CHROM. 4199

An evaluation of methanolysis for thin-layer chromatographic analysis of the monosaccharides in an immunologically active polysaccharide–peptide complex

Many attempts have been made up to date to separate sugar mixtures by thin-layer chromatography (TLC) on the various adsorbents such as cellulose[1–4]; Silica Gel G[5–12]; kieselguhr[9,13,14]; as well as silica gel or kieselguhr impregnated with boric acid[15–17], sodium tetraborate[15,18], acetate[10,19,20] or bisulfite[20]; but completely satisfactory results have not yet been obtained. Lately, to improve this separation technique, trimethylsilylation[21] and methylation[22] or both, have been introduced. These procedures, however, failed to achieve the expected results. Furthermore, we have often encountered difficulties in separating sugars such as glucose, galactose and mannose, which have the similar R_F values to each other, arising from the hydrolysis of the cell walls[23] and the immunologically active polysaccharide–peptide complex[24] of human pathogenic fungi which are now being studied by us. In our experience, a good separation of the sugar components has been achieved by methanolysis of the polysaccharide, instead of routine hydrolysis with hydrogen chloride in water.

This paper describes an evaluation of the methanolysis technique for the separation, on Silica Gel G plates, of the monosaccharides originating from an immunologically active polysaccharide–peptide complex of a dermatophyte, *Trichophyton mentagrophytes* causing trichophytosis in humans.

Experimental

Preparation of the chromatoplates. The glass plates were rinsed in a detergent overnight and washed several times with tap water. 30 g of Silica Gel G (E. Merck) were blended vigorously in a mortar for 1 min with 60 ml of distilled water. The homogeneous slurry was spread on to five glass plates (20 × 20 cm) with a thickness of 250 μ using a Yazawa applicator. The coated plates were dried at room temperature until the surface moisture had been removed, and then activated in an oven for 30 min at 120°; they were finally stored in a desiccator.

Preparation of the sample. The immunologically active polysaccharide–peptide complex was extracted with phenol–water by a modified method of WESTPHAL's[25], previously described in detail [24]; purified by gel filtration on a Sephadex G-100 column; dialysed and lyophilised. 10 mg of the white, fluffy material thus obtained was subjected to methanolysis as follows: the material was hydrolysed for 5 h at 100° in a sealed ampoule containing 3 ml of 3 % hydrogen chloride in dry methanol. The methanolysate was passed through an Amberlite IR-45 (OH^-) column to remove the excess hydrogen chloride, evaporated to dryness *in vacuo* and redissolved in 1 ml of pyridine for application to the thin-layer plate. The methyl glycosides of the representative hexoses, glucose, galactose and mannose, were prepared as standards in just the same manner as described above.

Development of the chromatoplates. Aliquots of the methanolysed sugars, dissolved in pyridine, were applied to the plates at a distance of 1.5 cm from the bottom. The glass development tank was lined with filter paper in order to saturate it with solvent.

The spotted chromatoplate was developed at room temperature using a solvent system of benzene–isopropanol–water (45:50:5), by an ascending technique for a distance of 10 cm above the sample origin. It takes about 50 min to complete the development. After the plate had been removed and dried at room temperature, the development was repeated once again with the same solvent system.

Detection of the spots. As naphthoresorcinol–sulfuric acid solution[26] had been shown to give the best results among the several spraying reagents tested, it was usually used in this work. In some cases, a 10% solution of sulfuric acid in methanol and anisaldehyde–sulfuric acid reagent[13] were also used.

Afterwards, the developed plate was dried in an oven at 50° until the odor of the developer had disappeared. The plate was sprayed with naphthoresorcinol–sulfuric acid reagent, and reheated at 100° until the methanolysed sugars appeared as the colored spots.

Results and discussion

Fig. 1 shows a chromatogram on Silica Gel G, developed with benzene–isopropanol–water (45:50:5) and sprayed with naphthoresorcinol reagent[26], in which the three standard hexoses were run individually or as a mixture, together with the methanolysed immunologically active polysaccharide. It can be clearly seen that the three methyl

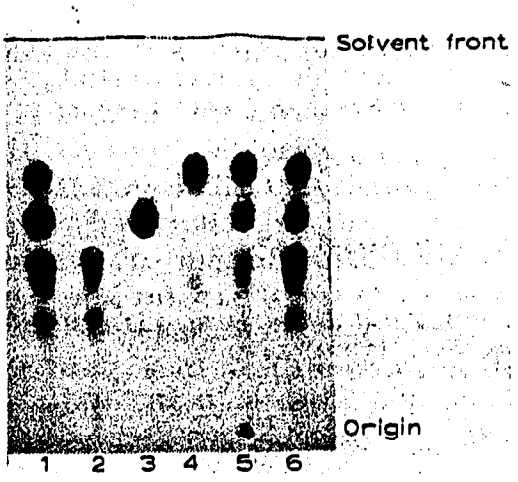

Fig. 1. Separation of some methyl glycosides and the methanolysed immunologically active polysaccharide–peptide complex on a thin layer of Silica Gel G by ascending double development. 1 = a mixture of methyl glucoside, methyl galactoside and methyl mannoside; 2 = methyl galactoside; 3 = methyl glucoside; 4 = methyl mannoside; 5 = methanolysate of the immunologically active polysaccharide–peptide complex; 6 = the same mixture as 1. Solvent system: benzene–isopropanol–water (45:50:5). Spots were visualized with naphthoresorcinol–sulfuric acid reagent[26].

glycosides: methyl glucoside, methyl galactoside and methyl mannoside, gave well defined spots and could be separated successfully. The last two glycosides showed one or two extra spots which might be due to α- and β-anomers of pyranose and furanose, and the identification of these spots is now in progress by gas chromatography. The detectable spot pattern indicated that this antigenic polysaccharide–peptide complex contained glucose and mannose, together with traces of galactose, although the com-

plicated problems of the undesired interaction products of sugars and amino acids[27,28], and of acid reversion[29] remain to be resolved.

Department of Biochemistry, Gifu University School of Medicine, Gifu (Japan)

YOSHINORI NOZAWA
HIROSHI UESAKA
HIDEAKI SUZUKI
YUKI ITO

1 A. SCHWEIGER, *J. Chromatog.*, 9 (1962) 374.
2 M. L. WOLFROM, D. L. PATIN AND R. M. DE LEDERKREMER, *J. Chromatog.*, 17 (1965) 488.
3 D. W. VOMHOF AND T. C. TUCKER, *J. Chromatog.*, 17 (1965) 300.
4 D. W. VOMHOF, J. TRUITT AND T. C. TUCKER, *J. Chromatog.*, 21 (1966) 335.
5 E. DEMOLE, *J. Chromatog.*, 6 (1961) 2.
6 G. PASTUSKA, *Z. Anal. Chem.*, 179 (1961) 355.
7 G. W. HAY, B. A. LEWIS AND F. SMITH, *J. Chromatog.*, 11 (1963) 479.
8 S. CHIBA AND T. SHIMOMURA, *Agr. Biol. Chem. (Tokyo)*, 31 (1967) 255.
9 R. F. POWNING AND H. IRZYKIEWICZ, *J. Chromatog.*, 29 (1967) 115.
10 V. A. DE STEFANIS AND J. G. PONTE, *J. Chromatog.*, 34 (1968) 116.
11 M. LATO, B. BRUNNELLI, G. CIUFFINI AND T. MEZZETTI, *J. Chromatog.*, 36 (1968) 191.
12 J. LEHRFELD, *J. Chromatog.*, 32 (1968) 685.
13 E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 351.
14 E. GUILLOUX AND S. BEAUGIRAND, *Bull. Soc. Chim. France*, (1965) 259.
15 H. JACIN AND A. R. MISHKIN, *J. Chromatog.*, 18 (1965) 170.
16 A. WEINSTEIN AND S. SEGAL, *Anal. Biochem.*, 20 (1967) 558.
17 M. LATO, B. BRUNELLI, G. CIUFFINI AND T. MEZZETTI, *J. Chromatog.*, 34 (1968) 26.
18 M. L. WOLFROM, R. M. DE LEDERKREMER AND G. SCHWAB, *J. Chromatog.*, 22 (1966) 474.
19 P. G. PIFFEIRI, *Anal. Chem.*, 37 (1965) 925.
20 YU. S. OVODOV, E. V. EVTUSHENKO, V. E. VASKOVSKY, R. G. OVODOVA AND T. F. SOLOV'EVA, *J. Chromatog.*, 26 (1967) 111.
21 J. E. KARKKAINEN, E. O. HAAHTI AND A. A. LEHTONEN, *Anal. Chem.*, 38 (1966) 1316.
22 M. GEE, *Anal. Chem.*, 35 (1963) 350.
23 Y. ITO, T. SETOGUTI AND Y. NOZAWA, *Japan. J. Med. Mycol.*, 9 (1968) 171.
24 Y. NOZAWA, T. NOGUCHI, H. UESAKA, T. HATTORI AND Y. ITO, *Japan. J. Med. Mycol.*, 9 (1968) 258.
25 O. WESTPHAL, O. LUDERITZ AND F. BISTER, *Z. Naturforsch.*, 76 (1952) 148.
26 G. PASTUSKA, *Z. Anal. Chem.*, 179 (1961) 427.
27 C. FRANCOIS, R. D. MARSHALL AND A. NEUBERGER, *Biochem. J.*, 83 (1962) 335.
28 J. P. DUSTIN, C. CZAJKOWSKA, S. MOORE AND E. J. PIGWOOD, *Anal. Chim. Acta*, 9 (1953) 256.
29 W. G. OVEREND, C. W. REES AND J. S. SEQUEIRA, *J. Chem. Soc.*, (1962) 3429.

Received April 15th, 1969

J. Chromatog., 43 (1969) 528–530

CHROM. 4214

Thin-layer chromatography of sugars, anthocyanins and anthocyanidins on Kieselgel G impregnated with basic lead acetate

Thin-layer chromatography has been widely used for the separation and identification of microgram quantities of sugars[1–35]. Kieselgel G and Kieselguhr G are the adsorbents most frequently used, but other substrates have also been proved satisfactory, *e.g.*, magnesium silicate[7], calcium silicate[8], plaster of Paris[30], cellulose[13,21–23,25,28,48] and polyamide[31]. To improve the separation of sugars, the adsorbent layers have been mostly prepared in buffer solutions of alkaline acetates or phosphates, and less often in borate[3,4,16,19,34,35] or sodium bisulphite[18].