Carbohydrate Research, 143 (1985) 229–232
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

TRIS [2-amino-2-(hydroxymethyl)propane-1,3-diol] behaves like an amino sugar in carbohydrate analysis

HUBERT KROTKIEWSKI

Department of Immunochemistry, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Czerska 12, 53-114 Wrocław (Poland)

(Received January 18th, 1985; accepted for publication, May 7th, 1985)

During the analysis of mono- and oligo-saccharides released from blood-group M and N glycopeptides by glycosidases from *Diplococcus pneumoniae*[1], an abundant component was found which showed the properties of a free amino sugar, but did not correspond to any known carbohydrate. This compound was identified as 2-amino-2-(hydroxymethyl)propane-1,3-diol (TRIS), which was derived from the enzyme sample dissolved in TRIS buffer.

Since TRIS is a frequently used component of buffer solutions, its carbohydrate-simulating properties in routine analytical methods have been studied.

When authentic TRIS (Koch–Light) was submitted to the procedure for transforming sugars into alditol acetates, it gave, in g.l.c. on OV-225, a symmetrical peak with T_{Xyl} 1.57 and T_{Gal} 0.64 (Fig. 1). The molecular response factor for this product, presumably TRIS tetra-acetate, was ~0.56 of the average response factor of the five sugars analysed (Fig. 1).

In p.e. at pH 5.4, TRIS moved as a single, compact spot towards the cathode with about twice the velocity of *N*-acetylneuraminic acid, which migrated towards the anode. Attempted *N*-acetylation of TRIS did not change its electrophoretic mobility, indicating that the amino group was not acetylated. TRIS can be *N*-acetylated[2] by using the more vigorous conditions applied for the acetylation of hydroxyl groups.

In p.c., TRIS migrated slightly slower and faster in solvents *B* and *C*, respectively (Table I), and in solvent *A* it gave a diffuse spot in the region of the neutral hexoses. TRIS could be detected with periodate–benzidine[3] or silver nitrate[4], but gave no response in the phenol–sulfuric acid reaction[5] and in the method of Ludowieg and Benmaman[6] for free aminohexoses.

Hydrolysates of glycoproteins may contain TRIS in addition to amino acids and amino sugars. The response of TRIS in the standard ninhydrin reaction[7] was therefore determined (Fig. 2). At relatively high concentration, TRIS gave a weak colour, similar to that of proline. The molar absorbances of TRIS, proline, and 2-amino-2-deoxyglucose, expressed as a percentage of the molar absorbance of

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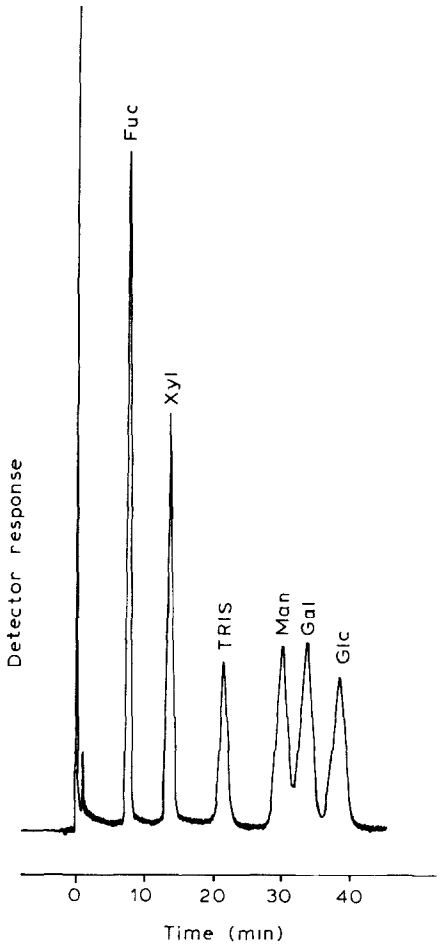

Fig. 1. Gas chromatogram of TRIS and some monosaccharides as alditol acetates in equimolar ratios (see Experimental).

TABLE I

MOBILITIES IN P.C OF TRIS AND SOME COMMON SUGARS

Solvent	R_F				
	TRIS	*Lactose*	*Galactose*	*Mannose*	*Fucose*
A (18 h)	[a]	0 55	1	1.30	1.48
B (15 h)	1.08	0 79	1	1.14	1.23
C (18 h)	1.15	0.72	1	1.12	1.24

[a] A diffuse spot between galactose and fucose.

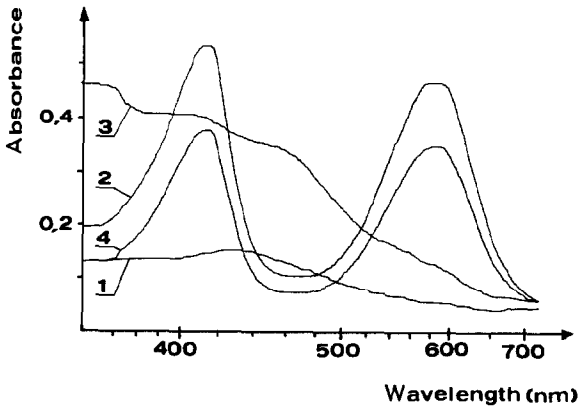

Fig. 2. Absorption spectra of the products formed in the standard ninhydrin reaction[7] (final volume, 7.0 mL) of 1, TRIS (9.75 μmol); 2, leucine (0.16 μmol); 3, proline (0.456 μmol); 4, GlcN (0.12 μmol).

leucine at 570 nm, were 0.21, 10.1, and 99.3%, respectively. Thus, the formation of colour by TRIS in the ninhydrin reaction is negligible.

The behaviour of TRIS in the commonly used analytical methods should be kept in mind when samples derived from TRIS-containing buffers are analysed for sugar components.

EXPERIMENTAL

General. — Descending chromatography was performed on Whatman No. 3 paper with *A*, 1-butanol–pyridine–water (6:4:3); *B*, ethyl acetate–pyridine–acetic acid–water (5:5:1:3); *C*, 1-propanol–ethyl acetate–water (6:1:3); and detection with silver nitrate[4]. Electrophoresis was performed on Whatman No. 3MM paper with pyridine–acetic acid buffer (pH 5.4, pyridine–acetic acid–water, 5:2:43) at 65 V/cm for 20 min, with detection using the periodic acid–benzidine reagent[3]. Neutral sugars were determined by the phenol–sulfuric acid method[5], hexosamines by the colorimetric method of Ludowieg and Benmaman[6], and amino acids and free amino sugars by the ninhydrin method[7]. G.l.c. of alditol acetates[8] was performed on a glass column (0.25 × 200 cm) packed with 3% OV-225 on Gas-Chrom Q (100–200 mesh) at 175°, using a Varian 2100 gas chromatograph equipped with a flame-ionisation detector. Absorption spectra were recorded by using a Zeiss Specord UV VIS spectrophotometer and 1-cm glass cuvettes. *N*-Acetylation was effected[9] with acetic anhydride in ethanol.

ACKNOWLEDGMENTS

The author thanks Professor E. Lisowska for valuable discussions. This investigation was supported by Grant 10.5 of the Polish Academy of Sciences.

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