

Note

Colorimetric determination of 3,6-anhydrogalactose in polysaccharides from red seaweeds

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Galactose and 3,6-anhydrogalactose are the major constituents of agar, carrageenan, and furcellaran¹. D-Xylose (0.2–1.8%) and 6-O-methyl-D-galactose (0.8–21%) are present in lower proportions in agar obtained from several Japanese seaweeds². Carrageenans from *Gigartina papillata*³ and *Iridaea membranacea*⁴ contain minor proportions of 6-O-methylgalactose and glucose.

The quantitative assay^{5,6} most commonly used for 3,6-anhydrogalactose involves reaction with the resorcinol reagent. Alternatively, Bolliger⁷ found that, upon treating with thymol in the presence of ferric chloride and hydrochloric acid, and heating, monosaccharides form deeply colored compounds that are insoluble in water, but soluble in chloroform. Patil *et al.*⁸ have standardized the conditions for the latter reagent for the determination of neutral and acidic carbohydrates.

We have now studied the possible application of this method to determining the 3,6-anhydrogalactose content of marine algal polysaccharides. 3,6-Anhydro-D-galactose does not exist in a crystalline form, and therefore, a standard solution thereof was obtained by hydrolysis of methyl 3,6-anhydro- α -D-galactopyranoside.

The optimal period for the development of the colored complex for 3,6-anhydro-D-galactose at 80° is illustrated in Fig. 1. The plot of absorbance against concentration for 3,6-anhydrogalactopyranose obeys Beer's law (see Fig. 2). Under the experimental conditions described, up to 350 μ g of D-galactose, 240 μ g of 6-O-methyl-D-galactose, and 50 μ g of D-xylose did not interfere. The absorbance of D-fructose was 55% of that of an equimolar amount of methyl 3,6-anhydro- α -D-galactopyranoside.

The major advantage of the present method over Yaphe's is lower interference by the aforementioned monosaccharides, but it entails use of a longer period of heating in order to ensure complete hydrolysis of the polysaccharides.

Table I shows the values obtained for 3,6-anhydrogalactose in samples of agar and carrageenan.

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TABLE I

CONCENTRATION OF 3,6-ANHYDROGALACTOSE IN ALGAL POLYSACCHARIDES

Polysaccharide	3,6-Anhydrogalactose (%)
Carrageenan (<i>Chondrus canaliculatus</i>)	13.9
Alkali-modified carrageenan ¹⁰ (<i>Chondrus canaliculatus</i>)	24.9
Agar (<i>Gracilaria lemaneiformis</i> , cystocarpic thalli)	23.5
Alkali-modified agar (<i>Gracilaria lemaneiformis</i> , cystocarpic thalli)	32.5
Agar (<i>Gracilaria lemaneiformis</i> , sterile thalli)	31.0
Alkali-modified agar (<i>Gracilaria lemaneiformis</i> , sterile thalli)	34.5

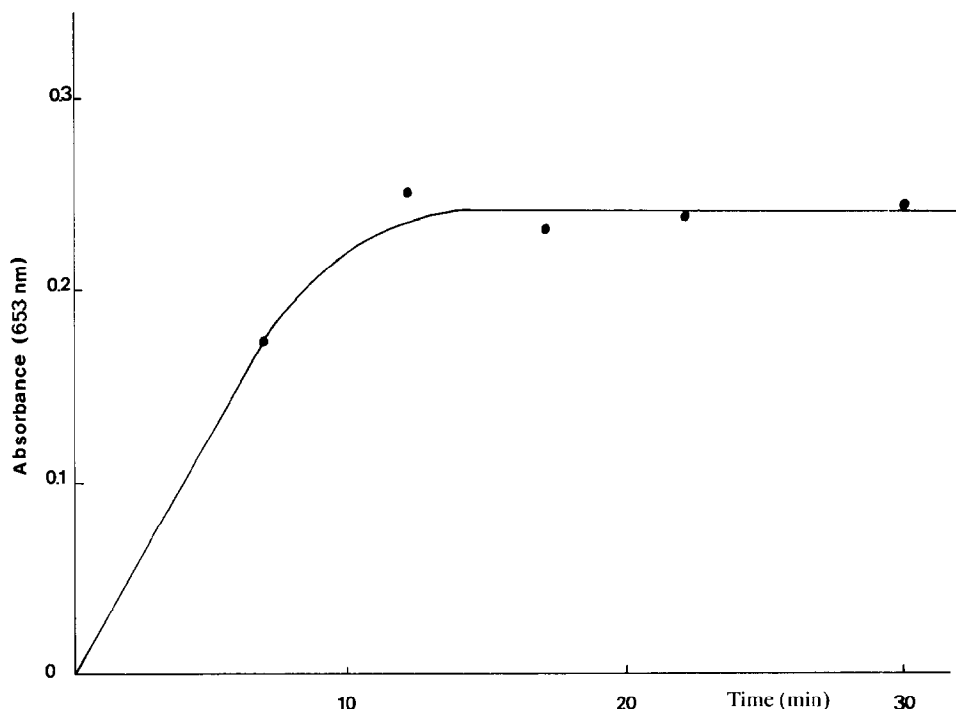


Fig. 1. Development of the colored complex, as shown by the absorbance at 653 nm, with time.

EXPERIMENTAL

Reagents. — These were *A*, thymol (5%) in ethanol; *B*, 0.5% of ferric chloride in conc. hydrochloric acid.

Solutions of sugars (10–200 μ g) in distilled water (2.0 mL) were well mixed with reagent *A* (0.5 mL), followed by reagent *B* (5.0 mL), in 25-mL, stoppered test-tubes equipped with stainless-steel springs. They were heated on a water bath during 13 min at 80°, and then rapidly cooled to room temperature. The mixtures were diluted with ethanol (10 mL), and the absorbance was determined at 635 nm

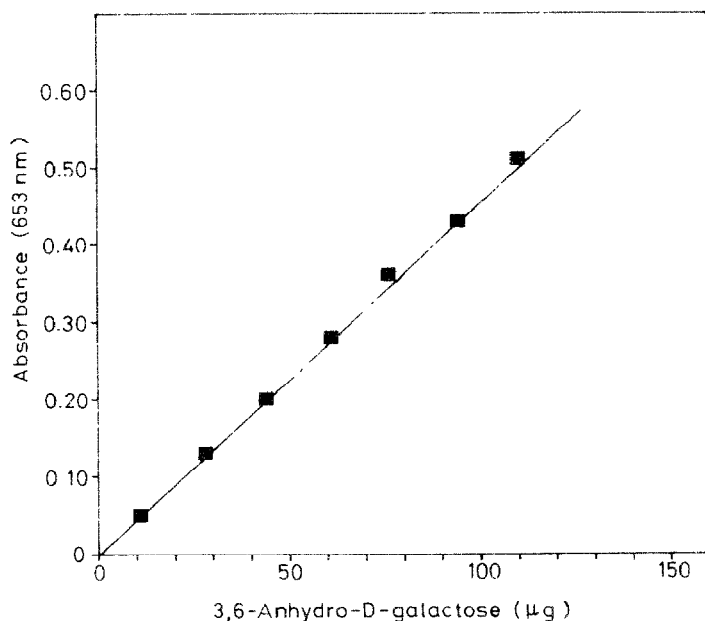


Fig. 2 Plot of absorbance at 653 nm versus concentration of 3,6-anhydro-D-galactose

with a Bausch and Lomb Spectronic 20 instrument. Standard curves for determination of the 3,6-anhydrogalactose content in polysaccharides (300 μg) were obtained with solutions of methyl 3,6-anhydro- α -D-galactopyranoside (12–120 μg). A correction factor of 0.92 was introduced for calculating the concentration of 3,6-anhydrogalactose. All of the measurements were conducted in triplicate. The standard curves were fitted to a straight line by using a least-squares, linear-regression method.

Methyl 3,6-anhydro- α -D-galactopyranoside was synthesized according to Lewis *et al.*⁹.

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