

Advances in Carbohydrate Chemistry and Biochemistry

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PREFACE

In this thirty-third volume of *Advances*, Haines (Norwich) surveys the relative reactivities of hydroxyl groups in carbohydrates, as regards esterification, etherification, and other reactions, in an article that updates that by Sugihara (Vol. 8); the author has correlated a vast amount of widely scattered literature in a way that should prove particularly helpful to the synthetic chemist seeking routes to specific, partially substituted intermediates. Also of particular interest to the synthetic chemist is the article by Hanessian and Pernet (Montreal); they have provided a comprehensive discussion of the synthesis of naturally occurring C-nucleosides, their analogs, and functionalized C-glycosyl precursors. Research on C-glycosyl compounds has made enormous strides since earlier articles were published by Haynes (Vols. 18 and 20), largely as a result of chemotherapeutic interest in such C-nucleosides as the formycin group. The reactions of D-glucofuranurono-6,3-lactone are collated and discussed by Dax and Weidmann (Graz); this compound is of considerable importance as a precursor for synthesis of various, useful carbohydrates, but earlier volumes of *Advances* had not furnished the detailed focus that is developed here on the chemistry of this particular lactone. Khan (Reading) has written a much-needed summary of the chemistry of sucrose, a subject that has burgeoned tremendously since its discussion by Levi and Purves in Vol. 4, largely as a result of efforts by the International Sugar Research Foundation to promote development of sucrose-based chemicals for potential, technological utilization. The comprehensive set of Tables of sucrose derivatives, included at the end of this Chapter, should prove particularly useful as a source of reference. Larm (Uppsala) and Lindberg (Stockholm) educe comparative information on the structures of twenty pneumococcal polysaccharides, thus greatly extending the information previously presented by How, Brimacombe, and Stacey in Vol. 19. Improved techniques for polysaccharide structure-determination, largely pioneered in Lindberg's laboratory, have greatly improved the speed and reliability of structural characterization, to the point that serological classification of micro-organisms on the basis of structures of their immunologically specific polysaccharides is becoming increasingly commonplace. Rexová-Benková and Markovič (Bratislava) describe the action pattern and specificity, occurrence and formation, purification, and assay of pectic enzymes, a topic last discussed by Kertesz and McColloch in Vol. 5. Since that time, there have been important developments, notably in the discovery of enzymes that split glycosidic linkages by β -elimination. Complementing our continuing series of bibliographic articles on the

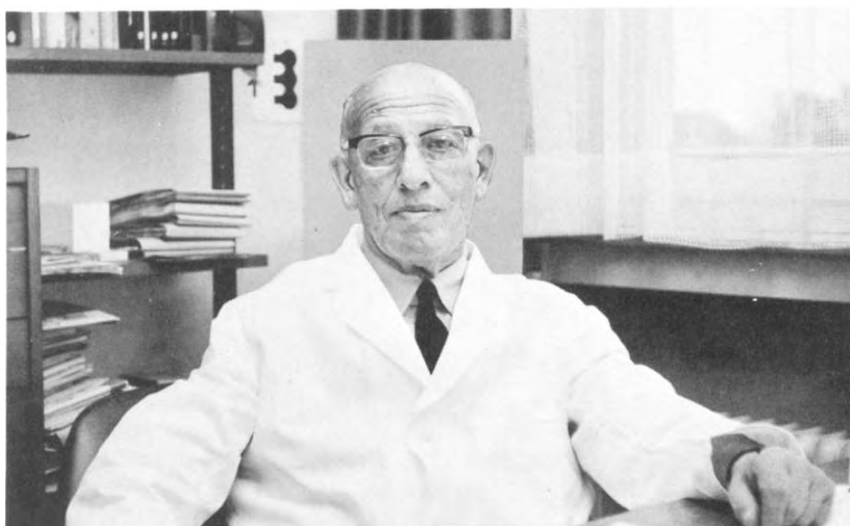
structures of simple carbohydrates as ascertained by x-ray, crystallographic methods (Jeffrey and Sundaralingam, Vols. 30-32), Marchessault and Sundararajan (Montreal) now provide a similar bibliography of crystal structures of polysaccharides determined during 1967-1974, thus updating the information in the article by Marchessault and Sarko in Vol. 22. An informative obituary describing the life and work of Alfred Gottschalk has been contributed by Neuberger (London).

The editors note with regret the passing of our friend Edward J. Bourne on November 30th, 1974.

The Subject Index was compiled by Dr. L. T. Capell.

Kensington, Maryland
Columbus, Ohio
June, 1976

R. STUART TIPSON
DEREK HORTON



Alfred Formick

1894-1973

ALFRED GOTTSCHALK

1894–1973

Alfred Gottschalk died in Tübingen on October 4, 1973, about six months before reaching the age of eighty. He was born in April, 1894, in Aachen (Aix-la-Chapelle), an old city that claims to be the birthplace of Charlemagne, and which is situated in a part of Germany now called the Land of North Rhine–Westphalia. His father was a prosperous merchant, and his background, that of a good German-Jewish middle-class family of the time. The home atmosphere seems to have been intellectually stimulating. Alfred was the third of four sons, and one of his brothers became a distinguished orientalist who, like Alfred, left Germany during the Nazi period, to return to that country after World War II. Alfred attended primary and secondary schools in Aachen, where he obtained his matriculation certificate (“Abitur”) in 1912. He decided to study medicine and, following the custom in many European countries, he studied at several universities, those of Munich, Freiburg, and Bonn. Alfred Gottschalk joined the German Army soon after the beginning of World War I, obtained a commission in 1915, and was decorated with the Iron Cross, 2nd Class. In 1920, he completed his medical studies at the University of Bonn, where he obtained the degree of Doctor of Medicine.

As a student, Alfred Gottschalk had already become interested in medical research, as shown by the fact that he participated in several investigations of a physiological kind before the war, and his first three papers were concerned with the effects of anoxia on peripheral nerves, especially those of cold-blooded animals. After graduating in medicine, Gottschalk spent a short time as an assistant in the Medical Poliklinik in the University of Frankfurt, but, for the rest of his life, his contact with patients was largely through his laboratory. He worked for a time with Lipschitz, who was Professor of Pharmacology in the University of Frankfurt. He then joined Nonnenbruch, in Würzburg, with whom he collaborated in several studies on amino acid metabolism in the liver. But the most important influence on Gottschalk’s scientific development must have been the personality of Carl Neuberg,¹ whom he joined in 1921. At

¹ For an obituary, see *Adv. Carbohydr. Chem.*, 13, 1–7 (1958).

that time, the Kaiser Wilhelm Institute for Biochemistry in Berlin-Dahlem was one of the most active centers in the world for research on the chemistry and biochemistry of carbohydrates, and it was led by Neuberg with vigor and imagination. The Director supplied the ideas, and he expected his staff to do, carefully and competently, the experiments designed by him, and to work hard. For the rest of his life, Gottschalk retained great respect for Neuberg, and he adopted, possibly unconsciously, the general principles of dealing with junior colleagues that he had seen in operation at Dahlem. In 1928, Gottschalk was appointed Director of the Chemical Institute of the Municipal Hospital in Stettin. This hospital, although not part of a university, provided good facilities for research, and Gottschalk became involved in investigations on clinical diabetes, whilst continuing with his old, more purely biochemical, studies.

In 1933, Hitler came to power in Germany, and a year or so later Gottschalk was dismissed from his post. Gottschalk had been born a Jew but, on marrying a Roman Catholic, he had adopted his wife's religion. Neither of these facts, nor his war record, made any significant difference to the way in which he was treated by the Nazis. During the next few years, he made a somewhat precarious living in Germany by private medical practice, but, after being incarcerated for a short time in 1938, he then left Germany and emigrated with his wife to England. He spent a short time as a visiting worker in the Biochemistry Department of the University of Liverpool, but conditions in England at the time were unfavorable for a middle-aged, refugee scientist trying to obtain a university position. In 1939, the Gottschalk family therefore emigrated to Australia, with the generous assistance of an English Catholic organization for helping victims of Nazi persecution. Gottschalk was accepted as a research scientist at the Walter and Eliza Hall Institute of Medical Research in Melbourne, which was then directed by Kellaway. At first, Gottschalk continued with his investigations of carbohydrate metabolism, until, in 1947, he was drawn by Burnet and his colleagues into work on the influenza virus and its interaction with red blood-cells. This problem stimulated his interest in glycoproteins, and the following 20 years constituted the most productive period in Gottschalk's scientific career. It may be noted that he was about 53 years old when he took up work on sialic acid.

Gottschalk was about 45 years old when he moved to Melbourne, and he adjusted himself quite well to a new and less formal society. He acquired a remarkable knowledge of the finer points of the English language, but his English speech always had a rather per-

sonal character which showed more than a trace of Rhenish German. Some years after he had settled in Melbourne, he became an Australian citizen. Gottschalk remained on the staff of the Hall Institute until 1957, when he had to retire because he had reached the age limit. However, Professor Fenner, F.R.S., Head of the Microbiology Department of the Australian National University in Canberra, offered him facilities to continue his research, and Gottschalk stayed in Australia until 1963. During his Australian period, Gottschalk had some difficult personal problems, and, as time went on, he concentrated more and more on his work and on biochemistry in general. Indeed, even at social occasions, it became difficult to converse with him for any length of time about a topic not related to biochemistry or to his book on "Glycoproteins." He now worked and lived exclusively for his science.

His important contributions to biochemistry were soon recognized. In 1949, the University of Melbourne conferred on Alfred Gottschalk the Degree of Doctor of Science: this was followed in two years by election to Fellowship in the Royal Australian Chemical Institute. In 1951, Gottschalk was awarded the David Syme Research Prize by the University of Melbourne, and, in 1954, he was honored by being elected a Fellow of the Australian Academy of Science.

In 1963, Alfred Gottschalk received an invitation to return to Germany and to join the Max-Planck-Institut für Virusforschung in Tübingen. He was at that time 69 years old, a time of life when most people have been retired a few years. Gottschalk could not, however, envisage retirement, and he accepted this invitation with alacrity. During the following ten years, he did some very good work in the glycoprotein field, inspiring a considerable number of younger colleagues, and exerting great influence all over the world by being an efficient editor of two editions of the book entitled "Glycoproteins." In 1966, Alfred Gottschalk was made an Honorary Professor in the Science Faculty of the University of Tübingen, and, in 1968, he was designated a Foreign Scientific Member of the Max-Planck Institute for Virus Research. In 1969, the University of Münster conferred upon Gottschalk the Honorary Degree of Doctor of Medicine.

When Alfred Gottschalk died, after an increasing fight against heart disease during the last years of his life, he was still in full possession of his considerable mental faculties. He had become interested in the history of biochemistry, but insofar as the present writer is aware, the writing of the expected book thereon was not completed. He had a varied life, full of stresses, but he had the satisfaction of having made important contributions to science. During his

24 years in Australia, he acquired new loyalties and new horizons, but he was pleased to return to his native land and to assist in the development of biochemistry in that country. By far his most important contribution to biochemistry was made in the last 27 years of his life. It must have been a source of great satisfaction to Alfred Gottschalk that his work was suitably recognized by the two countries with which he was most closely associated, namely, Germany and Australia.

The scientific work of Gottschalk falls into three, well-defined periods. His early work was mainly in the general area of the metabolism of carbohydrates and of biologically related compounds. Then, about 1947, he became involved in studies on the interaction between the influenza virion and the surface of the red cell, and this led him to his important work on sialic acid. During the last period, Gottschalk extended his interest to the general chemistry and biochemistry of glycoproteins.

While he was in Berlin-Dahlem, Gottschalk published, largely together with Carl Neuberg, a considerable number of papers, and he continued working in the field of fermentation and related problems after he moved, first to Stettin, and then to Melbourne. Neuberg's work on the fermentation of yeast was thus extended by Gottschalk to green plants and animal tissues. He investigated the formation of acetaldehyde in plant tissues and in animal metabolism under anaerobic conditions. He made some contributions to our knowledge of glycolysis in tumor cells and of the action of glycosidases. He also showed that the enzymic degradation of starch and glycogen to D-glucose can occur without the intermediate formation of maltose. Later, he investigated the metabolism of D-fructose in some detail, examining the equilibria of the different ring forms and anomers present in solution. He showed that D-fructopyranose cannot be metabolized, and that only the furanose form is "fermentable." Gottschalk also worked on the Pasteur effect, and on the action of some hormones on carbohydrate metabolism.

It had been known since 1941 that red blood-cells are agglutinated by fluids containing influenza virus. In 1942, G. K. Hirst proved that purified influenza virus is quickly and quantitatively adsorbed on red cells, but that, after the elapse of several hours, the virus particle, apparently intact, has come off the surface of the red cells. After this treatment, cells were unable to be agglutinated by influenza virus or to adsorb virus particles. The problem of the interaction of influenza virus with red cells was attacked in a comprehensive manner by Sir Macfarlane Burnet and his coworkers in Melbourne in the period of

1946-1947. They showed that partially purified preparations of "mucoprotein" from several sources were able to inhibit, specifically, the virus-induced agglutination of red cells. They also showed that the red cells could be "inactivated" not only by contact with the influenza virus but also by a purified enzyme obtained from culture filtrates of *Vibrio cholerae*. It seemed reasonable to conclude that a particular component present in a bound form on the surface of the red cell was specifically involved in the adsorption of the virus and was also concerned in the agglutination. It also seemed likely that the enzyme that had been found both in the influenza virus and in the *Vibrio* preparation acted by removing an active component from the red cell by hydrolytic action, thus abolishing the "receptor" property of the cell. The observation that "mucoproteins" act as inhibitors suggested that compounds, or chemical groupings, of carbohydrate structure might be involved, and it seemed appropriate to invite Gottschalk, who was by then a member of the Institute staff and who had extensive experience in this branch of chemistry, to tackle the chemical aspects of this problem.

In 1949, Gottschalk, together with P. E. Lind, showed that the loss of activity of ovomucin, one of the inhibitors of the red-cell agglutination, is closely associated with the liberation of a compound of low molecular weight. This "split product" behaved in many respects like an amino sugar, but unlike 2-amino-2-deoxy-D-glucose and -galactose, it was decomposed on heating with *M* hydrochloric acid within a few minutes at 100°. Investigations in several laboratories, but especially that of Gottschalk, over the next 10 years established the structure of this "split product," which was shown to be a sialic acid. It was also clearly demonstrated that the agglutino-gen-agglutinin reaction between the influenza virus and the red cell, and also the inhibition of this reaction by glycoproteins, involves a sialic acid grouping, and that the receptor-destroying enzyme is a neuraminidase.

In about 1936, sialic acid was discovered by Blix, who found it to be a component of submaxillary-gland proteins, and who described many of its properties. However, little notice was taken of this work at the time it was published. In 1941, Klenk, who was working on glycolipids of the brain, described a compound, later shown to be a methyl glycoside of sialic acid, that had been obtained by treatment of a lipid fraction with 5% methanolic hydrogen chloride at 105°. In 1954, Klenk and Faillard reported the first isolation of pure *N*-acetyl-neuraminic acid from animal sources.

In 1955, Gottschalk showed that, on relatively mild treatment with

alkali, *N*-acetylneuraminic acid gives pyrrole-2-carboxylic acid. He interpreted this reaction as being due to a reversal of aldol formation, followed by cyclization and rearrangement. Gottschalk had, in 1951, put forward a structure for sialic acids which we now believe was incorrect. However, later work by others, and himself, caused him to change his views, and he soon accepted the structure that is now considered correct. Gottschalk suggested that *N*-acetylneuraminic acid is formed by an aldol type of reaction between a 2-acetamido-2-deoxyhexose and pyruvic acid. Soon afterwards, in 1956, the reverse reaction, namely, a fission of *N*-acetylneuraminic acid catalyzed by alkali, was reported by Kuhn and Brossmer, and by Zilliken and Glick. At about the same time, Heimer and Meyer (1956) discovered an enzyme performing this fission, a specific aldolase that is formed by *Vibrio cholerae*. This aldol reaction was also the basis of the first synthesis of *N*-acetylneuraminic acid, carried out jointly by Cornforth and Gottschalk in 1957; these workers incubated 2-acetamido-2-deoxy-D-glucose with sodium oxalacetate at pH 11 and 20°, and isolated the sialic acid derivative in moderate yield. This synthesis appeared to prove the general structure of *N*-acetylneuraminic acid beyond reasonable doubt. However, shortly afterwards, Comb and Roseman (1958) investigated the action of a specific aldolase from *Clostridium perfringens*, and showed that the acetylhexosamine moiety has the *D-manno* configuration. The observation that the *D-gluco* derivative could be used effectively in the synthesis was explained by the ready occurrence of an epimerization between the two acetamido sugars at pH 11. The overall configuration of *N*-acetylneuraminic acid was established soon afterwards in the laboratory of R. Kuhn in Heidelberg, and that of the anomeric carbon atom was established later.

It is clear from this brief account that Alfred Gottschalk played an important, or even decisive, part in the establishing of the structure of sialic acid. In addition, Gottschalk produced the chemical basis and explanation for the important observations by Hirst, and, particularly, by Burnet on the interaction of animal cells with myxoviruses. He also played an important part in elucidating the action of neuraminidases. In this connection, mention should also be made of his work on a glycoprotein present in urine; this protein was discovered in 1952 by Tamm and Horsfall, who showed its inhibitory action on the agglutination of red cells by influenza virus. Gottschalk investigated the chemical properties of this protein, carried out an analysis of its sugar components, and, in particular, demonstrated the presence of sialic acid residues and their release from the protein by neuraminidase.

The glycoproteins produced by the submaxillary glands of several species were first investigated by Blix in 1956, but intensive work carried out in several laboratories, especially that of Gottschalk, has made this group one of the best known types of glycoproteins. The work of Gottschalk was mainly concerned with the glycoproteins of ox and sheep.

In a series of papers, Gottschalk showed that about half of these proteins consist of 2-acetamido-2-deoxy-D-galactose and *N*-acetylneuraminic acid residues, and that these two sugars are linked as disaccharidic, that is, as *N*-acetylneuraminosyl-(2 \rightarrow 6)-2-acetamido-2-deoxy-D-galactose, moieties to the peptide chain. Neuraminidase removes *N*-acetylneuraminic acid from the protein, showing that the residue is terminal; this indicates that the 2-acetamido-2-deoxy-D-galactose is associated directly with the peptide chain. Gottschalk also conducted extensive investigations on other chemical and physical properties of these proteins.

Later, Gottschalk, together with Buddecke, described an enzyme that specifically cleaves the linkage between the 2-acetamido-2-deoxy-D-galactose residue and the peptide chain; this enzyme was purified and fully characterized. It turned out to be a 2-acetamido-2-deoxy- α -D-galactosidase, and its attack on the submaxillary glycoprotein of sheep provided a method of preparing this protein free from its carbohydrate groups. This enzyme has also been widely applied in structural studies on glycoproteins, and, in particular, it was used to identify the anomeric type of the glycosidic linkage of a given 2-acetamido-2-deoxy-D-galactose residue.

In his earlier studies, Gottschalk (and, independently, Pigman) determined the amino acid composition of the glycoproteins of the submaxillary gland obtained from sheep and ox. Both of these proteins are very rich in L-alanine, glycine, L-serine, and L-threonine, and also contain moderately large proportions of L-aspartic acid and L-glutamic acid. The carbohydrate residues are removed from the peptide under relatively mild conditions, and, in 1961, Gottschalk assumed that the linkage was that of a glycosyl ester; this appeared even more probable when it was found that treatment with alkaline borohydride results in the reduction of some of the secondary carboxylic acid groupings of the L-aspartic and L-glutamic acid residues. In 1962, Hashimoto and Pigman pointed out that the glycoprotein obtained from submaxillary glands did not contain the expected number of aspartic and glutamic residues, and, in 1963, Anderson, Hoffman, and Meyer suggested that the proteoglycan from cartilage contained an O-glycosyl bond which linked the carbohydrate moiety to serine, and that treatment with alkali resulted in a β -

elimination. The three groups (of Meyer, Harbon, and Pigman) then demonstrated, in 1963–1964, that O-glycosyl bonds are present not only in proteoglycans but also in various glycoproteins, such as the blood-group substances and the glycoproteins produced by the submaxillary glands. In all these cases, alkali splits the linkage not by hydrolysis but by β -elimination. It was not until 1968 that Gottschalk accepted the view that there are no glycosyl ester bonds in the proteins produced by the salivary glands. He also soon accepted the view that the bond between the 2-amino-2-deoxy-D-galactose residue and the peptide in the submaxillary-gland glycoproteins is also a glycoside of this type, and, that the cleavage consists of a β -elimination. The reduction of the L-aspartic and L-glutamic acid residues was found to be due to the occurrence of an independent reaction that was somewhat unexpected. It is now accepted that glycoproteins are a special group of proteins having a peptide core to which oligosaccharide units are attached. This view is implicit in the work of Levene and others who had worked with ovalbumin and ovomucoid, but Gottschalk was the first to establish it for the glycoproteins of the mucin type, and he showed that, in substances which he investigated, one out of four amino acid residues carries an oligosaccharide side-chain. This concept, which was extended by him to other glycoproteins, was novel at the time and inspired much, or even most, of the later work in this field.

In November, 1960, the Elsevier Publishing Co. invited Gottschalk to write a multi-author book for the "BBA Library." The purpose of the book was to collect and review the knowledge then available on "mucoproteins." At that time, considerable interest in this field of biochemistry had just developed, as it was increasingly realized that glycoproteins are present in a great variety of tissues in a very large number of animal species. Gottschalk responded to this invitation by approaching some of the leading workers in this field to contribute to the book. His wide knowledge, the large number of personal contacts he had with biochemists, and his considerable critical faculties made Gottschalk an ideal editor. Almost all of the colleagues approached agreed to write their allotted chapters, and the whole plan was widely welcomed. Gottschalk showed himself to be a very strict and critical editor, who insisted that all of the information given should be comprehensive and accurate in every detail. He made many critical comments on the manuscripts submitted to him, and altogether he was not an easy task-master. He was insistent that the various authors should keep strictly to their time schedule agreed upon, and he did his best to avoid unnecessary repetition or contradiction between the various chapters.

The first edition of this book finally appeared in 1966, and it was an important landmark in the development of our knowledge of glycoproteins. A few years after the first edition had appeared, it was felt that some of the information given was out of date, and new knowledge was accumulating at a great rate. In 1967, it was decided to produce a second and enlarged edition, which saw the light of day in 1972. Again, the amount of effort that Gottschalk put into this venture was great, and he deserved considerable credit for having provided protein chemists with a most valuable work of reference. The publication of these two editions of "Glycoproteins" played an important part in the rate of advance of the subject.

ALBERT NEUBERGER

RELATIVE REACTIVITIES OF HYDROXYL GROUPS IN CARBOHYDRATES

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I. INTRODUCTION

A knowledge of the relative reactivities of hydroxyl groups in carbohydrates is fundamental to a thorough understanding of carbohydrate chemistry. It is now over twenty years since the subject was

last discussed in this Series,¹ and since that time, the development of separatory and analytical methods, in particular, gas-liquid chromatography (g.l.c.) and nuclear magnetic resonance (n.m.r.) spectroscopy, has allowed more-accurate measurements of product distributions, with a consequent increase in the reliability of deductions based on them. Over the past two decades, chemists have developed a greater appreciation of electronic and steric factors that influence the reactivity of organic molecules, and rationalization of selective reactivity amongst functional groups of the same type is aided by a consideration of these factors. The time, therefore, seems appropriate to reassess the subject and provide a summary that may stimulate further studies in this area.

The general arrangement of this article follows that of the previous one,¹ being divided into Sections dealing with important reactions of the hydroxyl group. Study of esterification (Section II) forms by far the largest part of the literature (since 1953) on selective reactions of carbohydrates. Etherification (Section III) has continued to be widely studied, especially that of polysaccharides, and some examples of the favored acetalation of primary hydroxyl groups are reported in Section IV. A new Section (V) has been added on the replacement of hydroxyl groups by halogen, a transformation of great importance for the selective introduction of a wide range of substituents. Selective oxidation (Section VI) has been developed mainly by an extensive study of molecular oxygen-metal catalyst systems. A Section (VII) on substituent migration is also included.

To bring further order to the large amount of literature, some Sections are subdivided into subsections dealing with reagent types, so that mechanistically related reactions are grouped together, and reagents may be profitably compared.

II. SELECTIVE ESTERIFICATION

The common methods of esterification utilize an acid chloride or an acid anhydride as the reagent, but other acid derivatives often show different selectivities, and these are also considered here. In view of the extensive articles on sulfonic esters of carbohydrates,²⁻⁴

(1) J. M. Sugihara, *Adv. Carbohydr. Chem.*, **8**, 1-44 (1953).

(2) R. S. Tipson, *Adv. Carbohydr. Chem.*, **8**, 107-215 (1953).

(3) D. H. Ball and F. W. Parrish, *Adv. Carbohydr. Chem.*, **23**, 233-280 (1968).

(4) D. H. Ball and F. W. Parrish, *Adv. Carbohydr. Chem. Biochem.*, **24**, 139-197 (1969).

which contain many references to relative reactivity, the majority of examples in this Section are drawn from studies on selective acylation.

1. Acid Chlorides and Acid Anhydrides

a. Influence of Steric and Polar Factors.—Although selective esterifications that differentiate between primary and secondary hydroxyl groups have long been known, relatively few attempts have been made to magnify the steric requirements of the transition state for esterification by increasing the bulk of the acid derivative, and thereby to enhance selectivity. 2,4,6-Triisopropylbenzenesulfonyl chloride shows a marked reluctance to react with even the primary hydroxyl groups in nucleotides,⁵ and this reagent finds considerable use in nucleotide coupling-reactions, which presumably proceed through mixed anhydrides as intermediates. Reaction of thymidine with pivaloyl (trimethylacetyl) chloride gave a high yield (82%) of the 5'-O-acyl derivative,⁶ and a similar result was obtained by using isobutoxycarbonyl chloride,⁷ both reagents showing greater selectivity than acetic anhydride in pyridine.⁸ Pivaloyl chloride has been shown to acylate 2'-O-benzyluridine⁹ and 2'-O-tetrahydropyran-2-yladenosine selectively at the primary position,¹⁰ and the superiority of this reagent was noted in the dimolar acylation of 3,4-O-isopropylidene-D-mannitol, when the 1,6-diester was found to be the sole product, whereas, on using benzoyl chloride, the yield of the 1,6-dibenzoate was poor and the reaction was not sufficiently selective.¹¹ 1-Adamantanecarbonyl chloride has been shown to react with nucleosides to yield, preponderantly, 5'-esters,¹² and the mixed anhydride of (triphenylmethoxy)acetic acid with 2,4,6-triisopropylbenzenesulfonyl chloride behaves similarly¹³; each acyl group in these esterifying agents may be regarded as a base-labile equivalent of the trityl group. Chlorodiphenylacetyl chloride reacts selectively (87%) with the primary hydroxyl group in thymidine, whereas, with

(5) R. Lohrmann and H. G. Khorana, *J. Am. Chem. Soc.*, **88**, 829-833 (1966).

(6) G. Weimann and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 4329-4341 (1962).

(7) R. L. Letsinger and K. K. Ogilvie, *J. Org. Chem.*, **32**, 2365-2366 (1967).

(8) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212-6222 (1958).

(9) B. E. Griffin, C. B. Reese, G. F. Stephenson, and D. R. Trentham, *Tetrahedron Lett.*, 4349-4354 (1966).

(10) B. E. Griffin and C. B. Reese, *Tetrahedron Lett.*, 2925-2931 (1964).

(11) S. J. Angyal and M. E. Evans, *Aust. J. Chem.*, **25**, 1495-1512 (1972).

(12) K. Gerzon and D. Kau, *J. Med. Chem.*, **10**, 189-199 (1967).

(13) E. S. Werstiuk and T. Neilson, *Can. J. Chem.*, **51**, 1889-1892 (1973).

chloroacetyl chloride, no substantial selectivity for acylation at O-5' was observed.¹⁴

Differential reactivity between secondary hydroxyl groups may also depend on steric factors. Unimolar *p*-toluenesulfonylation of methyl 4,6-*O*-ethylidene- α -D-mannopyranoside¹⁵ gave a good yield of the 3-*O*-*p*-tolylsulfonyl derivative, and no 2-isomer was detected. The hydroxyl group on C-3 occupies an equatorial position on the pyranoside ring, whereas the bond to that at C-2 is axial. A study of the unimolar *p*-toluenesulfonylation of the *trans*-fused methyl 4,6-*O*-benzylidene-D-glycopyranosides has shown that, where there is a choice between an axially and an equatorially attached hydroxyl group, it is the equatorial one that is selectively sulfonylated,^{16,17} in agreement with well established principles of conformational analysis. Similarly, 1,6-anhydro- β -D-mannopyranose is mainly esterified¹⁵ at the equatorial hydroxyl group on C-2, and, in cyclitol chemistry, a similar pattern of reactivity is generally observed. Thus, DL-1,4,5,6-tetra-*O*-substituted *myo*-inositols may be methanesulfonylated¹⁸ and *p*-toluenesulfonylated¹⁹ at HO-3 (*eq*) in high yields, and 1L-1,2,3,4-tetra-*O*-methyl-*chiro*-inositol is benzoylated selectively²⁰ at HO-5 (*eq*), rather than at HO-6 (*ax*).

2,4,6-Trimethylbenzenesulfonyl chloride has been shown²¹ to be much more selective for the monosulfonylation of a vicinal secondary diol (for example, methyl 4,6-*O*-benzylidene- α -D-glucopyranoside) than *p*-toluenesulfonyl chloride, and, apparently, 2,4,6-triisopropylbenzenesulfonyl chloride exhibits an even higher selectivity.²²

That steric factors alone cannot account for the reactivity of hydroxyl groups was indicated by the unimolar *p*-toluenesulfonylation of 1,4:3,6-dianhydro-D-glucitol.²³ The major product, the 5-*p*-toluenesulfonate (45%), is derived by reaction at the sterically hindered *endo*-hydroxyl group, and the *exo*-2-*p*-toluenesulfonate was isolated in only 12% yield. Although the more reactive HO-5 (*es*-

(14) A. F. Cook and D. T. Maichuk, *J. Org. Chem.*, **35**, 1940-1943 (1970).

(15) G. O. Aspinall and G. Zweifel, *J. Chem. Soc.*, 2271-2278 (1957).

(16) J. G. Buchanan and J. C. P. Schwarz, *J. Chem. Soc.*, 4770-4777 (1962).

(17) S. E. Creasey and R. D. Guthrie, *Carbohydr. Res.*, **22**, 487-490 (1972).

(18) T. Suami and S. Ogawa, *Bull. Chem. Soc. Jpn.*, **37**, 1238-1239 (1964).

(19) T. Suami, S. Ogawa, and S. Oki, *Bull. Chem. Soc. Jpn.*, **44**, 2820-2823 (1971).

(20) G. McCasland, M. O. Naumann, and L. J. Durham, *J. Org. Chem.*, **34**, 1382-1386 (1969).

(21) S. E. Creasey and R. D. Guthrie, *J. Chem. Soc. Perkin Trans. I*, 1373-1378 (1974).

(22) See reference 17 in Ref. 21.

(23) R. U. Lemieux and A. G. McInnes, *Can. J. Chem.*, **38**, 136-140 (1960).

terification rate ~ 1.4 times that of HO-2 towards *p*-toluenesulfonyl chloride in pyridine) is comparatively shielded, it is significant that it is strongly hydrogen-bonded intramolecularly to a ring-oxygen atom (O-4).

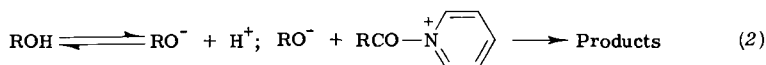
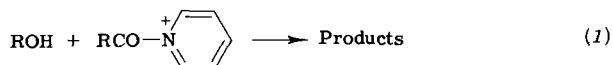
A study of the possible effects of intramolecular hydrogen-bonding on rates of esterification was initiated by Foster and coworkers.²⁴ By means of competitive reactions with *p*-phenylazobenzoyl chloride in pyridine, it was found that, although the ratio of esterification rate-constants for *trans*- (*eq*-OH) and *cis*- (*ax*-OH) 4-phenylcyclohexanol was 6.6:1, the ratio for *trans*- and *cis*-2-phenyl-1,3-dioxan-5-ol was 1:5.6. For the latter pair of compounds, infrared (i.r.) spectroscopy showed that, in dilute solution in carbon tetrachloride, the *cis* isomer has an absorption for bonded hydroxyl groups only at 3590 cm^{-1} , and presumably exists in that chair conformation in which HO-5 is involved in a strong, intramolecular hydrogen-bond with one or both of the ring-oxygen atoms. The *trans* isomer, however, has absorptions at 3633 (ϵ 79) and 3601 cm^{-1} (ϵ 26) for free and bonded hydroxyl groups. If intramolecular hydrogen-bonding occurs to any extent under the conditions of acylation, and if the rate-determining step in this reaction involves the attack of an un-ionized hydroxyl group on the acid derivative, the basicity of the oxygen atom should be enhanced, and the esterification facilitated.

An investigation of the unimolar acylation of 1,4:3,6-dianhydro-D-glucitol with *p*-phenylazobenzoyl chloride yielded results similar to those obtained in the earlier *p*-toluenesulfonylation study; the 5- and 2-esters were isolated in the ratio of 3:1. In an extension of this work, rates of acetylation of these and other alcohols in acetic anhydride-pyridine were measured.²⁵ In contrast to the esterifications involving acid halides in pyridine, no rate-enhancement effect attributable to intramolecular hydrogen-bonding was observed. The rate constants for reaction of *trans*- and *cis*-4-phenylcyclohexanol, and *trans*- and *cis*-2-phenyl-1,3-dioxan-5-ol were found to be 11.9, 4.0, 49.5, and 6.0 liter mol.⁻¹ sec.⁻¹, respectively; that is, the *trans* isomers are the more readily esterified of each pair, and the 1,3-dioxane derivatives react more readily than their cyclohexane counterparts. Furthermore, esterification of 1,4:3,6-dianhydro-D-glucitol with 1 molar equivalent of acetic anhydride in pyridine gave the 2- and 5-esters in the ratio of 1.7:1. Interestingly, a similar reaction in

(24) K. W. Buck, A. B. Foster, A. R. Perry, and J. M. Webber, *J. Chem. Soc.*, 4171-4177 (1963).

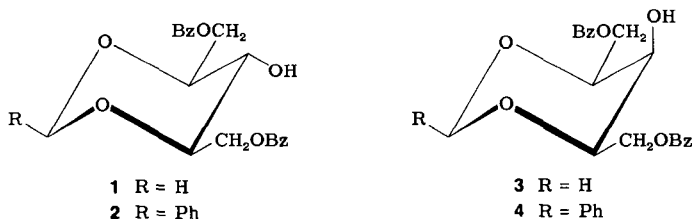
(25) K. W. Buck, J. M. Duxbury, A. B. Foster, A. R. Perry, and J. M. Webber, *Carbohydr. Res.*, **2**, 122-131 (1966).

the presence of pyridine hydrochloride gave the 2- and 5-esters in the ratio of 1:3.6, close to that obtained in the reactions with acid chlorides. Were the overall rate of reaction with acid halides in pyridine determined by process 1, and that with acetic anhydride by process 2, these results could be rationalized, as process 1 should be



increased by intramolecular hydrogen-bonding, and decreased by vicinal, electronegative substituents, whereas, in process 2, the reverse would apply.

Attractive though the argument is that enhanced reactivity is a result of intramolecular hydrogen-bonding,²⁶ it is open to criticism, as i.r. measurements on pyridine solutions of free sugars provide no evidence for intramolecular hydrogen-bonds.²⁷ Also, for solutions of alcohols plus pyridine in carbon tetrachloride, hydrogen bonding between pyridine and alcohols is predominant in dilute solution.²⁸ To clarify the role of intramolecular hydrogen-bonding in acylation reactions, Sugihara and coworkers²⁹ measured second-order rate-constants, k_2 , for the esterification of 1,5-di-*O*-benzoyl-2,4-*O*-methylene-ribitol (1) and -xylitol (3) and 1,5-di-*O*-benzoyl-2,4-*O*-benzylidene-ribitol (2) and -xylitol (4) with acid anhydrides and with acid chlo-



rides in pyridine. Towards acid anhydrides, compounds 1 and 2 (which, in their favored conformation, each have an equatorial hy-

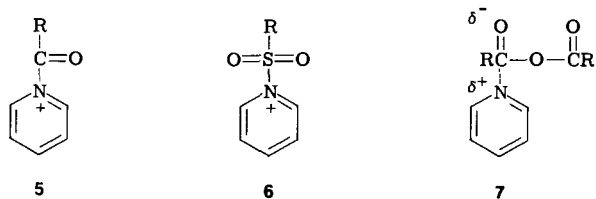
(26) For a review on the influence of the intramolecular hydrogen-bond on the reactivity of organic compounds, see I. D. Sadekov, V. I. Minkin, and A. E. Lutskiĭ, *Russ. Chem. Rev.*, **39**, 179-195 (1970).

(27) M. A. Kabayama and D. Patterson, *Can. J. Chem.*, **36**, 563-573 (1958).

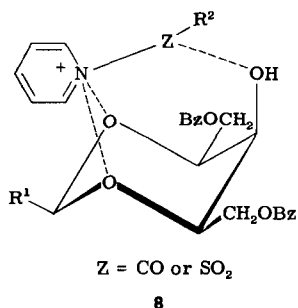
(28) T. J. V. Findlay and A. D. Kidman, *Aust. J. Chem.*, **18**, 521-530 (1965).

(29) J. M. Knoblich, J. M. Sugihara, and T. Yamazaki, *J. Org. Chem.*, **36**, 3407-3411 (1971).

droxyl group) are more reactive than compounds **3** and **4**, which each possess an axial hydroxyl group. However, in agreement with results of Foster and coworkers,^{24,25} compounds **3** and **4** showed greater reactivity than **1** and **2** towards acid chlorides in pyridine. For a series of *p*-substituted benzoyl and benzenesulfonyl chlorides, a plot of $\log k_2$ against substituent σ^+ values yielded straight lines of positive slope, ρ . The positive value of ρ was taken to imply that the degree of conjugation of the substituent with the reaction site decreases in going from substrate to activated complex in the rate-limiting step. The authors²⁹ proposed that polar interactions offer a more-reasonable explanation of the differences in reactivity than hydrogen-bonding effects. With the assumption that the reactive species in esterification with an acid chloride in pyridine is an acyl- or sulfonyl-pyridinium ion^{29a} (for example, **5** and **6**, respectively), and that that with an acid anhydride is an ion pair (**7**), the selectivity observed was rational-



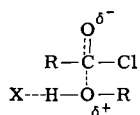
ized. Interaction of the positively charged nitrogen atom in **5** and **6** with the oxygen atoms in the 1,3-dioxane ring promotes reaction with the axial hydroxyl groups in compounds **3** and **4** by aiding the favorable alignment of the acyl (or sulfonyl) and hydroxyl functions, as in **8**. In contrast, with **1** and **2**, this type of cooperative interaction is



(29a) It should be noted that, although **6** is probably the reactive species in arenesulfonylations in pyridine (for example, using *p*-toluenesulfonyl chloride as the reagent), sulfonylations conducted in the presence of tertiary amines with alkane-sulfonyl chlorides (and bromides and anhydrides) that bear at least one α hydrogen atom probably proceed through a sulfene intermediate. For a summary, see J. F. King, *Acc. Chem. Res.*, **8**, 10-17 (1975).

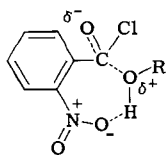
precluded. The lack of definitive, cationic character for **7**, formed from carboxylic acid anhydrides and pyridine, allows the usual steric considerations to dominate reactivity. In agreement with the observed, positive value of ρ found in the Hammett correlation, the contribution of conjugation with the reaction site by *para*-substituents in R_2 for **8** might be expected to be less than that of the same substituent in R of **5** or **6**. Numerous other examples of differential reactivity to these two types of reagent may be similarly rationalized.

An investigation pertinent to the subject of favored reactivity concerned the reactions of certain acyl halides with ethanol, in acetone or chloroform solution.³⁰ The mixed-order nature of some of these reactions suggested that every rate-determining transition-state contained the substrate, the nucleophile (ethanol), and an acceptor, X , for hydrogen bonding, as in **9**, suitable acceptors being an acetone molecule, a chloride ion, or another ethanol molecule. Significantly, the rate equation describing the reaction of *o*-nitrobenzoyl chloride with ethanol in chloroform contained second- and third-order terms. The former was attributed to a transition state (**10**) in which the acylating species itself acts as a proton acceptor. It is probable that, during acylation of a hydroxyl group that is intramolecularly hydrogen-bonded, a facile proton-transfer to the atom acting as hydrogen-bond acceptor (such as X in **11**) could lead to a rate of reac-

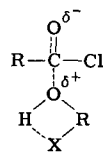


$X = \text{acceptor}$

9



10



11

tion increased over that of a similar, but nonbonded, hydroxyl group. Analogous considerations may apply to reactions of acyl halides in pyridine, where the acylating species is the acylpyridinium ion **5**. The possibility that intramolecular hydrogen-bonding does not persist in pyridine, and that the advantage of a bonded hydroxyl group would, therefore, be lost, invalidates this explanation of favored reactivity, but were hydrogen-bonding to pyridine hindered in the transition state, intramolecular hydrogen-bonding could conceivably assume importance.³¹

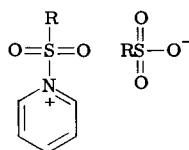
Although existence of any connection between an unusual reac-

(30) S. D. Ross, *J. Am. Chem. Soc.*, **92**, 5998-6002 (1970).

(31) J. M. Williams and A. C. Richardson. *Tetrahedron*, **23**, 1369-1378 (1967).

tivity of a hydroxyl group and its involvement in intramolecular hydrogen-bonding is still uncertain, attention will be drawn throughout this article to the co-occurrence of the two phenomena.

b. Pyranoid Derivatives of Monosaccharides.—An early study of the selective acylation of methyl 4,6-*O*-benzylidene- α -D-glucopyranoside with one molar equivalent of acetic anhydride, benzoic anhydride, methanesulfonic anhydride, or *p*-toluenesulfonic anhydride, or the corresponding acid chlorides, in pyridine showed a marked dependence of the product ratio on the reagent employed.³² Acid chlorides gave mainly 2-esters, whereas carboxylic anhydrides gave mainly 3-esters. However, both acetic anhydride (in the presence of pyridine hydrochloride) and sulfonic anhydrides favored the formation of 2-esters. The difference in reaction pattern between carboxylic and sulfonic acid anhydrides, and the similarity of the behavior of the latter to that of acid chlorides, may be a result of the readier formation of a sulfonylpyridinium sulfonate (**12**) from a sulfonic acid anhydride plus pyridine than of an acylpyridinium carboxylate from a carboxylic acid anhydride plus pyridine.



12

Horton and Lauterbach³³ developed a method, based on proton n.m.r. spectroscopy, for quantitatively determining the extent of acetylation in a partially substituted carbohydrate, thus avoiding problems of deacylation and migration during analysis. A study of the reaction of methyl 4,6-*O*-benzylidene- α -D-glucopyranoside with one equivalent of acetic anhydride in pyridine for 30 min at room temperature showed that it had proceeded to 67% of completion, and that it had achieved a degree of substitution (d.s.) at O-2 and O-3 of 0.3 and 0.37, respectively. A similar experiment on methyl 4,6-*O*-benzylidene- β -D-glucopyranoside showed^{33a} the d.s. at both O-2 and O-3 to be 0.5. The method was also applied to methyl α - and β -D-glucopyranoside (see later).

(32) R. W. Jeanloz and D. A. Jeanloz, *J. Am. Chem. Soc.*, **79**, 2579–2583 (1957).

(33) D. Horton and J. H. Lauterbach, *J. Org. Chem.*, **34**, 86–92 (1969).

(33a) J. H. Lauterbach, Ph.D. Dissertation, The Ohio State University, 1970.

Ethoxycarbonyl chloride is, by virtue of resonance involving the ester function, less reactive than acetyl chloride, and the reagent has found application for selective *O*-acylation in the steroid field.³⁴ With this reagent, methyl 4,6-*O*-benzylidene- α -D-glucopyranoside yielded³⁵ 2- and 3-esters in the ratio 24:1, and the related benzylthiocarbonyl chloride gave the 2-ester in 58% yield.³⁶

Unimolar esterifications of methyl 4,6-*O*-benzylidene- β -D-glucopyranoside are much less selective than those of the corresponding α -D-glucoside derivative. Reactions with methanesulfonyl chloride³⁷ or *p*-toluenesulfonyl chloride³⁷⁻³⁹ gave mixtures of the 2- and 3-mono- and 2,3-di-esters, with a slight preponderance of the 3-over the 2-ester. As the environment of HO-3, both sterically and electronically, is essentially the same in the α - and β -D-glucoside derivative, the difference in reactivity must stem from the greater reactivity of HO-2 in the α - compared to that in the β -D-glucoside derivative, rather than from the greater reactivity of HO-3 in the β - compared to that in the α -D-glucoside derivative. The results of competitive experiments confirmed this conclusion; reaction of a 1:1:1 mixture of methyl 4,6-*O*-benzylidene- α -D-glucopyranoside, methyl 4,6-*O*-benzylidene- β -D-glucopyranoside, and *p*-toluenesulfonyl chloride in pyridine gave 72% of unchanged β -D-glucoside derivative, 54% of unchanged α -D-glucoside derivative, and 37% of the 2-*p*-toluenesulfonate of the α -D-glucoside derivative.³⁷

Lack of selectivity in the reaction of the β -D-glucoside derivative with one molar equivalent of benzylthiocarbonyl chloride has also been noted: 40% of the 2,3-diester and 40% of the starting material were isolated.⁴⁰ Similarly, unimolar benzylation of phenyl 4,6-*O*-benzylidene- β -D-glucopyranoside gave only 9% of the 3-ester, together with 47% of the 2,3-diester.⁴¹ Acylation of benzyl 4,6-*O*-benzylidene- β -D-glucopyranoside with acetic anhydride-pyridine-pyridine hydrochloride yielded,⁴² in contrast to the reaction with the

- (34) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero, and T. Utne, *J. Am. Chem. Soc.*, **74**, 3309-3313 (1952).
- (35) W. M. Doane, B. S. Shasha, E. I. Stout, C. R. Russell, and C. E. Rist, *Carbohydr. Res.*, **4**, 445-451 (1967).
- (36) J. J. Willard, J. Sadowski, and W. Vitale, *Can. J. Chem.*, **41**, 1223-1230 (1963).
- (37) R. D. Guthrie, A. M. Prior, and S. E. Creasey, *J. Chem. Soc., C*, 1961-1966 (1970).
- (38) R. U. Lemieux, E. Fraga, and K. A. Watanabe, *Can. J. Chem.*, **46**, 61-69 (1968).
- (39) S. Stirn, O. Lüderitz, and O. Westphal, *Ann.*, **696**, 180-193 (1966).
- (40) J. J. Willard, J. S. Brimacombe, and R. P. Brueton, *Can. J. Chem.*, **42**, 2560-2567 (1964).
- (41) P. Rivaille and L. Szabó, *Bull. Soc. Chim. Fr.*, 716-721 (1963).
- (42) C. P. J. Glaudemans and H. G. Fletcher, Jr., *Carbohydr. Res.*, **7**, 480-482 (1968).

corresponding methyl α -D-glucoside derivative,³² the 3-acetate (14.4%) in excess of the 2-acetate (5%).

Methyl 4,6-O-benzylidene- α -D-altropyranoside showed little selectivity on reaction with a unimolar proportion of benzoyl chloride⁴³; 2- and 3-O-benzoyl and 2,3-di-O-benzoyl derivatives, together with starting material, were isolated in the molar ratios of 1:1.1:1.8:1. In contrast, benzoic anhydride in pyridine gave the 2-ester in 35% yield, with only a trace of the 3-benzoate.

From the results of many selective-esterification reactions, the greater reactivity of the 3-hydroxyl group in the α - and β -glycosides of 4,6-O-benzylidene-D-galactopyranose was recognized. Thus, methyl 4,6-O-benzylidene- α -D-galactopyranoside with benzoyl chloride,⁴⁴ *p*-toluenesulfonyl chloride,⁴⁵ or ethoxycarbonyl chloride⁴⁶ in pyridine gives the 3-esters in yields of 46, 40, and 59%, respectively. Selectivity for reaction at HO-3 appears to be even greater in the β - than the α -series. Thus, partial esterification of methyl 4,6-O-benzylidene- β -D-galactopyranoside with ethoxycarbonyl chloride⁴⁷ or *p*-toluenesulfonyl chloride⁴⁵ gave the 3-ester in yields of 65 and 58%, respectively. With one equivalent of benzoyl chloride in pyridine-dichloromethane, the benzyl β -D-galactopyranoside derivative gave 64-78% (based on diol reacted) of the 3-benzoate,⁴⁸ and the phenyl β -D-galactopyranoside derivative was reported to yield only the 3-benzoate (and starting material) on using benzoyl chloride as the reagent.⁴¹

It was suggested⁴⁸ that favored reaction at the equatorial 3-hydroxyl group in the β -series could be due to its involvement in hydrogen-bonding with the axial oxygen atom on C-4. Although similar bonding could occur between the equatorial 2-hydroxyl group and the oxygen atom of the equatorial methoxyl group on C-1, the latter disposition of groups appears, on i.r. spectroscopic evidence (note: $\Delta\nu$ values for *cis*- and *trans*-1,2-cyclohexanediols⁴⁹), less favorable towards bonding. Furthermore, the correlation of the enhanced reactivity of the 2-hydroxyl group in methyl 4,6-O-benzylidene- α -D-glucopyranoside with the presence of a *cis* OH-2-OMe-1 hydrogen-bond¹⁷ finds direct analogy with the enhanced reactivity of HO-3 in

(43) R. W. Jeanloz and D. A. Jeanloz, *J. Am. Chem. Soc.*, **80**, 5692-5697 (1958).

(44) M. Gyr and T. Reichstein, *Helv. Chim. Acta*, **28**, 226-233 (1945).

(45) E. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **28**, 1-17 (1945).

(46) A. C. Maehly and T. Reichstein, *Helv. Chim. Acta*, **30**, 496-507 (1947).

(47) F. Reber and T. Reichstein, *Helv. Chim. Acta*, **28**, 1164-1176 (1945).

(48) G. J. F. Chittenden and J. G. Buchanan, *Carbohydr. Res.*, **11**, 379-385 (1969).

(49) J. S. Brimacombe, A. B. Foster, M. Stacey, and D. H. Whiffen, *Tetrahedron*, **4**, 351-360 (1958).

the β -D-*galacto* derivative as being a result of a *cis* OH-3-O-4 hydrogen-bond. Unimolar benzylation of benzyl 4,6-*O*-benzylidene- β -D-galactopyranoside with benzoic anhydride afforded a mixture of the 2- and 3-*O*-benzoyl and 2,3-di-*O*-benzoyl derivatives,⁴⁸ further illustrating the difference in selectivity of acid anhydride and acid halide reagents.

Partial acetylation of methyl 4,6-*O*-benzylidene- α -D-mannopyranoside with 1.1 equivalents of acetic anhydride in pyridine, and separation of the reaction mixture on acid-treated silica gel to prevent acyl migration, gave the 2- and 3-*O*-acetyl and 2,3-di-*O*-acetyl derivatives together with starting material in molar ratios⁵⁰ of 1:5.3:3.5:1.2. Although it was claimed that no acyl migration occurred during the reaction, because samples of the pure products are stable in dry pyridine, the latter conditions do not replicate those of the reaction,^{50a} and isolation was preceded by the addition of water. Migration was found to be rapid in hydroxylic solvents containing base, and the ratios found may not therefore be kinetically controlled product-ratios. In a similar reaction in the β series, the 3-ester again preponderated. Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene- α -D-mannopyranoside has been prepared in 90% yield by treatment of the parent diol with benzoyl chloride in pyridine at low temperature.⁵¹

Esterification of several methyl 4,6-*O*-benzylidene-D-hexopyranosides with one molar equivalent of benzoyl chloride-triethylamine in an inert solvent was found to occur with good selectivity, mainly affording 2-benzoates from the α -D-*gluco*, α -D-*allo*, and α -D-*altro* compounds, and the 3-benzoate from the β -D-*gluco* compound.⁵²

1,6-Anhydro- β -D-glycopyranoses have proved to be useful compounds in selective-esterification studies, as they are rigid systems having predictable molecular geometries. Early work had shown the importance of steric factors in controlling reactivity. Thus, unimolar *p*-toluenesulfonylation of 1,6-anhydro-2-*O*-benzoyl- β -D-altrose⁵³

(50) P. J. Garegg, *Ark. Kemi*, **23**, 255-268 (1965).

(50a) It is most probably true, however, that acyl migration does not occur during the actual acetylation step, as *p*-toluenesulfonylation of the 2- and 3-acetates of methyl 4,6-*O*-benzylidene- α -D-mannopyranoside in pyridine proceeded without acetyl migration.⁵⁰ In addition, acetylation of partially acetylated derivatives of methyl α -³³ and β -D-glucopyranoside^{33a} with acetic anhydride-*d*₆, and investigation of the products by n.m.r. spectroscopy, gave no evidence for acetyl migration.

(51) F. R. Seymour, *Carbohydr. Res.*, **34**, 65-70 (1974).

(52) H. Hönig and H. Weidmann, *Carbohydr. Res.*, **39**, 374-378 (1975).

(53) F. H. Newth, *J. Chem. Soc.*, 441-447 (1956).

(HO-3 *eq*, HO-4 *ax*), and 1,6-anhydro- β -D-mannopyranose (HO-2 *eq*, HO-3 *ax*, HO-4 *ax*) and its 4-methyl ether,¹⁵ resulted in each case in favored sulfonylation at the equatorially oriented hydroxyl group. However, further studies have shown that the position occupied by the hydroxyl group on the ring can play an equal, or more important, part in controlling reactivity than its equatorial/axial nature, and the type of reaction is also important. Thus, partial benzylation of 2-acetamido-1,6-anhydro-2-deoxy- β -D-galactopyranose (HO-3 *ax*, HO-4 *eq*) with benzoyl chloride gave the 3- and 4-benzoates in the molar ratio of $\sim 4:3$, whereas methanesulfonyl chloride mainly yielded the 4-ester.⁵⁴ The claim that dimolar acetylation of 1,6-anhydro- β -D-galactopyranose (HO-2 *ax*, HO-3 *ax*, HO-4 *eq*) gave the 2,3-diacetate in 41% yield,⁵⁵ suggesting no enhanced reactivity of the equatorial hydroxyl group at C-4 over the axial hydroxyl groups at C-2 or C-3, has been questioned.^{55a} Isolation of the reaction products by chromatography on silica gel gave 2,3- and 2,4-diacetates in equal amounts, but either of these diacetates was found to undergo equilibration to form a 1:1 mixture of diacetates on contact with silica gel.^{55a} The danger of inferring relative reactivities of hydroxyl groups from yields of isolated products is thus apparent; n.m.r. determination of the relative proportions of the 2,3- and 2,4-diacetates in the acetylation mixture showed the latter compound to be the main diacetate produced in this reaction.

Considerable attention has been paid to selective reactivity of the hydroxyl groups in 1,6-anhydro- β -D-glucopyranose (HO-2 *ax*, HO-3 *ax*, HO-4 *ax*). Towards benzylation⁵⁶ and *p*-toluenesulfonylation^{56,57} with the respective acid chlorides, HO-2 and HO-4 showed enhanced reactivity over HO-3, and, with *p*-toluenesulfonyl chloride, the order of reactivity of the hydroxyl groups appears⁵⁷ to be $2 > 4 > 3$. It is noteworthy that benzyl chloroformate (benzyloxycarbonyl chloride) gives a higher yield of the 2,4-diester (74%) than benzoyl chloride (44%) under similar reaction-conditions⁵⁶; with the latter reagent, a considerable proportion of triester is also formed. The reaction of 2.5 molar equivalents of acetic anhydride in pyridine with 1,6-anhydro- β -D-glucopyranose shows little selectivity; the 2,4-,

(54) R. W. Jeanloz, *J. Am. Chem. Soc.*, **81**, 1956–1960 (1959).

(55) D. Shapiro, A. J. Acher, and E. S. Rachmann, *J. Org. Chem.*, **32**, 3767–3771 (1967).

(55a) M. E. Chacón-Fuertes and M. Martín-Lomas, *Carbohydr. Res.*, **42**, C4–C5 (1975).

(56) M. Cerny, V. Gut, and J. Pacák, *Collect. Czech. Chem. Commun.*, **26**, 2542–2550 (1961).

(57) R. W. Jeanloz, A. M. C. Rapin, and S. Hakomori, *J. Org. Chem.*, **26**, 3939–3946 (1961).

3,4-, and 2,3-diesters are formed⁵⁸ in the ratios 15:10:13. Significantly, *p*-toluenesulfonic anhydride shows the same selectivity as acid chlorides, giving the 2,4-di-*p*-toluenesulfonate in 79% yield.⁵⁹

An attempt has been made to relate selective esterification of the 1,6-anhydrohexopyranoses and their derivatives with the occurrence of intramolecular hydrogen-bonding in these compounds.⁵⁹ Reaction of 1,6-anhydro-3-deoxy- β -D-*xylo*-hexopyranose (HO-2 *ax*, HO-4 *eq*) with 3 molar equivalents of *p*-toluenesulfonyl chloride or 1.5 molar equivalents of benzoyl chloride in pyridine gave the 2-esters in yields of 70 and 63%, respectively. Infrared measurements in dilute solution showed that the starting diol exhibits hydroxyl absorption bands at 3610 and 3590 cm^{-1} , suggesting the presence of an intramolecular hydrogen-bond (2-OH \cdots O-5) and a free hydroxyl group. Interestingly, addition of five molar equivalents of pyridine did not affect the spectrum. This experiment provides a further example of the favored esterification of a sterically hindered hydroxyl group involved in hydrogen bonding. On the basis of $\Delta\nu$ values, hydrogen-bonding patterns in *O*-substituted derivatives of 1,6-anhydro- β -D-glucopyranose suggest that intramolecular hydrogen-bonding involving HO-2 or HO-4 is stronger than that involving HO-3, even if one of the former pair of hydroxyl groups is esterified, and the favored formation of the 2,4-diesters has been rationalized on this basis.

A report on the acetylation of 2,7-anhydro- β -D-*altro*-heptulopyranose (sedoheptulosan) with acetic anhydride further illustrates the low reactivity of an equatorial hydroxyl group on a bridged, pyranoid ring; under controlled conditions, 21% of the 1,3,5-tri-*O*-acetyl derivative may be isolated.⁶⁰

An interesting observation was made by Ferrier and coworkers⁶¹ during the acylation of 2,4-boronic esters of methyl α - and β -D-xylopyranosides, in both of which the pyranoid ring must be in the ${}^1C_4(D)$ conformation, and the 3-hydroxyl group must be axially attached. On treatment with benzoyl chloride in pyridine, the α -D-xyloside derivative gave 37% of the 3-benzoate and 19% of unreacted starting-material, but the β -D-xyloside derivative yielded

(58) D. Shapiro, Y. Rabinsohn, A. J. Acher, and A. Diver-Haber, *J. Org. Chem.*, **35**, 1464-1467 (1970).

(59) J. M. Macleod, L. R. Schroeder, and P. A. Seib, *Carbohydr. Res.*, **30**, 337-347 (1973).

(60) E. Zissis, *J. Org. Chem.*, **32**, 660-664 (1967).

(61) R. J. Ferrier, D. Prasad, A. Rudowski, and I. Sangster, *J. Chem. Soc.*, 3330-3334 (1964).

the 3-benzoate in 60% yield. Only in the β -D-xyloside derivative can HO-3 be intramolecularly hydrogen-bonded to the methoxyl group on C-1, and i.r. spectroscopy confirmed that the β anomer possesses a bonded hydroxyl group (3512 cm^{-1}), whereas the α anomer exhibits absorption for free hydroxyl groups only (3623 cm^{-1}).

The selective esterification of hydroxyl groups in glycopyranoses and simple glycopyranosides is of increasing interest in view of the synthetic utility of partially substituted derivatives of sugars. The most reactive of the secondary hydroxyl groups in an aldose is generally that forming part of the hemiacetal function, possibly as a result of the greater acidity of this hydroxyl group, and favored acylation at this site may be achieved.⁶² In the pentopyranoside series, selective acylation and sulfonylation of *arabino* and *xylo* stereoisomers have been studied. Treatment of benzyl⁶³ or methyl β -L-arabinopyranoside⁶⁴ with two molar equivalents of benzoyl chloride in pyridine affords the 2,3-diesters in yields of 65 and 66%, respectively. Predictably, unimolar *p*-toluenesulfonylation of methyl 2-O-benzoyl- β -L-arabinopyranoside gave the 3-*p*-toluenesulfonate,⁶⁵ and monobenzoylation of methyl 2-O-methyl- β -L-arabinopyranoside afforded the 3-benzoate, which, through methylation and hydrolysis, led to a convenient synthesis of 2,4-di-O-methyl-L-arabinose.⁶⁶ Unimolar benzoylation of benzyl α -D-xylopyranoside gave the 2-benzoate in 59% yield,⁶³ and dimolar benzoylation yielded the 2,4-diester (45%), 2,3-diester (27%), 2,3,4-triester (15%), and 2-ester (9%). No isomerization of benzyl 2,4-di-O-benzoyl- α -D-xylopyranoside to the 2,3-di-O-benzoyl compound was observed in pyridine containing pyridine hydrochloride, suggesting that the product distribution observed is a result of kinetic, not thermodynamic, control. As might be expected, reaction of methyl α -D-xylopyranoside with two molar equivalents of benzoyl chloride⁶³ or methanesulfonyl chloride⁶⁷ gave the 2,4-diesters as the major products, and unimolar methanesulfonylation⁶⁷ or *p*-toluenesulfonylation⁶⁸ afforded the 2-esters as the major, and 4-esters as the minor, products.

Analysis of the relative yields of products in these reactions suggests that the order of reactivity towards acid halides in pyridine

(62) M. Dejter-Juszynski and H. M. Flowers, *Carbohydr. Res.*, **28**, 61-74 (1973).

(63) T. Sivakumaran and J. K. N. Jones, *Can. J. Chem.*, **45**, 2493-2500 (1967).

(64) P. Kováč, *Carbohydr. Res.*, **20**, 418-420 (1971).

(65) E. J. Reist, L. V. Fisher, and D. E. Gueffroy, *J. Org. Chem.*, **31**, 226-229 (1966).

(66) P. Kováč and R. Palovčík, *Carbohydr. Res.*, **36**, 379-384 (1974).

(67) R. C. Chalk and D. H. Ball, *Carbohydr. Res.*, **28**, 313-325 (1973).

(68) J. G. Buchanan and R. Fletcher, *J. Chem. Soc., C*, 1926-1931 (1966).

is HO-2 > HO-4 > HO-3. This order is in agreement with reasoning advanced to explain results of selective esterification of methyl α -D-glucopyranoside (see later). The 2-hydroxyl group is the most reactive, as it is *cis* to the 1-alkoxyl substituent, and, in the 2-benzoate of a D-xylopyranoside, the 3-hydroxyl group suffers *gauche* interactions with a benzyloxy group and a hydroxyl group, whereas the 4-hydroxyl group is adjacent to a hydroxyl group and hydrogen atoms, and is less sterically hindered. Selective methanesulfonylation of methyl β -D-xylopyranoside⁶⁷ with 2 equivalents of the acid chloride, and chromatographic fractionation of the products, gave the tris(methanesulfonate) (6%), a difficultly resolvable mixture of bis(methanesulfonates), and the 4-methanesulfonate (10%); the last compound could be obtained in 38% yield by unimolar methanesulfonylation of the xyloside. Fractionation of the diester portion gave 44% of a compound judged by n.m.r. spectroscopy to be the 3,4-bis(methanesulfonate). The order of reactivity of hydroxyl groups therefore appears to be HO-4 > HO-3 > HO-2. Thus, the reactivity of HO-2 (which is *trans* to the methoxyl substituent on C-1) falls below that of HO-4, which, on steric considerations, appears the least hindered of the three hydroxyl groups.

Evidence of strong selectivity for reaction with acid chlorides at HO-2 in the α -D-xylopyranoside series was obtained on treatment of methyl 3-azido-3-deoxy- α -D-xylopyranoside with one molar equivalent of benzoyl chloride in pyridine; the 2-benzoate was isolated in 70% yield. The β anomer gave low yields of 2- and 4-esters on similar reaction.⁶⁹ The ratio of 2- to 3-acetates produced on reaction of benzyl 4-O-methyl- β -D-xylopyranoside with different acetylating reagents differs considerably.⁷⁰ With acetic anhydride-sodium acetate, the 2-hydroxyl group is the more reactive (ratio of 2- to 3-acetate, $\sim 2:1$), whereas the 3-hydroxyl group is the more reactive with acetic anhydride-perchloric acid (2- to 3-acetate, $\sim 1:3$). Acetic anhydride in pyridine, acetyl chloride in pyridine, and acetic anhydride in pyridine containing pyridine hydrochloride, all gave product ratios lying between these extremes.

On unimolar benzylation with the acid chloride in pyridine, methyl α -D-xylopyranoside gave the 3-benzoate in 46% yield,⁷¹ a result that might have been expected in view of the course of selec-

(69) T. Tsuchiya, K. Suo, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **43**, 531-537 (1970).

(70) P. J. Garegg, *Acta Chem. Scand.*, **16**, 1849-1857 (1962).

(71) S. A. Abbas, A. H. Haines, and A. G. Wells, *Carbohydr. Res.*, **42**, 362-364 (1975).

tive esterification of the configurationally related methyl α -D-mannopyranoside (see later).

Although there were early reports on the selective acylation^{72,73} and *p*-toluenesulfonylation⁷³ of methyl α -D-glucopyranoside, and the *p*-toluenesulfonylation of methyl α -D-galactopyranoside,⁷⁴ to yield 2,6-diester derivatives, further investigations of favored reactions of hexopyranosides were not undertaken until the 1960's. Methyl 2,6-di-*O*-(methylsulfonyl)- α -D-glucopyranoside (prepared in 51% yield by dimolar methanesulfonylation of methyl α -D-glucopyranoside⁷⁵) was found to be a convenient starting-material for the synthesis of 3,4-di- and 3,4,6-tri-*O*-methyl-D-glucose. A chromatographic investigation⁷⁶ of the products of unimolar methanesulfonylation of the methyl α -D-glucopyranoside showed, as expected, that the primary hydroxyl group is the most reactive, followed by the 2-hydroxyl group.

The results of a study of the di- and tri-molar methanesulfonylation of methyl α -D-galactopyranoside suggested, amongst the secondary hydroxyl groups, a reactivity order of HO-2 > HO-3 > HO-4, and, from the latter reaction, the 2,3,6-triester could be isolated in 30% yield; a similar reaction of methyl α -D-mannopyranoside gave the 2,3,6-triester in 41% yield, suggesting that the 4-hydroxyl group is the least reactive.⁷⁶ The difference in reactivity between the axial 2-hydroxyl and the equatorial 3-hydroxyl group in the *manno* series is illustrated by the results of treatment of methyl 6-*O*-trityl- α -D-mannopyranoside with one molar equivalent of *p*-toluenesulfonyl chloride; this afforded the 3-ester (in 51% yield) as the major mono-*p*-toluenesulfonate.⁷⁷ Dimolar methanesulfonylation of methyl β -D-glucopyranoside was much less selective than that of the α anomer, and the 4,6-di-*O*-(methylsulfonyl) derivative was the major product, in 13% yield.⁷⁶ A similar reaction with methyl β -D-galactopyranoside illustrated the resistance to esterification at the (axial) 4-hydroxyl group; the 3,6-diester was obtained in 26% yield.⁷⁶ Significantly, the 2-hydroxyl group in both of these β -D-glycosides appeared to be the least reactive.

Selective acylation of hexopyranosides under kinetic control has

(72) T. Lieser and R. Schweizer, *Ann.*, **519**, 271-278 (1935).

(73) J. Asselineau, *Bull. Soc. Chim. Fr.*, 937-944 (1955).

(74) P. A. Rao and F. Smith, *J. Chem. Soc.*, 229-232 (1944).

(75) A. K. Mitra, D. H. Ball, and L. Long, Jr., *J. Org. Chem.*, **27**, 160-162 (1962).

(76) R. C. Chalk, D. H. Ball, and L. Long, Jr., *J. Org. Chem.*, **31**, 1509-1514 (1966).

(77) V. L. N. Murty and I. R. Siddiqui, *Carbohydr. Res.*, **10**, 477-480 (1969).

been studied by using benzoyl chloride in pyridine. From tribenzoylation of methyl α -D-glucopyranoside,³¹ methyl α -D-mannopyranoside,³¹ and methyl α -D-galactopyranoside^{31,78} were obtained 2,3,6-tri-O-benzoyl derivatives in yields of over 50%, the same substitution pattern as that observed in the sulfonylation of the D-glucose and D-mannopyranoside. A study of dibenzoylation reactions suggested³¹ that the reactivity order for secondary hydroxyl groups is different for each pyranoside, being HO-2 > HO-3 > HO-4 for the D-glucoside, HO-3 > HO-2 > HO-4 for the D-mannoside, and HO-2, HO-3 > HO-4 for the D-galactoside. Because of low selectivity, the order of reactivity of HO-2 and HO-3 in the D-galactoside is uncertain, but the results of sulfonylation reactions suggested⁷⁶ HO-2 > HO-3. Dibenzoylation of methyl 6-deoxy- α -L-galactopyranoside,⁷⁹ methyl 6-deoxy- α -L-mannopyranoside,⁷⁹ and methyl 6-deoxy- α -D-glucopyranoside⁸⁰ led to good yields of the 2,3-di-O-benzoyl derivatives; the 4-hydroxyl group was, again, found to be the least reactive in this series.

The low reactivity of the 4-hydroxyl groups in α -galactopyranosides is understandable in view of their axial disposition, but, in the α -glucopyranosides and α -mannopyranosides, it may be attributable to steric hindrance through *gauche* interactions with HO-3 and a benzoyloxymethyl or methyl group at O-5 (acylation at the primary hydroxyl group being assumed to occur first). The 3-hydroxyl group suffers smaller steric interactions, with two *gauche*-disposed hydroxyl groups; or, assuming that HO-2 is esterified first, with a hydroxyl and a benzoyloxy group. The greater reactivity of HO-2 over HO-3 and HO-4 in the α -glucopyranosides has long been recognized,¹ and, although its origin is not yet clear, it would seem not to be due to the inductive effect of the anomeric center.³¹ However, as with other α -glucopyranoside derivatives,¹⁷ the presence of the *cis*-HO-2-MeO-1 grouping, favoring intramolecular hydrogen-bonding, may be correlated with the enhanced reactivity at O-2. In the α -mannopyranosides, any activation of HO-2 produced by its proximity to the anomeric center is apparently counteracted by its sterically unfavorable, axial orientation.

The rationalization just presented highlights an important point. For methyl α -D-glucopyranoside, deductions based on the analysis of yields of products probably lead to a measure of the relative reac-

(78) E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker, and L. Goodman, *J. Org. Chem.*, **30**, 2312-2317 (1965).

(79) A. C. Richardson and J. M. Williams, *Tetrahedron*, **23**, 1641-1646 (1967).

(80) Y. Kondo, K. Miyahara, and N. Kashimura, *Can. J. Chem.*, **51**, 3272-3276 (1973).

tivity of HO-2 compared to those of HO-3 and HO-4 in methyl α -D-glucopyranoside or methyl 6-O-benzoyl- α -D-glucopyranoside, or both, and the relative reactivity of HO-3 and HO-4 in methyl 2,6-di-O-benzoyl- α -D-glucopyranoside. A full, quantitative appreciation of relative reactivity requires a knowledge of the rate constants for reaction at a particular hydroxyl group for *each particular substitution* pattern existing elsewhere in the molecule. Only by carrying out a reaction to a d.s. low enough to ensure that only monosubstituted derivatives are formed may the relative reactivities of hydroxyl groups in a parent compound be truly determined from product distributions, and, under these conditions, accurate product analysis may prove difficult. A method has been described that uses standard, kinetic equations to evaluate ratios of the four rate-constants characterizing the reaction of a diol with an acylating or alkylating agent in an irreversible reaction.⁸¹ Analysis of the reaction of methyl 3-acetamido-3,6-dideoxy- β -D-glucopyranoside with acetyl chloride in pyridine showed that the ratio of second-order rate-constants, k_2/k_4 , for reaction at HO-2 and HO-4 (k_2 and k_4 , respectively) in the diol was 1.2:1; however, the ratio of the rate constants for the reaction of HO-2 in methyl 3-acetamido-4-O-acetyl-3,6-dideoxy- β -D-glucopyranoside and of HO-4 in the corresponding 2-O-acetyl derivative (k'_2 and k'_4 , respectively) was 3:1. Thus, there is a significant difference between the reactivity of a particular hydroxyl group in the diol and in its monoacetate; in this example, the enhancement of reactivity due to substitution in the β position is higher for HO-2 than for HO-4.

A similar kinetic approach, used to re-investigate the hydroxyl reactivities in methyl 4,6-O-benzylidene- α -D-glucopyranoside with acetic anhydride in pyridine,⁸² showed that the conclusion based on product distribution³² (namely, that HO-3 is more reactive than HO-2 in the initial stages of the reaction) is incorrect. A full analysis of the product ratios throughout the reaction led to the conclusion that accumulation of 3-acetate is caused by a marked decrease in the reactivity of HO-2 in the resulting methyl 3-O-acetyl-4,6-O-benzylidene- α -D-glucopyranoside compared to that of HO-3 in the corresponding 2-acetate, and that, in the "diol," the reactivities of the two hydroxyl groups are very similar.

An alternative method of quantifying reactivity in carbohydrates involves measurement of the d.s. at each hydroxyl group on treatment with a reagent. In an elegant approach to the problem, the

(81) J. Staněk, P. Chuchvalec, K. Čapek, K. Kefurt, and J. Jarý, *Carbohydr. Res.*, **36**, 273-282 (1974).

(82) J. Lehrfeld, *Carbohydr. Res.*, **39**, 364-367 (1975).

acetyl signals in the n.m.r. spectrum of methyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside were identified by means of specifically deuterated derivatives in which each of the acetyl groups was, in turn, replaced by a trideuterioacetyl group.³³ Methyl α -D-glucopyranoside was then treated with one molar equivalent of acetic anhydride in pyridine, and the mixed products were fully esterified by means of acetic anhydride-*d*₆ in pyridine. As it was ascertained that acetyl exchange does not occur under the conditions of acylation used, measurement of the relative peak-intensities for acetyl groups permitted determination of the degrees of substitution as C-2:C-3:C-4:C-6 = 0.2:0.2:0.2:0.4. A similar investigation on methyl β -D-glucopyranoside^{33a,82a} showed the relative distribution of acetyl groups to be O-2:O-3:O-4:O-6 = 0.19:0.14:0.17:0.50.

Many reports on selective esterification of other pyranoside derivatives have been made, and, in most instances, the patterns of reactivity observed are those that would be predicted from a consideration of the work already noted. Ethoxycarbonylation of methyl 6-*O*-methyl- α -D-mannopyranoside,⁸³ benzylation of methyl 2-benzamido-2-deoxy- α -D-glucopyranoside⁸⁴ and of methyl (methyl α -D-galactopyranosid)uronate,⁸⁵ and dimolar *p*-toluenesulfonylation of methyl 2-deoxy- α -D-*lyxo*-hexopyranoside⁸⁶ yielded, as the major products, derivatives unsubstituted at HO-4. Similarly, acetylation (and methanesulfonylation) of methyl 3,6-dideoxy- α -D-*xyl*o-hexopyranoside⁸⁷; acetylation,⁸⁸ methanesulfonylation,⁸⁸ *p*-toluenesulfonylation,⁸⁹ and benzylation⁸⁹ of methyl 3-acetamido-3,6-dideoxy- α -L-glucopyranoside; acetylation of methyl 3-acetamido-3,6-dideoxy- α -L-idopyranoside⁹⁰; acetylation of methyl 3-acetamido-3,6-dideoxy- α -D-gulopyranoside and methyl 3-acetamido-3,6-dideoxy- α -L-

(82a) D. Horton and J. H. Lauterbach, *Abstr. Pap. Am. Chem. Soc. Meet.*, **160**, CARB 7 (1970).

(83) E. G. Gros and E. M. Gruñeiro, *Carbohydr. Res.*, **23**, 148–151 (1972).

(84) M. W. Horner, L. Hough, and A. C. Richardson, *J. Chem. Soc., C*, 1336–1340 (1970).

(85) P. L. Gill, M. W. Horner, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, **17**, 213–215 (1971).

(86) J. S. Brimacombe and D. Portsmouth, *Carbohydr. Res.*, **1**, 128–136 (1965).

(87) K. Čapek, J. Čapková-Šteffková, and J. Jary, *Collect. Czech. Chem. Commun.*, **35**, 321–326 (1970).

(88) K. Čapek, J. Šteffková, and J. Jary, *Collect. Czech. Chem. Commun.*, **31**, 1854–1863 (1966).

(89) A. C. Richardson, *Carbohydr. Res.*, **4**, 415–421 (1967).

(90) K. Čapek, J. Šteffková, and J. Jary, *Collect. Czech. Chem. Commun.*, **33**, 781–787 (1968).

allopyranoside⁹¹; and methanesulfonylation (and acetylation) of methyl 3-acetamido-3,6-dideoxy- α -D-mannopyranoside,⁹² all with one molar proportion of the acid chloride, mainly yield the corresponding 2-esters. The same selectivity is not necessarily obtained when the acid anhydride in pyridine is used as the reagent.^{87,88,90}

The course of acid-catalyzed acetylations with acetic anhydride may depend markedly on the concentration of the acid and the type of acid. Reaction of 7-(3-deoxy-3-nitro- β -D-galactopyranosyl)theophylline with acetic anhydride in the presence of one molar equivalent of perchloric acid gave the 2',4',6'-triacetate in 89% yield, but, in the presence of only a trace of the acid, 82% of the 4',6'-diacetate was obtained.⁹³ Treatment of the *manno* isomer of the nucleoside with acetic anhydride-boron trifluoride gave the 4',6'-diacetate as a crystalline product (24%), whereas phosphoric acid as the catalyst yielded the 2',4',6'-triester (45%).

c. Furanoid Derivatives of Monosaccharides.—Because of the growing interest in nucleosides over the past thirty years, stemming initially from structural studies, and latterly from their chemical modification for biological testing, selective reactivity of the hydroxyl groups on the furanoid ring has received considerable attention. Favored esterification at the primary center can readily be achieved in 2-deoxy-D-*erythro*-pentofuranose derivatives, and new reagents for achieving this purpose have already been mentioned, but there are fewer reports of similar reactions of D-ribonucleosides.

The monoacylation of 5'-O-acylated ribonucleosides leads mainly to 3',5'-diesters, as for example, in the acylation of 5'-O-acetyladenosine,⁹⁴ 5'-O-acetyluridine,⁹⁵ or N⁶-benzoyl-5'-O-benzoylcytidine.⁹⁶ In contrast, unimolar sulfonylation favors formation of 2',5'-diesters, as in the *p*-toluenesulfonylation of 5'-O-acetyluridine^{95,97} and 5'-O-acetyladenosine.⁹⁸ It is pertinent that

(91) K. Čapek, J. Šteffková, and J. Jarý, *Collect. Czech. Chem. Commun.*, **35**, 107–115 (1970).

(92) K. Čapek, J. Šteffková, and J. Jarý, *Collect. Czech. Chem. Commun.*, **33**, 1750–1757 (1968).

(93) T. Nakagawa and T. Takamoto, *Bull. Chem. Soc. Jpn.*, **44**, 192–196 (1971).

(94) D. M. Brown, G. D. Fasman, D. J. Magrath, and A. R. Todd, *J. Chem. Soc.*, 1448–1455 (1954).

(95) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388–2393 (1956).

(96) D. H. Rammner and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 3112–3122 (1962).

(97) D. M. Brown, D. B. Parihar, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 3028–3035 (1958).

(98) A. R. Todd and T. L. V. Ulbricht, *J. Chem. Soc.*, 3275–3277 (1960).

benzoylation⁹⁹ and *p*-toluenesulfonylation¹⁰⁰ of 1-(5-*O*-benzoyl- β -D-lyxofuranosyl)uracil, which also contains a *cis*-2',3'-diol grouping, are remarkably selective for O-2'; the 2',5'-dibenzoate was isolated in 92% yield without recourse to chromatography. It was suggested that the formation of 3', 5'-diesters on acylation of ribonucleosides is a result of a facile, acyl migration leading to the thermodynamically more-stable 3',5'-diester under the processing conditions, rather than of a marked specificity¹⁰¹ for O-3'. Indeed, the greater nucleophilicity of HO-2' might be expected, in view of the enhanced reactivity it shows compared to HO-3' in alkylation reactions (see Section III).

The site of favored acylation in the *cis*-2',3'-diol system of nucleosides is of considerable interest, because amino acids are carried to the site of protein biosynthesis as¹⁰² the 2'- or 3'-(aminoacyl) derivative of the terminal adenosine residue of transfer ribonucleic acid (tRNA). From the partial acetylation of 5'-*O*-acetyluridine in pyridine with one molar equivalent of acetic anhydride during 30 min at 20°, 31% of an ~2:1 mixture of 2',5'- and 3',5'-di-*O*-acetyluridine was isolated.¹⁰³ Similar results were obtained on monoacetylation of 5'-*O*-trityladenosine and 5'-*O*-acetyladenosine. When these mixtures, produced under kinetic control, were refluxed in dry pyridine for one hour, ~1:3 mixtures of the 2'- and 3'-acetate were formed, representing the equilibrium proportions of these esters. Crystallization of the 2:1 or 3:1 mixtures of di-*O*-acetyluridines from absolute ethanol yielded pure 3',5'-diester in >70% yield. Favored acetylation at HO-2' was also observed with 1- β -D-xylofuranosyluracil and 1- β -D-lyxofuranosyluracil; interestingly, the 5'-ester was not the major product from either of these compounds.

Certain observations suggest that, towards certain acylating agents, the *cis*-2',3'-diol grouping in ribonucleosides may exhibit a greater reactivity than the primary hydroxyl group. From the reaction of thymidine with one molar equivalent of (*p*-nitrophenoxy)carbonyl chloride was isolated the 5'-ester in 33% yield, whereas uridine gave 72% of the 2',3'-cyclic carbonate with one molar equivalent of the reagent.¹⁰⁴ Another example involves the acylating agent 13, which, it is claimed, reacts with uridine in pyridine at 90° to yield 3'-*O*-

(99) T. Sasaki, K. Minamoto, and K. Hattori, *Tetrahedron*, **30**, 2689-2694 (1974).

(100) T. Sasaki, K. Minamoto, and K. Hattori, *J. Org. Chem.*, **38**, 1283-1286 (1973).

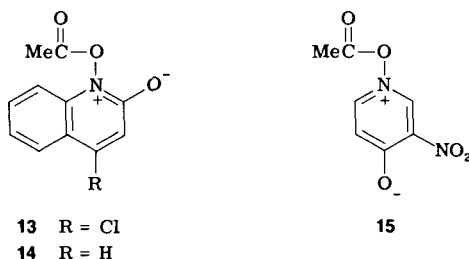
(101) A. M. Michelson, "Chemistry of the Nucleotides," Academic Press, London, 1963, p. 23.

(102) B. E. Griffin and C. B. Reese, *Proc. Natl. Acad. Sci. U.S.A.*, **51**, 440-444 (1961).

(103) G. A. R. Johnston, *Tetrahedron*, **24**, 6987-6993 (1968).

(104) R. L. Letsinger and K. K. Ogilvie, *J. Org. Chem.*, **32**, 296-300 (1967).

acetyluridine in 67% yield after chromatography¹⁰⁵; even with a large excess of **13**, the 3'-ester was the main product. Inosine gave 2'-(3')-acetylinosine in 33% yield. However, thymidine and 1- β -D-xylofuranosyluracil gave low yields of the 5'-acetate, and competitive acylation of an equimolar mixture of cyclohexanol and *cis*-1,2-cyclohexanediol with 2 molar proportions of **13** afforded a 1:6 molar mixture of the corresponding *mono*-O-acetyl derivatives, with the diol monacetate preponderating. Compound **14** also possesses the same high preference for reaction at a *cis*-diol grouping, but the related compound **15** acetylates uridine nonselectively. A tendency



for reaction at a vicinal-diol grouping is apparently shown by benzoic anhydride, which reacts with adenosine, but not 2'-deoxyadenosine, in aqueous solution, to give a mixture of mono- and di-O-benzoyl derivatives, the former consisting of a mixture of the 2'- and 3'-esters.¹⁰⁶

Product distributions obtained on esterification of nucleosides and nucleotides under basic conditions throw further light on factors affecting selective reactivity. *p*-Toluenesulfonylation of adenosine 5'-monophosphate in aqueous alkali yielded exclusively (in 54–61% yield) the 2'-*p*-toluenesulfonate.¹⁰⁷ Lack of reaction at HO-3' was attributed either to formation of a phosphoric *p*-toluenesulfonic anhydride, which sterically protected this hydroxyl group, or to the higher acidity of HO-2'. It has been shown that the acidic site (with pK_a 12.5) in adenosine is associated with the presence of both HO-2' and HO-3', as replacement of either of these by hydrogen, or of HO-2' by methoxyl, results in loss of this acidity.¹⁰⁸ Inductive effects, or the sta-

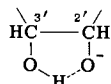
(105) Y. Mizuno, T. Itoh, and H. Tagawa, *Chem. Ind. (London)*, 1498–1499 (1965).

(106) L. N. Nikolenko, W. N. Nesawibatko, A. F. Usatyi, and M. N. Semjenowa, *Tetrahedron Lett.*, 5193–5195 (1970).

(107) M. Ikehara and S. Uesugi, *Tetrahedron Lett.*, 713–716 (1970).

(108) R. M. Izatt, L. D. Hansen, J. H. Rytting, and J. J. Christensen, *J. Am. Chem. Soc.*, 87, 2760–2761 (1965).

bilization of the anion by intramolecular hydrogen-bonding, as in 16, were proposed as possible explanations of this acidic site.



16

The result of the sulfonylation¹⁰⁷ just discussed suggests that, were a similar, hydrogen-bonded anion formed from adenosine 5'-monophosphate, attack at O-2' would be sterically favored, or that the proton involved in the intramolecular hydrogen-bond is not symmetrically placed and is bonded more strongly to O-3'. The effect of the bulk of a substituent at O-5' on the course of sulfonylation was examined through reaction of 8-bromoadenosine and its 5'-O-acetyl, 5'-O-benzoyl, and 5'-O-trityl derivatives with sodium hydride-triisopropylbenzenesulfonyl chloride in *N,N*-dimethylformamide.¹⁰⁹ In each instance, the 2'- and 3'-sulfonates were the main products; and, as the bulkiness of the 5'-substituent increased (acetyl < benzoyl < trityl), the ratio of 2':3'-ester increased (1.01:1, 1.33:1, and 2.84:1). Notably, even with 8-bromoadenosine, the 5'-ester was formed in only 18% yield, the main products being the 2'- and 3'-ester in yields of 38 and 44%, respectively.

The significant difference in reactivity sometimes observed when a hydroxyl group reacts in its un-ionized and ionized forms is nicely illustrated by the results of unimolar *p*-toluenesulfonylation of 9-(5-deoxy- β -D-xylofuranosyl)adenine.¹¹⁰ Reaction in pyridine with *p*-toluenesulfonyl chloride gave the 3'-*p*-toluenesulfonate, a result unexpected in view of the generally greater reactivity at HO-2' of nucleosides under these conditions, and of the steric hindrance supposedly suffered by HO-3'. Intramolecular hydrogen-bonding between HO-3' and N-3 of the purine ring could account for this reactivity pattern, and it was conjectured that formation of the dialkoxide would remove the possibility of hydrogen bonding, and thus, the cause of enhanced reactivity at HO-3' (compared to HO-2'). Under the latter conditions, steric factors predominated, and the 2'-*p*-toluenesulfonate was formed, as shown by the isolation of 9-(2,3-anhydro-5-deoxy- β -D-lyxofuranosyl)adenine resulting from internal displacement of the *p*-tolylsulfonyloxy group at C-2' by the anionic oxygen atom on C-3'.

(109) M. Ikehara and M. Kaneko, *Tetrahedron*, **26**, 4251-4259 (1970).

(110) E. J. Reist, V. J. Bartuska, D. F. Calkins, and L. Goodman, *J. Org. Chem.*, **30**, 3401-3403 (1965).

Esterification of simple, furanoid derivatives has illustrated the dependence of the reactivity of secondary hydroxyl groups upon the configurations at neighboring centers. Thus, although selective esterification of 1,2-*O*-isopropylidene- α -D-xylofuranose is readily achieved¹¹¹ at HO-5, benzoylation of 1,2-*O*-isopropylidene- α -D-ribofuranose¹¹² showed little regioselectivity. Because the secondary hydroxyl group is *exo* to the *cis*-fused, furanose-dioxolane ring-system in the D-xylofuranose derivative, and *endo* in the D-ribofuranose derivative, the pattern of reactivity observed is the opposite of that which might be predicted on steric reasoning. It is noteworthy that only in the D-ribofuranose derivative is HO-3 suitably situated to form an intramolecular hydrogen-bond to O-2. A further example of high selectivity is the *p*-toluenesulfonylation of methyl 6-*O*-trityl- α -D-mannofuranoside to yield 84% (based on the diol utilized) of the 2-*p*-toluenesulfonate.¹¹³ Methyl 6-*O*-trityl- β -D-galactofuranoside gave,¹¹⁴ in contrast, the 2-, 3-, and 5-esters and the 2,5-diester in the molar ratios of 6:5:9:8. The reactivity of the D-mannofuranoside derivative, which possesses a *cis*-2,3-diol grouping, thus resembles that of the D-ribonucleosides, but the D-galactofuranoside derivative, having a *trans*-2,3-diol grouping, does not show greater reactivity at HO-2.

d. Oligosaccharides and Polysaccharides.—The ready availability of many disaccharides, and the possibility of transforming them into compounds having commercial potentialities, has led to an interest in their selective reactions. Selective esterification of their primary hydroxyl groups may generally be performed; and, where these groups occupy nonidentical environments, significant differences in reactivity are frequently observed. Yields are often less than with monosaccharides. On treatment with 1.5 molar equivalents of methanesulfonyl chloride, trehalose afforded the 6-*O*- and 6,6'-di-*O*-(methylsulfonyl) derivatives in yields of 2.6 and 8%, respectively (after carbon-column chromatography). On treatment with (the bulkier) *p*-toluenesulfonyl chloride (4.7 molar equivalents), the 6,6'-diester was obtained (as its hexaacetate, in 39% yield¹¹⁵). From methyl β -maltoside, the 6,6'-bis(*p*-toluenesulfonate) and the 6- and 6'-*p*-toluenesulfonates were obtained in yields of 28, 1, and 18%, respec-

(111) C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5247–5252 (1958).

(112) N. A. Hughes and P. R. H. Speakman, *Carbohydr. Res.*, **1**, 341–347 (1966).

(113) I. R. Siddiqui and V. L. N. Murty, *Carbohydr. Res.*, **8**, 477–481 (1968).

(114) I. R. Siddiqui and B. Urbas, *Carbohydr. Res.*, **5**, 210–217 (1967).

(115) G. Birch and A. C. Richardson, *Carbohydr. Res.*, **8**, 411–415 (1968).

tively, on treatment with 1.1 molar equivalents of the acid chloride¹¹⁶; Wolfrom and coworkers synthesized the 6,6'-diester in 50% yield.¹¹⁷ Maltose has also been converted into the 6,6'-bis(*p*-toluenesulfonate), isolated in 45% yield as its hexaacetate.¹¹⁸

The trimolar *p*-toluenesulfonylation of sucrose has been re-investigated¹¹⁹ in view of a report¹²⁰ that reopened the question of the structure of a tri-*O-p*-tolylsulfonylsucrose previously designated as the 6,1',6'-triester.¹²¹ 6,1',6'-Tri-*O-p*-tolylsulfonylsucrose was obtained in 23% yield from the reaction mixture, after chromatography, as an amorphous powder which was converted in high yield (89%) into a crystalline pentabenzoate, also obtained by an independent route. Both the 6,1',6'-tris-(*p*-toluenesulfonate) and the 2,3,4,3',4'-penta-*O*-benzoyl-6,1',6'-tri-*O-p*-tolylsulfonylsucrose gave 3,6:1',4':3',6'-trianhydrosucrose on reaction with methanolic sodium methoxide, in contrast to the earlier report¹²¹ that a tri-*O-p*-tolylsulfonyl derivative of sucrose, obtained from the sucrose-*p*-toluenesulfonyl chloride reaction and thought to be mainly the 6,1',6'-triester, yielded 2,1':3,6:3',6'-trianhydrosucrose on treatment with sodium methoxide in methanol. It appears that the latter anhydride, if correctly identified, must have arisen not from the 6,1',6'-triester but from an as-yet-unknown tri-*O-p*-tolylsulfonyl derivative of sucrose, such as the 2,6,6'-triester.

A remarkable inertness towards acylation is shown by the secondary hydroxyl group on C-3 in maltose, lactose, and their methyl β -glycosides. Benzoylation of maltose with 10 molar equivalents of the acid chloride in pyridine gave¹²² the octabenzoate and the 1,2,6,2',3',4',6'-hepta-*O*-benzoyl derivative in the ratio of 5:6, and treatment of β -maltose monohydrate with 8.8 molar equivalents of acetyl chloride in pyridine-toluene at 0° gave¹²³ the 1,2,6,2',3',4',6'-heptaacetate and the octaacetate in the ratio of 27:10. Under similar conditions of benzoylation, cellobiose was converted into its oc-

(116) R. T. Sleeter and H. B. Sinclair, *J. Org. Chem.*, **35**, 3804-3807 (1970).

(117) M. L. Wolfrom, Y.-L. Hung, P. Chakravarty, G. U. Yuen, and D. Horton, *J. Org. Chem.*, **31**, 2227-2232 (1966).

(118) S. Umezawa, T. Tsuchiya, S. Nakada, and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **40**, 395-401 (1967).

(119) R. Khan, *Carbohydr. Res.*, **22**, 441-445 (1972); *Adv. Carbohydr. Chem. Biochem.*, **33**, 235-294 (1976).

(120) N. W. Isaacs, C. H. L. Kennard, G. W. O'Donnel, and G. N. Richards, *Chem. Commun.*, 360 (1970).

(121) R. U. Lemieux and J. P. Barrette, *Can. J. Chem.*, **38**, 656-662 (1960).

(122) I. M. E. Thiel, J. O. Deferrari, and R. Cadenas, *Ann.*, **723**, 192-197 (1969).

(123) W. E. Dick, Jr., B. G. Baker, and J. E. Hodge, *Carbohydr. Res.*, **6**, 52-62 (1968).

tabenzoate in 98% yield,¹²⁴ and, under the acetylation conditions, D-glucose was fully acetylated.¹²³ Hydrogen bonding between HO-3 and the oxygen atom of HO-2' was suggested¹²² as being responsible for the low reactivity of the former group in maltose, but this explanation must be too simplistic, because, in other cases, a correlation has been found between intramolecular hydrogen-bonding and enhanced reactivity towards acid chlorides (see Section II,1,a). A strong OH-3 · · · O-2', intramolecular hydrogen-bond could, however, cause the molecule to fold in such a way that access to HO-3 would be severely hindered, and molecular models of the favored conformation of maltose¹²⁵ and its methyl β -glycoside¹²⁶ indeed show that HO-3 (along with HO-2' and HO-6) is subject to considerable steric interaction.¹²⁷

α -Lactose hydrate may be benzoylated under Schotten-Bauman conditions at 0° to yield the 1,2,6,2',3',4',6'-hepta-*O*-benzoyl derivative in 24% yield,¹²⁸ but benzoylation in pyridine at 60° gives the octabenzoate. Selective esterification of methyl β -lactoside with the latter reagent at low temperatures has, however, been reported¹²⁹; treatment with 2.2 molar proportions of benzoyl chloride gave a complex mixture, from which the 3',6'-dibenzoate was isolated in 31% yield, and 5 molar equivalents of the chloride gave the 2,6,2',3',6'- and 2,6,3',4',6'-pentabenzoates, the 2,6,3',6'-tetrabenzoate, and the 3,6,6'-tribenzoate. Use of 6.5 molar equivalents of acid chloride afforded 33% of the 2,6,2',3',4',6'-hexabenzoate. A consideration of these results led to a reactivity order for the hydroxyl groups of 6' > 3' > 6 > 2 > 2', 4' > 3. The selective tetra-*O*-acetylation of methyl 6,6'-di-*O*-*p*-tolylsulfonyl- β -maltoside to give the substituted glycoside having the 3-hydroxyl group free has also been briefly reported (see reference 13 in Ref. 127). From the foregoing examples, it appears that, in methyl glycosides of (1 \rightarrow 4)-linked disaccharides, hydroxyl groups adjacent to the junction of the two rings (that is HO-3, HO-2', and HO-6) react sluggishly towards acid halides.

(124) J. O. Deferrari, I. M. E. Thiel, and R. A. Cadenas, *J. Org. Chem.*, **30**, 3053-3055 (1965).

(125) G. J. Quigley, A. Sarko, and R. H. Marchessault, *J. Am. Chem. Soc.*, **92**, 5834-5839 (1970).

(126) S. S. C. Chu and G. A. Jeffrey, *Acta Crystallogr.*, **23**, 1038-1049 (1967).

(127) P. L. Durette, L. Hough, and A. C. Richardson, *J. Chem. Soc. Perkin Trans. I*, 97-101 (1974).

(128) I. M. Vazquez, I. M. E. Thiel, and J. O. Deferrari, *Carbohydr. Res.*, **26**, 351-356 (1973).

(129) R. S. Bhatt, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, **32**, C4-C6 (1974).

Studies on the favored esterification of other oligosaccharides and of polysaccharides have continued. Mono-¹³⁰ and hexa-¹³¹O-*p*-tolylsulfonyl derivatives of cyclohexaamylose in which primary hydroxyl groups are esterified have been prepared, and optimal conditions have been ascertained for the maximal discrimination between primary and secondary hydroxyl groups in amylose by *p*-toluenesulfonyl chloride.¹³² Reaction of amylose with 90% formic acid at room temperature affords derivatives containing only one formyl group per D-glucosyl residue, and ~70% of these occupy¹³³ the primary positions at O-6. A thorough, kinetic analysis of the reactivities of hydroxyl groups in the D-glucopyranosyl residues of amylopectin towards homogeneous formylation by 90% formic acid has been made.¹³⁴ As expected, the velocity coefficient and equilibrium constant for the esterification of primary hydroxyl groups are higher than those for secondary groups, and measurements suggest that one secondary hydroxyl group is esterified rapidly to a low level of equilibrium, whereas the other is slowly esterified to a higher level of equilibrium. Earlier studies, conducted under homogeneous conditions, on the acetylation of partially acetylated celluloses had indicated that conditions that accelerate the reaction (for example, the presence of pyridine, or acids) can drastically decrease the reactivity of the primary relative to the secondary hydroxyl groups.¹³⁵ Partial acetylation studies on an amylopectin having a dextrose equivalent of ~15 showed¹³⁶ that 40% of substitution occurred at O-6, with the rest at O-2 and O-3. The relative reactivities of the hydroxyl groups in the D-glucosyl residues of cellulose have been discussed previously in this Series.¹³⁶

e. Cyclitols.—Many of the selective esterifications reported in cyclitol chemistry are readily explicable in terms of the greater steric availability of an equatorially compared to an axially attached hydroxyl group, and some examples have already been noted.^{18–20} A study of the *p*-toluenesulfonylation of partially sulfonylated derivatives of *myo*-inositol led to the reactivity sequences HO-1 and

(130) L. D. Melton and K. N. Slessor, *Carbohydr. Res.*, **18**, 29–37 (1971).

(131) W. Lautsch, R. Wiechert, and H. Lehmann, *Kolloid-Z.*, **135**, 134–136 (1954).

(132) R. L. Whistler and S. Hirase, *J. Org. Chem.*, **26**, 4600–4605 (1961).

(133) R. L. Whistler and H. J. Roberts, *J. Am. Chem. Soc.*, **81**, 4427–4429 (1959).

(134) S. P. Rowland, M. A. F. Brannan, H. J. Janssen, and P. F. Pittman, *Carbohydr. Res.*, **3**, 361–368 (1967).

(135) C. J. Malm, L. J. Tanghe, B. C. Laird, and G. D. Smith, *J. Am. Chem. Soc.*, **75**, 80–84 (1953).

(136) D. M. Jones, *Adv. Carbohydr. Chem.*, **19**, 219–246 (1964).

HO-3 > HO-4, and HO-6 > HO-5 > HO-2, based on the yields of reaction products isolated.¹³⁷ Formation of 1,3-di-*O-p*-tolylsulfonyl-*myo*-inositol in 59% yield by reaction of DL-1-*O-p*-tolylsulfonyl-*myo*-inositol with 1.2 molar equivalents of the acid chloride is a remarkably selective reaction, as four equatorial hydroxyl groups are available for reaction, but it is notable that, of these four, only HO-3 possesses a vicinal, *cis*, oxygen atom. Selective esterification has often been observed for cyclic acetal derivatives of the inositols, and several examples are known wherein a hydroxyl group that is *cis* to a vicinal oxygen atom shows enhanced reactivity over other hydroxyl groups in the molecule. Thus, on *p*-toluenesulfonylation¹³⁸ or benzylation¹³⁹ by the respective acid chloride in pyridine, DL-1,2-*O*-cyclohexylidene-*myo*-inositol yielded the 3-sulfonate and 3-benzoate in yields of 41 and 40%, respectively. Similarly, benzylation of both DL-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol¹⁴⁰ and DL-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol¹⁴¹ afforded the 3-benzoates as major products, and, on partial *p*-toluenesulfonylation¹⁴² and benzylation¹⁴³ respectively, DL-1,2:3,4-di-*O*-isopropylidene- and DL-1,2:3,4-di-*O*-cyclohexylidene-*epi*-inositol gave 5-esters in good yields.

The i.r. spectrum of DL-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol in dilute solution shows two bands in the hydroxyl-stretching region, at 3612 and 3562 cm⁻¹, representing $\Delta\nu$ values of 17 and 67 cm⁻¹, assuming that the value 3629 cm⁻¹ holds for a free, secondary hydroxyl group.¹⁴⁴ It was suggested that the diacetal exists in a chair conformation distorted by the *trans*-fusion of the 5,6-acetal ring, leading to a decrease in the distance between the *cis*-oxygen atoms on C-2 and C-3, and, therefore, to an increase in the strength of the 3-OH ··· O-2 hydrogen-bond; this is reflected in a high value of $\Delta\nu$ and correlates with the selective reaction at HO-3. It was deduced that DL-1,2:3,4-di-*O*-isopropylidene-*epi*-inositol exists in a skew conformation ($\Delta\nu$ values, 49 and 79 cm⁻¹), and, although the hydroxyl group involved in the stronger hydrogen-bond could not be

(137) T. Suami, S. Ogawa, and S. Oki, *Bull. Chem. Soc. Jpn.*, **44**, 2824-2826 (1971).

(138) T. Suami, S. Ogawa, T. Tanaka, and T. Otake, *Bull. Chem. Soc. Jpn.*, **44**, 835-841 (1971).

(139) T. Suami, S. Ogawa, K. Ohashi, and S. Oki, *Bull. Chem. Soc. Jpn.*, **45**, 3660-3667 (1972).

(140) S. J. Angyal, M. E. Tate, and S. D. Gero, *J. Chem. Soc.*, 4116-4122 (1961).

(141) N. B. Tarusova, V. S. Grosheva, S. P. Kozlova, R. B. Teplinskaya, and N. A. Preobrazhenskii, *Zh. Org. Khim.*, **4**, 967-971 (1968).

(142) S. J. Angyal and P. T. Gilham, *J. Chem. Soc.*, 3691-3699 (1957).

(143) S. J. Angyal and R. J. Hickman, *Carbohydr. Res.*, **20**, 97-104 (1971).

(144) S. J. Angyal and R. M. Hoskinson, *J. Chem. Soc.*, 2991-2995 (1962).

identified, a differential reactivity between the two is indicated if a hydrogen-bonding–reactivity correlation holds.

Selective esterifications have also been accomplished in the field of aminocyclitols and related antibiotic substances. The five equatorial substituents in 1,3-diamino-1,3-dideoxy-*myo*-inositol are acetylated on treatment with acetic anhydride in pyridine, 59% of 1,3-bis(acetamido)-4,5,6-tri-*O*-acetyl-1,3-deoxy-*myo*-inositol being isolated¹⁴⁵ after seven days at a reaction temperature of 5–10°. During studies on the synthesis of 3',4'-dideoxykanamycin B, a remarkable selectivity between equatorial hydroxyl groups in the diaminodideoxycyclitol residue and the 3-amino-3-deoxy-D-glucopyranosyl group of partially protected kanamycin B was observed; on reaction with benzoyl chloride in pyridine, the sole free hydroxyl group in the latter was selectively benzoylated in 96% yield.¹⁴⁶ Steric hindrance appears to be an overriding factor in this example; the unreactive hydroxyl group has substituted D-glucopyranosyl groups attached at both of the vicinal oxygen atoms.

The enhanced reactivity of the axial 5-hydroxyl group over that of the equatorial group on C-3 in methyl quinate towards acid chlorides in pyridine has been attributed¹⁴⁷ to intramolecular hydrogen-bonding of the former group to the *syn*-axially disposed oxygen atom on C-1.

f. Acyclic Derivatives.—Reports on the selective esterification of acyclic derivatives of carbohydrates have most often concerned sugar dithioacetals, and many examples from this area have been investigated by Zinner and coworkers.¹⁴⁸ Thus, acetylation of pentose and hexose dithioacetals with acetic anhydride–pyridine yielded¹⁴⁹ terminal acetates in yields of 20–60%, and benzoyl chloride in pyridine has been used to prepare the primary esters of dithioacetals of 2-deoxy-D-*erythro*-pentose,¹⁵⁰ D-glucose, D-galactose, D-mannose, D-arabinose, D-ribose, D-lyxose, and D-xylose.^{151,152} Terminal *p*-toluic

(145) T. Suami, S. Ogawa, H. Sano, and N. Kato, *Bull. Chem. Soc. Jpn.*, **44**, 1992–1994 (1971).

(146) S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Jpn.*, **45**, 3624–3628 (1972).

(147) D. Mercier, J. Cléophas, J. Hildesheim, A. M. Sépulchre, and S. D. Gero, *Tetrahedron Lett.*, 2497–2500 (1969).

(148) H. Zinner, K.-H. Stark, E. Michalzik, and K. Kristen, *Chem. Ber.*, **95**, 1391–1399 (1962) and earlier papers in this series.

(149) H. Zinner and K. Wessely, *Chem. Ber.*, **90**, 516–520 (1957).

(150) H. Zinner and H. Nimz, *Chem. Ber.*, **91**, 1657–1659 (1958).

(151) H. Zinner, K. Wessely, W. Bock, K. Rieckhoff, F. Strandt, and W. Nimmich, *Chem. Ber.*, **90**, 500–515 (1957).

(152) H. Zinner, W. Bock, and H.-P. Klöcking, *Chem. Ber.*, **92**, 1307–1313 (1959).

(*p*-methylbenzoic) esters of dithioacetal derivatives of carbohydrates have been recommended as superior, in both crystallinity and preparative yield, to the corresponding benzoates.¹⁵³

The results of selective *p*-toluenesulfonylation of pentose dialkyl dithioacetals illustrate an interesting dependence of reaction product on pentose configuration. Treatment of an arabinose derivative affords the 5-*p*-toluenesulfonate, but the *ribo*, *xylo* and *lyxo* derivatives give 2,5-anhydrides by cyclization of the 5-ester first formed.¹⁵⁴ These results have been rationalized in terms of the favored conformations adopted by the pentose chains, which, in the pentoses giving cyclized products, are considered to be sickle arrangements, by analogy with the peracetylated derivatives of dithioacetals of ribose and xylose. For the arabinose compound, a planar, extended conformation appears to be that having maximum stability, and the potential-energy barrier that must be surmounted to bring O-2 into a position suitable for attack at C-5 is seemingly greater than in the other pentose derivatives.

Several instances of differing reactivities amongst the secondary hydroxyl groups in dialkyl dithioacetals of aldoses have been noted. It has long been known that D-glucose diethyl dithioacetal reacts with benzoyl chloride under Schotten-Baumann conditions to give a 3,4,5,6-tetra-*O*-benzoyl derivative,¹⁵⁵ and, on reaction under similar conditions, D-xylose diethyl dithioacetal also shows a low reactivity of the 2-hydroxyl group, the 3,4,5-tribenzoate being formed in 29% yield.¹⁵⁶ On the other hand, with similar reagents, D-galactose diethyl dithioacetal yielded (at 0°) 52% of the 4,5,6-tri-*O*-benzoyl derivative.¹⁵⁷ A low reactivity at HO-2 towards acylation is not a general property of aldose dialkyl dithioacetals, however. From the reaction of 3-deoxy-D-*arabino*-hexose dimethyl dithioacetal with one molar equivalent of benzoyl chloride was isolated the 2,6-di-*O*-benzoyl derivative, and this diester could readily be prepared in 51% yield by use of 2 molar equivalents of the reagent.¹⁵⁸ In contrast, on treatment with one molar equivalent of benzoyl chloride under similar conditions, 3-deoxy-D-*xylo*-hexose dialkyl dithioacetals gave the 6-benzoates, and an increase in the proportion of benzoyl chloride did not afford definite dibenzoates.¹⁵⁹ The reason for this difference in reactivity is not yet clear, and further study seems warranted.

(153) H. Zinner and M. Pfeifer, *Chem. Ber.*, **94**, 2792-2797 (1961).

(154) J. Defaye and D. Horton, *Carbohydr. Res.*, **14**, 128-132 (1970).

(155) P. Brigl and H. Mühlischlegel, *Ber.*, **63**, 1551-1557 (1930).

(156) M. L. Wolfrom and W. von Bebenburg, *J. Am. Chem. Soc.*, **81**, 5705-5706 (1959).

(157) H. Bolliger and M. D. Schmid, *Helv. Chim. Acta*, **37**, 888-892 (1954).

(158) G. Rembarz, *Chem. Ber.*, **95**, 830-833 (1962).

(159) G. Rembarz and T. Reinhard, *J. Prakt. Chem.*, **26**, 79-82 (1964).

In experiments aimed at simplifying the synthesis of mono-, di-, and tri-glycerides, glycerol was treated with long-chain, fatty acid chlorides in chloroform containing enough *N,N*-dimethylformamide to achieve dissolution, and sufficient selectivity was claimed at each step to permit the preparation of 1,3-di- and 1,2,3-tri-*O*-acyl derivatives in each of which the acyl groups were different.¹⁶⁰ In an extension of this work, the interesting claim was made that monoacylation of glycerol could be aided by the addition of 1 molar equivalent of potassium thiocyanate, urethan, or triethyl phosphate, but the mechanism of their action was not made clear. In this way, 1-*O*-palmitoyl-DL-glycerol was prepared crystalline in 71% yield.¹⁶¹

2. *N*-Acylimidazoles

The chemistry of *N*-acylimidazoles has been developed largely through the work of Staab.¹⁶² Although these compounds have not yet found widespread use in the acylation of carbohydrates, some examples so far reported show that these reagents can be highly selective. Methyl 4,6-*O*-benzylidene- α -D-glucopyranoside reacts with one molar equivalent of *N*-benzoylimidazole in chloroform during 10 hours at the reflux temperature to yield the 2-*O*-benzoyl derivative in 78% yield.¹⁶³ On similar treatment, the corresponding *altro* compound also gave the 2-benzoate (48%); in this case, the starting "diol" was recovered in 44% yield.¹⁶⁴ Methyl 4,6-*O*-benzylidene- α -D-allopyranoside afforded the 2-benzoate as the major product,⁵² whereas the *manno*-“diol” gave the 2- and 3-benzoates in almost equal amounts.¹⁶⁵ Under the usual conditions of reaction, imidazole-catalyzed acyl migration ensures that esterification of such vicinal *cis*-diols is subject to thermodynamic control. Thus, heating under reflux of methyl 3-*O*-benzoyl-4,6-*O*-benzylidene- α -D-mannopyranoside in chloroform containing imidazole led to establishment of a 1:1 equilibrium between the 2- and 3-benzoates in one hour.¹⁶⁵ In contrast, on similar treatment, a *trans*-“diol” monoester, namely, methyl 3-*O*-benzoyl-4,6-*O*-benzylidene- α -D-glucopyranoside, was isomerized to the extent of only $\sim 10\%$ after 12 hours. Reaction of methyl 4,6-*O*-benzylidene- β -D-glucopyranoside led

(160) L. Hartman, *Nature (London)*, **176**, 1024 (1955).

(161) L. Hartman, *J. Chem. Soc.*, 3572–3575 (1957).

(162) H. A. Staab, *Angew. Chem.*, **74**, 407–423 (1962).

(163) F. A. Carey and K. O. Hodgson, *Carbohydr. Res.*, **12**, 463–465 (1970).

(164) N. L. Holder and B. Fraser-Reid, *Synthesis*, 83 (1972).

(165) S. A. Abbas and A. H. Haines, *Carbohydr. Res.*, **39**, 358–363 (1975).

to the formation of the 3-benzoate as the major product of 42% yield,⁵² but benzyl 4,6-*O*-benzylidene- β -D-galactopyranoside afforded the 3-ester in yields of 89–93%, and only trace amounts of unreacted, starting “diol” and 2-ester were detected by t.l.c.¹⁶⁶

The acylation of methyl α -D-glucopyranoside with *N*-(tri-*O*-methylgalloyl)imidazole has been reported.¹⁶⁷ Selective acylation was attempted in 1,4-dioxane, on the reasoning that, if intramolecular hydrogen-bonding plays a role in controlling selective reactivity of hydroxyl groups, the solvent should not disrupt such bonds, a criterion apparently satisfied by 1,4-dioxane.¹⁶⁸ Reaction of the glycoside and imidazolidine in the molar ratio of 2:5, with a catalytic amount of imidazol-1-ylsodium present, gave the 6-, 2,6-bis-, and 2,3,6-tris-(tri-*O*-methylgallic) esters in yields of 16, 27, and 21%, respectively; a ratio of 1:1 gave the 6-ester in 63% yield, and a 1:4 ratio gave the 2,3,6-triester in 65% yield. On acylation of methyl α -D-glucopyranoside with one molar equivalent of *N*-acetylimidazole in *N,N*-dimethylformamide, the d.s. values at O-2, O-3, O-4, and O-6 were^{168a} 0.30, 0.15, 0.20, and 0.35, respectively.

The greater selectivity in the acylation of carbohydrates often shown by *N*-acylimidazoles (compared to acid halides) in pyridine may possibly be associated with the lower reactivity of the former reagents, which may need several hours at $\sim 60^\circ$ to achieve reaction, in contrast to the often instantaneous acylation brought about by the latter reagents. That hydrogen bonding is not the sole cause of the selectivity shown by *N*-acylimidazoles is suggested by the fact that, on reaction with one molar equivalent of *N*-benzoylimidazole, 1,4:3,6-dianhydro-D-glucitol yields approximately equal amounts of the 2- and 5-esters.¹⁶⁵

Esterification of 5'-nucleoside derivatives with *N*-(aminoacyl)-imidazoles yields 2'- and 3'-(aminoacyl) derivatives and proceeds with apparently little selectivity^{169,170}; this is not surprising in view of the facile, base-catalyzed, ester migration that may occur in 2'/(3')-esters of the D-ribofuranose system (see Section VII,1).

N-p-Tolylsulfonylimidazole has been shown to exhibit a selectivity

(166) G. J. F. Chittenden, *Carbohydr. Res.*, **16**, 495–496 (1971).

(167) L. Birkofer and K. Idel, *Ann.*, 4–14 (1974).

(168) B. Åkermarck, *Acta Chem. Scand.*, **15**, 985–990 (1961).

(168a) J. Gelas and D. Horton, personal communication.

(169) B. P. Gottikh, A. A. Kraevskii, P. P. Purygin, T. L. Tsilevich, Z. S. Belova, and L. Rudzite, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2571–2573 (1967).

(170) B. P. Gottikh, A. A. Kraevskii, N. B. Tarusova, P. P. Purygin, and T. L. Tsilevich, *Tetrahedron*, **26**, 4419–4433 (1970).

similar to that of *N*-benzoylimidazole towards methyl 4,6-*O*-benzylidene- α -D-glucopyranoside.¹⁷¹

3. Esters

Transesterification between an alcohol and an ester occurs readily in the presence of base under anhydrous conditions. The preparation of carbohydrate esters of long-chain, fatty acids is of commercial interest, and methyl esters are often used as reagents. Selectivity for reaction at primary hydroxyl groups is invariably observed. Thus, it was claimed that several D-glucopyranosylamine derivatives yield 6-esters on treatment with methyl octadecanoate (stearate) in *N,N*-dimethylformamide in the presence of potassium carbonate plus zinc oxide.¹⁷² Sucrose and methyl tetradecanoate (myristate) in *N,N*-dimethylformamide containing a basic catalyst yield¹⁷³ 6- and 6'-tetradecanoates and other unidentified isomers in the relative proportions of 31:14:5, and only the thermodynamically favored products preponderate at any time throughout the reaction.¹⁷⁴ 1,2-*O*-Isopropylidene- α -D-glucofuranose reacts with methyl octadecanoate at 180° in the presence of potassium carbonate to afford the 6-octadecanoate.¹⁷⁵ Transesterification between methyl α -D-glucopyranoside and methyl dodecanoate (laurate), methyl hexadecanoate (palmitate), methyl octadecanoate, or methyl benzoate in the absence of a solvent, with sodium methoxide as the catalyst, gave¹⁷⁶ 6-esters in yields of 30–40%.

Differentiation between the secondary hydroxyl groups in methyl 4,6-*O*-benzylidene- α -D-glucopyranoside was observed on reaction with methyl benzoate–sodium methoxide at 200° for 45 minutes; the 2- and 3-esters and the 2,3-diester were formed¹⁷⁶ in the molar ratios of 55:23:10.

4. Acids

Direct esterification of D-glucose by heating with 50% acetic acid at 100° was reported to yield 26–30% of the 6-acetate after chromatography on cellulose, together with unchanged starting-material and

(171) D. R. Hicks and B. Fraser-Reid, *Synthesis*, 203 (1974).

(172) P. Spiess, Brit. Pat. 878,728 (1961); *Chem. Abstr.*, **57**, 13,864 (1962).

(173) R. U. Lemieux and A. G. McInnes, *Can. J. Chem.*, **40**, 2394–2401 (1962).

(174) R. U. Lemieux and A. G. McInnes, *Can. J. Chem.*, **40**, 2376–2393 (1962).

(175) K. Knoevenagel and R. Himmelreich, U.S. Pat. 3,171,832 (1965); *Chem. Abstr.*, **63**, 14,967 (1965).

(176) G. N. Bollenback and F. W. Parrish, *Carbohydr. Res.*, **17**, 431–438 (1971).

some diesters,¹⁷⁷ and, in a modification, unreacted D-glucose was removed by crystallization followed by fermentation.¹⁷⁸ Conversion of galactitol into 1-O-acetyl-DL-galactitol has also been achieved directly with this acid.¹⁷⁹ N-(Benzyloxycarbonyl)amino acids have been condensed with D-glucose in the presence of N,N'-dicyclohexylcarbodiimide, to yield¹⁸⁰ 6-O-(aminoacyl) derivatives in yields of 26–46%, and a similar reaction of adenosine with N-(benzyloxycarbonyl)phenylalanine afforded 70% of the 2'(3'),5'-diester.¹⁸¹ On treatment with the respective carboxylic acid plus diethyl diazodicarboxylate plus triphenylphosphine in 1,4-dioxane, thymidine was selectively esterified at the primary hydroxyl group, the 5'-acetate and 5'-benzoate being isolated in yields of 55 and 75%, respectively (after chromatography).¹⁸²

5. Acyl Azides

Acyl azides show selectivity for acylation at secondary (rather than primary) hydroxyl groups in nucleosides. Thus, on reaction with an excess of N-(benzyloxycarbonyl)glycylglycyl azide in 1,4-dioxane-water at 8° and pH 9, adenosine gave the 2'(3')-O-(aminoacyl) and 2',3'-di-O-(aminoacyl) derivatives.¹⁸³ Furthermore, adenosine and uridine were converted into a variety of 2'(3')-esters of aromatic amino acids by reaction with the appropriate acyl azide.¹⁸⁴

6. Acyl Cyanides

This class of acylating agents has thus far been but little used in carbohydrate chemistry. However, unimolar benzoylation of methyl 4,6-O-benzylidene- α -D-glucopyranoside, methyl 4,6-O-benzylidene- α -D-altropyranoside, and benzyl 4,6-O-benzylidene- β -D-galactopyranoside with benzoyl cyanide gave good yields of the corre-

(177) R. B. Duff, *J. Chem. Soc.*, 4730–4734 (1957).

(178) V. Bilik, *Chem. Zvesti*, **26**, 82–83 (1972); *Chem. Abstr.*, **76**, 154,047b (1972).

(179) L. Hough, J. K. N. Jones, and D. L. Mitchell, *Can. J. Chem.*, **37**, 725–730 (1959).

(180) N. K. Kochetkov, V. A. Derevitskaya, L. M. Likhoshervostov, N. V. Molodtsov, and G. G. Kara-Murza, *Tetrahedron*, **18**, 273–284 (1962).

(181) E. Ya. Dreiman, V. A. Dmitrieva, S. G. Kamzolova, Z. A. Shabarova, and M. A. Prokof'ev, *Zh. Obshch. Khim.*, **31**, 3899–3905 (1961).

(182) O. Mitsunobu, J. Kimura, and Y. Fujisawa, *Bull. Chem. Soc. Jpn.*, **45**, 245–247 (1972).

(183) V. N. Nezavibat'ko, L. N. Nikolenko, and M. N. Semenova, *Zh. Obshch. Khim.*, **41**, 2109 (1971).

(184) V. N. Nezavibat'ko, L. N. Nikolenko, T. I. Zubareva, and M. N. Semenova, *Dokl. Akad. Nauk SSSR*, **210**, 1355–1357 (1973).

sponding 2-, 2-, and 3-benzoate, respectively.¹⁶⁵ Although the selectivity shown appears similar to that of *N*-benzoylimidazole, the use of benzoyl cyanide can be advantageous in the preparation of esters that may undergo facile migration, as the reaction byproduct, namely, hydrogen cyanide, is less likely than imidazole to cause migration.

7. Inorganic Reagents

a. Phosphorylating Reagents.—The fundamental importance in biochemistry of carbohydrate phosphates has led to an extensive study of their preparation. Some syntheses involve selective phosphorylation of partially substituted carbohydrates, or of free sugars. With the various phosphorylating agents that have been used, favored reaction at primary hydroxyl groups is most often observed, showing that steric factors are important, but there are some notable exceptions.

An early synthesis of D-xylose 5-phosphate involved reaction of 1,2-*O*-isopropylidene- α -D-xylofuranose with phosphoryl chloride in pyridine,^{185,186} and a marked improvement in the preparation was achieved by using diphenyl phosphorochloridate as the phosphorylating species.^{187,188} The latter reagent also reacts selectively with benzyl β -D-ribofuranoside to afford the 5-(diphenylphosphate) in 73% yield,¹⁸⁹ and with methyl 2-deoxy- α,β -D-erythro-pentofuranoside to give¹⁹⁰ the 5-(diphenylphosphate) and 3,5-bis(diphenylphosphate) in the ratio of 4:1. The mixed reagent, phosphoryl chloride-trimethyl phosphate, originally utilized for selective phosphorylation of nucleosides (see later), showed useful specificity in converting *o*-nitrophenyl β -D-galactopyranoside into its 6-phosphate in 50% yield.¹⁹¹

A novel synthesis of D-glucose 6-phosphate (in 55% yield) has been reported; it involves alcoholysis of the cyclic phosphate of catechol with 1,2-*O*-isopropylidene- α -D-glucofuranose, followed by acid hydrolysis of the so-formed phosphoric diester.¹⁹² The reagent

(185) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 347–355 (1933).

(186) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **107**, 75–83 (1934).

(187) P. A. J. Gorin, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 582–583 (1955).

(188) J. L. Barnwell, W. A. Saunders, and R. W. Watson, *Can. J. Chem.*, **33**, 711–715 (1955).

(189) G. M. Tener and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 1999–2004 (1958).

(190) T. Ukita and K. Nagasawa, *Chem. Pharm. Bull.*, **7**, 655–657 (1959).

(191) W. Hengstenberg and M. L. Morse, *Carbohydr. Res.*, **10**, 463–465 (1969).

(192) T. Ukita and K. Nagasawa, *Chem. Pharm. Bull.*, **9**, 544–550 (1961).

has been applied to other polyhydroxy compounds, leading to phosphorylation at primary hydroxyl groups, as described later.

The selective phosphorylation of free sugars at the primary and the reducing centers has been achieved. D-Glucose 6-phosphate was prepared by direct reaction of D-glucose with meta-,¹⁹³ tetra-,¹⁹⁴ or poly-¹⁹⁵ phosphoric acid, and with phosphoryl chloride in sulfuric acid.¹⁹⁶ From the reaction of 2-amino-2-deoxy-D-glucose with metaphosphoric acid in acetonitrile was isolated the 6-phosphate,¹⁹⁷ but a more-satisfactory preparation utilized the reaction of 2-(anisylideneamino)-2-deoxy-D-glucose with diphenyl phosphorochloridate¹⁹⁸; selective phosphorylation of 2-(benzyloxycarbonyl)amino-2-deoxy-D-glucose with 2-cyanoethyl phosphate also appears to offer a satisfactory route to 2-amino-2-deoxy-D-glucose 6-phosphate.¹⁹⁹ It has been briefly noted that cyanoguanidine promotes the formation of D-ribose 5-phosphate from D-ribose plus orthophosphate,²⁰⁰ a reaction that could have had importance in prebiological chemistry, and related observations concern the formation of aldosyl phosphates in aqueous solution. D-Ribose and orthophosphate condense in aqueous solution at 25° and pH 7.0–8.8 in the presence of cyanogen or cyanamide to yield β -D-ribofuranosyl phosphate in 10 to 20% yield as the only sugar phosphate.²⁰¹ Involvement of HO-2 in the reaction is suggested by the unreactivity of 2-deoxy-D-*erythro*-pentose under the same conditions, and by the stereospecific formation of the β -D-ribofuranosyl ester. A correlation has been detected between the pK_a values of the anomeric hydroxyl groups of sugars and their tendency to undergo cyanogen-induced phosphorylation by orthophosphate.²⁰² For example, under similar conditions, D-ribose (pK_a 12.21), D-arabinose (pK_a 12.43), and 2-deoxy-D-*erythro*-pentose (pK_a 12.65) are phosphorylated to the extents of 20, 6, and 0%. A mechanism in-

(193) M. Viscontini and C. Olivier, *Helv. Chim. Acta*, **36**, 466–470 (1953).

(194) Y. Inoue, K. Onodera, and T. Ito, *Nippon Nogei Kagaku Kaishi*, **30**, 59–62 (1956); *Chem. Abstr.*, **51**, 3,466 (1957).

(195) J. E. Seegmiller and B. L. Horecker, *J. Biol. Chem.*, **192**, 175–180 (1951).

(196) T. Saito and J. Noguchi, *Nippon Kagaku Zasshi*, **82**, 469–471 (1961); *Chem. Abstr.*, **56**, 11,678 (1962).

(197) J. M. Anderson and E. Percival, *Chem. Ind. (London)*, 1018 (1954).

(198) F. Maley and H. A. Lardy, *J. Am. Chem. Soc.*, **78**, 1393–1397 (1956).

(199) F. Maley, *N.Y. State Dep. Health Ann. Rep. Div. Lab. Res.*, 63–64 (1962); *Chem. Abstr.*, **60**, 8,114 (1964).

(200) M. Calvin, *Proc. Roy. Soc. London, Ser. A*, **288**, 441–466 (1965).

(201) M. Halmann, R. A. Sanchez, and L. E. Orgel, *J. Org. Chem.*, **34**, 3702–3703 (1969).

(202) C. Degani, *Carbohydr. Res.*, **18**, 329–332 (1971).

volving initial protonation of the phosphorylating species, namely, cyano(imino)methyl phosphate, by the relatively acidic hydrogen atom of the anomeric hydroxyl group was suggested to account for the favored phosphorylation. Significantly, glycerol and nonreducing sugars are not phosphorylated under these conditions²⁰³; D-glucose affords α -D-glucopyranosyl phosphate (8–20%), β -D-glucopyranosyl phosphate (2–5%), and a phosphorylated disaccharide (3–34%).

A remarkably selective phosphorylation at HO-3 in L-ascorbic acid and its 5,6-O-isopropylidene derivative has been achieved with phosphoryl chloride in aqueous pyridine²⁰⁴; the use of acetone-pyridine as the solvent led to a much less selective reaction.

The selective phosphorylation of nucleosides has been the subject of considerable study. The ratios of monophosphorylated products obtained on treatment of adenosine with phosphoryl chloride was found to depend on the composition and alkalinity of the reaction medium.²⁰⁵ Anhydrous pyridine as the solvent led, after processing involving water, to a preponderance of the 5'-phosphate. On reaction in pyridine containing 0.1 molar equivalent of water to every mole of phosphoryl chloride, the 2'-phosphate preponderated. In aqueous barium hydroxide, no 5'-phosphate was formed; in no experiment, however, was a reasonable yield of phosphate obtained. Very selective esterification of HO-5' in unprotected nucleosides was achieved in a one-step procedure by reaction with phosphoryl chloride in trimethyl or triethyl phosphate, followed by processing involving the use of water.²⁰⁶ Concomitant formation of small proportions of 2'(3'),5'-diesters was greatly lessened by pretreating the phosphorylating agent with a small proportion of water, a result which is of interest in comparison to the effect of water on phosphorylation in pyridine.²⁰⁵

Reaction occurred at the primary hydroxyl group in thymidine on treatment with dibenzyl phosphorochloridate in acetonitrile containing pyridine,²⁰⁷ and the bulky bis(2,2,2-trichloroethyl) phosphorochloridate reacts²⁰⁸ with nucleosides to afford nucleoside 5'-[bis(2,2,2-trichloroethyl) phosphates] in yields of 40–70%; the

(203) C. Degani and M. Halmann, *J. Chem. Soc., C*, 1459–1465 (1971).

(204) H. Nomura, M. Shimomura, and S. Morimoto, *Chem. Pharm. Bull.*, **19**, 1433–1437 (1971).

(205) G. R. Barker and G. E. Foll, *J. Chem. Soc.*, 3798–3800 (1957).

(206) M. Yoshikawa, T. Kato, and T. Takenishi, *Bull. Chem. Soc. Jpn.*, **42**, 3505–3508 (1969).

(207) K. L. Agarwal and M. M. Dhar, *Experientia*, **21**, 432–433 (1965).

(208) A. Franke, K.-H. Scheit, and F. Eckstein, *Chem. Ber.*, **101**, 2998–3001 (1968).

latter derivatives are converted into nucleoside 5'-phosphates on treatment with zinc dust. Pyrophosphoryl chloride in *m*-cresol, *o*-chlorophenol, and a variety of other solvents selectively esterifies HO-5' in unprotected nucleosides, and 5'-phosphates were obtained after processing involving the use of water.²⁰⁹ Transesterification of nucleosides with tris(quinolin-8-yl) phosphate also affords a useful route to nucleoside 5'-phosphates.²¹⁰ Direct, selective phosphorylation of nucleosides at the primary hydroxyl group with 2-cyanoethyl phosphate plus *N,N'*-dicyclohexylcarbodiimide in pyridine is possible in the presence of a large excess of the nucleoside, a reaction of use in synthesizing ³²P-labelled nucleosides.²¹¹ Thymidine 5'-phosphate and uridine 5'-phosphate were obtained in yields of 47 and 28%, respectively, by treatment of the nucleoside with triphenylphosphine-dibenzyl hydrogen phosphate-diethyl azodicarboxylate in tetrahydrofuran, followed by hydrogenolysis²¹²; although yields were not high, no isomeric 2'- and 3'-phosphates were detected, and the sterically demanding nature of the reagent was confirmed by the observation that *l*-menthol was recovered unchanged on similar treatment. 2-(Dimethylamino)-4-nitrophenyl phosphate has been used to synthesize adenosine 5'-phosphate in 77% yield from the unprotected nucleoside.^{212a}

Selective reaction at the *cis*-2,3-diol grouping of unprotected D-ribonucleosides has occasionally been observed. Treatment of D-ribonucleosides with tris(tetramethylammonium) trimetaphosphate in *M* sodium hydroxide for 4 days at room temperature led to a mixture of nucleoside 2'- and 3'-phosphates in yields of >70%; no 5'-phosphate was detected.²¹³ Reaction of ethyl (trichloromethyl)phosphonate with nucleosides in *N,N*-dimethylformamide containing triethylamine, followed by basic hydrolysis of the reaction product, yielded 2'/(3')-phosphates in variable yields.²¹⁴ The participation of the *cis*-diol grouping in the reaction was suggested by the failure of thymidine or 2',3'-*O*-isopropylideneuridine to undergo reaction.

Trehalose 6-phosphate has been prepared in 28% yield through unimolar phosphorylation of the disaccharide with diphenyl phos-

(209) K. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, *J. Org. Chem.*, **34**, 1547-1550 (1969).

(210) H. Takaku and Y. Shimada, *Tetrahedron Lett.*, 1279-1282 (1974).

(211) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159-168 (1961).

(212) O. Mitsunobo, K. Kato, and J. Kimura, *J. Am. Chem. Soc.*, **91**, 6510-6511 (1969).

(212a) Y. Taguchi and Y. Mushika, *Tetrahedron Lett.*, 1913-1916 (1975).

(213) R. Saffhill, *J. Org. Chem.*, **35**, 2881-2883 (1970).

(214) A. Holý, *Tetrahedron Lett.*, 157-158 (1972).

phorochloridate²¹⁵; with 2 molar equivalents of the reagent, the 6-phosphate (in 24% yield) and 6,6'-diphosphate (33% yield) were obtained. Selective phosphorylation at the primary hydroxyl group in kanamycin was achieved in good yield by treating tetra-*N*-anisylidenekanamycin with diphenyl phosphorochloridate.²¹⁶ After removal of *N*-protecting groups, the 6'-(diphenyl phosphate) was isolated in 56% yield, and this was readily transformed by hydrogenolysis into kanamycin 6'-phosphate.

The alcoholysis of the cyclic phosphate of catechol by alditols can lead, after acid hydrolysis of intermediate, cyclic phosphates, to the selective formation of phosphoric esters of the primary hydroxyl groups in the alditols. Thus, erythritol and D-mannitol afford, after chromatographic purification of the reaction products, their 1-phosphates in yields of 31 and 38%, respectively.²¹⁷ The method was used to convert riboflavine into riboflavine 5'-phosphate.²¹⁸ 1-Deoxy-1-fluoro-L-glycerol has been converted into the 3-(dibenzyl phosphate) in 54% yield by selective reaction with dibenzyl phosphorochloridate.²¹⁹

b. Sulfating Reagents.—Important aspects of the relative reactivity of free sugars and glycosides towards sulfation have already been discussed in an article in this Series.²²⁰ In general, it has been found that sulfating agents react with hexoses and hexose derivatives having HO-6 free, to afford 6-sulfates initially, but polysulfation may then occur. No significant differences in the selectivity of the various reagents (for example, chlorosulfonic acid in pyridine or sulfuric acid, or the sulfur trioxide-pyridine complex in pyridine or *N,N*-dimethylformamide) appear to have been discovered, although, with the sulfur trioxide-pyridine reagent, reaction in *N,N*-dimethylformamide was claimed to minimize polysulfation of D-glucose (compared to the reaction in pyridine).²²¹

Interesting selectivity has been observed with some substituted carbohydrates. On treatment with sulfur trioxide-pyridine complex in *N,N*-dimethylformamide, methyl 4,6-*O*-benzylidene- α -D-

(215) D. L. MacDonald and R. Y. K. Wong, *Biochim. Biophys. Acta*, **86**, 390–392 (1964).

(216) S. Umezawa, K. Tatsuta, T. Tsuchiya, and E. Yamamoto, *Bull. Chem. Soc. Jpn.*, **40**, 1972–1974 (1967).

(217) T. Ukita and K. Nagasawa, *Chem. Pharm. Bull.*, **7**, 401–407 (1959).

(218) T. Ukita and K. Nagasawa, *Chem. Pharm. Bull.*, **7**, 465–468 (1959).

(219) W. J. Lloyd and R. Harrison, *Carbohydr. Res.*, **26**, 91–98 (1973).

(220) J. R. Turvey, *Adv. Carbohydr. Chem.*, **20**, 183–218 (1965).

(221) K. B. Guiseley and P. M. Ruoff, *J. Org. Chem.*, **26**, 1248–1254 (1961).

glucopyranoside afforded the 2-sulfate in 67% yield,²²² whereas, in the monosulfate fraction obtained on treatment of D-glucose with three molar equivalents of the same complex in pyridine, the 2-sulfate was not formed, even though the 3- and 4-sulfates were detected.²²³ Also, methyl 2-acetamido-6-O-acetyl-2-deoxy- α -D-galactopyranoside was selectively sulfated²²⁴ at the axially attached hydroxyl group, HO-4, in 68% yield, which contrasts with the known, low reactivity of HO-4 in D-galactose towards sulfation.²²³

Thymidine has been selectively sulfated at HO-5' by using chlorosulfonic acid in acetonitrile in the presence of pyridine.²⁰⁷

III. SELECTIVE ETHERIFICATION

In selective etherification, it is important to distinguish between reversible and irreversible reactions. The former class comprises etherifications with dimethyl sulfate, halogen compounds, oxirane (ethylene oxide), and diazoalkanes, whereas the latter class involves addition reactions of the Michael type of hydroxyl groups to activated alkenes. In this Section, irreversible and reversible reactions are described separately, and a further distinction is made in the former group by placing the rather specialized, diazoalkane-based alkylations in a separate subsection.

1. Dimethyl Sulfate, Halogen Compounds, and Oxirane

a. Selective Reactivity at Primary Hydroxyl Groups.—The differentiation of primary and secondary hydroxyl groups by use of the sterically demanding reagent chlorotriphenylmethane (trityl chloride) is well known,²²⁵ but the reactivity of secondary hydroxyl groups towards the reagent is also recognized. The ditrityl ether of uridine, first described by Levene and Tipson,²²⁶ was later shown to be the 2',5'-ditrityl ether,²²⁷ and the 3',5' isomer has been isolated in 27% yield from reaction of uridine with three molar equivalents of trityl chloride²²⁸; interestingly, under identical conditions, 6-azauridine²²⁸ and 6-azacytidine²²⁹ give 5'-trityl ethers as the sole

(222) K. B. Guiseley and P. M. Ruoff, *J. Org. Chem.*, **27**, 1479–1482 (1962).

(223) J. R. Turvey and T. P. Williams, *J. Chem. Soc.*, 2242–2246 (1963).

(224) S. Hirano, *Carbohydr. Res.*, **27**, 265–267 (1973).

(225) B. Helferich, *Adv. Carbohydr. Chem.*, **3**, 79–111 (1948).

(226) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **105**, 419–430 (1934).

(227) N. C. Yung and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 3060–3066 (1961).

(228) J. Žemlička, *Collect. Czech. Chem. Commun.*, **29**, 1734–1735 (1964).

(229) J. Beránek and J. Piřha, *Collect. Czech. Chem. Commun.*, **29**, 625–634 (1964).

products. Derivatives are known in which trityl groups are attached to vicinal oxygen atoms. From the reaction of uridine with trityl chloride, 2',3',5'-tri-*O*-trityluridine has been isolated in yields of 1.5 (Ref. 230), 4.2 (Ref. 231), and 2% (Ref. 232), in addition to 5'-*O*-trityl, and 2',5'- and 3',5'-di-*O*-trityl derivatives. 2',3',5'-Tri-*O*-trityl derivatives of *N*⁶-benzoyl-²³³ and *N*⁶-acetyl-cytidine²³⁴ have also been obtained in low yields. In all of these reactions, the 2',5'-diethers preponderated over the 3',5' isomers, but tritylation of 1-(5-*O*-trityl- β -D-arabinofuranosyl)uracil yielded the 3',5'-di-*O*-trityl derivative, presumably²³⁵ because of steric hindrance at the 2'-hydroxyl group.

The primary hydroxyl groups on C-6 of the D-glucopyranosyl group and C-6' of the D-fructofuranosyl group in sucrose appear to react selectively with trityl chloride in pyridine, four moles of the reagent per mole of sucrose affording, after chromatography, the 6,1',6'-tri- and 6,6'-di-*O*-trityl derivatives in yields of 58 and 30%, respectively.²³⁶ Dimolar tritylation of sucrose gave a mixture of mono-, di-, and tri-substituted derivatives, and the 6,6'-, 1',6'-, and 6,1'-di-*O*-trityl compounds were present²³⁷ in the ratios of 37:7:6, confirming the low reactivity of HO-1'.

The two primary hydroxyl groups in maltose and its derivatives show a large difference in reactivity. On selective tritylation, β -maltose,²³⁸ benzyl β -maltoside,²³⁹ and methyl 3-*O*-(methylsulfonyl)- β -maltoside¹²⁷ all gave mainly 6'-*O*-trityl compounds. Tritylation of cyclohexaamylose¹³⁰ and amylose^{132,240} yielded the expected 6-*O*-trityl derivatives.

A requirement for removal of protecting groups from primary hydroxyl groups under mild conditions during the synthesis of polynucleotides led to the development of modified trityl groups as protecting agents.²⁴¹ Mono-, di-, and tri-methoxytrityl ethers were prepared, and the introduction of each methoxyl group was found to

(230) J. F. Codington and J. J. Fox, *Carbohydr. Res.*, **3**, 124-127 (1967).

(231) H. U. Blank and W. Pfeleiderer, *Tetrahedron Lett.*, 869-870 (1967).

(232) A. F. Cook and J. G. Moffatt, *J. Am. Chem. Soc.*, **89**, 2697-2705 (1967).

(233) W. Hutzenlaub and W. Pfeleiderer, *Chem. Ber.*, **106**, 665-673 (1973).

(234) U. Brodbeck and J. G. Moffatt, *J. Org. Chem.*, **35**, 3552-3558 (1970).

(235) J. F. Codington, R. J. Cushley, and J. J. Fox, *J. Org. Chem.*, **33**, 466-468 (1968).

(236) L. Hough, K. S. Mufti, and R. Khan, *Carbohydr. Res.*, **21**, 144-147 (1972).

(237) T. Otake, *Bull. Chem. Soc. Jpn.*, **45**, 2895-2898 (1972).

(238) M. L. Wolfrom and K. Koizumi, *J. Org. Chem.*, **32**, 656-660 (1967).

(239) B. Helferich and W. Speicher, *Ann.*, **579**, 106-112 (1953).

(240) B. J. Bines and W. J. Whelan, *Chem. Ind. (London)*, 997-998 (1960).

(241) M. Smith, D. H. Rammner, I. H. Goldberg, and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 430-440 (1962).

increase the rate of ether hydrolysis in 80% acetic acid at room temperature by a factor of ~ 10 . However, the introduction of these substituents also increased the reactivity of the corresponding, substituted-trityl chloride towards secondary hydroxyl groups, resulting in a lower selectivity for the primary hydroxyl groups. The (mono-methoxy)trityl group has proved the most useful of these variants. A wide range of other substituted-trityl groups has been investigated with a view to retaining selectivity, but increasing ease of removal.²⁴²

Silyl groups containing bulky substituents have also been used as selective reagents for primary hydroxyl groups. Chlorotricyclohexylsilane reacts with sucrose to yield a trisubstituted derivative, tentatively identified by oxidation studies as the 6,1',6'-triether,²⁴³ and *tert*-butylchlorodimethylsilane,^{244,245} chlorotriisopropylsilane,²⁴⁶ and a range of other alkylchlorosilanes²⁴⁷ have been shown to react very selectively with primary hydroxyl groups in nucleosides. Chlorotriisopropylsilane also shows a remarkable selectivity between the two secondary hydroxyl groups in 5'-*O*-trityluridine; treatment of the substituted nucleoside with 1.2 molar equivalents of the chlorosilane gave the 2'-(triisopropylsilyl) ether in 70% yield, together with 25% of the 3'-isomer.²⁴⁶ The various alkylsilyl ethers of nucleosides show a range of hydrolytic stabilities, and the 3'-ethers of 2-deoxy-D-*erythro*-pentonucleosides are considerably more stable to aqueous acid than the 5'-isomers.²⁴⁷ These combined properties suggest that this class of compounds should have considerable potential in synthetic work.

b. Pyranoid Derivatives of Monosaccharides.—As in selective esterification studies, considerable use has been made of 4,6-cyclic acetal derivatives of alkyl aldopyranosides in attempting to determine some of the factors influencing the reactivity of secondary hydroxyl groups in carbohydrates. As models for the alkylation of cellulose, methyl 4,6-*O*-benzylidene- and 4,6-*O*-ethylidene- β -D-glucopyranoside were partially methylated with dimethyl sulfate—

(242) A. Taunton-Rigby, Y. H. Kim, C. J. Crosscup, and N. A. Starkovsky, *J. Org. Chem.*, **37**, 956–964 (1972).

(243) S. A. Barker, J. S. Brimacombe, M. R. Harnden, and J. A. Jarvis, *J. Chem. Soc.*, 3403–3406 (1963).

(244) K. K. Ogilvie and D. J. Iwacha, *Tetrahedron Lett.*, 317–319 (1973).

(245) K. K. Ogilvie, *Can. J. Chem.*, **51**, 3799–3807 (1973).

(246) K. K. Ogilvie, K. L. Sadana, E. A. Thompson, M. A. Quilliam, and J. B. Westmore, *Tetrahedron Lett.*, 2861–2864 (1974).

(247) K. K. Ogilvie, E. A. Thompson, M. A. Quilliam, and J. B. Westmore, *Tetrahedron Lett.*, 2865–2868 (1974).

19% aqueous sodium hydroxide (Haworth's procedure), the ethers hydrolyzed, and the products purified by chromatography and electrophoresis.²⁴⁸ Analysis of the reactions by use of Spurlin's approach²⁴⁹ suggested that the rate constants for methylation at HO-2 and HO-3 are approximately the same, but, as the proportion of 2,3-di-O-methyl derivative was always higher than that calculated, reactivity at one of the positions appeared to increase when the other was methylated. In contrast to methylation, reaction of 1-chloro-2-(diethylamino)ethane in aqueous sodium hydroxide with methyl 4,6-O-benzylidene- α - and - β -D-glucopyranoside was claimed²⁵⁰ to favor etherification of HO-3. The fact that the ratio of 2- to 3-substitution and the proportion of the disubstituted product are slightly higher for the β anomer was interpreted as being the result of a lower degree of steric interference between the methoxyl group on C-1 in the β anomer and the alkylating agent approaching HO-2. However, these speculations are of doubtful validity, in view of the relatively large proportion of disubstituted product formed, and the lack of a complete, kinetic analysis.

Novel, regioselective methylations of methyl 4,6-O-benzylidene- α - and - β -D-glucopyranoside have been achieved through their Cu(II) derivatives,²⁵¹ which were prepared by treating the disodium salts of the "diols" with cupric chloride. Treatment of these cupric salts with methyl iodide gave the 2- and 3-methyl ethers in yields of 19 and 73%, respectively, from the α -D-glucoside, and 16 and 78% from the β -D-glucoside. By contrast, reaction of the monosodium salts of the "diols" with methyl iodide in tetrahydrofuran led to mixtures in which the 2-methyl ether was in excess. This method of selective methylation was also applied to the anomeric methyl 2,3-di-O-methyl-D-glucopyranosides.²⁵² For the β -D-glucoside, a 72% yield of the 2,3,6-trimethyl ether was obtained, whereas, from the α anomer, the 2,3,6- or the 2,3,4-trimethyl ether could be obtained, in yields of 80 or 74% respectively, depending on the molar ratio of copper to glycoside in the copper derivative started with.

Reaction of methyl 4,6-O-benzylidene- α -D-glucopyranoside with methyl iodide²⁵³ or with tetra-O-acetyl- α -D-glucopyranosyl bro-

(248) I. Croon, *Acta Chem. Scand.*, **13**, 1235-1238 (1959).

(249) H. M. Spurlin, *J. Am. Chem. Soc.*, **61**, 2222-2227 (1939).

(250) E. J. Roberts and S. P. Rowland, *Carbohydr. Res.*, **4**, 509-511 (1967).

(251) E. Avela and B. Holmbom, *Acta Acad. Abo., Ser. B*, **31** (14), 14 pp. (1971).

(252) E. Avela, B. Melander, and B. Holmbom, *Acta Acad. Abo., Ser. B*, **31** (15), 13 pp. (1971).

(253) D. Trimmell, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydr. Res.*, **11**, 497-507 (1969).

mide,²⁵⁴ and of methyl 4,6-*O*-benzylidene- α -D-mannopyranoside with benzyl bromide in *N,N*-dimethylformamide,²⁵⁵ all in the presence of heavy-metal oxides, led predominantly to 2-substitution. However, on treatment with benzyl bromide-silver oxide reagent in *N,N*-dimethylformamide, the corresponding β -D-glucopyranoside derivative afforded a slight preponderance of the 3-*O*-benzyl derivative over its 2-isomer.²⁵⁶

It is of interest that reaction of methyl, benzyl, and *p*-nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy- β -D-glucopyranosides, and also the corresponding methyl α -D-glucopyranoside derivative, with (\pm)-2-chloropropionic acid gave, in preponderant yields, the respective 3-*O*-(D-1-carboxyethyl) derivatives²⁵⁷; only from the last-mentioned reaction was a significant amount of the 3-*O*-(L-1-carboxyethyl) derivative isolated.

In free aldoses, the 1-hydroxyl group is the most reactive towards alkylation in basic media, presumably as a result of its enhanced acidity (caused by the inductive effect of the ring-oxygen atom). Aldosides may, therefore, be prepared under basic (as well as the more-usual acidic) conditions, and the two methods may be complementary in affording a different anomer as the major product. Thus, methyl β -D-glucopyranoside was simply prepared, in 36% yield (isolated as its tetraacetate), by reaction of D-glucose with dimethyl sulfate-aqueous sodium hydroxide,²⁵⁸ in contrast to the facile formation of the α -D-glucoside under the Fischer glycosidation conditions. Similar alkaline methylation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucose afforded the β -D-glycoside in 70% yield²⁵⁹; on performing the reaction in dimethyl sulfoxide (instead of water), the α -D-glycoside, remarkably, was isolated in 86% yield. Some of the factors affecting the ratio of the two glycosides obtained on methyl glycosidation of sugar derivatives at O-1 have been investigated,^{260,261} and it appears that metal ions, intramolecular hydrogen-bonding, and the anomeric effect may all exert influences. The α : β ratio of the glyco-

(254) N. Yamaoka, T. Fujita, M. Kusaka, and K. Aso, *Nippon Nokei Kagaku Kaishi*, **38**, 5-9 (1964); *Chem. Abstr.*, **62**, 11,891 (1965).

(255) H. B. Borén, P. J. Garegg, and N. H. Wallin, *Acta Chem. Scand.*, **26**, 1082-1086 (1972).

(256) H. B. Borén, P. J. Garegg, L. Kenne, L. Maron, and S. Svensson, *Acta Chem. Scand.*, **26**, 644-652 (1972).

(257) R. W. Jeanloz, E. Walker, and P. Sinaý, *Carbohydr. Res.*, **6**, 184-196 (1968).

(258) D. M. Hall and O. A. Stamm, *Carbohydr. Res.*, **12**, 421-428 (1970).

(259) W. Roth and W. Pigman, *J. Am. Chem. Soc.*, **82**, 4608-4610 (1960).

(260) A. H. Haines, *Tetrahedron Lett.*, 1201-1202 (1969).

(261) A. H. Haines and K. C. Symes, *J. Chem. Soc., C*, 2331-2334 (1971).

sides produced depends on the proportions of the two anomeric forms of the reducing sugars present, the rate of interconversion of these anomeric forms, and also on the relative reactivities of their 1-hydroxyl groups. Significantly, it has been established that metal ions (for example Ca^{2+}) can produce profound changes in the composition of the mixture of glycosides obtained on treatment of D-allose with methanolic hydrogen chloride.^{262,263}

Comparisons of the relative reactivities of the hydroxyl groups in methyl α -D-glucopyranoside have been made by forming its mono-sodium salt in butyl alcohol, and treating the so-derived, anhydrous alkoxide with methyl iodide. An early investigation led to the isolation, after hydrolysis, of 2-O-methyl-D-glucose in small yield,²⁶⁴ but application of quantitative paper chromatography permitted a more-meaningful product-analysis to be made.²⁶⁵ The hydrolyzates contained D-glucose and mono-, di-, and tri-O-methyl-D-glucose in the molar ratios of 15:18:11:6, and the mono-O-methyl fraction contained the 2-, 3-, and 6-isomers in the ratios of 13:3:9. From the distribution of monosubstituted D-glucoses, relative rate-constants of 10:2:5 were calculated for substitution at HO-2, HO-3, and HO-6. The results of utilization of these values in the statistical treatment of Spurlin²⁶⁶ suggested that, when substitution occurs on the adjacent hydroxyl group, the modified, relative rate-constant for HO-2 is 8, and that for HO-3 is 6.7.

Haworth methylation of methyl β -D-glucopyranoside and its 4-benzyl and 4-(tetrahydropyran-2-yl) ethers was investigated in connection with partial-methylation studies on cellulose.²⁶⁷ For the unsubstituted glycoside, the ratios of relative rate-constants $k_2:k_3:k_4:k_6$ were estimated to be 8:2:1:8, and, for the 4-ethers, it was found that $k_6 > k_2 > k_3$; best agreements between calculated and experimental yields were found with the assumption that the rate constant for reaction at HO-3 is doubled when HO-2 is substituted. Later methylation studies,²⁶⁸ performed to low degrees of substitution, with analysis by gas-liquid chromatography, gave $k_2 > k_4 > k_3$ for the reactivity

(262) S. J. Angyal and K. P. Davies, *Chem. Commun.*, 500-501 (1971).

(263) M. E. Evans and S. J. Angyal, *Carbohydr. Res.*, **25**, 43-48 (1972).

(264) M. L. Wolfrom and M. A. El-Taraboulsi, *J. Am. Chem. Soc.*, **75**, 5350-5352 (1953).

(265) R. W. Lenz, *J. Am. Chem. Soc.*, **82**, 182-186 (1960).

(266) H. M. Spurlin, in "Cellulose and Cellulose Derivatives, Part II," E. Ott, H. M. Spurlin, and M. W. Grafflin, eds., Interscience, New York, 1954, pp. 673-712.

(267) A. N. de Belder, B. Lindberg, and O. Theander, *Acta Chem. Scand.*, **16**, 2005-2009 (1962).

(268) B. Norrman, *Acta Chem. Scand.*, **22**, 1623-1627 (1968).

sequence of secondary hydroxyl groups in methyl β -D-glucopyranoside, and indicated that the 6-hydroxyl groups in both methyl α - and β -D-glucopyranoside were by far the most reactive of all the hydroxyl groups.

Measurements on methyl α -D-glucopyranoside and its 6-benzyl and 6-(tetrahydropyran-2-yl) ethers, as well as on the (1 \rightarrow 6)-linked polysaccharides dextran and pustulan, revealed a reactivity sequence amongst secondary hydroxyl groups of HO-2 > HO-4 > HO-3, and it was concluded that the general pattern of reactivity in D-glucose polymers follows that shown by analogously substituted D-glucose derivatives. The relative reactivities of the hydroxyl groups in both anomers of methyl D-glucopyranoside towards *N,N*-diethylaziridinium chloride were found to be dependent on the concentration of the base in which the reaction was conducted,²⁶⁹ the extent of reaction at HO-6 remaining essentially constant with increasing concentration of the base, whereas that at the secondary hydroxyl groups decreased. From measurements on 2- and 3-O-substituted D-glucopyranosides,²⁷⁰ it appeared that substitution at HO-2 enhances the reactivity of HO-3 and HO-4 towards *N,N*-diethylaziridinium chloride in dilute base, but has little effect on the reactivity of HO-6. In contrast, substitution at HO-3 depressed the reactivity of HO-2 throughout the range of base concentrations used, and enhanced the reactivity of HO-6. These results were rationalized in terms of oxyanion stability, hydrogen-bonding possibilities, chelation of metal ions, and solvation of the oxyanions.

Methyl α -D-glucopyranoside may be converted,²⁷¹ in a remarkably selective reaction, into its 2,4,6-tribenzyl ether (in 62% yield) on treatment with three molar equivalents of sodium hydride in benzyl chloride at 110°, a substitution pattern that might have been predicted in view of the low, relative reactivity of HO-3 towards alkylation in the mechanistically related, Haworth procedure.²⁶⁸ Similar, selective benzylations have also been achieved on partially substituted derivatives of methyl α - and β -D-galactopyranoside²⁷² and on methyl 6-deoxy- α -L-galactopyranoside⁶²; in all of these, an unexpectedly high relative-reactivity of HO-4 (*ax*) compared to that of HO-3 (*eq*) was noted, indicating that steric factors are not the sole influence on reactivity in these cases. Nevertheless, the primary hydroxyl

(269) E. J. Roberts, C. P. Wade, and S. P. Rowland, *Carbohydr. Res.*, **17**, 393-399 (1971).

(270) E. J. Roberts, C. P. Wade, and S. P. Rowland, *Carbohydr. Res.*, **21**, 357-367 (1972).

(271) S. Koto, Y. Takebe, and S. Zen, *Bull. Chem. Soc. Jpn.*, **45**, 291-293 (1972).

(272) H. M. Flowers, *Carbohydr. Res.*, **39**, 245-251 (1975).

group in methyl 2,3-di-*O*-benzyl- α -D-galactopyranoside was found to be much more reactive than HO-4 towards benzylation.²⁷²

The d.s. at the various hydroxyl groups in methyl β -D-glucopyranoside on partial, Purdie methylation has been ascertained by n.m.r. spectroscopy.²⁷³ After reaction, the mixed products were fully methylated with trideuteriomethyl iodide, and the relative intensities of the signals for methyl protons were measured. Assignment of the signals, with the aid of specifically deuterated methyl ethers of the glycoside, allowed determination of the d.s. at the 2-, 3-, 4-, and 6-hydroxyl groups as 86, 40, 28, and 64%, respectively. In contrast, the relative reactivity of the hydroxyl groups in methyl β -D-glucopyranoside towards tetra-*O*-acetyl- α -D-glucopyranosyl bromide in the presence of silver oxide was found to be HO-6 \gg HO-3 > HO-4 > HO-2, although the differences in reactivity amongst the secondary groups were not large.²⁷⁴ The comparable reactivity of HO-4 to that of the other secondary groups is noteworthy in view of difficulties that have been experienced in synthesizing (1 \rightarrow 4)-linked disaccharides, but such difficulties may be due to steric hindrance by protecting groups rather than to inherently low reactivity.

Selective reactivity of the hydroxyl groups in methyl α -D-manopyranoside has been investigated under the methylation conditions of Haworth, of Kuhn (methyl iodide-silver oxide-*N,N*-dimethylformamide), and of Hakomori (methyl iodide-methylsulfinyl carbanion-dimethyl sulfoxide).²⁷⁵ For the first method, the reactivity order was HO-6 > HO-2 > HO-3 > HO-4; for the second, HO-2 > HO-3 > HO-4 \geq HO-6; and, for the third, HO-2 > HO-6 > HO-4 \geq HO-3. Although these orders were obtained from the overall, relative-substitution patterns of the hydroxyl groups (on considering all of the methyl ethers), and failed to take into account the effect that substitution at one hydroxyl group may have on the rate of substitution at another, they do, nevertheless, illustrate the strong dependence of selective reactivity on the reaction conditions. A similar investigation on methyl β -D-xylopyranoside showed that, towards Purdie methylation (methyl iodide-silver oxide), the reactivity sequence is HO-2 > HO-4 > HO-3, whereas, towards Hakomori methylation, it is²⁷⁶ HO-4 > HO-2 > HO-3.

(273) D. Gagnaire and L. Odier, *Carbohydr. Res.*, **11**, 33-41 (1969).

(274) A. M. Bills and J. W. Green, *J. Chem. Soc., B*, 716-720 (1967).

(275) N. Handa and R. Montgomery, *Carbohydr. Res.*, **11**, 467-484 (1969).

(276) Yu. S. Ovodov and E. V. Evtushenko, *Carbohydr. Res.*, **27**, 169-174 (1973).

Quantitative determination of the products from Haworth methylation of benzyl 4-*O*-methyl- β -D-xylopyranoside gave²⁷⁷ the ratio of rate constants $k_2:k_3$ as 3.2:1. Satisfactory agreement between predicted and observed product-ratios was found if it was assumed that, after methylation of HO-2, the reactivity of HO-3 increases by a factor of 3, but that methylation of HO-3 does not alter the reactivity of HO-2. The greater reactivity at HO-2 is, presumably, a result of its greater acidity, resulting from the inductive effect of two acetal oxygen atoms on C-1. When this group is ionized, the acidity of HO-3 should be decreased, but methylation at HO-2 removes the effect. Methylation at HO-3 should not, however, similarly affect HO-2.

c. Furanoid Derivatives of Monosaccharides.—There is considerable evidence that etherification of nucleosides and their derivatives may occur selectively at secondary hydroxyl groups. On Kuhn methylation of 2,2'-anhydro-1- β -D-arabinofuranosyluracil, alkylation in the sugar moiety occurred mainly at HO-3', and, during permethylation of 1- β -D-arabinofuranosyluracil, the results of monitoring by n.m.r. spectroscopy suggested that the 5'-hydroxyl group is present even after methylation at the secondary hydroxyl groups is complete.²³⁵ Reaction of uridine with benzyl bromide-sodium hydride in dimethyl sulfoxide gave *N*³-benzyl-2'-*O*-benzyluridine (33%), in addition to *N*³-benzyluridine²⁷⁸; 4-(methylthio)uridine and cytidine both showed selectivity for reaction at HO-2' under similar conditions.²⁷⁹ The monomethyl ether fraction obtained on partial methylation of cytidine in aqueous potassium hydroxide with dimethyl sulfate contained the 2', 3', and 5'-methyl ethers in²⁸⁰ the approximate ratios of 4:1:1. Under these alkaline conditions of reaction, all of the sugar hydroxyl groups are partly ionized. The pK_a of HO-5' has been estimated at 15–15.5 for adenosine,²⁸¹ whereas the *cis*-2',3'-diol grouping possesses¹⁰⁸ a pK_a of ~ 12.5 . The enhanced acidity of the vicinal-diol grouping is, presumably, responsible for the selective reactivity of nucleosides at HO-2' or HO-3' towards alkylating agents under basic conditions.

The interesting observation has been made that, although treatment of *N*⁶-trityl-5'-*O*-trityladenosine with benzyl chloride-sodium

(277) P. J. Garegg, *Acta Chem. Scand.*, **17**, 1343–1347 (1963).

(278) N. Imura, T. Tsuruo, and T. Ukiki, *Chem. Pharm. Bull.*, **16**, 1105–1109 (1968).

(279) K. Kikugawa, F. Sato, T. Tsuruo, N. Imura, and T. Ukita, *Chem. Pharm. Bull.*, **16**, 1110–1115 (1968).

(280) J. T. Kuśmierk, J. Giziewicz, and D. Shugar, *Biochemistry*, **12**, 194–200 (1973).

(281) J. B. Gin and C. A. Dekker, *Biochemistry*, **77**, 1413–1420 (1968).

hydride in 1,4-dioxane-benzene gives the 2',3'-dibenzyl ether as the main product, benzylation with benzyl bromide-potassium hydroxide in acetonitrile-1,4-dioxane containing 1-2 molar equivalents of water ceases, almost exclusively, at the monobenzylation stage, and favors the 2'-ether.²⁸²

d. Oligosaccharides and Polysaccharides.—Most of the reports on the partial alkylation of disaccharides have concerned sucrose. On treatment with methyl *p*-toluenesulfonate in tri-*O*-methylglycerol as the solvent, a trisodium salt of sucrose gave, amongst other compounds, a mixture of mono-*O*-methylsucroses, shown (by identification of its acid-hydrolysis products) to contain sucrose derivatives substituted on O-2 and O-6 of the D-glucosyl group and on O-1' and O-3' of the D-fructosyl group.²⁸³ This substitution pattern for D-glucose is in keeping with the known, relative reactivities of the hydroxyl groups in methyl α -D-glucopyranoside in alkaline media.²⁶⁵ In contrast, it was claimed²⁸⁴ that alkylation of a monosodium salt of sucrose with methyl iodide yielded a mixture of mono-*O*-methylsucroses containing each of the three isomers in which a primary hydroxyl group is methylated, but identification of the products was based only on chromatographic comparisons in one solvent system. Selective benzylation of sucrose under Kuhn conditions has been reported²⁸⁵ to proceed with reasonable selectivity, one molar equivalent of benzyl bromide giving the 2-, 3-, and 1'-, and 3'-benzyl ethers in the ratios of 86:1:3:10. Only the first of these compounds was obtained crystalline, and other identifications rested largely on chromatographic comparisons. The selective reaction at HO-2 of the D-glucosyl group and the apparent lack of etherification at the primary centers are noteworthy. The selective silylation of sucrose with chlorotricyclohexylsilane has been noted.²⁴³

Most of the selective-etherification studies on polysaccharides have been made with cellulose, and nearly all of them have involved quantitative separation of the D-glucose derivatives formed on hydrolysis of the partially substituted celluloses. In view of their stability, ethers of polysaccharides are particularly suited to this approach.

(282) A. Myles and W. Pfeleiderer, *Chem. Ber.*, **105**, 3327-3333 (1972).

(283) W. A. P. Black, E. T. Dewar, and D. Rutherford, *J. Chem. Soc.*, 3073-3077 (1959).

(284) F. Grundschober and V. Prey, *Monatsh. Chem.*, **92**, 1290-1293 (1961).

(285) E. Reinefeld and K.-D. Heincke, *Chem. Ber.*, **104**, 265-269 (1971).

The products are formed in kinetically controlled reactions, except in those instances, considered in the next subsection, where ethers result from the addition of a hydroxyl group to an activated alkene. The analytical method of Spurlin²⁶⁶ has often been used in order to evaluate relative rate-constants for reaction at the hydroxyl groups.

The relative reactivities of hydroxyl groups in cellulose have been studied under alkaline conditions with the following reagents: (1) dimethyl sulfate,²⁸⁶ (2) chloromethane,²⁸⁷ (3) chloroethane,^{288,289} (4) allyl sodium sulfate,²⁹⁰ (5) monochloroacetic acid,²⁹¹ (6) oxirane,²⁹²⁻²⁹⁵ (7) 1-chloro-2-(diethylamino)ethane,²⁹⁶ (8) 2-aminoethyl sodium sulfate,²⁹⁷ and (9) bis(2-chloroethyl)methylamine.²⁹⁸ The results are collected in Table I. The reaction with diazomethane (10 in Table I) under neutral conditions has also been investigated,²⁹⁹ and the findings are included therein in view of the irreversible nature of the methylation. The relative rate-constants reveal a consistently high reactivity of HO-2 relative to HO-3, and the former is comparable to, and in some cases greater than, that of the primary hydroxyl group, HO-6. This finding may be rationalized in terms of the high, relative acidity of HO-2, enhanced by its proximity to the anomeric center. The low reactivity of HO-3 is also apparent in all of the reactions.

The differences in reactivities of the three hydroxyl groups towards methylation do not seem to be affected by other cellulose chains in heterogeneous reactions, as similar ratios were found for both heterogeneous and homogeneous reactions.²⁸⁶ However, in work involving the repeated, heterogeneous methylation of cotton cellulose, the relative reactivities of the hydroxyl groups have been

(286) I. Croon and B. Lindberg, *Sven. Papperstidn.*, **60**, 843-849 (1957).

(287) I. Croon, *Sven. Papperstidn.*, **61**, 919-921 (1958).

(288) I. Croon and E. Flamm, *Sven. Papperstidn.*, **61**, 963-966 (1958).

(289) O. Ramnäs, *Acta Chem. Scand.*, **27**, 3139-3146 (1973).

(290) D. E. Hoiness, C. P. Wade, and S. P. Rowland, *Can. J. Chem.*, **46**, 667-672 (1968).

(291) I. Croon and C. B. Purves, *Sven. Papperstidn.*, **62**, 876-882 (1959).

(292) I. Croon and B. Lindberg, *Sven. Papperstidn.*, **59**, 794 (1956).

(293) I. Croon and B. Lindberg, *Sven. Papperstidn.*, **59**, 795-796 (1956).

(294) I. Croon and B. Lindberg, *Sven. Papperstidn.*, **59**, 797-798 (1956).

(295) I. Croon and B. Lindberg, *Sven. Papperstidn.*, **59**, 799 (1956).

(296) E. J. Roberts and S. P. Rowland, *Can. J. Chem.*, **45**, 261-265 (1967).

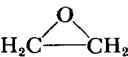
(297) E. J. Roberts and S. P. Rowland, *Can. J. Chem.*, **47**, 1571-1580 (1969).

(298) E. J. Roberts and S. P. Rowland, *Can. J. Chem.*, **48**, 1383-1390 (1970).

(299) I. Croon, *Sven. Papperstidn.*, **62**, 700-702 (1959).

TABLE I

Relative Reactivities of HO-2, HO-3, and HO-6 in Cellulose towards Various Reagents

Reagents	Relative rate-constants ^{a,b}			Notes	References
	k_2	k_3	k_6		
1. Me ₂ SO ₄	3.5	1	2	^c	286
2. MeCl	5	1	2		287
3. EtCl	4.5	1	2		288
	7	1	6.5		289
4. H ₂ C=CH—CH ₂ OSO ₃ ⁻ Na ⁺	3.5	1	5		290
5. ClCH ₂ CO ₂ H	2	1	2.5	^d	291
6. 	3	1	10	^{e,f}	292-295
7. Et ₂ NCH ₂ CH ₂ Cl	3.6	1	2.9		296
8. H ₂ NCH ₂ CH ₂ OSO ₃ ⁻ Na ⁺	4.6	1	7.1		297
9. MeN(CH ₂ CH ₂ Cl) ₂	8.2	1	3.6		298
10. CH ₂ N ₂	1.2	1	1.5		299

^a Normalized to $k_3 = 1$. ^b For entries 4 and 7-9, the numbers represent the relative distribution of the substituents between O-2, O-3, and O-6, at degrees of substitution of 0.07, 0.048, 0.14, and 0.034, respectively; thus, the relative rate-constants are only approximate. ^c The relative rate-constant for HO-3 was assumed to be doubled after methylation at HO-2. ^d The relative rate-constant at one of the secondary hydroxyl groups was assumed to be 0.3 when the other one is substituted. ^e The relative rate-constant for etherification of the 2-hydroxyethyl group is 10. ^f The relative rate-constants found are now thought to have been erroneous, as the formation of internal acetals^{300,301} was not taken into account.

rationalized in terms of fibril structure.³⁰² The low reactivity of HO-3 does not seem explicable in terms of steric hindrance from the hydroxymethyl group on C-5, as methylation of a (1 → 4)-xylan that lacks the latter grouping gave³⁰³ $k_2:k_3 = 2.5:1$. The possibility that the low reactivity of HO-3 in cellulose could partly result from a strong hydrogen-bond between this hydroxyl group and the ring-oxygen atom of a contiguous D-glucosyl residue³⁰⁴ was supported by the results of methylation studies on methyl 4,6-O-benzylidene- and 4,6-O-ethylidene-β-D-glucopyranoside, which showed $k_2:k_3$ to be 1.3:1 and 1.1:1, respectively.²⁴⁸ However, measurements²⁶⁷ on the

(300) E. J. Roberts and S. P. Rowland, *Can. J. Chem.*, **47**, 1592-1595 (1969).

(301) J. E. Höök and B. Lindberg, *Acta Chem. Scand.*, **22**, 2157-2160 (1968).

(302) S. Haworth, D. M. Jones, J. G. Roberts, and B. F. Sagar, *Carbohydr. Res.*, **10**, 1-12 (1969).

(303) I. Croon and T. E. Timell, *J. Am. Chem. Soc.*, **82**, 3416-3418 (1960).

(304) C. Y. Liang and R. H. Marchessault, *J. Polym. Sci.*, **37**, 385-395 (1959).

methylation of the 4-benzyl and 4-(tetrahydropyran-2-yl) ethers of methyl β -D-glucopyranoside, as model compounds for cellulose, showed a higher reactivity of HO-2 compared to that of HO-3, as found for cellulose. The low reactivity of HO-3 in cellulose need not, therefore, be ascribed to any special effects; the higher acidity of HO-2, coupled with the lower steric requirements for reaction at HO-6, would appear to explain the ratios observed.

The size of the reagent appears to exert an influence on substituent distribution; there is a progressive decrease in the relative degree of substitution on HO-2 on reaction with chloromethane, chloroethane, and 1-chloro-2-(diethylamino)ethane. In contrast to methylation with dimethyl sulfate and to aminoethylation, which are sluggish reactions, 2-(diethylamino)ethylation of cellulose proceeds rapidly at room temperature, and the effective reagent could be the *N,N*-diethylaziridinium cation. Aminoethylation of cellulose with aziridine (ethylenimine) was not successful, and this suggests that reaction with 2-aminoethyl sodium sulfate occurs without participation of the amino group; differing mechanisms for aminoethylation and 2-(diethylamino)ethylation are consistent with the differences in relative reactivities of hydroxyl groups observed in the reactions with the two reagents.

Cross-linking of cellulose, important in the production of minimum-care fabrics, has been studied by using bis(2-chloroethyl)methylamine.²⁹⁸ A surprisingly large degree of monosubstitution (43%) occurs, mainly at HO-2. Cross-linking of cellulose residues is favored between O-2 and O-6', suggesting that steric factors are important in the formation of the second bond in the cross-linking reaction.

Partial alkylations of other polysaccharides have received much less attention. Towards Haworth methylation, the primary hydroxyl groups in amylose were found²⁴⁸ to be the most reactive ($k_2:k_3:k_6 = 6:1:7$), and, as with cellulose,²⁸⁶ better agreement between observed and calculated percentages of products was obtained if it was assumed that the reactivity of HO-3 is doubled when the 2-hydroxyl group is substituted. Isolation of the monosubstituted products from hydrolysis of a partially methylated amylose (obtained by reaction of a sodio derivative with methyl iodide) afforded³⁰⁵ the 2-, 3-, and 6-methyl ethers in the ratios 13:5:6. Reaction of oxirane with waxy-maize starch (>99% of amylopectin) indicated³⁰⁶ a high, relative reactivity of HO-2 ($k_2:k_3:k_6 = 33:5:6$). In this work, account was

(305) B. J. Bines and W. J. Whelan, *J. Chem. Soc.*, 4232-4233 (1962).

(306) A. N. de Belder and B. Norrman, *Carbohydr. Res.*, **10**, 391-394 (1969).

taken of the cyclization of the 2-*O*-(2-hydroxyethyl) derivatives of D-glucose to afford the anomers of 1,2-*O*-ethylene-D-glucofuranose and -D-glucopyranose³⁰¹ during polysaccharide hydrolysis; earlier work on amylose,³⁰⁷ although indicating a high, relative reactivity of HO-2 as compared to that of HO-6, did not make this correction, and thus the estimated substitution at O-2 in the polysaccharide was too low. A similar, high reactivity of HO-2 was noted in the reaction of starch with oxirane.³⁰⁸

Partial, Haworth methylation of a partially hydrolyzed dextran (M_w 40,000), and estimation of the hydrolysis products at low d.s. values, gave³⁰⁹ $k_2:k_3:k_4 = 16:2:7$. Spurlin's analysis,²⁶⁶ with certain assumptions, was used to determine the change in the relative rate-constant k_3 during the reaction. The best fit with the experimental data was obtained when $k_a:k_b:k_c = 15:20:26$, where k_a , k_b , and k_c are, respectively, the relative rate-constants for HO-3 when HO-2, HO-4, and both HO-2 and HO-4 are methylated. *O*-(2-Hydroxyethyl)ation of a dextran of higher molecular weight (M_w 70,000) gave³⁰⁶ relative reactivities comparable to those observed on methylation, with $k_2:k_3:k_6 = 5.8:1:3$. Experiments on pustulan with partial methylation by means of dimethyl sulfate-aqueous sodium hydroxide to a low d.s. gave²⁶⁸ relative reactivities for the hydroxyl groups in an order similar to that for those measured for the dextran, with $k_2:k_3:k_4 = 9.3:1:3$.

e. Cyclitols.—In cyclitol chemistry, etherification at equatorial rather than axial hydroxyl groups has been reported, but other factors, such as the nature of the reagent, can exert marked influences on such selective reactivity. Thus, the equatorial hydroxyl group in DL-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol can be benzylated by using potassium hydroxide-benzyl chloride in benzene, to yield DL-1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol in 63% yield³¹⁰; only 1% of the axially substituted isomer was produced. However, reaction of the tetra-*O*-benzyl derivative with benzyloxymethyl chloride was not selective, and it was suggested that reactions in which the equatorial hydroxyl group in such derivatives of *myo*-inositol are mainly etherified are those which involve a transition state, reversibly formed, in which the carbon atom forming the new bond to the oxygen atom is attached to more atoms than it is in the final product (as, for example,

(307) C. E. Lott, Jr., and K. M. Brobst, *Anal. Chem.*, **38**, 1767-1770 (1966).

(308) H. C. Srivastava and K. V. Ramalingam, *Staerke*, **19**, 295-300 (1967); *Chem. Abstr.*, **67**, 101,230p (1967).

(309) B. Normman, *Acta Chem. Scand.*, **22**, 1381-1385 (1968).

(310) S. J. Angyal and M. E. Tate, *J. Chem. Soc.*, 6949-6955 (1965).

in S_N2 reactions and in esterification). Reactions proceeding through carbenium-ion intermediates [for example, the aforementioned reactions with benzyloxymethyl chloride, and the reaction of 3,4-dihydro-2*H*-pyran with hydroxyl groups in the presence of acid (see Section IV)] are very rapid, and insensitive to steric hindrance.

Two other *myo*-inositol derivatives have been selectively alkylated. Reaction of DL-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol with benzyl chloride-potassium hydroxide in benzene, followed by removal of the acetal groups, gave DL-1-*O*- and DL-4-*O*-benzyl-*myo*-inositol in the ratio of 5:2, whereas, under similar conditions, DL-1,2:5,6-*O*-cyclohexylidene-*myo*-inositol gave³¹¹ the same ethers in the ratio of 57:10. These results are not readily explicable in the absence of knowledge of the conformations adopted by the cyclic acetals.

Apparently, the solvent may also influence the selectivity during etherification. Reaction of 1L-1,2,3,4-tetra-*O*-benzyl-*chiro*-inositol, which possesses one equatorial and one axial hydroxyl group in its most-stable chair conformation, with benzyl chloride-potassium hydroxide in benzene gave an equatorial:axial substitution ratio for the penta-*O*-benzyl derivatives of 13:7, but, on reaction in benzyl chloride as the solvent, benzylation at the axial group predominated, the ratio then³¹² being 21:79. A similar tendency for alkylation at an axial hydroxyl group when benzyl chloride was the reaction medium was noted with 1L-3,4-di-*O*-benzyl-1,2-*O*-cyclohexylidene-*chiro*-inositol, the ratio of axial to equatorial benzylation being 21:4; in benzene, benzylation afforded almost equal amounts of the two isomers.

It is apparent that, in the aforementioned reactions of the tetra-*O*-benzyl-*myo*- and -*chiro*-inositol derivatives, reactivity is governed not solely by the axial or equatorial disposition of the hydroxyl groups but also by the configurations at centers neighboring these groups. Thus, the unreactive, axial HO-2 in the *myo*-inositol derivative is flanked by two equatorial (*cis*) substituents, but the more-reactive, axial hydroxyl group in the *chiro*-inositol derivative has one neighboring substituent equatorial (*cis*) and the other axial (*trans*), resulting in less steric hindrance than in the former compound.

f. Acyclic Derivatives.—The remarkably selective methylation achieved on treatment of D-glucose diethyl dithioacetal with methyl iodide-silver oxide at 0°, to give the 2-methyl ether in 51% yield, was reported to be unsuccessful when applied to dithioacetals of D-

(311) S. J. Angyal and A. F. Russell, *Aust. J. Chem.*, **22**, 391-404 (1969).

(312) S. J. Angyal and T. S. Stewart, *Aust. J. Chem.*, **19**, 1683-1691 (1966).

galactose, L-arabinose, L-rhamnose, and D-xylose.³¹³ A chromatographic investigation³¹⁴ confirmed that, from D-glucose diethyl dithioacetal, the only monoether formed is the 2-methyl, and showed that the apparent lack of reactivity of D-mannose, D-galactose, and L-arabinose dithioacetals towards Purdie methylation is merely due to their insolubility in methyl iodide. On conducting the reactions in tetrahydrofuran, the supposedly peculiar selectivity of the D-glucose derivative was no longer apparent. Under these conditions, L-arabinose diethyl dithioacetal gave, after acid hydrolysis of the mixture of reaction products, mainly 2-O-methyl-L-arabinose, a trace of 5-O-methyl-L-arabinose, and some more-highly methylated components.³¹⁵ D-Xylose diethyl dithioacetal afforded³¹⁶ the 2-, 3-, and 5-methyl ethers in the ratios of 20:10:1, and D-mannose diethyl dithioacetal gave³¹⁷ the 2-, 3-, and 6-methyl ethers in the ratios of 12:1:1. D-Galactose diethyl dithioacetal was exceptional in yielding the 2-, 3-, and 6-methyl ethers in approximately equal amounts.³¹⁸

The high tendency shown in several of these instances for methylation at O-2 could be the result of initial, electrophilic attack by methyl iodide at sulfur, leading to the formation of a methylsulfonium salt; that such a species might then transfer its methyl group to another nucleophilic site is given credence by the fact that the biological methylating agent S-adenosylmethionine functions in this way. Intramolecular, nucleophilic attack by O-2 on the methyl group of the sulfonium salt would lead, through a five-membered, transition state to a 2-methyl ether. Analogously, a six-membered transition-state would result from attack by O-3. It is noteworthy that 4-methyl ethers of the aldopentoses and aldohexoses and 5-methyl ethers of the aldohexoses, which, by this mechanism, would require seven- or eight-membered transition-states, respectively, have not been observed in these studies. The fact that the terminal hydroxyl group is found to be methylated in several instances is most reasonably seen as resulting from direct O-methylation at the relatively unhindered primary hydroxyl group.

2. Activated Alkenes

Although reaction of carbohydrates with such activated alkenes as acrylonitrile and methyl vinyl sulfone has industrial importance, the

(313) T. Lieser and E. Leckzyck, *Ann.*, **511**, 137-140 (1934).

(314) G. G. S. Dutton and K. Yates, *Can. J. Chem.*, **36**, 550-556 (1958).

(315) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **39**, 1797-1800 (1961).

(316) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **40**, 1899-1902 (1962).

(317) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **41**, 2500-2503 (1963).

(318) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **40**, 1146-1148 (1962).

positions of substitution in carbohydrates on partial etherification with these reagents have not been extensively investigated. As the reactions are reversible, and thus subject to thermodynamic control, the relative stability of the products is of overriding importance in deciding the product composition.

On treatment with acrylonitrile in 2% aqueous sodium hydroxide at 0°, tetrahydropyran-2-yl β -D-glucopyranoside gave the 2-, 3-, 4-, and 6-O-(2-cyanoethyl) ethers (together with some diethers) in yields that, on extrapolation to zero reaction, showed³¹⁹ $k_2:k_3:k_4:k_6$ to be in the ratios of 3:1:2:8; these values represent equilibrium, not rate, constants. The tendency for substitution at O-6 is a consequence of the greater stability of an ether derived from a primary (compared to a secondary) hydroxyl group, as a result of lower steric interactions in the former.

The reaction between cellulose and acrylamide was studied by quantitative, chromatographic separation of the substituted D-glucoses obtained on acid hydrolysis of the reaction product,³²⁰ followed by an analysis by Spurlin's method.²⁴⁹ Although, apparently, no check was made on the stability of the ethers to the conditions of hydrolysis, it might be expected that the ethers would isomerize only under basic conditions. The ratios of the relative equilibrium-constants for reaction at O-2, O-3, and O-6 were 9:1:19, and these are attributable to the high, relative stability of the primary ether, together with the low reactivity of O-3, also observed in rate-controlled reactions.

The sites of etherification of cellulose by methyl vinyl sulfone were investigated³²¹ by reaction to a d.s. of 0.11, followed by g.l.c. analysis of the products of hydrolysis. The ratios of 2-, 3-, and 6-ethers of D-glucose were found to be 20:3:100, with no evidence for di- (or higher-) substituted derivatives, and the overriding reactivity of the primary hydroxyl group is again noteworthy, in contrast to kinetically controlled reactions wherein 2-substitution is either favored over, or is comparable with, O-6-substitution. In an extension of this work,³²² methyl vinyl sulfone was generated in a variety of ways, and, under nonequilibrium conditions, the extent of 2- compared with 6-substitution in cellulose was achieved in ratios of up to 0.8:1; the ratio appears to be a function of the reagents employed,

(319) P. J. Garegg and J. Kubo, *Acta Chem. Scand.*, **18**, 207-212 (1964).

(320) G. F. Touzinsky, *J. Org. Chem.*, **30**, 426-428 (1965).

(321) S. P. Rowland, V. O. Cirino, and A. L. Bullock, *Can. J. Chem.*, **44**, 1051-1058 (1966).

(322) S. P. Rowland, A. L. Bullock, V. O. Cirino, and C. P. Wade, *Can. J. Chem.*, **46**, 451-457 (1968).

the reaction conditions, and the rate of diffusion of the reagents into the cellulose fibers. The cross-linking of cellulose with divinyl sulfone involves principally the primary hydroxyl groups,³²³ as would be expected from steric considerations, in contrast to cross-linking with bis(2-chloroethyl)methylamine, when (2 → 6')-links predominate.²⁹⁸

3. Diazoalkanes

Although it is generally understood that hydroxyl groups do not react with diazomethane unless they are appreciably acidic, cellulose reacts with diazomethane in moist ether to give, after acid hydrolysis, all seven of the O-methyl-D-glucoses possible, and the rate constants k_2 , k_3 , k_6 were calculated²⁹⁹ to be in the ratios of 2.4:2:3. The reaction of diazomethane with alcohols is facilitated by the presence of certain protonic acids (such as fluoroboric acid³²⁴) and Lewis acids (for example, boron trifluoride in the form of its diethyl etherate,³²⁵ and aluminum chloride³²⁶). The mechanism of methylation of aliphatic alcohols with diazomethane catalyzed by boron compounds, which may also operate for other Lewis acids, has been discussed.³²⁷

The presence of minute proportions (10 to 100 millimolar equivalents) of stannous chloride dihydrate was also found³²⁸ to catalyze, remarkably, the reaction of some D-glucopyranoside derivatives with diazomethane in methanol or methanol-*N,N*-dimethylformamide; without this catalyst, little methylation occurred. Methyl 4,6-O-benzylidene- α -D-glucopyranoside was reported to afford 93% of the 3-methyl ether, but alkylation of the β -D anomer was less selective and gave the 2- and 3-methyl ethers in yields of 34 and 52%, respectively. Reaction of methyl and phenyl α -D-glucopyranoside showed unprecedented selectivity, yields of 74 and 81% of the respective 3-methyl ethers being obtained, together with minor proportions of the 2,3-dimethyl ethers. Methyl and phenyl β -D-glucopyranoside gave 3-methyl ethers in yields of 54 and 47%, respectively, but, with these β -D-glucosides, 2,3-dimethyl ethers were simultaneously formed in much higher yields (48 and 44%, respectively) than with the α -D-

(323) V. O. Cirino, A. L. Bullock, and S. P. Rowland, *Carbohydr. Res.*, **17**, 67-78 (1971).

(324) M. Neeman, M. C. Caserio, J. D. Roberts, and W. S. Johnson, *Tetrahedron*, **6**, 36-47 (1959).

(325) E. Müller and W. Rundell, *Angew. Chem.*, **70**, 105 (1958).

(326) E. Müller, R. Heischkeil, and M. Bauer, *Ann.*, **677**, 55-58 (1964).

(327) C. E. H. Bawn and A. Ledwith, *Chem. Ind. (London)*, 1329-1331 (1958).

(328) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **18**, 677-686 (1970).

glucosides. Other Lewis acids (such as aluminum chloride hexahydrate, magnesium chloride hexahydrate, zinc chloride, and lead acetate trihydrate) were less satisfactory in promoting alkylation. The methylation of benzyl 4,6-*O*-benzylidene- β -D-galactopyranoside with diazomethane catalyzed by stannous chloride was also highly regioselective,³²⁹ the 2-methyl ether being formed in 91% yield; with boron trifluoride diethyl etherate as the catalyst, the 2,3-dimethyl ether was obtained in 84% yield. Involvement of HO-3 in intramolecular hydrogen-bonding to O-4 (which, it was suggested, is unaffected by stannous chloride, but prevented in the presence of boron trifluoride diethyl etherate) may explain these observations, as the unreactivity of strongly bonded hydroxyl groups towards diazomethane had been noted.²⁶

The first selective reaction involving HO-2' as the major site of reaction in an unprotected, naturally occurring D-ribonucleoside was claimed³³⁰ for the preparation of 2'-*O*-methyladenosine by the action of diazomethane on adenosine in aqueous 1,2-dimethoxyethane at 80°. Subsequent investigations by other workers^{281,331} showed that the mono-*O*-methyl fraction actually contains both the 2'- and the 3'-methyl ethers, in the ratio of 3:1, and that very small proportions of the 5'-methyl and 2',3'-dimethyl ethers are also formed. Selective methylation of the *cis*-2',3'-diol grouping of adenosine was to be expected under these conditions, in view of the fact that a pK_a of 12.5 has been associated with this grouping,¹⁰⁸ and also from a consideration of the mechanism of alkylation of hydroxyl groups by diazoalkanes. The apparently greater acidity of HO-2' is supported by the following order of elution of methyl ethers of adenosine from a strongly alkaline, ion-exchange resin [Dowex-1 2X (OH⁻)]: 2',3'-dimethyl (eluted first), 2'-methyl, 3'-methyl, and 5'-methyl.^{281,332}

The action of diazomethane in aqueous 1,2-dimethoxyethane on 4-methoxy-1- β -D-ribofuranosyl-2(1*H*)-pyrimidinone gave, after purification, the corresponding 2'-methyl ether in 37% yield.³³³ N.m.r. spectroscopy showed that the crude mono-*O*-methyl fraction contained the 2'- and 3'- isomers in the ratio of 7:2. The 2'-methyl ether proved to be a versatile intermediate, as acid hydrolysis yielded 2'-*O*-methyluridine, whereas reaction with ammonia or methylamine

(329) G. J. F. Chittenden, *Carbohydr. Res.*, **43**, 366-370 (1975).

(330) R. K. Robins and A. D. Broom, *J. Am. Chem. Soc.*, **87**, 1145-1146 (1965).

(331) D. M. G. Martin, C. B. Reese, and G. F. Stephenson, *Biochemistry*, **7**, 1406-1412 (1968).

(332) C. A. Dekker, *J. Am. Chem. Soc.*, **87**, 4027-4029 (1965).

(333) M. J. Robins and S. R. Naik, *Biochemistry*, **10**, 3591-3597 (1971).

gave, respectively, 2'-O-methylcytidine and a (previously unsynthesized) minor component of t-RNA, namely, N⁴-methyl-2'-O-methylcytidine.

The catalytic effect of stannous chloride dihydrate has been utilized in the methylation of nucleosides. Reaction of a suspension of adenosine in methanol containing 60 millimolar equivalents of this catalyst with diazomethane in 1,2-dimethoxyethane afforded³³⁴ quantitative mono-O-methylation at O-2' and O-3' in the ratio of 2:3. On the other hand, cytidine gave the 2'- and 3'-methyl ethers in the ratio of 4:1. In general, purine nucleosides gave their 3'-methyl ethers in somewhat greater proportions than their 2'-ethers, and pyrimidine nucleosides gave mixtures of 2'- and 3'-methyl ether in which the former preponderated.^{334a} The fact that the method appeared to be general for D-ribonucleosides, but ineffective for 2'-deoxynucleosides suggests that a metal complex, or a cyclic derivative, of the *cis*-2',3'-diol grouping may be involved. In novel extensions of this reaction, a series of nucleosides was treated with phenyldiazomethane to yield the 2'- and 3'-benzyl ethers,³³⁵ which are of some importance in the synthesis of polynucleotides, and with *o*-nitrophenyldiazomethane, to yield derivatives having photolabile protecting groups.³³⁶ In the former reactions, selective alkylation at HO-2' was shown by inosine, uridine, and cytidine, but adenosine showed selective reaction at HO-3'. Guanosine gave approximately equal amounts of both isomers, and the factors that control the ratios of 2'- to 3'-isomers in these reactions are not yet clear.

The effectiveness of a wide range of inorganic compounds in catalyzing the monomethylation of the *cis*-glycol system in ribonucleosides has been evaluated^{336a}; certain catalysts led to significant differences in the ratio of 2'- to 3'-methyl ethers. Concerning the mechanism of the catalytic action of stannous chloride dihydrate, it is significant that, in 2',3'-O-(dibutylstannylene)ribonucleosides, the dibutylstannylene function serves as an activating group for the 2'- and 3'-oxygen atoms in esterification and alkylation reactions.^{336b}

(334) M. J. Robins and S. R. Naik, *Biochim. Biophys. Acta*, **246**, 341-343 (1971).

(334a) M. J. Robins, S. R. Naik, and A. S. K. Lee, *J. Org. Chem.*, **39**, 1891-1899 (1974).

(335) L. F. Christensen and A. D. Broom, *J. Org. Chem.*, **37**, 3398-3401 (1972).

(336) D. C. Bartholomew and A. D. Broom, *J. Chem. Soc. Chem. Commun.*, **38** (1975).

(336a) M. J. Robins, A. S. K. Lee, and F. A. Norris, *Carbohydr. Res.*, **41**, 304-307 (1975).

(336b) D. Wagner, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, **39**, 24-30 (1974).

IV. SELECTIVE ACETALATION

In this Section, only those acetals formed by reaction of single hydroxyl groups in carbohydrate molecules will be considered.

Interaction of equimolar proportions of a vinyl ether and methyl α -D-glucopyranoside in *N,N*-dimethylformamide, under catalysis by *p*-toluenesulfonic acid, and analysis of the product mixture by methylation techniques, revealed that, although reaction times of several days afforded 4,6-*O*-alkylidene derivatives, much shorter times led to the formation of methyl 6-*O*-(1-alkoxyethyl)- α -D-glucopyranoside together with the 4,6-cyclic acetal.³³⁷ The actual vinyl ether used also influenced the product composition. After a 1- to 2-hour reaction-period with methyl vinyl ether, the glycoside gave methyl 6-*O*-(1-methoxyethyl)- α -D-glucopyranoside and methyl 4,6-*O*-ethylidene- α -D-glucopyranoside in approximately equal amounts, whereas the latter compound was apparently the major product when isopropyl vinyl ether was used under almost the same conditions. When the D-glucoside in about three molar excess was treated with methyl or ethyl vinyl ether in dimethyl sulfoxide in the presence of boron trifluoride etherate for 2 hours at 35°, the product contained a much higher proportion of the monosubstituted derivative; the 6-*O*-(1-methoxyethyl) and 6-*O*-(1-ethoxyethyl) derivatives formed 75 and 80%, respectively, of the crude reaction products, indicating a considerable favoring of reaction at the primary hydroxyl group.

As these acetals could be converted into the 4,6-*O*-ethylidene derivatives on treatment with acid, it was reasoned that use of a cyclic vinyl ether, namely, 3,4-dihydro-2*H*-pyran, might prevent this second process, thus leading to a more useful method of selective acetalation.³³⁸ An equimolar reaction with methyl α -D-glucopyranoside for 4 days in *N,N*-dimethylformamide led to utilization of 88% of the glycoside, and the 6-(tetrahydropyran-2-yl) ether constituted ~85% of the crude reaction-product. In contrast to the steric control apparent in this instance, reaction of 3,4-dihydro-2*H*-pyran with the axial and equatorial hydroxyl groups in DL-1,4,5,6-tetra-*O*-acetyl-*myo*-inositol was completely unselective,³³⁹ a fact that has been rationalized³¹⁰ in terms of the probable mechanism of these reactions.

(337) M. L. Wolfrom, A. Beattie, and S. S. Bhattacharjee, *J. Org. Chem.*, **33**, 1067-1070 (1968).

(338) M. L. Wolfrom, A. Beattie, S. S. Bhattacharjee, and G. G. Parekh, *J. Org. Chem.*, **33**, 3990-3991 (1968).

(339) S. J. Angyal and S. D. Gero, *J. Chem. Soc.*, 5255-5258 (1965).

Substitution at HO-5' in D-ribonucleosides has been achieved through acid-catalyzed transacetalation. On treatment with 2,2-dimethoxypropane in *N,N*-dimethylacetamide in the presence of bis-(*p*-nitrophenyl) phosphate, uridine gave, after chromatographic purification of the product, 5'-*O*-(1-methoxyisopropyl)uridine in 46% yield³⁴⁰; a minor product appeared to be the 2'-(3')-substituted nucleoside (on the basis of its hydrolytic behavior). The selectivity observed is in agreement with the greater readiness with which simple acetals undergo alkoxyl interchange with primary than with secondary alcohols.³⁴¹ However, the greater tendency to react at HO-5' is, presumably, dependent on the hydrogen-ion concentration, as, on treatment with the acetal of a carbonyl compound in *N,N*-dimethylformamide in the presence of hydrogen chloride, nucleosides afford 2',3'-*O*-alkylidene derivatives in good yield.³⁴²

Favored reaction at HO-5' in uridine 3'-phosphate through the formation of a mixed acetal of an aliphatic aldehyde with another alcohol has been claimed, but product isolation required chromatography on cellulose, and the yield reported³⁴³ was low (~23%).

A synthetically useful method for the transformation of a primary hydroxyl group in an unprotected nucleoside into a substituted-thiol group involves treatment of the nucleoside with 1.2 molar equivalents of 2-pyrimidinethiol and an excess of *N,N*-dimethylformamide dineopentyl acetal in acetonitrile at the reflux temperature³⁴⁴; this yields 5'-*S*-pyrimidin-2-yl-5'-thionucleosides. The reaction proceeds through acetal exchange of one alkoxyl group in the diacetal of *N,N*-dimethylformamide for the nucleoside residue, followed by attack of the sulfur atom in 2-pyrimidinethiol on C-5', instead of attack at the sterically hindered, methylene carbon atom of the neopentyl group. 5'-*O*-Tritylthymidine gave no reaction under similar conditions, illustrating lack of reactivity of its secondary hydroxyl group (that is, good selectivity of this reaction for primary hydroxyl groups).

V. SELECTIVE HALOGENATION

The selective replacement of hydroxyl groups in carbohydrates by halogen has mainly been achieved by the use of (a) sulfonyl chloride, (b) reagents based on phosphorus compounds, and (c) reagents of the

(340) A. Hampton, *J. Am. Chem. Soc.*, **87**, 4654-4655 (1965).

(341) W. L. Howard and N. B. Lorette, *J. Org. Chem.*, **25**, 525-530 (1960).

(342) S. Chládek and J. Smrť, *Collect. Czech. Chem. Commun.*, **28**, 1301-1308 (1963).

(343) S. M. Zhenodarowa and E. A. Sedelnikova, *Tetrahedron Lett.*, 3337-3340 (1966).

(344) A. Holý, *Tetrahedron Lett.*, 585-588 (1972).

N,N-dimethyl(halomethaniminium) halide type. The subject of deoxyhalogeno sugars has been discussed twice in this Series.^{345,346}

1. Sulfuryl Chloride

Helferich and coworkers reported the reaction of sulfuryl chloride in pyridine-chloroform with methyl α - and β -D-glucopyranoside,³⁴⁷ a trehalose,³⁴⁸ and a mannitol,³⁴⁸ to yield products in which some hydroxyl groups were replaced by chlorine atoms and others were engaged in cyclic sulfate formation. The product derived from the α -D-glucoside was initially thought³⁴⁹ to have chloro substituents on C-5 and C-6, but its correct structure was later shown to be that of methyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside 2,3-cyclic sulfate,^{350,351} inversion of configuration having occurred at C-4 during introduction of the chloro substituent. Further study showed³⁵² that, in these reactions, the chloro substituent is introduced through a bimolecular, nucleophilic displacement of a chlorosulfonyloxy group by a chloride ion, with concomitant inversion at a secondary center. As chlorosulfonyloxy groups are readily removed, the reagent offers a useful route to chlorodeoxy sugars. The d.s. achieved in these reactions is determined by steric and electronic effects in the individual sugar derivatives. Thus, reaction of methyl α -D-glucopyranoside with sulfuryl chloride in pyridine-chloroform solution at -70° yielded methyl α -D-glucopyranoside 2,3,4,6-tetra(chlorosulfate),³⁵² which, on treatment with one molar proportion of pyridine hydrochloride in chloroform, afforded methyl 6-chloro-6-deoxy- α -D-glucopyranoside 2,3,4-tri(chlorosulfate) in 65% yield; an excess of the hydrochloride gave methyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside 2,3-di(chlorosulfate) in 84% yield. The 6-chloro-6-deoxy-D-glucoside derivative could also be prepared³⁵² directly (in 59% yield) by reaction of methyl α -D-glucopyranoside with sulfuryl chloride at 0° , and, on further treatment with pyridine hydrochloride, this was converted into the 4,6-dichloro-4,6-dideoxy- α -D-galactoside derivative.

The facile displacement of the chlorosulfonyloxy group from the

(345) J. E. G. Barnett, *Adv. Carbohydr. Chem.*, **22**, 177-227 (1967).

(346) W. A. Szarek, *Adv. Carbohydr. Chem. Biochem.*, **28**, 225-306 (1973).

(347) B. Helferich, *Ber.*, **54**, 1082-1084 (1921).

(348) B. Helferich, A. Löwa, W. Nippe, and H. Riedel, *Ber.*, **56**, 1083-1087 (1923).

(349) B. Helferich, G. Sprock, and E. Besler, *Ber.*, **58**, 886-891 (1925).

(350) P. D. Bragg, J. K. N. Jones, and J. C. Turner, *Can. J. Chem.*, **37**, 1412-1416 (1959).

(351) J. K. N. Jones, M. B. Perry, and J. C. Turner, *Can. J. Chem.*, **38**, 1122-1129 (1960).

(352) H. J. Jennings and J. K. N. Jones, *Can. J. Chem.*, **43**, 2372-2386 (1965).

primary carbon atom is understandable from steric considerations. Although replacement of the 4-hydroxyl group by a chlorine atom takes place, with inversion, in both anomers of both methyl D-glucopyranoside and methyl D-galactopyranoside on treatment with sulfuryl chloride,³⁵² it does not occur with methyl α -D-mannopyranoside,³⁵² methyl 6-chloro-6-deoxy- α -D-mannopyranoside 2,3,4-tri(chlorosulfate) being the product of reaction. Similarly, methyl α -L-rhamnopyranoside gives only the tri(chlorosulfate) on treatment with sulfuryl chloride.³⁵³ The lack of reactivity towards bimolecular, nucleophilic displacement at C-4 in the latter glycosides is readily seen to be the result of steric interactions of the 1,3-diaxial type which would occur between a chloride ion approaching C-4 and the chlorosulfonyloxy substituent on C-2. A similar pattern of reactivity is observed in replacement reactions of 4-sulfonic esters of these glycosides.³⁵⁴

Reactivity at O-3, in addition to that at O-4 and O-6, was observed with methyl β -D-galactopyranoside, which, when treated with sulfuryl chloride, yielded methyl 3,4,6-trichloro-3,4,6-trideoxy- β -D-allopyranoside 2-(chlorosulfate) in 56% yield.³⁵² In contrast, under similar conditions, methyl α -D-galactopyranoside gave³⁵² methyl 4,6-dichloro-4,6-dideoxy- α -D-glucopyranoside 2,3-di(chlorosulfate). Further examples of the dependence of the reactivity on the configuration of C-1 are the conversion of methyl 4,6-O-benzylidene- β -D-glucopyranoside into methyl 4,6-O-benzylidene-3-chloro-3-deoxy- β -D-allopyranoside by sulfuryl chloride,³⁵² and of methyl 4,6-O-benzylidene- α -D-glucopyranoside, under similar conditions, into the 2,3-di(chlorosulfate).³⁵⁵

The negligible reactivity at C-3 in these α -D-glycosides may be rationalized as being due to unfavorable, 1,3-diaxial interactions that an incoming nucleophile at C-3 would experience with the axial methoxyl group on C-1. The orientation of substituents at C-4 can also have an important influence on the reactivity at C-3 of the pyranoside ring. As mentioned earlier, with sulfuryl chloride, methyl β -D-galactopyranoside undergoes chlorination at C-3, C-4, and C-6, to afford a D-*allo* derivative. However, methyl β -D-glucopyranoside gives methyl 4,6-dichloro-4,6-dideoxy- β -D-galactopyranoside³⁵² plus

(353) A. G. Cottrell, E. Buncel, and J. K. N. Jones, *Can. J. Chem.*, **44**, 1483-1491 (1966).

(354) L. Hough and A. C. Richardson, in "The Carbohydrates: Chemistry and Biochemistry," W. Pigman and D. Horton, eds., Academic Press, New York, 2nd Edition, 1972, Vol. IA, pp. 140-146.

(355) H. J. Jennings and J. K. N. Jones, *Can. J. Chem.*, **41**, 1151-1159 (1963).

methyl 3,6-dichloro-3,6-dideoxy- β -D-allopyranoside³⁵⁶; a 3,4,6-trichloro-substituted product was, apparently, not produced. A consideration of the respective transition states for S_N2 displacement at C-3 shows unfavorable dipolar and steric interactions in the 4,6-dichloro-4,6-dideoxy-D-galactoside derivative between the potential leaving-group at C-3 and the axial, chloro substituent at C-4, precluding further substitution. However, substitution at C-3 is possible if the substituent on C-4 is equatorially disposed, and the formation of methyl 3,6-dichloro-3,6-dideoxy- β -D-alloside shows that displacement at C-3 occurs only if this is the situation. Similar considerations apply in the reaction of methyl 3-chloro-3-deoxy- β -D-allopyranoside with sulfuryl chloride; methyl 3,6-dichloro-3,6-dideoxy- β -D-allopyranoside is formed after de(chlorosulfation), and no nucleophilic displacement of the chlorosulfonyloxy group at C-4 was observed.³⁵³

Throughout the series of glycosides studied, a common feature is the inertness of HO-2 towards replacement. The reason for this most probably lies in the inductive effect at C-2, caused by the two β -oxygen atoms attached to C-1.

Many other glycosides have been subjected to selective chlorination with sulfuryl chloride. Methyl β -L-arabinopyranoside afforded methyl 4-chloro-4-deoxy- α -D-xylopyranoside 2,3-di(chlorosulfate) in 29% yield, whereas the α -L anomer gave³⁵⁷ methyl 3,4-dichloro-3,4-dideoxy- β -D-ribofuranoside 2-(chlorosulfate) (30%). Methyl β -D-ribofuranoside was converted into methyl 3,4-dichloro-3,4-dideoxy- α -L-arabinopyranoside through the action of pyridine hydrochloride on its 2,3,4-tri(chlorosulfate).³⁵⁸ Methyl α -D-lyxopyranoside gave only the 2,3,4-tri(chlorosulfate),³⁵³ as would be expected from the disposition of its hydroxyl groups, similar to that in the rhamno- and manno-pyranosides. Methyl α -D-altropyranoside was transformed into the 6-chloro-6-deoxy 2,3,4-tri(chlorosulfate) derivative in 80% yield.³⁵³

An important step in the synthesis of paratose (3,6-dideoxy-D-ribohexose) involved the conversion of methyl 3-chloro-3-deoxy- β -D-allopyranoside into methyl 3,6-dichloro-3,6-dideoxy- β -D-allopyranoside in 33% yield.³⁵⁹

(356) D. M. Dean, W. A. Szarek, and J. K. N. Jones, *Carbohydr. Res.*, **33**, 383–386 (1974).

(357) H. J. Jennings and J. K. N. Jones, *Can. J. Chem.*, **43**, 3018–3025 (1965).

(358) S. S. Ali, T. J. Mepham, I. M. E. Thiel, E. Buncel, and J. K. N. Jones, *Carbohydr. Res.*, **5**, 118–125 (1967).

(359) E. H. Williams, W. A. Szarek, and J. K. N. Jones, *Can. J. Chem.*, **49**, 796–799 (1971).

With sulfuryl chloride, unsubstituted sugars yield glycosyl halide derivatives, and the reactivities of the (non-anomeric) secondary hydroxyl groups towards the reagent are often (but not always) similar to those in the corresponding alkyl glycosides. D-Galactose gives a mixture of products containing 3,4,6-trichloro-3,4,6-trideoxy-D-allosyl chloride 2-(chlorosulfate).³⁵² D-Mannose, however, affords³⁵² 6-chloro-6-deoxy- α -D-mannopyranosyl chloride 2,3,4-tri(chlorosulfate) which, in contrast to the corresponding derivative of the α -D-glycoside, undergoes displacement with chloride ion to form a tetrachlorotetradecy mono(chlorosulfate) of as-yet-unknown structure.³⁵² The configurationally related L-rhamnose gives L-rhamnopyranosyl chloride 2,3,4-tri(chlorosulfate); this was converted, by treatment with pyridine hydrochloride followed by de(chlorosulfation), into a dichlorodideoxy compound, supposedly 2,4-dichloro-2,4,6-trideoxy-L-galactose.³⁵³

D-Lyxose yielded a D-lyxosyl chloride 2,3,4-tri(chlorosulfate) which, on treatment with chloride ion, led to a dichlorodideoxy compound, most probably 2,4-dichloro-2,4-dideoxy-L-arabinose.³⁵³ D-Glucose gave a compound presumed to be 4,6-dichloro-4,6-dideoxy- α,β -D-galactosyl chloride 2,3-di(chlorosulfate),³⁶⁰ and D-xylose afforded a monochloromonodeoxy derivative formulated, on indirect evidence, as 4-chloro-4-deoxy-L-arabinopyranosyl chloride 2,3-di(chlorosulfate).³⁶⁰ 3,4-Dichloro-3,4-dideoxy- β -D-ribopyranosyl chloride 2-(chlorosulfate) was the major, and 4-chloro-4-deoxy- α -D-xylopyranosyl chloride 2,3-di(chlorosulfate) the minor, product from the reaction of L-arabinose with sulfuryl chloride at room temperature for 24 hours.^{357,361} It has been established that, on reaction with sulfuryl chloride at low temperature, crystalline α -D-xylopyranose and β -D-lyxopyranose afford, respectively, the 2,4,6-tri(chlorosulfate)s of β -D-xylopyranosyl chloride and α -D-lyxopyranosyl chloride,^{362,363} confirming that substitution at C-1 occurs by an S_N2 process on a 1-(chlorosulfuric) ester intermediate.

The reaction of sulfuryl chloride with disaccharides is of current interest. Although chlorine-containing products were isolated earlier from this reaction with sucrose,³⁵⁰ their structures were not elucidated. Since then, one tetrachlorotetradecy- and two penta-

(360) H. J. Jennings and J. K. N. Jones, *Can. J. Chem.*, **40**, 1408-1414 (1962).

(361) B. Coxon, H. J. Jennings, and K. A. McLauchlan, *Tetrahedron*, **23**, 2395-2412 (1967).

(362) H. J. Jennings, *Can. J. Chem.*, **46**, 2799-2805 (1968).

(363) H. J. Jennings, *Can. J. Chem.*, **47**, 1157-1162 (1969).

chloropentadeoxy-sucrose derivatives have been isolated³⁶⁴ in very low yield from the reaction at 50°; as expected, the pyranoid moiety contains chloro substituents at C-4 and C-6 and has the *galacto* configuration. The furanoid moiety carries chloro substituents at the primary centers (C-1' and C-6'), and also at C-4' in the two pentachloropentadeoxy compounds. From a reaction conducted at -78°, 6'-chloro-6'-deoxysucrose and 6,6'-dichloro-6,6'-dideoxysucrose were isolated in yields of 43 and 29%, respectively.³⁶⁵ Methyl β -maltoside has been converted³⁶⁶ into methyl 3,6-dichloro-4-O-(4,6-dichloro-4,6-dideoxy- α -D-galactopyranosyl)-3,6-dideoxy- β -D-allopyranoside, isolated in 48% yield as its acetate, on reaction with sulfuryl chloride for 2 hours at -70° and then for 24 hours at room temperature. The substitution pattern is in accordance with those observed in similar reactions of the component moieties of sucrose.

4,6-Dichloro-4,6-dideoxy- α -D-galactopyranosyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside was obtained in 72% yield by the reaction of α,α -trehalose with sulfuryl chloride.³⁶⁷

2. Phosphorus-based Reagents

Selective replacement of primary hydroxyl groups in carbohydrates by iodine atoms has been achieved by using the Rydon reagent, namely, methyltriphenoxyphosphonium iodide.³⁶⁸ Treatment of methyl 3,4-O-isopropylidene- β -D-galactopyranoside with the phosphonium salt in benzene for 48 hours at room temperature yielded 60% of the 6-deoxy-6-iodo derivative,³⁶⁹ and reaction of thymidine, uridine, and 2,2'-anhydrouridine in *N,N*-dimethylformamide afforded 5'-deoxy-5'-iodo derivatives in yields of 63, 65, and 31%, respectively.³⁷⁰

Reagents derived from triphenylphosphine may show similar, selective reactivity towards primary hydroxyl groups. Thus, after

(364) J. M. Ballard, L. Hough, and A. C. Richardson, *J. Chem. Soc. Chem. Commun.*, 1097-1098 (1972).

(365) J. M. Ballard, L. Hough, A. C. Richardson, and P. H. Fairclough, *J. Chem. Soc. Perkin Trans. I*, 1524-1528 (1973).

(366) P. L. Durette, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, **31**, 114-119 (1973).

(367) G. G. Birch, C.-K. Lee, and A. C. Richardson, *Carbohydr. Res.*, **36**, 97-109 (1974).

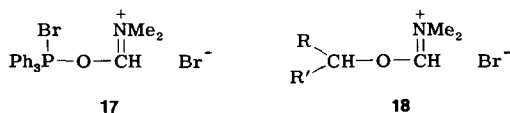
(368) S. R. Landauer and H. N. Rydon, *J. Chem. Soc.*, 2224-2234 (1953).

(369) N. K. Kochetkov and A. I. Usov, *Tetrahedron*, **19**, 973-983 (1963).

(370) J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **35**, 2319-2326 (1970).

heating methyl α -D-glucopyranoside in *N,N*-dimethylformamide with *N*-bromosuccinimide plus triphenylphosphine at 50°, methyl 6-bromo-6-deoxy- α -D-glucopyranoside was isolated as its triacetate in 66% yield.³⁷¹ Although, under similar conditions, the nucleosides uridine and inosine gave 5'-bromo-5'-deoxy compounds respectively isolated as the 2',3'-*O*-isopropylidene (70%) and 2',3'-diacetate (59%) derivatives, experiments with adenosine, cytidine, and *N*⁴-acetylcytidine did not lead to such high selectivity of the primary hydroxyl group. α,α -Trehalose could, however, be converted into its 6,6'-dideoxy-6,6'-dihalo derivatives on treatment with four molar equivalents of triphenylphosphine and the appropriate *N*-halosuccinimide in *N,N*-dimethylformamide,³⁷² and use of two molar equivalents afforded the 6-deoxy-6-halo derivatives in lower yield.

A report of work appearing to have potential application in carbohydrate chemistry described differentiation between primary and secondary hydroxyl groups in a simple procedure that uses triphenylphosphine dibromide in *N,N*-dimethylformamide.³⁷³ Although this reagent combination had previously been reported to convert primary and secondary alcohols into alkyl bromides,³⁷⁴ low temperatures of reaction (<0°) caused the replacement of a primary hydroxyl group by bromine, but the conversion of a secondary hydroxyl group into its formic ester. This reaction, coupled with a concurrently developed, facile procedure for the oxidation of alkyl bromides to carbonyl compounds,³⁷⁵ offers an excellent method for the oxidation of primary hydroxymethyl groups to aldehydes in the presence of secondary hydroxyl groups. The reaction most probably involves initial formation of complex 17 from triphenylphosphine dibromide and *N,N*-dimethylformamide³⁷⁶ followed by nucleophilic attack of the alcohol to form³⁷⁷ an *N,N*-dimethyl(alkoxymethaniminium) bromide (18).



(371) S. Hanessian, M. M. Ponpipom, and P. Lavallée, *Carbohydr. Res.*, **24**, 45-56 (1972).

(372) S. Hanessian and P. Lavallée, *Carbohydr. Res.*, **28**, 303-311 (1973).

(373) R. K. Boeckman and B. Ganem, *Tetrahedron Lett.*, 913-916 (1974).

(374) G. A. Wiley, R. L. Hershkowitz, B. M. Rein, and B. C. Chung, *J. Am. Chem. Soc.*, **86**, 964-965 (1964).

(375) B. Ganem and R. K. Boeckman, *Tetrahedron Lett.*, 917-920 (1974).

(376) H. J. Bestmann, J. Lienert, and L. Mott, *Ann.*, **718**, 24-32 (1968).

(377) M. E. Herr and R. A. Johnson, *J. Org. Chem.*, **37**, 310-312 (1972).

Nucleophilic attack by bromide ion at the alcohol-derived, oxygen-bearing carbon atom in **18** can lead to an alkyl bromide, and this course is sterically favored when the carbon atom is a primary center (**18**; R' = H, R = alkyl). When this carbon is a secondary center (**18**; R' = R = alkyl), nucleophilic attack is sterically less favored, and the species **18** survives at low temperature until processing involving the use of water causes hydrolysis to the formic ester. Reasonable, mechanistic pathways proceeding through intermediates of type **18** may be envisaged for other hydroxyl-to-halogeno conversions performed in *N,N*-dimethylformamide, described in this Section. As the cation in **18** is also considered to be the important intermediate in the replacement of hydroxyl groups by halogen that is brought about by *N,N*-dimethyl(halomethaniminium) halides (see Section V,3), the inclusion of the former reactions in this Section is somewhat arbitrary.

Mixtures of tertiary phosphines with carbon tetrahalides have been found to convert alcohols efficiently into alkyl halides, and some selectivity has been observed with nucleosides. On treatment with 2 molar equivalents of triphenylphosphine-carbon tetrabromide in *N,N*-dimethylformamide, uridine is transformed into its 5'-bromo-5'-deoxy derivative in 55% yield,³⁷⁸ but a related reaction with carbon tetrachloride afforded only 17% of 5'-chloro-5'-deoxyuridine, together with 68% of 2',5'-dichloro-2',5'-dideoxyuridine; retention of configuration at C-2 in the latter suggests the intermediacy of a 2,2'-anhydronucleoside intermediate. Thymidine gave a 73% yield of 5'-chloro-5'-deoxythymidine, and 5'-*O*-acetyluridine afforded 5'-*O*-acetyl-2'-chloro-2'-deoxyuridine in 38% yield under similar conditions. Triphenylphosphine in carbon tetrachloride has also been observed to cause selective replacement of HO-3 by chlorine, with inversion of configuration, in 5'-*O*-acetylinosine (in refluxing triethyl phosphate)³⁷⁹ and in a *C*- β -glycosyl derivative of D-erythrofurranose.³⁸⁰

The selective activation of the primary hydroxyl group in methyl α -D-glucopyranoside by reaction with carbon tetrachloride and tris(dimethylamino)phosphine in *N,N*-dimethylformamide at -40° has been reported.³⁸¹ An alkoxytris(dimethylamino)phosphonium

(378) J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **37**, 2289-2299 (1972).

(379) K. Haga, M. Yoshikawa, and T. Kato, *Bull. Chem. Soc. Jpn.*, **43**, 3922-3924 (1970).

(380) F. Nouaille, A.-M. S  pulchre, G. Lukacs, and S. D. Gero, *Compt. Rend., Ser. C*, **275**, 423-425 (1972).

(381) B. Castro, Y. Chapleur, B. Gross, and C. Selve, *Tetrahedron Lett.*, 5001-5004 (1972).

salt, with the alkoxyl group derived from HO-6, is formed, and reaction of this derivative with nucleophiles affords useful preparations of 6-substituted D-glucose derivatives.

The interesting observation has been made that 3'-amino-3'-deoxyadenosine as a suspension in phosphoryl chloride-triethyl phosphate at 4° is converted into the 5'-chloro-5'-deoxy derivative in 80% yield, but that predissolution of the nucleoside in triethyl phosphate, followed by treatment with phosphoryl chloride at 0°, yields the 5'-phosphate in 62% yield.³⁸²

In nearly all of the reactions described in this subsection, it appears that steric factors are the cause of the selectivities observed.

3. *N,N*-Dimethyl(methaniminium) Halide Derivatives

Substituted derivatives of *N,N*-dimethyl(methaniminium) halide (19) may be prepared by interaction of *N,N*-dimethylformamide with inorganic acid chlorides.³⁸³ For example, reaction of *N,N*-dimethylformamide with phosgene initially affords 20, which undergoes loss of carbon dioxide on warming, to yield^{384,385} *N,N*-dimethyl-(chloromethaniminium) chloride (21). Use of thionyl chloride instead of phosgene also leads to 21, but with corresponding loss of sulfur dioxide. Reactions of a substrate with these and similar acid chlorides in the presence of *N,N*-dialkylamides may, therefore, be the result of its interaction with an iminium salt, rather than of its direct interaction with the acid chloride. Such iminium halides as 21 are known to convert alcohols readily into alkyl halides,³⁸⁶ and the observation that methanesulfonyl chloride in *N,N*-dimethylformamide causes the highly selective replacement of primary hydroxyl groups in hexopyranosides by chlorine³⁸⁷ suggests that the glycosides probably react with the iminium salt 22 through a sterically demanding transition state to form an intermediate of type 23, which is

(382) M. Morr and M.-R. Kula, *Tetrahedron Lett.*, 23-24 (1974).

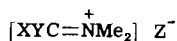
(383) H. Eilingsfeld, M. Seefelder, and H. Weidinger, *Angew. Chem.*, **72**, 836-845 (1960).

(384) G. Martin and M. Martin, *Bull. Soc. Chim. Fr.*, 1637-1646 (1963).

(385) M. L. Filleux-Blanchard, M. T. Quemeneur, and G. J. Martin, *Chem. Commun.*, 836-837 (1968).

(386) H. Eilingsfeld, M. Seefelder, and H. Weidinger, *Chem. Ber.*, **96**, 2671-2690 (1963).

(387) M. E. Evans, L. Long, Jr., and F. W. Parrish, *J. Org. Chem.*, **33**, 1074-1076 (1968).



- 19 X = Y = H; Z = Hal
 20 X = Cl; Y = H; Z = OCO·Cl
 21 X = Cl; Y = H; Z = Cl
 22 X = OSO₂Me; Y = H; Z = Cl
 23 X = OCH₂R; Y = H; Z = Cl
 24 X = OCHR¹R²; Y = H; Z = Cl

then attacked at the methylene carbon atom of the alkoxyl group by chloride ion. Methyl α -D-glucopyranoside, methyl β -D-glucopyranoside, and methyl α -D-mannopyranoside at 65° gave the respective 6-chloro-6-deoxy derivatives in yields of 97, 91, and 86%, respectively, but methyl α -D-xylopyranoside was recovered unchanged in 99% yield under analogous conditions. Evidence against a mechanism involving selective methanesulfonylation at HO-6, followed by SN2 attack by chloride ion, was the fact that methyl 6-O-(methylsulfonyl)- α -D-glucopyranoside was recovered quantitatively after treatment with the reagent, under conditions that yielded the 6-chloro-6-deoxy compound in 64% yield from the parent glycoside.

The selectivity of the methanesulfonyl chloride-*N,N*-dimethylformamide reagent towards alkyl glycopyranosides has been re-examined,³⁸⁸ and replacements at secondary positions have been found to occur, but at lower rates than at primary centers. In addition to the 6-chloro-6-deoxy derivative expected, methyl α -D-glucopyranoside gave methyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside, isolated as its diacetate in 8% yield, and the β -D anomer afforded a 2:1 mixture of methyl 3,6-dichloro-3,6-dideoxy- β -D-allopyranoside (31%) with methyl 4,6-dichloro-4,6-dideoxy- β -D galactopyranoside. Methyl 4,6-dichloro-4,6-dideoxy- α -D-glucopyranoside could be obtained in 37% yield from the reaction of methyl α -D-galactopyranoside with 20 equivalents of methanesulfonyl chloride in *N,N*-dimethylformamide for 4 days at 100°. The inversion of configuration occurring at secondary centers supports the intermediacy of *N,N*-dimethyl(alkoxymethaniminium) species of type 24. The reactivity pattern strongly resembles that observed in chlorosulfonylation (see Section V,1).

On treatment with 30 equivalents of methanesulfonyl chloride in *N,N*-dimethylformamide for 8 days at 65°, methyl β -maltoside gave a mixture of methyl 3,6-dichloro-4-O-(6-chloro-6-deoxy- α -D-glucopyranosyl)-3,6-dideoxy- β -D-allopyranoside, isolated in 46%

(388) R. G. Edwards, L. Hough, A. C. Richardson, and E. Tarelli, *Carbohydr. Res.*, **35**, 111-129 (1974).

yield as its tetraacetate, and methyl 3,6-dichloro-3,6-dideoxy-4-*O*-(4,6-dichloro-4,6-dideoxy- α -D-galactopyranosyl)- β -D-allopyranoside (8%, as its triacetate); the yield of the latter compound was improved by conducting the reaction at 100°. Milder conditions yielded the expected methyl 6,6'-dichloro-6,6'-dideoxy- β -maltoside [primed numbers refer to the carbon atoms of the D-glucosyl (nonreducing) group in the free sugar], but at no time was this compound present without the trichlorotrideoxy derivative, illustrating an abnormal reactivity of HO-3. The slow appearance of the tetrachlorotetradexo derivative also suggests that the reactivity of HO-4' is considerably less than that of HO-3. Treatment of benzyl β -cellobioside with the reagent also led to the introduction of chloro substituents at secondary (as well as primary) positions, affording 6,6'-dichloro-6,6'-dideoxy, 6,3',6'-, 6,4',6'-, and 3,6,6'-trichloro-trideoxy, and 3,6,3',6'- and 3,6,4',6'-tetrachloro-tetradexo derivatives, with inversion at the chiral centers.

Primary hydroxyl groups in unprotected nucleosides have been selectively replaced by halogen through reaction with *N,N*-dimethyl(chloromethaniminium) and *N,N*-dimethyl(bromomethaniminium) halides, uridine affording the 5'-chloro-5'-deoxy and 5'-bromo-5'-deoxy derivatives in yields of 80 and 75%, respectively.³⁸⁹ During an attempt to synthesize arsenic esters of nucleosides by interaction of arsenic trichloride or arsenic tribromide with nucleosides in *N,N*-dimethylformamide, replacement of hydroxyl groups by halogen was also observed, and *N,N*-dimethyl(halomethaniminium) species were proposed as the reactive intermediates responsible for halogenation.³⁹⁰ In *N,N*-dimethylacetamide, uridine reacted with arsenic trichloride to give 5'-*O*-acetyluridine as the preponderant product (43%), together with 2'-chloro-2'-deoxyuridine and its *O*-acetyl derivatives.³⁷⁸

The reagent methanesulfonyl chloride-*N,N*-dimethylformamide has been used to prepare 6-chloro-6-deoxyamylose of d.s. 0.8 from the polysaccharide,³⁹¹ but it was necessary first to convert the amylose into a reactive form by freeze-drying its aqueous solution.

The reaction of methyl α -D-glucopyranoside with sulfur monochloride in *N,N*-dimethylformamide to give the 6-chloro-6-deoxy derivative in 30–35% yield has been described³⁹²; it is possible that *N,N*-

(389) R. F. Dods and J. S. Roth, *Tetrahedron Lett.*, 165–168 (1969).

(390) R. F. Dods and J. S. Roth, *J. Org. Chem.*, **34**, 1627–1630 (1969).

(391) D. Horton, A. E. Luetzow, and O. Theander, *Carbohydr. Res.*, **27**, 268–272 (1973).

(392) H. B. Sinclair, *J. Org. Chem.*, **30**, 1283–1284 (1965).

dimethyl(chloromethaniminium) intermediates may also be involved in this reaction.

4. Other Reagents

That displacement of sulfonyloxy groups by chloride ion may occur during sulfonylation reactions in pyridine has long been recognized.² Whereas *p*-toluenesulfonylation of methyl α - and β -D-glucopyranosides in pyridine for 16 days at 27° led to the formation of tetra-*O*-*p*-tolylsulfonyl derivatives as major products, treatment at 75° gave methyl 6-chloro-6-deoxy-2,3,4-tri-*O*-*p*-tolylsulfonyl- α - and - β -D-glucopyranoside, respectively, together with the 4,6-dichloro-4,6-dideoxy-2,3-di-*O*-*p*-tolylsulfonyl-D-galactopyranoside derivatives.³⁹³ Methanesulfonylation at 75° followed a similar course for the α -D-glucopyranoside, but the β -D-glucopyranoside gave the expected 6-chloro-6-deoxy-2,3,4-tri-*O*-(methylsulfonyl) derivative and, curiously, methyl 4,6-dichloro-4,6-dideoxy- β -D-glucopyranoside; no D-*galacto* isomer of the latter compound was found. Methyl 2-acetamido-3-*O*-acetyl-6-chloro-2,6-dideoxy-4-*O*-(methylsulfonyl)- α -D-glucopyranoside was obtained in 12% yield as a by-product in the methanesulfonylation of the parent "diol" in pyridine at room temperature.³⁹⁴

In contrast to uridine,³⁸⁹ cytidine does not yield a 5'-chloro-5'-deoxy derivative on reaction with *N,N*-dimethyl(chloromethaniminium) chloride; instead 2,2'-anhydrocytidine is formed.³⁹⁵ However, thionyl chloride or bromide in hexamethylphosphoramide at room temperature achieves this selective replacement of the primary hydroxyl group of halogen in cytidine, and also in adenosine, in respective yields of 80 and 75% for the chloro compounds, and 55 and 30% for the bromo analogs.³⁹⁶

Reports by Mattocks^{397,398} that 2-acetoxy-2-methylbutanoyl chloride reacts with 1,2- and 1,3-diols to afford chloroacetates led to an investigation into use of this type of reagent for selective transformation of the vicinal diol grouping in ribonucleosides. Greenberg and Moffatt³⁹⁹ chose to use 2-acetoxy-2-methylpropanoyl chloride (which lacks the complication of a chiral center), and, firstly, found that *cis*-

(393) F. W. Parrish, F. H. Bissett, M. E. Evans, M. L. Bazinet, W. Yeomans, and L. Long, Jr., *Carbohydr. Res.*, **6**, 503-504 (1968).

(394) J. Hill and L. Hough, *Carbohydr. Res.*, **8**, 398-404 (1968).

(395) K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 867-870 (1970).

(396) K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 87-90 (1971).

(397) A. R. Mattocks, *J. Chem. Soc.*, 1918-1930 (1964).

(398) A. R. Mattocks, *J. Chem. Soc.*, 4840-4845 (1964).

(399) S. Greenberg and J. G. Moffatt, *J. Am. Chem. Soc.*, **95**, 4016-4025 (1973).

1,2-cyclopentanediol with 1.2–1.5 equivalents of the reagent in inert solvents gives *trans*-2-chlorocyclopentyl acetate in 65% (isolated) yield; by a g.l.c. determination, the true yield was found to be 95%. In contrast, *trans*-1,2-cyclopentanediol gives at least ten products! Similarly, *cis*-1,2-cyclohexanediol gives *trans*-2-chlorocyclohexyl acetate in high yield, whereas the isomeric *trans*-1,2-diol gives at least eleven products. Thus, the reaction appears to be specific for *cis*-diols on five- and six-membered rings, and to proceed with inversion of configuration at one of the centers.

The fact that 3-chloro- and 3-acetoxy-1,2-propanediol give,³⁹⁸ respectively, 2-acetoxy-1,3-dichloropropane and 1,2-diacetoxy-3-chloropropane with 2-acetoxy-2-methylbutanoyl chloride suggested that the regiospecificity is controlled by steric factors, but electronic factors may assume a dominant role in certain cases. Thus, 1-phenyl-1,2-ethanediol gave³⁹⁹ 2-chloro-2-phenylethyl acetate in 75% yield. Reaction of 5'-*O*-(*p*-nitrobenzoyl)uridine with four molar equivalents of 2-acetoxy-2-methylpropanoyl chloride (without solvent) for 45 minutes at 100° gave 3'-*O*-acetyl-2'-chloro-2'-deoxy-5'-*O*-(*p*-nitrobenzoyl)uridine in 84% yield. Reaction thus occurred with exclusive introduction of chlorine at C-2', and with overall retention of configuration. This result may be rationalized by invoking intramolecular participation of the carbonyl group at C-2 in the uracil residue at some stage in the reaction, to give a 2,2'-anhydronucleoside derivative, which is then attacked by chloride ion at C-2'. In the absence of solvent and after direct de-esterification of the crude product, uridine gave 2'-chloro-2'-deoxyuridine in 73% yield. In the presence of a solvent, uridine gave 5'-*O*-substituted derivatives of 2'-chloro-2'-deoxyuridine, but the nature of the substituent was solvent-dependent. A very short reaction-time in nitromethane led to the isolation of the hydrochloride of 1-(3-*O*-acetyl- β -D-arabinofuranosyl)-2,2'-anhydrouracil, supporting the mechanism already proposed and indicating that, in this solvent at least, reaction at the *cis*-diol grouping precedes that with HO-5'. Short-term reactions with uridine in *N,N*-dimethylformamide led to the isolation of 2'- and 3'-*O*-acetyl derivatives of uridine, indicating the intermediate formation of a 2',3'-acetonium ion in the reaction.

The reaction of adenosine with 2-acetoxy-2-methylpropanoyl chloride or the corresponding bromide is less regioselective than that of uridine,⁴⁰⁰ and gives 9-(2-*O*-acetyl-3-deoxy-3-halo- β -D-xylofuranosyl)- and 9-(3-*O*-acetyl-2-deoxy-2-halo- β -D-arabinofuranosyl)-adenine,

(400) A. F. Russell, S. Greenberg, and J. G. Moffatt, *J. Am. Chem. Soc.*, **95**, 4025–4030 (1973).

with compounds of the *D-xylo* configuration preponderating, in which the hydroxyl groups at C-5' are present as their 2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl ethers. Acid hydrolysis of the *D-xylo* compound obtained from reaction with 2-acetoxy-2-methylpropanoyl bromide, followed by catalytic hydrogenolysis of the product, afforded a facile route to 3'-deoxyadenosine (cordycepin).

Two groups of workers have reported modification of the nucleoside antibiotics tubercidin and formycin by reaction with 2-acetoxy-2-methylpropanoyl halides in acetonitrile.^{401,402} Tubercidin gave only the 2-*O*-acetyl-3-deoxy-3-halo- β -*D*-xylofuranosyl nucleoside,⁴⁰¹ substituted at O-5' with a trimethyldioxolanone moiety; this behavior is unlike that in the corresponding reactions of adenosine,⁴⁰⁰ guanosine,⁴⁰³ and inosine,⁴⁰³ where some formation of a 3-*O*-acetyl-2-chloro-2-deoxy- β -*D*-arabinofuranosyl isomer also occurred. Curiously, formycin with 2-acetoxy-2-methylpropanoyl bromide gives a mixture of 3-bromo-*xylo* and 2-bromo-*arabino* isomers in the ratio of 3:1 (as judged by n.m.r. analysis). Robins and coworkers⁴⁰² obtained essentially similar results in reactions of the two nucleosides with 2-acetoxy-2-methylpropanoyl iodide, which was formed, in the mixture, from the corresponding chloride plus sodium iodide.

On treatment with concentrated halogen acids, certain hexitols yield 1,6-dideoxy-1,6-dihalo compounds. The structure of the compound so obtained from galactitol, first reported by Bouchardat,⁴⁰⁴ was later verified by synthesis.⁴⁰⁵ Allitol is transformed⁴⁰⁶ into 1,4-anhydro-6-chloro-6-deoxy-DL-allitol and 1,4-anhydro-5,6-dichloro-5,6-dideoxy-DL-talitol on treatment with fuming hydrochloric acid at 100°.

Selective replacement of hydroxyl groups by bromine has been reported for some inositols and inosamines. DL-2-Amino-2-deoxy-*epi*-inositol hydrochloride plus acetyl bromide in acetic anhydride gave, after 6 hours at 150°, DL-4-acetamido-1,2,6-tri-*O*-acetyl-3,5-dibromo-3,4,5-trideoxy-*chiro*-inositol in 32% yield, the bromine atoms having been introduced with inversion.⁴⁰⁷ Under related conditions, DL-*epi*-

(401) T. C. Jain, A. F. Russell, and J. G. Moffatt, *J. Org. Chem.*, **38**, 3179-3186 (1973).

(402) M. J. Robins, J. R. McCarthy, Jr., R. A. Jones, and R. Menzel, *Can. J. Chem.*, **51**, 1313-1321 (1973).

(403) Unpublished work by J. C. Jain, A. F. Russell, and J. G. Moffatt; see reference 11 in Ref. 401.

(404) G. Bouchardat, *Ann. Chim. Phys.*, **27** (4), 145-210 (1872).

(405) K. Butler and W. A. W. Cummings, *J. Chem. Soc.*, 636-640 (1956).

(406) J. M. Ballard and B. E. Stacey, *Carbohydr. Res.*, **30**, 83-89 (1973).

(407) T. Suami, S. Ogawa, Y. Nakashima, and H. Sano, *Bull. Chem. Soc. Jpn.*, **40**, 2958-2963 (1967).

inositol gave⁴⁰⁸ two dibromodideoxy-inositols, DL-2,4-dibromo-2,4-dideoxy-*chiro*-inositol (4.2%, as its tetraacetate) and DL-1,4-dibromo-1,4-dideoxy-*chiro*-inositol (9.6%), and, in addition, two monobromomonodeoxy-inositols, DL-5-bromo-5-deoxy-*allo*-inositol (26%, as its pentaacetate) and DL-1-bromo-1-deoxy-*neo*-inositol (5.2%). The bromination of 2-amino-2-deoxy-*myo*-inositol hydrochloride and the *N*-acetyl derivatives of DL-1-amino-1-deoxy-*chiro*-inositol, DL-1-amino-1-deoxy-*muco*-inositol, 1,4-diamino-1,4-dideoxy-*neo*-inositol, and 1,5-diamino-1,5-dideoxy-*muco*-inositol with acetyl bromide in acetic anhydride have also been reported,⁴⁰⁹ and selective replacement of one, or two, hydroxyl groups by bromine was observed.

VI. SELECTIVE OXIDATION

1. Catalytic Oxidation

Over the past two decades, catalytic oxidation of carbohydrates has been extensively investigated, mainly by Heyns and coworkers, and the subject has been discussed.⁴¹⁰⁻⁴¹³ The catalyst of choice is usually platinum, in the form of platinum black or platinum suspended on carbon, and oxygen (or air) is passed through a vigorously stirred solution of the substrate containing the catalyst to effect reaction. Oxidations are most often conducted in water, but organic solvents have also been used. The mechanism of the oxidation is regarded as one of dehydrogenation, rather than one involving a peroxide intermediate.⁴¹⁰ Yields in catalytic oxidations vary from good to very low, but, even when they are low, selectivity and ease of isolation of the product make the method attractive.

Early observations by Heyns established that selective oxidation of carbohydrates is possible; for example, the hydroxymethyl group involving C-1 in L-sorbosepyranose could be converted into a carboxyl

(408) T. Suami, A. Suzuki, M. Uchida, and S. Yanagida, *Bull. Chem. Soc. Jpn.*, **42**, 2672-2676 (1969).

(409) T. Suami, S. Ogawa, S. Yanagida, and K. Nojima, *Bull. Chem. Soc. Jpn.*, **45**, 2593-2597 (1972).

(410) K. Heyns and H. Paulsen, *Angew. Chem.*, **69**, 600-608 (1957).

(411) K. Heyns and H. Paulsen, in "Newer Methods of Preparative Organic Chemistry," W. Foerst, ed., Academic Press, New York and London, 1963, Vol. 2, pp. 303-335.

(412) K. Heyns, H. Paulsen, G. Rüdiger, and J. Weyer, *Fortschr. Chem. Forsch.*, **11**, 285-374 (1969).

(413) K. Heyns and H. Paulsen, *Adv. Carbohydr. Chem.*, **17**, 169-221 (1962).

group in 65% yield.⁴¹⁴ A number of patents⁴¹⁵ on this process reflect the potential interest to industry of such selective oxidations. A general principle governing selective oxidation of polyhydroxy compounds is that a primary is usually oxidized more readily than a secondary carbon atom. The former can be converted into a carboxyl group in alkaline media, or, less satisfactorily, into an aldehyde group in neutral or slightly acidic media, and a secondary carbon atom is transformed into a ketone group in neutral or weakly acidic solution. Exceptions to the selectivity generally observed have, however, been noted. Catalytic oxidation of D-glucal resulted⁴¹⁶ in favored oxidation of the secondary allylic hydroxyl group on C-3, and sequential catalytic oxidation and reduction applied to methyl α - and β -D-galactopyranoside in unbuffered solutions led to the formation of methyl α - and β -D-fucopyranoside, respectively, a process rationalized as involving oxidation at C-4 as the first step.⁴¹⁷ Also, 2,7-anhydro- β -D-*altro*-heptulopyranose undergoes⁴¹⁸ catalytic oxidation at the axial, secondary hydroxyl group at C-5, rather than at the primary position at C-1.

Amongst secondary hydroxyl groups, selectivity is observed for oxidation at those which are axial in six-membered ring-systems, and *endo* in bicyclic systems. In both cases, the hydrogen atom geminal to the hydroxyl group is sterically less hindered to attack by the catalyst than that in the corresponding configurational isomers.

A secondary hydroxyl group forming part of the hemiacetal function of an aldose shows enhanced reactivity towards oxidation compared to the other secondary hydroxyl groups, and it may also be oxidized more readily than a primary alcohol group. Thus, oxidation of D-glucose⁴¹⁹ or D-galactose, D-mannose, D-xylose, and L-arabinose⁴²⁰ in the presence of one equivalent of alkali with platinum-on-carbon as the catalyst afforded the corresponding aldonic acids. In 2-ketoses, the primary hydroxyl group at C-1 is generally activated towards oxidation,⁴¹⁴ although evidence exists⁴²¹ that it may be less reactive

(414) K. Heyns, *Ann.*, **558**, 177-187 (1947).

(415) O. Dalmer and K. Heyns, German Pat. 692,897 (1940); *Chem. Abstr.*, **35**, 4,396 (1941); Canadian Pat. 381,575 (1939); *Chem. Abstr.*, **33**, 5,416 (1939); U.S. Pat. 2,189,778 (1940); *Chem. Abstr.*, **34**, 4,236 (1940); U.S. Pat. 2,190,377 (1940); *Chem. Abstr.*, **34**, 4,080 (1940).

(416) K. Heyns and H. Gottschalck, *Chem. Ber.*, **99**, 3718-3719 (1966).

(417) O. Gabriel, *Carbohydr. Res.*, **6**, 111-117 (1968).

(418) K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, **106**, 623-631 (1973).

(419) K. Heyns and R. Heinemann, *Ann.*, **558**, 187-192 (1947).

(420) K. Heyns and O. Stöckel, *Ann.*, **558**, 192-194 (1947).

(421) N. R. Trenner, U.S. Pat. 2,483,251 (1949); *Chem. Abstr.*, **44**, 3,521 (1950).

than the other primary hydroxyl group in the molecule. When the hemiacetal function of an aldohexose is suitably protected (for example, by glycoside formation), a primary hydroxyl group may readily be oxidized selectively, affording a valuable synthesis of uronic acids. Thus, methyl α -D-glucopyranoside gave, in 87% yield, methyl α -D-glucopyranosiduronic acid,⁴²² and many other similar examples are known.⁴¹³

Selective oxidation of the nucleosides uridine, thymidine, adenosine, and guanosine has been achieved,⁴²³ to afford the corresponding 5'-carboxylic acids, and the glycosides of amino sugars have been converted into uronic acid derivatives after protection of their amino groups.^{424,425} The favored, catalytic oxidation of one of the primary hydroxyl groups in benzyl β -maltoside provided a convenient synthesis of benzyl 4-O-(α -D-glucopyranosyluronic acid)- β -D-glucopyranoside, which was isolated as its hexaacetate or hexaacetate methyl ester.⁴²⁶ The oxidation of polysaccharides has been claimed to convert terminal, primary alcohol groups on nonreducing residues, side chains, or other sterically favored positions into carboxyl groups.^{427,428} Aldohexoses have been synthesized by subjecting hexitols to catalytic oxidation under neutral conditions. D-Mannose was obtained in 35% yield (as its phenylhydrazone) from D-manitol,⁴²⁹ and D-glucitol gave, as expected, D-glucose plus L-gulose⁴³⁰; isolation of the latter in 20% yield (as its 2-benzyl-2-phenylhydrazone) suggests that this may be a reasonable synthesis of the rare sugar.

Remarkable selectivity has been observed in the oxidation of molecules containing several secondary hydroxyl groups, such as aldopentopyranosides, 6-deoxyaldohexopyranosides, cyclitols, and various anhydro derivatives. As indicated previously, attack usually occurs at relatively hindered hydroxyl groups. When aqueous solutions of benzyl β -D-arabinopyranoside, benzyl β -D-ribosepyranoside,

(422) S. A. Barker, E. J. Bourne, and M. Stacey, *Chem. Ind. (London)*, 970 (1951).

(423) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149-1154 (1963).

(424) K. Heyns and H. Paulsen, *Chem. Ber.*, **88**, 188-195 (1955).

(425) K. Heyns and M. Beck, *Chem. Ber.*, **90**, 2443-2447 (1957).

(426) Y. Hirasaka, *Yakugaku Zasshi*, **83**, 960-965 (1963); *Chem. Abstr.*, **60**, 4,232 (1964).

(427) G. O. Aspinall and A. Nicolson, *J. Chem. Soc.*, 2503-2507 (1960).

(428) G. O. Aspinall and I. M. Cairncross, *J. Chem. Soc.*, 3998-4000 (1960).

(429) J. W. E. Glattfeld and S. Gershon, *J. Am. Chem. Soc.*, **60**, 2013-2023 (1938).

(430) K. Heyns and M. Beck, *Chem. Ber.*, **91**, 1720-1724 (1958).

benzyl α -D-lyxopyranoside, and benzyl α -D-xylopyranoside were treated with oxygen in the presence of platinum for several hours at 42°, the products of oxidation, obtained in yields of 20–30%, were, respectively, the corresponding β -D-*threo*-pentopyranosid-4-ulose, β -D-*erythro*-pentopyranosid-3-ulose, α -D-*threo*-pentopyranosid-3-ulose, and β -L-*threo*-pentopyranosid-4-ulose.⁴³¹

The products from the D-arabinoside and D-riboside are those to be expected if their favored conformations are ${}^1C_4(D)$ and ${}^4C_1(D)$, respectively. For the related methyl β -D-arabinopyranoside, complex-formation with cuprammonium⁴³² and its optical rotation^{433,434} indicate that it exists mainly in the ${}^1C_4(D)$ conformation in aqueous solution. Methyl β -D-ribofuranoside had been assigned the ${}^4C_1(D)$ conformation,⁴³² although the interpretation of the results has been questioned⁴³⁴ and n.m.r. measurements⁴³⁴ suggested that an ~1:1 mixture of the two chair conformers is actually present in aqueous solution. Selectivity for oxidation at C-3 in the D-lyxoside is surprising, as its n.m.r. spectrum in D₂O at 50° suggests that it exists preponderantly in the ${}^4C_1(D)$ conformation,⁴³¹ in which HO-2 has an axial disposition. In the ${}^1C_4(D)$ form, both HO-3 and HO-4 are axially disposed, and both should be equally susceptible to oxidative attack. However, reaction at C-3 in the ${}^1C_4(D)$ conformation has analogies in the oxidation of 1D-3-O-methyl-*chiro*-inositol [(+)-pinitol] and 1L-2-O-methyl-*chiro*-inositol [(–)-quebrachitol],⁴³⁵ in that, of two axial hydroxyl groups, only that which is *meta* and *trans* to an O-alkyl group is oxidized. The somewhat lower rate of oxidation of the D-xyloside is possibly a consequence of the necessity for oxidation to occur in the energetically unfavorable ${}^1C_4(D)$ conformation. Of the three axial hydroxyl groups in this conformer, oxidation at C-2 and C-4 might be predicted (by analogy with the oxidation of *muco*-inositol⁴³⁶), but an axial hydroxyl group vicinal to an equatorial O-alkyl group appears to be deactivated towards oxidation,⁴³⁵ and reaction at C-4 is thus not unreasonable. Other glycosides that have been oxidized to give products resulting from attack at supposedly axial hydroxyl groups include benzyl and methyl 6-deoxy- α -D-galactopyranoside,⁴³⁷ methyl

(431) K. Heyns, J. Lenz, and H. Paulsen, *Chem. Ber.*, **95**, 2964–2975 (1962).

(432) R. E. Reeves, *J. Am. Chem. Soc.*, **72**, 1499–1506 (1949).

(433) D. H. Whiffen, *Chem. Ind. (London)*, 964–968 (1956).

(434) S. J. Angyal, *Aust. J. Chem.*, **21**, 2737–2746 (1968).

(435) G. G. Post and L. Anderson, *J. Am. Chem. Soc.*, **84**, 471–478 (1962).

(436) See reference 98 in Ref. 413.

(437) K. Heyns, A. L. Baron, and H. Paulsen, *Chem. Ber.*, **97**, 921–925 (1964).

6-deoxy- α -L-mannopyranoside,⁴³⁸ and methyl 6-deoxy- β -D-allopyranoside.⁴³⁹

Insight into the stereoselectivity of oxidations with the platinum-oxygen system was obtained on oxidation of all eight of the 1,6-anhydro- β -D-alдохexopyranoses.⁴⁴⁰ Yields of the 1,6-anhydro- β -D-hexopyranosuloses ranged from 2.6 to 55%, and the following order of decreasing reactivity towards catalytic oxidation was found: HO-3(ax) > HO-4(ax) > HO-2(ax) > HO-4(eq) > HO-2(eq) > HO-3(eq). Noteworthy was the fact that 1,6-anhydro- β -D-idopyranose, possessing three equatorial hydroxyl groups, did undergo slow oxidation, mainly at HO-4 and partly at HO-2.

A study of the selective, catalytic oxidation of five 1,4-anhydrohexitols has been made.⁴⁴¹ With certain assumptions regarding the favored conformations of these substituted tetrahydrofurans, the following order of decreasing ease of oxidation was proposed: primary hydroxyl \approx quasi-axial secondary hydroxyl > quasi-equatorial secondary hydroxyl and side-chain secondary hydroxyl. In the 1,4:3,6-dianhydrohexitol series, only hydroxyl groups *endo* to the bicyclic system undergo attack,⁴⁴² and a similar selectivity was found for other compounds containing the 2,6-dioxabicyclo[3.3.0]octane ring-system.⁴⁴³ An interesting relationship between reactivity and configuration is demonstrated by the fact that 1,6-anhydro- α -D-galactofuranose is selectively oxidized at C-5 to afford the 5-ulose hydrate in 36% yield, whereas 1,6-anhydro- β -D-glucofuranose is inert towards catalytic oxidation⁴⁴⁴; this result is unusual, as, in the six-membered ring which is part of the fused-ring system of both compounds, the 5-hydroxyl group occupies an equatorial and an axial position in the *galacto* and *gluco* isomers, respectively. However, inspection of models reveals that steric approach to H-5 in the former compound is relatively facile, because, in relationship to the fused, five-membered ring, it occupies an *exo* position, whereas, in the latter compound, it is *endo*-disposed.

The cyclitols have provided much information on the relationship between the reactivities of secondary hydroxyl groups towards cata-

(438) J. S. Brimacombe and M. C. Cook, *J. Chem. Soc.*, 2663-2666 (1964).

(439) J. S. Brimacombe, M. C. Cook, and L. C. N. Tucker, *J. Chem. Soc.*, 2292-2294 (1965).

(440) K. Heyns, J. Weyer, and H. Paulsen, *Chem. Ber.*, **100**, 2317-2334 (1967).

(441) K. Heyns, E. Alpers, and J. Weyer, *Chem. Ber.*, **101**, 4199-4208 (1968).

(442) K. Heyns, W.-P. Trautwein, and H. Paulsen, *Chem. Ber.*, **96**, 3195-3199 (1963).

(443) K. Heyns, E. Alpers, and J. Weyer, *Chem. Ber.*, **101**, 4209-4213 (1968).

(444) K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, **104**, 2063-2070 (1971).

lytic oxidation and the molecular environment of these groups. The generally observed tendency, already noted, for oxidation at axially disposed groups is well illustrated by the behavior of *myo*-inositol⁴⁴⁵ and its *O*-methyl derivatives.⁴³⁵ *scyllo*-Inositol, in which all hydroxyl groups are equatorially disposed, is resistant towards catalytic oxidation. In inositols containing two axial hydroxyl groups, only one of them is oxidized, and, where these two axial groups are unlike (for example, in 1D-3-*O*-methyl-*chiro*-inositol or 1L-2-*O*-methyl-*chiro*-inositol), one of them is oxidized to the exclusion of the other.⁴³⁵ Oxidation of inositols possessing three axial hydroxyl groups leads similarly to the formation of monoketones only, and the reaction is very selective. Thus, with *muco*-inositol and *allo*-inositol, the axial hydroxyl group that is oxidized is that which is *meta* and adjacent to another axial hydroxyl group.⁴³⁶ Aminodeoxycyclitols may be selectively oxidized if their amino groups are protected as the *N*-acyl derivative,⁴⁴⁶ and this technique has found application in synthetic studies related to the synthesis of streptamine.^{446,447}

A study of the catalytic oxidation of six stereoisomers of 5-cyclohexene-1,2,3,4-tetrol revealed⁴⁴⁸ that, in all cases, an allylic hydroxyl group is selectively attacked, and a consideration of their favored conformations suggested that quasi-axial groups are selectively dehydrogenated before those that are quasi-equatorial.

A significant comparison has been drawn between the readiness of a hydroxyl group in a carbohydrate derivative to undergo catalytic oxidation and the ability of a suitable derivative of that hydroxyl group (for example, the *p*-toluenesulfonate) to undergo nucleophilic replacement.⁴¹⁸ Thus, of the pair of compounds 1,2:5,6-di-*O*-isopropylidene- α -D-glucofuranose and 1,2:5,6-di-*O*-isopropylidene- α -D-allofuranose, the former is inert to catalytic oxidation,⁴⁴⁹ and its 3-sulfonates are, with few exceptions, reluctant to undergo replacement. In contrast, the *allo* isomer is readily oxidized⁴⁴⁹ and its 3-sulfonic esters undergo⁴⁵⁰⁻⁴⁵² facile, SN2 reactions at C-3. Likewise, oxidation of the primary hydroxyl group in aldohexopyranose derivatives is the most difficult with those having the *galacto* configura-

(445) K. Heyns and H. Paulsen, *Chem. Ber.*, **86**, 833-840 (1953).

(446) K. Heyns and H. Paulsen, *Chem. Ber.*, **89**, 1152-1160 (1956).

(447) T. Suami and S. Ogawa, *Bull. Chem. Soc. Jpn.*, **40**, 1925-1929 (1967).

(448) K. Heyns, H. Gottschalck, and H. Paulsen, *Chem. Ber.*, **95**, 2660-2668 (1962).

(449) O. Theander, *Acta Chem. Scand.*, **18**, 2209-2216 (1964).

(450) D. T. Williams and J. K. N. Jones, *Can. J. Chem.*, **45**, 7-9 (1967).

(451) J. S. Brimacombe, J. G. H. Bryan, A. Husain, M. Stacey, and M. S. Tolley, *Carbohydr. Res.*, **3**, 318-324 (1967).

(452) A. B. Foster, R. Hems, and J. M. Webber, *Carbohydr. Res.*, **5**, 292-301 (1967).

tion,⁴¹⁷ and a low reactivity is also observed with replacement reactions at C-6 in galactopyranose derivatives.^{2,453-455}

Other replacement-oxidation reactivity correlations exist. Steric factors affecting these two types of reaction may, indeed, be very similar. Thus, the nucleophile in an SN2 reaction and the platinum catalyst in an oxidation can only approach the site of attack (the carbon atom possessing the leaving group and the hydrogen atom geminal to the hydroxyl group, respectively) from a sterically unhindered side. If this is not possible, both reactions will proceed only with difficulty. Not all effects observed in catalytic oxidation are interpretable on steric factors alone, however, and a dehydrogenation mechanism involving a polar transition-state (analogous to that in SN2 reactions) was suggested,⁴¹⁸ in which a hydride ion of the substrate is removed with simultaneous replacement by a hydroxyl group. It appears that intramolecular hydrogen-bonding may be a further factor influencing selectivity in catalytic oxidation.⁴⁵⁶

2. Oxidations Based on Dimethyl Sulfoxide

Although oxidations involving dimethyl sulfoxide have been widely used in carbohydrate chemistry, there are relatively few reports of selective oxidation with this reagent. Treatment of methyl 4,6-O-benzylidene- α -D-glucopyranoside with dimethyl sulfoxide-phosphorus pentaoxide afforded methyl 4,6-O-benzylidene- α -D-ribohexopyranosid-3-ulose in 11% yield, whereas, with the β -D-glucoside derivative, only starting-material was recovered.⁴⁵⁷ With dimethyl sulfoxide-acetic anhydride, methyl 4,6-O-benzylidene- α -D-glucopyranoside at room temperature gave a mixture of at least six products, from which methyl 2-O-acetyl-4,6-O-benzylidene- α -D-ribohexopyranosid-3-ulose could be crystallized directly in 24% yield.⁴⁵⁸

On treatment with dimethyl sulfoxide-acetic anhydride followed by sequential oximation, reduction, detritylation, and acid hydrolysis, a tetra-(6-O-trityl)-cyclohexaamylose was reported to afford 2-amino-2-deoxy-D-glucose, in addition to D-glucose, indicating⁴⁵⁹ that

(453) J. M. Sugihara and W. J. Teerlink, *J. Org. Chem.*, **29**, 550-554 (1964).

(454) S. Nadkarni and N. R. Williams, *J. Chem. Soc.*, 3496-3498 (1965).

(455) C. L. Stevens, P. Blumbergs, F. A. Daniher, D. H. Otterbach, and K. G. Taylor, *J. Org. Chem.*, **31**, 2822-2829 (1966).

(456) K. Heyns, G. Rüdiger, and H. Paulsen, *Chem. Ber.*, **105**, 1028-1031 (1972).

(457) Y. Kondo and F. Takao, *Can. J. Chem.*, **51**, 1476-1481 (1973).

(458) J. Defaye and A. Gadelle, *Carbohydr. Res.*, **35**, 264-269 (1974).

(459) K. Takeo, F. Yagi, and T. Kuge, *Kyoto Furitsu Daigaku Gakujutsu Hokoku, Nogaku*, 159-163 (1972); *Chem. Abstr.* **79**, 18,987t (1973).

selective oxidation had occurred at C-2. Analogous results were obtained when a similar series of reactions was applied to 6-*O*-tritylamylose.⁴⁶⁰ Of the total carbonyl content in the product of oxidation of cellulose with dimethyl sulfoxide-acetic anhydride, 47–62% was estimated to be present at C-6 as aldehyde groups, the remainder presumably being distributed⁴⁶¹ between C-2 and C-3. With dimethyl sulfoxide-acetic anhydride, the favored point of oxidative attack on the D-glucose residues of 6-*O*-tritylcellulose was⁴⁶² at C-2, and few residues were transformed into 2,3-dicarbonyl derivatives.

Useful syntheses of D- and L-lyxose from 1,3-*O*-benzylidene-D- and L-arabinitol have been achieved through the highly selective oxidation of the primary hydroxyl groups by dimethyl sulfoxide-dicyclohexylcarbodiimide.⁴⁶³ Oxidation of but one of the two (equivalent) hydroxyl groups in 1,3,4,6-tetra-*O*-benzyl-D-mannitol⁴⁶⁴ and 1,6-di-*O*-benzyl-2,5-*O*-methylene-D-mannitol⁴⁶⁵ was possible with dimethyl sulfoxide-acetic anhydride.

3. Glycol-cleaving Reagents

a. Sodium Metaperiodate and Periodic Acid.—The selectivity sometimes observed in periodate oxidation of polyhydroxy compounds can often be rationalized in terms of the ease of formation, the relative stabilities, or the rates of decomposition of the intermediate, cyclic esters through which these oxidations are presumed to proceed.^{466–470} With cyclic 1,2-glycols, oxidation of *cis* isomers is usually more rapid than for the *trans* isomers.^{471,472} In five-membered ring-systems, the dihedral angle between two vicinal hydroxyl groups can be smaller in *cis* than in *trans* isomers.

For six-membered-ring compounds in the chair conformations,

(460) M. L. Wolfrom and P. Y. Wang, *Carbohydr. Res.*, **12**, 109–114 (1970).

(461) S. L. Snyder, T. L. Vigo, and C. M. Welch, *Carbohydr. Res.*, **34**, 91–98 (1974).

(462) K. Bredereck, *Tetrahedron Lett.*, 695–698 (1967).

(463) T. A. Giudici and J. J. Griffin, *Carbohydr. Res.*, **33**, 287–295 (1974).

(464) R. K. Ness, H. W. Diehl, and H. G. Fletcher, Jr., *Carbohydr. Res.*, **13**, 23–32 (1970).

(465) T. B. Grindley, J. W. Bird, W. A. Szarek, and J. K. N. Jones, *Carbohydr. Res.*, **24**, 212–215 (1972).

(466) G. J. Buist and C. A. Bunton, *J. Chem. Soc.*, 1406–1413 (1954).

(467) G. J. Buist, C. A. Bunton, and J. H. Miles, *J. Chem. Soc.*, 4567–4574 (1957).

(468) G. J. Buist, C. A. Bunton, and J. H. Miles, *J. Chem. Soc.*, 4575–4579 (1957).

(469) G. J. Buist and C. A. Bunton, *J. Chem. Soc.*, 4580–4584 (1957).

(470) G. J. Buist, C. A. Bunton, and J. H. Miles, *J. Chem. Soc.*, 743–748 (1959).

(471) C. C. Price and M. Knell, *J. Am. Chem. Soc.*, **64**, 552–554 (1942).

(472) H. Klosterman and F. Smith, *J. Am. Chem. Soc.*, **74**, 5336–5339 (1952).

both *cis*- and *trans*-diols have similar dihedral angles, but deformation of the ring to decrease this angle is energetically less demanding for the former. Nevertheless, it is important to note that, for 1,2-cyclohexanediols, the equilibrium constant for formation of the cyclic intermediate is greater for the *trans* isomer,⁴⁷⁰ but the rate constant of breakdown of the *cis* intermediate is much the larger, and this factor constitutes the overriding influence on the rate of oxidation of these compounds. For polyhydroxycyclohexanes, other factors (for example, configuration^{473,474} and steric strain⁴⁷⁴) may influence their reactivity towards periodate oxidation. Temperature and acidity may also affect the relative rates of reaction of certain inositols with periodate.⁴⁷⁵ Certain spatial arrangements of three hydroxyl groups in a molecule, such as those found in 1,2-O-isopropylidene- α -D-glucofuranose⁴⁷⁶ and *cis,cis*-1,2,3-triols on six-membered rings,⁴⁷⁷ are conducive to the formation of reasonably stable, tridentate complexes at certain pH values, causing unusual stability of α -glycol groupings in such molecules towards cleavage by periodate.

Selective cleavage of the exocyclic, vicinal glycol groupings in the methyl D-gluco- and D-galacto-furanosides⁴⁷⁸ and methyl β -D-glycero-D-gulo-heptopyranoside⁴⁷⁹ has been observed, and D-galactono-1,4-lactone undergoes a similar reaction (to afford L-lyxuronic acid⁴⁸⁰). In contrast, on oxidation with one molar proportion of periodic acid, D-glycero-D-gulo-heptono-1,4-lactone undergoes favored attack at the *cis*, vicinal-diol grouping on the five-membered ring, rather than at either of the exocyclic glycol groupings, yielding D-arabinose in 70% yield.⁴⁸¹

The favored oxidative cleavage of a primary-secondary over a primary-tertiary vicinal diol grouping has been utilized in the synthesis of branched-chain sugar derivatives.^{482,483}

Periodic acid in dimethyl sulfoxide has been reported to show

(473) P. (F.) Fleury, J. E. Courtois, and A. Bieder, *Bull. Soc. Chim. Fr.*, 543-545 (1953).

(474) S. J. Angyal and D. J. McHugh, *J. Chem. Soc.*, 1423-1431 (1957).

(475) S. R. Sarfati and P. Szabó, *Carbohydr. Res.*, 11, 571-573 (1969).

(476) A. S. Perlin and E. von Rudloff, *Can. J. Chem.*, 43, 2071-2077 (1965).

(477) G. R. Barker and D. F. Shaw, *J. Chem. Soc.*, 584-593 (1959).

(478) O. Kjølberg, *Acta Chem. Scand.*, 14, 1118-1123 (1960).

(479) N. J. Antia and M. B. Perry, *Can. J. Chem.*, 38, 1917-1920 (1960).

(480) R. K. Hulyalkar and M. B. Perry, *Can. J. Chem.*, 43, 3241-3246 (1965).

(481) W. C. Griffiths, T. T. Galkowski, R. W. Kocon, and K. M. Reardon, *Carbohydr. Res.*, 13, 177-178 (1970).

(482) R. J. Woods and A. C. Neish, *Can. J. Chem.*, 32, 404-414 (1954).

(483) H. Paulsen and W. Stenzel, *Tetrahedron Lett.*, 25-28 (1974).

novel selectivity towards methyl aldopyranosides.⁴⁸⁴ Selective cleavage of the C-2-C-3 bond in the α -D-lyxoside, β -D-riboside, β -D-xyloside, and α -L-rhamnoside was observed, whereas rupture of the C-3-C-4 bond in the β -L-arabinoside and α -D-galactoside was found to occur. By contrast, no selectivity was observed in the oxidation of the α -D-glucoside and α -D-mannoside, or dextran. Most (but not all) of these reactions could be rationalized by assuming that vicinal hydroxyl groups in an axial-equatorial orientation are oxidized faster than those having the equatorial-equatorial disposition.

Selectivity in the oxidation of acyclic polyhydric alcohols by sodium metaperiodate has been noted.⁴⁸⁵ When treated with 0.1 molar equivalent of oxidant, aqueous solutions of D-mannitol, galactitol, and D-glucitol yielded products of cleavage at *threo*-glycol groupings, presumably because fewer nonbonded interactions are present in the cyclic intermediates derived from *threo*- than in those from *erythro*-diol groupings. Similar observations were made on the oxidation of hexitols with very low concentrations of periodic acid relative to substrate,^{486,487} but, at higher relative concentrations, rupture between primary and secondary alcohol groups occurred, to yield pentoses. Investigation of the partial, periodate oxidation of D-glucitol indicated⁴⁸⁸ that the order of susceptibility for C-C bond cleavage is 3,4 > 2,3 > 4,5 > 5,6 > 1,2.

b. Lead Tetraacetate.—The reaction of lead tetraacetate with carbohydrates has been discussed.⁴⁸⁹ Although, in the cyclopentane and cyclohexane series, *cis*-vicinal glycols generally react more rapidly than their *trans* isomers, suggesting that a cyclic intermediate is involved, *trans*-1,2-cyclopentanediol⁴⁹⁰ and *trans*-1,2-dimethyl-1,2-cyclopentanediol⁴⁹¹ both consume lead tetraacetate faster than their cyclohexanediol counterparts.^{491,492} Furthermore, some ditertiary vicinal-diols that cannot form cyclic esters are, nevertheless, cleaved by the reagent.^{490,493} In these compounds, it appears that glycol

(484) R. J. Yu and C. T. Bishop, *Can. J. Chem.*, **45**, 2195–2203 (1967).

(485) J. C. P. Schwarz, *J. Chem. Soc.*, 276–278 (1957).

(486) J. E. Courtois and M. Guernet, *Compt. Rend.*, **245**, 1273–1275 (1957).

(487) J. E. Courtois and M. Guernet, *Bull. Soc. Chim. Fr.*, 1388–1393 (1957).

(488) D. H. Hutson and H. Weigel, *J. Chem. Soc.*, 1546–1552 (1961).

(489) A. S. Perlin, *Adv. Carbohydr. Chem.*, **14**, 9–61 (1959).

(490) R. Criegee, E. Büchner, and W. Walther, *Ber.*, **73**, 571–575 (1940).

(491) P. Levesley, W. A. Waters, and A. N. Wright, *J. Chem. Soc.*, 840–845 (1956).

(492) R. Criegee, L. Kraft, and B. Rank, *Ann.*, **507**, 159–197 (1933).

(493) R. Criegee and H. Zogel, *Chem. Ber.*, **84**, 215–219 (1951).

cleavage with lead tetraacetate need not involve a cyclic intermediate, and an alternative mechanism may be operative,⁴⁹⁴ which is not, however, available to dissecondary glycols.⁴⁹⁵

An exocyclic, vicinal-diol grouping may be cleaved instead of a *trans* (but not a *cis*) vicinal-diol grouping on a furanoid ring.⁴⁹⁶ Oxidation of sucrose with one molar equivalent of lead tetraacetate led to attack mainly in the D-fructofuranosyl group, illustrating that cleavage at a *trans*, vicinal-glycol group on a furanose ring is favored over that on a pyranose ring; in contrast, periodate attacks the D-glucopyranosyl group more readily.⁴⁹⁷ Of particular importance is an extensive study on the oxidation of reducing sugars,^{498,499} which were shown to undergo selective cleavage at the α -hydroxy hemiacetal groups (C-1-C-2 bond in aldoses), to yield monoesters of a sugar having a shorter carbon chain. The fastest glycol-cleavage oxidations involve⁵⁰⁰ vicinal diols having a dihedral angle close to 0°, and, as reducing aldoses exhibit rates of oxidation in this extreme range, it has been suggested^{489,499} that they may be oxidized through their furanose forms, even though these may only form a minor part of the species in equilibrium in solution. This selective oxidation provides a simple method for directly preparing some relatively rare carbohydrates from readily available aldoses,^{501,502} and has also been adapted to provide a stepwise degradation of reducing disaccharides⁵⁰³ and higher oligosaccharides.⁵⁰⁴

The results of studies on the oxidation of alditols by lead tetraacetate suggest that a vicinal diol containing two secondary hydroxyl groups can be oxidized more readily than one consisting of a primary and a secondary hydroxyl group.⁵⁰⁵ If a tertiary hydroxyl group is part of a vicinal-diol grouping, the resistance of the grouping to lead tet-

(494) R. Criegee, E. Höger, G. Huber, P. Kruck, F. Martkscheffel, and H. Schellenberger, *Ann.*, **599**, 81-125 (1956).

(495) S. J. Angyal and R. J. Young, *J. Am. Chem. Soc.*, **81**, 5251-5255 (1959).

(496) R. Criegee, *Ann.*, **495**, 211-225 (1932).

(497) A. K. Mitra and A. S. Perlin, *Can. J. Chem.*, **37**, 2047-2052 (1959).

(498) A. S. Perlin and C. Brice, *Can. J. Chem.*, **33**, 1216-1221 (1955).

(499) A. S. Perlin and C. Brice, *Can. J. Chem.*, **34**, 541-553 (1956).

(500) R. E. Reeves, *Anal. Chem.*, **21**, 751 (1949).

(501) A. S. Perlin and C. Brice, *Can. J. Chem.*, **34**, 85-88 (1956).

(502) P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, **34**, 693-700 (1956).

(503) A. J. Charlson and A. S. Perlin, *Can. J. Chem.*, **34**, 1200-1208 (1956).

(504) F. W. Parrish, A. S. Perlin, and E. T. Reese, *Can. J. Chem.*, **38**, 2094-2104 (1960).

(505) P. F. Fleury, J. E. Courtois, and A. Bieder, *Bull. Soc. Chim. Fr.*, 118-122 (1952).

raacetate oxidation may be increased, and this difference in reactivity has been used to advantage in a synthesis of D-apiose.⁵⁰⁶

4. Miscellaneous Oxidants

a. Silver Carbonate on Celite.—The silver carbonate-on-Celite reagent, developed by Fétizon and Golfier,⁵⁰⁷ favors the oxidation of secondary over primary hydroxyl groups in simple polyhydric alcohols in benzene solution,⁵⁰⁸ and it has been found to exhibit considerable selectivity in the oxidation of carbohydrates. Oxidation of the allylic hydroxyl group at C-3 in D-glucal was achieved⁵⁰⁹ in yields of 60–80%, and selectivity for attack at the anomeric center in reducing aldoses has been noted. Thus, D-galactose with the reagent in aqueous solution at 80° afforded D-galactono-1,4-lactone⁵¹⁰, and 2,3:5,6-di-O-isopropylidene-D-mannofuranose, 2,3:5,6-di-O-isopropylidene-D-allofuranose, 2,3-O-isopropylidene-D-ribofuranose, and 3,4-O-isopropylidene-D-arabinopyranose were all converted by treatment in refluxing benzene into the corresponding aldonolactones.⁵¹¹ Partially protected 2-acetamido-2-deoxypyranoses were also selectively oxidized⁵¹² at C-1 in 1,4-dioxane, but elimination and epimerization were accompanying reactions.

Under certain conditions, cleavage of the bond between the anomeric carbon atom and its neighboring carbon atom in a free aldose is brought about by the reagent. After treatment of D-galactose in refluxing ethanol with the Fétizon reagent for 2 hours, D-lyxose (36%) was isolated, in addition to D-galacturonic acid (50%)⁵¹⁰. On treatment in methanol, D-xylose,⁵¹³ L-arabinose,⁵¹³ 3,4-O-isopropylidene-L-arabinose,⁵¹¹ 3-O-methyl-D-glucose,⁵¹⁴ L-sorbose,⁵¹⁵ and D-fructose⁵¹⁶ gave, after hydrolysis of intermediate esters, D-

(506) P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, **36**, 480–485 (1958).

(507) M. Fétizon and M. Golfier, *Compt. Rend., Ser. C*, **267**, 900–903 (1968).

(508) M. Fétizon, M. Golfier, and J. M. Louis, *Chem. Commun.*, 1102 (1969).

(509) J. M. J. Tronchet, J. Tronchet, and A. Birkhäuser, *Helv. Chim. Acta*, **53**, 1489–1490 (1970).

(510) M. Fétizon and N. Moreau, *Compt. Rend., Ser. C*, **275**, 621–623 (1972).

(511) S. Morgenlie, *Acta Chem. Scand.*, **26**, 2518–2522 (1972).

(512) N. Pravdič, B. Danilov, and H. G. Fletcher, Jr., *Carbohydr. Res.*, **36**, 167–180 (1974).

(513) S. Morgenlie, *Acta Chem. Scand.*, **26**, 1709–1710 (1972).

(514) S. Morgenlie, *Acta Chem. Scand.*, **25**, 2773–2774 (1971).

(515) S. Morgenlie, *Acta Chem. Scand.*, **26**, 2146–2147 (1972).

(516) S. Morgenlie, *Acta Chem. Scand.*, **27**, 1557–1564 (1973).

threose, L-erythrose, 2,3-O-isopropylidene-L-erythrose, 2-O-methyl-D-arabinose, L-threose, and D-erythrose, respectively.

In contrast to the oxidation of acyclic polyhydric alcohols in benzene,⁵⁰⁸ treatment of 1,2-O-isopropylidene- α -D-glucofuranose with silver carbonate-on-Celite in boiling methanol led to selective oxidation of the primary hydroxyl group, with the formation of the alduronic acid derivative in 68% yield.⁵¹⁷

A study⁵¹⁸ of the mechanism of oxidation of alcohols by the reagent suggested that a reversible, oriented adsorption of the alcohol onto the surface of the oxidant occurs, with the oxygen atom of the alcohol forming a coordinate bond to a silver ion, followed by a concerted, irreversible, homolytic shift of electrons to generate silver atoms, carbon dioxide, water, and the carbonyl compound. The reactivity of a polyhydroxy compound may not, it appears, be deduced from the relative reactivity of its component functions, as the geometry of the adsorbed state, itself affected by solvent polarity, exerts an important influence on the selectivity observed.⁵¹⁹

b. Chromium Compounds.—Chromium-based oxidants often oxidize polyhydric alcohols with some selectivity, but, generally, the yields of product are very low. On treatment with 1.5 molar equivalents of chromium trioxide in pyridine and removal of the ethylidene groups from the products, methyl 4,6-O-ethylidene- β -D-glucopyranoside gave methyl β -D-ribo-hexopyranosid-3-ulose as the principal, neutral product, but in only 5.4% yield.⁵²⁰ With the same reagent, methyl 4,6-O-benzylidene- β -D-galactopyranoside underwent attack at both C-2 and C-3 to a similar extent.⁵²¹ Reaction of methyl 6-O-trityl- α - and - β -D-glucopyranoside with the trioxide in acetone gave,⁵²² after detritylation and extensive chromatographic purification, methyl D-hexopyranosiduloses resulting from attack at C-2, C-3, and C-4. For both the α - and β -D-glucoside derivatives, oxidation at C-3 was favored, but methyl α - and β -D-ribo-hexopyranosid-3-ulose were obtained in yields of only 2 and 4.7%, respectively. Favored attack at C-3 under these conditions was

(517) S. Morgenlie, *Acta Chem. Scand.*, **27**, 2217–2218 (1973).

(518) F. J. Kakis, M. Fétizon, N. Douchkine, M. Golfier, P. Mourgues, and T. Prange, *J. Org. Chem.*, **39**, 523–533 (1974).

(519) M. Fétizon and P. Mourgues, *Tetrahedron*, **30**, 327–335 (1974).

(520) A. Assarsson and O. Theander, *Acta Chem. Scand.*, **12**, 1507–1511 (1958).

(521) E. Brimacombe, J. S. Brimacombe, B. Lindberg, and O. Theander, *Acta Chem. Scand.*, **15**, 437–438 (1961).

(522) O. Theander, *Acta Chem. Scand.*, **11**, 1557–1564 (1957).

also observed with methyl β -D-xylopyranoside.⁵²³ Methyl β -D-glucopyranoside has been partially oxidized with chromium trioxide in acetone,⁵²⁰ and with aqueous potassium dichromate-oxalic acid.⁵²⁴ In the former experiment, oxidation at C-3 again predominated, whereas, in the latter reaction, the yield of the product of oxidation at C-6, namely, methyl β -D-glucopyranoside, appeared to be the highest.

Chromium trioxide in pyridine selectively oxidizes the hydroxymethyl groups in thymidine, 2'-deoxyadenosine, 2'-deoxyguanosine, and 2'-deoxycytidine to carboxyl groups,⁵²⁵ but the partial liberation of the free, heterocyclic bases in the reactions suggested that oxidation at C-3 also occurs to some extent.

Chromic acid in acetone effects highly selective oxidation at C-5 in 6-deoxy-1,2-O-isopropylidene- β -L-idofuranose and 6-deoxy-1,2-O-isopropylidene- α -D-glucofuranose.⁵²⁶

c. Mercuric Acetate.—Secondary hydroxyl groups in unsubstituted alditols may be selectively oxidized by the action of mercuric acetate in methanol. Thus, after four weeks at 38°, ribitol afforded⁵²⁷ a mixture of DL-*erythro*-2-pentulose and DL-*erythro*-3-pentulose in the approximate ratio of 1:2, suggesting (unless the former compound is further oxidized) selectivity for oxidation at HO-3. On the other hand, on reaction in boiling methanol for several hours, D-arabinitol and xylitol gave D-*threo*-2-pentulose (11%) plus D-*threo*-3-pentulose (2.5%) plus D-*erythro*-2-pentulose (1.2%), and DL-*threo*-2-pentulose (12%) plus *erythro*-3-pentulose (1.6%), respectively.⁵²⁸ However, as considerable proportions of dehydration products were formed in the reactions at elevated temperature, the yields of particular pentuloses need not necessarily constitute a true indication of the extent of oxidation of the individual secondary hydroxyl groups. The reagent is not completely specific for oxidizing secondary hydroxyl groups, as reaction with glycerol yielded 1,3-dihydroxy-2-propanone plus DL-glyceraldehyde, and 1,2-ethanediol gave glycolaldehyde. It has

(523) E. Brimacombe, J. S. Brimacombe, and B. Lindberg, *Acta Chem. Scand.*, **14**, 2236–2239 (1960).

(524) B. Lindberg and O. Theander, *Acta Chem. Scand.*, **8**, 1870–1874 (1954).

(525) A. S. Jones, A. R. Williamson, and M. Winkley, *Carbohydr. Res.*, **1**, 187–195 (1965).

(526) D. E. Kiely, H. Walls, Jr., and R. Black, *Carbohydr. Res.*, **31**, 387–396 (1973).

(527) R. J. Stoodley, *Can. J. Chem.*, **39**, 2593–2601 (1961).

(528) L. Stankovič, K. Linek, and M. Fedoronko, *Carbohydr. Res.*, **10**, 579–583 (1969).

been claimed⁵²⁹ that mercuric acetate oxidizes D-mannitol to D-fructose in 30% yield, and D-glucitol to a mixture of D-fructose and L-sorbose in 20% yield. Reducing aldoses may be oxidized to the corresponding aldonic acids by the reagent.⁵²⁹

d. Potassium Ferrate.—In alkaline, aqueous media, this reagent is reported to convert methyl α -D-aldohexopyranosides into their corresponding methyl α -D-hexodialdo-1,5-pyranosides.⁵³⁰

e. *Acetobacter suboxydans*.—Microbiological oxidation by *Acetobacter suboxydans* has been used for many years as a highly selective method of introducing carbonyl groups into certain alditols, the sequence $\text{—CHOH—CHOH—CH}_2\text{OH}$ having the D-*erythro* configuration being converted into $\text{—CHOH—CO—CH}_2\text{OH}$ (see the Bertrand-Hudson rule^{531–533}). J. K. N. Jones and coworkers have used the technique to prepare 5-S-ethyl-5-thio-D-*threo*-2-pentulose⁵³⁴ and 6-S-ethyl-6-thio-L-sorbose¹⁷⁹ from 1-S-ethyl-1-thio-D-arabinitol and 1-S-ethyl-1-thio-D-glucitol, respectively. Several acetamidodeoxyketoses have also been prepared from the corresponding alditol derivatives.^{535–538}

VII. MIGRATION OF SUBSTITUENTS

Carbohydrate chemistry contains many examples of intramolecular migration of substituent groups. The largest number of these involve acyl groups, but migrations in phosphoric esters, and certain ethers have also been reported. Surprisingly, the topic has not been the subject of a comprehensive review, but there are several useful summaries thereof.^{539–542} In this Section, mainly those examples that have

- (529) G. N. Dorofeenko, *Ukr. Khim. Zh.*, **27**, 114–117 (1961); *Chem. Abstr.*, **55**, 20,970 (1961).
- (530) J. N. BeMiller, G. V. Kumari, and S. D. Darling, *Tetrahedron Lett.*, 4143–4146 (1972).
- (531) G. Bertrand, *Ann. Chim. Phys.*, **3** (8), 181–288 (1904).
- (532) G. Bertrand, *Compt. Rend.*, **126**, 762–765 (1898).
- (533) R. M. Hann, E. B. Tilden, and C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 1201–1203 (1938).
- (534) J. K. N. Jones and D. L. Mitchell, *Can. J. Chem.*, **37**, 1561–1566 (1959).
- (535) J. K. N. Jones, M. B. Perry, and J. C. Turner, *Can. J. Chem.*, **39**, 965–972 (1961).
- (536) J. K. N. Jones, M. B. Perry, and J. C. Turner, *Can. J. Chem.*, **39**, 2400–2410 (1961).
- (537) J. K. N. Jones, M. B. Perry, and J. C. Turner, *Can. J. Chem.*, **40**, 503–510 (1962).
- (538) J. C. Turner, *Can. J. Chem.*, **40**, 826–828 (1962).
- (539) E. L. Hirst and S. Peat, *Annu. Rep. Prog. Chem.*, **31**, 172–173 (1934).
- (540) W. A. Bonner, *J. Org. Chem.*, **24**, 1388–1390 (1959).

been reported since Sugihara's article¹ and which are of synthetic utility or mechanistic interest are considered.

1. Acyl Migration

The facile migration of acyl groups in partially acylated polyhydric alcohols was originally discovered, and correctly interpreted as proceeding through orthoacid intermediates, by Emil Fischer,⁵⁴³ and the intramolecular nature of the mechanism was substantiated by the results of a radioactive-labelling study.⁵⁴⁴ The rearrangement is both acid- and base-catalyzed, and can occur under the mildly alkaline conditions existing in some reactions (for example, Purdie methylation). Acyl groups generally rearrange from secondary to primary positions. Migrations of acyl groups involving each oxygen atom of an aldohexose (except O-6) as a site of migration origin have been observed,⁵⁴⁰ and the generalization⁵⁴⁵ that acyl groups migrate away from O-1 towards O-6 of an aldohexose is substantiated by many examples, but exceptions are known,⁴⁸ and there seems to be no theoretical basis for the observation. The rate of acyl migration can depend on (a) the solvent,^{546,547} (b) the alkalinity or acidity of the medium,⁵⁴⁷ and (c) the relative configurations at the two centers involved⁵⁴⁷; migration in a monoacyl derivative of a vicinal diol occurs more readily when the groups are *cis*- than when they are *trans*-disposed on a five- or six-membered ring, presumably because there is less strain in the orthoacid intermediate for the *cis* compounds.⁵⁴⁸

Acyl migration is a reversible process, and external factors (for example, solubility) can influence the equilibrium. Thus, after dissolution of methyl 2-O-benzoyl-4,6-O-benzylidene- α -D-glucopyranoside in acetone-aqueous sodium hydroxide, almost immediate crystallization of the 3-benzoate occurred, in 65% yield.⁵⁴⁹ Similarly, benzyl 3-O-benzoyl-4,6-O-benzylidene- β -D-galactopyranoside yielded the 2-

(541) R. U. Lemieux, in "Molecular Rearrangements," P. de Mayo, ed., Wiley, New York, 1964, Part 2, pp. 763-769.

(542) R. M. Rowell, *Carbohydr. Res.*, **23**, 417-424 (1972).

(543) E. Fischer, *Ber.*, **53**, 1621-1633 (1920).

(544) A. P. Doerschuk, *J. Am. Chem. Soc.*, **74**, 4202-4203 (1952).

(545) F. Brown, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 1125-1127 (1950).

(546) S. J. Angyal and G. J. H. Melrose, *J. Chem. Soc.*, 6494-6500 (1965).

(547) S. J. Angyal and G. J. H. Melrose, *J. Chem. Soc.*, 6501-6504 (1965).

(548) S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686-695 (1952).

(549) E. J. Bourne, A. J. Huggard, and J. C. Tatlow, *J. Chem. Soc.*, 735-741 (1953).

benzoate in 81% yield,⁴⁸ even though, under homogeneous conditions, the latter compound is only slightly more stable than the former. Although isomerization of the methyl 4,6-*O*-benzylidene- α -D-glucopyranoside 2- and 3-benzoates is rapid in the presence of hydroxide ions, imidazole in chloroform is less efficient in promoting the migration, and the 3-benzoate is isomerized to the extent of only ~10% after 12 hours in refluxing solution.¹⁶⁵ However, the 3-benzoate of methyl 4,6-*O*-benzylidene- α -D-mannopyranoside, which contains a *cis*, vicinal-diol grouping, is much more rapidly isomerized by the same reagent, equilibrium with the 2-isomer being attained in about 1 hour; in contrast, the 3-*O*-(2,6-dimethoxybenzoyl) and 3-*O*-(2,4,6-trimethylbenzoyl) derivatives are virtually unaffected under similar conditions,⁵⁵⁰ illustrating that the migratory tendencies of an aroyl group may be lessened by the introduction of suitable substituents.

Methyl α -D-mannopyranoside 2- and 3-acetates are also readily interconverted⁵⁰ on heating them in aqueous solution buffered at pH 5, 6, or 7. The isomerization of the 2- and 3-*O*-carbamoyl derivatives of methyl α -D-mannopyranoside under alkaline conditions has been studied, to provide information on the behavior of carbamoyl groups in bleomycin⁵⁵¹; in aqueous ethanol containing triethylamine, the 2- and 3-esters were present in the ratio of ~1:3. Migration of carbamoyl groups between the *cis*-disposed oxygen atoms at C-2 and C-3 of the carbohydrate component of novobiocin has also been noted.⁵⁵² Transfer of the carbamoyl group from O-3 to O-2 causes loss of antibiotic activity, and the equilibrium mixture contains 2- and 3-*O*-carbamoyl derivatives in the approximate ratio of 1:2. Isomerization of the 2-, 3-, and 4-hexanoates of lincomycin showed⁵⁵³ that, in both alkaline and acidic media, migration from O-3 to the *cis*-disposed O-4 is favored over that to the *trans*-disposed O-2.

The importance of relative configuration in influencing acyl migration was further illustrated during deacetylation of the anomers of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(2,4,6-trimethylbenzoyl)-D-glucopyranose. On treatment with methanolic ammonia⁵⁵⁴ or sodium methoxide,⁵⁵⁵ the β -D anomer (*trans*-O-1,O-2) gave the expected β -D-glucopyranosyl (2,4,6-trimethylbenzoate). In contrast, the α -D anomer

(550) S. A. Abbas and A. H. Haines, *Carbohydr. Res.*, **41**, 298–303 (1975).

(551) S. Omoto, T. Takita, K. Maeda, and S. Umezawa, *Carbohydr. Res.*, **30**, 239–247 (1973).

(552) J. W. Hinman, E. L. Caron, and H. Hoeksema, *J. Am. Chem. Soc.*, **79**, 5321–5322 (1957).

(553) T. O. Oesterling, *Carbohydr. Res.*, **15**, 285–290 (1970).

(554) H. B. Wood, Jr., and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **78**, 207–210 (1956).

(555) F. Micheel and G. Baum, *Chem. Ber.*, **88**, 2020–2025 (1955).

(*cis*-O-1,O-2) yielded 2-O-(2,4,6-trimethylbenzoyl)-D-glucose,⁵⁵⁶ resulting from deacetylation and subsequent acyl migration.⁵⁵⁷ Analogous results were obtained on alkaline deacetylation of 2,3,4,6-tetra-O-acetyl-1-O-(tri-O-acetylalloyl)- α - and - β -D-glucopyranose, and of 1-O-(*p*-acetoxybenzoyl)-2,3,4,6-tetra-O-acetyl- α - and - β -D-glucopyranose.^{558,559} A similar dependence of migration on configuration was shown in the pair of compounds 1-O-benzoyl- α - and - β -L-arabinofuranose, and 1-O-benzoyl- α - and - β -L-arabinopyranose.⁵⁶⁰ Both of the α -L-arabinose derivatives (*trans*-O-1,O-2) were stable in aqueous pyridine, whereas the β -L anomers readily underwent benzoyl migration to yield 2-O-benzoyl-L-arabinose.

Acyl migration has been observed many times during Purdie methylation of partially acylated carbohydrates. Further reports illustrate the supposedly "capricious" nature of acyl migration under alkylation conditions. For example, although methylation of methyl 2,3,4-tri-O-acetyl- α -D-glucopyranoside with methyl iodide-silver oxide gave the 2-methyl ether,⁵⁶¹ reaction of the corresponding β -D-glucoside derivative with these reagents in *N,N*-dimethylformamide (Kuhn methylation) yielded the 4-methyl ether in 45% yield.⁵⁶² Similarly, 4-methyl ethers were obtained from the corresponding ethyl⁵⁴² and phenyl⁵⁶³ β -D-glucopyranoside derivatives, although, for the latter compounds, isolation of 10% of the 3- and 29% of the 2-methyl ethers, respectively, suggested that a partial, secondary isomerization had occurred after the O-4 \rightarrow O-6 acyl migration. Benzylation of phenyl 2,3,4-tri-O-acetyl- β -D-glucopyranoside,⁵⁶⁴ and methylation of methyl 2,3,4-tri-O-(*N*-phenylcarbamoyl)- β -D-glucopyranoside⁵⁶⁵ was also accompanied by O-4 \rightarrow O-6 acyl migration.

Methyl 2,4,6-tri-O-acetyl- β -D-glucopyranoside afforded the 2-methyl ether in 66% yield on methylation under the Purdie conditions.⁵⁶⁶ A similar O-2 \rightarrow O-3 acyl rearrangement was observed on

(556) H. B. Wood, Jr., and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **78**, 2849-2851 (1956).

(557) C. Pedersen and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **82**, 3215-3217 (1960).

(558) O. T. Schmidt and H. Reuss, *Ann.*, **649**, 137-148 (1961).

(559) O. T. Schmidt and H. Schmadel, *Ann.*, **649**, 157-167 (1961).

(560) S. Tejima and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 2999-3004 (1963).

(561) W. N. Haworth, E. L. Hirst, and E. G. Teece, *J. Chem. Soc.*, 2858-2860 (1931).

(562) H. O. Bouveng, B. Lindberg, and O. Theander, *Acta Chem. Scand.*, **11**, 1788-1789 (1957).

(563) P. C. Wollwage and P. A. Seib, *J. Chem. Soc., C*, 3143-3155 (1971).

(564) P. A. Seib, *Carbohydr. Res.*, **8**, 101-109 (1968).

(565) H. O. Bouveng, *Acta Chem. Scand.*, **15**, 87-95 (1961).

(566) P. A. Finan and C. D. Warren, *J. Chem. Soc.*, 4214-4216 (1962).

methylation of 2,4,6-tri-*O*-acetyl- β -D-mannose, methyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl- β -D-mannopyranoside being the major product.⁵⁶⁷ Migration of acetyl groups in benzyl 2-*O*- and 3-*O*-acetyl-4-*O*-methyl- β -D-xylopyranoside in *N,N*-dimethylformamide containing silver oxide was found to be relatively slow, but, on Kuhn methylation, both of the monoacetates yielded⁷⁰ 2,4- and 3,4-dimethyl ethers in the same ratio of $\sim 2:1$, suggesting that products of reaction, possibly water,⁵⁴⁷ may greatly raise the rate of isomerization; related observations on the accelerating effect (on acyl migration) of small proportions of methyl iodide in chloroform-silver oxide mixtures have been made.⁵⁵⁰

Migration away from the anomeric center occurred when either 1,3,4,6-tetra-*O*-acetyl- α - or - β -D-glucopyranose was methylated under the Purdie conditions, to yield methyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside.⁵⁴⁰ That the acyl rearrangement occurred in both compounds is surprising, in view of the fact that only the α -D anomer of certain 1-*O*-aroyl derivatives of D-glucose undergo O-1 \rightarrow O-2 migration on treatment with base,⁵⁵⁴⁻⁵⁵⁹ but this difference in migratory aptitude of acetyl and aroyl groups may be rationalized in terms of a decreased reactivity of the carbonyl group in an aroyl residue towards nucleophilic attack, and, additionally, in the case of 2,4,6-trimethylbenzoyl derivatives,⁵⁵⁴⁻⁵⁵⁷ a decreased carbonyl reactivity due to steric hindrance. A comparison of the rates of migration of benzoyl and *p*-nitrobenzoyl groups from O-1 to O-2 in 1,3,5-tri-*O*-benzoyl- β -D-arabinofuranose and 1,3,5-tri-*O*-(*p*-nitrobenzoyl)- β -D-arabinofuranose, respectively, in acetone solution gave $t_{0.5}$ values of 814 and 27 minutes, respectively, illustrating that the *p*-nitro group aids nucleophilic attack at the ester carbonyl group.⁵⁶⁸

An interest in ester migration in the monoacyl derivatives of the vicinal-diol grouping in ribofuranosides stemmed from a desire to establish the position of attachment of the aminoacyl group to the terminal adenosine residue of aminoacyl-(t-RNA),⁵⁶⁹⁻⁵⁷¹ and also from the necessity of producing suitably protected intermediates for oligoribonucleotide syntheses. That a facile equilibrium can exist is shown by the fact that, on being kept, an ethanol solution of

(567) A. S. Perlin, *Can. J. Chem.*, **41**, 555-561 (1963).

(568) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, **29**, 3286-3290 (1964).

(569) C. S. McLaughlin and V. M. Ingram, *Biochemistry*, **4**, 1442-1447 (1965).

(570) C. S. McLaughlin and V. M. Ingram, *Biochemistry*, **4**, 1448-1456 (1965).

(571) B. E. Griffin, M. Jarman, C. B. Reese, J. E. Sulston, and D. R. Trentham, *Biochemistry*, **5**, 3638-3649 (1965).

2',5'- plus 3',5'-di-*O*-acetyluridine in the ratio of 1:1.57 deposited pure 3',5'-diester in almost quantitative yield.⁵⁷² The positions of equilibrium [given by k_1/k_2 (where k_1 and k_2 are the rate constants for the $O-2 \rightarrow O-3$ and $O-3 \rightarrow O-2$ migrations, respectively) and the rate constant for equilibration, $k_1 + k_2$] between 2',5'- and 3',5'-di-*O*-formyl-, -acetyl-, and -benzoyl-uridine have been measured⁵⁷² in anhydrous pyridine at 60°. In all instances, the 3',5'-diesters were marginally more stable than the 2',5'-isomers ($k_1/k_2 = 1.42, 1.61$, and 2.00), but the rates of equilibration differed strikingly. Although measurements were hampered by extreme sensitivity of the rate of equilibration to traces of water (note: the position of equilibrium between 2'- and 3'-*O*-acylnucleoside derivatives, as well as the rate of equilibration, can be solvent-dependent⁵⁷³), migratory aptitude was found to increase in the order: benzoyl < acetyl \ll formyl ($k_1 + k_2$ ratios = 1:18:670).

A comparison of formyl and acetyl migrations in 3'-*O*-acyladenosines in buffered dimethyl sulfoxide solution also showed that, under similar conditions, the formyl group equilibrates at by far the higher rate.⁵⁷¹ Significantly, it was reported that, for 2',5'-di-*O*-acetyluridines in pyridine at 20°, the *p*-methoxybenzoyl group is only 0.67 times as mobile as a benzoyl group.⁵⁷⁴ Apart from the use of the 2,6-dimethoxybenzoyl group to alleviate problems of acyl migration during methylation,⁵⁵⁰ the investigation of suitably substituted aroyl groups as nonmigrating, protecting groups has received little attention. The 2'- and 3'-pivalic esters of uridine are much less prone to interconversion than the corresponding acetyl derivatives. On heating the former esters at 50° in a 0.03 *M* solution of morpholine in deuteriomethanol, equilibrium (2':3'-ester = 1:1.37) was reached⁵⁷⁵ in about 5 days; under comparable (but not identical) conditions, equilibration of the acetates occurred in ~5 minutes.⁵⁷² The acetyl groups in 1-(2- and 3-*O*-acetyl- β -D-xylofuranosyl)uracil are considerably less mobile than those in the corresponding D-ribonucleosides.⁵⁷⁶ However, in refluxing pyridine, both isomers are isomerized into the 5'-*O*-acetyl derivative within 1 hour, illustrating

(572) C. B. Reese and D. R. Trentham, *Tetrahedron Lett.*, 2467-2472 (1965).

(573) D. P. L. Green and C. B. Reese, *Chem. Commun.*, 729-731 (1968).

(574) H. P. M. Fromageot, C. B. Reese, and J. E. Sulston, *Tetrahedron*, **24**, 3533-3540 (1968).

(575) J. Baker, M. Jarman, and J. A. Stock, *J. Chem. Soc. Perkin Trans. I*, 665-669 (1973).

(576) G. A. R. Johnston, *Tetrahedron Lett.*, 2679-2683 (1967).

the often-observed direction of migration from a secondary to a primary hydroxyl group.

Acyl migration can occur during detritylation reactions. Boiling of a solution of 2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-tri-*O*-tritylsucrose in glacial acetic acid containing a little water afforded a penta-*O*-acetyl-sucrose that, on methylation followed by deacetylation, gave 4,1',6'-tri-*O*-methylsucrose,⁵⁷⁷ suggesting an O-4 → O-6 acetyl transfer had occurred during detritylation or methylation, or both.⁵⁷⁸ Later work⁵⁷⁹ strongly suggested that migration had occurred in the former reaction, as detritylation with hydrogen bromide in acetic acid at low temperatures for a short time gave 2,3,4,3',4'-penta-*O*-acetylsucrose, which could be isomerized in acidic or basic media to 2,3,6,3',4'-penta-*O*-acetylsucrose, identical to the material obtained previously.⁵⁷⁷ These findings were substantiated by using n.m.r. spectroscopy to identify, unequivocally, the individual acetyl groups in the sucrose derivatives.⁵⁸⁰ An analogous acetyl migration from O-4 to O-6 took place on detritylation of 2,3,4,1',3',4'-hexa-*O*-acetyl-6,6'-di-*O*-tritylsucrose in boiling aqueous acetic acid,⁵⁸¹ but hydrogen bromide in acetic acid at low temperature has been successfully used to detritylate other sucrose derivatives without concomitant rearrangement.⁵⁸²

2,3,4,1',3',4'-Hexa-*O*-benzoyl-6,6'-di-*O*-tritylsucrose²³⁶ and 2,3,4,3',4'-penta-*O*-benzoyl-6,1',6'-tri-*O*-tritylsucrose,¹¹⁹ in contrast to the corresponding acetyl derivatives, may be detritylated in boiling, aqueous acetic acid without accompanying acyl migration. An O-6 → O-7 acetyl transfer was observed⁵⁸³ on detritylation of 1,2,3,4,6-penta-*O*-acetyl-7-*O*-trityl- β -D-*glycero*-D-*galacto*-heptose with hydrogen bromide in acetic acid at 10°.

In a study of isomerization in some partially acetylated derivatives of *myo*-inositol, weak bases were found to catalyze migration between all of the oxygen atoms, and *cis* and *trans* migrations occurred with almost equal facility.⁵⁴⁶ Equilibration of DL-2-*O*-acetyl-

(577) G. G. McKeown, R. S. E. Serenius, and L. D. Hayward, *Can. J. Chem.*, **35**, 28–36 (1957).

(578) G. G. McKeown and L. D. Hayward, *Can. J. Chem.*, **35**, 992–997 (1957).

(579) H. Bredereck, H. Zinner, A. Wagner, G. Faber, W. Greiner, and W. Huber, *Chem. Ber.*, **91**, 2824–2829 (1958).

(580) T. Suami, T. Otake, S. Ogawa, T. Shoji, and N. Kato, *Bull. Chem. Soc. Jpn.*, **43**, 1219–1223 (1970).

(581) R. Khan, *Carbohydr. Res.*, **25**, 232–236 (1972).

(582) T. Otake, *Bull. Chem. Soc. Jpn.*, **43**, 3199–3205 (1970).

(583) D. R. Strobach and L. Szabó, *J. Chem. Soc.*, 3970–3975 (1963).

1,4,5,6-tetra-*O*-methyl-*myo*-inositol with its 3-*O*-acetyl isomer in pyridine–water, and in chloroform in the presence of silver oxide, led to almost equal proportions of the two isomers, with only a slight preponderance of the one having its acetoxyl group in the equatorial position. In anhydrous pyridine at room temperature, no migration occurred in DL-1,3,4,5,6-penta-*O*-acetyl-*myo*-inositol, but low concentrations of water promoted rearrangements, an effect noted by others.⁵⁷² During methylation of DL-penta-*O*-acetyl-*myo*-inositols,⁵⁴⁷ it was found possible to suppress *trans*-acetyl migration almost completely by using neutral, dry silver oxide, a solvent of low polarity containing a low concentration of methyl iodide, and a low temperature; under these conditions, *cis* migration, although minimized, still occurred, and methylation was not complete.

Rearrangements have been noted during *p*-toluenesulfonylation reactions conducted in pyridine at elevated temperatures.⁵⁸⁴ DL-1,4,5,6-Tetra- and DL-1,3,4,5,6-penta-*O*-acetyl-*myo*-inositol gave, at 120°, the 1,3-di-*O*-*p*-tolylsulfonyl and 3-*O*-*p*-tolylsulfonyl derivatives, respectively. At room temperature, esterification was not accompanied by migration.

Studies of the acid-catalyzed isomerization of a range of mono-*O*-acylglycerol derivatives in ethanol⁵⁸⁵ and in chloroform⁵⁸⁶ showed that, at equilibrium, 1-*O*-acylglycerols preponderate over their 2-isomers in the ratio of ~9:1. The nature of the acyl group has a great influence on the rates of migration in ethanolic solution; for the series of 2-*O*-acylglycerols wherein the acyl groups were benzoyl, *p*-methoxybenzoyl, *p*-chlorobenzoyl, *p*-aminobenzoyl, triphenylacetyl, and 2,4,6-trimethylbenzoyl, these relative rates were in the ratios of 10:8:6:5.1:0.02:~0. Curiously, the addition of water to the medium greatly retarded the migration of the benzoyl group.

Aroyl migrations towards primary positions have been observed during the acid-catalyzed removal of acetal groups from 1,2,3,5-tetra-*O*-benzoyl-4,6-*O*-ethylidene-D-glucitol⁵⁸⁷ and 2,4-*O*-ethylidene-1,3-di-*O*-(*p*-nitrobenzoyl)erythritol.⁵⁸⁸

(584) S. J. Angyal, P. T. Gilham, and G. J. H. Melrose, *J. Chem. Soc.*, 5252–5255 (1965).

(585) O. E. Van Lohuizen and P. E. Verkade, *Recl. Trav. Chim. Pays-Bas*, **79**, 133–159 (1960).

(586) J. B. Martin, *J. Am. Chem. Soc.*, **75**, 5483–5486 (1953).

(587) M. Matsui, M. Okada, and M. Ishidate, *Chem. Pharm. Bull.*, **16**, 1288–1293 (1968).

(588) J. W. Van Cleve and C. E. Rist, *Carbohydr. Res.*, **4**, 95–96 (1967).

2. Phosphono Migration

Esters of phosphoric acid with polyhydroxy compounds can isomerize by an intramolecular process if they contain a free hydroxyl group in a sterically suitable position.⁵⁸⁹ The phenomenon was recognized by Levene and Raymond,^{185,186} who tried to prepare D-xylose 3-phosphate through phosphorylation of 5-O-benzoyl-1,2-O-isopropylidene- α -D-xylofuranose. Removal of the protecting groups by acid hydrolysis, and neutralization, afforded D-xylose 5-phosphate, suggesting a tendency for phosphono migration to occur towards a primary position. Confirmation of this tendency is found in the acid-catalyzed equilibrium between glycerol 1- and 2-phosphates,⁵⁹⁰⁻⁵⁹² which occurs⁵⁹³⁻⁵⁹⁶ at pH <3, and strongly favors the primary ester (ratio of 1- to 2-phosphate, $\sim 9:1$). The subject of phosphono migration assumed great importance during structural studies on the nucleotides,⁵⁹⁷ as nucleoside 2'- and 3'-phosphates are readily interconverted in acidic media, and they arise on alkaline hydrolysis of ribonucleic acid through nucleoside 2',3'-cyclic phosphate intermediates. It is clear that phosphono migration is an intramolecular process,⁵⁹⁸ and that it proceeds by way of a cyclic phosphate^{592,599} (which usually contains a five-membered ring). It is pertinent that 2'-, 3'-, and 4'-phosphates of 9- β -D-glucopyranosyladenine were not interconverted in refluxing 80% acetic acid or in trifluoroacetic anhydride,⁶⁰⁰ conditions under which ribonucleoside 2'- and 3'-phosphates are isomerized. In principle, cyclic phosphates of larger ring-sizes could be intermediates during rearrangements, but mild

(589) There has long been an inconsistency in the terminology often used to describe the phenomenon of migration in phosphoric esters. The term "phosphate migration" is incorrect, and, for monoesters of monophosphoric acid, the process is one of migration of the phosphono $[(HO)_2P(O)-]$ group [see *Chem. Abstr. Index Guide*, **76**, subsection H, paragraph 294, p. 131 I (1972)]. Although the term "phosphate migration" is well established, it will not be used here.

(590) M. C. Bailly, *Compt. Rend.*, **206**, 1902-1904 (1938).

(591) M. C. Bailly, *Compt. Rend.*, **208**, 443-445 (1939).

(592) P. E. Verkade, J. C. Stoppelenburg, and W. D. Cohen, *Recl. Trav. Chim. Pays-Bas*, **59**, 886-892 (1940).

(593) M. C. Bailly, *Bull. Soc. Chim. Fr.*, **9**, 314-339 (1942).

(594) M. C. Bailly, *Bull. Soc. Chim. Fr.*, **9**, 340-350 (1942).

(595) M. C. Bailly, *Bull. Soc. Chim. Fr.*, **9**, 405-420 (1942).

(596) M. C. Bailly, *Bull. Soc. Chim. Fr.*, **9**, 421-438 (1942).

(597) T. Ueda and J. J. Fox, *Adv. Carbohydr. Chem.*, **22**, 307-419 (1967).

(598) E. Chargaff, *J. Biol. Chem.*, **144**, 455-458 (1942).

(599) J. Baddiley, J. G. Buchanan, and L. Szabó, *J. Chem. Soc.*, 3826-3832 (1954).

(600) G. R. Barker and G. E. Foll, *J. Chem. Soc.*, 3794-3798 (1957).

treatment⁶⁰¹ of methyl α -D-mannopyranoside 4- and 6-phosphates with acid gave no evidence of phosphono migration between O-4 and O-6. It is, perhaps, relevant that, if a choice exists between five- and six-membered ring-closure in forming a cyclic phosphate, the former path is favored.⁶⁰²

In the inositol series, it has been shown^{603,604} that *myo*-inositol 1- and 2-phosphates are interconvertible in acid solution, migration occurring between the *cis*-disposed oxygen atoms. Although treatment with boiling, 80% aqueous acetic acid induces this interconversion, it appears that no *trans*-migration occurs. On the other hand, treatment with *M* hydrochloric acid at 80–85° induces phosphono migration from all positions,⁶⁰⁵ the migration being faster between *cis*- than between *trans*-disposed oxygen atoms. No migration in inositol phosphates occurs under alkaline conditions. Di- and tri-esters of phosphoric acid having suitably situated hydroxyl groups may also undergo migration under alkaline conditions. The basic hydrolysis of ribonucleic acid is an important example of this process in phosphoric diesters.⁶⁰⁶ Rearrangement of the phosphoric triester, 3,4,6-tri-*O*-acetyl- α -D-glucopyranosyl (dibenzyl phosphate) to 1,3,4,6-tetra-*O*-acetyl-2-deoxy- β -D-glucose-2-yl (dibenzyl phosphate) occurred in 93% yield during acetylation with acetic anhydride in triethylamine.⁶⁰⁷

3. Aryl Migration

The liberation of *p*-nitrophenoxide anion from *p*-nitrophenyl α -D-glucopyranoside on alkaline hydrolysis does not proceed by simple cleavage, but occurs⁶⁰⁸ only after prior migration of the *p*-nitrophenyl group, first to O-2, and then to O-3. A β -elimination then yields the phenoxide anion, and a saccharinic acid is produced on further reaction of the sugar residue. The migration most probably occurs through a *spiro*-Mesenheimer complex.⁶⁰⁹ Hydrolysis of the isomeric β -D-glucoside does not appear to follow this course, but involves an alternative, mixed-reaction pathway.

(601) T. N. Cawley and R. Letters, *Carbohydr. Res.*, **19**, 373–382 (1971).

(602) H. G. Khorana, G. M. Tener, R. S. Wright, and J. G. Moffatt, *J. Am. Chem. Soc.*, **79**, 430–436 (1957).

(603) T. Posternak, *Helv. Chim. Acta*, **42**, 390–393 (1959).

(604) D. M. Brown, G. E. Hall, and R. Letters, *J. Chem. Soc.*, 3547–3552 (1959).

(605) S. J. Angyal and M. E. Tate, *J. Chem. Soc.*, 4122–4128 (1961).

(606) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52–58 (1952).

(607) C. L. Stevens and R. E. Harmon, *Carbohydr. Res.*, **11**, 93–98 (1969).

(608) D. Horton and A. E. Luetzow, *Chem. Commun.*, 79–81 (1971).

(609) C. S. Tsai and C. Reyes-Zamora, *J. Org. Chem.*, **37**, 2725–2729 (1968).

SYNTHESIS OF NATURALLY OCCURRING C-NUCLEOSIDES, THEIR ANALOGS, AND FUNCTIONALIZED C-GLYCOSYL PRECURSORS

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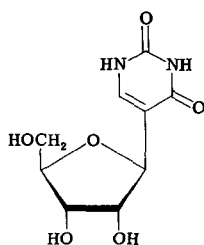
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I. INTRODUCTION

The C-nucleosides are a group of C-glycosylated heterocycles in which the anomeric carbon atom is attached to the heterocycle by a C-C bond. For a number of years after its discovery, pseudouridine¹ (1) was the only representative of this class of compound; it is found as a minor component in various transfer ribonucleic acids.² Since 1959, a number of other C-nucleosides have been isolated in rapid succession, mainly from fermentation sources, and have been found to exhibit a variety of interesting biological properties.³ Thus, pyraz-

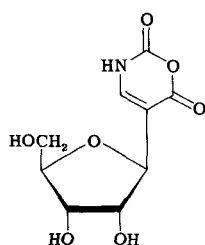
- (1) W. E. Cohn, *Biochim. Biophys. Acta*, **32**, 569-571 (1959); *J. Biol. Chem.*, **235**, 1488-1498 (1960).
- (2) For reviews, see E. Goldwasser and R. N. Heinriksen, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 399-416 (1966); R. W. Chambers, *ibid.*, **5**, 349-398 (1966).
- (3) R. J. Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, 1970.

omycin⁴ (7) has significant antiviral activity, and formycin^{5,6} (4), formycin B^{6,7} (5), and oxazinomycin⁸ (2) exhibit, in addition, antitumor activity. Showdomycin⁹⁻¹¹ (3) is reported to have antitumor, as well as antibacterial, activity. Indochrome BII^{12,13} (9), a component of a group of C-nucleosides containing a D-ribosyl group as the sugar component, and pseudouridine¹ are devoid of antibacterial, antitumor, or antiviral activities. Interestingly, the α anomer¹⁴ (8) of pyrazomycin, known as pyrazomycin B and isolated from fermentation sources, also exhibits antiviral properties.



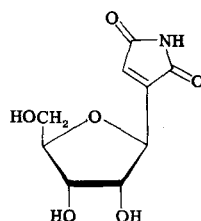
Pseudouridine

1



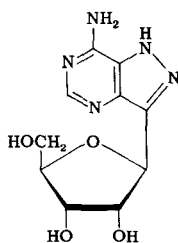
Oxazinomycin

2



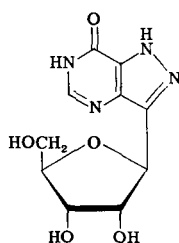
Showdomycin

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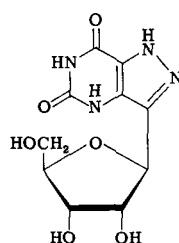
Formycin

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Formycin B

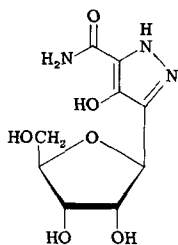
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Oxoformycin B

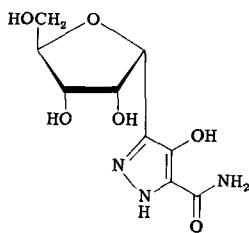
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- (4) K. Gerzon, D. C. DeLong, and J. C. Kline, *Pure Appl. Chem.*, **28**, 489-497 (1971).
- (5) M. Hori, E. Ito, T. Takita, G. Koyama, T. Takeuchi, and H. Umezawa, *J. Antibiot., Ser. A*, **17**, 96-99 (1964).
- (6) G. Koyama, K. Maeda, H. Umezawa, and Y. Iitaka, *Tetrahedron Lett.*, 597-602 (1966).
- (7) G. Koyama and H. Umezawa, *J. Antibiot., Ser. A*, **18**, 175-177 (1965).
- (8) K. Sasaki, Y. Kasakabe, and S. Ezumi, *J. Antibiot., Ser. A*, **25**, 151-154 (1972).
- (9) N. Nishimura, M. Mayma, Y. Komatsu, F. Kato, N. Shimaoka, and Y. Tanaka, *J. Antibiot., Ser. A*, **17**, 148-155 (1966).
- (10) Y. Nakagawa, H. Kano, Y. Tsukuda, and H. Koyama, *Tetrahedron Lett.*, 4105-4109 (1967).



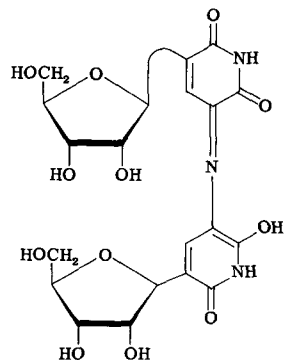
Pyrazomycin

7



Pyrazomycin B

8



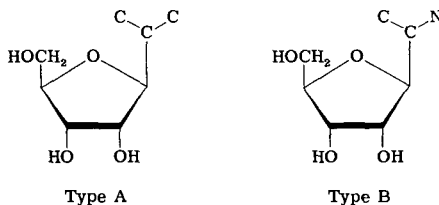
Indochrome BII

9

The biological importance of most of the naturally occurring C-nucleosides has prompted the exploration of synthetic routes leading to these compounds and their analogs. As synthetic targets, the C-nucleosides have deceptively simple structures, and the design of synthetic routes to them must take two important factors into account. Firstly, the methods selected for C-C bond formation at the anomeric center should be stereocontrolled, because the structures known thus far, are, with two exceptions,^{13,14} β -D-glycosyl compounds. Secondly, the carbon atom attached to the glycosyl group in a C-nucleoside precursor should be appropriately substituted or be amenable to substitution, so as to allow the elaboration of the other (non-sugar) heterocyclic portion of the molecule. For the purposes of planning a synthetic stratagem, C-nucleosides may be divided into two groups, based on the nature of the atoms that surround the carbon atom, in the nitrogenous heterocyclic portion, that is involved. Thus, in pseudouridine (1), oxazinomycin (2), showdomycin (3), and indochrome BII (9), this carbon atom is flanked by a carbon atom on both sides. In formycin (4), formycin B (5), oxoformycin B (6), and the pyrazomycins (7) and (8), the involved carbon atom of the pyrazole portion of the nitrogen heterocycle is flanked by a carbon atom and a nitrogen atom. These two groups of C-nucleosides may be described as belonging to a Type A and a Type B arrangement

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- (11) K. R. Damall, L. B. Townsend, and R. K. Robins, *Proc. Nat. Acad. Sci. U. S.*, **57**, 548-553 (1967).
 (12) H.-J. Knackmuss, G. Cosens, and J. Briaire, *Ann.*, **736**, 68-74 (1970).
 (13) H.-J. Knackmuss, *Angew. Chem. Int. Ed. Engl.*, **12**, 139-145 (1973).
 (14) G. E. Gutowsky, M. Chaney, H. D. Jones, R. L. Hamill, F. A. Davis, and R. D. Miller, *Biochem. Biophys. Res. Commun.*, **51**, 312-317 (1973).

of carbon atoms, respectively. These structural features should be considered in those instances in which the synthetic approach to the naturally occurring C-nucleosides ultimately involves the elaboration of the nitrogenous heterocyclic residue from anomerically functionalized C-glycosyl compounds.



This article is concerned with an outline, and an evaluation, of the synthetic methods leading to anomerically functionalized C-glycosyl derivatives and their utilization in the synthesis of naturally occurring C-nucleosides and their analogs. The section dealing with C-glycosyl derivatives will focus on preparative routes to 2,5-anhydro-D-aldoses, and their derivatives, homologs, and anomerically modified analogs, in which the terminal carbon atom is that in a hydroxymethyl group bearing a *cis* relationship to the carbon atom attached to C-1 of the C-glycosyl side-chain. Inasmuch as these compounds are intended as chemical precursors to the naturally occurring C-nucleosides and their analogs, examples in which the side-chain attached to C-1 is part of a saturated alkane will not be considered, as appropriate substitution cannot be readily effected. The discussion in Section II will therefore focus on the access to C- β -D-pentofuranosyl derivatives that are useful intermediates in the synthesis of C-nucleosides.

Viewed in a much broader context, C-aldopentofuranosyl derivatives may also be regarded as highly functionalized, chiral derivatives of substituted tetrahydrofuran. As such, they may be useful intermediates in the synthesis of a variety of compounds that contain a chiral, substituted tetrahydrofuran ring.

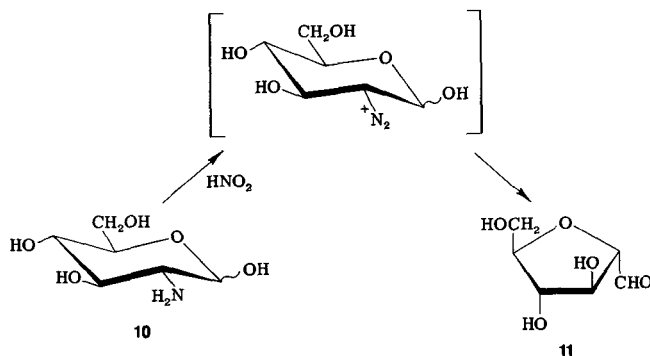
II. SYNTHESIS OF ANOMERICALLY FUNCTIONALIZED C- β -D-PENTOFURANOSYL DERIVATIVES

1. Intramolecular Cyclization of Acyclic Derivatives

a. **Deamination of Aminoaldoses and Aminoaldonic Acids.**—(i) **2-Amino-2-deoxyaldoses.** The deamination of 2-amino-2-deoxyaldoses and the corresponding acids with nitrous acid is one of the oldest

known reactions in carbohydrate chemistry, and it has been discussed in detail in this Series.^{15,16} In a number of cases, the reaction leads to 2,5-anhydroaldoses and 2,5-anhydroaldonic acids, respectively. For the purposes of evaluating practical approaches to the synthesis of C-nucleosides, it is not inappropriate to regard these compounds as anomERICALLY functionalized C-D-glycosyl compounds. It is of interest, therefore, to discuss synthetic routes to 2,5-anhydroaldoses, 2,5-anhydroaldonic acids, and their analogs that have the desired orientation of lateral side-chains, namely, *cis*-disposed hydroxymethyl and aldehyde, or carboxyl, groups.

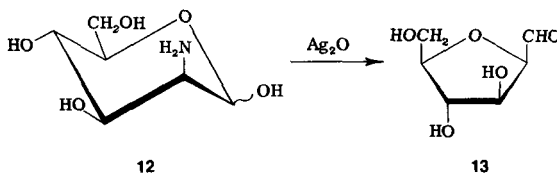
In analogy with the deamination of aminocyclohexanes,¹⁷⁻²¹ it has been found that the deamination, with nitrous acid, of 2-amino-2-deoxyaldohexoses in which the amino group has an equatorial orientation leads to 2,5-anhydrohexoses, as exemplified by the deamination of 2-amino-2-deoxy-D-glucose (10) to 2,5-anhydro-D-mannose²²⁻²⁵ (11).



The nitrous acid deamination of 2-amino-2-deoxy-D-mannose (12), in which the amino group is axially attached in the most favored con-

- (15) F. Shafizadeh, *Advan. Carbohydr. Chem.*, **13**, 9-61 (1958).
- (16) J. Defaye, *Advan. Carbohydr. Chem. Biochem.*, **25**, 181-228 (1970).
- (17) G. E. McCasland, *J. Amer. Chem. Soc.*, **73**, 2293-2295 (1951).
- (18) D. Y. Curtin and S. Schmukler, *J. Amer. Chem. Soc.*, **77**, 1105-1110 (1955).
- (19) A. Streitwieser, Jr., *J. Org. Chem.*, **22**, 861-869 (1957).
- (20) T. Taguchi, T. Matsuo, and M. Kojima, *J. Org. Chem.*, **29**, 1104-1106 (1964).
- (21) M. Chérest, H. Felkin, J. Sicher, F. Šipoš, and M. Tichý, *J. Chem. Soc.*, 2513-2520 (1965).
- (22) E. Fischer and F. Tiemann, *Ber.*, **27**, 138-147 (1894).
- (23) S. Akiya and T. Osawa, *Yakugaku Zasshi*, **74**, 1259-1262 (1954); *Chem. Abstr.*, **49**, 14,649 (1955).
- (24) B. C. Bera, A. B. Foster, and M. Stacey, *J. Chem. Soc.*, 4531-4535 (1956).
- (25) D. Horton and K. D. Philips, *Carbohydr. Res.*, **30**, 367-374 (1973).

formation,²⁶ leads to the formation of D-glucose as a result of the replacement of the amino group by a hydroxyl group, with inversion of configuration.^{26,27} Levene reported²⁷ that heating of 2-amino-2-deoxy-D-mannose (12) in the presence of silver oxide gave a crystalline compound that he considered to be 2,5-anhydro-D-glucose (13). Assuming that deamination with silver oxide proceeds by way



of a carbonium-ion mechanism, it could be postulated¹⁵ that the product is formed from the ${}^1\text{C}_4(\text{D})$ conformer, in which the amino group has the equatorial orientation.

In contrast to other 2,5-anhydroaldoses (which exhibit mutarotation, possibly due to the formation of hemiacetals²⁸), 2,5-anhydro-D-glucose does not show any mutarotation.²⁷ The importance of this compound as a potentially useful precursor to C-nucleosides warrants a reinvestigation of the deamination reaction, and the definitive proof of the structure of the compound. The readily accessible 2,5-anhydro-D-mannose (11) does not possess the *cis*-disposed side-chains at C-2 and C-5 that would be required of a synthetic precursor to the naturally occurring C-nucleosides, with the exception of α -pyrazomycin (8). The possibility of an inversion of the orientation of the aldehyde group in 11 by equilibration under basic conditions could be considered.

(ii) Deamination of 2-Amino-2-deoxyaldonic Acids and Lactones. Deamination of 2-amino-2-deoxyaldonic acids with nitrous acid has been shown to give 2,5-anhydroaldonic acids with overall retention of the configuration of C-2. The reaction parallels the deamination, with nitrous acid, of α -amino acids that leads to α -hydroxy acids with retention of configuration.^{19,29} Thus, the formation of 2,5-anhydro-D-gluconic acid²⁷ (15) from 2-amino-2-deoxy-D-gluconic acid (14) has

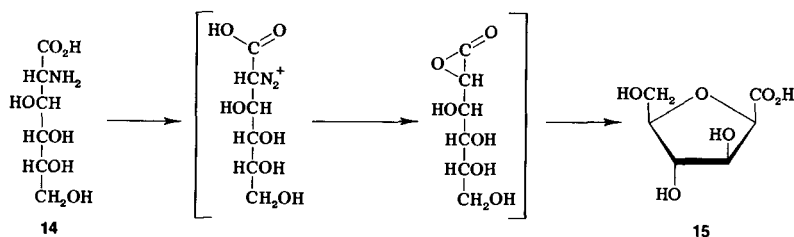
(26) D. Horton, J. S. Jewell, and K. D. Philips, *J. Org. Chem.*, **31**, 3843-3845 (1966); D. Horton, K. D. Philips, and J. Defaye, *Carbohydr. Res.*, **21**, 417-419 (1972).

(27) P. A. Levene, *J. Biol. Chem.*, **39**, 69-76 (1919); **59**, 135-139 (1924).

(28) A. B. Grant, *N. Z. J. Sci. Technol.*, **B37**, 509-521 (1956); *Chem. Abstr.*, **50**, 14,555 (1956).

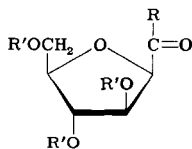
(29) P. Brewster, F. Hiron, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, *Nature*, **166**, 179-180 (1950).

been rationalized on the basis of participation by the carboxyl group, leading to an unstable α -lactone intermediate, followed by intramolecular cyclization.³⁰



2,5-Anhydrohexonic acids of this type have the desired *cis*-orientation of the side-chains at C-2 and C-5, and they may be regarded as versatile intermediates in the synthesis of C- β -D-pentofuranosyl heterocycles. Indeed, the synthesis of a C-nucleoside analog based on this approach has been reported³¹ (see Section IV,4,d). The inversion of configuration of C-3 in **15** would, on the other hand, lead to the *D*-ribo configuration, found in the natural C-nucleosides.

2,5-Anhydro-D-gluconic acid²⁷ (**15**) can be readily obtained by the deamination of 2-amino-2-deoxy-D-gluconic acid³² (**14**) in the presence of nitrous acid produced from silver nitrite-hydrochloric acid.³³ El Khadem and Swartz³¹ devised a chromatographic method for separating the product (**15**) from a small proportion of unchanged **14**. The methyl ester **16**, the crystalline amide **17**, and the dimethylamide **18** were prepared from the acid (**15**) by using standard procedures.³³



- 16** R = OMe, R' = H
17 R = NH₂, R' = H
18 R = NMe₂, R' = H

Deamination of the methyl ester (**19**) of 2-amino-2-deoxy-L-gluconic acid with nitrous acid gave methyl 2,5-anhydro-L-

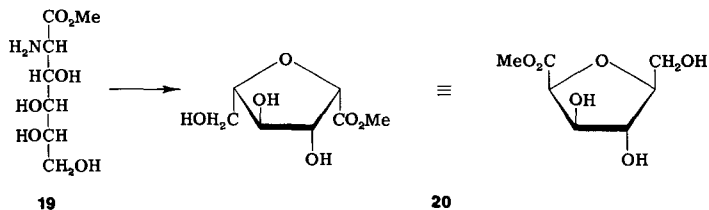
(30) A. B. Foster, *Chem. Ind.* (London), 627 (1955).

(31) H. S. El Khadem and D. L. Swartz, *Carbohydr. Res.*, **32**, c1-c3 (1974).

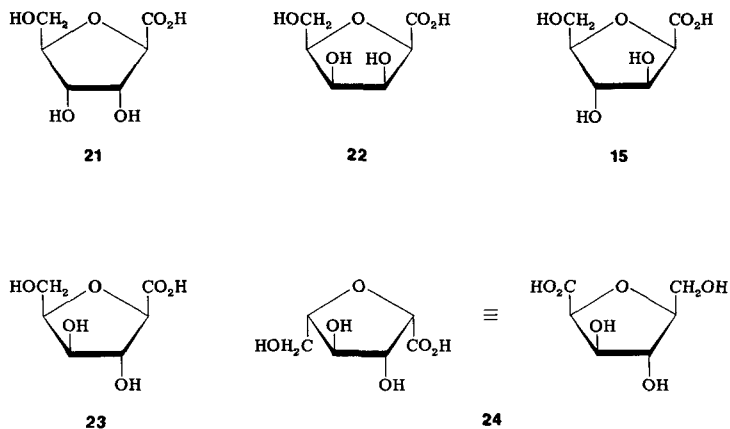
(32) K. Heyns and W. Koch, *Chem. Ber.*, **86**, 110-114 (1953).

(33) H. C. Cox, E. Hardegger, F. Kogl, P. Liechti, F. Lohse, and C. A. Salemnik, *Helv. Chim. Acta*, **41**, 229-234 (1958).

gluconate³⁴ (**20**). Although the *cis*-disposed side-chains are interchanged, compound **20** may still be regarded as a potential precursor to *C*- β -D-xylofuranosyl heterocycles, after appropriate chemical modification of the lateral side-chains.



Although the mechanistic implications were unknown to them, Tiemann^{22,35,36} and Levene^{27,37-39} and their associates had subjected several 2-amino-2-deoxyhexonic acids to deamination by means of nitrous acid; the corresponding 2,5-anhydrohexonic acids were further oxidized to the respective 2,5-anhydroaldaric acids, which enabled them to make structural correlations. Four of the eight possible 2,5-anhydro-D-aldonic acids have the *cis* disposition of the side chains required for possible synthesis of *C*-nucleosides. Of these, the *D-allo*⁴⁰ (**21**), *D-galacto*³⁷ (**22**), *D-gluco*²⁷ (**15**), and *L-gluco*³⁴ (**24**) iso-



(34) E. Hardegger and F. Lohse, *Helv. Chim. Acta*, **40**, 2383-2389 (1957).

(35) F. Tiemann, *Ber.*, **27**, 118-138 (1894).

(36) F. Tiemann and R. Haarmann, *Ber.*, **19**, 1257-1281 (1886).

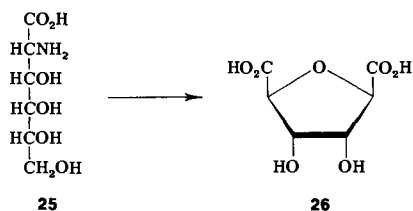
(37) P. A. Levene, *J. Biol. Chem.*, **31**, 609-621 (1917).

(38) P. A. Levene and F. B. LaForge, *J. Biol. Chem.*, **22**, 331-335 (1915).

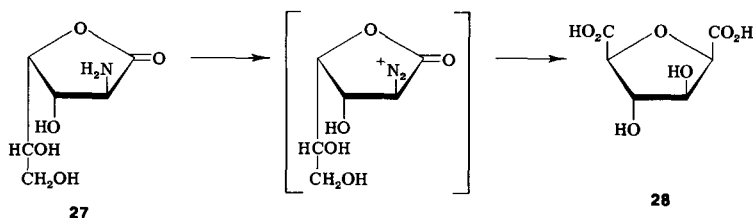
(39) P. A. Levene and F. B. LaForge, *J. Biol. Chem.*, **21**, 351-359 (1915).

(40) M. Bobek and J. Farkaš, *Collect. Czech. Chem. Commun.*, **34**, 247-252 (1969).

mers are known, and, with the exception of the *D-allo* isomer,⁴⁰ they have been obtained by the nitrous acid deamination method. The *D-gulo* isomer (23) is as yet unreported, but it could be structurally related to 24, in which the hydroxymethyl and carboxyl groups are interchanged. Levene and Clark⁴¹ also reported the preparation of 2,5-anhydro-*D*-allaric acid (26) by sequential deamination and oxidation of 2-amino-2-deoxy-*D*-allonic acid (25).



Deamination of 2-amino-2-deoxy-*D*-idonolactone (27) with nitrous acid led, after oxidation with nitric acid, to 2,5-anhydro-*D*-glucaric acid^{39,42} (28). As deamination of 2-amino-2-deoxy-*D*-idonic acid, followed by oxidation, gave 2,5-anhydro-*D*-idaric acid, the different behavior of the lactone 27 must be due to an attack on the incipient carbonium-ion at C-2 by a favorably disposed hydroxyl group in the molecule, such as at C-5.



In the light of our present knowledge of the mechanism of these deamination reactions, it can be understood why deamination of either 2-amino-2-deoxy-*D*-mannonic acid, or the corresponding lactone, gives 2,5-anhydro-*D*-mannonic acid.^{39,42}

b. Intramolecular Dehydration of Alditols.—One of the most direct and practical routes to 2,5-anhydroalditols^{16,43,44} containing *cis*-disposed chains consists in the acid-catalyzed dehydration of the corre-

(41) P. A. Levene and E. P. Clark, *J. Biol. Chem.*, **46**, 19–33 (1921).

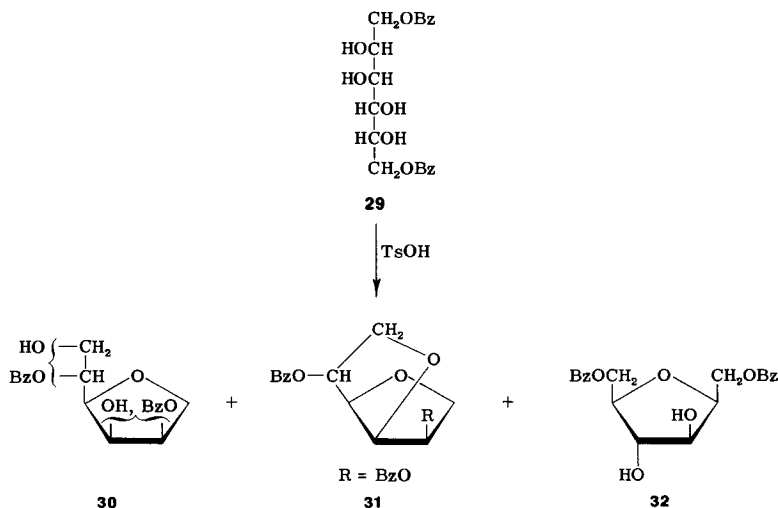
(42) P. A. Levene, *J. Biol. Chem.*, **36**, 73–87, 89–94 (1918).

(43) L. F. Wiggins, *Advan. Carbohydr. Chem.*, **5**, 191–228 (1950).

(44) S. Soltzberg, *Advan. Carbohydr. Chem. Biochem.*, **25**, 229–283 (1970).

spending alditols or their derivatives. Provided that one of the two hydroxymethyl groups can be chemically modified, and that appropriate transformations can be made in the ring portion in order to attain the desired configuration, these compounds can be used as ideal precursors to C-nucleosides.

Brigl and Grüner⁴⁵ reported the isolation of three products when 1,6-di-*O*-benzoyl-D-mannitol (**29**) was heated in boiling 1,1,2,2-tetrachloroethane in the presence of *p*-toluenesulfonic acid as the catalyst. These compounds were assigned mono- and di-anhydro structures, and were later shown by Hockett and coworkers^{46,47} to be 1,4-anhydro-D-mannitol dibenzoate (**30**), 1,4:3,6-dianhydro-D-mannitol dibenzoate (**31**), and 2,5-anhydro-1,6-di-*O*-benzoyl-D-glucitol (**32**). The latter compound, which can be readily isolated from the



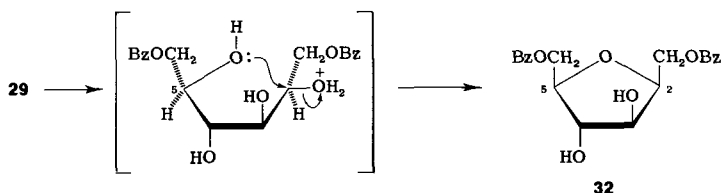
mixture by fractional recrystallization, was initially considered⁴⁵ to be 2,5-anhydro-1,6-di-*O*-benzoyl-D-mannitol, based on its behavior when oxidized by permanganate and its resistance to oxidation by lead tetraacetate. The inertness toward lead tetraacetate was ascribed⁴⁵ to the supposition that the vicinal hydroxyl groups on the ring had a *trans* relationship to each other. It was later shown by Hockett and coworkers⁴⁷ that the original ring assignment and the

(45) P. Brigl and H. Grüner, *Ber.*, **66**, 1945–1949 (1933); **67**, 1582–1589 (1934).

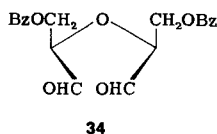
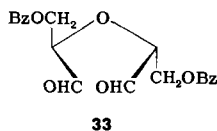
(46) R. C. Hockett, H. G. Fletcher, Jr., E. L. Sheffield, R. M. Goepp, Jr., and S. Soltzberg, *J. Amer. Chem. Soc.*, **68**, 927–930 (1946).

(47) R. C. Hockett, M. Zief, and R. M. Goepp, Jr., *J. Amer. Chem. Soc.*, **68**, 935–937 (1946).

location of the benzoyl groups were correct, but that compound **32** is an anhydroglucitol, not an anhydromannitol. Repetition of the lead



tetraacetate oxidation of **32** under standard conditions showed that one mole of oxidant per mole of substrate was, indeed, consumed. More important, a crystalline, optically inactive dialdehyde was isolated as the dihydrate, indicating that the parent compound was either a *meso* form or a racemic mixture. The dialdehyde **33**, resulting from 2,5-anhydro-1,6-di-*O*-benzoyl-*D*-mannitol, should be optically active, whereas the dialdehyde **34**, isolated from the oxidation



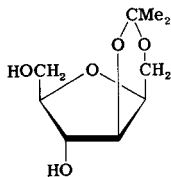
of the anhydrohexitol dibenzoate, should be optically inactive. These experiments led to the definitive assignment of Brigl and Grüner's⁴⁵ 2,5-anhydrohexitol as 2,5-anhydro-1,6-di-*O*-benzoyl-*D*-glucitol (**32**). It was, therefore, apparent that Walden inversion was taking place, during the anhydridization reaction.⁴⁸ The product (**32**) would result from an inversion, either at C-2 or C-5, as illustrated in the accompanying formulas.

Sugihara and Schmidt⁴⁹ reported the isolation of 2,5-anhydro-*D*-glucitol in crystalline form; its preparation on a relatively large scale has been described in the patent literature,⁵⁰ and consists in the thermal dehydration of *D*-mannitol. The process leads to the formation of 1,4-anhydro-*D*-mannitol, 1,5-anhydro-*D*-mannitol, 1,4:3,6-dianhydro-*D*-mannitol, and 2,5-anhydro-*D*-glucitol, which is isolated as the crystalline 1,3-*O*-isopropylidene derivative (**35**).

(48) R. C. Hockett, M. Conley, M. Yusem, and R. I. Mason, *J. Amer. Chem. Soc.*, **68**, 922-926 (1946).

(49) J. M. Sugihara and D. L. Schmidt, *J. Org. Chem.*, **26**, 4612-4615 (1961).

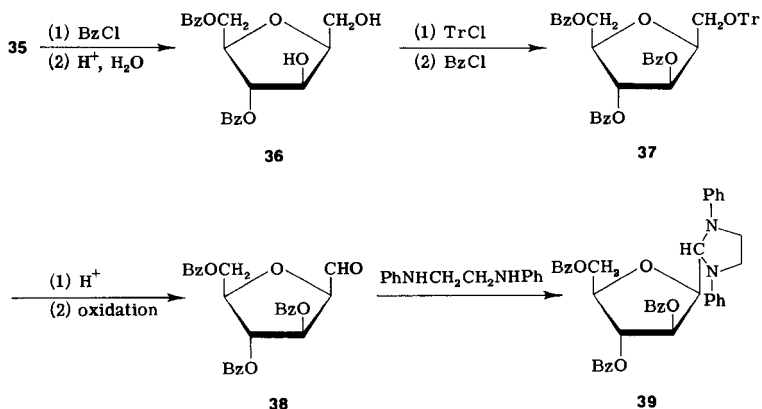
(50) L. A. Hartmann, U. S. Pat. 3,480,651 (1969); *Chem. Abstr.*, **72**, 79,420 (1970); U. S. Pat. 3,484,459 (1969); *Chem. Abstr.*, **72**, 101,059 (1970).



35

Compound **35** has also been obtained⁵¹ from **32**, by sequential debenzoylation, and acetalization with 2,2-dimethoxypropane. It has been used in the synthesis, in good overall yield, of versatile, anomerically functionalized precursors of C-nucleosides having the D-*arabino* or the D-*ribo* configuration.

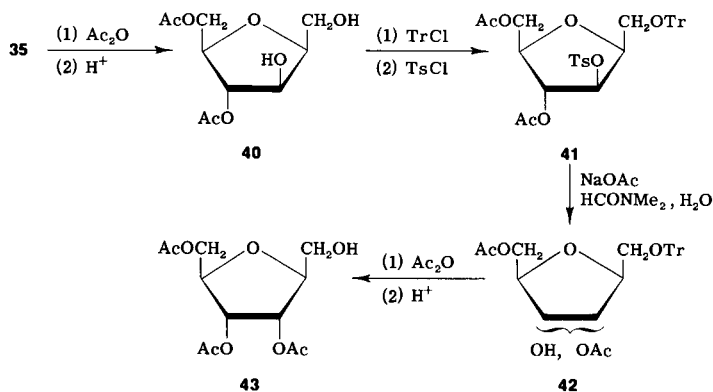
The aldehyde **38** was obtained from **35**, by way of **36** and **37**, by the carbodiimide–dimethyl sulfoxide oxidation procedure⁵² in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDAC)⁵³ and dichloroacetic acid. It was isolated in the form of its crystalline 1,3-diphenylimidazolidine derivative (**39**) by trapping the freshly prepared aldehyde **38** with *N,N'*-diphenylethylenediamine. (This reagent was developed by Wanzlick and Löchel⁵⁴ for the selective derivatization of aldehydes, and has been exploited for the isolation of nucleoside 5'-aldehydes⁵⁵ and other aldehydo derivatives of carbohydrates by Moffatt and co-workers.^{52(b)})



(51) S. Hanessian and G. Rancourt, *Abstr. Papers Amer. Chem. Soc. Meeting*, **169**, CARB 26 (1975).

(52)(a) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **87**, 5661–5670; 5610–5678 (1965); (b) G. H. Jones and J. G. Moffatt, *Methods Carbohydr. Chem.*, **6**, 315 (1972).

Starting with the same acetal (35), but proceeding by way of the diacetate 40, instead of the dibenzoate 36, it was possible to prepare,⁵¹ in high yield, the *p*-toluenesulfonate 41 by sequential tritylation and *p*-toluenesulfonylation. Compound 41 was then subjected to solvolysis in the presence of sodium acetate in moist *N,N*-dimethylformamide, and the product (42) was successively acetylated and detritylated to give 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-allitol (43). The



same sequence was performed in the benzoic ester series to give compounds 44 and 45. Oxidation of 45 in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride and dichloroacetic acid in dimethyl sulfoxide, followed by treatment with *N,N'*-diphenylethylenediamine, gave the known,⁵⁶ crystalline 2,5-anhydro-D-allose derivative (46). Compound 46 could also be obtained by oxidation of 43, followed by deacetylation and benzoylation.

Hough and Shute⁵⁷ reported that heating of 1-deoxyl-1-nitro-D-glycero-L-manno-heptitol (47) in aqueous solution led to the elimination of water and the formation of C-β-D-galactopyranosyltromethane (48) as the major product. After fractionation of the mother liquors on an anion-exchange resin, they obtained evidence for the formation, albeit in small amounts, of C-α-D-galactopyranosyl-

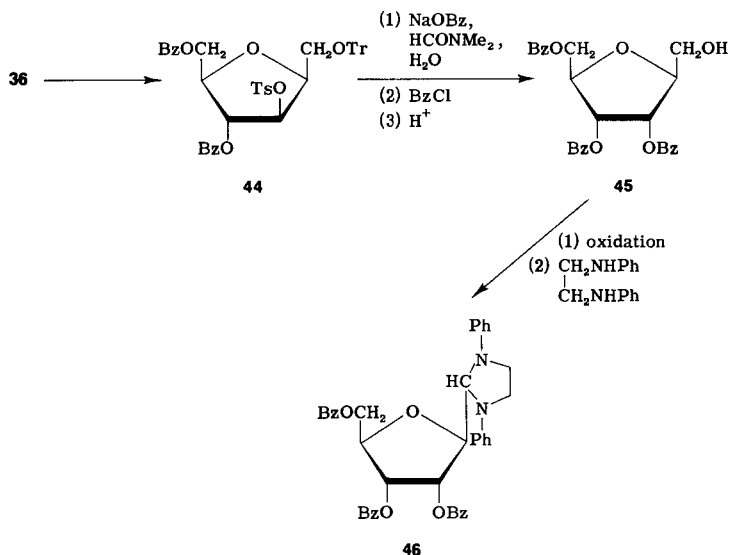
(53) A product of Bio-Rad Laboratories, Richmond, California.

(54) H. W. Wanzlick and W. Löchel, *Chem. Ber.*, **86**, 1463–1466 (1953).

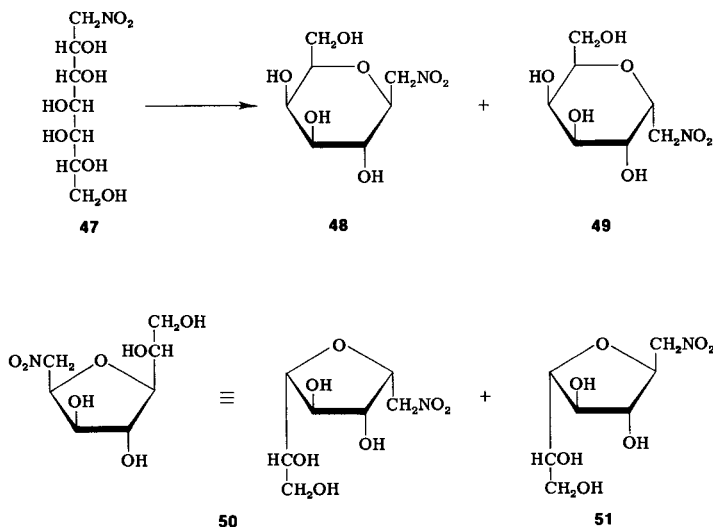
(55) N. P. Damodaran, G. H. Jones, and J. G. Moffatt, *J. Amer. Chem. Soc.*, **93**, 3812–3813 (1971).

(56) H. P. Albrecht, D. B. Repke, and J. G. Moffatt, *J. Org. Chem.*, **38**, 1836–1840 (1973).

(57) L. Hough and S. H. Shute, *J. Chem. Soc.*, 4633–4637 (1962).



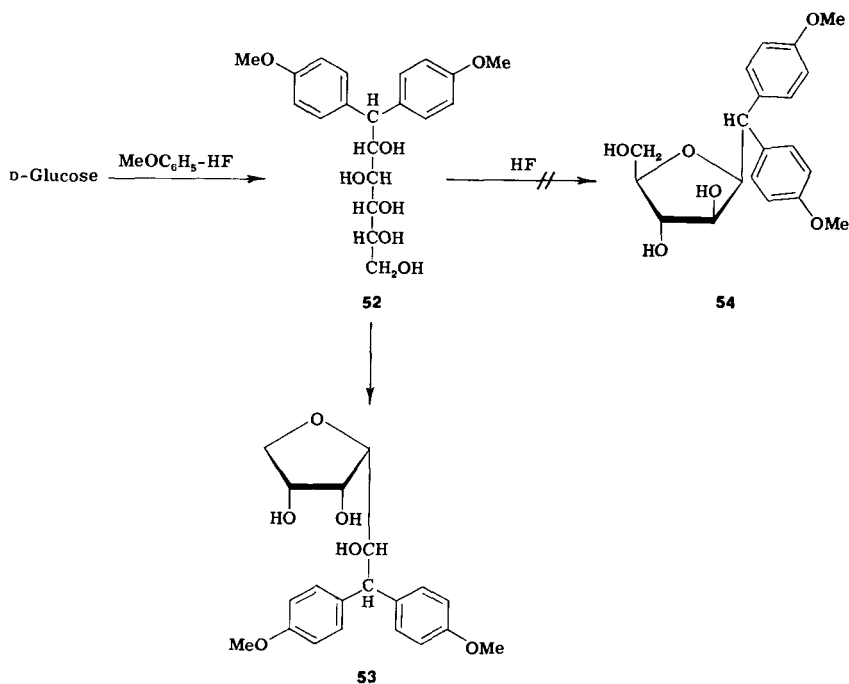
nitromethane (**49**) and the *C*- α - and *C*- β -D-galactofuranosylnitromethanes (**50** and **51**).



Of these products, only compound **50**, in which the side chains have a *cis* orientation, could be considered a potential precursor to a *C*- β -D-pentofuranosyl heterocycle. Its formation in small quantities, and the somewhat tedious isolation by ion-exchange chroma-

tography, precludes its utilization for the multi-step syntheses of C-nucleosides.

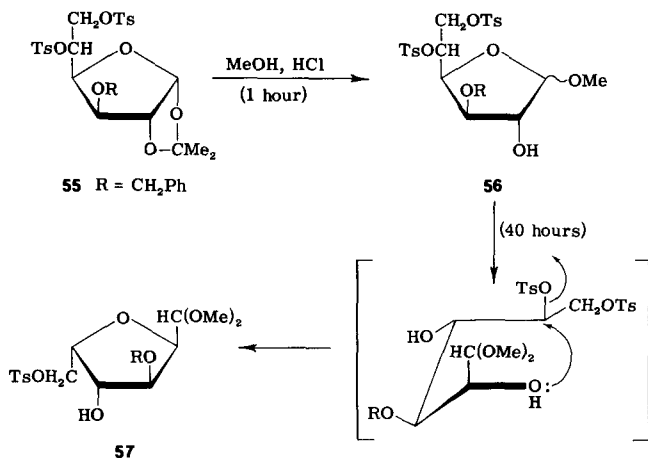
Micheel and Staněk⁵⁸ reported the formation of 1-deoxy-1,1-di-C-(*p*-methoxyphenyl)-D-glucitol (**52**), in 70% yield, when a mixture of D-glucose and anisole was heated in anhydrous liquid hydrogen fluoride. When heated in the presence of an excess of anisole and liquid hydrogen fluoride, compound **52** gave an anhydro compound that was proved to be the 3,6-anhydride **53**, not 2,5-anhydro-1-deoxy-1,1-di-C-(*p*-methoxyphenyl)-D-glucitol (**54**), as was initially suggested. The overall retention of configuration at C-3 in the product (**53**) indicates protonation of the 6-hydroxyl group followed by intramolecular attack by the 3-hydroxyl group.



c. Intramolecular Displacement of Sulfonate Groups.—The intramolecular displacement of a sulfonate group by a suitably situated hydroxyl group in acidic, basic, and neutral media constitutes another method for the preparation of 2,5-anhydroaldohexoses and

(58) F. Micheel and J. Stanek, Jr., *Tetrahedron Lett.*, 1609-1612 (1970); *Ann.*, **759**, 37-62 (1972); F. Micheel and H. Sobitzkat, *Carbohydr. Res.*, **30**, 71-81 (1973).

their analogs.¹⁶ The acid-catalyzed reaction has several precedents in the pentose and hexose series, and is considered to proceed by protonation of an oxygen atom of the sulfonate group, followed by intramolecular displacement by a γ -disposed hydroxyl group, with inversion of configuration of the carbon atom bearing the sulfonate group. In several of the known examples,¹⁶ 1,2-*O*-isopropylidene-*D*-aldofuranose derivatives containing a sulfonate group at C-5 have been solvolized in methanolic hydrogen chloride, as exemplified by the reaction of 3-*O*-benzyl-1,2-*O*-isopropylidene-5,6-di-*O*-*p*-tolylsulfonyl- α -*D*-glucofuranose⁵⁹ (**55**) to give 2,5-anhydro-3-*O*-benzyl-6-*O*-*p*-tolylsulfonyl-*aldehydo*-*L*-idose dimethyl acetal (**57**). The dimethyl



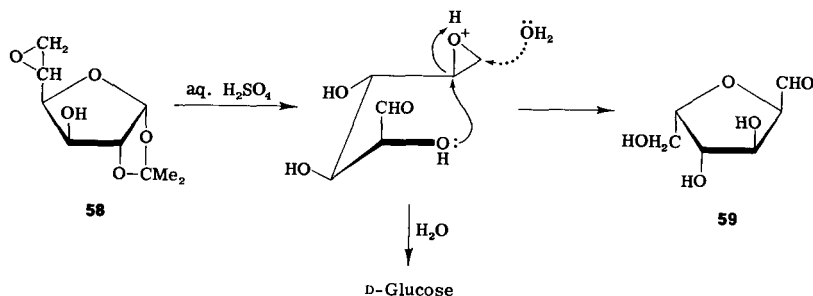
acetal intermediate, formed from the methyl glycoside **56**, undergoes intramolecular cyclization with inversion of configuration at C-5, to give **57**. A strong driving-force in these reactions is undoubtedly the stability of the resulting tetrahydrofuran ring, which does not revert back to acyclic products under the conditions of the reaction. Matsui and coworkers⁶⁰ utilized this method for the synthesis of several 2,5-anhydroaldose dimethyl acetals.

Intramolecular cyclization can also occur on treatment of epoxides under acidic conditions, although, when aqueous acids are used, a competing attack by water occurs,⁶¹ as in **58** \rightarrow **59**.

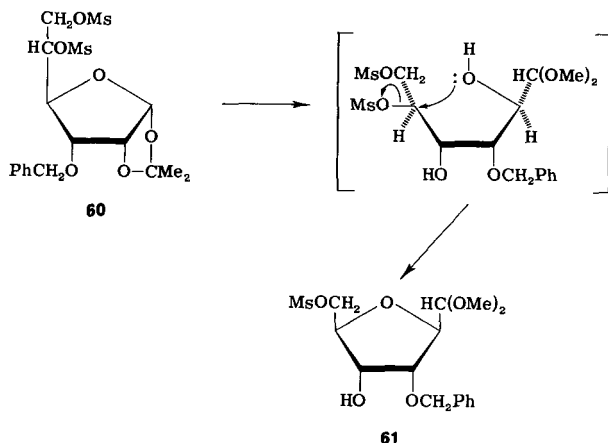
(59) J. Defaye and J. Hildesheim, *Tetrahedron Lett.*, 313-317 (1968); compare J. Defaye, D. Horton, and M. Muesser, *Carbohydr. Res.*, **20**, 305-318 (1971).

(60) T. Ogawa, M. Matsui, H. Ohrai, H. Kuzuhara, and S. Emoto, *Agr. Biol. Chem. (Tokyo)*, **36**, 1655-1657 (1972).

(61) C. A. Dekker and T. Hashizume, *Arch. Biochem. Biophys.*, **78**, 348 (1958).



The potentialities of this method are such that, with the proper choice of hexofuranose derivative, access can be gained to 2,5-anhydroaldoses in which the side chains have the *cis* orientation, as would be required for further elaboration into C-nucleosides. Matsui and coworkers⁶² reported the synthesis of modified C-nucleosides by acidic treatment of 3-*O*-benzyl-1,2-*O*-isopropylidene-5,6-di-*O*-(methylsulfonyl)- β -L-talofuranose (**60**), to give 2,5-anhydro-3-*O*-benzyl-6-*O*-(methylsulfonyl)-*aldehydo*-D-allose dimethyl acetal (**61**).

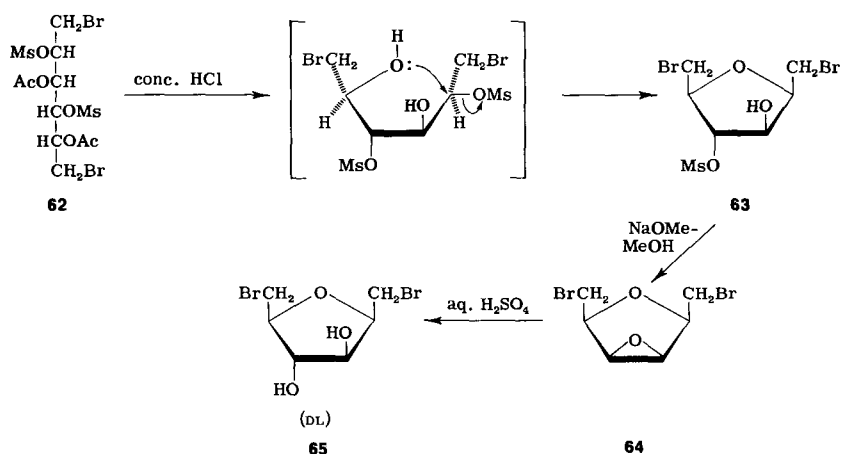


Intramolecular cyclization can also be effected with acyclic intermediates derived from such readily available alditols as D-mannitol. Kuszmann and Vargha⁶³ reported the formation of 2,5-anhydro-1,6-dibromo-1,6-dideoxy-4-*O*-(methylsulfonyl)-D-glucitol (**63**), in 73% yield, by boiling 3,5-di-*O*-acetyl-1,6-dibromo-1,6-dideoxy-2,4-di-*O*-

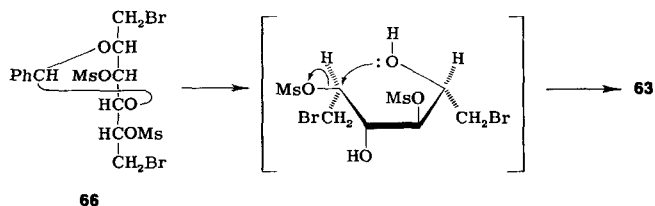
(62) T. Ogawa, M. Matsui, H. Ohrai, H. Kuzuhara, and S. Emoto, *Agr. Biol. Chem.* (Tokyo), **36**, 1449-1451 (1972).

(63) J. Kuszmann and L. Vargha, *Carbohydr. Res.*, **10**, 261-271 (1971).

(methylsulfonyl)-D-mannitol (**62**) in methanol containing concentrated hydrochloric acid. The formation of **63** undoubtedly involves an acid-catalyzed *O*-deacetylation, followed by intramolecular attack by the 5-hydroxyl group on C-2 (bearing the methanesulfonate group). The structure of **63** was proved by its transformation into the epoxide **64** by treatment with methanolic sodium methoxide. On treatment with aqueous acid, **64** gave a racemic mixture of the 2,5-anhydro-1,6-dibromo-1,6-dideoxyglucitols (**65**) which consumed one equivalent of periodate.



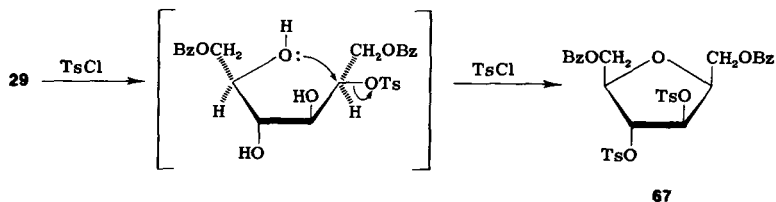
In a related study,⁶⁴ acid treatment of the benzylidene acetal **66** also gave the anhydroglucitol **63**.



Müller and Vargha⁶⁵ reported that treatment of 1,6-di-*O*-benzoyl-D-mannitol (**29**) with *p*-toluenesulfonyl chloride gave 2,5-anhydro-1,6-di-*O*-benzoyl-3,4-di-*O*-*p*-tolylsulfonyl-D-glucitol (**67**). The formation of this compound may be the result of a favored *p*-toluenesulfonylation at the 2(5)-hydroxyl group, followed by intramolecular cyclization and subsequent esterification by the excess of the reagent. This

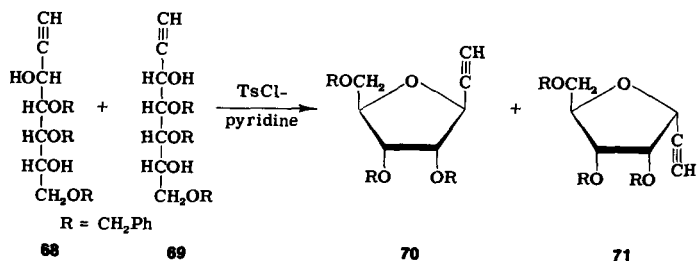
(64) J. Kuszmann and L. Vargha, *Carbohydr. Res.*, **17**, 309-318 (1971).

(65) A. Müller and L. Vargha, *Ber.*, **66**, 1165-1168 (1933).



mechanistic interpretation is based on precedents involving the formation of anhydroalditol derivatives⁶⁶ and 2,5-anhydropentose dialkyl dithioacetals.^{67,68} An exception in the case of the dithioacetals is the *D-arabino* isomer,⁶⁹ which gives the 5-*p*-toluenesulfonate expected. The internal displacement in the *D-lyxo*, *D-ribo*, and *D-xylo* isomers has been rationalized on the basis of favorable energy requirements in a non-extended, non-planar chain of carbon atoms in these molecules.⁷⁰ As the transition state for formation of the anhydro ring is approached, the substituent groups on C-2, C-3, and C-4 are sterically compatible in the aforementioned isomers. For the *D-arabino* dialkyl dithioacetals, a planar, zigzag conformation is expected to be the conformation of maximum stability, and an intramolecular, SN2 type of attack would not be a favored process.

Buchanan and coworkers⁷¹ found that treatment of the 7:3 mixture of the *D-altro* (68) and *D-allo* (69) hept-1-ynitol derivatives obtained from 2,3,5-tri-*O*-benzyl-*D*-ribofuranose and ethynylmagnesium bromide with 2.2 equivalents of *p*-toluenesulfonyl chloride at 60° gives the 1-*D*-ribofuranosylethyne derivatives (70 and 71) in 52 and 13%



(66) See, for example, Y. Rabinsohn and H. G. Fletcher, Jr., *J. Org. Chem.*, **32**, 3452–3457 (1967); A. Gateau, A.-M. Sepulchre, and S. D. Gero, *Compt. Rend.*, **C**, **273**, 1649–1651 (1971).

(67) H. Zinner, H. Brandhoff, H. Schmandke, H. Kristen, and R. Haun, *Chem. Ber.*, **92**, 3151–3155 (1959).

(68) J. Defaye, *Bull. Soc. Chim. Fr.*, 2686–2689 (1964).

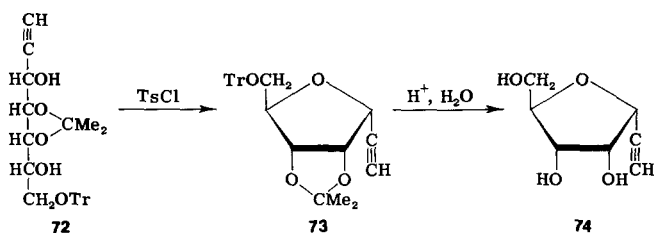
(69) H. Zinner, K. Wessely, and H. Kristen, *Chem. Ber.*, **92**, 1618–1623 (1959).

(70) J. Defaye and D. Horton, *Carbohydr. Res.*, **14**, 128–132 (1970); P. L. Durette and D. Horton, *Advan. Carbohydr. Chem. Biochem.*, **26**, 49–125 (1971).

(71) J. G. Buchanan, A. R. Edgar, and M. J. Power, *J. Chem. Soc. Perkin Trans. I*, 1943–1949 (1974); *Chem. Commun.*, 346–347 (1972); 501–502 (1975).

yields, respectively, after chromatographic purification. Under the same conditions, the pure *D-altro* diol (**68**) gave the two derivatives in 55 and 19% yields, respectively. It was anticipated⁷¹ that monosulfonylation at O-3 could be achieved by treatment of the mixed diols (**68** and **69**) with an equimolar amount of a sulfonyl chloride, but no reaction occurred under these conditions. Despite the relatively forcing sulfonylation conditions that were ultimately used, it is remarkable that disulfonates were only minor products (16%), and that the main reaction involved favored esterification at O-3, which was followed by intramolecular displacement of the sulfonate group, to give the tribenzyl ethers **70** and **71**. The configurational assignment of the latter compounds was based on optical rotational data, and by correlations with an alternative synthesis (see Section II,2,b).

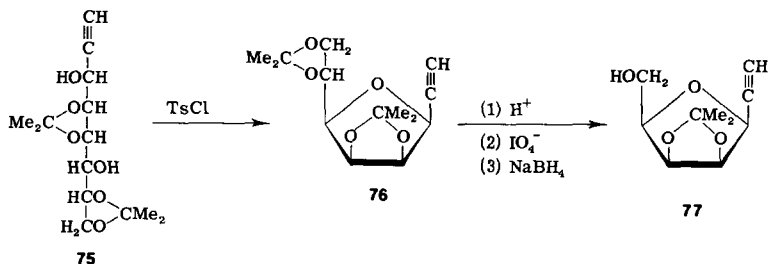
Cyclization⁷² of the *D-allo*-hept-1-ynitol derivative **72**, obtained from 2,3-*O*-isopropylidene-*D*-ribofuranose and ethynylmagnesium bromide, leads in very high yield to the amorphous α -*D*-ribosylethyne derivative **73**, which gave crystalline 1- α -*D*-ribofuranosylethyne (**74**) after acid hydrolysis.



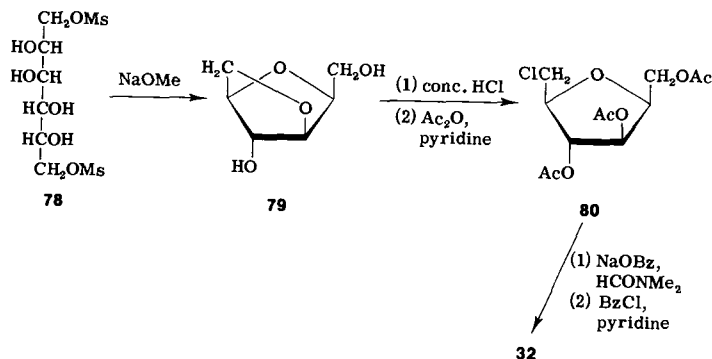
In the *D-manno* series, it was shown⁷² that the major product resulting from the reaction of 2,3:5,6-di-*O*-isopropylidene-*D*-mannofuranose with ethylmagnesium bromide is the *D-glycero-D-talo* isomer **75**, and not the *D-glycero-D-galacto* isomer, as was previously assumed.⁷³ Ring closure in the presence of *p*-toluenesulfonyl chloride gave the 1- β -*D*-mannofuranosylethyne derivative (**76**) in 75% yield; this was subsequently converted into the *D-lyxo* analog (**77**). These ring-closure products are versatile, anomERICALLY functionalized C-glycosyl compounds that can be effectively used as precursors to C-glycosyl heterocycles (see Section IV,3).

(72) J. G. Buchanan, A. D. Dunn, and A. R. Edgar, *Carbohydr. Res.*, **36**, c5-c7 (1974); *J. Chem. Soc. Perkin Trans. I*, 1191-1200 (1975); **68** (1976).

(73) W. S. Chilton, W. C. Lontz, R. B. Roy, and C. Yoda, *J. Org. Chem.*, **36**, 3222-3225 (1971).



Intramolecular displacement of primary sulfonyloxy or halide groups in derivatives of D-mannitol can also be brought about under basic conditions, albeit in low yield. Treatment of 1,6-di-*O*-(methylsulfonyl)-D-mannitol (**78**), or the corresponding dichloride derivative, with sodium methoxide gave 2,5:3,6-dianhydro-D-glucitol⁷⁴ (**79**). Treatment of the latter with hydrochloric acid at 100° in a sealed tube gave the 6-chloro-6-deoxy derivative (**80**), which was converted into the known 2,5-anhydro-1,6-di-*O*-benzoyl-D-glucitol⁴⁵⁻⁴⁷ (**32**). The sequence **78**–**80** is of interest in the context of C-β-D-nucleoside precursors, but it suffers from the fact that yields are low.

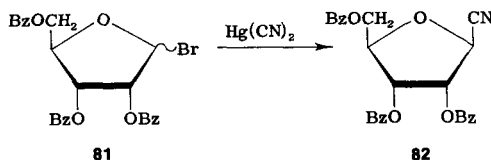


2. Formation of C–C Bonds at the Anomeric Center

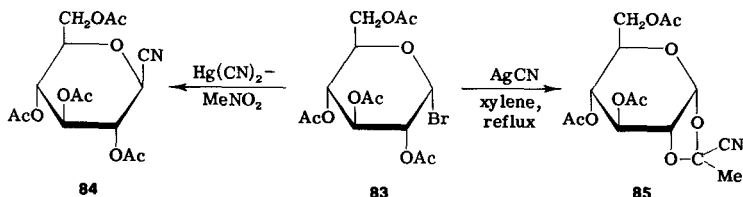
a. β-D-Pentofuranosyl Cyanides.—Glycosyl cyanides may be regarded as versatile, anomERICALLY functionalized intermediates for the synthesis of glycosylated heterocycles. Access to these compounds is relatively easy, and the cyano group can be subjected to successive hydrolysis and reduction, to give the corresponding anhydroaldonic acids and anhydroaldoses, respectively.

(74) L. Vargha and J. Kuszmann, *Carbohydr. Res.*, **8**, 157–163 (1968).

Bobek and Farkaš⁴⁰ reported the preparation of crystalline 2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl cyanide (**82**) from the corresponding bromide (**81**). Their work was based on initial experiences of Hel-



ferich and coworkers,⁷⁵⁻⁷⁷ and later work by Coxon and Fletcher,^{78,79} who had prepared β -D-aldopyranosyl cyanides from the reaction of acylated D-aldopyranosyl halides with mercuric cyanide. These investigators^{78,79} showed that 1,2-*O*-(1-cyanoalkylidene) derivatives could also be formed in these reactions, and that their formation was dependent on the nature of the salt and the solvent used. Thus, the product resulting from the reaction of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (**83**) with silver cyanide in boiling xylene was shown by Coxon and Fletcher⁷⁸ not to be the assumed⁸⁰ 1-cyanide (**84**), but actually 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-cyanoethylidene)- α -D-glucopyranose (**85**). However, with mercuric cyanide in nitromethane,⁷⁶ the expected nitrile (**84**) and the acetal (**85**) were formed in equal amounts, but in low yields.



It would be reasonable to assume that, in a solvent of high dielectric constant, such as nitromethane, the nitrile (**84**) is formed by direct attack of cyanide ion on an ion-pair (**86**) in which the bromide ion has the α -D orientation. Departure, assisted by metal ions, of the halide ion from **83** or **86**, with possible assistance by the lone pair of the ring-oxygen atom, would lead to an oxonium ion (**87**) that could

(75) B. Helferich and K. F. Wedemeyer, *Ann.*, **563**, 139-146 (1949).

(76) B. Helferich and K. L. Bellin, *Chem. Ber.*, **94**, 1158-1160 (1961).

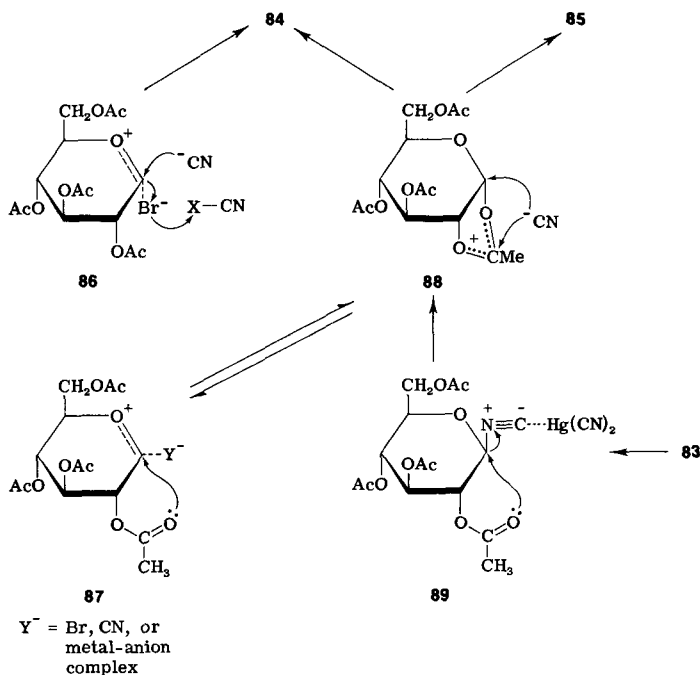
(77) B. Helferich and W. Ost, *Chem. Ber.*, **95**, 2612-2615 (1962).

(78) B. Coxon and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **85**, 2637-2642 (1963); **86**, 922-926 (1964).

(79) B. Coxon, *Tetrahedron*, **22**, 2281-2302 (1966).

(80) L. Buerger, *J. Amer. Chem. Soc.*, **56**, 2494-2495 (1934).

be internally attacked to give the 1,2-acetoxonium-ion^{81,82} intermediate (**88**). The latter is attacked by cyanide ion, by what appears to be a kinetically controlled reaction, to give the alkylidene derivative **85**.



The respective natures of the salt XY, the "gegen ion" Y, and the solvent play an important role in stabilizing such intermediates as **87** and **88**, which may exist as tightly, or loosely, ion-paired species, in different states of aggregation. In the presence of silver cyanide, the alkylidene product preponderates, presumably due to a much greater contribution from intermediate **88**. Both reaction products (**84** and **85**) were found to be stable under the conditions of the reaction; this supports the contention that they must have arisen from different reactive intermediates. Coxon and Fletcher⁷⁸ did not exclude the possibility that the alkylidene derivative **85** might have arisen from the isonitrile **89** by a rearrangement.

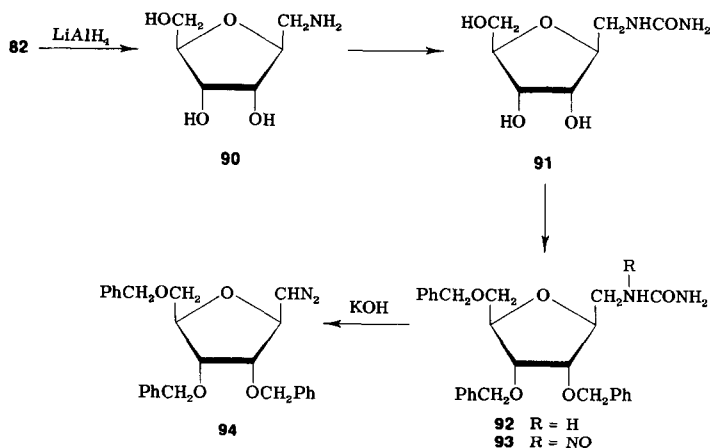
Alkylidene derivatives corresponding to **85** have not yet been reported in the D-ribofuranose series, as evidenced by the formation of the nitrile **82** in high yield.

(81) H. Paulsen, *Advan. Carbohydr. Chem. Biochem.*, **26**, 127-195 (1971).

(82) G. Wulff and G. Röhle, *Angew. Chem. Int. Ed. Engl.*, **13**, 157-170 (1974).

The β -D configuration was assigned⁴⁰ to **82** on the basis of the *trans* rule,⁸³ as exemplified by the reaction of glycosyl halides with heavy-metal salts of heterocyclic bases.^{83a} Unambiguous chemical proof was secured from the transformation of the nitrile into the corresponding acid (**21**).

The nitrile group in **82** has been transformed into other versatile functional groups, and the derivatives so obtained have been used in the synthesis of various naturally occurring C-nucleosides and their analogs. Reduction of **82** with lithium aluminum hydride gave the amine **90** which was, in turn, transformed⁸⁴ into the ureido and *N*-nitroso derivatives (**91–93**) by treatment with nitrourea, followed by benzylation, and nitrosation.⁸⁵ The diazo derivative **94**, obtained by treatment of **93** with alcoholic potassium hydroxide, was a key intermediate in the synthesis of formycin B and oxoformycin B (see Section III,2,a,b).



Reductive hydrolysis⁸⁶ of organic nitriles is known to lead to the corresponding aldehydes. Application of Backeberg and Staskun's⁸⁷ modification of this reaction to reduction of nitrile **82** was reported

(83) R. S. Tipson, *J. Biol. Chem.*, **130**, 55–59 (1939).

(83a) B. R. Baker, *Ciba Found. Symp., Chem. Biol. Purines*, **120** (1957).

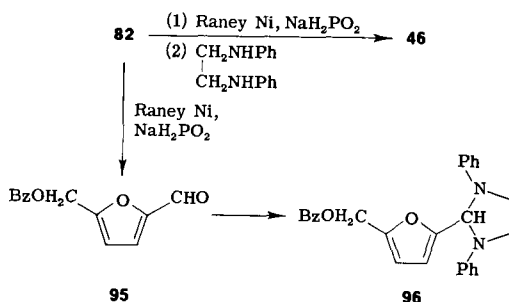
(84) J. Farkaš and F. Šorm, *Collect. Czech. Chem. Commun.*, **37**, 2798–2803 (1972); M. Bobek, J. Farkaš, and F. Šorm, *Tetrahedron Lett.*, 4611–4614 (1970).

(85) W. Kirmse and M. Buschhoff, *Chem. Ber.*, **100**, 1491–1506 (1967).

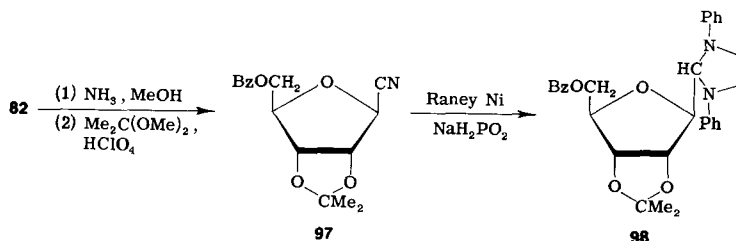
(86) E. Mosettig, *Org. React.*, **8**, 218–257 (1954), Houben-Weyl, "Methoden der Organische Chemie," Thieme Verlag, Stuttgart, 4th Edition, 1952, Vol. VII, Part 1, p. 299.

(87) O. G. Backeberg and B. Staskun, *J. Chem. Soc.*, 3961–3963 (1962).

by Montgomery⁸⁸ and Moffatt⁵⁶ and their coworkers. Thus, treatment of **82** with Raney nickel and sodium hypophosphite (NaH_2PO_2) in a mixture of pyridine, acetic acid, and water led to rapid conversion into a product shown⁸⁸ to be 5-(benzyloxymethyl)-2-furaldehyde (**95**). It was also shown⁵⁶ that the elimination reaction was actually taking place during the reductive hydrolysis, and that this undesirable, aromatization reaction could be avoided by conducting the reaction in the presence of *N,N'*-diphenylethylenediamine, thereby trapping the aldehyde formed. The desired 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allose could thus be isolated in good yield as the crystalline *N,N'*-diphenylimidazolidine derivative (**46**). Treatment of **95** with *N,N'*-diphenylethylenediamine gave the crystalline derivative **96**.



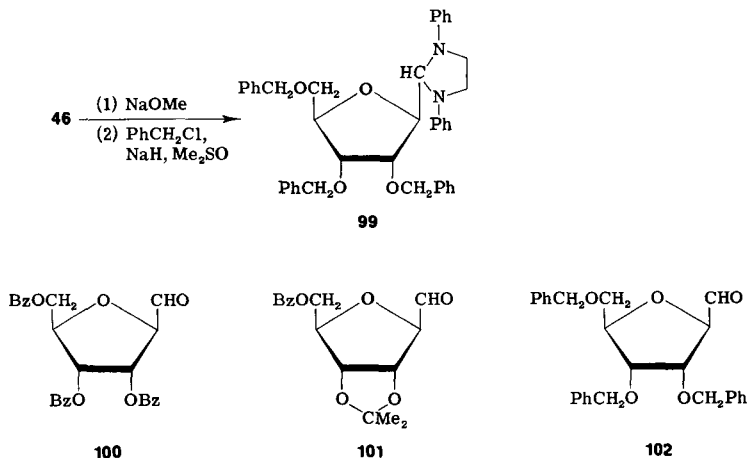
The elimination reaction could also be circumvented by replacement of the eliminable ester groups in **82** with an isopropylidene group, as in **97**. This compound was prepared^{56,89} by partial debenzoylation of **82** in the presence of methanolic ammonia, and subsequent treatment of the resulting diol with 1,3-dimethoxypropane and acetone in the presence of perchloric acid. Reductive hydrolysis of **97**, in the presence of *N,N'*-diphenylethylenediamine gave the imidazolidine derivative **98** in good yield.⁵⁶ Although the imidazo-



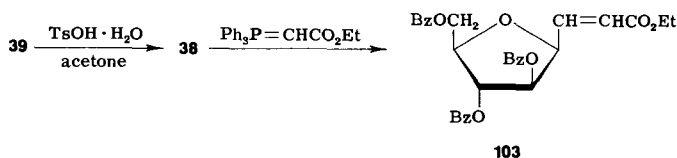
(88) J. A. Montgomery, K. Hewson, and A. G. Laseter, *Carbohydr. Res.*, **27**, 303-308 (1973).

(89) J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **7**, 443-445 (1970).

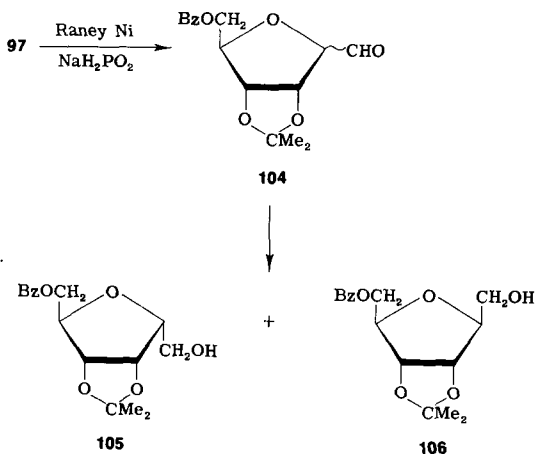
lidine ring in such compounds is readily hydrolyzed under mildly acidic conditions, it is very stable toward base, and so it was possible to prepare the benzyl ether **99**. Mild treatment with acid (for example, *p*-toluenesulfonic acid monohydrate in acetone) of compounds **46**, **98**, and **99** gave the 2,5-anhydro-D-allose derivatives **100**, **101**, and **102**, respectively; these have proved useful starting compounds for the synthesis of C-nucleosides.



The demonstration by Moffatt and coworkers^{55,56} that the formation of *N,N'*-diphenylimidazolidine derivatives of aldehydes can be extended to carbohydrates, and the mild conditions needed for regeneration of the aldehyde sugar derivatives, constitute an important contribution that has paved the way for the synthesis of several C-glycosyl heterocycles in the *D-ribo* series. The sequence has been extended to the *D-arabino* series,⁵¹ and it has been shown that treatment of freshly regenerated 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-glucose (**38**) with (ethoxycarbonylmethylene)phosphorane gives the expected alkene (**103**) in good yield. Compound **103** could be utilized as a chemical precursor for synthesis of C-glycosyl heterocycles having the *D-arabino* configuration.

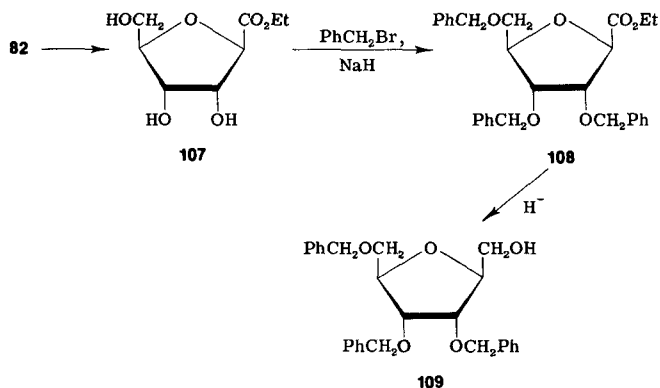


The free aldehydes **100–102** are chromatographically homogeneous syrups that can be converted into crystalline *tert*-butylcarbazones.⁵⁶ It has been recommended⁵⁶ that they be generated from the respective imidazolidines just prior to use. Previous experiences involving elimination and epimerization reactions of nucleoside 5'-aldehydes⁹⁰ justify the special precautions needed in the manipulation of 2,5-anhydroaldose derivatives that are also potentially subject to elimination and epimerization reactions. Indeed, Montgomery and coworkers⁸⁸ reported that reductive hydrolysis of **97** afforded a mixture of epimeric aldehydes (**104**), which could not be separated, but were reduced with sodium borohydride to the corresponding alcohols (**105** and **106**). Compound **105** was the preponderant isomer, and it was concluded that reduction of the nitrile gave primarily the epimerized aldehyde.



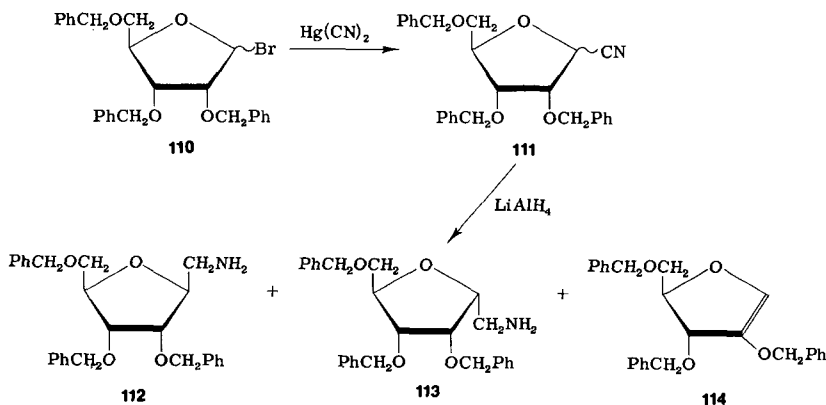
These authors⁸⁸ also sought an alternative route to a protected 2,5-anhydroallitol that would avoid epimerization. The nitrile **82** was hydrolyzed⁴⁰ to the acid **21**, and the latter was esterified to give **107**. Benzylation with benzyl chloride–sodium hydride in *N,N*-dimethylformamide gave a mixture of five products resulting from dehydration and ester interchange. The principal product (**108**), obtained in 43% yield by chromatographic separation, was reduced with sodium bis(2-methoxyethoxy)aluminum hydride, and the preferentially substituted anhydroallitol (**109**) was obtained in crystalline form. Access

(90) G. H. Jones and J. G. Moffatt, Jr., *Abstr. Papers Amer. Chem. Soc. Meeting*, **158**, CARB 16 (1969).



to compound **109**, which may be regarded as a versatile intermediate for the preparation of C-nucleosides, has been made easier by reduction of the crude mixture resulting from *O*-benzylation, followed by isolation by direct crystallization.⁸⁸

Unlike the behavior of **81**, treatment of 2,3,5-tri-*O*-benzyl-D-ribofuranosyl bromide⁹¹ (**110**) with mercuric cyanide gave an anomeric mixture of cyanides (**111**), which was reduced to an epimeric mixture of amines.⁹² Separation of this mixture by column chromatography gave the *D-allo* isomer (**112**), the *D-altro* isomer (**113**), and the glycal derivative (**114**). Compound **112** was, however, formed in moderate yield (18%), presumably because of a preponderance of the α anomer in the mixture of anomeric cyanides (**111**). Compound **112** was converted⁹² into the 1-ureido derivative (**92**) by treatment

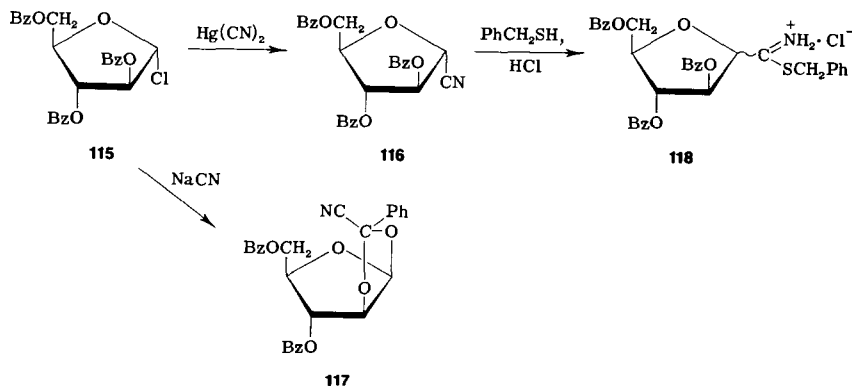


(91) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4605-4609 (1961).

(92) M. W. Winkley, *Carbohydr. Res.*, **31**, 245-254 (1973).

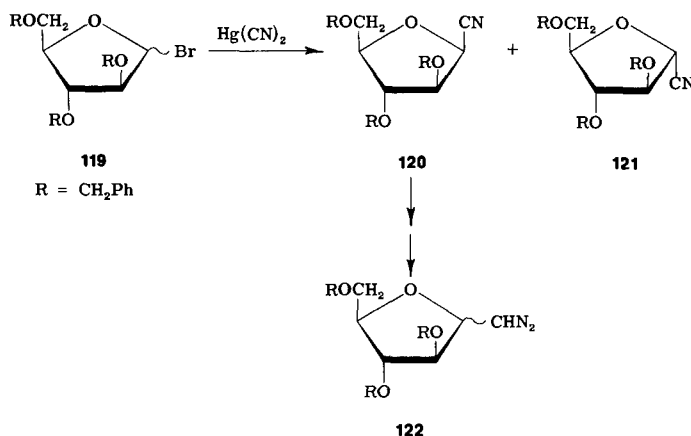
with nitrourea. Another route to the amine **112** involved a multi-stage synthesis starting from 3-O-benzyl-1,2:5,6-di-O-cyclohexylidene- α -D-glucofuranose.⁹³

The therapeutic importance of such D-arabinofuranosyl nucleosides^{3,94} as 1- β -D-arabinofuranosylcytosine⁹⁵ and 9- β -D-arabinofuranosyladenine⁹⁶ prompted several groups to explore synthetic routes to C- β -D-arabinofuranosyl nucleosides. Igolen and co-workers⁹⁷ reported the formation of 2,3,5-tri-O-benzoyl- α -D-arabinofuranosyl cyanide in 56% yield by treatment of the α -chloride (**115**) (or the bromide) with mercuric cyanide in nitromethane. Interestingly, when sodium cyanide was used, the alkylidene derivative **117** was formed in 34% yield, demonstrating once again⁷⁸ the influence of the nature of the salt in determining the site of attack on acyloxonium ions of sugars. Acid-catalyzed treatment of the nitrile **116** with α -toluenethiol gave a mixture of anomeric iminothioether derivatives (**118**) that were subsequently transformed into C- β -D-arabinofuranosylimidazoles. It is not clear whether anomerization took place prior, or subsequent, to iminothioether (thioformimide) formation.



- (93) T. Ogawa, Y. Kikuchi, M. Matsui, H. Ohrai, H. Kuzuhara, and S. Emoto, *Agr. Biol. Chem.* (Tokyo), **35**, 1825-1828 (1971).
- (94) S. S. Cohen, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 1-83 (1966).
- (95) D. T. Gish, R. C. Kelly, G. W. Camiener, and W. J. Wechter, *J. Med. Chem.*, **14**, 1159-1162 (1971), and references cited therein.
- (96) F. M. Schabel, *Chemotherapy*, **13**, 321-338 (1968); F. A. Miller, G. D. Dixon, J. Ehrlich, B. J. Sloan, and I. W. McLean, *Antimicrob. Ag. Chemother.*, 136-147 (1968).
- (97) G. Barnathan, T. Huynh Dinh, A. Kolb, and J. Igolen, *Compt. Rend.*, **274**, 2192-2193 (1972).

Goodman and coworkers⁹⁸ prepared the anomeric nitriles **120** and **121**, in approximately 85% yield, by treatment of the bromide **119** with mercuric cyanide in dry benzene, followed by chromatographic purification from non-nitrogenous contaminants. The β -D- anomer **120** was the preponderant product (4:1 ratio) as determined by ¹³C nuclear magnetic resonance spectroscopy. Interestingly, the ratio of the anomeric nitriles reflected, in reverse, the α : β ratio (3:17) of anomeric bromides, indicating the possibility of a direct displacement by the cyanide ion at the anomeric carbon atom. In nitromethane as the solvent, the proportion of the non-nitrogenous contaminant increased at the expense of the nitriles, and, with silver cyanide in xylene, the possible formation of an isonitrile, as previously suggested⁷⁸ in another series, was indicated, based on spectroscopic evidence.



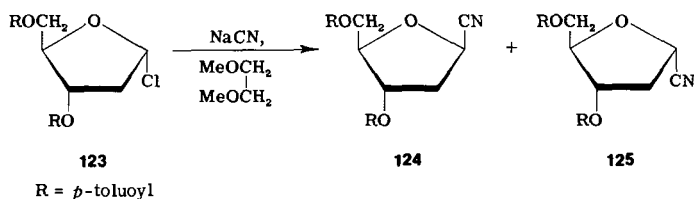
An independent synthesis of **120** was achieved by *O*-benzylation of 2,5-anhydro-D-gluconamide³³; however, undesirable side-reactions made this alternative approach impractical. Compound **120** was subsequently transformed into a C- β -D-arabinofuranosylated heterocycle by way of a 1,3-dipolar addition-reaction of the 1-diazo intermediate **122** to dimethyl acetylenedicarboxylate (see Section IV,2).

2-Deoxy-3,5-di-*O*-*p*-toluoyl- β -D-*erythro*-pentofuranosyl cyanide (**124**) has been prepared⁹⁹ in 85% yield from the corresponding chlo-

(98) E. M. Acton, A. N. Fujiwara, L. Goodman, and D. W. Henry, *Carbohydr. Res.*, **33**, 135-151 (1974).

(99) A. Kolb, T. Huynh Dinh, and J. Igolen, *Bull. Soc. Chim. Fr.*, 3447-3448 (1973); A. Kolb, C. Gouyette, T. Huynh Dinh, and J. Igolen, *Tetrahedron Lett.*, 2971-2974 (1973).

ride (**123**) and sodium cyanide in 1,2-dimethoxyethane at room temperature. Surprisingly, very low (<5%) yields of the anomeric nitriles **124** and **125** were formed in the presence of mercuric cyanide



in various solvents, the *p*-toluic ester of furfuryl alcohol¹⁰⁰ being the major contaminant in these reactions. It is also of interest to note the change in the ratio of anomeric nitriles with the nature of the solvent employed (see Table I). No nitriles were formed in the presence of mercuric cyanide in benzene. In such polar, aprotic solvents as ether, 1,4-dioxane, tetrahydrofuran, *N,N*-dimethylformamide, dimethyl sulfoxide, and hexamethylphosphoric triamide, the yields were rather poor. In 1,2-dimethoxyethane, the nitrile **124** was obtained in high yield and anomeric purity, presumably due, in part, to a favorable solvation of the cation by a bidentate-donor mechanism,¹⁰¹ thus increasing the effectiveness of cyanide ion as a nucleophile, in agreement with similar observations in another series.¹⁰² The anomeric configurations of nitriles **124** and **125** were assigned on the basis of spectroscopic and optical rotational data.

TABLE I
Influence of the Solvent in the Formation of **125** from **123**

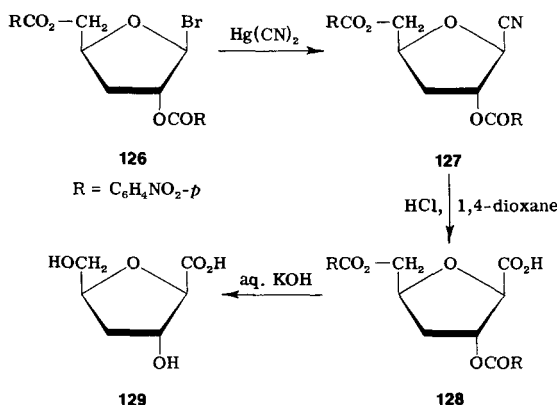
Solvent	Yield (%)	Ratio of β/α	Yield of furfuryl toluate (%)
Hexamethylphosphoric triamide (HMPT)	3	<1	42
HMPT-benzene	12	0.5	15
Dimethyl sulfoxide, 90°, 1 h	18	4	4
Acetone	70	9	2
1,2-Dimethoxyethane	85	2	2

(100) M. Prystas, J. Farkaš, and F. Šorm, *Collect. Czech. Chem. Commun.*, **28**, 3140-3143 (1963).

(101) H. E. Zaugg, *J. Amer. Chem. Soc.*, **83**, 837-840 (1961); H. D. Zook and T. R. Russo, *ibid.*, **82**, 1258-1259 (1960).

(102) S. Hanessian and A. G. Pernet, *Can. J. Chem.*, **52**, 1280-1293 (1974).

El Khadem and El Ashry¹⁰³ prepared 3-deoxy-2,5-di-*O*-(*p*-nitrobenzoyl)- β -D-*erythro*-pentofuranosyl cyanide (**127**) by treatment of the corresponding bromide (**126**) with mercuric cyanide in nitromethane. Unlike the behavior in the 2-deoxy series,⁹⁹ a very good yield (86%) of the nitrile was obtained under these conditions. The β -D configuration was assigned to **127**, based on the small $J_{2,3}$ coupling constant, and by analogy with the results of previous work.⁴⁰ Acid hydrolysis of **127** gave 2,5-anhydro-4-deoxy-3,6-di-*O*-(*p*-nitrobenzoyl)-D-*ribo*-hexonic acid (**128**), isolated in crystalline form, which was further transformed into the acid **129**, and subsequently utilized in the synthesis of a C-nucleoside analog of the antibiotic cordycepin (see Section IV,4,d).



b. Condensation Reactions with Organometallic and Related Reagents.—Hurd^{104–107} and Bonner¹⁰⁸ and their coworkers were the first to prepare and characterize the products resulting from the reaction of per-*O*-acylglycosyl halides with organomagnesium and organolithium compounds. For example, it was shown¹⁰⁷ that treatment of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide with an excess of phenylmagnesium bromide gave 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosylbenzene. Similar C-glycosylations were possible with the per-*O*-acylglycosyl halides of D-xylose,¹⁰⁷ D-mannose,¹⁰⁴ and lac-

(103) H. S. El Khadem and El S. H. El Ashry, *Carbohydr. Res.*, **32**, 339–348 (1974); **29**, 525–527 (1973).

(104) C. D. Hurd and R. P. Holysz, *J. Amer. Chem. Soc.*, **72**, 1732–1735 (1950).

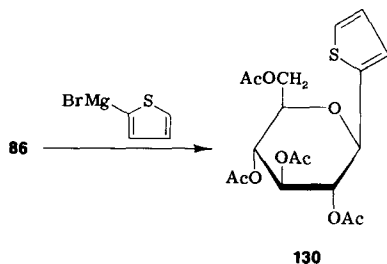
(105) C. D. Hurd and R. P. Holysz, *J. Amer. Chem. Soc.*, **72**, 1735–1738 (1950).

(106) C. D. Hurd and H. T. Miles, *J. Org. Chem.*, **29**, 2976–2979 (1964).

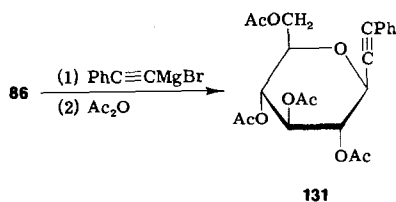
(107) C. D. Hurd and W. A. Bonner, *J. Amer. Chem. Soc.*, **67**, 1972–1976 (1945).

(108) W. A. Bonner, *Advan. Carbohydr. Chem.*, **6**, 251–290 (1951).

tose.¹⁰⁷ A variety of organomagnesium reagents could also be employed, as in the synthesis¹⁰⁷ of 2-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)thiophene (**130**).



The (more reactive) organolithium reagents also gave *C*-glycosyl derivatives,¹⁰¹ although the major product resulting from the reaction of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl chloride with phenyllithium was identified, after acetylation, as 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-2-*C*-phenyl-D-glucitol.¹⁰⁶ A similar observation has been reported by Ogura and Ogiwara,¹⁰⁹ who isolated the corresponding 2-*C*-ethynyl derivative, in low yield, from the reaction of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide with ethynylmagnesium bromide. The same compound had previously been isolated by Zelinski and R. E. Meyer,¹¹⁰ but its structure had remained unassigned. These authors¹¹⁰ reported the formation of 1-phenyl-2-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)ethyne (**131**) from the reaction of **86** with (phenylethynyl)magnesium bromide, followed by acetylation of the product.

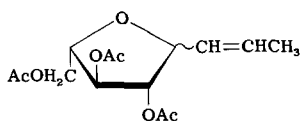
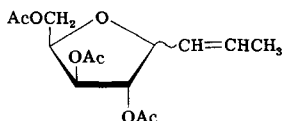


It is clear that the successful application of these *C*-glycosylations to the D-aldofuranose series, by treatment of the appropriate per-*O*-acylaldofuranosyl halides with organometallic reagents, could lead to synthetically useful *C*-glycosyl derivatives. Zhdanov and co-

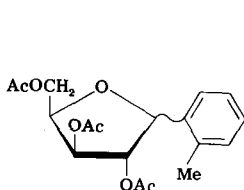
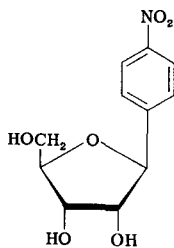
(109) H. Ogura and M. Ogiwara, *Chem. Pharm. Bull.* (Tokyo), **20**, 848-850 (1970).

(110) R. Zelinski and R. E. Meyer, *J. Org. Chem.*, **23**, 810-813 (1958).

workers¹¹¹ reported the formation of the acetylated 1-D-arabinofuranosyl- and 1-D-xylofuranosyl-1-propenes (**132**, **133**) in the

**132****133**

reaction of the respective per-*O*-acetylpenfuranosyl halides with allylmagnesium bromide. The acetylated D-xylofuranosyl-*o*-toluene (**134**) was obtained in 47% yield. Fox and coworkers¹¹² described the synthesis of compound **135**, a cyclic analog of chloramphenicol.

**134****135**

Applications of such C-glycosylations were studied by Buchanan and coworkers⁷¹; they reported the formation of the anomeric 2-(2,3,5-tri-*O*-benzyl-D-ribofuranosyl)ethynes (**70** and **71**) from treatment of the corresponding β -D chloride with ethynylmagnesium bromide. The major (68%) and minor (8%) products, which were separated by chromatography, were assigned the α and β anomeric configurations, respectively, based on their optical rotations. These authors also pointed out,⁷¹ that the preponderance of the α isomer (**71**) could be expected as the result of a direct attack on the anomeric carbon atom of the β -D chloride. Hydrogenation of the C-glycosyl compounds gave, in each case, a D-ribofuranosylethane whose optical rotation was also in agreement with Hudson's rules of isorotation,¹¹³ originally advanced for glycosides. Although the values of optical rotation reported for anomeric pairs of other C-glycosyl derivatives (see Sec-

(111) Yu. A. Zhdanov, G. N. Dorofeenko, and L. E. Zhivoglazova, *Dokl. Akad. Nauk SSSR*, **117**, 990-992 (1957); *Chem. Abstr.*, **52**, 8055 (1958); see also, *Chem. Abstr.*, **47**, 2710 (1953).

(112) R. S. Klein, M. P. Kotick, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, **36**, 4113-4116 (1971).

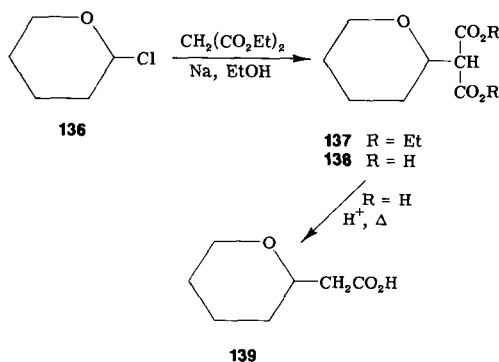
(113) C. S. Hudson, *J. Amer. Chem. Soc.*, **31**, 66-86 (1909).

tion II,2,c,i) also seem to comply with these rules, it is the opinion of the writers that independent, chemical proof as to the anomeric configuration of C-glycosyl compounds should be sought whenever possible. 3-(Ribofuranosyl)propiolates have also been prepared.^{113a}

Treatment of the ethyne derivative **71** with palladium chloride and mercuric chloride in the presence of carbon monoxide at a pressure of 1 atmosphere^{113b} gave methyl 2-(2,3,5-tri-*O*-benzyl- β -D-ribofuranosyl)maleate^{113c} in high yield. This product may be considered to be a useful precursor to showdomycin.

c. Condensation Reactions with Carbanions and Related Reagents.—(i) **Carbanions.** The condensation of carbanionic reagents with appropriate carbohydrate derivatives, such as *O*-substituted glycosyl halides, constitutes a direct method of C-glycosylation. An alternative approach is reaction with acyclic sugar derivatives, which leads to acyclic products substituted at C-1. Presumably, these could be cyclized to C-glycosyl derivatives.

There are very few precedents for the reaction of cyclic α -halo ethers with carbanions. Zelinski and coworkers¹¹⁴ and Schudel and Rice¹¹⁵ reported the preparation of diethyl DL-tetrahydropyran-2-ylmalonate (**137**) by treatment of 2-bromo- or 2-chloro-tetrahydropyran (**136**) with diethyl sodiomalonate. The product was subsequently converted into the malonic and acetic acid derivatives, **138** and **139**, respectively. The same sequence has also been reported by other workers.¹¹⁶

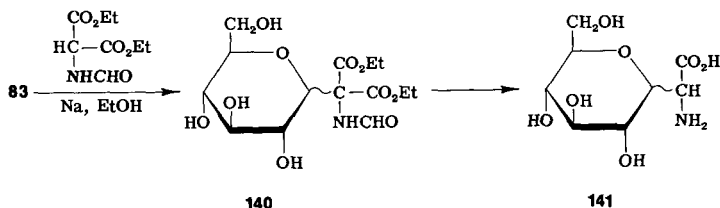


(113a) F. G. De Las Heras, S. Y.-K. Tam, R. S. Klein, and J. J. Fox, *J. Org. Chem.*, **41**, 84-90 (1976).

(113b) R. F. Heck, *J. Amer. Chem. Soc.*, **94**, 2712-2719 (1972).

(113c) J. G. Buchanan, A. R. Edgar, M. J. Power, and P. D. Theaker, *Carbohydr. Res.*, **38**, C22-C24 (1974).

Another precedent found in the carbohydrate series consisted in an attempt at C-glucosylation by this method. Treatment of 2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl bromide (**83**) with diethyl 2-formamidomalonate in the presence of sodium ethoxide in ethanol was reported to yield diethyl 2-formamido-2-D-glucopyranosylmalonate (**140**) in 14% yield.¹¹⁷ The latter was converted into a compound believed to be 2-D-glucopyranosylglycine (**141**). Analogous transformations were reported in the lactose series. The identity of these products as C-glycosyl compounds was not rigorously established. An alternative synthesis of **141** has been accomplished by Rosenthal and Brink.^{117a}



A systematic study of the reaction of O-acylglycosyl halides with carbanions showed that, under suitable conditions, C-glycosyl compounds could be obtained in high yield.¹¹⁸ Treatment of 2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl bromide with diethyl sodiomalonate, prepared from the diethyl ester plus sodium hydride in 1,2-dimethoxyethane, afforded crystalline diethyl 2-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl)malonate (**142**). With dibenzyl sodiomalonate, the corresponding dibenzyl ester (**143**) was obtained in high yield, and it was converted into the malonic (**144**) and acetic (**145**) acid derivatives by sequential hydrogenation and thermal decarboxylation. It was also shown that further functionalization of the carbon atom attached to C-1 in these compounds was possible, as exemplified by the formation of the α -bromo ester **146** by treatment of **144** with bromine in thionyl chloride,¹¹⁹ followed by esterification.

(114) R. P. Zelinski, N. G. Peterson, and H. R. Wallner, *J. Amer. Chem. Soc.*, **74**, 1504-1506 (1952).

(115) J. G. Schudel and R. V. Rice, U. S. Pat. 2,522,966 (1950); *Chem. Abstr.*, **45**, 6223 (1951).

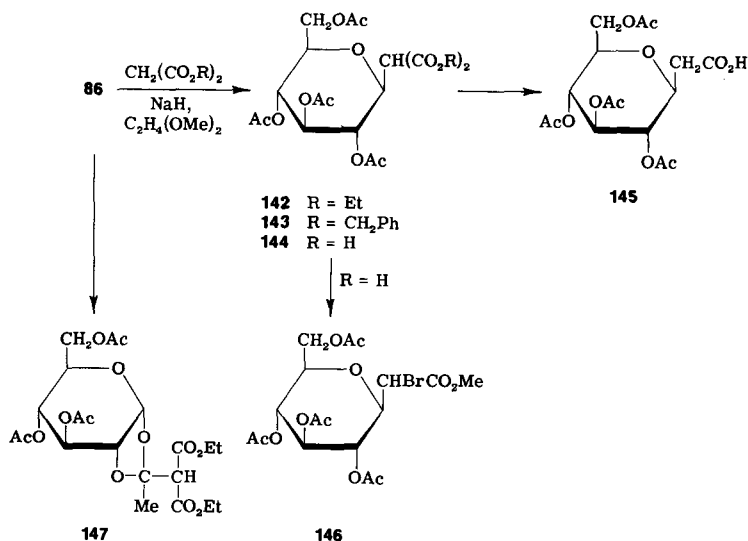
(116) J. Colonge, J. Dreux, and M. Coblenz, *Compt. Rend.*, **250**, 3202-3203 (1960).

(117) J. V. Kořtir and M. Queisnerová, *Chem. Listy*, **43**, 277-279 (1949); *Chem. Abstr.*, **45**, 553 (1951).

(117a) A. Rosenthal and A. Brink, *J. Carbohydr. Nucleos. Nucleot.*, **2**, 343-356 (1975).

(118) S. Hanessian and A. G. Pernet, *Can. J. Chem.*, **52**, 1266-1279 (1974); *Chem. Commun.*, 755-775 (1971).

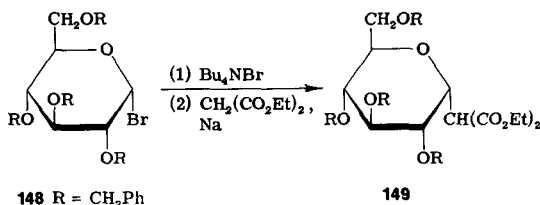
(119) E. Shwenk and D. Papa, *J. Amer. Chem. Soc.*, **70**, 3626-3627 (1948).



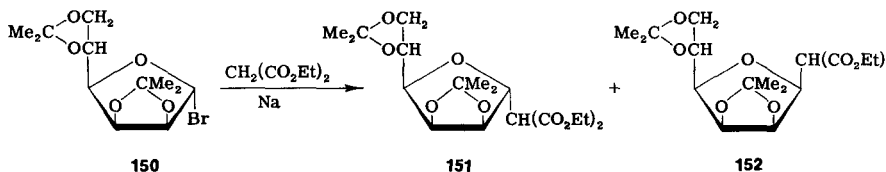
In a polar solvent such as *N,N*-dimethylformamide, an appreciable proportion of the acetal 147 was formed. The latter was the only product isolated from the reaction of 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl chloride with diethyl sodiomalonate in *N,N*-dimethylformamide. It is evident that, in these cases, predominant (if not exclusive) attack of the carbanion occurs at the dioxolenium carbon atom in the intermediate, acetoxonium ion. The nature of the solvent seems to play an important role in determining the sites of nucleophilic attack by the carbanion in such loosely or tightly ion-paired species as 86-88. The role of 1,2-dimethoxyethane as an effective solvent can be understood on the basis of its ability to form bidentate complexes¹⁰¹ with the cation, thus accentuating the nucleophilic character of the malonate carbanion. With 2,3,4,6-tetra-*O*-benzyl- α -D-glucopyranosyl bromide¹²⁰ (148) and diethyl sodiomalonate in diethyl malonate as the solvent, a mixture of anomeric diethyl 2-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)malonates was formed, presumably as a result of rapid anomerization, or rapid formation of an oxonium ion, followed by attack of the carbanion from the α and β sides in each instance. The proportion of the C- α -glycosyl compound was substantially increased by addition of an excess of tetrabutylammonium bromide to the reaction mixture prior to the addition of the carbanion, presumably because of an increase

(120) T. Ishikawa and H. G. Fletcher, Jr., *J. Org. Chem.*, **34**, 562-571 (1969); F. Weygand and H. Ziemann, *Ann.*, **687**, 179-198 (1962).

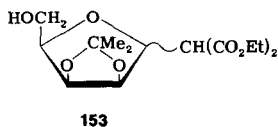
in the $\beta:\alpha$ ratio of the anomeric halides. It has been shown^{120,121} that the addition of halide ion to solutions containing *O*-benzylated α -D-glucopyranosyl halides, prior to the addition of an alcohol, increases the proportion of α -glycoside formed, relative to the β anomer. Compound **149** was transformed into diethyl 2-(2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl)malonate by hydrogenation and acetylation.



These model reactions were of great value in the extension of the C-glycosylation reaction with malonic esters to five-membered ring-systems. Treatment of 2,3:5,6-di-*O*-isopropylidene- α -D-mannofuranosyl bromide^{122,123} (**150**) with diethyl sodiomalonate led to an anomeric mixture of C-glycosyl compounds that could be separated by column chromatography, with the α (**151**) and β (**152**) anomers in



1:9 ratio.¹¹⁸ The mixture of anomers was also transformed into the 5,6-diol by selective acid hydrolysis of the 5,6-acetal group, and the product was converted into the *D*-lyxo analog (**153**) by standard methods. The anomeric configuration of compounds **151** and **152** was assigned by nuclear magnetic resonance data. It was also observed, as in the *D*-gluco series, that the optical rotational data of the respective anomers agreed well with Hudson's rules.¹¹³

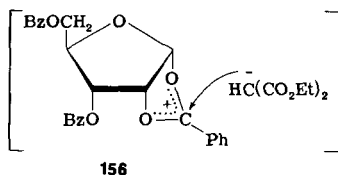


(121) R. U. Lemieux and A. R. Morgan, *J. Amer. Chem. Soc.*, **85**, 1889-1890 (1963).

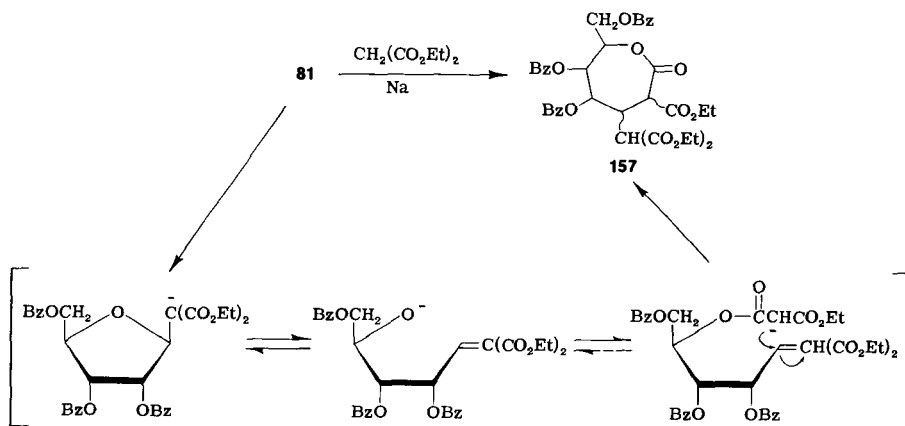
(122) J. B. Lee and T. J. Nolan, *Tetrahedron*, **23**, 2789-2794 (1967).

(123) S. Hanessian, M. M. Ponpipom, and P. Lavalley, *Carbohydr. Res.*, **24**, 45-56 (1972).

154 **155**



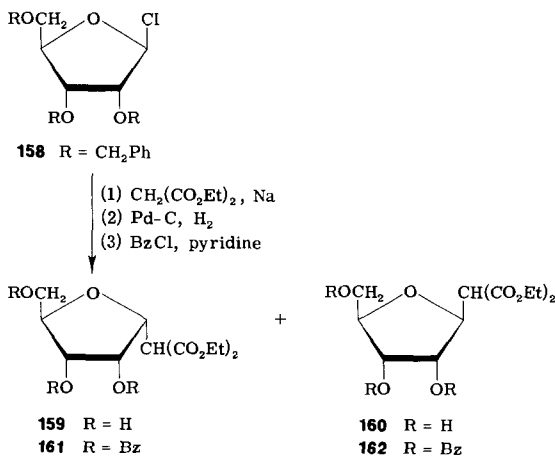
When the reaction was performed in diethyl malonate as the solvent, there were formed the acetal **156** and another product, which was assigned structure **157**. The same type of product was formed when dibenzyl malonate was the solvent. A plausible mechanism for



the formation of this product having such an unusual structure necessitates the initial formation of the desired β -D-glycosyl compound,

which could exist in equilibrium with its carbanion (or enolate anion). The latter is prone to a β -eliminative ring-opening, to give an unsaturated intermediate which is immediately transesterified with the excess of malonic ester, and this product undergoes ring closure to give the observed lactone (**157**). This hypothesis was experimentally verified by treatment of a mixture of the diethyl 2-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)malonates, obtained by another route, with sodium and diethylmalonate, in a reaction simulating the actual conditions of formation of **157**. The sole product was, indeed, the lactone **157**. It was, therefore, evident that formation of a C-glycosyl compound actually took place in the reaction of **81** with diethyl sodiomalonate, but that the products underwent further transformation in the reaction mixture.

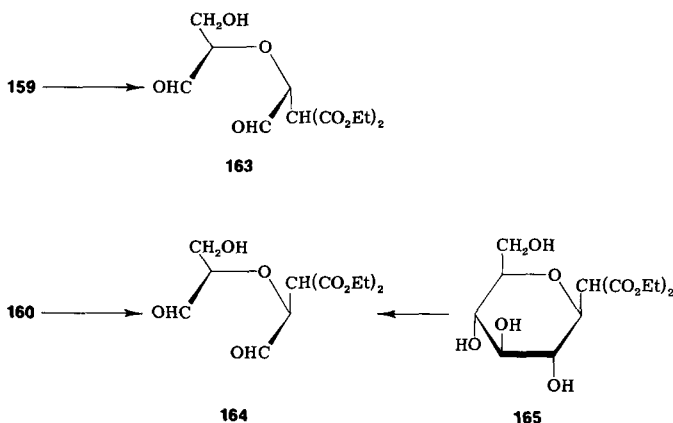
The formation of the desired C-glycosyl compounds was found to be much more favored when nonparticipating groups were present in the starting halide. Thus, treatment of 2,3,5-tri-*O*-benzyl- β -D-ribofuranosyl chloride¹²⁴ (**158**) with diethyl sodiomalonate gave a mixture of the expected C-glycosyl compounds that were isolated as the benzoates (**161** and **162**) in 44 and 46% yields, respectively. The



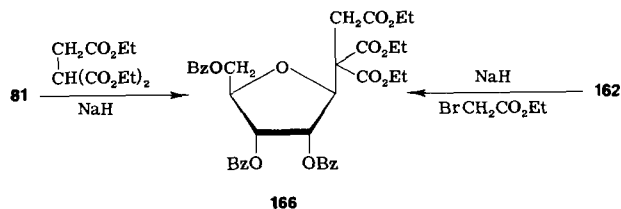
values of optical rotation for these compounds were $[\alpha]_{\text{D}} +71.6^\circ$ and $+21.8^\circ$ (CHCl_3), respectively, in agreement with Hudson's rules.¹¹³ Chemical proof of their anomeric, configurational assignments was obtained from a comparison of the optical rotations of the dialdehydes (**163** and **164**) obtained from **159** and **160**, respectively, with

(124) J. D. Stevens, R. K. Ness, and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1806-1810 (1968).

that of the dialdehyde derived from diethyl 2- β -D-glucopyranosylmalonate (**165**), of established anomeric configuration.¹¹⁸



Compounds **161** and **162** were found to be unstable in basic, protic media, presumably due to the presence of the acidic hydrogen atom of the malonic ester. As a result, their synthetic utility is somewhat limited. It was, however, found possible to alkylate **162** with ethyl bromoacetate in the presence of sodium hydride,¹²⁵ to give triethyl 1-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)-1,1,2-ethanetricarboxylate (**166**). This product was obtained in 20% yield by treatment of **81**

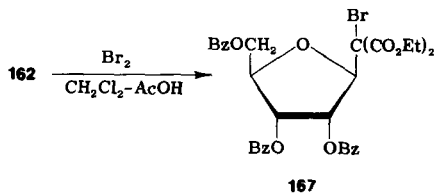


with triethyl sodioethanetricarboxylate in 1,2-dimethoxyethane; it constitutes a highly functionalized, versatile *C*- β -D-ribofuranosyl compound. Unlike compound **162**, it is stable under conditions of debenzoylation in the presence of sodium ethoxide, most probably because of the absence of an acidic proton.

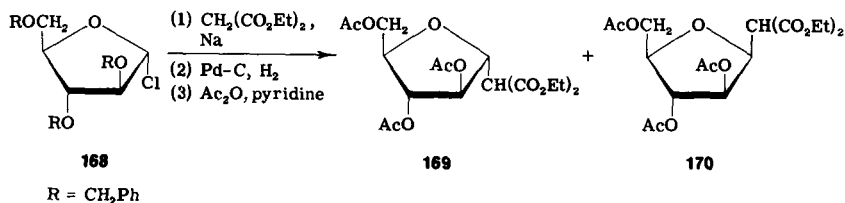
Compound **162** could be brominated to give the 2-bromomalonate analog (**167**). Reductive removal of bromine could be achieved catalytically,¹²⁶ or by treatment with triphenylphosphine.¹²⁵

(125) S. Hanessian, T. Ogawa, and Y. Guindon, unpublished results.

(126) T. Ogawa, A. G. Pemet, and S. Hanessian, *Tetrahedron Lett.*, 3543-3546 (1973).



C-Glycosylation was also effected in the *D-arabino* series, by treatment of 2,3,5-tri-*O*-benzyl- α -*D*-arabinofuranosyl chloride¹²⁷ (**168**) with diethyl sodiomalonate. The anomeric diethyl 2-*D*-arabinofuranosylmalonates were isolated as the acetates (**169** and **170**), and their anomeric configurations were determined by per-



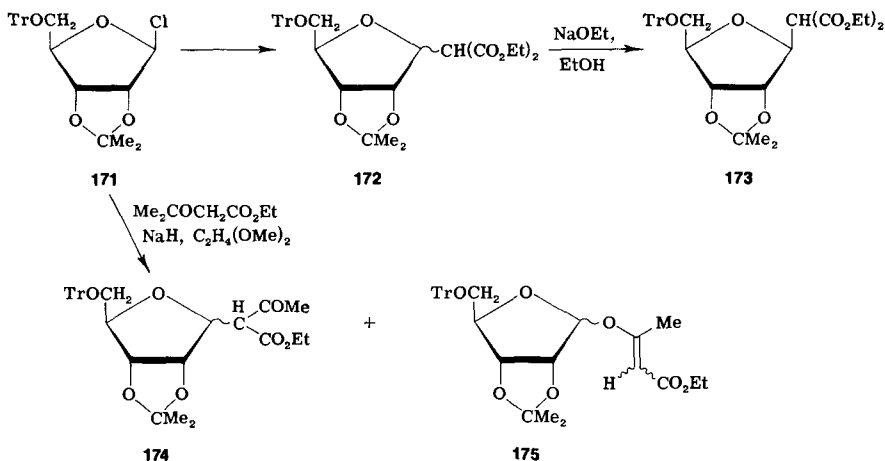
iodate oxidation studies, as in the *D-ribo* series. The optical rotations of the α (**169**) and β (**170**) anomers were $[\alpha]_D +28^\circ$ and -2° (CHCl_3), respectively, in agreement with Hudson's rules.¹¹³ It is of interest that both anomers were formed, in approximately equal amounts, from anomerically pure β -halide, and that the ratio did not change when the addition of the carbanion was preceded by the addition of an excess of tetrabutylammonium chloride. These results call for the intervention, at least in part, of oxonium ion intermediates, as anomerization is unlikely, and direct attack would only explain the formation of **170**. The possibility of anomerization of compound **170**, formed directly from **168**, cannot be excluded; however, no trace of ring-expansion products was encountered in this series, such as was observed in the *D-ribo* series.

Ohrui and Fox¹²⁸ reported that treatment of 2,3-*O*-isopropylidene-5-*O*-trityl- β -*D*-ribofuranosyl chloride (**171**) with diethyl sodiomalonate in 1,2-dimethoxyethane gave an anomeric mixture of C-glycosyl compounds (**172**) which, when allowed to equilibrate in ethanol containing sodium ethoxide, gave the β anomer (**173**) as the preponderant product. Similar observations were made with the C-

(127) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **87**, 2456-2461, 4636-4641 (1965).

(128) H. Ohrui and J. J. Fox, *Tetrahedron Lett.*, 1951-1954 (1973).

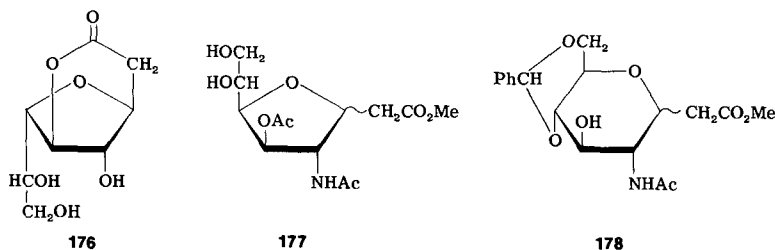
glycosyl compound **174**, which was accompanied by the glycoside (**175**). A subsequent study of this reaction has shown that the α anomers are, in fact, the thermodynamically more-stable products.^{128a}



(ii) **Substituted Methylene phosphoranes (Wittig Reaction).** The Wittig reaction¹²⁹ is one of the most useful methods for the controlled introduction of carbon-carbon bonds in organic molecules. It has found numerous applications in the area of carbohydrates, and the subject has been discussed in this Series.¹³⁰ Thus, using substituted methylenephosphoranes, or the phosphinoxy carbanions derived from dialkoxy-substituted methylenephosphonates (Wittig-Horner¹³¹ and Emmons-Wadsworth¹³² reactions), chain-extension and chain-branching reactions have been successfully performed at various

- (128a) H. Ohrui, G. H. Jones, J. G. Moffatt, M. L. Maddox, A. T. Christensen, and S. K. Byram, *J. Am. Chem. Soc.*, **97**, 4602-4613 (1975); see also, Ref. 118.
- (129) G. Wittig and G. Geissler, *Ann.*, **580**, 44-57 (1953); G. Wittig and U. Schöllkopf, *Chem. Ber.*, **87**, 1318-1330 (1954); G. Wittig and W. Haag, *ibid.*, **88**, 1654-1666 (1955); G. Wittig, H. D. Weidmann, and M. Schlosser, *ibid.*, **94**, 676-689 (1961); for some leading reviews, see A. Maercker, *Org. React.*, **14**, 270-490 (1965); H. J. Bestmann, in "Newer Methods of Preparative Organic Chemistry," W. Foerst, ed., Academic Press, New York, 1968, Vol. 5, pp. 1-60; A. W. Johnson, "Ylid Chemistry," Academic Press, New York, 1960, pp. 5-247; H. O. House, "Modern Organic Reactions," Benjamin, Menlo Park, Calif., 1972, pp. 682-707.
- (130) Yu. A. Zhdanov, Yu. E. Alexeev, and V. G. Alexeeva, *Advan. Carbohydr. Chem. Biochem.*, **27**, 227-299 (1972).
- (131) L. Horner, H. Hoffmann, H. G. Wippel, and G. Klahre, *Chem. Ber.*, **92**, 2499-2505 (1959).
- (132) W. S. Wadsworth and W. D. Emmons, *J. Amer. Chem. Soc.*, **83**, 1733-1738 (1961); for a review, see J. Boutagg and R. Thomas, *Chem. Rev.*, **74**, 87-99 (1974).

positions in cyclic and acyclic carbohydrate derivatives. The interaction of (alkoxycarbonylmethylene)phosphoranes with free sugars leads to the corresponding, chain-extended, unsaturated, acyclic products. The reaction may be accompanied by byproducts resulting from intramolecular cyclization on an alkenic carbon atom. Kochetkov and Dmitriev reported the formation of compounds **176**, **177**, and **178**



as byproducts in the reaction of D-galactose,¹³³ and 2-acetamido-2-deoxy-D-glucose and 2-acetamido-4,6-O-benzylidene-2-deoxy-D-glucose,¹³⁴ with (methoxycarbonylmethylene)triphenylphosphorane. Intramolecular cyclization at the 4-hydroxyl group is considered to be catalyzed by the phosphorane or triphenylphosphine oxide, and it is conceivable that C-glycosyl compounds of the types depicted, but with the desired, *cis* orientation of side chains, could be formed from the readily available, unsaturated, acyclic analogs.

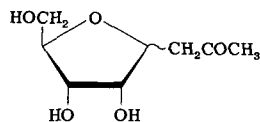
The Wittig reaction, or its phosphonate modification, can be a very useful reaction in the synthesis of anomERICALLY functionalized precursors to C-nucleosides, particularly when the ring-closure reaction leads to five-membered, anhydro derivatives, and occurs with a high degree of stereocontrol. Zhdanov and coworkers,¹³⁰ showed that the following five-membered, Wittig products (**179**–**182**) were formed from various free sugars and (*p*-methoxybenzoyl)- and (acetyl-methylene)-triphenylphosphoranes.

For 2,3:5,6-di-*O*-isopropylidene- α -D-mannofuranose (**183**), separation of the anomeric products (**184** and **185**) was possible,¹³⁵ and their structures were elucidated by periodate-oxidation studies. The configurational assignment for these products was based on the greater value of the specific rotation for the α anomer, as compared with that of the β anomer. The higher chromatographic mobility of one of the

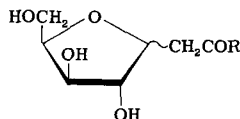
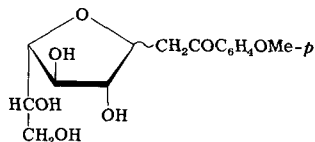
(133) N. K. Kochetkov and B. A. Dmitriev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 274–281 (1966); *Chem. Abstr.*, **64**, 19,734 (1966).

(134) B. A. Dmitriev and N. K. Kochetkov, *Dokl. Akad. Nauk SSSR*, **173**, 350–353 (1967); *Chem. Abstr.*, **67**, 54,381 (1967).

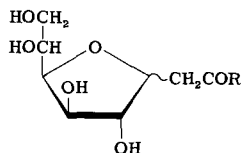
(135) Yu. A. Zhdanov and V. A. Polenov, *J. Gen. Chem. USSR*, **39**, 107–109 (1969).



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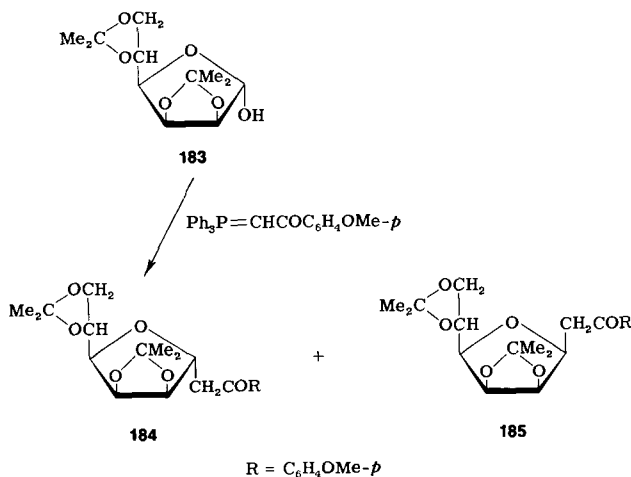
180 R = C₆H₄OMe-*p*
or R = Me

181



182

products was ascribed to a more compact structure, such as would obtain for the β anomer as against the α anomer. A correlation was also made between the relative differences in melting points of the anomers (184) and (185), such as is observed for other anomers.¹³⁶

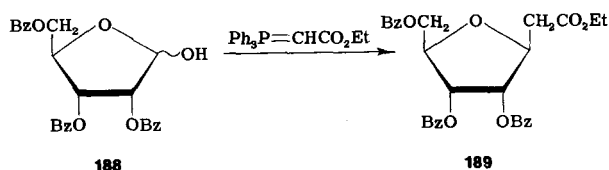
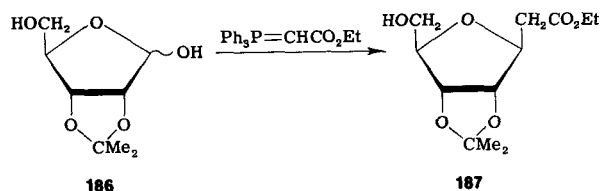


Ready access to 2-C-D-ribofuranosylacetates can be gained from the reaction of 2,3-O-isopropylidene-D-ribofuranose (186) with (ethoxycarbonylmethylene)triphenylphosphorane in boiling toluene.¹³⁷ The reaction proceeds with remarkable stereocontrol, to give crystalline ethyl 2-C-(2,3-O-isopropylidene- β -D-ribofuranosyl)acetate

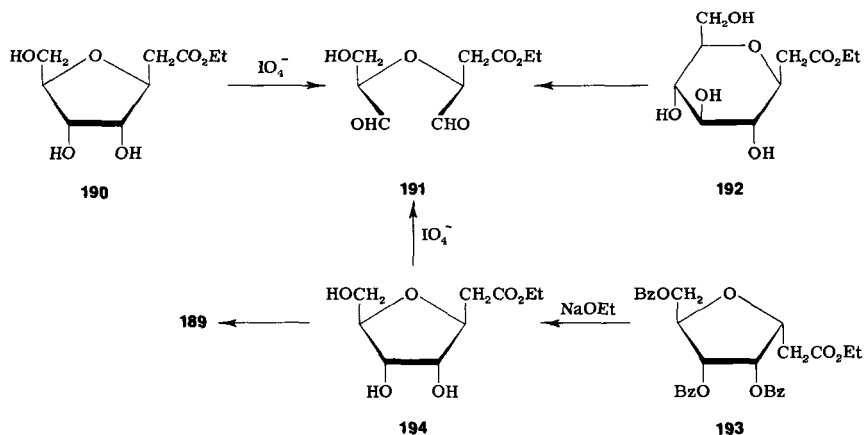
(136) Yu. A. Zhdanov and V. A. Polenov, *J. Gen. Chem. USSR*, **39** 1095-1097 (1969).

(137) S. Hanessian, T. Ogawa, and Y. Guindon, *Carbohydr. Res.*, **38**, C12-C14 (1974).

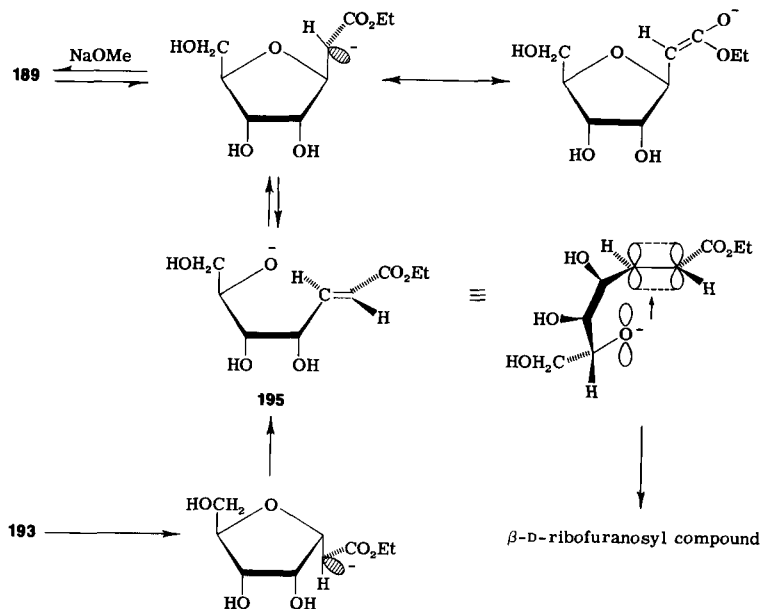
(187) in 65% yield. Treatment of 2,3,5-tri-*O*-benzoyl-D-ribofuranose (188) with the same phosphorane in *N,N*-dimethylformamide gave the β -C-glycosyl compound (189) in 38% yield. The structural rela-



tionship of the two C-glycosyl compounds was established by their mutual chemical interconversion. The anomeric configuration of compound 189 was assigned, based on periodate-oxidation studies of the debenzoylated product, 190, and comparison of the dialdehyde 191 with that obtained from ethyl 2-C- β -D-glucopyranosylacetate (192). Interestingly, a similar debenzoylation of the α anomer 193, obtained by another route, and periodate oxidation, also led to the dialdehyde 191. It appears that the debenzoylation of 193 was accompanied by essentially complete epimerization at C-1 of the D-ribofuranosyl group to give 194. In fact, debenzoylation of 193,



followed by benzoylation gave back the β -C-glycosyl compound **189**, not the expected **193**. Anomerization must occur, by way of a carbanionic or enolate ion intermediate that can undergo ring-opening (β -elimination) to give the unsaturated oxyanion **195**. In both anomers,



this process can be stereoelectronically favored, due to the anti-periplanar arrangement of the lone pair and the C-1-O bond. Evidently, in the ensuing ring-closure process, preponderant (if not exclusive) formation of the β anomer occurs by an α approach. Under the same conditions, the β anomer was not epimerized; this indicated that ethyl 2- β -D-ribofuranosylacetate is thermodynamically more stable than the α anomer. The presence of an added steric constraint, such as that in compound **187** may, however, lead to different results under similar, base-catalyzed, equilibrating conditions.¹³⁷⁻¹³⁹

Buchanan and coworkers^{113c} investigated the reaction of 2,3,5-tri-O-benzyl-D-ribofuranose with (ethoxycarbonylmethylene)triphenylphosphine in anhydrous 1,2-dimethoxyethane in the presence of a catalytic amount of benzoic acid. The alkenic products were isolated in high yield, and each was converted into an anomeric mix-

(138) H. Ohru, G. H. Jones, and J. G. Moffatt, personal communication; see also, Ref. 128a.

(139) S. Hanessian and Y. Guindon, unpublished observations.

ture of ethyl 2-(2,3,5-tri-*O*-benzyl-D-ribofuranosyl)acetates by treatment with sodium ethoxide; this could be separated into the respective anomers by chromatography. At this point, it is of interest to consider the influence of the solvent, the reaction conditions, and, possibly, the nature of the *O*-substituents in these Wittig reactions. In refluxing toluene¹³⁷ or in *N,N*-dimethylformamide, the main products are not alkenic, but cyclic β -D-ribofuranosyl derivatives arising from alkenic precursors. Obviously, there come into play kinetic and thermodynamic factors that lead to the divergence in the nature and stereochemistry of the products.

A synthesis of *C*-glycosyl α -amino acids by formylaminomethylenation of 1,4-lactones has been described.^{139a}

d. Condensation Reactions Catalyzed by Lewis Acids.—By virtue of its electronic character, the anomeric carbon atom, bearing electronegative substituents in various sugar derivatives, is prone to electrophilic substitution reactions. This property extends to carbon nucleophiles, and is best exemplified by the Friedel-Crafts type of reaction in the presence of aluminum chloride. Hurd and Bonner¹⁴⁰ were the first to report the successful preparation of 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosylbenzene from the treatment of the corresponding α -chloride with benzene in the presence of aluminum chloride. An undesirable side-reaction was the formation of 1,1-di-*C*-phenyl-1-deoxy-D-glucitol as a result of ring opening of the deacetylated *C*-glycosyl compound initially formed and further alkylation. Aluminum chloride was also found to catalyze the *C*-glycosylation of aldose peracetates.¹⁴¹ Thus, D-glucopyranosyl-, D-galactopyranosyl-, and D-xylopyranosylbenzene were prepared from the corresponding peracetates, albeit in low yields. The anomeric configuration in the D-*gluco* series was assigned on the basis of a comparison of the value of the optical rotation of α -D-glucopyranosylbenzene, obtained by another route,¹⁴² with that of the β anomer obtained by the Friedel-Crafts procedure.

Direct *C*-glucosylation can also be effected by boiling methyl α -D-glucopyranoside in benzene in the presence of aluminum chloride and boron trichloride. In this way, 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosylbenzene was prepared in 32% yield.¹⁴³ *C*-Glycosyla-

(139a) K. Bischofberger, R. H. Hall, and A. Jordaan, *J. Chem. Soc. Chem. Commun.*, 806–807 (1975).

(140) C. D. Hurd and W. A. Bonner, *J. Amer. Chem. Soc.*, **67**, 1664–1668 (1945).

(141) C. D. Hurd and W. A. Bonner, *J. Amer. Chem. Soc.*, **67**, 1759–1764 (1945).

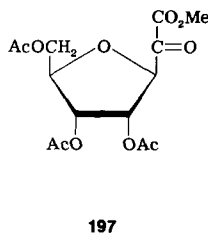
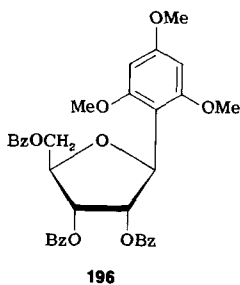
(142) W. A. Bonner and J. M. Craig, *J. Amer. Chem. Soc.*, **72**, 3480–3483 (1950).

(143) T. G. Bonner, E. J. Bourne, and S. McNally, *J. Chem. Soc.*, 761–767 (1962).

tions catalyzed by Lewis acids have also been extended to other aromatic compounds, including, among others, various azulenes¹⁴⁴ and Δ^8 -tetrahydrocannabinol.¹⁴⁵ Synthetically useful applications may be foreseen in the area of naturally occurring C-glycosyl compounds.¹⁴⁶

Boron trichloride appears to form with methyl β -D-ribofuranoside a complex which, after treatment with appropriate nucleophiles, such as sodium methoxide, or the carbanion derived from diethyl 2-(ethoxycarbonylmethyl)malonate, gives the corresponding α -glycosyl compound.¹⁴⁷

These acid-catalyzed C-glycosylations were successfully extended to the D-ribofuranose series by Šorm and coworkers,¹⁴⁸ who utilized the reaction in the first reported synthesis of showdomycin. Thus, treatment of 2,3,5-tri-O-benzoyl- β -D-ribofuranosyl bromide (81) with 1,2,5-trimethoxybenzene in the presence of zinc oxide gave 2,4,6-trimethoxy-1-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)benzene (196). Ozonolysis of the corresponding acetate derivative, followed by esterification, gave the highly functionalized C- β -D-ribofuranosyl derivative (197), which was used as a key intermediate in the synthesis of showdomycin (see Section III,1,b).



Kalvoda¹⁴⁹ studied the reaction of 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose (198) with various aromatic compounds in the presence of Lewis acids as catalysts, and prepared the corresponding D-ribofuranosylbenzenes, such as 199–201. It is of interest that anomeric mixtures were sometimes formed under essentially uniform conditions, and it is not yet clear whether the α anomer arises from

(144) W. Triebs, *Ann.*, **667**, 141–150 (1963).

(145) K. Bailey and D. Verner, *Chem. Commun.*, 89–90 (1972).

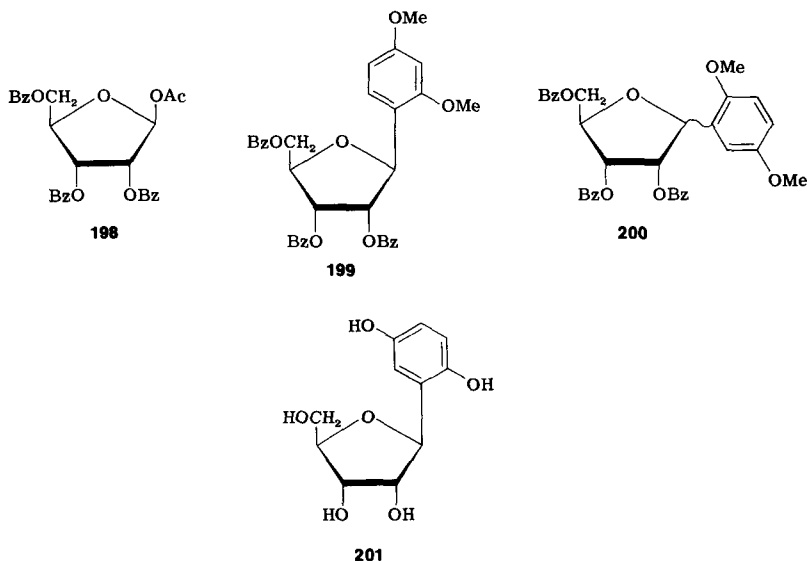
(146) L. J. Haynes, *Advan. Carbohydr. Chem.*, **18**, 227–258 (1963); **20**, 357–370 (1965).

(147) T. Ogawa and S. Hanessian, unpublished results.

(148) L. Kalvoda, J. Farkaš, and F. Šorm, *Tetrahedron Lett.*, 2297–2300 (1970).

(149) L. Kalvoda, *Collect. Czech. Chem. Commun.*, **38**, 1679–1692 (1973).

an acid-catalyzed, equilibration reaction, or from a direct attack on an "open," oxonium-ion type of intermediate.

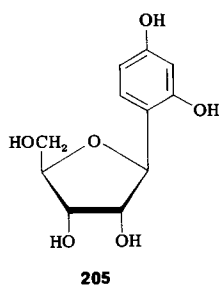
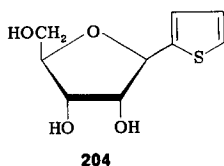
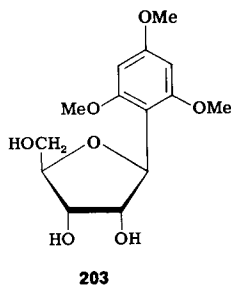
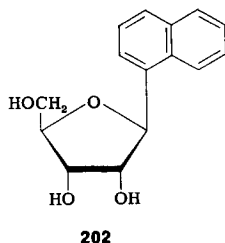


C-Glycosylation in the D-ribofuranose series can be effected under very mild conditions in the presence of stannic chloride as the catalyst, but the product may consist of anomeric mixtures. Thus, 2-β-D-ribofuranosylnaphthalene (**202**), 2,4,6-trimethoxy-1-β-D-ribofuranosylbenzene (**203**) and 2-β-D-ribofuranosylthiophene (**204**) were prepared from 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose and the appropriate aromatic compound.¹⁵⁰ Access to such aromatic C-glycosyl derivatives is, therefore, relatively easy, and it may be of interest in this connection to point out the antitumor action exhibited by 2,4-dihydroxy-1-β-D-ribofuranosylbenzene¹⁵¹ (**205**).

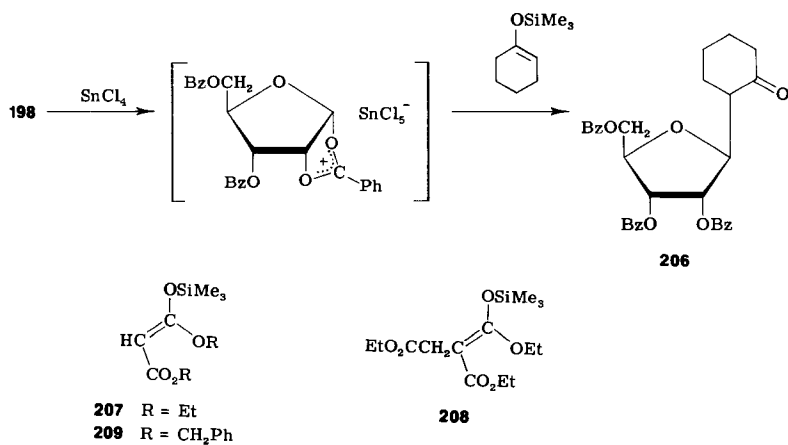
The scope of the acid-catalyzed formation of C-glycosyl compounds has been greatly expanded with the finding that enol ethers and ketene acetals can be used as the carbon source in electrophilic substitution reactions at the anomeric center.¹²⁶ Treatment of **198** with the trimethylsilyl enol ether derived from cyclohexanone, in the presence of stannic chloride, led to 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)cyclohexanone (**206**), presumably by way of the inter-

(150) H. Ohrui, H. Kuzuhara, and S. Emoto, *Agr. Biol. Chem.* (Tokyo), **36**, 1651-1653 (1972).

(151) R. A. Sharma, M. Bobek, and A. Bloch, *Abstr. Papers Amer. Chem. Soc. Meeting*, **166**, MEDI 7 (1973).

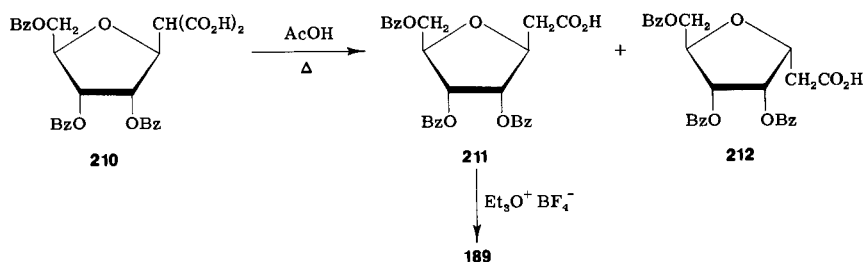


mediate, 1,2-benzoxonium ion. The *C*-β-*D*-ribofuranosyl derivatives **162** and **166**, and the *C*-β-*D*-glucopyranosyl derivative **142** were prepared likewise, by using the ketene acetals **207** and **208**, which could be readily obtained by treatment of the parent esters with sodium hydride, followed by *O*-alkylation of the corresponding enolate ions.¹⁵²

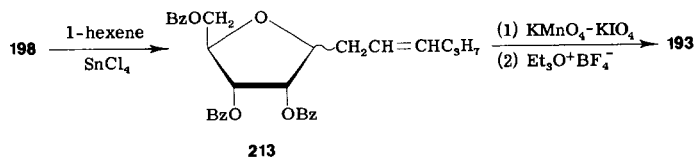


(152) Y. N. Kuo, F. Chen, C. Ainsworth, and J. Bloomfield, *Chem. Commun.*, 136–139 (1971).

By using the dibenzyl analog (209), it was possible to prepare dibenzyl 2-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)malonate in good yield. Catalytic hydrogenation gave the malonic acid derivative (210), which afforded the anomeric 2-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)acetic acids (211 and 212) on thermal decarboxylation. Presumably, decarboxylation was accompanied by opening of the ring, followed by acid-catalyzed closure to give a mixture of the anomers. It may be recalled¹³⁷ that treatment with sodium ethoxide of the ester derived from 212 led to preponderant, if not exclusive, epimerization to the β anomer (189). It is, therefore, of interest that equilibration takes place under acidic conditions.

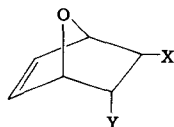


Another approach to functionalized C-glycosyl compounds consists in the condensation, catalyzed by stannic chloride, of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose (198) with terminal alkenes.^{126,153} Treatment of 198 with 1-hexene in the presence of stannic chloride gave a C-D-ribofuranosyl derivative (213) in high

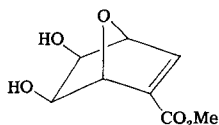
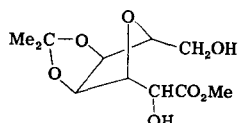


yield. Hydroxylation and oxidative cleavage with potassium permanganate-potassium periodate, followed by esterification with triethyloxonium fluoroborate, gave the α -C-glycosyl derivative (193). As occurrence of anomerization is unlikely under these conditions, it may be tentatively assumed that the initial C-glycosylation reaction gave the α anomer. It has already been established¹³⁷ that, under base catalysis, compound 193 is transformed into the β anomer (189).

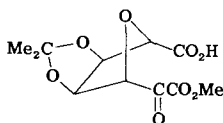
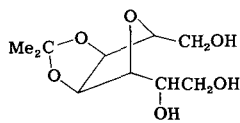
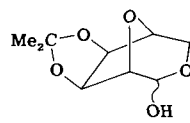
e. Miscellaneous Methods.—Access to anomerically functionalized, racemic, C-ribofuranosyl compounds has been gained through total synthesis.¹⁵⁴ The Diels-Alder product **214** obtained from furan and methyl 3-nitro-acrylate was transformed into the *cis*-diol **215** which, after acetalation followed by reduction, gave the methyl 3,6-anhydroheptonate **216**. Oxidation of **215** with ruthenium

**214**

X = NO₂; Y = CO₂Me
Y = CO₂Me; X = NO₂

**215****216**

tetraoxide, on the other hand, gave the crystalline keto acid derivative **217**. Reduction of the ozonide gave a mixture of epimeric triols (**218**), which was oxidized with periodate to give 2,5-anhydro-3,4-*O*-isopropylidene-DL-allose (**219**). Gensler and coworkers^{154a} described a multi-step synthesis of racemic methyl 2-(2,3-*O*-isopropylidene-β-ribofuranosyl)acetate from the readily available 2,3,4,4-tetrachloro-8-oxabicyclo[3.2.1]octa-2,6-diene.^{154b}

**217****218****219**

III. SYNTHESIS OF NATURALLY OCCURRING C-NUCLEOSIDES

1. Synthesis of C-Nucleosides in which the Glycosylic Carbon Atom is Attached to Two Carbon Atoms

This Section is concerned with the synthesis of C-nucleosides having the Type A arrangement of atoms attached to the glycosylic carbon atom, and covers the synthesis of pseudouridine (**1**), show-

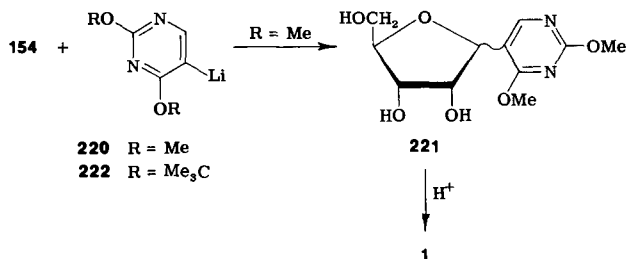
(154) G. Just and A. Martel, *Tetrahedron Lett.*, 1517-1520 (1973); see also, G. Just and K. Grozniger, *ibid.*, 4165-4168 (1974) for a correction.

(154a) W. J. Gensler, S. Chan, and D. B. Ball, *J. Amer. Chem. Soc.*, **97**, 436-437 (1975).

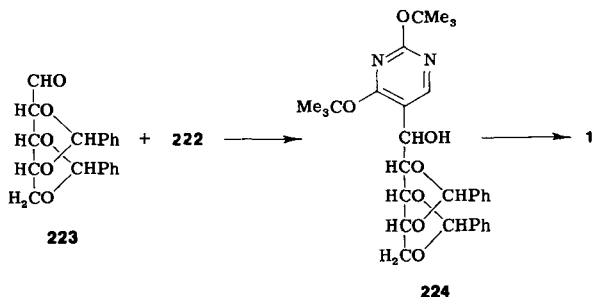
(154b) D. C. F. Law and S. W. Tobey, *J. Amer. Chem. Soc.*, **90**, 2376-2386 (1968).

domycin (2), and indochrome BII (9). We are not aware of any reported syntheses of oxazinomycin (2).

a. Synthesis of Pseudouridine.—The similarity of structure between pseudouridine (5- β -D-ribofuranosyluracil) (1) and uridine (1- β -D-ribofuranosyluracil) prompted the application of the method of synthesis of nucleosides to that of C-nucleosides. Shapiro and Chambers¹⁵⁵ were the first to report a chemical synthesis of pseudouridine (1). Condensation of 2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl chloride (154) with 2,4-dimethoxypyrimidin-5-yl lithium (220) afforded a mixture of the anomers of the intermediate C-nucleoside (221), which, after treatment with acid and chromatography, gave the desired pseudouridine in 2% yield. This product was accompanied by the α anomer and by the two anomeric C-pyranosyl nucleosides arising from acid-catalyzed ring-expansion.



A substantial improvement was reported by D. M. Brown and coworkers,¹⁵⁶ who used the more readily hydrolyzable *tert*-butyl pyrimidine derivative (222) in a condensation reaction with the *aldehydo*-D-ribose derivative 223. Acid hydrolysis of the epimeric mixture (224) gave pseudouridine and its α anomer in 18 and 8%



(155) R. Shapiro and R. W. Chambers, *J. Amer. Chem. Soc.*, **83**, 3920–3921 (1961).

(156) D. M. Brown, M. G. Burdon, and R. P. Slatcher, *Chem. Commun.*, 77–78 (1965);
D. M. Brown and M. G. Burdon, *J. Chem. Soc.*, 1051–1053 (1968).

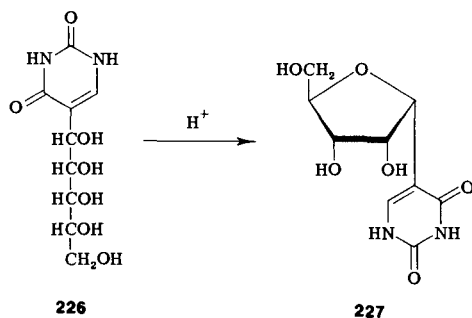
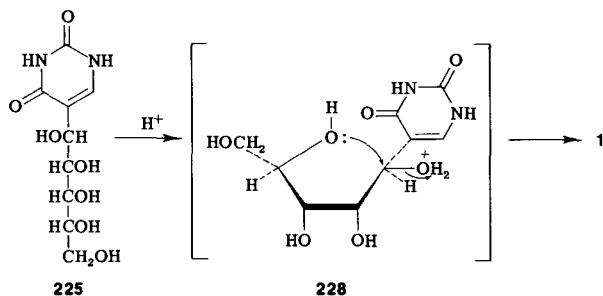
yield, respectively. It is of interest that, of the two possible anomeric pairs of products which might be formed during the cyclization of the debenzylidenated intermediate derived from **224**, only the five-membered ring-forms were obtained. A mechanistic interpretation for this cyclization reaction has been given by Moffatt and co-workers,¹⁵⁷ who were able to isolate the epimeric pentitols corresponding to **224** by chromatographic techniques. The assignment of configuration to these products was based primarily upon optical rotatory dispersion data reported in the literature for polyol derivatives of benzimidazoles,¹⁵⁸ pyrazoles,¹⁵⁹ triazines,¹⁶⁰ and other simple aromatic compounds.¹⁶¹ It has been recognized that various such heterocyclic polyols having S chirality at the newly created asymmetric carbon (OH on the right in the Fischer projection) exhibit positive Cotton-effects, whereas the reverse obtains when the chirality¹⁶² is R. Moffatt and coworkers¹⁵⁷ performed a systematic study of the stability of the epimeric pentitol derivatives (**224**) towards acid, and showed that, under mild conditions, the *tert*-butyl ether groups are the first to be cleaved. More-vigorous hydrolysis led to the cleavage of the ether and acetal groups, and the formation of the epimeric pentitols (**225** and **226**), which were separated by chromatography, and isolated in crystalline form. Their cyclization under mildly acid conditions proceeded in high yield, and revealed some interesting stereochemical aspects. Thus, when a suspension of the *D-altro* isomer (**225**) in hydrochloric acid was stirred at room temperature, almost exclusive formation of pseudouridine occurred. Acid treatment of the epimeric *D-allo* isomer (**226**), on the other hand, led preponderantly to the α anomer **227**, which could be induced to undergo partial anomerization under prolonged treatment with acid. The acid-catalyzed equilibration of the anomeric pseudouridines had been studied by Cohn¹ in his classical work on the structure of pseudouridine, and a mechanistic rationale had been proposed by Chambers and coworkers.¹⁶³ The conversions of the epimeric pentitols **225** and **226** into **1** and **227**, respectively, could involve an S_N2 type of displacement, by the 4'-hydroxyl group, of a protonated 1'-hydroxyl group, as shown by structure **228**, according to several precedents (see Section II,1). An alternative possibility, involving a solvent-stabilized, C-1' carbonium-ion intermediate, cannot be excluded.¹⁵⁷

(157) U. Lerch, M. G. Burdon, and J. G. Moffatt, *J. Org. Chem.*, **36**, 1507-1513 (1971).

(158) N. K. Richtmyer and C. S. Hudson, *J. Amer. Chem. Soc.*, **64**, 1612-1613 (1942).

(159) H. S. El Khadem, *J. Org. Chem.*, **28**, 2478 (1963); J. A. Mills, *Aust. J. Chem.*, **17**, 277-280 (1964).

(160) M. Bobek, J. Farkaš, and F. Šorm, *Collect. Czech. Chem. Commun.*, **32**, 3572-3580 (1967).



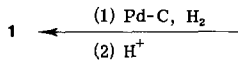
Asbun and S. B. Binkley¹⁶⁴ reported a synthesis of pseudouridine starting with the readily available 5-O-acetyl-2,3-O-isopropylidene-D-ribonolactone (**229**), which was allowed to react with 2,4-dibenzoyloxypyrimidin-5-yllithium (**222**) to give compound **230**. Reduction of the benzylic hydroxyl group with sodium borohydride to give **231**, followed by catalytic hydrogenolysis and acid-catalyzed hydrolysis and cyclization, gave pseudouridine (**1**) in 10% overall yield. Other D-aldonolactones were also condensed with the pyrimidine derivative, and the corresponding 5-β-D-alditol-1-yluracils were isolated. It may be noted that the cyclization conditions used by Asbun and Binkley¹⁶⁴ proved to be inefficient for the cyclization of **225** and **226**, and more-vigorous conditions were required in the latter reaction.¹⁵⁷ The reason for this difference is not apparent, as the

(161) H. S. El Khadem and Z. M. El Shafei, *Tetrahedron Lett.*, 1887-1889 (1963).

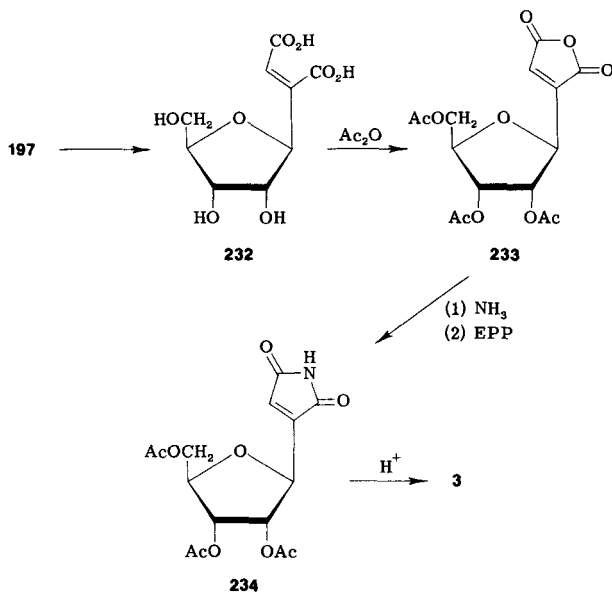
(162) W. S. Chilton and R. C. Krahn, *J. Amer. Chem. Soc.*, **84**, 4129-4132 (1967); **90**, 1318-1323 (1968); G. Lyle and M. J. Piazza, *J. Org. Chem.*, **33**, 2478-2484 (1968).

(163) R. W. Chambers, V. Kurkov, and R. Shapiro, *Biochemistry*, **2**, 1192-1203 (1963).

(164) W. A. Asbun and S. B. Binkley, *J. Org. Chem.*, **33**, 140-142 (1968).



3. Synthesis of showdomycin (3).—Showdomycin is structurally unique among the *C*-nucleosides of natural origin, in that it has an unusual heterocyclic aglycon. On the basis of chemical, and spectroscopic, structural studies, and, ultimately, by X-ray crystallographic examination, it was shown to be 2- β -D-ribofuranosylmaleimide^{10,11} (3). Synthetic approaches to showdomycin have taken into account its lability in base, attributable in part to a rapid, Michael type of intramolecular addition of the 5'-hydroxyl group to the double bond.¹⁰ The first synthesis of showdomycin was reported by Czech investigators,¹⁴⁸ who used compound 197 as the key intermediate. The remaining two carbon atoms of the maleimide ring were added by a Wittig reaction with (ethoxycarbonylmethylene)triphenylphosphine. The resulting mixture of esters was hydrolyzed, and the *cis*-acid **232** was cyclized to afford the maleic anhydride derivative (**233**). Treatment with ammonia, and cyclization of the resulting maleamic acid in the presence of ethyl polyphosphate (EPP) gave showdomycin triacetate (**234**) which, upon mild treatment with acid, gave crystalline showdomycin (3).



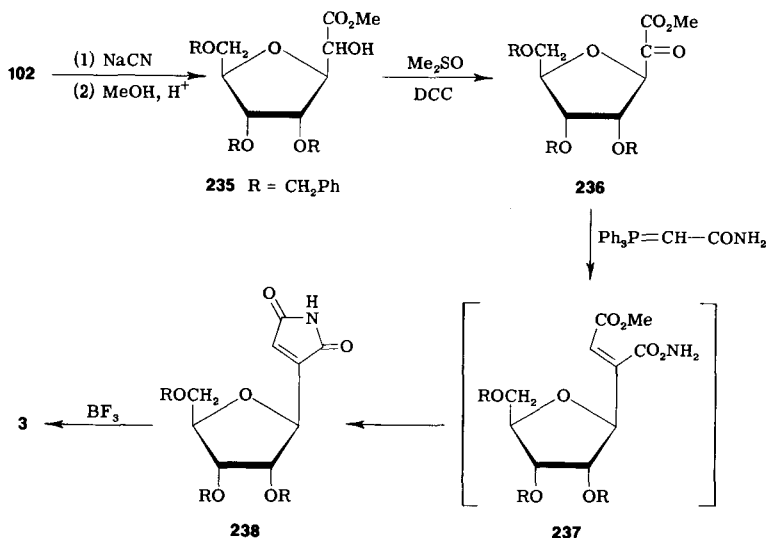
Trummlitz and Moffatt¹⁶⁵ described a much simplified synthesis of showdomycin that utilizes a keto ester intermediate similar to **197**. The 2,5-anhydro-*D*-allose derivative **102** was converted into the ester **235** by a cyanohydrin reaction, and **235** was oxidized to the keto ester **236**. The construction of the maleimide ring was achieved in a key, one-step Wittig reaction, utilizing (carbamoylmethylene)triphenylphosphine.¹⁶⁶ Presumably, a spontaneous cyclization of the intermediate, *cis*-oriented maleamic acid ester (**237**) had taken place in the formation of the showdomycin precursor (**238**). The debenzoylation step was achieved by treatment with boron trifluoride etherate or boron trichloride¹⁶⁷ to give crystalline showdomycin in high yield. Thus, in both of these two syntheses, the target compound was obtained by a judicious choice of starting compounds and protecting groups, so as to avoid treatment with base.

Showdomycin inhibits synthesis of nucleic acid. Thiols, such as cysteine and glutathione (among other compounds), reverse this inhibition, and it is considered that the interaction of the maleimide moiety with sulfhydryl groups within the cell or in the membrane may be responsible for the selective inhibition of enzymes by show-

(165) G. Trummlitz and J. G. Moffatt, *J. Org. Chem.*, **38**, 1841–1845 (1973).

(166) S. Trippett and D. M. Walker, *J. Chem. Soc.*, 3874–3876 (1959).

(167) T. G. Bonner, E. J. Bourne, and S. McNally, *J. Chem. Soc.*, 2929–2934 (1960).



domycin.¹⁶⁸ Another school¹⁶⁹ suggested that transport is the primary inhibitory target for the antibiotic. In this connection, it has been suggested¹⁷⁰ that 2-methyl-3- β -D-ribofuranosylmaleimide^{170a} should be a more effective antibiotic than showdomycin, due, in part, to more-effective binding with locally hydrophobic regions, and to a more selective reaction with target enzymes. The Roy-Burman and Visser¹⁷¹ showed that pre-incubation of showdomycin with cysteine completely removes its inhibitory effect on uridine 5'-(α -D-glucopyranosyl diphosphate) dehydrogenase.

c. Synthesis of Indochromes.—The indochromes^{12,13} (9, 239–241) are a group of water-soluble, blue compounds that are released into the culture liquid by bacteria that produce the pigment indigoidine.¹⁷² The structures of the main components, indochromes A and BII, have been determined, mainly by chemical degradation, by spectroscopic methods, and by synthesis.¹² The azaquinone portion

(168) Ref. 3, p. 393; see also, Y. Titani and Y. Tsuruta, *J. Antibiot., Ser. A*, **27**, 956–962 (1974).

(169) S. Roy-Burman, P. Roy-Burman, and D. Visser, *Biochem. Biophys. Res. Commun.*, **42**, 445–453 (1971).

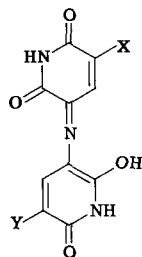
(170) T. Miyadera and E. M. Kosower, *J. Med. Chem.*, **15**, 534–537 (1972).

(170a) G. Trummlitz, D. B. Repke, and J. G. Moffatt, *J. Org. Chem.*, **40**, 3352–3356 (1975).

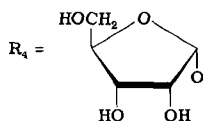
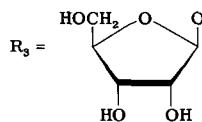
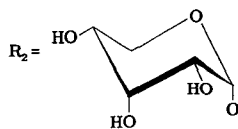
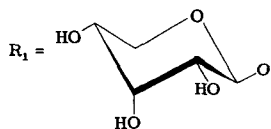
(171) S. Roy-Burman, P. Roy-Burman, and D. Visser, *Cancer Res.*, **28**, 1605–1610 (1968).

(172) H.-J. Knackmuss, G. Cosens, and M. P. Starr, *Eur. J. Biochem.*, **10**, 90–95 (1969).

is common to the indochromes, which seem to differ in the anomeric configuration and ring size of the D-ribosyl group. Thus, the linkages are β,β (pyranosyl), α,α (pyranosyl), β,β (furanosyl), and α,α (furanosyl) in indochromes A (**239**), BI (**240**), BII (**9**), and BIII (**241**), respectively. Indochrome BI appears to be a mixture of two components; indeed, the pure components of the pigment undergo equilibration in trifluoroacetic acid to regenerate the natural, isomeric mixture. The particularly deep-blue color of indochrome BII in weakly basic media has been attributed to strong, intramolecular hydrogen-bonding which gives rise to an extended, mesomerically stabilized structure.

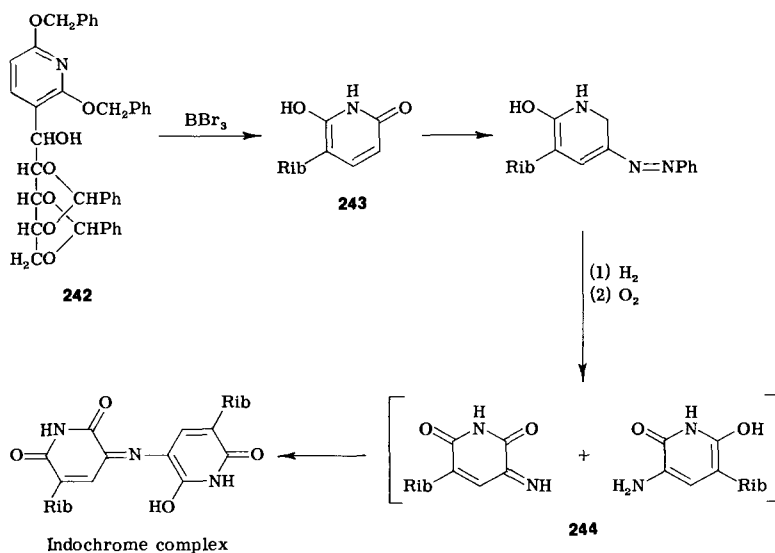


- 239** Indochrome A $X, Y = R_1$
240 Indochrome BI $X = R_1, Y = R_2,$
 and $X, Y = R_2$
9 Indochrome BII $X, Y = R_3$
241 Indochrome BIII $X, Y = R_4$



The synthesis¹² of indochrome A involved cyclization, catalyzed by boron tribromide, of the acyclic intermediate **242**, treatment of the resulting "deazauridine" **243** with benzenediazonium chloride, hydrogenation of the product, and autoxidation of the resulting 5-ben-

zeneazo intermediate. The anomeric mixture (**244**) thus formed was separated chromatographically.



2. Synthesis of C-Nucleosides in which the Glycosylic Carbon Atom is Attached to a Carbon Atom and a Nitrogen Atom

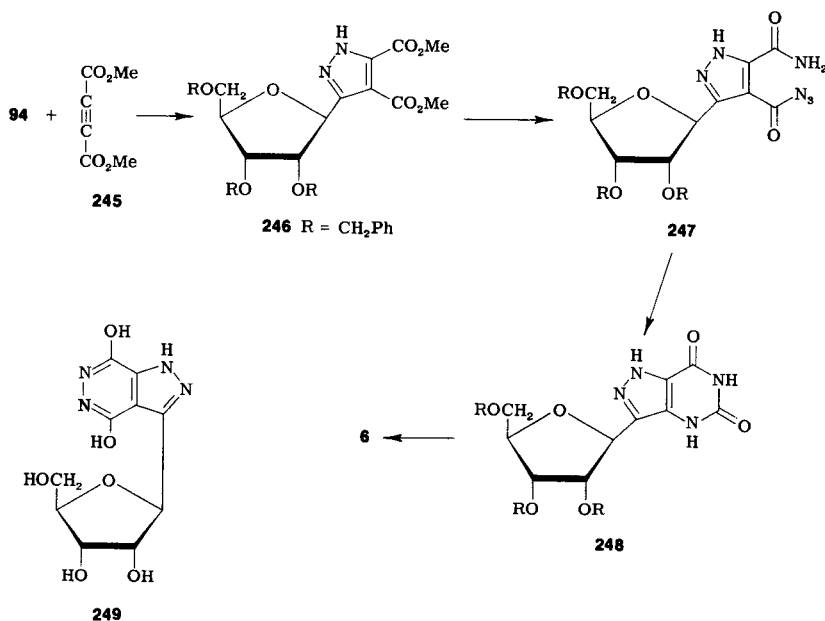
Grouped under this title are the C-nucleosides of Type B, such as the formycins (**4–6**) and the pyrazomycins (**7** and **8**). Interestingly, pyrazomycin (**7**) and formycin B (**5**) bear a strong structural resemblance, and except for the presence of a hydroxyl group instead of an amino group on the pyrazole ring, the former could be considered as a biosynthetically incomplete formycin. This may only be fortuitous, however, as the two antibiotics have distinctly different modes of action.³ Oxoformycin (**6**) is a metabolite of formycin and of formycin B. In fact, the latter compound may have also been formed in the culture liquids producing formycin, as a result of an enzymic deamination.^{7,173} The structures of the formycins^{3,5,7,174} and the pyrazomycins^{3,4,14} have been established by a combination of chemical and spectroscopic studies, and, ultimately, by synthesis. It is of interest

(173) H. Umezawa, T. Sawa, Y. Fukunaga, G. Koyama, M. Murase, M. Hamada, and T. Takeuchi, *J. Antibiot., Ser. A*, **18**, 178–181 (1965).

(174) R. K. Robins, L. B. Townsend, F. Cassidy, J. F. Geister, A. F. Lewis, and R. L. Miller, *J. Heterocycl. Chem.*, **3**, 110–114 (1966).

that, in addition to their respective biological activities, these C-nucleosides are useful biochemical tools.¹⁷⁵ Formycin, for example, is resistant toward enzymic cleavage of the glycosyl bond, presumably because of the greater stability of the C–C bond as compared to the C–N bond in nucleosides.

a. Synthesis of Oxoformycin B (6).—Oxoformycin B [3-β-D-ribofuranosylpyrazolo[4,3-*d*]-5,7(4*H*,6*H*)-pyrimidinedione (6)] was among the first natural C-nucleosides to be synthesized.⁸⁴ Cycloaddition of C-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)diazomethane (**94**) to dimethyl acetylenedicarboxylate (**245**) afforded the pyrazole derivative **246**, which was cyclized to tri-*O*-benzyl-oxoformycin B (**248**) by way of the intermediate azide **247**. Debenzylation by liquid ammo-

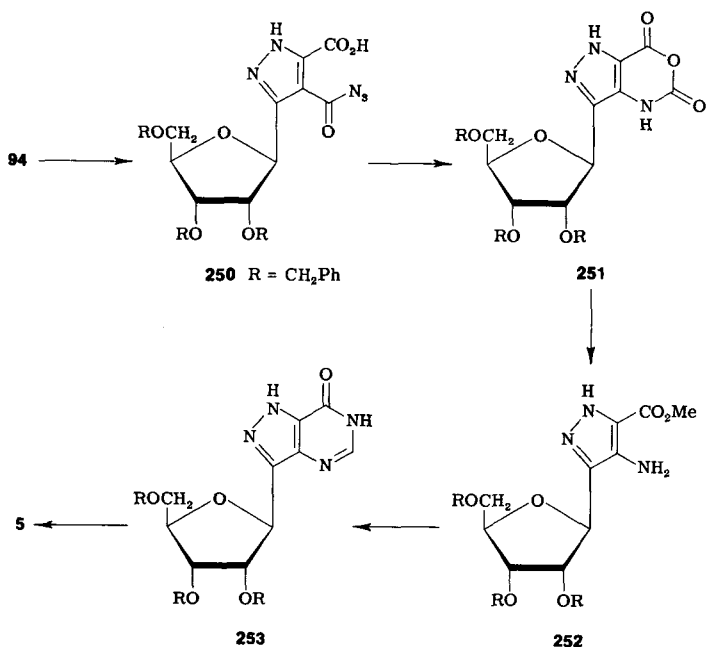


nia gave oxoformycin B (6). It will be recalled that the amino (**112**) and ureido (**92**) precursors are also available from other routes.^{92,93} The pyridazine C-nucleoside **249** was also prepared in the course of this work.

b. Synthesis of Formycin B (5).—The synthesis of formycin B [3-β-D-ribofuranosylpyrazolo[4,3-*d*]-7(6*H*)-pyrimidinone (5)] has also

(175) See Ref. 3, p. 367.

been accomplished¹⁷⁶ by the application of cycloaddition reactions¹⁷⁷ and by utilizing such pyrazole C-nucleosides as **246**. In order to avoid the formation of the oxoformycin nucleus during the Curtius rearrangement of the azide **247**, the azidocarboxylate **250** was used for the reaction. This led to the *N*-carboxylic anhydride **251**, probably by cyclization of the intermediate isocyanate. Opening of the anhydride ring and esterification gave the ester **252**, which was cyclized in the presence of formamide¹⁷⁸ to give tri-*O*-benzylformycin B (**253**); hydrogenolytic debenzylation then afforded formycin B (**5**).



By using the same sequence, these authors¹⁷⁸ also prepared 3-(2,3-*O*-isopropylidene- β -D-ribofuranosyl)pyrazolo[4,3-*d*]-7(6*H*)-pyrimidinone, which is an immediate precursor to a formycin B analog that lacks the 5'-hydroxymethyl group. The lower homolog corresponding to oxoformycin B has also been prepared; it was found to possess activity against leukemia L1210 in mice. Formycin B has

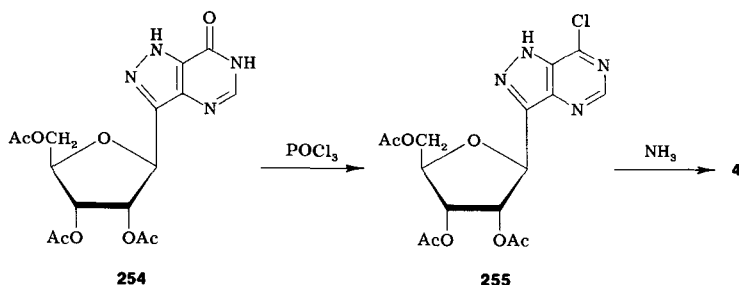
(176) E. M. Acton, K. J. Ryan, D. W. Henry, and L. Goodman, *Chem. Commun.*, 986-988 (1971).

(177) E. M. Acton, K. J. Ryan, and L. Goodman, *Chem. Commun.*, 313-314 (1970).

(178) R. K. Robins, L. B. Holum, and F. W. Furcht, *J. Org. Chem.*, **21**, 833-836 (1956).

also been obtained from formycin, by deamination in the presence of nitrous acid.⁷

c. Synthesis of Formycin (4).—A total synthesis of formycin {(7-amino-3- β -D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (4)} has not been reported as yet. However, it has been obtained from formycin B by chemical transformation.¹⁷⁹ Tri-*O*-acetylformycin B (254) was transformed into the 7-chloro derivative (255) by treatment with



phosphoryl chloride, and the latter derivative was treated with ammonia to give formycin (4). 7-Chloro- and other 7-substituted formycins have been utilized in the synthesis of various analogs (see Section IV,2).

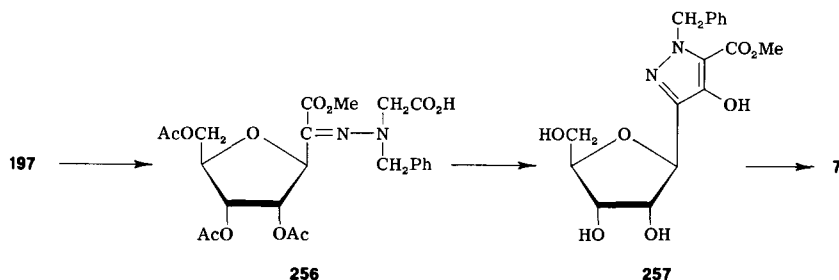
d. Synthesis of Pyrazomycin (7).—As with other naturally occurring C-nucleosides, the synthesis of pyrazomycin [4-hydroxy-3(5)- β -D-ribofuranosyl-5(3)-pyrazolecarboxamide] (7) was accomplished¹⁸⁰ by the construction of the heterocyclic moiety from an anomerically functionalized C-glycosyl intermediate; it was based on a general route to 3-substituted 4-hydroxypyrazole-5-carboxylic acids.¹⁸¹ The keto ester 197 was transformed into the hydrazone derivative (256); this was cyclized, and the product esterified to give the pyrazole derivative 257. Treatment with methanolic ammonia, followed by catalytic debenzoylation, gave⁴ crystalline pyrazomycin (7). A shorter synthesis of pyrazomycin and pyrazomycin B has also been reported.^{181a}

(179) R. A. Long, A. F. Lewis, R. K. Robins, and L. B. Townsend, *J. Chem. Soc., C*, 2443–2446 (1971).

(180) J. Farkaš, Z. Flegelová, and F. Šorm, *Tetrahedron Lett.*, 2279–2280 (1972).

(181) J. Farkaš and Z. Flegelová, *Tetrahedron Lett.*, 1591–1592 (1971).

(181a) S. De Bernardo and M. Weigle, *J. Org. Chem.*, **41**, 287–290 (1976).



IV. SYNTHESIS OF ANALOGS OF NATURALLY OCCURRING C-NUCLEOSIDES

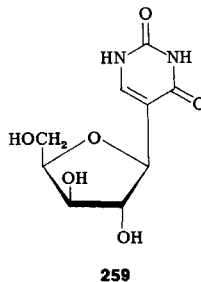
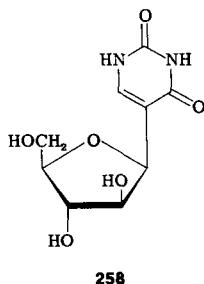
A significant number of synthetic analogs of C-nucleosides have been reported in the past decade. Of these, only the C-β-D-pentofuranosyl nucleosides are of immediate relevance to the subject matter of this article. A primary objective in the synthesis of analogs of a physiologically active substance is to achieve an increase in its biological and therapeutic efficacy. At the molecular level, this may be reflected in the potentiation or antagonism of one or more vital enzymic processes. A number of C-nucleosides are known to interfere in the biosynthesis of viral and bacterial nucleic acids by blocking certain key transformations in the metabolic pathways involving purine and pyrimidine nucleotides.^{3,4,182} The rationale for the synthesis of analogs of these naturally occurring C-nucleosides is, therefore, justified in the context of a general program aimed at developing more-specific and efficacious antibiotics and physiologically active compounds.

1. Synthetic Analogs of Pseudouridine

In the course of their studies of pseudouridine,¹⁶⁴ Asbun and S. B. Binkley¹⁸³ reported the synthesis of 5-β-D-arabinofuranosyl- and 5-β-D-xylofuranosyl-uracil (258 and 259) by the acid-catalyzed ring-closure of the corresponding alditol derivatives. The configuration at the anomeric carbon atom was determined on the basis of optical rotatory dispersion studies.

(182) P. Roy-Burman, "Analogues of Nucleic Acid Components," Springer-Verlag, New York, 1970.

(183) W. A. Asbun and S. B. Binkley, *J. Org. Chem.*, **31**, 2215-2219 (1966).



Šorm and coworkers¹⁸⁴ prepared several isomeric 6-aza-5-D-pentitol-1-yluracils (**260**) with the intention of effecting acid-catalyzed cyclizations to 5-D-pentofuranosyluracils. The *D-allo*, *D-altro*, *D-gluco*, and *D-galacto* analogs were prepared by way of the base-catalyzed cyclization of the corresponding heptulosonic acid thiosemicarbazones, followed by replacement of the sulfur atom of the thione group in the resulting 2,3,4,5-tetrahydro-6-pentitol-1-yl-3-thioxo-1,2,4-triazin-5-ones (**261**) by oxygen. Only the *D-allo* and *D-altro* configurations were amenable to acid-catalyzed cyclization. However, the cyclic product, previously assumed¹⁸⁵ to be the desired 6-azapseudouridine (**262**) was actually the 2',5'-anhydro analog (**263**).

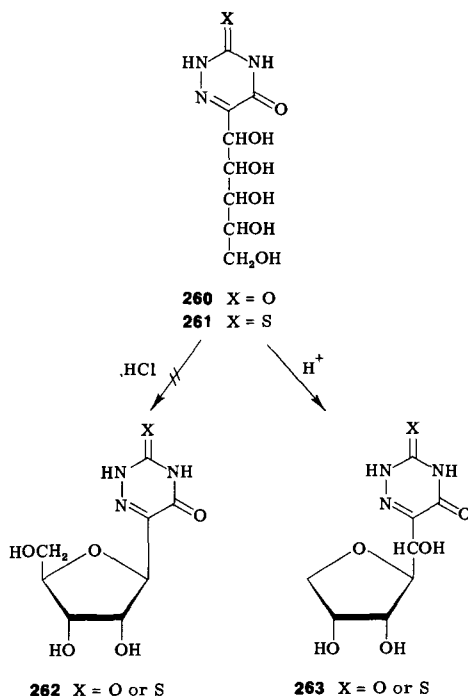
In contrast, the *D-gluco* and *D-galacto* isomers did not undergo cyclization, even under prolonged reaction-conditions. A striking influence of the configuration on the rates of cyclization of alditols in acid media has been observed by Baddiley and coworkers.¹⁸⁶ B. G. Hudson and R. Barker¹⁸⁷ made a systematic study of the mechanism of acid-catalyzed dehydration of tetritols and pentitols, and showed that the reaction leads primarily to the formation of five-membered rings, without inversion of configuration. The reaction seems to involve the intramolecular displacement of a protonated, primary hydroxyl group by a suitably disposed hydroxyl group in the chain, and it is also influenced by the dispositions of other hydroxyl groups in the molecule. It has been observed that, when the leaving group and its adjacent hydroxyl group are *gauche*-disposed, the cyclization reaction is retarded, because of hydrogen-bonding forces. The pres-

(184) M. Bobek, J. Farkaš, and F. Šorm, *Collect. Czech. Chem. Commun.*, **34**, 1673-1683 (1969).

(185) M. Bobek, J. Farkaš, and F. Šorm, *Tetrahedron Lett.*, 3115-3118 (1960).

(186) J. Baddiley, J. G. Buchanan, and B. Carss, *J. Chem. Soc.*, 4058-4063, 4138-4139 (1957).

(187) B. G. Hudson and R. Barker, *J. Org. Chem.*, **32**, 3650-3658 (1967).

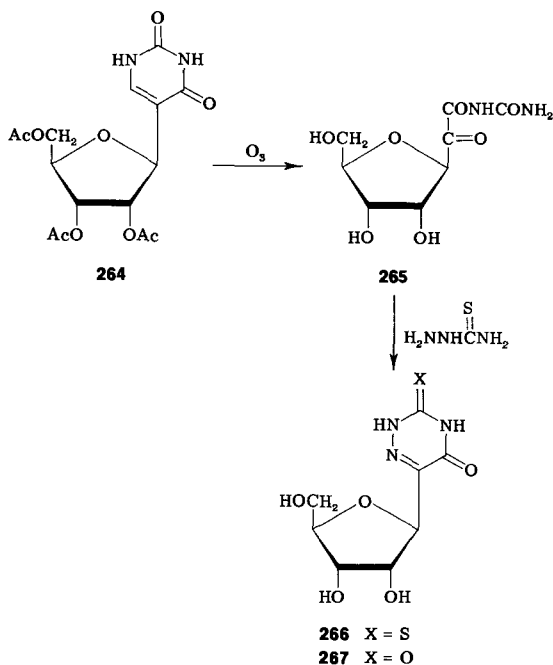


ence of a bulky substituent, such as the 6-azauracil ring in the vicinity of one of the hydroxyl groups, would introduce yet another factor in the transition state, as the intramolecular displacement by a hydroxyl group in such a molecule necessitates its favorable alignment with the carbon atom to be attacked. Based on these considerations, it can be understood why the *D-gluco* and *D-galacto* isomers (in which C-1' bears the bulky, nitrogen heterocycle residue, and interacts with suitably disposed hydroxyl groups in the chain) are relatively inert to acid-catalyzed cyclization, compared to the other isomers.

The formation of a 2',5'-anhydro ring, instead of the expected 1',4'-anhydro ring, in the case of the *D-allo* and *D-altro* isomers is in contrast to analogous, acid-catalyzed cyclizations of alditol-1-yluracils, which are known to give α - and β -pseudouridines.^{155-157,164} The fact that 1',4'-anhydro rings are not formed has been rationalized¹⁸⁴ on the basis of a lesser degree of stabilization of a carbonium ion adjacent to the 6-azauracil ring in **260** and **261**, compared to the uracil analogs **225** and **226**.

The synthesis of 6-azapseudouridine [5- β -D-ribofuranosyl]-6-aza-

uracil (**267**)] was accomplished¹⁸⁸ in an indirect way, by chemical transformation of pseudouridine. Thus, ozonolysis of tri-*O*-acetyl-pseudouridine (**264**), followed by base-catalyzed cyclization of the thiosemicarbazone derived from the ureide (**265**) gave the thioxo analog (**266**). Treatment with methyl iodide, and acid hydrolysis of the resulting methylthio derivative, gave 6-azapseudouridine (**267**).



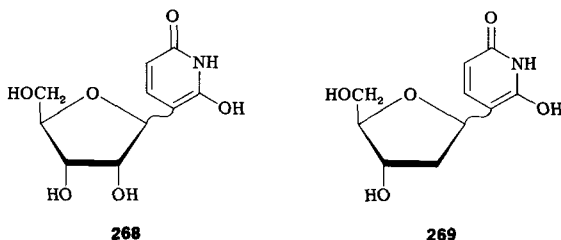
In their search for nucleoside derivatives that would inhibit thymidylate synthetase, Mertes and coworkers¹⁸⁹ reported their attempts to synthesize 5-deazapseudouridine and its 2'-deoxy analog. It was supposed, following the suggestion of B. R. Baker,¹⁹⁰ that the increased acidity of the 2,6-dihydroxypyrimidine ring in these products would lead to more effective inhibition of the enzyme. The condensation reaction between 2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl chloride (**154**) and 2,6-dibenzoyloxypyridin-3-ylcadmium led, after removal of protecting groups, to the C-nucleoside **268**, isolated as an anomeric

(188) M. Bobek, J. Farkaš, and F. Šorm, *Collect. Czech. Chem. Commun.*, **34**, 1690-1695 (1969).

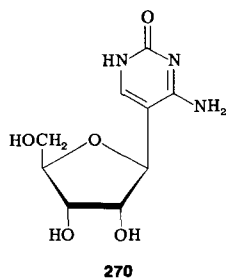
(189) M. P. Mertes, J. Zielinski, and C. Pillar, *J. Med. Chem.*, **10**, 320-325 (1967).

(190) B. R. Baker, *Cancer Chemother. Rept.*, **4**, 1 (1959).

mixture. The alkylidene derivative resulting from an attack on the 1,2-benzoxonium ion was the major product (25%). The 2'-deoxy analog (**269**) was likewise prepared, and isolated as an anomeric mixture. Unfortunately, both C-nucleosides were found to be unstable and to undergo rapid decomposition; this precluded the determination of anomeric configuration and ring size.



David and Lubineau¹⁹¹ reported the synthesis of pseudocytidine [5- β -D-ribofuranosylcytosine (**270**)] and its α anomer by a procedure analogous to that used in preparing pseudouridine.¹⁵⁵⁻¹⁵⁷ Thus, 2,4:3,5-di-O-benzylidene-*aldehydo*-D-ribose (**223**) was condensed with the dilithio derivative of 2-O,4-N-(trimethylsilyl)cytosine, and the resulting, epimeric, acyclic derivatives were subjected to acid-catalyzed cyclization. The anomeric configuration of the free C-nucleosides was ascertained by spectroscopic methods and by their transformation into α - and β -pseudouridine in the presence of nitrous acid. The anomeric 5-(β -D-ribofuranosyl)isocytosines have also been prepared by Fox and coworkers.^{191a}

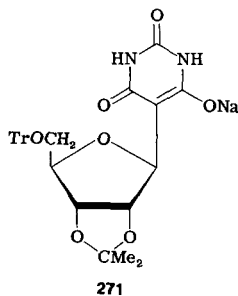


Ohrui and Fox¹²⁸ obtained the sodium salt of 5-(2,3-O-isopropylidene-5-O-trityl- β -D-ribofuranosyl)barbituric acid (**271**) by treatment of the corresponding C-malonyl derivative (**173**) with urea

(191) S. David and A. Lubineau, *Carbohydr. Res.*, **29**, 15-24 (1973).

(191a) C. K. Chu, K. A. Watanabe, and J. J. Fox, *J. Heterocycl. Chem.*, **12**, 817-818 (1975).

and sodium ethoxide. The conversion of the product into the free C-nucleoside has not been described by the authors. Compound **271** is an interesting example of the potential utility of C-glycosylmalonates in the synthesis of C-nucleoside analogs.¹⁰²



2. Synthetic Analogs of Formycin, Formycin B, and Oxoformycin B

Several derivatives of formycin in which the 7-amino group is replaced by other substituents, including halogens, have been prepared. Extensive studies on nucleoside interconversions in the purine¹⁹² series have shown that the 6-halogeno- and 6-(alkylthio)-purine nucleosides are versatile intermediates for nucleophilic-displacement reactions at C-6. The structurally equivalent, 7-substituted formycins behave in the same way. Thus, H. Umezawa and coworkers¹⁹³ prepared "7-(methylthio)formycin" [7-(methylthio)-3-β-D-ribofuranosylpyrazole[4,3-*d*]pyrimidine (**272**)] by the thiation of formycin B, followed by 5-methylation. Halogens and various substituted amino derivatives, exemplified by **273(a-c)** were prepared from **272** by displacement with appropriate nucleophiles. R. K. Robins, Townsend, and coworkers¹⁷⁹ recommended the use of the 7-chloro derivative **273a** for the preparation of 7-substituted formycins, because of readier displacements [as compared to the 7-(methylthio) analog].

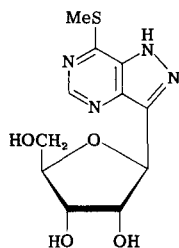
In connection with the cytokinin activity of *N*⁶-isopen-tenyladenosine,^{194,195} Leonard and coworkers¹⁹⁶ prepared the *N*⁷-

(192) J. A. Montgomery and H. J. Thomas, *Advan. Carbohydr. Chem.*, **17**, 301-308 (1962).

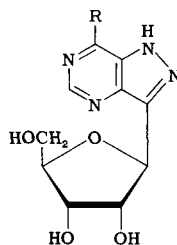
(193) S. Watanabe, G. Matsushashi, S. Fukatsu, M. Murase, G. Koyama, K. Maeda, and H. Umezawa, *J. Antibiot., Ser. A*, **19**, 93-96 (1966).

(194) F. Skoog, H. Q. Hamzi, A. M. Szwedkowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson, and R. N. Loepky, *Phytochemistry*, **6**, 1169-1192 (1967).

(195) M. J. Robins and E. M. Trip, *Biochemistry*, **12**, 2179-2187 (1973), and references cited therein.



272



273a R = Cl, Br, or I

273b R = NHMe, NMe₂, or NHOH273c R = NHNH₂, 273d R = NHCH₂CH=CMe₂

isopentenyl derivative (273d) of formycin by *N'*-alkylation of formycin with 3-methyl-2-butenyl bromide, followed by rearrangement to the *N*⁷-isomer. The same product was obtained,¹⁹⁵ in much improved yield and with higher melting point, by displacement of the 7-chlorine atom in 273a with 3-methyl-2-butenylamine. The analogous *N*⁷-benzylformycin was similarly prepared.

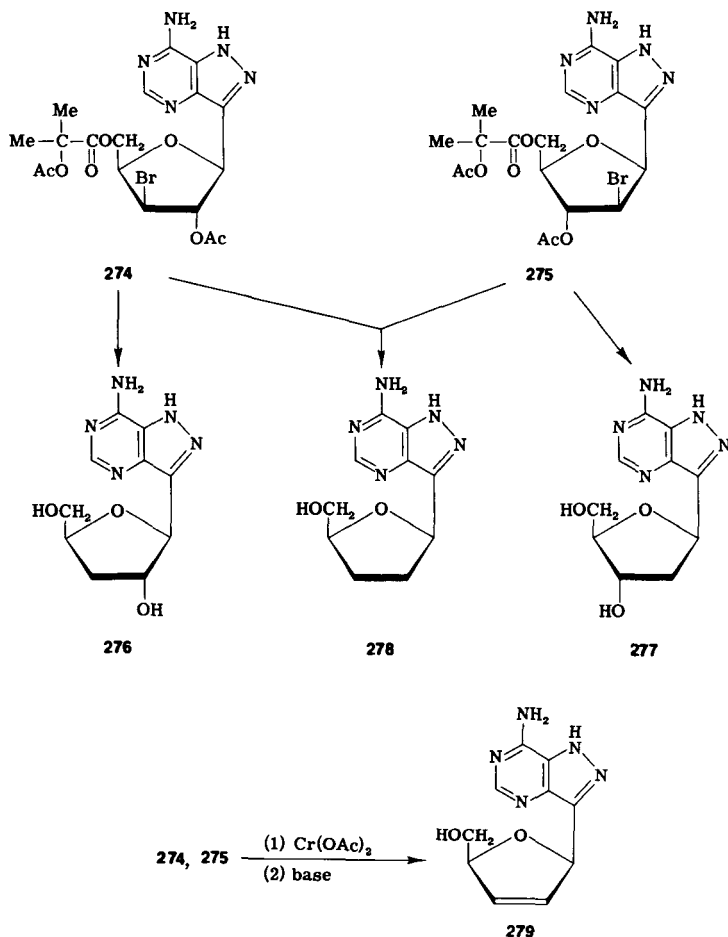
Moffatt and coworkers^{197,198} extended a halogenation reaction, described by Mattocks¹⁹⁹ for simple, *cis*-vicinal diols, to several nucleosides (such as adenosine, cytidine, formycin, and uridine). The reaction consists in the treatment of the diols with 2-acetoxyisobutyl halides, and proceeds by way of acetoxonium ions. A halogen atom is thus introduced into the nucleoside molecule, with retention or inversion of configuration depending on the nature of the nitrogenous heterocycle. Treatment of formycin with 2-acetoxyisobutyl bromide gave a 3:1 mixture of the bromides 274 and 275, which were ultimately transformed into the deoxyformycins (276 and 277). It is of interest that catalytic hydrogenolysis of the mixture of bromides led, after de-esterification, to the dideoxy analog 278. This unusual reduction occurs only when 2'- or 3'-*trans*-acetoxy groups are present, and has been explained^{197,198} on the basis of a palladium-catalyzed *trans*-elimination of the acetate group, giving a 2',3'-alkene that is reduced in the medium. In the presence of chromous acetate and ethylenediamine, the *trans*-acetoxy bromides (274 and

(196) S. M. Hecht, R. M. Bock, R. Y. Schmitz, F. Skoog, N. J. Leonard, and J. L. Occolowitz, *Biochemistry*, **10**, 4224-4228 (1971).

(197) S. Greenberg and J. G. Moffatt, *J. Amer. Chem. Soc.*, **95**, 4025-4040 (1973); A. F. Russell, M. Prystaš, E. K. Hamamova, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, **39**, 2182-2186 (1974).

(198) T. C. Jain, A. F. Russell, and J. G. Moffatt, *J. Org. Chem.*, **38**, 3179-3186 (1973).

275) were smoothly converted into the 2',3'-unsaturated analog (**279**), in agreement with the same type of well-known eliminations of halo esters^{200,201} and alkyl halides.²⁰²



The finding that the lower homolog of oxoformycin B, namely, 3-(2,3-O-isopropylidene-β-D-erythrofuransyl)pyrazolo[4,3-d]-

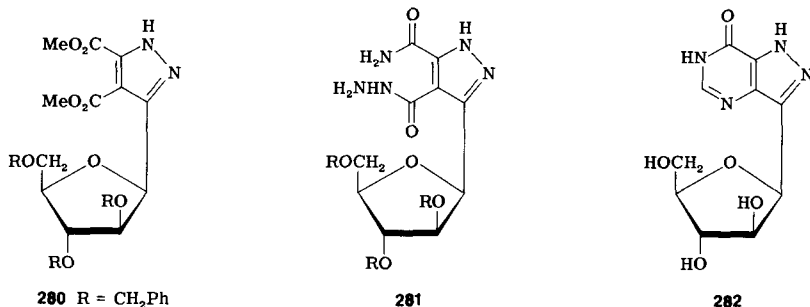
(199) A. R. Mattocks, *J. Chem. Soc.*, 1918–1930, 4840–4845 (1964).

(200) D. M. Singleton and J. K. Kochi, *J. Amer. Chem. Soc.*, **89**, 6547–6555 (1967); J. K. Kochi and D. M. Singleton, *ibid.*, **90**, 1582–1589 (1968); J. K. Kochi, D. M. Singleton, and L. J. Andrews, *Tetrahedron*, **24**, 3503–3515 (1968).

(201) For a review, see L. Kaplan, "Bridged Free Radicals," Dekker, New York, 1972.

(202) J. K. Kochi and J. W. Powers, *J. Amer. Chem. Soc.*, **92**, 137–146 (1970).

5,7(4*H*,6*H*)-pyrimidinedione, exhibits antileukemic activity in mice,^{96,176} and the knowledge of the therapeutic importance of β -D-arabinofuranosyl nucleosides,⁹⁴⁻⁹⁶ prompted the synthesis of the D-*arabino* isomer corresponding⁹⁸ to oxoformycin B. The 1-diazo sugar **112** was converted into the pyrazole diester **280** by a 1,3-dipolar addition reaction with dimethyl acetylenedicarboxylate. A Curtius reaction of the corresponding hydrazide (**281**), followed by rearrangement, ring-closure, and debenzylation, as in the synthesis of oxoformycin B itself,⁸⁴ gave the desired 3- β -D-arabinofuranosyl-pyrazolo[4,3-*d*]-5,7(4*H*,6*H*)-pyrimidinedione (**282**). The α anomer



was also formed in this sequence, and it could be separated earlier in the synthesis. The anomeric configurations of the free C-nucleosides were assigned on the basis of spectroscopic and circular dichroism studies. Unfortunately, when injected intraperitoneally, the product (**282**) was inactive against lymphoid leukemia L1210 implanted in mice.

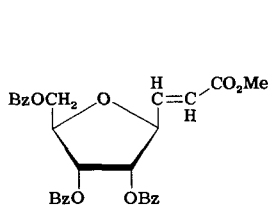
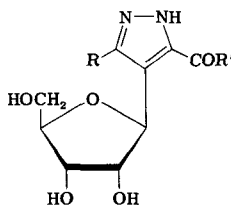
3. Synthetic Analogs of Pyrazomycin

Several C- β -D-pentofuranosylpyrazoles are known, but most of these derivatives have been prepared and utilized as intermediates in the synthesis of 3-glycosylpyrazolopyrimidines, such as the formycins and their analogs. Among these are the synthetic intermediates **246**, **250**, **257**, and **280**, which, for the most part, were obtained by 1,3-dipolar addition reactions.

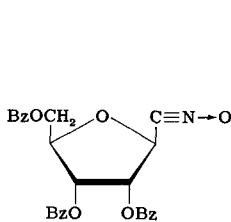
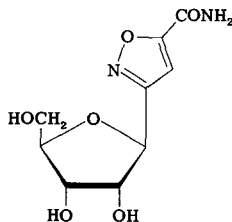
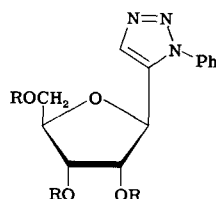
Moffatt and coworkers²⁰³ reported the synthesis of several 4- β -D-ribofuranosylpyrazoles, such as **284(a-c)**, by 1,3-dipolar cycloaddition of diazoalkanes to the alkenic C-glycosyl compound **283**, followed by dehydrogenation of the resulting pyrazolines. In view of the known biological activities of several nucleosides containing the

(203) H. P. Albrecht, D. Repke, and J. G. Moffatt, *J. Org. Chem.*, **39**, 2177-2184 (1974).

carboxamide group in the heterocyclic portion,²⁰⁴ the 4- β -D-ribofuranosylpyrazoles were converted into the corresponding mono- and di-carboxamide derivatives.

**283****284a** R = H, R' = OMe, OH, or NH₂**284b** R = CONH₂, R' = NH₂**284c** R = CO₂-*t*-Bu, R' = NH₂

Dipolar addition of ethyl propiolate to the nitrile oxide **285**, prepared by chlorination of the corresponding oxime, gave, after removal of protecting groups, the C-glycosyl-isoxazole²⁰⁵ (**286**). These reactions further demonstrate the utility of anomerically functionalized C- β -D-ribofuranosyl derivatives that can be prepared from the versatile aldehyde **100**.

**285****286****287**

Buchanan and coworkers⁷¹ prepared the β -D-ribofuranosyltriazole **287** by dipolar addition of benzyl azide to the β -D-ribofuranosylethyne **70**. Related analogs have been reported by others.^{113a,205a,205b} The anomeric 3-phenyl-4-D-ribofuranosylpyrazoles have been prepared by a dipolar, addition reaction.^{205c}

(204) See, for example, J. T. Witkowski, R. K. Robins, R. W. Sidwell, and L. N. Simon, *J. Med. Chem.*, **15**, 1150-1154 (1972), and references cited therein.

(205) H. P. Albrecht, D. B. Repke, and J. G. Moffatt, *Abstr. Papers Amer. Chem. Soc. Meeting*, **167**, CARB 15 (1974).

(205a) G. Just and M. Ramjeesingh, *Tetrahedron Lett.*, 985-988 (1975).

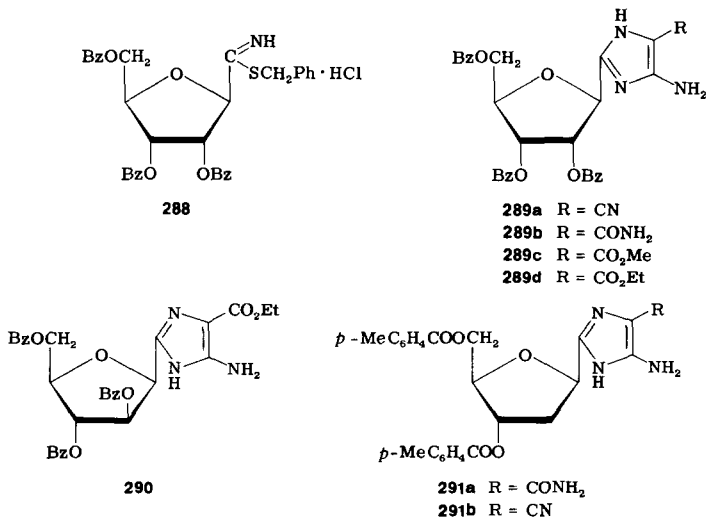
(205b) F. García González, J. Fernandez Bolaños, and J. Galbis Perez, *An. Quím.*, **70**, 1082-1087 (1974).

(205c) K. Arakawa, T. Miyasaka, and N. Namamishi, *Chem. Lett.*, 1305 (1974).

4. Synthesis of Other Analogs of C-Nucleosides

Grouped in this Section are the C-D-pentofuranosyl-imidazoles, -pyrazolopyrimidines, and -adenines. The last two analogs are positional isomers of formycin in which the heterocyclic moiety is attached to the sugar at an "unnatural" position. A rationale¹⁰³ for the synthesis of this type of analog is of interest; it was based on the possibility of a close structural similarity between a natural adenine nucleoside and synthetic analog with respect to available hydrogen-bonding sites.

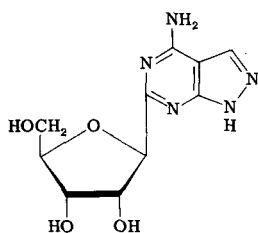
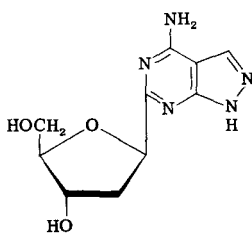
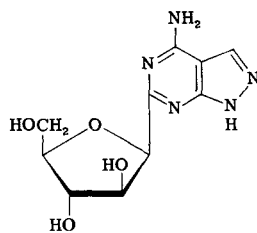
a. 2- β -D-Pentofuranosylimidazoles.—During the course of synthetic studies on C-nucleosides containing the adenine moiety, Igolen and coworkers^{99,206} prepared a series of O-benzoylated 2- β -D-ribofuranosylimidazoles bearing various substituents at C-5. Thus, a modified Pinner reaction²⁰⁷ on the nitrile **82** gave the corresponding thioformimidate (**288**), which was treated with 2-amino-2-cyanoacetic acid derivatives to give the corresponding 5-amino-2- β -D-ribofuranosylimidazoles (**289a-d**). The D-arabinofuranosyl analog (**290**) and the 2-deoxy- β -D-*erythro*-pentofuranosyl analog⁹⁹ (**291**) were similarly prepared, but they were not converted into the free C-nucleosides. However, 5-amino-2-(5-O-benzoyl- β -D-ribofuranosyl)-4-cyanoimidazole has been prepared by condensation of the 5-O-benzoyl monoester analog of **288** with 2-aminomalononitrile.²⁰⁸



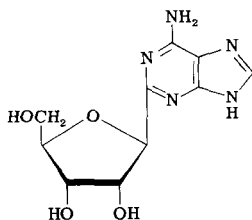
(206) J. Igolen, T. Huynh Dinh, A. Kolb, and C. Perreux, *Bull. Chim. Therap.*, 207-210 (1972); J. Igolen and T. Huynh Dinh, *Chem. Commun.*, 1267 (1971).

(207) M. Julia and T. Huynh Dinh, *Bull. Soc. Chim. Fr.*, 1303-1308 (1971).

b. **4-Amino-5-pentofuranosylpyrazolo[3,4-*d*]pyrimidines.**—A series of 4-amino-5-pentofuranosylpyrazolo[3,4-*d*]pyrimidines was prepared by Igolen and coworkers²⁰⁹ as potential antitumor agents, based on the finding that 4-aminopyrazolo[3,4-*d*]pyrimidine is active²¹⁰ against Adenocarcinoma 755. Condensation of 5-amino-4-cyanopyrazole with appropriate 1-thioformimide derivatives afforded anomeric mixtures of the corresponding *C*-nucleosides, which were separated by chromatography. The β -D anomers so obtained are represented by structures **292–294**. The 4-oxo analogs were also reported.²⁰⁹

**292****293****294**

c. **2- β -D-Pentofuranosyladenines.**—A series of 2- β -D-pentofuranosyladenines, exemplified by the *D-ribo* isomer (**295**), has been prepared by Igolen and coworkers^{99,208,211} by condensation of 5-amino-4-cyanoimidazole with appropriately substituted glycosyl thioformimidates. The β -D-arabinofuranosyl and 2-deoxy- β -D-*erythro*-pentofuranosyl analogs, corresponding to **295**, were also pre-

**295**

(208) T. Huynh Dinh, A. Kolb, C. Gouyette, and J. Igolen, *J. Heterocycl. Chem.*, **12**, 111–117 (1975).

(209) T. Huynh Dinh, A. Kolb, G. Barnathan, and J. Igolen, *Chem. Commun.*, 680–681 (1973); T. Huynh Dinh, A. Kolb, C. Gouyette, and J. Igolen, *J. Org. Chem.*, **40**, 2825–2830 (1975).

(210) R. K. Robins, *J. Med. Chem.*, **7**, 186–199 (1964).

pared. The anomeric configuration was determined by nuclear magnetic resonance spectroscopy and optical rotation data. Circular dichroism data enabled these authors²⁰⁸ to conclude that significantly different Cotton effects may be observed in the spectra of C- β -D-ribofuranosyladenines, depending on the position of attachment of the sugar moiety.²⁰⁸

d. 8- β -D-Pentofuranosyladenines.—The 8- β -D-ribofuranosyladenines are structurally (and, possibly, conformationally) quite close to such pyrazolopyrimidine C-nucleosides as formycin. In one of the two conformations possible (*syn* and *anti*), the hydrogen-bonding sites in the imidazolo-pyrimidine portion could occupy analogous sites in 9- β -D-pentofuranosyladenines, as suggested by El Khadem and El Ashry¹⁰³ for the C-nucleoside analog of cordycepin.²¹² It may be added that, in this conformation, or the alternative one, 8- β -D-pentofuranosyladenines can be stereochemically compared to formycin; hence, their relationship to cytidine and uridine with regard to the superposition of hydrogen-bonding sites. Such a comparison was made²¹³ between formycin 3'-phosphate, cytidine 3'-phosphate, and uridine 3'-phosphate; and it requires that the last two nucleotides have an *anti* conformation, and the first, a *syn* conformation. The effectiveness of such a relationship between natural and synthetic C-nucleosides, and its biological significance, may depend on at least the partial superposition of the sugar rings, particularly in the region of the 3'-phosphate groups.

Two methods are available for the synthesis of 8-C-glycosyladenines. A particularly mild method consists in the treatment of 5-amino-4-cyano-2-C-glycosylimidazoles, such as **289a**, with formamidine acetate.²¹⁴ In this way, Igolen and coworkers^{208,209,211} prepared the C-nucleosides **296** and **299**. An alternative synthetic approach to this class of compound involves the treatment of a 2,5-anhydrohexonic acid (such as **15** or **129**) with 4,5,6-triaminopyrimidine, followed by cyclization to the nitrogen he-

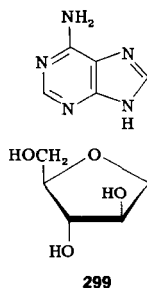
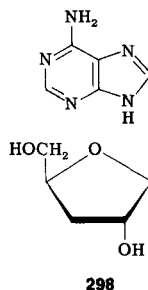
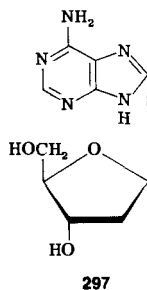
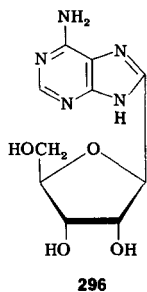
(211) A. Kolb, T. Huynh Dinh, and J. Igolen, *J. Carbohydr. Nucleos. Nucleot.*, **2**, 37-45 (1975).

(212) H. R. Bentley, K. G. Cunningham, and F. G. Spring, *J. Chem. Soc.*, 2301-2305 (1951); E. A. Kaczka, N. R. Trenner, B. Arison, and R. W. Folkers, *Biochem. Biophys. Res. Commun.*, **14**, 456-457 (1964).

(213) E. C. Taylor and R. W. Henders, *J. Amer. Chem. Soc.*, **87**, 1995-2003 (1965); E. C. Ferris and L. E. Orgel, *ibid.*, **87**, 4976-4977 (1965).

(214) D. C. Ward, W. Fuller, and E. Reich, *Proc. Nat. Acad. Sci. U. S.*, **62**, 581-588 (1969).

terocycle by a fusion reaction. El Khadem and coworkers^{31,103} thus prepared the cordycepin analog **298** and 8- β -D-arabinofuranosyladenine (**299**). The same approach was originally described by Bobek and Farkaš⁴⁰ in the D-*ribo* series. Comparison of the physical constants of this product with that obtained under milder conditions by Igolen and coworkers²⁰⁸ indicated, however, that the product described by the Czech workers⁴⁰ may actually be the α anomer. It was suggested²⁰⁸ that the high temperature ($\sim 220^\circ$) needed for cyclization of 4,6-diamino-5-(2,5-anhydro-D-*ribo*-hexonoyl)pyrimidine to the desired 8- β -D-ribofuranosyladenine may also cause anomerization.



The 2'-deoxy analog **297** has been prepared²¹⁵ according to the procedures developed in the D-*ribo* series.

(215) A. Kolb, C. Gouyette, T. Huynh Dinh, and J. Igolen, *Tetrahedron*, **31**, 2914-2920 (1975).

REACTIONS OF D-GLUCOFURANURONO-6,3-LACTONE*

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Technical University, A-8010 Graz, Austria*

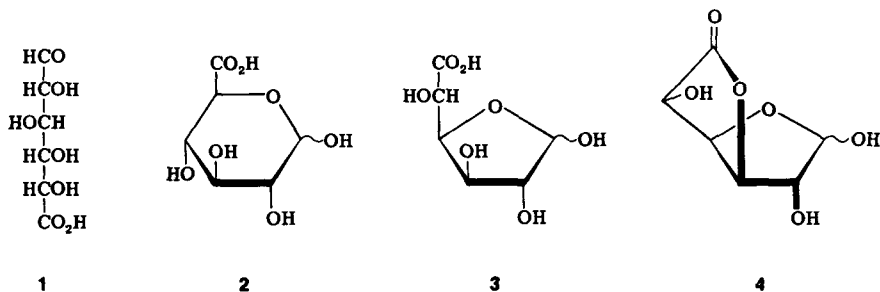
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* This article is dedicated to the former chairman of this Institute, Dr. A. v. Wacek, in honor of his 80th birthday.

† We express our gratitude for the financial support of this work to the "Fonds zur Förderung der wissenschaftlichen Forschung" in Vienna.

I. INTRODUCTION

D-Glucuronic acid principally forms three constitutional isomers—*aldehydo*-D-glucuronic acid (1), D-glucopyranuronic acid (2), and D-glucofuranuronic acid (3), all of which can cyclize further with formation of lactones of different stabilities. Thus far, D-glucofuranurono-6,3-lactone¹ (4), compound 2 (Ref. 2), derivatives of 3 (Ref. 3), as well as derivatives of D-glucopyranurono-6,3- (Ref. 4) and -6,1-lactones⁵ have been obtained.



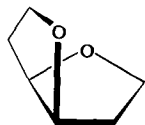
For the synthesis of D-glucuronic acid, methods of oxidation of suitable D-glucose derivatives have been devised during the past two decades; these procedures have been comprehensively reviewed by Marsh,⁶ Mehlretter,⁷ and Heyns and Paulsen.⁸ For special purposes, for example, for the preparation of 6-¹⁴C-labelled D-glucuronic acid, chain-extension reactions of 1,2-O-isopropylidene- α -D-xylo-pentodialdo-1,4-furanose by the cyanohydrin synthesis⁹ or by ethynylation¹⁰ are used, but these frequently yield mixtures of D-glucuronic acid and L-iduronic acid.

As the chemistry of D-glucuronic acid and its glycosides has already been reviewed,⁶ the present article is restricted to discussion

- (1) O. Schmiedeberg and H. Meyer, *Z. Physiol. Chem.*, **3**, 422 (1879).
- (2) F. Ehrlich and K. Rehorst, *Ber.*, **58**, 1989–1992 (1925).
- (3) L. Zervas and P. Sessler, *Ber.*, **66**, 1326–1329 (1933).
- (4) H. Wagner, G. Aurnhammer, H. Damninger, O. Seligmann, L. Pallos, and L. Farkas, *Chem. Ber.*, **105**, 257–261 (1972).
- (5) E. M. Fry, *J. Am. Chem. Soc.*, **77**, 3915–3916 (1955).
- (6) C. A. Marsh, in "Glucuronic Acid," G. J. Dutton, ed., Academic Press, New York and London, 1966, Chapter 1. See also, E. Anderson and L. Sands, *Adv. Carbohydr. Chem.*, **1**, 329–344 (1945); R. S. Teague, *ibid.*, **9**, 185–246 (1954).
- (7) C. L. Mehlretter, *Adv. Carbohydr. Chem.*, **8**, 231–249 (1953).
- (8) K. Heyns and H. Paulsen, *Adv. Carbohydr. Chem.*, **17**, 169–221 (1962).
- (9) J. C. Sowden, *J. Am. Chem. Soc.*, **74**, 4377–4379 (1952).
- (10) D. Horton and F. O. Swanson, *Carbohydr. Res.*, **14**, 159–171 (1970).

of the reactions of D-glucofuranurono-6,3-lactone, as they have been the subject of extensive investigations.

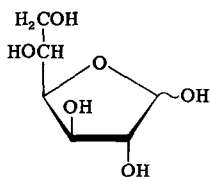
Fundamentally, D-glucofuranurono-6,3-lactone (4) contains the 2,6-dioxabicyclo[3.3.0]octane structure (5), just as do ido-, manno-, and



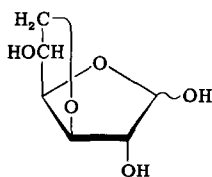
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gulo-furanurono-6,3-lactones. All of these compounds have the *threo* configuration at C-3-C-4, a condition necessary to formation of this bicyclic system. The same applies to 3,6-anhydrohexofuranoses, 3,6-anhydrohexono-1,4-lactones, hexaro-1,4:6,3-dilactones, hexodial-dodifuranoses, and 1,4:3,6-dianhydrohexitols.

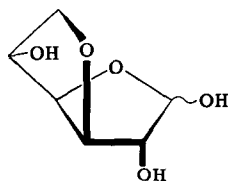
For the depiction of structural formulas of hexofuranoses, a combination of a three-dimensional, Haworth-perspective tetrahydrofuran ring with a Fischer projection of the C-5-C-6 side-chain is commonly used, as exemplified by formulas 3 and 6. With the formal closure of the second ring and formation of a 2,6-dioxabicyclo[3.3.0]octane system, however, the depiction of the C-6-C-3 ring, as in formula 7, also assumes three-dimensional geometry, and this does not correspond to the Fischer projection rule.¹¹ Consequently, structural representations of such bicyclic molecules should be as close as possible to the actual steric situation, as shown by structures 4 and 8.



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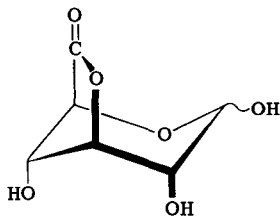


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Some differences between D-glucofuranurono-6,3-lactone (4) and D-glucose as regards their respective thermodynamic stabilities and their chemistry may be generalized as follows.

1. In contrast to the D-glucopyranose-D-glucofuranose equilibrium, in which the former preponderates, compound 4, because of its more favorable conformation, is thermodynamically more stable than D-glucopyranurono-6,3-lactone (9).

(11) *Chem. Eng. News*, **26**, 1623-1628 (1948); see p. 1628.



9

2. On account of the more "favorable" anomeric effect,¹² as well as of the larger nonbonding interactions of vicinal, synclinal substituents in furanoses as compared to pyranoses, β -D-glucofuranose,¹³ β -D-glucofuranurono-6,3-lactone, and their derivatives are of higher thermodynamic stability than their respective α -D anomers.

3. This free-energy difference between the anomers, which can be estimated by the ratios observed in glycosidation and anomerization equilibria, is larger in derivatives of **4** than in those of D-glucofuranose. This result can be attributed to the lessened conformational flexibility of bicyclic structures.

4. In addition to the aldehyde group, the lactone ring in **4** can also be subject to nucleophilic reactions in the course of which strong bases are likely to cause eliminations.

II. D-GLUCOFURANURONO-6,3-LACTONES PROTECTED AT C-1

1. D-Glucofuranosylurono-6,3-lactone Halides

Glycosyl halides, a very important group of carbohydrate derivatives, are commonly prepared¹⁴ from per-O-acylated sugars by reaction with hydrogen halides or halides of aluminum or titanium. The selection of the method depends mainly on the anomeric configuration of the substrate, the kind of its O-acyl groups, and the stability of the product to be prepared.

As generally expressed in Section I, derivatives of α -D-glucopyranose are thermodynamically more stable than the β -D anomers, whereas, for the D-glucofuranoses, the opposite stability of the anomers is observed. This regularity also applies to the respective glycosyl bromides and chlorides.

2,5-Di-O-acetyl- α -D-glucofuranosylurono-6,3-lactone bromide (**10**) was obtained in low yield by Neuberg and Neimann¹⁵ by interaction

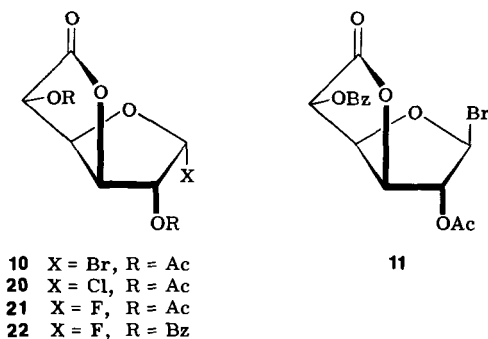
(12) P. L. Durette and D. Horton, *Adv. Carbohydr. Chem. Biochem.*, **26**, 112 (1971).

(13) J. W. Green, *Adv. Carbohydr. Chem.*, **21**, 95-142 (1966).

(14) W. Korytnyk and J. A. Mills, *J. Chem. Soc.*, 636-649 (1959).

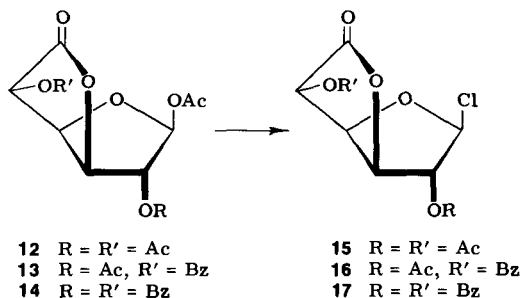
(15) C. Neuberg and W. Neimann, *Z. Physiol. Chem.*, **44**, 114 (1905).

of acetyl bromide with **4**. Compound **10** is obviously subject to rapid solvolysis, as its synthesis could not be reproduced¹⁶ in subsequent



investigations. However, Momose and coworkers¹⁷ obtained 2-*O*-acetyl-5-*O*-benzoyl- β -D-glucofuranosylurono-6,3-lactone bromide (**11**) on treatment of 1,2-di-*O*-acetyl-5-*O*-benzoyl- β -D-glucofuranurono-6,3-lactone (**13**) with hydrogen bromide in glacial acetic acid. Its β -D configuration was unequivocally established by infrared (i.r.) and nuclear magnetic resonance (n.m.r.) spectroscopy.

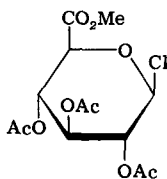
In contrast to hydrogen chloride, aluminum chloride almost exclusively reacts with sugars containing a 1-*O*-acetyl group and a 1,2-*trans* configuration of substituents, independent of the ring size¹⁴; in pyranoses, there is an additional limitation, as the conformation needs to be 1,2-*trans*-diequatorial. On treatment with aluminum chloride, 1,2,5-tri-*O*-acetyl- (**12**), 1,2-di-*O*-acetyl-5-*O*-benzoyl- (**13**), and 1-*O*-acetyl-2,5-di-*O*-benzoyl- β -D-glucofuranurono-6,3-lactone (**14**) yield¹⁷ 2,5-di-*O*-acetyl- (**15**) (Ref. 16), 2-*O*-acetyl-5-*O*-benzoyl- (**16**), and 2,5-di-*O*-benzoyl- β -D-glucofuranosylurono-6,3-lactone chloride (**17**), respectively, together with only minor propor-



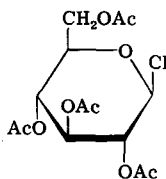
(16) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **101**, 173-177 (1933).

(17) A. Momose, K. Kamei, and Y. Nitta, *Chem. Pharm. Bull.*, **14**, 199-206 (1966).

tions of the α -D anomers. These compounds, like methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate chloride (18) and unlike 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl chloride (19), are quite unreactive¹⁷ to anomerization. 6-O-Acyl groups, absent from structures 15, 16, and 17, are requisite for participation in such configurational inversions.¹⁴



18



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In 1968, Goodman and coworkers¹⁸ succeeded in preparing the thermodynamically less stable 2,5-di-O-acetyl- α -D-glucofuranosylurono-6,3-lactone chloride (20) from 1,2,5 tri-O-acetyl- β -D-glucofuranurono-6,3-lactone (12) by a kinetically controlled reaction with titanium tetrachloride in chloroform. This agrees with the result obtained¹⁴ with methyl 1,2,3,4-tetra-O-acetyl- β -D-glucopyranuronate, whose reaction with aluminum chloride yields methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate chloride; titanium tetrachloride, however, leads to the α -anomeric chloride, exclusively.¹⁴

Finally, Hall and Steiner¹⁹ synthesized 2,5-di-O-acetyl- (21) and 2,5-di-O-benzoyl- β -D-glucofuranosylurono-6,3-lactone fluoride (22).

In summary, D-glucofuranosylurono-6,3-lactone halides exhibit the following properties. 1. Thermodynamic and solvolytic stability decreases in the series: fluoride, chloride, bromide. 2. 2,5-Di-O-acetyl-D-glucofuranosylurono-6,3-lactone halides are less stable than their 2,5-di-O-benzoyl or their 2-O-acetyl-5-O-benzoyl counterparts. 3. β -D-Glucofuranosylurono-6,3-lactone chlorides are subject to alcoholysis, with formation of alkyl β -D-glucofuranosidurono-6,3-lactones; under the same conditions the α -D anomers are nonreactive. 4. Like methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate chloride, 2,5-di-O-acyl- β -D-glucofuranosylurono-6,3-lactone chlorides are quite resistant¹⁴ to anomerization.

(18) I. Goodman, L. Salce, and G. H. Hitchings, *J. Med. Chem.*, **11**, 516-521 (1968).

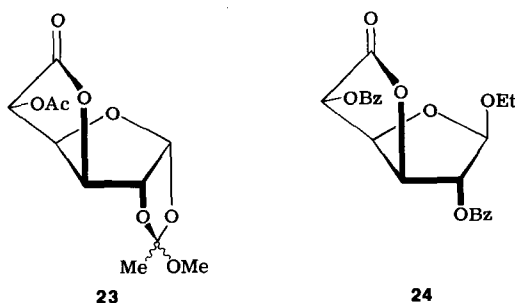
(19) L. D. Hall and P. R. Steiner, *Can. J. Chem.*, **48**, 2439-2443 (1970).

2. Alkyl and Aryl D-Glucofuranosidurono-6,3-lactones

The reaction of per-*O*-acylated D-glucosyl bromides and chlorides with alcohols by heterogeneous, or homogeneous, base catalysis is commonly used for the preparation of D-glucosides. By an S_N2 type of mechanism under Koenigs-Knorr conditions, α -D-glucopyranosyl halides form β -D-glucopyranosides, which are also obtained from the "unstable" β -D-glucopyranosyl chlorides through a neighboring-group participation.

Because of the particular structural features of compound 4, pointed out in Section I, the D-glucofuranosyluronic halide anomers not only have inverse thermodynamic stabilities with respect to those of D-glucopyranosyl halides but also show a different behavior towards alcohols. For instance, 2,5-di-*O*-acyl- α -D-glucofuranosylurono-6,3-lactone halides, which are difficult to prepare, do not react with alcohols, inasmuch as an *endo* approach of the reagent is inhibited.¹⁴ The β -bromides and -chlorides, however, just like β -D-glucopyranosyl chlorides, are subject to alcoholysis, with formation of β -D-glucofuranosidurono-6,3-lactones.¹⁶

Although the intermediate formation of acetoxonium ions is generally²⁰ assumed in reactions of 2-*O*-acetyl-D-glucosyl halides with alcohols in the presence of soluble bases, this mechanism is obviously not valid for 2-*O*-benzoyl-D-glucosyl halides. Here, a direct reaction^{21,22} of a "solvent-separated ion-pair" or "intimate ion-pair" with alcohol to give the β -glycoside is more probable. Through such a mechanism, the formation of 5-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)- α -D-glucofuranurono-6,3-lactone²³ (23) by reaction of



(20) G. Wulff and G. Röhle, *Angew. Chem.*, **86**, 173-187 (1974).

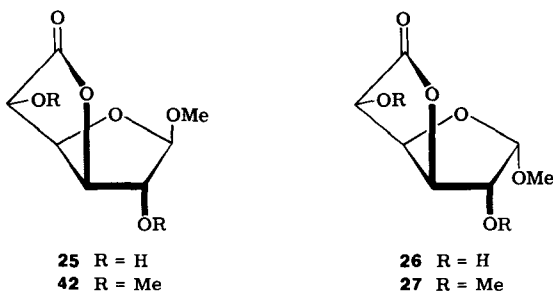
(21) A. F. Diaz, I. Lazdins, and S. Winstein, *J. Am. Chem. Soc.*, **90**, 1904-1905 (1968).

(22) D. J. Raber, J. M. Harris, R. E. Hall, and P. v. R. Schleyer, *J. Am. Chem. Soc.*, **93**, 4821-4828 (1971).

(23) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **110**, 707-717 (1935).

2,5-di-*O*-acetyl- β -D-glucofuranosylurono-6,3-lactone chloride (15) with methanol may be reasonably explained. 2,5-Di-*O*-benzoyl- β -D-glucofuranosylurono-6,3-lactone chloride (17), however, reacts with ethanol to afford ethyl 2,5-di-*O*-benzoyl- β -D-glucofuranosidurono-6,3-lactone¹⁶ (24).

Methyl D-glucofuranosidurono-6,3-lactones may be synthesized by direct glycosidation of 2 or 4 with methanol in the presence of a mineral acid. Thus, by treatment of 4 with methanol-hydrogen chloride at room temperature, Peat and coworkers²⁴ (for the first time) obtained methyl β -D-glucofuranosidurono-6,3-lactone (25), which, at elevated temperature, affords an anomeric mixture of methyl (methyl D-glucopyranosid)uronates. By application of cation-exchange resins, instead of soluble acids, in this glycosidation, higher yields of methyl D-glucofuranosidurono-6,3-lactones from both compounds 4 (Refs. 25 and 26) and 2 (Ref. 27) are obtained. Under these conditions, the thermodynamically less stable methyl α -D-glucofuranosidurono-6,3-lactone (26) may be isolated in low yield. The two anomers form an equilibrium,²⁵ in the ratio of 4:1, that can be reached from either side.



Methyl α -D-glucofuranosidurono-6,3-lactone (26) may be obtained exclusively by reaction of 4 with trimethyl orthoformate in the presence of boron trifluoride etherate.²⁸ Its 2,5-dimethyl ether (27) is formed by methyl iodide-silver oxide methylation^{29,30} (Purdie-Ir-

(24) L. N. Owen, S. Peat, and W. J. G. Jones, *J. Chem. Soc.*, 339-344 (1941).

(25) E. M. Osman, K. C. Hobbs, and W. E. Walston, *J. Am. Chem. Soc.*, **73**, 2726-2729 (1951).

(26) J. E. Cadotte, F. Smith, and D. Spriestersbach, *J. Am. Chem. Soc.*, **74**, 1501-1504 (1952).

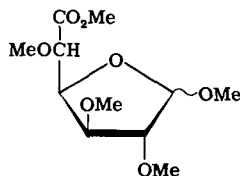
(27) G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310-3315 (1955).

(28) M. L. Wolfrom, J. W. Spoor, and R. A. Gibbons, *J. Org. Chem.*, **22**, 1513-1514 (1957).

(29) J. Pryde and R. T. Williams, *Biochem. J.*, **27**, 1205 (1933).

(30) R. E. Reeves, *J. Am. Chem. Soc.*, **62**, 1616-1617 (1940).

vine³¹) of **4**. With methyl iodide, barium oxide, and barium hydroxide in *N,N*-dimethylformamide (Kuhn-Trischmann³²), however, **4** reacts with formation of methyl (methyl 2,3,5-tri-*O*-methyl-D-glucofuranosid)uronates³³ (**28**).

**28**

Transacetalation of 5-*O*-substituted 1,2-*O*-alkylidene-D-glucofuranurono-6,3-lactones is a reaction particularly suited to the synthesis of selectively protected D-glucofuranosidurono-6,3-lactones.³⁴

Because direct glycosidation of **4** with phenols is not possible, indirect methods must be used for the preparation of aryl D-glucofuranosidurono-6,3-lactones (**29**). In addition, aryl 2,5-di-*O*-acetyl-D-glucofuranosidurono-6,3-lactones (**30**), obtained³⁵⁻³⁷ from the reaction of 1,2,5-tri-*O*-acetyl-D-glucofuranurono-6,3-lactones with phenols, can only be deacetylated by such multi-step procedures as (1) ammonolysis of **30** to afford aryl D-glucofuranosiduronamides (**31**), followed by amide hydrolysis and lactonization,^{35,37} or (2) reduction of **30** with lithium aluminum hydride, and subsequent oxidation of the intermediate aryl D-glucofuranosides³⁸ (**32**) (see Scheme 1).

3. 1,2-*O*-Alkylidene- α -D-glucofuranurono-6,3-lactones

The simultaneous protection of two hydroxyl groups by condensation of sugars with carbonyl compounds to give 1,3-dioxolanes or 1,3-dioxanes is also applicable to D-glucuronic acid. Principally, for the preparation of *O*-alkylidene- α -D-glucofuranuronic acid derivatives, two methods are available: 1, direct condensation of **2** or **4** with

(31) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021-1037 (1903).

(32) R. Kuhn and H. Trischmann, *Chem. Ber.*, **96**, 284-287 (1963).

(33) G. Roglić and D. Keglević, *Croat. Chem. Acta*, **44**, 229-242 (1972); *Chem. Abstr.*, **77**, 88,797p (1972).

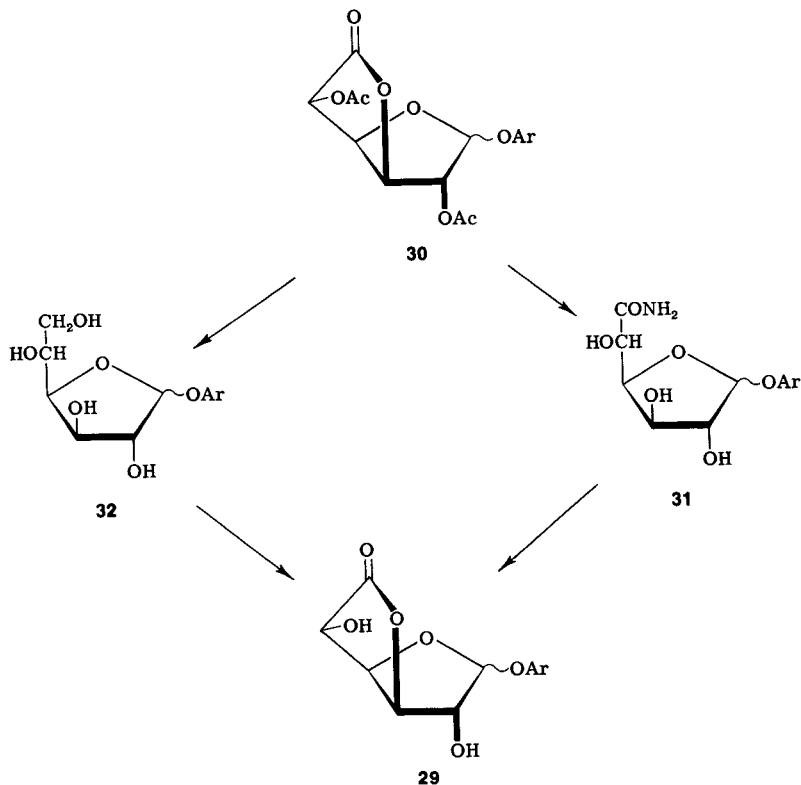
(34) H. Weidmann, *Monatsh. Chem.*, **96**, 766-773 (1965).

(35) K. C. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **74**, 5605-5608 (1952).

(36) K. C. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **75**, 1042-1044 (1953).

(37) M. Ishidate and M. Matsui, *Yakugaku Zasshi*, **82**, 662-669 (1962); *Chem. Abstr.*, **58**, 4,639e-h (1963).

(38) K. Kato, K. Yoshida, and H. Tsukamoto, *Chem. Pharm. Bull.*, **12**, 664-669 (1964).



Scheme 1

aldehydes or ketones, and, 2, oxidation of 1,2-*O*-alkylidene- or 1,2:3,5-di-*O*-alkylidene- α -D-glucofuranoses to the corresponding acids, followed by lactonization of the reaction products of the former.

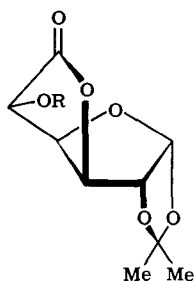
1,2-*O*-Isopropylidene- α -D-glucofuranurono-6,3-lactone (33), frequently used as the starting material for various syntheses, is prepared by reaction of **2** or **4** with acetone and a catalytic amount of sulfuric acid^{24,39} or in the presence of a cation-exchange resin.⁴⁰ Later investigations have shown that the use of absolute acetone, or the addition of 2,2-dimethoxypropane as a water scavenger, causes severe damage to the resin, resulting in lower yields and impure products.⁴¹

(39) M. Fieser, L. F. Fieser, E. Toromanoff, Y. Hirata, H. Heyman, M. Tefft, and S. Bhattacharya, *J. Am. Chem. Soc.*, **78**, 2825-2832 (1956).

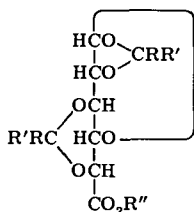
(40) H. Weidmann, *Ann.*, **679**, 178-186 (1964).

(41) H. Weidmann, unpublished results.

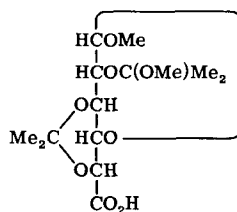
Thus, on treatment of **4** with acetone, 2,2-dimethoxypropane, and a strong acid, Lee⁴² obtained methyl 1,2:3,5-di-*O*-isopropylidene- α -D-glucofuranuronate (**34**) exclusively, although in low yield. In the absence of acetone, **4** reacts with 2,2-dimethoxypropane in the presence of an acid to form a product having the interesting structure **35**. For the synthesis⁴³ of 1,2-*O*-cyclohexylidene- α -D-glucofuranurono-6,3-lactone⁴⁴ (**36**) from **4** and cyclohexanone, sulfuric acid is the only catalyst generally employed.



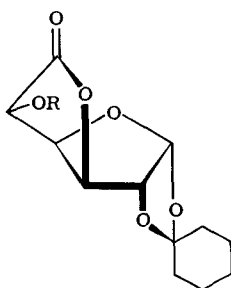
- 33** R = H
44 R = Me
45 R = Bzl
50 R = Ms
52 R = Ts



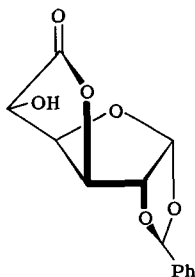
- 34** R = R' = R'' = Me
37 R = R'' = H, R' = Ph



35



- 36** R = H
46 R = Bzl
51 R = Ms



38

Interesting results were obtained in reactions of **2** with benzaldehyde in the presence of zinc chloride as the catalyst⁴⁵. Whereas

(42) C. H. Lee, *Carbohydr. Res.*, **22**, 230-232 (1972).

(43) H. Paulsen and D. Stoye, *Chem. Ber.*, **99**, 908-919 (1966).

(44) M. Ishidate and M. Okada, *Yakugaku Zasshi*, **71**, 1163-1165 (1951); *Chem. Abstr.*, **46**, 4,997b (1952).

(45) R. H. Shah, *Carbohydr. Res.*, **12**, 43-56 (1970).

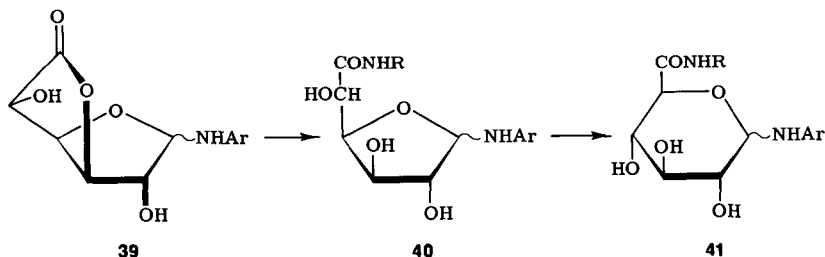
1,2:3,5-di-*O*-benzylidene- α -D-glucofuranuronic acid (**37**) could be isolated after 6 hours, 1,2-*O*-(*S*)-benzylidene- α -D-glucofuranurono-6,3-lactone (**38**) was the preponderant product after 48 hours.

The method of oxidation⁶⁻⁸ of 1,2-*O*-alkylidene- and of 1,2:3,5-di-*O*-alkylidene- α -D-glucofuranoses with formation of D-glucofuranuronic acid derivatives^{3,46} is of minor synthetic utility.

4. (D-Glucofuranosylamine)urono-6,3-lactones

(D-Glucofuranosylamine)uronic acids attained biological significance after nucleoside antibiotics containing hexuronic acid residues were isolated from natural sources; examples are gougerotin and blasticidin S.

Condensation of **4** with primary aromatic amines (for example, aniline,⁴⁷ 2- and 4-nitroaniline,^{47,48(a),49} 4-aminoazobenzene,⁴⁷ 4-aminobenzenesulfonamide,⁴⁷ 2-nitro-4,5-dimethylaniline^{48(a)}) yielded the corresponding (*N*-aryl-D-glucofuranosylamine)urono-6,3-lactones (**39**). All of these compounds are subject to ready oxidation on exposure to air. The strongly positive optical rotations observed indicate preponderant formation of α anomers; this was demonstrated^{48(a)} by n.m.r. analysis of the condensates of **4** with 2-nitroaniline or 2-nitro-4,5-dimethylaniline. Formation of Schiff bases was excluded. Subsequent reaction⁴⁸ of (*N*-aryl-D-glucofuranosylamine)urono-6,3-lactones (**39**) with ammonia or primary aliphatic amines leads to formation of (*N*-aryl-D-glucopyranosylamine)uronamides (**40**), which isomerize to (*N*-aryl-D-glucopyranosylamine)uronamides (**41**) in the presence of acids.



(46) D. Drehfahl and F. M. Matschke, *Chem. Ber.*, **82**, 484-487 (1949).

(47) S. Takitani, *Chem. Pharm. Bull.*, **9**, 222-226 (1961).

(48)(a) T. Kishikawa, *Chem. Pharm. Bull.*, **17**, 2494-2501 (1969); (b) H. Nakajima, *Yakugaku Zasshi*, **81**, 1094-1099 (1961); *Chem. Abstr.*, **56**, 4,849a (1962).

(49) Y. Nitta, J. Ide, and N. Ogikubo, Japan. Patent 21.067 (1963), 21.068 (1963); *Chem. Abstr.*, **60**, 3,082b (1964).

Reaction⁵⁰ of **4** with ammonia or strongly nucleophilic, primary and secondary amines yields mixtures of D-glucopyranuronamides and (D-glucopyranosylamine)uronamides.

Treatment of **4** with glycinonitrile (aminoacetonitrile) affords^{50(a)} (N-cyanomethyl-D-glucofuranosylamine)urono-6,3-lactone. Its negative optical rotation is an indication of the β -D configuration of this compound.

III. ETHERIFICATIONS

In carbohydrate chemistry, the preparation of ethers that are stable in the presence of acids, bases, and aqueous alkali is an important analytical and synthetic tool. The methods used for the etherification of hydroxyl groups⁵¹ generally employ reactions of unprotected sugars and glycosides with methyl, allyl, benzyl, triphenylmethyl, and alkylsilyl halides in the presence of a variety of aqueous and nonaqueous bases.

By methylation of D-glucofuranurono-6,3-lactone (**4**) with methyl iodide in the presence of silver oxide, Pryde and Williams²⁹ (for the first time) obtained "trimethyl glucurone," whose structure was later proved³⁰ to be that of methyl 2,5-di-O-methyl- α -D-glucofuranosidurono-6,3-lactone (**27**). This result clearly established the dioxabicyclo[3.3.0]octane structure of **4**, for which a pyranoid form had previously been assumed. On treatment with hydrochloric acid in methanol, compound **27** [which can also be prepared²⁶ from methyl α -D-glucofuranosidurono-6,3-lactone (**26**) by Purdie-Irvine methylation] anomerizes³⁰ with formation of methyl 2,5-di-O-methyl- β -D-glucofuranosidurono-6,3-lactone^{24,25} (**42**). For the synthesis of 2,5-di-O-alkyl-D-glucofuranosidurono-6,3-lactones, alkylation procedures employing aqueous, strong bases cannot be used, as hydrolytic opening of the lactone ring invariably occurs. Thus, reaction of **27** with dimethyl sulfate in the presence of aqueous alkali gives⁵² methyl 2,3,5-tri-O-methyl- α -D-glucofuranosiduronic acid. Its methyl ester (the α anomer of **28**) is obtained on treatment either with etheral diazomethane or methyl iodide-silver oxide,⁵² or, in admixture

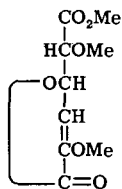
(50)(a) J. Ide, *Yakugaku Zasshi*, **85**, 213-220 (1965); *Chem. Abstr.*, **63**, 1,848g (1965);
(b) Y. Nitta, J. Ide, and H. Takahashi, *Yakugaku Zasshi*, **82**, 1563-1566 (1962);
Chem. Abstr., **59**, 733d (1963); (c) J. Ide, *Yakugaku Zasshi*, **85**, 226-231 (1965);
Chem. Abstr., **63**, 1,848g (1965).

(51) J. F. W. McOmie, *Adv. Org. Chem.*, **3**, 216-218 (1963).

(52) F. Smith, *J. Chem. Soc.*, 584-587 (1944).

with its β anomer, from **4** directly³³ by a Kuhn-Trischmann methylation. The same procedure converts⁵³ **33** into methyl 1,2-*O*-isopropylidene-3,5-di-*O*-methyl- α -D-glucofuranuronate.

Methyl iodide-silver oxide methylation²⁹ of **4**, its glycosides,⁵⁴ and 1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone²⁴ (**33**) invariably gives a side product, whose structure was shown³⁰ to be that of 4-deoxy-2,5-di-*O*-methyl-L-*threo*-hex-4-enaro-6,3-lactone 1-methyl ester (**43**). Its proportion depends on the structure of the substrate,⁵⁴

**43**

the reaction temperature,³⁰ the presence of water,⁵⁵ and the sequence of addition of the reagents.³⁰ Whereas Peat and coworkers²⁴ described the exclusive formation of **43** from **33**, under unspecified conditions, 1,2-*O*-isopropylidene-5-*O*-methyl- α -D-glucofuranurono-6,3-lactone (**44**) is the preponderant product⁵⁵ on methylation of **33** under anhydrous conditions. Compound **44** is also accessible from **33** by using³⁴ moist, ethereal diazomethane, first applied by Schmidt and Kraft⁵⁶ for the methylation of D-mannaro-1,4:6,3-dilactone.

There are distinct correlations between the mechanism of formation of compound **43**, the eliminations frequently observed with derivatives of aldaric acids, and the reducing power of alkyl D-glucofuranosidurono- and 1,2-*O*-alkylidene- α -D-glucofuranurono-6,3-lactones toward complexed copper(II) solutions. These phenomena are discussed in Section VII.

Benzyl and triphenylmethyl ethers of carbohydrates are preferred over methyl ethers when selective removal of protecting groups is important. The relatively high nucleophilic activity of the 5-hydroxyl group in glycosides and 1,2-*O*-alkylidene derivatives of **4** permits its benzylation and triphenylmethylation under mild conditions. Thus, treatment of **33** (Ref. 34) and **36** (Ref. 57) with benzyl bromide and

(53) K. Heyns and W. Baltes, *Chem. Ber.*, **99**, 3477-3479 (1966).

(54) K. Dax, N. Gassner, and H. Weidmann, to be published.

(55) J. K. N. Jones, *Can. J. Chem.*, **34**, 310-312 (1956).

(56) O. T. Schmidt and H. Kraft, *Ber.*, **74**, 33-49 (1941).

(57) W. Timpe, K. Dax, N. Wolf, and H. Weidmann, *Carbohydr. Res.*, **39**, 53-60 (1975).

silver oxide in nonpolar solvents yields their respective 5-benzyl ethers (45 and 46). Triphenylmethylation⁵⁴ of 33 was achieved with chlorotriphenylmethane in pyridine at elevated temperature.

IV. ESTERIFICATIONS

Esters of 4, which are used as substrates for the preparation of D-glucofuranosylurono-6,3-lactone halides (see Section I,1) or aryl D-glucofuranosidurono-6,3-lactones (see Section I,2), are prepared by reaction of 4 with acid halides or anhydrides in the presence of a basic or an acidic catalyst.

Specifically, in reactions of 4 with acetic anhydride in the presence of zinc chloride⁵⁸ or boron trifluoride^{17,35} as the catalyst, 1,2,5-tri-*O*-acetyl- β -D-glucofuranurono-6,3-lactone (12) is the preponderant product; in the presence of pyridine,⁵⁸ however, acetylation of 4 by acetic anhydride leads to the favored formation of the corresponding α -D anomer. In contrast to acetylations, in benzylation reactions, the ratio of anomers apparently changes. Thus, on treatment of 4 with benzoyl chloride in pyridine, Momose and coworkers¹⁷ isolated 1,2,5-tri-*O*-benzoyl- β -D-glucofuranurono-6,3-lactone in a yield of 50%.

In view of the great preponderance of the β -D anomer in crystalline D-glucofuranurono-6,3-lactone (4), the results obtained from its acetylation with acetic anhydride-pyridine disagree with observations⁵⁹ suggesting that anomeric ratios are not altered during acylation in pyridine.

Selective esterification, particularly *p*-toluenesulfonylation and methanesulfonylation, of one, or two, of several hydroxyl groups in carbohydrate molecules is an important reaction for constitutional and configurational alterations.

In the series of esters of D-glucofuranurono-6,3-lactone (4), methyl 2,5-di-*O*-(methylsulfonyl)-⁶⁰ (47), methyl 2,5-di-*O*-*p*-tolylsulfonyl-⁵⁴ (48), and methyl 5-*O*-benzyl-2-*O*-(methylsulfonyl)- β -D-glucofuranosidurono-6,3-lactone⁶⁰ (49), as well as 1,2-*O*-isopropylidene-5-*O*-(methylsulfonyl)-⁶¹ (50), 1,2-*O*-cyclohexylidene-5-*O*-(methylsulfonyl)-⁴³ (51), and 1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl- α -D-glucofuranurono-6,3-lactone⁵⁵ (52), have been prepared. All of these com-

(58) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **100**, 743-748 (1933).

(59) J. Staněk, M. Černý, J. Kocourek, and J. Pacák, "The Monosaccharides," Publishing House of the Czechoslovak Academy of Sciences, Prague, 1963, p. 177.

(60) H. Weidmann, D. Wewerka, and N. Wolf, *Monatsh. Chem.*, **99**, 509-521 (1968).

(61) L. D. Hall, L. Hough, and R. A. Pritchard, *J. Chem. Soc.*, 1537-1545 (1961).

pounds are valuable intermediates for the synthesis of selectively protected D-glucofuranose derivatives (see Section IX).

Mixed acetylated-benzoylated D-glucofuranurono-6,3-lactones, well suited for the preparation of D-glucofuranosylurono-6,3-lactone halides, were obtained^{16,62} by the following reactions. Acetolysis of 5-O-benzoyl-1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactone led to formation of 1,2-di-O-acetyl-5-O-benzoyl- α - and - β -D-glucofuranurono-6,3-lactone (**12** and its anomer). 1-O-Acetyl-2,5-di-O-benzoyl- α - and - β -D-glucofuranurono-6,3-lactone (**13** and its anomer) were synthesized by selective hydrolysis of methyl 2,5-di-O-benzoyl- β -D-glucofuranosidurono-6,3-lactone followed by acetylation of the anomeric hydroxyl group.

A more versatile procedure for the synthesis of mixed 1,2,5-triesters of **4**, or of 2,5-diesters of **25**, starts from the readily accessible 5-O-benzyl-1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactone³⁴ (**45**) (see Section IX).

Interesting results were obtained in an investigation of equimolar acylations⁶³ of both anomers of methyl D-glucofuranosidurono-6,3-lactone (**25** and **26**). Whereas their reactions^{16,60,64-66} with acetic anhydride, acetyl chloride, or benzoyl chloride in the presence of pyridine showed no selectivity, ethoxy- and benzyloxy-carbonyl chloride, respectively, under the same conditions exhibited an appreciable selectivity for the 5-hydroxyl group. Thus, methyl 5-O-(ethoxycarbonyl)- (**53**) and 5-O-(benzyloxycarbonyl)- β -D-glucofuranosidurono-6,3-lactone (**54**) could be obtained in yields of 50–60%, together with minor proportions of the corresponding 2,5-di-O-acylated derivatives. For the anomer **26**, in which the difference in reactivity between the 2- and 5-hydroxyl groups is, because of strong hydrogen-bonding (see Section V), less than in **25**, formation of larger proportions of the respective 2,5-di-O-acylated product, besides the 5-O-substituted products (**55** and **56**), was observed. 2-O-Acylated derivatives of the anomers **25** and **26** that have sufficient solubility in nonpolar solvents for use in i.r. investigations

(62) Y. Nitta, A. Momose, and K. Kamei, Japan. Patent 20,288 20,289, 20,290 (1967), 15,112 (1967); *Chem. Abstr.*, **68**, 78,559e, 78,560f, 78,561g, 105,499b (1968).

(63) H. Weidmann, K. Dax, and D. Wewerka, *Monatsh. Chem.*, **101**, 1831–1840 (1970).

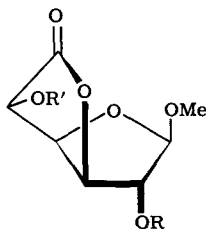
(64) V. Prey and A. Szabolcs, *Monatsh. Chem.*, **89**, 350–357 (1958).

(65) Y. Nitta, J. Ide, A. Momose, and Y. Nakajima, *Yakugaku Zasshi*, **82**, 578–583 (1962); *Chem. Abstr.*, **57**, 4,201a (1962).

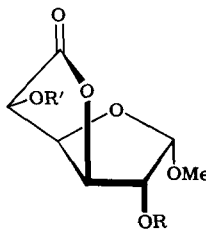
(66) Y. Nitta, J. Ide, A. Momose, and M. Kuwada, *Yakugaku Zasshi*, **82**, 790–794 (1962); *Chem. Abstr.*, **57**, 14,601i (1962).

of hydrogen bonding (see Section V) were obtained in the following way.

Treatment of **25** and **26**, respectively, in pyridine with equimolar amounts of benzyloxycarbonyl chloride at low temperature, followed by addition of an excess of ethoxycarbonyl chloride, yielded the corresponding methyl 5-*O*-(benzyloxycarbonyl)-2-*O*-(ethoxycarbonyl)- β - (**57**) and - α -D-glucofuranosidurono-6,3-lactone (**58**). Hydrogenolysis of the benzyloxycarbonyl group resulted in formation of methyl 2-*O*-(ethoxycarbonyl)- β - (**59**) (70%) and - α -D-glucofuranosidurono-6,3-lactone (**60**) (30%), respectively, both in crystalline form. The β -D



- 47** R = R' = Ms
48 R = R' = Ts
49 R = Ms, R' = Bzl
53 R = H, R' = CO₂Et
54 R = H, R' = CO₂Bzl
57 R = CO₂Et, R' = CO₂Bzl
59 R = CO₂Et, R' = H
61 R = H, R' = Bzl



- 55** R = H, R' = CO₂Et
56 R = H, R' = CO₂Bzl
58 R = CO₂Et, R' = CO₂Bzl
60 R = CO₂Et, R' = H

anomer **59** could also be synthesized from **45** by transacetalation, (ethoxycarbonyl)ation of the intermediate methyl 5-*O*-benzyl- β -D-glucofuranosidurono-6,3-lactone⁶⁰ (**61**), and hydrogenolysis of the benzyl group. With 1-benzoylimidazole,⁶⁷ compound **25** yielded⁵⁴ methyl 5-*O*-benzoyl- β -D-glucofuranosidurono-6,3-lactone exclusively. The structural causes of these phenomena are discussed in Section V.

V. STRUCTURE-REACTIVITY CORRELATIONS.

CONFORMATION OF D-GLUCOFURANOSIDURONO-6,3-LACTONES

Conformational analysis consists in investigations concerned with the determination of molecular shapes, commonly described by bond angles and bond lengths. Among the various methods generally used⁶⁸ for the estimation of these parameters, X-ray analysis provides

(67) H. A. Staab, *Angew. Chem.*, **74**, 407-423 (1962).

(68) See Ref. 12, Section 2.

the most accurate results; its application is limited, however, to crystalline, solid compounds. Conformations in solution⁶⁹ (determining reactivities as related to structures) are most frequently elucidated by spectroscopic and polarimetric methods. Because the coupling constants of vicinal hydrogen atoms may be approximately related⁷⁰ to dihedral angles, n.m.r. spectroscopy has proved to be a most useful tool.^{68,71}

In compounds containing a pyranoid ring, the situation is comparatively simple.^{69,72} In these, the two chairlike conformations, ${}^4C_1(D) \equiv {}^1C_4(L)$ and ${}^1C_4(D) \equiv {}^4C_1(L)$, need only be considered, as they have the lowest free-energy among the various conformations. In addition, the free-energy difference between the two chair conformations is, usually, of a magnitude strongly favoring one of these conformations, so that an unequivocal conformational assignment from n.m.r. data is possible.

As the magnitudes of the conformational-interconversion energies of cyclohexane and of pyranoid sugar derivatives are known to be quite similar, by analogy, the energy barriers for furanoid sugar derivatives⁷³ are expected to differ little from those of cyclopentane. Because of the larger number⁷⁴ of possible conformations of furanoses as compared to pyranoses, and because of the low energy-differences by which they are separated, it has been proposed⁷³ that the results of conformational analysis of furanoid sugars in solution be interpreted in terms of conformational equilibria between various, rapidly interconverting forms.

Because of the smaller *endocyclic* angles in furanoses, as compared to pyranoses, the Karplus equation,⁷⁰ even after modification,⁷⁶⁻⁷⁹ does not allow accurate calculation of the dihedral angles

(69) S. J. Angyal, *Angew. Chem.*, **81**, 172-182 (1969).

(70) M. Karplus, *J. Chem. Phys.*, **30**, 11-15 (1959).

(71) L. D. Hall, *Adv. Carbohydr. Chem.*, **19**, 51-93 (1964).

(72) See Ref. 12, Section 4.

(73) See Ref. 12, Section 5.

(74) Only two conformations for tetrahydrofuran (3T_4 and 1E , oxygen at position 1) are possible,⁷⁵ provided that the following presuppositions are made: 1, the C-C-C angle is equal to the C-C-O angle; 2, the C-O-C angle is larger than the C-C-C angle; 3, all C-C bonds are of equal length; and 4, the two C-O bonds are of identical length differing from that of C-C bonds.

(75) H. Hönig and H. Weidmann, unpublished results.

(76) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699-3705 (1962).

(77) L. D. Hall, *Chem. Ind. (London)*, 950-951 (1963); compare *Chem. Commun.*, 505-508 (1973).

(78) R. U. Lemieux, J. D. Stevens, and R. R. Fraser, *Can. J. Chem.*, **40**, 1955-1959 (1962).

from the spin-spin couplings observed. In addition, the degree of puckering in furanoses is not precisely described by the terms "twist" (*T*) or "envelope" (*E*) conformation,⁷⁷ commonly used for conformational assignments for furanoid sugars.

In *ortho*-annelated, bicyclic, furanoid sugar derivatives, because of lessened conformational flexibility, only a limited number of conformations is thermodynamically favored. The preponderance of the 3T_2 conformation found for (furanoid) 1,2-*O*-isopropylidene- α -D-glucoside and - β -L-ido-furanoses,^{76,80} 1,2-*O*-benzylidene- α -D-glucofuranose,⁸¹ and 1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl- α -D-glucofuranurono-6,3-lactone⁷⁶ (**52**) confirms this.

The 60-MHz, p.m.r. spectra of compounds **25**, **26**, and **33** cannot be fully analyzed, inasmuch as, in parts of the spectra, the magnitudes of the differences of the chemical shifts and that of the spin-spin coupling-constants (splittings) are very similar. In order to avoid possible conformational changes by *O*-protection⁸² in the compounds to be analyzed, compounds **25**, **26**, and **33** were investigated⁸⁴ in⁸⁵ pyridine-*d*₅ by 220-MHz, p.m.r. spectroscopy.

From the data compiled in Table I, it may be concluded that com-

TABLE I

Proton Coupling-Constants^a of Methyl β - (**25**) and α -D-Glucofuranosidurono-6,3-lactone (**26**), and 1,2-*O*-Isopropylidene- α -D-glucofuranurono-6,3-lactone (**33**)

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
25	< 0.5	< 0.5	4.7	6.5
26	4.2	< 1	~3	4.3
33	3.8	< 0.5	2.9	4.1

^a Coupling constants, *J* (Hz), obtained by 220-MHz proton magnetic resonance spectroscopy in pyridine-*d*₅. Values from Ref. 84.

(79) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870-2871 (1963).

(80) R. J. Abraham, K. A. McLauchlan, L. D. Hall, and L. Hough, *Chem. Ind. (London)*, 213-214 (1962).

(81) B. Coxon, *Carbohydr. Res.*, **8**, 125-134 (1968).

(82) In the aforementioned compounds, functional derivatization, described⁸³ to increase the dispersion of their n.m.r. spectra, caused⁶³ enhanced rate of lactone hydrolysis (apparent from their electrolytic conductivities).

(83) L. D. Hall, *Adv. Carbohydr. Chem. Biochem.*, **29**, 11-40 (1974), Section 2.

(84) H. Weidmann and K. Dax, *Monatsh. Chem.*, **102**, 877-884 (1971).

(85) Pyridine-*d*₅ was found to show the most favorable solvent-induced changes of the chemical shifts for the three compounds; in so far as a "first-order" analysis of the 60-MHz spectra was possible, the observed "splittings" gave no indication for solvent-induced, conformational changes.

pounds **26** and **33** exist in the 3T_2 conformation, although the small coupling-constants, $J_{2,3}$, allow only a rough estimate to be made of the degree of puckering. This result is in agreement with previous conformational assignments⁷⁶ for a derivative of **33**. The smaller dihedral angle between the C-1-H and C-2-H bonds in **26** as compared to that in **33** proves the existence of a lower degree of twisting in this part of its tetrahydrofuran ring, most likely due to hydrogen bonding⁸⁴ from the 2-hydroxyl group to the anomeric oxygen atom. The other coupling-constants ($J_{2,3}$, $J_{3,4}$, and $J_{4,5}$), and, consequently, the projection angles, are essentially identical in both compounds, from which a high degree of conformational similarity of their lactone rings is inferred.

In contrast to these compounds, the coupling constants obtained from n.m.r. measurements of **25** might, on first sight, appear to be consistent with the 3T_2 , as well as with the E_2 and 3E conformations, respectively. However, the following considerations clearly favor the 3T_2 conformation, with a more moderate puckering of the tetrahydrofuran ring.

1. The E_2 conformation requires very small dihedral angles between the hydrogen atoms on C-3 and C-4, as well as those on C-4 and C-5, and this is not verified by the values resulting from the corresponding coupling-constants.

2. For the E^3 conformation, discussed by Hall and Steiner¹⁹ for derivatives of β -D-glucofuranosylurono-6,3-lactone fluoride, the value of $J_{1,2}$ is too small. In addition, in such a conformation, the distance between the proton of the 2-hydroxyl group and the ring-oxygen atom of the furanoid ring is too large to account for the hydrogen bonding observed⁸⁴ in compound **54** (see Table II).

The different conformations of the lactone rings in **25**, **26**, and **33** were found⁸⁴ to be decisive for the phenomena described in Sections VI and VII. Two interdependent factors can be assumed to be responsible for the different stabilities observed of their lactone rings towards nucleophiles, namely, ring strain and electron delocalization, the latter of which is known⁸⁶ to cause substantial stabilization in esters and lactones. This resonance participation was proved⁸⁷ by the shortened C-6-O-3 bond-length, found to be 134 pm by X-ray analysis of β -D-glucofuranurono-6,3-lactone; for this compound, the E^1 conformation of the furanose and the E_5 conformation of the lactone ring were described.⁸⁷

(86) R. Huisgen and H. Ott, *Tetrahedron*, **6**, 253-267 (1959).

(87) S. H. Kim, G. A. Jeffrey, R. D. Rosenstein, and P. W. R. Corfield, *Acta Crystallogr.*, **22**, 733-743 (1967).

The coupling constants $J_{3,4}$ and $J_{4,5}$ (see Table I) prove a lesser degree of puckering in the lactone ring of **25** as compared to those in **26** and **33**; hence, the extent of resonance stabilization in **25** can be assumed to be larger than in **26** and **33**. Assuming identical transition states in nucleophilic, lactone ring-opening reactions⁸⁸ of **25**, **26**, and **33** (see Section VI), the preceding reasoning explains the comparatively low reactivity of **25**. As the lactone conformations in **26** and **33** are practically identical, explanations giving possible causes for the rather small differences in their observed reactivity (see Sections VI and VII) have not yet been forthcoming.

The different reaction-periods needed⁵⁷ for eliminations of the anomeric methyl D-glucofuranosidurono-6,3-lactones (see Table IV) must be attributed to differences in C-5-H acidity. For a possible explanation,⁸⁹ it may be assumed that the electron-withdrawing property of the molecular vicinity also depends on the degree of electron

TABLE II
Infrared Measurements and Hydrogen Bonding⁸⁴

Compound	ν^a	$\Delta\nu^b$	$r_{O\cdots H}$ (calc.) ^c	$r_{O\cdots H}$ (model) ^d
59	3576	53	2.15	2.2
33	3582	47	2.25	2.3
60	3586	43	2.3	2.3
54	3585	44	2.3	2.4
56	3552	77	1.9	2.0

^a O-H stretching frequencies in cm^{-1} . ^b Deviation (in cm^{-1}) from the "standard" frequency for O-H stretching (3629 cm^{-1}). ^c O-H distances calculated. ^d O-H distances estimated from Dreiding stereomodels.

(88) No participation of resonance in the sp^3 -hybridized transition state.

(89) Differences in carbonyl stretching-frequencies⁹⁰⁻⁹² and ^{13}C -H coupling constants^{93,94} of cyclanones are related^{90,95} to different C-H acidities dependent on ring size, and were interpreted⁹³ in terms of bond-angle-dependent hybridization. In i.r. measurements,^{54,66} various derivatives of compound **25** consistently showed higher lactone carbonyl stretching-frequencies than those of compound **26**.

(90) H. Shechter, M. J. Collis, R. Dessy, Y. Okuzumi, and A. Chen, *J. Am. Chem. Soc.*, **84**, 2905-2910 (1962).

(91) P. v. R. Schleyer and R. D. Nicholas, *J. Am. Chem. Soc.*, **83**, 182-187 (1961).

(92) C. S. Foote, *J. Am. Chem. Soc.*, **86**, 1853-1854 (1964).

(93) C. S. Foote, *Tetrahedron Lett.*, 579-583 (1963).

(94) J. N. Shoolery, *J. Chem. Phys.*, **31**, 1427-1428 (1959).

(95) A. Schriesheim, R. J. Muller, and C. A. Rowe, Jr., *J. Am. Chem. Soc.*, **84**, 3164-3168 (1962).

delocalization. As the extent of resonance stabilization decreases, the $-I$ action increases.

The i.r. data⁸⁴ contained in Table II show different degrees of hydrogen-bonding between the 5-hydroxyl group and the tetrahydrofuran oxygen atom, decreasing in the order: **59** > **33** > **60**. This result is in complete agreement with the different puckering of their lactone rings deduced from n.m.r. measurements. Whereas compound **54** exhibits moderate hydrogen-bonding of the 2-hydroxyl group to the tetrahydrofuran oxygen atom, the 2-hydroxyl group in **56**, as expected, bonds to the anomeric oxygen atom (see the acylation reactions in Section IV).

VI. REACTIONS INVOLVING THE LACTONE RING OF DERIVATIVES OF D-GLUCOFURANURONO-6,3-LACTONE

1. Hydrolysis Reactions

Compound **4** exhibits considerable stability towards hydrolytic, lactone ring-opening.^{96,97} Thus, after 24 hours at 37°, 0.02 molar aqueous solutions of **4** contain only 5% of **2** and, at room temperature, equilibrium between **2** and **4** is only reached after several weeks.⁹⁶

Figure 1 is a plot of the electrolytic conductivity of 0.05 molar aqueous solutions of compounds **25**, **26**, and **33** against time,⁶³ showing a strong dependence of the rate of hydrolysis on the structure.^{25,98} This behavior primarily results from differences in the conformations of **25**, **26**, and **33**, which have different degrees of lactone ring-puckering (see Section V). As a corollary, methyl β -D-glucofuranosiduronic acid lactonizes more rapidly⁹⁹ than 1,2-O-isopropylidene- α -D-glucofuranuronic acid.

For the preparation of **2** by aqueous, alkaline hydrolysis of the lactone ring in **4**, precautions against Lobry de Bruyn-Alberda van Ekenstein rearrangements,¹⁰⁰ and even against decomposition¹⁰¹ of

(96) Y. Imai and Y. Hirasaka, *Yakugaku Zasshi*, **80**, 1139-1142 (1960); *Chem. Abstr.*, **54**, 22,581e (1960).

(97) T. Yamana, Y. Mizukami, and S. Asahi, *Yakugaku Zasshi*, **84**, 696-699 (1964); *Chem. Abstr.*, **61**, 13,132f (1964).

(98) E. Tomita and Y. Nitta, *Yakugaku Zasshi*, **87**, 495-499 (1967); *Chem. Abstr.*, **67**, 108,910u (1967).

(99) M. Ishidate, Y. Imai, Y. Hirasaka, and K. Umemoto, *Chem. Pharm. Bull.*, **13**, 173-176 (1965).

(100) I. R. Siddiqui and C. B. Purves, *Can. J. Chem.*, **41**, 382-386 (1963).

(101) W. Hach and D. G. Benjamin, *J. Am. Chem. Soc.*, **76**, 917-918 (1954).

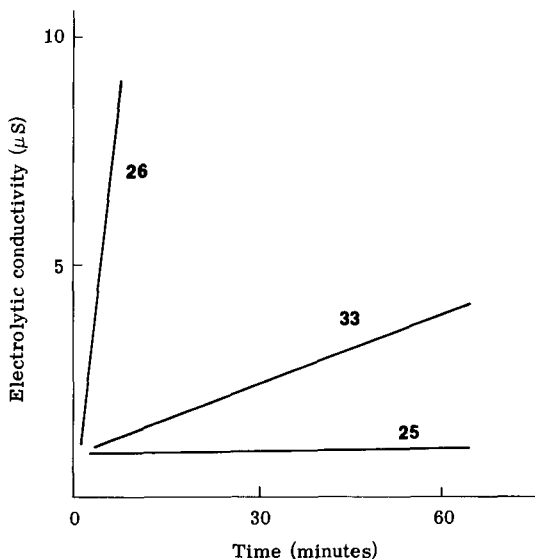


FIG. 1.—Electrolytic Conductivity of 0.05 *M* Aqueous Solutions of Methyl β - (25) and α -D-Glucufuranosidurono-6,3-lactone (26), and 1,2-*O*-Isopropylidene- α -D-glucufuranurono-6,3-lactone (33), Against Time.⁶³

the substrate, need to be taken. To avoid such side reactions, either addition¹⁰¹ of **4** to an excess of aqueous alkali followed by rapid acidification, or the use⁹⁹ of strong anion-exchange resins, is recommended. Because of its lower solubility,¹⁰² compound **4** is the only product isolated on evaporation of aqueous solutions of **2**, or of mixtures of **2** and **4**.

Following the methods of epimerization of aldonic acids, F. G. Fischer and H. Schmidt¹⁰³ described the formation of L-iduronic acid by isomerization of different salts of D-glucuronic acid at elevated temperature. This result could not be confirmed in later investigations; instead, Carlsson and coworkers,^{104,105} employing the same conditions, isolated D-mannuronic, D-altruronic, and D-alluronic acid, as well as D-*lyxo*-5-hexulosonic, L-*ribo*-5-hexulosonic, and L-*ribo*-4-hexulosonic acid. Furthermore, when treated with a slight excess of aqueous alkali, compound **4** was described¹⁰⁰ as affording D-mannuronic acid and D-*lyxo*-5-hexulosonic acid, only.

(102) See Ref. 6, p. 23.

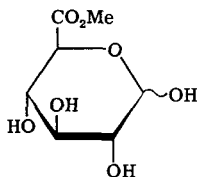
(103) F. G. Fischer and H. Schmidt, *Chem. Ber.*, **92**, 2184–2188 (1959).

(104) B. Carlsson, O. Samuelson, T. Popoff, and O. Theander, *Acta Chem. Scand.*, **23**, 261–267 (1969).

(105) B. Carlsson and O. Samuelson, *Acta Chem. Scand.*, **23**, 318–319 (1969).

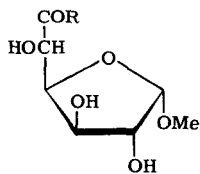
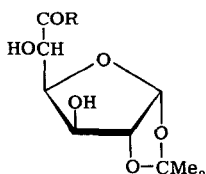
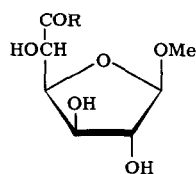
2. Alcoholysis Reactions

As with its hydrolytic, lactone ring-opening, compound **4** reacts^{106,107} rather slowly with methanol, with formation of an equilibrium mixture containing methyl D-glucopyranuronate (**62**) and **4** in the ratio of $\sim 3:1$. Compound **62** is formed much more rapidly and in high yield, however, on treatment^{27,108} of **4** with methanol in the

**62**

presence of a catalytic amount of sodium methoxide. Similarly, ethyl D-glucopyranuronate can be obtained¹⁰⁹ by treatment of **4** with sodium ethoxide in ethanol. Reaction of **62** with methanol under acidic conditions yields²⁷ mixtures of the anomers of methyl D-glucofuranosidurono-6,3-lactone; extension of the reaction period gives rise²⁷ to formation of the anomeric methyl (methyl D-glucopyranosid)uronates.

In accordance with the different stabilities of **25**, **26**, and **33** toward hydrolytic, lactone ring-opening, on reaction with methanol catalyzed by weak bases, compounds **26**, **33**, and **25**, in that order, afford¹¹⁰ decreasing proportions of the corresponding methyl D-glucofuranuronate derivatives **63**, **64**, and **65**. All of these compounds

**63** R = OMe**66** R = NH₂**64** R = OMe**67** R = NH₂**65** R = OMe**68** R = NH₂

(106) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **111**, 347-353 (1935).

(107) C. T. Bishop, *Can. J. Chem.*, **31**, 134-144 (1953).

(108) O. Touster and V. H. Reynolds, *J. Biol. Chem.*, **197**, 863-868 (1952).

(109) Y. Hirasaka and K. Umemoto, *Yakugaku Zasshi*, **82**, 1676-1678 (1962); *Chem. Abstr.*, **59**, 1,739c (1963).

(110) H. Weidmann and K. Dax, unpublished results.

were not isolated, inasmuch as evaporation of their methanolic solutions invariably re-forms¹¹⁰ the respective starting-material. This result is not in agreement with the finding of Tamura and coworkers,¹¹¹ who obtained methyl 5-*O*-acetyl-1,2-*O*-isopropylidene- α -D-glucofuranuronate on treatment of 5-*O*-acetyl-1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone with methanol in the presence of an anion-exchange resin, acetic acid-triethylamine, or sodium acetate.

3. Ammonolysis, Aminolysis, and Hydrazinolysis Reactions

This subsection deals exclusively with reactions of alkyl and aryl D-glucofuranosidurono-6,3-lactones, 1,2-*O*-alkylidene- α -D-glucofuranurono-6,3-lactones, and their respective derivatives, as reactions of 4 with nitrogenous bases constitute the subject matter of Section I,4. In reactions with ammonia and primary amines,^{39,112} in agreement with their observed different rates of hydrolysis and methanolysis, compounds 25, 26, and 33 show differences in lactone reactivity, and also give different yields of the corresponding furanoid amides. Whereas methyl α -D-glucofuranosiduronamide²⁵ (66) and 1,2-*O*-isopropylidene- α -D-glucofuranuronamide^{25,39} (67) could be obtained in pure, crystalline state, methyl β -D-glucofuranosiduronamide²⁴ (68), particularly in larger-scale preparations⁴¹ from 25, was obtained as a syrup contaminated by colored impurities.

A large number of investigations have dealt with ammonolysis reactions of esters of alkyl and aryl D-glucofuranosidurono-6,3-lactones, as well as of those of 1,2-*O*-alkylidene- α -D-glucofuranurono-6,3-lactones. Whereas liquid ammonia at -70° , and saturated methanolic solutions of ammonia at room temperature, generally remove *O*-acyl groups simultaneously with ammonolysis of the lactone, ammonia in chloroform is a more selective reagent. Thus, on treatment⁶⁰ of 5-*O*-acetyl-1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone with a saturated solution of ammonia in chloroform at 0° , a 50% yield of 3-*O*-acetyl-1,2-*O*-isopropylidene- α -D-glucofuranuronamide was obtained. Under the same conditions, 5-*O*-benzoyl-1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone gave⁶⁰ 3-*O*-benzoyl-1,2-*O*-isopropylidene- α -D-glucofuranuronamide in low, variable

(111) (a) I. Matsunaga and Z. Tamura, *Chem. Pharm. Bull.*, **17**, 1383-1389 (1969); (b) T. Kinoshita, M. Ishidate, and Z. Tamura, *ibid.*, **14**, 986-990 (1966); (c) M. Ishidate, Z. Tamura, and T. Kinoshita, *ibid.*, **10**, 1258-1259 (1962).

(112) J. Ide, *Yakugaku Zasshi*, **85**, 220-226 (1965); *Chem. Abstr.*, **63**, 1,848g (1965).

yields. Also, under these conditions, acyl-migration reactions were observed⁶⁰ with methyl 2,5-di-*O*-acetyl- and 2,5-di-*O*-benzoyl- β -D-glucofuranosidurono-6,3-lactone, to form the respective methyl 2,3-di-*O*-acetyl- β -D-glucofuranosiduronamides. In view of these results, formation³⁷ of phenyl 5-*O*-acetyl- β -D-glucofuranosidurono-6,3-lactone by treatment of phenyl 2,5-di-*O*-acetyl- β -D-glucofuranosidurono-6,3-lactone with methanolic ammonia followed by acid hydrolysis is difficult to explain.

Treatment of methyl 2,5-di-*O*-(ethoxycarbonyl)- α -D- and - β -D-glucofuranosidurono-6,3-lactone and 5-*O*-(ethoxycarbonyl)-1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone with this chloroform-ammonia reagent gives⁶³ methyl 2,5-di-*O*-(ethoxycarbonyl)- α -D- and methyl 2-*O*-(ethoxycarbonyl)- β -D-glucofuranosiduronamide, and 5-*O*-(ethoxycarbonyl)-1,2-*O*-isopropylidene- α -D-glucofuranuronamide, respectively. The loss of the 5-*O*-acyl group from the β -D compound is a result of the extended reaction-time necessary for complete opening of the lactone ring.

A very low yield of 1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl- α -D-glucofuranuronamide was obtained¹¹³ on ammonolysis of the corresponding 5-*O*-*p*-tolylsulfonyl lactone (52) with ammonia in methanol at low temperature. In a similar reaction of 1,2-*O*-isopropylidene-5-*O*-(methylsulfonyl)- α -D-glucofuranurono-6,3-lactone (50), formation of substantial proportions of 1,2-*O*-isopropylidene-5-*O*-(methylsulfonyl)- α -D-glucofuranuronamide could be observed¹¹⁰ in the reaction mixture; however, on evaporation, the starting material was reformed.

On reaction with hydroxylamine in the presence of appropriate bases, such derivatives of D-glucofuranurono-6,3-lactones as 25, 26, and 33 form¹¹⁴ *N*-hydroxyamides. On the other hand, when treated with hydroxylamine without base catalysis, compound 4 yields¹¹⁵ *aldehydo*-D-glucurono-6,3-lactone oxime.

In contrast to aldoses, ketoses, and compound 2, which, on treatment with phenylhydrazine, form phenylosazones, compound 4, under the same conditions, yields¹¹⁶ the phenylhydrazone of *aldehydo*-D-glucuronic phenylhydrazide (69). In a series of papers, Momose and coworkers¹¹⁷⁻¹²⁰ described the synthesis of *N*-

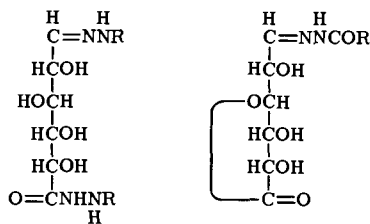
(113) J. Yoshimura, J. Hashimoto, and H. Ando, *Bull. Chem. Soc. Jpn.*, **46**, 1272-1275 (1973).

(114) This reaction is commonly used in a color test for lactones and methyl esters.

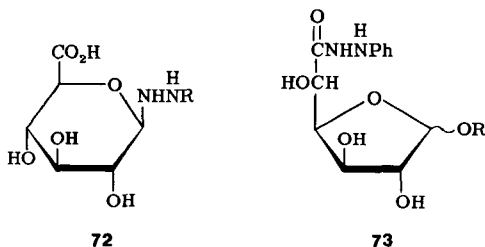
(115) G. Giemsa, *Ber.*, **33**, 2996-2998 (1900).

(116) M. Akagi, S. Tejima, and M. Haga, *Chem. Pharm. Bull.*, **10**, 98-100 (1962).

(117) Y. Nitta and A. Momose, *Yakugaku Zasshi*, **82**, 1046-1050 (1962); *Chem. Abstr.*, **58**, 5,630a (1963).

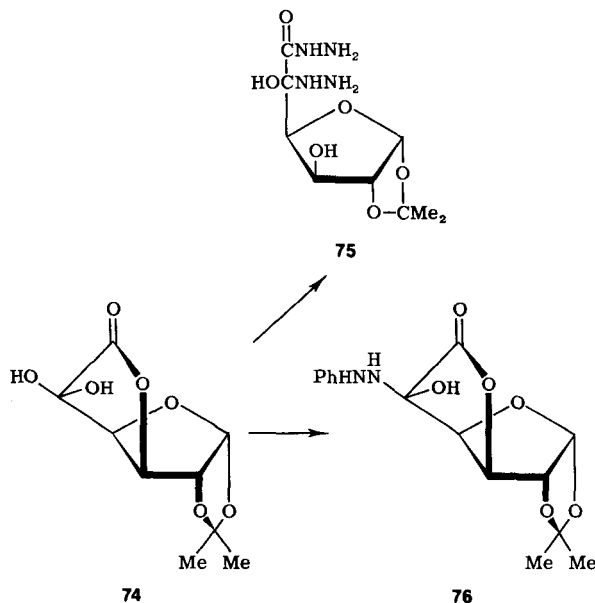
**69** R = Ph**70****71** R = acyl

acylhydrazones (**70**) of *aldehydo*-D-glucurono-6,3-lactone by reactions of **4** with a variety of hydrazides of benzoic, nicotinic, and isonicotinic acids; the products were expected to be of tuberculo-static activity. The rate of formation of the *N*-isonicotinoylhydrazone of *aldehydo*-D-glucurono-6,3-lactone by treatment of **4** with isonicotinoylhydrazones of various ketones was also investigated.¹¹⁷ When heated in aqueous solution, *N*-acylhydrazones of *aldehydo*-D-glucurono-6,3-lactone are subject to disproportionation, with formation¹¹⁸⁻¹²⁰ of *N*-acylhydrazones of *N*-acyl-*aldehydo*-D-glucuronic hydrazide (**71**). (*N*-Acyl-β-D-glucopyranosylhydrazine)uronic acids (**72**), which are transient intermediates in these reactions, suffer decarboxylation, yielding¹¹⁸ *N*-acylhydrazones of 2-furaldehyde. By reactions¹²¹ of methyl, ethyl, and butyl D-glucofuranosidurono-6,3-lactone with phenylhydrazine (and with a number of its derivatives substituted in the aromatic nucleus), D-glucofuranosiduronic *N*-phenylhydrazides (**73**) were obtained that exhibited hepato-protective activity.

**72****73**

- (118) Y. Nitta and A. Momose, *Yakugaku Zasshi*, **82**, 947-951 (1962); *Chem. Abstr.*, **58**, 5,773d (1963).
 (119) A. Momose, *Yakugaku Zasshi*, **82**, 952-955 (1962); *Chem. Abstr.*, **58**, 5,773f (1963).
 (120) A. Momose, *Yakugaku Zasshi*, **82**, 1050-1056 (1962); *Chem. Abstr.*, **58**, 5,630b (1963).
 (121) K. Okui, R. Kaifu, R. Nagashima, and Y. Hinohara, Ger. Offen. 2,124,044 (1972); *Chem. Abstr.*, **77**, 152,517b (1972).

On treatment with hydrazine or phenylhydrazine, the hydrate of 1,2-*O*-isopropylidene- α -D-xylo-5-hexulofuranurono-6,3-lactone³⁴ (**74**) forms¹²² the hydrazino compounds **75** and **76**, respectively. Whereas, because of its lower nucleophilicity, phenylhydrazine does not affect



the lactone carbonyl function, hydrazine simultaneously cleaves the lactone ring with formation of the hydrazide. Compounds **75** and **76** both differ¹²² in their reactions from those observed with aminoalcohols. The reactions of the 1,2-*O*-alkylidene-5-*O*-(methylsulfonyl)- α -D-glucofuranurono-6,3-lactones with hydrazine⁴³ take a different course, justifying their discussion in the context of elimination reactions (see Section VI,5).

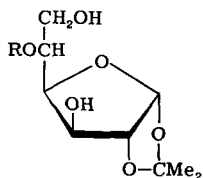
4. Reaction with Complex, Metal Hydrides

The importance of reactions with complex, metal hydrides in carbohydrate chemistry is well documented by a vast number of publications that deal mainly with reduction of carbonyl groups, *N*- and *O*-acyl functions, lactones, azides, and epoxides, as well as with reactions of sulfonic esters. With rare exceptions, lithium aluminum hydride and lithium, sodium, or potassium borohydride are the

(122) H. Paulsen and H. Kuhne, *Carbohydr. Res.*, **13**, 289–292 (1970).

reagents most widely employed.¹²³ Their original selectivities can be modified, to meet the requirements of many specific problems, by the pH, the solvent, the temperature, derivatization, or addition of Lewis acids.

Wolfrom and Wood¹²⁴ (for the first time) demonstrated the reduction of aldonolactones by sodium borohydride at controlled pH-values, to form aldoses. Later, alkyl D-glucofuranosides¹²⁵ and 1,2-O-isopropylidene- α -D-glucofuranose⁹ were prepared from alkyl D-glucofuranosidurono-6,3-lactones and from **33**, respectively, by means of the same reagent. Reduction¹²⁶ of alkyl 2,5-di-O-alkyl-D-glucofuranosidurono- and of 5-O-alkyl-1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactones with lithium aluminum hydride allows the synthesis^{34,55,110} of O-alkylated derivatives of D-glucofuranose. Whereas O-acyl groups are usually removed simultaneously with lactone reduction, esters of *p*-toluene- and methane-sulfonic acid are rather stable under controlled conditions. On treatment of 1,2-O-isopropylidene-5-O-*p*-tolylsulfonyl- α -D-glucofuranurono-6,3-lactone (**52**) with lithium aluminum hydride at low temperature, Irimajiri and coworkers¹²⁷ succeeded in preparing 1,2-O-isopropylidene-5-O-*p*-tolylsulfonyl- α -D-glucofuranose (**77**) in a yield of 48%. Previous attempts⁶¹ to obtain **77**, employing various complex metal hydrides, were unsuccessful. In a re-investigation of the reduction of *p*-



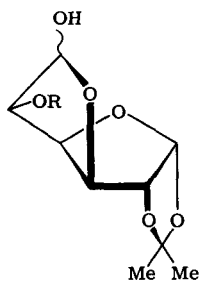
77 R = Ts

78 R = Ms

toluenesulfonic and methanesulfonic esters of **25** (Ref. 110) and **33** (Ref. 128), lithium borohydride in tetrahydrofuran was found to

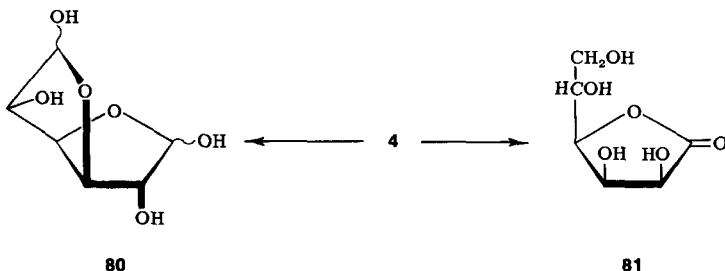
- (123) For reviews, see J. Malek and M. Černý, *Synthesis*, 217-234 (1972), and E. Schenker, in "Neuere Methoden der präparativen organischen Chemie," W. Foerst, ed., Verlag Chemie, Weinheim/Bergstraße, 1966, Vol. IV, pp. 173-293.
- (124) M. L. Wolfrom and H. B. Wood, Jr., *J. Am. Chem. Soc.*, **73**, 2933-2934 (1951).
- (125) D. D. Phillips, *J. Am. Chem. Soc.*, **76**, 3598-3599 (1954).
- (126) S. Roseman, *J. Am. Chem. Soc.*, **74**, 4467-4468 (1952).
- (127) T. Irimajiri, H. Yoshida, T. Ogata, and S. Inokawa, *Bull. Chem. Soc. Jpn.*, **43**, 3242-3245 (1970).
- (128) K. Dax, I. Macher, and H. Weidmann, *J. Carbohydr. Nucleos. Nucleot.*, **1**, 323-336 (1974).

be the preferred reagent. Under these conditions, compound **77**, as well as 1,2-*O*-isopropylidene-5-*O*-(methylsulfonyl)- α -D-glucofuranose (**78**), could be prepared in yields of up to 80%. The ready accessibility of these compounds permitted substantial improvements to be made in the synthesis of L-idose (see Section IX). Similarly, methyl 2,5-di-*O*-*p*-tolylsulfonyl- β -D-glucofuranoside was formed from methyl 2,5-di-*O*-*p*-tolylsulfonyl- β -D-glucofuranosidurono-6,3-lactone¹¹⁰ (**48**). When a tetrahydrofuran solution of lithium borohydride is added to a solution of **52** in the same solvent at 0°, 1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl- α -D-gluc-1,4:6,3-difuranose¹²⁷ (**79**) is the preponderant product. By application¹²⁸ of lithium tri-*tert*-

**79**

butoxyaluminum hydride to **52**, compound **79** was exclusively formed.

Although reaction of **4** with sodium borohydride under slightly acidic conditions forms¹²⁹ D-gluc-ohexodialdose¹³⁰ (**80**), L-gulono-1,4-lactone¹³¹ (**81**) may be obtained¹¹⁰ from **4** in neutral solution if appropriate precautions are taken to avoid formation of D-glucitol.

**80****81**

(129) D. L. MacDonald and H. O. L. Fischer, *J. Am. Chem. Soc.*, **78**, 5025-5026 (1956).

(130) F. G. Fischer and H. Schmidt, *Chem. Ber.*, **93**, 658-662 (1960).

(131) M. L. Wolfrom and K. Anno, *J. Am. Chem. Soc.*, **74**, 5583-5584 (1952).

By applying the same conditions, Bakke and Theander¹³² synthesized L-ascorbic acid from *aldehydo-L-threo*-hex-4-enurono-6,3-lactone obtained by hydrolysis of 1,2-*O*-isopropylidene- α -D-xylo-5-hexulofuranurono-6,3-lactone (74). 1,2-*O*-Isopropylidene- α -D-glucofuranose-5,6,6'- d_3 and 1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone-5-*d* were prepared by treatment of 74 with sodium borodeuteride¹³³ and zinc borodeuteride,¹¹⁰ respectively.

5. Elimination Reactions

Base-catalyzed elimination-reactions appear to be a very common feature of carbohydrate-derived, carboxylic acid derivatives,¹³⁴ although their real nature, and particularly their relationship to various unusual phenomena observed with D-glucuronic acid derivatives, had not been fully recognized before 1973. The opening statement of Section IV,3 of the article in Vol. 28 dealing with the dehydration of glycuronic acids¹³⁵ clearly characterized the situation three years ago: "The mechanism of the aqueous alkaline degradation of uronic acids, of their lactones, and of their esters is unknown." From the earliest investigations concerned with the elucidation of the structure and the chemistry of D-glucuronic acid, its unexpected behavior in alkaline solutions had been observed.

Thus, Goebel and Babers²³ reported the unusual reducing ability of D-glucosiduronolactones as compared to "true" glycosides towards Fehling solution, and Peat and coworkers,²⁴ as well as Osman and coworkers,²⁵ observed the non-stoichiometric consumption of aqueous alkali by compound 25, a property attributed to the formation of unsaturated products. Pryde and Williams,²⁹ for the first time, described the isolation of an unsaturated by-product from the methylation of D-glucuronolactone with methyl iodide-silver oxide. This compound, also obtained²⁴ from 33 by Purdie-Irvine methylation under unspecified conditions, was found to be⁵² 4-deoxy-2,5-di-*O*-methyl-L-*threo*-hex-4-enaro-6,3-lactone methyl ester (43). This compound is readily accessible from D-glucaric acid derivatives by treatment either with diazomethane¹³⁶ or methyl iodide-silver oxide.¹³⁷

(132) J. Bakke and O. Theander, *Chem. Commun.*, 175-176 (1971).

(133) D. W. Mackie and A. S. Perlin, *Can. J. Chem.*, **43**, 2921-2924 (1965).

(134) J. Kiss, *Adv. Carbohydr. Chem. Biochem.*, **29**, 229-303 (1974).

(135) M. S. Feather and J. F. Harris, *Adv. Carbohydr. Chem. Biochem.*, **28**, 161-224 (1973); see Section IV,3, p. 206.

(136) O. T. Schmidt, H. Zeiser, and H. Dippold, *Ber.*, **70**, 2402-2415 (1937).

(137) F. Smith, *J. Chem. Soc.*, 510-517 (1944).

In a very thorough investigation of the reactions of hexaric acid lactones, and particularly dilactones, with sodium methoxide, F. Smith^{138,139} invariably obtained unsaturated products, at that time thought to be formed by "isomerization and enolization." Hence, for alkyl D-glucofuranosidurono-6,3-lactones, also of bicyclic structure, similar reactions were believed^{24,25} to account for their unusual behavior in alkaline solutions.

During the study of reduction of alkyl D-glucofuranosidurono-6,3-lactones and 1,2-O-alkylidene- α -D-glucofuranurono-6,3-lactones with complex, metal hydrides, an unexpected result was obtained¹⁴⁰ that explains the general course of formation of unsaturated products from derivatives of hexofuranuronic acids. Whereas sodium borohydride in protic solvents reduces such compounds as **25**, **26**, **33**, and their derivatives to the corresponding D-glucofuranoses (see Section VI,4), the same reagent in aprotic dipolar solvents¹⁴¹ converted¹⁴⁰ 5-O-benzyl-1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactone (**45**) into 2-O-benzyl-3-deoxy-L-*threo*-hex-2-enono-1,4-lactone¹⁴² (**83**). The yields from this reaction depend⁵⁷ on the donor number¹⁴³ of the solvent, as is evident from the results given in Table III. The results of

TABLE III

Reaction of 5-O-Benzyl-1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactone (**45**) with Sodium Borohydride in Various Aprotic, Dipolar Solvents to Yield 2-O-Benzyl-3-deoxy-L-*threo*-hex-2-enono-1,4-lactone⁵⁷ (**83**)

Solvent	DN ^a	A ^b Yield (%)	B ^b Yield (%)	C ^b Yield (%)
Hexamethylphosphoric triamide	38.8	79 (10)	81 (1.5)	72 (0.75)
Dimethyl sulfoxide	29.8	65 (24)	73 (8)	55 (0.75)
N,N-Dimethylacetamide	27.8	65 (8)	71 (0.75)	47 (0.5)
N,N-Dimethylformamide	26.6	63 (16)	71 (1.5)	37 (1)
Tetramethylene sulfone	14.8	^c (3)	^c (1.5)	^c (0.5)

^a Donor number.¹⁴³ ^b Key: A, at 25°; B, at 45°; C, at 65°. Reaction time (hours) in parentheses. ^c 5-O-Benzyl-1,2-O-isopropylidene- α -D-glucofuranose exclusively.

(138) D. Heslop and F. Smith, *J. Chem. Soc.*, 637-642 (1944).

(139) F. Smith, *Adv. Carbohydr. Chem.*, **2**, 79-106 (1946), Section 5.

(140) H. Weidmann, W. Timpe, and N. Wolf, *Carbohydr. Res.*, **25**, 67-70 (1972).

(141) R. O. Hutchins, D. Hoke, J. Keogh, and D. Koharski, *Tetrahedron Lett.*, 3495-3498 (1969).

(142) *Chem. Abstr.* nomenclature: 3(5*H*)-Furanone, 5-(1,2-dihydroxyethyl)-3-(phenyl-methoxy)-[S-(*R*⁺,*R*⁺)]-.

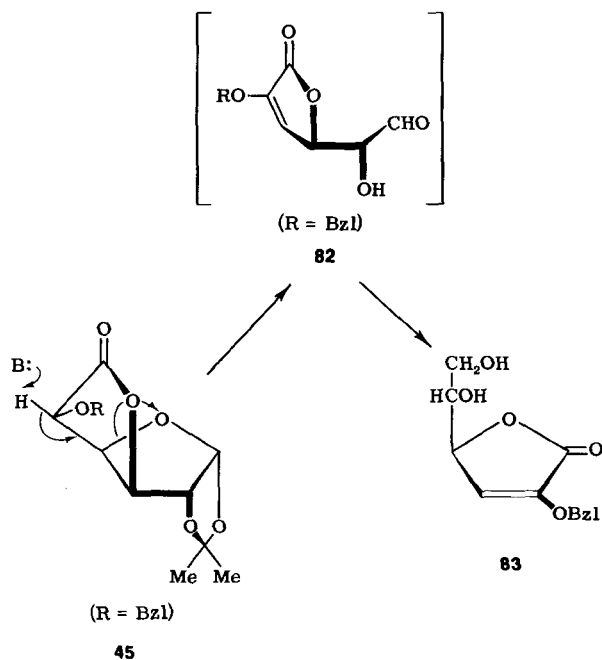
(143) H. Liebig, *Chem.-Ztg.*, **95**, 301-308 (1971).

TABLE IV

Reactions of Derivatives of Hexofuranurono-6,3-lactones with Sodium Borohydride in Hexamethylphosphoric Triamide Yielding Derivatives of Hex-2-enono-1,4-lactones⁵⁷

Compound	Time (hours)	Yield (%)	Product
45	1.5	81	83
46	0.5	80	83
61	18	69	83
Anomer of 61	10	67	83
42	6	45	{ 3-deoxy-2,5-di-O-methyl-L-threo- hex-2-enono-1,4-lactone
27	2	25	
86	4.5	57	{ 2-O-benzyl-3-deoxy-5-O-methyl-L- threo-hex-2-enono-1,4-lactone
Anomer of 86	2.5	57	

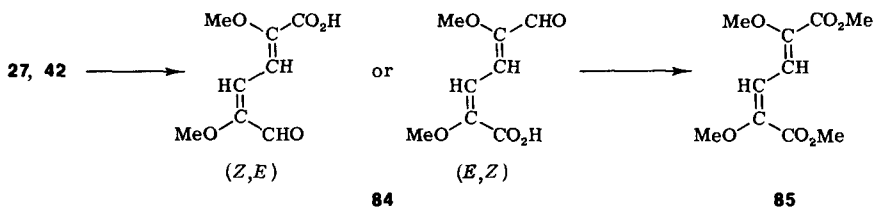
these investigations, summarized in Table IV, clearly demonstrate the generality of this reaction.



The sequence of reactions leading to compound 83 by an elimination that liberates the aldehyde 82, which is immediately reduced to 83, is depicted. This result also explains the formation of 43 by Purdie-Irvine methylations of 4 (Ref. 29) and 33 (Ref. 24); in both, moist silver oxide is the base, and it initiates elimination followed

by oxidation of the intermediate aldehyde group to a carboxylic group which is then esterified.

Because of the reducing or oxidizing properties of the basic reagents just described, the aldehyde initially formed by elimination is only a transient intermediate. Attempts to isolate it by treatment of the anomeric methyl 2,5-di-*O*-methyl-*D*-glucofuranosidurono-6,3-lactones (**27**, **42**) with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU)¹⁴⁴ invariably yielded the doubly eliminated 2,5-dimethoxy-6-oxo-2,4-hexadienoic acid¹⁴⁵ (**84**). As shown in Scheme 2, oxidation of **84** with silver oxide in alkaline solution, followed by esterification, gave dimethyl (*Z,E*)-2,5-dimethoxy-2,4-hexadienedioate¹⁴⁸ (**85**), found to be identical with a product previously obtained by Posternak and coworkers¹⁴⁹ from *D*-glucaric acid.



Scheme 2

In order to decide whether compound **85** is formed from the (*Z,E*) or the (*E,Z*) conformer of **84**, additional information appeared to be necessary. As depicted in Scheme 3, the transformations⁵⁴ of methyl 5-*O*-benzyl-2-*O*-methyl- β -*D*-glucofuranosidurono-6,3-lactone (**86**) to dimethyl (*Z,E*)-2-methoxy-5-(phenylmethoxy)-2,4-hexadienedioate (**87**) conclusively demonstrate that the site of the *E* disposition arises

(144) H. Oediger and F. Möller, *Angew. Chem.*, **79**, 53-54 (1967).

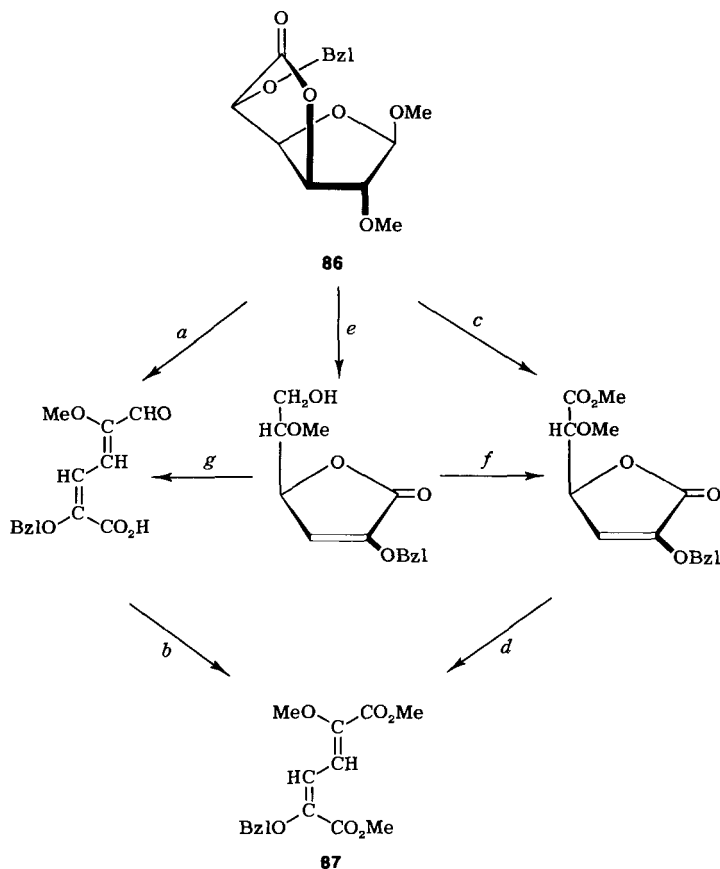
(145) Yoshimura and coworkers¹¹³ anticipated the formation of a compound of this constitution from an elimination of methyl (methyl 2,3,5-tri-*O*-methyl- α -*D*-glucofuranosid)uronate employing potassium *tert*-butoxide. However, the n.m.r. data reported are not consistent^{146,147} with this constitution.

(146) G. P. Newsoroff and S. Sternhell, *Tetrahedron Lett.*, 6117-6122 (1968).

(147) D. H. Williams and I. Fleming, "Spektroskopische Methoden in der organischen Chemie," Georg Thieme Verlag, Stuttgart, 2. Aufl., 1971, pp. 125-131.

(148) K. Dax and H. Weidmann, *Int. Symp. Carbohydr. Chem. VIIth*, Bratislava, Czechoslovakia, August 5-9, 1974.

(149) A. Gabbai, A. Malera, D. Janjic, and T. Posternak, *Helv. Chim. Acta*, **49**, 168-174 (1966).



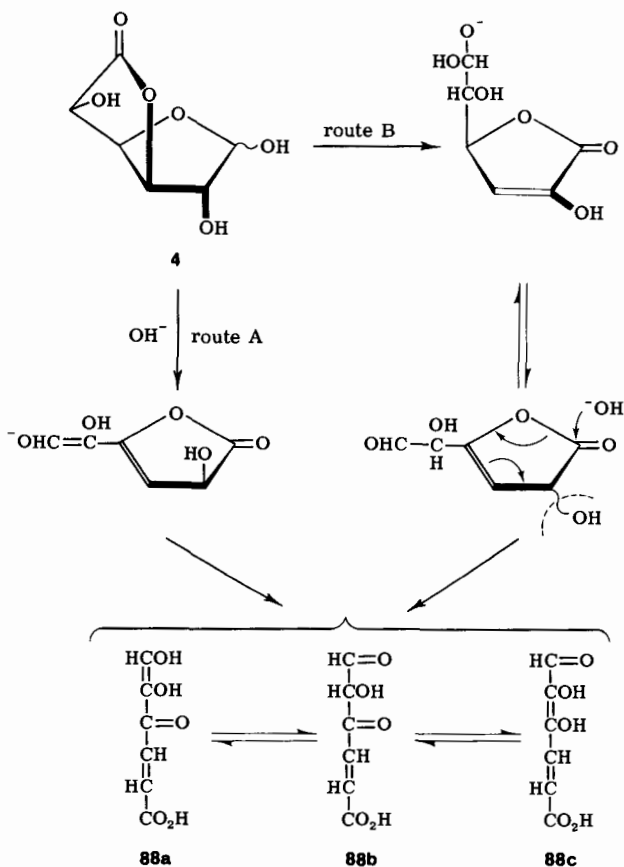
Scheme 3

Transformations of Methyl 5-O-Benzyl-2-O-methyl- β -D-glucofuranosidurono-6,3-lactone (**86**) to Dimethyl (Z,E)-2-Methoxy-5-(phenylmethoxy)-2,4-hexadienedioate⁵⁴ (**87**). (*a*) Elimination employing DBU; (*b*) oxidation with silver oxide-sodium hydroxide followed by diazomethane esterification; (*c*) acidic glycoside cleavage, oxidation by dimethyl sulfoxide-acetic anhydride with formation of 5-O-benzyl-2-O-methyl-D-glucaro-1,4:6,3-dilactone, elimination by using DBU, followed by short treatment with diazomethane; (*d*) elimination by DBU with subsequent diazomethane esterification; (*e*) sodium borohydride in hexamethylphosphoric triamide; (*f*) catalytic oxidation followed by short treatment with diazomethane; (*g*) dimethyl sulfoxide-sulfur trioxide-pyridine-triethylamine.¹⁵⁰⁾

(150) G. M. Cree, D. W. Mackie, and A. S. Perlin, *Can. J. Chem.*, **47**, 511-512 (1969).

from the lactone part of **86**. In these eliminations, derivatives of **26** invariably react at higher rates than derivatives of the corresponding β anomer⁵⁴ (see Section V).

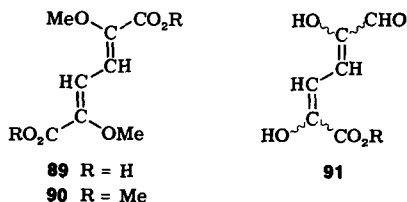
When investigating the formation of color in reactions of D-glucurono-6,3-lactone with alkali, Ishidate and coworkers¹⁵¹ treated **4** with methanolic potassium hydroxide in *N,N*-dimethylformamide, and obtained a crystalline, strongly reducing, yellow compound in low yield. From various degradation reactions¹⁵¹ and polarographic studies,¹⁵² the tautomeric structures (**88 a, b, and c**) were assigned to this compound.



Scheme 4

Because the structure of this compound,¹⁵¹ as well as the course of its formation, as originally proposed (see Scheme 4, route A) and as revised¹³⁵ (Scheme 4, route B), are not in agreement with the general considerations for elimination reactions, a re-examination of this result was indicated. Although the findings of the n.m.r. analysis⁵⁴ (δ 9.15, s, 1 H, aldehydic; 6.64, d, $J \sim 12$ Hz, 1 H, vinylic; 6.40, d, $J \sim 12$ Hz, 1 H, vinylic), as well as the reported¹⁵¹ zero optical rotation, are compatible with the constitution of tautomer **88c**, further reactions with this compound disproved the structural assignments.

Following the general practice of structure elucidation by relating compounds of unknown structure to those with unequivocal constitution, this yellow "reductone" (Ref. 151), prepared as described, was first treated⁵⁴ with diazomethane for enol and carboxyl protection. When subjected to silver oxide oxidation in alkaline solution,¹⁵³ the intermediate, whose n.m.r. analysis showed aldehydic (δ 9.3, s, 1 H), vinylic (7.07 and 6.72; d and 1 H each, $J \sim 12$ Hz), and methyl protons [3.97 (3 H) and 3.85 (6 H)], formed crystalline (Z,Z)-2,5-dimethoxy-2,4-hexadienedioic acid (**89**) showing melting point and n.m.r. data identical with those reported¹⁴⁹ for previous preparations. Final diazomethane esterification yielded⁵⁴ dimethyl (Z,Z)-2,5-dimethoxy-2,4-hexadienedioate¹⁴⁹ (**90**), unequivocally identified. Hence, formation of **89** and **90** cannot be visualized as having originated from structures **88 a, b, or c**. Thus, the constitution of Ishidate's yellow "reductone" must be revised to be that of 2,5-dihydroxy-6-oxo-2,4-hexadienoic acid (**91**). The exclusive isolation¹⁵⁴ of the



(1963); (b) M. Kawata, Y. Mizutani, N. Shinriki, M. Kimura, and M. Ishidate, *ibid.*, **18**, 50-54 (1970).

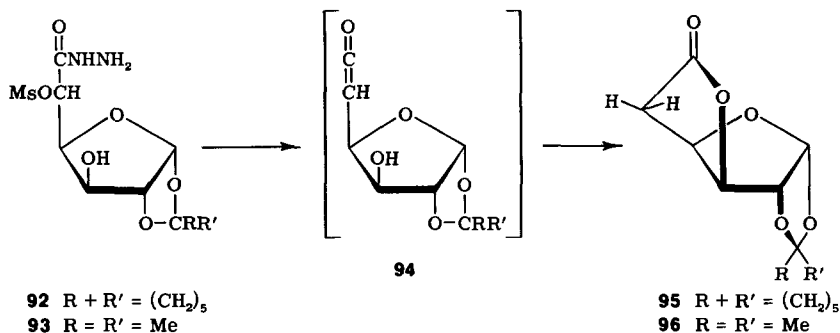
(152) M. Kawata, Y. Mizutani, N. Shinriki, M. Kimura, and M. Ishidate, *Chem. Pharm. Bull.*, **18**, 55-60 (1970).

(153) J. A. Pearl, *Org. Synth.*, **30**, 101-103 (1950).

(154) The crude product isolated from the reaction mixture clearly shows the presence of mixtures of (Z,Z)- and (E,Z)-isomers. Compound **91** could be obtained under the same conditions from **25** in superior yields.

(*Z,Z*)-stereoisomer depends solely on the preponderance of this conformation among the possible conformers, interconvertible by keto-enol equilibria.

The fragmentation of 1,2-*O*-cyclohexylidene-⁴³ (92) and 1,2-*O*-isopropylidene-5-*O*-(methylsulfonyl)- α -D-glucofuranuronic hydrazone¹¹⁰ (93) to yield the corresponding 5-deoxy-D-*xyl*o-hexofuranurono-6,3-lactones 95 and 96, with transient formation of ketenes (94), is a special case of elimination caused by bases.



Unsuccessful attempts⁴¹ to employ a Zemplén degradation reaction for the formation of 1,2-*O*-isopropylidene- α -D-*xyl*o-pentodialdo-1,4-furanose from 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene- α -D-glucofuranuronitrile can also be explained by a rapid elimination competing with the degradation.

VII. REDUCING ABILITY OF ALKYL D-GLUCOFURANOSIDURONIC AND 1,2-*O*-ALKYLIDENE- α -D-GLUCOFURANURONIC ACID DERIVATIVES

All experimental results discussed in the preceding subsection (VI,5) have the same general cause, which may be summarized as follows. On treatment with bases of low nucleophilicity, carbonyl compounds, lactones, esters, and nitriles, containing at least one hydrogen atom at the α - and an appropriate leaving-group at the β -position, give rise to α,β -unsaturated derivatives. This elimination, most probably occurring through an Elcb mechanism,¹⁵⁵ is a common feature¹³⁴ of lactones and esters of aldonic, uronic, and aldaric acids. From this generalization, the close relationship between reactions of glycolactones and alkyl D-glucofuranosidurono- or 1,2-*O*-

(155) J. N. BeMiller and G. V. Kumari, *Carbohydr. Res.*, **25**, 419-428 (1972).

alkylidene- α -D-glucofuranurono-6,3-lactones with bases is immediately obvious (see Section VI,5).

F. Smith¹³⁹ correctly attributed the reducing ability of aldarodilactones and of aldarolactone esters towards complexed Cu(II) in alkaline solution to the formation of enols. Consequently, for a long time, the formation of unsaturated products was thought to be exclusively responsible for the reducing power of the title compounds. In fact, Goebel and Babers,²³ who first observed this "unusual reducing property" with methyl 2,5-di-O-acetyl- β -D-glucofuranosidurono-6,3-lactone, explicitly excluded the participation of a "terminal aldehydic group" in this reaction.

The fact, however, that 2,5-di-O-methyl-D-mannaro-1,4:6,3-dilactone,⁵⁶ in contrast to 1,2-O-isopropylidene-5-O-methyl- α -D-glucofuranurono-6,3-lactone³⁴ (44), does not reduce Benedict solution, constituted early evidence that D-glucofuranurono-6,3-lactones form more than one reducing functionality. The elimination reaction in alkyl D-glucofuranosidurono- and 1,2-O-alkylidene- α -D-glucofuranurono-6,3-lactones, initiated by proton abstraction at C-5 (see 45 \rightarrow 83 by way of 82), is invariably accompanied by the liberation of the aldehyde 82. Thus, the reducing ability of derivatives of D-glucofuranuronic acid toward Fehling or Benedict reagent is caused by formation of enolic, as well as aldehydic, functions.¹⁵⁶ It is this dualism, long unrecognized, that distinguishes hexofuranuronic from aldaric acids in reactions with bases.

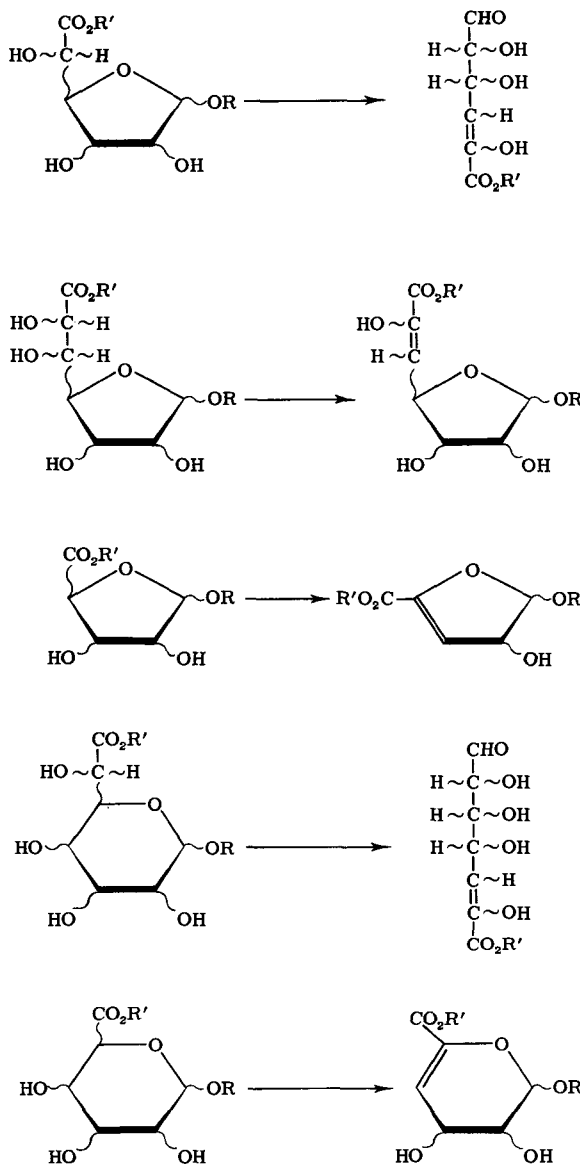
From the reactions shown in Scheme 5, it is obvious that only those uronic acid derivatives whose elimination proceeds with the formation of enolic or aldehydic groups, or both, afford products capable of reducing the Cu(II) ion. Although such structures can be expected from hexo- and hepto-furanuronic, as well as from heptopyranuronic, acid derivatives, glycosides of pentofuranuronic and of hexopyranuronic acid derivatives do not exhibit reducing properties. However, in view of this generalization, the zero reducing power observed for compound 26 requires a different explanation.

A re-examination of the behavior of compounds 25, 26, and 33 towards base, under equal conditions of concentration and temperature, revealed⁴⁰ the following quantitative differences. Whereas compound 25 reduces (the strongly alkaline) Fehling¹⁵⁷ as well as (the weakly basic) Benedict solution,¹⁵⁸ compound 33 reduces the latter only. Compound 26, however, as reported,²⁵ shows no reducing

(156) Most probably, the aldehyde liberated causes further elimination.

(157) H. Fehling, *Ann.*, **72**, 106-113 (1849).

(158) S. R. Benedict, *J. Biol. Chem.*, **5**, 485 (1909).



Scheme 5

power towards either reagent. This result, which is in full agreement with the different rates of lactone hydrolysis⁶³ of **25**, **26**, and **33** (see Section VI,1), can only be explained⁵⁴ in the following way. In all of these substrates, the proton at C-5 competes with the lactone car-

bonyl group for the hydroxide ion. In cases of comparatively weak or intermediate carbonyl activity, as in **25** or **33**, the proton abstraction and lactone hydrolysis have comparable rates at appropriate pH values, so that elimination with formation of the reducing species can occur. In compound **26**, however, lactone saponification is the more rapid reaction. In all instances, elimination is completely inhibited, and the reducing power is lost as soon as the carboxylate anion is formed.

After Yoshimura and coworkers,¹¹³ from their experiments with nineteen different uronic acid derivatives protected at O-1, confirmed the formation of **84** (see Section VI,5), the same authors attributed the observed reducing ability to a "cupric ion catalyzed β -elimination." The exceptionally low values of oxidation-reduction equivalents found for α derivatives of D-glucofuranurono-6,3-lactones and methyl D-glucofuranuronates were thought to be only accounted for by assuming an intermediate substrate-L-tartaric acid-Cu(II) complex coordination. However, considering that complexing of Cu(II) ion with fully O-methylated substrates is most unlikely, the explanation given in the preceding paragraph fully accounts for the different reducing abilities of anomeric substrates.

VIII. OXIDATION REACTIONS

Aldoses may be oxidized by a variety of reagents, among which, bromine in buffered solution,¹⁵⁹ iodine in the presence of aqueous alkali,¹⁶⁰ and oxygen or air in the presence of a platinum catalyst¹⁶¹ are those most commonly used. All of these methods allow the preparation of aldono-1,4- and -1,5-lactones, respectively, in various yields. In contrast to these reagents, silver carbonate on Celite¹⁶² and dimethyl sulfoxide-acetic anhydride,¹⁶³ respectively, are of more limited applicability. The former can only be used in nonpolar solvents, and requires protection of at least the 2-hydroxyl group in order to avoid overoxidation. The dimethyl sulfoxide-acetic anhydride reagent, known to be quite unselective, also oxidizes primary and secondary alcoholic groups.

(159) H. S. Isbell, *Bur. Stand. J. Res.*, **8**, 1692 (1932).

(160) R. L. Colbran and T. P. Nevell, *J. Chem. Soc.*, 2427-2429 (1957).

(161) K. Heyns, H. Paulsen, G. Rüdiger, and J. Weyer, *Fortschr. Chem. Forsch.*, **11**, 285-374 (1969).

(162) S. Morgenlie, *Acta Chem. Scand.*, **26**, 2518-2522 (1972), and previous work cited therein.

(163) Y. Rabinsohn and H. G. Fletcher, Jr., *J. Org. Chem.*, **32**, 3452-3457 (1967).

The oxidation of **4**, although of minor synthetic importance, can be best accomplished with bromine in aqueous solution,¹⁶⁴ or oxygen in the presence of a catalyst¹⁶⁵; with both reagents, mixtures of D-glucaro-1,4- and -6,3-lactones,¹⁶⁶ arising from rapid hydrolysis of the intermediate D-glucaro-1,4:6,3-dilactone, are obtained.¹⁶⁵ 2,5-Di-O-methyl- and 5-O-benzyl-2-O-methyl-D-glucaro-1,4:6,3-dilactone are the sole products formed⁵⁴ in the oxidation of the corresponding D-glucofuranurono-6,3-lactones with dimethyl sulfoxide-acetic anhydride.

As glycoloses are continually gaining importance as intermediates in carbohydrate reactions, an ever-increasing number of reagents for their preparation has become available.¹⁶⁷ In the following description, methods and results of investigations dealing with the oxidation of alkyl D-glucofuranosidurono- and 1,2-O-alkylidene- α -D-glucofuranurono-6,3-lactones, respectively, are described.

As discussed in Sections III and IV, the 5-hydroxyl group in such substrates as **25** and **33** shows a pronounced reactivity in alkylation and acylation reactions. Oxidation of **33** to 1,2-O-isopropylidene- α -D-xylo-5-hexulofuranurono-6,3-lactone (**74**) can be readily accomplished by means of activated manganese dioxide,^{34,168} chromium trioxide in glacial acetic acid,¹³³ dimethyl sulfoxide-phosphorus pentaoxide,¹⁶⁹ oxygen in the presence of platinum,¹⁷⁰ ruthenium tetroxide,¹⁷¹ dimethyl sulfoxide-chlorine,¹¹⁰ and dimethyl sulfide-N-chlorosuccinimide.¹¹⁰ The high yield¹⁷¹ of **74** from 1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactone 5-nitrate clearly shows the influence of the neighboring carbonyl group.

As hydroxyl groups in *exo* positions of bicyclic systems are known¹⁶¹ to be unaffected by catalytic dehydrogenation, compounds **25** and **26** were selectively oxidized¹⁷¹ to the respective anomers of methyl D-xylo-5-hexulofuranosidurono-6,3-lactone. As a corollary of this selectivity, 5-O-protected alkyl D-glucofuranosidurono-6,3-lactones resist¹⁷¹ oxidation with oxygen in the presence of platinum catalysts. The method¹⁷² of base-induced elimination of nitric esters

(164) C. S. Hudson and H. S. Isbell, *J. Am. Chem. Soc.*, **51**, 2225-2229 (1929).

(165) See Ref. 6, p. 46.

(166) F. Smith, *J. Chem. Soc.*, 633-636 (1944).

(167) R. W. Butterworth and S. Hanessian, *Synthesis*, 70-88 (1971).

(168) H. Weidmann and G. Olbrich, *Tetrahedron Lett.*, 725-726 (1965).

(169) K. Onodera, S. Hirano, and N. Kashimura, *Carbohydr. Res.*, **6**, 276-285 (1968).

(170) K. Heyns, E. Alpers, and J. Weyer, *Chem. Ber.*, **101**, 4209-4213 (1968).

(171) K. Dax and H. Weidmann, *Carbohydr. Res.*, **25**, 363-370 (1972).

(172) W. D. Emmons and J. P. Freeman, *J. Am. Chem. Soc.*, **77**, 4415-4416 (1955).

of such substrates suffers from lack of acidity of the hydrogen atom on C-2. Dimethyl sulfoxide-phosphorus pentaoxide proved to be¹⁷¹ the preferred reagent for the oxidation of 5-*O*-substituted methyl D-glucofuranosidurono-6,3-lactones. In oxidations of methyl 5-*O*-benzyl- β -D-glucofuranosidurono-6,3-lactone (**61**) employing ruthenium dioxide-sodium metaperiodate,¹⁷³ partial loss of the 5-*O*-benzyl group with formation of benzoic acid was observed.¹⁷¹

IX. SYNTHESSES STARTING FROM D-GLUCOFURANURONO-6,3-LACTONE

Because the lactone ring of alkyl D-glucofuranosidurono- and 1,2-*O*-alkylidene- α -D-glucofuranurono-6,3-lactones can be readily reduced (see Section VI,4), these compounds are equivalent to 3,6-disubstituted derivatives of D-glucofuranose, in which the 5-hydroxyl group is readily accessible. In addition, transacetalation of 5-*O*-substituted 1,2-*O*-alkylidene- α -D-glucofuranurono-6,3-lactones liberates the 2-hydroxyl group, allowing the synthesis of derivatives containing different substituents at O-2 and O-5, respectively. The accessibility of either the 2- or the 5-hydroxyl group opens up the possibility for selective configurational inversions, with formation of derivatives of various hexofuranurono-6,3-lactones and hexofuranoses.

1. Derivatives of D-Glucofuranurono-6,3-lactone Containing Different Substituents

As mentioned in Section IV, 5-*O*-benzyl-1,2-*O*-isopropylidene- α -D-glucofuranurono-6,3-lactone (**45**) has proved to be a very useful starting-material for various syntheses. Hydrolysis of its isopropylidene group, followed by Purdie-Irvine methylation yielded methyl 5-*O*-benzyl-2-*O*-methyl- α -D-glucofuranosidurono-6,3-lactone.⁵⁷ The corresponding β anomer was obtained⁵⁷ by transacetalation of **45** with methanol, yielding **61** (Ref. 60), and subsequent methyl iodide-silver oxide methylation. Mixed 2,5-diester of **25** were obtained⁶⁰ by esterification of **61**, followed by hydrogenolysis of the 5-*O*-benzyl group, and esterification of the 5-hydroxyl group. Alternatively, compound **25**, after (benzyloxycarbonyl)ation at O-5 (see Section IV), esterification of the 2-hydroxyl group, hydrogenolysis, and esterification of the 5-hydroxyl group, gives the same results.⁶³

(173) H. Nakata, *Tetrahedron*, 19, 1959-1963 (1963).

2. Methyl β -D-Mannofuranosidurono-6,3-lactone

This compound, previously prepared¹⁷⁴ in low yield from alginic acid, has been synthesized¹²⁸ from **61** by oxidation at C-2, and stereo-specific reduction of the intermediate methyl 5-*O*-benzyl- β -D-*arabino*-2-hexulofuranosidurono-6,3-lactone. Hydrogenolysis of the 5-*O*-benzyl group yielded the title compound in a superior yield.

3. L-Idose

The simplified preparations¹²⁸ of 5-*O*-sulfonylated derivatives of 1,2-*O*-isopropylidene- α -D-glucofuranose (**77** and **78**) (see Section VI,4) was the key to substantial improvements in the synthesis of L-idose.

4. Derivatives of 5-Deoxy-D-xylo-hexofuranose

Derivatives of 5-deoxy-D-xylo-hexofuranurono-6,3-lactone may be obtained by starting from 1,2-*O*-alkylidene-5-*O*-(methylsulfonyl)- α -D-glucofuranurono-6,3-lactones by two different procedures.

a. Base-catalyzed Fragmentation⁴³ Employing Hydrazine (see Section VI,6).—This procedure was simplified⁵⁴ by using hydrazine hydrate to form the intermediate 1,2-*O*-alkylidene-5-deoxy- α -D-xylo-hexofuranuronic hydrazide, which was converted into the corresponding lactone by treatment with *N*-bromosuccinimide.¹⁷⁵

b. Replacement of the 5-(Methylsulfonyl)oxy Group by Bromide.—This was followed by reductive cleavage of the carbon-bromine bond.¹²⁷

5. L-Ascorbic Acid

Bakke and Theander¹³² described an interesting, new synthesis of L-ascorbic acid by one-step oxidation of 1,2-*O*-isopropylidene- α -D-glucofuranose to 1,2-*O*-isopropylidene- α -D-xylo-5-hexulofuranurono-6,3-lactone hydrate (**74**), followed by hydrolysis of the isopropylidene group, and specific, borohydride reduction of the aldehyde group liberated.

6. Amino and Diamino Sugars

Hydrogenation of derivatives of glycurononitriles, readily accessible by lactone ammonolysis, and dehydration of the intermediate

(174) H. W. H. Schmidt, *Tetrahedron Lett.*, 235–240 (1967).

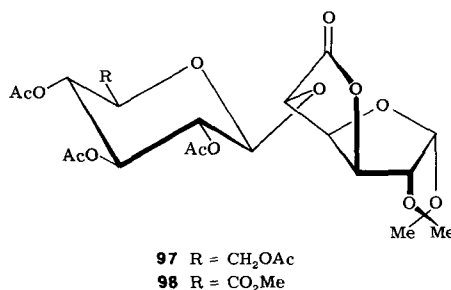
(175) H. T. Cheung and E. R. Blout, *J. Org. Chem.*, **30**, 315–316 (1965).

uronamides, is a general method¹⁷⁶ for the introduction of terminal amino groups. By this procedure, 6-amino-6-deoxy-D-glucose was prepared,⁴⁰ starting from **33**. The synthesis of methyl 3,6-diamino-3,6-dideoxy- β -D-altrofuranoside could be accomplished¹⁷⁷ by starting with **25**. The 6-amino-6-deoxy function was formed by the same principle, but the 3-amino group was introduced by stereoselective ammonolysis of the intermediate methyl 6-amino-2,3-anhydro-6-deoxy- β -D-mannofuranoside.

Starting from 5-O-acetyl-1,2-O-isopropylidene- α -D-glucofuranurono-6,3-lactone, Kinoshita and coworkers¹⁷⁸ achieved the synthesis of 3-amino-3-deoxy-1,2-O-isopropylidene- α -D-allofuranuronic acid by the following sequence of reactions: methanolysis of the lactone ring, oxidation at C-3 to form methyl 5-O-acetyl-1,2-O-isopropylidene- α -D-ribo-3-hexulofuranuronate, followed by stereospecific reduction of the oxime of the latter.

Catalytic hydrogenation of the oxime of D-glucurono-6,3-lactone leads¹⁷⁹ to simultaneous isomerization with formation of L-gulono-1,6-lactam.

Compound **33** also lends itself to the synthesis of 1,5-linked disaccharides. Thus, its condensation¹⁸⁰ with 2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl bromide, and with methyl 2,3,4-tri-O-acetyl- α -D-glucopyranosyluronate bromide, respectively, yields the corresponding β -D-linked disaccharides (**97** and **98**).



Compounds **97** and **98** can be subjected¹⁸⁰ to selective lactone ammonolysis under conditions not affecting the ester groups. Reduction

(176) H. Weidmann and H. K. Zimmerman, Jr., *Ann.*, **641**, 132-137, 138-142 (1968).

(177) H. Weidmann, E. Wildschek, D. Wewerka, and N. Wolf, *Abstr. Pap. Am. Chem. Soc. Meet.*, **154**, D25 (1967).

(178) A. Tsuji, T. Kinoshita, and M. Maeda, *Chem. Pharm. Bull.*, **16**, 539-543 (1968).

(179) H. Weidmann and E. Fauland, *Ann.*, **679**, 192-194 (1964).

(180) M. Appenroth (1970), and K. R. Leipert (1973), Doctor's theses, Technical University, Graz, Austria.

of **97** and **98** with sodium borohydride is similarly selective, affording disaccharides of 1,2-*O*-isopropylidene- α -D-glucofuranose. The application of the glycuronitrile procedure to **97** and **98** allows¹⁸⁰ the synthesis of disaccharides containing 6-amino-6-deoxy, as well as 6'-amino-6'-deoxy, functions.

THE CHEMISTRY OF SUCROSE

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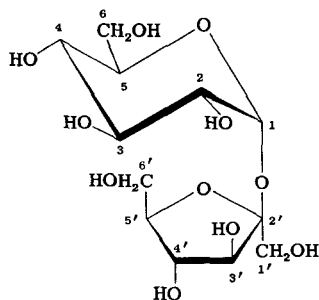
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I. INTRODUCTION

Sucrose is one of the leading world-commodities: its current annual production in all forms exceeds ninety million tons. The potential of this regenerable, almost ubiquitous, natural product as a chemical raw-material has been extensively explored. However, the actual commercial success achieved has, so far, been insignificant. This can be attributed primarily to the lack of understanding of the basic chemistry of sucrose. During the last decade, efforts have, therefore, been concentrated on the study of the fundamental aspects of the chemistry of this molecule. The development of improved, or modern, synthetic methods and analytical techniques has led to the preparation and characterization of a large number of sucrose derivatives on which its commercial utilization may hopefully be based.

Three surveys on the chemistry of sucrose have appeared during the past five years.¹⁻³ Nevertheless, as progress in this field has been rapid, a further article on this subject is now justified. In this Chapter, an attempt has been made to collate information on the reactions of sucrose, and to illustrate some of the physical methods that have contributed to the characterization of sucrose derivatives. In addition, some of the potential commercial applications of compounds derived from sucrose have been considered briefly.

II. STRUCTURE AND SYNTHESIS



Sucrose*

(β-D-Fructofuranosyl α-D-glucopyranoside)

1

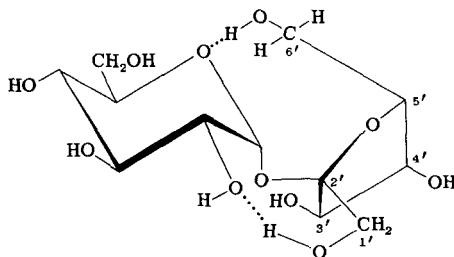
* The numbering of the carbon atoms (when the trivial name is used) is shown in formula 1. Three of the hydroxyl groups (at C-6, C-1', and C-6') are primary, and the rest are secondary.

- (1) L. Hough, in "Sugar," J. Yudkin, J. Edelman, and L. Hough, eds., Butterworth, London, 1972, pp. 49-59.

Sucrose (1), systematically named β -D-fructofuranosyl α -D-glucopyranoside, is a nonreducing disaccharide. An excellent article on the structure and configuration of sucrose has appeared in this Series.⁴ Synthesis of sucrose by enzymic methods has been achieved through glycosylation involving free D-fructose and either D-glucose 6-phosphate^{5,6} or D-glucose.⁷ The structure was finally confirmed by Lemieux and Huber in 1953 by chemical synthesis.⁸ Treatment of 3,4,6-tri-O-acetyl-1,2-anhydro- α -D-glucopyranose with 1,3,4,6-tetra-O-acetyl-D-fructofuranose in a sealed tube for 104 h at 100° gave octa-O-acetylsucrose in 5.5% yield.⁸ Several other chemical syntheses have since then been reported.⁹⁻¹¹ However, in none of these syntheses⁹ has the yield exceeded 6.6%.

Such physical methods as X-ray crystallography, neutron diffraction, and nuclear magnetic resonance (n.m.r.) spectroscopy have proved of immense value in determining the configuration and the conformation of the sucrose molecule. An X-ray diffraction study of sucrose sodium bromide dihydrate by Beevers and Cochran¹² confirmed the chemically assigned, relative configurations of the asymmetric carbon atoms of the molecule. A similar investigation of sucrose itself was somewhat less satisfactory.¹³ Neutron diffraction has been used to elucidate the precise molecular structure of sucrose in the crystal.¹⁴ Seven of the hydroxyl groups in the anhydrous crystal are hydrogen-bonded, including two intramolecular bonds (O-2---H—O-1' and O-5---H—O-6') as shown in 1a. Although the 4-hydroxyl group does not participate in hydrogen bonding, its hydrogen atom has been found to be loosely fixed in position through two fairly close contacts with oxygen atoms in other sucrose molecules.¹⁴

-
- (2) R. Khan, in "Molecular Structure and Function of Food Carbohydrates," G. G. Birch and L. F. Green, eds., Applied Science Publishers Ltd., London, 1973, pp. 33-49.
 - (3) K. J. Parker, *Sucr. Belge*, **93**, 15-27 (1974).
 - (4) I. Levi and C. B. Purves, *Adv. Carbohydr. Chem.*, **4**, 1-35 (1949).
 - (5) W. Z. Hassid, M. Doudoroff, and H. A. Barker, *J. Am. Chem. Soc.*, **66**, 1416-1419 (1944).
 - (6) W. Z. Hassid and M. Doudoroff, *Adv. Enzymol.*, **10**, 123-143 (1950).
 - (7) H. Kauss, *Z. Naturforsch.*, **17b**, 698-699 (1962).
 - (8) R. U. Lemieux and G. Huber, *J. Am. Chem. Soc.*, **75**, 4118 (1953).
 - (9) H. Tsuchida and M. Komoto, *Agric. Biol. Chem.*, **29**, 239-242 (1963).
 - (10) R. K. Ness and H. G. Fletcher, Jr., *Carbohydr. Res.*, **17**, 465-470 (1971).
 - (11) B. Fraser-Reid, *Int. Symp. Carbohydr. Chem. VIIth, Bratislava* (1974).
 - (12) C. A. Beevers and W. Cochran, *Proc. Roy. Soc. London, Ser. A*, **190**, 257-272 (1947).
 - (13) C. A. Beevers, T. R. R. McDonald, J. H. Robertson, and F. Stern, *Acta Crystallogr.*, **5**, 689-690 (1952).
 - (14) G. M. Brown and H. A. Levy, *Science*, **141**, 921-923 (1963).



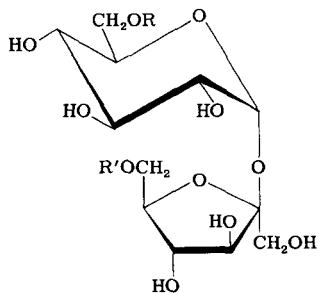
1a

III. ETHERS

1. Trityl Ethers

Trityl ethers of carbohydrates have attracted considerable interest, because primary hydroxyl groups may be readily and selectively protected by the trityl group, and subsequently regenerated under mild conditions.¹⁵ The tritylation of sucrose has been widely investigated. Somewhat selective tritylation of sucrose has been achieved with 1.2 molar equivalents of chlorotriphenylmethane in pyridine for 96 h at room temperature, to give a mixture containing monotrityl ethers and higher ethers in the ratio¹⁶ of 1:1. The mono-*O*-tritylsucroses were isolated from the mixture by countercurrent distribution. Crystallization of the resulting syrup, and fractional recrystallization of the product afforded 6-*O*-tritylsucrose (**2**), with m.p. 99–101°, in 35% yield. After further chromatography on silica gel, the residue gave 6'-*O*-tritylsucrose (**3**) as an amorphous powder in 36% yield. It was later found that the fraction containing the two mono-*O*-tritylsucroses could conveniently be isolated in over 50% yield from the original reaction-mixture¹⁶ by eluting from a column of silica gel with 1:1 dichloromethane–acetone.¹⁷

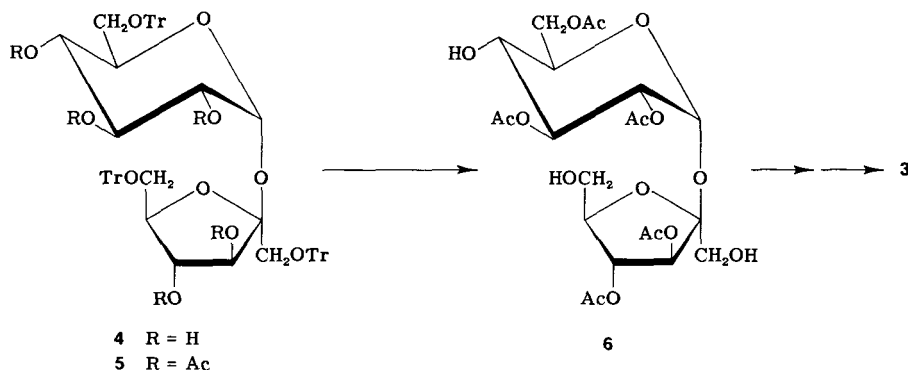
An unambiguous synthesis of 6'-*O*-tritylsucrose was subsequently



2 R = Ph₃C, R' = H

3 R = H, R' = Ph₃C

achieved by Buchanan and coworkers.¹⁸ 6,1',6'-Tri-*O*-tritylsucrose^{19,20} (4), prepared in 66% yield, was converted into the corresponding pentaacetate (5) by treatment with acetic anhydride and pyridine. Detritylation of compound 5 in boiling, aqueous acetic acid occurred, with the expected O-4 → O-6 migration of the acetyl group,²¹⁻²³ to give crystalline 2,3,6,3',4'-penta-*O*-acetylsucrose (6) in 43% yield. Treatment of 1 mole of 6 with 1.52 moles of chloro-



triphenylmethane in pyridine for 20 h at 50°, followed by catalytic de-esterification with sodium methoxide in methanol, gave the ether 3 in 52% yield.¹⁸

Synthesis of 2,3,4,1',3',4',6'-hepta-*O*-acetyl-6-*O*-tritylsucrose (9) has also been achieved by an unambiguous route.²⁴ Treatment of 2,3,1',3',4',6'-hexa-*O*-acetyl-4,6-*O*-benzylidenesucrose²⁵ (7) with aqueous acetic acid for 6 min at 100° gave 2,3,1',3',4',6'-hexa-*O*-acetylsucrose (8) which, on tritylation with chlorotriphenylmethane in pyridine followed by conventional acetylation with acetic anhydride, afforded²⁴ compound 9.

(15) B. Helferich, *Adv. Carbohydr. Chem.*, **3**, 79-106 (1948).

(16) T. Otake, *Bull. Chem. Soc. Jpn.*, **43**, 3199-3205 (1970).

(17) R. Khan, Brit. Pat. Application (1973).

(18) J. G. Buchanan, D. A. Cummers, and D. M. Turner, *Carbohydr. Res.*, **21**, 283-292 (1972).

(19) K. Josephson, *Ann.*, **472**, 230-240 (1929).

(20) G. G. McKeown, R. S. E. Serenius, and L. D. Hayward, *Can. J. Chem.*, **35**, 28-36 (1957).

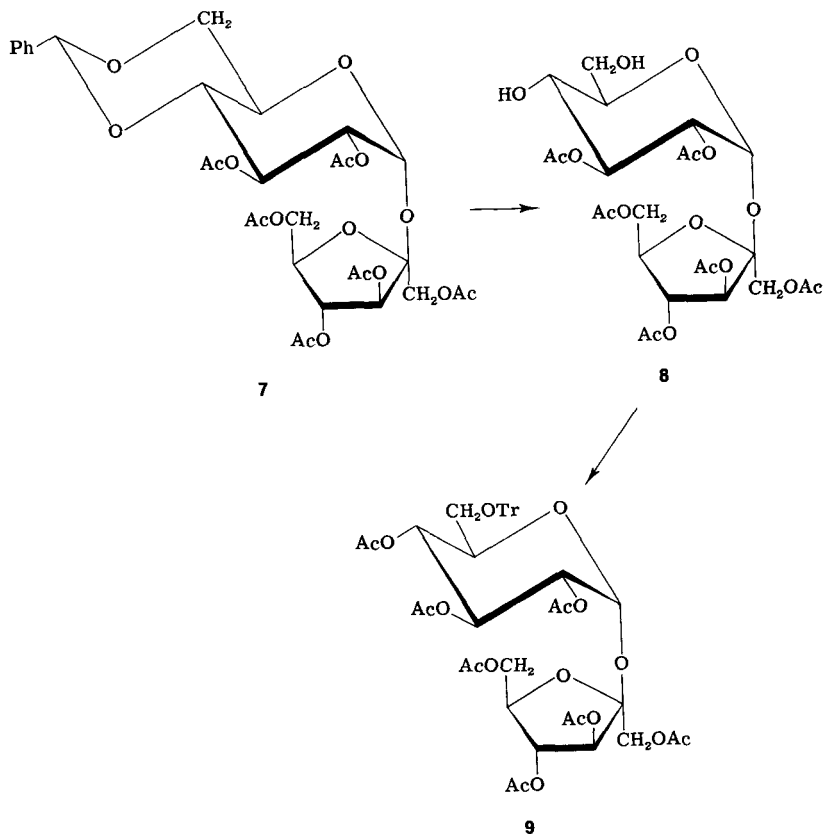
(21) R. U. Lemieux and J. P. Barrette, *J. Am. Chem. Soc.*, **80**, 2243-2246 (1958).

(22) H. Bredereck, H. Zinner, A. Wagner, G. Faber, W. Greiner, and W. Huber, *Chem. Ber.*, **91**, 2824-2829 (1958).

(23) T. Suami, T. Otake, S. Ogawa, T. Shoji, and N. Kato, *Bull. Chem. Soc. Jpn.*, **43**, 1219-1223 (1970).

(24) R. Khan and M. R. Jenner, unpublished data.

(25) R. Khan, *Carbohydr. Res.*, **32**, 375-379 (1974).

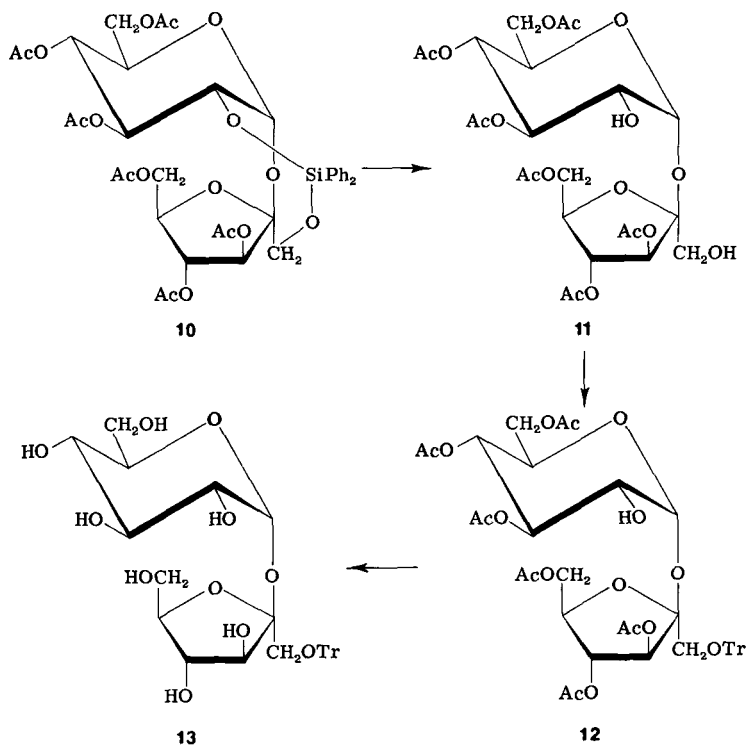


Selective tritylation of the primary hydroxyl groups at C-6 and C-6' was indicated by the absence of 1'-*O*-tritylsucrose (13) from the fraction containing the mono-*O*-tritylsucroses.¹⁶ Synthesis of compound 13 by unambiguous routes has been achieved.^{26,27} Treatment of 3,4,6,3',4',6'-hexa-*O*-acetyl-2,1'-*O*-(diphenylsilyl)sucrose (10) with aqueous acetic acid gave 3,4,6,3',4',6'-hexa-*O*-acetylsucrose (11) which, on being heated with chlorotriphenylmethane in pyridine for 24 h at 60°, afforded 3,4,6,3',4',6'-hexa-*O*-acetyl-1'-*O*-tritylsucrose (12) in 95% yield. De-esterification of 12 with sodium methoxide in methanol gave compound 13 in 90% yield.²⁷

Tetramolar tritylation of sucrose in pyridine for 2 days at room temperature gave, after column chromatography on silica gel, 6,6'-di-

(26) T. Otake, *Bull. Chem. Soc. Jpn.*, **47**, 1938–1944 (1974).

(27) M. R. Jenner and R. Khan, *Brit. Pat. Application* (1974); unpublished data.

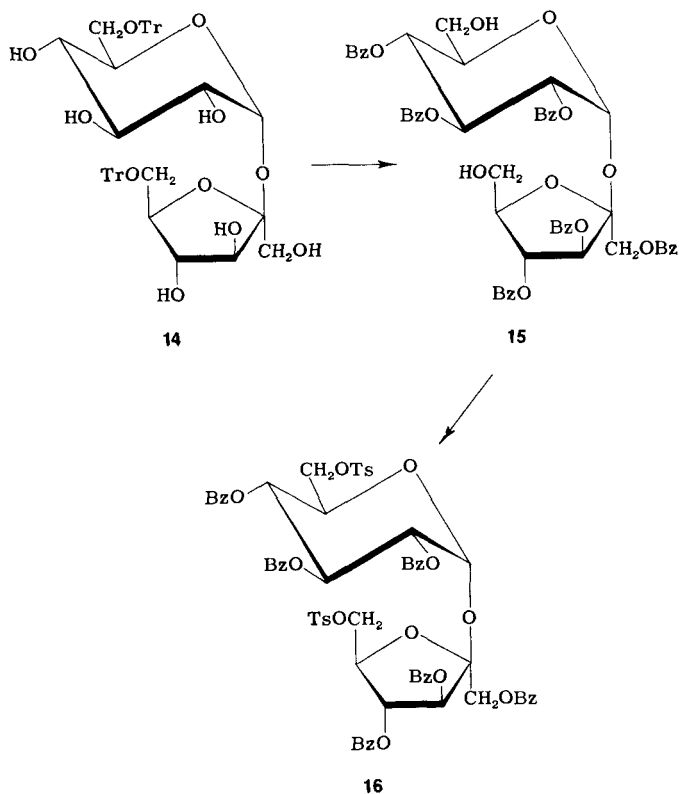


O-tritylsucrose (**14**) and 6,1',6'-tri-*O*-tritylsucrose (**4**) in yields of 30 and 58%, respectively.²⁸ The structure of the crystalline dinitrile ether **14** was ascertained by converting it into the known²⁹ 2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-di-*O*-*p*-tolylsulfonysucrose (**16**). On treatment with benzoyl chloride in pyridine followed by detritylation, either with hydrogen bromide in glacial acetic acid at 0° or boiling aqueous acetic acid, compound **14** gave 2,3,4,1',3',4'-hexa-*O*-benzoylsucrose (**15**). No migration of the benzoyl group during the de-etherification reaction was observed. The reaction of **15** with *p*-toluenesulfonyl chloride in pyridine gave the 6,6'-disulfonate (**16**) and this confirmed the location of the trityl groups in **14**.

When 1 mole of sucrose was treated with 2 moles of chlorotriphenylmethane in pyridine for 96 h at room temperature, it gave, after counter-current distribution and column chromatography, mono-*O*-tritylsucroses, di-*O*-tritylsucroses, and 6,1',6'-tri-*O*-trityl-

(28) L. Hough, K. S. Mufti, and R. Khan, *Carbohydr. Res.*, **21**, 144–147 (1972).

(29) C. H. Bolton, L. Hough, and R. Khan, *Carbohydr. Res.*, **21**, 133–143 (1972).

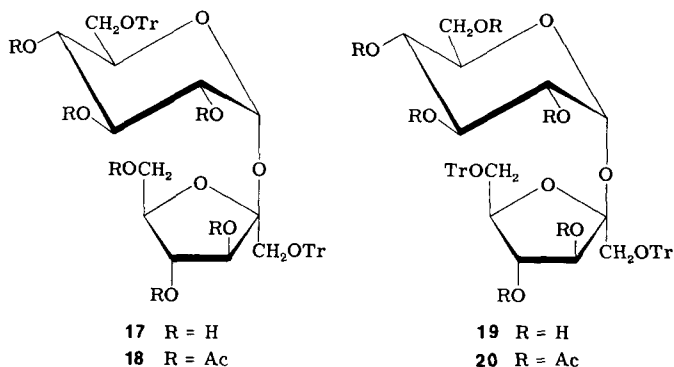


sucrose (4) fractions in yields of 27, 37, and 17%, respectively.³⁰ The fraction containing the di-*O*-tritylsucroses afforded, after fractional recrystallization, 6,6'-di-*O*-tritylsucrose (14) and 6,1'-di-*O*-tritylsucrose (17) in 74 and 12% yield, respectively. The residue gave, after further chromatography on silica gel, 1',6'-di-*O*-tritylsucrose (19) in 14% yield. The structures of 17 and 19 have been confirmed on the basis of proton nuclear magnetic resonance (¹H n.m.r.) spectra of their respective peracetates (18 and 20). An unambiguous synthesis of 2,3,4,3',4',6'-hexa-*O*-acetyl-6,1'-di-*O*-tritylsucrose (18) has been achieved.³¹ Treatment of 3,3',4',6'-tetra-*O*-acetyl-2,1':4,6-di-*O*-isopropylidenesucrose³² with aqueous acetic acid gave 3,3',4',6'-tetra-*O*-acetylsucrose which, on heating with chlorotriphenylmethane in pyridine for 48 h at 65°, afforded 3,3',4',6'-tetra-*O*-acetyl-6,1'-di-*O*-tri-

(30) T. Otake, *Bull. Chem. Soc. Jpn.*, **45**, 2895-2898 (1972).

(31) R. Khan, unpublished data.

(32) R. Khan and K. S. Mufti, *Carbohydr. Res.*, **43**, 247-253 (1975).



tylsucrose. The ditrityl ether gave **18** in 75% yield on conventional acetylation with acetic anhydride in pyridine. Compound **20** has also been synthesized unambiguously.³³ The sucrose pentaacetate **6** was treated with chlorotriphenylmethane in pyridine for 20 h at 100°, to give, after acetylation with acetic anhydride, 2,3,4,6,3',4'-hexa-*O*-acetyl-1',6'-di-*O*-tritylsucrose (**20**) in 55% yield.³³

2. Methyl Ethers

The value of methylation studies in structural determination of carbohydrates is well known. Methylation of sucrose has generally been achieved by the use of dimethyl sulfate-sodium hydroxide,^{34,35} methyl iodide-silver oxide-acetone,²⁰ sodium hydride-methyl iodide-*N,N*-dimethylformamide,³⁵ or diazomethane-boron trifluoride etherate.^{36,37} The last method (already applied to monosaccharides^{38,39}) has been found particularly useful for sucrose, because it proceeds without concomitant migration of acyl groups. The reaction of 2,3,6,1',3',4',6'-hepta-*O*-acetylsucrose (**21**) and 2,3,4,6,1',3',4'-hepta-*O*-acetylsucrose (**22**) with diazomethane in dichloromethane in the presence of a catalytic proportion of boron trifluoride etherate for 0.5 h at -5° gave the corresponding 4-methyl (**23**) and 6'-methyl (**24**)

(33) J. G. Buchanan and D. M. Cummmerson, *Carbohydr. Res.*, **21**, 293-296 (1972).

(34) E. G. V. Percival, *J. Chem. Soc.*, 648-653 (1935).

(35) H. Bredereck, G. Hagelloch, and E. Hambsch, *Chem. Ber.*, **87**, 35-37 (1954).

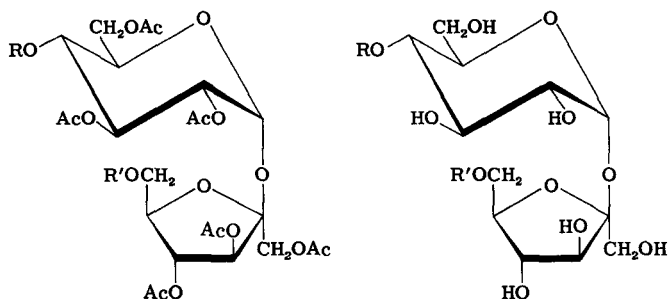
(36) M. G. Lindley, Ph.D. Thesis, University of Reading, England (1974).

(37) M. G. Lindley, G. G. Birch, and R. Khan, *Carbohydr. Res.*, **43**, 360-365 (1975).

(38) E. G. Gros and S. M. Flematti, *Chem. Ind. (London)*, 1556-1557 (1966).

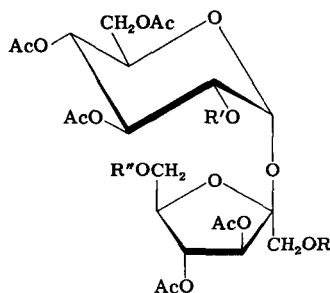
(39) J. O. Deferrari, E. G. Gros, and I. O. Mastronardi, *Carbohydr. Res.*, **4**, 432-434 (1967); E. G. Gros and I. O. Mastronardi, *ibid.*, **10**, 318-321 (1969); W. E. Dick, B. G. Baker, and J. E. Hodge, *ibid.*, **6**, 52-62 (1968); C. P. J. Glaudemans and H. G. Fletcher, Jr., *ibid.*, **7**, 480-482 (1968); P. A. Seib, *ibid.*, **8**, 101-109 (1968).

ethers.^{36,37} Little or no migration of acetyl groups was observed. Catalytic de-esterification of **23** and **24** with sodium methoxide in methanol gave the free methyl ethers (**25**) and (**26**), respectively. Likewise, treatment of 2,3,4,6,3',4'-hexa-*O*-acetylsucrose (**27**) and 3,4,6,3',4',6'-hexa-*O*-acetylsucrose (**28**) with diazomethane-boron trifluoride etherate in dichloromethane gave the expected 1',6'-dimethyl³⁷ (**29**) and 2,1'-dimethyl⁴⁰ (**30**) ethers, respectively. Compound **30** constitutes the first example of a crystalline, methyl ether



- 21** R = H, R' = Ac
22 R = Ac, R' = H
23 R = Me, R' = Ac
24 R = Ac, R' = Me

- 25** R = Me, R' = H
26 R = H, R' = Me



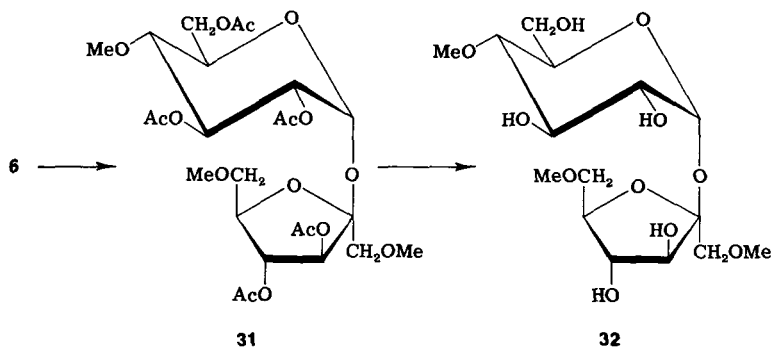
- 27** R = R'' = H, R' = Ac
28 R = R' = H, R'' = Ac
29 R = R'' = Me, R' = Ac
30 R = R' = Me, R'' = Ac

derivative of sucrose.⁴⁰

Synthesis of 4,1',6'-tri-*O*-methylsucrose (**32**) has been achieved by treating 2,3,6,3',4'-penta-*O*-acetylsucrose (**6**) with methyl iodide and

(40) R. Khan and M. R. Jenner, unpublished data.

silver oxide in acetone.²⁰ De-esterification of the corresponding 4,1',6'-trimethyl ether (31) with a basic ion-exchange resin gave the free ether 32, whose structure was confirmed by acid hydrolysis



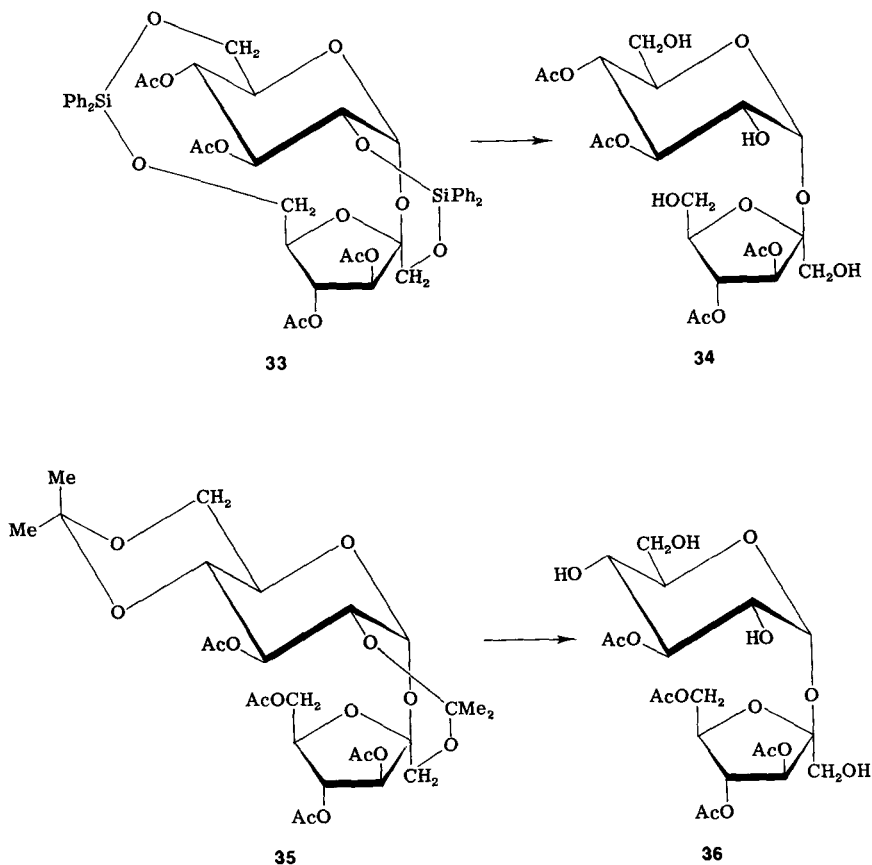
and identification of the resulting *O*-methylhexoses. An alternative synthesis of 32 in 82% yield has been achieved by methylation of compound 6 with diazomethane–boron trifluoride etherate.³⁷ The reaction of the addition compound of sucrose and potassium hydroxide ($\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot 3\text{KOH}$) with dimethyl sulfate was described by E. G. V. Percival as giving 6,1',6'-tri-*O*-methylsucrose.³⁴ An unambiguous synthesis of this compound was subsequently reported.³⁷ Methylation of 2,3,4,3',4'-penta-*O*-acetylsucrose with diazomethane–boron trifluoride etherate gave, after chromatographic separation, the corresponding 6,1',6'-trimethyl ether which, on de-esterification, afforded free 6,1',6'-tri-*O*-methylsucrose in 77% yield.

Octa-*O*-methylsucrose has been prepared by treating sucrose with either dimethyl sulfate–sodium hydroxide, or sodium hydride and methyl iodide in *N,N*-dimethylformamide.³⁵

IV. ESTERS

1. Acetates, Benzoates, and 3-Benzoylpropionates

Trityl ethers and acetals of sucrose have generally been used as precursors for the synthesis of partially acylated derivatives of sucrose. Deacetalation of 3,4,3',4'-tetra-*O*-acetyl-2,1':6,6'-di-*O*-(diphenylsilyl)sucrose (33) and 3,3',4',6'-tetra-*O*-acetyl-2,1':4,6-di-*O*-isopropylidenesucrose (35) with aqueous acetic acid for 25 min at 50° gave 3,4,3',4'-tetra-*O*-acetylsucrose²⁷ (34) and 3,3',4',6'-tetra-*O*-acetylsucrose³² (36), respectively. Synthesis of 2,3,4,3',4'-penta-*O*-acetyl-

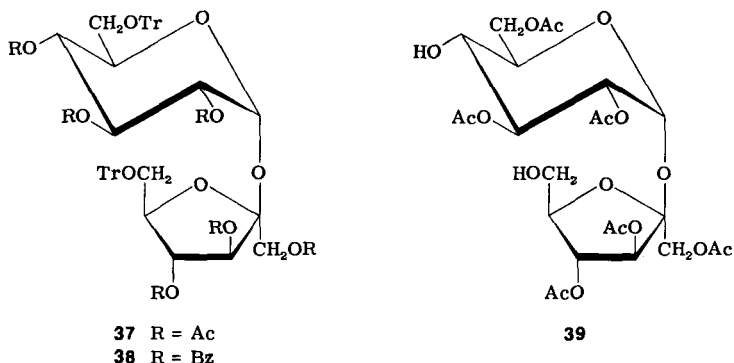


sucrose²² and 2,3,4,3',4'-penta-*O*-benzoylsucrose⁴¹ from their corresponding 6,1',6'-tri-*O*-tritylsucrose derivatives has been achieved by treatment with hydrogen bromide in glacial acetic acid in a mixture of chloroform and glacial acetic acid for 5–10 min at 0°. When detritylation of 2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-tri-*O*-tritylsucrose was conducted in boiling, aqueous acetic acid, it resulted in acetyl migration from O-4 → O-6, probably by way of the 4,6-ortho-ester, to give^{18,21–23} 2,3,6,3',4'-penta-*O*-acetylsucrose (6). Similar detritylation of 2,3,4,3',4'-penta-*O*-benzoyl-6,1',6'-tri-*O*-tritylsucrose with boiling, aqueous acetic acid gave 2,3,4,3',4'-penta-*O*-benzoylsucrose, with little or no ester migration.⁴¹

Similar results were obtained when detritylation of 2,3,4,1',3',4'-hexa-*O*-acetyl-6,6'-di-*O*-tritylsucrose (37) and 2,3,4,1',3',4'-hexa-*O*-

(41) R. Khan, *Carbohydr. Res.*, **22**, 441–445 (1972).

benzoyl-6,6'-di-*O*-tritylsucrose (**38**) was studied. De-etherification of **37** in boiling, aqueous acetic acid occurred, with the expected *O*-4 → *O*-6 migration of the acetyl group, to give⁴² 2,3,6,1',3',4'-hexa-*O*-acetylsucrose (**39**). Under similar conditions of detritylation, compound **38** afforded 2,3,4,1',3',4'-hexa-*O*-benzoylsucrose (**15**), suggesting that, unlike acetyl substituents, the benzoyl groups of compound **38** do not migrate.²⁸



Selective de-esterification of octa-*O*-acetylsucrose with alumina has been reported.^{43,44} A solution of octa-*O*-acetylsucrose in chloroform was placed on a column of silica gel (Laporte Type H) and allowed to stay in contact therewith for 44 h. The column was then eluted with ethyl acetate, to afford 2,3,4,6,1',3',4'-hepta-*O*-acetylsucrose¹⁸ (**22**), 2,3,6,1',3',4',6'-hepta-*O*-acetylsucrose⁴⁵ (**21**), and 2,3,4,6,1',3',6'-hepta-*O*-acetylsucrose in yields of 9, 2.7, and 6%, respectively.⁴⁴

Preparation of compounds **21** and **22** has been achieved in much better yields from a crude mixture of mono-*O*-tritylsucrose heptaacetates by the following reaction sequence.⁴⁵ Detritylation of the mixture with hydrogen bromide in glacial acetic acid–chloroform–glacial acetic acid at 0° gave a mixture of sucrose heptaacetates from which compound **22** crystallized out in 30% yield. The resulting syrup gave, on successive tritylation, detritylation (with boiling, aqueous acetic acid), retritulation, and chromatographic separation on silica gel, compound **21** in 5% overall yield, based on sucrose.⁴⁵ Prepara-

(42) R. Khan, *Carbohydr. Res.*, **25**, 232–236 (1972).

(43) J. M. Ballard, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, **24**, 152–153 (1972).

(44) J. M. Ballard, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, **34**, 184–188 (1974).

(45) R. Khan and K. J. Parker, Brit. Pat. Application (1973).

tion of octa-*O*-acetylsucrose by conventional methods has been reported.⁴⁶⁻⁵²

The potential of the 3-benzoylpropionyl group for the protection of hydroxyl groups has been demonstrated both for nucleosides^{53,54} and for sugars.^{55,56} The reaction of 2,3,4,3',4'-penta-*O*-benzoylsucrose and 2,3,4,1',3',4'-hexa-*O*-benzoylsucrose (15) with 3-benzoylpropionic acid and *N,N'*-dicyclohexylcarbodiimide in pyridine for 24 h at room temperature gave the corresponding 6,1',6'-tri-*O*-(3-benzoylpropionyl) and 6,6'-di-*O*-(3-benzoylpropionyl) derivatives.⁵⁶ The 3-benzoylpropionic esters were readily cleaved on treatment with hydrazine hydrate-acetic acid-pyridine. The reaction of simple esters of 3-benzoylpropionic acid with hydrazine hydrate-acetic acid-pyridine is known to give 4,5-dihydro-6-phenyl-3-pyridazinone.⁵⁷

2. Sulfonates

The value of sugar sulfonates in structural determination and as synthetic intermediates is well known.^{58,59} The selective *p*-toluenesulfonylation of sucrose has been widely investigated.^{29, 41,60-65} Trimolar *p*-toluenesulfonylation of sucrose to give a tri-*O*-*p*-tolylsulfonysucrose was first reported by Hockett and Zief.⁶⁰ On the basis of the elemental analysis and the greater reactivity of primary as compared to secondary hydroxyl groups, its structure was assumed to be that of 6,1',6'-tri-*O*-*p*-tolylsulfonysucrose (40). Subsequent examination by Bragg and J. K. N. Jones⁶¹ showed that,

(46) P. Schutzenberger, *Compt. Rend.*, **61**, 485-486 (1865).

(47) A. Herzfeld, *Z. Ver. Deut. Zucker-Ind.*, **37**, 422-423 (1887).

(48) C. S. Hudson and J. M. Johnson, *J. Am. Chem. Soc.*, **37**, 2748-2753 (1915).

(49) G. J. Cox, J. H. Ferguson, and M. L. Dodds, *Ind. Eng. Chem.*, **25**, 968-970 (1933).

(50) R. P. Linstead, A. Rutenberg, W. G. Dauben, and W. L. Evans, *J. Am. Chem. Soc.*, **62**, 3260-3263 (1940).

(51) A. Lemon, *Compt. Rend.*, **214**, 84-87 (1942).

(52) I. A. Wolf, *J. Am. Chem. Soc.*, **67**, 1623-1624 (1945).

(53) R. L. Letsinger, M. H. Caruthers, P. S. Miller, and K. K. Ogilvie, *J. Am. Chem. Soc.*, **89**, 7146-7147 (1967).

(54) R. L. Letsinger and P. S. Miller, *J. Am. Chem. Soc.*, **91**, 3356-3359 (1969).

(55) R. Belorizky, G. Excoffier, D. Gagnaire, J. P. Uille, M. Vignon, and P. Vottero, *Bull. Soc. Chim. Fr.*, 4749-4753 (1972).

(56) R. D. Guthrie, T. J. Lucas, and R. Khan, *Carbohydr. Res.*, **33**, 391-395 (1974).

(57) T. Curtis, *J. Prakt. Chem.*, **50**, 529 (1895).

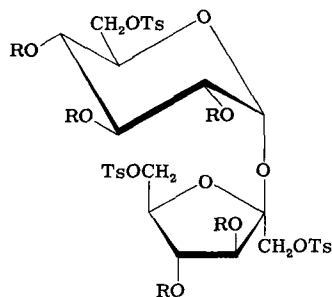
(58) R. S. Tipson, *Adv. Carbohydr. Chem.*, **8**, 107-215 (1953).

(59) D. H. Ball and F. W. Parrish, *Adv. Carbohydr. Chem.*, **23**, 233-279 (1968); *Adv. Carbohydr. Chem. Biochem.*, **24**, 139-167 (1969).

(60) R. C. Hockett and M. Zief, *J. Am. Chem. Soc.*, **72**, 1839-1840 (1950).

(61) P. D. Bragg and J. K. N. Jones, *Can. J. Chem.*, **37**, 575-578 (1959).

indeed, the tri-*O-p*-tolysulfonylsucrose consisted mainly of compound **40**. The trisulfonate fraction⁶⁰ was subjected to methylation, followed by *O*-de-*p*-tolysulfonylation and hydrolysis, and identification of the resulting methylated hexoses.⁶¹ However, this conclusion⁶¹ has been questioned by Lemieux and Barrette,⁶² who re-examined the trimolar *p*-toluenesulfonylation reaction of sucrose in pyridine to give a mixture of products containing penta-, tetra-, tri-, and di-*O-p*-tolysulfonylsucroses in the molar ratios of 0.05:0.33:1:1, respectively. A tri-*O-p*-tolysulfonylsucrose was isolated in 29% yield after chromatography on silicic acid, and was shown to be mainly, 6,1',6'-tri-*O-p*-tolysulfonylsucrose (**40**). The structural assignment was substantiated on the basis that, on treatment with sodium methoxide in methanol, it gave 2,1':3,6:3',6'-trianhydrosucrose in 77.4% yield.⁶² This contention⁶² by Lemieux and Barrette has subsequently been criticized⁴¹ as having no basis (see Section V). Compound **40** has been synthesized unambiguously.⁶⁶ Detritylation of 2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-tri-*O*-tritylsucrose (**5**) with hydrogen bromide in glacial acetic acid at 0° gave 2,3,4,3',4'-penta-*O*-acetylsucrose which, on treatment with *p*-toluenesulfonyl chloride in pyridine, afforded the corresponding 6,1',6'-trisulfonate (**41**). Catalytic de-esterification of **41** gave 6,1',6'-



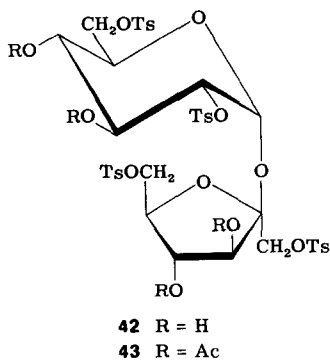
40 R = H

41 R = Ac

- (62) R. U. Lemieux and J. P. Barrette, *Can. J. Chem.*, **37**, 1964–1969 (1959); **38**, 656–662 (1960).
- (63) V. Kollonitsch, "Sucrose Chemicals," Kline, The International Sugar Research Foundation, Washington, D.C., 1970, (a) pp. 70–77; (b) p. 83; (c) p. 86; (d) pp. 94–97; (e) pp. 84–86; (f) pp. 173–191; (g) pp. 192–205; (h) pp. 30–33; (i) pp. 153–154; (j) pp. 151–155; (k) pp. 162–163; (l) pp. 220–223.
- (64) R. Neumann and J. A. Ibara, *J. Prakt. Chem.*, **314**, 365–366 (1972).
- (65) J. M. Ballard, L. Hough, and A. C. Richardson, unpublished data.
- (66) T. Suami, N. Kato, M. Kawamura, and T. Nishimura, *Carbohydr. Res.*, **19**, 407–411 (1971).

tri-*O-p*-tolylsulfonylsucrose (**40**) as an amorphous powder.⁶⁶ A direct synthesis of **40** has also been described⁴¹; treatment of 1 mole of sucrose with 3 moles of *p*-toluenesulfonyl chloride in pyridine at 0°, as described by Lemieux and Barrette,⁶² gave, after chromatography on silica gel, compound **40** in 23% yield.⁴¹

Tetramolar *p*-toluenesulfonylation of sucrose in pyridine at -40° has been reported by Jones and coworkers to give a chromatographically homogeneous material believed to be 2,6,1',6'-tetra-*O-p*-tolylsulfonylsucrose.^{63(a)} However, no proof of its structure was offered. Hough and coworkers carried out this reaction at 0° and isolated, after chromatography on silica gel, a crude tetrasulfonate in 60% yield and compound **40** in 33% yield.⁶⁵ On additional chromatography on silica gel, the tetrasulfonate fraction gave 2,6,1',6'-tetra-*O-p*-tolylsulfonylsucrose (**42**) in 32% yield. The structure of **42** was established on the basis of its mass-spectral data and chemical reactivity. Compound **42** was also synthesized from 3,4,3',4'-tetra-*O*-acetylsucrose (**34**) by treatment with *p*-toluenesulfonyl chloride in pyridine to give 3,4,3',4'-tetra-*O*-acetyl-2,6,1',6'-tetra-*O-p*-tolylsulfonylsucrose (**43**). Catalytic de-esterification of **43** with sodium methoxide



in methanol gave⁶⁷ compound **42**.

3. Chlorosulfates

The reaction of carbohydrates with sulfuryl chloride was first studied by Helferich and coworkers,⁶⁸⁻⁷⁰ and the work was extended by J.

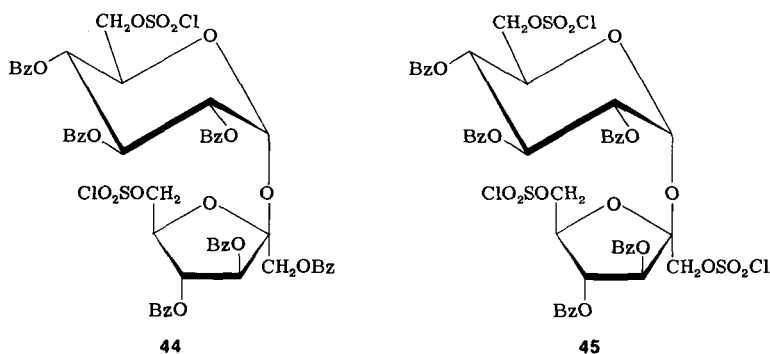
(67) R. Khan and M. R. Jenner, unpublished data.

(68) B. Helferich, *Ber.*, **54**, 1082-1084 (1921).

(69) B. Helferich, A. Löwa, W. Nippe, and H. Riedel, *Ber.*, **56**, 1083-1087 (1923).

(70) B. Helferich, G. Sprock, and E. Besler, *Ber.*, **58**, 886-891 (1925).

K. N. Jones and his colleagues⁷¹⁻⁷⁵ to give chlorodeoxy derivatives. The reaction of sucrose with sulfuryl chloride was first reported by Jones and coworkers, who isolated a mixture of di- and tri-chlorodeoxy sucrose disulfates.⁷¹ On the basis of their results on methyl α -D-glucopyranoside, it was suggested that the D-glucopyranosyl group of sucrose had been largely converted into the 4,6-dichloro-4,6-dideoxy-2,3-di-O-sulfo- α -D-galactopyranosyl system.⁷¹ This reaction has been reinvestigated by Hough and coworkers, and various characterized chlorodeoxy derivatives of sucrose have been isolated^{76,77} (see Section VII,2). With a view to studying the nucleophilic displacement-reaction, various chlorosulfate derivatives of sucrose have been prepared.⁷⁸ Treatment of 2,3,4,1',3',4'-hexa-O-benzoylsucrose with sulfuryl chloride in a mixture of chloroform and pyridine at -75° gave 2,3,4,1',3',4'-hexa-O-benzoylsucrose 6,6'-bis(chlorosulfate) (44). Similar reaction of 2,3,4,3',4'-penta-O-benzoylsucrose with sulfuryl chloride afforded the corresponding 6,1',6'-tris(chlorosulfate) (45) in crystalline form. Chloro-



sulfate groups are readily cleaved to give the corresponding hydroxyl groups with retention of configuration.⁷⁴ Treatment of 2,3,4,3',4'-

(71) P. D. Bragg, J. K. N. Jones, and J. C. Turner, *Can. J. Chem.*, **37**, 1412-1416 (1959).

(72) J. K. N. Jones, M. B. Perry, and J. C. Turner, *Can. J. Chem.*, **38**, 1122-1129 (1960).

(73) H. J. Jennings and J. K. N. Jones, *Can. J. Chem.*, **40**, 1408-1414 (1962); **41**, 1151-1159 (1963); **43**, 2372-2386, 3018-3025 (1965).

(74) A. G. Cottrell, E. Buncel, and J. K. N. Jones, *Chem. Ind. (London)*, 552 (1966); *Can. J. Chem.*, **44**, 1483-1491 (1966).

(75) H. Parolis, W. A. Szarek, and J. K. N. Jones, *Carbohydr. Res.*, **19**, 97-105 (1971).

(76) J. M. Ballard, L. Hough, and A. C. Richardson, *Chem. Commun.*, 1097-1098 (1972).

(77) J. M. Ballard, L. Hough, A. C. Richardson, and P. H. Fairclough, *J. Chem. Soc. Perkin Trans. I*, 1524-1528 (1973).

(78) R. Khan, *Carbohydr. Res.*, **25**, 504-510 (1972).

penta-*O*-benzoyl-6,6'-dichloro-6,6'-dideoxysucrose 1'-chlorosulfate in methanol with sodium iodide in aqueous methanol gave 2,3,4,3',4'-penta-*O*-benzoyl-6,6'-dichloro-6,6'-dideoxysucrose in 89% yield.⁷⁸

4. Other Esters

a. Sulfates and Sulfites.—Sulfuric and sulfurous acid esters of sucrose have received very little attention in the past. Preparation of sucrose sulfates by treating sucrose with sulfur trioxide-*N,N*-dimethylformamide, sulfonyl chloride (see Sections IV,3 and VII,2), or chlorosulfonic acid has been reported.^{63(b)} However, apart from the sulfonyl chloride reaction, none of the reaction products have been rigidly characterized. A reinvestigation of these reactions is still needed. Reaction of sucrose with thionyl chloride in pyridine has been claimed to give dichloro-dideoxysucrose disulfite. A similar claim has been made for preparation of a polymeric sulfite by the action of thionyl chloride in either pyridine at 15° or *N,N*-dimethylformamide-5-ethyl-2-methylpyridine.^{63(b)} The reaction of sucrose with thionyl chloride in a mixture of acetic acid and acetic anhydride has been reported to give, in addition to some monochloro-monodeoxysucrose heptaacetate, partially acetylated chlorodeoxysucroses.^{63(c)} In order to obtain characterized derivatives, it would be of interest to investigate these reactions with partially esterified derivatives of sucrose.

b. Carbonates.—Synthesis of sucrose carbonates was attempted with a view to bridging the two hexose residues. Although no such bridging has been observed, the potential value of sucrose carbonates as synthetic-resin intermediates has been recognized.^{79,80} Treatment of sucrose with an alkyl chloroformate at 0° in an aqueous, alkaline medium gave partially substituted *O*-(alkoxycarbonyl)sucrose.⁸⁰ A tri-*O*-(ethoxycarbonyl)sucrose has been prepared in 75–80% yield by treating 1 mole of sucrose with 5 moles of ethyl chloroformate and 2 *M* sodium hydroxide at 0°. The tri-*O*-(ethoxycarbonyl)sucrose has been shown to polymerize at 150°/12 torr, to afford a poly(sucrose carbonate). Octa-*O*-(ethoxycarbonyl)sucrose has been prepared⁷⁹ in 97% yield by treating the partly esterified derivative (d.s. 5.7) with ethyl chloroformate in pyridine. The *O*-(alkoxycarbonyl)sucroses show inertness towards invertase action, and more resistance than octa-*O*-acetylsucrose to acid hydrolysis.⁷⁹ The lack of

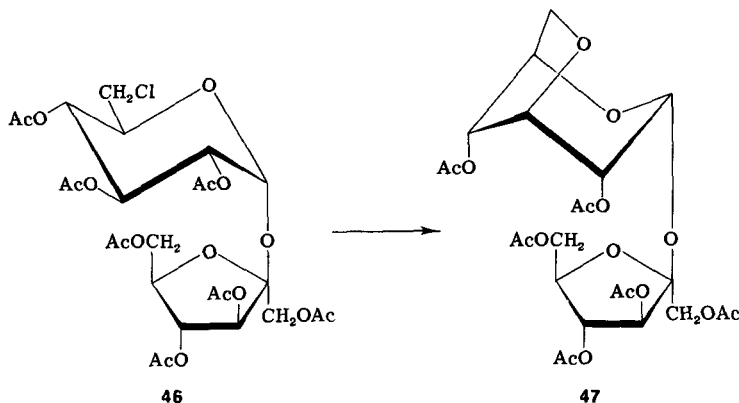
(79) R. S. Theobald, *J. Chem. Soc.*, 5359–5364 (1961).

(80) R. S. Theobald, *J. Chem. Soc.*, 5365–5370, 5370–5376 (1961).

acid sensitivity of the interglycosidic linkages in these derivatives is presumably due to steric inhibition in the transition state.

V. ANHYDRO DERIVATIVES

Internal-displacement reactions to give sugar anhydrides are well known, and anhydro derivatives of sucrose have been prepared by treatment of the respective sulfonates or chlorides with a base. Buchanan and coworkers described the synthesis of 3',6'-anhydrosucrose by treating 2,3,4,6,1',3',4'-hepta-*O*-acetyl-6'-*O*-(*p*-nitrophenylsulfonyl)sucrose with sodium ethoxide in ethanol for 2 h under reflux.¹⁸ Similar treatment of 2,3,4,1',3',4',6'-hepta-*O*-acetyl-6-chloro-6-deoxysucrose (46) with sodium methoxide in methanol, followed by acetylation, gave crystalline 2,4,1',3',4',6'-hexa-*O*-acetyl-3,6-anhydrosucrose (47) in 83% yield.⁸¹ The ¹H n.m.r. spectrum of 47

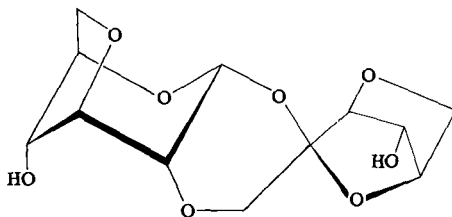


showed that the hexopyranoside moiety was constrained in the ¹C₄(D) conformation. Synthesis of 1',4':3',6'-dianhydrosucrose³³ and 3,6:3',6'-dianhydrosucrose²⁹ has been achieved by alkaline alcoholysis of 2,3,4,6,3',4'-hexa-*O*-acetyl-1',6'-di-*O*-(methylsulfonyl)sucrose and 6,6'-di-*O*-*p*-tolylsulfonylsucrose, respectively.

The structure of a tri-*O*-*p*-tolylsulfonylsucrose, claimed by Lemieux and Barrette (see Section IV,2) to be 6,1',6'-tri-*O*-*p*-tolylsulfonylsucrose, was based on its conversion into 2,1':3,6:3',6'-trianhydrosucrose⁶² (48). This contention⁶² has, however, been criticized as having no basis.⁴¹ Richards and coworkers⁸² synthesized

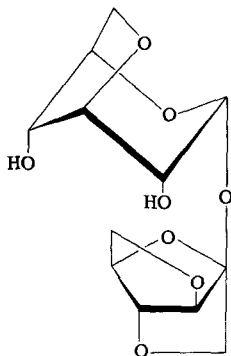
(81) R. Khan, M. R. Jenner, and K. S. Mufti, *Carbohydr. Res.*, **39**, 253–262 (1975).

(82) N. W. Isaacs, C. H. L. Kennard, G. W. O'Donnell, and G. N. Richards, *Chem. Commun.*, 360 (1970).



48

2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-tri-*O*-*p*-tolylsulfonylsucrose (41) by an unambiguous route; on treatment with sodium methoxide in methanol, as described by Lemieux and Barrette,⁶² compound 41 gave⁶² a different trianhydride, namely, 3,6:1',4':3',6'-trianhydrosucrose (49). The structure of 49 was determined by X-ray



49

crystallography. They⁶² offered two possible explanations for their results: either that compound 48 arose from an isomer of 6,1',6'-tri-*O*-*p*-tolylsulfonylsucrose (40), or that the *O*-acyl groups exert a conformational influence on the course of the reaction.⁶² Subsequent investigation of the alkaline alcoholysis of compound 40 and its corresponding pentabenzoate derivative gave 49 as the only isolable product in 65 and 70% yield, respectively.⁴¹ These results supported the fact that the tri-*O*-*p*-tolylsulfonylsucrose erroneously identified by Lemieux and Barrette as 6,1',6'-tri-*O*-*p*-tolylsulfonylsucrose 40 could not have been the precursor of the trianhydride 48, and must have arisen from an unknown tri-*O*-*p*-tolylsulfonylsucrose, such as 2,6,6'-tri-*O*-*p*-tolylsulfonylsucrose. An unambiguous synthesis of the trianhydride 48 would, therefore, be of interest. The alkaline alcoholysis of 2,3,6,3',4'-penta-*O*-acetyl-4,1',6'-tri-*O*-*p*-tolylsulfonylsucrose has

been reported to proceed with inversion of configuration at C-4, to give 1,4:3,6-dianhydro- β -D-fructofuranosyl 3,6-anhydro- α -D-galactopyranoside.²¹

VI. CYCLIC ACETALS

The reaction of sucrose with paraldehyde in the presence of freshly fused zinc chloride has been claimed to give 4,6:1',3':4',6'-tri-*O*-ethylidenesucrose and 4,6:1',3':4',6'-tri-*O*-ethylidene-2,3-*O*-(oxidodiethylidene)sucrose.^{63(d)} However, the structures of these acetals have not been rigorously characterized. In view of the acid-lability of sucrose, it would be desirable to conduct the acetalation reaction either in slightly basic media or under conditions in which hydrolysis of the glycosidic linkage of the sucrose molecule is prevented. The first synthesis of a characterized, cyclic acetal derivative of sucrose was reported by Khan.²⁵ On treatment with α,α -dibromotoluene in pyridine for 1.5 h at 85°, followed by conventional acetylation with acetic anhydride, sucrose gave, after chromatography on silica gel, 2,3,1',3',4',6'-hexa-*O*-acetyl-4,6-*O*-benzylidenesucrose (7) in 35% yield.²⁵

The reaction of sucrose with a combination of 2,2-dimethoxypropane-*N,N*-dimethylformamide-*p*-toluenesulfonic acid has been exploited to give various, interesting, cyclic acetal derivatives.^{32,83-85} This combination of reagents for acetonation is known to give strained, and otherwise inaccessible, acetals of monosaccharides.⁸⁶⁻⁸⁹ Treatment of sucrose with 2,2-dimethoxypropane in *N,N*-dimethylformamide in the presence of a catalytic proportion of *p*-toluenesulfonic acid for 80 min at room temperature afforded a mixture containing 4,6-*O*-isopropylidenesucrose, 2,1':4,6-di-*O*-isopropylidenesucrose and some unreacted sucrose. Conventional acetylation of the reaction mixture with acetic anhydride in pyridine gave, after column chromatography on silica gel, 2,3,1',3',4',6'-hexa-*O*-acetyl-4,6-*O*-isopropylidenesucrose (50) and 3,3',4',6'-tetra-*O*-acetyl-2,1':4,6-di-*O*-isopropylidenesucrose (35) in yields of 55 and 15%, respectively.^{32,83} Compound 35 probably constitutes the first example in carbohydrate chemistry of an eight-membered cyclic acetal.

(83) K. S. Mufti and R. Khan, Brit. Pat. Application (1974).

(84) R. Khan, unpublished data.

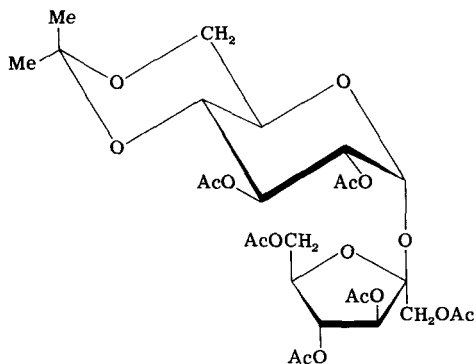
(85) R. Khan and M. R. Jenner, unpublished data.

(86) M. E. Evans and F. W. Parrish, *Tetrahedron Lett.*, 3805-3807 (1966).

(87) M. E. Evans, F. W. Parrish, and L. Long, Jr., *Carbohydr. Res.*, **3**, 453-462 (1967).

(88) A. Hasegawa and H. G. Fletcher, Jr., *Carbohydr. Res.*, **29**, 209-222 (1973).

(89) A. Hasegawa and M. Nakajima, *Carbohydr. Res.*, **29**, 239-245 (1973).



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Its stability parallels that of the 1,3-dioxepane ring in the 3,4-*O*-benzylidene or 3,4-*O*-isopropylidene derivative of 1,6-di-*O*-benzoyl-2,5-*O*-methylene-*D*-mannitol, in which the seven-membered acetal ring is flexible enough to accommodate the torsional angle between projected *trans* C—O bonds of 41° or less.⁹⁰ The yield of the diacetal **35** was improved to 50% when the reaction of sucrose was performed with the aforementioned combination of reagents³² for 80 min at 40° (Ref. 91). The reaction of 6,6'-dichloro-6,6'-dideoxysucrose with 2,2-dimethoxypropane-*N,N*-dimethylformamide-*p*-toluenesulfonic acid at room temperature, gave, after acetylation, and chromatography of the product on silica gel, 6,6'-dichloro-6,6'-dideoxy-2,1'-*O*-isopropylidenesucrose as the major, and 6,6'-dichloro-6,6'-dideoxy-2,1':3,4-di-*O*-isopropylidenesucrose as the minor, product.⁸⁵

Use of dimethoxydiphenylsilane in combination with *N,N*-dimethylformamide-*p*-toluenesulfonic acid to introduce silicon into the sucrose molecule has been exploited.²⁷ On treatment with this combination of reagents, sucrose gave, after chromatographic fractionation of the acetylated mixture, 3,4,6,3',4',6'-hexa-*O*-acetyl-2,1'-*O*-(diphenylsilyl)sucrose (**10**) and 3,4,3',4'-tetra-*O*-acetyl-2,1':6,6'-di-*O*-(diphenylsilyl)sucrose (**33**) in 45 and 10% yield, respectively.²⁷ With a view to introducing other heteroatoms, such as phosphorus or nitrogen, into the sucrose molecule, such reagents as dimethoxyphenylphosphine, *N,N*-dimethylformamide dimethyl acetal, and 3,3-dimethoxypropylamine in combination with *N,N*-dimethylformamide-*p*-toluenesulfonic acid are of interest.

(90) J. F. Stoddart and W. A. Szarek, *J. Chem. Soc., B*, 13, 437–442 (1971).

(91) R. Khan, unpublished data.

VII. HALIDES

1. Nucleophilic-displacement Reactions of Sulfonates

Bimolecular, nucleophilic-displacement reactions of sugar sulfonates have been reviewed in this Series.^{58,59} The value of these reactions in the preparation of deoxyhalo sugars has been emphasized by Hanessian.⁹²

The application of bimolecular, nucleophilic substitution (S_N2) reactions to sucrose sulfonates has been found of significant value in synthesizing deoxyhalosucroses and in determining the relative reactivity of the various hydroxyl groups in the sucrose molecule. The reaction of octa-*O*-(methylsulfonyl)sucrose with sodium iodide in butanone for 24 h under reflux gave 6,6'-dideoxy-6,6'-diiodo-2,3,4,1',3',4'-hexa-*O*-(methylsulfonyl)sucrose in 75% yield.²⁹ Little or no reaction was observed at C-1', probably because of unfavorable, polar interactions in the transition state. A summary of the steric and polar factors in nucleophilic-displacement reactions of sugar sulfonates has been provided by Richardson.⁹³ The replacement of sulfonyloxy groups at a chiral center has been achieved by the use of high-boiling, aprotic solvents of high dielectric constants, such as *N,N*-dimethylformamide and hexamethylphosphoric triamide. Use of hexamethylphosphoric triamide, in particular, allows the reaction to be performed at a comparatively low temperature with minimal decomposition.

On benzoylation with benzoyl chloride in pyridine at room temperature, 6,6'-di-*O-p*-tolylsulfonylsucrose gave²⁹ significant proportions of a monochloromonodeoxy derivative (16%) and 6,6'-dichloro-6,6'-dideoxysucrose (7%); this indicated a difference between the reactivities at O-6 and O-6'. This behavior was also observed in transesterification reactions of sucrose.^{94,95} With a view to confirming this observation,²⁹ the nucleophilic-substitution reaction of 2,3,4,1',3',4'-hexa-*O*-acetyl-6,6'-di-*O-p*-tolylsulfonylsucrose (**51**) was investigated.⁹⁶ On treatment with sodium chloride in hexamethylphosphoric triamide for 14 h at 85°, compound **51** gave, after chromatographic separation, 2,3,4,1',3',4'-hexa-*O*-acetyl-6-chloro-6-deoxy-6'-*O-p*-tolylsulfonylsucrose (**52**) plus 2,3,4,1',3',4'-hexa-*O*-

(92) S. Hanessian, *Adv. Chem. Ser.*, **74**, 159–201 (1968).

(93) A. C. Richardson, *Carbohydr. Res.*, **10**, 395–402 (1969).

(94) E. Reinfeld and S. Klaudinos, *Zucker*, **21**, 330–338 (1968).

(95) R. U. Lemieux and A. G. McInnes, *Can. J. Chem.*, **40**, 2394–2401 (1962).

(96) L. Hough and K. S. Mufti, *Carbohydr. Res.*, **25**, 497–503 (1972).

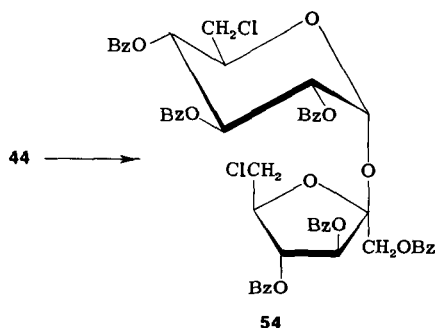
(97) L. Hough and K. S. Mufti, *Carbohydr. Res.*, **27**, 47-54 (1973).

mixture and its derivatives. The reaction of octa-*O*-(methylsulfonyl)-sucrose with sodium bromide in hexamethylphosphoric triamide for 2 h at 85° gave,⁹⁷ after chromatographic separation, 6-bromo-6-deoxy-1,3,4-tri-*O*-(methylsulfonyl)- β -D-fructofuranosyl 4,6-dibromo-4,6-dideoxy-2,3-di-*O*-(methylsulfonyl)- α -D-galactopyranoside in 39% yield, in addition to 6,6'-dibromo-6,6'-dideoxy-2,3,4,1',3',4'-hexa-*O*-(methylsulfonyl)sucrose (36%). These results indicated that the reactivity of the sulfonyloxy groups in the sucrose sulfonates is in the order: C-6 \approx C-6' > C-4.

2. Sulfuryl Chloride Reactions

The reaction of sulfuryl chloride with carbohydrates to give chloro-deoxy derivatives has been reviewed briefly in this Series.⁹⁸ The reaction of sulfuryl chloride with monosaccharides has been shown to afford products in which the secondary hydroxyl groups are replaced by chlorine with inversion of configuration.⁶⁸⁻⁷⁵ Jones and coworkers reported that the reaction of methyl α -D-glucopyranoside with sulfuryl chloride and pyridine in chloroform at room temperature proceeds by way of the 4,6-bis(chlorosulfate) by an SN2 process, with chloride as the nucleophile, to give methyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside 2,3-bis(chlorosulfate).⁷⁴

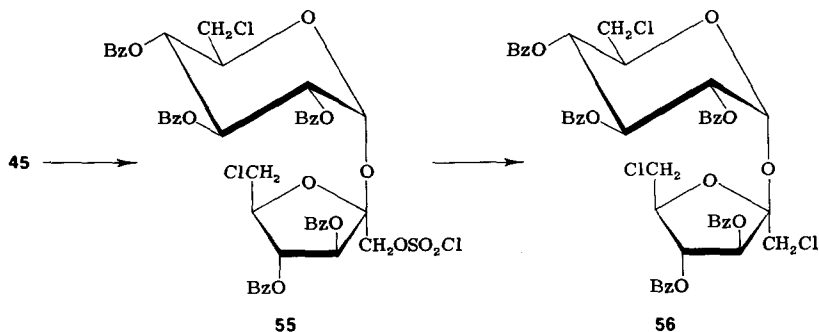
However, based on the following evidence, a question has been raised about the "assumed" SN2 character of the displacement reaction.⁷⁸ The reaction of 2,3,4,1',3',4'-hexa-*O*-benzoylsucrose 6,6'-bis(chlorosulfate) (**44**) with pyridinium chloride in chloroform for 4 h at 55° gave 2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-dichloro-6,6'-dideoxy-sucrose (**54**) in 96% yield. When compound **44** was heated under reflux with butanone alone, compound **54** (35%) was the only iso-



(98) W. A. Szarek, *Adv. Carbohydr. Chem. Biochem.*, **28**, 225-306 (1973).

lable product. The value of the chlorosulfonyloxy substituent (OSO_2Cl) as a leaving group has been demonstrated by kinetic studies with alkyl chlorosulfates.⁹⁹⁻¹⁰² However, when compound **44** was heated with sodium azide under reflux in butanone, the only isolable product was the 6,6'-dichloride **54**. The azide derivative expected in the reaction mixture was not detected. Similarly, on treatment with either pyridinium chloride in chloroform or butanone under reflux, 2,3,4,3',4'-penta-*O*-benzoylsucrose 6,1',6'-tris-(chlorosulfate) (**45**) gave 2,3,4,3',4'-penta-*O*-benzoyl-6,6'-dichloro-6,6'-dideoxysucrose 1'-(chlorosulfate) (**55**) in 76 and 40% yield, respectively. The higher reactivity of the chlorosulfate group on C-6 and C-6' in **45** is comparable to the nucleophilic displacement of the sulfonyloxy groups in the sulfonate derivatives of sucrose.^{29,96,97} When compound **45** was heated under reflux with an excess of sodium azide in butanone, it afforded, as observed previously, the dichloride **55** in 83% yield. The fact that compounds **54** and **55** were obtained instead of the corresponding azides indicated effective competition by the chloride ion, which could only have arisen from the chlorosulfonyloxy groups of the precursors (**44** and **45**). These observations suggested that the reaction is intramolecular, similar to the $\text{S}_{\text{N}}\text{i}$ process, but with inversion of configuration.⁷⁸

Displacement of the 1'-chlorosulfate group in compound **55** has been achieved by the use of a dipolar, aprotic solvent. When compound **55** was heated in hexamethylphosphoric triamide at 95°, it gave, after chromatography on silica gel, 2,3,4,3',4'-penta-*O*-benzoyl-6,1',6'-trichloro-6,1',6'-trideoxysucrose (**56**) in 63% yield.⁷⁸ The yield of **56** was raised to 88% when compound **55** was treated with sodium chloride in hexamethylphosphoric triamide for 24 h at 95°. It is of interest that a similar displacement of the sulfonyloxy group at C-1' has so far been unsuccessful.



The reaction of sulfuryl chloride with sucrose was originally studied by Jones and his colleagues^{63(e),71} (see Section IV,3), and the work was extended by Hough and coworkers.^{76,77} Treatment of sucrose with sulfuryl chloride in a mixture of pyridine and chloroform at -78° gave, essentially, a poly(chlorosulfate) product which, on dechlorosulfation with barium carbonate and a catalytic amount of sodium iodide in methanol, afforded, after dry-column chromatography, 6,6'-dichloro-6,6'-dideoxysucrose in 29% yield, and 6'-chloro-6'-dideoxysucrose in 43% yield.^{76,77} When the reaction was conducted for 2 h at -78° and then for 2 h at room temperature, it gave a complex mixture from which 4,6-dichloro-4,6-dideoxy-2,3-di-*O*-sulfo- α -D-galactopyranosyl 3,4-anhydro-1,6-dichloro-1,6-dideoxy- β -D-*ribo*-hexulofuranoside was isolated in 17% yield. Treatment of sucrose with sulfuryl chloride in pyridine for 48 h at 50° yielded a tarry mixture which, on chromatographic separation, gave, in addition to the 3,4-anhydride, 4,6-dichloro-4,6-dideoxy-2,3-di-*O*-sulfo- α -D-galactopyranosyl 1,4,6-trichloro-1,3,4,6-tetra-deoxy- β -D-*glycero*-hex-3-enofuranoside and 4,6-dichloro-4,6-dideoxy-2,3-di-*O*-sulfo- α -D-galactopyranosyl 1,4,6-trichloro-1,4,6-trideoxy- β -D-hexulofuranoside,⁷⁷ of unknown chirality at C-3 of the last-mentioned moiety.

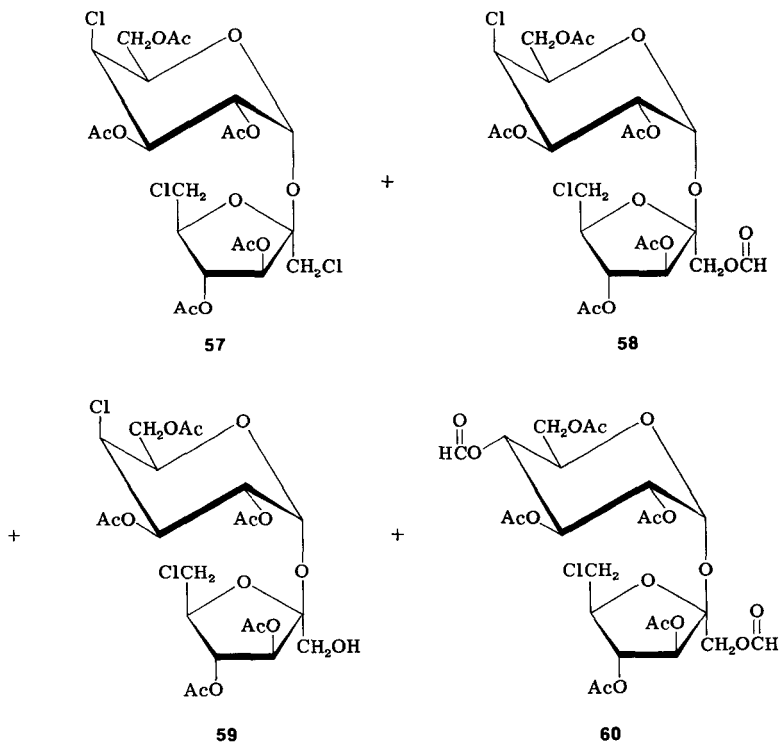
3. Reaction with the Methanesulfonyl Chloride-*N,N*-Dimethylformamide Complex

Use of methanesulfonyl chloride in *N,N*-dimethylformamide permits selective replacement of primary hydroxyl groups in hexopyranosides,^{103,104} methyl β -maltoside,¹⁰⁴ and sucrose¹⁰⁵ by chlorine. However, under forcing conditions, substitution at a chiral center has also been observed.¹⁰⁴ An attempt to rationalize the reaction of this combination of reagents with alcohols has been made.¹⁰⁴ The initial step, slow and presumably rate-limiting, is the formation of iminium ion $(\text{Me}_2\text{N}^+=\text{CHOSO}_2\text{Me})\text{Cl}^-$, which then reacts with an alcohol (ROH) to give an intermediate $(\text{Me}_2\text{N}^+=\text{CHOR})\text{Cl}^-$. Bimolecular, nucleophilic, substitution at the alkyl (R) group by chloride ion af-

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- (99) H. K. Hall, Jr., *J. Am. Chem. Soc.*, **78**, 1450-1454 (1956).
(100) E. Buncl and J. P. Millington, *Can. J. Chem.*, **43**, 547-555, 556-564 (1965).
(101) E. Buncl, *Chem. Rev.*, **70**, 323-337 (1970).
(102) E. C. F. Ko and R. E. Robertson, *Can. J. Chem.*, **50**, 434-437 (1972).
(103) M. E. Evans, L. Long, Jr., and F. W. Parrish, *J. Org. Chem.*, **33**, 1074-1076 (1968).
(104) R. G. Edwards, L. Hough, A. C. Richardson, and E. Tarelli, *Tetrahedron Lett.*, 2369-2370 (1973).
(105) R. Khan, K. S. Mufti, and K. J. Parker, Brit. Pat. Application (1973).

fords the chlorodeoxy product. The last step of the S_N2 reaction was found by Long and coworkers not to be rate-limiting.¹⁰³

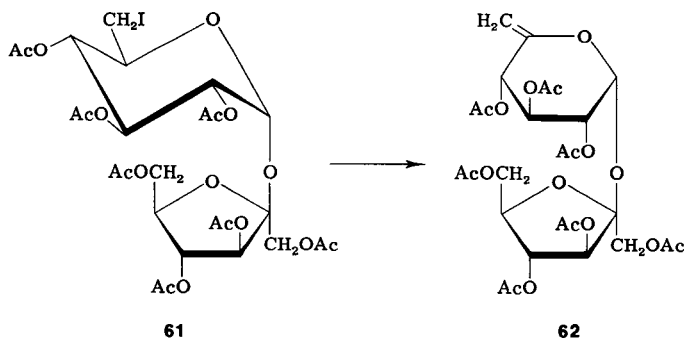
The reaction of sucrose with methanesulfonyl chloride and *N,N*-dimethylformamide was found to give 6,6'-dichloro-6,6'-dideoxy-sucrose as the major product, and 6-chloro-6-deoxy- β -D-fructofuranosyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside and 6,1',6'-trichloro-6,1',6'-trideoxysucrose as the minor products.^{105,106} The application of this reaction has been extended to various, partially acylated derivatives of sucrose.⁸¹ Treatment of 2,3,6,3',4'-penta-*O*-acetylsucrose (**6**) with methanesulfonyl chloride in *N,N*-dimethylformamide, initially for 2 h at -5° and then for 24 h at 90° , gave, after chromatography on silica gel, 3,4-di-*O*-acetyl-1,6-dichloro-1,6-dideoxy- β -D-fructofuranosyl 2,3,6-tri-*O*-acetyl-4-chloro-4-deoxy- α -D-galactopyranoside (**57**), 3,4-di-*O*-acetyl-6-chloro-6-deoxy-1-*O*-formyl- β -D-fructofuranosyl 2,3,6-tri-*O*-acetyl-4-chloro-4-deoxy- α -D-galactopyranoside (**58**), 3,4-di-*O*-acetyl-6-chloro-6-deoxy- β -D-fructofuranosyl 2,3,6-tri-*O*-acetyl-4-chloro-4-deoxy- α -D-galactopyranoside (**59**), and 2,3,6,3',4'-penta-*O*-acetyl-6'-chloro-6'-deoxy-4,1'-di-*O*-formylsucrose (**60**) in yields of 6.2, 19.8, 19, and 19.7%, respectively.



Similar reaction of 2,3,1',3',4',6'-hexa-*O*-acetylsucrose with methanesulfonyl chloride in *N,N*-dimethylformamide gave two products, which were separated on silica gel and characterized as 1,3,4,6-tetra-*O*-acetyl- β -D-fructofuranosyl 2,3-di-*O*-acetyl-4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside and 2,3,1',3',4',6'-hexa-*O*-acetyl-6-chloro-6-deoxy-4-*O*-formylsucrose. The formation of formic esters during the reaction of sugars with methanesulfonyl chloride-*N,N*-dimethylformamide by way of hydrolysis of the $(\text{Me}_2\text{N}^+=\text{CHOR})\text{Cl}^-$ complex has been recognized.¹⁰³ The formyl group in 2,3,4,3',4'-penta-*O*-benzoyl-6,6'-dichloro-6,6'-dideoxy-1'-*O*-formylsucrose was selectively removed by treatment with an anion-exchange resin to give 2,3,4,3',4'-penta-*O*-benzoyl-6,6'-dichloro-6,6'-dideoxysucrose.⁸¹

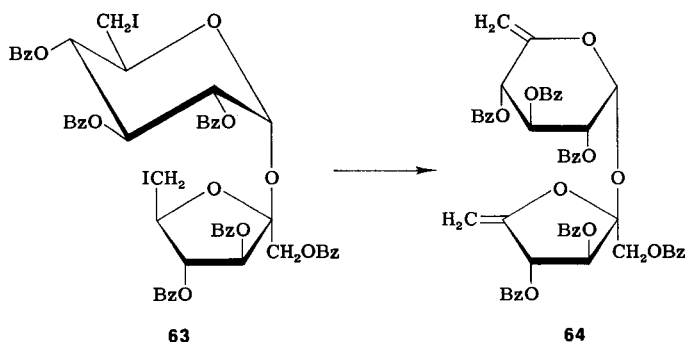
VIII. UNSATURATED DERIVATIVES

The potential importance of unsaturated sugars as synthetic and biological intermediates is widely recognized. During the past decade, considerable interest has been shown in the synthesis and reactions of this class of compound.^{107,108} The reaction of suitably protected 6-bromo-6-deoxy- or 6-deoxy-6-iodo-aldohexopyranoses with silver fluoride in pyridine to give 6-deoxyhex-5-enose vinyl ethers was first reported by Helferich and Himmen.¹⁰⁹ The reaction of silver fluoride and pyridine with sucrose derivatives containing deoxyhalogeno groups, to afford exocyclic vinyl ethers, has been reported.^{29,96,97,110} Treatment of 2,3,4,1',3',4',6'-hepta-*O*-acetyl-6-deoxy-6-iodosucrose (**61**) with silver fluoride and pyridine for 24 h at room temperature gave a dark-brown mixture which was purified, by extraction with ether followed by chromatography on silica gel, to give¹¹⁰ 1,3,4,6-tetra-*O*-acetyl- β -D-fructofuranosyl 2,3,4-tri-*O*-acetyl-6-deoxy- α -D-xylo-hex-5-enopyranoside (**62**). A similar reaction of



(106) R. Khan, unpublished data.

2,3,4,6,1',3',4'-hepta-*O*-acetyl-6'-deoxy-6'-iodosucrose removed the asymmetry at C-5' by the expected elimination of hydrogen iodide, to give 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl 1,3,4-tri-*O*-acetyl-6-deoxy- β -D-*threo*-hex-5-enofuranoside.¹¹⁰ On being shaken with silver fluoride in pyridine for 24 h at room temperature, 2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-dideoxy-6,6'-diiodosucrose (**63**) gave crystalline 1,3,4-tri-*O*-benzoyl-6-deoxy- β -D-*threo*-hex-5-enofuranosyl 2,3,4-tri-*O*-benzoyl-6-deoxy- α -D-*xyl*-hex-5-enopyranoside²⁹ (**64**). The



¹H n.m.r.spectrum of compound **64** showed the well-known allylic coupling¹¹¹ between the protons on C-4 and C-6 and C-4' and C-6'. On reacting with silver fluoride and pyridine, 2,3,4,1',3',4'-hexa-*O*-acetyl-6,6'-dibromo-6,6'-dideoxysucrose, like the 6,6'-diiodo derivative (**63**), gave the corresponding 6,6'-dideoxy-5,5'-diene derivative.⁹⁶

IX. DEOXY DERIVATIVES

The deoxy sugars, particularly those bearing a terminal deoxy function, are an important class of compounds that frequently occur, often as methyl ethers, in glycosides and polysaccharides of plants and of products of microbiological origin. Deoxy sugars may be prepared by reductive dehalogenation of deoxyhalogeno derivatives of carbohydrates.^{92,112} Synthesis of terminal-deoxy derivatives of su-

(107) (a) R. J. Ferrier, *Adv. Carbohydr. Chem.*, **20**, 67-137 (1965); (b) *Adv. Carbohydr. Chem. Biochem.*, **24**, 199-266 (1969).

(108) L. Hough, R. Khan, and B. A. Otter, *Adv. Chem. Ser.*, **74**, 120-140 (1968).

(109) B. Helferich and E. Himmen, *Ber.*, **61**, 1825-1835 (1928); **62**, 2136-2141 (1929).

(110) R. Khan and M. R. Jenner, unpublished data.

(111) S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15-46 (1964).

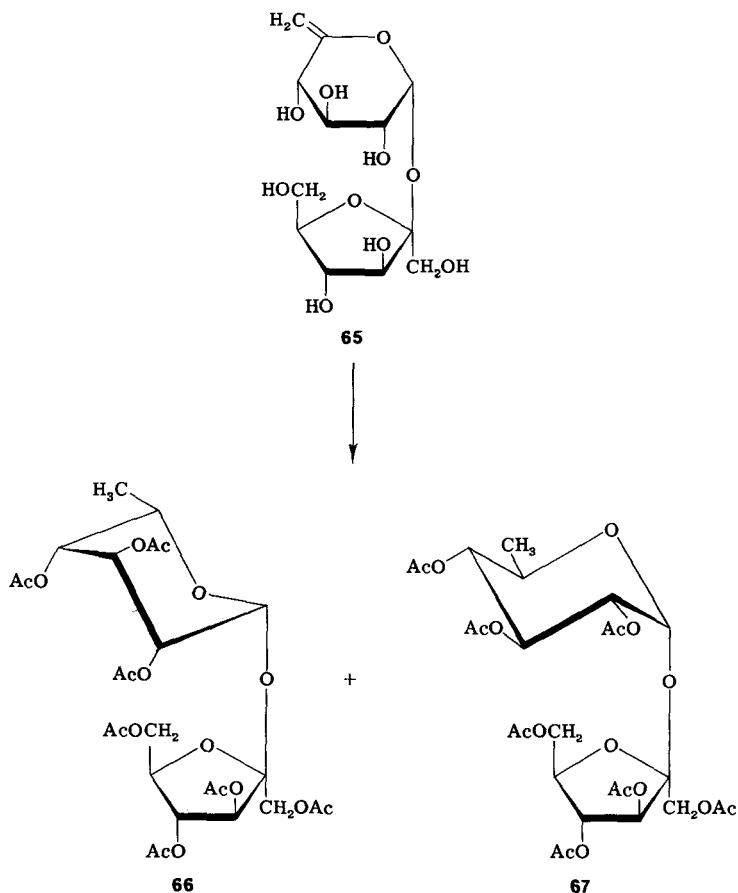
(112) S. Hanessian, *Adv. Carbohydr. Chem.*, **21**, 143-207 (1966).

crose has been achieved by catalytic reduction either of the respective halides or the exocyclic vinyl ethers.^{29,66,97,110} The reductive dehalogenation of sucrose halides has been achieved with freshly prepared, Raney nickel catalyst by shaking in methanol in an atmosphere of hydrogen in the presence of barium carbonate,²⁹ by refluxing in ethanol alone,²⁹ or by boiling in methanol in the presence of barium carbonate and hydrazine hydrate.^{97,110} The last method has been found the most suitable. Treatment of 2,3,4,6,1',3',4'-hepta-*O*-acetyl-6'-deoxy-6'-iodosucrose with freshly prepared Raney nickel T-4 as the catalyst¹¹³ in methanol in the presence of barium carbonate and hydrazine hydrate for 1 h under reflux gave the corresponding 6'-deoxy derivative.¹¹⁰ Synthesis of 2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-dideoxysucrose was achieved by catalytic hydrogenation of 2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-dideoxy-6,6'-diiodosucrose.²⁹ The structure of the dideoxy compound was confirmed by its ¹H n.m.r. spectrum, in which the methyl groups on C-5 and C-5' appeared²⁹ as doublets at δ 1.2 and 1.47.

Reduction of pyranoid exocyclic vinyl ethers has been found to give both 6-deoxy-D- and -L-hexoses.¹⁰⁸ In the hydrogenation of 1,2,3,4-tetra-*O*-acetyl-6-deoxy- β -D-*xylo*-hex-5-enopyranose, the proportion of each isomer was found to be dependent on the catalyst and on the substituents in the vinyl ether.¹⁰⁸ In a similar investigation, Umezawa and coworkers showed that, on catalytic hydrogenation, methyl 2,3,4-tri-*O*-acetyl- α -D-*xylo*-hex-5-enopyranoside gave, exclusively, methyl 2,3,4-tri-*O*-acetyl-6-deoxy- β -L-idopyranoside.¹¹⁴ Reduction of 1,3,4,6-tetra-*O*-acetyl- β -D-fructofuranosyl 2,3,4-tri-*O*-acetyl-6-deoxy- α -D-*xylo*-hex-5-enopyranoside (**62**) with palladium-on-charcoal in a mixture of ethyl acetate and methanol afforded 1,3,4,6-tetra-*O*-acetyl- β -D-fructofuranosyl 2,3,4-tri-*O*-acetyl-6-deoxy- β -L-idopyranoside (**66**) as the only isolable product (in 46% yield).¹¹⁰ Similar hydrogenation of the free 6-deoxy-5-eno derivative (**65**) of sucrose, followed by acetylation and chromatographic separation gave, in addition to compound (**66**) (46%), 2,3,4,1',3',4',6'-hepta-*O*-acetyl-6-deoxysucrose (**67**) in 10% yield. The structure of compound **66** was supported by its ¹H n.m.r. spectrum. The derived first-order coupling-constants ($J_{1,2}$ 2.0, $J_{2,3}$ 3.5, $J_{3,4}$ 3.5, and $J_{4,5}$ 2.5 Hz) revealed an *a,e,e,e* arrangement of H-1, H-2, H-3, and H-4. This indicated a ¹C₄(L) conformation for the β -L-idopyranoside moiety in **66**, which

(113) S. Nishimura, *Bull. Chem. Soc. Jpn.*, **32**, 61-64 (1959).

(114) D. Ikeda, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **44**, 2529-2537 (1971).



was in agreement with the findings of Umezawa and his colleagues.¹¹⁴ Reduction of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl 1,3,4-tri-*O*-acetyl-6-deoxy- β -D-*threo*-hex-5-enofuranoside in ethyl acetate-methanol in the presence of palladium-on-charcoal gave 2,3,4,6,1',3',4'-hepta-*O*-acetyl-6'-deoxysucrose in 99% yield.¹¹⁰

X. NITROGEN-CONTAINING COMPOUNDS

1. Azides

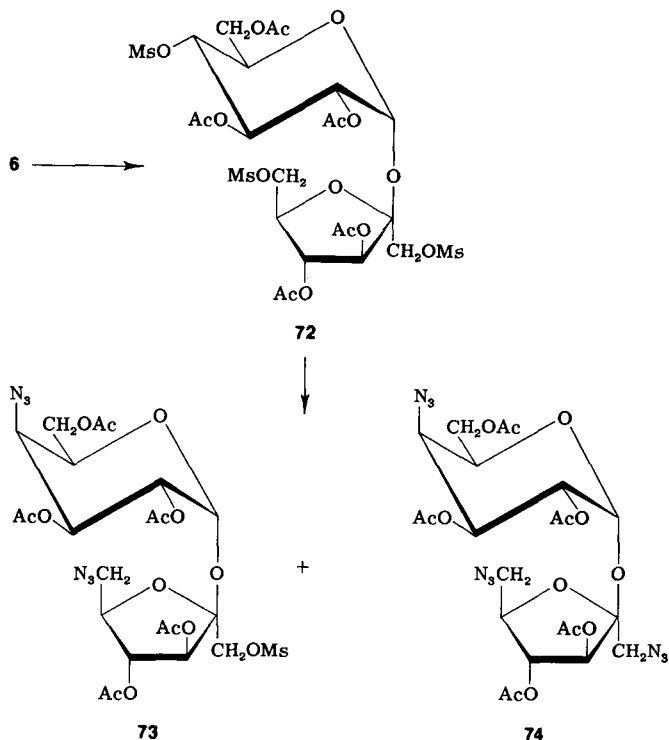
Azidodeoxy sugars are useful intermediates in the synthesis of aminodeoxy sugars. Nucleophilic-displacement reactions of sulfonate and deoxyhalo derivatives of sucrose with sodium azide have been used for the preparation of sucrose azides. The reaction of

2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-di-*O*-*p*-tolylsulfonylsucrose with sodium azide in *N,N*-dimethylformamide for 3 days at 130° gave 6,6'-diazido-2,3,4,1',3',4'-hexa-*O*-benzoyl-6,6'-dideoxysucrose in 48% yield.²⁹ The nucleophilic-displacement reaction of octa-*O*-(methylsulfonyl)sucrose (68) with azide ion, under controlled conditions, exhibited the selectivity expected.^{29,97} The reaction of compound 68 with sodium azide in 10:1 (v/v) butanone-*N,N*-dimethylformamide for 48 h at 105–108° gave 6,6'-diazido-6,6'-dideoxy-2,3,4,1',3',4'-hexa-*O*-(methylsulfonyl)sucrose (69) in 75% yield.²⁹ When the reaction was performed in the same solvents but in 1:2 (v/v) ratio, at 130°, it afforded, in addition to compound 69, a triazide derivative²⁹ (20%), whose structure was subsequently established⁹⁷ as that of 6-azido-6-deoxy-1,3,4-tri-*O*-(methylsulfonyl)- β -D-fructofuranosyl 4,6-diazido-4,6-dideoxy-2,3-di-*O*-(methylsulfonyl)- α -D-galactopyranoside (70). Treatment of compound 68 with sodium azide in hexamethylphosphoric triamide for 48 h at 90° gave 70 in 60% yield plus a tetraazide identified, according to its ¹H n.m.r. spectrum, as 1,6-diazido-1,6-dideoxy-3,4-di-*O*-(methylsulfonyl)- β -D-fructofuranosyl 4,6-diazido-4,6-dideoxy-2,3-di-*O*-(methylsulfonyl)- α -D-galactopyranoside (71) in 10% yield.⁹⁷

Umezawa and coworkers reported the synthesis of a sucrose triazide that they erroneously characterized as 6,1',6'-triazido-6,1',6'-trideoxysucrose.¹¹⁵ The structure of this triazide has been reassigned¹¹⁶ as 1,6-diazido-1,6-dideoxy- β -D-fructofuranosyl 4-azido-4-deoxy- α -D-galactopyranoside, based on the following unambiguous synthesis. 2,3,6,3',4'-Penta-*O*-acetylsucrose (6) was obtained by detritylation of 2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-tri-*O*-tritylsucrose (5) with boiling aqueous acetic acid.²⁰ A similar method of detritylation of compound 5 was used by Umezawa and coworkers; however, they ignored the fact that it gave compound 6 (instead of 2,3,4,3',4'-penta-*O*-acetylsucrose, which they mistakenly assumed). On treatment with methanesulfonyl chloride in pyridine, compound 6 afforded the corresponding 4,1',6'-trimethanesulfonate (72). The reaction of compound 72 with sodium azide in hexamethylphosphoric triamide for 20 h at 85° gave, after chromatographic separation, 3,4-di-*O*-acetyl-6-azido-6-deoxy-1-*O*-(methylsulfonyl)- β -D-fructofuranosyl 2,3,6-tri-*O*-acetyl-4-azido-4-deoxy- α -D-galactopyranoside (73) plus 3,4-di-*O*-acetyl-1,6-diazido-1,6-dideoxy- β -D-fructofuranosyl 2,3,6-tri-*O*-acetyl-4-azido-4-deoxy- α -D-galactopyranoside (74) in yields of 24.2 and

(115) S. Umezawa, T. Tsuchiya, S. Nakada, and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **40**, 395–401 (1967).

(116) L. Hough and K. S. Mufti, *Carbohydr. Res.*, **29**, 291–296 (1973).



2. Amines

Amino sugars are components of many antibiotics¹¹⁸ and bacterial polysaccharides,¹¹⁹ and are therefore of considerable interest. Amino-deoxysucroses have generally been prepared by catalytic hydrogenation of the corresponding azides. Synthesis of 2,3,4,1',3',4',6'-hepta-O-acetyl-6-amino-6-deoxysucrose¹²⁰ and 3-acetamido-3-deoxy- α -D-allopyranosyl β -D-fructofuranoside¹²¹ has been achieved by catalytic hydrogenation of the corresponding azidodeoxy derivatives. 6,6'-Diamino-6,6'-dideoxysucrose has been prepared by hydrogenation of 6,6'-diazido-6,6'-dideoxysucrose over palladium-on-charcoal.¹²² It is of interest that a similar hydrogenation of the corresponding, perace-

(118) J. D. Dutcher, *Adv. Carbohydr. Chem.*, **18**, 259-308 (1963).

(119) N. Sharon, in "The Amino Sugars," E. A. Balazs and R. W. Jeanloz, eds., Vol. IIA, Academic Press, New York, 1965, pp. 1-45.

(120) I. Jezo, *Chem. Zvesti*, **25**, 364-368 (1971).

(121) I. Jezo, *Chem. Zvesti*, **25**, 369-374 (1971).

(122) R. Khan, K. S. Mufti, and K. J. Parker, Brit. Pat. Application (1973).

tylated derivative was not successful, probably because of steric interference between the acetyl substituent and the catalyst surface. Similarly, when hydrogenation of 6,1',6'-triazido-2,3,4,3',4'-penta-*O*-benzoyl-6,1',6'-trideoxysucrose was attempted, no reaction occurred. The catalytic hydrogenation proceeded smoothly with the free azide, 6,1',6'-triazido-6,1',6'-trideoxysucrose, to give 6,1',6'-triamino-6,1',6'-trideoxysucrose in 67% yield.¹¹⁷ *N*-Acetylation of the triamine with acetic anhydride in methanol gave 6,1',6'-tris(acetamido)-6,1',6'-trideoxysucrose. With acetic anhydride and pyridine, the 6,1',6'-triamine and the corresponding 6,1',6'-triacetamide gave 6,1',6'-tris(acetamido)-2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-trideoxysucrose (m.p. 99–101°; $[\alpha]_D +37^\circ$). The structure of this compound was confirmed on the basis of its ¹H n.m.r. spectrum. The first-order coupling-constants ($J_{1,2}$ 3.5, $J_{2,3}$ 10.0 $J_{3,4}$ 10.0 Hz) revealed the α -D-*gluco* configuration and the ⁴C₁(D) conformation for the hexopyranoside moiety.¹¹⁷ Umezawa and coworkers¹¹⁵ reported the synthesis of a tris(acetamido)trideoxysucrose pentaacetate (m.p. 142–143°; $[\alpha]_D +45^\circ$) which they erroneously characterized as 6,1',6'-tris(acetamido)-2,3,4,3',4'-penta-*O*-acetyl-6,1',6'-trideoxysucrose (see Section X,1).

XI. MISCELLANEOUS COMPOUNDS

1. β -D-Fructofuranosyl α -D-Galactopyranoside

Enzymic synthesis of β -D-fructofuranosyl α -D-galactopyranoside has been achieved¹²³ by using raffinose as the D-fructosyl donor to D-galactose in a levansucrase system. The first chemical synthesis of β -D-fructofuranosyl α -D-galactopyranoside was achieved in 62% yield by Khan⁴² in 1972 by the following reaction sequence: 2,3,4,1',3',4'-hexa-*O*-acetyl-6,6'-di-*O*-tritylsucrose (37) \rightarrow 2,3,6,1',3',4'-hexa-*O*-acetyl-6'-*O*-tritylsucrose \rightarrow 2,3,6,1',3',4'-hexa-*O*-acetyl-4-*O*-(methylsulfonyl)-6'-*O*-tritylsucrose \rightarrow 2,3,6,1',3',4',6'-hepta-*O*-acetyl-4-*O*-(methylsulfonyl)sucrose \rightarrow β -D-fructofuranosyl α -D-galactopyranoside.^{116,124} Interestingly, the inversion of the configuration of C-4 in the sucrose molecule to give β -D-fructofuranosyl α -D-galactopyranoside caused almost complete loss of sweetness.¹²⁵

(123) D. S. Feingold, G. Avigad, and S. Hestrin, *J. Biol. Chem.*, **224**, 295–307 (1957).

(124) P. H. Fairclough, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, **40**, 285–298 (1975).

(125) M. G. Lindley, G. G. Birch, and R. Khan, *J. Sci. Food Agric.*, **27**, 140–144 (1975).

2. Phosphates

Synthesis of sucrose 6'-phosphate by an enzymic method using uridine 5'-(α -D-glucopyranosyl diphosphate) plus D-glucose 6-phosphate has been reported.¹²⁶⁻¹³¹ The first, unambiguous, chemical synthesis of sucrose 6'-phosphate was achieved by Buchanan and coworkers.¹⁸ The reaction of 2,3,4,6,1',3',4'-hepta-O-acetylsucrose, prepared by five steps of synthesis, with cyanoethyl phosphate in pyridine gave a crude product from which sucrose 6'-phosphate was isolated as the barium salt.

3. Ketonic Derivatives

Microbial oxidation of sucrose by *Agrobacterium tumefaciens* to give β -D-fructofuranosyl α -D-ribo-hexosid-3-ulose has been reported.¹³²⁻¹³⁵ Chemical oxidation of suitably protected derivatives of sucrose is of interest. The reaction of 2,3,6,1',3',4',6'-hepta-O-acetylsucrose with either ruthenium tetroxide in carbon tetrachloride, or dimethyl sulfoxide-acetic anhydride, gave 1,3,4,6-tetra-O-acetyl- β -D-fructofuranosyl 2,3,6-tri-O-acetyl- α -D-xylo-hexosid-4-ulose in 70% yield.¹³⁶ Oxidation of 2,3,4,1',3',4',6'-hepta-O-benzoylsucrose with potassium permanganate in aqueous acetone gave the corresponding 6-carboxylic acid which, on treatment with diazomethane, afforded the corresponding 6-methyl ester.¹³⁷

XII. POTENTIAL, CHEMICAL UTILIZATION

1. Surfactants and Surface-coating Agents

The long-chain, fatty acid esters of sucrose are non-ionic, nontoxic, and biodegradable, and compare well in overall performance with other surface-active compounds in detergency, emulsification, and

- (126) L. F. Leloir and C. E. Cardini, *J. Biol. Chem.*, **214**, 157-165 (1955).
- (127) R. C. Bean and W. Z. Hassid, *J. Am. Chem. Soc.*, **77**, 5737-5738 (1955).
- (128) D. P. Burma and D. C. Mortimer, *Arch. Biochem. Biophys.*, **62**, 16-28 (1956).
- (129) J. Mendicino, *J. Biol. Chem.*, **235**, 3347-3352 (1960).
- (130) A. J. Keys and R. V. Martin, *Biochem. J.*, **88**, 44P-45P (1963).
- (131) M. D. Hatch, *Biochem. J.*, **93**, 521-526 (1964).
- (132) M. J. Bernaerts and J. De Ley, *Biochim. Biophys. Acta*, **30**, 661-662 (1958).
- (133) S. Fukui, R. M. Hochster, R. Durbin, E. E. Grebner, and D. S. Feingold, *Bull. Res. Council. Isr. Sect. IIA*, **4**, 262-268 (1963).
- (134) S. Fukui and R. M. Hochster, *J. Am. Chem. Soc.*, **85**, 1697-1698 (1963).
- (135) W. M. Kurowski and S. J. Pirt, *J. Gen. Microbiol.*, **68**, 65-69 (1971).
- (136) R. Khan, unpublished data.
- (137) E. Tarelli, L. Hough, and A. C. Richardson, unpublished data.

related properties. Preparation of such esters of sucrose has been widely investigated.^{63(f),138-147} A comprehensive collection of the U.S. patents relating to the preparation and application of sucrose fatty acid esters has been published.¹⁴¹ A well-known and effective method of preparation is by transesterification of sucrose with methyl esters of long-chain fatty acids or unsaturated, drying-oil acids.

The transesterification of sucrose has been performed with a fatty acid ester of a volatile alcohol in the presence of an alkaline catalyst in a dipolar, aprotic solvent.¹⁴² The reaction of sucrose (293 mmoles) with methyl dodecanoate (293 mmoles) in *N,N*-dimethylformamide in the presence of sodium methoxide in a pressure bomb for 8 h at 130° gave, after solvent extraction and crystallization, sucrose mono(dodecanoate) (m.p. 72–80°; $[\alpha]_D +52^\circ$) in ~50% yield.¹⁴² Commercialization of these sucrose esters has so far been limited, in part because of the use of expensive solvents, and, in part, because solvent remaining in the product makes it unsuitable for use as a food emulsifier. In view of this situation, methods have been developed in which the use of toxic and expensive solvents has been avoided.

The transesterification reaction of sucrose has been conducted in the presence of a suitable emulsifying agent.¹⁴⁶ A mixture of sucrose, sodium oleate, and methyl stearate is heated with propylene glycol, which is an emulsifying agent, at ~130° with constant stirring. The reaction mixture is evaporated under diminished pressure to remove the traces of moisture along with 10% of the propylene glycol, and then treated with anhydrous potassium carbonate at 110° under di-

- (138) L. Osipow, F. D. Snell, W. C. York, and A. Finchler, *Ind. Eng. Chem.*, **48**, 1459–1462 (1956).
- (139) L. Osipow, F. D. Snell, D. Marra, and W. C. York, *Ind. Eng. Chem.*, **48**, 1462–1464 (1956).
- (140) K. J. Parker, R. Khan, and K. S. Mufti, Brit. Pat. Application (1973); R. Khan and K. S. Mufti, Ger. Offen. 2,412, 374; *Chem. Abstr.*, **82**, 100,608r (1975).
- (141) J. C. Colbert, "Sugar Esters: Preparation and Application," Noyes Data Corporation, New Jersey, 1974, p. 30.
- (142) H. B. Hass, F. D. Snell, W. C. York, and L. I. Osipow, U. S. Pat. 2,893,990 (1959); *Chem. Abstr.*, **53**, 19,422c (1959).
- (143) H. B. Hass, U. S. Pat. 2,970,142 (1961); *Chem. Abstr.*, **55**, 12,885g (1961).
- (144) H. Von Brachel and M. Schön, Brit. Pat. 1,188,614; *Chem. Abstr.*, **73**, 15,168s (1970).
- (145) L. I. Osipow and W. Rosenblatt, U. S. Pat. 3,644,333; *Chem. Abstr.*, **73**, 66,862w (1970).
- (146) L. I. Osipow and W. Rosenblatt, Brit. Pat. 1,180,103; *Chem. Abstr.*, **71**, 51,470b (1969).
- (147) R. O. Feuge, T. J. Weiss, and T. H. Zeringue, Jr., Brit. Pat. 1,308,234; *Chem. Abstr.*, **75**, 110,553 (1971).

minated pressure until essentially all of the propylene glycol has distilled off. The rate of reaction has been found to be of the same general order as in homogeneous solutions. Water has also been used to form a transparent emulsion, which is removed during or prior to the addition of the esterification catalyst.¹⁴²

Transesterification reaction of sucrose with long-chain fatty acid esters has been effectively performed in a melt as the reaction medium.¹⁴¹ According to this process, sucrose and sodium stearate are mixed with a small proportion of water to afford a (homogeneous) solution. Most of the water is then removed by heating the mixture at 100–125°, and a catalyst is added. The mixture is now treated with a fatty acid ester while the remaining water is being distilled off at 150°/60 torr. The reaction is allowed to continue for 3 h at that temperature and pressure, to give a mixture containing mono-, di-, and tri-esters in the ratios of 26:15:9, respectively. This method has shown advantage over other methods because it is high-yielding and does not require an additional step for removing the solvent.

Unsaturated fatty acid esters^{63(g),143,148–151} and allyl ethers of sucrose^{63(h),152–156} have shown promise as surface coating-agents. The essential requirement of such a material is a high degree of substitution, and, ideally, an octasubstituted ester derivative should be the most suited for this purpose. However, to accomplish such a high degree of substitution economically on a commercial scale does not yet appear feasible.

2. Plastics and Polymers

Work on sucrose-based plastics and polymers, sponsored by the International Sugar Research Foundation, has been reviewed. The

- (148) "Engineering and Pilot Plant Data for the Commercial Production of Sucrose Esters for the Ink, Paint, and Protective Coatings Industry," Sugar Research Foundation, Inc., New York, 1963.
- (149) "The Chemistry of Preparation of Sucrose Esters for the Ink, Paint, and Protective Coating Industry," Sugar Research Foundation, Inc., New York, 1963.
- (150) E. G. Bobalek, T. J. Walsh, and H. H. Chiang, *Off. Dig. Fed. Soc. Paint Technol.*, **33**, 453–468 (1961).
- (151) E. G. Bobalek and A. P. De Mendoza, *Off. Dig. J. Paint Eng.*, **35**, 1013–1035 (1963).
- (152) C. G. Tomocko and R. Adams, *J. Am. Chem. Soc.*, **45**, 2698–2701 (1923).
- (153) P. L. Nichols, Jr., and E. Yanovsky, *J. Am. Chem. Soc.*, **67**, 46–49 (1945).
- (154) P. L. Nichols, Jr., A. N. Wrigley, and E. Yanovsky, *J. Am. Chem. Soc.*, **68**, 2020–2022 (1946).
- (155) P. L. Nichols, Jr., and E. Yanovsky, *Sugar*, **42**, 28–29 (1947).
- (156) B. D. Jones, Ph.D. Thesis, University of Birmingham, England (1964).

commercial outcome has so far not been very encouraging, but their potential as hydrophilic polymers has been recognized. The hydrophilicity of conventional resins can be modified by copolymerization or graft polymerization reactions with sucrose or its derivatives. The copolymerization of 6,1',6'-tri-*O*-(*p*-vinylbenzoyl)sucrose with styrene and methyl methacrylate has been investigated by Jenkins and his colleagues.^{157,158} The incorporation of sucrose into the polystyrene system has been found to impart a certain amount of biodegradability and an increase in the glass-transition temperatures.^{157,158} The copolymerization of sucrose with 3-chloropropylene oxide affords a product which is marketed as "Ficol" and is used for chromatography.¹⁵⁹ Sucrose has also been used in other polymer-forming systems, such as phenolic resins,⁶³⁽ⁱ⁾ amino-resins of the sucrose-melamine-formaldehyde and sucrose-urea-formaldehyde types,^{63(j),160,161} and polyurethan foams.^{63(k)}

3. Agricultural Chemicals, and Pharmaceuticals

Sucrose is synthesized by almost every green plant in the world, and is assimilated by most organisms. It is an early product of photosynthesis, and acts as the main agent for translocating carbon from the photosynthetic centers to the rest of the plant. It therefore appears logical to conceive that suitably modified sucrose derivatives could find application as selective pesticides, specific poisons, or growth regulators. It is known that the effectiveness of a pesticidal compound depends upon its water dispersibility and cell permeability, which could be achieved by incorporating sucrose molecules into the toxic moieties of compounds known to be biologically active. Under the aegis of The International Sugar Research Foundation, about a hundred derivatives of sucrose have been systematically screened for their biological properties.^{63(l)} However, despite many optimistic claims, none of these derivatives have achieved any commercial success.

The transport across cell walls is often a major problem for therapeutic agents. The possibility of designing drugs chemically linked to sucrose, which would act as a carrier, offers a means of overcoming this limitation.

(157) A. D. Jenkins, R. D. Guthrie, and T. J. Lucas, Brit. Pat. Application (1974).

(158) T. J. Lucas, Ph.D. Thesis, University of Sussex, England, 1973.

(159) Pharmacia Chemicals, Uppsala, Sweden.

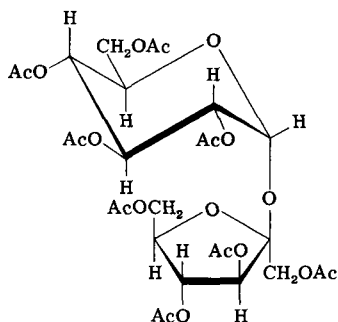
(160) W. Flavell, *Int. Sugar Res. Conf., 1st, Brussels*, 1970.

(161) W. Flavell and G. L. Redfearn, in "Sugar," J. Yudkin, J. Edelman, and L. Hough, eds., Butterworth, London, 1971, pp. 69-79.

XIII. PHYSICAL METHODS

1. Nuclear Magnetic Resonance Spectroscopy

The value of ^1H n.m.r. spectroscopy in determining the structures of carbohydrates is well recognized. In this Section, some of the important features observed in the 100-MHz, ^1H n.m.r. spectra of sucrose derivatives will be discussed, and the potential of ^{13}C nuclear magnetic resonance spectroscopy will be very briefly indicated. Horton and his colleagues¹⁶² discussed the high resolution, ^1H n.m.r. spectra of octa-*O*-acetylsucrose (75). The chemical shifts and cou-



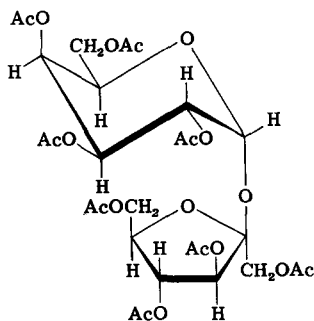
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pling constants were determined with the aid of double-resonance techniques, the multiple-solvent method, and a high magnetic field. Discrete signals for H-1, H-2, H-3, H-4, H-3', and H-4' at δ 5.85, 5.01, 5.78, 5.67, 5.29, and 5.52, respectively, were observed. The first-order coupling-constants derived ($J_{1,2}$ 3.7, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5, and $J_{4,5}$ 9.7 Hz) confirmed the α -D-*gluco* configuration and $^4\text{C}_1(\text{D})$ conformation of the hexopyranosyl moiety. The first-order coupling-constants ($J_{3',4'}$ 5.5 and $J_{4',5'}$ 5.1 Hz) were in agreement with a favored conformation for the tetra-*O*-acetyl-D-fructofuranosyl group (in octa-*O*-acetylsucrose) that has C-2', C-3', C-5', and O-5' approximately in one plane, with C-4' displaced above that plane.

The orientation of the acetoxyl group at C-4 of the pyranoside moiety in 1,3,4,6-tetra-*O*-acetyl- β -D-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (76), derived from 2,3,6,1',3',4',6'-hepta-*O*-acetyl-4-*O*-(methylsulfonyl)sucrose (see Section XI,1), was confirmed on the basis of its ^1H n.m.r. spectrum.⁴² The derived first-order coupling-constants ($J_{1,2}$ 3.4, $J_{2,3}$ 10.5, $J_{3,4}$ 3.0, and $J_{4,5}$ 3.0 Hz)

(162) W. W. Binkley, D. Horton, and N. S. Bhacca, *Carbohydr. Res.*, **10**, 245-258 (1969).

revealed an *e,a,a,e* arrangement of H-1, H-2, H-3, and H-4, respectively, in agreement with the α -D-*galacto* configuration and ${}^4C_1(D)$ conformation in compound **76**. Similarly, the structure of 1,3,4,6-



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tetra-*O*-acetyl- β -D-fructofuranosyl 2,3,4-tri-*O*-acetyl-6-deoxy- β -L-idopyranoside (**66**) was confirmed¹¹⁰ from its 1H n.m.r. spectrum (see Section IX). High-resolution, 1H n.m.r. spectroscopy has proved of immense value in identifying the position of isolated hydroxyl groups in sucrose derivatives. In the 1H n.m.r. (100 MHz) spectrum of 2,3,6,1',3',4',6'-hepta-*O*-acetylsucrose (**21**), separate signals for H-1, H-2, H-3, H-3', and H-4' were observed, as expected, at δ 5.64, 4.3, 5.42, 5.5, and 5.37, respectively.⁴⁵ The fact that the signal of H-4 shifted upfield from where it usually occurs ($\delta \sim 5.29$) in fully acetylated derivatives indicated the location of the free hydroxyl group in compound **21**. Addition of trichloroacetyl isocyanate to the solution in chloroform-*d* resulted in the appearance of a singlet at δ 8.78 due to the imino proton, thereby confirming the presence of a single hydroxyl group in compound **21**. The upfield shift of resonances due to protons on adjacent carbon atoms bearing acetal-oxy,^{25,27,32,83} methoxyl,^{36,37} and methylsulfonyloxy³² groups, in comparison with the corresponding acetoxyl substituents, has also been observed. In relation to the 1H n.m.r. spectrum of octa-*O*-acetylsucrose (**75**), the resonances due to H-2 and H-4 in 3,3',4',6'-tetra-*O*-acetyl-2,1':4,6-di-*O*-isopropylidenesucrose (**35**) appeared³² at a higher field, namely, at δ 3.85 and 3.75, respectively, thereby indicating the involvement of O-2 and O-4 in the presumed acetal-type of linkage in compound **35**. High-resolution, 1H n.m.r. spectra of the peracetylated trityl ethers of sucrose have been described.²⁶ Introduction of trityl ether groups into the sucrose molecule results in a deshielding of the protons on the furanoid and pyranoid rings.²⁶ In the spectra of 1'- and 6'-*O*-trityl- and 1',6'-di-*O*-trityl-sucrose, the

ring protons of the hexopyranose moiety have been found²⁶ to resonate at a field slightly higher than those for the corresponding protons in octa-*O*-acetylsucrose (75).

In an article published in this Series,¹⁶³ Hall demonstrated the potential of lanthanide shift-reagents in solving the hidden-resonance problem of ¹H n.m.r. spectra of carbohydrates. The importance of this technique with sucrose derivatives is indicated by the following example.¹⁶⁴ In a normal, ¹H n.m.r. (100 MHz) spectrum of 2,3,4,1',3',4',6'-hepta-*O*-acetyl-6-chloro-6-deoxysucrose (46), the overlapping signals due to H-3, H-3', and H-4' appear between δ 5.32 and 5.52, and could not be interpreted on a first-order basis. However, when a lanthanide shift-reagent, tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,5-octanedione)europium(III) was added to the chloroform-*d* solution in various concentrations, it caused the ring protons to shift downfield. Discrete signals for H-3, H-3', and H-4' were then obtained that allowed confirmation of the supposed structure of compound 46.

¹³C Nuclear magnetic resonance (c.m.r.) spectra of α -D-glucopyranose,^{165,166} β -D-fructofuranose,¹⁶⁷ sucrose,¹⁶⁸ raffinose,¹⁶⁸ stachyose,¹⁶⁸ nystose,¹⁶⁹ and 1-kestose¹⁶⁹ have been described. The c.m.r. spectrum of sucrose has been measured in aqueous solution with tetramethylsilane as the internal standard.¹⁶⁸ The chemical-shift values observed for sucrose (see Table I) almost equal the sum of the values for α -D-glucopyranose and β -D-fructofuranose. The signals for the anomeric carbon atoms have been identified conclusively at the lower end of the spectrum, as expected, with C-2 of the D-fructofuranosyl group in sucrose at the lowest field. The ¹³C nuclei of the primary hydroxymethyl groups appeared at the higher-field end of the spectrum. The c.m.r. spectrum of 2,3,4,1',3',4'-hexa-*O*-acetyl-6,6'-dichloro-6,6'-dideoxysucrose (53) (see Table I) in chloroform-*d* in a 10-mm sample-tube, with tetramethylsilane as the internal standard, was recorded with a Varian CFT-20, ¹³C n.m.r. spectrometer.¹⁷⁰ The tentative assignments given in Table I for compound 53 are based on earlier interpretations¹⁶⁵⁻¹⁶⁸ of the spectra of α -D-glucopyranose, β -D-

(163) L. D. Hall, *Adv. Carbohydr. Chem. Biochem.*, **29**, 11-40 (1974).

(164) T. Yüceer and R. Khan, unpublished data.

(165) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355-1361 (1970).

(166) A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, **48**, 2596-2606 (1970).

(167) D. Doddrell and A. Allerhand, *J. Am. Chem. Soc.*, **93**, 2779-2781 (1971).

(168) A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, **93**, 2777-2779 (1971).

(169) W. W. Binkley, D. Horton, N. S. Bhacca, and J. D. Wander, *Carbohydr. Res.*, **23**, 301-306 (1972).

(170) R. Khan, unpublished data.

TABLE I
 Values^a of ¹³C N.m.r. Chemical Shifts

Assignments ^b	Chemical shift of ¹³ C resonances of			
	α -D-Glucopyranose ^{c,d,e}	β -D-Fructofuranose ^{c,e}	Sucrose ^{c,f}	Compound 53
D-Fructosyl C-2		101.3	103.4	104.53
D-Glucosyl C-1	92.0		91.8	90.42
D-Fructosyl C-3 or C-4		80.4	81.1	81.44
C-4 or C-3		75.4	76.4	76.53
C-5		74.4	73.9	76.13
D-Glucosyl C-2	72.8		72.5	70.26
C-3 or C-5	71.5		72.1	69.78
C-5 or C-3	71.3		70.9	69.57
C-4	69.6		69.1	69.50
D-Glucosyl C-6, and D-fructosyl C-1 and C-6	60.8	62.8 62.2	62.2 61.4 60.1	62.49
				44.17
				43.3
Ac				20.54

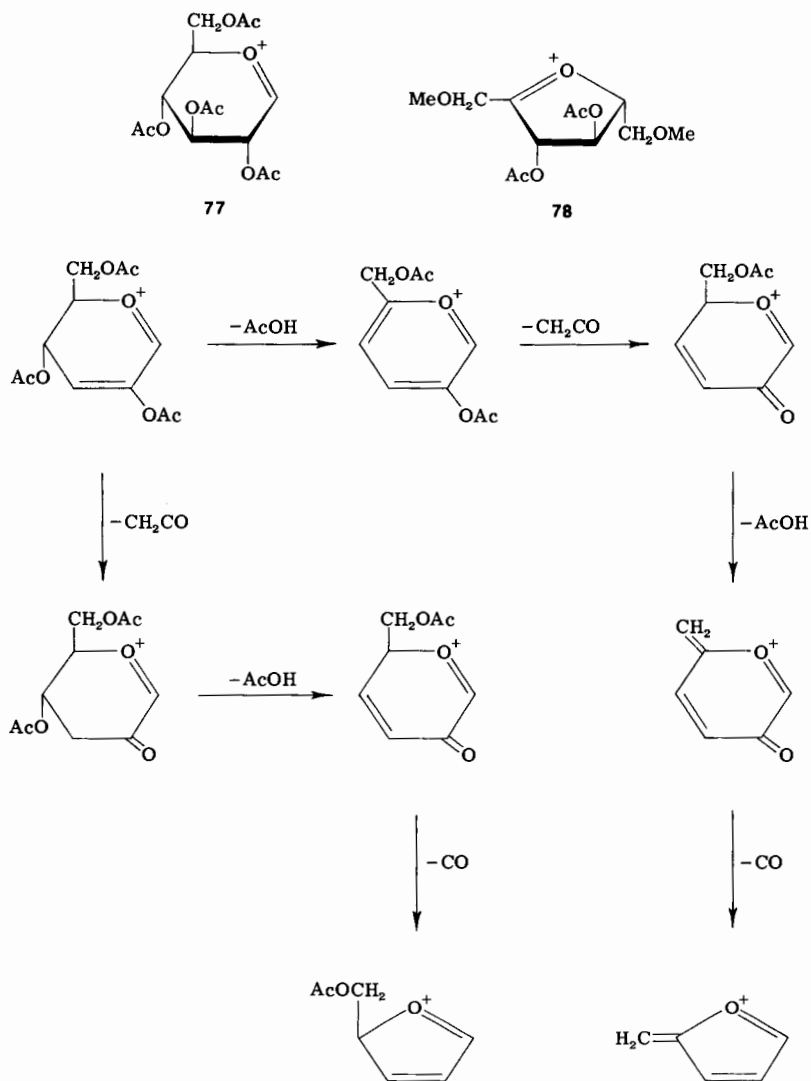
^a Chemical shifts are expressed in p.p.m. downfield of the ¹³C resonance of tetramethylsilane. ^b Except for the general anomeric-carbon and primary (CH₂OH) carbon assignments, these are tentative. ^c Measured in water. ^d Data from Ref. 165. ^e Data from Ref. 167. ^f Data from Ref. 168.

fructofuranose, and sucrose. The chemical-shift values ($\delta_{\text{Me}_4\text{Si}}$) of most of the methine carbon atoms in **53** compare well with those for sucrose, except for the two hydroxymethyl (¹³CH₂O-) groups on C-5 and C-5'. The resonances due to C-6 and C-6' underwent drastic chemical-shifts on chlorination, -17.23 and -16.8 p.p.m. with respect to sucrose. By a simple method of deduction, the resonance position for C-1' in the spectrum of **53** was identified at 62.49 p.p.m. Although most of the assignments are at present tentative, the potential of this technique in the structural determination of sucrose derivatives has been demonstrated.

2. Mass Spectrometry

Mass spectrometry has proved an important and versatile technique in carbohydrate chemistry.¹⁷¹⁻¹⁷⁵ High-resolution, electron-im-

- (171) N. K. Kochetkov and O. S. Chizhov, *Adv. Carbohydr. Chem.*, **21**, 39-93 (1966).
 (172) O. S. Chizov and N. K. Kochetkov, *Methods Carbohydr. Chem.*, **6**, 540-554 (1972).



Scheme 1

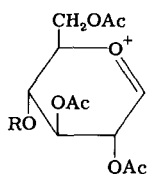
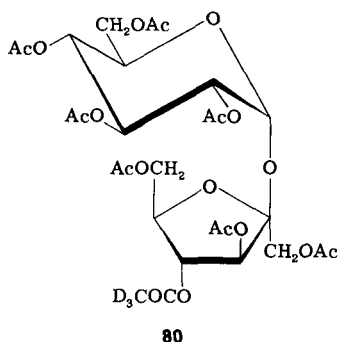
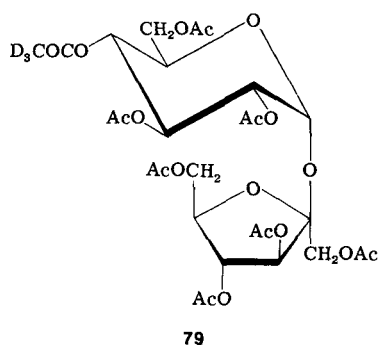
- (173) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Holden-Day, San Francisco, 1964, Vol. II, pp. 203-240.
- (174) N. K. Kochetkov, O. S. Chizov, and A. F. Bochkov, *MTP Int. Rev. Sci., Org. Chem. Ser., 1*, Butterworth, London, 1973, Vol. 7, pp. 147-190.
- (175) J. Lönngren and S. Svensson, *Adv. Carbohydr. Chem. Biochem.*, **29**, 41-106 (1974).

pact, mass spectrometry of sucrose derivatives has been studied by Horton and his colleagues.¹⁷⁶ The initial fragmentation was shown to proceed by localized ionization at a single glycosyl residue to give hexopyranosyl and ketofuranosyl cations, and subsequent fragmentation within that residue. Unlike octa-*O*-acetylsucrose, the per(trimethylsilyl) ether of sucrose showed a small, but distinct, molecular-ion peak. However, use of peracetate derivatives of sucrose for mass-spectral study has generally been preferred, because of the ease of preparation and purification. In the case of sucrose derivatives, the cleavage of the D-fructosidic bond is the most favored, initial fragment, as this leads to a tertiary carbonium ion. The mass spectrum of 2,3,4,6,3',4'-hexa-*O*-acetyl-1',6'-di-*O*-methylsucrose (**29**) has been studied, and, based on earlier information,¹⁷⁷⁻¹⁷⁹ a probable pattern of fragmentation has been suggested³⁶ (see Scheme 1). Compound **29** underwent cleavage at the interglycosidic linkage to give ions at *m/e* 331 and 275 due to hexopyranosyl (**77**) and ketofuranosyl (**78**) cations, respectively. The loss of acetic acid from compound **77** gave an ion at *m/e* 271 that underwent further fragmentation with the loss of either acetic acid, ketene, acetic acid, and carbon monoxide, or ketene, two molecules of acetic acid, and carbon monoxide. The ketofuranosyl cation (**78**) gave peaks at *m/e* 215, 173, 155, and 141. No attempt will be made to propose here a decomposition pattern for **78**, as very little is known about the way in which the ketofuranosyl cations fragment. Extensive labelling experiments are needed in order to confirm these results.

The fragmentation pattern of 2,3,6,1',3',4',6'-hepta-*O*-acetyl-4-*O*-(trideuterioacetyl)sucrose (**79**) and 2,3,4,6,1',3',6'-hepta-*O*-acetyl-4'-*O*-(trideuterioacetyl)sucrose (**80**) has been described.⁴⁴ The mass spectrum of compound **79** contains fragment ions corresponding to the two oxycarbonium ions (**81**) and (**82**). Likewise, for **80**, ions **83** and **84** were observed. For compound **79**, the hexopyranosyl cat-

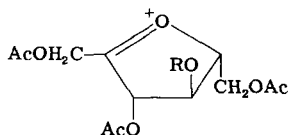
- (176) W. W. Binkley, R. C. Dougherty, D. Horton, and J. D. Wander, *Carbohydr. Res.*, **17**, 127-144 (1971).
- (177) L. Hough, A. K. Palmer, and A. C. Richardson, *J. Chem. Soc. Perkin Trans. I*, 2513-2517 (1972); 784-788 (1973).
- (178) J. P. Kamerling, J. F. G. Vliegthart, J. Vink, and J. J. De Rider, *Tetrahedron*, **27**, 4275-4288 (1971).
- (179) K. G. Das and B. Thayumanavan, *Org. Mass Spectrom.*, 1063-1069 (1972).
- (180) R. K. Ness and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **74**, 5344-5346 (1952).
- (181) M. E. Tate and C. T. Bishop, *Can. J. Chem.*, **41**, 1801-1806 (1963).
- (182) T. Iwashige and H. Saeki, *Chem. Pharm. Bull.*, **15**, 1803-1806 (1967).
- (183) R. K. Ness, H. W. Diehl, and H. G. Fletcher, Jr., *Carbohydr. Res.*, **13**, 23-32 (1970).

ion (81) underwent loss of acetic acid (m/e 334 \rightarrow 274), whereas loss of trideuterioacetic acid (m/e 334 \rightarrow 271) was not detected. A similar loss of acetic acid from the ketofuranosyl cation (82) was not noted. Similarly, for compound 80, no loss of acetic acid or trideuterioacetic acid was observed from the ketofuranosyl cation (84) (m/e 334 \rightarrow 274, m/e 334 \rightarrow 271, respectively). These results suggested that the decomposition pattern for the ketofuranosyl ion (82) is different from that for the hexopyranosyl ion (83), which affords 85 by loss of a molecule of acetic acid.



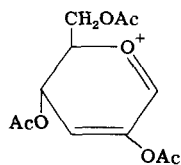
81 R = COCD₃ (m/e 334)

83 R = Ac (m/e 331)



82 R = Ac (m/e 331)

84 R = COCD₃ (m/e 334)



85

XIV. TABLES OF PROPERTIES OF SUCROSE DERIVATIVES

The following Tables constitute a list of most of the known, characterized derivatives of sucrose. The names of the solvents used for measuring the specific rotations are abbreviated as follows: A, acetone; C, chloroform; Dm, dichloromethane; E, ethanol; M, methanol; Mf, *N,N*-dimethylformamide; P, pyridine; and W, water.

TABLE II
 Trityl Ethers

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
1'-O-Trityl-	193-196	+70.4 (M)		26
3,4,6,3',4',6'-hexa-O-acetyl-	67-69	+64.8 (C)	¹ H n.m.r., m.s.	27
2-O-acetyl-		+64.8 (C)	¹ H n.m.r., m.s.	26,27
2-O- <i>p</i> -tolylsulfonyl-	87-89	+59.6 (C)	¹ H n.m.r.	27
6-O-Trityl-	99-101	+51.7 (Mf)	i.r., u.v.	16
3,3',4',6'-tetra-O-acetyl-		+44.5 (C)		32
2,1'-di-O-acetyl-		+45.2 (C)	¹ H n.m.r.	25
hepta-O-acetyl-	103-104		¹ H n.m.r., i.r.	16
6'-O-Trityl	221-223	+40.1 (C)		18
2,3,6,3',4'-penta-O-acetyl-	146-148	+27.1 (C)		124
1'-O-acetyl-		+19.9 (C)		42
4-O-acetyl-	118	+58.5 (C)	¹ H n.m.r., i.r.	18
4-O-(methylsulfonyl)-	85-87	+29.4 (C)	¹ H n.m.r.	42
4-O- <i>p</i> -tolylsulfonyl-	82-84	+31.3 (C)	¹ H n.m.r.	42
4,1'-di-O-(methylsulfonyl)-	101-103	+51.8 (C)	¹ H n.m.r.	124
6,1'-Di-O-trityl-	133		i.r., u.v.	30
3,3',4',6'-tetra-O-acetyl-		+48.2 (C)	¹ H n.m.r.	32
2,4-di-O-acetyl-	101-104	+84 (C)	¹ H n.m.r., i.r.	30,31
2,4-di-O-(methylsulfonyl)-	189-190	+50.7 (C)	¹ H n.m.r.	32
1',6'-Di-O-trityl-	129		i.r., u.v.	30
2,3,6,3',4'-penta-O-acetyl-	105-107	+46.9 (C)	¹ H n.m.r.	124
4-O-acetyl-	95-97	+65.3 (C)		30,124
4-O-(methylsulfonyl)-	111-114	+37.4 (C)	¹ H n.m.r.	124
6,6'-Di-O-trityl-	134-136	+43 (C)		28,30
hexa-O-acetyl-	104-105	+64.6 (C)	¹ H n.m.r.	28
hexa-O-benzoyl-	107-110	+3 (C)	¹ H n.m.r.	28
6,1',6'-Tri-O-trityl-	128-130	+62.2 (C)		18,20
penta-O-acetyl-	235-236	+68.9 (C)		22
penta-O-benzoyl-	87-90	+19 (C)	¹ H n.m.r.	28

TABLE III
Methyl Ethers

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
4- <i>O</i> -Methyl-		+49.6 (W)		37
hepta- <i>O</i> -acetyl-		+49.4 (C)	^1H n.m.r., m.s.	37
6'- <i>O</i> -Methyl-		+53.4 (W)		37
hepta- <i>O</i> -acetyl-		+59.1 (C)	^1H n.m.r., m.s.	37
2,1'-Di- <i>O</i> -methyl-	86-88			
hexa- <i>O</i> -acetyl-		+59.8 (C)	^1H n.m.r., m.s.	40
1',6'-Di- <i>O</i> -methyl-		+70 (W)		37
hexa- <i>O</i> -acetyl-		+59.8 (C)	^1H n.m.r., m.s.	37
4,6-Di- <i>O</i> -methyl-		+61.4 (W)		37
hexa- <i>O</i> -acetyl-		+63 (C)	^1H n.m.r., m.s.	37
4,6'-Di- <i>O</i> -methyl-		+44.8 (W)		37
hexa- <i>O</i> -acetyl-		+61.9 (C)	^1H n.m.r., m.s.	37
6,6'-Di- <i>O</i> -methyl-		+63.6 (W)		37
hexa- <i>O</i> -benzoyl-		+21.5 (C)	^1H n.m.r.	37
4,1',6'-Tri- <i>O</i> -methyl-		+67.1 (W)		20,37
penta- <i>O</i> -acetyl-		+60.1 (C)	^1H n.m.r., m.s.	20,37
6,1',6'-Tri- <i>O</i> -methyl-		+69 (W)		37
penta- <i>O</i> -acetyl-		+52 (C)	^1H n.m.r., m.s.	34,37
2,4,6,1'-Tetra- <i>O</i> -methyl-				
tetra- <i>O</i> -acetyl-		+56.2 (C)	^1H n.m.r., m.s.	40
Octa- <i>O</i> -methyl-		+70.1 (C)		35

TABLE IV
Acetates, Benzoates, and 3-Benzoylpropionates

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
3,4,3',4'-Tetra-O-acetyl-	154-156	+46 (C)	¹ H n.m.r.	27
2-O-acetyl-	133-134	+52.5 (C)		66
6-O-acetyl-		+40.1 (C)		33
1'-O-acetyl-	160	+49.5 (C)	¹ H n.m.r.	18
1',6'-di-O-acetyl-		+61.3 (C)	¹ H n.m.r., i.r.	66
6,1',6'-tri-O-acetyl-	111-113	+66.5 (C)	¹ H n.m.r., m.s.	27
3,3',4',6'-Tetra-O-acetyl-	121-123	+58.6 (C)	¹ H n.m.r.	32
2,1'-di-O-acetyl-		+28.4 (C)	m.s.	25
6-O-acetyl-		+48.8 (C)	¹ H n.m.r., m.s.	45
		+46.3		43,44
4,6-di-O-acetyl-	118-119	+51 (C)	¹ H n.m.r., m.s.	27
2,3,6,3',4'-Penta-O-acetyl-	154-156	+22 (C)		18,20
1'-O-acetyl-		+32.1 (C)		124
2,3,4,6,1',3',6'-Hepta-O-acetyl-		+54.3 (C)	¹ H n.m.r.	44
Octa-O-acetyl-	69	+59.6 (C)	¹ H n.m.r.	50,162,176
Hepta-O-acetyl-2-O-				
(3-benzoylpropionyl)-	90-92	+39.4 (C)	¹ H n.m.r.	27
Hepta-O-acetyl-4-O-				
(trideuterioacetyl)-	84-87		m.s.	44
Hepta-O-acetyl-4'-O-				
(trideuterioacetyl)-	85-88		m.s.	44
2,3,4,3',4'-Penta-O-benzoyl-		+2.1 (C)		41
6,1',6'-tri-O-				
(3-benzoylpropionyl)-	68-70	+22 (C)	¹ H n.m.r.	56
2,3,4,1',3',4'-Hexa-O-benzoyl-		+23 (C)		28
6,6'-di-O-				
(3-benzoylpropionyl)-	72-74	+19 (C)	¹ H n.m.r.	56
2,3,1',3',4',6'-Hexa-O-benzoyl-	124-125	+58.6 (C)	¹ H n.m.r.	83
Octa-O-benzoyl-	60-63	+32.6 (C)		180

TABLE V
Sulfonates and Chlorosulfates

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
2- <i>O-p</i> -Tolylsulfonyl- hepta- <i>O</i> -acetyl-	140-141	+69.8 (P)		27
3,4,3',4'-tetra- <i>O</i> -acetyl-		+51.6 (C)	¹ H n.m.r., m.s.	27
6,1',6'-tri- <i>O</i> -benzoyl-	75-78	+55 (C)	¹ H n.m.r., m.s.	65
4- <i>O</i> -(Methylsulfonyl)- 2,3,6,1',3',4'-hexa- <i>O</i> - acetyl-		+22.1 (C)		42
hepta- <i>O</i> -acetyl-	94-95	+25.2 (C)	¹ H n.m.r., m.s.	42
4- <i>O-p</i> -Tolylsulfonyl- 2,3,6,1',3',4'-hexa- <i>O</i> - acetyl-		+20.6 (C)		42
hepta- <i>O</i> -acetyl-		+24.5 (C)	¹ H n.m.r.	42
6- <i>O-p</i> -Tolylsulfonyl- 2,3,1',3',4',6'-hexa- <i>O</i> - acetyl-	53-55	+49.2 (C)	¹ H n.m.r.	110
hepta- <i>O</i> -acetyl-		60.5 (C)	¹ H n.m.r., m.s.	110
6'- <i>O-p</i> -Tolylsulfonyl- hepta- <i>O</i> -acetyl-	46-49	+47.8 (C)	¹ H n.m.r.	110
2,1'-Di- <i>O</i> -(methylsulfonyl)- hexa- <i>O</i> -acetyl-		+49.6 (C)	¹ H n.m.r., m.s.	27
2,1'-Di- <i>O-p</i> -tolylsulfonyl- hexa- <i>O</i> -acetyl-		+51.8 (C)	¹ H n.m.r., m.s.	27
4,1'-Di- <i>O</i> -(methylsulfonyl)- hexa- <i>O</i> -acetyl-	61-64	+41.7 (C)		124
2,4-Di- <i>O</i> -(methylsulfonyl)- 3,3',4',6'-tetra- <i>O</i> -acetyl- hexa- <i>O</i> -acetyl-		+39.9 (C)	¹ H n.m.r., m.s.	32
		+44.5 (C)	¹ H n.m.r., m.s.	32
4,6'-Di- <i>O</i> -(methylsulfonyl)- hexa- <i>O</i> -acetyl-	63-65	+47.8 (C)		124
6,6'-Di- <i>O-p</i> -tolylsulfonyl- hexa- <i>O</i> -acetyl-	112-114	+60 (P)		29
hexa- <i>O</i> -benzoyl-	64-67	+55 (Dm)	¹ H n.m.r.	29
	93-96	+26.3 (Dm)		29
4,1',6'-Tri- <i>O</i> - (methylsulfonyl)- penta- <i>O</i> -acetyl-	56-58	+36.1 (C)	¹ H n.m.r.	116
4,1',6'-Tri- <i>O-p</i> - tolylsulfonyl- penta- <i>O</i> -acetyl-	85-91			21
		+41 (C)		41
6,1',6'-Tri- <i>O-p</i> - tolylsulfonyl- penta- <i>O</i> -acetyl-		+39.4 (C)		66
penta- <i>O</i> -benzoyl-	135.5-136.5	+68 (C)	¹ H n.m.r., i.r.	66
	87-90	+19 (C)	¹ H n.m.r.	41
6,1',6'-Tri- <i>O</i> - (methylsulfonyl)- penta- <i>O</i> -benzoyl-	99-101	+27 (C)	¹ H n.m.r.	41

(Continued)

TABLE V (Continued)

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
2,4,6,1'-Tetra- <i>O</i> - (methylsulfonyl)- tetra- <i>O</i> -acetyl-	85-86	+39.9 (C)	¹ H n.m.r., m.s.	32
2,6,1',6'-Tetra- <i>O</i> - <i>p</i> - tolylsulfonyl- tetra- <i>O</i> -acetyl-	71-74	+43.3 (C) +63.3 (C)	¹ H n.m.r., m.s.	65,67 65,67
Octa- <i>O</i> -(methylsulfonyl)-	205-206	+39.9 (C)		29
Octa- <i>O</i> - <i>p</i> -tolylsulfonyl-	82-86	+41.78 (A)		60
1'-Chlorosulfate				
6,6'-dichloro-6,6'- dideoxy- penta- <i>O</i> -benzoyl-	136-138	+11.5 (C)	¹ H n.m.r.	78
6,6'-Bis(chlorosulfate) hexa- <i>O</i> -benzoyl-		+31.1 (C)	¹ H n.m.r.	78
6,1',6'-Tris(chlorosulfate) penta- <i>O</i> -benzoyl-	133-134	+19.8 (C)	¹ H n.m.r.	78

TABLE VI
Anhydro Derivatives

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
3,6-Anhydro- hexa- <i>O</i> -acetyl-	158-160	+24 (C)	¹ H n.m.r., m.s.	81
3',6'-Anhydro-	146	+104 (M)		18
1',4':3',6'-Dianhydro-	184-185			33
3,6:3',6'-Dianhydro- tetra- <i>O</i> -acetyl-	69-72	+32.5 (C)	¹ H n.m.r.	29
2,1':3,6:3',6'-Trianhydro- di- <i>O</i> -acetyl-	163-164.5	+117 (C)		62
di- <i>O</i> -methyl-	181.5-182.5	+128.6 (C)		21
di- <i>O</i> - <i>p</i> -tolylsulfonyl-	179-181	+140 (C)		62
1',4':3,6:3',6'-Trianhydro- di- <i>O</i> -acetyl-	164.5-166			62
	194-196	+95 (W)		41,82
	183-185	+116 (C)	¹ H n.m.r.	41

TABLE VII
 Cyclic Acetals

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
2,1'-O-Isopropylidene- 3,4,3',4'-tetra-O-acetyl- 6,6'-dichloro-6,6'- dideoxy-	154	+9.5 (C)		85
4,6-O-Benzylidene- hexa-O-acetyl-	155-157	+44.3 (C)	¹ H n.m.r.	25
4,6-O-Isopropylidene- hexa-O-acetyl-		+46 (C)	¹ H n.m.r., m.s.	83
hexa-O-benzoyl-	168-170	+45.7 (C)	¹ H n.m.r.	83
2,1':4,6-Di-O-isopropylidene- tetra-O-acetyl-	85-87	+12.8 (C)	¹ H n.m.r.	32,91
2,1':3,4-Di-O- isopropylidene- 3',4'-di-O-acetyl- 6,6'-dichloro-6,6'- dideoxy-	191-193	-19.5 (C)	¹ H n.m.r., m.s.	85
2,1'-O-(Diphenylsilyl)- hexa-O-acetyl-	142-144	+60.6 (C)	¹ H n.m.r., m.s.	27
2,1':6,6'-Di-O- (diphenylsilyl)- tetra-O-acetyl-	234-236	+9.6 (C)	¹ H n.m.r., m.s.	27

TABLE VIII

Halides

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
1'-Deoxy-1'-iodo- hexa-O-acetyl- 2-O-(methylsulfonyl)-		+52.2 (C)	¹ H n.m.r., m.s.	27
6-Chloro-6-deoxy- 2,3,1',3',4',6'-hexa-O-acetyl- 4-O-acetyl-	96-99	+46 (W)	¹ H n.m.r., m.s.	81
4-O-formyl-	112-114	+45 (C)	¹ H n.m.r., m.s.	81
4-O-benzoyl-	54-56	+69 (C)	¹ H n.m.r., m.s.	81
hepta-O-benzoyl- hexa-O-acetyl- 6'-O-benzoyl-	93-95	+69 (C)	¹ H n.m.r.	81
		+16 (C)	¹ H n.m.r.	81
6-Deoxy-6-iodo- hepta-O-acetyl-		+61.9 (C)	¹ H n.m.r.	96
	79-81	+61.8 (C)	¹ H n.m.r., m.s.	110
		+58.8 (C)		66
6'-Bromo-6'-deoxy- hexa-O-acetyl- 4-O-(methylsulfonyl)-	49-51	+32.2 (C)	¹ H n.m.r.	124
6'-Chloro-6'-deoxy- hepta-O-acetyl- penta-O-acetyl- 4,1'-di-O-formyl-	116-117	+47.3 (W)	¹ H n.m.r.	81
		+56.3 (C)	¹ H n.m.r.	77
		+38 (C)	¹ H n.m.r., m.s.	81
6'-Deoxy-6'-iodo- hepta-O-acetyl-	78-80	+38.4 (C)	¹ H n.m.r., m.s.	110
6,6'-Dichloro-6,6'-dideoxy- hexa-O-acetyl- 2,3,4,3',4'-penta-O- benzoyl-	85-88	+55 (W)		96,105
	117-118	+55 (C)	¹ H n.m.r., m.s.	96,105
hexa-O-benzoyl-	140-142	-11 (C)	¹ H n.m.r.	81
hexa-O-(methylsulfonyl)-	165-167	+9 (C)	¹ H n.m.r.	29,78
6,6'-Dibromo-6,6'-dideoxy- hexa-O-benzoyl- hexa-O-(methylsulfonyl)-	139-140	+51.5 (A)	¹ H n.m.r.	97
	176-178	+0.3 (C)	¹ H n.m.r.	96
	171-173	+39.5 (A)	¹ H n.m.r.	97
6,6'-Dideoxy-6,6'-diiodo- hexa-O-benzoyl- hexa-O-(methylsulfonyl)- penta-O-acetyl- 1'-O- <i>p</i> -tolylsulfonyl- tetra-O-acetyl-	178-179	+1.6 (C)	¹ H n.m.r.	29
	218-219	+41.8 (A)	¹ H n.m.r.	29
	69-71	+32 (C)	¹ H n.m.r., i.r.	66
2,1'-di-O- <i>p</i> -tolylsulfonyl-	69-72	+44.5 (C)	¹ H n.m.r., m.s.	65
6,1',6'-Trichloro-6,1',6'- trideoxy- penta-O-benzoyl-		+52.5 (A)		78
	90-92	+3.9 (C)	¹ H n.m.r.	78

TABLE IX
Unsaturated Derivatives

Compound	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
Tri- <i>O</i> -acetyl-6-deoxy- α -D- <i>xylo</i> -hex-5-enopyranoside, tetra- <i>O</i> -acetyl- β -D-fructofuranosyl		+31.6 (C)	^1H n.m.r., m.s.	110
Tri- <i>O</i> -acetyl-6-deoxy- β -D- <i>threo</i> -hex-5-enofuranoside, tetra- <i>O</i> -acetyl- α -D-glucopyranosyl	158–160	+59.9 (C)	^1H n.m.r., m.s.	110
Tri- <i>O</i> -benzoyl-6-deoxy- α -D- <i>xylo</i> -hex-5-enopyranoside, tri- <i>O</i> -benzoyl-6-deoxy- β -D- <i>threo</i> -hex-5-enofuranosyl	145–147	–5 (C)	^1H n.m.r.	29
6-Deoxy-tri- <i>O</i> -(methylsulfonyl)- α -D- <i>xylo</i> -hex-5-enopyranoside, 6-deoxy-tri- <i>O</i> -(methylsulfonyl)- β -D- <i>threo</i> -hex-5-enofuranosyl	110–114	+13.7 (A)		29

TABLE X
Deoxy Derivatives

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
1'-Deoxy-hexa- <i>O</i> -acetyl-2- <i>O</i> -(methylsulfonyl)-	118–120	+44.2 (C)	^1H n.m.r.	27
6-Deoxy-hepta- <i>O</i> -acetyl-	47–51	+90 (C) +62 (C)	^1H n.m.r., i.r. ^1H n.m.r., m.s.	66 110
6'-Deoxy-hepta- <i>O</i> -acetyl-	135–137	+59.9 (C)	^1H n.m.r., m.s.	110
6,6'-Dideoxy-hexa- <i>O</i> -benzoyl-	118–120	+19.6 (C)	^1H n.m.r.	29
hexa- <i>O</i> -(methylsulfonyl)-penta- <i>O</i> -acetyl-	191–192	+44.4 (C)	^1H n.m.r.	29
1'- <i>O</i> - <i>p</i> -tolylsulfonyl-		+60.3 (C)	^1H n.m.r., i.r.	66

TABLE XI
Nitrogen-containing Compounds

Derivative of sucrose	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
6,6'-Diazido-6,6'-dideoxy-	163-174	+78.8 (W)	i.r.	122
		+62.8 (W)		96
hexa-O-acetyl-		+5.8 (C)	¹ H n.m.r.	96
hexa-O-benzoyl-	75-78	+42.5 (Dm)	¹ H n.m.r.	29
hexa-O-(methylsulfonyl)-	189-190	+41.4 (P)	¹ H n.m.r.	29
6,1',6'-Triazido-6,1',6'-trideoxy-		+64 (A)	i.r.	117
penta-O-benzoyl-	58-60	+31 (C)	¹ H n.m.r., i.r.	117
penta-O-(methylsulfonyl)-	72-74	+55 (C)	¹ H n.m.r., i.r.	117
6,6'-Diamino-6,6'-dideoxy-	105-110	+51.6 (W)	i.r.	122
6,6'-Bis(acetamido)-6,6'-dideoxy-	85-88	+44.5 (M)	i.r.	122
hexa-O-acetyl-	86-89	+70.5 (C)	¹ H n.m.r.	122
6,1',6'-Triamino-6,1',6'-trideoxy-	121-125	+68.5 (W)	i.r.	117
6,1',6'-Tris(acetamido)-6,1',6'-trideoxy-	107-110	+3 (M)	i.r.	117
penta-O-acetyl-	99-101	+37 (C)	¹ H n.m.r., i.r.	117

TABLE XII
***β*-D-Fructofuranosyl *α*-D-Galactopyranoside Derivatives**

Compound	M.p. (degrees)	[<i>α</i>] _D , degrees (solvent)	Spectra	References
<i>α</i> -D-Galactopyranoside, <i>β</i> -D-fructofuranosyl	177–179	+82.3 (W)		42,116,124
octa- <i>O</i> -acetyl-		+52.6 (C)	¹ H n.m.r.	42
octa- <i>O</i> -benzoyl-	79–81	+52.6 (C)		42
<i>α</i> -D-Galactopyranoside, 1- <i>O</i> -(methylsulfonyl)- <i>β</i> - D-fructofuranosyl	158–161	+70.1 (W)		116
Tri- <i>O</i> -acetyl-4- <i>O</i> -benzoyl- <i>α</i> -D-galactopyranoside, tri- <i>O</i> -acetyl-1- <i>O</i> - benzoyl- <i>β</i> -D- fructofuranosyl	59–61	+54.6 (C)	¹ H n.m.r.	124
tri- <i>O</i> -acetyl-6- <i>O</i> - benzoyl- <i>β</i> -D- fructofuranosyl	64–66	+70.4 (C)	¹ H n.m.r.	124
tri- <i>O</i> -acetyl-1- <i>O</i> - (methylsulfonyl)- <i>β</i> -D- fructofuranosyl	64–66	+59.8 (C)	¹ H n.m.r.	124
di- <i>O</i> -acetyl-6- <i>O</i> -benzoyl- 1- <i>O</i> -(methylsulfonyl)- <i>β</i> -D-fructofuranosyl		+15.6 (C)	¹ H n.m.r.	116
4-Azido-4-deoxy- <i>α</i> -D- galactopyranoside, <i>β</i> -D-fructofuranosyl	116–119	+80.1 (E)		124
1-azido-1-deoxy- <i>β</i> -D- fructofuranosyl		+77.4 (E)		124
6-azido-6-deoxy- <i>β</i> -D- fructofuranosyl		+63.8 (E)		124
Tri- <i>O</i> -acetyl-4-azido-4- deoxy- <i>α</i> -D- galactopyranoside, tetra- <i>O</i> -acetyl- <i>β</i> -D- fructofuranosyl	97–99	+47.4 (C)	¹ H n.m.r.	124
tri- <i>O</i> -acetyl-1-azido-1- deoxy- <i>β</i> -D- fructofuranosyl		+46 (C)	¹ H n.m.r.	124
tri- <i>O</i> -acetyl-6-azido-6- deoxy- <i>β</i> -D- fructofuranosyl		+41.3 (C)	¹ H n.m.r.	124
di- <i>O</i> -acetyl-6-azido-6- deoxy-1- <i>O</i> - (methylsulfonyl)- <i>β</i> -D- fructofuranosyl	135–178	–7.1 (C)	¹ H n.m.r.	116
di- <i>O</i> -acetyl-1,6-diazido- 1,6-dideoxy- <i>β</i> -D- fructofuranosyl	57–59	+40.1 (C)	¹ H n.m.r.	116

(Continued)

TABLE XII (Continued)

Compound	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
4-Azido-4-deoxytri- <i>O</i> - (methylsulfonyl)- α -D- galactopyranoside, 1,6-diazido-1,6-dideoxy- di- <i>O</i> -(methylsulfonyl)- β -D-fructofuranosyl	63-65	+73.3 (A)	^1H n.m.r.	116
4,6-Diazido-4,6-dideoxy- di- <i>O</i> -(methylsulfonyl)- α - D-galactopyranoside, 6-azido-6-deoxytri- <i>O</i> - (methylsulfonyl)- β -D- fructofuranosyl	151-153	+45.2 (A)		97
1,6-diazido-1,6-dideoxy- di- <i>O</i> -(methylsulfonyl)- β -D-fructofuranosyl	171-173	+45.7 (A)		97
Tri- <i>O</i> -acetyl-4-bromo-4- deoxy- α -D- galactopyranoside, tri- <i>O</i> -acetyl-6-bromo-6- deoxy- β -D- fructofuranosyl	53-55	+40.8 (C)	^1H n.m.r.	124
4,6-Dibromo-4,6-dideoxy- di- <i>O</i> -(methylsulfonyl)- α - D-galactopyranoside, 6-bromo-6-deoxy-tri- <i>O</i> - (methylsulfonyl)- β -D- fructofuranosyl	140-141	+54.8 (A)	^1H n.m.r.	97
4-Chloro-4-deoxy- α -D- galactopyranoside, β -D-fructofuranosyl	106-108	+84.6 (E)		124
6-chloro-6-deoxy- β -D- fructofuranosyl		+71 (E)		81
1,6-dichloro-1,6-dideoxy- β -D-fructofuranosyl		+68.2 (E)		124
Tri- <i>O</i> -acetyl-4-chloro-4- deoxy- α -D- galactopyranoside, tetra- <i>O</i> -acetyl- β -D- fructofuranosyl	74-76	+56.8 (C)		124
tri- <i>O</i> -acetyl-6-chloro-6- deoxy- β -D- fructofuranosyl	103-104	+77 (C)	^1H n.m.r.	81
3,4-di- <i>O</i> -acetyl-6-chloro- 6-deoxy- β -D- fructofuranosyl		+53.5 (C)	^1H n.m.r.	81
di- <i>O</i> -acetyl-6-chloro-6- deoxy-1- <i>O</i> -formyl- β -D- fructofuranosyl		+67 (C)	^1H n.m.r., m.s.	81

TABLE XII (Continued)

Compound	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
di- <i>O</i> -acetyl-1,6-dichloro- 1,6-dideoxy- β -D- fructofuranosyl	92-94	+66.8 (C) +69 (C)	^1H n.m.r. ^1H n.m.r.	124 81
di- <i>O</i> -acetyl-1,6-di- <i>O</i> -trityl- β -D-fructofuranosyl	101-103	+73.4 (C)		124
Di- <i>O</i> -acetyl-4,6-dichloro- 4,6-dideoxy- α -D- galactopyranoside, tetra- <i>O</i> -acetyl- β -D- fructofuranosyl	78-80	+103 (C)	^1H n.m.r., m.s.	81
3,6-Anhydro- α -D- galactopyranoside, 1,4:3,6-dianhydro- β -D- fructofuranosyl	191-192.5	+137.5 (W)		21
di- <i>O</i> -acetyl-	137.5-138.5	+94.3 (C)		21
di- <i>O</i> -methyl-	105-106	+48.6 (C)		21

TABLE XIII
Miscellaneous Derivatives

Compound	M.p. (degrees)	$[\alpha]_D$, degrees (solvent)	Spectra	References
6,6'-Dideoxy-6,6'- di(thiocyanato)sucrose				
hexa-O-acetyl-	169-171	+71 (C)	¹ H n.m.r.	29
hexa-O-benzoyl-	92-95	+31.9 (C)	¹ H n.m.r.	29
hexa-O-(methylsulfonyl)-	177-180	+68.5 (C)	¹ H n.m.r.	29
Octa-O-benzylsucrose		+31.6 (C)		181
		+38.6 (C)		182,183
Tri-O-acetyl-6-deoxy- β -L- idopyranoside, tetra-O-acetyl- β -D- fructofuranosyl	176-179	-1.5 (C)	¹ H n.m.r.	110
3,4-Anhydro-1,6-dichloro-1,6- dideoxy- β -D- <i>ribo</i> -hexulo- furanoside, 4,6-dichloro- 4,6-dideoxy-2,3-di-O-sulfo- α -D-galactopyranosyl	146-148	+68.5 (C)	¹ H n.m.r., m.s.	77
1,4,6-Trichloro-1,3,4,6-tetra- deoxy- β -D- <i>glycero</i> -hex-3- enofuranoside, 4,6-di- chloro-4,6-dideoxy-2,3-di- O-sulfo- α -D-galacto- pyranosyl	111-112	+79 (C)	¹ H n.m.r., m.s.	77
1,4,6-Trichloro-1,4,6-trideoxy- β -D-hexulofuranoside, 4,6- dichloro-4,6-dideoxy-2,3- di-O-sulfo- α -D-galacto- pyranosyl	107-111		¹ H n.m.r., m.s.	77

THE PNEUMOCOCCAL POLYSACCHARIDES: A RE-EXAMINATION

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I. INTRODUCTION

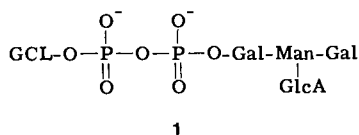
The pneumococcal polysaccharides were treated in a previous Volume of this Series.¹ The immunological properties of these polysaccharides have been well studied,² thanks to extensive work by Heid-

(1) M. J. How, J. S. Brimacombe, and M. Stacey, *Adv. Carbohydr. Chem.*, **19**, 303-358 (1964).

(2) M. Heidelberger, *Fortschr. Chem. Org. Naturst.*, **18**, 501-536 (1960).

elberger and coworkers. These polysaccharides and their antisera have also been used in the immunological characterization of other polysaccharides. Structural studies of these polysaccharides are, therefore, of special importance, and the present article summarizes results of such studies that have been obtained since the previous article was completed. Some pneumococcal polysaccharides have also been discussed in a review of bacterial exopolysaccharides.³

Studies of a great number of extracellular polysaccharides from Gram-positive and Gram-negative bacteria indicate that they are composed of oligosaccharide repeating-units.³ In the biosynthesis of these polysaccharides, the repeating unit, linked to the pyrophosphate of an isoprenoid C₅₅ alcohol glycosyl-carrier lipid (GCL), is first formed by stepwise transfer of glycosyl groups from "sugar nucleotides." The repeating unit is then transferred from this intermediate (for example 1) to the polymer. The synthesis of a bacterial



polysaccharide therefore requires a considerable number of highly specific enzymes, increasing in number with the complexity of the repeating unit. The repeating unit seems to be rather small, di- to hexa-saccharide units having been demonstrated for several polysaccharides.³ The hexasaccharide is the largest repeating-unit that has been firmly established, but the presence of an octasaccharide repeating-unit has been indicated.⁴ Tentative structures in which large repeating-units are proposed should, in the opinion of the present authors, be regarded with some doubt. Several such structures have been suggested for pneumococcal polysaccharides. Major features proposed in these structures are most probably correct. Observations that have been interpreted as being due to minor structural features and that necessitated the assumption of a large repeating-unit may, however, be without structural significance.

The purification of a pneumococcal polysaccharide may be difficult, and the polysaccharide material is sometimes contaminated by a cell-wall component known as the C-substance. The methods for structural analysis of polysaccharides (which, like several of the pneumococcal polysaccharides, contain amino sugar and uronic acid

(3) I. W. Sutherland, *Adv. Microbiol. Physiol.*, **8**, 143-213 (1972).

(4) Unpublished results from the authors' laboratory.

residues) are not always adequate, especially when results of quantitative, rather than qualitative, significance are needed. Sometimes, it may even be difficult to obtain satisfactory, quantitative analysis of the component sugars.

Some extracellular polymers from bacteria are composed of oligosaccharide repeating-units linked to each other by means of phosphoric diester linkages. Such polymers will also be discussed in this article and they will, for convenience, be referred to as polysaccharides. Their biosynthesis is most probably analogous to that of the teichoic acids,^{5,6} and involves the polymerization of a repeating unit, linked to a glycosyl-carrier lipid. In this reaction, however, the oligosaccharide together with a phosphate group is transferred to the polymer. In addition to the reducing-sugar residues, residues of glycerol phosphate or ribitol phosphate may be part of the polymer. These residues are transferred to the lipid phosphate from cytidine 5'-(1-deoxy-D-glycerol-1-yl pyrophosphate) and cytidine 5'-(5-deoxy-D-ribitol-5-yl pyrophosphate), respectively. Unless there are other, not-yet-revealed biosynthetic routes, the repeating units of these polymers should terminate with a 1-deoxy-D-glycerol-1-yl phosphate, a 5-deoxy-D-ribitol-5-yl phosphate, or a glycosyl phosphate residue. The latter should have the same anomeric configuration as in the corresponding "sugar nucleotide." These biosynthetic considerations are useful as complementary evidence in structural studies of polysaccharides containing phosphoric diesters, as has been demonstrated by Baddiley and coworkers. Several examples will be given.

The pneumococci all belong to different types of the same species, namely, *Streptococcus pneumoniae*. This was earlier called *Diplococcus pneumoniae*, but has been renamed.⁷ There are some 80 different types of pneumococcus, and two systems of nomenclature, the Danish, used in Europe, and Eddy's, used in the United States.⁸ Tables correlating the Danish and American designations have been published.⁸ The Danish system, with Arabic numerals and common abbreviations, such as Ph1 for Type 1 and S1 for its type-specific, capsular polysaccharide, will be used in this article.

When the previous article on pneumococcal polysaccharides¹ was

(5) A. R. Archibald and J. Baddiley, *Adv. Carbohydr. Chem.*, **21**, 323-375 (1966).

(6) J. Baddiley, *Acc. Chem. Res.*, **3**, 98-105 (1970).

(7) D. H. Bergey, "Manual of Determinative Bacteriology," Williams and Williams, Baltimore, MD, 8th Edition, 1974, p. 499.

(8) F. Kauffmann, E. Lund, and B. E. Eddy, *Int. Bull. Bacteriol. Nomencl. Taxon.*, **10**, 31-40 (1960).

published, complete structures for S3 and S8 had been determined. Considerable structural information was also available for S2, S5, S6, S14, S18, and S34. As will be seen from the following, progress has since been considerable, but nevertheless, a lot yet remains to be done.

II. STRUCTURAL STUDIES

1. Type 1 Capsular Polysaccharide

A crude sample of S1, containing both C-substance and polyglutamate, was purified by chromatography on DEAE-Sephadex.⁹ The polysaccharide contained D-glucose, 2-amino-2-deoxy-D-glucose, a 2-amino-2-deoxy-galactose, and D-galacturonic acid residues, and O-acetyl groups. About 50% of the amino sugar residues were N-acetylated. Only traces of D-glucose were released on acid hydrolysis, unless S1 was first N-acetylated, indicating that an amino sugar is linked to D-glucose. Original S1 did not consume periodate, but about 1 molecule per 4 sugar residues was consumed after O-deacetylation. Some partially characterized oligosaccharides were obtained by graded hydrolysis of carboxyl-reduced S1, and by deamination of original S1.

2. Type 2 Capsular Polysaccharide

Type 2 capsular polysaccharide is composed of L-rhamnose, D-glucose, and D-glucuronic acid residues in the proportions 3:2:1. Earlier studies of S2 are summarized in Ref. 1. The studies discussed here have, to some extent, given conflicting evidence. Heidelberger and coworkers¹⁰ studied the inhibition of the type 2-anti type 2 system with different O-(D-glucopyranosyluronic acid)-D-glucoses, and observed that an α -D-(1 \rightarrow 6)-linked acid, isomaltobiouronic acid, was more efficient than the others tested. These studies, in conjunction with earlier results, strongly suggested the presence of a terminal isomaltobiosyluronic group in S2. Stacey and coworkers^{11,12} studied the structure of S2 by using induced enzymes and periodate

(9) R. C. E. Guy, M. J. How, M. Stacey, and M. Heidelberger, *J. Biol. Chem.*, **242**, 5106-5111 (1967).

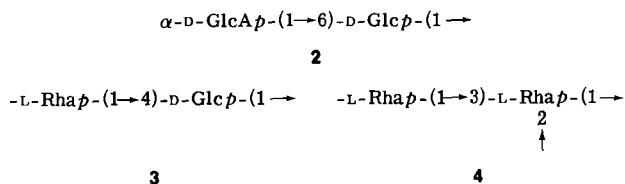
(10) M. Heidelberger, N. Roy, and C. P. J. Glaudemans, *Biochemistry*, **8**, 4822-4824 (1969).

(11) S. A. Barker, P. J. Somers, M. Stacey, and J. W. Hopton, *Carbohydr. Res.*, **1**, 106-115 (1965).

(12) S. A. Barker, P. J. Somers, and M. Stacey, *Carbohydr. Res.*, **3**, 261-270 (1967).

oxidation. From the results of these studies, and from previous methylation analyses,¹ they proposed a dodecasaccharide repeating-unit for S2. This structure, in conflict with the results just discussed, contains a terminal, β -D-(1 \rightarrow 4)-linked acid residue, that of cellobiouronic acid.

Methylation analysis of original and carboxyl-reduced S2 indicated a simpler structure.¹³ It demonstrated that S2 contains terminal D-glucuronic acid, 6-O-substituted D-glucose, 4-O-substituted D-glucose, 3-O-substituted L-rhamnose, and 2,3-di-O-substituted L-rhamnose in the molar proportions 1:1:1:2:1, in agreement with a hexasaccharide repeating-unit. Partial hydrolysis of S2, followed by borohydride reduction, and carboxyl reduction with lithium aluminum deuteride, yielded isomaltitol dideuterated at C-6 in the D-glucose residue. The presence of a terminal, isomaltobiosyluronic group (2), as indicated by Heidelberger and coworkers,¹⁰ was thereby confirmed. Partial, acid hydrolysis, during which essentially only L-rhamnosidic linkages should be cleaved, followed by methylation analysis, demonstrated the presence of the partial structures 3 and 4.



Carboxyl-reduced, fully acetylated S2 was treated with chromium trioxide in acetic acid.¹⁴ Acetylated glycopyranosides in which the "aglycon" group occupies an equatorial position in the most stable chair form (generally the β anomers) are oxidized to esters of 5-hexulosonic acids during this treatment. The other anomers (generally the α anomers) are reasonably stable under these conditions.^{15,16} Sugar analysis of the oxidized product showed that one L-rhamnosyl residue is β -L-linked, and the five other sugar residues are α -D- or α -L-linked. The oxidized product was subjected to methylation analysis. The methylation was performed by the method devised by Hakomori,¹⁷ with sodium methylsulfinyl carbanion and methyl iodide in

(13) O. Larm, B. Lindberg, S. Svensson, and E. A. Kabat, *Carbohydr. Res.*, **22**, 391-397 (1972).

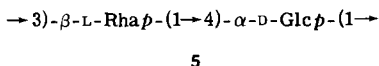
(14) O. Larm, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **31**, 120-126 (1973).

(15) S. J. Angyal and K. James, *Aust. J. Chem.*, **23**, 1209-1221 (1970).

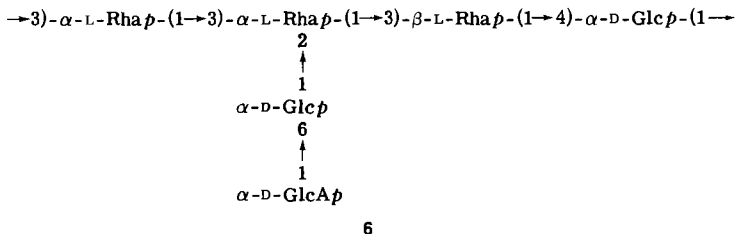
(16) J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, **26**, 661-666 (1972).

(17) S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205-208 (1964).

dimethyl sulfoxide. Ester groups are cleaved during this treatment, and new hydroxyl groups become exposed. Comparison between this analysis and that of carboxyl-reduced S2 demonstrated that half of the 2,4-di-*O*-methyl-*L*-rhamnose had disappeared, and that 2,3,6-tri-*O*-methyl-*D*-glucose was replaced by 2,3,4,6-tetra-*O*-methyl-*D*-glucose. The presence of the structural element **5** was, consequently, demonstrated.



The structural elements **2**, **4**, and **5** might be combined into different, alternative structures. One of these, **6**, was proved to be correct by subjecting S2 to two consecutive degradations, the results of which were monitored by methylation analyses.¹⁸ In the first degradation, fully methylated S2 was treated first with base, and then with



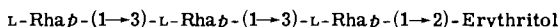
acid under mild conditions,¹⁹ whereupon, the terminal glycosyluronic acid residue was eliminated, and the hydroxyl group on C-6 of the penultimate D-glucosyl residue became exposed. This hydroxyl group was replaced by a 6-deoxy-6-C-*p*-tolylsulfinyl group by successive treatment with *p*-toluenesulfonyl chloride, sodium iodide, and sodium *p*-toluenesulfinate.²⁰ Upon treatment of the thus-modified polysaccharide with base, the new terminal group was eliminated, and the hydroxyl group on C-2 of the originally branching L-rhamnosyl residue became exposed. Only about 30% of the D-glucosyl groups were eliminated, probably because of incomplete *p*-toluenesulfonylation, or incomplete replacement of the *p*-tolylsulfonyloxy group, or both. The results, however, demonstrated that the penultimate D-glucosyl residue is linked to O-2 of the branching L-rhamnosyl residue.

(18) L. Kenne, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **40**, 69-75 (1975).

(19) B. Lindberg, J. Lönngren, and J. L. Thompson, *Carbohydr. Res.*, **28**, 351-357 (1973).

(20) B. Lindberg and H. Lundström, *Acta Chem. Scand.*, **20**, 2423–2426 (1966).

Nuclear magnetic resonance (n.m.r.) studies of methylated S2 and of the methylated product obtained after elimination of the uronic acid confirmed the presence of one β -L-linkage and five α -D- or α -L-linkages in the original polysaccharide. Smith degradation of S2 yielded the tetrasaccharide 7, also in agreement with the structure proposed. The tetrasaccharide showed $[\alpha]_D -11^\circ$, in good agreement with the calculated value, assuming one β -L-linkage and two α -L-linkages.



7

3. Type 4 Capsular Polysaccharide

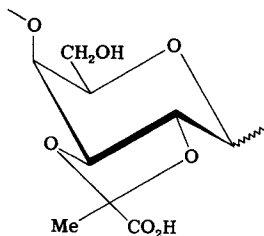
A purified sample of S4 contained D-galactose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-mannose, a 2-acetamido-2,6-dideoxygalactose (*N*-acetylglucosamine), and pyruvic acid in²¹ the molar proportions 3:3:2:3:3. The tentative assignment of the D configuration to the fucosamine, based upon the m.p. of its hydrochloride, is irrelevant. The pyruvic acid, previously demonstrated to be²² a component of S4, was split off when S4 was treated with acid, but not on treatment with base, and was consequently assumed to be linked as an acetal. The investigation of S4 was continued with periodate-oxidation studies.²³ Original S4 consumed only traces of periodate. Most of the pyruvic acid could be removed on treatment with 0.01 *M* hydrochloric acid at 100° for 30 min. The modified polysaccharide consumed periodate, with simultaneous disappearance of D-galactose. On borohydride reduction of the product, followed by acid hydrolysis, D-threitol was obtained; and, when the hydrolysis was performed under mild conditions, oligosaccharides containing threitol were obtained. These results indicated that the pyruvic acid is linked to a D-galactopyranosyl residue which is further substituted at O-4. Methylation analyses²⁴ of S4 before and after removal of the pyruvic acid residues yielded 6-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-galactose, respectively. The pyruvic acid is, consequently, linked to O-2 and O-3 of the D-galactopyranosyl residue, as in 8. Pyruvic acid is generally linked to O-4 and O-6 of hexo-

(21) J. D. Higginbotham and M. Heidelberger, *Carbohydr. Res.*, **23**, 165-173 (1972).

(22) M. Heidelberger, W. F. Dudman, and W. Nimmich, *J. Immunol.*, **104**, 1321-1328 (1970).

(23) J. D. Higginbotham and M. Heidelberger, *Carbohydr. Res.*, **27**, 297-302 (1973).

(24) J. Y. Lew and M. Heidelberger, unpublished results.



8

pyranosyl residues. Pyruvic acid linked to vicinal positions has also been observed, both to *cis* positions (O-3 and O-4 in D-galactopyranose²⁵) and *trans* positions (O-3 and O-4 in L-rhamnose²⁶). The *trans*-fusion accounts for the ease with which the pyruvic acid residues in S4 are hydrolyzed with acid.

The difficulties inherent in obtaining correct sugar analyses for a polysaccharide containing three different amino sugars are obvious. It therefore seems possible that S4 is composed of tetrasaccharide repeating-units, containing one residue each of the four different sugar components.

4. Type 5 Capsular Polysaccharide

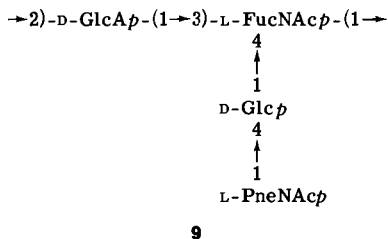
In addition to the unusual sugar 2-acetamido-2,6-dideoxy-L-talose (*N*-acetyl-L-pneumosamine), S5 contains D-glucose, D-glucuronic acid, and 2-acetamido-2-deoxy-L-fucose residues¹ The proportions of these sugars in the polysaccharide do not seem to have been determined. The polysaccharide has been further investigated by Stacey and coworkers.²⁷ The major components found on methylation analysis were 2,3,6-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-glucuronic acid. A complex mixture of hexosamine derivatives was also obtained. Upon periodate oxidation of S5, most of the 2-acetamido-2-deoxy-L-fucosyl residues were, however, resistant. From the results of these and previous studies, it was suggested that S5 contains the partial structure 9. This structure, proposed in a survey,²⁸ must be regarded as tentative only; for example, it does not explain the extreme lability of S5 towards alkali.

(25) P. J. Garegg, B. Lindberg, T. Onn, and T. Holme, *Acta Chem. Scand.*, **25**, 1185-1194 (1971).

(26) Yuen-Min Choy and G. G. S. Dutton, *Can. J. Chem.*, **52**, 684-687 (1974).

(27) S. A. Barker, S. M. Bick, J. S. Brimacombe, M. J. How, and M. Stacey, *Carbohydr. Res.*, **2**, 224-233 (1966).

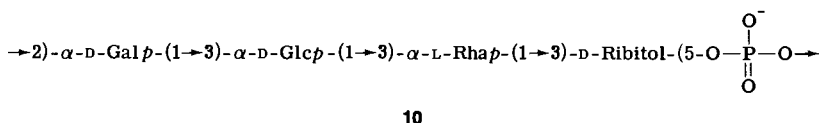
(28) S. A. Barker and P. J. Somers, in "The Carbohydrates; Chemistry and Biochemistry," W. Pigman and D. Horton, eds., Academic Press, New York, 2nd Edition, 1970, Vol. IIB, p. 582.



(L-PneNAc = *N*-acetyl-L-pneumosamine
= 2-acetamido-2, 6-dideoxy-
L-talosyl)

5. Type 6 Capsular Polysaccharide

A repeating unit for S6 was proposed by Rebers and Heidelberg,²⁹ as discussed in Ref. 1. The only features not determined were the structure and configuration of a ribitol phosphate moiety. From biosynthetic considerations,⁶ the phosphate group should be linked to O-5 of D-ribitol, and the structure should, therefore, be that depicted in 10.



6. Type 7 Capsular Polysaccharide

Earlier structural studies on S7 were rendered difficult, as preparations of this polysaccharide were contaminated, especially by the C-substance. A sample purified by precipitations with ammonium sulfate, followed by chromatography on a DEAE-cellulose column, was prepared by Tyler and Heidelberg.³⁰ The material contained D-galactose, D-glucose, L-rhamnose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose in the molar proportions 4:2:3:2:2. Samples prepared from specific precipitates with antisera showed somewhat different proportions of the same sugars. The L-rhamnosyl and 2-acetamido-2-deoxy-D-galactosyl residues were not oxidized by periodate. In addition to these sugars, erythritol, threitol, and glycerol were obtained on hydrolysis of the periodate oxidized-borohydride reduced polysaccharide. The formation of the tetritols demonstrates the presence of D-glucosyl and D-galactosyl

(29) P. A. Rebers and M. Heidelberg, *J. Am. Chem. Soc.*, **83**, 3056-3059 (1961).

(30) J. M. Tyler and M. Heidelberg, *Biochemistry*, **8**, 1384-1392 (1968).

residues, substituted at O-4 in the original polysaccharide. Cross-reactions and inhibition experiments indicated that the polysaccharide contains terminal β -D-galactopyranosyl groups, possibly linked to O-4 or O-6 of D-glucose.

Somewhat different proportions of the parent sugars, 3.5:2.3:3.0:-1.1:1.0, were obtained by Bishop and coworkers,³¹ also after rigorous purification of the product. (The value 2.1 for 2-acetamido-2-deoxy-D-glucose was erroneously given in the abstract of Ref. 29.) These proportions are in good agreement with the proportions between total sugars, 2-acetamido-2-deoxy sugars, and 6-deoxy sugars, as determined from the proportions between total protons, *N*-acetyl protons, and *C*-methyl protons in the 100-MHz, proton n.m.r. spectrum.

Hydrolysis of the fully methylated polysaccharide yielded 3,4-di-*O*-methyl-L-rhamnose (5.9), 2,4-di-*O*-methyl-L-rhamnose (21.5), 2,3,4,6-tetra-*O*-methyl-D-galactose (12.3), 2,3,6-tri-*O*-methyl-D-glucose (21.8), 2,3,4-tri-*O*-methyl-D-galactose (9.4), 3,4-di-*O*-methyl-D-galactose (8.9), 2-deoxy-3,4,6-tri-*O*-methyl-2-(methylamino)-D-glucose (4.6), 2-deoxy-3,4-di-*O*-methyl-2-(methylamino)-D-glucose (6.7), and 2-deoxy-3-*O*-methyl-2-(methylamino)-D-galactose (8.9%). Periodate-oxidation studies gave essentially the same results as reported by Tyler and Heidelberger,³⁰ and these agreed with the results of the methylation analysis. On time-lapse hydrolysis, D-galactose was the first monomeric sugar released, followed by L-rhamnose. That the polysaccharide contains terminal D-galactopyranosyl groups is evident from the methylation analysis, and the release of L-rhamnose was interpreted as an indication that this D-galactosyl group is linked to L-rhamnose. A possible, tentative structure for a repeating unit (11) which could account for the results was proposed.

Contiguous D-glucosyl residues in this repeating unit were assumed, as D-glucose was released when the polysaccharide was treated with cellulase. A polysaccharide having the proposed structure should be hardly hydrolyzed by cellulase, and furthermore, di- and higher oligo-saccharides, but not D-glucose, are released by the action of this enzyme. The assignment of this structural feature seems, therefore, to be unfounded.

Even though some important structural features of S7 have been revealed, further experiments are needed in order to provide the complete structure. The anomeric nature of most of the sugar residues, for example, has not yet been determined.

(31) A. S. Chaudhari, C. T. Bishop, and R. J. Fielder, *Carbohydr. Res.*, **25**, 161-172 (1968).

7. Type 9N Capsular Polysaccharide

The structure of S9N (9N in the Danish system is equivalent to 9 in the American system,⁸ as used in Refs. 32 and 33) has been investigated by Heidelberger and coworkers.^{32,33} A purified polysaccharide contained D-glucose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-mannose, 2-acetamido-2-deoxy-D-galactose, and D-glucuronic acid in the proportions 33:23:20:0.7:22. The possibility that the low percentage of 2-acetamido-2-deoxy-D-galactose derived from a contaminant was considered. On partial hydrolysis of S9N, 3-O-(α -D-glucopyranosyluronic acid)-D-glucose and a number of other, only partially characterized, oligosaccharides were formed. The former strongly inhibited the homologous precipitation in anti-Pn9N serum. On periodate oxidation of S9N, most of the D-glucuronic acid and about half of the D-glucose residues were oxidized. A number of oligosaccharides were isolated after Smith degradation of S9N, and these were partially characterized. They all contained erythritol, evidently from a D-glucosyl residue substituted at O-4. A possible, tentative structure for a repeating unit (12) of S9N was proposed. This, in the opinion of the present authors, is based upon insufficient evidence.

It seems possible that S9N is composed of pentasaccharide repeating-units, with two D-glucosyl residues and one residue each of 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-mannose, and D-glucuronic acid. There is, furthermore, no evidence against branching in the molecule. The property of 3-O-(α -D-glucopyranosyluronic acid)-D-glucose as a strong inhibitor may indicate instead that this residue is terminal. Further studies are needed before a complete structure of S9N can be proposed.

8. Type 10A Capsular Polysaccharide

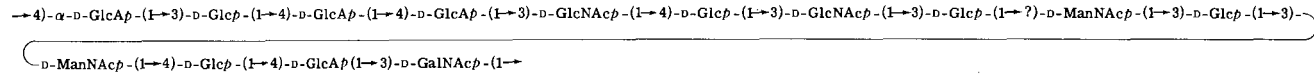
The structure of S10A has been investigated by Baddiley and coworkers.^{34,35} The polysaccharide is composed of D-galactose, 2-acetamido-2-deoxy-D-galactose, ribitol, and phosphate in the molar proportions 4:1:1:1. A hexasaccharide and its *N*-deacetylated derivative were obtained from S10A by treatment first with base and then

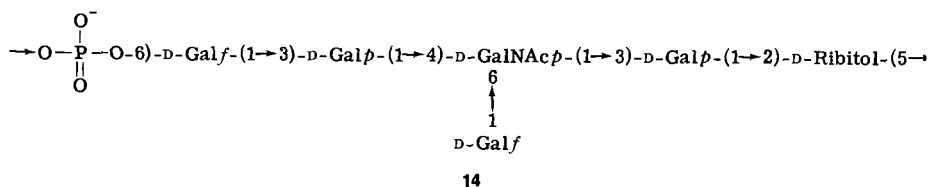
(32) J. D. Higginbotham, A. Das, and M. Heidelberger, *Biochem. J.*, **126**, 225–231 (1972).

(33) A. Das, J. D. Higginbotham, and M. Heidelberger, *Biochem. J.*, **126**, 233–236 (1972).

(34) E. V. Rao, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **100**, 801–810 (1966).

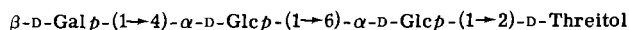
(35) E. V. Rao, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **100**, 811–814 (1966).





9. Type 11A Capsular Polysaccharide

The structure of S11A has been investigated by Baddiley and coworkers.³⁷ The polymer contains D-glucose, D-galactose, and glycerol residues, and phosphate and O-acetyl groups in the molar proportions 2:2:1:1:2. The serological activity is associated with the O-acetyl groups. On treatment of S11A with base, glycerol 1-phosphate and a phosphorus-free polysaccharide were produced. On methylation analysis of the latter, comparable amounts of 2,3,4-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-galactose, and 2,4,6-tri-O-methyl-D-galactose were obtained. 2-O-β-D-Galactopyranosyl-D-erythritol was obtained on Smith degradation of the phosphorus-free polysaccharide. Smith degradation of the deacetylated but phosphorus-containing polysaccharide yielded the same component plus erythritol phosphate, demonstrating that the glycerol phosphate is linked to O-4 of a D-glucosyl residue. On Smith degradation of original S11A, followed by dephosphorylation, the erythritol galactoside and 2-O-α-D-glucopyranosyl-D-threitol were obtained. On similar treatment, but using only 2.0 molecules of periodate per tetrasaccharide repeating-unit, a tetrasaccharide was obtained. The structure of this product (15) was determined by chemical and enzymic methods.

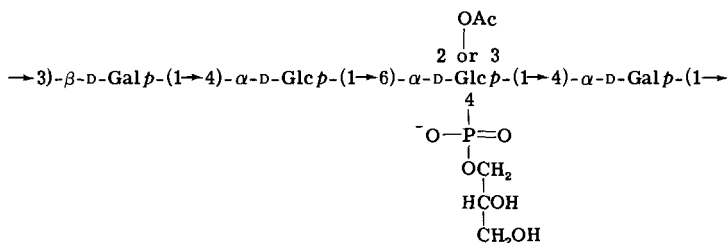


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From the combined evidence, the structure of the repeating unit (16) of S11A was proposed. The anomeric natures of three hexosyl residues were determined in the fragmentation studies reported. The anomeric nature of the remaining D-galactosyl residue was taken to be α, considering the specific rotation of S11A. One O-acetyl group was assigned to O-2 or O-3 of the D-glucose residue carrying the glycerol phosphate side-chain, as this residue was resistant to periodate oxidation before deacetylation. The position of the other O-

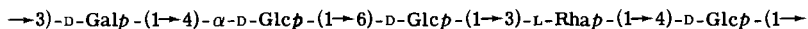
(37) D. A. Kennedy, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **115**, 37-45 (1969).

acetyl group was not ascertained, and the absolute configuration of the glycerol phosphate was not determined. From biosynthetic considerations, it should, however, be a D-glycerol 1-phosphate residue, as indicated in 16.



16

There is strong cross-reaction between S11A and S18. The partial structures proposed for S18 (Refs. 1 and 38), 17 and 18, with glycerol phosphate and the O-acetyl group linked to positions not yet determined, are also remarkably similar to that of S11A.



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10. Type 12 Capsular Polysaccharide

Purified S12, obtained by precipitation with concanavalin A or by ion-exchange chromatography, contains D-glucose, D-galactose, 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2,6-dideoxy-L-galactose (*N*-acetyl-L-fucosamine) in³⁹ the molar proportions 2:1:1:1. Kojibiose (2-*O*- α -D-glucopyranosyl-D-glucose) was isolated⁴⁰ from a partial hydrolyzate of S12. This established the presence of kojibiose as a structural entity in the polysaccharide, a feature that had previously been indicated by immunochemical studies. Methylation analysis⁴¹ of S12 gave equimolecular amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-galactose. The combined evidence therefore indicates that S12 is

(38) S. Estrada-Parra and M. Heidelberger, *Biochemistry*, **2**, 1288-1294 (1963).

(39) J. A. Cifonelli, P. Rebers, M. B. Perry, and J. K. N. Jones, *Biochemistry*, **5**, 3066-3072 (1966).

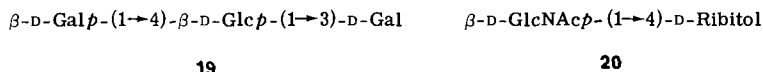
(40) I. J. Goldstein, J. A. Cifonelli, and J. L. Duke, *Biochemistry*, **13**, 867-870 (1974).

(41) J. L. Duke, I. J. Goldstein, and J. A. Cifonelli, *Carbohydr. Res.*, **37**, 81-88 (1974).

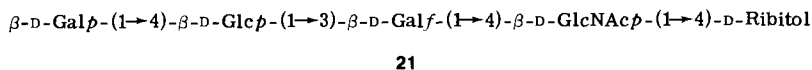
composed of pentasaccharide repeating-units in which terminal kojibiose and D-galactose residues are linked to a backbone of amino sugar residues.

11. Type 13 Capsular Polysaccharide

Baddiley and coworkers⁴² have studied the structure of S13, which is composed of D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and ribitol residues, and phosphate groups in the molar proportions 2:1:1:1:1. O-Acetyl groups are also present. A neutral pentasaccharide was obtained by hydrolysis with alkali, followed by enzymic dephosphorylation. On mild, acid hydrolysis, this yielded two main products, a trisaccharide (19) and a disaccharide (20), the structures of which were determined by conventional methods.



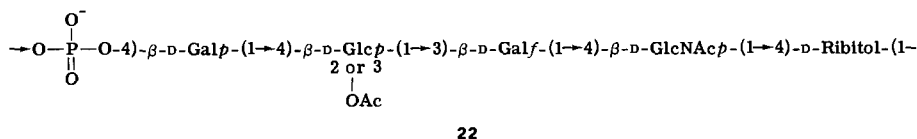
L-Arabinose was formed on periodate oxidation, borohydride reduction, and mild, acid hydrolysis of the pentasaccharide, demonstrating that it contains a D-galactofuranosyl residue. The amino sugar residue was resistant to periodate oxidation but was oxidized after N-deacetylation, showing that this residue is substituted at O-4, not at O-3. The structure of the pentasaccharide 21 was thereby established. It showed $[\alpha]_D -36^\circ$, indicating that all sugar residues are β -D-linked.



Acid hydrolysis of S13 produced phosphates of ribitol and D-galactose. Alkaline hydrolysis, however, occurred unidirectionally, as subsequent acid hydrolysis yielded D-galactose 3- and 4-phosphate, only. Alkaline hydrolysis of phosphoric diesters tends to proceed with participation of a vicinal hydroxyl group, with intermediate formation of a cyclic phosphate.⁵ The phosphate linkage was, therefore, located at O-1 of the ribitol residue, which is further substituted at O-2. The L configuration was assigned to this residue, in agreement with the presumed mode of biosynthesis. As the D-galactopyranosyl residue in S13 was oxidized by periodate, and threitol and its phosphate were formed on subsequent borohydride reduction and acid

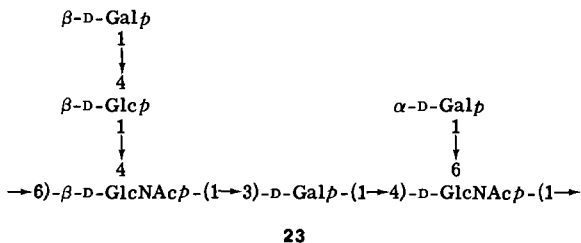
(42) M. J. Watson, J. M. Tyler, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **130**, 45-54 (1972).

hydrolysis, it was concluded that the phosphate was linked to O-4 of this residue. The D-glucosyl residue in S13 was found resistant to periodate oxidation before, but not after, *O*-deacetylation, indicating that an *O*-acetyl group is linked to O-2 or O-3 of this residue. From the combined evidence, structure **22** was proposed for the repeating unit of S13. The only questionable feature of this structure is the exact location of the *O*-acetyl groups.



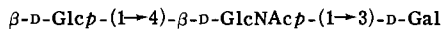
12. Type 14 Capsular Polysaccharide

As a result of structural studies¹ of S14, which contains D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-glucose, a rather complex repeating-unit containing 12 sugar residues was proposed. A simple, hexasaccharide repeating-unit (**23**) was subsequently suggested.²⁸

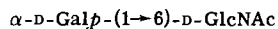


This was based upon a methylation analysis, reported in Ref. 1, which essentially yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,4,6-tri-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-glucose, and 2-amino-2-deoxy-3-*O*-methyl-D-glucose in the molar proportions of 2:1:1:2, and upon enzymic hydrolysis⁴³ with induced enzymes, as an induced β -D-galactosidase released D-galactose and a trisaccharide (**24**). After several treatments, a nondialyzable residue remained which was composed of equal parts of D-galactose and 2-acetamido-2-deoxy-D-glucose. On treatment with an induced 2-acetamido-2-deoxy- β -D-glucosidase, the residue yielded the disaccharide **25**. Even if the oligosaccharides **24** and **25** are components of the repeating unit **23**,

(43) S. A. Barker, G. I. Pardoe, M. Stacey, and J. W. Hopton, *Nature*, **204**, 938-939 (1964).



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25

it is difficult to see how a non-dialyzable core having the aforementioned composition could be formed on cleavage of β -D-galactopyranosidic linkages. Furthermore, some previously reported structural features¹ do not fit into this structure, which should be regarded as tentative only.

A methylation analysis of S14, performed in the authors' laboratory,⁴⁴ gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, and 2,3,6-tri-O-methyl-D-glucose in the proportions 1:1:1. Although these are the same components as previously reported, the proportions are different. In addition to these sugars, 2-deoxy-3-O-methyl-2-(methylamino)-D-glucose was also obtained.

The immunological properties of a modified S14, obtained on treatment with D-galactose oxidase and subsequent oxidation of the aldehyde group to a carboxyl group with chlorite, have been investigated.⁴⁵

13. Type 18A Capsular Polysaccharide

The structure of S18A was studied by Heidelberger and co-workers.⁴⁶ It is composed of D-galactose, D-glucose, a rhamnose, a 2-acetamido-2-deoxyglucose, and glycerol residues, and phosphate groups in the approximate molar proportions 4:7:2:2:2. The D-galactosyl, rhamnosyl, and 2-acetamido-2-deoxy-D-glucosyl residues are not oxidized by periodate. Like S11A and S18, polysaccharide S18A seems to be a polysaccharide proper, with substituents of glycerol phosphate. Unlike these polysaccharides, it does not contain O-acetyl groups. Its serological properties resemble those of O-deacetylated S18.

14. Type 19 Capsular Polysaccharide

Pure S19 was isolated with the aid of zone electrophoresis,⁴⁷ and was subjected to structural investigations.^{48,49} The polymer, which

(44) Unpublished results from the authors' laboratory.

(45) S. Estrada-Parra and I. Gomez, *Immunochemistry*, **9**, 1095-1101 (1972).

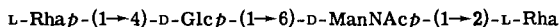
(46) M. Heidelberger, S. Estrada-Parra, and R. Brown, *Biochemistry*, **3**, 1548-1550 (1964).

(47) T. Miyazaki, T. Yadomae, and J. K. N. Jones, *J. Biochem. (Tokyo)*, **68**, 755-758 (1970).

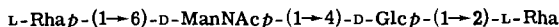
(48) T. Miyazaki and T. Yadomae, *Carbohydr. Res.*, **16**, 153-159 (1971).

(49) T. Miyazaki and T. Yadomae, *Chem. Pharm. Bull. (Tokyo)*, **18**, 1249-1253 (1970).

was sensitive to acid and to base, showed $[\alpha]_D +37^\circ$, and contained L-rhamnose, D-glucose, 2-acetamido-2-deoxy-D-mannose, and phosphate in the molar proportions 2:1:1:1. On treatment with acid phosphate, followed by dilute alkali, two tetrasaccharides were produced. Both contained the same sugars as S19, and in the same proportions. One was neutral and the other acidic, containing a phosphoric ester group. Structural studies of the neutral polysaccharide, by use of methylation analysis and periodate oxidation as the main methods, indicated the partial structure **26** or **27**.



26



27

Unpublished results⁵⁰ lend support to the latter alternative (**27**). The anomeric nature of the sugar residues and the position to which the phosphate group is linked were not determined.

The type-specific polymers (from pneumococci) that contain phosphate groups generally also contain an alditol (glycerol or ribitol) residue. The only known exception is S19, which therefore occupies a unique position among the pneumococcal polysaccharides. Polymers consisting of reducing sugar residues and phosphate groups are, however, known to be produced by other bacteria, for example, a *Micrococcus* species.⁵¹ In agreement with the biosynthetic considerations discussed in the Introduction, the phosphate group should be linked to C-1 of the reducing L-rhamnose residue in **26** or **27**. As the L-rhamnosyl phosphate most probably derives from TDP-L-rhamnose, in which the L-rhamnosyl group has the β -L configuration, it should consequently have the same configuration in S19.

15. Type 23 Capsular Polysaccharide

Analysis of S23 showed that it is composed⁵² of residues of L-rhamnose (35), D-galactose (31), and D-glucose (32%). Some phosphorus (3%) was also found. Immunochemical studies indicated that the polymer contains terminal L-rhamnosyl groups.

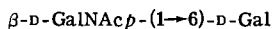
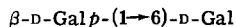
(50) T. Miyazaki, personal communication.

(51) M. D. Partridge, A. L. Davison, and J. Baddiley, *Biochem. J.*, **121**, 695-700 (1971).

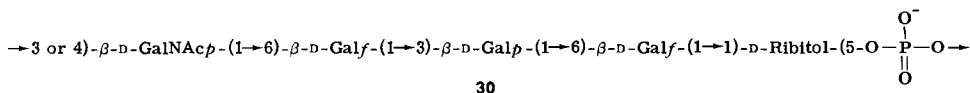
(52) M. Heidelberger, J. M. Davie, and R. M. Krause, *J. Immunol.*, **99**, 794-796 (1967).

16. Type 29 Capsular Polysaccharide

The structure of S29 has been investigated by Baddiley and co-workers.⁵³ It contains D-galactose, 2-acetamido-2-deoxy-D-galactose, and ribitol residues, and phosphate groups in the molar proportions 3:1:1:1. Alkaline hydrolysis, followed by treatment with alkaline phosphatase, yielded a basic pentasaccharide and its N-acetylated analog. On deamination with nitrous acid, the former yielded 2,5-anhydro-D-talose and a tetrasaccharide, demonstrating that the 2-amino-2-deoxy-D-galactose residue occupies a terminal position in the pentasaccharide. Methylation analysis of the tetrasaccharide yielded 1,2,3,4-tetra-O-methylribitol, 2,3,5,6-tetra-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, and 2,3,5-tri-O-methyl-D-galactose. On methylation analysis of the neutral pentasaccharide, no 2,3,5,6-tetra-O-methyl-D-galactose but an increased proportion of 2,3,5-tri-O-methyl-D-galactose was obtained, demonstrating that the 2-amino-2-deoxy-D-galactosyl group is linked to O-6 of a D-galactofuranosyl residue. The pentasaccharide contains two D-galactofuranosyl residues, the glycosidic linkages of which are preferentially cleaved on acid hydrolysis. Partial, acid hydrolysis of the neutral polysaccharide thus yielded ribitol and the disaccharides **28** and **29**. From these experiments, the sequence of the sugar residues in

**28****29**

the pentasaccharide was established. The negative specific rotation of the pentasaccharide, $[\alpha]_D -47^\circ$, indicated that all sugar residues are β -D-linked. Periodate-oxidation studies of the pentasaccharide and of S29 confirmed these results, and further demonstrated that the ribitol residue in S29 is substituted at O-1 and O-5 by a sugar residue and a phosphoric diester grouping, respectively, and that this grouping is also linked to O-3 or O-4 of the 2-amino-2-deoxy-D-galactosyl residue. The configuration of the ribitol residue was assigned on biosynthetic grounds. The only uncertainty in the structure of S29 (**30**) is, therefore, whether the phosphate group is linked to O-3 or O-4 of the 2-amino-2-deoxy-D-galactosyl residue.

**30**

(53) E. V. Rao, M. J. Watson, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **111**, 547-556 (1969).

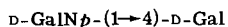
17. Type 31 Capsular Polysaccharide

Polysaccharide S31 is composed of L-rhamnose, D-galactose, and glucuronic acid residues in⁵⁴ the proportions 2:2:1. As the polysaccharide is sensitive to acid hydrolysis, it may contain glycofuranosidic residues, most probably D-galactofuranosyl residues. On acid hydrolysis, an aldobiouronic acid composed of a glucosyluronic acid group and a D-galactose residue was produced.

18. Type 33B Capsular Polysaccharide

The structure of S33B was investigated by M. J. Watson.⁵⁵ It contains D-galactose, D-glucose, 2-acetamido-2-deoxy-D-galactose, and ribitol residues, and phosphate groups in the molar proportions 3:1:1:1:1. On dephosphorylation, first with alkali and then with alkaline phosphatase, a neutral hexasaccharide and its *N*-deacetylated analog were liberated. Methylation analysis of the former gave a 1,2,3,5-tetra-*O*-methylribitol, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,3,6-tri-*O*-methyl-D-galactose. The amino sugar and a branching D-galactosyl residue were not accounted for in this analysis.

Acid hydrolysis of the basic hexasaccharide yielded the disaccharide **31**. Its mobility in paper chromatography lay between those of the corresponding (1 → 3)-linked (from S10A) and β-(1 → 6)-linked (from S29) isomers, which is why it was assumed to be (1 → 4)-linked. The (1 → 2)-linked isomer was excluded, as the D-galactose residue that is part of **31** carries a D-galactopyranosyl group linked to O-2 in the original polysaccharide, as will be discussed.

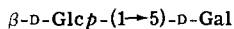


31

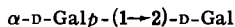
Several oligosaccharides were obtained on partial, acid hydrolysis of the neutral polysaccharide. Disaccharide **32**, containing a nonreducing β-D-glucopyranosyl group, should be (1 → 4)- or (1 → 5)-linked (according to the methylation analysis). As it was formed in good yield by hydrolysis under mild conditions, the D-galactose residue was assumed to be furanosidic and consequently substituted at O-5. In the galactobiose **33**, the nonreducing group is α-D-linked and, as indicated by color reactions, the linkage is (1 → 2).

(54) N. Roy, W. R. Carroll, and C. P. J. Glaudemans, *Carbohydr. Res.*, **12**, 89-96 (1970).

(55) M. J. Watson, *Biochem. J.*, **137**, 603-606 (1974).



32



33

Structure **34** was proposed for a trisaccharide composed of D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-galactose residues. The D-glucosyl group and the D-galactosyl residues are the same as in disaccharide **32**. That the 2-acetamido-2-deoxy-D-galactose residue is linked to O-3 was indicated by color reactions and the fact that this



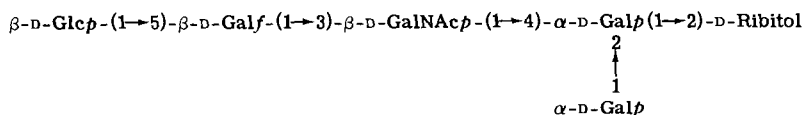
34

residue, in S33B, is not oxidized by periodate. The isolation of this trisaccharide in good yield is remarkable, and indicates that the glycosidic linkages of the pyranoid 2-acetamido-2-deoxy-D-galactosyl residue and the furanoid D-galactosyl residue are hydrolyzed at comparable rates. Structure **35** was proposed for a second, nonreducing trisaccharide.



35

The D-galactosyl residues are the same as in disaccharide **33**, and the fact that ribitol is substituted at O-2 was evident from the methylation analysis. Finally, a nonreducing tetrasaccharide was obtained, containing D-galactose, 2-acetamido-2-deoxy-D-galactose, and ribitol residues in the proportions 2:1:1. From these results, and periodate-oxidation studies, structure **36** was proposed for the neutral hexasaccharide.



36

The formation of ethylene glycol phosphate and glycerol phosphate on Smith degradation of S33B demonstrated that the phosphate group is linked to O-1 of ribitol and to O-6 of a hexopyranosyl residue, oxidizable between C-3 and C-4. The latter residue could be either of the terminal hexosyl groups in **36**. The configuration of the ribitol residue, with the phosphate group at O-5 of D-ribitol, was assumed for biosynthetic reasons.

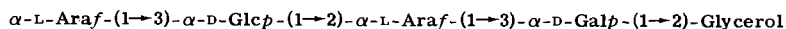
The anomeric natures of two sugar residues in **36** are evident from the characterization of disaccharides **32** and **33**. The other anomeric

linkages in **36** were assigned on account of the optical rotation of the hexasaccharide, $[\alpha]_D +51^\circ$. In this assignment, the author must have assumed that the D-galactofuranosyl residue is β -D-linked; this seems to be a reasonable assumption, as, to the best of our knowledge, α -D-galactofuranosides have not been conclusively demonstrated in Nature. The reason why it was assumed that the 2-acetamido-2-deoxy-D-galactosyl residue is β -D-linked and the D-galactosyl residue linked to ribitol is α -D-linked (and not the other way around) is, however, less obvious. Also, some other structural features of S33B seem, in the opinion of the present authors, to be less firmly established, and structure **36** for the hexasaccharide repeating-unit should be regarded as tentative only.

19. Type 34 Capsular Polysaccharide

Earlier studies of S34, which is composed of D-galactose, D-glucose, and ribitol residues and phosphate groups in the proportions 3:1:1:1, were summarized in Ref. 1. As a result of these studies, it was proposed that S34 contains pentasaccharide repeating-units, united by phosphoric diester linkages. The structure of the pentasaccharide, except for the configurations of some glycosidic linkages, was determined. The O-acetyl groups present in S34 were not located.

This structure was confirmed, and the configurations of all the linkages were determined, in complementary studies.⁵⁶ Periodate oxidation-borohydride reduction of deacetylated S34, followed by dephosphorylation, produced a pentasaccharide (**37**) in which the L-arabinofuranose and glycerol moieties derive from D-galactofuranosyl and ribitol residues, respectively. Oligosaccharide **37**



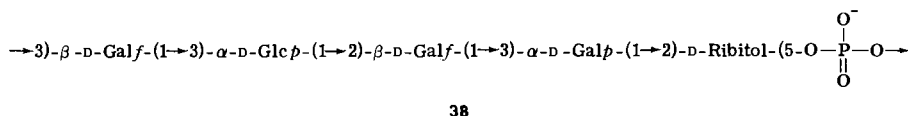
37

was subjected to three consecutive degradations, each removing only the terminal sugar residue. Each degradation involved periodate oxidation followed by treatment with 1,1-dimethylhydrazine. The procedure is analogous to the Barry degradation,⁵⁷ in which phenylhydrazine is used. The final product was 2-O- α -D-galactopyranosylglycerol. From the optical rotations of the original and the degraded materials, the configurations of all glycosidic linkages were established.

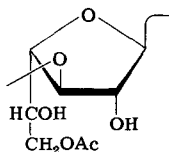
(56) J. R. Dixon, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **100**, 507-511 (1966).

(57) P. S. O'Colla, *Methods Carbohydr. Chem.*, **5**, 382-392 (1965).

The periodate-oxidation studies also demonstrated that the phosphate group in S34 is linked to O-5 of the 2-O-substituted ribitol and to O-2 or O-3 of the terminal β -D-galactofuranosyl residue. Acid hydrolysis of S34 yielded D-galactose 2- or 3-phosphate.⁵⁸ These two compounds were synthesized by unambiguous methods. The 2-phosphate was essentially unchanged after treatment with 0.1 M hydrochloric acid at 100°, but the 3-phosphate, and also the D-galactose phosphate from S34, was partially converted into another phosphate, probably the 4-phosphate. The phosphate group is, consequently, linked to O-3 of the terminal β -D-galactofuranosyl residue, and **38** is the structure of the repeating unit of deacetylated S34. The ribitol residue in **38** was, from biosynthetic arguments, assumed to have the D configuration.



On periodate oxidation of S34, part of the internal β -D-galactofuranosyl residues is not oxidized, but all these residues are oxidized after deacetylation. The O-acetyl groups, about one per two pentasaccharide repeating-units, are therefore located on O-5 or O-6 of these D-galactofuranosyl residues.⁵⁹ As methyl 6-O-acetyl- β -D-galactofuranoside strongly inhibits the precipitation of S34 by the anti-Pn34 system,⁶⁰ it was concluded that the O-acetyl groups are most probably linked to O-6 of the internal β -D-galactofuranosyl residues, as in **39**.



Methyl 3-O- β -D-galactofuranosyl- α -D-glucopyranoside was also synthesized. Although representative of a partial structure in S34, it

(58) G. J. F. Chittenden, W. K. Roberts, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **109**, 597-602 (1968).

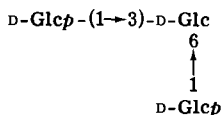
(59) J. R. Dixon, W. K. Roberts, G. T. Mills, J. G. Buchanan, and J. Baddiley, *Carbohydr. Res.*, **8**, 262-265 (1968).

(60) N. Roy and C. P. J. Glaudemans, *Carbohydr. Res.*, **8**, 214-218 (1968).

did not inhibit the homologous precipitation reaction of S34 with its rabbit serum.⁶¹

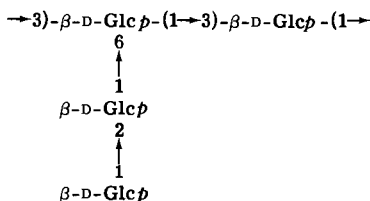
20. Type 37 Capsular Polysaccharide

Polysaccharide S37 is unique among the pneumococcal polysaccharides, as it contains a single constituent sugar, D-glucose.⁶² The polysaccharide consumes 0.95 molecule of periodate per D-glucose residue, with simultaneous release of approximately 0.3 molecule of formic acid. Smith degradation of S37 yielded a polymeric backbone of (1 → 3)-linked D-glucosyl residues, resistant to further oxidation. Two oligosaccharides, 2-O-β-D-glucopyranosyl-D-glucose (sophorose) and a trisaccharide (from its reactions with periodate, tentatively identified as 40) were isolated after partial, acid hydrolysis.



40

As a result of these studies, tentative structure 41 for a repeating unit of S37 was proposed. Eventually, some residues could have a second sophorosyl end-group, linked to the other (1 → 3)-linked D-glucosyl residue in 41, a proposal that the present authors consider



41

less likely. It was assumed that all four D-glucosyl units in 41 are β-D-linked, but experimental evidence has only been given for one of these, the terminal residue in the side chain.

21. The C-Substance

The C-substance is a species-specific antigen of *Streptococcus pneumoniae* that often contaminates the type-specific capsular poly-

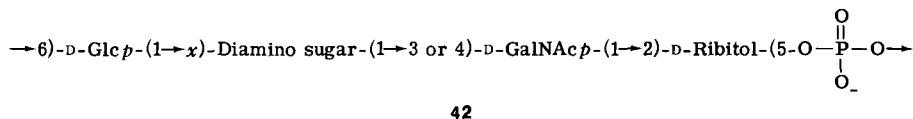
(61) C. P. J. Glaudemans, *Carbohydr. Res.*, **10**, 213-219 (1969).

(62) J. C. Knecht, G. Schiffman, and R. Austrian, *J. Exp. Med.*, **132**, 475-487 (1970).

saccharides. It has been demonstrated^{63,64} that it is a cell-wall teichoic acid; for it, the name C-teichoic acid has been proposed. It is composed of equimolecular proportions of ribitol phosphate, 2-acetamido-2-deoxy-D-galactose, D-glucose, and a 2-acetamido-4-amino-2,4,6-trideoxyhexose. The enzymic synthesis of a nucleoside of this diamino sugar has been investigated.⁶⁵ It further contains a choline phosphate residue, but less than one molar equivalent. Deamination of the 4-amino sugar moiety proceeds with partial cleavage of both the glycosidic linkage and the linkage to the substituent at C-3. D-Glucitol, ribitol, and their phosphates were thus produced on deamination of C-substance, followed by borohydride reduction and acid hydrolysis. On deamination and acid hydrolysis of C-substance that had been pretreated with alkali, D-glucose but no D-glucose phosphate was produced. As the alkaline hydrolysis of a phosphoric diester preferably occurs with assistance of a vicinal hydroxyl group, with the intermediate formation of a cyclic phosphate, it was therefore concluded that the phosphate group is linked to O-6 of D-glucose and, for biosynthetic reasons, to O-5 of D-ribitol.

On deamination, C-substance that had been treated with alkali and phosphatase yielded the disaccharide 2-O-(2-acetamido-2-deoxy-D-galactosyl)-D-ribitol. Periodate oxidation of C-substance had revealed that the ribitol residue is substituted at O-2 and O-5.

From the combined evidence, the tentative structure **42** was proposed for the repeating unit of C-substance. The choline phosphate residue is possibly linked to O-3 of the 2-acetamido-2-deoxy-D-galactose.



Analysis of the so-called F-substance showed that it contains ~6% of fatty acids and, in addition to these, the same components as the C-substance. The earlier observation¹ that these substances are closely related was thus confirmed.

III. CONCLUSION

Structural studies of some 25 of the ~80 different capsular polysaccharides from pneumococci have been reported. Complete structures

(63) D. E. Brundish and J. Baddiley, *Biochem. J.*, **110**, 573-582 (1968).

(64) M. J. Watson and J. Baddiley, *Biochem. J.*, **137**, 399-404 (1974).

(65) J. Distler, B. Kaufman, and S. Roseman, *Arch. Biochem. Biophys.*, **116**, 466-478 (1966).

have been proposed for S2, S3, S6, S8, and S34. For three others, namely, S11A, S13, and S29, only minor structural features are still unknown. For most of the pneumococcal polysaccharides, however, the structures are unknown or only partially known. Considering the importance of these compounds in immunochemistry, this is an unsatisfactory situation. It is the hope of the present authors that this article may stimulate further work in this field.

PECTIC ENZYMES

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I. INTRODUCTION

Since the reviews on pectic enzymes by Kertesz and McColloch¹ and Deuel and Stutz² appeared, research in this field has provided a number of new findings. Pectic enzymes catalyzing the splitting of glycosidic bonds in pectic substances by β -elimination were discovered.^{3,4} A number of homogeneous or highly purified enzyme preparations have been obtained, their action pattern characterized, and some of their molecular properties described. Considerable attention

- (1) Z. I. Kertesz and R. J. McColloch, *Advan. Carbohydr. Chem.*, **5**, 79–102 (1950).
- (2) H. Deuel and E. Stutz, *Advan. Enzymol.*, **20**, 341–382 (1958).
- (3) P. Albersheim, H. Neukom, and H. Deuel, *Helv. Chim. Acta*, **43**, 1422–1426 (1960).
- (4) C. W. Nagel and R. H. Vaughn, *Arch. Biochem. Biophys.*, **93**, 344–352 (1961).

has been devoted to the study of the role of pectic enzymes in physiological and pathological changes of plants, as well as to the study of conditions of production of pectic enzymes by micro-organisms. Some of the findings were treated critically in earlier reviews.⁵⁻¹⁰

Early studies in this field often suffered from the disadvantage that insufficiently purified enzymes were used. The existing methods, such as fractional salting-out with ammonium sulfate, precipitation with organic solvents, dialysis, and heat inactivation of contaminating enzymes, yielded inhomogeneous and, in many cases, unspecific preparations. Application of modern separation methods, such as gel-permeation and ion-exchange chromatography, electrophoresis, and adsorption, in various combinations, made possible the separation of even complex mixtures of enzymes, and isolation of highly purified, specific, and often homogeneous, enzyme preparations.

Application of new analytical methods, in particular of different types of electrophoresis, has also provided new data on the molecular properties of these enzymes and has set more rigorous criteria of their purity and homogeneity.

It is the purpose of this article to present some of the aspects of the mode of action, properties, and function of pectic enzymes.

II. CLASSIFICATION

The group of pectic enzymes includes pectinesterase, which catalyzes the de-esterification of pectin, and depolymerizing enzymes which catalyze the splitting of glycosidic α -(1 \rightarrow 4) bonds of the D-galacturonan chain of the pectin molecule.

According to the IUPAC-IUB Enzyme Nomenclature,¹¹ pectinesterase belongs to the carboxyl ester hydrolases (EC 3.1.1.11) and has the systematic name pectin pectyl-hydrolase. The literature also contains the expressions pectin methylesterase, pectin demethoxylase, and pectin methoxylase for the same enzyme. The old name pectase,

(5) H. Neukom, *Schweiz. Landwirtsch. Forsch.*, **2**, 112-122 (1963).

(6) A. G. J. Voragen and W. Pilnik, *Z. Lebensm. Untersuch.-Forsch.*, **142**, 346-359 (1970).

(7) F. M. Rombouts and W. Pilnik, *Crit. Rev. Food Technol.*, **3**, 1-26 (1972).

(8) R. K. S. Wood, *Ann. Rev. Plant. Physiol.*, **11**, 299-322 (1960).

(9) D. F. Bateman and R. L. Millar, *Ann. Rev. Phytopathol.*, **4**, 119-146 (1966).

(10) P. Albersheim, T. Jones, and P. D. English, *Ann. Rev. Phytopathol.*, **7**, 171-194 (1969).

(11) IUPAC-IUB, "Enzyme Nomenclature," Elsevier, Amsterdam, 1973.

TABLE I

Classification of Depolymerizing Pectic Enzymes According to Neukom⁵

Mechanism	Substrate	Action pattern	
		Random	Terminal
Hydrolysis	pectate	endopolygalacturonase	exopolygalacturonase
	pectin	endopolymethylgalacturonase	exopolymethylgalacturonase
β -Elimination	pectate	endopectate lyase	exopectate lyase
	pectin	endopectin lyase	exopectin lyase

which does not properly describe the mode of action, as well as the name pectolipase are no longer used.

The depolymerases catalyzing the hydrolytic cleavage of the α -(1 \rightarrow 4)-glycosidic bonds in the D-galacturonan moiety of pectic substances were divided by Demain and Phaff¹² into four groups, depending on (a) the preference of the enzyme for pectate or pectin, and (b) the random or terminal splitting of the glycosidic bonds. At the same time, they proposed a unified nomenclature for these enzymes. The enzymes preferring pectate were called polygalacturonases, and those preferentially degrading the highly esterified substrates were called polymethylgalacturonases. The prefixes endo- and exo- used in connection with either of the foregoing names denoted a random- or a terminal-action pattern, respectively.

After the discovery of enzymes splitting glycosidic bonds of pectin and pectate by β -elimination,^{3,4} Neukom⁵ extended the classification system for the depolymerizing enzymes, taking into account, also, the mechanism of cleavage of the glycosidic bonds (see Table I). According to this classification, the depolymerizing enzymes are divided into eight groups, four being the original hydrolases and the other four, analogous groups operating by a β -elimination mechanism. The latter enzymes were designated pectate lyase and pectin lyase, or transeliminases with the prefix endo- or exo- with respect to the action pattern.

Investigations of pectic enzymes conducted during the past few years have shown that the classification system due to Neukom⁵ is no longer adequate. Thus, the hypothetical enzymes degrading highly

(12) A. L. Demain and H. J. Phaff, *Wallerstein Lab. Commun.*, **20**, 119-140 (1957).

TABLE II
Nomenclature of Pectic Depolymerases

Preferred substrate	Action pattern	Name	Modified ^a EC systematic name ¹¹	EC No.
<i>Hydrolases</i>				
D-Galacturonan	random	endo-D-galacturonanase	poly-(1 → 4)-α-D-galactosiduronate glycanohydrolase	3.2.1.15
D-Galacturonan	terminal	exo-D-galacturonanase	poly-(1 → 4)-α-D-galactosiduronate glycanohydrolase	3.2.1.67
D-Galacturonan	penultimate bonds	D-galacturonandigalacturono hydrolase	poly-(1 → 4)-α-D-galactosiduronate digalacturonohydrolase	3.2.1.82
Oligo-D-galactosiduronate	terminal	oligo-D-galactosiduronate hydrolase		
<i>Lyases</i>				
D-Galacturonan	random	endopectate lyase	poly-(1 → 4)-α-D-galactosiduronate lyase	4.2.2.2
D-Galacturonan	penultimate bonds	exopectate lyase	poly-(1 → 4)-α-D-galactosiduronate exolyase	4.2.2.9
Oligo-D-galactosiduronate	terminal	oligo-D-galactosiduronate lyase	oligo-D-galactosiduronate lyase	4.2.2.6
Poly(methyl D-galactosiduronate)	random	pectin lyase	poly(methyl D-galactosiduronate) lyase	4.2.2.10

^a Modified to conform with accepted carbohydrate nomenclature.

esterified substrates by the terminal action-pattern of hydrolysis (exopolymethylgalacturonase) and of β -elimination (exopectin lyase) have not yet been found. Hatanaka and Ozawa¹³ described a new type of hydrolase cleaving penultimate glycosidic bonds starting at the nonreducing end of the molecule of pectic acid, and releasing di-D-galactosiduronate as the sole reaction-product. They suggested the names "digalacturonopolygalacturonase" or "polygalacturonide digalacturonohydrolase" for this enzyme. It was later found that some hydrolases, as well as lyases having the terminal action-pattern, degrade oligo-D-galactosiduronates, in preference to the polymeric substrates, at a rate inversely proportional to the chain length of the substrate.¹⁴⁻¹⁷ Hence, some authors called these enzymes "oligogalacturonide hydrolase" and "oligogalacturonide lyase," respectively. All of these facts were taken into consideration when compiling the classification shown in Table II.

Enzymes catalyzing the hydrolytic cleavage of glycosidic bonds of D-galacturonans are called D-galacturonanases, instead of the earlier term polygalacturonases, in agreement with the preferred name of the substrate. Enzymes having the terminal-action pattern, where the preferred substrate is D-galacturonan, belong to the exo-D-galacturonanases, but those preferentially splitting the oligo-D-galactosiduronates are designated oligo-D-galactosiduronate hydrolases. The lyases having the terminal-action pattern are differentiated in a similar way.

All enzymes already mentioned, except oligo-D-galactosiduronate hydrolase, are included in the Enzyme Nomenclature of the IUPAC-IUB Enzyme Commission,¹¹ and their code numbers and suitably modified, systematic names are used herein.

III. SUBSTRATES

Pectin and pectic acid, which are the natural substrates of pectic enzymes, are branched heteropolysaccharides in which the backbone contains L-rhamnose residues and α -D-(1 \rightarrow 4)-linked residues of D-galactopyranosiduronic acid. The neutral sugars D-galactose and L-arabinose, and sometimes D-xylose and L-fucose, form the side

(13) C. Hatanaka and J. Ozawa, *Nippon Nogei Kagaku Kaishi*, **43**, 764-772 (1969).

(14) F. Moran, S. Nasuno, and M. P. Starr, *Arch. Biochem. Biophys.*, **125**, 734-741 (1968).

(15) S. Hasegawa and C. W. Nagel, *Arch. Biochem. Biophys.*, **124**, 513-520 (1968).

(16) C. W. Nagel and S. Hasegawa, *J. Food Sci.*, **33**, 378-382 (1968).

(17) C. Hatanaka and J. Ozawa, *Agr. Biol. Chem. (Tokyo)*, **34**, 1618-1624 (1970).

chains of the pectin molecule.¹⁸⁻²⁴ The carboxyl groups of the D-galactopyranosiduronic acid residues are partly esterified with methanol. Some of the secondary alcohol groups at C-2 and C-3 are acetylated. The degree of esterification, the proportion of neutral saccharides, and the degree of polymerization (d.p.) are the principal elements of heterogeneity of pectic compounds of varied origins.

When studying the mode of action and some of the properties of pectic enzymes, oligo-D-galactosiduronates and some of their derivatives are used as substrates. These are prepared from partial hydrolyzates (obtained by acid, alkaline, or enzymic treatment) by precipitation as salts of copper or strontium,^{25,26} or by separation by paper chromatography,²⁷ chromatography on DEAE-cellulose,²⁸ Dowex-1 (Refs. 29 and 30), or gel chromatography.³¹ For large-scale preparation of oligomeric substrates, Van Houdenhoven and coworkers³² used the endo-D-galacturonanase of *Saccharomyces fragilis* bound to a support of Sepharose 4B. Filtration of a solution of the substrate through a column of the bound enzyme continuously released the fragments, which were then separated on columns of BioGel P2 and P4.

Valuable information on the action pattern of some enzymes was obtained by using oligo-D-galactosiduronate-L-galactonolactones (from oligo-D-galactosiduronates reduced at the reducing end) and esterified oligo-D-galactosiduronates prepared according to McCready and Seegmiller,³³ as well as oligo-D-galactosiduronates oxidized with periodic acid.³⁴

(18) A. J. Barrett and D. H. Northcote, *Biochem. J.*, **94**, 617-627 (1965).

(19) G. O. Aspinall, J. W. T. Craig, and J. L. Whyte, *Carbohydr. Res.*, **7**, 442-452 (1968).

(20) M. J. Foglietti and F. Percheron, *Carbohydr. Res.*, **7**, 146-155 (1968).

(21) V. Zitko and C. T. Bishop, *Can. J. Chem.*, **43**, 3206-3214 (1965).

(22) G. O. Aspinall and R. S. Fanshawe, *J. Chem. Soc.*, 4215-4225 (1961).

(23) G. O. Aspinall, B. Gestetner, J. A. Molloy, and M. Uddin, *J. Chem. Soc.*, 2554-2559 (1968).

(24) R. G. Ovodova, V. E. Vaskovsky, and J. S. Ovodov, *Carbohydr. Res.*, **6**, 328-332 (1968).

(25) A. L. Demain and H. J. Phaff, *Arch. Biochem. Biophys.*, **51**, 114-121 (1954).

(26) H. J. Phaff and B. S. Luh, *Arch. Biochem. Biophys.*, **36**, 231-232 (1952).

(27) A. L. Demain and H. J. Phaff, *J. Biol. Chem.*, **210**, 381-393 (1954).

(28) C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.*, **13**, 89-102 (1966).

(29) C. W. Nagel and S. Hasegawa, *Arch. Biochem. Biophys.*, **118**, 590-595 (1967).

(30) H. Derungs and H. Deuel, *Helv. Chim. Acta*, **37**, 657-659 (1954).

(31) L. Rexová-Benková, *Chem. Zvesti*, **24**, 59-62 (1970).

(32) F. E. A. van Houdenhoven, P. J. G. M. de Witt, and J. Visser, *Carbohydr. Res.*, **34**, 233-239 (1974).

IV. PECTINESTERASES

1. Action Pattern and Specificity

On the basis of existing views, pectinesterase can be characterized as an enzyme highly specific for the D-galacturonan structure. Even a partial reduction of the carboxyl groups of pectin (D-galactopyranosiduronate to D-galactose residues) brings about a marked inhibition of the pectinesterase activity.³⁵ Methyl esters of alginic acid and gum tragacanth are not de-esterified by its action.^{36,37} The pectinesterase does not hydrolyze the methyl ester of D-galactopyranuronic acid, the mono- and di-methyl esters of its dimer, and the triester of its trimer, but readily attacks the polymethyl esters of D-galacturonans having a degree of polymerization of 10 or more.³³ So far, the chain length of the oligo-D-galacturonan methyl esters at which the action of pectinesterase sets in has not been established.

The specificity of pectinesterase is not so marked with respect to the alcohol moiety of the ester, as it hydrolyzes ethyl esters of D-galacturonans at a rate ~6–16% that of de-esterification of the methyl esters.³⁶ *Citrus natsudaoidai* pectinesterase de-esterifies the ethyl esters of pectic acid at a rate 1/5th to 1/7th of that for the methyl esters; the propyl and allyl esters are attacked at 1/20th to 1/80th of the rate.³⁸ The tomato, citrus, alfalfa, and papaya pectinesterases do not hydrolyze the glycol and glycerol esters of pectin.³⁹

Enzymic de-esterification of the methyl esters of pectin proceeds linearly along the chain of the molecule, giving rise to blocks of free carboxyl groups.^{40–44} The same conclusion was reached on the basis of the high stability-constant of calcium pectinates prepared by par-

(33) R. M. McCready and C. G. Seegmiller, *Arch. Biochem. Biophys.*, **50**, 440–450 (1954).

(34) E. F. Jansen and L. R. MacDonnell, *Arch. Biochem.*, **8**, 97–112 (1945).

(35) J. Solms and H. Deuel, *Helv. Chim. Acta*, **38**, 321–329 (1955).

(36) L. R. MacDonnell, R. Jang, E. F. Jansen, and H. Lineweaver, *Arch. Biochem.*, **28**, 260–273 (1950).

(37) H. J. Phaff, *Arch. Biochem.*, **13**, 67–81 (1947).

(38) M. Manabe, *Nippon Nogei Kagaku Kaishi*, **47**, 385–390 (1973).

(39) H. Deuel, *Helv. Chim. Acta*, **30**, 1523–1534 (1947).

(40) T. H. Schultz, H. Lotzkar, H. S. Owens, and W. D. Maclay, *J. Phys. Chem.*, **49**, 554–563 (1945).

(41) R. Speiser, C. R. Eddy, and C. H. Hills, *J. Phys. Chem.*, **49**, 563–579 (1945).

(42) R. Speiser, M. J. Copley, and G. C. Nutting, *J. Phys. Chem.*, **51**, 117–133 (1947).

(43) C. H. Hills, H. H. Mottern, G. C. Nutting, and R. Speiser, *Food Technol.*, **3**, 90–94 (1949).

(44) W. Heri, H. Neukom, and H. Deuel, *Helv. Chim. Acta*, **44**, 1945–1949 (1961).

tial enzymic de-esterification of highly esterified pectin, as compared with the stability constant of pectin de-esterified in an alkaline medium.⁴⁵

A hypothesis on the action of pectinesterase reported in reviews,^{2,46} according to which this enzyme catalyzes the de-esterification of the methyl ester groups of pectin that are adjacent to free carboxyl groups, received experimental support by Solms and Deuel,³⁵ who examined the effect of orange pectinesterase on pectins partly de-esterified either enzymically or in alkaline medium. These authors³⁵ did not exclude the possibility of initiation of the enzyme reaction from either end of the pectin molecule. The problem was excellently solved by Macmillan and coworkers,⁴⁷⁻⁴⁹ who studied the mode of action of tomato and microbial pectinesterases. The fundamental requirement was the preparation of highly purified, enzymically homogeneous compounds, without which the studies could not be performed. In the case of tomato pectinesterase, it was necessary to remove the endo-D-galacturonanase present,⁵⁰ the action of which would interfere with the investigation of the site of initial attack.⁴⁷ The substrate used was highly esterified pectin [degree of esterification (d.e.) of 95.8%, d.p. 33] which contained approximately one free carboxyl group per molecule. This substrate was treated with highly purified, pectinesterase-free, exopectate lyase produced by *Clostridium multifementans*,⁵¹ which specifically degrades de-esterified D-galacturonans by sequentially splitting O-(4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid)-(1 → 4)-D-galactopyranuronic acid from the reducing end of the chain. The effect of the lyase was negligible in comparison with its activity on the de-esterified substrate. After adding the pectinesterase, the activity of the lyase immediately increased, suggesting that most of the tomato pectinesterase activity occurred at the reducing end of the molecules (see Fig. 1). By employing an experimental procedure whereby the action of both enzymes could be monitored simultaneously, it was found that, on using different amounts of the enzymes, the ratio between the initial enzyme rates was constant and independent of the ratio of added enzymes. Hence, it followed that the initial activity of the lyase was

(45) R. Kohn, I. Furda, and Z. Kopec, *Collect. Czech. Chem. Commun.*, **33**, 264-269 (1968).

(46) H. Lineweaver and E. F. Jansen, *Advan. Enzymol.*, **11**, 267-295 (1951).

(47) M. Lee and J. D. Macmillan, *Biochemistry*, **9**, 1930-1934 (1970).

(48) M. Lee, L. Miller, and J. D. Macmillan, *J. Bacteriol.*, **103**, 595-600 (1970).

(49) L. Miller and J. D. Macmillan, *Biochemistry*, **10**, 570-576 (1971).

(50) M. Lee and J. D. Macmillan, *Biochemistry*, **7**, 4005-4010 (1968).

(51) L. Miller and J. D. Macmillan, *J. Bacteriol.*, **102**, 72-78 (1970).

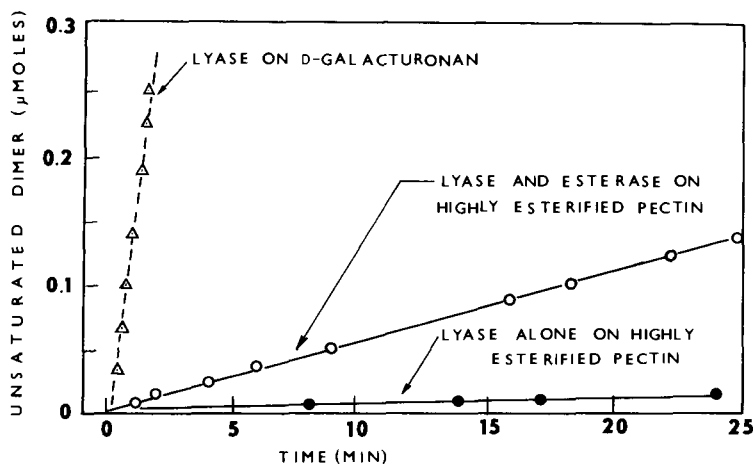


FIG. 1.—Effect⁴⁷ of Tomato Pectinesterase on the Activity of Pectate Lyase Acting on Highly Esterified Pectin. [Reaction mixtures contained 0.5% of D-galacturonan (broken line) or poly(D-galacturonic acid methyl ester) methyl glycoside (solid lines), 5 mM CaCl₂, 33 mM phosphate buffer (pH 7.0), and 0.25 unit of lyase in a final volume of 3.0 ml. In addition, the reaction mixture represented by the middle curve contained 0.031 unit of tomato pectinesterase in a final volume of 3.3 ml.] Reprinted, with permission, from M. Lee and J. D. Macmillan, *Biochemistry*, 9, 1930–1934 (1970). Copyright by the American Chemical Society.

limited by the action of pectinesterase. The final reaction-product inhibits the action of pectinesterase. When both enzymes, pectinesterase and lyase, were present, the inhibition of pectinesterase was lower than during the action of pectinesterase alone, due to the removal of the terminal acidic groups by the action of the lyase (see Fig. 2). The results of this experiment supported the previous findings that blocks of the carboxyl groups de-esterified by the action of pectinesterase are situated near the reducing end. The conclusion to be drawn from this work was that about half of the tomato pectinesterase activity is initiated near the reducing end of the molecule of highly esterified pectin, and that the remaining activity occurs at some secondary locus or loci, probably near to free carboxyl groups.⁴⁷

By using the same experimental procedure, the action pattern of pectinesterase produced by *Clostridium multifementans*⁴⁸ was examined. As none of the separation procedures used were suitable for removing the pectinesterase from exopectate lyase,⁵¹ a specific lyase was obtained by inactivating the pectinesterase by heating for 30 minutes at 38° and pH 7.0; under these conditions, the activity of the lyase was retained. All the pectinesterase preparations used were contaminated with the lyase, which could not be differentially inac-

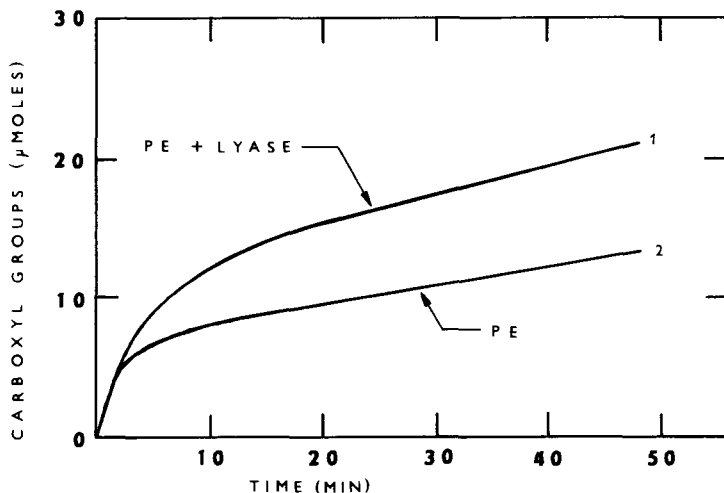


FIG. 2.—Activity⁴⁷ of Tomato Pectinesterase (PE) in the Presence (1) and Absence (2) of Exopectate Lyase. [Reaction mixtures contained 0.125% of poly(D-galacturonic acid methyl ester) methyl glycoside, 0.5 mM CaCl₂, 2.8 units of pectinesterase, and, in the reaction mixture represented by curve 1, 1.5 unit of lyase.] Reprinted, with permission, from M. Lee and J. D. Macmillan, *Biochemistry*, 9, 1930–1934 (1970). Copyright by the American Chemical Society.

tivated. The lyase obtained in that way was tested for action on highly esterified pectin, which was found not to be a suitable substrate, and it became active only after addition of the pectinesterase; then, both activities followed the same course, and the rate-limiting factor for the lyase activity was, apparently, the action of pectinesterase. In contrast with tomato pectinesterase, where only half of the activity occurred at the reducing ends of the molecules, the *Clostridium multifementans* pectinesterase showed a different pattern of attack on highly esterified pectin chains, and all of its activity occurred at the reducing ends of the molecules.

In further work on this series, the same methods were used for the examination of the action pattern of highly purified pectinesterase produced by *Fusarium oxysporum* f. sp. *vasinfectum*.⁴⁹ This pectinesterase was found to affect highly esterified pectin by a mechanism similar to that of tomato pectinesterase, that is, more than half of the enzymic activity occurred at the reducing ends of the molecules, and the rest attacked a different locus or loci of the pectin chains. These conclusions were supported by a comparison of the effect of clostridial lyase on pectin partly de-esterified in an alkaline solution with its effect on pectin partly de-esterified by *Fusarium oxysporum* pectinesterase. The lyase did not act on the randomly

alkali-de-esterified pectin during the first phase of the reaction, and its action only started after 100 minutes. Substrate de-esterified with pectinesterase was immediately suitable for the action of the exopectate lyase.

The action of pectinesterase does not proceed to the complete de-esterification of pectin, but stops at a certain degree of esterification, concerning the value of which there are discrepancies in the literature, apparently due to (a) errors in the analytical methods used, as well as (b) the inhomogeneity of the enzymes and substrates. For tomato pectinesterase, final values of methoxyl content of 1.8% (Ref. 52) were found, as well as of 0.4–0.6% (Ref. 53), for alfalfa pectinesterase 0.5% (Ref. 54), and for the action of *Pseudomonas prunicola* pectinesterase, there was reported a removal of 75% of the methoxyl groups from different types of pectins,⁵⁵ corresponding to final values of 0.6–1.6% for methoxyl content. For a highly purified form of *Coniothyrium diplodiella* pectinesterase, 60% hydrolysis of the methyl ester was found,⁵⁶ and, for orange pectinesterase, Solms and Deuel³⁵ obtained a final, constant value of esterification of 10–11%, corresponding to 1.6–1.7% methoxyl content of the pectins used as substrates.

In the action of pectinesterase, evidently, end-product inhibition takes place.⁵⁷ For highly purified tomato pectinesterase, Lee and Macmillan⁵⁰ found an inhibition constant $K_i = 7$ mM D-galactopyranosiduronate units, the inhibition being competitive (see Fig. 3).

The apparent Michaelis constants for pectinesterase are usually given in percentage of pectin, which constitutes a poorly defined substrate, so that the quantitative value of these data is limited. For pectinesterases of various origins, K_m values of 0.04–0.24% of pectin were found.^{36,58–62} For orange pectinesterase, by using pectins having

- (52) C. H. Hills, J. W. White, Jr., and G. L. Baker, *Proc. Inst. Food Technol.*, **3**, 47–58 (1942).
- (53) C. H. Hills, C. L. Ogg, and R. Speiser, *Ind. Eng. Chem.*, **17**, 507–510 (1945).
- (54) E. F. Jansen, S. W. Waisbrot, and E. Rietz, *Ind. Eng. Chem.*, **16**, 523–524 (1944).
- (55) G. B. Mills, *Biochem. J.*, **44**, 302–305 (1949).
- (56) A. Endo, *Agr. Biol. Chem. (Tokyo)*, **28**, 757–764 (1964).
- (57) H. Lineweaver and G. A. Ballou, *Arch. Biochem.*, **6**, 373–387 (1945).
- (58) L. R. MacDonnell, E. F. Jansen, and H. Lineweaver, *Arch. Biochem.*, **6**, 389–401 (1945).
- (59) M. Holden, *Biochem. J.*, **40**, 103–108 (1946).
- (60) C. H. Hills and H. H. Mottern, *J. Biol. Chem.*, **168**, 651–663 (1947).
- (61) M. Manabe, *Agr. Biol. Chem. (Tokyo)*, **37**, 1487–1491 (1973).
- (62) H. Nakagawa, Y. Yanagawa, and H. Takehana, *Agr. Biol. Chem. (Tokyo)*, **34**, 998–1003 (1970).

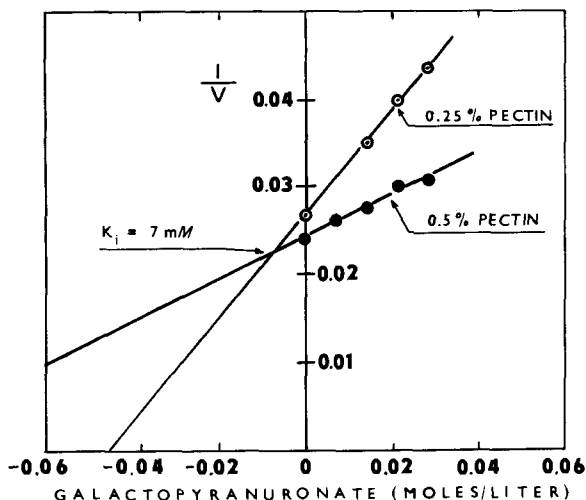


FIG. 3.—Inhibition⁵⁰ of Pectinesterase by D-Galacturonan. [Reaction mixtures contained either 0.5 or 0.25% of pectin, N.F., 0.05 M NaCl, various amounts of D-galacturonan, and 40 units of pectinesterase in a total volume of 20 ml. The pH was 7.0 and the temperature was 30°.] Reprinted, with permission, from M. Lee and J. D. Macmillan, *Biochemistry*, 7, 4005-4010 (1968). Copyright by the American Chemical Society.

different d.e. values, the K_m values corresponded to 1.55–4.05 mM of ester³⁵; for tomato pectinesterase, a K_m value of 4 mM D-galactopyranosiduronate residues was reported⁵⁰; and for *Clostridium multifementans* pectinesterase, the apparent K_m was 2.5 mM methyl D-galactopyranosyluronate residues.⁴⁸ The last form of expression for K_m seems to be the most suitable.

The activity of pectinesterase is affected by pH, temperature, presence of salts and various inhibitors.

The original view that pectinesterases from higher plants differ from those of microbial origin in the pH optimum, which, for fungal pectinesterases, lies in the acid region,^{1,8,46} has only limited validity. All pectinesterases (thus far characterized) of higher plants have their pH optima in the region from pH 7 to 9, but, for some of the microbial pectinesterases, pH optima in the alkaline region were found too (see Table III). The value of pH optima >8 must be accepted with caution, because, at that pH, simultaneous alkaline (random) de-esterification of pectin begins to set in; a more suitable substrate for the action of pectinesterase is thereby formed, so that even the subtraction of values obtained by alkaline de-esterification without pectinesterase may not provide the true value for the origi-

TABLE III

pH Optima of Some Plant and Microbial Pectinesterases, and Concentrations of Na⁺ and Ca²⁺ Causing Maximum Activation

Origin	Purification	pH optimum	Optimal concentration		References
			NaCl (mM)	CaCl ₂ (mM)	
Plant					
Tomato	crude	7.0–7.8	100	n.d. ^a	58,63,64
	pure	7.5–8.5	50	5	50
	pure	8.0	100	5	62
Orange	crude	7.5	100	5	58,63
Apple	crude	7.0	n.d. ^a	n.d. ^a	63
Tobacco	crude	7.5	n.d. ^a	n.d. ^a	63
Citrus	pure	8.0	n.d. ^a	n.d. ^a	61
Alfalfa	crude	7.0	130	20	57,61
Banana	pure	6.0–9.5	n.d. ^a	20	65
Snap beans	crude	8.2	200	50	66
Bean hypocotyls	crude	7.0	100	n.d. ^a	67
Southern peas	crude	7.5–8.0	250	n.d. ^a	68
<i>Avena</i> coleoptile	crude	n.d. ^a	100	n.d. ^a	69
Papaya	crude	7.5	200	n.d. ^a	70
Turnip	crude	7.5–8.5	100	n.d. ^a	71
Microbial					
Pectinol (commercial preparation)	crude	4.3–5.0	100	n.d. ^a	63,72
<i>Aspergillus niger</i>	pure	4.5	n.d. ^a	n.d. ^a	73
<i>Monilia laxa</i>	pure	4.0	n.d. ^a	n.d. ^a	74
<i>Coniothyrium diploidiella</i>	pure	4.5–5.0	100	25	56
<i>Fusarium oxysporum</i>	crude	5.0	n.d. ^a	n.d. ^a	75
<i>Sclerotinia libertiana</i>	pure	4.8–5.2	150	5	76
<i>Rhizoctonia solani</i>	crude	6.0–7.0	no effect	n.d. ^a	67
<i>Diplodia gossypina</i>	crude	6.5	n.d. ^a	3	77
<i>Cercospora herpotrichoides</i>	crude	7.5	85	n.d. ^a	78
<i>Pellicularia filamentosa</i>	crude	7.0	n.d. ^a	n.d. ^a	79
<i>Acrocylindrium</i>	pure	7.0–7.5	100	10	80
<i>Pseudomonas prunicola</i>	crude	7.8	50	n.d. ^a	55
<i>Clostridium multifementans</i>	pure	9.0	50	n.d. ^a	51

^a n.d. = not determined.

(63) R. J. McColloch and Z. I. Kertesz, *Arch. Biochem.*, **13**, 217-229 (1947).

(64) H. R. Pithawala, G. R. Savur, and A. Sreenivasan, *Arch. Biochem.*, **17**, 235-248 (1948).

(65) H. O. Hultin and A. S. Levine, *Arch. Biochem. Biophys.*, **101**, 396-402 (1963).

(66) J. P. Van Buren, J. C. Moyer, and W. B. Robinson, *J. Food Sci.*, **27**, 291-294 (1962).

nal substrate. There is also a possibility of inactivation of some pectinesterases at pH values above 9 (see Ref. 51).

The presence of salts of univalent and bivalent cations increases, by several-fold, the activity of pectinesterases from higher plants, which is minimal in the absence of salts.^{38,50,57,60,63,64,66,69,70} The activating effect of salts on pectinesterases of microbial origin is not so great, an increase by 1.5- to 2-fold being reported.^{51,56,63,76-78,80} Table III shows the concentrations of sodium chloride and calcium chloride that caused the maximal activation of pectinesterases of plant and microbial origin. The mechanism of activation has not yet been satisfactorily explained.

Older papers report an unusual resistance of pectinesterases to chemical agents.⁶³ Diisopropyl fluorophosphate had a negligible inhibitory effect on microbial pectinesterase,⁵⁵ and with highly purified tomato pectinesterase, this or any other inhibitor of serine-type esterases had no effect, even at higher concentrations.⁸¹ In contrast with older data,⁶³ however, iodine showed an increasing inhibitory effect in parallel with the degree of purification of tomato pectinesterase.⁸¹ An inhibition of the action of pectinesterases was found to be caused by detergents, but here, fungal pectinesterase displayed a greater resistance than pectinesterases from higher plants.⁶³ The inhibition by anionic detergents (both fatty acid salts and alkyl sulfates) of tomato pectinesterase activity reached a maximum at the C₁₄ chain-length.⁸² Inactivation by dodecyl sodium sulfate was observed with banana pectinesterase,⁶⁵ and was used for examining the dif-

(67) D. F. Bateman, *Phytopathology*, **53**, 197-204 (1963).

(68) J. L. Collins, *J. Food Sci.*, **35**, 1-4 (1970).

(69) K. T. Glasziou, *Physiol. Plantarum*, **12**, 670-680 (1959).

(70) L. W. S. Chang, L. L. Morita, and H. Y. Yamamoto, *J. Food Sci.*, **30**, 218-222 (1965).

(71) F. Kiermeier, *Ann.*, **561**, 232-237 (1948).

(72) V. B. Fish and R. B. Dustman, *J. Amer. Chem. Soc.*, **67**, 1155-1157 (1945).

(73) A. Koller, Dissertation No. 3774, ETH, Zürich (1966).

(74) A. Slezárik and L. Rexová, *Biologie*, **22**, 407-413 (1967).

(75) P. E. Waggoner and A. E. Dimond, *Phytopathology*, **45**, 79-87 (1955).

(76) S. Oi and Y. Satomura, *Agr. Biol. Chem. (Tokyo)*, **29**, 936-942 (1965).

(77) C. Wang Sy-ying and J. A. Pinckard, *Phytopathology*, **61**, 1118-1124 (1971).

(78) C. Hänsler, G. Menke, and F. Grossmann, *Experientia*, **27**, 1022 (1971).

(79) K. R. Barker and J. C. Walker, *Phytopathology*, **52**, 1119-1125 (1962).

(80) H. Kimura, F. Uchino, and S. Mizushima, *Agr. Biol. Chem. (Tokyo)*, **37**, 1209-1210 (1973).

(81) O. Markovič and J. Patočka, unpublished results.

(82) G. J. Miller and R. J. McColloch, *Biochem. Biophys. Res. Commun.*, **1**, 91-93 (1959).

ferences between the individual forms of tomato pectinesterase.⁸³ The inhibitory effect of tannin on pectinesterases was also studied; a 1 mM concentration had no effect⁵⁵ on *Pseudomonas prunicola* enzyme, whereas a 50 μ M concentration caused 100% (reversible) inhibition of tomato pectinesterase.⁸⁴ The inhibitory effect of sucrose, glycerol, and D-glucose on papaya pectinesterase was characterized as noncompetitive,⁷⁰ and various fractions of banana pectinesterase displayed different sensitivities to the inhibitory effect of sucrose.⁸⁵

2. Occurrence and Formation

Pectinesterases have been found to be present in all the species of higher plants tested—in their fruits, leaves, stems, and roots. A substantial proportion of pectinesterase is bound to the cell wall and is released on treatment with salt solutions, as well as on shifting the pH toward the alkaline region.^{86–88} On the basis of diffusion of pectinesterase from the segments of *Avena* coleoptile, it was found that the enzyme is localized in the space that certainly includes the cell wall⁶⁹; this is in agreement with the findings of Jansen and co-workers⁸⁷ that the main part of pectinesterase from *Avena* coleoptile passes into the extraction medium immediately after the pH has been adjusted to 7.5. The pectinesterase is assumed to possess the same binding site as, for example, α -chymotrypsin, which is also bound by the cell wall of *Avena* coleoptile. The mechanism of binding of pectinesterase to the cell wall of *Avena* coleoptile is different from that to the cell wall of oranges,⁸⁶ where a binding of the enzyme–substrate type is assumed, and where pectinesterase is released only after de-esterification of the cell-wall pectin. Differences in the binding of these pectinesterases are apparently also associated with the content of pectic substances in cell walls (oranges contain 39% of pectic substances, whereas *Avena* coleoptiles contain only 5%).^{86,87} At least three types of binding site were assumed for the tomato cell-walls.⁸⁸ One was presumed to be specific for pectinesterase, another for β -D-fructofuranosidase, and the third, nonspecific, with a greater affinity for pectinesterase.

A number of plant pathogens, fungi, and bacteria are known to

(83) R. Pressey and J. K. Avants, *Phytochemistry*, **11**, 3139–3142 (1972).

(84) C. B. Hall, *Nature*, **312**, 717–718 (1966).

(85) H. O. Hultin, B. Sun, and J. Bulger, *J. Food Sci.*, **31**, 320–327 (1966).

(86) E. F. Jansen, R. Jang, and J. Bonner, *Food Res.*, **25**, 64–72 (1960).

(87) E. F. Jansen, R. Jang, and J. Bonner, *Plant Physiol.*, **35**, 567–574 (1960).

(88) H. Nakagawa, K. Sekiguchi, N. Ogura, and H. Takehana, *Agr. Biol. Chem.* (Tokyo), **35**, 301–307 (1971).

produce pectinesterase that passes into the culture medium or into the host's internal medium, together with other pectolytic and cellulolytic enzymes.^{37,49,56,67,74-80,89-95} Microbial pectinesterases are mostly inducible enzymes, their production being induced by the presence of pectin or pectic acid, and also by D-galactopyranuronic acid, galactaric acid, gum tragacanth,³⁷ and D-glucose,⁹⁶ as well as by O-(carboxymethyl)cellulose (CM-cellulose),⁹⁰ as the source of carbon in the culture medium.

3. Purification

Much attention has now been devoted to the purification and characterization of tomato pectinesterase, apparently because of the high content thereof in the ripe fruits.

Lee and Macmillan⁵⁰ obtained a highly purified pectinesterase from tomatoes (Heinz variety 135). After extraction with 5% sodium chloride solution, and fractional salting-out with ammonium sulfate, the main fraction of D-galacturonanase was adsorbed onto a calcium phosphate gel. Further separation on a column of DEAE-cellulose led to the removal of a substantial proportion of extraneous protein, and triple-recycling chromatography on a column of Sephadex G-75 (Superfine) resulted in a final product that contained only traces of D-galacturonanase. The product was characterized as homogeneous with respect to molecular size and sedimentation properties; its sedimentation coefficient ($s_{20,w}$) was estimated to be 3.08 S. On the basis of staining for lipids, as well as of the identification of lipids in the aqueous and ether phases after extraction of purified pectinesterase, a lipoprotein character was envisaged for tomato pectinesterase.

Almost simultaneously, a pectinesterase was isolated from tomatoes of the Immuna variety.⁹⁷ After extraction with 2% sodium chloride at pH 7.8, and fractional salting-out with ammonium sulfate, chromatography on DEAE-Sephadex A-50 removed a substantial proportion of colored contaminants and accompanying acid compo-

(89) J. Wallace, J. Kuc, and E. B. Williams, *Phytopathology*, **52**, 1004-1009 (1962).

(90) D. F. Bateman and S. V. Beer, *Phytopathology*, **55**, 204-211 (1965).

(91) D. A. Bush and R. C. Codner, *Phytochemistry*, **7**, 863-869 (1968).

(92) J. A. Meyer, E. D. Garber, and S. G. Shaeffer, *Botan. Gaz.*, **125**, 298-300 (1964).

(93) A. F. Perley and O. T. Page, *Can. J. Microbiol.*, **17**, 415-420 (1971).

(94) G. Weste, *Phytopathol. Z.*, **67**, 327-336 (1970).

(95) M. K. Abo-el-Dahab, *Phytopathology*, **54**, 597-601 (1964).

(96) R. Paquin and L. J. Coulombe, *Can. J. Botany*, **40**, 533-541 (1962).

(97) O. Markovič and A. Slezárik, *Collect. Czech. Chem. Commun.*, **34**, 3820-3825 (1969).

nents. Separation, on a column of Sephadex G-75, of the fraction having pectinesterase activity yielded 6 peaks, the third of which (having maximal pectinesterase activity) was further fractionated on a column of CM-Sephadex C-50 by using a linear concentration and a pH gradient of a phosphate-sodium chloride buffer. A freeze-drying step and desalting on Sephadex G-25 (Fine) were inserted after every purification step, and these treatments increased the purity of the final product. The homogeneity of the resulting pectinesterase was checked by disc electrophoresis. The sedimentation constant, the approximate molecular weight, and the amino acid content were determined. The value of the sedimentation coefficient was later corrected⁹⁸; under more suitable conditions, a value of 3.25 S was found, and the molecular weight determined by sedimentation equilibrium was 27,800.

By means of gel electrophoresis on cross-linked, hydrolyzed starch,⁹⁹ with simultaneous checking for proteins, lipids, and pectinesterase activity, it was found, however, that the product isolated after the separation on CM-Sephadex C-50 constitutes but one of five multiple forms of tomato pectinesterase, and is the one present in preponderant proportion⁹⁸ (see Fig. 4). The accompanying lipid and sugar components were separated from this pectinesterase form in the course of the purification procedure. After analysis of the hydrolyzate of the final product for fatty acids, as well as for carbohydrate components, it was possible to exclude the possibility of a lipoprotein,⁵⁰ as well as glycoprotein,¹⁰⁰ character of this form of tomato pectinesterase.

Delincée and Radola¹⁰⁰ used a commercial preparation, as well as fresh tomatoes, for the preparation, purification, and characterization of tomato pectinesterase. The tomatoes were pressed and then homogenized directly with ammonium sulfate at 70% saturation. The precipitate obtained was extracted with 0.3 M phosphate and repeatedly salted out with ammonium sulfate, and the product was separated on a column of Sephadex G-75. The pattern of separation was similar to that in preceding work.^{50,97} A detailed study of the size properties of pectinesterase was conducted by gel-filtration and sedimentation analysis.¹⁰⁰ By column and thin-layer gel-filtration on Sephadex G-75, the approximate molecular weight of a number of preparations of tomato pectinesterase was determined, values of 24,000 and 27,000 being obtained. A possible interaction of the

(98) O. Markovič, *Collect. Czech. Chem. Commun.*, **39**, 908-913 (1974).

(99) O. Markovič and Ľ. Kuniak, *J. Chromatogr.*, **91**, 873-875 (1974).

(100) H. Delincée and B. J. Radola, *Biochim. Biophys. Acta*, **214**, 178-189 (1970).

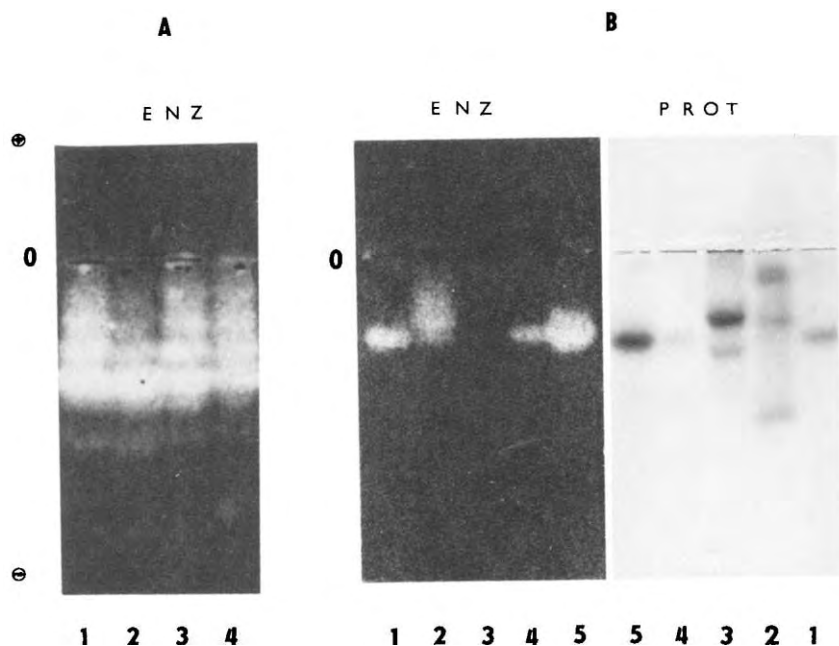


FIG. 4.—Monitoring of the Multiple Molecular Forms of Tomato Pectinesterase by Starch-gel Electrophoresis.⁹⁸ [ENZ, detection of pectinesterase activity by paper print with pectin and Bromothymol Blue; PROT, protein staining with nigrosin; O, origin. Key: A, 1: crude tomato extract after ammonium sulfate salting-out, and dialysis; 2: pectinesterase fraction from column of DEAE-Sephadex A-50; 3 and 4: pectinesterase fractions from column of Sephadex G-75. B, Two parts of the same gel after horizontal slicing: 1, 500 μ g of the isolated form of pectinesterase from a column of CM-Sephadex C-50 with 175 mM phosphate-sodium chloride buffer; 2, active fraction at 150 mM buffer; 4 and 5, 250 μ g and 1 mg of the isolated form of pectinesterase, respectively.]

enzyme with the dextran gel was precluded by using thin-layer chromatography on Bio-Gel P-60, by which the molecular weight of the tomato pectinesterase was estimated to be 28,000. Ultracentrifugation analysis of the enzyme gave a value of $s_{20,w}$ of 3.3 S, and the molecular weight determined by sedimentation equilibrium was 26,300. The frictional ratio was calculated to be 1.17, indicating relative molecular symmetry. By using thin-layer, isoelectric focusing on Sephadex G-75 with carrier ampholytes, the isoelectric point of tomato pectinesterase was found to be 8.4.

The pectinesterase from tomatoes of the Hikari variety^{62,101} was purified, after extraction with 0.25 M phosphate buffer of pH 8.0, by (101) H. Nakagawa, Y. Yanagawa, and H. Takehana, *Agr. Biol. Chem.* (Tokyo), 34, 991-997 (1970).

fractional salting-out with ammonium sulfate and subsequent separation on columns of DEAE-cellulose and Sephadex G-100. The resulting product was found to have a $s_{20,w}$ value of 3.17 S by sedimentation analysis. The composition of the amino acids was determined; in contrast with pectinesterase from tomatoes var. Immuna,⁹⁷ cystine, methionine, and histidine were not present.

Four forms of pectinesterase were found in different tomato varieties (Marion, Homestead, and Pixie)⁸³ by separation on columns of DEAE-Sephadex A-50, using elution with 150 mM sodium chloride at pH 6.0. By gel filtration on columns of Sephadex G-100, the molecular weights of the individual forms were found to be 22,000–27,000, and 35,000.

In a study of the pectinesterase from bananas,^{64,85,102} three pectinesterase fractions were obtained after respective extraction with water, 150 mM sodium chloride, and 150 mM sodium chloride of pH 7.5. The fractions obtained were further purified by fractional salting-out with ammonium sulfate, and chromatography on columns of DEAE- and CM-cellulose. A 50-fold purification was achieved, and the individual, purified fractions were characterized with respect to different effects of cations, inhibition by sucrose, and reaction kinetics.

By use of starch-gel electrophoresis, the total extract of bananas, and the fractions obtained after separation on DEAE-Sephadex A-50, were found to contain six multiple forms of pectinesterase having electrophoretic patterns different from those of tomato pectinesterase.¹⁰³

Relatively less attention has been devoted to the isolation of pectinesterase from oranges.^{35,36} Jansen and coworkers⁸⁷ described a purification of pectinesterase by adsorption on the cell walls of oranges; this adsorption is not specific, but serves to provide a 7.5-fold increase of specific activity.

In the purification of pectinesterase from the fruits of *Citrus natsudaidai*,⁶¹ fractional salting-out with ammonium sulfate was followed by chromatography on a column of DEAE-cellulose and by separation of the active fraction on Sephadex G-100. A preparation (purified solution) having a specific activity 460-fold greater than that of the original extract was obtained. Its homogeneity was checked by disc electrophoresis, and its amino acid content was determined and fundamental, kinetic data were obtained.

(102) H. O. Hultin and A. S. Levine, *J. Food Sci.*, **30**, 917–921 (1965).

(103) O. Markovič, K. Heinrichová, and B. Lenkey, *Collect. Czech. Chem. Commun.*, **40**, 769–774 (1975).

Among the microbial pectinesterases, the one from *Coniothyrium diplodiella*⁵⁶ was purified by repeated chromatography on columns of Duolite A-2 and DEAE-cellulose. Two electrophoretically homogeneous forms of pectinesterase were obtained, having an identical pH optimum and the same behavior towards pectins of different d.e.

The pectinesterase produced by *Sclerotinia libertiana*⁷⁶ was purified on columns of Duolite A-2, Amberlite CG-50, and CM-cellulose. The final product was purified 266-fold, its sedimentation coefficient was calculated to be 4.41 S, and zone electrophoresis in starch gel showed a slight contamination of this product.

By use of a crude preparation obtained after the cultivation of *Aspergillus niger*,¹⁰⁴ pectinesterase was purified by repeated chromatography on DEAE-cellulose, using gradient elution. The homogeneity of the product was checked by free electrophoresis, sedimentation analysis, and determination of the N-terminal amino acid (phenylalanine).

Macmillan and coworkers^{51,105,106} purified pectinesterase produced by *Clostridium multifementans* by using practically all of the available methods and materials (calcium phosphate gel, DEAE-cellulose, DEAE-, QEAE-, CM-, and SE-Sephadex, Sephadexes G-75, G-100, G-150, and G-200, Sepharose 4B, and zonal centrifugation). However, they could not separate pectinesterase from exopectate lyase, and, hence, they postulated that either (a) a complex of the two enzymes having an apparent molecular weight of 400,000 exists, or (b) the two enzymes are identical in their molecular species. On the basis of the mode of action of this pectinesterase in comparison with that of those from tomatoes and from *Fusarium oxysporum*, the existence of a complex of pectinesterase and exopectate lyase in *Clostridium multifementans* appears to be the more probable.

Miller and Macmillan⁴⁹ purified pectinesterase produced by *Fusarium oxysporum* f. sp. *vasinfectum* by chromatography on DEAE-Sephadex A-25, Sephadex G-75, CM-Sephadex C-50, and CM-cellulose. The homogeneity of the pectinesterase obtained was confirmed by disc electrophoresis; an apparent molecular weight of 35,000 was estimated by its behavior on a column of Sephadex G-75 (Superfine).

The pectinesterase produced by a strain of *Acrocyldrium*⁸⁰ was

(104) Ľ. Rexová-Benková and A. Slezárik, *Collect. Czech. Chem. Commun.*, **31**, 122-129 (1965).

(105) J. D. Macmillan and R. H. Vaughn, *Biochemistry*, **3**, 564-572 (1964).

(106) J. D. Macmillan, H. J. Phaff, and R. H. Vaughn, *Biochemistry*, **3**, 572-578 (1964).

purified by chromatography on Duolite CS-101 and on CM- and DEAE-cellulose. The product obtained showed no D-galacturonanase activity and moved in the ultracentrifuge as a single peak having a sedimentation coefficient of 5.4 S.

4. Assay

The original test for the presence of pectinesterase was the formation of gel after an enzymic de-esterification of pectin in the presence of Ca^{2+} ions. This behavior was observed by Frémy¹⁰⁷ more than 100 years ago, after he found the presence of "pectase" in carrots.

For the estimation of pectinesterase, Kertesz¹⁰⁸ applied the titration of enzymically liberated carboxyl groups in the presence of acid-base indicators. These indicators were later replaced by glass electrodes of a pH-meter, and continuous titration at constant pH was introduced.^{34,57} This method could be used when working in the region at pH ~ 7.0 . For pectinesterases having pH optima in the acid region, a modification of the titration method was developed⁷²; continuous titration at the pH optimum of the enzyme is used, and then the reaction mixture is quickly titrated to pH 7.0, which is the inflection point of the titration curves for pectin and for D-galactopyranuronic acid.

To determine the activity of pectinesterases having pH optima near 7.0, various modifications of continuous titration are now used (autotitrators with recording pH-meters, constant temperature vessels provided with magnetic stirring, and flushing with nitrogen).^{50,70,100}

The substrate commonly employed is 0.5–1.0% of pectin in 50–200 mM sodium chloride (20–60 ml) at a suitable pH and 30°. The pectinesterase activity is mostly expressed in micro-equivalents of ester hydrolyzed per minute at the given pH and temperature. The use of a variety of units of activity, which was complicated and prevented ready comparison of the results of various authors, has now been discontinued.

Methods utilizing the determination of enzymically released methanol, either after distillation,^{59,64} or directly in the reaction mixture,¹⁰⁹ just like the method of manometric determination of the carbon dioxide released from hydrogen carbonate buffer,⁵⁵ have not found

(107) E. Frémy, *Ann. Chim. Phys.*, **24**, 5–58 (1848).

(108) Z. I. Kertesz, *J. Biol. Chem.*, **121**, 589–597 (1937).

(109) P. J. Wood and I. R. Siddiqui, *Anal. Biochem.*, **39**, 418–428 (1971).

wide application; they lack the greatest advantage of titration methods, namely, their continuous character.

For a quick but informative determination of pectinesterase activity, a very advantageous method, based on the registration of change of pH during action of the enzyme in the region of pH 7.5–7.0, has been devised.¹¹⁰ In this region, the change of the reaction rate in relation to the pH is negligible, and the enzymic activity can be determined with an accuracy of $\pm 5\%$ within 2 minutes, with a consumption of only 3 ml of substrate solution.

During a study of the biosynthesis of pectin substances, a sensitive micromethod for the assay of pectinesterase activity was developed¹¹¹ that uses a biosynthetically prepared [¹⁴C]methyl-labelled pectin as the substrate; after enzymic de-esterification, the substrate remaining is precipitated with an excess of methanol and, after centrifugation, the [¹⁴C]methanol present in the supernatant liquor is counted in a liquid scintillation counter in order to assess the pectinesterase activity.

Another method is based on the same principle,¹¹² in which the [¹⁴C]labelled methyl ester of D-galacturonan is prepared by esterification of pectic acid with [¹⁴C]diazomethane. In the course of the enzymic de-esterification, aliquots are removed, and the unreacted substrate is precipitated with acidified ethanol or 1-propanol. After centrifugation, the labelled methanol in the supernatant liquor is determined in a liquid scintillation counter. An advantage of this method lies in the possibility of using, as substrates, short-chain oligo-D-galactosiduronates partially esterified with [¹⁴C]methanol. These substrates, beginning with the trisaccharide, are not soluble in 1:4 80% phenol–diethyl ether, which is used for the extraction of enzymically released, labelled methanol.

Qualitative tests for the presence of pectinesterase, based on a color change of such indicators as Bromothymol Blue or Bromocresol Green, after de-esterification of pectin, are simple and very useful; these tests are used as drop tests when checking chromatographic columns^{85,97,100} or for a qualitative assay of pectinesterase in culture filtrates, using the cup-plate method.¹¹³

To detect pectinesterase activity *in situ*, the print technique has

(110) L. P. Somogyi and R. Romani, *Anal. Biochem.*, **8**, 498–501 (1964).

(111) H. Kauss, A. L. Swanson, R. Arnold, and W. Odzuck, *Biochim. Biophys. Acta*, **192**, 55–61 (1969).

(112) Y. Milner and G. Avigad, *Anal. Biochem.*, **51**, 116–120 (1973).

(113) J. E. Dingle, W. W. Reid, and G. L. Solomons, *J. Sci. Food Agr.*, **4**, 149–155 (1953).

been used¹⁰⁰; this employs paper impregnated with pectin and Bromothymol Blue after chromatography on thin layers of Sephadex,^{100,103} as well as after starch-gel electrophoresis, when examining the multiple forms of pectinesterase from tomatoes^{98,99} and bananas.¹⁰³

V. D-GALACTURONANASES

1. Action Pattern and Specificity

D-Galacturonanases catalyze the hydrolytic cleavage of the glycosidic α -D-(1 \rightarrow 4) bonds of nonesterified D-galactopyranosiduronic residues, and hence, pectic acid and D-galacturonans having a low d.e. are the substrates of preference. Random splitting of internal bonds of the D-galacturonan chain, which is catalyzed by endo-D-galacturonanases, results in a pronounced diminution in the viscosity of a substrate solution at a low degree of splitting of glycosidic bonds. The primary reaction-products are higher oligo-D-galactosiduronates.

Although the decrease in viscosity and the liberation of oligo-D-galactosiduronates as reaction products are the features common to the activity of all endo-D-galacturonanases, the mode of action of these enzymes is not identical. Differences in the ratio of lowering of viscosity to the number of hydrolyzed glycosidic bonds observed for various endo-D-galacturonanases (in particular, when the same polymeric substrate is used) indicate differences in their mode of action. An even more pronounced difference is seen in the action pattern during the last phases of the reaction, when the enzyme attacks oligo-D-galactosiduronates formed in the course of the reaction. These differences are displayed not only by enzymes of different origin but even by enzymes produced by the same biological species.

For three endo-D-galacturonanases, isolated from the culture filtrate of *Coniothyrium diplodiella*,¹¹⁴⁻¹¹⁶ that differed in the extent of degradation of sodium pectate, a 50% decrease of viscosity of the substrate solution corresponded to the splitting of 3, 4, and 10% of the glycosidic bonds, respectively. A similar difference was found between two endo-D-galacturonanases isolated from tomatoes¹¹⁷; one

(114) A. Endo, *Agr. Biol. Chem.* (Tokyo), **28**, 551-558 (1964).

(115) A. Endo, *Agr. Biol. Chem.* (Tokyo), **28**, 535-542 (1964).

(116) A. Endo, *Agr. Biol. Chem.* (Tokyo), **28**, 543-550 (1964).

(117) R. Pressey and J. K. Avants, *Biochim. Biophys. Acta*, **309**, 363-369 (1973).

of the enzymes, having a molecular weight of 44,000, caused a sharp diminution of the viscosity of the substrate solution, typical for the random splitting of glycosidic bonds, whereas the other (molecular weight 84,000) caused a much slower decrease in viscosity at the same degree of degradation of the substrate, indicating the release of smaller products in the course of the whole reaction, but the degradation was not of the terminal-cleavage type.

An unusual course of degradation of high-molecular substrates was observed with the endo-D-galacturonanase produced by *Colletotrichum lindemuthianum*.¹¹⁸ A pronounced decrease in viscosity indicated a random splitting of glycosidic bonds, but, as the preponderant products throughout the reaction were D-galactopyranuronate and its dimer and trimer, it was assumed that the enzyme initially attacks substrate molecules randomly, and that the attack then progresses from that site along the chain, releasing the aforementioned reaction-products until it reaches a barrier, such as a branch point or a neutral sugar residue; then, the enzyme attacks another molecule.

The characterization of the end products of the degradation of high-molecular substrates, as well as experiments in which the effect of enzymes on oligomeric substrates was investigated, have contributed significantly to the elucidation of the mode of action of endo-D-galacturonanases.

From the commercial preparation Pectinex, Koller⁷³ separated two endo-D-galacturonanases differing in pH optimum and in the activity on lower oligo-D-galactosiduronates. Tetra(D-galactosiduronic acid) was the lowest oligomeric substrate hydrolyzed by the enzyme of pH optimum 5.5, whereas the other enzyme (pH optimum 4.0) degraded trisaccharide also. The activity of endo-D-galacturonanases isolated from the culture media of *Acrocyldrium*¹¹⁹ and *Aspergillus foetidus*¹²⁰ is restricted to substrates containing at least four D-galactopyranosiduronate residues. The monomer through the trimer are the end products of the reaction that is also catalyzed by the endo-D-galacturonanase isolated from a culture extract of *Aspergillus japonicus*,¹²¹ the extracellular enzyme of *Colletotrichum lindemuthianum*,¹¹⁸ and the endo-D-galacturonanase isolated from apples infected with *Penicillium expansum*.¹²²

(118) P. D. English, A. Maglothlin, K. Keegstra, and P. Albersheim, *Plant Physiol.*, **49**, 293–297 (1972).

(119) H. Kimura, F. Uchino, and S. Mizushima, *J. Gen. Microbiol.*, **74**, 127–137 (1973).

(120) J. Brooks and W. W. Reid, *Chem. Ind. (London)*, 325–326 (1955).

(121) S. Ishii and T. Yokotsuka, *Agr. Biol. Chem. (Tokyo)*, **36**, 1885–1893 (1972).

(122) T. R. Swinburne and M. E. Corden, *J. Gen. Microbiol.*, **55**, 75–87 (1969).

Many endo-D-galacturonanases are even able to degrade tri(D-galactosiduronic acid), for example, the enzymes produced by *Erwinia carotovora*,¹²³ *Aspergillus saitoi*,¹²⁴ *Geotrichum candidum*,¹²⁵ *Coniothyrium diplodiella*,^{115,116} and *Corticium rolsii*,¹²⁶ but some of the enzymes degrade trisaccharide at a much lower rate than higher oligosaccharides. Endo-D-galacturonanase from *Saccharomyces fragilis* degrades trisaccharide at about 1/50th of the rate for the tetrasaccharide.²⁷ The ratio of the degradation rates of tri- and tetrasaccharide by extracellular endo-D-galacturonanase of *Aspergillus niger* is 1:20 (Ref. 127). On the other hand, the difference in the rate of splitting of these two oligomers by a tomato endo-D-galacturonanase is very small.¹²⁸ None of the endo-D-galacturonanases described so far hydrolyze di(D-galactosiduronic acid).

The preferred substrates of all endo-D-galacturonanases are the high-molecular D-galacturonans. The rate of splitting of the glycosidic bonds decreases with the shortening of the substrate chain.^{27,127,129,130} During a study of the kinetics of splitting of D-galacturonans by endo-D-galacturonanase of *Saccharomyces fragilis*, Demain and Phaff²⁷ observed three rate-phases in the reaction. During the first, linear phase, polymeric molecules and higher oligo-D-galactosiduronates down to the pentasaccharide are split, until 25% of the glycosidic bonds are broken. In the second phase (1/44th as fast), tetrasaccharide is split to trisaccharide and D-galactopyranuronate, and in the last (extremely slow) phase, trisaccharide is degraded to dimer and monomer. The decrease in the rate of cleavage of the glycosidic bonds with the shortening of the substrate chain, the extremely slow degradation of tri(D-galactosiduronic acid), and the immunity of di(D-galactosiduronic acid) to the action of endo-D-galacturonanase were ascribed to a protective effect of one of the terminal units of D-galactopyranosiduronate in the substrate molecule.^{131,132} Demain and Phaff²⁷ attributed the protective effect to the terminal group at the nonreducing end, on the basis of the finding

(123) S. Nasuno and M. P. Starr, *J. Biol. Chem.*, **241**, 5298-5306 (1966).

(124) M. Yamasaki, T. Yasui, and K. Arima, *Agr. Biol. Chem. (Tokyo)*, **30**, 1119-1128 (1966).

(125) I. Barash and Z. Eyal, *Phytopathology*, **60**, 27-30 (1970).

(126) A. Kaji and T. Okada, *Arch. Biochem. Biophys.*, **131**, 203-209 (1969).

(127) Ľ. Rexová-Benková, *Eur. J. Biochem.*, **39**, 109-115 (1973).

(128) D. S. Patel and H. J. Phaff, *Food Res.*, **25**, 47-57 (1960).

(129) H. J. Phaff and A. L. Demain, *J. Biol. Chem.*, **218**, 875-884 (1956).

(130) B. S. Luh, S. J. Leonhard, and H. J. Phaff, *Food Res.*, **21**, 448-455 (1956).

(131) D. S. Patel and H. J. Phaff, *J. Biol. Chem.*, **234**, 237-241 (1959).

(132) P. J. Mill and R. Tuttobello, *Biochem. J.*, **79**, 57-64 (1961).

that, in low-molecular substrates, bonds in the vicinity of the reducing end are preferentially broken.

The difference in the effect of various endo-D-galacturonanases on oligomeric substrates [in particular, the difference in the rate of degradation of tri(D-galactosiduronic acid) and in the action pattern toward the tetrasaccharide] indicates, however, that it is not the substrate, but rather, the properties of the enzyme (in particular, the character of its active center) that constitute the determining factor.

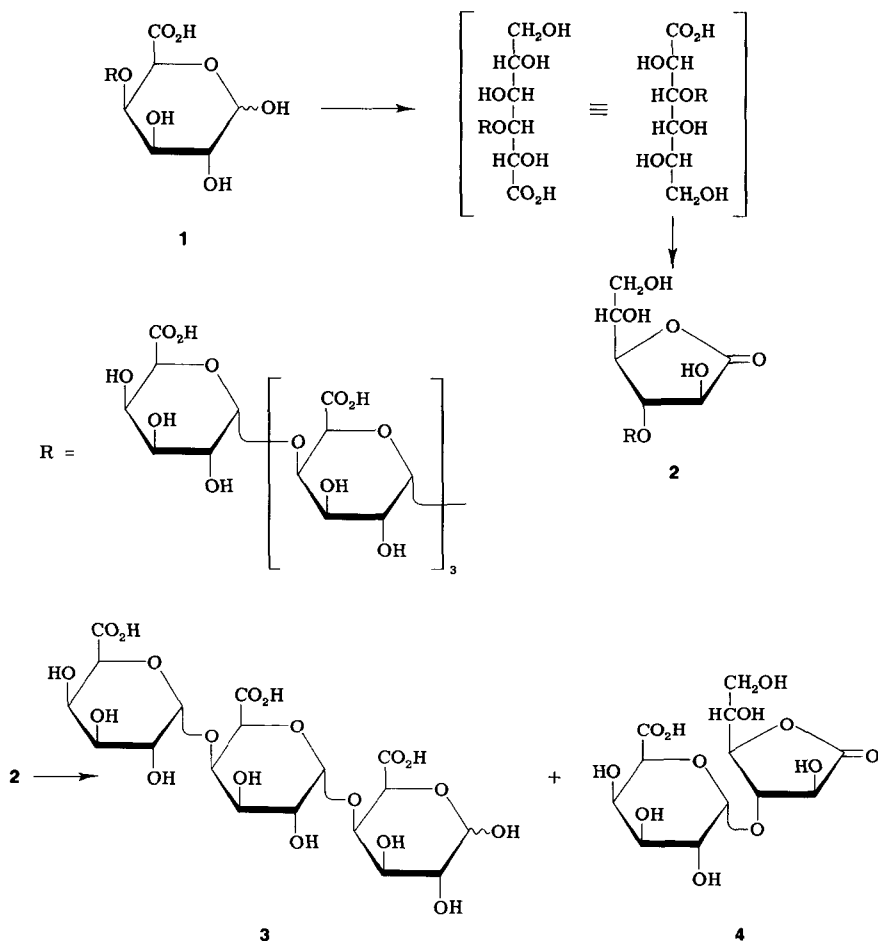
Three different patterns of action towards low-molecular substrates are known with various endo-D-galacturonanases (see Scheme 1).

SCHEME 1
Action Patterns of Endo-D-galacturonanases^a

Action pattern	E-S Complexing
(A)	
(B)	
(C)	

^a Key: (GalpA), ● = D-galactopyranosiduronic acid unit; E = enzyme; S = substrate; U = binding subsite; ↑ = catalytic site.

Action pattern A occurs for endo-D-galacturonanases of *Saccharomyces fragilis*,^{27,131} *Aspergillus niger*,^{127,132,133} and *Acrocylin-drium*.¹¹⁹ This type of degradation is characterized by the specific cleavage of tetra(D-galactosiduronic acid) to trisaccharide and D-galactopyranuronate, and by extremely slow splitting,^{27,127} or complete inability to degrade the trisaccharide.^{119,133} The tetra(D-galactosiduronic acid) 3-glycoside (2) of L-galactonolactone [obtained by reduction of the reducing end of penta(D-galactosiduronic



acid) (1)] is degraded specifically to tri(D-galactosiduronic acid) (3) and a nonreducing derivative (4) of di(D-galactosiduronic acid).^{27,127}

(133) A. Koller and H. Neukom, *Eur. J. Biochem.*, **7**, 485-489 (1969).

The tri(D-galactosiduronic acid) 3-glycoside of L-galactonolactone is resistant to the activity of these enzymes. Penta(D-galactosiduronic acid) (1) whose reducing-end unit had been oxidized by periodate was split by the enzyme of *Acrocylindrium* to oxidized dimer and tri(D-galactosiduronic acid) (3).¹¹⁹

The activity of tomato endo-D-galacturonanase follows action pattern B (see Scheme 1), which is characterized by an alternative cleavage of tetra(D-galactosiduronic acid) and by a relatively rapid degradation of trisaccharide.¹²⁸ In the tetrasaccharide, bond 1 is split* faster than bond 2. Bond 3 is not broken by the enzyme. Both the reduced and oxidized tetra(D-galactosiduronic acid) derivatives are hydrolyzed by this enzyme at bond 2.

Action pattern C (see Scheme 1) was observed with the extracellular endo-D-galacturonanase produced by *Erwinia carotovora*.¹²³ It differs from the first two types in a specific hydrolysis of penta(D-galactosiduronic acid) (1) to trisaccharide and disaccharide, and in having only two alternative ways for cleavage of hexasaccharide. Both bonds are split in the trisaccharide 3. The pentasaccharide is split five times faster than the tetrasaccharide.

The action pattern and the specificity of enzymes acting on homopolymeric substrates are determined by the nature of the active center.¹³⁴⁻¹³⁷ Hence, it may be assumed that the active centers of enzymes exhibiting the aforementioned action-patterns are different.

By use of the extracellular endo-D-galacturonanase of *Aspergillus niger*, the action pattern and the kinetics with oligomeric substrates (disaccharide through hexasaccharide) and their reduced derivatives were investigated, and the results were interpreted with respect to the size of the substrate binding-site.¹²⁷ This enzyme is characterized by action-pattern A, with an extremely slow degradation of tri(D-galactosiduronic acid) (3). It was found that the trisaccharide 3 acts as a competitive inhibitor of the degradation of higher oligomeric substrates. The disaccharide does not serve as substrate, nor does it cause inhibition. The kinetic constants V and K_m change with the substrate chain-length; the K_m values tend to decrease, whereas the V values tend to increase with increasing chain-length of the substrate. The greatest difference in V values was found between the

* The bonds are numbered from the reducing end towards the nonreducing one.

(134) J. A. Thoma, C. Brothers, and J. Spradlin, *Biochemistry*, **9**, 1768-1775 (1970).

(135) J. Robyt and D. French, *Arch. Biochem. Biophys.*, **100**, 451-467 (1963).

(136) Y. Nitta, M. Misushima, K. Hiromi, and S. Ono, *J. Biochem.* (Tokyo), **69**, 567-576 (1971).

(137) J. F. Robyt and D. French, *J. Biol. Chem.*, **245**, 3917-3927 (1970).

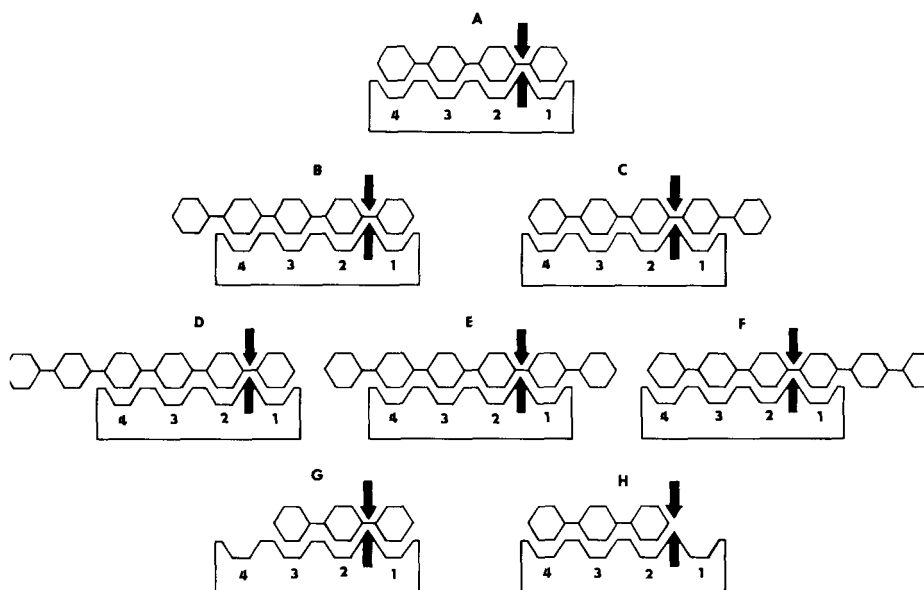


FIG. 5.—Schematic Illustration of the Enzyme-Substrate Complexes of *A. niger* Endo-D-galacturonanase with Oligogalacturonic Acids.¹²⁷ [A, tetra(D-galactosiduronic acid) complex; B and C, penta(D-galactosiduronic acid) complexes; D, E, and F, hexa(D-galactosiduronic acid) complexes; G, tri(D-galactosiduronic acid) productive complex; H, tri(D-galactosiduronic acid) nonproductive complex; 1-4, binding subsites; \bigcirc , D-galactopyranosiduronic acid units; —, glycosidic α -D-(1 \rightarrow 4) bonds; \downarrow catalytic groups of endo-D-galacturonanase.]

trisaccharide 3 and the tetrasaccharide. On the basis of (a) the action pattern, (b) the number of productive complexes, and (c) the difference in the values of catalytic constants for trisaccharide and tetrasaccharide, enzyme-substrate complexing was assumed as schematically illustrated in Fig. 5. A tendency of the substrate to occupy as much as possible of the binding site, and the absolute specificity for the hydrolysis of tetra(D-galactosiduronic acid) as a consequence of the unique way of combination with the binding site of the enzyme (A), were also taken into consideration.

According to this model, the active site of *Aspergillus niger* endo-D-galacturonanase is composed of four subsites, and the catalytic groups are situated between subsites 1 and 2. With penta(D-galactosiduronic acid) (B and C) and hexa(D-galactosiduronic acid) (D, E, and F), two and three productive complexes occur, respectively. With tri(D-galactosiduronic acid), two ways of interaction with the binding site of the enzyme are presumed (G and H). The interaction with subsites 1-3 leads to a productive complex, whereas the alterna-

tive complexing with subsites 2–4 leads to a nonproductive complex (H) in which the substrate does not interact with the catalytic groups of the enzyme, but blocks a great part of the binding site and, therefore, acts as a competitive inhibitor. With high-molecular substrates, the formation of a complex involving any of the segments of the four D-galactopyranosiduronate residues may be assumed; this results in a random splitting of the substrate molecule.

The number of productive complexes with individual oligomeric substrates given by the expression $(n - m + 1)$ (see Ref. 136) is also in accordance with the concept of a binding-site capable of interacting with four D-galactopyranosiduronate residues.

The orientation of the substrate molecule in the complex with the enzyme was characterized on the basis of the action pattern for the tetra(D-galactosiduronic acid) 3-glycoside (2) of L-galactonolactone. The specific cleavage of bond 2 indicates that the substrate is oriented in such a way that the D-galactopyranosiduronate residue at the reducing end of the segment forming the complex is bound at subsite 1.

According to this model of the active site, the catalytic groups are located eccentrically, similarly to the situation for *Bacillus subtilis* alpha-amylase.¹³⁵ According to Robyt and French,¹³⁷ this arrangement of the active center ensures a firmer binding of the residues at the C-1 side of the glycosidic bond broken, and so facilitates distortion of the conformation of the reacting sugar residue toward a half-chair conformation, which is typical for the SN2 mechanism of acid-catalyzed cleavage of glycosidic bonds proceeding with retention of configuration.

With the endo-D-galacturonanase of *Aspergillus niger*, another locus of contact with the substrate is envisaged on the basis of kinetic values and frequency of splitting of glycosidic bonds of hexa- to octa-saccharide (and of their reduced derivatives), the locus taking part in the interaction of the enzyme with substrates of higher d.p. This locus is separated from the "primary" binding site, and apparently binds the sixth D-galactopyranosiduronate residue, counting from the broken glycosidic bond towards the nonreducing end.¹³⁸

A similar interpretation of action patterns B and C (Scheme 1), and of the number of productive complexes, indicates that, in the corresponding enzymes, there is a binding site composed of three (B) or five (C) subsites having the catalytic groups situated between the first and second (B) and the second and third (C) subsites. Another indication of a binding site containing three subsites for enzymes of

(138) Ľ. Rexová-Benková, *Abstr. Commun., Meeting FEBS, 9th, Budapest, 1974*, 75.

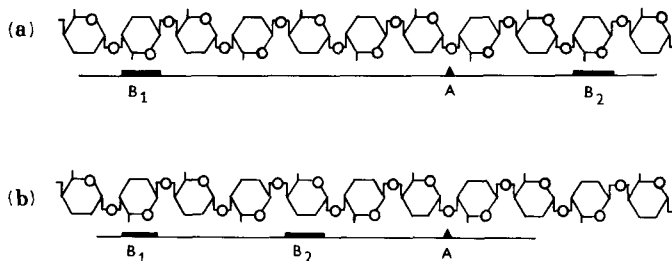


FIG. 6.—Schematic Illustration of Two Possibilities for Enzyme-Substrate Complexes of *A. niger* Endo-D-galacturonanase.¹³³ [B₁ and B₂, binding sites; A, active groups of endo-D-galacturonanase.]

the B type is the ability to form productive complexes with oxidized and reduced derivatives of tetra(D-galactosiduronic acid) observed for tomato endo-D-galacturonanase, resulting in splitting of bond 2 (Ref. 128).

On the basis of accumulation of trisaccharide and hexasaccharide as preponderant products of the cleavage of D-galacturonans, Koller and Neukom^{73,133} also considered the possibility that, in endo-D-galacturonanase of *Aspergillus niger* purified from the commercial preparation Pectinex, the binding groups for substrate are separated from the catalytic center by a distance of three and six D-galactopyranosiduronate units, respectively (see Fig. 6). As the degradation of substrate was directly proportional to the content of free carboxyl groups, they suggested that the substrate is attached to the binding site through carboxyl groups.

A three-unit segment adjacent to the glycosidic bond that is split was considered by Kimura and coworkers¹¹⁹ to be essential for the action of the endo-D-galacturonanase from *Acrocyllindrium*.

On the basis of the effect of pH on the activity of *Aspergillus niger* endo-D-galacturonanase, Koller⁷³ considered that essential groups for the catalytic reaction are the imidazole group of the histidine and the carboxyl group of the glutamic or aspartic acid residues of the enzyme, or alternatively, the carboxyl group of the D-galactopyranosiduronic acid residues of the substrate; however, no direct evidence for the role of these groups in the enzyme reaction was offered. Koller⁷³ further rejected the view of Solms and Deuel¹³⁹ as to the function of the secondary alcoholic groups at C-2 and C-3 in the formation of the enzyme-substrate complex by showing that acetylation of 70% of these groups had no pronounced effect on the rate of the enzyme reaction.

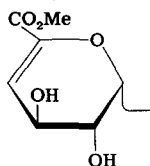
(139) J. Solms and H. Deuel, *Helv. Chim. Acta*, **34**, 2242-2249 (1951).

For *Aspergillus niger* extracellular endo-D-galacturonanase, the role of histidine in the enzyme reaction was investigated by the method of photo-oxidative inactivation, catalyzed by Methylene Blue.¹⁴⁰ The inactivation of the enzyme was paralleled by the decomposition of histidine. The similarity of pH profiles, as well as the values of the rate constants of enzyme inactivation ($4.0 \times 10^{-2} \text{ min}^{-1}$) and of decomposition of histidine ($3.9 \times 10^{-2} \text{ min}^{-1}$), indicate that one of the five histidine residues present in the molecule of the enzyme¹⁴¹ is essential for its activity.

A role of the carboxyl group of the substrate and the amino group of the enzyme in the formation of the enzyme-substrate complex was assumed by Nyeste and coworkers¹⁴² on the basis of an effect of blocking the amino groups of the D-galacturonanase of *Botrytis cinerea*.

Exo-D-galacturonanases catalyze the hydrolytic cleavage of the terminal α -D-(1 \rightarrow 4) bonds of D-galacturonan chains, releasing D-galactopyranuronic acid as the product. In contrast with endo-D-galacturonanases, exo-D-galacturonanases also split the di(D-galactosiduronate), which is thus a suitable substrate for differentiation of the activity of these two enzymes. With high-molecular substrates, the terminal action-pattern is displayed by a substantial rise in the reducing groups, accompanied by a slow decrease in the viscosity of the substrate solution. A 50% decrease in viscosity corresponds to the cleavage of approximately 30–45% of the glycosidic bonds.^{143–145} Splitting of the bonds may proceed from the reducing, or from the nonreducing, end of the substrate molecule.

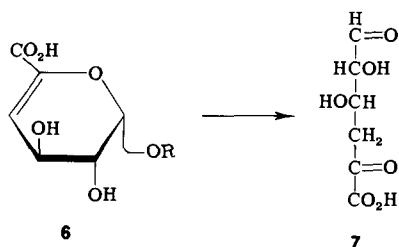
Plant and microbial exo-D-galacturonanases catalyze the splitting of glycosidic bonds, starting at the nonreducing end. These enzymes do not attack substrates containing, at the nonreducing end, a 4-deoxy-L-*threo*-hex-4-enopyranosyluronate group (5) (4,5-unsaturated



5

- (140) Ľ. Rexová-Benková and A. Slezárik, *Collect. Czech. Chem. Commun.*, **35**, 1255–1260 (1970).
- (141) Ľ. Rexová-Benková and A. Slezárik, *Collect. Czech. Chem. Commun.*, **33**, 1965–1967 (1968).
- (142) L. Nyeste, J. Holló, and S. Juhasz, *Nahrung*, **8**, 373–382 (1964).
- (143) H. Kimura and S. Mizushima, *Agr. Biol. Chem. (Tokyo)*, **37**, 2589–2593 (1973).
- (144) A. Endo, *Agr. Biol. Chem. (Tokyo)*, **28**, 639–645 (1964).
- (145) I. Barash and E. Angel, *Israel J. Botany*, **19**, 599–608 (1970).

“D-galacto”pyranosyluronate group)** formed by the alkaline β -eliminative cleavage or by the action of lyases.^{15,146,147} Exceptions are the enzyme isolated by Hasegawa and Nagel¹⁴⁸ from the cell extract of an aerobic *Bacillus* strain and the exo-D-galacturonanase isolated from an extract of *Aspergillus niger* mycelium¹⁴⁹⁻¹⁵¹ which also split bonds adjacent to the unsaturated terminal group of 6, releasing 4-deoxy-L-threo-hexos-5-ulosuronic acid (7) which is formed from 4,5-



unsaturated hexopyranuronate.¹⁵⁰ Reduction or oxidation of the residue at the reducing end of the polymeric substrate has no effect on the course and rate of degradation.¹⁵² With oligomeric substrates, however, this modification results in a diminution of the rate of substrate degradation and in immunity of the bond adjacent to the modified residue towards the action of exo-D-galacturonanase.¹⁴³

Exo-D-galacturonanases do not degrade pectic acid completely,^{147,153,154} their activity being restricted to the α -D-(1 \rightarrow 4) bonds between the D-galactopyranosiduronate residues. Older data indicating 100% degradation of pectic acid by fungal exo-D-galacturonanase^{155,156} are attributable to an insufficiently purified en-

** All oligosaccharides containing this group (5) at their nonreducing end will be termed “unsaturated.”

(146) C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.*, **13**, 161-174 (1966).

(147) C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.*, **14**, 197-201 (1969).

(148) S. Hasegawa and C. W. Nagel, *Nature*, **213**, 207-208 (1967).

(149) C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.*, **13**, 175-183 (1966).

(150) C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.*, **15**, 1-13 (1969).

(151) C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.*, **15**, 15-23 (1969).

(152) R. Pressey and J. K. Avants, *Plant Physiol.*, **52**, 252-256 (1973).

(153) P. J. Mill, *Biochem. J.*, **99**, 562-565 (1966).

(154) P. J. Mill, *Biochem. J.*, **99**, 557-561 (1966).

(155) A. Ayres, J. Dingle, A. Phipp, W. W. Reid, and G. L. Solomons, *Nature*, **170**, 834-836 (1952).

(156) H. Saito, *Nippon Nogei Kagaku Kaishi*, **28**, 869 (1954).

zyme preparation (contaminated with endo-D-galacturonanase). The degree of degradation varies over a wide range, depending on the source of the pectin substrate. Exo-D-galacturonanase from carrot degraded the pectate from bast ramie, orange peel, carrot root, citrus, bast hemp, and peach flesh by 56.1, 52.1, 51.3, 48.9, 12.7, and 6.7%, respectively.¹⁵⁷

The activity of some enzymes having the terminal-action pattern depends on the d.p. of the substrate. Exo-D-galacturonanase isolated from the carrot splits native pectate more slowly than a partially degraded substrate.^{152,157} A more pronounced difference was observed with the mercury-dependent exo-D-galacturonanase purified from an extract of *Aspergillus niger* mycelium, for which the ratio of degradation rates of pectate and di(D-galactosiduronic acid) was 1:85 (Ref. 154). With another exo-D-galacturonanase isolated from the same source, the substrates were split at the rate ratio of 1:11 (Ref. 153). Hatanaka and Ozawa¹⁵¹ reported an *Aspergillus niger* enzyme that splits substrates having the unsaturated unit at the nonreducing end, the ratios of splitting rates for D-galacturonan, di(D-galactosiduronic acid), and its unsaturated analogue being 1:6.3:0.09. The rate of splitting of oligomeric substrates by the *Bacillus* sp. enzyme decreased in the following order of the d.p.: $3 > 4 > 5 > 2 >$ acid-soluble pectic acid.¹⁵ Exo-D-galacturonanase purified from the extract of *Acrocylindrium* mycelium splits the disaccharide only 3.5 times faster than the polymeric substrate, and the values of *V* for the two substrates were very similar.¹⁴³ For exo-D-galacturonanase formed *in vivo* by *Penicillium digitatum*, the chain length did not determine the rate of substrate cleavage,¹⁴⁵ at a d.p. of 2, 3, 12.7, or 112. Enzymes clearly preferring low-molecular substrates^{148,152-154} are classified as oligo-D-galactosiduronate hydrolases.

In none of the cases mentioned were substrates that were polymerized to different degrees used at equimolar concentrations, so that, in some cases, it is not clear to what extent the differences in the rate of cleavage reflect the effective concentration of the terminal bonds (as assumed by Mill¹⁵⁴) and to what extent they reflect the differences in the enzyme reaction-mechanism. More satisfactory information would be obtainable by comparing the values of the maximum velocities.

It appears that the degradation of D-galacturonans by exo-D-galacturonanase proceeds by a multi-chain mechanism. Pressey and Avants¹⁵² investigated the course of degradation of pectic acid by peach exo-D-galacturonanase by using gel chromatography on Seph-

(157) C. Hatanaka and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **28**, 627-632 (1964).

adex G-100. As the degradation progressed, the substrate peak diminished, and was shifted towards higher elution volumes. This indicates that the enzyme does not remain associated with the first substrate molecule, but that it splits different molecules continuously. The results of Mill¹⁵⁴ are in agreement with this statement; he examined, by paper chromatography, the course of cleavage of tri(D-galactosiduronic acid) by the enzyme from *Aspergillus niger* mycelium.

Using a cell extract of *Erwinia aroideae*, Hatanaka and Ozawa^{13,158} partly purified an enzyme having a terminal-action pattern that splits penultimate glycosidic bonds of D-galacturonan with the release of di(D-galactosiduronic acid) as the sole reaction-product. Replacement of the D-galactopyranosyluronate group at the nonreducing end of the substrate molecule by its 4,5-unsaturated analogue resulted in a decrease of the rate of cleavage of substrate. The unsaturated trisaccharide was split to D-galactopyranuronate and unsaturated dimer, which indicates that the splitting of the glycosidic bonds proceeds from the nonreducing end of the substrate molecule.

All the known plant and microbial exo-D-galacturonanases degrade the substrate starting from the nonreducing end of the molecule, and the rate of splitting of the glycosidic bonds is either independent of, or inversely proportional to, the d.p. of the substrate. The contrary is true for the exo-D-galacturonanase found in the gastrointestinal tract of some species of plant pathogenic insects. Courtois and coworkers, working with *Pyrrhocoris apterus*¹⁵⁹ and with *Rhagium inquisitor*,¹⁶⁰ found enzymes having the terminal-action pattern, the activities of which were proportional to the d.p. of the substrates. The inability of the enzyme from both sources to degrade substrates modified at the reducing end of the molecule indicates that the degradation starts at the reducing end.

In 1952, Seegmiller and Jansen¹⁶¹ described an enzyme capable of degrading highly esterified D-galacturonans by the endo-action pattern, although it was inactive toward de-esterified substrates. In the classification of Demain and Phaff¹² and Neukom,⁵ the enzymes having this substrate specificity were designated as polymethylgalacturonases. After the discovery of pectin lyase by Deuel and coworkers,³ it became apparent that, for most enzymes specifically

(158) C. Hatanaka and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **33**, 116–118 (1969).

(159) J. E. Courtois, F. Percheron, and M. J. Foglietti, *Compt. Rend.*, **266**, 164–166 (1968).

(160) M. J. Foglietti, V. Levaditou, J. E. Courtois, and C. Chararas, *Compt. Rend.*, **165**, 1019–1022 (1971).

(161) C. G. Seegmiller and E. F. Jansen, *J. Biol. Chem.*, **195**, 327–336 (1952).

degrading highly esterified substrates, the mechanism of β -elimination is operative. Therefore, the actual existence of "polymethylgalacturonase" is now doubted.^{7,162,163}

Only a few enzymes have been discovered that do not degrade the highly esterified substrates by the β -eliminative mechanism. Koller⁷² used chromatography on cellulose phosphate to separate, from the commercial preparation Pectinex, a fraction that degraded 95% esterified pectin at pH 4 and 7. D-Galacturonanase and pectinesterase also present in the fraction were inactivated by heat. In the course of the degradation, there was no increase of absorbance at 235 nm typical of β -eliminative cleavage, and, hence, Koller characterized the enzyme as a polymethylgalacturonase.

An enzyme having its pH optimum at 7 and the same activity was isolated from the filtrate of a surface culture of *Aspergillus niger*.¹⁰⁴ The homogeneity of the preparation was verified by free electrophoresis, ultracentrifugation, and determination of lysine as the N-terminal amino acid. In the course of degradation of pectin, 96.8% esterified, there was no increase of absorbance at 235 nm. As with commercial polymethylgalacturonase, this enzyme was more heat-stable than endo-D-galacturonanase; after heating for 4 h at 50°, some 54% of the original activity was retained.¹⁶⁴

Tani¹⁶⁵ described a hydrolase produced by *Glousporium kaki hori* which degraded highly esterified pectin at pH 4.5, the degradation products showing no absorbance at 235 nm.

In all three examples, β -elimination was monitored by measuring the absorbance at 235 nm (with high-molecular substrates). Voragen¹⁶⁶ pointed out that use of these substrates, which, at higher concentrations, can form viscous, optically dense solutions, could prevent the measurement of absorbance at 235 nm and thus cause an error in enzyme classification. The existence of polymethylgalacturonase is not definite, and revision of some of the results may be necessary.

Generally, D-galacturonanases resemble other glycan hydrolases in having their pH optimum in a weakly acidic region. The pH optimum of most endo-(as well as exo-)D-galacturonanases lies between pH 4.0 and 6.5 (Refs. 16, 73, 115, 116, 119, 121, 124, 143,

(162) F. M. Rombouts, Doctoral Thesis, Wageningen (1972).

(163) W. Pilnik, F. M. Rombouts, and A. G. J. Voragen, *Chem. Mikrobiol. Technol. Lebensm.*, **2**, 122–128 (1973).

(164) Ľ. Rexová-Benková, *Collect. Czech. Chem. Commun.*, **32**, 4504–4509 (1967).

(165) T. Tani, *Proc. Conf. Gamagori, Japan*, 40–45 (1967).

(166) A. G. J. Voragen, Doctoral Thesis, Wageningen (1972).

153, 157, 167, and 168). An exception is the unusually acid-stable endo-D-galacturonanase purified from the culture filtrate of *Corticium rolfsii*, which is active between pH 1.5 and 3.0 (Ref. 169), the optimum lying at pH 2.5 (Ref. 126). With most endo-D-galacturonanases, the pH optimum was found to depend on the d.p. of the substrate. With decreasing d.p., the pH optimum of these enzymes was shifted towards the acidic side. Yeast endo-D-galacturonanase shows the highest activity with high-molecular substrates at pH 4.4, whereas, with tri- and tetra-saccharide, the reaction proceeds most rapidly at pH 3.5 (Ref. 27). A similar shift of the pH optimum was found with the extracellular endo-D-galacturonanases of *Geotrichum candidum*¹²⁵ and *Aspergillus niger*,¹³² and with an endo-D-galacturonanase purified from peaches.¹⁵²

With endo-D-galacturonanases isolated from tomatoes, the pH optimum is influenced not only by the substrate size but also by the presence of sodium chloride.^{117,170} Pressey and Avants¹⁷⁰ examined the effect of sodium chloride on the dependence between pH and activity, using substrates having different d. p. over the range of molecular weight from 1,400 to 1,000,000. In the absence of sodium chloride, native pectic acid was hydrolyzed optimally at pH 5.0. For substrates having average molecular weights of 11,000, 9,000, and 3,200, the pH optima were 4.5, 4.3, and 4.2, respectively. With all substrates, addition of sodium chloride to the reaction medium resulted in an increase in activity and a shift of the pH optimum to lower pH values. The activating effect was most pronounced at low pH values. A similar effect on the pH optimum was shown by cesium chloride (CsCl) and sodium nitrate. One of the reasons for the higher pH optimum with high-molecular substrates is, according to Pressey and Avants,¹⁷⁰ an aggregation of these substrates at low pH values as a consequence of the formation of hydrogen bonds between the macromolecules. The shift of the pH optimum towards the acidic side after addition of salts is attributed to a disruption of these bonds. Low-molecular substrates are soluble and do not associate at low pH.

2. Occurrence and Formation

Endo-D-galacturonanases and exo-D-galacturonanases are produced by most plant pathogens, such saprophytic fungi as *As-*

(167) A. H. Fielding and R. J. W. Byrde, *J. Gen. Microbiol.*, **58**, 73-84 (1969).

(168) M. C. Wang and N. T. Keen, *Arch. Biochem. Biophys.*, **141**, 749-757 (1971).

(169) A. Kaji and T. Ohsaki, *Nippon Nogei Kagaku Kaishi*, **45**, 520-528 (1971).

(170) R. Pressey and J. K. Avants, *J. Food Sci.*, **36**, 486-489 (1971).

pergillus,^{121,154,171,172} *Penicillium*,^{122,145,173-175} *Monilia*,⁷⁴ *Rhizopus*,¹⁷⁶ *Sclerotinia*,¹⁶⁷ *Coniothyrium*,¹¹⁴ some bacteria,^{123,158,177-181} and some yeasts.^{27,182} They may occur, either separately or together, in several plant organs (fruits, stems, leaves). In fruits, they play an important role in the ripening process.¹⁸³⁻¹⁸⁶ Courtois and coworkers found D-galacturonanase activity in the digestive tract of some insect species parasitic on forest trees,¹⁸⁷ poplars,^{188,189} conifers,¹⁹⁰⁻¹⁹² and eucalypti.¹⁹³ Supposedly, the enzymes play a role in nutrition, and in the boring of passages by the insect under the bark, where the saliva-excreted D-galacturonanase facilitates the decomposition of pectin in the intercellular spaces of the wood.^{188,189,194}

- (171) M. Yamasaki, T. Yasui, and K. Arima, *Agr. Biol. Chem.* (Tokyo), **30**, 142-148 (1966).
- (172) R. Tuttobello and P. J. Mill, *Biochem. J.*, **79**, 51-57 (1961).
- (173) H. A. Melonk and C. E. Homer, *Can. J. Microbiol.*, **18**, 1065-1072 (1972).
- (174) E. D. Garber, *Phytopathology*, **59**, 147-152 (1967).
- (175) E. D. Garber and L. Beraha, *Can. J. Botany*, **44**, 1645-1650 (1966).
- (176) R. A. Cappellini, *Phytopathology*, **56**, 734-737 (1966).
- (177) K. Okamoto, C. Hatanaka, and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **27**, 596-597 (1963).
- (178) J. Preiss and G. Ashwell, *J. Biol. Chem.*, **238**, 1571-1576 (1963).
- (179) C. W. Nagel and R. H. Vaughn, *Arch. Biochem. Biophys.*, **94**, 328-332 (1961).
- (180) M. H. Bilimoria, *J. Indian Inst. Sci.*, **48**, 53-64 (1966).
- (181) S. Nasuno and M. P. Starr, *Phytopathology*, **56**, 1414-1415 (1966).
- (182) A. Thuillier, C. Chararas, and J. E. Courtois, *Compt. Rend.*, **262**, 2757-2759 (1966).
- (183) G. E. Hobson, *Biochem. J.*, **92**, 324-332 (1964).
- (184) S. Hasegawa, V. P. Maier, H. P. Kaszycki, and J. K. Crawford, *J. Food Sci.*, **34**, 527-529 (1969).
- (185) R. Pressey, D. M. Hinton, and J. K. Avants, *J. Food Sci.*, **36**, 1070-1073 (1971).
- (186) D. Raymond and H. J. Phaff, *J. Food Sci.*, **30**, 266-273 (1965).
- (187) J. E. Courtois, C. Chararas, M. M. Debris, and H. Laurant-Hubé, *Bull. Soc. Chim. Biol.*, **47**, 2219-2231 (1965).
- (188) C. Chararas, J. E. Courtois, and H. Laurant-Hubé, *Ann. Pharm. Fr.*, **25**, 257-264 (1967).
- (189) J. E. Courtois, C. Chararas, M. M. Debris, and H. Laurant-Hubé, *Symp. Intern. Chim. Biochim. Lignine, Cellulose, Hemicelluloses*, Grenoble (1964).
- (190) J. E. Courtois, C. Chararas, and M. M. Debris, *Compt. Rend.*, **256**, 2608-2609 (1961).
- (191) C. Chararas, J. E. Courtois, M. M. Debris, and H. Laurant-Hubé, *Bull. Soc. Chim. Biol.*, **45**, 383-395 (1963).
- (192) A. Le Fay, J. E. Courtois, A. Thuillier, C. Chararas, and S. Lambin, *Compt. Rend.*, **268**, 2968-2970 (1969).
- (193) C. Chararas, J. E. Courtois, A. Thuillier, A. Le Fay, and H. Laurant-Hubé, *Compt. Rend. Soc. Biol.*, **166**, 304-308 (1972).
- (194) J. E. Courtois, C. Chararas, M. M. Debris, and H. Laurant-Hubé, *Ann. Pharm. Fr.*, **22**, 549-558 (1964).

By use of starch-gel electrophoresis^{175,195} and disc electrophoresis¹⁹⁶ and various separation methods, D-galacturonanases were found to occur as multiple molecular forms.^{167,173-175,184,197}

In micro-organisms, the production of D-galacturonanases is influenced by the conditions of cultivation, in particular by the composition of the culture medium, and by some other factors. Thus, the production of the pectic enzyme system in *Erwinia aroideae* growing in a pectin medium was stimulated by a low-molecular compound present in carrot extract¹⁹⁸⁻²⁰⁰; this compound has not been characterized in detail. A similar effect was found with the polyamines spermidine and putrescine, as well as with acetic acid and butanoic acid.²⁰⁰ Omission of D-glucose and increase in the concentration of pectin as the source of carbon in the culture medium of *Aspergillus niger* favored the production of exo-D-galacturonanase at the expense of endo-D-galacturonanase.¹⁵⁴ Likewise, the growth of *Colletotrichum lindemuthianum* in the presence of pectin as the sole source of carbon resulted in a selective production of exo-D-galacturonanase.²⁰¹

Some micro-organisms produce D-galacturonanases constitutively, whereas, with others, their production depends on the presence of pectic substances in the growth medium. The first type produce D-galacturonanases even in the absence of pectic compounds, but addition of pectin, pectate, or D-galactopyranuronic acid to the medium brings about an increase in production.^{171,172,202} The increase of extracellular endo-D-galacturonanase in *Foetidus candidum* after addition of sodium pectate to the culture medium is attributed to an increased secretion of the enzyme, rather than to its increased production.²⁰³

In the second group, the D-galacturonanase-synthesizing system can be induced either by substrate or by the end product of substrate

(195) J. W. Bennett and E. D. Garber, *Phytopathol. Z.*, **68**, 164-169 (1970).

(196) H. Stegemann, *Z. Physiol. Chem.*, **348**, 951-952 (1967).

(197) S. S. Hagar and G. A. McIntyre, *Can. J. Botany*, **50**, 2479-2488 (1972).

(198) H. Tomizawa, K. Izaki, and H. Takahashi, *Agr. Biol. Chem. (Tokyo)*, **34**, 1064-1070 (1970).

(199) T. Tsuchida, K. Nakamura, Y. Fujii, and H. Takahashi, *Agr. Biol. Chem. (Tokyo)*, **32**, 1355-1361 (1968).

(200) T. Tsuchida, K. Nakamura, Y. Fujii, and H. Takahashi, *Agr. Biol. Chem. (Tokyo)*, **32**, 1395-1397 (1968).

(201) K. Keegstra, P. D. English, and P. Albersheim, *Phytochemistry*, **11**, 1873-1880 (1972).

(202) W. A. Ayers, G. C. Papavizas, and R. D. Lumsden, *Phytopathology*, **59**, 786-791 (1969).

(203) I. Barash, *Phytopathology*, **58**, 1364-1371 (1968).

degradation.^{95,118,176,204} The synthesis of endo-D-galacturonanase by *Verticillium albo-atrum* is specifically induced by D-galactopyranuronic acid, but, at higher concentrations, catabolic repression also occurs.²⁰⁵ A similar situation was observed with *Pyrenochaeta terrestris*, where D-glucose served both as inducer and repressor.²⁰⁶

3. Purification

The development of modern separation techniques has affected the purification procedures employed for D-galacturonanases. Fractional precipitation with ammonium sulfate and with organic solvents are now used only in combination with new separation techniques. To separate fractions having D-galacturonanase activity, adsorption to pectate or calcium pectate gel has been used in several instances.^{157,207}

Purification of endo-D-galacturonanase from a submerged culture of *Aspergillus niger*¹³² constituted one of the first attempts at obtaining a homogeneous, pectic enzyme. Repeated chromatography on CM-cellulose led to a 400-fold purified preparation which was homogeneous in paper electrophoresis. However, its ability to split di(D-galactosiduronic acid) indicated contamination with exo-D-galacturonanase.

To separate and isolate extracellular endo-D-galacturonanase, pectinesterase, and polymethylgalacturonase from a surface culture of *Aspergillus niger*, repeated chromatography on DEAE-cellulose was employed.¹⁰⁴ The homogeneity of the enzymes was demonstrated by sedimentation analysis and free electrophoresis, and, in the case of polymethylgalacturonase, also by demonstrating lysine to be the N-terminal amino acid. The sedimentation and diffusion constants and the amino acid content indicated a molecular weight of 35,000 for endo-D-galacturonanase.¹⁴¹ Endo-D-galacturonanase having a close molecular weight (35,500) was isolated¹²¹ from the extract of a culture of *Aspergillus japonicus* by gel chromatography on SE-Sephadex and Sephadex G-100. The homogeneity of the preparation was demonstrated by electrophoresis on poly(acrylamide) gel.

An endo-D-galacturonanase was isolated from the culture medium of *Aspergillus saitoi* by gel-permeation chromatography on Sepha-

(204) H. W. Mussell, *Dissertation Abstr.*, **29**, 4064-B, Purdue Univ., 1968 (1969).

(205) R. M. Cooper and R. K. S. Wood, *Nature*, **246**, 309-311 (1973).

(206) J. C. Horton and N. T. Keen, *Phytopathology*, **56**, 908-916 (1966).

(207) D. S. Patel and H. J. Phaff, *Food Res.*, **25**, 37-46 (1960).

dexes G-100, G-75, and G-50. The preparation was homogeneous in free electrophoresis.¹²⁴

Preparative electrophoresis on Sephadex G-25 (Ref. 168) or double isoelectric focusing,²⁰⁸ preceded by chromatography on Sephadex G-75, CM-cellulose, and calcium phosphate, was used for the isolation of endo-D-galacturonanase from the filtrate of a *Verticillium albo-atrum* culture. The homogeneity was confirmed in both cases by electrophoresis on poly(acrylamide) gel. The molecular weight of the enzyme was close to the values found for *Aspergillus* endo-D-galacturonanases.

The only pectic enzyme thus far obtained in crystalline form is the endo-D-galacturonanase from *Acrocylindrium*.²⁰⁹ Crystallization of the enzyme from a solution of ammonium sulfate was preceded by chromatography on calcium phosphate, Duolite CS 101, and DEAE-cellulose, and by starch-gel electrophoresis.

In all of the examples mentioned, multi-step purification procedures were needed in order to obtain a specific preparation of endo-D-galacturonanase. Rexová-Benková and Tibenský²¹⁰ obtained a specific preparation of extracellular endo-D-galacturonanase from a mixture of pectolytic enzymes of *Aspergillus niger* by affinity chromatography on pectic acid cross-linked by epichlorohydrin. The enzyme was bound selectively to the cross-linked substrate at pH 4.2 (the pH optimum of the enzyme), and was subsequently released from the column by elution with a buffer at pH 6.0 (see Fig. 7). The active-site-directed mechanism was confirmed here by the finding that cross-linked pectic acid acts as a competitive inhibitor of endo-D-galacturonanase, as well as on the basis of identity of the values of the inhibition constant and the association constant on the enzyme-cross-linked pectic acid complex formed in the absence of soluble substrate.²¹¹

The method was used in a modified form for the purification of endo-D-galacturonanase from tomatoes.²¹² Elution from the column by use of a linear pH gradient led to the separation of pectinesterase and of three multiple molecular forms of endo-D-galacturonanase dif-

(208) H. W. Mussell and B. Strouse, *Can. J. Biochem.*, **50**, 625-632 (1972).

(209) F. Uchino, Y. Kurono, and S. Doi, *Agr. Biol. Chem. (Tokyo)*, **30**, 1066-1068 (1966).

(210) Ľ. Rexová-Benková and V. Tibenský, *Biochim. Biophys. Acta*, **268**, 187-193 (1972).

(211) Ľ. Rexová-Benková, *Biochim. Biophys. Acta*, **276**, 215-220 (1972).

(212) Ľ. Rexová-Benková and O. Markovič, *Abstr. Intern. Symp. Carbohydr. Chem. Biochem.*, 7th, Bratislava, 1974.

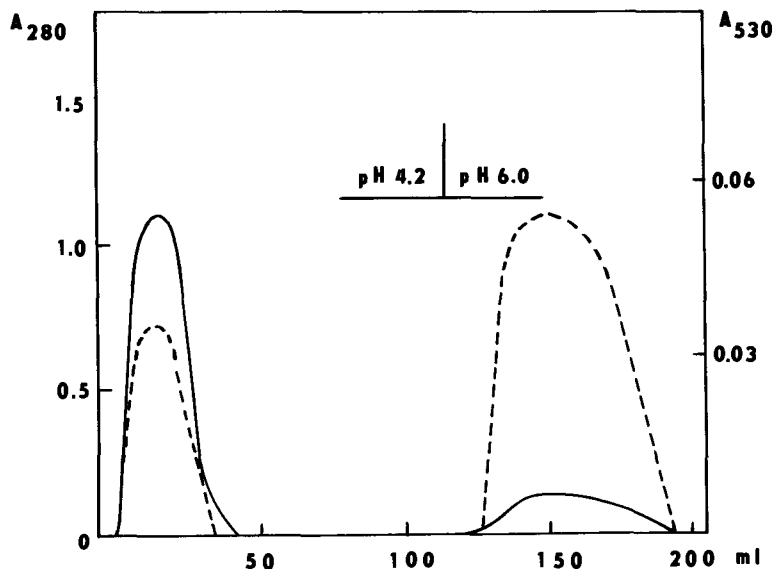


FIG. 7.—Isolation of Endo-D-galacturonanase on a Column of Cross-linked Pectic Acid.²¹⁰ [The Column (1.4 cm \times 12 cm) was equilibrated with 0.1 M acetate buffer (pH 4.2). The first peak was eluted with the equilibrating buffer. Endo-D-galacturonanase was eluted with 0.1 M acetate buffer (pH 6.0). ---, D-galacturonanase activity in 1 ml of eluate per min, determined by Somogyi reagent. The activity is expressed as change in absorbance at 530 nm. —, absorbance at 280 nm.]

fering in their mobility in starch-gel electrophoresis. Two of these forms, those of molecular weight 44,000 and 84,000, had already been separated by Pressey and Avants¹¹⁷ by chromatography on DEAE-Sephadex A-50.

Multiple molecular forms of D-galacturonanases have also been isolated from other sources. Two highly purified forms of exo-D-galacturonanase^{153,154} were obtained from the mycelial extract of *Aspergillus niger* by use of triple rechromatography on DEAE-cellulose. Fractionation with ammonium sulfate, and chromatography on Duolite CS 101 and A-2 and on DEAE-cellulose, led to the isolation of three forms of extracellular endo-D-galacturonanase and one exo-D-galacturonanase from the culture filtrate of *Coniothyrium diplodiella*.^{114–116} The exo-D-galacturonanase was further purified by repeated zone electrophoresis on a column of cellulose.¹⁴⁴ Two forms of *Colletotrichum lindemuthianum* endo-D-galacturonanase having molecular weights of 78,000 and 62,000 were isolated by a purification procedure involving gel chromatography and chromatography on agarose.¹¹⁸

4. Assay

Methods of determining the activity of depolymerizing pectic enzymes have not changed much over the years. The activity of D-galacturonanases is usually determined on the basis of measuring, during the course of the enzyme reaction, (a) the rate of increase of reducing groups, and (b) the decrease of viscosity of the substrate solution.

To determine the reducing groups, many authors use titration with hypiodite according to Willstätter and Schudel²¹³ as described by Phaff.²¹⁴ The method is based on the oxidation of the reducing group of reducing saccharides with iodine, and titration of unreacted iodine with sodium thiosulfate. The results of estimating the reducing saccharides by this method agree satisfactorily with the results of acidimetric determination of carboxyl groups.²¹⁵ However, the method is laborious, and requires large amounts of enzyme, as well as of substrate. A photolorimetric modification of the method was developed for micro-quantities by Mill and Tuttobello.¹³²

Some authors estimate the reducing groups by a colorimetric method with the 3,5-dinitrosalicylic acid-phenol reagent, according to Deuel and coworkers.²¹⁶ The method is not suitable for substrates having a high degree of esterification, because of the alkalinity of the reaction medium. Under these conditions, glycosidic bonds are simultaneously split by β -elimination, so that the amount of reducing groups estimated does not correspond to the amount released by the enzyme. Likewise, oligomeric substrates, also nonesterified, undergo partial hydrolysis under the reaction conditions.²¹⁵ From this point of view, it is preferable to use the photolorimetric estimation of reducing groups according to Nelson and Somogyi; this uses a cuprous reagent,²¹⁷ which gives identical standard readings for D-galactopyranuronic acid, as well as for the oligomers.¹²⁷ But here again, because of the alkaline reaction-medium, use of the method is restricted to nonesterified substrates.

When *esterified* substrates are used, the method of choice appears to be oxidation with chlorous acid according to Launer and Tomi-

(213) R. Willstätter and G. Schudel, *Ber.*, **51**, 780-781 (1918).

(214) H. J. Phaff, *Methods Enzymol.*, **8**, 646 (1966).

(215) A. G. J. Voragen, F. M. Rombouts, M. J. Hooydonk, and W. Pilnik, *Lebensm. Wiss. Technol.*, **4**, 7-11 (1971).

(216) E. Borel, F. Hostetter, and H. Deuel, *Helv. Chim. Acta*, **35**, 115-120 (1952).

(217) M. Somogyi, *J. Biol. Chem.*, **195**, 19-23 (1952).

matsu.²¹⁸⁻²²⁰ The oxidation proceeds in an acid medium, generally according to the following equation.



For enzymic assays, a modification by Voragen and coworkers is recommended.²¹⁵

The activity of exo-D-galacturonanase is sometimes determined, on the basis of the amount of released D-galactopyranuronate, either by Dische's carbazole method in the modification of McComb and McCready²²¹ or with the aid of the NADH-linked system of D-galactopyranuronate isomerase-dehydrogenase according to Nagel and Hasegawa.²²² By the action of this enzyme system, D-galactopyranuronate is transformed to D-tagaturonic acid and finally to D-altronic acid. The rate of oxidation of NADH is recorded at 340 nm.

Viscosimetric determination of activity is used only with pectic enzymes displaying the random-action pattern. The activity is mostly expressed as the time required for attaining a 50% decrease of viscosity (in s) or as the amount of enzyme required for attaining a certain decrease of viscosity per unit time.²²³

Pilnik and coworkers¹⁶³ used the value of specific viscosity η_s expressed as $\eta_s = (t - t_b)/t_b$, where t is the flow time of the reaction mixture, and t_b , the flow time of the buffer solution. The activity is determined graphically, by plotting reciprocal specific viscosities against reaction time. The slopes of the straight lines resulting provide the values of the activity.

Avigad and Milner²²⁴ used a turbidimetric method based on the measurement of residual turbidity caused by complexing the acid polysaccharide with such quaternary ammonium detergents as cetylpyridinium bromide. The activity unit was expressed as the amount of enzyme bringing about a decrease of absorbance at 400 nm of 0.01/min.

In addition to the aforementioned quantitative methods, the activity of pectic depolymerases is often identified by the cup-plate method.¹¹³ Cups are cut out from solidified agar containing the substrate, and are filled with the enzyme solution. After elapse of a cer-

(217) H. F. Launer and Y. Tomimatsu, *Anal. Chem.*, **26**, 382-386 (1954).

(219) H. F. Launer and Y. Tomimatsu, *Anal. Chem.*, **31**, 1385-1390 (1959).

(220) H. F. Launer and Y. Tomimatsu, *Anal. Chem.*, **31**, 1569-1574 (1959).

(221) E. A. McComb and R. M. McCready, *Anal. Chem.*, **24**, 1630 (1952).

(222) C. W. Nagel and S. Hasegawa, *Anal. Chem.*, **21**, 411-415 (1967).

(223) B. L. Gosh and R. G. Bose, *Current Sci. (India)*, **42**, 854-855 (1973).

(224) G. Avigad and Y. Milner, *Israel J. Chem.*, **5**, 175-180 (1967).

tain incubation period, the zones of degraded substrate are stained with iodine.

VI. LYASES

1. Action Pattern and Specificity

The discovery of enzymes that catalyze the cleavage of α -D-(1 \rightarrow 4) glycosidic bonds of D-galacturonans by the mechanism of β -elimination constitutes one of the most significant contributions to the elucidation of enzymic degradation of pectic substances. In a commercial preparation of pectinase, Deuel and coworkers³ found an enzyme specifically splitting highly esterified pectin by this mechanism, and, from a culture filtrate of *Bacillus polymyxa*, Nagel and Vaughn⁴ purified a lyase specifically degrading pectic acid and low-esterified D-galacturonans.

It had been known that the glycosidic bonds of esterified D-galacturonans are split by a mechanism of β -elimination either in an alkaline or in a neutral medium (pH 6.8) at higher temperatures.^{225,226} Deuel and coworkers²²⁷ showed that, under these conditions, the D-galacturonan chain is split, with the production of sugar conjugates containing, at the nonreducing end, a 4-deoxy-L-*threo*-hex-4-enopyranosyluronate group (5). An excellent article by Kiss²²⁸ on β -eliminative degradations of glycuronans has appeared in this Series.

The basis of β -elimination is the removal of the hydrogen atom from C-5, which is activated by the electron-withdrawing group at C-6, in the presence of a suitable proton-acceptor as catalyst (8) (see Scheme 2). The unstable, intermediate ion formed (9a,b) is stabilized by a splitting of the C-O bond in the β position. The loss of substituents at C-4 and C-5 gives rise to a double bond (5). The free carboxyl groups at C-6 cannot sufficiently activate the hydrogen atom at C-5 in an alkaline medium and, hence, the nonenzymic β -elimination, in contrast to the enzymic one, is restricted to esterified D-galacturonans, and the degree of degradation is determined by the degree of esterification.

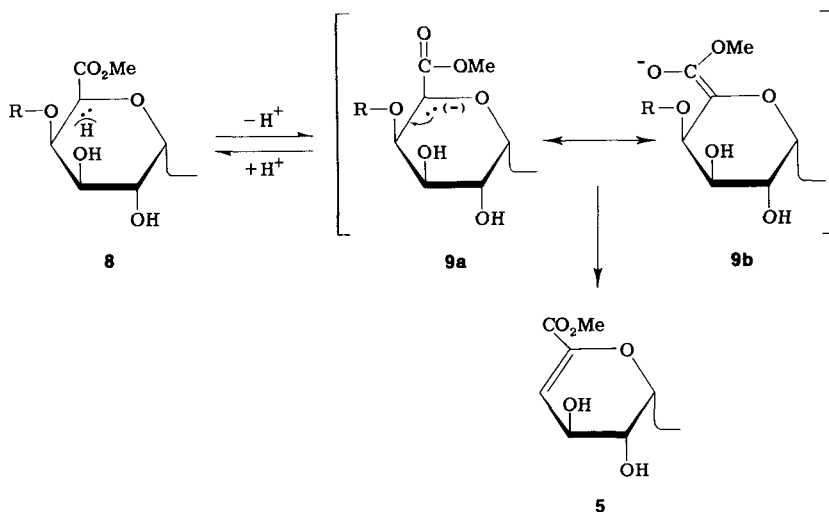
Conjugation of the double bond with the carboxyl group on C-5 in the cleavage products brings about the strong absorption at 235 nm.

(225) B. Vollmert, *Makromol. Chem.*, **5**, 101-109 (1950).

(226) H. Neukom and H. Deuel, *Chem. Ind. (London)*, 683 (1958).

(227) P. Albersheim, H. Neukom, and H. Deuel, *Arch. Biochem. Biophys.*, **90**, 46-51 (1960).

(228) J. Kiss, *Advan. Carbohydr. Chem. Biochem.*, **29**, 229-303 (1974).



Scheme 2

Another characteristic property of the products of β -eliminative cleavage of pectic compounds is the reaction with thiobarbituric acid after previous cleavage with periodate, giving rise to red condensation products having²²⁹ an absorption maximum at 547–550 nm. Both properties are used for the identification of β -eliminative cleavage of pectic substances.

In the course of degradation of nonesterified and low-esterified substrates by pectate lyases, the glycosidic bonds can be split either at random or terminally, starting from the reducing end of the molecule. On the other hand, in the course of splitting of highly esterified substrates by pectin lyases, only the random-action pattern is operative. So far, no pectin lyase is known that can split the glycosidic bonds terminally.

The optimum substrate of endopectate lyase was considered to be de-esterified D-galacturonan. However, Bateman²³⁰ and Sherwood²³¹ described lyases that could split nonesterified substrate, but that displayed a higher activity with partly esterified substrates. On the basis of this observation, Pilnik and coworkers^{162,163,232} studied the effect of the degree of esterification of D-galacturonans having a statistical distribution of the esterified units on the kinetics of the reaction

(229) A. Weissbach and J. Hurwitz, *J. Biol. Chem.*, **234**, 705–709 (1959).

(230) D. F. Bateman, *Phytopathology*, **56**, 238–244 (1966).

(231) R. T. Sherwood, *Phytopathology*, **56**, 279–286 (1966).

(232) A. G. J. Voragen, F. M. Rombouts, and W. Pilnik, *Lebensm. Wiss. Technol.*, **4**, 126–129 (1971).

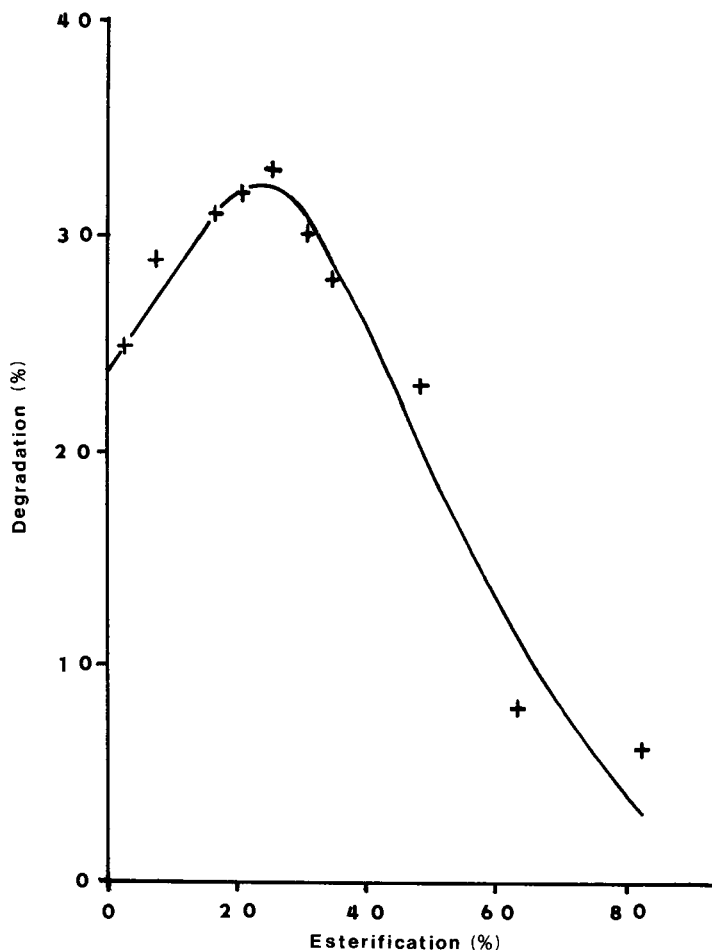


FIG. 8.—Degradation Limit¹⁶² of Substrates Having Different Degrees of Esterification, with Pectate Lyase of *Arthrobacter*, strain 547.

catalyzed by endopectate lyase from *Arthrobacter* and *Bacillus subtilis*, and they found that, for (a) pectate lyase of one *Arthrobacter* strain, the optimum substrate is 21% esterified D-galacturonan, (b) the lyase of *Bacillus polymyxa*, it is 26% esterified substrate, and (c) the enzyme from the other *Arthrobacter* strain, the optimum substrate was esterified to the extent of 44%. For these substrates, the highest value of V and of $1/K_m$ were found, as well as the highest degree of substrate degradation (see Figs. 8 and 9). The lyases differed from a pectin lyase preferentially splitting highly esterified substrates in that they also degraded D-galacturonans esterified with

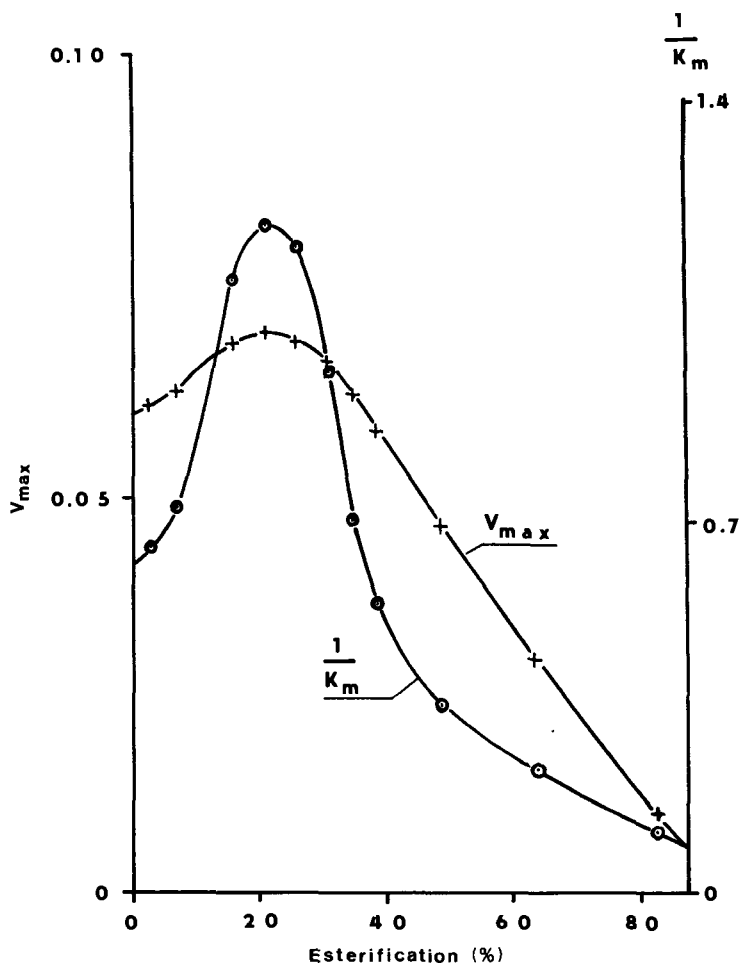


FIG. 9.—Influence¹⁶² of Degree of Esterification of Pectin Substrate on V and $1/K_m$ of Pectate Lyase of *Arthobacter*, strain 547.

glycol. Pilnik and coworkers suggested that both types of enzyme be considered as pectin lyase, and that they be distinguished according to their preference for highly esterified and low-esterified pectin. Rombouts¹⁶² considered, as a possible reason for the higher values of V and $1/K_m$ with esterified substrates, the formation of two different enzyme-substrate complexes, a catalytic role being played in one of them by free carboxyl groups only, but in the other, by esterified groups also. The decrease of activity at the higher degree of esterification was attributed to the formation of unproductive com-

plexes. The dependence of the value of V on the content of ester groups indicates, however, that the effect of the methyl ester or glycol ester group may consist in its direct participation in the catalytic reaction. It is possible that these groups (unless they are present in such a proportion as to prevent the formation of the complex with the enzyme) may facilitate the activation of the hydrogen atom at C-5 of the D-galactopyranosiduronate residue directly participating in the β -elimination process, similarly to the situation with nonenzymic, β -eliminative splitting of glycosidic bonds.

The hydroxyl groups at C-2 and C-3 are not essential for the catalytic reaction. McNicol and Baker²³³ showed that the endopectate lyases of *Bacillus sphaericus* and *Bacillus polymyxa* degrade the Vi antigen, the bacterial-surface polysaccharide containing α -D-(1 \rightarrow 4)-linked residues of 2-acetamido-3-O-acetyl-2-deoxy-D-galactopyranuronate, in the same way as its O-deacetylated derivative and D-galacturonan.

Different endopectate lyases differ in their action pattern toward polymeric and oligomeric substrates. A common feature of all these enzymes is the splitting of the glycosidic bonds of pectic acid and low-esterified D-galacturonans up to a certain degree of randomness, but the percentage of split glycosidic bonds corresponding to a 50% diminution in viscosity of the substrate solution varies.^{162,234-237} The extracellular, endopectate lyase of *Arthrobacter*, isolated by Rombouts,¹⁶² splits high-molecular substrates less randomly; the degree of randomness, depending on the pH and the temperature, increases with increasing temperature and with decreasing pH (from 9.1 to 7.0). The temperature and pH effects were regarded by Rombouts as caused by the presence of multiple molecular forms of endopectate lyase, differing in pH optimum and in activation energy. Another possible cause may be a multiple attack on the substrate.

On the basis of the action pattern toward oligomeric substrates, two groups of endopectate lyases can be distinguished (see Scheme 3). The main feature of group A is the specific splitting of an unsaturated tetrasaccharide at the central bond, as well as the ability to split the trisaccharide and its unsaturated analogue. The principal, final product of enzyme action operating according to this action pattern is

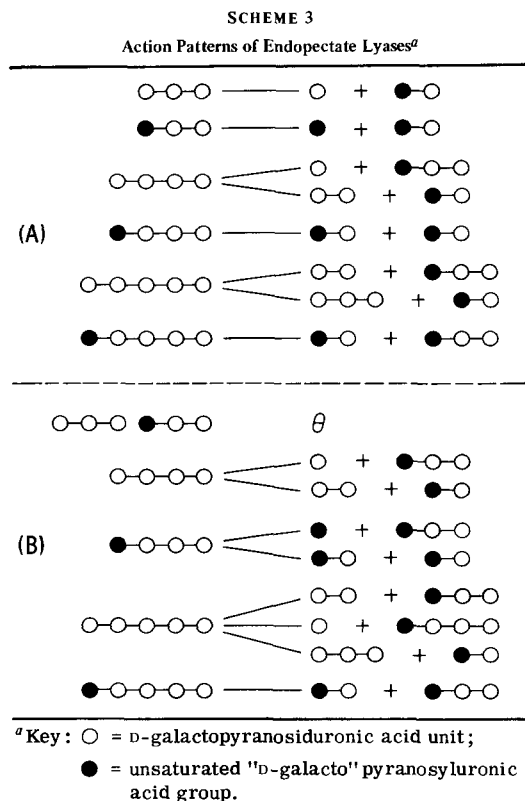
(233) L. A. McNicol and E. E. Baker, *Biochemistry*, **9**, 1017-1023 (1970).

(234) S. Hasegawa and C. W. Nagel, *J. Food Sci.*, **31**, 838-845 (1966).

(235) M. S. Mount, D. F. Bateman, and H. G. Bahsam, *Phytopathology*, **60**, 924-931 (1970).

(236) S. Nasuno and M. P. Starr, *Biochem. J.*, **104**, 178-185 (1967).

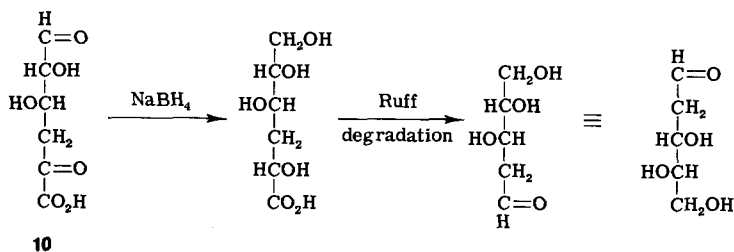
(237) C. W. Nagel and T. M. Wilson, *Appl. Microbiol.*, **20**, 374-383 (1970).



a disaccharide which was characterized by Hasegawa and Nagel²³⁸ as *O*-(4-deoxy-β-*L*-*threo*-hex-4-enopyranosyluronic acid)-(1 → 4)-D-galactopyranuronic acid ("unsaturated disaccharide"). Splitting of the unsaturated trisaccharide releases the unsaturated disaccharide and a compound, not absorbing in the ultraviolet region at 235 nm, which reacts with (a) thiobarbituric acid to afford a chromogen having an absorption maximum at 548–550 nm, and (b) *o*-phenylenediamine. On the basis of these properties, Nagel and Anderson²³⁹ characterized this compound as 4-deoxy-*L*-*threo*-hexos-5-ulosuronic acid (10). In the same way, it had been characterized by Preiss and Ashwell,¹⁷⁸ who identified the orientation of the hydroxyl groups at C-2 and C-3 on the basis of its conversion by reduction and Ruff degradation into 2-deoxy-*L*-*threo*-pentose (11) (see Scheme 4).

(238) S. Hasegawa and C. W. Nagel, *J. Biol. Chem.*, **237**, 619–621 (1962).

(239) C. W. Nagel and M. M. Anderson, *Arch. Biochem. Biophys.*, **112**, 322–330 (1965).



Scheme 4

For endopectate lyases, the rate of cleavage of glycosidic bonds and the affinity of the enzyme for the substrate depend on the chain length,^{240,241} as with endo-D-galacturonanase. The frequency of splitting of bonds 2 and 3 in tetra(D-galactosiduronic acid) is different. Endopectate lyase of *Bacillus polymyxa* splits²⁴⁰ bond 3 1.4 times faster than bond 2.

Action pattern A was observed with extracellular, endopectate lyase of *Bacillus polymyxa*,^{4,240} with extra- and intra-cellular lyase of *Erwinia carotovora*,²⁴¹ with extracellular enzyme of *Xanthomonas campestris*,²³⁶ and with lyase produced by *Bacteroides ruminicola*²⁴² isolated from the rumen fluid of sheep.²⁴³

Action pattern B was observed with endopectate lyase isolated from the culture medium of the anaerobic strain of *Bacillus* sp.^{29,234} The main end-product of the action of these enzymes is "unsaturated tri(D-galactosiduronic acid)" formed by preferential cleavage of bond 3 of tetra(D-galactosiduronic acid) and of its unsaturated analog, as well as of penta(D-galactosiduronic acid). Replacement of the group at the nonreducing end by its unsaturated analogue (5) results in a decrease in the rate of splitting of the substrate; the "unsaturated tetrasaccharide" is split at only 30% of the rate of splitting of the saturated tetrasaccharide.

Nagel and Hasegawa²⁹ studied the frequency of splitting of glycosidic bonds of oligomeric substrates, and showed that, in both the saturated and the unsaturated tetrasaccharide, the enzyme attacks preferentially at bond 3 rather than at bond 2, but that the unsaturated group decreases the degree of preference from 26:1 to 1.6:1. The ratio of splitting rates of bonds 2, 3, and 4 in penta(D-galactosiduronic acid) is 1:19:13. The values of V and K_m change, the

(240) M. M. Anderson and C. W. Nagel, *Nature*, **203**, 649 (1964).

(241) F. Moran, S. Nasuno, and M. P. Starr, *Arch. Biochem. Biophys.*, **123**, 298-306 (1968).

(242) M. Wojciechowicz, *Acta Microbiol. Polon.*, Ser. A, **4**, 189-196 (1972).

(243) M. Wojciechowicz, *Acta Microbiol. Polon.*, Ser. A, **3**, 45-56 (1971).

former in proportion to, the latter inversely to, the chain length of the substrate.

Characteristic properties of endopectate lyases are the high pH optimum, and a requirement for Ca^{2+} ions in order to maintain catalytic activity. The pH optimum of various endopectate lyases ranges from 8.0 to 9.5 (Refs. 4, 178, 234, 236, 243). Besides activation by Ca^{2+} ions, the optimal concentration of which is 1 mM,^{234,236,244} strontium salts were also considered in the case of *Bacillus* sp. lyase.²³⁴ The enzyme from *Pseudomonas* sp. was also partly activated by magnesium chloride,¹⁷⁸ and for the lyase of *Clostridium felsineum*, salts of other bivalent cations had an activating effect as well.²⁴⁵ (Ethylenedinitrilo)tetraacetic acid completely inactivated all of the lyases mentioned. The activity of endopectate lyase from *Pseudomonas* was also lessened in the presence of sodium chloride, potassium chloride, and dipotassium hydrogen phosphate (K_2HPO_4).

Exopectate lyase splits the penultimate α -D-(1 \rightarrow 4)-glycosidic bonds of D-galacturonans, starting at the reducing end. "Unsaturated di(D-galactosiduronic acid)" is the product of the reaction. So far, only two enzymes of this type have been investigated. From soil, Ng and Vaughn²⁴⁶ isolated the anaerobic bacteria *Clostridium multifementans* and *Clostridium butyricum*, which produce an exopectate lyase. Macmillan and Vaughn¹⁰⁵ partly purified this enzyme from the filtrate of an aerobic culture of *Clostridium multifementans*. The terminal type of degradation was confirmed by showing that, throughout the reaction, the only product is "unsaturated di(D-galactosiduronic acid)." Likewise, the 22.5% of split bonds, corresponding to a 50% diminution in viscosity, demonstrated the gradual shortening of the substrate chains.²⁴⁷ In contrast with endopectate lyases, the rate of splitting of glycosidic bonds does not depend on the size of the substrate. The rates of splitting of substrates having d.p. 3, 4, and 12 are almost identical. Tri(D-galactosiduronic acid) is the smallest substrate undergoing degradation. Evidence for the attack at the reducing end was the finding by Macmillan and co-workers²⁴⁷ that the product of splitting of tri(D-galactosiduronic acid) is an "unsaturated disaccharide" and D-galactopyranuronate.

An enzyme having the same action-pattern was found in *Erwinia*

(244) M. P. Starr and F. Moran, *Science*, **135**, 920-921 (1962).

(245) L. S. Kapitonova, N. A. Rodionova, and R. V. Fenixova, *Biokhimiya*, **38**, 1054-1061 (1973).

(246) H. Ng and R. H. Vaughn, *J. Bacteriol.*, **85**, 1104-1113 (1963).

(247) J. D. Macmillan, H. J. Phaff, and R. H. Vaughn, *Biochemistry*, **3**, 572-578 (1964).

aroideae.²⁴⁸ In contrast to other pectate lyases, the enzyme is strikingly activated by Na^+ (and less by Ca^{2+}) ions.²⁴⁹

The activity of exopectate lyase of *Clostridium multifementans* depends on the presence of bivalent cations of Ca, Ba, Sr, Mg, and Mn. The activation constant with calcium chloride is 0.06 mM. In a later stage of substrate degradation, an inhibition by Ca^{2+} was observed at concentrations above 0.5 mM; this was attributed by Macmillan and Vaughn¹⁰⁵ to a binding of calcium to the carboxyl group of two different molecules of substrate and to the inability of exopectate lyase to split the substrate as this barrier is approached.

The pH optimum of both exopectate lyases lies in the alkaline region. The pH optimum of exopectate lyase of *Erwinia aroideae* is influenced by univalent cations. The enzyme activated by K^+ and NH_4^+ has its pH optimum at 8.0, but, in the presence of Na^+ ions, it is shifted towards the alkaline region.

Lyases preferentially splitting the terminal glycosidic bonds of oligomeric substrates and their unsaturated analogues from the reducing end, giving rise to 4-deoxy-L-threo-hexos-5-ulosuronic acid (10), were partly purified from the cell extracts of *Erwinia*^{17,250} and a strain of *Pseudomonas*.²⁵¹ Hence, they are classified as oligo-D-galactosiduronate lyases. Moran and coworkers²⁵⁰ found this enzyme in the cell extract of *Erwinia carotovora*. The enzyme splits di(D-galactosiduronic acid) 400 times faster than the high-molecular D-galacturonan. The affinity of the enzyme and the rate of splitting were higher with the "unsaturated disaccharide" than with the saturated one.

Hatanaka and Ozawa¹⁷ purified, from the cell extract of *Erwinia aroideae*, an enzyme preferring the "unsaturated disaccharide" as substrate, and classified it as an "unsaturated oligo-D-galactosiduronate lyase."

The pH optimum of oligo-D-galactosiduronate lyases is lower than that of exopectate lyases. Both of these lyases have a pH optimum at 7.0. They differ from the other pectate lyases in not being activated by Ca^{2+} ions. An exception is the oligo-D-galactosiduronate lyase of *Pseudomonas* sp.²⁵¹ The oligo-D-galactosiduronate lyase found in a

(248) K. Okamoto, C. Hatanaka, and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **28**, 331-336 (1964).

(249) C. Hatanaka and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **37**, 593-597 (1973).

(250) F. Moran, S. Nasuno, and M. P. Starr, *Arch. Biochem. Biophys.*, **125**, 734-741 (1968).

(251) C. Hatanaka and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **35**, 1617-1624 (1971).

strain of *Erwinia*²⁵² is activated by Co^{2+} and Mn^{2+} ions. The optimum substrate for this enzyme is tetra(D-galactosiduronic acid). The rate of splitting of substrates having different d.p. values decreased in the order: tetrasaccharide, acid-soluble pectic acid, D-galacturonan, trisaccharide. Likewise, for the oligo-D-galactosiduronate lyase from *Pseudomonas* sp.,²⁵¹ the optimum substrate was the tetrasaccharide, the rate of splitting decreasing to trisaccharide, to disaccharide, and to D-galacturonan. With this enzyme, no difference in the rate of splitting of saturated and unsaturated analogs was found.

Pectin lyases preferentially split the highly esterified D-galacturonans by a random-action pattern, with production of esterified, unsaturated oligo-D-galactosiduronates.³ The extent of degradation of the substrate and the affinity for the enzyme decrease with decreasing d.e.^{166,232} An important factor affecting the enzymic activity is the distribution of esterified groups in D-galacturonan chains. Voragen¹⁶⁶ studied the kinetics of action of pectin lyase on pectins having different d.e. values and different distributions of the esterified D-galactopyranosiduronate residues; he found the lyase activity to be higher towards enzymically de-esterified substrates (which have a blockwise distribution of the esterified groups) than towards substrates having the same d.e. but a statistical distribution of these units (de-esterified in an alkaline solution). The size of the reaction products increases with decreasing d.e. for randomly esterified substrates. Most of the pectin lyases thus far described do not attack pectate,^{3,253} exceptions being the pectin lyase from *Rhizoctonia solani*²³⁰ and the enzyme purified from the culture medium of *Fusarium solani* f. *phaseoli*,²³¹ which are known to degrade pectate as well.

The rate of splitting of glycosidic bonds of highly esterified substrates decreases with decreasing d.p. The products of partial degradation of pectin by heat were broken down by pectin lyase (a commercial preparation) at a much lower rate.²⁵⁴ D-Galacturonan methyl ester, and fully esterified hexa-, penta-, and tetra-(D-galactosiduronic acid) were degraded by pectin lyase of *Aspergillus fonsecaeus*²⁵⁵ at a ratio of rates of 100:2.6:0.65:0.049. Fully esterified tetrasaccharide was the smallest substrate degraded by the enzyme.

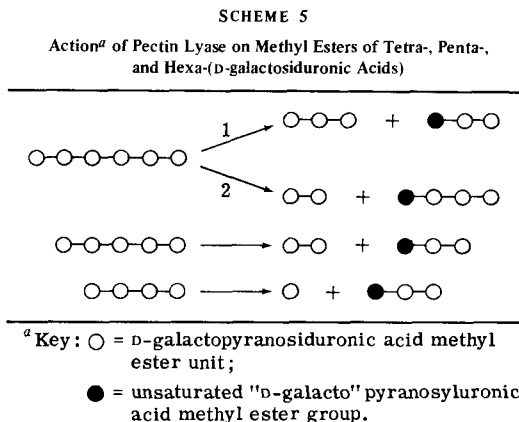
Analysis of the products of splitting of esterified tetra- through hexa-(D-galactosiduronic acid) showed pectin lyase to be unable to split the first and the second bonds, starting from the reducing end of

(252) C. Hatanaka and J. Ozawa, *Agr. Biol. Chem.* (Tokyo), **36**, 2307–2313 (1972).

(253) R. D. Edström and H. J. Phaff, *J. Biol. Chem.*, **239**, 2403–2407 (1964).

(254) P. Albersheim and W. Killias, *Arch. Biochem. Biophys.*, **97**, 107–115 (1962).

(255) R. D. Edström and H. J. Phaff, *J. Biol. Chem.*, **239**, 2409–2415 (1964).



the molecules²²⁵ (see Scheme 5). The same action-pattern was observed with the pectin lyase of a commercial preparation.¹⁶⁶ With tetra- and penta-saccharide, bond 3 is split specifically. In the fully esterified hexasaccharide, bond 3 is split preferentially, the rate of splitting of bond 4 being much lessened.

The d.e. and the distribution of the esterified residues of D-galactopyranosiduronic acid also affect the pH optimum of pectin lyases. With highly esterified substrates, the pH optimum ranges with various pectin lyases from 5.1 to 6.6 (Refs. 4, 166, 253, 256). With decreasing d.e., the pH optimum is shifted to lower values, more pronouncedly with substrates having a random distribution of esterified residues of D-galactopyranosiduronic acid than with substrates having a blockwise distribution.¹⁶⁶

The affinity of pectin lyase for the substrate depends on its d.e. Voragen and coworkers²³² observed that, for pectin lyase from a commercial preparation, $1/K_m$ values decreased as the d.e. decreased. On the other hand, the values of V did not depend on the d.e. The effect of the d.e. on the affinity of the enzyme for the substrate was attributed to the lower content of reactive sites in the less esterified substrates. Values of $1/K_m$ increased with decreasing pH. Voragen and coworkers²³² contended that the charged groups of the enzyme, or of the substrate, play a role in the formation of the enzyme-substrate complex.

Ca^{2+} ions activate most pectin lyases,^{166,253,257} their activating effect depending on the pH and the d.e. The effect of Ca^{2+} ions is much lessened with the highly esterified substrates^{255,257} and is also pH-

(256) R. Amado, Dissertation No. 4536, ETH, Zürich (1970).

(257) S. Ishii and T. Yokotsuka, *Agr. Biol. Chem.* (Tokyo), **36**, 146-153 (1972).

dependent. For the pectin lyase of a commercial preparation, calcium had an activating effect at pH 7.5–8.5, whereas, at lower pH (6.5–5.8), it was inhibitory, and, at pH 5.3–4.3, it had no effect on enzyme activity. A similar effect was displayed¹⁶⁶ by Mg^{2+} and by Sr^{2+} .

A number of hypotheses have been advanced regarding the function of ions. Edstrom and Phaff²⁵³ attributed the activating effect to a decrease of the negative charge of partly esterified pectins due to binding of calcium to free carboxyl groups. The same mechanism was considered by Ishii and Yokotsuka.²⁵⁷ Another possibility is the function of calcium ions in replacement of missing methoxyl groups.^{166,255}

2. Occurrence and Formation

Pectate lyases are produced by bacteria and by fusaria.^{258,259} Most of the bacterial pectate lyases are produced inductively.^{4,95,260–262} *Clostridium multifementans* produces endopectate lyase on substrates of pectin origin, with the exception of D-galactopyranuronate. With oligo-D-galactosiduronates, the production of enzyme is directly proportional to the chain length of the substrate.¹⁰⁵ *Fusarium solani* f. sp. *cucurbitae* produces endopectate lyase in the presence of pectate, but not in the presence of pectin as the carbon source. The enzyme production is catabolically repressed²⁶² by D-galactose, D-glucose, and D-galactopyranuronic acid, if any of these is present at a concentration greater than 0.025%.

Zucker and Hankin²⁶³ compared the production of endopectate lyase by the pathogenic *Erwinia carotovora* and the saprophytic *Pseudomonas fluorescens*. The pathogen produces a small proportion of the enzyme constitutively, whereas the saprophyte shows only the inducible synthesis, and also differs from the pathogen in the fact that it requires some 10 to 20 generations in which to adapt to the substrate. The authors²⁶³ considered that the constitutive enzyme serves the pathogen only for initial attack of the substrate. The products formed then induce the synthesis of a new enzyme which further catalyzes cleavage of substrate.

A constitutive production of endopectate lyase was observed in

(258) G. C. Papavizas and W. A. Ayers, *Phytopathology*, **56**, 1269–1273 (1966).

(259) R. L. Millar, *Phytopathology*, **55**, 130 (1965).

(260) A. Fuchs, *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **31**, 323–340 (1965).

(261) M. P. Starr and S. Nasuno, *J. Gen. Microbiol.*, **46**, 425–433 (1967).

(262) J. G. Hancock, C. Eldridge, and M. Alexander, *Can. J. Microbiol.*, **16**, 69–74 (1970).

(263) M. Zucker and L. Hankin, *J. Bacteriol.*, **104**, 13–18 (1970).

Aeromonas liquefaciens, the enzyme synthesis being catabolically repressed.²⁶⁴

All the pectin lyases so far described are of fungal origin and are secreted extracellularly. Production of pectin lyase by *Rhizoctonia solani*, *Botrytis cinerea*, and *Fusarium oxysporum* f. *lycopersici* was induced by pectate with D-glucose,²⁶⁵ pectin showing a lower inductive effect.²³¹

3. Purification

Most of the bacterial endopectate lyases and oligo-D-galactosiduronate lyases already described were prepared by simple purification procedures, including fractional salting-out with ammonium sulfate and a single-step chromatography on modified celluloses,^{234,239,241} but the degree of purification was not reported. A 66-fold purified endopectate lyase was obtained from the cultivation medium of *Xanthomonas campestris* by precipitation with acetone, extraction of the precipitate, and chromatography on DEAE- and CM-cellulose.²³⁶

The highest degree of purification was achieved with extracellular endopectate lyase from the anaerobic bacterium *Clostridium felsineum*.²⁴⁵ A 225-fold purified preparation, homogeneous in disc electrophoresis, was obtained by precipitation with ethanol, and chromatography on CM-cellulose and on Sephadex G-200. Its molecular weight (determined by gel chromatography) was 105,000.

A purification procedure including all of the separation principles then known led to a 156-fold purification of exopectate lyase of *Clostridium multifementans*, but pectinesterase could not be removed.^{51,105}

Pectin lyases have been obtained from commercial preparations of pectic enzymes and from culture filtrates of some fungi. On starting with Pectinol R-10, the first pectin lyase was purified¹²⁶ by chromatography on DEAE-cellulose and Sephadexes G-75 and G-50. To purify the pectin lyase from Pectinex preparation, repeated chromatography on hydroxylapatite was employed, followed by²⁵⁶ preparative, discontinuous electrophoresis on poly(acrylamide) gel and chromatography on Sephadex G-50. A pectin lyase homogeneous in disc electrophoresis was obtained⁶ from a commercial preparation after adsorption onto calcium phosphate, and chromatography on DEAE-Sephadex A-25 and on Sephadex G-100.

(264) E. J. Hsu and R. H. Vaughn, *J. Bacteriol.*, **98**, 172-181 (1969).

(265) R. T. Sherwood, *Phytopathology*, **54**, 907 (1964).

Adsorption on calcium pectate and calcium phosphate, and chromatography on DEAE-cellulose, were used for the purification of pectin lyase from *Aspergillus fonsecaeus*.²⁵³ Two forms of pectin lyase (having pH optima at 7.3 and 8.3) were isolated²⁶⁶ from the culture filtrate of *Sclerotinia fructigena* by chromatography on Sephadex G-75 and CM-Sephadex C-50.

A pectin lyase having a molecular weight of 32,000 was isolated from the culture medium of *Aspergillus sojae* by chromatography on DEAE-Sephadex and on CM-cellulose, and by repeated chromatography on SE-Sephadex.²⁵⁷

4. Assay

Methods used for the determination of lyase activity are based on measurement of the rate of formation of 4,5-unsaturated glycosiduronate products, either on the basis of estimating the increment of absorbance at 235 nm, or by use of the thiobarbituric acid test.³ The first technique makes possible the direct measurement of changes in absorbance of the reaction mixture during the enzyme reaction. A unit of pectin lyase activity is defined as the amount of enzyme catalyzing an increase in absorbance at 235 nm of the reaction mixture by 0.555 per min at pH 5.2. The millimolar absorption coefficient of the unsaturated products is²⁶⁷ $5.55 \text{ M}^{-1}\text{cm}^{-1}$. Calculation of the unit of activity for exopectate lyase is based on the value of the millimolar absorption coefficient for the unsaturated di(D-galactosiduronic acid) of $4.6 \text{ M}^{-1} \text{ cm}^{-1}$ at pH 8.0. When estimating the activity of endopectate lyase, a millimolar absorption coefficient of $4.8 \text{ M}^{-1} \text{ cm}^{-1}$ is used as the standard, the activity unit being defined²⁶⁸ as the amount of enzyme catalyzing β -eliminative splitting of 1 microequivalent of glycosidic bond per min at pH 8.3.

4-Deoxy-L-*threo*-hexos-5-ulosuronic acid (10), the product of the action of oligo-D-galactosiduronate lyase, shows no absorption at 235 nm; therefore, its activity is estimated on the basis of the decrease in ultraviolet absorption at this wavelength, using "unsaturated di(D-galactosiduronic acid)" as the substrate.

The periodate-thiobarbituric acid test developed by Waravdekar and Saslaw²⁶⁹ is used in the modification of Weissbach and Hurwitz²²⁹ for identification of the products of β -eliminative cleavage.

(266) R. J. W. Byrde and A. H. Fielding, *J. Gen. Microbiol.*, **52**, 287-297 (1968).

(267) P. Albersheim, *Methods Enzymol.*, **8**, 628-631 (1966).

(268) J. D. Macmillan and H. J. Phaff, *Methods Enzymol.*, **8**, 632-635 (1966).

(269) V. S. Waravdekar and L. D. Saslaw, *Biochim. Biophys. Acta*, **24**, 439 (1957).

Exposure to periodate yields 3-formylpyruvic acid, which reacts with thiobarbituric acid, giving rise to red condensation products having an absorption maximum at 545–550 nm. Certain workers have used the thiobarbituric acid test without previous oxidation of the “unsaturated oligo-D-galactosiduronates” with periodic acid.^{3,29,239,260} Even here, the products of reaction with thiobarbituric acid have an absorption maximum at 550 nm.

In addition to the foregoing methods, all of the methods reported in Section V,4 can be used for the assay of lyase activity.

VII. PECTIC ENZYMES IN PLANT PHYSIOLOGY AND PATHOLOGY

The conversion of protopectin, the water-insoluble parent pectic substance, into soluble pectin and pectate and, further, into their cleavage products, is one of the mechanisms playing a role in the plant during its maturation, as well as in the process of infection of the plant. In the plant, protopectin has the function of an intercellular adhesive and, hence, its conversion into the soluble form results in a disruption of tissue rigidity, and in cell separation that is reflected in softening and subsequent liquefaction of the plant material.

Even though the whole mechanism of these conversions is not yet completely known, there is ample evidence that one of the important roles is played by pectic enzymes of the plant or of the pathogen, the first being involved in the physiological changes, and the second in pathological alteration of the plant.

The parallelism observed between the development of pectinesterase and endo-D-galacturonanase activity on the one hand, and the formation of water-soluble pectin observed in the course of fruit maturation on the other, indicates that these two processes are closely related. Thus, in tomatoes, both activities begin to increase exponentially in the course of ripening to the orange-colored stage and continue to rise further through the red stage, up to the overripe stage.^{183,270} No endo-D-galacturonanase is present in unripe fruit, and only a small amount of pectinesterase has been found. In fruit suffering from “blotchy” ripening, the two activities are also rather depressed. A similar correlation between the degree of ripening and the activity of the two enzymes has been observed for dates,^{184,271}

(270) G. E. Hobson, *Biochem. J.*, **86**, 358–365 (1962).

(271) H. A. Al-Jasim and K. S. Al-Delaimy, *J. Sci. Food Agr.*, **23**, 915–917 (1972).

peaches,¹⁸⁵ bananas,¹⁰² and pears.²⁷² In avocados, only endo-D-galacturonanase activity was observed to increase,¹⁸⁶ and the level of pectinesterase was almost equal in young and in ripe fruit.²⁷³ Correlation between ripeness and endo-D-galacturonanase activity was observed, even in a single fruit, for date and avocado; the riper parts contained higher enzyme activity than the unripe.^{184,186}

It is not yet known whether the increase of these activities in the course of fruit ripening is due to a direct synthesis of the enzymes, or whether conversion of inactive precursors into the active enzymes is involved. There is also the possibility of (a) an effect of inhibitors present in unripe fruit on the activity of endo-D-galacturonanase, and (b) disappearance of such inhibitors in the course of ripening.¹⁸⁴ For pectinesterase, it has been speculated that plants may be able to regulate the activity of this enzyme by metabolic control of the amount and proportion of certain, naturally occurring, fatty acids.⁸²

Much attention has been devoted to study of the role of pectic enzymes in plant pathology. Two comprehensive reviews have appeared during the past ten years.^{9,10} Hence, only some of the fundamental aspects and some subsequent observations will be discussed here.

A plant disease caused by a plant pathogen is a complicated process wherein a number of factors play a part. Its extent and course are especially determined by the ability of the pathogen to attack the plant and the ability of the plant to mobilize its protective mechanism against the infection.

According to Albersheim and coworkers,¹⁰ the mechanism of induction of pathogeny is associated with an interaction, between the pathogen and the cell-wall saccharides of the host, which influences the production of enzymes degrading its cell walls. The production of enzyme is regulated both by the pathogen and the host.

A direct role of pectic enzymes produced by the pathogen in the process of infection is indicated by (a) the ability of all known pathogens to produce pectic enzymes, (b) the correlation between the production of these enzymes and the virulence observed in most pathogenic isolates,^{79,274-278} (c) the presence of pectic enzymes pro-

(272) C. W. Nagel and M. E. Patterson, *J. Food Sci.*, **32**, 294-297 (1967).

(273) G. Zauberman and N. M. Schiffmann, *Plant Physiol.*, **49**, 864-865 (1972).

(274) H. W. Mussell and R. J. Green, *Phytopathology*, **60**, 192-195 (1970).

(275) J. M. Wells, *Phytopathology*, **58**, 1598-1602 (1968).

(276) G. P. Singh and A. Husain, *Phytopathology*, **54**, 1100-1101 (1964).

(277) J. H. Chan and W. E. Sackston, *Can. J. Botany*, **50**, 2449-2453 (1972).

(278) H. Ljunggren and G. Fahraens, *J. Gen. Microbiol.*, **26**, 521-528 (1961).

duced by the pathogen in the infected plant-tissue,^{89,279-284} and (d) the correlation between the extent of disease symptoms and the activity of pectic enzymes in the host-pathogen combination.²⁷⁴

Production of pectic enzymes, in particular of those having a random-action pattern, by the pathogen is one of the important prerequisites of successful infection. These enzymes cause a weakening of the cell walls by cleavage of protopectin, whereby the penetration of the pathogen into the plant is made possible.²⁷⁸ Cleavage of protopectin was originally attributed to the specific enzyme protopectinase,^{2,8} but the use of highly purified preparations of pectic enzymes has shown that the decomposition of cell walls is due to the action of a *system* of enzymes,²⁸⁵ among them pectinesterase,^{79,91,281,286-288} endo-D-galacturonanase,^{118,279,285,289} endopectate lyase,^{235,263,281,290} and pectin lyase.^{91,266} On the other hand, Karr and Albersheim,²⁹¹ working with Pectinol R-10, isolated a wall-modifying enzyme which differed from pectin and pectate depolymerases in that it liberated (from the plant tissue) water-soluble polymers only, but degraded pectic acid to only a limited extent. The authors²⁹¹ postulated that the enzyme only splits bonds of importance for the structural integrity of the tissue.

The ability of micro-organisms to produce pectic enzymes *in vitro* constitutes no proof of their pathogenicity. Some micro-organisms produce pectic enzymes on synthetic-nutrient media, but do not always possess the ability to produce them *in vivo*. An important role is here played by the susceptibility or resistance of the plant to the effect of the pathogen. Production of D-galacturonanase and pectinesterase by *Fusarium oxysporum* f. *lycopersici* was found to be much higher on susceptible than on resistant tomato-stems.²⁸⁷ Likewise,

- (279) F. D. Calonge, A. H. Fielding, R. J. W. Byrde, and O. A. Akinferon, *J. Exp. Botany*, **20**, 350-357 (1969).
- (280) J. G. Hancock, *Phytopathology*, **56**, 975-979 (1966).
- (281) L. M. Unbehaun and L. D. Moore, *Phytopathology*, **60**, 304-308 (1970).
- (282) T. Curren, *Can. J. Botany*, **47**, 791-794 (1969).
- (283) J. H. Chan and W. E. Sackston, *Can. J. Botany*, **48**, 1073-1077 (1970).
- (284) I. Barash and S. Khazzam, *Phytochemistry*, **9**, 1189-1197 (1970).
- (285) D. F. Bateman, *Phytopathology*, **53**, 1178-1186 (1963).
- (286) J. G. Hancock, R. L. Millar, and J. W. Lorbeer, *Phytopathology*, **54**, 932-935 (1964).
- (287) D. C. Deese and M. A. Stahmann, *Phytopathology*, **52**, 255-260 (1962).
- (288) W. K. Smith, *J. Gen. Microbiol.*, **18**, 33-41 (1958).
- (289) W. A. Ayers, G. C. Papavizas, and R. D. Lumsden, *Phytopathology*, **59**, 925-930 (1969).
- (290) M. T. Turner and D. F. Bateman, *Phytopathology*, **58**, 1509-1515 (1968).
- (291) A. L. Karr and P. Albersheim, *Plant Physiol.*, **46**, 69-80 (1970).

isolates from resistant stems produced less D-galacturonanase than isolates from susceptible stems.²⁷⁴ One of the causes may consist in the chemical composition of the cell walls of the resistant stems, which represses or controls the production of pectic enzymes.²⁷⁴

In bean hypocotyl, the increase of resistance in the course of ripening was found to be accompanied²⁹² by a decrease in the methoxyl content from 0.5 to 0.2%, while the content of calcium increased from 0.38 to 1.92%. An increased content of calcium was observed in a resistant variety of cucumber hypocotyl.²⁹³ These results suggest that one of the protective mechanisms of the plant against infection is the conversion of pectic acid into calcium pectate, which is resistant to the action of pectic enzymes.

This view is supported by other findings. The severity of tomato wilt after infection with *Fusarium oxysporum* f. *lycopersici* in calcium-deficient mutants was extremely high, whereas the disease was suppressed at high concentrations of calcium.²⁹⁴ Calcium-deficient plants contained more water-soluble pectin than normal plants.²⁹⁵ In bean hypocotyls infected with *Rhizoctonia solani*, an accumulation of ⁴⁵Ca was observed near the lesions.²⁹⁶ In those regions, the tissue was more deeply macerated than healthy tissue. Together with the increase of calcium content, an increased respiration near the site of infection was observed. On the basis of these results, Bateman²⁹⁶ advanced the following hypothesis as to the protective mechanism of the plant toward infection. The increased rate of respiration at the sites of infection results in an accumulation of calcium and, possibly, of other cations which activate the pectinesterase of the host cell-walls. Pectinesterase then de-esterifies pectin in the affected zone. The pectic acid thus formed gives, with calcium or other multivalent cations, insoluble pectates which are then resistant to the action of depolymerases.

Besides this protective mechanism, there is the possibility of the effect of other inhibitors, such as oxidized phenolic compounds,²⁹⁷⁻³⁰⁰

(292) D. F. Bateman and R. D. Lumsden, *Phytopathology*, **55**, 734-738 (1965).

(293) A. Mahadevan, J. Kuć, and E. B. Williams, *Phytopathology*, **55**, 1000-1003 (1965).

(294) M. E. Corden, *Phytopathology*, **55**, 222-224 (1965).

(295) L. V. Edgington, M. E. Corden, and A. E. Dimond, *Phytopathology*, **51**, 179-182 (1961).

(296) D. F. Bateman, *Phytopathology*, **54**, 438-445 (1964).

(297) J. W. Byrde, *Hort. Sci.*, **32**, 227-238 (1957).

(298) M. Cole, *Nature*, **181**, 1596-1597 (1958).

(299) M. Cole and R. K. S. Wood, *Ann. Botany*, **25**, 435-452 (1961).

(300) F. Grossmann, *Phytopathol. Z.*, **45**, 1-20 (1962).

chlorogenic and caffeic acid,³⁰¹ condensed polymers of catechols, and leucoanthocyanidines.^{302,303} The sweet-potato tissue was found to contain a heat-labile inhibitor of microbial, pectin depolymerases which is precipitable with ammonium sulfate.³⁰⁴ Inhibitors having similar properties were found in beans, cabbage, cucumbers, onions, pears, plums, and strawberries.^{305,306} A phytohemagglutinin-like substance inhibiting fungal D-galacturonanase was isolated from the cell walls of red kidney-bean hypocotyls, tomato stems, and a suspension of cultivated sycamore cells.³⁰⁷

- (301) S. S. Patil and A. E. Dimond, *Phytopathology*, **57**, 492-496 (1967).
- (302) T. A. Bell, J. L. Etchells, and W. W. G. Smart, Jr., *Botan. Gaz.*, **126**, 40-45 (1965).
- (303) T. A. Bell, J. L. Etchells, F. C. Williams, and W. L. Porter, *Botan. Gaz.*, **133**, 220-223 (1973).
- (304) I. Uritani and M. A. Stahmann, *Phytopathology*, **51**, 277-285 (1961).
- (305) W. Bock, M. Krause, and G. Dongowski, *Nahrung*, **14**, 375-381 (1970).
- (306) W. Bock, G. Dongowski, and M. Krause, *Nahrung*, **16**, 787-799 (1972).
- (307) P. Albersheim and A. J. Anderson, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1815-1819 (1971).

BIBLIOGRAPHY OF CRYSTAL STRUCTURES OF POLYSACCHARIDES 1967-1974

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I. INTRODUCTION

In Volume 22 of this Series, the data known at that time on the crystalline structures of polysaccharides were reviewed.¹ Since then, an extensive literature has accumulated: new homopolysaccharides, as well as complex polysaccharides, have been studied, and the classical structures have been re-examined under a variety of conditions that yielded much more accurate structures. Instrumental improvements, such as rotating-anode generators and toroidal reflecting-cameras, now lessen the time of exposure of the specimen to the X-rays, with corresponding improvements in the diffractograms. Advances in electron-diffraction techniques using single crystals of a polymer allow new insights into the base-plane dimensions and the symmetry elements of the unit cell.

The problems associated with deriving a polysaccharide structure from diffraction data remain. As the number of parameters needed to describe a segment of the chain is far greater than the number of diffraction spots, it is necessary to start with a model that is reasonable on steric grounds. Then, by comparison between observed and calculated diffraction data, the original postulate is modified to bring

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(1) R. H. Marchessault and A. Sarko, *Adv. Carbohydr. Chem.*, **22**, 421-482 (1967).

it to the best possible agreement. This model-building exercise is now greatly facilitated by the availability of high-speed, digital computers, and increasing improvement in computing strategy is found. Measurement of distances between atoms on a scale model by use of a hand ruler to explore short contacts has become obsolete.

The pioneering efforts to analyze polysaccharide conformations mathematically were those of D. W. Jones,² who presented the *virtual bond method*, and of Ramachandran,^{3,4} who introduced the ϕ, ψ *method*. The former requires a knowledge of the helix symmetry and the pitch. A rotation of the sugar residue is performed about the "virtual bond" joining the linkage atoms, for example, O-1 and O-4 in a (1 \rightarrow 4)-linked polysaccharide. Contiguous residues are generated subject to the constraints imposed by the helix symmetry and the pitch. The steric feasibility is evaluated by computing the interatomic distances between the residues. Following the development of this method for cellulose, by Jones,² the conformations of (1 \rightarrow 4)- β -D-xylan,⁵ O-acetylamylose,⁶ and amylose⁷ have been studied by this approach. A complete description of the virtual-bond method has been presented.⁸

On the other hand, the ϕ, ψ method examines the rotations of contiguous residues about the glycosidic bonds, for example, C-1-O and O-C-4' in a (1 \rightarrow 4)-linked polysaccharide. This method scans the entire conformational space available to a polymer. The analysis can thus proceed either with a regular, helical structure, or a random conformation. The ϕ, ψ method has been used quite extensively in the conformational analysis of polysaccharides.⁹⁻²⁶ The (ϕ, ψ) steric

- (2) D. W. Jones, *J. Polym. Sci.*, **32**, 371-394 (1958).
- (3) G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, in "Aspects of Protein Structure," G. N. Ramachandran, ed., Academic Press, London, 1967, pp. 121-135.
- (4) G. N. Ramachandran, in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, eds., Freeman, San Francisco, 1968, pp. 77-87.
- (5) W. J. Settineri and R. H. Marchessault, *J. Polym. Sci., Part C*, **11**, 253-264 (1965).
- (6) A. Sarko and R. H. Marchessault, *J. Am. Chem. Soc.*, **89**, 6454-6462 (1967).
- (7) A. D. French and V. G. Murphy, *Carbohydr. Res.*, **27**, 391-406 (1973).
- (8) P. R. Sundararajan and R. H. Marchessault, *Can. J. Chem.*, **53**, 3563-3566 (1975).
- (9) V. S. R. Rao, P. R. Sundararajan, C. Ramakrishnan, and G. N. Ramachandran, in "Conformation of Biopolymers," G. N. Ramachandran, ed., Academic Press, London, 1967, pp. 721-737.
- (10) D. A. Rees and R. J. Skerrett, *Carbohydr. Res.*, **7**, 334-348 (1968).
- (11) J. Blackwell, A. Sarko, and R. H. Marchessault, *J. Mol. Biol.*, **42**, 379-383 (1968).
- (12) D. A. Rees, *J. Chem. Soc., B*, 217-226 (1969).
- (13) P. R. Sundararajan and V. S. R. Rao, *Biopolymers*, **8**, 305-312 (1969).
- (14) P. R. Sundararajan and V. S. R. Rao, *Biopolymers*, **8**, 313-323 (1969).

map is now available for the disaccharide segment of most of the known polysaccharides. The use of semi-empirical, potential-energy functions to estimate the nonbonded, coulombic, and hydrogen-bond interaction-energies leads to a quantitative correlation of the several helical and statistical conformations of polysaccharides.

In the next phase of the stereochemistry of polysaccharides, a significant advance was made when Arnott²⁷ introduced the method of *linked-atom, least-squares analysis* to this area. This method allows a chain to be generated, with a certain amount of flexibility in the ring geometry. The average bond lengths, bond angles, and torsion angles, and their standard deviations, as derived from the crystal-structure results on small molecules, serve as the input data. The atomic coordinates are then calculated. The geometrical parameters are varied, within the limits of their standard deviations, to meet the constraints, such as the ring closure, the repeat distance along the chain, and hydrogen-bonding criteria. At about the same time, novel methods^{28,29} became available to pack the chains in the crystalline lattice. Application of these two procedures improved the degree of precision in the structure analysis of polysaccharides.³⁰⁻³² Of course, the prerequisite for such analyses is a reasonably good understanding of the geometrical features of the related mono- and disaccharides. Accurate structure-data based on single-crystal crys-

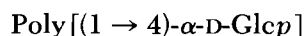
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- (15) P. R. Sundararajan and V. S. R. Rao, *Biopolymers*, **9**, 1239-1247 (1970).
 - (16) D. A. Rees and R. J. Skerrett, *J. Chem. Soc., B*, 189-193 (1970).
 - (17) C. V. Goebel, W. L. Dimpfl, and D. A. Brant, *Macromolecules*, **3**, 644-654 (1970).
 - (18) B. K. Sathyanarayana and V. S. R. Rao, *Biopolymers*, **10**, 1065-1615 (1971).
 - (19) D. A. Rees and W. E. Scott, *J. Chem. Soc., B*, 469-479 (1971).
 - (20) D. A. Rees and A. W. Wight, *J. Chem. Soc., B*, 1366-1372 (1971).
 - (21) B. K. Sathyanarayana and V. S. R. Rao, *Biopolymers*, **11**, 1379-1394 (1972).
 - (22) S. M. Gabbay, P. R. Sundararajan, and R. H. Marchessault, *Biopolymers*, **11**, 79-94 (1972).
 - (23) P. R. Sundararajan and R. H. Marchessault, *Can. J. Chem.*, **50**, 792-794 (1972).
 - (24) P. R. Sundararajan, R. H. Marchessault, G. J. Quigley, and A. Sarko, *J. Am. Chem. Soc.*, **95**, 2001-2008 (1973).
 - (25) A. Sarko and R. Muggli, *Macromolecules*, **7**, 486-494 (1974).
 - (26) R. H. Marchessault and P. R. Sundararajan, *Pure Appl. Chem.*, **42**, 399-415 (1975).
 - (27) S. Arnott and W. E. Scott, *J. Chem. Soc. Perkin Trans. II*, 324-335 (1972).
 - (28) D. E. Williams, *Acta Crystallogr., Sect. A*, **25**, 464-470 (1969).
 - (29) P. Zugenmaier and A. Sarko, *Acta Crystallogr., Sect. B*, **28**, 3158-3166 (1972).
 - (30) W. T. Winter and A. Sarko, *Biopolymers*, **13**, 1447-1460 (1974).
 - (31) W. T. Winter and A. Sarko, *Biopolymers*, **13**, 1461-1482 (1974).
 - (32) S. Arnott, W. E. Scott, D. A. Rees, and C. G. A. McNab, *J. Mol. Biol.*, **90**, 253-267 (1974).

tallography of carbohydrates and oligosaccharides has contributed immensely to this advancing field of knowledge.^{26,33,34}

In this article, we have collected the crystal-structure data on polysaccharides reported during the period 1967–1974. In addition to the unit-cell dimensions, the significant features of the structures are also mentioned. The chain axis in most publications is along the *c* direction of the unit cell. (In recent publications, the authors have found it convenient to visualize the chain axis as being the *z* axis of the coordinate system, and hence have chosen to relate it to the *c* dimension of the unit cell.) Only in those instances in which the *b* axis of the unit cell is along the chain axis is it so stated in what follows. Helical symmetry, when represented by the notation *n*(*h*), means that there are *n* residues per turn, *h* being the projected height of the repeating unit onto the helix axis. The reliability factor, *R*, which is a rough estimate of the accuracy of the structure, is given if the authors reported it. In such cases, $R = \Sigma |I_{\text{obs}}^{1/2} - I_{\text{cal}}^{1/2}| / \Sigma I_{\text{obs}}^{1/2}$. In some instances, this is also called the “error function.” In the title to each abstract, a common name or descriptive title for the polysaccharide described is given at the left, and the chemical formula at the right.

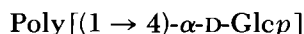
II. AMYLOSE AND OTHER α -D-GLYCANS

1. V-Amylose^{35,36}



The unit-cell dimensions are: for the anhydrous form, *a* = 13.0 Å (1.30 nm), *b* = 22.5 Å (2.25 nm), and *c* = 7.90 Å (790 pm); and for the hydrate, *a* = 13.7 Å (1.37 nm), *b* = 23.8 Å (2.38 nm), and *c* = 8.05 Å (805 pm). The space group is P2₁2₁2₁ for both forms. A continuous expansion of helix diameter as a function of the water content is observed.

2. V-Amylose^{36,37}



The space group is P2₁2₁2₁, with *a* = 13.61 Å (1.361 nm), *b* = 23.60 Å (2.360 nm), and *c* = 8.01 Å (801 pm). Comparison was made of the hydrous and dehydrated structures, and it was proposed

(33) G. A. Jeffrey and R. D. Rosenstein, *Adv. Carbohydr. Chem.*, **19**, 7–22 (1964).

(34) G. A. Jeffrey and M. Sundaralingam, *Adv. Carbohydr. Chem. Biochem.*, **30**, 445–466 (1974); **31**, 347–371 (1975); **32**, 353–384 (1976).

(35) H. F. Zobel, A. D. French, and M. E. Hinkle, *Biopolymers*, **5**, 837–845 (1967).

(36) A. D. French and B. Zaslow, *J. Chem. Soc. Chem. Commun.*, 41–42 (1972).

(37) B. Zaslow, V. G. Murphy, and A. D. French, *Biopolymers*, **13**, 779–790 (1974).

that a net rotation of 30° of the helices about their axes accompanies the hydrate-anhydrous transition. Molecules of water of hydration were not located. The error function, considering only the equatorial reflections, is 16%, and for all of the observed and the unobserved reflections, it is 76%.

3. Anhydrous V-amylose³⁰ Poly[(1 \rightarrow 4)- α -D-Glcp]

The space group is $P2_12_12_1$, with $a = 12.97 \text{ \AA}$ (1.297 nm), $b = 22.46 \text{ \AA}$ (2.246 nm), and $c = 7.91 \text{ \AA}$ (791 pm). A left-handed, 6-fold, helical conformation was proposed, with intra-chain hydrogen-bonds OH-2---OH-3' and OH-2---OH-6. A corner-to-center chain, OH-2---OH-2 hydrogen-bond was also suggested. With four molecules of water, the R factor is 29.7%.

4. Amylose-dimethyl sulfoxide complex³⁸ Poly[(1 \rightarrow 4)- α -D-Glcp]

Alternate "up" and "down" helices, having six residues per turn, pack in a pseudotetragonal cell with $a = b = 19.21 \text{ \AA}$ (1.921 nm) and $c = 8.12 \text{ \AA}$ (812 pm). The space group is $P2_12_1$. There are 8 molecules of dimethyl sulfoxide in the cell, three of which are inside the helix.

5. Amylose-halogenated hydrocarbon complex³⁹ Poly[(1 \rightarrow 4)- α -D-Glcp]

Amylose complexes (wet precipitates) were prepared with: fluoro-benzene, 1,1,2,2-tetrachloroethane, 1,1,2,2-tetrabromoethane, bromoform, and *tert*-butyl alcohol. The conformation and packing of the amylose chains complexed with halogen-substituted hydrocarbons are the same as found in the complex with *tert*-butyl alcohol, namely, 7-fold helices packed in a pseudo-hexagonal array. The diffraction maxima at 14.2, 14.5, and 16.0 \AA (1.42, 1.45, and 1.60 nm) were interpreted as being due to the halogen atoms, located inside the amylose helix, at specific sites.

6. Amylose-1-butanol complex⁴⁰ Poly[(1 \rightarrow 4)- α -D-Glcp]

Both hydrated and anhydrous forms are found. For the hydrate, $a = 13.70 \text{ \AA}$ (1.370 nm), $b = 25.80 \text{ \AA}$ (2.580 nm), and $c = 8.10 \text{ \AA}$ (810 pm). For the anhydrous form, $a = 13.20 \text{ \AA}$ (1.320 nm), $b = 27.0 \text{ \AA}$

(38) A. D. French and H. F. Zobel, *Biopolymers*, **5**, 457-464 (1967).

(39) R. R. Bumb and B. Zaslow, *Carbohydr. Res.*, **4**, 98-101 (1967).

(40) M. E. Hinkle and H. F. Zobel, *Biopolymers*, **6**, 1119-1128 (1968).

(2.70 nm), and $c = 7.92 \text{ \AA}$ (792 pm). The space group is $P2_12_12_1$ for both forms. The complexes are unstable in the open air, and revert to V-amylose hydrate on standing. The change of unit-cell dimensions as a function of the hydrous-anhydrous transition of amylose-1-butanol was discussed.

7. Amylose-potassium bromide complex⁴¹ Poly[(1 \rightarrow 4)- α -D-Glcp]

The unit cell is tetragonal, with $a = b = 10.7 \text{ \AA}$ (1.07 nm) and $c = 16.1 \text{ \AA}$ (1.61 nm). The amylose helix is left-handed, with four D-glucose residues per turn. Both ions are located in a water-like environment. The atoms O-2, O-3, and O-4 from D-glucose residues on adjacent chains coordinate around K^+ . The R factor is 41%.

8. Amylose-ethylenediamine complex⁴² Poly[(1 \rightarrow 4)- α -D-Glcp]

The unit cell is tetragonal, with a symmetry approximating $P2_12_12_1$. The cell dimensions are $a = b = 18.87 \text{ \AA}$ (1.887 nm) and $c = 7.99 \text{ \AA}$ (799 pm). The helix diameter is 13.3 \AA (1.33 nm). One ethylenediamine molecule for every two D-glucose residues is indicated. The location of the ethylenediamine molecule in the lattice was discussed. The structure is almost identical to that of the amylose-dimethyl sulfoxide complex.

9. V-Amylose-1-naphthol complex⁴³ Poly[(1 \rightarrow 4)- α -D-Glcp]

Electron diffraction by lamellar, single crystals leads to a two-dimensional, tetragonal unit-cell with $a = b = 22.9 \text{ \AA}$ (2.29 nm). From X-ray diffraction data obtained from a film of sedimented, lamellar crystals, it was found that the c axis spacing (7.8 \AA ; 780 pm) is equivalent to that in 6-fold and 7-fold amylose helices. The true helical diameters of the 1-butanol, isopropyl alcohol, and 1-naphthol complexes were calculated from experimental data. The ratios of 6:7:8 indicated that the 1-naphthol complex has eight D-glucose residues per turn. The diversity of helical orientations in V-amylose crystals was discussed.

10. Amylose-dimethyl sulfoxide complex⁴⁴ Poly[(1 \rightarrow 4)-D-Glcp]

(41) J. J. Jackobs, R. R. Bumb, and B. Zaslow, *Biopolymers*, **6**, 1659-1670 (1968).

(42) T. D. Simpson, *Biopolymers*, **9**, 1039-1047 (1970).

(43) Y. Yamashita and K. Monobe, *J. Polym. Sci., Part A-2*, **9**, 1471-1481 (1971).

(44) T. D. Simpson, F. R. Dintzis, and N. W. Taylor, *Biopolymers*, **11**, 2591-2600 (1972).

The unit cell is orthogonal, with $a = 30.23 \text{ \AA}$ (3.023 nm), $b = 28.18 \text{ \AA}$ (2.818 nm), and $c = 7.91 \text{ \AA}$ (791 pm), containing amylose having a 7-fold helical structure. Dimethyl sulfoxide and amylose complex in at least two crystalline conformations: 6-fold and 7-fold helices. The proportion of water present influences the conformation resulting. The two polymorphs are distinguished by reflections of spacings 12.74 and 10.26 \AA (1.274 and 1.026 nm) in the 7-fold, and 13.47 \AA (1.347 nm) and 9.48 \AA (948 pm) in the 6-fold, helical structures.

11. Amylose–dimethyl sulfoxide complex^{31,45} Poly[(1 → 4)- α -D-Glcp]

The space group is $P2_12_12_1$. The unit cell is pseudotetragonal, with $a = b = 19.17 \text{ \AA}$ (1.917 nm), and $c = 24.39 \text{ \AA}$ (2.439 nm), with two antiparallel chains per cell. The amylose chain is a left-handed 6(–1.355) helix, with three turns per crystallographic repeat. One molecule of dimethyl sulfoxide for every three D-glucose residues is located inside the helix. An additional 4 molecules of dimethyl sulfoxide and 8 of water are located in the interstices. The interstitial dimethyl sulfoxide is the source of additional layer-lines that are not consistent with the 8.13 \AA (813 pm) amylose repeat. The overall R factor is 35%, and, for the layer lines with amylose contribution alone, it is 29%.

12. B-Amylose¹¹ Poly[(1 → 4)- α -D-Glcp]

The unit cell is orthorhombic, with $a = 15.9$ (or 31.8) \AA (1.59 or 3.18 nm), $b = 9.1 \text{ \AA}$ (910 pm) (or 18.2 \AA ; 1.82 nm), and $c = 10.4 \text{ \AA}$ (1.04 nm). The helices are left-handed, with a 6-fold screw-axis. It was proposed that, in V-amylose, an intra-chain hydrogen-bond of the type OH-2---OH-6 exists between the i^{th} and $(i + 6)^{\text{th}}$ residues, and, upon conversion into B-amylose, the hydrogen bond is of the type OH-2---(H₂O)---OH-6.

13. Nageli amylodextrin^{46,47} Poly[(1 → 4)- α -D-Glcp]

Amylodextrins from waxy-maize starch (A type) and potato starch (B type) retain the same diffraction pattern as that of the parent starch. On separation of a starch to give an amylo-dextrin, the

(45) W. T. Winter and A. Sarko, *Biopolymers*, **11**, 849–852 (1972).

(46) K. Kainuma and D. French, *Biopolymers*, **10**, 1673–1680 (1971).

(47) K. Kainuma and D. French, *Biopolymers*, **11**, 2241–2250 (1972).

sharpness and intensity of the diffraction patterns are either retained, improved, or developed, depending on the parent starch. Failure of the B-structure to collapse during drying is interpreted to mean that water is not intercalated between turns of a helix or otherwise required in order to maintain the packing geometry. A model for B-starch was proposed that employs intertwined (double) helices, packed in a unit cell with $a = 12.0 \text{ \AA}$ (1.20 nm), $b(\text{fiber axis}) = 10.48 \text{ \AA}$ (1.048 nm), $c = 16.25 \text{ \AA}$ (1.625 nm), and $\beta = 96.5^\circ$.

14. O-Acetylamylose I⁶ Poly[(1 \rightarrow 4)-Ac- α -D-Glcp]

The unit cell is pseudohexagonal, with $a = 10.87 \text{ \AA}$ (1.087 nm), $b = 18.83 \text{ \AA}$ (1.883 nm), and $c = 52.53 \text{ \AA}$ (5.253 nm). The chain contains 14 monomers in three turns of the left-handed helix. Antiparallel packing of the chains yields the best fit with the X-ray data and the least number of close contacts. The overall R factor is 58%, and it is 30% for the zero, third, and sixth layer reflections alone.

15. Mycodextran (nigeran)²⁴ Poly[(1 \rightarrow 3)- α -D-Glcp-(1 \rightarrow 4)- α -D-Glcp]

Stacks of lamellar, single crystals were used to obtain a fiber diagram. The unit-cell dimensions are $a = 17.6 \text{ \AA}$ (1.76 nm), $b = 6.85 \text{ \AA}$ (685 pm), and $c = 13.4 \text{ \AA}$ (1.34 nm), with a two-fold screw-axis along the chain. Intramolecular hydrogen-bonds of the type OH-2---OH-3' between the residues having the α -D-(1 \rightarrow 4)-linkage and OH-2---OH-4' between the residues having the α -D-(1 \rightarrow 3)-linkage were proposed. In the crystal, the conformation of the chain is that of a "corrugated ribbon."

III. CELLULOSE AND OTHER β -D-GLYCANS

1. Sodiocelluloses and their regeneration Poly[(1 \rightarrow 4)- β -D-Glcp] complexes⁴⁸

The products formed by the decomposition of sodiocellulose by aqueous reagents and alcohols were studied. For the sodiocellulose prepared from native cellulose by aqueous treatment, the spacing of the 101 reflection varied considerably with the experimental conditions (the greatest being 9.24 \AA ; 924 pm). Although the 101 and 002 reflections did not vary in spacing, their relative intensities were af-

(48) J. O. Warwicker and A. C. Wright, *J. Appl. Polym. Sci.*, **11**, 659-671 (1967).

fect. For $d_{101} = 9.24 \text{ \AA}$ (924 pm), the unit-cell, base dimensions are $a = 10.02 \text{ \AA}$ (1.002 nm), $c = 10.20 \text{ \AA}$ (1.020 nm), and $\beta = 52.05^\circ$. Similar results were obtained for sodiocelluloses from mercerized ramie and from fortisan. The following cell-dimensions were obtained for sodiocelluloses washed in alcohols.

(1) Treated with methanol: (i) wet, $a = 9.74 \text{ \AA}$ (974 pm), $c = 10.01 \text{ \AA}$ (1.001 nm), $\beta = 53.3^\circ$; (ii) dried in air, $a = 8.57 \text{ \AA}$ (857 pm), $c = 9.32 \text{ \AA}$ (932 pm), $\beta = 59.4^\circ$; (iii) dried at 110° , $a = 8.22 \text{ \AA}$ (822 pm), $c = 9.14 \text{ \AA}$ (914 pm), $\beta = 61.6^\circ$.

(2) Treated with ethanol: (i) wet, $a = 13.4 \text{ \AA}$ (1.34 nm), $c = 12.5 \text{ \AA}$ (1.25 nm), $\beta = 40.1^\circ$; (ii) dried in air, $a = 10.9 \text{ \AA}$ (1.09 nm), $c = 10.75 \text{ \AA}$ (1.075 nm), $\beta = 48.3^\circ$; (iii) dried at 110° , $a = 9.28 \text{ \AA}$ (928 pm), $c = 9.73 \text{ \AA}$ (973 pm), $\beta = 55.6^\circ$.

(3) Treated with 1-propanol: (i) wet, $a = 9.26 \text{ \AA}$ (926 pm), $c = 9.59 \text{ \AA}$ (959 pm), $\beta = 57^\circ$; (ii) dried in air, $a = 12.11 \text{ \AA}$ (1.211 nm), $c = 11.6 \text{ \AA}$ (1.16 nm), $\beta = 43.9^\circ$.

(4) Treated with 1-butanol: (i) wet, $a = 12.11 \text{ \AA}$ (1.211 nm), $c = 11.6 \text{ \AA}$ (1.16 nm), $\beta = 43.9^\circ$; (ii) dried in air, $a = 9.28 \text{ \AA}$ (928 pm), $c = 9.73 \text{ \AA}$ (973 pm), $\beta = 55.6^\circ$.

In all these cases, $b(\text{fiber axis}) = 10.3 \text{ \AA}$ (1.03 nm).

2. Single crystals of cellulose⁴⁹

Poly[(1 \rightarrow 4)- β -D-Glcp]

Single crystals of cellulose were prepared by regeneration (saponifying cellulose formate and cellulose acetate solutions). The unit cell is monoclinic, with $a = 10.4 \text{ \AA}$ (1.04 nm), $b(\text{chain direction}) = 10.3 \text{ \AA}$ (1.03 nm), $c = 10.4 \text{ \AA}$ (1.04 nm), and $\beta = 60^\circ$. Whereas the angle β is close to that found for celluloses II and III, the lattice dimensions are close to those of water cellulose [$a = 10.3 \text{ \AA}$ (1.03 nm), $b = 10.3 \text{ \AA}$ (1.03 nm), $c = 9.98 \text{ \AA}$ (998 pm)]. Two water molecules per D-glucose residue are contained in the crystal.

3. Algal cellulose⁵⁰

Poly[(1 \rightarrow 4)- β -D-Glcp]

The unit cell of cellulose from *Chaetomorpha melagonium* is monoclinic, with $a = 16.43 \text{ \AA}$ (1.643 nm), $b(\text{fiber axis}) = 10.33 \text{ \AA}$ (1.033 nm), $c = 15.70 \text{ \AA}$ (1.570 nm), and $\beta = 96.97^\circ$. In base-plane projection, each of the Meyer-Misch subcells that make up the super-lattice are identical. All equatorial reflections can be indexed by using a one-chain unit-cell, meaning that every single chain has

(49) S. Munekata and H. Sobue, *J. Polym. Sci., Part B*, **5**, 1043-1045 (1967).

(50) I. A. Nieduszynski and E. D. T. Atkins, *Biochim. Biophys. Acta*, **222**, 109-118 (1970).

the same equatorial projection. Eight possible 8-chain unit-cells were suggested, each containing sheets of chains in the same sense, alternating with sheets of chains of opposite sense.

4. Cellulose I⁵¹

Poly[(1 → 4)-β-D-Glcp]

A monoclinic unit-cell with $a = 8.2 \text{ \AA}$ (820 pm), b (fiber axis) = 10.30 \AA (1.030 nm), $c = 7.90 \text{ \AA}$ (790 pm), and $\beta = 83.3^\circ$ is used. The distance between the terminal oxygen atoms in the cellobiose unit is taken to be 10.3912 \AA (1.03912 nm). A left-handed, helical structure, with seven cellobiose residues in a pitch of 72.1 \AA (7.21 nm) was proposed. The packing arrangement involves the central reversed and corner chains, and a relative shift between them of 0.25 repeat length along the b axis.

5. *Valonia* cellulose²⁵

Poly[(1 → 4)-β-D-Glcp]

The unit cell is monoclinic, with $a = 15.76 \text{ \AA}$ (1.576 nm), $b = 16.42 \text{ \AA}$ (1.642 nm), $c = 10.34 \text{ \AA}$ (1.034 nm), and $\gamma = 96.8^\circ$, containing 8 chains. The chains with a twofold screw-axis along their axes, pack with parallel polarity. The OH-3'---O-5 and OH-2---O-6' intramolecular hydrogen-bonds and OH-6---OH-3 intermolecular hydrogen-bond occur. The R factor is 32%. A new triclinic cell with $a = 9.41 \text{ \AA}$ (941 pm), $b = 8.15 \text{ \AA}$ (815 pm), $c = 10.34 \text{ \AA}$ (1.034 nm), $\alpha = 90^\circ$, $\beta = 57.5^\circ$, and $\gamma = 96.2^\circ$ was proposed. Based on packing analysis, an extensively hydrogen-bonded, antiparallel structure was proposed for cellulose II, and the interconversion of celluloses I and II in terms of their stability was discussed.

6. Native cellulose^{52,53}

Poly[(1 → 4)-β-D-Glcp]

The unit cell is monoclinic with $a = 16.34 \text{ \AA}$ (1.634 nm), $b = 15.72 \text{ \AA}$ (1.572 nm), $c = 10.38 \text{ \AA}$ (1.038 nm), and $\gamma = 97^\circ$. The space group is $P2_1$. The cell contains disaccharide segments of 8 chains. The parallel packing of the chains is favored. There are two intra-chain hydrogen-bonds, OH-3---O-5' and OH-6---OH-2'. An intermolecular hydrogen-bond of the type OH-6---OH-3 lies along the a axis. The R factor is 21.5%. Arguments were presented against regular chain-folding in native cellulose.

(51) A. Viswanathan and S. G. Shenouda, *J. Appl. Polym. Sci.*, **15**, 519-535 (1971).

(52) K. H. Gardner and J. Blackwell, *Biopolymers*, **13**, 1975-2001 (1974).

(53) K. H. Gardner and J. Blackwell, *Biochim. Biophys. Acta.* **343**, 232-237 (1974).

7. Tri-*O*-acetylcellulose-nitromethane complex⁵⁴ Poly[(1 → 4)-Ac-β-D-Glcp]

Lamellar, single crystals of cellulose triacetate, precipitated from nitromethane with butyl alcohol, were studied by X-ray and electron diffraction. Only the crystals containing the mother liquor, or moistened with nitromethane, showed rich diffraction details. From stretched and annealed fibers, it was found that the unit cell is tetragonal, with $a = b = 21.15 \text{ \AA}$ (2.115 nm), and $c = 41.36 \text{ \AA}$ (4.136 nm).

8. (1 → 4)-β-D-Xylan^{55,56} Poly[(1 → 4)-β-D-Xylp]

For the typical "hemicellulose" pentosan, a major component of hardwoods, three types of unit cell are found: (a) the dry cell, $a = b = 8.8 \text{ \AA}$ (880 pm), $c = 14.85 \text{ \AA}$ (1.485 nm), and $\gamma = 120^\circ$; (b) the hydrate (one water molecule per residue), $a = b = 9.16 \text{ \AA}$ (916 pm), $c = 14.85 \text{ \AA}$ (1.485 nm), and $\gamma = 120^\circ$; and (c) the dihydrate (two water molecules per residue, at 100% relative humidity), $a = b = 9.64 \text{ \AA}$ (964 pm), $c = 14.85 \text{ \AA}$ (1.485 nm), and $\gamma = 120^\circ$. For the hydrate, the chains are antiparallel in the space group $P3_221$. The 3(−4.95) helices are stabilized by their interaction with chains of water molecules. The *R* factor is 20%. Changes in the X-ray diagram with relative humidity were discussed.

9. *O*-Acetylxyln²² Poly[(1 → 4)-Ac-β-D-Xylp]

The unit cell is monoclinic, with $a = 7.64 \text{ \AA}$ (764 pm), $b = 12.44 \text{ \AA}$ (1.244 nm), $c = 10.31 \text{ \AA}$ (1.031 nm), and $\gamma = 85^\circ$. The unit cell contains two chains, each with a twofold screw-axis along its axis. Intermolecular forces are responsible for assuming of a twofold screw by the helix of *O*-acetylxyln.

10. (1 → 3)-β-D-Xylan⁵⁷⁻⁵⁹ Poly[(1 → 3)-β-D-Xylp]

This polysaccharide is the cell-wall material of siphonous green algae. The unit cell is hexagonal, with $a = c = 15.4 \text{ \AA}$ (1.54 nm),

(54) A. Kuppel, H. Bittiger, and E. Husemann, *Kolloid-Z.*, **250**, 623-624 (1972).

(55) I. A. Nieduszynski and R. H. Marchessault, *Nature (London)*, **232**, 46-47 (1971).

(56) I. A. Nieduszynski and R. H. Marchessault, *Biopolymers*, **11**, 1335-1344 (1972).

(57) E. D. T. Atkins and K. D. Parker, *Nature (London)*, **220**, 784-785 (1968).

(58) E. D. T. Atkins, K. D. Parker, and R. D. Preston, *Proc. Roy. Soc. London, Ser. B*, **173**, 209-221 (1969); *Chem. Abstr.*, **71**, 971 (1969).

(59) E. D. T. Atkins and K. D. Parker, *J. Polym. Sci., Part C*, **28**, 69-81 (1969).

$b(\text{fiber axis}) = 6.12 \text{ \AA}$ (612 pm), and $\beta = 120^\circ$. The space group is $P6_2$. Six D-xylose residues form a right-handed helix of pitch 18.36 \AA (1.836 nm), three such helices intertwining. There is one triple helix per cell. The OH-2 groups point roughly towards the center of the triple helix, so that, through operation of the threefold axis, a triad of OH-2 groups is generated to form interchain hydrogen-bonds with each other. There is one stoichiometrically bound water molecule per residue. The R factor is 29%.

11. Mannans I and II⁶⁰Poly[(1 \rightarrow 4)- β -D-Manp]

Codium cell-walls contain no crystalline mannan unless they are treated with boiling water. An orthorhombic unit-cell with $a = 7.21 \text{ \AA}$ (721 pm), $b(\text{fiber axis}) = 10.27 \text{ \AA}$ (1.027 nm), and $c = 8.82 \text{ \AA}$ (882 pm) was derived. Cell-wall material treated with 12–14% potassium hydroxide gave a different diagram, corresponding to mannan II. In a water-saturated atmosphere, the unit cell for mannan II is monoclinic, with $a = 18.8 \text{ \AA}$ (1.88 nm), $b(\text{fiber axis}) = 10.2 \text{ \AA}$ (1.02 nm), $c = 18.7 \text{ \AA}$ (1.87 nm), and $\beta = 57.5^\circ$.

12. Mannan I⁶¹Poly[(1 \rightarrow 4)- β -D-Manp]

The cell walls of the treated algae (*Codium*), give a fiber diagram corresponding to an orthorhombic unit-cell with $a = 8.82 \text{ \AA}$ (882 pm), $b = 7.21 \text{ \AA}$ (721 pm), and $c = 10.27 \text{ \AA}$ (1.027 nm). The space group is $P2_12_12_1$. An intrachain hydrogen-bond of the type OH-3---O-5' and an interchain hydrogen-bond OH-2---O-5 were proposed, and these were supported by the results of polarized-infrared spectroscopy. The same unit-cell is valid for the vegetable matter from ivory nut. The glucomannan from white pine was treated successively with reagents of increasing hydrolytic strength, and changes in crystallization were monitored by the X-ray powder patterns. Except for a reflection of spacing 7.7 \AA (770 pm), the d spacings are compatible with the lattice dimensions of mannan I.

13. O-Acetylmannan⁶²Poly[(1 \rightarrow 4)-Ac- β -D-Manp]

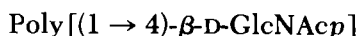
The fiber repeat is 15.24 \AA (1.524 nm). The threefold screw symmetry of the chain was confirmed by the observation of a third layer-line, meridional reflection.

(60) E. Frei and R. D. Preston, *Proc. Roy. Soc. London, Ser. B*, **169**, 127–145 (1968); *Chem. Abstr.*, **68**, 57,399 (1968).

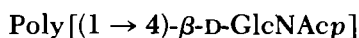
(61) I. A. Nieduszynski and R. H. Marchessault, *Can. J. Chem.*, **50**, 2130–2138 (1972).

(62) H. Bittiger and R. H. Marchessault, *Carbohydr. Res.*, **18**, 469–470 (1971).

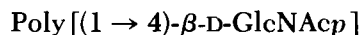
IV. GLYCOSAMINOGLYCANS (AMINO POLYSACCHARIDES)

1. β -Chitin^{63,64}

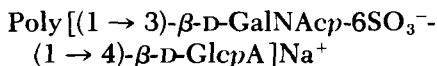
The pogonophore tubes of certain marine diatoms contain β -chitin as a mixture of two phases, A and B. For the anhydrous A form, $a = 4.85 \text{ \AA}$ (485 pm), $b = 10.38 \text{ \AA}$ (1.038 nm), $c = 9.26 \text{ \AA}$ (926 pm), and $\beta = 97.5^\circ$. The chains are packed parallel to each other in the space group $P2_1$. The R factor is 31%. Two distinct hydrates of the A form have unit-cell dimensions $a = 4.8 \text{ \AA}$ (480 pm), $b = 10.4 \text{ \AA}$ (1.04 nm), $c = 10.5 \text{ \AA}$ (1.05 nm), $\beta = 97^\circ$; and $a = 4.8 \text{ \AA}$ (480 pm), $b = 10.4 \text{ \AA}$ (1.04 nm), $c = 11.1 \text{ \AA}$ (1.11 nm), $\beta = 97^\circ$. For the two hydrates in the B form, these are $a = 9.67 \text{ \AA}$ (967 pm), $b = 10.4 \text{ \AA}$ (1.04 nm), $c = 21.1 \text{ \AA}$ (2.11 nm), $\beta = 99.5^\circ$; and $a = 4.78 \text{ \AA}$ (478 pm), $b = 10.1 \text{ \AA}$ (1.01 nm), $c = 23.3 \text{ \AA}$ (2.33 nm), and $\beta = 94^\circ$. The fiber axis is b .

2. Diatom chitin⁶⁵

A highly crystalline form of chitin is found in the spines of certain diatoms. The unit cell is monoclinic, with $a = 4.8 \text{ \AA}$ (480 pm), b (fiber axis) $= 10.32 \text{ \AA}$ (1.032 nm), $c = 9.83 \text{ \AA}$ (983 pm), and $\beta = 112^\circ$. The space group is $P2_1$, with one chain per unit cell. An intramolecular hydrogen-bond of the type OH-3---O-5' and two intermolecular hydrogen-bonds involving a hydroxymethyl group and the amide hydrogen atom were proposed.

3. α -Chitin⁶⁶

The chitobiose unit has been treated as a rigid body, and by using the full-matrix, least-squares, rigid-body, refinement procedure, the structure was refined to an R factor of 40.7%. Visually estimated intensities were used. The structure was found to be free from short contacts, and to be stabilized by an intrachain OH-3---O-5' hydrogen-bond and one interchain N-H---O hydrogen-bond.

4. Chondroitin 6-sulfate⁶⁷

(63) J. Blackwell, K. D. Parker, and K. M. Rudall, *J. Mol. Biol.*, **28**, 383-385 (1967).

(64) J. Blackwell, *Biopolymers*, **7**, 281-298 (1969).

(65) N. E. Dweltz, J. R. Colvin, and A. G. McInnes, *Can. J. Chem.*, **46**, 1513-1521 (1968).

(66) C. Ramakrishnan and N. Prasad, *Biochim. Biophys. Acta*, **261**, 123-135 (1972).

(67) E. D. T. Atkins, R. Gaussen, D. H. Isaac, V. Nandanwar, and J. K. Sheehan, *J. Polym. Sci., Part B*, **10**, 863-865 (1972).

The unit cell is orthorhombic, with $a = 12.1 \text{ \AA}$ (1.21 nm), b (fiber axis) $= 28.5 \text{ \AA}$ (2.85 nm), $c = 14.4 \text{ \AA}$ (1.44 nm), or monoclinic, with $a = 12.1 \text{ \AA}$ (1.21 nm), b (fiber axis) $= 28.5 \text{ \AA}$ (2.85 nm), $c = 9.3 \text{ \AA}$ (930 pm), and $\beta = 93^\circ$. A threefold helical structure, with three disaccharide units per turn, repeating every 28.5 \AA (2.85 nm), was proposed. Fibers of the free acid form show a lessened repeat of 18.6 \AA (1.86 nm), with a twofold helical conformation for the chain.

5. Chondroitin 6-sulfate^{68,69} Poly[(1 \rightarrow 3)- β -D-GalNAcp-6SO₃⁻-(1 \rightarrow 4)- β -D-GlcpA]Na⁺

This polymer crystallizes in three polymorphs. The threefold helical structure packs in a trigonal unit-cell with $a = 14.3 \text{ \AA}$ (1.43 nm) and $c = 28.7 \text{ \AA}$ (2.87 nm). The 8-fold helical structure occurs in a tetragonal unit-cell with $a = 13.8 \text{ \AA}$ (1.38 nm) and $c = 78.2 \text{ \AA}$ (7.82 nm). Axial periodicity in both cases is similar [$h = 9.6 \text{ \AA}$ (960 pm) and 9.8 \AA (980 pm), respectively], but the helix twist-angle is different (120 and 45° , respectively). Distribution of the charged side-groups in these helices was discussed. An orthorhombic form, with a twofold helical structure, has a repeat of 18.6 \AA (1.86 nm).

6. Chondroitin 4-sulfate⁷⁰ Poly[(1 \rightarrow 3)- β -D-GalNAcp-4SO₃⁻-(1 \rightarrow 4)- β -D-GlcpA]Na⁺

The repeat distance is 28.5 \AA (2.85 nm). The molecule is a helix having three disaccharide units with a projected height of 9.5 \AA (950 pm).

7. Dermatan sulfate⁶⁹⁻⁷¹ Poly[(1 \rightarrow 3)- β -D-GalNAcp-4SO₃⁻-(1 \rightarrow 4)- α -L-IdopA]Na⁺

Three crystalline forms of dermatan sulfate are known. (i) Initial crystallization of the sodium salt occurs in a tetragonal unit-cell with $a = b = 12.5 \text{ \AA}$ (1.25 nm) and $c = 74.4 \text{ \AA}$ (7.44 nm). The chains are 8-fold helices. The space group is $P4_32_12$ or $P4_12_12$. (ii) On lowering the pH, a trigonal form is obtained, with $a = b = 14.2 \text{ \AA}$ (1.42 nm) and $c = 28.5 \text{ \AA}$ (2.85 nm). The chains are threefold helices. (iii) Annealing leads to an orthorhombic form with $a = 18.6 \text{ \AA}$ (1.86 nm),

(68) S. Arnott, J. M. Guss, D. W. L. Hukins, and M. B. Mathews, *Science*, **180**, 743-745 (1973).

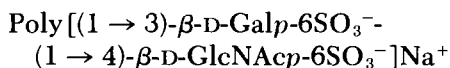
(69) S. Arnott, J. M. Guss, D. W. L. Hukins, and M. B. Mathews, *Biochem. Biophys. Res. Commun.*, **54**, 1377-1383 (1973).

(70) E. D. T. Atkins and T. C. Laurent, *Biochem. J.*, **133**, 605-606 (1973).

(71) E. D. T. Atkins and D. H. Isaac, *J. Mol. Biol.*, **80**, 773-779 (1973).

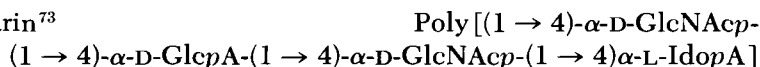
$c = 15.6 \text{ \AA}$ (1.56 nm), and b (fiber axis) = 19.4 \AA (1.94 nm). The chains are twofold helices.

8. Keratan sulfate⁷²



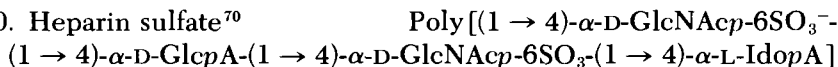
The molecules are helices having a twofold screw-axis, with an axial rise per disaccharide residue of 9.45 \AA (945 pm). The axes of the chains are parallel, and about equally spaced, but are not further organized into crystalline arrays. A hydrogen bond was proposed between the OH-3 group of the 2-acetamido-2-deoxy-D-glucose residues and O-5 of the D-galactose 6-sulfate residues.

9. Heparin⁷³



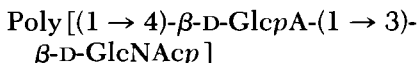
The sodium salt of heparin crystallizes in a triclinic unit-cell with $a = 13.0 \text{ \AA}$ (1.30 nm), $b = 10.2 \text{ \AA}$ (1.02 nm), $c = 15.9 \text{ \AA}$ (1.59 nm), $\alpha = 104^\circ$, $\beta = 96^\circ$, and $\gamma = 116^\circ$. The tetrasaccharide repeating-unit has no internal symmetry. Adjacent chains are packed parallel to each other. The merits of the models having the uronic acid residues in the ${}^1C_4(L)$ and ${}^4C_1(L)$ conformations were discussed.

10. Heparin sulfate⁷⁰



The repeat distance is 18.6 \AA (1.86 nm), with a meridional reflection on the first layer-line. A tetrasaccharide repeating-unit was favored. It was suggested that the linkage between the 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid residues is alternately $\alpha\text{-D-(1} \rightarrow 4)$ and $\beta\text{-D-(1} \rightarrow 4)$.

11. Hyaluronic Acid⁷⁴



The planar acetyl groups lie close to planes perpendicular to the helix axis, and project at angles of 120° apart. This allows the chains to hydrogen-bond side by side in a variety of trigonal and hexagonal arrangements, invariably trapping columns of water molecules. The

(72) S. Arnott, J. M. Guss, D. W. L. Hukins, I. C. M. Dea, and D. A. Rees, *J. Mol. Biol.*, **88**, 175-184 (1974).

(73) I. A. Nieduszynski and E. D. T. Atkins, *Biochem. J.*, **135**, 729-733 (1973).

(74) E. D. T. Atkins and J. K. Sheehan, *Nature (London) New Biol.*, **235**, 253-254 (1972); *Chem. Abstr.*, **7**, 2165 (1972).

chains of the two individual building-units of hyaluronic acid are held together by the acetamido groups hydrogen-bonding in a regular, well ordered manner.

12. Hyaluronic Acid⁷⁵ Poly[(1 → 4)-β-D-GlcpA-(1 → 3)-β-D-GlcNAcp]

The unit cell is orthorhombic, with $a = 11.4 \text{ \AA}$ (1.14 nm), $b = 9.8 \text{ \AA}$ (980 pm), and $c = 33.7 \text{ \AA}$ (3.37 nm). A double helix was proposed in which two identical, left-handed strands are antiparallel to one another. Each strand has four disaccharide residues per pitch length.

13. Hyaluronate⁷⁶ Poly[(1 → 4)-β-D-GlcpA-(1 → 3)-β-D-GlcNAcp-6SO₃⁻]Na⁺

Sodium hyaluronate crystallizes in a hexagonal unit-cell with $a = 11.7 \text{ \AA}$ (1.17 nm) and $c = 28.5 \text{ \AA}$ (2.85 nm). Another form, produced by annealing, packs in a hexagonal unit-cell with $a = 18.7 \text{ \AA}$ (1.87 nm) and $c = 28.5 \text{ \AA}$ (2.85 nm). The conformation of the chain is a threefold helix. The significance of the side-chain interaction was discussed, and different packing schemes were proposed for the two types of unit cell.

14. Hyaluronate⁶⁸ Poly[(1 → 4)-β-D-GlcpA-(1 → 3)-β-D-GlcNAcp-6SO₃⁻]Na⁺

A low-humidity form crystallizes in a tetragonal unit-cell with $a = 9.9 \text{ \AA}$ (990 pm) and $c = 33.9 \text{ \AA}$ (3.39 nm). A regular, fourfold helix is indicated. The higher-humidity form is orthorhombic, with $a = 9.8 \text{ \AA}$ (980 pm), $b = 11.4 \text{ \AA}$ (1.14 nm), and $c = 33.7 \text{ \AA}$ (3.37 nm).

15. Hyaluronate⁷⁷ Poly[(1 → 4)-β-D-GlcpA-(1 → 3)-β-D-GlcNAcp-6SO₃⁻]K⁺

The unit cell is orthorhombic, with $a = 11.0 \text{ \AA}$ (1.10 nm), $b = 9.9 \text{ \AA}$ (990 pm), and $c = 33.0 \text{ \AA}$ (3.30 nm). The molecule is a double helix, each strand containing four disaccharide units per pitch length. On

(75) I. C. M. Dea, R. Moorhouse, D. A. Rees, S. Arnott, J. M. Guss, and E. A. Balazs, *Science*, **179**, 560-562 (1973).

(76) E. D. T. Atkins, C. F. Phelps, and J. K. Sheehan, *Biochem. J.*, **128**, 1255-1263 (1972).

(77) E. D. T. Atkins and J. K. Sheehan, *Science*, **179**, 562-564 (1973).

annealing under tension, the X-ray pattern corresponds to a system of antiparallel, single-stranded, left-handed, threefold helices. An intermediate form gives a pattern for which $a = 10.4 \text{ \AA}$ (1.04 nm), $b = 9.0 \text{ \AA}$ (900 pm), and $c = 37.1 \text{ \AA}$ (3.71 nm). In this case, the projected height of the disaccharide unit is 9.3 \AA (930 pm). A scheme of transitions of the various conformational states of hyaluronate was proposed.

V. GLYCURONANS [POLY(GLYCOSIDURONIC ACIDS)]

1. L-Guluronan [poly(L-guluronic acid)]⁷⁸⁻⁸⁰ Poly[(1 → 4)- α -L-GulpA]

The unit cell is orthorhombic, with $a = 8.6 \text{ \AA}$ (860 pm), b (fiber axis) = 8.7 \AA (870 pm), and $c = 10.7 \text{ \AA}$ (1.07 nm). The space group is $P2_12_12_1$. The chain with the twofold screw axis has an intramolecular hydrogen-bond of the type OH-2---O-6. All of the intermolecular hydrogen-bonds involve water molecules and all of the oxygen atoms, except the bridge oxygen atoms. The L-guluronic acid residues are in the ${}^1C_4(L)$ conformation. Intensive drying leads to a poor diffraction pattern with $a = 7.7 \text{ \AA}$ (770 pm), b (fiber axis) = 8.7 \AA (870 pm), and $c = 10.6 \text{ \AA}$ (1.06 nm).

2. D-Mannuronan [poly(D-mannuronic acid)]^{78,79,81} Poly[(1 → 4)- β -D-ManpA]

Two disaccharide segments are contained in an orthorhombic unit-cell with $a = 7.6 \text{ \AA}$ (760 pm), b (fiber axis) = 10.4 \AA (1.04 nm), and $c = 8.6 \text{ \AA}$ (860 pm). The space group is $P2_12_12_1$. A sheet-like structure, with OH-3---O-5' intrachain, and OH-6---OH-3 and OH-2---O-5 intersheet, hydrogen-bonds was proposed. The R factor is 23%.

(78) E. D. T. Atkins, W. Mackie, and E. E. Smolko, *Nature (London)*, **225**, 626-628 (1970).

(79) E. D. T. Atkins, W. Mackie, K. D. Parker, and E. E. Smolko, *J. Polym. Sci., Part B*, **9**, 311-316 (1971).

(80) E. D. T. Atkins, I. A. Nieduszynski, W. Mackie, K. D. Parker, and E. E. Smolko, *Biopolymers*, **12**, 1879-1887 (1973).

(81) E. D. T. Atkins, I. A. Nieduszynski, W. Mackie, K. D. Parker, and E. E. Smolko, *Biopolymers*, **12**, 1865-1878 (1973).

VI. SULFATED POLYSACCHARIDES

1. ι -Carrageenan^{32,82} Poly[(1 \rightarrow 4)-3,6-An- α -D-Galp-2SO₃⁻-
(1 \rightarrow 4)- β -D-Galp-4SO₃⁻] Ca^{2+} or Sr^{2+}

The Ca^{2+} and Sr^{2+} salts of ι -carrageenan have isomorphous crystal structures with a trigonal unit cell, $a = b = 13.73 \text{ \AA}$ (1.373 nm), $c = 13.28 \text{ \AA}$ (1.328 nm), and $\gamma = 120^\circ$. The molecule consists of a double helix in which two, identical, righthanded, threefold, helical chains of pitch 26.56 \AA (2.656 nm) intertwine. One chain is translated axially by 13.2 \AA (1.32 nm) relative to the other. Up-pointing and down-pointing chains are distributed randomly among the molecular sites, and the space group of this statistical structure is P3_212 . The R factor is 35% for the Ca^{2+} salt, and 34% for the Sr^{2+} salt.

2. κ -Carrageenan⁸² Poly[(1 \rightarrow 4)-3,6-An- α -D-Galp-(1 \rightarrow 3)- β -D-Galp-4SO₃]

The fiber axis repeat distance is 24.6 \AA (2.46 nm). The molecule is a double helix. The individual chains contain 3 disaccharide residues in one complete turn of pitch 24.6 \AA (2.46 nm).

3. Agarose (agaran)⁸³ Poly[(1 \rightarrow 3)- β -D-Galp-(1 \rightarrow 4)-3,6-An- α -L-Galp]

Agarose and its *O*-methyl, sulfate, *O*-(2-hydroxyethyl), and *O*-(carboxyethylidene) derivatives give diffraction diagrams corresponding to a common molecular structure. A double helix having an axial periodicity of 9.5 \AA (950 pm) was proposed. Each chain in the double helix is a left-handed, threefold helix of pitch 19.0 \AA (1.90 nm), and it is translated axially, relative to its partner, by 9.5 \AA (950 pm).

(82) N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, **45**, 85-99 (1969).

(83) S. Arnott, A. Fulmer, W. E. Scott, I. C. M. Dea, R. Moorhouse, and D. A. Rees, *J. Mol. Biol.*, **90**, 269-284 (1974).

AUTHOR INDEX

Numbers in parentheses are reference numbers and indicate that an author's work is referred to, although his name is not cited in the text.

A

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