Thin-layer chromatography of sugar mercaptals and sulphates

Difficulty in obtaining adequate resolution by the standard paper chromatographic techniques[1–5], of the monosaccharide diethyl dithioacetals (mercaptals) obtained during mercaptolysis of polysaccharide mucilages, has lead to the development of the thin layer procedure described. A related problem in the study of seaweed mucilages is the identification of hexose monosulphates produced during acidic fragmentation of the mucilage and this has only been partly solved by paper chromatography and paper electrophoresis. Paper chromatography in solvents giving a fair degree of separation often involves long development times and discrete spots are difficult to obtain. Furthermore, the preparation of new sugar sulphates and an interest in their chemical reactions has also emphasised the need for alternative, faster, means of chromatographic analysis. The solvent of REES[6] has been successfully applied to thin-layer chromatography (TLC) using plates coated with a mixture of silica gel and kieselgur.

Experimental

Resolution of the sugar mercaptals was achieved on 20 × 30 cm plates, coated with Kieselgel G (Merck) to an indicated thickness of 0.4 mm, and activated at 110° for one hour, with the solvent benzene–ethanol (100:15) for 110 min. The spots were detected by spraying with the mixture *p*-anisidine HCl (1 g), conc. sulphuric acid (5 ml) and butanol (100 ml), and heating at 100° for 10 min.

For the separation of the isomeric hexose sulphates, plates (20 × 30 cm), were coated with a mixture of silica gel and kieselgur (Merck, Kieselgel G and Kieselgur G in the proportion 1:2) and developed with ethyl methyl ketone saturated with water containing 1 % (w/v) cetylpyridinium chloride (CPC) for 90 min. The plates were activated at 110° for one hour before use and the sugar sulphates were detected by spraying with the diphenylamine aniline reagent of BAILEY AND BOURNE[7].

Results

The rates of migration of the sugar mercaptals relative to 3,6-anhydrogalactose mercaptal (R_{AG}) were determined for double development in the solvent benzene–ethanol (100:15); these are listed in Table I and illustrated in Fig. 1.

The R_F (relative to the slower of the two solvent fronts) of various isomeric hexose sulphates were determined with the solvent previously described; these are listed in Table II and illustrated in Fig. 2.

TABLE I

Sugar	R_{AG}*
Xylose	0.065
Xylose mercaptal	0.29
Galactose mercaptal	0.17
6-O-Methyl galactose mercaptal	0.55
3,6-Anhydrogalactose mercaptal	1.00

* See text.

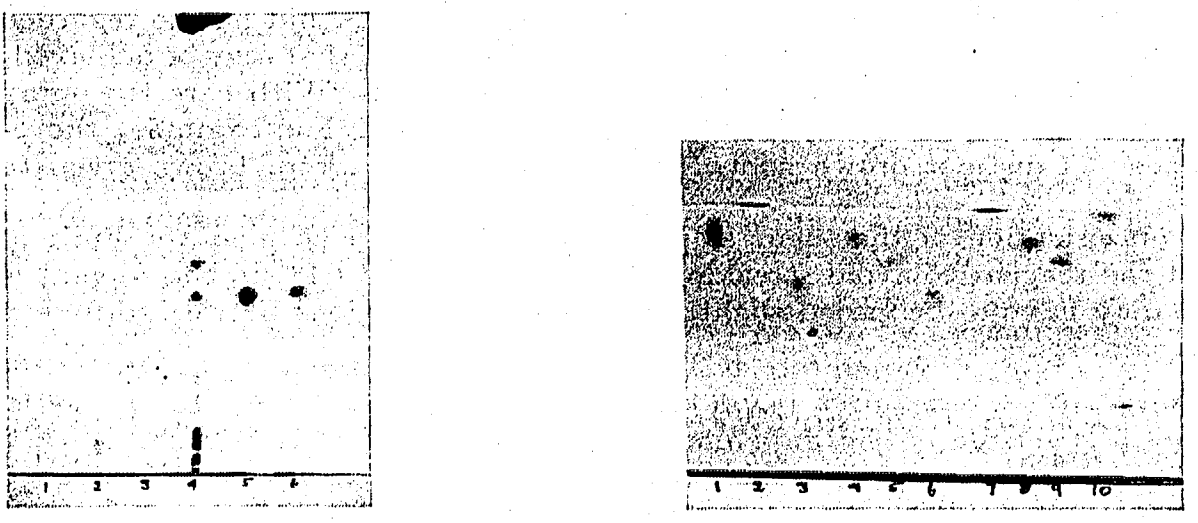

Fig. 1. Thin-layer chromatography of sugar mercaptals. 1 = 6-O-methyl-D-galactose mercaptal; 2 = D-galactose mercaptal; 3 = D-xylose and D-xylose mercaptal; 4 = mercaptolysis products from *Laurencia pinnatifida* mucilage; 5 = 3,6-anhydro-D-galactose mercaptal; 6 = mixture of 1, 2, 3, and 5.

Fig. 2. Thin-layer chromatography of hexose sulphates. 1 = Glucose 2-(barium sulphate); 2 = glucose 3-(barium sulphate); 3 = glucose 6-(barium sulphate); 4 = galactose 2-(barium sulphate); 5 = galactose 3- and 6-(barium sulphate); 6 = galactose 6-(barium sulphate); 7 = galactose 2,3-di(barium sulphate); 8 = galactose 4-(sodium sulphate); 9 = galactose 3- and 4-(sodium sulphate) and galactose; 10 = glucose 3- and 4-(sodium sulphate).

Discussion

The diethyl dithioacetals of the monosaccharides xylose, galactose, 6-O-methyl galactose and 3,6-anhydrogalactose are easily distinguished by a single development and the procedure is sufficiently rapid for the monitoring of mercaptolysis reactions.

In the procedure described for the separation of isomeric hexose sulphates the 2- and 4-sulphated mono-sulphates cannot be distinguished. However, a recent paper by PAINTER[8] describes the chromatographic resolution of these isomers. The cation associated with the sulphate grouping does not appear, in this solvent at any

TABLE II

Sugar	R_F*
Glucose 2-(barium sulphate)	0.91
Glucose 3-(barium sulphate)	1.00
Glucose 6-(barium sulphate)	0.73
Galactose 2-(barium sulphate)	0.90
Galactose 3-(barium sulphate)	0.81
Galactose 6-(barium sulphate)	0.70
Galactose 2,3-di(barium sulphate)	1.00
Galactose 4-(sodium sulphate)	0.90
Galactose 3-(sodium sulphate)	0.82
Glucose 4-(sodium sulphate)	0.90
Galactose	0.44
Xylose	0.68

* Refers to the slower of the two solvent fronts.

rate, to affect the relative R_F values significantly. R_F values should not be regarded as absolute, but merely an indication of the degree of separation that may be achieved; comparison should always be made with authentic specimens. With care the spray reagent specified may provide further help in identification. For example, the 2-isomers appear as distinct brown spots. The remainder vary from brown/green to blue.

The concentration of the sugar sulphate applied should be near the limit of detection of the reagent to avoid streaking. The proportion of CPC in the solvent is critical and may have to be varied slightly to allow for variations between different commercial samples.

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Detection of the polythionates on paper chromatograms

Tests have been described for the detection of the polythionates in microgram quantities on paper chromatograms[1,2]. These tests are not always suitable when detection must be followed by elution from the paper for further studies such as measurements of radioactivity. The dithionate ion can be especially difficult to detect in view of its chemical stability. In the test described by POLLARD, MCOMIE AND JONES[1] the chromatograms formed are permanent, whereas in the method of GARNIER AND DUVAL[2] the spots cannot be eluted after detection by the reagents used in a form suitable for further studies. The need for an alternative method of detection arose out of our work on the sulphur metabolism of the thiobacilli.

Although dithionates are very stable, they can be hydrolysed. The salts can only be hydrolysed slowly at elevated temperatures but the free acid can be hydrolysed at 50° quite rapidly[3] providing the basis for a test applicable on filter paper:

$$H_2S_2O_6 + H_2O \longrightarrow H_2SO_3 + H_2SO_4$$

By spraying the paper with a mixture of hydrochloric acid and hydrogen peroxide followed by gentle warmth the dithionic and other acids are converted to sulphuric