Carbohydrate Research, 32 (1974) 171–174

Note

Color reactions of 2-amino-2-deoxy-D-mannose and 2-acetamido-2-deoxy-D-mannose*

JOHN HYNAN, GARY JOHNSON, AND YUAN CHUAN LEE**

Department of Biology and McCollum–Pratt Institute, Johns Hopkins University, Baltimore, Maryland 21218 (U. S. A.)

(Received July 11th, 1973; accepted July 31st, 1973)

The phenol–sulfuric acid method[1] for determination of carbohydrates is a simple, sensitive, accurate method widely used for various types of sugar analysis. The method had been believed to be unaffected by presence of 2-amino-2-deoxy sugars[2]. However, in the course of the chemical synthesis of β-D-(1 → 4)-linked *O*-D-galactosylhexosamines[3], we found that *O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-mannose gives a color yield higher than would be expected from the D-galactosyl group alone. Subsequent studies on the color reaction of 2-acetamido-2-deoxy-D-mannose showed that this *N*-acetylhexosamine indeed produces color under the normal conditions of the phenol–sulfuric acid method.

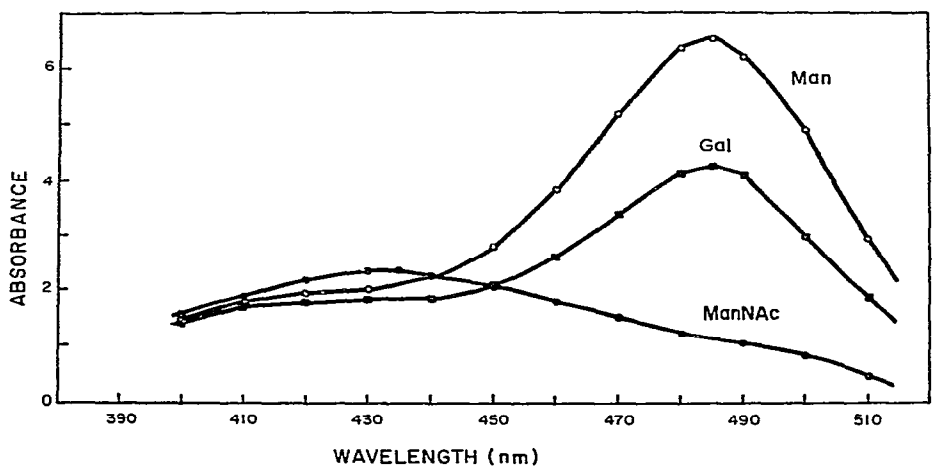

Fig. 1. Absorption spectra of colored products formed from D-mannose (—○—), D-galactose (—■—), and 2-acetamido-2-deoxy-D-mannose (—●—) in the phenol–sulfuric acid method. (Absorbance values were calculated for 1 μmole of each sugar.)

*Supported by USPHS NIH Research Grant AM9970. Contribution No. 740 from the McCollum–Pratt Institute, Johns Hopkins University.
**Recipient of USPHS NIH Research Career Development Award KO4 AM 70,148.

As shown in Fig. 1, when applied to 2-acetamido-2-deoxy-D-mannose, the phenol–sulfuric acid method yields a yellow color having maximum absorption at 435 nm. The molar absorbance of the color produced by 2-acetamido-2-deoxy-D-mannose was ~25% of that produced by neutral sugars at 480 nm (see Fig. 2).

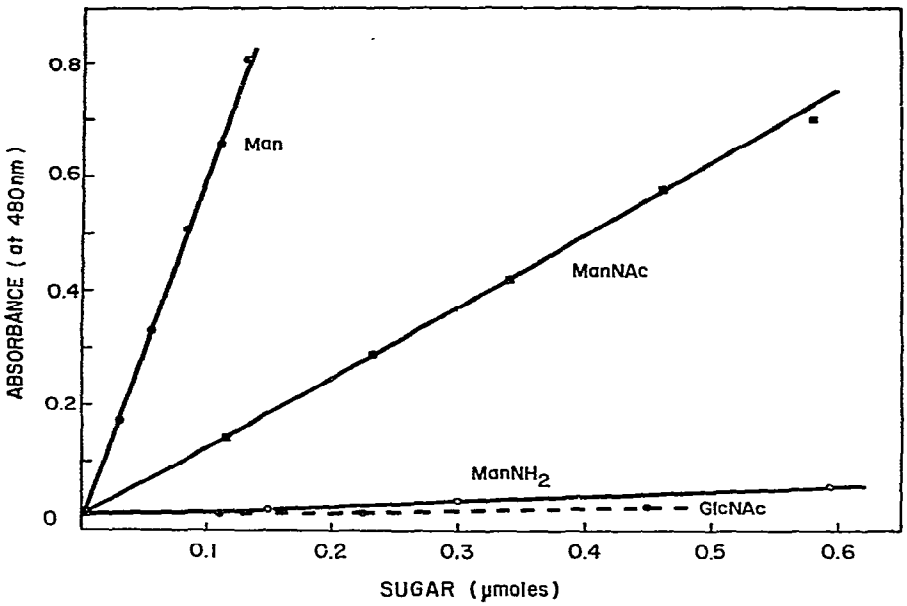

Fig. 2. Standard curves for D-mannose (—●—), 2-acetamido-2-deoxy-D-mannose (—■—), 2-amino-2-deoxy-D-mannose (—○—), and 2-acetamido-2-deoxy-D-glucose (---●---).

Under identical conditions, a negligible amount of color was obtained from 2-acetamido-2-deoxy-D-glucose and -galactose* and 2-amino-2-deoxy-D-mannose, -glucose*, and -galactose*. Under the conditions of the orcinol–sulfuric acid method[4], however, the color yield from 2-acetamido-2-deoxy-D-mannose was immeasurably low. Likewise, the color obtained from 2-acetamido-2-deoxy-D-mannose in the cysteine–sulfuric acid method was negligible[5].

It is thus apparent that the estimation of neutral sugars in polysaccharides containing 2-acetamido-2-deoxy-D-mannose will afford high values, unless the contribution due to 2-acetamido-2-deoxy-D-mannose is taken into account. In contrast, no such correction is necessary when the orcinol–sulfuric acid assay is employed**.

The foregoing facts are given in Table I, which presents the relative absorbances obtained by phenol–sulfuric acid and orcinol–sulfuric acid assays of 2-acetamido-2-

*Standard curves for 2-acetamido-2-deoxy-D-galactose and 2-amino-2-deoxy-D-glucose and D-galactose, although not included in Fig. 2, fall on or near the standard curves for 2-amino-2-deoxy-D-mannose and 2-acetamido-2-deoxy-D-glucose.

**2-Acetamido-2-deoxy-D-mannose produces substantial color when the orcinol–sulfuric acid reaction-mixture is incubated at 100°, instead of 80° as prescribed by Winzler[4].

deoxy-D-mannose, D-galactose, and *O*-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-mannose.

TABLE I

COMPARISON OF ABSORBANCE BY TWO COLORIMETRIC METHODS

	Absorbance[a]	
	Phenol–sulfuric acid	*Orcinol–sulfuric acid*
D-Gal	3.81	1.58
D-ManNAc	1.23	0.002
β-D-Gal-(1→4)-D-ManNAc	5.12	1.59

[a]Value produced by 1 μmole of the sugar.

These findings may explain some of the enigmatic results found in the literature dealing with the sugar-composition analysis of materials known to contain 2-acetamido-2-deoxy-D-mannose residues. In two such studies[6,7], the investigators found discrepancies in the analysis for neutral-sugar composition when this was determined on (*a*) intact polysaccharide and (*b*) partially or fully hydrolyzed samples, by the phenol–sulfuric acid assay or the cysteine–sulfuric acid assay. Incongruities in the neutral-sugar composition of *Pneumococcus* Type IV capsular polysaccharide as determined by the cysteine–sulfuric acid method have been attributed to interference by fucosamine[7], and discrepancies in results given by the phenol–sulfuric acid method were recorded by the authors without any explanation. In view of our findings, it is now clear that the high values for neutral-sugar composition given by the phenol–sulfuric method were attributable to the interference by the color produced by 2-acetamido-2-deoxy-D-mannose.

It is also apparent that the phenol–sulfuric acid method can be useful for the measurement of 2-acetamido-2-deoxy-D-mannose when neutral sugars and uronic acids are absent from the samples. In such situations, the phenol–sulfuric acid method provides a means for rapid determination of 2-acetamido-2-deoxy-D-mannose.

EXPERIMENTAL

Unless otherwise specified, the sugars used in this study were commercial products, and were used without further purification. 2-Acetamido-2-deoxy-D-mannose was kindly provided by Dr. Saul Roseman. *O*-*β*-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-mannose was prepared by chemical modification of lactose[3].

All color reactions were conducted in test tubes (13 × 100 mm); absorbances were determined with a Bausch and Lomb Spectronic 20 colorimeter. A modified phenol–sulfuric acid assay[8] was used in this work. The orcinol–sulfuric acid method of Winzler[4] and the cysteine–sulfuric acid method of Dische and Danilchenko[5] were

so scaled down that the color of the entire reaction-mixture could be read directly in the test tubes.

REFERENCES

1 M. DUBOIS, K. A. GILLIS, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
2 R. MONTGOMERY, *Biochim. Biophys. Acta*, 48 (1961) 591.
3 G. JOHNSON AND Y. C. LEE, *Abstr. Papers Amer. Chem. Soc. Meeting*, 165 (1973) CARB 34.
4 R. J. WINZLER, *Methods Biochem. Anal.*, 2 (1955) 290.
5 Z. DISCHE AND A. DANILCHENKO, *Anal. Biochem.*, 21 (1967) 119.
6 T. MIYAZAKI AND T. YADOMAE, *Carbohyd. Res.*, 16 (1971) 153.
7 J. D. HIGGINBOTHAM AND M. HEIDELBERGER, *Carbohyd. Res.*, 23 (1972) 165.
8 J. MCKELVY AND Y. C. LEE, *Arch. Biochem. Biophys.*, 132 (1969) 99.