Thin Layer Chromatography of Sialyloligosaccharides

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Several methods have been described for the separation of carbohydrates by thin layer chromatography (TLC). Stahl and Kaltenbach [1] used layers of Kieselguhr G (Merck AG, Germany) buffered with acetate, and Pastuska [2] introduced the impregnation of Silica Gel G (Merck AG, Germany) with boric acid for the separation of simple sugars. Kieselguhr G impregnated with phosphate buffer,[3] mixtures of silica gel and alumina oxide,[4] celite-starch,[5] cellulose powder,[6] and silica gel impregnated with borate or bisulfite have also been used for TLC of carbohydrates.[7,8]

Granzer [9] separated *N*-acetyl-, *N*-glycolyl-, and *N,O*-diacetylneuraminic acid by TLC on silica gel G in propanol-water or propanol-ammonia solvent mixtures. Klenk *et al.* differentiated between *N*-acetylneuraminic acid and a proposed dimer thereof in butanol-pyridine-water 6:5:6 (v/v).[10] No suitable technique has been available, however, for the separation of more complex sialic acid-containing oligosaccharides by TLC. The behaviour of these compounds in different solvent systems was therefore investigated.

Materials and methods. Chemicals and solvents were of analytical grade. The solvents were redistilled before use. A sample of *N*-acetylneuraminic acid (NANA) was obtained from Dr. L. Svennerholm. Two isomers of monosialyllactose, 3′MSL (*N*-acetylneuraminyl-(2→3)-galactopyranosyl-(1→4)-glucopyranoside) and 6′MSL (*N*-acetylneuraminyl-(2→6)-galactopyranosyl-(1→4)-glucopyranoside and a disialyllactose, DSL (*N*-acetylneuraminyl-(2→8)-*N*-acetylneuraminyl-(2→3)-galactopyranosyl-(1→4)-glucopyranoside), were prepared as sodium salts from cow colostrum by a gel filtration method.[11] Commercial samples of lactose, glucose, and galactose were used.

Chromatography was performed on Silica Gel G (Merck AG, Germany) on 20 × 20 cm glass plates. The plates were prepared by mixing 32 g gel and 64 ml distilled water or 0.1 N boric acid. The gel was spread by a Quickfit apparatus (No. 8CR, Quickfit & Quartz Ltd., Stone, England) in a layer 0.25 mm thick. Before use the plates were activated for 30 min at 110°C. The chromatography was performed at 21° in a 21 × 11 × 21 cm glass tank. The inside of the tank was covered with

Table 1. R_F-Values of sialyloligo-

Solvent[a]	Propanol-13 N NH_3-H_2O 6:1:2	Propanol-1 N NH_3-H_2O 6:2:1	Propanol-H_2O 6:3	Propanol-0.1 M HCl 6:3	Propanol-0.3 M formic acid 6:3
TLC-plate	Silica gel	Silica gel	Silica gel	Silica gel	Silica gel
Time, h	3.5	3.5	3.5	3.5	3.5
3′MSL	0.20	0.28	0.38	0.35	0.19
6′MSL	0.13	0.22	0.33	0.30	0.15
DSL	0.11	0.19	0.24	0.24	0.19
NANA	0.13	0.18	0.15[b]	0.22[b]	0.21[b]
Lactose	0.13	0.19	0.46	0.46	0.38
Glucose	0.24	0.33	0.54	0.54	0.49
Galactose	0.21	0.27	0.50	0.53	0.42

[a] The composition of solvent is given as volume ratio.
[b] Tailing of spots.

filter paper to obtain an atmosphere saturated with solvent. The chromatograms were developed by spraying with resorcinol [12] or anisaldehyde reagents.[13]

The solvents investigated were modifications of those previously used for paper chromatography of sugars, including propanol-water, propanol-ammonia-water, butanol-pyridine-water, acetone-methanol-water, acetone-methanol-butanol, ethyl acetate-pyridine-acetic acid-water, isopropanol-butanol-water and butanol-acetic acid-water mixtures.

Results and discussion. The R_F-values for 3'MSL, 6'MSL, DSL, NANA, lactose, glucose, and galactose in 11 different solvents are listed in Table 1.

Propanol-water, propanol-ammonia, isopropanol-butanol-water, and butanol-pyridine-water mixtures proved to be the most efficient of the solvents tested for the separation of sialyloligosaccharides. The tendency to tail was very slight in these solvents. The best general solvent for separation of the tested salts of sialyloligosaccharides was propanol-water 6:3 (v/v). The addition of dilute hydrochloric acid or ammonia instead of water gave lower R_F-values and reduced the separation between DSL and NANA. A mixture of propanol-13 N ammonia-water 6:1:2 (v/v) gave, however, the best resolution between 3'MSL and 6'MSL. This solvent could also be used for the preparative separation of these two sugars in column chromatography on silica gel.[11] Butanol-pyridine-water 6:5:6 (v/v) gave a group separation between sialyloligosaccharides and NANA. Isopropanol-butanol-water mixtures separated sialyloligosaccharides. This type of solvent has already been described for the separation of neutral mono- and oligosaccharides on boric acid-impregnated silica gel.[14] For the present purpose the polarity of the solvent was increased, the composition isopropanol-butanol-water 5:3:3 (v/v) proving most efficient. The separation of lactose, glucose, and galactose was still good.

Pastuska [15] has reported that double bonds appear during chromatography of neutral sugars. It was suggested that this was due to a separation of the open-chain and cyclic forms of the sugars. Weicker and Brossmer [16] indicated that aminosugars form from monosaccharides during TLC on silica gel in solvents containing a high concentration of ammonia. Neither of these effects could be demonstrated with the solvents listed above.

With the exceptions mentioned above, analytical separations of sialic acids and sialic acid-containing oligosaccharides have been performed by paper chromatographic or electrophoretic methods. The detection of sialic acid on paper by resorcinol-TCA, Ehrlich [17] or thiobarbituric acid [18] spray

saccharides on TLC at 21°C.

Isopropanol-butanol-0.3 M formic acid 5:3:3	Isopropanol-butanol-H_2O 5:3:3	Isopropanol-butanol-H_2O 5:3:2	Butanol-HAc-H_2O 5:4:1	Butanol-pyridine-H_2O 6:5:6	Ethyl acetate-pyridine-H_2O-HAc 5:5:3:1
Silica gel borate	Silica gel borate	Silica gel borate	Silica gel	Silica gel	Silica gel
3	4	4	3	3	2.5
0.24	0.22	0.11	0.22[b]	0.66[b]	0.33
0.19	0.17	0.06	0.22[b]	0.63[b]	0.29
0.17	0.11[b]	0.04	0.18[b]	0.61	0.19[b]
0.18[b]	0.06[b]	0.03	0.29	0.34[b]	0.31[b]
0.37	0.33	0.28	0.38	0.63	0.49
0.47	0.44	0.42[b]	0.47	0.66	0.61
0.44	0.39	0.37	0.44	0.63	0.55

reagents may, however, raise considerable technical problems. The TLC method presented here gives a faster separation and increased sensitivity, besides permitting the use of more aggressive spray reagents for detection of the sugars.

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Anchimerically Assisted Sulfoxide Reactions

I. On the Reduction of 2-(Ethylsulfinyl)-cyclohexene-1-carboxylic Acid in Acidic Iodide Solution

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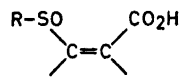
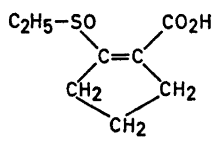
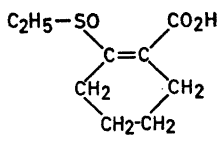
For the study of carboxyl-assisted sulfoxide reductions [1] 1,2-disubstituted cycloalkenes are of great interest. Heretofore, only one system of this kind, 2-(ethylsulfinyl)-cyclopentene-1-carboxylic acid (I), has been kinetically investigated with respect to the reduction of the sulfoxide group by means of iodide in acid solution.[2] It was found that I was much more slowly reduced than open-chain compounds of the same type, as represented below:



This fact was interpreted as due to an increase of ring strain during the formation of a bicyclic acyloxysulfonium ion intermediate from I. If this interpretation were correct, we thought that the same would not occur for the reduction of the next higher homologue, *i.e.* 2-(ethylsulfinyl)-cyclohexene-1-carboxylic acid (II), and we now wish to report briefly the preparation and the kinetic study of II.



I



II

The reduction of II was carried out at 25.0 ± 0.02°C in 50 % (v/v) acetic acid with sodium perchlorate added, $[ClO_4^-]$ = 0.250 M, to keep the ionic strength