Journal of Chromatography, 79 (1973) 373–374

CHROM. 6634

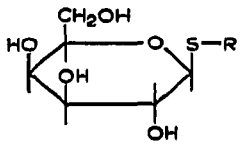
Note

Paper and thin-layer chromatography of 1-thio-β-D-galactopyranosides

The bacterial *lac* operon is induced by a number of compounds, many of which are not substrates of the induction products[1]. Such compounds are called "inducteurs gratuits" and most of them are 1-thio-β-D-galactopyranosides[2]. By following the effect of variously modified thiogalactosides on the induction, it may be possible to establish the structural requirements of the repressor.

TABLE I

PAPER AND THIN-LAYER CHROMATOGRAPHY OF 1-THIO-β-D-GALACTOPYRANOSIDES[a]



No.	*R*	R_F			*Colour of spot after TLC*
		Paper chromatography	*TLC*		
			A	*B*	
I	$-CH<^{CH_3}_{CH_3}$	0.57	0.70	—	brown
II	$-CH_2CH_2CH_2OH$	0.35	0.42	—	brown
III	$-CH_3$	0.37	0.54	—	brown
IV	$-CH_2CH_2OH$	0.21	0.26	—	brown
V	$-CH_2CHOHCH_3$	0.32	0.43	—	orange–brown
VI	$-CH_2CH_2CH_2OH$	0.31	0.33	—	yellow–brown
VII	$-CH_2CH_2CN$	0.30	0.46	—	orange–brown
VIII	$-CH_2CH_2CH_2SNa$	0.15	0.37	0.79	red–violet
IX	Lactose	0.03	0.00	—	black
X	$-CH_2CH_2OCH_3$	0.40	0.51	—	orange–brown
XI	$-CH_2CH_2SNa$	0.16	0.29	0.71	red–violet
XII	$-CH_2CHOHCH_2Cl$	0.39	0.50	—	red–brown
XIII	$-CH_2CH_2SCN$	0.10	0.21	0.75	red–violet
XIV	$-CH_2CH_2CONH_2$	0.14	0.10	0.58	red–brown
XV	$-CH_2CH(O)CH_2$	0.28	0.32	—	orange–brown
XVI	$-CH_2CH_2-$	0.09	0.46	—	orange–brown
XVII	$-CH_2CH_2COCH_3$	0.30	0.48	—	violet
XVIII	$-(CH_2)_7CH_3$	—	0.84	—	violet
XIX	$-CH_2CHOHCH_2-$	0.03	0.09	0.51	grey
XX	$-CH_2CH_2NH_2 \cdot HCl$	0.07	0.02	—	yellow
XXI	$-CH_2CH_2COOCH_3$	0.41	0.62	—	orange–brown
XXII	$-CH_2COOH$	0.16	0.52	—	violet
XXIII	$-CH_2CH_2COONa$	0.25	0.62	—	yellow
XXIV	$-Na$	—	0.37	—	yellow–brown
XXV	Glucose	0.10	0.12	—	black
XXVI	Galactose	0.10	0.12	—	black

[a] Compounds are listed in descending order of their inducing capacity for the *lac* operon.

Some of the inducing thiogalactosides were found to be inhibitors[2] of β-galactosidase, the product of the *lac* operon induction. It was interesting to determine whether the inducers–inhibitors are also substrates of the enzyme. For this study[2], and for synthetic purposes, chromatographic methods for the separation of the compounds in question were developed.

Methods and results

Paper chromatography was carried out on Whatman No. 1 filter-paper with the upper phase of solvent consisting of *n*-butanol–acetic acid–water (4:1:5). The carbohydrates and their derivatives were detected with ammoniacal silver nitrate solution[3].

All the thin-layer chromatographic (TLC) experiments were carried out on commercially available Silufol silica gel sheets (Cavalier, Czechoslovakia). The solvents used were (A) chloroform–ethanol (6:4) and (B) chloroform–methanol (2:8). The separated compounds were detected[4] by spraying the plates with a 5% solution of anisaldehyde in concentrated sulphuric acid, which produced yellow spots on a white background. Spots of characteristic colours were obtained by heating the plates for 2 min at 80° after the sulphuric acid treatment (Table I). By this method, it was possible to detect 1–3 μg of the compounds; the maximum amount of a compound that could be separated by TLC was *ca.* 200 μg.

Owing to the alkyl substitutions, the R_F values of the thiogalactosides were sufficiently high (Table I). The R_F values obtained by paper chromatography were not significantly affected by the extraction of thiogalactosides from bacterial cell-free preparations. Deproteinization of the incubation mixtures with $Ba(OH)_2$ was used routinely[5] and no further desalting was necessary.

TLC is suitable for determining the purity of thiogalactosides and their acetylated derivatives during synthetic procedures.

We thank Dr. M. ČERNÝ of the Charles University for his advice.

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Received January 3rd, 1973