

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<sub>a</sub>, 210 mg., exhibiting a yellow or ketose indicator color; I<sub>b</sub>, 544 mg., brown (aldose); I<sub>c</sub>, 281 mg., pink (uronic acid); I<sub>d</sub>, 676 mg., yellow (ketose; Seliwanoff test also positive); I<sub>e</sub>, 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<sup>55</sup>) were run parallel and simultaneously on the same sheets. From this it was evident that zone I<sub>b</sub> probably contained allose and that zone I<sub>d</sub> probably contained psicose. When the material from zone I<sub>b</sub>,  $[\alpha]_D^{25} +4.3^\circ$  (*c* 2.24, water), was maintained in a desiccator, it partially crystallized. The first portion that crystallized was removed with methanol, which

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

dissolved the sirup. These crystals were DL-allose, m.p. 180°; X-ray powder diffraction data identical with those<sup>17</sup> 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<sub>d</sub> 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,<sup>46,56</sup> 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<sup>17</sup> 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.

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

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

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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 D-glucitol and by conversion to (DL + D)-*xyl*o-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,<sup>1</sup> 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.<sup>2</sup> No definitive characterizations for these non-fermentable products, however, were forthcoming until the isolation by Wolfrom and Schumacher<sup>3</sup> 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.<sup>4</sup>

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)<sup>5</sup> on D-glucose. It already had been noted that the strong base resin Amberlite IRA-400<sup>6</sup> 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.<sup>6</sup> 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<sup>3</sup> 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,  $[\alpha]_D^{25} 12^\circ$  in water, as that reported for the product from D-fructose and potassium hydroxide.<sup>3</sup> 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

(6) J. C. Sowden, *THIS JOURNAL*, **76**, 4487 (1954); see also L. Rebenfeld and E. Pacsu, *ibid.*, **76**, 4370 (1953).

(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.

racemate with regard to this isomer. Products of either sign of rotation could be obtained at will by seeding a synthetically prepared DL-mixture of the phenylosotriazoles of zero rotation with the appropriate antipode.<sup>7</sup> Although preferential crystallization of antipodes by seeding was among the early methods of resolution, it is still encountered only infrequently.

Because of its relatively great solubility in water, the (DL + D)-*xylo*-hexose phenylosotriazole failed to crystallize from the reaction medium when prepared in accordance with the directions for L-*xylo*-hexose phenylosotriazole.<sup>8</sup> Isolation of the derivative was achieved through adsorption on and elution from activated carbon, which has a large capacity for the substance. Elution of a phenylosotriazole from carbon with acetone has been employed previously, when the derivative failed to crystallize even after removal of copper and sulfate ions.<sup>9</sup> This prior removal of copper sulfate was found to be unnecessary in the present instance. The inorganic salts passed through the column of adsorbent with the initial aqueous effluent, whereas most of the colored impurities were retained tenaciously on the carbon when the osotriazoles were eluted with acetone. The simplicity of this method of isolation and purification makes it worth emphasis for manipulation of the more soluble phenylosotriazoles. Yields by this adsorption-elution technique, however, were not improved in the case of the less soluble, enantiomeric D- and L-*xylo*-hexose phenylosotriazoles over the yield previously recorded.<sup>8</sup>

The finding of L-hexoses among the reaction products of D-hexoses calls for re-emphasis of the possibilities of fragment recombination as a means of isomerization in alkaline media. As pointed out by Wolfrom and Schumacher,<sup>3</sup> it is likely that L-sorbose is produced in this reaction by aldolization of appropriate fragments (L-glyceraldehyde and dihydroxyacetone) rather than by an enolization-deenolization sequence involving the hexose 4,5-enediol. If fragment recombination produces the L-sorbose, then L-fructose also is to be expected from the reaction since the two sugars are formed in nearly equal amounts by the condensation of glyceraldehyde with itself or with dihydroxyacetone in aqueous alkali.<sup>10</sup> It is striking that thus far only the D-isomers of glucose, mannose and particularly of fructose have been reported from the action of alkali on any of them. With regard to specifically isotope-labeled sugars, D-glucose-1-C<sup>14</sup> has been shown to retain the label in carbon-1 during subjection to mild alkaline treatment for the purpose of preparing the epimeric sugars.<sup>11</sup> However, additional evidence for fragment recombination in the

alkaline isomerization of the sugars is found in the distribution of label in C<sup>14</sup>-D-glucosaccharin obtained from D-mannose-1-C<sup>14</sup> by the action of lime-water.<sup>12</sup>

### Experimental

**Isomerization of D-Glucose and Elimination of Known Components of the Reaction Mixture.**—Solutions of D-glucose (20%) were exposed to a strong base resin (Amberlite XE-98)<sup>6</sup> at 40–60° for 48 hours, the dry weight of the resin being 40% that of the sugar. Fermentable sugars and ionic substances were removed by fermentation with yeast followed by ion-exchange. After concentration at reduced pressure, the demineralized solution was preserved by xylene and refrigeration.<sup>13</sup> Titration of an aliquot of the solution indicated the presence of no free acids and only a trace of lactones. The non-aldose content of the non-fermentable material was estimated to be about 65% from the amount of residue remaining after removal by ion-exchange of the acids formed by oxidation of an aliquot with bromine.<sup>14</sup>

**Chromatography and Crystallization of Sorbose.**—The deionized solution from 200 g. of D-glucose (250 ml. containing 62.5 g. of solids) was chromatographed on a carbon<sup>15</sup>-Celite<sup>16</sup> (1:1) column (95 × 450 mm.; 1500 g. of adsorbent) by the method of Whistler and Durso,<sup>17</sup> with water as developer. The sirups, weighing about 30 g., from fractions in the range 3 to 4.7 l. of effluent, deposited crystals (total, 4.9 g.), some in a short while and some on standing, after suitable adjustment of the concentrations in methanol-ethanol. The bulk of the crystals (4.1 g.) were obtained from fractions in the range 3.5 to 4.2 l. of effluent. The crystalline material remained unoxidized when treated with bromine and gave a positive Seliwanoff test for ketose.

The various crops of crystals, as initially isolated, showed specific optical rotations in the range 12.4 to 18° in water. Repeated recrystallizations of the various crystalline fractions from methanol, ethanol, water and combinations of these solvents spread the rotations over the range 9 to 35°, whereas the melting points of all fractions remained in the range 150–160°.

**Preparation and Resolution of the Phenylosotriazole.**—Crystals of specific rotation 12° were converted to the phenylosazone, m.p. 164–165°, yield 577 mg., from 300 mg. of sugar (97% of theory).

*Anal.* (DL + D)-Phenylosazone: Calcd. for C<sub>18</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>: C, 60.4; H, 6.19; N, 15.6. Found: C, 60.5; H, 5.98; N, 15.5.

The phenylosazone was converted to the phenylosotriazole according to the directions for the preparation of L-*xylo*-hexose phenylosotriazole.<sup>8</sup> The product did not crystallize on concentration, and the water solution was passed successively through three 1-g. columns of Darco.<sup>15</sup> Two such columns were found to be necessary and sufficient for complete adsorption and subsequent recovery of the phenylosotriazole. The product was eluted with acetone, which was then evaporated under a stream of air. Recrystallization from water after treatment with a trace of Darco yielded 290 mg. of light brown crystals. Three additional recrystallizations gave colorless material (123 mg.) of m.p. 158.5–159° and  $[\alpha]_D^{25}$  47° in pyridine, in agreement with the reported values<sup>8</sup> for L-*xylo*-hexose phenylosotriazole, except for the opposite sign of rotation. These constants were not changed by further recrystallization from water nor from acetone. The melting point was depressed to 141–142° by admixture with the authentic L-isomer but was undepressed by the authentic D-isomer.

*Anal.* (DL + D)-Phenylosotriazole: Calcd. for C<sub>12</sub>H<sub>15</sub>N<sub>3</sub>O<sub>4</sub>: C, 54.3; H, 5.70; N, 15.8. Found: C, 54.5; H, 5.37; N, 15.6.

(12) J. C. Sowden and Dorothy J. Kuenne, *ibid.*, **75**, 2788 (1953).

(13) Processing to this point was performed by the Corn Products Refining Co., Argo, Ill., and we are indebted to Mr. J. B. Gottfried of that company for the non-fermentable material.

(14) According to the procedure of C. S. Hudson and H. S. Isbell, *THIS JOURNAL*, **51**, 2225 (1929).

(15) Darco G-60, a product of the Darco Department, Atlas Powder Co., New York, N. Y.

(16) A siliceous filter-aid produced by Johns-Manville Co., New York, N. Y.

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

(7) The authentic L- and D-*xylo*-hexose phenylosotriazoles used for comparison were prepared, respectively, from commercial L-sorbose and from the mixture of D-idose and D-gulose obtained by application of the nitromethane synthesis to D-xylose (J. C. Sowden and H. O. L. Fischer, *THIS JOURNAL*, **69**, 1048, 1963 (1947)).

(8) W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **67**, 939 (1945).

(9) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, *ibid.*, **74**, 2210 (1952).

(10) E. Schmitz, *Ber.*, **46**, 2327 (1913); H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **19**, 519 (1936); W. G. Berl and C. E. Feazel, *THIS JOURNAL*, **73**, 2054 (1951).

(11) H. G. Hers, J. Edelman and V. Ginsburg, *ibid.*, **76**, 5160 (1954); see also A. A. Bothner-Bay and M. Gibbs, *ibid.*, **72**, 4805 (1950).

The third mother liquor in the above series of recrystallizations deposited, upon evaporation of the solvent, a nearly colorless crystalline residue (89 mg.), m.p. 142–144° and  $[\alpha]^{25}_D - 17^\circ$  in pyridine. This material was triturated with hot ethyl acetate, and the insoluble residue was recrystallized from water to a maximum m.p. 158.5–159° and  $[\alpha]^{25}_D - 47^\circ$  in pyridine. These constants are in agreement with those of *L*-xylo-hexose phenylosotriazole.<sup>8</sup> The melting point was depressed to 140–141° by admixture with the authentic *D*-isomer but was undepressed by the authentic *L*-isomer.

**Resolution of Authentic *DL*-xylo-Hexose Phenylosotriazole by Seeding.**—Amounts of 150 mg. each of the authentic *D*- and *L*-xylo-hexose phenylosotriazoles were dissolved together in acetone and the solvent evaporated. The melting point (140.5–141°) of the residue was unchanged by recrystallization from 3 ml. of water. The recovered material (290 mg.) was optically inactive.

A solution of 277 mg. of the racemate in 13 ml. of hot water was cooled slightly and seeded with the *D*-enantiomorph. Crystals formed first in the region around the seed crystals as the solution cooled slowly to room temperature. The crystals (163 mg.) removed after a short time showed m.p. 141–142.5° and  $[\alpha]^{25}_D 13^\circ$  in pyridine (calculated composition: 64% *D*, 36% *L*). The mother liquor with an additional 1 ml. of wash water was seeded with the *L*-enantiomorph and cooled to 0°. The resulting crystals (67 mg.) showed m.p. 150.5–152° and  $[\alpha]^{25}_D - 23^\circ$  in pyridine (calculated composition: 20% *D*, 80% *L*).

When the compositions (calculated from optical rotations) and melting points from this and the preceding sec-

tion were plotted against each other, it was apparent that the composition of minimum melting point was near to 50:50. However, that the racemate is a true compound was verified by a 2° depression of its melting point on admixture with small amounts of either enantiomorph.

**Reduction to *DL*-Glucitol.**—An amount of 500 mg. of (*DL* + *D*)-sorbitose,  $[\alpha]^{25}_D 12^\circ$ , was hydrogenated in solution in 50% ethanol for 9 hours at 90° and 1500 p.s.i. of hydrogen, with Adams platinum oxide catalyst. After filtration, concentration and addition of methanol, there was obtained 185 mg. of crystals showing m.p. 125–135°. Recrystallization from water with the addition of dioxane gave 117 mg. of product with m.p. 136–138°; X-ray powder diffraction data<sup>18</sup>: 9.01 (2),<sup>19</sup> 5.90 (3), 4.38 (1), 3.88 (4), 3.59 (5), 2.62 (6), 2.81, 2.74, 2.46, 2.37, 2.33, 2.10, 1.99, 1.94, 1.63. These properties are in agreement with those reported for *DL*-glucitol.<sup>20</sup> Acetylation of 40 mg. of the hexitol gave 77 mg. of *DL*-glucitol hexaacetate, m.p. 117–117.5°.<sup>20</sup>

**Acknowledgment.**—The authors wish to thank the Sugar Research Foundation, New York, N. Y. for their support of this work.

(18) We are indebted to Mr. Eugene McLaren of this Department for the X-ray powder diffraction data.

(19)  $\text{CuK}\alpha$  radiation; interplanar spacings, Å.; order of intensities estimated visually, (1) most intense.

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

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

## The Carbohydrates of the Gramineae. VII. The Constitution of a Water-soluble Hemicellulose of the Endosperm of Wheat (*Triticum vulgare*)<sup>1,2</sup>

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The constitution of a water-soluble hemicellulose from wheat flour has been investigated by methylation studies. The methylated hemicellulose gives upon hydrolysis 2,3,5-tri-*O*-methyl-*L*-arabinose (13 moles), 2,3-di-*O*-methyl-*D*-xylose (19 moles), 2-*O*-methyl-*D*-xylose (6 moles) and *D*-xylose (4 moles). The highly branched structure of this hemicellulose is similar to that of the hemicellulose from the "squeegee" fraction of wheat flour and other hemicelluloses of the endosperm of the *Gramineae*.

Water-soluble pentosans from the endosperm of wheat have been variously described<sup>3–7</sup> but only in the cases of the polysaccharide associated with wheat  $\beta$ -amylase<sup>6</sup> and of the araboxylan of wheat flour<sup>7</sup> have constitutional studies been made.

This paper is concerned with the constitutional study of a hemicellulose extracted from wheat flour with water at room temperature. The wheat flour was first treated with boiling 82% ethanol in order to inactivate any enzymes, a procedure which has not always been applied to studies of this type.<sup>7</sup> Following an extensive extraction of the resulting wheat flour with 70% ethanol to remove the lower molecular weight sugars and glucofructosans, which have been studied separately,<sup>8,9</sup> the flour was

extracted with water in a Waring blender at room temperature and precipitated from the aqueous extract with ethanol. In the light of present knowledge such a vigorous extraction procedure is not to be recommended since water-soluble polyglucosans are produced by the action of the Waring blender on the wheat starch,<sup>10</sup> and these make it difficult to isolate the pure hemicellulose component. It has been shown previously<sup>2</sup> that the water-soluble components of wheat flour cannot be separated by fractional precipitation from an aqueous solution with ethanol. Consequently, the acetate of the mixture of water-soluble polysaccharides was subjected to fractional precipitation from a solution in pyridine and acetone with ether and petroleum ether. Even after repeated fractional precipitation in this manner a complete separation of the hexosans from the pentosans was not achieved (Tables I and II). However, the principal product from the fractionation of the acetates,  $[\alpha]^{25}_D - 91^\circ$  in pyridine, upon deacetylation by heating an acetone solution with 15% sodium hydroxide, was found to give a polysaccharide,  $[\alpha]^{25}_D - 94^\circ$  in 2% sodium hydroxide, which was largely pentosan in character.

(10) T. J. Schoch, *Tappi*, **35**, 22A (1952).

(1) This paper, No. 3303, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, is part of a report of research done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service.

(2) Part VI, K. A. Gilles and F. Smith, *Cereal Chem.*, in press.

(3) M. E. Freeman and R. A. Gortner, *Cereal Chem.*, **9**, 506 (1932).

(4) R. Geoffrey, *Bull. soc. chim. biol.*, **19**, 60 (1937).

(5) A. Wróblewski, *Ber.*, **30**, 2289 (1897).

(6) L. H. Ford and S. Peat, *J. Chem. Soc.*, 856 (1941).

(7) A. S. Perlin, *Cereal Chem.*, **28**, 382 (1951).

(8) R. Montgomery and F. Smith, *ibid.*, **31**, 490 (1954).

(9) R. Montgomery and F. Smith, unpublished work.