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PROTON AND CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES ON METHYL (METHYL D-GALACTOSID)URONATES AND THEIR PER-*O*-ACETYL DERIVATIVES

BETTY MATSUHIRO, ALBERTO B. ZANLUNGO,
Departamento de Química, Facultad de Ciencia, Universidad Técnica del Estado, Av. Ecuador 3469, Santiago (Chile)

AND GUY G. S. DUTTON
Department of Chemistry, The University of British Columbia, Vancouver, B.C. V6T 1Y6 (Canada)

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ABSTRACT

The ^{1}H- and ^{13}C-n.m.r. spectra of the anomeric methyl (methyl D-galactosid)-uronates, as well as the ^{1}H-n.m.r. spectra of their acetyl derivatives, were analyzed. The spectra of the unacetylated D-galactopyranosiduronates showed good correlation with those of the corresponding anomeric D-galactopyranuronic acids and their methyl esters, and with those of the anomeric methyl D-galactopyranosides. From the values of the chemical shifts and coupling constants, it was concluded that the anomeric methyl (methyl D-galactopyranosid)uronates and their corresponding peracetates are in the $^4C_1$(D) conformation. The chemical shifts in the ^{13}C-n.m.r. spectra show good correlation with those of the methyl D-galactosides. The signals of the furanose derivatives appear at fields lower than those of the corresponding pyranose compounds.

INTRODUCTION

D-Galacturonic acid is widely distributed among homo- and hetero-polysaccharides, and may occur either as the free acid or as the methyl ester. This acid is, for example, present in pectins[1], and certain gums and mucilages[2], and is a component of ~10% of the capsular polysaccharides of the genus *Klebsiella*, where it replaces the more common D-glucuronic acid[3]. Despite this, there have been few n.m.r. studies on D-galacturonic acid and its derivatives. Schmidt and Neukom[4] discussed certain p.m.r. parameters for the anomeric methyl (methyl D-galacto-furanosid)uronates (**5** and **7**), and Tjan *et al.*[5] published the p.m.r. spectra of D-galacturonic acid and its methyl ester, together with those of their polymer-homologous series.

We now report data obtained from the ^{1}H- and ^{13}C-n.m.r. spectra of the anomeric methyl (methyl D-galactopyranosid)uronates (**1** and **3**), their peracetates (**2** and **4**), and the corresponding furanosides (**5** and **7**) and their acetates (**6** and **8**).

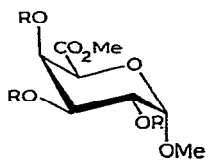
Whereas D-galacturonic acid has not been found in Nature in the furanose form, the corresponding, neutral hexose has been so encountered, *e.g.*, in *Klebsiella* serotypes K12 (ref. 6) and K41 (ref. 7), and the latter may be converted into the uronic acid by oxidation, as has been achieved by Aspinall and colleagues[8].

TABLE I

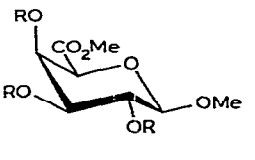
CHEMICAL SHIFTS (δ) FOR COMPOUNDS 1–8[a]

Protons	*Compound*							
	1	3	5	7	2	4	6	8
-OCOCH_3					1.96 2.05 2.07	1.97 2.03 2.08	2.10 2.14 2.22	2.07 2.07 2.18
-OCH_3	3.42	3.59	3.43	3.40	3.42	3.54	3.40	3.32
-COOCH_3	3.83	3.83	3.81	3.83	3.72	3.72	3.78	3.76
H-1	4.92	4.37	4.87	5.01	5.14	4.42	5.05	4.93
H-2	3.84[b]	3.55[b]	4.15	4.07	5.22[b]	5.22[b]	5.09	5.04
H-3	3.90[b]	3.75[b]	4.29	4.20	5.40[b]	5.06[b]	5.57	5.00
H-4	4.35	4.28	4.13	4.32	5.75	5.68	4.38	4.48
H-5	4.65	4.48	4.38	4.53	4.57	4.31	5.27	5.38

[a]Compounds **1**, **3**, **5**, and **7** in deuterium oxide, and compounds **2**, **4**, **6**, and **8** in chloroform-*d*. Chemical shifts of compounds **1**, **2**, **6**, and **7** were measured at 220 MHz; those of compounds **3** and **4** were measured from their 100-MHz spectra; and those of compounds **5** and **8**, at 270 MHz. A spectrum of compound **1** was also recorded at 400 MHz. [b]Calculated as the AB part of an XABM subsystem.



1 R = H
2 R = Ac



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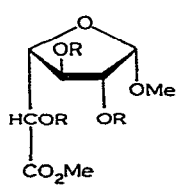

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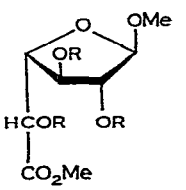
6 R = Ac



7 R = H
8 R = Ac

RESULTS AND DISCUSSION

The chemical shifts of the anomeric methoxyl group (see Table I) in compounds **1–5** and **7** are in agreement with those reported for methyl glycopyranosides[9,10] (axial: δ 3.30–3.48, equatorial: 3.50–3.64) and for methyl glycofuranosides[11] (α: δ 3.40–3.49, β: 3.37–3.48). For the acetates **6** and **8**, the signal for **6** is at δ 3.40, at the limit of the range, and, for **8** it is at δ 3.32, a little below the usual value.

The change in the chemical shift of a particular proton on going from compound **1** to compound **3** ($\alpha \rightarrow \beta$) is always positive (H-1: +0.55; H-2: +0.29; H-3: +0.15; H-4: +0.07; H-5: +0.17 p.p.m.), and these values agree well with those obtained from the anomeric D-galactopyranoses[12,13] and the anomeric D-galactopyranuronic acids[5,12]. Due to the similarity in structure of **1** to that of α-D-galactopyranuronic acid, and its methyl ester, and of **3** to the β anomers, the chemical shifts of the same ring-protons in these compounds are very close, with the sole exception of H-1. Likewise, compounds **1** and **3** are closely related to the anomeric methyl galactopyranosides, and, again, the differences in the chemical shifts of H-1, H-2, and H-3 between these pairs are only 0.03–0.10 p.p.m.. The relative values of the chemical shifts of the ring protons of compounds **1** and **3** correspond with those expected for monosaccharides having the D-*galacto* configuration in the $^4C_1$(D) conformation.

More-significant changes are to be seen in the signal of H-5, which, in the spectrum of compound **3**, is situated at the lowest field, and, in those of compounds **1**, **5**, and **7**, H-5 is the second-lowest field-signal, after H-1. Similar low-field values for H-5 have been reported for uronic and glycosiduronic acids[14,15], the reason being the greater deshielding effect of the carboxyl group in relation to the hydroxymethyl group, when bonded to C-5. Literature data[16] on several aliphatic compounds show that the deshielding effect of carboxyl and methoxycarbonyl groups are similar, even though the former always bring the α-proton signal to a slightly lower field.

TABLE II

$^3J_{H,H}$ VALUES (Hz) OF COMPOUNDS **1–8**[a]

Protons	*Coupling constants*	*Compound*							
		1	**3**	**5**	**7**	**2**	**4**	**6**	**8**
H-1	$J_{1,2}$	3.5(d)	7.5(d)	4.5(d)	2.2(d)	3.5(d)	7.2(d)	4.5(d)	<1.0(d)
H-2	$J_{2,3}$	10.0[b]	10.2[b]	7.8(dd)	4.0(dd)	10.7[b]	10.4[b]	7.2(dd)	2.2(d)
H-3	$J_{3,4}$	3.0[b]	3.2[b]	7.8(t)	6.0(dd)	3.4[b]	3.2[b]	6.5(dd)	10.8(dd)
H-4	$J_{4,5}$	1.5(dd)	1.5(dd)	3.6(dd)	2.7(dd)	1.5(dd)	1.4(dd)	3.9(dd)	5.7(dd)
H-5	$J_{5,4}$	1.5(d)	1.5(d)	3.6(d)	2.7(d)	1.5(d)	1.4(d)	3.9(d)	5.7(d)

[a]Compounds **1**, **3**, **5**, and **7** in deuterium oxide, and compounds **2**, **4**, **6**, and **8** in chloroform-*d*.
[b]AB part of an XABM subsystem.

The values of the coupling constants are reported in Table II, and, as with the chemical shifts, the values for the ring protons in compounds **1** and **3** are in very good agreement with the $^4C_1$(D) conformation.

The magnitude of the $J_{4,5}$ value for compounds **1** and **3** is similar to that of those for the same coupling in galactopyranuronic acid (1.5–1.6 Hz), and quite close to the somewhat lower $J_{4,5}$ value for the anomeric methyl D-galactopyranosides, as well as the D-galactopyranoses[13]. The relatively low value of $J_{4,5}$ for all of these compounds may be explained through the effect of the joint, antiperiplanar arrangement of the hydroxyl group on C-4 and H-5 (ref. 17), and of the ring-oxygen atom and H-4 (ref. 18). In fact, for a similar relationship between H-1 and H-2 in pyranoses, the reported value[19] of $J_{1,2}$ is 1.0–1.5 Hz. Izumi[12] explained these results by assuming that, for galactopyranoses and their derivatives, the dihedral angle defined by H-4 and H-5 is much larger than that between H-3 and H-4 ($J_{3,4}$ 3.2–3.7 Hz), causing a distortion of the chair conformation.

For the anomeric furanosides **5** and **7**, it is found that $J_{1,2}$ (see Table II) agrees with the values reported for methyl glycosides having a *cis* ($J_{1,2}$ 3.4–4.2 Hz), and a *trans* ($J_{1,2}$ 0–2 Hz) relationship between these protons[11,20].

The chemical shifts of the ring protons of the acetylated derivatives **2** and **4** (see Table I) are those expected on the basis of (*a*) their $^4C_1$ conformations, and (*b*) the shifts to lower field produced by acetylation of the hydroxyl groups on C-2, C-3, and C-4. The shifts for the H-2, H-3, and H-4 resonances lie between 1.31 and 1.67 p.p.m., whereas that of H-1 has a much lower value (0.05–0.22 p.p.m.), and the resonance signal of H-5 moves upfield (0.08–0.17 p.p.m.).

TABLE III

ASSIGNMENTS OF SIGNALS IN ^{13}C-N.M.R. SPECTRA OF COMPOUNDS **1**, **3**, **5**, AND **7**

Compound	*Chemical shifts*[a]							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CO_2CH_3	OCH_3
1	100.43	68.46	69.62	70.88	71.34	172.02	53.63	56.35
(*α*)	(−0.07)	(−0.94) [+0.24][b]	(−0.98) [−2.26][b]	(+0.48)	(−0.58)			(+0.05)
3	104.23	70.88	73.01	70.41	74.88	171.25	53.56	57.10
(*β*)	(−0.67)	(−0.92)	(−0.89)	(+0.61)	(−1.32)			(−1.2)
5	102.85	76.88	74.17	82.60	71.17	174.73	53.74	56.35
(*α*)	(−0.25)	(−0.52)	(−1.33)	(+0.30)	(−2.53)			(+0.25)
7	109.27	81.65	76.71	84.27	70.13	174.27	53.48	55.71
(*β*)	(−0.03)	(−0.25)	(−1.09) +6.47][c]	(+0.27) [−6.29][c]	(−1.87)			(−0.39)

[a]Chemical shifts, and the figures in parentheses, which represent the difference of the shift of a given signal from that of the corresponding methyl galactoside, are in p.p.m. [b]The values in square brackets are the differences that arise if the assignments of C-2 and C-3 are reversed. [c]As in footnote *b*, but for reversal of C-3 and C-4.

For compound **2**, the 100-MHz spectrum shows the signal for H-3 as a complex multiplet, due to virtual coupling[21] with H-1. This signal changes into the B part of an MBAX subsystem, when the spectrum is recorded at 220 MHz.

It is interesting that, in analogy with the case of compound **2**, the 100-MHz spectrum of compound **6** shows for H-3 a complex multiplet that is due to virtual, long-range coupling with H-1. In the 220-MHz spectrum, H-3 appears as a doublet of doublets which is amenable to first-order analysis.

Spin-decoupling studies were performed on compounds **1**, **2**, **6** and **8**, in order to confirm the assignments made.

The chemical shifts of the ^{13}C signals of compounds **1–8** are presented in Table III. The low-field signal in the spectrum of each compound is readily assignable to C-1 nuclei. For each furanose derivative, the signal is located downfield from that of the configurationally related pyranose derivative. This characteristic was pointed out in earlier ^{13}C-n.m.r. studies[22–25] of furanoses. Perlin[23] showed that each ^{13}C nucleus of a methyl furanoside is less shielded by an average of 3 to 5 p.p.m., in relation to the corresponding methyl pyranoside.

Small shift-differences (0.03–0.67 p.p.m.) were observed for the anomeric carbon nuclei of methyl (methyl D-galactosid)uronates, compared with the published data[26] for the corresponding methyl galactosides.

Assignments for the C-2 to C-5 nuclei of **1** and **3** are based on the data reported by Gorin and Mazurek[26] for the derivatives of D-glucuronic acid. Replacement of CH_2OH by CO_2CH_3 on C-5 causes shielding of C-5 (0.92 and 1.32 p.p.m.) comparable with the value (0.8 and 1.3 p.p.m.) found for methyl (methyl α-D-glucopyranosid)-uronate and its anomer. Downfield shifts (0.94 and 0.61 p.p.m., respectively) for C-4 are produced for compounds **1** and **3**. The downfield displacement in these cases is less than the value observed for the aldopyranose derivatives (1.8 and 1.5 p.p.m.) that bear an equatorial instead of an axial hydroxyl group.

These assignments were confirmed by selective, proton-decoupling techniques. For compound **1**, selective irradiation of H-4 and H-5 confirmed the assignments made for C-4 and C-5. Irradiation of H-2 and H-3 was conducted simultaneously, as, at 80 MHz, the signals of these protons are very close. Under these conditions, two singlets previously assigned to C-2 and C-3 appeared in the ^{13}C-n.m.r. spectrum. The signal at 69.62 p.p.m. was definitely assigned to C-3, and that at 68.46 p.p.m. to C-2, on the assumption that the difference ($\Delta\delta$) between the corresponding signal of methyl α-D-galactopyranoside fits the general trend. If the assignments are reversed, values of $\Delta\delta = +0.24$ p.p.m. for C-2, and $\Delta\delta = -2.26$ p.p.m. for C-3, are obtained, which are not in accordance with the values for $\Delta\delta$ reported by Gorin and Mazurek, and also found in this work.

For compound **3**, selective irradiations of H-3, H-4, and H-5 confirmed the assignments for C-2, C-3, C-4, and C-5. For compound **7**, the C-2, C-3, C-4, and C-5 signals follow the same trend as found with methyl α-D-galactofuranoside. Selective irradiation of H-2 and H-5, and simultaneous irradiation of signals due to H-3 and H-4 (which are close at 80 MHz), confirmed these assignments.

Comparison of the chemical shifts of the C-2 to C-5 nuclei of **5** with those of methyl α-D-galactofuranoside[26] showed that replacement of CH_2OH by CO_2H on C-5 produces an upfield displacement of 2.53 p.p.m. for C-5, but no marked difference in the shift was observed for C-4. For C-3, the upfield shift (1.33 p.p.m.) is more pronounced than with the configurationally related pyranoside **1**. No selective, irradiation experiments were performed on this compound.

EXPERIMENTAL

Compounds **1**, **3**, **5**, *and* **7**. — A solution of dry D-galacturonic acid (11.0 g) in anhydrous methanol (800 mL) containing 1% of dry hydrogen chloride was boiled under reflux for 18 h, cooled, and made neutral with lead carbonate. The suspension was filtered, the filtrate evaporated *in vacuo*, and the resulting syrup dissolved in boiling ethanol, to give crystals on cooling; these were recrystallized from ethyl acetate, affording plates (2.17 g) of methyl (methyl α-D-galactopyranosid)uronate, **1**, m.p. 150–151°, $[\alpha]_D^{20}$ +138.6° (*c* 1.00, methanol); lit.[27] m.p. 147°, $[\alpha]_D$ +128° (water).

From the mother liquor of the recrystallization, crystals, m.p. 188–190°, (0.280 g) were isolated. Recrystallization from the same solvent afforded methyl (methyl β-D-galactopyranosid)uronate, **3**, m.p. 192–194°, $[\alpha]_D^{22}$ −44.8° (*c* 1.15, methanol); lit.[28] m.p. 194°, $[\alpha]_D$ −45.6°.

The first ethanolic filtrate was evaporated *in vacuo* to a syrup (8.70 g) which was chromatographed on a column (77 × 4 cm) of silica gel (Merck). It was eluted with 19 : 1 ethyl acetate–methanol (3.0 L), and 5-mL fractions were collected. Fractions 110–140 afforded syrup **7** (2.61 g), which showed only one spot in t.l.c. in 19 : 1 ethyl acetate–methanol; $[\alpha]_D^{22}$ −112.8° (*c* 1.38, methanol); ^{1}H-n.m.r. (D_2O): δ 4.87 (s, H-1); lit.[4] for methyl (methyl β-D-galactofuranosid)uronate, $[\alpha]_D$ −107.3°; ^{1}H-n.m.r. (D_2O): δ 4.90 (s, H-1).

In t.l.c., fractions 150–200 (1.47 g) showed a mixture of two compounds. Fractions 170–200, which contained a preponderance of the slower-moving component, were evaporated to dryness, and chromatographed on preparative, t.l.c. plates precoated with silica gel (Merck 60 F_{254}), with a concentrating zone. Development was performed with 9 : 1 ethyl acetate–methanol.

The slower-moving zone was stripped off, extracted with methanol, the extract evaporated to a syrup, and the syrup chromatographed in the same way. Again, the slower-moving zone was stripped off, extracted with methanol, and the extract evaporated to a syrup; this was dissolved in 1 : 1 ethyl acetate–hexane, and the solution gave needles of methyl (methyl α-D-galactofuranosid)uronate, **5**, m.p. 64–66°, $[\alpha]_D^{23}$ +92.7° (*c* 1.17, methanol); ^{1}H-n.m.r. (D_2O): δ 4.85 (d, H-1, $J_{1,2}$ 4.0 Hz); lit.[29] m.p. 68°, $[\alpha]_D$ +83.4°; ^{1}H-n.m.r. (D_2O): δ 4.78 (d, H-1, $J_{1,2}$ 3.8 Hz).

Compounds **2**, **4**, **6**, *and* **8**. — The acetyl derivatives were prepared as reported[4,30,31].

N.m.r. spectra. — P.m.r. spectra (100, 220, 270, and 400 MHz) were recorded,

and integrated, with Varian XL-100, Varian HR-220, Nicolet–Oxford H-270, and Bruker WH-400 spectrometers, for 6–8% solutions. The spectra of compounds **3**, **5**, and **7** were for solutions in deuterium oxide containing acetone as the internal reference, and those of compounds **2**, **4**, **6**, and **8**, in chloroform-*d* containing 1% of tetramethylsilane as the internal standard. Chemical shifts are given on the δ scale. Coupling constants were measured from 100- or 250-MHz, sweep-width spectra. The ^{13}C-n.m.r. spectra were recorded at 20 MHz, with a Bruker WP-80 spectrometer equipped with a Fourier-transform accessory employing a ^{2}H-lock. The spectra were recorded with complete proton-decoupling. Solutions of concentration 60–100 mg/mL were made up in deuterium oxide, with 1,4-dioxane added as the internal reference. The chemical shifts obtained relative to 1,4-dioxane were corrected, in order to relate them to tetramethylsilane.

The selective decoupling was accomplished by use of monochromatic irradiation at the resonance of a given proton, and the frequency was determined from ^{1}H-n.m.r. spectra obtained for the same sample as had been used for the ^{13}C-n.m.r. spectra. For compound **1**, the protons irradiated were H-1, H-4, and H-5; for **2**, H-4 and H-5; for **6** and **8**, H-3 and H-4.

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