ELSEVIER

Carbohydrate Research 300 (1997) 199–206

CARBOHYDRATE
RESEARCH

Determination of the absolute configuration of monosaccharides by ^{1}H NMR spectroscopy of their per-*O*-(*S*)-2-methylbutyrate derivatives

William S. York *, Stephen Hantus, Peter Albersheim, Alan G. Darvill

Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology, The University of Georgia, 220 Riverbend Road, Athens, GA 30602-4712 USA

Received 25 September 1996; accepted 28 January 1997

Abstract

An empirical method was developed to determine the absolute configuration of monosaccharides, based on high-field ^{1}H NMR spectroscopy of their per-*O*-(*S*)-2-methylbutyrate (SMB) derivatives. The SMB derivatives of the D and L forms of a given monosaccharide are diastereomers, allowing them to be distinguished on the basis of differences in their ^{1}H NMR chemical shifts. The reproducibility of these chemical shift differences may allow the absolute configuration of a wide range of monosaccharides to be routinely determined by comparing the spectra of their SMB derivatives to standard spectra in a data base, making it unnecessary to prepare and analyze new standards for each analysis. The derivatization procedure uses inexpensive, easy-to-handle reagents and is virtually complete. Application of the method to three complex glycans provided an unambiguous determination of the absolute configurations of their constituent monosaccharides. © 1997 Elsevier Science Ltd.

Keywords: Absolute configuration; Monosaccharides; NMR Spectroscopy; Diastereomers

1. Introduction

The structural characterization of a complex glycan is not complete until the absolute configurations of its glycosyl residues have been established. Nevertheless, assignment of this important structural parameter is often neglected either because (i) naturally occurring sugars exist predominantly as either the D configuration (e.g., D-glucose) or the L configuration (e.g., L-arabinose), and glycosyl residues are assumed (sometimes incorrectly) to have the predominant configuration, or (ii) established methods for determining the absolute configuration can be time consuming and/or give ambiguous results. Thus, more reliable and facile methods for determining the absolute configuration of monosaccharides would be valuable tools for elucidating the complete structures of complex glycans.

Several methods have been developed to determine the absolute configurations of monosaccharides. These methods are based on (i) 'anomalous scattering' in X-ray crystallography [1], (ii) exciton coupling in circular dichroic spectroscopy [2–4], (iii) optical rotation [5], (iv) enantioselective GLC [6], and

---

* Corresponding author.

PII S0008-6215(97)00050-5

(v) NMR spectroscopy [7] or (vi) chromatography [8–11] of diastereomeric products formed with chiral derivatizing agents. A frequently used chromatographic method [8,9] involves the separation of diastereomeric alkyl glycosides prepared by solvolysis of the glycan with a chiral alcohol. This empirical method requires at least one of the enantiomeric forms of the monosaccharide to be available as a standard. In order to obtain unambiguous results by this method, it is sometimes necessary to optimize the conditions for solvolysis and chromatography, or to experiment with different chiral alcohols (e.g., *sec*-butyl versus *sec*-octyl) and/or chemical derivatives (e.g., trimethylsilyl versus acetyl).

NMR methods can, in principle and in favorable cases, provide the absolute configuration of individual chiral carbons without referring to the spectra of standard molecules. For example, individual ^{1}H NMR resonances in secondary alcohols derivatized with Mosher's reagent [(*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride] [7] have different chemical shifts depending on the absolute configuration of the alcoholic carbon. These chemical shift effects have been attributed to shielding by the phenyl substituent of the acid moiety of the ester and correlated to the absolute geometry of the alcohol [7]. This type of analysis would be more complicated for per-*O*-acylated monosaccharides wherein the shielding effects of several esters would contribute to the chemical shift of each proton and wherein the interaction of the bulky acyl groups may lead to non-ideal ester geometry [12]. Thus, it is likely that the routine configurational analysis of monosaccharides by ^{1}H NMR spectroscopy of their per-*O*-α-methoxy-α-trifluoromethylphenylacetyl esters would have to be based on the preparation of suitable standards. However, Mosher's reagent is expensive and water sensitive. Furthermore, it is possible that the bulky phenyl substituent of Mosher's reagent could interfere with complete derivatization of some monosaccharides.

We report here a convenient alternative method for determining the absolute configuration of monosaccharides based on high-field ^{1}H NMR spectroscopy of their per-*O*-(*S*)-2-methylbutyrate (SMB) derivatives.

2. Experimental

Complex glycans.—The L-galactose-containing oligoglycosyl alditol (XXJGol) was isolated from endoglucanase-treated jojoba seed amyloid as described [13]. A mixture of β-L-arabinose-containing oligosaccharides (Fraction LE-3 in reference [14]) was prepared from endoglucanase-treated tomato xyloglucan. The cell-wall arabinogalactan of *Mycobacterium smegmatis* [15] was a gift of Dr. Michael McNeil (Department of Microbiology, Colorado State University).

*Preparation of per-*O*-(*S*)-2-methylbutyrate (SMB) derivatives.*—Glycans (i.e., oligosaccharides, oligoglycosyl alditols, and polysaccharides, 0.5–1.0 mg) were hydrolyzed in trifluoroacetic acid (2 N, 200 μL, 121 °C, 90 min), and the solvent was evaporated under a stream of dry air. Initially, standard D- and L-sugars (1.0 mg) were exposed to these hydrolysis conditions to confirm that they did not lead to significant degradation of the monosaccharides. Subsequently, standards were not treated with acid. (*S*)-(+)-2-Methylbutyric anhydride (100 μL, Aldrich) and pyridine (100 μL, Aldrich) were added to the hydrolyzed glycans and standard monosaccharides, and the reactions were incubated at 121 °C for 4 h in a tube sealed with a Teflon-lined screw cap. The reagents were evaporated ($\sim$ 8 h under a stream of dry air), and toluene (300 μL) was added and evaporated. The residues were dissolved in CH_2Cl_2 (1 mL) and extracted three times with 2 M Na_2CO_3 (2 mL) and then with water (2 mL). During the final water extraction, the lower (CH_2Cl_2) phase in each sample, containing the SMB derivatives, was transferred to a clean glass tube, concentrated to syrup by evaporation, and then dried by adding and evaporating neat 2-propanol (300 μL).

The oligoglycosyl alditol XXJGol contained small amounts of residual borate due to a $NaBH_4$ reduction step in its preparation. This borate, which could interfere with the acylation reaction [16], was removed after the hydrolysis step by alternately adding and evaporating 9:1 methanol:acetic acid a total of 4 times and then alternately adding and evaporating neat methanol 2 times.

NMR spectroscopy.—SMB derivatives were dissolved in acetone-d_6, and their ^{1}H NMR spectra were recorded with a Bruker DRX-600 spectrometer operating at a sample temperature of 298 K. Chemical shifts were measured relative to internal acetone-d_5 (δ 2.050). 1D NMR spectra were recorded as 16384 TPPI data points with a spectral width of 10 ppm (6 kHz). No zero filling was necessary, as the resolution of these spectra (8192 real data points, 0.7 Hz/point, 0.0012 ppm/point) was sufficient to assign chemical shifts to within 0.001 ppm. Double-quantum filtered COSY spectra [17] (512 free induction decays, each acquired as 32 or 48 transients of 4096 data points)

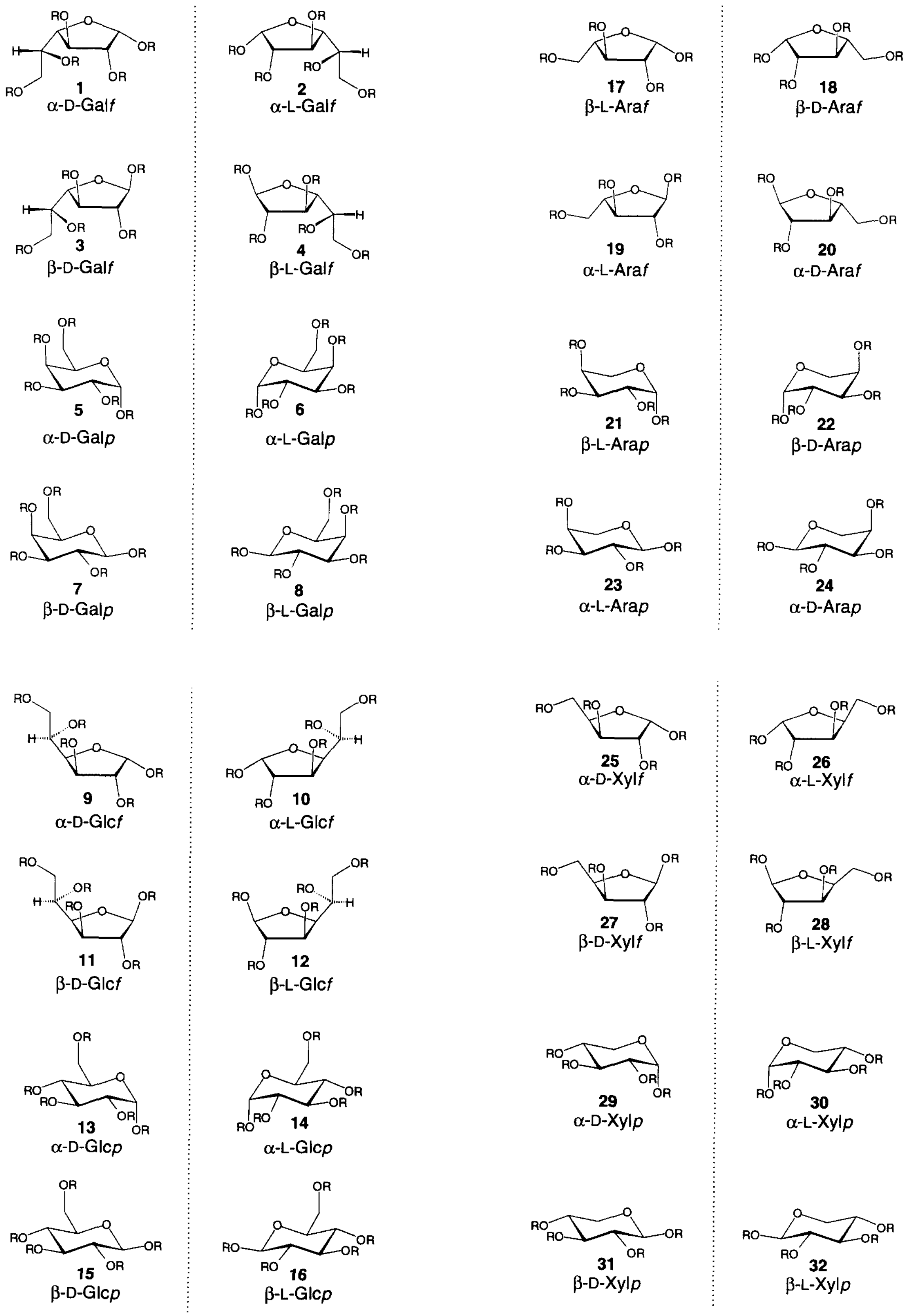

Fig. 1. Structures of compounds **1–32** [R = (*S*)-2-methylbutyrate]. The dotted line represents a mirror-image plane for the sugar moiety, but due to the presence of the chiral 2-methylbutyrate substituents, structures 'reflected' in this plane are diastereomers rather than enantiomers.

were recorded with a spectral width of 7 ppm, employing the time-proportional phase increment method [18] in both dimensions. The COSY spectra were used only to assign resonances, and accurate chemical shifts and coupling constants were measured from the 1D spectra.

3. Results and discussion

^{1}H NMR spectra of SMB monosaccharides.—The SMB derivatives of the D and L forms of several neutral monosaccharides (Fig. 1) were prepared (see **Experimental**), and their ^{1}H NMR spectra were recorded (Figs. 2 and 3). The deshielding of protons attached to *O*-acylated carbons [19] resulted in a greater chemical shift dispersion than in the native monosaccharides, facilitating the assignment of the ^{1}H-resonances (Tables 1 and 2). Internal acetone-d_5 (δ 2.050), always present in acetone-d_6, is a convenient chemical shift standard easily recognized by its characteristic coupling pattern (line intensities 1:2:3:2:1, $^2J_{H,D}$ 2.2 Hz). However, acetone is hygroscopic and care must be taken to keep the samples dry when using acetone-d_5 as a reference. For example, samples left on the bench for 3 weeks absorbed ~ 0.5% H_2O, resulting in an apparent change of -0.003 ppm for all resonances of the SMB derivatives. This change was attributed to the effects of dissolved water on the chemical shift of the acetone-d_5 resonance used to calibrate the spectrum. This problem is easily recognized, because H_2O produces a characteristic resonance which occurs at δ 2.80 at very low water concentrations and is shifted downfield as the water concentration increases. Occasionally, an HDO resonance, approximately 0.03 ppm upfield of the H_2O resonance, is also present due to exchange with acetone-d_6. Samples that have absorbed enough water to significantly affect the calibration (i.e., containing a large H_2O resonance with $\delta > 2.820$) can be dried by evaporating the acetone-d_6 and adding and evaporating 0.5 mL of 2-propanol before redissolving the sample in dry acetone-d_6. It is also possible, *but not recommended*, to adjust the calibration to account for the effects of dissolved water. Calibration of the spectrum with acetone-d_5 using:

$$\delta(\text{acetone-}d_5) = 2.050 + (0.03 \times (\Delta\delta_{\text{wa}} - 0.750))$$

where $\Delta\delta_{\text{wa}} = \delta(H_2O) - \delta(\text{acetone-}d_5)$, provides chemical shift values for the SMB derivatives that are usually within 0.001 ppm of those measured in dry acetone-d_6 using δ (acetone-d_5) = 2.050. However, it is not necessary to perform such a correction for spectra of water-free samples recorded at a well-defined sample temperature (298 K), as chemical shifts measured from these spectra are extremely reproducible.

Anomeric proton resonances of the SMB derivatives were identified by their characteristic downfield shifts [20] and coupling patterns [14,21,22], and by 2D NMR spectroscopy (see below). This analysis

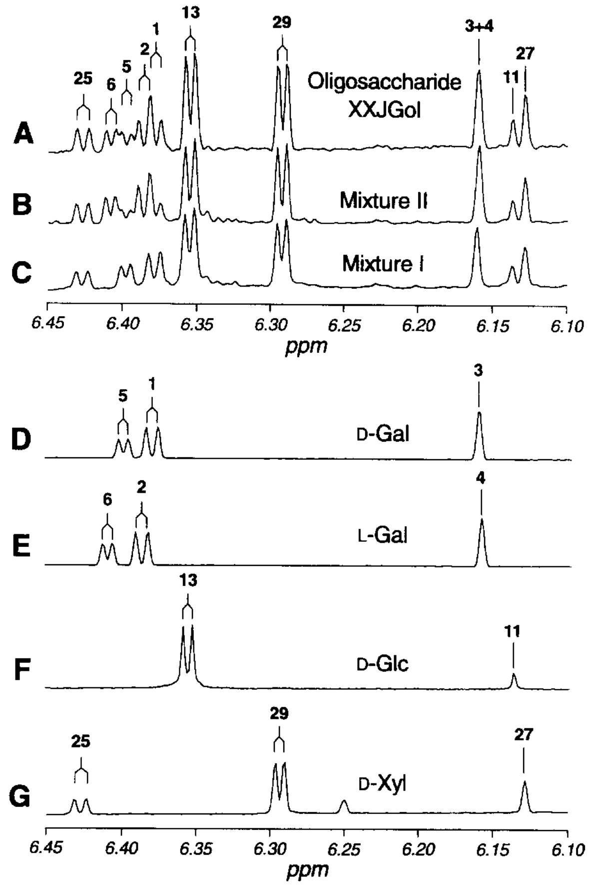

Fig. 2. Partial 600 MHz ^{1}H NMR spectra of per-*O*-(*S*)-2-methylbutyrate (SMB) derivatives of monosaccharides found in jojoba xyloglucan. The reduced oligosaccharide XXJGol was hydrolyzed, the resulting monosaccharides were derivatized with (*S*)-2-methylbutyric anhydride, and the ^{1}H NMR spectrum of the SMB derivatives was recorded (Panel A). The spectra of the SMB derivatives of D-Gal, L-Gal, D-Xyl, D-Glc and D-Glcol in the ratios 2:0:3:3:1 (Mixture I, Panel C) and 1:1:3:3:1 (Mixture II, Panel B) and SMB derivatives of individual standard D and L monosaccharides (Panels D–G) are also shown for comparison. This analysis demonstrates that XXJGol contains both D-Gal and L-Gal. This conclusion is confirmed by the chemical shifts of resonances (e.g., H-1 of β-pyranosyl forms) in other regions of the spectrum. Derivatives (Table 1) giving rise to the anomeric proton resonances are indicated numerically.

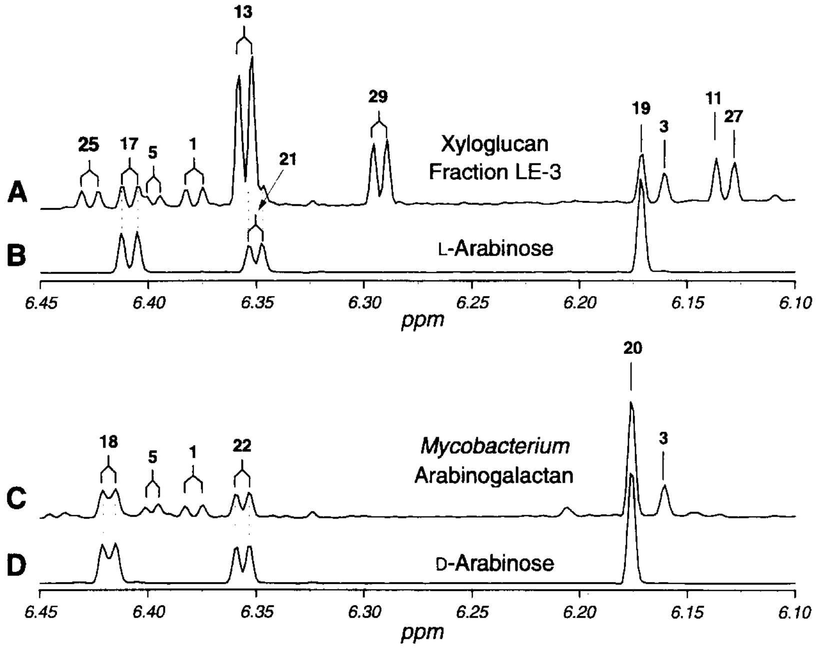

Fig. 3. Partial 600 MHz ^{1}H NMR spectra of per-*O*-(*S*)-2-methylbutyrate (SMB) derivatives of monosaccharides from tomato xyloglucan fraction LE-3 (Panel A), and *M. smegmatis* arabinogalactan (Panel C). The SMB derivatives of standard L-Ara (Panel B) and D-Ara (Panel D) are shown for comparison. This analysis demonstrates that tomato xyloglucan oligosaccharides contain L-Ara and *M. smegmatis* arabinogalactan contains D-Ara. In addition, tomato xyloglucan contains D-Xyl and D-Glc, and *M. smegmatis* arabinogalactan contains D-Gal (see Table 1 and Fig. 2). Derivatives (Table 1) giving rise to the anomeric proton resonances are indicated numerically.

revealed the presence of four cyclic forms (α-furanose, β-furanose, α-pyranose, and β-pyranose) for each monosaccharide. COSY spectra [17] of the SMB derivatives of D-Gal, L-Gal, D-Ara, and L-Ara were recorded. The spin systems corresponding to the four ring forms of D-Gal and L-Gal were fully assigned (Table 2), but unambiguous assignment of the SMB derivatives of D-Ara and L-Ara was not achieved, due to the effects of strong coupling. For example, H-1 of **18** appears to be broadened (Fig. 3) due to virtual coupling [20] with H-3, which is strongly coupled to H-2.

This analysis established that the diastereomeric SMB derivatives of D and L monosaccharides can be distinguished by small, but significant, chemical shift differences in their ^{1}H NMR spectra ($\Delta\delta$ values in Table 1). Many of the chemical shift differences (Tables 1 and 2) are greater than the line width (typically < 2.5 Hz, which is < 0.004 ppm at 600 MHz), allowing individual lines in the spectra of the SMB derivatives of mixtures of D and L monosaccharides to be resolved. Most of the chemical shift differences exhibit systematic trends as illustrated in Table 1, wherein the derivatives of each monosaccharide are listed in the same order with respect to the absolute configurations of C-1 and C-2. For example, changing the configuration from 1*R*,2*R* to 1*S*,2*S* consistently results in a deshielding ($+0.005 < \Delta\delta < +0.011$) of H-1 of the listed furanose derivatives. The conversion 1*S*,2*R* to 1*R*,2*S* is unusual, producing only a small change ($-0.002 < \Delta\delta < +0.004$) in the chemical shift of H-1 of the listed furanose derivatives. Further study is required to determine whether systematic chemical shift effects of (*R*)- or (*S*)-2-methylbutyration could be utilized to determine the absolute configuration of monosaccharides for which no authentic D and L standards are available. Nevertheless, the chemical shift effects described above can be used as the basis for an empirical method based on standard monosaccharides of known configuration.

The use of SMB derivatives to determine the absolute configurations monosaccharides from xyloglucans.—The nonasaccharide XXJG [23] is a subunit of the xyloglucan of the fucose-deficient *Arabidopsis thaliana* mutant *mur1*. XXJG is closely related to XXFG, a nonasaccharide subunit of a wide range of cell-wall xyloglucans [24]. Although the terminal L-Fuc*p* residue of XXFGol is replaced by an L-Gal*p* residue in XXJGol, both oligosaccharides are biologically active in plants [23]. The absolute configuration of the terminal Gal residue of XXJG was initially

Table 1
Anomeric proton resonances of per-*O*-(*S*)-2-methylbutyryl derivatives of selected monosaccharides

Compound	Sugar GEOMETRY	$^3J_{1,2}$ [a]	δ H-1 [b]	Δδ [c]
1	α-D-Gal*f* (1*R*,2*R*)	4.6	6.379	+0.007
2	α-L-Gal*f* (1*S*,2*S*)	5.0	6.386	
3	β-D-Gal*f* (1*S*,2*R*)	< 2	6.161	−0.001
4	β-L-Gal*f* (1*R*,2*S*)	< 2	6.160	
5	α-D-Gal*p* (1*R*,2*R*)	3.8	6.398	+0.010
6	α-L-Gal*p* (1*S*,2*S*)	3.7	6.408	
7	β-D-Gal*p* (1*S*,2*R*)	7.5	5.941	−0.012
8	β-L-Gal*p* (1*R*,2*S*)	8.0	5.929	
9	α-D-Glc*f* (1*R*,2*R*)	4.7	6.494	+0.005
10	α-L-Glc*f* (1*S*,2*S*)	4.7	6.499	
11	β-D-Glc*f* (1*S*,2*R*)	< 2	6.137	−0.002
12	β-L-Glc*f* (1*R*,2*S*)	< 2	6.135	
13	α-D-Glc*p* (1*R*,2*R*)	3.6	6.355	+0.005
14	α-L-Glc*p* (1*S*,2*S*)	3.7	6.360	
15	β-D-Glc*p* (1*S*,2*R*)	8.5	5.942	−0.010
16	β-L-Glc*p* (1*R*,2*S*)	8.5	5.932	
17	β-L-Ara*f* (1*R*,2*R*)	4.1	6.409	+0.009
18	β-D-Ara*f* (1*S*,2*S*)	4.1	6.418	
19	α-L-Ara*f* (1*S*,2*R*)	< 2	6.172	+0.004
20	α-D-Ara*f* (1*R*,2*S*)	< 2	6.176	
21	β-L-Ara*p* (1*R*,2*R*)	3.6	6.350	+0.006
22	β-D-Ara*p* (1*S*,2*S*)	3.7	6.356	
23	α-L-Ara*p* (1*S*,2*R*)	6.5	5.817	−0.007
24	α-D-Ara*p* (1*R*,2*S*)	6.6	5.810	
25	α-D-Xyl*f* (1*R*,2*R*)	4.6	6.427	+0.011
26	α-L-Xyl*f* (1*S*,2*S*)	4.7	6.438	
27	β-D-Xyl*f* (1*S*,2*R*)	< 2	6.128	+0.001
28	β-L-Xyl*f* (1*R*,2*S*)	< 2	6.129	
29	α-D-Xyl*p* (1*R*,2*R*)	3.6	6.293	+0.002
30	α-L-Xyl*p* (1*S*,2*S*)	3.7	6.295	
31	β-D-Xyl*p* (1*S*,2*R*)	7.3	5.850	−0.011
32	β-L-Xyl*p* (1*R*,2*S*)	7.4	5.839	

[a] Apparent first-order coupling constant in Hz.
[b] Chemical shift (ppm).
[c] Chemical shift difference (ppm) for diastereomeric pair (e.g., per-*O*-(*S*)-2-methylbutyryl α-D-Gal*p* versus per-*O*-(*S*)-2-methylbutyryl α-L-Gal*p*).

established [23], after considerable effort, using the chromatographic method of Gerwig et al. [8] on closely related oligosaccharides.

XXJG was prepared from the amyloid xyloglucan of jojoba seeds [13]. In order to facilitate its structural determination, XXJG was converted to the oligoglycosyl alditol XXJGol by reduction with $NaBH_4$. XXJGol consists of Gal, Xyl, Glc and Glcol in a 2:3:3:1 ratio. Two standard mixtures, consisting of the SMB derivatives of D-Gal, L-Gal, D-Xyl, D-Glc and D-Glcol in the ratios 2:0:3:3:1 (Mixture I) and 1:1:3:3:1 (Mixture II) were prepared and analyzed along with the individual D and L monosaccharide standards described above. The 600 MHz ^1H NMR spectrum of the SMB derivatives of the monosaccharide constituents of XXJGol (see **Experimental**, Fig. 2A) was nearly indistinguishable from that of Mixture II (Fig. 2B), but clearly distinguishable from that of Mixture I (Fig. 2C). This analysis confirmed that D-Gal and L-Gal residues are present in a 1:1 ratio in XXJGol and that the Xyl and Glc residues have the D configuration.

The xyloglucan secreted by suspension-cultured tomato cells contains an unusual trisaccharide side chain, β-Ara*f*-(1 → 3)-α-Ara*f*-(1 → 2)-α-Xyl*p* [14]. All previously characterized Ara*f* residues in xyloglucans have the α-L configuration, and so the detection of a β-Ara*f* residue in tomato xyloglucan was unexpected. The absolute configuration of the β-Ara*f* residue in this side chain has not been previously established. Therefore, oligosaccharides obtained from tomato xyloglucan were hydrolyzed and SMB derivatives of their monosaccharide constituents were prepared. For example, oligosaccharide Fraction LE-3 (reference [13]), in which ~ 6% of the glycosyl residues were α-Ara*f* and ~ 3% were β-Ara*f*, was analyzed. The ^1H NMR spectrum (Fig. 3A) of the SMB derivatives obtained from LE-3 contained resonances corresponding to L-Ara, D-Gal, D-Xyl, and D-Glc. Prior to hydrolysis and derivatization, approximately one-third of the Ara in LE-3 consisted of β-Ara*f* residues. Had the β-Ara*f* residues been in the D configuration, several resolved resonances due to the SMB derivatives of D-Ara (e.g., H-1 of **18** and **20**, Fig. 3D), would have been observed in the spectrum. However, no resonances corresponding to D-Ara were detected, indicating that both the α-Ara*f* and β-Ara*f* residues in LE-3 are in the L configuration (Fig. 3).

The use of SMB derivatives to determine the absolute configurations monosaccharides of the arabinogalactan from Mycobacterium smegmatis.—The cell walls of Mycobacteria contain an arabinogalactan comprised of D-Ara*f* and D-Gal*f* residues [15,25]. The arabinogalactan was hydrolyzed and SMB derivatives of the resulting monosaccharides were prepared. The ^1H NMR spectrum of these derivatives (Fig. 3C) included resonances corresponding to D-Ara and D-Gal, confirming previously reported results [25] obtained using the chromatographic method of Gerwig et al. [8].

Table 2
NMR parameters for (S)-2-methylbutyrate derivatives of galactose

	Compound number and sugar geometry							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	α-D-Gal*f*	α-L-Gal*f*	β-D-Gal*f*	β-L-Gal*f*	α-D-Gal*p*	α-L-Gal*p*	β-D-Gal*p*	β-L-Gal*p*
δ H-1	6.379	6.386	6.161	6.160	6.398	6.408	5.941	5.929
$^3J_{1,2}$	4.6	5.0	~ 1	~ 1	3.8	3.7	7.5	8.0
δ H-2	5.421	5.463	5.166	5.180	5.336	5.343	5.353	5.351
$^3J_{2,3}$	7.4	7.5	~ 1	~ 1	10.9	11.0	10.3	10.2
δ H-3	5.674	5.656	5.181	5.187	5.489	5.494	5.380	5.387
$^3J_{3,4}$	6.5	7.1	5.2	5.1	3.5	4.0	3.0	3.2
δ H-4	4.343	4.358	4.462	4.462	5.597	5.611	5.511	5.516
$^3J_{4,5}$	6.3	6.0	5.0	4.7	~ 1	~ 1	~ 1	~ 1
δ H-5	5.332	5.328	5.420	5.425	4.590	4.582	4.439	4.432
$^3J_{5,6}$	4.0	4.3	4.4	4.3	7.3	7.2	6.5	6.3
$^3J_{5,6'}$	6.3	6.2	7.0	7.3	5.9	6.0	6.2	6.0
δ H-6	4.417	4.432	4.406	4.412	4.130	4.131	4.188	4.207
δ H-6′	4.107	4.110	4.188	4.207	4.089	4.055	4.082	4.044
$^3J_{6,6'}$	−11.8	−12.0	−11.7	−11.7	−11.7	−11.7	−11.3	−11.2

4. Conclusions

We have developed a convenient alternative method for determining the absolute configuration of monosaccharides based on ^{1}H NMR spectroscopy of their per-*O*-(*S*)-2-methylbutyrate derivatives. The derivatization reaction is simple and complete, and requires no expensive reagents. The method does depend on access to a high-field (≥ 500 MHz) NMR spectrometer to achieve adequate resolution of resonances arising from each pair of diastereomers. However, this should not be a problem for most laboratories performing structural analysis of complex glycans, which frequently involves the use of high-field NMR spectroscopy.

In all of the cases described herein, 1D NMR analysis was sufficient to unambiguously determine the absolute configuration of each monosaccharide in the mixture. Although we performed 2D NMR analysis in order to fully assign the resonances of all four SMB derivatives of D- and L-galactose, such a thorough analysis would not be necessary for the routine application of the method. We believe that the method will prove to be very robust because each monosaccharide gives rise to many resonances (e.g., a total of 28 multiplets for the four derivatives of an aldohexose), and almost all of these resonances (not just the anomeric resonances shown in Figs. 2 and 3) are likely to be affected by the absolute configuration of the monosaccharide (see Table 2). Thus, the method may be applicable to almost any monosaccharide for which suitable standards are available. In addition, the chirally induced chemical shift effects are very reproducible, and eventually this may make it unnecessary to isolate or synthesize rare or unusual monosaccharide standards if the spectra of their SMB derivatives have been recorded elsewhere and submitted to a widely accessible spectral data base. This is not the case for chromatographic methods, which require frequent column calibration with authentic standards.

The method described here may not, in every case, provide an unambiguous determination of the absolute configurations of all of the constituents of a complex mixture of monosaccharides, where signal overlap may be a problem. It is yet to be determined whether it would be more efficient in such cases to use other methods (see ref [1–11]) or to modify the method described here by, for example, using different NMR solvents or applying 2D NMR experiments.

Acknowledgements

This research was supported by United States Department of Energy (DOE) grant DE-FG02-96ER-20220, and by the DOE-funded (DE-FG05-93ER20097) Center for Plant and Microbial Complex Carbohydrates. The authors thank Dr. Michael McNeil (Dept. of Microbiology, Colorado State University) for providing the Mycobacterium arabinogalactan, Dr. John Glushka for helpful discussions, and Dennis Warrenfeltz for technical support and maintenance of the NMR spectrometers.

References

[1] J.M. Bijvoet, A.F. Peerdeman, and A.J. van Bommel, *Nature*, 168 (1951) 271–272.

[2] N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, CA, 1996.

[3] Y. Shiomi, M. Saisho, K. Tsugagoshi, and S. Shinkai, *J. Chem. Soc., Perkin Trans. 1*, (1993) 2111–2117.

[4] J. Frelek, Z. Pakulski, and A. Zamojski, *J. Carbohydr. Chem.*, 12 (1993) 625–639.

[5] S.F. Mason, *Molecular Optical Activity and the Chiral Discriminations*, Cambridge University Press, Cambridge, 1982.

[6] H. Bretting, G. Jacobs, I. Benecke, W. Konig, and J. Thiem, *Carbohydr. Res.*, 139 (1985) 225–236,

[7] J.A. Dale and H.S. Mosher, *J. Am. Chem. Soc.*, 95 (1973) 512–519.

[8] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.

[9] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.

[10] Y. Nishida, C. Bai, H. Ohrui, and H. Meguro, *J. Carbohydr. Chem.*, 13 (1994) 1003–1008.

[11] P. Schneider, M.J. McConville, and M.A.J. Ferguson, *J. Biol. Chem.*, 269 (1994) 18332–18337.

[12] I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, 56 (1991) 1296–1298.

[13] S. Hantus, W.S. York, P. Albersheim, and A.G. Darvill, (1977), unpublished work.

[14] W.S. York, V.S.K. Kolli, R. Orlando, P. Albersheim, and A.G. Darvill. *Carbohydr. Res.*, 285 (1996) 99–128.

[15] M. Daffe, M. McNeil, and P.J. Brennan, *Carbohydr. Res.*, 249 (1993) 383–389.

[16] P. Albersheim, D.J. Nevins, P.D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.

[17] M. Rance, O.W. Sorensen, G. Bodenhausen, G. Wagner, R.R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 117 (1983) 479–485.

[18] D. Marion and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 113 (1983) 967–974.

[19] C.R. Narayanan and M.S. Parkar, *Indian J. Chem.*, 9 (1971) 1019–1020.

[20] S.W. Homans, *Prog. NMR Spectrosc.*, 22 (1990) 55–81.

[21] S.J. Angyal, *Carbohydr. Res.*, 77 (1979) 37–50.

[22] B.A. Wolucka, M. McNeil, E. de Hoffmann, T. Chojnacki, and P.J. Brennan, *J. Biol. Chem.*, 269 (1994) 23328–23335.

[23] E. Zablackis, W.S. York, M. Pauly, S. Hantus, W.-D. Reiter, C.C.S. Chapple, P. Albersheim, and A.G. Darvill, *Science*, 272 (1996) 1808–1810.

[24] P. Albersheim, J. An, G. Freshour, M.S. Fuller, R. Guillen, K.-S. Ham, M.G. Hahn, J. Huang, M. O'Neill, A. Whitcombe, M.V. Williams, W.S. York, and A.G. Darvill, *Biochem. Soc. Trans.*, 22 (1994) 374–378.

[25] M. Daffe, P.J. Brennan, and M. McNeil, *J. Biol. Chem.*, 265 (1990) 6734–67443.