[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Chromatography of Sugars and Related Polyhydroxy Compounds[1]

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The method of chromatographic analysis was first applied in the carbohydrate field by Reich,[4] who separated the *p*-phenylazobenzoates of α-D-glucose and β-D-fructose from each other on both alumina and silica columns. This was extended by Coleman and associates[5] to a longer list of the sugar *p*-phenylazobenzoates, as well as to the *p*-phenylazobenzoate derivatives of methyl glycosides and of partially acetylated and methylated sugars, employing adsorption on both Magnesol and silicic acid columns. Mertzweiller, Carney and Farley[6] reported the chromatography of the *p*-phenylazobenzoates from mixtures of 3-methyl-D-glucose, 2,3-dimethyl-D-glucose, 2,3,6-trimethyl-D-glucose and 2,3,4,6-tetramethyl-D-glucose by adsorption on silica columns. Jones[7] recorded the separation of methyl tetramethyl-D-glucopyranoside and methyl 2,3,6-trimethyl-D-glucoside by means of a flowing chromatogram on an aluminum column. Bell[8] separated 2,3,4,6-tetramethyl-D-glucose, 2,3,6-trimethyl-D-glucose and dimethyl-D-glucose on a silica column by a modified process of chromatography termed "partition chromatography," first used by Martin and Synge[9] with the N-acetylamino acids. Bell employed the Molisch reagent to detect the adsorption zones. Norberg, Auerbach and Hixon[10] reported the separation of 2,3-dimethyl-D-glucose, 2,3,6-trimethyl-D-glucose and 2,3,4,6-tetramethyl-D-glucose by adsorption on a column of fibrous alumina, using ultraviolet light to detect the adsorption zones. McNeely, Binkley and Wolfrom[11] resolved eight pairs of fully acetylated sugars and sugar alcohols by adsorption on columns of Magnesol, employing aqueous alkaline potassium permanganate as the brush or streak reagent. This method was later applied to some industrial cane sugar products.[12]

The work of Hayashi,[13] which has been cited in the chromatographic literature, is perhaps not pertinent since no Tswett chromatographic techniques were involved. Tiselius[14] has devised a flowing chromatogram on charcoal whereby aqueous solutions of sugar mixtures may be resolved into their components by following changes in the indices of refraction of the solutions. The method involves the use of specialized and expensive equipment.

We wish to report herein the details of a method for the chromatography of the sugars and related polyhydroxy compounds in their unsubstituted form.

The selectivity required of an adsorbent is arbitrary and we may set the standard of good selectivity at any desired level. We have chosen for the purposes of this work the separation of a mixture of sorbitol[15] and D-mannitol as the requisite criterion. It can be seen that adsorptive selectivity arises essentially from the difference in adsorptive strength shown by the adsorbent toward the different sugars. With these considerations in mind, we have found that a variety of domestic commercial clays are very suitable. A representative sample is Florex XXX (Floridin Company, Warren, Pennsylvania). For rough separations, the following adsorbents are also useful: Super-Filtrol, Special Filtrol, Magnesium Silicate 34, alumina and Magnesol.

We have arranged a number of carbohydrates and related compounds into a chromatographic adsorption series (Table I). The compounds are listed in descending order of adsorptive strength as determined by the position of the adsorbed zone after development with the specified developer. Substances written in the same sequence are not separable under the conditions specified but might be separable under other conditions of development. Reference to this series will then indicate whether the method will be able to separate a mixture of several of these carbohydrates, on the assumption that admixture of the components will not interfere with their respective adsorptive strengths.

The chromatography was performed with organic solvents, which usually contained a certain amount of water, depending on the compounds being chromatographed and the organic solvent used. Usually ethyl alcohol or isopropyl alcohol was employed, the concentrations being

(1) A preliminary report of this work has appeared in THIS JOURNAL, **67**, 1865 (1945).

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(4) W. S. Reich, *Compt. rend.*, **208**, 589, 748 (1939); *Biochem. J.*, **33**, 1000 (1939).

(5) G. H. Coleman, A. G. Farnham and A. Miller, THIS JOURNAL, **64**, 1501 (1942); G. H. Coleman and C. M. McCloskey, *ibid.*, **65**, 1588 (1943); G. H. Coleman, D. E. Rees, R. L. Sundberg and C. M. McCloskey, *ibid.*, **67**, 381 (1945).

(6) J. K. Mertzweiller, D. M. Carney and F. F. Farley, *ibid.*, **65**, 2367 (1943).

(7) J. K. N. Jones, *J. Chem. Soc.*, 333 (1944).

(8) D. J. Bell, *ibid.*, 473 (1944).

(9) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

(10) Ethelda J. Norberg, I. Auerbach and R. M. Hixon, THIS JOURNAL, **67**, 342 (1945).

(11) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

(12) W. W. Binkley, Mary G. Blair and M. L. Wolfrom, *ibid.*, **67**, 1789 (1945).

(13) F. Hayashi, *J. Biochem. (Japan)*, **16**, 1 (1932).

(14) A. Tiselius, *Advances in Colloid Sci.*, **1**, 81 (1942); A. Tiselius, *Kolloid Z.*, **105**, 101 (1943); A. Tiselius and L. Hahn, *ibid.*, **105**, 177 (1943).

(15) By sorbitol we denote the common form obtainable by the reduction of D-glucose.

those indicated in Table I for the various classes. These different concentrations of developers arose from the fact that as the adsorptive strength of the various sugars decreased as we proceeded down the series, progressively weaker developers became necessary for their development. Increased temperature and humidity decreased adsorptive strength and capacity but did not affect adsorptive selectivity.

TABLE I

CHROMATOGRAPHIC ADSORPTION SERIES OF CARBOHYDRATES AND SOME RELATED SUBSTANCES

Arranged in decreasing order of adsorptive strength

Adsorbent: 1.68 g. of 5:1 mixture of Florex XXX-Celite.
Column: 0.9 × 6 cm. of adsorbent.
Adsorbate soln.: 1 mg. in 0.5 cc. of developer shown below.
Developer: noted below following class heading.

Class I (10 cc. of 70% ethyl alcohol[a])
Potassium acid D-glucosaccharate, D-glucosamine hydrochloride, D-chondrosamine hydrochloride
(*dextro*)-Tartaric acid
D-Gluconic acid, ammonium D-gluconate, sodium D-gluconate
D-Arabonic acid, L-arabonic acid, potassium D-arabonate, citric acid
D-Mannonic acid
α-D-Galacturonic acid

Class II (10 cc. of 90% ethyl alcohol)
Lactitol, melibiitol
L-Iditol, *meso*-inositol (m. p. 225°), stachyose, Schardinger α-dextrin
Lactose
Sorbitol,[15] dulcitol, L-perseulose, D-perseulose, Schardinger β-dextrin, (*levo*)-malic acid
Raffinose
Xylitol
D-Mannitol, D-talitol, gentiobiose

Class III (5 cc. of 90% ethyl alcohol)
D-Gulose
Melibiose, D-mannose, D-ribose, 3,6-anhydrosorbitol, D-psicose
Cellobiose
1-Desoxy-sorbitol
Ascorbic acid
D-Arabitol
Allitol

Class IV (4 cc. of 95% ethyl alcohol)
Maltose, D-galactose, D-fructose, melezitose, D-mannoheptulose, trehalose, D-gluco-D-*gulo*-heptose
L-Fucitol, turanose, 1,5-anhydro-D-mannitol, 1,4-anhydro-D-mannitol, D-lyxose
D-Rhamnitol, L-rhamnitol, 2-desoxy-sorbitol, D-glucoheptulose
L-Arabinose, D-arabinose, adonitol, quercitol, diethylene glycol, dipentaerythritol
Sucrose, erythritol, L-altrose
D-Glucuronic acid γ-lactone
L-Sorbose, succinic acid
Anhydroenneaheptitol
D-Glucose, glycerol

Class V (5 cc. of 90% isopropyl alcohol)
L-Allose
1,4:3,6-Dianhydro-D-mannitol, amygdalin
α-D-Galacto-meta-saccharinic acid
Propylene glycol β-D-glucoside
L-Fucose

Class VI (6 cc. of 97% isopropyl alcohol)
Methyl α-D-glucopyranoside, methyl β-D-glucopyranoside
Methyl α-D-mannopyranoside, D-xylose, D-glucono-δ-lactone
1,4-Anhydro-sorbitol, dipropylene glycol, pentaerythritol, 1,5:3,6-dianhydro-D-mannitol, 1,4:3,6-dianhydro-sorbitol
D-Glucono-γ-lactone

Class VII (4 cc. of 97% isopropyl alcohol)
L-Rhamnose, salicin
Ethylene glycol
Propylene glycol
Phloridzin
1,4:3,6-Dianhydro-L-iditol

[a] Made by adding 30 cc. of water to 70 cc. of absolute ethanol. Other dilutions were made similarly except that 95% ethyl alcohol was the azeotrope.

Detection of the adsorption zones on the extruded column was effected by streak reagents, of which the following were used: aqueous alkaline potassium permanganate,[11] congo red or other indicators for acids and 1,6-dichlorophenolindophenol for ascorbic and other acids.

Elution was performed with water or ethanol–water mixtures. In addition to the usual or flask method of elution, a second method, designated column elution, was especially adapted for this investigation since the use of the flask method with clays was both time and labor-consuming. The method was simply to repack the sectioned moist adsorbate into another column and to remove the adsorbed component from the column with a strong developer.

The sensitivity of this chromatographic procedure was sufficient to detect easily 0.01 mg. of D-mannitol contained in 0.05 cc. of 95% ethyl alcohol on a 0.35 × 4 cm. column of adsorbent. A mixture containing 98% sorbitol and 2% D-mannitol was separated. Examples are given of typical separations and recoveries in nearly quantitative yields of binary mixtures consisting of D-mannitol and sorbitol; sucrose and raffinose; dipentaerythritol from crude pentaerythritol. A mixture of D-glucose and sorbitol was separated and each component analytically recovered in quantitative yields. The delactonization of D-glucono-γ-lactone and D-glucono-δ-lactone into free D-gluconic acid was easily followed. A ternary mixture[16] of 1,4:3,6-dianhydro-sorbitol, 1,4:

(16) H. G. Fletcher, Jr., and R. M. Goepp, Jr., THIS JOURNAL, **67**, 1042 (1945).

3,6-dianhydro-D-mannitol and 1,4:3,6-dianhydro-L-iditol was separated into three zones. Lemon juice was chromatographed and the ascorbic acid was concentrated into a definite zone. A five-component system consisting of α-D-galacturonic acid, D-galactose, D-glucose, D-xylose and L-rhamnose, a mixture which may conceivably be obtainable in a hemicellulose hydrolyzate, was chromatographed into five zones. About 1 mg. of the mixture was sufficient for the purpose so that the usefulness of the method in the analysis of the hydrolyzate of a complex polysaccharide can be perceived.

Experimental

A. General Procedure

Adsorbents.—The following adsorbents were of approximately equal adsorptive selectivity: (1) Florex XXX and (2) Floridin XXX-Floridin Co., Warren, Pennsylvania; (3) Attapulgus Clay (and types A and AA) (through 200 mesh)—Attapulgus Clay Co., Attapulgus, Georgia; (4) Bleaching Clay 260—Industrial Minerals and Chemical Co., Berkeley, California; (5) J Neutrol—Filtrol Corp., Los Angeles, California; (6) Lloyd's Reagent—Eli Lilly and Co., Indianapolis, Indiana. Of the above, numbers 4, 5 and 6 had the greatest adsorptive strength; number 2 the weakest; while numbers 1 and 3 were intermediate. In our work we have standardized on Florex XXX and this will be understood to be the adsorbent used in all of the following descriptions. A filter-aid (Johns-Manville Celite 535) was used to improve the flow-rate. The use of a filter-aid was chosen in preference to using a coarser grade of adsorbent because the finer material gave sharper zones. An amount of filter-aid of from 17-34% of the weight of the clay was suitable. A lower percentage gave very slow flow-rates while a higher percentage resulted in a decreased capacity for the column. We have standardized our procedure on the 17% mixture and this mixture will be understood whenever the term adsorbent is employed.

The clay and the filter-aid as received from the suppliers contain considerable impurities. In case the only objective of an experiment is the detection of different zones on the column these impurities are of no importance. They do not affect the adsorptive properties of the adsorbent. However, if the objective is the chromatographic separation of a mixture with the subsequent recovery of each of the various adsorbates, then the adsorbent should be cleaned. This is done conveniently by packing the adsorbent into a percolator or into a large size chromatographic tube and allowing the following solvents to percolate successively through, under water-pump vacuum: one column-length of 90% ethyl alcohol, (one column-length of liquid is that amount of liquid just sufficient to wet the entire column); one column-length of water; one column-length of acetone; one column-length of benzene; and finally 1.5 column-lengths of acetone. The adsorbent, after extrusion, is layered on a glass plate and air-dried, after which it may be sifted through an ordinary kitchen flour sifter to break up any large aggregates, and then stored for use. This treatment does not change the adsorptive quality of the material in any manner.

Initial Solutions.—The carbohydrate should first be dissolved in the water and the organic solvent then added. In cases where the solubility of the sample is too small in the organic solvent, a higher concentration may be achieved by keeping the solution hot. The hot solution may then be poured on the chromatographic column. The heat will soon be absorbed by the column, and the succeeding large volume of cold developer will normalize the situation.

Developers.—We have found the following solvents to be useful as developers: methyl alcohol, ethyl alcohol, isopropyl alcohol, *n*-butyl alcohol, *s*-butyl alcohol, isobutyl alcohol, *t*-butyl alcohol, acetone, dioxane, pyridine, cellosolve, methylcellosolve and mixtures of benzene and ethyl alcohol. The addition of water or acids increases the developing power of these solvents, the less hydrophilic ones requiring more water for the same effect. We have standardized on ethyl and isopropyl alcohols, using water to increase the developing power when necessary. The strength of the developer to be used depends upon the components to be analyzed. The adsorption series (Table I) will serve as a general guide. The definition of the exact concentration of the developers listed therein was necessary in determining the relative positions of the substances in the series. Once this is established, these concentrations are to be taken only as guides, and considerable latitude is allowable in the volume and strength of the developer. The closer the components are in the series, the stricter the conditions become, and in the case of neighbors the concentration should be kept to within at least 3% of the stated strength. In general, it was found that the strength of developer to be used should be such that two to five column-lengths of developer would give adequate development along the column. In cases where components are in different classes, the developer for the most weakly adsorbed component (lowest in Table I) should be used. It is possible that an occasional reversal in order may occur on changing the nature of the developer from ethyl alcohol to isopropyl alcohol. In many cases the difference in adsorptive strengths in multiple mixtures may be so great that in order to accommodate both extremes a single column will not suffice, in which case flowing chromatograms and multiple passes will have to be employed. This will be the case also if the number of components in a mixture is very large.

The solvents used need not be especially purified. When x% alcohol is specified, such a solution is made up of x cc. of absolute alcohol and $100 - x$ cc. of water.

Chromatographic Adsorption and Extrusion.—The developed column is extruded and streaked with the reagent (see below), after which it is sectioned with a sharp, thin-bladed knife according to the zones and the streak mark removed. The zones are then ready for elution. These zones will be referred to as the adsorbates. Extrusion of large columns from glass tubes is sometimes difficult. Dr. W. W. Binkley of this Laboratory has found that extrusion properties are improved by the employment of aluminum tubes, especially if they be initially sharply jarred by dropping from a short height.

Streak Reagents. (1) **Alkaline Permanganate.**—One part of sodium hydroxide and 0.1 part of potassium permanganate were dissolved in 10 parts of water. This streak reagent may be applied with a camel-hair brush, or, if desired, a brush made from No. 85 Fiberglas (Fiberglas Corp., Toledo, Ohio) which is more resistant to the reagent but suffers from being brittle. This streak reagent will disclose the presence of any permanganate-oxidizable substances on the column by its change in color from green to tan. Since the developers used may also react, reliance will have to be placed upon their differences in rates of oxidation. The polyhydroxy compounds will react much faster with the reagent than the developer. Thus, the zones of carbohydrates will be disclosed momentarily (a matter of about thirty seconds) and then will disappear as the remainder of the streak becomes tan-colored. In some cases it will be necessary to partially dry the column before the zones will appear, in which case the column will have to be handled very carefully since it will crumble easily. A thin, uniform brush mark gives the best results. The streak is difficult to read and considerable experience is required to note the position of the zones.

(2) **Acid-base Indicators.**—These are the usual indicators as prepared for analytical laboratory use. We have found congo red and some of the available universal indicators to be useful.

(3) **2,6-Dichlorophenolindophenol.**—An amount of 5 mg. of this reagent was dissolved in 20 cc. of water. It is more stable if kept in the refrigerator. This indicator turns from blue to pink in the presence of acids and is decolorized by ascorbic acid.

Elution. (1) **Flask Elution.**—The adsorbate was placed in sufficient water so that a thin suspension of the

earth was obtained. After being stirred for a short time, the suspension was centrifuged and the centrifugate decanted. This was repeated three more times, the centrifugates combined, then concentrated to a small volume and ethanol added to effect crystallization. It should be noted that although the centrifugates may appear perfectly clear, there may be nevertheless a considerable amount of adsorbent in them which on concentration will gradually flocculate. Even though the solution be taken to dryness, the addition of water to redissolve the carbohydrate may cause peptization. As ethanol is added to this solution, further flocculation will occur. The addition of ethanol should be adjusted so that the flocculated adsorbent may be filtered before the carbohydrate crystallizes. Decolorization with a small amount of charcoal may be necessary. Methanol may be used to extract the sugar from the dried residue. Microscopic observation is necessary to determine that crystals of carbohydrate are perfectly free of amorphous adsorbent. Failure to observe these precautions will inevitably result in a mixture of carbohydrate and adsorbent.

(2) **Column Elution.**—The moist adsorbate was repacked into a chromatographic column, and the column was developed with a sufficient volume of a strong developer so that the adsorbed component was entirely removed from the column. Usually this amount was about five column-lengths of a developer containing about 15% more water than that used for the initial chromatogram. The first 10% of filtrate to come through was immediately added back to the top of the column. After the complete development the filtrate was treated in exactly the same manner as in the flask elution method and with the same precautions. A considerable saving in time may be achieved by allowing the filtrate to drip into a distilling flask, thus carrying out the elution and concentration simultaneously.

Effect of Temperature and Humidity.—Experiments carried out at 0, 24 and 50° showed that apparently both adsorptive strength and adsorptive capacity decreased slightly as the temperature rose. However, the adsorptive selectivity remained the same. Rising humidity showed the same apparent effect as rising temperature.

Sensitivity.—An amount of 0.1 mg. of D-mannitol in 0.5 cc. of 95% ethanol (*c*, 0.02 g. per 100 cc. soln.) was detectable on a 0.9 × 6 cm. column. An amount of 0.2 mg. of D-mannitol was easily detectable in the following concentrations in 95% ethanol: *c*, 0.1; *c*, 0.02; *c*, 0.01 and *c*, 0.005. An amount of 0.01 mg. of D-mannitol in 0.05 cc. of 95% ethanol (*c*, 0.02) was easily detectable on a 0.35 × 4 cm. column.

B. Examples[17]

Analysis of a Mixture of D-Glucose and Sorbitol.[15]—This particular example represents a semi-micro quantitative determination after the constituents have been established either by chromatography or other methods. A mixture containing 1 mg. of sorbitol and 0.9 mg. of D-glucose in 0.5 cc. of 95% ethanol was chromatographed on a 0.9 × 6 cm. column with 4 cc. of 95% ethanol. The individual zones were eluted with six portions of water (total *ca.* 15 cc.). The combined eluates from each zone were titrated, those from the upper zone for sorbitol by periodate oxidation and those from the lower for D-glucose by a reducing sugar determination. The results showed 100% D-glucose and 99.7% sorbitol. A separate chromatogram showed that the sorbitol zone was non-reducing toward Fehling solution.

D-Mannitol and Dulcitol.—A mixture containing 100 mg. each of D-mannitol and dulcitol in 25 cc. of 95% ethanol was chromatographed on a 3.3 × 20 cm. column with 350 cc. of 90% ethanol as developer; the time required was two hours and fifteen minutes. Each zone was eluted with four portions of water. The combined centrifuged eluates from each zone were concentrated to a small volume and alcohol added to the point of crystallization. The first crop of D-mannitol gave a recovery of 90% with m. p. 166–167°; a second crop gave a further 6% with the same melting point. The accepted value for the melting point of D-mannitol is 166°. The first crop of dulcitol gave 95% recovery with m. p. 187–188°; accepted value 188°.

Sucrose and Raffinose.—A mixture containing 1.5 g. of sucrose and 0.5 g. of raffinose pentahydrate in 100 cc. of 95% ethanol was chromatographed on a 3.3 × 6.7 cm. column with 100 cc. of 95% ethanol as developer; the time required was thirty minutes. This amount of developer removed the sucrose entirely from the column, the filtrate containing the sucrose being collected in the receiver. The first 15 cc. of filtrate was discarded. The filtrate was concentrated and ethanol added to effect crystallization. The first crop of sucrose gave a recovery of 95% (m. p. 188–189°; $[\alpha]^{28}D$ + 66.5°, *c* 3.95, water) and a second crop a further 2.1% (m. p. 188–189°). The accepted values for the constants of sucrose are: m. p. 188°; $[\alpha]^{20}D$ + 66.5° (water). The extruded column was streaked with alkaline permanganate and the raffinose zone was eluted with four portions of water. The eluate on concentration and crystallization with ethanol gave a recovery of 87% on the first crop; $[\alpha]^{28}D$ + 122° (*c* 3.3, water) after oven-drying to yield the anhydrous form; accepted value, $[\alpha]^{20}D$ + 123°. A further 8% of crystals of slightly lower purity was recovered from the mother liquor.

Separation of a Small Amount of Dipentaerythritol from a Large Amount of Pentaerythritol.—An amount of 2.0 g. of pentaerythritol (m. p. 184–237°) containing dipentaerythritol was dissolved in 8 cc. of hot water and 152 cc. of absolute isopropyl alcohol added to this solution. The resultant solution was chromatographed on a 5.1 × 11.6 cm. column with 1000 cc. of 97% isopropyl alcohol as developer. This amount of developer developed the pentaerythritol zone off the column; the time required was three hours. The first 50 cc. of filtrate was discarded and the rest was concentrated to give a first crop of 1.26 g. of crystalline pentaerythritol with m. p. 263°. A second crop gave 0.36 g. with m. p. 259–260°. A third crop gave 0.09 g., m. p. 259–260°. The accepted value for the melting point of pentaerythritol is 263°. The extruded column was streaked with alkaline permanganate and the moist zone containing dipentaerythritol was repacked into another chromatographic tube and the dipentaerythritol removed from the column with 500 cc. of 80% ethanol. The filtrate on concentration gave a first crop of crystals weighing 190 mg. with m. p. 220–221°. A second crop gave 45 mg. with m. p. 219–220°. The dipentaerythritol content of the original mixture was thus 12%. The accepted value for the melting point of dipentaerythritol is 221°.

1,4:3,6-Dianhydro-D-mannitol, 1,4:3,6-Dianhydrosorbitol, and 1,4:3,6-Dianhydro-L-iditol.—A mixture (19.4 mg.) consisting of these three components[16] in 2.5 cc. of 95% ethanol was chromatographed on a 1.9 × 18 cm. column with 28 cc. of absolute ethanol as developer. The chromatogram showed three zones, separated by interzones of about 4 cm.

Sorbitol (98%) and D-Mannitol (2%).—A mixture containing 180 mg. of sorbitol and 3.6 mg. of D-mannitol in 10 cc. of 95% ethanol was chromatographed on a 3.3 × 20 cm. column with 350 cc. of 90% ethanol as developer. The column showed two zones, a very small bottom zone (D-mannitol) and a very large upper zone, separated by about 4 mm.

Lemon Juice.—Lemon juice, freshly obtained by grinding the fleshy portion of the fruit with acid-washed sand, was centrifuged. One cc. of the centrifugate was taken and 7.0 cc. of absolute ethanol added to it. The mixture was centrifuged and 1.0 cc. of the centrifugate was chromatographed on a 0.9 × 6 cm. column with 2 cc. of 95% ethanol as developer. The 2,6-dichlorophenolindophenol streak showed an ascorbic acid zone at the 3.9–4.3 cm. (measured from the top of the column) region. The alkaline permanganate streak showed the presence of at least four zones, and indicated that the ascorbic acid zone was probably contaminated. The congo red streak showed the presence of a large amount of acid above the ascorbic acid zone.

(17) All melting points are corrected values. Column dimensions cited are those of the packed adsorbent.

Decitrated Lemon Juice.—Decitrated lemon juice was obtained in a manner similar to the above example except that the grinding was performed in the presence of calcium carbonate. The ground extract was centrifuged and 5 cc. of the centrifugate was taken and 35 cc. of absolute ethanol added to it. The mixture was centrifuged and 30 cc. of this centrifugate was chromatographed with 6 cc. of 90% ethanol as additional developer. This amount of developer resulted in developing most of the components below the ascorbic acid zone off the column. The 2,6-dichlorophenolindophenol streak showed the ascorbic acid zone to be at the 3.4–6.2 cm. region. The congo red streak showed the presence of three acid zones above the ascorbic acid zone.

Delactonization of D-Gluconolactones.—It was found that the rates of delactonization of D-glucono-γ-lactone and D-glucono-δ-lactone may be followed by means of this chromatographic method. A solution of each of the lactones was made by dissolving 0.1 g. of the material in 0.5 cc. of water. At definite time intervals, 0.05 cc. of the solution was added to 0.95 cc. of absolute ethanol. The addition of this amount of ethanol to the solution "quenched" the delactonization to such an extent that the rate was practically zero. An amount of 0.05 cc. of this alcoholic solution was then chromatographed on a 0.9 × 5 cm. column with 2.0 cc. of 95% ethanol as developer. It was found that within three minutes the solution of D-glucono-δ-lactone showed the presence of free acid and that this rapidly increased over a period of about three hours. The solution of D-glucono-γ-lactone did not show the presence of any free acid zone after standing for six hours, but did after standing for one day.

α-D-Galacturonic Acid, D-Galactose, D-Glucose, D-Xylose and L-Rhamnose.—Approximately 0.2 mg. of each of these compounds in 0.5 cc. of 95% isopropyl alcohol was chromatographed on a 0.9 × 9.5 cm. column with 7 cc. of 94% isopropyl alcohol as developer. The streaked column showed that the five components had been resolved into five separate zones as follows, measured in cm. from the top of the column: α-D-galacturonic acid, 0–0.3; D-galactose, 0.7–1.4; D-glucose, 2.0–2.8; D-xylose, 3.7–4.9; L-rhamnose, 6.0–7.5. The order follows by reference to the adsorption series, assuming that no reversals in order occurred on changing the developer from ethyl alcohol to isopropyl alcohol. In this case, separate chromatograms of each component showed that no reversals had occurred.

Summary

1. A method for the chromatographic analysis of the unsubstituted sugars and related compounds has been established.

2. A number of sugars and related compounds have been arranged in a chromatographic adsorption series.

3. A mixture of D-glucose and sorbitol was separated and each zone analyzed to show a quantitative separation.

4. The following mixtures were separated and their components recovered in nearly quantitative yields: D-mannitol and dulcitol; sucrose and raffinose; pentaerythritol and dipentaerythritol.

5. The separation of the components of the following mixtures was demonstrated qualitatively: three dianhydrohexitols; sorbitol (98%) and D-mannitol (2%); ascorbic acid in lemon juice; D-gluconic acid and its lactones; a five-component sugar mixture.

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D-Gluco-L-*tagato*-octose[1]

BY M. L. WOLFROM AND ALVA THOMPSON[2]

In previous communications of this series we have reported the development of a general method for the preparation of ketoses from the lactones of the sugar acids possessing one less carbon atom. We now wish to describe the application of this method to the synthesis of the first eight-carbon atom ketose, D-gluco-L-*tagato*-octose from D-gluco-D-*gulo*-heptono-γ-lactone (the D-α-glucoheptonolactone of E. Fischer[3]). The nomenclature which we have used for the new ketose is an extension of the device introduced by Hudson[4] for aldoses of higher-carbon content.

The immediate starting point for our work was the crystalline D-gluco-D-*gulo*-heptonic acid hexaacetate monohydrate.[5] The course of reaction in this synthesis runs: D-gluco-D-*gulo*-heptonic acid monohydrate (I) → sodium D-gluco-D-*gulo*-heptonate hexaacetate trihydrate (II) → D-gluco-D-*gulo*-heptonyl chloride hexaacetate (III) → 1-diazo-1-desoxy-*keto*-D-gluco-L-*tagato*-octose hexaacetate (IV) → *keto*-D-gluco-L-*tagato*-octose hexaacetate (V) → D-gluco-L-*tagato*-octose (VI). All members of this series were crystalline with the exception of the acid chloride (III) and the keto-octose (VI). The latter was isolated as an amorphous solid characterized by its known phenylosazone, *keto*-acetate, elementary analysis and rotation. It was not fermented by yeast. It failed to give a Seliwanoff ketose reaction, although D-glucoheptulose exhibits a weak Seliwanoff test.

The solubility of the sodium salt (II) was unusual. It was soluble in water, benzene and chloroform. These solubilities allow of some comparisons in optical rotatory power. The salt possessed a molecular rotation $[M]^{20}D$ in chloroform of +11,600° while that of the free acid[5a] in the same solvent was +3000°. This difference might be interpreted to indicate that this hydrated salt ionized in chloroform solution.

(1) Paper No. 9 in the series entitled "The Action of Diazomethane upon Acylic Sugar Derivatives;" previous communication, M. L. Wolfrom and A. Thompson, THIS JOURNAL, **68**, 791 (1946).

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(3) (a) E. Fischer, *Ann.*, **270**, 64 (1892); (b) *cf.* H. Kiliani, *Ber.*, **19**, 767 (1886).

(4) C. S. Hudson, THIS JOURNAL, **60**, 1537 (1938); "Advances in Carbohydrate Chem.," **1**, 12, 28 (1945).

(5) (a) M. L. Wolfrom, M. Konigsberg and D. I. Weisblat, THIS JOURNAL, **61**, 574 (1939); (b) G. B. Robbins and F. W. Upson, *ibid.*, **62**, 1074 (1940).