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

Chromatography of Sugars and their Derivatives

BY L. W. GEORGES,[1] R. S. BOWER[1] AND M. L. WOLFROM

The use of chromatographic methods for separating sugars and their derivatives has shown a marked expansion since the discovery by Reich[2] that a mixture of the *p*-phenylazobenzoates of D-fructose and D-glucose yielded two visible bands on a chromatogram developed on both silica and alumina. In this Laboratory, we have been concerned with the development of chromatographic methods for sugar separations, employing the general brush or streak technique established by Zechmeister and co-workers.[3] With non-aqueous solvents as developers, the acetates of the sugars and sugar alcohols were chromatographed on "Magnesol," a commercial hydrated magnesium acid silicate, using an aqueous solution of alkaline permanganate as the streak indicator.[4] Later, it was found[5] that the sugars, sugar alcohols and glycosides could be directly chromatographed on a number of adsorbents with developers, such as ethanol or propanol-2, to which were added varying amounts of water. Certain types of native clays, such as Florex XXX, were the preferred adsorbents. One of the advantages of using the unacetylated sugars was that there was no substituent group to mask the character of the sugar residue. It was also possible to eliminate the preliminary work involved in preparing the acetates, a procedure which frequently leads to a multiplicity of isomers and which can be quite tedious and in some instances not very desirable or feasible.

From the standpoint of laboratory manipulations, the above-mentioned clay chromatographic technique suffers from two disadvantages. These are the poor extrusion properties of the clay columns[5c] and the difficulty of reading the indicator streak. We wish to report herein the use of an adsorbent that overcomes these difficulties and which can be used for the chromatographic separation of either the unsubstituted polyhydroxy

TABLE I

CHROMATOGRAPHIC ADSORPTION SERIES OF SOME SUGARS AND DERIVATIVES (ARRANGED IN DECREASING ORDER OF ADSORPTIVE STRENGTH)

Adsorbent: 0.9 × 11 cm.[a] of 5:1 mixture of Silene EF[f]–Celite[g]

Adsorbate soln.: noted below under group headings

Developer: noted below following class headings

Group I. Sugars, Sugar Alcohols and Glycosides (0.5 cc. of 90%[b] dioxane[c] followed immediately by a soln. of 2 mg. subst. in 0.2 cc. of 90% dioxane)

Class 1. (10 cc. of 90% dioxane)

α-D-Galacturonic acid
Lactose monohydrate
Lactitol
Dulcitol
Melezitose, raffinose pentahydrate, gentiobiose, D-gluco-D-*gulo*-heptose
Sucrose, maltose monohydrate, cellobiose, D-glucitol (sorbitol)
D-Galactose, D-mannitol
D-Glucose, D-fructose, D-mannose, L-sorbose
L-Fucitol
L-Arabinose

Class 2. (5 cc. of 90% dioxane)

L-Fucose
D-Xylose, L-rhamnose monohydrate
Methyl α-D-glucopyranoside

Group II. Acetylated Sugars and Acetylated Sugar Alcohols (0.5 cc. of benzene followed immediately by a soln. of 2 mg. subst. in 0.5 cc. of benzene)

Class 1. (15 cc. of 250:1[d] benzene–ethanol[e])

Raffinose hendecaacetate
β-Melibiose octaacetate, sucrose octaacetate
β-Maltopyranose octaacetate

Class 2. (15 cc. of 500:1 benzene–ethanol)

keto-D-Fructose pentaacetate
D-Glucitol (sorbitol) hexaacetate, D-mannitol hexaacetate
β-D-Glucopyranose pentaacetate
α-D-Arabinopyranose tetraacetate

Class 3. (15 cc. of 1000:1 benzene–ethanol)

α-L-Fucose tetraacetate

Group III. Methylated Sugars (0.5 cc. of benzene followed immediately by a soln. of 2 mg. subst. in 0.5 cc. of absolute chloroform)

Class 1. (15 cc. of 100:1 benzene–ethanol)

2,3-Dimethyl-D-glucose
2,3,6-Trimethyl-D-glucose

Class 2. (12.5 cc. of 250:1 benzene–ethanol)

2,3,4,6-Tetramethyl-D-glucose

[a] Column dimensions are those of the adsorbent. [b] Prepared by diluting 90 cc. of absolute dioxane with 10 cc. of water. [c] Purified by the procedure of K. Hess and H. Frahm, *Ber.*, **71B**, 2627 (1938), as modified by L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., New York, N. Y., 1941, p. 369. [d] Volume ratio. [e] Benzene was thiophene-free and ethanol was absolute. [f] *Cf.* ref. 6. [g] *Cf.* ref. 7.

(1) Research Associate (L. W. G., Project 203, Corn Industries Research Foundation; R. S. B., Project 212, Ordnance Department) of The Ohio State University Research Foundation.

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

(3) L. Zechmeister, L. de Cholnoky and (Mlle.) E. Ujhelyi, *Bull. soc. chim. biol.*, **18**, 1885 (1936).

(4) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(5) (a) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., *ibid.*, **67**, 1865 (1945); (b) **68**, 1449 (1946). *Cf.* this reference for a review of other published work.

(5c) The Scientific Glass Apparatus Co., Bloomfield, New Jersey, has made for us some experimental chromatographic tubes with a slight taper that overcome these extrusion difficulties.

TABLE II

CHROMATOGRAPHIC SEPARATION OF SOME SUGAR (AND DERIVATIVES) PAIRS ON SILENE EF[f]–CELITE[g] (5:1)

Substance	Wt., mg.	Adsorbate soln. Solvent	Cc.	Size of column of adsorbent, cm.	Developer	Position of zones, cm. from top	Recovery, %	$[\alpha]_D$ (20–25°, c 4)[d] Found	Acc'pt.	Solvent
D-Glucitol (sorbitol)	100	90% dioxane[a]	30	5.1[h] × 24.0	1800 cc. of 92%	5.0–10.5	78[c]	+ 10°	+ 10°	Chloroform
D-Mannitol	100				dioxane[a]	15.0–21.5	90[c]	+ 25.5	+ 26	Chloroform
Raffinose pentahydrate	800	90% ethanol	33	5.1 × 25.5	500 cc. of 95%	1.5– 4.7	83	+104	+105	Water
Sucrose	800				propanol-2	11.2–21.0	80	+ 66	+ 66.5	Water
D-Galactose	500	90% dioxane	20	4.4 × 19.5	218 cc. of 92%	2.3– 4.4	78	+ 77	+ 80	Water
L-Rhamnose monohydrate	500				dioxane	13.7–18.0	80	+ 8.6	+ 8.2	Water
Maltose monohydrate	500	85% dioxane	20	4.4 × 17.0	300 cc. of 92%	1.0– 3.3	78	+130	+130	Water
D-Glucose	500				dioxane	4.5–6.8	87	+ 51	+ 52.5	Water
D-Glucitol (sorbitol) hexaacetate	250	Benzene	33	5.1 × 26.0	1800 cc. of benzene-ethanol	9.0–14.8	75	+ 10.7[e]	+ 10	Chloroform
D-Mannitol hexaacetate	250				(1000:1)[b]	16.5–20.0	71	+26	+ 26	Chloroform

[a] *Cf.* Table I, notes *b* and *c*. [b] *Cf.* Table I, note *e*. [c] Recovered and characterized as the hexaacetate. [d] Values for the mutarotating sugars were read at equilibrium. [e] Rechromatographed. *Cf.* experimental portion for details. Value after initial separation was +11.8°. [f] *Cf.* ref. 6. [g] *Cf.* ref. 7. [h] Diameter.

compounds or their acetylated or methylated derivatives. This adsorbent is "Silene EF,"[6] a commercial synthetic, hydrated calcium acid silicate. Developers used with the sugars, sugar alcohols and glycosides were dioxane and the lower molecular weight alcohols, to which were added various amounts of water. The columns all extruded easily, various lots of adsorbents showing no differences in this respect. When the extruded column was brushed with the alkaline permanganate indicator, the streak showed the positions of the adsorbed zones more distinctly and sharply than with any other combination of adsorbent and developer that has been tried in this Laboratory. This was especially true in the dioxane–water system. A number of typical sugars, sugar alcohols and glycosides are arranged in a chromatographic series in Table I, group I. That a change in the developer may sometimes be advantageous, is illustrated by the data depicted in Fig. 1, wherein a separation on Silene EF, of sucrose and raffinose is shown to be best accomplished by development with propanol-2 and water. With the acetylated and methylated derivatives (*cf.* Table I, groups II and III), the benzene–ethanol mixtures employed previously[4] as developers, were found to be suitable.

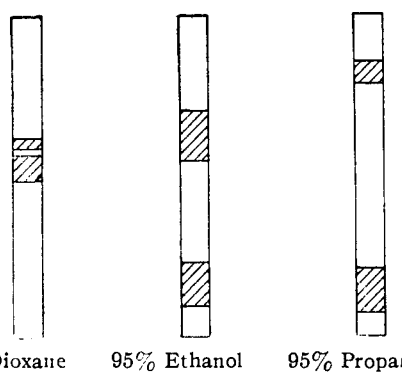

Fig. 1.—Separation of sucrose–raffinose zones on Silene EF–Celite (5:1) with different developers. An amount of 0.5 cc. of the developer was placed on each column, followed immediately by a solution of 2 mg. each of sucrose and raffinose pentahydrate in 0.5 cc. of the developer. Development was then effected with 10 cc. of the developer noted (5 cc. in case of the 95% ethanol).

In Table I, compounds listed in the same line were not separable under the conditions cited. That a separation may be effected under other conditions, is illustrated by the cited separation of D-glucitol (sorbitol) hexaacetate and D-mannitol hexaacetate (Table II and experimental portion). Three pairs (in equal admixture) of sugars were separated and the products were isolated and identified (Table II). D-Glucitol (sorbitol) and D-mannitol were likewise readily separated and identified by acetylation of the separated zone material (Table II and experimental part).

Experimental

The adsorbent, Silene EF, is a commercial, synthetic hydrated calcium acid silicate.[6] It was used in admixture (5:1) with a filter-aid.[7] It contains a considerable amount of water-soluble materials but is used without purification. Different lots vary considerably in their adsorptive properties. The extrusion characteristics of this adsorbent are excellent and were not found to vary with different lots.

The streak reagent was a freshly prepared 1% solution of potassium permanganate in 2.5 *N* sodium hydroxide. The zones turned brown and exhibited good boundary lines. With the purified dioxane–water developer, the zones were particularly well delineated and the interzones were dark pink in color and persisted for a relatively long period of time.

Further general manipulative details are cited in a previous publication.[5b] Procedures for two typical separations listed in Table II are given below.

Separation of D-Galactose and L-Rhamnose.—Only those details not cited in Table II will be given. The sectioned zones (with the streak removed) were each eluted portion-wise with a total of 200 cc. of water at room temperature, filtered through a sintered glass funnel containing a layer of Celite 535,[7] and to the eluate was added immediately an excess (20 cc.) of a 10% neutral lead acetate aqueous solution. The precipitate that formed was removed by filtration. The excess lead ion was precipitated with hydrogen sulfide and the filtered (decolorizing charcoal) solution was concentrated under reduced pres-

(6) A product of the Columbia Chemical Division, Pittsburgh Plate Glass Co., Barberton, Ohio.

(7) Celite 535, a siliceous filter-aid manufactured by Johns-Manville Co., New York, N. Y.

sure at 40–45° to a volume of *ca.* 50 cc. This solution was passed slowly over a 1.6 cm. diam. column containing 50 g. of cation exchange resin (Amberlite IR-100[8]) and the effluent was passed in like manner over a similar column of anion acceptor resin (Amberlite IR-4[8]). The resultant solution was concentrated under reduced pressure to a crystalline residue which was generally contaminated with a small amount of silica. The latter was removed by treatment with a small amount of water followed by filtration. The filtrate, after solvent removal, yielded the crystalline sugar in the amount and purity listed in Table II.

Separation of D-Glucitol (Sorbitol) Hexaacetate and D-Mannitol Hexaacetate.—These compounds were not separable under the conditions listed in Table I (group II, class 2). Their separation was effected under the conditions given in Table II. Elution of each zone was effected with 100 cc. of acetone followed by filtration and washing with a further 300 cc. of acetone. Solvent removal yielded the crystalline acetates. The material from the D-glucitol hexaacetate zone was rechromatographed on a 4.4 × 22.5 cm. column of the same adsorbent and developed with 1250 cc. of benzene–ethanol (1000:1). The material in the main zone was recovered as described above. The small second zone was discarded.

(8) A product of the Resinous Products and Chemical Co., Philadelphia, Pennsylvania.

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Summary

The suitability of a synthetic hydrated calcium acid silicate (Silene EF) for the chromatography of sugars and their derivatives (alcohols, glycosides, acetates and methyl ethers) is demonstrated. The streak (toward the alkaline permanganate indicator employed) and extrusive properties of this adsorbent are especially favorable.

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Isosucrose Synthesis

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The development of a chromatographic method for the separation of the sugar acetates[2] and an improved procedure for the preparation of 1,3,4,6-D-fructofuranose tetraacetate led us to an investigation of the condensation products formed between 1,3,4,6-D-fructofuranose tetraacetate and 2,3,4,6-D-glucopyranose tetraacetate with phosphoric anhydride.[3] Employing laborious and difficult crystallization techniques, Irvine and Stiller had found no sucrose in the products from this reaction. It was considered desirable to search the condensates for sucrose by employing these new chromatographic techniques.

Isosucrose octaacetate was crystallized from the products of this condensation. Adsorption of the residual material on "Magnesol"[2] led to the detection and isolation of a zone which yielded more isosucrose octaacetate. Adsorption of the material from this zone on Silene EF[4] under conditions established for the separation of sucrose and isosucrose octaacetates gave only one zone, that of isosucrose octaacetate. The material from the isosucrose octaacetate zone of the Magnesol chromatogram was deacetylated and allowed to react with freshly prepared diazo-uracil[5] (specific for sucrose); the results were negative.

(1) Research Associate of the Sugar Research Foundation, Inc. (Project 190 of The Ohio State University Research Foundation.) The laboratory assistance of Miss Eloise Carpenter is gratefully acknowledged. Preliminary work on this problem had been carried out in this Laboratory by Mrs. Ruth Haverstock Ness.

(2) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(3) J. C. Irvine and E. T. Stiller, *ibid.*, **54**, 1079 (1932).

(4) L. W. Georges, R. S. Bower and M. L. Wolfrom, *ibid.*, **68**, 2169 (1946).

(5) H. W. Raybin, *ibid.*, **55**, 2603 (1933); **59**, 1402 (1937).

1,3,4,6-D-Fructofuranose tetraacetate was prepared by the acetolysis of inulin triacetate following the procedure recommended by Irvine and Stiller[3] except that it was found that pure acetyl bromide produced only a negligible degree of hydrolysis. The addition of hydrogen bromide to the reaction mixture caused nearly complete acetolysis in less than three hours at 25° and a 54–58% yield of 1,3,4,6-D-fructofuranose tetraacetate resulted. It is thus apparent that hydrobromic acid accidentally introduced by moisture contamination is the active agent in this frequently used hydrolytic reagent.

Experimental

Ten grams of inulin triacetate (dried for two days under reduced pressure at 45°) was dissolved in a mixture of 100 cc. of glacial acetic acid (dried over anhydrous calcium sulfate) and 12 cc. of acetyl bromide (freshly prepared). The specific rotation (sodium D-line, 23°) of the reaction solution changed from −40 to −1° in three and one-half to four hours at 30°. Irvine and Stiller[3] stated that a final value of +80° was attained in about three hours.

Ten grams of inulin triacetate (dried in air at room temperature) was dissolved in a mixture of 100 cc. of glacial acetic acid and 20 cc. of acetyl bromide. Then 20 cc. of 30–32% hydrogen bromide in glacial acetic acid solution was added and the mixture was allowed to stand for two to two and one-half hours at 25°. The specific rotation of the reaction solution changed from −40 to +83° during this reaction period. The reactants were then poured into a 3-liter beaker surrounded with ice and 100 g. of finely crushed ice was added to the beaker followed by 10 g. of sodium acetate. A total of 1.5 liters of distilled water was then added and the *p*H of the solution adjusted to 5.5 with sodium bicarbonate (*ca.* 170 g.). The neutralized solution was extracted 10 times with 100-ml. portions (total 1 liter) of chloroform. The extracts were dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure at 40–45°. The average yield of