

THE ALKALINE OXIDATION OF CELLULOSE. II
CHROMATOGRAPHY OF ACETYLATED
CARBOHYDRATE ACIDS

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In the course of an investigation of the composition of oxycellulose prepared by peroxide in presence of alkali (1) it was necessary to develop a chromatographic method for separating acetylated carbohydrate acids. The method developed is described in this paper.

The experimental technique commonly referred to as chromatographic analysis has been extensively investigated as to its applications to carbohydrates, by Wolfrom and his coworkers. McNeeley, Binckley, and Wolfrom (2) were able to achieve separation of sugar acetates on Magnesol using aqueous alkaline permanganate as the brush reagent. Georges, Bower, and Wolfrom (3) applied the brush technique to the chromatography of sugars on Silene as the adsorbent. Bower and Wolfrom (4) have separated sugar acids, as the acetylated amides, on Silene EF, a hydrated sodium aluminum silicate. Hoffman and Wolfrom (5) used acid washed Silene EF for chromatography of the unacetylated sugar acids. An excellent review of the literature and of practical laboratory procedures has been written by Binckley and Wolfrom (6).

After investigating several adsorbents it was found that Mallinckrodt silicic acid, Analytical Reagent Grade, effected separation of several different types of acetylated carbohydrate derivatives when benzene solutions of either acetic acid or acetone were used as developers. An undesirable feature of silicic acid noted is that the adsorbent has poor filtration properties, but this may be corrected by mixing in Celite, an inactive diatomaceous earth.

The quantitative work on chromatographic analysis carried out in this investigation is summarized in table I. All runs were made on 70 g. columns of 3 silicic acid/1 Celite. The loading ordinarily used was much below capacity. Based on run E, 10 mg. of material per gram of adsorbent is quite practical even for two compounds which are relatively difficult to separate. The strength and amount of developer used depends not only on the nature of the compounds being chromatographed, but also on the amount of moisture in the silicic acid. The silicic acid-Celite adsorbent was made up by mixing the undried silicic acid with the Celite.

EXPERIMENTAL

Standard chromatographic procedure. A fairly typical separation is described below.

A typical chromatographic tube, 35 mm. diameter, was fitted to a one-liter suction flask, connected to a water aspirator pump. Seventy grams of a mixture of three parts Mallinckrodt A.R. silicic acid to one part Celite was poured into the tube with the ap-

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paratus under a slight vacuum. Under full vacuum the sides of the tube were tapped with a cork ring until all of the adsorbent was settled. After breaking the vacuum slowly, a wad of cotton was placed on the top of the column to prevent turbulence from disturbing

TABLE I
CHROMATOGRAPHIC SEPARATIONS

COMPOUND	WEIGHT IN MG.	DEVELOPER	POSITION	% RECOV- ERY	M. P., °C	
					Found	Lit.
A. Gluconic acid pentaacetate and Arabonic acid tetraacetate	127	500 ml. 15/1 benzene- acetic acid	Upper	87	110-111.5	110-111(7)
	120		Lower	91	135-135.5	135-136(8)
B. Gluconic acid pentaacetate and D- α -Glucoheptonolactone pentaacetate	127	300 ml. 30/1 benzene- acetic acid	Upper	81	111-111.5	110-111(9)
	163		Lower	83	130-131	128(9)
C. Galactonamide pentaacetate and Galactonic acid pentaacetate	135	600 ml. 20/1 benzene- acetic acid	Upper	82	164-164.5	165-166(10)
	142		Lower	83	131-131.5	131-132(7)
D. Pentaacetylgluconic phenylhydrazide and Gluconic acid pentaacetate	129	600 ml. 20/1 benzene- acetic acid	Upper	81	153-153.5	152-154(11)
	134		Lower	84	111-111.5	110-111(7)
E. Gluconamide pentaacetate and Pentaacetylgluconic phenylhydrazide	388	700 ml. 20/1 benzene- acetic acid	Upper	87	183-184	184-185(10)
	402		Lower	82	152-153	152-154(11)
F. D- α -Glucoheptonolactone pentaacetate and Glucononitrile pentaacetate	123	300 ml. 50/1 benzene- acetic acid	Upper	88	130-131	128(9)
	134		Lower	82	82-83	84(12)

the adsorbent. Gluconic acid pentaacetate, 127 mg., and D- α -glucoheptonolactone tetraacetate, 163 mg., were dissolved in 20 ml. of U.S.P. chloroform. After pre-wetting the adsorbent column with 50 ml. of benzene, the chloroform solution was added, and the column was developed with 300 ml. of a developer made up of thirty parts of benzene to one part acetic acid. Following the development of the column, full vacuum was applied,

and the chromatogram was dried for a few minutes and extruded with the aid of a wooden dowel.

A stripe of alkaline permanganate (1% potassium permanganate, 20% sodium hydroxide) was painted the length of the column using a glass wool brush. As soon as the two well-defined zones were located, the column was sectioned with a spatula, discarding the non-reducing zones. After removal of the indicator stripe, the two reducing zones were eluted with 200 ml. of acetone each, allowed to stand for about an hour, filtered on a sintered glass funnel, and the adsorbent was washed with two 500-ml. portions of acetone.

The acetone was removed from each eluate by evaporation in stream of air. After drying completely, each flask was washed with chloroform. The chloroform was filtered to remove silica, which is difficult to remove entirely from the acetone solution. After evaporation of the chloroform and drying the residues in a vacuum desiccator, the solid material in each flask was recrystallized from a hot mixture of toluene and petroleum ether.

The material isolated from the top zone of the column melted at 111–111.5°, and therefore was gluconic acid pentaacetate; recovery: 81%, literature m.p. (8) 110–111°.

The crystalline material from the lower zone melted at 130–131°. Upson and Bartz (10) have reported the melting point of D- α -glucoheptonolacton tetraacetate as 128°; recovery: 83%.

Experience with tetraacetyl gluconic acid indicated that if there were a free hydroxyl in the acetylated lactone as reported (10), the hydroxylactone acetate would have been much more strongly adsorbed, and therefore, more slowly developed than was observed. Analytical data indicate that the lactone is the pentaacetate. Found, C: 49.15, 48.80; H: 5.17, 5.23; mol. wt. 422, 398. Theory for D- α -glucoheptonolactone pentaacetate requires C: 48.80, H: 5.30, and mol. wt. 418. Theory for the tetraacetate requires C: 47.87, H: 5.36, and mol. wt. 376.

Results obtained in separating several mixtures of several derivatives of sugar acids are shown in table I.

SUMMARY

1. A chromatographic method for the separation of acetylated carbohydrate acids and derivatives has been developed.
2. The order of adsorption, on silicic acid, of various acetylated carbohydrate derivatives has been determined to be amides > phenyl hydrazides > acids > lactones > nitriles.

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REFERENCES

- (1) *J. Org. Chem.*, preceding paper.
- (2) McNEELEY, BINCKLEY, AND WOLFROM, *J. Am. Chem. Soc.*, **67**, 527 (1945).
- (3) GEORGES, BOWER, AND WOLFROM, *J. Am. Chem. Soc.*, **68**, 2169 (1946).
- (4) BOWER AND WOLFROM, unpublished results.
- (5) HOFFMAN, Thesis, The Ohio State University (1948).
- (6) BINCKLEY AND WOLFROM, *Chromatography of Sugars and Related Substances*, Scientific Report Series, No. 10, Sugar Research Foundation, Inc., New York (1948).
- (7) HURD AND SOWDEN, *J. Am. Chem. Soc.*, **60**, 235 (1938).
- (8) ROBBINS AND UPSON, *J. Am. Chem. Soc.*, **62**, 1074 (1940).
- (9) UPSON AND BARTZ, *J. Am. Chem. Soc.*, **53**, 4226 (1931).
- (10) ROBBINS AND UPSON, *J. Am. Chem. Soc.*, **60**, 1788 (1938).
- 11) MAJOR AND COOK, *J. Am. Chem. Soc.*, **58**, 2476 (1936).
- (12) ZEMPLEN AND KISS, *Ber.*, **60**, 165 (1927).