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STUDIES OF THE DISTRIBUTION OF THE D-GALACTOSYL SIDE-CHAINS IN GUARAN

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ABSTRACT

The distribution of the D-galactosyl side-chains in guaran has been studied by a specific degradation procedure. A methylated guaran derivative having only HO-6 unsubstituted was subjected to an oxidation–β-elimination–mild acid hydrolysis procedure, whereby the D-galactosyl side-chains and the unbranched D-mannosyl residues were degraded, leaving intact only branched D-mannosyl residues having one or more, adjacent, branched D-mannosyl residues. Methylation analysis of the degradation mixture showed that the D-galactosyl side-chains in guaran were distributed mainly in pairs or triplets.

INTRODUCTION

The plant-seed galactomannans are composed of a (1→4)-linked β-D-mannan backbone to which are attached α-D-galactopyranosyl units at O-6 of certain D-mannopyranosyl residues[1]. In order to understand the properties of galactomannans, it is necessary to know the distribution of the D-galactopyranosyl units along the D-mannan backbone. Studies by enzymic methods[2] have indicated that the galactomannans have the D-galactopyranosyl side-chains distributed in large blocks. Studies of guaran[3] by periodate-oxidation techniques in combination with methylation analysis indicated that the D-galactopyranosyl side-chains are attached in a near-random arrangement to the D-mannan backbone. A recent investigation[4] of the distribution of the D-galactopyranosyl side-chains in the galactomannans guaran and locust-bean gum, using a specific degradation method, indicated that they are alternately disposed in guaran and located in blocks in locust-bean gum. We now report on the distribution of the D-galactopyranosyl side-chains in guaran and the use of a novel, specific method of degradation.

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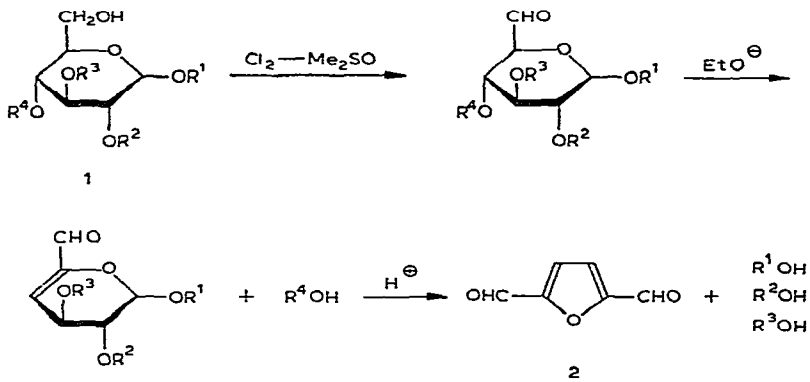
RESULTS AND DISCUSSION

The glycosidic linkage of a methylated hexopyranoside having an unsubstituted hydroxyl group at position 2, 3, 4, or 6 can be cleaved by applying the sequence of reactions oxidation, β-elimination, and mild hydrolysis with acid[5–8]. The sequence of reactions for a hexopyranoside having HO-6 unsubstituted is shown in **1**→**2**.



R^1, R^2, R^3, R^4 = alkyl or glycosyl group

In guaran, the D-galactopyranosyl side-chains and the unsubstituted D-mannopyranosyl residues of the backbone have HO-6 unsubstituted, as indicated by the results of methylation analysis in Table I. By subjecting guaran to the sequence tritylation, methylation (Hakomori), and detritylation, and then subjecting the product (methylated guaran *A*) to the same sequence of reactions (*cf.* Ref. 9), methylated guaran *B* was obtained in which most of the primary hydroxyl groups were unsubstituted. The products formed on hydrolysis of methylated guarans *A* and *B* are shown in Table I, together with the products after *A* and *B* had been methylated. The small proportions of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-mannose obtained from *A* indicated that tritylation of HO-6 in the precursor was

TABLE I

METHYL ETHERS[a] FROM THE HYDROLYSATES OF MODIFIED GUARANS

Modified guaran	*2,3,4,6-Me*$_4$-*Gal* (%)	*2,3,4-Me*$_3$-*Gal* (%)	*2,3,6-Me*$_3$-*Man* (%)	*2,3-Me*$_2$-*Man* (%)
Methylated guaran	35.6		25.1	39.3
Partially methylated guaran *A*	2.2	22.1	5.8	59.4 (10.5)[b]
Methylated *A*	36.8	2.1	28.3	32.8
Partially methylated guaran *B*	3.8	30.8	5.4	60.0
Methylated *B*	35.8		25.0	39.2

[a]Analysis by g.l.c.–m.s.[10] of the alditol acetates. [b]This figure represents di-*O*-methylgalactoses and mono-*O*-methylmannoses.

extensive. However, the 10.5% yield of di-*O*-methyl-D-galactose and mono-*O*-methyl-D-mannose reflected the extent of tritylation of secondary positions.

Further methylation of *A* gave products (Table I) similar to those obtained after methylation of guaran, demonstrating that no appreciable degradation had occurred in the detritylation stage. The ratio of secondary to primary hydroxyl groups in *A* was ~1:6 compared to ~3:1 in guaran. The products (Table I) obtained on hydrolysis of *B* indicated that there was negligible tritylation of secondary hydroxyl groups in the precursor, and that 90% of the D-galactopyranosyl residues and (1→4)-linked D-mannosyl residues had HO-6 unsubstituted. Methylation analysis of *B* indicated that no significant degradation occurred in the second detritylation stage.

The partially methylated guaran derivative *B* admixed with methylated dextran (as internal standard) was oxidised with a chlorine–methyl sulphoxide complex[6]. The oxidised product *C* was hydrolysed and the resulting partially methylated sugars were analysed (Table II). The results showed that most of the unsubstituted primary hydroxyl groups in *B* had been oxidised; the recovery of oxidised material was >90%. Reduction of *C* with sodium borodeuteride, followed by hydrolysis, and analysis of the resulting sugars by g.l.c.–m.s. of the alditol acetates showed that the primary hydroxyl groups had been oxidised to aldehyde groups, as only one deuterium was permanently introduced in the reduction step. Moreover, conversion of the primary hydroxyl groups into aldehyde groups was quantitative.

TABLE II

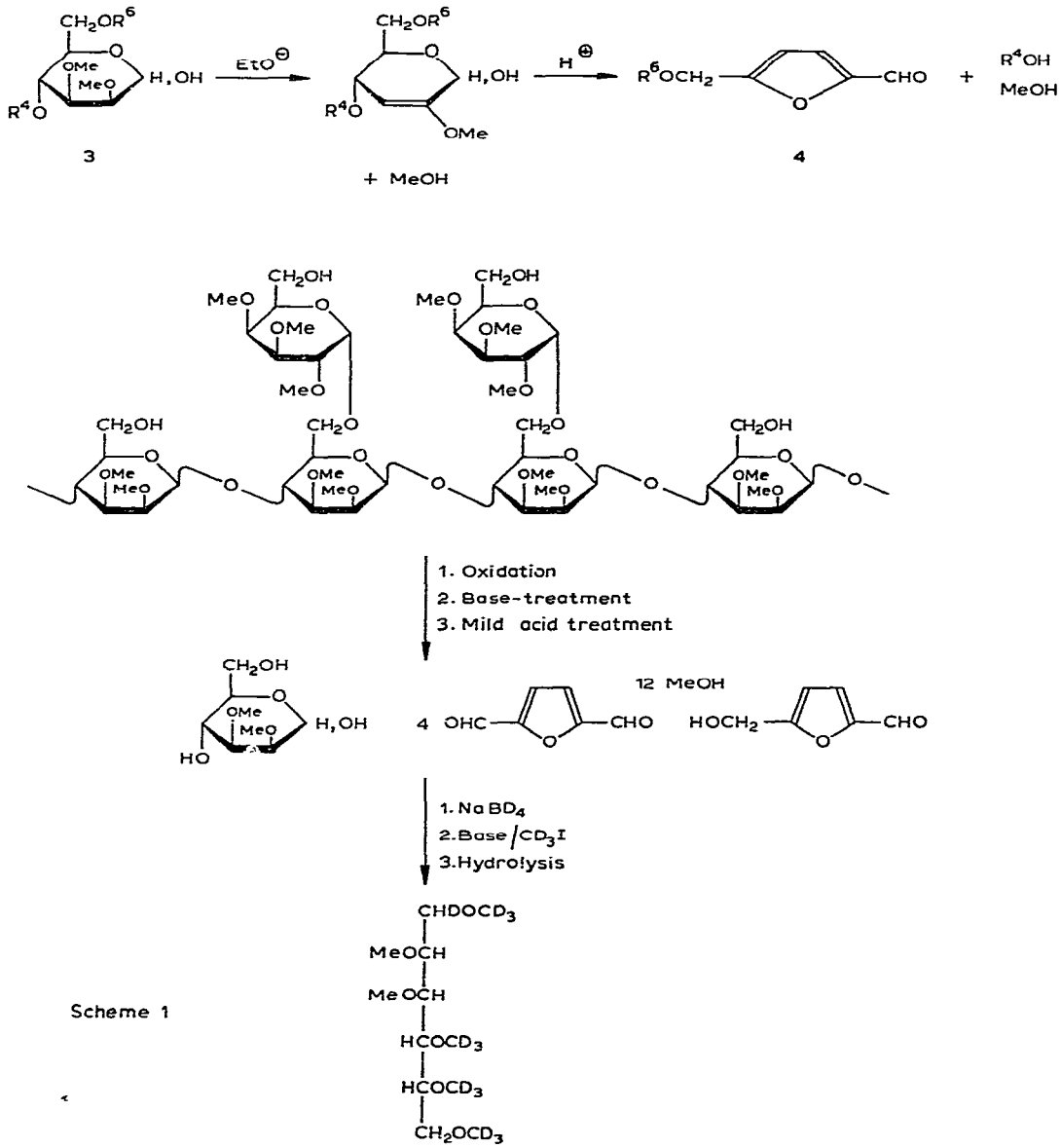
METHYL ETHERS FROM THE HYDROLYSATES OF MODIFIED GUARANS

Modified guaran	*2,3,4,6-Me*$_4$-*Gal* (%)[a]	*2,3,4*,6*-Me*$_4$-*Man*[b] (%)[a]	*2,3,6-Me*$_3$-*Man* (%)[a]	*2,3,4-Me*$_3$-*Gal* (%)[a]	*2,3-Me*$_2$-*Man* (%)
B	3.1	0	5.9	32.6	58.4
C	2.8	0	4.7	0	33.0
C, degraded, reduced ($NaBD_4$)	5.5	0	3.5	0	20.4[c]
C, degraded, reduced ($NaBD_4$), trideuteriomethylated	3.9	8.6	0	0	0

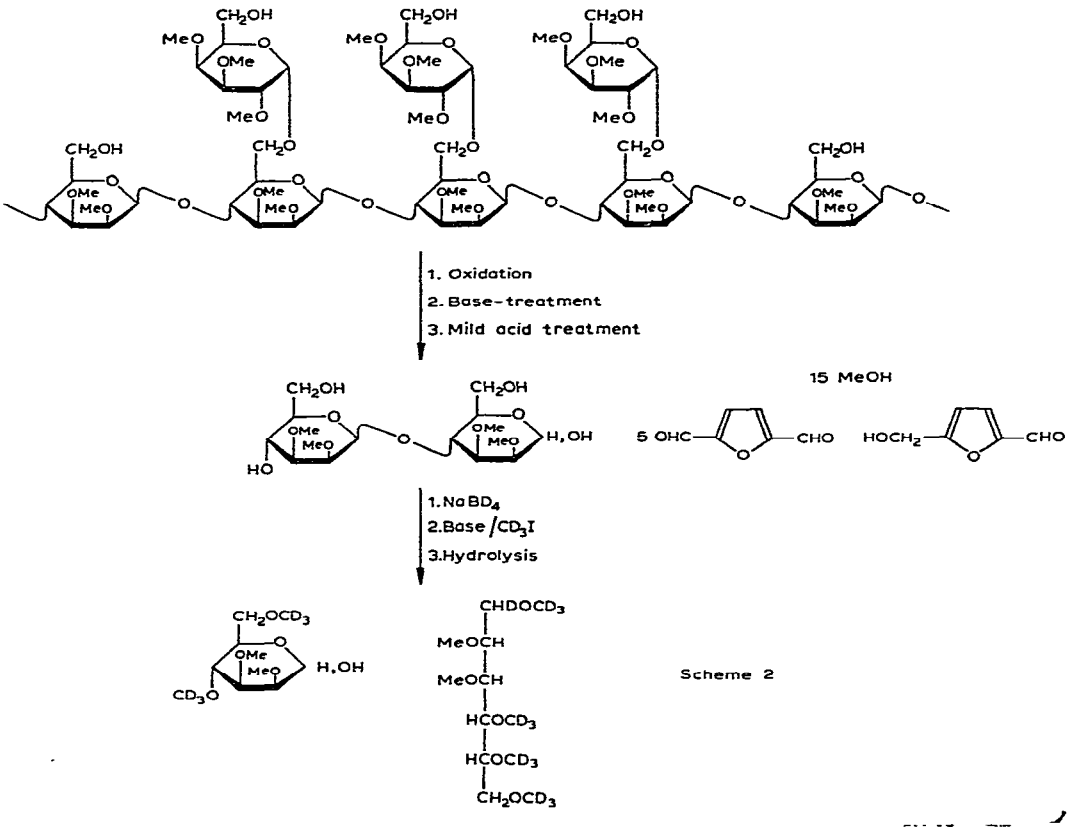
[a]The molar percentages have been calculated against the internal standard so that the methyl ethers obtained for *B* total 100% designates positions of trideuteriomethyl groups. [c]Labelled, tc the extent of 65%, with a deuterium atom at C-1.

The oxidised guaran *C* (together with the internal standard) was treated with sodium methoxide to effect the β-elimination [*cf.* **1**→**2**; when a methylated D-mannopyranose residue is released, it will be further degraded[11] (**3**→**4**)]. The product, which was not isolated, was treated with acetic acid at 100°, which results in further degradation of the α,β-unsaturated, keto(aldehydo) sugars, and then reduced with sodium borodeuteride and hydrolysed before and after trideuteriomethylation. Analysis of the resulting methylated sugars (Table II) showed that, of the original 35.8% of branch

points (Table I), 20.4% survived the degradation procedure, and 13.3% of these contained a deuterium atom at C-1 and thus constituted a reducing end. Thus, as shown in the annexed schemes, most of the D-galactopyranosyl side-chains are arranged in pairs or triplets in the proportions ~1:2. The formation of 8.6% of 2,3-di-*O*-methyl-4,6-di-*O*-trideuteriomethyl-D-mannose (the hexamethyl ether is too volatile be to analysed) is also consistent with this conclusion. The accuracy of the



Scheme 1



methodology is limited and may not detect a small proportion of isolated D-galactosyl side-chains. The presence of D-galactosyl side-chains in blocks larger than three can be excluded, because such an arrangement would furnish 2,3-di-*O*-methyl-D-mannose after hydrolysis of the reduced ($NaBD_4$) and trideuteriomethylated, degraded material.

The precise arrangements of the structures in the annexed schemes cannot be deduced from this investigation, but studies of the interaction of galactomannans with carrageenan[1] suggest that there are heavily substituted areas and areas free of substitution.

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at <40° (bath). G.l.c. was conducted on a glass column (200 × 0.3 cm) containing 3% of OV-225 on Chromosorb (80–100 mesh) or a glass-capillary column (25 m × 0.25 mm) wall-coated with SP-1000 (LKB Products, Sweden). A Perkin–Elmer model 900 gas chromatograph with flame-ionization detector was used. Peak areas were

measured with a Hewlett–Packard 3370 B electronic integrator. For g.l.c.–m.s., a Perkin–Elmer 270 gas chromatograph–mass spectrometer fitted with an OV-225 S.C.O.T. column was used. Hydrolyses were performed with 90% formic acid for 1 h at 100°, followed by treatment with 0.25M sulphuric acid for 16 h at 100°.

Guaran, isolated[12] from guar flour (Sigma Chemical Company, St. Louis, Missouri, U.S.A.), contained D-galactose and D-mannose in the molar proportions 36:64.

Partially methylated guarans A *and* B. — To a suspension of guaran (5 g) in methyl sulphoxide (60 ml) were added pyridine (200 ml) and trityl chloride (75 g). The mixture was kept for 72 h at 40° and then dialysed against methanol, and the tritylguaran (9.4 g) recovered was dried over phosphorus pentaoxide *in vacuo*.

To a solution of tritylguaran (9.4 g) in methyl sulphoxide (300 ml) in a sealed vessel (under nitrogen) was added 2M sodium methylsulphinylmethanide (150 ml). The mixture was stirred for 2 h and kept overnight at room temperature. Methyl iodide (75 ml) was added portionwise with external cooling. The resulting solution was stirred for 1 h, poured into water, dialysed, and concentrated to dryness. The residue was dissolved in chloroform, and light petroleum (b.p. 60–71°) was added to precipitate the methyltritylguaran (11.5 g).

To a suspension of methyltritylguaran (11.5 g) in methanol (1.9 l) was added 4M hydrochloric acid (25.5 ml), and the mixture was stirred for 18 h at room temperature, neutralised with saturated aqueous sodium hydrogen carbonate, dialysed, and concentrated to dryness. The residue was eluted from a column (1 m × 5 cm) of Sephadex LH 20 with chloroform–acetone (2:1). The partially methylated guaran *A* (5.5 g) was eluted with the void volume.

To a solution of *A* (5.5 g) in dichloromethane (275 ml) were added pyridine (138 ml) and trityl chloride (59.5 g). The solution was kept for 48 h at 40°, and then dialysed against methanol and concentrated to dryness. The residue was dissolved in chloroform, and light petroleum was added to precipitate the trityl derivative (7.3 g), which was methylated and then detritylated, as described above, to yield partially methylated guaran *B* (4.5 g).

Oxidation of partially methylated guaran B *and degradation of the product.* — The oxidising agent[6] was prepared under anhydrous conditions at −45° by dropwise addition of methyl sulphoxide (9 ml) to a stirred solution of chlorine in dichloromethane (25 ml). A solution of a mixture of *B* (100 mg) and fully methylated dextran (50 mg, internal standard) in dichloromethane (5 ml) was then added dropwise. The mixture was stirred at −45° for 6 h and then triethylamine (7 ml) was added dropwise. The mixture was allowed to attain room temperature and, after a further 15 min, lyophilised. The residue was partitioned between chloroform and water, and the chloroform phase was concentrated to dryness to give *C*.

To a solution of *C* in dichloromethane (10 ml) was added methanolic M sodium methoxide (5 ml). The solution was kept at room temperature for 1.5 h, and then neutralised with acetic acid and treated with 50% acetic acid for 16 h at 100°. The degraded material, isolated by partition between chloroform and water, was reduced

with sodium borodeuteride in ethanol, and portions were hydrolysed, or methylated with trideuteriomethyl iodide.

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