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Paper chromatography of higher esters of fatty acids with saccharose

Analyses of the samples taken in the course of the reaction of methyl esters of fatty acids with saccharose in dimethylformamide or dimethylsulfoxide[1–14], and those of the finished product for determining the mono- and diester content, are based on the specific rotation of the butanol solution[3]. This method is not reliable, assuming the presence of only two components, the mono- and diester. In fact, the reaction is more intricate, as higher esters of saccharose or esters of fatty acids together with split products of saccharose, especially glucose, form comparatively easily, as shown by earlier work studying the kinetics of the reesterification of saccharose dipalmitate with saccharose[15].

These classic methods may serve as orienting tests, but they do not comply with the requirements for accurate determinations of the composition of the product or of more detailed studies of the reaction. Therefore our attention was called to analytical studies of higher and lower esters of saccharose.

The determination of mono- and diesters of fatty acids with saccharose by paper chromatography has been described in an earlier paper[16]. A mixture of the substances or the reaction solution was separated on Whatman No. 1 paper by eluting with the system benzene–*n*-propanol (10:3, v/v). The descending technique was used and for a good separation an atmosphere of 90 % relative moisture in the chamber was very important.

On drying, the paper was washed in a petroleum ether solution of paraffin, m.p. 50° (concentration 10 g/l) and on evaporating the solvent in a drier, the spots were detected by dipping the paper into a water bath at 27° for 2 min. The mono- and diesters emulsified the paraffin and the spots became transparent. The sensitivity of the method was between 0.55 and 1 μg of mono- and diester.

The higher esters of saccharose showed a much lower emulsifying ability. The monoesters of glucose and fructose behaved similarly. Although those esters could be

identified qualitatively, this method did not ensure a reliable quantitative determination and was inconvenient for other esters. Therefore we tried to develop a new chromatographic method for the determination of higher esters of saccharose than the mono- and diesters.

Experimental

Materials. The esters of palmitic, stearic and myristic acids with saccharose, used in this experiment, were formed by the reaction of the methyl esters of the fatty acids with saccharose in dimethylformamide[3]. The methyl ester purity was 99–99.5 % (GLC). The saccharose was pure, furnished by Lachema. The esters of saccharose were isolated from the reaction mixture by *n*-butanol, *n*-hexane and acetone extractions.

With ordinary methods[1–3,6] of isolation, *e.g.* by means of solvents, it is not possible to obtain pure esters of saccharose and, as in the previous work, adsorption on a silica gel column was used for isolating pure tri-, tetra-, penta- and hexaesters.

A mixture of esters of saccharose with palmitic acid containing approximately 70 % of tri-, tetra-, penta-, hexa-, hepta- and octa-derivatives, was separated on silica gel Lachema CH (average diameter of pores, 80 Å) by eluting with chloroform and acetone in varying proportions. Fractions of successively lower degree of esterification of saccharose were taken. The method will be described in another paper.

Elementary analyses showed a good agreement with theoretical values. The figures found for triester were:

C = 67.42 %, H = 11.09 % (theoretical C = 68.14 %, H = 10.68 %); for tetraester

C = 69.62 %, H = 11.01 % (theoretical C = 70.43 %, H = 11.04 %); for hexaester

C = 73.30 %, H = 11.10 % (theoretical C = 73.17 %, H = 11.48 %).

The pentaester was not analysed since the amount available was too small for an elementary analysis. The esters were chromatographically pure.

Procedure. Portions between 20 and 80 μg of higher esters in a chloroform solution were applied to 20 F 1 paper (Spezialpapierfabrik, Niederschlag). The mono- and diesters were first separated on silica gel with an eluting mixture of chloroform–acetone (80:20), as they interfered with the reliable determination of triesters and, when present in larger amounts, the spots partially overlapped. Other substances, like saccharose and the remaining solvents, did not interfere.

The paper was eluted by the descending technique with a benzene–*n*-propanol mixture (10:1). Dishes of water were placed in the chromatography chamber, or the whole of the bottom was covered with water to get an optimal atmosphere of approximately 90 % relative moisture at 20°. The elution time was 6–7 h.

The chromatograms were dried first in hot air and then in a drier at 90° for 15 min. For detection purposes the different solubilities of the saccharose esters in various solvents[17] were used. The hydrophobic power of the substances was increased by immersing the paper in a petroleum ether solution of paraffin (m.p. 50°), concentration 5 g/l. The solvent was allowed to evaporate and the chromatogram was again dried in a drier for 10 min at 90°.

The treated chromatogram was dipped into a water bath containing 40 % methanol at 20° ± 1°. To maintain this temperature a Wobsser ultrathermostat (Medingen) was used. In the regions of the higher esters of saccharose with fatty acids

white spots appeared and the surrounding paper became transparent. For quantitative determinations it is of great importance to maintain the prescribed temperature of the bath and the immersion time from 45 to 60 sec. These conditions vary according to the type of chromatographic paper used, for example, with Whatman No. 1 it is necessary either to increase the amount of the bound nonpolar phase by changing the concentration of the paraffin solution from 5 g/l to 10 g/l or to decrease the detection time to 20–30 sec.

The developed paper was dried between two filter papers and the spots were outlined (see Fig. 1). The spacings between the spots increased regularly with increasing amounts of acyl groups in the molecules, as shown by the corresponding R_F values, which for the triester, tetraester, pentaester and hexaester were found to be 0.04, 0.19, 0.37 and 0.54, respectively. On the basis of these regular spacings it was possible to classify the other spots with the R_F values 0.71 and 0.87 as the heptaester and octaester, respectively. The mean difference of R_F values of the spots was 0.17. The R_F values found with the 20 F 1 paper were practically the same as those with Whatman No. 1.

In contrast to the system used for the analyses of mono- and diesters where the R_F values were independent of the type of the fatty acid, the R_F values of the higher fatty acid esters of saccharose differed slightly according to the type of the fatty acid. The R_F values of all esters slightly increased from stearic acid to lauric acid. *E.g.* the R_F values of the saccharose tristearate and trimyristate were 0.02 and 0.08, respectively (Fig. 2). A similar relationship was found also for other esters, the values ranging from 0.02 to 0.04.

The detection of the spots may be repeated on drying.

Quantitative determination. The area of the spots was shown to be influenced by

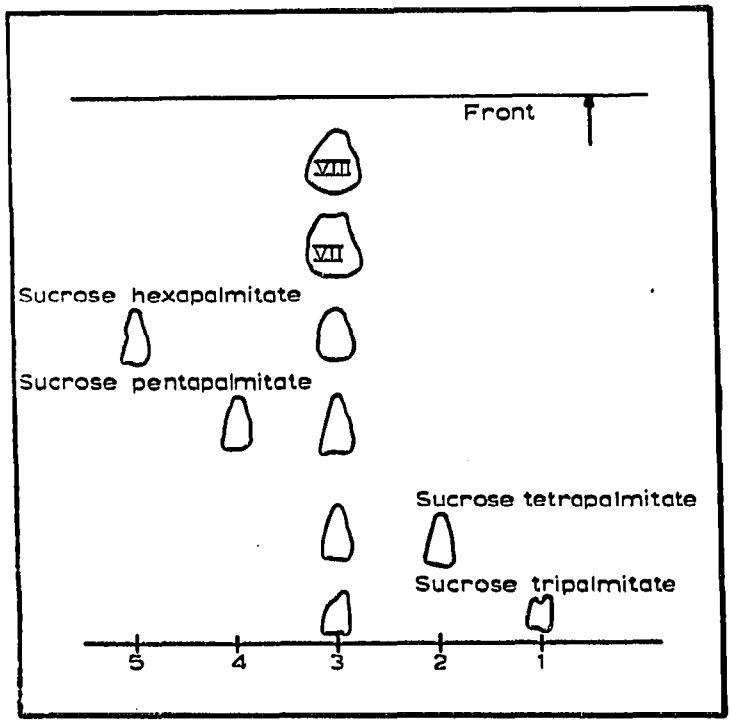

Fig. 1. Paper chromatogram of simple and mixed samples of sucrose esters.

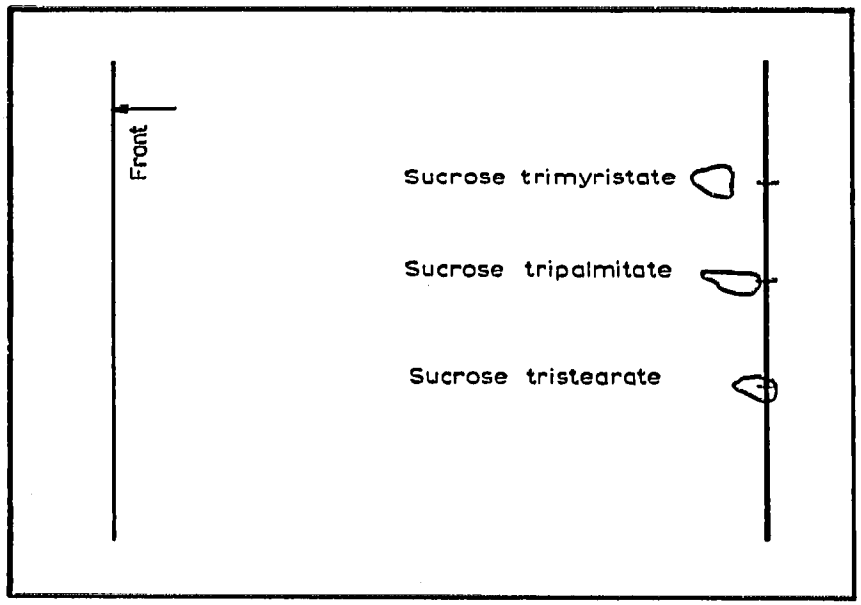

Fig. 2. Paper chromatogram of the triesters of myristic, palmitic and stearic acids with sucrose.

a number of factors, the most important of which are the temperature of the bath, the detection time and the paraffin concentration. For the quantitative determination of unknown samples it is essential to maintain constant conditions, likewise in the case of calibration. The dependence of the area of the spots on the concentration was examined for the tri-, tetra- and pentaesters. The concentrations were between 5 and 70 μg. It was found that the area of the spot was directly proportional to the concentration. Sets of measurements of 10–20 runs were statistically evaluated and formulated by the equation of the regression line[18].

$$A = b_A \cdot c + a$$

where A is the area of the spot in cm^2, c is the concentration of the substance in micrograms, and a and b_A are constants for the straight line in question. The relations for

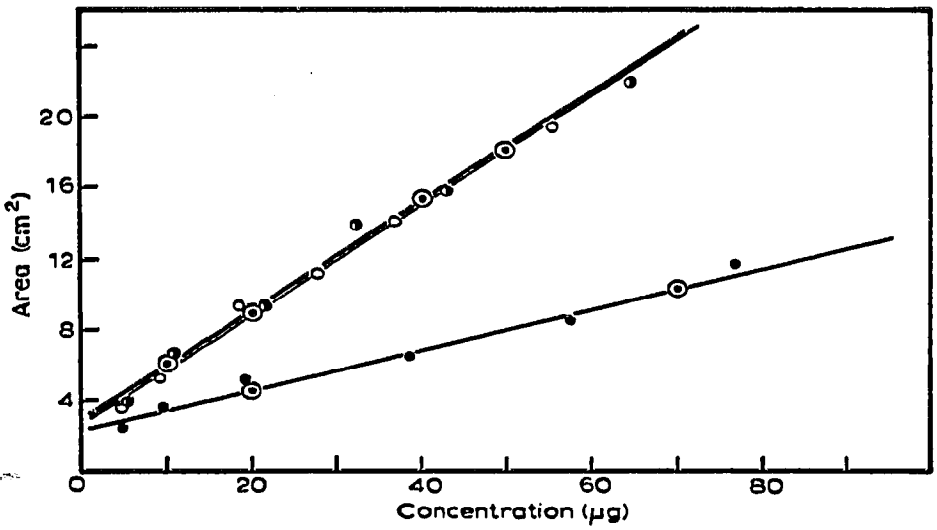

Fig. 3. Plot of area spots *vs.* concentration of sucrose tri-, tetra- and pentapalmitate. (●) Tripalmitate, (○) tetrapalmitate, (◐) pentapalmitate, (⊙) calculated value.

the tri-, tetra- and pentaesters are shown in Fig. 3. The values in the figure are arithmetic means of the measurements made, the encircled points showing the values of the regression line. The corresponding equations were: $A = 0.115 \cdot c + 2.25$ for the triester; $A = 0.307 \cdot c + 2.79$ for the tetraester; and $A = 0.305 \cdot c + 3.01$ for the pentaester.

The sensitivity of the method is fairly high, as we can see from Fig. 1, ranging from 1 to 3 μg. Thus it is possible to detect with certainty 1–3 % of the substance in the mixture.

The accuracy of the method, assuming the conditions mentioned were strictly observed, is adequate. The error of the determination did not exceed $\pm$ 10 % of the results with concentrations up to 20 μg, and $\pm$ 5 % with higher concentrations.

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