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Thin-layer chromatography of substituted methyl β-maltosides

During the synthesis[1] of substituted methyl β-maltosides, extensive recourse was made to chromatography. This note records the chromatographic behavior of these maltoside derivatives; both thin-layer (TLC) and dry-column[2] chromatography are reported.

Experimental

Thin-layer plates (0.25 mm thickness) were prepared with a Quickfit Instrument* apparatus from a slurry of Silica Gel G (25 g) and water (50 ml). Before use, the plates were air dried, horizontally, for 16 h. Solvents were purified by distillation. Plates were developed until the solvent front had ascended 13 cm above the spotting site. Benzene–absolute ethanol (2:1) was used to develop the unacylated maltosides, and toluene–methanol (50:1) for the acylated maltosides as well as for multiple ascents. Spots were detected by spraying with a solution of ethanol–water–concentrated sulfuric acid (10:5:1) and heating until charred. All spots turned black upon charring except the deoxy-maltosides, which charred dark brown after changing from various shades of yellow. Iodine vapor also located the *p*-tolylsulfonyl (tosyl) maltosides when a nondestructive method was needed.

Dry-column chromatography was used to isolate the tosylated maltosides (compounds 3, 7, and 11, Table I). A 3-g sample of the partially tosylated reaction mixture of methyl β-maltoside was introduced onto the dry column by first dissolving the mixture in a slurry consisting of 15 g of Davison Grade 12 silica gel and 50 ml 95% ethanol. After the ethanol had evaporated, the residue was introduced on top of a dry Silica Gel G column (200 g, 4 × 40 cm) and the column was developed with

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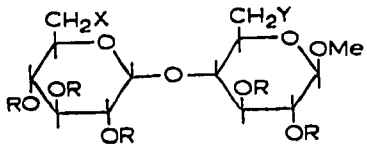
TABLE I

R_F VALUES OF SUBSTITUTED METHYL β-MALTOSIDES



Compound No.	R	X	Y	R_F values × 100	
				Solvent 1[a]	Solvent 2
1	H	OH	OH		12
2	Ac	OAc	OAc	20	
3	H	OTs	OTs		79
4	Ac	OTs	OTs	25	
5	Ac	I	I	45	
6	Ac	H	H	37	
7	H	OTs	OH		42
8	Ac	OTs	OAc	25	
9	Ac	I	OAc	33	
10	Ac	H	OAc	30	
11	H	OH	OTs		35
12	Ac	OAc	OTs	25	
13	Ac	OAc	I	34	
14	Ac	OAc	H	30	
15	Ac	OTs	I	34	
16	Ac	OTs	H	31	
17	Ac	OBz	H	38	

[a] Solvent 1 = toluene–methanol (50:1). Solvent 2 = benzene–absolute ethanol (2:1). Ac = acetyl, Bz = benzoyl, Ts = *p*-tolylsulfonyl.

benzene–absolute ethanol (2:1). Fractions (1–2 ml) were collected, monitored, and appropriately combined. Their R_F values are given in Table I.

Discussion

Synthesis of these substituted maltosides began with the partial tosylation of methyl β-maltoside (compound 1) to give a mixture of mono- and disubstituted derivatives, along with a large amount of starting material. Small amounts of tri, tetra, and higher orders of substitution were also present. To separate this mixture a modification of the dry-column chromatographic technique of LOEV AND GOODMAN[2] proved useful. Where LOEV AND GOODMAN halted elution when the dry column was fully developed, we simply modified the technique by continuing the elution and collecting fractions, as is usually done in wet-column chromatography. Excellent separation between compound 3 and higher order of substitution was obtained. Compound 3 overlapped only slightly with compounds 7 and 11, which eluted as a mixture. Sufficient separation between compounds 7 and 11 was realized when pure compound 11 crystallized in the last few fractions collected. Compound 1 remained on the column and was removed by eluting with 95% ethanol. The corresponding iodides (compounds 5, 9, and 13) were prepared quantitatively by heating under reflux with sodium iodide the respective acetylated tosyl products (compounds 4, 8, and 12), which were prepared by reaction of acetic anhydride–pyridine with compounds 3, 7, and 11. Toluene–methanol (50:1) separated well every reaction sequence in the acetylated series.

With the substituted maltosides the order of migration varied, *viz.* deoxy-iodo > deoxy > tosyl > acetate and the sequence was found to be general; for example, compound 5 > 6 > 4 > 2. This sequence occurred also with 6-substituted methyl α- and β-glucopyranosides[3]. EVANS *et al.*[4] report for 6-chloro-6-deoxy and 6-deoxy methyl D-glucopyranosides a sequence deoxy > chlorodeoxy > hydroxy. With both the acetylated and unacetylated chlorodeoxy and deoxy glucosides in several solvent systems we observed the sequence chlorodeoxy > deoxy > acetate (or hydroxyl). No explanation can be offered for this difference.

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Thin-layer gel filtration of modified proteins on 4% fine pearl-condensed agar

The possibility of preparing fine pearl agar suitable for thin-layer gel filtration has been reported in some preliminary results[1]. The major advantages of this thin-layer technique are reduced running time and very small sample size; the latter is especially important if we are working with high molecular weight substances which have been prepared by complicated and difficult isolation methods.

The fractionation experiments to test the feasibility of thin-layer gel filtration on agar gel were performed with nonprotein material–Blue Dextran 2000.

In the present study we tried to use the same thin-layer method for simple orientation estimation of aggregate and molecular sizes of modified and denatured proteins of an experimental blood expander "Modified Bovine Serum" (MBS)[2–4]. This protein material is a heterogeneous and complex mixture of modified serum protein and gelatin, together with their aggregates and degradation products.

Gel filtration on agar, resp. agarose gel, which has been introduced by POLSON[5], was shown to be more suitable for fractionation of MBS than gel filtration on Sephadex G-200. Dextran gel has too rigid a structure and its network detains only a smaller part of the comparatively large molecules and aggregates of MBS.

Materials and methods

"Modified Bovine Serum" was a standard preparation, batch No. "DG 472", prepared by heat denaturation and formolation of bovine serum in the presence of