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PROPERTIES OF ^{13}C-N.M.R. SPECTRA OF *O*-(1-CARBOXYETHYLIDENE) DERIVATIVES OF METHYL β-D-GALACTOPYRANOSIDE: MODELS FOR DETERMINATION OF PYRUVIC ACID ACETAL STRUCTURES IN POLYSACCHARIDES

PHILIP A. J. GORIN, MYTOSK MAZUREK,
Prairie Regional Laboratory, National Research Council Saskatoon, Saskatchewan (Canada)
HELENA S. DUARTE,
Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Recife (Brazil)
MARCELLO IACOMINI, AND JOSÉ H. DUARTE
Departamento de Bioquímica, Universidade Federal do Paraná, Curitiba (Brazil)

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ABSTRACT

^{13}C-N.m.r. spectra were recorded of compounds containing *O*-(1-carboxyethylidene) groups linked to galactopyranose and fucopyranose derivatives. These compounds are useful as aids in determination of the positions and configurations of pyruvic acid acetal substituents in polysaccharides. Chemical shifts of non-protonated acetal carbons depend on whether the acetal ring is 5-membered (δ_c 107–109.5) or 6-membered (δ_c 100.5–102.4). The C-3 signals of 3,4-(1-carboxyethylidene) acetals are typical, being at $\sim\delta_c$ 81 and in the case of the barium salt of the methyl β-D-galactopyranoside derivative. The exact value depends on the configuration, whether it is as in **6** (δ_c 81.1) or **5** (δ_c 80.4). The CH_3 signals of proton-n.m.r. spectra are also diagnostically useful, falling at δ 1.97 and 2.07 respectively. (The foregoing shift-values are pH-dependent). The pyruvylated galactan from the snail, *Pomacea lineata*, was shown to contain some residues that could be assigned a structure corresponding, in the positions of acetal substitution and acetal configuration, to structure **6**. Compound **6** (barium salt) is of interest as its ^{13}C-n.m.r. spectrum lacks non-protonated carbonyl and acetal carbon resonances, when obtained by the usual procedures. While this is principally because of long T_1 values, the non-protonated acetal carbon signals are comparatively broad, possibly through slow conformational interchange. In the case of the carbonyl resonance, the lack of sensitivity is because of a low n.O.e. value of 1.4, approximately one half that of other carbon atoms in the molecule.

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TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS OF 3,4-*O*- AND 4,6-*O*-SUBSTITUTED ACETAL DERIVATIVES OF HEXOPYRANOSIDES

Compound	*Carbonyl*	*Non-protonated acetal carbon atoms*	*C-1*	*CH_3 of acetal*
Methyl 6-*O*-benzyl-3,4-*O*-[1-(hydroxymethyl)ethylidene]-β-D-galactopyranoside (3) (D_2O)		111.88	103.86	21.94
Methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranoside from 3 ($CDCl_3$)	169.96	106.47	103.31	23.60
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (6) (D_2O)	178.41	107.24	103.68	24.53
Barium salt of 6 (D_2O, 70°)	178.49	109.60	104.31	24.98
Methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranoside from 4 ($CDCl_3$)	170.05	106.06	103.23	23.49
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (5) (D_2O)	175.37	107.44	103.79	24.06
Barium salt of 5 (D_2O, 70°)		109.45	104.47	25.05
Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (D_2O)		112.93	103.96	28.38
				26.62
Major isomer of methyl 3,4-*O*-[1-(hydroxymethyl)ethylidene]-β-D-fucopyranoside (D_2O, 70°)		111.81	104.47	22.56
Methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-fucopyranoside ($CDCl_3$)	170.05	106.02	103.18	16.54
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside (8) (D_2O)	174.76	106.81	103.61	23.99
Barium salt of 8 (D_2O, 70°)	178.60	109.21	104.11	24.89
Minor isomer of methyl 3,4-*O*-[1-(hydroxymethyl)ethylidene]-β-D-fucopyranoside		111.64	104.48	24.15
Methyl 3,4-*O*-isopropylidene-β-D-fucopyranoside		111.93	104.50	28.86
				27.09
4,6-*O*-(1-Carboxyethylidene)-β-D-galactopyranoside (Ba salt; D_2O, 70°)	177.32	102.39	97.76	26.96
			94.43	
3,6-Anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose dimethyl acetal (D_2O)	175.03	100.46	105.74	26.12
			103.25	
Barium salt of foregoing (D_2O)	177.05	101.87	105.75	26.31
			103.21	
Methyl 4,6-*O*-isopropylidene-β-D-galactopyranoside (D_2O)		100.73	104.46	29.45
				19.05

RESULTS AND DISCUSSION

Carbon-13 nuclear magnetic resonance spectroscopy may be used to determine the ring size of cyclic acetals. Stoddart[1] reported that methylene acetals containing 5-, 6-, and 7-membered rings gave acetal carbon signals showing typical chemical shifts. Differences were reported between acetal resonances of 1,2-*O*-isopropylidene-ethanediol (δ_c 108.5), 1,3-*O*-isopropylidene-1,3-propanediol (δ_c 97.7), and 1,4-*O*-isopropylidene-1,4-butanediol (δ_c 100.9)[2]. Comparable values been observed for other isopropylidene derivatives, and have been used in assignment of ring size[3,4]. Five-membered *O*-[1-(hydroxymethyl)ethylidene] rings, having a non-protonated acetal carbon resonance at δ_c 110, are distinguishable from those of six-membered acetals[5] having δ_c 99.0–101.5. There is also a correlation between configuration and the ^{13}C shift of CH_3 resonances of certain 6-membered acetal groups, for example 4,6-*O*-[1-(acetoxymethyl)ethylidene], 4,6-*O*-(1-carboxyethylidene), and 4,6-*O*-[1-(methoxycarbonyl)ethylidene] derivatives of methyl galactopyranoside give signals at δ_c 24.7–26.0 when the methyl group is equatorial and δ_c 15.1–18.3 when it is axial[6] to the 1,3-dioxane ring. It was observed that differences of configuration in 3,4-*O*-[1-(acetoxymethyl)ethylidene] and 3,4-*O*-[1-(hydroxymethyl)ethylidene] derivatives causes only small differences in CH_3 shift[5,6]. These small differences have been attributed to conformational mobility of the ring and to the quasiequatorial and quasiaxial dispositions of the methyl groups[3].

The present study concerns the determination of various ^{13}C-n.m.r. and proton-n.m.r. spectral parameters of compounds structurally related to 3,4-*O*- and 4,6-*O*-(1-carboxyethylidene)-D-galactopyranosyl residues, which occur in certain polysaccharides[7–11]. These include chemical shifts (Tables I and II), spin–lattice relaxation times (Table III), nuclear Overhauser enhancements (Table IV), and, for a few signals, line broadening.

TABLE II

CHEMICAL SHIFTS OF METHYL ACETAL PROTONS IN PROTON N.M.R. SPECTRA OF 3,4-*O*-(1-CARBOXYETHYLIDENE) DERIVATIVES

Compound	*Chemical shifts of CH_3 of acetal, δ in p.p.m.*
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (6) (D_2O)	1.94
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (5) (D_2O)	2.04
Barium salt of 6 (D_2O)	1.87
Barium salt of 5 (D_2O)	1.97
Barium salt of 6 (D_2O, 70°)	1.97
Barium salt of 5 (D_2O, 70°)	2.07
Barium salt of pyruvylated galactan of *Pomacea lineata*	1.99

TABLE III

SPIN–LATTICE RELAXATION TIMES, T_1, OF CARBON ATOMS IN VARIOUS ACETALS

Compound	*Spin–lattice relaxation times, T_1, in s (chemical shifts, δ_C, in parentheses)*					
	Carbonyl	*Acetal nonprotonated carbon atom*	*Monoprotonated carbon atoms*	*C-6*	*OCH_3*	*Acetal CH_3*
Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (D_2O, 34°)		10.4 (112.03)	0.79–0.82 (73.94–103.96)	0.51 (62.02)	2.2 (58.16)	0.91, 0.68 (28.38) (26.62)
Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (D_2O, 52°)		16.8	1.4	0.85	3.4	1.2, 1.0
Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (D_2O, 70°)		23.3	2.0–2.1	1.2	5.1	1.7, 1.5
Methyl 6-*O*-β-D-galactopyranosyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (D_2O, 34°)		5.7 (112.10)	0.33–0.54 (69.80–103.96)	0.40 (62.04)	1.5 (58.39)	0.66, 0.51 (28.36) (26.63)
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (6, Ba salt, D_2O)	10.8 (178.41)	10.0 (109.04)	0.44–0.72 (72.12–103.68)	0.44 (62.09)	1.6 (58.20)	0.70 (24.53)
3,6-Anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl-L-galactose dimethyl acetal	5.2 (175.03)	6.8 (100.46)	0.32–0.44 (66.93–105.74)	0.11, 0.18 (74.11) (66.23)	1.6 (56.81) (56.10)	0.51 (26.12)
Ba salt of foregoing (D_2O)	7.7 (177.15)	5.8 (101.87)	0.22–0.34 (67.23–105.75)	0.11–0.22 (62.97) (74.11)	1.5, 1.4 (56.85) (56.10)	0.47 (26.31)
Methyl ester of foregoing (acetone-d_6)	7.7 (171.04)	6.5 (99.01)	0.22–0.38 (66.44–105.32)	0.11 (65.90, 74.56)	1.7, 1.1 (54.04) (52.59) (55.33)	0.57 (26.04)
Major isomer of methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-fucopyranoside ($CDCl_3$)	26.2 (170.05)	15.04 (105.87)	0.79–1.01 (68.52–103.14)	0.77 (16.47)	2.4, 3.0 (56.76) (52.73)	0.67 (23.46)
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside (8) (Ba salt, D_2O)	13.1 (178.48)	11.7 (108.66)	0.44–0.60 (69.92–103.48)	0.57 (16.87)	1.76 (58.12)	0.60 (24.54)

TABLE IV

N.O.e. VALUES OF CARBON ATOMS IN VARIOUS ACETALS

Compound	*N.O.e. values of carbon nuclei (chemical shifts, δ_c, of signals in parentheses)*					
	Carbonyl	*Acetal nonprotonated carbon atom*	*Monoprotonated carbon atoms*	*C-6*	*OCH_3*	*Acetal CH_3*
Methyl 4,6-*O*-isopropylidene-β-D-galactopyranoside (D_2O)		2.9 (100.73)	2.8–3.3 (67.34–104.46)	3.3 (63.39)	2.6 (58.17)	2.9, 2.8 (29.45) (19.05)
4,6-*O*-(1-Carboxyethylidene)-α, β-D-galactose (barium salt, D_2O)	1.2 (177.15)	— (101.86)	2.4–2.9 (66.15–97.18)	2.6 (63.20)		2.85 (26.40)
3,6-Anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose dimethyl acetal (barium salt, D_2O)	2.5 (177.15)	2.6 (101.87)	2.5–3.1 (67.23–105.75)	2.1, 2.6 (65.96) (74.11)	2.2, 2.1 (56.85) (56.85)	2.5 (26.31)
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**) (barium salt, D_2O)	1.4 (178.41)	2.3 (109.04)	2.6–3.1 (72.12–103.68)	3.1 (62.09)	2.5 (58.20)	2.6 (24.53)
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside (**8**) (barium salt, D_2O)	2.4 (178.48)	2.5 (108.66)	2.6–3.0 (69.92–103.48)	2.6 (16.87)	2.6 (58.12)	2.9 (24.54)
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**) (acid form, D_2O)	1.3 (174.66)	3.3 (107.22)	2.4–3.3 (71.89–103.68)	3.3 (61.92)	3.0 (58.12)	3.3 (23.95)
Methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside (**8**) (acid form, D_2O)	1.5 (176.76)	2.2 (106.81)	2.4–3.1 (69.56–103.61)	2.7 (16.70)	2.3 (58.07)	3.4 (23.99)

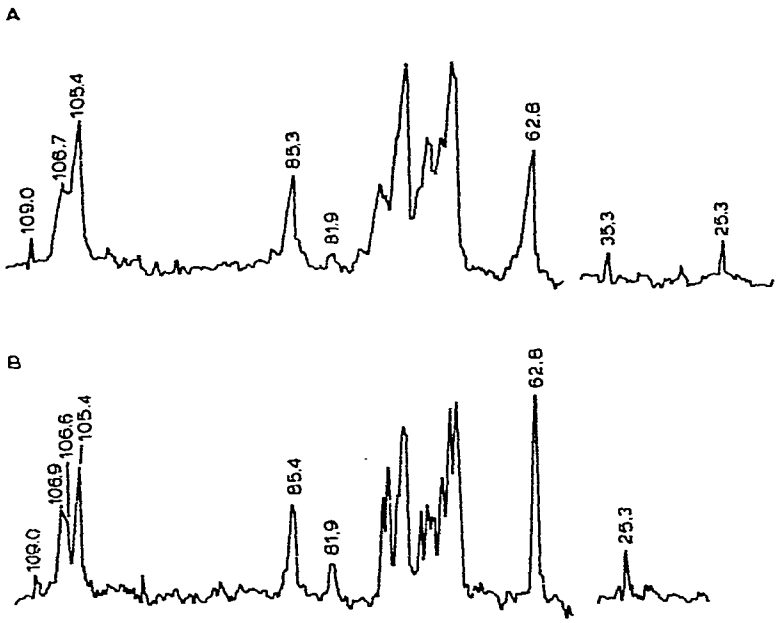
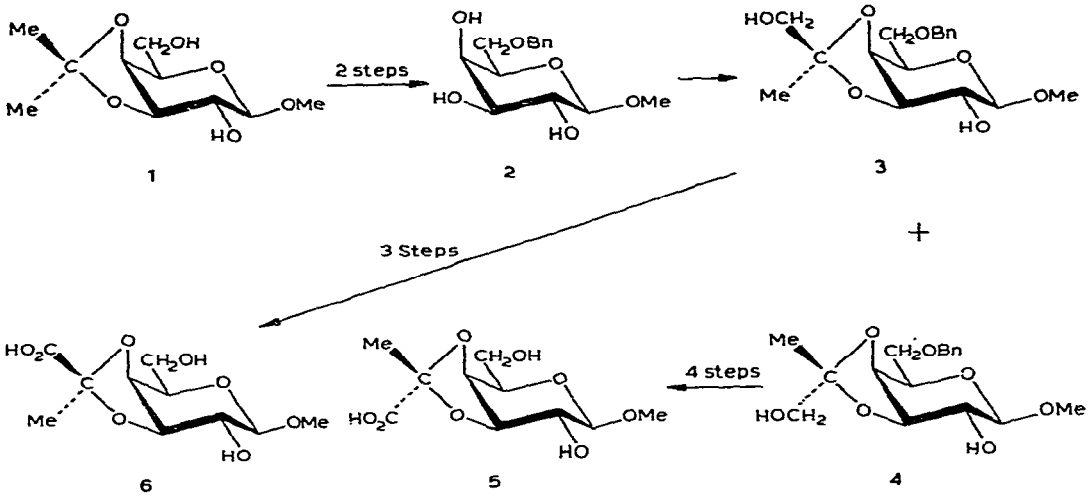


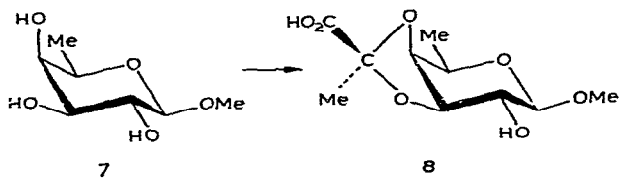
Fig. 1. ^{13}C-N.m.r. spectra of β-D-galactopyranan from *Pomacea lineata* (A) and material obtained by 2 successive Smith degradations (B). Obtained as D_2O solutions at 70°; numerical values are chemical shifts expressed as δ_c in p.p.m.

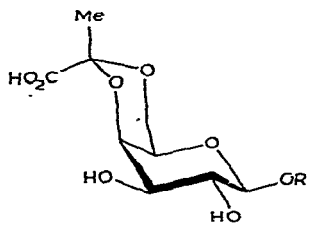
With the aid of chemical-shift values, it is possible to determine the position of substitution and configuration of pyruvic acid acetals in a β-D-galactopyranan from *Pomacea lineata* (formerly called *Ampullarius lineata*, SP1X, 1827). Characteristic of such substitution were signals of non-protonated acetal carbon atoms, C-3 of acetal-substituted β-D-galactopyranosyl units, and acetal CH_3 groups (Fig. 1, A). The C-3 and CH_3 signals became comparatively larger in the spectra of polysaccharides obtained on successive Smith degradations, incorporating mild hydrolytic conditions (for example Fig. 1,B).

The model compounds used in the spectral investigations and preparative routes are as follows. These include the 2 isomers (**5** and **6**) of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside. Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**1**) was selectively mono-*O*-benzylated with benzyl bromide and silver oxide in *N*,*N*-dimethylformamide[12] to give the 6-benzyl ether. This was partially hydrolyzed and the resulting methyl 6-*O*-benzyl-β-D-galactopyranoside (**2**) was treated with 2-oxopropyl acetate containing sulfuric acid to give the 2 isomeric 3,4-*O*-[1-(acetoxymethyl)ethylidene] derivatives. These were deacetylated, providing two methyl 6-*O*-benzyl-3,4-*O*-[1-(hydroxymethyl)ethylidene]-β-D-galactopyranosides (**3** and **4**), which were formed in a ratio of ~3.3:1 as indicated by the areas of CH_3 signals in the ^{13}C-n.m.r. spectrum at δ_c 21.94 and 23.60. According to the results of Garegg and colleagues[6], based on an unpublished crystallographic determination, the major signal at δ_c 21.94 having the lower shift-value should correspond to CH_3

of the 1-(hydroxymethyl)ethylidene group having the *endo*-hydroxymethyl configuration, as in 3. The major isomer was purified by crystallization and was oxidized with oxygen in the presence of platinum giving the sodium salt of the 3,4-*O*-(1-carboxyethylidene) derivative, which was converted into the acid form and thence with diazomethane into the methyl ester. This was purified by chromatography on a column of silicic acid. Hydrogenolysis with palladium on charcoal removed the *O*-benzyl group, providing the major isomer of methyl 3,4-*O*-[1-(methoxycarbonyl)-







R = 4-*O*-Substituted 3,6-anhydro-L-galactose dimethyl acetal

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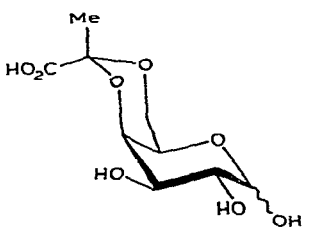

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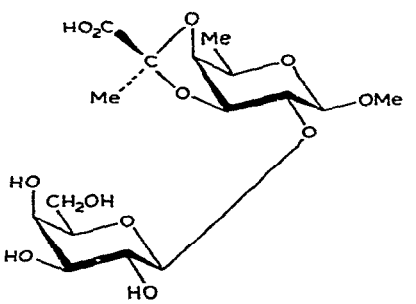

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ethylidene]-β-D-galactopyranoside. The methyl ester of the minor isomer was prepared starting from the mother liquor obtained from crystallization of **3**. A similar series of reactions was conducted, followed by column chromatography on silicic acid, of the mixture of methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranosides. Each methyl ester was converted into the barium salt of the 3,4-*O*-(1-carboxyethylidene) derivative by the action of cold, aqueous barium hydroxide and thence to the free acids **5** and **6** by using a cation-exchange resin.

A similar series of reactions was conducted starting from methyl β-D-fucopyranoside **7**. This was converted into methyl 3,4-*O*-[1-(hydroxymethyl)ethylidene]-β-D-fucopyranosides in a ratio of ~5:1, according to the ^{13}C-n.m.r. signals of acetal CH_3 groups at δ_c 22.56 and 24.15. Each isomer was obtained in crystalline and the major component was converted into methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-fucopyranoside, further transformed into its corresponding 3,4-*O*-(1-carboxyethylidene) derivative (barium salt) and free acid **8**.

The chemical shifts of the non-protonated acetal carbon atom of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6** and **5**) are δ_c 107.24 and 107.44, respectively, close to the value of 109 p.p.m. characteristic of 5-membered *O*-isopropylidene rings[3,4]. These shifts are pH-sensitive, being δ_c 109.07 and 108.92, respectively, for the barium salts. As would be expected, these signals are downfield from the C-2″ resonance of the 6-membered cyclic acetal of 3,6-anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose dimethyl acetal[7] (**9**) which lies at δ_c 100.44 (free acid) and at δ_c 101.71 (barium salt). Dependence on pH is observed with acetal CH_3 resonances of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranosides (**6** and **5**), which fall respectively (room temperature) at δ_c 23.97 and 24.06 (free acid) and at δ_c 24.55 and 24.58 (barium salt).

Shifts of CH_3 groups may be used to characterize the size of the acetal ring in polysaccharides. Thus, the barium salt of the pyruvylated galactan[13] of the albumen gland of a snail, *Pomacea lineata*, gives a CH_3 signal at δ_c 24.6, close to values of δ_c 24.6–25.2, reported for polysaccharides from bacteria that contain 5-membered (1-carboxyethylidene) groups[5]. These values, as would be expected, differ from those of 6-membered diastereomers of methyl 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside, which lie at[5,6] δ_c 17.2 and 26.1, that of δ_c 26.44 for 4,6-*O*-(1-carboxyethylidene)-α,β-D-galactose (**10**, barium salt), δ_c 27.1 for its parent polysaccharide, that of δ_c 26.26 for the barium salt of 3,6-anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose dimethyl acetal (**9**), and similar values for other structurally related polysaccharides. The small difference of 0.03 p.p.m. between the CH_3 resonances of diastereomers of the barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside is insufficient to allow the assignment of configuration of the acetal in the pyruvylated galactan of *Pomacea lineata*, already mentioned. In contrast, the C-3 signal is configurationally dependent, having δ_c 81.12 with **6** and δ_c 80.44 with **5**. As the C-3 (pyruvylated) signal of the galactan is at δ_c 81.9, the configuration of the acetal ring present in **6** is favored. This conclusion is confirmed using proton-n.m.r. spectroscopy.

The barium salt of compound **6** in D_2O at 70° gives a CH_3 signal at δ 1.97, whereas that of diastereomer **5** is at δ 2.07. The *O*-(1-carboxyethylidene) group in the galactan gives a CH_3 signal at δ 1.99, corresponding to the configuration of **6**, opposite to that reported in various bacterial polysaccharides[5]. (Shift values of these and related compounds are summarized in Table II). These signals are at lower field than the δ 1.41 reported for a 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside group in a polysaccharide[14] and other values of diastereomers of the 6-membered acetals[5,6].

Factors affecting signal shape in ^{13}C-n.m.r. spectra of O-(*1-carboxyethylidene*) *derivatives of galactopyranose and fucopyranose.* — 1. *Line broadening.* ^{13}C-N.m.r. spectra of the barium salts of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranosides (**6** and **5**), obtained with conventional acquisition-time, pulse width, and sweep width lacked signals corresponding to carbonyl and acetal non-protonated carbon atoms. Although this property arises mainly from expectedly high T_1 values (see later), an unusual broadening occurred of signals of non-protonated acetal carbon atoms in spectra of certain compounds. For example, methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**, barium salt) gave a spectrum showing a C-2′ signal at δ_c 109.6 having a wider line-width at half-height of 2.5 Hz than that of the mono-protonated carbon, which was 1.7 Hz wide. Generally, a non-protonated carbon atom has a longer T_2 value and therefore a narrower line-width than a mono-protonated carbon atom[15]. Increasing the temperature to 70° led to each signal having equal line-widths of 1.0 Hz, indicating that the signal broadening may be due to the presence of more than one conformer of the acetal rings and that interconversion is slow on the n.m.r. time-scale at room temperature, but faster at 70°.

The acid form of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**) also gives a ^{13}C-n.m.r. spectrum whose non-protonated acetal carbon resonance has a greater line-width (2.4 Hz) than those of the carbonyl carbon atom (0.6 Hz) and C-1 (1.3 Hz). Respective line-widths for the acid form of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside **8** are 3.9, 1.7, and 1.0 Hz. A similar broadening occurs with the C-2″ signal (δ_c 107.01, 6.0 Hz) of the acid form of methyl 3,4-*O*-(1-carboxyethylidene)-2-*O*-β-D-galactopyranosyl-β-D-fucopyranoside (**11**), which is wider than C-1 and C-1′ signals (δ_c 102.50, 101.90; line width, both 2.5 Hz).

Broadening was also observed with the C-2′ signal of 4,6-*O*-(1-carboxyethylidene)-α,β-D-galactose (**10**, barium salt). At room temperature, the observed line widths at half-height were 28 Hz (C-2′, δ_c 102.06) and 2.0 Hz (C-1, δ_c 97.20), whereas at 70° they were 16 and 2 Hz, respectively. However, in these spectra, the C-2′ signal is a composite arising from α and β anomers, thus leading to line-width estimates that are possibly too high. The aforementioned line-width phenomenon does not occur with such other *O*-(1-carboxyethylidene) derivatives as methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**5**, barium salt) and the barium salt and free acid forms of 3,6-anhydro-4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose dimethyl acetal (**9**).

2. T_1 values. These values were measured by the Freeman–Hill modification of

the inversion-recovery method[16] and, as expected, those obtained for the barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**) varied greatly depending on the carbon atom, being 11 s and 10 s for the carboxyl carbon and non-protonated acetal carbon atom, respectively, and 0.44–0.72 s for other carbon atoms, with the exception of the $-OCH_3$ carbon atoms. Although T_1 values were not obtained at 70° for this compound, a comparable T_1 value of 23 s for non-protonated carbon atoms was obtained with methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside. Other T_1 values are recorded for various acetals in Table III.

Increase in molecular weight leads to increased correlation times and lower T_1 values. This occurs on going from methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside, whose C-2′ and C-1 atoms, at room temperature, have T_1 values of 10.4 and 0.82 s, respectively, to methyl 6-*O*-β-D-galactopyranosyl-3,4-*O*-isopropylidene-β-D-galactopyranoside, whose corresponding values are 5.7 and 0.47 s. The T_1 values of methyl groups of acetals decrease from 0.91 and 0.68 s to 0.66 and 0.51 s. In the case of the ^{13}C-spectrum of the pyruvylated galactan[13], obtained at 70°, the carbonyl signal does not appear, and the acetal carbon signal at δ_c 109.0 is small in comparison with the acetal CH_3 signal at δ_c 25.3 and the C-3 signal of a pyruvylated β-D-galactopyranosyl residue at δ_c 81.9 (Fig. 1,A). It thus appears that the smallness of the non-protonated C-2′ signal is due to its comparatively long T_1 value.

3. *N.O.e. values.* Normally with small molecules, the increase in intensity of a ^{13}C resonance as a result of proton decoupling[17], namely the n.O.e. value, approaches the maximum[17,18] of 2.988. To attain the maximum value, ^{13}C relaxation must occur entirely by ^{13}C-H dipole–dipole interactions, and the effective correlation-times for rotational reorientation must be in a low range[17], typical of small molecules. N.O.e. values for carbon atoms in cholesteryl chloride and sucrose are ~3, but smaller values were obtained for C-4 and C-5 of adenosine 5′-monophosphate, indicating that relaxation mechanisms, other than dipole–dipole interactions, were important[17]. This appears to be a contributing factor for the decreased intensity of the C-1′ carbonyl signal of the barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**). N.O.e. values were determined by comparison of the signal-integral to noise ratio obtained in a conventional spectrum to one obtained by the anti-gated technique[18,20]. The C-1′ atom had an n.O.e. value of 1.4, lower than the 2.3–3.1 observed for other carbon atoms. In the case of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside (**8**, barium salt), the n.O.e. was higher for C-1′, being 2.4 (compared with 2.5–3.0 for other carbon atoms). Thus, low n.O.e. values may contribute to the smallness of carbonyl signals at C-1′, which is the carbon most remote from neighbouring protons, but have little effect on the size of the non-protonated acetal carbon at C-2′, which resembles other non-protonated carbon atoms whose n.O.e. is[17] ~3 (n.O.e. values are given in Table IV).

The ^{13}C-n.m.r. spectra of the acid forms of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (**6**) and methyl 3,4-*O*-(1-carboxyethylidene)-β-D-fucopyranoside (**8**) also displayed carbonyl resonances however low n.O.e. values of 1.3 and 1.5, respectively (Table IV).

EXPERIMENTAL

General methods. — ^{13}C-N.m.r. spectra were obtained by using a Varian XL-100 n.m.r. spectrometer incorporating Fourier transform. For T_1 and n.O.e. determinations, degassed 20% solutions were used. Carbon-13 chemical shifts are expressed as δ_c relative to external tetramethylsilane (Me_4Si), whose resonance was determined in a separate experiment as an offset from that of D_2O. Proton shifts are for D_2O solutions, being relative to external Me_4Si contained in a capillary. The values are ~0.41 (33°) and ~0.50 p.p.m. (70°) higher than those reported when sodium 2,2-dimethyl-2-silapentane-5-sulfonate is used as internal standard.

Methyl 3,4-O-[1-(hydroxymethyl)ethylidene]-β-D-fucopyranosides. — Methyl β-D-fucopyranoside (7, 7.5 g) was shaken in 2-oxopropyl acetate[21] (75 mL) containing sulfuric acid (0.3 mL) and Baker 3 Å molecular sieve (20 g). The sugar completely dissolved after 2 h. The mixture was then added to aqueous sodium hydrogencarbonate containing ice, which was then extracted with ethyl acetate. The extract was evaporated to a syrup that was dissolved in 0.1M methanolic sodium methoxide (30 mL) which, after 30 min, was evaporated and the resulting residue deionized with Amberlite IR-120 (H^+) and Dowex 1-XB (HCO_3^-) resins. The product crystallized from ethyl acetate, providing the major isomer of methyl 3,4-*O*-[1-(hydroxymethyl)-ethylidene]-β-D-fucopyranoside, (1.87 g), m.p. 175–178°, $[\alpha]_D^{25}$ +13° (*c* 0.6 methanol); ^{13}C-n.m.r. (D_2O, 70°): δ_c 111.81, 104.47, 80.66, 78.08, 74.16, 70.42, 67.59, 58.46, 22.56, and 17.19.

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.75. Found: C, 51.11; H, 7.76.

The mother liquor of the foregoing crystallization was examined by t.l.c. on silica gel (solvent: chloroform–ethanol, 9:1 v/v), which showed the presence of the previously crystallized product (R_F 0.3) and slower-moving material (R_F 0.25). Column chromatography on silicic acid (eluant: 50:1 (v/v) chloroform–methanol) provided more of the foregoing material (0.16 g from ethyl acetate) and the minor isomer of methyl 3,4-*O*-[1-hydroxymethyl)ethylidene]-β-D-fucopyranoside (0.25 g from ethyl acetate), m.p. 131–133°, $[\alpha]_D^{25}$ +17° (*c* 0.7, water); ^{13}C-n.m.r. (D_2O, 70°): δ_c 111.64, 104.48, 80.97, 79.15, 74.50, 70.43, 66.85, 58.50, 24.15, and 17.10. Comparison of the CH_3 signals at δ_c 22.56 and 24.15 in the original mixture of isomers formed in the reaction with 2-oxopropyl acetate showed that they were present in a ratio of ~5:1.

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.75. Found: C, 51.27; H, 7.70.

Methyl 3,4-O-[1-(methoxycarbonyl)ethylidene]-β-D-fucopyranoside. — A solution of the major methyl 3,4-O-[1-(hydroxymethyl)ethylidene]-β-D-fucopyranoside (1.50 g) in water (10 mL) was added to a wet preparation of platinum, freshly prepared by hydrogenation of Adams' platinum oxide catalyst (1.50 g). The mixture was maintained at 90° and oxygen bubbled through with sufficient force to keep the catalyst suspended. A solution of sodium hydrogencarbonate (0.59 g) in water (18 mL) was added to the mixture in portions of 3 mL each h. After 8 h, the solution was treated with Amberlite IR-120 (H^+), filtered, and lyophilized. The resulting

O-(1-carboxyethylidene) derivative was esterified with ethereal diazomethane. Examination by t.l.c. [silica gel, 12:1 (v/v) chloroform–ethanol] showed a trace of starting material (R_F 0.3) and the title compound (R_F 0.8). Column chromatography on silicic acid (chloroform) provided the required material (0.95 g) as a syrup, $[\alpha]_D^{25}$ +3° (*c* 1.0, methanol); ^{13}C-n.m.r. ($CDCl_3$): δ_c 107.05, 105.87, 103.14, 80.43, 77.18; 77.10, 68.52; 56.76, 52.73, 23.46, and 16.47.

Anal. Calc. for $C_{11}H_{18}O_7$: C, 50.37; H, 6.92. Found: C, 50.21; H, 6.89.

Methyl 3,4-O-(1-carboxyethylidene)-β-D-fucopyranoside: free acid **8** *and barium salt, derived from the major* O-*(1-hydroxymethyl)ethylidene derivative.* — Methyl 3,4-*O*-[1-(*methoxycarbonyl)ethylidene*]-β-D-fucopyranoside, derived from the major 3,4-[1-hydroxymethyl)ethylidene] acetate, was dissolved in water containing an excess of barium hydroxide and, after 18 h at room temperature, Dry Ice was added, and the mixture was filtered and the filtrate evaporated. The residue was extracted with water and the mixture filtered and the filtrate re-evaporated. The resulting barium salt of **8** had $[\alpha]_D^{25}$ −9° (*c* 0.3, water); ^{13}C-n.m.r. (D_2O): δ_c 178.49, 108.66, 103.48, 80.51, 78.09, 72.00, 69.92, 58.12, 24.54, and 16.89; in D_2O at 70°: δ_c 178.60, 109.21, 104.11, 81.03, 78.52, 72.53, 70.39, 58.47, 24.89, and 17.31.

The barium salt was converted into the free acid **8** having $[\alpha]_D^{25}$ −8° (*c* 0.3, water) by treatment in water with Amberlite IR-120 (H^+) followed by filtration and lyophilization, ^{13}C-n.m.r. (D_2O): δ_c 174.76, 106.81, 103.61, 81.13, 78.67, 71.78, 69.56, 58.07, 23.99, and 16.70.

Anal. Calc. for $C_{10}H_{16}O_7$: C, 48.38; H, 6.50. Found: C, 48.27; H, 6.31.

Methyl 6-O-benzyl-β-D-galactopyranoside (**2**). — Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**1**, 8.9 g) was partly *O*-benzylated in *N,N'*-dimethylformamide (30 mL) containing silver oxide (18.0 g), which was shaken and to which 1.3 molar equivalents of benzyl bromide (5.8 mL) was added. After 18 h, the mixture was diluted with dichloromethane, the mixture filtered, and the filtrate evaporated to a syrup that was treated in 80% aqueous acetic acid (100 mL) for 1 h at 100° in order to remove the *O*-isopropylidene group. The product, on examination by t.l.c. [silica gel, 9:1 (v/v) chloroform–water] showed 3 spots having R_F 0.0, 0.25, and 0.6. It was partitioned between benzene and water, and the aqueous layer found to contain materials corresponding to spots having R_F 0.0 and 0.25. This material, following evaporation, was dissolved in ethyl acetate (100 mL), ether (100 mL) was then added, and the solution was kept for 24 h at 4°. The mother liquor was decanted from the precipitate and evaporated to a syrup that crystallized from 1:1 ethyl acetate–hexane (150 mL) providing compound **2** (3.5 g), m.p. 104°, $[\alpha]_D^{25}$ −15° (*c* 1.2, ethanol); ^{13}C-n.m.r. ($CDCl_3$): δ_c 138.10, 128.50, 128.06, 127.78, 104.18, 73.72 (2 carbons), 73.68 (2 carbons), 71.51, 69.43, 69.29, and 57.15.

Anal. Calc. for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 58.93; H, 7.11.

The material having R_F 0.6 just described was methyl 2,6-di-*O*-benzyl-β-D-galactopyranoside, and from ether–hexane it had m.p. 81–82°, $[\alpha]_D^{25}$ −13° (*c* 0.6, ethanol); yield 1.6 g.

Anal. Calc. for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.47; H, 6.81.

Methyl 6-O-benzyl-3,4-O-[1-(hydroxymethyl)ethylidene]-β-D-galactopyranoside (**3**). — Compound **2** (4.2 g) was converted into its 3,4-[1-(hydroxymethyl)ethylidene acetals by the method already outlined in the fucopyranoside series. The product contained 2 isomers, which on t.l.c. [silica gel, 9:1 (v/v) chloroform–ethanol] had R_F 0.55 (major, **3**) and 0.50 (minor, **4**). According to the intensities of ^{13}C-n.m.r. CH_3 signals (D_2O) at δ_c 21.94 and 23.60, they were present in the ratio of ~3.3:1. Crystallization from ether gave the major isomer **3** (1.20 g), m.p. 116°, $[\alpha]_D^{25}$ −13° (*c* 0.6, ethanol); ^{13}C-n.m.r. (D_2O): δ_c 138.32, 129.92, 129.71, 129.58, 111.88, 103.86, 80.07, 75.07, 74.28, 74.72, 73.72, 72.63, 70.01, 66.70, 58.19, and 21.94. The material (1.13 g) in the mother liquor was recovered.

Anal. Calc. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.73; H, 6.97.

Methyl 3,4-O-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranosides. — The major product from the preceding experiment (**3**, 0.90 g) was successively oxidized with Pt/O_2 and esterified with diazomethane as described previously. The silicic acid chromatography procedure provided a fraction (0.37 g) corresponding to methyl 6-*O*-benzyl-3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranoside, which was hydrogenolyzed with 5% palladium-on-charcoal in acetic acid. The mixture was filtered and the filtrate lyophilized giving methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranoside (from ethyl acetate–ether), m.p. 152–153°, $[\alpha]_D^{25}$ −8° (*c* 1.0, methanol); yield 0.25 g; ^{13}C-n.m.r. ($CDCl_3$): δ_c 169.96, 106.47, 103.31, 80.56, 74.82, 73.18, 71.41, 62.26, 57.20, 52.95, and 23.60.

Anal. Calc. for $C_{11}H_{18}O_8$: C, 47.48; H, 6.52. Found: C, 47.65; H, 6.59.

The mother liquor from the preparation of crystalline compound **3** contained the minor isomer (**4**), and the syrupy mixture (1.13 g) was oxidized with Pt/O_2 and then converted into its methyl ester. The product was partitioned between chloroform and water and the material present in the chloroform layer was hydrogenolyzed with 5% palladium-on-charcoal in acetic acid. T.l.c. [silica gel, 9:1 (v/v) chloroform–water] showed the presence of three components having R_F 0.50, 0.55, and 0.60. Column chromatography on silicic acid [50:1 (v/v) chloroform–methanol] provided the component having R_F 0.60, which was the other isomer of methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-galactopyranoside which, from ether–hexane, had m.p. 147–149°, $[\alpha]_D^{25}$ −13° (*c* 0.5, methanol); yield 0.07 g; ^{13}C-n.m.r. ($CDCl_3$): δ_c 170.05, 106.96, 103.23, 79.26, 75.82, 74.06, 73.24, 62.12, 57.21, 52.64, and 23.49.

Anal. Calc. for $C_{11}H_{18}O_8$: C, 47.48; H, 6.52. Found: C, 47.74; H, 6.47.

Methyl 3,4-O-(1-carboxyethylidene)-β-D-galactopyranoside (**6**), *free acid and barium salt, derived from the major isomer* **3**. — The barium salt of **6**, $[\alpha]_D^{25}$ −6° (*c* 0.3, water), was prepared by the action of aqueous barium hydroxide on the 3,4-*O*-[1-(methoxycarbonyl)ethylidene] derivative prepared from **3**; ^{13}C-n.m.r. (D_2O): δ_c 178.40, 109.07, 103.71, 80.63, 75.45, 74.16, 72.15, 62.12, 58.22, and 24.55; (D_2O, 70°): δ_c 178.49, 109.60, 104.31, 81.12, 76.00, 74.62, 72.63, 63.64, 58.56, and 24.98. The acid from **6** had $[\alpha]_D^{25}$ −5° (*c* 0.3, water); ^{13}C-n.m.r. (D_2O): δ_c 174.41, 107.24, 103.68, 80.42, 74.26, 74.04, 71.92, 61.94, 58.13, and 24.5.

Anal. Calc. for $C_{10}H_{16}O_8$: C, 45.45; H, 6.10. Found: C, 45.13; H, 6.34.

Methyl 3,4-O-(1-carboxyethylidene)-β-D-galactopyranoside (**5**), *free acid and barium salt derived from the minor isomer* **4**. — The barium salt of **5** had $[\alpha]_D^{25}$ −6° (*c* 0.3, water); ^{13}C-n.m.r. (D_2O): δ_c 178.84, 108.92, 103.85, 79.88, 76.00, 74.54, 74.17, 61.94, 58.15, and 24.58 (D_2O, 70°): δ_c 109.45, 104.47, 80.44, 76.46, 74.93, 74.71, 62.50, 58.54, and 25.05. The free acid had $[\alpha]_D^{25}$ −10° (*c* 0.2, water); ^{13}C-n.m.r. (D_2O): δ_c 175.37, 107.44, 103.79, 80.40, 76.64, 74.30, 74.01, 61.88, 68.20, and 24.06.

Anal. Calc. for $C_{10}H_{16}O_8$: C, 45.45; H, 6.10. Found: C, 45.09; H, 6.43.

Methyl 6-O-β-D-galactopyranosyl-3,4-O-isopropylidene-β-D-galactopyranoside. — 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (0.50 g) was added in five portions to a shaken mixture of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (0.50 g) in dichloromethane (10 mL) containing silver carbonate (2.5 g) and Baker 3 Å molecular sieve (5 g). After 2 h, the mixture was diluted with dichloromethane, which was filtered and the filtrate evaporated to a syrup. The product was deacetylated with methanolic sodium methoxide and after 30 min the solution was made neutral with carbon dioxide, evaporated, and the resulting residue deionized in water with ion-exchange resins. The material contained galactose and the required substance, with a mobility equal to that of rhamnose on a paper chromatogram [40:11:19 (v/v/v) 1-butanol–ethanol–water], column chromatography on cellulose [10:1 (v/v) acetone–water] provided methyl 6-*O*-β-D-galactopyranosyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (0.41 g), $[\alpha]_D^{25}$ +20° (*c* 0.5, water); ^{13}C-n.m.r. (D_2O): δ_c 112.10, 104.55, 103.96, 79.94, 76.31, 75.08, 73.88, 73.83, 73.20, 71.90, 69.93, 69.80, 62.04, 58.39, 28.36, and 26.63.

Anal. Calc. for $C_{16}H_{28}O_{11}$: C, 48.48; H, 7.12; Found: C, 48.23; H, 7.01.

Methyl 2-O-β-D-galactopyranosyl-3,4-O-[1-(methoxycarbonyl)ethylidene]-β-D-fucopyranoside (**11**). — 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.5 g) was condensed with methyl 3,4-*O*-[1-(methoxycarbonyl)ethylidene)-β-D-fucopyranoside (0.35 g) by the procedure already described. After treatment with diazomethane, the product was examined by t.l.c. on silica gel [4:1 (v/v) chloroform–ethanol), and spots having R_F 0.8 (starting ester) and R_F 0.15 (product) were detected. Column chromatography on cellulose [solvents: benzene–methanol, 10:1, 4:1, and 3:1 (v/v), successively] provided methyl 2-*O*-β-D-galactopyranosyl-3,4-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-fucopyranoside (136 mg), $[\alpha]_D^{25}$ +4° (*c* 0.3, methanol).

Anal. Calc. for $C_{17}H_{28}O_{12}$: C, 48.11; H, 6.65. Found: C, 48.43; H, 6.83.

The barium salt of the 3,4-*O*-(1-carboxyethylidene) derivative **11** was prepared by alkaline hydrolysis; ^{13}C-n.m.r. (D_2O): δ_c 177.31, 108.41, 102.74, 101.95, 80.46, 78.37, 77.43, 76.10, 73.92, 72.12, 69.91, 62.11, 57.64, 24.65, and 16.79; (D_2O, 70°): δ_c 108.98, 103.26, 102.73, 80.83, 78.73, 78.19, 76.52, 74.59, 71.82, 70.50, 62.59, 58.03, 25.06, and 17.23.

The derived free acid **11** had $[\alpha]_D^{25}$ −7° (*c* 0.3, water); ^{13}C-n.m.r. (D_2O): δ_c 174.80, 107.01, 102.50, 101.90, 81.23, 78.92, 76.70, 76.20, 73.93, 71.98, 69.81, 69.64, 62.02, 57.69, 24.09, and 16.67.

Methyl 4,6-O-isopropylidene-β-D-galactopyranoside. — Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (4.0 g) was partially hydrolyzed in 80%

aqueous acetic acid (80 mL) for 1 h at 100° and the solution evaporated. The residue was shaken with acetone (200 mL) containing sulfuric acid (0.2 mL) and anhydrous copper sulfate (50 g) for 2 h, pyridine (10 mL) then added and the solution filtered and evaporated. The product was deacetylated in 0.1M methanolic sodium methoxide (20 mL) and the solution was evaporated and the residue deionized in aqueous solution with ion-exchange resins. The products contained methyl β-D-galactopyranoside as a contaminant of its 4,6-isopropylidene acetal and the mixture was chromatographed on a column of silicic acid [eluant: 25:1 (v/v) chloroform–methanol]. The resulting methyl 4,6-*O*-isopropylidene-β-D-galactopyranoside, from ethyl acetate–ether, had m.p. 161–163°, $[\alpha]_D^{25}$ −17° (*c* 0.7, ethanol); yield 56%; ^{13}C-n.m.r. (D_2O): δ_c 104.46, 100.73, 72.63, 71.43, 69.58, 67.34, 63.39, 58.17, 29.45, and 19.05.

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.55. Found: C, 51.27; H, 7.65.

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