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Note

Separation and quantitation of enantiomeric 3,6-anhydrogalactoses by conversion to the corresponding diastereomeric acetylated *sec*-butyl 3,6-anhydrogalactonates

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Abstract

A method to determine the proportion of both enantiomers of 3,6-anhydrogalactose in a sample has been developed using methyl 3,6-anhydro-α-D-galactoside as a model compound. It involves an oxidative hydrolysis to obtain the corresponding aldonic acids, which are further converted to the acetylated diastereomeric *sec*-butyl esters. These are separated and quantified by GLC using a standard column for carbohydrate analysis; the procedure has been extended to seaweed polysaccharides. © 1998 Elsevier Science Ltd. All rights reserved

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Polysaccharides composed of alternating units of β-D-galactose and 3,6-anhydro-α-galactose are commonly found in red seaweeds [1].

It is well known that in agars the 3,6-anhydrogalactose units belong to the L-series, while in carrageenans they are in the D-form. Recently, some polysaccharide fractions containing both isomers of this sugar have been isolated from red seaweeds belonging to the Gigartinaceae, known to be carrageenophytes [2–4]. Therefore, structure elucidation of these polysaccharides should include determination of the absolute configuration of 3,6-anhydrogalactose.

Several methods have been developed to assign the D or L configuration of sugars. Some of them involve acid hydrolysis of the polysaccharide to obtain the free monosaccharides, which are then treated with a chiral reagent to give diastereomeric compounds. These compounds are then analyzed using GLC with non-chiral columns [5–11], or high-field ^{1}H NMR spectroscopy [12]. However, it must be kept in mind that during the acid hydrolysis performed under the usual conditions [13], degradation of 3,6-anhydrogalactose occurs; thus these methods cannot be applied to this sugar.

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In other methods [14,15], monosaccharide enantiomers are separated as different sugar derivatives using enantioselective GLC, but none of them refers to the 3,6-anhydrogalactose.

^{1}H and ^{13}C NMR spectroscopy, either of a polysaccharide or of the oligosaccharides obtained by its partial hydrolysis, are sometimes useful to determine the structure of a polysaccharide, including the absolute configuration of 3,6-anhydrogalactose [16–19]. However, spectra of polysaccharide samples that contain both isomers of this sugar are usually too complex to be completely resolved.

A qualitative evidence of the presence of both isomers of 3,6-anhydrogalactose may be obtained by partial reductive hydrolysis of the polysaccharide sample and detection of the acetylated derivatives of agarobiitol and/or carrabiitol by GLC–MS [20].

Herein we report a method to determine the proportion of both enantiomers of 3,6-anhydrogalactose in a sample that involves an oxidative hydrolysis to obtain the corresponding aldonic acids, which are stable in the reaction medium. The 3,6-anhydrogalactonic acids are further converted to the acetylated diastereomeric *sec*-butyl esters that are separated and quantified by GLC using a standard column for carbohydrate analysis.

This method was developed using methyl 3,6-anhydro-α-D-galactoside as a model compound, which was submitted to a mild acid hydrolysis (0.5 M TFA, 7.5 h at 65 °C) in the presence of bromine as oxidant to give 3,6-anhydrogalactonic acid. Since there was no reaction between 3,6-anhydrogalactonic acid and *sec*-butanol when hydrogen chloride was used as catalyst [21], esters were prepared by converting the aldonic acid to the aldonyl chloride as an intermediate step. The reaction between the aldonyl chloride and racemic *sec*-butanol yielded the diastereomeric *sec*-butyl esters, which were subsequently acetylated and analyzed by GLC–MS.

The chromatogram gave two very well-resolved peaks (separation factor 1.009) with equivalent areas (Fig. 1). The mass spectra of these peaks were identical and showed the characteristic fragmentation pattern of the 3,6-anhydro ring [22] as well as a signal at m/z 57 derived from the sec-butyl group (see Experimental). Comparing the results obtained with (*S*)-(+)-*sec*-butanol and the racemic alcohol, the GLC retention times relative to acetylated *myo*-inositol of the derivatives of both enantiomers were assigned (D: 0.709; L: 0.715).

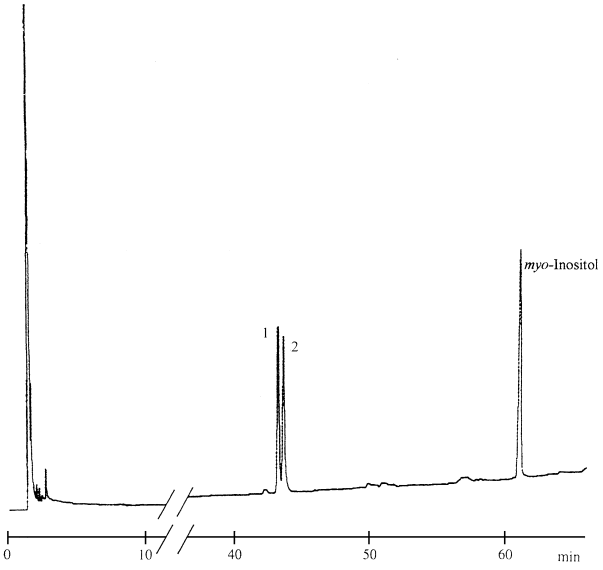

Fig. 1. Gas chromatogram of diastereomeric acetylated *sec*-butyl 3,6-anhydrogalactonates and *myo*-inositol; (1) (*S*)-*sec*-butyl 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactonate and (2) (*R*)-*sec*-butyl 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactonate [equivalent to (*S*)-*sec*-butyl 2,4,5-tri-*O*-acetyl-3,6-anhydro-L-galactonate].

This method was also applied to commercial κ-carrageenan and agarose which are known to contain only the D- and the L-isomer of 3,6-anhydro-α-galactose, respectively. In order to extend the procedure to a polysaccharide, after the oxidative treatment, the sample was submitted to further acid hydrolysis (2 M TFA, 2 h at 121 °C) in absence of bromine. After reduction, the sample was treated as described above. As expected, the chromatogram showed the signals of the corresponding acetylated *sec*-butyl 3,6-anhydrogalactonate and acetylated galactitol.

A low proportion of the acetylated *sec*-butyl galactonate was also detected, indicating that during the oxidative hydrolysis some galactosidic linkages were also cleaved. The retention time relative to acetylated *myo*-inositol was 0.829. The assignment of this peak was based in the fact that the mass spectrum of this compound was identical to that of the acetylated derivative of the product obtained by reaction between D-galactono-1,4-lactone and (*S*)-(+)-*sec*-butanol.

This method was also applied to a complex polysaccharide fraction (Fs) obtained from cystocarpic *Gigartina skottsbergii*. It has been reported previously [3] that Fs contained 61.4% of galactose and 22.5% of 3,6-anhydrogalactose as major sugar

constituents. Besides, the ratio of 3,6-anhydro-D-:-L-galactose, estimated from the ^{13}C NMR spectrum, was 1:2. Derivatization of Fs to the acetylated *sec*-butyl 3,6-anhydrogalactonates and further analysis by GLC gave the corresponding diastereomeric esters in a peak ratio (32:68), which confirmed the earlier determination from the ^{13}C NMR spectrum.

1. Experimental

Preparation of 3,6-anhydrogalactonic acid.—Methyl 3,6-anhydro-α-D-galactoside (2 mg) was dissolved in 0.5 M TFA solution (1 mL) [23] containing Br_2 (20 μL). The mixture was heated in a vial at 65 °C for 7.5 h [24]. After cooling, the sample was concentrated to dryness under a stream of dry air at 45 °C and further coevaporated with water (2×0.5 mL).

Conversion of 3,6-anhydrogalactonic acid to the peracetylated aldonyl chloride.—The residue was dissolved in 1:1 (v/v) TFA:Ac_2O (0.4 mL) and heated at 50 °C for 1 h in a vial. The solution was evaporated to dryness under stream of dry air at 45 °C. The aldonyl chloride was synthesized by the modified method of Wolfrom and Wood [25]. Toluene (1 mL) and $SOCl_2$ (0.25 mL) were added to the acetylated product, and the mixture, which was placed in a round-bottom flask, was refluxed for 30 min. After cooling, the solution was evaporated.

Conversion of the peracetylated aldonyl chloride to the sec*-butyl 3,6-anhydrogalactonate.*—The residue was dissolved in racemic *sec*-butanol (100 μL) and heated at 70 °C for 1 h. The solution was evaporated to dryness, and the mixture was reacetylated as described above. The same procedure was carried out with (*S*)-(+)-*sec*-butanol.

Derivatization of the polysaccharides.—The same procedure was carried out with a commercial κ-carragenan (Sigma), agarose (K&K Lab) and Fs [3] but, in these cases, after the oxidative treatment, hydrolysis of the polysaccharide was completed by adding a 2 M TFA solution (0.5 mL) and heating 2 h at 121 °C. After cooling, the acid was eliminated as described above.

Reduction with sodium borohydride.—The mixture obtained in the previous step was dissolved in 0.5 M Na_2CO_3 solution (120 μL) and kept at room temperature for 1.5 h and then treated with $NaBH_4$ (2 mg) for 1.5 h. Excess $NaBH_4$ was decomposed by dropwise addition of 25% AcOH until bubbling stopped. To remove the sodium ions, the solution was poured onto a column (7 mm i.d.×6.6 cm) of Amberlite IR-120 (H^+) and eluted with water. The eluate was evaporated to dryness and further coevaporated with MeOH (5×0.5 mL) [26].

After reduction, the sample was treated as described for the standard 3,6-anhydrogalactonic acid.

Preparation of sec*-butyl galactonate.*—Commercial D-galactono-1,4-lactone (2 mg) was dissolved in (*S*)-(+)-*sec*-butanol (100 μL) placed in a vial and heated at 70 °C for 2 h. The mixture was evaporated to dryness and acetylated as described before.

GLC.—GLC was carried out on a Hewlett–Packard 5890A gas–liquid chromatograph equipped with a flame ionization detector and fitted with fused-silica column (0.25 mm i.d.×30 m) WCOT-coated with a 0.20 μm film of SP-2330. Chromatography was performed programmed from 150 °C (5 min hold) to 240 °C at 1.5 °C min^{-1}. N_2 was used as carrier at a flow rate of 1 mL min^{-1}; the split ratio was 80:1, and the head pressure 15 psi. Injector and detector temperatures were 245 °C.

GLC–MS.—GLC–MS was performed on a HP 5890A gas chromatograph equipped with the SP-2330 column (see above) interfaced to a Trio-2 VG Masslab mass spectrometer working at 70 eV. He was used as carrier gas. The *sec*-butyl 3,6-anhydrogalactonate showed a mass spectrum with characteristic peaks: *m/z* (%) 287 (3), 259 (3), 245(3), 198 (5), 187 (33), 157 (12), 142 (4), 127 (26), 115 (12), 98 (9), 97 (17), 85 (56), 73 (5), 69 (10), 57 (15), 43 (100).

Methyl 3,6-anhydro-α-D-galactoside.—This compound was prepared as described by Lewis et al. [27], but for the synthesis of methyl 6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside, the reaction mixture was kept at −10 °C for 12 h, and then at 2 °C for 29 h.

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