[CONTRIBUTION FROM THE DIVISION OF PLANT BIOLOGY, CARNEGIE INSTITUTION OF WASHINGTON]

d-Sorbitol: A New Source, Method of Isolation, Properties and Derivatives

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The rare sugar alcohol *d*-sorbitol has been found to occur in large quantities in Toyon berries, the fruits of *Photinia arbutifolia, Lindl.* The sorbitol is readily isolated by slight modifications of the methods which have already been used for its preparation from other natural sources (Method I)[1] or by the use of pyridine as a solvent from which to effect its crystallization (Method II). By this latter method *d*-sorbitol may be separated from reducing sugars which are quite soluble in pyridine,[2] and from other hexitols which are much less soluble in pyridine and which in contrast with sorbitol separate without pyridine of crystallization.

d-Sorbitol, which is very soluble in hot pyridine and but slightly soluble in cold pyridine (about 1 g. per 100 ml. at 0°), crystallizes with one mole of pyridine. Removal of the pyridine leaves anhydrous sorbitol, melting point 89–93°. Recrystallized from ethanol, monoamylamine and ethyl lactate, or prepared from benzal-, triacetone-, or hexacetyl-sorbitol, or from glucose by hydrogenation, *d*-sorbitol is anhydrous and melts between 89 and 93°.[3] Within the limits of experimental error these different preparations were found to have the same specific rotations at several wave lengths and to have identical crystal structures as shown by x-ray diffraction patterns of the powdered crystals.

Experimental

Isolation of *d*-Sorbitol. Method I.—Toyon berries were heated for one-half hour in one-third their weight of boiling water and extracted in a hand press. The pulp was agitated with warm water and again pressed dry. After three such extractions the combined extracts were filtered with charcoal and siliceous earth and fermented with yeast at 30–32° for four days. The fermented juice was centrifuged, evaporated under reduced pressure and the residue extracted with seven portions of absolute ethanol (each portion equal to five times the weight of the residue). Evaporation of the ethanol left a colorless, fluid gum. Acylation of a small portion of the gum obtained from 6.12 kg. of fresh berries indicated a sorbitol content of 144 g. or 2.4%. Crystallization of the gum from ethanol followed by concentration and crystallization of the mother liquors yielded 117 g. of *d*-sorbitol.

Method II.—Dried Toyon berries were extracted for twenty hours with three times their weight of ethanol (85%) at 60°. After five extractions the extracts were evaporated and the residue dissolved in ten times its weight of water. This solution was fermented with yeast at 37° for four days, decolorized with charcoal and evaporated to dryness at reduced pressure. The residual gum was dissolved in three times its weight of pyridine, from which a crop of colorless crystals containing one mole of pyridine was obtained. (Subsequent experiments indicate that the gum obtained upon evaporation of the alcoholic extract can be crystallized directly from pyridine, thus eliminating the fermentation.) These were recrystallized from pyridine. By combining and concentrating the mother liquors a small second crop of crystals was recovered. Fresh berries yielded 2.7% of *d*-sorbitol.

Purification of *d*-Sorbitol. Recrystallization from Ethanol.—*d*-Sorbitol, prepared by Method I (62.0 g.), was recrystallized twice from absolute ethanol, then twice from 90% ethanol. The product (32.0 g.) contained no ash and did not reduce Benedict's solution; m. p. 91–93° after drying over phosphorus pentoxide at 1 mm. and 66° for twelve hours.

Anal. Calcd. for $C_6H_{14}O_6$: C, 39.56; H, 7.69. Found: C, 39.57, 39.60; H, 7.74, 7.76.

The recrystallized sorbitol did not gain in weight or change in melting point when permitted to stand exposed to the air for several days during the damp winter months.

The optical rotations of the crystals in aqueous solution at the following wave lengths were: C, 8.34: 6678, −1.8°; 5876, −2.0°; 5461, −2.2°; 4358, −3.4°.[4]

The optical rotation of a supersaturated solution of *d*-sorbitol in pyridine was nearly four times the rotation of an aqueous solution of *d*-sorbitol: C, 2.45: 6678, −6.5°; 5876, −8.0°; 5780, −8.6°; 5461, −9.8°; 4358, −17.6°.

Recrystallization from Pyridine. *d*-Sorbitol Pyridine Compound.—*d*-Sorbitol (4.5 g.) was recrystallized successively from pyridine (100 ml., 33 ml., 26 ml. and 20 ml.). The white, fibrous crystals were dried over calcium chloride at room temperature and 25 mm. for twenty hours; m. p. 89° corrected; weight, 2.6303 g. These crystals hold pyridine very tenaciously for they lost only 0.0064 g. when dried over concentrated sulfuric acid at room

(1) (a) Boussingault, *Compt. rend.*, **74**, 939 (1872); (b) Embden and Griesbach, *Z. physiol. Chem.*, **91**, 251 (1914); (c) Tutin, *Biochem. J.*, **19**, 416 (1925); (d) Vincent and Delachanal, *Compt. rend.*, **108**, 147 (1889); (e) *ibid.*, **108**, 354 (1889); (f) *ibid.*, **109**, 676 (1889); (g) *ibid.*, **114**, 486 (1892).

(2) Holty, *J. Phys. Chem.*, **9**, 764 (1905).

(3) Widely divergent values have been reported for the melting point of *d*-sorbitol. Beilstein, "Handbuch der organischen Chemie," (1918), 4te. Aufl., Bd. I., p. 533; Hitzemann and Tollens, *Ber.*, **22**, 1048 (1889); Pfanstiehl and Black, *Ind. Eng. Chem.*, **13**, 685 (1921); Riiber, Sörensen, and Thorkelsen, *Ber.*, **58**, 964 (1925).

(4) The optical rotations were determined in 2-dm. tubes at 20°. The concentration, "C," is reported in g. per 100 ml. of solution. The monochromatic light was obtained from a helium hot cathode arc and a mercury arc using Zeiss filters supplemented with copper sulfate and methylene blue filters. The intensity of the light at 4358 Å. was such that specific rotations at this wave length are not more accurate than those at longer wave lengths.

temperature and 1 mm. for forty-eight hours. After drying over sulfuric acid at 84° and 1 mm. for forty-eight hours the crystals lost 0.7958 g. of pyridine or 0.9995 mole of pyridine per mole of sorbitol. The dried sorbitol melted at 88–89°, corrected, to a clear liquid. Optical rotation of the dried crystals in aqueous solution: C, 9.92: 6678, −1.8°; 5876, −2.1°; 5780, −2.1°; 5461, −2.6°; 4358, −4.3°.

The pyridine-free sorbitol, recovered from the solution used for the determination of the optical rotation, was converted into triformal-sorbitol, m. p. 213–214° corrected; optical rotation in pyridine solution: C, 1.395: 6678, −21.8°; 5876, −28.9°; 5780, −30.1°; 5461, −33.0°; 4358, −51.3°.

In the course of preparing the sorbitol pyridine compound it was observed that large transparent crystals of m. p. 76–77° were occasionally obtained rather than the fibrous crystals of m. p. 89° described above. The lower melting point was due to absorbed pyridine rather than to differences in the crystal structure of the two compounds as demonstrated by grinding and drying the crystals and by means of x-ray diffraction patterns of the powdered crystals. The pyridine-free sorbitol, obtained by drying the large crystals, melted at 90–93° corrected, to a turbid, opaque liquid which did not become clear until heated to 150–160°. When heated in vacuum (1–2 mm.) the crystals melted to a clear liquid at 91–92° corrected.

The sorbitol pyridine compound exhibits the sharp burning taste characteristic of pyridine. After removal of the pyridine the sorbitol resumes its sweet cooling taste.

Although the sorbitol pyridine compound holds pyridine very tenaciously in a dry atmosphere, this is not the case when water vapor is present. It has been observed that the sorbitol pyridine compound, exposed to moist air, slowly liberates pyridine. After several days the pyridine volatilizes completely, leaving the sorbitol in the form of a granular cake. And what is most surprising, the residual sorbitol is anhydrous (m. p. 90°).

When the sorbitol pyridine compound is dissolved in water the pyridine is set free as evidenced by the fact that it is quickly removed by distillation of the solution under reduced pressure or upon the addition of reagents which form insoluble compounds with pyridine. The optical rotation of the sorbitol pyridine compound in aqueous solution likewise indicates that the pyridine is not combined: C, 14.87: 6678, −1.1°; 5876, −1.4°; 5780, −1.4°; 5461, −1.6°; 4358, −2.5°.

Recrystallization from Other Solvents.—*d*-Sorbitol was recrystallized from ethyl lactate, from monoamylamine, and from monoamylamine by the addition of petroleum ether. The sorbitol crystallized as clusters of needles and as bundles of needles which were solvent free. Each preparation melted at 90–93° corrected. The crystals from monoamylamine frequently melted to a turbid liquid which did not become clear until heated to 108–118° or heated at 1–2 mm. to 90–92°. Attempts to recrystallize *d*-sorbitol from glacial acetic acid led to the formation of gels from which the sorbitol failed to crystallize.

Distillation of *d*-Sorbitol.—*d*-Sorbitol could not be vaporized below its melting point at 1–2 microns pressure. At 200° the sorbitol vaporized quite rapidly, condensing at 20° to a colorless, viscous liquid. The distillate was recrystallized from ethyl lactate; m. p. 91–93° corrected.

***d*-Sorbitol from Sorbitol Derivatives.**—In order to eliminate the possibility that the *d*-sorbitol isolated from Toyon berries might be contaminated by some substance not readily removed by crystallization, *d*-sorbitol was prepared by hydrolysis of several of its derivatives and crystallized as described above. *d*-Sorbitol prepared by the hydrolysis of benzal-sorbitol[1e] and of sorbitol hexaacetate[1c] was identical with that obtained from Toyon berries in respect to melting point, optical rotation, melting point of the pyridine compound and melting point and optical rotation of the triformal derivative. Of these two methods for the purification of sorbitol the former proved by far the more convenient and efficient.

***d*-Sorbitol from Triacetone-*d*-sorbitol.**—Although not previously described, it was found that sorbitol could be prepared easily by the hydrolysis of triacetone-sorbitol, as shown by the following experiment. *d*-Sorbitol pyridine (1.27 g.) was obtained by the hydrolysis of triacetone-sorbitol (3.0 g.) with water (6 ml.) and acetic acid (3 ml.) at 100°; m. p. 85–86° corrected. Found: 0.953 mole of pyridine per mole of *d*-sorbitol; m. p. pyridine-free sorbitol, 87–89° corrected. Optical rotation in aqueous solution and melting point and optical rotation of the triformal derivative were identical with the values reported above.

***d*-Sorbitol from Triformal-*d*-sorbitol.**—Attempts to prepare *d*-sorbitol by the hydrolysis of triformal-sorbitol in the presence of acetic and hydrochloric acids gave unsatisfactory results. *d*-Sorbitol was slowly decomposed by the action of strong hydrochloric acid although no levulinic acid, as determined with 2,4-dinitrophenylhydrazine,[5] was produced.

***d*-Sorbitol from Glucose.**—By way of reaffirming the configurational relationship of naturally occurring sorbitol, *d*-sorbitol was prepared from *d*-glucose by the action of aluminum amalgam in the presence of ammonium hydroxide.[6] The sorbitol was isolated as the pyridine compound; m. p. 85° corrected. Found 1.004 mole of pyridine per mole of *d*-sorbitol. The pyridine-free sorbitol melted at 87–89° corrected. The optical rotation in aqueous solution and the optical rotation of the triformal derivative in pyridine solution were identical with the values reported above.

Derivatives of *d*-Sorbitol. *d*-Sorbitol Hexaacetate.—*d*-Sorbitol hexaacetate, prepared and recrystallized as described by Tutin, melted at 99° as previously recorded.[7] However, crystallization of the compound from isoamyl acetate and drying at 66° and 1 mm. or crystallization from ethanol raised the melting point to 101° corrected. When distilled in high vacuum the sorbitol hexaacetate melted at 101–102° corrected.

Anal. Calcd. for $C_{18}H_{26}O_{12}$: C, 49.77; H, 5.99. Found: C, 49.84, 49.75; H, 5.97, 5.90.

Optical rotation in acetone solution: C, 3.344: 6678, +7.5°; 5876, +9.1°; 5780, +9.5°; 5461, +10.6°; 4358, +18.7°.

Optical rotation in pyridine solution: C, 7.696: 6678, +5.7°; 5876, +7.1°; 5780, +7.2°; 5461, +8.5°; 4358, +15.2°.

(5) Strain, *J. Biol. Chem.*, **102**, 151 (1933).

(6) Nanji and Paton, *J. Chem. Soc.*, **125**, 2474 (1924).

(7) Asahina and Shinoda, *Chem. Abs.*, **24**, 1704 (1930); Refs. 1c, 1e.

Optical rotation in dioxane solution: C, 5.192: 6678, +4.4°; 5876, +5.7°; 5780, +6.0°; 5461, +6.6°; 4358, +11.1°.

Benzal-*d*-sorbitol.—The slightly soluble compound obtained from *d*-sorbitol and benzaldehyde in the presence of acids has been reported to melt from 163 to 200°.[8] It has been the writer's experience that derivatives prepared in the presence of hydrochloric acid at room temperature have higher melting points (195–196.5°) than derivatives prepared at lower temperatures (5°) or higher temperatures (70°).

Triacetone-*d*-sorbitol.—Triacetone-sorbitol was prepared from sorbitol and acetone in the presence of hydrogen chloride as described by Speier,[9] m. p. 33–45°. Instead of removing the hydrogen chloride with lead carbonate and silver oxide as recommended by Speier, it was found that this acid could be removed by shaking the solution with calcium oxide in the presence of calcium chloride; yield, 28%; b. p. 140° uncorrected, at 1–2 mm.; m. p. 45–46°. Optical rotation in pyridine solution: C, 3.243: 6678, +5.3°; 5876, +6.3°; 5780, +6.3°; 5461, +6.6°; 4358, +9.1°. Optical rotation in ethanol (absolute) solution: C, 2.874: 6678, +13.2°; 5876, +14.2°; 5780, +14.8°; 5461, +15.8°; 4358, +25.9°.

Triformal-*d*-sorbitol.—Triformal-*d*-sorbitol, prepared by heating equal parts by weight of *d*-sorbitol, formalin (40%) and concentrated hydrochloric acid as described by Schulz and Tollens,[10] melted at 206–208° corrected after recrystallization from ethanol; m. p. 206°,[10] 208–209°.[6] In the course of preparing triformal-sorbitol it was found that the substitution of an equal weight of polyoxymethylene for the formalin produced a more rapid reaction and nearly doubled the yield of triformal-sorbitol. After recrystallization from ethanol (90%) the triformal-sorbitol melted at 213° corrected and after sublimation at 1 mm. and 200° it melted at 213–214° corrected.

Anal. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.42. Found: C, 49.54, 49.60; H, 6.45, 6.40.

Optical rotation in pyridine solution: C, 1.206: 6678, −22.0°; 5876, −27.8°; 5780, −28.2°; 5461, −32.8°; 4358, −49.8°.

Triformal-*d*-sorbitol from Triacetone-*d*-sorbitol.—Triacetone-sorbitol (0.19 g.), polyoxymethylene (0.19 g.) and concentrated hydrochloric acid (0.19 ml.) were heated on the water-bath for ten minutes. The resulting solid mass of crystals was cooled, extracted with water and recrystallized from ethanol (95%); weight, 0.070 g.; m. p. 213° corrected; m. p., mixed with authentic triformal-sorbitol, 213–214° corrected.

Triformal-*d*-sorbitol from the Sorbitol Pyridine Compound.—Triformal-sorbitol (0.23 g., m. p. 213° corrected) was obtained by heating the sorbitol pyridine compound (1.0 g.), polyoxymethylene (1.0 g.) and concentrated hydrochloric acid (1.0 ml.) and recrystallizing the product from ethanol.

Action of Pyridine on *d*-Sorbitol.—Unlike many polyhydroxy compounds, *d*-sorbitol is not epimerized[11] by heating in pyridine solution as demonstrated by the fact that 85% of the sorbitol could be recovered after heating with 5 times its weight of pyridine in a sealed tube at 165° for four days.

Crystallization of Hexitols from Pyridine.—Inositol and dulcitol were slightly soluble in boiling pyridine, mannitol and pentaerythritol were moderately soluble, and resorcinol and phloroglucinol were extremely soluble even in cold pyridine. The first four compounds crystallized without pyridine of crystallization.

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Summary

d-Sorbitol may be conveniently prepared from California Christmas or Toyon berries.

A new method for the isolation of *d*-sorbitol from natural sources is reported.

d-Sorbitol and other hexitols are easily crystallized from pyridine. The sorbitol forms crystals containing one mole of pyridine of crystallization.

Some of the properties of the sorbitol pyridine compound are described.

The melting points of highly purified preparations of *d*-sorbitol have been determined. Anhydrous sorbitol, prepared from natural sources, from benzal-sorbitol, triacetone-sorbitol, sorbitol hexaacetate and from *d*-glucose, has been found to melt at 89–93°. It was observed that large crystals of *d*-sorbitol frequently melted to a turbid viscous liquid which did not become clear until heated to 110–150° or unless heated to 90–93° at 1–2 mm. pressure. This phenomenon may account for the high melting point attributed to *d*-sorbitol by earlier workers.

The specific rotations of the sorbitol preparations used for the melting point determinations were identical. The average values obtained at different wave lengths for aqueous solutions containing 8–10 g. of *d*-sorbitol per 100 ml. of solution were: 6678, −1.75°; 5876, −2.05°; 5780, −2.08°; 5461, −2.5°; 4358, −4.0°.

Some properties of triacetone-sorbitol, tri-

(8) Davis, Slater and Smith, *Biochem. J.*, **20**, 1155 (1926); Fischer, *Ber.*, **27**, 1524 (1894); Hass and Hill, *Biochem. J.*, **26**, 987 (1932); Lobry de Bruyn and van Ekenstein, *Rec. trav. chim. Pays-Bas*, **18**, 151 (1899); Meunier, *Ann. chim. phys.*, [6] **22**, 412 (1891); *Compt. rend.*, **111**, 49 (1890).

(9) Speier, *Ber.*, **28**, 2531 (1895).

(10) Schulz and Tollens, *ibid.*, **27**, 1892 (1894).

(11) Armstrong, "The Simple Carbohydrates and Glucosides," (1919), 3d ed., pp. 10, 55; Levene and Hill, *J. Biol. Chem.*, **102**, 563 (1933).

formal-sorbitol, benzal-sorbitol and sorbitol hexaacetate are described.

Triacetone-sorbitol and the sorbitol pyridine compound are easily converted into triformal-sorbitol. This reaction provides a ready means for the identification of *d*-sorbitol and many of its derivatives. The average values of the specific rotations of triformal-sorbitol determined in pyridine solution (about 1.5 g. per 100 ml. solution) were: 6678, −21.9°; 5876, −28.6°; 5780, −29.5°; 5461, −33.0°; 4358, −51.0°.

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Derivatives of Piperazine. II. Utilization in Identification of Fatty Acids

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Additional derivatives similar to that previously described with malonic acid[1] have now been prepared and data regarding them are given in Table I.

TABLE I

YIELDS, PROPERTIES AND COMPOSITION OF PIPERAZINE DERIVATIVES

Piperazonium	Yield, %	M. p., °C., corr.	Cryst. solv.	Neut. equiv. Calcd.	Neut. equiv. Found	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1-Acid oxalate	90	Not under 300	Water	176.1	178.1	40.88	6.87	15.91	40.98	6.94	15.82
1-Acid succinate	90	205 −206 (dec.)	Alcohol (95%)	204.1	203.7	47.02	7.90	13.72	47.13	7.91	13.84
1-Acid adipate	83	244 −245 (dec.)	Alcohol (50%)	232.1	229.4	51.69	8.62	12.07	51.77	8.74	12.17
1-Acid sebacate	82	166 −168	Water	288.2	284.3	58.27	9.79	9.72	58.10	9.92	9.72
1,4-Di-acid glutarate	77	152	Alcohol (95%)	116.7	117.0	47.97	7.48	8.00	47.72	7.86	8.29
1,4-Di-monochloroacetate	68	145 −146	Alcohol (95%)	275.1	275.1	Cl, 25.78		10.18	Cl, 25.48		10.16
1,4-Di-trichloroacetate	75	121 −121.5	Alcohol (95%)	412.8	411.5	Cl, 51.53		6.79	Cl, 51.34		6.83
1,4-Di-acetate	71	208.5–209	*n*-Butanol	206.1	203.1			13.59			13.43
1,4-Di-propionate	50	124 −125	Dioxane	234.2	232.9			11.96			12.07
1,4-Di-butyrate	88	121 −122	Dioxane	262.2	260.0			10.69			10.64
1,4-Di-valerate	85	112.5–113	Dioxane	290.2	293.4			9.65			9.80
1,4-Di-caproate	65	111 −111.5	Acetone	318.3	316.0			8.80			8.69
1,4-Di-heptoate	72	95 − 96	Acetone	346.3	343.2			8.09			8.09
1,4-Di-isobutyrate	90	89.5– 90	Dioxane	262.2	260.1			10.69			10.55
1,4-Di-isovalerate	67	139 −140	Acetone	290.2	290.9			9.65			9.68
1,4-Di-lactate	60	96 − 96.5	Cellosolve	266.2	266.6			10.52			10.65

All of these derivatives are hydrolyzed by refluxing in hydrochloric acid. The resulting piperazine dihydrochloride is identified by conversion into 1,4-dinitrosopiperazine.[2] The therapeutic properties of these compounds are being investigated.

The salts of piperazine hexahydrate have been found to be helpful for the identification of fatty acids. They are easily prepared and purified, the time required for the entire operation being less than an hour. They crystallize well, melt at convenient temperatures, and possess neutralization equivalents that can be easily determined. In preparing the salts for analytical purposes 0.05 mole of piperazine hexahydrate is added to 0.1 mole of the acid. Upon stirring, the entire solution solidifies. The solid mass is washed with ether and recrystallized from the appropriate solvent. For qualitative organic analysis an excess of the acid is used. If immediate crystallization does not occur, this may be secured by warming the solution or adding ether. After filtration, drying on a porous plate and washing with ether, the melting points of the salts thus obtained were within a few degrees of those of the highly purified products.

These derivatives are soluble in water, alcohol, hot acetone, hot cellosolve (monoethyl ether of ethylene glycol) and hot dioxane (diethylene oxide). They are insoluble in ether and hexane. Neutralization equivalent determinations yield values that indicate that one molecule of acid is hydrolyzed off of the 1,4-piperazonium compound. These have been determined on 0.3 to 0.5 g. samples of the highly purified products after drying over phosphorus pentoxide for a week. They are given in Table I. For qualitative organic analysis purposes, however, melting point and mixed melting point determinations suffice.

(1) Pollard and Adelson, THIS JOURNAL, **56**, 150 (1934).
(2) Ladenburg, *Ber.*, **24**, 2401 (1891).