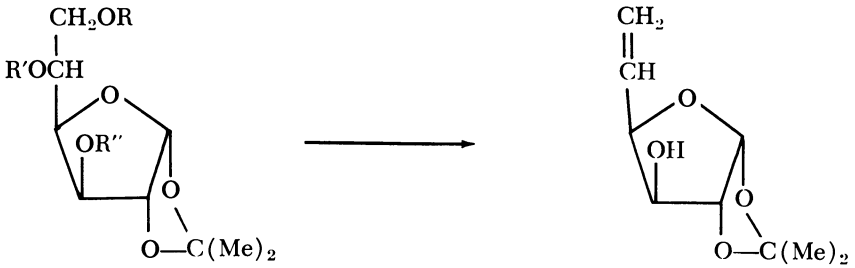
A chapter on deoxy sugars (*43*) recently prepared by this author contains various methods for preparing the halodeoxy sugars. These were mentioned briefly as they were used as intermediates in the synthesis of deoxy sugars. The introduction of halogen atoms in sugars constitutes one of the earlier contributions by chemists to synthetic carbohydrate chemistry. Halosugars were considered to be ideal precursors for terminal deoxy sugars. Unfortunately their potential utility for the preparation of other sugar derivatives has been relatively unexplored. While some of the well-established methods for introducing halogen atoms in sugars have been widely expanded, several new ones have become available within the past five years. This paper will be concerned with updating some of the well-known procedures and with outlining some of the newer methods. A critical appraisal of these with regard to practicality, merits, disadvantages, and general suitability as intermediates for synthetic work will be given.

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

The Preparation of Halodeoxy Sugars by Displacement of Sulfonate Esters

This reaction was first applied in the sugar field by Freudenberg and Raschig (*40*) and is being used up to the present day essentially under the same conditions. Tipson (*103*) reviewed the literature extensively in 1953, and the subject is being brought up-to-date in the same series (*5*). While primary sulfonates in sugars were converted into iodides with ease, most secondary sulfonate esters were looked upon as relatively unreactive substances, and this property was used as a diagnostic feature for the presence of the former group in a molecule. This generalization still holds true with a notable exception of the reactivity of sulfonate esters at C–4 in pyranose sugars. These have been found to react with various nucleophiles with remarkable ease compared with other secondary sulfonates in a pyranose ring. This finding has been put to good advantage in the synthesis of several biologically important aminodeoxy and deoxy sugars. Sodium iodide has been the choice reagent because of its solubility properties. The reaction is usually performed in a sealed tube at temperatures ranging from 80°-130°C. in such solvents as acetone, pentanedione, acetic anhydride, 2-butanone, etc.

From the many examples reported in the literature (*103*) it is clear that the reaction conditions do not affect such groups as esters, acetals,

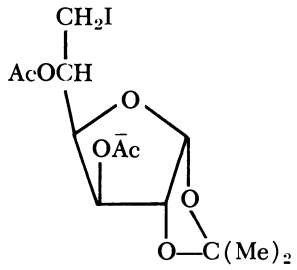


1a, R = Ts
R', R'' = H

2

1b, R, R' = Ts
R'' = H

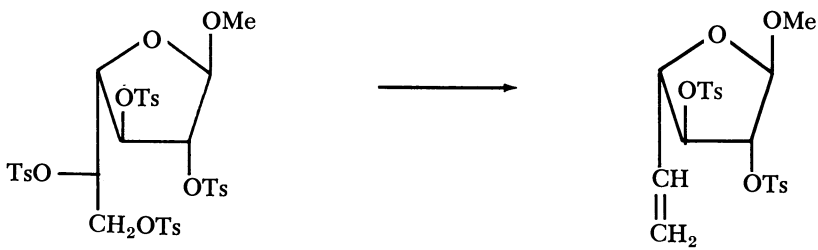
1c, R, R', R'' = Ms



1a

3

and anhydro functions. Side reactions during the displacement reaction are not prevalent although it should be noted that in certain sulfonate esters the reaction takes a totally different course. For example, 1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-D-glucofuranose. (**1a**) is transformed into the 5,6-unsaturated derivative **2** in the presence of sodium iodide within 2 hours (*10*). When the reaction was performed in refluxing acetic anhydride, the product was the expected 6-deoxy-6-iodo derivative **3** (*8*). The formation of terminal unsaturated derivatives from the reaction of vicinal primary-secondary sulfonates of the type **1b** and **1c** with sodium iodide was recognized by Tipson and Cretcher (*104*). The reaction of **1b** and **1c** with sodium iodide in acetone afforded in both cases the corresponding 5,6-unsaturated sugars rather than the iodo analogs (*66*). Similarly, treatment of **4** with sodium iodide in acetone gave the



4 **5**

5,6-unsaturated derivative **5** with no introduction of iodine in the molecule (*6*). Other examples involving the formation of unsaturated sugar derivatives (*36*) and various other side reactions have been recorded (*103*).

Another deviation from the normal displacement reaction of primary tosylates occurs in nucleoside derivatives (*39, 81*) where cyclonucleosides and anhydronucleosides are formed by participation of a nitrogen atom (as in purine nucleosides) and oxygen atom (as in pyrimidine nucleosides), respectively. Iodonucleosides can result from these reactions only if these cyclic compounds are prone to attack by iodide ion. Several new examples of unexpected reactions during the solvolysis of sulfonate esters in sugar derivatives have been recorded in the past few years (*2, 4, 5, 7, 15, 44, 62, 63, 94*).

Metal halide salts other than sodium iodide have been used sparsely to prepare halodeoxy sugars from sulfonate esters. Lithium chloride (*107*) and lithium bromide (*33*) have found limited application. Potassium fluoride (dihydrate) in absolute methanol has been used (*51, 52*) to introduce fluorine atoms in terminal positions of various D-glucose derivatives. The reaction is conducted in sealed tube systems and requires

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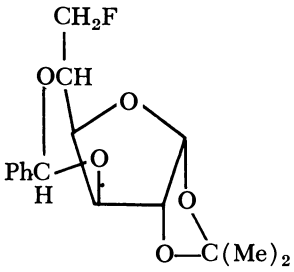
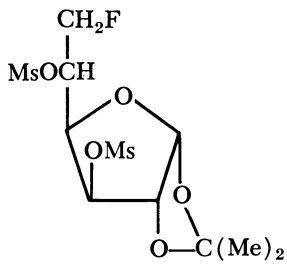
heating periods up to 15 hours at 100°C. Some of the 6-deoxy-6-fluoro-D-glucose derivatives which were made by this method in the early 1940's are illustrated.



6



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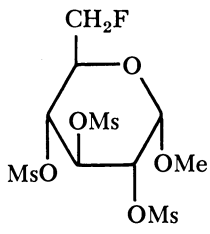

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It should be noted that whereas the reaction of **1c** with sodium iodide in acetone forms (*10*) the 5,6-unsaturated derivative **2c,** a substitution reaction takes place when potassium fluoride is used. Employing somewhat higher temperatures, Kent and co-workers (*68, 102*) have observed that methoxyl groups are introduced in the molecule in some cases, without incorporation of fluorine. Methylation could be avoided if other solvents such as ethylene glycol or glycerol were used instead of methanol. Note that *N,N*-dimethylformamide, which is a very unusual solvent for nucleophilic displacement reactions, was found unsatisfactory in the displacement of methyl 2,3-*O*-isopropylidene-5-*O*-methylsulfonyl-D-ribofuranoside with potassium fluoride (*69*). The yield of the desired 5-fluoro derivative was much better when methanol was used instead (150°-156°C., 18 hours, sealed system) (*69*). Because potassium fluoride is alkaline (owing to a strong tendency for H–bond formation), care should be taken to protect hydroxyl groups in the molecule to prevent possible anhydro ring formation. Attempted formation of fluoro sugars through displacement reactions of sulfonates have produced anhydro sugars (*74*) instead.

The use of tetra-*n*-butylammonium fluoride (*54*) in an aprotic solvent such as acetonitrile may be more advantageous. Foster and colleagues (*19, 37*) have effected an S_N2 type of reaction using this reagent in the conversion of 1,2:5,6-di-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-D-allofuranose into the C–3 epimeric fluorodeoxy derivative. Note that whereas potassium fluoride is ineffective in displacing secondary sulfonate esters in sugars, tetra-*n*-butylammonium fluoride is capable of effecting a displacement with Walden inversion even in a *furanose* drivative.

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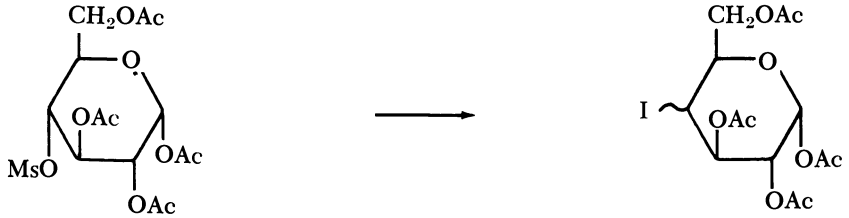
Perhaps the earliest report of the replacement of a sulfonate ester attached to a secondary carbon atom in a sugar derivative was that of Helferich (*53*). Under quite drastic conditions (sodium iodide in acetone, 105°C., 72 hours, sealed system) the 4-mesylate derivative **9** was converted into a crystalline 4-deoxy-4-iodo sugar derivative **10** in 46% yield. Although the position of the iodine atom was established, the configuration at C–4 was not known.



9 **10**

In another study Hess and Stenzel (*59*) have reported that treatment of methyl α-D-glucoside (**11**) with a 6*M* equivalent of tosyl chloride in pyridine for two days at 75°C. afforded a crystalline "monochloro" derivative in which the C–4 tosyloxy function was assumed to have been replaced preferentially. Further reaction of this product with sodium iodide in acetone at 105°C. for 24 hours afforded a 59% yield of the crystalline methyl 4-chloro-4,6-dideoxy-6-iodo-hexoside derivative. When **11** was treated with a 6*M* excess of tosyl chloride in pyridine at 80°C. for four days, the product (54%) was presumed to be crystalline methyl 4,6-dichloro-4,6-dideoxy-2,3-di-*O*-*p*-tolysulfonyl-α-D-glucopyranoside, which on the basis of modern mechanistic concepts is shown in the more probable configuration (D-*galacto*) as **13**. Reaction of this product (2 grams) with sodium iodide in acetone at 130°C. for 24 hours afforded 1.2 grams of starting material which was identified by melting point and mixed melting point. It was also unaffected with zinc and acetic acid, or sodium and benzene. Essentially the same chlorodeoxy sugars could be obtained in the corresponding β-anomeric glycoside series.

The chlorinating agent was implied to be the pyridine hydrochloride formed during the reaction since treatment of either compounds with pyridine hydrochloride at 95°C. for 48 hours afforded **13** in about 80% yield. The same reagent had no effect on compound **11** which implies that chloride ion is attacking the initially formed **12**. The formation of **13** was validated by comparison with a methyl 4,6-dichloro-4,6-dideoxy hexoside (now established as an α-D-galactoside) although the original D-*gluco* configuration was assumed (*59*) to have been maintained. The

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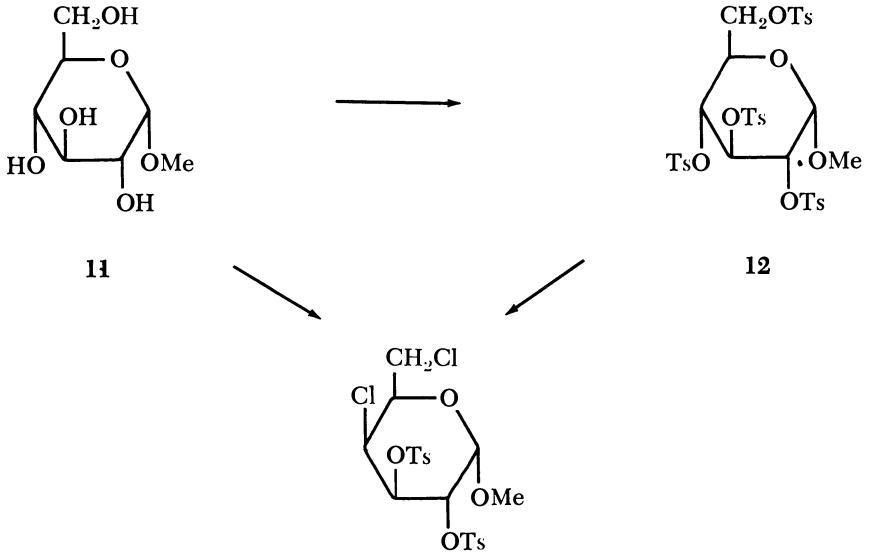
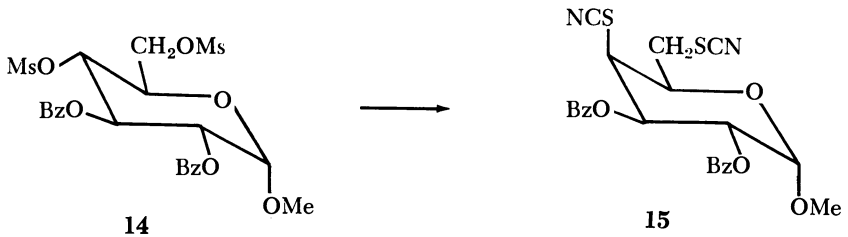


11 12 13

observations concerning the relative reactivities of C–4 and C–6 tosylates as well as some tentative conclusions regarding the stereochemistry of the products should be verified by repetition of this work and by correlating the reaction products with known structures. The reported ease of preparing compound **13** from **11** in fairly good yield (54%) makes this rather neglected reaction an attractive synthetic route to 4,6-disubstituted hexoses and should present some obvious implications in synthetic work. Other examples are known where at slightly elevated temperatures excess tosyl chloride will afford chlorinated sugars (*53, 103*).

These observations reported in the mid-30's on the relative reactivities of the C–4 and C–6 tosylates toward a nucleophile such as sodium iodide, lay dormant until 1963, when it was found (*60*) that treatment of methyl 2,3-di-*O*-benzoyl-4,6-di-*O*-methylsulfonyl-α-D-glucopyranoside (**14**) with potassium thiocyanate at 130°C. for 48 hours afforded a 40% yield of the corresponding 4,6-dithiocyanato derivative **15**.



14 15

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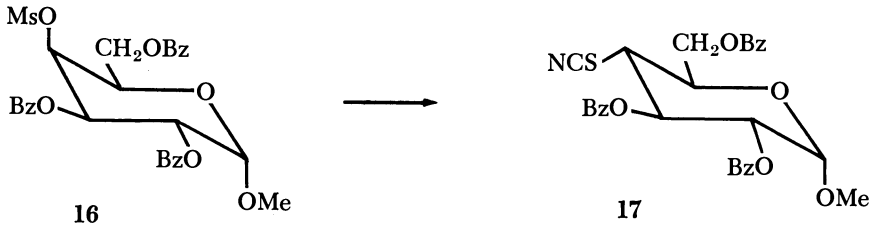
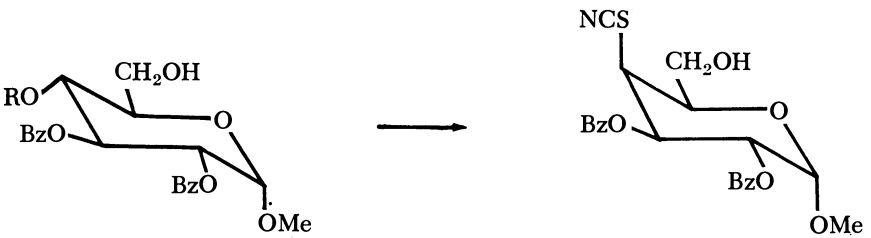
Product **15** was eventually transformed into methyl 4,6-dideoxy-α-D-*xylo*-hexopyranoside by reductive desulfurization. A related reaction is the formation (*41*) of the 4-thiocyanato derivative **17** in 47% yield from **16** (140°C., 22 hours in *N*,*N*-dimethylformamide). This has been suggested as a good route to 4-deoxy-D-*xylo*-hexoses compared with the available methods (*43*).



16 **17**

The reactivity at the C–4 position of hexose and hexoside sulfonates has been demonstrated in the *gluco* and *galacto* series and could undoubtedly be extended to other sugars as well. In another example (*25*), methyl 2,3-di-*O*-benzoyl-4-*O*-*p*-tolysulfonyl-α-D-glucopyranoside (**18a**) was treated with potassium thiocyanate in *N*,*N*-dimethylformamide at 140°C. for 9 hours to give the C–4 epimeric thiocyanato derivative **19** in 34% yield. The corresponding 4-*p*-bromobenzenesulfonate (**18b**) however, afforded a 55% yield of **19** in only 2½ hours of reaction time.

It is of interest now to note the somewhat unpredictable effects of the aglycon portion, the substituents at C–2, C–3, and C–6, and the nature of the nucleophile in related displacement reactions. Owen and



18a, R = CH_3—C$_6$H$_4$—SO_2—

18b, R = Br—C$_6$H$_4$—SO_2—

19

Ragg (*85*) reported that no displacement of the tosyloxy function could be effected by treatment of methyl 2,3,6-tri-*O*-benzoyl-4-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (**20a**) (or the 4-methylsulfonyl derivative **20b**)

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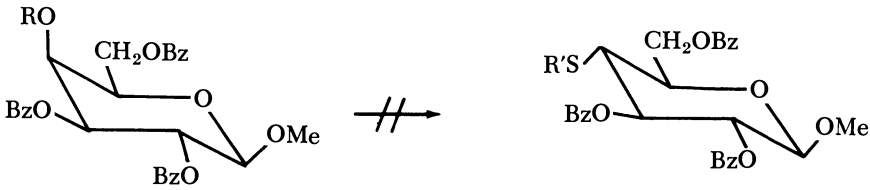
with either potassium thiolacetate or potassium thiocyanate in *N,N*-dimethylformamide at 105°C. and 140°C., respectively, for periods up to 50 hours.



20a, R = CH_3—⟨○⟩—SO_2—

20b, R = CH_3SO_2—

21

R′ = CN, $COCH_3$

The lack of reactivity at C–4 cannot be solely attributed to the presence of the bulky benzoate functions at C–2 and C–3 since the corresponding tri-*O*-acetate was found to be equally unreactive. Essentially the same reaction is successful with **16** which differs from **20** only in the configuration at C–1. In remarkable contrast to the behavior of **20**, the corresponding 2,3-di-*O*-methyl derivative **22** reacts with potassium thiolacetate in *N,N*-dimethylformamide to give after only 3 hours at 110°C. a good yield of **23** in which both C–4 and C–6 have been substituted. When **22** was treated with sodium iodide in acetone under conditions which are known to replace primary sulfonates, both sulfonates were replaced. Thus, as the authors put it, a situation existed where the secondary sulfonate was being displaced too easily. Optimum conditions for the selective replacement of the C–6 sulfonate were found to be 98°C. for 48 hours where a 21% yield of the crystalline **24** could be obtained. Such behavior is in general agreement with other observations (*103*) concerning the lower reactivity of the primary sulfonyloxy groups in derivatives of galactose as compared with glucose analogs. A study (*82*) of the effects of substituents in 6-*O*-*p*-tolylsulfonyl-D-galactose and galactoside derivatives on the rates of displacement by sodium iodide in acetone, *N,N*-dimethylformamide and methylsulfoxide, showed some retarding influence when ketal groups were present at C–3 and C–4. Nevertheless, in a 2,3,4-tri-*O*-methyl derivative the rate of reaction was still much lower than in the D-*gluco* analog. The conclusion is that the C–4 substituent in the galactose structure has only a minor influence in retarding the displacement reaction at C–6. This low reactivity has been

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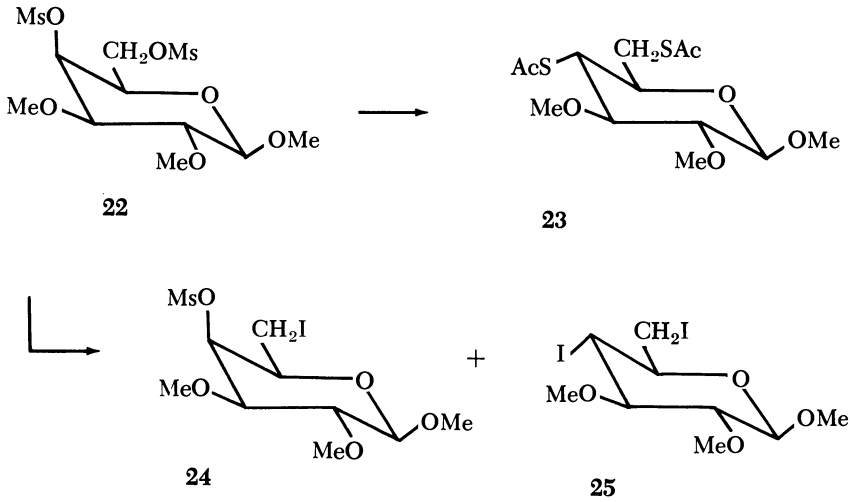
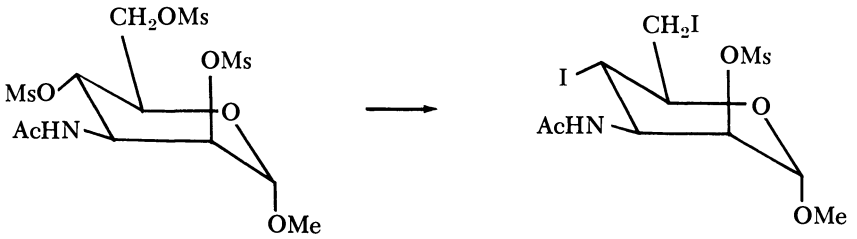
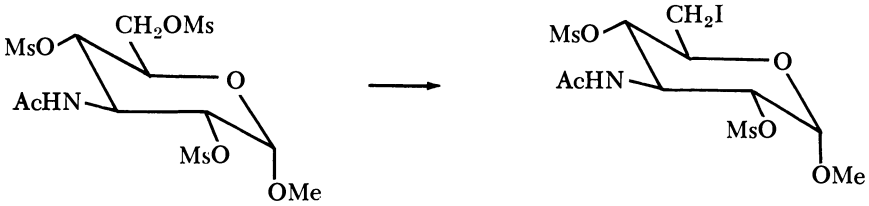
attributed (*101*) to an electronic effect exerted by the lone pairs of electrons of the ring oxygen and the axial C–4 oxygen which tend to repel the approaching charged nucleophile.



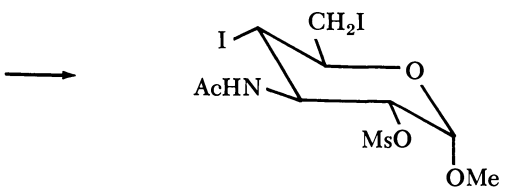
Iododeoxy sugars have been valuable intermediates for the synthesis of deoxy and aminodeoxy sugars of biological interest (*30, 45*). An approach (*87*) to the synthesis of desosamine (3-dimethylamino-3,4,6-trideoxy-D-*xylo*-hexose) involved the reaction of methyl 3-acetamido-3-deoxy-2,4,6-tri-*O*-methylsulfonyl-α-D-mannopyranoside (**26**) with sodium iodide in 2-butanone under reflux for 28 hours. There was rapid formation of sodium methanesulfonate-sodium iodide complex (NaOMs • NaI) and the crystalline di-iodo derivative **27** was subsequently isolated in approximately 60% yield. Because of the participating ability of the acetamido function, the replacement of the mesyloxy group at C–4 by iodine was effected with overall retention of configuration, although conclusive proof was not given. The reaction was also successful with the C–2 epimeric D-*gluco* analog **28**, however a period of 50 hours was required to produce the di-iodo derivative **30** (40%). The 6-deoxy-6-iodo derivative **29** was assumed to have been initially formed and subsequently reacted during the extended reaction time. The involvement of the acetamido group in the reaction leading to **30** was supplemented by the formation of water soluble by-products, presumably oxazolinium salts. The configuration at C–4 in **30** was once again assumed to be unperturbed (D-*gluco*). Although no kinetics were performed, the experimental data on the above two reactions demonstrates that the rate of the displacement reaction at C–4 is somewhat dependent on the configuration of C–2.



26 27

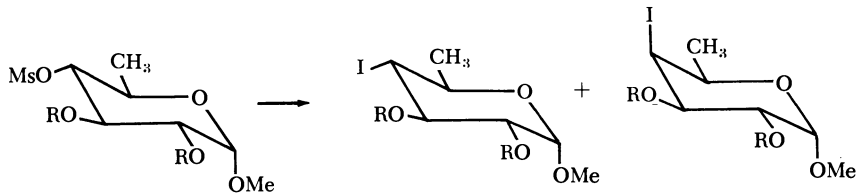


28 29



30

No quantitative data were available on the reactivity at C–4 in hexose sulfonates until the studies of Stevens and co-workers (*95*). It was shown that when methyl 6-deoxy-2,3-di-*O*-benzyl-4-*O*-methylsulfonyl-α-D-glucopyranoside (**31**) was allowed to react with sodium iodide in pentane-



31 R = CH_2Ph 32 33

dione at 125°C. for 3 hours, both C–4 epimeric deoxyiodo compounds **32** (D-*gluco*) and **33** (D-*galacto*) were formed in the average yields of 9% and 34%, respectively. Assuming Walden inversion, the expected product would have been the 4,6-dideoxy-4-iodo galactoside (**33**). The crystalline **32** and **33** had optical rotations consistent with the assigned structures and were each transformed into the known 4-azido-4,6-dideoxy derivatives. When treated under the same conditions, the C–4 epimeric mesylate derivative of **31** gave a 39% yield of **32** and a 4% yield of **33.** Further studies revealed that **32** and **33** could be interconverted in the presence of sodium iodide in acetone at temperatures as low as 60° to 80°C., conditions in which **31** remained unaffected. The kinetics of the interconversion **32**⇆**33** was studied using radioactive iodide ions. By using a large excess of ^{131}I, its incorporation into **32** and **33** becomes an irreversible process since in the early stages of the displacement the excess ^{131}I prevents the reverse reaction—*i.e.*, the liberation of non-labeled iodine. From the absolute rate constants it was concluded that **33** (axial C–4 substituent) was reacting 2.8 times (at 62.8°C.) and 2.4 times (at 82°C.) faster than **32** (equatorial C–4 substituent), corresponding to a ground state energy difference of about 0.8 kilocalories per mole.

It is evident from these observations that the products resulting from the reaction of isolated C–4 sulfonate esters of pyranoses with nucleophiles should be carefully analysed. Consideration should be given to the conformation of the expected product, the nature of the nucleophile, that of the leaving group and to the geometry of the transition state (whether resembling the starting material or the product). Epimerization at C–4 during displacement reactions becomes more significant in those cases where the leaving group is of low nucleophilicity (sulfonate) and the attacking anion has a high order of nucleophilicity (*61*). In addition, it should be noted that when the entering nucleophile assumes an axial disposition further reaction of this "initial" product with excess reagent can take place, leading eventually to epimeric products. The thermodynamically more stable "equatorial" C–4 epimer usually predominates in such mixtures. Experience has shown that relatively short times are required for such displacement reactions with nucleophiles such as azide, iodide, and thiocyanate.

Attempted selective displacement (*96*) of the primary tosylate function in **34** with sodium iodide in refluxing 2-butanone led to the 6-deoxy-6-iodo derivative **35** in 32% yield only, while the di-iodo derivative **36** was formed in 45% yield. These results are to be compared with those reported by Owen and Ragg (*85*) who observed no reaction with either potassium thiolacetate or potassium thiocyanate in the corresponding β-series.

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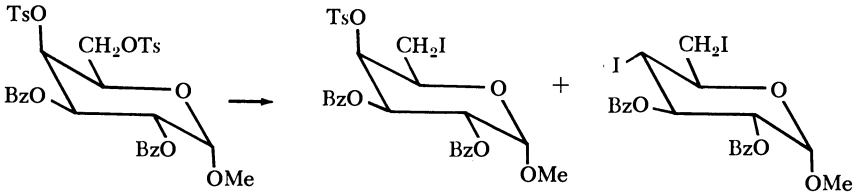
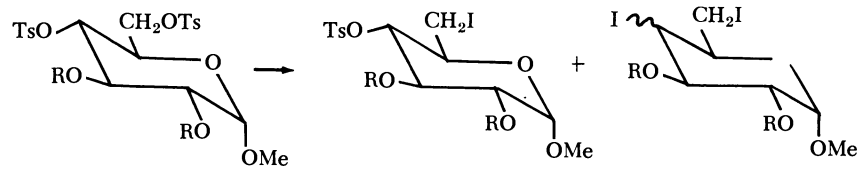


34 35 36

The D-*gluco* analog **37** reacted with sodium iodide in refluxing 2-butanone to give the crystalline 6-deoxy-6-iodo derivative **38** in 82% yield (*97*). Only 11% of the mixed di-iodo derivative **39** was formed in this case, which reflects on the higher order of reactivity at C–4 in **34** compared to **37**.



37 38 39

R = CH_2Ph

In view of the unexpected effects of the C–2 and C–3 substituents on the reaction of C–4 sulfonates, it is worthwhile to point out the observations made with some 2,3-dideoxy derivatives. Treatment of ethyl 2,3-dideoxy-4,6-di-*O*-methylsulfonyl-D-*erythro*-hexopyranoside (**40**) with sodium iodide and acetone at 115°C. caused the displacement of the C–6 mesylate group selectively to give **41.** Catalytic hydrogenation then gave the corresponding ethyl 4-*O*-methylsulfonyl-2,3,6-trideoxy-α-D-*erythro*-hexoside in good overall yield (83%) (*72*).

In the original work (*72*), the authors stated that heating of **42** with excess sodium iodide did not result in further exchange. The extensive studies of Stevens and co-workers (*96, 97*) on the displacement reactions of compounds much related to **40,** indicate that the C–4 sulfonate group can indeed be displaced by various nucleophiles. In fact compound **42** and its C–4 epimer (**43**) (D-*threo*) have been subjected to displacement reactions with benzoate (*38*), acetate and azide (*98*) ions to give the corresponding C–4 inverted products.

It is of interest to note that treatment (*23*) of the 2,3-unsaturated analog **44** with sodium benzoate in *N,N*-dimethylformamide affords compounds **45** and **46** (3:1) with inversion at C–4. The selective formation

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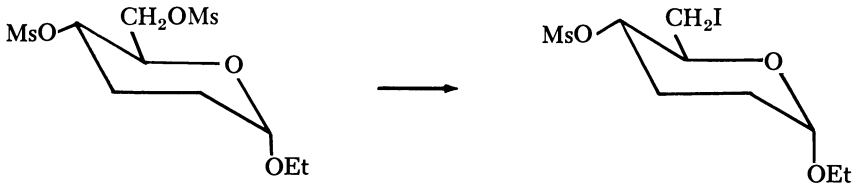
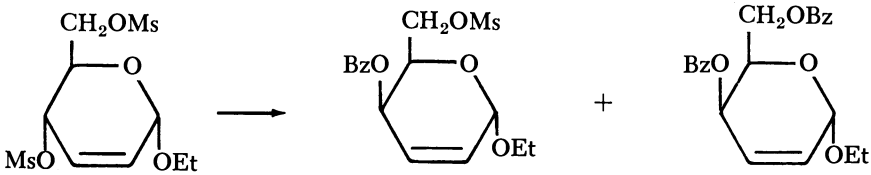


40 **41**

42 **43**

of **45** is undoubtedly the result of the allylic type activation at C–4. Starting with the epimeric D-*threo* isomer the two products corresponding to **45** and **46** (D-*erythro*) were formed (*23*) in equal amounts. Selective displacement with iodide ion at room temperature had already been reported in the case of **44** in 1950 (*72*).



44 **45** **46**

The replacement of primary sulfonate esters by halides has found application in the chemical modification of antibiotics. Thus tri-*N*-carbobenzoxy kanamycin was selectively tosylated to give the 6-*O*-*p*-tolylsulfonyl derivative **47**, which, reacting with sodium iodide in acetone-dioxane in a sealed tube in a boiling water bath for 15 minutes, gave the 6-deoxy-6-iodo derivative **48a** in 94% yield (*105*). The bromo analog **48b** was prepared by using dry lithium bromide and extending the reaction time to 2 hours. The 6-chloro-6-deoxy derivative **48c** was obtained likewise using lithium chloride and heating for 5 hours. All of these

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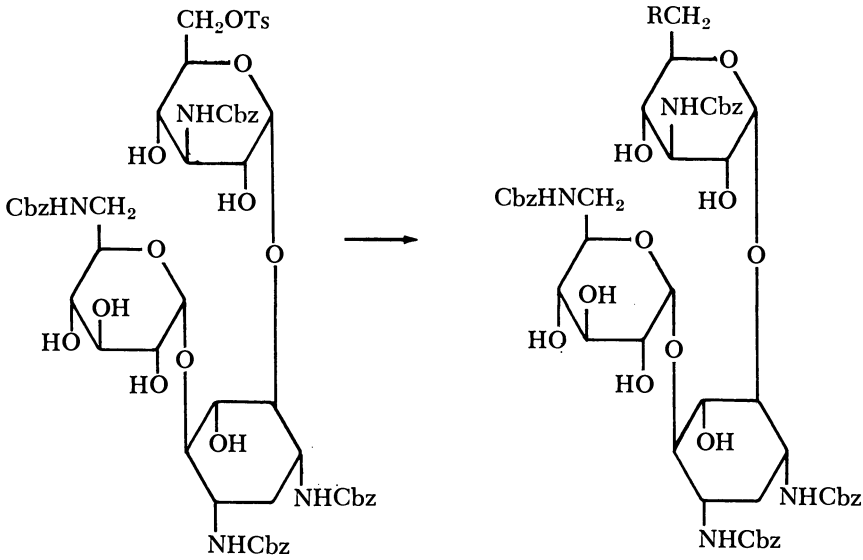
6-halo derivatives were then reduced to 6-deoxy derivatives which were deblocked to give 6-deoxy kanamycin. This and the unblocked chloro-deoxy kanamycin (from **48c**) possessed strong inhibitory activity against several bacteria (*105*).



47

48a, R = I

48b, R = Br

48c, R = Cl

The Preparation of Halodeoxy Sugars Using Phosphorus-Containing Reagents

Rydon and co-workers (*73*) have shown that the reaction of simple alcohols with triphenylphosphite methiodide and triphenylphosphite dihalides gives alkyl halides according to the general scheme.

$$(PhO)_3PMeI + ROH \longrightarrow RI + PhOH + MePO(OPh)_2$$
$$(PhO)_3P\ hal_2 + ROH \longrightarrow R\ hal + PhOH + hal\ PO(OPh)_2$$

In addition to simple halides, the method was used to prepare cholesteryl iodide (30%) and cyclohexyl iodide (34%) from the corresponding alcohols, thus demonstrating the applicability of the reaction to cyclic secondary alcohols. An early adaptation to carbohydrates was reported by Lee and El Sawi (*75*). They claimed that treatment of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (**49**) with triphenylphosphite methiodide

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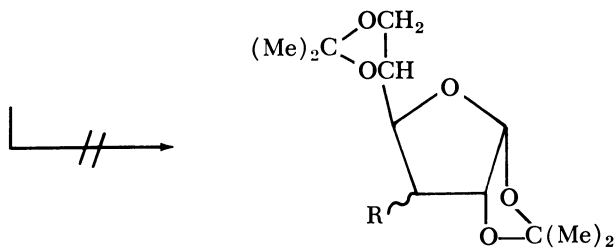
afforded the 3-deoxy-3-iodo derivative **50** which was ultimately transformed into a 3-deoxy-D-*arabino*-hexose derivative. The reaction has been re-examined, and its scope has been expanded in the carbohydrate area by Kochetkov and co-workers (*70*). The Russian investigators showed that whereas the reaction has potential utility in synthetic work, the identities of the reaction products should be considerd with scrutiny since certain functional groups such as acetals and esters tend to rearrange during the reaction. This indeed was the case when **49** was treated with triphenylphosphite dibromide or triphenylphosphite methiodide, for in both cases the products were not the expected 3-halo derivatives **50**, but the rearranged 6-halo derivatives **51**.

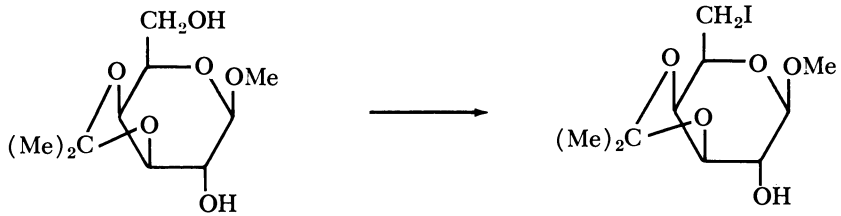
49 **51**, R = Br, I



50

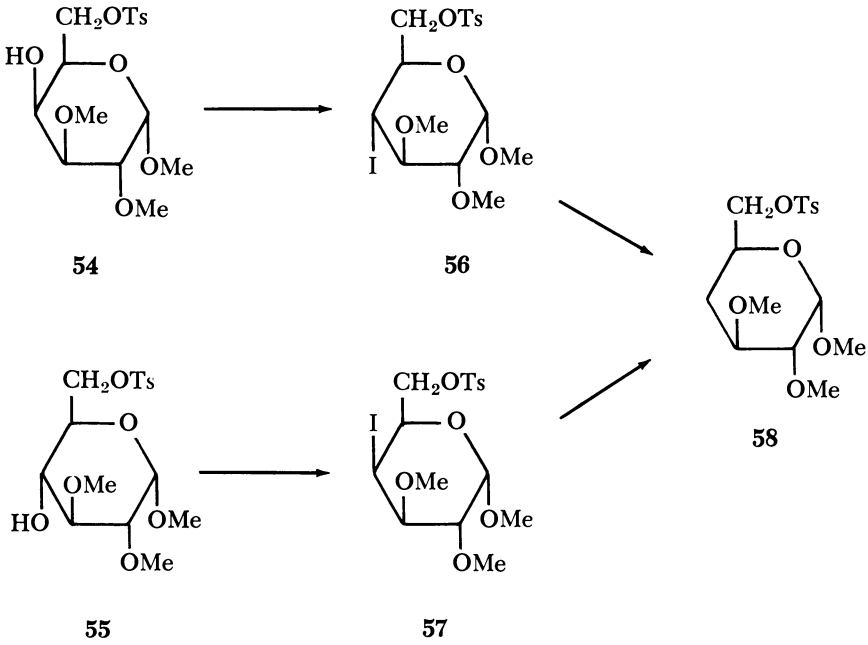
The reaction is quite susceptible to steric effects since hindered secondary hydroxyl groups were found to be unreactive. The method can therefore be used to selectively replace a primary hydroxyl group by halogen in the presence of more hindered secondary hydroxyl groups in the same molecule. An example (*70*) is the reaction of **52** with triphenylphosphite methiodide which affords the 6-deoxy-6-iodo derivative **53** (60%) in which the C–2 hydroxyl group remains intact.

Non-hindered secondary hydroxyl groups have been shown, however, to react under the same conditions giving the corresponding halo derivatives. These direct replacements of hydroxyl groups by halogens are



52 53

assumed to take place with Walden inversion at the ring carbon. Proof for such an S_N2 type reaction is found in the following interesting example (*70*). The C–4 epimeric glycosides **54** (D-*galacto*) and **55** (D-*gluco*) reacted with triphenylphosphite methiodide to give the C–4 epimeric iodo derivatives **56** and **57**, respectively, which were reduced to the same 4-deoxy glycoside derivative **58**.



54 56 58 55 57

Whereas 1,2-*O*-isopropylidene-5,6-di-*O*-methyl-D-glucofuranose was found to be unreactive towards triphenylphosphite dibromide, triphenylphosphite methiodide or phosphorus pentachloride, the related methyl 2,5,6-tri-*O*-methyl-β-D-glucofuranoside (**59**), in which the hindrance caused by the ketal group is absent, reacted with triphenylphosphite methiodide to give the 3-deoxy-3-iodo derivative **60** in 31% yield.

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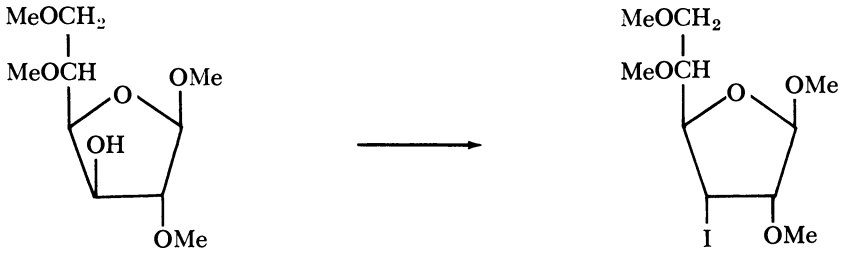
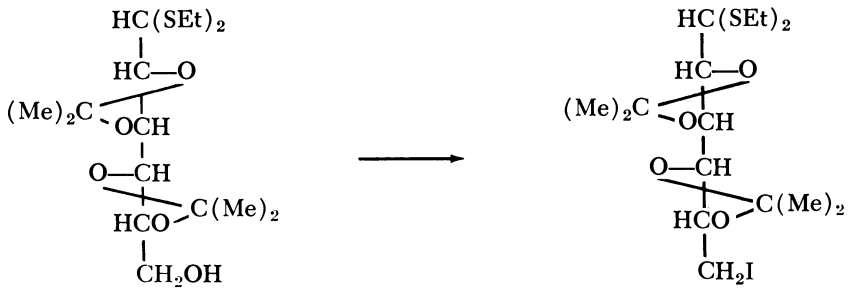


59 **60**

The above two examples demonstrate the versatility of this method of replacing secondary hydroxyl groups by halogens (*70*). Although the yields are only moderate it is of special interest to note that replacement of a secondary hydroxyl group by halide with Walden inversion is possible in a furanose derivative such as **59**. This method of halogen introduction at C–4 and related ones to be discussed shortly seem to be preferable to the tosylate displacement technique. Configurational effects are important in such displacements and the initial C–4 halides may react further to give mixed products (epimeric at C–4). For the purpose of obtaining deoxy sugars, however, the stereochemistry of the deoxyhalo intermediate is irrelevant.

The reaction was found to be adaptable to dithioacetal derivatives also (*70*). Thus the product from the treatment of **61** with triphenylphosphite methiodide was the expected 6-deoxy-6-iodo derivative **62** with no noticeable migration of ketal groups.



61 **62**

For **63**, however, where a participating function is present in the vicinity of C–4, a rearranged product **65** was formed (*70*) in addition to the expected **64**. The formation of **65** has been rationalized on

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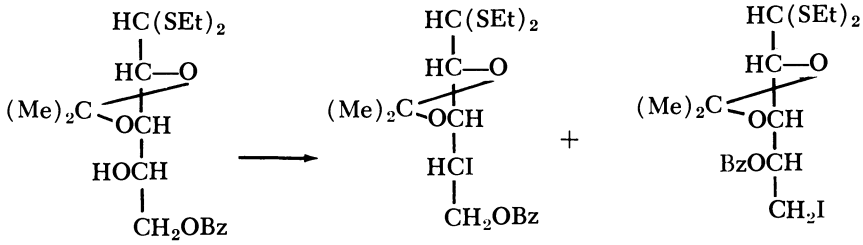
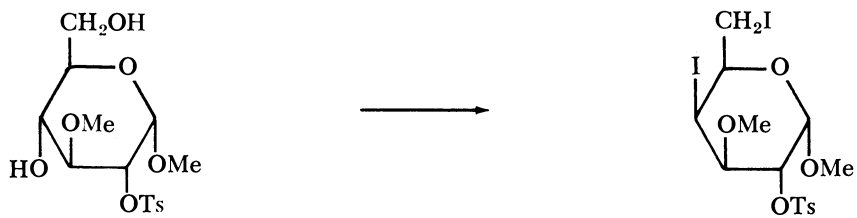


63 64 65

the basis of intramolecular benzoxonium ion formation in the product **64**, with loss of iodide ion from C–4, followed by attack of the latter at the less hindered C–5 atom. The fact that the by-product was actually **65** and not the isomeric 4-*O*-benzoyl-5-deoxy-5-iodo-D-xylose derivative (resulting from actual participation of the ester grouping in a complexed form of **63** rather than **64**) was corroborated by its transformation into a derivative of known configuration (L-*arabino*).

As previously stated, the preparative use of this reaction is, in some cases, limited by the rearrangement of protective groups such as ketals and esters and also by steric hindrance. The first limitation can be circumvented by using *O*-methyl ethers and tosyloxy functions rather than ketals, since migration is prevented in those cases. Furthermore, the absence of bulky groups such as ketals will frequently eliminate the crowding effect imparted on a neighboring hydroxyl group. A mechanism has been proposed (*70*) to account for the migration of ketal groups which takes place with retention of configuration at the open hydroxyl groups.

This method of direct introduction of halogen atoms in sugars using phosphorus reagents has been employed in the synthesis (*71*) of the biologically derived dideoxy sugar, chalcose (4,6-dideoxy-3-*O*-methyl-D-*xylo*-hexose). Both the C–6 and C–4 hydroxyl groups in **66** could be substituted by iodine atoms in one step to give **67** (85% yield) which was subsequently converted into chalcose by standard procedures.



66 67

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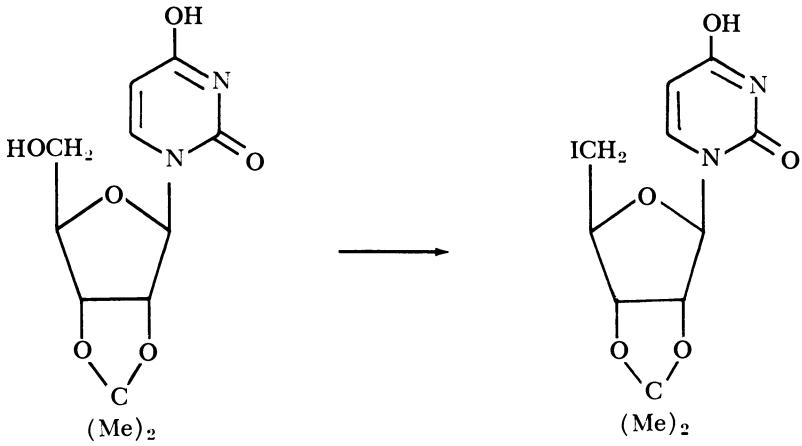
The reaction has been extended (*106*) to the nucleoside field and provides a means for the direct iodination of suitably protected nucleosides. Thus treatment of 2,3-*O*-isopropylidene uridine (**68**) with triphenylphosphite methiodide in *N*,*N*-dimethylformamide at room temperature afforded the corresponding crystalline 5-deoxy-5-iodo analog **69** in 77% yield.



68 **69**

In a similar way, 5-*O*-acetylthymidine was converted into the 3-deoxy-3-iodo derivative **72** in 55% yield. In this case, the replacement of the hydroxyl group by iodine was presumed to have taken place by retention of the configuration at C–3. The first intermediate in the reaction was proposed to be the phosphonate (**70**) which rapidly collapses to an *O*-3-cyclonucleoside (**71**) and the latter is subsequently attacked by iodide ion to give the product **72**. It was also observed (*106*) that treatment of nucleosides containing a *cis* vicinal diol grouping such as 5-*O*-acetyluridine with triphenylphosphite methiodide failed to provide iodinated products but gave phosphonate derivatives instead.

Despite several attractive features in this method of direct halogen introduction and the obvious applications in the synthesis of deoxy sugars, its uses have not been further exploited by other groups of workers. Some new related methods have become available which reportedly eliminate the difficulties previously encountered such as rearrangement, unreactivity due to steric hindrance, and phosphonate ester formation. The reaction is based on the observation (*28*) that triethylphosphine reacts with ethanol and carbon tetrachloride to give ethyl chloride, chloroform, and triethylphosphite. In a new adaptation (*76*, *77*) of this

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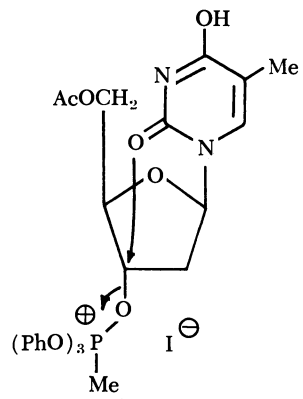

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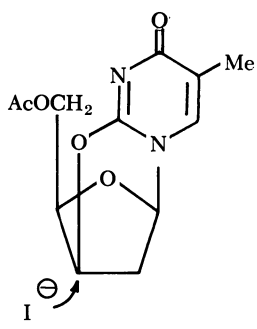

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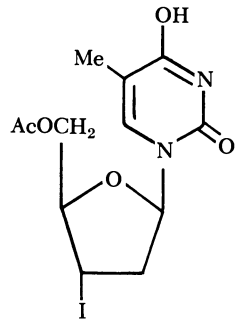
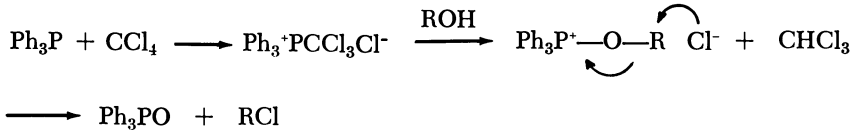


72

reaction it was found that treatment of various alcohols with triphenylphosphine in carbon tetrachloride at reflux temperature gave the corresponding chloro derivatives in high yields. Exclusion of moisture from the reaction mixture assures neutral conditions. The attachment of halo groups onto secondary carbon atoms, is presumed to take place with inversion of configuration while the neighboring carbon atoms are unaffected. Certain α-hydroxy esters were converted into α-halo esters using this method (*76, 77*).



The only recorded example using this method in the sugar series is the chlorination of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**73**) which affords in addition to the expected 6-chloro-6-deoxy derivative **74a**, a 5,6-unsaturated derivative **75** as well. These products were separated by silica gel column chromatography; no yields were given.

The potential utility of this method in the synthesis of chlorodeoxy sugars cannot be fully appraised at the present time owing to the lack of sufficient examples.

The synthesis of halodeoxy sugars has also been achieved by reaction of sugar phosphorodiamido and phosphonamido derivatives with alkyl halides (*83*). Heating equimolar amounts of 6-(tetraethylphosphorodiamido)-1,2:3,4-di-*O* isopropylidene-D-galactose with methyl iodide (and benzyl bromide) at 140°C. for 4 hours afforded the 6-deoxy-6-iodo (**74b**) (75%) and 6-bromo-6-deoxy (**74c**) (56%) derivatives, respectively.

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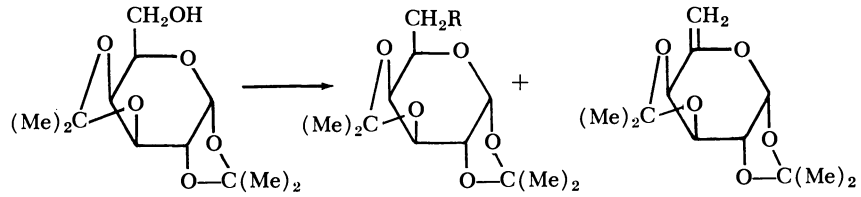

73 74a, R = Cl 74b, R = I 74c, R = Br 74d, R = F 75

Under the same conditions the 6-(*N*,*N*-diethyl-*P*-methylphosphoramido) analog and ethyl chloroacetate gave a 38% yield of the 6-chloro-6-deoxy derivative **74a**. With ethyl fluoroacetate, a 90% yield of 6-fluoro analog **74d** was obtained. Migration of ketal groups in some derivatives has been observed (*86*) during these reactions. Treatment of the 3-dipropylphosphinite derivative of **49** (obtained by reaction of **49** with Pr_2PNEt_2 at 100°C., 12 hours) with benzyl chloride (110°C., 1 hour) afforded 6-chloro-6-deoxy-1,2:3,5-di-*O*-isopropylidene-D-glucofuranose (33%). Further work is obviously needed using such phosphorus-containing reagents to establish the possible applications to the synthesis of sugars containing halogen atoms in various positions in the ring.

The Preparation of Chlorodeoxy Sugars by Reaction with Sulfuryl Chloride

In 1921 Helferich (*54*) reported that the reaction of methyl α-D-glucopyranoside (**11**) with sulfuryl chloride in pyridine afforded a methyl 4,6-dichloro-4,6-dideoxy hexoside 2,3-cyclic sulfate (**76**). Subsequent papers (*55*, *56*) involved the extension of this reaction to other glycosides and sugar derivatives. Thus a method had become available for the direct replacement of the hydroxyl groups by chlorine atoms in sugar molecules. Although the positions of the chlorine atoms were known in Helferich's original reaction product, the actual stereochemistry at C–4 was not established conclusively until almost forty years later (*11*, *67*). The method was based on the ability or inability of the reaction products to engage in epoxide formation as shown in the scheme below. Reaction of **11** and **77** with sulfuryl chloride in pyridine followed by cleavage of the respective cyclic sulfates gave the methyl 4,6-dichloro-4,6-dideoxy glycosides (**78**) and (**79**), respectively, which were found to be different. Upon treatment with alkali, only **79** gave the known epoxide **80**, thus establishing the *trans* arrangement of substituent at C–4—C–3 (D-*gluco*) in the former. The Helferich products **76** and **78** therefore had the D-*galacto* configuration.

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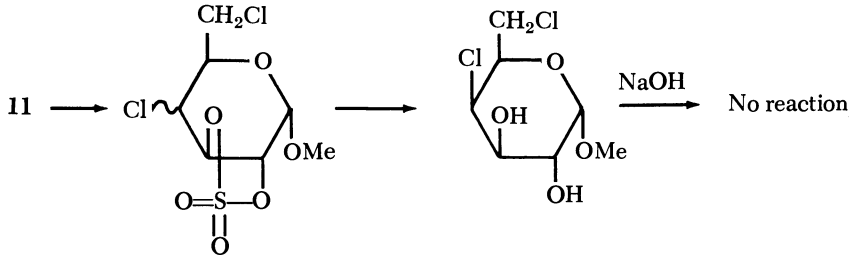
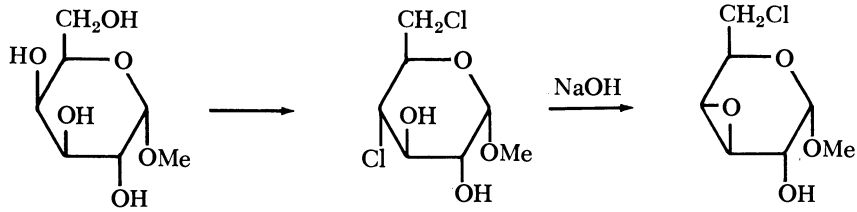
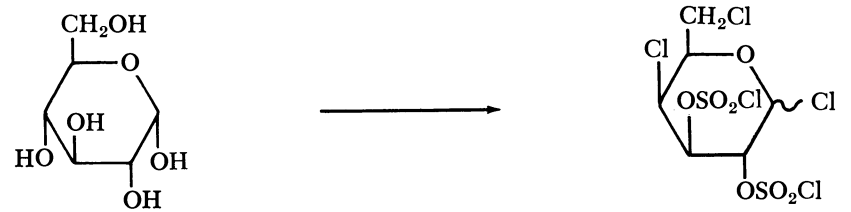


76 78



77 79 80

As in the displacement reactions of sulfonates, C–4 was once again the most reactive center among the ring carbon atoms and was substituted by a chlorine atom with inversion of configuration. Application of this reaction to the methyl pentosides also showed a remarkable selectivity in replacing the C–4 hydroxyl group. The unreacted hydroxyl groups usually formed chlorosulfate esters or cyclic esters depending on their spatial disposition. Changes in the reaction conditions were necessary to adapt this reaction to sugars in the free form (*64*), and it was found that lowering the reaction temperature to —70°C. afforded in the case of D-glucose (**81**), 4,6-dichloro-4,6-dideoxy-D-galactopyranosyl chloride 2,3-dichlorosulfate (**82**). The fact that glycosyl chloride formation



81 82

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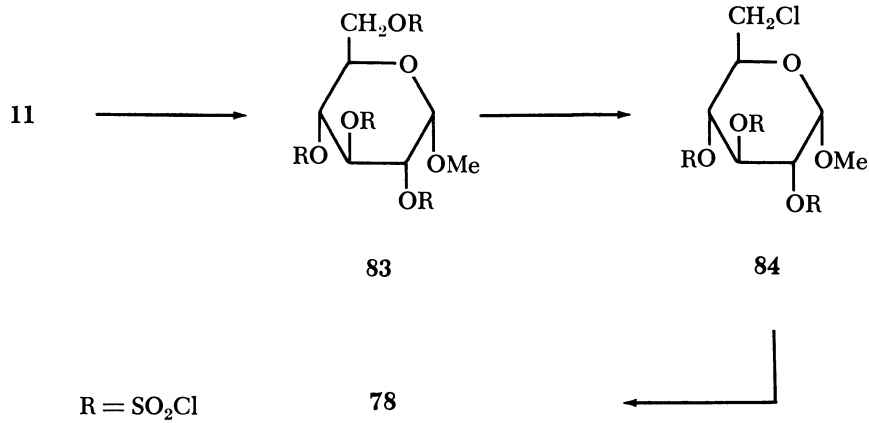
had taken place was shown by converting **82** into **78**. Under these modified conditions, a 2,3-dichlorosulfate rather than a cyclic sulfate was formed. Similarly, reaction with D-xylose afforded a product which was formulated as 4-chloro-4-deoxy-L-arabinofuranosyl chloride 2,3-dichlorosulfate. Maltose afforded a 4,6,6′-trichloro-4,6,6′-trideoxy "maltosyl" chloride tetrachlorosulfate as expected. Treatment of the 2,3-dichlorosulfate derivatives with pyridine at temperatures ranging from 0° to 60°C. for 8 hours afforded the 2,3-cyclic sulfates initially obtained when the sulfuryl chloride-pyridine reaction was carried out at normal temperatures. Dechlorosulfation was effected by treatment of the esters with sodium iodide in 6% aqueous methanol at room temperature (*65*).

A selective introduction of a chlorine atom at C–6 could be achieved (*65*) by treating **11** (and the β-anomer) with sulfuryl chloride in pyridine at −70°C. followed by treatment of the resulting tetrachlorosulfate (**83**) with one mole of pyridine hydrochloride which gave **84**. Heating of **84** with pyridine hydrochloride in chloroform resulted in further substitution by chloride to give **78** after dechlorosulfation. The above illustrated transformations support the proposed mechanism (*65*) for



the reaction which is considered to proceed through the initial formation of the tetrachlorosulfate ester (**83**), followed by an attack by the chloride ion from the pyridine hydrochloride. It would be of interest to induce sugar chlorosulfates to undergo fragmentation in the absence of pyridine. Substitution by chlorine in such a case would be expected to proceed with possible retention of configuration (S_Ni) (*61*) at the chlorosulfate-bearing carbon.

Although the pronounced reactivity at the C–4 position is evident, examples of the formation of polychlorinated sugars are available. Thus treatment of D-galactose (**85**) with sulfuryl chloride in pyridine followed

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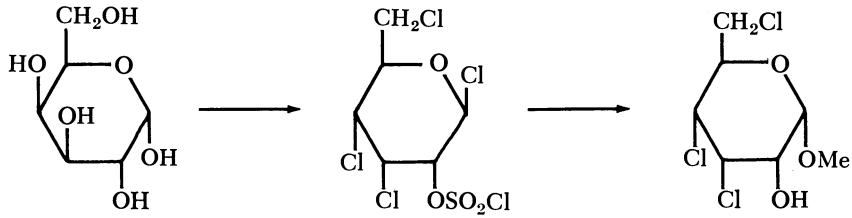
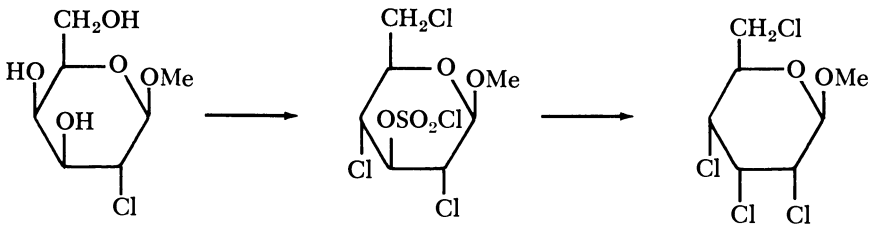
by glycosidation of the intermediate glycosyl halide (**86**) gave an 11% yield of methyl 3,4,6-trichloro-3,4,6-trideoxy-α-D-allopyranoside (**87**). The same product could be obtained from **78** by treatment with sulfuryl chloride in a mixture of pyridine and chloroform. The crystalline free sugar from **87** could be obtained by mild acid hydrolysis.



85 86 87

By a suitable choice of chloro substituent and starting glycoside such as in **88**, a tetrachloro tetradeoxy glycoside (**90**) was synthesized (*26*).



88 89 90

The *allo* configuration was assigned on the expectation that the last chlorosulfate group at C–3 in **89** was also displaced by a Walden inversion.

According to Jones and co-workers (*26*, *27*, *64*, *65*) the extent of substitution of hydroxyl functions by chlorine appears to be a factor of the steric and deactivating effects of neighboring groups in the molecule. For example, substitution by chlorine at C–4 in derivatives having the *manno* configuration is not observed because of unfavorable 1,3-diaxial interactions with the approaching chloride ion. Such an interaction is also reflected in the formation of a 4,6-dichloro derivative from **77** while under the same conditions the β-anomer affords a 3,4,6-trichloro derivative. In the former case it is argued that the rearside approach of chloride ion to C–3 would be hindered by the α-methoxyl group. This can be contrasted with the fact that both methyl α- and β-D-glucosides give only

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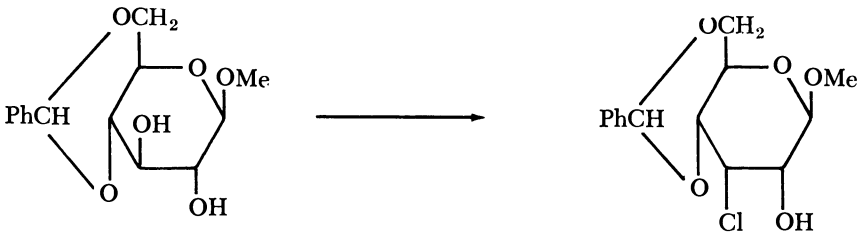
the methyl 4,6-dichloro-4,6-dideoxy-α and β-D-galactosides, respectively. A deactivating effect of the axial C–4 chloro group is considered to be the cause for the lack of reactivity at C–3 in those cases. In support of this view, methyl 4,6-*O*-benzylidene-β-D-glucoside **91** undergoes facile substitution at C–3 by chloride ion, to give methyl 4,6-*O*-benzylidene-3-chloro-3-deoxy-β-D-allopyranoside (**92**) (*65*). The latter can be obtained crystalline in 47% yield (*46*) and can serve as a precursor to 3-substituted D-glucose derivatives, such as 3-amino-3-deoxy-D-glucose (kanosamine).



91 **92**

The conformational aspects of the substitution reactions with sulfuryl chloride have been summarized (*27*).

An attractive feature in this reaction is the possibility of direct substitution and formation of unblocked sugar derivatives containing one or more chlorodeoxy function in essentially two steps. Another facet is the formation of methyl 4,6-dichloro-4,6-dideoxy-hexosides from certain methyl glycosides in one step. Such compounds could be valuable intermediates in the synthesis of dideoxy and diamino sugars of biological importance.

The Preparation of Bromodeoxy Sugars from O-Benzylidene Acetals

Cyclic benzylidene acetals have been utilized in carbohydrate chemistry for the temporary protection of favorably situated hydroxyl groups. A new facet in the chemistry of such acetals has been unveiled (*43*, *46*, *48*) which renders them valuable synthetic intermediates as well. The reaction of benzylidene acetals derived from various sugars with *N*-bromosuccinimide (NBS) has been found to give monobenzoylated bromodeoxy sugar derivatives. Thus treatment (*47*) of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**93**) (R = H) with 1.1 equivalents of NBS in a refluxing mixture of carbon tetrachloride and tetrachloroethane containing barium carbonate for 2 hours afforded a 60% yield of methyl 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside (**94**). This

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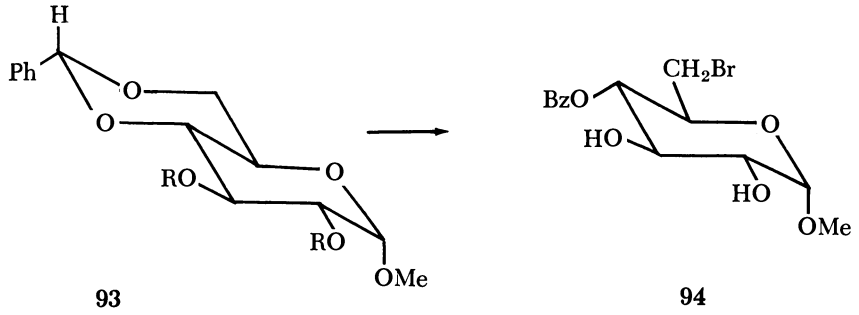
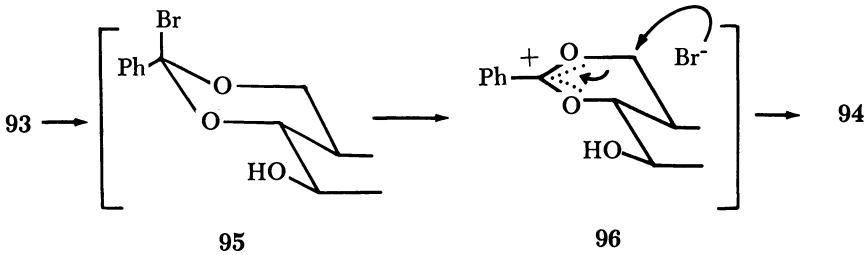


93 94

R = H, Bz, Ac, Ms, etc.

product possesses the combined advantages of a good leaving group at C–6, a selectively esterified hydroxyl group at C–4, and it is an ideal precursor to various 6-substituted D-glucoses. A plausible mechanism for this reaction can be envisaged to involve initial abstraction of the benzylic hydrogen atom by bromine atoms formed from the reagent, to give



95 96

an unstable bromoacetal (**95**), which would be expected to collapse giving the benzoxonium ion (2-phenyl-1,3-dioxolenium ion) (**96**) and bromide ion. The oxonium ion would then undergo ring opening as a result of the attack of bromide ion at C–6, to give the bromo ester derivative **94**. The 6-bromo-4-benzoate derivative was the preponderant if not the exclusive product in this and other reactions involving 4,6-*O*-benzylidene acetals. This selectivity can be explained in terms of steric effects and the preference for attack at the primary carbon atom. Although an overall radical type mechanism can be considered the results available so far can be better rationalized on the basis of an ionic termination process. The possibility of both mechanisms operating in certain cases cannot be excluded.

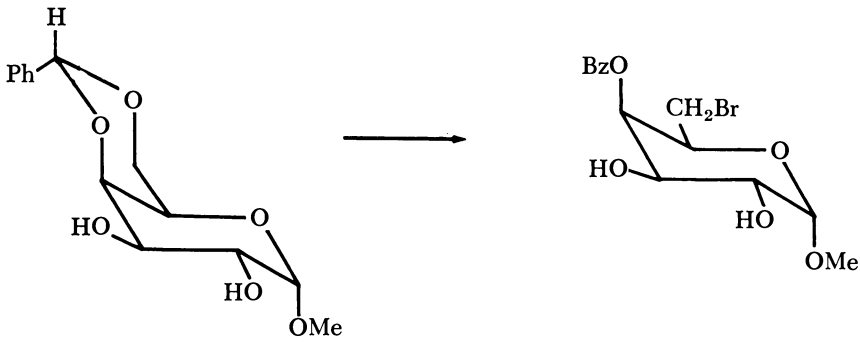
The light-catalyzed reaction between benzaldehyde diethyl acetal and NBS was previously shown (*78*) to produce ethyl benzoate, but the ethyl

bromide could not be detected. However, with *O*-benzylidene cyclohexane 1,2-diol the *trans*-2-bromocyclohexylbenzoate could be obtained (*89*).

Current views (*100*) on the mechanism of bromination by NBS invoke the formation of molecular bromine and bromine atoms in low concentration, which subsequently act as the brominating agent. The bromination reaction was studied in detail in this laboratory under a variety of conditions using **93** (R = Ms) as a model. The product **94** (R = Ms) was indeed formed (42%) when NBS was substituted by 1.1 equivalents of bromine which was added at a slow rate to the reaction mixture. The yield was 68% when benzoyl peroxide was used as a catalyst. Using NBS alone or in the presence of reagents such as barium carbonate, pyridine, or *s*-trinitrobenzene, the yield was 60–70%.

Subsequent to the announcement (*47*) of this reaction, a similar transformation of 4,6-*O*-benzylidene acetals was reported (*35*) in which a free radical initiator was used. Under these conditions a blocked derivative such as **93** (R = Bz) was transformed in 76% yield into the corresponding **94**. Selective formation of the 6-bromo derivative was observed as in the initial studies (*47*).

This indirect method of selective benzoylation at C–4 is of special interest in the *galacto* series (*47*). Reaction of methyl 4,6-*O*-benzylidene-α-D-galactopyranoside **97** with NBS afforded the corresponding 6-bromo-4-benzoate (**98**) in over 90% yield. The axial hydroxyl group at C–4



97 **98**

was thus selectively benzoylated in the presence of the equatorial C–2 and C–3 hydroxyl groups. The reaction is equally successful with 2,3-disubstituted derivatives in the β-series. Another distinguishing feature in this reaction is the convenient route to 6-bromo-6-deoxy-galactose derivatives from the readily available acetals. This feature is of preparative significance since alternative methods for the introduction of halogen

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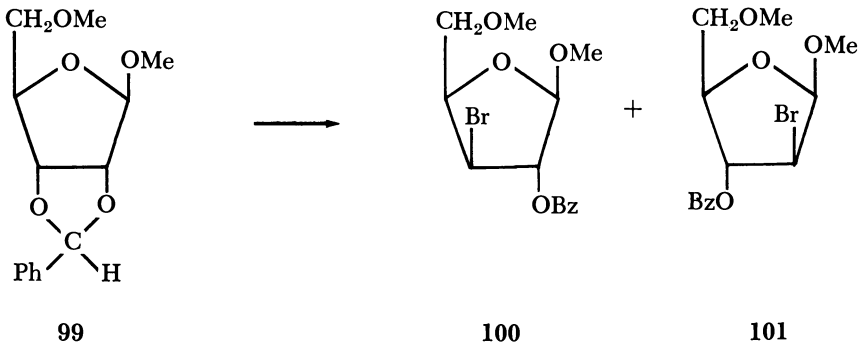
atoms at C–6 in the galactose structure, such as by the displacement of tosylates with metal halides, present some difficulties (*103*) (*see* the discussion on the displacement of tosylates in this paper).

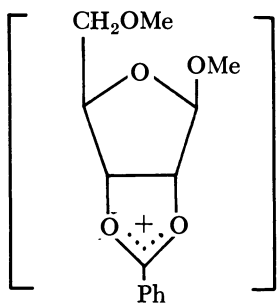
The presence of hydroxyl groups in the benzylidene sugars does not interfere with the reaction and by-products are usually minor. Suitable solvents other than carbon tetrachloride, include benzene and tetrachloroethane. Epoxide, amide, and other commonly encountered functionalities in sugar derivatives are unaffected under the reaction conditions. The corresponding 6-bromo-4-benzoates are valuable intermediates for the synthesis of 6-, 2,6-, 3,6-substituted and related sugar derivatives (*46*).

Application of the ring opening reaction to disaccharide derivatives such as 4,6:4′, 6′-di-*O*-benzylidene-α,α-trehalose affords the corresponding 6,6′-dibromo-4,4′-dibenzoate in good yield.

Unlike the 4,6-*O*-benzylidene derivatives, methyl 2,3-*O*-benzylidene-5-*O*-methyl-β-D-ribofuranoside (**99**) (*9*) in which the acetal spans two secondary hydroxyl groups, reacts with NBS to give (*48*) the isomeric bromobenzoates **100** and **101**. These are formed in a ratio of 2:1, and,



99 **100** **101**



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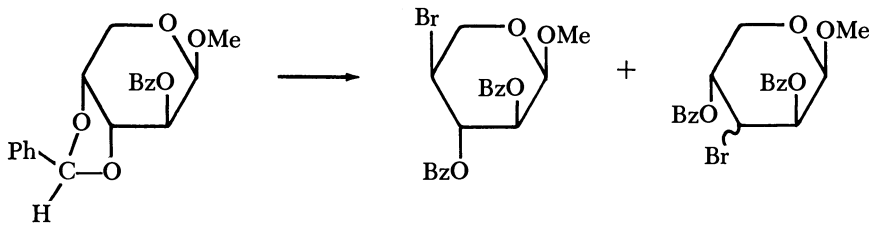
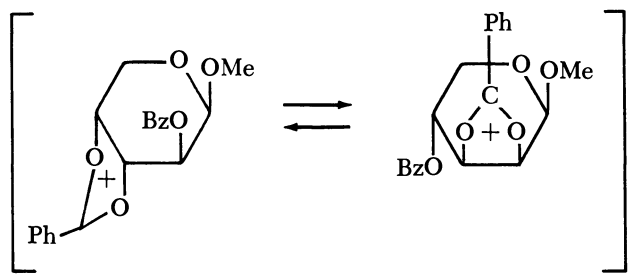
although they cannot be separated by column chromatography on silicic acid, they afford upon catalytic reduction and debenzoylation, 2- and 3-deoxypentosides which are readily separable. It is thus seen that when the acetal ring carbons have a similar steric environment, attack at both sites in an intermediate such as **102** (or its radical counterpart) is possible. The predominance of the 3-bromo isomer (**100**) over **101** is not unexpected and parallels epoxide ring opening reactions by nucleophiles in the *ribo* series.

Another internal acetal, methyl 2-*O*-benzoyl-3,4-*O*-benzylidene-β-D-arabinopyranoside **103** (*84*), behaves in an analogous manner, except that the isomeric 4-bromo (**104**) and 3-bromo (**105**) benzoate derivatives are formed in a ratio of 1:1. The identity of **104** (L-*xylo*) can be deduced from its mode of formation from a presumed benzoxonium ion (**106**)



103 **104** **105**



106 **107**

and from it subsequent reactions (such as reduction, etc.). The 3-bromo isomer (**105**) can, however, have the D-*lyxo* or the D-*arabino* configurations depending on whether it is formed from the intermediate **106** or a rearranged benzoxonium ion (**107**). None of the 2-bromo isomer that could result from **107** could be detected. The NBS reaction was extended (*48*) to a third and last type of common *O*-benzylidene acetals, namely those formed from a secondary hydroxyl group situated on a ring and

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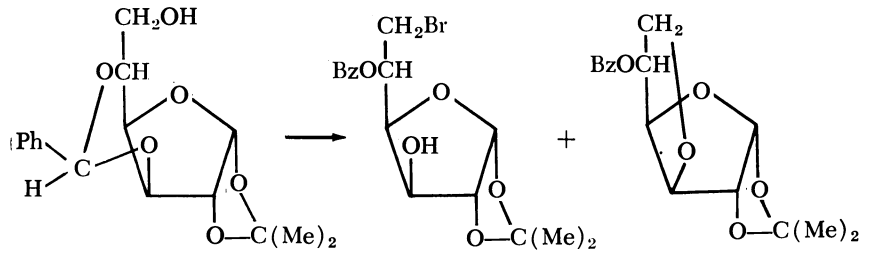
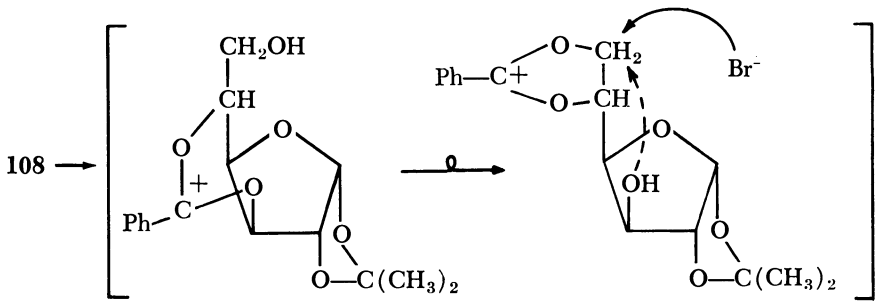
another on a side chain. The readily available 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-D-glucofuranose (**108**) (*14*) when reacting with NBS under the usual conditions afforded the two products **109** and **110** in a ratio of 3:2 respectively (*48*). The structures of these unexpected products were proved by appropriate conversion into known crystalline derivatives and by spectral data. The expected reaction course would have been the attack of bromide ion (or a bromine atom) at C–3 and/or C–5



108 **109** **110**

in the initial benzoxonium ion (**111**) (or its radical counterpart). Molecular models show that the C–6 hydroxyl group is in favorable position for



111 **112**

an intramolecular attack on the benzylic carbon in the charged oxonium ion (**111a**) (a three-dimensional representation of **111**). Such a process could lead to a charged ortho ester-type intermediate which collapses to the rearranged benzoxonium ion (**112**). The primary carbon atom is then attacked by bromide ion and also intramolecularly by the C–3 hydroxyl group to give the observed products. The anhydro derivative (**110**) could not be obtained from **109** under simulated reaction conditions, and it must be formed from **111** during the initial reaction. It is because of such observations involving intramolecular participation of hydroxyl (and ester) groups, that at least in those cases, an ionic termination step

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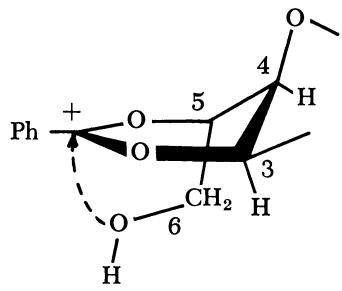
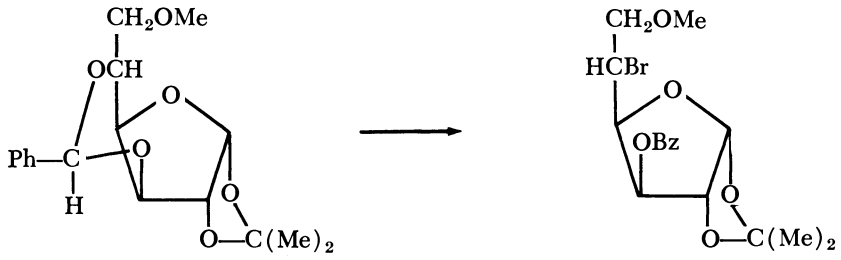


111a

is favored over the alternative radical-type process. In other words, a benzoxonium ion intermediate is more likely to undergo such internal rearrangements than its radical counterpart. In support of this explanation, the crystalline 6-*O*-methyl derivative **113** derived from **108** was found to give, under the same conditions, a single bromobenzoate derivative which from its spectral properties and further transformations is most probably **114** (*48*). In the absence of a participating group at



113 114

C–6, the benzoxonium ion from **113** is attacked by bromide ion preferentially and selectively at the less hindered acyclic C–5 position, rather than the C–3 ring position.

Because the NBS reactions affords trans oriented bromobenzoate derivatives at C–2 and C–3 (or other ring positions) from the appropriate sugar acetals, an indirect synthesis of aryloxonium salts becomes possible. In a model experiment (*46*) methyl β-D-ribofuranoside was converted into methyl 2,3-*O*-*p*-anisylidene-5-deoxy-β-D-ribofuranoside through sequential acetal formation, mesylation, and reduction of the crystalline C–5 mesylate ester with lithium aluminum hydride. In designing this experiment an ideal substrate was sought, containing a nonparticipating C–5 function and a favorably substituted acetal function which would be expected to confer a higher stability to the anticipated

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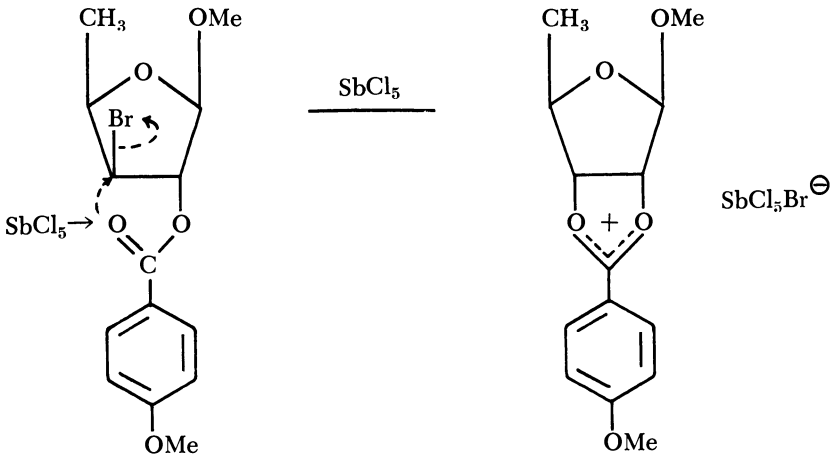
carboxonium salt. The presumed anion $SbCl_5Br^{\ominus}$, being devoid of nucleophilic character would also contribute to the stability. Reaction of the acetal with NBS afforded a mixture of 2- and 3-bromobenzoates (**115**) (shown as a 3-bromo-2-benozate for simplicity) which was treated with a solution of antimony pentachloride in dichloromethane at room temperature. An oily salt, presumably **116** could be isolated upon adding carbon tetrachloride. The possible synthetic applications of such intermediates was demonstrated by treatment of **116** with sodium azide in acetonitrile, where a mixture of *trans* (2,3) azido benzoates was isolated as a chromatographically homogeneous sirup.



115 **116**

These observations cover the general scope of the reaction of NBS with *O*-benzylidene acetals. The conclusions that can be derived based on the compounds studied so far are the following: (1) Benzylidene acetals spanning primary and secondary ring carbons (4,6-*O*-benzylidene hexopyranosides) afford invariably a preponderance of the corresponding 6-bromo-4-benzoates (an exception is found for methyl 4,6-*O*-benzylidene-β-D-galactopyranoside). (2) Internal acetals formed from secondary hydroxyl groups attached to the sugar ring are opened to give isomeric bromobenzoates. The proportions of such isomers seem to be dependent mainly on steric and conformational factors. (3) Acetals joining a secondary hydroxyl group on a side chain with one on a ring, afford predominantly if not exclusively the acyclic bromide. (4) The presence of nearby participating functions (ester, hydroxyl) to the benzoxonium ion (or its radical counterpart) will often lead to rearranged intermediates. If a primary carbon is now involved in the charged specie, attack will

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invariably occur at that carbon atom. Thus the direction of ring opening can often be altered to different extents by placing the proper type of functionality on the adjacent hydroxyl groups. Mass spectral data on the deoxy sugars derived from the various bromo derivatives are reported in a separate paper (*29*).

Miscellaneous Methods

Several other methods for the introduction of halo atoms at C–6 in hexose derivatives have been known since the late 1920's. One of the earlier methods involved the reaction of methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside with phosphorus pentachloride which resulted only in an 8% overall yield of methyl 6-chloro-6-deoxy-α-D-glucoside (*57*). In contrast, the reaction of methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-altropyranoside with phosphorus tribromide and bromine afforded a 73% yield of the corresponding 6-bromo-6-deoxy derivative (*91*).

The 1-*O*-trityl group in 3,4,5,6-tetra-*O*-acetyl-1-*O*-trityl-D-*arabino*-hexulose was replaced by chlorine upon treatment with phosphorus pentachloride in chloroform at 0°C. for 30 minutes to give the corresponding crystalline 1-chloro derivative without affecting the carbonyl group (*12*). This reaction has been scarcely used probably because of the limitations arising from the fact that hydroxyl groups have to be blocked. In an attempt to remove the trityl group from a peracylated aldose dithioacetal derivative with chloroform saturated with hydrogen bromide, Wolfrom and co-workers (*108*) obtained the corresponding ω-bromodeoxy derivative. Phosphorus pentachloride in chloroform at reflux temperature has been shown to replace the primary hydroxyl groups in 2,3,4,5-tetra-*O*-benzoyl-mannitol by chlorine (*80*).

Treatment of 1,4:3,6-dianhydro-D-glucitol with boron trichloride gives 1,6-dichloro-1,6-dideoxy-D-glucitol (*20*). Although methyl 6-chloro-6-deoxy-α-D-glucopyranoside (isolated as the tribenzoate) could be isolated from the reaction of methyl 3,6-anhydro-α-D-glucopyranoside with boron trichloride (*21*), the application to the isomeric furanoside derivative led to complex results.

A novel route to halo sugars (and deoxy sugars) was described by Brown and Jones (*16*, *17*) and is based on the observation that alkyl hydrazines are oxidized to the corresponding alkyl iodides by iodine in aqueous potassium iodide solution. Treatment of 3-deoxy-3-hydrazino-1,2:5,6-di-*O*-isopropylidene-D-allofuranose (**117**) with the above mentioned reagent afforded the 3-deoxy-3-iodo derivative **118** in addition to the 3,3′-diiodo derivative **119**. The product was exclusively **118** when iodine itself was used as reagent. With *N*-bromosuccinimide, the 3-bromo-3-deoxy derivative corresponding to **118** was formed. The authors proposed a D-*gluco* configuration for **118** on the basis of NMR data. Although

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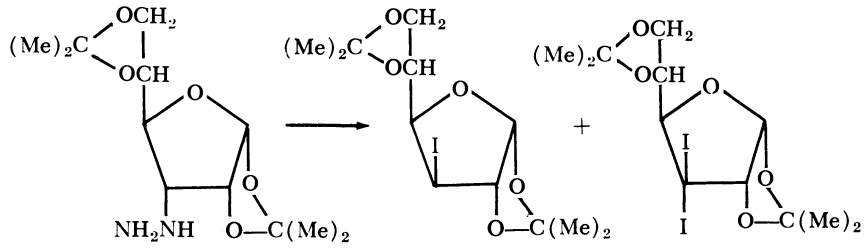
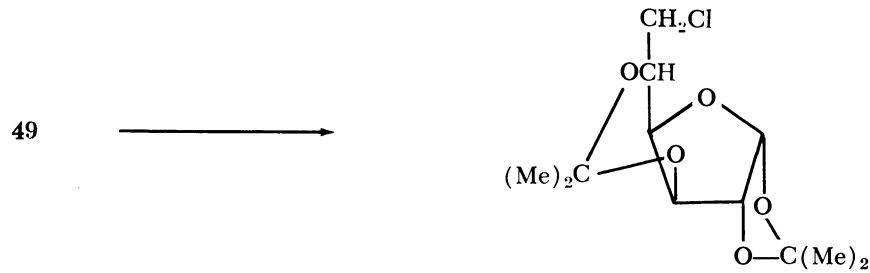
this reaction has some interesting mechanistic implications, its preparative value is somewhat limited by the fact that other hydrazino sugar derivatives are not as readily available as **117**.



117 **118** **119**

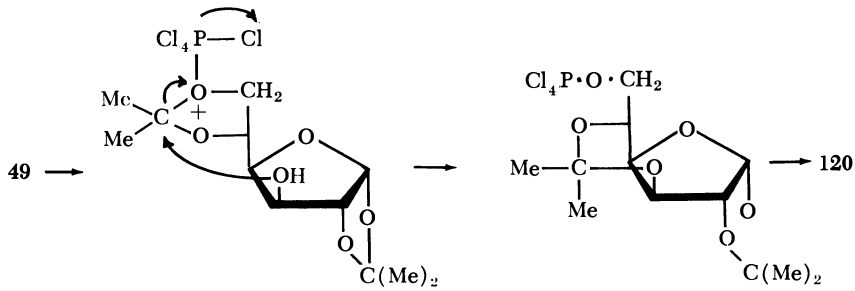
According to Kent and co-workers (*109*) *N*-(2-chloro-1,1,2-trifluoroethyl)-diethylamine ($CHClFCF_2NEt_2$) reacts with 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**73**), in the presence of anhydrous potassium fluoride to give a mixture of products. Employing preparative thin layer chromatography, the 6-chloro derivative (**74a**) could be isolated from such a mixture. It should be noted that the 6-fluoro derivative was not formed. Reaction of the amine with sugar derivatives containing secondary hydroxyl groups afforded esters which could be hydrolyzed with dilute alkali to regenerate the hydroxyl group. No halo sugar derivatives were formed in those cases. Compound **74a** has been prepared in 66% yield (*18*) by the pyridine-catalyzed decomposition of the 6-*O*-chloroformyl ester of **73**. Displacement of the corresponding 6-*O*-tosyl ester by treatment with lithium chloride in *N*,*N*-dimethylformamide afforded a 96% yield of **74a** (*18*).

The reaction of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (**49**), with phosphorus pentachloride was reported in 1926 by Allison and Hixon (*1*) who formulated the product as 3-chloro-3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (2% yield). A reinvestigation (*93*) of the

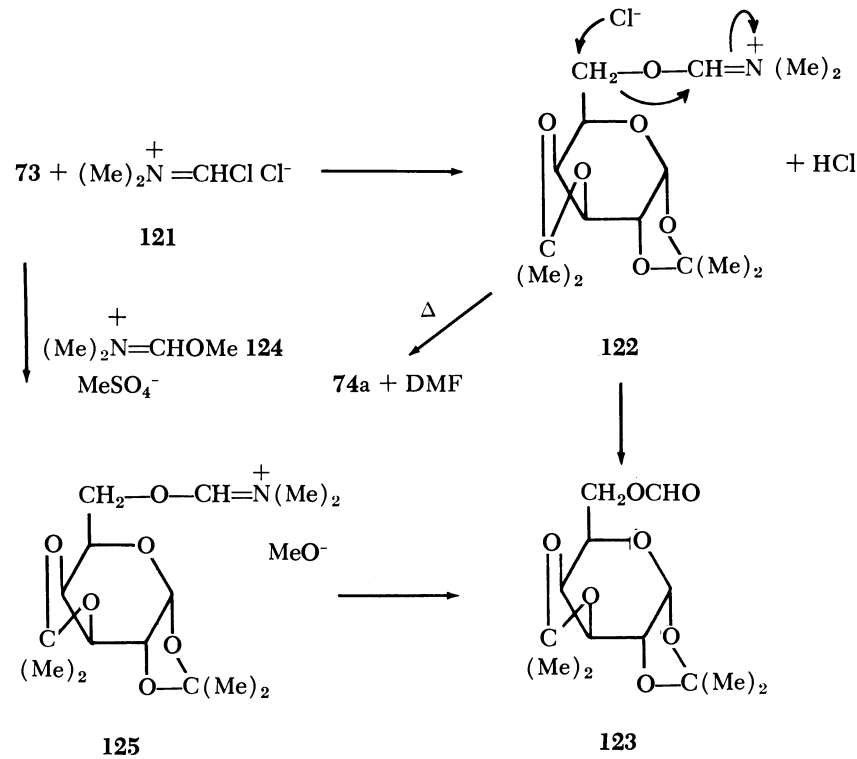


120

reaction showed that the product (low yield) was actually the isomeric 6-chloro-6-deoxy-1,2:3,5-di-*O*-isopropylidene-D-glucofuranose (**120**). Migration of the 5,6-*O*-isopropylidene group to the 3,5-position was explained on the basis of an S_Ni' mechanism of chlorination. An alternative pathway was proposed more recently by Baddiley, J., Buchanan, and Hardy, *J. Chem. Soc.*, **414**, 2180 (1961) and is depicted in the sequence shown below:

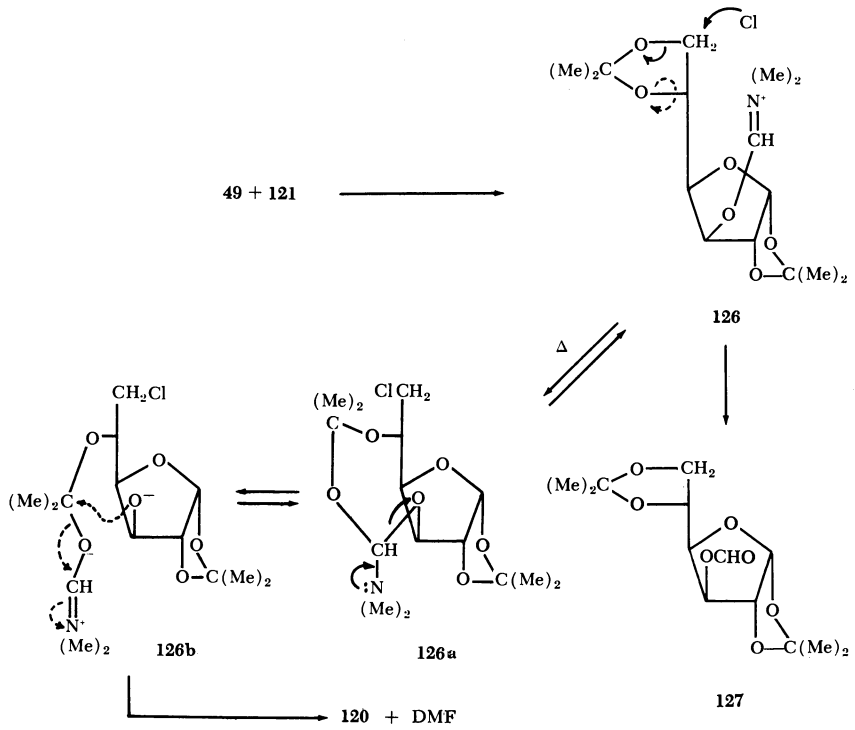


It should be noted that the product **120** has been prepared (*50*) at best in only 14% yield from **49** by the above procedure.



A new synthesis (*46*, *49*) of chlorodeoxy sugars involves the thermal decomposition in solution of *O*-imino ester chlorides formed from sugar derivatives containing isolated hydroxyl groups and (chloromethylene) dimethylammonium chloride (**121**) (*3*, *31*, *32*). Thus treatment of **73** with an equimolar amount or a slight excess of **121** in a chlorinated solvent such as 1,1,2-trichloroethane, at room temperature, affords the intermediate **122** which can be isolated. Treatment of **122** with aqueous bicarbonate solution affords the 6-*O*-formate ester (**123**) in high yield. Refluxing the original solution for 3 to 4 hours with exclusion of moisture affords the 6-chloro derivative **74a** in 86-90% yield. A noteworthy feature in this reaction is the formation of *N*,*N*-dimethylformamide as the other product. The formate ester (**123**) could also be obtained (*46*, *49*) upon treatment of **73** with methoxymethylene) dimethylammonium methylsulfate (**124**) (*13*) and subsequent decomposition of the presumed intermediate **125** with aqueous bicarbonate.

For **49**, the reaction was highly successful in that a 3-*O*-formate ester (**127**) was indeed formed in a reaction period of 1 to 2 hours at room temperature with **121** followed by usual processing. Refluxing the solution for 3 to 4 hours afforded a chlorodeoxy sugar in 69-71% yield which proved to be **120** rather than the expected 3-chloro derivative (D-*allo*).



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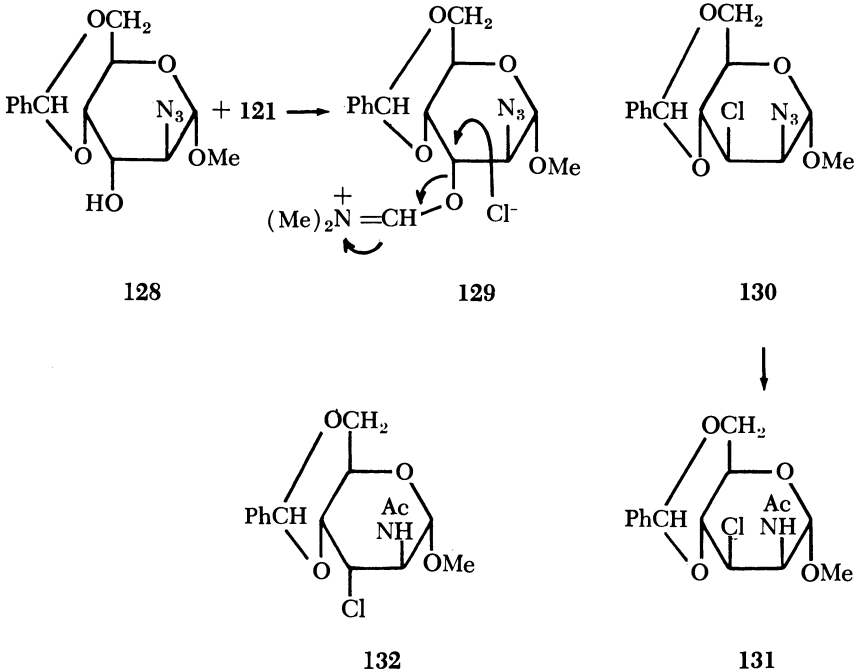
The formation of **120** can be explained by attack of chloride ion at C–6 (rather than the more hindred *endo* side at C–3) as depicted in the intermediate **126**, with concomitant migration of the ketal function to the 3,5-position. This procedure provides a convenient route to 6-substituted D-glucose derivatives from the readily accessible **49**. Since rotation about the C–5—0 bond is required for the migration of the ketal, the reaction most likely is not of a concerted nature and may proceed *via* **126a–126b**.

Treatment of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (**128**) (*42*) with **121** followed by refluxing and processing afforded a chloro derivative as a sirup in 70% yield (*46*, *49*). Reduction of this product with an excess of Raney-nickel in methanol containing acetic anhydride afforded a crystalline product, m.p. 179°C., which is formulated as the D-*manno* analog **131**. The actual product is most likely methyl azido-4,6-*O*-benzylidene-3-chloro-2,3-dideodxy-α-D-mannopyranoside (**130**) resulting from attack of chloride ion at C–3 with inversion of configuration in the intermediate **129**. Had the chlorination proceeded



128 **129** **130**

132 **131**

with retention of configuration at C–3, then the product of reduction and *N*-acetylation would have been the known (*22*) D-*altro* analog **132**. The reaction could conceivably be extended to the preparation of other esters

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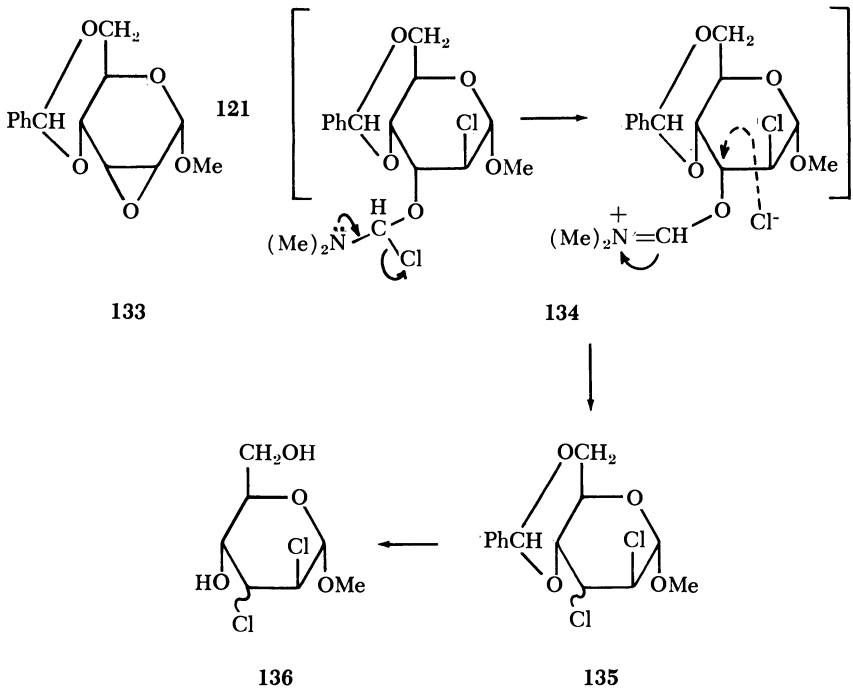
as well as using the appropriate amide chlorides such as (chloroethyl) dimethylammonium chloride (*31*, *32*), $(Me)_2^+N = CCH_3Cl\ Cl^-$ which would give acetate esters. This has been done with **73**.

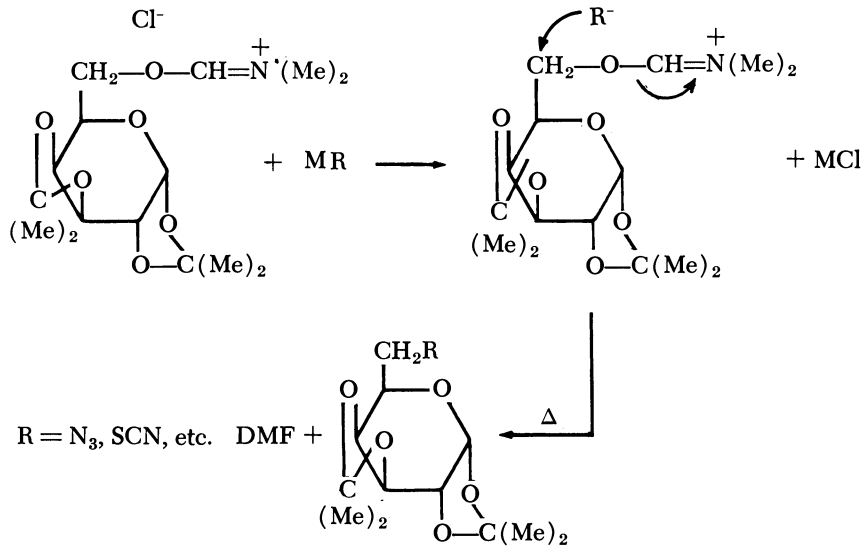
Another useful application of this reaction was demonstrated (*46*, *49*) in the opening of epoxide rings in suitably blocked sugar derivatives by the highly electrophilic reagent **121**. When **121** was allowed to react with methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allyopyranoside (**133**) (*90*, *91*) in tetrachloroethane at room temperature overnight, and the resulting solution was subsequently heated at 110°C. for 2 hours, a 2,3-dichloro derivative **135** was formed in 97% yield. Mild acid hydrolysis or hydrogenolysis over palladium-on-carbon afforded a crystalline methyl 2,3-dichloro-2,3-dideoxy-α-D-hexopyranoside (**136**). The reaction can be visualized as an initial attack of chloride ion on the epoxide function to give presumably an intermediate having the *altro* or *gluco* configuration. The liberated C–3 or C–2 oxygen anion respectively would then attack the reagent giving an intermediate such as **134** (shown here in the more probable *altro* configuration) which on heating decomposes with attack



of a second chloride ion to give the product. The same sequence has been postulated (*110*) in the reaction of several aliphatic cyclic ethers with **121**.

It would be reasonable to expect that the decomposition of the *N,N*-dimethylimino ester chlorides proceeds *via* a bimolecular mechanism already demonstrated for the thermal decomposition of simple imino ester salts (*79*). In the carbohydrate series, where an isolated secondary hydroxyl group is involved, such a process would result in chlorodeoxy sugar derivatives with overall inversion of configuration, provided that the approach of the chloride ion is not sterically hindered. Further experiments are in progress in this laboratory utilizing additional model substance to establish the scope and stereochemical course of the chlorination reaction.

An interesting ramification is the transformation that could be conceivably effected with the intermediate *N,N*-dimethylimino ester chlorides themselves through exchange reactions with other anions. Thus the exchange of chloride for azide or thiocyanate, and subsequent heating of the new salt would be expected to provide the corresponding azidodeoxy or thiocyanate sugar derivatives, respectively. If such exchange reactions are indeed realized then a method would be available to replace hydroxyl groups in sugar derivatives directly essentially in one step and without proceeding through displacement reactions of the corresponding sulfonate esters. A hypothetical example is shown below.



Sinclair (*92*) has described an improved method for the preparation of methyl 6-chloro-6-deoxy-α-D-glucopyranoside (**137**) from methyl α-D-glucopyranoside (**11**). The reaction was effected with sulfur monochloride S_2Cl_2 in *N,N*-dimethylformamide at room temperature and the

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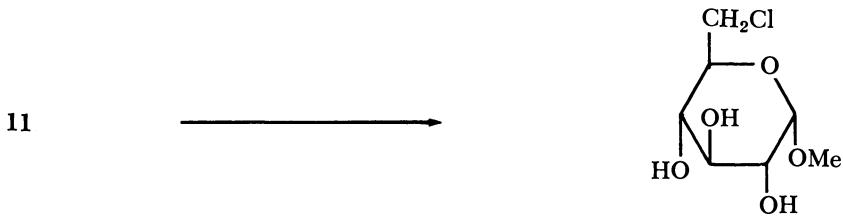

137

crystalline **137** was obtained in 32% yield after chromatography over Darco-Celite. Compound **137** can also be obtained in higher yield (*34*) from the reaction of **11** with methanesulfonyl chloride in *N,N*-dimethylformamide. The reaction presumably proceeds by initial selective imino ester formation at C–6 from **11** and a complex formed from the reagent and the solvent, followed by the subsequent decomposition of the intermediate at slightly elevated temperatures to give the product.

A recent chapter on "halogenated carbohydrates" contains a comprehensive review of the subject (*see* J. E. C. Barnett, *Advan. Carbohydrate. Chem.* **22**, 177 (1967)).

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10

Mass Spectrometry in Carbohydrate Chemistry

Glycosides and *O*-Isopropylidene Ketals of Deoxy Sugars

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*The mass spectra of methyl 3-deoxy-β-*D-threo*-pentopyranoside, methyl 4-deoxy-β-*D-threo*-pentopyranoside, and 5-deoxy-β-*D-xylo*-furanoside are discussed and compared; fragmentation paths are sufficiently different to allow identification on the basis of their mass spectra. On the other hand, the mass spectra of methyl 2- and 3-deoxy-5-O-methyl-β-*D-erythro*-pentofuranosides do not exhibit fragmentation differences. The mass spectra of 3-deoxy-1,2:5,6-di-O-isopropylidene-*D-xylo*-hexofuranose, 5-deoxy-1,2-O-isopropylidene-*D-xylo*-hexofuranose, and 6-deoxy-1,2-O-isopropylidene-*D-glucofuranose show prominent differences, even between the 5- and 6-deoxy isomers. The interpretation of the spectra was aided by metastable-ion peaks, mass spectra of D_2O-exchanged analogs, and the mass spectrum of an O-isopropylidene derivative prepared with acetone-d_6.*

In a single-focusing, magnetic-deflection mass spectrometer, gaseous molecules are ionized by techniques such as electron bombardment (*4*, *25*, *29*), photo-ionization (*4*, *25*, *29*), field ionization (*2*, *4*, *25*, *29*), and chemical ionization (*32*, *33*). The ionized molecules, called molecular ions, can decompose by fragmentation and/or rearrangement to form ions of mass lower than the mass of the original molecule (*4*, *5*, *30*). Since the sample pressure in the ionization region is on the order of 10^{-5} mm.Hg, except for chemical ionization, all reactions of molecular ions are gas phase and unimolecular. These positive ions are accelerated out of the ionization region in an electric field and subsequently deflected

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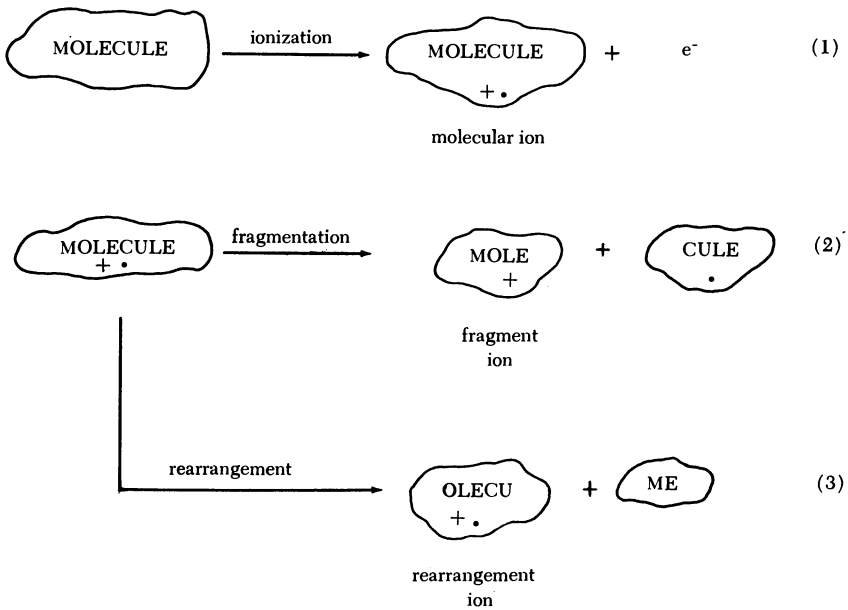
in a magnetic field (*4*, *25*, *29*). This causes the ions to separate according to their mass-to-charge ratios, m/e, and a mass spectrum consists of a record of the detection of these m/e–groups. Instruments of this type can resolve, for example, $C_{15}H_{18}O_3^{+\bullet}$ (nominally m/e 246) from $C_{11}H_{25}N_3O_3^{+\bullet}$ (nominally, m/e 247); but they cannot resolve $C_{15}H_{18}O_2^{+\bullet}$ (exactly, m/e 246.2043) from $C_{16}H_{22}O_2^{+\bullet}$ (exactly, m/e 246.2407), although commercial high-resolution, double-focusing instruments are available which can (*31*).

Processes occurring upon ionization and notations used for odd-electron ions ($^{+\bullet}$) and even-electron ions ($^{+}$) are illustrated in Equations 1, 2, and 3.



Only the positively charged species are accelerated out of the ionization region; neutral radicals—*e.g.*, CULE • in Equation 2, and molecules—*e.g.*, ME in Equation 3, produced by fragmentation and rearrangement, and un-ionized sample are pumped away.

Metastable-ion peaks are useful for establishing fragmentation routes (*4*, *5*, *25*, *29*, *30*). If ion_1 fragments to ion_2 after acceleration in the electric field and before deflection in the magnetic field, ion_2 is not found at the mass-to-charge ratio corresponding to either ion_1 or ion_2. It is detected as a broad, low-intensity peak, called a metastable-ion peak, and designated m^*, at approximately the square of the mass of ion_2 divided by the mass of ion_1 (Equations 4 and 5). These peaks are particularly

important in establishing that ion_2 comes from ion_1, especially if ion_2 can also be postulated to form by an alternate route. The presence of a metastable-ion peak can be taken as positive evidence that at least some of the ion_2's came from ion_1's, but the absence of a metastable peak cannot be used as an indication that such a direct fragmentation is not occurring.

$$[ion_1]^+ \rightarrow [ion_2]^+ + \text{a neutral species} \quad (4)$$

$$m^* \cong \frac{(\text{mass of } ion_2)^2}{(\text{mass of } ion_1)} \quad (5)$$

In the interpretation of a mass spectrum, careful consideration must be given to stabilization of positive charge in the ions and to the stability of the radicals and molecules formed upon rearrangement and fragmentation. The structure of the sample molecule can often be revealed by studying the *m/e*–groups which are formed in greatest abundance from unimolecular reactions upon electron impact (*4*, *5*, *8*, *9*, *25*, *29*, *30*). The information gleaned from a mass spectrum is particularly useful when combined with NMR, infrared, and ultraviolet spectral data, (*35*). The sample size required for a useful mass spectrum is usually no more than a few tenths of a milligram and can be in the nanogram quantity. The versatility of mass spectrometry has greatly been extended by a gas-chromatography-mass-spectrometry combination which allows the recording of the mass spectra of compounds as they come off the gas chromatography column.

Mass Spectra of Carbohydrates

A number of reviews of mass spectra of carbohydrates have been published from which references to the original papers are available (*4*, *9*, *11*, *24*, *26*). The application of mass spectrometry to this field was initially limited by the relatively low volatility of free carbohydrates and by the complex spectra obtained from some derivatives. These limitations have been partially overcome by new inlet techniques and by pioneering studies on classes and derivatives in order to understand the characteristic fragmentations and rearrangements of the molecular ions of a wide range of carbohydrates.

The mass spectra of free carbohydrates and their glycosides, obtained by ionization upon electron impact, are limited in their usefulness for structural studies. Peaks corresponding to molecular ions are generally not observed due to extensive fragmentation to ions of low *m/e* (*4*, *9*, *11*, *24*, *26*). In contrast, positive ions produced by field ionization do not give fragment spectra as characteristic as do those produced by electron impact, but the molecular ion peaks are intense, often the most intense in the spectra (*3*).

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The mass spectra of per-*O*-acetylated and per-*O*-methylated carbohydrates generally do not exhibit molecular-ion peaks (*4, 9, 11, 24, 26*). Their spectra are characteristic of ring size and substitution, although stereochemical differences influence relative peak intensities rather than fragmentation paths. Amino sugars, deoxy sugars (*6, 27*), anhydro sugars, and some disaccharides have been studied as well as sugars in furanose, pyranose, and acyclic forms.

O-Isopropylidene derivatives of carbohydrates form structural isomers from carbohydrates which themselves are epimers. Since structural isomers often fragment differently whereas epimers do not, mass spectra of these derivatives may permit interpretation in terms of stereochemistry. Although molecular-ion peaks are not observed, the molecular weight can be determined readily from a relatively intense "M-CH_3" peak, resulting from loss of a methyl radical from a 1, 3-dioxolane ring (*12*).

Diethyl dithioacetal derivatives of carbohydrates are generally crystalline compounds which are easily prepared from sugars in the combined or free form. In contrast to the mass spectra of the carbohydrate derivatives discussed above, the mass spectra of diethyl dithioacetals allow direct determination of molecular weight from molecular-ion peaks of 5-20% relative intensity. Major fragments are formed which are characteristic of substitution on C–1, C–2, C–3, and C–4. Since the molecules are acyclic, complications due to ring forms are not found. The use of dialkyl dithiocetals also allows the determination of the partial or total configuration of the parent sugar by the application of the MacDonald-Fischer degradation (*28*). This and other features make these very suitable carbohydrate derivatives for such studies.

From the mass spectra of nucleosides, it is possible to recognize fragmentations involving the sugar moiety and fragmentations involving the base (*7*). The mass spectra of adenosine (*7*), 2′-deoxyadenosine (*7*), and 3′-deoxyadenosine (*21*) contain significant peaks from which the presence of 2′-deoxy or 3′-deoxy functions can be readily recognized. The mass spectra of the nucleoside antibiotic puromycin and its derivatives have been recorded (*16*).

The positive-ion mass spectrum of somalin, a steroid linked to a 2, 6-dideoxy-3-*O*-methylhexopyranoside, exhibits a very minute molecular-ion peak when obtained by electron impact (*1, 36*). On the other hand, the negative-ion mass spectrum of somalin contains an intense peak one mass unit lower than the molecular ion (*1*). (Negative-ion mass spectra are obtained upon electron impact by accelerating and deflecting negative ions produced by electron capture, as illustrated for an alcohol in Equation 6). A capability for study of positive and negative ions formed

$$R - O - H + e^- \rightarrow R - O^- + H\cdot \qquad (6)$$

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by field ionization represents a versatile mass spectrometry laboratory for applications to carbohydrate chemistry.

Mass Spectra of Deoxy-Sugar Derivatives

The deoxy sugars (*18*) occupy a unique area in the domain of biologically important sugars. They have been implicated in the mechanisms of various biological functions and have been known in some cases to be responsible for the biological properties of the parent substances. Until recently, one of the commonly used techniques for the characterization of various deoxy sugars, whether derived from biological sources or through synthesis, was the application of specific colorimetric tests (*15*). Although relatively small amounts of material are required for these tests, a preliminary mild acid hydrolysis step to the free sugar is necessary.

Other methods of identification include the customary preparation of derivatives, comparisons with authentic substances whenever possible, and periodate oxidation. Lately, the application of nuclear magnetic resonance spectroscopy has provided an elegant approach to the elucidation of structures and stereochemistry of various deoxy sugars (*18*). Microcell techniques can provide a spectrum on 5-6 mg. of sample. The practicing chemist is frequently confronted with the problem of having on hand a few milligrams of a product whose structure is unknown. It is especially in such instances that a full appreciation of the functions of mass spectrometry can be developed.

Until now, studies of deoxy sugars by this technique have utilized three types of derivatives, namely acetylated sugars, methylated methyl glycosides, and dialkyl dithioacetals. Limited studies on the former group comprise a 6-deoxyhexose (*6*) and two isomeric, 3, 4-dideoxypentoses (*37*). The mass spectra of four methylated methyl hexopyranosides having a deoxy function at C–2, C–3, C–4, and C–6, respectively, were examined by Kochetkov and colleagues (*26*, *27*). It was shown that useful information could be secured from the fragmentation patterns regarding the position of the deoxy function in these derivatives. Finally, the commonly encountered members of deoxy and dideoxy sugars in synthetic work and in biological substances were investigated in the form of their dialkyl dithioacetals (*13*). Not only could the fragmentation patterns be easily interpreted and correlated with the respective structures, but significant differences were apparent in these spectra which allowed definite conclusions to be made regarding the position of deoxy functions and other substituents. Like the methylated glycosides, these deriavtives should also be adaptable to gas chromatographic analysis (*40*).

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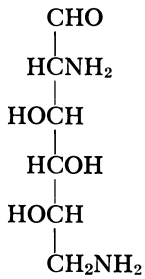
This tool has been of great value in the elucidation of the structures of some important biologically-derived amino (*14*) and deoxy (*13*) sugars in the form of their dialkyl dithioacetals. Tedious degradation reactions which would require both time and valuable material could be avoided in many cases by resorting to mass spectrometry. The antibiotic sugars (22) paramose (**1**), mycinose (**2**) and chalcose (**3**) were, for example, studied by mass spectrometry (*13, 14*).



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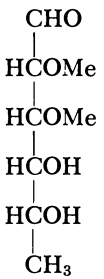

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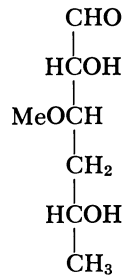

3

Discussion of the Mass Spectra of Compounds 4–11

The present work involves the study of methyl glycosides and *O*-isopropylidene ketals of various isomeric deoxy sugars by mass spectrometry. Several of the compounds selected for the present study have free hydroxyl groups, and interpretation of their mass spectra shows the scope of the study of these and related deoxy sugar derivatives by mass spectrometry without prior substitution of all hydroxyl groups. Some of the candidates (compounds **4, 7, 8** and **10**) are structurally related to biologically-derived deoxy sugars.

The following compounds were obtained from the ring opening reaction (*18, 19, 20*) of suitable *O*-benzylidene acetals with *N*-bromosuccinimide, followed by catalytic reduction of the respective isomeric bromobenzoates and, finally, debenzoylation: methyl 2-deoxy-5-*O*-methyl-β-D-*erythro*-pentofuranoside (**7**), methyl 3-deoxy-5-*O*-methyl-β-D-*erythro*-pentofuranoside (**8**), methyl 3-deoxy-β-D-*threo*-pentopyranoside (**4**) methyl 4-deoxy-β-D-*threo*-pentopyranoside (**5**), methyl 5-deoxy-D-xylofuranoside (**6**) and 6-deoxy-1, 2-*O*-isopropylidene-D-glucofuranose (**10**). Except for the crystalline compound **10**, the deoxy sugar derivatives were all chromatographically homogeneous liquids which had chemical and nuclear magnetic resonance spectral characteristics compatible with the respective structures. It should be noted that compounds **4** and **5** can be easily differentiated using periodate oxidation data. However, discrimination between the two structures can be made easily from their mass spectra using only minute amounts of sample. Presumably this method

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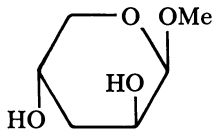
could also be useful in differentiating the corresponding 2-deoxy analog which like **5** would be oxidized by periodate.



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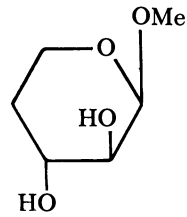

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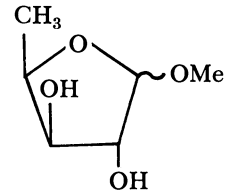

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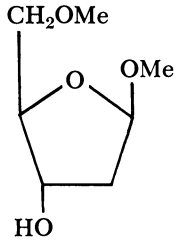

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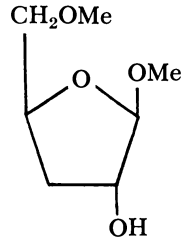

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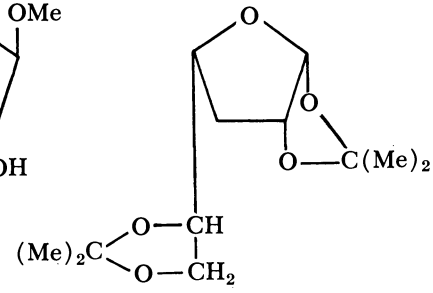

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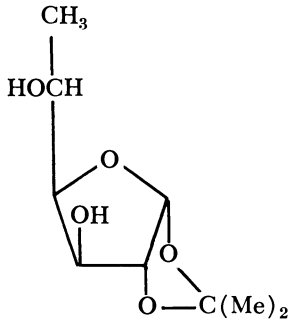

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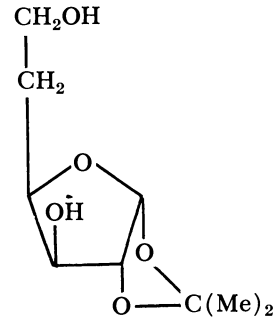

11

The remaining derivatives, 3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-*xylo*-hexofuranose (*34,39*) (**9**) and 5-deoxy-1,2-*O*-isopropylidene-D-*xylo*-hexofuranose (*23, 41*) (**11**), together with the previously mentioned **10** comprise

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a group of three isomeric deoxy-hexofuranoses. Their differentiation by mass spectral analysis is particularly significant, since the physical constants of compounds **10** (*17*, *38*) m.p. 89°C., $[\alpha]_D$ —26°, those of **11,** (*10*) m.p. 89°–90°C., 94–96 $[\alpha]_D$ —10° and those of the isomeric 3-deoxy-1, 2-*O*-isopropylidene-D-*ribo*-hexofuranose (*10*), m.p. 84°C., are very similar.

Experimental. The mass spectra in Figures 1–8 are positive-ion spectra produced by electron impact and were obtained from a single-focusing, magnetic deflection Atlas CH4 Mass Spectrometer. The ionizing potential was 70 e.v. and the ionizing current 18μ a. An enamel reservoir heated to 120°C. was used from which the sample was leaked into the ion source.

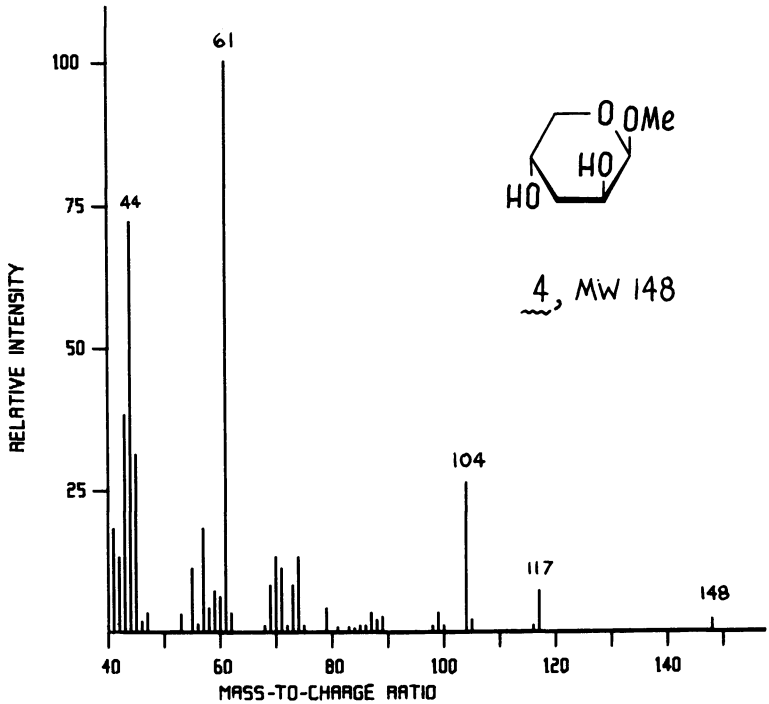
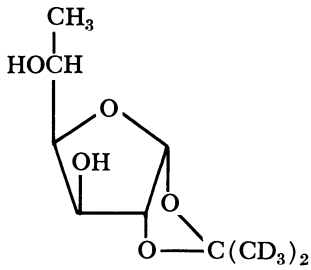


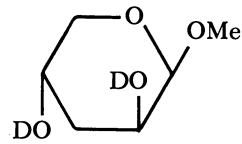
*Figure 1. Mass spectrum of methyl 3-deoxy-β-*D-threo-*pentopyranoside* **(4).**

The compounds were also run with D_2O leaking into the ion source from a stainless steel reservoir. The resulting mass spectra show partial exchange of the hydroxyl protons with deuterium. From the shifts of the peaks in the mass spectra of the deuterated analogs, the number of exchangeable hydrogens retained in each fragment can be determined.

Compound **10** was also prepared using acetone-d_6. Peak shifts between the mass spectra of compounds **10** and **10a** will be used to interpret the fragmentation of compound **10.**

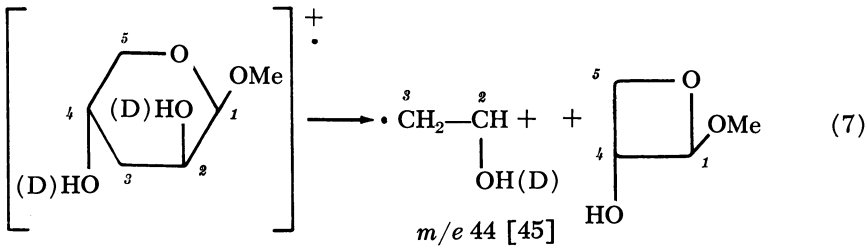


10a

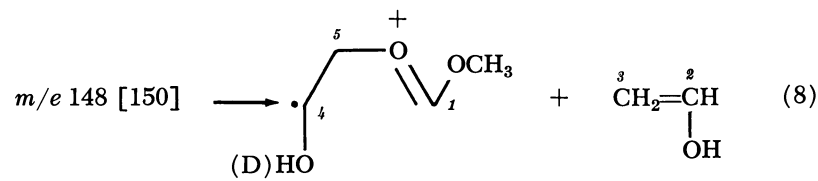


4a

The Mass Spectra of Sugars 4 (Figure 1), 5 (Figure 2), and 6 (Figure 3). In the mass spectra of methyl 3-deoxy-β-D-*threo*-pentopyranoside (**4**, Figure 1) and its D_2O-exchanged analog (**4a**, mass spectrum not shown), molecular-ion peaks are found at *m/e* 148 and *m/e* 150 respectively. Loss of the C–1 methoxyl group, a commonly observed (*4, 9, 11, 24, 26*) fragmentation of methyl glycosides, accounts for the fragmentation to *m/e* 117 [119]. The peaks at *m/e* 104 [105] and *m/e* 44 [45] can be explained by loss of a two-carbon portion of the ring, possibly C–2–C–3, C–3–C–4, or C–4–C–5, as proposed in Equations 7 and 8 for loss of C–2–C–3. (The value in brackets here and hereafter represents the location of the peak in the mass spectrum of the D_2O-exchanged analog.)



m/e 148 [150] *m/e* 44 [45] (7)



m/e 148 [150] *m/e* 104 [105] (8)

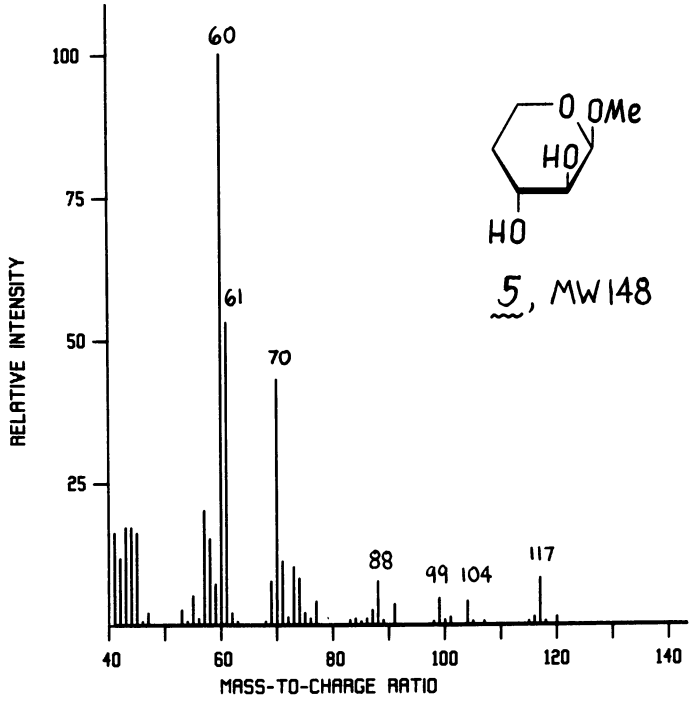


Figure 2. Mass spectrum of methyl 4-deoxy-β-D-threo-pentopyranoside **(5).**

(The structures shown for ions, such as in Equations 7 and 8, are entirely speculative since the mass spectrum gives only relative abundance of the various *m/e*–groups formed. Chemical reasoning, metastable-ion peaks, peak shifts in the deuterated analogs, and published mass spectra of similar compounds are the bases for the proposed modes of fragmentation and for the postulated structures.)

The most striking difference between the mass spectra of methyl 3-deoxy- and 4-deoxy-β-D-*threo*-pentopyranosides, **4** (Figure 1) and **5** (Figure 2), respectively, is the prominence of *m/e* 104 and 44 in the former and of *m/e* 60 [62] in the latter. The loss of C–2–C–3 from **5** leads to *m/e* 60 (*see* Equation 9), whereas its loss from **4** leads to *m/e* 44 (*see* Equation 7). The peak at *m/e* 104 [105] in Figure 2 shows that the elimination of C–3–C–4 also occurs from isomer **5.**

The shift of *m/e* 88 in Figure 2 to *m/e* 90 in the mass spectrum (not shown) of the D_2O-exchanged analog **5a** indicates that this fragment has the part of the original molecule shown in structure **12** rather than the part in structure **13**, both of which add to 88. This assignment is

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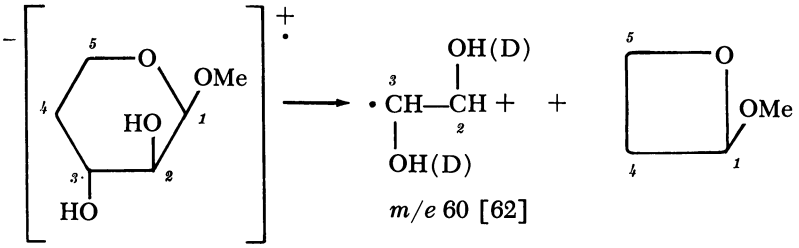
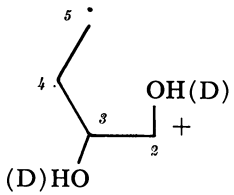


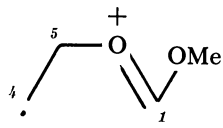
(9)

from **5**

supported by the presence of a metastable-ion peak at *m/e* 55.8 (calculated 55.7), for the loss of 18 mass units, a molecule of water, from *m/e* 88 (*see* Equation 10).



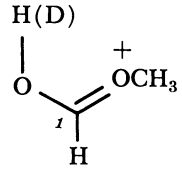
12, *m/e* 88 [90]



13

$$m/e\ 88 \xrightarrow{m^*} m/e\ 70 + H_2O \qquad (10)$$

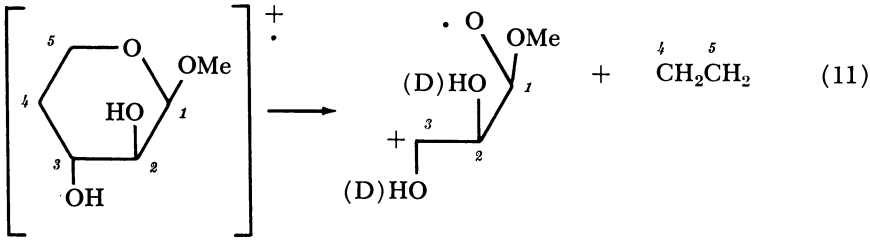
The peaks at *m/e* 61 in Figures 1 and 2 shift to *m/e* 62 in the mass spectra of the D_2O-exchanged compounds **4a** and **5a**. A stable ion (**14**) containing C–1, the ring oxygen, and a rearranged hydrogen can be proposed for this fragment. The shifts to *m/e* 62 suggest that the rearranged proton comes from one of the hydroxyl groups.



14, *m/e* 61 [62]

No molecular-ion peak is observed in the mass spectrum of isomer **5**, although the fragment at *m/e* 117 [119] is present from loss of the C–1

methoxyl group. An important, but low intensity, peak at *m/e* 120 [122] apparently arises from loss of C–4–C–5 as shown in Equation 11.



(11)

m/e 120 [122]

The 5-deoxy isomer, methyl 5-deoxy-β-D-*xylo*-furanoside (**6**), gives a mass spectrum (Figure 3) which contains no molecular-ion peak, but does contain peaks at *m/e* 117 [119] and *m/e* 116 [117] for the losses of the C–1 methoxyl group and a molecule of methanol, respectively.

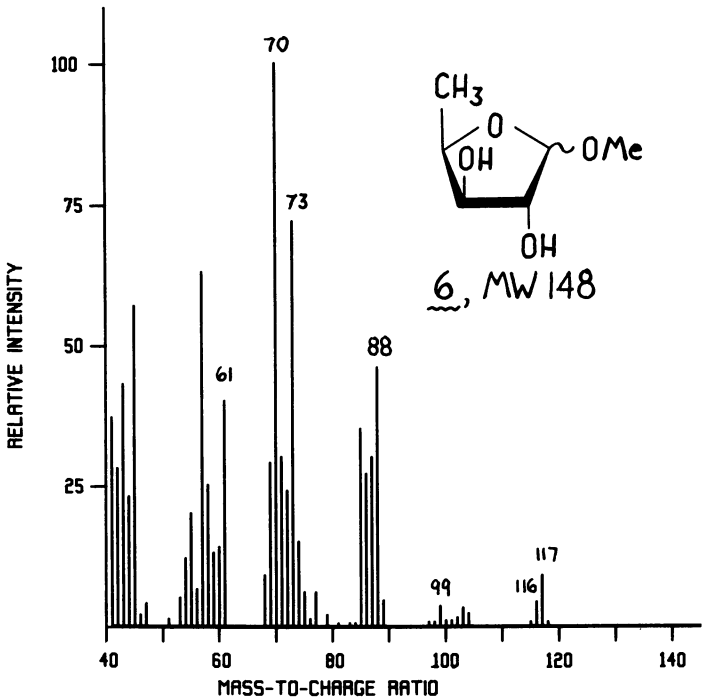
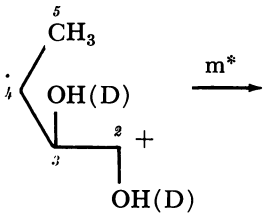


Figure 3. Mass spectrum of methyl 5-deoxy-β-D-xylofuranoside **(6).**

Losses of a methyl radical and a molecule of water from the fragment at *m/e* 88 [90] are supported by the presence of metastable-ion peaks at *m/e* 60.6 (calculated for 88 → 73, 60.5) and *m/e* 55.8 (calculated for 88 → 70, 55.7). Plausible interpretations of these peaks are given in Equations 12 and 13. The loss of a methyl radical from the ion at *m/e* 88 is prominent in Figure 3, but not in Figures 1 and 2, making it characteristic of the 5-deoxy furanoid structure.



m/e 73 [75] + CH_3· (12)

m/e 88 [90]

m*

m/e 70 [71] + H_2O (13)

An interesting sidelight is found in the loss of water from *m/e* 117 [119] in Figures 1-3 to give *m/e* 99 [99]. A metastable-ion peak at *m/e* 83.9 (calculated, 83.8) is found for this transition. The lack of peak shift for *m/e* 99 indicates that water is lost between the two hydroxyl groups and that the loss doesn't involve ring hydrogens. For this to occur unimolecularly, the rings would have to open for the atoms involved to approach one another sterically. The shift of *m/e* 116 in Figure 3 to *m/e* 117 in the mass spectrum of the D_2O-exchanged analog **6a** shows that the loss of methanol from the molecular ion of **6** occurs between the C–1 substituent and a hydroxyl group.

An examination of the mass spectra of these isomeric compounds and of their D_2O-exchanged analogs leads to the conclusions that the spectra can be interpreted in terms of the structures **4–6** and that structural differences lead to extensive differences in fragmentation. These mass spectra now can be used for identification purposes and as models to aid in the interpretation of the mass spectra of similar compounds, possibly of unknown structure.

The Mass Spectra of Sugars 7, (Figure 4) and 8 (Figure 5). Whereas the mass spectrum of methyl 5-deoxy-β-D-xylofuranose (**6**, Figure 3) showed no prominent peaks from cleavage of the C–4–C–5 bond to lose the C–5 substituent, the mass spectra of methyl 2-deoxy-5-*O*-methyl-β-D-*erythro*-pentofuranoside (**7**, Figure 4) and methyl 3-deoxy-5-*O*-methyl-β-D-*erythro*-pentofuranoside (**8**, Figure 5), and of their D_2O-exchanged

analogs, have intense peaks from such a cleavage. This cleavage, illustrated in Equations 14 and 15, gives rise to stable ions at *m/e* 117 [118] and *m/e* 45 [45], depending on which carbon the charge is retained.

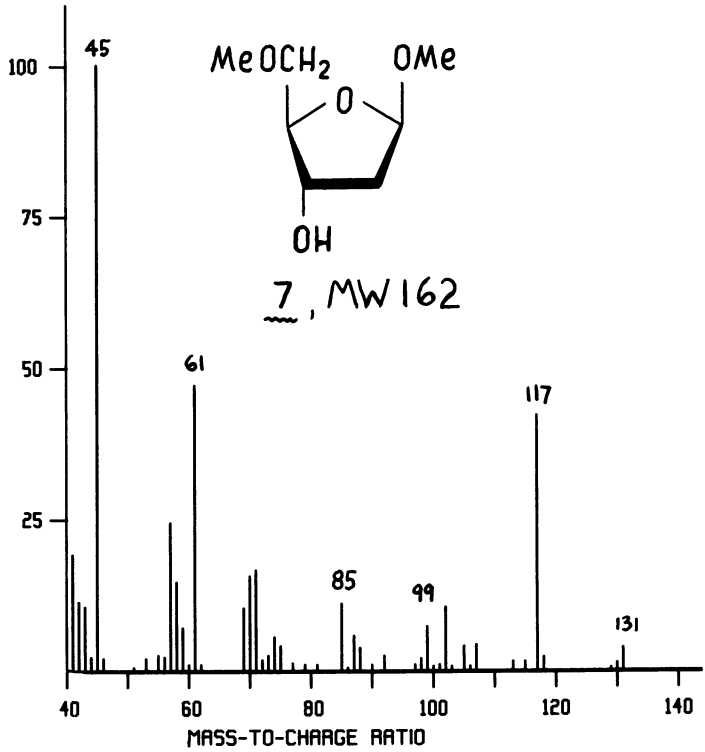
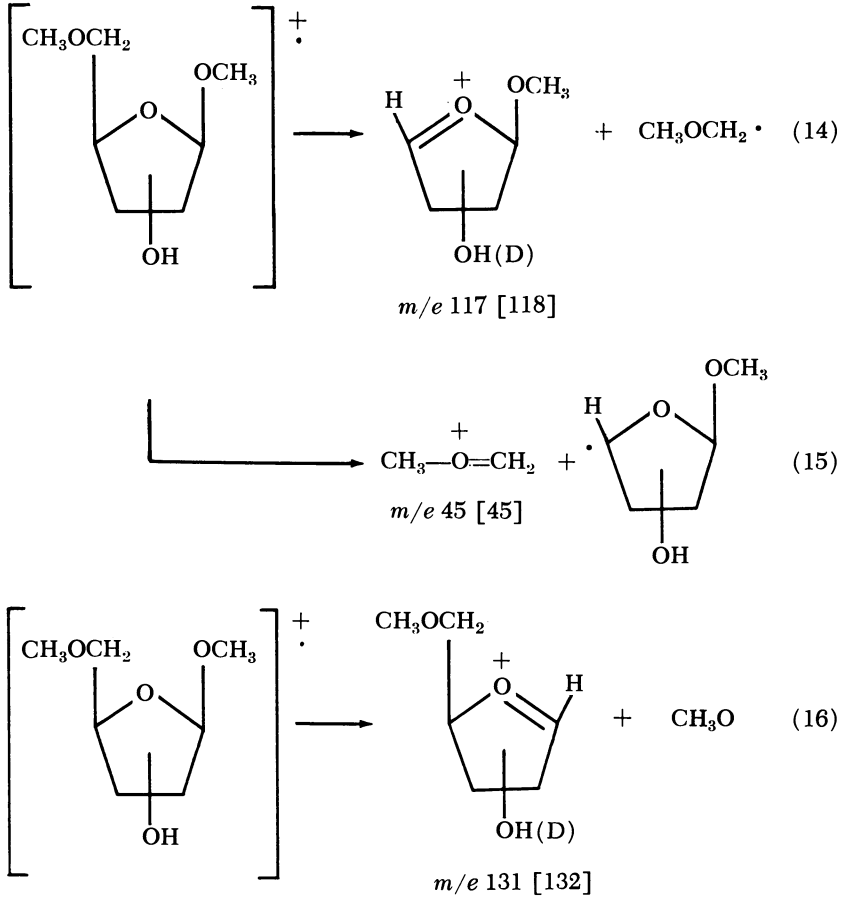


Figure 4. Mass spectrum of methyl 2-deoxy-5-O-methyl-β-D-erythro-pentofuranoside **(7).**

No molecular-ion peak is observed from either isomer. The loss of a methoxyl group to give *m/e* 131 is shown in Equation 16 to be from the C–1 position, on the basis of analogy to reported studies using per-*O*-methylated sugars with a methoxyl-d_3 substituent on C–1 (*24*).

The similarity between Figures 4 and 5 is a reflection of the fact that C–2 and C–3 and their hydroxyl substituents and deoxy functions do not trigger the major fragmentations of these molecules upon electron impact. A few minor relative intensity differences can be observed, but they are not of a large enough magnitude or of such a nature as to be useful in distinguishing between the 2- and 3-deoxy isomers.

Metastable-ion peaks at *m/e* 61.8 (calculated, 61.7) and *m/e* 83.8 (calculated, 83.8) relate the peaks at *m/e* 85 and 99 to *m/e* 117, respectively, by loss of methanol and of water.



These mass spectra illustrate a major difference between mass spectrometry and other spectroscopic methods of analysis. In other techniques, functional groups can be recognized, and the wavelength of the energy they absorb is dependent only on their immediate environment, spatial as well as bond-linked. However, a mass spectrum is a composite of the probabilities of all the possible bond cleavages and formations (in rearrangements) in molecular ions and fragment ions. The final appearance of a spectrum does not depend on isolated functional groups acting independently, but depends on the molecule fragmenting as a unit.

The Mass Spectra of O-Isopropylidene Ketals 9 (Figure 6), 10, (Figure 7), and 11 (Figure 8). Three fragmentations characteristic of *O*-isopropylidene ketals are loss of a methyl radical from the ketal ring, cleavage of bonds adjacent to the ketal ring, and loss of acetone (*12*).

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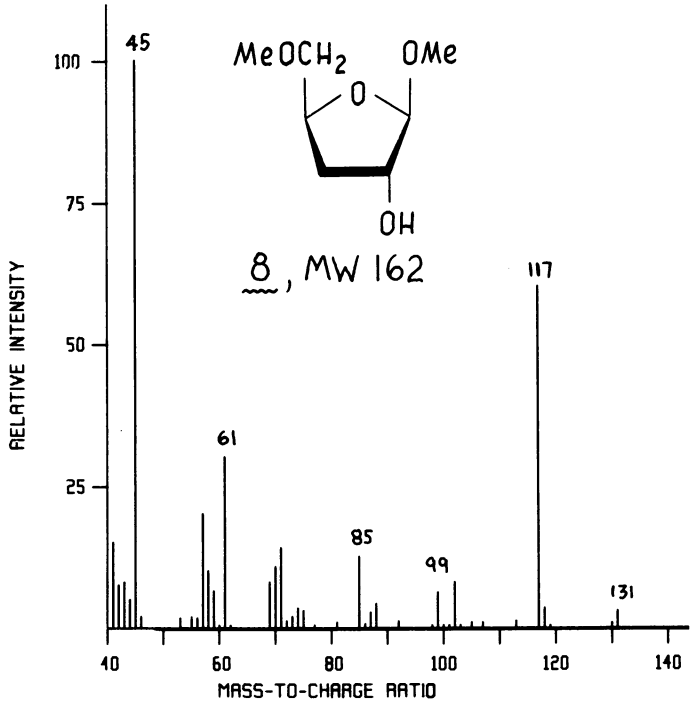

*Figure 5. Mass spectrum of methyl 3-deoxy-5-O-methyl-β-*D-erythro-*pentofuranoside* **(8).**

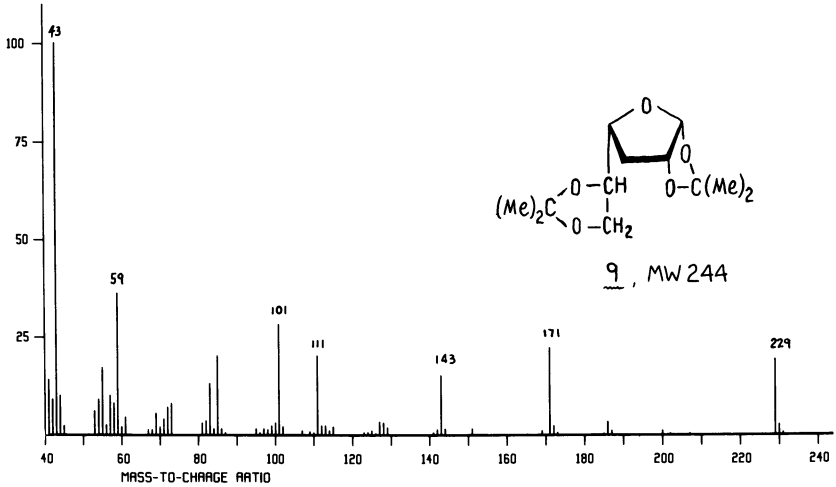

*Figure 6. Mass spectrum of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-*D*-xylo-hexofuranose* **(9).**

Molecular-ion peaks are not generally observed, but molecular weight can be readily determined from a relatively intense "M-15" ($M-CH_3$)

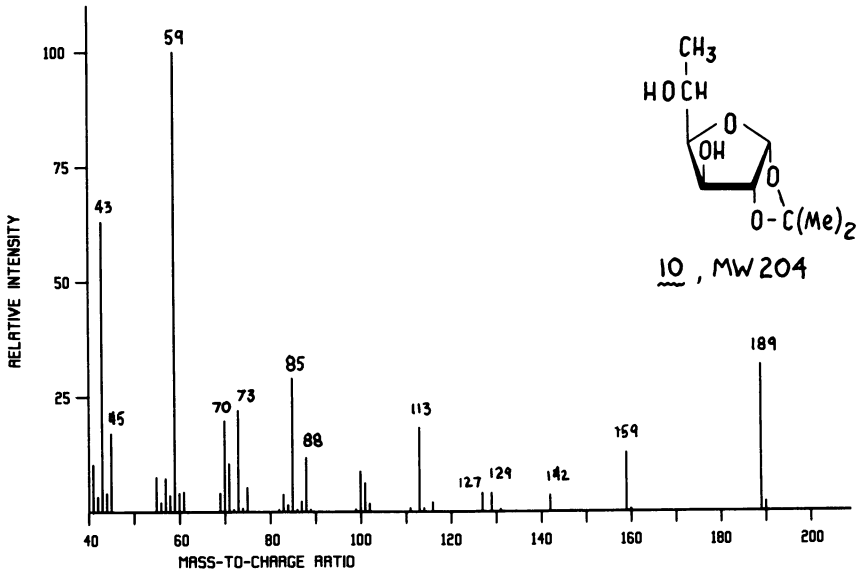

Figure 7. Mass spectrum of 6-deoxy-1,2-O-isopropylidene-D-glucofuranose **(10).**

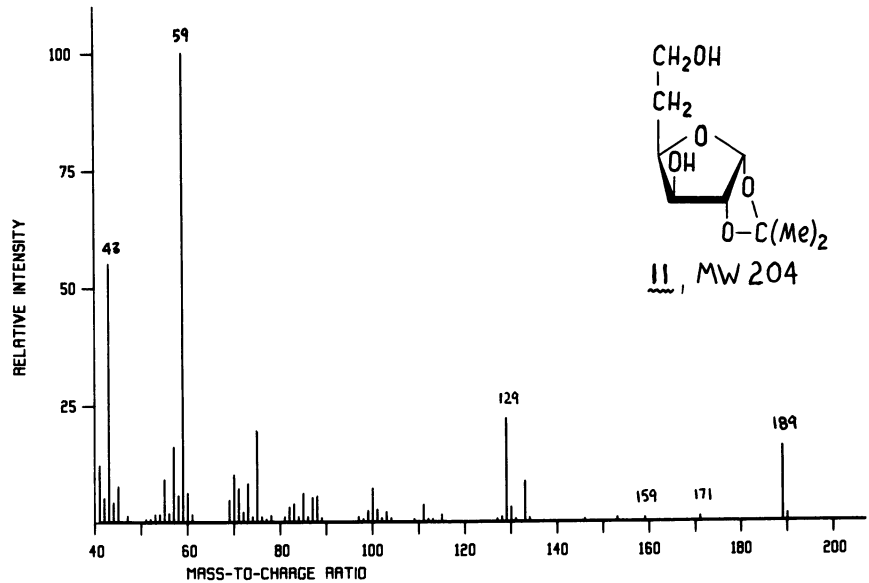

Figure 8. Mass spectrum of 5-deoxy-1,2-O-isopropylidene-D-xylo-hexofuranose **(11).**

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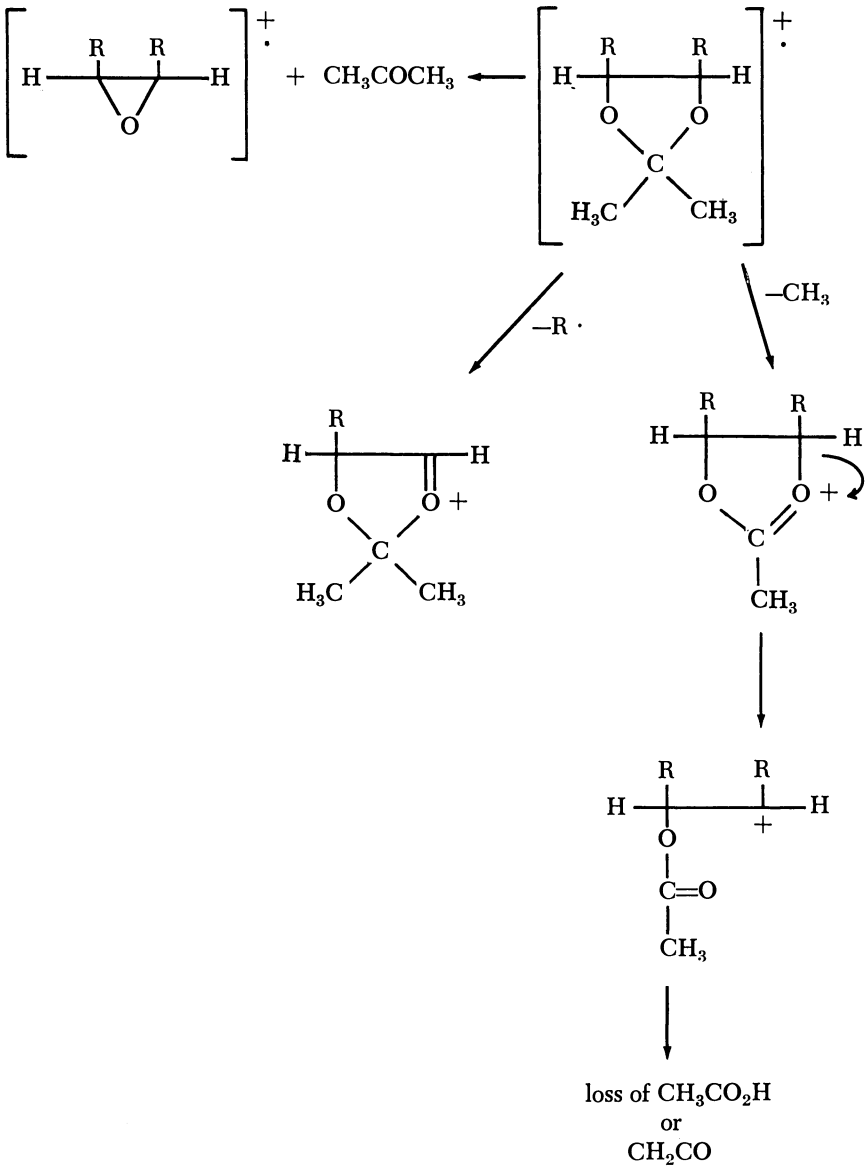
peak. The fragment formed by loss of a methyl radical fragments further by loss of acetic acid or ketene. Scheme 1 contains equations to illustrate these characteristic paths, although the structures are speculative.

Scheme 1



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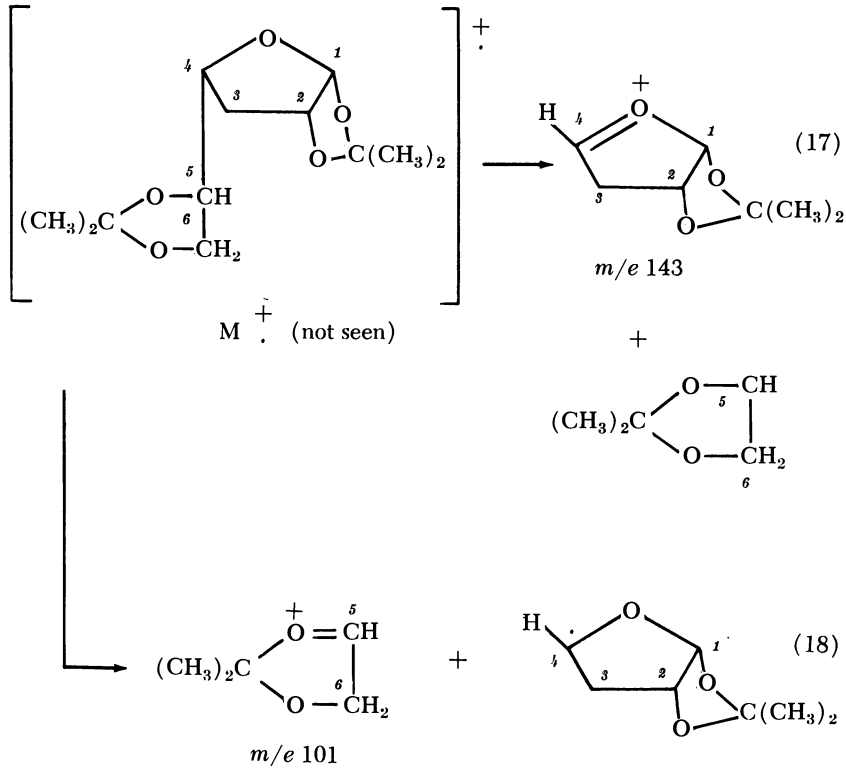
In the mass spectrum (Figure 6) of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-*xylo*-hexofuranose (**9**) the fragmentations described above are found at *m/e* 229, 171, 143, 111, and 101. The fragments at *m/e* 143 and 101 arise by cleavage of C–4–C–5 with charge retention on C–4 and C–5, respectively (*see* Equations 17 and 18). Scheme 2 summarizes the losses of a methyl group, acetone from the second cyclic ketal function, and



acetic acid which account for the peaks at *m/e* 229, 171, and 111. Metastable-ion peaks at *m/e* 127.8 (calculated, 127.7) and *m/e* 72.3 (calculated, 72.1) support the sequence in Scheme 2. Mass spectra of *O*-isopropylidene ketals commonly exhibit intense peaks at *m/e* 59 and 43 which deuterium labeling has shown to be protonated acetone and the acetyl ion (*12*).

6-Deoxy-D-glucose forms a mono-*O*-isopropylidene ketal, 6-deoxy-1,2-*O*-isopropylidene-D-glucofuranose (**10**), whereas 3-deoxy-D-*xylo*-hexose forms a di-*O*-isopropylidene derivative **9**. This difference is easily recognized from the corresponding mass spectra, Figures 7 and 6 respectively, by mass difference as well as by fragmentation paths. To help unravel

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Scheme 2

molecular ion **9** → *m/e* 229 + $CH_3\cdot$

↓ m*

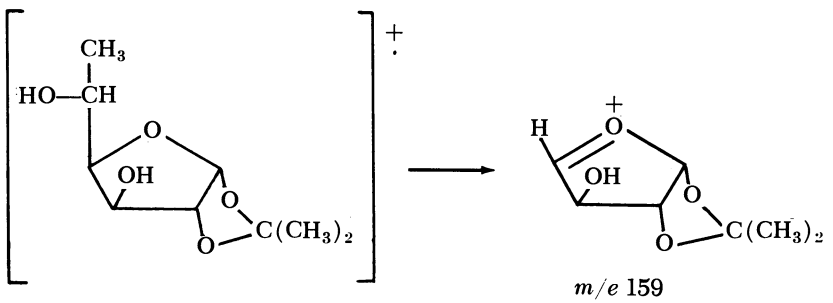
m/e 171 + CH_3COCH_3

↓ m*

m/e 111 + CH_3CO_2H

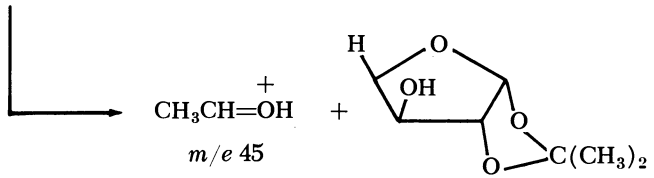
these paths, the mass spectra of **10a** (compound **10** prepared with acetone-d_6) and of the D_2O-exchanged analog of **10** were obtained.

Once again no molecular-ion peak is seen, but the "M–CH_3" peak is prominent at *m/e* 189 in Figure 7. An important peak at *m/e* 159 from C–4–C–5 cleavage with charge retention on C–4 establishes the presence of a furanose ring and of a 6-deoxy function. Charge retention on C–5 leads to the ion at *m/e* 45 (*see* Equations 19 and 20).



m/e 159

molecular ion **10** (not seen) + $CH_3\dot{C}HOH$ (19)



m/e 45

(20)

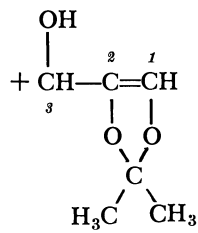
In the mass spectrum (Figure 8) of the corresponding ketal of 5-deoxy-D-*xylo*-hexose, 5-deoxy-1,2-*O*-isopropylidene-D-*xylo*-hexofuranose (**11**), the peak from C–4–C–5 cleavage, *m/e* 159, is of minor relative intensity. Since the ions at *m/e* 159 are the same from both isomers, **10** and **11**, the intensity difference must be attributable to the lower stability of the primary radical formed from C–5 of **11** compared with the secondary radical from 10:

$$HOCH_2 - CH_2 \cdot \quad vs \quad CH_3\dot{C}HOH.$$

Actually, this decreased probability of fragmentation to primary radicals is pronounced in the mass spectra of diethyl dithioacetal derivatives of deoxy sugars and is particularly useful there also for locating the position of deoxy functions (*13*).

Detailed discussions of some of the remaining peaks in Figures 7 and 8 and in the mass spectra of **10a** and the D_2O-exchanged analogs is of more interest to the mass spectrometrist than to the carbohydrate chemist. The probable origins of these peaks will be discussed here, however, because there will be occasions when the carbohydrate chemist must dig into a spectrum in order to satisfy himself that he has interpreted the spectrum in terms of a correct structure.

To illustrate the value of the mass spectra of the labeled compounds, the peaks at *m/e* 129 in Figures 7 and 8 will be considered first. These peaks could be from the loss of acetic acid (60 mass units) from *m/e* 189, or the loss of water (18 mass units) from *m/e* 189 followed by loss of ketene (42 mass units); structure **15**, containing C–1–C–2–C–3 less a rearranged hydrogen atom from C2, is another possibility. The composition of this ion could be important for confirming the presence of a 3-hydroxyl group.



15, *m/e* 129

In the mass spectra of the D_2O-exchanged analog of **10** and of the d_6-analog (**10a**), *m/e* 129 fails to shift in the former and shifts to *m/e* 135 in the latter. This is inconsistent with the possibilities suggested in the above paragraph and with **15** which contains a hydroxyl group, but is consistent with structure **16** or its equivalent. Structure **16** retains

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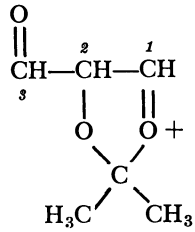
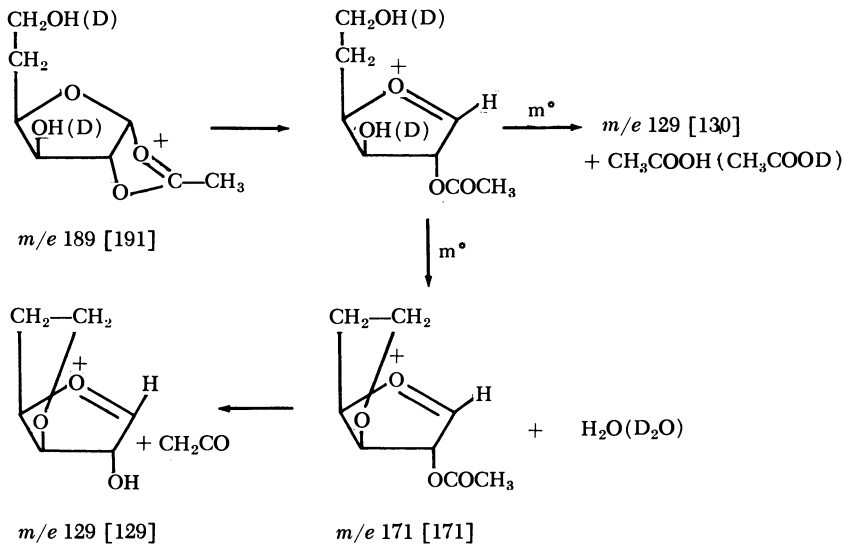
C–1–C–2–C–3 but has transferred the C-3-hydroxyl hydrogen, rather than a C–2 ring hydrogen as shown in **15**.



16

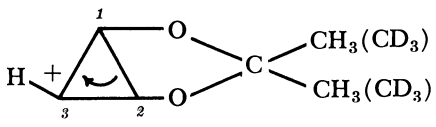
On the other hand, a metastable-ion peak at *m/e* 88.1 (calculated, 88.0) is present in the mass spectrum of **11** (Figure 8) for the formation of *m/e* 129 from *m/e* 189, by loss of acetic acid. In the mass spectrum of the D_2O-exchanged analog, *m/e* 129 partially shifts to *m/e* 130 and partially stays at *m/e* 129. Metastable-ion peaks are also present at *m/e* 154.8 (calculated, 154.7) and *m/e* 97.3 (calculated, 97.3) for the loss of water from *m/e* 189 followed by the loss of ketene, to give an ion at *m/e* 129. Since *m/e* 171 from the loss of water remains at *m/e* 171, the loss of water must involve the hydroxyl hydrogens. Scheme 3 is an attempt to summarize this in terms of structures which are entirely

Scheme 3



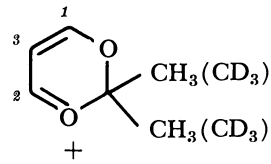
speculative. To complicate matters further, structure **16** is also consistent with the lack of shift of *m/e* 129.

Peaks at *m/e* 113 and 85 have been found in the mass spectra (*12*) of other *O*-isopropylidene ketals of sugars, as well as in Figure 7. Since these shift to *m/e* 119 and to *m/e* 88 and 91 in the mass spectrum of **10a** as they did for the d_6-analogs in Reference 12, the structures, **17, 18**, and **19** from Reference 12 are shown as possible explanations. The peak at *m/e* 85 (91) could alternatively be from *m/e* 113 (*119*) by loss of carbon monoxide (28 mass units) from the six-membered-ring of structure **17b.**



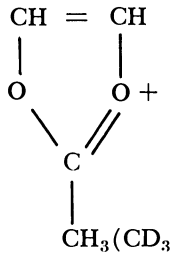
17a

or

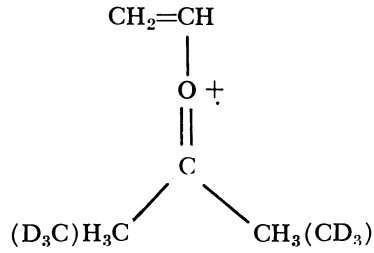


17b

m/e 113 (119)



18, *m/e* 85 (88)

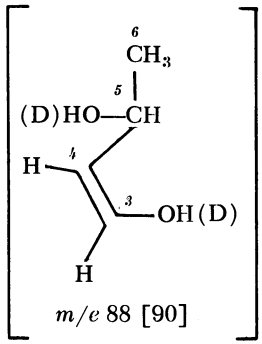


19, *m/e* 85 (91)

Peaks at *m/e* 88, 73, and 70 in Figure 7 shift to *m/e* 90, 75, and 70 in the mass spectrum of the D_2O-exchanged analog and fail to shift in the mass spectrum of the d_6-analog **10a**. Equations 21 and 22 are consistent with this data.

In Figure 7 the peak at *m/e* 142, which shifts to *m/e* 148 in the mass spectrum of **10a** and remains at *m/e* 142 in the spectrum of the D_2O-exchanged analog, probably arises by the loss of the C-3 and C-4 side chains. This ion could fragment further by eliminating a methyl radical from the ketal to give an ion at *m/e* 127 which shifts to *m/e* 130.

This detailed interpretation of the mass spectrum in Figure 7 in terms of structure **10** is included to illustrate the advantages and limitations



$$\xrightarrow{m^*} m/e\ 70\ [70] + H_2O\ (D_2O) \qquad (21)$$

$$m/e\ 88\ [90] \longrightarrow m/e\ 73\ [75] + CH_3^{\bullet} \qquad (22)$$

of mass spectrometry in structural problems. Generally, the most that can be hoped for from the mass spectrum of an unknown compound is that a structure can be proposed which explains at least the major fragments in a rational manner consistent with the body of data collected from mass spectra of known structures. A mass spectrum is most useful when considered in conjunction with other spectral and chemical data gathered on the sample. It should be emphasized again that one gets relative abundances of various *m/e*-groups from a mass spectrum and any attempt to translate these data to structures must remain speculation. However, deuterium labeling does indicate which portions of the original molecule remain in a fragment.

Acknowledgment

The authors thank D. H. Murray and E. J. Hedgley for samples of compounds **9** and **11**, respectively. The support of grants GM-12328 and AI-07570 from the National Institutes of Health, U. S. Public Health Service, is acknowledged by D. C. DeJongh. The mass spectrometer was purchased by Wayne State University under Grant CP-1476 from the National Science Foundation.

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Studies of Deoxy Sugars by Proton Magnetic Resonance Spectroscopy

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Recent advances in the instrumental aspects of nuclear magnetic resonance spectroscopy have made feasible more detailed studies of the proton resonance spectra of carbohydrate derivatives. In the present discussion, the potential applications of frequency swept nuclear magnetic double resonance experiments are described and are illustrated with respect to deoxy sugars. The stereospecific dependences of the proton resonance parameters of pyranose carbohydrate derivatives are discussed, and it is shown that both geminal and long-range proton-proton coupling constants exhibit regular stereospecific dependences.

During the past few years proton magnetic resonance (P.M.R.) spectroscopy has become firmly established as one of the most powerful physico-chemical tools available for investigating the structures and conformations of carbohydrate derivatives (*24, 40, 44*). It has also become apparent that the more sophisticated P.M.R. instrumentation, which is now becoming widely available, is leading to more detailed analyses of the P.M.R. spectra of carbohydrates than was formerly possible. For example, the original 40 MHz investigation (*38*) of fully acetylated pyranose carbohydrates has now been repeated at 100 MHz by Lemieux and Stevens (*41*), who have detected several additional stereospecific dependences which could not be deduced from the original spectra. Hanessian (*32*) has recently published a brief summary of P.M.R. studies of some deoxy sugars. In the present discussion we shall outline some of the experiments which may be performed with the aid of "frequency-sweep" spin decoupling. We shall also describe several quite new stereospecific dependences which will find use in the elucidation of configurational and conformational problems associated with carbohydrate derivatives in general and with deoxy sugars in particular.

In Deoxy Sugars; Hanessian, S.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

Experimental

All P.M.R. spectra were measured with a Varian HA 100 spectrometer operating in the "frequency-sweep" mode with tetramethylsilane as the reference for the internal lock. The double and triple resonance experiments were performed using a Hewlett Packard 200 CD audio-oscillator and a modified Hewlett Packard 200 AB audio-oscillator (*vide infra*). Spectra were measured using whichever sweep width was required to ensure adequate resolution of the multiplets under investigation, generally 250 or 100 Hz, and sweep rates were selected as necessary. Extensive use was made of the "Difference 1" and "Difference 2" calibration modes of the instrument, both for the decoupling experiments and for the calibration of normal spectra.

All derivatives used were prepared by essentially standard literature procedures and had physical constants in accord with previously reported values. Furthermore, the P.M.R. spectra were in each case consistent with the assigned structures. All solutions were concentrated under reduced pressure and m.p.'s are uncorrected. (I) 2-Deoxy-D-*arabino*-hexopyranose was a commercial sample from Pfanstiehl Lab. Inc., Waukegan, Illinois and was used without further purification. (II) 3, 4, 6-Tri-*O*-acetyl-D-glucal (**1**) was a commercial sample from Aldrich Chem. Co., Milwaukee, Wisconsin and was purified by distillation and recrystallized three times from aqueous ethanol. (III) 1, 3, 4, 6-tetra-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranose (**4**) was prepared by the method of Bonner (*11*) while the corresponding β-anomer (**5**) was synthesized following the procedure of Overend, Stacey, and Stanek (*47*). (IV) 5, 6-Dideoxy-1, 2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose (**20**) was provided by A. Rosenthal and G. Khan of this Department.

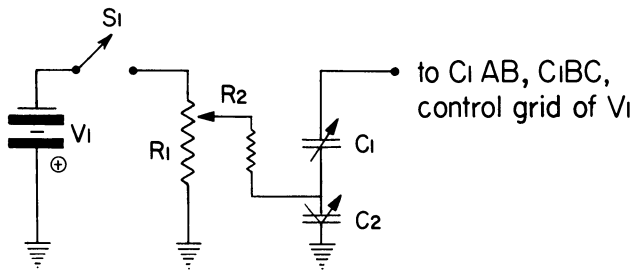
Fine-frequency Control for Hewlett Packard 200 AB Audio Oscillator. Although the power output of this oscillator is entirely satisfactory, the frequency selection is not sufficiently sensitive or "resetable" for spin-decoupling experiments. The simple circuit modification shown below in Figure 1 remedies this shortcoming by providing a "fine-frequency" control when the oscillator is used in the *ca.* 2,500 Hz range. The variable capacitor C_1 allows the Helipot to attain any chosen range: in our case C_1 was adjusted to give one-cycle-per-second-per-turn.

Applications of Nuclear Magnetic Double Resonance (N.M.D.R.)

In this section we shall outline some of the potential applications of N.M.D.R. to problems in carbohydrate chemistry. A brief discussion will be given of both the "spin-decoupling" (*3*, *23*) and the "spin-tickling" (*3*, *21*) methods together with an indication of their respective advantages. Since excellent reviews of the N.M.D.R. method have been published (*8*, *9*) it is only necessary here to mention a few relevant items of nomenclature.

N.M.D.R. experiments can be broadly subdivided into two categories (*8*, *9*). The first is "homonuclear" in which the two nuclei involved are of the same nuclear species—*e.g.* $^1H - \{^1H\}$, in this formulation the nuclear species being observed is indicated first, while the species ($\times$) being irradiated is indicated $\{\times\}$. The second is "heteronuclear," in

which two different nuclear species are involved—*e.g.* $^1H - \{^{19}F\}$. We shall only be concerned here with homonuclear decoupling of the type $^1H - \{^1H\}$. Any such experiment requires two radiofrequency (R.F.) fields whose intensities can be separately controlled. (These two radio frequencies are generally obtained by audio-modulation of the spectrometer R.F. unit. *See* References *26* and *33* for a qualitative introduction and for references to the development of this approach.) One of these, usually termed the "decoupling field," is used to irradiate a chosen resonance, and its intensity (measured in Hz) is arranged to be at least as great as the total width of the resonance to be irradiated (ΣJ). The second R.F. field, termed the "observing field" is used to observe the other resonances under optimum conditions, as in the measurement of a normal spectrum. By convention, the respective frequencies of the "decoupling" and "observing" R.F. fields are called "ω_2" and "ω_1", and their respective intensities are referred to as "H_2" and "H_1".



V1 = 2.8 V mercury battery
R1 = 10 K, 10 turn helipot
R2 = 470 K, 1/2 watt
C1 = 5 - 50 pf, ceramic
C2 = 20 pf varicap
S1 = ganged to power switch

Figure 1. Circuit diagram showing a fine-frequency control for a Hewlett-Packard 200 AB Audio-oscillator.

In the case of a "field-sweep" N.M.D.R. experiment (*22*, *26*), the frequency separation between the two R.F. fields (ω_2–ω_1) is set approximately equal to the chemical shift separation between the proton which is to be observed and that which is to be irradiated. Then the spectrum

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is recorded by slowly scanning the static magnetic field (H_o) of the spectrometer. Since this is by definition a "field-sweep" experiment, it follows that both ω_1 and ω_2 are scanned during the course of the experiment and this has several serious disadvantages. First, ω_2 does not remain centered at all times on the resonance which we wish to irradiate, and to offset this H_2 has to be made much greater than ΣJ. Second, it is only possible in the course of one experiment to decouple one particular resonance from one other; if a resonance is spin-coupled to "n" other nuclei, then a total of "n" field-sweep decoupling experiments are required.

A preferable experimental arrangement, which eliminates both of the above disadvantages, is the so-called "frequency-sweep" N.M.D.R. experiment. Here H_o is kept constant at all times, ω_2 is located on the center of the resonance to be irradiated and then the spectrum is observed by slowly sweeping ω_1 through the spectrum. Since ω_2 remains at all times on the center of the resonance to be decoupled, this experiment enables one to remove simultaneously all of the couplings caused by that particular resonance. All of the experiments discussed below were performed under these conditions.

For either experiment we can consider that ω_2 causes the irradiated protons to flip back and forth between their two spin-states so rapidly that they no longer couple with other protons in the same molecule. An alternative rationale can be couched in terms of the decoupling field equalizing the populations of the two energy levels of the irradiated protons, which is qualitatively equivalent to "saturating" that resonance. (Although neither of these two models is strictly correct, they do at least provide a simple rationale for the N.M.D.R. experiment.)

The above introduction has been based on the premise that the major requirement for the N.M.D.R. experiment is the selected removal of one or more spin couplings from a spectrum. Occasionally this type of experiment is either undesirable, or impossible to perform, and then it is often feasible to make use of a so-called "spin-perturbation" or "spin-tickling" experiment (3). In this case a comparatively weak decoupling field is applied to a single line (transition) of a particular resonance. This perturbs the energy levels associated with that particular line, which results in the "doubletting of the lines" of other resonances which are connected with the irradiated energy level (*vide infra*). Although this experiment thus results in a spectrum which is more complex than the original spectrum, interpretation is usually straightforward. Experimentally, spin-tickling is easier to perform than spin-decoupling because the strength of H_2 has only to be of the order of the line width (1 Hz, say), and as a result, spurious beat patterns, etc., are less intense than those encountered for larger values of H_2.

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The most obvious utility of the spin-decoupling technique is for confirming spectral assignments and many such applications have already been made to problems in the carbohydrate area. This technique also provides an indirect method for obtaining the chemical shift of resonances which cannot be observed directly because they are obscured by other overlapping resonances. The method is only applicable if the "hidden" resonance (*A*, say) is spin coupled to some other resonance (*X*, say) which is itself clearly resolved. If this is the case then the *X* resonance can be monitored while a decoupling field of sufficient strength is positioned within the suspected vicinity of hidden resonance (*A*). The decoupling field is moved until a maximal decoupling of the *X* resonance is observed, at which point it is assumed that the decoupling field is located symmetrically over the hidden resonance, which is thus located. Lemieux and Stevens (*42*) used this general approach (under field-sweep conditions) during their interesting study of aqueous solutions of hexoses and pentoses. However, this method has several intrinsic disadvantages. First of all, the frequency separation required for optimal decoupling is larger than the actual chemical shift separation between the two spin coupled resonances. This arises from a general Bloch-Siegert shift and although a correction can be made for this phenomenon, this is somewhat bothersome. (Bloch-Siegert shifts (*10*) are most clearly demonstrated during frequency-sweep spin decoupling experiments and studies of 1, 6-anhydro-hexopyranose derivatives have revealed several interesting examples (*22*, *27*).) A second shortcoming, namely, incomplete decoupling, arises when the hidden-resonance is itself highly multiple because of spin coupling with several nuclei. Optimal spin decoupling can only be obtained on the Varian HA-100 if the resonance to be irradiated is less than *ca.* 20 Hz wide. If the resonance is any wider, only partial decoupling can be obtained, and it is then difficult to decide just when optimal decoupling has been achieved. Fortunately, both of these potential hazards can be simultaneously eliminated if a spin-tickling procedure is used to solve the hidden resonance problem. Bloch-Siegert shifts, which depend in part upon the field strength of the decoupling field, are effectively minimized in spin-tickling experiments which utilize rather weak (*ca.* 1 Hz) decoupling fields. Moreover, decoupling-power requirements are no longer important since it is not necessary to irradiate the whole multiplet, but just a single line. A further positive advantage accrues from the utilization of spin-tickling insofar that such an experiment results in an indirect determination of each of the individual transitions of the hidden resonance and thus yields all of the spin coupling information contained in the hidden resonance. Difficulties are experienced unless the hidden resonance is chemically shifted from all other resonances to which it is coupled (*vide infra*).

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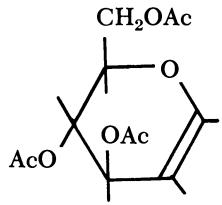
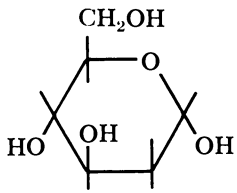
The two general types of N.M.D.R. experiment outlined above can be clearly illustrated with reference to 3, 4, 6-tri-*O*-acetyl-D-glucal (**1**).



1

A full discussion of the spectrum of this derivative has been reported previously (*26*) and Figure 2A shows the partial spectrum of the H_1 and H_2 resonances of this derivative. When the H_3 resonance is irradiated under frequency sweep conditions with a strong decoupling field, the couplings of this resonance with both H_1 and H_2 are simultaneously removed, which leaves them as the simple "AB"-quartet shown in Figure 2B. Now spin tickling experiments may be performed in which first the high field (Figure 2C), and then the low field (Figure 2D) transitions of the "decoupled" H_2 resonance are irradiated. In both cases each of the two H_1 transitions is perturbed to a doublet. Since the above experiments were performed under frequency-sweep conditions a characteristic "zero-beat" pattern is observed which indicates the precise position of the decoupling field. Clearly this "spin-tickling" technique offers an extremely accurate means for determining the position of any particular transition and we shall now discuss an application of this method to the "hidden-resonance" problem.

For reasons which will be discussed later, we were interested in obtaining a detailed analysis of the P.M.R. spectrum of both the α-(2) and β-(3) anomers of 2-deoxy-D-*arabino*-hexopyranose in D_2O solution.



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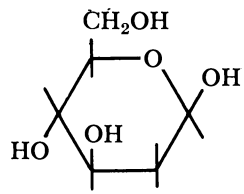

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The P.M.R. spectrum of an equilibrated aqueous solution of 2-deoxy-D-*arabino*-hexopyranose was first measured by Lenz and Heeschen (*37*) who reported values for the chemical shifts of the anomeric protons and

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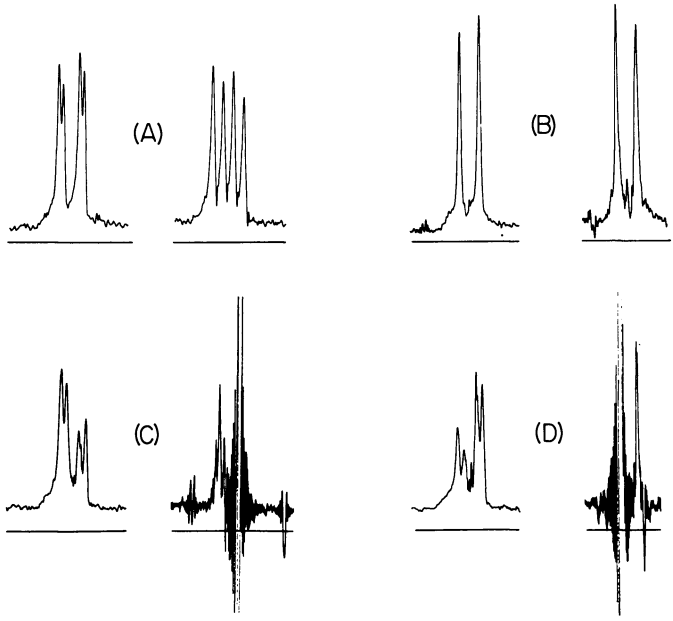

Figure 2. Partial 100 MHz P.M.R. Spectrum of 3,4,6-tri-O-acetyl-D-glucal (**1**) *measured for a chloroform -d solution: (A), normal spectrum of the H_1 and H_2 resonances respectively: (B) frequency sweep spin-decoupled spectrum of the H_1 and H_2 resonances, with a strong decoupling field centred on the H_3 resonance: (C), as in (B) above, but with an additional weak radiofrequency field centred on the high field transition of the H_2 resonance: (D), as in (B) above, but with a weak radiofrequency field centred on the low field transition of the H_2 resonance.*

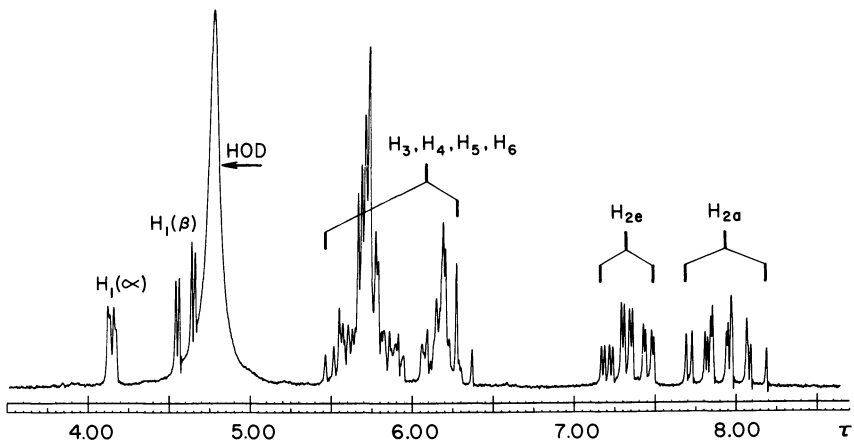

*Figure 3. 100 MHz P.M.R. Spectrum of an equilibrated solution of 2-deoxy-D-*arabino*-hexopyranose in D_2O.*

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for the H_1, H_2 coupling constants. As can be seen in Figure 3 the normal 100 MHz spectrum of an equilibrated solution is quite well resolved, and in particular the H_2 resonance near 7.5 τ exhibit an interesting multiplicity. It was possible completely to assign this spectral region by strongly irradiating in turn each of the two anomeric proton resonances. The effect of these two experiments is shown in Figure 4, where Figure 4B shows the normal spectrum of the H_1 and H_2 resonances, Figure 4A shows the effect of irradiating $H_1(\alpha)$ and Figure 4C the effect of irradiating $H_1(\beta)$. The assignments for the H_2 resonances follows by inspection.

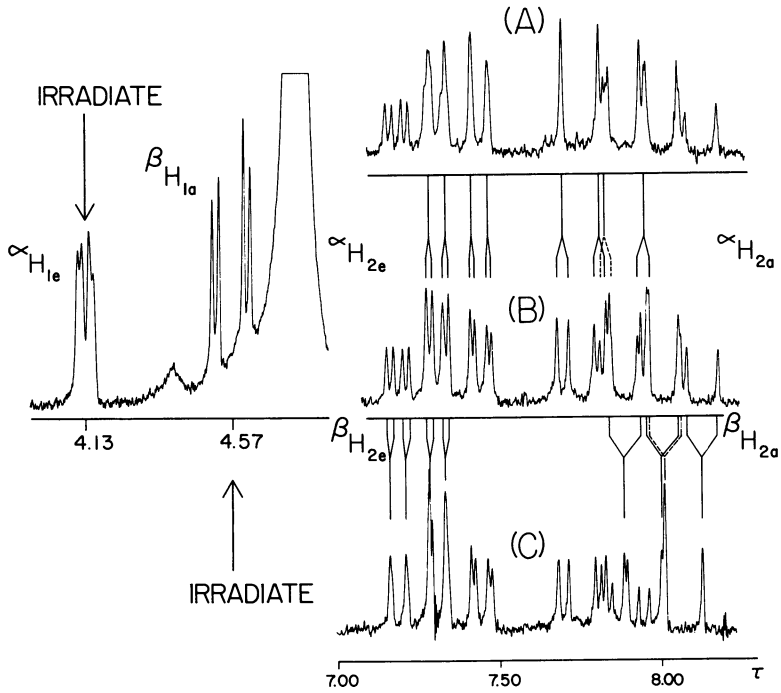

*Figure 4. Partial 100 MHz P.M.R. spectra of an equilibrated solution of 2-deoxy-*D*-arabino-hexopyranose in D_2O solution: (B) normal resonance; (A) with irradiation of $H_1(\alpha)$; (C) with irradiation of $H_1(\beta)$.*

It should be noted that it would have been totally impossible to have effected these decoupling experiments by the "field-sweep" method; ω_1 has to be scanned *ca.* 100 Hz to observe the H_2 resonances which of course would require ω_2 to be scanned by an equal amount. Since it is not possible to obtain a decoupling field of this magnitude, the above experiment would merely result in partial decoupling of the H_2 resonances.

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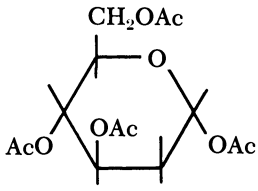
Having obtained in the above fashion an assignment for the various H_2 resonances, attempts were then made to obtain by indirect methods the chemical shifts of both of the H_2 resonances, which were hidden beneath the unresolved band at *ca.* 5.7 τ. A strong decoupling field was successively located at different positions within this area until perturbations were observed in the H_2 region. It was not possible to place sufficient power into the decoupling field to effect complete decoupling owing to the large band width of the H_3 resonance (*ca.* 25 Hz) wide. Hence the H_3 resonances could not be detected with any certainty. Some progress was made in solving this problem, by attempting spin-tickling experiments. Here, a weak field was applied to the H_3 lines and the perturbations induced into the H_2 resonances were observed. Unfortunately, the transitions of the H_3 and H_4 resonances are somewhat overlapped and as a result clearly defined spin-tickling was not obtained. However, sufficient of the H_3 transitions were located in this way to show that for the α-anomer, $J_{3,4}$ is *ca.* 9.1 Hz.

Comments upon the Stereospecific Dependences of the P.M.R. Parameters of Carbohydrates

The study of Lemieux and co-workers (*38*, *39*) which first delineated the stereospecific dependences of the P.M.R. parameters of pyranose derivatives has since been confirmed by many workers (*24*, *40*, *44*). In the following section we shall discuss some of the more recently recognized stereospecific dependences, dealing first with the dependences of coupling constants and then with those of chemical shifts. Reference will be made to the P.M.R. parameters of the α-(2) and β-(3) anomers of 2-deoxy-D-*arabino*-hexopyranose derivatives which were obtained in experiments outlined in the previous section, together with the parameters of the α-(4) and β-(5) anomers of 1, 3, 4, 6-tetra-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranose.



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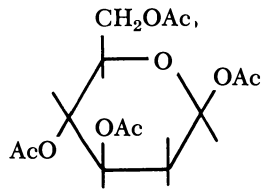
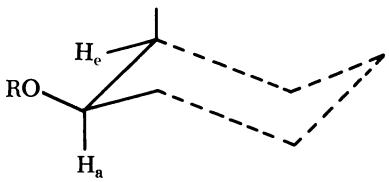


5

Vicinal Coupling Constants

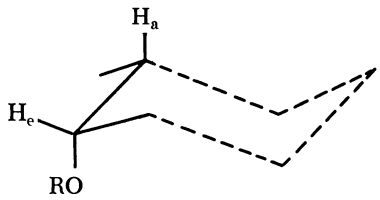
It is now generally realized that the angular dependence of vicinal coupling constants is considerably more complex than was at first appreciated (*24*, *38*, *39*) and that care must be exercised in utilizing

dihedral angles which are based upon the Karplus equation (*34, 35*). The early empirical modifications (*2, 37, 43*) of this equation allowed for the so-called "electronegativity" dependence (*1, 36, 53*) and to this extent are still satisfactory. However, they do not allow for the more recently observed "configurational dependence." Williams and Bhacca (*52*) studied the vicinal coupling constants of a series of steroidal alcohols and their acetates, and found that the gauche coupling constants could be divided into two groups as shown in (**6**) and (**7**). They considered that



6, R = H or Ac

$J_{a,e} = 5.5 \pm 1.0$ Hz.



7, R = H or Ac

$J_{e,a} = 2.5$—3.2 Hz.

all of the derivatives had ring *A* in an undeformed chair conformation and suggested that their results were solely consistent with a "configurational" dependence involving the relative orientation of the C–O bond. Since then, other workers have reported similar dependences for the vicinal couplings of cyclohexanes (12), bicycloheptanes (18), and pyranose carbohydrates (*15, 18*). This type of dependence had been reported several years earlier by Anet during his studies of cyclohexanols (*4*) and camphane-2, 3-diols (*5*). Several studies in this laboratory have also furnished results which are consistent with the regular occurrence of a configurational dependence. This is particularly noteworthy for derivatives of 1, 6-anhydro-hexopyranoses, which are all conformationally rigid. Since that subject will be reported elsewhere (*27*) we shall confine the present discussion to the vicinal couplings of the derivatives of 2-deoxy-D-*arabino*-hexopyranose, which are summarized in Table I. It is significant that the individual couplings of the free sugars are in most instances identical with those of the corresponding tetra-*O*-acetates. This is in accord with the suggestion of Williamson (*53*), that a change from —OH to —$OCOCH_3$ should have no significant influence upon the magnitude of any associated couplings. The close similarity of the two sets of couplings also implies that the free sugars have the same conformations as the acetates. It can be seen that the various *vicinal* couplings between protons

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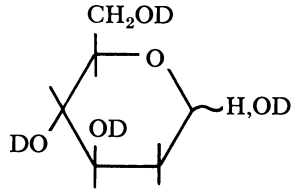
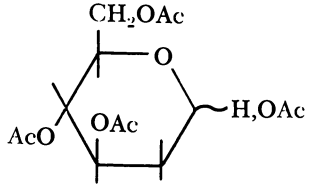
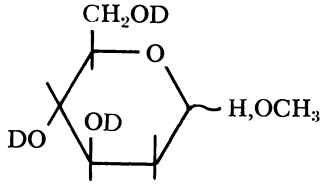
Table I. The Coupling Constants of Derivatives

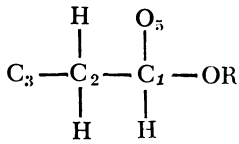
Compound	Anomer	1,2a	1,2e
	α **2** [b]	3.5	1.4
	β **3** [b]	9.5	2.0
	α **4** [c]	3.6	1.5
	β **5** [c]	9.8	2.4
	α [c, d]	3.8	1.4
	β [c, d]	9.9	1.9

[a] Unresolved multiplet.

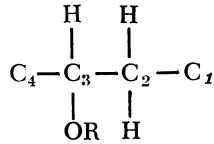
[c] For a $CDCl_3$ solution.

[e] Indirect measurement from a spin-tickling experiment.

which nominally have a gauche relationship, vary within the range 1.4 to 5.1 Hz. The overall lower magnitude of the $J_{1,2}$ values as compared with the $J_{2,3}$ couplings can be related to an electronegativity dependence, since the C–1, C–2 fragment (**8**) has two associated oxygen substituents where-



8



9

as the C–2, C–3 fragment (**9**) has only one. However, this still does not account for the differential between the various $J_{1,2}$ values, which do

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of 2-Deoxy-D-*arabino*-hexopyranose

Coupling Constant (Hz)							
2a,3	*2a,2e*	*2e,3*	*3,4*	*4,5*	*5,6₁*	*5,6₂*	*6₁,6₂*
13.1	11.5	5.1	*ca.* 9 [5]	[a]	[a]	[a]	[a]
12.5	11.5	5.0	[a]	[a]	[a]	[a]	[a]
13.8	11.0	5.0	9.4	9.4	2.5	4.8	12.8
12.4	11.0	5.0	9.4	9.4	2.3	4.6	12.2
12.6	11.4	5.0	[a]	[a]	[a]	[a]	[a]
12.5	11.0	5.0	[a]	[a]	[a]	[a]	[a]

[b] For a D_2O solution.

[d] *See* Ref. 39.

not appear to be consistent with any regular conformational deformation. For example in compounds (**2, 3, 4** and **5**) we find "*e, a*" couplings of 3.5 and 3.6 Hz; "*e, e*" couplings of 1.4 and 1.5 Hz and "*a, e*" couplings of 2.0 and 2.4 Hz, all of which correspond to dihedral angles of *ca.* 60°. Possibly these differences constitute a further example of the configurational dependence. (Compare Ref. *15* for a similar discussion).

Booth (*12*) has suggested that the magnitude of this configurational dependence is maximal when the electronegative substituent is co-planar with one of the protons involved in the vicinal coupling, and this explanation is consistent with most of the data previously published; it also explains why $J_{1e,2e}$ is the smallest of the $J_{1,2}$ values shown in Table I. While this rationale is of some use experimentally we wish to draw attention to an assumption which is implicit in all attempts to relate vicinal couplings with dihedral angles, and which may be the source of the above configurational dependence. It is always assumed that the ring-

carbon atoms of a carbohydrate derivative have precisely tetrahedral symmetry, so that calculated inter-proton dihedral angles can be directly related to the inter-carbon dihedral angles, and thence to the ring conformation. X-ray diffraction studies of the bond angles and bond lengths of carbohydrates, which have been reviewed by Sundaralingham (*51*), suggest that the precise hybridization of each carbon atom of a carbohydrate ring system depends upon the orientation of the substituent attached to that carbon. If these findings apply as generally to carbohydrates in the liquid phase as they do in the solid phase, then it is obvious that the couplings between a pair of vicinal protons can be expected to depend (a), upon their nominal dihedral separation (Karplus dependence) and (b) upon the orientation of the substituent with respect to the ring. In any event, it does seem that there is a regular configurational dependence for vicinal couplings and a reasonable degree of caution must be exercised in using such couplings as the basis for detailed conformational analyses. It is sensible, if such an approach is used, solely to define the symmetry of a conformation. In this respect it should be noted that the large vicinal couplings between trans-diaxial protons and the near-zero coupling between protons separated by a dihedral angle of 90°, can both lead unequivocally to the symmetry of a conformation, even though they cannot by themselves define the conformation quantitatively.

In view of the possible ambiguities which can attend conformational assignments based on vicinal coupling constants, it is fortunate that both "long-range" and geminal couplings each exhibit stereospecific dependences.

Long-range Coupling Constants

It has been recognized (*50*) for some time that in a fully saturated system, spin coupling can occur between protons which are separated by four bonds, as in the system H–C–C–C–H. The observation (*25*) that, for 1, 6-anhydrohexopyranose derivatives, the preferred stereochemistry for such couplings requires the two protons to have a "1, 3-diequatorial" orientation ($^4J_{e,e}$) has since been substantiated by studies of other pyranose derivatives. As a result, it has been assumed that the observation of any long-range coupling whatsoever implies that the protons involved have a 1, 3-diequatorial stereo-relationship. The results summarized in Table II and discussed below show that long-range couplings are in fact far more widespread than previously anticipated; however, they can still be used with some confidence for stereochemical assignments (*30*). In most of the cases discussed below, the couplings could be measured directly and their source subsequently confirmed by "frequency-sweep" N.M.D.R. experiments. Very small couplings which

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Table II. Signs of Proton-Proton Constants of Carbohydrate Derivatives

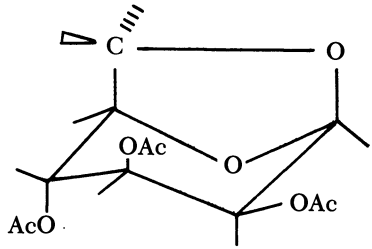
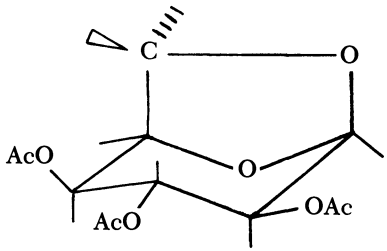
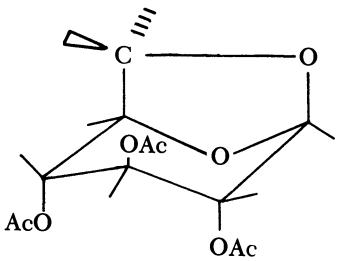
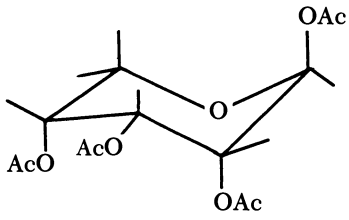
Compound	*Long Range Couplings (Hz)*
 **10**[b]	$J_{1,3} = +1.6$; $J_{3,5} = +1.5$ $J_{2,4} = \|0.45\|$; $J_{1,5}$ *ca.* $\|0.1\|$
 **11**[d]	$J_{4,6_2} = +1.2$; $J_{1,6_1} = J_{1,6_2} = \|0.15\|$
 **12**[c]	$J_{1,3} = \|1.2\|$; $J_{2,4}\|1.25\|$; $J_{3,5}\|1.5\|$
 **13**[c]	$J_{1,3} = \|0.1\|$; $J_{1,5_e} = \|0.2\|$; $J_{1,5_a} = \|0.1\|$ $J_{1,4} = \|0.1\|$; $J_{2,4} = +0.8$ $J_{3,5_e} = \|0.45\|$

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Table II. (Continued)

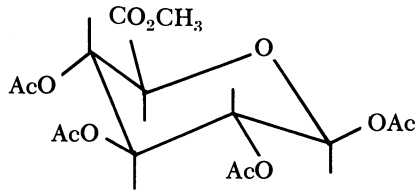
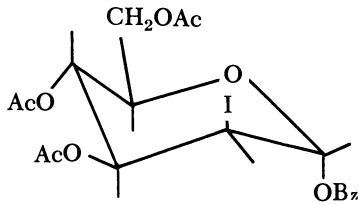
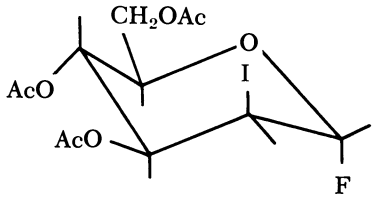
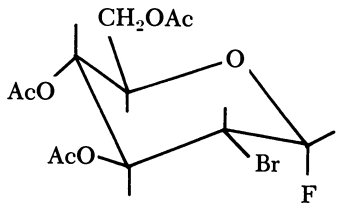
Compound	*Long Range Couplings (Hz)*
	$J_{2,4} = +0.8$
14[b, c]	
	$J_{1,3} = -0.65$; $J_{1,5} = \|0.65\|$
15[c]	
	$J_{1,3} = -0.50$; $J_{2,4} = -0.45$ $J_{1,5} = \|0.2\|$; $J_{1,4} = \|0.1\|$
16[c]	
	$J_{1,3} = -0.4$
17[b]	

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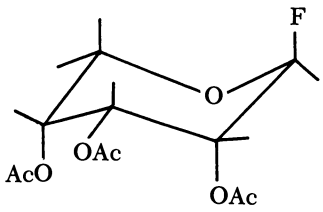
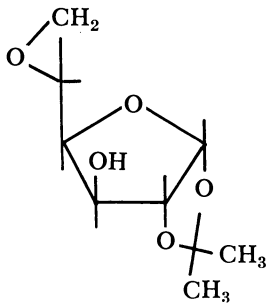
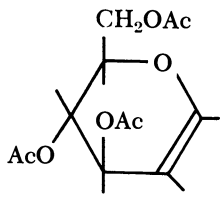
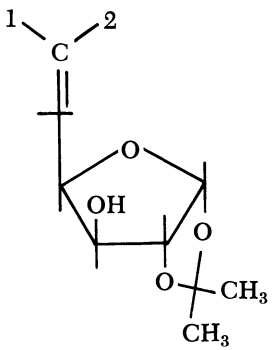
Table II. (Continued)

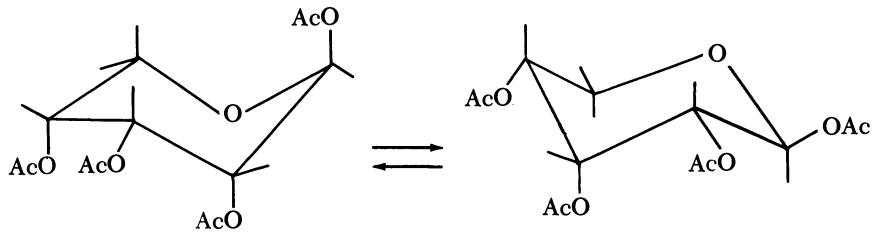
Compound	*Long Range Couplings (Hz)*
 **18**[c]	$J_{2,4} = \|1.0\|$
 **19**[c]	$J_{4,6} = -0.27$
 **1**[c]	$J_{1,3} = -1.3$; $J_{3,5} = \|0.7\|$ $J_{2,4} = \|0.5\|$
 **20**[b]	$J_{4,6_1} = -1.4$; $J_{4,6_2} = -1.6$

[a] *See* also Ref. 27 for a preliminary account.
[b] For a chloroform-d solution.
[c] For an acetone-d_6 solution.
[d] For a benzene-d_6 solution.

could not be entirely resolved were detected by careful comparisons of line-widths, both before and during spin-decoupling. In all cases the virtual coupling limits of Musher and Corey (*46*) were exceeded. In spite of this, all values are subject to small systematic errors since it is difficult to obtain an accurate measure of any coupling which is only partially resolved.

Inspection of the values given in Table II indicates that the "planar-M" or "1-3-diequatorial" ($^4J_{e,e}$) disposition is indeed the preferred arrangement for long-range couplings in the pyranose system, where it leads to splitting 1.2 to 1.6 Hz (compounds **10, 11, 12, 18**). However, smaller couplings can occur for protons having a 1, 3-axial : equatorial disposition ($^4J_{a,e}$) in which case the splitting is 0.5 ± 0.2 Hz (compounds **10, 13,** and **15**). With one possible exception, we have observed no examples of $^4J_{a,a}$ couplings which are, therefore, either extremely uncommon or extremely small (<0.1 Hz). The only possible example is the $J = +0.8$ splitting of methyl 1, 2, 3, 4-tetra-*O*-acetyl-β-D-glucuronate (**14**); although the spectrum of this derivative exceeds the virtual coupling limits of Musher and Corey (*46*), this splitting is solvent dependent (0.45 Hz in acetone and 0.8 Hz in chloroform) and thus there is still the possibility that this splitting is entirely a second order perturbation.

The spectrum of 1, 2, 3, 4-tetra-*O*-acetyl-β-D-ribopyranose (**13**) in acetone-d_6 solution (Figure 5), exhibits a particularly large selection of long-range couplings. It may well be that the intermediate values of some of these couplings are consistent with an earlier suggestion (*41*) that this derivative is undergoing a rapid chair-chair conformational inversion as depicted below. This would certainly have the merit of



13a 13b

rationalizing the value of the $J_{2,4}$ splitting (+0.8 Hz) which is abnormally small for a $^4J_{e,e}$ coupling. The spectrum of this compound is also of some interest insofar as it shows couplings of 0.2 and 0.1 Hz between H_1 and the H_5 protons, that is, through the ring oxygen; this was the first such coupling which we had ever observed. However, we have since found

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that such couplings are in fact quite common; in general the $^4J_{e,e}$ coupling through the ring oxygen is smaller {compounds **10, 12** (*ca.* 0.1 Hz)}, than the $^4J_{e,a}$ counterpart {compounds **15, 16** (*ca.* 0.5 Hz)}.

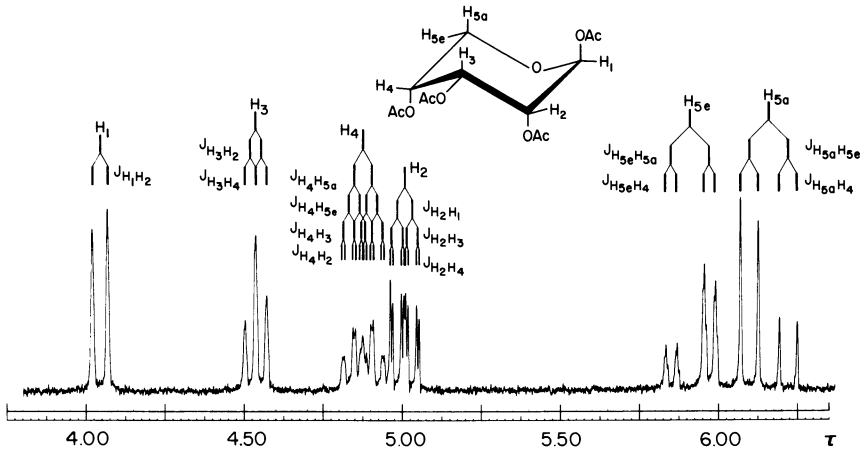

*Figure 5. Partial 100 MHz P.M.R. Spectrum of 1,2,3,4-tetra-O-acetyl-β-D-ribopyranose (**8**) in acetone-d_6 solution.*

In addition to the above mentioned ^4J couplings, we have now observed several examples of couplings across five bonds—*i.e.* ^5J. For example, the ribopyranose derivative (**13**) shows a coupling of *ca.* 0.1 Hz between H_1 and H_4. Further examples occur in the spectrum of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**12**), where $J_{1,4}$ = *ca.* 0.1 Hz and $J_{2,5}$ = 0.5 Hz. Whether it is significant that these couplings all occur between equatorially oriented protons must await further studies.

Several furanose derivatives studied have also revealed a selection of long-range couplings, and the determination of their stereospecificity, if any, is actively under investigation in this laboratory (*31*) since it offers a potentially useful approach to the study of furanose conformations. Again, it is found that ^4J coupling can occur across the ring oxygen.

The widespread occurrence of long-range couplings in both furanose and pyranose derivatives explains why so many of the P.M.R. spectra of carbohydrate derivatives are apparently poorly resolved, even when the resolution of the spectrometer is above reproach. For example, the H_1 resonance of the 1,6-anhydro-D-glucose derivative (**12**) is coupled to all of the other six ring protons. A further example of the line-broadening effect follows a consideration of the spectrum of 5,6-dideoxy-5,6-epithio-1,2-*O*-isopropylidene-β-L-idofuranose for which the half-height

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width of the H_1 resonance was found to be 0.9 Hz broader than that of the H_5 resonance.

While the above studies were in progress results from dioxane derivatives were published (*19,94*) which support all of the comments and cautions we have made above. Additional reports have been made of a long-range coupling across a ring oxygen in an unsaturated pentopyranose derivative (*17*) and in a saturated furanose derivative (*20*).

Thus far, emphasis has been laid upon long-range couplings in saturated derivatives, which have been shown to be of common occurrence. For unsaturated sugars, long-range couplings are likely to be even more widespread and of some general interest. We shall not discuss such couplings in detail and shall only mention several isolated examples from our own laboratory. The spectrum of 3,4,6-tri-*O*-acetyl-D-glucal (**1**) exhibits two couplings in addition to those previously described; H_3 and H_5 are coupled by 0.7 Hz while H_2 and H_4 are coupled by 0.5 Hz. The spectrum (Figure 6) of 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose (**20**) shows two long-range couplings across the double bond, between H_4 and the H_6 protons of −1.4 and −1.6 Hz.

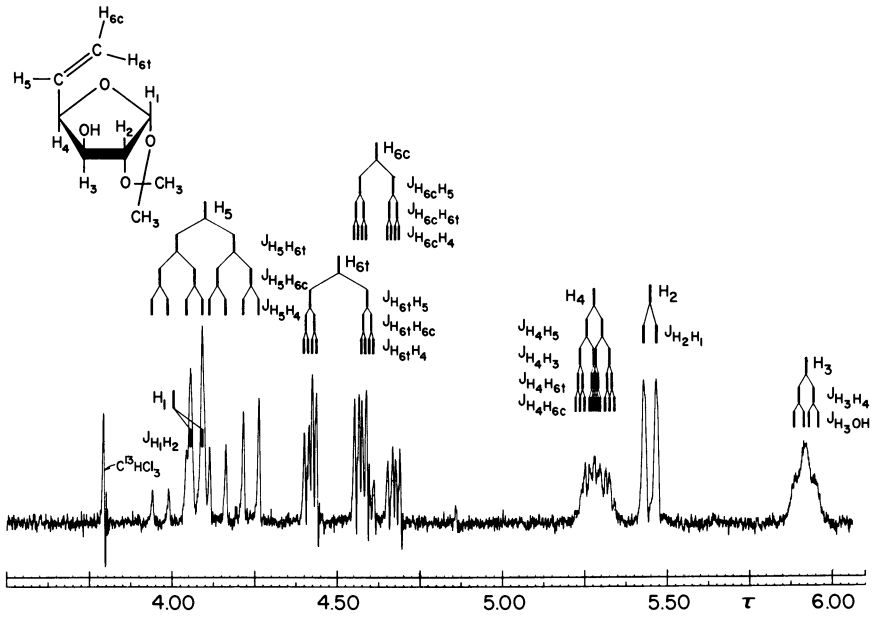

Figure 6. Partial 100 MHz P.M.R. Spectrum of 5,6-Dideoxy-1,2-O-isopropylidene-α-D-xylo-hex-5-enofuranose (**20**) *in $CHCl_3$ solution.*

From a study (*29*) of the relative signs of various ^1H, ^1H coupling constants of carbohydrate derivatives it is evident that the couplings

of epoxide derivatives are similar in character to those of unsaturated derivatives, whereas episulfides are comparable in some respects with "sp^3" systems. It is hardly surprising therefore to find that the 5,6-epoxide (**19**) shows a negative long-range coupling between H_4 and H_6 whereas the episulfide does not show a long-range coupling.

There is one further aspect of the stereospecificity of ^4J couplings which deserves mention; that is that the signs of these couplings show a regular stereospecific dependence (29,30). We have found that all $^4J_{e,e}$ couplings determined are positive in sign whereas the $^4J_{e,a}$ couplings are negative; no exceptions have been found during the course of twelve separate determinations. This suggests an interesting and potentially useful method for making configurational assignments. These findings are not in agreement with the calculations of Barfield (*6*), who predicts that both $^4J_{e,e}$ and $^4J_{a,e}$ should be positive in sign and have magnitudes of approximately +1.1 and +0.5 Hz respectively, while $^4J_{a,a}$ couplings should be −0.3 Hz. It should be noted that Barfield's calculations are only concerned with the "through-bond" (indirect) coupling mechanism, and purposely ignore any possible contribution from "through-space" coupling (the so-called "direct" mechanism).

It is evident from the above discussion that more-detailed studies of the P.M.R. spectra of carbohydrate derivatives will reveal an ever-increasing number of long-range couplings. Studies in this laboratory are directed towards the investigation of the occurrence of these couplings in the spectra of both furanose and pyranose derivatives.

Geminal Coupling Constants

Comparatively little interest has been shown by organic chemists in the magnitudes of geminal coupling constants, although P.M.R. spectroscopists themselves were at one time most interested in them from a theoretical standpoint. This lack of attention is rather surprising in view of the fact that Barfield and Grant (*7*) mentioned in 1963 that the magnitude of geminal couplings was dependent upon the relative orientation of an adjacent π-bond. More recently, Pople and Bothner-By (*48*) and also Bothner-By (*13*) and Cookson and co-workers (*14*) have demonstrated the dependences of geminal couplings upon the relative orientation of nearby electronegative substituents. The first published example of this dependence in the carbohydrate field is that of Coxon (*16*) for the C,5-methylene protons of pentopyranose derivatives. We had also independently made a similar observation during our studies of pentopyranosyl fluorides and the dependence is briefly summarized in (**A**) and (**B**) below. It can be seen from the projected valency diagrams that when the adjacent electronegative substituent is oriented

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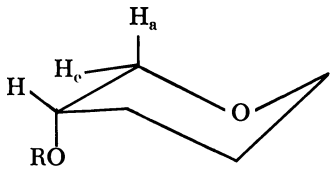
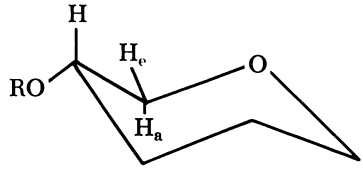
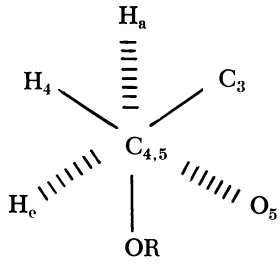
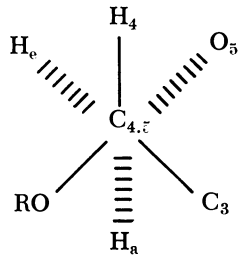




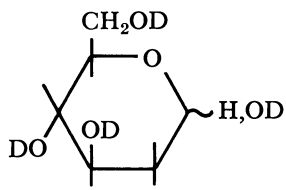
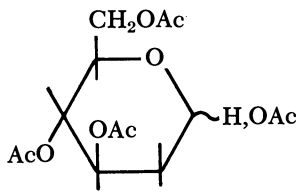


A



B

Table III. The Chemical Shifts of Derivatives

Compound	*Anomer*	*1*	*2a*
	α **2** [b]	4.13	7.81
	β **3** [b]	4.57	8.03
	α **4** [c]	3.75	8.07
	β **5** [c]	4.13	8.19

[a] Unresolved multiplet.
[c] For a $CDCl_3$ solution.

between the methylene protons the geminal coupling is smaller than when it is oriented externally to those protons. Typical ranges which we have observed for these geminal couplings are as follows: *Pentopyranose Derivatives:* axial OR_4 substituent, $J_{5e,5a} = 13.3 \pm 0.3$ Hz; equatorial OR_4 substituent, $J_{5e,5a} = 11.2 \pm 0.5$ Hz. *2-Deoxy-*D-arabino-*hexopyranose Derivatives:* axial OR_4 substituent, $J_{2e,2a} = ca.$ 13.8 Hz; equatorial OR_1 substituent, $J_{2e,2a} = ca.$ 12.4 Hz.

The fact that the absolute magnitudes of these two sets of coupling constants are different is also expected on the basis of the Pople, Bothner-By treatment (*48*). It would clearly be of some considerable interest if a general study were to be made of the stereospecificity of this geminal dependence since this may lead to another method for determining carbohydrate conformations.

Ring Proton Chemical Shifts

One of the most interesting stereospecific dependences which followed from the original study (*38,39*) of O-acetylated hexopyranose and pentopyranose derivatives was that of the anomeric proton shifts. In general it was found that the axially oriented anomeric protons gave resonances to higher field than the equatorially oriented protons. Many subsequent studies of pyranose derivatives (*24,40*) and of inositol derivatives (*43*)

of 2-Deoxy-D-*arabino*-hexopyranose

Chemical Shifts (τ-values)

2e	*3*	*4*	*5*	*6₁*	*6₂*	*OR*
7.37	5.6 [d]	[a]	[a]	[a]	[a]	
7.24	5.8 [d]	[a]	[a]	[a]	[a]	
7.73	4.68	4.94	5.95	5.68	5.97	7.89, 7.95 7.98, 8.00
7.67	4.87	5.02	6.22	5.68	5.95	7.92, 7.96 7.99, 8.01

[b] For a D_2O solution.

[d] Obtained indirectly by double-resonance.

have amply confirmed the generality of this dependence for protons attached to carbohydrate rings at positions other than the anomeric center although several apparent exceptions to the above "rule" were also noted. These "exceptions" were subsequently incorporated into a broader rationale of ring proton shifts by Lemieux and Stevens (*41*), in which it was recognized that ring proton shifts were dependent—(a) upon the orientation of the particular proton with respect to the ring and (b) upon the relative orientation of other electronegative substituents attached to the ring system. These substituent dependences appear to be quite systematic for the anomeric proton shifts of fully *O*-acetylated pyranose sugars in chloroform solution (*41*) and of free sugars in aqueous solution (*42*). They are also stereoregular for fully *O*-acetylated inositol derivatives in chloroform solution, where they have been used (*45*) as supporting evidence for configurational assignments. However, no data is yet available to show if these empirical relationships apply to other less symmetrically substituted carbohydrate derivatives nor have any studies delineated if they apply for solvents other than chloroform or water. It seems improbable that these rules will apply for any derivative containing an aromatic substituent, but this is in itself a fruitful area for future research.

It is of interest to consider the validity of the above configurational dependences when applied to the chemical shifts found for the derivatives listed in Table III. Consider first the free sugars (**2**) and (**3**). Inspection of the C_2–methylene proton shifts show that changing the anomeric hydroxyl function from an equatorial to an axial orientation—*i.e.* β- $\rightarrow$ α-anomer, causes H_{2a} to shift downfield by 0.22 p.p.m., while H_{2e} shifts by +0.13 p.p.m. (We shall adopt the convention that "—x" corresponds to a downfield shift and "+x" to an upfield shift.) Assuming ideal chair symmetry, these shifts can be compared respectively with those of the α- and β-anomers (*42*) of D-glucose and D-mannose. For D-glucose the H_{2a} chemical shift change $\beta \rightarrow \alpha$ is —0.28 p.p.m., which is in fair agreement with the value listed above for H_{2a}. However, for D-mannose the H_{2e} change $\beta \rightarrow \alpha$ is +0.02 which is in poor agreement with the shift found for H_{2e} above. A similar set of comparisons can be made for the corresponding fully -*O*-acetylated derivatives. Using the H_2 shifts for the acetates (**4**) and (**5**) indicates that for H_{2a} a $\beta \rightarrow \alpha$ change induces a shift of —0.12 p.p.m. while for H_{2e} the corresponding shift is +0.06 p.p.m. Again these values can be compared with those from the corresponding fully acetylated D-glucose and D-mannose derivatives which are (*41*):- H_{2a} ($\beta \rightarrow \alpha$) = —0.20 p.p.m. and H_{2e} ($\beta \rightarrow \alpha$) = +0.20 p.p.m. Thus the sense of these chemical shift changes are in accord with the results from the hexa- and penta-pyranose acetates, but their magnitudes are seriously different.

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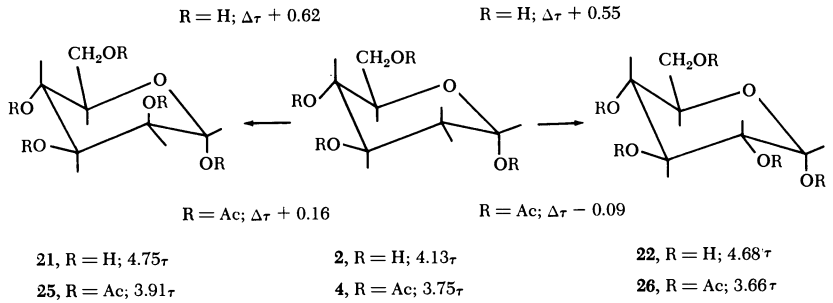
These comparisons between the shifts of derivatives of D-glucose and D-mannose and those of the corresponding derivatives of 2-deoxy-D-*arabino*-hexopyranose can be extended to the anomeric proton shifts, as summarized in Table IV. It will be recalled that the shifts discussed by Lemieux and Stevens correspond to the inversion of an acetoxy (or hydroxy) function from an equatorial to an axial orientation. The shifts listed in Table IV. correspond to the replacement of a hydrogen substituent by an acetoxy (or hydroxy) substituent. Thus it can be seen from this Table that the +0.25 p.p.m. shift difference between the H_{1e} shifts of fully acetylated D-glucose and D-mannose arises from a +0.09 p.p.m. contribution which follows the removal of the equatorially oriented acetoxy group and a +0.16 p.p.m. shift from the introduction of the axially oriented acetoxy group. It is not worthwhile continuing these detailed comparisons at this juncture because of the paucity of the

Table IV. Comparison of Anomeric Proton (H_1) Chemical Shifts for Free and Acetylated D-gluco and D-mannopyranoses With Those of Related 2-Deoxy Sugar Derivatives. Effect of Replacing an Axial or an Equatorial C_2-Methylene Proton With a Hydroxy or Actetoxy Substituent

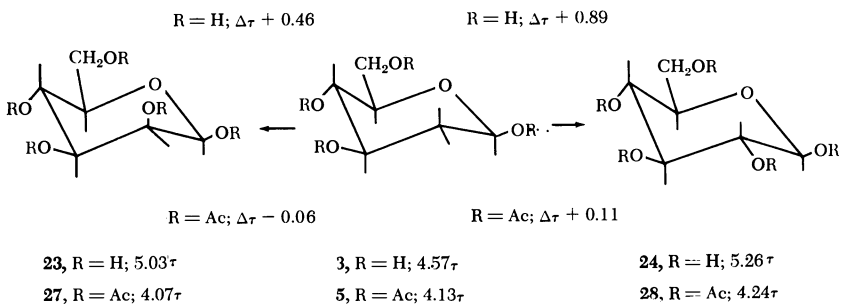


21, R = H; 4.75τ
25, R = Ac; 3.91τ

2, R = H; 4.13τ
4, R = Ac; 3.75τ

22, R = H; 4.68τ
26, R = Ac; 3.66τ

β-anomers:



23, R = H; 5.03τ
27, R = Ac; 4.07τ

3, R = H; 4.57τ
5, R = Ac; 4.13τ

24, R = H; 5.26τ
28, R = Ac; 4.24τ

experimental data; however, this is clearly a valuable and interesting approach to the study of the stereospecificity of ring proton shifts and further comparisons based on these lines should be most rewarding.

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