Quantitative Estimation of Sucrose Esters of Palmitic Acid[1]

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ABSTRACT

Thin layer chromatography was adapted for direct quantitative estimation of sucrose esters of palmitic acid. Urea-phosphoric acid spray was used to detect the sucrose moiety of the various esters. The photometrically determined density of each spot on the thin layer plate was found to be proportional to its sucrose content. Ester content was then obtained by multiplying sucrose found by the factor, mol. wt. ester/mol. wt. sucrose. Ester mixtures were prepared by interesterifying sucrose with various proportions of methyl palmitate in dimethylformamide solution. Positional isomers were observed at each level of substitution but could not be adequately separated from each other for quantitative evaluation.

INTRODUCTION

Several analytical methods have been applied to the qualitative measurement of sucrose esters of fatty acids. Most quantitative measurements have relied on elution of esters from thin layer chromatography (TLC) plates, paper strips or chromatographic columns followed by reaction of the esters with a color-producing reagent (1-4).

Qualitative TLC plates normally show the existence of numerous spots which have been identified by previous investigators as positional isomers of sucrose mono-, di- and triesters. Linow et al. (5) have reported 11 separate ester spots for a sucrose palmitate preparation on a one-dimensional TLC plate. Wachs and Gerhardt (6) originally found seven ester spots, specifically one mono-, four di- and three triesters, using one-dimensional TLC but were able to separate and observe seven mono-isomers by two-dimensional development.

York et al. (7) have reported that about 80% of the fatty acid of the monoester is found on the glucose portion of the sucrose molecule. Gee and Walker (8) have determined that the 6 position of sucrose is most frequently esterified with the 4 and 1 position also showing appreciable substitution. The 6′ position, which is in the fructose moiety, was observed to be relatively lower in activity.

Acylation of various combinations of the eight hydroxyls of sucrose with one fatty acid yields 255 different esters, 8 mono-, 28 di-, 56 tri-, etc. Available methods of separation and detection by TLC were considered to be inadequate for quantitatively analyzing mixtures of sucrose esters, even those which were relatively simple. Some published methods were deemed to be tedious, particularly those requiring the recovery of esters from individual spots. Other methods were not specific for sucrose esters or did not give quantitative results. The current work was undertaken to improve the quantitative procedure for sucrose esters to better evaluate ester distribution and isomer formation.

EXPERIMENTAL PROCEDURES

TLC plates were prepared from silica gel containing calcium sulfate binder (Adsorbosil-1) and activated by heating at 110 C for 1 hr. Plate size was 20 x 20 cm. Sucrose esters were applied as chloroform solutions to give 50-250 μg per application. Two or three levels of each ester mixture were applied to each TLC plate. High levels of application about 250 μg permitted the accurate determination of ester components present in low concentration, while low levels about 50 μg were required for good separation of components present in high concentrations.

Plates were developed with a mixture of toluene-ethyl acetate-95% ethanol (2:1:1 v/v) when mono- through tetraesters were to be separated. Further separation of lower esters was achieved by two-dimensional TLC in which the developing solvent was a mixture of chloroform-methanol (4:1 v/v) (9). The present method for quantitative evaluation was not suitable for measurement of two-dimensional TLC plates.

Higher esters were separated by developing the plates with a solution of petroleum ether-ethyl ether-glacial acetic acid (75:25:1 v/v). This solution was a modification of that used by Malins and Mangold (10). The proportion given was required for the degree of separation desired. Separation of the mono- through octaesters on a single plate was not achieved. The change in polarity and solubility of the esters over this range was too large.

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TABLE I

Calculations for TLC Analysis of Sucrose Palmitate Standard[a]

Ester	Spot area	Mol. wt. of ester	Product, area X mol. wt.	Ester, wt. %	Ratio mol. wt. sucrose to mol. wt. ester	Sucrose on plate[b] Wt. %	Wt., μg
Free sucrose	11	342	3762	0.76	1.000	0.8	0.4
Mono	414	581	240534	48.30	0.588	28.4	14.2
Di	284	819	232596	46.70	0.418	19.5	9.8
Tri	15	1058	15870	3.15	0.323	1.0	0.5
Tetra	4	1296	5184	1.03	0.264	0.3	0.1

[a]Amount spotted on TLC plate, 50 mg.
[b]Amount of sucrose (μg) per unit area is 0.0344.

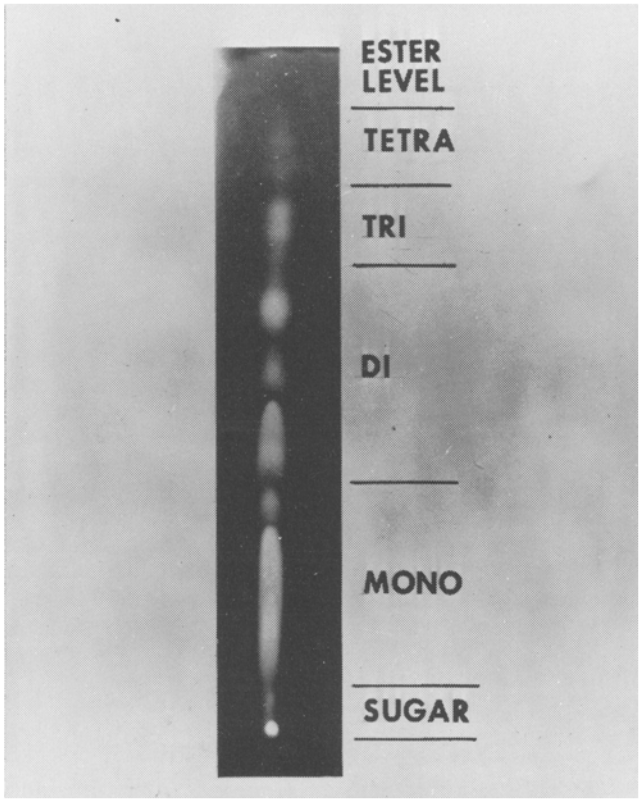

FIG. 1. One-dimensional separation of sucrose palmitate product.

Sucrose ester spots were visualized by spraying the air-dried, developed plates to saturation with a solution of urea (1 g), 85% phosphoric acid (4.5 ml), and water-saturated *n*-butanol (48 ml) (11); drying the plates in air; and then heating them in an oven at 110 C for 30 min. Spots of the lower esters appeared after about 10 min of heating. But the higher esters required the longer time to develop the characteristic color.

A slate gray spot appeared wherever the sucrose moiety was present. Amounts of color bodies were measured quantitatively with a Photovolt Model 530 Thin Layer Recording Densitomer. Areas under the peaks were determined with a planimeter and recorded as the densities of the corresponding spots.

FIG. 3. Two-dimensional separation of sucrose palmitate product.

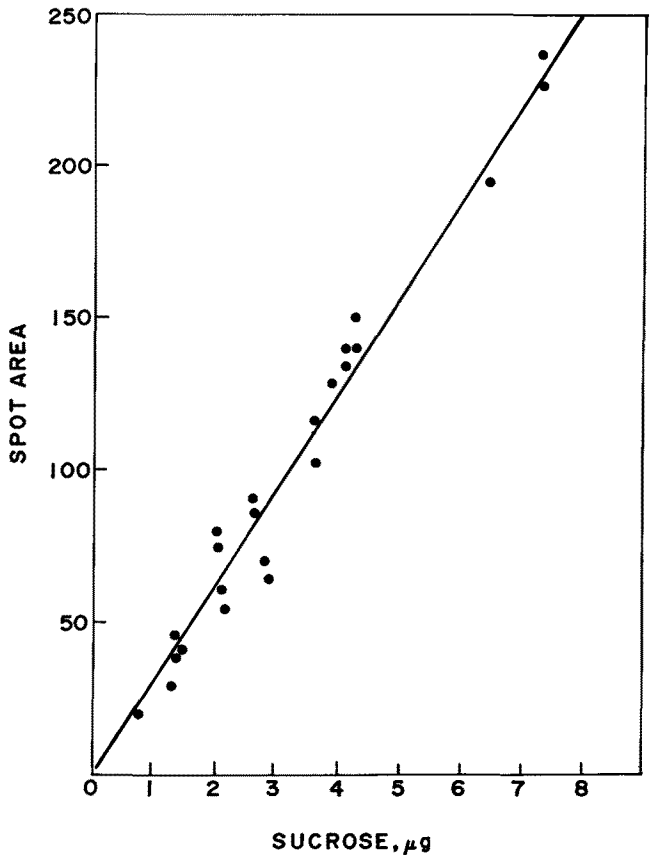

FIG. 2. Relationship between area under curve of densitometer recording of spot on TLC plate and sucrose content of that spot.

Low intensity spots barely detectable by visual observation which contained about 0.3 μg of the sucrose moiety were easily measured by the densitomer. Such spots were found to be more intense and observable under UV light. Fluorescence of the sucrose-urea complex, however, was not proportional to the sucrose content, making quantitative measurements under UV light impractical.

The intensity of the urea-detected spots faded slowly on standing, reaching a minimum level, about one half of their original value, after about three days. Fading was found to be proportional to the sucrose content since plates analyzed when freshly prepared and after one week storage gave the same values for total ester and percentage composition of the different esters.

Other indicators were less satisfactory than was the urea-phosphoric acid solution. Anthrone and naphthoresorcinol reagents did not always react with sucrose esters to yield colored products. Fluorescence indicators such as dichlorofluoroscein and Rhodamine B were not selective for sucrose esters. They also gave variable fluorescent backgrounds which interfered with quantitative evaluation of the detected spots. This observation also was made by Ranny (3).

The sucrose esters were prepared using the basic method of Osipow et al. (12) but following the specific laboratory procedure given by the Sugar Research Foundation (13). This method uses the anhydrous conditions considered to be essential for reproducibility (14). The mole ratio of sucrose to methyl palmitate was varied to yield sucrose esterified at different average levels.

Sucrose ester products were separated into fractions by the silica gel column procedure of Mima and Kitamore (2). The elution curves were determined by weighing the solvent-free residues of each fraction collected. Purity of

TABLE II

Composition of Sucrose Palmitate Preparations[a]

	Mole ratio, methyl palmitate-sucrose, %							
	1:1		2:1			4:1		
Ester	TLC	Calc.	TLC	Col.	Calc.	TLC	TLC	Calc.
Mono	43.5	49.2	16.0	17.3	16.0	0.6		1.3
Di	35.7	34.7	33.0	30.3	30.0	11.0		6.9
Tri	16.4	12.8	24.5	52.4[b]	29.4	16.5	22.8[c]	17.9
Tetra	4.4[d]	2.8	26.4[d]		17.1	71.9[d]	32.1	27.4
Penta		0.4			6.1		27.1	25.9
Hexa					1.4		16.5	15.0
Hepta					0.2		1.5	4.9
Octa					0.0		Trace	0.7

[a]Mono-, di- and tripalmitates separated on TLC plates by development with solution of toluene-ethyl acetate-95% ethanol (10:5:5 v/v). Tetrapalmitates through octapalmitate separated with solution of petroleum ether-ethyl ether-acetic acid (75:25:1 v/v). Calculated values based on random interesterification.

[b]Tripalmitates through octapalmitate treated as a composite.

[c]Mono- through tripalmitates treated as a composite.

[d]Tetrapalmitates through octapalmitate treated as a composite.

the isolates with respect to degree of acylation was assessed by TLC.

The ratio of protons in the sucrose and palmitic acid moieties in some of the fractions collected was measured by NMR. This established the level of esterification of the various fractions. The identity of these fractions agreed with that given by Kinoshita (15). Figure 1 is a photograph of a TLC plate of a typical sucrose palmitate product, developing solvent, toluene, ethyl acetate and ethanol.

RESULTS AND DISCUSSION

Mixtures of known amounts of purified sucrose mono-, di-, tri- and tetrapalmitates were developed on TLC plates. The weight of the equivalent sucrose in each ester was calculated. Figure 2 shows that a plot of weight of sucrose vs. spot density (peak area) is a straight line function. [The weight of a given sucrose ester in a chromatographed mixture can therefore be found by determining the equivalent amount of sucrose and multiplying it by the factor, mol. wt. ester/mol. wt. sucrose.]

A purified sample of mixed sucrose palmitates was used as an arbitrary standard. A known weight of this mixture was applied to each TLC plate along with ester mixtures to be analyzed in order to correct for plate-to-plate variations in color density. Each peak area of the standard was multiplied by the molecular weight of the corresponding ester to convert relative quantity of sucrose to relative quantity of specific ester. Ester proportions then were converted to percentage composition and to weight of ester at each spot. Weight of ester at each spot of the standard was converted to sucrose weight through multiplication by the factor, mol. wt. sucrose/mol. wt. ester. The density of the individual spot could then be correlated with weight of sucrose at that spot (Table I).

Unknown ester mixtures were analyzed by measuring spot density of each ester type (mono-, di-, triester, etc.), converting the results to weight of sucrose, and multiplying the weights obtained by the factor mol. wt. ester/mol. wt. sucrose. This gave ester weights which can be recalculated as percentage composition.

In these calculations isomers at each level of acylation were measured as a unit. Composition of the ester mixture was expressed in terms of mono-, di-, triesters, etc. Results obtained by averaging replicate determinations of three different sucrose palmitate preparations are given in Table II along with theoretical values for the random distribution of the acyl groups during the interesterification reaction.

Tetra- and higher esters gave a single spot with the solution of toluene, ethyl acetate and ethanol. Preparations containing measurable amounts of penta- and higher esters could only be evaluated as composites of these esters with this solvent. Higher esters were separated with petroleum ether-ethyl ether, which did not separate the mono-, di- and triesters. The latter then were evaluated as a composite.

Comparison of the data reveals a small deviation from random distribution which may be due to analytical error. Such deviation is more likely to be due to the differences in reactivity between the various sucrose hydroxyl groups. Weight yields of mono- and diesters separated by chromatographic column agreed well with those calculated from TLC plates. Total ester weights calculated from TLC plates were also found to approximate the weight of ester preparation applied to the plate. Occasional weight discrepancies could be accounted for by the presence of small amounts of nonsucrose bearing material.

Calculations for random distribution were based on the existence of eight active hydroxyl groups on sucrose. The calculated compositions would not have differed materially if only six or seven active hydroxyls had been postulated. Attempts to prepare high levels of hepta- and octaesters by the Osipow procedure as given were not successful. Further work is in progress to produce higher esters to evaluate their distribution patterns.

The existence of a large number of isomers was confirmed by the use of a two-dimensional TLC plate. Figure 3 shows a two-dimensional separation of a preparation in which the mole ratio of sucrose-methyl palmitate was 1:1. The plate was photographed under UV light.

Columns eluted with acetone in chloroform according to the method of Bares (16) for isolation of higher esters did not give anticipated results. Weights obtained for each peak did not correspond to previously observed distribution patterns. TLC analysis of the isolates showed them to consist entirely of mixed esters. Results by Bares and Zajic (17) for their sucrose ester preparations by the Osipow process were reported not to follow random distribution patterns although they carried the reactions to equilibrium. It would seem that this discrepancy was due to the analytical procedure rather than the preparative process.

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