THIN-LAYER CHROMATOGRAPHY OF SUCROSE ESTERS AND MIXTURES OF RAFFINOSE AND SUCROSE*

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(Received February 12th, 1962)

Widespread interest in the development of sucrose as a raw material for industrial chemicals has been stimulated by the development of sucrose esters as surface-active agents. These esters are synthesized by an alkaline transesterification procedure, whereby the reaction is terminated at a time favoring mono- or di-esterification of sucrose. Methods have been lacking to determine the composition of these esterification products, except by an average saponification equivalent value. A simple isolation procedure for determination of specific products has not been successfully developed for the reaction mixture, which usually contains a mixture of monoester, diester and unreacted sucrose. The average saponification equivalent value gives only a rough approximation of ester distribution.

Partially substituted sugar derivatives can be separated on silica columns[1,2]. With proper choice of solvents, the separation results in fractions of different degrees of substitution. As a routine technique for product evaluation, this procedure is time consuming and tedious. We have reported the composition of sucrose monostearate by gas chromatography[3]. This method is useful for structural composition data, but requires time to prepare sugar derivatives which have gas chromatographic mobility. The development of thin-layer chromatography with silica gel[4] suggested a rapid method to study product composition of these sugar esters and sucrose. During the period of our investigation, PASTUSKA[5] reported the successful use of thin-layer chromatography for carbohydrate separations. The technique of chromatography on chromatostrips as developed by MILLER AND KIRCHNER[4] provides a rapid and simple method for thin-layer chromatographic separation without elaborate equipment. Thin-layer chromatography on chromatostrips was extended to include the separation of sucrose and raffinose as part of a general study of separation of sugars.

EXPERIMENTAL

Qualitative analysis

Chromatostrips were prepared by coating pieces of single-weight window-glass, 1/2 in. × 5 1/2 in. in size, with silica gel*** Merck, No. 7729, Germany, containing 5%

* This paper was presented in part at the 12th General Meeting of the American Society of Sugar Beet Technologists, Denver, Colo., Febr. 5–8, 1962.

** A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

*** Obtained from E. Merck A.G., Darmstadt, Germany. Reference to a company or product by name does not imply approval or recommendation of the product by the Department of Agriculture to the exclusion of others that may also be suitable.

starch binder as described by MILLER AND KIRCHNER[4] and modified by APPLEWHITE *et al.*[6]. Fluorescent zinc salts were not added to the coating materials because the esters could be located with a spray of a 0.2 % solution of dichlorofluorescein in 95 % ethanol.

Two microliter spots containing 10–200 μg of sucrose esters dissolved in chloroform were applied at a distance of 1.5 cm from the bottom of the strip. A mark was made at a distance of 10 cm from the point of sample application so that replicate strips would migrate the same distance for R_F comparisons. The chromatostrips were irrigated by ascending migration of 1 ml of solvent mixture contained in a stoppered test tube. A number of different solvents were tried and a solvent mixture of toluene, ethyl acetate, and 95 % ethyl alcohol (10:5:5, by vol.) gave the best separations of sucrose esters (Fig. 1, strips 1–5). The chromatostrips were dried in air and

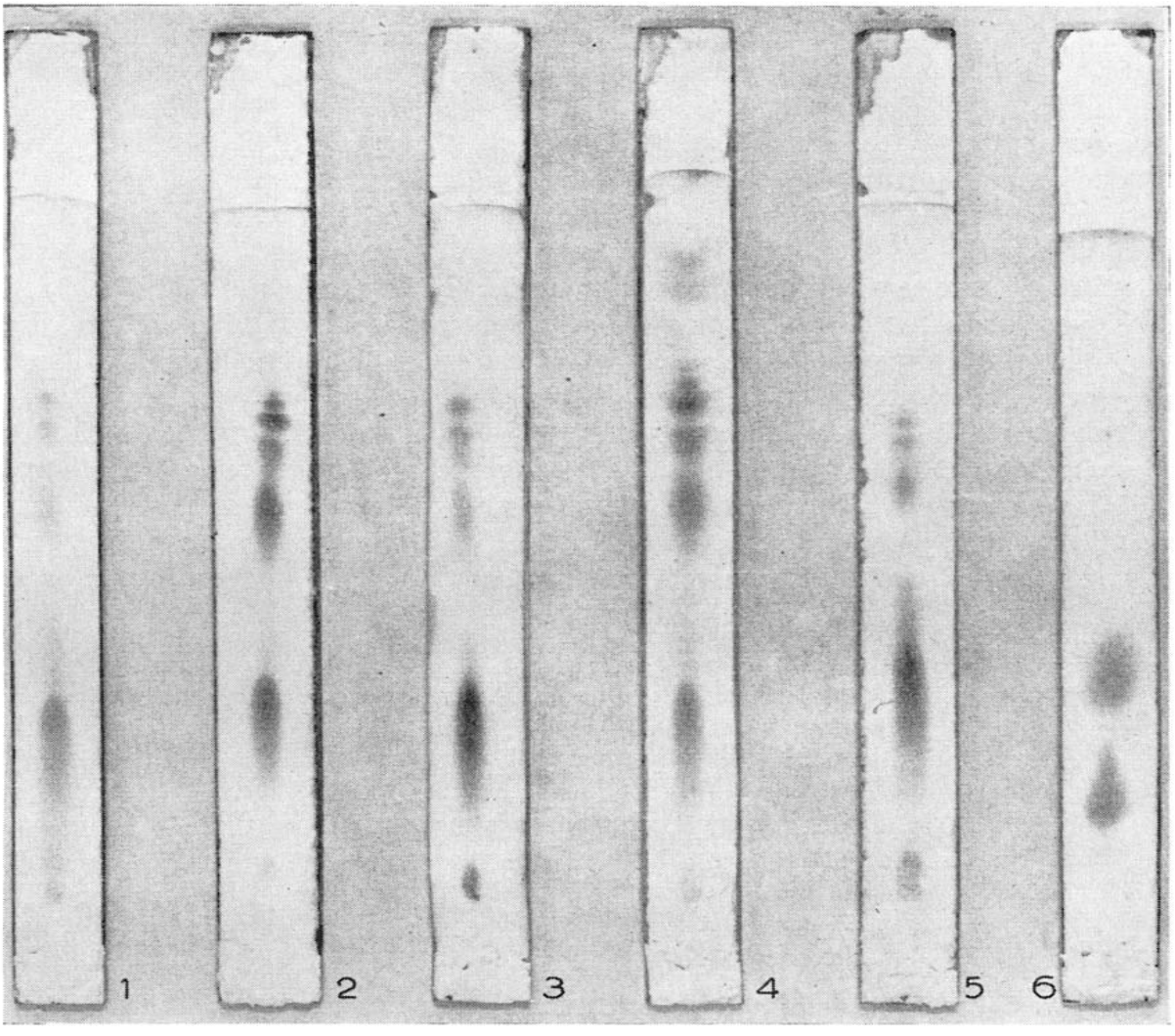

Fig. 1. Developed chromatostrips showing separations of sucrose esters, and sucrose and raffinose. The strips were prepared from silica gel G (for thin-layer chromatography, Merck), sprayed with dilute sulfuric acid, and heated at 110°. Sucrose esters chromatographed were: 1. Sucrose monopalmitate (200 μg); 2. Sucrose dipalmitate (200 μg); 3. Sucrose monolaurate (200 μg); 4. Sucrose lardate (200 μg); and 5. Sucrose tallowate (200 μg). Chromatostrip 6 shows the separation of 35 μg sucrose from 35 μg raffinose. See text for identification of components.

sprayed with the dichlorofluorescein indicator solution. The sucrose esters appeared as yellow spots on an orange background, when illuminated with a long-wave U.V. lamp. The time required for analysis was about 30 min.

Aqueous solutions of sucrose and raffinose mixtures were resolved on chromatostrips using a solvent composed of isopropyl alcohol–toluene–ethyl acetate–water (10:2:5:2.5, by vol.). The highly polar solvents migrate slowly on silica gel, and a period of 1–2 h was necessary for solvent migration of 10 cm. For sucrose and raffinose, the irrigated chromatostrips were sprayed after drying with a naphthoresorcinol–phosphoric acid indicator (5 vols. of 0.2 % naphthoresorcinol in acetone plus 1 vol. of 9 % phosphoric acid in water). The sugar-containing spots were revealed after a 5–10 min heating period at 110°. A typical separation is shown in Fig. 1, chromatostrip 6.

Quantitative analysis

The speed and ease of separation of sucrose-containing components on silica gel chromatostrips suggested the further application of this technique to obtain quantitative data.

Replicate chromatostrips were prepared as described for qualitative analysis so that each strip contained 100–200 μg of sample. After solvent irrigation, one strip was sprayed with the appropriate indicator to locate the fractions present. The R_F values of the various separated materials were noted on the sprayed strips and corresponding areas were scraped from the unsprayed chromatostrips. The silica gel sugar fractions were transferred to 10 mm filter tubes containing coarse fritted discs and each was eluted with 5 1-ml portions of dimethylformamide. The dimethylformamide solutions were evaporated to dryness *in vacuo* at 50–60° in a rotary evaporator. Blanks for unknowns were prepared from blank areas of irrigated chromatostrips and were treated in the same way as the sugar-containing areas. The evaporated residues were analyzed directly for ketose by the colorimetric method of ROE[7] and measured at 490 mμ on a Bausch and Lomb Spectronic 20 colorimeter. Standard samples of sucrose gave recoveries within 10 % of the applied amount and are shown in Table I.

TABLE I

EFFICIENCY OF RECOVERY OF SUCROSE FROM DEVELOPED SILICA GEL CHROMATOSTRIPS PREPARED WITH STARCH BINDER

Sucrose applied (μg)	*Sucrose recovered (μg)*
100	101
80	74
60, 60	60, 63
40	37
20	19

Analyses for sugar esters were made in a like manner except the residues were saponified with 1 ml of 0.2 *N* sodium hydroxide in 90 % ethanol by heating for 5 min at 60° in a water bath followed by addition of the resorcinol–hydrochloric acid reagents for ketose color development. Some typical results for commercial sucrose esters are

shown in Table II. All samples were measured against sucrose as a standard. The precision of the sucrose ester analysis was considered satisfactory for the purpose of this investigation. Seven replicate analyses were made on commercial samples of sucrose monopalmitate and on sucrose dipalmitate. The major constituent, monoester, in the sucrose monopalmitate averaged 75%, with a standard error of 2.3%. The diester content of sucrose dipalmitate averaged 66% with a standard error of 1.9%.

TABLE II

COMPOSITION OF SOME SUCROSE ESTER SAMPLES ANALYZED BY QUANTITATIVE THIN-LAYER CHROMATOGRAPHY WITH SILICA GEL

Ester	% *Sucrose*	% *Monoester*	% *Diester*
Sucrose monolaurate	8	81	11
Sucrose monopalmitate	2	84	14
Sucrose dipalmitate	–	36	64

DISCUSSION

The resolution of the sugar esters was better on silica gel than on paper. The silica gel acts as a strong adsorbent for hydroxyls, thereby adding to the separation power achieved by liquid partition. In Fig. 1 the chromatostrips 1, 2, 3, 4, 5 show separations of several sucrose ester samples. The solvent mixture used gave separations as follows: unsubstituted sucrose remained at the origin; sucrose monoester appeared as a long spot; and the sucrose diesters used here resolved into a series of four to five spots. Chromatostrip 4 shows two other fast moving spots which may represent substitution of greater degree than diesterification. If desired, adjustment of solvent composition could be made to study the more highly esterified products. Chromatostrip 6 shows the separation of sucrose and raffinose with raffinose as the slower migrating sugar.

For qualitative examination the same type of resolution resulted when either starch or calcium sulfate was used as a binder for the silica gel. Chromatostrips prepared with starch are recommended as they are easy to handle and can be marked with a pencil. However, chromatostrips prepared with silica gel G (Merck, Germany)* containing calcium sulfate as a binder have the advantage that they can be sprayed with dilute sulfuric acid and heated at 110° for 5–10 min to reveal spots. For the sucrose esters, the dichlorofluorescein indicator was preferred over the corrosive acid spray. However, the heated sulfuric acid spray reagent is a much more general indicator than dichlorofluorescein.

Ten to 200 μg amounts of sample can be detected by this technique, depending upon the number of components present. Samples exceeding 200 μg form diffuse spots which tail, due to overloading of the strip; samples of about 100 μg are useful for both qualitative and quantitative analysis.

The distinct separation of sucrose from raffinose and sucrose monoesters and diesters, made it possible to remove fractions from the chromatostrips for quantitative colorimetric measurement. The quantitative measurements were made for

* Obtained from Brinkmann Instruments, Inc., Great Neck, L. I., N. Y.

monoesters or diesters collectively, as no attempt was made to measure each of the resolved diester spots individually. A yellow color is eluted from the silica gel which contributes to color absorbance at 490 mμ. A blank determination was, therefore, included in the procedure to correct for this impurity, but the preparation of the chromatostrips is not uniform enough to prevent some variation in absolute measured sucrose values.

The resorcinol–hydrochloric acid reagents of ROE[7] were used to develop the characteristic ketose color. A specific ketose reagent was considered necessary under the conditions described to avoid difficulties from glucose-like impurities in starch. Although an aqueous solution of the starch binder used gave a ketose reaction with these reagents, the ketose color precursor from the starch was not eluted with dimethylformamide, and did not interfere in the optical density measurement. Silica gel containing sugar gave low values from known samples when eluted with water. Samples eluted with dimethylformamide gave better recoveries. The silica gel G strips gave poor recovery of sucrose materials even after dimethylformamide elution. Values were consistently low, due either to mechanical losses (silica gel G chromatostrips are weakly held together and tend to form loose powders) or to the fact that calcium sulfate binder may contribute strong adsorption forces.

ACKNOWLEDGEMENT

I wish to thank Dr. T. H. APPLEWHITE for his helpful suggestions on the technique of silica gel chromatostrips.

SUMMARY

The technique of qualitative and quantitative silica gel chromatography on glass strips has been adapted to separate mixtures of sucrose esters, and sucrose and raffinose. Solvent mixtures are described which give rapid, definitive separations of these compounds. The separated components can be eluted from the silica gel and measured for sucrose content by the colorimetric procedure of ROE.

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