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Abstract

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Chromatography of Monosaccharide Derivatives in a Thin Layer of Aluminum Oxide

When the method of paper chromatography is used for the identification and separation of substituted carbohydrates possessing hydrophobic properties, certain limitations arise, the chief of which is a decrease in the resolving power of the method, so that derivatives similar in structure cannot be separated with sufficient clarity; in a number of cases serious difficulties arise in detecting substances on paper.

Chromatography in an unfixed thin layer of aluminum oxide has recently been rapidly gaining a firm place as a highly effective analytical method; it has already been applied to such classes of natural compounds as, for example, steroids ([1],[2]). This method also renders invaluable service in the separation, establishment of individuality, and identification of various carbohydrate derivatives. In view of the fact that up to now there have been no reports in the literature on the broad use of this type of chromatography in carbohydrate chemistry, in this article we summarize the experience accumulated in our laboratory in the chromatography of monosaccharide derivatives in a thin layer of aluminum oxide.

Chromatography in an unfixed thin layer of aluminum oxide is especially useful for the study of relatively low-polarity, fully or partially substituted carbohydrates. Along with the advantages of the usual methods of analytical chromatography, this method has a number of additional merits, of which the following should first be noted: high resolving power for many classes of compounds; short chromatographing time (10–20 min.); universality of solvent systems and of the method of detecting spots; extreme simplicity of the apparatus; and the possibility of rapidly selecting conditions for preparative adsorption chromatography. The working technique is analogous to that used for other compounds ([1–4]) and is briefly as follows. Onto a glass plate about 20 cm long, a layer of sifted aluminum oxide is poured; the required thickness (~1 mm) and width are imparted to it by passing along the plate a glass tube with two rubber rings fitted onto it. The substances are applied at a distance of 3–4 cm from the lower edge of the plate, 1.5–2 cm apart from one another. Development is carried out by the ascending method, placing the plate at an angle of 15–20° in a closed chamber. Chromatography lasts about 20 min., after which the still-moist plate is sprayed from an atomizer with concentrated H_2SO_4 and heated under an infrared lamp. The substances are detected as sharp black spots on a white background.

To obtain maximally reproducible values of R_f, aluminum oxide of constant activity should be used. In our work we used aluminum oxide of the grade "for chromatography,"activity grade II according to Brockmann, without preliminary treatment. The greatest resolving power of the method is achieved with the use of strictly airtight chromatographic chambers; experience shows, however, that in everyday work simpler chambers covered with ordinary glass may be used (the R_f values given below were obtained in such chambers). The amounts of substances that must be applied to the chromatogram in order to obtain–

obtaining a clear result, can easily be selected after two or three trials. The sensitivity of the method for detecting spots is so great that these amounts usually amount to several tens of micrograms. It should be noted that when solutions of substances in strongly polar solvents are applied to the plates (considerably exceeding in polarity the solvent system used for development), the values of R_f may be overestimated.

Detection of spots with conc. H_2SO_4 is characterized by very high sensitivity and great universality. In addition, many compounds, such as halogen derivatives, trityl ethers, cardiac glycosides, etc., give spots on chromatograms colored in characteristic colors. Other reagents may also be used for detecting substances. The most critical part of the work is the selection of the solvent for chromatography. In the present case, as in any chromatographic method, depending on the nature of the compounds, solvent systems of varying polarity must be used; combinations of petroleum ether, benzene, chloroform, ethyl acetate, and methanol are usually used. For chromatography of fully substituted monosaccharides containing no free hydroxyl groups, we used a petroleum ether –benzene mixture (1 : 1); the R_f values in this system lie within the range 0.3–0.6. If these compounds are chromatographed in benzene, the R_f values increase to 0.5–0.9. In chromatography of acetone or benzylidene derivatives and full acetates of the α-forms of monosaccharides, we used a chloroform–methanol mixture (95 : 5) as the solvent system; the R_f values obtained lie in the interval 0.4–0.6. When selecting solvent systems for incompletely substituted monosaccharides, it should be borne in mind that with the appearance of each new hydroxyl group the polarity of the substance increases sharply. If substances with one free hydroxyl group can be chromatographed in chloroform or its mixtures with ethyl acetate, then for investigation of compounds with two or more hydroxyl groups it is necessary to use mixtures of chloroform with methanol.

It must be taken into account that the R_f values obtained in chromatography in a thin layer of aluminum oxide (including those given in this article) are of a more approximate nature than analogous data obtained in paper chromatography, which is a drawback of the method under consideration. Although no special study of this question has yet been carried out, it is obvious that the magnitude of R_f, in addition to the quality of the adsorbent, also depends on the thickness of the adsorbent layer, temperature, degree of tightness of the chamber, and other factors. In this connection, in order to obtain reproducible

R_f values it is necessary, as far as possible, to adhere more carefully to standard chromatographic conditions.

Chromatography in a thin layer of aluminum oxide has been used by us very widely for solving the following problems: identification of compounds (by comparison on one chromatogram with a reference substance); control of individuality; qualitative assessment of the composition of complex mixtures isolated from natural objects, obtained as a result of reactions, etc.; selection of conditions for preparative chromatography on aluminum oxide; qualitative investigation of fractions obtained in preparative chromatography. In addition, comparison of substances of unknown structure with known reference compounds made it possible in a number of cases to obtain valuable information about their structure. Extension of the method to other, as yet uninvestigated, classes of compounds generally encounters no limitations of a fundamental nature. It may be assumed in advance, however, that when working with highly polar compounds, for example with free monosaccharides, it is more expedient to use chromatography on paper or in a thin layer of silica gel. Certain difficulties are also encountered when working with substances labile to aluminum oxide. But in view of the very small pro-

the duration of chromatography in a thin layer, transformations occurring on the adsorbent can, in a number of cases, be disregarded.

Using chromatography in an unfixed thin layer of alumina, we studied various derivatives of monosaccharides or compounds containing a monosaccharide structural unit. Data on the chromatographic behavior of those classes of compounds that we investigated in the largest number of representatives are given below. The most commonly used solvent systems were: petroleum ether—benzene 1 : 1 (A), benzene (B), chloroform (V), chloroform—methanol 19 : 1 (G), chloroform—methanol 49 : 1 (D), benzene—chloroform 2 : 3 (E), benzene—methanol 9 : 1 (Zh), ethyl acetate (Z), and chloroform—methanol 1 : 1 (I).

Completely protected derivatives of monosaccharide mercaptals (the substances contain no free hydroxyl groups). Recommended solvent systems: A, B, and V. The R_f values obtained were: diethyl mercaptal of 2,4-3,5-di-*O*-benzylidene-*D*-xylose, 0.85 (B); 2,4-3,5-di-*O*-benzylidene-*D*-ribose, 0.85 (B); 2,3-4,5-di-*O*-isopropylidene-*D*-xylose, 0.6 (A), 0.85 (B); 2,3-4,5-di-*O*-isopropylidene-*L*-arabinose, 0.85 (B); 2-*O*-benzoyl-3,4-5,6-di-*O*-isopropylidene-*D*-glucose, 0.30 (B); 4-*O*-benzoyl-2,3-5,6-di-*O*-isopropylidene-*D*-glucose, 0.55 (B); 6-iodo-6-deoxy-2,3-4,5-di-*O*-isopropylidene-*D*-galactose, 0.80 (B); 2,3,4,5,6-penta-*O*-benzoyl-*D*-glucose, 0.75 (B); 2,3,4,5,6-penta-*O*-acetyl-*D*-glucose, 0.5 (B); 2,3,4,5-tetra-*O*-acetyl-*L*-arabinose, 0.3 (B), 0.7 (V); dibenzyl mercaptal of 6-iodo-6-deoxy-2,3-4,5-di-*O*-isopropylidene-*D*-galactose, 0.85 (A), 0.90 (B); ethylene mercaptal of 2,3,4,5,6-penta-*O*-acetyl-*D*-glucose, 0.4 (B).

Completely protected derivatives of hexitols and cyclic forms of monosaccharides are chromatographed in the same solvent systems A, B, and V. R_f values for the compounds studied: 2,5-diiodo-2,5-dideoxy-1,4-3,6-

dianhydro-*L*-ioditol, 0.90 (B); 1,6-diiodo-1,6-dideoxy-2,3,4,5-di-*O*-benzylidene-*D*, *L*-dulcitol, 0.68 (A), 0.87 (B); 1,6-dideoxy-2,3-4,5-di-*O*-isopropylidene-*D*, *L*-dulcitol, 0.7 (B); 1,6-dideoxy-2,3,4,5-di-*O*-benzylidene-*D*, *L*-dulcitol, 0.75 (B); 1,2-3,4-5,6-tri-*O*-isopropylidene-*D*-mannitol, 0.6 (B); 1,2,5,6-tetra-*O*-benzoyl-3,4-*O*-isopropylidene-*D*-mannitol, 0.58 (B), 0.85 (V); 1,2-5,6-di-*O*-isopropylidene-3-*O*-tosyl-*D*-glucofuranose, 0.8 (V); 6-*O*-trityl-2,3,4-tri-*O*-acetyl-α-methyl-*D*-glucopyranoside, 0.30 (B); 2,3-5,6-di-*O*-isopropylidene-α-ethylthio-*D*-mannofuranoside, 0.70 (B).

Derivatives of aldose forms of carbohydrates are chromatographed in solvent systems B, V, G, and D. R_f values for the compounds studied: 2,3-4,5-di-*O*-isopropylidene-aldehydo-*L*-arabinose, 0.6 (G); *p*-nitrophenylhydrazone of 2,3-4,5-di-*O*-isopropylidene-aldehydo-*L*-arabinose, 0.6 (B); 2,3-4,5-di-*O*-isopropylidene-aldehydo-*D*-xylose, 0.7 (G); 2,4-3,5-di-*O*-benzylidene-aldehydo-*D*-ribose, 0.1 (V); 2,4-3,5-di-*O*-benzylidene-aldehydo-*D*-xylose, 0.9 (chloroform–methanol 9 : 1); 2,3,4,5-tetra-*O*-acetyl-aldehydo-*L*-arabinose, 0.45 (D), 0.8 (G); 2,3,4,5,6-penta-*O*-acetyl-aldehydo-*D*-glucose, 0.1 (V); 2,3,4,5,6-penta-*O*-benzoyl-aldehydo-*D*-glucose, 0.7 (D).

Partially protected carbohydrate derivatives containing one free hydroxyl group. Along with solvent systems V and G, systems E, Zh, and Z may be recommended. R_f values for the compounds studied: dibenzyl mercaptal of 2,3-4,5-di-*O*-isopropylidene-*D*-galactose, 0.1 (B), 0.35 (E); 3,4-5,6-di-*O*-isopropylidene-*D*-mannose, 0.3 (E); 3,4-5,6-di-*O*-isopropylidene-*D*-glucose, 0.25 (E); 2,3-5,6-di-*O*-isopropylidene-*D*-glucose, 0.15 (E); 2,3-*O*-isopropylidene-5-*O*-benzoyl-*L*-arabinose, 0.10 (B), 0.35 (V); diethyl mercaptal of 2,3-4,5-di-*O*-isopropylidene-*D*-galactose, 0.10 (B), 0.15 (E), 0.4 (V); 3,4-5,6-di-*O*-isopropylidene-*D*-mannose, 0.20 (B); 3,4-5,6-di-*O*-isopropylidene-*D*-glucose, 0.15 (B), 0.30 (V), 0.75 (Zh); 2,3-5,6-di-*O*-isopropylidene-*D*-glucose, 0.10 (B), 0.23 (V), 0.55 (Zh); 2,3-*O*-

isopropylidene-5-*O*-benzoyl-*L*-arabinose, 0.40 (B); dimethyl acetal of 3,4-5,6-di-*O*-isopropylidene-*D*-mannose, 0.50 (Zh); 3,4-*O*-isopropylidene-6-iodo-6-deoxy-β-methyl-*D*-galactopyranoside, 0.35 (Zh), 0.6 (G); 3,4-*O*-isopropylidene-β-methyl-*D*-fucoside, 0.35 (Zh); 1,2-5,6-di-*O*-isopropylidene-*D*-glucofuranose, 0.2 (B); 2,3-5,6-di-*O*-cyclohexylidene-*D*-mannofuranose, 0.70 (Z).

Partially protected carbohydrate derivatives containing two or more free hydroxyl groups. Recommended solvent systems–B, G, Z, and also I. R_f values for the compounds studied: 1,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-*D*-mannitol, 0.15 (B); 1,2,5,6-tetra-*O*-benzoyl-*D*-mannitol, 0.7 (I); 3,4-*O*-isopropylidene-*D*-mannitol, 0.3 (I); 1,4-3,6-dianhydro-*D*-mannitol, 0.30 (B); 1,4-3,6-dianhydro-*D*-sorbitol, 0.25 (B), 0.35 (G); 2,3-5,6-di-*O*-cyclohexylidene-*D*-mannitol, 0.6 (Z); 3,4-*O*-isopropylidene-β-methyl-*D*-galactopyranoside, 0.40 (G).

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Note: Figure translations are in progress. See original paper for figures.