stand at 5° for 5 days. It was then processed as above. The crude product weighed 1.4 g. Recrystallization from ethanol at room temperature gave 0.55 g. of unreacted aceto-bromo compound. The mother liquor was diluted with water and refrigerated for 3 days. The product was collected on a filter and recrystallized from ethanol at room temperature to give 0.5 g. (11%) of methyl (*o*-chlorophenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate; m.p. 151–152°, $[\alpha]^{24}D$ −62.8° (*c* 1, $CHCl_3$); literature values[17] m.p. 151–152°, $[\alpha]^{20}D$ −65.0° (*c* 1, $CHCl_3$).

Phenyl β-D-Glucopyranosiduronic Acid.—Three hundred milligrams (0.73 mmole) of methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate was dissolved in 20 ml. of methanol containing 0.3 ml. of 0.5 *N* barium methoxide. The solution was refrigerated for 3 days. Barium was removed by addition of the calculated amount of dilute sulfuric acid. The mixture was filtered and the filtrate was concentrated to a crystalline residue (150 mg., 75%). The residue was suspended in boiling benzene and enough ethanol added to obtain a homogeneous solution. The acid obtained on cooling had a sharp melting point 163–164° with a preliminary softening at 120°. The preliminary melt could be eliminated by recrystallizing from boiling ethanol–benzene or, better, from ethyl acetate. Found: $[\alpha]^{25}D$ −90.0° (*c* 1, H_2O), literature values[24] m.p. 161–162°, $[\alpha]^{18}D$ −90.5° (*c* 1.6, H_2O).

2-Naphthyl β-D-Glucopyranosiduronic Acid.—One gram (0.022 mole) of methyl (2-naphthyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate was suspended in 50 ml. of methanol. A homogeneous system was obtained on addition of 0.5 ml. of 0.5 *N* barium methoxide. The solution was refrigerated for 24 hr. and barium was then removed as barium sulfate by addition of the calculated amount of sulfuric acid. The salt was removed by filtration and the filtrate was evaporated to dryness in an air stream. The residue was crystallized from water to give 360 mg. (52.0%) of 2-naphthyl β-D-glucopyranosiduronic acid; m.p. 151.5–152°, $[\alpha]^{24}D$ −100° (*c* 1, ethanol); literature values[25] m.p. 149–150°, $[\alpha]^{22}D$ −97° (ethanol).

Methyl (Methyl Gentisyl β-D-Glucopyranosid)-uronate.—Two hundred mg. (0.42 mmole) of methyl (methyl gentisyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate was dissolved in 20 ml. of methanol and 0.2 ml. of 0.5 *N* barium methoxide was added to the solution. The solution was refrigerated overnight and then treated with a calculated amount of sulfuric acid. Barium sulfate was removed by filtration and the filtrate concentrated to crystals. The crude material (120 mg., 82%) was recrystallized from ethanol at room temperature to constant melting point 180–181.5°, $[\alpha]^{28}D$ −83.4 (*c* 1.4, CH_3OH). *Anal.*[33] Calcd. for $C_{14}H_{18}O_{10}$: C, 50.28; H, 5.03; CH_3O, 17.32. Found: C, 50.60; H, 5.15; CH_3O, 17.16.

Methyl Glucofuranosidurono-γ-lactones from Glucuronic Acid and Methyl Glucuronate (III → IV, II → IV, Chart 1).—Twenty-five grams (0.129 mole) of glucuronic acid was stirred in 500 ml. of methanol with 12 g. of Nalcite HCR cation-exchange resin at room temperature for 24 hr. Analysis of the liquor by Schoorl's method indicated 13% reducibles as dextrose.[35] After filtration and washing resin with methanol, combined liquors were concentrated under reduced pressure and the resulting light yellow sirup crystallized spontaneously on addition of ethanol; yield 15.9 g. (65%) in two crops. The combined crops were recrystallized from ethanol to give 9.7 g. of prisms; m.p. 138–140°, $[\alpha]^{25}D$ −55.0° (*c* 1, H_2O) and giving no depression of melting point with methyl β-D-glucofuranosidurono-γ-lactone.[35–37] Needles obtained from mother liquor, m.p. 148°; $[\alpha]^{25}D$ +147.0° (*c* 1, H_2O) gave no depression of melting point with methyl α-D-glucofuranosidurono-γ-lactone.[35,36] When the reaction was interrupted at 16 hr. a 50% yield of crystalline methyl α- and methyl β-D-glucofuranosidurono-γ-lactone was obtained. Acetylation of the sirup obtained from mother liquors yielded 5% of methyl tetra-*O*-acetyl-β-D-glucopyranuronate; m.p. 176°, $[\alpha]^{25}D$ +8.50° (*c* 1, $CHCl_3$) (III → II, Chart 1).

Methyl glucuronate (11.8 g., 0.0567 mole) was similarly treated with methanol in the presence of Nalcite HCR; 3.6 g. of methyl α- and methyl β-D-glucofuranosidurono-γ-lactones (33%) were isolated in crystalline form. As a control, methyl glucuronate from the same batch was acetylated with pyridine–acetic anhydride and the crystalline acetylated esters were isolated in 90% yield.

Reaction IV → V is included in Chart 1 to complete the known reactions in this series.[37]

Acknowledgment.—The authors wish to express their appreciation of the able advice offered by Dr. R. H. Tennyson during the course of this work. Acknowledgment is also made to Mr. R. R. Johnson for general technical assistance.

(35) Methyl β-D-glucofuranosidurono-γ-lactone has about 15% the reducing value of dextrose: Elizabeth M. Osman, K. C. Hobbs and W. E. Walston, THIS JOURNAL, **73**, 2726 (1951).

(36) J. E. Cadotte, F. Smith and D. Spriestersbach, *ibid.*, **74**, 1501 (1952).

(37) L. N. Owen, S. Peat and W. J. G. Jones, *J. Chem. Soc.*, 339 (1951).

ARGO, ILLINOIS

[CONTRIBUTION FROM THE CEREAL CROPS SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Preparation of Panose by the Action of NRRL B-512 Dextransucrase on a Sucrose–Maltose Mixture[2]

BY MARY KILLEY, R. J. DIMLER AND J. E. CLUSKEY

RECEIVED NOVEMBER 12, 1954

A new method is presented for the preparation of panose involving the action of culture filtrates of *Leuconostoc mesenteroides* NRRL B-512, containing dextransucrase, upon a sucrose–maltose mixture. A preliminary study of the effects of variations in carbohydrate and enzyme concentration and in reaction temperature provided a basis for selection of suitable conditions for limiting the reaction mainly to panose formation. The most important variable was found to be the ratio of maltose to sucrose, while the total carbohydrate concentration had a smaller but significant effect. The preparative procedure developed included separation of the synthesized panose from the yeast-treated reaction mixture by chromatography on a carbon–Celite[3] column. The resulting product crystallized readily and recrystallization did not change its properties.

Panose, a 4-α-isomaltopyranosyl-D-glucose, was crystallized first by Pan[4] as a product of the transglucosylation action of an *Aspergillus niger* culture filtrate on maltose. From a preparative standpoint this method had the disadvantage that the culture filtrate had to be freshly prepared in order to avoid marked decreases in the yield of panose. We present here an alternative method for preparation of panose which avoids this difficulty and which yields a crystalline product that does not require recrystallization.

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented at the Midwest Regional Meeting of the American Chemical Society, Omaha, Nebr., November, 1954.

(3) Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

(4) S. C. Pan, L. W. Nicholson and P. Kolachov, THIS JOURNAL, **73**, 2547 (1951).

This method is based upon the action of an enzyme(s), dextransucrase, which is present in the culture filtrates of *L. mesenteroides* NRRL B-512. Dextransucrase catalyzes the formation of dextran, a predominantly α-1,6-linked polymer of glucose, from sucrose by transfer of glucosyl units with sucrose acting as donor and probably as the initial acceptor. Studies of the acceptor specificity of dextransucrase[5] have shown that maltose can serve as acceptor with sucrose as donor. The first product to be formed appeared chromatographically to be panose, which in turn appeared to act as acceptor leading to the formation of oligosaccharides.

Further studies of the action of NRRL B-512 dextransucrase on mixtures of sucrose and maltose were undertaken, therefore, to establish that panose is the trisaccharide formed and to determine whether suitable conditions could be found for the preparative synthesis of panose. It was felt that the normal course of action of dextransucrase, *i.e.*, the formation of dextran, could be diverted almost completely to the following path of panose synthesis by use of proper amounts of maltose and sucrose and under suitable conditions of temperature, time and enzyme concentrations

Sucrose (glycosyl donor) +

maltose (glycosyl acceptor) $\xrightarrow[\text{sucrase}]{\text{dextran-}}$ panose + fructose

A preliminary study of the effect of these variables, applying methods of statistical analysis, confirmed this belief and revealed that, within limits of control achieved, the yield of panose is dependent mainly on the ratio of maltose to sucrose.

A highly satisfactory procedure for preparative synthesis of panose was developed using these studies as a basis for the selection of conditions and incorporating the use of a carbon–Celite[6,7] column to obtain a more easily crystallized product. The resulting panose corresponded in properties with the product reported by Pan[4] and was isolated in yields of 67% of theory based on the sucrose used.

A major advantage of the preparative procedure using dextransucrase is the stability of the enzyme system.[8] This procedure also provides a means of preparing panose selectively labeled with radioactive glucose units by using either labeled sucrose or labeled maltose.

Methods and Materials

Enzyme Preparation.—A culture filtrate of *L. mesenteroides* NRRL B-512 containing 57 dextransucrase units/ml. was used.[8,9] The age of the filtrate apparently has no detrimental effect upon the reaction, the culture filtrate having been stored for 9 months. Presence of 7,950 μg. of carbohydrate/ml. in the filtrate was taken into account in any calculations.

Maltose.—Maltose hydrate used for large-scale preparation, 95% pure by quantitative paper chromatography, was prepared by β-amylase degradation of waxy-corn starch and used without recrystallization. Maltose hydrate used to test optimum panose conditions was prepared by the same method, but had been recrystallized 4 times; ($[\alpha]^{25}D$ 129.6 (*c* 4, water; *l*, 1)).

Preparation of Carbon–Celite Column.—A column 7.5 cm. × 33 cm. was packed with a mixture of Norit SG and Celite 501 as described by Jeanes, *et al.*[7]

Enzymatic Synthesis and Measurement of Products.—The reaction was initiated by addition of sucrose to a maltose–dextransucrase mixture. Sucrose was added in the form of a concentrated solution rather than the solid state in order to avoid a high local concentration of the sucrose. Total volume of each mixture was 10 ml. The *p*H of all combinations was 5. The course of the reaction was followed by measuring the disappearance of sucrose. When 90 ± 3% of the sucrose had been utilized, the reaction was stopped by heating on a steam-bath and the products of enzyme activity were separated by paper chromatography in butanol:methyl Cellosolve:water (2:1:1) solvent and the carbohydrates determined by a quantitative anthrone method.[10] A combination of a modified Schlemmer's test and an anthrone determination for easily hydrolyzable fructose units was used[11,12] to determine the disappearance of sucrose. This determination is based upon the fact that reducing sugars are destroyed by boiling with milk of lime and do not give the usual sugar reactions after this treatment.

Ten ml. of the solution to be tested, containing not over 2% sugar, is mixed in a test-tube with 1 ml. of 10% milk of lime and the tube is placed in a boiling water-bath for 10–12 minutes. The tube is centrifuged and the supernatant pipetted off and tested for sucrose by the modified anthrone method.

Preparation of Panose.—The choice of conditions for this preparation is discussed under Results and Discussion. A concentrated solution of sucrose (20 g.) was added to a solution containing 200 g. of maltose and 5,000 units of dextransucrase (100 ml.) and the resulting mixture quickly diluted to 1,000 ml. After incubation at 25° at a *p*H of 5.0 for a period of approximately 17 hours, the reaction mixture was analyzed for the amount of sucrose remaining and the reaction then stopped by heating on a steam-bath. A quantitative paper chromatogram showed 27 g. of panose to be present in the mixture (theory 30 g. for complete reaction of sucrose). This solution was treated with a suspension of washed yeast cells (2.5 g. of bakers' compressed yeast/g. carbohydrate), *p*H 5.0, for approximately 24 hours at 30°.[13,14] The yeast was removed completely by centrifugation and washed at least twice with distilled water. A quantitative chromatogram on the combined supernatants showed no spot other than panose and oligosaccharides and showed 25.3 g. of panose present. The supernatants were evaporated *in vacuo* to a thick sirup which was then dissolved in 40 ml. of water and 1 ml. absolute alcohol. The mixture was placed on a carbon–Celite column and the various fractions separated by elution with 2.5, 5, 10 and 15% ethanol, respectively (see Fig. 1). Percentage of carbohydrates in each fraction was determined by semi-quantitative anthrone determination and selected fractions were subjected to qualitative paper chromatography. The major portion of panose was removed from the column by the 10% ethanol. Those fractions containing the panose were collected and evaporated to a sirup *in vacuo*. The sirup was dissolved in 10 ml. of water and 40 ml. of methanol was added. The solution, seeded with panose, was allowed to remain overnight in an atmosphere of methanol, *i.e.*, placed in a desicca-

(5) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko C. A. Hoffman, E. S. Sharpe and R. W. Jackson, *J. Biol. Chem.*, **200**, 793 (1953).

(6) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(7) Allene Jeanes, C. A. Wilham, R. W. Jones, H. M. Tsuchiya and C. E. Rist, *ibid.*, **75**, 5911 (1953).

(8) V. E. Sohns, J. P. Rogovin, H. F. Conway and C. T. Langford, "Pilot-Plant Production of Clinical-Sized Dextran by Acid Hydrolysis of the Enzymatically Synthesized High Polymer," AIC-372, Northern Utilization Research Branch, Peoria, Ill., 1954.

(9) H. M. Tsuchiya, H. J. Koepsell, J. Corman, G. Bryant, M. O. Bogard, V. H. Feger and R. W. Jackson, *J. Bact.*, **64**, 521 (1952).

(10) R. J. Dimler, W. C. Schaefer, C. S. Wise and C. E. Rist, *Anal. Chem.*, **24**, 1411 (1952).

(11) C. A. Browne and F. W. Zerban, "Sugar Analysis," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 733.

(12) C. S. Wise, R. J. Dimler, H. A. Davis and C. E. Rist, *Anal. Chem.*, **27**, 33 (1955).

(13) J. E. Hodge and H. A. Davis, "Selected Methods for Determining Reducing Sugars," AIC-333, Northern Regional Research Laboratory, Peoria, Ill., 1952, p. 19.

(14) If a source of maltose is not readily obtainable, recovery of the maltose may be desired. This can be accomplished by foregoing yeast treatment and fractionating two or three times on the carbon Celite column.

tor containing a dish of methanol instead of desiccant. Crystals were removed by filtration, the mother liquor evaporated to a sirup, and treated as above to obtain a second crop of crystals. Final yield was 20 g. (67%, based on sucrose used). Properties of the crystals are as follows: m.p. 221° dec.; $[\alpha]^{25}D$ (c 0.76, water) +162° mutarotates down to +154°; reducing power toward copper–tartrate–phosphate reagent,[13,15] calculated as glucose, 42%; non-fermentable with bakers' compressed yeast. Recrystallization from methanol and water did not change these constants. Corresponding properties reported by Pan[3] are as follows: m.p. 213° dec.; $[\alpha]^{25}D$ (c 2.2, water) +154° (mutarotates downward); reducing power, 40%; non-fermentable. The product was identified further by an X-ray comparison with the compound prepared by Pan[16] and the two substances were found to be identical.

Results and Discussion

Optimum Conditions for Panose Synthesis.—This study was not intended to be a comprehensive study of conditions for optimum panose synthesis, but merely to serve as a basis for interpretation and prediction of the direction which such a study must take. Variables studied were temperature, enzyme concentration, maltose concentration and maltose:sucrose ratio. The pH was not included as a variable, because Koepsell and Tsuchiya[17] have found the optimum pH for activity stability of dextransucrase to have rather narrow limits around pH 5.0. Each variable was studied at two levels which were carefully chosen from available knowledge of the enzyme and from data obtained from a test run. Since a statistical analysis of the data was desired, the levels were selected and the experiment designed so that individual effects and interactions of factors could be obtained from a minimum amount of data. All possible combinations of variables were studied, amounting to sixteen experiments. Each experiment was run in duplicate or triplicate. The set of sixteen was divided into two blocks of eight each, and each block performed on different days. The blocks were so chosen that the error introduced by running experiments at different times was canceled.[18]

Each experiment was stopped at as close to 90% completion as possible. Because of length of time necessary (at least 1 hour) for the sucrose test, it was not always practicable to stop at exactly 90%. The values, therefore, represent a range of 90 ± 3%.

Results of these experiments are shown in Table I. Values for panose and oligosaccharides, *i.e.*, the chromatographically slower moving or immobile saccharides, are calculated on the basis of sucrose. Theoretically, for every gram of sucrose, 1.5 g. of panose should be formed. Weights of oligosaccharides were treated as though the material were panose. Values given are averages of duplicate or triplicate determinations.

A study of Table I will show that in certain experiments, especially those involving the 10:4 ratio of maltose:sucrose, the percentages of panose plus oligosaccharides do not add up to 90%. That this probably was caused by other enzymatic activities in the culture filtrate used was shown by incubating the enzyme with sucrose alone under the same conditions. Analysis showed that a large portion (approximately 60%) was broken down to glucose and fructose instead of yielding dextran and fructose.

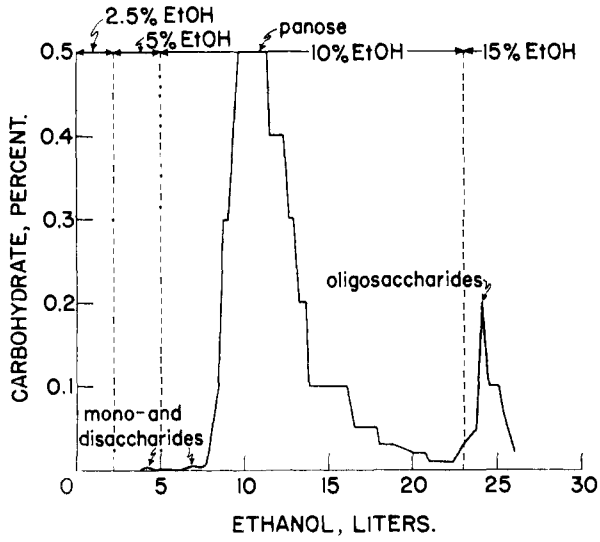

Fig. 1.—Fractionation of carbohydrate mixture on carbon–Celite column.

TABLE I

STUDIES OF OPTIMUM CONDITIONS FOR PANOSE SYNTHESIS. YIELDS[19] OF PANOSE AND OLIGOSACCHARIDES OBTAINED UNDER VARIOUS COMBINATIONS OF CONDITIONS

	Enzyme units/ml. incubation mixture: 5 units, Maltose, 5%	5 units, Maltose, 20%	20 units, Maltose, 5%	20 units, Maltose, 20%
	Temperature, 0°			
Panose[a]	70	90	77	96
Oligosaccharides[a]	7	8	12	12
Panose[b]	65	64	62	74
Oligosaccharides[b]	20	16	21	17
	Temperature, 25°			
Panose[a]	80	90	83	84
Oligosaccharides[a]	12	9	8	10
Panose[b]	53	55	52	59
Oligosaccharides[b]	23	26	29	26

[a] Maltose:sucrose = 10:1. [b] Maltose:sucrose = 10:4.

It can be seen that in other experiments the total of panose and oligosaccharides goes well over 90%. Maltose alone was incubated with the enzyme under these conditions with the result that some products corresponding to panose and polysaccharides were formed (approximately 5%). This, plus the fact that less than 10% sucrose remained when the reaction was stopped, seems to account for the discrepancy.

Statistical methods were used to evaluate the effects and interactions of the variables. This analysis provided an objective approach with more adequate allowance for the uncontrolled variability of results than is possible by visual inspection of Table I. Discussion of these methods has been omitted because detailed discussions can be found elsewhere.[20,21] The statistical test used in this case

(15) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(16) The sample of reference panose was generously supplied by Dr. Pan.

(17) H. J. Koepsell and H. M. Tsuchiya, *J. Bact.*, **63**, 293 (1952).

(18) K. A. Brownlee, *Ann. N. Y. Acad. Sci.*, **52**, 820 (1950).

(19) Expressed as percentages of theoretical weight of panose.

(20) W. L. Gore, "Statistical Methods for Chemical Experimentation," Interscience Publishers, Inc., New York, N. Y., 1952.

(21) W. J. Youden, "Statistical Methods for Chemists," John Wiley and Sons, Inc., New York, N. Y., 1951.

is "analysis of variance." Analysis of the foregoing data is summarized in Table II.

TABLE II

ANALYSIS OF VARIANCE BASED ON YIELD OF PANOSE OBTAINED DURING STUDIES OF OPTIMUM CONDITIONS[22]

Source of variance	Sum of squares	Degrees of freedom	F ratio
Factor E (enz. concn.)	25	1	1.06
Factor M (maltose concn.)	306	1	12.97*
Factor T (temp.)	110	1	4.66
Factor R (maltose:sucrose ratio)	2162	1	91.61**
EM interaction	4	1	0.17
ET interaction	25	1	1.06
ER interaction	0	1	
MT interaction	56	1	2.37
MR interaction	56	1	2.37
TR interaction	156	1	6.6*
Subtotal	2900	10	
Error	118	5	
Total	3018	15	

Those factors and interactions in Table II marked with a double asterisk have a major effect upon the reaction, while those marked with a single asterisk have a smaller effect. It can be seen that the maltose:sucrose ratio exerts a very real effect. Maltose concentration and temperature–ratio interaction also have some effect, although much smaller than the ratio. These observations are compatible with the knowledge of the enzyme action. The smaller the ratio of maltose:sucrose, the greater the chance for the formation of dextran. However, as the ratio increases, chance of dextran formation becomes increasingly smaller. The total carbohydrate concentration would normally be expected to have an effect, within limits, on enzyme action.

Values for the incubation time necessary for each of the 16 samples to reach 90% sucrose utilized are given in Table III. It is obvious from inspection of this table that temperature and concentration of carbohydrate and enzyme all influence the rate of reaction.

TABLE III

TIME REQUIRED IN HOURS FOR 90% SUCROSE TO BE UTILIZED

Maltose:sucrose	Enzyme units/ml. incubation mixture: 5 units, Maltose, 5%	5 units, Maltose, 20%	20 units, Maltose, 5%	20 units, Maltose, 20%
	Temperature, 0°			
10:1	19	42	6	17
10:4	25	67	6.5	18.5
	Temperature, 25°			
10:4	5.5	17	3.5	5
10:1	4	17	2	4.5

We have chosen the following conditions: 5 units of enzyme/ml., 20% maltose concentration, 10:1 maltose:sucrose ratio, and a temperature of 25°. These conditions were chosen on the basis of practicality and convenience for our own purposes.

Acknowledgments.—The authors wish to express their gratitude to Dr. H. M. Tsuchiya for his suggestion of the possibility of using dextransucrase for panose synthesis and for a generous supply of NRRL B-512 culture filtrate. They also express their appreciation to H. F. Zobel for the X-ray analysis.

PEORIA, ILLINOIS

(22) F ratios are obtained by dividing each factor and interaction mean squares by the error mean square (mean square = sum of squares/degrees of freedom). Critical values of F are from 0.15 to 6.6 for confidence limits of 90%. Any factor or interaction falling within this range has little or no effect on the formation of panose. The interactions between three and four factors are of no significance and are included in the error.

None of the experimental effects are estimated with very great precision, but the analysis indicates which variables and interactions are most important and should be selected for further study.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Action of Alkali on D-Fructose[1]

BY M. L. WOLFROM AND J. N. SCHUMACHER

RECEIVED SEPTEMBER 3, 1954

In an attempt to interpret in part the nature of the alkaline defecation process in cane sugar-house work, an aqueous solution of D-fructose was heated for 24 hr. at *p*H 8 (initial) in the presence of aconitate ion. Chromatography on clay of the deionized and fermented reaction mixture led to the isolation of D-glucuronic acid, allitol and galactitol (these two after reduction with hydrogen and nickel at low pressure and room temperature) and of (DL + D)-sorbose, identified in part by reduction and isolation of DL-glucitol, L-glucitol, DL-iditol and L-iditol (the last three as their hexaacetates). Subsequent isolative cellulose sheet chromatography led to the separation of (DL + D?)-allose and of DL-*ribo*-hexose phenylosazone of indicative DL-psicose origin. While the origin of the D-glucuronic acid is obscure, it is considered that the others probably arise by the reverse aldolization of D-fructose to trioses followed by their recombination by aldolization.

In continuation of our studies on the composition of cane final molasses and the chemical reactions leading to its formation, it was established through model systems that a potent color-forming system, present under simulated mill conditions, is that of D-fructose and alkali.[2] In the cane sugar mill defecation process, the reducing sugars present are exposed to the action of hot alkali in the presence of aconitate ion. It was the objective of the present work to investigate further this isolated D-fructose–aconitate alkaline system to obtain some indication of its nature through the isolation, on a crystalline basis, of some of the components of this complex reaction mixture. Accordingly, following simulated mill conditions,[3] a slightly alkaline (*p*H 8.00, 3.05 equiv. of potassium hydroxide) aqueous

(1) A preliminary account of this work has been published in *Science*, **119**, 587 (1954), and in *El Crisol*, **6**, 67 (1952).

(2) M. L. Wolfrom, W. W. Binkley and J. N. Schumacher, *Ind. Eng. Chem.*, in press.

(3) W. W. Binkley and M. L. Wolfrom, *Advances in Carbohydrate Chem.*, **8**, 291 (1953).