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^{13}C- AND ^{1}H-N M R. SPECTROSCOPY OF PERMETHYLATED α- AND β-D-GALACTOPYRANOSES

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ABSTRACT

The complete assignment of the ^{13}C- and ^{1}H-n m r spectra of the permethylated α- and β-D-galactopyranoses was performed with the aid of specific trideuteriomethylation, heteronuclear spin-decoupling, and spectrum simulation. The n m.r data are discussed and compared with those of the permethylated glucopyranoses Identification of partially methylated galactoses, *e g*, as obtained in the methylation analysis of carbohydrates, can be carried out by conversion of the free hydroxyl functions into ^{2}H- or ^{13}C-labelled methoxyl groups, and comparison of the n m r spectra of the resulting permethyl ethers with those of reference compounds

INTRODUCTION

In the course of studies of the n m r. spectroscopy of carbohydrate derivatives, we described[1,2] the complete interpretation of the ^{13}C- and ^{1}H-n m r spectra of solutions of permethylated α- and β-D-glucose in acetonitrile-d_3 It was found that the OMe resonances are well resolved in the ^{13}C- (25 2 MHz) and the ^{1}H- (100 MHz) n m r spectra The positions of one or more labelled (^{13}C or ^{2}H) OMe groups could be determined unequivocally These observations made possible the application of n m r spectroscopy in permethylation analysis The structure of partially methylated glucoses, which are obtained in permethylation analysis, can be deduced by n m r spectroscopy if the free hydroxyl functions are converted into labelled OMe groups The principal advantage of this method is that the number of reference compounds is restricted to a maximum of four for each monosaccharide, namely, the pyranose and furanose permethyl ethers, because the isotope effects on the shifts are negligible For the general application of this method in the analysis of oligo- and polysaccharides, it is essential that the assignment of the OMe resonances in the permethylated derivatives of the constituent monosaccharides is known

We now report on the n m r spectra of the permethylated galactopyranoses The OMe resonances in the ^{13}C- and ^{1}H-n m r spectra of these compounds were assigned by specific labelling The carbon resonances of the sugar skeleton were identified by heteronuclear, off-resonance spin-decoupling The values of the skeleton proton resonances were established from 300-MHz spectra of the perdeuterio-

methylated compounds Accurate ^{1}H-n m r. parameters were obtained by computer simulation of the spectra.

^{1}H-N m r spectral data of the OMe groups and the anomeric protons of the permethylated galactopyranoses dissolved in benzene-d_6 have been reported by Rathbone[3–5] The chemical shifts of the OMe groups, when benzene-d_6 is used as solvent, are concentration-dependent; this dependence is negligible in acetonitrile-d_3 Therefore, the spectra recorded in acetonitrile-d_3 are more suitable for identification purposes

RESULTS AND DISCUSSION

^{13}C-N m r *spectra of permethylated α- and β-D-galactopyranoses*

Chemical shifts of the OMe groups The OMe resonances were identified by comparison of the spectra of permethylated galactopyranoses that were selectively labelled with OCD_3 groups (Table I)

Changing the position of MeO-1 from equatorial to axial produces a significant, upfield shift of the MeO-1 signal (1 5 p p m) and of the MeO-2 signal (2 1 p p m), whereas the resonance positions of MeO-3, MeO-4, and MeO-6 remain almost unaltered (Fig 1) The relative differences in chemical shifts between the anomers are similar to those found for the per-*O*-methyl α- and β-D-glucopyranoses[1 2]

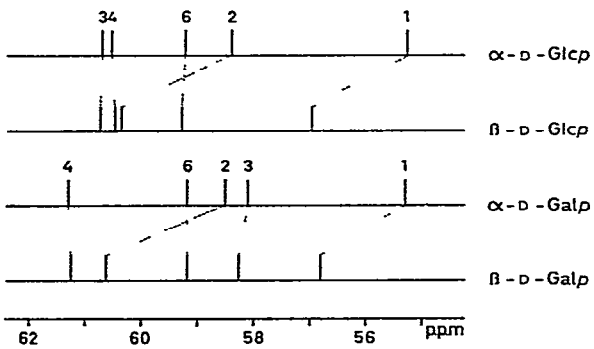

Fig 1 Correlation of the OMe carbon resonances of permethylated α- and -β-D-glucopyranoses and -galactopyranoses

Comparison of the spectra of the per-*O*-methyl derivatives of the galactopyranoses and the corresponding glucopyranoses show that the axial MeO-4 substituent (galactose) resonates ~0 8 p p m downfield from the equatorial MeO-4 group (glucose) (Fig 1) A much stronger effect is observed on the neighbouring MeO-3 group, for which the signal is shifted upfield ~2 5 p p m upon moving the MeO-4 group from an equatorial to an axial position The chemical shifts of MeO-1, MeO-2, and MeO-6 are almost identical in the corresponding derivatives of both monomers

TABLE I

^{13}C N M R DATA (25 2 MHZ, ACETONITRILE-d_3) CHEMICAL SHIFTS (δ) OF THE OMe GROUPS IN PERMETHYLATED α- AND -β-D-GALACTOPYRANOSES

Starting compound[a]	*Permethyl ether with OCH_3 substituent in positions(s)*	*MeO-1*		*MeO-2*		*MeO-3*		*MeO-4*		*MeO-6*	
		α	β	α	β	α	β	α	β	α	β
Me D Gal*p*	1 2 3 4 6	55 29	56 79	58 53	60 62	58 08	58 26	61 31	61 26	59 17	59 20
Me D-Gal*p*	1	55 29	56.82	—[b]	—	—	—	—	—	—	—
6-*O*Me D Gal*p*	6	—	—	—	—	—	—	—	—	59.19	59 23
2,6-Di-*O*Me-α-D-Gal*p*	2 6	—	—	58 53	—	—	—	—	—	59 19	—
Me 2,6 di-*O*Me-β-D-Gal*p*	1 2 6	—	56.82	—	60 65	—	—	—	—	—	59 21
Me 2,3-di-*O*Me-D-Gal*p*	1 2 3	55 32	56 84	58 53	60 63	58 13	58 28	—	—	—	—

[a]The compounds were methylated with CD_3I, but α-D-Gal*p* and β-D-Gal*p* were also methylated with CH_3I [b]— = Missing signal due to the introduction of an OCD_3 group in this position

Chemical shifts of the skeleton carbon atoms The carbon atoms of the sugar skeleton resonate downfield from the OMe groups They were assigned by off-resonance ^{13}C-{^{1}H} spin-decoupling[18] (Table II). However, H-2 and H-3 in the α-D anomer could not be decoupled individually The corresponding carbon resonances were identified by comparison with the spectra of the per-*O*-methyl derivatives of β-D-galactopyranose and of α- and β-D-glucopyranose

TABLE II

^{13}C-N M R DATA (25 2 MHz, ACETONITRILE-d_3) CHEMICAL SHIFTS (δ) OF THE SKELETON CARBONS IN PERMETHYLATED α- AND β-D-GALACTOPYRANOSE

Anomer	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α	98 80	78 78	80 97	77 25	69 88	72 34
β	105 26	81 50	84 65	76 10	73 86	72 01

Change from equatorial to axial position of MeO-1 produces a significant, upfield shift of the resonances of C-1, C-2, C-3, and C-5 (Fig 2). The chemical shifts of C-4 and C-6, which are more remote from the anomeric centre, are only slightly influenced. The relatively great, upfield shifts of C-3 and C-5 in the α-D anomer result from 1,3-diaxial interactions of C-1 and its axial OMe group with C-3 and C-5 and their axial protons These interactions give rise to H_{ax}–^{13}C bond polarizations[6,7] It is concluded that similar differences exist between the anomers of the permethylated galactopyranoses as between the permethylated glucopyranoses[1].

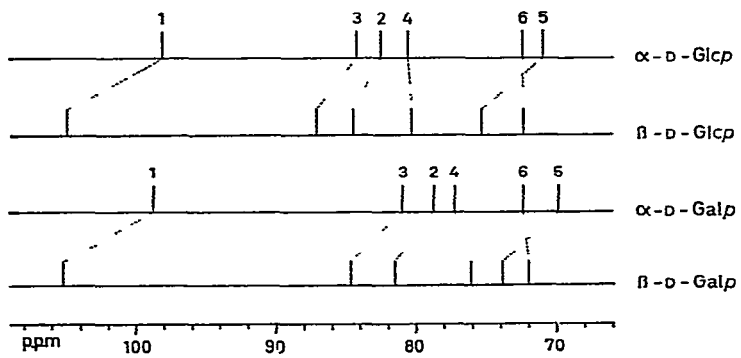

Fig 2 Correlation of the skeleton carbon resonances of permethylated α- and -β-D-glucopyranoses and -galactopyranoses

Comparison of the spectra of the corresponding anomers of permethylated galactose and glucose show (Fig 2) that the resonances of C-2–5 in the galactoses are shifted upfield by 1 1–4 4 p p m The shifts of C-3 and C-4 are produced by the epimerization of C-4; the shift of C-2 results from the diaxial interaction between

C-2–H-2 and C-4–OMe (*ax*) The positions of the C-1 and C-6 resonances are almost identical for the glucose and galactose derivatives For C-1, this is to be expected because this carbon atom is relatively remote from C-4, but C-6 could be shifted upfield as a result of steric interaction between MeO-4 (*ax*) and the substituent at C-5 However, this effect is very small

^{1}H-N m r spectra of permethylated α- and β-D-galactopyranoses

Chemical shifts of the OMe groups From the specifically trideuteriomethylated galactoses mentioned in Table I, 100-MHz ^{1}H-n m r spectra were recorded for solutions in acetonitrile-d_3. By comparison of the OMe singlets in these spectra, the signals were identified (Table III)

TABLE III

^{1}H-N M R DATA (100 MHZ, ACETONITRILE-d_3) CHEMICAL SHIFTS (δ) OF THE OMe GROUPS IN PERMETHYLATED α- AND -β-D-GALACTOPYRANOSE

Anomer	*MeO-1*	*MeO-2*	*MeO-3*	*MeO-4*	*MeO-6*
α	3 28	3 35	3 39	3 43	3 30
β	3 39	3 43	3 40	3 42	3 30

The chemical shifts of MeO-3, MeO-4, and MeO-6 are hardly affected by anomeric change[3] (Fig 3) The substantial, upfield shifts of MeO-1 and MeO-2 for the α-D anomer in both the ^{1}H- and ^{13}C-n m r spectra point to anisotropic and solvation effects

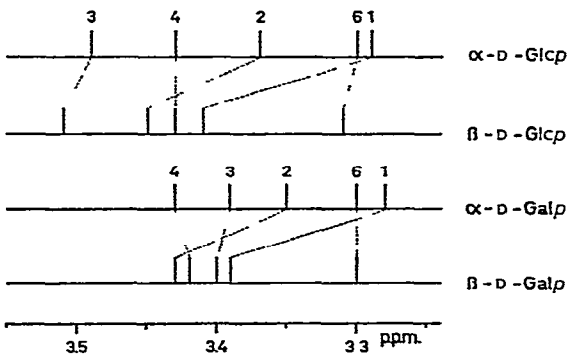

Fig 3 Correlation of the OMe proton resonances of permethylated α- and -β-D-glucopyranoses and -galactopyranoses

The chemical shifts of the OMe groups at positions 1, 2, 4, and 6 are almost similar to those in the corresponding permethylated glucopyranose anomers[1]. However, the chemical shift of MeO-3 is strongly influenced[8] by the epimerization on C-4, as was also observed in the ^{13}C-n m r spectra of these compounds

Chemical shifts and coupling constants of the skeleton protons To determine the chemical shifts and coupling constants of the sugar-skeleton protons, 300-MHz spectra were recorded for solutions of the perdeuteriomethylated galactopyranoses in acetonitrile-d_3 These spectra are rather complex, due to small $\Delta\delta/J$ values In the spectrum of the α-D anomer, the coupling constants $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ could not be determined accurately because H-2 and H-3 apparently have the same chemical shifts. The complexity of the spectrum arising from this collapse was such that a complete analysis of the H-1–H-4 part of the spectrum could not be accomplished. In the spectrum of the β-D anomer, H-5, H-6, and H-6′ form an ABC sub-system which could not be analyzed The 220-MHz spectra of solutions of the compounds in benzene-d_6 were then examined Additional information was thereby obtained about the coupling constants, although the values are not identical with those for acetonitrile-d_3, as a consequence of different effects of solvation on the conformation of the compounds The experimental p m r data for each solvent were refined by computer simulation The values of coupling constants and chemical shifts obtained are given in Table IV

TABLE IV

[1]H-N M R PARAMETERS[a] (δ p p m , J Hz) OF THE SKELETON PROTONS IN PERMETHYLATED α- AND -β-D-GALACTOPYRANOSE

Anomer	*Solvent*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*
α	Acetonitrile-d_3	4 78	3 42	3 44	3 64	3 76	3 48	3 42
α	Benzene-d_6	4 81	3 88	3 65	3 55	3 95	3 57	3 66
β	Acetonitrile-d_3	4 09	3 05	3 15	3 59	—[b]	—[b]	—[b]
β	Benzene-d_6	4 15	3 67	2 98	3 44	3 33	3 49	3 64
		$J_{1\,2}$	$J_{2\,3}$	$J_{3\,4}$	$J_{4\,5}$	$J_{5\,6}$	$J_{5\,6}$	$J_{6\,6}$
α	Acetonitrile-d_3	~2 2	~11 0	~1 6	1 5	6 0	6 7	−9 7
α	Benzene-d_6	3 7	10 0	3 1	1 5	5 7	6 9	−9 2
β	Acetonitrile-d_3	7 5	9 7	3 0	0 8	—[b]	—[b]	—[b]
β	Benzene-d_6	7 5	9 5	3 1	1 0	5 5	7 5	−9 2

[a]Determined at 300 MHz for solutions in acetonitrile-d_3, and at 220 MHz for solutions in benzene-d_6
[b]Complex multiplet between 3 45 and 3 53 p p m

Some regularities in the chemical shifts of the skeleton atoms are observed The increased shielding of H-1, H-2, H-3, and H-5 on changing from the α- to the β-D anomer is accompanied by a decrease in the shielding of the carbon atoms to which they are directly attached This points to changes in bond polarizations over the pyranoid rings[9]

Comparison with the spectral data of the corresponding glucose derivatives[1] shows that the epimerization at C-4 results in a strong, downfield shift of the signal for H-4 (which is moved from an axial to an equatorial position) and less-pronounced downfield shifts of the signals for H-2, H-3, and H-5 Consequently, the chemical

shifts of H and C in positions 2–5 are affected inversely by change in position of the 4-OMe substituent The resonance positions of H-1 and H-6,6′ are almost identical to those in the glucose derivatives

The OMe resonances of permethylated α- and β-D-galactopyranose in the ^{1}H- as well as in the ^{13}C-n m r spectra could be derived straightforwardly from the spectra of a series of permethyl derivatives bearing OCD_3 groups at different positions. In both types of spectra, the signals of the OCD_3 groups are missing, the absence of an OCD_3 carbon resonance is due to ^{13}C-D spin–spin splittings and to the absence of contributions for nuclear Overhauser effects ^{13}C-Labelling[1 2] can also be used, which intensifies the carbon resonances of the labelled OMe groups

The ^{13}C- and ^{1}H-n m r patterns of the five OMe resonances and also those of the ring atoms are typical for the permethylated galactopyranoses, as they differ significantly from those of other permethylated aldohexoses[2]

When acetonitrile-d_3 was used, the chemical shifts of the OMe-protons are almost independent of the concentration This is an advantage over using benzene-d_6 as solvent[3–5], although the δ range in which the signals for the OMe groups occur is somewhat smaller than for benzene-d_6 The relative positions of the chemical shifts of MeO-1 and MeO-6 in permethylated α-D-galactopyranose are reversed on changing from acetonitrile-d_3 to benzene-d_6 Thus, for identification purposes, *e g*, in permethylation analysis of carbohydrates, only data for a single solvent should be compared

EXPERIMENTAL

The monosaccharide derivatives were permethylated according to the method of Kuhn[10], using MeI or CD_3I (99% D, Merck) as appropriate The separation of α and β anomers was effected by t l c on Silica Gel G (Merck), using hexane–acetone (6 4) and detection with u v light after spraying with 1% Morin in methanol, followed by extraction from the silica gel with chloroform

Trideuteriomethyl 6-O-methyl-2,3,4-tri-O-trideuteriomethyl-α- and β-D-galactopyranoside — 6-*O*-Methyl-α-D-galactopyranose yields mainly the furanoid forms on permethylation[10] To obtain the pyranoid forms, the compound (160 mg) was treated (85°, 24 h) with CD_3OH containing 4% of HCl, and the product was methylated with CD_3I T l c of the product yielded the β-furanoside (R_F 0 61, 41 mg), the β-pyranoside (R_F 0 54, 56 mg) contaminated with a small amount of the α-furanoside, and the α-pyranoside (R_F 0 46, 98 mg)

Trideuteriomethyl 2,6-di-O-methyl-3,4-di-O-trideuteriomethyl-α- and β-D-galactopyranoside — Methyl β-D-galactopyranoside was converted into the 3,4-*O*-isopropylidene derivative[11] (for p m r data, see Ref 12) This compound was methylated[10] in the presence of Drierite, and the product was purified by distillation (0 2 mmHg, 125–130°) (For p m r data, see Ref 13)

Methyl 3,4-*O*-isopropylidene-2,6-di-*O*-methyl-β-D-galactopyranoside was hydrolysed[14] with 90% trifluoroacetic acid to give methyl 2,6-di-*O*-methyl-β-D-

galactopyranoside {b p 140–150°/0 005 mmHg, $[\alpha]_D$ −24° (*c* 6 8, chloroform), for p m r data, see Ref 13}. The product was subjected in sequence to methylation with CD_3I, acid hydrolysis (95°, 20 h) with Amberlite IR-120(H^+) resin, and methylation with CD_3I T l c then yielded the title compounds in the ratio 4 6

*Methyl 2,3-di-*O*-methyl-4,6-di-*O*-trideuteriomethyl-α- and β-*D*-galactopyranoside.* — Methyl α-D-galactopyranoside was converted into the 4,6-*O*-benzylidene derivative[15] (for p m r. data, see Ref 13), and then methylated[10] in the presence of Drierite to give methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-galactopyranoside, which was crystallised from ether P m r data ($CDCl_3$, 60 MHz) δ 5 2 ($J_{1,2}$ ~2 5 Hz, H-1), 3 56 (MeO-1), 3 62 (MeO-2,3), 5 70 (PhC*H*), 7 3–7 9 (Ph), 3 5–4.6 (remaining protons) Hydrolysis[14] of the compound in 90% trifluoroacetic acid, followed by treatment with 4% methanolic HCl and then methylation with CD_3I, gave the title anomers, which were separated by t l c

Perdeuteriomethylated α- and β-D-galactopyranoses — These anomers were prepared by methylation of methyl α-D-galactopyranoside with CD_3I, followed by hydrolysis, remethylation with CD_3I, and separation of the anomers by t l c

N m r spectroscopy. — ^{1}H-N m r spectra were recorded on a Varian HA-100 spectrometer (Organic Chemical Institute TNO, Utrecht), a Varian HR-220 spectrometer (TNO Central Laboratories, Delft), or a Varian HA-300 spectrometer (Laboratory of N m r spectroscopy, University of Ghent, Belgium) The instruments were operated in the field-sweep mode at a probe temperature of ~25° Solutions (5–20%) of the galactose derivatives in acetonitrile-d_3 or in benzene-d_6 were used Chemical shifts are given relative to that of Me_4Si on the δ scale, with an accuracy of 0 01 p p m The accuracy of the coupling constants is ~0.1 Hz.

The theoretical spectra were calculated from the initial, experimental parameters in an interactive, iterative procedure with the spin-simulation program SIMEQ[16], using a 16 k Varian 620 i computer coupled with a Varian XL-100 spectrometer The proton systems were treated as seven-spin (ABCDEFG) systems consisting of H-1,2,3,4,5,6,6′. All vicinal coupling constants were taken as positive, but the geminal coupling constant $J_{6,6}$ was taken as negative[17]

Proton-noise-decoupled ^{13}C-n m r. spectra of deuterium-labelled galactose derivatives in acetonitrile-d_3 were recorded at 25 2 MHz on a Varian XL-100-15 FT spectrometer operating in the deuterio-lock mode at ~30° Chemical shifts are given relative to that of internal Me_4Si (δ scale), with an accuracy of 0 04 p p m

Assignment of the resonances of the ring carbons is based on a special, off-resonance ^{13}C-{^{1}H} spin-decoupling technique[18] Various single frequencies were irradiated at intervals of 10 Hz in the range of the resonance frequencies of the protons H-1 up to and including H-6′ From the plot of the resonance frequencies in the partially decoupled, Fourier Transform ^{13}C-n m r spectra against the irradiated frequencies, and with the aid of the ^{1}H-n m r. frequencies of the ring protons, the assignment of the carbon resonances was made

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