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PREFACE

In this volume, Wander and Horton (Columbus) provide a detailed discussion of the dithioacetals of sugars, compounds of considerable utility in the synthetic chemistry of carbohydrates because of the large number of reactions that are possible at the dithioacetal group and because of the hydroxyl groups of the acyclic sugar chain that are capable of selective protection or chemical transformation. The article complements that on the monothio derivatives of sugars by Horton and Hutson in Volume 18. Neuberg's chapter in Volume 4 (1949) on biochemical reductions at the expense of sugars has been updated, as regards the utilization of sugars by yeasts, in an extensive article by Barnett (Norwich) that delineates the true complexity of the processes so frequently oversimplified through unjustified generalizations in standard biochemical texts. Whistler, Bushway, and Singh (Lafayette), in collaboration with Nakahara and Tokuzen (Tokyo), have collected the information currently available on the sources, structures, and mode of action of noncytotoxic polysaccharides that display antitumor properties. This aspect of cancer chemotherapy has received scant attention elsewhere, and the chapter serves to bring together into common focus the extensive, but widely scattered, literature on the subject. Dekker and Richards (Townsville) describe the occurrence, purification, properties, and mode of action of hemicellulases in an article that complements and extends our previous chapters on enzymes of interest to carbohydrate chemists. The present chapter treats, in fact, four major enzyme types, acting on L-arabinans, D-galactans, D-mannans, and D-xylans, from the standpoint of their action on the complex polysaccharide group known collectively as hemicelluloses. Jeffrey (Upton) and Sundaralingam (Madison) continue our series of bibliographic articles, initiated in Volume 30, on carbohydrate structures by describing those that have been ascertained by crystallographic methods during 1974. As a new feature, they have introduced projection diagrams, produced by means of computer graphics, for depiction of the conformation of each of the organic molecules or ions. The obituary article, by Ballou and Barker (Berkeley), describes the fascinating career of Zev Hassid, and particularly discusses his contributions to our knowledge of glycosyl esters of nucleoside pyrophosphates and the biosynthesis of polysaccharides.

The editors note with profound regret the passing on October 29, 1975, of our friend and erstwhile mentor Sir Edmund Hirst, a member of the Executive Committee of *Advances* from 1948 to 1950 and of our Board of Advisors from 1950 to 1952, an Associate Editor for the British Isles from 1953 to 1954, a member of the Board of Advisors for the British Isles from 1955 to 1968, a member of the Board of Advisors for the British Commonwealth from 1969 to 1974 (Vol. 29), and a member of the Board of Advisors from 1974 (Vol. 30) until his death.

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

Kensington, Maryland   R. STUART TIPSON
Columbus, Ohio   DEREK HORTON
February, 1976

WILLIAM ZEV HASSID

1899–1974

Zev (Ze'ev) Hassid was born in Jaffa, Palestine, probably on October 1st, 1899, although he seemed uncertain about the date of his birth and sometimes gave the year as 1897 or 1901. The name William was added after he came to the United States. His parents, Mordecai and Esperanza Hassid (Chassid) were born in Poland, but were Russian citizens at the time of his birth. His father was a lumber merchant who took lumber from Russia to Palestine. When Zev was 4 years old, the family moved from Palestine to a farm in the vicinity of Kremenetz in the Russian Ukraine, and his childhood was spent in this rural environment. He often wandered in the adjacent woods and fields, hunted for birds' nests, and associated with shepherds, so much so that his father scolded him and said that if he did not spend more time on his studies he would qualify for nothing more than a herdsman.

Russian and Yiddish were Zev's native languages. Little is known about his early education, except that he was required to study traditional Jewish religious material. He did not accept this instruction readily and, in later life, rebelled by disassociating himself from most formal religious activities. Nevertheless, his early training was evidenced by a familiarity with and an occasional quotation from the Scriptures.

In 1912, Hassid was sent back to Palestine with a group of Jewish children, to continue his education in a Hebrew language school. His parents hoped that he would be admitted to the "Gymnasia Hertzlia" in Tel Aviv, but instead, he was sent to the recently founded Agricultural High School in the Jewish settlement of Petah-Tikva. The curriculum included the Hebrew, French, and Arabic languages, Hebrew religious studies, history, geography, and considerable science, plus professional studies in soils, plant nutrition, subtropical horticulture, animal husbandry, laboratory practice in analytical methods, and field experience in agricultural techniques. He completed these studies in 1916.

With the outbreak of World War I, Hassid was cut off from communication with his parents, who had remained in Russia. Consequently, his only income was what he could earn himself. While

still in school, and afterwards, he worked as a laborer in the orange groves and other farms near Petah-Tikva, Nikva-Israel, and Ben Shemen. His income was small and his food and clothing were correspondingly poor. For a considerable period, he lived on bread, watery soup, pumpkin porridge, and oranges; he was often hungry. His clothing was worn and ragged. Once, his shoes were stolen and he had to go barefoot for a considerable time. During this period, Hassid was frequently ill. He suffered repeated attacks of malaria and dysentery; he also contracted typhoid fever, which he barely survived.

Palestine was controlled by the Turks until the British Army invaded the country in 1917. Following the Balfour Declaration in the same year, many young Jews joined the British Army to help liberate the country from the Turks and create conditions favorable for the establishment of a Jewish homeland. Hassid volunteered for army service early in 1918, partly for patriotic reasons and partly to improve his standard of living. Initially, he was rejected by the army because of his poor health, but finally, through the intervention of James de Rothschild, an officer in the British Army, he was accepted into the 38th Brigade of the Royal Fusiliers (1st Judeans), later referred to as the Jewish Legion. At this time, he became a British subject and served for two years in the army, mainly as a clerk at supply depots and at General Headquarters, 3rd Echelon. He never engaged in combat; however, on at least one occasion, he was close enough to the front to be within artillery range; a large shell landed close to him, but failed to explode. He also guarded prisoners and supplies in transit. The latter duty required him to travel as far as Beirut in Lebanon and Alexandria in Egypt. At the railway station in Beirut, he once witnessed the ceremonial arrival of Lawrence of Arabia. In Alexandria, he first heard of the University of California from a fellow soldier, Assaf Gur (Grazovsky), who had studied there.

When Hassid completed his military service with the rank of corporal, in August, 1920, he was awarded the British War Medal, and the Victory Medal bearing the inscription "The great war for civilization." His superior officer provided him with the following recommendation: "Corporal Hassid has been employed as a clerk in the Battalion Orderly Room and at Records, 3rd Echelon. He is a conscientious, painstaking worker, reliable and capable, and as a soldier has borne an exemplary character."

After leaving the army, Hassid decided to use the savings accumulated from his pay to go to California to study agronomy at the University there, with the intention of returning ultimately to Pales-

tine to assist in the development of scientific agriculture. He travelled by way of Paris, New York, and Chicago, staying briefly in each city, and finally arrived in Berkeley, California, in late 1920. His funds were almost exhausted, so he supported himself by doing odd jobs in stores and restaurants in Berkeley in the winter, and by working as a farm hand in the vicinity of Fresno in the San Joaquin Valley in the spring and summer. He also taught Hebrew in the local synagogue.

Hassid first registered at the University of California in August, 1921. However, his knowledge of English was so limited that he could not follow lectures well enough to take notes, and even reading textbooks required more time than he could spare between jobs. So, after a week of frustration and mounting tension, he took a leave of absence from the University and moved to Fresno, where he spent two years as a student in Fresno State College, majoring in Letters and Science with an emphasis on Chemistry, French, and Mathematics. The following year, he enrolled at the Southern Branch of the University of California at Los Angeles. His course grades were about average; he even failed one course in quantitative analysis and had to repeat it later. His undistinguished record during this period probably resulted from his inadequate command of English and the fact that he had to earn a living while attending school.

In August, 1924, Hassid returned to the Berkeley campus of the University of California. He majored first in chemistry, but later changed to general literature, the field in which he obtained the Bachelor of Arts degree in December, 1925. Following graduation, he immediately started graduate studies in the School of Education, and, in December, 1926, he received a Certificate of Completion with majors in chemistry and general literature and minors in mathematics and physics. In the same month, Hassid obtained a General Secondary School Credential from the State Board of Education, but he apparently never taught in public schools.

By September, 1927, and possibly earlier, Hassid took a position as a research assistant in the Division of Plant Nutrition of the Agricultural Experiment Station under Prof. D. R. Hoagland. His main duties were the routine analysis of plant materials and soils for a variety of inorganic constituents, but he also obtained experience in growing plants in culture solutions and in studying the absorption of various nutrients. This work renewed Hassid's interest in plant research, and, in August, 1928, he again enrolled in the University, this time as a graduate student in Plant Nutrition. During the following

two years, while still working half-time or more, he took courses in botany, plant physiology, and plant nutrition, and prepared a Master of Science thesis dealing with the structure of the four cyclic isomers of penta-O-acetyl-D-galactose. Prof. Hoagland formally supervised his thesis research, but the nature of the problem clearly indicates that it was inspired and guided mainly by Prof. Walter H. Dore, who was interested in the structure of carbohydrates and had applied X-ray diffraction methods for this purpose. After receiving the Master's degree in Plant Nutrition in August, 1930, Hassid started to prepare for a Doctorate in Plant Physiology. While visiting the beaches south of San Francisco, he observed the abundant, fleshy, marine algae which appeared to consist largely of polysaccharides, and he decided to investigate the structure of the major component. This was the subject of his Ph.D. thesis, which was completed and accepted in December, 1934, with Prof. Dore as the chairman of the committee. However, Hassid had worked almost independently, using methods, developed by Haworth, for determination of carbohydrate structure which were unfamiliar to Dore. Prof. T. D. Stewart of the Chemistry Department, a member of the thesis committee, was particularly impressed by the clear results and logical presentation of Hassid's thesis, and his enthusiastic reaction helped Hassid to obtain an appointment the following year as a Junior Chemist in the Division of Plant Nutrition of the Agricultural Experiment Station in Berkeley.

The circumstances of Hassid's appointment as a Junior Chemist are amusing, and illustrative of the method of making appointments in 1935. Prof. Hoagland lectured on and taught laboratory courses in plant biochemistry during the Fall term. Hassid had served for several years as a teaching assistant in the laboratory course, and Hoagland, who directed an active research program and served as Chairman of the Division, had come to depend on him for the preparation of reagents, the setting up of equipment, and much of the instruction. But, after receiving his Ph.D. in 1934, Hassid began to look for a better position than was provided by his assistantship. In the early summer of 1935, he obtained an offer of a position elsewhere, and told Prof. Hoagland that he was planning to leave before the beginning of the Fall term. Hoagland was upset at this news, because he was preparing to attend a Botanical Congress in Amsterdam and would not return to Berkeley until the beginning of that term, when he would have no experienced assistant for the laboratory course. He finally asked Hassid whether he would stay on if he received an Experiment Station appointment. Hassid agreed. Hoag-

land then consulted with Dean Hutchison, who found that the funds available were insufficient to provide the usual starting-salary of $2,000 per year, but that he could offer $1,800. Hassid accepted this, although it was considerably below what he was offered outside the University. He never regretted his decision. In 1939, he received the additional title of Instructor, and so was launched upon his academic career.

Hassid's independent scientific research began with an investigation of the ethanol-extractable carbohydrates in the marine alga *Irideae laminarioides*, and this work led step by step to an interest in the biochemistry of carbohydrates that he sustained in one form or another for almost 35 years. In the initial study, he identified dulcitol (galactitol) as a major component of this plant extract, and observed that reducing sugars, or sugars that became reducing on acid hydrolysis, were noticeably absent. He then purified and characterized an abundant polysaccharide from the same organism, and showed it to be a sulfated galactan. From methylation studies, he was able to conclude that the galactosyl residues were probably joined by $(1 \rightarrow 4)$-glycosidic linkages, and that the hydroxyl group on C-6 was probably esterified with the sulfate. These results led him to speculate that the metabolism in this alga might involve a relationship between galactitol and galactan analogous to that between D-glucose and starch in higher plants, but he never followed up this idea.

Hassid's study of the structure of the algal galactan was the first of a long series of investigations of polysaccharides. Many of the preparations were provided by colleagues with whom he was always happy to collaborate. C. B. Lipman called his attention to a very viscous substance produced from D-mannitol by an unidentified bacterium that had been isolated "from a mud brick taken from a wall in an old Roman village which was built about 400 A.D. in the western desert of Egypt." In a paper with W. L. Chandler (1937), Hassid characterized this viscous material as a polysaccharide containing about 10 glucose residues. In the following years, he published papers dealing with the molecular structure of canna starch (with W. H. Dore), dog-liver glycogen (with I. L. Chaikoff), the dextran formed from sucrose by *Betacoccus arabinosaceus* (with H. A. Barker), an insoluble polysaccharide derived from *Saccharomyces cerevisiae* (with M. A. Joslyn and R. M. McCready), and glycogen and starch derived from sweet corn (*Zea mays*) (with R. M. McCready).

The existence of the enzyme phosphorylase, which converts glycogen and inorganic phosphate into α-D-glucosyl phosphate, had

been demonstrated by C. F. and G. T. Cori in 1936, and the reversal of this reaction was reported in 1939 by W. Kiessling. Hassid and R. M. McCready (1941) undertook the structural analysis of the biosynthetic polysaccharide produced, and showed that it had starchlike properties, but that the molecules were unbranched, in contrast to the highly branched natural polymer. In a short review for *Chronica Botanica,* published in 1942, Hassid related the prevalent view that "the enzyme phosphorylase, and not amylase as had been previously assumed, is chiefly responsible for the synthesis and breakdown of starch in the plant." McCready and Hassid developed a convenient method for preparing pure α-D-glucosyl phosphate on a relatively large scale, thus making this important compound readily available for biochemical studies. They also developed a procedure for determining the relative proportions of amylose and amylopectin in starch, based upon the large difference in the absorption coefficients of iodine complexes of the two components. The absorption coefficients were found to correlate well with the degree of hydrolysis of the components by *beta*-amylase. Their results supported the conclusion of K. H. Meyer that amylose consists of long, unbranched chains of D-glucosyl residues.

This experience with carbohydrates prepared Hassid for an important collaborative effort with S. Ruben and M. D. Kamen (1939) constituting the first application of a radioactive carbon in the study of photosynthesis. The short-lived, ^{11}C-isotope (20.5 minutes half-life) had become available through Kamen's association with the Radiation Laboratory at the University in Berkeley, and a study was carried out to determine the distribution of the label from [^{11}C]carbon dioxide when fed to plant leaves in the light, or after they had been kept in the dark for various periods of time. Although some of the label was incorporated into carbohydrate, the results indicated that most of it was present in the plant in a water-soluble, noncarbohydrate form. In an extension of this work, the green alga *Chlorella pyrenoidosa* was utilized in place of barley leaves, allowing a much more efficient incorporation of the radiocarbon label. Studies of the kinetics of incorporation in the light and in the dark, and on the reversibility of the reaction, were performed. Although it was concluded that "the greater fraction if not all the C^*O_2 has been reduced to C^*OOH," no specific identification of the initial product of photosynthesis was made, other than that there were "at least one alcoholic hydroxyl and one carboxyl group in the active molecules." A molecular weight of "at least 200" for the major, radioactive product was estimated from the results of diffusion measurements. The

product was later identified as "phosphoglyceric acid" (glyceric acid 3-phosphate) by M. Calvin and his associates.

Other than a brief collaboration with S. Aronoff, A. Benson, and M. Calvin (1947) on the distribution of label in photosynthesizing plant-tissue, this short sojourn by Hassid in the field of photosynthesis was not continued, and his involvement appears to have been based more on an interest in carbohydrate structural analysis than in the fundamentals of carbon fixation in plants. However, it is apparent from the later turn of events that this introduction to the utility of radioactive-tracer techniques for the elucidation of biochemical processes had a strong influence on his development.

Hassid's initial appointment as a Junior Chemist in the Agricultural Experiment Station was followed by promotion to the academic staff as an Instructor in Plant Nutrition in 1939 and, finally, as Assistant Professor in 1941. At about this time, he developed an association with his colleagues H. A. Barker and M. Doudoroff that grew into a close scientific collaboration and a lifelong friendship. His first joint study with Barker concerned the structure of an extracellular $(1 \rightarrow 6)$-dextran produced by the bacterium *Leuconostoc mesenterioides* when grown on sucrose. Less than 3 years later, he was involved with Doudoroff and N. Kaplan in the beginning of an important study on the biosynthesis of sucrose. Doudoroff had been interested in the bacterium *Pseudomonas saccharophila*, because it oxidizes sucrose faster than the component monosaccharides D-glucose and D-fructose; this was shown to result from the presence of an enzyme that converts sucrose into α-D-glucosyl phosphate and D-fructose. Doudoroff, Kaplan, and Hassid found that this reaction can be reversed, leading to the formation of sucrose (as detected by the production of a nonreducing substance that yielded reducing sugar on acid hydrolysis). That it was indeed sucrose was proved by Hassid, Doudoroff, and Barker in 1944, when they prepared 2.5 grams of the crystalline disaccharide by the action of sucrose phosphorylase on a mixture of 15 grams each of α-D-glucosyl phosphate and D-fructose, and showed that its properties were identical with those of commercial sucrose.

The enzymic synthesis of sucrose resulted in some publicity that came to the attention of officials of the Coca Cola Company, who were having difficulty in obtaining sucrose because of wartime rationing. The company sent a representative to Berkeley to ascertain whether commercial quantities of sucrose could be made by the enzymic method. Hassid and his associates were away on vacation at the time, so the Coca Cola emissary discussed the problem with Prof.

Hoagland and reported that his company was prepared to provide $500,000 for research on this enzyme if a commercial process for the synthesis of sucrose seemed feasible. Unfortunately, Prof. Hoagland was pessimistic about the possibility of sweetening Coca Cola by this method, and so further support of research on sucrose phosphorylase was left to the University, the U. S. Public Health Service, and the National Science Foundation.

Later studies on sucrose phosphorylase showed that it could transfer D-glucose to L-sorbose and to D-*threo*-pentulose to form nonreducing, disaccharide analogs of sucrose, and to L-arabinose to yield 3-*O*-α-D-glucopyranosyl-L-arabinose. The mechanism of the phosphorylase reaction was investigated by isotope-exchange reactions with ^{32}P-orthophosphate, which was shown to exchange into nonradioactive α-D-glucosyl phosphate. With such alternative monosaccharide acceptors as L-sorbose, the enzyme was shown to transfer D-glucose from sucrose in the *absence* of orthophosphate. Clearly, the reaction involved a D-glucosyl–enzyme intermediate that could be formed either from α-D-glucosyl phosphate or from an α-D-glucosyl derivative such as sucrose.

Near the end of the 1940s, Hassid's research began to take a new direction as he concentrated on the synthesis of radiocarbon-labelled sugars and on the chemical preparation of sugar phosphates. E. W. Putman was a major collaborator in developing methods for preparing labelled sugars from plant tissue after their biosynthesis by the photosynthetic fixation of [^{14}C]carbon dioxide. They applied the new methods of paper chromatography to the preparation of uniformly ^{14}C-labelled carbohydrates of high specific activity, including D-glucose, D-fructose, D-galactose, sucrose, and starch. Hassid generously supplied radioactive sugars both to his colleagues and to many scientists throughout the country before they became commercially available. These studies on the preparation of labelled sugars were extended to investigations of the route by which the carbon dioxide, once fixed as glyceric acid 3-phosphate, was converted into various carbohydrates, as well as the processes by which labelled hexose was taken up and utilized for the synthesis of sucrose and cellulose. In 1947, he was promoted to the rank of Professor of Plant Biochemistry.

At about this time, Hassid was joined by V. Ginsburg and E. F. Neufeld, and an active program evolved dealing with the role of "sugar nucleotides" (glycosyl esters of nucleoside pyrophosphates) in the interconversion of carbohydrates in higher plants. In the initial studies, they were joined by P. K. Stumpf, who had been ap-

pointed to the Department of Plant Nutrition in 1948, and who, as an undergraduate student at Harvard University, had worked with D. E. Green on the purification of potato phosphorylase. Although Stumpf is best known for his investigations on lipid metabolism in plants, his earlier studies in sugar metabolism were influential in the development of Hassid's interests in this field. The discovery by L. F. Leloir (1951) of "uridine diphosphate D-glucose" [uridine 5'-(α-D-glucopyranosyl pyrophosphate)] and the demonstration that this compound serves as a D-glucosyl donor for the synthesis of certain disaccharides, focussed attention on the "sugar nucleotides" as intermediates in the interconversion of carbohydrates, and Hassid directed his concern to these compounds in higher plants. With Ginsburg and Stumpf (1956), he investigated the occurrence of uridine 5'-pyrophosphate esters of D-glucose, D-galactose, D-xylose, and L-arabinose in the mung bean (*Phaseolus aureus*), the latter two derivatives being found for the first time in Nature. The same source yielded the uridine 5'-pyrophosphate esters of 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid (with J. Solms and D. S. Feingold), whereas the guanosine 5'-pyrophosphate esters of L-galactose and D-mannose were later identified in the red alga *Phorphyra perforata* (with J. C. Su), and guanosine 5'-(D-mannopyranosyluronic acid pyrophosphate) was isolated from the brown alga *Fucus gardneri* (with T. J. Lin).

These studies on the natural occurrence of "sugar nucleotides" in plants were paralleled by investigations of their biosynthesis by the pyrophosphorylase reaction. A series of papers with Neufeld, Putman, Feingold, Ginsburg, and others, delineated the presence in higher plants of pyrophosphorylases that formed the respective uridine 5'-(hexosyl pyrophosphate) from the reaction of uridine 5'-triphosphate with the 1-phosphoric esters of α-D-galactose, α-D-xylose, β-L-arabinose, α-D-glucuronic acid, α-D-galacturonic acid, and 2-acetamido-2-deoxy-α-D-glucose. Because these studies required the use of glycosyl phosphates as substrates, and because such esters were not generally available at the time, considerable effort was devoted to the improvement of published syntheses and the development of new ones for the preparation of glycosyl phosphates. Later studies dealt with the enzymic phosphorylation of several sugars, including D-galactose, L-arabinose, D-glucuronic acid, and D-galacturonic acid (with Neufeld, Feingold, and others). In 1959, he transferred to the Department of Biochemistry.

Hassid's international reputation naturally attracted senior scientists from around the world to Berkeley to work in his laboratory.

One of these was Winifred M. Watkins from the Lister Institute of Preventive Medicine in London, who is noted for her studies on the structure and biosynthesis of the blood-group antigens. In 1961, Watkins and Hassid undertook a study of the biosynthesis of lactose in mammary tissue. It had been claimed by J. E. Gander, W. E. Petersen, and P. D. Boyer in 1957 that bovine mammary tissue contains enzymes that convert uridine 5′-(D-galactosyl pyrophosphate) and α-D-glucosyl phosphate into lactosyl phosphate, and that the latter is hydrolyzed to free lactose. However, as uridine 5′-(D-galactosyl pyrophosphate) and α-D-glucosyl phosphate are in ready equilibrium by way of uridine 5′-(α-D-glucosyl pyrophosphate), D-[14C]glucose might be expected to be incorporated equally into the two parts of lactose by this pathway, an expectation that was contrary to observations recorded by other workers. Watkins and Hassid reinvestigated this matter, and established that uridine 5′-(D-galactosyl pyrophosphate) and free D-glucose are the precursors of lactose in lactating guinea-pig and bovine mammary tissue, and that the product is lactose, not lactosyl phosphate. In 1965, he became Professor Emeritus.

In addition to these results on lactose synthesis, Watkins and Hassid made the important observation that mammary tissue contains an enzyme activity that transfers a D-galactosyl group to 2-acetamido-2-deoxy-D-glucose. Noting the occurrence in milk of oligosaccharides that contain this "lactosamine" residue, and finding that different mammary-gland preparations gave different relative proportions of [14C]lactose and 2-acetamido-2-deoxy-[14C]lactose when incubated with uridine 5′-(D-[14C]galactosyl pyrophosphate), they concluded that "different enzymes are responsible for the synthesis of the two compounds." This conclusion, and a later one by H. Babad and Hassid (1966) that the soluble, purified, lactose synthetase from milk is "very labile to further purification," proved to be incorrect, for it was subsequently found by K. E. Ebner and others (1967) that the mammary-gland β-D-galactosyltransferase is under the control of the specificity-altering protein α-lactalbumin. In the presence of α-lactalbumin, the favored acceptor is D-glucose, and lactose is the product, whereas, in its absence, 2-acetamido-2-deoxy-D-glucose acts as the acceptor to yield 2-acetamido-2-deoxylactose. The observations of Watkins and Hassid can be explained by the presence of variable proportions of α-lactalbumin in their preparations of lactose synthetase, and the results of Babad and Hassid as constituting the first successful fractionation of these two proteins.

The existence of enzymes that interconvert "sugar nucleotides" without degrading the molecule had become recognized by the latter

part of the 1950s, and Hassid turned to the study of some of these reactions in plants. With Neufeld and Feingold, he demonstrated the enzymic conversion of uridine 5'-(D-glucopyranosyluronic acid pyrophosphate) into the nucleotide derivatives of D-galacturonic acid, D-xylose, and D-arabinose. In a later study, the enzyme activities that catalyze these reactions were separated, so that it was possible to show the 4-epimerization of uridine 5'-(D-glucopyranosyluronic acid pyrophosphate) to the D-galactosyluronic acid derivative as an isolated step, and to demonstrate the decarboxylation of uridine 5'-(D-glucopyranosyluronic acid pyrophosphate) to uridine 5'-(D-xylosyl pyrophosphate). An enzyme activity was also found that epimerizes the D-xylosyl derivative to the L-arabinosyl derivative.

Throughout his career, Hassid was concerned with the fundamental question of how the sugar that is formed in a photosynthesizing plant is converted into such disaccharides as sucrose and into such polysaccharides as starch and cellulose. His earliest experiments dealt with the infiltration of radiolabelled sugars into plant leaves, but they later became more sophisticated, with the utilization of well defined "sugar nucleotides" as specific donors in cell-free, enzyme systems. It was known that uridine 5'-(α-D-glucosyl pyrophosphate) is a precursor of a (1 → 4)-β-D-glucan in *Acetobacter xylinum* (L. Glaser, 1957) and of the (1 → 4)-α-D-glucan glycogen in liver (Leloir, 1957). Feingold, Neufeld, and Hassid (1958) reported the synthesis of a (1 → 3)-β-D-glucan (callose) from this "sugar nucleotide" by a digitonin-treated, particulate transferase from mung bean, the product apparently being identical to laminaran, whereas R. A. Dedonder and Hassid (1964) observed formation of a (1 → 2)-β-D-glucan in *Rhizobium japonicum*. In a similar way, uridine 5'-(D-xylosyl pyrophosphate) was shown to be the precursor of a (1 → 4)-β-D-xylan in asparagus (*Asparagus officinalis*). Hassid also investigated the synthesis of alginic acid (with T.-Y. Lin) and of pectin (with C. L. Villemez), and the methylation of the latter polysaccharide to afford the methyl ether and ester derivatives (with H. Kauss).

This interest in polysaccharide formation in plants led Hassid naturally to an investigation of the biosynthesis of cellulose, and to, perhaps, the culminating point of his scientific career. Even today the study of cellulose biosynthesis in higher plants is fraught with technical difficulties that have hindered the definition of this system in the same detail that has been possible with other polysaccharide-forming reactions. In 1964, A. D. Elbein, G. A. Barber, and Hassid reported the formation of cellulose from guanosine 5'-(α-D-glucopyran-

osyl pyrophosphate) by the action of a particulate enzyme-preparation from mung-bean seedlings. In contrast to the finding of Glaser with a bacterial system, the plant synthetase was found to have no activity with uridine 5'-(α-D-glucopyranosyl pyrophosphate). The polysaccharide product was characterized primarily on the basis of its alkali-insolubility, which was similar to that of cellulose, and on the formation of radioactive cellobiose and cello-oligosaccharides by acid hydrolysis and by acetolysis. The study was complicated by the concomitant formation of a D-glucomannan from endogenous guanosine 5'-(D-mannosyl pyrophosphate). However, a soluble-enzyme system that produced cellulose was eventually obtained (with H. M. Flowers and K. K. Batra, 1969), and this product was only slightly contaminated by the D-glucomannan.

Some controversy concerning the biosynthesis of cellulose arose when D. O. Brummond and A. P. Gibbons (1964) reported that uridine 5'-(α-D-glucopyranosyl pyrophosphate) is a precursor of a cellulose-like polymer in *Lupinus albus*, and L. Ordin and M. A. Hall (1967) observed a similar reaction in *Avena sativa*. These reports stimulated Hassid to restudy the roles of both uridine 5'-(α-D-glucopyranosyl pyrophosphate) and guanosine 5'-(α-D-glucopyranosyl pyrophosphate) in the synthesis of alkali-insoluble polysaccharides in *Phaseolus aureus*. It was eventually established, with H. M. Flowers and Batra (1968), that the polymer formed with uridine 5'-(α-D-glucopyranosyl pyrophosphate) in *L. albus* is an alkali-insoluble (1 → 3)-β-D-glucan, not cellulose, whereas that produced by *A. sativa* is a mixed (1 → 3)-β-D- and (1 → 4)-β-D-glucan. C. M. Tsai and Hassid (1971) succeeded in separating the two enzymic activities of *A. sativa* that produce the two polysaccharides, and they found that the type of D-glucan formed is dependent on the concentration of the "sugar nucleotide" donor in the system.

From this brief survey, it is seen that there were few features of carbohydrate metabolism in plants that escaped Hassid's touch, and much that we now know about the role of "sugar nucleotides" in the interconversion of carbohydrates in plants is a direct result of his persistent effort. From the incorporation of labelled precursors into monosaccharides, to the conversion of the monosaccharides into their glycosyl phosphates, to the action of the pyrophosphorylases in the synthesis of glycosyl esters of nucleoside pyrophosphates, to the interconversion of the resulting "sugar nucleotides," to the polymerization of the activated monosaccharides to yield disaccharides and the homopolysaccharides, and, finally, to the modification of the polysaccharides by methylation — in summary, to almost every aspect

of carbohydrate metabolism—Hassid contributed his full and de-
voted attention. Neither did his efforts slacken with age, for he con-
tinued working and writing and thinking as though these topics were
among the most important things in life, and, to him, they were. He
also believed in the importance of communication as a force in scien-
tific progress, for he was a prolific writer of reviews[1] and contributed
heavily to books and serials dealing with carbohydrates, the total of
such review articles numbering almost 50.

Hassid's many contributions on the structure and synthesis of plant
carbohydrates were recognized by a number of honors and awards.
He received the first Sugar Research Award (1945) of the National
Academy of Sciences (jointly with M. Doudoroff and H. A. Barker),
the Charles Reid Barnes Honorary Life Membership Award of the
American Society of Plant Physiologists (1964), and the C. S. Hudson
Award of the Division of Carbohydrate Chemistry of the American
Chemical Society (1967). He was elected to membership in the Na-
tional Academy of Sciences (1958) and the American Academy of
Arts and Sciences (1969), and he was honored at the 6th Interna-
tional Symposium on Carbohydrate Chemistry (1972) as one of three
outstanding, senior American carbohydrate chemists. He was elected
Chairman of the Division of Carbohydrate Chemistry (1949–1950) of
the American Chemical Society, and he served as a member of
numerous editorial boards, including those of the *Journal of Biologi-
cal Chemistry, Annual Review of Biochemistry, Carbohydrate Re-
search, Phytochemistry,* and *Analytical Biochemistry.*

Perhaps as a result of his severe childhood illnesses and early dep-
rivations, Hassid never enjoyed really robust health. For many years,
he suffered from the effects of high blood-pressure, and he had
debilitating attacks of hyperthyroidism and hepatitis. In his early
sixties, he experienced a rather severe coronary occlusion from
which he never fully recovered. As he got older, he developed
increasing coronary complications that finally resulted in his death
on April 28th, 1974. He left many friends who will remember him as
a friendly, gentle, and soft-spoken person, but one who on rare oc-
casions, when sufficiently annoyed, could display a strong temper.
His personal warmth and generosity, coupled with his sincerity and
modesty, attracted many friends whom he treasured and often
regarded as somewhat larger than life. He took pride in the ac-

(1) See, for example, W. Z. Hassid and M. Doudoroff, *Advan. Carbohydr. Chem.,* **5,**
29–48 (1950); E. F. Neufeld and W. Z. Hassid, *ibid.,* **18,** 309–356 (1963); H. Nikaido
and W. Z. Hassid, *Advan. Carbohydr. Chem. Biochem.,* **26,** 351–483 (1971).

complishments of his colleagues and students, and spent much time and effort in helping to further their careers and in nominating them for promotions, awards, and honors of various sorts. He never tired or stinted in helping those who he felt were deserving.

Hassid married Lila Berlin Fenigston in 1936. They had no children, but any void this may have created in their lives was filled by the many friends who shared the warm hospitality of their home in the Berkeley hills. Lila was a gracious and vivacious hostess and an accomplished violinist, who made their home a center for friendly social gatherings and for the performance of chamber music. She also had a talent for making fine English translations of Yiddish poetry which were either published in Jewish periodicals or presented orally in a series of radio programs. Lila's appreciation of and contributions to the arts provided a happy counterpoint to the scientific life of her husband.

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DITHIOACETALS OF SUGARS

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15

I. INTRODUCTION

The chemistry of dithioacetals of sugars dates back to the classic paper[1] by Emil Fischer, in 1894, in which he described the conversion of various aldoses into their diethyl dithioacetals by the action of ethanethiol plus concentrated hydrochloric acid; by simple experiments, he outlined in this paper the main aspects of the reactions of the dithioacetals, thereby providing the starting point for much of the subsequent work on their chemistry. The literature on dithioacetals of sugars has been summarized by Reid[2] and reviewed more extensively by Staněk and colleagues[3]; the essential elements of the subject have been sketched by Wolfrom[4] as part of a discussion of the chemistry of acyclic sugar derivatives.

Dithioacetals and their derivatives comprise the largest recorded class of acyclic carbohydrate derivatives. They are versatile intermediates in synthesis not only because a multitude of transformations are possible at the dithioacetal group, but also because all of the hydroxyl groups of the sugar chain are available for chemical transformation or selective protection.[5] Of particular importance is the fact that they provide a route for kinetically controlled ring-closure of the sugar under neutral conditions by the action of mercury(II) salts, thus providing a useful route of access to furanosides; Green[6] has discussed this aspect in detail in this Series. A generally applicable method of chain descent for sugars proceeds by degradation of disulfone intermediates formed from the corresponding dithioacetals, and analysis of mass-spectrometric fragmentation of dithioacetals has provided a very useful tool in the structural elucidation of sugars on a micro scale; these and other aspects will be developed in succeeding Sections of this Chapter. The scope of this Chapter is restricted to the dithioacetals and their direct chemical transformations, and serves to complement earlier Chapters[7,8] that treated the chemistry of thio sugar derivatives in which bivalent sulfur replaces one of the

(1) E. Fischer, *Ber.*, **27**, 673–679 (1894).
(2) E. E. Reid, "Organic Chemistry of Bivalent Sulfur," Chemical Publishing Co., Inc., New York, 1960, Vol. III, pp. 349–361.
(3) J. Staněk, M. Černý, J. Kocourek, and J. Pacák, "The Monosaccharides," Academic Press, New York, 1963, pp. 589–605.
(4) M. L. Wolfrom, in "The Carbohydrates: Chemistry and Biochemistry," W. Pigman and D. Horton, eds., Academic Press, Inc., New York, 1972, Vol. IA, pp. 356–359.
(5) J. M. Sugihara, *Advan. Carbohydr. Chem.*, **8**, 1–44 (1953).
(6) J. W. Green, *Advan. Carbohydr. Chem.*, **21**, 95–142 (1966).
(7) A. L. Raymond, *Advan. Carbohydr. Chem.*, **7**, 129–145 (1945).
(8) D. Horton and D. H. Hutson, *Advan. Carbohydr. Chem.*, **18**, 123–199 (1963).

oxygen atoms in a sugar molecule, but which mentioned dithio-acetals only to the extent of their involvement in the formation of monothio sugars and their derivatives. Appended to this Chapter is a set of Tables that lists the melting points and specific rotations of sugar dithioacetals and certain derivatives.

Although dithioacetals are odorless, an inherent practical disadvantage of their preparation (and any other process wherein lower thiols are employed as reagents or are formed as products) is the generally disagreeable scent of the lower thiols. This handicap has been pointed up with singular color[9] by Malisoff and coworkers, and regarded more soberly by numerous authors; 1-hexanethiol[10] and 1-octanethiol[9] have been reported to possess acceptable bouquets, but data on their derivatives are essentially nonexistent, depriving the investigator using these thiols of the benefits of prior reports. Most of the early examples were prepared from ethanethiol, but α-toluenethiol has enjoyed considerable vogue in later years. It has been suggested that the lower volatility of the latter mercaptan diminishes the intensity of the stench, but this temporary advantage may be outweighed by its extraordinary persistence. Perhaps the most realistic compromise is a dry note by Wolfrom and Karabinos[11] that the ". . . odor of the ethyl mercaptan can be minimized . . . by the use of good fume cupboards."

II. FORMATION OF DITHIOACETALS

The acid-catalyzed reaction of simple aldehydes and ketones with a thiol (benzenethiol) was first demonstrated by Baumann,[12] who characterized the products as diphenyl dithioacetals; two years later, in 1887, Mylius extended[13] this reaction to natural-product chemistry by preparing the diphenyl dithioacetal of a steroidal ketone. Thwarted in his attempts[14,15] to prepare acetals by the reaction of sugars with alcoholic acid, Emil Fischer predicted by analogy that Baumann's reaction[12] would convert sugars into thioglycosides; however, in his definitive[1] paper on the reactions of aldoses with thiols, Fischer observed that, in all of the examples from which he was able

(9) W. M. Malisoff, E. M. Marks, and F. G. Hess, *Chem. Rev.*, **7**, 493–547 (1930).

(10) Z. El-Hewe[i]hi, *Chem. Ber.*, **86**, 862–866 (1953).

(11) M. L. Wolfrom and J. V. Karabinos, *J. Amer. Chem. Soc.*, **67**, 500–501 (1945).

(12) E. Baumann, *Ber.*, **18**, 883–892 (1885).

(13) F. Mylius, *Ber.*, **20**, 1968–1989 (1887).

(14) E. Fischer, *Ber.*, **26**, 2400–2412 (1893).

(15) E. Fischer, *Ber.*, **28**, 1145–1167 (1895).

to isolate a product (after relatively brief exposure of the sugar to the thiol plus concentrated hydrochloric acid), two equivalents of the thiol had combined with one equivalent of an aldose to yield a crystalline dithioacetal.

Water-soluble and low-melting dithioacetals (for example, derivatives of D-xylose) were virtually inaccessible to Fischer, as his technique of isolation relied on spontaneous separation of a crystalline product after dilution of the reaction mixture with water. Alternative isolative methods that were subsequently developed to deal with such problems of solubility include the use of lead carbonate,[16] barium carbonate,[17] or an anion-exchange resin[18] to remove the acid catalyst, with subsequent removal of all or part of the diluent by evaporation, and the use of chloroform[19] or ether[20] to remove the excess of thiol from the water layer and induce crystallization of the product.

Although the original paper reported[1] isolation of diethyl dithioacetals of only one pentose, four hexoses, and a heptose, this reaction has subsequently been generalized, with examples ranging from trioses[21] to octoses.[22,23]

Mercaptalation of simple, organic, carbonyl compounds is considered[24] to proceed by way of electrophilic addition of one thiol molecule, followed, for favorable examples, by bimolecular displacement of water from the protonated monothiohemiacetal intermediate, and, finally, deprotonation of the dithioacetal thus formed; each step is presumed to be reversible, and Bethell and Ferrier[25] demonstrated an example in which a diethyl dithioacetal reacted with benzenethiol in the presence of acid to form, in 80% yield, a diastereoisomeric mixture of ethyl phenyl dithioacetals. It is also known

(16) M. L. Wolfrom, M. R. Newlin, and E. E. Stahly, *J. Amer. Chem. Soc.*, 53, 4379–4383 (1931).

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

(18) H. Zinner, *Chem. Ber.*, 86, 495–496 (1953).

(19) W. G. Overend, M. Stacey, and J. Staněk, *J. Chem. Soc.*, 2841–2845 (1949).

(20) B. Berrang, D. Horton, and J. D. Wander, *J. Org. Chem.*, 38, 187–192 (1973).

(21) H. W. Arnold and W. L. Evans, *J. Amer. Chem. Soc.*, 58, 1950–1952 (1936).

(22) R. M. Hann, W. D. Maclay, and C. S. Hudson, *J. Amer. Chem. Soc.*, 61, 1270–1271 (1939).

(23) R. M. Hann, A. T. Merrill, and C. S. Hudson, *J. Amer. Chem. Soc.*, 66, 1912–1921 (1944).

(24) E. Campaigne, in "Organic Sulfur Compounds," N. Kharasch, ed., Pergamon Press, New York, 1961, Vol. I, pp. 134–145; E. Campaigne and J. R. Leal, *J. Amer. Chem. Soc.*, 76, 1272–1275 (1954).

(25) G. S. Bethell and R. J. Ferrier, *J. Chem. Soc. Perkin I*, 1033–1037 (1972).

that other functional groups present in the carbonyl compound can influence[26] the course of the reaction.

$$\underset{|}{\overset{|}{C}}=O \;+\; RSH \;\underset{\longleftarrow}{\overset{H^+}{\rightleftharpoons}}\; \underset{\overset{+}{O}H_2}{>}C\underset{}{\overset{SR}{<}} \;\underset{RSH}{\overset{-H_2O}{\rightleftharpoons}}\; >C\underset{SR}{\overset{\overset{\overset{H}{+}}{SR}}{<}} \;\underset{}{\overset{-H^+}{\rightleftharpoons}}\; >C\underset{SR}{\overset{SR}{<}}$$
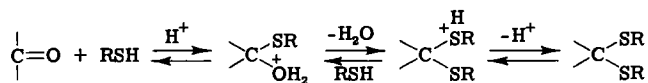
1. Influence of Different Thiols

Numerous thiols have been found suitable for preparing dithio-acetals of sugars; ethanethiol[1] and α-toluenethiol[27] have been used most commonly, but small, aliphatic thiols function interchangeably in this reaction. Condensation with 1,10-decanedithiol produces a dimeric species (1), assumed to possess a 26-membered ring,[28]

$$
\begin{array}{c}
S(CH_2)_{10}S \\
\diagup \qquad\qquad \diagdown \\
RCH \qquad\qquad HCR \\
\diagdown \qquad\qquad \diagup \\
S(CH_2)_{10}S
\end{array}
$$
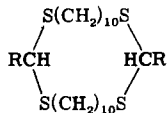
R = polyhydroxyalkyl

1

whereas monomeric dithioacetals are readily formed during reaction with 1,2-ethanedithiol,[27] 1,3-propanedithiol,[27] or 1,2-dithioglycerol.[28] Benzenethiol has been shown[29] to be less nucleophilic than aliphatic thiols, and Fischer stated[1] categorically that neither it nor hydrogen sulfide would react with sugars to form dithioacetals; subsequently, it was established[30,31] that the reaction of benzenethiol with hexoses does proceed, albeit very slowly, to give the appropriate diphenyl dithioacetals, whereas the corresponding reaction of pentoses[32,33] proceeds at a somewhat greater rate.

The relative nucleophilicities of benzenethiol and α-toluenethiol

(26) J. J. Ritter and M. J. Lover, *J. Amer. Chem. Soc.*, 74, 5576–5577 (1952), and references cited therein.

(27) W. T. Lawrence, *Ber.*, 29, 547–552 (1896).

(28) B. Gauthier and C. Vaniscotte, *Bull. Soc. Chim. Fr.*, 30–35 (1956).

(29) M. Moura Compos and H. Hauptmann, *J. Amer. Chem. Soc.*, 74, 2962–2964, 3179–3180 (1952).

(30) E. Zissis, A. L. Clingman, and N. K. Richtmyer, *Carbohydr. Res.*, 2, 461–469 (1966).

(31) S. Takahashi, M. Kurabayashi, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), 15, 1657–1661 (1967).

(32) D. Horton and J. D. Wander, *Carbohydr. Res.*, 13, 33–47 (1970).

(33) D. Horton and J. D. Wander, *Carbohydr. Res.*, 15, 271–284 (1970).

were established by examination of competitive reactions[29] between the two thiols; however, 1,2-ethanedithiol displaces either of these thiols under equilibration conditions, because cyclization to the 1,3-dithiolane ring-system produces a more stable dithioacetal,[29] a condition not shared by the medium–large ring that monomeric dithioacetals of 1,10-decanedithiol would form.

Whenever possible, the formation of heterocycles containing only sulfur atoms as heteroatoms prevails from condensations with reagents containing mixed functional groups; thus, 1,2-dithioglycerol converts aldoses into 1,3-dithiolane-4-methanol[28] derivatives (2). The

$$RCH \overset{\displaystyle SCH_2}{\underset{\displaystyle SCH}{\big|}}$$

CH$_2$OH

(L form)

2
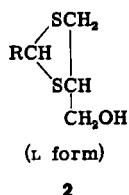
nucleophilicity of other heteroatoms is also enhanced by the possibility of generating a cyclic product; o-aminobenzenethiol[34] (3), 2-(ethylamino)ethanethiol,[35] and the amino acid cysteine[36] undergo a 1:1 condensation with aldoses in an acidic medium to produce thiazolidine derivatives, for example, 4, to the complete exclusion of dithioacetals.

3   **4**
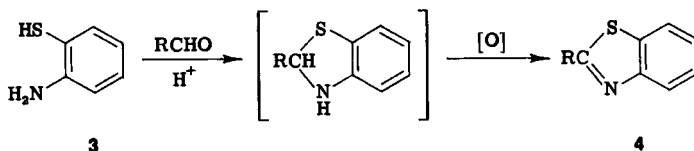
2. Influence of Stereochemical Features

It is apparent that the reaction of sugars with thiols is influenced by interactions between the numerous functional groups present, as changes in the nature of the reactants are reflected in variations in the rate or course (or both) of the mercaptalation. D-Glucose reacts with an excess of ethanethiol in hydrochloric acid during four hours at 0° to form the diethyl dithioacetal in fair yield,[1] whereas it reacts at

(34) L. Sattler, F. W. Zerban, G. L. Clark, and C.-C. Chu, J. Amer. Chem. Soc., 73, 5908–5909 (1951).

(35) T. Takatori and T. Taguchi, Yakugaku Zasshi, 88, 527–534 (1968); Chem. Abstr., 69, 87,392 (1968).

(36) M. P. Schubert, J. Biol. Chem., 130, 601–603 (1939); G. Ågren, Enzymologia, 9, 321–328 (1941).

room temperature with an equimolecular proportion of the thiol to give only ethyl 1-thio-α-D-glucopyranoside.[37,38] A reinvestigation[39] of this reaction by paper chromatography indicated that D-glucose diethyl dithioacetal is formed rapidly under these conditions, but that it undergoes a gradual conversion, presumably into thiopyranosides, during a few hours. A good yield of D-glucose diphenyl dithioacetal is obtained after eleven days[30] of reaction; in general, however, optimal yields of dithioacetals from D-glucose are obtained after short times of reaction.

D-Mannose diethyl dithioacetal is isolated[40] in 63% yield from D-mannose by treatment under the foregoing conditions for five minutes, whereas a 31% yield of ethyl thiopyranosides results[41] after 16 hours at room temperature. Paper-chromatographic monitoring of this reaction as a function of time verified[39] that production of thioglycosides occurs as a competing, presumably subsequent, process, and established that the rate of this second reaction is much lower than for the corresponding process involving D-glucose. Differing reports on the presence[39] or absence[41] of dithioacetal in the product mixture after extended reaction suggested that the dithioacetal is probably present to only a minor extent. Studies on D-galactose similarly revealed that the dithioacetal can be isolated satisfactorily,[35,42] or converted further into a mixture of thiopyranosides[41] on extended reaction; only a trace of the dithioacetal could be detected[39] in the reaction mixture after 24 hours, suggesting that D-galactose diethyl dithioacetal undergoes the secondary reaction at a rate intermediate between those of the D-*gluco* and D-*manno* analogues. The reported[30] reaction-times providing satisfactory conversion of D-mannose (overnight) and D-galactose (24 hours) into their respective diphenyl dithioacetals indicate that D-galactose is much more reactive towards mercaptalation than D-glucose, although possibly somewhat less so than D-mannose. Minimization of interference by thioglycoside formation during mercaptolyses[42-44] is ac-

(37) E. Pacsu and E. J. Wilson, Jr., *J. Amer. Chem. Soc.*, **61**, 1930–1931 (1939).

(38) P. Brigl, K. Gronemeier, and A. Schulz, *Ber.*, **72**, 1052–1059 (1939).

(39) M. L. Wolfrom, D. Horton, and H. G. Garg, *J. Org. Chem.*, **28**, 1569–1572 (1963).

(40) P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **74**, 695–699 (1927).

(41) J. Fried and D. E. Walz, *J. Amer. Chem. Soc.*, **71**, 140–143 (1949).

(42) M. L. Wolfrom, *J. Amer. Chem. Soc.*, **52**, 2464–2473 (1930).

(43) C. Araki and S. Hirase, *Bull. Chem. Soc. Jap.*, **26**, 463–467 (1953).

(44) M. L. Wolfrom, J. C. Sowden, and E. N. Lassettre, *J. Amer. Chem. Soc.*, **61**, 1072–1076 (1939); M. L. Wolfrom, C. S. Smith, and A. E. Brown, *ibid.*, **65**, 255–259 (1943).

complished by maintaining the mixture at, or slightly below, 0° throughout the reaction.

The conversions of 3-amino-3-deoxy-D-allose,[45] 3-amino-3-deoxy-D-glucose,[45] 3-amino-3-deoxy-D-mannose,[39] and 3-amino-3,6-dideoxy-D-mannose[46] into their respective diethyl dithioacetals were accompanied by rapid formation of substantial proportions of thioglycosides. Paper-chromatographic data[39] indicated that the composition of products formed from 3-amino-3-deoxy-D-mannose after about four hours remained essentially unaltered over extended periods of reaction; these results were interpreted[39] in terms of a rapid, kinetically controlled reaction to form the dithioacetal, followed by slower processes transforming the dithioacetal into a mixture of products whose composition, given time to stabilize, is determined by the relative thermodynamic stabilities of the components. Ethyl 3-amino-3,6-dideoxy-1-thio-β-D-mannopyranoside, which was separated from the mixture of products formed during mercaptalation of 3-amino-3,6-dideoxy-D-mannose, reproduced the original distribution of products under conditions of the mercaptalation, thereby proving[46] the equilibrium character of the conversions involving thioglycosides.

L-Arabinose diethyl dithioacetal appears to possess greater thermodynamic stability than the corresponding thioglycosides, as no other product is detected under conditions of mercaptalation, even after extended periods of reaction[41]; it was reported,[10] however, that the acid-catalyzed action of 1-hexanethiol upon L-arabinose produces only a thio-L-arabinoside of undetermined anomeric configuration and ring size. D-Ribose can be converted, in satisfactory yields, into appropriate dithioacetals by the action of an excess of any of several aliphatic thiols in hydrochloric acid.[47] Unspecified yields of the diphenyl dithioacetal and an uncharacterized product containing one or more phenyl tri-O-acetyl-1-thio-D-ribosides have been reported (as the syrupy peracetates[48]) from consecutive reaction of D-ribose with benzenethiol in hydrochloric acid, and with acetic anhydride in pyridine (in the presence of unreacted benzenethiol and acid); under fairly extended, conventional conditions,[37] crystalline D-ribose diphenyl dithioacetal is formed in fair yield, together with a minor

(45) J. Jarý, Z. Kefurtová, and J. Kovář, Collect. Czech. Chem. Commun., 34, 1452–1458 (1969).
(46) M. von Saltza, J. D. Dutcher, J. Reid, and O. Wintersteiner, J. Org. Chem., 28, 999–1004 (1963).
(47) H. Zinner, Chem. Ber., 83, 275–277 (1950).
(48) Z. El-Hewehi, Chem. Ber., 91, 2039–2044 (1958).

proportion of an unidentified mixture of thioglycosides. Reaction of
D-ribose with one molar equivalent of methanethiol in concentrated
hydrochloric acid generates[49] the dithioacetal as the main product,
together with some thiopyranosides and minor traces of thiofurano-
sides (plus unreacted D-ribose); the analogous reaction in M hydro-
chloric acid also produces[49] mainly the dithioacetal, although thio-
furanosides are the more abundant minor product, thiopyranosides
being present only in trace amounts. Direct formation of the thiogly-
cosides from D-ribose was invoked in the latter reaction, as dithio-
acetals of D-ribose are stable in M acid; the mechanism of formation
was not speculated upon in the report.[49]

D-Xylose likewise forms dialkyl[16] and diaryl[32] dithioacetals under
conventional conditions, without significant competition from forma-
tion of thioglycosides. By methods employing radioactive-tracer
techniques, it has been shown[50] that, in 40:10:1 ethanethiol-N,N-
dimethylformamide–hydrochloric acid, rapid formation of thiofurano-
sides is gradually (24 hours) displaced by conversion into the dithio-
acetal; small proportions of thiopyranosides are present as minor
constituents throughout the reaction. The effect of the change of sol-
vent is uncertain, but the reaction medium is probably responsible
for the accompanying, slow (several days), partial conversion of
the dithioacetal into 2-(ethylthio)-3-(ethylthiomethyl)furan (5). 2-
Furaldehyde diethyl dithioacetal was not converted into 5 under the
reaction conditions, and two possible routes, each having a 5-mem-
bered-ring episulfonium ion as a common intermediate, were of-
fered[50] in order to rationalize the formation of 5.
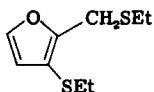
5

3. Sugars Other than Aldoses

Despite a report[11] asserting that, under conditions of mercaptala-
tion, deoxyaldoses are insufficiently stable to permit a product to be
isolated, a number[19,51–64] of deoxyaldose dialkyl dithioacetals have

(49) C. J. Clayton, N. A. Hughes, and S. A. Saeed, *J. Chem. Soc.* (*C*), 644–648 (1967).
(50) R. J. Ferrier, L. R. Hatton, and W. G. Overend, *Carbohydr. Res.*, 6, 87–96 (1968).
(51) D. R. Swan and W. L. Evans, *J. Amer. Chem. Soc.*, 57, 200–202 (1935).
(52) R. E. Deriaz, W. G. Overend, M. Stacey, E. G. Teece, and L. F. Wiggins, *J. Chem. Soc.*, 1879–1883 (1949).
(53) I. W. Hughes, W. G. Overend, and M. Stacey, *J. Chem. Soc.*, 2846–2849 (1949).

been prepared in satisfactory yield under the conventional conditions; the assertion of unreactivity or instability, or both,[1,11] appears to be correct, however, for ketoses in which the carbonyl group is involved in a ring. The fact that ketoses are merely decomposed under conditions that convert aldoses into dithioacetals provides a convenient technique for the isolation[65] of aldoses from admixtures with expendable ketoses.

Acylation[66] of D-fructose leads[67] to substantial yields of a completely esterified, acyclic derivative (6) in which the ketonic center exists as a free carbonyl group, and provides an indirect route for the synthesis of ketose dialkyl dithioacetals, as 6 reacts with two equivalents of thiol under acid catalysis to produce[68] the acylated dithioacetal 7, which can subsequently be saponified to yield the free dithioacetal[69] (8) of the ketose.

$$
\begin{array}{ccccc}
CH_2OAc & & CH_2OAc & & CH_2OH \\
| & & | & & | \\
C{=}O & & EtSCSEt & & EtSCSEt \\
| & EtSH & | & NaOMe & | \\
AcOCH & \xrightarrow{\quad} & AcOCH & \xrightarrow{\quad} & HOCH \\
| & ZnCl_2 & | & & | \\
HCOAc & & HCOAc & & HCOH \\
| & & | & & | \\
HCOAc & & HCOAc & & HCOH \\
| & & | & & | \\
CH_2OAc & & CH_2OAc & & CH_2OH \\
\\
\mathbf{6} & & \mathbf{7} & & \mathbf{8}
\end{array}
$$
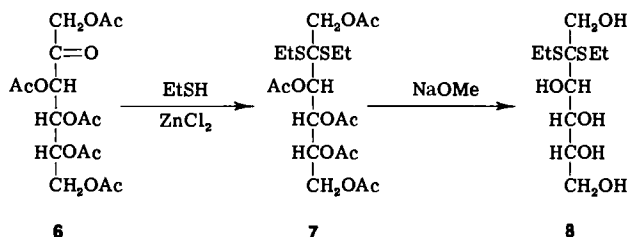
Aldosulose derivatives having unprotected ketonic functions have been converted into similarly substituted ethylene dithioacetals by the action of 1,2-ethanedithiol in the presence of boron trifluoride. 1,6-Anhydro-2,4-dideoxy-β-D-glycero-hexopyranos-3-ulose[70] (9)

(54) P. W. Kent, *Nature*, **166**, 442 (1950).

(55) W. G. Overend, F. Shafizadeh, and M. Stacey, *J. Chem. Soc.*, 671–677 (1950).

(56) H. R. Bolliger, *Helv. Chim. Acta*, **34**, 989–991 (1951).

(57) A. B. Foster, W. G. Overend, and M. Stacey, *J. Chem. Soc.*, 974–979 (1951).

(58) R. L. Mann and D. O. Woolf, *J. Amer. Chem. Soc.*, **79**, 120–126 (1957).

(59) G. Rembarz, *Chem. Ber.*, **93**, 622–625 (1960).

(60) H. Zinner, B. Ernst, and F. Kreienbring, *Chem. Ber.*, **95**, 821–824 (1962).

(61) H. Zinner, G. Wulf, and R. Heinatz, *Chem. Ber.*, **97**, 3536–3540 (1964).

(62) C. C. Bhat, K. V. Bhat, and W. W. Zorbach, *Carbohydr. Res.*, **10**, 197–212 (1969).

(63) E. Fischer and H. Herborn, *Ber.*, **29**, 1961–1967 (1896).

(64) O. Ruff, *Ber.*, **35**, 2360–2370 (1902).

(65) See, for example, B. Görlich, *Ann.*, **634**, 192–196 (1960).

(66) C. S. Hudson and D. H. Brauns, *J. Amer. Chem. Soc.*, **37**, 2736–2745 (1915).

(67) E. Pacsu and F. V. Rich, *J. Amer. Chem. Soc.*, **54**, 1697–1698 (1932).

(68) P. Brigl and R. Schinle, *Ber.*, **66**, 325–330 (1933).

(69) M. L. Wolfrom and A. Thompson, *J. Amer. Chem. Soc.*, **56**, 880–882 (1934).

(70) J. Pecka and M. Černý, *Collect. Czech. Chem. Commun.*, **38**, 132–142 (1973).

and methyl 2,3,6-tri-*O*-benzoyl-α-D-*xylo*-hexopyranosid-4-ulose[71] (**10**) were thus converted into the dithioacetals **11** and **12**, respectively. The major product of reaction of **10** with ethanethiol, a less potent nucleophile, is a bis(diethyl dithioacetal) derivative, whereas similar treatment of the corresponding benzyl β-pyranoside (**13**) and β-pyranosyl bromide (**14**) affords[72] as the principal products the thioglycoside dithioacetal **15** and the glycosyl bromide **16**, respectively.

H₂C——O / O / X' (structure 9/11)

X CH₂OBz / O / BzO / BzO / R' / R (structure 10–16)

9 X = O
11 X = −SCH₂CH₂S−

10 X = O, R = OMe, R' = H
12 X = −SCH₂CH₂S−, R = OMe, R' = H
13 X = O, R = H, R' = OCH₂Ph
14 X = O, R = H, R' = Br
15 X = (SEt)₂, R = SEt, R' = H
16 X = (SEt)₂, R = H, R' = Br
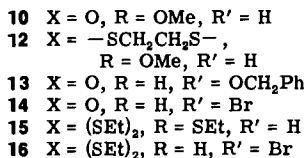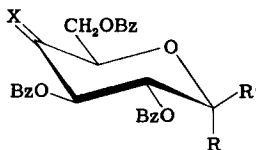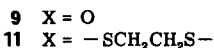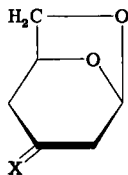
As long as the aldehydic (or ketonic) center is preserved, sugar derivatives containing more-highly oxidized functions can be converted into dithioacetals under similar conditions. As expected from early studies on the reactivity of simple dicarbonyl[73] compounds, pentodialdoses[74] and hexodialdoses[75,76] (in which the aldehydic centers are well separated) undergo mercaptalation at both termini to form α,ω-bis(dialkyl dithioacetals), whereas aldosuloses, in which the intrinsic[1] unreactivity of the ketonic function adjacent to C-1 is compounded by steric[73] interactions, form only a 1,1-(dialkyl dithioacetal)[77] under the usual conditions; an example of the latter product has also been prepared indirectly[78] by addition of ethanesulfenyl chloride to 3,4,5,6-tetra-*O*-acetyl-1-deoxy-1-diazo-*keto*-D-fructose

(71) J. F. Batey, C. Bullock, J. Hall, and J. M. Williams, *Carbohydr. Res.*, **40**, 275–283 (1975).
(72) J. M. Williams, *Abstr. Int. Carbohydr. Symp. VIth*, 1972.
(73) T. Posner, *Ber.*, **33**, 2983–2993 (1900); **35**, 493–505 (1902); A. Schönberg and O. Schütz, *Ann.*, **454**, 47–53 (1927).
(74) D. L. MacDonald and H. O. L. Fischer, *J. Amer. Chem. Soc.*, **77**, 4348–4350 (1955).
(75) C. E. Ballou and H. O. L. Fischer, *J. Amer. Chem. Soc.*, **75**, 3673–3675 (1953).
(76) D. L. MacDonald and H. O. L. Fischer, *J. Amer. Chem. Soc.*, **78**, 5025–5026 (1956).
(77) S. Bayne, *Proc. Chem. Soc.*, 170 (1958).
(78) F. Weygand, E. Klieger, and H. J. Bestmann, *Chem. Ber.*, **90**, 645–648 (1957).

(17), followed by displacement of the halogen with sodium ethane-thioxide, and subsequent saponification. Substitution of benzene-sulfenyl chloride in the sequence resulted in the formation[78] of the (mixed) ethyl phenyl dithioacetal **19**, evidently by way of the inter-mediate **18**.
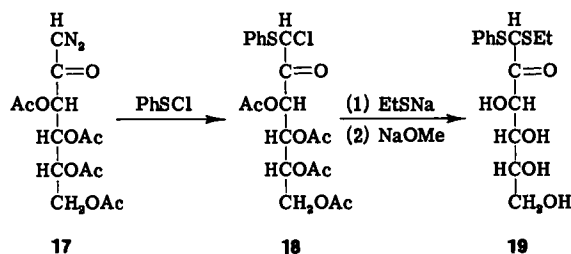
D-Glucuronic acid[79] and its 6,3-lactone[80-82] react with thiols to af-ford the dithioacetal of the lactone; in alkali, the product opens to give the sodium salt,[81] but the lactone ring re-forms upon neutral-ization. The same conditions convert D-galacturonic acid into its dithioacetal,[83] which lactonizes upon warming,[84] but re-opens in alkali. N-Acetylneuraminic acid has similarly been converted[85] into 5-acetamido-5-deoxy-D-*glycero*-D-*galacto*-nonulosono-1,4-lactone 2-(diethyl dithioacetal); the reactivity of this ketose is presumably related to the presence (potential or realized) of the lactone func-tionality.

Mercaptalation of D-galacturonic acid in the presence of an alcohol results[86] in concomitant esterification; the product from the analo-gous reaction of D-glucuronic acid was not[81] identified. Diazo-methane also converts D-galacturonic acid dithioacetals into the cor-responding[83] methyl esters. Ethanethiolysis of the muramic acid analogue **20** affords the internally esterified dithioacetal[87] **21** in 71% yield.
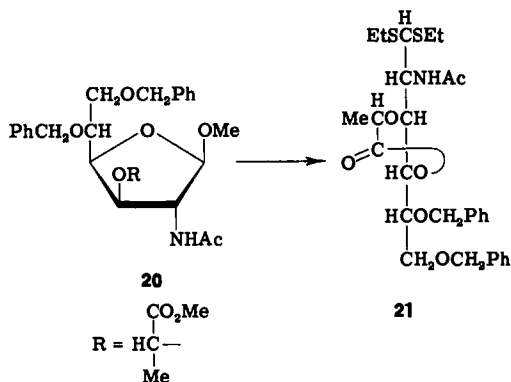
Aminodeoxy sugars[46,88,89] generally undergo a poor reaction with acidic thiols, eventually yielding a small proportion of the corresponding dithioacetal (as the amine hydrochloride); the effect is strongest[39] for 2-amino-2-deoxyaldoses.[81,90-95] Protonation of the strongly basic amino group presumably occurs immediately (under acid catalysis), and the field effect from this positive center suppresses[39,93] further acid-catalyzed processes involving the protonated aminodeoxy sugar. The use of fuming hydrochloric acid as the catalyst[92] facilitates the reaction somewhat, but, in general (unless the presence of the free amino group in the dithioacetal is essential), substantial improvements, both in the length of time needed to complete the reaction and in the yields realized, are achieved by acylating the amino group[81,91,96] prior to mercaptalation; this modification failed with 3-benzamido-3,6-dideoxy-D-mannose, but gave satisfactory results with the 3-acetamido analogue.[46]

4. Influence of Pre-existing Substituents in the Sugar; Reactions Proceeding through Episulfonium Ions as Intermediates

Substituents present in the sugar derivative at the time of mercaptalation may lead to any of three consequences, namely: (a) the reaction proceeds without disturbance to the substituent(s), (b) the reaction proceeds, but the substituent undergoes hydrolysis, or (c) the course of the reaction is altered. Deoxy sugars and free or N-substituted[97] aminodeoxy sugars are common examples of the first group, as are deoxyhalogenoaldoses,[98-100] sugars having O-methyl[101-103] and

(79) H. Zinner, C.-G. Dässler, and G. Rembarz, Chem. Ber., 91, 427–430 (1958).

(80) C. Neuberg, Ber., 33, 3315–3323 (1900).

(81) M. L. Wolfrom and K. Onodera, J. Amer. Chem. Soc., 79, 4737–4740 (1957).

(82) F. García González, J. Fernández-Bolaños, and M. Repetto Jiménez, An. Real Soc. Españ. Fis. Quim., B55, 743–748 (1959).

(83) H. A. Campbell and K. P. Link, J. Biol. Chem., 120, 471–479 (1937).

(84) H. Zinner, W. Thielebeule, and G. Rembarz, Chem. Ber., 91, 1006–1011 (1958).

(85) R. Kuhn and R. Brossmer, Angew. Chem., 69, 534 (1957).

(86) R. J. Dimler and K. P. Link, J. Amer. Chem. Soc., 62, 1216–1219 (1940).

(87) J.-C. Jacquinet and P. Sinaÿ, Carbohydr. Res., 34, 139–150 (1974).

(88) M. L. Wolfrom, Y.-L. Hung, and D. Horton, J. Org. Chem., 30, 3394–3400 (1965).

(89) S. David and A. Veyrières, Carbohydr. Res., 13, 203–209 (1970).

(90) P. W. Kent, Research (London), 3, 427–428 (1950).

(91) M. L. Wolfrom and K. Anno, J. Amer. Chem. Soc., 74, 6150–6151 (1952).

(92) M. W. Whitehouse, P. W. Kent, and C. A. Pasternak, J. Chem. Soc., 2315–2317 (1954).

(93) L. Hough and M. I. Taha, J. Chem. Soc., 3311 (1957).

(94) T. H. Haskell and S. Hanessian, J. Org. Chem., 28, 2598–2604 (1963).

O-benzyl[104] substituents (although 2-O-benzyl derivatives may be somewhat sensitive to conditions[105]), 3,6-anhydro sugars,[43] and sulfonic[106,107] esters; however, Veksler[96] reported the concomitant formation of a supposed 3,6-anhydro bridge during mercaptalation of some derivatives of 6-O-p-tolylsulfonyl-D-galactose.

Under the general conditions[1] (hydrochloric acid as the catalyst), the second class includes acid-sensitive substituents, such as acetals[93,104,108,109] and glycosides[37]; 1,5-anhydro-2-deoxy-D-lyxo-hex-1-enitol (D-galactal) is converted into 2-deoxy-D-lyxo-hexose diethyl dithioacetal by treatment with ethanethiol plus hydrochloric acid.[110] In the presence of a cation-exchange resin, 3-deoxy-1,2-O-isopropylidene-β-L-threo-pentodialdo-1,4-furanose reacts with α-toluenethiol (and other thiols) to afford the corresponding 1,2-O-isopropylidene 5-(dibenzyl dithioacetal)[110a] in almost quantitative yield. Interglycosidic linkages may or may not survive during mercaptalation; in the former event, dithioacetals of disaccharides[16,111] arise, although it is difficult to envisage the supposedly intact products[112–114] that were reported from mercaptalation of the *nonreducing* disaccharide sucrose; Sugihara[115] later described the isolation of di-

(95) S. David and A. Veyrières, *Carbohydr. Res.*, 10, 35–48 (1969).

(96) V. I. Veksler, *Zh. Obshch. Khim.*, 31, 989–993 (1961).

(97) A. Wickstrøm and J. K. Wold, *Acta Chem. Scand.*, 14, 1419–1423 (1960).

(98) E. Fischer, B. Helferich, and P. Ostmann, *Ber.*, 53, 873–886 (1920).

(99) F. Micheel and F. Suckfüll, *Ann.*, 502, 85–98 (1933).

(100) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, B53, 377–380 (1957).

(101) M. L. Wolfrom and L. W. Georges, *J. Amer. Chem. Soc.*, 59, 601–603 (1937).

(102) J. M. Sugihara and M. L. Wolfrom, *J. Amer. Chem. Soc.*, 71, 3509–3510 (1949).

(103) L. Hough and A. C. Richardson, *J. Chem. Soc.*, 5561–5563 (1961).

(104) E. F. L. J. Anet, *Carbohydr. Res.*, 7, 84–85 (1968).

(105) O. T. Schmidt and E. Wernicke, *Ann.*, 558, 70–80 (1946).

(106) F. Micheel and H. Ruhkopf, *Ber.*, 70, 850–853 (1937).

(107) A. B. Foster, W. G. Overend, M. Stacey, and L. F. Wiggins, *J. Chem. Soc.*, 2542–2546 (1949).

(108) E. J. Bourne, G. P. McSweeney, M. Stacey, and L. F. Wiggins, *J. Chem. Soc.*, 1408–1414 (1952).

(109) B. Coxon and L. Hough, *J. Chem. Soc.*, 1463–1469 (1961).

(110) J. L. Barclay, A. J. Cleaver, A. B. Foster, and W. G. Overend, *J. Chem. Soc.*, 789–790 (1956).

(110a) H. Zinner and R. Reck, *J. Prakt. Chem.*, 315, 179–184 (1973).

(111) S. Hirase and C. Araki, *Bull. Chem. Soc. Jap.*, 27, 105–112 (1954).

(112) Y. Uyeda and J. Kamon, *Bull. Chem. Soc. Jap.*, 1, 179–180 (1926).

(113) Y. Maeda and Y. Uyeda, *Bull. Chem. Soc. Jap.*, 1, 181–182 (1926).

(114) Y. Uyeda, *Bull. Chem. Soc. Jap.* 4, 264–265 (1929).

(115) J. M. Sugihara, *Abstr. Pap. Amer. Chem. Soc. Meet.*, 112, 13Q (1945).

thioacetals of D-glucose on using the same general conditions, noting that 1-butanethiol affords the optimal recovery of D-glucose derivatives, whereas increasingly bulky (large or branched) thiols react progressively more poorly with sucrose.

"Overmercaptalation" also leads, on occasion, to incorporation of additional[10,116] alkylthio residues along the side chain. The alternative, commoner occurrence, hydrolysis to monosaccharide fragments, has utility as an analytical technique; the identities of monosaccharide components of polysaccharides[43,117−119] (and of other condensed, natural products[54,58,95,120]) have been established by "mercaptolysis" (hydrolysis in an acidic medium containing a thiol) followed by characterization of the aldose dithioacetals thus obtained, and the degree of polymerization (number of monosaccharide residues present in the macromolecule) of a polysaccharide can be estimated[44] by analysis of the rate of conversion of the sample into monosaccharide dithioacetal(s). Fragments derived by mercaptolysis were used in elucidation[121] of the carbohydrate components of streptomycin.

Carboxylic esters fall largely into the third category. 2,3,4,5,6-Penta-O-acetyl[69] (and -benzoyl[122])-*aldehydo*-D-glucose (**22** and **23**, respectively) undergo zinc chloride-catalyzed condensation with ethanethiol to afford peracylated dithioacetals (**24** and **25**, respectively), and peracetylated aldopyranoses react with thiols in the presence of zinc(II) chloride[123,124] or *p*-toluenesulfonic acid[125] to afford mainly acetylated alkyl 1-thioaldopyranosides; however, the free aldehyde group in the former examples is exceptionally reactive, whereas the latter reaction is considered to proceed by way of a 1,2-acetoxonium-ion intermediate **26** that assists in the departure of the acetoxyl group from C-1 and undergoes subsequent opening through nucleophilic attack by thiol at C-1.

22 R = Ac
23 R = Bz

24
25

26
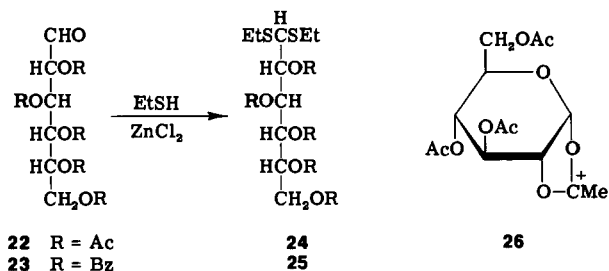
(116) J. Staněk and J. Šáda, *Collect. Czech. Chem. Commun.*, 14, 540–550 (1949).
(117) E. E. Percival, *Chem. Ind.* (London), 1487 (1954).
(118) A. N. O'Neill, *J. Amer. Chem. Soc.*, 77, 2837–2843 (1955).
(119) T. J. Painter, *Can. J. Chem.*, 38, 112–118 (1960).

The presence of carboxylic ester groups, especially fewer than a full complement, in sugars undergoing acid-catalyzed reactions with thiols facilitates the introduction of additional thio substituents into the polyhydroxyalkyl chain. Byproducts obtained in the zinc chloride-catalyzed thiolation of aldopyranose peracetates with ethanethiol contain a third ethylthio group.[123,124] Their formation may be compactly rationalized as occurring by attack of ethanethiol at C-2 of **26**; the 2-S-ethyl-2-thio compound thus formed would be unable to form a 1,2-acetoxonium ion, and would accordingly be susceptible to mercaptalation. Support is lent to this hypothesis by the observation[123] that one of the two thiohexose dithioacetals is identical with a 2-S-ethyl-2-thiohexose diethyl dithioacetal that had been prepared as a tetra-O-benzoyl derivative by Brigl and coworkers.[126,127] (The other product was assumed,[124] correctly,[128] to be the epimeric thioether.)

Brigl's compound was originally prepared[126] by the action of ethanethiol and hydrochloric acid on 3,4,5,6-tetra-O-benzoyl-*aldehydo*-D-glucose; under these conditions, 3,4,5,6-tetra-O-benzoyl-D-glucose diethyl dithioacetal (**27**) is formed[126] first, replacement of the 2-hydroxyl group occurring in a subsequent, slower process. The trithio compound was arbitrarily assigned the structure 3,4,5,6-tetra-O-benzoyl-2-S-ethyl-2-thio-D-glucose diethyl dithioacetal. The same product has been reprepared several times[128-131] from **27** by essentially the same method,[132] as well as by treatment of pentabenzoyl derivatives of cyclic D-glucoses with ethanethiol in an acidic medium.[127] Conversion[127] of the trithio product into D-*arabino*-hexosulose phenylosazone identified it as a 2-thiohexose of the D-*gluco* or D-*manno* series; alternatively, saponification followed by demercaptalation in the presence of an excess of barium carbonate[127] converted the product into 2-S-ethyl-2-thio-D-glucopyranose (**31**), whose

(120) D. L. MacDonald and C. A. Knight, *J. Biol. Chem.*, **202**, 45–50 (1953).

(121) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, *J. Amer. Chem. Soc.*, **68**, 2096–2101, 2679–2684 (1946).

(122) P. Brigl and H. Mühlschlegel, *Ber.*, **63**, 1551–1557 (1930).

(123) R. U. Lemieux, *Can. J. Chem.*, **29**, 1079–1091 (1951).

(124) R. U. Lemieux and C. Brice, *Can. J. Chem.*, **33**, 109–119 (1955).

(125) M. L. Chawla and O. P. Bahl, *Carbohydr. Res.*, **32**, 25–29 (1974).

(126) P. Brigl, H. Mühlschlegel, and R. Schinle, *Ber.*, **64**, 2921–2934 (1931).

(127) P. Brigl and R. Schinle, *Ber.*, **65**, 1890–1895 (1932).

(128) D. Horton, L. G. Magbanua, and J. M. J. Tronchet, *Chem. Ind.* (London), 1718–1719 (1966).

(129) H. R. Bolliger and M. D. Schmid, *Helv. Chim. Acta*, **34**, 1597–1600.

(130) B. Berrang and D. Horton, *Chem. Commun.*, 1038–1039 (1970).

(131) See also: D. Horton, *Pure Appl. Chem.*, **42**, 301–325 (1975).

(132) H. R. Bolliger, *Methods Carbohydr. Chem.*, **1**, 186–189 (1962).

structure was subsequently established[133] by n.m.r. spectroscopy. Demercaptalation of the trithio product by use of exactly two molar equivalents of sodium hydrogen carbonate, instead of the excess of barium carbonate, was shown[128] to produce 2-S-ethyl-2-thio-D-mannopyranose (32), also identified by n.m.r. spectroscopy; careful re-examination of the sequence involving barium carbonate revealed that *both* epimeric 2-thiopyranosides were thus formed,[128] and a subsequent study[130] proved that **32** is formed first, but undergoes spontaneous conversion into **31** at pH 8. Further proof that Brigl's product is 3,4,5,6-tetra-O-benzoyl-2-S-ethyl-2-thio-D-mannose diethyl dithioacetal (**29**) was obtained[130] by saponification of **29**, followed by partial hydrolysis of the dithioacetal function to yield ethyl 2-S-ethyl-1,2-dithio-α-D-mannofuranoside (**30**), whose structure had already been established unequivocally[134] by X-ray crystallography; the structure of the deacylated trithio product **33** was subsequently verified by X-ray crystallography.[135,136]
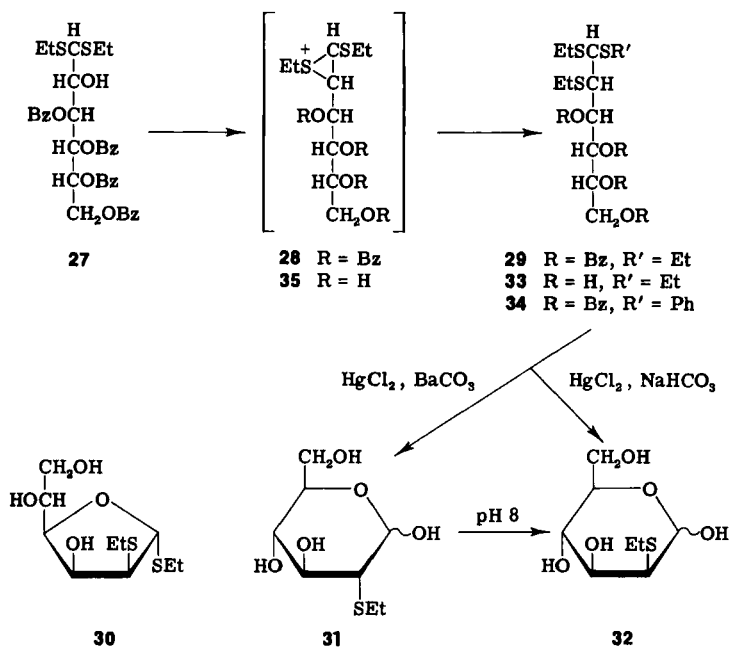
(133) A. E. El Ashmawy, D. Horton, L. G. Magbanua, and J. M. J. Tronchet, *Carbohydr. Res.*, **6**, 299–309 (1968).

(134) J. Defaye, T. Nakamura, D. Horton, and K. D. Philips, *Carbohydr. Res.*, **16**, 133–144 (1971); J. Defaye, A. Ducruix, and C. Pascard-Billy, *Bull. Soc. Chim. Fr.*, 4514–4515 (1971); A. Ducruix and C. Pascard-Billy, *Acta Crystallogr.*, **B28**, 1195–1201 (1972).

It is highly probable that the initial step in the overmercaptalation of **27** involves formation of a 2,3-acyloxonium ion; however, formation of the 1,2-episulfonium ion **28** as an intermediate in the replacement of the 2-hydroxyl group has been established[130] through the conversion of **27** into 3,4,5,6-tetra-*O*-benzoyl-2-*S*-ethyl-2-thio-D-mannose ethyl phenyl dithioacetal (**34**) by the action of benzenethiol and an acid catalyst. The presence of an ethylthio group on C-2 in **34** was further established by conversion of **34** into 3,4,5,6-tetra-*O*-benzoyl-2-*S*-ethyl-2-thio-D-mannose dimethyl acetal.

Deamination (see Section III,4) of 2-amino-2-deoxy-D-glucose diethyl dithioacetal at pH 0 and 5 respectively affords **31** (Refs. 128 and 133) and **30** (Ref. 134); a similar 1,2-episulfonium ion (**35**) was invoked[131] as an intermediate in the formation of both products, attack occurring sequentially by the second sulfur atom and by solvent water in the former reaction, and by O-4 of the sugar in the latter example.

3,4,5-Tri-*O*-benzoyl-D-xylose diethyl dithioacetal reacts with ethanethiol plus acid to form a 2-*S*-ethyl-2-thio-D-pentose diethyl dithioacetal[137] of undetermined stereochemistry; in view of the common stereochemical arrangements about C-1–C-4 in the D-*gluco* and D-*xylo* structures, this product probably has the D-*lyxo* arrangement.

Ethanethiolysis of 3,5,6-tri-*O*-benzoyl-D-glucofuranose affords a product that Brigl and Schinle[127] presumed to be 3,5,6-tri-*O*-benzoyl-2,4-di-*S*-ethyl-2,4-dithio-D-glucose. Bethell and Ferrier[25] subsequently identified this product as 4,5,6-tri-*O*-benzoyl-2,3-di-*S*-ethyl-2,3-dithio-D-allose diethyl dithioacetal (**36**). One of the isolable[138] intermediates of this reaction is 3,5,6-tri-*O*-benzoyl-2-*S*-ethyl-2-thio-D-mannose diethyl dithioacetal (**38**); furthermore, methanethiolysis of 3,5,6-tri-*O*-benzoyl-2-*S*-ethyl-2-thio-D-mannofuranose in a mixture of chloroform and trifluoroacetic acid affords 4,5,6-tri-*O*-benzoyl-3-*S*-ethyl-2-*S*-methyl-2,3-dithio-D-allose dimethyl dithioacetal (**39**). From these observations, it was proposed[25,138] that the original reaction proceeds, by way of the bridged acetoxonium ion **37**, through a sequence of displacements involving episulfonium ions.

H		H		H
RSCSR				EtSCSEt
HCSR				EtSCH
HCSEt		BzO⌐ ⟍ ⟍		BzOCH
HCOBz	←	BzO⌐	← H⁺ EtSH	HCOH
HCOBz		⁺C		HCOBz
CH₂OBz		Ph		CH₂OBz

36 R = Et   **37**   **38**
39 R = Me
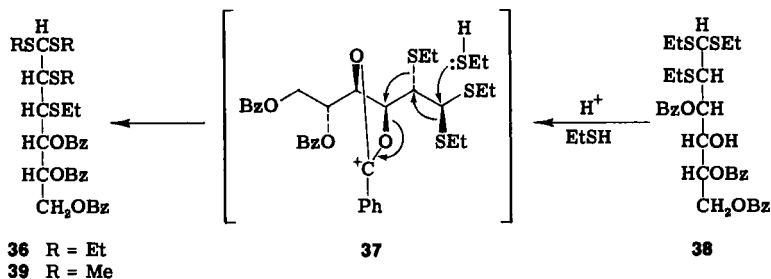
Testing the limits of this type of participating reaction, Bethell and Ferrier[139] treated 3-O-benzoyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose with a mixture of ethanethiol and trifluoroacetic acid in chloroform; the product, ethyl 4-O-benzoyl-2,3,6-tri-S-ethyl-1,2,3,6-tetrathio-α-D-mannopyranoside (40), was identified spectroscopically, and its formation signalled that the 6-hydroxyl group combines with the 4-O-benzoyl group of the intermediate 42 to form the six-membered-ring benzoxonium ion 41, thereby interrupting the sequence of vicinal-displacement reactions. Methanethiolysis of 3-O-benzoyl-2-S-ethyl-5,6-O-isopropylidene-2-thio-α-D-glucofuranose afforded the 6-S-ethyl product 43, indicating that the alkylthio groups migrate down the carbon chain in tandem, and that the final sulfur migration is from C-3 to C-6.

40 R = Et
43 R = Me
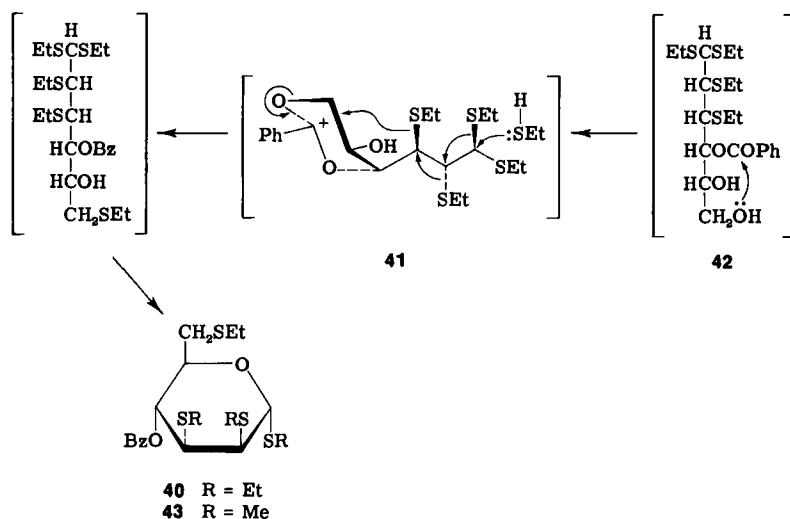
Treatment of keto-D-psicose pentaacetate with ethanethiol plus zinc chloride produces a syrupy compound that undergoes hydrogenolysis to afford an optically inactive dideoxyhexitol; from this evidence of symmetry, Wolfrom and coworkers[140] concluded that a third

(135) A. Ducruix, C. Pascard-Billy, D. Horton, and J. D. Wander, *Carbohydr. Res.*, 29, 276–279 (1973).
(136) A. Ducruix and C. Pascard-Billy, *Acta Crystallogr.*, B30, 1056–1063 (1974).
(137) M. L. Wolfrom and W. von Bebenburg, *J. Amer. Chem. Soc.*, 82, 2817–2819 (1960).
(138) G. S. Bethell and R. J. Ferrier, *J. Chem. Soc. Perkin I*, 2873–2878 (1972).
(139) G. S. Bethell and R. J. Ferrier, *J. Chem. Soc. Perkin I*, 1400–1405 (1973).
(140) M. L. Wolfrom, B. W. Lew, and R. M. Goepp, Jr., *J. Amer. Chem. Soc.*, 68, 1443–1448 (1946).

ethylthio group had been introduced at C-5; however, extended ethanethiolysis of 1,3,4,5,6-penta-O-acetyl-keto-D-fructose and -L-sorbose was later shown[141] to produce the respective 1,2,2-trithio products in fair yield. A D-gluco-heptulose hexaacetate of unspecified tautomeric identity was reported[142] to form an unidentified dithioglycoside. In contrast, however, 2,3,4,6-tetra-O-acetyl-D-glucopyranose[143] and 2,3,4,5-tetra-O-benzoyl-D-glucose[144] react with ethanethiol plus acid to give only tetra-O-acyl diethyl dithioacetals.

In the presence of ethanethiol and zinc chloride (or, better, boron trifluoride), tetra-O-acetyl-α-L-arabinopyranose undergoes conversion into 2,3,4-tri-O-acetyl-5-S-ethyl-5-thio-L-arabinose diethyl dithioacetal[145] (44). Equally stereospecific migration of an ethylthio group occurs in the alkaline hydrolysis of 5-O-p-tolylsulfonyl-L-arabinose diethyl dithioacetal (see Section III,2); the thioglycosides thus formed were converted into 44 by acetylation and subsequent treatment with ethanethiol plus hydrochloric acid in ether; a 1,5-episulfonium ion (45 or 46, respectively) was postulated[146] as an intermediate in these two processes, nucleophilic attack occurring at C-1 in both examples. Evidence for the existence of this intermediate was found in the reaction of 2,3,4-tri-O-acetyl-L-arabinose dimethyl dithioacetal with ethanethiol plus zinc chloride; migration of a methylthio group in the formation of 2,3,4-tri-O-acetyl-5-S-methyl-5-thio-L-arabinose ethyl methyl dithioacetal (47) thus produced was established[147] by the presence in its mass spectrum of a major fragment (see Section VI,2) corresponding to +CH(SMe,SEt), and by its conversion into an independently prepared derivative of 5-S-methyl-5-thio-L-arabinose. It was later reported[148] that 5-O-p-tolylsulfonyl-L-arabinose dibenzyl dithioacetal reacts with sodium iodide in acetone to yield mainly the cyclic dithioacetals benzyl 1,5-dithio-α,β-L-arabinopyranoside (49), which were considered to arise by favored loss of the readily displaceable benzyl group from the intermediate episulfonium ion 48.
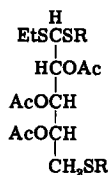
44 R = Et
47 R = Me
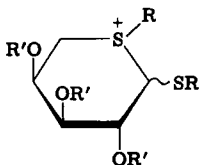
45 R = Et, R' = Ac
46 R = Et, R' = H
48 R = CH₂Ph, R' = H
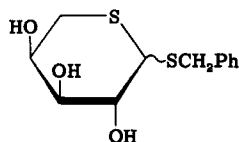
49

(141) G. S. Bethell and R. J. Ferrier, Carbohydr. Res., 34, 194–199 (1974).
(142) M. L. Wolfrom and A. Thompson, J. Amer. Chem. Soc., 56, 1804–1806 (1934).

Conversion of tetra-*O*-acetyl-β-D-ribopyranose into methyl 1-thio-β-D-ribopyranoside, by the action of methanethiol and zinc chloride, with subsequent deacetylation, is accompanied by the formation of substantial proportions of methyl 1,5-dithio-β-D-ribopyranoside (**55**) and 4-*S*-methyl-4-thio-L-lyxose dimethyl dithioacetal (**53**), plus a trace of 5-*S*-methyl-5-thio-D-ribose dimethyl dithioacetal (**56**). Although similar treatment of tetra-*O*-acetyl-β-D-ribofuranose for a "short time" produced the expected methyl 1-thio-β-D-
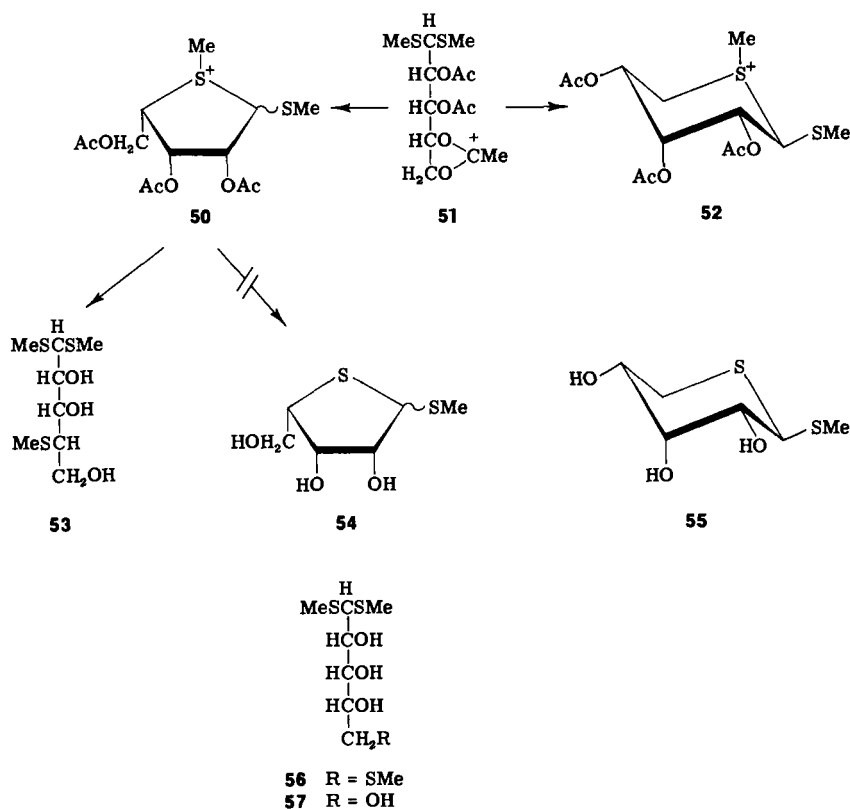
(143) M. L. Wolfrom, S. W. Waisbrot, D. I. Weisblat, and A. Thompson, *J. Amer. Chem. Soc.*, **66**, 2063–2065 (1944).
(144) M. D. Schmid and H. R. Bolliger, *Helv. Chim. Acta*, **37**, 884–887 (1954).
(145) M. L. Wolfrom and T. E. Whiteley, *J. Org. Chem.*, **27**, 2109–2110 (1962).
(146) N. A. Hughes and R. Robson, *J. Chem. Soc.* (C), 2366–2368 (1966).
(147) N. A. Hughes and R. Robson, *Chem. Commun.*, 1383–1384 (1968).
(148) J. Harness and N. A. Hughes, *Chem. Commun.*, 811 (1971).

ribofuranoside in low yield, the major product of the reaction was D-ribose dimethyl dithioacetal (57); after extended reaction, however, the products included a decreased proportion of 57, together with a distribution of 53, 55, and 56 that was similar to that obtained from the pyranose, except that no thioglycosides were formed. These results were rationalized[149] as proceeding, by way of isomeric tri-O-acetyl dithioacetals, to the common intermediate 51, which is activated for internal, nucleophilic attack, with inversion, at either C-4 or C-5; subsequent, intramolecular, nucleophilic attack at the methyl group or at the anomeric center of either of the episulfonium ions (50 and 52) gives rise to acyclic or cyclic products, respectively.

The nonformation of dithio-α-D-ribopyranosides was interpreted as being due to the presence of a 1,3-diaxial interaction between the 3-acetoxyl group and the nonreacting methylthio group on C-1, which destabilizes the transition state leading to formation of the axial anomer.[149] In solution, furthermore, acetylated dithioacetals[32,33,150] are known, from n.m.r. spectroscopy (see Section VI,1), to favor conformations free from such interactions, and to twist away from the almost universal, planar, zigzag form to avoid such an interaction; the conformation about C-1–C-4 required for cyclization of the acyclic intermediate 51 to the α-pyranoside series is also destabilized by the presence of such an eclipsed, 1,3-interaction[151,152] between the pair of substituents just mentioned. The failure of 54 to be formed from 50 suggests that 50 may open in a first-order process to afford a mesomerically stabilized, acyclic ion that subsequently combines with methanethiol to give 53; as 54 bears some steric analogy to 2,5-anhydro derivatives of D-arabinose (in having three adjacent substituents on the same side of a five-membered ring), the rationale[153] that steric compression between three substituents militates against formation of 2,5-anhydrides from dithioacetals of D-arabinose should apply equally well to the stability of 54 or, more importantly, of 50. It was noted,[147] perhaps significantly, that, whereas 2,3,4-tri-O-acetyl-L-arabinose dimethyl dithioacetal reacts with ethanethiol to afford an isolable, major product, the corresponding reaction of the diethyl analogue with methanethiol produces an intractable, "complex mixture"; the particular, solvent or reactant properties of individual thiols merit further study.

(149) N. A. Hughes, R. Robson, and S. A. Saeed, *Chem. Commun.*, 1381–1383 (1968).
(150) D. Horton and J. D. Wander, *Carbohydr. Res.*, 10, 279–288 (1969).
(151) H. S. El Khadem, D. Horton, and T. F. Page, Jr., *J. Org. Chem.*, 33, 734–740 (1968).
(152) D. Horton, P. L. Durette, and J. D. Wander, *Ann. N. Y. Acad. Sci.*, 222, 884–914 (1973).
(153) J. Defaye and D. Horton, *Carbohydr. Res.*, 14, 128–132 (1970).

5. Nucleophilic Reactions

It has been demonstrated[20,32] that nucleophilic, chain-extension reactions starting from dithioacetals are unworkable when a polar substituent is present at the position adjacent to the carbanionic site, because (intramolecular) β-elimination of the polar substituent occurs faster than any intermolecular, nucleophilic attack (see Section III,5); however, Corey and Seebach[154] found that 2-lithio-1,3-dithiane (58) is a stable nucleophile suitable for the introduction of formyl groups (after hydrolysis) into organic molecules having electrophilic carbon atoms, and the extension of this reaction into synthetic carbohydrate chemistry has provided a versatile means of effecting chain-extension or -branching.

Gero and coworkers[155-157] treated methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside (59) with 58 to afford[155,156] the branched-chain dithioacetal 60 by diaxial opening of the epoxide ring; the corresponding D-*manno* epoxide reacted similarly to give 61. The terminal epoxide 62 was attacked exclusively at the primary position to give[156,157] 6-deoxy-1,2-O-isopropylidene-α-D-*gluco*-heptodialdo-1,4-furanose 7-(trimethylene dithioacetal) (63). It was subsequently demonstrated[158] that 58 displaces halogen and sulfonic ester groups from primary positions to afford satisfactory yields of chain-extended products, as in the conversion of 1-deoxy-2,4-O-ethylidene-1-iodo-D-erythritol (64) into 2-deoxy-D-*erythro*-pentose trimethylene dithioacetal (65).
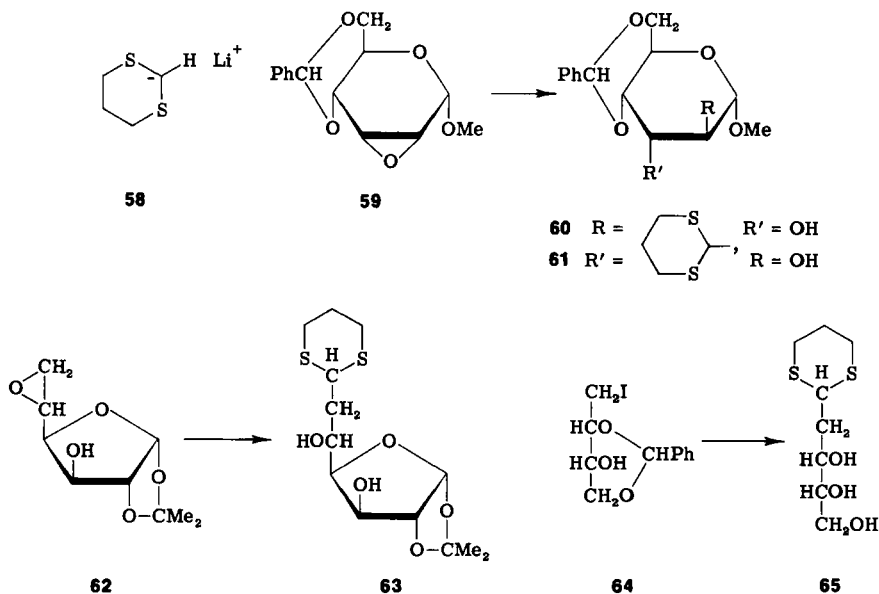
Concurrently, Paulsen and his coworkers[159,160] developed a synthetic approach to branched-chain sugars having a hydroxyl group at the branch position by the addition of **58** to a range of sugar derivatives having exposed carbonyl groups. Thus, 5-deoxy-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranos-3-ulose (**66**) was converted into 5-deoxy-3-*C*-(1,3-dithian-2-yl)-1,2-*O*-isopropylidene-β-L-ribofuranose (**67**), from which L-streptose was liberated[159] by hydrolysis of the protecting groups, and methyl 3,4-*O*-isopropylidene-β-D-*erythro*-pentofuranosulose (**68**) was converted, by subsequent deprotection and reduction of the newly introduced formyl group, into the methyl β-D-pyranoside[159] of D-hamamelose (**69**), attack occurring in both examples from the less-hindered side. Paralleling the Kiliani synthesis, **58** combines with 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-arabinose to give[159] a mixture of 3,4:5,6-di-*O*-isopropylidene-D-glucose trimethylene dithioacetal and some of the D-*manno* analogue.

66   **67**   **68**   **69**
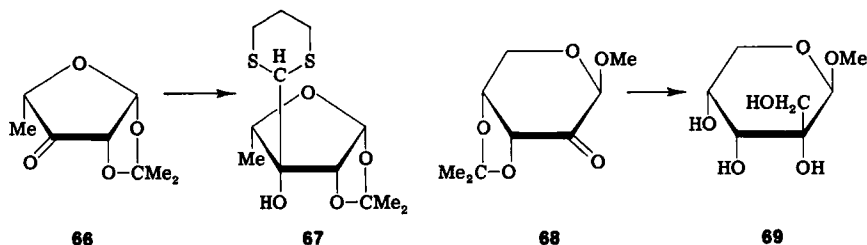
Replacement of **58** by its 2-methyl homologue **70** leads to the introduction of protected *C*-acetyl groups. A key step in the constitutional synthesis[160] of the methyl glycoside (**73**) of D-aldgarose was the addition of **70** to methyl 2-*O*-benzyl-4,6-dideoxy-β-D-*erythro*-hexopyranosid-3-ulose (**71**) to form a separable mixture of methyl 2-*O*-benzyl-4,6-dideoxy-3-*C*-(2-methyl-1,3-dithian-2-yl)-β -D-*ribo*-hex-

(154) E. J. Corey and D. Seebach, *Angew. Chem.*, **77**, 1134–1135 (1965); D. Seebach, *Synthesis*, **1**, 17–36 (1969).

(155) A. M. Sepulchre, G. Lukacs, G. Vass, and S. D. Gero, *C. R. Acad. Sci., Ser. C*, **273**, 1180–1182 (1971).

(156) A. M. Sepulchre, G. Lukacs, G. Vass, and S. D. Gero, *Angew. Chem.*, **84**, 111–112 (1971).

(157) A. M. Sepulchre, G. Lukacs, G. Vass, and S. D. Gero, *Bull. Soc. Chim. Fr.*, 4000–4007 (1972).

(158) A. M. Sepulchre, G. Vass, and S. D. Gero, *Tetrahedron Lett.*, 3619–3620 (1973).

(159) H. Paulsen, V. Sinnwell, and P. Stadler, *Angew. Chem.*, **84**, 112–113 (1972); *Chem. Ber.*, **105**, 1978–1988 (1972).

(160) H. Paulsen and H. Redlich, *Angew. Chem.*, **84**, 1100–1102 (1972); H. Paulsen, *Staerke*, **25**, 389–396 (1973).

opyranoside (**72**) and its 3-epimer. Subsequently, Gero and co-workers[161] successfully added **70** to a plethora of carbonyl sugars, including 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose and 2,3-*O*-isopropylidene-5-*O*-(tetrahydropyran-2-yl)-D-ribono-1,4-lactone (**74**); the latter reaction effects net reduction of the carboxyl group, to afford the 2,3-diulose 2-(trimethylene dithioacetal) **75**. Reaction of these anions with Schiff-base derivatives provides a route to sugars having amino groups at the branch point, whereas the action of 2-lithio-2-(trimethylsilyl)-1,3-dithiane converts sugars having free carbonyl groups into propane-1,3-diyl dithioacetal derivatives.[162]
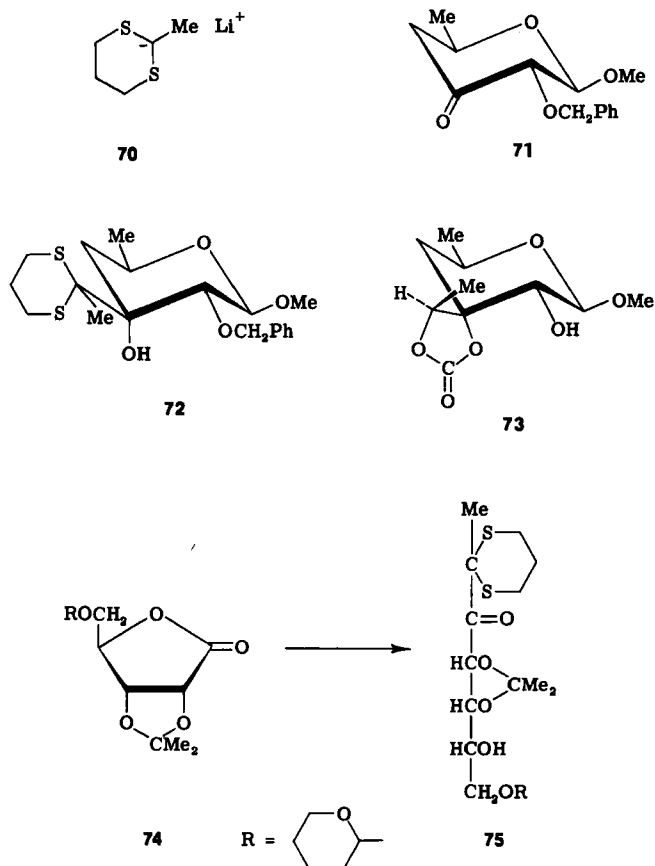
(161) A. M. Sepulchre, A. Gateau-Olesker, G. Lukacs, G. Vass, and S. D. Gero, *Tetrahedron Lett.*, 3945–3948 (1973).

(162) H. Paulsen, *Abstr. Int. Symp. Carbohydr., VIth*, 1972.

Whereas the configuration of such branched-chain sugars as **60** and **61**, which have a hydrogen atom attached to the branch point, is readily determined by measurements of vicinal, proton–proton spin-coupling values in their nuclear magnetic resonance (n.m.r.) spectra, those examples having a hydroxyl group at the (quaternary) branch point present a more difficult problem. For crystalline products, such as **67**, single-crystal X-ray crystallography[163] offers a solution, and sterically induced shifts of ^{13}C n.m.r. signals may be analyzed in the context of a series of related examples to deduce[156,164,165] the stereochemistry of the molecule. A third method, lanthanide-shifted ^{1}H n.m.r. spectroscopy,[166,167] is advantageous, in that it requires only a small sample (not necessarily crystalline), uses routine equipment, and can be accomplished with a single reference-sample, which may oftentimes be a synthetic precursor of the branched-chain product.

The latter method is valid for branched-chain products that adopt the same conformation as their precursors, as it is predicated on the idea that a paramagnetic ion will bind similarly to configurationally and conformationally related molecules (for example, **76** and **77**), and differently to unrelated molecules (for example, **78** and **79**). The
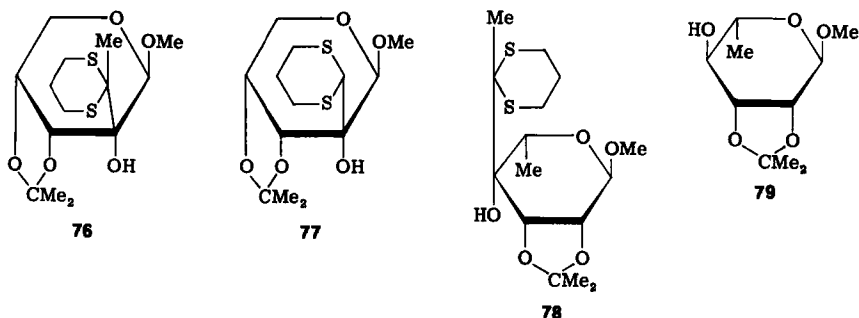
lanthanide-ion concentration-dependence of chemical shifts of these four compounds is displayed in Figs. 1–4, respectively, and casual inspection reveals that, whereas all of the corresponding pairs of

(163) W. Depmeier, O. Jarchow, P. Stadler, V. Sinnwell, and H. Paulsen, *Carbohydr. Res.*, 34, 219–224 (1974).

(164) G. Lukacs, A. M. Sepulchre, A. Gateau-Olesker, G. Vass, S. D. Gero, R. D. Guthrie, W. Voelter, and E. Breitmaier, *Tetrahedron Lett.*, 5163–5166 (1972).

(165) A. M. Sepulchre, B. Septe, G. Lukacs, S. D. Gero, W. Voelter, and E. Breitmaier, *Tetrahedron*, 30, 905–915 (1974).

(166) S. D. Gero, D. Horton, A. M. Sepulchre, and J. D. Wander, *Tetrahedron*, 29, 2963–2972 (1973).

(167) S. D. Gero, D. Horton, A. M. Sepulchre, and J. D. Wander, *J. Org. Chem.*, 40, 1061–1066 (1975).
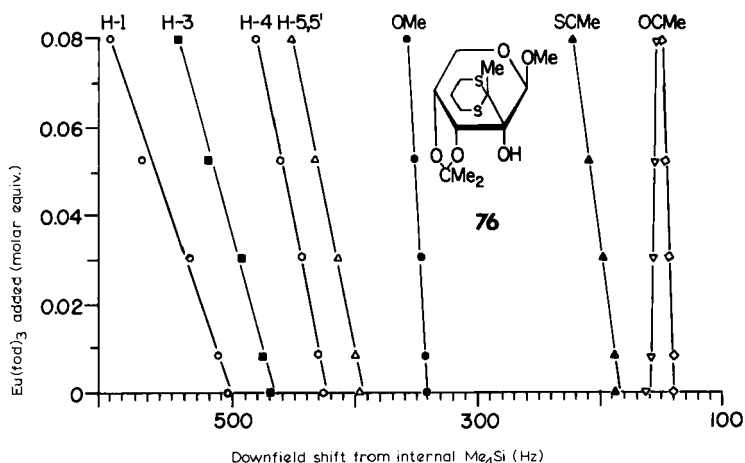
FIG. 1.—Chemical-shift Values Measured for Solutions of Methyl 3,4-O-Isopropylidene-2-C-(2-methyl-1,3-dithian-2-yl)-β-D-ribopyranoside (**76**) in the Presence of Different Concentrations of Eu(fod)$_3$.
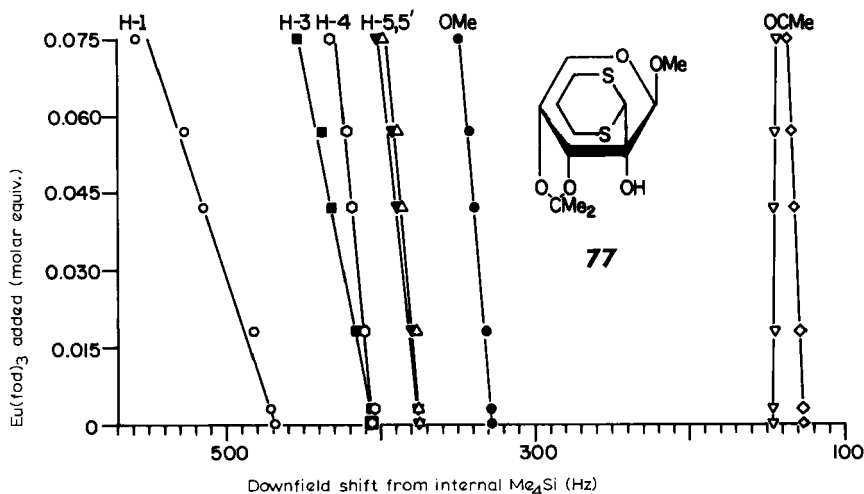
FIG. 2.—Chemical-shift Values Measured for Solutions of Methyl 2-C-(1,3-Dithian-2-yl)-3,4-O-isopropylidene-β-D-ribopyranoside (**77**) in the Presence of Different Concentrations of Eu(fod)$_3$.

lines in Figs. 1 and 2 have similar slopes (indicating conformational and, therefore, configurational congruence), major discrepancies are evident between corresponding pairs of lines (particularly of the H-3 and the OH signals) in Figs. 3 and 4, which, therefore, may be inferred to have arisen from conformationally (and, probably, configu-
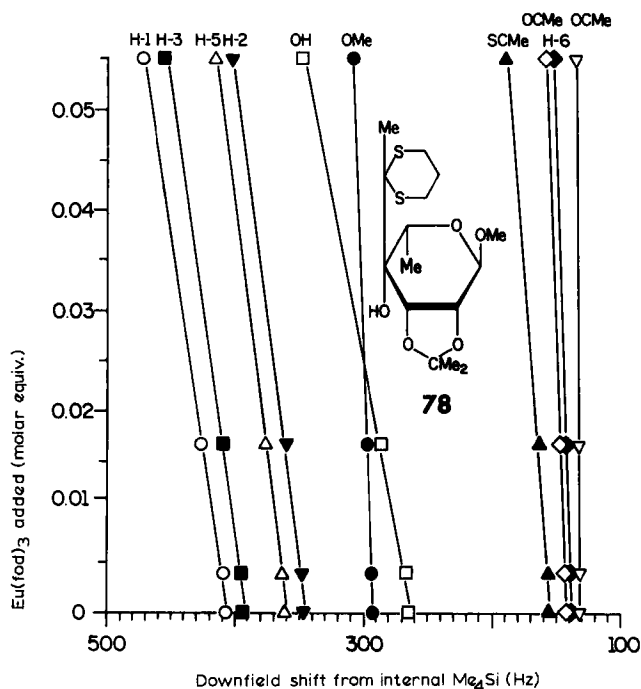
FIG. 3.—Chemical-shift Values Measured for Solutions of Methyl 6-Deoxy-2,3-O-isopropylidene-4-C-(2-methyl-1,3-dithian-2-yl)-α-L-talopyranoside (78) in the Presence of Different Concentrations of Eu(fod)₃.
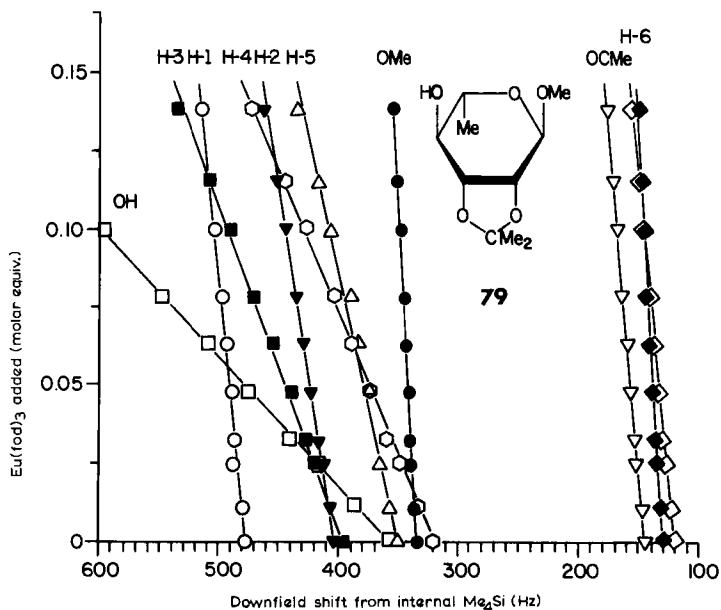
FIG. 4.—Chemical-shift Values Measured for Solutions of Methyl 6-Deoxy-2,3-O-isopropylidene-α-L-mannopyranoside (79) in the Presence of Different Concentrations of Eu(fod)₃.

rationally) different molecules. Linear regression-analysis[167] of data thus generated against the expression:

$$\Delta\delta_{ui} = k\Delta\delta_{ri}, \tag{1}$$

in which $\Delta\delta_{ui}$ and $\Delta\delta_{ri}$ represent the change in chemical shift of the ith proton of the configurational unknown and the reference molecule, respectively, and k is a constant, gives $k = 1 \pm 0.2$ and a correlation coefficient $(r) > 0.9$ for congruent pairs of molecules; conversely, values outside these limits are consonant with conformational differences arising from opposite stereochemistry of the hydroxyl groups in the two molecules.

6. Miscellaneous Reactions

Addition of ethanesulfenyl chloride to 3,4,5,6-tetra-O-acetyl-1-deoxy-1-diazo-keto-D-fructose (17) gave a 1-chloro-1-S-ethyl-1-thio derivative that reacted[78] with ethanethioxide ion to form the 1-(diethyl dithioacetal) 80. 2,3,4,5,6-Penta-O-acetyl-1-bromo-1-S-ethyl-1-thio-D-galactitol 81, prepared by controlled brominolysis (see Section IV,2) of the corresponding diethyl dithioacetal, reacted with ethanethioxide ion[168] and, in separate reactions, with benzothiazolethione[169] and with thiourea[168] to give, respectively, the ethyl methyl dithioacetal 82, the benzothiazol-2-yl methyl dithioacetal 83, and the pseudothiouronium salt 84, which is formally a dithioacetal; decomposition of the latter product afforded[168] the dithiohemiacetal 85.

80   81   82 R = Me

83 R =

84 R = $(NH_2)_2C^+-$
85 R = H
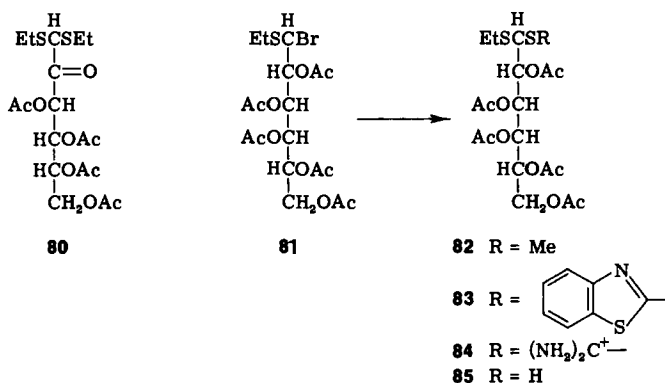
(168) H. Zinner, R. Kleeschätzky, and M. Schlutt, J. Prakt. Chem., 313, 855–860 (1971).

(169) H. Zinner and M. Schlutt, J. Prakt. Chem., 313, 1181–1184 (1971).

The ketenic dithioacetals **87** and **89** were prepared[170] from 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*xylo*-pentodialdo-1,4-furanose   (**86**) and from 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (**88**), respectively, by the action of carbon disulfide, tributylphosphine, and dimethyl butynedioate.

86 X = O   **88**

87 X =

                                                          89
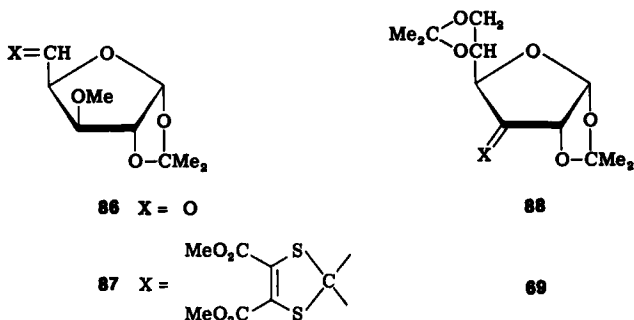
Acid hydrolysis of 1,2:5,6-di-*O*-isopropylidene-3,4-dithio-D-iditol was found[171] to result in acetal migration, to afford the dithioacetal 3,4-S-isopropylidene-3,4-dithio-D-iditol (**90**). The action of phosphorus pentasulfide on **91** gives the corresponding 3-thione, which exists as the dimeric[172] 1,3-dithietane **92**.

G=O

91

90

G =
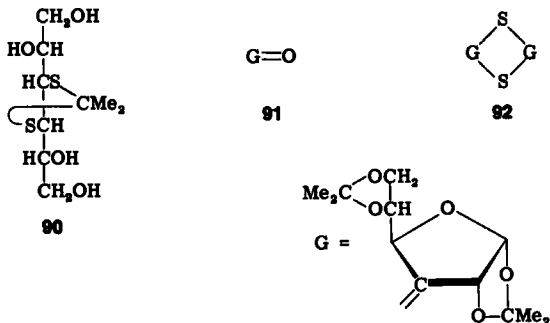
                    III. REACTIONS OF HYDROXYL AND OTHER
                    GROUPS IN DITHIOACETALS OF SUGARS

In general, hydroxyl (and other) groups on the substituted-alkyl side-chain of sugar dithioacetals are unexceptional in their reactions; it is evident, especially from exhaustive studies by Zinner and co-workers (see Ref. 110a and earlier papers), that the nature of the

(170) J. M. J. Tronchet, T. Nguyen-Xuan, and M. Rouiller, *Carbohydr. Res.*, **36**, 404–407 (1974).
(171) G. E. McCasland and A. B. Zanlungo, *Carbohydr. Res.*, **17**, 475–477 (1971).
(172) J. N. Dominguez and L. N. Owen, *Carbohydr. Res.*, **22**, 225–226 (1972).

alkyl portion of the alkylthio groups causes no predictable alteration in the course of these reactions, although proclivities for crystallization may vary.

1. Formation of Ethers and Esters (Except Sulfonates)

In the presence of sufficient excess of reagent, complete acylation[122,173] occurs; in general, reaction proceeds mainly at primary hydroxyl groups during partial acylation, and ω-monosubstituted acetic,[174] benzoic,[175] p-nitrobenzoic,[176] and p-toluic[177] esters can routinely be prepared from dithioacetals. It has been stated[176] that this terminal acylation occurs under kinetic control; however, 4-O-benzoyl-2-deoxy-D-erythro-pentose ethylene dithioacetal spontaneously rearranges to the 5-O-benzoyl isomer in organic solvents containing traces of heavy-metal salts.[178] Conversion of D-glucose diethyl dithioacetal into a borate complex prior to benzoylation results in the same 6-benzoate[179]; dithioacetals of 3-deoxy-D-lyxo-hexose afford 2,6-di-O-benzoyl derivatives[180] under these conditions. The 2-hydroxyl group of D-xylose diethyl dithioacetal and D-glucose diethyl dithioacetal appears less reactive towards benzoylation, as the respective 3,4,5-tri-[181] and 3,4,5,6-tetra-O-benzoyl[122,182] (27) derivatives are formed by reaction with controlled proportions of benzoyl chloride; an analogous conversion of D-galactose diethyl dithioacetal into its 4,5,6-tribenzoate was reported to occur under Schotten–Baumann conditions.[183] A tetra-O-acetyl-D-glucose dithioacetal (93) has been directly prepared from the parent dithioacetal,[184] and found to be identical to, or readily interconvertible into, products obtained by detritylation of 2,3,4,5-tetra-O-acetyl-6-O-trityl-D-glucose diethyl dithioacetal and by controlled mercaptolysis of 2,3,4,6-tetra-O-

(173) W. Schneider and J. Sepp, Ber., 49, 2054–2057 (1916).
(174) H. Zinner and K. Wessely, Chem. Ber., 90, 516–520 (1957).
(175) P. Brigl and W. Zerrweck, Z. Physiol. Chem., 229, 117–124 (1934); T. Lieser and R. Schweizer, Ann., 519, 271–277 (1935).
(176) H. Zinner, K. Wessely, W. Bock, K. Rieckhoff, F. Strandt, and W. Nimmich, Chem. Ber., 90, 500–515 (1957).
(177) H. Zinner and M. Pfeifer, Chem. Ber., 94, 2792–2797 (1961).
(178) H. Zinner and F. Schneider, Chem. Ber., 95, 2295–2301 (1962).
(179) P. Brigl and H. Grüner, Ann., 495, 60–83 (1932).
(180) G. Rembarz, Chem. Ber., 95, 830–833 (1962).
(181) M. L. Wolfrom and W. von Bebenburg, J. Amer. Chem. Soc., 81, 5705–5706 (1959).
(182) P. Brigl and R. Schinle, Ber., 63, 2884–2887 (1930).
(183) H. R. Bolliger and M. D. Schmid, Helv. Chim. Acta, 37, 888–892 (1954).
(184) R. U. Lemieux and H. F. Bauer, Can. J. Chem., 32, 362–365 (1954).

acetyl-D-glucopyranose[143]; considerable lability appears to charac-
terize the acetyl groups of 93, which has been converted into
ethyl 2,3,5,6-tetra-O-acetyl-1-thio-α-D-glucofuranoside[143] and into a
2-O-methyl derivative[184] (94). Formation of the former derivative

93   94   27
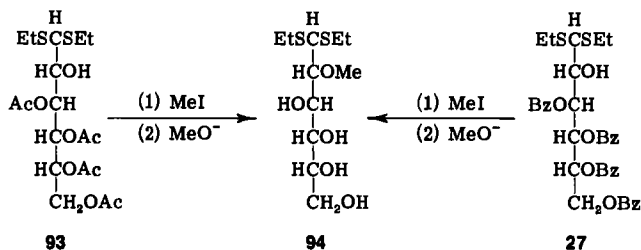
would seem to provide more driving force for rearrangement, and,
by analogy to the corresponding tetrabenzoate, the 3,4,5,6 arrange-
ment of substituents in 93 seems the more probable. In general the
choice of catalysis is not critical in these acylation reactions. Deacyl-
ation by the action of methoxides[182,185] or methanolic ammonia[16,69]
proceeds without complications.

Trityl ethers of these acyclic dithioacetals are formed very readily
and specifically at primary alcoholic positions; the facility of this con-
version is such that derivatization of D-ribose[186] and 2-deoxy-D-
erythro-pentose,[187] which proceeds poorly with the free sugars, is ef-
fected in yields exceeding 50% by a sequence of reactions involving
mercaptalation, tritylation, acetylation, deacetylation, and demer-
captalation. Acetylation followed by purification and subsequent sa-
ponification was recommended[188] as necessary, although Bristow and
Lythgoe reported[189] the direct isolation of 5-O-trityl-D-arabinose di-
ethyl dithioacetal by extraction and crystallization. D-Sorbose dithio-
acetals form 1,6-ditrityl ethers, although exactly one equivalent of
chlorotriphenylmethane derivatizes, exclusively, the primary hy-
droxyl group remote from the dithioacetal group[190]; 1-O-trityl groups
in these ketose compounds are replaced by acetyl groups during
acetylation with acetic anhydride in pyridine. Detritylation of dithio-
acetal derivatives proceeds normally in hydrogen bromide–acetic

(185) P. Chang and B. Lythgoe, J. Chem. Soc., 1992–1993 (1950).
(186) H. Zinner, Chem. Ber., 86, 496–500 (1953).
(187) H. Zinner, H. Nimz, and H. Venner, Chem. Ber., 91, 638–641 (1958).
(188) H. Zinner, H. Brandner, and G. Rembarz, Chem. Ber., 89, 800–813 (1956).
(189) N. W. Bristow and B. Lythgoe, J. Chem. Soc., 2306–2309 (1949).
(190) H. Zinner and U. Schneider, Chem. Ber., 96, 2159–2164 (1963).

acid, to regenerate the alcohol, or, in hydrogen bromide–chloroform, to afford the corresponding bromodeoxy compound.[191]

Graduated selectivity as a function of configuration is observed in the reactivity[5] of alcoholic groups along the side chain toward methylation. The classical example is D-glucose diethyl dithioacetal, which had early been shown[192] to undergo conversion into only one monomethyl derivative (94). Brigl and Schinle[182] prepared 94 indirectly, by methylating and subsequently saponifying the tetrabenzoate (29) of the parent dithioacetal, and established its identity with the known[193] 2-O-methyl-D-glucose. A later investigation[194] revealed that the exceptional solubility of D-glucose diethyl dithioacetal in methyl iodide expedites the etherification, but it was found that, even in poorer solvents, the specificity of the process remains. The reactivity of the 2-hydroxyl group of a number of D-glucose derivatives is known[5] to be singular, so that the role of the dithioacetal group in this conversion is uncertain; mercaptolysis of partially methylated starch (d.s. 1.05) affords[102] a good yield (52% overall; 70% of isolated, crude material) of the 2-methyl ether 94.

Partial methylation of L-arabinose diethyl dithioacetal was found[195] to proceed selectively at O-2, but a small percentage of the 5-methyl ether was also detected. This selectivity progressively diminishes in the formation of the corresponding derivatives of D-mannose,[196] D-xylose,[17] and D-galactose,[197] although methylation at O-2 invariably predominates, with O-3 and O-ω increasing in reactivity through the series; the proportion of more-extensively methylated products increases in the same order, whereas secondary hydroxyl groups more remote than O-3 form no monomethyl ethers. Sulfur participation in the attack upon methyl iodide is a distinct possibility, and alkylation of sulfur has been suggested[198] as a competing reaction in the attempted methylation of 2,3,5-tri-O-benzoyl-D-arabinose diethyl dithioacetal. Permethylation was accomplished fairly satisfactorily,[40,199] and specific methylation of partially protected derivatives has been

(191) M. L. Wolfrom, J. L. Quinn, and C. C. Christman, *J. Amer. Chem. Soc.*, **57**, 713–717 (1935).
(192) P. E. Papadakis, *J. Amer. Chem. Soc.*, **52**, 2147–2149, 3465 (1930).
(193) P. Brigl and R. Schinle, *Ber.*, **62**, 1716–1723 (1929).
(194) G. G. S. Dutton and K. Yates, *Can. J. Chem.*, **36**, 550–556 (1958).
(195) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **39**, 1797–1800 (1961).
(196) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **41**, 2500–2503 (1963).
(197) G. G. S. Dutton and Y. Tanaka, *Can. J. Chem.*, **40**, 1146–1148 (1962).
(198) H. G. Fletcher, Jr., and H. W. Diehl, *J. Org. Chem.*, **30**, 2321–2323 (1965).
(199) P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **69**, 175–180 (1926).

judiciously employed both in synthesis[105] and in structure determination;[182,200] simultaneous displacement of substituents already present has been reported[201] to occur under forcing conditions, but the exact nature of this conversion is as yet uncertain.

Other derivatives of hydroxyl groups, shown to form without apparent complications, include phenylurethans,[202] mixed[203] and cyclic[204] carbonates, diphenylphosphoric[205] esters, and terminal 1-adamantoates[206]; cyclic sulfites[204] are produced by the action of thionyl chloride.

2. Formation and Reactions of Sulfonic Esters

2,5-Anhydroaldopentose dithioacetals are the sole products from treatment of dithioacetals of D-ribose,[207] D-xylose,[207] and D-lyxose[208] with sulfonyl chlorides in pyridine; D-*glycero*-L-*manno*-heptose diethyl dithioacetal yields a 4,7-anhydride[209] under the same conditions; however, dithioacetals of D-arabinose,[210,211] L-arabinose,[211–213] D-glucose,[213,214] D-mannose,[215–217] D-*glycero*-D-*gulo*-heptose,[209] 2-deoxy-D-*erythro*-pentose,[218] and 3-deoxy-D-*xylo*-hexose[60] are reported to afford sulfonic esters at the primary alcohol group with facility.

(200) O. T. Schmidt and E. Wernicke, *Ann.*, **556**, 179–186 (1944).

(201) O. T. Dalley and R. J. McIlroy, *J. Chem. Soc.*, 555–557 (1949).

(202) E. Hardegger, E. Schreier, and Z. El-Hewehi, *Helv. Chim. Acta*, **33**, 1159–1164 (1950).

(203) H. Zinner and H. Schmandke, *Chem. Ber.*, **94**, 1304–1310 (1961).

(204) H. Zinner, G. Sych, and F. Schneider, *Chem. Ber.*, **93**, 468–472 (1960).

(205) J. L. Barclay, A. B. Foster, and W. G. Overend, *J. Chem. Soc.*, 2505–2511 (1955).

(206) B. F. West, K. V. Bhat, and W. W. Zorbach, *Carbohydr. Res.*, **8**, 253–261 (1968).

(207) H. Zinner, H. Brandhoff, H. Schmandke, H. Kristen, and R. Haun, *Chem. Ber.*, **92**, 3151–3155 (1959).

(208) J. Defaye, *Bull. Soc. Chim. Fr.*, 2686–2689 (1964).

(209) F. Micheel, H. Pfetzing, and G. Pirke, *Carbohydr. Res.*, **3**, 283–294 (1966).

(210) H. Zinner, K. Wessely, and H. Kristen, *Chem. Ber.*, **92**, 1618–1623 (1959).

(211) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B58**, 717–720 (1962).

(212) P. A. Levene and J. Compton, *J. Biol. Chem.*, **116**, 189–202 (1936).

(213) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B62**, 1069–1072 (1966).

(214) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B54**, 303–308 (1958).

(215) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B55**, 693–696 (1959).

(216) W. W. Zorbach and C. O. Tio, *J. Org. Chem.*, **26**, 3543–3545 (1961).

(217) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B58**, 721–724 (1962).

(218) H. Zinner and H. Wigert, *Chem. Ber.*, **92**, 2893–2896 (1959).

The behavior of D-galactose dialkyl dithioacetals is uncertain; Spanish workers reported the isolation of 6-O-(methylsulfonyl)[217] and 6-O-p-tolylsulfonyl[214] derivatives from initially cooled reactions, although Veksler claimed[96] to have repeated the work under these conditions, and to have found only an anhydro product (whose reported elemental analysis, although unsatisfactory for the anhydride dithioacetal, bore no resemblance whatsoever to that calculated for the ester); subsequently, Zinner and coworkers[219] reported that lower reaction-temperatures and use of arylsulfonyl halides promote cyclization, whereas low yields (25–30%, varying slightly with the alkyl group of the alkanethiol) of methanesulfonates can be isolated after only an initial cooling of the reaction mixture. The formation of the two products by competing reactions of a common intermediate was invoked[219] to rationalize the failure of pyridine or alkali to cyclize previously isolated samples of the sulfonates. Mercaptalation of 6-O-p-tolylsulfonyl-D-galactose affords the corresponding dithioacetal[106] in 83% yield.

It has been pointed out[153] that, for each of those pentoses forming a 2,5-anhydride, one of the three substituents attached to the tetrahydrofuran ring is located *trans* with respect to the other two, whereas the *arabino* anhydride would have all three groups on the same side; accordingly, it was suggested that steric compression between these three groups (C-1 and the 3- and 4-hydroxyl groups) destabilizes the transition state that would lead to cyclization to the latter product. Although this rationalization was predicated[153] upon cyclization subsequent to formation of the sulfonic ester, it applies equally well to a concerted process involving the common intermediate just noted. This generalization appears to be extendable to higher sugars; the derivatives of D-glucose, D-mannose, and D-*glycero*-D-*gulo*-heptose possess D-*arabino* stereochemistry along the four terminal positions of the side chain, and are esterified, whereas the derivatives of D-*glycero*-L-*manno*-heptose and D-galactose, both D-*lyxo* at the terminus, undergo cyclization.

ribo   *xylo*
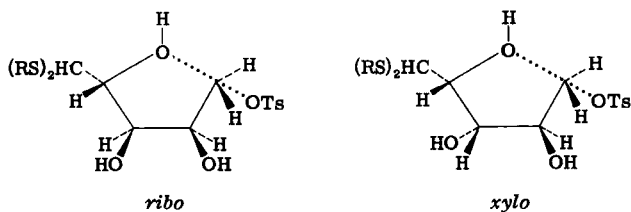
(219) H. Zinner, K.-H. Stark, E. Michalzik, and H. Kristen, *Chem. Ber.*, **95**, 1391–1399 (1962).

lyxo   arabino

                                (cyclization not observed)

Van Es[220] treated a series of partially methylated derivatives of D-xylose diethyl dithioacetal (O-2 was substituted and O-5 was unsubstituted throughout) with a solution of *p*-toluenesulfonyl chloride in pyridine, and observed that those examples (**95** and **96**) having a hydroxyl group at C-4 cyclize readily, by way of a (presumed) sequential, multiple-participation mechanism, to afford ethyl 2-S-ethyl-1,2-dithio-D-lyxofuranoside (**98**) and its 3-methyl ether (**99**), respectively, by attack of O-4 at C-1 of the episulfonium ion **100**, whereas the 4-O-methylated dithioacetal **97** reacted with the same reagents under forcing conditions to afford the alkene **101**, a more-energetic process of elimination occurring through loss of H-2, because the 4-O-methyl group is unable to participate in cyclization. 5-O-*p*-Tolylsulfonyl-L-arabinose diethyl dithioacetal reacts under forcing, alkaline conditions to produce[146] ethyl 5-S-ethyl-1,5-dithio-α,β-L-arabinofuranoside (**102**), which was interpreted as arising through the intermediate formation of the 1,5-episulfonium ion **103**, with subsequent attack of O-4 at C-1.

95 R = R' = H
96 R = Me, R' = H
97 R = R' = Me

98 X = OH
99 X = OMe

100

102   **103**   **101**

Uncomplicated conversion of secondary alcoholic groups into sulfonic esters has been accomplished in such diverse systems as 2,4-*O*-benzylidene-D-glucurono-6,3-lactone dialkyl dithioacetals,[221] 5-*O*-benzoyl-2,4-*O*-benzylidene-D-ribose dipropyl dithioacetal,[203] and 5-deoxy-2,3-*O*-isopropylidene-D-arabinose diethyl dithioacetal and its 5-azido analogue,[222] and in the further reaction of the di-isobutyl dithioacetals of D-ribose and D-xylose, after cyclization, to form[223] 2,5-anhydro-3,4-di-*O*-*p*-tolylsulfonyl-D-ribose di-isobutyl dithioacetal (**104**) and its D-xylose analogue (**105**), respectively. The D-lyxose analogue (**106**) of **105** is formed similarly.[223a] The failure[198] of 2,3,5-tri-*O*-benzyl-D-arabinose diethyl dithioacetal to react with *p*-toluenesulfonyl chloride indicates that limits on the generality of this reaction exist; in view of the fact that 2,3,5-tri-*O*-benzyl-D-arabinose dibenzyl acetal forms[198] a 4-*O*-*p*-tolylsulfonyl derivative, it is clear that these limitations are not yet clearly understood.

Bimolecular, nucleophilic processes involving displacement of sulfonyloxy groups with inversion of configuration have been reported to occur without distinction in displacements by iodide,[100,209,211,213–215,217] thiocyanate,[215] thiolacetate,[224] and azide[225,226] ions; however, azidolysis of 5-azido-5-deoxy-2,3-*O*-isopropylidene-4-*O*-(methylsulfonyl)-D-arabinose diethyl dithioacetal affords, in addition to the 4,5-diazido-4,5-dideoxy-L-xylose product, a trace (<2% yield) of a second 4,5-diazido-4,5-dideoxy product, in which the D-*arabino* stereochemistry is preserved, and formation of a 4,5-azidonium-ion intermediate was invoked[222] to explain the formation of the latter product. An elegant demonstration[227] of the positions of acetalation in 3,4-*O*-isopropylidene-D-xylose dimethyl dithioacetal (**107**) included establishing of the identity of the 5-*O*-benzoyl derivative (**108**) of **107** (prepared by direct, partial benzoylation) with the product obtained by mono-*p*-toluenesulfonylation of **107** and subsequent displacement of the sulfonyloxy group from the initial prod-

(220) T. van Es, *Carbohydr. Res.*, **37**, 373–380 (1974).

(221) H. Zinner and C.-G. Dässler, *Chem. Ber.*, **93**, 1597–1608 (1960).

(222) S. Hanessian, *Carbohydr. Res.*, **1**, 178–180 (1965).

(223) J. Defaye and J. Hildesheim, *Bull. Soc. Chim. Fr.*, 940–943 (1967).

(223a) J. Defaye, *Bull. Soc. Chim. Fr.*, 2099–2102 (1968).

(224) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B60**, 519–522 (1964).

(225) S. Hanessian and T. H. Haskell, *J. Org. Chem.*, **28**, 2604–2610 (1963).

(226) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Soc. Real Españ. Fis. Quím.*, **B65**, 415–418 (1969).

(227) H. Zinner and J. Milbradt, *Carbohydr. Res.*, **3**, 389–402 (1967).

uct (109) by benzoate ion; as the products of (*a*) direct condensation and (*b*) inversion proved identical, the carbon center involved was necessarily primary. Replacement of a terminal bromodeoxy group by iodide has also been reported.[191]
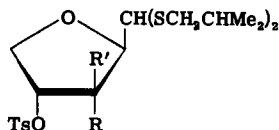
104 R = OTs, R' = H
105 R = H, R' = OTs
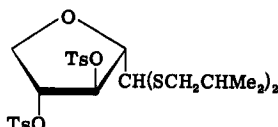
106
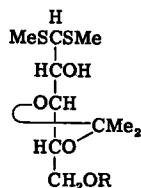
107 R = H
108 R = Bz
109 R = Ts

Reduction of azides with lithium aluminum hydride produces[222,225] the corresponding amines, whereas application of the same reagent to sulfonic esters affords deoxy sugars by hydrogenolysis of ω-sulfonyloxy groups[60,216,218]; saponification competes with the latter process on about an equal basis[210] in 5-*O*-*p*-tolylsulfonyl-D-arabinose dithioacetals, and prevails[203] for secondary sulfonates.

The endocyclic disulfonate groups in 104 (*ribo*, 3,4-*cis*) and 106 (*lyxo*, 3,4-*trans*) are converted[223,223a] by the action of sodium iodide–*N,N*-dimethylformamide–zinc dust into the corresponding alkenes, namely, 2,5-anhydro-3,4-dideoxy-D-*glycero*(or L-*glycero*)-pent-3-enose di-isobutyl dithioacetals (110 from 104; 110a from 106). In contrast, the *xylo* disulfonate (105) reacts by migration of one of the alkylthio groups, giving a mixture of three furan derivatives, formulated[227a] (correcting an earlier, erroneous assignment[50,223]) as 111 (major), 111a (minor), and 111b (trace), respectively.
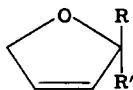
110   R = CH(SCH₂CHMe₂)₂ , R' = H
110a R = H, R' = CH(SCH₂CHMe₂)₂
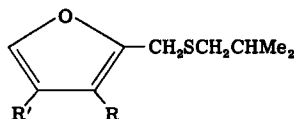
111   R = H, R' = SCH₂CHMe₂
111a R = H, R' = H
111b R = SCH₂CHMe₂ , R' = H

3. Formation and Reactions of Alkylidene Acetals

A substantial body of literature has been devoted to the study of condensation reactions of dithioacetals with aldehydes and ketones. Although phosphoric anhydride has been used as a catalyst for these

(227a) P. Angibeaud, J. Defaye, H. Franconie, and M. Muesser, to be published.

reactions, zinc chloride or copper(II) sulfate plus a trace of sulfuric acid are more commonly accepted. Some earlier reports presented erroneous conclusions about substitution patterns present in cyclic acetal derivatives of dithioacetals, as a consequence of misconceptions concerning, for example, the ability of 2-O-methylhexoses to afford osazones,[228,229] the homogeneity of crystalline products,[229] the purity of reagents,[230] the influence of available sulfur atoms upon the apparent extent of glycol-cleavage oxidation,[231] and a paucity of independently prepared, suitably substituted[5] samples for comparison; later studies have shown[232-234] that, in general, 1,3-dioxolane derivatives result from condensations of dithioacetals (and acetals) with ketones, and 1,3-dioxane derivatives form the corresponding processes with aldehydes, in the order[235,236] predicted by the Hann–Hudson rule[237,238] for substitution of alditols. Thus, dithioacetals of D-ribose,[233,236] D-xylose, and D-lyxose[236] form 2,4:3,5-dibenzylidene acetals, and D-ribose,[239] D- or L-arabinose,[32,240,241] and D-xylose[20,227,242] dithioacetals afford 2,3:4,5-di-isopropylidene acetals, although a crystalline 2,4:3,5-di-O-isopropylidene derivative of D-xylose diethyl dithioacetal has also been reported.[243] Steric constraints[237] cause L-arabinose diethyl dithioacetal to form[236,241] a 2,3:4,5-dibenzylidene derivative. Hexose dithioacetals having the D-*gluco*,[228] D-*galacto*,[230]

(228) J. Munro and E. G. V. Percival, *J. Chem. Soc.*, 640, 873–874 (1936); see also, H. Zinner and R. Heinatz, *J. Prakt. Chem.*, 312, 561–570 (1970).

(229) E. Pacsu, *J. Amer. Chem. Soc.*, 58, 2076 (1936).

(230) E. Pacsu, S. M. Trister, and J. W. Green, *J. Amer. Chem. Soc.*, 61, 2444–2448 (1939).

(231) E. J. C. Curtis and J. K. N. Jones, *Can. J. Chem.*, 38, 1305–1315 (1960).

(232) E. Pacsu and S. M. Trister, *J. Amer. Chem. Soc.*, 63, 925–928 (1941).

(233) D. J. J. Potgieter and D. L. MacDonald, *J. Org. Chem.*, 26, 3934–3938 (1961).

(234) H. Zinner and G. Wulf, *Chem. Ber.*, 102, 180–185 (1969); see also, earlier papers in this series.

(235) H. Zinner and F. Schneider, *Chem. Ber.*, 95, 2769–2774 (1962).

(236) H. Zinner, B. Richard, M. Blessmann, and M. Schlutt, *Carbohydr. Res.*, 2, 197–203 (1966); H. Zinner and H. Banz, *J. Prakt. Chem.*, 314, 428–436 (1972).

(237) R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.*, 66, 1909–1912 (1944); 70, 765–770 (1948).

(238) S. A. Barker, E. J. Bourne, and D. H. Whiffen, *J. Chem. Soc.*, 3865–3870 (1952); S. A. Barker and E. J. Bourne, *Advan. Carbohydr. Chem.*, 7, 137–207 (1952).

(239) M. A. Bukhari, A. B. Foster, J. Lehmann, J. M. Webber, and J. H. Westwood, *J. Chem. Soc.*, 2291–2295 (1963).

(240) G. N. Bollenback and L. A. Underkofler, *J. Amer. Chem. Soc.*, 72, 741–745 (1950).

(241) C. F. Huebner, R. A. Pankratz, and K. P. Link, *J. Amer. Chem. Soc.*, 72, 4811–4812 (1950); D. Horton and M. Sakata, *Carbohydr. Res.*, in press.

(242) T. van Es, *Carbohydr. Res.*, 32, 370–374 (1974).

(243) D. G. Lance and J. K. N. Jones, *Can. J. Chem.*, 45, 1533–1538 (1967).

and D-*manno* configurations are reported to give isopropylidene derivatives at the 2,3:5,6 and 3,4:5,6, 4,5, and 3,4:5,6 positions, respectively.

Where condensation may occur with two equivalents of the carbonyl compound, an acetal ring involving a primary hydroxyl group is generally favored[244,244a]; dithioacetals of D-ribose,[244a] D-arabinose,[244] and D-xylose[243] undergo partial reaction with acetone to afford mixtures of products in which the 4,5-monoisopropylidene derivative preponderates. Dithioacetals of 2-deoxy-D-*erythro*-pentose[235] and of 3-deoxy-D-*xylo*-hexose[234] form 3,4- and 4,5-monoisopropylidene acetals, respectively, in exception to the generalization that primary hydroxyl groups are favored sites of attachment. The faster-forming ring of a diacetalated product can be selectively hydrolyzed,[236,241] usually in aqueous acid. Positional integrity of other substituents appears to be retained during partial hydrolysis, although 3,4-O-benzylidene-2-deoxy-D-*erythro*-pentose ethylene dithioacetal (prepared indirectly) rearranges spontaneously[178] to the normal (3,5) isomer upon exposure to acid; methylation of 2,3:4,5-di-O-isopropylidene-D-xylose diethyl dithioacetal was reported by Dalley and McIlroy,[201] but van Es[242] has disputed this claim.

Condensation reactions with symmetrical ketones (acetone, cyclohexanone) or formaldehyde create no new asymmetric centers at the acetal group, whereas condensation of aldehydes or unsymmetrical ketones across two nonequivalent hydroxyl groups generates a new asymmetric center at each such acetal function.[245] Although the thermodynamically favored epimer is generally presumed to be the sole product formed, methyl D-galacturonate dimethyl dithioacetal and 6-O-benzoyl-D-galactose dimethyl dithioacetal are reported to condense with benzaldehyde to give epimeric pairs of products differing only in their stereochemistry about the acid-labile (unidentified) acetal position.[246] Field effects from amino and acetamido groups do not appear to influence the course of acetalation reactions.[247]

In the presence of catalytic amounts of *p*-toluenesulfonic acid, D-galactose diethyl dithioacetal (112) reacts[248] with *tert*-butyl vinyl ether to afford the 5,6-O-ethylidene derivative 113 in 56% yield; Wolfrom and Parekh[248] proposed that the reaction proceeds by initial, electrophilic addition of O-6 to the double bond, followed by pro-

(244) S. B. Baker, *J. Amer. Chem. Soc.*, 74, 827–828 (1952).

(244a) D. G. Lance, W. A. Szarek, and J. K. N. Jones, *Can. J. Chem.*, 47, 2889–2891 (1969).

(245) J. A. Mills, *Advan. Carbohydr. Chem.*, 10, 1–53 (1955).

(246) H. Zinner and W. Thielebeule, *Chem. Ber.*, 93, 2791–2803 (1960).

tonation of the alkoxyl group and subsequent attack by O-5 to form the dioxolane ring. This mechanism is consistent with the observed[248] conversion of 114 (prepared by reaction of ethyl vinyl ether with the 2,3,4,5-tetraacetate of 112) into 113 under the same conditions.

112	+	113	114
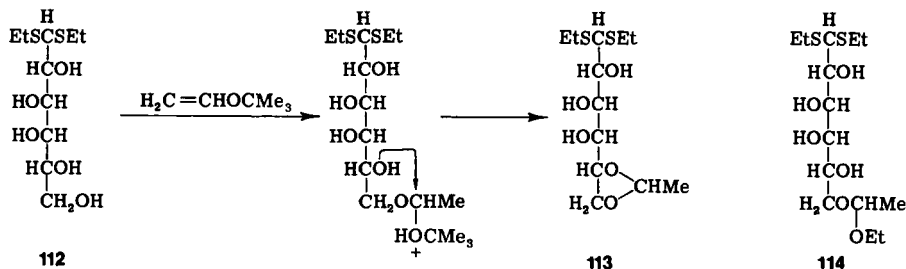
Conversions involving other carbonyl and carboxyl functions within the molecule are effected without disturbance to the dithioacetal group. Methyl 2,3:4,5-di-O-benzylidene-D-galacturonate dialkyl dithioacetals have been converted into the corresponding derivatives of D-galactose by reduction with lithium aluminum hydride,[246] whereas lithium borohydride has been employed to reduce partially substituted D-glucurono-6,3-lactone dithioacetals[221,249] and 2,4-O-benzylidene-D-*xylo*-pentodialdose 1-(diethyl dithioacetal)[249]; D-glucuronic acid phenylhydrazide dialkyl dithioacetals have been prepared[79,82] from the corresponding derivatives of D-glucurono-6,3-lactone plus phenylhydrazine, and similar reaction with benzylamine was reported[82] to produce an amide.

4. Reactions of Amino Groups

Amino groups in dithioacetals undergo normal substitution reactions. Conventional reactions have been reported to occur with acetic anhydride,[88,90,91] benzoyl chloride,[46,92] 1-fluoro-2,4-dinitrobenzene,[250] p-toluenesulfonyl chloride,[247] and benzyloxycarbonyl chloride[92]; the reported[92] application of sodium chloride and methyl iodide to the conversion of 2-amino-2-deoxy-D-glucose diethyl dithioacetal hydrochloride into a pentamethyl derivative is presumed to be an error. Although crude 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal was in one instance converted in low yield into a poorly

(247) J. Yoshimura and T. Sato, *Nippon Kagaku Zasshi*, 80, 1479–1483 (1959); *Chem. Abstr.*, 55, 5,355d (1961).
(248) M. L. Wolfrom and G. G. Parekh, *Carbohydr. Res.*, 11, 547–557 (1969).
(249) H. Zinner, K.-H. Rohde, and A. Mattheus, *Ann.*, 677, 160–165 (1964).
(250) M. L. Wolfrom, H. G. Garg, and D. Horton, *J. Org. Chem.*, 29, 3280–3283 (1964).

crystalline tetraacetate[251] of undetermined[91,184] structure, subsequent
attempts[90,91] to repeat the reaction produced only the corresponding
pentaacetate (in significantly better yield).

The product from diazotization of 2-amino-2-deoxy-D-glucose
diethyl dithioacetal at pH 5 in aqueous acetic acid, originally[252] con-
sidered to be 2,5-anhydro-D-glucose diethyl dithioacetal, was sub-
sequently identified[134] as ethyl 2-S-ethyl-1,2-dithio-α-D-man-
nofuranoside (30). The principal product from a similar diazotization
reaction, at pH 0 in aqueous solution, is 2-S-ethyl-2-thio-D-glucose[133]
(31). These conversions were rationalized[133] by postulation of a *trans*-
1,2-episulfonium ion (115), which forms with inversion at C-2 as the
diazonium ion collapses; subsequent attack at C-1 by the 4-hydroxyl
group produces 30. In order to rationalize the apparent retention of
configuration at C-2 in 31, intramolecular attack by the second
ethylthio group was suggested, subsequent attack at C-1 by solvent
water on the isomeric ion 116 preserving the 2-S-ethyl-2-thio-D-
gluco configuration; however, although steric access at C-1 is more
favorable in 116 than in 115, there exists the alternative possibility
that the reaction proceeds directly from 115 to a derivative of 2-S-
ethyl-2-thio-D-mannose (32), which was later[130] shown to be readily
converted into the observed product (31). However, the latter hy-
pothesis seems to be contraindicated by the observation[253] that 2-S-
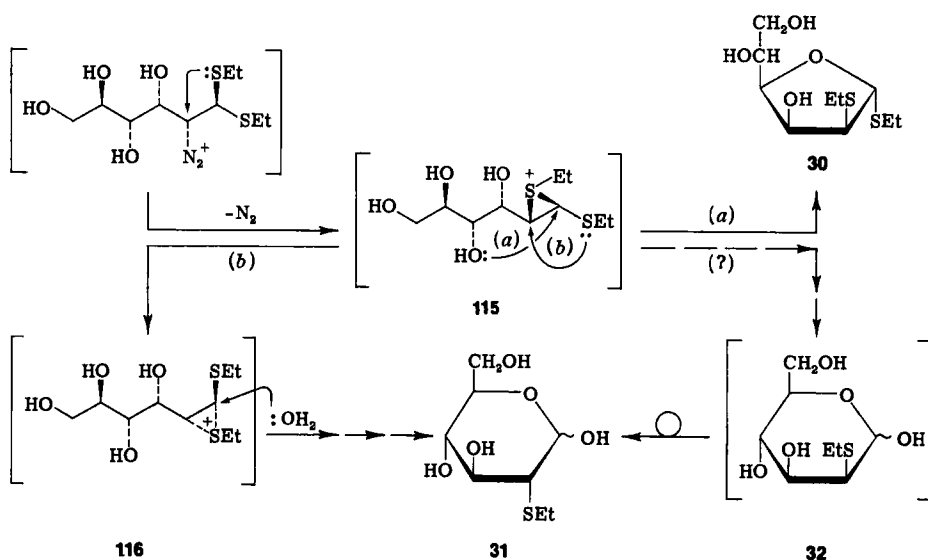
(251) M. L. Wolfrom, R. U. Lemieux, and S. M. Olin, *J. Amer. Chem. Soc.*, 71,
2870–2873 (1949).

ethyl-2-thio-D-mannose (32) was not detectable in the deamination products from 2-amino-2-deoxy-D-glucose diethyl dithioacetal at pH 0 under conditions where epimerization of 32 to 31 did not occur. Deamination of 2-amino-2-deoxy-D-glucose ethylene dithioacetal at pH 5.6 affords (after acetylation) a mixture of three products, tentatively identified[131,253] as the triacetates of 1,2-S-ethylene-1,2-dithio-α-D-manno-furanose and -pyranose, and of 2-deoxy-D-*arabino*-hexono-1,4-lactone; the former two are readily explained by attack of O-4 and O-5, respectively, on an episulfonium intermediate related to 115, whereas the latter may have arisen by 1,2-hydride migration in the same intermediate, with subsequent hydrolysis.

Diazotization of 2,5-anhydro-3,4-dideoxy-3,4-epimino-L-arabinose di-isobutyl dithioacetal effects removal of the nitrogen atom to give[254] 2,5-anhydro-3,4-dideoxy-D-*glycero*-pent-3-enose di-isobutyl dithioacetal (110).

5. Reactions with Bases

Emil Fischer reported[1] that aldose dithioacetals are weak acids, and that D-glucose diethyl dithioacetal reacts with bases to form stable, crystalline salts. In a fully protected derivative, for example, 2,3:4,5-di-O-isopropylidene-D-arabinose diphenyl dithioacetal (117), the proton bonded to C-1 would be expected to be the most acidic, and, indeed, treatment of 117 with such strong bases as butyllithium, potassium *tert*-butoxide, or dimethylsulfinyl carbanion does remove the C-1 proton.[32] β-Elimination of good leaving-groups from the carbon atom adjacent to a dithioacetal group was demonstrated[255] in aliphatic examples; however, the stability of the 1,1-bis(phenylthio)vinyl moiety appears to be quite high, as deprotonation of 117 results in essentially synchronous elimination of the isopropylidenated oxygen atom from C-2, immediate expulsion of a molecule of acetone giving the presumed intermediate 118. Reaction of the alkoxide ion 118 with water generates the ketene dithioacetal 2-deoxy-4,5-O-isopropylidene-D-*erythro*-pent-l-enose diphenyl dithioacetal (119), a syrup that was originally identified from its n.m.r. spectrum, whereas treatment of 118 with iodomethane or dimethyl sulfate affords the corresponding, syrupy 3-methyl ether (120), which was completely characterized; *p*-nitrobenzoylation of 119 gave[32] a

(252) J. Defaye, *Bull. Soc. Chim. Fr.*, 1101–1103 (1967).

(253) P. Angibeaud, C. Bosso, J. Defaye, and D. Horton, *Abstr. Pap. Amer. Chem. Soc. Meet.*, 168, CARB-6 (1974).

crystalline derivative (121). Under similar conditions, 2,3:4,5-di-*O*-isopropylidene-D-xylose diphenyl dithioacetal was converted, in good yields, into the diastereoisomeric alcohol (122), 3-methyl ether (123), and crystalline 3-*p*-nitrobenzoate (124), respectively,[20] and the 1,1-bis(ethylthio) analogues (125 and 126, respectively) were subsequently prepared from the corresponding diethyl dithioacetal derivatives of D-arabinose and D-xylose, the former by reaction with (*a*) dimethylsulfinyl carbanion and (*b*) iodomethane,[32] and the latter by treatment with (*a*) potassium hydroxide in refluxing tetrahydrofuran and (*b*) dimethyl sulfate.[242]

$$
\begin{array}{cccc}
\underset{\text{PhSCSPh}}{\overset{\text{H}}{\Big\downarrow}} &
\underset{\text{CH}}{\overset{\text{PhS}\diagdown_C\diagup\text{SPh}}{\Big\|}} &
\underset{\text{CH}}{\overset{\text{PhS}\diagdown_C\diagup\text{SPh}}{\Big\|}} &
\underset{\text{CH}}{\overset{\text{EtS}\diagdown_C\diagup\text{SEt}}{\Big\|}} \\
\end{array}
$$

117	118 R = O⁻	122 R = OH	125 R = OMe, R' = H
	119 R = OH	123 R = OMe	126 R = H, R' = OMe
	120 R = OMe	124 R = OCOC₆H₄NO₂-*p*	
	121 R = OCOC₆H₄NO₂-*p*		
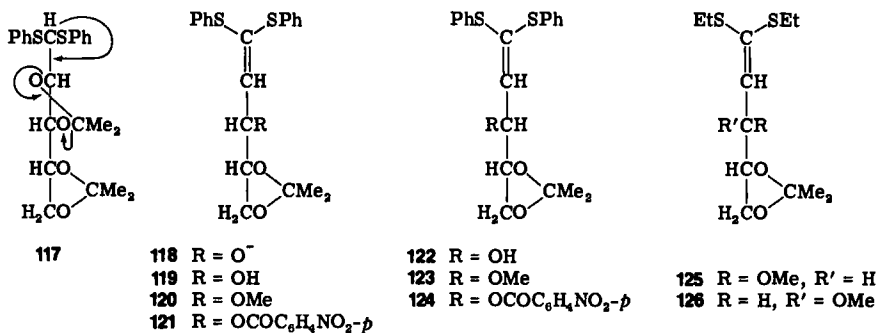
The reactivity of 120 is much less than that of either a normal alkene or a dithioacetal. Attempts to hydrogenolyze (see Section IV,3) or reduce 120 with Raney nickel, to hydrolyze the dithioacetal (see Section IV,1) with mercury(II) chloride (even in the presence of an overwhelming excess of the reagent), and to ozonize the double bond at −78°, produce only the starting material; an analytical sample was prepared by extended treatment of the crude product with a concentrated, aqueous, alkaline solution of potassium permanganate at the reflux temperature.[20] Acetolysis, ozonolysis, or brominolysis of 120 at room temperature affords diphenyl disulfide, and extended oxidation with hydrogen peroxide in acetone, with peroxypropionic acid, and with peroxyacetic acid, produces benzenesulfonic acid, methyl phenyl sulfone, and an uncharacterized explosive, respectively; the products occur as intractable mixtures, and the yields are invariably low.

Acid hydrolysis under milder conditions gave isolable products derived from the pentose portion of 120. The action of 2*M* hydrochlo-

(254) J. Cléophax, S. D. Gero, and R. D. Guthrie, *Tetrahedron Lett.*, 567–570 (1967); J. Cléophax, J. Hildesheim, A. M. Sepulchre, and S. D. Gero, *Bull. Soc. Chim. Fr.*, 153–156 (1969).

(255) E. Rothstein, *J. Chem. Soc.*, 1550–1553 (1940).

ric acid during 24 hours at the reflux temperature allowed sub-
sequent column-chromatographic isolation of a 9% yield of an unsat-
urated bis(phenyl thioether) having the elemental composition
$C_4H_5O(SPh)_2$, whereas a solution of **120** in concentrated hydrochloric
acid underwent conversion during 20 minutes at room temperature
(and subsequent acetylation) into a mixture of products, including 5-
O-acetyl-2-deoxy-3-S-phenyl-3-thio-D-*threo*-pentono-1,4-lactone (**127**,
8% yield) and its 3-epimer **128** (5% yield).

Thermodynamic control of the ratio of **127** to **128** formed was
shown by the conversions of **119,121,122**, and **124**, under identical
conditions, into mixtures of the epimers in which no significant dif-
ference in the proportions could be discerned.[20] The formation of **127**
and **128** may be rationalized as proceeding by the following steps: (*a*)
acid-catalyzed hydrolysis of the 4,5-acetal group and loss of the 3-
substituent from **119–124**, (*b*) attack of O-4 at C-1 of the resulting
allylic carbonium ion, (*c*) hydrolysis of one phenylthio group, (*d*)
migration of the other to C-3 by an internal, S$_N$2 type of process, and
(*e*) addition of water at C-1.

127 R = SPh, R' = H
128 R = H, R' = SPh
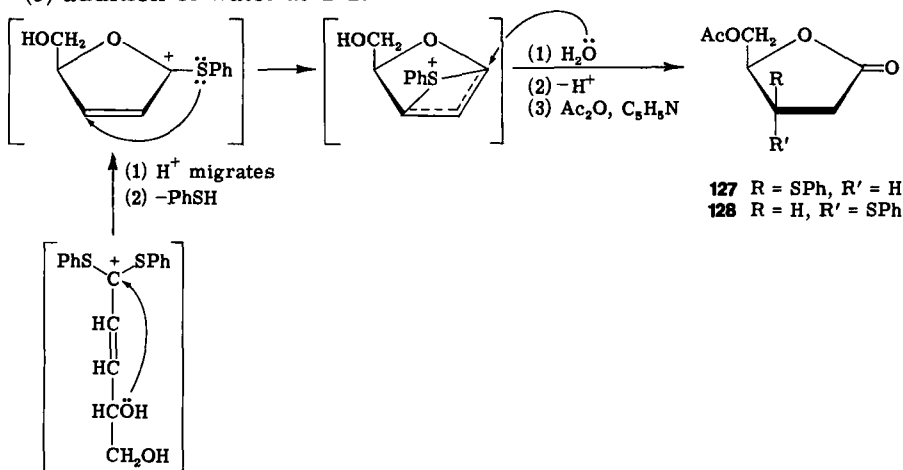
Treatment of 2-deoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-
ose diethyl dithioacetal (**128a**) with butyllithium in tetrahydrofuran
produced a carbanion that is stable by virtue of having no electronega-
tive substituent at C-2; this anion subsequently reacted with iodo-
methane to afford[255a] 1,3-dideoxy-4,5:6,7-di-*O*-isopropylidene-D-
arabino-heptulose diethyl dithioacetal (**128b**) in 82% yield (see
Section II,5). This reaction provides a useful route to 1,3-dideoxy-
2-ketoses.

(255a) D. Horton and R. A. Markovs, *Abstr. Pap. Amer. Chem. Soc. Meet.*, **170**,
CARB-6 (1975).

R
EtSCSEt
|
CH₂
|
OCH
⌐—OCH
⌐   ⟋CMe₂
HCO
|
HCO
| ⟍CMe₂
H₂CO⟋

128a R = H
128b R = Me
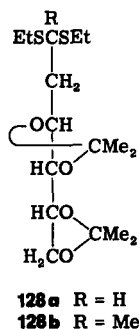
IV. REACTIONS OF THE DITHIOACETAL GROUP

1. Replacement of Alkylthio Groups by Action of Mineral and Lewis Acids

In his pioneering study on these compounds, Emil Fischer[1] reported that the parent sugar can be regenerated from D-glucose diethyl dithioacetal by the action of mineral acid, mercury(II) chloride, or silver(I) nitrate. The former conversion, in effect, partially reverses the acid-catalyzed formation-reaction (see Sections I and II,2), some greater degree of complication being evident from the observations that (a) both D-glucose and a 1-thiopyranoside are formed in this reaction,[37] and (b) ethyl 1-thio-α-D-glucofuranoside (a likely initial product of hydrolysis) undergoes a series of rearrangements at 100° in dilute acid, initially affording the β anomer, but gradually rearranging[256] to the stable 1-thiopyranosides. Equilibration of 6-acet-amido-6,8-dideoxy-D-*erythro*-D-*galacto*-octose dimethyl dithioacetal with 2M hydrochloric acid during 6 hours at room temperature affords[257] a 15% yield of the ethyl 1-thio-α-pyranoside. The hydrolyses catalyzed by metal ion proceed because of the great affinity of the sulfur atom for such "soft" acids as mercury(II) and silver(I); relatively stable, crystalline, 1:1 adducts[202] of dithioacetals with mercury(II) chloride can be isolated soon after mixing the reactants, the hydrolysis reaction apparently occurring more slowly. The action of silver(I) benzoate has been shown[258] to convert D-glucose diethyl dithioacetal into 2-O-benzoyl-D-glucose. Thallium(III) trifluoroace-

(256) E. Pacsu and E. J. Wilson, Jr., *J. Amer. Chem. Soc.*, **61**, 1450–1454 (1939).
(257) G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *J. Chem. Soc. (C)*, 2218–2224 (1970).
(258) C. Pedersen and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **82**, 3215–3217 (1960).

tate[259] and ammonium cerium(IV) nitrate[260] have been used to hydrolyze dithioacetals of simple aliphatic and aromatic carbonyl compounds.

The course of reactions with mercury(II) chloride is subject to control by experimental conditions, and by substitutive and stereochemical constraints within the particular dithioacetal undergoing hydrolysis. Much of the synthetic versatility of dithioacetals derives from this control, and various aspects have been reviewed in detail in this Series[6-8] and elsewhere.[261]

At neutral pH, hydrolysis is extremely slow, but the reaction proceeds to completion with minimal interference from side reactions; as an increase in the acidity of the medium not only accelerates the rate of hydrolysis but also introduces competing reactions and displaces the equilibrium towards the dithioacetal, the optimal conditions for the demercaptalation reaction are a compromise[176] between the rate and the yield of the reaction. The identity of the thiol also influences the rate of mercury(II) chloride-catalyzed hydrolysis, diphenyl dithioacetals reacting[30] very readily and ethylene dithioacetals slowly[188,235] or not at all,[72] the reverse of their relative rates of formation.

Total hydrolysis of the dithioacetal group by excess amounts of mercury(II) chloride occurs in aqueous acetone; the dithioacetal is thus available as a protecting group for the carbonyl carbon atom of sugars, and it has been widely used (a) as a direct means of purification, for example, with D-altrose,[262] D-glycero-D-ido-heptose,[263] and D-idose,[264] (b) to facilitate such difficult reactions as the tritylation[187,189] of pentoses, and (c) as a protecting group, as in the preparation of ω-deoxyaldoses.[60,216,265,266] Other substituents present in the molecule are generally unaffected during the reaction; thus 2-O-methyl-[182] and 6-O-benzoyl-D-glucose,[175] 2-S-ethyl-2-thio-D-mannose,[128,130] and 5-S-ethyl-5-thio-L-arabinose[146] were prepared by hydrolysis of their respective diethyl dithioacetals. The presence of other substituents

(259) T. L. Ho and C. M. Wong, Can. J. Chem., 50, 3740–3741 (1972).
(260) T. L. Ho, H. C. Ho, and C. M. Wong, Chem. Commun., 791 (1972).
(261) E. Pacsu, Methods Carbohydr. Chem., 2, 354–367 (1963).
(262) E. Sorkin and T. Reichstein, Helv. Chim. Acta, 28, 940–941 (1945).
(263) J. W. Pratt, N. K. Richtmyer, and C. S. Hudson, J. Amer. Chem. Soc., 75, 4503–4507 (1953).
(264) N. K. Richtmyer and C. S. Hudson, J. Amer. Chem. Soc., 57, 1716–1721 (1935).
(265) G. Rembarz, J. Prakt. Chem., [4] 19, 319–323 (1963).
(266) W. W. Zorbach and J. P. Ciaudelli, J. Org. Chem., 30, 451–452 (1965).

may influence the tautomeric form of the product, as with the last-mentioned example, 5-trityl ethers,[187,189] 5-acetates,[174] 5-*p*-toluates,[177] and 3,5-isopropylidene acetals,[235] all of which are constrained to cyclize as furanoses.

a. Hydrolyses Affording Acyclic Acetals and *aldehydo* Tautomers.—Hydrolysis of aldose dithioacetals having the 4- and 5-hydroxyl groups protected affords the interesting, acyclic tautomers, in which the aldehydic center exists as a free carbonyl group. Demercaptalation of an impure preparation of D-glucose diethyl dithioacetal pentaacetate (24) was first attempted in 1918 by Schneider and coworkers.[267] Shortly afterwards, Levene and Meyer reported demercaptalation reactions affording syrupy 2,3,4,5,6-pentamethyl-*aldehydo* derivatives of D-glucose,[199] D-galactose, and D-mannose,[40] and Wolfrom[268] successfully prepared crystalline *aldehydo*-D-glucose pentaacetate (129) by the action of mercury(II) chloride on a crystalline preparation of 24. In the following year, Brigl and Mühlschlegel[122] prepared the corresponding pentabenzoate (130),

<pre>
        H   H
     EtSCSEt   C=O
        | |
      HCOAc   HCOR
        | |
      AcOCH   ROCH
        | |
      HCOAc   HCOR
        | |
      HCOAc   HCOR
        | |
      CH2OAc   CH2OR

        24   129   R = Ac
                        130   R = Bz
</pre>
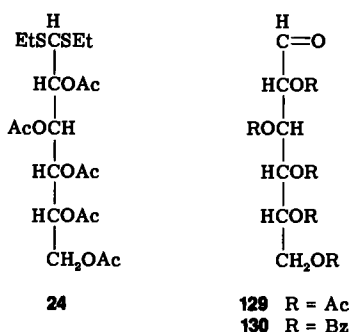
and, within five years, the *aldehydo* acetates of D-galactose,[42] L-arabinose,[269] D-xylose,[16] L-fucose,[270] D-*glycero*-L-*gluco*-heptose,[271] and D-*glycero*-D-*galacto*-heptose,[272] as well as 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose[69] and 3,4,5,6-tetra-*O*-benzoyl-*aldehydo*-D-glucose, had been obtained by the same route.[126] *aldehydo*-Acetates subsequently prepared in the same way were those of the remaining D-pentoses,[273-275] a few higher aldoses,[276,277] methyl 2,3,4,5-tetra-*O*-

(267) W. Schneider, J. Sepp, and O. Stiehler, *Ber.*, 51, 220–234 (1918).
(268) M. L. Wolfrom, *J. Amer. Chem. Soc.*, 51, 2188–2193 (1929).
(269) M. L. Wolfrom and M. R. Newlin, *J. Amer. Chem. Soc.*, 52, 3619–3623 (1930).
(270) M. L. Wolfrom and J. A. Orsino, *J. Amer. Chem. Soc.*, 56, 985–987 (1934).
(271) R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.*, 56, 2080 (1934).
(272) E. Montgomery and C. S. Hudson, *J. Amer. Chem. Soc.*, 56, 2463–2464 (1934).
(273) M. L. Wolfrom and F. B. Moody, *J. Amer. Chem. Soc.*, 62, 3465–3466 (1940).

acetyl-D-galacturonate,[86] some 2-deoxy-[61,110,278] and 3-deoxy-al-doses,[279] and 2-acetamido-2-deoxy-D-glucose[90,92]; however, the latter report was disputed by workers[280] who claimed to have isolated the unsaturated derivative **131**, as well as the oxazoline **132** (presumed to be an intermediate in the formation of **131** from 2-acetamido-3,4,5,6-tetra-O-acetyl-2-deoxy-D-glucose diethyl dithioacetal) under the conditions used by Kent and coworkers.[90,92] Bethell and Ferrier[138] reported a similar decomposition of 4,5,6-tri-O-benzoyl-2,3-di-S-ethyl-2,3-dithio-D-allose diethyl dithioacetal (**36**) to afford **133** upon demercaptalation.

| **131** | **132** | **133** |
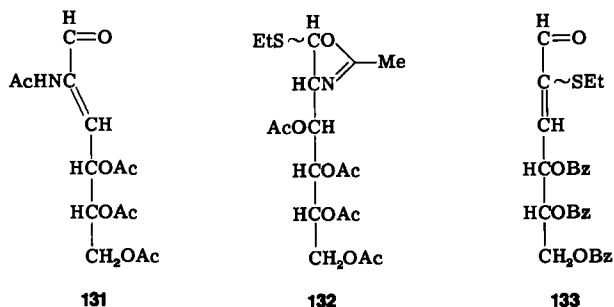
The chemical potentialities of these acyclic aldehyde forms soon overshadowed their novelty. Micheel and Suckfüll[99] hydrolyzed 2,3,4,5-tetra-O-acetyl-6-deoxy-6-iodo-D-galactose diethyl dithioacetal, initially obtaining a derivative of the 6-hydroxyaldehyde **134**, which slowly cyclized to afford 2,3,4,5-tetra-O-acetyl-D-galactoseptanose (**135**), characterized[281] as the pentaacetate; attempted performance of a similar transformation with 2,3,4,5-tetra-O-benzoyl-6-deoxy-6-iodo-D-glucose diethyl dithioacetal resulted in O-5 → O-6 acetyl migration, to give[191] a glucopyranose derivative. Gätzi and Reichstein[282] treated

(274) M. L. Wolfrom, D. I. Weisblat, W. H. Zophy, and S. W. Waisbrot, *J. Amer. Chem. Soc.*, **63**, 201–203 (1941).

(275) H. Zinner, *Chem. Ber.*, **83**, 418–420 (1950).

(276) R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.*, **59**, 1898–1900 (1937).

(277) M. L. Wolfrom, M. Konigsberg, and F. B. Moody, *J. Amer. Chem. Soc.*, **62**, 2343–2349 (1940).

(278) H. Zinner, H. Nimz, and H. Venner, *Chem. Ber.*, **90**, 2696–2699 (1957).

(279) G. Rembarz, *Wiss. Z. Univ. Rostock, Math.-Naturwiss. Reihe*, **10**, 29–37 (1961); *Chem. Abstr.*, **55**, 19,805 (1961).

(280) R. E. Harmon, G. Wellman, and S. K. Gupta, *Abstr. Pap. Amer. Chem. Soc. Meet.*, **163**, CARB 24 (1972).

(281) F. Micheel and F. Suckfüll, *Ann.*, **507**, 138–143 (1933); *Ber.*, **66**, 1957–1958 (1933).

(282) K. Gätzi and T. Reichstein, *Helv. Chim. Acta*, **21**, 914–925 (1938).

2,3:4,5-di-O-isopropylidene-D-arabinose diethyl dithioacetal with mercury(II) chloride, obtaining the syrupy aldehydo sugar derivative 136; the protecting groups of 136 are stable to nucleophiles, so that reaction of 136 with methylmagnesium iodide occurs only at the aldehyde group, affording a mixture (137) of derivatives of 6-deoxy-D-

$$
\begin{array}{c}
\text{H} \\
\text{C=O} \\
| \\
\text{HCOAc} \\
| \\
\text{AcOCH} \\
| \\
\text{AcOCH} \\
| \\
\text{HCOAc} \\
| \\
\text{CH}_2\text{OH} \\
\textbf{134}
\end{array}
$$

135

$$
\begin{array}{c}
\text{H} \\
\text{C=O} \\
| \\
\text{OCH} \\
| \quad \text{CMe}_2 \\
\text{HCO} \\
| \\
\text{HCO} \\
| \quad \text{CMe}_2 \\
\text{H}_2\text{CO} \\
\textbf{136}
\end{array}
$$

$$
\begin{array}{c}
\text{Me}_2\text{C} \quad \text{OCH}_2 \\
\quad \text{OCH} \\
| \\
\text{OCH} \\
| \quad \text{CMe}_2 \\
\text{HCO} \\
| \\
\text{CHOH} \\
| \\
\text{Me} \\
\textbf{137}
\end{array}
$$
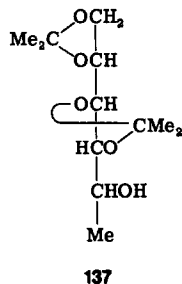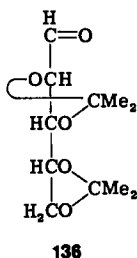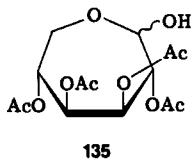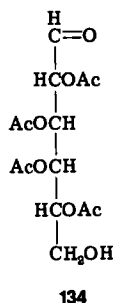
mannitol and 6-deoxy-L-gulitol. Nucleophilic additions to suitably protected aldehydo (or keto) groups have subsequently been shown to be a completely general reaction, encompassing, for example, such nucleophiles as active methylene compounds, Wittig reagents,[283] and 2-lithio-1,3-dithiane (58; see Section II,5). The action of diazomethane upon *aldehydo*-aldose acetates generally affords, after reduction, the 1-deoxyketose derivative having the same configuration.[274]

Aldehydo derivatives having incomplete protection, or several different types of protecting group, have been extensively studied since the 1950's, principally by Zinner's group. Such examples as 3,6-anhydro-D-galactose,[118]   2,5-anhydro-D-ribose,[207]   and   2,4-O-ethylidene-D-glucurono-6,3-lactone[221] are sterically unable to cyclize through C-1 upon hydrolysis of the respective dithioacetals, whereas dithioacetals of 4,5-O-isopropylidene-D-galactose,[230] and other examples having unprotected hydroxyl groups,[126,233,284] undergo demercaptalation to afford free aldehydes for want of an unprotected hydroxyl group suitably situated to form a 5- or 6-membered ring; conversely, the existence of a demercaptalated product free from major, steric constraints in the aldehydo form is evidence[249,285] that the appropriate hydroxyl groups are protected. The 2,3:4,5-bis(cyclic carbonates) of D-arabinose dialkyl dithioacetals are resistant[204] to hydrolysis by mercury(II) chloride, whereas the corresponding reac-

(283) N. K. Kochetkov and B. A. Dmitriev, *Tetrahedron*, 21, 803–815 (1965).
(284) H. Zinner and E. Wittenburg, *Chem. Ber.*, 94, 1298–1303 (1961).
(285) H. Zinner, W. Rehder, and H. Schmandke, *Chem. Ber.*, 95, 1805–1811 (1962).

tion of 2-deoxyaldose dithioacetals is considerably accelerated[286] due to a favorable inductive effect.

In a reaction medium containing mercury(II) chloride plus an alcohol (instead of aqueous acetone), dithioacetals having those hydroxyl groups protected that would take part in glycosyl ring formation are solvolyzed to afford acetals, which may also be prepared from the corresponding aldehydo derivatives by treatment with an alcohol plus an acid catalyst.[40,199] D-Galactose diethyl dithioacetal pentaacetate (138) reacts with methanolic mercury(II) chloride to give the corresponding acylated dimethyl acetal 139, which may be saponified to afford the base-stable dimethyl acetal (140) of D-galactose.[287] 2-Deoxy-D-*arabino*-hexose diethyl dithioacetal triacetate[56] and 3,4,5-tri-O-benzoyl-2-S-ethyl-2-thio-D-lyxose(or -xylose) diethyl dithioacetal[137] are similarly converted into dimethyl acetals without disturbance to the other substituents, and 4,5-O-isopropylidene-D-galactose diethyl dithioacetal affords the corresponding dimethyl acetal in 70% yield[230] under similar conditions.

H H H
EtSCSEt   MeOCOMe   MeOCOMe
HCOAc   HCOAc   HCOH
AcOCH   HgCl₂,   AcOCH   Ba(OMe)₂   HOCH
AcOCH   MeOH,   AcOCH   HOCH
HCOAc   HgO   HCOAc   HCOH
CH₂OAc   CH₂OAc   CH₂OH

138   139   140
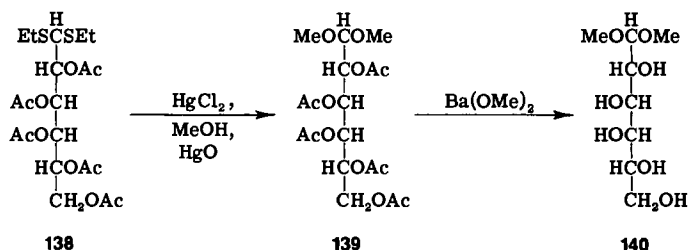
The aspects of synthetic utility are reversed between aldehydo derivatives and acetals, in that the carbonyl group of the former is exceedingly reactive, whereas, in the latter, the hydroxyl groups and their substituents may undergo base-catalyzed, reductive, or oxidative transformations without involvement of the (protected) carbonyl group. Thus, 6-deoxy-4,5-O-isopropylidene-D-galactose dibenzyl dithioacetal was converted, in steps, into the dimethyl acetal; this was selectively benzylated at O-2, the ether methylated at O-3, and the product deprotected to afford digitalose (3-O-methyl-D-fucose)[105]; 4,5,6-tri-O-benzoyl-D-galactose diethyl dithioacetal underwent (*a*) conversion into the dimethyl acetal, (*b*) di-O-methylation, and (*c*) deprotection, to afford 2,3-di-O-methyl-D-galactose[183]; 3,4,5,6-tetra-O-benzoyl-2-S-ethyl-2-thio-D-mannose diethyl dithioacetal was converted into the dimethyl acetal prior to treatment with Raney nickel (see Section IV,3) in order to effect selective reduction[129] of C-2. In

(286) H. Zinner, H. Nimz, and H. Venner, *Chem. Ber.*, 91, 148–150 (1958).
(287) H. A. Campbell and K. P. Link, *J. Biol. Chem.*, 122, 635–640 (1938).

planning syntheses using dithioacetals, benzyl groups are of strategic[104] value, because they resist the action of acids and bases, but may be removed by neutral hydrogenolysis after conversion of the dithioacetal into the corresponding acetal (or other derivative).

b. **Formation of Glycosides and Thioglycosides.** — Schneider and coworkers[173,267] treated D-glucose diethyl dithioacetal with one molar equivalent of mercury(II) chloride in hot water, obtaining, after neutralization, an ethyl 1-thio-D-glucoside in 65% yield. Pacsu and coworkers[256,288] repeated the preparation in the presence of mercury(II) oxide (to neutralize hydrochloric acid as fast as it was formed), and proposed an α-D-furanoside structure for the product. Oxidation by periodic acid afforded[143] one equivalent of formaldehyde and no formic acid, verifying that the product is ethyl 1-thio-α-D-glucofuranoside. The diethyl dithioacetals of 2-acetamido-2-deoxy-D-glucose,[289] D-glucuronamide,[290] and D-glucurono-6,3-lactone[291] underwent conversion into the corresponding ethyl 1-thio-α-D-furanoside derivatives by the same procedure, the last being isolated as the sodium salt, and the first being accompanied by a small proportion of the β anomer as a byproduct. Similarly, partial demercaptalation of D-ribose dialkyl dithioacetals affords the corresponding alkyl 1-thio-α-D-ribofuranosides[292] in ~70% yields.

Although Schneider and coworkers[267] and Green and Pacsu[288] were unable to isolate ethyl 1-thio-α-D-galactofuranoside by partial demercaptalation of D-galactose diethyl dithioacetal, Wolfrom and coworkers[293] accomplished a chromatographic isolation of this syrupy product (as the crystalline peracetate) in 44% yield. Chromatographic analysis of the products of this reaction revealed that the thiofuranoside and the completely hydrolyzed aldose are formed in approximately equal amounts; similar analysis of products from an incomplete demercaptalation reaction of 2-acetamido-2-deoxy-D-galactose diethyl dithioacetal (141) showed[294] that the ethyl 1-thio-α-

(288) J. W. Green and E. Pacsu, J. Amer. Chem. Soc., 59, 1205–1212 (1937).

(289) M. L. Wolfrom, S. M. Olin, and W. J. Polglase, J. Amer. Chem. Soc., 72, 1724–1729 (1950).

(290) Y. Nitta, A. Momose, and M. Takagi, Yakugaku Zasshi, 82, 567–573 (1962); Chem. Abstr., 58, 4,638d (1963).

(291) Y. Nitta and A. Momose, Yakugaku Zasshi, 82, 574–577 (1962); Chem. Abstr., 58, 4,639c (1963).

(292) H. Zinner, A. Koine, and H. Nimz, Chem. Ber., 93, 2705–2712 (1960).

(293) M. L. Wolfrom, Z. Yosizawa, and B. O. Juliano, J. Org. Chem., 24, 1529–1530 (1959).

(294) M. L. Wolfrom and Z. Yosizawa, J. Amer. Chem. Soc., 81, 3474–3476 (1959).

D-furanoside (after acetylation, 144, 32%) and the free sugar (142, 24%) are formed in almost equal amounts, minor components being the starting material (12%), the 1-thio-β-D-pyranoside 143 (4%), and the 1-thio-β-D-furanoside (after acetylation, 145, 3%).

| 141 | 142 R, R' = H, OH | 144 R = H, R' = SEt |
| | 143 R = SEt, R' = H | 145 R = SEt, R' = H |
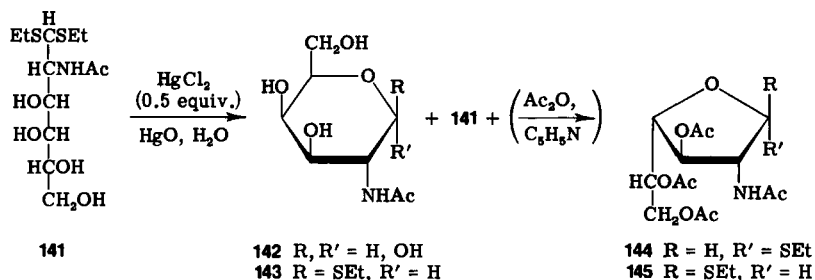
Whereas ethyl 1-thio-β-D-arabinofuranoside has not been prepared directly, partial hydrolysis of 5-O-benzoyl-D-arabinose diethyl dithioacetal affords, in 38% yield, the corresponding 5-O-benzoyl thioglycoside, whence the desired[295] product may be liberated by saponification; partial hydrolysis of 5-S-ethyl-5-thio-L-arabinose diethyl dithioacetal affords the ethyl 1,5-dithio-β-L-furanoside. Partial demercaptalation of D-lyxose diethyl dithioacetal gives the free sugar plus detectable traces of a 1-thiofuranoside, whereas dithioacetals of D-xylose and D-mannose undergo complete hydrolysis to afford only the aldose plus unreacted starting-material[292]; however, 2-S-ethyl-2-thio-D-mannose diethyl dithioacetal[130] (33) undergoes conversion[296] into ethyl 2-S-ethyl-1,2-dithio-α-D-mannofuranoside[129] (30) in 60–70% yield. Dithioacetals of 2-deoxy-D-*arabino*-hexose[19] and 2-deoxy-D-*erythro*-pentose[286] undergo complete hydrolysis only, presumably due to the absence of an inductively withdrawing group[19,177,286,297] at C-2; this accelerates the (presumed) second[288] step of the total hydrolysis-sequence, namely, removal of the remaining alkylthio group from the (intermediate) thiofuranoside; the effect is stronger for derivatives of ketoses, as 6-O-benzoyl-1-deoxy-D-psicose diethyl dithioacetal undergoes complete[298] demercaptalation in warm

(295) E. J. Reist, P. A. Hart, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, 81, 5176–5180 (1959).

(296) M. L. Wolfrom, W. von Bebenburg, and A. Thompson, *J. Org. Chem.*, 26, 4151–4153 (1961); D. Horton and M. Sakata, *Carbohydr. Res.*, 39, 67–78 (1975).

(297) M. L. Wolfrom, D. I. Weisblat, and A. R. Hanze, *J. Amer. Chem. Soc.*, 66, 2065–2068 (1944).

(298) E. J. Reist, P. A. Hart, B. R. Baker, and L. Goodman, *J. Org. Chem.*, 27, 1722–1727 (1962).

50% aqueous acetic acid, whereas 4-*O*-benzoyl-3,5-*O*-benzylidene-2-deoxy-D-*erythro*-pentose diethyl dithioacetal can be deacetalated in warm 80% acetic acid without disturbance[235] of the thio groupings.

Green[6] has generalized that, from each reaction, the favored 1-thiofuranoside appears to be that having the 1,2-*cis* arrangement of polar substituents.

At reflux, the action of 3 equivalents of mercury(II) chloride in methanol converts the diethyl dithioacetal of D-glucose into a 5:1 mixture of methyl β- and α-D-glucopyranoside[299]; extension of this reaction to the dibenzyl dithioacetals of L-arabinose, L-rhamnose, and D-galactose afforded, as major products, the methyl β-L-pyranoside of the L-pentose and the methyl α-pyranosides of the two hexoses, respectively.[300]

Pacsu and Green[301] subsequently discovered that the action of the mercury(II) salt plus an acid acceptor in an alcohol offers a synthetic access to furanosides; thus, D-galactose diethyl dithioacetal reacts with 3 equivalents of mercury(II) chloride plus mercury(II) oxide in ethanol to afford[288,301] ethyl β-D-galactofuranoside in 70% yield. Good yields of methyl α-(plus some β-)D-mannofuranoside,[302] ethyl α-(and β-)L-rhamnofuranoside,[303] methyl α-D-lyxofuranoside,[304] and methyl 2-acetamido-2-deoxy-D-glucofuranoside[305] have also been obtained by the same method. The reaction is, however, not completely general, as in reactions of the appropriate dithioacetals under these conditions, (*a*) alkyl 1-thio-α-D-glucofuranosides are stable,[288] (*b*) ethyl α-L-arabinofuranoside is formed[306] in low yield, and (*c*) dimethyl acetals of D-fructose[307,308] (~70% yield at −80°), L-rhamnose,[309] and 2-*O*-methyl-D-mannose[232] may be isolated in significant yields; methyl D-fructofuranosides preponderate in demercaptalations of the D-fructose dithioacetals at room temperature, and ethyl α-L-rhamnofuranoside is the major product from demercaptalation of L-rhamnose dithioacetals in ethanol. Zorbach and coworkers[62] found that

(299) E. Pacsu, *Ber.*, **58**, 509–513 (1925).

(300) E. Pacsu and N. Ticharich, *Ber.*, **62**, 3008–3012 (1929).

(301) E. Pacsu and J. W. Green, *J. Amer. Chem. Soc.*, **58**, 1823–1824 (1936).

(302) A. Scattergood and E. Pacsu, *J. Amer. Chem. Soc.*, **62**, 903–910 (1940).

(303) J. D. Geerdes, B. A. Lewis, R. Montgomery, and F. Smith, *Anal. Chem.*, **26**, 264–266 (1954).

(304) M. Nys and J. P. Verheijden, *Bull. Soc. Chim. Belg.*, **69**, 57–62 (1960).

(305) M. W. Whitehouse and P. W. Kent, *Tetrahedron*, **4**, 425–429 (1958).

(306) J. W. Green and E. Pacsu, *J. Amer. Chem. Soc.*, **60**, 2056–2057 (1938).

(307) E. Pacsu, *J. Amer. Chem. Soc.*, **60**, 2277–2278 (1938).

(308) E. Pacsu, *J. Amer. Chem. Soc.*, **61**, 1671–1675 (1939).

(309) J. W. Green and E. Pacsu, *J. Amer. Chem. Soc.*, **60**, 2288–2289 (1938).

yields of methyl 2-deoxy-D-*erythro*-pentopyranosides afforded by demercaptalation of the corresponding diethyl or dibenzyl dithioacetal are inferior to those from direct, acid-catalyzed glycosidation.

Benzyl 1-thio-lactoside and ethyl lactoside, prepared from lactose dibenzyl dithioacetal[116] by the preceding methods, are necessarily pyranosides (owing to the substitution on O-4). Stevens and co-workers[310] prepared ethyl 2,3:4,5-di-*O*-isopropylidene-1-thio-β-D-glucoseptanoside by partial demercaptalation of 2,3:4,5-di-*O*-isopropylidene-D-glucose diethyl dithioacetal.

Pacsu[307] speculated that mercury(II) chloride-catalyzed demercaptalation reactions proceed by way of an initially formed monothioacetal, which subsequently suffers displacement of one of the geminal substituents to become either (*a*) an acetal, (*b*) a glycoside, or (*c*) a thioglycoside, further steps possibly following; although this theory has not been conclusively[297] disproved, neither does proof exist that it is either correct or universal. By using mercury(II) acetate in a mixture of acetic acid and acetic anhydride, E. P. Painter[311] succeeded in replacing one of the alkylthio groups of several sugar dithioacetals by an acetoxyl group, and obtained unequal amounts of the diastereoisomeric monothioacetals, an observation that he rationalized in terms of favored avenues of approach to a regionally planar, sulfonium intermediate; the results from studies of the rate of exchange of ^{14}C-labelled acetoxyl-groups[312] were in accord with this formulation, as was an analysis of mutarotation kinetics.[311] Later, it was found that, upon exposure to the initial reaction-conditions, each epimeric monothioacetal mutarotates[313] to the same, equilibrium condition.

Complete acetolysis of the alkylthio groups to afford 1,1-diacetates had early been accomplished by Pirie[314] by using acetic anhydride–sulfuric acid. This reaction has since been shown[82,245,273,315] to be not only completely general, but also more forcing[204] than the action of mercury(II) chloride or bromine. Geminal diacetates may be prepared by the action of acetic anhydride–pyridine[273] or acetic anhydride–sulfuric acid,[314] although *aldehydo*-D-*glycero*-D-*galacto*-hep-

(310) J. P. Beale, N. C. Stephenson, and J. D. Stevens, *Chem. Commun.*, 484–486 (1971); C. J. Ng and J. D. Stevens, unpublished data.

(311) E. P. Painter, *Tetrahedron*, **21**, 1337–1347 (1965); *Can. J. Chem.*, **42**, 2018–2022 (1964).

(312) N. H. Kurihara and E. P. Painter, *Can. J. Chem.*, **44**, 1773–1782 (1966).

(313) E. P. Painter and N. H. Kurihara, *Can. J. Chem.*, **45**, 1467–1473, 1475–1483 (1967).

(314) N. W. Pirie, *Biochem. J.*, **30**, 374–376 (1936).

tose hexaacetate is reportedly[269] unaffected by the latter reagent. The action of lead tetraacetate in acetic acid during 90 hours effects replacement of the benzylthio groups from D-glucose dibenzyl dithioacetal pentaacetate to afford isolable[316] amounts of 1,1,2,3,4,5,6-hepta-O-acetyl-D-glucose.

2. Replacement of Alkylthio Groups by Halogen Atoms

It has long been known that, in protic solvents, dithioacetals are converted quantitatively into the aldoses by reaction with iodine[317] or chlorine[318]; quantitative cleavage of dithioacetals is also effected[319] by solutions of potassium bromate plus potassium bromide and, less straightforwardly, by bromine.[28] This cleavage is synthetically useful; Gauthier and Vaniscotte[28] converted D-glucose ethylene dithioacetal pentaacetate into *aldehydo*-D-glucose pentaacetate (139), in 60% yield, by treatment of the dithioacetal with an exactly equivalent amount of bromine; Weygand and coworkers[320] subsequently effected the conversion 24 → 129 in 80% yield by action of a twofold excess of bromine, demonstrating that yields from the corresponding conversion of the D-*manno* analogue are almost constant (~70%) for proportions of bromine between a twofold and sixfold excess. Unprotected D-mannose diethyl dithioacetal reacts with a twofold excess of bromine in methanol during 3 minutes at 0°, and during 2.5 hours under reflux, to afford methyl α,β-D-mannofuranoside and methyl α-D-mannopyranoside, respectively, in good[321] yields. Wolfrom and Inouye[322] found that the action of bromine in methanol is far superior to that of mercury(II) chloride in effecting partial demercaptalation of half-gram amounts of 2-deoxy-5-O-(p-nitrobenzoyl)-2-(trifluoroacetamido)-D-arabinose diethyl dithioacetal to afford the corresponding ethyl 1-thio-α,β-D-glycofuranosides.

The action of limited amounts of active halogen compounds in aprotic solvents converts protected dithioacetals into 1-halo-1-thioal-

(315) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B63**, 487–490 (1967).

(316) E. J. Bourne, W. M. Corbett, M. Stacey, and R. Stephens, *Chem. Ind.* (London), 106–107 (1954).

(317) B. Holmberg, *J. Prakt. Chem.*, [2] **135**, 57–100 (1932).

(318) S. W. Lee and G. Dougherty, *J. Org. Chem.*, **5**, 81–85 (1940); B. Gauthier and J. Maillard, *C. R. Acad. Sci.*, **236**, 1890–1892 (1953).

(319) F. Weygand and H. J. Bestmann, *Chem. Ber.*, **90**, 1230–1234 (1957).

(320) F. Weygand, H. J. Bestmann, and H. Ziemann, *Chem. Ber.*, **91**, 1040–1043 (1958).

(321) R. Kuhn, W. Baschang-Bister, and W. Dafeldecker, *Ann.*, **641**, 160–176 (1961).

(322) M. L. Wolfrom and S. Inouye, *Carbohydr. Res.*, **41**, 117–133 (1975).

ditol derivatives in which the carbonyl center is asymmetrically[4,277] substituted. Thus, D-galactose diethyl dithioacetal pentaacetate (138) reacts with phosphoryl chloride,[323] acetyl bromide,[324] bromine (in ether),[325] or (1-chloro[or bromo]methyl) methyl ether[326] to afford a highly reactive 1-S-ethyl-1-halo-1-thioalditol (81 or 146, depending

<pre>
      H   H   H
   EtSCBr   EtSCSEt   EtSCCl
    | | |
   HCOAc   AcBr, or   HCOAc   POCl₃ or   HCOAc
    | | |
  AcOCH   Br₂—Et₂O, or   AcOCH   MeOCH₂Cl   AcOCH
    | ◄───────────── | ─────────► |
  AcOCH   MeOCH₂Br   AcOCH   AcOCH
    | | |
   HCOAc   HCOAc   HCOAc
   CH₂OAc   CH₂OAc   CH₂OAc

    81   138   146
</pre>
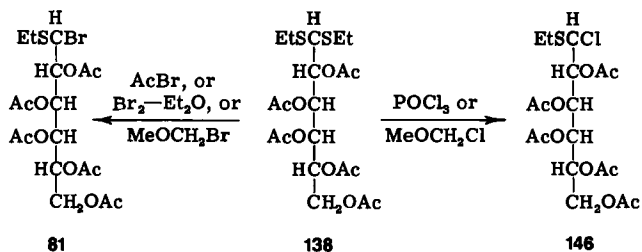
upon the halogen atom in the reagent); 146 has also been prepared[324] by the action of ethereal hydrogen chloride on 1,2,3,4,5,6-hexa-O-acetyl-D-galactose S-ethyl monothioacetal, and a number of oxygenated analogues[4,277,323,324] have been prepared by the action of these and related reagents on protected *aldehydo*-aldose and acetal derivatives. The stability of the α-bromothioethers declines with increasing size[327] of the S-alkyl group, and, whereas 2-acetamido-3,4,5,6-tetra-O-acetyl-1-bromo-2-deoxy-1-S-ethyl-1-thio-D-glucitol (147) decomposes[250] rapidly, the 2-(2,4-dinitroanilino) analogue 148 is relatively stable; attempted preparation of the 1-chloro analogue (149) of 148 by the action of chlorine on the corresponding dithioacetal afforded the 1-S-(1,1-dichloroethyl)-1-thioalditol 150.

<pre>
          H
        RSCX
          |
        HCNHR'
          |
        AcOCH
          |
        HCOAc
          |
        HCOAc
        CH₂OAc
</pre>
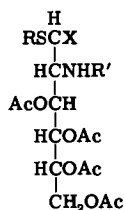
147 R = Et, R' = Ac, X = Br
148 R = Et,
       R' = C₆H₃-2, 4-(NO₂)₂,
       X = Br
149 R = Et,
       R' = C₆H₃-2, 4-(NO₂)₂,
       X = Cl
150 R = CCl₂Me,
       R' = C₆H₃-2, 4-(NO₂)₂,
       X = Cl

(323) M. L. Wolfrom and D. I. Weisblat, *J. Amer. Chem. Soc.*, 62, 878–880 (1940).

Aprotic halogenolysis may proceed by initial S-halogenation to generate a tertiary sulfonium group that suffers subsequent displacement by the companion halide ion, affording the α-halo-thioether plus an alkanesulfenyl halide; the inductive withdrawing, and inferior resonance-donating, effects of the halogen substituent preclude facile replacement of the second thio group.[250] If cyclohexene is used to trap any excess of bromine, the sulfenyl halide byproduct reacts to form a *trans*-1-(alkylthio)-2-chlorocyclohexane[328] that may complicate isolation of the desired products, so that excess amounts of bromine are to be avoided, or removed by evaporation.

$$\underset{\text{RSCSR}}{\overset{\text{H}}{|}} + X_2 \longrightarrow \left[\overset{X}{\underset{\text{HCSR}}{\overset{+}{\underset{|}{SR}}}} \; X^- \right] \longrightarrow \underset{\text{C(SR)X}}{\overset{\text{H}}{|}} + \text{RSX}$$

R = alkyl or aryl, X = Br or Cl
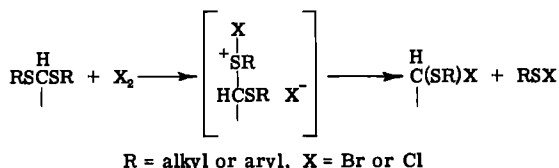
The different reactivities of the two substituents on C-1 allow selective replacement and conversion of them. Alkyl monothioacetals [proposed as intermediates in mercury(II)-catalyzed demercaptalation reactions — see Section IV,1,b] have been prepared from α-bromothioethers by the combined action of an alcohol and silver(I) carbonate; the introduction of S-nucleophiles is discussed in Section II,6. Reduction of 81 by lithium aluminum hydride effects hydrogenolysis of the carbon–halogen bond, whereas the action of Raney nickel on the derived S-ethyl O-methyl monothioacetal specifically cleaves the carbon–sulfur bond to afford the pentaacetate of 1-O-methyl-D-galactitol.[327]

The deprotected, epimeric products (151) of condensation of 81 with 6-acylamido-9-(chloromercuri)purine are formally related to 9-glycosyladenines, and Wolfrom and coworkers[329] coined the term "acyclic nucleoside analogues" to describe such compounds. A

(324) M. L. Wolfrom, D. I. Weisblat, and A. R. Hanze, *J. Amer. Chem. Soc.*, **62**, 3246–3250 (1940).

(325) F. Weygand, H. Ziemann, and H. J. Bestmann, *Chem. Ber.*, **91**, 2534–2537 (1958).

(326) I. Farkaš, M. Menyhárt, R. Bognár, and H. Gross, *Chem. Ber.*, **98**, 1419–1426 (1965).

(327) H. Zinner, R. Kleeschätzky, and P. Neels, *Chem. Ber.*, **98**, 1492–1497 (1965).

(328) J. Defaye, D. Horton, S. S. Kokrady, and Z. Machon, *Carbohydr. Res.*, **43**, 265–280 (1975).

(329) M. L. Wolfrom, A. B. Foster, P. McWain, W. von Bebenburg, and A. Thompson, *J. Org. Chem.*, **26**, 3095–3097 (1961).

series of studies on these derivatives describes a number of acyclic nucleoside analogues prepared by combining various[330-332] pentoses and hexoses with the common purines and pyrimidines, and some

151
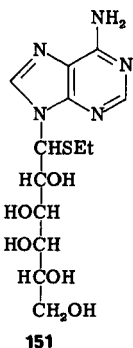
examples exhibit activity as antimetabolites.[332] Acyclic nucleoside derivatives of 2-amino-2-deoxy-D-glucose have also been prepared[250,333] by this route. Whereas attempts to cyclize these acyclic analogues to afford glycosyl-purines and -pyrimidines have failed, such derivatives of pentoses having a 5-membered 2,5-anhydro ring (termed[334] "homonucleosides"), which have been prepared[332,334] by the same methods from the corresponding 2,5-anhydropentose dithioacetals, may form spontaneously[328] upon treatment of acyclic pentose nucleoside analogues (for example, **152**; but not the D-*arabino* isomer—see Section II,2) with *p*-toluenesulfonyl chloride in pyridine.

152
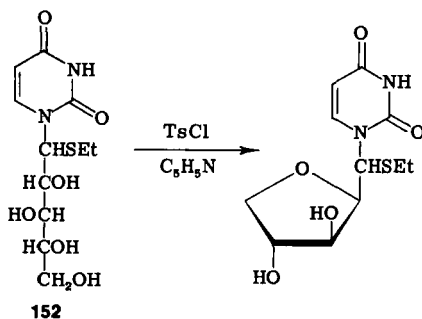
(330) M. L. Wolfrom, P. McWain, H. B. Bhat, and D. Horton, *Carbohydr. Res.*, **23**, 296–300 (1972); see also, earlier papers in this series.

(331) D. Horton and S. S. Kokrady, *Carbohydr. Res.*, **24**, 333–341 (1972).

(332) For a detailed discussion, see: D. Horton, D. C. Baker, and S. S. Kokrady, *Ann. N. Y. Acad. Sci.*, **255**, 131–150 (1975).

Although, before 1940, Wolfrom and coworkers[277] drew attention to the question of configurational assignment of acyclic molecules having asymmetric substitution at C-1, no specific assignments were made until many years later, when the acyclic nucleoside analogues were examined by n.m.r. spectroscopy, optical rotatory correlations, and X-ray crystallography. From nuclear magnetic resonance studies[31,32,135,145,152] of aldose dithioacetals (see Section VI,1) and related compounds, it is known that (a) the acyclic side-chain generally adopts an extended, planar, zigzag arrangement as its favored conformation, unless parallel interactions of substituents on next-but-one carbon positions (as in *ribo* or *xylo* configurations) destabilize it, and (b) tetrahedrally hybridized end-groups seem to favor the rotamer having that group in the extended position more than does a trigonally hybridized substituent having the corresponding arrangement; it might thus be expected[152,335] that the conformation depicted for **153**, in which the approximately tetrahedral sulfur atom adopts the extended position (and H-1 and H-2 are antiparallel), would be more stable (compared to available rotamers) than that conformation depicted for its epimer (**154**) and, therefore, that the mean dihedral angle, which determines the observed value of the spin-coupling interaction ($J_{1,2}$) between H-1 and H-2, would be larger for **153** [(1-R)] than for **154** [(1-S)]. Firm proof for a key example was furnished by X-ray crystallographic analysis[336] of (1R)-2,3,4,5-tetra-O-acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-S-ethyl-1-thio-D-arabinitol (**155**), a
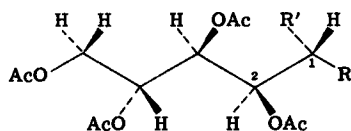
153 R = SEt, R' =

154 R' = SEt, R =
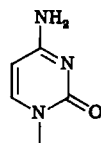
155 R = SEt, R' =
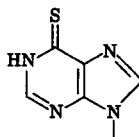
(333) M. L. Wolfrom and P. J. Conigliaro, *Carbohydr. Res.*, **20**, 369–374 (1971).
(334) J. Defaye and Z. Machon, *Carbohydr. Res.*, **24**, 235–245 (1972).

determination that also validated structure assignments as to the position of substitution on the heterocycle, its tautomeric form, the extended shape of the sugar chain, and the gauche disposition of the heterocyclic residue. A similar, crystallographic analysis was performed[337] with a pyrimidine example, namely (1R)-1-S-ethyl-1-(5-fluorouracil-1-yl)-1-thio-D-arabinitol, thus providing key reference points for chemical and spectral correlations of C-1 configuration throughout the series.

3. Hydrogenolysis of Carbon–Sulfur Bonds

The use of Raney nickel to effect reductive cleavage of C–S bonds was innovated by Bougalt and coworkers,[338] and developed by du Vigneaud and coworkers[339]; application of this reaction to carbohydrates was initiated by Hudson and coworkers,[340] who transformed 2,3,4,6-tetra-O-acetyl-1-thio-D-glucopyranose into 1,5-anhydro-D-glucitol; and, shortly thereafter, Wolfrom and Karabinos[341] prepared the pentaacetates of 1-deoxy-D-glucitol and 2-deoxy-D-arabino-hexitol by hydrogenolysis of diethyl dithioacetal pentaacetates (24 and 7) of D-glucose and D-fructose, respectively. The generality of this reaction as a synthetic route to deoxy sugars has been established by numerous extensions of the method in preparations of derivatives of 1-deoxyalditols[240,342–344] and nonterminal-deoxy sugars,[70,74] although occasionally, supposed aberrations have been reported. The numerous applications of desulfurization by Raney nickel in the chem-

(335) D. Horton, S. S. Kokrady, and J. D. Wander, unpublished results.

(336) D. C. Baker, A. Ducruix, D. Horton, and C. Pascard-Billy, *Chem. Commun.*, 729–730 (1974).

(337) A. Ducruix, D. Horton, R. A. Markovs, and C. Pascard-Billy, unpublished data.

(338) J. Bougault, E. Cattelain, and P. Chabrier, *Bull. Soc. Chim. Fr.*, [5] 5, 1699–1712 (1938); [5] 7, 780–781, 781–789 (1940).

(339) V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy, and S. A. Harris, *J. Biol. Chem.*, 146, 475–485 (1942); R. Mozingo, D. E. Wolf, S. A. Harris, and K. Folkers, *J. Amer. Chem. Soc.*, 65, 1013–1016 (1943).

(340) N. K. Richtmyer, C. J. Carr, and C. S. Hudson, *J. Amer. Chem. Soc.*, 65, 1477–1478 (1943).

(341) M. L. Wolfrom and J. V. Karabinos, *J. Amer. Chem. Soc.*, 66, 909–911 (1944).

(342) E. Zissis and N. K. Richtmyer, *J. Amer. Chem. Soc.*, 75, 129–131 (1953); 76, 5515–5522 (1954); A. E. El-Ashmawy and D. Horton, *Carbohydr. Res.*, 1, 164–170 (1965).

(343) E. Zissis and N. K. Richtmyer, *J. Amer. Chem. Soc.*, 74, 4373–4377 (1952).

(344) E. Zissis, N. K. Richtmyer, and C. S. Hudson, *J. Amer. Chem. Soc.*, 73, 4714–4716 (1951).

istry of carbohydrates have been reviewed by Fletcher and Richt-
myer[345] in this Series.

```
      H
   EtSCSEt                  Me              CH₂OAc           CH₂OAc
     |                       |                |                |
    HCOAc                  HCOAc           EtSCSEt            CH₂
     |                       |                |                |
   AcOCH    Raney Ni       AcOCH            AcOCH            AcOCH
     |   ───────────→        |                |    ──────────→   |
    HCOAc   aq. EtOH        HCOAc            HCOAc            HCOAc
     |                       |                |                |
    HCOAc                  HCOAc            HCOAc            HCOAc
     |                       |                |                |
   CH₂OAc                  CH₂OAc           CH₂OAc           CH₂OAc
     24                                       7
```
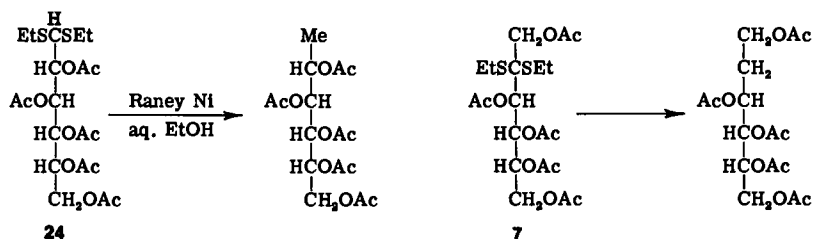
Raney nickel in aqueous ethanol normally acts to substitute
hydrogen atoms in place of all of the alkyl-(or aryl- or other)thio
groups present in a dithioacetal, without disturbing other substi-
tuents; thus, the 2-S-ethyl-2-thio-D-pentose diethyl dithioacetal[137]
156, 5-S-ethyl-5-thio-D-arabinose diethyl dithioacetal[145] (**157**), and
2,3,4,5-tetra-O-acetyl-6-S-acetyl-6-thio-D-galactose diethyl dithioace-
tal or its 6-thiocyanato analogue[346] (**158**) react with Raney nickel to

```
                                                H
                                             EtSCSEt
         H                  H                   |
      EtSCSEt            EtSCSEt               HCOAc
        |                  |                     |
       CHSEt             HOCH                  AcOCH
        |                  |                     |
      HOCH               HCOH                  AcOCH
        |                  |                     |
      HCOH               HCOH                  HCOAc
        |                  |                     |
     CH₂OH              CH₂SEt                 CH₂SCN
       156                157                    158
```
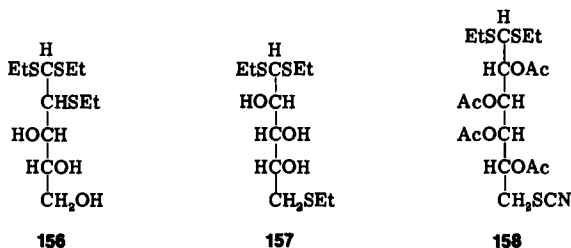
afford the respective dideoxyalditols. On being kept in the reaction
mixture, 1,5-dideoxy-L-arabinitol (prepared from the enantiomorph
of **157**) undergoes gradual epimerization into a mixture containing
ribo and *xylo* stereoisomers.[347] Other, general cautions pertain to the
catalyst, which is pyrophoric in the dry state and must accordingly be
handled and disposed of with care, and which tends to adsorb[240]
carbohydrate materials very tenaciously (especially in larger-scale
reactions), requiring thorough extraction to remove the product from
the surface of the catalyst.

Certain compounds, such as ethyl 2-S-ethyl-2-thio-α-D-man-

(345) H. G. Fletcher, Jr., and N. K. Richtmyer, *Advan. Carbohydr. Chem.*, 5, 1–28
    (1950).
(346) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc.
    Españ. Fis. Quím.*, B62, 1065–1068 (1966).
(347) J. Harness and N. A. Hughes, *J. Chem. Soc. Perkin I*, 38–41 (1972).

nofuranoside (30) triacetate, give complex mixtures upon attempted desulfurization by Raney nickel; in contrast, 1,3,5,6-tetra-O-acetyl-2-S-ethyl-2-thio-α,β-D-mannofuranoside undergoes desulfurization readily, to afford the 2-deoxy analogue.[134]

Occasionally, during hydrogenolysis, a labile substituent is displaced from the position adjacent to the dithioacetal grouping. This behavior was first observed by Wintersteiner and coworkers[46] in the reduction of 3-benzamido-2,4,5-tri-O-benzoyl-3,6-dideoxy-D-mannose diethyl dithioacetal (159) to afford a mixture of 3-benzamido-2,4,5-tri-O-benzoyl-1,3,6-trideoxy-D-mannitol (160) and 3-benzamido-4,5-di-O-benzoyl-1,2,3,6-tetradeoxy-D-*arabino*-hexitol (161); the latter product was interpreted as arising through intramolecular displacement of the 2-benzoyloxy group by an alkylthio group, to form a 1,2-episulfonium intermediate (162) that subsequently underwent

	159			160			161			162
	H									H
	EtSCSEt			Me			Me			EtSC⁺
	BzOCH	Raney Ni →	BzOCH		+	CH₂			HC〉SEt
	BzHNCH			BzHNCH			BzHNCH			HC
	HCOBz			HCOBz			HCOBz			BzHNCH
	HCOBz			HCOBz			HCOBz			HCOBz
	Me			Me			Me			HCOBz
										Me
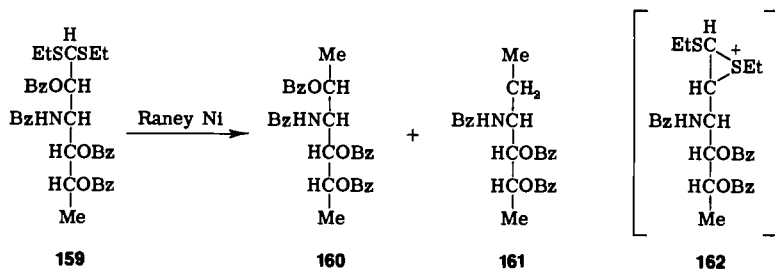
reduction to give 161. This rationalization is consistent with the later observation[88] that the extent of hydrogenolysis of the substituent on C-2 of 3,6-bis(acetamido)-3,6-dideoxy-D-altrose diethyl dithioacetal is significantly increased by per-O-acetylation of the substrate, as this increases the lability of the 2-hydroxyl group to nucleophilic displacement prior to the reduction; however, 3,4,5,6-tetra-O-acetyl-D-*arabino*-hexosulose 1-(diethyl dithioacetal) was desulfurized without disturbance to the keto group.[78]

Treatment of D-galactose diethyl dithioacetal (112) with an aged preparation of Raney nickel or with a limited amount of the fresh reductant affords[348] the corresponding 1-S-ethyl-1-thioalditol (163) as the major product; the same process occurs for D-*gluco*, L-*arabino*, D-*manno*, and 6-deoxy-L-*manno* analogues, and complete reduction of 163 to the deoxyalditol 164 was accomplished by further hydrogenolysis. The stepwise conversion of a methanolic solution of 112 into 163 (isolable in 60% yield), and thence into 164 (isolable in fair

(348) J. K. N. Jones and D. L. Mitchell, *Can. J. Chem.*, **36**, 206–211 (1958); B. Lindberg and L. Nordén, *Acta Chem. Scand.*, **15**, 958 (1961).

yield) was later accomplished[349] by ultraviolet photolysis, the sequential conversion of **163** into the sulfoxide **165**, and, thence, into galactitol (**166**) competing as a minor, second process. Photolysis of solutions of the peracetylated diethyl dithioacetals of D-arabinose, D-ribose, D-xylose, and D-glucose in alcoholic solvents under an atmosphere of nitrogen likewise gives high yields (80–87%) of the respective per-O-acetyl-1-S-ethyl-1-thioalditols.[350,350a]

H EtSCSEt	CH₂SEt	Me	O↑ CH₂SEt	CH₂OH
HCOH	HCOH	HCOH	HCOH	HCOH
HOCH	HOCH	HOCH	HOCH	HOCH
HOCH	HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH	HCOH
CH₂OH	CH₂OH	CH₂OH	CH₂OH	CH₂OH
112	**163**	**164**	**165**	**166**
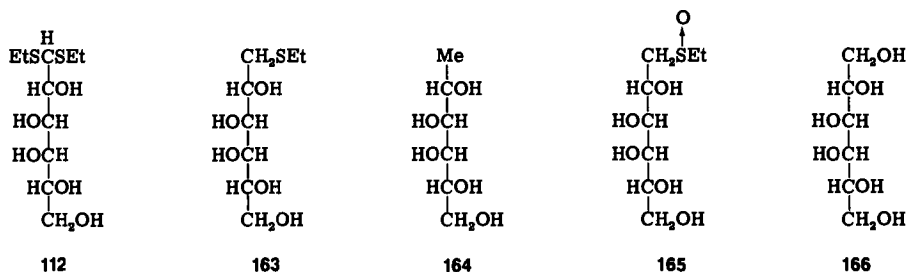
Deoxy sugar derivatives prepared by hydrogenolysis of dithioacetals have been singularly useful in the correlation or elucidation of configurational relationships. This reaction contributed to the identification of streptose,[121,351] and the identity of the sugar component of hygromycin was determined with the aid of this reduction; reduction of hygromycin with sodium borohydride, and subsequent mercaptolysis, gave 6-deoxy-L-galactose diethyl dithioacetal (**167**), whereas the sequence of reactions: mercaptolysis, hydrogenolysis, and a second mercaptolysis yielded a dideoxyhexose derivative (**169**) that liberated propanal upon glycol cleavage. It was educed[58] from this observation that a carbonyl group is present at C-5 of **168**, which must therefore have the D-*arabino* configuration, because it affords an L-*galacto* product upon reduction of the carbonyl group containing C-5. Hydrogenolysis of the diethyl dithioacetals (**170 and 94**, respectively) of 2-acetamido-2-deoxy-D-glucose and 2-O-methyl-D-glucose yielded 1-deoxyalditols that underwent glycol cleavage and

(349) D. Horton and J. S. Jewell, *J. Org. Chem.*, **31**, 509–513 (1966).

(350) D. Horton and J. S. Jewell, *Abstr. Pap. Amer. Chem. Soc. Meet.*, Winter, 1966, Phoenix, C-11.

(350a) K. Matsuura, Y. Araki, and Y. Ishido, *Bull. Chem. Soc. Jap.*, **46**, 2261–2262 (1973).

(351) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, and K. Folkers, *J. Amer. Chem. Soc.*, **68**, 2405–2406 (1946); I. R. Hooper, L. H. Klemm, W. J. Polglase, and M. L. Wolfrom, *ibid.*, **68**, 2120–2121 (1946); **69**, 1052–1056 (1947).

subsequent, mild oxidation to afford the *N*-acetyl derivative (**171**) of L-alanine[251] and *O*-methyl-L-lactic acid[352] (**172**), which were of the same (thus proved) configuration as derivatives of the respective natural products.

$$
\begin{array}{ccccccc}
\text{H} & & \left[\begin{array}{c}\text{H}\end{array}\right. & & \left[\begin{array}{c}\text{H}\end{array}\right. & & \text{H} \\
\text{EtSCSEt} & & \text{C=O} & & \text{C=O} & & \text{EtSCSEt} \\
\text{HOCH} & & \text{HOCH} & & \text{HOCH} & & \text{HOCH} \\
\text{HCOH} & \xleftarrow[\text{(2) EtSH,}]{\text{(1) NaBH}_4} & \text{HCOH} & \xrightarrow[\text{HCl}]{\text{EtSH}} & \text{HCOH} & \xrightarrow[\text{(2) EtSH,}]{\text{(1) Raney Ni}} & \text{HCOH} \\
\text{HCOH} & \text{HCl} & \text{HCOH} & & \text{HCOH} & \text{HCl} & \text{CH}_2 \\
\text{HOCH} & & \text{C=O} & & \text{EtSCSEt} & & \text{Me} \\
\text{Me} & & \left.\text{Me}\right] & & \left.\text{Me}\right] & & \\
\textbf{167} & & \textbf{168} & & & & \textbf{169}
\end{array}
$$

$$
\begin{array}{ccccc}
\text{H} & & \text{Me} & & \text{CO}_2\text{H} \\
\text{EtSCSEt} & & \text{HCR} & & \text{RCH} \\
\text{HCR} & & \text{HOCH} & \xrightarrow[\text{(2) Br}_2\text{—H}_2\text{O}]{\text{(1) IO}_4^- \text{ or Pb(OAc)}_4} & \text{Me} \\
\text{HOCH} & \xrightarrow{\text{Raney Ni}} & \text{HCOH} & & \\
\text{HCOH} & & \text{HCOH} & & \textbf{171} \quad \text{R = NHAc} \\
\text{HCOH} & & \text{CH}_2\text{OH} & & \textbf{172} \quad \text{R = OMe} \\
\text{CH}_2\text{OH} & & & & \\
& \textbf{170} \quad \text{R = NHAc} & & & \\
& \textbf{94} \quad \text{R = OMe} & & &
\end{array}
$$

The observation[353] that 1-deoxy-D-mannitol (prepared by hydrogenolysis[353] of D-mannose diethyl dithioacetal) is identical to 6-deoxy-D-mannitol (prepared[354] by reduction at C-6) is of fundamental interest in stereochemistry, because it offers chemical verification of the "principle of equivalent symmetry," that is, that the C-1 and C-6 positions, which are symmetrically interchangeable, are chemically indistinguishable. The identity of "L-α-fucohexitol" (derived from 6-deoxy-L-galactose by the Fischer–Kiliani procedure) with 1-deoxy-D-*glycero*-D-*galacto*-heptitol (prepared by hydrogenolysis of the corresponding dithioacetal) proved[355] that the former is 7-deoxy-L-*glycero*-D-*manno*-heptitol.

(352) M. L. Wolfrom, R. U. Lemieux, S. M. Olin, and D. I. Weisblat, *J. Amer. Chem. Soc.*, **71**, 4057–4059 (1949).

(353) N. K. Richtmyer and C. S. Hudson, *J. Amer. Chem. Soc.*, **72**, 3880–3882 (1950).

(354) W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Amer. Chem. Soc.*, **68**, 628–632 (1946).

(355) E. Zissis, N. K. Richtmyer, and C. S. Hudson, *J. Amer. Chem. Soc.*, **72**, 3882–3884 (1950).

V. Oxidation Reactions

1. Oxidation of Hydroxyl Groups in the Sugar Residue

4,5-O-Isopropylidene-D-fucose dibenzyl dithioacetal consumes[200] slightly less than one equivalent of periodic acid (in aqueous methanol) or of lead tetraacetate (in benzene), affording isolable amounts of glyoxal dibenzyl dithioacetal from a large-scale reaction. Somewhat later, it was reported[241] that 2,3-O-benzylidene-L-arabinose diethyl dithioacetal reacts with as many as four molar equivalents of lead tetraacetate in acetic acid; however, limited oxidation of the monoacetal afforded almost one equivalent of formaldehyde (isolated as the dimedon derivative), from which the location of the acetal group was deduced. Zinner and coworkers[356] obtained a trace amount of formaldehyde from the reaction of 2,4-O-benzylidene-D-xylose diethyl dithioacetal with lead tetraacetate, reasoning incorrectly that the 4- and 5-hydroxyl groups were unsubstituted; the correct structure was educed by Curtis and J. K. N. Jones[231] from nonoxidative transformations. Zinner and Wittenburg[284] subsequently recommended that isolated yields of formaldehyde–dimedon be observed to exceed 40% before glycol-cleavage oxidation results (for a dithioacetal) be interpreted as arising from a terminal, vicinal-diol grouping, and reliable analytical data have been obtained by accurate, semiquantitative determination[200,241,244,357,358] of products obtained from controlled, glycol-cleavage oxidations in favorable solvents.

One mole of D-*manno*-hexodialdose 1,6-bis(diethyl dithioacetal) rapidly consumes seven moles of sodium metaperiodate, to give four moles of formic acid, further oxidation occurring at a diminished rate[75]; D-galactose diethyl dithioacetal consumes in excess of seven equivalents of the same reagent.[359] Okui[360] described the consumption of ten molar equivalents of this oxidant by the diethyl dithioacetals of D-glucose and other hexoses, reporting that the analogous 2-deoxy and 2-(benzyloxycarbonylamino)-2-deoxy derivatives are oxidized to stable 3,3-bis(alkylsulfonyl)propanal derivatives;

(356) H. Zinner, G. Rembarz, H.-W. Linke, and G. Ulbricht, *Chem. Ber.*, **90**, 1761–1768 (1957).

(357) H. Zinner, G. Rembarz, and H. P. Klöcking, *Chem. Ber.*, **90**, 2688–2696 (1957).

(358) H. Zinner and J. Milbradt, *Carbohydr. Res.*, **2**, 470–479 (1966).

(359) A. N. O'Neill, *J. Amer. Chem. Soc.*, **77**, 6324–6326 (1955).

(360) S. Okui, *Yakugaku Zasshi*, **75**, 1262–1266 (1955); *Chem. Abstr.*, **50**, 8,463d (1955).

however, Hough and Taha[361] found that the three-carbon fragment reacts, not by S-oxidation, but by cleavage at the C-1–C-2 bond to generate a two-carbon unit that consumes the sixth (and final) equivalent of oxidant. The C-1–C-2 cleavage occurs readily for 2-acylamido-2-deoxy derivatives, but not for dibenzyl dithioacetals; Wolfrom and Usdin[362] isolated ethyl trithio-orthoglyoxylate [(EtS)$_3$CCHO] from the oxidation of D-galactose diethyl dithioacetal by a solution of lead tetraacetate in dry 1,4-dioxane.

S. B. Baker[238] found that the 4,5-O-isopropylidene derivative of D-arabinose dibenzyl dithioacetal undergoes stoichiometric oxidation by lead tetraacetate in benzene, whereas the 2,3-benzoate of this dithioacetal consumes a large excess of the same oxidant in acetic acid. He attributed the latter behavior to the proximity of the glycol unit to the dithioacetal group, but Stacey and coworkers[316] showed that the consumption of lead tetraacetate by solutions of D-glucose dibenzyl dithioacetal pentaacetate in acetic acid is accelerated by addition of water, and retarded by addition of benzene, to the reaction medium; Zinner and coworkers[363] subsequently demonstrated that ω-benzoates of aldose dithioacetals undergo rapid glycol-cleavage by lead tetraacetate, but that the rate of overoxidation increases as the polarity of the reaction medium is increased. Wolfrom and Winkley[364] cleaved 2-acetamido-2-deoxy-3,4-O-isopropylidene-D-glucose diethyl dithioacetal with lead tetraacetate in 5:1 benzene–chloroform, and Chipman and coworkers[365] later effected selective cleavage at the C-5–C-6 bond of 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose diethyl dithioacetal by the action of 1.3 equivalents of sodium periodate in water during 5 minutes at 0°, subsequent reduction with borohydride affording fair yields of derivatives of 2-amino-2-deoxy-D-xylose in both examples.

In some instances, it may be more practical to remove the dithioacetal group prior to the oxidation, either by hydrogenolysis, as in the conversion of 170 into 171 (see Section IV,3), or by demercaptalation.[180,234,284]

Following earlier reports[366] of unreactivity of *Acetobacter suboxydans* towards hexose diethyl dithioacetals, Bollenback and Un-

(361) L. Hough and M. I. Taha, *J. Chem. Soc.*, 3994–3997 (1957).
(362) M. L. Wolfrom and E. Usdin, *J. Amer. Chem. Soc.*, 75, 4619 (1953).
(363) H. Zinner, W. Bock, and H.-P. Klöcking, *Chem. Ber.*, 92, 1307–1313 (1959).
(364) M. L. Wolfrom and M. W. Winkley, *J. Org. Chem.*, 31, 1169–1173 (1966).
(365) P. van Eikeren, W. A. White, and D. M. Chipman, *J. Org. Chem.*, 38, 1831–1836 (1973).

derkofler[240] found that this organism displays "an oxidative specifi-
city of uncertain character" towards some aldose dithioacetals.
J. K. N. Jones and coworkers[367] subsequently developed an oxida-
tion procedure using *A. suboxydans*, whereby aldose dialkyl di-
thioacetals undergo conversion into aldos-(ω − 1)-ulose 1-(dialkyl
dithioacetals), in yields ranging from 45% [D-mannose diethyl
dithioacetal → D-*lyxo*-hexos-5-ulose 1-(diethyl dithioacetal)] to 80%
[D-arabinose dimethyl dithioacetal → D-*threo*-hexos-4-ulose 1-(di-
methyl dithioacetal)]; although a D-*erythro* arrangement of the
glycerol-1-yl terminus was held[367] to be a requirement for the
organism's action, D-galactose diethyl dithioacetal is enzymically
converted[368] into the glycos-5-ulose derivative in 60% yield during
54 days. Dimethyl sulfoxide–acid anhydride oxidizing mixtures[369]
do not affect the dithioacetal group, so that this oxidant may prove
useful in conversions of partially protected dithioacetal derivatives.

2. Oxidation of Sulfur Atoms; The MacDonald–Fischer Degradation

The first example of S-oxidation of a sugar dithioacetal was the ob-
servation by Emil Fischer[1] that four oxygen atoms are taken up by D-
glucose diethyl dithioacetal during treatment with potassium
permanganate. Gauthier and Vaniscotte[28] re-examined this oxidation
with a number of different dithioacetals of D-glucose, and noted that
isolation of the crystalline disulfones (**173**) thus formed could be a
very delicate process; use of only one equivalent of permanganate ef-
fects addition of a single oxygen atom to the dithioacetal group, to
form a mono-sulfoxide (**174**). The action of hydrogen peroxide[28,370] in
acetic acid converts dithioacetals into the corresponding disulfoxides
(**175**), or, in the presence of ammonium molybdate, into disulfones.[370]
(Note, however, that an unidentified product so prepared from pen-
tose dialkyl dithioacetals has been reported[371] to detonate spontane-
ously "*mit großer Heftigkeit*.") Kuhn and coworkers[321] pointed out
that such partial S-oxidation creates new asymmetric centers, both at
C-1 and at singly oxygenated sulfur atoms, designated by asterisks in

(366) R. M. Hann, E. B. Tilden, and C. S. Hudson, *J. Amer. Chem. Soc.*, 60, 1201–1203
     (1938); B. Iselin, *J. Biol. Chem.*, 175, 997–998 (1948).
(367) D. T. Williams, J. K. N. Jones, N. J. Dennis, R. J. Ferrier, and W. G. Overend,
     Can. J. Chem., 43, 955–959 (1965).
(368) D. T. Williams and J. K. N. Jones, *Can. J. Chem.*, 45, 741–744 (1967).
(369) K. Onodera, S. Hirano, and N. Kashimura, *Carbohydr. Res.*, 6, 276–285 (1968).
(370) H. Zinner and K.-H. Falk, *Chem. Ber.*, 88, 566–572 (1955).
(371) H. Zinner and K.-H. Falk, *Chem. Ber.*, 89, 2451–2454 (1956).
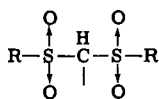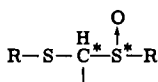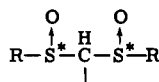
$$173 \qquad 174 \qquad 175$$

174 and **175**, and they prepared three, isomeric disulfoxides from D-galactose dibenzyl dithioacetal. In contrast with the parent dithioacetals, the monosulfoxides are weakly basic, and the latter undergo rapid disproportionation in the presence of dilute mineral acid to liberate the aldose plus a disulfide; the disulfoxides derived from D-galactose are acid-stable, but undergo a base-catalyzed, retroaldol type of reaction to afford bis(benzylsulfinyl)methane and D-lyxose.[321]

MacDonald and H. O. L. Fischer[372] oxidized D-glucose diethyl dithioacetal pentaacetate (**24**) and its D-*manno* isomer with monoperoxyphthalic acid in ether, isolating not the epimeric disulfones but a common product, to which they assigned the structure 3,4,5,6-tetra-O-acetyl-1,2-dideoxy-1,1-bis(ethylsulfonyl)-D-*arabino*-hex-1-enitol (**176**). Ammonolysis of **176** and reacetylation afforded **177**, which was also prepared by oxidation of 2-acetamido-3,4,5,6-tetra-O-acetyl-2-deoxy-D-glucose diethyl dithioacetal, whereas the action of hydrazine in methanol on **176** produced a retroaldol reaction (and saponification) affording D-arabinose (**179**, 40% yield) and bis(ethylsulfonyl)methane (**178**). The latter reaction accords with earlier ob-

servations by Rothstein[373] in the aliphatic series, and it compares very favorably with the traditional methods[374] for effecting chain-descent, proceeding in three, mild, generally high-yielding steps; subsequent, minor modifications have enhanced its utility. MacDonald and H. O. L. Fischer[375] employed peroxypropionic acid in 1,4-dioxane to oxidize unacetylated D-mannose diethyl dithioacetal, treating the crude product with dilute, aqueous ammonia to prepare **179** in 83% overall yield; Zinner and Falk[371] espoused the use of acetone as the solvent for the oxidation step. Peroxyacetic acid may also be used, but an unidentified product, so prepared from a ketenic dithioacetal (**120**), detonated[20] spontaneously, after crystallizing, during concentration of a solution under vacuum.
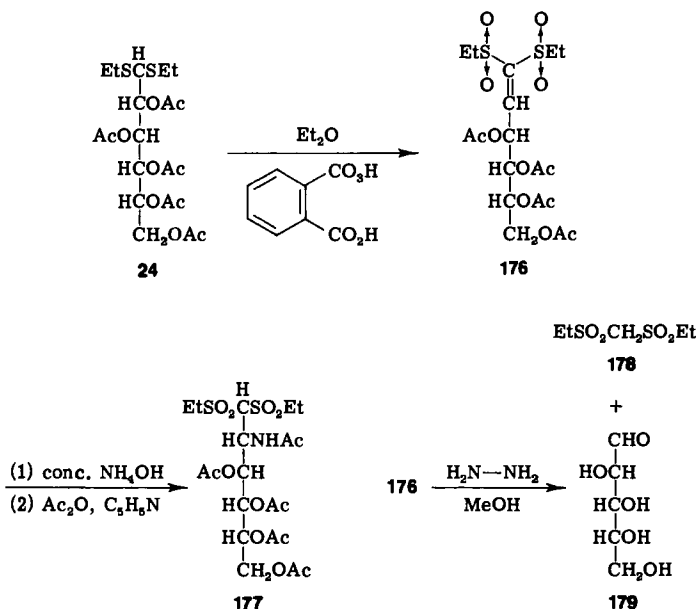
This reaction was quickly extended to the degradation of pentoses,[376–378] ketoses,[74,379] and inososes.[74] Oxidation of D-fructose diethyl dithioacetal (**8**) with peroxypropionic acid affords the disulfone **180**, which is unable to suffer dehydration in the same way as the preceding example, and which decomposes[74,379] in dilute, aqueous solutions of ammonia to give, initially, formaldehyde, **178**, and the tetrose D-erythrose (**181**). Formaldehyde and **178** also combine[379] to afford 1,1,3,3-tetrakis(ethylsulfonyl)propane. Similar treatment of *myo*-inosose-2 diethyl dithioacetal (**182**) proceeds by way of the cyclic sulfone **183**, which suffers degradation at both carbon-carbon bonds to the sulfonylated carbon atom, to yield[74] *xylo*-pentodialdose (**184**).
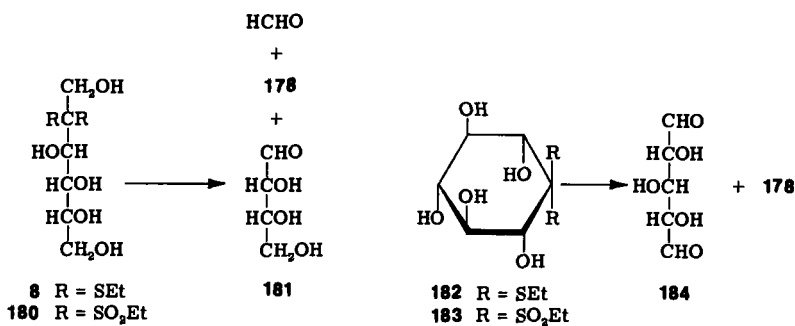
(372) D. L. MacDonald and H. O. L. Fischer, *J. Amer. Chem. Soc.*, 74, 2087–2090 (1952).

(373) E. Rothstein, *J. Chem. Soc.*, 1560–1565 (1940).

(374) See: L. Hough and A. C. Richardson, in "The Carbohydrates: Chemistry and Biochemistry," W. Pigman and D. Horton, eds., Academic Press, New York, 1972, Vol. IA, pp. 113–163.

Oxidation of 2-amino-2-deoxy-D-glucose diethyl dithioacetal hydrochloride by peroxypropionic acid was accompanied by loss of ammonia, and the product was the same as that prepared from the D-glucose dithioacetal; however, the 2-acetamido-2-deoxy analogue was similarly oxidized in methanol at −10° to afford a 2-amino disulfone (as the propionate salt) that was acetylated to give 177, whereas the analogous oxidation in water at room temperature gave a high yield of ammonium ethyl sulfite, presumably by S → O migration of the ethyl group.[380] MacDonald–Fischer degradation of 6-deoxy-L-galactose[381] affords 5-deoxy-L-lyxose in ~60% yield; degradation of the diaminohexose paromose gave 5-acetamido-5-deoxy-L-xylose, from which datum, plus optical rotation values, the parent sugar was identified[94] as 2,6-diamino-2,6-dideoxy-L-idose. 2-Acetamido-2-deoxy-D-ribose and 4-acetamido-4-deoxy-L-erythrose were prepared from the diethyl dithioacetals of 3-acetamido-3-deoxy-D-allose[382,383] (or -D-altrose[109,381]) and 5-acetamido-5-deoxy-L-arabinose,[384] respectively, by oxidation with peroxypropionic acid; a portion of the 3-acetamido sulfones eliminated 178 spontaneously (presumably by autocatalysis) to give the desired pentose, whereas the 4-acetamido tetrose was isolated after mild ammonolysis of a crystalline intermediate.

In initially characterizing 176, MacDonald and H. O. L. Fischer[372] observed that it does not react with tetranitromethane or bromine–acetic acid, but that it forms red solutions in pyridine and that it slowly consumes osmium tetraoxide; the disulfone prepared from 6-deoxy-L-galactose[381] underwent hydrogenation in the presence of Raney nickel. After reporting that disulfones prepared from pentoses[377] completely dehydrate, and those from 6-deoxy-L-mannose[385] dehydrate to some extent, both affording 1-alkenes analogous to 176,

(375) D. L. MacDonald and H. O. L. Fischer, *Biochim. Biophys. Acta*, 12, 203–206 (1953).
(376) L. Hough and T. J. Taylor, *Chem. Ind.* (London), 575–576 (1954).
(377) L. Hough and T. J. Taylor, *J. Chem. Soc.*, 1212–1218 (1955).
(378) C. E. Ballou, H. O. L. Fischer, and D. L. MacDonald, *J. Amer. Chem. Soc.*, 77, 5967–5970 (1955).
(379) E. J. Bourne and R. Stephens, *J. Chem. Soc.*, 4009–4013 (1954).
(380) L. Hough and M. I. Taha, *J. Chem. Soc.*, 3564–3572 (1957).
(381) R. Kuhn, W. Bister, and W. Dafeldecker, *Ann.*, 628, 186–192 (1959).
(382) B. Coxon and L. Hough, *Chem. Ind.* (London), 1249–1250 (1959).
(383) B. Coxon and L. Hough, *J. Chem. Soc.*, 1643–1649 (1961).
(384) S. Hanessian and T. H. Haskell, *Heterocycl. Chem.*, 1, 57 (1964).
(385) L. Hough and T. J. Taylor, *J. Chem. Soc.*, 3544–3548 (1955).

Hough and Taylor[386] found that the disulfone prepared from D-glucose (or 2-amino-2-deoxy-D-glucose[380] or D-mannose[385]) diethyl dithioacetal undergoes spontaneous dehydration to yield a solid that forms a pale-yellow solution in pyridine, and that consumes only two equivalents of periodate, affording one equivalent of formic acid; they assigned the structure 2,6-anhydro-1-deoxy-1,1-bis(ethylsulfonyl)-D-mannitol (185) to this solid, which they presumed was formed by nucleophilic attack of O-6 on the double bond of the unsaturated intermediate 186. The stereochemistry at C-2 was assigned on
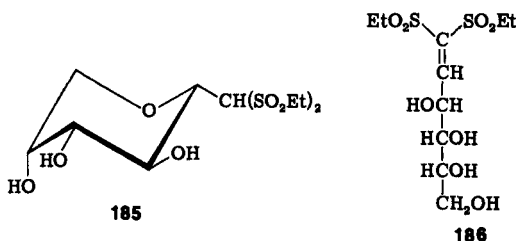
the basis of optical rotation values and from predictions of conformational stability. Recrystallization of 1-deoxy-1,1-bis(ethylsulfonyl)-D-galactitol (prepared by oxidation[370] with hydrogen peroxide) affords the 4-epimer of 185, which has also been isolated directly from the analogous oxidation with peroxypropionic acid,[386,387] and 187 was characterized as one of the products from oxidation[109,382,383] of the diethyl dithioacetals of 3-acetamido-3-deoxy-D-allose and -D-altrose. Farrington and Hough[388] later reported that the acyclic alkene produced by oxidation of D-arabinose (as well as other pentose) diethyl dithioacetal exists only transiently, and that it undergoes cyclization to afford the 2,5-anhydro derivative 188. The corresponding heptose-derived disulfones cyclize to 2,6-anhydrides[389] whose structures were deduced from n.m.r. data.
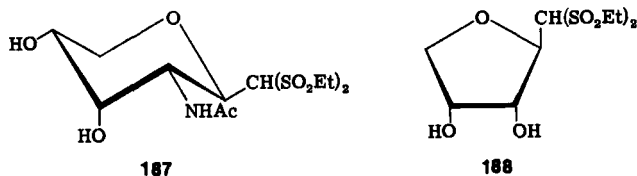
(386) L. Hough and T. J. Taylor, *Chem. Ind.* (London), 1018–1019 (1954); *J. Chem. Soc.*, 970–980 (1956).
(387) R. Barker and D. L. MacDonald, *J. Amer. Chem. Soc.*, 82, 2297–2301 (1960).
(388) A. Farrington and L. Hough, *Carbohydr. Res.*, 16, 59–64 (1971).
(389) L. D. Hall, L. Hough, S. H. Shute, and T. J. Taylor, *J. Chem. Soc.*, 1154–1160 (1965).

Hough and Richardson[390] proposed that the loss of **178** from the 2,6-anhydro disulfone **189** occurs by dissociation of the C-1–C-2 bond to give the oxonium ion **190**, which would be expected to adopt a conformation approximating the half-chair form illustrated, plus the bis(alkylsulfonyl)methyl anion, which subsequently react with solvent to yield D-lyxose and **178**, respectively. In accord[391] with this rationalization, (*a*) the C-methyl homologue (**191**) of **189** is not de-

189 R = EtSO₂CHSO₂Et (with H above)

191 R = EtSO₂CSO₂Et (with Me above)
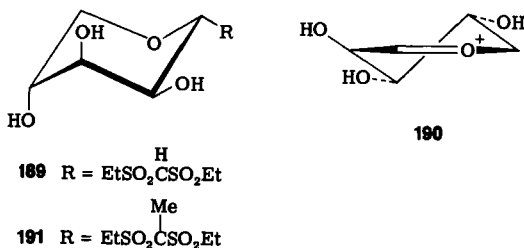
graded by ammonolysis, presumably due to the inductive effect of the methyl group [which would destabilize the 1,1-bis(alkylsulfonyl)ethyl anion], and (*b*) the 3,4-isopropylidene acetal of **189** is stable to ammonolysis, whereas the 4,5-isopropylidene acetal of **185** undergoes degradation to 3,4-*O*-isopropylidene-D-arabinose; the latter observation follows from the prediction that the oxonium ion (**192**) that would be formed, were heterolysis to occur from the former acetal, would be severely distorted away from the necessary coplanarity of C-2–C-1–O-5–C-5 by flattening of C-1–C-2–C-3–C-4 already present due to the acetal group, whereas the corresponding ion (**193**) from the latter acetal, having no two consecutive atoms common to both flattened regions, can fulfil its geometric requirements by adopting the "boat" conformation shown. Cleavage of the 3-methyl ether of **185** to afford 2-*O*-methyl-D-arabinose proves that the vicinal hydroxyl group does not participate in the dissociation.[103] The kinetics of the degradation reaction are not simple, being inter-

192   **193**   **194**
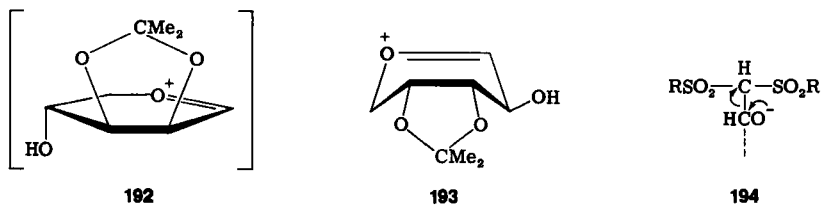
(390) L. Hough and A. C. Richardson, *Proc. Chem. Soc.*, 193–194 (1959).
(391) L. Hough and A. C. Richardson, *J. Chem. Soc.*, 1024–1032 (1962).

mediate between first- and second-order, and the reaction is acceler-
ated by an increase in the polarity of the solvent.[392] The faster[383,387]
heterolysis reaction of the intact disulfones (173) was rationalized[383]
as occurring through deprotonation of the 2-hydroxyl group, followed
by attack of the newly released electron-pair at C-2 of the anion 194
to displace C-1 (as an anion) and generate a carbonyl group.

VI. SPECTROSCOPIC PROPERTIES OF DITHIOACETALS

1. Nuclear Magnetic Resonance Spectroscopy

The nuclear magnetic resonance (n.m.r.) spectra of aldose dithio-
acetals dissolved in pyridine-d_5 or dimethyl sulfoxide-d_6 generally
exhibit extensively overlapping multiplets under which most of the
sugar-proton resonances lie. Peracetylation of the dithioacetal, how-
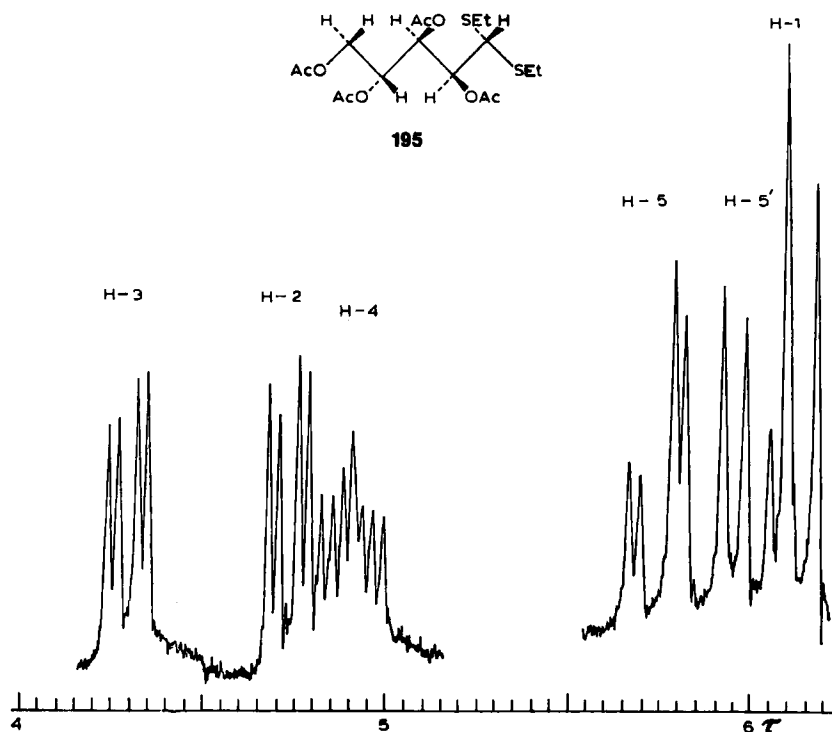
FIG. 5.—The Low-field Portion of the N.m.r. Spectrum of Tetra-O-acetyl-D-
arabinose Diethyl Dithioacetal (195) in Chloroform-d at 100 MHz.

(392) L. Hough and A. C. Richardson, *J. Chem. Soc.*, 1019–1023 (1962).
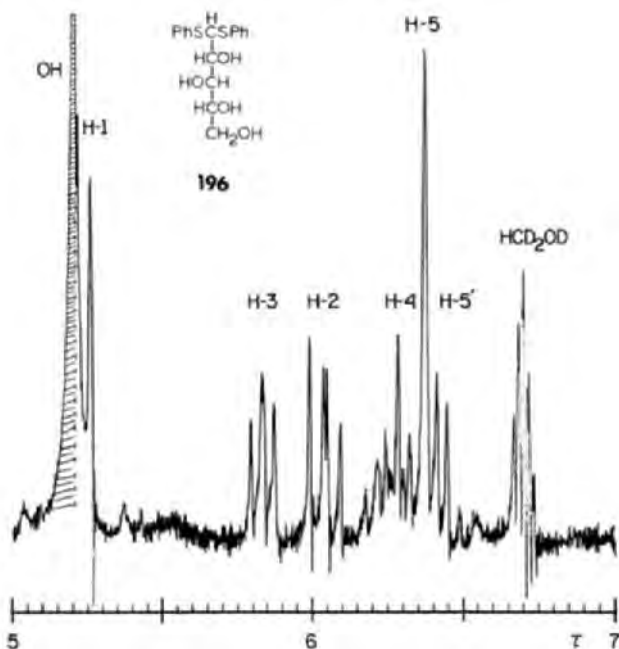
FIG. 6—Proton N.m.r. Spectrum at 100 MHz of D-Xylose Diphenyl Dithioacetal (196) in Methanol-d_4.

ever, leads to differential changes in shielding of the various protons in the molecule, so that well separated n.m.r. signals are generally observed[32,33,150] for all of the sugar protons, even at 60 MHz. The 100-MHz n.m.r. spectrum of the carbohydrate protons of a solution of D-arabinose diethyl dithioacetal tetraacetate (195) in chloroform-d is depicted in Fig. 5. Coupling constants and chemical shifts have been extracted on a first-order basis from the n.m.r. spectra of a representative group of peracetylated pentose and 6-deoxyhexose diethyl[150] and diphenyl[32,33] dithioacetals; both the ethylthio groups and the methylene protons of the ethylthio groups of the diethyl dithioacetals are diastereotopic, and their signals exhibit slight magnetic nonequivalence.[150,393]

It was subsequently found that solutions of some of the unprotected pentose diethyl and diphenyl dithioacetals in methanol-d_4 give essentially completely separated signals (see Fig. 6), whereas the n.m.r. spectral dispersion of others can be augmented by incremental additions of a paramagnetic lanthanide cation (see Fig. 7)

(393) R. J. Ferrier and L. R. Hatton, Carbohydr. Res., 5, 132–139 (1967).
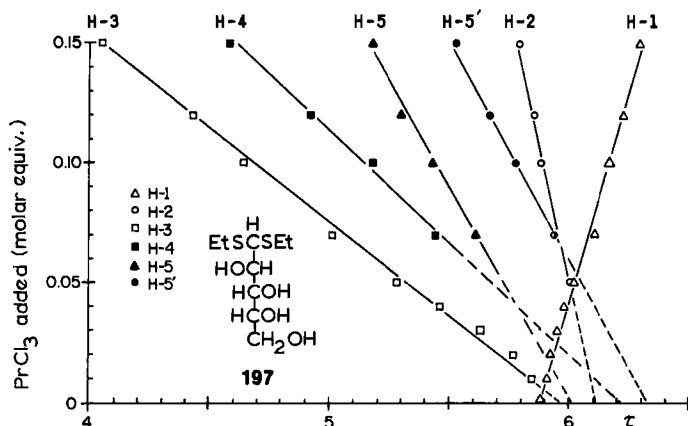
FIG. 7—Plot of the Chemical Shifts of the Proton Resonances of D-Arabinose Diethyl Dithioacetal (197) vs. the Relative Concentration of PrCl$_3$ · 6D$_2$O added. (Values for the unperturbed chemical-shifts were estimated by extrapolation back to zero concentration of Pr^{3+}.)

or pyridine-d_5, and a partial set of coupling values has been tabulated for the series of compounds.[394] Use of the paramagnetic, Lewis-acid complex tris[6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato]europium(III) in deducing the configuration of branched-chain trimethylene dithioacetals is discussed in Section II,5. Copper(II) ions have been reported[395] to cause selective broadening in the resonances of protons adjacent to sulfur atoms, by paramagnetic enhancement of relaxation.

Spin-coupling data so obtained have been used for determining the most probable conformations of the dithioacetals and other acyclic sugar derivatives, on the basis of the well founded[396] assumption[397] that vicinal pairs of protons in an antiparallel arrangement have spin-coupling interactions of ~9 Hz, and *gauche*-related (60° dihedral angle) pairs are characterized by coupling values of ~2 Hz. Recognizing that (*a*) the interproton, dihedral angle is not uniquely

(394) D. Horton and J. D. Wander, *J. Org. Chem.*, 39, 1859–1863 (1974).
(395) E. V. E. Roberts, J. C. P. Schwarz, and C. A. McNab, *Carbohydr. Res.*, 7, 311–319 (1968).
(396) L. D. Hall, *Advan. Carbohydr. Chem.*, 19, 51–93 (1964); P. L. Durette and D. Horton, *Advan. Carbohydr. Chem. Biochem.*, 26, 49–125 (1971); P. L. Durette, D. Horton, and J. D. Wander, *Advan. Chem. Ser.*, 117, 147–176 (1973); see also, Ref. 152.
(397) M. Karplus, *J. Chem. Phys.*, 30, 11–15 (1959); *J. Amer. Chem. Soc.*, 85, 2870–2871 (1963).

determined by a measured coupling-constant, and (b) the acyclic, carbohydrate side-chain is in constant, rapid motion and not constrained to exist exclusively in staggered, rotameric forms, the present authors[32,33,150,394] deduced that the extended, planar, zigzag form (illustrated for 195) is the favored (but not necessarily exclusive) conformation for essentially[398] every example that does not have a parallel, eclipsed, 1,3-interaction between large, polar substituents in this conformation; for examples not meeting this criterion (for instance, 197, or other members of the *ribo* or *xylo* series), an equilibrium between sickle conformations, achieved by rotation through 120° about one (or more) of the C–C bonds internal to the chain in order to relieve the destabilizing, eclipsed interaction(s), was shown[32,33,150] to be more consistent with coupling data observed for members of the *ribo* and *xylo* series. Analysis of this type has been extended to other, acyclic derivatives (see Ref. 394 for a complete list of examples), although, in some cases, the limitations on quantitative interpretation appear to have been exceeded.[399]

In general, no qualitative differences are observed in the n.m.r. spectra corresponding to the sugar protons of diethyl and diphenyl dithioacetals, except for increased deshielding of the H-1 resonance [and replacement of alkyl(thio) by aryl(thio) protons], so that similar conformations are indicated. An exception to this generalization is provided by the dithioacetals of D-ribose, of which the diphenyl compounds exhibit coupling constants indicative of rotation about the C-3–C-4 bond into one favored sickle form,[32,394] whereas the coupling data recorded for the diethyl analogues[150,394] indicate rotation about the C-2–C-3 bond into the other twisted conformer, and it was suggested[152,394] that this phenomenon derives from instability of rotational states about the C-2–C-3 and C-3–C-4 bonds, rather than from a decided energetic favoring of one sickle form. The apparent equilibrium between sickle conformations in examples not favoring the planar, zigzag arrangement may be rationalized in this context as a time-averaging of less-disfavored rotameric states, and it was later shown[400] that this phenomenon may be strongly temperature-dependent. Thus, the 250-MHz n.m.r. spectrum of tetra-*O*-acetyl-D-ribose di-isobutyl dithioacetal in acetone-d_6 at 25° shows couplings of $J_{1,2}$ 6.0, $J_{2,3}$ 6.5, $J_{3,4}$ 4.0, and $J_{4,5\,anti}$ 7.5 Hz, indicating a mixture of two

(398) H. (S.) El Khadem, D. Horton, and J. D. Wander, *J. Org. Chem.*, 37, 1630–1635 (1972).

(399) J. B. Lee and B. F. Scanlon, *Tetrahedron*, 25, 3413–3428 (1969).

(400) J. Defaye, D. Horton, and M. Muesser, unpublished data.

sickle conformations derived by rotation about C-2–C-3 or about C-3–C-4 to conformers having no parallel 1,3-interactions. At low temperature ($-100°$ in acetone-d_6 or $-125°$ in 2-chloropropene-d_5), the second of these rotamers appears to be essentially the exclusive form, as indicated by changes[400] in the observed couplings to $J_{1,2}$ 3.5, $J_{2,3}$ 8.5, $J_{3,4}$ 2.5, and $J_{4,5\ anti}$ 9.0 Hz. Aldose dithioacetals adopting sickle conformations in solution, such as D-ribose diethyl and diphenyl dithioacetals, also exist in sickle conformations in the crystalline state, although not necessarily in the one most favored in solution.[401] A parallel examination of 2-S-ethyl-2-thio-D-mannose diethyl dithioacetal (33) by n.m.r. spectroscopy[135] and by X-ray crystallography[135,136] revealed that the favored conformation in both the solid state and in solution is the planar, zigzag form illustrated (33a), even though S-2 lies between the two, bulky sulfur atoms on C-1 in this conformation; the nonextended rotational state about the O-5–O-6 bond is a fairly common occurrence in these conformations in solution, presumably because no major factors operate at the hydroxymethyl group to favor either the twisted or the extended rotamer.
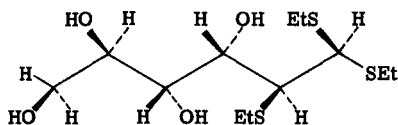
33a

Information contained in the 100-MHz n.m.r. spectrum of 2-deoxy-4,5-O-isopropylidene-3-O-(p-nitrobenzoyl)-D-erythro-pent-1-enose diphenyl dithioacetal (121) allows deduction[32] of the pattern of substitution, because the vinylic resonance (a doublet at δ 5.97) is coupled to a lower-field signal (H-3, δ 6.25), which, by virtue of coupling to H-2, must be H-3, and, by virtue of being powerfully deshielded, must bear the p-nitrobenzoyl group; the remaining oxygen atoms (O-4 and O-5) are, therefore, the heteroatoms of the dioxolane ring.

The conformational flexibility associated with acyclic molecules constitutes a formidable impediment to the determination, by n.m.r. spectroscopy, of configurational relationships that may often be readily apparent in cyclic derivatives of the same sugar.

2. Mass Spectrometry

Whereas the extreme conformational flexibility of acyclic dithioacetals limits the amount of structural information available from

n.m.r. spectroscopy (relative to a cyclic analogue), this condition may have the opposite effect on the mass spectrum of these derivatives, which tend to be simple and instructive. The destructive nature of this technique is offset by the minimal (< 1 μg) sample requirement. Reviews of this subject are included in several treatises[402] on applied mass spectrometry.

Detailed assignment has been made of the patterns of fragmentation subsequent to electron-impact ionization of fully acetylated aldose and ketose diethyl dithioacetals.[403] A similar study of the corresponding, peracetylated aldose ethylene dithioacetals[404] revealed that the extreme stability of the 2-(1,3-dithiolanium) cation suppresses other fragmentation pathways, thereby severely limiting the amount of information available about the parent sugar. The decomposition of both of these classes of derivative suffers complication by processes (loss of ketene, acetic acid, or acetic anhydride) that are characteristic of acetates in general, and is therefore of little value in structure elucidation.

Frequently, unprotected dithioacetals are sufficiently volatile for analysis by direct introduction into the source, and DeJongh[405] established that the mass spectra of free dithioacetals are relatively uncomplicated; fragmentation of the unprotected dithioacetal produces a few, characteristic fragments, which makes it an exceptionally useful derivative for characterization by mass spectrometry. The most informative ions in this decomposition are as follows.

(a) *The Molecular Ion* ($M^{\ddot{+}}$). The species resulting from ionization of a sulfur atom is stabilized relative to oxygenated analogues, and the dialkyl dithioacetals exhibit recognizably large molecular ions[405] which define the molecular weight; the same is generally true of diphenyl,[32] but not ethylene,[404] dithioacetals.

(b) *The Bis(alkylthio)methyl Cation* (198). Scission of the C-1–C-2 bond of $M^{\ddot{+}}$ affords 198, which is generally the base peak in the mass spectrum, and serves to identify the alkyl groups of the parent thiol.

(401) A. Ducruix, D. Horton, C. Pascard-Billy, and J. D. Wander, unpublished data.

(402) N. K. Kochetkov and O. S. Chizhov, *Advan. Carbohydr. Chem.*, 21, 39–93 (1966); H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of National Products by Mass Spectrometry," Holden–Day, San Francisco, 1964, pp. 203–240; S. Hanessian, *Methods Biochem. Anal.*, 19, 105–228 (1971); D. C. DeJongh, in "The Carbohydrates: Chemistry and Biochemistry," W. Pigman and D. Horton, eds., Academic Press, New York, 2nd Edition, 1976, Vol. IB, in press.

(403) D. C. DeJongh, *J. Amer. Chem. Soc.*, 86, 3149–3154 (1964).

(404) D. C. DeJongh, *J. Amer. Chem. Soc.*, 86, 4027–4030 (1964).

(405) D. C. DeJongh, *J. Org. Chem.*, 30, 1563–1570 (1965).

$$
\begin{array}{c}
\overset{H+\cdot}{EtS\underset{|}{C}SEt} \\
\underset{|}{C}HOH \\
(\underset{|}{C}HOH)_n \\
\underset{|}{C}H_2OH \\
M^{+\cdot}
\end{array}
\longrightarrow
\underset{\textbf{198}}{EtS\overset{H}{\underset{}{C}}=\overset{+}{S}Et}
$$
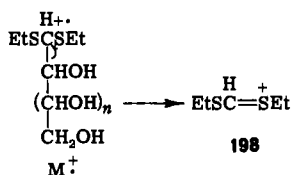
The thiol of choice appears to be ethanethiol; for aldose diethyl dithioacetals, this fragment has m/e 135. Failure of this ion to exhibit major intensity is diagnostic of either a 2-deoxyaldose or a ketose.[405,406] Hughes and Robson[147] identified 5-S-methyl-5-thio-L-arabinose ethyl methyl dithioacetal from the occurrence of this fragment [$^+$CH(SMe,SEt)] at m/e 121, and Berrang and Horton[130] identified 2-S-ethyl-2-thio-D-mannose ethyl phenyl dithioacetal by the occurrence of the corresponding fragment [$^+$CH(SEt,SPh)] at m/e 183.

(c) *The Bis(alkylthio)allyl Cation* (199). Scission of the C-3–C-4 bond of M^+ and synchronous or subsequent elimination of H-1 plus the 2-substituent produce an extensively delocalized, allylic cation

$$
\begin{array}{c}
\overset{H+\cdot}{EtS\underset{|}{C}SEt} \\
\underset{|}{C}HOH \\
(\underset{|}{C}HR \\
(\underset{|}{C}HOH)_n \\
\underset{|}{C}H_2OH
\end{array}
\xrightarrow{-H_2O}
\underset{\textbf{199}}{
\begin{array}{c}
EtS\overset{+}{\underset{}{C}}SEt \\
\underset{||}{C}H \\
\underset{}{C}HR
\end{array}}
$$
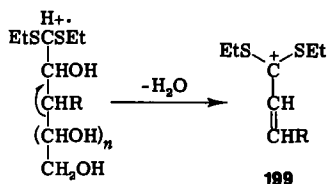
(199), which DeJongh[405] termed "Fragment A." The mass number and intensity of this fragment are instructive about C-2, C-3, and C-4 substituents. Fragment 199 appears at m/e 177 in the mass spectrum of aldose diethyl dithioacetals having a hydroxyl group at C-3, at m/e 161 in 3-deoxy sugars, and at m/e 191 in 3-O-methyl derivatives; 199 is not prominent in the mass spectra of 2- and 4-deoxy sugar dithioacetals, because the former has no substituent to eliminate, whereas C-3–C-4 scission in the latter produces a (disfavored) primary free-radical site at C-4.

(d) "*Fragment B*" (200). This species, formed by the loss of both alkylthio groups and one hydrogen atom from M^+, contains all of the

200

(406) D. C. DeJongh and S. Hanessian, *J. Amer. Chem. Soc.*, **88**, 3114–3119 (1966).
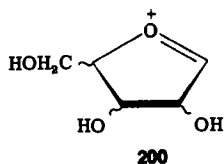
original substituents of the sugar before its mercaptalation; **200** echoes the molecular ion in indicating the identity, but not the location, of substituents.

(*e*) *The (Alkylthio)ethyl Cation* (**201**). Scission of the C-2–C-3 bond and one C–S bond of $M^{\ddagger}$, and transfer of one proton to the C-1–C-2 portion produces **201** ("Fragment C"). The abundance of **201** is

$$\begin{bmatrix} \begin{matrix} CHSEt \\ \| \\ CHR \end{matrix} + H \end{bmatrix}^{+}$$
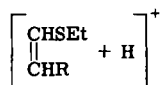
201

minimal for 3-deoxy sugars, whereas it occurs at m/e values decreased by 16, or increased by 14, daltons for 2-deoxy and 2-*O*-methyl derivatives, respectively.

The dependence of the m/e values and intensities of these fragments upon the position of deoxygenation is summarized in Table I, which illustrates the patterned uniqueness of these fragments.

The presence and location of acetamido groups is also readily deducible from the fragmentation of acetamidodeoxy sugar dialkyl dithioacetals.[407] The appearance of a prominent molecular ion appears to be indicative of a 2-acetamido group, as is an abundant peak corresponding to loss of $AcNH_2$; **199** appears at m/e 218 $(177 + 59 - 18)$ in the presence of a 3-acetamido group. Failing the appearance of the molecular ion, the molecular weight can be deter-

TABLE I

Major Fragments Observed in the Mass-spectral Decomposition of
Deoxyaldose Diethyl Dithioacetals[405]

	Position of deoxygenation								
	Hexose								Pentose
Ion	None	2	3	5	6	2,3	2,6	3,6	4,6	5
$M^{\dagger}$	286	270	270	270	270	254	254	254	254	240
$^{+}CH(SEt)_2$	135	*a*	135	135	135	*a*	*a*	135	135	135
199	177	*a*	161	177	177	*a*	*a*	161		177
200	163	147	147	147	147	131	131	131	131	117
201	105	89	*a*	105	105	*a*	89		105	105

a Observed at only minor intensity.

(407) D. C. DeJongh and S. Hanessian, *J. Amer. Chem. Soc.*, **87**, 3744–3751 (1965).

mined from $M^{\ddagger} - H_2O$ and $M^{\ddagger} - 2H_2O$ ions, for which prominent metastable ions provide verification.

Location of substituents is often facilitated by the occurrence of a series of ions arising by scission, in turn, of each of the carbon–carbon bonds that do not involve a methylene group along the chain; the differences in mass number of these fragments identify the substituents of each atom in turn. Thus, 4,5-bis(acetamido)-4,5-dideoxy-L-xylose diethyl dithioacetal (202) exhibits a molecular ion at m/e

202
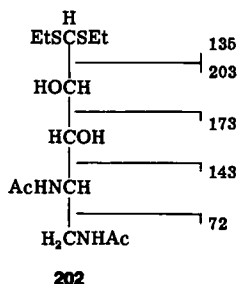
338, together with fragments at m/e 203, 173, 143, and 72 (as well as others), the differences being 30 ($\overset{2}{C}HOH$), 30 ($\overset{3}{C}HOH$), and 71 ($\overset{4}{C}HNHAc$) mass numbers, respectively; m/e 72 is $[CH_2NHAc]^+$. DeJongh and Hanessian[407] described a satisfactory micromethod for the preparation of dithioacetals.

As with acetylation, the introduction of isopropylidene acetal groupings appears to complicate the fragmentation pathways just outlined. Prominent molecular ions are observed for 2,3:4,5-di-*O*-isopropylidene-D-ribose diphenyl dithioacetal and its D-*arabino*[32] isomer, together with abundant peaks 15 mass numbers smaller ($- \cdot CH_3$ from isopropylidene), and a fragment at m/e 101 [4-(2,2-dimethyl-1,3-dioxolanium) ion[408]]; appearance of the latter ion permits assignment of one isopropylidene group at O-4 and O-5, allowing determination of the positions of the acetal rings. The $^+CH(SPh)_2$ ion is of minor intensity. The mass spectrum of 5,6-*O*-ethylidene-D-galactose diethyl dithioacetal exhibits major peaks at m/e 135 [$^+CH(SEt)_2$] and m/e 87 (the next lower homologue of m/e 101), the latter permitting immediate and confident assignment of the positions of substitution.[248]

Isobutane-mediated chemical ionization (c.i.) of D-glucose diethyl dithioacetal produces[409] initially an unstable proton-capture ion

(408) D. C. DeJongh and K. Biemann, *J. Amer. Chem. Soc.*, **86**, 67–74 (1964).
(409) D. Horton, J. D. Wander, and R. L. Foltz, *Carbohydr. Res.*, **36**, 75–96 (1974).

[MH]$^+$, one mass number larger (*m/e* 287) than the original molecule, which loses one molecule, or two molecules, each of ethanethiol and water to form the six major ions in the spectrum. Substitution of ammonia as the reagent gas diminishes the intensity of the latter fragments, the principal ions being an intact ion [M + NH$_4$]$^+$ formed by capture of the ammonium ion, and fragments resulting from loss of one molecule or two molecules of ethanethiol. C.i. (isobutane) of the corresponding pentaacetate produces an unstable [MH$^+$] ion that eliminates ethanethiol and undergoes a series of acetate-decomposition reactions in which all five acetate groups may be accounted for; c.i. (ammonia) affords a stable [M + NH$_4$]$^+$ ion plus a prominent fragment corresponding to loss of one ethylthio group, one acetate group, and the charged species (H$^+$ or NH$_4$$^+$) that was captured in the ionization step.[409] There is thus considerable simplification in the fragmentation processes following c.i.; however, the absence of products resulting from cleavage of skeletal bonds may render the technique of value in examining such products as oligosaccharide dialkyl dithioacetals produced by mercaptolysis of polysaccharides. When fragmentation of carbon chains is a desired process, the electron-impact mode is the more useful ionization technique.

3. Other Spectroscopic Methods

The infrared spectral absorptions of the bonds involving sulfur atoms in sugar dithioacetals are of interest primarily to demonstrate the absence of the more characteristic, strong sulfone[410] [7.3–7.6 and 8.6–9.0 μm] or sulfoxide[410] [9.45 and 9.7 μm (hydrogen-bonded)] absorptions.[349] The ultraviolet (u.v.) spectra of carbohydrate dialkyl dithioacetals exhibit relatively weak absorptions near 212 (ϵ_{mM} 1–2) and 230–245 nm (ϵ_{mM} ~0.6),[349] which have been assigned in simpler systems[411] to photoexcitation of the carbon–sulfur bond and to the formation of a photoexcited state involving geminal interaction between the sulfur atoms, respectively; for the diphenyl derivatives, the intense absorption of the aromatic groups prevents useful examination of this region.[32,33] Both of the u.v. absorptions of aldose dialkyl dithioacetals suffer dissymmetric perturbation due to the asymmetry

(410) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden–Day, San Francisco, 1962, pp. 54–55.
(411) H. H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," Wiley, New York, 1962, pp. 474–481; see also, B. Wladislaw, P. R. Olivato, and O. Sala, *J. Chem. Soc.* (B), 2037–2040 (1971).

present in the secondary alcoholic centers; this produces two (or three) Cotton effects at 212 and 230–245 nm, respectively, in the optical rotatory dispersion spectrum, and corresponding extrema in the circular dichroism spectra, the sign of the latter apparently correlating with the configurations[412] at C-2–C-4.

VII. General Considerations

Dithioacetals generally[188,356] form well defined crystals having sharp melting points, as do their acetates,[356] and these properties led Wolfrom and Karabinos[11] and Hardegger and coworkers[202] to propose the use of these normally optically active derivatives as a means of characterizing aldoses through comparison of physical properties. Such refinements as the use of higher thiols[202,413] (which appears to result in diminished sensitivity of physical properties to configuration)[414] and $HgCl_2$ complexes,[202,413] and conversion into p-nitrobenzoate[188,202,413] or phenylurethan[202,356] derivatives, have also been proposed as methods of characterization. An obvious but valid point that was raised in one of these discussions[202] is that the (symmetrically disubstituted) dithioacetal derivative contains exactly the same asymmetric configurational features as the hypothetical, acyclic sugar from which it is formed; in contrast, conversion of an aldose into a phenylosazone removes the asymmetry at C-2 (this is not true of 2-ketoses, wherein C-2 is already trigonally hybridized), whereas ring formation by quaternization of the carbonyl center results in generation of an additional asymmetric feature. Accordingly, it is to be expected that optimal economy should be available in working with dithioacetals, in that they contain all of the essential configurational information, but no extraneous stereochemical data.

This consideration is invalid in two circumstances. The first results from the use of optically active thiols in forming the dithioacetals, which introduces the intrinsic, asymmetric elements of the thiolyl residues. This "exception" was contrived by Czech workers[415] as a scheme to resolve racemic sugar mixtures after converting them into diastereoisomers[416] by condensation with (+)-2-methyl-1-butanethiol;

(412) M. K. Hargraves and D. L. Marshall, Carbohydr. Res., 29, 339–344 (1973).

(413) Z. El-Hewe[i]hi, Chem. Ber., 86, 781–784 (1953).

(414) H. Zinner, Chem. Ber., 84, 780–784 (1951).

(415) E. Votoček and V. Veselý, Ber., 47, 1515–1519 (1914); Z. Zuckerind. Boehm., 40, 207–211 (1916).

(416) L. Pasteur, "Researches on the Molecular Asymmetry of Natural Products (1860)," Alembic Club Reprints–No. 14, The University of Chicago Press, Chicago, 1902.

the procedure was successful for DL-arabinose, but failed to resolve 6-deoxy-DL-galactose.[415]

The second condition predictably increasing the number of asymmetric centers is observed in dithioacetals having two, different, thiolyl groups attached to the same carbon atom. Cyclic dithioacetals of this type can be prepared directly, that is, by condensation with 1,2-dithioglycerol[28] (in which C-2 also happens to be asymmetrically substituted), as in the conversion of D-glucose into 203, whereas the

CH₂OH

HC

HCOH

HOCH

HCOH

HCOH

CH₂OH

203
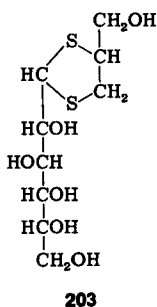
acyclic analogues have been prepared only by indirect routes involving, in two examples, intramolecular migration during nucleophilic displacement of an alkylthio group from C-1 of a symmetrical dithioacetal[130,147] (discussed in Section II,4), in a third example, conversion of a diazo sugar into an α-chlorothioether by action of an alkanesulfenyl chloride and subsequent displacement of the chloride substituent by sodium thiophenoxide[78] (see Section II,6), and, in a fourth example, acid-catalyzed exchange[25] of one of the thio groups of a diethyl dithioacetal by benzenethiol. Derivatives possessing analogous, asymmetric features are readily prepared (see Section IV,2) from dithioacetals through initial replacement of one alkylthio group by a halogen atom; although the new asymmetry was specifically considered[277] more than 30 years ago, no definitive treatise on this subject has appeared. The most practical basis for a nomenclature for identifying such "anomeric" centers appears to be specification of the order of substitution according to the Cahn–Ingold–Prelog sequence rule.

Hudson observed similarities in the rotatory power of some configurationally related examples; he was, however, unable to generate workable "isorotation rules" for the dithioacetals[276,417]; later advances in conformational analysis[32,33,150,152,344] have revealed that the conformational uniformity implicit in the "isorotation" treatment is not

(417) R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.*, **59**, 548–551 (1937).

found in these derivatives. J. K. N. Jones and coworkers[418] reported that, although satisfactory separations of protected aldose diethyl dithioacetals may be accomplished by gas chromatography, uncertainties of yield in the preparation, and the occurrence of occasional minor byproducts, prevent the use of these derivatives to quantitate mixtures of sugars.

D-Glucose diethyl dithioacetal, whose crystalline form is monoclinic,[419] is inert to the action of D-glucosidases[420]; the hydrochloride of its 2-amino-2-deoxy analogue gives negative Molisch, Elson–Morgan, and Dische–Borenfreund tests, but is ninhydrin-positive.[92] Several N-substituted 6-amino-6-deoxy-D-glucose ethylene dithioacetal derivatives were prepared as unsuccessful, antimicrobial, therapeutic agents.[97]

The sequence of reactions: mercaptalation–purification–demercaptalation has been employed with exemplary success as a method[19,262–264] of achieving difficult isolations (for example, of D-idose[262]). A patent has been issued whereby noxious odors are removed from hydrocarbon distillates by treatment with D-glucose and acid.[421]

VIII. TABLES OF DATA

The following Tables record the melting points and specific rotations of sugar dithioacetals and certain derivatives.

(418) D. G. Lance and J. K. N. Jones, *Can. J. Chem.*, 45, 1995–1998 (1967); D. T. Williams and J. K. N. Jones, *ibid.*, 44, 412–415 (1966).
(419) Y. Krouvine and C. Stora, *C. R. Acad. Sci. Ser. C.*, 220, 328–330 (1945).
(420) E. Fischer and H. Thierfelder, *Ber.*, 27, 2031–2037 (1894).
(421) H. E. Bode, U. S. Pat. 2,160,116 (1939); *Chem. Abstr.*, 33, 7,547 (1939).
(422) C. Neuberg, *Ber.*, 33, 2243–2254 (1900).
(423) M. Smrž and J. Farkas, *Collect. Czech. Chem. Commun.*, 33, 3803–3808 (1968).
(424) G. W. Kenner, H. J. Rodda, and A. R. Todd, *J. Chem. Soc.*, 1613–1620 (1949).
(425) T. Lieser and E. Leckzyck, *Ann.*, 511, 137–140 (1934).
(426) W. J. Humphlett, *Carbohydr. Res.*, 7, 431–441 (1968).
(427) R. Allerton, W. G. Overend, and M. Stacey, *J. Chem. Soc.*, 255–257 (1952).
(428) L. Goodman, *Chem. Commun.*, 219–220 (1968).
(429) H. Kaufmann and T. Reichstein, *Helv. Chim. Acta*, 50, 2280–2287 (1967).
(429a) J. Defaye, R. L. Foltz, D. Horton, and M. Muesser, *Abstr. Colloq. Spectrosc. Intern., 18th,* Grenoble, Sept. 15–19, 1975, No. 175.
(430) L. H. Welsh and G. L. Keenan, *J. Amer. Chem. Soc.*, 64, 183–186 (1942).
(431) T. Lennartz, *Ber.*, 75, 833–853 (1942).
(432) E. Pacsu, *Ber.*, 57, 849–853 (1924).
(433) F. Kipnis and J. Ornfelt, *J. Amer. Chem. Soc.*, 71, 2270 (1949).

(434) J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *An. Real Soc. Españ. Fis. Quím.*, **B56**, 719–722 (1960).

(435) M. Černý, J. Pacák, and J. Staněk, *Chem. Ind.* (London), 945–946 (1961); M. Černý and J. Pacák, *Collect. Czech. Chem. Commun.*, **26**, 94–105 (1962).

(436) A. F. Cook and W. G. Overend, *J. Chem. Soc.* (C), 1549–1556 (1966).

(437) D. J. Ball, A. E. Flood, and J. K. N. Jones, *Can. J. Chem.*, 37, 1018–1021 (1959).

(438) These data were inadvertently omitted from Ref. 270.

(439) O. T. Schmidt, W. Mayer, and A. Distelmaier, *Ann.*, **555**, 26–41 (1943).

(440) J. Němec, Z. Kefurtová, K. Kefurt, and J. Jarý, *Collect. Czech. Chem. Commun.*, 33, 2097–2110 (1968).

(441) Z. El-Hewehi and F. Runge, *Wiss. Z. Martin-Luther-Univ.*, 8, 1013–1024 (1959); *Chem. Abstr.*, **55**, 11,432 (1962).

(442) J. Jarý and K. Kefurt, *Collect. Czech. Chem. Commun.*, 31, 2059–2067 (1966).

(443) N. K. Kochetkov and B. A. Dmitriev, *Izv. Akad. Nauk SSSR*, 669–677 (1964).

(444) D. A. Rosenfeld, N. K. Richtmyer, and C. S. Hudson, *J. Amer. Chem. Soc.*, 73, 4907–4910 (1951).

(445) R. K. Hulyalkar, J. K. N. Jones, and M. B. Perry, *Can. J. Chem.*, 41, 1490–1492 (1963).

(446) R. M. Hann, A. T. Merrill, and C. S. Hudson, *J. Amer. Chem. Soc.*, 57, 2100–2103 (1935).

(447) M. Teuber, R. D. Bevill, and M. J. Osborn, *Biochemistry*, 7, 3303–3308 (1968).

(448) B. Lindberg and H. Meier, *Acta Chem. Scand.*, 16, 543–547 (1962).

(449) W. D. Maclay, R. M. Hann, and C. S. Hudson, *J. Amer. Chem. Soc.*, 60, 1035–1040 (1938).

(450) H. Zinner and W. Rehpenning, *Carbohydr. Res.*, 5, 176–183 (1967).

(451) H. Zinner and H. Kristen, *Chem. Ber.*, 97, 1654–1658 (1964).

(452) F. G. Fischer and H. Schmidt, *Chem. Ber.*, 93, 658–662 (1960).

(453) H. Saeki, T. Iwashige, O. Ohki, K. Furuya, and M. Shirasaka, *Ann. Sankyo Res. Lab.*, 19, 137–143 (1967); *Chem. Abstr.*, 68, 96,075 (1968).

(454) F. A. Kuehl, Jr., R. L. Peck, C. E. Hoffhine, Jr., and K. Folkers, *J. Amer. Chem. Soc.*, 70, 2325–2330 (1948).

(455) M. N. Mirzayanova, L. P. Davydova, and G. I. Samokhvalov, *Zh. Obshch. Khim.*, 40, 693–697 (1970).

(456) W. Schroeder, B. Bannister, and H. Hoeksema, *J. Amer. Chem. Soc.*, 89, 2448–2453 (1967).

(457) A. B. Grant, *N. Z. J. Sci. Technol. Sect. B*, 37, 509–521 (1956).

(458) S. B. Baker, *Can. J. Chem.*, 33, 1102–1108 (1955).

TABLE II

Physical Constants of Dithioacetals and Per-*O*-acetylated Dithioacetals of Unsubstituted Monosaccharides

Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	
Triose								
D-Glyceraldehyde	ethyl	syrup						21
Pentoses								
D-Arabinose	methyl	122.5	−2.7	MeOH	84	+34.1	MeOH	188
	ethyl	125–126	0.0	py	80	+30.0	Chf	274
			−11.0	MeOH		+34.9	MeOH	188,282,377
	1-propyl	133–134	−12.7	MeOH				120,188
	2-propyl	134.5	−24.4	MeOH	82	+51.9	MeOH	188
	2-methyl-1-propyl	128.5	−14.8	MeOH				188
	1-butyl	113.5	−11.5	MeOH				188
	(+)-2-methyl-1-butyl	118–120						415
	3-methyl-1-butyl	121–124						415
	phenyl	186.5–187	+24.0	py				32
	benzyl	149.0	+18.6	py				108,188,238
	ethylene	154.5	+11.4	py	100	+35.9	MeOH	188
DL-Arabinose	3-methyl-1-butyl	113–115						415
	1-pentyl	125–130	0.0	?				422
L-Arabinose	methyl	121.5–122.5	+2.1	MeOH				414
	ethyl	125–125.5	+9.9	MeOH				414
			−5.0	py				1,194
					79–80	−29.9	Chf	37,269
	1-propyl	128.0	+29.0	?				113
	2-propyl	133.5–134.5	+24.4	MeOH				414

	2-methyl-1-propyl	125.5–126.5	+12.9	MeOH				*114*,414
	1-butyl	111.5	+14.0	?				112
	(+)-2-methyl-1-butyl	114–116						415
	3-methyl-1-butyl	121–124						415
	1-pentyl	132–134	+27.5	EtOH				422
	phenyl	186–186.8	−25.9	py	82–83	−60.3	py	32
	benzyl	144.0	−18.9	py				27,300
	2-phenylethyl	188.0	+253.1	py				413
	ethylene	154.0						27
					100–102	−37.0	py	213
	trimethylene	150.0						27
D-Lyxose	methyl	103.5	+4.0	MeOH	99.0	+38.1	MeOH	188
			+6.0	EtOH		+37.5	EtOH	202
	ethyl	104.0	+20.3	MeOH	38.5	+50.3	MeOH	188
			+41.0	water		+40.5	Chf	273
			+41.0	water		+40.5	Chf	273
	1-propyl	101.5–102.0	+17.9	MeOH				120,*188*
	2-propyl	78.0	+46.9	MeOH	61.0	+66.5	MeOH	188
	2-methyl-1-propyl	66–67	+9.5	MeOH				188,*208*
	1-butyl	75.5	+16.1	MeOH				188
	phenyl	63–64	−79.0	EtOH	syrup	−30.0	Chf	33
	benzyl	105.0	−26.0	py	104.0	+174.0	Chf	202
			−1.5	EtOH		+203.0	EtOH	202
			+4.8	MeOH		+198.0	MeOH	188
	ethylene	142.0	+17.5	MeOH				188
			+12.5	EtOH	83.0	+39.0	EtOH	202
L-Lyxose	ethyl	101–102	−41.5	water				423
D-Ribose	methyl	76–76.5	−9.6	MeOH				18,47
			−9.5	EtOH				202
			−21.0	water				202
	ethyl	82–83	−41.5	water				239,424
			−25.9	MeOH				18,47,377

(*continued*)

TABLE II (*continued*)

104

Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	
					49.5–50	+25.1	Chf	275
						+27.7	MeOH	275
	1-propyl	85–85.5	−23.1	MeOH				47,120
	2-propyl	97–97.5	−48.6	MeOH				18,47
					102–103	+12.9	Chf	275
						+18.7	MeOH	275
	2-methyl-1-propyl	83.5–84.0	−18.6	MeOH				47
	phenyl	101.5–102.0	+42.3	py	syrup	+79.8	Chf	32,48
	benzyl	80.0	−19.0	EtOH	75.0	−115.0	EtOH	47,54,202
			−20.0	MeOH		−97.0	Chf	47,54,202
						−113.0	MeOH	275
	ethylene	108.0	−21.0	py				18,47,202
			−25.0	MeOH				18,47,202
			−29.0	EtOH				18,47,202
D-Xylose	methyl	64–66	−1.4	MeOH				18
					71–72	+16.0	MeOH	356
	ethyl	63–65	−30.8	water	46–48	+12.5	Chf	16,17,18,50, 231,356,377
	1-propyl	syrup	+9.0	MeOH	syrup	+15.9	MeOH	356
	2-propyl	54–55	+10.8	MeOH	96.0	−14.0	MeOH	356
	2-methyl-1-propyl	syrup	+14.7	MeOH				356
	1-butyl	syrup	+13.8	MeOH	syrup	+11.0	MeOH	356
	phenyl	100–101.5	−8.0	EtOH	90–91	+54.5	EtOH	32
	benzyl	77–78	−129.5	MeOH				356,425
	ethylene	76–77	−5.9	MeOH				363

DL-Xylose	ethyl				47.0	0.0	Chf	426
L-Xylose	ethyl	63–64	+70.0	Chf	49–50	−13.0	Chf	426
Deoxypentoses								
5-Deoxy-D-arabinose	methyl	72.0	−15.0	MeOH				210
	ethyl	109.0	−27.0	MeOH				210
	1-propyl	100.0	−22.6	MeOH				210
	2-propyl	83.0	−33.5	MeOH				210
	2-methyl-1-propyl	84.0	−25.4	MeOH				210
	1-butyl	84.0	−19.2	MeOH				210
5-Deoxy-L-arabinose	ethyl	108–109	+11.9	py				51,64,212
2-Deoxy-D-*erythro*-pentose	methyl	59–60	+1.5	py	syrup	+24.4	py	278
	ethyl	syrup	−10.9	py	syrup	+10.2	py	278
	1-propyl	syrup	−10.6	py	syrup	+10.5	py	278
	2-propyl	58–59	−41.7	py	syrup	−2.7	py	278
	2-methyl-1-propyl	syrup	−17.1	py	syrup	−0.9	py	278
	1-butyl	syrup	−12.8	py	syrup	+6.5	py	278
	benzyl	66–67	+12.8	Chf				54
					syrup	−81.6	py	278
	ethylene	97.5	−38.7	py	syrup	−3.9	py	278
	trimethylene	123–124	−35.0	MeOH	syrup	+6.3	Chf	158
2-Deoxy-L-*erythro*-pentose	ethyl	syrup	+8.8	EtOH				52
	benzyl	66–70	−12.0	Chf				52
3-Deoxy-L-*erythro*-pentose	benzyl	syrup	+18.2	Chf				427
2,5-Dideoxy-D-*erythro*-pentose	methyl	97–98	−26.2	Chf	syrup	+22.2	Chf	218

(continued)

TABLE II (continued)

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Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	[α]_D (degrees)	Solvent[a]	M.p. (degrees)	[α]_D (degrees)	Solvent[a]	
2,3-Dideoxy-D-*glycero*-pentose	benzyl	70–71	−23.0	Chf				428
2,3-Dideoxy-L-*glycero*-pentose	benzyl	74.0	+20.4	Chf				427
2-Deoxy-D-*threo*-pentose	methyl	61–62	+9.6	MeOH	syrup	+24.2	MeOH	61
	ethyl	syrup	+15.9	MeOH	syrup	+20.8	MeOH	61
	1-propyl	59–60	+9.0	MeOH	syrup	+25.2	MeOH	61
	2-propyl	syrup	−12.5	MeOH	syrup	+21.6	MeOH	61
	2-methyl-1-propyl	syrup	−11.2	MeOH	syrup	+27.3	MeOH	61
	1-butyl	syrup	+3.6	MeOH	syrup	+29.1	MeOH	61
	ethylene	71.0	+33.5	MeOH	syrup	+39.3	MeOH	61
3-Deoxy-L-*threo*-pentose	2-propyl	syrup	+11.1	py				110a
	1-butyl	syrup	+8.6	py				110a
Hexoses								
D-Allose	ethyl	95–96	−30.8	MeOH				429
D-Altrose	ethyl	103–106	+4.4	py				343
			+12.3	water				343
					90–91	+29.6	Chf	429a
	benzyl	121–122	+39.4	py				264
L-Altrose	benzyl	121–122	−39.2	py				264
D-Galactose	methyl	160–161	+5.3	MeOH				414
					111–112	+13.6	Chf	327
	ethyl	142–143	−3.5	py				1,22,42,43, 111,118,119, 359
			+6.0	EtOH				
			−4.8	water				

			+6.9	MeOH				414
					76.5–77 80.5–81 90.5–91 trimorphic	+7.3	Chf	42,43,69,430
	1-propyl	129.0	+27.5	?				113
					60–62	+4.2	Chf	327
	2-propyl	137–138	+8.3	MeOH				414
	2-methyl-1-propyl	129.0	+41.2	?				114
		129.5–130.5	+12.9	MeOH				414
		50–52	−2.7	Chf				327
	1-butyl	123.0	+12.7	?				112
					43–45	+5.7	Chf	327
	(+)-2-methyl-1-butyl	123–124						415
	3-methyl-1-butyl	122–123						415
	1-hexyl	128.0	−2.8	py				10
	1-heptyl	126–127	−101.7	py				48
	phenyl	173–174	−31.5	py				30,48
	benzyl	144.0	−26.4	py				27,300,361
	p-chlorobenzyl	136.0	−51.0	py				48
	2-phenylethyl	145.0	+26.1	py				413
	ethylene	149.0						27
					188–189	+16.0	py	315
	decamethylene	172 ("not sharp")						48
DL-Galactose	ethyl	127.0	0.0	water	112–113	0.0	Chf	43,117
			0.0	py				43,117
D-Glucose	methyl	161.0	−20.8	M NaOH	83.0	+38.7	C₂H₂Cl₄	267
	ethyl	127–128	−29.8	water				1
			−8.8	MeOH				65,414
					46–48	+10.9	Chf	69
						+17.4	C₂H₂Cl₄	267,268
	1-propyl	147.0	+41.0	?				113,267
	2-propyl	126–127	−16.1	MeOH				414

(continued)

TABLE II (*continued*)

Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	
	2-methyl-1-propyl	140–141	−4.6	MeOH				114,*414*
	1-butyl	124.0	+27.0	?				112
	(+)-2-methyl-1-butyl	138–139						415
	3-methyl-1-butyl	142–144						415
	1-pentyl	138–142						1
	1-hexyl	104.0	−106.7	py				10
	1-octyl	113.8–114.0	+4.1	AcOH				28
	1-(9-octadecenyl)	104.0						431
	phenyl	155–157	+1.5	py				30
	benzyl	139.0	−98.4	py				27,432
			−98.8	EtOH				116
					64.0	+31.8	$C_2H_2Cl_4$	267
	2-phenylethyl	148.0	+27.1	py				413
			+5.3	AcOH				28
	2-thenyl	130–131						433
	ethylene	143.0	−10.6	water	100.9–101.1	+5.0	Chf	27,28
	trimethylene	130.0						27
	1-(hydroxymethyl)-ethylene	155–156	+13.2	AcOH				28
	1-(acetoxymethyl)-ethylene				101.3–101.5	+25.1	Chf	28
L-Gulose	ethyl	85.5–86	+20	MeOH				429a
D-Idose	benzyl	38–40	+139.1	Chf				262
L-Idose	ethyl	96–98	−7.4	MeOH	55.5–57	−4.2	Chf	429a
D-Mannose	methyl	144.5–145.0	+4.0	MeOH				1,216,*414*
	ethyl	133.5–134.0	+17.4	MeOH				414
			0	py				194,*302*

		mp	[α]	solvent	mp	[α]	solvent	Ref.
	1-propyl	125.0	+31.0	?	51–52	+31.2	Chf	314
								113
	2-propyl	143–144	+38.0	MeOH				414
	2-methyl-1-propyl	111–112	+12.2	MeOH				268,414
	1-butyl	117.0	+16.5	?				112
	1-hexyl	123.0	−5.8	py				10
	phenyl	138–139	−30.0	py				30
	benzyl	126.0						232
					72–75	+50.0	py	434
	2-phenylethyl	132.0						
		188.0	−11.9	py				413
	ethylene	153–154	+12.9	water				27
					130–131	+34.0	py	215,434
D-Talose	ethyl	92–93	+16.0	MeOH				368,429a
					72.5–73	+25.4	Chf	429a
Deoxyhexoses								
2-Deoxy-D-arabino-hexose	ethyl	135–136	+13.1	MeOH	76–77	+28.6	MeOH	56,110,361
						+35.2	Chf	56,110,361
			+10.0	EtOH	76.0	+35.0	MeOH	205
	benzyl	154.0	−40.0	EtOH	63.0	+18.2	MeOH	19
3-Deoxy-D-arabino-hexose	methyl	82.0	−38.6	MeOH	syrup	−5.5	Chf	59
	ethyl	syrup	−38.0	MeOH	syrup	0.0	Chf	59
	1-propyl	syrup	−20.6	MeOH	syrup	+4.8	Chf	59
	2-propyl	syrup	−19.9	MeOH	syrup	+11.7	Chf	59
	2-methyl-1-propyl	syrup	−12.4	MeOH	syrup	+5.4	Chf	59
	1-butyl	syrup	−18.3	MeOH	syrup	+6.3	Chf	59
4-Deoxy-D-arabino-hexose	benzyl	103–105	+146.0	EtOH				435

TABLE II (continued)

Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	
4-Deoxy-L-arabino-hexose	ethyl	81–82	−2.6	MeOH				436
2,6-Dideoxy-D-arabino-hexose	ethyl	83–84	+11.8	Chf				266
	benzyl	88–91						266
3,6-Dideoxy-D-arabino-hexose	methyl	syrup	−14.9	MeOH	syrup	+7.2	MeOH	265
5,6-Dideoxy-D-arabino-hexose	ethyl	108–109						58
5,6-Dideoxy-L-arabino-hexose	ethyl	110.0						437
2,3-Dideoxy-D-erythro-hexose	ethyl	syrup						406
6-Deoxy-D-galactose	ethyl	167–168.5						415
	(+)-2-methyl-1-butyl	140–142						415
	3-methyl-1-butyl	151–152.5						415
	benzyl	184.0	−27.8	py				105,200
6-Deoxy-DL-galactose	3-methyl-1-butyl	160–162						415
6-Deoxy-L-galactose	ethyl	167–168.5						415
					99–100	+5.0	Chf	438
	(+)-2-methyl-1-butyl	136–138.5						415
	3-methyl-1-butyl	151–152.5						415

	benzyl	184.0	+27.8	py				439
	ethylene	191–191.5						415
6-Deoxy-L-glucose	ethyl	97–98	+47.1	water				63,344
2-Deoxy-D-*lyxo*-hexose	ethyl	107–108	+40.0	MeOH				55,205
					syrup	+31.5	Chf	110
			−45.0	EtOH				57
	benzyl	106–107	−38.0	py				57
3-Deoxy-L-*lyxo*-hexose	methyl	37–40	+48.0	Chf				440
		46.5–48.0						440
2,6-Dideoxy-L-*lyxo*-hexose	ethyl	101–102	−12.6	Chf				31
	phenyl	85–88						31
3,6-Dideoxy-L-*lyxo*-hexose	ethyl	syrup						406
6-Deoxy-D-mannose	methyl	161.5–162.5	−8.0	MeOH				216
6-Deoxy-L-mannose	methyl	156.5–157	+4.4	MeOH				414
	ethyl	136.5–137	−10.5	MeOH				1,414
					59–61	−42.0	Chf	11,415
	1-propyl	130.0	+10.0	?				113
	2-propyl	96–97	−31.0	MeOH				414
	2-methyl-1-propyl	112.5–113.5	−6.3	MeOH				114,414
	1-butyl	119.0	+16.5	?				112
	3-methyl-1-butyl	108–110.5						415
	1-hexyl	117.0						48
	1-heptyl	106.0	−106.4	py				48
	phenyl	124–125.5	+74.8	EtOH	syrup	+31.0	Chf	33

(*continued*)

TABLE II (continued)

Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	
	benzyl	125.0	+35.3	py				27,300
	2-phenylethyl	135.0	+20.0	py				441
	ethylene	169.0						27
2-Deoxy-D-*ribo*-hexose	ethyl	93–96	+0.1	THF				62
	benzyl	146.5–149.0	+55.5	THF				62
3-Deoxy-D-*ribo*-hexose	methyl	74–75	+47.0	Chf				440
2,6-Dideoxy-D-*ribo*-hexose	ethyl	40–41						406
3-Deoxy-D-*xylo*-hexose	methyl	77.0	+35.5	MeOH	47.0	+43.1	Chf	60,234
	ethyl	syrup	+21.1	MeOH				60
	2-propyl	syrup	+21.3	MeOH				60
	1-butyl	syrup	+17.3	MeOH				60
	benzyl	51–53	+31.6	Chf				442
	ethylene	83.5	+32.7	MeOH	83.5	+40.4	Chf	234
4-Deoxy-D-*xylo*-hexose	benzyl	106–108	−85.0	EtOH				436
5-Deoxy-D-*xylo*-hexose	ethyl	56–57						406
4,6-Dideoxy-D-*xylo*-hexose	ethyl	101–102						406

Higher Aldoses

D-*glycero*-D-*galacto*-Heptose	ethyl	188–190	−9.2	py				272
			−11.9	water	77.0	−2.2	Chf	272
D-*glycero*-L-*gluco*-Heptose	ethyl	135–136	+5.3	MeOH				389
			+37.8	water	105	+27.0	Chf	271
	benzyl	146–147	+73.8	py	82–83	+9.2	Chf	417
D-*glycero*-D-*gulo*-Heptose	ethyl	155–156	−7.7	py				*1,344*
			−30.5	water				*1,344*
					99–100	−12.0	Chf	277
D-*glycero*-D-*ido*-Heptose	benzyl	130–131	+71.4	py				263
L-*glycero*-L-*ido*-Heptose	benzyl	128.0	−78.5	py				443
D-*glycero*-D-*manno*-Heptose	ethyl	151–153	+31.1	water				444
		155–156	+29.6	water				445
D-*glycero*-L-*manno*-Heptose	ethyl	204–205	−9.7	py	145–146	+5.6	Chf	276,389
L-*glycero*-D-*manno*-Heptose	ethyl	202–203	+10.2	py				447
D-*glycero*-D-*talo*-Heptose	ethyl	165–166						448
D-*erythro*-L-*galacto*-Octose	benzyl	157.0	+15.8	py				23
D-*threo*-L-*galacto*-Octose	ethyl	214.0	−3.2	py	106.0	+29.9	Chf	22
	benzyl	208–209	+18.5	py	88–89	−29.6	Chf	449

Ketoses

D-*erythro*-Pentulose	methyl	syrup	+12.8	MeOH	syrup	+37.7	MeOH	450

(continued)

TABLE II (continued)

Parent sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	
	ethyl				syrup	+9.6	MeOH	450
	1-propyl				syrup	+4.7	MeOH	450
	2-propyl				syrup	+3.4	MeOH	450
D-Fructose	ethyl	65–67	+35.8	MeOH	83.0	+20.0	Chf	69,379
			+16.5	water				306
	benzyl				110–111	+40.1	Chf	379
L-Sorbose	methyl	syrup	−37.3	Chf	116.0	−8.2	Chf	190
	ethyl	syrup	−27.4	Chf	94–95	−12.3	Chf	190
1-Deoxy-D-*ribo*-hexulose	ethyl	47–48	+12.0	EtOH	syrup			298
1,3-Dideoxy-D-*erythro*-hexulose	methyl	syrup	−37.8	Chf				206
Aldos-2-uloses (1,1-dithioacetals)								
D-*arabino*-Hexosulose	ethyl	110–112	+48.7	Chf	99–100	+32.0	Chf	78
			−6.3	water				77
	benzyl	151–152	+50.0	EtOH				77
	ethyl, phenyl				109–110	−109.5	Chf	78
D-*lyxo*-Hexosulose	ethyl	166–167[c]	−136.0	water				77
L-*xylo*-Hexosulose	ethyl	117–118	+69.0	water				77
	benzyl	136–137	+66.5	EtOH				77

Other Aldosuloses

D-*threo*-Pentos-4-ulose (1,1)	ethyl	120–121	−17.3	MeOH				367
L-*arabino*-Hexos-5-ulose (1,1)	ethyl	syrup	−13.0	water				368
D-*xylo*-Hexos-5-ulose (1,1)	ethyl	syrup	−30.0	water				367
D-*lyxo*-Hexos-5-ulose (1,1)	ethyl	syrup	+28.0	water				367

Dialdoses
[bis(dithioacetals)]

D-*threo*-Tetrodi-aldose	methyl	116.0	+5.5	MeOH				358,451
L-*threo*-Tetrodi-aldose	methyl	118.0	−4.4	MeOH				227
D-*gluco*-Hexodi-aldose	ethyl	80–81	+8.0	acetone	70–71	−13.5	MeOH	76,452
D-*manno*-Hexodi-aldose	ethyl	109–111	+26.9	EtOH				75

Branched-chain Dialdose [3¹-(dithioacetal)]

5-Deoxy-3-*C*-formyl-L-lyxose	trimethylene		−56.0	MeOH				163

a Chf = chloroform; py = pyridine; THF = tetrahydrofuran. *b* The reference number in roman type is that of that from which the constants were taken. *c* Reported as 116–167°.

TABLE III

Physical Constants of Dithioacetals and Per-O-acetylated Dithioacetals of Disaccharides and of Alduronic Acid,
Nitrogenous, Halo, Thio, and Anhydro Derivatives of Monosaccharides

Sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent	
Disaccharides								
3,6-Anhydro-4-O-β-D-galacto-pyranosyl-D-galactose	ethyl	116–117	+14.0	water	121–121.5	+12.0	Chf	119
3,6-Anhydro-4-O-D-galacto-pyranosyl-L-galactose	ethyl	171–172	−8.5	water	101–103.5	−11.8	Chf	111
			−20.9	MeOH		−22.1	PhH	111
			−51.7	py		−16.7	EtOH	111
4-O-β-D-Galactopyranosyl-D-glucose	butyl	106.0	+23.6	?				112
	benzyl	128.0	−38.2	EtOH	145	+26.2	EtOH	116
4-O-α-D-Glucopyranosyl-D-glucose	ethyl				122–122.5	+87.5	Chf	16
	1-propyl	146.0	+25.0	?				113
	2-methyl-1-propyl	140.0	+13.2	?				114
	1-butyl	126.0	+12.0	?				112
2-Acetamido-4-O-(2-acet-amido-2-deoxy-α-D-gluco-pyranosyl)-2-deoxy-D-glucose	ethyl	152–159	−13.4	EtOH				365
Alduronic Acid Derivatives								
D-Galacturonic acid	methyl	166.0	+11.2	MeOH				246
	ethyl	132.5	+17.0	MeOH				83
	1-propyl	124–125	+21.5	MeOH				84

	1-butyl	122.0	+24.5	MeOH				84
methyl ester	methyl	163.0	+6.9	MeOH	133	+21.3	Chf	246
	ethyl	133–134	+17.8	EtOH	112.5–113.5	+20.5	Chf	83,86
	1-butyl	124–125	+21.8	MeOH				84
	benzyl	192–193.5			129–131			82
	ethylene	164–166	+1.0	?	159–160	0.0	?	82
ethyl ester	methyl	143.0	+5.6	MeOH	145	+9.5	Chf	246
	ethyl	128–129	+15.7	EtOH	80–81	+11.0	Chf	86
	ethylene	132–134			151–153	−13.0	py	82
sodium salt	methyl		−17.9	water				84
	ethyl		−13.6	water				83
	1-propyl		−8.0	water				84
	1-butyl		−12.2	water				84
phenylhydrazide	methyl	180.0	−11.8	py				84
	ethyl	156.0	−24.8	MeOH				84
	1-propyl	154.0	−22.1	MeOH				84
	2-propyl	127–128	−7.5	MeOH				84
	1-butyl	144.0	−18.9	MeOH				84
6,3-lactone	methyl	102.0	+46.2	MeOH				246
					108	+11.1	MeOH	84
	ethyl	81.0	+39.3	MeOH				84
			+36.0	water				83
	1-propyl	syrup	+41.5	MeOH	syrup	+12.9	MeOH	84
	2-propyl	syrup	+53.4	MeOH	80	+17.7	MeOH	84
Sodium D-glucuronate	ethyl	115–118	−37.0	water				81
D-Glucuronamide	methyl	146–147	−32.0	water	129–130	−16.4	Chf	290
	ethyl	131–132	−33.0	water				81
			−30.0	water	134–136	−6.0	Chf	290
	2-propyl	85–87	−49.6	water	121–122	+3.0	EtOH	290
	ethylene	121–122.5						82
N-Benzyl-D-glucuronamide	ethylene	181–182.5						82

TABLE III (continued)

Sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent	
D-Glucuronic acid phenylhydrazide	ethyl	142.0	−26.6	MeOH				79
	1-propyl	131.0	−12.5	MeOH				79
	2-propyl	121.0	−27.7	MeOH				79
	1-butyl	121.0	−20.3	MeOH				79
	benzyl	153.0	−96.3	MeOH				79
	ethylene	182–184	−9.8	py				82
6,3-lactone	methyl				101	+73.7	MeOH	79
	ethyl	141–143	+169.0	EtOH	113–114	+58.0	Chf	81
		amorphous	−16.1	MeOH	110–112	+53.9	MeOH	79
	1-propyl				90	+53.1	MeOH	79
	2-propyl				127	+31.1	MeOH	79
	1-butyl	syrup	−20.6	MeOH				79
	benzyl	163.0	−349.6	py				79
	ethylene	186–188	−4.0	py	195–196			82
Aminodeoxy Sugars								
2-Acetamido-2-deoxy-DL-glyceraldehyde	ethylene	137–138						95
2-Amino-2-deoxy-DL-glyceraldehyde	ethylene	60–63						95
3-Amino-2,3-dideoxy-D-glycero-tetrose	ethyl	syrup	+2.2	MeOH				89
5-Acetamido-5-deoxy-L-arabinose	ethyl	133–134	+90.0	Chf				225

4-Acetamido-4,5-dideoxy-D-xylose	ethyl	103–105	+16.0	MeOH				222
4,5-Diacetamido-4,5-dideoxy-L-xylose	ethyl	136–137						222
3-Acetamido-3-deoxy-D-allose	ethyl	160.0	−24.5	MeOH	125	+30.0	Chf	382,383
6-Amino-6-deoxy-D-allose hydrochloride	ethyl	syrup						45
3-Acetamido-3-deoxy-D-altrose	ethyl	146–147	+26.2	MeOH	110			109,382
3,6-Diacetamido-3,6-dideoxy-D-altrose	ethyl	101–102	+22.0	MeOH	161–162	+45.0	Chf	88
3,6-Diamino-3,6-dideoxy-D-altrose	ethyl	186.0	+31.0	water				88
2-Acetamido-2-deoxy-D-galactose	ethyl	164–165	−39.0	EtOH				293
			−52.0	MeOH				293
					137–139	−17.0	Chf	81,92
6-Acetamido-6-deoxy-D-galactose	methyl	176–182.5	0.0	EtOH				453
	ethyl	174–175						407
2-Amino-2-deoxy-D-galactose	ethyl	155–157	+24.0	water				81
6-Benzamido-6-deoxy-D-galactose	benzyl	154.5–155.0	+37.0	MeOH				96
2-(Benzyloxycarbonyl)amino-2-deoxy-D-galactose	ethyl	59–61	+33.0	Chf				92
2-Acetamido-2-deoxy-D-glucose	ethyl	129.5–130.5	−35.0	water	75–77	+1.0	Chf	91
	1-propyl	120–121						407
	ethylene	168–169						95
2-Amino-2-deoxy-D-glucose	ethyl	109–110	−21.0	water				91
hydrochloride	ethyl	75–76	−18.0	water				90,92,380

TABLE III (*continued*)

Sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent[a]	M.p. (degrees)	$[\alpha]_D$ (degrees)	Solvent	
6-Amino-6-deoxy-D-glucose hydrochloride	ethyl	119–121	−25.5	water				45
2-Benzamido-2-deoxy-D-glucose	ethyl	130–131	−18.0	EtOH				92
2-(Benzyloxycarbonyl)amino-2-deoxy-D-glucose	ethyl	94.0	−28.0	EtOH				92
6-Deoxy-6-(dibenzylamino)-D-glucose	ethylene	137–138	+4.9	Chf				97
2-Deoxy-2-(2,4-dinitroanilino)-D-glucose	ethyl				94–96	−153.0	Chf	250
6-Deoxy-6-morpholino-D-glucose	ethylene	120–121	−6.9	Chf				97
6-Deoxy-6-piperidino-D-glucose	ethylene	104–105	−24.1	Chf				97
6-Deoxy-6-pyrrolidino-D-glucose	ethylene	113–114	−18.2	Chf				97
2-Acetamido-2-deoxy-D-gulose	ethyl	147–148						407
2-Acetamido-2-deoxy-D-idose	ethyl	100–101						407
3-Acetamido-3,6-dideoxy-L-idose	ethyl	138–139						407
	1-propyl	150–151						407
2,6-Diacetamido-2,6-dideoxy-L-idose	ethyl	138–139	−21.6	MeOH				94
	benzyl	82–84	−12.5	MeOH	91–93			94
2,6-Diamino-2,6-dideoxy-L-idose dihydrochloride	benzyl	225–230	−126.0	MeOH				94

3-Amino-3,6-dideoxy-D-mannose hydrochloride	ethyl	111.5–113.0	+2.5	water	46
3-Acetamido-3-deoxy-D-*ribo*-hexose	ethyl	91–92			407
3,5-Bis(benzamido)-2,3,5-trideoxy-D(or L-)*xylo*-hexodialdose	(tetra)ethyl	146–146.5			454
3-Acetamido-3-deoxy-D-*glycero*-D-*galacto*-heptonic acid	ethyl	150–152	+5.3	water	455
6-Acetamido-6,8-dideoxy-D-*erythro*-D-*galacto*-octose	methyl	178–179			257
		179–179.5			456
6-Amino-6,8-dideoxy-D-*erythro*-D-*galacto*-octose	methyl	142–144			456
5-Acetamido-3,5-dideoxy-D-*glycero*-D-*ido*-nonulosono-1,4-lactone	ethyl	124–125	−83.0	MeOH	85
Anhydro sugars					
2,5-Anhydro-D-lyxose	2-methyl-1-propyl	100–101	+46.8	Chf	208
2,5-Anhydro-D-ribose	1-propyl	67–70	−91.4	MeOH	207
	2-methyl-1-propyl	89–90	−85.6	MeOH	207
2,5-Anhydro-D-xylose	2-methyl-1-propyl	94.0	+11.5	Chf	207
	benzyl	98.0	+159.6	Chf	207
3,6-Anhydro-D-galactose	ethyl	112–113	−10.0	water	119,359
			+26.8	py	118
			+21.0	EtOH	117
3,6-Anhydro-L-galactose	ethyl	110–111	+14.1	water	43,111
			−21.0	EtOH	43,111
		syrup	−26.3	py	43,111

TABLE III (continued)

Sugar	Radical of thiol	Dithioacetals			Peracetates			References[b]
		M.p. (degrees)	[α]D (degrees)	Solvent[a]	M.p. (degrees)	[α]D (degrees)	Solvent	
2,5-Anhydro-D-mannose	ethyl	syrup	+60.4	acetone				457
			+102.2	acetone	syrup	+74.2	Chf	252
1,6-Anhydro-2,4-dideoxy-β-D-glycero-hexopyranos-3-ulose	ethylene	69–70	−102.0	Chf				70
Azidodeoxy sugars								
5-Azido-5-deoxy-L-arabinose	ethyl	100–101	+83.3	Chf				225
	ethylene	98–102	+11.0	py	72–76	−34.0	py	226
6-Azido-6-deoxy-D-galactose	ethyl	144–146	−6.0	py				226
	ethylene	185–187	−18.5	py	151.5–153.0	+26.0	py	226
6-Azido-6-deoxy-D-glucose	ethylene	107–109	−8.0	py	103–105	−11.0	py	226
6-Azido-6-deoxy-D-mannose	ethylene	170–172	+10.0	py	120–123	+65.0	py	226
Deoxyhalogeno sugars								
5-Deoxy-5-iodo-L-arabinose	ethylene				110–110.5			211,213
6-Bromo-6-deoxy-D-galactose	ethyl				110–111			191
6-Bromo-6-deoxy-D-glucose	ethyl	107.0	+5.0	water				
6-Deoxy-6-iodo-D-galactose	ethyl	123.0	+6.9	py	109–110	−2.3	Chf	99,214,217
	ethylene	135–136						214
					166–167			217
6-Deoxy-6-iodo-D-glucose	ethyl	96–97	−13.0	py				100
	benzyl	107 (dec.)	−67.0	py				100
	ethylene	127–129	−5.0	py	114–115	−10.6	py	100,213

6-Deoxy-6-iodo-D-mannose	ethylene				144–145.5	+37.0	py	215
7-Deoxy-7-iodo-D-*gulo*-heptose	ethyl				80.0	−19.0	Chf	209
Thio sugars								
5-S-Acetyl-5-thio-L-arabinose	ethylene				135–136	−86.0	py	224
5-S-Ethyl-5-thio-L-arabinose	ethyl	65–67	−16.0	py				145,*146*
2,3-Di-S-ethyl-2,3-dithio-D-allose	ethyl	syrup	−2.0	EtOH				25
6-S-Acetyl-6-thio-D-galactose	ethyl				120–122.5	+10.0	py	224
	ethylene				195–196.5	+53.5	py	224
6-S-Ethyl-6-thio-D-galactose	ethyl	157–158	−6.2	py	77.5–78.0	−4.9	Chf	458
6-S-Acetyl-6-thio-D-glucose	ethylene				148–150	+22.0	py	224
2-S-Ethyl-2-thio-D-glucose	ethyl	110–110.5						224
6-S-Acetyl-6-thio-D-mannose	ethyl				86–89	+47.0	py	224
	ethylene				100–102	+85.0	py	224
2-S-Ethyl-2-thio-D-mannose	ethyl	101–102	+2.3	acetone				123,*130*
3-Deoxy-2-S-methyl-2-thio-L-(*xylo* or *lyxo*)-hexose	methyl	80.5–81.5	−54.2	Chf				440

a Chf = chloroform; py = pyridine. *b* The reference number in roman type is that of that from which the constants were taken.

THE UTILIZATION OF SUGARS BY YEASTS[1]

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*"J'ai communément employé dix livres de levure en pâte pour un quintal de sucre, &
une quantité d'eau égale à quatre fois le poids du sucre: ainsi la liqueur fermentes-
cible se trouvoit composée ainsi qu'il suit: je donne ici les résultats de mes expéri-
ences tels que je les ai obtenus, & en conservant même jusqu'aux fractions que m'a
données le calcul de réduction."* (Lavoisier, 1789).[3]

*"We should also like to re-emphasize the differences which may exist between yeast
strains and to stress the dangers of simple generalizations when using the term
'yeast'."* (Burger, Bacon, Bacon, and Millbank, 1965).[4]

I. INTRODUCTION

Even recent works on systematic carbohydrate chemistry state that
a given sugar is "not fermentable by ordinary yeasts" or is "slowly
fermented by yeasts." Such statements are often startlingly irrele-
vant, untrue, only partly true, or so imprecise as to be unhelpful. The
subject is important, as yeasts may conveniently be used to manipu-
late transformations of sugars or to remove unwanted sugars from
mixtures. By applying present knowledge and by advancing it, ex-
isting technological applications could be greatly extended. It is with

(3) [A. L.] Lavoisier, "Traité Élémentaire de Chimie," Cuchet, Libraire, Paris, 1789,
    p. 143.
(4) M. Burger, E. E. Baçon, J. S. D. Bacon, and J. W. Millbank, *Nature* (London), **205**,
    622–623 (1965).

such contexts in mind that, in this Chapter, something of what is known about the breakdown of sugars by yeasts is outlined.

In common with other living organisms, for biosynthetic processes necessary for growth, yeasts obtain energy from sugars by breaking them down. The energy set free is stored as the "high energy" phosphate derivative adenosine 5'-triphosphate (ATP) that is synthesized as the sugar is catabolized. In catabolism, glycosidic bonds are hydrolyzed to yield component monosaccharides. Hexoses and pentoses are degraded to glyceraldehyde 3-phosphate, and this to the acetyl group of acetyl coenzyme A; this acetyl group can be oxidized to carbon dioxide and water. The precise course of events depends on the circumstances, such as the availability of oxygen (that is, whether the conditions are aerobic or anaerobic). A low concentration of oxygen is often important for obtaining a high yield of ethanol from certain sugars. Particularly in initial and terminal reactions, differences are found between different yeasts, and such relatively minor biochemical differences are often of considerable practical importance.

Nearly all of these chemical transformations are catalyzed enzymically within the plasmalemma (inside the yeast cell-wall), which is the semipermeable membrane that encloses the protoplasm. Many of these enzymes are present in the cells, even under widely differing conditions of their growth, but a number of processes are involved in enzymic regulation and, thus, in the regulation of sugar catabolism. Some enzymes may be synthesized only in response to the presence of the sugar or of a structurally similar compound. Contrariwise, enzyme synthesis may be repressed by an increase in the concentration of ATP or of some other end-product. These enzyme inductions (for a review, see Ref. 5) and repressions (for a review, see Ref. 6) are two important kinds of control in metabolic regulation.

The utilization of sugars by yeasts has long been turned to account in making leavened bread, wine, and beer. An early industrial exploitation of microbial biochemistry was the manufacture of glycerol during the First World War by the addition of sulfite to a fermenting mixture of yeast and sugar [7-9]; for a review, see Ref. 10. Since then, a

(5) M. H. Richmond, in "Essays in Biochemistry," P. N. Campbell and G. D. Greville, eds., Academic Press, London, 1968, Vol. 4, pp. 105–154.

(6) K. Paigen and B. Williams, *Advan. Microbial Physiol.*, 4, 251–324 (1970).

(7) C. Neuberg and E. Reinfurth, *Biochem. Z.*, 89, 365–414 (1918).

(8) C. Neuberg and E. Reinfurth, *Biochem. Z.*, 92, 234–266 (1918).

(9) W. Connstein and W. Lüdecke, *Ber.*, 52, 1385–1391 (1919).

strain of *Torulopsis magnoliae* has been isolated which, without interference with metabolism, and without forming more than traces of other alditols, yields glycerol from up to 40% of the sugar utilized.[11] Patents have been taken out for the production by yeasts of other alditols, such as D-arabinitol[12-14]; for a review, see Ref. 15.

Sugars may be removed from citrus molasses, in preparing flavonoids, by *Saccharomyces cerevisiae*,[16] or from vitamin C concentrate by *Saccharomyces uvarum (carlsbergensis)*.[17] In certain tropical situations, *Kluyveromyces fragilis* may prove valuable to be grown as a source of protein, partly because of this yeast's ability to utilize the D-glucitol of surplus coconut milk (from the fruit of *Cocos nucifera*) which causes pollution when indiscriminately disposed of.[18] Yeasts are also commercial sources of enzymes of sugar metabolism, such as β-D-fructofuranosidase (EC 3.2.1.26)[19,20] or β-D-galactosidase (EC 3.2.1.23); for a review, see Ref. 21.

These are examples of a range of uses to which yeasts have already been put in relation to their utilization of sugars. This range will probably be much extended. As two examples: (*i*) *Sporobolomyces singularis* is capable of glycosyl transfers, to form such compounds as O-D-galactosyllactose and O-D-galactobiosyllactose from lactose[22,23];

(10) A. Harden, "Alcoholic Fermentation," Longmans, Green and Co., London, 4th Edition, 1932.

(11) G. J. Hajny, W. F. Hendershot, and W. H. Peterson, *Appl. Microbiol.*, 8, 5–11 (1960).

(12) O. Lavin and J. W. Holloway, Brit. Pat. 822,154 (1959); *Chem. Abstr.*, 54, 10,238a (1960).

(13) O. Lavin and J. W. Holloway, U. S. Pat. 2,934,474 (1960); *Chem. Abstr.*, 54, 16,738i (1960).

(14) J. C. J. Graham, Brit. Pat. 870,622 (1961); *Chem. Abstr.*, 55, 27,765d (1961).

(15) J. F. T. Spencer, *Progr. Ind. Microbiol.*, 7, 1–42 (1968).

(16) J. M. Sudarsky and R. A. Fisher, U. S. Pat. 2,984,601 (1961); *Chem. Abstr.*, 55, 18,007b (1961).

(17) P. Morse, U. S. Pat. 3,012,942 (1961).

(18) M. E. Smith and A. T. Bull, *Proc. Soc. Gen. Microbiol.*, 1, 24 (1973).

(19) W. Frommer, F. Ziegler, and E. Rauenbusch, Ger. Pat. 1,264,374 (1968); *Chem. Abstr.*, 70, 46,198u (1969).

(20) Most of the names used in this Chapter for enzymes are those recommended by the Commission on the Nomenclature and Classification of Enzymes, in "Comprehensive Biochemistry," M. Florkin and E. H. Stotz, eds., Elsevier, Amsterdam, 3rd Edition, 1973, Vol. 13. However, the Editors have altered some of the names to conform with established nomenclature for carbohydrates.

(21) J. S. Harrison, in "The Yeasts," A. H. Rose and J. S. Harrison, eds., Academic Press, London, 1970, Vol. 3, pp. 529–545.

(22) P. A. J. Gorin, J. F. T. Spencer, and H. J. Phaff, *Can. J. Chem.*, 42, 1341–1344 (1964).

(ii) yeasts may be used to remove specific sugars from mixtures, or a specific enantiomer from a racemate. Compared with bacteria or molds, yeasts are often remarkably easy to handle. Like bacteria, they may be homogeneous in culture, but they are usually easier to remove by centrifuging or filtering. Yeasts tend to be able to grow at a pH lower than those for bacteria, and also in the presence of many antibiotics. Consequently, it is usually practicable to keep cultures of yeasts free from fast-growing, contaminating micro-organisms. With a knowledge of the differing ability of over 400 species to utilize about 25 sugars, alditols, or glycosides,[24] the yeasts already offer a considerable technological potential.

The ability of yeasts to utilize sugars is not only of potential value; it can also be a nuisance.[25,26] Yeasts are notorious as spoilers of foods that contain a high concentration of one or more sugars, such as honey, maple syrup, sugar cane, and confectionery. The capacity of the yeasts *Saccharomyces bisporus* and *Saccharomyces rouxii* to ferment honey,[27,28] which is composed of 70 to 80% of hexoses,[29] is a feat of outstanding physiological interest in view of the remarkably high osmotic forces which the yeasts must withstand.[30–32]

II. THE DIFFERENT KINDS OF YEAST

Yeasts are fungi having a marked tendency to exist as separated cells. A work on the identification of yeasts[24] lists 434 species, classified into 41 genera. By and large, the genera are distinguished from each other by (i) their microscopical appearance, (ii) the way new cells are formed from old ones in growth, and (iii) whether or not sexual reproduction has been observed and, if so, the characteristics of that reproduction. The genera are listed in Table I.

Practical considerations may make such anatomical and sexual fea-

(23) P. A. J. Gorin, J. F. T. Spencer, and H. J. Phaff, *Can. J. Chem.*, 42, 2307–2317 (1964).
(24) J. A. Barnett and R. J. Pankhurst, "A New Key to The Yeasts," North-Holland Publishing Co., Amsterdam, 1974.
(25) M. Ingram, in "The Chemistry and Biology of Yeasts," A. H. Cook, ed., Academic Press, New York, 1958, pp. 603–633.
(26) H. W. Walker and J. C. Ayres, in Ref. 21, pp. 463–527.
(27) A. A. v. Richter, *Mycolog. Centralbl.*, 1, 67–76 (1912).
(28) A. G. Lochhead and D. A. Heron, *Can. Dept. Agr. Bull.*, No. 116 (1929).
(29) I. R. Siddiqui, *Advan. Carbohyd. Chem. Biochem.*, 25, 285–309 (1970).
(30) M. Ingram, *Symp. Soc. Gen. Microbiol.*, 7, 90–133 (1957).
(31) W. J. Scott, *Advan. Food Res.*, 7, 83–127 (1957).
(32) H. Ōnishi, *Advan. Food Res.*, 12, 53–94 (1963).

TABLE I

Alphabetic List of Yeast Genera and Some of their Characteristics

Genus (and approx. number of species)	Utilization of sugars		Comments	References
	Anaerobic	Aerobic		
Aessosporon (1)	none	several	a pink yeast, formerly named *Sporobolomyces salmonicolor*, having oval or elongate budding-cells; filamentous	33–35
Ambrosiozyma (5)	weak	several	some renamed, filamentous species of *Pichia* and *Endomycopsis* having budding and splitting cells of various shapes	36,37
Arthroascus (1)	none	D-glucose and a few other sugars are used weakly	formerly *Endomycopsis javanensis*; elongate budding-cells; markedly filamentous	37,38
Brettanomyces (9)	most ferment several sugars, often forming acetic acid	several	budding cells of varied shape; filamentous	24,39
Bullera (3)	none	several	spherical or oval budding-cells; not filamentous	24,40
Candida (104)	varied	varied	budding cells of varied shape; filamentous	24,41
Citeromyces (1)	ferments D-glucose, sucrose, and raffinose	several	oval or spherical budding-cells; not filamentous	42
Cryptococcus (19)	none	varied, but all use *myo*-inositol	spherical, oval, or elongate budding-cells, usually with a polysaccharide capsule; not filamentous	24,43
Cyniclomyces (1)	weak	few sugars	formerly *Saccharomycopsis guttulata*; occurs in alimentary canal of rabbits; grows in complex medium between 30 and 40°; difficult to maintain; oval or cylindrical cells with polar budding; not filamentous	33,44

Debaryomyces (10)	weak or none	varied	various shapes of budding cell; not filamentous	24,45
Dekkera (2)	fermentation occurs	use some sugars	formerly *Brettanomyces* species; various shapes of budding cells; not filamentous	46
Endomycopsis (10)	weak or none	varied	various shapes of budding cell; markedly filamentous. Genus no longer accepted; species placed in *Saccharomycopsis, Ambrosiozyma, Arthroascus, Guilliermondella, Hansenula, Hormoascus, Pichia,* and the filamentous (non-yeast) genus *Endomyces*	37,38,44
Filobasidium (2)	varied	many sugars	oval budding-cells; markedly filamentous	24,47
Guilliermondella (1)	ferments D-glucose slowly	few sugars	formerly *Endomycopsis selenospora;* oval budding-cells, markedly filamentous	37,38
Hanseniaspora (3)	ferments D-glucose and sometimes, cellobiose	few sugars	lemon shaped or oval cells with bipolar budding; not filamentous	48
Hansenula (28)	varied	varied	spherical, oval, or elongate budding-cells; some species are filamentous	24,49
Hormoascus (1)	weak	α- and β-D-glucopyranosides, D-xylose, D-ribose, alditols	formerly *Endomycopsis platypodis;* spherical budding-cells; markedly filamentous	37,38
Kloeckera (4)	few sugars	few sugars	lemon shaped, oval, or elongate cells with bipolar budding; not filamentous	50
Kluyveromyces (19)	all ferment some sugars	varied	13 species were once classified as *Saccharomyces;* spherical, oval, or elongate budding-cells; some species form filaments	24,51

(continued)

TABLE I (*continued*)

Genus (and approx. number of species)	Utilization of sugars		Comments	References
	Anaerobic	Aerobic		
Leucosporidium (7)	weak or none	varied	oval or elongate budding-cells; markedly filamentous	52
Lipomyces (3)	none	many sugars	oval or spherical budding-cells; older cells usually have a large, fat globule; not filamentous	53
Lodderomyces (1)	weak	several sugars	spherical, oval, or cylindrical budding-cells; filamentous	54
Metschnikowia (5)	varied	many sugars	spherical, oval, or cylindrical budding-cells; not filamentous	55
Nadsonia (2)	both ferment some sugars	varied	lemon shaped, oval, or elongate cells with bipolar budding; not filamentous	56
Nematospora (1)	usually weak	few sugars	a plant pathogen; budding cells of various shapes; filamentous	57
Oosporidium (1)	none	few sugars	formerly *Trichosporon margaritiferum*; budding cells of various shapes; not filamentous	58
Pachysolen (1)	few sugars	several sugars	spherical or oval budding-cells; not filamentous	59
Pichia (45)	varied	varied	various shapes of budding cell; most are filamentous	24,60
Pityrosporum (3)	none	? (these yeasts are difficult to cultivate)	isolated from mammals; require lipids; spherical or oval cells with monopolar budding; not filamentous	61
Rhodosporidium (2)	none	many sugars	pink yeasts; spherical, oval, or elongate budding-cells; some are filamentous	62

Rhodotorula (10)	none	usually several sugars	common aerial contaminants; spherical, oval, or elongate budding-cells; not filamentous	24,63
Saccharomyces (42)	all species ferment some sugars	varied	spherical, oval, or elongate budding-cells; some are a little filamentous	24,64
Saccharomycodes (1)	ferments D-glucose, sucrose, and raffinose	uses β-D-glucopyranosides, sucrose, and raffinose	large, lemon-shaped or elongate cells with bipolar budding; not filamentous	65
Saccharomycopsis either A			see *Cyniclomyces*	
or B (4)	weak or none	varied	formerly species of *Endomycopsis* and *Candida;* oval or elongate budding-cells; filamentous	24,37,44,66,67
Schizoblastosporion (1)	none	few sugars	oval or cylindrical cells with bipolar budding; not filamentous	68
Schizosaccharomyces (4)	all species ferment some sugars	only aldohexoses, β-D-fructofuranosides, and α-D-glucopyranosides	spherical or cylindrical cells reproduce by fission; one species is filamentous	69
Schwanniomyces (4)	all species ferment some sugars	varied	oval, spherical, or elongate budding-cells; not filamentous	70
Selenotila (2)	some sugars fermented	use many sugars	crescentic, oval, or spherical budding-cells; not filamentous	24,71
Sporodiobolus (2)	none	use several sugars	pink yeasts, oval or elongate budding-cells; filamentous	72
Sporobolomyces (11)	none	varied	pink yeasts, oval or elongate budding-cells; some are filamentous	24,33
Sterigmatomyces (5)	none	varied	spherical or oval cells with buds on sterigmata; not filamentous	24,73,74
Sympodiomyces (1)	none	several	spherical or oval cells with conidia; filamentous	24,75

TABLE I (*continued*)

Genus (and approx. number of species)	Utilization of sugars		Comments	References
	Anaerobic	Aerobic		
Syringospora (3)	some sugars fermented	varied	name given recently to 3 species of *Candida;* round, oval, or elongate budding-cells; filamentous	76
Torulopsis (51)	varied	varied	spherical, oval, or elongate budding-cells; not filamentous	24,77
Trichosporon (12)	varied	varied	spherical, oval, or elongate budding-cells; markedly filamentous	24,78
Trigonopsis (1)	none	few sugars	three-cornered or oval cells; not filamentous	79
Wickerhamia (1)	some sugars fermented	few sugars	oval or elongate cells with bipolar budding; not filamentous	80
Wickerhamiella (1)	none	few sugars	name given recently to *Torulopsis domercqii;* small, oval, or spherical budding-cells; not filamentous	81
Wingea (1)	some sugars fermented	many sugars	formerly *Pichia robertsii;* spherical or oval budding-cells; not filamentous	82

(33) H. J. Phaff, in "The Yeasts. A Taxonomic Study," J. Lodder, ed., North-Holland Publishing Co., Amsterdam, 2nd Edition, 1970, pp. 831–862.

(34) J. P. van der Walt, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 36, 49–55 (1970). In subsequent references to this journal in this Chapter, the "abbreviation" of the title will be shortened to *A. v. L., J. Microbiol. Serol.*

(35) J. W. Fell, in "Recent Trends in Yeast Research," D. G. Ahearn, ed., School of Arts and Sciences, Georgia State University, Atlanta, Georgia, 1970, pp. 49–66.

(36) J. P. van der Walt, *Mycopathol. Mycol. Appl.*, 46, 305–316 (1972).

(37) J. A. von Arx, *A. v. L., J. Microbiol. Serol.*, 38, 289–309 (1972).

(38) N. J. W. Kreger-van Rij, in Ref. 33, pp. 166–208.

(39) J. P. van der Walt, in Ref. 33, pp. 863–892.

(40) H. J. Phaff, in Ref. 33, pp. 815–821.

(41) N. van Uden and H. Buckley, in Ref. 33, pp. 893–1087.

(42) L. J. Wickerham, in Ref. 33, pp. 121–127.

(43) H. J. Phaff and J. W. Fell, in Ref. 33, pp. 1088–1145.

(44) J. P. van der Walt and D. B. Scott, *Mycopathol. Mycol. Appl.*, 43, 279–288 (1971).

(45) N. J. W. Kreger-van Rij, in Ref. 33, pp. 129–156.

(46) J. P. van der Walt, in Ref. 33, pp. 157–165.

(47) L. Rodrigues de Miranda, *A. v. L., J. Microbiol. Serol.*, 38, 91–99 (1972).

(48) H. J. Phaff, in Ref. 33, pp. 209–225.

(49) L. J. Wickerham, in Ref. 33, pp. 226–315.

(50) H. J. Phaff, in Ref. 33, pp. 1146–1160.

(51) J. P. van der Walt, in Ref. 33, pp. 316–378.

(52) J. W. Fell and H. J. Phaff, in Ref. 33, pp. 776–802.

(53) W. C. Slooff, in Ref. 33, pp. 379–402.

(54) J. P. van der Walt, in Ref. 33, pp. 403–407.

(55) M. W. Miller and N. van Uden, in Ref. 33, pp. 408–429.

(56) H. J. Phaff, in Ref. 33, pp. 430–439.

(57) L. do Carmo-Sousa, in Ref. 33, pp. 440–447.

(58) L. do Carmo-Sousa, in Ref. 33, pp. 1161–1166.

(59) L. J. Wickerham, in Ref. 33, pp. 448–454.

(60) N. J. W. Kreger-van Rij, in Ref. 33, pp. 455–554.

(61) W. C. Slooff, in Ref. 33, pp. 1167–1186.

(62) J. W. Fell, H. J. Phaff, and S. Y. Newell, in Ref. 33, pp. 803–814.

(63) H. J. Phaff and D. G. Ahearn, in Ref. 33, pp. 1187–1223.

(64) J. P. van der Walt, in Ref. 33, pp. 555–718.

(65) H. J. Phaff, in Ref. 33, pp. 719–724.

(66) L. J. Wickerham, C. P. Kurtzman, and A. I. Herman, in Ref. 35, pp. 81–92.

(67) D. Yarrow, *A. v. L., J. Microbiol. Serol.*, 38, 357–360 (1972).

(68) N. J. W. Kreger-van Rij, in Ref. 33, pp. 1224–1228.

(69) W. C. Slooff, in Ref. 33, pp. 733–755.

(70) H. J. Phaff, in Ref. 33, pp. 756–766.

(71) D. Yarrow, *A. v. L., J. Microbiol Serol.*, 35, 418–420 (1969).

(72) H. J. Phaff, in Ref. 33, pp. 822–830.

(73) J. W. Fell, in Ref. 33, pp. 1229–1234.

(74) R. W. M. Buhagiar and J. A. Barnett, *J. Gen. Microbiol.*, 77, 71–78 (1973).

(75) J. W. Fell and A. C. Statzell, *A. v. L., J. Microbiol. Serol.*, 37, 359–367 (1971).

(76) J. P. van der Walt, *Mycopathol. Mycol. Appl.*, 40, 231–243 (1970).

(77) N. van Uden and M. Vidal-Leiria, in Ref. 33, pp. 1235–1308.

(78) L. do Carmo-Sousa, in Ref. 33, pp. 1309–1352.

tures important when selecting a yeast for its chemical abilities. For example, a yeast that is particularly liable to grow filamentously may be easier to filter than another growing as separate cells. On the other hand, it may be more difficult to assess the mass of the filamentous yeast photometrically and also, perhaps, to control its growth. In addition, the exact mode of sexual reproduction may well affect the choice of yeast if a series of mutants is required to carry out different chemical changes; this is because it may be important to be able to do tests of genetical segregation in order to determine the nature of the changes. Information on such biological features of each species is given in Lodder's great taxonomic work,[83] and characteristics of more recently described species are outlined in another monograph.[24] Yeast systematics has been reviewed by Kreger-van Rij.[84,85]

Within genera that are fairly homogeneous morphologically, such as *Saccharomyces* or *Torulopsis*, the species differ from each other notably in regard to which compounds they can utilize for growth or "fermentation." On the other hand, within some genera, such as *Candida* or *Trichosporon*, many species differ from each other greatly, both nutritionally and morphologically.

Each species is made up of one or more strains. For example, by November, 1972, only one strain of *Brettanomyces abstinens* had been reported. By contrast, very many strains are known of the baking and brewing species, *Saccharomyces cerevisiae:* the 1970 edition of the catalogue of the National Collection of Yeast Cultures (NCYC)[86] lists 357 strains of this species. A strain is a culture and its subcultures, derived from a single isolated cell (*cf.,* Refs. 87 and 88).

(79) W. C. Slooff, in Ref. 33, pp. 1353–1357.

(80) H. J. Phaff, in Ref. 33, pp. 767–771.

(81) J. P. van der Walt and N. V. D. W. Liebenberg, A. v. L., J. Microbiol. Serol., 39, 121–128 (1973).

(82) J. P. van der Walt, in Ref. 33, pp. 316–378.

(83) J. Lodder, ed., "The Yeasts. A Taxonomic Study," North-Holland Publishing Co., Amsterdam, 2nd Edition, 1970.

(84) N. J. W. Kreger-van Rij, in Ref. 21, 1969, Vol. 1, pp. 5–78.

(85) N. J. W. Kreger-van Rij, in "The Fungi. An Advanced Treatise," G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman, eds., Academic Press, New York, 1973, Vol. IVA, pp. 11–32.

(86) National Collection of Yeast Cultures, Catalogue of Cultures Maintained at the Brewing Industry Research Foundation, Nutfield, Surrey, 1970.

(87) R. E. Buchanan, R. S. John-Brooks, and R. S. Breed, J. Gen. Microbiol., 3, 444–462 (1949).

(88) S. T. Cowan, "A Dictionary of Microbial Taxonomic Usage," Oliver and Boyd, Edinburgh, 1968, p. 99.

The descendants of the original cell should be produced asexually, that is to say, by vegetative cell-division. Thus, all sub-cultures of a single strain should have the same genetic constitution, except insofar as mutation occurs amongst them, and changes in abilities to utilize sugars are, indeed, sometimes observed in cultures of yeasts.[89,90] So, microbial strains are comparable to named varieties of cultivated higher plants, such as the apple (*Malus pumila*), Bramley's Seedling.

Most yeast strains are specified by number. For example, two strains of *Saccharomyces cerevisiae* are NCYC 77 and CBS (Centraalbureau voor Schimmelcultures) 1234. In a research publication, strain numbers should always be cited, because, firstly, there are often relevant, intraspecific differences between strains and, secondly, although taxonomic authorities often change the names of the yeasts, the strain number does not change.

For much work on chemical activities of micro-organisms, it is important to use strains having known relevant genetical characteristics. Unfortunately, however, strains of only two species, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, have as yet been subjected to extensive genetic analysis, and neither species can utilize a wide range of exogenous sugars.

The information given in this Chapter is based chiefly on studies with relatively few kinds of yeast, the most popular of which have been *Saccharomyces cerevisiae*, *Saccharomyces uvarum* (synonymous with *Saccharomyces carlsbergensis*), *Candida utilis* (that is, *Torulopsis* or *Torula utilis*), and *Kluyveromyces* (*Saccharomyces*) *fragilis*.[91]

III. THE COMPARTMENTS OF YEAST CELLS

The salient, anatomical structures of a yeast cell (see Fig. 1) are (*i*) the cell wall, (*ii*) the plasmalemma, (*iii*) the vacuole, (*iv*) the nucleus, and (*v*) the endoplasmic reticulum. There are also a number of small organelles, such as mitochondria. The membranes that enclose each of these components form functional compartments of the cells, whose activities require that various internal, chemical changes should be separated physically. The parts of the cells are described

(89) R. Scheda and D. Yarrow, *Arch. Mikrobiol.*, **55**, 209–225 (1966).
(90) R. Scheda and D. Yarrow, *Arch. Mikrobiol.*, **61**, 310–316 (1968).
(91) The names of yeasts used in this Chapter are those given by Lodder[83] or Barnett and Pankhurst.[24] When the name given here differs from that used by the author whose work is cited, the latter name is given in parentheses.
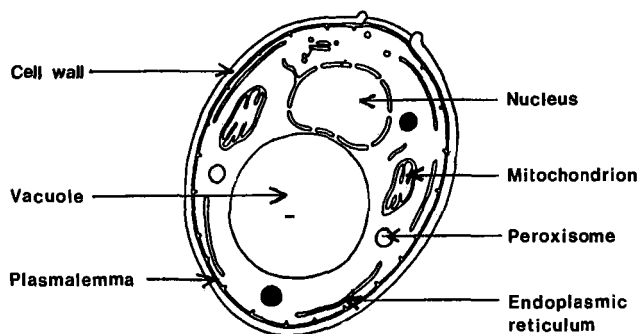
FIG. 1.—Anatomy of a Yeast Cell (from Matile[92]).

next, but only to make clear their various roles relevant to the way in which yeasts utilize sugars. The cytology of yeasts has been authoritatively reviewed in Ref. 93.

1. The Cell Wall

The cell walls of yeasts maintain the characteristic shapes of the cells. The walls vary in thickness with the kind of yeast, as well as with the age, nutrition, and treatment of the cells. Yeast cell-walls may be only[94] ~ 70 nm or as much[95] as 200 nm thick, constituting about 30% of the dry mass of the cells.[96] Yeast cell-walls probably form a filter through which very large molecules cannot pass. For example, dextran molecules having a molecular weight higher than 4,500 were found not to penetrate the isolated walls of bakers' yeast.[97] Ottolenghi,[98] however, has shown that a strain of Saccharomyces can take up bovine albumin.

The cell wall of Saccharomyces cerevisiae has a structure of crossed molecules of β-D-linked D-glucan, which gives the wall its strength and the cell its shape. This glucan is embedded in other polysaccharide(s) or in glycoprotein. Thus, yeast cell-walls consist mainly of polysaccharide, with relatively little protein, lipid, or min-

(92) P. Matile, A. v. L., J. Microbiol. Serol., 35 (Suppl.), 59–70 (1969).
(93) P. Matile, H. Moor, and C. F. Robinow, in Ref. 21, 1969, Vol. 1, pp. 219–302.
(94) H. Moor and K. Mühlethaler, J. Cell Biol., 17, 609–628 (1963).
(95) H. Hagedorn, Protoplasma, 58, 250–268 (1964).
(96) G. Falcone and W. J. Nickerson, Science, 124, 272–273 (1956).
(97) P. Gerhardt and J. A. Judge, J. Bacteriol., 87, 945–951 (1964).
(98) P. Ottolenghi, C. R. Trav. Lab. Carlsberg, 36, 95–111 (1967).

eral. All of these components vary from one yeast to another, as, for example, has been found for the amino acids of cell-wall proteins of 30 yeasts.[99] Quantitatively minor components of the cell wall may be of major importance functionally; this is probably true for the protein, for example, as mannan–protein complexes may have essential, enzymic roles.

Usually, the polysaccharide is chiefly D-glucan and D-mannan, although small proportions of other monosaccharide residues may be present. Usually, too, the D-glucan is mainly β-D-(1→ 3)-linked, but, in some yeasts, an α-D-(1 → 3)-linked D-glucan is also present and may preponderate. In the β-D-(1 → 3)-linked D-glucan, some of the units carry branches on C-6, whereas the mannans have a more highly ramified structure. This structure is often based on an α-D-(1 → 6)-linked backbone chain having branches on nearly every secondary carbon atom, and which usually contain α-D-(1 → 2)- and α-D-(1 → 3)-links between the residues, although mannans having β-D-links also occur. D-Glucan that is β-D-(1 → 3)-linked, apart from where it branches, ordinarily forms most of the inner part of the wall. Some of this glucan may contain a small proportion of chains formed by β-D-(1 → 6) links. Chitin usually composes a few percent of the total content of the wall. The mannan is usually in the outer region of the wall; protein and phosphate are connected with this fraction in particular.

The constituents together form a complex network, and any of the separable polysaccharide fractions may be absent, or vary qualitatively and quantitatively from one kind of yeast to another. In particular, there are qualitative differences between the mannans of various species. Major structural differences are also found, because, unlike ascosporogenous yeasts, such as *Saccharomyces* species, basidiomycetous yeasts[100] appear to have multilayered walls.[101,102]

The composition of yeast cell-walls was reviewed very fully by Phaff,[103] later by Bonaly,[103a] and the yeast mannans by Ballou.[104]

(99) D. J. Stewart and S. Widanapatirana, *Microbios*, 9, 167–172 (1974).

(100) These comprise the following genera: *Aessosporon, Leucosporidium, Rhodosporidium, Sporidiobolus, Filobasidium, Rhodotorula, Cryptococcus, Sterigmatomyces, Sporobolomyces,* and *Bullera.*

(101) N. J. W. Kreger-van Rij and M. Veenhuis, *A. v. L., J. Microbiol. Serol.*, 37, 253–255 (1971).

(102) N. J. W. Kreger-van Rij and M. Veenhuis, *J. Gen. Microbiol.*, 68, 87–95 (1971).

(103) H. J. Phaff, in Ref. 21, 1971, Vol. 2, pp. 135–210.

(103a) R. Bonaly, *Sci. Pharm. Biol. Lorraine*, 2, 25–40 (1974).

(104) C. E. Ballou, *Advan. Enzymol. Relat. Areas Mol. Biol.*, 40, 239–270 (1974).

Work on glucan and mannan structures is described in Refs. 105–108a. Taylor and Cameron[109] have reviewed the preparation and quantitative analysis of fungal cell-walls.

Walls can be disintegrated by means of enzymic preparations, such as those from digestive juices of the snail,[110] from *Cytophaga johnsonii*,[111–113] or from other micro-organisms.[103] If this wall break-down is conducted in a buffered solution containing a high concentration of solute, such as 0.8 *M* $MgSO_4$ or 0.6 *M* KCl, the resultant "spheroplasts" or "protoplasts" are cells, respectively lacking most or all of the cell wall, held together by their plasmalemmata (for reviews, see Refs. 110, 114, and 115.) Protoplasts have many of the characteristics of intact cells, but they are spherical and burst in solutions having low osmotic pressures at which the corresponding, intact cells may function optimally. In suitable culture-media, protoplasts may synthesize incomplete or complete new cell-walls (for a review, see Ref. 116); in the former case, a glucan is synthesized by *Saccharomyces cerevisiae* which is different from that of normal walls in ultrastructure and in constitution.[117]

2. The Plasmalemma

The plasmalemma, cytoplasmic membrane, or cell membrane is ~8 nm thick.[118] It is the protective barrier of the part of the cell

(105) R. Sentandreu and M. V. Elorza, in "Yeast, Mould and Plant Protoplasts," J. R. Villanueva, I. García-Acha, S. Gascón, and F. Uruburu, eds., Academic Press, London, 1973, pp. 187–204.

(106) D. J. Manners, A. J. Masson, and J. C. Patterson, *Biochem. J.*, 135, 19–30 (1973).

(107) D. J. Manners, A. J. Masson, and J. C. Patterson, *J. Gen Microbiol.*, 80, 411–417 (1974).

(108) C. E. Ballou and W. C. Raschke, *Science*, 184, 127–134 (1974).

(108a) T. Nakajima and C. E. Ballou, *J. Biol. Chem.*, 249, 7679–7684 (1974).

(109) I. E. P. Taylor and D. S. Cameron, *Annu. Rev. Microbiol.*, 27, 243–259 (1973).

(110) J. R. Villanueva and I. García-Acha, *Methods Microbiol.*, 4, 665–718 (1971).

(111) J. S. D. Bacon, B. D. Milne, I. F. Taylor, and D. M. Webley, *Biochem. J.*, 95, 28C–30C (1965).

(112) J. K. Baird and W. L. Cunningham, *Biochem. J.*, 125, 32P–33P (1971).

(113) J. S. D. Bacon, in Ref. 105, pp. 61–74.

(114) J. R. Villanueva, in Ref. 85, G. C. Ainsworth and A. S. Sussman, eds., Academic Press, New York, 1966, Vol. II, pp. 3–62.

(115) J. R. Villanueva, I. García-Acha, S. Gascón, and F. Uruburu, eds., "Yeast, Mould and Plant Protoplasts," Academic Press, London, 1973.

(116) O. Nečas, *Bacteriol. Rev.*, 35, 149–170 (1971).

(117) D. R. Kreger and M. Kopecká, in Ref. 115, pp. 117–130.

(118) E. Vitols, R. J. North, and A. W. Linnane, *J. Biophys. Biochem. Cytol.*, 9, 689–699 (1961).

within the cell wall, and it controls the entry and exit of solutes. In *Saccharomyces cerevisiae*[119,120] and *Candida utilis*,[121] the plasmalemma contains 40–50% of protein, ~40% of lipid, and 5% of carbohydrate which may have come from remains of the cell walls. The structure of the plasmalemma in yeast cells is reviewed in Refs. 93, 122, and 123. Arnold[124] calculated that the width of the periplasmic space (between the plasmalemma and the cell wall) is ~10 nm, and the volume about 1 fl.

3. The Vacuole

The vacuole, often the largest organelle, is limited by a single membrane, the *tonoplast*. There is controversy about what the vacuole does. Three functions are attributed to it, namely, (*i*) osmotic regulation; (*ii*) storage of such compounds as amino acids, purines, sugars, and phosphate; and (*iii*) that of a *lysosome*, a compartment in which breakdown reactions of such macromolecules as proteins and nucleic acids are separated from active synthesis in the cytoplasm. The subject is reviewed in Refs. 93 and 122.

4. The Endoplasmic Reticulum

In the cytoplasm, the endoplasmic reticulum is a complex system of double membranes with a lumen ~20 nm wide. These membranes are so fine that they are only visible by electron microscopy. They are probably associated with nearly every organelle, having connections with the nuclear membrane, the plasmalemma, and the mitochondria (for a review, see Ref. 93). It would be surprising were the endoplasmic reticulum not an important structure for metabolic compartmentation.

5. The Other Organelles

The nucleus, mitochondria, and other organelles are functionally comparable to those of many other organisms. The nucleus carries nearly all of the inherited characteristics of each yeast cell, control-

(119) A. A. Boulton, *Exp. Cell Res.*, 37, 343–359 (1965).

(120) R. P. Longley, A. H. Rose, and B. A. Knights, *Biochem. J.*, 108, 401–412 (1968).

(121) C. García Mendoza and J. R. Villanueva, *Biochim. Biophys. Acta*, 135, 189–195 (1967).

(122) K. Hunter and A. H. Rose, in Ref. 21, 1971, Vol. 2, pp. 211–270.

(123) R. Marchant and D. G. Smith, *Biol. Rev. Cambridge Phil. Soc.*, 43, 459–480 (1968).

(124) W. N. Arnold, *Physiol. Chem. Phys.*, 5, 117–123 (1973).

ling both its anatomical and physiological potentialities. The mitochondria are spherical, rod-shaped, or thread-like, and they may be branched. They are 0.3 to 1 μm in diameter, and up to ~5 μm long. As in plants and animals, the mitochondria contain many enzyme systems concerned with the oxidation of substrates, and electron-transporting systems connected with transferring the free energy of oxidative reactions to give ATP. Mitochondria are self-replicating and contain nucleic acids. Peroxisomes,[125,126] fragile bodies that allow of a certain metabolic compartmentation, are found in yeasts.[127] All of these organelles are of major importance in the functioning of yeast cells, but their operation is beyond the scope of this Chapter.

IV. CRITERIA FOR THE UTILIZATION
OF SUGARS BY YEASTS

What is meant when we say that a yeast utilizes a sugar? Or, rather, how do we decide that a yeast is utilizing a sugar? Criteria for utilization are (*i*) aerobic growth of the yeast in the presence, but not in the absence, of the sugar; (*ii*) a higher rate of oxygen uptake by the yeast with the sugar than without it; and (*iii*) a higher output of carbon dioxide and ethanol with it than without it.

1. Aerobic Growth

An increase both in the total mass of the yeast and in the number of yeast cells, occurring together, is here called "growth." Certain conditions, external to the yeast cells, are necessary for their rapid and sustained growth. These conditions usually include the presence in aqueous solution of (*i*) a source of carbon, such as a sugar; (*ii*) a source of nitrogen, such as ammonia; (*iii*) oxygen; (*iv*) salts, such as phosphate; and (*v*) elements in trace amounts, such as manganese. Many yeasts also require an external supply of one or more vitamins, such as biotin.

With a knowledge of their particular requirements, it should be practicable to make for most yeasts a chemically defined medium in which there can be nearly maximal rates of growth. Often, however, faster growth occurs in a medium such as that containing malt extract, yeast extract, and peptone, with D-glucose as the main source of

(125) C. J. Avers, *Sub-Cell. Biochem.*, 1, 25–37 (1971).
(126) P. J. Rogers and P. R. Stewart, *J. Gen. Microbiol.*, 79, 205–217 (1973).
(127) C. J. Avers and M. Federman, *J. Cell Biol.*, 37, 555–559 (1968).

carbon.[128] The optimal temperature for growth of yeasts is often[129] about 25°, and the optimal pH may lie between 4 and 6.

Every strain of yeast may be expected to grow at a different rate, which will change if any of the conditions just mentioned are changed. Many yeasts can, under some conditions, double their mass in as little as two hours, and a number can grow even faster.

The ability of a yeast to utilize a compound as the sole source of carbon for growth may be tested in a chemically defined medium that lacks any organic compound, except perhaps for certain vitamins. The organic compound to be tested may be added to the medium at a concentration of, for example, 50 mM. Practical aspects of studying the aerobic growth of micro-organisms are summarized in Ref. 130.

2. Aerobic Respiration

The term "respiration" is used here for the whole process by which yeasts oxidize organic compounds, liberating energy that goes into building up molecules of ATP. The external signs of respiration are (i) disappearance of the organic substrate from the aqueous medium in which the yeast is suspended, (ii) uptake of oxygen by the yeast, and (iii) formation by the yeast of carbon dioxide.

If yeast from an actively growing culture is centrifuged, washed in buffer or water and, after re-centrifugation, resuspended in a suitable buffer, the yeast will usually take up oxygen and give off carbon dioxide quite fast, for example, ~ 5 μmoles/mg of dry weight of yeast/hour at 25°. If this washed yeast is aerated in the buffer at 25° for about two hours, its rate of oxygen uptake will often fall from 5 to, for example, 0.1 or 0.2, as the endogenously stored carbohydrate is oxidized mainly to carbon dioxide and water. Added to such starved yeast, D-glucose will usually stimulate respiration, to give a respiratory rate of the same order as that of the unstarved yeast. Thus, it is convenient to measure the rate of respiration of an organic substrate with washed, starved yeast (see, for example, Ref. 131). With unstarved yeast, the endogenous rate (that is, without added substrate) is usually so high as to mask any effect of the addition of an external source of carbon.

(128) L. J. Wickerham, *U. S. Dept. Agr., Tech. Bull.*, **1029**, 1–56 (1951).
(129) J. L. Stokes, in Ref. 21, 1971, Vol. 2, pp. 119–134.
(130) G. G. Meynell and E. Meynell, "Theory and Practice in Experimental Bacteriology," University Press, Cambridge, 2nd Edition, 1970.
(131) J. A. Barnett and H. L. Kornberg, *J. Gen. Microbiol.*, **23**, 65–82 (1960).

TABLE II

Rates of Respiration[a] on D-Glucose, Cellobiose, or
Salicin as Substrates, by Intact, Washed,
Starved Cells of the Yeast *Kloeckera
africana* (Strain NCYC 26)[b]

	Carbon source for growth	
Respiratory substrate	Cellobiose	D-Glucose
D-Glucose	4.9	4.6
Cellobiose	4.9	<0.1
Salicin	4.6	<0.1

[a] The rates are given as μmoles of oxygen con-
sumed/mg of dry weight of yeast/hour. [b] J. A. Barnett,
unpublished results.

The rate at which a given sugar is respired by a washed, starved
suspension of yeast depends on (*i*) the strain of yeast, (*ii*) the com-
position of the medium in which the yeast has been grown, in-
cluding the source of carbon, and (*iii*) the stage of growth at which
the yeast is harvested. Table II illustrates an effect of varying the
carbon source for growth on the ability of a yeast to respire different
substrates: here, respiration of the β-D-glucopyranosides cellobiose
and salicin was either *induced* by cellobiose in the growth medium
or *repressed* by D-glucose, or both. Induction or repression may
operate by affecting the synthesis of one or more catabolic enzymes,
in this case probably a β-D-glucosidase (EC 3.2.1.21), or a carrier
that takes the substrate across the plasmalemma into the cell. When
studying the mechanisms responsible for the utilization of a com-
pound by a given strain of yeast, it may be important to be able to
compare induced cells with those that are non-induced or repressed.

3. Anaerobic Fermentation

Many yeasts can utilize certain sugars and derivatives of sugars
anaerobically. This anaerobic utilization is here called "fermenta-
tion." The formation of gas, presumably carbon dioxide, is usually
the most obvious sign of fermentation.

Saccharomyces cerevisiae has been reported capable of slow, an-
aerobic growth.[132-139] From results of experiments with a baking

(132) F. Windisch, Z. *Physiol. Chem.*, 179, 88–98 (1928).
(133) F. Windisch, *Biochem. Z.*, 246, 332–382 (1932).

yeast,[138] the maximum anaerobic growth-rate can be estimated as a doubling time of about 6 hours for about 5 generations. However, another baking strain has since been said to grow fast anaerobically with a doubling time of as little as one hour on D-glucose,[140,141] going through 23 generations in 38 hours.[142] This strain was said to grow more slowly under aerobic conditions.[143] Sols and his colleagues have discussed[144] the main pathways by which D-glucose may be catabolized in anaerobically growing yeast.

4. Other Criteria for Utilization

Other criteria for utilization of sugars that may be employed are (*i*) acidification of the medium in which the yeast is suspended, (*ii*) disappearance from the medium of the sugar being tested, and (*iii*) incorporation of carbon-14 or tritium from a so-labelled sugar into the yeast.

V. THE SUGARS UTILIZED BY YEASTS

Table III shows the numbers of yeast species able or unable to utilize each of various sugars and their derivatives. This information comes chiefly from a survey[83] made for the purpose of classifying and identifying yeasts. A detailed summary has been given by Barnett and Pankhurst.[24] The tests used to provide this information were crude and unquantitative, but the results constitute by far the widest survey ever done, both for numbers of yeasts and for compounds surveyed. The results given as positive or negative should be repeatable; those that seemed unreliable have been listed in Table III under the "?" category. However, it must be emphasized that there is a considerable difference between a yeast that doubles its mass in

(134) M. C. Brockmann and T. J. B. Stier, *J. Cellular Comp. Physiol.*, **29**, 1–14 (1947).
(135) T. J. B. Stier, R. E. Scalf, and C. J. Peter, *J. Cellular Comp. Physiol.*, **36**, 159–163 (1950).
(136) P. Slonimski, *Actualités Biochim.*, **17**, 1–203 (1953); *Chem. Abstr.*, **49**, 12,618*i* (1955).
(137) J. White and D. J. Munns, *Wallerstein Lab. Commun.*, **14**, 199–221 (1951).
(138) J. White, "Yeast Technology," Chapman and Hall, London, 1954.
(139) M. H. David and B. H. Kirsop, *J. Gen. Microbiol.*, **77**, 529–531 (1973).
(140) E. R. Tustanoff and W. Bartley, *Biochem. J.*, **91**, 595–600 (1964).
(141) W. Bartley and V. Broomhead, *Biochem. J.*, **121**, 461–467 (1971).
(142) E. R. Tustanoff and W. Bartley, *Can. J. Biochem.*, **42**, 651–665 (1964).
(143) E. S. Polakis and W. Bartley, *Biochem. J.*, **97**, 284–297 (1965).
(144) A. Sols, C. Gancedo, and G. De la Fuente, in Ref. 21, 1971, Vol. 2, pp. 271–307.

TABLE III

Abilities of 434 Species of Yeast to Utilize Sugars and their Derivatives
Aerobically or Anaerobically[a]

Sugar	Aerobic growth[b]			Anaerobic fermentation[b]		
	+	−	?	+	−	?
D-Glucose	434	0	0	229	132	73
D-Galactose	214	160	60	54	300	80
L-Sorbose	109	204	121	0	434	0
Sucrose	264	136	34	109	272	53
Maltose	226	167	41	40	332	62
Cellobiose	217	168	49	12	296	126
α,α-Trehalose	279	98	57	34	229	171
Lactose	47	348	39	6	422	6
Melibiose	51	362	21	17	337	80
Raffinose	148	234	52	67	286	81
Melezitose	153	231	50	6	297	131
D-Xylose	214	157	63	0	434	0
L-Arabinose	111	246	77	0	434	0
D-Arabinose	32	314	88	0	434	0
D-Ribose	86	240	108	0	434	0
L-Rhamnose	70	321	43	0	434	0
Glycerol	259	62	113	0	434	0
Erythritol	94	324	16	0	434	0
Ribitol	164	181	89	0	434	0
Galactitol	27	369	38	0	434	0
D-Mannitol	287	86	61	0	434	0
D-Glucitol	267	91	76	0	434	0
Methyl α-D-glucopyranoside	143	222	69	5	229	200
myo-Inositol	32	382	20	0	434	0
Arbutin	113	100	221	0	0	434
Salicin	206	167	61	0	0	434

[a] Information derived from the compilation by Barnett and Pankhurst.[24] [b] The symbol +: sugar utilized; −: sugar not utilized. Under the symbol "?" are those species for which a definite + or − cannot be given for one or more of the following reasons: (i) some strains of the species are + and others are −; (ii) utilization was described by the original authors with an equivocating qualification, such as "weak"; or (iii) there is no information on that species. Figures in the Table are the numbers of the species.

one hour and another that takes, for example, 20 hours under the same conditions. Both yeasts might be listed as "+".

Table III indicates, for example, that (i) all species of yeast utilize D-glucose, and more than half of them can do so anaerobically; (ii) more than half the species utilize aerobically any one of the following: cellobiose, D-glucitol, glycerol, maltose, D-mannitol, sucrose, or

trehalose; and (*iii*) fewer than 10% of species utilize D-arabinose, galactitol, or *myo*-inositol.

The information in Table III also indicates, for example, that 314 species of yeast are known that utilize D-glucose aerobically, but do not similarly utilize D-arabinose. Hence, any of these yeasts might be useful for removing D-glucose from a mixture of D-glucose and D-arabinose. The names of these 314 yeasts can be found from a table in the monograph of Barnett and Pankhurst,[24] from which it is also possible to select a yeast with a more stringent specification, such as the combination D-glucose +, D-arabinose −, D-xylose +, L-arabinose −, and so on. However, should it be required to select one from a large number of yeasts (*a*) it is recommended that, if practicable, a strain should be chosen that some workers have already put to practical use, and (*b*) advice can usually be obtained from a national yeast-culture collection where thousands of strains are handled regularly.

Yeasts are known that utilize sugars or alditols other than those listed in Table III, such as D- and L-arabinitol, D-fructose, D-mannose, xylitol, and L-xylose. In 1905, Armstrong[145] stated that, without exception, yeasts which ferment D-glucose, also ferment D-fructose and D-mannose; this was confirmed by the later surveys of Stelling-Dekker,[146] Lodder,[147] and Diddens and Lodder.[148] All of the many yeasts that Lodder[147,148] subjected to growth-tests utilized, aerobically, D-fructose and D-mannose, as well as D-glucose.

VI. The Entry of Sugars into Yeast Cells

1. Introduction

Certain glycosides are hydrolyzed outside the plasmalemma. With this exception, the first step in sugar utilization is movement of the sugar across the plasmalemma, a process that generally occurs by means of a carrier associated with the plasmalemma. Without such carriers, the plasmalemma is impermeable to sugars.[149] These carri-

(145) E. F. Armstrong, *Proc. Roy. Soc., Ser. B*, **76**, 600–605 (1905).
(146) N. M. Stelling-Dekker, *Verhandel. Koninkl. Ned. Akad. Wetenschap. Afdel. Natuurk., Sect. II*, **28**, 1–547 (1931).
(147) J. Lodder, "Die Anaskosporogenen Hefen," N. V. Noord-Hollandsche Uitgeversmaatschappij, Amsterdam, 1934.
(148) H. A. Diddens and J. Lodder, "Die Anaskosporogenen Hefen. Zweite Hälfte," N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, 1942.
(149) W. D. Stein, "The Movement of Molecules Across Cell Membranes," Academic Press, New York, 1967.

ers are like enzymes in the following respects. (a) Where inves-
tigated, the carriers have been shown to be proteins. (b) The carriers
form complexes with their substrates. (c) The carriers have varying
degrees of substrate specificity, including stereospecificity. (d) They
show saturation kinetics. (e) Many are inducible and repressible. (f)
They are under direct, genetical control.

To understand clearly the results of research on the uptake of
sugars by yeasts, it is important to appreciate the following general-
izations. (i) Sugars that are not metabolized by a yeast may enter its
cells, and many studies on sugar transport have been made with non-
metabolizable sugars in order to avoid the problem of separating ef-
fects of transport from those of metabolism. One example is that of L-
sorbose uptake by *Saccharomyces cerevisiae*[150]; other examples are
provided by non-utilizable, substituted sugars, made by synthetic
chemists, such as deoxy and thio sugars. (ii) There may be more than
one mode of entry for a given sugar into a particular yeast, and this
could be occasioned by the simultaneous presence of two or more
carriers having the same, or overlapping, substrate specificities. (iii)
One or more of such carriers in a particular yeast may be inducible,
only being formed by the yeast as a functioning system in response
to the presence, external to the yeast, of the sugar to be carried, or of
some structurally comparable compound. (iv) Each carrier may be
under separate genetical control, which is probably also separable
from that concerned with other processes involving utilization of the
sugar taken in by that carrier. Hence, for any sugar utilization, it may
be possible to obtain a series of mutants, each with a single genetical
difference from the wild type, affecting a carrier, or one of the cata-
bolic enzymes. (v) Sugar transport is often studied by using either
metabolic inhibitors or inhibitors of transport.[151] Most compounds
that inhibit the transport of sugars do so by competing externally for
combination with the carriers, or by so modifying them that internal
release is impaired. Transport of a given sugar may be inhibited by
other sugars that are transported on the same carrier, by sugar ana-
logues that are bound by the carrier, although not released, or by
structurally dissimilar compounds. (vi) Because of experimental dif-
ficulties in making direct measurements of the affinity of a carrier for
many sugars, this is commonly performed by testing the capacity of

(150) V. P. Cirillo, *Trans. N. Y. Acad. Sci.*, **23**, 725–734 (1961).
(151) V. P. Cirillo, in "Metabolic Inhibitors. A Comprehensive Treatise," R. M. Hoch-
    ster, M. Kates, and J. H. Quastel, eds., Academic Press, New York, 1972, Vol.
    III, pp. 47–68.

such sugars to inhibit the uptake of certain non-metabolized sugars. Inhibition provides a measure of the affinity of the inhibiting sugar for the carrier. (*vii*) Transport carriers may generally seem to have wider substrate specificities than enzymes. However, this difference is probably only apparent. The number of substrates for a carrier is more properly compared with that for an enzyme in addition to its structurally related competitive inhibitors; this is because the transporting system may depend solely on the binding of carrier to substrate rather than on its chemical transformation. (*viii*) Carrier-mediated transport of sugars has been studied in spheroplasts, the cell walls having been digested away enzymically. (*ix*) The carriers under discussion here take sugars from solution between the cell wall and the plasmalemma, across the plasmalemma, and into the cytoplasm. All such sugars do not necessarily become available to every relevant, enzymic system of the cell, some of which systems are enclosed in further membrane-bounded structures, such as the vacuole. (*x*) The importance of the carriers is underlined by cases of crypticity; that is, when the cells fail to utilize a sugar, despite being fully equipped with the intracellular enzymes to do so.

The transport of sugar into yeasts is usually studied by incubating a suspension of yeast in buffer, at an appropriate temperature, with the sugar under investigation in solution. The suspension is sampled at timed intervals, and the yeast cells can be separated from the solution either with a miniature, high-speed centrifuge or by rapid filtration through a cellulose nitrate filter having suitable pore-size. Either (*a*) disappearance from the solution, or (*b*) uptake by the yeast may be measured. The yeast can be washed quickly with ice-cold water or buffer, as this does not usually remove appreciable amounts of intracellular sugar. The latter may be estimated chemically after breaking up the cells in, for example, hot aqueous ethanol. Most estimations are done with radioactively labelled sugars.

2. Kinds of Transport

Carrier-mediated movement of sugars across the plasmalemma of yeasts involves the combination of the sugar with a protein on one side of the plasmalemma, followed by release of the sugar into the cytoplasm on the other side. Such movement is described either as (*i*) *facilitated diffusion*, when the movement requires no metabolic energy, or (*ii*) *active transport*, which involves the expenditure of metabolic energy. Sugars entering yeast cells by active transport may be accumulated within the cells to a concentration many hundred times the external level. This subject has been reviewed by

Cirillo,[152] Rothstein,[153] Divies and Morfaux,[154] Kotyk and Janáček,[155] Suomalainen and Oura,[156] and Jennings.[157]

A third kind of sugar-uptake system occurs in bacteria (for reviews, see Refs. 158–162), namely *group translocation*, and some authorities consider that this occurs in yeasts, too.[163] In certain bacteria, uptake of sugar is coupled to its phosphorylation and to energy-yielding metabolism; it involves the following overall reaction.

Sugar + enolpyruvate phosphate → sugar phosphate + pyruvate

Two enzymic reactions have been identified in this process, which effects the entry of D-fructose, D-glucose, D-mannose, L-sorbose, D-glucitol, and D-mannitol.

To sum up, the ways in which sugars are likely to enter yeasts may be as follows (*cf.*, Ref. 159).

	Outside plasmalemma	Inside plasmalemma
Facilitated diffusion	S ——————→	s
Active transport	s ——————→	S
Group translocation	s ——————→	S-P

(*s*, sugar at low concentration; **S**, sugar at high concentration; S-P, sugar phosphate)
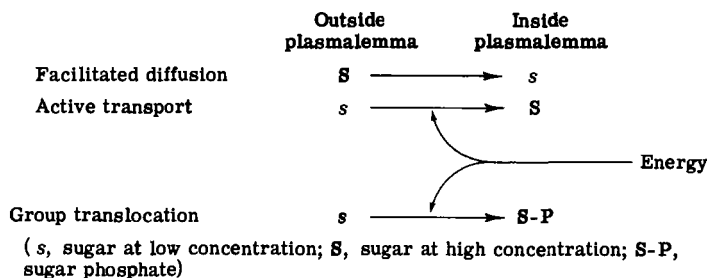
3. Monosaccharides

There have been extensive studies of the entry of monosaccharides into *Saccharomyces cerevisiae*, although the number and nature of

(152) V. P. Cirillo, *Annu. Rev. Microbiol.*, 15, 197–218 (1961).
(153) A. Rothstein, in "The Fungi," G. C. Ainsworth and A. S. Sussman, eds., Academic Press, New York, 1965, Vol. I, pp. 429–455.
(154) C. Divies and J. N. Morfaux, *Ann. Technol. Agr.*, 17, 355–377 (1968).
(155) A. Kotyk and K. Janáček, "Cell Membrane Transport," Plenum Press, New York, 1970.
(156) H. Suomalainen and E. Oura, in Ref. 21, 1971, Vol. 2, pp. 3–74.
(157) D. H. Jennings, *Trans. Brit. Mycol. Soc.*, 62, 1–24 (1974).
(158) S. Roseman, in "Metabolic Pathways," L. E. Hokin, ed., Academic Press, New York, 3rd Edition, 1972, Vol. 6, pp. 41–89.
(159) S. Roseman, in "The Molecular Basis of Biological Transport," J. F. Woessner and F. Huijing, eds., Academic Press, New York, 1972, pp. 181–215.
(160) H. L. Kornberg, *Proc. Roy. Soc., Ser. B*, 183, 105–123 (1973).
(161) H. L. Kornberg, *Symp. Soc. Exp. Biol.*, 27, 175–193 (1973).
(162) A. Kepes, *Biochimie*, 55, 693–702 (1973).

the carriers is still controversial. Differences in opinion may be related both to the techniques and to the strains of yeast used by different authors. According to Kotyk and coworkers[164–167] and Cirillo,[168,169] entry is chiefly by facilitated diffusion, and phosphorylation is not involved, whereas van Steveninck and coworkers[170–175] held that group translocation involving phosphorylation within the plasmalemma also occurs and is important. Van Steveninck suggested[174] that the sugar and carrier form a complex with a polyphosphate group that acts as a donor, so that phosphorylated sugar is released inside the plasmalemma.

Kotyk and coworker[165,166] described three monosaccharide carriers in *Saccharomyces cerevisiae*. One transports all of the monosaccharides tested. The second is more specific for sugars that are similar structurally to D-glucose, the affinities of carrier for the sugar being lower the more there are changes of orientation of hydroxyl groups from those of D-glucose. The relative importance of the positions decreases in the following order: C-1 > C-3 > C-4 > C-5 > C-2[165,168,176] (see Table IV). These studies gave information about

TABLE IV

Estimates of Affinities of the High-Specificity Carrier of Bakers'
Yeast for Some Monosaccharides

Monosaccharide	Half-saturation constant, K_m (mM)	
	Cirillo[168]	Kotyk[165]
D-Glucose	5–9	4–6
2-Deoxy-D-*arabino*-hexose	5	4–5
D-Fructose	25	17
D-Mannose	50	27
D-Xylose	40	95–170
D-Arabinose	250	75–155
D-Lyxose	250	80–110
L-Xylose	>2,000	200–600
L-Rhamnose	>2,000	1,000

(163) J. van Steveninck, *Biochim. Biophys. Acta*, 203, 376–384 (1970).
(164) A. Kotyk, *Folia Microbiol.* (Prague), 10, 30–35 (1965).
(165) A. Kotyk, *Folia Microbiol.* (Prague), 12, 121–131 (1967).
(166) A. Kotyk and D. Michaljaničová, *Folia Microbiol.* (Prague), 13, 212–220 (1968).
(167) A. Kotyk and D. Michaljaničová, *Biochim. Biophys. Acta*, 332, 104–113 (1974).
(168) V. P. Cirillo, *J. Bacteriol.*, 95, 603–611 (1968).
(169) V. P. Cirillo, *J. Bacteriol.*, 95, 1727–1731 (1968).

the effects of substitutions, as well as of changes in configuration. The only single change of a group (at one of the six carbon atoms) able to abolish binding was methylation of the anomeric hydroxyl group (methyl α-D-glucopyranoside). The hydroxyl group at C-2 does not appear to contribute to binding, as the carrier has the same affinity for 2-deoxy-D-*arabino*-hexose ("2-deoxy-D-glucose") as for D-glucose itself. With the surprising exception of D-fructose, many molecular changes produced a complete loss of affinity (L-xylose and L-rhamnose). The acyclic forms of D-glucose and D-fructose are identical from C-3 to C-6, and are readily interconverted, so, possibly, it is wholly or partly the acyclic forms of these sugars that are bound. The third carrier subserves an active transport of D-arabinose, D-galactose, D-glucose, D-ribose and D-xylose when those sugars are supplied exogenously at low concentration (0.02 to 2.0 mM).[166] Horák and Kotyk[177] isolated a D-glucose-binding lipoprotein from membranar material, presumably plasmalemma, of *Saccharomyces cerevisiae*. The lipoprotein bound sugars for which the main D-glucose carrier is specific, but not D-galactose or L-arabinose. The molecular weight of the carrier protein or binding site was $\sim$5,000, and that of the lipoprotein $\sim$35,000. The authors[177] considered that there are at least 3×10^6 binding sites per cell.

The monosaccharide carriers of *Saccharomyces cerevisiae*, and also of *Kluyveromyces* (*Saccharomyces*) *fragilis*, appear from polarimetric measurements to have a higher affinity for the α-D anomers of D-glucose, D-mannose, and D-xylose than for the corresponding β anomers.[178] Intracellularly, α-D-glucopyranose is a slightly better substrate of hexokinase than the β anomer.[179]

Differences, in some wine yeasts, of the affinities of a carrier for certain monosaccharides have led to some fascinating studies over many years. Although bakers' and brewers' yeasts are among many that ferment D-glucose more quickly than D-fructose in an equimolecular mixture of the two sugars,[180–182] Dubourg,[183] in 1897, described

(170) J. van Steveninck and A. Rothstein, *J. Gen. Physiol.*, 49, 235–246 (1965).

(171) A. Rothstein and J. van Steveninck, *Ann. N. Y. Acad. Sci.*, 137, 606–623 (1966).

(172) J. van Steveninck and E. C. Dawson, *Biochim. Biophys. Acta*, 150, 47–55 (1968).

(173) J. van Steveninck, *Biochim. Biophys. Acta*, 163, 386–394 (1968).

(174) J. van Steveninck, *Arch. Biochem. Biophys.*, 130, 244–252 (1969).

(175) J. van Steveninck, *Biochim. Biophys. Acta*, 274, 575–583 (1972).

(176) C. F. Heredia, A. Sols, and G. De la Fuente, *Eur. J. Biochem.*, 5, 321–329 (1968).

(177) J. Horák and A. Kotyk, *Eur. J. Biochem.*, 32, 36–41 (1973).

(178) R. Ehwald, P. Sammler, and H. Göring, *Folia Microbiol.* (Prague), 18, 102–117 (1973).

(179) M. Salas, E. Viñuela, and A. Sols, *J. Biol. Chem.*, 240, 561–568 (1965).

preferential fermentation of D-fructose by "Sauternes" yeast (see also Refs. 184–188), later identified as *Saccharomyces bailii (elegans)*.[182] Other yeasts that selectively ferment D-fructose include *Torulopsis stellata (bacillaris)*,[182] which often initiates the fermentation of the sweet, white wines of Sauternes.[189] Sound, mature wine-grapes contain about equal amounts of D-glucose and D-fructose,[190,191] but the Sauternes wines are made from grapes rotted by the mold *Botrytis cinerea (pourriture noble*, noble rot), which uses D-glucose preferentially.[190] Hence, there is more D-fructose than D-glucose in Sauternes must, which may contain 40% or more (w/v) of hexose. By contrast to *Torulopsis stellata, Kloeckera apiculata* (the yeast that starts the fermentation of most clarets[189]) utilizes the two sugars at about the same rate.[182] Investigation of the selective utilization of D-fructose by a "Sauternes yeast" showed that (*i*), unlike intact yeast, broken cells ferment D-glucose preferentially,[192] and (*ii*) a carrier at the plasmalemma, common for D-glucose and D-fructose, appeared to have a greater affinity for D-fructose.[193.]

There is general agreement that D-galactose enters non-induced cells of *Saccharomyces cerevisiae* by facilitated diffusion, by way of the general monosaccharide carrier of wide specificity, although it has a low affinity for D-galactose.[169,170,172,175,194–197] However, the na-

(180) [A. P.] Dubrunfaut, *Ann. Chim.* (Paris), [3] **21**, 169–178 (1847).

(181) E. Bourquelot, *Ann. Chim.* (Paris), [6] **9**, 245–275 (1886).

(182) E. Peynaud and S. Domercq, *Ann. Inst. Pasteur*, **89**, 346–351 (1955).

(183) E. Dubourg, *Rev. Viticult.*, **8**, 467–472 (1897).

(184) U. Gayon and E. Dubourg, *Compt. Rend.*, **110**, 865–868 (1890).

(185) U. Gayon and E. Dubourg, *Z. Zuckerind.*, **40**, 479–482 (1890).

(186) A. Fernbach, M. Schoen, and M. Mori, *Compt. Rend.*, **184**, 551–553 (1927).

(187) H. Sobotka and M. Reiner, *Biochem. J.*, **24**, 1783–1786 (1930).

(188) A. Gottschalk, *Wallerstein Lab. Commun.*, **10**, 109–118 (1947).

(189) S. Domercq, "Étude et classification des levures de vin de la Gironde," Institut National de la Recherche Agronomique, Paris, 1956.

(190) J. Ribéreau-Gayon and E. Peynaud, "Traité d'Oenologie," Librairie Polytechnique Ch. Béranger, Paris, 1960, Vol. 1.

(191) E. Peynaud and P. Ribéreau-Gayon, in "The Biochemistry of Fruits and Their Products," A. C. Hulme, ed., Academic Press, London, 1971, Vol. 2, pp. 171–205.

(192) A. Gottschalk, *Biochem. J.*, **40**, 621–626 (1946).

(193) A. Sols, *Biochim. Biophys. Acta*, **20**, 62–68 (1956).

(194) M. Burger, L. Hejmová, and A. Kleinzeller, *Biochem. J.*, **71**, 233–242 (1959).

(195) V. P. Cirillo, *Abh. Deut. Akad. Wiss. Berlin, Kl. Med.*, **6**, 153–159 (1967); *Chem. Abstr.*, **67**, 106,206v (1967).

(196) A. Kotyk and C. Haškovec, *Folia Microbiol.* (Prague), **13**, 12–19 (1968).

(197) A. Sols, in "Aspects of Yeast Metabolism," A. K. Mills and H. Krebs, eds., Blackwell Scientific Publications, Oxford, 1968, pp. 47–66.

ture of the inducible D-galactose-carrier in *Saccharomyces cerevisiae* was a further source of controversy between van Steveninck,[172,175] on the one hand, who favored phosphorylation and active transport, and Cirillo and coworkers[169,198,199] and Kotyk and coworkers,[167] on the other, who considered that the induced transport of D-galactose occurs by facilitated diffusion and does not involve phosphorylation. Cirillo and coworkers[169,198] found that the inducible, D-galactose transport-system, which depends on the *GAL2* gene, also allows entry, by facilitated diffusion, of the nonmetabolized D-galactose analogues D-fucose and L-arabinose. "Transportless" mutants transport neither D-galactose nor L-arabinose above the non-induced rate.[198] The report that L-arabinose induces D-galactose transport[169] was later invalidated by the finding that this induction depends on ~1% of D-galactose as an impurity in the L-arabinose.[200]

Van Steveninck[172,175] reported that, with two other strains of *Saccharomyces cerevisiae*, D-galactose-induced cells transport D-galactose actively, by a process involving phosphorylation. Employing ^{14}C-labelled D-galactose, he exploited the time interval between induction of active transport and induction of the metabolic system for D-galactose. Because the phosphorylated fraction was labelled faster than the free, intracellular sugar, he concluded that a D-galactose phosphate is the precursor of intracellular D-galactose. The affinity for D-galactose was high with induced cells ($K_m \sim 5$ mM) and low with D-glucose-grown cells ($K_m \sim 600$ mM).

Haškovec and Kotyk[201] tried to isolate the D-galactose carrier from D-galactose-induced *Saccharomyces cerevisiae*. They found a fraction of homogenized yeast-cells, containing much membrane material and sedimenting at 40,000 g, that bound D-galactose with a dissociation constant of 0.2 to 0.6 mM. The fraction did not phosphorylate D-galactose.

Transport of "deoxy-D-glucoses" into *Saccharomyces cerevisiae* has been studied.[173,202] 4-Deoxy-D-*xylo*-hexose, which is both "4-deoxy-D-glucose" and "4-deoxy-D-galactose," appears to enter by both D-glucose and D-galactose carriers. D-Galactose competes very little for uptake by D-glucose-grown cells, but shows marked competition[202] in the case of "D-galactose-adapted cells."

(198) S. Kuo, M. S. Christensen, and V. P. Cirillo, *J. Bacteriol.*, 103, 671–678 (1970).
(199) S. Kuo and V. P. Cirillo, *J. Bacteriol.*, 103, 679–685 (1970).
(200) F. Azam, S. C. Kuo, and V. P. Cirillo, *J. Bacteriol.*, 106, 915–919 (1971).
(201) C. Haškovec and A. Kotyk, *Eur. J. Biochem.*, 9, 343–347 (1969).
(202) A. Kotyk and D. Michaljaničová, *Proc. Intern. Symp. Yeasts, 4th*, Part I, p. 293 (1974).

Unlike *Saccharomyces cerevisiae*, a strain of *Rhodotorula glutinis* (*gracilis*), takes up monosaccharides by unequivocally active transport.[203-207] The carrier appears to have wide substrate-specificity, transporting D-fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, D-ribose, D-xylose, and L-xylose. D-Xylose may be concentrated by the cells as much as a thousand-fold.[203]

The uptake of D-ribose by *Rhodotorula glutinis* has been interpreted as a mechanism resembling simple diffusion,[208] the rate of entry being proportional to the external concentration of D-ribose, between 5 μM and 5 mM. On the other hand, comparable results for D-ribose entry into *Pichia fermentans*, a non-utilizer of D-ribose, might be explained differently.[209] The apparently linear plot of the rate of entry of D-ribose against its concentration, up to 32 mM, could correspond to the lower part of a rectangular hyperbola representing the activity of a carrier-mediated system having a very low affinity for D-ribose, possibly the D-glucose carrier. *Pichia pinus*, which utilizes D-ribose for growth and respiration, apparently has two carriers for D-ribose, that in D-ribose-grown yeast having a higher affinity for D-ribose (apparent K_m 0.1 mM) than that in succinate-grown yeast ($K_m \sim 4$ mM). It is striking that the acyclic derivative ribitol inhibits D-ribose uptake by succinate- or D-ribose-grown *Pichia pinus*, although not that by *Pichia fermentans*. This observation may be compared to the equally odd report that the D-glucose carrier of *Saccharomyces cerevisiae* has some affinity for D-mannitol.[210] It is relevant to ask: in what molecular form does D-ribose cross the plasmalemma of *Pichia pinus*? Although there is probably <1% of *aldehydo*-D-ribose in solution,[211] the state of the sugar in water does not correspond in any way to the "availability" of any one form of the sugar; this is because very little energy would be required to open the pyranose ring, and this energy might well become available at the plasmalemma from an interaction between the sugar and part of the membrane. The effects of change of temperature on the rate of uptake of D-ribose by *Pichia fermentans* are shown in Fig. 2. The

(203) A. Kotyk and M. Höfer, *Biochim. Biophys. Acta*, **102**, 410–422 (1965).
(204) M. Höfer and A. Kotyk, *Folia Microbiol.* (Prague), **13**, 197–204 (1968).
(205) M. Höfer, *J. Membrane Biol.*, **3**, 73–82 (1970).
(206) M. Höfer, *Arch. Mikrobiol.*, **80**, 50–61 (1971).
(207) M. Höfer, *J. Theor. Biol.*, **33**, 599–603 (1971).
(208) J. Horák and A. Kotyk, *Folia Microbiol.* (Prague), **14**, 291–296 (1969).
(209) J. A. Barnett, *J. Gen. Microbiol.*, **90**, 1–12 (1975).
(210) W. A. Maxwell and E. Spoerl, *J. Bacteriol.*, **105**, 753–758 (1971).
(211) S. J. Angyal and V. A. Pickles, *Aust. J. Chem.*, **25**, 1695–1710 (1972).
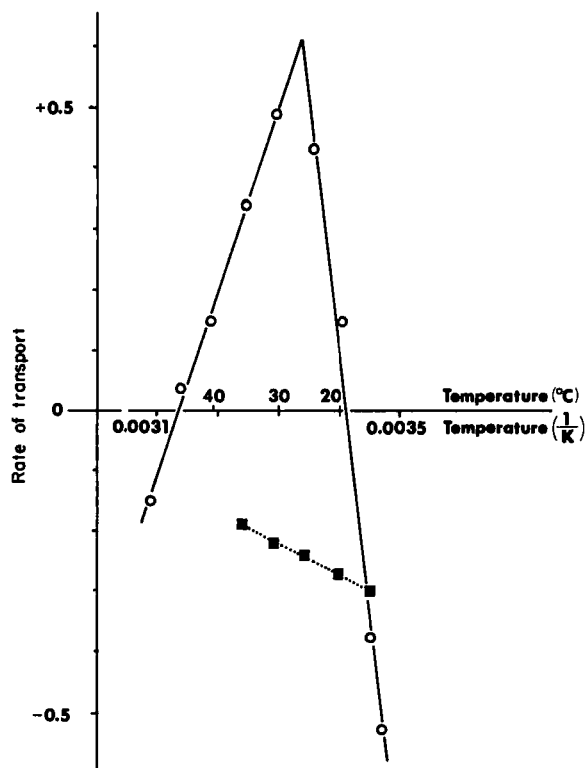
FIG. 2.—Temperature Dependence of Net Transport of D-Xylose into *Rhodotorula glutinis (gracilis)* [—○—] (Heller and Coworkers, 1974)[212] and of D-Ribose into *Pichia fermentans* [----■----] (Barnett, 1975).[209] [Rate of transport = $\log_{10}$ (μg of sugar/mg of dry weight of yeast/min).]

temperature relations of the entry of D-xylose into *Rhodotorula glutinis (gracilis)* are very different (see Fig. 2). This yeast does not take up monosaccharides measurably at temperatures below 15°; between 15 and 30°, the apparent activation-energy for entry is about 126 kJ.-deg. mol^{-1}, and, between 30 and 50°, there is a decrease in the rate of transport. The Arrhenius plot in Fig. 2 shows that the two straight lines obtained intersect at the optimal temperature of ~28° for the uptake of D-xylose.[212,213]

Haškovec and Kotyk[214] studied transport into a strain of *Torulopsis*

(212) K. B. Heller, U. Winter, and M. Höfer, *Proc. Intern. Symp. Yeasts, 4th*, Part I, pp. 285–286 (1974).
(213) K. Heller and M. Höfer, *Z. Physiol. Chem.*, 354, 225 (1973).
(214) C. Haškovec and A. Kotyk, *Folia Microbiol.* (Prague), 18, 118–124 (1973).

candida of the monosaccharides D-galactose, L-sorbose, and D-xylose, as well as that of a number of alditols, namely, erythritol, D- and L-arabinitol, ribitol, xylitol, D-glucitol, and D-mannitol. The yeast was grown on succinate as the sole source of carbon, and so the carriers could be considered non-induced. The authors reported[214] that the substrates enter by active transport by means of four carriers: (*i*) the first has a high affinity for alditols; (*ii*) the second has a high affinity for the monosaccharides; (*iii*) the third has a low affinity both for alditols and monosaccharides; and (*iv*) the fourth is specific to erythritol and D-ribose, for both of which it has a high affinity.

A strain of *Rhodotorula glutinis* (*gracilis*) takes up a number of alditols (D-glucitol, D-mannitol, erythritol, D-arabinitol, L-arabinitol, ribitol, and xylitol) by the monosaccharide carrier.[215,216] Both ribitol and L-arabinitol induced a second alditol-transporting system having a high specificity for pentitols. D-Glucose inhibited the uptake of D-arabinose, ribitol, and D-xylose.[216a]

4. Glycosides

Emil Fischer's view that yeasts always hydrolyze disaccharides before fermenting them[217–219] was disputed by Willstätter in the nineteen-twenties (for a review, see Ref. 220). Willstätter and Oppenheimer[221] showed that lactose-fermenting yeasts may ferment lactose more rapidly than they ferment D-glucose, D-galactose, or a mixture of the two. Such observations led them to conclude that the first metabolic step is not necessarily hydrolytic. These two views, initial hydrolysis on the one hand, and "direct" fermentation on the other, were a subject for dispute until Gottschalk[222] suggested that the entry of a disaccharide across the cell membrane might be the rate-limiting step of that sugar's catabolism. This is the current view.

Indeed, although some oligosaccharides are hydrolyzed by certain

(215) R. Klöppel and M. Höfer, *Proc. Intern. Symp. Yeasts, 4th*, Part I, pp. 291–292 (1974).
(216) R. Klöppel and M. Höfer, *Zentralbl. Bakteriol., Parasitenk., Infektionskr. Hyg., Abt. 1: Orig., Reihe A*, **228**, 211–217 (1974).
(216a) M. Höfer, O. Seletzky-Hild, and P. Dahle, *Zentralbl. Bakteriol., Parasitenk., Infektionskr. Hyg., Abt. 1: Orig., Reihe A*, **228**, 199–203 (1974).
(217) E. Fischer, *Ber.*, **27**, 3479–3483 (1894).
(218) E. Fischer, *Z. Physiol. Chem.*, **26**, 60–87 (1898).
(219) E. Fischer and P. Lindner, *Ber.*, **28**, 3034–3039 (1895).
(220) J. Leibowitz and S. Hestrin, *Advan. Enzymol.*, **5**, 87–127 (1945).
(221) R. Willstätter and G. Oppenheimer, *Z. Physiol. Chem.*, **118**, 168–188 (1922).
(222) A. Gottschalk, *Wallerstein Lab. Commun.*, **12**, 55–67 (1949).

yeasts outside the plasmalemma, and are transported into the cell as monosaccharides, glycosides may, alternatively, enter the cells intact. De la Fuente and Sols[223] found that the anaerobic utilization of β-D-fructofuranosides by *Saccharomyces cerevisiae*, and that of α-D-galactopyranosides by *Saccharomyces uvarum* (*carlsbergensis*) involves hydrolysis outside the plasmalemma. On the other hand, the utilization of maltose and other α-D-glucopyranosides by *Saccharomyces cerevisiae*, and that of β-D-galactopyranosides by *Kluyveromyces* (*Saccharomyces*) *fragilis*, involves transport of the intact glycoside, which is cleaved inside the plasmalemma. Sucrose may be hydrolyzed externally as a β-D-fructofuranoside, or transported and subjected to α-D-glucosidase activity within. However, a strain of *Rhodotorula glutinis*, grown on D-glucose in the presence of yeast extract, does not transport intact sucrose into its cells.[223a]

Such α-D-glucopyranosides as maltose, methyl α-D-glucopyranoside, or ethyl 1-thio-α-D-glucopyranoside enter the cells of D-glucose-grown *Saccharomyces cerevisiae* by facilitated diffusion, or by active transport into cells grown on methyl α-D-glucopyranoside[224–229]; entry into *Saccharomyces uvarum* (*carlsbergensis*) is comparable.[230] Unlike monosaccharides, lactose, maltose, melezitose, melibiose, and raffinose are reported not to enter yeasts of various species that do not utilize them[225]; maltotriose may penetrate *Saccharomyces cerevisiae* by a separate carrier of high specificity.[231] Van Steveninck[163] studied the active transport of methyl α-D-glucopyranoside into maltose-grown cells of *Saccharomyces cerevisiae*. The K_m for entry was 8 mM methyl α-D-glucopyranoside, and, unlike the results in another report,[229] the K_m did not change with "deadaptation," that is, incubation with D-glucose. Entry of methyl α-D-glucopyranoside appeared to involve its phosphorylation, followed by intracellular dephosphorylation. With another strain, Kotyk and Michaljaničová[167] found no evidence for such phosphorylation.

Okada and Halvorson's[228,232] discussion of their studies on the

(223) G. de la Fuente and A. Sols, *Biochim. Biophys. Acta*, **56**, 49–62 (1962).

(223a) S. Janda and M. von Hedenström, *Arch. Microbiol.*, **101**, 273–280 (1974).

(224) J. J. Robertson and H. O. Halvorson, *J. Bacteriol.*, **73**, 186–198 (1957).

(225) G. Harris and C. C. Thompson, *J. Inst. Brewing*, **66**, 213–217 (1960).

(226) G. Harris and C. C. Thompson, *Biochim. Biophys. Acta*, **52**, 176–183 (1961).

(227) H. Okada and H. O. Halvorson, *Biochim. Biophys. Acta*, **82**, 538–546 (1964).

(228) H. Okada and H. O. Halvorson, *Biochim. Biophys. Acta*, **82**, 547–555 (1964).

(229) C. P. M. Görts, *Biochim. Biophys. Acta*, **184**, 299–305 (1969).

(230) R. A. de Kroon and V. V. Koningsberger, *Biochim. Biophys. Acta*, **204**, 590–609 (1970).

(231) G. Harris and C. C. Thompson, *J. Inst. Brewing*, **66**, 293–297 (1960).

transport of ethyl 1-thio-α-D-glucopyranoside or methyl α-D-glucopyranoside into *Saccharomyces cerevisiae* is particularly interesting. They suggested that active transport depends on an inducible coupling between the energy metabolism of the cell and the carrier responsible for facilitated diffusion. This kind of organization may be widespread.[157] A further suggestion was that the initial fate of D-glucose formed hydrolytically from maltose may be the synthesis of trehalose (not glycolysis), and that the anabolic and catabolic trehalose pathway could be coupled, or be partly identical, with the transport system.[167,233]

Suggestions have been made that the ability of cells to concentrate certain exogenously supplied metabolites depends on their movement being coupled to the flow of cations, such as $Na^{\oplus}$, $K^{\oplus}$, or, more particularly, $H^{\oplus}$ across the plasmalemma.[234,235] A strain of *Saccharomyces uvarum* (*carlsbergensis*) grown on maltose absorbed with it two to three equivalents of protons.[236,237] The accelerated rate of proton uptake increased to a maximum value at ~ 4 mM maltose (K_m 1.6 mM). The uptake of protons was accelerated when the yeast was incubated with methyl α-D-glucopyranoside, turanose, or sucrose (the latter, presumably in this instance, hydrolyzed within the plasmalemma), but not with D-glucose, D-galactose, or 2-deoxy-D-*arabino*-hexose, which entered by facilitated diffusion. Another yeast, a strain of *Kluyveromyces* (*Saccharomyces*) *fragilis*, absorbed extra protons in the presence of lactose, which it catabolizes. Furthermore, the plasmalemma of *Rhodotorula glutinis* (*gracilis*) is reported to contain an energy-linked, $H^{\oplus}$ pump directly connected with the active transport of monosaccharides.[238]

VII. THE CATABOLISM OF D-GLUCOSE

Although the intermediary metabolism of carbohydrates is described in standard textbooks of biochemistry, the main steps are given here, particularly in order to make clear how yeasts may differ

(232) H. O. Halvorson, H. Okada, and J. Gorman, in "The Cellular Functions of Membrane Transport," J. F. Hoffman, ed., Prentice–Hall, Inc., Englewood Cliffs, New Jersey, 1964, pp. 171–191.

(233) F. K. Zimmermann, N. A. Khan, and N. R. Eaton, *Mol. Gen. Genet.*, **123**, 29–41 (1973).

(234) P. Mitchell, *Advan. Enzymol. Relat. Areas Mol. Biol.*, **29**, 33–87 (1967).

(235) F. M. Harold, *Bacteriol. Rev.*, **36**, 172–230 (1972).

(236) A. Seaston, C. Inkson, and A. A. Eddy, *Biochem. J.*, **134**, 1031–1043 (1973).

(237) R. Brocklehurst, G. Carr, P. Earnshaw, and A. A. Eddy, *Proc. Soc. Gen. Microbiol.*, **1**, 47 (1974).

(238) P. C. Misra and M. Höfer, *FEBS Lett.*, **52**, 95–99 (1975).
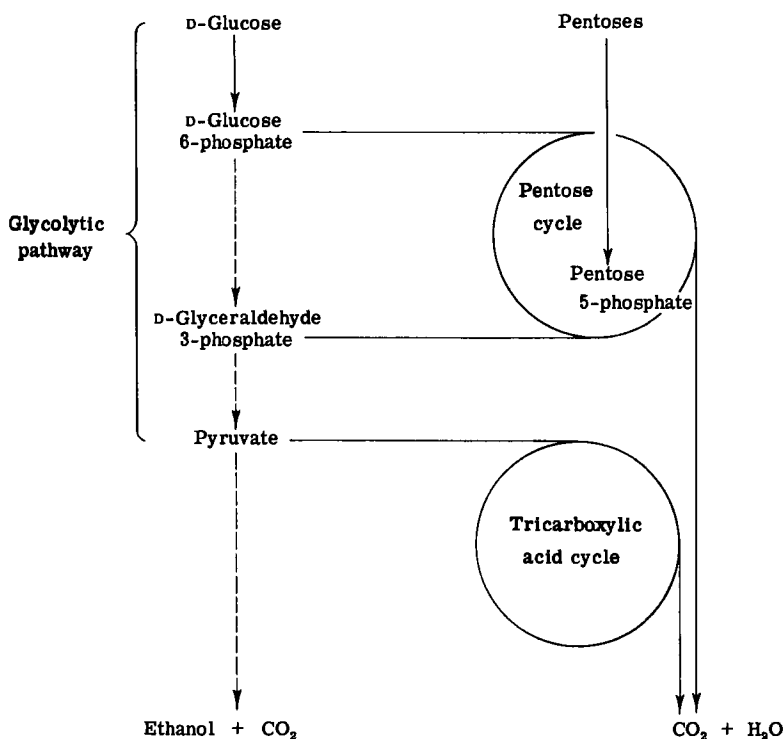
FIG. 3.—Interrelationships of the Central Pathways of Carbohydrate Catabolism in Yeasts.

from each other both in their ability to utilize sugars and in the way in which they use them. The catabolism of carbohydrates by yeasts involves a few distinct reaction-sequences, or central pathways, and the ability to utilize a carbohydrate usually depends on the cells' abilities to convert the substrate into intermediary metabolites of one of these pathways. A yeast may fail to utilize a sugar because (*i*) the sugar does not enter the cells, (*ii*) the yeast lacks one or more enzymes necessary to convert the substrate into an intermediary metabolite of a central pathway, or (*iii*) the appropriate central pathway is inoperative from lack of one or more enzymes that control its reactions. Interrelationships of the central pathways are shown in Fig. 3.

All species of yeast can catabolize D-glucose, and the reaction sequences responsible for this metabolism are also those of the breakdown of other sugars. So, the breakdown of D-glucose will be considered first. Probably, most yeasts break D-glucose down to pyruvate by the glycolytic (or Embden–Meyerhof) pathway (see

D-Glucose

ATP ⟶

hexokinase (EC 2.7.1.1)

ADP ⟵

D-Glucose 6-phosphate

D-glucose phosphate isomerase (EC 5.3.1.9)

D-Fructose 6-phosphate

or other phosphate donor ⎰ ATP ⟶

6-phosphofructokinase (EC 2.7.1.11)

ADP ⟵

D-Fructose 1,6-diphosphate

fructose diphosphate aldolase (EC 4.1.2.13)

1,3-Dihydroxy-2-propanone phosphate

+

triosephosphate isomerase (EC 5.3.1.1)

D-Glyceraldehyde 3-phosphate

$NAD^{\oplus}$ + orthophosphate ⟶

glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12)

NADH ⟵

1,3-Di-O-phosphonoglycerate

ADP ⟵

phosphoglycerate kinase (EC 2.7.2.3)

ATP ⟵

3-O-Phosphonoglycerate

phosphoglycerate phosphomutase (EC 5.4.2.1)

2-O-Phosphonoglycerate

H_2O ⟵

enolase (EC 4.2.1.11)

Enolpyruvate phosphate

ADP ⟶

pyruvate kinase (EC 2.7.1.40)

ATP ⟵

Pyruvate

FIG. 4.—The Glycolytic Pathway.

Fig. 4), the overall reaction of which may be summarized as follows.

D-Glucose + 2 ADP + 2 NAD$^\oplus$ + 2 P$_i$ → 2 pyruvate + 2 ATP
$$+ \; 2 \; NADH + 2 \; H^\oplus + 2 \; H_2O$$
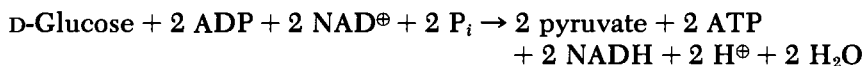
1. The Anaerobic Catabolism of D-Glucose

Over half the known species of yeasts can ferment D-glucose anaerobically (see Table III). *Saccharomyces cerevisiae* ferments both α- and β-D-glucopyranose at about the same rate.[176] In the anaerobic fermentation of sugars, yeasts convert the pyruvate produced by glycolysis into ethanol by way of acetaldehyde.

Pyruvate

pyruvate decarboxylase
(EC 4.1.1.1)

Acetaldehyde + CO$_2$

NADH + H$^\oplus$

alcohol dehydrogenase
(EC 1.1.1.1)

NAD$^\oplus$
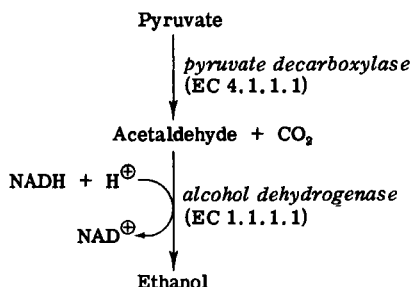
Ethanol

In practice, the decarboxylation of pyruvate to form acetaldehyde and carbon dioxide, by the action of pyruvate decarboxylase, is irreversible, and occurs as several steps.[239,240] Acetaldehyde is reduced to ethanol by NAD$^\oplus$-linked alcohol dehydrogenase.

The overall equation for alcoholic fermentation is

$$C_6H_{12}O_6 \rightarrow 2 \; C_2H_5OH + 2 \; CO_2.$$
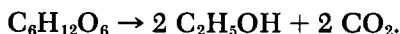
This equation is commonly attributed to Gay-Lussac,[241] but he simply found that 100 parts by weight of sugar is converted in fermentation into 51.34 parts by weight of alcohol and 48.66 parts of carbon dioxide. The empirical formula for glucose was not found until 1843, and the molecular formula, until 1868.[242]

The fate of the carbon atoms of D-glucose is as follows.

(239) H. Holzer and K. Beaucamp, *Biochim. Biophys. Acta*, 46, 225–243 (1961).
(240) L. O. Krampitz, I. Suzuki, and G. Greull, *Fed. Proc.*, 20, 971–977 (1961).
(241) [L. J.] Gay-Lussac, *Ann. Chim.* (Paris), [1] 95, 311–318 (1815).
(242) W. Pigman and D. Horton, in "The Carbohydrates: Chemistry and Biochemistry," W. Pigman and D. Horton, eds., Academic Press, New York, 2nd Edition, 1972, Vol. IA, pp. 1–67.

	Atom No.	Atom No.
	1 CH_3	6 CH_3
(6) CH_2OH	2 CH_2OH	5 CH_2OH
structure	+	+
	3 CO_2	4 CO_2
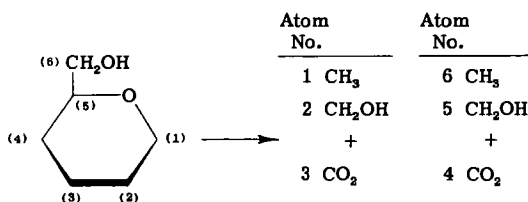
Some of the 1,3-dihydroxy-2-propanone phosphate (see Fig. 4) may be reduced to glycerol 3-phosphate, which is then hydrolyzed by a specific phosphatase to glycerol, and this is eliminated from the cells.[243] Certain yeasts are notorious for producing acid: *Saccharomyces bailii* (*Zygosaccharomyces acidifaciens*) anaerobically,[244] and *Brettanomyces bruxellensis* and *Brettanomyces claussenii* aerobically.[245] In bakers' yeast, the acetic acid may be formed by the action of aldehyde dehydrogenase on acetaldehyde.[246]

$$MeCHO + NAD^{\oplus} + OH^{\ominus} \rightarrow MeCO^{\ominus}_2 + NADH + H^{\oplus}$$
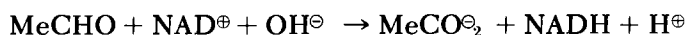
2. The Aerobic Catabolism of D-Glucose

Under aerobic conditions, yeasts convert most of the pyruvate produced by glycolysis into acetylcoenzyme A. The oxidative decarboxylation of pyruvate involves three enzymes and five coenzymes, and the net equation is as follows.

$$Pyruvate + NAD^{\oplus} + CoA\text{-}SH \rightarrow acetyl\text{-}S\text{-}CoA + NADH + H^{\oplus} + CO_2$$
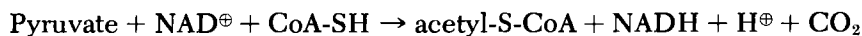
Acetylcoenzyme A supplies the main fuel for the tricarboxylic acid cycle (see Fig. 5), which is the principal route by which carbohydrate is oxidized to carbon dioxide and water. The effect of one turn of the cycle is the simple oxidation of a molecular unit of acetate.

$$MeCO_2H + 2\ O_2 \rightarrow 2\ CO_2 + 2\ H_2O$$
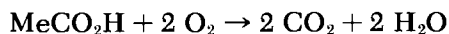
Another central pathway by which yeasts may catabolize D-glucose is the pentose cycle (see Fig. 6), the initial stages of which are (*i*) the phosphorylation of D-glucose, followed by (*ii*) oxidation of D-glucose 6-phosphate to 6-*O*-phosphono-D-gluconate. The net result of the operation of this cycle is the complete oxidation of D-glucose.

(243) C. Gancedo, J. M. Gancedo, and A. Sols, *Eur. J. Biochem.*, 5, 165–172 (1968).
(244) W. J. Nickerson and W. R. Carroll, *Arch. Biochem.*, 7, 257–271 (1945).
(245) M. T. J. Custers, "*Onderzoekingen over het Gistgeslacht Brettanomyces*," Thesis, De Technische Hoogeschool te Delft, 1940.
(246) S. Black, *Arch. Biochem. Biophys.*, 34, 86–97 (1951).

FIG. 5.—The Tricarboxylic Acid Cycle.

$$C_6H_{12}O_6 + 7\ H_2O + 12\ NADP^\oplus + ATP \rightarrow 6\ CO_2$$
$$+ 12\ NADPH + 12\ H^\oplus + ADP + P_i$$
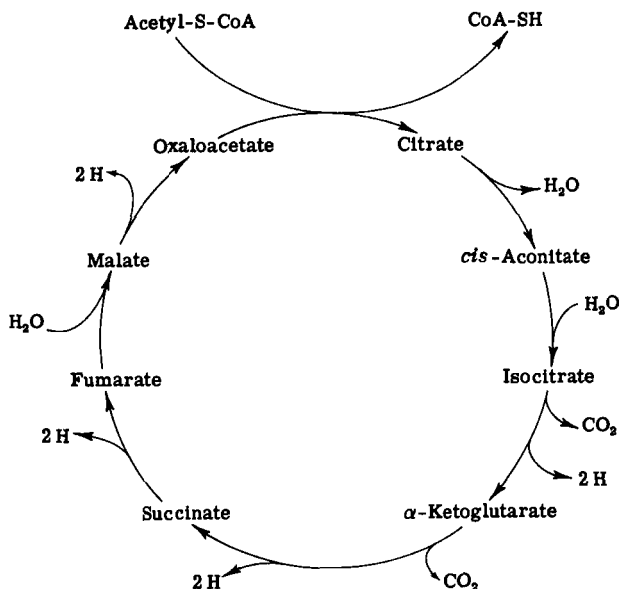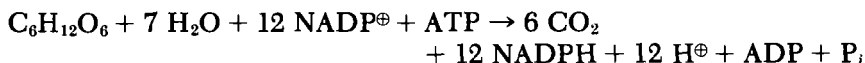
By studying the initial rates at which $^{14}CO_2$ is formed from (*i*) D-glucose-*1-14C* and (*ii*) D-glucose-6-*14C*, aerobically growing cells of *Saccharomyces cerevisiae* were shown to catabolize about 12% of D-glucose by means of the pentose cycle, and the rest glycolytically.[247-249] Although the results of examination of resting cells of another strain of *Saccharomyces cerevisiae* suggested that there was no significant oxidation of D-glucose by way of the pentose cycle,[250] an earlier analysis[251] of the specific activities of the metabolic products (acetate and ethanol, as well as CO_2) had indicated that resting cells of *Saccharomyces cerevisiae* break down up to 17% of its ca-

(247) C. H. Wang, C. T. Gregg, I. A. Forbusch, B. E. Christensen, and V. H. Cheldelin, *J. Amer. Chem. Soc.*, 78, 1869–1872 (1956).

(248) C. H. Wang, I. J. Stern, C. M. Gilmour, S. Klungsoyr, D. J. Reed, J. J. Bialy, B. E. Christensen, and V. H. Cheldelin, *J. Bacteriol.*, 76, 207–216 (1958).

(249) S. L. Chen, *Biochim. Biophys. Acta*, 32, 470–479 (1959).

(250) N. R. Eaton and H. P. Klein, *Biochem. J.*, 67, 373–381 (1957).

(251) H. J. Blumenthal, K. F. Lewis and S. Weinhouse, *J. Amer. Chem. Soc.*, 76, 6093–6097 (1954).

D-Glucose

ATP

ADP

D-Glucose 6-phosphate ←———————————→ D-Fructose 6-phosphate

NADP$^{\oplus}$

NADPH

6-O-Phosphono-D-
glucono-1, 5-lactone

H$_2$O

6-O-Phosphono-D-gluconate   D-Erythrose
                                               4-phosphate

NADP$^{\oplus}$

NADPH

CO$_2$

D-$erythro$-Pentulose
5-phosphate   D-Glyceraldehyde
                                          3-phosphate

D-$threo$-Pentulose 5-phosphate ←

D-Ribose 5-phosphate ←————————→ D-Sedoheptulose 7-phosphate
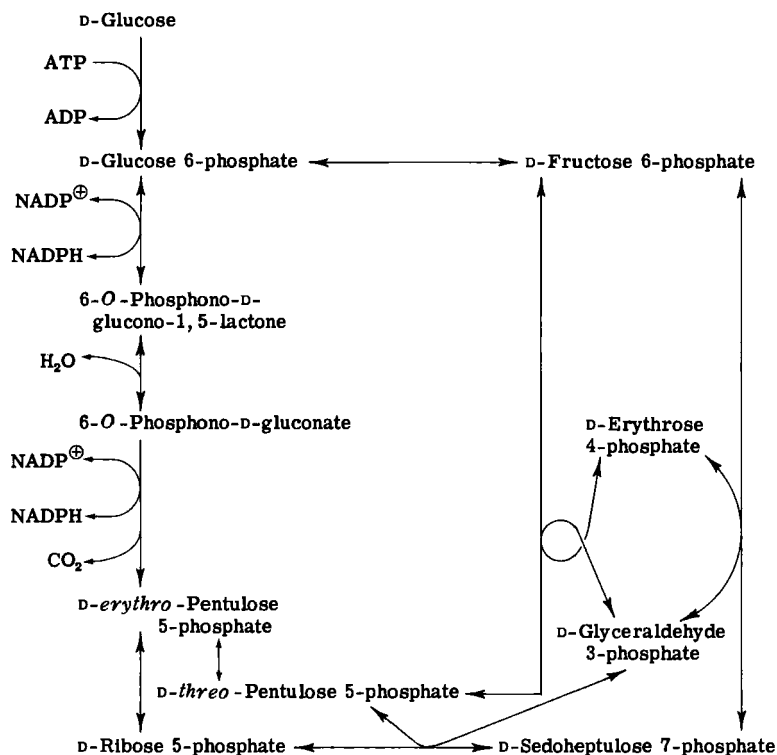
FIG. 6.—The Pentose Cycle.

tabolized D-glucose by the pentose cycle. A later estimate[252] is 9.7%, and subsequent authors[253] have given even lower figures and have criticized the methodology of the earlier work. Comparable figures are 35% for *Candida (Torulopsis) utilis*[251] and 50% for *Candida albicans.*[254] According to Horecker and coworkers,[255] *Saccharomyces cerevisiae* forms pentose phosphate from D-glucose chiefly by the non-oxidative steps of the pentose cycle, that is, from D-fructose 6-phosphate, through sedoheptulose (D-*altro*-heptulose) 7-phosphate,

(252) H. Simon and R. Medina, Z. *Naturforsch. B,* **23,** 326–329 (1968).

(253) J. M. Gancedo and R. Lagunas, *Plant Sci. Lett.,* **1,** 193–200 (1973); *Chem. Abstr.,* **78,** 156,416m (1973).

(254) F. W. Chattaway, R. Bishop, M. R. Holmes, F. C. Odds, and A. J. E. Barlow, *J. Gen. Microbiol.,* **75,** 97–109 (1973).

(255) B. L. Horecker, O. M. Rosen, J. Kowal, S. Rosen, B. Scher, C. Y. Lai, P. Hoffee, and T. Cremona, in Ref. 197, pp. 71–103.

to D-ribose 5-phosphate. *Candida utilis*, on the other hand, forms much pentose phosphate by the oxidative part of the cycle. These differences between the two species correspond with the fact that *Candida utilis* has a higher activity of the enzymes of the pentose cycle.[255] The control of this cycle is not yet fully understood. It may be quantitatively controlled by such factors as (*i*) variations in the amount of D-glucose 6-phosphate dehydrogenase (EC 1.1.1.49),[256] and (*ii*) negative feed-back control, that enzyme being inhibited by D-erythrose 4-phosphate and glyceraldehyde 3-phosphate.[257] Although D-glucose 6-phosphate dehydrogenase of brewers' yeast contains bound NADP⊕, and is inhibited by NADPH, the enzyme is insensitive to oxidized glutathione,[258] which counteracts the inhibition in mammalian tissue.[259] The regulatory properties of D-glucose 6-phosphate dehydrogenase have been reviewed by Bonsignore and De Flora.[260]

3. The Inability to Utilize D-Glucose Anaerobically

All species of yeasts that can use a sugar anaerobically can also do so aerobically, although the converse is not true. Pink, carotenoid-containing yeasts, of which the genus *Rhodotorula* is best known, are incapable of utilizing sugars anaerobically.[147] Thus, with these yeasts, unlike with *Saccharomyces cerevisiae* and *Candida utilis*, the pentose cycle appears to play a major role in the normal catabolism of carbohydrates. Several strains of the genus *Rhodotorula* have been found[261-263] to be deficient in a key enzyme of the glycolytic pathway, namely, 6-phosphofructokinase (EC 2.7.1.11), by the action of which D-fructose 6-phosphate is phosphorylated to D-fructose 1,6-diphosphate (see Fig. 4). However, some other nonfermentative (entirely aerobic) yeasts have no such deficiency (see Table V), and there is no evidence that they lack a glycolytic pathway. A strain of one of such species, *Torulopsis candida*, has been found to be

(256) C. B. Osmond and T. Ap Rees, *Biochim. Biophys. Acta,* **184,** 35–42 (1969).
(257) G. F. Domagk, R. Chilla, and K. M. Doering, *Life Sci.,* **13,** 655–662 (1973).
(258) R. H. Yue, E. A. Noltmann, and S. A. Kuby, *J. Biol. Chem.,* **244,** 1353–1364 (1969).
(259) L. V. Eggleston and H. A. Krebs, *Biochem. J.,* **138,** 425–435 (1974).
(260) A. Bonsignore and A. De Flora, *Curr. Top. Cell. Regul.,* **6,** 21–62 (1972).
(261) R. J. Brady and G. H. Chambliss, *Biochem. Biophys. Res. Commun.,* **29,** 343–348 (1967).
(262) M. Höfer, J.-U. Becker, K. Brand, K. Deckner, and A. Betz, *FEBS Lett.,* **3,** 322–324 (1969).
(263) J. M. Gancedo and C. Gancedo, *Arch. Mikrobiol.,* **76,** 132–138 (1971).

<div align="center">TABLE V</div>

<div align="center">Phosphofructokinase Activity in Various Yeasts Grown on D-Glucose</div>

Yeast	Specific activity[b]	Activities[a]		
		PFK[c]	GPDH[d]	Ratio of GPDH/PFK
Fermentative				
Saccharomyces cerevisiae	64	0.178	0.260	1.46
Torulopsis glabrata	—	0.060	0.371	6.18
stellata	—	0.064	0.271	4.23
Hansenula anomala	10	0.074	0.892	12.05
Candida salmanticensis[e]	20	—	—	—
Nonfermentative				
Torulopsis candida 1[f]	—	0.033	0.375	11.36
2	—	0.045	0.538	11.96
Endomycopsis javanensis	—	0.042	1.136	27.05
Rhodotorula glutinis	<5	<0.001	0.466	>450
minuta	<5	<0.001	0.450	>450
rubra[g]	<5	<0.001	0.450	>450
pilimanae	—	<0.001	0.482	>450
Pichia vini	12	—	—	—
membranaefaciens[h]	33	—	—	—

[a] From Brady and Chambliss.[261] The ratio is given here to permit comparing one yeast with another. [b] Milliunits/mg of protein (Gancedo and Gancedo[263]). [c] PFK = phosphofructokinase. [d] GPDH = D-glucose 6-phosphate dehydrogenase. [e] Given as *Torulopsis salmanticensis*. [f] Given as *Torulopsis famata*. [g] Given as *Rhodotorula mucilaginosa*. [h] Kreger-van Rij[60] recorded very weak fermentation of D-glucose by some of the 62 strains examined.

without alcohol dehydrogenase activity,[264] and this lack could account for its inability to ferment D-glucose. In this context, it is interesting that an ethanol-utilizing strain of the non-fermenting yeast *Lipomyces starkeyi*[265] grown aerobically on ethanol has high, but on D-glucose, very low, alcohol dehydrogenase activity, 47 and 2 nmoles of NADH/mg of protein/min, respectively. Similar observations have been reported for *Rhodotorula glutinis* (*gracilis*).[266]

One strain of *Rhodotorula glutinis* (*gracilis*) has been investigated further,[267] by using D-glucose specifically ¹⁴C-labelled at different

(264) J. A. Barnett, *J. Gen. Microbiol.*, **52**, 131–159 (1968).
(265) H. M. C. Heick and M. Barrette, *Biochim. Biophys. Acta*, **212**, 8–12 (1970).
(266) A. Guerritore and G. M. Hanozet, *Ital. J. Biochem.*, **22**, 244–257 (1973).
(267) M. Höfer, K. Brand, K. Deckner, and J.-U. Becker, *Biochem. J.*, **123**, 855–863 (1971).

positions, and measuring the distribution of the label in various fractions of the cells and their metabolites. This yeast appears to break down D-glucose through the pentose cycle solely: (i) 20% of the D-glucose was directly decarboxylated to pentose phosphate, and (ii) 80% was catabolized by way of the non-oxidative part of the pentose cycle. The authors[267] suggested that the pentose phosphates are split into C_2 fragments and glyceraldehyde 3-phosphate, which can then be oxidized by the tricarboxylic acid cycle. Two other strains of unspecified *Rhodotorula* species have been reported to be unlike *Candida utilis* and *Saccharomyces cerevisiae*, in that they are rich in enzymes of the pentose cycle and poor in those of the glycolytic pathway.[268] Following subsequent work with another strain of *Rhodotorula glutinis*, it has been suggested that (a) this yeast catabolizes D-glucose glycolytically[268a] and (b) a 6-phosphofructokinase is present,[268b] although it is unstable and, unlike that of *Saccharomyces cerevisiae*,[268c] it is activated by phosphate.

4. The Control of Glycolysis: Pasteur Effects

The change in free energy for the anaerobic conversion of D-glucose into ethanol, given by

$$C_6H_{12}O_6 \rightarrow 2 \text{ EtOH} + 2 \text{ CO}_2, \quad \Delta G' = -235 \text{ kJ} \quad (\text{Ref. 269}),$$

is much less than that for the aerobic oxidation of D-glucose, given by

$$C_6H_{12}O_6 + 6 \text{ O}_2 \rightarrow 6 \text{ CO}_2 + 6 \text{ H}_2\text{O}, \quad \Delta G' = -2{,}873 \text{ kJ} \quad (\text{Ref. 269}).$$
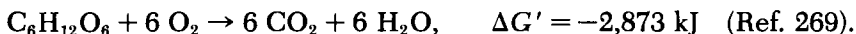
So, when there is a change from anaerobic to aerobic conditions, there is a diminution in the consumption of D-glucose. This is termed the Pasteur effect. The action of oxygen in diminishing carbohydrate breakdown, and in decreasing the accumulation of the products of anaerobic metabolism, was first described by Pasteur,[270] who found[271] that, in the presence of air, the ratio of weight of yeast

(268) M. Nakagawa and C. Tatsumi, *Nippon Nogei Kagaku Kaishi*, 42, 330–336 (1968); *Chem. Abstr.*, 69, 74,672s (1968).

(268a) M. J. Mazón, J. M. Gancedo, and C. Gancedo, *Proc. Intern. Symp. Yeasts, 4th*, Part I, p. 31 (1974).

(268b) M. J. Mazón, J. M. Gancedo, and C. Gancedo, *Biochem. Biophys. Res. Commun.*, 61, 1304–1309 (1974).

(268c) W. Atzpodien and H. Bode, *Eur. J. Biochem.*, 12, 126–132 (1970).

(269) H. A. Krebs, H. L. Kornberg, and K. Burton, *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.*, 49, 212–298 (1957).

(270) L. Pasteur, *Compt. Rend.*, 52, 1260–1264 (1861).

(271) L. Pasteur, "Études sur la Bière," Gauthier-Villars, Paris, 1876.

formed to sugar used was 1:4, but, without air, could be as high as 1:176. He considered[271] that the rate of decomposition of sugar was less in the presence than in the absence of air, and, 49 years later, this was shown to be true by Meyerhof.[272] Meyerhof found that oxygen has a considerable effect on the fermentation by some yeasts, although for others the effect was very small (cf., also, Ref. 273): in air, the ratio of moles of D-glucose oxidized to moles of D-glucose fermented varied from 1:80, for a bottom brewery yeast, to 4:1 for an unidentified yeast.

Much has been published on the controversial subject of the control of glycolysis. The following brief summary of some of the controls responsible for the Pasteur effect in yeasts is based mainly on a review by Sols and coworkers[144] (see also, Fig. 7). (i) Isocitrate dehydrogenase (NAD⊕) (EC 1.1.1.41), one of the controlling enzymes of the tricarboxylic acid cycle (see Fig. 5), catalyzes the reaction

$$\text{isocitrate} + \text{NAD}^{\oplus} \rightarrow \alpha\text{-ketoglutarate} + CO_2 + \text{NADH} + H^{\oplus}$$

and depends for its activity on allosteric activation[275] by AMP. When the energy level of the cell is high, the ratio of the concentration of AMP to that of ATP is low, and, hence, the concentration of citrate increases. (ii) 6-Phosphofructokinase is responsible for phosphorylating D-fructose 6-phosphate to D-fructose 1,6-diphosphate. In the glycolytic pathway (see Fig. 4), this enzyme is the site of the Pasteur effect, as it is inhibited allosterically by citrate and ATP produced by the tricarboxylic acid cycle, and activated[179,276-280] by AMP (for a review, see Refs. 281 and 281a). Inhibition of 6-phosphofructokinase raises the concentration of D-glucose 6-phosphate, and this could facilitate the formation of reserves of polysaccharide. (iii) Thus,

(272) O. Meyerhof, Biochem. Z., 162, 43–86 (1925).
(273) A. Chassang-Douillet, J. Ladet, H. Boze, and P. Galzy, Z. Allgem. Mikrobiol., 13, 193–199 (1973).
(274) G. Cohen, "The Regulation of Cell Metabolism," Hermann, Paris, 1968.
(275) J. A. Hathaway and D. E. Atkinson, J. Biol. Chem., 238, 2875–2881 (1963).
(276) E. Viñuela, M. L. Salas, and A. Sols, Biochem. Biophys. Res. Commun., 12, 140–145 (1963).
(277) A. Ramaiah, J. A. Hathaway, and D. E. Atkinson, J. Biol. Chem., 239, 3619–3622 (1964).
(278) A. Betz and B. Chance, Arch. Biochem. Biophys., 109, 585–594 (1965).
(279) M. L. Salas, J. Salas, and A. Sols, Biochem. Biophys. Res. Commun., 31, 461–466 (1968).
(280) E. G. Afting and D. Ruppert, Arch. Biochem. Biophys., 156, 720–729 (1973).
(281) T. E. Mansour, Curr. Top. Cell. Regul., 5, 1–46 (1972).
(281a) A. Ramaiah, Curr. Top. Cell. Regul., 8, 297–345 (1974).

Exogenous D-glucose

Endogenous D-glucose

P_i --------------→

D-Glucose 6-phosphate

D-Fructose 6-phosphate

P_i --------------→

$NH_4^{\oplus}$ ------------→

D-Fructose 1,6-diphosphate

Pyruvate

Citrate + ATP ◄──────── AMP

NAD-isocitrate
dehydrogenase

α-Ketoglutarate
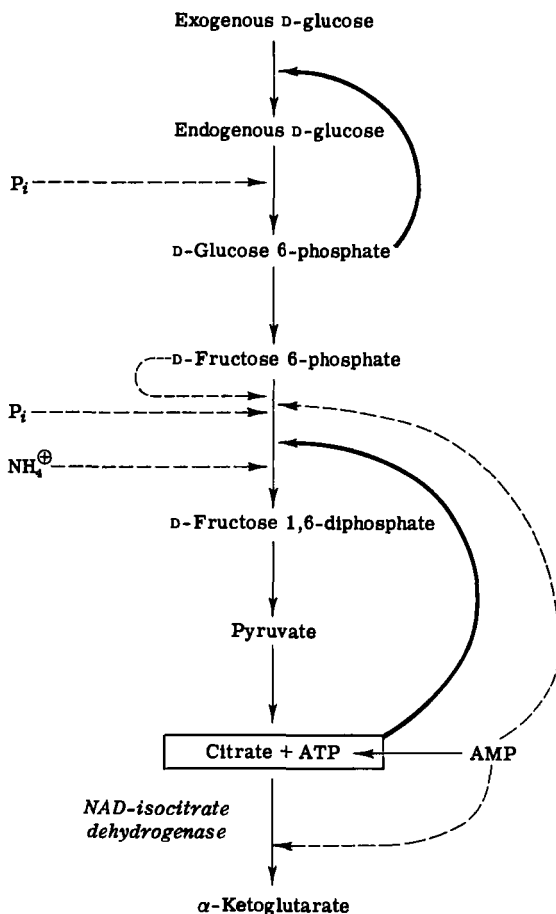
FIG. 7.—The Control of Glycolysis (after Cohen[274]). (Points of inhibition are indicated by black arrows, and of activation by dotted arrows. Published by permission of the copyright owners.)

although the allosteric behavior of 6-phosphofructokinase may be responsible for inhibiting glycolysis, it does not explain the rapid lowering of the rate of utilization of D-glucose. To achieve this, Sols and coworkers[144] suggested that there is allosteric feedback inhibition, by D-glucose 6-phosphate, of the transport of D-glucose into the cells across the plasmalemma.[282-284] As long ago as 1935, Dixon and

(282) A. Kotyk and A. Kleinzeller, *Biochim. Biophys. Acta*, 135, 106–111 (1967).
(283) F. Azam and A. Kotyk, *FEBS Lett.*, 2, 333–335 (1969).
(284) J. U. Becker and A. Betz, *Biochim. Biophys. Acta*, 274, 584–597 (1972).

Holmes[285] suggested that the Pasteur effect involves changes in cell permeability, limiting the rate at which D-glucose reaches the cell's enzymes. Even in an entirely aerobic yeast, *Rhodotorula glutinis* (*gracilis*), the entry of monosaccharides at the plasmalemma is now proposed as a regulatory process in their metabolism.[286]

Meyerhof's observations,[272] already mentioned, which showed that the presence of air does not greatly lessen the glycolytic formation of ethanol by all fermentative yeasts, have been amply confirmed by a number of authors, including Bartley and coworkers.[143,287] They found that a concentration of exogenous D-glucose of 0.9% represses the formation of mitochondrial structures and respiratory enzymes in their strain of *Saccharomyces cerevisiae*. Mitochondria were formed in cells grown on 0.09% of D-glucose or 0.9% of D-galactose, and, in the presence of D-glucose, synthesis of respiratory enzymes was less than with D-galactose.

Aerated growth of *Saccharomyces cerevisiae* on D-glucose in batch culture may occur in two phases. Firstly, the D-glucose is chiefly catabolized to ethanol and, secondly, when the concentration of D-glucose is low, growth occurs primarily by aerobic utilization of the ethanol.[288–295a] High concentrations of D-glucose may lower oxidative catabolism, with a corresponding increase in the production of ethanol.[296]

Under certain conditions, only about half of the D-glucose taken up by anaerobic *Saccharomyces uvarum* (*carlsbergensis*) may be metabolized to ethanol and glycerol[297]; most of the D-glucose remaining

(285) K. Dixon and E. Holmes, *Nature* (London), 135, 995–996 (1935).
(286) M. Höfer and J. Becker, *Zentralbl. Bakteriol., Parasitenk., Infektionskr. Hyg., Abt. 1: Orig., Reihe A*, 220, 374–379 (1972).
(287) E. S. Polakis, W. Bartley, and G. A. Meek, *Biochem. J.*, 97, 298–302 (1965).
(288) W. H. Swanson and C. E. Clifton, *J. Bacteriol.*, 56, 115–124 (1948).
(289) W. D. Maxon and M. J. Johnson, *Ind. Eng. Chem.*, 45, 2554–2560 (1953).
(290) M. Lemoigne, J. P. Aubert, and J. Millet, *Ann. Inst. Pasteur*, 87, 427–439 (1954).
(291) B. Ephrussi, P. P. Slonimski, Y. Yotsuyanagi, and J. Tavlitzki, *C. R. Trav. Lab. Carlsberg, Ser. Physiol.*, 26, 87–102 (1956).
(292) W. Krause, *Arch. Mikrobiol.*, 52, 266–281 (1965).
(293) K. Beran and J. Zemanová, *Biotechnol. Bioeng.*, 11, 853–862 (1969).
(294) H. K. von Meyenburg and A. Fiechter, in "Yeasts," *Proc. Symp. Yeasts, 2nd, Bratislava, 16–21 July, 1966*, A. Kocková-Kratochvílová, ed., Vydavatel'stvo Slovenskej Akadémie Vied, Bratislava, 1969, pp. 377–385.
(295) C. Beck and H. K. von Meyenburg, *J. Bacteriol.*, 96, 479–486 (1968).
(295a) S. Haarasilta and E. Oura, *Eur. J. Biochem.*, 52, 1–7 (1975).
(296) P. P. Slonimski, *Proc. Intern. Congr. Biochem., 3rd, Brussels*, 242–252 (1956).
(297) A. Betz, *Physiol. Plant.*, 19, 1049–1054 (1966).

TABLE VI

Rates of Oxidative Respiration and Non-Oxidative Fermentation by Yeasts
Growing Aerobically in 3% D-Glucose[a]

Yeast	μl of gas/10^7 yeast cells/10 min	
	Oxygen uptake	Carbon dioxide evolved by fermentation
Saccharomyces italicus	0.0	94.5
uvarum (carlsbergensis)	0.0	68.2
bayanus (oviformis)	0.0	61.2
Torulopsis dattila	0.0	52.0
Schizosaccharomyces pombe	0.0	40.6
Saccharomyces chevalieri	1.2	90.8
bayanus ("pasteurianus")	1.9	58.7
cerevisiae (turbidans)	3.6	68.2
cerevisiae	4.8	78.0
Brettanomyces lambicus	1.2	9.3
Torulopsis colliculosa	10.7	39.2
Saccharomyces kloeckerianus (Debaryomyces globosus)	12.3	22.2
Nematospora coryli	21.1	29.2
Torulopsis sphaerica[b]	25.7	3.5
Kluyveromyces (Saccharomyces) fragilis	24.5	1.9
Pichia fermentans	24.3	1.3
Candida tropicalis	27.7	0.9
Schwanniomyces occidentalis	9.1	0.0
Candida (Torulopsis) sake	13.3	0.0
Trichosporon fermentans	15.5	0.0
Candida lambica (monosa)	21.0	0.0
Hansenula anomala	24.1	0.0
Candida utilis	30.0	0.0

[a] Based on results of De Deken.[301] [b] A form of *Kluyveromyces lactis*.

is incorporated directly into insoluble polysaccharide.[298] These observations accord with others made on *Saccharomyces cerevisiae*. Aerobically grown, brewing strains of *Saccharomyces cerevisiae* may form reserve carbohydrate as < 10% of the dry weight, although, when they are grown almost anaerobically, glycogen and trehalose constitute 40% of the dry weight. Ethanolic fermentation of these reserves by the brewing yeasts was found to be faster aerobically

(298) A. Betz and R. Hinrichs, *Eur. J. Biochem.*, **5**, 154–157 (1968).

than anaerobically,[299] but fermentation by a baking strain was the same under both conditions.[300]

When grown in aerobic conditions on 3% D-glucose, yeasts having the ability to ferment sugars anaerobically may vary in their behavior, from those that are completely oxidative under these conditions, such as *Candida utilis*, to others that are completely fermentative, such as *Schizosaccharomyces pombe*.[301] These results are shown in Table VI, although the precise significance of the figures may be in doubt, because no details were given[301] regarding the phase of growth to which each figure applied. Thus, it is certain that the many researches on strains of *Saccharomyces cerevisiae* do not provide a thorough description of the behavior of other fermentative yeasts. Another case in point is the genus *Brettanomyces;* yeasts of this genus exhibit[245] a "negative Pasteur effect," that is, fermentation of D-glucose with the production of ethanol is faster in the presence of oxygen than in its absence (for a review, see Ref. 302).

VIII. The Catabolism of Other Hexoses

1. D-Fructose and D-Mannose

It is D-fructofuranose, not D-fructopyranose, that is utilized, at least by bakers' yeast.[303,304] As with D-glucose, the initial step in the intracellular utilization of either D-fructofuranose[305] or D-mannose is phosphorylation by the constitutive hexokinase[306] (see Ref. 307 for a review). The D-fructose 6-phosphate formed is an intermediate of both the glycolytic pathway and the pentose cycle. D-Mannose phosphate isomerase (EC 5.3.1.8) effects the conversion of D-mannose 6-phosphate into D-fructose 6-phosphate,[308,309] or D-mannose 6-phosphate is epimerized to D-glucose 6-phosphate.[308]

(299) V. E. Chester, *Biochem. J.*, **86**, 153–160 (1963).
(300) V. E. Chester, *Biochem. J.*, **92**, 318–323 (1964).
(301) R. H. De Deken, *J. Gen. Microbiol.*, **44**, 149–156 (1966).
(302) T. O. Wikén, in Ref. 197, 1968, pp. 133–153.
(303) A. Gottschalk, *Aust. J. Exp. Biol. Med. Sci.*, **21**, 133–137 (1943).
(304) A. Gottschalk, *Aust. J. Exp. Biol. Med. Sci.*, **23**, 261–266 (1945).
(305) M. W. Slein, G. T. Cori, and C. F. Cori, *J. Biol. Chem.*, **186**, 763–780 (1950).
(306) L. Berger, M. W. Slein, S. P. Colowick, and C. F. Cori, *J. Gen. Physiol.*, **29**, 379–391 (1946).
(307) D. L. Purich, H. J. Fromm, and F. B. Rudolph, *Advan. Enzymol. Relat. Areas Mol. Biol.*, **39**, 249–326 (1973).
(308) M. W. Slein, *J. Biol. Chem.*, **186**, 753–761 (1950).
(309) E. Noltmann and F. H. Bruns, *Biochem. Z.*, **330**, 514–520 (1958).

2. D-Galactose

The ability of yeasts to utilize D-galactose (unlike that for D-glucose, D-fructose, or D-mannose) has long been known to depend on the carbon source on which the yeast is grown.[310,311] In 1900, Dienert[312] showed that the rate of D-galactose fermentation by yeasts depends upon the sugar that had been present in the medium on which the yeast had grown. Transferring yeast grown on D-galactose to a D-glucose medium led to a loss of D-galactose-fermenting ability. Different yeasts were found to differ in their behavior. However, generally, D-galactose, lactose, or melibiose in the growth medium produced a yeast having high activity against D-galactose; sucrose and maltose produced low activity; and yeasts grown on D-glucose or D-fructose were almost incapable of fermenting D-galactose.

At equilibrium, D-galactopyranose is present in aqueous solution as about 27% α anomer and 73% β anomer,[312a] and transport into cells has been reported as not specific for either form for *Saccharomyces cerevisiae* and *Kluyveromyces (Saccharomyces) fragilis*, grown on complex media.[312b] Within the cells, aldose 1-epimerase (EC 5.1.3.3) converts β-D-galactose into the α anomer[312b] for which galactokinase activity is highly specific.[325]

The reactions by which yeasts convert D-galactose into D-glucose 6-phosphate, an intermediate of both the glycolytic pathway and the pentose cycle, are shown in Fig. 8. In this route of D-galactose catabolism, the presence of certain important enzymes is known to be affected by the carbon source in the growth medium. Galactokinase (EC 2.7.1.6), first identified from *Kluyveromyces (Saccharomyces) fragilis*,[313] is present in D-galactose-grown, but not D-glucose-grown, yeast; this has been found both for *Kluyveromyces fragilis*[314–319] and for "Dutch top yeast" [*Saccharomyces cerevisiae?*].[320] In *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*, hexosyl phos-

(310) [F.] Dienert, *Compt. Rend.*, 128, 569–571 (1899).

(311) A. Harden and R. V. Norris, *Proc. Roy. Soc., Ser. B.* 82, 645–649 (1910).

(312) F. Dienert, *Ann. Inst. Pasteur*, 14, 139–189 (1900).

(312a) S. J. Angyal, *Angew. Chem. Int. Ed. Engl.*, 8, 157–226 (1969).

(312b) P. Sammler, R. Ehwald, and H. Göring, *Folia Microbiol.* (Prague), 19, 479–488 (1974).

(313) R. Caputto, L. F. Leloir, and R. E. Trucco, *Enzymologia*, 12, 350–355 (1948).

(314) R. E. Trucco, R. Caputto, L. F. Leloir, and N. Mittelman, *Arch. Biochem.*, 18, 137–146 (1948).

(315) R. Caputto, L. F. Leloir, R. E. Trucco, C. E. Cardini, and A. C. Paladini, *J. Biol. Chem.*, 179, 497–498 (1949).

(316) C. E. Cardini and L. F. Leloir, *Arch. Biochem. Biophys.*, 45, 55–64 (1953).
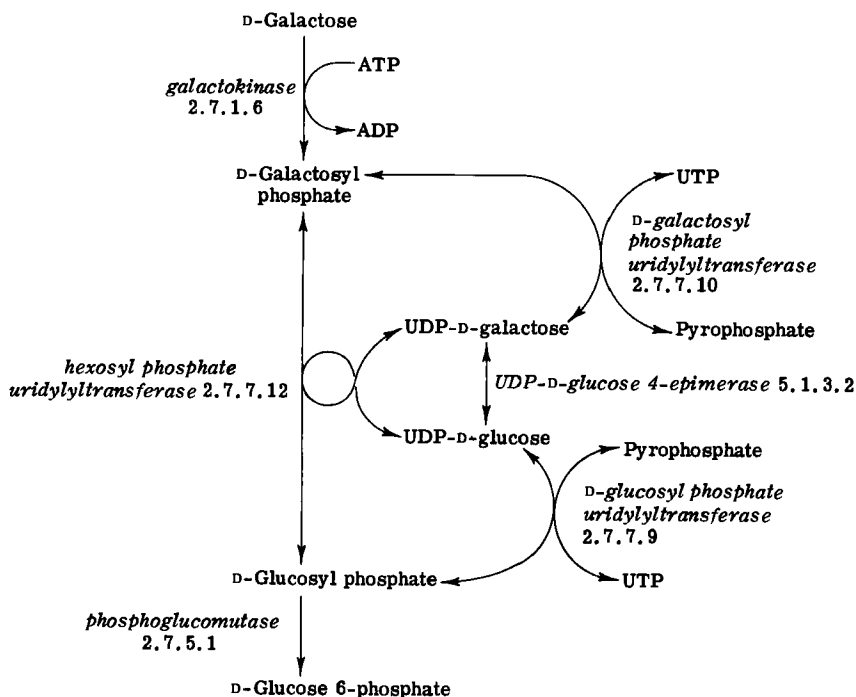
FIG. 8.—Enzymic Reactions Concerned with the Conversion of D-Galactose into D-Glucose 6-Phosphate. (Enzyme Commission numbers are given for each enzyme.)

phate uridylyltransferase (EC 2.7.7.12) and UDP-D-glucose 4-epimerase (EC 5.1.3.2) are present if the growth is on D-galactose, but are absent or almost absent with D-glucose as the growth substrate[317,321] (see Table VII). However, there was one report that the epimerase is active in D-glucose-grown yeasts.[322] D-Glucosyl phosphate uridylyltransferase (EC 2.7.7.9) is found in yeasts grown on either D-galactose or D-glucose.[317,321] The metabolism of D-galactose

(317) H. de Robichon-Szulmajster, Biochim. Biophys. Acta, 29, 270–272 (1958).
(318) F. Alvarado, Biochim. Biophys. Acta, 41, 233–238 (1960).
(319) M. R. Heinrich, J. Biol. Chem., 239, 50–53 (1964).
(320) J. F. Wilkinson, Biochem. J., 44, 460–467 (1949).
(321) H. de Robichon-Szulmajster, Science, 127, 28–29 (1958).
(322) G. T. Mills, E. E. B. Smith, and A. C. Lochhead, Biochim. Biophys. Acta, 25, 521–528 (1957).
(323) H. de Robichon-Szulmajster, Ann. Technol. Agr., 10, 81–142 (1961).
(324) B. G. Adams, J. Bacteriol., 111, 308–315 (1972).
(325) S. M. Howard and M. R. Heinrich, Arch. Biochem. Biophys., 110, 395–400 (1965).

TABLE VII

Presence of Enzymes of the D-Galactose Pathway in Yeasts Grown on
D-Glucose or D-Galactose

Enzyme	Galactokinaseless strain of Saccharomyces cerevisiae[321]		Kluyveromyces fragilis[317]	
	Carbon source in growth medium			
	D-Glucose	D-Galactose	D-Glucose	D-Galactose
	Specific activities of enzymes (in nmoles of substrate reacted/minute/mg of protein)			
Galactokinase (EC 2.7.1.6)	0	0	0	26,000
Hexosyl phosphate uridylyl-transferase (EC 2.7.7.12)	0.01	8.96	10	3,680
UDP-D-glucose 4-epimerase (EC 5.1.3.2)	0	5.70	0	333,000
D-Glucosyl phosphate uridylyl-transferase (EC 2.7.7.9)	9.17	12.50	1,770	2,220

by yeasts was reviewed by de Robichon-Szulmajster,[323] and Table VIII summarizes information on the D-galactose pathway.

Genetical control of the utilization of D-galactose, in *Saccharomyces cerevisiae* (or hybrids of this with other species), involves at least three regulatory genes[342] and five structural genes (see

(326) H. C. Douglas and D. C. Hawthorne, *Genetics*, 49, 837–844 (1964).
(327) J. Bassel and H. Douglas, *J. Bacteriol.*, 95, 1103–1110 (1968).
(328) H. M. Kalckar, B. Braganca, and A. Munch-Petersen, *Nature* (London), 172, 1038 (1953).
(329) R. E. Trucco, *Nature* (London), 174, 1103–1104 (1954).
(330) R. G. Hansen and R. A. Freedland, *J. Biol. Chem.*, 216, 303–307 (1955).
(331) F. Azam, S. Kuo, and V. P. Cirillo, *J. Bacteriol.*, 106, 915–919 (1971).
(332) L. F. Leloir, *Arch. Biochem. Biophys.*, 33, 186–190 (1951).
(333) W. L. Salo, J. H. Nordin, D. R. Peterson, R. D. Bevill, and S. Kirkwood, *Biochim. Biophys. Acta*, 151, 484–492 (1968).
(334) R. A. Darrow and R. Rodstrom, *Biochemistry*, 7, 1645–1654 (1968).
(335) R. A. Darrow and R. Rodstrom, *J. Biol. Chem.*, 245, 2036–2042 (1970).
(336) A. Munch-Petersen, *Acta Chem. Scand.*, 9, 1523–1536 (1955).
(337) E. E. McCoy and V. A. Najjar, *J. Biol. Chem.*, 234, 3017–3021 (1959).
(338) H. C. Douglas, *Biochim. Biophys. Acta*, 52, 209–211 (1961).
(339) A. Tsoi and H. C. Douglas, *Biochim. Biophys. Acta*, 92, 513–520 (1964).
(340) P. Bevan and H. C. Douglas, *J. Bacteriol.*, 98, 532–535 (1969).
(341) H. C. Douglas, D. P. Grindall, and H. Talbott, *J. Bacteriol.*, 99, 287–290 (1969).
(342) H. C. Douglas and D. C. Hawthorne, *Genetics*, 54, 911–916 (1966).

Table IX) (for reviews, see Refs. 363–366). *GAL1*, *GAL7*, and *GAL10* are closely linked[326] structural genes for (*i*) galactokinase,[321] (*ii*) hexosyl phosphate uridylyltransferase,[326,327] and (*iii*) UDP-D-glucose 4-epimerase,[326] respectively. The wild-type yeast synthesizes these three enzymes simultaneously in the presence of D-galactose.[321] *GAL5* is the structural gene for the electrophoretically major form of phosphoglucomutase,[338–340] and *GAL2* is the structural gene for the carrier responsible for the entry of D-galactose.[169,323,345] Mutants of any of these five structural genes fail to utilize D-galactose, although in each case only one enzyme, or the carrier, is affected.

3. 2-Deoxy-D-*arabino*-hexose

2-Deoxy-D-*arabino*-hexose ("2-deoxy-D-glucose") is not a substrate for the growth, respiration, or fermentation of yeasts. However, it is metabolized, although the extent to which the hexose ring is cleaved is small. The compound is phosphorylated by hexokinase, with ATP as the donor.[366a] 2-Deoxy-D-*arabino*-hexose is employed as an ana-

(343) K. W. van de Poll, A. Kerkenaar, and D. H. J. Schamhart, *J. Bacteriol.*, 117, 965–970 (1974).

(344) P. K. Maitra, *J. Bacteriol.*, 107, 759–769 (1971).

(345) H. C. Douglas and F. Condie, *J. Bacteriol.*, 68, 662–670 (1954).

(346) Ø. Winge and C. Roberts, *C. R. Trav. Lab. Carlsberg*, 24, 263–315 (1948).

(347) S. Spiegelman, M. Sussman, and B. Taylor, *Fed. Proc.*, 9, 120 (1950).

(348) Ø. Winge and C. Roberts, *C. R. Trav. Lab. Carlsberg*, 25, 141–171 (1952).

(349) H. O. Halvorson, S. Winderman, and J. Gorman, *Biochim. Biophys. Acta*, 67, 42–53 (1963).

(349a) F. K. Zimmermann and N. R. Eaton, *Mol. Gen. Genet.*, 134, 261–272 (1974).

(350) N. R. Eaton and F. K. Zimmermann, unpublished results.

(351) N. A. Khan and N. R. Eaton, *Mol. Gen. Genet.*, 112, 317–322 (1971).

(352) C. C. Lindegren and G. Lindegren, *J. Gen. Microbiol.*, 15, 19–28 (1956).

(353) A. M. A. ten Berge, G. Zoutewelle, and K. W. van de Poll, *Mol. Gen. Genet.*, 123, 233–246 (1973).

(354) A. M. A. ten Berge, G. Zoutewelle, K. W. van de Poll, and H. P. J. Bloemers, *Mol. Gen. Genet.*, 125, 139–146 (1973).

(355) A. M. A. ten Berge, G. Zoutewelle, and R. B. Needleman, *Mol. Gen. Genet.*, 131, 113–121 (1974).

(356) Ø. Winge and C. Roberts, *C. R. Trav. Lab. Carlsberg*, 18, 419–459 (1957).

(357) D. C. Hawthorne, *Heredity*, 12, 273–284 (1958).

(358) C. C. Lindegren and G. Lindegren, *Genetica*, 26, 430–444 (1953).

(359) C. C. Lindegren, D. D. Pittman, and B. Ranganathan, *Proc. Intern. Genet. Symp. Jap. Cytologia Suppl.*, 42–50 (1957).

(360) S. Ouchi and C. C. Lindegren, *Can. J. Genet. Cytol.*, 5, 257–267 (1963).

(361) Y. L. Hwang, G. Lindegren, and C. C. Lindegren, *Can. J. Genet. Cytol.*, 6, 373–380 (1964).

(362) P. Ottolenghi, *C. R. Trav. Lab. Carlsberg*, 38, 213–221 (1971).

TABLE VIII

Publications on Enzymes of the D-Galactose Pathway in Yeasts

Enzyme (and Enzyme Commission No.)	*Saccharomyces cerevisiae*	*Kluyveromyces fragilis*
Galactokinase (2.7.1.6)	Wilkinson (1949)[320] kinase present in extracts of D-galactose-grown yeast; de Robichon-Szulmajster (1958)[321] kinase under control of gene *GAL1*; Adams (1972)[324] kinase induction and repression	Caputto and coworkers (1948),[313] Trucco and coworkers (1948),[314] and Caputto and coworkers (1949)[315] kinase in extracts of D-galactose-adapted yeast; Cardini and Leloir (1953)[316] extracts have more kinase when yeast is grown on lactose than on D-glucose; de Robichon-Szulmajster (1958)[317] extracts have activity when yeast is grown on D-galactose, not on D-glucose; Alvarado (1960)[318] kinase substrate specificity and K_m; Heinrich (1964)[319] partial purification and properties of kinase; Howard and Heinrich (1965)[325] anomeric specificity of kinase
Hexosyl phosphate uridylyltransferase (2.7.7.12)	de Robichon-Szulmajster (1958)[321] transferase in D-galactose-grown yeast, very little in D-glucose-grown yeast; Douglas and Hawthorne (1964),[326] Bassel and Douglas (1968)[327] transferase under control of gene *GAL7*	Kalckar and coworkers (1953),[328] Trucco (1954),[329] and Hansen and Freedland (1955)[330] extracts of yeast catalyze reversible uridylyl transfer from UDP-D-glucose to D-galactosyl phosphate; Mills and coworkers (1957)[322] and de Robichon-Szulmajster (1958)[317] transferase in D-galactose-grown yeast; very little in D-glucose-grown yeast

UDP-D-glucose 4-epimerase (5.1.3.2)	de Robichon-Szulmajster (1958)[321] epimerase in D-galactose-grown, not D-glucose-grown, yeast; Douglas and Hawthorne (1964)[326] epimerase under control of gene GAL10; Azam and coworkers (1971)[331] epimerase inactivated by L-arabinose	Leloir (1951)[332] epimerase in D-galactose-adapted yeast; Mills and coworkers (1957)[322] epimerase in both D-galactose-grown and D-glucose-grown yeast; de Robichon-Szulmajster (1958)[317] epimerase in D-galactose-grown, not D-glucose-grown, yeast; Salo and coworkers (1968)[333] and Darrow and Rodstrom (1968, 1970)[334,335] purification and properties of epimerase
D-Galactosyl phosphate uridylyltransferase (2.7.7.10)		Kalckar and coworkers (1953)[328] transferase present in yeast extracts
Glucosyl phosphate uridylyltransferase (2.7.7.9)	de Robichon-Szulmajster (1958)[321] transferase in D-galactose-grown yeast	Munch-Petersen (1955)[336] transferase purification, properties, and reaction mechanism; de Robichon-Szulmajster (1958)[317] transferase in D-glucose-grown and D-galactose-grown yeast
Phosphoglucomutase (2.7.5.1)	McCoy and Najjar (1959)[337] purified mutase, and gave properties and reaction mechanism; Douglas (1961),[338] Tsoi and Douglas (1964),[339] and Bevan and Douglas (1969)[340] mutase under control of gene GAL5	Douglas and coworkers (1969)[341] 5 or 6 electrophoretic variants of mutase characteristics of *Kluyveromyces fragilis*, *K. lactis*, and *K. marxianus*

TABLE IX

**Known Genes Concerned with the Utilization of Sugars by Some *Saccharomyces*
Species and Interspecific Hybrids[a]**

Utilization affected	Genes	Role of genes	References
D-Glucose, D-fructose, and D-mannose	*FDP80*	regulator for hexosediphosphatase	343
D-Glucose	*PGI1*	mutant is deficient in D-glucose phosphate isomerase	344
	GAL1	structural gene for galactokinase	321
	GAL2	structural gene for D-galactose carrier	323,345
	GAL3	"long-term adaptation" to D-galactose	346,347
	GAL4	regulator of *GAL1*, *GAL7*, and *GAL10*	326
	GAL5	structural gene for phosphogluco-mutase	338,339
D-Galactose	*GAL7*	structural gene for galactosyl phosphate uridylyltransferase	326,327
	GAL10	structural gene for UDP-D-glucose 4-epimerase	326
	GAL80	repressor: recessive mutants synthesize (constitutively) enzymes controlled by *GAL1*, *GAL7*, and *GAL10*	342
	GAL81	operator gene for *GAL4*: permits constitutive synthesis of enzymes controlled by *GAL4*	342
	MAL1	? regulator of α-D-glucosidase synthesis	348–350
	MAL2	regulator of α-D-glucosidase synthesis: some mutants are constitutive, others are non-utilizers	348–350
	MAL3	? regulator of α-D-glucosidase synthesis	348–350
	MAL4	regulator of α-D-glucosidase synthesis: constitutive for α-D-glucosidase synthesis and resistant to catabolite repression; non-utilizing mutants have been found	348–351
Maltose	*MAL5*		349,352
	MAL6	? regulator of α-D-glucosidase and maltose carrier; recessive mutants found: (*i*) maltose non-utilizers, (*ii*) constitutive utilizers	349,353–355
	DSF6, *DSF7*, *DSF17*, *DSF21*, *DSF24*, *DSF28*	mutants do not utilize maltose, even in the presence of *MAL4*; ? maltose carrier affected	233

TABLE IX (continued)

Utilization affected	Genes	Role of genes	References
Melibiose	MEL1	structural gene for α-D-galactosidase	352,356
Methyl α-D-gluco-pyranoside	MGL1	structural or regulator gene for oligo-(1 → 6)-D-glucosidase; utilization of methyl α-D-glucopyranoside in presence of MGL2	350,357
	MGL2	? structural gene for carrier for methyl α-D-glucopyranoside	227
	MGL3	structural or regulator gene for oligo-(1 → 6)-glucosidase; utilization of methyl α-D-glucopyranoside in presence of MGL2	350,357
Melezitose and turanose	MLZ1	control of certain α-D-glucopyrano-sidase activities	358–361
Sucrose and raffinose	SUC1 to SUC5	polymeric structural genes for β-D-fruc-tofuranosidase	348,362
	SUC80	β-D-fructofuranosidase production resistant to repression by hexose	362a,500

a Strains carrying most of these genes are listed in the 1975 Catalogue of the Yeast Genetic Stock Center (Curators, John Bassel and Rebecca Contopoulou), Donner Laboratory, University of California, Berkeley, California 94720.

logue of D-glucose, particularly for experiments on uptake and phosphorylation, in which processes it is a substrate of the same mechanisms as those for D-glucose. In 1953, Overend and Stacey[367] reviewed the chemistry of the 2-deoxy sugars, but subsequent treatises on carbohydrate chemistry have not dealt with 2-deoxy-D-*arabino*-hexose in detail.

In non-growing, cell suspensions, 2-deoxy-D-*arabino*-hexose strongly inhibits anaerobic fermentation and aerobic respiration.[368-373] The sugar inhibits growth, and lyses growing cells, of

(362a) B. K. Ghosh, B. Montenecourt, and J. O. Lampen, *J. Bacteriol.*, 116, 1412–1420 (1973).
(363) H. de Robichon-Szulmajster, *Ann. Technol. Agr.*, 10, 185–256 (1961).
(364) R. K. Mortimer and D. C. Hawthorne, in Ref. 21, 1969, Vol. 1, pp. 385–460.
(365) L. H. Hartwell, *Annu. Rev. Genet.*, 4, 373–396 (1970).
(366) H. C. Douglas and D. C. Hawthorne, *J. Bacteriol.*, 109, 1139–1143 (1972).
(366a) E. M. Bessell, A. B. Foster, and J. H. Westwood, *Biochem. J.*, 128, 199–204 (1972).
(367) W. G. Overend and M. Stacey, *Advan. Carbohyd. Chem.*, 8, 45–105 (1953).
(368) F. B. Cramer and G. E. Woodward, *J. Franklin Inst.*, 253, 354–360 (1952).

Pichia farinosa, Saccharomyces cerevisiae,[374] and *Schizosaccharo-myces pombe,*[374–376a] because of damage to the cell walls.

Saccharomyces cerevisiae incorporates 2-deoxy-D-*arabino*-hexose into uridine[377,378] and guanosine[379] nucleotides, and forms 2,2'-dideoxy-α,α-trehalose (2-deoxy-α-D-*arabino*-hexopyranosyl 2-deoxy-α-D-*arabino*-hexopyranoside)[380] and 2-deoxy-α,α-trehalose (2-deoxy-α-D-*arabino*-hexopyranosyl α-D-*arabino*-hexopyranoside), probably by the following reactions.[381]

2-Deoxy-D-*arabino*-hexose

$$\rightarrow \text{2-deoxy-D-}arabino\text{-hexose 6-phosphate} \qquad (i)$$

2-Deoxy-D-*arabino*-hexose 6-phosphate + UDP-D-glucose

$$\leftrightarrow \text{2-deoxy-}\alpha,\alpha\text{-trehalose 6-phosphate} \qquad (ii)$$

$$\rightarrow \text{2-deoxy-}\alpha,\alpha\text{-trehalose} + \text{P}_i \qquad (iii)$$

In addition, 2-deoxy-D-*arabino*-hexose is incorporated into the cell-wall mannan, and this may cause a lesser degree of branching of the mannan molecules.[382] It was suggested that high, internal concentrations of 2-deoxy-D-*arabino*-hexose 6-phosphate may block the synthesis of cell-wall polysaccharides and glycoproteins by inhibiting D-glucose phosphate isomerase (EC 5.3.1.9) and, hence, the formation of D-mannose 6-phosphate from D-fructose 6-phosphate.[383]

(369) G. E. Woodward, F. B. Cramer, and M. T. Hudson, *J. Franklin Inst.*, **256**, 577–587 (1953).

(370) E. Hofmann, H. Mucke, and I. Behne, *Naturwissenschaften*, **49**, 214–215 (1962).

(371) C. F. Heredia, G. de la Fuente, and A. Sols, *Biochim. Biophys. Acta*, **86**, 216–223 (1964).

(372) M. E. Feldheim, H. W. Augustin, and E. Hofmann, *Biochem. Z.*, **344**, 238–255 (1966).

(373) E. R. Blakley, T. Myoda, and J. F. T. Spencer, *Can. J. Biochem.*, **44**, 927–935 (1966).

(374) B. F. Johnson, *J. Bacteriol.*, **95**, 1169–1172 (1968).

(375) R. K. Poole and D. Lloyd, *Arch. Mikrobiol.*, **88**, 257–272 (1973).

(376) R. Megnet, *J. Bacteriol.*, **90**, 1032–1035 (1965).

(376a) P. Biely, V. Farkaš, and Z. Krátký, *Biologia* (Bratislava), **29**, 919–925 (1974); *Chem. Abstr.* **82**, 70,203k (1975).

(377) P. Biely and Š. Bauer, *Biochim. Biophys. Acta*, **121**, 213–214 (1966).

(378) P. Biely and Š. Bauer, *Collect. Czech. Chem. Commun.*, **32**, 1588–1594 (1967).

(379) P. Biely and Š. Bauer, *Biochim. Biophys. Acta*, **156**, 432–434 (1968).

(380) V. Farkaš, Š. Bauer, and J. Zemek, *Biochim. Biophys. Acta*, **184**, 77–82 (1969).

(381) J. Zemek and Š. Bauer, *Biochim. Biophys. Acta*, **264**, 393–397 (1972).

(382) P. Biely, Z. Krátký, and Š. Bauer, *Biochim. Biophys. Acta*, **255**, 631–639 (1972).

(383) S. C. Kuo and J. O. Lampen, *J. Bacteriol.*, **111**, 419–429 (1972).

The action of 2-deoxy-D-*arabino*-hexose on *Schizosaccharomyces pombe* may be quite different from that on *Saccharomyces cerevisiae*,[375] and it has been exploited to aid in disrupting the cells[384,385] and in selecting auxotrophic mutants.[386]

IX. THE CATABOLISM OF CERTAIN GLYCOSIDES

The first step in the utilization, by yeasts, of the most common glycosides is either (*i*) their passage intact across the plasmelemma (see Section VI,4), or (*ii*) their initial hydrolysis outside the plasmalemma, followed by entry of some or all of the components. Hence, the inability of a yeast to utilize one of the common oligosaccharides may usually be attributed either to lack of an appropriate glycosidase or to its inaccessibility to the substrate because of lack of an appropriate, membrane carrier.

It is appropriate to record here a well-founded, experimental observation that was once considered[220] inconsistent with these generalizations and with other information in this Chapter. In 1940, Kluyver and Custers[387] established the correctness of some earlier reports that certain yeasts can utilize particular disaccharides aerobically, but not anaerobically, although they can use the component hexoses anaerobically. This is true for the utilization of maltose by *Torulopsis dattila*,[77,387,388] and of lactose by *Candida intermedia* (*Blastodendrion intermedium*).[41,387] Anaerobic utilization is, presumably, blocked at the level of transport across the plasmalemma; this possibility could readily be investigated.

1. β-D-Fructofuranosides

The β-D-fructofuranosidase of *Saccharomyces cerevisiae* catalyzes the hydrolysis of sugars having a terminal, unsubstituted β-D-fructofuranosyl group.[389] Most of the studies have been conducted with sucrose or raffinose as the substrate; these are cleaved as on p. 184. The relative rates on page 184 have been found for the hydrolysis of sucrose, raffinose, and stachyose by purified β-D-fructofuranosidases

(384) H. C. Birnboim, *J. Bacteriol.*, **107**, 659–663 (1971).
(385) F. Foury and A. Goffeau, *J. Gen. Microbiol.*, **75**, 227–229 (1973).
(386) R. Megnet, *Mutat. Res.*, **2**, 328–331 (1965).
(387) A. J. Kluyver and M. T. J. Custers, A. v. L., *J. Microbiol. Serol.*, **6**, 121–162 (1940).
(388) F. Hoeke, *Chem. Weekblad*, **36**, 237–241 (1939).
(389) K. Myrbäck, *Enzymes* (2nd Edition), **4**, 379–392 (1960).

Sucrose → D-Glucose + D-Fructose

Raffinose → Melibiose + D-Fructose
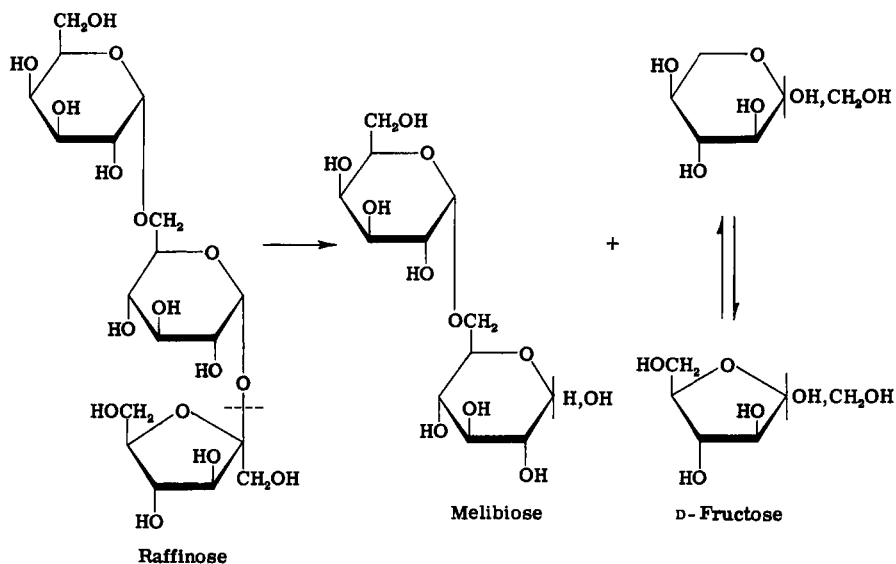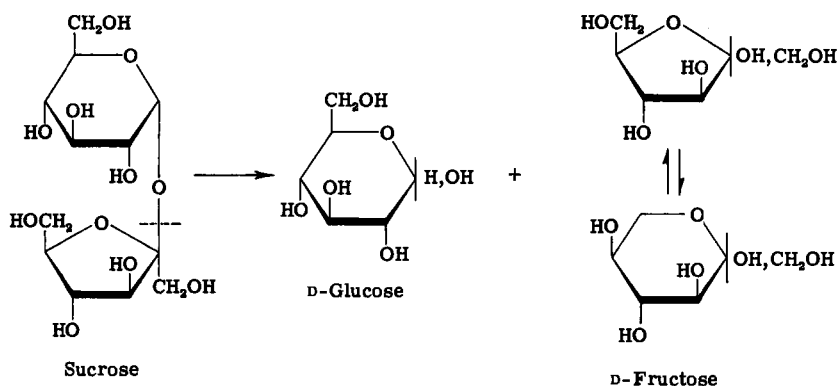
of bakers' and brewers' yeast (presumably *Saccharomyces cerevisiae*).[390]

Yeast	Sucrose	Raffinose	Stachyose
Bakers'	100	23.0	6.8
Brewers'	100	12.6	3.1

The structures of these oligosaccharides are as follows.

(390) M. Adams, N. K. Richtmyer, and C. S. Hudson, *J. Amer. Chem. Soc.*, **65**, 1369–1380 (1943).

Sucrose

O-α-D-Galactosyl-(1→6)-*O*-α-D-galactosyl-(1→6)-*O*-α-D-glucosyl β-D-fructofuranoside

Raffinose

Stachyose
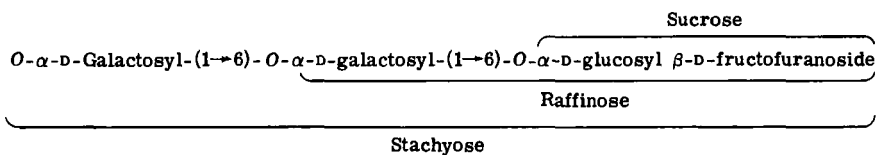
Weidenhagen[391] found that yeast β-D-fructofuranosidase hydrolyzes neither methyl nor phenyl β-D-fructofuranoside. On the other hand, (*i*) others reported sucrose to be hydrolyzed only 13.5 times faster than methyl β-D-fructofuranoside,[392] and (*ii*) yeast β-D-fructofuranosidase preparations have been shown to (a) form methyl β-D-fructofuranoside from sucrose plus methanol by trans-D-fructosylation,[393] and (b) hydrolyze ethyl β-D-fructofuranoside and *N*-(*p*-nitrophenyl)-β-D-fructofuranosylamine.[394] Weidenhagen's enzyme[391] quantitatively converted highly purified inulin into D-fructose, although the living yeast (*Saccharomyces cerevisiae?*) did not attack inulin. The arrangement of the sugar residues in inulin is as follows.[395]

$$\text{Fru}f\text{-}[2\text{-}(\rightarrow 1\text{-Fru}f\text{-}2\text{-})_n \rightarrow 1]\text{-Fru}f\text{-}(2 \rightarrow 1)\text{-Glc}p$$
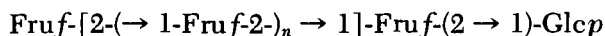
where Fru*f* = β-D-fructofuranosyl group or residue, and Glc*p* = α-D-glucopyranose residue. Yeast β-D-fructofuranosidase was discussed in valuable reviews by Neuberg and Mandl,[396] Gottschalk,[397] Myrbäck,[389] and Lampen.[398]

Some yeasts release much of their β-D-fructofuranosidase into the medium,[399–401] but the enzyme is retained by intact cells of other yeasts, such as *Saccharomyces cerevisiae*, with which most of the relevant work has been done. On the other hand, even with this yeast, raffinose and sucrose are hydrolyzed by β-D-fructofuranosidase out-

(391) R. Weidenhagen, *Z. Zuckerind.*, **82**, 912–922 (1932).
(392) C. B. Purves and C. S. Hudson, *J. Amer. Chem. Soc.*, **56**, 702–707 (1934).
(393) J. S. D. Bacon, *Biochem. J.*, **50**, xviii (1952).
(394) A. Baseer and S. Shall, *Int. J. Biochem.*, **2**, 503–506 (1971).
(395) G. O. Aspinall, E. Percival, D. A. Rees, and M. Rennie in "Rodd's Chemistry of Carbon Compounds," S. Coffey, ed., Elsevier, Amsterdam, 2nd Edition, 1967, Vol. 1, Part F, pp. 596–714.
(396) C. Neuberg and I. Mandl, *Enzymes*, **1**, Part 1, 527–550 (1950).
(397) A. Gottschalk, in "Handbuch der Pflanzenphysiologie," W. Ruhland, ed., Springer-Verlag, Berlin, 1958, Vol. VI, pp. 87–124.
(398) J. O. Lampen, *Enzymes* (3rd Edition), **5**, 291–305 (1971).
(399) A. Guilliermond, "The Yeasts," Wiley, New York, 1920.
(400) L. J. Wickerham, *Arch. Biochem. Biophys.*, **76**, 439–448 (1958).
(401) R. G. Dworschack and L. J. Wickerham, *Appl. Microbiol.*, **9**, 291–294 (1961).

side the plasmalemma, so that the D-fructose and D-glucose liberated enter the cytoplasm as monomers. The consequent similarity between the β-D-fructofuranosidase activity of intact and disintegrated cells has long been recognized.[402] The following findings are evidence that this activity occurs outside the permeability barrier of intact cells. (i) The effect, on the β-D-fructofuranosidase activity of intact cells, of varying the pH is the same as that for the enzyme in solution, but this is not true for the whole fermentation system.[403–405] (ii) Uranyl ions have little effect on the β-D-fructofuranosidase activity of intact cells, although the ions greatly lessen fermentation[406] by inhibiting sugar transport across the plasmalemma.[153] (iii) The kinetics of the hydrolysis of sucrose by intact cells are consistent with the view that hydrolysis occurs outside the plasmalemma.[407] (iv) Hexose liberated by the β-D-fructofuranosidase of intact cells can be trapped by adding exogenous hexokinase; the hexose phosphates formed do not enter the cells across the plasmalemma.[223] (v) With the disintegration of the cell wall in spheroplast or protoplast formation, there is a simultaneous liberation of β-D-fructofuranosidase activity into the medium,[408,409] the resulting spheroplasts being correspondingly low in activity.[409,410] (vi) Isolated cell-walls, obtained by mechanical disintegration of the cells, contain much β-D-fructofuranosidase.[409] (vii) Cells that utilize sucrose may be converted into spheroplasts that cannot do so, although they can use D-glucose.[409]

It is possible that the β-D-fructofuranosidase is held in the periplasmic space, between the plasmalemma and the cell wall, as suggested by Best[407] and others.[411–412a] Should this be so, the cell wall must act as a barrier to the loss of the enzyme molecules from the cell; they would remain partly trapped in the structural framework of the cell wall, producing a physiologically advantageous con-

(402) H. Euler and S. Kullberg, Z. Physiol. Chem., 71, 14–30 (1911).

(403) R. Willstätter and F. Racke, Ann., 425, 1–135 (1921).

(404) K. Myrbäck, Z. Physiol. Chem., 139, 30–38 (1924).

(405) B. J. Wilkes and E. T. Palmer, J. Gen. Physiol., 16, 233–242 (1932).

(406) D. J. Demis, A. Rothstein, and R. Meier, Arch. Biochem. Biophys., 48, 55–62 (1954).

(407) J. B. Best, J. Cellular Comp. Physiol., 46, 29–52 (1955).

(408) M. Burger, E. E. Bacon, and J. S. D. Bacon, Nature (London), 182, 1508 (1958).

(409) D. D. Sutton and J. O. Lampen, Biochim. Biophys. Acta, 56, 303–312 (1962).

(410) J. Friis and P. Ottolenghi, C. R. Trav. Lab. Carlsberg, 31, 259–271 (1959).

(411) M. Burger, E. E. Bacon, and J. S. D. Bacon, Biochem J., 78, 504–511 (1961).

(412) W. N. Arnold, J. Bacteriol., 112, 1346–1352 (1972).

(412a) N. M. Mityushova and A. M. Ugolev, Dokl. Akad. Nauk SSSR, 195, 503–506 (1970); Chem. Abstr., 74, 73,018z (1971).

centration of enzyme.[410] Alternatively, the β-D-fructofuranosidase may be within the cell wall, below the outer layer of mannan, attached to the wall by D-mannose–phosphoric diester bridges or held there by hydrogen bonds.[413] These two hypotheses may not be mutually exclusive. Most of the β-D-fructofuranosidase of "fresh bakers' yeast" (Ref. 412) or of brewers' yeast[414] may be released into solution by mechanical disruption. This fact appears to be consistent with (i) earlier observations of the soluble nature of the enzyme[415,416] and (ii) the presence of only a small proportion of the total enzyme in the cell-wall material.[412,417] However, some of the apparently contradictory observations have been made on different strains and, furthermore, the proportion and precise location of the enzyme may vary with the growth medium used and with the exact stage of growth of the yeast,[413,418–421] and these factors have not always been carefully controlled.

When intact cells of *Kluyveromyces (Saccharomyces) fragilis* are treated with 2-mercaptoethanol, they may release most of their β-D-fructofuranosidase, and this dissolves in the suspending medium. Davies and coworkers[422–424] suggested that (i) this release is achieved by breakdown of disulfide bridges in a mannan–protein complex of the outer region of the cell wall, and (ii) the enzyme is not bound to the cell wall, but is retained in enzyme-impermeable structures within the wall. However, according to Phaff,[103] the enzyme of *Kluyveromyces fragilis*[419–426] is inulinase (EC 3.2.1.7)[427–429] and not β-

(413) J. O. Lampen, *A. v. L., J. Microbiol. Serol.*, **34**, 1–18 (1968).
(414) N. J. Williams and A. Wiseman, *Biochem. Soc. Trans.*, **1**, 1299–1301 (1974).
(415) E. Fischer, *Ber.*, **27**, 2985–2993 (1894).
(416) R. Willstätter and F. Racke, *Ann.*, **427**, 111–141 (1922).
(417) I. McMurrough and A. H. Rose, *Biochem. J.*, **105**, 189–203 (1967).
(418) R. Willstätter, C. D. Lowry, and K. Schneider, *Z. Physiol. Chem.*, **146**, 158–180 (1925).
(419) R. Davies, *Biochem. J.*, **55**, 484–497 (1953).
(420) A. Davies, *J. Gen. Microbiol.*, **14**, 109–121 (1956).
(421) R. Weimberg and W. L. Orton, *J. Bacteriol.*, **91**, 1–13 (1966).
(422) R. Davies and P. A. Elvin, *Biochem. J.*, **93**, 8P–9P (1964).
(423) R. Davies, *Abh. Deut. Akad. Wiss. Berlin, Kl. Med.*, **6**, 195–198 (1967); *Chem. Abstr.*, **70**, 65,506j (1969).
(424) D. K. Kidby and R. Davies, *Biochim. Biophys. Acta*, **201**, 261–266 (1970).
(425) R. Davies, *J. Gen. Microbiol.*, **66**, 37–53 (1971).
(426) V. V. Yurkevich and N. S. Kovaleva, *Dokl. Akad. Nauk SSSR*, **207**, 1233–1235 (1972).
(427) H. E. Snyder and H. J. Phaff, *A. v. L., J. Microbiol. Serol.*, **26**, 433–452 (1960).
(428) H. E. Snyder and H. J. Phaff, *J. Biol. Chem.*, **237**, 2438–2441 (1962).
(429) G. Avigad and Š. Bauer, *Methods Enzymol.*, **8**, 621–628 (1966).

D-fructofuranosidase (EC 3.2.1.26). The ratio of rate of hydrolysis of inulin to that of sucrose is much higher for inulinase than for the β-D-fructofuranosidase of *Saccharomyces cerevisiae*. The smaller size of the enzyme of *Kluyveromyces fragilis* than that of *Saccharomyces cerevisiae*,[430] and the excretion of the enzyme by *Kluyveromyces fragilis*,[427] combined with its ability to utilize inulin as the sole source of carbon,[51,431] are further evidence of the enzyme's identity as inulinase, as the cell-wall is presumably impermeable to inulin.[432] Another inulin-utilizing yeast, *Candida kefyr*, also produces an enzyme having both inulinase and β-D-fructofuranosidase activity.[433] β-D-Fructofuranosidase activity has also been studied with *Saccharomyces chevalieri (paradoxus)* and *Kluyveromyces (Saccharomyces) lactis*.[426,434,435] In these yeasts, too, "sucrase" and inulinase activities could not be separated. The enzyme was released[436] quite readily from *Saccharomyces chevalieri* by "osmotic shock."

In the light of these studies on both *Kluyveromyces fragilis* and *Saccharomyces cerevisiae*, Kidby and Davies[430] proposed the cell-wall structure shown in Fig. 9. Unlike Lampen's[413] suggestions, (*i*) the enzyme (inulinase or β-D-fructofuranosidase) is not chemically bonded to the wall, and (*ii*) the outer part of the wall has sulfide bridges as part of a barrier to large molecules. The following findings support these suggestions. (*a*) For *Kluyveromyces fragilis*, the electrophoretic mobility of the enzyme is the same regardless of whether it is released by the action of 2-mercaptoethanol on the cells or by sonic oscillation.[430] (*b*) 1,4-Dithiothreitol releases β-D-fructofuranosidase from *Saccharomyces cerevisiae*[437] and from *Saccharomyces uvarum (carlsbergensis)*.[438]

The physiology of the intracellular movement of yeast β-D-fructofuranosidase in its formation has been the subject of various studies. Clearly, the enzyme is synthesized somewhere inside the

(430) D. K. Kidby and R. Davies, *J. Gen. Microbiol.*, **61**, 327–333 (1970).

(431) M. Sacchetti, *Arch. Mikrobiol.*, **4**, 427–446 (1933).

(432) E. J. Conway and M. Downey, *Biochem. J.*, **47**, 347–355 (1950).

(433) H. Negoro, *Hakko Kogaku Zasshi*, **51**, 879–886 (1973).

(434) V. V. Yurkevich, N. S. Kovaleva, and K. H. Baker, *Fiziol. Rast.*, **19**, 937–945 (1972).

(435) V. V. Yurkevich and N. S. Kovaleva, *Biol. Nauki*, **15**, 98–103 (1972).

(436) J. Schwencke, G. Farías, and M. Rojas, *Eur. J. Biochem.*, **21**, 137–143 (1971).

(437) W. L. Smith and C. E. Ballou, *Biochem. Biophys. Res. Commun.*, **59**, 314–321 (1974).

(438) A. Sommer and M. J. Lewis, *J. Gen. Microbiol.*, **68**, 327–335 (1971).
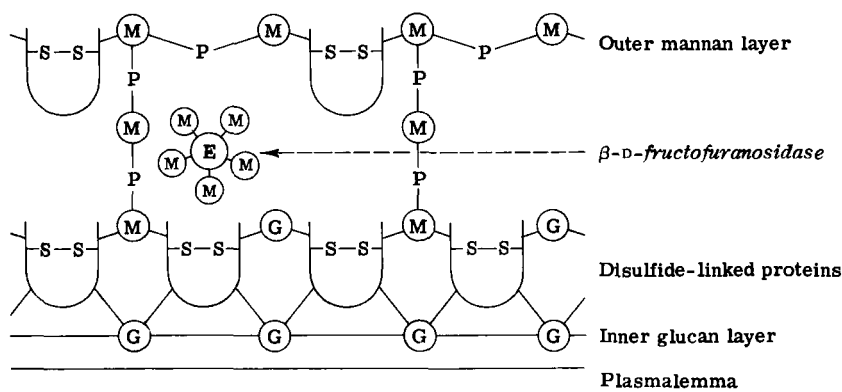
FIG. 9.—Diagram of Hypothetical Structure of Yeast Cell-wall (from Kidby and Davies[430]). (Phosphoric diester links are represented by —P—.)

plasmalemma, and moves outside it[410]; in common with other exoen-zymes, this external β-D-fructofuranosidase is associated with much carbohydrate.[439–441] This β-D-fructofuranosidase, outside the plasma-lemma, is a glycoprotein, containing 50 to 70% of D-mannose[441–443] that may be linked glycosidically to a serine part of the protein as short oligosaccharides[443] and to asparagine through 2-acetamido-2-deoxy-D-glucose residues as polysaccharide chains consisting of resi-dues of up to 70 monomers.[444,444a] The enzyme has[441] a molecular weight of 270,000. Removal of the mannan affects neither the activity nor the stability of the enzyme.[445] By using gel filtration on Sephadex G-200 columns, Gascón and Ottolenghi[446] detected a form of the en-zyme of low molecular weight occurring inside the plasmalemma, and predicted correctly that this form would be found to be free from carbohydrate. The molecular weight (135,000) and specific activity are similar to those of the protein moiety of the external enzyme,

(439) E. H. Fischer, L. Kohtès, and J. Fellig, Helv. Chim. Acta, 34, 1132–1138 (1951).
(440) K. Myrbäck and W. Schilling, Enzymologia, 29, 306–314 (1965).
(441) N. P. Neumann and J. O. Lampen, Biochemistry, 6, 468–475 (1967).
(442) B. Andersen and O. S. Jørgensen, Acta Chem. Scand., 23, 2270–2276 (1969).
(443) H. Greiling, P. Vögele, R. Kisters, and H. D. Ohlenbusch, Z. Physiol. Chem., 350, 517–518 (1969).
(444) N. P. Neumann and J. O. Lampen, Biochemistry, 8, 3552–3556 (1969).
(444a) T. Nakajima and C. E. Ballou, J. Biol. Chem., 249, 7685–7694 (1974).
(445) A. L. Tarentino, T. H. Plummer, Jr., and F. Maley, J. Biol. Chem., 249, 818–824 (1974).
(446) S. Gascón and P. Ottolenghi, C. R. Trav. Lab. Carlsberg, 36, 85–93 (1967).

although the two forms differ in their amino acid composition.[447,448] High concentrations of D-glucose repress the formation of β-D-fructofuranosidase,[409,410,446,447,449,450] and the small-molecular (internal) form of the enzyme is responsible for most, or all, of the β-D-fructofuranosidase activity of repressed cells. In *Schizosaccharomyces pombe*, adenosine 3',5'-bis(phosphate) appears to be concerned with the regulation of the synthesis of catabolite-sensitive enzymes, such as β-D-fructofuranosidase and α-D-glucopyranosidase.[450a]

Five, genetically distinct, β-D-fructofuranosidases have been described for strains of *Saccharomyces* hybrids. The behavior and kinetics of each enzyme are very similar.[362,451] W. L. Smith and Ballou have purified the mannan–protein β-D-fructofuranosidases of three strains of *Saccharomyces cerevisiae* whose cell walls have differences in mannan structure.[452] By use of immunochemical methods, they found that the structure of each β-D-fructofuranosidase mannan is similar to that of the cell wall of the corresponding strain only. Mutations affecting the structure of the one also produced similar changes in the other.

To explain the movement of β-D-fructofuranosidase from the cytoplasm into the periplasmic space,[453–455] various mechanisms involving vesicles of the endoplasmic reticulum or of vacuolar material have been proposed. In spheroplasts, most of the large form of the enzyme that is present is in the vacuoles and the small form is in the cytosol. A current view is that the β-D-fructofuranosidase is secreted through the plasmalemma, where glycosylation occurs.[456,456a] Subsequent work[456b] has demonstrated a continuous range of molecular forms, from the "small" carbohydrate-free enzyme to the "heavy" form containing 50% of carbohydrate. The presence of this range

(447) S. Gascón and J. O. Lampen, *J. Biol. Chem.*, **243**, 1567–1572 (1968).

(448) S. Gascón, N. P. Neumann, and J. O. Lampen, *J. Biol. Chem.*, **243**, 1573–1577 (1968).

(449) F. Dodyk and A. Rothstein, *Arch. Biochem. Biophys.*, **104**, 478–486 (1964).

(450) S. Gascón and P. Ottolenghi, *C. R. Lab. Carlsberg*, **39**, 15–24 (1972).

(450a) G. Schlanderer and H. Dellweg, *Eur. J. Biochem.*, **49**, 305–316 (1974).

(451) P. Ottolenghi, *Eur. J. Biochem.*, **18**, 544–552 (1971).

(452) W. L. Smith and C. E. Ballou, *Biochemistry*, **13**, 355–361 (1974).

(453) P. Beteta and S. Gascón, *FEBS Lett.*, **13**, 297–300 (1971).

(454) R. A. Holley and D. K. Kidby, *Can. J. Microbiol.*, **19**, 113–117 (1973).

(455) S. Gascón, P. S. Lazo, F. Moreno, and A. G. Ochoa, in Ref. 105, pp. 157–166.

(456) J. Meyer and P. Matile, *Proc. Intern. Symp. Yeasts, 4th*, Part 1, 211 (1974).

(456a) J. Meyer and P. Matile, *Arch. Microbiol.*, **103**, 51–55 (1975).

(456b) F. Moreno, A. G. Ochoa, S. Gascón, and J. R. Villanueva, *Eur. J. Biochem.*, **50**, 571–579 (1975).

may be the result of the sequential addition of D-mannose to the small form of enzyme during its secretion, culminating in the formation of the heavy enzyme outside the plasmalemma. 2-Deoxy-D-*arabino*-hexose inhibits the glycosylation and secretion of β-D-fructofuranosidase in *Saccharomyces cerevisiae*,[456b] so that the small form accumulates inside the plasmalemma. On the other hand, cycloheximide {3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]pentanedioimide} inhibits synthesis but not glycosylation of the enzyme protein; hence the large form is present and not the small.[456b]

Certain yeasts only utilize sucrose after a delay of three or four weeks;[456c] this late utilization appears to result from changes in the cells themselves and not from genetic mutation followed by selection.[456d] These yeasts are found notably among some *Saccharomyces* species, such as *Saccharomyces rouxii*,[64] that tolerate high concentrations of sugar.[456c,456d] An investigation of a strain of this species has suggested that, with aging, changes occur in the plasmalemma or in the membrane of some further compartment within the cell, so that intracellular β-D-fructofuranosidase is released.[456e] However, this work does not explain that, whereas a number of strains of *Saccharomyces rouxii* have been found to utilize sucrose after a delay, none of the 55 strains examined could use raffinose.[64]

2. α-D-Glucopyranosides

a. Action of α-D-Glucosidase and Oligo-(1 → 6)-D-glucosidase.— Sucrose is a double glycoside, being both β-D-fructofuranosyl α-D-glucopyranoside and α-D-glucopyranosyl β-D-fructofuranoside.
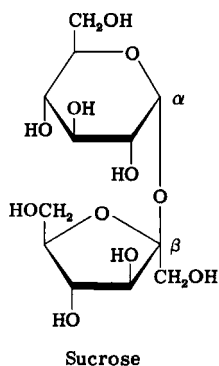
Sucrose

(456c) M. P. Scarr, *J. Gen. Microbiol.*, 5, 704–713 (1951).
(456d) D. Pappagianis and H. J. Phaff, A. v. L., *J. Microbiol. Serol.*, 22, 353–370 (1956).
(456e) W. N. Arnold, *J. Bacteriol.*, 120, 886–894 (1974).

Hence, sucrose may be hydrolyzed by either a β-D-fructofuran-osidase or an α-D-glucopyranosidase (EC 3.2.1.20),[457,458] and Weiden-hagen[459] showed that an α-D-glucosidase of *Saccharomyces cerevisiae* hydrolyzes sucrose at pH 6.9, although not at pH 4.7, which is optimal for β-D-fructofuranosidase. Another example is *Candida tropicalis* which, like many other yeasts,[24] utilizes sucrose but not raffinose.[41] An unfractionated extract of this yeast hydro-lyzed sucrose, maltose, and methyl α-D-glucopyranoside, but not raffinose.[460] The same yeast forms an *alpha*-amylase (EC 3.2.1.1) that is active against maltose and isomaltose, but not sucrose.[461,462] Khan and his colleagues[463] described the utilization of sucrose by means of an α-D-glucosidase in an interspecific *Saccharomyces* hybrid lacking β-D-fructofuranosidase.

α-D-Glucopyranosides that are utilized, as the sole source of carbon, by yeasts include maltose, melezitose, methyl α-D-glucopyranoside, sucrose, and α,α-trehalose. Maltotriose, which constitutes 15 to 20% of the fermentable carbohydrate of brewers' wort[464] is utilized by a number of *Saccharomyces* species.[465,466] In addition, some yeasts can utilize turanose[223,467,468] or palatinose.[467,468] The structures of these compounds are shown in Table X.

In yeasts, α-D-glucosidase activity occurs within the plasma-lemma,[223,409] although further work may possibly reveal some ex-ternal α-D-glucosidases, as for the mold *Mucor rouxii*.[469] Indeed, for *Saccharomyces uvarum (logos)*, an enzyme having α-D-glucosidase activity is reported as being, like β-D-fructofuranosidase, a glycopro-tein having[470] a molecular weight of 270,000. Previous estimates of

(457) R. Weidenhagen, *Ergeb. Enzymforsch.*, **1**, 168–208 (1932).

(458) R. Weidenhagen, *Ergeb. Enzymforsch.*, **2**, 90–103 (1933).

(459) R. Weidenhagen, *Naturwissenschaften*, **16**, 654–655 (1928).

(460) T. Sawai, *Botan. Mag.* (Tokyo), **69**, 177–185 (1956).

(461) T. Sawai, *J. Biochem.* (Tokyo), **45**, 49–56 (1958).

(462) T. Sawai, *J. Biochem.* (Tokyo), **48**, 382–391 (1960).

(463) N. A. Khan, F. K. Zimmermann, and N. R. Eaton, *Mol. Gen. Genet.*, **123**, 43–50 (1973).

(464) I. C. MacWilliam, *J. Inst. Brewing*, **74**, 38–54 (1968).

(465) R. B. Gilliland, *C. R. Trav. Lab. Carlsberg*, **26**, 139–148 (1956).

(466) D. J. Millin and D. G. Springham, *J. Inst. Brewing*, **72**, 388–393 (1966).

(467) D. S. Hwang and C. C. Lindegren, *Nature* (London), **203**, 791–792 (1964).

(468) D. S. Hwang and C. C. Lindegren, *Nature* (London), **205**, 880–883 (1965).

(469) E. Reyes and J. Ruiz-Herrera, *Biochim. Biophys. Acta*, **273**, 328–335 (1972).

(470) S. Chiba, T. Saeki, and T. Shimomura, *Agr. Biol. Chem.* (Tokyo), **37**, 1823–1829 (1973).

the molecular weights of yeast α-D-glucosidases had lain[471-473] between 60,000 and 100,000.

One *Saccharomyces* interspecific hybrid (*i*) utilizes maltose or methyl α-D-glucopyranoside, but not sucrose; (*ii*) accumulates sucrose intracellularly; and (*iii*) contains sucrose-hydrolyzing enzyme, supposedly identical to that hydrolyzing maltose and methyl α-D-glucopyranoside.[474] Avigad's[474] explanation was that the enzyme is in an intracellular compartment, impermeable to sucrose in the cytosol.

The utilization of α-D-glucopyranosides by yeasts is commercially important. For example, brewing yeasts are reported to utilize the sugars of brewers' wort, sucrose, monosaccharides, maltose, and maltotriose, in that order.[475] Yet, knowledge of the α-D-glycosyl hydrolases of yeasts is not considerable, probably because there have been difficulties in (*i*) purifying the enzymes,[476] and (*ii*) obtaining yeasts that have been subjected to extensive, genetical analysis. Even now, this is only possible for two species, namely, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.[364,365] (*iii*) Furthermore, there have been failures to ensure that the yeast grew in a medium fully defined chemically and also, apparently, to appreciate that the precise stage of growth may seriously affect the enzymic content of the yeast.

Studies on the specificities of purified α-D-glycosyl hydrolases of about eight kinds of yeast are summarized in Table XI. The enzymes of *Saccharomyces cerevisiae*, namely, α-D-glucosidase and oligo-$(1 \rightarrow 6)$-D-glucosidase (EC 3.2.1.10), have been examined the most thoroughly.

The α-D-glucosidase of *Saccharomyces cerevisiae* is similar to that of *Saccharomyces italicus*. The enzyme is highly specific for the sugar moiety, having no activity when it is modified by (*i*) inversion of the configuration at C-2 (α-D-mannopyranosyl) or C-4 (α-D-galactopyranosyl), (*ii*) substitution on the 6-hydroxyl group (raffinose), or (*iii*) other substitutions or replacements on C-2 to C-6. Yeast α-D-glucosidase forms complexes with trehalose or isomaltose, but does not hydrolyze them. The oligo-$(1 \rightarrow 6)$-D-glucosidase of *Saccharomyces cerevisiae* hydrolyzes methyl α-D-glucopyranoside,

(471) H. Halvorson and L. Ellias, *Biochim. Biophys. Acta*, **30**, 28–40 (1958).
(472) N. A. Khan and N. R. Eaton, *Biochim. Biophys. Acta*, **146**, 173–180 (1967).
(473) J. O. Lampen, S. C. Kuo, and F. R. Cano, in Ref. 105, pp. 143–156.
(474) G. Avigad, *Biochim. Biophys. Acta*, **40**, 124–134 (1960).
(475) A. W. Phillips, *J. Inst. Brewing*, **61**, 122–126 (1955).
(476) E. H. Fischer and E. A. Stein, *Enzymes*, 2nd Edition, **4**, 301–312 (1960).

α-D-Glucopyranoside	Structure
Maltose 4-*O*-α-D-Glucopyranosyl-D- glucopyranose	
Sucrose β-D-Fructofuranosyl α-D-glucopyranoside	
Methyl α-D-glucopyranoside	
Isomaltose 6-*O*-α-D-Glucopyranosyl- D-glucopyranose	
Turanose 3-*O*-α-D-Glucopyranosyl- D-fructopyranose	

TABLE X (*continued*)
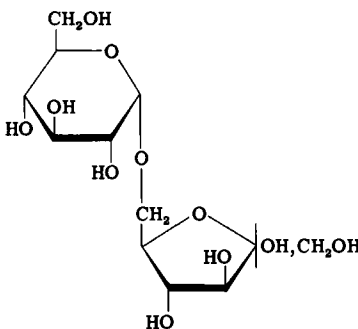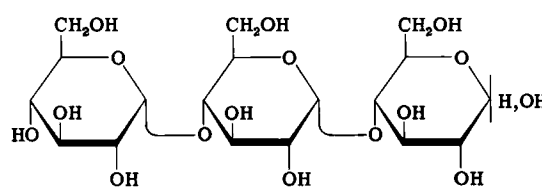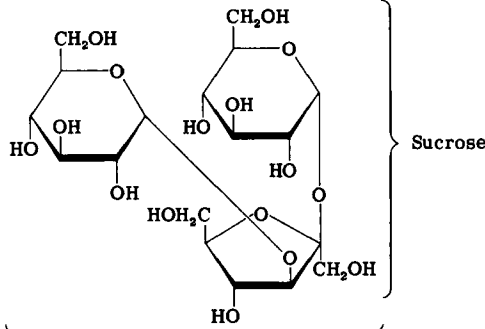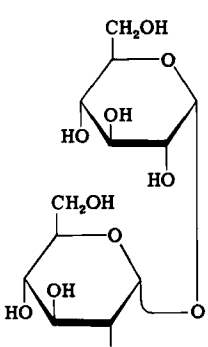
α-D-Glucopyranoside	Structure
Palatinose 6-*O*-α-D-Glucopyranosyl- D-fructofuranose	
Maltotriose *O*-α-D-Glucopyranosyl-(1→4)- *O*-α-D-glucopyranosyl-(1→4)- D-glucopyranose	
Melezitose *O*-α-D-Glucopyranosyl-(1→3)- *O*-β-D-fructofuranosyl α-D-glucopyranoside	
α, α-Trehalose α-D-Glucopyranosyl α-D-glucopyranoside	

TABLE XI

Substrate Specificity of Yeast Enzymes Having α-D-Glucosidase Activity

Enzyme source	Name of enzyme	Substrate[a]									References
		Sucrose	Maltose	Melezitose	MeαGlc[b]	Maltotriose	PNPG[c]	Turanose	PhαGlc[d]	Isomaltose	
Saccharomyces cerevisiae	α-D-glucosidase	+	+	?	−/?	+	+	+	+	−	347,459,472,477–479
	oligo-(1 → 6)-D-glucosidase	+	−		+		+		−	+	347,472,480–483
uvarum			+		−						477
			+		+	+		+	+	+	470,484
italicus	α-D-glucosidase	+	+	+	?		+	+	+		471,485
bayanus	α-D-glucosidase		+	?	+						486
	oligo-(1 → 6)-D-glucosidase		−	+	+			+			486
Saccharomyces interspecific hybrids		+	+				+	+			349
		+	+	+	−		+	?			487
		+	+	−	−	+	+				487
		?	−	−	+		?	−			487,488
Schizosaccharomyces octosporus		−	+								489
pombe		−	+		?	+		+	+		490
Candida tropicalis	*alpha*-amylase	−	+							+	461,462

[a] Key: + = hydrolyzed; − = not hydrolyzed; ? = slow hydrolysis. [b] Methyl α-D-glucopyranoside. [c] p-Nitrophenyl α-D-glucopyranoside.
[d] Phenyl α-D-glucopyranoside.

isomaltose, and panose, but not maltose. Thus, although the α-D-glucosidases are specific for the hydrolysis of maltose, and the oligo-(1 → 6)-D-glucosidases for methyl α-D-glucopyranoside and isomaltose, both kinds of enzyme share common substrates, namely, sucrose and p-nitrophenyl α-D-glucopyranoside. However, there is evidence of the existence in *Schizosaccharomyces octosporus*,[489] *Schizosaccharomyces pombe*,[490] *Candida tropicalis*,[461,462] *Candida solani*,[491] and *Candida (Procandida) stellatoidea*[492] of enzymes that hydrolyze maltose but not sucrose.

Induction of the two α-D-glucosyl hydrolases of *Saccharomyces cerevisiae* was studied by means of antisera specific for each enzyme. The antisera quantitatively precipitated the appropriate enzyme, leaving the other fully active.[232] Maltose induces both the α-D-glucosidase and the oligo-(1 → 6)-D-glucosidase[493]; methyl α-D-glucopyranoside or ethyl 1-thio-α-D-glucopyranoside induced the latter but not the former enzyme. In *Saccharomyces bayanus (oviformis)*, maltose induced α-D-glucosidase, but methyl α-D-glucopyranoside induced both α-D-glucosidase and oligo-(1 → 6)-D-glucosidase; ethyl 1-thio-α-D-glucopyranoside was found to be a poor inducer for this yeast.[486] Some reports make difficult the confident in-

(477) A. H. Cook and A. W. Phillips, *Arch. Biochem. Biophys.*, **69**, 1–9 (1957).

(478) A. W. Phillips, *Arch. Biochem. Biophys.*, **80**, 346–352 (1959).

(479) S. Chiba, S. Sugawara, T. Shimomura, and Y. Nakamura, *Agr. Biol. Chem. (Tokyo)*, **26**, 787–793 (1962).

(480) G. Terui, H. Okada, and Y. Oshima, *Technol. Rept. Osaka Univ.*, **9**, 237–259 (1959).

(481) G. Terui, H. Okada, and Y. Oshima, *Hakko Kogaku Zasshi*, **37**, 177–189 (1959); *Chem. Abstr.* **54**, 15,520d (1960).

(482) J. Gorman and H. Halvorson, *Methods Enzymol.*, **8**, 562–565 (1966).

(483) N. A. Khan and R. H. Haynes, *Mol. Gen. Genet.*, **118**, 279–285 (1972).

(484) S. Chiba, T. Saeki, and T. Shimomura, *Agr. Biol. Chem. (Tokyo)*, **37**, 1831–1836 (1973).

(485) H. Halvorson, *Methods Enzymol.*, **8**, 559–562 (1966).

(486) B. Axelrod, H. Ashe, T. Fukui, and D. E. Lewis, *Rec. Chem. Progr.*, **28**, 121–133 (1967).

(487) T. M. Yau and C. C. Lindegren, *Biochem. Biophys. Res. Commun.*, **27**, 305–308 (1967).

(488) S. Hestrin and C. C. Lindegren, *Nature (London)*, **165**, 158 (1950).

(489) E. Hofmann, *Biochem. Z.*, **272**, 417–425 (1934).

(490) S. Chiba and T. Shimomura, *Agr. Biol. Chem. (Tokyo)*, **29**, 540–547 (1965).

(491) E. K. Novák, *Acta Microbiol. Acad. Sci. Hung.*, **10**, 7–10 (1963).

(492) E. K. Novák, F. Kevei, B. Oláh, and J. Zsolt, *Acta Biol. Acad. Sci. Hung.*, **16**, 137–140 (1965).

(493) J. Ouwehand and R. van Wijk, *Mol. Gen. Genet.*, **117**, 30–38 (1972).

terpretation of the results in terms of induction and repression; for example, often, when maltose-grown yeast is compared solely with that grown on D-glucose. As D-glucose often suppresses enzyme synthesis,[6,494] the amount of enzyme in uninduced, non-repressed yeast would in such cases be unknown.

Studies on the genetics of the α-D-glucosidase activity of yeasts, particularly of *Saccharomyces cervisiae*, reviewed in 1969 by Mortimer and Hawthorne,[364] have since been extended by Khan and coworkers,[233,351,463,483,495–495c] by ten Berge and (or) coworkers for *Saccharomyces uvarum* (*carlsbergensis*),[353,355,493,496] and by Naumov[497,498] for other *Saccharomyces* species. The genetical control of these D-glucosidases is remarkably complex; that for α-D-glucosidase may be summarized as follows. (*i*) Each of at least five unlinked loci controls the ability to form inducible α-D-glucosidase and behaves as a single gene. These polymeric genes are designated *MAL1*, *MAL2*, *MAL3*, *MAL4* and *MAL6* (see Table IX; for a review, see Ref. 364). (*ii*) As α-D-glucosidase is present in very small proportions in strains that lack any known *MAL* genes, the genes are probably not structural genes. These strains do not utilize maltose.[353,495] (*iii*) In response to the presence of maltose in the culture medium, a single *MAL* gene is usually sufficient to increase the α-D-glucosidase above this low basal level, so that there is enough α-D-glucosidase for the utilization of maltose[351]; this assumes that α-D-glucosidase synthesis is not being repressed, for example, by D-glucose. (*iv*) The proportion of α-D-glucosidase in maltose-grown cells has been stated to be directly proportional to the number of *MAL* genes present.[499] (*v*) Strains having one or more of the *MAL* genes usually form inducible and repressible α-D-glucosidase. (*vi*) Gene *MAL4* is exceptional; in its presence, α-D-glucosidase is constitutive, and resistant to catabolite repression.[233] (Mutants have also been obtained for a *Saccharomyces* strain having β-D-fructofuranosidase formation that is

(494) B. Magasanik, *Cold Spring Harbor Symp. Quant. Biol.*, **26**, 249–256 (1961).

(495) N. A. Khan, F. K. Zimmermann, and N. R. Eaton, *Mol. Gen. Genet.*, **124**, 365–367 (1973).

(495a) N. A. Khan, *Mol. Gen. Genet.*, **136**, 55–61 (1975).

(495b) R. Needleman and N. R. Eaton, *Mol. Gen. Genet.*, **133**, 135–140 (1974).

(495c) N. A. Khan, *Mol. Gen. Genet.*, **133**, 363–365 (1974).

(496) A. M. A. ten Berge, *Mol. Gen. Genet.*, **115**, 80–88 (1972).

(497) G. I. Naumov, *Genetika*, **6** (7), 120–125 (1970).

(498) G. I. Naumov, *Genetika*, **6** (10), 121–124 (1970).

(499) F. Rudert and H. O. Halvorson, *Bull. Res. Counc. Israel, Sect. A*, **11**, 337–344 (1967).

resistant to repression by hexoses.[500]) (*vii*) With a strain carrying an allele of gene *MAL4*, forming constitutive α-D-glucosidase even in the presence of D-glucose, Khan and coworkers[233] isolated 19 mutants that affected the utilization of maltose, but not the synthesis of α-D-glucosidase. Thus, in addition to the *MAL* genes for α-D-glucosidase, at least seven genes (*DSF*) are concerned with maltose utilization, and, perhaps, with its entry into the cells. (*viii*) Even if there are none of the polymeric *SUC* genes for β-D-fructofuranosidase, *MAL*-mutants for constitutive α-D-glucosidase, or genes *MAL4* or *MAL2*, allow the yeast to utilize sucrose by means of an α-D-glucosidase.[463] Yeasts lacking *SUC* but having *MAL* genes do not ordinarily utilize sucrose unless α-D-glucosidase synthesis is constitutive, because it is not inducible by sucrose. Furthermore, another gene, *SSF* (sucrose specific fermentation) is necessary for sucrose utilization to occur without *SUC* genes, even if α-D-glucosidase synthesis is constitutive.[463]

In *Saccharomyces cerevisiae*, utilization of methyl α-D-glucopyranoside and isomaltose is controlled by at least three complementing, linked genes,[357] each gene being designated *MGL*. The gene *MGL2* is reported to be responsible for the entry of methyl α-D-glucopyranoside into the cells.[227] The oligo-(1 → 6)-D-glucosidases produced by yeasts having either of two complementing gene-pairs, *MGL1* and *MGL2*, or *MGL3* and *MGL2*, have the same molecular weight, and substrate and serological specificities, but differ in specific activity, affinity for substrate, and heat stability.[483] Enzyme induction by methyl α-D-glucopyranoside requires the presence of *MGL1* or *MGL3*.

b. Action of α,α-Trehalase (EC 3.2.1.28).—Over 60% of the known yeast species can utilize exogenous α,α-trehalose as the sole source of carbon (see Table III), and even yeasts that do not utilize trehalose may contain α,α-trehalase.[501] α,α-Trehalase acts within the plasmalemma,[502] and is specific for α,α-trehalose,[501,503] each molecule of which is hydrolyzed to two of D-glucose. The enzyme has been isolated from various yeasts,[504–508] including *Candida tropicalis*[509]

(500) B. S. Montenecourt, S.-C. Kuo, and J. O. Lampen, *J. Bacteriol.*, 114, 233–238 (1973).
(501) G. Avigad, O. Ziv, and E. Neufeld, *Biochem. J.*, 97, 715–722 (1965).
(502) N. O. Souza and A. D. Panek, *Arch. Biochem. Biophys.*, 125, 22–28 (1968).
(503) K. Myrbäck, *Ergeb. Enzymforsch.*, 10, 168–190 (1949).
(504) E. Fischer, *Ber.*, 28, 1429–1438 (1895).
(505) A. Kalanthar, Z. *Physiol. Chem.*, 26, 88–101 (1898).
(506) A. Bau, *Wochschr. Brau.*, 16, 305–306 (1899).

and *Saccharomyces uvarum (carlsbergensis)*,[510] and has been purified from *Saccharomyces cerevisiae*[511] and from a hybrid yeast.[501] α,α-Trehalase activity is also found in *Nematospora coryli* and *Saccharomyces kloeckerianus (Debaryomyces globosus)*.[509]

α,α-Trehalose can act as a storage carbohydrate in yeasts (for reviews, see Refs. 512 and 513), and may constitute up to 15% of the dry weight of bakers' yeast.[514-516] Thus, yeast extract may contain various proportions of α,α-trehalose and so, for this reason at least, it is unsuitable for adding to culture media that are intended to be chemically defined, particularly with respect to the source of carbon.[517] Storage trehalose may be formed from D-glucose by way of D-glucose 6-phosphate,[518] and also from other sugars, such as D-galactose[363] and D-xylose.[518a,518b] The action of α,α-trehalose phosphate synthase (UDP-forming) (EC 2.4.1.15)[519] is followed by that of a phosphatase.

UDP-D-Glc + D-glucose 6-phosphate → UDP + α,α-trehalose
                                      phosphate → α,α-trehalose + P
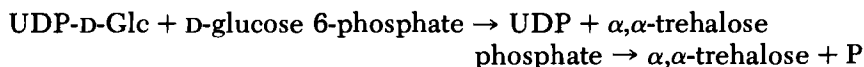
Regulation of the synthesis and breakdown of storage trehalose is probably quite complex.[144,474,502,520-529]

(507) P. Lindner, *Wochschr. Brau.*, **28**, 61–64 (1911).

(508) K. Myrbäck and B. Örtenblad, *Biochem. Z.*, **291**, 61–69 (1937).

(509) T. M. Lukes and H. J. Phaff, *A. v. L., J. Microbiol. Serol.*, **18**, 323–335 (1952).

(510) J. Krüger and B. Hess, *Arch. Mikrobiol.*, **61**, 154–158 (1968).

(511) A. Panek and N. O. Souza, *J. Biol. Chem.*, **239**, 1671–1673 (1964).

(512) W. E. Trevelyan, in Ref. 25, pp. 369–436.

(513) D. J. Manners, in Ref. 21, 1971, Vol. 2, pp. 419–439.

(514) G. Tanret, *Compt. Rend.*, **192**, 1056–1058 (1931).

(515) K. Myrbäck, *Sv. Kem. Tidskr.*, **48**, 55–61 (1936).

(516) K. Myrbäck and B. Örtenblad, *Biochem. Z.*, **288**, 329–337 (1936).

(517) R. J. Bouthilet, N. E. Neilson, E. M. Mrak, and H. J. Phaff, *J. Gen. Microbiol.*, **3**, 282–289 (1949).

(518) L. F. Leloir and E. Cabib, *J. Amer. Chem. Soc.*, **75**, 5445–5446 (1953).

(518a) S. Janda, I. Beneš, V. Procházková, and R. Tauchová, *Collect. Czech. Chem. Commun.*, **37**, 1762–1763 (1972).

(518b) S. Janda and A. Kotyk, *Folia Microbiol.* (Prague), **17**, 461–470 (1972).

(519) E. Cabib and L. F. Leloir, *J. Biol. Chem.*, **231**, 259–275 (1958).

(520) K. M. Brandt, *Biochem. Z.*, **309**, 190–201 (1941).

(521) H. Suomalainen and S. Pfäffli, *J. Inst. Brewing*, **67**, 249–254 (1961).

(522) A. D. Panek, *Arch. Biochem. Biophys.*, **98**, 349–355 (1962).

(523) A. D. Panek, *Arch. Biochem. Biophys.*, **100**, 422–425 (1963).

(524) A. D. Panek, *J. Bacteriol.*, **99**, 904–905 (1969).

(525) H. Blennemann, S. Janocha, H. Keller, and H. Netter, *Z. Physiol. Chem.*, **231**, 164–179 (1963).

3. β-D-Glucopyranosides

β-D-Glucopyranosides are hydrolyzed by many yeasts,[530,531] about half the species known being able to utilize cellobiose, for example, as the sole carbon source for aerobic growth (see Table III).

Neither *Saccharomyces cerevisiae* nor *Saccharomyces uvarum*, the two species which, together, include bakers' and brewers' yeasts, utilizes cellobiose, arbutin, or salicin.[64] However, it has long been supposed that such yeasts contain β-D-glucosidase (EC 3.2.1.21). In 1894, Emil Fischer[415] reported that beer yeast can decompose amygdalin into mandelonitrile and D-glucose. Then, in 1905, Henry and Auld,[532] in attempting to use "ordinary pressed yeast" to remove D-glucose from cyanogenetic D-glucosides, repeated Fischer's[415] studies and reported further that the yeast, or extracts from it, hydrolyze salicin and arbutin, too. Since then, various authors[533-536] have found hydrolytic activity by such yeasts, or by preparations from them, against the same substrates and, also, cellobiose. In addition, yeasts of the following species can split phlorizin (phloretin-2-yl β-D-glucopyranoside): *Candida albicans, Candida guilliermondii, Candida pseudotropicalis (Torula cremoris), Cryptococcus albidus, Kluyveromyces lactis (Torulopsis sphaerica)*, and *Rhodotorula rubra (mucilaginosa)*.[537]

Some detailed studies have been made on β-D-glucosidases of the following: (*i*) *Rhodotorula minuta* (misnamed "*Saccharomyces cerevisiae*"),[538,539] (*ii*) *Kluyveromyces (Saccharomyces) lactis*,[540-546] (*iii*)

(526) T. Savioja and J. K. Miettinen, *Acta Chem. Scand.*, 20, 2451–2455 (1966).
(527) M. T. Küenzi and A. Fiechter, *Arch. Mikrobiol.*, 64, 396–407 (1969).
(528) M. H. Miguez da Rocha Leão and A. D. Panek, *An. Acad. Brasil. Cienc.*, 44, 571–576 (1972).
(529) J. B. van der Plaat and P. van Solingen, *Biochem. Biophys. Res. Commun.*, 56, 580–587 (1974).
(530) J. A. Barnett and T. Swain, *Nature* (London), 177, 133–134 (1956).
(531) J. A. Barnett, M. Ingram, and T. Swain, *J. Gen. Microbiol.*, 15, 529–555 (1956).
(532) T. A. Henry and S. J. M. Auld, *Proc. Roy. Soc., Ser. B,* 76, 568–580 (1905).
(533) A. Bau, *Wochschr. Brau.*, 34, 29–31 (1917).
(534) C. Neuberg and E. Färber, *Biochem. Z.,* 78, 264–272 (1917).
(535) R. Weidenhagen, *Z. Zuckerind.*, 79, 591–602 (1929).
(536) R. Weidenhagen, *Z. Zuckerind.*, 80, 11–24 (1930).
(537) N. P. Jayasankar, R. J. Bandoni, and G. H. N. Towers, *Phytochemistry*, 8, 379–383 (1969).
(538) J. D. Duerksen and H. O. Halvorson, *J. Biol. Chem.*, 233, 1113–1120 (1958).
(539) J. D. Duerksen and H. O. Halvorson, *Biochim. Biophys. Acta*, 36, 47–55 (1959).
(540) A. Herman and H. O. Halvorson, *J. Bacteriol.*, 85, 895–900 (1963).

TABLE XII

β-D-Glucosidases: Estimates of Molecular Weight and Molecules per Yeast Cell

Source of enzyme (yeast)	State	Number of molecules of enzyme/cell	Molecular weight	References
Rhodotorula minuta	non-induced	2,700⎤	300,000	538
	induced	164,000⎦		
Kluyveromyces hybrid	constitutive	54,000	315,000	548,552
Saccharomyces italicus (a non-utilizer of β-D-glucosides)	non-inducible	400		560
Kaplan's unidentified yeast	induced	41,000	313,000	558

Kluyveromyces fragilis,[547] (*iv*) *Kluyveromyces dobzhanskii*,[547] (*v*) a hybrid of *Kluyveromyces fragilis* and *Kluyveromyces dobzhanskii*,[548–552] and (*vi*) a yeast of unknown identity, called[553–558] "*Saccharomyces cerevisiae*." This identity is questioned, because this yeast,[555] but not *Saccharomyces cerevisiae*,[64] can grow on cellobiose as the sole source of carbon.[559.]

The β-D-glucosidases studied were intracellular (cytoplasmic) enzymes of high molecular weight, about 300,000 (see Table XII), but

(541) A. Herman and H. O. Halvorson, *J. Bacteriol.*, **85**, 901–910 (1963).
(542) G. L. Marchin and J. D. Duerksen, *J. Bacteriol.*, **96**, 1181–1186 (1968).
(543) G. L. Marchin and J. D. Duerksen, *J. Bacteriol.*, **96**, 1187–1190 (1968).
(544) G. L. Marchin and J. D. Duerksen, *J. Bacteriol.*, **97**, 237–243 (1969).
(545) M. Tingle and H. O. Halvorson, *Genet. Res.*, **19**, 27–32 (1972).
(546) M. Tingle and H. O. Halvorson, *J. Bacteriol.*, **110**, 196–201 (1972).
(547) L. W. Fleming and J. D. Duerksen, *J. Bacteriol.*, **93**, 135–141 (1967).
(548) A. S. L. Hu, R. Epstein, H. O. Halvorson, and R. M. Bock, *Arch. Biochem. Biophys.*, **91**, 210–218 (1960).
(549) A. M. MacQuillan and H. O. Halvorson, *J. Bacteriol.*, **84**, 23–30 (1962).
(550) A. M. MacQuillan and H. O. Halvorson, *J. Bacteriol.*, **84**, 31–36 (1962).
(551) J. D. Duerksen and L. W. Fleming, *Biochem. Biophys. Res. Commun.*, **12**, 169–174 (1963).
(552) L. W. Fleming and J. D. Duerksen, *J. Bacteriol.*, **93**, 142–150 (1967).
(553) J. G. Kaplan, *J. Cell. Biol.*, **19**, 38A (1963).
(554) J. G. Kaplan, *Fed. Proc.*, **23**, 210 (1964).
(555) J. G. Kaplan, *J. Gen. Physiol.*, **48**, 873–886 (1965).
(556) J. G. Kaplan and W. Tacreiter, *J. Gen. Physiol.*, **50**, 9–24 (1966).
(557) A. N. Inamdar and J. G. Kaplan, *Fed. Proc.*, **25**, 508 (1966).
(558) A. N. Inamdar and J. G. Kaplan, *Can. J. Biochem.*, **44**, 1099–1108 (1966).
(559) The yeast was neither identified nor deposited with a culture collection (J. G. Kaplan, personal communication, 1974).
(560) H. O. Halvorson, *Wallerstein Lab. Commun.*, **23**, 5–12 (1960).

nonetheless they were free from detectable carbohydrate.[542] On the other hand, the β-D-glucosidase activity of the *Kluyveromyces* hybrid could have been due[552] "to a population of different hybrid enzyme molecules." Table XIII gives information on the specificities of the β-D-glucosidases of three yeasts. As is well known for β-D-glucosidases in general,[561] these yeast enzymes have wide substrate-specificities for the aglycon.

It has not yet been shown unequivocally that yeasts contain different β-D-glucosidases that differ markedly in their specificity for hydrolyzing the common β-D-glucopyranosides, cellobiose, aesculin, arbutin, and salicin, as has been proposed,[531] although this could be true.[540,541,556,558] The enzymes have been distinguished from each other serologically and by their affinities for 1-thio-β-D-glycopyranosides that do not serve as substrates.[547] The β-D-glucosidase of *Rhodotorula minuta* was found to be inducible, particularly strongly by methyl β-D-glucopyranoside or methyl or ethyl 1-thio-β-D-glucopyranoside.[538,539] On the other hand, the β-D-glucosidases in *Kluyveromyces fragilis* and *Kluyveromyces dobzhanskii* were found to be constitutive.[547,548] Synthesis of β-D-glucosidase appears to be repressed at high, but induced at low, concentrations of D-glucose. After adding 10 mM or 100 mM D-glucose to strains of *Kluyveromyces lactis*, β-D-glucosidase synthesis fell to about 40% of the level of the control; with the *Kluyveromyces* hybrid, synthesis was repressed to <1% of the control.[549] However, D-glucose in low concentrations acted as an inducer, maximum induction being achieved at 1 mM for one strain of *Kluyveromyces lactis*, and 1 μM for the other.[549]

Kaplan and Tacreiter[556] found that their unidentified yeast, cellobiose-grown, incorporated carbon-14 of [14C]-labelled methyl β-D-glucopyranoside when the sugar moiety but not the methyl group was labelled, and they drew the following conclusions. (*i*) Methyl β-D-glucopyranoside does not enter the cells, but is split at the plasma-lemma. (*ii*) The yeast had two β-D-glucosidase systems, firstly, the internal enzyme (see Table XIII) which was active largely against aryl β-D-glucopyranosides and, secondly, a "surface β-D-glucosidase" which differed from the internal enzyme by actively hydrolyzing methyl β-D-glucopyranoside and cellobiose.[556] However, these suggestions would have been strengthened had the following been established: that (*a*) the [14C]methanol, released from the D-glucoside, is not washed from inside the cells; (*b*) comparable experiments with [14C]cellobiose show that this sugar is hydrolyzed out-

(561) J. B. S. Haldane, "Enzymes," Longmans, Green and Co., London, 1930.

TABLE XIII

Specificities of β-D-Glucosidases of Three Yeasts[a]

Substrate	Amount of induction $\left(\dfrac{\Delta E/\Delta \text{ mass}}{\text{value for control}}\right)$		Maximum rate of hydrolysis (μmoles/mg of protein/min)			Enzymic affinity (K_i)		
	Rh	Kl	Rh	Kl	U	Rh	Kl	U
Cellobiose (4-O-β-D-glucopyranosyl-D-glucopyranose)	1.9	1.4	1.2		6	0.15 M		
Gentiobiose (6-O-β-D-glucopyranosyl-D-glucopyranose)					1			
Salicin [2-(hydroxymethyl)phenyl β-D-glucopyranoside]	2.7	5.5	8.2	8.3	11	2.6 mM	320 μM	1.0 mM
Arbutin (hydroquinone β-D-glucopyranoside)	1.1	3.3	18.7	13.2	15	1.7 mM	260 μM	220 μM
Aesculin (7-hydroxycoumarin-6-yl β-D-glucopyranoside)	1.0	6.4	6.4	6.7	3	14 μM	59 μM	
Amygdalin [O-β-D-glucopyranosyl-(1 $\rightarrow$ 6)-β-D-glucopyranosyl-O-CH(CN)Ph]	1.0	6.3	0	0	7	14 mM	1.0 mM	7.7 mM
Methyl β-D-glucopyranoside	71		3.3		4	5.6 mM		
Allyl β-D-glucopyranoside	4.3		14.5			680 μM		
Phenyl β-D-glucopyranoside	2.3		16.4		17	1.6 mM		270 μM
p-Nitrophenyl β-D-glucopyranoside			12.6		40	80 $\mu M[K_m]$	80 $\mu M[K_m]$	95 $\mu M[K_m]$
3-Phenylpropyl β-D-glucopyranoside	2.9		19.6			90 μM		
Phenyl 1-thio-β-D-glucopyranoside	1.1		0			4.5 mM	3.3 mM	
Methyl 1-thio-β-D-glucopyranoside	59		0			880 μM		
Ethyl 1-thio-β-D-glucopyranoside	57		0			220 μM		
D-Glucose	0		—			8.5 mM	8.8 mM	6.7 mM
Control (no addition)	1.0	1.0						

[a] Rh = *Rhodotorula minuta* (Refs. 538, 539, and 548); Kl = *Kluyveromyces* interspecific hybrid (Ref. 548); U = unidentified yeast (Refs. 557 and 558).

side the plasmalemma, for example, by trapping[223]; (c) particulate fractions of the cells should have appropriate β-D-glucosidase activity. However, attempts to isolate such active fractions failed.[557,558]

4. α-D-Galactopyranosides

Melibiose is utilized unequivocally by only 51 of 434 yeast species (see Table III). Unlike top (ale) yeasts (*Saccharomyces cerevisiae*), bottom (lager) yeasts (*Saccharomyces uvarum*) have long been known to be able to use melibiose (6-*O*-α-D-galactopyranosyl-D-glucose)[219,562] and to be a good source of α-D-galactosidase (EC 3.2.1.22). As a β-D-fructofuranosidase, this enzyme acts outside the plasmalemma,[223,563,564] hydrolyzing melibiose to D-galactose and D-

D-Galactose   +   D-Glucose

Melibiose
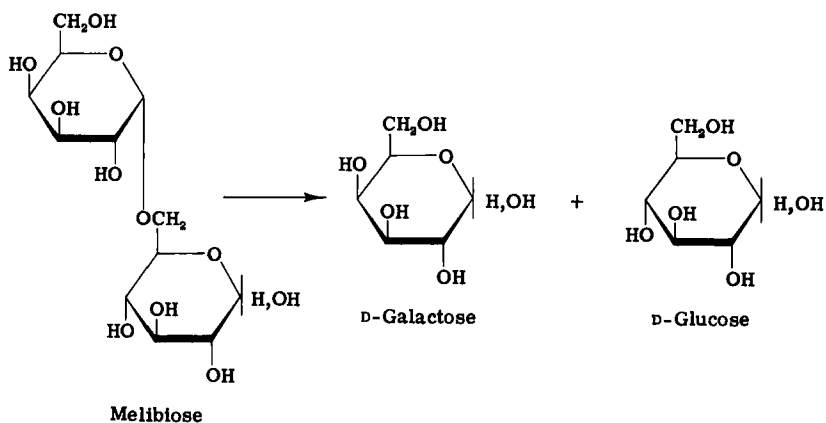
glucose, or raffinose to D-galactose and sucrose.[565] An estimate of the molecular weight of the enzyme[455] is >250,000. Preparations of it from brewers' bottom yeast can polymerize D-galactose, forming 6-*O*-α-D-galactopyranosyl-D-galactose and (1 → 3)-α-D-, (1 → 4)-α-D-, and (1 → 5)-α-D-galactobioses.[566,567] The α-D-galactosidases have been reviewed by Dey and Pridham.[568]

(562) A. Bau, *Chem.-Ztg., Chem. App.*, 19, 1873–1874 (1895).
(563) G. de la Fuente and A. Sols, *Abstr. Intern. Congr. Biochem, 4th, Vienna*, 131 (1958).
(564) J. Friis and P. Ottolenghi, *C. R. Trav. Lab. Carlsberg*, 31, 272–281 (1959).
(565) V. I. Isajev, *Chem. Listy*, 21, 101–107, 141–147, 191–202 (1927); *Chem. Abstr.*, 22, 97 (1928).
(566) M. J. Clancy and W. J. Whelan, *Biochem. J.*, 76, 22P–23P (1960).
(567) M. J. Clancy and W. J. Whelan, *Arch. Biochem. Biophys.*, 118, 724–729 (1967).
(568) P. M. Dey and J. B. Pridham, *Advan. Enzymol. Relat. Areas Mol. Biol.*, 36, 91–130 (1972).
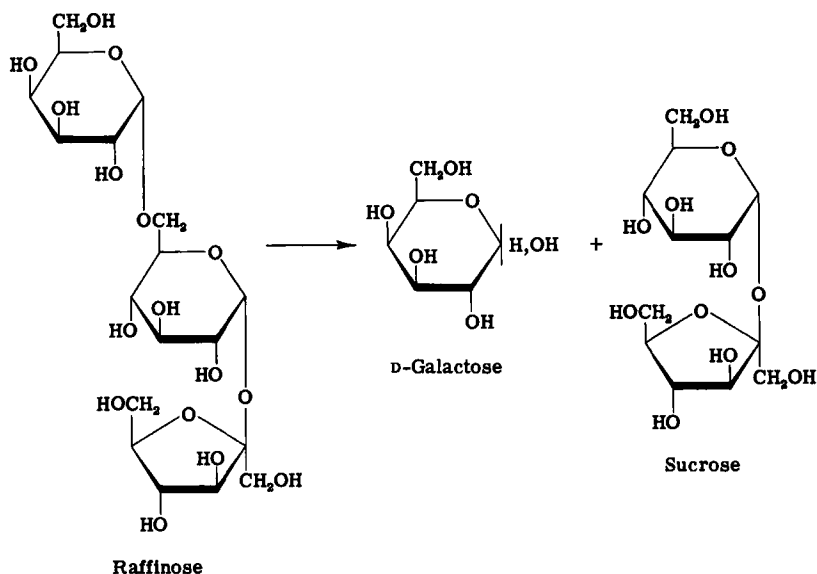
Raffinose

Øjvind Winge and his colleagues at the Carlsberg Laboratorium made various hybrids of *Saccharomyces uvarum* which, according to their genotype, could utilize all, part, or none of the raffinose molecule[356,569,570] (see Table XIV). Thus, (*i*) yeasts lacking genes for both α-D-galactosidase (*MEL*) and β-D-fructofuranosidase (*SUC*) did not hydrolyze raffinose; (*ii*) yeasts having *SUC* genes, but no *MEL* genes, utilized the D-fructofuranosyl group, so that melibiose remained; (*iii*) yeasts having a *MEL* gene, but no *SUC* gene, hydrolyzed raffinose to sucrose plus D-galactose, which were utilized only if genes *MAL* and *GAL* were present, respectively. Table XIV instances eight such combinations.

Correspondingly, yeasts of different species utilize different parts of the raffinose molecule,[571] or none. The following examples of each kind of utilization for species of *Saccharomyces* come from Reference 64. (*i*) Neither *Saccharomyces bisporus* nor *Saccharomyces rouxii* utilizes raffinose. (*ii*) *Saccharomyces cerevisiae* and *Saccharomyces fermentati* can each utilize solely the D-fructofuranosyl group of raffinose after the initial action of β-D-fructofuranosidase. (*iii*) *Saccharomyces oleaginosus* (*italicus* var.

(569) Ø. Winge and C. Roberts, *Nature* (London), **177**, 383–384 (1956).
(570) M. Losada, *C. R. Trav. Lab. Carlsberg*, **18**, 460–482 (1957).
(571) N. J. W. Kreger-van Rij, *Symp. Soc. Gen. Microbiol.*, **12**, 196–211 (1962).

TABLE XIV

Hydrolysis and Utilization of Raffinose by Cultures of *Saccharomyces uvarum* of Various Genotypes[570]

	Genotype							
	MAL	MEL	SUC	MEL GAL	MEL MAL	MEL SUC	MEL MAL GAL	MEL SUC GAL
Enzymes present[a]								
α-D-Glucosidase	+	−	−	−	+	−	+	−
α-D-Galactosidase	−	+	−	+	+	+	+	+
β-D-Fructofuranosidase	−	−	+	−	−	+	−	+
? Galactokinase[b]	−	−	−	+	−	−	+	+
Sugars produced by hydrolysis of raffinose[c]								
D-Fructose	O	O	PU	O	PU	PU	PU	PU
D-Glucose	O	O	O	O	PU	PU	PU	PU
D-Galactose	O	P	O	PU	P	P	PU	PU
Sucrose	O	P	O	P	O	O	O	O
Melibiose	O	O	P	O	O	O	O	O

[a] Key: + = enzyme present; − = enzyme not present. [b] The gene *GAL*, for D-galactose utilization, may refer to one or more genes controlling different enzymes of the Leloir pathway.[323] [c] Key: O = sugar not produced; P = sugar produced, but not utilized, by yeast; PU = sugar produced, and utilized, by yeast.

melibiosi)[572] and *Saccharomyces oleaceus,* having α-D-galactosidase activity without β-D-fructofuranosidase or α-D-glucosidase activity, utilize only the D-galactosyl group of raffinose. The genetics of melibiose utilization has been examined with hybrids of *Saccharomyces oleaginosus, Saccharomyces uvarum, Saccharomyces chevalieri,* and *Saccharomyces italicus.*[573,574] (*iv*) Both *Saccharomyces uvarum* and *Saccharomyces kluyveri* utilize the whole raffinose molecule. Although strains of *Saccharomyces uvarum* have been shown to possess both α-D-galactosidase and β-D-fructofuranosidase activity, other yeasts, not similarly investigated, might possibly utilize raffinose completely by the combined action of α-D-galactosidase and α-D-glucosidase. (*v*) *Saccharomyces eupagycus* and *Saccharomyces inusitatus* apparently hydrolyze both melibiose and sucrose, using all but the D-galactosyl group of raffinose as they cannot utilize D-galactose. (*vi*) *Saccharomyces hienipiensis*[575] and *Saccharomyces norbenesis*[576] each utilizes melibiose, but they do not normally use raffinose, sucrose, or D-galactose.

5. β-D-Galactopyranosides

Only just over 10% of yeast species utilize lactose (see Table III), and most of these species also utilize D-galactose. β-D-Galactosidase (EC 3.2.1.23) activities have been studied chiefly for two species, namely, *Kluyveromyces fragilis*[221,223,313,577−587] and *Kluyveromyces lactis.*[545,588]

Some of the earlier publications were particularly concerned with the fact that lactose is utilized faster than either of its component monosaccharides, D-glucose or D-galactose, by *Kluyveromyces (Saccharomyces) fragilis,*[221,579] *Candida pseudotropicalis (Torula cremoris), Candida kefyr (Torula lactosa),*[579] and other yeasts.[589] This was held to show that hydrolysis is not the first step in lactose utilization. However, after Gottschalk's critical review,[222] later authors could explain these and other observations by suggesting that the β-

(572) N. van Uden and L. Assis-Lopes, *Port. Acta Biol., Ser. A,* **4,** 323–327 (1957); *Chem. Abstr.,* **51,** 18,092*i* (1957).

(573) C. Roberts, A. T. Ganesan, and W. Haupt, *Heredity,* **13,** 499–517 (1959).

(574) W. Haupt and H. Alps, *Arch. Mikrobiol.,* **45,** 179–187 (1963).

(575) J. Santa María, *J. Gen. Microbiol.,* **28,** 375–384 (1962).

(576) J. Santa María, A. v. L., *J. Microbiol. Serol.,* **29,** 329–343 (1963).

(577) C. Neuberg and E. Hofmann, *Biochem. Z.,* **256,** 450–461 (1932).

(578) E. Hofmann, *Biochem. Z.,* **256,** 462–474 (1932).

(579) K. Myrbäck and E. Vasseur, *Z. Physiol. Chem.,* **277,** 171–180 (1943).

D-galactosidase is enclosed in an osmotic barrier. This explanation applies, for example, to the greater dependence on pH value of lactose hydrolysis by disrupted *Kluyveromyces (Saccharomyces) fragilis* than by intact cells.[583] Accordingly, a system of stereospecific transport across the plasmalemma is now recognized to be the initial requirement for the utilization of β-D-galactosides by intact cells of *Kluyveromyces fragilis.*[223,584]

β-D-Galactosidases have been purified from *Kluyveromyces (Saccharomyces) lactis*[588] and *Kluyveromyces (Saccharomyces) fragilis,*[587] that for the latter having a molecular weight estimated at > 500,000. Lactose and *o*-nitrophenyl β-D-galactopyranoside are substrates. The enzyme is difficult to purify, because of its instability, which has been studied with material from *Kluyveromyces fragilis.*[585,586]

Sporobolomyces singularis is unusual in being able to utilize lactose but not D-galactose.[33,590] This observation led to the finding of marked transglycosylation activities in this yeast, the β-D-galactosyl group of lactose being transferred to the secondary hydroxyl group of each of a number of acceptor molecules to form such new oligosaccharides as *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose and *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl - (1 → 4)-*O*-β-D-galactopyranosyl - (1 → 4)-D - glucose.[22,23] Such transglycosylations from lactose by material from yeasts had been recorded previously,[582,591] four *O*-β-D-galactosyloligosaccharides* being synthesized by preparations from *Kluyveromyces (Saccharomyces) fragilis.*[592,593]

(580) E. Hofmann and H. Scheck, *Biochem. Z.,* 319, 522–528 (1949).
(581) B. van Dam, J. G. Revallier-Warffemius, and L. C. van Dam-Schermerhorn, *Neth. Milk Dairy J.,* 4, 96–114 (1950).
(582) H. R. Roberts and E. F. McFarren, *J. Dairy Sci.,* 36, 620–632 (1953).
(583) A. Davies, *J. Gen. Microbiol.,* 14, 425–439 (1956).
(584) R. Davies, *J. Gen. Microbiol.,* 37, 81–98 (1964).
(585) G. Szabó and R. Davies, *J. Gen. Microbiol.,* 37, 99–112 (1964).
(586) G. Szabó and J. Rózsa, *Acta Microbiol. Acad. Sci. Hung.,* 12, 91–102 (1965).
(587) A. K. Kulikova, A. S. Tikhomirova, and R. V. Feniksova, *Biokhimiya,* 37, 405–409 (1972).
(588) L. Biermann and M. D. Glantz, *Biochim. Biophys. Acta,* 167, 373–377 (1968).
(589) M. Rogosa, *J. Biol. Chem.,* 175, 413–423 (1948).
(590) H. J. Phaff and L. do Carmo-Sousa, *A. v. L., J. Microbiol. Serol.,* 28, 193–207 (1962).
(591) J. H. Pazur, *J. Biol. Chem.,* 208, 439–444 (1954).
(592) J. H. Pazur, J. M. Marsh, and C. L. Tipton, *J. Biol. Chem.,* 233, 277–279 (1958).
(593) J. H. Pazur, M. Shadaksharaswamy, and A. Cepure, *Arch. Biochem. Biophys.,* 94, 142–147 (1961).

X. The Catabolism of Pentoses and Alditols

There are various, interrelated reasons why the abilities of yeasts to utilize pentoses and alditols should be associated. (*i*) Pentoses and pentitols may be reversibly interconverted by dehydrogenases; (*ii*) catabolic routes may be shared; and (*iii*) many of the alditol dehydrogenases have particularly wide substrate-specificity, so that a single enzyme may act on pentitols and pentoses, as well as on hexitols and hexoses.

In principle, the first step by which yeasts could convert an aldopentose into an intermediate of the pentose cycle might be (*a*) an epimerization, (*b*) a conversion into the corresponding ketose by way of the enediol, (*c*) conversion by oxidation and reduction, or (*d*) phosphorylation. For example, D-xylose might be expected to be catabolized initially, either by isomerization to D-*threo*-pentulose, or reduction to xylitol which would then be oxidized to D-*threo*-pentulose. D-*threo*-Pentulose is phosphorylated to give D-*threo*-

pentulose 5-phosphate, an intermediary metabolite of the pentose cycle.
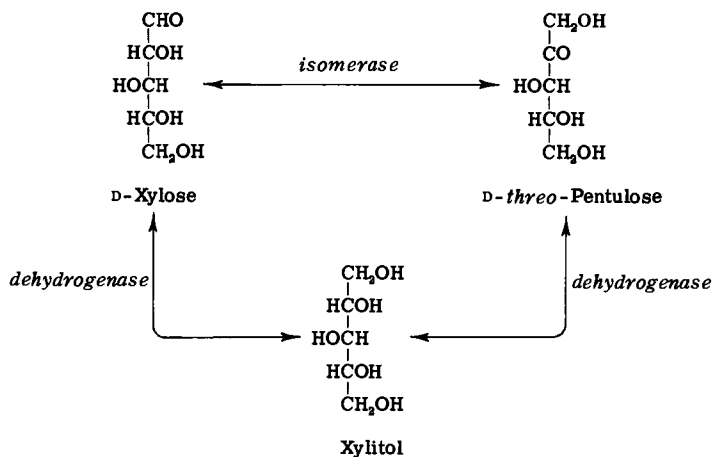
Again, in principle, alditols might be oxidized to the corresponding ketoses by $NAD^{\oplus}$- or $NADP^{\oplus}$-linked dehydrogenases, or to the aldoses by $NADP^{\oplus}$-linked dehydrogenases. Alternatively, the aldoses might be phosphorylated. Aldoses and ketoses may be phosphorylated, and alditol phosphates oxidized to aldose or ketose phosphates. These compounds are likely to be catabolized by the reactions of the Embden–Meyerhof glycolytic pathway or of the pentose cycle.

$$
\begin{array}{l}
\text{CH}_2\text{OH} \\
| \\
\text{CHOH} \\
| \\
\text{R}
\end{array}
\; + \; \text{NAD(P)}^{\oplus} \;
\xrightleftharpoons[\text{dehydrogenase}]{}
\;
\begin{array}{l}
\text{CH}_2\text{OH} \\
| \\
\text{CO} \\
| \\
\text{R}
\end{array}
\; + \; \text{NAD(P)H} \qquad (i)
$$

Alditol   Ketose

$$
\begin{array}{l}
\text{CH}_2\text{OH} \\
| \\
\text{CHOH} \\
| \\
\text{R}
\end{array}
\; + \; \text{NADP}^{\oplus} \;
\xrightleftharpoons[\text{dehydrogenase}]{}
\;
\begin{array}{l}
\text{CHO} \\
| \\
\text{CHOH} \\
| \\
\text{R}
\end{array}
\; + \; \text{NADPH} \qquad (ii)
$$

Alditol   Aldose

$$
\begin{array}{l}
\text{CH}_2\text{OH} \\
| \\
\text{CHOH} \\
| \\
\text{R}
\end{array}
\; + \; \text{ATP} \;
\xrightarrow{\text{kinase}}
\;
\begin{array}{l}
\text{CH}_2\text{OPO}_3\text{H}_2 \\
| \\
\text{CHOH} \\
| \\
\text{R}
\end{array}
\; + \; \text{ADP} \qquad (iii)
$$

Alditol   Alditol phosphate
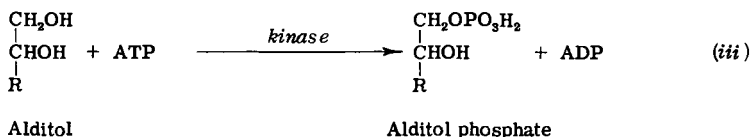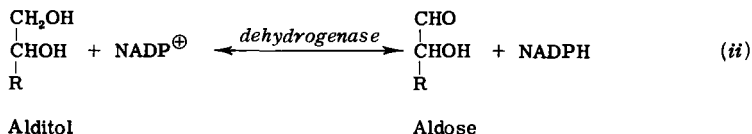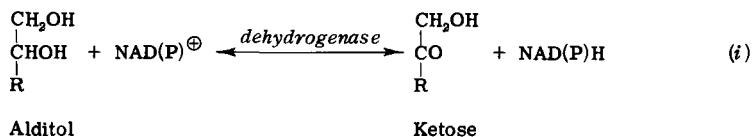
There are valuable reviews on the metabolism of pentoses,[594–596] alditols,[597–600] or both,[601,602] and of both by yeasts.[603]

Table XV summarizes the reactions of alditols described for various species of yeast. Further publications on the subject, not referred to in Table XV, include those on *Candida tropicalis,*[621–625]

(594) W. A. Wood, in "The Bacteria," I. C. Gunsalus and R. Y. Stanier, eds., Academic Press, New York, 1961, Vol. 2, pp. 59–149.
(595) W. A. Wood, *Annu. Rev. Biochem.*, 35, 521–588 (1966).
(596) Y. N. Karasevich, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 30, 231–242 (1965).
(597) N. L. Edson, *Rept. Aust. N. Z. Assoc. Advan. Sci.*, 29, 281–299 (1953); *Chem. Abstr.*, 48, 2,152a (1954).
(598) O. Touster and D. R. D. Shaw, *Physiol. Rev.*, 42, 181–225 (1962).
(599) V. H. Cheldelin, C. H. Wang, and T. E. King, in "Comparative Biochemistry," M. Florkin and H. S. Mason, eds., Academic Press, New York, N. Y., 1962, Vol. IIIA, pp. 427–502.
(600) D. H. Lewis and D. C. Smith, *New Phytol.*, 66, 143–184 (1967).
(601) B. L. Horecker, "Pentose Metabolism in Bacteria," Wiley, New York, 1962.
(602) S. Hollmann and O. Touster, "Non-Glycolytic Pathways of Metabolism of Glucose," Academic Press, New York, 1964.
(603) J. A. Barnett, in Ref. 85, G. C. Ainsworth and A. S. Sussman, eds., Academic Press, New York, 1968, Vol. III, pp. 557–595.
(604) L. A. Veiga, M. Bacila, and B. L. Horecker, *Biochem. Biophys. Res. Commun.*, 2, 440–444 (1960).
(605) L. A. Veiga, *J. Gen. Appl. Microbiol.*, 14, 65–78 (1968).
(606) L. A. Veiga, *J. Gen. Appl. Microbiol.*, 14, 79–87 (1968).
(607) Y. N. Karasevich, *Mikrobiologiya*, 38, 465–467 (1969).
(608) M. Chakravorty, L. A. Veiga, M. Bacila, and B. L. Horecker, *J. Biol. Chem.*, 237, 1014–1020 (1962).

TABLE XV

Summary of Coenzyme-linked Oxido-reductions in the Metabolism of Alditols by Yeasts

Yeast	Reactions[a]	
	NAD⊕-linked	NADP⊕-linked
Candida albicans	For D-xylose-grown yeast, extracts catalyze xylitol ↔ D-*threo*-pentulose.[604,606] Purified enzyme also (*a*) reduces NAD⊕ with D-glucitol or ribitol, and (*b*) oxidizes NADH with D-*erythro*-pentulose or D-fructose.[606]	For D-xylose-grown yeast, extracts catalyze (*i*) xylitol ↔ D-xylose, (*ii*) L-arabinitol ↔ L-arabinose,[604,605] (*iii*) ribitol ↔ D-ribose, (*iv*) galactitol ↔ D-galactose. Purified enzyme also (*a*) oxidizes NADPH with D-glucose, and (*b*) reduces NADP⊕ with D-glucitol, erythritol, or D-mannitol.[605]
tropicalis	For L-arabinitol-grown yeast, extracts reduce NAD⊕ with L-arabinitol.[607]	For L-arabinitol-grown yeast, extracts reduce NADP⊕ with L-arabinitol.[607]
utilis	For D-xylose-grown yeast, extracts catalyze oxidation of NADH with D- or L-*threo*-pentulose, and reduction of NAD⊕ with xylitol.[620] Purified enzyme catalyzes (*i*) xylitol ↔ D-*threo*-pentulose, (*ii*) ribitol ↔ D-*erythro*-pentulose, (*iii*) D-mannitol ↔ D-fructose, (*iv*) D-glucitol ↔ D-fructose, (*v*) D-*glycero*-D-*gluco*-heptitol ↔ sedoheptulose,[601,608] and oxidizes (*vi*) D-altritol, (*vii*) L-*glycero*-D-*gluco*-heptitol, (*viii*) L-glucitol, (*ix*) L-iditol, and (*x*) D-arabinitol.[609] This activity may be constituted in all or part of three enzymic components separated electrophoretically, catalyzing I (*i*), (*ii*), (*iv*), and (*ix*); II (*i*), (*ii*), (*ix*), and (*x*); III (*i*), (*iii*), and (*iv*).[264]	For D-xylose-grown yeast, extracts catalyze oxidation of NADPH with D-xylose.[620] Purified enzyme catalyzes xylitol ↔ D-xylose, reduction of D-glyceraldehyde, D-erythrose, L-arabinose, D-ribose, D-galactose, and D-glucose, and oxidation of galactitol, L-arabinitol, D-glucitol, ribitol, erythritol, and glycerol.[610,611]
Pichia delftensis	For succinate-grown yeast, extracts catalyze D-glucitol ↔ D-fructose + L-*xylo*-hexulose, D-mannitol ↔ D-fructose, D-arabinitol → *threo*-pentulose, and reduction of NAD⊕ with xylitol or erythritol.[264]	

	For D-glucitol-grown yeast, extracts catalyze **D-glucitol** ↔ **D-fructose** + L-*xylo*-hexulose, **D-mannitol** ↔ **D-fructose**, D-arabinitol → *threo*-pentulose, erythritol → *glycero*-tetrulose, and reduction of NAD$^{\oplus}$ with L-iditol, **ribitol**, or xylitol.[264]	For D-glucitol-grown yeast, extracts catalyze reduction of NADP$^{\oplus}$ with **D-mannitol**.[264]
farinosa (miso)	For D-glucose-grown yeast, partly purified enzyme catalyzes **erythritol** → ? *glycero*-tetrulose, D-arabinitol ↔ D-*threo*-pentulose, xylitol → *threo*-pentulose, **ribitol** → *erythro*-pentulose, **D-glucitol** → fructose, galactitol → ? tagatose, **D-mannitol** → fructose.[612]	
membranae-faciens	For succinate-grown yeast, extracts catalyze D-glucitol → fructose, erythritol → *glycero*-tetrulose, reduction of NAD$^{\oplus}$ with ribitol, xylitol, or D-arabinitol, and oxidation of NADH with **D-fructose** or L-*xylo*-hexulose.[264]	For succinate-grown yeast, extracts catalyze reduction of NADP$^{\oplus}$ with **D-mannitol**.[264]
Rhodotorula glutinis	For D-xylose-grown yeast, extracts catalyze oxidation of NADH with D- and L-*threo*-pentulose.[620]	For D-xylose-grown yeast, extracts reduce D-xylose in the presence of NADPH.[620]
? *Rhodotorula* species (strain Rh-110)	Partly purified enzyme catalyzes reduction of NAD$^{\oplus}$ with xylitol or D-glucitol.[268]	Partly purified enzyme catalyzes oxidation of NADPH with D-xylose.[268]
Saccharomyces bailii (acidifaciens)	For D-glucitol-grown yeast, extracts catalyze reduction of NAD$^{\oplus}$ with **D-glucitol**, L-iditol, ribitol, or xylitol, and oxidation of NADH with **D-fructose** or L-*xylo*-hexulose.[264]	For D-glucitol-grown yeast, extracts catalyze reduction of NADP$^{\oplus}$ with **D-glucitol** or **D-mannitol**.[264]
bisporus (mellis)	Extracts catalyze reduction of NAD$^{\oplus}$ with xylitol, **D-glucitol**, ribitol, or galactitol, and oxidation of NADH with D- or L-*threo*-pentulose.[613]	Extracts catalyze D-arabinitol ↔ D-*erythro*-pentulose, and reduction of NADP$^{\oplus}$ with xylitol.[613]
cerevisiae	Extracts catalyze reduction of NAD$^{\oplus}$ with D-mannitol.[614]	
rouxii	For D-glucose-grown yeast, a purified enzyme catalyzes xylitol ↔ D-*threo*-pentulose, and crude extracts catalyze reduction of NAD$^{\oplus}$ with ribitol.[615]	For D-glucose-grown yeast, a purified enzyme catalyzes D-arabinitol ↔ D-*erythro*-pentulose, and crude extracts catalyze reduction of NADP$^{\oplus}$ with xylitol.[615]
uvarum	Extracts catalyze NAD$^{\oplus}$ reduction with D-mannitol.[616]	

TABLE XV (*continued*)

Yeast	Reactions[a]	
	NAD⊕-linked	NADP⊕-linked
? *Schwanniomyces occidentalis*[b]	For *myo*-inositol-grown yeast, extracts catalyze xylitol ↔ D-*threo*-pentulose.[617,618]	For *myo*-inositol-grown yeast, extracts catalyze xylitol ↔ L-*threo*-pentulose.[617,618]
Torulopsis candida	For succinate-grown yeast, extracts catalyze **D-glucitol** → fructose, L-iditol → *xylo*-hexulose, **ribitol** → *erythro*-pentulose, xylitol → *threo*-pentulose, and oxidation of NADH with **D-fructose** or L-*xylo*-hexulose.[264]	For succinate-grown yeast, extracts catalyze **D-glucitol** → *xylo*-hexulose + glucose, **D-mannitol** → fructose + mannose, **ribitol** → ribose, xylitol → xylose + *threo*-pentulose, D-arabinitol → arabinose + *threo*-pentulose, and oxidation of NADPH with **D-fructose**, L-*xylo*-hexulose, D-ribose, **D-xylose**, or **L-arabinose**.[264]
	For D-mannitol-grown yeast, extracts catalyze reduction of NAD⊕ with **D-glucitol**, L-iditol, **ribitol**, or xylitol, and oxidation of NADH with **D-fructose** or L-*xylo*-hexulose.[264]	For D-mannitol-grown yeast, extracts catalyze reduction of NADP⊕ with **D-glucitol** or **D-mannitol**, and oxidation of NADPH with **D-fructose**, L-*xylo*-hexulose, **D-xylose**, or **L-arabinose**.[264]
	For D-glucitol-grown yeast, extracts catalyze **D-glucitol** → fructose + *xylo*-hexulose, **D-mannitol** → fructose, xylitol → *threo*-pentulose, and D-arabinitol → *threo*-pentulose, reduction of NAD⊕ with L-iditol or **ribitol**, and oxidation of NADH with **D-fructose** or L-*xylo*-hexulose.[264]	For D-glucitol-grown yeast, extracts catalyze **D-glucitol** → *xylo*-hexulose + glucose, **D-mannitol** → fructose + mannose, **ribitol** → ribose, xylitol → xylose + *threo*-pentulose, and D-arabinitol → arabinose + *threo*-pentulose, and oxidation of NADPH with **D-fructose**, L-*xylo*-hexulose, **D-glucose**, **D-mannose**, **D-galactose**, D-ribose, **D-xylose**, D-arabinose, or **L-arabinose**.[264]
	For erythritol-grown yeast, extracts catalyze reduction of NAD⊕ with **D-glucitol**, L-iditol, **ribitol**, xylitol, or erythritol, and oxidation of NADH with **D-fructose** or L-*xylo*-hexulose.[264]	For erythritol-grown yeast, extracts catalyze reduction of NADP⊕ with **D-glucitol**, **D-mannitol**, xylitol, or D-arabinitol, and oxidation of NADPH with **D-fructose**, L-*xylo*-hexulose, or **D-mannose**.[264]
		For D-xylose-grown yeast, extracts catalyze D-arabinose → D-arabinitol; D-lyxose → D-arabinitol; and L-xylose → xylitol.[619]

[a] Names of compounds printed in bold type are of those known to be utilizable by the species. [b] The identity of this yeast is in doubt, as the species is authoritatively stated not to utilize *myo*-inositol.[70]

Candida utilis,[626-629] *Metschnikowia pulcherrima*,[630,631] and *Saccharomyces uvarum*.[632,633]

In practice, the initial stages of catabolism of alditols and pentoses by yeasts seem generally to involve $NAD^{\oplus}$- or $NADP^{\oplus}$-linked oxidations and reductions. On the other hand, many bacteria catabolize pentoses by isomerizations and phosphorylations,[634] and some yeasts do so too. D-Xylose isomerase has been reported in strains of *Candida utilis*[635] and in *Rhodotorula glutinis (gracilis)*, for which it is necessary for the utilization of D-xylose.[636] In addition, ribokinase has been detected in *Metschnikowia pulcherrima*.[637] Figures 10 and 11 show the various routes by which yeasts are considered to catabolize pentitols and pentoses, or hexitols and hexoses. Other pentoses utilized by some yeasts include D-*erythro*-pentulose,[638] D-lyxose,

(609) M. Chakravorty and B. L. Horecker, *Methods Enzymol.*, 9, 163–166 (1966).

(610) B. M. Scher and B. L. Horecker, *Arch. Biochem. Biophys.*, 116, 117–128 (1966).

(611) B. M. Scher and B. L. Horecker, *Methods Enzymol.*, 9, 166–170 (1966).

(612) H. Onishi and N. Saito, *Agr. Biol. Chem.* (Tokyo), 26, 245–251 (1962).

(613) R. Weimberg, *Biochem. Biophys. Res. Commun.*, 8, 442–445 (1962).

(614) J. B. Wolff and N. O. Kaplan, *J. Bacteriol.*, 71, 557–564 (1956).

(615) J. M. Ingram and W. A. Wood, *J. Bacteriol.*, 89, 1186–1194 (1965).

(616) D. Müller, *Enzymologia*, 3, 26–28 (1937).

(617) A. Sivak and O. Hoffmann-Ostenhof, *Biochem. Z.*, 336, 229–240 (1962).

(618) P. Dworsky and O. Hoffmann-Ostenhof, *Biochem. Z.*, 343, 394–398 (1965).

(619) Y. N. Karasevich, *Mikrobiologiya*, 40, 389–397 (1971).

(620) C. Chiang and S. G. Knight, *Nature* (London), 188, 79–81 (1960).

(621) Y. N. Karasevich, *Mikrobiologiya*, 39, 1040–1045 (1970).

(622) Y. N. Karasevich and A. P. Ipatova, *Mikrobiologiya*, 34, 966–971 (1965).

(623) Y. N. Karasevich and A. P. Ipatova, *Mikrobiologiya*, 36, 386–389 (1967).

(624) Y. N. Karasevich and A. P. Ipatova, *Mikrobiologiya*, 37, 201–206 (1968).

(625) I. K. Shakhova, *Mikrobiologiya*, 42, 99–106 (1973).

(626) A. C. Arcus and N. L. Edson, *Biochem. J.*, 64, 385–405 (1956).

(627) M. Chakravorty, M. Bacila, L. A. Veiga, and B. L. Horecker, *Fed. Proc.*, 20, 84 (1961).

(628) H. Horitsu and M. Tomoeda, *Agr. Biol. Chem.* (Tokyo), 30, 962–966 (1966).

(629) H. Horitsu, M. Tomoeda, and K. Kumagai, *Agr. Biol. Chem.* (Tokyo), 32, 514–517 (1968).

(630) Y. N. Karasevich, *Dokl. Akad. Nauk SSSR*, 182, 957–959 (1968).

(631) Y. N. Karasevich, *Mikrobiologiya*, 39, 748–754 (1970).

(632) D. Müller, *Biochem. Z.*, 268, 152–157 (1934).

(633) J. B. Wilhelmsen, *Enzymologia*, 23, 259–266 (1961).

(634) R. P. Mortlock, D. D. Fossitt, and W. A. Wood, *Proc. Nat. Acad. Sci. U. S.*, 54, 572–579 (1965).

(635) M. Tomoeda and H. Horitsu, *Agr. Biol. Chem.* (Tokyo), 28, 139–143 (1964).

(636) M. Höfer, A. Betz, and A. Kotyk, *Biochim. Biophys. Acta*, 252, 1–12 (1971).

(637) Y. N. Karasevich and V. S. Ivoilov, *Mikrobiologiya*, 41, 581–585 (1972).

(638) T. Suzuki and H. Onishi, *A. v. L., J. Microbiol. Serol.*, 36, 227–232 (1970).

FIG. 10. — Possible Routes of Pentitol and Pentose Catabolism by Yeasts (after Barnett[903]).

Hexitols   **Aldoses**   **Ketoses**   **Hexose phosphates**

$$\xrightarrow[\textit{dehydrogenase}]{\textit{NADP}}$$ D-Mannose $\xrightarrow{\textit{kinase}}$ D-Mannose 6-phosphate

D-Mannitol $\xrightarrow[\textit{dehydrogenase}]{\textit{NAD(P)}}$

isomerase

D-Fructose $\xrightarrow{\textit{kinase}}$ D-Fructose 6-phosphate

D-Glucitol $\xrightarrow[\textit{dehydrogenase}]{\textit{NAD(P)}}$

GLYCOLYTIC PATHWAY or PENTOSE CYCLE

isomerase   *epimerase*

$\xrightarrow[\textit{dehydrogenase}]{\textit{NADP}}$ D-Glucose $\xrightarrow{\textit{kinase}}$ D-Glucose 6-phosphate

$\xrightarrow[\textit{dehydrogenase}]{\textit{NAD(P)}}$ L-*xylo*-Hexulose

Leloir pathway

Galactitol $\xrightarrow[\textit{dehydrogenase}]{\textit{NADP}}$ D-Galactose $\xrightarrow{\textit{kinase}}$ D-Galactosyl phosphate

FIG. 11.—Possible Routes of Hexitol and Hexose Catabolism by Yeasts (after Barnett[603]).

and L-xylose,[631] although a number of yeasts investigated do not use L-xylose.[81,639-641]

Yeasts that do not utilize certain alditols or pentitols may nonetheless appear equipped enzymically to do so. For example, although *Candida utilis* does not use D-glucitol,[41] extracts contain active dehydrogenases that convert D-glucitol into D-fructose[264,608] or D-glucose,[610,642] both of which it can utilize. Possibly, D-glucitol does

$$
\begin{array}{ccccc}
CH_2OH & & CH_2OH & & CHO \\
| & & | & & | \\
CO & & HCOH & & HCOH \\
| & & | & & | \\
HOCH & \underset{NADH}{\overset{NAD^{\oplus}}{\rightleftarrows}} & HOCH & \underset{NADPH}{\overset{NADP^{\oplus}}{\rightleftarrows}} & HOCH \\
| & & | & & | \\
HCOH & & HCOH & & HCOH \\
| & & | & & | \\
HCOH & & HCOH & & HCOH \\
| & & | & & | \\
CH_2OH & & CH_2OH & & CH_2OH \\
\text{D-Fructose} & & \text{D-Glucitol} & & \text{D-Glucose}
\end{array}
$$
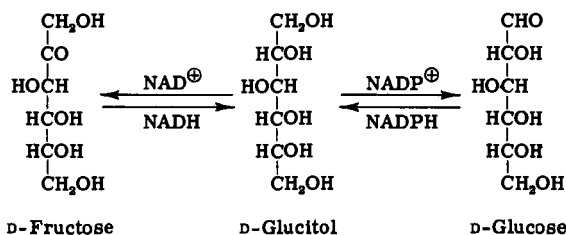
not cross the plasmalemma of this yeast,[264] or the dehydrogenases might be subject to some further compartmentation. Similarly, *Candida albicans* cannot use galactitol,[41] although this yeast contains an enzyme capable of oxidizing galactitol to D-galactose,[605] on which it can grow aerobically.[41]

$$
\begin{array}{ccc}
CH_2OH & & CHO \\
| & & | \\
HCOH & & HCOH \\
| & & | \\
HOCH & \underset{NADP^{\oplus}}{\overset{NADPH}{\rightleftarrows}} & HOCH \\
| & & | \\
HOCH & & HOCH \\
| & & | \\
HCOH & & HCOH \\
| & & | \\
CH_2OH & & CH_2OH \\
\text{Galactitol} & & \text{D-Galactose}
\end{array}
$$
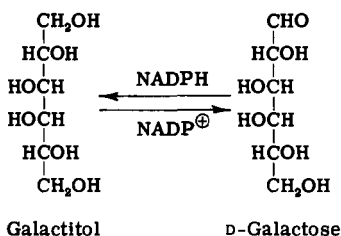
Under aerobic conditions, certain yeasts can convert 20 to 40% of the D-galactose they take up into galactitol, which is excreted and can be isolated from the culture medium.[643] These yeasts include strains of *Hansenula anomala*, *Pichia farinosa*, *Candida diddensii* (*polymorpha*), and *Torulopsis versatilis*, not one of which species,

(639) J. P. van der Walt, A. v. L., *J. Microbiol. Serol.*, 28, 81–84 (1962).
(640) J. P. van der Walt, A. v. L., *J. Microbiol. Serol.*, 29, 319–322 (1963).
(641) J. P. van der Walt, A. v. L., *J. Microbiol. Serol.*, 30, 273–280 (1964).
(642) B. L. Horecker, in "International Symposium on Metabolism, Physiology and Clinical Use of Pentoses and Pentitols," B. L. Horecker, K. Lang, and Y. Takagi, eds., Springer-Verlag, Berlin, 1969, pp. 5–25.
(643) H. Onishi and T. Suzuki, *J. Bacteriol.*, 95, 1745–1749 (1968).

strikingly, utilizes galactitol, although they use D-galactose.[41,49,77] Thus, in these cases, D-galactose may be regarded as being used inefficiently, much of it being converted into galactitol, which serves no obvious function for the yeast, unless to facilitate some enzymic activity.[644] Many yeasts, grown on D-glucose or other sugars, form various alditols, particularly glycerol, erythritol, D-arabinitol, or D-mannitol. The enzymic basis for production of D-arabinitol from D-glucose by *Saccharomyces rouxii* has been investigated,[615] and some yeasts form large proportions of alditols, such as erythritol, from *n*-alkanes.[645–645c] Spencer[15] reviewed the subject of alditol production by yeasts, which is of commercial interest. It is also worth noting that, unlike intracellular lipids, the lipids formed outside the plasmalemma by certain yeasts, notably *Rhodotorula graminis* and *Rhodotorula glutinis*, are made up of fatty acid esters of alditols other than glycerol, such as mannitol or arabinitol[646] (for a review, see Ref. 647).

XI. THE CATABOLISM OF *myo*-INOSITOL

Of 434 yeast species, 32 can utilize *myo*-inositol as the sole source of carbon for aerobic growth (see Table III). Half of those species that use *myo*-inositol belong to the genus *Cryptococcus*.[24] Most of the research on the biochemistry of this utilization has been performed by Hoffmann-Ostenhof and his colleagues[617,618,648–652] with an organism that they called *Schwanniomyces occidentalis*. Unfortunately, its true identity is in doubt, because, unlike their strain, this species does not utilize *myo*-inositol for aerobic growth.[70,653] When grown on *myo*-inositol, extracts of this organism had an inducible, but unstable, enzyme that oxidized *myo*-inositol to D-glucuronate. As shown, D-glucuronate may then be converted into D-*threo*-pentulose, which is catabolized by the reactions of the pentose cycle. On the

myo-Inositol   D-Glucuronic acid   L-Gulonic acid
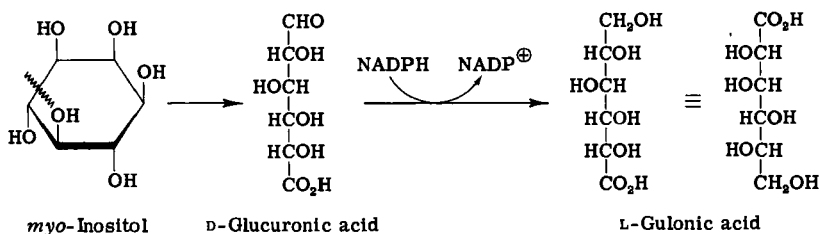
(644) A. D. Brown and J. R. Simpson, *J. Gen. Microbiol.*, 72, 589–591 (1972).
(645) K. Hattori and T. Suzuki, *Agr. Biol. Chem.* (Tokyo), 38, 581–586 (1974).
(645a) K. Hattori and T. Suzuki, *Agr. Biol. Chem.* (Tokyo), 38, 1203–1208 (1974).

$$\text{NAD}^{\oplus} \quad \text{NADH} \longrightarrow \underset{\substack{\text{L-}xylo\text{-3-Hexulos-}\\ \text{onic acid}}}{\begin{array}{c} CO_2H \\ | \\ HOCH \\ | \\ CO \\ | \\ HCOH \\ | \\ HOCH \\ | \\ CH_2OH \end{array}} \quad \xrightarrow{\;CO_2\;} \quad \underset{\text{L-}threo\text{-Pentulose}}{\begin{array}{c} CH_2OH \\ | \\ CO \\ | \\ HCOH \\ | \\ HOCH \\ | \\ CH_2OH \end{array} \equiv \begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ CO \\ | \\ CH_2OH \end{array}}$$

$$\text{NADPH} \quad \text{NADP}^{\oplus} \longrightarrow \underset{\text{Xylitol}}{\begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}} \quad \text{NAD}^{\oplus} \quad \text{NADH} \longrightarrow \underset{\text{D-}threo\text{-Pentulose}}{\begin{array}{c} CH_2OH \\ | \\ CO \\ | \\ HOCH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}}$$
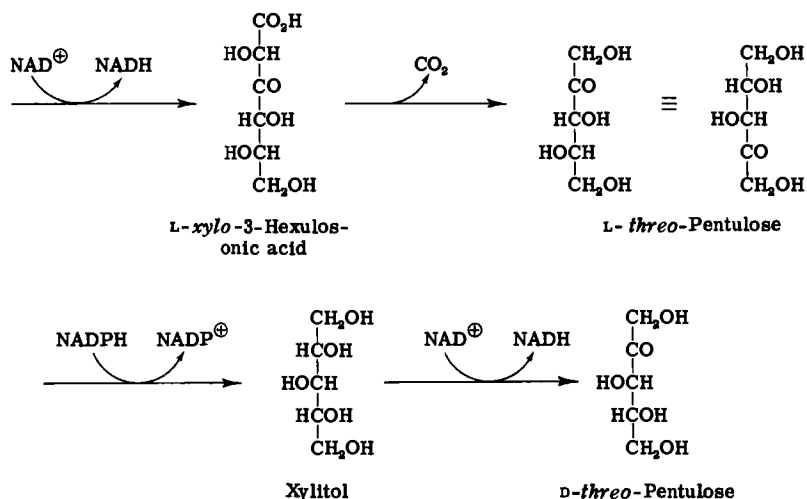
other hand, the initial step of *myo*-inositol catabolism by a strain of *Cryptococcus melibiosum* seems to be an $NAD^{\oplus}$-linked oxidation to *myo*-inosose-2.[654]

For normal growth with different sources of carbon, many yeasts require *myo*-inositol in small quantities, as it is important in lipid metabolism.[122] Accordingly, strains of *Kloeckera apiculata* (*brevis*),[655,656] *Saccharomyces uvarum* (*carlsbergensis*),[657,658] *Schizo-*

(645b) K. Hattori and T. Suzuki, *Agr. Biol. Chem.* (Tokyo), **38**, 1875–1881 (1974).

(645c) K. Hattori and T. Suzuki, *Agr. Biol. Chem.* (Tokyo), **38**, 2419–2424 (1974).

(646) M. H. Deinema, *Proc. Symp. Yeasts, 2nd, Bratislava, 1966*, 479–482 (1969).

(647) F. H. Stodola, M. H. Deinema, and J. F. T. Spencer, *Bacteriol. Rev.*, **31**, 194–213 (1967).

(648) R. G. Janke, C. Jungwirth, I. B. Dawid, and O. Hoffmann-Ostenhof, *Monatsh. Chem.*, **90**, 382–395 (1959).

(649) C. Jungwirth, A. Sivak, O. Hoffmann-Ostenhof, and R. G. Janke, *Monatsh. Chem.*, **92**, 72–74 (1961).

(650) A. Sivak and O. Hoffmann-Ostenhof, *Biochim. Biophys. Acta*, **53**, 426–428 (1961).

(651) P. Dworsky and O. Hoffmann-Ostenhof, *Acta Biochim. Polon.*, **11**, 269–277 (1964).

(652) P. Dworsky and O. Hoffman-Ostenhof, *Monatsh. Chem.*, **98**, 993–996 (1967).

(653) This was confirmed for 10 strains by D. Yarrow (1974), personal communication. Professor Hoffmann-Ostenhof (personal communication) received his yeast, under the name of *Schwanniomyces occidentalis*, from the late Professor A. Janke.

(654) M. Vidal-Leiria and N. van Uden, *Biochim. Biophys. Acta*, **293**, 295–303 (1973).

(655) P. R. Burkholder, I. McVeigh, and D. Moyer, *J. Bacteriol.*, **48**, 385–391 (1944).

(656) W. B. Emery, N. McLeod, and F. A. Robinson, *Biochem. J.*, **40**, 426–432 (1946).

saccharomyces pombe,[656] and *Saccharomyces cerevisiae*[86] are used to assay *myo*-inositol.

XII. GENERALIZATIONS AND SPECULATIONS

The information in Sections VI to XI depends on the study of relatively few species and strains, but, in this Section, an attempt will be made to provide some generalizations, applicable to most yeasts, regarding the way in which they utilize sugars. These generalizations must necessarily be partly speculative and, as such, must eventually be subjected to experimental verification by the enzymic examination of selected yeasts. It is hoped that this Section will stimulate such investigations.

Certain simple, general facts about sugar utilization by yeasts have long been appreciated.[659] (*i*) A yeast that ferments a sugar anaerobically can nearly always also use it for aerobic growth, although the converse is not true. (*ii*) Generally, if a yeast ferments any sugar anaerobically, it will ferment D-glucose, D-mannose, and D-fructose. (*iii*) Pink (carotenoid-containing) yeasts of the genera *Rhodotorula*, *Sporobolomyces*, *Cryptococcus*, or *Rhodosporidium* do not ferment sugars anaerobically; the reason is obscure.

However, in 1966, Barnett[660,661] proposed a method for deducing the routes by which yeasts convert sugars into intermediates of the central metabolic pathways. This method involves examining associations between the results of the tests carried out by taxonomists when they determine the abilities of many strains to utilize different substrates. Barnett and Pankhurst[24] tabulated the abilities of 434 species of yeast to utilize 31 compounds that are sugars or their derivatives; this survey was based on the work of a number of authors who examined ~4,500 strains for taxonomic purposes. The detailed results analyzed here are for the quantal ability of 497 *strains* of yeasts to grow aerobically on 23 compounds. The yeasts included 250 strains from the genera *Pichia, Endomycopsis,* and *Debaryomyces*,[662] 112 strains of *Candida* species, and 135 from miscellaneous yeasts.[663]

(657) A. E. Wiles, *J. Inst. Brewing*, **56**, 183–193 (1950).

(658) R. H. Smith, *J. Gen. Microbiol.*, **5**, 772–780 (1951).

(659) A. J. Kluyver, *Ann. Zymol.*, Ser. II, **1**, 48–61 (1931); *Chem. Abstr.*, **27**, 4342 (1933).

(660) J. A. Barnett, *Nature* (London), **210**, 565–568 (1966).

(661) J. A. Barnett, *J. Gen. Microbiol.*, **42**, i–ii (1966).

(662) N. J. W. Kreger-van Rij, unpublished results.

(663) W. Slooff and D. Yarrow, unpublished results.

The method proposed for scrutinizing these results is analogous to that by which information on biosynthetic pathways has been obtained from auxotrophic mutants. The principle of the latter technique is as follows. Consider a sequence of metabolic reactions wherein a is converted into d through intermediates b and c, by means of enzymes $e1$, $e2$, and $e3$.

$$a \xrightarrow[e1]{} b \xrightarrow[e2]{} c \xrightarrow[e3]{} d$$
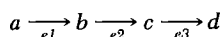
Mutant yeasts that are deficient in any of these enzymes have different capabilities. Thus, without $e1$, the yeast can metabolize b, c, or d, but not a; without $e2$, it can metabolize c or d, but not a or b; and so on. Hence, for such metabolic sequences, if the requirements of two mutant strains are satisifed by the same substrate x, and of only one of the strains by substrate y, then y is the precursor[664] of x. Even if such work was often incomplete in terms of its detailed enzymology, nevertheless it opened up new fields of intermediary metabolism.[665]

1. Associations of Abilities of Yeasts to Utilize Glycosides

a. β-D-Glucopyranosides.—A very simple example of this method of scrutiny is given by referring to the utilization of the β-D-glucopyranosides cellobiose and salicin. The results of testing the abilities of 496 strains to grow aerobically on these compounds is as follows.

		Salicin	
		−	+
Cellobiose	−	188	11
	+	9	288

This table shows that (*i*) 188 strains used neither substrate, and 288 used both cellobiose and salicin; (*ii*) 9 strains used cellobiose, but not salicin; (*iii*) 11 strains used salicin, but not cellobiose. Thus, with only 20 exceptions (4%), a yeast that utilizes salicin also utilizes cellobiose, and conversely. Provided that the β-D-glucosidases are equally accessible to the β-D-glucopyranosides, this result is to be

(664) A. M. Srb and N. H. Horowitz, *J. Biol. Chem.*, 154, 129–139 (1944).

(665) J. B. S. Haldane, "The Biochemistry of Genetics," Allen and Unwin, London, 1954.

expected in view of the insensitivity of β-D-glucosidases to the aglycon of their substrates (see Section IX,3). The 20 strains that provide exceptions have not yet been investigated further.

Another 132 such associations between pairs of substrates are given in Tables XVI and XVII. Results comparable to those for cellobiose and salicin are shown for two other interrelationships between β-D-glucopyranosides, namely (i) cellobiose and arbutin, and (ii) salicin and arbutin (see Table XVI, squares Gi and Gh). The associations of abilities of yeasts to utilize a number of other substrates are considered next.

b. β-D-Fructofuranosides and α-D-Glucopyranosides. — Investigations into the utilization of raffinose have usually shown it to be hydrolyzed initially, outside the plasmalemma, by a β-D-fructofuranosidase which also hydrolyzes sucrose (see Section IX). Hence, yeasts that utilize raffinose would be expected to utilize sucrose too, although there are a few exceptional yeasts having α-D-galactosidase activity, but no β-D-fructofuranosidase or α-D-glucosidase activity. Many yeasts might be expected to utilize sucrose by means of an α-D-glucosidase, but not to hydrolyze raffinose. The findings with the 497 strains investigated are consistent with these predictions, only two yeasts being reported able to utilize raffinose but not sucrose (see Table XVI, square Ad).

Assuming that sucrose and maltose are equally accessible to their hydrolytic enzymes, further analysis confirmed that yeasts unable to utilize raffinose may utilize sucrose by means of a maltose-hydrolyzing enzyme. Here, three substrates are treated simultaneously, namely, sucrose, raffinose, and maltose, which is not possible from the information given in Table XVI. Thus, yeasts that are [sucrose +, raffinose +] are divided into those that are maltose + or maltose −; yeasts that are [sucrose +, raffinose −] are similarly divided, and so on. Only four yeasts that were [sucrose +, raffinose −] were reported to be maltose −.

Substrate	Sucrose	+	+	−	−
	Raffinose	+	−	+	−
Maltose { +		164	127	2	23
{ −		29	4	0	147
Σ		193	131	2	170

Twenty-three strains may have possessed an α-D-glucosidase incapable of hydrolyzing sucrose, either because of (i) restricted substrate-specificity, or (ii) inaccessibility of the enzyme owing to the

TABLE XVI

Abilities of 497 Yeast Strains to Utilize Pairs of Substrates: Glycosides, D-Galactose, and L-Rhamnose[a]

		Sucrose − +	Maltose − +	Trehalose − +	Raffinose − +	Melezitose − +	Meα-D-Glc − +	Arbutin − +	Salicin − +	Cellobiose − +	Melibiose − +	Lactose − +	D-Galactose − +	Row
Maltose	−	147 33												b
	+	25 291												
Trehalose	−	106 59	113 52											c
	+	66 264	68 262											
Raffinose	−	170 132	152 150	111 191										d
	+	2 193	29 166	54 140										
Melezitose	−	170 121	181 110	158 133	192 100									e
	+	2 204	0 206	7 198	111 95									
Meα-D-Glc	−	165 76	180 62	138 102	182 60	224 18								f
	+	6 248	0 253	27 227	120 134	67 187								
Arbutin	−	98 77	104 70	120 55	116 59	161 14	138 37							g
	+	59 220	59 221	31 247	168 112	112 168	85 194							
Salicin	−	114 83	121 75	138 59	132 65	181 16	157 40	167 14						h
	+	58 240	60 239	27 270	171 128	111 188	85 213	8 265						
Cellobiose	−	115 83	123 75	136 63	136 63	181 18	155 44	167 17	188 11					i
	+	57 242	58 241	29 268	167 132	111 188	87 210	8 263	9 288					
Melibiose	−	168 248	176 240	160 256	298 119	272 141	227 189	163 230	181 235	181 236				j
	+	4 76	5 75	5 74	4 76	16 64	15 64	12 49	16 63	18 62				
Lactose	−	168 275	171 272	161 281	287 157	280 164	229 214	171 241	192 251	196 248	393 50			k
	+	3 50	10 43	4 49	15 38	11 42	12 40	3 39	5 47	3 50	23 30			
D-Galac- tose	−	130 106	126 111	136 101	177 60	186 51	160 76	111 108	131 106	129 108	234 2	235 2		l
	+	42 219	55 205	29 230	126 135	106 155	82 178	64 172	66 193	70 191	183 78	209 51		
L-Rham- nose	−	159 241	167 233	157 242	246 155	272 129	219 180	171 198	193 206	195 206	349 52	368 32	191 210	m
	+	12 84	14 82	8 88	56 40	19 77	22 74	3 82	3 93	3 93	67 28	75 21	46 50	
Column		A	B	C	D	E	F	G	H	I	J	K	L	

[a] Figures in each square do not always total 497, because some results are missing.

TABLE XVII

Abilities of 497 Yeast Strains to Utilize Pairs of Substrates: Pentoses, Alditols, D-Galactose, and L-*xylo*-Hexulose[a]

		D-Arabinose − +	L-Arabinose − +	Erythritol − +	Galactitol − +	D-Mannitol − +	Ribitol − +	D-Ribose − +	D-Glucitol − +	L-Sorbose − +	D-Xylose − +	Row
L-Arabinose	−	336 13										o
	+	96 51										
Erythritol	−	331 25	297 58									p
	+	101 39	52 88									
Galactitol	−	414 43	342 114	243 114								q
	+	18 21	7 32	13 26								
D-Mannitol	−	144 0	140 4	140 4	144 0							r
	+	288 64	209 142	216 136	313 39							
Ribitol	−	226 7	217 16	222 11	226 7	137 96						s
	+	206 57	132 130	134 129	231 32	7 256						
D-Ribose	−	334 9	294 48	308 35	332 11	141 202	233 117					t
	+	98 55	55 98	48 104	124 28	3 149	0 145					
D-Glucitol	−	140 0	137 3	138 2	140 0	133 7	136 4	137 3				u
	+	292 64	212 143	218 138	317 39	11 345	97 259	206 149				
L-Sorbose	−	283 23	246 59	230 75	300 5	135 170	191 114	236 69	133 172			v
	+	150 41	103 88	126 65	157 34	9 182	42 149	107 84	7 184			
D-Xylose	−	206 2	206 2	182 26	208 0	123 85	155 53	192 16	123 85	174 34		w
	+	227 62	143 145	174 114	249 39	21 267	78 210	151 137	17 271	132 157		
D-Galactose	−	224 13	211 26	194 43	237 0	109 128	167 70	200 37	110 127	206 31	141 96	l
	+	209 51	138 121	162 97	220 39	35 224	66 193	143 116	30 229	100 160	67 193	
Column		N	O	P	Q	R	S	T	U	V	W	

[a] Figures in each square do not always total 497, because some results are missing.

presence of a permeability barrier. Twenty-nine yeasts presumably had an operative β-D-fructofuranosidase, but no accessible α-D-glucosidase. Of the yeasts that utilized sucrose (193 + 131 = 324), 193 strains, that is 60%, used raffinose too. It may be inferred that each of these had an operative β-D-fructofuranosidase. Conversely, 40% of these yeasts probably hydrolyzed sucrose by means of an α-D-glucosidase.

The results shown next are consistent with the view that enzymes hydrolyzing melezitose also split sucrose and maltose. With only two exceptions, yeasts using melezitose also used sucrose and maltose. All the strains that utilized melezitose also used maltose (see also, Table XVI,Be).

Substrate	Sucrose	+	+	–	–
	Maltose	+	–	+	–
Melezitose {	+	204	0	2	0
	–	87	33	23	147

The marked association between melezitose and α,α-trehalose utilizations (see Table XVI, square Ce) was unexpected. With only seven (1.4%) exceptions, yeasts that used melezitose were also reported to use α,α-trehalose. No such association is apparent between α,α-trehalose and maltose (see Table XVI, square Bc). These findings suggest that, in this group of yeasts, melezitose-hydrolyzing enzymes also hydrolyze α,α-trehalose, although this conclusion is not borne out by the experimental work described in Section IX. Either, many yeasts differ in this respect from those so far examined in detail, or some other interpretation of this association must be found.

2. Associations of Abilities to Utilize Pentoses and Alditols

a. D-Ribose and Ribitol. — The most likely routes for D-ribose and ribitol catabolism by yeasts are as follows (see Section X).

A   D-Ribose → ribitol → D-*erythro*-pentulose →
          D-*erythro*-pentulose 5-phosphate → pentose-cycle reactions

B   Ribitol → D-ribose → D-ribose 5-phosphate →
                                             pentose-cycle reactions

C   a Ribitol → D-*erythro*-pentulose, and so on, and
     b D-Ribose → D-ribose 5-phosphate, and so on.

Were (*i*) scheme A invariably true and (*ii*) D-ribose and ribitol

equally accessible to the relevant enzymic system,[665a] yeasts able to utilize D-ribose should also use ribitol. The converse would not be true, as D-ribose reductase might be missing and, in such cases, ribitol could be utilized, although not D-ribose. The figures in Table XVII (square St) are consistent with scheme A. The extreme rarity of yeasts that utilize D-ribose but not ribitol for aerobic growth is inexplicable in terms of either schemes B or C. Furthermore, the utilization of D-ribose is highly associated with that of D-glucitol and D-mannitol (see Table XVII, squares Tu and Rt). This finding indicates that D-ribose catabolism involves alditol dehydrogenase activity. The further fact that most of the yeasts utilizing D-glucitol also use D-mannitol, and *vice versa,* (see Table XVII, square Ru) suggests that the first step in the catabolism of D-ribose, D-mannitol, and D-glucitol may be mediated by the same aldose reductase.

b. D-Xylose and L-Arabinose. — Again, yeasts that utilize L-arabinose and not D-xylose are rare[660,666] (see Table XVII, square Ow); this is explicable if (*i*) the catabolic routes are as follows (see Section X):

L-Arabinose → L-arabinitol → L-*threo*-pentulose → xylitol →
 D-*threo*-pentulose → D-*threo*-pentulose 5-phosphate →
 pentose-cycle reactions

D-Xylose → xylitol, and so on;
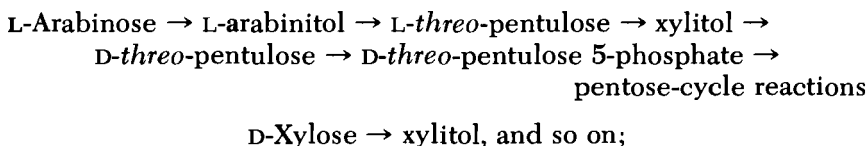
(*ii*) both initial reductions are due to the same enzyme; and (*iii*) both substrates are equally accessible to the enzymic system.[665a] If, contrary to this scheme, many yeasts were to break down D-xylose after an initial phosphorylation, or following isomerization (see Section X), the results in Table XVII, square Ow would not be expected.

Further inferences may be made from the associations between other pairs of substrates; Krzanowski and Barnett[667] discussed the interpretation and handling of the numbers in these tables of association. It should be borne in mind that interpretation of some associations in terms of catabolic routes may prove quite incorrect. Rather, the associations might result from close genetic linkage; this is likely to apply to the associations between D-galactose and lactose or melibiose (see Table XVI, squares Kl and Jl).

(665a) An alternative condition could be that ribitol (not D-ribose) is always free to enter the yeasts, perhaps on the D-glucose carrier (A. P. Sims, personal communication). This might also apply to D-xylose.
(666) S. Windisch, *Brauwissenschaft*, 10, 203–207 (1948).
(667) W. J. Krzanowski and J. A. Barnett, in preparation.

These speculations depend on the fact that there is a restricted number of enzymes and, consequently, of catabolic routes. The approach outlined in this Section shows that it may be practicable to know (*i*) what is happening in hundreds of species, and (*ii*) which yeasts are exceptional and so of particular interest for study. The speculations must be subjected to verification in the laboratory by the examination of selected yeasts, and will only prove useful if they provide a basis for experimental investigation of the utilization of sugars by yeasts.

XIII. ADDENDUM*

1. Cell Walls

An autoradiographic study[668] of the pattern of incorporation of D-[1-³H]mannose into the mannan of growing *Saccharomyces cerevisiae* has shown that (*i*) whilst the small new bud is spherical, new D-mannan is formed uniformly over the whole surface of the bud; (*ii*) this phase is followed by polarized growth at the tip of the bud; and (*iii*), finally, there is further formation of D-mannan over the whole bud. The ellipsoidal shape of the adult cell is a result of the polarized tip-growth combined with the nonpolarized (spherical) growth. The wall of the mother cell remains largely unlabelled. Once it is inserted into the wall,[669] there is no turnover of D-mannan, although about 10% of the D-mannan formed by the cells is found in the growth medium. This D-mannan may be liberated because (*a*) the growing cell-walls fail to trap all that is formed, or (*b*) it has a nonstructural role.[669] The arrangement of the side chains in the D-mannan of *Saccharomyces cerevisiae*,[670] and immunological characteristics of the mannans of *Saccharomyces*[671,672] and *Kluyveromyces*,[672] have been studied, as has the incorporation of 2-

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* With the receipt of the galley proofs, the opportunity was taken to outline some additional work and to make certain reappraisals.

(668) V. Farkaš, J. Kovařík, A. Košinová, and Š. Bauer, *J. Bacteriol.*, 117, 265–269 (1974).
(669) Z. Krátký, P. Biely, and Š. Bauer, *Biochim. Biophys. Acta*, 404, 1–6 (1975).
(670) L. Rosenfeld and C. E. Ballou, *Biochem. Biophys. Res. Commun.*, 63, 571–579 (1975).
(671) J. Šandula and A. Vojtková-Lepšíková, *Folia Microbiol.* (Prague), 19, 94–101 (1974).
(672) J. Šandula, A. Kocková-Kratochvílová, and D. Šikl, *J. Gen. Microbiol.*, 83, 339–347 (1974).

acetamido-2-deoxy-D-glucose into the side chains of the D-mannan of *Kluyveromyces lactis*.[673]

The formation of cell-wall D-glucan has been examined[674] in *Saccharomyces cerevisiae* and also, the β-D-glucanases associated with the cell walls of *Schizosaccharomyces versatilis*,[675] *Pichia polymorpha*,[676,677] *Cryptococcus albidus*, and *Candida utilis*.[677] Galactomannan, α-D-glucan, and β-D-glucan of the cell wall of *Schizosaccharomyces pombe* have also been investigated.[678] The polysaccharides of cell walls of four species of *Cryptococcus* differ from those of four species of *Rhodotorula*.[679] Those of the *Cryptococci* are mainly of the α-D-(1 → 3)-linked D-glucan; the *Rhodotorulae* contain glucomannans having D-glucose and D-mannose residues in the ratio of ∼1:3.

A particularly interesting publication has appeared[680] on the inhibition of biosyntheses of cell-wall polysaccharide and glycoprotein in *Saccharomyces cerevisiae* by 2-deoxy-D-*arabino*-hexose. In addition to its specific effects, it lowers production of energy by the cells, and interferes with the control of enzyme synthesis. 2-Deoxy-D-*arabino*-hexose is incorporated into the wall D-glucan, but the extent of both the incorporation and the inhibition of the formation of D-glucan and D-mannan depends on whether D-glucose or D-mannose is the main source of carbon in the growth medium. During growth on D-glucose, 2-deoxy-D-*arabino*-hexose inhibits the formation of D-mannan more than of D-glucan, whereas D-glucan formation is suppressed in a D-mannose medium. The composition of the walls depends on the carbon source, and is probably affected by the direction of the interconversion of D-glucose 6-phosphate and D-mannose 6-phosphate (which is influenced by the supply of exogenous hexose).

Work on microbial polysaccharides was reviewed in Ref. 681.

(673) W. L. Smith, T. Nakajima, and C. E. Ballou, *J. Biol. Chem.*, 250, 3426–3435 (1975).

(674) R. Sentandreu, M. V. Elorza, and J. R. Villanueva, *J. Gen. Microbiol.*, 90, 13–20 (1975).

(675) G. H. Fleet and H. J. Phaff, *J. Biol. Chem.*, 249, 1717–1728 (1974).

(676) T. G. Villa, V. Notario, and J. R. Villanueva, *Arch. Microbiol.*, 104, 201–206 (1975).

(677) J. R. Villanueva, V. Notario, T. Santos, and T. G. Villa, *Abstr. Intern. Symp. Yeast Other Protoplasts, 4th*, 18 (1975).

(678) D. A. Bush, M. Horisberger, I. Horman, and P. Wursch, *J. Gen. Microbiol.*, 81, 199–206 (1974).

(679) N. P. Elinov, G. A. Vitovskaya, V. G. Kaloshin, and T. M. Kolotinskaya, *Biokhimiya*, 39, 787–792 (1974).

(680) Z. Krátký, P. Biely, and Š. Bauer, *Eur. J. Biochem.*, 54, 459–467 (1975).

(681) R. J. Sturgeon, *Carbohydr. Chem.*, 7, 253–293 (1975).

2. Storage Carbohydrates

In Section IX,2, some mention was made of the role of α,α-trehalose as a storage carbohydrate in yeasts. Studies on its metabolic control have shown that extracts of bakers' yeast can be separated into two protein fractions, (i) neither of which can split α,α-trehalose, (ii) but, recombined and incubated with $Mg^{2\oplus}$, ATP, and adenosine 3′,5′-monophosphate (cAMP), they form active α,α-trehalase.[682] Work on the other major carbohydrate-reserve in yeasts, namely, glycogen,[683] was reviewed in Ref. 513; later work has been described on the regulation of glycogen synthesis by *Saccharomyces cerevisiae*[684,685] and *Candida tropicalis*.[686]

3. Sugar Transport

A study of uptake of L-sorbose by *Saccharomyces cerevisiae* has provided evidence of some compartmentation within the cells,[687] the accessibility of the compartment to L-sorbose being under metabolic control. Work on the effects of change of temperature on the entry of monosaccharides into *Rhodotorula glutinis (gracilis)*[212,213] has been extended[688]: for both the transport of D-xylose into this yeast and of D-ribose into *Pichia pinus*,[209] there is a "break" in the Arrhenius plot (see Fig. 2). Uptake of three pentitols and three hexitols by an unspecified strain of *Saccharomyces cerevisiae*,[689] a yeast that does not utilize alditols, may possibly occur by simple diffusion, transport being insensitive to changes in temperature, pH, and the presence of 2,4-dinitrophenol or of uranyl ions; or, alternatively, apparent transport may result from adsorption of the alditols on the surfaces of the yeast cells. On the other hand, the entry of erythritol was affected by changes in temperature.

(682) P. van Solingen and J. B. van der Plaat, *Biochem. Biophys. Res. Commun.*, **62**, 553–560 (1975).

(683) Dr H.-J. Vohmann kindly drew attention to the lack of mention of glycogen as a reserve.

(684) L. B. Rothman and E. Cabib, *Biochemistry*, **8**, 3332–3341 (1969).

(685) E. Cabib, L. B. Rothman-Denes, and K. Huang, *Ann. N. Y. Acad. Sci.*, **210**, 192–206 (1973).

(686) O. Käppeli, H. Aeschbach, H. Schneider, and A. Fiechter, *Eur. J. Appl. Microbiol.*, **1**, 199–211 (1975).

(687) E. Spoerl, S. H. Benedict, S. N. Lowery, J. P. Williams, and J. P. Zahand, *J. Membr. Biol.*, **20**, 319–340 (1975).

(688) K. B. Heller and M. Höfer, *J. Membr. Biol.*, **21**, 261–271 (1975).

(689) D. S. Canh, J. Horák, A. Kotyk, and L. Říhová, *Folia Microbiol.* (Prague), **20**, 320–325 (1975).

An interesting paper has appeared on the uptake of 2-deoxy-D-*arabino*-hexose by an unspecified strain of *Kluyveromyces* (*Saccharomyces*) *fragilis*.[690] This publication constitutes a defence and extension of the view[163,170-175] that phosphorylation is associated with the active transport of sugars into yeasts (see Section VI,3 and 4). From pulse-labelling experiments, the authors concluded[690] that such group translocation occurs with the entry of 2-deoxy-D-*arabino*-hexose into *Kluyveromyces fragilis*. The yeast was pre-incubated with unlabelled sugar, and then a pulse was given of negligble amounts of 2-deoxy-D-[¹⁴C]*arabino*-hexose of high specific activity. Examination of extracts from the cells indicated that the specific activity of the intracellular 2-deoxy-D-*arabino*-hexose 6-phosphate increased faster than that of the unphosphorylated sugar. The authors considered that deviations from these results obtained by them[690] and others (see, for example, Ref. 167) resulted from the artifact of adsorption of sugar on the filters which were analyzed with the yeast. This adsorption made it seem that, initially, the specific activity of the free sugar increased faster than that of the phosphorylated form. Be that as it may, this new work does not explain why L-sorbose is taken up by *Saccharomyces cerevisiae* in competition with D-glucose,[691] but is apparently not phosphorylated. Nor is it explained why galactokinaseless *Saccharomyces cerevisiae* neither metabolizes D-galactose by transport-associated phosphorylation nor accumulates D-galactose by an active process.[199] The work by Jaspers and van Steveninck[690] draws attention to more than one critical, methodological problem of studying the uptake of sugars by yeasts. For example, these workers reported[690] that there is little difference in uptake between yeast under aerobic from that under anaerobic conditions. Yet they were using yeast at 10% wet weight/volume, that is, about 20 mg dry weight of yeast/ml of suspension. No evidence was given of the possibility of an aerobic incubation under these conditions, which would probably be largely anaerobic with actively metabolizing yeast. Furthermore, in common with much work on sugar transport by yeasts (see, for example, Refs. 150, 163, 165-169, 173-175, 198, 199, 203, 205, 206, and 688), the study of Jaspers and van Steveninck[690] was made with yeast harvested at an unspecified phase of growth and then thoroughly washed. Sugar uptake may vary markedly with the growth phase,[692] and the rate of uptake of 2-deoxy-

(690) H. T. A. Jaspers and J. van Steveninck, *Biochim. Biophys. Acta*, **406**, 370–385 (1975).
(691) J. van Steveninck, *Biochim. Biophys. Acta*, **150**, 424–434 (1968).
(692) R. Serrano and G. DelaFuente, *Mol. Cell. Biochem.*, **5**, 161–171 (1974).

D-*arabino*-hexose by another yeast, namely, *Pichia pinus*, falls exponentially in starving yeast, immediately after washing.[693] In other species, too, the characteristics of transport of D-glucose appear to change with the starvation of yeast initially growing exponentially.[693] These changes may be accounted for by (*i*) an initial de-inhibition of uptake, (*ii*) loss of supply of energy, and (*iii*) modification and destruction of the carrier.

4. Hexoses

Respiration of *Saccharomyces cerevisiae*, but not of *Saccharomyces rouxii* (which tolerates high concentrations of sugar, see Section I), is repressed in a medium having a water activity of 0.95, due to the presence of poly(ethylene glycol), and containing 24 or 36% (w/v) of D-glucose.[694] A mutant of *Saccharomyces rouxii* grows optimally on D-glucose in the presence of 60% of sucrose,[695] at which concentration it grows twice as fast as in 40% of sucrose. A mutant of *Saccharomyces uvarum* (*carlsbergensis*) has been isolated[696] that grows normally on D-galactose, ethanol, or glycerol, but not on D-glucose, D-fructose, D-mannose, or sucrose, probably because hexosediphosphatase (EC 3.1.3.11) is not inactivated. During D-glucose–ethanol diauxic growth of *Saccharomyces cerevisiae* (see Section VII,4), the glyoxylate cycle[131] operates only after the D-glucose disappears.[697] 6-Phosphofructokinase of bakers' yeast is activated by low, and inhibited by high, concentrations of D-fructose[698]; and, for *Saccharomyces uvarum* (*carlsbergensis*), a separate catabolic route for D-fructose might partly bypass the control step at 6-phosphofructokinase.[699] A study has been made[700] of hexokinase and glucokinase of a strain of *Rhodotorula glutinis*.

On D-galactose catabolism, work has been published on the role of

(693) J. A. Barnett and A. P. Sims, in preparation.
(694) A. D. Brown, *J. Gen. Microbiol.*, 86, 241–249 (1975).
(695) T. Y. Koh, *J. Gen. Microbiol.*, 88, 101–114 (1975).
(696) K. W. van de Poll, A. Kerkenaar, and D. H. J. Schamhart, *J. Bacteriol.*, 117, 965–970 (1974).
(697) S. Haarasilta and E. Oura, *Eur. J. Biochem.*, 52, 1–7 (1975).
(698) A. Betz, U. Röttger, and K. H. Kreuzberg, *Arch. Microbiol.*, 103, 123–126 (1975).
(699) A. Betz and J. U. Becker, *Physiol. Plant.*, 33, 285–289 (1975).
(700) M. J. Mazón, J. M. Gancedo, and C. Gancedo, *Arch. Biochem. Biophys.*, 167, 452–457 (1975).

the *GAL3* (Ref. 701) and *GAL4* (Refs. 701 and 702) loci of *Saccharomyces cerevisiae* (see Table IX). UDP-D-glucose 4-epimerase (Table VIII) may have a regulatory role in the Leloir pathway (see Fig. 8), giving classical, hyperbolic kinetics with UDP-D-galactose, but allostericity with UDP-D-glucose.[703] This enzyme has a low rate of activity at low concentrations of UDP-D-glucose but, under these conditions, D-glucose 6-phosphate is a strong activator.

5. Glycosides

Partly purified β-D-fructofuranosidase of a strain of *Kluyveromyces (Saccharomyces) fragilis* hydrolyzes sucrose and raffinose with a pH optimum of 4.2, but of 5.2 for inulin.[704] Furthermore, the hydrolysis of sucrose was found to be competitively inhibited by raffinose, but not by inulin, so two enzymes may be responsible for the β-D-fructofuranosidase activity of this yeast.

MAL2, a positive regulatory gene for the synthesis of α-D-glucosidases by *Saccharomyces cerevisiae* (see Table IX), forms a protein which, in combination with maltose (or a metabolic derivative thereof), stimulates synthesis of α-D-glucosidase.[705] A non-Mendelian character has been described[706] that affects the utilization of maltose by *Saccharomyces cerevisiae*. Studies have been made[707] on the synthetic reactions of two purified enzymes of *Saccharomyces bayanus (oviformis)* having α-D-glucosidase activities (see Table XI). These enzymes release the α anomer of D-glucose from *p*-nitrophenyl α-D-glucopyranoside, and use the α (but not the β) anomer for the synthesis of disaccharides. However, competitive inhibition by D-glucose is independent of its anomeric form. For *Saccharomyces uvarum (logos)*, the carbohydrate moiety of purified α-D-glucosidase[470] contains D-mannose and D-galactose[708] in the ratio of 2 : 1.

(701) S. Tsuyumu and B. G. Adams, *Genetics*, **77**, 491–505 (1974).

(702) A. J. S. Klar and H. O. Halvorson, *Mol. Gen. Genet.*, **135**, 203–212 (1974).

(703) M. Ray and A. Bhaduri, *J. Biol. Chem.*, **250**, 4373–4375 (1975).

(704) M. Takahashi and S. Soutome, *Utsunomiya Daigaku Nogakubu Gakujutsu Hokoku*, 9, 95–98 (1975); *Chem. Abstr.*, **83**, 39,304r (1975).

(705) F. K. Zimmermann and N. R. Eaton, *Mol. Gen. Genet.*, **134**, 261–272 (1974).

(706) D. H. J. Schamhart, A. M. A. ten Berge, and K. W. van de Poll, *J. Bacteriol.*, **121**, 747–752 (1975).

(707) H. L. Lai and B. Axelrod, *Biochim. Biophys. Acta*, **391**, 121–128 (1975).

(708) T. Saeki, S. Chiba, and T. Shimomura, *Agr. Biol. Chem.* (Tokyo), **39**, 551–552 (1975).

6. Alditols

There is an inducible catabolic route for pentitols in *Rhodotorula glutinis (gracilis)*.[709] A further study has been published[710] on the production of erythritol and D-mannitol by *n*-alkane-grown *Candida zeylanoides*.

(709) M. Höfer, P. Dahle, and R. Klöppel, *Biochem. Soc. Trans.*, 3, 1083–1084 (1975).
(710) K. Hattori and T. Suzuki, *Agr. Biol. Chem.* (Tokyo), 39, 57–61 (1975).

NONCYTOTOXIC, ANTITUMOR POLYSACCHARIDES

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I. Introduction

Interest in host-mediated, antitumor action of noncytotoxic polysaccharides stemmed from dissatisfaction with current cancer chemotherapy experiments. In these experiments, countless numbers of chemical compounds are being tested for their tumoricidal activities, with the aim of attaining a total kill of cancer cells. It has been the experience of clinicians, as well as of laboratory experimenters, however, that successful total kill of cancer cells is apt to include the host too. Hence, the enhancement or potentiation of host resistance emerged as a possible means of inhibiting tumor growth without

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harming the host. Starting from this point of view, extensive studies have been made on noncytotoxic and host-mediated antitumor polysaccharides extracted from different botanical sources. These studies are still in progress in many laboratories, and the role of the polysaccharides as immunopotentiators is being especially debated.

The purpose of the present article is to summarize the available information in this area and to indicate the present status of the problem.

II. Bacterial Polysaccharides

It has been known for more than a century that human malignant growths sometimes undergo regression following an acute, bacterial infection.[1-7] The earliest antitumor polysaccharide investigated was isolated in 1943 from *Serratia marcescens* and became known as Shear's polysaccharide.[8] Upon intraperitoneal (i.p.) injection, the polysaccharide caused extensive hemorrhage to Sarcoma 37 tumors in mice. Although the tumors became largely necrotized, the surviving tumor cells eventually caused the deaths of most of the animals. Due to the toxicity and other side-effects of the polysaccharide, clinical tests were not performed.[9,10] Further work showed that biological properties of the polysaccharide differed for preparations from different strains of organisms.[11,12]

The original, Shear preparation obtained from *S. marcescens*, grown on a D-glucose medium, was composed of aldohexose, 6-deoxyhexose, hexosamine, and phospholipids.[13,14] Creech and coworkers[15,16]

(1) N. N. Busch, *Berlin. Klin. Wochschr.*, 5, 137–139 (1868).

(2) N. N. Fehleisen, *Deut. Med. Wochschr.*, 8, 553–554 (1882).

(3) C. H. H. Spronck, *Ann. Inst. Pasteur*, 6, 683–707 (1892).

(4) S. P. Beebe and M. Tracy, *J. Amer. Med. Assoc.*, 49, 1493–1498 (1907).

(5) A. Gratia and R. Linz, *Compt. Rend. Soc. Biol.*, 108, 427–435 (1931).

(6) W. B. Coley, *Ann. Surg.*, 97, 434–460 (1933).

(7) H. C. Nauts, G. A. Fowler, and F. H. Bogatko, *Acta Med. Scand.*, 145, Suppl. 276, 1–103 (1953).

(8) M. J. Shear and F. C. Turner, *J. Nat. Cancer Inst.*, 4, 81–97 (1943).

(9) H. J. Creech, M. A. Hamilton, E. T. Nishimura, and R. F. Hankwitz, Jr., *Cancer Res.*, 8, 330–336 (1948).

(10) H. J. Creech, M. A. Hamilton, I. C. Diller, E. T. Nishimura, and M. J. Shear, *Cancer Res.*, 7, 716 (1947).

(11) A. Perrault and M. J. Shear, *Cancer Res.*, 7, 714–715 (1947).

(12) H. J. Creech, M. A. Hamilton, and I. C. Diller, *Cancer. Res.*, 8, 318–329 (1948).

(13) J. L. Hartwell, M. J. Shear, and J. R. Adams, Jr., *J. Nat. Cancer Inst.*, 4, 107–122 (1943).

(14) H. Kahler, M. J. Shear, and J. L. Hartwell, *J. Nat. Cancer Inst.*, 4, 123–129 (1943).

found considerable variation in the nitrogen, phosphorus, and carbohydrate composition of *S. marcescens* polysaccharides, depending upon the strain, the composition of the medium, and the duration of culture growth. Fractionation of Shear's polysaccharide with hot picric acid led to the isolation of a glucan and of a polysaccharide containing 2-amino-2-deoxy-D-glucose,[17] but no biological activity was present. It was suggested that the active polysaccharide preparation consisted of a polysaccharide–lipid complex.[18]

Adams and coworkers[19] fractionated a polysaccharide–lipid complex obtained from *S. marcescens* grown in a D-glucose medium. A lipopolysaccharide obtained on extraction with aqueous phenol could be further fractionated by ultracentrifugation and complexing with Cetavlon. The fractions differed in their sugar compositions and antitumor activity against Sarcoma 37 in mice (see Table I). The fraction that contained D-glucose, D-mannose, and 2-amino-2-deoxy-D-glucose and had high solubility in dilute sodium chloride and in aqueous acetone was active even at 40–80 μg/mouse. Its content of 2-amino-2-deoxy-D-glucose and its solubility properties suggested that it contained lipid. Creech and coworkers[15,16] isolated from *Serratia marcescens* a polysaccharide preparation, containing D-glucose and 2-amino-2-deoxy-D-glucose, that was active at 40–90 μg/mouse, but that became much less active on alkali treatment. Srivastava and Adams[20] examined the structure of similar fractions, and concluded that the polysaccharides were composed of a main chain of D-glucosyl and D-mannosyl residues joined together by $(1 \rightarrow 3)$-α-D-glycosidic bonds, with some D-glycosyl residues carrying branches at O-2 and O-4 that terminated in either D-glucosyl or D-mannosyluronic acid groups.

Adams and coworkers[21] also examined the chemical composition and antitumor activity of polysaccharide fractions obtained from the Temple University strain of *S. marcescens* grown in a medium containing sucrose. Some fractions were obtained from bacterial cells by repeated extraction with aqueous phenol, followed by digestion with

(15) H. J. Creech, L. H. Kohler, H. F. Havas, R. L. Peck, and J. Andre, *Cancer Res.*, 14, 817–823 (1954).

(16) H. J. Creech and R. F. Hankwitz, Jr., *Cancer Res.*, 14, 824–829 (1954).

(17) P. Rathgeb and B. Sylvén, *J. Nat. Cancer Inst.*, 14, 1099–1108 (1954).

(18) P. Rathgeb and B. Sylvén, *J. Nat. Cancer Inst.*, 14, 1109–1117 (1954).

(19) H. C. Srivastava, E. Breuninger, H. J. Creech, and G. A. Adams, *Can. J. Biochem. Physiol.*, 40, 905–918 (1962).

(20) H. C. Srivastava and G. A. Adams, *Can. J. Biochem.*, 40, 1415–1425 (1962).

(21) H. J. Creech, E. R. Breuninger, and G. A. Adams, *Can. J. Biochem.*, 42, 593–603 (1964).

TABLE I

Composition and Antitumor Activity[a] of Some Fractions from *Serratia marcescens*[19]

Composition	N (%)	Uronic acid (%)	Dose (μg/mouse)	Tumor area (cm², on day 15)	Mice with complete regression (% on day 45)
Control			none	4.6	4
Glc, Man, GlcN	0.51		40–80	1.4	64
Glc, Man	0.25	36.6	400–900	1.3	61
Glc, Man		6.2	2,400–6,000	0.7	73
Glc, Man, Gal, GlcN			1,200–4,000	2.2	46

[a] Sarcoma 37 tumors were implanted subcutaneously in mice, and only those mice that developed a tumor of 1.4–1.6 cm² in area in 8–10 days were used for tests. Various fractions were administered intraperitoneally in a single dose.

trypsin and ultracentrifugation, and one fraction was isolated from defatted, bacterial cells. The fractions extracted by phenol contained D-glucose, L-rhamnose, heptose, and 2-amino-2-deoxy-D-glucose, but although almost free from lipid, they were contaminated with nucleic acid; they were active at 40–80 and 100–200 μg/mouse, respectively, whereas the fraction isolated from defatted cells, containing D-mannose, D-glucose, and a small proportion of 2-amino-2-deoxy-D-glucose, only had comparable activity at a dose of 200–400 μg/mouse. As simple extraction with chloroform–methanol completely removed the lipids, the authors inferred that the lipids were not covalently bound to the polysaccharide. Infrared spectra, resistance to enzymic hydrolysis, and the results of periodate oxidation suggested that the principal, interlinking bonds were (1 → 3)-α-D-glycosidic with, perhaps, a few (1 → 6) bonds. Thus, the fractions isolated by Creech and coworkers[15,16,21] and Srivastava and coworkers[20] differed chemically, although the fractions were antitumor active in similar test systems.

Serratia marcescens polysaccharide has been reported to be active against a number of other tumors, such as Sarcoma 180 (Ref. 22), Ehrlich carcinoma,[22] Guerin carcinoma,[23] Sarcoma M-1 (Ref. 23), and Walker carcinosarcoma.[24] Navashin and coworkers[25] found that

(22) Z. V. Ermoléva, G. E. Vaisberg, A. I. Braude, I. V. Ravich, T. V. Golosova, and N. A. Pasternak, *Antibiotiki*, 10, 134–137 (1965); *Chem. Abstr.*, 62, 16,814 (1965).
(23) I. G. Veksler, *Antibiotiki*, 13, 72–77 (1968); *Chem. Abstr.*, 68, 67,578 (1968).
(24) S. M. Navashin, I. P. Fomina, and T. G. Terenteva, *Dokl. Akad. Nauk SSSR*, 158, 981–983 (1964); *Chem. Abstr.*, 62, 3,283 (1965).

animals developed resistance to this polysaccharide, and that continued administration was then without effect. However, if the polysaccharide was given periodically, an increase in activity and a diminution in toxicity were observed. Nishimura and Baum[26,27] reported that S. marcescens polysaccharide produced inhibition of Ehrlich ascites carcinoma in Strong A mice when administered intraperitoneally (30 μg, three times daily), whereas administration of 20 μg of the polysaccharide every 5 hours had no effect on tumor growth. Sugiura[28] reported inhibition of Ehrlich ascites carcinoma with Shear's preparation on daily i.p. administration; however, no effect on solid Ehrlich tumor was observed. Belkin and coworkers[29] noticed a marked, cellular swelling and cell vacuolization of Sarcoma 37 ascites cells in CAF mice by i.p. injection of S. marcescens polysaccharide in a dose range of 1–2 μg/g. The polysaccharide also showed a marked effect on Toshida ascites sarcoma in Marshall-520 rats at a dose of 5–8 μg/g, whereas it showed no effect on various other ascites tumors, such as Sarcoma 180, Ehrlich carcinoma, Krebs-2 carcinoma (all three in CAF albino mice), Leukemia L1210, and a lymphocytic neoplasm P-288 (both in CDBA mice).[29] The differences in composition and test systems employed may explain the various activities of different S. marcescens polysaccharide preparations. Murphy–Sturm lymphosarcoma in rats has also shown regression due to S. marcescens polysaccharide.[30] Prior conditioning of CAF mice with 200 μg of Bordetella pertussis or S. marcescens lipopolysaccharide per mouse inhibited the transplantation of Sarcoma 180 ascites tumors in mice.[31] Polysaccharides from Pseudomonas pseudomalle were active when administered before or after implantation, or with tumor cells.[32]

Polysaccharide complexes from Escherichia coli,[33–37] Staphylo-

(25) S. M. Navashin, I. P. Fomina, and T. G. Terenteva, Antibiotiki, 10, 1011–1017 (1965); Chem. Abstr., 64, 10,254 (1966).

(26) E. T. Nishimura and J. H. Baum, A. M. A. Arch. Pathol., 63, 352–366 (1957).

(27) E. T. Nishimura and J. H. Baum, A. M. A. Arch. Pathol., 63, 513–522 (1957).

(28) K. Sugiura, Cancer Res., 13, 431–441 (1953).

(29) M. Belkin, W. G. Hardy, A. Perrault, and H. Sato, Cancer Res., 19, 1050–1062 (1959).

(30) M. de Los Angeles Contreras, U. S. At. Energy Comm., UR-664 (1965); Chem. Abstr., 63, 10,547 (1965).

(31) S. Malkiel and B. J. Harris, Cancer Res., 21, 1461–1464 (1961).

(32) E. P. Efimtseva, Y. V. Galaev, E. M. Gubarev, and V. I. Efremenko, Antiobiotiki, 12, 375–380 (1967); Chem. Abstr., 67, 31,274 (1967).

(33) M. Ikawa, J. B. Koepfli, S. G. Mudd, and C. Niemann, J. Nat. Cancer Inst., 13, 157–166 (1952).

coccus aureus,[38-41] *Streptococcus*,[42-48] *Klebsiella*,[49,50] *Pseudomonas*,[51-56] *Acetobacter xylinum*,[57,58] *Proteus vulgaris*,[59] *Bacillus subtilis*,[60] *Bacillus brevis*,[61] *Salmonella typhimurium*,[62] *Salmonella enteritides*,[63] *Brucella abortus*,[64] *Shigella paradysen-*

(34) M. Ikawa, J. B. Koepfli, S. G. Mudd, and C. Niemann, *J. Nat. Cancer Inst.*, 14, 1195-1201 (1954).

(35) M. Ikawa, J. B. Koepfli, S. G. Mudd, and C. Niemann, *J. Amer. Chem. Soc.*, 75, 1035-1038 (1953).

(36) P. Klarner, Z. *Krebsforsch.*, 62, 291-296 (1958).

(37) I. I. Maiko, M. B. Kaganskaya, O. Ya. Rashba, M. O. Shvaiger, T. P. Mandrik, and O. S. Zherebilo, *Mikrobiol. Zh.* (Kiev), 33, 548-552 (1971); *Chem. Abstr.*, 76, 42,007 (1972).

(38) E. Mihich and E. Neter, *Proc. Soc. Exp. Biol. Med.*, 106, 97-101 (1961).

(39) U. Iwao, *J. Antibiot.* (Tokyo), *Ser. B*, 22, 287 (1969).

(40) I. Umezawa, *J. Antibiot.* (Tokyo), *Ser. B*, 23, 287-297 (1970).

(41) E. Mihich, O. Westphal, O. Lüderitz, and E. Neter, *Proc. Soc. Exp. Biol. Med.*, 107, 816-819 (1961).

(42) J. Mikolasek, *Neoplasma*, 19, 507-518 (1972).

(43) M. Yoshimura, M. Akiyama, and S. Kigoshi, *Kanazawa Daigaku Gan Ken Kyusho Nepo*, 3, 200-211 (1970); *Chem. Abstr.*, 73, 43,830 (1970).

(44) W. Koehler, O. Prokop, M. Von Ardenne, and O. Kuehnemund, *Ger. Pat.* 2,039,637 (1971); *Chem Abstr.*, 75, 67,468 (1971).

(45) H. Schmitz, W. T. Bradner, A. Gourevitch, B. Heinemann, K. E. Price, J. Cein, and I. R. Hooper, *Cancer Res.*, 22, 163-166 (1962).

(46) S. Koshimura, S. Shoin, and H. Okamoto, *Jap. Pat.* 34,754 (1971); *Chem. Abstr.*, 76, 23,833 (1972).

(47) H. Okamoto, *Fr. Pat.* 2,003,018 (1971); *Chem. Abstr.*, 72, 118,788 (1970).

(48) H. Okamoto, S. Shoin, and S. Koshimura, *Ger. Pat.* 2,043,971 (1971); *Chem. Abstr.*, 75, 150,319 (1971).

(49) J. R. Crook, W. K. Otto, and R. S. Jones, *Proc. Soc. Exp. Biol. Med.*, 109, 552-556 (1962).

(50) S. Tomioka, S. Hata, T. Oishi, M. Naiki, and K. Wakabayashi, *Jap. Pat.* 71 18,597 (1971); *Chem. Abstr.*, 75, 74,902 (1971).

(51) M. O. Shvaiger, T. P. Mandrik, M. B. Kaganskaya, O. Ya. Rashba, and L. S. Kunitskaya, *Mikrobiol. Zh.* (Kiev), 32, 105-110 (1970); *Chem Abstr.*, 73, 33,758 (1970).

(52) K. Aoyama, F. Miyazawa, H. Kurisu, S. Hatta, H. Arai, Y. Fujita, M. Urabe, Y. Sakai, and T. Aoki, *Eisei Shikensho Hokoku*, 74, 361-374 (1956); *Chem. Abstr.*, 51, 8,198 (1957).

(53) G. M. Fukui, J. Y. Homma, and C. Abe, *Jap. J. Exp. Med.*, 41, 489-492 (1971).

(54) J. Y. Homma and C. Abe, *Jap. J. Exp. Med.*, 42, 23-34 (1972).

(55) Y. Kojima, J. Y. Homma, and C. Abe, *Jap. J. Exp. Med.*, 41, 493-496 (1971).

(56) M. Soeda, K. Fukuhara, and S. Kawashima, *Toho Igakkai Zasshi*, 8, 1605-1607 (1961); *Chem. Abstr.*, 58, 11,855 (1963).

(57) Z. V. Ermoléva, G. E. Vaisberg, A. I. Braude, T. I. Afanaseva, N. I. Givental, N. M. Furer, I. P. Fomina, S. M. Navashin, I. V. Ravich, E. A. Vedmina, T. V. Golosova, and N. N. Zabolotskaya, *Antibiotiki*, 6, 618-623 (1961); *Chem. Abstr.*, 57, 2793 (1962).

teriae,[65] *Lactobacillus*,[66] *Streptomyces*,[67] and others[68] were reported active against solid tumors. Polysaccharides from *E. coli* mutant K_m were nontoxic, and showed activity against a number of tumors, such as Ehrlich carcinoma, Sarcoma 37, lymphadenoma NK/Ly, lymphosarcoma Li0-1, and Sarcoma K230 (Ref. 37). Pretreatment of mice with *Proteus vulgaris* lipopolysaccharide (100 µg/mouse, i.p.) increased the tumor weight of subsequently implanted Sarcoma 180 or Ehrlich carcinoma, whereas they lessened the tumor weight when administered after tumor implantation.[59] This lipopolysaccharide (100 µg/mouse) gave the maximal effect when administered 2 days after tumor implantation (60% inhibition), but when given on the 3rd or 4th day after tumor implantation, the inhibitory effect was lowered by as much as 30%. The tumor-inhibitory activity of *P. vulgaris* lipopolysaccharide on solid tumors is given in Table II. The lipopolysaccharide showed maximal activity when administered intracutaneously (i.c.), even at nontoxic doses (100 µg/mouse). This lipopolysaccharide also caused an increase in the survival times of rats inoculated with Yoshida ascites carcinoma. The control rats died in 15 days, whereas 60% of rats given 5 mg/kg three times (on days 2, 4, and 6) survived for 30 days. A polysaccharide isolated by partial hydrolysis of *P. vulgaris* lipopolysaccharide inhibited the growth of isologous solid tumors [methylcholanthrene-induced Sarcoma (CB1) in C34/He mice and a radiation-induced adenocarcinoma (1095) in XVII strain mice] when it was administered at 100 µg/mouse (i.p.) 3–4 weeks before tumor grafting. However, when administered 1–5 days before tumor grafting, the tumor incidence increased. Acet-

(58) Z. V. Ermoléva, I. V. Ravich, S. M. Navashin, A. I. Braude, I. P. Fomina, T. G. Terenteva, N. V. Pokidova, and V. I. Boiko, *Antibiotiki*, 7, 571–581 (1962); *Chem. Abstr.*, 61, 7555 (1964).

(59) D. Mizuno, O. Yoshioka, M. Akamatu, and T. Kataoka, *Cancer Res.*, 28, 1531–1537 (1968).

(60) D. G. Zatula, S. R. Reznik, A. T. Slabospitskaya, and N. B. Markova, *Mikrobiol. Zh.* (Kiev), 33, 201–205 (1971); *Chem. Abstr.*, 75, 74,326 (1971).

(61) R. Watanabe, A. Matsuo, T. Ogushi, and K. Hatano, *Jap. Pat.* 71 21,465 (1971); *Chem. Abstr.*, 75, 87,082 (1971).

(62) B. L. Wasilauskas and J. A. Cameron, *Cancer*, 27, 217–222 (1971).

(63) H. R. Strausser and L. A Bober, *Cancer Res.*, 32, 2156–2159 (1972).

(64) R. Kozima, *Jap. Pat.* 71 17,150 (1971); *Chem. Abstr.*, 75, 62,185 (1971).

(65) P. A. Zahl and C. Bjerknes, *Proc. Soc. Exp. Biol. Med.*, 54, 329–332 (1943).

(66) T. Kagawa, *Jap. Pat.* 72 02,554 (1972); *Chem. Abstr.*, 76, 139,075 (1972).

(67) Y. Seikizawa, S. Inouye, and K. Kagino, *J. Antibiot.* (Tokyo), Ser A, 15, 236–241 (1962); *Chem. Abstr.*, 61, 10,003 (1964).

(68) B. E. Aizemann, *Mikrobiol. Zh. Akad. Nauk Ukr. RSR*, 27, 61–67 (1965); *Chem. Abstr.*, 64, 11,710 (1966).

TABLE II

Effect of *Proteus vulgaris* Lipopolysaccharide on Solid
Ehrlich Carcinoma and[59] Sarcoma 180

Total dose (μg/mouse × number of doses)	Route of injection[a]	Ehrlich carcinoma		Sarcoma 180	
		Survivors	Inhibition ratio (%)	Survivors	Inhibition ratio (%)
Control		9/9	0	10/10	0
100 × 7	i.p.	9/9	65.3		
100 × 3	i.p.			9/10	42.8
100 × 3	i.v.	5/5	71.1		
100 × 3	i.c.	5/5	85.4	10/10	74.7
100 × 3	i.c.	9/9	81.3		
10 × 3	i.c.	9/9	79.2	10/10	45.1

[a] Key: i.c., intracutaneous; i.p., intraperitoneal; i.v., intravenous. Lipopolysaccharide was injected either daily for seven days, or for three days on days 2, 4, and 6 after tumor inoculation. Mice used were dd/Y (inbred strain). Tumors were removed and weighed on the 14th day after implantation. Inhibition ratio (%) was calculated on the basis of the tumor weight given by the authors.

oxane (a polysaccharide complex from *Acetobacter xylinum*) containing D-glucose, D-mannose, ribose, and rhamnose, was effective against solid Sarcoma 180, showing 50–80% inhibition, but it was only moderately effective against Ehrlich ascites tumors when administered in a single, prophylactic dose. This polysaccharide was nontoxic.[57]

From these results, it might be predicted that all endotoxic lipopolysaccharides would be active against solid tumors. Lipopolysaccharides are composed of lipid A, core polysaccharide, and species-specific side-chains. Lipid A, a common constituent of all lipopolysaccharides, is isolated as an insoluble material by gentle hydrolysis of certain lipopolysaccharides. The role of lipid A in tumor regression is not yet clear, as it is active in some tumor systems and inactive in others. Lipid A from *Escherichia coli* 08 inhibited the growth both of Ehrlich ascites and Sarcoma 37 tumors in mice.[69] Mihich and coworkers[41] found that lipid A from *E. coli* 0.111:B4 endotoxin, in a single, intravenous (i.v.) or i.p. dose of 200 μg/mouse produced hemorrhagic necrosis in 90–100% of Sarcoma 180 solid tumors in HaICR Swiss mice, whereas the lipopolysaccharide itself was effective to the same extent at a dose level of 40 μg/mouse. These authors[41] suggested that lipid A, although

(69) N. Kasai, Y. Aoki, T. Watanabe, T. Odaka, and T. Yamamoto, *Jap. J. Microbiol.*, 5, 347–366 (1961); *Chem. Abstr.*, 58, 6106 (1963).

active, is much less effective than the lipopolysaccharide on a weight basis. The activity of this lipid A preparation was probably not due to residual lipopolysaccharide, as Mihich and coworkers[41] found less than 0.01% of residual lipopolysaccharide in their preparation. Pretreatment of mice with lipid A from *Bordetella pertussis* did not retard the growth of transplanted Sarcoma 180, whereas the lipopolysaccharide did retard tumor growth.[31] Wasilauskas and Cameron[62] examined the action of various lipopolysaccharides and molecular fragments isolated from the rough and smooth strains of *Salmonella typhimurium* against Sarcoma 37 in mice. The lipopolysaccharide was found to have the following structure.

O-Specific polysaccharide—Glc — Gal — Glc — (Hep)$_2$ — (KDO)$_3$ — lipid A
                                                  | |
                                                 GlcN   Gal
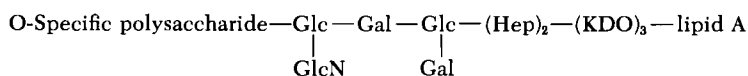
Ability to cause tumor hemorrhage decreased as carbohydrate was sequentially removed from the polysaccharide end of the lipopolysaccharide. Lipopolysaccharides and fragments were active at a dose of 25 μg/mouse, but lipid A, common antigen, O-antigen, and "KDO" (3-deoxy-D-*manno*-octulosonic acid) were inactive. These results indicated that, to be active, lipid A requires, at least, attachment of the "KDO" units. However, lipid A may be active against other tumors or, perhaps at a different dose-level, against Sarcoma 37. It is becoming apparent that many of the biological properties of lipopolysaccharides are due to the lipid A component. For biological tests, the lipid has been made water-soluble by complexing with bovine serum albumin. The solubility of lipid A may explain the activity found when lipid A is bound to the "KDO" trisaccharide.

Polysaccharides may be active components in undefined aqueous extracts (from sonicated *E. coli*) which inhibited ascites tumors, such as Sarcoma 180, Ehrlich carcinoma, and MM$_2$ in ddD mice.[70] Likewise, polysaccharides may be implicated in the activity of heat-killed *Corynebacterium parvum* against tumors in mice.[71-73]

III. FUNGAL AND LICHEN POLYSACCHARIDES

Crude, fungal extracts from a number of fungi[74-77] and various strains of Basidiomycetes[78-83] have been reported to be active against experimental tumors, and, in some cases, they showed definite pal-

(70) T. Watanabe, *Jap. J. Exp. Med.*, **36**, 453–455 (1966).
(71) M. F. A. Woodruff and J. L. Boak, *Brit. J. Cancer*, **20**, 345–355 (1966).
(72) B. N. Halpern, G. Biozzi, C. Stiffel, and D. Mouton, *Nature*, **212**, 853–854 (1966).
(73) D. W. Weiss, R. S. Bonhag, and K. B. DeOme, *Nature*, **190**, 889–891 (1961).

liative action on some non-operable, human tumors.[74] A number of polysaccharides have been isolated from fungal sources, and found active.[84–111] Crude polysaccharides composed mainly of β-D-(1 → 3)-linked D-glucose and small proportions of D-galactose and D-man-

(74) A. Catero, S. B. Azevedo, and A. E. Area Leao, *Acta Unio Intern. Contra Cancrum*, 7, 448–455 (1951); *Chem. Abstr.*, 48, 9556 (1954).

(75) Z. T. Mankowski, *Proc. Soc. Appl. Bacteriol.*, 83, 25–85 (1955).

(76) Z. T. Mankowski, I. C. Diller, and M. E. Fisher, *Cancer. Res.*, 17, 382–386 (1957).

(77) R. Sevilla-Santos, *J. Philippine Pharm. Assoc.*, 50, 133–139 (1964); *Chem. Abstr.*, 63, 3357 (1965).

(78) F. J. Gregory, E. M. Healy, H. P. L. Agersbory, Jr., and H. Warren, *Mycologia*, 58, 80–90 (1966).

(79) E. H. Lucas, R. L. Ringler, R. U. Byerrum, J. A. Stevens, D. A. Clarke, and C. C. Stock, *Antibiot. Chemotherapy*, 7, 1–4 (1957).

(80) J. F. Ronald, Z. F. Chimielewiez, B. A. Weiner, A. M. Gross, O. P. Boeing, J. V. Luck, T. J. Bardos, H. C. Reilly, K. Sugiura, C. C. Stock, E. H. Lucas, R. U. Byerrum, and J. A. Stevens, *Science*, 132, 1897 (1960).

(81) T. O. Yoshida, J. A. Rising, and W. J. Nungester, *Proc. Soc. Exp. Biol. Med.*, 111, 676–679 (1962).

(82) T. Ikekawa, M. Nakanishi, N. Uehara, G. Chihara, and F. Fukuoka, *Gann*, 59, 155–158 (1968).

(83) S. Shibata, Y. Nishikawa, C. F. Mei, F. Fukuoka, and M. Nakanishi, *Gann*, 59, 159–161 (1968).

(84) T. Kamasuka, Y. Momoki, and S. Sakai, *Gann*, 59, 443–445 (1968).

(85) T. Ikekawa, N. Uehara, Y. Maeda, M. Nakanishi, and F. Fukuoka, *Cancer Res.*, 29, 734–735 (1969).

(86) S. Otsuka, S. Ueno, C. Yoshikumi, F. Hirose, Y. Omura, T. Wada, T. Fujii, and E. Takahashi, *Ger. Pat.* 2,050,719 (1971); *Chem. Abstr.*, 75, 4030 (1971).

(87) N. Komatsu, S. Kikumoto, K. Kimura, S. Sakai, T. Kamasuka, Y. Momoki, T. Yamamoto, S. Takada, and J. Sugayama, *Ger. Pat.* 1,915,687 (1969); *Chem. Abstr.*, 72, 41,748 (1970).

(88) T. Kamasuka, Y. Momoki, S. Sakai, S. Takada, T. Yamamoto, J. Sugayama, N. Komatsu, S. Kikumoto, and K. Kimura, *Jap. Pat.* 71 41,596 (1971); *Chem. Abstr.*, 76, 71,022 (1972).

(89) T. Ikekawa, Y. Yoshioka, M. Emori, T. Sano, and F. Fukuoka, *Cancer Chemotherapy Rep.*, 57, 85–86 (1973); *Chem. Abstr.*, 78, 13,639 (1973).

(90) S. Otsuka, S. Ueno, C. Yoshida, F. Hirose, M. Ohara, T. Fujii, and T. Sawada, *Jap. Pat.* 71 17,149 (1971); *Chem. Abstr.*, 75, 62,175 (1971).

(91) T. Sasaki, Y. Arai, T. Ikekawa, G. Chihara, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), 19, 821–826 (1971).

(92) K. Mitsugi, M. Okunishi, and J. Hamuro, *Ger. Pat.* 2,138,329 (1972); *Chem. Abstr.*, 76, 152,057 (1972).

(93) G. Chihara, Y. Maeda, J. Hamuro, T. Sasaki, and F. Fukuoka, *Nature*, 222, 687–688 (1969).

(94) G. Chihara, J. Hamuro, Y. Y. Maeda, Y. Arai, and F. Fukuoka, *Cancer Res.*, 30, 2776–2781 (1970).

(95) F. Fukuoka, *Fr. Pat.* 2,020,160 (1970); *Chem. Abstr.*, 74, 130,370 (1971).

(96) G. Chihara, J. Hamuro, Y. Monura, Y. Suzuki, and F. Fukuoka, *Jap. Pat.* 72 37,002 (1972); *Chem. Abstr.*, 78, 16,452 (1973).

nose have been isolated from fruit bodies and mycelia, and from culture fluids of *Cereosporie cryptomeriae, Chaetomium cochlides, Cladosporium fulvum, Cochliobolus sativus, Flammulina velutipes, Lentinus edodes, Pholiota namenko, Poria cocos, Pyrensphora teres,* and *Sclerotinia sclerotiorum.* Such polysaccharides have shown activity against solid tumors. They are analogous to $(1 \rightarrow 3)$-β-D-glucans, but contain a small proportion of D-mannose. Similar polymers have been isolated from *Crepidotus* culture-filtrates[97,99]; the glucan therefrom was most effective by i.p. administration, and least by subcutaneous (s.c.) administration, against solid Sarcoma 37, Sarcoma 180, and Ehrlich carcinoma, whereas no response was seen against AH-13 ascites hepatoma, ascites sarcoma, Ehrlich carcinoma, and syngeneic tumors.[98] The *Crepidotus* glucan caused inhibition of solid tumors in mice at a dose of 1 mg/kg, regardless of whether the glucan was administered 7 days before or 7 days after tumor implantation.[99] Polysaccharides from *Pullularia*, a yeast-like fungus, also showed activity against solid Ehrlich tumor.[106,107] Polysaccharides

(97) Y. Yamamura, Y. Naka, K. Iokumura, and J. Sawada, *Jap. Pat.* 71 33,199 (1971); *Chem. Abstr.*, 76, 12,829 (1972).

(98) H. Nakayoshi, *Nippon Saikingaku Zasshi*, 23, 7–12 (1968); *Chem. Abstr.*, 69, 17,912 (1968).

(99) H. Nakayoshi, W. T. Watanabe, Y. Yamamura, and M. Ono, *Jap. J. Exp. Med.*, 38, 437–442 (1968).

(100) N. Komatsu, S. Okubo, S. Kikumoto, K. Kimura, G. Saito, and S. Sakai, *Gann*, 60, 137–144 (1969).

(101) N. Komatsu, S. Okubo, and S. Kikumoto, *J. Antibiot.* (Tokyo), Ser. B, 23, 157–160 (1970); *Chem. Abstr.*, 73, 129,539 (1970).

(102) T. Miyajima, S. Yoshizumi, S. Kikumoto, and H. Takahashi, *Seito Gijutsu Kenkyukaishi*, 22, 35–44 (1970).

(103) S. Sakai, S. Takada, T. Kamasuka, K. Momoki, and J. Sugayama, *Gann*, 59, 507–512 (1968).

(104) P. P. Singh, R. L. Whistler, R. Tokuzen, and W. Nakahara, *Carbohydr. Res.*, 37, 245–247 (1974).

(105) J. Johnson, Jr., S. Kirkwood, A. Misaki, T. E. Nelson, J. V. Scaletti, and F. Smith, *Chem. Ind.* (London), 820–822 (1963).

(106) P. A. Bulanov and A. A. Malama, *Dokl. Akad. Nauk Belorussk.* SSR, 9, 270–272 (1965); *Chem. Abstr.*, 63, 8919 (1965).

(107) A. G. Lobanok and P. A. Bulanov, *Dokl. Akad. Nauk Belorussk.* SSR, 10, 430–433 (1966); *Chem. Abstr.*, 65, 14,066 (1966).

(108) S. Ukai, K. Hirose, T. Kiho, C. Hara, T. Irikura, T. Kanechika, and Y. Hasegawa, *Chem. Pharm. Bull.* (Tokyo), 20, 2293–2294 (1972).

(109) T. Irikura, Y. Hasegawa, T. Kanechika, K. Hirose, and S. Ukai, *Jap. Pat.* 72 16,613 (1972); *Chem. Abstr.*, 78, 20,173 (1973).

(110) T. Irikura, Y. Hasegawa, T. Kanechika, K. Hirose, and S. Ukai, *Jap. Pat.* 72 16,612 (1972); *Chem. Abstr.*, 78, 20,174 (1973).

(111) Y. Yoshioka, T. Ikekawa, M. Noda, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), 20, 1175–1180 (1972).

from *Tremella* differ from other fungal polysaccharides in chemical composition, being composed of D-xylose, D-mannose, and D-glucuronic acid,[108–110] but they, too, show activity.

Antitumor glucans isolated from fungi differ widely in their structures. Linear $(1 \rightarrow 3)$-β-D-glucans have been isolated from *Lentinus edodes*,[93–95] *Coriolus*,[92] *Crepidotus*,[99] *Janoderma applanatum*,[91] and *Phellinus linteus*.[91] β-D-$(1 \rightarrow 4)$-Linked D-glucans from *Lentinus edodes*,[93,94] and β-D-$(1 \rightarrow 3)$- and -$(1 \rightarrow 4)$-linked D-glucans from *Janoderma applanatum* and *Phellinus linteus*[93] have been reported to show antitumor activity. Exceptionally high antitumor activity against Sarcoma 180 has been demonstrated by a $(1 \rightarrow 3)$-β-D-glucan having a β-D-glucopyranosyl group linked $(1 \rightarrow 6)$ to every 3rd or 4th residue of the main chain. Such a polysaccharide, schizophyllan, has been obtained from *Schizophyllum commune*.[100–102] The scleroglucan (also termed polytetran and polytran) from *Sclerotium glucanicum*,[104,105] and sclerotinan from *Sclerotinia sclerotiorum*,[103] are of interest. The antitumor activities of several pure glucans are given in Table III. Schizophyllan has been examined in detail; it showed high activity when injected by the i.p. or i.v. route, but had low activity by the s.c. route.[100] Schizophyllan inhibited solid Sarcoma 180 (see Table III), Sarcoma 37 (87% inhibition at a dose of 1.25–5 mg/kg), and Yoshida sarcoma in rats (74% inhibition at a dose of 0.7 mg/kg). Subcutaneously implanted, spontaneous, mammary carcinomas in Swiss mice were not responsive, but Friend virus disease was 37% inhibited with schizophyllan at a dose of 50 mg/kg (0% at lower doses). Schizophyllan showed some interesting effects on the survival rate of mice intraperitoneally implanted with Sarcoma 180 ascites tumor cells. Whereas pretreatment of the mice with polysaccharide was without effect, combined pre- and post-treatment, and post-treatment, resulted in the survival of 57 and 43% of the mice, respectively. No ascites fluid was observed in the surviving mice, but s.c. solid tumors were seen in a few mice. On the other hand, schizophyllan had no effect on the survival of mice intraperitoneally implanted with Sarcoma 37, Ehrlich carcinoma, or Yoshida sarcoma ascites tumors.[100]

Pachyman, a glucan obtained from *Poria cocos*, was inactive,[112] whereas pachymaran, obtained by debranching pachyman through periodate oxidation and mild hydrolysis, was found effective against mouse sarcoma.[112,113] O-(Carboxymethyl)ation of pachyman gave an

(112) G. Chihara, J. Hamuro, Y. Maeda, Y. Arai, and F. Fukuoka, *Nature*, **225**, 943–944 (1970).

(113) Ajinomoto Co., Inc., *Brit. Pat.* 1,313,373 (1973); *Chem. Abstr.*, **79**, 42,808 (1973).

TABLE III

Antitumor Activity of Some Fungal Glucans Against Subcutaneously Implanted Sarcoma 180 in Mice[a]

Glucan	Source	Linkages	Dose (mg/kg × number)	Route of injection	Complete regression	Inhibition ratio (%)
Lentinan[93]	*Lentinus edodes*	$(1 \rightarrow 3)$-β-D	25 × 10	i.p.	2/9	73.0
			5 × 10	i.p.	7/10	97.5
			1 × 10	i.p.	6/10	95.1
			0.2 × 10	i.p.	6/10	78.1
Schizophyllan[100]	*Schizophyllum commune*	$(1 \rightarrow 3)$-β-D, $(1 \rightarrow 6)$-β-D	5 × 10	i.p.	4/10	89
			1 × 10	i.p.	7/10	81
			0.5 × 10	i.p.	7/10	82
			5 × 4	i.v.	5/10	100
			1 × 4	i.v.	4/10	96
			10 × 10	s.c.	4/10	82
			1 × 10	s.c.	0/10	11
Pachymaran[112]	pachyman from *Poria cocos*	$(1 \rightarrow 3)$-β-D	5 × 10	i.p.	4/9	96
Scleroglucan[104]	*Sclerotium glucanicum*	$(1 \rightarrow 3)$-β-D, $(1 \rightarrow 6)$-β-D	50 × 10	i.p.	2/10	41.2
			5 × 10	i.p.	5/10	88.2
			0.5 × 10	i.p.	7/10	91.6
Fraction LC-I[93]	*Lentinus edodes*	$(1 \rightarrow 4)$-β-D, $(1 \rightarrow 6)$-β-D	30 × 10	i.p.	8/9	96.5
			15 × 10	i.p.	10/10	100
			5 × 10	i.p.	8/10	99.0

[a] Treatment with glucans was started 24 h after tumor implantation, and the results were recorded after 5 weeks.

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antitumor-active derivative.[114,115] O-(Carboxymethyl)ation of pachymaran gave a derivative that was active against Sarcoma 180, Ehrlich carcinoma, MM-102 carcinoma in C3H mice, and CCM adenocarcinoma in SWM/MS mice.[116]

Various glucans, such as pustulan, lichenan, and isolichenan, isolated from lichens, have been found active against solid sarcomas in mice. Pustulan-like glucans containing β-D-$(1 \rightarrow 6)$-linkages have been isolated from *Gyrophera esculenta* and *Lassalia papulose*,[117–120] but these glucans differed from pustulan in containing 4–10% of acetyl groups situated at O-3. Deacetylation lowered the activity, and replacement of O-acetyl groups by O-methyl groups, or complete acetylation of the glucan, yielded inactive products.[120] Similar, partially acetylated glucans have been isolated from *Umbilicaria sp.*,[121] and lichenan [a $(1 \rightarrow 3),(1 \rightarrow 4)$-$\beta$-D-glucan] and isolichenan [a $(1 \rightarrow 3),(1 \rightarrow 4)$-$\alpha$-D-glucan] have been isolated from *Cetraria sp.*[117,122,123] An antitumor lichenan has also been isolated from *Alectoria sp.*[124] Other lichens that have yielded antitumor glucans are *Parmelia caperata, Clandomia mitus, Usnea baylei*,[125] and *Evernia prunastri.*[124] These glucans were active against Sarcoma 180 solid tumors, and inactive against ascites tumors. The antitumor activity, composition, and structure of various glucans isolated in pure form from lichens are given in Table IV.

(114) S. Takada, J. Sugayama, and S. Sakai, *Jap. Pat.* 70 39,521 (1970); *Chem. Abstr.*, 74, 115,869 (1971).
(115) J. Hamuro, F. Fukuoka, and G. Chihara, *Ger. Pat.* 2,152,059 (1971); *Chem. Abstr.*, 77, 70,421 (1972).
(116) J. Hamuro, Y. Yamashita, Y. Ohsaka, Y. Y. Maeda, and G. Chihara, *Nature*, 233, 486–487 (1971).
(117) F. Fukuoka, M. Nakanishi, S. Shibata, Y. Nishikawa, T. Takeda, and M. Tanaka, *Gann*, 59, 421–432 (1968).
(118) S. Shibata, Y. Nishikawa, T. Takeda, and M. Tanaka, *Chem. Pharm. Bull.* (Tokyo), 16, 2362–2369 (1968).
(119) Y. Nishikawa, *Farumashia*, 5, 543–549 (1969).
(120) Y. Nishikawa, T. Takeda, S. Shibata, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), 17, 1910–1916 (1969).
(121) Y. Nishikawa, M. Tanaka, S. Shibata, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), 18, 1431–1434 (1970).
(122) Y. Nishikawa, S. Kobayashi, S. Shibata, F. Fukuoka, and S. Maekawa, *Seikagaku*, 41, 632 (1969).
(123) S. Shibata, Y. Nishikawa, and F. Fukuoka, *Jap. Pat.* 71 17,147 (1971); *Chem. Abstr.*, 75, 67,474 (1971).
(124) T. Takeda, M. Funatsu, S. Shibata, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), 20, 2445–2449 (1972).
(125) S. Shibata, Y. Nishikawa, M. Tanaka, F. Fukuoka, and M. Nakanishi, *Z. Krebsforsch.*, 71, 102–104 (1968).

Antitumor Activity of Lichen Glucans Against Subcutaneously
Implanted Sarcoma 180 in Mice[a]

Glucan	Source	Linkages	Dose (mg/kg)	Complete regression	Inhibition ratio (%)
Partially[120]	Gyrophera	$(1 \rightarrow 6)$-β-D	200	8/10	99.1
O-acetylated	esculenta		150	7/10	97.3
pustulan			100	4/10	91.3
Pustulan[120]	Gyrophera esculenta	$(1 \rightarrow 6)$-β-D	150	1/8	85.5
Lichenan[117]	Cetraria	$(1 \rightarrow 3)$-, $(1 \rightarrow 4)$-β-D	200	5/6	99.1
	islandica		150	8/8	100
			100	8/10	99.7
Lichenan[124]	Evernia prunastri	$(1 \rightarrow 3)$-, $(1 \rightarrow 4)$-β-D	200	10/10	100
Isolichenan[117]	Cetraria	$(1 \rightarrow 3)$-, $(1 \rightarrow 4)$-α-D	200	6/8	99.6
	islandica		150	7/8	98.9

[a] Glucans were administered i.p., daily for ten days, starting 24 h after tumor implantation, and the results were recorded after 5 weeks.

IV. YEAST POLYSACCHARIDES

Zymosan, composed of essentially intact, yeast cell-walls, and microscopically similar to living yeast, has been reported to be active against solid tumors in mice.[76,126–137] Diller and coworkers[133] exam-

(126) R. Lewisohn, *Proc. Soc. Exp. Biol. Med.*, 43, 558–561 (1940).

(127) R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger, and D. Lasglo, *Amer. J. Pathol.*, 17, 251–260 (1941).

(128) R. Lewisohn, *Cancer Res.*, 1, 799–806 (1941).

(129) Z. T. Mankowski, Y. Yamashita, and I. C. Diller, *Proc. Soc. Exp. Biol. Med.*, 96, 79–80 (1957).

(130) W. T. Bradner, D. A. Clarke, and C. C. Stock, *Cancer Res.*, 18, 347–351 (1958).

(131) W. T. Bradner and D. A. Clarke, *Cancer Res.*, 19, 673–678 (1959).

(132) I. C. Diller, Z. T. Mankowski, and L. T. Harris, *Cancer Res.*, 14, 848–852 (1954).

(133) I. C. Diller, Z. T. Mankowski, and M. E. Fisher, *Cancer Res.*, 23, 201–208 (1963).

(134) I. C. Diller, M. E. Fisher, and D. Gable, *Proc. Soc. Exp. Biol. Med.*, 117, 107–110 (1964).

(135) W. T. Bradner, D. A. Clarke, and C. C. Stock, *Proc. Amer. Assoc. Cancer Res.*, 2, 191 (1957).

(136) Z. T. Mankowski, I. C. Diller, and W. J. Nickerson, *Proc. Amer. Assoc. Cancer Res.*, 2, 324 (1958).

(137) I. C. Diller and Z. T. Mankowski, *Acta Unio Intern. Contra Cancrum*, 16, 584–587 (1960).

ined yeast hydroglucan in detail, and found that given intraperitoneally, it was active against subcutaneously implanted Sarcoma 37, Sarcoma 180, and Krebs-2 carcinoma in ICR1 albino mice, whereas ascites tumors, carcinoma 755, and rhabdomyosarcoma MC-1a were not responsive. Hydroglucan produced regression in Sarcoma 37 tumors on i.v., i.p., intramuscular (i.m.), or subcutaneous (s.c.) injection, and the effect decreased in this order.[133] Zymosan was also effective against Sarcoma M-1 (Refs. 138 and 139), Brown–Pearce tumors[140,141] in rabbits, and a transplanted, benzopyrene-induced tumor[142] in rats. Immunization with zymosan also produced resistance to tumor implantation, and zymosan inhibited metastasis of Ehrlich ascites tumors, and lowered the count of tumor nodes in mouse lung.[24] Belkin and coworkers[29] found that a soluble fraction from bakers' yeast, at a dose of 50–100 μg/g, induced marked cell swelling and vacuolization in ascites Sarcoma 37 in CAF mice, whereas zymosan at a dose of 250–500 μg/g produced only a moderate effect.

Mannan fractions are obtained from the hot-water extract of yeast cells, and glucan fractions are obtained by alkaline extraction of the residue. Mankowski and coworkers[136,137] and Riggi and DiLizio[143] considered that a glucan was the active component of yeast cellwalls. A water-insoluble glucan from *Saccharomyces cerevisiae* produced 57–60% inhibition of the growth of solid Sarcoma and Ehrlich carcinoma, and 46% inhibition of Walker carcinoma.[24] It has now been shown that the mannans obtained from yeasts are also quite active.[144–150] Depending on the species and the method of fractionation, mannans or glucomannans, or both, are obtained. Their antitumor

(138) V. Liepa and K. F. Bass-Shadkhan, *Protivoopukholevye Soedin. 5-Nitrofuranovogo Ryada*, 267–276 (1972); *Chem. Abstr.*, 78, 92,717 (1973).
(139) V. Liepa and K. F. Bass-Shadkhan, *Protivoopukholevye Soedin. 5-Nitrofuranovogo Ryada*, 79–84 (1972); *Chem. Abstr.*, 78, 119,290 (1973).
(140) G. Zakenfels, L. Lejina, D. Leja, and V. K. Demidova, *Protivoopukholevye Soedin. 5-Nitrofuranovogo Ryada*, 73–78 (1973); *Chem. Abstr.*, 78, 119,289 (1973).
(141) I. Nagy, M. Ato, M. Koszoru, and K. Majorossy, *Arch. Ital. Patol. Clin. Tumori*, 7, 19–30 (1964); *Chem. Abstr.*, 62, 3255 (1965).
(142) E. Matthies, K. Pfordte, and W. Ponsold, *Arch. Geschwulstforsch.*, 41, 110–113 (1973); *Chem. Abstr.*, 79, 51,692 (1973).
(143) S. J. Riggi and N. R. Di Luzio, *Fed. Proc.*, 20, 265 (1961).
(144) S. Suzuki, H. Hatsukaiwa, H. Sunayama, M. Uchiyama, F. Fukuoka, M. Nakanishi, and S. Akiya, *Gann*, 60, 65–69 (1969).
(145) S. Suzuki, M. Suzuki, H. Hatsukaiwa, H. Sunayama, T. Suzuki, M. Uchiyama, F. Fukuoka, M. Nakanishi, and S. Akiya, *Gann*, 60, 273–277 (1969).
(146) S. Oka, N. Kumano, K. Sato, K. Tamari, K. Matsuda, H. Hirai, T. Oguma, K. Ogawa, S. Kiyooka, and K. Miyao, *Gann*, 60, 287–293 (1969).

activities and compositions are given in Table V. These data show that the mannans are more active than the glucan fraction. The activity of the glucomannan fraction seems to depend upon the content of D-glucose and, perhaps, the linkages. Thus, a glucomannan fraction from *Candida utilis* was inactive, whereas a fraction from *C. albicans* B-792 was highly active. It was suggested that the difference in activity was due to the β-D-glucosyl residues present in the latter fraction, whereas α-D-glucosyl residues are present in the (less active) *C. utilis* fraction.[144] Yeast mannans are highly branched, with $(1 \rightarrow 6)$-α-D-mannosyl groups in the main chains that carry branches of α-D-$(1 \rightarrow 6)$-linked manno-oligosaccharides joined to the main chain by α-D-$(1 \rightarrow 2)$-links. Some branches bear D-glucosyl residues at their terminals.[150] The structural chemistry of various yeast and fungal polysaccharides has been reviewed by Gorin and Spencer.[151]

The mannans, by i.p. injection, were well tolerated in mice, but showed high toxicity by i.v. administration.[150] The O-(carboxymethyl) derivative was not toxic by i.v. administration, and still showed high activity.[150,152] Antitumor polysaccharides have also been obtained from *Trametes*.[153]

Clinical trials of an O-(carboxymethyl)mannan have been reported by Oka and coworkers[154] on 11 patients having advanced lung cancer. O-(Carboxymethyl)mannan was intravenously administed twice weekly, the total dose averaging 10 g. Although a one-year observation period was insufficient to permit critical evaluation of the efficiency of this treatment on these critical cases (in terms of tumor shadow in chest X-rays), these workers[154] pointed out that some patients survived longer than had been expected. No undesirable side-effects on liver or kidneys were found.

(147) S. Oka, N. Kumano, S. Kato, K. Sato, K. Tamari, K. Matsuda, K. Ogawa, T. Saeki, and N. Moritani, *Jap. Pat.* 71 16,914 (1971); *Chem. Abstr.*, **75**, 62,178 (1971).

(148) S. Oka, N. Kumano, S. Kato, K. Sato, K. Tamari, K. Matsuda, K. Ogawa, T. Saeki, and N. Moritani, *Jap. Pat.* 71 16,193 (1971); *Chem. Abstr.*, **75**, 62,179 (1971).

(149) S. Oka, N. Kumano, S. Kato, K. Sato, K. Tamari, K. Matsuda, K. Ogawa, T. Saeki, and N. Moritani, *Jap. Pat.* 71 17,148 (1971); *Chem. Abstr.*, **75**, 62,183 (1971).

(150) N. Kumano, *Sci. Rept. Res. Inst. Tohoku Univ. Ser. C*, **19**, 89–102 (1972); *Chem. Abstr.*, **78**, 79,497 (1973).

(151) P. A. J. Gorin and J. F. T. Spencer, *Advan. Carbohydr. Chem.*, **23**, 367–417 (1968).

(152) S. Oka, N. Kumano, and K. Kurita, *Gann*, **63**, 365–369 (1972).

(153) S. Otsuka, S. Ueno, C. Yoshida, F. Hirose, T. Fujii, and M. Ohara, *Jap. Pat.* 71 16,911 (1971); *Chem. Abstr.*, **75**, 62,182 (1971).

(154) S. Oka, N. Kumano, K. Kurita, K. Sato, K. Tamari, K. Matsuda, H. Hirai, K. Ogawa, T. Oguma, S. Kiyooka, T. Ogawa, and K. Miyao, *Proc. Intern. Congr. Chemother. VIIth Prague*, 167–168 (1971).

TABLE V

Antitumor Activity of Yeast Polysaccharides Against Subcutaneously Implanted Sarcoma 180 in Mice[a]

Polysaccharide	Yeast	Composition	Dose (mg/kg × number)	Route	Regression	Inhibition ratio (%)
Glucan[145]	S. cerevisiae	83% Glc	150 × 10	i.p.	4/9	88.6
Mannan[145]	S. cerevisiae	99% Man	150 × 10	i.p.	7/10	95.9
			100 × 10	i.p.	7/8	100.0
Mannan[144]	C. albicans	99% Man	200 × 1	i.p.	4/9	64.8
			150 × 9			
Glucomannan[144]	C. albicans	63% Man, 37% Glc	200 × 1	i.p.	6/8	99.1
			150 × 9			
Glucomannan[144]	C. utilis	56.5% Man, 43.5% Glc	200 × 10	i.p.	0/7	9.1
Glucomannan[150]	C. utilis	92% Man, 8% Glc	100 × 10	i.p.	8/10	98.8
			100 × 10	i.v.	3/10	100.0
O-(Carboxymethyl)-mannan[150]	C. utilis	92% Man, 8% Glc	100 × 10	i.p.	5/10	91.7
			100 × 10	i.v.	7/10	98.2

[a] Polysaccharides were administered i.p. or i.v. in the appropriate dose, starting 24 h after tumor implantation, and the results were recorded after 5 weeks.

V. PLANT POLYSACCHARIDES

Belkin and coworkers[29] examined various crude, plant-polysaccharide preparations, and found that many produce hemorrhagic necrosis in solid Sarcoma 37; they also produced a progressive increase in cell volume and cell vacuolization of ascites tumor-cells. Polysaccharide preparations from golden rod (*Solidago* sp., whole plant), burdock root (*Arcticum lappa*), *Aucancua carmizulis* (seed), *Bryonia alba*, and *B. diocca* (root) showed marked effect on solid Sarcoma 37 and ascites tumor-cells at low dosages. Only the golden-rod preparation induced cell vacuolization and swelling in ascites forms of Sarcoma 37, Sarcoma 180, Ehrlich carcinoma, Krebs-2 carcinoma, Hepatoma 134, P-288 (a tetraploid, lymphocytic neoplasm induced in BDA/2 mice), Yoshida sarcoma, and Leukemia L1210. The other polysaccharides showed a constant effect only on Sarcoma 37 and Yoshida sarcoma ascites cells. An aqueous extract of *Calendula officinalis* (marigold) showed activity against Sarcoma 180 in mice and was nontoxic.[155]

Gum arabic prevented tumor "takes" if animals were treated with gum arabic before tumor implantation.[156,157] Bamboo polysaccharide was quite effective in preventing tumor "takes" when given intraperitoneally, starting 10 days before s.c. tumor implantation of Ehrlich carcinoma. Starch and degraded starches ("dextrins") had no inhibitory effect.[157]

Osswald[158] found that gum tragacanth, gum arabic, and O-(carboxymethyl)cellulose (CMC) could either increase the metastasis or decrease the tumor "takes" of Ehrlich ascites carcinoma in female NMRI mice. The effect depended upon the dose, route of injection, and molecular size of the polysaccharide. When gum tragacanth was intraperitoneally injected in a single dose of 100 mg/kg, 6 hours before tumor implantation, only 5 out of 15 mice developed tumors, but if given 24 hours before tumor implantation, 12 out of 15 mice developed tumors. With O-(carboxymethyl)cellulose (100 mg/kg, i.p.) and gum arabic (400 mg/kg, i.p.), all mice showed tumor growth, but the mice receiving polysaccharide 6 hours before tumor implantation showed less metastasis than the mice receiving polysaccharide 24 hours before tumor implantation. The i.v. administration of CMC (50 mg/kg, once daily for three days) had a more pronounced effect on

(155) P. Manolov, T. Boyadzhiev, and P. Nikolov, *Eksp. Med. Morfol.*, 3, 41–45 (1964); *Chem. Abstr.*, 62, 9652 (1965).

(156) N. Hasselgreen, *Amer. J. Cancer*, 34, 280–281 (1938).

(157) W. Nakahara, F. Fukuoka, Y. Maeda, and K. Aoki, *Gann*, 55, 283–288 (1964).

(158) H. Osswald, *Arzneim.-Forsch.*, 18, 1495–1498 (1968).

tumor growth, allowing only 1 out of 15 mice to have a tumor, whereas, by i.p. administration, 4 out of 15 mice had a tumor. The i.v. administration of gum arabic caused less metastasis than the i.p. administration. CMC of M.W. ~ 290,000 produced fewer metastases than CMC samples of lower molecular weight.[158]

O-Methylcellulose (MC) produced regression of subcutaneously implanted Murphy–Sturm lymphosarcoma in Sprague–Dawley rats, with the effect depending upon the starting time of the treatment and the time of tumor implantation.[159] MC (2 mg/rat) was injected three times a week as long as the rats remained alive. When treatment with MC (900 mg, total) was started 10 days before tumor implantation, 95% of the tumors in the rats showed complete regression. If the treatment (700 mg, total) was started one day before tumor implantation, 67% of the rats had regression, and if treatment (550 mg, total) was started 8 days after tumor implantation, 67% regression was again observed. In all cases, complete regression occurred between days 17 and 21. In splenectomized rats, MC lost its tumor-inhibitory effect, indicating an indirect action of MC on the tumor.[159]

Gum tragacanth (2.5 mg/mouse) inhibited the growth of Landschütz ascites tumors in C+ mice, and, when intraperitoneally administered at a dose of 10 mg/mouse, prolonged the longevity of tumor-bearing mice by a factor of two.[160] Total inhibition with gum tragacanth was also obtained for Bp8 ascites tumor in C+ mice at a dose of 2.5 mg/mouse, but it had no effect on subcutaneously implanted, solid C+ leukemia in C+ mice. Oral administration of gum tragacanth had no effect on the tumors. Samples of different commercial grades differed in their activity. Indian gum tragacanth powders from Sterculia urens, and Persian flake powders from Astragalus gummifera Labillardiere, were noninhibitory, whereas Persian ribbon powders from A. gummifera from a different habitat showed inhibition at a dose of 2 mg/mouse; this could, perhaps, explain the inactivity of gum tragacanth reported by Oettel and Wilhelm.[161] Belkin and coworkers[29] found that Sarcoma 37 ascites cells in CAF mice were not affected by citrus pectin, gum karaya, gum ghatti, gum tragacanth, soluble starch, and glycogen, even at high doses, and gum acacia showed some inconsistent effects.

Hemicelluloses from bamboo,[157,162-169] sugar-cane bagasse,[170] rice

(159) A. Lazar and D. C. Lazar, J. Nat. Cancer Inst., 28, 1255–1267 (1962).

(160) E. M. F. Roe, Nature, 184, 1891 (1959).

(161) H. Oettel and G. Wilhelm, Cancer Res., Suppl. No. 2, 143 (1955).

(162) S. Sakai, G. Saito, J. Sugayama, T. Kamasuka, S. Takeda, and T. Takano, J. Antibiot. (Tokyo) Ser. B, 16, 387–391 (1963); Chem. Abstr., 61, 1122 (1964).

stalks,[171,172] corn stalks,[172,173] and wheat straw[171,172,174] caused regression of solid Sarcoma 180 in mice, but not of ascites tumors. Hemicellulose from the leaves of bamboo grass inhibited solid Sarcoma 180 and Ehrlich carcinoma, with resorption and necrosis occurring without hemorrhage,[167] but ascites tumors and Friend virus disease were unaffected. Hemicellulose B from wheat straw (100 mg/kg), highly active against solid Sarcoma 180, produced only a slight effect on Ehrlich carcinoma, and none on the autologous grafts of spontaneous, mammary adenocarcinoma.[174] Hemicellulose A from wheat straw, and hemicellulose B from soybean, sunflower stalk, and slash pine, had no antitumor activity.[174] An arabinoglucuronoxylan, isolated from wheat-straw hemicellulose B by complexing with Fehling solution, was completely devoid of activity, whereas the arabinoglucoxylan obtained from the supernatant liquor was highly active (see Table VI), even[175] at a dose of 25 mg/kg. The results of enzymic and acid hydrolysis and Smith degradation showed that the active component of wheat-straw hemicellulose B contained a glucan. By fractionation of bamboo-leaf hemicellulose on a cellulose column, followed by precipitation as a copper complex, Suzuki and coworkers[163] obtained a highly active arabinoxylan, whereas the supernatant

(163) S. Suzuki, T. Saito, M. Uchiyama, and S. Akiya, *Chem. Pharm. Bull.* (Tokyo), 16, 2032–2039 (1969).

(164) T. Tanaka, F. Fukuoka, and W. Nakahara, *Gann*, 56, 529–536 (1965).

(165) M. Iio and K. Yamafugi, *Kyushu Daigaku Nogakubu Gakugei Zasshi*, 23, 113–118 (1968); *Chem. Abstr.*, 69, 66,025 (1968).

(166) S. Suzuki and T. Saito, *Tohoku Yakka Daigaku Kenkyu Nempo*, 14, 13–17 (1967); *Chem. Abstr.*, 71, 111,067 (1969).

(167) S. Sakai, G. Saito, J. Sugayama, T. Kamasuka, T. Takano, and S. Takada, *Gann*, 55, 197–203 (1964).

(168) T. Mizuno and F. Shibata, *Shizuoka Daigaku Nogakubu Kenkyu Hokoku*, 18, 113–118 (1968); *Chem. Abstr.*, 71, 98,979 (1969).

(169) J. Sugayama, T. Kamasuka, S. Takada, T. Takano, G. Saito, and S. Sakai, *J. Antibiot.* (Tokyo) Ser. A, 19, 132–136 (1966); *Chem. Abstr.*, 65, 6131 (1966).

(170) T. Tanaka, *Gann*, 58, 1–4 (1967); S. Oka, N. Okamura, S. Kato, K. Tamari, K. Matsuda, and M. Shida, *ibid.*, 59, 35–42 (1968).

(171) J. Sugayama, T. Hachisuka, T. Takano, S. Takada, G. Saito, and S. Sakai, *Jap. Pat.* 65 24,789 (1965); *Chem. Abstr.*, 64, 7978 (1966).

(172) T. Kamasuka, J. Sugayama, S. Takai, T. Yamamoto, K. Momoki, and S. Sakai, *Jap. Pat.* 69 13,957 (1969); *Chem. Abstr.*, 71, 105,204 (1969).

(173) B. Larsen and E. B. Thorling, *Acta Pathol. Microbiol. Scand.*, 75, 229–237 (1969).

(174) W. Nakahara, R. Tokuzen, F. Fukuoka, and R. L. Whistler, *Nature*, 216, 374–375 (1967).

(175) P. P. Singh, H. Gremli, R. L. Whistler, R. Tokuzen, and W. Nakahara, unpublished results.

TABLE VI

Antitumor Activity[a] and Composition of Some Plant Hemicelluloses

Hemicellulose	Source	Composition	Dose (mg/kg)	Complete regression	Inhibition ratio (%)
Wheat-straw hemicellulose B[173]	wheat straw	Xyl 70%, Ara 17%, Glc 7.5%, Gal 3%, and uronic acid 2%	100 50	8/10 4/10	99.5 88.3
Arabinoglucurono-xylan[181]	insoluble copper complex obtained on treatment of wheat-straw hemicellulose B with Fehling solution	Xyl, Ara, uronic acid, and Gal	100 5	0/10 0/10	41.8 12.8
Arabinoglucoxylan[174]	supernatant liquor from treatment of wheat-straw hemicellulose B with Fehling solution	Xyl, Ara, and Glc 45%	25 5	8/10 1/9	95.2 78.6
Arabinoxylan[163] (p-Fr-I-A)	insoluble copper complex obtained on treatment of a bamboo-leaf hemicellulose fraction with Fehling solution	Xyl 63.4%, Glc 19.5%, Ara 17.1%, Gal (traces)	200	8/10	95.2
Arabinoxylan[163] (Fr-I-B)	supernatant liquor obtained from the above treatment of bamboo-leaf hemicellulose with Fehling solution	Xyl 29.1%, Ara 26.3%, Glc 12.2%, Gal 32.4%	200	3/9	93.2
Bagasse poly-saccharide[170]	sugar-cane bagasse	Xyl, Ara, Man, Gal, Glc (traces)	100 75 10		92 67 74.6

[a] Sarcoma 180 tumors were subcutaneously implanted in mice, and polysaccharides were administered intraperitoneally, daily for 10 days, starting 24 h after tumor implantation. The results were recorded after 5 weeks (4 weeks in the case of the bagasse polysaccharide).

liquor gave a fraction of only moderate activity at a dose of 200 mg/kg. This behavior contrasts with the results obtained with wheat-straw hemicellulose B, which yielded an active fraction from the copper supernatant liquor, and the insoluble complex gave an inactive arabinoglucuronoxylan. The composition and antitumor activity of these fractions from wheat-straw hemicellulose B, bamboo-grass hemicellulose, and bagasse hemicellulose are given in Table VI.

VI. OTHER POLYSACCHARIDES

Algin (sodium alginate from kelp) and mesogloian (a crude polysaccharide preparation from *Mesogloia divaricata*) produced marked cell-swelling and vacuolization of Sarcoma 37 ascites tumor cells in CAF mice.[29] From molasses, a polysaccharide preparation composed of hexosyl and pentosyl residues inhibited sarcoma and Ehrlich tumors.[176] Water-soluble polysaccharides from the tissues of Strong A mice produced mitotic arrest of Ehrlich cells when administered to CAF mice.[176] A polysaccharide component from bull seminal-plasma inhibited Novikoff cell-transplantation.[177] From Sarcoma 180 tumor cells, Makari[178] isolated cancer antigens that he called "tumor polysaccharide substance." This substance induced immunity when injected in small doses, but produced immunoparalysis in large doses.[178]

Various polysaccharide derivatives, such as DEAE-dextran,[173,179–182] heparin and heparin salts,[183–188] dextran sulfates,[187–189]

(176) E. T. Nishimura, T. R. Harwood, J. H. Baum, and P. B. Putong, *A. M. A. Arch. Pathol.*, **65**, 88–97 (1958); *Chem. Abstr.*, **52**, 13,067 (1958).

(177) B. Sheid, *Experientia*, **27**, 691–692 (1971); *Chem. Abstr.*, **75**, 74,217 (1960).

(178) J. G. Makari, *Nature*, **205**, 1178–1183 (1965).

(179) E. B. Thorling and B. Larsen, *Acta Pathol. Microbiol. Scand.*, **75**, 237–246 (1969).

(180) J. S. Manning, A. J. Hackett, and N. B. Darby, Jr., *Appl. Microbiol.*, **22**, 1162–1163 (1971).

(181) F. Kuzuya, *Igaku No Ayumi*, **78**, 643–644 (1971); *Chem. Abstr.*, **76**, 121,751 (1972).

(182) K. Bolewski, *Farm. Polska*, **26**, 443–449 (1970); *Chem. Abstr.*, **74**, 51,781 (1971).

(183) G. Gallinotto, *Atti Acad. Med. Lombarda*, **18**, 376–377 (1963); *Chem. Abstr.*, **61**, 4854 (1962).

(184) G. Csaba and J. Korosi, *Ger. Pat.* 1,129,148 (1962); *Chem. Abstr.*, **57**, 16,729 (1962).

(185) A. Landsberger, *Med. Welt*, 1222–1223 (1963); *Chem. Abstr.*, **59**, 10,592 (1963).

(186) A. Landsberger and H. H. Briese, *Zentr. Allgem. Pathol. Anat.*, **106**, 129–133 (1964); *Chem. Abstr.*, **62**, 15,315 (1965).

(187) K. Suemasu and S. Ishikawa, *Gann*, **61**, 125–130 (1970).

(188) H. Nitani, K. Suemasu, and T. Miwa, *Ger. Pat.* 1,951,822 (1971); *Chem. Abstr.*, **75**, 40,413 (1971).

and thiosemicarbazide derivatives of periodate-oxidized polysac-
charides,[190] have also shown inhibition of certain tumors. Heparin in
a single, i.v. dose of 50 mg/rat caused diminution in mortality of rats
that had been intravenously injected with Walker carcinoma cells,
and it also lessened pulmonary metastasis.[183] DEAE-dextran in-
creased the survival time of rats bearing implanted Yoshida sarcoma;
a similar effect was noted if the sarcoma cells were incubated with
DEAE-dextran prior to inoculation.[173,181] This inhibition of tumor
growth was reversible, as the effect of DEAE-dextran could be pre-
vented by subsequent incubation with dextran sulfate.[173] Similar ef-
fects were obtained in *in vivo* studies.[179] The inhibitory effect of
DEAE-dextran on tumor growth, and the reversal by dextran sulfate,
were demonstrated with four different experimental tumor-systems:
NJA leukemia and JBI ascites tumors (both in C3H/A mice), a plas-
mocytoma in Balb/c mice, and Yoshida ascites tumors in Wistar
rats.[173] Sulfated, degraded laminaran inhibited the growth of Sarcoma
180 when injected into the growing tumors at the site of the trans-
plantation.[191] Chlorite-oxidized oxyamylose (COAM) lessened both
tumor occurrence and death due to intramuscularly induced Mo-
loney sarcoma virus when administered in an appropriate dose at 7
or 13 days after the virus, or when injected in repeated doses on al-
ternate days from 7 until 19 days, or from 13 until 23 days, after virus
challenge.[192] COAM, however, did not cause regression of es-
tablished tumors.[192] COAM has also been reported to cause regres-
sion of spontaneous, mammary carcinoma in C3H mice when the
COAM was administered neonatally.[193]

VII. STRUCTURE AND ANTITUMOR ACTIVITY

Because of numerous possibilities for inter-sugar linkages, polysac-
charides (even if they are composed of only one kind of monosac-
charide residue) can possess complex structures. They also form
secondary structures, depending upon the conformations of the com-
ponent sugar residues, the molecular weight, and the inter- and intra-
chain hydrogen-bonding. The situation becomes more complicated

(189) A. Landsberger, *Klin. Wochschr.*, **40**, 542 (1962); *Chem. Abstr.*, **57**, 6572 (1962).
(190) V. C. Berry, M. L. Conalty, J. E. McCormick, R. S. McElhinney, and J. F.
      O'Sullivan, *Proc. Roy. Irish Acad.*, **64B**, 269–284 (1966).
(191) B. Jollès, M. Remington, and P. S. Andrews, *Brit. J. Cancer*, **17**, 109–115 (1963).
(192) E. De Clercq and P. De Sommer, *Eur. J. Cancer*, **8**, 535–540 (1972).
(193) A. Billiau, R. Leyten, M. Vandeputte, and P. De Sommer, *Life Sci.*, **10**, 643–647
      (1971).

when polysaccharides are composed of two or more kinds of mono-saccharide residues. Although the fundamental chemical structure has, in many cases, been well documented, knowledge of the physical structure is still fragmentary. Polysaccharides having antitumor action differ greatly in their composition and, hence, in their chemical and physical structures. Antitumor activity appears to be possessed by a wide range of glycans extending from homopolymers (such as glucans, mannans, and glucomannans) to highly complex glycans (such as gums, hemicelluloses, and bacterial lipopolysaccharides). Although it is difficult to draw relationships between structure and antitumor activity of complex polysaccharides, some possible relationships can be drawn from comparison of the behavior of simpler glycans and, especially, of the homoglycans. The D-glucans listed in Table VII were investigated as essentially pure polymers, and can be compared, as they were tested under similar conditions against Sarcoma 180 in mice. To a lesser degree, comparison can be made with other glycans examined by other biological tests.

In general, it may be concluded that soluble D-glucans are active antitumor agents, particularly if they are mainly linear, without excessively long branches, and if they are not quickly hydrolyzed by humoral D-glucanases. Thus, glycogen, starch, and "dextrins" are inactive. Nigeran from *Asperigillus japonica* is also inactive, possibly because it is an α-D-glucan whose units are joined by $(1 \rightarrow 3)$- and $(1 \rightarrow 4)$-links, and, although the linkages mainly alternate, there are some regions of adjacent $(1 \rightarrow 4)$-linkages that permit cleavage by amylases. Isolichenan is also an α-D-glucan, but it has a higher proportion $(3:2)$ of $(1 \rightarrow 3)$-linkages.[194] Consequently, it may be presumed that, if humoral amylase can hydrolyze certain $(1 \rightarrow 4)$-linkages, the residual chains of $(1 \rightarrow 3)$-linked residues may be long enough to effect a host response and otherwise contribute to tumor regression.

In general, the $(1 \rightarrow 3)$-β-D-glucans, and β-D-glucans having a preponderance of, or, perhaps, long stretches of, $(1 \rightarrow 3)$-linkages in the main chain, are active. β-D-Glucans containing mainly $(1 \rightarrow 6)$-linkages have less activity. As a completely uniform, $(1 \rightarrow 3)$-linked glucan would be insoluble in water, it is not unexpected that active large polysaccharides with continuous $(1 \rightarrow 3)$-linkages in the main chain also have numerous, single-unit side-chains of β-D-glucopyranosyl residues joined $(1 \rightarrow 6)$ that provide solubility in water. A good example is scleroglucan. Pachyman from *Poria cocos*, a tree-rot fungus, is a water-insoluble D-glucan and it is tumor inactive.

(194) N. B. Chanda, E. L. Hirst, and D. J. Manners, *J. Chem. Soc.*, 1951–1958 (1957).

TABLE VII

Comparison and Antitumor Activity[a] of Some D-Glucans

D-Glucan	Linkages	$[\alpha]_D$ (degrees)	D.p.	Dose (mg/kg)	Complete regression	Inhibition ratio (%)
Lentinan[93]	$(1 \rightarrow 3)$-β-D	+19.5 to +21.5 (NaOH)	5,800–6,500	5	7/10	97.5
Pachyman[103]	$(1 \rightarrow 3)$-β-D and a few $(1 \rightarrow 2)$-β-D or $(1 \rightarrow 6)$-β-D	+21.5 (NaOH)	700	50 10	2/9 0/8	41 55
Lichenan[124]	$(1 \rightarrow 3)$ and $(1 \rightarrow 4)$-β-D (ratio 3:1)	+12.0 (H$_2$O)	60	200	10/10	100
Isolichenan[124]	$(1 \rightarrow 3)$ and $(1 \rightarrow 4)$-α-D (ratio 3:2)	+197.0 (H$_2$O)	40–50	150	7/8	98.9
LC-I from *Lentinus edodes*[93,117]	$(1 \rightarrow 4)$ and $(1 \rightarrow 6)$-β-D			15	10/10	100
Scleroglucan	$(1 \rightarrow 3)$ and $(1 \rightarrow 6)$-β-D (ratio 3–4:1)	−1 (NaOH)	110	0.5	7/10	91.6
Nigeran (*Aspergillus japonicus*)[93,104,106]	$(1 \rightarrow 3)$ and $(1 \rightarrow 4)$-α-D (ratio 1:1)	+220 (NaOH)	300–350	50	0/10	57
Glycogen-like D-glucan from *Pellicularia filamentosa*[103]		+250		100	inactive	

[a] Antitumor activity was tested on subcutaneously implanted Sarcoma 180 in mice. The glucans were injected intraperitoneally 10 times (once a day), starting 24 h after tumor implantation, and the results were recorded after 4 to 5 weeks.

On Smith degradation, the product is partially or totally soluble in water, and it exhibits high antitumor activity.[112,113] Cellulose is inactive, but O-methylcellulose and O-(carboxymethyl)cellulose are active. Pustulan from the lichen *Umbilicaria pustulata* is a $(1 \rightarrow 6)$-β-D-glucan that has some natural solubility due to partial acetylation; it has some antitumor activity, but loses[120] this when acetyl groups are removed and the solubility decreases. In general, however, it may be concluded that $(1 \rightarrow 6)$-linked D-glucans are less effective antitumor agents than $(1 \rightarrow 3)$-linked D-glucans, and that those of moderately high molecular weight are more effective than those of low molecular weight.[195] It is understandable that such generalizations as these are very tenuous and are not supported by firm data; moreover, some unexplained exceptions are evident.

The dextrans tested are inactive unless derivatized with 2-(diethylamino)ethyl groups.[196] Their inactivity has not yet been explained.

Some activity is seen with certain D-mannans and glucomannans (see Table VIII). Fragments of yeast mannans showed some activity, and the fractions of highest molecular weight were the most active.[195]

Injection of urea along with lentinan and pachymaran causes loss in their antitumor activity,[197] suggesting that conformational change might be implicated with activity, although this point has not been validated. Optical rotations of polysaccharides in water differ from those in urea solution, and, although this indication of conformational change may be important, it is, perhaps, more likely that other factors explain the effect of added urea. The finding that polysaccharides can change the α-helix of bovine serum albumin[198] or guinea-pig serum[199] shows that injected polysaccharides may have significant effects on protein structure and cell-wall character.

The importance of acetyl groups to the activity of certain bacterial polysaccharides and, especially, to their immunological specificity has been established.[200-203] In addition to raising the solubility,

(195) S. Oka, N. Kumano, K. Sato, K. Tamari, K. Matsuda, H. Hirai, T. Oguma, K. Ogawa, S. Kiyooka, and K. Miyao, *Proc. Intern. Congr. Chemother. 6th*, 1, 122–124 (1969); *Chem. Abstr.*, 74, 123,331 (1971).
(196) E. B. Thorling, B. Larsen, and H. Nielsen, *Acta Pathol. Microbiol. Scand.*, 79, 81–90 (1971).
(197) J. Hamuro, Y. Y. Maeda, Y. Arai, F. Fukuoka, and G. Chihara, *Chem. Biol. Interactions*, 3, 69–71 (1971).
(198) J. Hamuro and G. Chihara, *Nature*, 245, 40–41 (1973).
(199) T. Okuda, Y. Yoshioka, T. Ikekawa, G. Chihara, and K. Nishioka, *Nature New Biol.*, 238, 59–60 (1972).
(200) O. T. Avery and W. F. Goebel, *J. Exp. Med.*, 58, 731–755 (1933).

acetyl groups can significantly alter the molecular conformation. Thus, Katz[204] suggested that acetyl groups present in gluco-mannans from pine strongly affect the physical properties. These groups increase the solubility in water, and change the molecular orientation and the development of lateral order; there are many instances where solubility and conformational changes have been produced by derivatization of polysaccharides.

Roe and coworkers[205-207] pointed out that gum tragacanth, although negatively charged because of its carboxyl groups, becomes attached to acetic cell-surfaces within 10 minutes after i.p. injection. Yet the antitumor activity of hemicelluloses cannot be correlated with their carboxyl content.

VIII. MODE OF ANTITUMOR ACTION

The nature of the antitumor action of a polysaccharide is not entirely clear, but certain bacterial polysaccharides may directly attack tumors, as evidenced by the resulting intratumoral hemorrhage and necrosis. On the other hand, most of the polysaccharides from other botanical sources cannot be shown to exert any direct action on tumor cells. Their antitumor action must, therefore, be considered to be dependent upon the reaction of the host; that is, their effect is host-mediated. It is possible that, in some instances, these two types of action may be interwoven. The discussion in this Section covers only those polysaccharides that effect a host-mediated reaction.

Several workers have reported that bacterial, antitumor agents cannot have a direct effect on tumor cells, as the bacterial polysaccharide-preparations that produced hemorrhage and necrosis in solid tumors had no effect on tumor cells *in vitro*[208] or in tissue culture.[209,210] Seligman and coworkers[211] found that only a small propor-

(201) N. Roy and C. P. J. Glaudemans, *Carbohydr. Res.*, 8, 214–218 (1968).

(202) J. R. Dixon, W. K. Roberts, G. T. Mills, J. G. Buchanan, and J. Baddiley, *Carbohydr. Res.*, 8, 262–265 (1968).

(203) S. Estrada-Parra and M. Heidelberger, *Biochemistry*, 2, 1288–1294 (1963).

(204) G. Katz, *Tappi*, 48, 34–41 (1965).

(205) W. Galbraith, E. Mayhew, and E. M. F. Roe, *Brit. J. Cancer*, 16, 163–169 (1962).

(206) E. Mayhew and E. M. F. Roe, *Brit. J. Cancer*, 18, 528–536 (1964).

(207) E. Mayhew and E. M. F. Roe, *J. Roy. Microscop. Soc.*, 84, 475–483 (1965).

(208) P. A. Zahl, *J. Nat. Cancer Inst.*, 11, 279–288 (1950).

(209) J. R. McConnell, S. F. Hallett, and M. J. Shear, *Cancer Res.*, 7, 716 (1947).

(210) E. Y. L. Lasfargues, D. R. A. Wharton, and J. C. Difine, *Cancer Res.*, 11, 425–427 (1951).

(211) A. M. Seligman, M. J. Shear, J. Leiter, and B. Sweet, *J. Nat. Cancer Inst.*, 9, 13–18 (1948).

TABLE VIII

Properties and Antitumor Activity[a] of Some Polysaccharides Containing Mainly (1 → 6)-Linkages

Polysaccharide	Sugar composition	Linkages	$[\alpha]_D$ (degrees)	D.p.	Dose (mg/kg × number)	Complete regression	Inhibition ratio (%)
Mannan[144] from C. albicans	Man	(1 → 6)- and (1→2)-α-D	+38.5 (water)	50	200 × 1 150 × 9	4/9	64.8
Glucomannan[144] from C. albicans	Man 1.7, Glc 1.0	(1 → 6)- and (1→2)-α-D	+15.0 (water)	45	200 × 1 150 × 9	6/8	99.1
Glucomannan[144] from C. utilis	Man 1.3, Glc 1.0	(1 → 6)- and (1→2)-α-D	+71.5	56	200 × 10	0/7	9.1
Glucomannan[150] from C. utilis	Man 23, Glc 2	(1 → 6)- and (1→2)-α-D	+74.8	160	100 × 10	8/10	100.0
Partially acetylated pustulan[120]	Glc	(1 → 6)-β-D	−37.5 (NaOH)	120	150 × 10	7/10	97.3
Pustulan[120]	Glc	(1 → 6)-β-D	−37.4 (NaOH)	120	150 × 10	1/8	85.5
Dextran A[103]	Glc			3,000		inactive	
Dextran B[103]	Glc			12,000		inactive	

[a] Antitumor activity was tested against subcutaneously implanted Sarcoma 180 in mice. Daily intraperitoneal treatment was started 24 h after tumor implantation, and the results were recorded after 5 weeks.

tion of polysaccharides tagged with radioiodine reached the tumor site, and suggested that there was no direct action of the polysaccharide on the tumors.

1. Effect on Blood Supply

As bacterial toxins produced hemorrhagic necrosis in the tumors, it was considered that the mechanism of action of these polysaccharides might be due to their effect on the blood supply to the tumor. In fact, it was established that a lowering in blood pressure resulted from the injection of bacterial polysaccharides in human patients and in mice having tumors.[212,213] Hence, earlier workers directed considerable effort towards determining the effect of bacterial polysaccharides on the vascular supply to tumors.

Algire and coworkers[214,215] also studied the vascular reactions of normal and malignant tissues *in vivo*, and the effect of peripheral hypotension on transplanted tumors. They concluded that any agent or condition that lowers the blood pressure in arteries supplying a tumor, also interferes with the circulation within the tumor. According to them, if this hypotensive state could be maintained, damage to the tumor tissue would occur from the resulting ischemia. Thus, histamine, known to induce hypotension, was found to induce a partial destructive effect on the tumor cells. Similar circulatory effects were produced by Shear's polysaccharides.[216] Youngner and Algire[217] observed that mechanical obstruction to tumor blood-supply could duplicate the action of bacterial polysaccharide in producing hemorrhage and tissue damage. Diller and coworkers[218] found that administration of S. *marcescens* polysaccharide led to acceleration of cortical, secretory activity. Algire and coworkers[214] suggested that hypotension, and, consequently, the tumor-necrotizing property, is characteristic of the bacterial polysaccharides. They found that, although polysaccharides affect the circulation of both normal and neoplastic tissue, only the tumor tissues were necrotized; this was possibly due to the unique blood-supply of the tumor. Later, Shear

(212) T. Sack and A. M. Seligman, *J. Nat. Cancer Inst.*, 9, 19–34 (1948).
(213) L. V. Beck, D. Berkowitz, and B. Seltzer, *Cancer Res.*, 8, 162–168 (1948).
(214) G. H. Algire, F. Y. Legallais, and H. D. Parks, *J. Nat. Cancer Inst.*, 8, 53–62 (1947).
(215) G. H. Algire and F. Y. Legallais, *J. Nat. Cancer Inst.*, 12, 399–421 (1951).
(216) G. H. Algire, F. Y. Legallais, and B. F. Anderson, *J. Nat. Cancer Inst.*, 12, 1279–1296 (1952).
(217) J. S. Youngner and G. H. Algire, *J. Nat. Cancer Inst.*, 10, 565–579 (1949).
(218) I. C. Diller, B. Blauch, and L. V. Beck, *Cancer Res.*, 8, 591–605 (1948).

and coworkers[219] employed continuous-recording instruments for the direct measurement of carotid-artery blood-pressure in mice given intramuscular implants of Sarcoma 37, and found that, on administration of Shear's polysaccharide and other tumor-damaging substances, there was no correlation between systematic changes in blood pressure and hemorrhagic necrosis in the tumors.

2. Effect on Cell Volume and Vacuolization

Belkin and coworkers[29] reported that various, crude preparations from higher plants produced damage in Sarcoma 37 ascites tumor-cells by increasing the cell volume and the neoplasmic cell vacuolization. These workers suggested that, as a result of the administration of polysaccharide, the membranes of the ascites cell and its endoplasmic reticulum acquired increased permeability to water and solutes; consequently, the cells imbibed water and swelled until they were microscopically visible as vacuoles. For most polysaccharides tested, this phenomenon took two days or more. Golden-rod polysaccharide was most effective, and the vacuoles were visible within 4–8 hours of administration. When this phenomenon had progressed to full effect, the number of vacuolated cells had increased to about 70% of the total cell population. During this time, the cells increased in volume to as much as 90 times that of controls. These phenomena of cell swelling and vacuolization primarily involved the cytoplasm and, presumably, caused considerable damage to the cells. The vacuoles were optically empty, and cytochemical tests for the vacuolar contents were negative for fat, glycogen and other polysaccharides, and amino acids. Interestingly, several of the polysaccharides that induced hemorrhagic necrosis in solid Sarcoma 37 failed to produce swelling and vacuolization of the ascites cells. Belkin and coworkers[29] found that these polysaccharides did not affect the tumor cells in *in vitro* systems. This result emphasized the importance of the host in the pharmacological trinity of malignant cells, polysaccharide, and host on producing this phenomenon. The authors pointed out[29] various possibilities by which the host could be causing this phenomenon, two of which follow.

1. The polysaccharide molecules could be absorbed into the general circulation from the peritoneal cavity of the host, which then activates the polysaccharide molecule. The active moiety then dif-

(219) M. J. Shear, B. Achinstein, and S. N. Pradhan, *J. Nat. Cancer Inst.*, 21, 585–594 (1958).

fuses back into the peritoneal cavity, and directly affects tumor cells. Similar ideas have been expressed by other workers.

2. The polysaccharide molecules, or some portion of them, could stimulate an organ or organ system (for example, the adrenal, liver, or reticuloendothelial system), which then secretes a substance, probably very much different from the polysaccharide, which acts on the tumor cells. However, Belkin and coworkers[29] found that the polysaccharides from golden rod, hydrangea, or clover do not stimulate the reticuloendothelial system.

3. Effects on Immune Tumor Response

Malkiel and Harris[31] found that prior conditioning of the mouse with *Bordetella pertussis* lipopolysaccharide influenced the tumor "takes." According to them, the reticuloendothelial activity was stimulated to increase antibody response. Rejection of tumors might then result through an antibody–antigen reaction in which the tumor cells act as an antigen. The Lazars[159] showed that O-methylcellulose stimulated the reticuloendothelial system and led to the inhibition of Murphy–Sturm lymphosarcoma in rats. O-Methylcellulose did not show an effect in the splenectomized animals, suggesting that it could directly affect the tumors. As injections of O-methylcellulose were known to produce hyper-*gamma*-globulinemia, it was suggested that inhibition of tumor may be due to an alteration of the immune responses in the treated animals, such as to enhance tumor rejection. The effect of O-methylcellulose differed from that of gum arabic, which had no effect on the successfully transplanted tumors, but prevented the "tumor takes" if the animal was pretreated with gum arabic.[156] Calmette–Guerin bacillus[220] and O-methylcellulose[159] showed similar responses to tumors in mice. The tumors grew for 7 to 10 days, and then regressed. This bacillus also stimulated the phagocytic function of the reticuloendothelial system.

Sasaki and coworkers[221] examined the radiographic distribution of pachymaran in various tissues (such as adrenal glands, colon, duodenum, heart, jejunum, kidney, liver, lung, lymph nodes, spleen, stomach, and thymus) and concluded that the reaction was mainly observed in the cells classified as reticuloendothelial.

According to Mizuno and coworkers,[59] the reticuloendothelial system was strongly stimulated by the intracutaneous injection of the lipopolysaccharide of *Proteus vulgaris*, and a marked antitumor effect

(220) L. J. Old, D. A. Clarke, and B. Benacerraf, *Nature*, 184, 291–292 (1959).
(221) T. Sasaki, J. Hamuro, G. Chihara, and M. Amano, *Gann*, 61, 589–591 (1970).

was obtained on solid Ehrlich carcinoma and sarcoma. According to them,[59] these two effects were parallel. The *P. vulgaris* lipopolysaccharide, although nontoxic, was still antitumor active, and did not produce a hemorrhagic reaction in tumors. The *P. vulgaris* lipopolysaccharide also showed against tumor cells a slight, direct, cytocidal activity that led the authors[59] to believe that two factors could be involved in the antitumor effect of the lipopolysaccharide; one, a marked stimulation of the reticuloendothelial system, and the other, a slight cytocidal effect of the lipopolysaccharide itself.

Benacerraf and coworkers[222] found that splenic hyperplasia was induced by repeated injections of zymosan. Diller and coworkers[133] noticed a marked increase in the number of macrophages in the splenic tissue after a few hours of hydroglucan injection. It was conjectured that these phagocytic cells migrated to the tumor bed and were active in the tumor-cell destruction. This marked increase in the phagocytic cells could not be observed in either C3H or C57HL mice, whose tumors were not regressed.

Tokuzen[223] compared the local, cellular reactions to tumor grafts in mice treated with some antitumor polysaccharides. She noted that, in early stages, antitumor polysaccharides caused an extensive outpouring of lymphoid cells, mostly macrophages, in the immediate vicinity of the Sarcoma 180 grafts, and that this was followed by invasion into the graft by connective-tissue cells. These reactions were absent, or slight, in untreated controls, and negative for the inactive polysaccharides.

Weiss and coworkers[73] discussed evidence for the assumption that the ability of cancer cells to grow in the host revealed the failure of antibody formation or of phagocytic capability of the reticuloendothelial system.

The mechanism of action of yeast polysaccharides differed from that of bacterial polysaccharides. For example, some of the bacterial polysaccharides caused extreme hemorrhagic reaction within the tumors, whereas zymosan did not produce hemorrhage in the tumors. Diller and coworkers[133] and Arndt and coworkers[224] found that hydroglucan did not elicit Schwartzmann reaction in the skin of mice in which it induced tumor destruction. The histological changes, indicative of an alarm reaction, that were produced as a result of treatment

(222) B. Benacerraf, G. J. Thorbecke, and D. Jacoby, *Proc. Soc. Exp. Biol. Med.,* **100,** 796–799 (1959).

(223) R. Tokuzen, *Cancer Res.,* **31,** 1590–1593 (1971).

(224) W. F. Arndt, W. T. Bradner, and H. A. Schneider, *Proc. Soc. Exp. Biol. Med.,* **100,** 765–767 (1959).

with bacterial agents[225] did not appear during treatment with hydroglucan or zymosan.[222] Also, there was no microscopically demonstrable effect on the adrenals, such as was observed by Diller and coworkers[218] in response to bacterial polysaccharides.

Nakahara and coworkers[157] found that pretreatment with sasa polysaccharide was equally as effective as treatment after implantation, and this led them to consider that the mechanism of action must be an indirect or a host-mediated reaction. To determine the effect of antitumor polysaccharide on the host defense-systems, detailed studies were conducted by this group. Nakahara and coworkers[164] found that treatment, with sasa polysaccharide, of mice with or without tumors raised the plasma-protein level, especially that of the globulin fraction. Inactive polysaccharides failed to show any increase in the plasma-protein level under similar conditions. These authors[164] conjectured that the elevation of the level of the globulin fraction by sasa polysaccharide could be related to its antitumor effect. Interestingly, they found that the plasma from tumors of mice treated with sasa polysaccharide showed a slight antitumor effect on subcutaneously implanted Ehrlich carcinoma. This apparent, passive immunity needs confirmation before it can be accepted. Plasma from normal or tumor-bearing mice did not show any inhibitory effect. The authors[164] were of the opinion that plasma hyperglobulinemia altered the immune response in the treated animals. However, they found no increase of antibody-forming ability, and this suggested that antibody formation may not be related to the mechanism of homologous-tumor resistance. Increase in circulating *gamma*-globulin due to the appearance of a new *gamma*-globulin fraction, and changes in serum lipoproteins, were also noted on administration of O-methylcellulose.[226] Lowering of albumins and enhancement of *beta*- and *gamma*-globulins were also produced by injection of zymosan into rats carrying Sarcoma M-1.

Similarly, Tanaka[227] found that formation of serum antibody for sheep erythrocytes was unaffected by bagasse polysaccharide, another antitumor polysaccharide. Bagasse polysaccharide did not elicit hyperplasia of the reticuloendothelial system in mice, and thus there seemed to be no apparent correlation between carbon clearance and administration of bagasse polysaccharide. Considering that the

(225) A. J. Donnelly, H. F. Havas, and M. E. Broesbeck, *Cancer Res.*, 18, 149–154 (1958).
(226) M. Frohlich, V. Balazas, K. Kovacs, and A. Benko, *Nature*, 183, 1119–1120 (1959).
(227) T. Tanaka, *Gann*, 58, 451–457 (1967).

serum factor could be involved in the antitumor activity, she examined the properties of the serum obtained from mice treated with bagasse polysaccharide. The serum from mice in which the tumors regressed on administration of bagasse polysaccharide showed, *in vivo*, a slightly inhibitory effect during the growth of subcutaneously implanted Sarcoma 180. The serum from the control mice treated with bagasse polysaccharide showed no inhibitory effect. The results were comparable to those obtained with sasa polysaccharide.[164] The validity of the slightly inhibitory effect needs further examination; so, also, do the specificity and immunochemical properties of this serum factor. The complement titer increased in the mice in which the tumors regressed on treatment with polysaccharide, but decreased in control mice with tumors and in mice in which the tumors did not regress after polysaccharide treatment. It was not certain whether the increase in the serum-complement level was due to the decrease in anticomplement activity or to the increase of the complement component. Tanaka[227] proposed that the increase in the hemolytic activity of complement component in whole serum should be considered to be one of the factors involved in the mode of tumor-inhibitory activity. Okuda and coworkers[199] reported that some correlation exists between the antitumor activity and the anticomplementary activity of polysaccharides from some Basidiomycetes. They suggested that the inactivation of the C3 component of complement by the polysaccharides *in vitro* might have occurred by way of the alternative pathway or C3-activator system. It was not clear whether or not the inactivation of C3 by the polysaccharides *in vitro* had a direct relationship with the host-mediated tumor-inhibition *in vivo*.

Arai and coworkers[228] showed that administration of an immunosuppressive agent along with lentinan in animals lowered the activity of lentinan, thus suggesting that lentinan exerts its activity through stimulation of an unspecified, immune response. Maeda and Chihara[229] found a loss in the effect of lentinan administered in neonatally thymectomized mice, and the activity decreased considerably on administration of antilymphocyte serum that had a selective, immunosuppressive effect and that especially suppressed cell-mediated, immune responses, with little effect on humoral, immune responses. The fact that lentinan lost its activity in thymectomized mice, and also lost its activity when injected with antilymphocyte serum in non-thymectomized mice, indicated that the mode of action of lentinan was part of the thymus-derived, immune mechanism, in

(228) Y. Arai, H. Tanooka, J. Sekini, and F. Fukuoka, *Gann*, **62**, 131–134 (1971).
(229) Y. Y. Maeda and G. Chihara, *Nature*, **229**, 634 (1971).

which small lymphocytes played an important part. According to these authors,[229] the regression of tumors was due to the stimulation of cell-mediated responses. Donner and coworkers[230] evaluated the effect of a *Proteus vulgaris* polysaccharide on the growth of solid tumors. Inbred mice carrying a 3-methylcholanthrene-induced sarcoma, or an X-ray-induced adenocarcinoma, showed an increase in tumor growth when the bacterial polysaccharide was injected into the animal a few days before the tumor graft. However, if the polysaccharide was injected two to three weeks before tumor implantation, tumor development was delayed. Neutralization of tumor cells with serum showed that a soluble substance responsible for increasing tumor growth was temporarily elaborated after the administration of the polysaccharide. The nature of this soluble substance was not clear. The Hellstroms[231] proposed that "blocking antibodies," which protect the tumors from the host–cellular immune processes, are present in the sera of tumor-bearing animals. According to Donner and coworkers,[230] it was possible that administration of the polysaccharide altered the kinetics of cell-mediated and humoral immune-reactions and caused an early and increased production of circulating antibodies, resulting in an enhancement of tumor growth. Their results also suggested rapid decline in the production of blocking antibodies and a subsequent increase in the cell-mediated, immune response leading to a decrease in tumor growth.

D-Glucans from fungi show high activity at low dosage, and lower activity at higher dosage. It was suggested that this dosage-dependent activity of the D-glucans bears some relationship to the immunobiological reactions in the host.[93,94,104]

Makari[178] isolated cancer antigens from Sarcoma 180 tumor cells, and found that they were polysaccharide materials which he called "Tumor–Polysaccharide Substances" (TPS). TPS was found to induce immunity when injected in small dosage, whereas in large dosage it produced immunoparalysis. Makari assigned TPS the key role in the immunological concept of cancer. He speculated that, due to chronic and prolonged exposure to various carcinogenic agents, the normal polysaccharide substance, which was believed to surround normal cells, was modified into a new, abnormal polysaccharide substance. He considered that all carcinogenic agents, although different, have one property in common, namely, the ability to stimulate in host tissues the endogenous formation of tumor-specific, antigenic, polysaccharide substances. The significance of TPS in rela-

(230) M. Donner, D. Tottier, and C. Burg, *Eur. J. Cancer*, 8, 141–147 (1972).
(231) K. E. Hellstrom and I. Hellstrom, *Advan. Cancer. Res.*, 12, 167–223 (1969).

tion to immunity and susceptibility to cancer was demonstrated by the fact that newborn mice were immunized when given a small dosage of TPS, whereas mice given a large dosage became immunoparalyzed and showed an increased death rate. Mice receiving TPS showed marked plasma–cellular reaction, suggesting that TPS was the antigen concerned with the response against tumors. Makari[178] even went so far as to suggest the distinct possibility of utilizing small doses of TPS as a vaccine to stimulate immune response against tumors in mice.

The effect of antitumor polysaccharides on the level of properdin,[232–234] a serum protein that participates in many immune reactions, has also been investigated. Pillemer and coworkers[232,235] showed that the injection of zymosan caused marked alterations in the serum properdin level of experimental animals. Injection of large doses of zymosan caused temporary depression of properdin level, and injection of small doses caused transient increases. Studies in experimental animals also suggested a relationship between properdin levels and the growth of transplantable tumors. Herbut and Kraemer[236] postulated that the properdin system might be one of the natural, defense factors against neoplasms. Stock and coworkers[130] found that mouse Sarcoma 180 grew faster in mice treated with large doses of zymosan (which depressed the properdin levels of these mice), but grew less well in mice treated with a smaller dose of zymosan (which elevated the properdin level). However, none of these results have demonstrated the direct involvement of properdin in tumor inhibition. Stock and coworkers[130] showed that a host-mediated phenomenon was responsible for the loss of tumors in zymosan-treated mice. The authors considered that the properdin system might be involved. Diller and coworkers[133] studied the effect of yeast polysaccharides (zymosan and hydroglucan), but could not establish involvement of the properdin system in the host-defense process. These authors found[133] only a two-fold, statistically insignificant, increase in the serum-properdin titers following hydroglucan administration, and suggested that antitumor action of hydroglucan was independent of alterations in the serum-properdin levels.

Polysaccharides from *Pseudomonas aeruginosa*[55] and other bact-

(232) L. Pillemer and D. A. Ross, *Science*, 121, 732–733 (1955).
(233) M. Landy and L. Pillemer, *J. Exp. Med.*, 103, 823–833 (1956).
(234) L. Pillemer, L. Blum, I. H. LePow, D. A. Ross, E. W. Todd, and A. C. Wardlaw, *Science*, 120, 279–284 (1954).
(235) L. Pillemer, M. Landy, and M. J. Shear, *J. Exp. Med.*, 106, 99–110 (1957).
(236) P. A. Herbut and W. H. Kraemer, *Cancer Res.*, 16, 408–412 (1956).

eria,[237-240] and mannans[241] from yeast, are known to be interferon inducers, and, interestingly, these polysaccharides are also known to possess tumor-inhibitory properties. Nagy and coworkers[242] noted a 30–60% increase in the serum-properdin level after zymosan administration. The properdin level initially dropped in all animals after injection with living tumor-cells, and later rose to high levels in the surviving animals. Pfordte and Ponsold[243] considered properdin to have a function in an unspecific, defense system, and showed that zymosan stimulated this system. The role of the serum-properdin system in anticancer defense of the body has been discussed by Cron.[244] A direct, inhibitive effect of a properdin fraction from guinea pig was shown by Oravec and Cambelova.[245] They found that an asparaginase-free, properdin fraction inhibited tumor cells *in vivo* and *in vitro*. This fraction inhibited Ehrlich ascites tumor-cells and 6C3HED tumor-cells in mice, and the inhibition depended upon the properdin units present in the fraction.

4. Role of Cell-membrane Contact Inhibition

It is of interest that lentinan differs in its effects from other antitumor polysaccharides, such as zymosan. According to Maeda and coworkers,[246] it did not stimulate conventional responses in normal mice: blood-leukocyte counts were not affected, carbon-clearance activity was not increased, humoral immune-responses (such as antibody production against a heterologous antigen) were not stimulated, delayed cutaneous hypersensitivity in guinea pigs was not promoted, and the survival of skin allografts in mice was not prolonged. Lentinan appears only to be an immunopotentiator in the sense that it induces marked, host-mediated activity in causing regression of Sarcoma 180, in which thymus and thymus-derived T cells participate. Also interesting in this connection is the fact

(237) W. R. Stinebring and J. S. Youngner, *Nature*, 204, 712 (1964).
(238) J. S. Youngner and W. R. Stinebring, *Science*, 144, 1022–1023 (1964).
(239) J. S. Youngner and W. R. Stinebring, *Nature*, 208, 456–458 (1965).
(240) M. Ho, *Science*, 146, 1472–1474 (1967).
(241) V. Lackovic, L. Borecky, D. Sikl, L. Masler, and S. Bauer, *Proc. Soc. Exp. Biol. Med.*, 134, 874–879 (1970).
(242) I. Nagy, M. Koszoru, K. Majorossy, and J. Vajda, *Acta Unio Intern. Contra Cancrum*, 20, 1241–1243 (1964).
(243) K. Pfordte and W. Ponsold, *Neoplasma*, 18, 173–177 (1971).
(244) J. Cron, *Neoplasma*, 17, 155–168 (1970).
(245) C. Oravec and J. Cambelova, *Neoplasma*, 16, 677–678 (1969).
(246) Y. Y. Maeda, J. Hamuro, Y. Yamada, K. Ishimura, and G. Chihara, *Immunopotentiation: Ciba Found. Symp.*, 18, 259–281 (1973).

that the greatly lessened activity of T cells in tumor-bearing mice could be restored to normal by the administration of lentinan, as shown by Takatsu and coworkers.[247]

Lentinan is peculiar in that it very markedly inhibits Sarcoma 180, but has no effect on any other transplanted tumor thus far tested. The reason for this unique phenomenon is as yet unknown.

DEAE-dextran showed *in vitro*[248] and *in vivo*[179] inhibitory effect on ascites tumor-cells. It was suggested that the action of DEAE-dextran was, in some way, connected with changes in the cellular membrane. Ambrose and coworkers[249] had shown that tumor cells have a strongly negative surface charge. DEAE-dextran could be bound to these charges, and the "neutralization" of part of the negative charge on the cell surface could establish cell-to-cell contact, allowing the cells to receive signals to stop dividing. The cells were not immediately killed by incubation with DEAE-dextran, as it was possible to revert them to "normal" activity by subsequent incubation with dextran sulfate, which neutralized the surface charge. Thorling and coworkers[196] later found that the neutralization of cell-surface charge was dependent both upon molecular weight and degree of substitution (d.s.). The effect was greatest for a dextran of high molecular weight (2,000,000) and highest d.s. (50%), and was negligible for a DEAE-dextran with a molecular weight of 64,000 and a d.s. of 15%. Suemasu and Ishikawa[187] showed that heparin or dextran sulfate, added to Sato lung carcinoma *in vitro*, increased the electrophoretic mobility of the cells, probably due to the binding of heparin to the cell membrane. Roe and coworkers[205,206] showed that gum tragacanth became attached to the ascites cell-surface within 10 minutes of i.p. injection into tumor-bearing mice, and caused metaphase block within one hour. They did not observe penetration of gum tragacanth into the cells until 6 hours or more after the treatment. In later work, they showed that potassium and sodium levels remained normal during treatment with gum tragacanth, thus precluding imbalance of these ions as an explanation for the tumor-inhibitory effect.[250] Purification of the soluble, tumor-inhibitory portions of gum tragacanth showed that cytotoxic activity was retained in the fraction of highest negative charge, suggesting that the antitumor effect could be related to the negative charge of the polysaccharide.[250]

(247) K. Takatsu, T. Hamaoka, and M. Kitagawa, *Proc. Jap. Cancer Assoc.*, 31, 209 (1972).
(248) B. Larsen and K. Olsen, *Eur. J. Cancer*, 4, 157–162 (1968).
(249) E. J. Ambrose, A. M. James, and J. H. B. Lowick, *Nature*, 177, 576–577 (1956).
(250) E. M. F. Roe, H. Smyth, and E. Flahavan, *Cancer Res.*, 32, 2067–2074 (1972).

Mehrishi[251] found that the negative charge of the tumor cell-membrane increased on treatment with both the polyanions, poly I: poly C (tumor growth-inhibitory) and chondroitin sulfate (tumor growth-promoting), and he was of the opinion that to attribute both tumor-inhibitory and tumor growth-promoting activities to the high electron-density of the tumor cell was an oversimplification.

IX. EFFECT ON AUTOCHTHONOUS TUMORS

All of the host-mediated, antitumor polysaccharides so far noted have been isolated and identified, based on a bio-assay system normally using Sarcoma 180 in mice. The allogeneic, tumor-graft rejection-mechanism is the basis of this system.

One of the most important questions concerning the antitumor effect of these polysaccharides is whether they can inhibit the growth of autochthonous tumors. Transplanted tumors, be they syngeneic or allogeneic, are artifacts produced in the laboratory, and are not necessarily truly comparable with tumors that arise spontaneously within the body of the host (autochthonous tumors).

Tokuzen and Nakahara[252] described experiments in which the effect of some plant polysaccharides (GE-3, lentinan, and wheat-straw hemicellulose B) was tested on spontaneous, mammary adenocarcinoma of Swiss albino mice. Tumors of suitable sizes were surgically removed as completely as possible, and a small piece, about 1 mm in diameter, was returned to the site of operation as an autograft, in order to imitate incomplete removal and to ensure local recurrence. The result showed that none of the materials listed had *any* effect, as evaluated by the rate of local recurrence ensured by autograft of the tumor after surgical removal, length of postoperative survival, frequency of new tumors developing away from the site of the original tumors, and the frequency of lung metastasis.

As mentioned, Tokuzen[223] examined local, cellular reactions around Sarcoma 180 grafts during the process of regression under polysaccharide treatment, and found a massive outpouring of lymphoid cells one week after tumor implantation. Autochthonous grafts of spontaneous, mammary tumors, not at all inhibited by polysaccharides, called forth no local, lymphoid-cell infiltration.[252]

The question arose as to what would happen to autochthonous grafts if a local reaction were induced around them such as occurred around Sarcoma 180 under polysaccharide treatment. Tokuzen and

(251) J. N. Mehrishi, *Nature*, 228, 365–367 (1970).
(252) R. Tokuzen and W. Nakahara, *Arzneim.-Forsch.*, 21, 269–271 (1971).

Nakahara[253] attempted to answer this question by implanting autochthonous grafts mixed with Sarcoma 180, and following with the usual polysaccharide treatments. The critical point in their experiment was whether or not the induced, allogeneic, graft-rejection mechanism could discriminate the autochthonous cells from the cells of allogeneic Sarcoma 180. The local reaction induced against an allogeneic-tumor graft (Sarcoma 180) was capable of so involving autochthonous, tumor-graft implanted (mixed with it) that the mixed graft became completely suppressed in the majority of instances. The real, immunologic mechanism responsible for the destruction of autochthonous tumor-graft and simultaneously introduced Sarcoma 180 under treatment with polysaccharide is not clear. Histologic events accompanying and leading to the tumor destruction and disappearance can be described as essentially lymphoid-cell infiltration, followed by formation of granulation tissue, which finally became completely absorbed. The prominent, local, lymphoid-cell reaction suggested, but did not prove, the essential role of it in the process. Whatever be the true mechanism of the rejection reaction, it failed to recognize the autochthonous graft as "self" and to tolerate it, when mobilized primarily against allogeneic graft which was "not self." Another point of interest was local recurrence at the site of operation, and development of new tumors in other parts of the mammary gland took place quite frequently, even when autochthonous grafts regressed completely. Obviously, the destruction of autochthonous graft was a local affair, and it did not produce sufficient systemic resistance to prevent the development of mammary tumors elsewhere.

Despite the fact that all of the so-called antitumor polysaccharides hitherto discovered proved totally without effect on autochthonous, animal tumors, some relationships may exist between the mechanism of allogeneic, transplanted-tumor rejection and the suppression of autochthonous tumors, as indicated by experiments of Tokuzen and Nakahara.[253,254]

The polysaccharides so far examined, although strongly active in suppressing transplanted tumors, do not seem eligible for clinical trial in human cancer therapy. Therefore, to search for new polysaccharides more strongly antitumor-active than those thus far discovered, and, especially, ones that may have an inhibiting action on autochthonous tumors, stands as a challenge for further studies on antitumor polysaccharides.

(253) R. Tokuzen and W. Nakahara, Cancer Res., 33, 645–647 (1973).
(254) R. Tokuzen and W. Nakahara, Z. Krebsforsch., 81, 239–242 (1974).

HEMICELLULASES: THEIR OCCURRENCE, PURIFICATION, PROPERTIES, AND MODE OF ACTION

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I. INTRODUCTION

The hemicellulases, as a group of enzymes, must be defined and classified according to their substrates, the hemicelluloses, a rather indefinite group that has been variously defined, for example, as those polysaccharides, soluble in alkali, that are associated with the cellulose of the plant cell-wall. The hemicelluloses are usually classified according to the sugar residues present, for example, as D-galactan, D-mannan, and D-xylan. L-Arabinans are often associated with the pectic polysaccharides from a number of plant sources, but are usually included in the hemicellulose group. Most hemicelluloses, however, occur, not as homoglycans, but as heteroglycans, containing different types of sugar residues, often as short appendages linked to the main, backbone chain. Their structures have been discussed in detail by Aspinall[1,2] and Timell.[3,4]

The terms "hemicellulases" or hemicellulose-degrading enzymes refer to those enzymes (glycan hydrolases, EC 3.2.1) that specifically degrade only hemicelluloses, and do not include the glycosidases (for example, α-L-arabinofuranosidase, α- and β-D-galactosidases, α- and β-D-mannosidases, and β-D-xylosidases), which, in addition to their activity on glycosides of low molecular weight, are also frequently capable of hydrolyzing the short-chain or monosaccharide appendages from the main, backbone chain of hemicelluloses. Typical hemicellulases, therefore, are the L-arabinanases, D-galactanases, D-mannanases, and D-xylanases.

It is the intention of this article to survey the literature on hemicellulases from 1950 to 1973, and to deal, in detail, only with those enzyme preparations that have, by the usual criteria of purity employed in protein purification procedures, been shown to be homogeneous. The article will, therefore, exclude most of the work published on hemicellulases in which crude or partially purified enzyme prepara-

(1) G. O. Aspinall, *Advan. Carbohyd. Chem.*, 14, 429–468 (1959).
(2) G. O. Aspinall, "Polysaccharides," Pergamon Press, Oxford, 1970, p. 103.
(3) T. E. Timell, *Advan. Carbohyd. Chem.*, 19, 247–295 (1964).
(4) T. E. Timell, *Advan. Carbohyd. Chem.*, 20, 409–483 (1965).

tions were used. Such studies suffer the disadvantage that the observations may result from the action of more than one enzyme component and, therefore, give misleading information on properties and action patterns of the enzymes.

A comprehensive review on D-xylanases and their degradation of D-xylans was written by Sörensen[5] in 1957, and since then, brief reviews of the same subject have been written in English by Timell[6] and in Japanese by Fukui[7] (1961) and Sasaki[8] (1971). Work on D-xylanases from 1957 onwards will, therefore, be discussed in detail in this article.

The work of Courtois and his associates deserves specific mention in recognition of their elucidation of the structures of legume galactomannans by employing various mannanases of different origins. However, because these workers employed impure enzyme preparations in their studies, their work has not been discussed in detail in the Section dealing with the mode of action of D-mannanases.

II. L-ARABINANASES

1. Introduction

L-Arabinanases are hydrolytic enzymes capable of degrading L-arabinans. Purified enzymes have been isolated[9-11] that hydrolyze both the α-L-(1 → 3)-linked L-arabinofuranosyl appendages of sugar-beet L-arabinan, and the α-L-(1 → 5)-linked L-arabinofuranosyl residues of the main, "linear" chain. Enzymes of this kind have not yet been classified by the Enzyme Commission of the International Union of Biochemistry, and we have described them as (1 → 5)-(1 → 3)-α-L-arabinan arabinanohydrolases.

The same purified preparations have also been found[9-11] to hydrolyze the (1 → 3)-α-L-arabinofuranosyl side-chain linkages of L-arabino-D-xylans and gum arabic, as well as liberating L-arabinose from phenyl α-L-arabinofuranoside. Enzymes having the latter type of specificity are usually classified as glycosidases. However, as they

(5) H. Sörensen, *Acta Agr. Scand., Suppl.,* 1, 1957, 85 pp.
(6) T. E. Timell, *Svensk Papperstidn.,* 65, 435–447 (1962).
(7) S. Fukui, *Tampakushitsu Kakusan Koso,* 6, 90–99 (1961).
(8) T. Sasaki, *Ehime Daigaku Nogakubu Kiyo,* 15, 71–136 (1971).
(9) A. Kaji, K. Tagawa, and K. Matsubara, *Agr. Biol. Chem.* (Tokyo), 31, 1023–1028 (1967).
(10) A. Kaji and K. Tagawa, *Biochim. Biophys. Acta,* 207, 456–464 (1970).
(11) A. Kaji and O. Yoshihara, *Biochim. Biophys. Acta,* 250, 367–371 (1971).

TABLE I

Sources of L-Arabinanases

Organism	References
(a) Bacteria	
Clostridium felsineum var. *sikokianum*	12
(b) Fungi	
Alternaria solani	13
Aspergillus awamori	14
japonicus	15
niger	9,10,13–19
oryzae	14,20
Botryosphaeria ribis	21
Botrytis allii	13
cinerea	22,23
fabae	13
tulipae	13
Byssochlamys fulva	24
Chaetomium sp.	15
Cladosporium sp.	15
cucumerinum	13
Colletotrichum lindemuthianum	13
Coniophora puteana	25
Coniothyrium diplodiella	22,26
Corticium centrifugum	27
rolfsii	11,27–29
Fusarium sp.	15
oxysporum f. *lupini*	13
lycopersici	13
pisi	13
Gloeosporium kaki	22
Glomerella cingulata	13
Hypochnus centrifugus	27
Monascus sp.	15
Monilia fructigena	13
Mucor sp.	15
Mycosphaerella pinodes	13
Neurospora sp.	15
Penicillium sp.	15
camemberti	13
digitatum	30
expansum	23
Phycomyces sp.	15
Phytophthora palmivora (Butl.)	31
Rhizopus sp.	15
nigricans	13
solani	23
tritici	32
Sclerotinia fructigena (Aderh. & Ruhl.)	23,33,34
libertiana	22
sclerotiorum	15,23,35
rolfsii	27

TABLE I (*continued*)

Organism	References
Tramates gibbosa	25
Trichoderma viride	13
(c) Animals (invertebrates)	
Helix aspera	20
(d) Plants	
Apple, barley, carrot, cucumber, pea, peach, potato, radish, tomato	36
Stylosanthes humilis (seed)	37
Trifolium subterranum, Daliak (seed)	37

(12) A. K. Yanabuki, H. Taki, Y. Oyama, and T. Okada, *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, 15, 40–44 (1963).

(13) A. Fuchs, J. A. Jobsen, and W. M. Wouts, *Nature*, 206, 714–715 (1965).

(14) A. Kaji, H. Taki, A. Shimasaki, and T. Shinkai, *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, 15, 34–39 (1963).

(15) K. Tagawa, *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, 21, 186–195 (1970).

(16) Y. Misawa, M. Matsubara, and M. Hatano, *Nippon Shokuhin Kogyo Gakkaishi*, 14, 394–399 (1967).

(17) K. Tagawa and A. Kaji, *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, 15, 45–49 (1963).

(18) K. Tagawa and G. Terui, *Hakko Kogaku Zasshi*, 46, 693–700 (1968).

(19) A. Kaji, K. Tagawa, and T. Ichimi, *Biochim. Biophys. Acta*, 171, 186–188 (1969).

(20) M. V. Tracey, *J. Sci. Food Agr.*, 15, 607–611 (1964).

(21) J. H. McClendon, G. F. Somers, and J. W. Heuberger, *Phytopathology*, 50, 258–261 (1960).

(22) A. Kaji, K. Tagawa, and K. Motoyama, *Nippon Nogei Kagaku Kaishi*, 39, 352–357 (1965).

(23) R. J. W. Byrde and A. H. Fielding, *Nature*, 205, 390–391 (1965).

(24) W. W. Reid, *Biochem. J.*, 50, 289–292 (1952).

(25) L. Jurasek and R. Sopko, *Drevar. Vyskum*, No. 2, 71–83 (1962).

(26) A. Kaji and K. Tagawa, *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, 16, 143 (1965).

(27) A. Kaji and O. Yoshihara, *Agr. Biol. Chem.* (Tokyo), 34, 1249–1253 (1970).

(28) A. Kaji and K. Tagawa, *Nippon Nogei Kagaku Kaishi*, 40, 325–330 (1966).

(29) A. Kaji and O. Yoshihara, *Appl. Microbiol.*, 17, 910–913 (1969).

(30) D. A. Bush and R. C. Codner, *Phytochemistry*, 7, 863–869 (1968).

(31) O. A. Akinrefon, *New Phytol.*, 67, 543–556 (1968).

(32) J. H. McClendon and J. H. Kreisher, *Anal. Biochem.*, 5, 295–312 (1963).

(33) A. H. Fielding and R. J. W. Byrde, *J. Gen. Microbiol.*, 58, 73–84 (1969).

(34) F. Laborda, A. H. Fielding, and R. J. W. Byrde, *J. Gen. Microbiol.*, 79, 321–329 (1973).

(35) J. G. Hancock, *Phytopathology*, 57, 203–206 (1967).

(36) J. Ozawa and K. Okamoto, *Rept. Ohara Inst. Agr. Res.*, 40, 103–106 (1952).

(37) R. F. H. Dekker and G. N. Richards, unpublished results.

also attack the main-chain linkages of L-arabinan, they must also be classified as L-arabinanases (that is, as glycan hydrolases) to distinguish them from α-L-arabinofuranosidases (α-L-arabinofuranoside arabinohydrolase, EC 3.2.1.55), which hydrolyze simple glycosides or the α-L-arabinofuranosyl appendages from L-arabinose-containing polysaccharides, but which are not expected to attack the main chain of L-arabinans [for example, (1 → 5)-L-arabinan].

Because no distinction has been made in the literature concerning the two types of enzyme, this Section will discuss those enzymes capable of hydrolyzing L-arabinans and will include most of the reported "α-L-arabinofuranosidases" that, on this basis, should have been classified as L-arabinanases.

2. Occurrence

L-Arabinanases have been reported to be produced by the bacterium *Clostridium felsineum* var. *sikokianum*,[12] saprophytic and phytopathogenic fungi, the snail, and plants (see Table I). Rumen bacteria[38–42] and protozoa,[43–47] and caecal bacteria (sheep)[42] also produce enzymes capable of liberating L-arabinose from arabino-xylans[38–47] and are probably α-L-arabinofuranosidases. Clarke and co-workers[39] showed that the cell-free enzyme preparations from three strains of the rumen bacterium *Butyrivibrio fibrisolvens* were capable of releasing L-arabinose from sugar-beet L-arabinan and arabinoxylan, but the presence of only small proportions of L-arabinose in the L-arabinan hydrolyzate suggests the action of an L-arabinofuranosidase.

L-Arabinanases have been found in terrestrial plants, but had not been reported to be produced by germinating seeds. They have, however, now been found[37] to be produced in germinating seeds of a

(38) B. H. Howard, G. Jones, and M. R. Purdom, *Biochem. J.*, 74, 173–182 (1960).
(39) R. T. J. Clarke, R. W. Bailey, and B. D. E. Gaillard, *J. Gen. Microbiol.*, 56, 79–86 (1969).
(40) B. D. E. Gaillard, R. W. Bailey, and R. T. J. Clarke, *J. Agr. Sci.*, 64, 449–454 (1965).
(41) B. A. Dehority, *Appl. Microbiol.*, 15, 987–993 (1967).
(42) R. W. Bailey and J. C. MacRae, *J. Agr. Sci.*, 75, 321–326 (1970).
(43) R. W. Bailey, R. T. J. Clarke, and D. E. Wright, *Biochem. J.*, 83, 517–523 (1962).
(44) R. W. Bailey and B. D. E. Gaillard, *Biochem. J.*, 95, 758–766 (1965).
(45) R. W. Bailey and R. T. J. Clarke, *Nature*, 199, 1291–1292 (1963).
(46) M. Naga and K. El-Shazly, *J. Gen. Microbiol.*, 53, 305–315 (1968).
(47) A. R. Abou-Akkada, J. M. Eadie, and B. H. Howard, *Biochem. J.*, 89, 268–272 (1963).

tropical and a temperate legume, *Stylosanthes humilis* and *Trifolium subterranum* (Daliak), respectively, degrading sugar-beet L-arabinan to L-arabinose and L-arabinose oligosaccharides of degree of polymerization (d.p.) ⩾ 2.

Most L-arabinanases of fungal origin are usually secreted extracellularly into the medium in which the organism is grown, but intracellular L-arabinanases have also been found to exist, for example, within the mycelial cells of *Sclerotinia fructigena*.[34]

Fungal, extracellular L-arabinanases have been shown to be inductive and constitutive. Several saprophytic fungi were found by Fuchs and coworkers[13] to produce L-arabinanase inductively but not constitutively, whereas several phytopathogenic fungi were found capable of producing L-arabinanases by induction when grown on L-arabinan, and constitutively when these organisms were grown on D-glucose as the sole carbon source. Tagawa and Terui[18] and Kaji and Yoshihara[29] found that L-arabinose, but not the D enantiomorph, could also induce the production of L-arabinanases in *Aspergillus niger*, but found that the yield of enzyme was considerably lower than when the organism was grown on L-arabinan. They suggested that the decreased yield of L-arabinanase by induction on L-arabinose may have been due to the latter's acting as a catabolic repressor in a similar way to the induction of cellulase by cellobiose, as reported by Mandels and Reese.[48]

L-Arabinanases from the snail were found to be present[20] in the digestive juices of the stomach. The origin of their secretion is not yet known.

3. Purification

a. **Assay Procedure.**— Substrates commonly used in the assay procedure for L-arabinanases include L-arabinan (sugar beet), phenyl α-L-arabinofuranoside, and *p*-nitrophenyl α-L-arabinofuranoside.

The assay procedure usually involves measurement of the increase in reducing end-groups liberated during hydrolysis of L-arabinan by the Nelson–Somogyi method[49,50] using alkaline copper solutions,[9–11,15,22] the Sumner method[51] using 3,5-dinitrosalicylic acid in

(48) M. Mandels and E. T. Reese, *J. Bacteriol.*, **79**, 816–826 (1960).
(49) N. Nelson, *J. Biol. Chem.*, **153**, 375–380 (1944).
(50) M. Somogyi, *J. Biol. Chem.*, **160**, 61–68 (1945); **195**, 19–23 (1952).
(51) J. B. Sumner, *J. Biol. Chem.*, **62**, 287–290 (1925).

alkaline solution,[24,52] or the hypoiodite method.[14] Other assays involve estimation of the amount of phenol[23,52] or p-nitrophenol[10,31,34] liberated from phenyl or p-nitrophenyl α-L-arabinofuranoside by measurement of the absorbance at 270 and 400 nm, respectively. The agar "cup-plate" diffusion technique[53] has also been applied to the quantitative determination of L-arabinanase activity; this method is dependent on the linear relationship between the diameter of the zone and log(amount of enzyme).

b. Definition of the L-Arabinanase Unit of Activity.—An L-arabinanase unit of activity is defined as that amount of the enzyme that causes the liberation of reducing end-groups (or phenol and its derivatives) corresponding to the formation of 1 μmole of L-arabinose per minute under defined conditions of temperature, ionic strength, and pH.

c. Separation and Purification of L-Arabinanases.—Large volumes of culture fluid containing extracellularly secreted L-arabinanases have been concentrated by various precipitation procedures by utilization of one of the following precipitants: I, acetone[13,24,31,32]; II, methanol[52]; III, Rivanol (6,9-diamino-2-ethoxyacridinium lactate)[17,22,54]; IV, tannic acid[33] (use of this precipitant has also been shown[13,31] to cause considerable inhibition of the enzyme); and V, ammonium sulfate (most common; see, for example, Refs. 9–11).

Intracellular L-arabinanases[34] have been extracted from the mycelial cells of *Sclerotinia fructigena* by grinding the mycelium with an abrasive such as sand in a mortar at 0° and extracting with a buffer.

Subsequent purification techniques have included: ion-exchange chromatography on DEAE-cellulose,[9,10,14,22,54] DEAE-Sephadex,[9-11] ECTEOLA-cellulose,[30,33] QAE-Sephadex,[11] calcium phosphate,[52] cellulose phosphate,[32,52] CM-cellulose,[12] CM-Sephadex,[33] hydroxylapatite,[9] and SE-Sephadex[9-11]; gel-permeation chromatography on Sephadex G-75 (Refs. 31 and 33), G-100 (Refs. 9–11 and 34), G-200 (Refs. 11 and 55), and Biogel P-300 (Ref. 34); isoelectric focusing at pH 3–10, with sucrose as the anticonvection stabilizer[34]; zone electrophoresis on cellulose acetate[33] or starch[56] as the carrier

(52) H. Gremli and H. Neukom, *Carbohyd. Res.*, **8**, 110–112 (1968).

(53) J. Dingle, W. W. Reid, and G. L. Solomons, *J. Sci. Food Agr.*, **4**, 149–155 (1953).

(54) A. Kaji and K. Tagawa, *Nippon Nogei Kagaku Kaishi*, **38**, 580–584 (1964).

(55) K. Tagawa, *Hakko Kogaku Zasshi*, **48**, 740–746 (1970).

(56) A. Kaji, H. Taki, O. Yoshihara, and A. Shimasaki, *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, **12**, 265–268 (1961).

medium; heat denaturation[10,11]; and crystallization from ammonium sulfate solution.[10]

   d. Criteria of Enzyme Homogeneity.—Many of the L-arabinanases reported in the literature have been only partially purified, and, in some cases, the enzyme preparations have been reported as "pure" but the authors have not demonstrated homogeneity of their preparations. Only three L-arabinanase preparations have been shown to be homogeneous by one or more of the following, commonly accepted criteria: poly(acrylamide) gel electrophoresis,[11] moving-boundary electrophoresis,[10] or ultracentrifugation.[9,11,19]

4. Physicochemical Properties

   L-Arabinanase preparations from most fungal sources (see Refs. 9–15, 22, 27, 31, 33, 34, 52, and 54, and Table I) have been shown to have optimal activity at pH values lying between 2.5 and 6.0. For example, the purified preparations from two strains of *Aspergillus niger*[9,10] showed optimal activity at pH 3.8–4.0, and were stable between pH 3.0 and 8.5. The enzyme produced by *Corticium rolfsii*[11] had, however, a pH optimum at 2.5, and was stable over a wider pH range (1.5–10.0); this enzyme was found to be remarkably stable to very low pH values (1.1–2.5) when kept at 30°, being only completely inactivated after 24 h at pH 1.1, and after 8 days at pH 1.5. It lost only 20 and 40% of its original activity when kept for 20 days at pH 2.5 and 2.0, respectively. *Corticium rolfsii* has also been found to produce other carbohydrate-metabolizing enzymes, for example, an endo-galacturonanase[57] and a β-D-galactosidase,[58] that show this remarkable tolerance to low pH values.

   The L-arabinanase preparation of *Aspergillus niger*[10,15,55] has been shown to be thermostable above 70° when kept at pH values ranging from 6.5 to 8.0, and to lose only 40 and 80% of its original activity when kept at pH 6.0, for 10 min at 70 and 98°, respectively. Another characteristic feature of this enzyme was that, at 50°, it was stable over a range of pH from 3.0 to 8.5, with no appreciable loss of activity. However, at 60°, the enzyme was found to be stable in two pH regions: 3.5–4.8 and 6.5–8.0, but, at pH 4.8–6.5, up to 70% of the original activity was lost. Such results suggested the possibility of the existence of two enzymes; however, these possibilities were excluded, because the enzyme preparation showed only a single

(57) A. Kaji and T. Okada, *Arch. Biochem. Biophys.*, 131, 203–209 (1969).
(58) A. Kaji and T. Ichimi, *Appl. Microbiol.*, 18, 1036–1040 (1969).

component (*a*) in moving-boundary electrophoresis at six different pH values, and (*b*) by gel-permeation chromatography. A study of the kinetics of the heats of inactivation of this enzyme at pH 4.0 and 7.0 suggested that the inactivation of the enzyme (activation energy of 554 and 88.2 kJ.mol^{-1}, respectively) at these pH values was due to some kind of conformational change of the enzyme protein, and that the thermostability at these pH values was, therefore, attributable to the specific conformation of the enzyme molecule. The pH–thermostability relationship was found[15] to be characteristic of the *Aspergillus niger* L-arabinanase only, and was not detectable in enzyme preparations from *Aspergillus japonicus* or *Sclerotinia sclerotiorum* that were also rigorously examined for this unusual pH–stability phenomenon.

Extra- and intra-cellular (EC and IC, respectively) "arabinan-degrading enzymes" have been shown[34] to be produced by *Sclerotinia fructigena*. Fractionation by isoelectric focusing (pH range 3–10) resolved the EC enzyme preparation into 2 fractions [isoelectric point (pI) 3.0 and 6.5], and the IC preparation into 3 fractions (pI 3.0, 4.5, and 6.5), each having L-arabinanase activity. The different enzyme fractions were not examined for homogeneity, but comparative studies of their physicochemical properties (see Table II) showed that the EC enzymes of pI 3.0 and 6.5 were identical to the IC enzymes of the same pI. Enzymes having pI values of 3.0 and 6.5 were found to be optimally active and stable at low pH values, and, in this respect, were similar to other L-arabinanases from phytopathogenic fungi. By contrast, however, the IC enzyme of pI 4.5 was more stable and showed optimum activity at neutral pH values

TABLE II

Some Properties of "Arabinan-degrading Enzymes" of *S. fructigena*[34]

Source of enzyme	pI[a]	Optimum pH	Stable at pH	Temp. (°C) of complete thermal inactivation
Culture fluid	3.0	3.0–4.0	3–8	60
(EC[b])	6.5	5.0	3–8	55
Mycelium	3.0	3.0–4.0	3–8	60
(IC[c])	4.5	5.7–6.0	6–9	60
	6.5	5.0	3–8	55

[a] pI denotes the isoelectric point. [b] EC denotes extracellular enzymes. [c] IC denotes intracellular enzymes.

TABLE III

The Isoelectric Points and Molecular Weights of Several
L-Arabinanases of Different Fungal Origin

Source of enzyme	pI	Molecular weight[a]	References
Aspergillus niger (K1)	3.6	53,000	10,55
Phytophthora palmivora	—[b]	15,850	
	—	63,100	31
Sclerotinia fructigena	—	30,500	
	—	53,700	33
	3.0	200,000	
	4.5	350,000	34
	6.5	40,000	
Commercial preparation (Pectinol R-10)	3.3	—	52

[a] Determined from gel-permeation chromatographic data. [b] — indicates not determined.

and above (see Table II). All enzymes were thermally inactivated at temperatures above 60° during 10 min at pH 7.0.

The pI values of several L-arabinanases of different fungal origin have been reported, and are listed in Table III.

Like such other polysaccharide-degrading enzymes as cellulases,[59,60] galactanases, D-mannanases, and D-xylanases (see Sections III, IV, and VI for references), L-arabinanases have been shown to be relatively small molecules with molecular weights (M.W.) ranging from 15,850 to 63,100 (see Table III). Two "arabinan-degrading enzymes" produced by *Sclerotinia fructigena*[34] were, however, found to be rather large molecules with a respective M.W. of 200,000 and 350,000. These enzymes are probably better regarded as glycosidases, that is, α-L-arabinofuranosidases, than as polysaccharases (arabinanases), by analogy with data derived from the M.W. of other glycosidases (see Refs. 61–63). Evidence to support this statement is derived from the work of Eriksson and his associates,[61,62] who dem-

(59) G. Keilich, P. Bailey, and W. Liese, *Wood Sci. Technol.*, 4, 273–283 (1970).
(60) K. E. Eriksson and B. Pettersson, *Int. Biodeterior. Bull.*, 7, 115–119 (1971).
(61) E. Ahlgren, K. E. Eriksson, and O. Vesterberg, *Acta Chem. Scand.*, 21, 937–944 (1967).
(62) E. Ahlgren, K. E. Eriksson, and O. Vesterberg, *Acta Chem. Scand.*, 21, 1193–1200 (1967).
(63) P. M. Dey and J. B. Pridham, *Advan. Enzymol.*, 36, 91–130 (1972).

onstrated that the molecules of fungal glycosidases are generally larger than those of the related, polysaccharide-degrading enzymes.

The amino acid composition of the crystalline L-arabinanase preparation isolated from *Aspergillus niger* showed[10] relatively small proportions of proline and methionine, and rather larger proportions of serine and threonine; this highly purified preparation was also found to contain some carbohydrate material (its composition was not reported) and it is, therefore, probably a glycoprotein.

L-Arabinanases from *A. niger* have been shown[19,55] to be inhibited or inactivated by certain heavy-metal ions, such as Hg^{2+} and Ag^+. Fe^{3+} was also found to be slightly inhibitory (26%), whereas other metal cations (Mn^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, and Co^{2+}) did not exert any significant effect on the activity. Urea (6 M) irreversibly denatured the *A. niger* enzyme.[55]

The crude, enzyme preparation from *Phytophthora palmivora*[31] was shown to be activated by Ca^{2+} and by some phenolic compounds (such as L-epicatechol) and inhibited by L-arabinose and various plant phenolic analogues, especially the leucoanthocyanins.

The highly purified, crystalline L-arabinanase preparation from *A. niger*[10] was found to be capable of hydrolyzing both the (1 → 3)- and (1 → 5)-α-L-arabinofuranosyl linkages of L-arabinan.[64] An investigation[65] of the kinetic properties of this enzyme revealed that one active site participated in the hydrolysis of both types of linkage. The Michaelis constant (K_m) and the maximum velocity (V_m) values are shown in Table IV. The V_m values indicate that the α-L-(1 → 3)-linkages are hydrolyzed faster than the α-L-(1 → 5)-linkages. L-Arabinose was shown to act as a competitive inhibitor in the hydrolysis of phenyl α-L-arabinofuranoside. The inhibition constant (K_i) of the arabinose–enzyme complex was found to be 19.5 mM L-arabinose (that is, 2.93 mg of L-arabinose/ml). In a two-substrate system, both L-arabinan and (1 → 5)-L-arabinan were found to inhibit, competitively, the hydrolysis of phenyl α-L-arabinofuranoside, the K_i values being 0.46 and 22.0 mg/ml, respectively; these values are of the same order of magnitude as the K_m values (see Table IV), and suggest that the same active site is involved in the hydrolysis of both types of linkage in L-arabinan. Other evidence, presented by Tagawa[65] to support the validity of a single active site, was based on the energy of activation (Arrhenius method), which showed no significant difference between each of the three different substrates

(64) K. Tagawa and A. Kaji, *Carbohyd. Res.*, 11, 293–301 (1969).
(65) K. Tagawa, *Hakko Kogaku Zasshi*, 48, 730–739 (1970).

TABLE IV

K_m and V_m Values of Some L-Arabinanases of Different Fungal Origin

		K_m Substrates[a]			V_m (μmoles of Ara/mg of protein/min) Substrates[a]		
Source of arabinanase	References	PhLAra (mM)	A (mg/ml)	1,5 A (mg/ml)	PhLAra	A	1,5 A
Aspergillus niger	9,19	4.48	0.26	—	—	—	—
A. niger (K1)	10,65	4.70	0.26	20.40	420	38.7	12.7
Corticium rolfsii	11	2.86	8.47	28.60	124	53	16.7
Phytophthora palmivora	31	0.65[b]					
Sclerotinia fructigena I	34	0.12[c]			—		
II		0.19[c]			—		
III		1.20[c]			—		

[a] PhLAra = phenyl α-L-arabinofuranoside, A = L-arabinan, Ara = arabinose, 1,5 A = (1 → 5)-L-arabinan (see Ref. 64). [b] This enzyme preparation was found to consist of two arabinanases, and the K_m value given is that of the combined enzymes at pH 4.0 (at pH 4.7, K_m was 1.55 mM). [c] This is the value of K_m at the optimum pH of each of these enzymes (see Table II). The substrate used was p-nitrophenyl α-L-arabinofuranoside.

[48.7 kJ.mol^{-1} for phenyl α-L-arabinofuranoside, 46.6 for L-arabinan and 48.3 for (1 → 5)-L-arabinan], and on the effect of pH on the V_m value for each of the three substrates. In the latter, the agreement between the V_m–pH profiles of L-arabinan and (1 → 5)-L-arabinan indicated that the same ionizable groups are involved in the hydrolysis of both linkages.

The kinetic parameters of other L-arabinanases of fungal origin are also shown in Table IV. The crude, enzyme preparation of *Phytophthora palmivora*[31] was found to be competitively inhibited by an arabinogalactan having a K_i value of 5.83 mM.

5. Mode of Action

Two types of L-arabinanase have been detected, namely, exo and endo.

a. Endo-L-arabinanases.—L-Arabinanases of the endo type have been found to be produced by the bacterium *Clostridium felsineum*,[12] various fungi (for example, *Botrytis cinerea*,[22] *Coniothyrium diplodiella*,[26] *Gloeosporium kaki*,[22] and *Sclerotinia sclerotiorum*[22]) and germinating seeds[37] of *Stylosanthes humilis* and *Trifolium subterranum* (Daliak). These enzymes have not been purified, and the preparations used for substrate studies have usually been the culture fluid or the ammonium sulfate precipitate. The crude, enzyme preparations from fungi other than those just mentioned may also produce the endo-enzyme, but failure to detect such enzymes may be due to the "masked action," in these preparations, of exo-enzymes which are likely to degrade any arabino-oligosaccharides arising from endo-enzyme action on arabinan. The nature of the products arising from endo-attack on L-arabinan is not yet known, as there have been no reports in which end-products have been isolated or chemically characterized.

The action of the L-arabinanase(s) extracted from germinating seeds[37] on sugar-beet L-arabinan yielded L-arabinose and an L-arabinose oligosaccharide (probably a disaccharide) as major products of hydrolysis, with smaller proportions of L-arabinose oligosaccharides of d.p. > 2.

b. Exo-L-arabinanases.—Most of the L-arabinan-degrading enzymes studied have been of the exo type, degrading L-arabinan completely to L-arabinose.

The highly purified enzyme-preparations of *Aspergillus niger*[9,10,19,64,65] and *Corticium rolfsii*[11] have been shown to be capable of hydrolyzing both the (1 → 3)- and (1 → 5)-α-L-arabinofuranosyl

residues of beet L-arabinan, and the $(1 \rightarrow 3)$-α-L-arabinofuranosyl residues of wheat L-arabino-D-xylan and gum arabic. These enzymes are also capable of liberating L-arabinose from phenyl α-L-arabinofuranoside, and are specific for the furanoside, the pyranoside being resistant to attack.

The *A. niger* enzyme hydrolyzes both types of linkage of L-arabinan at one active site, and the substrate is attacked from the nonreducing end by a multi-chain mechanism[10,65] (see Greenwood and Milne[66] for a general discussion of single- and multi-chain mechanisms).

In its attack on L-arabinan, the *A. niger* enzyme[10,65] was found to hydrolyze the substrate rapidly to the extent of 30%; thereafter, attack was slow. This initial, rapid hydrolysis of L-arabinan corresponds to the favored attack on the α-L-$(1 \rightarrow 3)$-linked L-arabinofuranosyl residues, leaving a mainly linear $(1 \rightarrow 5)$-α-L-arabinan which was slowly, and eventually, completely, hydrolyzed to L-arabinose. Tagawa and Kaji[64] utilized this unique property of their enzyme preparation to prepare from the parent L-arabinan a linear $(1 \rightarrow 5)$-L-arabinan which was subsequently purified and characterized. In a similar way, wheat arabinoxylan yielded a predominantly linear $(1 \rightarrow 4)$-β-D-xylan.

Gremli and Neukom,[52] using a partially purified L-arabinanase preparation isolated from Pectinol R-10, found that their preparation would degrade sugar-beet L-arabinan to the extent of only 50%, whereas the crude preparation completely hydrolyzed the polymer to L-arabinose, D-galactose, and L-rhamnose. The limit-arabinan, not attacked by the enzyme, was found to be of high M.W., and total hydrolysis with acid revealed L-arabinose, D-galactose, and L-rhamnose in the ratios 5:3:1, whereas the parent L-arabinan contained these sugars in the ratios of 15:3:1. The failure of the partially purified enzyme to hydrolyze L-arabinan completely suggests that the D-galactose and L-rhamnose residues are situated within the molecular framework of L-arabinan, and that they block exo action. However, these residues are susceptible to attack by the crude enzyme-preparation, presumably because of the action of other enzymes. These observations supported the view of Hough and Powell[67] that D-galactose and L-rhamnose residues are constituents of the L-arabinan molecule, and that they are concentrated in the inner parts of the molecule.

(66) C. T. Greenwood and E. A. Milne, *Advan. Carbohyd. Chem.*, **23**, 281–366 (1968).
(67) L. Hough and D. B. Powell, *J. Chem. Soc.*, 16–22 (1960).

The production of an "enzyme-resistant limit-arabinan" (containing D-galactose and L-rhamnose residues) from a highly purified, sugar-beet L-arabinan preparation was not observed by Kaji and his associates.[64,65]

III. D-GALACTANASES

I. Introduction

D-Galactanases are hydrolytic enzymes capable of degrading D-galactans and L-arabino-D-galactans. Two types of D-galactanase have been reported[68-71] that are specific for (1 → 3)- and (1 → 4)-β-D-galactopyranosyl linkages. Enzymes of this type have not yet been classified by the Enzyme Commission of the International Union of Biochemistry, and we have therefore assigned to these D-galactanases the following systematic names: (1 → 3)-β-D-galactan galactanohydrolase and (1 → 4)-β-D galactan galactanohydrolase. Both enzymes have been shown[68-71] to degrade D-galactan randomly, to afford D-galactose and D-galacto-oligosaccharides, and they are therefore endo-D-galactanases. An "exo-(1 → 4)-β-D-galactanase" has been reported to be produced by *Sclerotium rolfsii*.[72]

2. Occurrence

D-Galactanases have been reported to be produced by *Bacillus subtilis*, by a rumen anaerobic bacterium, by fungi, and by plants (see Table V). D-Galactanases are inductive, and those of microbial origin are usually produced extracellularly in response to the carbon source of the culture medium.

(68) S. Emi and T. Yamamoto, *Agr. Biol. Chem.* (Tokyo), **36**, 1945–1954 (1972).
(69) S. Emi, J. Fukumoto, and T. Yamamoto, *Agr. Biol. Chem.* (Tokyo), **35**, 1891–1898 (1971).
(70) Y. Hashimoto, Y. Tsujisaka, and J. Fukumoto, *Nippon Nogei Kagaku Kaishi*, **43**, 831–836 (1969).
(71) Y. Hashimoto, *Nippon Nogei Kagaku Kaishi*, **45**, 147–150 (1971).
(72) H. D. van Etten and D. F. Bateman, *Phytopathology*, **59**, 968–972 (1969).
(73) J. H. McClendon, G. F. Somers, and J. W. Heuberger, *Phytopathology*, **50**, 258–261 (1960).
(74) N. J. King, *Biochem. J.*, **100**, 784–792 (1966).
(75) J. Ozawa, *Rept. Ohara Inst. Agr. Res.*, **40**, 110–112 (1952).
(76) A. L. J. Cole, *Phytochemistry*, **9**, 337–340 (1970).
(77) M. Knee and J. Friend, *J. Gen. Microbiol.*, **60**, 23–30 (1970).
(78) M. Knee and J. Friend, *Phytochemistry*, **7**, 1289–1291 (1968).
(79) W. W. Reid, *J. Sci. Food Agr.*, **1**, 234–240 (1950).

TABLE V

Sources of D-Galactanases

Organism	References
Bacteria	
Bacillus subtilis var *amylosacchariticus*	68
subtilis (K-50)	69
Rumen bacteria	
Butyrivibrio fibrisolvens (PC/103)	39
fibrisolvens (PC/104)	39
Fungi	
Botryosphaeria ribis	73
Byssochlamys fulva	24
Coniophora cerebella	74
puteana	25
Gibberella saubinetti	75
Gloesoporium kawakamii	75
Helminthosporium oryzae	75
Penicillium expansum	75
Phytophthora infestans	76–78
Piricularia oryzae	75
Rhizopus niveus	70,71
tritici	32,75
Sclerotinia cinerea	75
sclerotiorum	35
Sclerotium rolfsii	72
Trametes gibbosa	73
Various micro fungi	36
Various phytopathogenic fungi	13
Plants	79

3. Purification

a. Assay Procedure.—Substrates commonly used in the assay of D-galactanase activity include coffee-bean arabinogalactan,[68–71] soybean arabinogalactan,[68,69] larch arabinogalactan,[74] larch galactan,[39] lupin D-galactan,[32,39,72,78] potato D-galactan,[76,78] and pectic acid fractions rich in D-galactose.[24,74,77]

The assay procedures usually involve the measurement of the increase in reducing end-groups by the Nelson–Somogyi,[70,74,76,78] Shaffer–Somogyi,[68,69] or the Sumner[24] method.

b. Definition of the D-Galactanase Unit of Activity.—The unit of D-galactanase activity is usually defined as that amount of enzyme that causes the liberation of reducing end-groups corresponding to the

formation of 1 μmole of D-galactose per minute under defined conditions of temperature, ionic strength, and pH.

c. Concentration of Culture Solutions.—Precipitants that have been used to concentrate D-galactanases present in large volumes of culture fluid include ammonium sulfate,[68-70,76,78] isopropyl alcohol,[68,69] acetone,[24,32,68] and Rivanol.[70]

d. Separation and Purification of D-Galactanases.—Mixtures of D-galactanases have been fractionated and purified by using the most modern protein-purification techniques.[80] Ion-exchange chromatography has been used with anion exchangers [DEAE-Sephadex[69,70] and Duolite A-2 (Refs. 68 and 69)] and cation exchangers (cellulose phosphate,[32] CM-cellulose,[68] and SE-Sephadex[68,69]). Gel-permeation chromatography has been used with Sephadexes G-75 (Refs. 68 and 69) and G-100 (Refs. 70 and 76). Isoelectric focusing[68,69] at pH 3 to 10 was used with sucrose as the anticonvection stabilizer. Affinity-binding chromatography[70] has been used to remove a D-mannanase contaminant from D-galactanase F-III during purification. Crystallization from cold acetone solution[68,69] has also been utilized.

e. Criteria of Purity.—Several D-galactanases[68,70] from different sources have been purified by a combination of the purification techniques just outlined; the products behaved as single, symmetrical peaks in gel-permeation chromatography and isoelectric focusing. However, the enzyme elaborated by *Bacillus subtilis* (K-50) is the only preparation[69] that has been shown to be homogeneous (for example, by ultracentrifugation).

4. Physicochemical Properties

a. (1 → 4)-β-D-Galactanases.[68,69]—Purified (1 → 4)-β-D-galactanases from *Bacillus subtilis*[68,69] showed optimal activity at pH 6–7, and at 55–60°, and were found to be stable over a pH range of 5.0–10.5. They were completely inactivated when kept at 65–70° for 15 minutes at pH 7.5–8.0. The D-galactanases elaborated by *B. subtilis* var. *amylosacchariticus*[68] (namely, D-galactanases I–III) were found to be stabilized by Ca^{2+}, and sensitive to such metal chelators as (ethylenedinitrilo)tetraacetate (EDTA) (see Table VI).

On the other hand, the D-galactanase from *B. subtilis* (K-50) showed no dependency towards metal ions.[69]

The pI and M.W. values for several D-galactanases from *B. subtilis* are given in Table VII. These enzymes are considered to be neutral (pI 6.88) and alkaline (pI 7.96–8.90) glycan hydrolases, and their

(80) *Methods Enzymol.*, **22** (1971).

TABLE VI

The Effect of Ca²⁺ on the Activity and Stability of Several Purified
(I → 4)-β-D-Galactanases from *B. subtilis* var. *amylosacchariticus*[68]

Galactanase	pH optimum		Temperature optimum (°C)		pH stability		Complete thermal inactivation (°C)	
	Ca²⁺ᵃ	EDTAᵇ	Ca²⁺	EDTA	Ca²⁺	EDTA	Ca²⁺	EDTA
I	7.0	7.0	60	45	5.5–10.5	inactive	70	50
II	6.0	6.0	60	50	5.5–10.5	inactive	70	50
III	6.5	6.5	60	50	5.5–10.5	inactive	65	50

ᵃ Calcium acetate (2 mM). ᵇ EDTA (2 mM).

sizes (as suggested by the M.W.) are comparable to those of other polysaccharases; for examples, see Sections II, IV, and VI.

The K_m values for the various D-galactanases from *B. subtilis* are shown in Table VII; they further demonstrate that the enzymes are different.

The purified enzyme-preparation from[69] *B. subtilis* K-50 was inactivated by Fe^{2+}, Fe^{3+}, Ag^+, and Hg^{2+}. The sedimentation coefficient of this enzyme was found to be 3.35.

b. (1 → 3)-β-D-Galactanases.[70] — Four purified (1 → 3)-β-D-galactanases produced by the fungus *Rhizopus niveus*[70] showed optimal activity at pH 5.0 and 40°, and were found to be stable over a pH range of 3.0 to 9.0; they were completely inactivated when kept at 65–70° for 10 minutes at pH 5.0. No other physical properties were reported for these enzymes.

TABLE VII

Some Properties of (1 → 4)-β-D-Galactanases from *Bacillus subtilis*[68,69]

Strain	Enzyme	pI	M.W. × 10⁴	K_m (mg/ml)
Amylosacchariticus (1043)	I	6.88	35ᵃ	0.43
	II	7.96	36ᵃ	1.07
	III	8.90	32ᵃ	0.86
K-50	A	8.39	37ᵇ	1.13

ᵃ Based on gel-permeation chromatographic data. ᵇ Calculated from sedimentation-equilibrium data.

5. Mode of Action

a. (1 → 4)-β-ᴅ-Galactanases. — The purified ᴅ-galactanase prepara-
tions (G I-III) from *B. subtilis* var. *amylosacchariticus*[68] were found
to be specific for (1 → 4)-β-ᴅ-galactopyranosyl linkages; they do not
attack coffee arabinogalactan, which contains mainly β-ᴅ-(1 → 3)-
linked ᴅ-galactopyranosyl residues (see Refs. 71 and 81 for the struc-
ture of coffee arabinogalactan).

The three ᴅ-galactanases degraded soybean arabinogalactan to ᴅ-
galactose, galactobiose, and galactotriose as major products, with
trace amounts of mixed oligosaccharides containing both ʟ-arabinose
and ᴅ-galactose residues. Arabinose was not detected in the enzymic
hydrolyzates, although the arabinogalactan was degraded to the ex-
tent of 28–34%.

The ᴅ-galactanase preparation from *B. subtilis* K-50 was also
found[69] to be specific for (1 → 4)-β-ᴅ-galactopyranosyl linkages, de-
grading   soybean   arabinogalactan   to   galactobiose   [*O*-β-ᴅ-Gal*p*-
(1 → 4)-ᴅ-Gal*p*] as the only major product after 24 h of hydrolysis,
with smaller proportions of ᴅ-galactose and mixed oligosaccharides
containing both ʟ-arabinose and ᴅ-galactose. At this stage, the re-
ducing power corresponded to 33% hydrolysis of the polysaccharide.
These results are rather unusual, in that the hydrolyzates contained
no major proportions of ʟ-arabinose or arabinose–galactose oligosac-
charides, and, as soybean arabinogalactan has been found[82] to consist
of two ʟ-arabinose residues per 4 to 5 ᴅ-galactose residues in the
main chain, it would be expected that a higher yield of arabinose–
galactose oligosaccharides would result from such a hydrolysis. From
the results presented by Emi and coworkers,[69] it is difficult to class-
ify this enzyme as either endo or exo.

A crude preparation of a (1 → 4)-β-ᴅ-galactanase from *Sclerotium
rolfsii*[72] hydrolyzed *Lupinus albus* ᴅ-galactan to ᴅ-galactose only;
this result indicates that this preparation may contain an exo-enzyme.

b. (1 → 3)-β-ᴅ-Galactanases. — Four   ᴅ-galactanases   produced   by
Rhizopus niveus were purified by Hashimoto and coworkers.[70] These
enzyme preparations degraded coffee arabinogalactan to various ex-
tents ranging from 6.5–14.0%.

The action pattern of only one of these ᴅ-galactanases, namely, ᴅ-
galactanase F-III, has been studied[71] in detail. This preparation

(81) M. L. Wolfrom and D. L. Patin, *J. Org. Chem.*, **30**, 4060–4063 (1965).
(82) M. Morita, *Agr. Biol. Chem.* (Tokyo), **29**, 564–573, 626–630 (1965).

TABLE VIII

Degradation Products Arising[71] from the Enzymic Degradation of
Coffee Arabinogalactan by Galactanase F-III

Product of hydrolysis[a]	D.p.	Relative yield (%)	Ratio of arabinose to galactose
Arabinose	1	13.1	—
Galactose	1	12.6	—
Oligosaccharide I	2	0.9	1:1
II	2	22.3	—
III	3	0.8	1:2
IV	4	0.9	1:3
V	4	5.7	1:3

[a] In order of decreasing chromatographic mobility.

degrades coffee arabinogalactan to L-arabinose, D-galactose, galac-tobiose, and a series of mixed arabinogalacto-oligosaccharides (see Table VIII). The presence of L-arabinose in the digests indicates either that the purified galactanase F-III preparation contains an "arabinofuranosidase" contaminant, or that it is a D-galactanase having multisubstrate specificity. Multisubstrate specificity has been demonstrated with highly purified xylanases which were shown to be capable of hydrolyzing both types of linkage in arabinoxylans (see Section VI).

The structures of several oligosaccharides (see Table VIII) arising from enzymic degradation of coffee arabinogalactan have been deter-mined[71] by periodate oxidation and by using the specificities of purified preparations of β-D-galactosidase and of D-galactanase (F-III) from *Rhizopus niveus*. The hydrolysis products arising from the specific enzymolysis of these oligosaccharides are summarized in Table IX.

Galactobiose [II, O-β-D-Gal$p$-(1 → 6)-D-Gal$p$] is not attacked by D-galactanase F-III, demonstrating that the enzyme is specific only to (1 → 3)-β-D-galactopyranosyl linkages. The enzyme was also capable of removing L-arabinofuranose from arabinogalactosides I, III, and V, but did not liberate any L-arabinose from oligosaccharide IV (arab-inose:galactose = 1:3). Partial hydrolysis of IV with acid did not yield arabinose, confirming that, in this oligosaccharide, the L-arabinose residue is present in the pyranoid form. Enzymic degrada-tion by D-galactanase F-III of oligosaccharide IV yielded galac-tobiose and an arabinose–galactose disaccharide of unknown linkage, which was resistant to further enzymic attack.

TABLE IX

Products of Hydrolysis Resulting[71] from Enzymic and Acid Hydrolysis of Arabinogalacto-oligosaccharides I–V

Arabino-galacto-oligo-saccharides[a]	Hydrolysis products arising from			Periodate consumed (moles/mole of substrate)		Structure proposed
	Partial acid hydrolysis[b]	Galactanase F-III hydrolysis	β-D-Galactosidase hydrolysis	Calculated	Found	
I (AG)	n.d.	Ara + Gal	no attack	2	n.d.	O-α-L-Ara$f$-(1 → 3)-D-Gal$p$
II (G_2)	n.d.	no attack	Gal	5	4.27	O-β-D-Gal$p$-(1 → 6)-D-Gal$p$
III (AG$_2$)	n.d.	Ara + G$_2$(II)	no attack	4	3.8	O-α-L-Ara$f$-(1 → 3)-O-β-D-Gal$p$-(1 → 6)-D-Gal$p$
IV	G$_2$(II)	(A–G)[c] + G$_2$(II)	no attack		n.d.	O-?-L-**Ara**p-(1 → ?)-O-β-D-Gal$p$-(1 → 3)-O-[β-D-Gal$p$-(1 → 6)]-D-Gal$p$
V	Ara + G$_2$ + AG$_2$	Ara + Gal + G$_2$(II) + AG$_2$(III)	Gal + AG$_2$(III)	4	4.8	O-α-L-Ara$f$-(1 → 3)-O-β-D-Gal$p$-(1 → 6)-D-Gal$p$-(1 → 3)-D-Gal$p$

[a] See Table VIII. [b] Hydrochloric acid (0.0–2.0 M) for 20 h at 4°. Ara = L-arabinose (A), Gal = D-galactose (G), n.d. = not determined.
[c] (A–G) = unknown linkage, but A is in the pyranoid form.

The derivation of the structures of the oligosaccharides I–V is summarized in Table IX, and this approach, when combined with the results of earlier methylation studies,[81] suggests that coffee-bean arabinogalactan consists mainly of a "linear" backbone chain of

$$
\begin{array}{c}
\alpha\text{-Ara}f \\
1 \\
\downarrow \\
3
\end{array}
$$

$$
\begin{array}{cccccc}
\alpha\text{-Ara}f & \text{?-Ara}p & \beta\text{-Gal}p & & \beta\text{-Gal}p & \beta\text{-Gal}p \\
1 & 1 & 1 & & 1 & 1 \\
\downarrow & \downarrow & \downarrow & & \downarrow & \downarrow \\
3 & \text{?} & 6 & & 6 & 6
\end{array}
$$

$-\beta\text{-Gal}p\text{-}(1\rightarrow3)\text{-}\beta\text{-Gal}p\text{-}(1\rightarrow3)\text{-}\beta\text{-Gal}p\text{-}(1\rightarrow3)\text{-}\beta\text{-Gal}p\text{-}(1\rightarrow3)\text{-}\beta\text{-Gal}p\text{-}(1\rightarrow3)\text{-}\beta\text{-Gal}p\text{-}(1\rightarrow3)\text{-Gal}p\cdot$
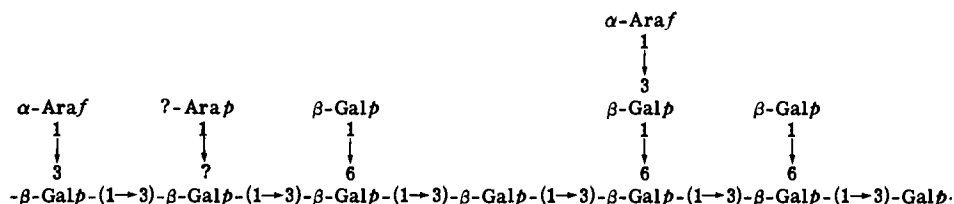
Coffee-bean Arabinogalactan

[β-D-(1→3)-Linked D-galactopyranosyl residues and α-L-(1→3)-linked L-arabinofuranosyl groups are labile to enzyme F-III; and β-D-(1→6)-linked D-galactopyranosyl groups (or residues) and ?-L-(1→?)-linked L-arabinopyranosyl groups are stable to F-III.]

(1 → 3)-β-D-galactopyranosyl residues carrying (1 → 3)-α-L-arabinofuranosyl and (1 → 6)-β-D-galactopyranosyl groups as side chains, as well as an occasional, interposed, (1 → 6)-β-D-galactopyranosyl entity as illustrated. The frequency of occurrence of additional L-arabinopyranosyl side-branch units in the polysaccharide chain is not yet known.

IV. D-MANNANASES

1. Introduction

D-Mannanases [(1 → 4)-β-D-mannan mannanohydrolases, endo-D-mannanases, EC 3.2.1.78] are hydrolytic enzymes capable of hydrolyzing the (1 → 4)-β-D-mannopyranosyl linkages of D-mannans and D-galacto-D-mannans.[83–86] The highly purified enzyme-preparations from *Bacillus subtilis*[83] and *Aspergillus niger*[85] have, however, also been shown to be capable of degrading the D-gluco-D-mannans of konjac (*Amorphophallus konjac*) and arum root, yielding D-glucose, D-mannose, and a series of manno- and glucomanno-oligosaccharides. The presence, in the enzymic digests, of D-glucose and

(83) S. Emi, J. Fukumoto, and T. Yamamoto, *Agr. Biol. Chem.* (Tokyo), **36**, 991–1001 (1972).
(84) K. E. Eriksson and M. Winell, *Acta Chem. Scand.*, **22**, 1924–1934 (1968).
(85) Y. Tsujisaka, K. Hiyama, S. Takenishi and J. Fukumoto, *Nippon Nogei Kagaku Kaishi*, **46**, 155–161 (1972).
(86) Y. Hashimoto and J. Fukumoto, *Nippon Nogei Kagaku Kaishi*, **43**, 317–322 (1969).

Organism	References
Bacteria	
Aerobacter mannanolyticus	87,88
Bacillus aroideae	89
mesentericus	90
subtilis	83,91–93
subtilis var. *amylosacchariticus*	68
Sporocytophaga myxococcoides	94
Rumen bacteria	
Streptococcus sp.	95
Rumen protozoa (mixed)	96
Fungi	
Aspergillus awamori	97
fumigatus	93,98
giganteus	93
luchuensis	93
nidulans	93,98
niger	59,84,97,99–101
niger van Tieghem	85
oryzae	91,97,102
phoenicis	98
wentii	59
Botryosphaeria ribis	73
Botrytis cinerea	98
Cephalosporium sp.	103
Chaetomium globosum	59,93,104
Chrysosporium lignorum	61,105
Cladosarum olivaceum	93
Collybia velutipes	104
Coniophora cerebella	74,104
Epicoccum yuccae	93
Fomes annosus	61
igniarius	104
marginatus	104
Fusarium culmorum	98
oxysporum	98
Fusicoccum sp.	93
Gelatinosporium sp.	93
Gloeophyllum sepiarium	106
Lenzites saepiaria	104
Memnoniella echinata	93
Merulius silvester	104
Myrothecium roridum	98
verrucaria	98,107
Paecilomyces varioti	93
Penicillium chrysogenum	98,108
expansum	93
funiculosum	93
isariiforme	93
notatum	98,108
ochro-chloron	93

TABLE X (*continued*)

Organism	References
paxillus	109
piscarium	93
verruculosum	93
wortmanni	93
Phellinus igniarus	106
Polyporus betulinus	104
schweinitzii	59
Puccinia graminis var. *tritici*	109(b)
Rhizopus niveus	86,101
tritici	32
Schizophyllum commune	104
Sclerotium rolfsii	72
Sporotrichum pruinosum	93
Stereum sanguinolentum	61
Trametes versicolor	104
Trichoderma viride	93,104
Trichurus spiralis	93

Mycorrhiza fungi

Amanita muscaria	106
Leccinum scabrum	106
Suillus bovinus	106
luteus	106
Xerocomus badius	106

Plants

(a) **Terrestrial**

Amorphophallus konjac (tuber)	110
Asparagus officinalis (seed)	111
Barley (seed)	103,112
Cyamopsis tetragonolobus (seed)	103,112–114
Leucaena glauca (seed)	115,116
Medicago sativa (seed)	116
Phoenix dactylifera (seed)	117
Trigonella foenum-graecum (seed)	118–121
Vicia sativa (seed)	122

(b) **Marine (algae)**

Cladophora rupestris	123
Laminaria digitata	123
Rhodymenia palmata	123
Ulva lactuca	123

Animals (invertebrates)

Astacus fluviatilis Fabr.	124
Helix pomatia	93,125
Homarus vulgaris M-E	124
Gypsoma aceriana	126
Melasoma populi	126
Melolontha vulgaris (Hanneton)	127
Polyphylla fullo	126
Rhagium inquisitor L.	128
Sciapteron tabaniformis	126

(87) K. Inoue and N. Inoue, *Ann. Rept. Nat. Inst. Nutrition* (Tokyo), 52 (1956); 67–69 (1958).

(88) S. Innami, *Agr. Biol. Chem.* (Tokyo), **25**, 155–163 (1961).

(89) T. Otsuki, *Botan. Mag.* (Tokyo), **34**, 173 (1937).

(90) M. Mayeda, *J. Biochem.* (Tokyo), 1, 131–137 (1922).

(91) P. Z. Allen and W. J. Whelan, *Biochem. J.*, **88**, 69–70 (1963).

(92) J. E. Courtois and P. Le Dizet, *Bull. Soc. Chim. Biol.*, **50**, 1695–1710 (1968).

(93) E. T. Reese and Y. Shibata, *Can. J. Microbiol.*, 11, 167–183 (1965).

(94) S. Clermont, M. Charpentier, and F. Percheron, *Bull. Soc. Chim. Biol.*, **52**, 1481–1495 (1970).

(95) P. P. Williams and R. N. Doetsch, *J. Gen. Microbiol.*, **22**, 635–644 (1960).

(96) R. W. Bailey and B. D. E. Gaillard, *Aust. J. Biol. Sci.*, **22**, 267–269 (1969).

(97) A. A. Kuznetsov and B. N. Stepanenko, *Prikl. Biokhim. Mikrobiol.*, 7, 651–658 (1971).

(98) H. Lyr and E. Novak, *Z. Allegem. Mikrobiol.*, 2, 86–98 (1961).

(99) A. A. Kuznetsov and B. N. Stepanenko, *Biokhimya*, **32**, 558–563 (1967).

(100) Y. Misawa, M. Matsubara, M. Hatano, M. Hara, and T. Inuzuka, *Nippon Shokuhin Kogyo Gakkaisshi*, 14, 286–291 (1967).

(101) Y. Hashimoto, *Nippon Nogei Kagaku Kaishi*, **44**, 287–292 (1970).

(102) A. A. Kuznetsov and B. N. Stepanenko, *Biokhimya*, **32**, 368–376 (1967).

(103) R. L. Whistler, W. H. Eoff, and D. M. Doty, *J. Amer. Chem. Soc.*, **72**, 4938–4949 (1950).

(104) H. Lyr, *Z. Allegem. Mikrobiol.*, 3, 25–36 (1963).

(105) K. E. Eriksson and W. Rzedowski, *Arch. Biochem. Biophys.*, **129**, 683–688 (1969).

(106) G. Ritter, *Z. Allegem. Mikrobiol.*, 4, 295–312 (1964).

(107) P. Kooiman, *Enzymologia*, 18, 371–384 (1957).

(108) H. O. Bouveng, T. Iwasaki, B. Lindberg, and H. Meier, *Acta Chem. Scand.*, 17, 1796–1797 (1963).

(109) (a) J. E. Courtois and P. Le Dizet, *Bull. Soc. Chim. Biol.*, **45**, 743–747 (1963); (b) C. F. van Sumere, C. van Sumere-De Preter, and G. A. Ledingham. *Can. J. Microbiol.*, 3, 761–770 (1957).

(110) N. Sugiyama, H. Shimahara, T. Andoh, and M. Takemoto, *Agr. Biol. Chem.* (Tokyo), **37**, 9–17 (1973).

(111) R. Goldberg, *Compt. Rend.*, **261**, 5605–5608 (1965).

(112) C. van Sumere, *Naturwissenschaften*, 40, 582 (1953).

(113) S. R. Lee, *Soul Taehakkyo Nonmunjip, Chayon Kwahak, Saengnongge*, 17, 7–22 (1966).

(114) J. Sehgal, H. S. Nainawatee, and B. M. Lal, *Biochem. Physiol. Pflanz.*, **164**, 423–428 (1973).

(115) J. W. Hylin and K. Sawai, *J. Biol. Chem.*, **239**, 990–992 (1964).

(116) J. E. Courtois and P. Le Dizet, *Bull. Soc. Chim. Biol.*, **52**, 15–22 (1970).

(117) J. Tampion, *Enzymologia*, **42**, 253–255 (1972).

(118) J. S. Grant-Reid and H. Meier, *Planta*, **112**, 301–308 (1973).

(119) A. Sioufi, F. Percheron, and J. E. Courtois, *Phytochemistry*, 9, 991–999 (1970).

(120) S. Clermont-Beaugiraud and F. Percheron, *Bull. Soc. Chim. Biol.*, **50**, 633–640 (1968).

(121) S. Beaugiraud and F. Percheron, *Compt. Rend.*, **259**, 3879–3881 (1964).

(122) J. E. Courtois and P. Le Dizet, *Ann. Pharm. Franc.*, **27**, 495–504 (1969).

D-manno-oligosaccharides containing a glucosyl group at the nonreducing end or a glucose residue at the reducing end, or both, indicates that these enzymes[83,85] are also capable of hydrolyzing the -Man-(1 → 4)-Glc- and -Glc-(1 → 4)-Man- bonds, as well as the -Man(1 → 4)-Man- linkages.

2. Occurrence

D-Mannanases have been reported to be produced by various species of bacteria, including bacteria from human intestines (for example, *Aerobacter mannanolyticus*[87,88]) and from the rumen. Other sources include the rumen protozoa, various fungi (including saprophytic, phytopathogenic, and mycorrhiza fungi), marine algae, germinating terrestrial plant-seeds, and various invertebrates (for example, insects, crustaceans, and the snail) (see Table X).

Enzymes degrading konjac glucomannan have also been found (see Ref. 88) to be produced by the following invertebrates: crab, earshell, earthworm, lobster, sea-urchin, snail, and turbo.

There is no experimental evidence to indicate that D-mannanases are produced by the mucosal cells of the vertebrate gastrointestinal tract, and any degradation of D-mannans that occurs within this tract is likely to be due to D-mannanases of bacterial or protozoal origin.

In germinating seeds (such as those of clover, fenugreek, and lucerne[129]), the cells of the aleurone layer have been shown to be responsible for the *in vivo* synthesis, and secretion into the endosperm storage-tissue, of the extracellular D-mannanases and related enzymes that are responsible for the metabolism of the reserve galactomannan.

D-Mannanases of microbial origin have been reported[88,93,98,104-106,130] to be both inductive and constitutive enzymes, usually being secreted extracellularly into the medium in which the

(123) W. A. M. Duncan, D. J. Manners, and A. G. Ross, *Biochem. J.*, 63, 44–51 (1956).
(124) P. Kooiman, *J. Cellular Comp. Physiol.*, 63, 197–201 (1964).
(125) F. L. Myers and D. H. Northcote, *Biochem. J.*, 69, 54P (1958).
(126) M. M. Debris, C. Chararas, and J. E. Courtois, *Compt. Rend. Soc. Biol.*, 158, 1238–1240 (1964).
(127) J. E. Courtois, F. Petek, and M. A. Kolahi Zanouzi, *Compt. Rend. Soc. Biol.*, 156, 565–566 (1962).
(128) V. Levaditou, C. Chararas, M. J. Foglietti, and J. E. Courtois, *Compt. Rend. Soc. Biol.*, 165, 796 (1971).
(129) J. S. Grant-Reid and H. Meier, *Planta*, 106, 44–60 (1972).
(130) P. J. Bailey, W. Liese, R. Roesch, G. Keilich, and E. G. Afting, *Biochim. Biophys. Acta*, 185, 381–391 (1969).

micro-organism is cultured. However, there are some exceptions, namely, the bacterial D-mannanases produced by *Sporocytophaga myxococcoides*[94] and *Aerobacter mannanolyticus*,[87] which were found to be intracellular. Rumen mannanases of protozoal[96] origin are also produced intracellularly. Several pure cultures of rumen bacteria (obligative anaerobic *Streptococci*) isolated by P. P. Williams and Doetsch[95] were, on the other hand, found to be secreted extracellularly. The same authors[95] also examined several non-rumen bacteria, but could not detect any extracellular D-mannanases therein. This does not, however, exclude the possibility that these micro-organisms do not produce these enzymes intracellularly.

Courtois and his associates[128] examined various tracts (head, gastric pouch, and middle and posterior intestines) of the digestive system of the xylophagic insect *Rhagium inquisitor*, and found that the activity of enzymes degrading glucomannans was highest in the gastric pouch and the posterior intestine. Whether these enzymes are produced by secretory cells of the digestive tract is not known, as whole segments of these tracts were ground and extracted with isotonic saline solution in the preparation stage. This type of treatment would also cause inclusion of any protozoal (symbiont)-derived enzymes, which are known to be responsible for the degradation of polysaccharides by insects. D-Mannanases have also been found in the soluble extract prepared from the digestive tract of a snail (*Helix pomatia*).[125]

D-Mannanases present in the digestive juices of the stomachs of the crustaceans *Astacus fluviatilis* and *Homarus vulgaris* were found[124] to be produced by the hepatopancreatic gland (which secretes into the stomach) and not by bacterial symbionts within the stomach.

3. Purification

a. Assay Procedure. — Substrates commonly used in the assay procedures for D-mannanase activity include $(1 \rightarrow 4)$-β-D-mannan and galacto- and gluco-mannans from various different plant sources, as shown in Table XI.

The assay procedures usually involve either measurement of the increase in reducing end-groups by the Nelson,[94,97] Somogyi,[83,85,86,115] or Sumner[84,93] methods, or measurement of the decrease in viscosity.[59,84,88,94,97,109(a),115,117,130]

b. Definition of the D-Mannanase Unit of Activity. — When measured by increase in reducing end-groups, the unit of D-mannanase

<div align="center">TABLE XI</div>

D-Mannans from Various Terrestrial Plant Sources That Have Been Used as Substrates in Assay Procedures of D-Mannanase Activity, and in Action Pattern and Structural Studies using D-Mannanases

Plant source	D-Mannose to D-galactose ratio	References[b]
a. Galactomannans		
Ceratonia siliqua (Carob)	3.88	59,92–94,109(a),112,*116,* 122,124
Coffee bean	45.0	83,85,86,101,109(a)
Cyamopsis tetragonolobus (guar)	2.00	32,59,72,73,83–85,*92,* 93–95,103,105,113,122
Genista scoparia	1.59	92,122
Gleditschia ferox	3.90	92,116,122
Leucaena glauca	1.33[a]	115
Medicago sativa (lucerne)	1.00	92,116
Sesbania grandiflora	2.03[a]	122
Soybean	2.07	*83,85*
Trifolium pratense (red clover)	2.00	96
repens	1.07	92,116,122
Trigonella foenum-graecum (fenugreek)	1.00	118,*122*
b. Glucommanans		
Amorphophallus konjac (konjac)		83,87,88,93, 107,110
Arum root		85
Asparagus officinalis		111
Eremurus fuscus		102
regelii		97,132
spectabilis		132
Phaseolus aureus		133,134
Picea abies (Norwegian spruce)		74,108
Pinus banksiana (jack pine)		135
strobus		59
Tubera salep (salep orchid)		128
c. $(1 \rightarrow 4)$-β-D-Mannans		
Corozo		94,120–122
Orchis maculata		117
Phoenix canariensis		94,120,122
Phytelephas macrocarpa (ivory nut)		59,93,96,104,106,124,132, 136
Tubera salep		59,91,92,109(b),130

[a] See Ref. 131. [b] Italicized reference numbers refer to those references from which the mannose:galactose ratios were compiled.

activity is usually defined as that amount of enzyme which causes the liberation of reducing end-groups corresponding to the liberation of 1 μmole of D-mannose per minute under defined conditions of temperature, ionic strength, and pH. Where viscosity measurements have been used to determine the activity, the activity is expressed in terms of arbitary units based on the change in viscosity (see, for example, Ref. 137).

c. **Extraction of D-Mannanases from Germinating Seeds.** — A typical procedure for extracting polysaccharases from germinating seeds is as follows.

The seeds are sterilized by washing with sodium hypochlorite solution (1%, v/v) or mercuric chloride (0.1%, w/v) to prevent microbial growth during germination. The seeds are next thoroughly washed with water, left to soak in water for 24 h, and then allowed to germinate at 25–30° in covered Petri dishes containing moist filter-paper.

After 5–15 days, depending on the time needed for germination, the seeds are collected, stored frozen if necessary, and hand-ground (mortar and pestle, with acid-washed sand as an abrasive) or homogenized in buffer (0.1M, pH 6.0–7.5, containing 1–2 mM EDTA) or isotonic saline at 4°, and left to stand overnight. The enzymes are then isolated from the extract by standard procedures.

d. **Separation and Purification of D-Mannanases.** — D-Mannanases have been purified by ion-exchange chromatography on DEAE-cellulose,[110] DEAE-Sephadex,[59,83–86,93] Duolite A-2 (Ref. 83), and SE-Sephadex[83,86]; by gel-permeation chromatography on Bio-gel P-150 (Ref. 84), Sephadex G-75 (Refs. 59, 61, and 83–85), and Sephadex G-100 (Refs. 59, 86, 101, and 110); by isoelectric focusing (pH 3–10) in 1,2-ethanediol (75% v/v)[61,84,105] or sucrose (50% w/v)[83,85]; by zone electrophoresis on Sephadex G-25 (Ref. 61), Sephadex G-75 (Ref. 85), or poly(acrylamide)[84]; and by moving-boundary electrophoresis.[121] Affinity-binding chromatography[83] has been used to remove *alpha*-amylases from a D-mannanase preparation by binding to raw corn-starch, and D-mannanases have been crystallized from ammonium sulfate[115] and acetone[83] solutions.

(131) J. S. Grant-Reid and H. Meier, Z. *Pflanzenphysiol.*, 62, 89–92 (1970).

(132) A. A. Kuznetsov and B. N. Stepanenko, *Prikl. Biokhim. Mikrobiol.*, 8, 26–33 (1972).

(133) J. S. Heller and C. L. Villemez, *Biochem. J.*, 128, 243–252 (1972).

(134) A. D. Elbein and W. Z. Hassid, *Biochem. Biophys. Res. Commun.*, 23, 311–318 (1966).

(135) O. Perila and C. T. Bishop, *Can. J. Chem.*, 39, 815–826 (1961).

(136) R. Somme, *Acta Chem. Scand.*, 24, 72–76 (1970).

(137) K. E. Almin and K. E. Eriksson, *Biochim. Biophys. Acta*, 139, 238–247 (1967).

e. **Criteria of Purity.** — D-Mannanase preparations from *Aspergillus niger* have been shown to be homogeneous by the criteria of ultracentrifugation[84] and zone electrophoresis.[84,85] The crystalline D-mannanase preparation from *Bacillus subtilis*[83] behaved as a single, symmetrical peak on isoelectric focusing, and was considered to be pure. Other D-mannanase preparations claimed to be "pure" (for example, from *Rhizopus niveus*,[86] *Amorphophallus konjac*,[110] *Leucaena glauca*,[115] and *Trigonella foenum-graecum*[121]) have not been shown to be homogeneous by the usual criteria of purity.

4. Physicochemical Properties

The D-mannanase produced by *Bacillus subtilis*[83] was found to show a dependency towards Ca^{2+} for the maintenance of activity and stability. In the presence of Ca^{2+}, this enzyme preparation showed optimal activity at pH 6.0 and 60°, and was stable at pH values of 5.0–9.5. The enzyme was completely inactivated when kept at 70° for 15 min at pH 7.5. In the presence of EDTA, however, the enzyme showed optimal activity at pH 6.0 and 40°, was stable only at pH values of 6.0 to 7.5, and was completely inactivated at 55°. Temperature and pH properties of D-mannanases (from other sources) not dependent on metal cofactors are summarized in Table XII, together with the pI and M.W. values for several D-mannanases. These enzymes are considered to be acid glycan hydrolases, and their sizes (M.W. range, $22–47 \times 10^4$) are comparable to those of other polysaccharases (see, for example, Sections II, III, and VI). Other physical properties of D-mannanases that have been measured include the partial specific volume (0.72 $cm^3.g^{-1}$, for the *Aspergillus niger*[84] enzyme) and the sedimentation coefficient (3.85, for the *A. niger*[84] enzyme).

Values of K_m for D-mannanase have been reported for the *Bacillus subtilis*[83] enzyme (2.8 mg/ml for coffee-bean galactomannan, 5.9 mg/ml for soybean galactomannan, and 3.4 mg/ml for konjac glucomannan), and the *Amorphophallus konjac*[110] enzyme (714 $\mu g/ml$ for konjac glucomannan of M.W. 1.12×10^6).

The amino acid composition was determined for the D-mannanase preparation from *Aspergillus niger*,[84] and revealed that this preparation contained a high proportion of aromatic and acidic amino acid constituents. The D-mannanase preparation from *Bacillus subtilis*[83] was found to contain 5 moles of tryptophan and 9 moles of tyrosine per mole of the enzyme.

D-Mannanase preparations[101] from *Rhizopus niveus* and *Aspergillus niger* are activated by certain salts (such as NaCl, KCl,

TABLE XII

Some Properties of D-Mannanases from Different Microbial and Plant Sources

Source	pH optimum	pH stability	Temperature optimum (°C)	Temperature of complete thermal inactivation (°C)	pI	M.W. × 10⁴
Amorphophallus konjac[110]	4.7	4.0–8.0	40	80		
Aspergillus niger[84]	3.0,3.8	2.0–9.2	65	80	4.10	42[a]
niger van Tieghem[85]	3.5	4.0–7.0		80	3.40	
wentii[59]						42–47[b]
Bacillus subtilis[83]	6.0[c]	5.0–9.5[c]	60[c]	70[c]	5.24	22[d]
Chrysosporium lignorum[61,105]					4.12	
Fomes annosus[61]					3.90–4.20	
Leucaena glauca[115]	5.3					
Polyporus schweinitzii[59]						35–37[b]
Rhizopus niveus[86]	5.5	2.5–10.0	40			
Stereum sanguinolentum[61]					3.58	
Trigonella foenum-graecum[121]	5.3					

[a] Based on sedimentation equilibrium data. [b] Based on gel-permeation chromatographic data (Bio Gel P-60 and P-100). [c] In the presence of calcium acetate (2 mM). [d] Based on gel-permeation chromatographic data (Sephadex G-75).

Na_2SO_4, and $NaNO_3$) and by acetate ion. Calcium chloride activates the *A. niger* enzyme, but has no effect on the D-mannanase from *R. niveus*.[101] Copper ions ($CuSO_4$) at concentrations of 100 μM to 10 mM were found[95] to inhibit a partially purified D-mannanase preparation produced by a rumen *Streptococcus* isolate.

The crude enzyme-preparation from *Cyamopsis tetragonolobus* (guar)[114] was found to be inhibited by gibberellic acid and by D-galactose (0.28 M), but D-mannose showed no significant inhibition. D-Galactose produced ~80% inhibition of the crude enzyme-preparation, but this value was probably at least partly due to inhibition of α-D-galactosidases, which are known[113] to be produced by germinating guar-seeds.

5. Mode of Action

a. Bacterial D-Mannanases. — The D-mannanase system of *Bacillus subtilis*[83] is the only bacterial enzyme of this type that has been purified, and studied in any great detail. This enzyme was shown[83] to be a typical endo-enzyme, and to degrade various D-galacto- and D-gluco-D-mannans to D-mannose, D-manno-oligosaccharides of d.p. 2–8, and oligosaccharides of mixed constitution containing either D-galactose or D-glucose residue(s), in addition to those of mannose (see Table XIII). Bacterial D-mannanases of the exo type have not yet been detected.

TABLE XIII

Degradation Products from the Action of *B. subtilis* D-Mannanase[83] on Galacto- and Gluco-mannans

Source	Type of mannan	Degradation products[a]	
		Major	Minor
Soybean	galactomannan	M_2, M_3, GM_2, GM_3	M, M_4, M_5
Guar	galactomannan	GM, GM_2, GM_3	M_2–M_5
Coffee	galactomannan	M_2–M_4, GM_3, GM_4	M, M_5–M_8, GM_2
Konjac	glucomannan	M_2–M_4, $GlcM_1$, $GlcM_2$	M, M_5, $GlcM_3$

[a] Key: M = mannose; G = galactose; Glc = glucose; M_x = manno-oligosaccharides of d.p. x; GM_m = galactomanno-oligosaccharides containing m D-mannose residues; $GlcM_n$ = glucomanno-oligosaccharides containing n D-mannose residues. The d.p. values of these mixed oligosaccharides, with the exception of coffee galactomannosides GM_3 and GM_4, were compiled from the relative R_F values of these oligosaccharides on the chromatograms.

The D-manno-oligosaccharides of d.p. 2–8 resulting from enzymic hydrolysis of coffee-bean galactomannan were shown[83] to belong to a homologous series of β-D-(1 → 4)-linked D-mannose residues. On acid hydrolysis, and enzymic degradation with a β-D-mannosidase preparation from *Rhizopus niveus*, they yielded D-mannose as the only hydrolysis product. Two mixed oligosaccharides, containing D-mannose and D-galactose, were also isolated from the coffee galactomannan hydrolyzate, and, on acid hydrolysis, yielded D-mannose and D-galactose in the ratio of 3 : 1 and 4 : 1, respectively. Enzymic hydrolyzates of konjac glucomannan also yielded a mixed oligosaccharide, which was isolated and shown[83] to consist of equimolar amounts of D-glucose and D-mannose residues.

The mode of action of *Bacillus subtilis*[83] D-mannanase on D-manno-oligosaccharides is as shown. It indicates preferential attack on the

$$M_4 \quad M - M \downarrow M \downarrow M^*$$

$$M_5 \quad M - M - M \Downarrow M \downarrow M^*$$

$$M_6 \quad M - M - M \downarrow M \Downarrow M \downarrow M^*$$

$$M_7 \quad M - M - M - M \Downarrow M \downarrow M - M^*$$

$$M_8 \quad M - M - M - M \Downarrow M \downarrow M - M - M^*$$
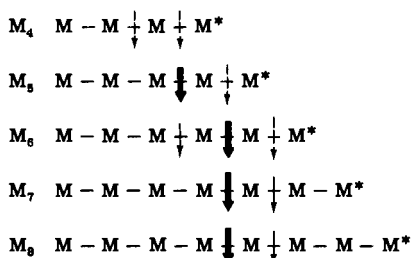
[where M = β-D-(1→4)-linked D-mannopyranosyl residues, $\Downarrow$ = linkages readily attacked by D-mannanase, $\downarrow$ = linkages moderately attacked by D-mannanase, $\vdots$ = linkages slowly attacked by D-mannanase, and the asterisk indicates the reducing-end residue]

D-mannose chain at the 3rd and 4th (1 → 4)-β-D-mannopyranosyl linkages from the nonreducing end of the molecule. Mannobiose (M_2) and mannotriose (M_3) were not attacked, whereas mannotetraose (M_4) was slowly degraded to M_2 and M_3, with D-mannose appearing after 24 h of hydrolysis.

b. **Fungal D-Mannanases.**—D-Mannanases of fungal origin have been shown[59,84–86,101] to degrade D-mannans in a random manner, and to be of the endo type. Products arising from the enzymic degradation of several D-mannans are shown in Table XIV; they indicate that most D-mannans are degraded to manno-oligosaccharides of d.p. $\geqslant 2$ and mixed D-mannose oligosaccharides containing D-galactose (from galactomannan) or D-glucose (from glucomannans). Mixed oligosaccharides resulting from enzymic hydrolysis of galactoglucomannans

TABLE XIV

Degradation Products Arising from the Action of D-Mannanases of
Different Fungal Origins on Various D-Mannans

Source of D-mannanase	References	Source of D-mannan[a]	Degradation products
Aspergillus niger	84	guar	M_2, M_3
A. niger van Tieghem	85	soybean	M_2–M_4
		guar	M_1–M_4, mixed oligosaccharides[b]
		coffee	M_1–M_3, mixed oligosaccharides[b]
		arum root	M_1–M_3, mixed oligosaccharides[c], Glc
Rhizopus niveus	86	coffee	M_2–M_5, mixed oligosaccharide[b] (GM_6)
Aspergillus wentii and *Polyporus schweinitzii*	59	salep	M_1, manno-oligosaccharides of d.p. $\geqslant 2$
		ivory nut	M_1, manno-oligosaccharides of d.p. $\geqslant 2$
		locust bean	M_1, manno-oligosaccharides of d.p. $\geqslant 2$, mixed oligosaccharides[b]
		guar	manno-oligosaccharides of d.p. $\geqslant 2$, mixed oligosaccharides[b]
		Pinus strobus	manno-oligosaccharides of d.p. $\geqslant 2$, mixed oligosaccharides[c]

^{a} See Table XI for a description of the type of mannan. ^{b} Containing both D-mannose and D-galactose residues. ^{c} Containing both D-mannose and D-glucose residues.

(for example, from *Pinus strobus*[59]) are likely to contain D-galactose in addition to D-glucose and D-mannose; for example:

$$-\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}$$
$$6$$
$$\uparrow$$
$$1$$
$$\alpha\text{-D-Gal}p$$
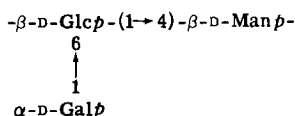
D-Mannanase preparations from *Aspergillus niger*[84,101] were found to degrade manno-oligosaccharides of d.p. $\geqslant 3$, mainly to D-mannose and M_2, but M_2 was not further degraded. The D-mannanase preparation from *Rhizopus niveus*[86] degraded coffee-bean galactomannan to M_2, M_3, M_4, M_5, and a mixed oligosaccharide of

mean d.p. 7 (GM_6). D-Mannose was not liberated during the course of the hydrolysis. Enzymic degradation of manno-oligosaccharides of d.p. $\geqslant 6$ were not investigated, but it appears[86] that, if produced in the hydrolyzate, they would be rapidly degraded to M_2 to M_5. The failure of this enzyme to degrade manno-oligosaccharides of d.p. $\leqslant 5$ indicates that the number of binding sites at the active center must be >5 for effective formation of the enzyme–substrate complex and for productive hydrolysis. The failure of D-mannanase to attack GM_6 suggests that the D-galactosyl substituent interferes with the mode of binding at the active center, thereby preventing the formation of a productive complex.

Clarke and Stone[138] reported that a highly purified $(1 \rightarrow 4)$-β-D-glucan hydrolase (EC 3.2.1.4) preparation from *A. niger*, not claimed to consist of a single protein entity, was also capable of randomly degrading ivory-nut mannan and konjac glucomannan. The activity of this D-glucanase preparation in degrading D-mannan may have been due to a D-mannanase contaminant, as the preparation was not shown to be homogeneous; or, alternatively, the D-glucanase may be able to tolerate the inversion of the configuration of C-2, and therefore be capable of degrading D-mannan.

The effect, on enzymic hydrolysis by the D-mannanases of *B. subtilis*[83] and *A. niger* van Tieghem,[85] of the degree of branching of three different galactomannans revealed (see Table XV), not surprisingly, that the least-branched polysaccharide is the most susceptible to attack, whereas those containing a higher proportion of D-galactose (that is, more highly branched) are hydrolyzed least. The unusually

TABLE XV

The Effect of the Degree of Branching on the Extent of Hydrolysis of Various Galactomannans by D-Mannanases from *B. subtilis* and *A. niger* van Tieghem[85]

Source of galactomannan	Mannose to galactose ratio	Source of D-mannanase	
		B. subtilis	*A. niger*
		Extent of hydrolysis (%)[a]	
Soybean hull	~2.00	n.d.[b]	12.0
testa	2.09	27.0	n.d.
Guar gum	~2.00	5.0	12.0
Coffee bean	45.00	36.0	58.0

[a] After 24 h. [b] n.d. = not determined.

(138) A. E. Clarke and B. A. Stone, *Biochem. J.*, 96, 802–807 (1965).

high degree of hydrolysis of the galactomannan from soybean testa (despite the apparent relatively high degree of branching), may be associated with major differences in structure, such as the presence of branches containing more than one D-galactose residue (compare, carob galactomannan[92]) or differences in the regularity of spacing of the branches. Further interpretation of these results must, therefore, await more-detailed knowledge of the polysaccharide structures.

c. D-Mannanases of Plant Origin. — An unusual but interesting D-mannanase preparation (galactomannan depolymerase) was isolated from germinated *Leucaena glauca* seeds by Hylin and Sawai.[115] The purified, crystalline preparation rapidly depolymerized *L. glauca* galactomannan (d.p. 150) into fragments of considerable size, with no concomitant release of reducing sugars even upon prolonged incubation. However, with partially purified enzyme-preparations of *L. glauca* extracts, these depolymerized fragments were susceptible to further enzymic attack, probably by other enzymes present (such as α-D-galactosidase, β-D-mannosidase, and D-mannanase), yielding D-galactose, D-mannose, O-α-D-Gal$p$-(1 → 6)-D-Man$p$, and oligosaccharides of mixed constitution.

A D-mannanase preparation from germinated fenugreek seeds[120] (free from α-D-galactosidase and β-D-mannosidase) degraded D-mannans from corozo and *Phoenix canariensis* mainly to M_2 and M_3 with trace amounts of D-mannose. M_2 and M_3 were not degraded by this enzyme, and M_4 was hydrolyzed mainly to M_2 accompanied by some M_3 and traces of D-mannose. M_5 was degraded mainly to M_2 and M_3, with trace amounts of D-mannose. The mode of action of this enzyme[120] on manno-oligosaccharides of d.p. 2–5 is as follows.

M_2 M – M*   not attacked

M_3 M – M – M*   not attacked

M_4 M – M $\mid$ M $\mid$ M*

M_5 M – M $\mid$ M $\mid$ M $\mid$ M*
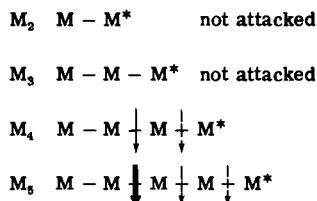
[where M = β-D-(1→4)-linked D-mannopyranosyl residues, M* indicates the reducing-end residue, $\mid$ = linkages rapidly cleaved by D-mannanase, $\mid$ = linkages moderately cleaved by D-mannanase, and $\mid$ = linkages slowly cleaved by D-mannanase]

The action on konjac glucomannan of a purified D-mannanase

preparation from germinated *Amorphophallus konjac* tubers was investigated by Sugiyama and coworkers.[110] They found that this enzyme preparation rapidly decreased the viscosity of konjac glucomannan (M.W. 1.12×10^6), but that this was accompanied by the production of less than 2% of total reducing sugars. This effect may arise from the hydrolysis of limited, enzyme-susceptible regions in the glucomannan, and may indicate some degree of block-copolymer structure in the polysaccharide. Certainly, a further investigation of the products from this degradation would provide valuable information on the original polysaccharide structure.

Konjac glucomannan was randomly degraded to D-mannose, D-glucose, a component co-chromatographing with cellobiose, and a series of manno- and glucomanno-oligosaccharides. The presence of D-glucose in these digests indicates that the enzyme is also capable of cleaving the Man$p$-$(1 \rightarrow 4)$-Glc$p$ and Glc$p$-$(1 \rightarrow 4)$-Man$p$ linkages of glucomannan, as is the D-mannanase preparation of *A. niger*[85]; the presence of a component of R_F corresponding to that of cellobiose suggests, however, that *A. konjac* D-mannanase may not be capable of hydrolyzing cellobiose [O-β-D-Glc$p$-$(1 \rightarrow 4)$-D-Glc$p$].

D-Mannanases of the exo type have not been detected in plant sources.

6. Oligosaccharide Degradation Products Arising from Enzymic Hydrolysis of Galacto- and Gluco-mannans

Endo-D-mannanases degrading galacto- and gluco-mannans usually liberate a series of mixed oligosaccharides, in addition to the linear $(1 \rightarrow 4)$-β-D-manno-oligosaccharides. Several of the former oligosaccharides have been isolated, and characterized (usually by methylation), and are described next.

a. **Galactomanno-oligosaccharides.**—The galactomanno-oligosaccharides constitute a series of $(1 \rightarrow 4)$-β-D-mannopyranosyl residues carrying, on O-6, either a single D-galactopyranosyl group, or, alternatively, a single D-galactopyranosyl group and an O-α-D-Gal$p$-$(1 \rightarrow 6)$-D-Gal$p$ unit (see, for example, Ref. 92). Several such galactomanno-oligosaccharides, of d.p. ranging from 2 to 14, have been characterized; they are listed in Table XVI. The D-mannanase preparations used in both cases were partially purified, and the oligosaccharides obtained may be the result of the action of several enzymes.

b. **Glucomanno-oligosaccharides.**—Several glucomanno-oligosaccharides of d.p. 2–5 isolated from enzymic hydrolyzates of gluco-

TABLE XVI

Structures of Several Galactomanno-oligosaccharides Isolated from
Enzymic Hydrolyzates of Galactomannans

Source of galactomannan	Source of enzyme	Oligosaccharide structure	References
Leucaena glauca	*L. glauca*	O-α-D-Gal$p$-(1 → 6)-D-Man$p$	115
Ceratonia siliqua (carob)	*Bacillus subtilis*	O-α-D-Gal$p$-(1 → 6)-O-β-D-Man$p$-(1 → 4)-D-Man$p$, O-α-D-Gal$p$-(1 → 6)-O-β-D-Man$p$-(1 → 4)-D-Man$p$-(1 → 4)-D-Man$p$, a pentasaccharide[a] (Man/Gal = 4:1), a hexa- and a heptasaccharide (Man/Gal = 4:2, and 4:3, respectively),[b] a galactomanno-oligosaccharide[c] of d.p. 14	92

[a] Structure not characterized by methylation, but considered to belong to the same homologous series as the trimer and tetramer. [b] Structures not characterized by methylation, but may contain either 2 single D-Gal$p$ residues or a D-Gal$p$-(1 → 6)-D-Gal$p$ residue. [c] This oligosaccharide was shown by methylation to be of the following structure.

β-Man$p$1 → 4Man$p$1 → 4Man$p$1 → 4Man$p$1 → 4Man$p$1 ⌐
      6   6
      ↑   ↑
      1   1
   α-Gal$p$ α-Gal$p$
                                6
                                ↑
                                1
                           α-Gal$p$
└→ 4Man$p$1 → 4Man$p$1 → 4Man$p$1 → 4Man$p$1 → 4Man$p$
                         6
                         ↑
                         1
                    α-Gal$p$
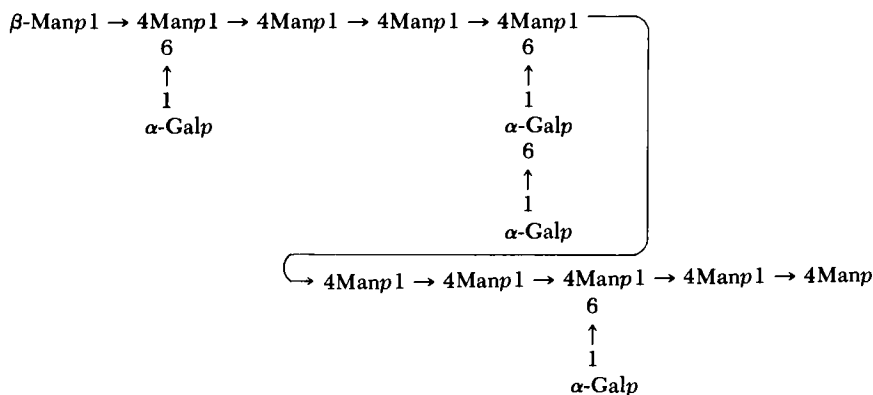
mannans are shown in Table XVII. The D-mannanase preparations used have been crude enzyme mixtures, and the oligosaccharides resulting may not derive from the action of a single enzyme component. The glucomanno-oligosaccharides constitute a series of linear β-D-(1 → 4)-linked D-mannopyranosyl residues with interposed (1 → 4)-β-D-glucose residues.

TABLE XVII

Structures of Several Glucomanno-oligosaccharides Isolated from
Enzymic Hydrolyzates of Glucomannans

Source of glucomannan	Source of enzyme	Oligosaccharide structure	$[\alpha]_D$ (degrees)
Picea abies Karst[108]	commercial "cellulase" from *Penicillium chrysogenum-notatum*	O-β-D-Glcp-(1→4)-D-Manp	6.0
		O-β-D-Manp-(1→4)-D-Glcp	18.6
		O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Glcp	n.d.
		O-β-D-Glcp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Manp	n.d.
		O-β-D-Manp-(1 → 4)-O-β-D-Glcp-(1 → 4)-D-Manp	n.d.
Pinus banksiana Lamb[135]	commercial "hemi-cellulase"	O-β-D-Glcp-(1→4)-D-Manp	5.5
		O-β-D-Manp-(1→4)-D-Glcp	12.0
		O-β-D-Manp-(1 → 4)-O-β-D-Glcp-(1 → 4)-D-Glcp	9.5
		O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Glcp	n.d.
		O-β-D-Glcp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Manp	n.d.
Phaseolus aureus[134]	fungal mannanase	O-β-D-Glcp-(1 → 4)-D-Manp, a tri-, tetra-, and penta-saccharide[a] containing Man/Glc in the ratio of 2 : 1, 3 : 1, and 4 : 1, respectively	n.d. n.d.
Amorphophallus konjac[139]	*Trichoderma* sp. (crude and purified "cellulase" preparations)	O-β-D-Manp-(1→4)-D-Glcp	17.0
		O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Glcp	−6.8
		O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Glcp	−14.4
		O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Glcp	−21.3
		O-β-D-Glcp-(1→4)-D-Manp	n.d.
		O-β-D-Glcp-(1 → 4)-O-β-D-Manp-(1 → 4)-D-Manp	n.d.

[a] Not characterized chemically.

(139) K. Kato, T. Watanabe, and K. Matsuda, *Agr. Biol. Chem.* (Tokyo), 34, 532–539 (1970).

V. $(1 \rightarrow 3)$-β-D-XYLANASES

1. Introduction

$(1 \rightarrow 3)$-β-D-Xylanases are hydrolytic enzymes [$(1 \rightarrow 3)$-β-D-xylan xylanohydrolases] capable of hydrolyzing $(1 \rightarrow 3)$-β-D-xylans to D-xylose [exo-$(1 \rightarrow 3)$-β-D-xylanases, EC 3.2.1.72], or D-xylose and D-xylo-oligosaccharides [endo-$(1 \rightarrow 3)$-β-D-xylanases, EC 3.2.1.32].

2. Occurrence

$(1 \rightarrow 3)$-β-D-Xylanases have been reported to be produced by several strains of bacteria from marine environments,[140,141] such as sea water and marine-bottom sediments, and by green, brown, and red algae (seaweeds). The enzymes have also been isolated from terrestrial fungi, for example, *Aspergillus batatae*,[142] *Chaetomium globosum*,[142] and *Irpex lacteus*.[143] These bacteria and fungi were found to produce both $(1 \rightarrow 3)$- and $(1 \rightarrow 4)$-β-D-xylanases, which were secreted extracellularly.

Of the 64 strains of $(1 \rightarrow 3)$-β-D-xylan-decomposing marine bacteria (unidentified) examined, at least 40 possessed potent $(1 \rightarrow 3)$-β-D-xylanase activity, and 36 strains were also capable of hydrolyzing $(1 \rightarrow 4)$-β-D-xylans (see Table XVIII).

Several fungi, when cultured on wheat bran[142] (for example, *Aspergillus batatae* and *Chaetomium globosum*), glucose[142] (for example, *C. globosum*), and cellulose[143] (for example, *Irpex lacteus*) were found to produce $(1 \rightarrow 3)$-β-D-xylanases constitutively, in addition to $(1 \rightarrow 4)$-β-D-xylanases. A study of the comparative rates of production of D-xylanases by *Chaetomium globosum* showed[142] that $(1 \rightarrow 4)$-β-D-xylanases attained maximum activity after 3 days of growth, whereas the $(1 \rightarrow 3)$-β-D-xylanases attained optimal production after 5 days.

3. Purification

Substrates used in the procedure for the assay of $(1 \rightarrow 3)$-β-D-xylanase activity have normally been $(1 \rightarrow 3)$-β-D-xylans from marine

(140) H. Fujisawa and M. Murakami, *Nippon Suisan Gakkaishi*, **36**, 741–747 (1970).

(141) H. Fujisawa and M. Murakami, *Nippon Suisan Gakkaishi*, **37**, 119–123 (1971).

(142) S. Fukui, T. Suzuki, K. Kitahara, and T. Miwa, *J. Gen. Appl. Microbiol.* (Tokyo), **6**, 270–280 (1960).

(143) K. Nisizawa, I. Morimoto, N. Handa, and Y. Hashimoto, *Arch. Biochem. Biophys.*, **96**, 152–157 (1962).

TABLE XVIII

Number of (1 → 3)-β-D-Xylan-decomposing Marine Bacterial Strains Possessing
(1 → 3)- and (1 → 4)-β-D-Xylanase Activity[141]

Sources of bacterial strains	Number of strains of (1 → 3)-β-D-xylan-decomposing bacteria examined	Number of strains of (1 → 3)-β-D-xylan decomposing bacteria with potent	
		(1→3)-β-D-Xylanase activity	(1→4)-β-D-Xylanase activity
Seaweeds	30	18	18
Bottom sediments	27	17	15
Seawater	7	5	3
Total	64	40	36

algae (for example, *Halimeda cuneata*,[140,141] *Cauperpa cuneata*,[142] and *Cauperpa brachypus*[143]). Attempts to purify (1 → 3)-β-D-xylanase have utilized ammonium sulfate precipitation,[142] acetone precipitation,[142] and zone electrophoresis[143] at pH 8.7 with starch as the carrier medium.

The (1 → 3)-β-D-xylanase from *Chaetomium globosum*[142] was purified by several fractionation steps employing ammonium sulfate and acetone, and the "purified" preparation resulting was shown to be free from α-amylase, cellulase, and (1 → 4)-β-D-xylanase activities, and to have undergone a 106-fold purification. Whether this preparation was homogeneous was not indicated, but it has been used in study of mode of action on (1 → 3)-β-D-xylan.

The (1 → 3)-β-D-xylanase system of *Irpex lacteus*[143] was resolved into nine components by zone electrophoresis at pH 8.7, but each component was found to be contaminated with (1 → 3)- and (1 → 4)-β-D-glucanase, amylase, and (1 → 4)-β-D-xylanase activities. None of these components were purified further.

4. Properties

The physicochemical properties of (1 → 3)-β-D-xylanases have not yet been reported.

5. Mode of Action

The "purified" (1 → 3)-β-D-xylanase preparation from *Chaetomium globosum*[142] degraded (1 → 3)-β-D-xylan (*Cauperpa cuneata*,

d.p. = 40–50, M.W. = 5×10^3 to 6×10^3) to yield only D-xylose. The absence of any D-xylo-oligosaccharides from the enzymic hydrolyzate suggests that the enzyme may be of the exo type.

$(1 \rightarrow 3)$-β-D-Xylo-oligosaccharides of d.p. 3–6 were degraded mainly to xylose and some xylobiose (X_2), and X_2 was slowly degraded to xylose. The rate of attack of $(1 \rightarrow 3)$-β-D-xylanase on these xylo-oligosaccharides was in the following order: xylohexaose $\gg$ xylopentaose $>$ xylotetraose $>$ xylotriose.

$(1 \rightarrow 3)$-β-D-Xylan was hydrolyzed within 1 hour to a limit of 93–95%, and the incomplete hydrolysis was associated with the production, in the hydrolyzate, of X_2 (which, as already mentioned, is only slowly degraded to D-xylose). $(1 \rightarrow 3)$-β-D-Xylanases of the endo type have not been detected.

VI. $(1 \rightarrow 4)$-β-D-XYLANASES

1. Introduction

In this Section, the term "xylanase" will refer to those enzymes capable of hydrolyzing the $(1 \rightarrow 4)$-β-D-xylopyranosyl linkages of the $(1 \rightarrow 4)$-β-D-xylans, namely, arabinoxylan, arabinoglucuronoxylan, arabino-4-O-methyl-D-glucuronoxylan, and glucuronoxylan. D-Xylanases of this type have been assigned the Enzyme Commission numbers 3.2.1.8 [$(1 \rightarrow 4)$-β-D-xylan xylanohydrolase, endo-xylanase] and 3.2.1.37 [$(1 \rightarrow 4)$-β-D-xylan xylohydrolase, exo-xylanase].

2. Occurrence

D-Xylanases have been reported to occur in bacteria from marine and terrestrial environments, fungi (saprophytes, phytopathogens, and mycorrhiza), rumen bacteria and protozoa, ruminant caecal bacteria, insects, snails, crustaceans, marine algae, and germinating seeds of terrestrial plants (see Table XIX). Their presence has, however, not been demonstrated in vertebrate animal tissues (for example, the mucosal lining of the gastrointestinal tract), and such animals, including man, non-ruminants, and ruminants, appear to rely upon D-xylanases of microbial flora (for example, in the intestines, rumen, and caecum) and fauna (for example, rumen protozoa) in their digestive tracts to degrade D-xylans in the diet.

In the invertebrate crustaceans *Astacus fluviatilis* and *Homarus vulgaris*, the hepatopancreas gland has been shown[124] to be a source of D-xylanase in their digestive juices. D-Xylanases have also been

TABLE XIX

Sources of D-Xylanases

Organism	References
Bacteria	
(a) Terrestrial environment	
Actinomycetes bacteria	144
Bacillus sp.	145,146
polymyxa	147
subtilis	148,149,[a]150,151
Cellvibrio fulvus	5
Clostridium sp.	5
Microbispora rosea	152[a]
Micromonospora chalcea	5
Nocardia corallina	152
Sporocytophaga myxococcoides	5,94
Streptomyces sp.	152,153[a]
Streptomyces (QMB-814)	154
albogriseolus	152,153
albus	5
olivaceus	153
xylophagus	153,155,156
(b) Marine environment (bacterial species not yet identified)	157
Fungi	
Agaricus bisporus	158
Alternaria acremonium	153
Aspergillus	159
batatae	160
niger	8,59,60,130,159,161–171
niger van Tieghem	172
oryzae	20,146,173
sojae	174
terreus	175
wentii	59
Botryosphaeria ribis	73
Cephalosporium acremonium	153
sacchari	176
Ceratocystis paradoxa	176
Cercospora melonis	153
Chaetomium sp.	153
globosum	5,59,177
globosum var. *affine*	170,171
trilaterale	153,178,179
Chrysosporium lignorum	61,105
Colletotrichum trifolii	180

TABLE XIX (*continued*)

Organism	References
Collybia velutipes	170,171
Coniophora cerebella	170,171,181
puteana	25
Cytospora sp.	182
Diplodia viticola	183
Echinodontium tsugicola	153
Fomes annosus	61
igiarius	170,171
marginatus	170,171
Fusarium sp.	159,184
roseum	185
Gibberella sabinetti	153
Gloeophyllum saepiarium	106,170,171
Glomerella cingulata	153
Helminthosporium sp.	184,186
Irpex lacteus	185,187
Lenzites saepiaria	170,171
Macrosporium bataticola	153
Marasmius sacchari	176
Merulius lacrymans	170,171
silvester	170,171
Myrothecium verrucaria	107,185,188
Neurospora sp.	189
Oxiporus sp.	190
Penicillium sp.	159,184,191,192
cyclopium	193
digitatum	194
funiculosum	185,186,193
janthinellum	5
janthinellum (Biourge)	195
pinophilum	185
rugulosum	193
verruculosum	186,193
viridicatum	185
Pericularia oryzae	196
Phellinus igniarus	106,170,171
Polyporus betulinus	170,171
schweinitzii	59
Poria sp.	197
Rhizoctonia solanii	72
Rhizopus sp.	184
niveus	166
tritici	32
Schizophyllum commune	153,170,177,198
Sclerotinia sclerotiorum	35

(*continued*)

TABLE XIX (continued)

Organism	References
Sclerotium rolfsii	72
Stereum sanguinolentum	61,199
Trametes gibbosa	25
pini	153
versicolor	170,171
Trichoderma sp.	184
koningi	200,201
viride	166,169–171,185,190,202–206
lignorum	192
Verticillium dahliae	207
lateritium	207
Mycorrhiza fungi	
Amanita muscaria	106
Leccinum scabrum	106
Paxillus involutus	106
Suillus aeroginascens	106
bovinus	106
luteus	106
tridentinus	106
variegatus	106
Xerocomus badius	106
Mesophilous and thermophilous fungi	
isolated from barley kernels	208,[a] 209[a]
Various other basidiomycetes fungi	210[a]
Rumen bacteria	
Bacillus firmus	148
Bacteroides	211,212
amylogenes	38,211
ruminicola	41
succinogenes	41
Butyrivibrio sp.	38,213
fibrisolvens	38–40
Clostridium sp.	213
Ruminococcus albus	41,213,214
flavefaciens	41,213
Mixed rumen bacteria	40,42,215
Ruminant (sheep) mixed caecal bacteria	42
Rumen ciliates (protozoa)	
Epidinium ecaudatum (Crawley)	43,44
Entodinium sp.	44,216
Eremoplastron bovis	44,45
Eudiplodinium medium	46
Polyplastron multivesiculatum	
(Dogiel and Fedorowa)	47
Mixed rumen micro-organisms	42,217,218

TABLE XIX (continued)

Organism	References
Animals (invertebrates)	
Anthaxia corinthia	126
Astacus fluviatilis Fabr.	124
Gypsonoma aceriana	126
Helix aspera	20
pomatia	125
Homarus vulgaris M-E	124
Ipstypographus L.	219
Melanophila picta	126
Melanoplus bivittatus	220
Polyphylla fullo	126
Rhagium inquisitor L.	221
Sciapteron tabaniformis	126
Terrestrial plants (seeds)	
Barley, maize, oats, rye, wheat	222
Trifolium subterranum (Daliak)	37
Stylosanthes humilis (Townsville Stylo)	37
Marine plants (algae)	
Cladophora rupestris	123
Laminaria digitata	123
Rhodymenia palmata	123
Ulva lactuca	123

ᵃ These references contain a comprehensive list of bacterial and fungal strains and species that have been screened for the production of D-xylanase, but that are too numerous to include in this Table.

(144) N. A. Tiunova, R. V. Feniksova, N. Kobzeva, V. D. Kuznetsov, L. I. Kostycheva, N. A. Rodionova, I. Itomlenskite, and D. G. Pirieva, Prikl. Biokhim. Mikrobiol., 7, 456–461 (1971); Chem. Abstr., 75, 95,576c (1971).
(145) W. M. Fogarty and O. P. Ward, Biochem. Soc. Trans., 1, 260–262 (1973).
(146) S. Fukui, J. Gen. Appl. Microbiol. (Tokyo), 4, 39–50 (1958).
(147) W. M. Fogarty and P. J. Griffin, Biochem. Soc. Trans., 1, 263–265 (1973).
(148) M. Inaoka, Mem. Ehime Univ. Sect. VI, 95–103, 117–122 (1961).
(149) M. Takahashi and Y. Hashimoto, Hakko Kogaku Zasshi, 41, 181–186 (1963).
(150) H. Lyr, Z. Allegem. Mikrobiol., 12, 135–142 (1972).
(151) M. Takahashi and Y. Hashimoto, Hakko Kogaku Zasshi, 41, 116–119 (1963).
(152) I. Kusakabe, T. Yasui, and T. Kobayashi, Nippon Nogei Kagaku Kaishi, 43, 145–153 (1969).
(153) H. Iizuka and T. Kawaminami, Agr. Biol. Chem. (Tokyo), 33, 1257–1263 (1969).
(154) H. R. Goldschmid and A. S. Perlin, Can. J. Chem., 41, 2272–2277 (1963).
(155) H. Iizuka and T. Kawaminami, Agr. Biol. Chem. (Tokyo), 29, 520–524 (1965).
(156) T. Kawaminami and H. Iizuka, Agr. Biol. Chem. (Tokyo), 33, 1787–1789 (1969).
(157) H. Fujisawa and M. Murakami, Nippon Suisan Gakkaishi, 35, 677 (1969), and references cited therein.

(158) K. Hashimoto and Z. Takahashi, *Toyo Shokuhin Kogyo Tanki Daigaku, Toyo Shokuhin Kenkyusho Kenkyu Hokokusho*, 9, 320–326 (1970).

(159) M. A. Shcherbakov and Z. P. Tyurina, *Izv. Akad. Nauk Moldavsk. SSR, Ser. Biol. Khim. Nauk*, 86–87 (1972); *Chem. Abstr.*, 78, 146,274z (1973).

(160) S. Fukui and M. Sato, *Bull. Agr. Chem. Soc. Japan*, 21, 392–393 (1957).

(161) N. A. Rodionova, R. V. Feniksova, and I. Itomlenskite, *Kim. Drev.*, 8, 127–134 (1971).

(162) T. Iwamoto, T. Sasaki, and M. Inaoka, *Mem. Ehime Univ.*, 17, 13–25 (1973).

(163) T. Iwamoto, T. Sasaki, and M. Inaoka, *Mem. Ehime Univ.*, 17, 185–197 (1973).

(164) M. Claeyssens, F. G. Loontiens, K. Kersters-Hilderson, and C. K. De Bruyne, *Enzymologia*, 40, 177–198 (1971).

(165) T. Sasaki and M. Inaoka, *Mem. Ehime Univ.*, 12, 149–156 (1967).

(166) M. Sugiura, T. Ogiso, R. Hatano, and Y. Tanaka, *Gifu Yakka Daigaku Kiyo*, 17, 117–124 (1967).

(167) M. Sugiura, M. Ito, and M. Aoyama, *Yakugaku Zasshi*, 90, 1258–1261 (1970).

(168) R. V. Feniksova, N. A. Rodionova, I. Itomlenskite, I. V. Ulezlo, and N. Y. Kobzeva, *Otkrytiya, Izobret. Prom. Obraztsy, Tovarnye Znaki*, 50, 56–57 (1973); *Chem. Abstr.*, 78, 134,513p (1973).

(169) F. J. Simpson, *Can. J. Microbiol.*, 5, 99–107 (1959).

(170) H. Lyr, *Arch. Mikrobiol.*, 33, 266–282 (1959).

(171) H. Lyr, *Arch. Mikrobiol.*, 35, 258–278 (1960).

(172) J. Fukumoto, Y. Tsujisaka, and S. Takenishi, *Nippon Nogei Kagaku Kaishi*, 44, 447–456 (1970).

(173) A. K. Kundu, D. Mukherjee, K. R. Das, and A. K. Das Gupta, *Indian J. Exp. Biol.*, 6, 129–130 (1968).

(174) S. Nakayama and R. Takeda, *Hakko Kyokaishi*, 27, 319–332 (1966).

(175) Y. M. Khokhlova and D. Y. Ismailova, *Prikl. Biokhim. Mikrobiol.*, 9, 203–208 (1973); *Chem. Abstr.*, 79, 50,164h (1973).

(176) R. F. H. Dekker and G. N. Richards, *Carbohyd. Res.*, 38, 257–265 (1974).

(177) J. Varadi, *Biodeterior. Mater., Proc. Int. Biodeterior. Symp.*, 2nd, 129–135 (1971).

(178) T. Kawaminami and H. Iizuka, *Hakko Kogaku Zasshi*, 48, 169–176 (1970).

(179) T. Kawaminami and H. Iizuka, *Hakko Kogaku Zasshi*, 48, 161–168 (1970).

(180) J. G. Hancock and R. L. Millar, *Phytopathology*, 55, 356–360 (1965).

(181) N. J. King and D. B. Fuller, *Biochem. J.*, 108, 571–576 (1968).

(182) C. Gairola and D. Powell, *Phytopathol. Z.*, 72, 305–314 (1971).

(183) G. A. Strobel, *Phytopathology*, 53, 592–596 (1963).

(184) A. Y. Strikevskaya, *Fermenty Nar. Khoz. Med.*, 159–161 (1971); *Chem. Abstr.*, 76, 11,832t (1972).

(185) J. A. Gascoigne and M. M. Gascoigne, *J. Gen. Microbiol.*, 22, 242–248 (1960).

(186) V. I. Bilai, A. Y. Strikevskaya, V. V. Lizak, and G. V. Taradii, *Eksp. Mikol.*, 3–7 (1968); *Chem. Abstr.*, 71, 88,866m (1969).

(187) K. Nisizawa, I. Morimoto, N. Handa, and Y. Hashimoto, *Arch. Biochem. Biophys.*, 96, 152–157 (1962).

(188) M. A. Hulme and D. W. Stranks, *J. Gen. Microbiol.*, 69, 145–155 (1971).

(189) M. Dirdjohadipoetro, K. Hayashi, and M. Funatsu, *J. Fac. Agr. Kyushu Univ.*, 15, 257–267 (1969).

(190) M. Sinner, N. Parameswaran, H. H. Dietrichs, and W. Liese, *Holzforschung*, 27, 36–42 (1973).

(191) A. Y. Strikevskaya, *Fermenty. Med., Pishch. Prom. Sel. Khoz.*, 244–245 (1968); *Chem. Abstr.*, 71, 100,406w (1969).

(192) V. I. Bilai, N. M. Pidoplichko, A. Y. Strikevskaya, and A. A. Vyun, *Eksp. Mikol.*, 8–14 (1968); *Chem. Abstr.*, 71, 88,662s (1969).

(193) A. Y. Strikevskaya, *Eksp. Mikol.*, 24–27 (1969); *Chem. Abstr.*, 71, 90,043j (1969).

(194) A. L. J. Cole and R. K. S. Wood, *Phytochemistry*, 9, 695–699 (1970).

(195) S. Takenishi and Y. Tsujisaka, *Hakko Kogaku Zasshi*, 51, 458–463 (1973).

(196) K. Sumizu, M. Yoshikawa, and S. Tanaka, *J. Biochem.* (Tokyo), 50, 538–543 (1961).

(197) J. Comtat and F. Barnoud, *Compt. Rend., C*, 277, 61–64 (1973).

(198) J. Varadi and L. Jurasek, *Drevar. Vyskum*, No. 1, 7–13 (1970).

(199) K. E. Eriksson and B. Pettersson, *Int. Biodeterior. Bull.*, 7, 115–119 (1971).

(200) M. Dirdjohadipoetro, S. Hashimoto, K. Hayashi, and M. Funatsu, *J. Fac. Agr. Kyushu Univ.*, 15, 269–275 (1969).

(201) N. Toyama, *Hakko Kogaku Zasshi*, 38, 81–88 (1960).

(202) K. Nomura, T. Yasui, S. Kiyooka, and T. Kobayashi, *Hakko Kogaku Zasshi*, 46, 634–640 (1968).

(203) S. Toda, H. Suzuki, and K. Nisizawa, *Hakko Kogaku Zasshi*, 49, 499–521 (1971).

(204) T. Nisizawa, H. Suzuki, and K. Nisizawa, *J. Biochem.* (Tokyo), 70, 387–393 (1971).

(205) S. Hashimoto, T. Muramatsu, and M. Funatsu, *Agr. Biol. Chem.* (Tokyo), 35, 501–508 (1971).

(206) K. Kulp, *Cereal Chem.*, 45, 339–350 (1968).

(207) Z. Safiyazov and T. S. Shulmanl, *Dokl. Vses. Akad. Sel'skokhoz. Nauk*, 19–20 (1973); *Chem. Abstr.*, 79, 39,927p (1973).

(208) B. Flannigan, *Brit. Mycol. Soc. Trans.*, 55, 277–281 (1970).

(209) B. Flannigan and P. N. Sellars, *Brit. Mycol. Soc. Trans.*, 58, 338–341 (1972).

(210) M. Kawai, *Nippon Nogei Kagaku Kaishi*, 49, 529–534 (1973).

(211) R. E. Hungate, "The Rumen and its Microbes," Academic Press, New York (1966).

(212) D. J. Walker, *Aust. J. Agr. Res.*, 12, 171–175 (1961).

(213) S. G. Kock and A. Kistner, *J. Gen. Microbiol.*, 55, 459–462 (1969).

(214) A. Kistner and L. Gouws, *J. Gen. Microbiol.*, 34, 447–458 (1964).

(215) B. H. Howard, *Biochem. J.*, 67, 643–651 (1957).

(216) R. W. Bailey and R. T. J. Clarke, *Nature*, 198, 787 (1963).

(217) R. J. Beveridge and G. N. Richards, *Carbohyd. Res.*, 29, 79–87 (1973).

(218) D. J. Walker, *Aust. J. Biol. Sci.*, 20, 799–808 (1967).

(219) J. E. Courtois, C. Chararas, and M. M. Debris, *Compt. Rend.*, 252, 2608–2609 (1961).

(220) K. W. Talmadge and P. Albersheim, *J. Insect Physiol.*, 15, 2273–2283 (1969).

(221) V. Levaditou, C. Chararas, M. J. Foglietti, and J. E. Courtois, *Compt. Rend. Soc. Biol.*, 165, 796–799 (1971).

(222) I. A. Preece and M. MacDougall, *J. Inst. Brew.*, 64, 489–500 (1958).

detected in the digestive juices of the snail,[20,125] but their source is not yet known.

Various xylophagic insects have also been found to contain D-xylanases. However, the origin of these enzymes has not been determined, and they may be derived either from cells of the digestive tract, or from protozoal symbionts inhabiting the digestive tract.

In germinating seeds, it is reasonable to assume that the cells of the aleurone layer are likely to be responsible for the *in vivo* synthesis, and secretion into the endosperm storage tissue, of the extracellular enzymes which are responsible for the metabolism of the reserve polysaccharides. The seed of the tropical legume *Stylosanthes humilis* does not store the characteristic reserve polysaccharides (such as starch and galactomannan) common to other legume seeds, but has been shown[223] to contain large proportions of an arabinoxylan which is, presumably, a source of metabolizable energy during germination. In such seeds, therefore, the D-xylanase system must play a significant physiological role, and it has been demonstrated[37] that, during germination, D-xylanase activity is greater than that of any other carbohydrase component.

Most of the bacteria and fungi listed in Table XIX produce D-xylanases that are secreted extracellularly. However, some microorganisms (for example, rumen bacteria[38-41,212-214] and protozoa,[43-47,216] *Sporocytophaga myxococcoides*,[94] and *Aspergillus niger*[162]) also produce D-xylanases intracellularly.

There is considerable confusion and conflict as to whether the D-xylanases of bacteria and fungi are generally produced inductively or constitutively in response to the carbon source on which they are grown. Thus, Lyr,[98,104,170,171,224] using several species of wood-rotting fungi, demonstrated that D-xylanase was always produced extracellularly, no matter what polymer (for example, cellulose, pectin, D-xylan, starch, or D-mannan) was used as the carbon source (see Table XX). However, in this work, Lyr employed a medium containing malt extract, which may have influenced the production of D-xylanases. Several other workers have also demonstrated that wood-rotting fungi produce D-xylanases when cultured on cellulose,[61,105,107,126,176,183,199] or D-glucose.[94,130,188,225] Furthermore, Eriksson and his associates found that D-xylanase was produced by *Stereum sanguinolentum*[226] and *Chrysosporium lignorum*[105] when a cellulose preparation free from D-xylan was used as the sole source of carbon. They further showed that the inclusion of yeast extract in the growth medium had no influence on the production of D-xylanase. Other carbon sources that have been reported to produce constitutive D-xylanases from fungi include glycerol,[107] sophorose[204] [O-β-D-Glc$p$-

(223) C. Pang Way and G. N. Richards, *Aust. J. Chem.*, 24, 1041–1048 (1971).
(224) H. Lyr, *Arch. Mikrobiol.*, 34, 189–203 (1959).
(225) H. Lyr, *Arch. Mikrobiol.*, 34, 418–433 (1959).
(226) B. Bucht and K. E. Eriksson, *Arch. Biochem. Biophys.*, 124, 135–141 (1968).

TABLE XX

Enzyme Production by a Cross-section of Several Wood-rotting Fungi
Grown on Various Carbon Sources[171]

Carbon source	Cellulase	D-Xylanase	Pectinase	Amylase
		Enzyme activity (units/ml)		
Pectin	168	1112	7588	1037
Cellulose	1444	802	629	—
D-Xylan	14	452	105	868
Starch	212	1158	469	2448
D-Glucose	68	469	186	901

$(1 \rightarrow 2)$-D-Glc$p$], D-mannan (ivory nut,[104] guar[105]), L-arabinose,[169,183] and D-xylose.[169,183]

The production of D-xylanase activity by fungi cultured on cellulose may also be due to the action of a cellulase component having multisubstrate activity. Cellulases have always been reported to be adaptive enzymes,[226] and the multiplicity of such enzymes may imply that this pseudo-xylanase activity is produced constitutively when fungi are grown on cellulose as the sole source of carbon. Cellulases of this type have been shown[203] also to attack D-xylan even when they are highly purified (for example, cellulase F-2 from *Trichoderma viride*[203]).

On the contrary, however, both Lyr[224] and Ritter,[106] from their investigation of the course of enzyme secretion by the organisms during growth on various carbon sources, have demonstrated that fungal cellulases and D-xylanases are different enzymes. These results conclusively demonstrate that the D-xylanases are constitutive. However, work by Björndal (cited in Refs. 105 and 199) has revealed the presence of a D-xylan in the mycelium of *Stereum sanguinolentum*, and suggests that formation of D-xylanase by this organism, when grown on cellulose, may be self-induced, that is, D-xylanase is induced for the lysis of the old cell-wall material, which then supplies the organism with an endogenous supply of energy. This finding therefore indicates that D-xylanase may also be an adaptive enzyme (that is, induced). Several other workers have also reported[61,149,156,169,183,185,196,225] that xylanases were induced when fungi were grown on media containing D-xylan as the carbon source.

In conclusion, from the evidence just presented, it appears that, in fungi, there is often no strict differentiation between adaptive and constitutive D-xylanases.

Bacterial D-xylanases appear to be usually adaptive,[145,149,156,227] but they have also been shown to be produced constitutively (see, for example, Refs. 94 and 150).

3. Purification

a. Assay Procedure.—Hemicelluloses from different terrestrial or marine (for example, *Rhodymenia palmata*[164]) plant sources have usually been used in the assay procedure for D-xylanase activity. The most commonly used substrates are arabinoxylan,[179,218,228] arabinoglucuronoxylan,[44,176,228] glucuronoxylan,[176,181] and "xylan" (Refs. 183, 189, and 203). The last two substrates are usually sparingly soluble in water, and are solubilized by treatment with alkali (*M* sodium hydroxide) followed by neutralization.[181,203] Soluble D-xylan derivatives that have been used include *O*-(carboxymethyl)-D-xylan[167,229] and *O*-(2-hydroxyethyl)-D-xylan.[200,205]

Assay procedures usually involve measurement of the increase in reducing end-groups by the Nelson–Somogyi (see, for example, Refs. 176 and 228) and Sumner reducing methods (for example, Ref. 199). Other assay procedures are based on the following methods: (*i*) loss in weight of recovered substrate after dialysis, or following precipitation with ethanol (for example, Ref. 41), (*ii*) decrease in viscosity (for example, Ref. 164), and (*iii*) measurement of clearance zones in hemicellulose agar (for example, the "cup-plate" assay[182]).

b. Definition of the D-Xylanase Unit of Activity.—The unit of D-xylanase activity is usually defined as that amount of enzyme which causes the liberation of reducing end-groups corresponding to the formation of 1 μmole of D-xylose per minute under defined conditions of pH, ionic strength, and temperature. Where the molecular weight of the polymer substrate is known, the unit of activity can be expressed as the number of bonds per polymer molecule broken per unit of time in the initial stage of the degradation process (see, for example, Ref. 199).

c. Concentration of Culture Solutions.—Extracellularly secreted microbial D-xylanases present in culture solutions have been concentrated by employing one (or a combination) of the following precipitants: (*i*) ethanol,[161,181] (*ii*) acetone,[183,218,230] (*iii*) alumina,[196]

(227) F. J. Simpson, *Can. J. Microbiol.*, 2, 28–38 (1956).
(228) R. F. H. Dekker and G. N. Richards, *Carbohyd. Res.*, 39, 97–114 (1975).
(229) G. Hrazdina and H. Neukom, *Biochim. Biophys. Acta*, 128, 402–403 (1966).
(230) M. Takahashi and Y. Hashimoto, *Hakko Kogaku Zasshi*, 41, 181–186 (1963).

(*iv*) Rivanol,[8,165] (*v*) adsorption onto ion-exchangers, such as DEAE-Sephadex,[8] (*vi*) affinity binding to insoluble D-xylan[231] at 5°, and (*vii*) ammonium sulfate (the most commonly used precipitant[228]).

d. Extraction of Intracellular D-Xylanases. — Intracellular D-xylanases (for example, from bacteria,[94] fungal mycelia,[162] and rumen bacteria and protozoa) have been extracted by employing the following techniques: (*i*) homogenization in water or buffer[162] followed by extraction with buffer[44,215] or 1-butanol,[38] (*ii*) disruption of the microbial cell-wall by grinding in buffer with Ballotini glass beads in, for example, a Nossal Cell Disintegrator,[39,40,42,44–47,216] (*iii*) lysis of the microbial cell-wall by using a detergent (for example, Triton X-100, see Ref. 94) or indole–buffer reagent,[216] (*iv*) a French press,[42] (*v*) preparation of an acetone powder[232] of the mixed micro-organisms (for example, rumen bacteria and protozoa) and extracting with water, and (*vi*) sonication.[217]

e. Separation and Purification of D-Xylanases. — Xylanases have been separated and purified by using conventional, protein-purification techniques. These include: **A. Ion-exchange chromatography.** — (*i*) Anion exchangers: DEAE-cellulose,[44,155,158,178,181,183,218,233,234] DEAE-Sephadex,[8,59,162,163,172,189,195,197,199,205,228,235–237] and Duolite A-2 (Ref. 230); (*ii*) Cation exchangers: Amberlite resins CG-50 (Ref. 203) and IRC-50 (XE-64) (Refs. 189 and 200), CM-cellulose,[145,158] cellulose phosphate,[32] Cellex-CM,[228] CM-Sephadex,[205] SE-Sephadex,[164,172,195] Duolite C-10 (Ref. 230), and hydroxylapatite.[189,229] **B. Gel-permeation chromatography.** — (*i*) Sephadex G-75 (Refs. 8, 60, 61, 162, 163, 178, 199, 229, 233, and 237), G-100 (Refs. 8, 44, 59, 163, 172, 195, 203, and 234), and G-150 (Ref. 8); (*ii*) Bio Gel P-100 (Ref. 181), P-150 (Refs. 197, 199, and 238), and P-200 (Ref. 236). **C. Isoelectric focusing.** - Using the following as an anticonvection stabilizer: (*i*) ethanediol (75% v/v)[60,61,105,226,238]; (*ii*) sucrose (50% w/v).[228,236] **D. Zone elec-**

(231) S. Kijooka, T. Kobayashi, and T. Yasui, Jap. Pat. 7,125,708; *Chem. Abstr.*, **75**, 128,511*h* (1971).

(232) D. J. Walker and M. F. Hopgood, *Aust. J. Agr. Res.*, **12**, 651–660 (1971).

(233) J. Varadi, V. Necesany, and P. Kovacs, *Drevar. Vyskum*, **16**, 147–159 (1971).

(234) M. Inoue, S. Okada, and J. Fukumoto, *Nippon Nogei Kagaku Kaishi*, **44**, 1–7 (1970).

(235) T. Sasaki and M. Inaoka, *Mem. Ehime Univ.*, **12**, 157–164 (1967).

(236) R. F. H. Dekker and G. N. Richards, *Carbohyd. Res.*, **42**, 107–123 (1975).

(237) H. H. Dietrichs, *Mitt. Bundesforschungsanstalt Forst- Holz-wirtsch.*, No. 93, 153–168 (1973).

(238) K. E. Eriksson and W. Rzedowski, *Arch. Biochem. Biophys.*, **129**, 689–695 (1969).

trophoresis.–Using the following media: (*i*) Sephadex G-25 (Refs. 60, 61, and 164); (*ii*) paper[239]; and (*iii*) poly(acrylamide) gel.[162,197,199,233] E. Affinity binding to D-xylan.[203] F. Crystallization: from acetone[230] and ammonium sulfate.[204]

f. **Criteria of Purity.**—D-Xylanase preparations have been examined for homogeneity by ultracentrifugation,[172,195,199,205,234] moving-boundary electrophoresis,[155,172] paper electrophoresis,[160,203,229,239] and poly(acrylamide) gel electrophoresis.[181,183,205,228,233,236]

4. Physicochemical Properties

D-Xylanases of fungal origin are generally most active at pH 3.5–5.5, and are stable over a wide range of pH, usually from 3 to 10. They usually show optimal, thermal activity at ~50°, and tend to be rather thermostable, often being totally inactivated only at temperatures greater than 65° (see Table XXI). The D-xylanase system (HC II) produced by the fungal phytopathogen *Ceratocystis paradoxa*[236] (see Table XXI) is rather unusual, in that it is highly stable to heat (temperature optimum of 80°, and stable from 0 to 60°), being completely inactivated only after 1 hour at 100° at pH 5.5. Even in the presence of EDTA, this enzyme maintained its thermostability, suggesting that polyvalent metal ions may not be involved. It is rather unusual to find such extreme thermal stability in an enzyme from a mesophilic organism. Bacterial D-xylanases (for example, from *Bacillus subtilis*[230] and *Streptomyces xylophagus*[155]) have somewhat higher pH optima than those from fungal origin, and are stable at pH 5.0–7.3.

The molecular weights of D-xylanase preparations of different origins (see Table XXII), like those of the other hemicellulases (see Sections II, III, and IV), are relatively low, ranging from 16,000 to 38,000. The pI values reported for several D-xylanases are also shown in Table XXII, and, with the exception of the *Ceratocystis paradoxa* D-xylanase I (Ref. 228) (pI 9.17), are mainly acid glycanases.

Whether this is true for the other purified D-xylanase preparations is not yet known, as, in most cases, the pI values have not been reported, but it appears equally possible that neutral (for example, *Fomes annosus*,[61] pI = 7.0), and alkaline (for example, *Ceratocystis paradoxa*,[228] pI = 9.17) D-xylanases also exist. The K_m values of several fungal D-xylanases have been reported (see Table XXII) and

(239) M. Takahashi and Y. Hashimoto, *Hakko Kogaku Zasshi*, 41, 186–189 (1963).

TABLE XXI

pH and Temperature Properties of D-Xylanases

Source of enzyme	pH optimum	pH stability	Temperature optimum (°C)	Temperature of complete thermal inactivation (°C)
Bacillus subtilis[230]	6.0–6.2	5.0–7.0	37–45	70
Streptomyces xylophagus[155]	6.2	5.3–7.3	55–60	70
Agaricus bisporus[158] A[a]	5.4	4.5–6.5	45–50	65
B[b]	5.0	3.5–6.0	50	70
Aspergillus niger[162] I[c]	4.5	—[d]	50	60
A. niger II	5.5	—	50	60
A. niger[234] III	4.5–5.0	2.0–9.0	50	80
A. niger van Tieghem[172] I	5.5	3.5–10.0	—	60
A. niger van Tieghem II	5.0	4.0–10.0	—	>75
A. niger van Tieghem III	3.5	3.0–8.0	—	>75
Ceratocystis paradoxa[228,236] I	5.5	—	40	65
C. paradoxa II	5.1	5.0–10.0	80	100
Diplodia viticola[183]	3.8	—	—	—
Penicillium janthinellum[195] I	5.3	5.0–8.0	—	70
P. janthinellum II	4.7	4.0–9.0	—	80
P. janthinellum III	4.7	5.0–9.0	—	80
Schizophyllum commune[233]	4.8–5.4	—	—	65
Trichoderma viride[203]	5.5–6.0	3.0–7.0	—	90
T. viride[205]	3.5	2.0–7.0	50	65
Commercial[229] "cellulase"	4.5	4.0–11.0	—	80

[a] Xylanase isolated from wheat-bran culture. [b] Xylanase isolated from mushroom fruiting body. [c] Indicates different types of xylanases isolated. [d] — Indicates not specified.

range from 0.27–14.00 mg of hemicellulose per ml. Obviously, these values are dependent on the detailed structure of the hemicellulose used. D-Xylanases have been reported to be inhibited by sulfhydryl reagents, metal ions, glycerol, 1,2-ethanediol, and various sugars (see Table XXIII). Experiments using several sulfhydryl reagents, namely, *p*-chloromercuribenzoate, iodine, and iodoacetic acid, have shown that the D-xylanase from *Bacillus subtilis*[239] requires certain -SH groups for activity. Glycerol and 1,2-ethanediol, at concentrations greater than 60% (v/v), strongly inhibit the action of *Aspergillus niger* xylanase.[162] Furthermore, the inhibition caused by 1,2-ethanediol was found[162] to be reversible. Metal-ion inhibitors of D-xylanase from *Bacillus subtilis*,[239] *Agaricus bisporus*,[158] *Ceratocystis paradoxa*,[236] and *Trichoderma viride*[205] are shown in Table XXIII.

TABLE XXII

Molecular Weight, Isoelectric Point, and K_m Values of
D-Xylanases of Different Fungal Origin

Source of enzyme	M.W.[a] × 10³	pI	K_m (mg/ml)
Aspergillus niger[60]	—[b]	3.90,4.50	—
Aspergillus wentii[59]	25–28	—	—
Ceratocystis paradoxa I[228]	—	9.17	4.24
C. paradoxa II[236]	—	4.50	0.27
Coniophora cerebella[181]	34–38	—	—
Chrysosporium lignorum[61]	—	4.44,6.00	—
Fomes annosus[61]	—	4.10–4.60,7.00	—
Polysporus schweinitzii[59]	35	—	—
Schizophyllum commune[233]	—	—	14.00
Stereum sanguinolentum[61]	—	3.62,4.30	—
S. sanguinolentum[199]	21.6[c]	—	—
Trichoderma viride[203]	16	—	2.5
Commercial[229] "cellulase"	—	4.5	1.0

[a] Calculated from gel-permeation chromatographic data. [b] — Indicates not specified.
[c] Calculated by the ultracentrifugation method (M.W. = 23.9 × 10³ from the amino acid composition).

The Hg^{2+} ion was found to be the most potent inhibitor, and its effect on enzymic activity suggests reaction with thiol groups. However, Hg^{2+} is also known to react with the amino and imidazolium groups of histidine,[240] and with peptide linkages,[241] and may be capable of coordinating with carboxyl and amino groups.[242] Other metal ions (see Table XXIII) partially inhibit D-xylanase action.[158,239] Certain sugars [for example, L-arabinose, D-xylose, xylotriose (X_3), and xylopentaose (X_5)] have also been reported to be inhibitory towards D-xylanases (see Table XXIII). Saccharide X_5 was found to be a competitive inhibitor of Trichoderma viride D-xylanase,[203] with an inhibition constant (K_i) of 134 μg/ml. The D-xylanase from Ceratocystis paradoxa[228,236] was also found to be inhibited at high substrate concentrations (>10 mg of hemicellulose B per ml). D-Xylanases from Bacillus subtilis[230] and Agaricus bisporus[158] have been reported to be activated by calcium chloride and sodium chloride, respectively. However the activation of Bacillus subtilis D-xylanase

(240) R. B. Simpson, J. Amer. Chem. Soc., 83, 4711–4717 (1961).
(241) W. Haarmann, Biochem. Z., 314, 1–17 (1943).
(242) J. L. Webb, "Enzyme and Metabolic Inhibitors," Academic Press, New York, 1966, Vol. 2.

TABLE XXIII

Inhibitors of D-Xylanases

Inhibitor	Source of D-xylanase	References
Sulfhydryl reagents (p-chloromercuribenzoate, iodine, iodoacetic acid)	*Bacillus subtilis*	239
Ethanediol, glycerol	*Aspergillus niger*	162
Metal ions		
Hg^{2+}, Ag^+, Cu^{2+}, Fe^{2+}, Fe^{3+}	*Bacillus subtilis*	239
Mn^{2+}, Zn^{2+}, Mg^{2+}	*Agaricus bisporus*	158
Hg^{2+}	*Trichoderma viride* and *Ceratocystis paradoxa*	205,236
Sugars		
L-Arabinose, D-xylose, and xylotriose (Xyl_3)	*C. paradoxa*	228
D-Xylose	*C. paradoxa*	236
Xylopentaose (Xyl_5)	*T. viride*	203

by calcium chloride did not increase the stability of the enzyme.[230]

The D-xylanase system of *Stereum sanguinolentum*,[199] the only D-xylanase for which an amino acid composition has as yet been published, was found to contain a high proportion of acidic and aromatic amino acid residues. The M.W., as determined from the amino acid composition, is 23,900, compared with 21,600 as calculated from ultracentrifugation data. Other physical parameters that have been determined[199] for this D-xylanase include the sedimentation coefficient [2.8S, which is similar to that reported for a D-xylanase isolated from *Trichoderma viride*,[205] namely, 2.1S], the partial specific volume (0.71 $cm^3.g^{-1}$), and the molar extinction coefficient (6.25×10^4). Activation energies have been reported for D-xylanases from *Schizophyllum commune*[233] (E_A 28.6 $kJ.mol^{-1}$) and from a commercial "cellulase" preparation[229] (E_A 34.0 $kJ.mol^{-1}$).

Several fungal glycan hydrolases, (for example, *alpha*-amylase from *Aspergillus oryzae*,[243] glucamylases I and II from *Aspergillus niger*,[244] L-arabinanase from *A. niger*,[10] and cellulases F-1 and F-2 from *Trichoderma viride*[203]) have been shown to contain covalently linked carbohydrate residues as components of their molecular structure. Such enzymes have been referred to by Pazur and his associates, as *glycoenzymes*.[245] A D-xylanase isolated from *Trichoderma*

(243) H. Hanabusa, T. Ikenaka, and S. Akabori, *J. Biochem.* (Tokyo), **42**, 55–62 (1955).
(244) J. H. Pazur, H. R. Knull, and A. Cepure, *Carbohyd. Res.*, **20**, 83–96 (1971).
(245) J. H. Pazur, H. R. Knull, and D. L. Simpson, *Biochem. Biophys. Res. Commun.*, **40**, 110–115 (1970).

viride[203] has also been shown to be a glycoenzyme, and it was found to contain 16.8% of carbohydrate. However, the monosaccharide composition of the carbohydrate moiety was not reported. The D-xylanase preparation from *Schizophyllum commune*[233] failed to respond to the glycoprotein staining procedure, and is not considered to be a glycoenzyme.

Purified, crystalline preparations have been reported for the D-xylanases from *Trichoderma viride*[205] and *Bacillus subtilis*[230]; they were crystallized from ammonium sulfate and acetone solutions, respectively.

5. Mode of Action

This Section will be concerned with (*i*) the specificities of D-xylanases of bacterial and fungal origin, and (*ii*) the "hemicellulase" systems of rumen bacteria and protozoa, and it will include the xylobiases (β-D-xylosidases), xylo-oligosaccharidases, α-L-arabinofuranosidases, and D-xylanases.

a. Bacterial D-Xylanases.—The D-xylanases produced by *Bacillus subtilis*[230] and *Streptomycetes xylophagus*[155] have been shown to be mainly of the endo enzyme type. The *B. subtilis* D-xylanase[230] degraded D-xylan, liberating initially arabino- and xylo-oligosaccharides of d.p. $\geqslant 2$, and, on prolonged hydrolysis (24 h), these yielded D-xylose in addition to the products already mentioned. Xylobiose (X_2) was not further degraded. The *S. xylophagus* D-xylanase[155] degraded D-xylan in a similar way, but did not liberate L-arabinose. D-Xylose was produced on prolonged incubation (> 5 h), and, together with X_2, constituted the major end-products of hydrolysis. Disaccharide X_2 was not further degraded by this enzyme.

b. Fungal D-Xylanases.—D-Xylanases are potentially of either the exo or the endo type. There are many claims in the literature to isolation of exo-D-xylanases from fungi, but such enzymes are difficult to distinguish from β-D-xylosidases, as both are capable of degrading D-xylo-oligosaccharides of d.p. $\geqslant 2$, but, by definition, only the former should attack D-xylan. The two types of enzyme can be clearly distinguished from one another, however, by nuclear magnetic resonance spectroscopic analysis, which determined,[246] for example, the configuration of the glycose residue released. Retention of configuration indicates a glycosidase, and inversion of configuration, an exo-glycanase.

D-Xylanases having action patterns of the exo type have been claimed to be produced extracellularly by *Aspergillus batatae*,[160] *A.*

niger,[164,165,235] and *Coniophora cerebella,*[181] but they have not been so extensively purified as the endo-D-xylanase preparations. It is not certain whether these partially purified enzyme preparations are true exo-D-xylanases, because the fungi also produce endo-D-xylanases. In fact, the same results would be obtained if the enzyme preparations consisted of a mixture of a β-D-xylosidase and an endo-D-xylanase. Therefore, unless an exo-D-xylanase preparation has been properly shown to be homogeneous, claims of exo-action properties must be interpreted with caution.

Fungi have been the most common source of endo-D-xylanases (see Table XIX), and enzyme preparations from them have been more extensively studied than those from any other genera. The endo-D-xylanases can be divided into two groups, namely, (*i*) those that liberate L-arabinose from the enzymic hydrolysis of arabinoxylans and arabinoglucuronoxylans (that is, the "arabinose-liberating xylanases"), and (*ii*) those that do not liberate L-arabinose from these substrates (that is, the "non-arabinose-liberating xylanases"). Both groups are, of course, also capable of degrading glucuronoxylans and D-xylans.

(i) **The "Arabinose-liberating Endo-D-xylanases."** D-Xylanases, by definition, are glycan hydrolases capable of hydrolyzing $(1 \rightarrow 4)$-β-D-xylopyranosyl linkages of the hemicellulosic D-xylans. However, several highly purified xylanase preparations have also been shown to be capable of hydrolyzing the $(1 \rightarrow 3)$-α-L-arabinofuranosyl branch points of arabinoxylans.[8,128,158,163,247,248] These enzymes have been isolated from *Agaricus bisporus,*[158] *Aspergillus niger,*[8,163] several different strains of *A. niger,*[162,247,248] *Ceratocystis paradoxa,*[228] and *Diplodia viticola.*[183] The crude enzyme preparation from *D. viticola*[183] was capable of liberating L-arabinose from corn-cob and grape arabinoxylans, but when purified, degraded the D-xylans to the xylo-oligosaccharides of d.p. 2–5, yielding no arabinose. It appears, therefore, that the crude enzyme-preparation contains either two different D-xylanases, one of which is capable of hydrolyzing the arabinose substituent, or a D-xylanase and an α-L-arabinofuranosidase (or an arabinanase; see, for example, Ref. 10). The last-mentioned type of enzyme has been found to be produced by several other phy-

(246) P. A. J. Gorin, J. F. T. Spencer, and D. E. Eveleigh, *Carbohyd. Res.*, 11, 387–398 (1969).

(247) Y. Tsujisaka, S. Takenishi, and J. Fukumoto, *Nippon Nogei Kagaku Kaishi*, 45, 253–259 (1971).

(248) S. Takenishi and Y. Tsujisaka, *Agr. Biol. Chem.* (Tokyo), 37, 1385–1391 (1973).

topathogenic fungi,[15] and shown[64] to be capable of hydrolyzing the α-L-arabinofuranoside branch points of arabinoxylans.

The xylanase produced by *Agaricus bisporus*[158] degraded rice-straw hemicellulose, initially to L-arabinose and the xylo-oligosaccharides of d.p. $\geq$ 2. D-Xylose was liberated upon prolonged hydrolysis.

The D-xylanase system of *Aspergillus niger*[8,162,163] was found to consist of two different D-xylanases (I and II). D-Xylanase I degraded arabinoxylan (rice straw) to D-xylose, L-arabinose, and a mixture of xylo-oligosaccharides of d.p. 2–5. Disaccharide X_2 and xylotriose (X_3) were not attacked, but the arabinoxylo-oligosaccharide AX_3 was hydrolyzed to X_3 and L-arabinose. The multiplicity of these D-xylanases produced by *Aspergillus niger* was further investigated by Iwamoto and coworkers.[162] They examined the effect of the carbon source [D-xylan, D-xylose, L-arabinose, O-(carboxymethyl)cellulose, and D-glucose], culture age, and strain specificity, and found that, in each case, both types of D-xylanase (I and II) were produced. Both D-xylanases were produced within the fungal mycelium (that is, intracellularly), as well as being secreted extracellularly into the growth medium. Fractionation of the crude enzyme-preparation by gel-permeation chromatography (on Sephadex G-75), ion-exchange chromatography (on DEAE-Sephadex), and zone electrophoresis [on poly(acrylamide) gel] showed, in each instance, that two D-xylanases existed, and that one of them *always* liberated L-arabinose from the degradation of arabinoxylan.[162]

Aspergillus niger van Tieghem[247,248] produced three D-xylanases, namely, D-xylanase I, II, and III, two of which (II and III) degraded rice-straw arabinoxylan to D-xylose, L-arabinose, and a mixture of arabinoxylo- and xylo-oligosaccharides as follows.

Enzyme[247,248]	Degradation products[a]	Degree of hydrolysis (%)
Xylanase II	Xyl, Ara, X_2, X_3, AX_5	48
Xylanase III	Xyl, Ara, X_2, AX_3, AX_4, AX_5	34

[a] Key: AX_m = branched arabinoxylo-oligosaccharides of d.p. = m, and X_n = linear xylo-oligosaccharides of d.p. = n.

D-Xylanase II did not attack X_2, but liberated L-arabinose from arabinoxylobiose (AX_2). Arabinoxylotriose (AX_3) was hydrolyzed to L-arabinose and X_3, and the latter was further degraded to xylose and X_2. D-Xylanase II also attacked phenyl α-L-arabinofuranoside, liberating L-arabinose, but it would not attack the β anomer.[248]

Ceratocystis paradoxa has been shown[228,236] to produce several D-xylanases, one of which (HC I; see Ref. 228) was found to liberate L-arabinose from spear grass (*Heteropogon contortus*) and sugar-cane bagasse hemicellulose B samples (arabino-4-*O*-methylglucuronoxylans), and from wheat-endosperm arabinoxylan. Initial attack of this enzyme on these substrate samples liberated a mixture of arabinoxylo- and xylo-oligosaccharides. L-Arabinose appeared after 2 h, and D-xylose and an "insoluble degraded-hemicellulose" appeared on prolonged incubation. The latter may arise as a result of the progressive removal of L-arabinose and uronic acid residues, leaving a "xylan" of low L-arabinose and uronic acid content that eventually precipitates from solution.

Degradation products arising from the hydrolysis of arabinoxylo- and xylo-oligosaccharides of d.p. 3–6 and 2–5, respectively, by the action of a D-xylanase (HC I) from *Ceratocystis paradoxa* are shown in Table XXIV. Xylotetraose (X_4) was the smallest xylo-oligosaccharide attacked, and both it and X_5 yielded X_2 and X_3. The mechanism proposed[228] for the hydrolysis of X_4 by HC I is as follows.

$$X_2 + E$$
$$\uparrow H_2O$$
$$X_4 \rightleftharpoons E \sim X_4 \rightleftharpoons E \sim X_2 + X_2$$
$$X_4 \updownarrow$$
$$E \sim X_6$$
$$\swarrow H_2O \searrow$$
$$2X_3 + E \qquad X_2 + X_4 + E$$
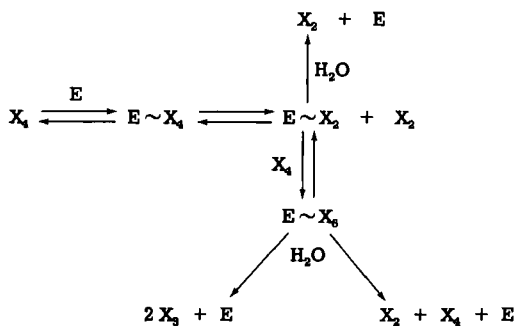
(where E = enzyme, and E $\sim X_2$, E $\sim X_4$, and E $\sim X_6$ = enzyme–substrate complexes)

D-Xylanase HC I degraded[228] the arabinoxylo-oligosaccharides (see Table XXIV) to L-arabinose and the parent xylo-oligosaccharides which, if larger than X_3, were subsequently further hydrolyzed. The hexasaccharide AX_5 also yielded some AX_3, which was subject to further degradation to L-arabinose and X_3. This finding was rather surprising, as the rate and product studies with xylo-oligosaccharides suggest that the binding site of D-xylanase HC I requires a chain of at least five D-xylose residues for the formation of a complex amenable to rapid hydrolysis, and that the active site is situated within the binding subsites, as illustrated by the following model for the active site of the enzyme.[228]

Catalytic
site
↓

Binding
subsites

| X | X | X | X | X |
| 5 | 4 | 3 | 2 | 1 |

(where X = a D-xylose residue)
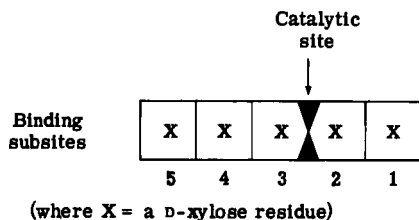
The relatively rapid attack on AX_5 to produce AX_3 plus X_2 suggests, therefore, that the presence of the L-arabinose substituent may not interfere with the binding of the β-D-$(1 \rightarrow 4)$-linked D-xylose chain to the binding site. There is evidence[249] that the L-arabinose substituent in AX_5 is probably present on the nonreducing-end D-xylosyl group, but the foregoing argument is valid no matter where the L-arabinose is situated on the xylose chain.

The arabinosidase activity of D-xylanase HC I was suppressed when the enzyme was pre-incubated with X_3 prior to attack on hemicellulose B. The presence of X_3 did not, however, prevent further scission of the D-xylan chain, which suggests that participation of two active sites is involved in the hydrolysis of hemicellulose B by the action of xylanase HC I.

A comparison of the properties and mode of action of several "arabinose-liberating xylanases" is summarized in Table XXV; it indicates that each enzyme is different from the others. *Aspergillus niger* van Tieghem D-xylanases II and III (Refs. 172 and 247) failed

TABLE XXIV

Degradation Products Arising from the Hydrolysis of Arabinoxylo- and
Xylo-oligosaccharides by a D-Xylanase (HCI) from *C. paradoxa*

Oligosaccharide[a]	End-products of hydrolysis
AX_2	A, X_2
AX_3	A, X_3
AX_4	A, X_2, X_3
AX_5	A, AX_3, X_2, X_3
X_2	—[b]
X_3	—
X_4	X_2, X_3
X_5	X_2, X_3

[a] A = L-arabinose, X = D-xylose. [b] — Indicates no attack.

(249) R. F. H. Dekker and G. N. Richards, *Carbohyd. Res.*, 43, 335–344 (1975).

TABLE XXV

Comparison of the Properties and Mode of Action of Purified D-Xylanases that Liberate L-Arabinose from Hemicelluloses

Microbial origin	Optimal pH	Temperature stability (°C)	Source of hemicellulose[a]	Products of hydrolysis	Attack on sugar-beet L-arabinan[a]	Products of attack[b] on		References
						AX$_3$	X$_3$	
Aspergillus niger van Tieghem sp. enzyme II	5.0	0–60	rice-straw arabinoxylan	A, X$_1$, X$_2$, X$_3$, AX$_5$	no	A, X$_1$, X$_2$, X$_3$	X$_1$, X$_2$	172,247,248
Enzyme III	3.5	0–50	rice-straw arabinoxylan	A, X$_1$, X$_2$, AX$_3$, X$_3$, AX$_4$, AX$_5$	no	n.s.	n.s.	172,247
Aspergillus niger	4.5	0–60	"xylan" (n.s.)	A, X$_2$, AX$_3$, X$_3$, X$_4$	n.s.	A, X$_3$	no	163
Ceratocystis paradoxa	5.5	0–46	spear-grass hemi-cellulose B	A, X$_1$, X$_2$, X$_3$, AX$_4$, X$_4$, AX$_5$, X$_5$	yes	A, X$_3$	no	228
Agaricus bisporus	5.4	0–45	rice-straw hemi-cellulose	A, X$_1$, X$_2$, X$_3$, X$_4$–X$_8$	n.s.	n.d.	n.d.	158

[a] n.s. = not specified. [b] n.d. = not determined.

to degrade sugar-beet L-arabinan, whereas *Ceratocystis paradoxa* xylanase HC I (Ref. 228) slowly attacked the α-L-(1 → 3)-linked L-arabinofuranosyl linkages of beet L-arabinan, releasing L-arabinose. Table XXV also shows that both D-xylanases I (*A. niger*[163]) and HC I (*C. paradoxa*[228]) failed to degrade X_3, but liberated L-arabinose from AX_3, whereas D-xylanase II (*A. niger* van Tieghem[172,247,248]) was capable of attacking X_3, and, hence, after removal of L-arabinose from AX_3, it subsequently yielded D-xylose and X_2.

(ii) **The "Non-arabinose-liberating Endo-xylanases."** This group of D-xylanases, constituting the majority of the known fungal endo-D-xylanases, usually degrades arabinoxylan and other D-xylans to D-xylose, D-xylo-oligosaccharides, and, in some cases, oligosaccharides containing both L-arabinose and D-xylose. These enzymes, in highly purified form, have been isolated from various strains of *Aspergillus niger*,[8,162,163,247,248] *Ceratocystis paradoxa*,[236] *Diplodia viticola*,[183] *Stereum sanguinolentum*,[199] and *Trichoderma viride*[203]; the degradation products arising from their attack on D-xylans are summarized in Table XXVI.

Most of these D-xylanase preparations, with the exception of that from *Diplodia viticola*, degraded D-xylan randomly, liberating xylose as well as oligosaccharides. The absence of D-xylose from the enzymic hydrolyzates from the latter source may be due to the fact that samples were only assayed in the early stages of hydrolysis (24 h).

The partially purified D-xylanase of *Coniophora cerebella*[181] degraded poplar 4-*O*-methylglucuronoxylan to D-xylose, a mixture of D-xylo-oligosaccharides of d.p. 2–5, and an enzyme-resistant polymer that was not further investigated. The enzymic digests also afforded a series of acidic oligosaccharides, the fastest-moving component of this series migrating on paper chromatograms behind the acidic tetrasaccharide (1), previously isolated by Timell[6] from birch 4-*O*-methylglucuronoxylan.

$$\beta\text{-D-Xyl}p\text{-}(1\rightarrow4)\text{-}\beta\text{-D-Xyl}p\text{-}(1\rightarrow4)\text{-D-Xyl}p$$
$$2$$
$$\uparrow$$
$$1$$
$$4\text{-}O\text{-Me-}\alpha\text{-D-Glc}p\text{A}$$

$$1$$
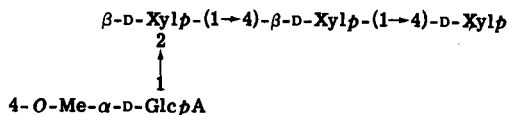
(where 4-*O*-Me-α-D-Glc*p*A = 4-*O*-methyl-α-D-glucuronic acid)

The marine algal D-xylan obtained from *Rhodymenia palmata* has been shown[250,251] to be composed of β-D-(1 → 3)- and β-D-(1 → 4)-linked D-xylose residues in a single D-xylan chain, and it is suscep-

TABLE XXVI

The Degradation Products Arising from the Hydrolysis of D-Xylans by
Endo-D-xylanases of Fungal Origin

Source of D-xylanase	Source of D-xylan[a]	Degradation products[b]	References
Aspergillus niger van Tieghem			
D-xylanase I	rice straw	X, AX_2, X_2, AX_3, AX_4	172,247,248
D-xylanase II	rice straw	X, X_2, X_3, (A–X), X_4	8
A. niger			
D-xylanase B	corn cob	X, X_2, X_3, (A–X), X_4	162
D-xylanase II	rice straw	X, X_2, (A–X), X_3, X_4	163
Ceratocystis paradoxa, D-xylanase HC II	spear grass, wheat endosperm	X, AX_2, X_2, AX_3, X_3, AX_4, X_4, AX_5, X_5	236
Commercial "cellulase"	wheat straw	X, X_2–X_5	229
Diplodia viticola	corn cob, grape	X_2–X_5	181
Stereum sanguinolentum	*Rhodymenia palmata*	X_2, X_3, (X_4–X_{10})	150
Trichoderma viride	Commercial xylan (n.s.)	X, X_2–X_5	203

[a] n.s., not specified. [b] (A–X) denotes an arabinoxylo-oligosaccharide, the d.p. of which was not specified. (X_4–X_{10}) denotes a mixture of xylo-oligosaccharides (of d.p. ranging from 4 to 10) that contain both β-D-(1 → 3)- and -(1 → 4)-linked D-xylopyranose residues, usually in the ratio of 1:2.

tible to attack by fungal D-xylanases. The partially purified D-xylanase preparation from *Aspergillus niger*[164] degraded the algal D-xylan to D-xylose and a mixture of xylo-oligosaccharides of d.p. 2–5. The purified D-xylanase preparation of *Stereum sanguinolentum*[250] degraded *Rhodymenia palmata* D-xylan to X_2, X_3, and a series of higher oligosaccharides (X_4–X_{10}) of mixed β-D-(1 → 3)- and β-D-(1 → 4)-linkages, usually in the ratio of 1:2. Attack is believed to occur at the β-D-(1 → 4)-linkage only when it is flanked on both sides by other β-D-(1 → 4)-linkages. Björndal and coworkers[250] suggested that the decreasing yields of X_6 to X_4 indicate that a β-D-(1 → 3)-

(250) H. Björndal, K. E. Eriksson, P. J. Garegg, B. Lindberg, and B. Swan, *Acta Chem. Scand.*, 19, 2309–2315 (1965).
(251) V. C. Barry, J. E. McCormick, and P. W. D. Mitchell, *J. Chem. Soc.*, 3692–3696 (1954).

linkage not only inhibits the cleavage of an adjacent linkage, but also lessens the rate of cleavage of the linkage two residues removed. This enzyme did not hydrolyze X_2 and X_3, but degraded higher xylo-oligosaccharides to give mixtures of di- and tri-saccharides.[250]

At high concentrations of the substrate, this enzyme was capable of synthesizing oligosaccharides of higher d.p. from X_4 and X_5 (transglycosylation), but this effect could be prevented by using low concentrations of substrate (for example, 0.01%). In a later paper, Eriksson and Pettersson[199] reported that a highly purified D-xylanase preparation isolated from *Stereum sanguinolentum* hydrolyzed a series of xylo-oligosaccharides of d.p. $\geqslant 3$, mainly to D-xylose and X_2. This later preparation was, therefore, capable of hydrolyzing X_3, and it appears that this fungal organism produces at least two different D-xylanases.

The D-xylanase isolated from *Trichoderma viride*[203] appears to be similar in its mode of attack on D-xylan to one of the D-xylanases from *Stereum sanguinolentum*. This enzyme degraded xylo-oligosaccharides of d.p. 2–5 to a mixture of D-xylose and X_2, with traces of X_3. In both cases, however, X_2 remained unattacked. Another D-xylanase which appears to be similar in its mode of action is that isolated from *Aspergillus niger*.[163] This enzyme degraded X_3 and xylotriitol to D-xylose and X_2, and to xylitol and X_2, respectively. The release of xylitol suggests that attack occurs mainly at the reducing end of the molecule. The same D-xylanase[163] did not attack an arabinoxylotriose (AX_3) or X_2.

Ceratocystis paradoxa D-xylanase HC II (Ref. 236) degraded wheat endosperm arabinoxylan and spear-grass hemicellulose B, initially to X_2 and a series of mixed arabinoxylo- and xylo-oligosaccharides of d.p. $\geqslant 3$. D-Glucose and a D-glucose disaccharide (probably cellobiose) appeared in the hydrolyzates of hemicellulose B; they are products arising from degradation of a minor β-D-linked glucan component (either a degraded cellulose, or another type of glucan; see, for example, Ref. 252) associated with the hemicellulose B preparation. This enzyme preparation was also found[236] to be capable of attacking O-(carboxymethyl)cellulose, yielding D-glucose and cellobiose as the main products of hydrolysis. D-Xylose and AX_2 appeared in the hemicellulose hydrolyzates after 4 h, and L-arabinose was not detected. Prolonged incubation progressively decreased the mean d.p. of the oligosaccharides liberated, and, after 24 h, a degraded hemicellulose was precipitated whose intrinsic viscosity, and, hence,

(252) A. J. Buchala and K. C. B. Wilkie, *Naturwissenschaften*, **57**, 496 (1970).

presumably, its molecular weight, was considerably lower than that of the original hemicellulose B (49.3 as compared to 66.9 ml.g^{-1}). The absence of any oligosaccharide products containing more than one L-arabinose residue (for example, A_2X_3) is significant, and indicates that, in the portions of the hemicellulose molecules attacked by this enzyme, there are no instances where L-arabinofuranose substituents occur on contiguous D-xylose residues of the hemicellulose backbone.[236]

Trisaccharide X_3 was the lowest homologue attacked, liberating D-xylose and X_2, whereas AX_3 yielded AX_2 and D-xylose.[236] The mode of action of *Ceratocystis paradoxa* xylanase HC II on arabinoxylo- and xylo-oligosaccharides is as shown; it indicates that attack occurs at the reducing end of the oligosaccharide chain.

$$
\begin{array}{ll}
\overset{\displaystyle A}{\underset{\displaystyle |}{}} & \\
X - X \mid X & X - X \mid X \\[2em]
\overset{\displaystyle A}{\underset{\displaystyle |}{}} & \\
X - X \mid X \mid X & X - X \mid X \mid X \\[2em]
\overset{\displaystyle A}{\underset{\displaystyle |}{}} & \\
X - X \mid X \mid X \mid X & X - X \mid X \mid X \mid X
\end{array}
$$
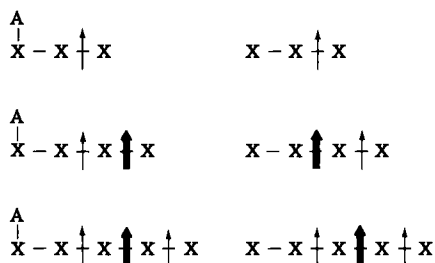
[where X = β-D-(1→4)-linked D-Xyl$p$, A = α-L-(1→3)-linked L-Ara$f$, ⬆ indicates linkages

readily attacked by D-xylanase HC II, and ⬆ indicates linkages attacked at a lower rate]

The relative rates of hydrolysis of xylo-oligosaccharides by D-xylanase HC II (Ref. 236) suggested that the binding site of the enzyme is effectively filled by a chain of four D-xylose residues, as illustrated, with the catalytic site situated between subsites 1 and 2. Apparently, the same "fit" can also be achieved by AX_4, as it is

Catalytic
site
↓

Binding
subsites | X | X | X | X | Binding
site

4   3   2   1
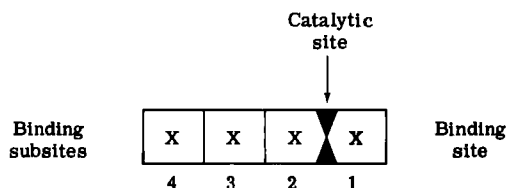
hydrolyzed even more rapidly than X_4. It appears, therefore, that the presence of the L-arabinose substituent may not impede binding of the enzyme to the hemicellulose in the vicinity of the substituent, although, of course, the D-xyloside linkage to the "right" of the substituent, namely, 2, is resistant to hydrolysis (that is, AX_2 is stable).

$$-\beta\text{-}\text{D}\text{-}\text{Xyl}p\text{-}(1\rightarrow 4)\text{-}\text{D}\text{-}\text{Xyl}p\text{-}$$
$$3$$
$$\uparrow$$
$$1$$
$$\alpha\text{-}\text{L}\text{-}\text{Ara}f$$
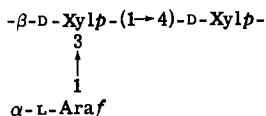
2

In this respect, an analogy may be drawn with the mode of action of barley-malt *alpha*-amylase on amylopectin,[253] which produces the oligosaccharide panose [*O*-α-D-Glc*p*-(1 → 6)-*O*-α-D-Glc*p*-(1 → 4)-D-Glc*p*], which is resistant to further hydrolysis. It appears, therefore, that the foregoing conclusions are compatible with the rather unusual suggestion that the more highly substituted regions of the hemicellulose may be preferentially attacked by D-xylanase HC II (see next).

The degraded hemicellulose (IH) which precipitated from solution during the course of enzymic hydrolysis of spear-grass hemicellulose B contained smaller proportions of L-arabinose, D-glucose, D-galactose, and uronic acid than the original material. The lowering in L-arabinose content resulted from the formation of arabinose–xylose oligosaccharides, as no free L-arabinose was liberated. When IH was redissolved, it was subject to further attack by D-xylanase HC II, liberating the same oligosaccharides as those arising from attack on the original hemicellulose B,[236] and, upon prolonged incubation, the hemicellulose precipitate again formed, which, when solubilized, was again attacked by D-xylanase HC II, yielding similar products, including the precipitate. The production of the IH precipitate during enzymic hydrolysis is evidently a time-dependent, retrogradation phenomenon which appears to be favored by a lowering in the proportion of non-xylose constituents. It is not surprising to find that the less-substituted D-xylan chains retrograde the more rapidly. However, in order to explain these observations, it must be concluded, rather surprisingly, that the enzyme tends to attack selectively the hemicellulose molecules (or portions of the molecules) which are most substituted with arabinose and uronic acid residues, leaving a less substituted xylan of lower molecular weight which retrogrades and thus becomes resistant to further attack.

c. **The Rumen Microbial Hemicellulase System.**—The hemicellulases of rumen micro-organisms include the α-L-arabinofuranosidase, xylobiase, xylo-oligosaccharidase, and D-xylanase systems. This group is also likely to include the α-D-glucosiduronases (the α-D-

(253) W. J. Whelan, *Staerke*, 12, 358–364 (1960).

glucosiduronate glucuronohydrolases), which are capable of hydrolyzing the α-D-(1 → 2)-linked D-glucopyranosyluronic groups (often containing a methoxyl group at C-4) of glucuronoxylans. However, enzymes having this specificity have been neither sought nor detected in rumen microbial systems, and so will not be discussed further.

The hemicellulases of rumen micro-organisms have been isolated from pure culture isolates of (a) bacteria, for example, *Bacillus firmus*,[148] *Bacteroides amylogenes*,[38] *Butyrivibrio fibrosolvens*,[38,40] and *Ruminococcus flavefaciens*,[41] and (b) protozoa, for example, *Epidinium ecaudatum*,[43,44] an *Entodinium* sp.,[44,216] *Eudiplodinium medium*,[46] *Eremoplastron bovis*,[44,45] and *Polyplastron multivesiculatum*.[47] They have also been found in cell-free preparations of mixed bacteria,[40,42,215] protozoa,[43] and mixtures of both organisms.[42,217,218,254,255]

In most cases, there was detected a xylobiase (β-D-xyloside xylohydrolase, β-D-xylosidase) which hydrolyzed X_2 and D-xylose oligosaccharides of d.p. 3–6 to D-xylose. A "xylosidase" has also been partially purified from *Epidinium ecaudatum*[44] and shown to degrade D-xylose oligosaccharides of d.p. 3–5 to xylose and X_2. This enzyme was separated from other hemicellulases by fractionation on Sephadex G-100 and DEAE-cellulose; it did not degrade X_2 or D-xylan, and was classified[44] as a "xylodextrinase."

α-L-Arabinofuranosidases (EC 3.2.1.55, α-L-arabinofuranoside arabinohydrolase) that hydrolyze the α-L-(1 → 3)-linked arabinose branch-points of arabinoxylans[38,42] are found to be produced by most of the aforementioned preparations of rumen micro-organisms.

The D-xylanases of rumen microbial origin are all of the endo type, and degrade D-xylan, arabinoxylan, hemicellulose B, and xylo-oligosaccharides of d.p. ⩾3, mainly to X_2 and D-xylose; X_2 remains unattacked. The D-xylanases were found[38,42] to have a higher affinity for linear D-xylans than for the branched hemicelluloses, namely, arabinoxylan and a branched B fraction from *Trifolium pratense* (red clover). The presence of L-arabinose, D-galactose, and uronic acid substituents on the D-xylan backbone thus appears to inhibit the action of these D-xylanases. The D-xylan was not effectively hydrolyzed until the side-group substituents had mostly been removed, for example, by arabinofuranosidase action on arabinoxylan.[44]

(254) J. H. Pazur, T. Budovich, E. W. Shuey, and C. E. Georgi, *Arch. Biochem. Biophys.*, **70**, 419–425 (1957).

(255) D. J. Walker and M. F. Hopgood, *Aust. J. Agr. Res.*, **12**, 651–660 (1961).

The different types of rumen hemicellulases therefore appear to work in a synergistic manner in degrading arabinoxylan to D-xylose and L-arabinose. The action of α-L-arabinofuranosidase was shown by Bailey and Gaillard[44] to precede the action of the xylosidase(s) and xylanase(s). In this way, the D-xylans are effectively degraded to D-xylose and L-arabinose, which can then be utilized[41] by the "fermenting microbial population" in the rumen. This type of hemicellulase system is, therefore, very different from that already described from the fungal pathogen *Ceratocystis paradoxa*,[228,236] in which the D-xylanase preferentially attacked the branched hemicellulose.

6. Oligosaccharides Isolated and Characterized from Enzymic Hydrolyzates of Various D-Xylans

a. Arabinoxylans and Arabinoglucuronoxylans.—Several arabinose–xylose oligosaccharides have been isolated from the enzymic hydrolyzates of arabinoxylan by the action of microbial D-xylanases. These mixed oligosaccharides were first observed by Bishop and Whitaker,[256] who used a "purified" cellulase preparation (containing xylanase activity) from the fungus *Myrothecium verrucaria* to degrade wheat-straw xylan. A series of arabinose–xylose oligosaccharides of d.p. 3–7 was isolated, and one of these, the trisaccharide AX₂, was characterized by periodate oxidation and methylation, and its structure found[257] to be O-α-L-Araf-(1 → 3)-O-β-D-Xylp-(1 → 4)-D-Xylp (3). This then provided the first direct evidence that L-arabinosyl groups were covalently linked to β-D-(1 → 4)-linked D-xylose residues in arabinoxylan.

$$\beta\text{-D-Xyl}p\text{-}(1{\rightarrow}4)\text{-D-Xyl}p$$
$$3$$
$$\uparrow$$
$$1$$
$$\alpha\text{-L-Ara}f$$

$$3^{249,257,258}$$
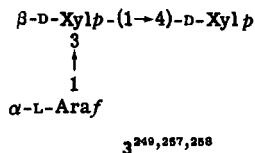
Aspinall and coworkers[258] also found a trisaccharide produced in enzymic hydrolyzates of rye-flour and cocksfoot-grass arabinoxylan by use of a commercial "hemicellulase" preparation. The trisaccharides from both hydrolyzates were characterized by chemical

(256) C. T. Bishop and D. R. Whitaker, *Chem. Ind.* (London), 119 (1955).
(257) C. T. Bishop, *J. Amer. Chem. Soc.*, 78, 2840–2841 (1956).
(258) G. O. Aspinall, I. M. Cairncross, R. J. Sturgeon, and K. C. B. Wilkie, *J. Chem. Soc.*, 3881–3885 (1960).

methods, and were found to have the same structure (3) as the trisaccharide earlier isolated by Bishop.[257]

Goldschmid and Perlin,[259] using an enzyme preparation that contained a D-xylanase, isolated from *Streptomyces* QMB 814, to degrade wheat-flour arabinoxylan, isolated a tetrasaccharide (AX₃) which was shown by chemical methods to be O-α-L-Araf-$(1 \rightarrow 3)$-O-[β-D-Xylp-$(1 \rightarrow 4)$]-β-D-Xylp-$(1 \rightarrow 4)$-D-Xylp (4).

$$\beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}\beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}D\text{-}Xyl p$$
$$3$$
$$\uparrow$$
$$1$$
$$\alpha\text{-}L\text{-}Ara f$$

4
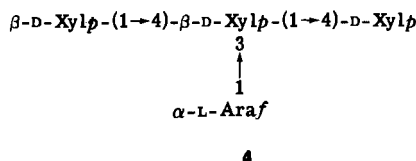
Takenishi and Tsujisaka[248] used a highly purified D-xylanase preparation (xylanase I) isolated from *Aspergillus niger* to hydrolyze rice-straw arabinoxylan, and found two arabinoxylo-oligosaccharides, AX₂ and AX₃, which migrated *ahead* of the corresponding linear xylo-oligosaccharides, namely, X₂ and X₃, on paper chromatograms. These oligosaccharides were isolated, and characterized both by chemical and enzymic techniques, and their structures were shown to be O-β-D-Xylp-$(1 \rightarrow 4)$-O-[α-L-Araf-$(1 \rightarrow 3)$]-D-Xylp (5), and O-β-D-Xylp-$(1 \rightarrow 4)$-D-Xylp-$(1 \rightarrow 4)$-O-[α-L-Araf-$(1 \rightarrow 3)$]-D-Xylp (6).

$$\beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}D\text{-}Xyl p \qquad \beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}\beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}D\text{-}Xyl p$$
$$3 \qquad\qquad\qquad\qquad\qquad\qquad 3$$
$$\uparrow \qquad\qquad\qquad\qquad\qquad\qquad \uparrow$$
$$1 \qquad\qquad\qquad\qquad\qquad\qquad 1$$
$$\alpha\text{-}L\text{-}Ara f \qquad\qquad\qquad\qquad\qquad \alpha\text{-}L\text{-}Ara f$$

5[248]   **6**[248]
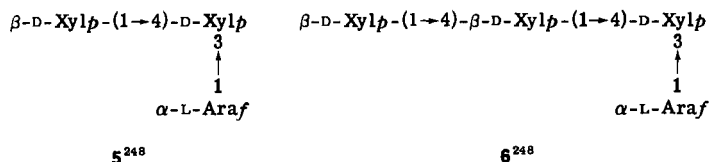
Using a purified D-xylanase preparation from *Ceratocystis paradoxa* (xylanase HC II), Dekker and Richards[249] isolated two arabino-xylo-oligosaccharides (AX₂ and AX₃) which also migrated *ahead* of the corresponding xylo-oligosaccharides (X₂ and X₃) on paper chromatograms, and which were characterized by methylation and enzymic techniques. Their structures were identified as O-α-L-Araf-$(1 \rightarrow 3)$-O-β-D-Xylp-$(1 \rightarrow 4)$-D-Xylp (AX₂, 3) and O-α-L-Araf-$(1 \rightarrow 3)$-O-β-D-Xylp-$(1 \rightarrow 4)$-D-Xylp-$(1 \rightarrow 4)$-D-Xylp (AX₃, 7).

$$\beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}\beta\text{-}D\text{-}Xyl p\text{-}(1\rightarrow4)\text{-}D\text{-}Xyl p$$
$$3$$
$$\uparrow$$
$$1$$
$$\alpha\text{-}L\text{-}Ara f$$

7
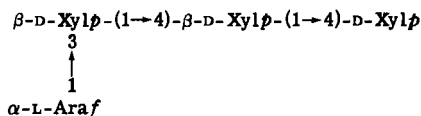
Beveridge and Richards[217] used mixed, rumen microbial, cell-free, enzyme preparations to hydrolyze spear-grass hemicellulose B, and found a series of neutral oligosaccharides (d.p. 2–6) in which the arabinose-containing xylo-oligosaccharides migrated *ahead* of the corresponding D-xylose oligosaccharides on paper chromatograms. This observation on chromatographic mobility on paper is in agreement with that observed by Tsujisaka and coworkers[247,248] and Dekker and Richards,[249] but differs from that of the earlier groups of workers (see, for example, Refs. 256–259) who found the converse, namely, that the arabinoxylo-oligosaccharides migrated *behind* the corresponding xylo-oligosaccharides on paper, with use of similar solvent-systems.

An arabinoxylo-oligosaccharide that migrated *ahead* of X_2 on paper chromatograms was found in enzymic hydrolyzates of rye-flour and cocksfoot-grass arabinoxylans,[258] and of wheat-straw xylan,[5] but its structure was not determined. It appears, however, that this component was probably the disaccharide O-α-L-Ara$f$-$(1 \rightarrow 3)$-D-Xyl$p$ (AX). Higher, mixed arabinoxylo-oligosaccharides (d.p. > 4) have also been reported to be present in enzymic hydrolyzates of hemicellulosic D-xylans, but their structures have not yet been determined.[5,215,217,222,228,236,256,258-260]

The origin and known structures of several arabinoxylo-oligosaccharides isolated from the enzymic hydrolyzates of hemicellulosic D-xylans are summarized in Table XXVII.

Several xylo-oligosaccharides of d.p. 2–7 have been isolated from enzymic hydrolyzates of terrestrial-plant D-xylans, arabinoxylans, arabino-4-O-methylglucuronoxylans, and 4-O-methylglucuronoxylans by the action of endo-xylanases,[5,6,38-40,42-44,47,181,215-217,222,228,236,247-250, 254,256,258-261] and have been shown to constitute a series of β-D-$(1 \rightarrow 4)$-linked D-xylo-oligosaccharides.

b. Rhodymenia palmata D-Xylan.— The D-xylan (rhodymenan) isolated from the marine alga *R. palmata* has been shown to be susceptible to attack by the D-xylanases of rumen bacteria,[215] and of the plant pathogen *Stereum sanguinolentum.*[250] D-Xylanase preparations from these organisms were found to degrade rhodymenan to D-xylose, a series of β-D-$(1 \rightarrow 4)$-linked xylo-oligosaccharides of d.p.

(259) H. R. Goldschmid and A. S. Perlin, *Can. J. Chem.*, **41**, 2272–2277 (1963).
(260) R. W. Bailey, "Oligosaccharides," Pergamon Press, Oxford, 1965, p. 41, and references cited thereon.
(261) R. L. Whistler and C. C. Tu, *J. Amer. Chem. Soc.*, **74**, 3609–3612 (1952).

TABLE XXVII

The Origin and Structures of Arabinoxylo-oligosaccharides of d.p. 3–4 Isolated from the Enzymic
Hydrolyzates of Various D-Xylans

Enzyme preparation	Origin of enzyme	Source of xylan	Oligosaccharide structure	$[\alpha]_D$ (degrees)	References
Cellulase	*Myrothecium verrucaria*	wheat straw	3	−19.3	257
"Hemicellulase"	commercial preparation	cocksfoot grass	3	−14.9	258
		rye flour	3	−15.3	258
D-Xylanase	*Streptomyces* QMB 814	wheat flour	4	−75	259
Rumen cell-free enzymes	Mixed bovine-rumen bacteria and protozoa	spear grass (hemi-cellulose B)	unknown, now thought to be[a] 7	−70	217
D-Xylanase I	*Aspergillus niger*	rice straw	5	−80	248
			6	−84	248
D-Xylanase HC II	*Ceratocystis paradoxa*	spear grass (hemi-cellulose B)	3	−17.3	249
			7	−73.6	249

[a] Proposed structure (see Refs. 228, 236, and 249) from the results of the action of the endo-D-xylanases (HC I and HC II) from *Ceratocystis paradoxa* on this oligosaccharide.

≥ 2, and a series of xylo-oligosaccharides, of d.p. ≥ 2, containing both β-D-(1 → 3)- and β-D-(1 → 4)-linkages. Several of the latter components have been isolated, and their structures characterized both by using chemical techniques,[215,250] and the specificity of a β-D-xylosidase (from *Cyamopsis tetragonoloba*, guar bean).[250] Xylo-oligosaccharides of this kind, of d.p. 2–6, were found to contain only one β-D-(1 → 3)-linkage per molecule; their structures are summarized in Table XXVIII.

c. **Glucuronoxylans.**—The acidic xylo-oligosaccharides (that is, xylo-oligosaccharides containing a covalently linked 4-O-methyl-D-glucuronic acid substituent) have not been studied in as much detail as the neutral oligosaccharides from hemicelluloses. However, several glycuronic acids have been isolated from enzymic hydrolyzates of wood 4-O-methylglucuronoxylans by using commercial enzyme

TABLE XXVIII

Structures of Xylo-oligosaccharides Isolated from Enzymic
Hydrolyzates of *Rhodymenia palmata* D-Xylan

Source of D-xylanase	D.p.	Structures of xylo-oligosaccharides	$[\alpha]_D$ (degrees)	References
Rumen bacteria	2	O-β-D-Xylp-(1 → 3)-D-Xylp	−18.4	215
	3	O-β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)-D-Xylp	−52.0	215
	4	suspected to be: O-β-D-Xylp-(1 → 4)-O-[β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)]-D-Xylp	−56.7	215
Stereum sanguinolentum	4	O-β-D-Xylp-(1 → 4)-O-[β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)]-D-Xylp		250
	4	O-β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)-D-Xylp-(1 → 4)-D-Xylp		250
	5	O-β-D-Xylp-(1 → 4)-O-[β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)-D-Xylp-(1 → 4)]-D-Xylp	−144[a]	250
	5	O-β-D-Xylp-(1 → 4)-O-β-D-Xylp-(1 → 4)-O-[β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)]-D-Xylp	−144[a]	250
	6	O-β-D-Xylp-(1 → 4)-O-β-D-Xylp-(1 → 4)-O-[β-D-Xylp-(1 → 3)-O-β-D-Xylp-(1 → 4)-O-β-D-Xylp-(1 → 4)]-D-Xylp	−154[a]	250

[a] Optical rotation at 436 nm.

preparations [for example, *Trichoderma viride* "cellulase" (Refs. 237 and 262) (Onozuka, Japan), *Oxiporus* "cellulase" (Ref. 237) (Merck A.G., Germany), and "pectinase" (Nutritional Biochemical Corp., U.S.A.)], a partially purified D-xylanase preparation from *Coniophora cerebella*,[181] and "purified" D-xylanases[237,262,263] from commercial "cellulases" (from, for example, Onozuka, and Merck).

The "pectinase" preparation used by Timell[6] to degrade white-birch (*Betula papyrifera* Marsh) 4-*O*-methylglucuronoxylan yielded D-xylose, xylo-oligosaccharides of d.p. 2–6, and a series of glycuronic acid oligosaccharides, extending from aldotetrao- to an aldo-octao-uronic acid. The mono-, bio-, and trio-uronic acids were not produced. The acidic oligomers (d.p. 4–8) were isolated, and characterized by chemical methods, and were shown to be *O*-4-*O*-methyl-α-D-Glc*p*A-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p*-(1 → 4)-D-Xyl*p* (**1**), *O*-4-*O*-methyl-α-D-Glc*p*A-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p*-(1 → 4)-D-Xyl*p*-(1 → 4)-D-Xyl*p* (**8**), and *O*-4-*O*-methyl-α-D-Glc*p*A-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p*-(1 → 4)-D-Xyl*p*-(1 → 4)-D-Xyl*p* (**9**). The heptao- and octao-uronic acids were not characterized, but are thought to be of the same homologous series. The general structure of these glycuronic acid–xylose oligosaccharides can be represented[6] by the following, general formula.

$$\beta\text{-}D\text{-}Xyl p\text{-}(1{\rightarrow}4)\text{-}\beta\text{-}D\text{-}Xyl p\text{-}(1{\rightarrow}4)_n\text{-}D\text{-}Xyl p$$
$$2$$
$$\uparrow$$
$$1$$
$$4\text{-}O\text{-}Me\text{-}\alpha\text{-}D\text{-}Glc p A$$

$$\mathbf{1}\ n = 1$$
$$\mathbf{8}\ n = 2$$
$$\mathbf{9}\ n = 3$$
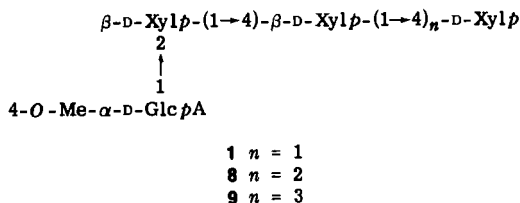
The D-xylanase preparation[263] from Merck "cellulase" degraded red-beech (*Fagus sylvatica* L.) 4-*O*-methylglucuronoxylan to (mainly) D-xylose, xylo-oligosaccharides of d.p. ≥2, 4-*O*-methyl-glucuronic acid, and aldotetraouronic acid, whereas a xylanase from Onozuka "cellulase" (Ref. 263) produced D-xylose, xylo-oligo-saccharides of d.p. 2–10, 4-*O*-methylglucuronic acid, and a series of glycuronic acids of d.p. 3–8. The D-xylanase from *Coniophora cerebella*[181] degraded poplar (*Populus tremuloides* Michx)

(262) M. Sinner, H. H. Dietrichs, and M. H. Simatupang, *Holzforschung*, **26**, 218–228 (1972).

(263) M. Sinner, *Mitt. Bundesforschungsanstalt Forst- Holz-wirtsch.*, No. 93, 257–266 (1973).

4-O-methylglucuronoxylan to a series of acidic oligomers of d.p. ≥ 4, in addition to D-xylose and xylo-oligosaccharides of d.p. 2–5. The glycuronic acids were not characterized chemically, but their constitution may be the same as that of those oligomers identified by Timell.[6]

BIBLIOGRAPHY OF CRYSTAL STRUCTURES OF CARBOHYDRATES, NUCLEOSIDES, AND NUCLEOTIDES[*] 1974

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I. Introduction

The format is similar to that for the previous bibliographies for 1970–1972 (Ref. 1) and for 1973 (Ref. 2). Where possible, we have added projection diagrams to illustrate the conformation of the organic molecule or ion. These diagrams were produced, by means of computer graphics, directly from the unit-cell dimensions and the atomic coordinates of the molecules in the crystal structures. For the carbohydrates, carbon and hydrogen atoms are shown as small, solid circles and other atoms by the appropriate symbol. The CRYSNET software was used to rotate[3] the molecular diagrams so as to corre-

(1) G. A. Jeffrey and M. Sundaralingam, *Advan. Carbohydr. Chem. Biochem.*, **30**, 445–466 (1974).

(2) G. A. Jeffrey and M. Sundaralingam, *Advan. Carbohydr. Chem. Biochem.*, **31**, 347–371 (1975).

(3) T. F. Koetzle, L. C. Andrews, F. C. Bernstein, and H. J. Bernstein, "CRYSNET. A Network for Crystallographic Computing," in ACS *Advan. Chem. Ser.* "Computer Networking and Chemistry," to be published.

spond as closely as possible to the conventional, conformational diagrams used in the carbohydrate literature. For the nucleosides and nucleotides, the ORTEP programs[4] were used, with the following atom designations: carbon, open circle; hydrogen, small open circle; oxygen, solid circle; nitrogen, hatched circle; sulfur, dotted circle; and halogen, cross-hatched circle.

II. Data for Carbohydrates

$C_6H_6BaO_4S \cdot 2H_2O$ Barium 2-O-sulfato-L-ascorbate dihydrate[5]
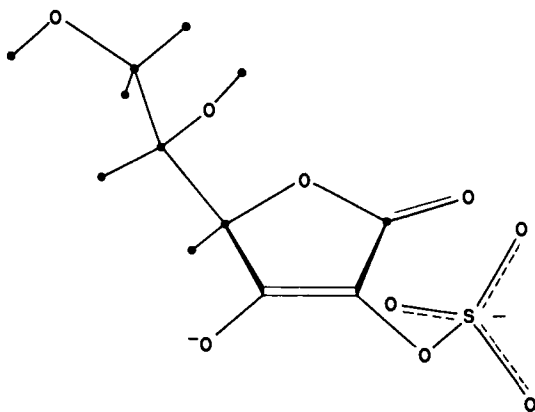
P1; $Z = 1$; $D_x = 2.431$; $R = 0.008$ for 1,223 intensities. There is good agreement with the corresponding, structural features of the L-ascorbate anion.[6] The ring is planar within ±0.03 Å (3 pm), and the shapes of the side chains are similar. The O-4–C-4–C-5–C-6 and C-4–C-5–C-6–O-6 torsion angles are 55 and 161°, respectively. The ester S–O and C–O lengths of 1.628 (162.8 pm) and 1.389 A (138.9 pm) are respectively longer and shorter than observed in other sulfuric ester anions. The Ba^{2+} ion has ten oxygen nearest-neighbors between 2.75 (275 pm) and 3.06 Å (306 pm), three of which are from water molecules.

$2(C_6H_7O_6)Ca \cdot 2H_2O$ Calcium L-ascorbate dihydrate[7]

(4) C. K. Johnson, ORTEP, Oak Ridge National Laboratory, Report ORNL-3794 (1965).

(5) B. W. McClelland, Acta Crystallogr., **B30**, 178–186 (1974).

(6) J. Hvoslef, Acta Crystallogr., **B25**, 2214–2223 (1969).

(7) R. A. Hearn and C. E. Bugg, Acta Crystallogr., **B30**, 2705–2711 (1974); J. Hvoslef and K. E. Kjellevold, ibid., **B30**, 2711–2716 (1974).
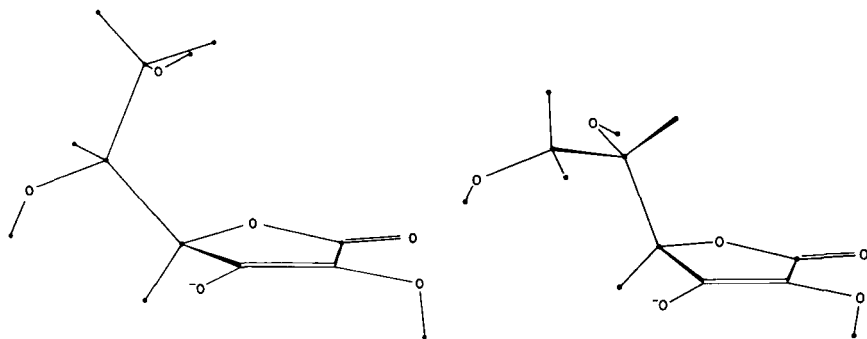
P2$_1$; Z = 2; D$_x$ = 1.771, 1.773; R = 0.038 for 1,321 intensities, 0.036 for 2,283 intensities. The proton attached to O-3 is that removed on salt formation. The ring is planar within ±0.05 Å (5 pm). The shapes of the side chains of the two ions are different, with O-4–C-4–C-5–O-5 torsion angles of 56 and 170°, respectively. The other conformational angle of the side chain, O-5–C-5–C-6–O-6, is 47° in both molecules. The calcium ion is eight-coordinated, in a distorted, square anti-prism, by six oxygen atoms from three L-ascorbate ions and by two water molecules.

C$_6$H$_{10}$Na$_3$O$_{10}$P · 2H$_2$O   Trisodium 6-*O*-phosphono-D-gluconate dihy-
drate[8]
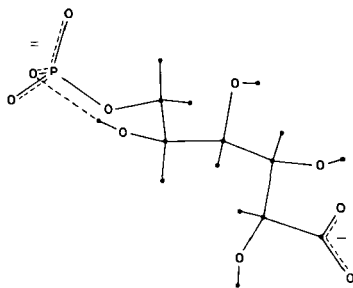
P2$_1$; Z = 2, D$_x$ = 1.89; R = 0.034 for 1,878 intensities. The acyclic, 6-*O*-phosphono-D-gluconate ion has a sickle carbon-chain confor-mation, which is different from those in the two polymorphic forms of potassium D-gluconate monohydrate,[9] but is the same as that in

(8) G. D. Smith, A. Fitzgerald, C. N. Caughlan, K. A. Kern, and J. P. Ashmore, *Acta Crystallogr.*, **B30**, 1760–1765 (1970).
(9) N. C. Panagiotopoulos, G. A. Jeffrey, S. J. La Placa, and W. C. Hamilton, *Acta Crystallogr.*, **B30**, 1421–1430 (1974).

D-glucitol.[10] The P–O-6–C-6–C-5 torsion angle is −102°. There is an intramolecular hydrogen-bond between O-5–H and one of the phosphate oxygen atoms. The three sodium ions have three different coordinations, namely, distorted tetrahedral, tetragonal pyramidal, and octahedral.

$C_6H_{11}NO_6$ α-D-Glucopyranuronamide[11]
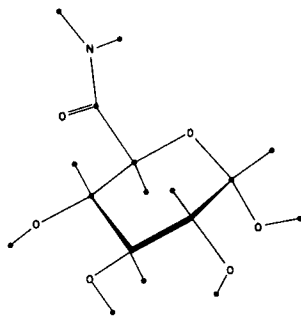
$P2_1$; Z = 2; D_x = 1.67; R = 0.051 for 699 intensities. The conformation is $^4C_1$, with torsion angles of 55–62°. The amide angle, O–C–C–O, is 144°. The anomeric C–O bond is shorter [1.394 Å (139.4 pm)] and the ring bond C-5–O-5 is longer [1.432 Å (143.2 pm)] than the mean, 1.420 Å (142.0 pm).

The atomic coordinates, diagrams, and torsion angles given in the paper referred to the L enantiomer. To obtain the D molecule, the sign of the y coordinates and of the torsion angles should be reversed.

$C_6H_{11}KO_7 \cdot H_2O$ Potassium D-gluconate monohydrate (Form A)[9]
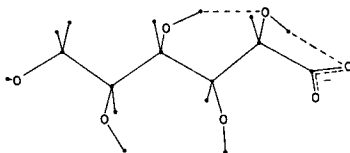
$P2_12_12_1$; Z = 4, D_x = 1.701; R = 0.034 for 2,821 neutron intensities. The D-gluconate ion has the extended-chain conformation, with an intramolecular hydrogen-bond between the *syn*-diaxial hydroxyl

(10) Y.-J. Park, G. A. Jeffrey, and W. C. Hamilton, *Acta Crystallogr.*, **B27**, 2393–2401 (1971).

(11) J. L. Flippen and R. D. Gilardi, *Acta Crystallogr.*, **B30**, 537–539 (1974).

groups, O-4–H $\cdots$ O-2–H. There is also a bifurcated hydrogen-bond, with a weak, intramolecular interaction between O-2–H and a carboxylate oxygen atom. The H $\cdots$ O bond distances range from 1.666 to 2.121 Å (166.6 to 212.1 pm) and the O–H $\cdots$ O angles from 107 to 177°. The K ions are eight-coordinated within 3.3 Å (330 pm) by six hydroxyl oxygen atoms and two water oxygen atoms. The carboxylate oxygen atoms are not in the first coordination sphere of the cation.

$C_6H_{11}KO_7 \cdot H_2O$ Potassium D-gluconate monohydrate (Form B)[9]
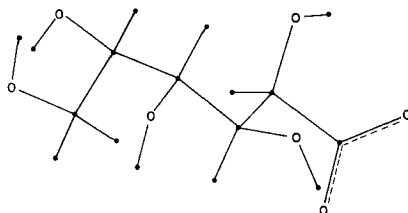
P2$_1$; Z = 2; D$_x$ = 1.786; R = 0.033 for 2,704 neutron intensities. The D-gluconate ion has a sickle chain-conformation, different from that in D-glucitol. All the hydrogen bonding is intermolecular, with H $\cdots$ O distances between 1.805 and 2.051 Å (180.5 and 205.1 pm) and O–H $\cdots$ O angles between 159 and 178°. The K ions are nine-coordinated within 3.3 Å (330 pm), with the closest oxygen atom being that of the carboxylate group at 2.68 Å (268 pm).

$C_6H_{12}O_5$ Methyl β-D-ribopyranoside[12]
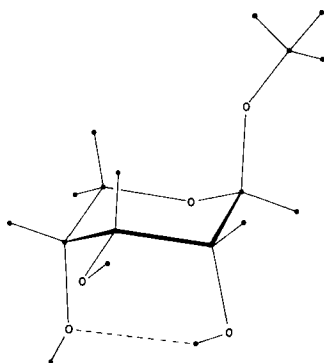
(12) A. Hordvik, *Acta Chem. Scand.*, **B28**, 260–271 (1974).

$P2_12_12_1$; $Z = 4$; $D_x = 1.479$; $R = 0.07$ for 660 intensities. The conformation is $^1C_4$, with three axially attached hydroxyl groups, two of which, O-2-H and O-4-H, are linked by an intramolecular hydrogen-bond. Similar intramolecular hydrogen-bonding has been observed in the crystal structures of methyl 1-thio-α-D-ribopyranoside and methyl 1,5-dithio-α-D-ribopyranoside.[13] This is the same crystal structure as that given in a preliminary report,[14] which did not include the atomic parameters.

$C_6H_{12}O_3S \cdot 0.25H_2O$ Methyl 1,5-dithio-α-D-ribopyranoside tetartohydrate,[15] m.p. 63–65°C
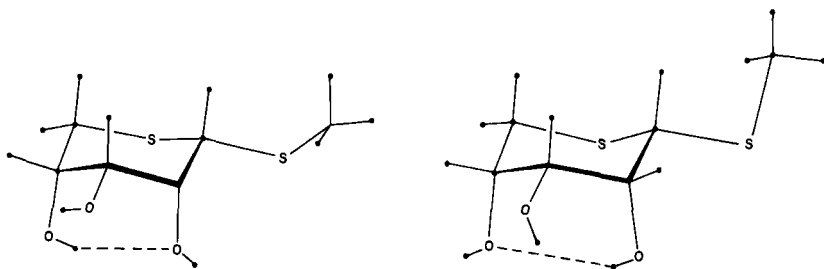
C2; $Z = 8$; $D_x = 1.457$; $R = 0.032$ for 1,535 intensities. The crystals contain two symmetry-independent molecules, both of which have the $^1C_4$ conformation, but differ in the orientation of the thio-glycosidic bonds. The torsion angle S-5-C-1-S-1-C-6 is 159° in one molecule and 82° in the other. As in methyl β-D-ribopyranoside, there are intramolecular hydrogen-bonds between O-2-H and O-4-H. The O-H · · · O direction of the hydrogen bonds is opposite in the two molecules. There are small, but possibly significant, differences in the S-C bond lengths.

The atomic coordinates and torsion angles reported in this paper referred to the L enantiomer.

$C_6H_{12}O_3S_3$ Methyl 1,5-dithio-β-D-ribopyranoside,[16] m.p. 111–113°C

$P2_1$; $Z = 4$; $D_x = 1.464$; $R = 0.049$ for 1,092 intensities. The crystal has two symmetry-independent molecules, both of which have the $^4C_1$ conformation and the same orientation of the thio-glycosidic bonds. The torsion angles S-5-C-1-S-1-C-7 are +66°, +67°. There

(13) R. L. Girling and G. A. Jeffrey, Carbohydr. Res., 27, 257–260 (1973).
(14) V. J. James and J. D. Stevens, Carbohydr. Res., 21, 334–335 (1972).
(15) R. L. Girling and G. A. Jeffrey, Acta Crystallogr., B30, 327–333 (1974).
(16) R. L. Girling and G. A. Jeffrey, Acta Crystallogr., B30, 327–333 (1974).

appear to be significant differences, of the order of 0.04 Å (4 pm), in the C–S bond lengths.

The atomic coordinates reported referred to the L enantiomer.
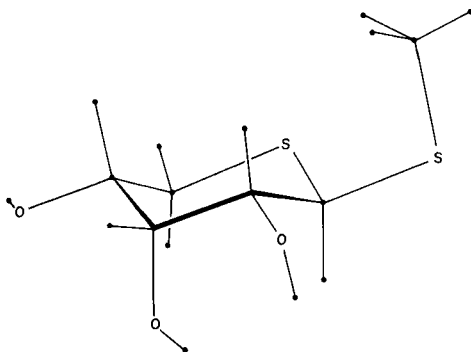
$C_6H_{12}O_6 \cdot CaCl_2 \cdot 2H_2O$ β-D-Fructopyranose · calcium chloride, dihydrate[17]
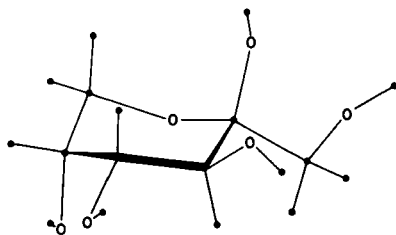
$P2_1$; Z = 2; D_x = 1.68; R = 0.036 for 864 intensities (data were poor and limited). The conformation of the pyranose is $^2C_5$, with considerable distortion. The ring torsion-angles range from 38 to 64°, corresponding to a flattening of the ring at C-2. The Ca^{2+} ion is seven-coordinated to a pentagonal bipyramid of oxygen atoms. The $Ca^{2+} \cdots O$ distances are 2.33 to 2.48 Å (233–248 pm). There appears to be considerable strain in the structure, in contrast to the crystal structures of other calcium halide complexes.

$2(C_6H_{12}O_6) \cdot CaCl_2 \cdot 3H_2O$ Bis(β-D-fructopyranose) · calcium chloride, trihydrate[18]

(17) D. C. Craig, N. C. Stephenson, and J. D. Stevens, *Cryst. Struct. Commun.*, **3**, 277–282 (1974).

(18) D. C. Craig, N. C. Stephenson, and J. D. Stevens, *Cryst. Struct. Commun.*, **3**, 195–200 (1974).
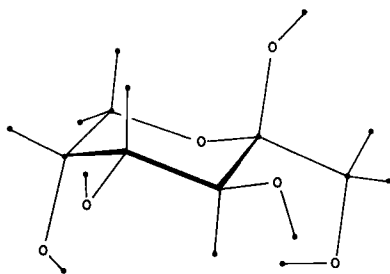
C2; Z = 2; D_x = 1.61; R = 0.026 for 1,119 intensities. The conformation of the pyranose is $^2C_5$, with ring torsion-angles of 51 to 56°. The Ca^{2+} ion is eight-coordinated in a distorted, square anti-prism, with Ca $\cdots$ O distances of 2.45 to 2.50 Å (245–250 pm). The crystal structure is isomorphous with that of the $SrCl_2$ complex.[19]

$C_6H_{13}O_9P \cdot H_2O$ *myo*-Inositol 2-phosphate monohydrate[20]
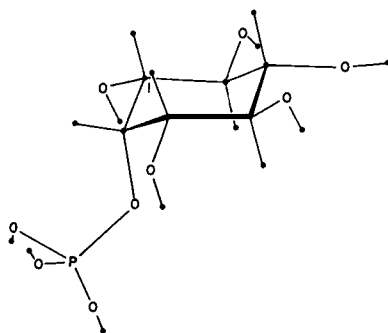
$P2_1/c$; Z = 4; D_x = 1795; R = 0.045 for 1,680 intensities. The molecule has a chair conformation, with the ester group axially attached, and ring torsion-angles of 55 to 60°. The orientation of the phosphate group is asymmetric with respect to the *m* symmetry of the cyclitol moiety, with a C-1-C-2-O-2-P torsion angle of −124°.

$C_8H_{14}N_2O_4S$ 1-Methyl-4,5-(1,2-α-L-glucofurano)imidazolidine-2-thione[21]

$P2_12_12_1$; Z = 4; D_x = 1.41; R = 0.074 for 1,154 intensities. The conformation of the furanose is T_3. The imidazolidine ring is planar,

(19) P. F. Eiland and R. Pepinsky, *Acta Crystallogr.*, **3**, 160–161 (1950).

(20) C. S. Yoo, G. Blank, J. Pletcher, and M. Sax, *Acta Crystallogr.*, **B30**, 1938–1987 (1974).

(21) R. Jiménez-Garey, A. López-Castro, and R. Márquez, *Acta Crystallogr.*, **B30**, 1801–1805 (1974).

and this plane makes a dihedral angle of 104° with the mean plane of the atoms of the sugar ring. The positions of the hydrogen atoms were not determined. The α-D form was actually studied.
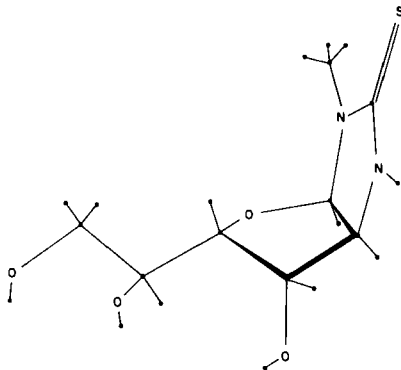
$C_8H_{15}NO_6$ 2-Acetamido-2-deoxy-α-D-galactose[22]
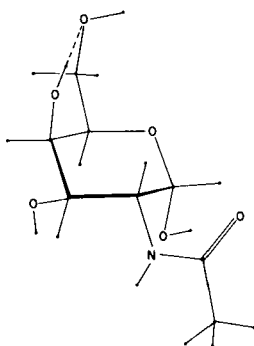
$P2_1$; $Z = 2$; $D_x = 1.38$; $R = 0.066$ for 893 intensities. The conformation is $^4C_1$, with torsion angles between 56 and 60°. The N-acetyl torsion angle, C-1–C-2–N-2–C-7, is 83°. The O-5–C-5–C-6–O-6 torsion angle is −64°. There is an intramolecular hydrogen-bond between the axial O-4–H and O-6. The O–H $\cdots$ O angle is 158°, and the H $\cdots$ O distance is 2.07 Å (207 pm).

$C_9H_{14}O_5$ 1,6-Anhydro-3,4-O-isopropylidene-β-D-talopyranose[23]

$P2_12_12_1$; $Z = 4$; $D_x = 1.416$; $R = 0.045$ for 1,171 intensities. The conformation of the pyranose is very distorted, $^3C_0$, with ring torsion-angles from 25 to 78°. The conformations of the anhydro

(22) R. D. Gilardi and J. L. Flippen, *Acta Crystallogr.*, **B30**, 2931–2933 (1974).
(23) N. C. Panagiotopoulos, *Acta Crystallogr.*, **B30**, 1402–1407 (1974).

and isopropylidene rings are ^0E. The plane of the four carbon atoms of these rings makes dihedral angles of 88 and 34°, respectively, with the reference plane of the $^3C_0$ pyranose ring. There are small, but apparently significant, variations in the C–O bond lengths of the order of 0.015 Å (1.5 pm).
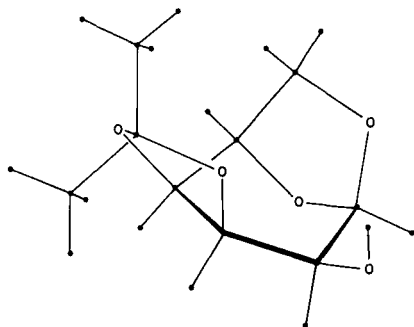
$C_9H_{16}O_4S_2$ 5-Deoxy-3-C-formyl-β-L-lyxofuranose 3^1-(trimethylene dithioacetal),[24] m.p. 128°C
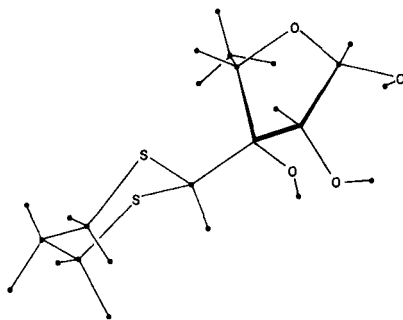
P2$_1$; Z = 2; D$_x$ = 1.458; R = 0.04 for 1,071 intensities. The conformation of the furanose is $^2T_3$ (176.2°), with the dithiane ring and the 4-C-methyl group equatorially attached. The dithiane ring has a chair conformation. The molecules are hydrogen-bonded in infinite chains.

$C_{11}H_{15}ClO_7$ 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl chloride[25]

(24) W. Depmeier, O. Jarchow, P. Stadler, V. Sinnwell, and H. Paulsen, *Carbohydr. Res.*, 34, 219–226 (1974).
(25) G. Kothe, P. Luger, and H. Paulsen, *Carbohydr. Res.*, 37, 283–292 (1974).
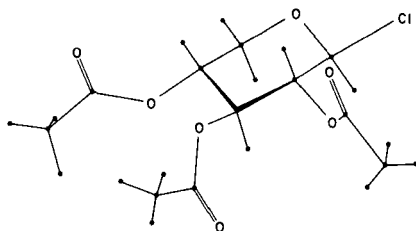
$P2_12_12_1$; $Z = 4$; $D_x = 1.38$; $R = 0.039$ for 1,101 intensities. The conformation is $^4C_1$, flattened at C-3, with torsion angles of 53–64° and all substituents equatorially attached. The acetoxyl torsion-angles, C-1-C-2-O-2-C(acetyl), C-2-C-3-O-3-C(acetyl), C-3-C-4-O-4-C(acetyl) are 124°, −111°, and 109°, respectively. The C–Cl bond length is normal, 1.754 Å (175.4 pm).

$C_{11}H_{15}N_3O_7$ Tri-O-acetyl-α-D-arabinopyranosyl azide[26]
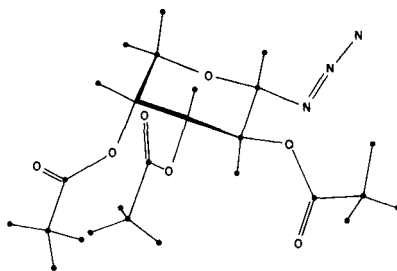
$P2_12_12_1$; $Z = 4$, $D_x = 1.34$; $R = 0.044$ for 1,524 intensities. The conformation of the pyranose is $^1C_4$, with the azide group equatorially attached. The ring torsion-angles are 49 to 63°. The azide torsion-angle O–C–N–N is 76°. The azide group is not linear, 173°, and the N–N bonds are unequal, 1.245 Å (124.5 pm), 1.136 Å (113.6 pm).
    The atomic coordinates refer to the L enantiomer.

$C_{11}H_{18}O_8$ trans-O-β-D-Glucopyranosyl methyl acetoacetate,[27] m.p. 186°C

$P2_12_12_1$; $Z = 4$; $D_x = 1.400$; $R = 0.052$ for 1,586 intensities. The conformation of the pyranose is $^4C_1$. The glycosidic torsion-angle, O-5-C-1-O-1-C-7, is −62°. The methyl acetoacetate group is not exactly planar, owing to steric repulsion. The reversal in optical ro-

(26) P. Luger and H. Paulsen, Chem. Ber., 107, 1579–1589 (1974).
(27) J. Ruble and G. A. Jeffrey, Carbohydr. Res., 38, 61–69 (1974).

tation in different solutions was explained by conformational changes on dissolution.
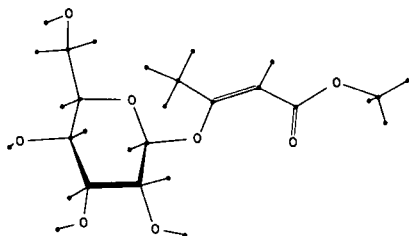
$C_{11}H_{19}NO_8 \cdot H_2O$ *N*-Acetylmuramic   acid,   2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranose hydrate[28]
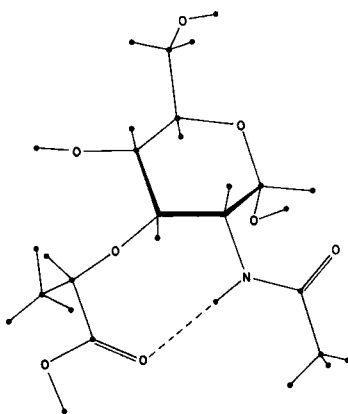
$P2_12_12_1$; $D_x = 1.389$; $R = 0.079$ for 1,063 intensities. The conformation of the pyranose is $^4C_1$, with ring torsion-angles between 49.5 and 62°. The *trans* peptide link in the *N*-acetyl group is 7° from planar. The configuration of the lactyl carbon atom is D. The O-5–C-5–C-6–O-6 torsion angle is −59°. There is an intramolecular hydrogen-bond between the N–H of the *N*-acetyl group and the C=O of the lactyl group. The C-1–O-1 bond is short, 1.38 Å (138 pm).

$C_{12}H_{16}O_8$ 2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose[29]

(28) J. R. Knox and N. S. Murthy, *Acta Crystallogr.*, **B30**, 365–371 (1974).
(29) F. Leung and R. H. Marchessault, *Can. J. Chem.*, **52**, 2516–2521 (1974).
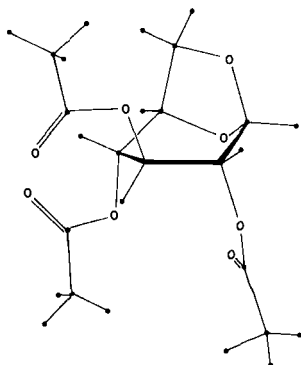
$P2_12_12_1$; $D_x = 1.44$; $R = 0.048$ for 1,408 intensities. The conformation of the pyranose is $^1C_4$ and that of the anhydro ring is E_0. The ring torsion-angles are close to those in the unacetylated molecule,[30] ranging from 31° around C-3 to 75° around O-5. The acetoxyl groups are close to planar with torsion angles C-1–C-2–O-2–C(acetyl), C-2–C-3–O-3–C(acetyl), and C-3–C-4–O-4–C(acetyl) of 154°, −156°, and 145°, respectively,

$C_{12}H_{18}O_8$ Methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside[31]
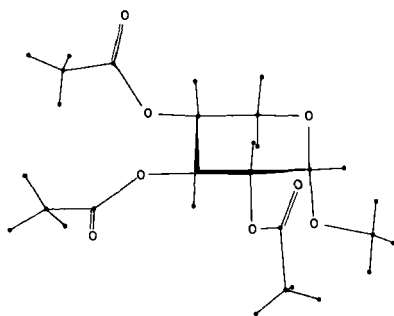
$P4_12_12_1$; $Z = 8$, $R = 0.037$; number of observations not reported. The conformation is $^4C_1$, with normal torsion-angles of 56.5 to 59.8°. The glycosidic angle, O-5–C-1–O-1–C-6, is 74° The acetoxyl torsion-angles, C-1–C-2–O-2–C(acetyl), C-2–C-3–O-7–C(acetyl), and C-3–C-4–O-4–C(acetyl), are 86°, −141°, and 131°, respectively. The C-1–O-1 bond is 1.402 Å (140.2 pm).

(30) Y.-J. Park, H. S. Kim, and G. A. Jeffrey, *Acta Crystallogr.*, B27, 220–227 (1971).
(31) V. J. James and J. D. Stevens, *Cryst. Struct. Commun.*, 3, 27–30 (1974).

$C_{12}H_{20}O_4S_2$ 5-Deoxy-3-C-formyl-1,2-O-isopropylidene-β-L-lyxo-
furanose 3^1-(trimethylene dithioacetal),[24] m.p. 141°C
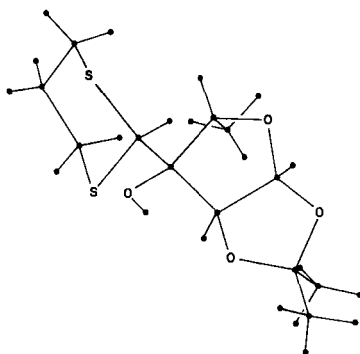
P2$_1$; Z = 2; D_x = 1.363; R = 0.035; number of intensities not re-
ported. The conformation of the furanose is $^3T_4$ (44.5°), with the
dithiane ring and the 4-C-methyl groups *trans*-diaxial. The dioxo-
lane ring is $^oT_6$. The dithiane ring is a chair, with torsion angles of
60 to 68°. The molecules are hydrogen-bonded in infinite chains.

$C_{12}H_{22}O_{11}$ β-Lactose; O-β-D-galactopyranosyl-(1 → 4)-β-D-gluco-
pyranose[32]
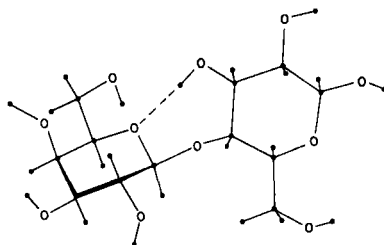
P2$_1$; Z = 2; D_x = 1.586; R = 0.068 for 1,595 reflections (film mea-
surements). The conformation of both moieties is $^4C_1$. The linkage
bonds are 70.7°, 108.0° (Gal → Glc). There is an intramolecular
hydrogen-bond between O-3–H of the D-glucose residue and the
ring-oxygen atom of the D-galactosyl group. The D-glucose ring-ox-
ygen atom accepts an intermolecular hydrogen-bond. The linkage
oxygen atoms are not hydrogen-bonded. Both anomeric bonds are
short, 1.402 Å (140.2 pm), 1.388 Å (138.8 pm). There was no evi-

(32) K. Hirotsu and A. Shimada, *Bull. Chem. Soc. Jap.*, **47**, 1872–1879 (1974).

dence of co-crystallization with a minor proportion of the other anomer, such as was observed for α-lactose monohydrate,[33] and the calcium chloride[34] and calcium bromide[35] heptahydrate complexes of α-lactose.

$C_{12}H_{26}O_4S_3$ 2-S-Ethyl-2-thio-D-mannose diethyl dithioacetal[36]
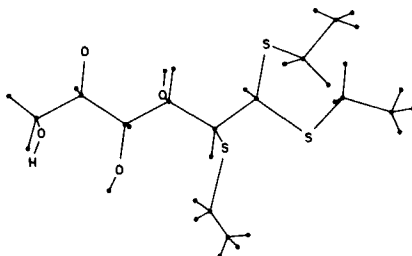
P2$_1$; Z = 4; D$_x$ = 1.25; R = 0.065 for 2,505 intensities. This is a more-detailed report of an earlier publication.[37] The C–S bond lengths range from 1.782 Å (178.2 pm) to 1.845 Å (184.5 pm) in the two symmetry-independent molecules. The hydrogen positions were only partially reported.

$C_{13}H_{13}ClN_2O_3S$ 1-(p-Chlorophenyl)-4-α-D-erythrofuranosyl-4-imidazoline-2-thione[38]
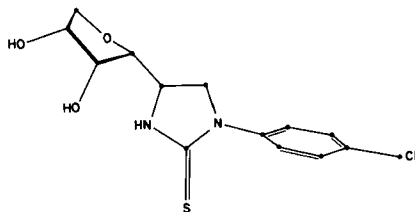
(33) D. C. Fries, S. T. Rao, and M. Sundaralingam, *Acta Crystallogr.*, **B27**, 994–1005 (1971).
(34) C. E. Bugg, *J. Amer. Chem. Soc.*, **95**, 908–913 (1973).
(35) W. J. Cook and C. E. Bugg, *Acta Crystallogr.*, **B29**, 907–909 (1973).
(36) A. Ducruix and C. Pascard-Billy, *Acta Crystallogr.*, **B30**, 1056–1063 (1974).
(37) A. Ducruix, C. Pascard-Billy, D. Horton, and J. D. Wander, *Carbohydr. Res.*, **29**, 276–279 (1973).
(38) S. Pérez-Garrido, A. Conde, and R. Márquez, *Acta Crystallogr.*, **B30**, 2348–2352 (1974).

$P2_12_12_1$; $Z = 4$; $D_x = 1.53$; $R = 0.073$ for 861 intensities. The conformation of the furanose is $^1T_0$ ($-62°$). The imidazoline and phenyl rings are planar within ±0.03 Å (±3 pm). The dihedral angles between the furanose, imidazoline, and phenyl rings, as defined by the torsion angles O–C–C–N-2, C-7–N–C–C-3, are $-63°$, $-134°$. No hydrogen positions were reported.

$C_{13}H_{18}O_9$ 1,2,3,4-Tetra-O-acetyl-α-D-arabinopyranose[39]
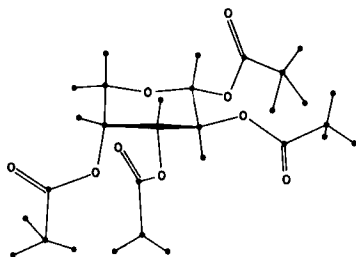
$P2_12_12_1$; $Z = 4$; $R = 0.038$; number of observations not reported. The conformation is $^1C_4$, flattened at C-3, with ring torsion-angles of 45 to 65°. The acetoxyl torsion-angles C-1–C-2–O-2–C(acetyl), C-2–C-3–O-3–C(acetyl), C-3–C-4–O-4–C(acetyl), and O-5–C-1–O-1–C(acetyl), are $-111°$, $161°$, $144°$, and $88°$, respectively.

There are errors in the signs of the z-coordinates of O-7 and O-8, which have to be interchanged.

$C_{13}H_{18}O_9$ 1,2,3,4-Tetra-O-acetyl-β-D-arabinopyranose,[40] m.p. 86°C
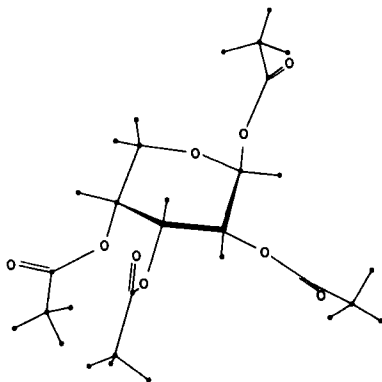
(39) V. J. James and J. D. Stevens, Cryst. Struct. Commun., 3, 187–190 (1974).
(40) V. J. James and J. D. Stevens, Cryst. Struct. Commun., 3, 19–22 (1974).

P2$_1$; Z = 2; R = 0.039; number of observations not reported. The conformation is $^1C_4$, with axially attached acetyl groups on O-1 and O-4. The ring torsion-angles range from 51 to 61°. The acetoxyl torsion-angles C-1–C-2–O-2–C(acetyl), C-2–C-3–O-3–C(acetyl), C-3–C-4–O-4–C(acetyl), and O-5–C-1–O-1–C(acetyl) are −88°, 122°, 109°, and −69°, respectively.

$C_{13}H_{22}O_6$ Methyl 2,3:4,5-di-O-isopropylidene-α-D-alloseptanoside[41]
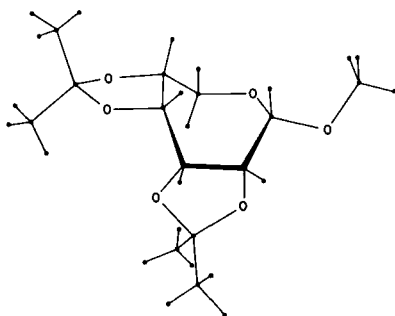
P6$_1$; Z = 6; D$_x$ = 1.24; R = 0.026 for 1423 reflections. The conformation is close to $^{4,5}B^1$. The ethylidene rings are ^cE and ^oE.

$C_{14}H_{20}N_2O_7$ 1-(Tri-O-acetyl-α-D-xylopyranosyl)imidazole[42]
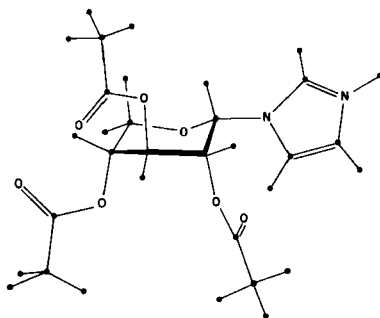
P2$_1$2$_1$2$_1$; Z = 4; D$_x$ = 1.36; R = 0.041 for 1,533 intensities. The conformation of the pyranose is a distorted $^1C_4$, flattened at C-3 and C-4 owing to *syn*-diaxial repulsion of axial acetoxyl groups at C-2

(41) D. C. Craig, N. C. Stephenson, and J. D. Stevens, *Cryst. Struct. Commun.* 3, 77–81 (1974).
(42) P. Luger, G. Kothe, and H. Paulsen, *Chem. Ber.*, 107, 2626–2634 (1974).

and C-4. The ring torsion-angles range from 38 to 67°. The plane of the imidazole ring is perpendicular to the reference plane of the pyranose ring.

$C_{16}H_{24}O_{10}$ 3,4,6-Tri-O-acetyl-1,2-O-(1-exo-ethoxyethylidene)-α-D-glucopyranose[43]
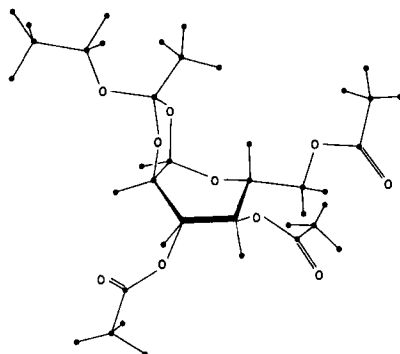
This is a more-detailed crystallographic report of an earlier publication.[44]

$C_{18}H_{32}O_{16}$ 1-Kestose; O-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl α-D-glucopyranoside[45]

Correction of a typographical error. In Table I of the original paper,[46] the x coordinate of C-3′ should read −779 instead of −9. All numbers derived by using this parameter are correct.[47]

$C_{26}H_{21}BrO_7$ Tri-O-benzoyl-β-D-xylopyranosyl bromide[48]

P2$_1$2$_1$2$_1$; Z = 4; D$_x$ = 1.44; R = 0.040 for 2,686 intensities. The conformation of the pyranose is $^1C_4$, with all substituents axially attached. The ring is flattened at C-1 and C-2 owing to syn-diaxial repulsions, and the ring torsion-angles range from 37 to 60°. There is a large disproportion between the C–O ring-bond lengths, with C-2–O-1 shorter by 0.09 Å (9 pm).

(43) J. A. Hertman, G. F. Richards, and L. Schroeder, *Acta Crystallogr.*, B30, 2322 (1974).
(44) J. A. Hertman and G. F. Richards, *Carbohydr. Res.*, 28, 180–182 (1973).
(45) G. A. Jeffrey and Y.-J. Park, *Acta Crystallogr.*, B30, 837 (1974).
(46) G. A. Jeffrey and Y.-J. Park, *Acta Crystallogr.*, B28, 257–267 (1972).
(47) See *Advan. Carbohydr. Chem. Biochem.*, 30, 455 (1974).
(48) P. Luger, P. L. Durette, and H. Paulsen, *Chem. Ber.*, 107, 2615–2625 (1974).
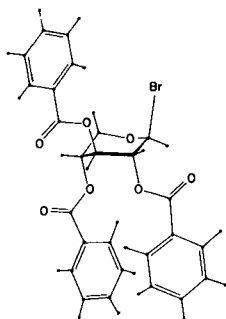
$C_{36}H_{60}O_{30} \cdot 6H_2O$ Cyclohexaamylose hexahydrate; α-cyclodextrin[49]

$P2_12_12_1$; $Z = 4$, $D_x = 1.493$, $R = 0.060$ for 4,558 intensities. The six α-D-glucosyl residues form a truncated cone. All six residues have similar dimensions and the conformation of each is $^4C_1$. The linkage bonds O-5-C-1-O-4'-C-4' and C-1-O-4'-C-4'-C-3' are similar, 101 to 113°, 121 to 135°, except for one residue which is rotated more nearly normal to the torus axes, resulting in linkage torsion-angles 88 and 117°, 90 and 170°. Two of the water molecules are hydrogen-bonded within the torus, and four are outside, between molecules. Of the six primary alcohol groups, two have O-5-C-5-C-6-O-6 torsion angles of +69°, and the other four are −63 to −72°. Those having the +sc orientation are hydrogen-bonded to the inner water molecules.

$C_{36}H_{60}O_{30} \cdot C_3H_8O \cdot 4.8H_2O$ Cyclohexaamylose–1-propanol · 4.8 hydrate[50]

$P2_12_12_1$; $Z = 4$; $D_x = 1.468$; $R = 0.070$ for 5,303 intensities. This inclusion compound is of the cage type, in contrast to the potassium acetate adduct, which is of the channel type. The cyclohexaamylose molecule has the form of a truncated cone, with the larger diameter formed by the O-2-H and O-3-H groups. The 1-propanol guest molecules are 2-fold disordered. It is not known whether they are hydrogen-bonded to the cyclohexaamylose. The D-glucosyl residues have the $^4C_1$ conformation. The torsion angles of the linkage bonds, as defined by O-4-C-1-O-4-C-4, C-1-O-4-C-4-O-4 range between 159 and 171°, 150 and 184°, respectively. The hydrogen bonding is uncertain, but there ap-

(49) P. C. Manor and W. Saenger, *J. Amer. Chem. Soc.*, **96**, 3630–3639 (1974).
(50) W. Saenger, R. K. McMullan, J. Fayos, and D. Mootz, *Acta Crystallogr.*, **B20**, 2019–2028 (1974).

pears to be intramolecular hydrogen-bonding between O-2-H and O-3-H groups on adjacent D-glucosyl residues.

III. DATA FOR NUCLEOSIDES AND NUCLEOTIDES

$C_8H_{12}N_4O_5$ 6-Azacytidine[51]; 5-amino-2-β-D-ribofuranosyl-*as*-triazin-3(2*H*)-one
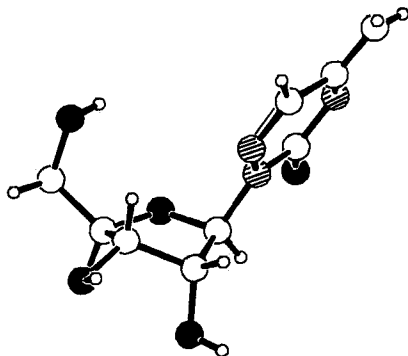
P$2_12_12_1$; Z = 4; D_x = 1.55; R = 0.031 for 1,557 intensities. The conformation of the D-ribosyl group is $^3T_2$ (15°), the glycosyl disposition is in the so-called "high anti" range (99°), and the exocyclic C-4'–C-5' bond torsion-angle is −61°.

There are typographical errors in the atom labels, with two C-2' atoms described. The second C-2' should be C-3', and C-3' → C-4', C-4' → C-5', C-5' → O-4', O-1' → O-2', O-2' → O-3', O-3' → O-5', O-5' → H-5, H-5 → H(N-4), H(N-4) → H'(N-4), H'(N-4) → H-1', and H-1' → H-2'.

$C_{10}H_{11}N_5O_2$ 9-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)adenine[52]
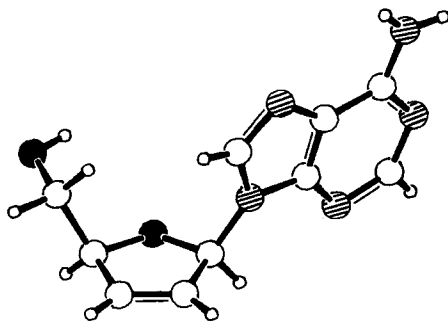
$P2_12_12_1$; $Z = 4$; $D_x = 1.42$; $R = 0.045$ for 1,860 intensities. The presence of the 2,3 double bond constrains the sugar to the $^4T_0$ ($-112°$) conformation. The glycosyl torsion-angle is *anti* ($73°$), and the exocyclic C-4'–C-5' bond torsion-angle is $63°$. The bases exhibit considerable overlap.

$C_{10}H_{11}N_5NaO_7P \cdot 4H_2O$ Guanosine 3',5'-cyclic monophosphate, sodium salt, tetrahydrate (cyclic GMP)[53]
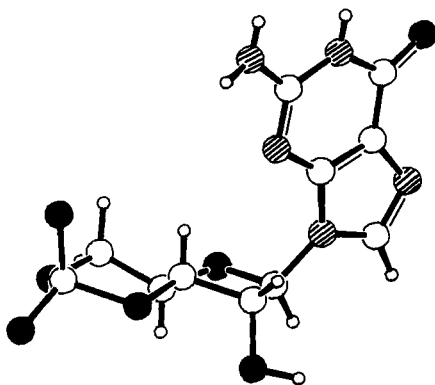
$P2_12_12_1$; $Z = 4$; $D_x = 1.665$; $R = 0.034$ for 1,637 intensities. The conformation of the D-ribosyl group is $_4T^3$ ($43°$), the glycosyl disposition is *syn* ($-102°$), the exocyclic C-4'–C-5' torsion angle is $-175°$. The phosphate ring is locked into a chair conformation, being most puckered about the C-3'–C-4' bond of the D-ribosyl residue and flattened at the phosphate end. The phosphate ring torsion-angles range from 44 to $69°$. The Na^+ ion is coordinated to six water molecules at distances ranging from 2.32 to 2.68 Å (232 to 236 pm). There is no direct linkage between the positively charged, metal ion and the anionic oxygen atoms of the phosphate. The sodium octahedra share edges, to form an infinite column parallel to the b axis. The nucleotides are packed in head-to-tail fashion, with the bases exhibiting stacking interactions that mainly involve the adjacent pyrimidine rings. Thus, the crystal structure is formed by columns of nucleotides and sodium–water octahedra.

$C_{10}H_{12}N_4O_4$ Nebularine; 9-β-D-ribofuranosylpurine[54]

(51) P. Singh and D. J. Hodgson, *Biochemistry*, 13, 5445–5452 (1974).
(52) W. L. B. Hutcheon and M. N. G. James, *Acta Crystallogr.*, **B30**, 1777–1782 (1974).
(53) A. K. Chwang and M. Sundaralingam, *Acta Crystallogr.*, **B30**, 1233–1240 (1974).
(54) T. Takeda, Y. Ohashi, and Y. Sasada, *Acta Crystallogr.*, **B30**, 825–827 (1974).
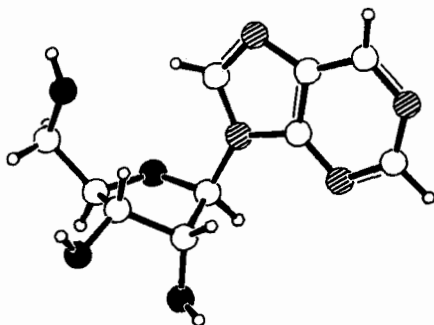
P2$_1$; Z = 2; D$_x$ = 1.54; R = 0.073 for 1,218 intensities. The conformation of the D-ribosyl group is $^3T_2$ (4°), the glycosyl torsion angle is *anti* (14°), and the exocyclic C-4'–C-5' bond torsion-angle is −76°.

C$_{10}$H$_{12}$N$_5$Na$_2$O$_7$P · 4H$_2$O   2'-Deoxyguanosine 5'-phosphate, disodium salt, tetrahydrate[55]
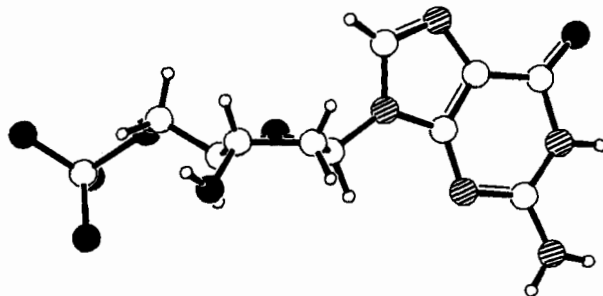
P2$_1$; Z = 2; D$_x$ = 1.646; R = 0.037 for 1,196 intensities. The conformation of the D-ribosyl group is $^0T_4$ (84°) and the glycosyl disposition is *anti* (54°). The exocyclic C-4'–C-5' bond torsion-angle is 62°, an orientation rarely observed in nucleotides. One of the Na$^+$ ions is octahedrally coordinated in a manner involving four water molecules, O-3 of the sugar, and O-6 of the base. The other Na$^+$ ion is pentacoordinated in a manner involving two water molecules, O-6 of the base, and two of the anionic oxygen atoms of the phosphate group. The polyhedra involving the two Na$^+$ ions share a common edge.

(55) D. W. Young, P. Tollin, and H. R. Wilson, *Acta Crystallogr.*, **B30**, 2012–2018 (1974).

$C_{10}H_{13}N_4O_8P \cdot CH_3OH \cdot H_2O$ Inosine 5'-phosphate, methanolate, monohydrate[56]
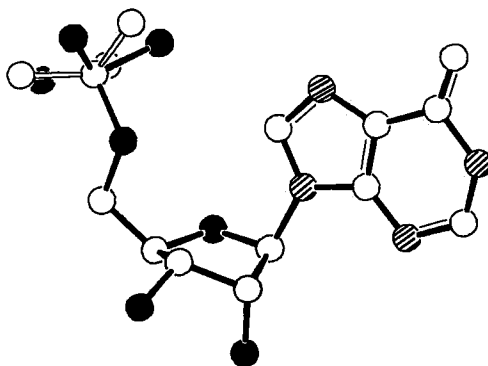
$P2_12_12_1$; $Z = 4$; $D_x = 1.535$; $R = 0.108$ for 1,873 intensities. The conformation of the D-ribosyl group is $^3T_2$ (11°), the glycosyl disposition is *anti* (20°), and the exocyclic C-4'–C-5' bond torsion-angle is −66°. The phosphate oxygen atoms, as well as the methanol and water molecules, are disordered.

The published coordinates referred to the L enantiomer.

$C_{10}H_{13}N_5O_4$ 9-β-D-Arabinofuranosyladenine (Ara-A)[57]
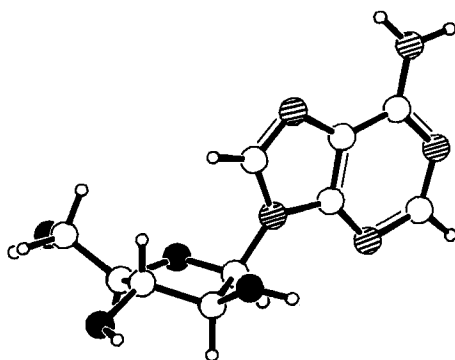
$P2_12_12_1$; $Z = 4$; $D_x = 1.576$; $R = 0.032$ for 1,085 intensities. The conformation of the arabinosyl group is $^3T_4$ (25°), the glycosyl dis-

(56) N. Nagashima, K. Wakabayashi, T. Matzuzaki, and Y. Iitaka, *Acta Crystallogr.*, B30, 320–326 (1974).
(57) G. Bunick and D. Voet, *Acta Crystallogr.*, B30, 1651–1660 (1974).

position is *anti* (58°), and the exocyclic C-4'–C-5' bond torsion angle is 62°.

$C_{10}H_{13}N_5O_4 \cdot HCl$ 9-β-D-Arabinofuranosyladenine   hydrochloride (Ara-A $\cdot$ HCl)[58,59]
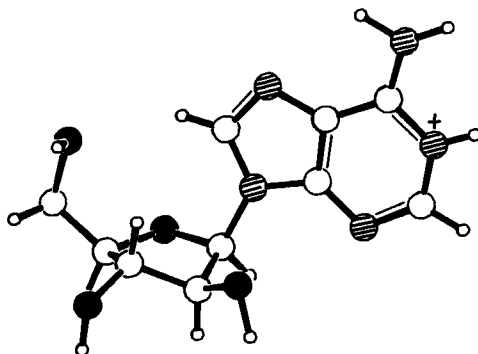
$P2_1$; $Z = 2$; $D_x = 1.560$; two independent investigations: $R = 0.031$ for 862 intensities[58]; $R = 0.045$ for 1,681 intensities.[59] The conformation of the D-arabinosyl group is $^3T_2$ (9°), the glycosyl disposition is *anti* (30° and 29°), and the exocyclic C-4'–C-5' bond torsion-angle is −62° in both cases.

The published coordinates referred to the L enantiomer.

$C_{10}H_{13}N_5O_4 \cdot HBr \cdot H_2O$ Formycin hydrobromide, monohydrate[60]
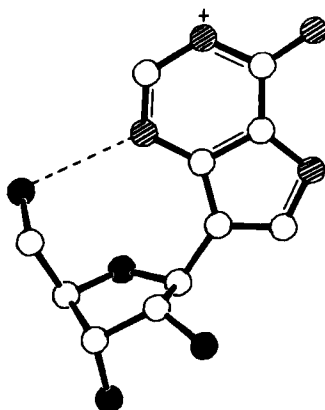
(58) A. K. Chwang, M. Sundaralingam, and S. Hanessian, *Acta Crystallogr.*, **B30**, 2273–2277 (1974).

(59) T. Hata, S. Sato, M. Kaneko, B. Shimizu, and T. Tamura, *Bull. Chem. Soc. Jap.*, **47**, 2758–2763 (1974).

P1; Z = 1; $D_x = 1.78$; R = 0.083 for 1,475 intensities. The conformation of the D-ribosyl group is $_3T^2$ (183°) and the C-glycosyl disposition is *syn* (−149°). There is an intramolecular hydrogen-bond between the 5'-hydroxyl group and N-3 of the base. The exocyclic C-4'–C-5' bond exhibits the seldom-observed torsion-angle of 47° (when involved in this type of hydrogen bond).

$C_{10}H_{14}N_2O_8PS \cdot (C_2H_5)_3NH$ Uridine 3'-thiophosphate, methyl ester, triethylammonium salt[61]

Molecule 1   Molecule 2
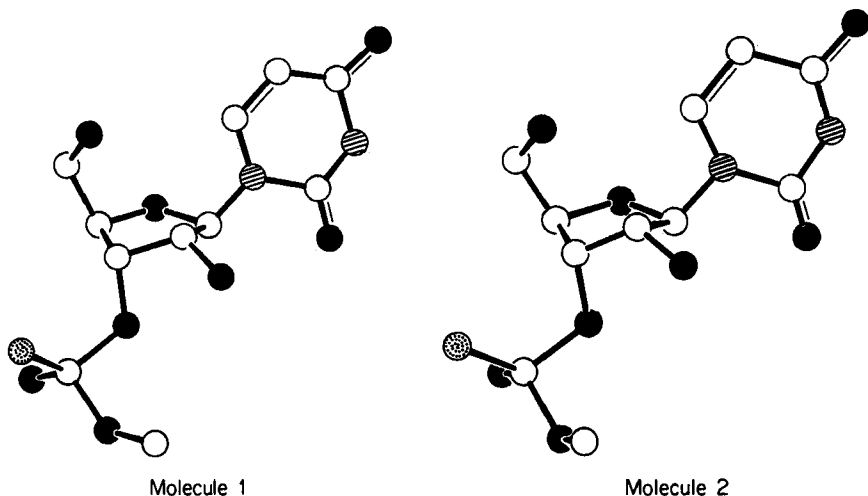
$P2_1$; Z = 4; $D_x = 1.490$; R = 0.191 for 2,243 intensities. There are two symmetry-independent molecules. The conformations of the D-ribosyl groups are $^2T_3$ (165°) and ^2E(162°), the glycosyl dispositions are *anti* (44° and 38°), and the exocyclic C-4'–C-5' bond torsion-angles are −79° and −71°. The dispositions around the P–O–3' and P–O–5' ester bonds are −145° and 46° in molecule 1, and −145° and 70° in molecule 2. The marked disorder of the triethylammonium cations precludes ascertaining the location of the atoms of the alkyl groups.

$C_{10}H_{14}N_3O_5 \cdot CH_3OSO_3H \cdot H_2O$ 3-Methylcytidine methosulfate, monohydrate[62]

(60) G. Koyama, H. Umezawa, and Y. Iitaka, *Acta Crystallogr.*, **B30**, 1511–1516 (1974).
(61) W. Saenger, D. Suck, and F. Eckstein, *Eur. J. Biochem.*, **46**, 559–567 (1974).
(62) E. Shefter, S. Singh, T. Brennan, and P. Sackman, *Cryst. Struct. Commun.*, **3**, 209–213 (1974).
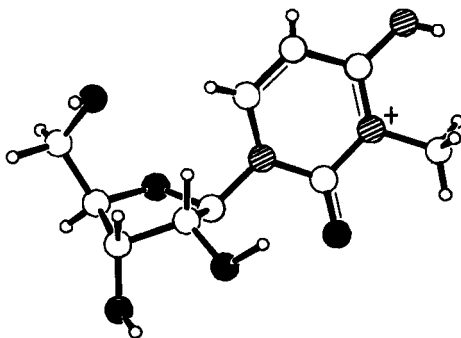
$P2_12_12_1$; $Z = 4$; $D_x = 1.51$; $R = 0.076$ and 1,176 intensities. The conformation of the D-ribosyl group is $^2T_3$ (169°), the glycosyl disposition is *anti* (45°), and the orientation about the exocyclic C-4′–C-5′ bond is −57°.

$C_{12}H_{14}N_2O_5$ 2,5′-Anhydro-2′,3′-*O*-isopropylideneuridine[63]
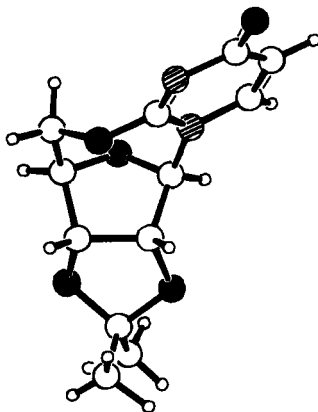
$P2_1$; $Z = 2$; $D_x = 1.474$; $R = 0.049$ for 828 intensities. The conformation of the D-ribosyl group is $_0T^4$ (−106°), the glycosyl torsion-angle is constrained to the *syn* range (−114°) by the C-2–O-2–C-5′ bridge. Similarly, the bridge constrains the exocyclic C-4′–C-5′ bond torsion-angle (O-1′–C-4′–C-5′–O-2) to the *gauche* conformation (−73′), thus placing O-2 approximately over the middle of the furanose ring. The O-2′–C-2′–C-3′–O-3′ torsion angle is −8°, and

(63) P. C. Manor, W. Saenger, D. B. Davies, K. Jankowski, and A. Rabczenko, *Biochim. Biophys. Acta*, **340**, 472–483 (1974).

the isopropylidene ring is in approximately an envelope conformation.

$C_{13}H_{18}N_4O_5S \cdot H_2O$ 2-(Ethylthio)-8-methylinosine monohydrate[64]
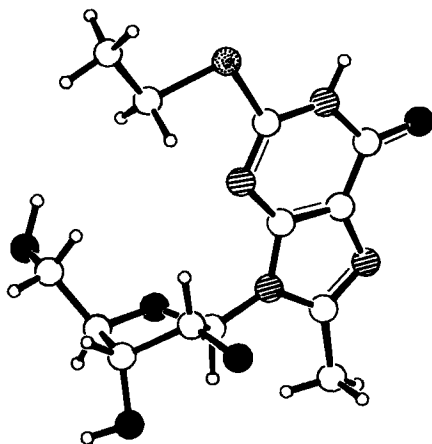
$P2_1$; $Z = 2$; $D_x = 1.536$; $R = 0.065$ for 1,387 intensities. The conformation of the D-ribosyl group is $^2T_1$ (153°), the glycosyl disposition is *syn* (−110°), and the exocyclic C-4′–C-5′ bond torsion-angle is 62°. The nucleotides are stacked in head-to-tail fashion involving the pyrimidine rings, and the sulfur atom lies over the imidazole ring.

The published sign of the *z*-coordinate of H-2 (C-12) is incorrect; it should be *minus*.

$C_{24}H_{36}N_4O_4$ Cytosamine triacetate; 1-*N*-[2,3,6-trideoxy-
4-*O*-(4,6-dideoxy-4-dimethylamino-α-D-glucopy-
ranosyl)-β-D-*erythro*-hexopyranosyl]cytosine
triacetate[65]

$P2_12_12_1$; $Z = 4$; $D_x = 1.231$; $R = 0.063$ for 2,411 intensities. The glycosyl disposition is *anti* (43°). The pyranoid moieties are in the favored chair conformation; the torsion angles about the ring bonds range from 51.0 to 63.4° in ring A (attached to the base), and 54.2 to 63.5° (in ring B). The torsion angles about the glycosidic C–O bonds are 79.5 and −162.5°, respectively, for C-3′–C-4′–O–C-1 and C-5′–C-4′–O–C-1, and 67.9 and -169.3°, respectively, for

(64) N. Nagashima and K. Wakabayashi, *Acta Crystallogr.*, **B30**, 1094–1099 (1974).
(65) J. Sygusch, F. Brisse, and S. Hanessian, *Acta Crystallogr.*, **B30**, 40–47 (1974).

C-4–O–C-1–O-5 and C-4′–O–C-1–C-2. The lengths of the C–O
bonds (141.3 and 144.0 pm) are unequal, the anomeric bond being
shorter. Both of the ring C–O bonds are also unequal, the shorter
bonds involving the anomeric carbon atom. The only hydrogen
bond in the structure is the inter-base hydrogen-bond involving
the amino nitrogen atom (N-4) and the carbonyl oxygen atom (O-2).
The cytosine ring and the N-acyl group are essentially coplanar.
The two acetyl groups are also planar.
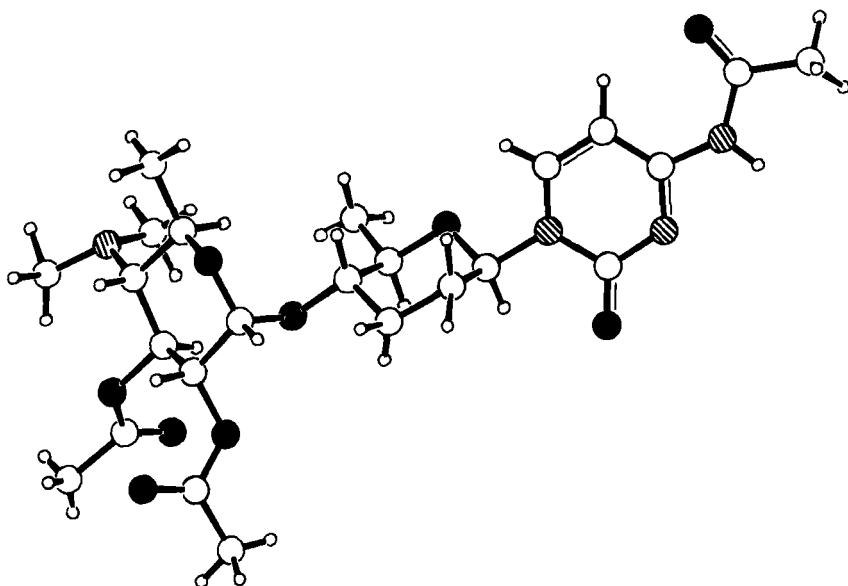
IV. Preliminary Communications

1. Carbohydrates

$2(C_6H_9O_7)Ca \cdot 4H_2O$	Calcium α-D-galacturonate tetrahydrate[66]
$3(C_6H_9O_7)CaNa \cdot 6H_2O$	Calcium sodium α-D-galacturonate hexahydrate[67]
$C_6H_{12}Cl_2O_4$	1,6-Dichloro-1,6-dideoxy-D-mannitol[68]
$C_6H_{13}BrO_5$	1-Bromo-1-deoxy-D-mannitol[68]
$C_6H_{13}ClO_5$	1-Chloro-1-deoxy-D-mannitol[68]

(66) S. Thanomkul and J. Hjortas, *Eur. Crystallogr. Meeting Abstr.*, 336 (1974).
(67) J. Hjortas, B. Larsen, and S. Thanomkul, *Acta Chem. Scand.*, **B28**, 269–270 (1974).
(68) J. C. Wallace, *Amer. Crystallogr. Ass. Meeting Abstr.*, 241 (1974).

$C_8H_{12}N_2O_3S$	4-D-Erythrofuranosyl-1-methyl-4-imidazoline-2-thione[69]
$C_8H_{15}NO_6$	2-Acetamido-2-deoxy-D-mannose[70]
$C_8H_{18}O_{10}S$	1,6-Di-*O*-(methylsulfonyl)-D-mannitol[68]
$C_9H_{16}O_7$	Methyl 6-*O*-acetyl-β-D-glucopyranoside[71]
$C_9H_{16}O_7$	Methyl 6-*O*-acetyl-β-D-galactopyranoside[72]
$C_{17}H_{27}N_3O_5S_2$	8-Azido-8-deoxy-1,2:3,4-di-*O*-isopropylidene-6,7-*S*-trimethylene-6,7-dithio-L-*threo*-α-D-*galacto*-octo-pyranose[73]
$C_{18}H_{32}O_{16} \cdot H_2O$	Melezitose monohydrate; *O*-α-D-glucopyranosyl-(1 → 3)-β-D-fructofuranosyl α-D-glucopyranoside monohydrate[74]

2. Nucleosides and Nucleotides

$C_9H_{12}N_6O_4$ 8-Azaadenosine[75]

$P2_12_12_1$; $Z = 4$; $D_x = 1.643$; $R = 0.049$ for 1,029 intensities. The conformation of the D-ribosyl group is $^2T_1$, the glycosyl disposition is "high" *anti*, and the exocyclic C-4'–C-5 bond torsion-angle is g^+.

$C_{10}H_{11}N_4NiO_8P \cdot 7H_2O$ Inosine nickel 5'-monophosphate, heptahydrate[76]

$P2_12_12_1$; $Z = 4$; $D_x = 1.591$; $R = 0.087$ for 1,471 intensities. The conformation of the D-ribosyl group is C-3'-*endo*, the glycosyl disposition is *anti*, and the exocyclic C-4'–C-5' bond torsion-angle is g^-. The Ni atom is octahedrally coordinated; one of these ligands involves N-7, and the remaining five are water molecules. Two

(69) E. Moreno, A. Conde, and R. Márquez, *Eur. Crystallogr. Meeting Abstr.*, 320 (1974).

(70) A. Neuman, H. Gillier-Pandraud, and F. Longchambon, *Eur. Crystallogr. Meeting Abstr.*, 324 (1974).

(71) P. J. Garegg, K. B. Lindberg, and C. G. Swahn, *Acta Chem. Scand.*, **B28**, 269–270 (1974).

(72) B. Lindberg, P. J. Swahn, and C. G. Swahn, *Acta Chem. Scand.*, **B27**, 380–381 (1973).°

(73) A. Gateau-Olesker, S. D. Gero, C. Pascard-Billy, C. Riche, A.-M. Sepulchre, G. Vass, and N. A. Hughes, *J. Chem. Soc. Chem. Commun.*, 811–812 (1974).

(74) K. Hirotsu and A. Shimada, *Chem. Lett. Jap.*, 83–86 (1973).

(75) P. Singh and D. J. Hodgson, *J. Amer. Chem. Soc.*, 96, 5276–5278 (1974).

(76) G. R. Clark and J. D. Orbell, *Chem. Commun.*, 139–140 (1974).

° Omitted from the 1973 bibliography.

of these molecules are also involved in intramolecular hydrogen-bonds to two phosphate oxygen atoms.

$C_{10}H_{11}N_4O_8PZn \cdot H_2O$ Inosine zinc 5'-monophosphate, monohydrate[77]

$P2_12_12_1$; Z = 4; D_x = 1.966; R = 0.042 for 1,623 intensities. The conformation of the D-ribosyl group is C-3'-*endo*, the glycosyl disposition is *anti*, and the exocyclic C-4'–C-5' bond torsion-angle is g^+. The Zn atom has a distorted, tetrahedral coordination involving N-7 of the base and the phosphate oxygen atom of three neighboring nucleotide molecules.

$C_{10}H_{12}N_2O_6$ Thymidine-5'-carboxylic acid[78]

$P2_1$; Z = D_x = 1.504; R = 0.039 for 2,224 intensities. There are two symmetry-independent molecules. The conformations of the 2'-deoxy-D-*erythro*-pentofuranosyl groups are $^2T_3$ (172°,165°), the glycosyl dispositions are *anti* (44°,53°), and the exocyclic C-4'–C-5' bond torsion-angles are −21 and −32°. The carboxyl groups are hydrogen-bonded to the carbonyl oxygen atom (O-4) and the ring nitrogen atom (N-3) of neighboring molecules, forming cyclic dimers.

$C_{10}H_{12}MnN_5O_8P \cdot 8H_2O$ Guanosine manganese 5'-monophosphate, octahydrate[79]

C2; Z = 4; D_x = 1.766. The glycosyl disposition is *anti*. The manganese atom is coordinated to N-7 of the base and to five water molecules. There are intramolecular hydrogen-bonds between two phosphate oxygen atoms and two of the coordinated water molecules. A third water molecule coordinated to the metal atom is intramolecularly hydrogen-bonded to the carbonyl oxygen atom (O-6) of the base. There is no direct, metal–phosphate hydrogen-bonding.

$C_{10}H_{12}N_5NiO_8P \cdot 8H_2O$ Guanosine nickel 5'-monophosphate, octahydrate[80]

(77) P. deMeester, D. M. L. Goodgame, T. J. Jones, and A. C. Skapski, *Biochim. Biophys. Acta*, **353**, 392–394 (1974).
(78) D. Suck, W. Saenger, and W. Rohde, *Biochim. Biophys. Acta*, **361**, 1–10 (1974).
(79) P. deMeester, D. M. L. Goodgame, T. J. Jones, and A. C. Skapski, *Biochem. J.*, **139**, 791–792 (1974).
(80) P. deMeester, D. M. L. Goodgame, A. C. Skapski, and B. T. Smith, *Biochim. Biophys. Acta*, **340**, 113–115 (1974).

C2; $Z = 4$; $D_x = 1.828$; $R = 0.076$ for 1,023 intensities. This compound is isostructural with its manganese analog.

$C_{10}H_{15}N_3O_5$ 2'-O-Methylcytidine[81]

$P2_12_12_1$; $Z = 8$; $D_x = 1.428$; $R = 0.069$ for 2,460 intensities. There are two symmetry-independent molecules. The conformations of the D-ribosyl groups are $^2T_3$, the glycosyl dispositions are *anti*, and the exocyclic C-4'–C-5' bond torsion-angles are g^-. The methyl group in molecule 1 is disordered, and the C-1'–C-2'–O-2'–Me torsion angles are 94° (66%), 182° (33%). In molecule 2, this torsion angle is 167°.

$C_{11}H_{16}N_4O_5 \cdot 1.5H_2O$ Coformycin sesquihydrate; 3-β-D-ribofuranosyl-6,7,8-trihydroimidazo[4,5-*d*][1,3]diazepin-8(R)-ol[82]

$P2_12_12_1$; $Z = 4$; $D_x = 1.49$; $R = 0.057$ for 1,021 intensities. The conformation of the D-ribosyl group is $_1T^2$, the glycosyl disposition is *anti*, and the exocyclic C-4'–C-5' bond torsion-angle is g^-. The base moiety is a modified purine ring with the "original" pyrimidine ring expanded to a seven-membered ring which assumes a puckered form.

$C_{13}H_{15}FN_2O_7$ 3',5'-Di-O-acetyl-2'-deoxy-2'-fluorouridine[83]

$P2_1$; $Z = 2$; $D_x = 1.464$; $R = 0.039$ for 1,237 intensities. The conformation of the D-ribosyl group is $_4T^3$ (38°), the glycosyl disposition is *syn* ($-108°$), and the exocyclic C-4'–C-5' bond torsion-angle is 167°. There is an inter-base hydrogen-bond involving N-3-H and O-4.

$C_{15}H_{20}N_6O_8$ N-(9-β-D-Ribofuranosylpurin-6-ylcarbamoyl)-L-threonine[84]

$P2_1$; $Z = 2$; $D_x = 1.56$; $R = 0.04$ for 2,036 intensities. The conformation of the D-ribosyl group is $^2T_1$, the glycosyl disposition is

(81) B. Hingerty, P. J. Bond, R. Langridge, and F. Rottman, *Biochem. Biophys. Res. Commun.*, **61**, 875–881 (1974).
(82) H. Nakamura, G. Koyama, Y. Iitaka, M. Ohno, N. Yagaisawa, S. Kondo, K. Maeda, and H. Umezawa, *J. Amer. Chem. Soc.*, **96**, 4327–4328 (1974).
(83) D. Suck, W. Saenger, P. Main, G. Germain, and J.-P. Declercq, *Biochim. Biophys. Acta*, **361**, 257–265 (1974).
(84) R. Parthasarathy, J. M. Ohrt, and G. B. Chheda, *Biochem. Biophys. Res. Commun.*, **60**, 211–218 (1974).

anti, and the exocyclic C-4'–C-5' bond torsion-angle is g^-. The nitrogen atom of the L-threonyl residue forms intramolecular, bifurcated hydrogen-bonds to the base ring-nitrogen N-1 and the threonyl hydroxyl oxygen atom.

$C_{20}H_{19}H_7O_6Os$ Adenosine bis(pyridine) osmate(VI)[85]

P2$_1$2$_1$2$_1$; Z = 4; D$_x$ = 1.872; R = 0.018 for 2,243 intensities. The conformation of the D-ribosyl group is $^2T_3$ (166°), the glycosyl disposition is *syn*, and the exocyclic C-4'–C-5' bond torsion-angle is g^-. The Os atom bridges HO-2 and HO-3 of the D-ribosyl group.

(85) J. F. Conn, J. J. Kim, F. L. Suddath, P. Blattmann, and A. Rich, *J. Amer. Chem. Soc.*, **96**, 7152–7153 (1974).

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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.

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