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

TABLE II

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

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

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

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

TABLE III

TIME REQUIRED IN HOURS FOR 90% SUCROSE TO BE UTILIZED

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

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

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

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

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

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

The Action of Alkali on D-Fructose[1]

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

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

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

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

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

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

solution 0.2 *M* in D-fructose and 0.2 *M* in *trans*-aconitate was heated for 24 hr.[4] at 100°. The final *p*H was 6.15. Table I shows the rate of coloration and *p*H change occurring in the solution. The aconitic acid initially added was the *trans* form. In solution this is known to undergo some change to the *cis* isomer.[3,5] The equilibria involved are not presently defined but are functions of *p*H and temperature.

TABLE I

RATE OF CHANGE IN *p*H AND COLOR OF A SOLUTION OF D-FRUCTOSE (*c* 12) HEATED AT 100° WITH AN EQUIMOLAR QUANTITY OF TRIPOTASSIUM *trans*-ACONITATE

Time, hr.	$T_{490m\mu}$, %[a]	*p*H[b]	Time, hr.	$T_{490m\mu}$, %[a]	*p*H[b]
0	98	8.00[c]	4	19	6.50
1	92	7.50	7	7	6.38
2	50	6.85	9.5	4	6.32
3	27	6.65	24	0	6.15

[a] Lumetron photoelectric colorimeter (model 400). [b] Beckman *p*H meter (model G) standardized at *p*H 7.00 against phosphate buffer. [c] An amount of 3.05 equiv. of potassium hydroxide required (see ref. 2).

The amber colored reaction solution was deionized with exchange resins and excess D-fructose was removed by yeast fermentation. The resultant sugar mixture was chromatographed on clay with 90% ethanol–water as developer.[6] From the effluent there was obtained a crystalline material, m.p. 158.5–159.5°, $[\alpha]^{25}D$ +12.4° (water).

The above apparently homogeneous substance possessed the analysis and properties of a ketohexose but its constants were not those exhibited by any of the known eight 2-ketohexoses. It was at first considered that the substance might be one of the 3-ketohexoses postulated by Nef[7] to be present in alkaline solutions of hexoses. Reduction with hydrogen and Raney nickel catalyst at high pressure, led to the separation of the readily crystallizable DL-glucitol[8] (a racemic compound), identified by melting point, mixed melting point, and X-ray powder diffraction data. Further identification was effected through its crystalline hexaacetate which was compared with an authentic specimen[8] by melting point, and by its X-ray powder diffraction lines. Fractional crystallization of the acetylated mother liquor material from the isolation of DL-glucitol led to the separation of the crystalline hexaacetate of L-glucitol,[8] D-iditol[9] and DL-iditol. Identification was made through comparison with authentic specimens by melting point behavior and by their X-ray powder diffraction lines. DL-Iditol hexaacetate was unrecorded but was synthesized from known material. The X-ray powder diffraction data characterize DL-glucitol hexaacetate and DL-iditol hexaacetate as true racemic compounds.

The above mixture of alditols could not have arisen through the reduction of any 3-ketohexose but it could have been formed by the reduction of a mixture of DL-sorbose and D-sorbose. Finally, the X-ray powder diffraction data of the isolated crystals were compared with those of authentic specimens of DL-sorbose[10] and D-sorbose[10–12] (Table II). From these data it can be determined that DL-sorbose (m.p. 159–162°) is a true racemic compound, in verification of the conclusion arrived at by Adriani[13] on the basis of solubility data. Our isolated crystals were mainly DL-sorbose and from their optical rotation, +12.4°, the proportion of D-sorbose to L-sorbose was very closely 2:1.[14]

TABLE II

X-RAY[a] POWDER DIFFRACTION INTERPLANAR SPACINGS FOR DL-SORBOSE, D-SORBOSE AND CRYSTALLINE MATERIAL FROM EFFLUENT OF CLAY CHROMATOGRAM

d-Spacings,[d] Å.					
DL-Sorbose[b]	Isolate	D-Sorbose[c]	DL-Sorbose[b]	Isolate	D-Sorbose[c]
4.44	4.55	4.49	3.84	3.82	3.28
3.18	3.20	5.22	3.70	3.71	2.40
6.27	6.11	6.21	2.16	2.17	1.82
2.39	2.40	3.70	5.61	5.61	1.74
5.18	5.25	3.21	2.72	2.75	2.64
			1.81	1.81	

[a] CuKα radiation. [b] M.p. 159–162°. [c] M.p. 162–165°. [d] Arranged in decreasing order of intensities; estimated visually.

The principal zone material from the original clay chromatogram was a sirup that was mainly ketose in character (30% apparent aldohexose by hypoiodite oxidation) and was slightly dextrorotatory (+3°). Reduction[14a] of the crude zone material to the alditol state was effected in methanol (97%) solution at room temperature with hydrogen (20 p.s.i.) and a large excess of active Raney nickel catalyst prepared according to Pavlic and Adkins.[15] From the reduced product there was isolated a mixture of allitol and galactitol (dulcitol), the separation and identification of which was effected through their crystalline hexaacetates.

Following the general technique of Flood, Hirst and Jones,[16] the crude, unreduced main zone material, from the original clay chromatogram, was chromatographed on cellulose sheets, development being effected with 1-butanol:ethanol:water and indication with *p*-anisidine. A total of 2.75 g. of the zone material was so chromatographed, about 70 sheets being required. The material was a complex mixture and was further separated into 5 zones which by indicator color were presumably mainly ketose (zones I_a and I_d), aldose (zone I_b) and uronic acid (zones I_c and I_e, small in amount).

The material from the aldose zone I_b ($[\alpha]D$ +4.3°) deposited crystals which by melting point

(4) The time factor was exaggerated over mill conditions.

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

(6) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., THIS JOURNAL, **68**, 1449 (1946).

(7) J. U. Nef, *Ann.*, **403**, 208 (1914).

(8) M. L. Wolfrom, B. W. Lew, R. A. Hales and R. M. Goepp, Jr., THIS JOURNAL, **68**, 2342 (1946).

(9) G. Bertrand and A. Lanzenberg, *Bull. soc. chim.*, [3] **35**, 1073 (1906).

(10) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **19**, 5 (1900).

(11) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *ibid.*, **16**, 262 (1897).

(12) M. L. Wolfrom, S. M. Olin and E. F. Evans, THIS JOURNAL, **66**, 204 (1944).

(13) J. H. Adriani, *Rec. trav. chim.*, **19**, 183 (1900).

(14) Mary Grace Blair and J. C. Sowden (personal communication) likewise have determined, by a different method, that a mixture of DL-sorbose and D-sorbose is formed by the action of alkali upon D-glucose; THIS JOURNAL, **77**, 3323 (1955).

(14a) Yvonne Khouvine, *Compt. rend.*, **204**, 983 (1937).

(15) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

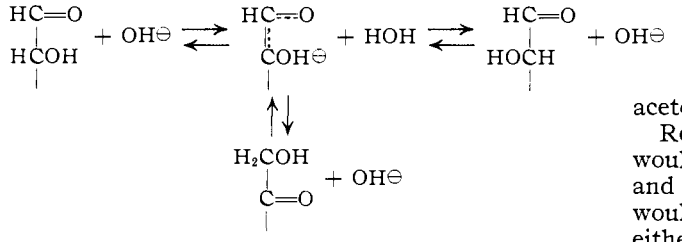
(16) A. E. Flood, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1679 (1948).

behavior and X-ray powder diffraction data, in comparison with authentic specimens,[17] established the material as a mixture of DL-allose with another form of allose, probably D-allose.

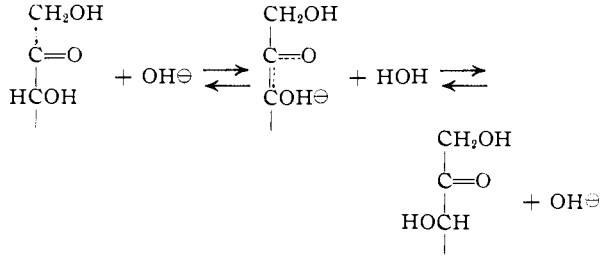
The material from the ketose zone I_d formed a crystalline phenylosazone which by melting point behavior and X-ray powder diffraction lines, in comparison with authentic specimens,[17] was mainly DL-*ribo*-hexose phenylosazone[18] admixed with other crystalline material which may have been D (or L-)-*ribo*-hexose phenylosazone. Since this originated from a zone that was apparently ketose in nature, this constitutes presumptive, but not definitive, evidence for the presence of the ketohexose psicose. A definitive identification of D-psicose as a product of the action of ammonium hydroxide upon D-glucose, has been reported by Hough, Jones and Richards.[19]

While the above outlined experimental results were obtained in a study designed to elucidate certain aspects of cane final molasses formation, they also can be considered as a contribution to the interpretation of the action of alkali upon reducing sugars. The identification of crystalline sodium D-glucuronate monohydrate and the paper chromatographic evidence for the presence of uronic acids in the reaction mixture, is difficult to reconcile with such a premise.

The initial effect induced upon reducing sugars by alkali is the well-established interconversion through the 1,2-enediolate ion,[20] involving the subtraction and addition of protons in the Lowry sense.[21,22]



It can be postulated that the 2-ketose so formed may undergo 2,3-enediolate formation and the production of D-sorbose from D-galactose[10] seems best so explained.[20]



Further effects of alkali produce disproportionation with fragmentation. In the hexose series this leads to the formation of such three-carbon fragments as pyruvaldehyde,[20,23,24] DL-lactic acid,[20,23,24] acetol,[24a] diacetyl[24b] and reductone.[25,26] Disproportionation, without apparent fragmentation, produces the various types of saccharinic acids. That a fragmentation with a recombination of fragments may be involved here has been proposed by Sowden and Kuenne.[27]

In previous work from this Laboratory, it had been demonstrated that DL-glucitol[8] and allitol[28] were formed in the alkaline electroreduction of D-glucose concomitant with the formation of D-mannitol and D-glucitol (sorbitol). It was proposed[8] at the time that dehydrogenation at the electrode surface may have produced new carbonyl groups which were subsequently reduced to alditols. The like production of members of the L-series in the present instance cannot be so explained. If they were formed by progressive enolization down the carbon chain, it would necessitate the assumption of 4,5-enediols. It appears more probable that equilibria are concerned which involve reverse aldolization. The conception of a reverse aldolization to interpret sugar fragmentation was proposed by Bernier and Evans[29,30] and has been utilized by others.[24,31,32]

By a reverse aldolization, probably involving an enediolate ion, D-fructose can give rise to dihydroxyacetone and D-glyceraldehyde. The latter can be interconverted to L-glyceraldehyde. Recombination by aldolization in the *trans* fashion can then give rise to DL-sorbose and DL-fructose. Since D-sorbose is in excess, the direct recombination in the D-form must predominate. That sorbose and fructose can be formed by the alkali-catalyzed aldolization of dihydroxyacetone and glyceraldehyde has been established.[33,34]

Recombination by aldolization, in the *cis* form would give rise to D-tagatose, L-tagatose, D-psicose and L-psicose. Interconversion of the latter two would yield D-allose and L-allose. Reduction of either D-tagatose or L-tagatose would produce galactitol (dulcitol) while allitol could arise from any of the allose or psicose enantiomorphs.

The ketose fraction obtainable by the action of alkali upon D-fructose thus is shown to be complex

(17) M. L. Wolfrom, J. N. Schumacher, H. S. Isbell and F. L. Humoller, THIS JOURNAL, **76**, 5816 (1954).

(18) Nomenclature of J. C. Sowden, *ibid.*, **69**, 1047 (1947).

(19) L. Hough, J. K. N. Jones and E. L. Richards, *J. Chem. Soc.*, 2005 (1953); see also F. W. Zerban and L. Sattler, *Ind. Eng. Chem.*, **34**, 1180 (1942).

(20) W. L. Evans, *Chem. Revs.*, **31**, 537 (1942); J. C. Sowden and R. Schaffer, THIS JOURNAL, **74**, 505 (1952).

(21) M. L. Wolfrom with W. L. Lewis, *ibid.*, **50**, 837 (1928).

(22) T. M. Lowry, *J. Chem. Soc.*, **127**, 1371 (1925).

(23) W. L. Evans, *Chem. Revs.*, **6**, 281 (1929).

(24) R. Montgomery, *Sci. Rept. Series, Sugar Research Foundation* New York, N. Y., **11** (1949).

(24a) A. Emmerling and G. Loges, *Ber.*, **16**, 837 (1883); O. Baudisch, *Biochem. Z.*, **89**, 279 (1918).

(24b) R. Nodzu and R. Goto, *Bull. Chem. Soc. Japan*, **11**, 381 (1936).

(25) H. von Euler and H. Hasselquist, "Reduktone," Ferdinand Enke, Stuttgart, 1950.

(26) J. Moura Goncalves and Aida Hasson, *Anais acad. brasil. cienc.*, **24**, 221 (1952); *C. A.*, **47**, 2647 (1953).

(27) J. C. Sowden and Dorothy J. Kuenne, THIS JOURNAL, **75**, 2788 (1953).

(28) M. L. Wolfrom, B. W. Lew and R. M. Goepp, Jr., *ibid.*, **68**, 1443 (1946).

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

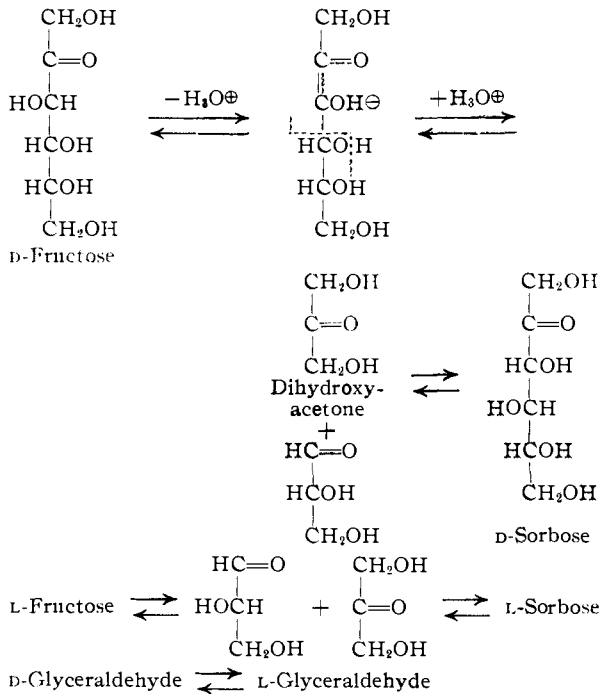
(30) W. L. Evans, *J. Org. Chem.*, **1**, 6 (1936).

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

(32) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 1784 (1954).

(33) E. Schmitz, *Ber.*, **46**, 2327 (1913); W. G. Berl and C. E. Feazel, THIS JOURNAL, **73**, 2054 (1951).

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



and accordingly other ketoses than D-fructose can be predicted in the ketose fraction[21] arising from D-glucose by the action of alkali, in agreement with the data of other workers.[35] The assumption of Gottfried and Benjamin[35] and others,[36] however, that di-D-fructose dianhydrides are formed in these alkaline solutions, is not supported by the experimental facts.

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Experimental

Treatment of D-Fructose with Hot Alkali in an Aconitate Solution and Isolation of Product.—A solution of 34.8 g. (0.2 mole) of *trans*-aconitic acid in 100 ml. of water was adjusted to just beyond neutrality[2] by adding potassium hydroxide (3.05 equiv.) until a *p*H of 8.00 was attained. An amount of 38 g. (0.211 mole) of D-fructose was dissolved in this solution and diluted to 317 ml. to yield a resultant concentration of 12 g. of D-fructose per 100 ml. of solution. The resultant solution was then heated at 100° for 24 hr. and the *p*H and color changes effected are shown in Table I.

The amber colored reaction mixture was cooled to room temperature, diluted with water to a total volume of 3000 ml. and deionized by successive passage through 67 × 7 cm. (diam.) columns of Amberlite IR-120[37] and Duolite A-4.[38] The final effluent was brought to neutrality with sodium hydroxide. Excess D-fructose was largely removed by one fermentation for 120 hr. at 28–30° with 22 g. of starch-free baker's yeast. The fermented solution was filtered with suction through Celite[39] and the colorless, neutral filtrate was dewatered at 45–50° under 28 mm. pressure to a sirup; yield 8.62 g. (dried to constant weight over phosphorus pentoxide).

Chromatography on Clay of the Non-fermented Residue.—The above sirup (8.62 g.) was dissolved in 100 ml. of abs. methanol and the solution was divided into 3 equal parts. Each of the parts (2.87 g. in 33.3 ml.) was diluted to 100 ml. with abs. methanol and added to the top of a tapered glass column[40] containing a 23 × 7.5 cm. (diam.) adsorbent column of 600 g. of Florex XXX[41]–Celite[42] (7:1 by wt.) prewashed with 5000 ml. of 95/5[43] ethanol/water and conditioned further with 100 ml. of abs. methanol. Development was effected 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 hr., the exposed column area was streaked with alkaline permanganate indicator (1 part of potassium permanganate in 100 parts of 2.5 *N* sodium hydroxide), employing a fine-tipped glass pipet. Three zones were indicated: a top zone at 18–45 mm. from the column top; a middle zone at 67–201 mm. (hereafter designated zone I); and a bottom zone at 210–230 mm. (bottom of column). The sectioned top zone and bottom zone materials were each eluted with 1500 ml. of 70/30 ethanol/water and the zone I material with 3000 ml. of the same solvent. Solvent removal under reduced pressure from each zone elution and from the effluent, yielded sirups; yield (from the 3 chromatograms combined): 0.290 g. from the column top, 0.838 g. from the top zone, 4.09 g. from zone I, 0.630 g. from the bottom zone, 2.12 g. from the effluent, 7.97 g. total (92.5% recovery).

An amount of 40 g. of the D-fructose was fermented with yeast and the fermentation residue was chromatographed as described above. No zones were detected on the column and only glycerol was found in the effluent.

Isolation of DL-Sorbose and D-Sorbose Mixture from the Effluent of the Clay Chromatogram.—The sirup from the effluent produced crystals on storage in a desiccator over calcium chloride. A small amount of abs. ethanol was added to facilitate crystallization. The ethanol solution was removed by decantation and the residual crystals were dissolved in 4 ml. of water and treated twice with 15 mg. of decolorizing carbon (Darco G-60[44]) for 5 min. at 50° with subsequent suction filtration through acid-washed asbestos fiber followed by a final gravity filtration through analytical filter paper (to remove siliceous material). A final product was obtained on solvent removal under reduced pressure; m.p.[45] 158.5–159.5°, $[\alpha]^{25}D$ +12.4° (*c* 2.9, water); X-ray powder diffraction data listed in Table II.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.71. Found: C, 40.09; H, 6.79.

Characterization of the DL-Sorbose and D-Sorbose Mixture by Reduction to a Mixture of DL-Glucitol, DL-Iditol, D-Iditol and L-Glucitol; Their Separation and Identification.—An amount of 500 mg. of the above isolated sorbose mixture was dissolved in 50 ml. of water and reduced with 2 g. of Raney nickel[15] at an initial pressure of 19 atm. of hydrogen by heating at 80° for 3 hr. A small amount of ethanol was added to the contents of the cooled bomb to coagulate the catalyst which was then removed by filtration. The filtrate gave negative Benedict and Molisch tests and was dewatered under reduced pressure and the resultant sirup was dried to constant weight over phosphorus pentoxide; yield 470 mg. The sirup partially crystallized when maintained over phosphorus pentoxide for several weeks, whereupon crystallization was facilitated by the addition of

(35) J. C. Sowden and R. Schaffer, THIS JOURNAL, **74**, 499 (1952); J. B. Gottfried and D. G. Benjamin, *Ind. Eng. Chem.*, **44**, 141 (1952).

(36) L. Sattler and F. W. Zerban, *ibid.*, **37**, 1133 (1945).

(37) A cation exchange resin manufactured by the Resinous Products and Chemical Co., Philadelphia, Pa.

(38) An anion exchange resin manufactured by the Chemical Process Co., Redwood City, Calif.

(39) No. 535, a siliceous filter-aid manufactured by the Johns-Manville Co., New York, N. Y.

(40) Manufactured by the Scientific Glass Co., Bloomfield, N. J.

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

(42) Ref. 39; No. 545; material passing an 80 (per inch) mesh screen was employed.

(43) Volume ratios before mixing.

(44) A product of the Darco Department, Atlas Powder Co., New York, N. Y.

(45) All melting points are corrected.

methanol; yield 220 mg. (fraction A). Solvent removal from the mother liquor left a sirup that was dried over phosphorus pentoxide; yield 240 mg. (fraction B).

A portion of the above crystalline material (fraction A) was recrystallized twice from water–methanol to yield fluffy white needles, m.p. 132–134° (135–137° recorded[8] for DL-glucitol); X-ray powder diffraction data (identical with those of an authentic specimen): 5.91 (2),[46] 4.41 (1), 3.87 (3), 3.58 (4), 2.78, 2.63, 2.36, 2.13, 1.92, 1.64.

Acetylation of a portion (100 mg.) of fraction A at 80° for 1 hr., with 5 ml. of acetic anhydride and 50 mg. of powdered, twice-fused sodium acetate, gave after hydrolysis of the excess anhydride with 25 g. of crushed ice, followed by extraction with chloroform, a crystalline acetate from benzene–petroleum ether (b.p. 30–60°); yield 151 mg., m.p. 113–114° and 114–115° on admixture with an authentic specimen (m.p. 116–117°) of DL-glucitol hexaacetate[8]; X-ray powder diffraction data[46] (identical with those of an authentic specimen): 6.19, 5.20 (1), 4.35 (2), 3.87, 3.47 (3), 3.20 (4), 3.01, 2.20, 1.92, 1.68.

Fraction B (240 mg.) above was acetylated as described for fraction A except that the aqueous reaction mixture was not extracted with chloroform and produced crystals, on standing overnight at 3°, which were twice crystallized from benzene–petroleum ether (b.p. 30–60°); yield 340 mg. (fraction C), m.p. 155–163°.

The filtrate from the above material (fraction C before recrystallization), was neutralized to *p*H 6 with solid sodium bicarbonate and extracted with five 10-ml. portions of chloroform. Solvent removal from the combined extracts gave a sirup; yield 104 mg. When this sirup was dissolved in benzene and petroleum ether was added, crystallization occurred on standing. These crystals (fraction D) were recrystallized from benzene–petroleum ether; m.p. 117–119° undepressed on equal admixture with an authentic specimen of D-iditol hexaacetate (m.p. 121–122° recorded[9]), m.p. 160–162° in equal admixture with an authentic specimen of L-iditol hexaacetate[47] (see below); X-ray powder diffraction data[46] (identical with those of an authentic specimen of D-iditol hexaacetate): 7.34 (3), 6.51, 5.56 (5), 4.46 (1), 4.07 (4), 3.56 (2), 3.01, 2.74, 2.22, 1.87.

Solvent removal from the mother liquor of fraction D above 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 and the separated solid (partially crystalline) was removed by decantation. This process was repeated until on the fourth repetition the separated solid was entirely crystalline. There was so obtained a small amount of L-glucitol hexaacetate, yield 5 mg., m.p. 93–96°, m.p. 94–96° in equal admixture with an authentic specimen (m.p. 98–99°)[8] of L-glucitol hexaacetate; X-ray powder diffraction data[46] (identical with those of an authentic specimen): 6.92 (3), 5.05 (1), 4.40 (5), 3.72, 3.41 (2), 3.20, 2.93 (4), 2.64, 2.36, 2.09. When this product was recrystallized from benzene–petroleum ether with an equal amount of D-glucitol (sorbitol) hexaacetate, DL-glucitol hexaacetate was obtained; m.p. 111–115° (116–117° recorded[8]); X-ray powder diffraction data identical with those cited above for an authentic specimen of DL-glucitol hexaacetate.

Fraction C above (m.p. 155–163°) was washed with abs. ethanol (evaporation of this extract yielded D-iditol hexaacetate) and when recrystallized from benzene–petroleum ether gave white, fluffy needles, m.p. 160–162°, m.p. 162–163° on admixture with an authentic specimen of DL-iditol hexaacetate (m.p. 162–163°) described below; X-ray powder diffraction data identical with those described below.

DL-Iditol Hexaacetate.—L-Iditol hexaacetate was prepared, according to Khouvine and Arragon,[48] by the catalytic reduction (with subsequent acetylation) of *keto*-L-sorbose pentaacetate in abs. ethanol with Raney nickel[15] and hydrogen (7 p.s.i.) at room temperature; m.p. 118–119° (121–122° recorded[47]). D-Iditol hexaacetate was prepared in the same manner from *keto*-D-sorbose pentaacetate.[49]

Equal amounts (100 mg.) of the D- and L-forms of iditol hexaacetate were recrystallized thrice from benzene–petroleum ether to yield DL-iditol hexaacetate, m.p. 162–163°; X-ray powder diffraction data[46]: 7.80 (3), 6.81 (5), 5.52 (4), 4.41 (2), 3.91, 3.48 (1), 2.75, 2.34, 2.15, 1.89.

Isolation and Characterization of Sodium D-Glucuronate Monohydrate.—The top zone material (838 mg.) from the clay chromatogram was dissolved in 5 ml. of water and treated with carbon to remove siliceous material, as described above for the effluent substance. The sirup obtained on solvent removal under reduced pressure was crystallized at room temperature from abs. methanol by the addition of abs. ethanol followed by recrystallization from water–ethanol; yield 42 mg., m.p. 150° (dec. with frothing), unchanged on admixture with an authentic specimen of sodium D-glucuronate monohydrate of like melting point behavior, $[\alpha]^{24}D$ +21.5° (*c* 1.42, water) (+21.3° found for an authentic sample); X-ray powder diffraction data[46] (identical with those of authentic material): 7.34, 5.45 (4), 4.25 (1), 3.54, 3.24 (2), 2.95, 2.53 (3), 2.30, 2.14 (5), 1.77, 1.62.

Identification of Allitol and Galactitol in the Reduction Product from Zone I of the Clay Chromatogram.—The above-described zone I material (4.09 g.) gave a strong Seliwanoff ketose reaction and showed an apparent aldohexose content of 30.4% by the Cajori hypoiodite assay[50]; $[\alpha]^{28}D$ +3.2° (*c* 9.04, water).

An amount of 810 mg. of the zone I material was dissolved in 5 ml. of water, diluted to 150 ml. with abs. methanol and reduced with 4 g. of Raney nickel[15] at room temperature for 21 hr. under a pressure of 20 p.s.i. of hydrogen. The non-reducing (to Benedict solution) sirup obtained on solvent removed from the filtered reaction mixture was crystallized from methanol–ethanol and recrystallized from water–ethanol; yield 750 mg. (fraction E), m.p. 130–140°. X-Ray powder diffraction data characterize this material as a mixture of allitol and galactitol (dulcitol): 4.65,[46,51] 5.88, 3.41,[52] 3.06, 247, 6.38,[52] 2.74, 2.01, 4.41, 3.25,[52] 2.21, 2.28, 1.95[52]; allitol: 4.67,[46,51] 5.84, 3.37, 3.04, 2.48, 2.72, 2.02, 4.39, 3.28, 2.20, 2.28; galactitol: 4.74,[46,51] 3.65, 3.43, 4.20, 3.54, 6.43, 2.79, 2.47, 2.88, 1.95, 3.23.

An amount of 100 mg. of fraction E above was acetylated with 5 ml. of acetic anhydride and 500 mg. of twice-fused sodium acetate by heating to 100° to effect solution followed by 45 min. at 80°. The cooled reaction mixture was poured onto 50 g. of crushed ice, neutralized to *p*H 6 with sodium bicarbonate, and extracted with four 10-ml. portions of chloroform. The sirup obtained on solvent removed from the combined chloroform extracts was freed of acetic acid by repeated additions of benzene with subsequent removal under reduced pressure. Crystals formed in the resultant sirup on standing and were removed by filtration after the addition of 95% ethanol. They were recrystallized from ethanol–petroleum ether to yield prisms of galactitol (dulcitol) hexaacetate of low melting point, m.p. 140–150° (171° recorded[53]); X-ray powder diffraction data[46] (identical with those of an authentic specimen of galactitol hexaacetate): 6.86 (4), 4.87 (1), 4.48, 3.95 (5), 3.66, 3.43 (2), 3.19 (3), 2.65, 2.25, 1.95.

The 95% ethanol mother liquor from the above crystallization, slowly crystallized on standing for several weeks in a desiccator over calcium chloride and phosphorus pentoxide. Prisms were obtained on recrystallization from ethanol–petroleum ether; m.p. 56–57°, m.p. 58–59° on admixture with an authentic specimen of allitol hexaacetate (m.p. 61°)[54]; X-ray powder diffraction data[46] (identical with those of an authentic specimen): 6.81, 5.54, 5.11, 4.65 (1), 3.99 (3), 3.72, 3.51 (2), 3.10 (4), 2.61 (5), 2.09.

Isolation of DL-Allose and D(?)-Allose and Indication of DL-Psicose in Zone I Material by Paper Strip Chromatography.—Following the general technique of Flood, Hirst and Jones,[18] 5 ml. of a 6% aqueous solution of the zone I material (300 mg.), from the above-described clay chromatogram, was placed on 8 tapered sheets (5.5 × 18 in.) of Whatman No. 1 paper and this was repeated until 2.75 g. had been added to *ca.* 70 sheets. The descending chromatograms were developed in a cabinet with 4/1.1/1.9 1-butanol/ethanol/water (vol. ratio) for 40–45 hr. Side-strip

(46) CuKα radiation; interplanar spacings, A.; relative intensity measured visually, (1) most intense.

(47) G. Bertrand, *Ann. chim. phys.*, [8] **3**, 243 (1904).

(48) Yvonne Khouvine and G. Arragon, *Bull. soc. chim.*, [5] **5**, 1410 (1938); see also F. B. Cramer and E. Pacsu, THIS JOURNAL, **59**, 1467 (1937).

(49) M. L. Wolfrom, S. M. Olin and E. F. Evans, *ibid.*, **66**, 204 (1944).

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

(51) Arranged in approximate decreasing order of intensities.

(52) Lines produced by galactitol.

(53) G. Bouchardat, *Ann. chim. phys.*, [4] **27**, 152 (1872).

(54) J. Wiemann, *Ann. chim.*, [11] **5**, 316 (1936).

indication was effected on the dried sheets with *p*-anisidine hydrochloride in 1-butanol. Elution was effected with water. Sirupy materials from 5 indicated zones, in descending order from the strip top, were isolated: I_a, 210 mg., exhibiting a yellow or ketose indicator color; I_b, 544 mg., brown (aldose); I_c, 281 mg., pink (uronic acid); I_d, 676 mg., yellow (ketose; Seliwanoff test also positive); I_e, 202 mg., pink (uronic acid); lost in indicator strips, *ca.* 550 mg.; total recovery, 2.46 mg. (90%).

Small portions of each of the above zones were rechromatographed in the same manner. Known samples of crystalline L-allose and amorphous D-psicose (prepared through the crystalline *keto*-D-psicose pentaacetate[55]) were run parallel and simultaneously on the same sheets. From this it was evident that zone I_b probably contained allose and that zone I_d probably contained psicose. When the material from zone I_b, $[\alpha]^{25}D$ +4.3° (*c* 2.24, water), was maintained in a desiccator, it partially crystallized. The first portion that crystallized was removed with methanol, which dissolved the sirup. These crystals were DL-allose, m.p. 180°; X-ray powder diffraction data identical with those[17] of an authentic specimen of DL-allose. The mother liquor sirup, from the above crystallization, deposited a second crop of crystals on standing, m.p. 138–145°.

An amount of 200 mg. of the zone I_d material, 400 mg. of phenylhydrazine hydrochloride and 600 mg. of sodium acetate were heated in 4 ml. of water for 20 min. at 98°. On cooling to 0°, yellow-brown crystals separated which were recrystallized from abs. ethanol to yield yellow crystals, m.p. 177–181° dec.; X-ray powder diffraction data: 10.14,[46,56] 8.80m, 7.75, 7.03, 6.19, 5.70m, 5.05, 4.60vs, 4.39s, 4.21m, 3.96m, 3.75m, 3.56, 3.44, 3.30s, 3.16m, 3.03, 2.84, 2.71, 2.44, 2.22, 1.97. These data agree closely with those reported[17] for an authentic sample of DL-*ribo*-hexose phenylosazone and show the preparation to be essentially that.

None of the other zones yielded crystalline material and no further identifications were made.

(55) M. L. Wolfrom, A. Thompson and E. F. Evans, THIS JOURNAL, **67**, 1793 (1945).

(56) vs, very strong; s, strong; m, medium.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Isomerization of D-Glucose to D- and L-Sorbose by a Strong Base Resin

BY MARY GRACE BLAIR AND JOHN C. SOWDEN

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(DL + D)-Sorbose has been isolated by carbon chromatography and crystallization from the neutral, non-fermentable portion of the reaction products of D-glucose and a strong base resin. Identification was effected through reduction to DL-glucitol and by conversion to (DL + D)-*xylo*-hexose phenylosotriazole, the latter being resolved into the pure antipodes by routine recrystallization.

In a previous report from this Laboratory regarding the action of alkali on D-glucose,[1] attention was directed to the discrepancy, rapidly growing with time, between the initial amount of sugar and the sum of the amounts of D-glucose, D-mannose and D-fructose in dilute sodium hydroxide at 35°. The product was shown to include an appreciable quantity of neutral, non-fermentable carbohydrate, much of which was ketonic. D-Psicose and a hypothetical 3-ketohexose already had been suggested as components of analogous mixtures obtained under related conditions by various workers.[2] No definitive characterizations for these non-fermentable products, however, were forthcoming until the isolation by Wolfrom and Schumacher[3] of (DL + D)-sorbose, (DL + D(?))-allose, D-glucuronic acid, galactitol (after reduction) and probably DL-psicose from the reaction of aconitate buffered potassium hydroxide on D-fructose. In addition, D-psicose has been isolated from the reaction mixture produced by the action of aqueous ammonia on D-glucose.[4]

The present report concerns the isolation of a mixture of D- and L-sorbose, the former predominating, which was formed by the action of a strong base resin (Amberlite XE-98)[5] on D-glucose. It already had been noted that the strong base resin Amberlite IRA-400[5] behaves similarly to aqueous sodium hydroxide in the catalysis of the Lobry de Bruyn–Alberda van Ekenstein isomerization of D-glucose to D-mannose and D-fructose.[6] Hence, the finding of (DL + D)-sorbose as an easily-isolable substance both from the reaction of D-glucose with Amberlite XE-98 and of D-fructose with potassium hydroxide[3] is additional evidence in the correlation of the behavior of the resinous bases and aqueous alkali.

It is surprising that the first crop of sorbose crystals isolated in our study had the same optical rotation, $[\alpha]^{25}D$ 12° in water, as that reported for the product from D-fructose and potassium hydroxide.[3] The identity of these constants is seemingly fortuitous, however, since our second crop showed a somewhat larger rotation.

The total amount of sorbose which was formed in the reaction has not been estimated. Crystallization occurred after only a rough chromatographic separation on carbon of the non-fermentable residue, and there was isolated an amount of mixed, crystalline sorboses corresponding to 2.5% of the original D-glucose or 7.8% of the non-fermentable residue. The average composition of the crystals was estimated from the rotations to be 68% D- and 32% L-sorbose.

The enantiomorphs were identified through an extraordinary resolution of their mixed phenylosotriazoles, which occurred spontaneously upon routine recrystallization of this derivative, the optical rotation of the first few mother liquors being opposite in sign to that of the isolated crystals. The optically active forms are much less soluble than the racemate. Hence, the isomer in excess began to crystallize first and continued by depleting the

(1) J. C. Sowden and R. Schaffer, THIS JOURNAL, **74**, 499 (1952).

(2) For references to the earlier literature, see 3 and 4.

(3) M. L. Wolfrom and J. N. Schumacher, *El Crisol*, **6**, 67 (1952); *Science*, **119**, 587 (1954); THIS JOURNAL, **77**, 3318 (1955).

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

(5) A product of Rohm and Haas Co., Philadelphia, Pa.

(6) J. C. Sowden, THIS JOURNAL, **75**, 4487 (1954); see also L. Rebenfeld and E. Pacsu, *ibid.*, **75**, 4370 (1953).