THE ACTION OF ALKALI ON D-FRUCTOSE

AND

THE FRACTIONATION OF FLORIDA BLACKSTRAP MOLASSES

DISSERTATION

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I. INTRODUCTION AND STATEMENT OF THE PROBLEM

In the milling of cane juice, the reducing sugars present, D-glucose and D-fructose can undergo a Lobry de Bruyn and Alberda van Ekenstein transformation. In addition, these sugars, as well as the sucrose that is present, undergo color formation with the amino and organic acids present in the cane juice with ultimate formation of sugar high polymers. D-Fructose has been found to produce the most color with these acids.

The purpose of this work was to find what simple products are produced when D-fructose is treated with aconitic acid (the main organic acid present in cane juice) under simulated conditions. In addition, the purpose of this work was to fractionate further the Florida blackstrap molasses as completely as possible with the hope of isolating and identifying the sugar high polymer precursor that may be present in the molasses.

II. HISTORICAL

A. The Action of Alkali on D-Fructose

One of the most important rearrangements which reducing monosaccharides undergo in alkaline solutions is that described by Lobry de Bruyn and Alberda van Ekenstein (1), in a series of papers published

(1) C.A.Lobry de Bruyn and W.Alberda van Ekenstein, Rec. trav. chim., 14, 203(1895); 15, 92(1896); 16, 257, 262, 282(1897); 18, 147(1899); 19, 1(1900).

in the 1890's. They showed that in mildly alkaline solutions D-glucose was converted into D-mannose, as well as into D-fructose, and these three sugars were readily interconvertible in mildly alkaline solutions. They demonstrated that the almost optically inactive sirup which they obtained by the action on D-glucose of potassium, sodium, or ammonium hydroxides, could be formed equally well by the use of lime, magnesia, sodium carbonate, or potassium carbonate. They thus concluded that it was a matter of the hydroxyl group and not the nature of the special alkaline material used; however, it was believed that the particular base used had a pronounced effect on the product of the reaction. Lobry de Bruyn and Alberda van Ekenstein (1) showed that when D-glucose was treated with lead hydroxide, they obtained D-mannose only, and furthermore, they discovered that D-fructose, in the presence of lead hydroxide, was not converted into D-glucose or D-mannose. Later, Kuzin (2) found that calcium hydroxide at room temperatur

(2) A. Kuzin, Ber., 69, 1041-49(1936.

gave D-mannose but no D-fructose, whereas sodium hydroxide gave D-fructose but practically no D-mannose. Gottfried and Benjamin (3)

(3) J. B. Gottfried and D. G. Benjamin, Ind. Eng. Chem., 44, 141-5(1952).

however, have shown that the percent ketose produced by the action of base on D-glucose solutions is essentially independent of the type of base, but is dependent on the concentration of base. Different bases affect the velocity of the reaction but not the nature or proportion of the products determined, at least within the reaction temperature range used of 70° to 130°.

More extensive rearrangement of the sugars in alkaline solution leads to the formation of the so-called saccharins and saccharinic acids. The term "saccharinic acids" was applied to those acids obtained through the rearrangement of either an aldose or a ketose when these latter compounds were subjected to alkaline conditions, a chemical change which always yielded a deoxy acid, thus showing that an internal oxidation-reduction had taken place. The lactones of these deoxy acids were named "saccharins". In 1839, Peligot (4)

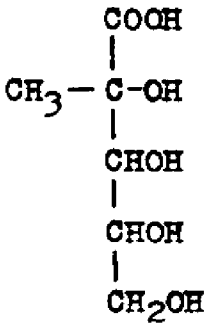
(4) E. Peligot, Ann., 30, 75(1839).

reported the isolation of an acidic substance from among the products of the action of alkalies on D-glucose. However, it remained for Scheibler (5) and Kiliani(6) to show that D-fructose, invert sugar

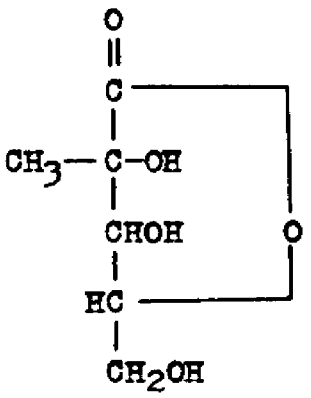
(5) C. Scheibler, Ber., 13, 2212(1880).

(6) H. Kiliani, Ber., 15, 701(1882).

and lactose could be used as well as D-glucose, in the preparation of this acid and its lactone, and as a result of their efforts they formulated these compounds as follows.



Saccharinic acid



Saccharin

Wolfrom and Lewis (7) established donditions for the inter-

(7) M. L. Wolfrom and W. Lee Lewis, J. Am. Chem. Soc., 50, 837-54 (1928).

conversion of D-glucose with the formation of negligible amounts of saccharinic acids. By allowing a molar solution of D-glucose in clear lime water, saturated at 35° to attain polarimetric equilibrium, there was obtained: D-glucose, 63.4 %; D-mannose, 2.4%; D-fructose, 30.9%; and other substances (probably saccharinic acids), 3.3%. In the work of Lobry de Bruyn and Alberda van Ekenstein (1) saccharinic acid was formed to the extent of 20-30% of the sugar used.

Lobry de Bruyn and Alberda van Ekenstein reported the formation, in addition to D-glucose, D-mannose, and D-fructose, of a sedond ketose and a substance which they called glutose (later believed to be a "3-ketohexose"). An extensive series of experiments

was carried out by Spoehr and Strain (8) on the formation of "glu-

(8) H. H. Spoehr and H.H.Strain, J. Biol. Chem., 85, 365 (1929-30).

tose" from hexoses and invert sugar with lead hydroxide and found great variations in the yields of non-fermentable residues, in their reducing power, and in the aldose content. They also showed that "glutose phenylosazone" was a mixture of phenylhydrazine derivatives. Sattler and Zerban (9), without offering any valid experimental proof,

(9) L. Sattler and F. W. Zerban, Ind. Eng. Chem., 37, 1133 (1945).

considered the glutose mixture as a complex mixture containing fructosans. When a 50% D-glucose solution in 0.2N sodium hydroxide solution was heated for ten hours at 48°, there was produced 21% D-fructose, 3% D-mannose and 2% of a ketose-like substance (10), supposedly the same substance

(10) Tatsuo Takeshima, Repts. Sci. Research Inst. (Japan), 24, 306, 312, 318 (1948). See also S. M. Cantor and K. C. Hobbs, U. S. Patent 2,354,664 (August, 1944).

found by Lobry de Bruyn and Alberda van Ekenstein. This substance increased in amount with higher pH, higher temperatures, and if D-fructose was used in place of D-glucose as the starting material. A second ketose-like material was said to occur if D-mannose was acted upon. Pure D-fructose (9) was partly destroyed by long heating of its solution and after fermentation of the residual D-fructose there remained an unfermentable sirup. Paper chromatography (11)

(11) F. W. Zerban, L. Sattler, Geraldine Rosenthal and A. Glauback, Sugar, 47, No. 2, 33 (1952).

of this sirup showed a spot characteristic of a ketose and since it was believed to move at the same rate as D-psicose, it was considered to be D-psicose. This sirup also was claimed to yield a phenylosazone (12) which was stated to give the same x-ray diffraction data as that

(12) G. L. Clark, J. O. Gardner, L. Sattler and F. W. Zerban, Sugar, 47, No.5, 40(1952).

of the phenylosazone prepared from the alcohol-soluble sirup obtained from distillery slops and presumed(12) to be D-allulosephenylhydrasone. Schneider and Erlemann(13) heated D-fructose or D-glucose solutions

(13) F. Schneider and G. A. Erlemann, Zucker-Beihefte No.3, 40-43(1951). Chem. Abstracts, 46, 381e(1952). Int. Sug. J., 55, 80(1953).

at pH 7.5 and 90° and three outstanding spots were obtained when the products were chromatographed on paper. These were identified as the original sugar, the other of the two (and possibly some mannose), and a third as yet incompletely characterized product, but believed to be psicose. The same results were also obtained when D-fructose was heated with lead hydroxide. Using the procedure of Sattler and Zerban, (10), DeWhalley, Alban, and Gross (14) heated D-fructose solutions

(14) H. DeWhalley, N. Alban, and D. Gross, Analyst, 76, 287(1951). Int. Sugar J., 54, 11(1952).

and removed the excess D-fructose by fermentation. Chromatography of the residue showed many spots similar to those found in raw cane juice. Chemical fractionation of the residue, followed by chromatographic treatment of the separate fractions, showed that some of the apparently

simple fractions were in reality very complex. On the chromatogram there was found (a) three dianhydrides (diheterolevulosans) of fructose, (b) a compound believed to be D-allulose (D-psicose), and (c) a "monomeric anhydride of fructose" (no evidence for this assumption was cited). Hough, Jones and Richards (15) treated a D-glucose solution with

(15) L. Hough, J.K.N.Jones and E.L.Richards, J. Chem. Soc., 2005 (1953).

ammonia for two days at 37° and after working up the product, the resulting sirup was fractionated on a cellulose column. One fraction yielded a small amount of sirup, $[\alpha]_D$ +2.0°, which showed the same color reactions on paper as a ketohexose and moved at the same rate as psicose. Other investigators (16, 17, 18) indicated the presence

(16) W. Coltof, Biochem. Z., 243, 191 (1931).

(17) L. Sattler and F. W. Zerban, Sugar, 39, No. 12, (1944).

(18) G. L. Clark, Hung Kao, L. Sattler and F. W. Zerban, Ind. Eng. Chem., 41, 530 (1949).

of glycerol, methylglyoxal, acetol and acetoin and a product yielding D-glucose phenylosazone in the unfermentable residue from the action of alkali on D-glucose.

Rebenfeld and Pacsu (19) found that when D-glucose or D-fructose

(19) L. Rebenfeld and E. Pacsu, J. Am. Chem. Soc., 75, 4370 (1953).

solutions were allowed to stand in contact with a strongly basic anion exchange resin, Amberlite IRA-400 (OH form), D-glucose was slowly converted to fructose and D-fructose slowly converted to glucose. When equilibrium was attained (212 hr.), 30% of the D-glucose was

converted to fructose and 55% of the D-fructose was converted to glucose.

Heidt and Colman (20) found that D-glucose, D-fructose and

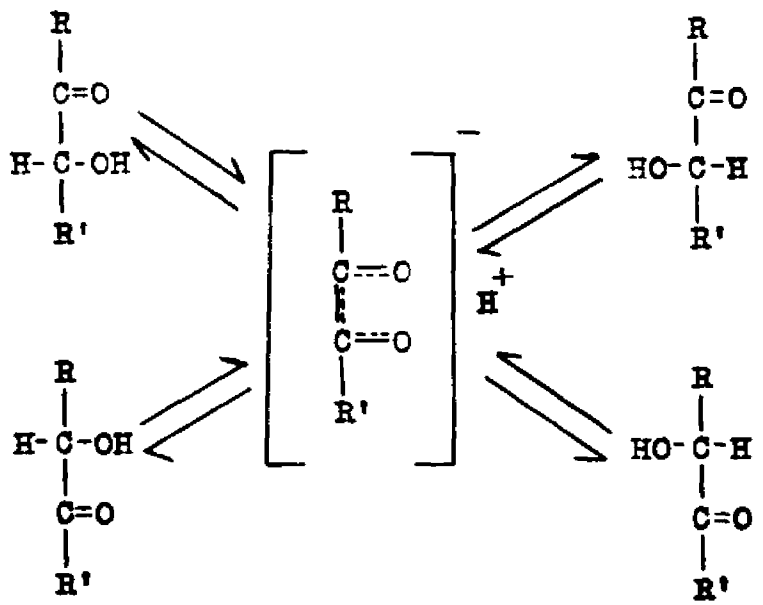
(20) L. J. Heidt and Catherine M. Colman, J. Am. Chem. Soc., 74, 4711 (1952).

invert sugar were degraded into non-reducing substances (70%) when heated (.0014, .0042, and .0126 mole/liter respectively) with sodium carbonate or sodium bicarbonate (0.4 moles/liter) at 100° for ten minutes. It was also shown that the half-life for the degrading of D-fructose into non-reducing material was six minutes at 100° at pH 10 and 28 minutes at pH 9.3.

The basic concept of the reaction mechanism of the Lobry de Bruyn and Alberda van Ekenstein isomerization reactions postulates enediol(21)

(21) J. U. Nef, Ann., 357, 294-312 (1907); 376, 1-119 (1910); 403, 204-383 (1913).

intermediates as illustrated in the scheme:



(R= H, CH_2OH, etc.; R'= H, CH_2OH, etc.)

This concept was confirmed by Wolfrom and Lewis (7) and is now the generally accepted mechanism. The isolation of D-glucose from heated D-fructose solutions (22) indicates that the enolization may be either

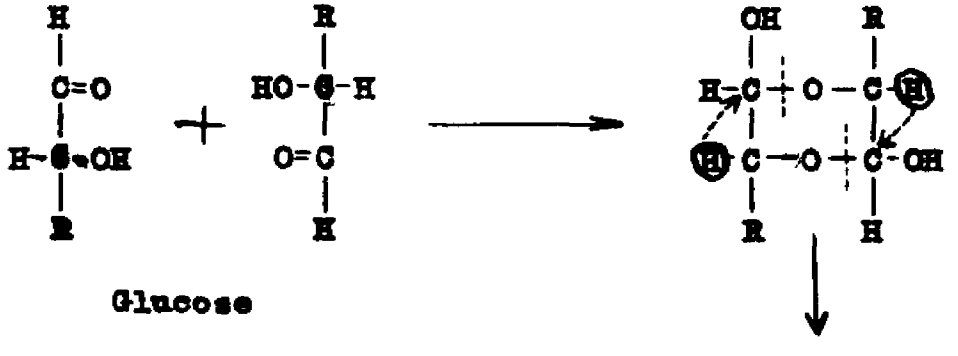
(22) M. L. Wolfrom and W. L. Shilling, J. Am. Chem. Soc., 73, 3557 (1951).

base or acid catalyzed. Presumably a high pH favors enolization while a low pH favors anhydride formation.

Doubt was cast upon the assumption of an intermediary enediol when Fredenhagen and Bonhoeffer (23) reported experiments in which

(23) H. Fredenhagen and K. F. Bonhoeffer, Z. physik. chem., A 181, 392 (1938).

D-glucose was kept in a medium of deuterium oxide (heavy water). The repeated formation of enediols would under these circumstances be expected to result in the eventual equilibration of the hydrogen atoms bound directly to carbon atoms 1 and 2 with the deuterium of the aqueous environment. No significant incorporation of deuterium could be detected at 25°, while at 35° non-reproducible results were obtained. On this basis, Fredenhagen and Bonhoeffer rejected the enediol intermediate and to explain the reaction they proposed the following mechanism:



Glucose

Fructose

$R = -(CHOH)_3-CH_2OH$

In 1942, Goto (24) also rejected the enediol intermediate when finding

(24) K. Goto, J. Chem. Soc. Japan, 63, 217 (1942).

an 85% conversion of D-glucose to D-fructose in alkaline D_2O without introduction of carbon bound deuterium into the product.

In an effort to find evidence for a mechanism that would explain this reported carbohydrate isomerization in heavy water without exchange of carbon-bound hydrogen by deuterium, Bothner-By and Gibbs (25)

(25) A. A. Bothner-By and M. Gibbs, J. Am. Chem. Soc., 72, 4805 (1950).

employed 1-C^{14}-D-glucose to explore the possibility of rearrangement of the carbon skeleton during the isomerization by alkali and found that such rearrangement does not occur. Topper and Stetten (26)

(26) Y. J. Topper and DeWitt Stetten, Jr., J. Biol. Chem., 189, 191-202.

have reported that in contradiction to the observation of Fredenhagen and Bonhoeffer, and Goto, carbon deuterium is, indeed, incorporated during the alkaline isomerization of D-glucose in heavy water. An expansion of the classical enediol mechanism to include cis- and trans-

enediols with individual functions also was proposed by Topper and Stetten to explain their experimental observations. Thus they pictured the transformation of D-glucose to D-mannose as occurring via the following steps: D-glucose $\rightleftharpoons$ trans-enediol $\rightleftharpoons$ D-fructose $\rightleftharpoons$ cis-enediol $\rightleftharpoons$ D-mannose. Sowden and Schaffer (27) also obtained evidence

(27) J.C.Sowden and R. Schaffer, J. Am. Chem. Soc., 74, 499, 505 (1952).

that carbon-bound deuterium is incorporated into the sugar products during the isomerization of D-glucose in heavy water. Their results are in agreement with the classical mechanism for the alkaline isomerization of reducing sugars. By comparison of the extents of deuterium incorporation into the D-fructose and D-mannose, and by examination of the distribution of deuterium on the D-fructose carbon chain, they concluded that D-fructose is not necessarily an intermediate in the conversion of D-glucose to D-mannose by aqueous alkali. This finding disagrees with that of Topper and Stetten (26).

The preparation (28, 29) of D-fructose, D-glucose, and sucrose

(28) S. Udenfriend and M. Gibbs, Science, 110, 708 (1949).

(29) M. Wolochow, F. W. Putnam, M. Daudoroff, W. F. Hassid and H. A. Barker, J. Biol. Chem., 180, 1237 (1949).

containing radioactive C^{14} promises new developments in the chemistry and biology of these compounds as well as in the mechanism of their reactions in aqueous alkali.

B. Modern Methods of Separation

The similarity of the sugars in physical and chemical properties has made it difficult to separate them when mixed, and the lack of suitable methods for the separation of sugars, as well as their determination, on a micro scale has delayed the progress of many investigations in complex sugar reactions as well as in plant and animal physiology. In 1939, Reich (30) made a new approach to the problem

(30) W. S. Reich, Biochem. J., 33, 1000 (1939).

of the separation of the sugars. He converted a mixture of D-glucose and D-fructose into the corresponding colored p-phenylazobenzoyl derivatives and showed that their separation could be observed visually by chromatography on a column of silica or alumina. Since the description of these experiments, many workers have utilized the principle of adsorption chromatography for the separation of the sugars and related substances. This method of separation was utilized by Freudenberg and Boppel (31) and other workers (32, 33) to separate the

(31) K. Freudenberg and H. Boppel, Ber., 73, 609 (1940).

(32) J. K. Mertzweiler, D. M. Carney and F. F. Farley, J. Am. Chem. Soc., 65, 2367 (1943).

(33) G. H. Coleman, A. G. Farnham and A. Miller, J. Am. Chem. Soc., 64, 1501 (1942).

p-phenylazobenzoyl derivatives of the fully and partially methylated sugars on alumina.

McNeely, Binkley and Wolfrom,(34) have used Magnesol, a commercial

(34) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, J. Am. Chem. Soc., 67, 527 (1945).

hydrated magnesium acid silicate, as an adsorbent for the separation of mixtures of the sugar acetates. Since these substances are colorless it is necessary to extrude the column of Magnesol and locate the position of the sugar derivatives by streaking the side of the column with permanganate solution. Lew, Wolfrom and Goepp (35)

(35) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., J. Am. Chem. Soc., 67, 1865 (1945).

applied this procedure to the chromatography of unsubstituted sugars and related compounds on Florex XXX (a fuller's earth type clay). The method has been utilized to separate the components of cane juice and cane final molasses (36), diheterolevulosans (37) which were ob-

(36) W. W. Binkley and M. L. Wolfrom, J. Am. Chem. Soc., 72, 4778 (1950).

(37) M. L. Wolfrom and Mary Grace Blair, J. Am. Chem. Soc., 70, 2406 (1948).

tained by refluxing concentrated aqueous solutions of D-fructose, and many other sugar mixtures. Using hydrated calcium acid silicate and a similar technique, Georges, Bower and Wolfrom (38) were able to sep-

(38) L. W. Georges, R. S. Bower and M. L. Wolfrom, J. Am. Chem. Soc., 68, 2169 (1946).

arate mixtures of sugar acetates and of methylated sugars.

The chromatographic separation of starch into its components, amyl-

ose and amylopectin, has been achieved by Tanret (38a) and Pacsu

(38a) C. Tanret, Compt. rend., 158, 1353 (1914).

and Miller (39) while Ashford, Evans, and Hibbert (40) have shown

(39) E. Pacsu and J. W. Miller, J. Am. Chem. Soc., 62, 1168 (1941).

(40) W. R. Ashford, T. H. Evans and H. Hibbert, Can. J. Res., B. 24, 246 (1946).

that polysaccharides can be separated on adsorbents such as magnesium oxide and calcium carbonate. Wolfrom and coworkers (41) separated

(41) E. E. Dickey and M. L. Wolfrom, J. Am. Chem. Soc., 71, 825 (1949); M. L. Wolfrom and J. C. Dacons, ibid, 74, 5331, (1952).

the products of acetolysis of cellulose by chromatography on Silene E. F. and Magnesol, and characterized the oligosaccharides so obtained through the alloheptaose member.

Tiselius (42) separted carbohydrate mixtures by passing aqueous

(42) A. Tiselius, Advances in Colloid Science, 1, 81 (1942).

solutions up through a carbon column and measuring the refractive indices of the various zones as they passed from the column. It was not possible to separate compounds of the same molecular weight, but the procedure was used mainly as a means of separating mono- from di- and the latter from trisaccharides. This procedure of carbon chromatography was used (43) to isolate isomaltose and gentiobiose from the acid hydrolys

(43) R.L.Whistler and D. F. Durso, J.Am.Chem.Soc., 72, 677(1950); M. L. Wolfrom, A. Thompson, A.N.O'Neill and T.T. Galkowski, J. Am. Chem. Soc., 74, 1062 (1952).

of amylopectin and from each of the reversion reactions (using same acid concentration, time and temperature of heating, but subsituting varying initial concentrations of D-glucose for amylopectin). This was accomplished by removing the D-glucose from the polymeric sugars by means of a carbon column followed by acetylation and chromatographic separation of the acetate mixture on a magnesol-celite column. Later, Thompson, Wolfrom and Quinn (43a) employing the technique, established

(43a) A. Thompson, M. L. Wolfrom and E. J. Quinn, J. Am. Chem. Soc., 75, 3003 (1953).

that the 6-α-D-glucopyranosyl linkage is preformed in the amylpectin molecule. The aicd hydrolysis product of amylopectin and the product obtained on the reversion reaction of D-glucose was chromatographed on a carbon column which separated the D-glucose from the disaccharides. Acetylation of the disaccharides and chromatographic separation of the acetate mixture on a magnesol-celite column gave a large quantity of β-isomaltose octaacetate in the case of amylopectin but only a negligible amount in the case of the reversion reaction of D-glucose. This indicates that the 6-α-D-glucopyranosyl linkage is actually preformed in the starch molecule and is only in a negligible part a product of the reversion under the hydrolytic conditions employed.

With the introduction of partition chromatography, a further method for the separation of the sugars became available. Bell (44), who was

(44) D. J. Bell, J. Chem. Soc., 473 (1944).

the first to apply this new procedure, showed that 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose could be separated

from one another and from 2,3-di-*O*-methyl-D-glucose by partition chromatography on a column of silica gel with water as the stationary phase, using first chloroform and then chloroform/1-butanol as the mobile phase. A further advance came with the application of paper partition chromatography to the analysis of complex mixtures of simple sugars. This method enables detailed studies of the composition of complex polysaccharides, using only minute amounts of material. Originally, this method of paper partition chromatography was developed by Consden, Gorden and Martin (45) for the hydrolysis of protein and

(45) R. Consden, A. H. Gorden and A. J. P. Martin, Biochem. J., 38, 224 (1944).

peptide hydrolysates, but in 1946, it was applied by Partridge (46) to

(46) S. M. Partridge, Biochem. J., 42, 238 (1948).

the separation of the sugars. It was shown that small quantities (Ca. 10 mg.) of sugar mixtures could be separated, and their components identified by virtue of the difference in partition of the sugars between water held by the cellulose fibers of the paper(the stationary phase) and a solvent such as butanol saturated with water (the mobile phase. The procedure consists in placing a spot of a sugar solution near the top of a sheet or strip of filter paper, the top of which is immersed in a suitable solvent mixture contained in a trough. The trough and paper are then placed in a container so that the paper hangs vertically from the trough and the atmosphere which surrounds it is saturated with the vaper of the solvent. The solvent is then allowed to advance down the paper for a desired peried of time, when the paper

is removed, dried and the sugars located by a spray reagent. The sugars are identified by the rate at which they move, under standard conditions, down the face of the paper. In this way Partridge was able to detect glucose, fructose and sucrose in apple juice; fructose, glucose and inositol in the foetal blood of sheep; and glucose only in the ethanolic-extract of fresh egg white.

Since the development by Partridge of the separation of sugars by means of paper partition chromatography, many investigators have used this method for the isolation and identification of sugar mixtures. Better solvents and spray reagents have been developed. Jermyn and Isherwood (47) claimed that improved separation for any pair of

(47) M. A. Jermyn and F. A. Isherwood, Biochem. J., 44, 402 (1949).

sugars could be obtained with solvents of three-component systems such as ethyl acetate/acetic acid/water and ethyl acetate/pyridine/water. Hough, Jones and Wadman (48) claimed that hydrolysis of

(48) L. Hough, J. K. N. Jones and W. H. Wadman, J. Chem. Soc., 1702 (1950).

ethyl acetate occurs, making reproducible results difficult. They recommend instead, for the rapid and efficient separation of sugars and their methylated derivatives, miscible solvents such as butanol/ethanol/water, butanol/pyridine/water and butanol/glacial acetic acid/water. The solvent system has been even extended to four components by Albon and Gross (49) using a 5/3/3/1 mixture of 1-butanol/pyridine/water/

(49) N. Albon and D. Gross, Analyst, 75, 454 (1950).

benzene for separating raffinose from raw beet sugars. In addition to butanol/pyridine/water solvents, Jeanes, Wise and Dimler (50) used

(50) Allene Jeanes, C. S. Wise, and R. J. Dimler, Anal. Chem., 23. 415 (1951).

fusel oil(b.p. 121-29)/pyridine/water mixture, and found that it was possible to dry off a chromatogram and then continue development with the same solvent again in the same direction, thus making it possible to follow the separation of a mixture of substances. Hough, Jones and Wadman (48) have improved the water-saturated 1-butanol separations by raising the working temperature within the chromatographic chamber to 37°. The separation of sugars and methylated sugars is quicker and the spots are more compact. Counsell, Hough and Wadman (51) reported in

(51) J. N. Counsell, L. Hough, and W. H. Wadman, Research (London), 4, 143-44 (1951).

addition to quicker and more precise separations at elevated temperatures, the advantage of handling higher concentrations of materials.

Partridge (46), in his original work, detected the reducing sugars by spraying the paper with ammoniacal silver nitrate solution, which gave dark brown spots. The non-reducing ketoses were detected by spraying with trichloroacetic acid to produce furfural which in turn reacted with a phenol such as naphthoresorcinol, giving colored spots. Hough, Jones and Wadman (48) investigated thoroughly the use of many spraying reagents, including the phosphate, phthalate and the trichloroacetic salts of aniline for detection and identification, by characteristic colors, of many different types of sugars. They also list colors

given by the various groups of sugars with the following spray reagents: p-anisidine hydrochloride in butanol, diphenylamine trichloroacetate, dimethylaniline, α-naphthylamine trichloroacetate, orcinol or resorcinol in 1-butanol with a little hydrochloric acid, and urea hydrochloride or anthraquinone with hydrochloric acid. By making use of the fact that 2,3,4-tri-*O*-methyl-D-xylose gives a red-brown and 2,3,5-tri-*O*-methyl-L-arabinose a bluish black color, with the p-aminodimethylaniline, Bozys, Cuendet, Ehrenthal, Koch and Smith (52) showed that xylans of wheat straw and corn cobs contain

(52) L. A. Bozys, L. S. Cuendet, I. Ehrenthal, R. Koch, and F. Smith, Nature, 166, 520 (1950).

terminal xylose units as well as terminal arabinose units. Buchanan, Dekker and Long (53) made use of the oxidation of the sugar with sodium

(53) J. G. Buchanan, C. A. Dekker and A. G. Long, J. Chem. Soc., 3162 (1950).

periodate followed either by Schiff's reagent for detecting the aldehyde produced or by potassium iodite for detecting the formic acid produced (iodine being liberated). An aqueous solution of potassium permanganate (1%) containing 2% sodium carbonate was used by Pacsu, Mora and Kent (54).

(54) E. Pacsu, T. P. Mora and P. W. Kent, Science, 110, 446 (1949).

Bright yellow bands were obtained on a purple background, gradually turning to grey bands on a brown background. This technique indicated all the sugars and glycosides examined except the methylated derivatives.

In addition to the wide use of color reagents for the identification

of sugars as described above, and in some in conjunction with their ultraviolet fluorescence, the R_F (55)values are used by many workers

(55) $R_F = \frac{\text{distance from starting line to center of spot}}{\text{distance solvent front has moved}}$

as an aid to identification. Comparison of the color and R_F values of unknown sugars with known standards run alongside reinforces the identification. Instead of the solvent front, use has been made of fast moving sugars for the measurement of R values. For example, Brown and co-workers (56) used tetra-*O*-methyl-D-glucose for measuring the

(56) F. Brown, E. L. Hirst, L. Hough, J. K. N. Jones and W. H. Wadman, Nature, 161, 720 (1948).

R_G (57) values of methylated sugars, and Forsyth (58) used xylose for

(57) $R_G = \frac{\text{distance from starting line to center of spot}}{\text{distance tetramethyl D-glucose has moved.}}$

(58) W. G. C. Forsyth, Biochem. J., 46, 141 (1950).

measuring the R_X values of the sugars in hydrolysates of soluble soil polysaccharides.

The separation of sugar mixtures was further advance by Flood, Hirst and Jones (59) who standardized the procedure of eluting the

(59) A. E. Flood, E. L. Hirst and J. K. N. Jones, J. Chem. Soc., 1679 (1948).

sugars from the paper sheets. The sugar mixture was placed on a series of spots on the starting line and the chromatogram developed. The sheet was then dried and a strip (containing one of the spots) was

cut off and the position of the sugars on the strip indicated by a spray reagent. The strip was then placed alongside the remaining sheet and transverse strips were cut out as indicated by the spots on the sprayed strips. The sugar was then removed from the transverse strips by extraction with water, and the amount of sugar determined by use of Smogyi's copper reagent. The use of this technique has permitted the isolation and identification of sugars present in plants and also the study of some of the problems of the sugar industry. Using this procedure, Hirst, Hough and Jones (60) obtained various

(60) E. L. Hirst, L. Hough and J. K. N. Jones, J. Chem. Soc., 928 (1949).

methylated derivatives of glucose from the hydrolysis products of methylated maize starch, waxy-maize starch, glycogen and araban. By using large sheets (47 x 57 cm.), applying the sugar mixture in a continuous streak across the sheet (a mechanical device was used to insure uniform distribution), and developing the sheets in a chromatocab, Lemieux and Bauer (61) obtained the mono-, di- and tri-*O*-methyl-D-

(61) R. U. Lemieux and H. Bauer, Can. J. Chem., 31, 814 (1953).

glucose from the hydrolysis of tri-*O*-methylcellulose. By re-chromatography of the mono-*O*-methyl-D-glucose, it was found to be a mixture of the 2-, 3-, and 6-*O*-methyl-D-glucose and the di-*O*-methyl-D-glucose was found to be a mixture of 2,6- and 2,3-*O*-dimethyl-D-glucose. In a similar manner, Pazur and Gordon (62) obtained pure inulobiose on the

(62) J. H. Pazur and A. L. Gordon, J. Am. Chem. Soc., 75, 3458 (1953).

partial hydrolysis of inulin. Gilles, Meredith and Smith(63) also

(63) K. A. Gilles, W. V. S. Meredith and F. Smith, Cereal Chem., 29, 314 (1952).

isolated D-glucose, D-xylose and L-arabinose from the acid hydrolysate of the water-soluble polysaccharides obtained by extracting barley flour with water. The determination of raffinose in raw beet sugars was accomplished by Albon and Gross (64) by spotting the raw beet sugar

(64) N. Albon and D. Gross, Analyst, 75, 454 (1950).

on filter paper strips along with a spot of pure raffinose at each end. After development, the raffinose spots at each end were cut off, the position of the raffinose detected, and then replaced in their original positions on the remainder of the chromatogram paper. A transverse strip enclosing the possible raffinose was separated and extracted with water. On solvent removal, crystals characteristic of raffinose were isolated.

In order to separate larger amounts of sugars than is possible by paper chromatography, Hough, Jones and Wadman (65) introduced the

(65) L. Hough, J. K. N. Jones and W. H. Wadman, Nature, 162, 448 (1948).

cellulose column. The cellulose columns were packed with a powder obtained by rubbing ashless tablet clippings through a sieve. The solvent mixture usually employed was 1-butanol saturated with water and containing 1 per cent ammonia. (The use of ammonia is not without its dangers since ammonia reacts with sugars). The effluent from the

column was collected, and drops of eluate from each fraction were spotted across the top of a sheet of paper in order and developed in the usual manner. Thus the degree of separation could be determined and fractions containing the pure sugars could be selected. A mixture of L-rhamnose, D-ribose, L-arabinose and D-galactose was separated by this method in a yield of approximately 95%, which was sufficient for the determination of physical constants and the preparation of derivatives. To obtain reproducible results, Hough, Jones and Wadman (66)

(66) L. Hough, J. K. N. Jones and W. H. Wadman, J. Chem. Soc., 2511(1949).

found that the column needs to be packed evenly and very tightly, and described a method of testing the suitability of the cellulose by using a mixture of dyes. Employing this procedure, Hirst, Hough and Jones, (67) separated the sugar mixture obtained on the partial hy-

(67) E. L. Hirst, L. Hough and J. K. N. Jones, Nature, 163, 177 (1949).

drolysis of the gum exudate of the tropical tree Sterculia setigera into its components. Their results are tabulated in Table I.

Hough, Jones and Hirst (68) obtained D-galactose, L-rhamnose

(68) L. Hough, J. K. N. Jones and E. L. Hirst, Nature, 165, 34-5 (1950).

and 3-*O*-methyl-D-galactose by partition chromatography on powdered cellulose columns, of the product obtained on acid hydrolysis of slippery elm mucilage. In the same manner, Easterby and Jones (69)

(69) D. G. Easterby and J. K. N. Jones, Nature, 165, 614 (1950).

TABLE I

Cellulose Column Chromatography of the Hydrolysis Product of the Gum Exudate of the Tropical Tree *Sterculia Setigera*.

Fraction	Wgt. in mgm.	$[\alpha]_D \cdot H_2O$	R_G values of sugars present	Sugars present
I	15	12°	0.385	Ketose
II	413	9.4°	0.30	L-Rhamnose
III	145	8.0°	0.17; 0.13	Aldose D-Tagatose
IV	373	34°	0.17; 0.13 0.07	Aldose, D-Tagatose & D-Galactose
V	640	80.5°	0.07	D-Galactose

separated the components of a sugar mixture obtained by the acid hydrolysis of linseed mucilage. Table II gives a summary of their results. In the presence of an enzyme preparation from peas, DL-lactaldehyde combines with triose phosphate, to give optically active methylpentose sugars. By cellulose powder chromatography Hough and Jones (70) separated two deoxy sugars, namely 6-deoxy-

(70) L. Hough and J. K. N. Jones, J. Chem. Soc, 4047 (1952).

D-fructose and 6-deoxy-L-sorbose.

A more recent method developed for the separation of sugars and sugar derivatives is ionophoresis. The migration of substances on the surface of paper under the influence of applied electrical potentials has been observed for many years, but Haugaard and Kroner (71)

(71) G. Haugaard and T. D. Kroner, J. Am. Chem. Soc., 70, 2135 (1948).

first reported the use of ionophoresis in conjunction with partition chromatography as a method for separating amino acids. Jaenicke (72)

(72) L. Jaenicke, Naturwissenschaften, 39, 86 (1952).

only recently applied it to carbohydrates. The migration of certain neutral sugar derivatives, in ionophoresis, occurs at an alkaline pH in the presence of borate ions. Sugar molecules, containing hydroxyl groups suitably situated can form complexes with the borate ions. Differences in properties of borate complexes are utilized in the separation of sugars and sugar derivatives on chromatograms. A series of ionophoreses in borate buffers was carried out by Consden

TABLE II

Cellulose Column Chromatography of the Sugar Components Obtained from the Acid Hydrolysis of Linseed Mucilage

Fraction	Wgt. in mgm.	$[\alpha]_D$, H_2O	R_G values of sugars present	Sugars present
I	68	+ 8	0.30	L-Rhamnose
II	21	-	0.21 0.17	Methylpentose D-xylose
III	368	+ 25	0.17	D-xylose
IV	119	+ 98	0.135	L-Arabinose
V	50	-	0.135 0.075	L-Arabinose L-Galactose
VI	101	- 70	0.075	L-Galactose

and Stanier (73) on D-fructose, L-sorbose, D-glucose, D-galactose,

(73) R. Consden and W. M. Stanier, Nature, 169, 783 (1952).

D-mannose, L-arabinose, D-ribose, L-rhamnose, raffinose and cellobiose. They found that the sugar migrated towards the anode.

As an index of migration of the borate ion-carbohydrate complexes in filter paper ionophoresis, the term M_G has been suggested by Foster (74) where for any substance

$$M_G = \frac{\text{True distance of migration of the substance}}{\text{True distance of migration of D-glucose.}}$$

(74) A. B. Foster, Chem. and Ind., 828 (1952).

The true distances of migration are those corrected for movement due to electroendosmotic flow by reference to the movement of 2,3,4,6-tetra-O-methyl-D-glucose, which does not form a complex with borate ions. The ionophoretic behavior of a range of sugar derivatives in borate buffers has been reported by Foster and Stacey (75), who point out

(75) A. B. Foster and M. Stacey, J. Appl. Chem., 3, 19 (1953).

the advantages of ionophoresis. One advantage is that certain sugars or their derivatives which cannot be resolved by partition chromatography, can be readily separated by ionophoresis. Secondly, separation may be carried out in as short a time as one hour.

Woodin (76) applied ionophoresis to the hydrolyzate of the

(76) A. M. Woodin, Biochem. J., 51, 319 (1952).

mucopolysaccharide of cornea and identified the following sugars: galactose, galactosamine, and glucosamine. Foster (77) extended

(77) A. B. Foster, J. Chem. Soc., 205, 982 (1953).

the application of innophoresis to certain disaccharides, and moreover investigated the mode of interaction of borate ions with carbohydrates in the alkaline media (pH 10-12) used.

C. Cane Sugar House Work

1. Introduction

The production of sucrose from sugar cane is revealed in the earliest record of modern civilization (78, 79). Avequin (80)

(78) Noel Deer, History of Sugar, Chapman and Hall, London, 1949, Vol. I, p. 12.

(79) E. O. von Lippmann, Geschichte des Zuckers, J. Springer, Berlin, 2nd ed., 1929.

(80) J. B. Avequin, J. chim. med. pharmacie toxicologie, 2 1, 132 (1836).

introduced the first application of the scientific method to the process. The isolation of sucrose from the sugar cane has been a problem to the sugar industry for a long time. By studying the composition of cane juice and molasses, the production of sucrose from the sugar cane has been greatly improved. A detailed discussion of the composition of cane juice and cane final molasses has been presented by Binkley and Wolfrom (81).

(81) W. W. Binkley and M. L. Wolfrom, Advances in Carbohydrates, 8, 291 (1953).

The present discussion will be concerned only with those phases of the subject which seem most pertinent to the immediate problem.

2. Composition of Cane Juice

Cane juice is an aqueous solution circulating in the living plant and carrying materials required for growth and metabolism. It is extremely complex and distinguishes itself from the other plant juices by its characteristically high content of sucrose.

Binkley and Wolfrom (82) found that cane juice contained 0.3-

(82) W. W. Binkley and M. L. Wolfrom, J. Am. Chem.Soc., 68, 1720 (1946).

3.0% reducing sugars. The acetylation of "lyophilized" normal cane juice solids followed by chromatography on a magnesium silicate of these acetates led to the isolation and proper identification of these sugars as D-glucose and D-fructose. The analysis (83) for

(83) C. A. Browne and F. W. Zerban, Physical and Chemical Methods of Sugar Analysis, John Wiley and Sons, Inc., New York, 3rd ed., 1941.

sucrose, D-glucose and D-fructose in solution is extensively employed in industry. It depends upon the fact that sucrose is a non-reducing disaccharide of rather high optical rotation, $[\alpha]_D + 66^\circ$, and that on acid hydrolysis to its reducing sugar components, the mixture is levorotatory because of the high equilibrium levorotation, $[\alpha]_D -92^\circ$, displayed by D-fructose over that of D-glucose, $[\alpha]_D + 52.5^\circ$. The fact that D-fructose is highly tautomeric and changes its equilibrium rotation markedly with temperature is also employed in analysis.

Other carbohydrates in cane juice are the soluble polysaccharides vaguely classified under the terms "hemicelluloses, soluble gums and pectins". Cane juice is a rich source of the cyclic alcohol myo-inositol. It was isolated from cane juice by chromatography on fuller's earth clay (84). Uronic acids have been detected in cane juice (

(84) W. W. Binkley, Mary Grace Blair and M. L. Wolfrom, J. Am. Chem. Soc., 67, 1789 (1945).

(85) K. U. Lefevre and B. Tollins, Ber. 40, 4513 (1907); A. D. Dickson, H. Otterson and K. P. Link, J. Am. Chem. Soc., 52 775 (1930).

By decarboxylation on heating with hot mineral acids and determining the amount of carbon dioxide evolved, Browne and Phillips (86) found

(86) C. A. Browne and M. Phillips, Intern. Sugar J., 41, 430 (1939).

that Louisiana cane juice had a uronic acid content of 0.44% of the ash-free solids.

The principal organic acid of cane juice is aconitic acid. Its presence was first detected by Behr (87) in 1877, but its proper

(87) A. Behr, Ber., 10, 351 (1877).

identification was not recorded until 1919 (88). Other acids present

(88) C. S. Taylor, J. Chem. Soc., 115, 886 (1919).

(based only on qualitative tests and analytical methods) are malic, succinic, glycolic, formic and oxalic. The recent isolation from cane juice with ion exchange resins and chromatography on silic acid of

sufficient amounts of these acids, has permitted the adequate identification of fumaric and succinic acids in addition to aconitic acid (89,90). Aconitic acid exhibits geometric isomerism and exists

(89) C. S. Marvel and R. D. Rands, J. Am. Chem. Soc., 72, 2642 (1950).

(90) E. J. Roberts, C. A. Fort and L. F. Martin, Abstracts Papers Am. Chem. Soc., 124, 10D (1953).

in cis and trans forms (91). These are interconvertible in solution,

(91) R. E. Miller and S. M. Cantor, Advances in Carbohydrate Chem., 6, 231 (1951).

temperature and pH being factors (92). The pH of cane juice lies in

(92) J. A. Ambler and E. J. Roberts, J. Org. Chem., 13, 399 (1948).

the range 5.2-6.2 where the equilibrium would favor the cis form (92).

The most abundant amino acid of cane juice is L-asparagine. It was first isolated and identified by Zerban (93), who also iden-

(93) F. W. Zerban, 8th Intern. Congr. Pure Applied Chem., 8, 103 (1912); C. A. 6, 3337 (1912).

tified L(dextro)-glutamine and tyrosine. Application of amino acid paper chromatography to the cations and anions removedfrom cane juice by ion exchange resins indicated the presence also of leucine (or isoleucine), valine, γ-aminobutyric acid, alanine, glycine, serine, glutamic and aspartic acids, and probably lysine and glutamine(94, 95).

(94) G. N. Kowkabany, W. W. Binkley and M. L. Wolfrom, Abstracts Papers 12th Intern. Congr. Pure Applied Chem., 166(1951); Agr. Food Chem., 1, 84 (1953).

(95) O. E. Pratt and L. F. Wiggins, Proc. Brit. West Indies Sugar Technol., 29 (1949).

Spot enhancement with the proper known amino acids was used to support the findings of these chromatograms.

3. Milling of Sugar Cane

The sugar cane is a reed or grass and is heavy and bulky. Massive equipment is needed to handle it. The harvested cane must be moved to the raw sugar mill as quickly as possible to prevent the deterioration of the sugar juices. The cane is first washed to remove soil and stones picked up with the cane. It is then cut into shorter lengths. This cane is crushed in a series of grooved rollers, which exert hundreds of tons of pressure. The juice runs into tanks, and the cane fiber becomes drier and drier as it moves from one set of crushers to the next. When the mass of material reaches the final set of rollers, it is sprayed with hot water to remove the last bit of sugar. The fiber, known as bagasse, which finally emerges, is so dry that it can be taken immediately to the furnace room to be used as fuel.

The thin juice recovered from the crushing constitutes about 80 percent of the weight of the cane but it has only 10 to 15 per cent of the sugar. Calcium hydroxide is added to bring the pH of the juice to 7.5-8.0 and the mixture is heated to 220°F. and maintained around 200° F. for several hours. This is the defecation process and results in a clarification of the liquid with the precipitation of suspended materials, proteins, waxes and fats. After passing

through the settlers the pH is approximately 7. The muds from the settlers are removed with rotary (continuous) filters, and are subsequently discarded or used for fertilizer. The clarified juice is heated to 225°F. in the first of a bank (usually four) of multiple effect vacuum evaporators for a period of ten minutes or less and for longer periods at lower temperatures in subsequent evaporators as the juice is concentrated to a sirup. This sirup is further concentrated in vacuum pans where the sugar is crystallized. During these processes the mother liquors (molasses) are recirculated and fresh defecated juice is added. When the recovery of sucrose is no longer economically feasible, the mother liquor is known as the final or blackstrap molasses. The resulting mixture of crystals and molasses, known as massecuite, flows to crystallizers where, upon cooling, additional sugar crystallizes on grains already formed.

From the crystallizers the mixture is poured into rapidly spinning wire mesh baskets called centrifugals. The motion throws off most of the sirup, leaving crystals behind. The raw sugar, as it empties from the centrifugal baskets, is light brown in color because some molasses still clings to the crystals. The raw sugar is then sent to the refineries to be purified.

The raw sugar entering the refinery is crushed and treated with sirup forming a semi-liquid substance called magma. The magma is washed in centrifugal machines to remove the thin film of molasses which surrounds the raw sugar crystals. The washed crystals are dissolved in warm water, chemically treated, and forced through pressure filters. This treatment removes all non-sugars and impurities,

and the brownish color which still remains is removed by treating with bone-char. Following filtration, the colorless liquid sugar is concentrated and crystallized in vacuum pans. Crystallizing the sugar in these pans requires considerable skill by sugar boilers, because it is important that sugar consist of uniform crystals of desired size. Finally the crystals are removed from the vacuum pans and centrifuged to remove the last traces of sirup from the sugar.

4. Composition of Cane Blackstrap Molasses

The term "blackstrap" originated in the Dutch sugar industry from black "stroop" meaning black sirup (96). The molasses obtained in

(96) Noel Deer, Intern. Sugar J., 47, 123 (1945).

the early stages of sucrose production has a pleasant, palatable flavor and is used in the preparation of edible molasses.

As in cane juice, sucrose is also the principal sugar of cane blackstrap molasses. However the ratio of sucrose to reducing sugars has dropped from 10-15/1 in the juice to 1.5-2.5/1 in the molasses. Patents have been issued for the recovery of sucrose from molasses, but general acceptance of these processes by the industry is still lacking. Some of the methods depend on the removal from molasses of the reducing sugars and other impurities by lime (97), invertase-free yeast (98)

(97) E. E. Battelle, U. S. Patents 1,044,003 (1913), 1,044,004 (1913).
(98) H. DeF. Olivarius, U. S. Patents 1,730,473 (1929), 1,788,628 (1931).

or barium hydroxide (99) and on the removal of the sugar polymers by

(99) A. L. Holven, U. S. Patents 1,878,144 (1933), 1,878,145 (1933).

adsorption on fuller's earth clay (100). Other methods are based

(100) M. L. Wolfrom and W. W. Binkley, U. S. Patent 2,504,169 (1950); J. Am. Chem. Soc., 69, 664 (1947).

upon the use of solvents (101) and of ion exchange resins (102).

(101) J. H. Payne, U. S. Patent 2,501,914 (1950).

(102) N. V. Octrooien Maatschappij, Activit Dutch Patent 58,496 (1946); Chem. Abstracts 41, 4667 (1947).

The significant simple sugars are D-glucose and D-fructose. Binkley and Wolfrom (36), using the chromatographic procedure of Lew, Wolfrom and Goepp (103), isolated D-glucose by the chromatography of Cuban

(103) B. W. Lew, M. L. Wolfrom and R. M. Goepp Jr., J. Am. Chem. Soc., 68, 1449 (1946).

blackstrap molasses on fuller's earth clay. D-Fructose was obtained by McNeely, Binkley and Wolfrom (34) as a sirup which yielded crystalline acetates of the β-D-pyranose and ketc- forms of this sugar after acetylation and chromatography on magnesium acid silicate. Table III gives the constituents of Cuban final molasses.

Zerban and Sattler (104) claimed the presence of D-psicose

(104) F. W. Zerban and L. Sattler, Ind. Eng. Chem., 34, 1180 (1942).

(D-ribohexulose, D-allulose) in distillery slops (from the fermentation

TABLE III

Sugar Constituents of Cuban Final Molasses [a]

Constituent	Analytical, %[b]	Chromatographic, %[b]
Sucrose	35.3[c]	30.5
Apparent glucose	18.3[c]	
D-glucose		6.0
D-fructose		1.4
Total for D-Glucose and D-Fructose		7.4
Solids	86.5	
Ash (sulfate)	9.2	

a W. W. Binkley and M. L. Wolfrom, J. Am. Chem. Soc., 72, 4778 (1950).

b Basis whole molasses

c A survey of 100 raw mill blackstrap molasses samples collected during two seasons from Cuba, Puerto Rico, Dominican Republic, Haiti, Hawaii, and Peru was made by the New York Sugar Trade Laboratory in 1947. This showed an average sucrose content, by analytical methods, of 36.38%, invert 20.64% and water 13.06%.

of cane molasses), but sharp experimental support is lacking. Wolfrom and Blair (37) and Sattler and Zerban (9) have obtained diheterolevulosans (difructose dianhydrides) by refluxing concentrated aqueous solutions of D-fructose. Chromatography of Cuban blackstrap molasses on a pilot plant scale chromatogram on fuller's earth clay did not reveal the presence of these substances (105). Binkley and Wolfrom (36)

(105) Report No. 12 (June 30, 1950) by Project 190 of The Ohio State University Research Foundation to the Sugar Research Foundation, Inc

isolated myo-inositol from Cuban final molasses by chromatography on fuller's earth clay. The myo-inositol content (0.251 %) of this molasses was determined by fermentation with yeast, acetylation of the unfermentable residue, chromatography on magnesium acid silicate of the acetylated residue and the isolation of crystalline myo-inositol hexaacetate. A small amount of D-mannitol as the crystalline hexaacetate was obtained from the molasses by these chromatographic procedures by Binkley and Wolfrom (36). Extension of these chromatographic techniques to the unfermentable residue of Cuban molasses led to the isolation of erythritol and D-arabitol (as their respective acetates) as trace constituents (36); the yeast may be the source of these substances. The uronic acid content of Louisiana molasses has been shown by Browne and Phillips (86) to be 2% by quantitative estimates based on the ash-free solids.

Balch, Broeg and Ambler (106) have shown that Louisiana molasses

(106) R. T. Balch, C. B. Broeg and J. A. Ambler, Sugar, 40, No. 10, 32 (1945); 41, No. 1, 46 (1946).

is a rich source of aconitic acid and contains more than 6% of this acid in some cases. Recently the commercial production of this acid from molasses has been initiated in Louisiana (107). Malic, citric

(107) L. Godchaux II, Sugar J., 11, 3 (April, 1949).

and lactic acid as well as formic acid have been adequately identified from molasses by Nelson (108) as their crystalline hydrazides or

(108) E. K. Nelson, J. Am. Chem. Soc., 51, 2808 (1929).

as their zinc salts. Marvel and Hager (109) found capric, lauric,

(109) C. S. Marvel and F. D. Hager, J. Am. Chem. Soc., 46, 726 (1924).

myristic and palmitic acids in a fraction (Bauer oil) of fermented Cuban molasses, but these acids are considered to be products of bacteriological action and are not normal constituents of unfermented molasses.

A surprisingly large fraction, 60-70%, of the nitrogen content in molasses (0.3 to 1.4% in the cane blackstraps of North America) is in a relatively simple form. Payne (110) isolated aspartic acid (as the

(110) J. H. Payne, Hawaiian Planters Record, 50, No. 2, 65 (1946).

free acid), glutamic acid (as the hydrochloride), lysine (as the picrate) and the purines, guanine (as the hydrochloride) and xanthine (as the free base) from Hawaiian molasses with the aid of ion exchange resins. The amino acids from Florida molasses were resolved by Kowkabany, Binkley and Wolfrom (94) with paper chromatography, employing spot

enhancement with known specimens; the presence of asparagine, aspartic acid, glutamic acid, γ-aminobutyric acid, alanine, glycine, leucine (or isoleucine) and valine were substantiated. The principal bearers of complex nitrogen are the sugar polymers which contain up to 1.7% of this element (111). How this nitrogen is incor-

(111) W. W. Binkley and M. L. Wolfrom, unpublished work.

porated into the polymer structure is not presently known.

III. EXPERIMENTAL

A. The Action of Alkali on D-Fructose

1. The Action of Heat on D-Fructose in an Alkali Medium with a Potassium Aconitate Buffer.

An amount of 34.8g. (0.2 mole) of trans-aconitic acid (112)

(112) Obtained from Eastman Kodak Company, Rochester, N.Y. Recrystallized from acetic acid.

was dissolved in 100 ml. of water and the pH of the resulting solution was adjusted to 8.0 with a 6N potassium hydroxide solution. An amount of 38g. (0.211 mole) of D-fructose (113) was dissolved in the alkaline

(113) Obtained from the Sugar Research Foundation Pilot Plant at Boulder, Colorado, and recrystallized once from water/ethanol according to Polarimetry, Saccharimetry and the Sugars, circular of the National Bureau of Standards C440, U. S. Department of Commerce, Washington, D. C., 1942, p. 404.

aconitate solution and water was added until the concentration of the solution was 12%. The resulting solution was then heated at 100° (114)

(114) All temperatures are in degrees Centigrade.

for 24 hours (115). The reaction was followed by taking the pH at

(115) This is an average of the time and temperature used in the processing of cane juice.

various intervals and by measuring the per cent transmission of light through the solution with a Lumetron colorimeter (116), Table IV.

Table IV

Rate of Color Formation and pH Change at 100° in the Reaction of trans-Aconitic Acid with D-Fructose.

Total Time, hours	Per cent transmission at 490 mμ [a]	pH
0	98	8.00
1	92	7.50
2	50	6.85
3	27	6.65
4	19	6.50
7	7	6.38
9.5	4	6.32
24	0	6.15

a Measured with a Lumetron photoelectric colorimeter (Model 400).

(116) Lumetron Photoelectric Colorimeter, model 400, Wave length 4900 Angstrom units.

The amber colored reaction mixture was diluted up to 3 liters with distilled water and was deionized by one passage through a 67cm. x 7cm. (diam.) column of Amberlite IR-100 (117) and one passage through

(117) A cation exchange resin produced by the Resinous Products and Chemical Company, Philadelphia, Pa.

a 67cm. x 7cm. (diam.) column of Duolite A-4 (118). Excess D-fructose

(118) An anion exchange resin manufactured by the Chemical Process Company, Redwood City, California.

was largely removed by one fermentation at 28-30° with 22g. of Fleischmann's starch-free baker's yeast for a period of 120 hours. The fermentation solution was filtered with suction through celite(119)

(119) No. 535, a siliceous filter aid produced by the Johns-Manville Company, New York, N. Y.

and the colorless neutral solution was dewatered at 45-50° and ca. 28 mm. pressure to yield 8.62g. of sirup (dried to constant weight over $CaCl_2$ and P_2O_5).

2. Chromatographic Analysis of the Obtained Sirup.

The sirup obtained was dissolved in 100ml. of absolute methanol and the solution was divided into three equal parts. Each of the parts (2.87g. in 33.3ml.) was diluted up to 100 ml. with abso-

lute methanol and added to the top of a tapered glass column (120) containing a 23 cm. x 7.5 cm. (diam.) adsorbent column of

(120) Tapered glass tubes may be obtained from the Scientific Glass Company, Bloomfield, N. J.

600 g. of Florex XXX (121)/Celite(122) (5/1 by wgt.) prewashed with

(121) A fuller's earth type of clay produced by the Floridin Company, Warren, Pa.

(122) No. 545, previously passed through an 80 mesh screen.

5 liters of 95/5: ethanol/water and conditioned further with 100 ml. of absolute methanol. The column was developed with 1500 ml. of 90/10:ethanol/water. The extruded adsorbent column was wrapped with aluminum foil to leave an exposed area 15 mm. wide running down the length of the column. After drying at room temperature for 20 hours, the exposed area was streaked with alkaline permanganate (1 part of potassium permanganate in 100 parts of 2.5N sodium hydroxide) indicator, employing a fine tipped glass pipet. Three zones were indicated as shown in Figure 1: a top zone at 18–45 mm. from the column top; a middle zone at 67–201 mm. (hereafter referred to as Zone I); and a bottom zone 210–230 mm. (bottom of column). The sectioned top zone and bottom zone materials each were eluted with 1500 ml. of 70/30:ethanol/water and the Zone I material with 3000 ml. of the same solvent. The removal of solvent from each zone elution and from the effluent under reduced pressure yielded sirups. Yield data are recorded in Table V.

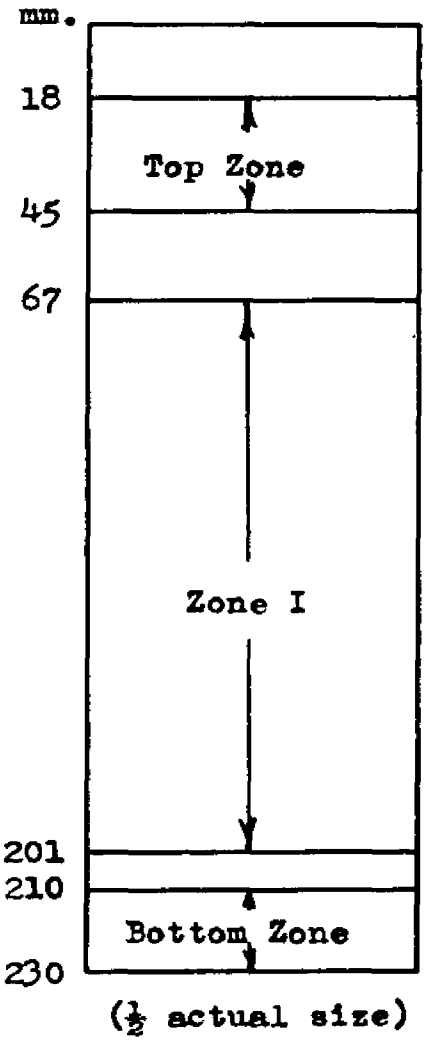


Figure 1. Chromatogram on Fuller's Earth Clay of the Non-fermentable Products of the Reaction at 100° of D-Fructose with trans-Aconitic Acid at pH 8.0

TABLE V

Yield Data from the Chromatography of the Sirup Obtained from the Action of Alkali on D-Fructose in a Potassium Aconitate Buffer.

ZONE	SAMPLE			
	1	2	3	Total
Top of Column	0.110g.	0.080g.	0.100g.	0.290g.
Top Zone	0.305	0.270	0.263	0.838
Zone I	1.39	1.35	1.35	4.09
Bottom Zone	0.21	0.22	0.20	0.63
Effluent	0.74	0.70	0.68	2.12
			Total	7.968

3. Isolation and Characterization of DL-Sorbose and D-Sorbose from the Effluents.

a. Isolation of DL-Sorbose and D-Sorbose Mixture from the Effluents.

The effluents when stored in a desiccator over calcium chloride produced crystals. A small amount of absolute ethanol was added to facilitate crystallization. The ethanol solution was decanted off and the substance was recrystallized by dissolving in 4 ml. of distilled water and treating with decolorizing carbon (15 mg. of Darco G-60) for 5 minutes at 50°. This mixture was filtered through a small pad of acid-washed abbestos fiber in a semi-micro Buchner funnel. A second charcoal treatment was applied and the colorless filtrate was then filtered by gravity through analytical filter paper. Pure crystalline material was obtained on solvent removal; m.p. 158.5-159.5° (123), $[\alpha]_D^{25}$ 12.40° (C 2.9, water). X-ray powder diffraction

(123) All melting points are corrected and were taken on a Fischer-Johns apparatus.

characteristics for this mixture are given in Table VI along with the x-ray powder diffraction data for DL-sorbose and D-sorbose.

Anal. Calcd. for $C_{12}H_{14}O_4$: C, 39.99; H, 6.72

Found (124) : C, 40.09; H, 6.79

(124) Microanalysis by Dr. E. W. D. Huffman, 505 Majestic Bldg., Denver, Colorado.

TABLE VI

X-ray Powder Diffraction Interplanar Spacings of DL-Sorbose, D-Sorbose and Fraction I

DL-Sorbose d-spacings Å.	Fraction I d-spacings Å.	D-Sorbose d-spacings Å. a
4.44	4.55	4.49
3.18	3.20	5.22
6.27	6.11	6.21
2.39	2.40	3.70
5.18	5.25	3.21
3.84	3.82	3.28
3.70	3.71	2.40
2.16	2.17	1.82
5.61	5.61	1.74
2.72	2.75	2.64
1.81	1.81	

a Arranged in decreasing order of intensities

b. The Identification of the DL-Sorbose and D-Sorbose Mixture.

(1.) Catalytic Reduction of the DL-Sorbose-D-Sorbose Mixture

An Amount of 500 mg. of the sorbose mixture was dissolved in 50 ml. of water and placed in a 300 ml. hydrogenation bomb. Approximately 2 g. of Raney nickel prepared according to the method of Parli and Adkins (125) was added and the bomb was sealed tightly

(125) A. A. Parli and H. Adkins, J. Am. Chem. Soc., 68, 1471 (1946).

and was flushed out twice with hydrogen. A hydrogen pressure of 19 atm. was applied to the bomb and the bomb was shaken continuously at a temperature of 80° for 3 hours. The bomb was cooled and its contents removed. A small amount of ethanol was added to facilitate the coagulation of the Raney nickel, which was then removed by gravitational filtration. The filtered solution gave negative Benedict and Molisch tests. The solvent was removed by evaporation under reduced pressure at 50° and the resulting sirup was dried over phosphorous pentoxide to a constant weight of 470 mg. The sirup partially crystallized when it was kept over phosphorous pentoxide for several weeks; the addition of methanol facilitated further crystallization, whereupon the crystals were separated by filtration through a semi-micro Buchner funnel and dried. The crystalline substance weighed 220 mg. and was designated Fraction A. Removal of the solvent from the mother liquor after drying over calcium chloride and phosphorous pentoxide yielded 240 mg. of sirup. This sirup was designated Fraction B.

(2) Identification of Fraction A as DL-Glucitol.

The crystalline material was recrystallized twice from a water-methanol solution, giving fluffy white needles, m.p. 132-34°. The literature (126) cites 135-37° for DL-glucitol. The x-ray powder

(126) M. L. Wolfrom, B. W. Lew, R. A. Hales and R. M. Goepp, Jr., J. Am. Chem. Soc., 68, 2342 (1946).

diffraction characterization for Fraction A was identical with that of DL-glucitol (Table VII).

Acetylation of a portion (100 mg.) of the crystalline substance with 5 ml. of acetic anhydride and 50 mg. of powdered, anhydrous (twice fused) sodium acetate at 80° (no decomposition) for one hour and extraction with chloroform, after hydrolysis of the anhydride excess with 25 g. of crushed ice, yielded a crystalline hexaacetate (151 mg.) from benzene/pet. ether (b.p. 30-60°); m.p. 113-14°. The literature (126) cites 116-17° as melting point for DL-glucitol hexaacetate; mixed m.p. with an authentic sample (m.p. 116-17°) 114-15°. The x-ray spacings for the crystalline hexaacetate were found to be identical with those of DL-glucitol hexaacetate (Table VII)

(3) Identification of Fraction B as a Mixture of DL-Iditol, D-Iditol, and L-Glucitol.

Fraction B (240 mg.) was acetylated with 7 ml. of acetic anhydride and 100 mg. of powdered, anhydrous sodium acetate for one hour at 90°. Solution took place at 80°. After cooling to room temperature, the colorless solution was poured onto 50 g. of crushed ice and the

TABLE VII

X-ray Powder diffraction Interplanar Spacings of DL-Glucitol and DL-Glucitol hexaacetate.

DL-Glucitol, d-spacings, Å.	DL-Glucitol Hexaacetate d-spacings Å.
5.91 (2)	6.19
4.41 (1)[a]	5.20 (1)
3.87 (3)	4.35 (2)
3.58 (4)	3.87
2.78	3.47 (3)
2.63	3.20 (4)
2.36	3.01
2.13	2.20
1.92	1.92
1.64	1.68

a Relative intensity estimated visually, (1) most intense line.

mixture was stirred for one hour. On standing overnight in the refrigerator at 3±3°, a crystalline hexaacetate separated. The hexaacetate was filtered by gravitation and recrystallized twice from benzene/petroleum ether to yield 340 mg. of white needles, m.p. 157-63°. The filtrate was neutralized with sodium bicarbonate to pH 6 and extracted with five 10 ml. portions of chloroform. Solvent removal yielded a sirup (104 mg.). When this sirup was dissolved in benzene and petroleum ether was added, crystallization occurred on standing. The crystals were removed from the mother liquor by filtration and recrystallized from benzene/petroleum ether; m.p. 117-19°. An equal mixture of this compound with L-iditol hexaacetate melted at 160-62°, but when an equal mixture of D-iditol hexaacetate was used instead of the L-isomer the melting point was 117-18°. This compound exhibited the same x-ray powder diffraction characteristics as D-iditol hexaacetate (Table VIII). Evaporation of the solvent from the mother liquid produced a sirup. The sirup was dissolved in benzene and petroleum ether was added drop by drop until a faint cloudiness appeared. The solvent was allowed to evaporate slowly. This treatment with benzene and petroleum ether was repeated 5 times to give finally a small amount (3-5 mg.) of a crystalline hexaacetate; m.p. 93-96°. The literature (126) cites 98-99° for L-glucitol. The melting point for an equal mixture of this hexaacetate and L-glucitol hexaacetate was 94-96°. This acetate showed x-ray powder diffraction characteristics identical with those of L-glucitol hexaacetate (Table VIII). When this compound was recrys-

tallized with an equal amount of D-glucitol hexaacetate, a crystalline compound was obtained, m.p. 130-33°, which had the same x-ray data as that of DL-glucitol hexaacetate (Table VII).

The crystalline acetate, m.p. 155-63°, obtained from the aqueous solution on acetylation of Fraction B was washed with absolute ethanol and when recrystallized from benzene/petroleum ether gave white fluffy needles; m.p. 160-62°. (The ethanol solution on evaporation yielded D-iditol hexaacetate). This acetate was believed to be DL-iditol hexaacetate; however, DL-iditol hexaacetate at that time was unknown. In order to have an authentic sample to compare, DL-iditol hexaacetate was prepared as follows.

Penta-O-acetyl-keto-D-sorbose (127) (0.9 g.) was dissolved in

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(127) M. L. Wolfrom, S. M. Olin and E. F. Evans, J. Am. Chem. Soc., 66, 204 (1944).

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150 ml. of absolute ethanol and placed in a 400 ml. hydrogenation flask. Approximately 3 g. of Raney nickel (125) was added and the flask connected to a power hydrogenation apparatus and flushed out with hydrogen. A pressure of 7 lb. of hydrogen was applied and reduction was carried out over a period of 21 hours. The Raney nickel was filtered from the solution and the solvent was removed by evaporation under reduced pressure. Acetylation of the resulting sirup with 10 ml. of acetic anhydride and 100 mg. of anhydrous sodium acetate at 80° for one hour gave an oil when the reaction mixture was poured onto 100 g. of crushed ice. The oil was recovered by decantation of the aqueous solution and crystallized from benzene/petroleum ether. On recrystallization from ether/petroleum ether 0.8 g. of D-iditol

hexaacetate, m.p. 118-19°, was obtained.

L-Iditol hexaacetate was prepared in the same manner as the D-iditol hexaacetate except that 1.5 g. of the penta-O-acetyl-keto-L-sorbose (128) was used; yield 1.25 g., m.p. 119-20°.

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(128) G. Arragon, Compt. rend., 196, 1733 (1933); H. H. Schlubach and J. Vorwerk, Ber., 66, 1251 (1933).

An amount of 100 mg. of L-iditol hexaacetate and 100 mg. of D-iditol hexaacetate was dissolved in 1 ml. of benzene and crystallization induced by the addition of petroleum ether. Two recrystallizations from benzene/petroleum ether yielded pure crystalline DL-iditol hexaacetate, m.p. 162-63°.

The mixed melting point of the acetate believed to be DL-iditol hexaacetate and the authentic DL-iditol hexaacetate was 162-63°, and it exhibited the same x-ray powder diffraction data as that of DL-iditol hexaacetate (Table VIII).

4. The Isolation and Characterization of Sodium D-Glucuronate.

Traces of siliceous matter from the adsorbent mixture remained in the top zone material, and to remove this impurity the zone material was dissolved in 5 ml. of water and treated with decolorizing carbon (100 mg. of Darco G-60) for 5 minutes at 50°. The mixture was passed through a small pad of acid-washed asbestos fiber in a semi-micro Büchner funnel and filtered by gravitation through analytical filter paper. The solvent from the clear filtrate was removed by evaporation under reduced pressure and the resulting sirup was dissolved in absolute

TABLE VIII

X-ray Powder Diffraction Interplanar Spacings of DL-Iditol Hexaacetate, D-Iditol Hexaacetate and L-Glucitol Hexaacetate

DL-Iditol Hexaacetate d-spacings Å.	D-Iditol Hexaacetate d-spacings Å.	L-Glucitol Hexaacetate d-spacings Å.
7.80 (3)[a]	7.34 (3)	6.92 (3)
6.81 (5)	6.51	5.05 (1)
5.52 (4)	5.56 (5)	4.40 (5)
4.41 (2)	4.46 (1)	3.72
3.91	4.07 (4)	3.41 (2)
3.48 (1)	3.56 (2)	3.20
2.75	3.01	2.93 (4)
2.34	2.74	2.64
2.15	2.22	2.36
1.89	1.87	2.09

a Relative intensity, estimated visually; (1) most intense.

methanol. Absolute ethanol was added until the appearance of a faint turbidity; crystallization occurred on standing at room temperature. Recrystallization from absolute methanol/absolute ethanol yielded pure white fluffy needles (.42 mg.); m.p. 150° (dec.). These gave a positive Molisch and Benedict test, and a negative Seliwanoff test. An aqueous solution of the crystalline material was passed through a small column of Amberlite IR-100 (117), after which it showed acidic properties. A sample of the original material when placed on a piece of aluminum foil and ignited gave a large ash residue.

Since the crystalline substance was reducing and showed acidic properties after passage through the ion exchange resin, the substance was believed to be a salt of a glycuronic acid. Sodium D-glucuronate monohydrate had the same melting point, 150° (dec.), as the crystalline material, except that it frothed. The frothing was due to the liberation of the one molecule of water. The crystalline material was then recrystallized from water/ethanol, giving white needles; m.p. 150° (dec.) with frothing. A mixed melting point with the authentic sodium D-glucuronate monohydrate was 150° (dec. and frothing). The optical rotation of the recrystallized material, $[\alpha]^{25}\underline{D}$ +21.48° (C, 1.42 H_2O) was the same as that found for sodium D-glucuronate, $[\alpha]^{25}\underline{D}$ +21.32° (C, 3.01 H_2O). The x-ray diffraction data for the drystalline substance was identical with that of sodium D-glucuronate (Table IX).

5. The Characterization of the Zone I Material.

a. Reduction of Zone I Material.

The sirup obtained from the elution of Zone I (Table V) gave

TABLE IX

X-ray Powder Diffraction Interplanar Spacings of Sodium D-Glucuronate

d-spacings Å.	
7.34	
5.45	(4)[a]
4.25	(1)
3.54	
3.24	(2)
2.95	
2.53	(3)
2.30	
2.14	(5)
1.77	
1.62	

a Relative intensities estimated visually; (1) most intense.

positive Benedict, Molisch and Seliwanoff tests: $[\alpha]^{28}\underline{D}$ + 3.21° (c 904, water.

Reduction of the sirup (.810 g.) was carried out by dissolving it in 5 ml. of water, diluting to 150 ml. with absolute methanol, and placing the solution in a hydrogenation flask. Raney nickel (4 g.), prepared fresh according to the method of Parli and Adkins (125), was added and the flask flushed out twice with hydrogen. A pressure of 20 lb. of hydrogen was then applied and the flask and its contents were continuously shaken for 21 hours. The Raney nickel was filtered from the solution and the filtrate was evaporated to a sirup under reduced pressure. On treating the sirup with methanol and ethanol, crystallization occurred. The crystalline material was filtered by suction, and recrystallized from water/ethanol; yield 750 mg., m.p. 130-40°. The x-ray powder diffraction data for this crystalline material was characteristic for allitol but it also showed some characteristic lines for dulcitol (Table X).

Exploratory chromatography of the crystalline material on the Florex XXX/Celite mixture indicated no separation of zones using ethanol/water developers.

An amount of 100 mg. of the crystalline material was acetylated with 5 ml. of acetic anhydride and 0.5 g. of twice-fused sodium acetate. The reaction mixture was heated to 100° at which time all of the material went into solution, and maintained at 80° for an additional 45 minutes. The solution was cooledand poured onto 50g, of crushed ice, neutralized with sodium bicarbonate, and extracted four times

TABLE X

X-ray Powder Diffraction Interplanar Spacings of Allitol, Dulcitol and of the Reduction Product of Zone I.

Allitol d-spacings Å.	Reduction mixture d-spacings Å.	Dulcitol d-spacings Å.
4.67[a]	4.65	4.74
5.84	5.88	3.65
3.37	3.41*	3.43
3.04	3.06	4.20
2.48	2.47	3.54
2.72	6.38*	6.43
2.02	2.74*	2.79
4.39	2.01	2.47
3.28	4.40	2.88
2.20	3.25*	1.95
2.28	2.21	3.23
	2.28	
	1.95*	

a Arranged in decreasing order of intensity.

* Lines also present in dulcitol.

with 10 ml. portions of Chloroform. The chloroform was removed by evaporation under reduced pressure and traces of acetic acid were removed by repeated additions of benzene with subsequent removal under reduced pressure. On standing, the sirup acetate partially crystallized. The crystals were removed by dissolving the remaining portion of sirup in 95% ethanol and filtering. The crystals were recrystallized from ethanol/petroleum ether, giving prisms, m.p. 140-50°. The x-ray diffraction data were found to be identical with those of dulcitol hexaacetate (Table XI). The melting point for dulcitol (galactitol) hexaacetate given in the literature (129)

(129) G. Bouchardat, Ann. chim. phys., (4) 27, 168 (1872).

is 171°.

The remaining portion of the sirup acetate, which was dissolved in 95% ethanol, slowly crystallized when allowed to stand in a desiccator for several weeks. On recrystallization from ethanol/petroleum ether, prisms were obtained; m.p. 56-57°. The literature (130) cites 61°

(130) J. Wiemann, Ann. Chim. (11), 5, 316 (1936); M. L. Wolfrom, B. W. Lew and R. M. Goepp, Jr., J. Am. Chem. Soc., 68, 1443 (1946).

for allitol hexaacetate. A melting point of 58-59° was observed when mixed with an authentic sample of allitol hexaacetate. The x-ray diffraction data were identical with those of an authentic sample of allitol hexaacetate (Table XI).

TABLE XI

X-ray Powder Diffraction Interplanar Spacings for Allitol Hexaacetate and Dulcitol Hexaacetate.

Allitol Hexaacetate d-spacings Å..	Dulcitol Hexaacetate d-spacings Å.
6.81	6.86 (4)
5.54	4.87 (1)
5.11	4.48
4.65 (1)[a]	3.95 (5)
3.99 (3)	3.66
3.72	3.43 (2)
3.51 (2)	3.19 (3)
3.10 (4)	2.65
2.61 (5)	2.25
2.09	1.95

a Relative intensities estimated visually; (1) most intense.

b. Attempt to Prepare a Crystalline Derivative of the Zone I Material.

(1) Acetylation of the Zone I Material.

An Amount of 590 mg. of the Zone I material was acetylated in the same manner as that described for D-psicose (131). The sirup

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(131) W. W. Binkley and M. L. Wolfrom, J. Am. Chem. Soc., 70, 3940 (1948).

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was placed in a 100 ml. round bottomed flask along with 10 ml. of acetic anhydride and 0.30 g. of freshly fused zinc chloride. The reaction mixture was continuously stirred and kept at 0° for 24 hours. The contents were poured onto 100 g. of crushed ice and after complete hydrolysis of the excess acetic anhydride, the aqueous solution was neutralized to pH 6 with sodium bicarbonate and extracted four times with 15 ml. of chloroform. The solvent was removed to yield 1.18 g. of the sirupy acetate. The sirup, dissolved in 20 ml. of benzene, was added at the top of a 170 x 44 mm. (diam.) column of 100 g. of 5 parts of Magnesol/1 part of Celite (by wgt.), prewet with benzene. The chromatogram was developed with 500 ml. of 100 parts of benzene/1 part of ethanol (by vol.). When the column was extruded and streaked with the alkaline permanganate reagent, it showed only one zone. Elution of the zone with 500 ml. of benzene, and removal of the solvent yielded 1.02 g. of the sirupy acetate. The sirup could not be induced to crystallize.

(2) Preparation of a Diisopropylidene Derivative

The method of Steiger and Reichstein (132) was used in an

(132) Marguerite Steiger and T. Reichstein, Helv. Chim. Acta, 18, 790 (1935).

attempt to prepare a crystalline isopropylidene derivative. An amount of 2 g. of the dried sirup was shaken for 48 hours with 3 g. of anhydrous copper sulfate, 90 ml. of acetone and 0.1 ml. of concentrated sulfuric acid. It was then filtered and washed with acetone and the solution was shaken for 2 hours with 1 g. of powdered potassium hydroxide. After filtration, the acetone was distilled off and the residue was dissolved in ether. The ether solution was shaken thoroughly 3 times with 5 ml. portions of a 20% potassium carbonate solution, and was then dried with sodium sulfate. The ether was removed by distillation, and the residue was distilled in high vacuum (0.2 mμ) at 105°. An amount of 1.80 g. of a slightly colored oil was obtained. All attempts to induce crystallization failed to give a crystalline derivative.

(3) Preparation of an Osazone and Osotriazole.

The Zone I material (0.20 g.) was placed in a test tube along with 0.40 g. of phenylhydrazine hydrochloride, 0.60 g. of crystalline sodium acetate and 4 ml. of water with 0.5 ml. of a saturated sodium bisulfite solution. The test tube and its contents were place in a beaker of boiling water and allowed to react for 20 minutes. The test tube was removed and cooled in an ice bath; the osazone

crystallized and was removed by filtration. The osazone was recrystallized from ethanol/water: when it (light yellow in color) was allowed to dry it turned dark brown with decomposition. When the osazone (produced on a second attempt) was recrystallized 3 times with ethanol/water and allowed to dry, a product, yellow orange in color, was obtained: m.p. 159-63°(dec.). The x-ray data for this compound are recorded in Table XII along with the x-ray data of the phenylosazone prepared from an authentic sample of D-psicose.

The method of Haskins, Hann and Hudson (133) for the preparation

(133) W. F. Haskins, R. M. Hann, and C. S. Hudson, J. Am. Chem. Soc., 67, 939 (1945).

of a crystalline osotriazole was then applied to the above phenylosazone. The osazone prepared fresh from 500 mg. of the Zone I material was mixed with 30 ml. of water and 0.42 g. of copper sulfate pentahydrate. The mixture was heated to boiling under a reflux condenser for thirty minutes, allowed to cool to room temperature and filtered. The filtrate was concentrated in vacuo to almost dryness, diluted with 1 ml. of absolute alcohol and allowed to stand at 5° for 24 hours. No crystalline compound was obtained.

c. Oxidation of the Zone I Material with Standard Iodine Solution

An Amount of 54.6 mg. of Zone I material was subjected to the method of Cajori (134) for the oxidation of aldoses. The substance

(134) F. A. Cajori, J. Biol. Chem., 54, 617 (1922).

TABLE XII

X-ray Powder Diffraction Interplanar Spacings of L-Psicose Phenylosazone and the Phenylosazone Prepared from Zone I Material.

L-Psicose Phenylosazone d-spacings Å.		Phenylosazone From Zone I Material d-spacings Å.	
10.26		10.40	
8.72		8.80	
7.68	(3)[a]	7.66	
6.91		5.63	
6.51		4.91	(3)
5.22		4.61	(5)
4.89	(1)	4.36	(1)
4.42	(4)	3.89	(4)
4.04		3.56	
3.88	(5)	3.25	(2)
3.58		2.87	
3.22	(2)	2.44	
2.84			
2.44			

a Relative intensity estimated visually; (1) most intense.

was dissolved in 10 ml. of water; 2 ml. of a 15% sodium carbonate solution and 10 ml. of a standardized iodine in potassium iodide solution (0.1014 N) were added. The solution was shaken thoroughly and placed in the dark at room temperature for 30 minutes. The solution was then acidified with 10% sulfuric acid and titrated with standardized sodium thiosulfate (0.05034 N) using a starch indicator. From the titration, 1.81 ml. of the 0.1014 N iodine, or 23.29 mg., had been used; calculated for aldose, 16.61 mg. (or 30.42%) from equation (1). D-Fructose was run as a blank and showed no uptake

(1) $C_6H_{12}O_6 + 2I + Na_2CO_3 \longrightarrow C_6H_{12}O_7 + 2\,NaI + CO_2$

of the iodine solution.

d. Fractionation of the Zone I Material into its Components.

A paper chromatogram was prepared as follows. An aqueous solution (2%) of the Zone I material was added with a micro pipet, a drop at a time, to a designated spot 3 inches from one end of a 5.5 by 18 inch sheet of Whatman No. 1 filter paper (the opposite end of the filter paper was cut to a point starting on the long edge, 1.5 inch from each corner and proceeding to the midpoint of the short edge; this facilitated the flow of the developer off the paper). The paper was allowed to dry between each addition. A total of 4 additions was found sufficient. The sheet was hung on separate racks in the usual apparatus for descending paper chromatography (45, 135). The apparatus was placed in a cylindrical glass jar

(135) University Apparatus Co., Berkeley Chromatography Division, Berkeley, California.

(44 x 24 cm. (diam.)) which had been conditioned by placing a mixture of 4/1.1/1.9: 1-butanol/ethanol/water into the bottom of the jar. The developer, whose composition was the same as that of the conditioning solvent mixture, was added and the chromatogram was developed for 40 hours. The chromatogram was removed from the jar and dried in air. The chromatogram was sprayed uniformly with a solution of 1-butanol saturated with p-anisidine hydrochloride. The 1-butanol was allowed to evaporate at room temperature and the sheet was then heated at 100° until the spots had developed fully (usually 2-4 minutes). The results are shown in Figure 2.

In an attempt to separate larger amounts, the Zone I material was chromatographed on a cellulose column. A column (1 x 14 inch) was packed with cellulose (136) according to the method of Hough,

(136) Ashless Whatman No. 1 cellulose powder, standard grade. Obtained from Kauffman Lattimer Company, Columbus, Ohio.

Jones and Wadman (66). The packed column was prewashed with a mixture of 1-butanol, saturated with water and containing 1% ammonia, until the solvent coming through contained no more color (usually about 24 hours). In order to test the efficiency of the column, a synthetic mixture composed of L-rhamnose, L-arabinose and D-galactose (60 mg. of each) was chromatographed. The sugar mixture was added to the top of the column as a sirup and developed with the same solvent used for the prewashing. The rate of flow was regulated so that 10 ml. of solvent passed through the column every half hour. An automatic fraction cutter (137) was used to collect the fractions.

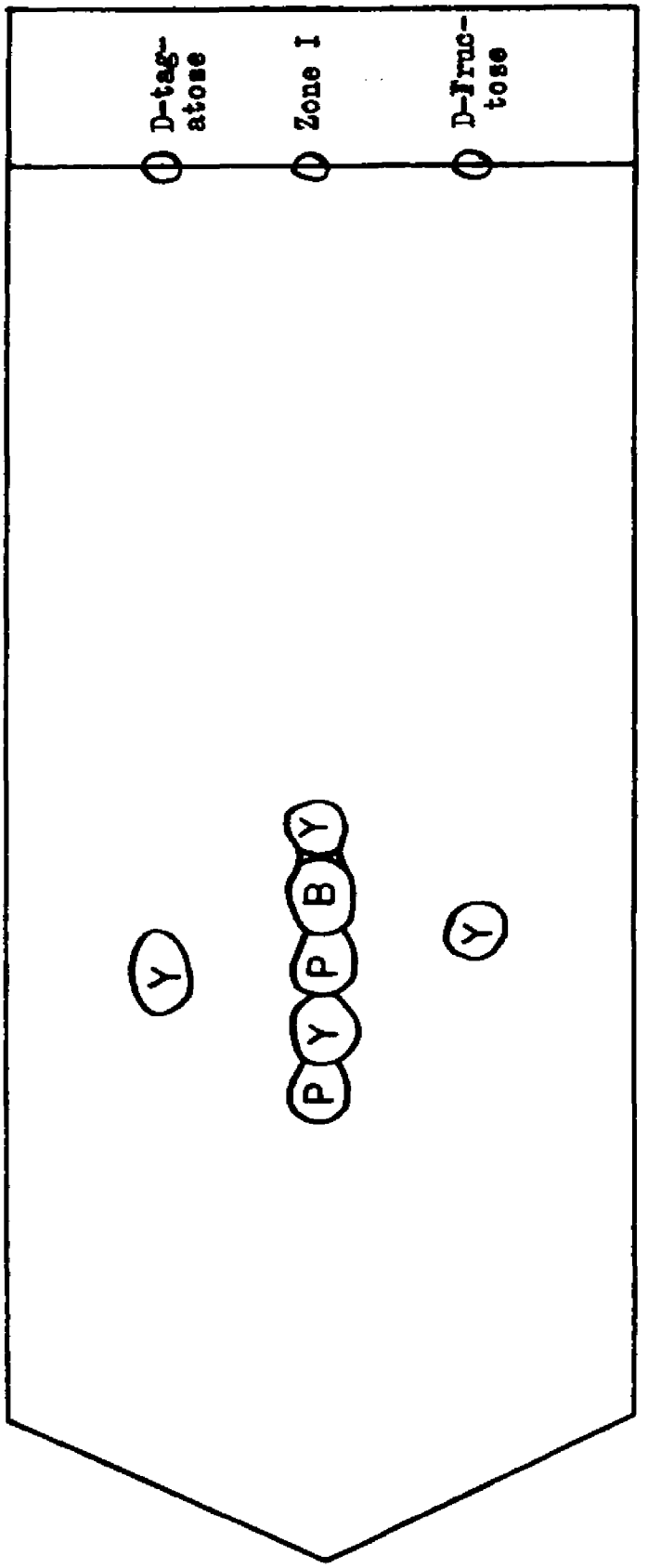

Figure 2- Sugar Paper Chromatography of the Zone I Material.
Scale ½ actual size. Y= yellow; B = brown; P = pink.

(137) Automatic fraction cutter, designed by Roy L. Whistler, Department of Agricultural Chemistry, Purdue University, Lafayette, Ind.

The fractions containing the sugars were selected by placing five drops from each fraction on a sheet of Whatman No. 1 paper containing a designated spot for each fraction. The paper was sprayed with p-anisidine hydrochloride in 1-butanol. The sugars were recovered in about 90-95% yield, as pure specimens.

In the same manner, 200 mg. of the Zone I material was chromatographed on the column. When the fractions were examined, some resolution was noticed, but it was not sufficient to be of much value.

Since the Zone I material could not be resolved on a clay column or on the cellulose column, but could be sufficiently resolved on paper strips, the method of Flood, Hirst and Jones (59) was used for eluting sugars from the paper sheets, with the following changes. An amount of 5 ml. of a 6% solution of the Zone I material was added with a micro pipet, to 8 sheets (5.5 x 18 inches) of Whatman No. 1 paper in a continuous streak (3/16 inch wide centered 3 inches from one end of the paper). Care was taken to obtain as uniform distribution as possible. About ten applications to each sheet were required to transfer the 5 ml. of the solution to the sheets. The sheets (four at a time) were placed in the developing jar and developed for 40-44 hours with 1-butanol/ethanol/water: 4/1.1/1.9 (volume ratio). The sheets were removed from the jar and dried in the hood at room temperature. A one-half inch strip from each side of each sheet was cut off and sprayed with p-anisidine hydrochloride in 1-butanol. The strips

were first dried at room temperature and then at 100° in an oven saturated with water vapor for 10 minutes. After locating the position of the sugars on the strips, the strips were placed back, alongside the sheets in their original position, and transverse strips were cut out (see Figure 3) as indicated by the spots on the sprayed strips. The sugar was then removed from the transverse strips as follows. The bottom plate from a 10 inch (diam.) desiccator was removed and the desiccator was fitted with a stand on top of which was place a small petri dish containing about a quarter inch of water. Two strips of Whatman No. 1 paper were placed in the dish, about one inch of each strip being hung over the outside edge from opposite sides. The transverse strips were attached to the part of the strips hanging over the outside edge. The transverse strips were beveled at the bottom in order to obtain quicker elution. The first few drops of water coming off the transverse strip contained practically all of the material. The solvent from the combined elutions of each zone was removed at 50° under reduced pressure. Yield data from these zones are recorded in Table XIII. All of the zone materials were sirups except Zone I_b, which partially crystallized.

e. Rechromatography of Zones I_a, I_b, I_c, I_d and I_e on Paper.

An aqueous 2% solution of each zone material was prepared, and three drops (one at a time) of each solution was added to a designated spot on the sheet of Whatman No. 1 filter paper. Solutions (2%) of known sugars were also prepared and spotted on the sheet inbetween the zone spots. The chromatograms were placed in the developing jar and

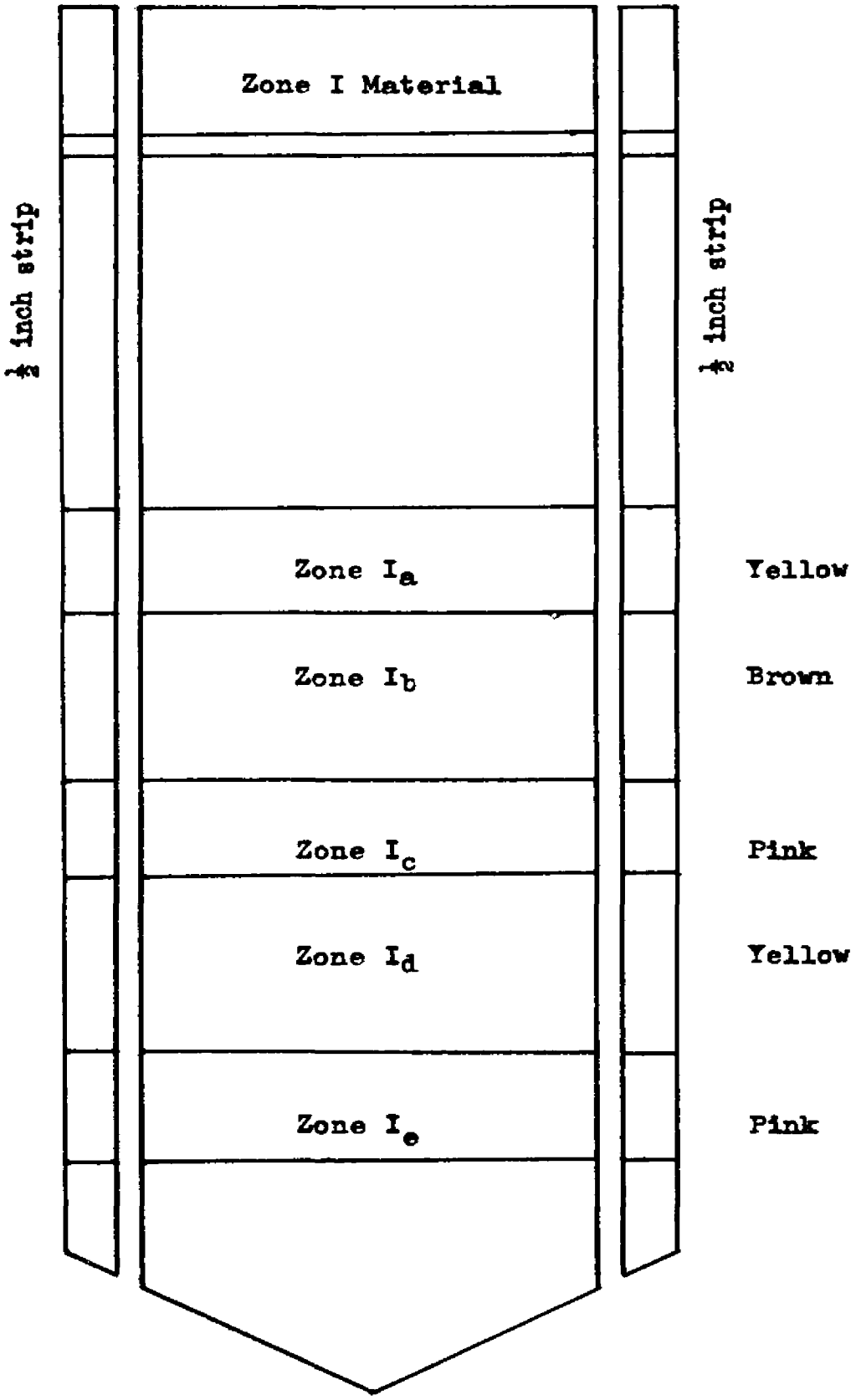

Figure 3- Zones Produced on the Fractionation of the Zone I material on Filter Paper. Scale ½ actual size.

TABLE XIII

Yield Data from the Resolution of the Zone I Material on Paper Sheets

		Weight, g.
Before Resolution		2.75 g.
Zone I_a		0.210
I_b		0.544
I_c		0.281
I_d		0.676
I_e		0.202
	Total [a]	1.913

a Approximately one-fifth (0.55 g.) of the Zone I material was contained in strips cut off which were used in the location of the zones.

developed with 1-butanol/ethanol/water; 4/1.1/1.9 (vol. ratio) for 40 hours. The chromatograms were sprayed with p-anisidine hydrochloride in 1-butanol, and after drying in air for one-half hour, were dried at 100° for 10 minutes in an oven saturated with water vapor. Figures 4 and 5 show a picture of the results obtained.

f. Identification of Zone I_b as DL-Allose and D-Allose. Dimorphism of Allose.

When the Zone I_b material, $[\alpha]^{25}$ D +4.32 (C, 2.24 water), was allowed to stand in the desiccator, a few crystals formed. The crystals were separated from the mother liquor sirup by dissolving the sirup in methanol. The crystalline material had a melting point of 180°. Upon standing, the mother liquor sirup grew more crystals, however the melting point of these newly formed crystals was 138-45°. It was believed that the first crystals obtained were DL-allose and on further crystallization the D-allose crystallized out to lower the melting point. Since DL-allose was unknown it was prepared by crystallizing together equal amounts of D-allose(138)

(138) Sample obtained from H. S. Isbell, U. S. Department of Commerce, National Bureau of Standards, Washington, 25, D.C.

and L-allose (139). On recrystallization the crystalline product,

(139) Sample obtained from F. L. Humoller, Loyola University, Chicago 12, Illinois.

m.p. 180°, exhibited the same x-ray date (Table XIV) as that of the Zone I_b material melting at 180°. The x-ray data of the Zone I_b

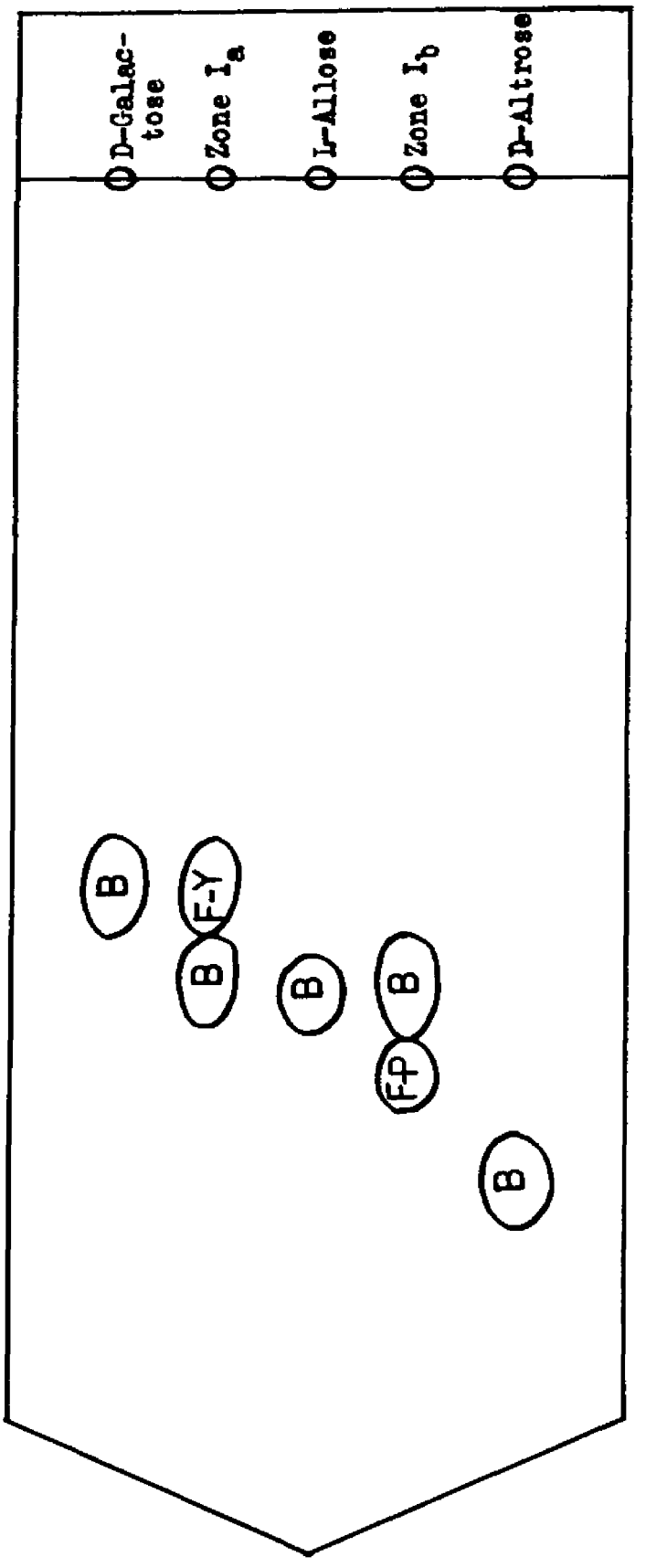

Figure 4— Paper Chromatography of the Zones Collected from the Fractionation of Zone I on Paper Strips. Scale ½ Actual Size. B= brown; F-Y= faint spot, yellow in color; F-P= faint spot, pink in color.

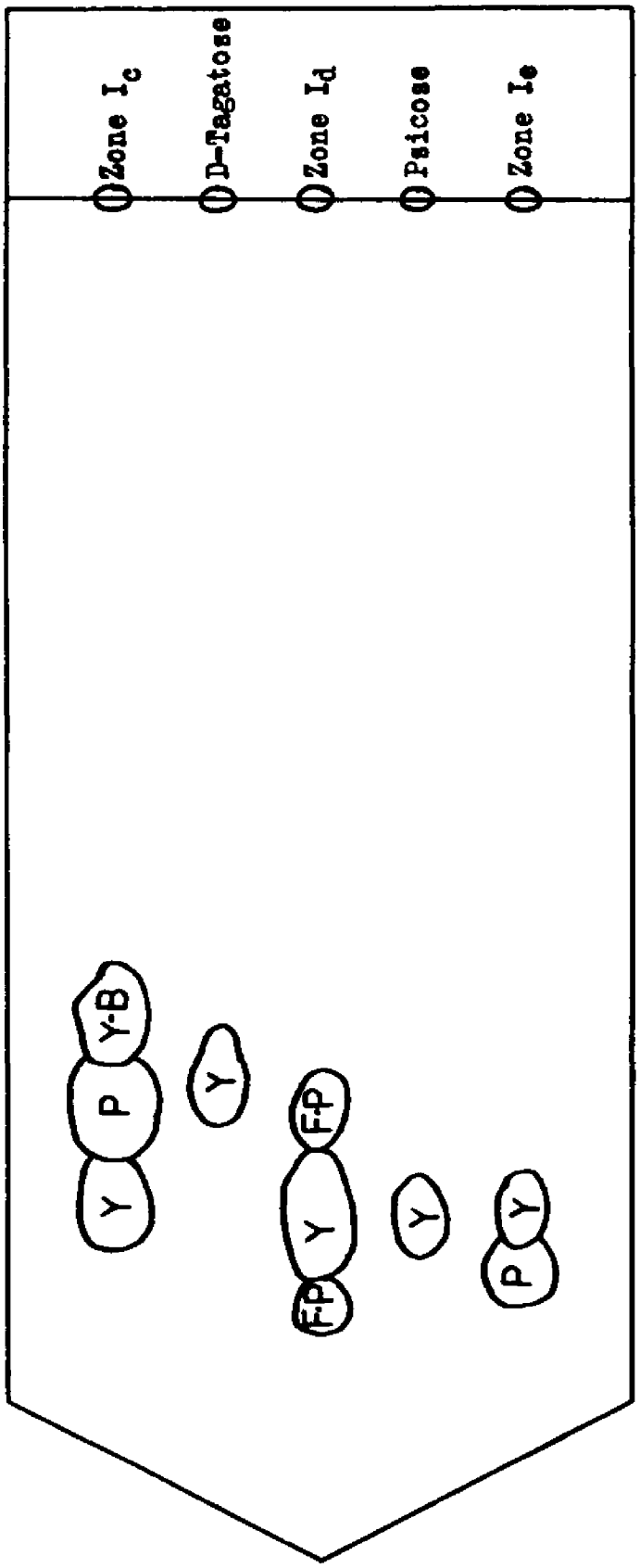

Figure 5- Paper Chromatography of the Zones Collected from the Fractionation of Zone I on Paper Strips. Scale $\frac{1}{2}$ actual size. P= pink; Y= Yellow; F-P= faint spot, pink; Y-B = yellow brown.

TABLE XIV

X-ray Powder Diffraction Interplanar Spacings of DL-Allose, D-Allose and L-Allose.

DL-Allose d-spacings Å.	D-Allose (m.p. 141°) d-spacings Å.	L-Allose (m.p. 128°) d-spacings Å.
6.35	6.39	14.09
5.94 (2)[a]	5.96 (3)	8.34
5.88	4.26 (1)	7.18
4.31 (1)	3.67	5.43
3.94	3.22	4.60 (2)
3.75 (4)	3.11	4.21 (3)
2.96	2.90 (2)	4.02 (1)
2.87 (3)	2.69 (4)	3.61 (4)
2.51	2.50	3.14
2.46	2.46 (5)	2.89
2.32 (5)	2.36	2.72
2.22	2.01	2.40 (5)
2.10	1.92	2.30
1.92	1.88	2.00
	1.69	1.91

a Relative intensity estimated visually; (1) most intense.

material, melting at 138-45°, was very similar to that of DL-allose. The lines were broader, due to the fact that the D-allose x-ray data are very close to that of DL-allose.

The D-allose furnished by Isbell had a melting point of 141° and the x-ray diffraction data of this compound were very different from those of L-allose, m.p. 128°, supplied by Humoller(Table XIV). Both of these compounds were the β-form, thus excluding anomerism and establishing the fact that allose is dimorphous. About two months after this comparison, L-allose (139) was found to be transformed to the lower energy form, m.p. 141°, and now exhibiting the same x-ray diffraction data as the D-allose furnished by Isbell (138). When L-allose, m.p. 141°, was recrystallized from an ethanol/water solution, the melting point obtained was 130-31° and again exhibited the x-ray diffraction data as shown for L-allose (m.p. 128°) in Table XIV. On standing the melting point slowly raised toward 141°.

g. Preparation of DL-Psicose Phenylosazone and Preparation of the Phenylosazone of the Zone I_d Material.

Equal amounts (3 mg.) of D-psicose phenylosazone (140) and

(140) Prepared from D-psicose by M. L. Wolfrom, A. Thompson and E. F. Evans, J. Am. Chem. Soc., 67, 1793 (1945).

L-psicose phenylosazone (140a) were mixed together and dissolved in

(140a) Prepared from L-allose obtained from F. L. Humoller (139).

hot alcohol. The resulting solution was filtered and to the filtrate water was added. On standing the ethanol-water solution grew crystals.

The crystals were collected on a filter and dried; m.p. 177-80° (dec.), x-ray powder diffraction data recorded in Table XV.

An amount of 0.20 g. of the Zone I_d material was placed in a test tube along with 0.40 g. of phenylhydrazine hydrochloride, 0.60 g. of sodium acetate and 4 ml. of water. The test tube was heated in a boiling water bath for 20 minutes, at which time the tube was removed and cooled in an ice bath. The phenylosazone crystallized out as yellow-brown crystals, which on recrystallization from absolute ethanol gave yellow crystals, m.p. 177-81°(dec.). The x-ray diffraction data of this substance contained all the characteristic lines of DL-psicose phenylosazone plus a few extra lines, see Table XV.

B. Fractionation of Florida Blackstrap Molasses.

1. Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay.

One hundred grams of Florida blackstrap molasses (141) was

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(141) This final molasses was collected at the Clewiston, Florida, mill of the United States Sugar Corporation on January 6, 1951 and had been stored at ca. 5°.

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fractionated on fuller's earth clay according to the method of Binkley and Wolfrom (36), with the following changes; The molasses was diluted with 40 ml. of distilled water and a smooth paste prepared by the addition of 55 g. of a mixture of 5 Florex XXX/1 Celite (No. 545). This paste was suspended in 2000 ml. of absolute ethanol with good agitation. The resulting suspension was poured onto an 11-12 cm. by 7-9 cm. (diam.)

TABLE XV

X-ray Powder Diffraction Interplanar Spacings of DL-Psicose Phenylosazone and of the Phenylosazone Prepared from the Zone I_d Material.

DL-Psicose Phenylosazone d-spacings Å.	Phenylosazone of Zone I_d Material d-spacings Å.
10.13	10.22
8.82	8.80 (2)
7.76	7.75
7.20	7.03
5.72 (2)[a]	5.70 (4)
5.01	5.05
4.66 (1)	4.60 (1)
4.42 (5)	4.39 (5)
4.23 (3)	4.21
3.76	3.96*
3.63	3.75
3.27	3.55
3.16 (4)	3.30 (3)
2.84	3.16
2.45	2.87
2.00	2.44
	2.22*
	1.97

a Relative intensity estimated visually; (1) most intense.

* Lines not present in DL-psicose phenylosazone.

column of 250 g. of 5 Florex XXX/1 Celite (no. 545), prewet with 2 liters of absolute ethanol. When the supernatant liquor had moved down to the top of the bed formed by the settling of the suspension, 5 liters each of 95/5, 80/20, 50/50: ethanol/water (volume ratios before mixing) and water, were allowed to percolate through the bed. The apparatus was operated under a reduced pressure of 10-25 mm. to increase the rate of percolation. The column effluents were concentrated at 48-50° under reduced pressure to a volume slightly less than desired for analysis. The final adjusted volume for the 95/5 fraction was 4000 ml. and for each of the 80/20, 50/50 and water fractions it was 500 ml.

Solids Analysis. The quartz sand procedure (142) was used

(142) Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, p. 557 (1945).

for the determination of solids in the molasses fraction. Pure quartz sand was digested with hydrochloric acid for 16 hours, washed free from acid, dried first in air and then in a muffle furnace at 400° for two hours. About 20 g. of the prepared sand and a short stirring rod were placed in an aluminum dish (55 mm. in diameter and 40 mm. in depth), fitted with a cover. The pan and its contents were dried in an oven at 108° for two hours, cooled in a desiccator and weighed immediatly. An aliquot portion of the fraction was added to the weighed pan and mixed thoroughly with the sand. It was then heated on a steam bath and stirred every 2 or 3 minutes until the mass became too stiff to manipulate readily. The pan and its contents, along with the cover, were dried at 70° for 18 hours under a pressure of 50 mm.

The cover was placed tightly on the pan, and after cooling to room temperature, was weighed immediately. Analyses were always run in triplicate. Results are shown in Table XVI.

2. Dialysis with Cellulose Membrane of the 80/20 and 50/50: Ethanol/water and Water Fractions from the Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay.

Each of the adjusted volumes of the molasses fractions was poured into a cellophane membrane bag (143)(12 in. length, 2.25 in. diam., 0.0023 in. wall thickness), and was covered with a layer of toluene (3-5 mm. thick). The bag and its contents were lowered into a 4 liter glass jar containing 3800 ml. of water covered with a layer of toluene. The contents of the membrane bag and the distilled water in the glass jar were stirred continuously throughout the dialysis. The distilled water in the jar was changed after 24 hours, and the dialysis was continued for an additional 40 hours. When the dialysis was completed, the solution of dialyzed material was concentrated under reduced pressure at 48-50° to a volume of 50 ml. less than its volume before dialysis and the volume was then adjusted with water to its original volume (500 ml.). The solids content of this solution was then determined as described above and recorded in Table XVII. The solution of the material passing through the membrane bag (dialyzable portion) was concentrated also under

(143) Catalog number 70160C, Central Scientific Co., Chicago, Ill. The membrane is supplied in the tubular form. A 26 in. length of this tube was allowed to soak in distilled water for 15-20 minutes at room temperature and the bag was formed by tying two overhand knots at one end of the tube.

TABLE XVI

Solids in the Fractions Obtained from the Fractionation on Fuller's Earth Clay of Florida Blackstrap Molasses.

	Solids, % whole molasses
Before Fractionation	78.50 g.
Fraction	g.
95 ethanol / 5 water	51.36
80 ethanol / 20 water	9.68
50 ethanol / 50 water	11.92
water	4.32
Total	77.28

TABLE XVII

Distribution of Solids Produced by the Dialysis with Cellophane Membrane of 80/20 and 50/50: Ethanol/Water and Water Fractions from the Fractionation on Fuller's Earth Clay of Florida Blackstrap Molasses.

	Material in Solution Added to Membrane Bag, g.	Material Remaining in Membrane Bag after Dialysis, g.	Material Passing Through Membrane Bag During the Dialysis, g.
80 ethanol 20 water	9.10	1.20 (13.2%)	8.03 (88.2%)
50 ethanol 50 water	11.27	2.05 (18.2%)	9.33 (82.8%)
water	4.06	0.92 (22.6%)	3.02 (74.4%)

reduced pressure at 48-50° to a volume of 250 ml. for the 80/20: ethanol/water and water fractions and 500 ml. for the 50/50: ethanol/water fraction. The solids contents of these solutions were then determined and are recorded in Table XVII.

3. Deionization of the Dialyzable Portions of the 80/20 and 50/50: Ethanol/Water Fractions from the Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay.

The 80/20 and 50/50: ethanol/water and water fractions were deionized by passage through a column of Amberlite IR-120 (118), and then through a column of Duolite A-4 (119). The material passing through both columns was considered as the non-ionic portion. The cations were removed from the Amberlite column by passing a 10% solution of hydrochloric acid through the column, and washing with distilled water until the effluent was almost neutral to litmus. The anions were removed from the Duolite column by passing a 5% solution of sodium hydroxide through the column and washing until the effluent was colorless. The solvents from the cationic, anionic and non-ionic fractions were removed under reduced pressure and the fractions were dried and weighed. Yields are presented in Table XVIII.

4. Amino Acid Chromatography of the Deionized Fractions.

A total of 5 applications of a 2% solution prepared from the cationic, anionic and non-ionic fractions of the dialyzable portions of the 80/20 and 50/50: ethanol/water and water fractions obtained

TABLE XVIII

Weight of Ionic Fractions Produced by the Deionization of the Dialyzable Portions of the 80/20 and 50/50: Ethanol/Water and Water Fractions from the Fractionation on Fuller's Earth Clay of Florida Blackstrap Molasses.

	Weight of Fraction Before Deionization g.	Cations[a] g.	Anions g.	Non-ionic g.
80 ethanol 20 water	8.10	10.30	2.14	2.71
50 ethanol 50 water	9.39	6.94	3.10	0.50
water	3.02	2.62	1.86	0.23

a Obtained as the chlorides or as the hydrochlorides.

from the fractionation of Florida blackstrap molasses on Fuller's earth clay, were made on Whatman No. 1 filter paper. Several amino acids (L-asparagine, L-aspartic acid and L-glutamic acid) were also spotted on the chromatograms. The chromatograms were placed in the developing jar for descending paper chromatography, and developed for 22 hours with a mixture of 1-butanol saturated with water containing 80 ml. of 99.5% acetic acid per 500 ml. of solution. The chromatograms were removed from the developing jar, dried in air at room temperature for 24 hours and sprayed with ninhydrin solution (0.25% ninhydrin in water saturated with 1-butanol). The sprayed sheets were allowed to remain at room temperature for 24 hours, at which time spot formation was at its maximum intensity. The results of these chromatograms are shown in Figure 6.

5. Organic Acid Chromatography of the Deionized Fractions

Paper chromatograms of the cationic, anionic and non-ionic fractions of the dialyzable portions of the 80/20 and 50/50: ethanol/water and water fractions were prepared as described above in section B-4. The chromatograms were developed for 22 hours with 1-butanol saturated with a water solution containing 80 ml. of 99.5% acetic per 500 ml. of solution. The chromatograms were dried in air for 24 hours and then sprayed with a solution of brom-phenol-blue (40 mg. of brom-phenol-blue in 100 ml. of 95/5: ethanol/water and adjusted to a purple tint (pH 5.5) with sodium hydroxide solution.) . The organic acids showed up as yellow spots on a purple-blue background. Results are shown in Figure 7.

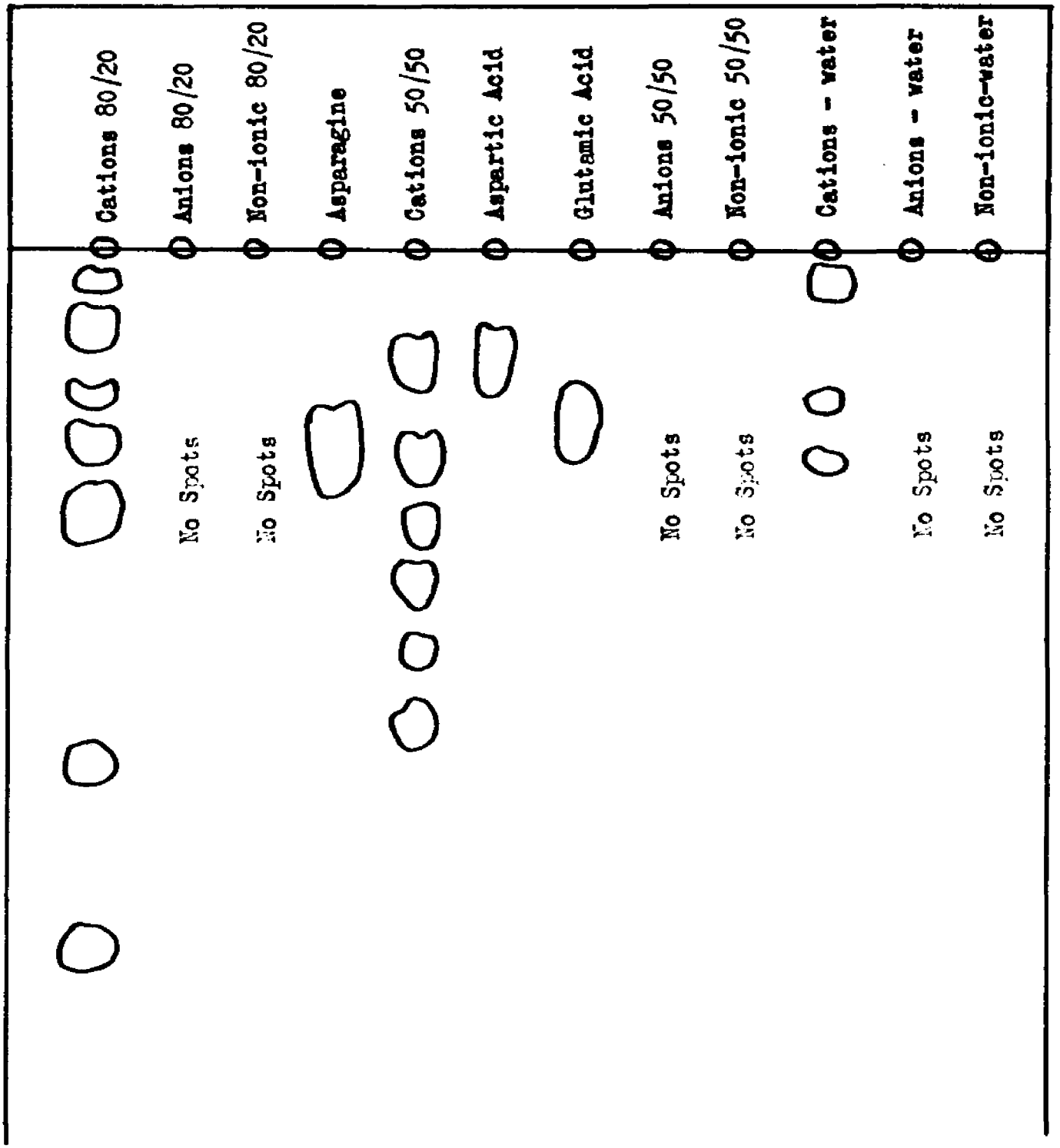

Figure 6- Amino Acid Paper Chromatogram of the Deionized Dialyzable Portions of the 80/20 and 50/50:Ethanol/Water and Water Fractions from the Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay. Scale ½ actual size.

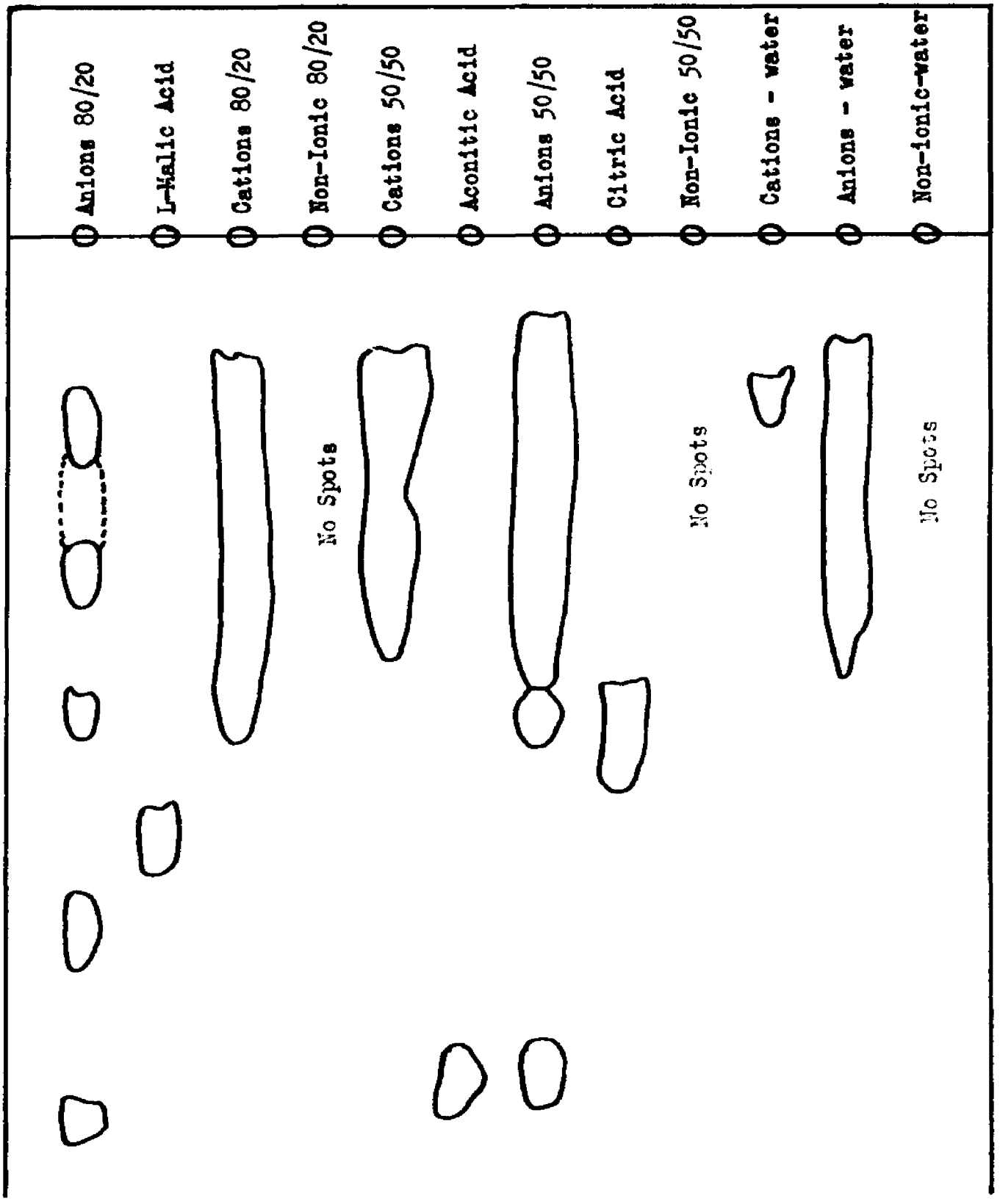

Figure 7- Organic Acid Paper Chromatogram of the Deionized Dialyzable Portions of the 80/20 and 50/50:Ethanol/Water and Water Fractions from the Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay Scale ½ Actual Size.

6. Sugar Paper Chromatography of the Non-ionic Portions of the Deionized Fractions

A paper chromatogram of the non-ionic portions obtained by deionization of the dialyzable fractions of the 80/20 and 50/50: ethanol/water and water fractions obtained from the fractionation of Florida molasses was prepared as follows. Five applications of a 5% solution of each non-ionic fraction of the 80/20 and 50/50: ethanol/water and water fractions were placed on a sheet of Whatman No. 1 filter paper (18 by 5½ inch). D-Fructose, D-glucose, D-glucurono-γ-lactone and sucrose were also added to the paper by placing two drops of a 2% solution of each sugar on the paper (several extra drops of the sucrose solution were needed to obtain a good spot with the spray reagent). The chromatogram was placed in the developing jar and developed for 40 hours with a mixture composed of 1-butanol/water/ethanol:4/1.9/1.1. The chromatogram was removed from the tank, dried, sprayed with p-anisidine hydrochloride in 1-butanol and dried in an oven at 100° for ten minutes. The spots were located and are shown in Figure 8.

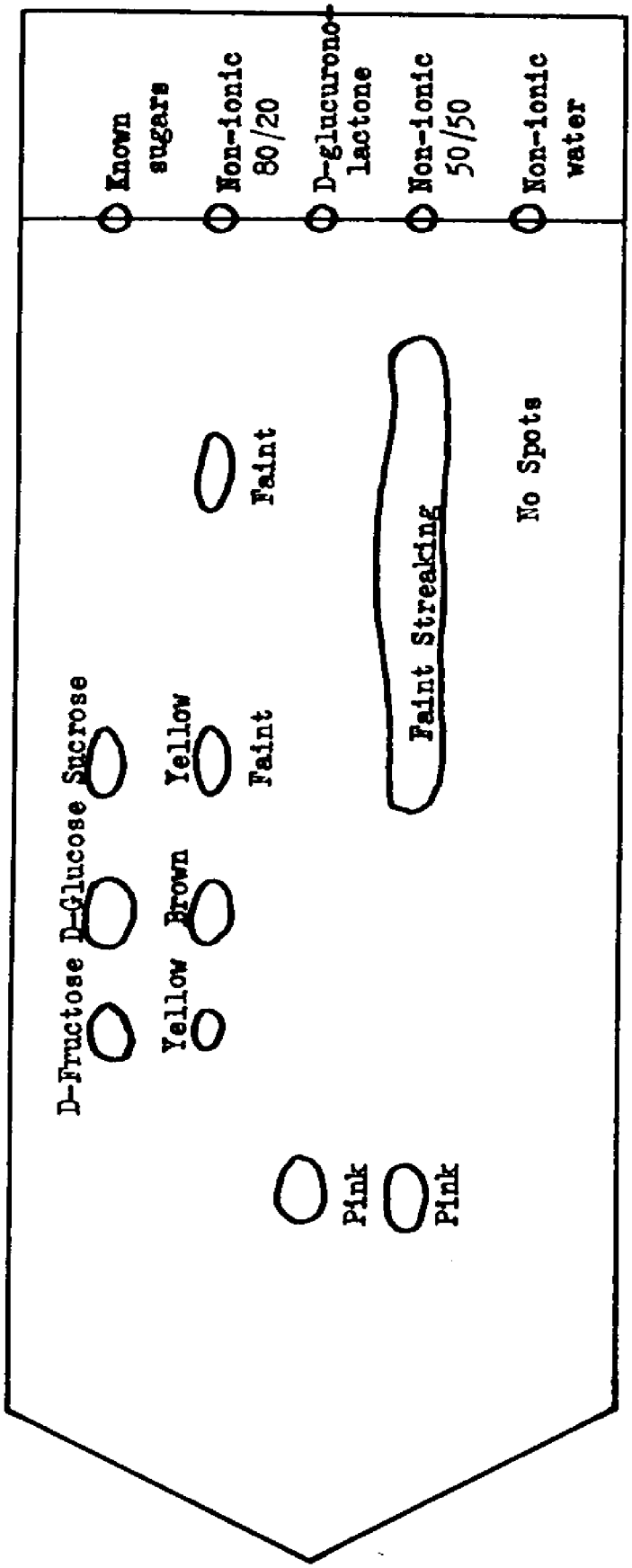

Figure 8- Sugar Paper Chromatogram of the Non-ionic Portions of the Deionized Dialyzable Portions of the 80/20 and 50/50: Ethanol/Water and Water Fractions from the Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay. Scale $\frac{1}{2}$ Actual Size

IV DISCUSSION OF RESULTS

A. The Action of Heat on D-Fructose in an Alkaline Medium with a Potassium Aconitate Buffer

1. General

In previous work (144), it was found that D-fructose is the greatest color producer of the cane juice sugars. Comparable model experiments with D-glucose and sucrose at their estimated concentrations in cane juice, showed that this sugar yielded the most color when allowed to react with trans-aconitic acid and L-asparagine under a simulated mill heating schedule. Furthermore, molasses-like substances were obtained from D-fructose and D-glucose with trans-aconitic acid under simulated mill condition; amino acids were not essential ingredients for color formation. The color of the melassigenic products was an amber from the aconitic acid reactions and a brown from the amino acid products. The reaction of D-fructose with trans-aconitic acid appeared to involve fewer complications than the corresponding reactions with L-asparagine.

D-Fructose is considered to be the source of several simple sugars reported as present in cane molasses, more particularly in the distillery slop from cane molasses. Zerban and Sattler (9, 104) claimed that D-psicose and several of the diheterolevulosans were found in distillery slop. The chromatography of the appropriate fractions of Cuban blackstrap molasses on a four meter column of fuller's earth clay did not

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(144) J. N. Schumacher (with M. L. Wolfrom), M. Sc. Thesis, The Ohio State University, 1952.

yield these sugars (145). The action of calcium hydroxide on D-glu-

(145) W. W. Binkley and M. L. Wolfrom, Sugar Research Foundation, Member Rept. 25, 21 (1950).

cose, which exhibits a prototropic shift yielding 30% of apparent D-fructose (7), did not yield any detectable D-psicose (146).

(146) Report No. 14 (July 13, 1951) to the Sugar Research Foundation by Project 190 of the Ohio State University Research Foundation, p. 46.

The present experiment is an investigation of the simple non-fermentable (yeast) organic substances produced by the action of heat on an equal molar mixture of trans-aconitic acid and D-fructose adjusted to a pH of 8.0 with potassium hydroxide (at this pH the aconitic acid is present as the di-potassium aconitate). The reaction products were deionized and decolorized with ion exchange resins; the fermentable sugars were removed with yeast. The nonfermented residue was a golden colored sirup. Use was made of the modern methods of separation to make possible an adequate separation of the reaction products. The use of x-ray powder diffraction analysis was an important aid in identifying crystalline compounds from the carbohydrate mixtures.

Isolated from the reaction mixture as crystalline compounds were DL-sorbose and D-sorbose, and DL-allose and D-allose. Also found was D-glucuronic acid, isolated as the sodium salt. The formation of sorbose and allose from D-fructose is the first case cited of such a transformation.

2. Proposed Mechanism for the Isomerization of D-Fructose

The simplest isomerization reaction of the reducing sugars is the Lobry de Bruyn and Alberda van Ekenstein transformation (1). The interpretation of the mechanism of this transformation is postulated on the formation of an intermediate enediol. However, in addition to enolization, cleavage of the carbon chain also occurs. Nef (21) has elucidated that cleavage of the carbon chain may take place to give: (1) formaldehyde and aldopentoses, (2) glycolaldehyde and aldotetroses, or (3) dihydroxyacetone and glyceraldehyde. The cleavage of the carbon chain probably takes place through a reversed aldol condensation, which was first proposed by Bernier (147) who studied

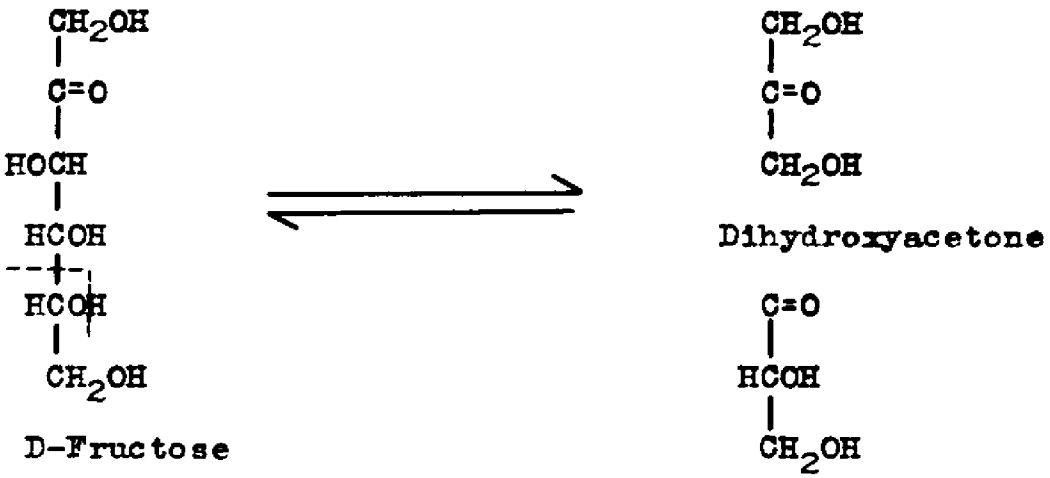
(147) C. Bernier, Ph. D. Dissertation, The Ohio State University, 1935.

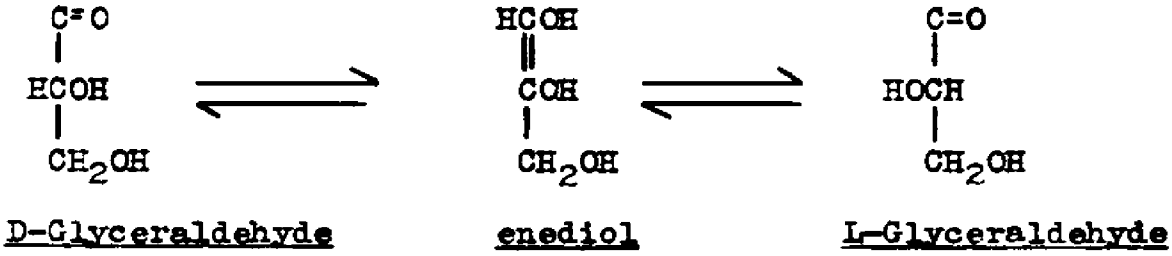
the mechanism for the reaction of alkali on reducing sugars. Pigman and Goepp (148) have also proposed a reversed aldol condensation for

(148) W. W. Pigman and R. M. Goepp, Jr., Chemistry of the Carbohydrates, Academic Press, Inc., New York, 1948, p. 78.

the fragmentation of the carbon chain.

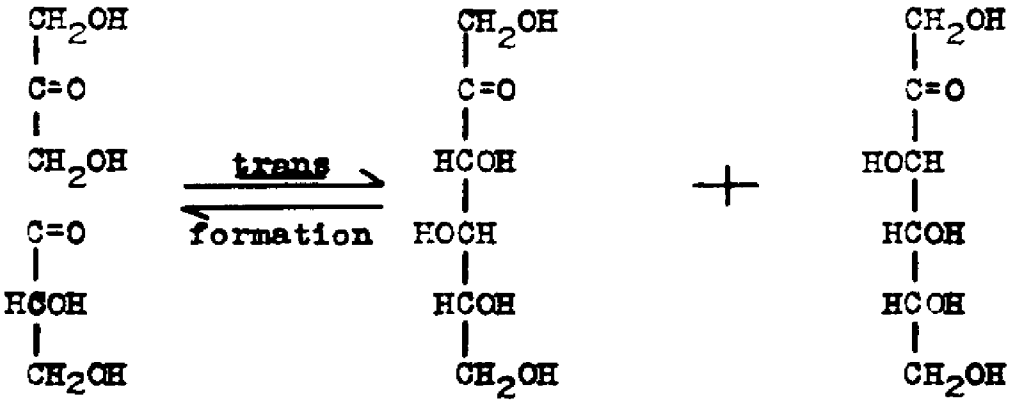
Applying a reversed aldol condensation to D-fructose we could obtain D-glyceraldehyde and dihydroxyacetone. The D-glyceraldehyde may isomerize through the corresponding enediol to the corresponding aldose, namely L-glyceraldehyde. Dihydroxyacetone may then take part in an aldol condensation with D-glyceraldehyde to produce D-fructose



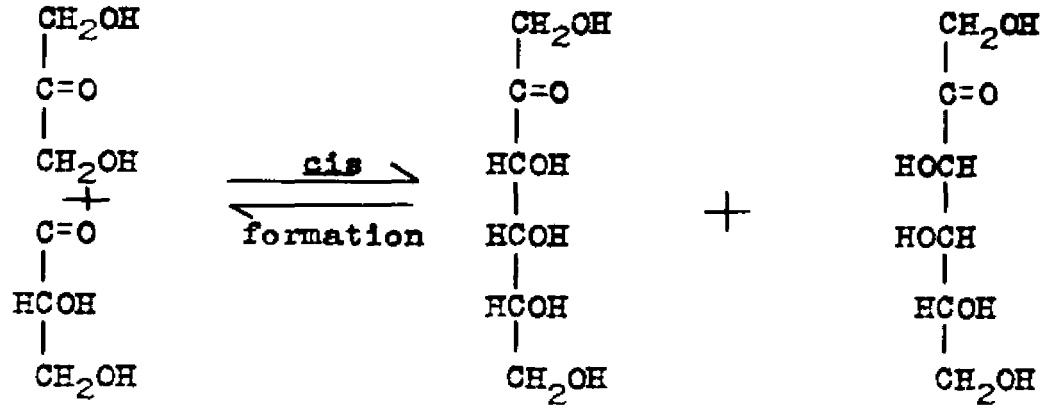


D-Glyceraldehyde enediol L-Glyceraldehyde

and D-sorbose if the hydroxyl groups for the two new asymmetric centers form trans-, and D-psicose and D-tagatose if the hydroxyl groups for the two new asymmetric centers form cis.

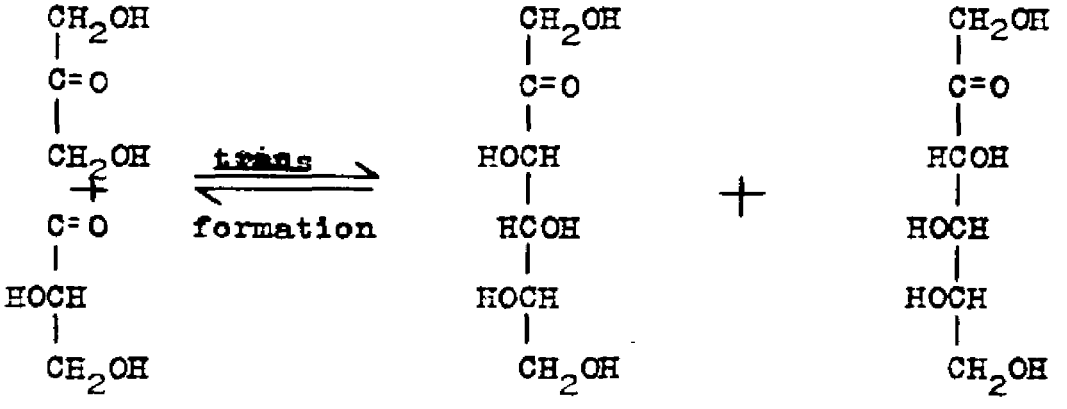


D-Glyceraldehyde D-Sorbose D-Fructose

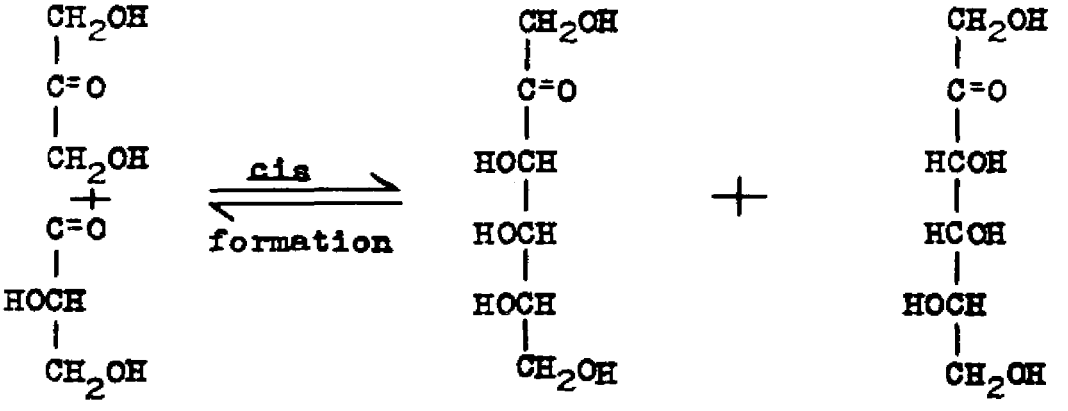


D-Glyceraldehyde D-Psicose D-Tagatose

In a similar manner, dihydroxyacetone may condense with L-glyceraldehyde to form L-fructose, L-sorbose, L-psicose and L-tagatose.



L-Glyceraldehyde L-Sorbose L-Fructose



L-Glyceraldehyde L-Psicose L-Tagatose

These newly formed ketohexoses may now isomerize through their corresponding enediols to the corresponding aldohexoses.

Since the L-form of glyceraldehyde was formed from the D-form, the D-glyceraldehyde was present in greater amounts and consequently had more opportunity to condense with dihydroxyacetone. The D-form of the newly formed ketose would as a result be expected to be formed in excess of the L-form.

The aldol condensation of glyceraldehyde and dihydroxyacetone to form ketohexoses has been demonstrated. Fischer and Baer (149)

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(149) H. O. L. Fischer and E. Baer, Helv. Chim. Acta, 19, 519 (1936).

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reported a synthesis of D-fructose and D-sorbose from D-glyceraldehyde alone, or from D-glyceraldehyde and dihydroxyacetone. The D-glyceraldehyde was allowed to stand at room temperature for two hours in the presence of barium hydroxide. The ratio of D-fructose to D-sorbose was 1:1 in a 90 to 95% yield. Fischer and Baer stated that there must be a conversion in part of the D-glyceraldehyde to dihydroxyacetone, and when these two substances in the ratio of 1:1 were allowed to react, the time was shortened from 2 hours to 40 minutes. They did not find and D-psicose or D-tagatose in the reaction mixture. Schmitz (150)

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(150) E. Schmitz, Ber., 13, 2327 (1913).

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reported formation of acrose through the action of 0.1 per cent barium hydroxide upon DL-glyceraldehyde for 24 hours. As a result of this experiment, Schmitz obtained DL-fructose (α-acrose) as crystals,

and crystalline DL-sorbose (ρ-acrose). No other isomers were found under these mild conditions: they may have been present in small amount.

B. Isolation and Characterization of DL-Sorbose and D-Sorbose.

1. Chromatographic Separation of DL-Sorbose and D-Sorbose

The chromatographic procedures of Lew, Wolfrom and Goepp(103) provided a very convenient method of separation for the DL-sorbose (racemic compound) and D-sorbose mixture from the reaction product. The sorbose mixture, being loosely held by the clay adsorbent, moved off the column into the effluent. Sorbose is very readily crystallizable and crystallized out of the effluent as a mixture of DL-sorbose and D-sorbose. The specific rotation of this mixture was $+12.40^{\circ}$ and since the specific rotation of D-sorbose is $+43.4^{\circ}$, the per cent D-sorbose and DL-sorbose in the mixture can be calculated from the equation

$$X(43.4^{\circ}) + (1-X)(0^{\circ}) = 12.40^{\circ}$$

$$43.4\ X = 12.40$$

$$X = 28.57\% \text{ D-sorbose}$$

From this equation it was found that the mixture was composed of 28.57 per cent D-sorbose and 71.43 per cent DL-sorbose, or 64.6 per cent D-sorbose and 35.3 per cent L-sorbose.

2. Catalytic Reduction of DL-Sorbose and D-Sorbose

Due to the fact that on reduction of a ketohexose a new asymmetric center is formed, two sugar alcohols (alditols) should arise. If both

of these sugar alcohols can be isolated and identified, the structure of the ketose will be established, since the pair of sugar alcohols are specific for a particular ketose. Reduction of DL-sorbose and D-sorbose should produce DL-glucitol, L-glucitol, DL-iditol and D-iditol wherein L-glucitol and D-iditol are specific for D-sorbose, and D-glucitol and L-iditol are specific for L-sorbose.

Since glucitol and iditol are inseparable on the clay column, and their acetates are likewise inseparable on a Magnesol column, these types of column chromatography were unsuitable for their separation. Differences in their crystallization properties enabled their separation by (126) fractional crystallization. DL-glucitol (a racemic compound) being readily crystalline (in contrast to D- or L-glucitol), crystallized out of the reduction product. By acetylation of the remaining portion with sodium acetate in acetic anhydride, DL-iditol hexaacetate crystallized out of the aqueous solution on hydrolysis of the acetylation mixture. Extraction of this aqueous solution with chloroform yielded D-iditol hexaacetate and L-glucitol hexaacetate, which were separated by repeated fractional crystallization from benzene-petroleum ether. Use was made of their melting points and mixed melting points with authentic samples of the known carbohydrates as well as of their x-ray powder diffraction data.

3. X-ray Powder Diffraction Characterization of DL-Sorbose and D-Sorl

DL-Sorbose crystallizes as a racemic compound, and the x-ray data for this compound should be different from that for D-sorbose. Inspection of the x-ray data tabulated in Table VI (page 47), shows

that D-sorbose has many lines whose d-spacings are very close to those present in DL-sorbose. However, the order of intensities is not the same and DL-sorbose contains extra lines. The DL- and D-sorbose mixture has lines that are in both DL-sorbose and D-sorbose but since DL-sorbose is present in excess, the pattern represents the x-ray data of DL-sorbose more than that of D-sorbose.

C. Formation and Isolation of Sodium D-Glucuronate

More extensive rearrangement of sugars in alkaline solution leads to the formation of saccharinic acids. Since these acids are deoxy acids, an internal oxidation-reduction takes place. If D-glucose (produced from the enolization of D-fructose) was subjected to intermolecular oxidation-reduction it could be conceived that carbon number six of D-glucose would be most susceptible to attack, one molecule of D-glucose being oxidized to D-glucuronic acid and the second molecule being reduced to 6-deoxy-D-glucose.

Since the lactones of glycuronic acids are not held by Amberlite IR-120 and Duilote A-4 deionizing resins, D-glucuronic acid (as its lactone) would pass through the deionizing resin columns. If the aqueous solution in the anion column happened to be on the alkaline side, the D-glucuronic lactone would pass off the column as the salt. The isolation of sodium D-glucuronate illustrates this point. Since the anion column was regenerated with sodium hydroxide, incomplete washing would leave the column on the alkaline side, enabling the formation of sodium D-glucuronate.

Uronic acids are synthesized either by reduction of the mono-

lactones of aldaric acids or by oxidation of the primary alcoholic groups of sugars or derivatives. D-glucuronic acid has been prepared (151)

(151) A. Jolles, Biochem. Z., 34, 242 (1911).

in small yield by the oxidation of D-glucose with hydrogen peroxide without catalyst at 37°. The isolation of D-glucuronic acid from the action of alkali on D-fructose has never been accomplished. Browne and Phillips (86) estimated the presence of 0.44% uronic acids, based on the ash-free solids, of Louisiana cane juice and 2 per cent uronic acids in the Louisiana molasses. The uronic acids in the cane juice are derived from the cane fiber and the pectins in the cane juice. The increase in the uronic acid content of the molasses is due to the concentration of the cane juice uronic acids in the molasses, but could also be due to the formation of the uronic acid during the milling of the juice.

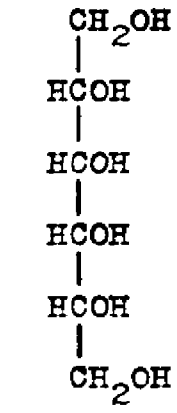
D. Fractionation and Characterization of the Zone I Material

1. Catalytic Reduction of the Zone I Material

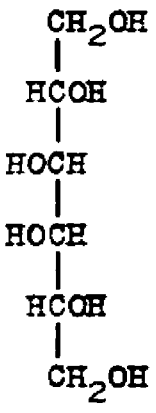
Upon reduction of the Zone I material with Raney nickel at high temperature and pressure, there were produced two crystalline sugar alcohols (crystallized as a mixture) and a small amount of a non-reducing sirup. The x-ray data for this crystalline mixture had all the main lines of allitol and some lines of dulcitol (galactitol). Acetylation of this mixture gave allitol hexaacetate, m.p. 56-57°, and dulcitol hexaacetate, m.p. 140-50°. The low melting point of dulcitol

hexaacetate is probably due to contamination with a small amount of allitol hexaacetate.

Since allitol and dulcitol are epimers around two different carbons, these alditols can not arise from the same hexose.



Allitol



Dulcitol

Allitol (a meso form) can arise from the reduction of D- or L-psicose or D- or L-allose, and dulcitol (a meso form) can arise on reduction of D- or L-tagatose or D- or L-galactose. Allitol and dulcitol could also arise from the reduction of a 3-ketohexose.

2. Paper Chromatography versus Cellulose Powder Chromatography

The use of cellulose powder column chromatography provides an excellent means for crude separation of a large sample of a carbohydrate mixture. Substances which move readily on the column are easily separated from substances moving more slowly; however, the method is very inadequate for substances moving at nearly the same rate. The use of smaller amounts of the carbohydrate mixture gives better separations, but the technique loses its purpose, requires more

work, and consumes more time.

The greater resolving power or paper strips enables adequate separation of carbohydrate substances moving at nearly the same rate. The disadvantage of the procedure is that the separation is limited to small amounts of material. The amount of time and work needed to run and isolate carbohydrates from paper strips is about equal to the time and work needed to run a cellulose powder column. Large amounts of carbohydrate mixtures can now be separated more adequately, by employing large sheets of filter paper run in a chromatocab than by cellulose powder columns.

3. Formation of DL-Allose and D-Allose

a. General

As was postulated in the above mechanism (Section A-2 of Discussion), D-fructose can be converted by fragmentation and recombination of the fragments into D- and L-psicose with the D-form being formed in excess. Under the conditions of the reactions, the newly formed DL-psicose and D-psicose may isomerize through their corresponding enediols to give DL-allose and D-allose, with D-allose forming a racemic compound.

The x-ray diffraction data of the phenylosazone obtained from the Zone I_d material (Table XV, page 78) very closely represent the x-ray data given by DL-psicose phenylosazone. All of the lines that are characteristic of DL-psicose phenylosazone are also present in the x-ray pattern of the phenylosazone of Zone I_d; however, the order of intensities is somewhat changed. This difference in intensities

as well as the presence of extra lines can be attributed to the fact that Zone I_d was heterogeneous (See Figure 5, page74) which would also make the phenylosazone heterogeneous. Furthermore, since DL-allose and D-allose were obtained, the presence of DL-psicose and D-psicose would be expected. Due to the fact that DL-psicose phenylosazone forms a racemic compound, the x-ray diffraction data of this compound were different from those of D- or L-psicose phenylosazone (see Tables XII and XV, pages 64 and 78). If Zone I_d does contain a mixture of DL-psicose and D-psicose, the x-ray diffraction data of Zone I_d phenylosazone would contain lines characteristic of the phenylosazone of both DL-psicose and D-psicose. Since DL-psicose was believed to be present in a large excess, the x-ray pattern would represent more closely the pattern of DL-psicose phenylosazone, but would also contain some of the more intense lines of D-psicose phenylosazone. The slight deviation of the Zone I_d phenylosazone x-ray pattern from the x-ray pattern of DL-psicose phenylosazone can be attributed to these facts.

This formation of DL-allose and D-allose as well as D- and L-psicose have previously never been obtained from the unfermentable sirup produced by the action of alkali on D-fructose. Wolfrom, Lew, Hales and Goepp, Jr. (126) obtained DL-glucitol and allitol from the alkaline electroduction of D-glucose. This reduction procedure produces mainly D-mannitol, but after the removal of D-mannitol, DL-glucitol and allitol were obtained. Zerban, Sattler, Rosenthal and Blauback (11) reported the isolation of an unfermentable sirup from the action of heat on D-fructose which on paper chromatography

exhibited a ketose spot characteristic of psicose. This sirup also yielded a phenylosazone (12) which gave the same x-ray diffraction data as that of the phenylosazone, m.p. 178, dec. 183°(which they proceeded to call D-psicosephenylosazone), prepared from the alcohol-soluble sirup obtained from distillery slops. The x-ray data which they record (only seven lines are given, indicating a poor pattern) very closely resembles the x-ray data given by the phenylosazone of the Zone I_d material (Table XV, page 78), indicating that they actually had DL-psicose phenylosazone in an impure state. Hough, Jones and Richards (15) obtained a small amount of sirup, specific rotation + 2.0°, by fractionation of the product obtained on treating D-glucose with ammonia on a cellulose column. This sirup showed the color reactions of a ketohexose and moved at the same rate as D-psicose.

b. Dimorphism of Allose and the Synthesis of DL-Allose

The x-ray pattern of L-allose, m.p. 128°, prepared by Humoller was different than that of D-allose, m.p. 141°, prepared by Isbell. The D-allose prepared by Isbell had a melting point of 128° when first prepared, but after remaining in the file for some time, it was transformed to the lower energy form. The optical rotation, determined by Isbell (152) showed the compound to be still in the beta-form. The

(152) H. S. Isbell, private communication.

transformation of the L-allose sample, m.p. 128 to the higher melting form was probably catalyzed by the Zone I_b material. Since the Zone I_b

was found to be a mixture of DL-allose and D-allose, the presence of crystalline L-allose, m.p. 141°, is highly probable, and could thus nucleate the lower melting form and cause its transformation.

Recrystallization of equal amounts of D-allose and L-allose produced DL-allose, a racemic compound. The x-ray data for this racemate are different from those of either optically active form. This report of the synthesis of DL-allose is the first record of its synthesis.

E. Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay

1. General

Fractionation of whole molasses on fuller's earth clay has become a convenient method for the isolation and quantitative estimation of the sugar high polymers from molasses. Cuban and blackstrap molasses and Louisiana blackstrap molasses have been fractionated (153)

(153) Report No. 2 (Feb. 12, 1951) on USDA Contract A-1$_g$-32692 Project of the Ohio State University Research Foundation, pp. 8 and 14.

by this method. The 50/50:ethanol/water and water fractions are the high polymer fractions and include 16.24% of the original molasses (A small amount of the sugar high polymers may be present also in the 80/20: ethanol/water fraction). The separation of the simple sugars (95 ethanol/ 5 water fraction) from the nitrogen-containing substances was nearly complete; the 80/20:ethanol/water fraction also contained small amounts of simple sugars.

2. Dialysis with Cellulose Membrane of the 80/20 and 50/50: Ethanol/Water and Water Fractions from the Fractionation of Florida Blackstrap Molasses on Fuller's Earth Clay

The use of dialysis as a primary method for the isolation of the sugar polymers from blackstrap molasses was delayed because the membrane would retain the insoluble substances of molasses along with the polymers. The use of this principle as a secondary or auxiliary fractionation offers definite advantages. Dialysis is a mild physical method of separation; the removal of the simple organic and inorganic impurities as well as any low molecular weight sugar polymer that may be present, from the sugar high molecular weight polymers should be quantitative.

The distribution of the solids of these fractions produced by dialysis is shown in Table XVII (page 82). The portion of these fractions passing through the membrane was greater than expected, indicating a low concentration of the sugar high polymers.

3. Deionization of the Dialyzable Portions of the 80/20 and 50/50:Ethanol/Water and Water Fractions with Ion Exchange Resins.

a. General

The dialyzable portions of the 80/20 and 50/50:ethanol/water and water fractions from the fractionation of Florida blackstrap molasses with fuller's earth clay were allowed to pass through the cation exchange resin column and then the anion exchange resin column to separate the cationic and anionic constituents and to obtain the non-ionic portion. The cationic constituents were regenerated from the cation

exchange resin with hydrochloric acid; the anionic constituents were regenerated from the anion exchange resin with sodium hydroxide and passed through the cation exchange resin a second time to remove the sodium ion. The yield of the cationic (as the hydrochlorides or as the chlorides), the anionic and the non-ionic constituents are shown in Table XVIII (page 84).

b. Amino Acid Paper Chromatography of the Deionized Fractions from the Dialyzable Fractions of Florida Blackstrap Molasses

The cationic, anionic and non-ionic constituents of each dialyzable portion of the 80/20 and 50/50:ethanol/water and water fractions were chromatographed separately on paper. The chromatograms were developed with 1-butanol saturated with a water mixture containing 80 ml. of 99.5% acetic acid per 500 ml. of water; the amino acid spots were located with ninhydrin reagent. Several amino acids (asparagine, glutamic acid and aspartic acid) were also run as controls. As shown in Figure 6 (page 86), the cationic constituents of the 80/20 and 50/50:ethanol/water and water fractions contained the majority of the amino acids, with the water fraction containing only three faint spots. The anionic and non-ionic constituents from all three fractions did not give any spots with ninhydrin.

The deionization of the prepared molasses fractions seems to provide a very good method for the isolation of amino acids in molasses.

Previous work (94) found the 80/20 and 50/50:ethanol/water fractions from the fractionation of Florida blackstrap molasses on fuller's earth clay to contain the majority of the amino acids.

However, the fractions were not deionized and the amino acids were not separated. By paper chromatography (94) the 80/20:ethanol/water fraction produced spots indicating the presence of glycine, alanine, γ-aminobutyric acid, valine and leucine (or isoleucine); the 50/50: ethanol/water fraction, aspartic acid, glutamic acid and asparagine. Figure 6 (page 86) shows that the anionic portion of the 50/50:ethanol/ water fraction contains three spots indicating the presence of aspartic acid, glutamic acid and asparagine, but in addition it shows four other spots. These extra spots are probably amino acids that are present in the 80/20:ethanol/water fraction, which were not completely removed in the original fractionation.

c. Organic Acid Paper Chromatography of the Deionized Fractions from the Dialyzable Fractions of Florida Blackstrap Molasses

The cationic, anionic and the non-ionic constituents of each dialyzable portion of the 80/20 and 50/50:ethanol/water and water fractions were chromatographed separately on paper. The chromatograms were developed with 1-butanol saturated with a water mixture containing 80 ml. of 99.5% acetic acid per 500 ml. of water. Several organic acids (L-malic, aconitic and citric acid) were run as controls. The chromatograms were sprayed with brom-phenol-blue in a 95/5:ethanol/water solution. Examination of Figure 7 (page 87) shows that the anionic portion of the 80/20:ethanol/water fraction has five distinct spots with a faint smearing between the two slowest moving spots. Since amino acids can give the same type of spot with brom-

phenol-blue, they were shown to be absent in this fraction when it failed to develop any spots with ninhydrin (Figure 6, page 86). The anionic portion of the 50/50:ethanol/water fraction gave a spot which moved at the same rate as aconitic acid, and a second spot moving at the same rate as citric acid. Also there was produced a long streak which is due to the sugar polymer present. Since the polymer is known to be acidic it would be expected to react with the brom-phenol-blue. The anions of the water fraction show only a streak, which is also due to the sugar polymer. The continuous streak shown by the cations of the 80/20 and 50/50:ethanol/water fractions and the one spot in the water fraction was probably due to the amino acids present in these fractions.

As shown by the anionic portions of the 50/50:ethanol/water and water fractions any low molecular weight sugar polymer would have to be present in these fractions. Since the polymer is acidic it would remain attached to the anion exchange resin and on regeneration it would be present in the anionic portions of these fractions.

d. Sugar Paper Chromatography of the Non-Ionic Portions of the Deionized Dialyzable Fractions from Florida Blackstrap Molasses

The non-ionic constituents of each dialyzable portion of the 80/20 and 50/50:ethanol/water and water fractions were chromatographed along with D-glucose, D-fructose, sucrose and D-glucurono-γ-lactone. The chromatogram was developed with a mixture of 1-butanol/water/ethanol:4/1.9/1.1 and the sugars were indicated with

p-anisidine hydrochloride solution. The non-ionic portion of the 80/20:ethanol/water fraction gave three spots, one moving at the same rate as D-fructose, another moving at the same rate as D-glucose and a third at the same rate as D-sucrose. In previous work (154) definite spots for these sugars in the 80/20 fraction

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(154) Report No. 4 (July 25, 1951) on USDA Contract A-1$_s$-32692, Project 427 of the Ohio State University Research Foundation, pp. 15-17.

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were not obtained, although the presence of sucrose in the 80/20: ethanol/water fraction was demonstrated previously (36). The non-ionic portion of the water fraction did not produce any spots with p-anisidine hydrochloride, but the cationic portion of the 50/50: ethanol/water fraction gave a spot characteristic of a uronic acid and moving at the same rate as D-glucuronic acid γ-lactone.

V. SUMMARY

1. The action of potassium hydroxide at 100° on D-fructose with a potassium aconitate buffer (pH 8.6, dipotassium aconitate) has been investigated. The reaction product was deionized on ion exchange resins and the nonfermented residue was chromatographed on fuller's earth clay. Two major zones and a minor zone were produced. A crystalline substance was obtained from one of the major zones (column effluent) and a second crystalline substance from the minor zone.

2. Reduction of the crystalline substances isolated from the column effluent yielded D-iditol, L-glucitol, DL-iditol and DL-glucitol. Acetylation and fractional crystallization of the acetates were employed in separating these sugar alcohols. X-ray diffraction data were used to characterize them.

3. DL-Iditol hexaacetate was prepared and characterized for the first time.

4. The crystalline material present in the minor zone was identified as sodium D-glucuronate by its melting point, optical rotation and x-ray powder diffraction data.

5. Attempts to prepare a crystalline derivative of the column major zone (Zone I), failed to give a definitive derivative in each case.

6. Catalytic reduction of Zone I gave a mixture of allitol and dulcitol.

7. Acetylation of the Zone I reduction mixture gave the hexa-acetate of allitol and of dulcitol (galactitol), identified by their melting points and x-ray diffraction data.

8. Column chromatography failed to resolve further the Zone I material.

9. Partition chromatography on paper sheets resolved the Zone I material into five distinct zones.

10. DL-Allose and D-allose were isolated from one of the Zone I fractions by elution from paper strips. Identification was made by melting point, x-ray diffraction data and optical rotation.

11. DL-Allose was prepared for the first time.

12. The dimorphism of allose, supported by x-ray data, is described.

13. A second Zone I fraction showed on sugar paper chromatography a spot characteristic of a ketose and moving at the same rate as psicose.

14. Florida blackstrap molasses was fractionated on fuller's earth clay by adsorbing a thick sirup of the cane juice solids on clay by extracting the mixture while it was resting on a bed of clay with 95/5, 80/20, 50/50:ethanol/water and water, respectively.

15. The 80/20, 50/50:ethanol/water and water fractions from the fractionation of Florida blackstrap molasses on fuller's earth clay were dialyzed with a cellulose membrane. The per cent of these fractions passing through the membrane was determined.

16. The dialyzable portions of the 80/20, 50/50:ethanol/water and water fractions were deionized by passage through ion exchange resins. The cationic constituents were regenerated with hydrochloric acid. The anionic constituents were regenerated with sodium hydroxide and decationized by passage again through a cation exchange resin.

17. The ionic and non-ionic constituents were subjected to amino acid, organic acid and sugar paper chromatography. Amino acids were found only in the cationic portions of the 80/20 and 50/50:ethanol/water fractions and to a small extent in the cationic portions of the water fractions. The anionic portion of the 80/20:ethanol/water fraction showed the presence of five different organic acids, while the anionic portion of the 50/50:ethanol/water fraction showed a spot moving at the same rate as aconitic acid. The sugar high polymer appeared to be concentrated in the anionic portions of the 50/50:ethanol/water and water fractions. The non-ionic portion of the 80/20:ethanol/water fraction contained spots moving at the same rate as D-fructose, D-glucose and sucrose plus a very slow moving spot. The non-ionic portion of the 50/50:ethanol/water fraction exhibited a spot characteristic of a uronic lactone and moving at the same rate as D-glucurono-γ-lactone.

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In August, 1950, I came to the Ohio State University in order to begin study toward an advanced degree. I held an Ohio State University Research Foundation assistantship with the U. S. Department of Agriculture from August 1950 to September 1951. In October 1951, I accepted a teaching assistantship which lasted till March, 1952. From April to October, 1952, I was awarded a DuPont Research Assistantship, and it was during this period (August, 1952) that I received the degree of Master of Science.

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