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Preliminary communication

The structure of 5-membered acetal groups of pyruvic acid in the D-galactan of the snail *Pomacea lineata**

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A β-D-galactopyranan, isolated from the albumen gland of the snail *Pomacea lineata* (formerly *Ampullarius lineata* SPIX, 1827), contains 3,4-*O*-(1-carboxyethylidene) groups, as shown by the liberation of pyruvic acid on acid hydrolysis and by methylation data[1]. The ^{13}C-n.m.r. spectrum of the D-galactan (salt form) contains minor signals (for solutions in D_2O at 70°) at δ_C 109.0 (non-protonated ketal carbon), 81.9 (C-3 of β-D-galactopyranosyl unit substituted by acetal group), and 25.3 (CH_3 of acetal group). The size of the signals, relative to that of the signal at δ_C 85.3 (*O*-glycosylated C-3), increased in spectra of products of successive Smith degradations (mild hydrolytic conditions). However, the carbonyl signal was not observed, and the signal for the non-protonated acetal carbon remained very small, owing to long, spin-lattice relaxation times, T_1. The position of substitution of the acetal groups, and in particular their configuration, were determined by use of the ^{13}C- and ^{1}H-n.m.r. spectra of model diastereomers of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside.

The model compounds were prepared as follows: Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**1**) was mono-*O*-benzylated with benzyl bromide and silver oxide in *N*,*N*′-dimethylformamide to give the 6-*O*-benzyl derivative, which was partially hydrolyzed with 80% aqueous acetic acid at 100° into methyl 6-*O*-benzyl-β-D-galactopyranoside (**2**) in 32% yield, m.p. 104° (from ethyl acetate–hexane), $[\alpha]_D^{25}$ −15° (*c* 1.2, ethanol). Treatment of **2** with acetoxyacetone containing sulphuric acid gave a mixture of 3,4-*O*-acetoxyisopropylidene derivatives, which were *O*-deacetylated into methyl 6-*O*-benzyl-3,4-*O*-hydroxyisopropylidene-β-D-galactopyranosides (**3** and **6**; ratio ~3:3:1). These show CH_3 signals at δ_C 21.94 and 23.50, respectively. According to a study by Garegg *et al.*[2] of methyl 3,4-*O*-hydroxyisopropylidene-β-D-galactopyranosides, where the configurations were determined by crystallography, the major signal at higher field, δ_C 21.94, should

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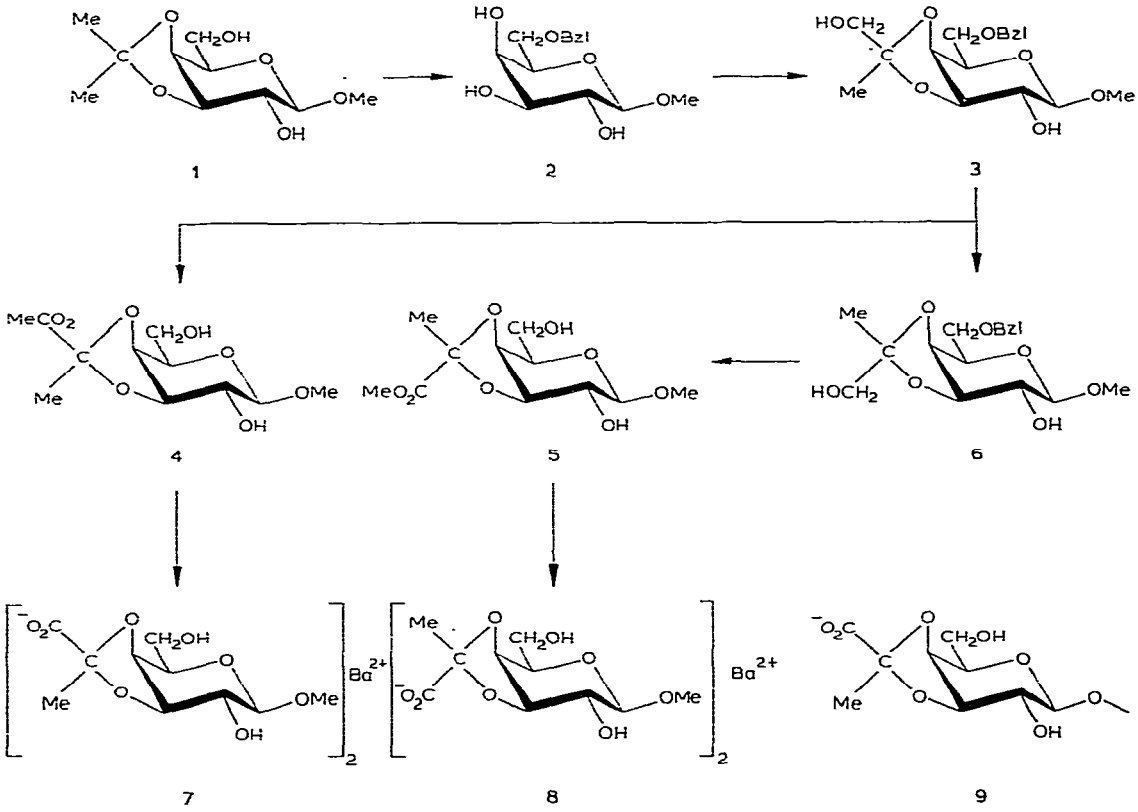

have an *endo* hydroxymethyl group, as in 3. Compound 3, obtained in 24% yield, had m.p. 116° (ether) and $[\alpha]_D^{25}$ −13° (*c* 0.6, ethanol); ^{13}C-n.m.r. (D_2O): δ 111.88 (non-protonated acetal carbon), 103.86 (C-1), 80.07 (C-3), and 21.94 (CH_3). Compound 3 was oxidized with platinum in the presence of oxygen in aqueous sodium hydrogencarbonate solution at 90° to give the sodium salt of the 3,4-*O*-(1-carboxyethylidene) derivative, and this was converted to the acid form, and thence to the methyl ester with diazomethane. This was purified by silicic acid colum chromatography (eluent: chloroform) and hydrogenolyzed in acetic acid solution with 5% palladium-on-charcoal, to provide methyl 3,4-*O*-(1-methoxycarbonylethylidene)-β-D-galactopyranoside (4) (yield: 35% based on 3; 8% based on 2), m.p. 152–153° (ethyl acetate–diethyl ether), $[\alpha]_D^{25}$ −8° (*c* 1.0, methanol); ^{13}C-n.m.r. ($CDCl_3$): δ 169.96 (carbonyl), 106.47 (non-protonated acetal carbon), 103.31 (C-1), 80.56 (C-3), and 23.60 (CH_3). Compound 4 was converted into the barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (7) with cold, aqueous barium hydroxide; ^{13}C-n.m.r. (D_2O, 70°): δ 178.49 (carbonyl), 109.60 (non-protonated acetal carbon), 104.31 (C-1), 81.12 (C-3), and 24.98 (CH_3); ^{1}H-n.m.r. (D_2O, 70°): δ 1.97 (CH_3).

The minor methyl 3,4-*O*-(1-methoxycarbonylethylidene)-β-D-galactopyranoside (5) was prepared by use of a similar series of reactions, starting from the mother liquor

obtained by crystallization of 3. Compound 5 was freed from impurities by silicic acid column chromatography (eluent: 50:1, v/v, chloroform–methanol) (yield, 1% based on 2), m.p. 147–149°, $[\alpha]_D^{25}$ –13° (*c* 0.5, methanol); ^{13}C-n.m.r. ($CDCl_3$): δ 170.05 (carbonyl), 106.06 (non-protonated acetal carbon), 103.23 (C-1), 79.26 (C-3), and 23.49 (CH_3). The derived barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (8) was prepared with aqueous barium hydroxide; ^{13}C-n.m.r. (D_2O, 70°): δ 109.45 (non-protonated acetal carbon), 104.47 (C-1), 80.44 (C-3), and 25.05 (CH_3); ^{1}H-n.m.r. (D_2O, 70°): δ 2.07 (CH_3).

The ^{13}C- and ^{1}H-n.m.r. spectra of the snail D-galactan were interpreted as follows: The 5-membered acetal ring is indicated by the signal of the non-protonated ketal carbon at δ_C 109.0, close to the values of 109.60 and 109.45 for 7 and 8, and different from δ_C 102.39 for the barium salt of 4,6-*O*-(1-carboxyethylidene)-β-D-galactose. Non-protonated acetal carbon atoms of 5-membered rings give ^{13}C signals at fields lower than those of 6-membered rings and are readily distinguishable[3–5]. The D-galactan gives a CH_3 acetal signal at δ_C 24.6, close to the values of 24.98 and 25.05 for the 3,4-*O*-(1-carboxyethylidene) derivatives 4 and 7. It differs from the values reported for isomers[2,6] of 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside at δ_C 17.2 and 26.1, and at δ_C 27.1 for a polysaccharide containing units with a related structure[7]. Similar values were observed for the barium salts of 4,6-*O*-(1-carboxyethylidene)-α,β-D-galactose (δ_C 26.44) and 3,6-anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-α,β-D-galactopyranosyl]-L-galactose dimethyl acetal (δ_C 26.26).

The snail D-galactan contains (1→3)- and (1→6)-linked β-D-galactopyranosyl residues according to methylation data[8], and the former linkage is evidenced by an *O*-glycosylated, C-3 signal at δ_C 85.3, close to that of C-3′ of β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc, which is observed[9] at δ_C 83.6. The minor signal of the D-galactan at δ_C 81.9 arises from C-3 of units containing 3,4-*O*-(1-carboxyethylidene) substituents, since it corresponds to C-3 signals of 7 and 8 at δ_C 81.12 and 80.44, respectively.

TABLE I

SHIFTS OF N.M.R. SIGNALS, DEPENDENT ON ACETAL CONFIGURATION, IN COMPOUNDS CONTAINING *O*-1-(-CARBOXYETHYLIDENE) SUBSTITUENTS IN β-D-GALACTOPYRANOSYL UNITS

Compound	*Chemical shift (p.p.m.)*[a]	
	C-3 of unit substituted by acetal group	*Proton of CH_3*
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (7)	81.12	1.97
Diastereomer of 7 (8)	80.44	2.07
Pyruvylated unit in D-galactan (9)	81.9	1.99

[a]Tetramethylsilane as the external standard.

No conclusions could be reached on the configuration of the acetal group of the snail D-galactan by consideration of the ^{13}C chemical shift of CH_3 resonances of diastereomers of the barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (7 and 8), which are only 0.07 p.p.m. apart. However, the C-3 signal is configurationally dependent (Table I), and the minor C-3 resonance of the D-galactan at δ_C 81.9 is close to 81.12 of the C-3 signal of 7. The suggested structure 9 having an *endo* carboxyl group was confirmed by ^1H-n.m.r. data, since the barium salt of 7 (D_2O, 70°) gave a CH_3 signal at δ 1.97, close to the value of 1.99 obtained for the D-galactan. In contrast, the corresponding signal of 8 was observed at δ 2.07 (Table I). For routine determination of (1-carboxyethylidene) structures in polysaccharides, ^1H- is superior to ^{13}C-n.m.r. spectroscopy, because of its sensitivity and because the CH_3 proton resonances also depend on the ring size of the acetal, whether it is 5- or 6-membered. Resonances of the latter compounds are observed at δ 1.4–1.5 with the carboxyl group in the axial position, as for 4,6-*O*-substituents on β-D-galactopyranosyl units [7,10]. For equatorial carboxyl substituents, it would appear that the CH_3 resonances would be at a field lower by ~0.2 p.p.m.

The results herein are of interest as Garegg et al.[6] found that 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl units in bacterial polysaccharides have an acetal configuration corresponding to that of 8 (*i.e., S*). The various shifts of 3,4-*O*-hydroxyisopropylidene derivatives of the polysaccharides, obtained by reduction, were compared with those of model methyl 3,4-*O*-hydroxyisopropylidene-D-galactopyranosides. It would be of interest to compare the chemical shifts of the proton CH_3 signals of the salt forms of these polysaccharides with those of the snail polysaccharide, under the same spectral conditions.

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