Carbohydrate Research, 176 (1988) 300–305
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

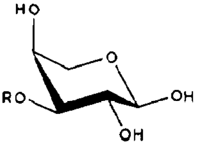
Note

Structural aspects of 3-*O*-α-D-galactopyranosyl-L-arabinose and the corresponding substituted L-arabinitol

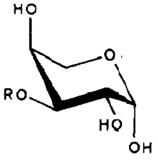
HEIDI I. JOÃO, GRAHAM E. JACKSON, NEIL RAVENSCROFT, AND ALISTAIR M. STEPHEN
Departments of Inorganic and Organic Chemistry, University of Cape Town, Rondebosch 7700 (South Africa)

(Received June 25th, 1987; accepted for publication in revised form, October 10th, 1987)

Complete ^{1}H- and ^{13}C-n.m.r. spectral assignments of the signals for 3-*O*-α-D-galactopyranosyl-L-arabinose (**1**) in D_2O solution have been made by using a combination of high-resolution 1D n.m.r. and 2D correlation experiments (COSY[1] and HETCOR[2]) at 200 MHz. The disaccharide was isolated in the form of two hepta-*O*-methyl derivatives (from arabic acid[3], derived in turn from gum arabic, Turc. variety[4]) in low yield as a syrup, and from the gum exudate of stems of *Acacia cyanophylla*[5]. It was also obtained, as a crystalline product in unusually high yield, from a polysaccharide found in *Watsonia* corm-sacs[6–8]. A sample of this preparation[6] was used in our n.m.r. investigation.



1 (α-anomer)
R = α-D-Gal*p*



1 (β-anomer)

The starting points for the characterization of the sugar were the anomeric-proton signals at δ 4.56 ($J_{1,2}$ 7.7 Hz) and 5.26 ($J_{1,2}$ 2.5 Hz), and 5.16 ($J_{1,2}$ 3.4 Hz) which were assigned to H-1 of α-L-Ara*p*, β-L-Ara*p*, and α-D-Gal*p*, respectively, on the basis of chemical shifts and coupling constants[9]. Integration of the proton spectrum gave a relative ratio of 3:2 for the α- and β-pyranosyl forms of the L-arabinose residue, which is also the ratio found for the free sugar[10], thus implying that a large substituent on O-3 does not affect the equilibrium established by mutarotation[cf.,11]. This ratio of intensities assisted in the assignment of the other ^{1}H and the ^{13}C resonances. Evidence for the furanose forms of the reducing-sugar residue was found, as for free L-arabinose[10], but to an extent of only ~9%. This

0008-6215/88/$ 03.50

discussion relates therefore to the substituted Ara*p* anomers, a comment following which concerns the minor Ara*f* contribution to the spectra.

From the assignments of the anomeric protons, made by using the HETCOR spectrum (see Fig. 1), the signals of the anomeric carbon atoms follow. In addition, the proton–proton (COSY) and the HETCOR spectra allow the assignments of H-2 and C-2 of α-L-Ara*p*, β-L-Ara*p*, and α-D-Gal*p*. Being the position of linkage, the signal for C-3 of L-Ara*p* is shifted downfield, out of the normal range for ring-carbon atoms[12]. On the basis of intensity, the resonances at δ 77.71 and 74.48 were assigned to α-L-Ara*p* C-3 and β-L-Ara*p* C-3, respectively.

The APT experiment[13] was used to assign the signals of α-D-Gal*p* C-6, and α- and β-L-Ara*p* C-5, these being the only methylene carbon atoms. The remaining

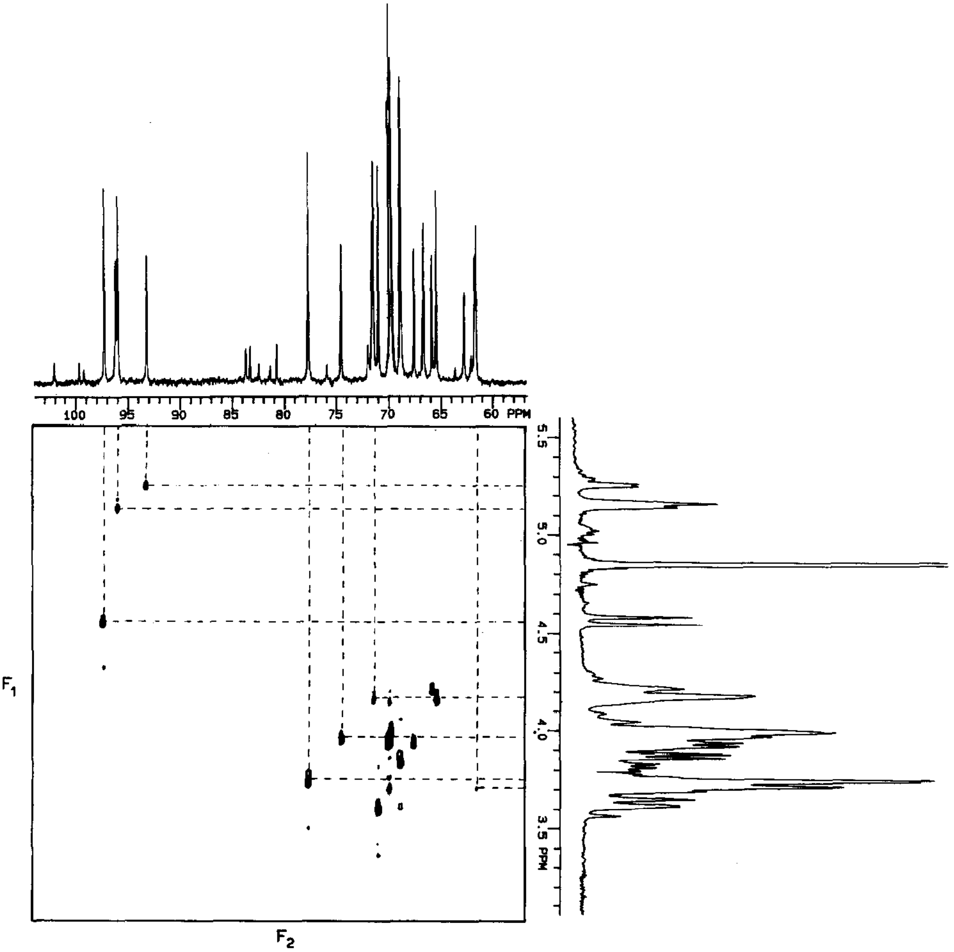


Fig. 1. Heteronuclear ^{1}H–^{13}C shift-correlation map (HETCOR) of **1**, recorded at 200 MHz and 297 K, using $J_{C,H}$ = 140 Hz. The respective ^{13}C and ^{1}H one-dimensional spectra are displayed along the F_2 and F_1 axes. Some of the H,C correlations are indicated.

carbon resonances were identified by comparison with the literature[12]. The final assignments are given in Table I and Fig. 2. Using the HETCOR experiments, complete elucidation of the proton spectrum was possible (see Table II). These assignments were consistent with the coupling scheme shown by the COSY spectrum, a possible conformational representation of **1** being shown as Fig. 3. Whereas, for β-L-Ara*p*, no preference is apparent for[13] $^4C_1$ *vs.* $^1C_4$, the α-D-Gal*p* substituent on equatorial O-3 appears to anchor the conformation in the $^4C_1$ mode, which is the one exclusively expected for both α-L-Ara*p*[14] and 3-*O*-α-D-Gal-L-Ara*p*.

Interestingly, the effect of equilibration is expressed throughout the molecule, as some (C-1, C-5, and C-6) of the galactosyl carbon resonances are twinned in the appropriate ratio. Corresponding carbon signals have been observed as twinned for a (1→3)-linked disaccharide, in dimethyl sulfoxide solution[15]. Whereas twinning of the anomeric carbon signal of the nonreducing moiety of a disaccharide in which the reducing residue is substituted by glycosylation at O-2 has been observed[11,16], the present example is remarkable in that the linkage is to O-3, and both C-5 and C-6 are implicated, as well as C-1. Twinning was removed upon reduction of the disaccharide with $NaBD_4$. Resonances (^1H and ^{13}C) of the reduced disaccharide were fully assigned (see Tables I and II) by using procedures already described. The signal of L-Ara*p* C-1 appeared as low-intensity triplet due to ^2H coupling.

L-Ara*f* anomers were present in insufficient amount to give cross-peaks in the 2D spectra of 3-*O*-α-D-galactopyranosyl-L-arabinose. The proton signals at δ 5.32 ($J_{1,2}$ 4.7 Hz) and 5.29 ($J_{1,2}$ 1.6 Hz) are attributable to H-1 of the 3-*O*-substituted β- and α-L-Ara*f*, respectively. The chemical shifts and splittings are similar to those obtained for unsubstituted arabinose (δ 5.27 and 5.26, and $J_{1,2}$ 4.1 and 1.0 Hz)[17]. The galactosyl substituent has a small effect on the equilibrium between pyranose and furanose forms; for the substituted arabinose the furanose contribution is ~9%, whereas a value of 4.5% has been quoted for free arabinose[17]. The furanose

TABLE I

^{13}C ASSIGNMENTS OF 3-*O*-α-D-GALACTOPYRANOSYL-L-ARABINOSE (**1**) AND THE CORRESPONDING SUBSTITUTED L-ARABINITOL

Compound		*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1 (α form)	α-Gal*p*	95.95	68.87	69.85	69.73	71.38	61.52
	α-Ara*p*	97.19	70.94	77.71	65.44	66.62	
1 (β form)	α-Gal*p*	96.14	68.87	69.85	69.73	71.49	61.64
	β-Ara*p*	93.14	67.58	74.48	65.85	62.76	
1 (α form)[a]	α-Ara*f*	102.0	80.7	83.2[b]	83.6[b]	62.0	
1 (β form)[a]	β-Ara*f*	[c]	75.8	81.3[d]	82.4[d]	63.6	
1 (reduced)	α-Gal*p*	99.49	69.19	69.92	69.92	72.57	61.75
	Ara-ol	63.20	71.41	78.65	72.24	62.95	

[a]Only C-1 of Gal*p* could be assigned (see text). [b]Assignments might have to be reversed. [c]Obscured by C-1 of α-Gal*p* attached to Ara*p* (see Fig. 2). [d]Assignments might have to be reversed.

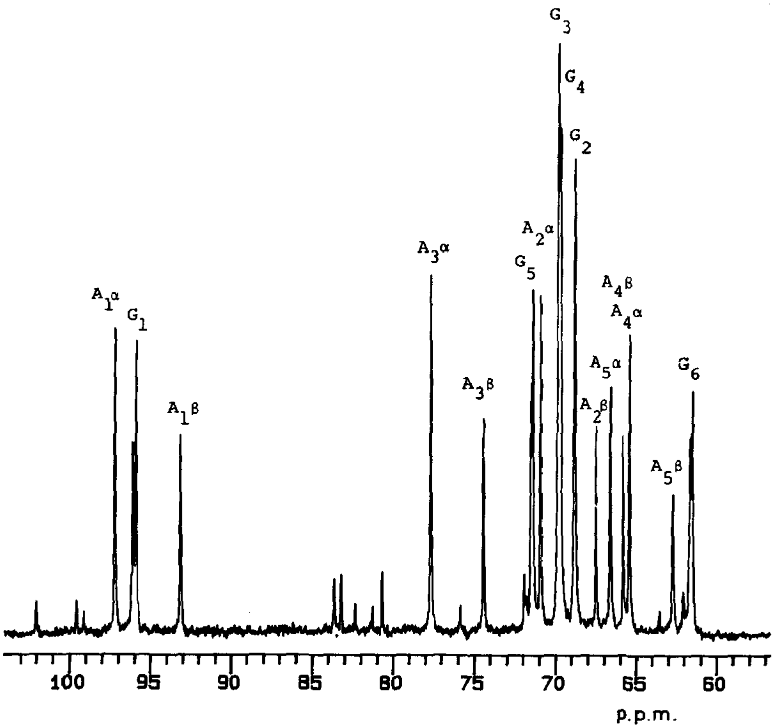


Fig. 2. $^{13}C\{^1H\}$ spectrum of **1** recorded at 50.3 MHz and 297 K (compare Fig. 3).

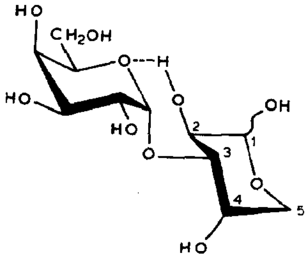


Fig. 3. A possible conformation for disaccharide **1**, on the basis of the exo-anomeric effect. The conformation of L-Ara*p*, here substituted at O-3 by α-D-Gal*p*, is in conformity with n.m.r. assignments.

forms cause the attached α-D-Gal*p* group to experience a different electronic environment, resulting in the appearance of a broad signal at δ 5.02 due to H-1 of the α-D-Gal*p* (*cf.*, δ 5.16 for H-1 of α-D-Gal*p*, where L-Ara is pyranosidic). The correctness of the interpretation was supported by recording the spectrum at 80°, at which temperature the percentage of L-Ara*f* anomers (H-1 at δ 5.27 and 5.26) and the intensity of the signal at δ 5.02 were increased. This observation of the twinning of proton signals emanating from a glycosyl group linked to a reducing sugar had been reported *e.g.*, for an oligosaccharide containing a mannosyl group attached to O-3 of galactose as the reducing-terminal sugar[18].

TABLE II

^{1}H ASSIGNMENTS OF 3-*O*-α-D-GALACTOPYRANOSYL-L-ARABINOSE (**1**) AND THE CORRESPONDING SUBSTITUTED L-ARABINITOL

Compound		*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
1 (α and β forms)	α-Gal*p*	5.16	3.88	3.88–3.96	3.96–4.0	4.18	3.75
	α-Ara*p*	4.56	3.64	3.75	4.18	3.68–3.75	
	β-Ara*p*	5.26	3.95	3.87	4.22	3.94–4.0	
1 (reduced)	α-Gal*p*	5.16	3.85	3.98	3.84	3.96	3.68
	Ara-ol	[a]	3.72	3.80	3.96	3.74	

[a]Not detected, due to fast relaxation resulting from geminal deuterium.

The presence of the L-Ara*f* anomers in the disaccharide was most evident from the ^{13}C-n.m.r. spectrum (see Fig. 2), which showed signals of low intensity (unlabelled) for all the carbon atoms of α- and β-L-Ara*f* (see Table I), with the position of glycosylation being revealed by the downfield shift of C-3. The only observable effect of the α- and β-L-Ara*f* forms on the attached α-D-Gal*p* group was the downfield shift of the anomeric carbon atom (δ 99.6 and 99.1, respectively; *cf.*, Table I for the chemical shifts of α-D-Gal*p*, where L-arabinose is pyranosidic). These signals were modified as expected upon reduction of the disaccharide.

EXPERIMENTAL

The disaccharide (**1**) was isolated from a sample of *Watsonia* corm-sac polysaccharide, and identified as previously described[6]. Confirmation was obtained by using the Morrison procedure[19], whereby the peracetylated alditol of arabinose and the peracetate of galactononitrile were characterized by g.l.c. analysis (capillary column of DB-225 at 215°). The position of linkage was confirmed by methylation analysis of the NaBD$_4$-reduced disaccharide. G.l.c.–m.s. of the derived alditol acetates showed two peaks at retention times 0.40 (56%) and 1.00 (44%), corresponding to the acetylated derivatives of C-1-deuterated 1,2,4,5-tetra-*O*-methylarabinitol and 2,3,4,6-tetra-*O*-methylgalactitol, respectively. E.i.-m.s. of permethylated, borodeuteride-reduced **1** showed the following diagnostic[20,21] fragments: *m/z* 219 (bA$_1$), 187 (bA$_2$), 155 (bA$_3$); 252 (aJ$_1$), 192 (aJ$_2$); 46, 381, 349; and 45, 382, 350 (ald).

A solution of **1** in D$_2$O was freeze-dried, and the process was repeated three times, in order to replace exchangeable hydrogen atoms with deuterium. Spectra (^{1}H- and ^{13}C-n.m.r.) were recorded for solutions in D$_2$O (286 mg/mL) with a Varian VXR-200 spectrometer and with acetone as an internal reference (δ 2.21 for ^{1}H, and 31.0 for ^{13}C). Coupling constants were determined by using a diluted solution.

ACKNOWLEDGMENTS

We thank the Foundation for Research Development of the Council for Scientific and Industrial Research and the Council of the University of Cape Town for their financial support.

REFERENCES

1 A. BAX, R. FREEMAN, AND G. A. MORRIS, *J. Magn. Reson.*, 42 (1981) 164–168.
2 A. BAX, *J. Magn. Reson.*, 53 (1983) 517–520.
3 F. SMITH, *J. Chem. Soc.*, (1939) 744–753.
4 J. K. N. JONES, *J. Chem. Soc.*, (1953) 1672–1675.
5 A. J. CHARLSON, J. R. NUNN, AND A. M. STEPHEN, *J. Chem. Soc.*, (1955) 269–273.
6 D. H. SHAW, A. M. STEPHEN, AND (in part) A. O. FULLER, *J. Chem. Soc.*, (1965) 2287–2289.
7 D. H. SHAW AND A. M. STEPHEN, *Carbohydr. Res.*, 1 (1966) 400–413.
8 D. H. SHAW AND A. M. STEPHEN, *S. Afr. Ind. Chem.*, 18 (1964) 65.
9 R. U. LEMIEUX AND J. D. STEVENS, *Can. J. Chem.*, 44 (1966) 249–262.
10 J. F. STODDART, *Stereochemistry of Carbohydrates*, Wiley, New York, 1971, pp. 160–192.
11 E. PETRÁKOVÁ AND P. KOVÁČ, *Chem. Zvesti*, 35 (1981) 551–566.
12 J. H. BRADBURY AND G. A. JENKINS, *Carbohydr. Res.*, 126 (1984) 125–156.
13 S. L. PATT AND J. N. SHOOLERY, *J. Magn. Reson.*, 46 (1982) 535–539.
14 R. J. FERRIER AND P. M. COLLINS, *Monosaccharide Chemistry*, Penguin Books, London, 1972.
15 R. E. HOFFMAN, J. C. CHRISTOPHIDES, D. B. DAVIES, AND C. J. LAWSON, *Carbohydr. Res.*, 153 (1986) 1–16.
16 T. USUI, N. YAMAOKA, K. MATSUDA, K. TUZIMURA, H. SUGIYAMA, AND S. SETO, *J. Chem. Soc., Perkin Trans. 1*, (1973) 2425–2432.
17 S. J. ANGYAL AND V. A. PICKLES, *Aust. J. Chem.*, 25 (1972) 1695–1710.
18 G. G. S. DUTTON, H. PAROLIS, J.-P. JOSELEAU, AND M.-F. MARAIS, *Carbohydr. Res.*, 149 (1986) 411–423.
19 I. A. MORRISON, *J. Chromatogr.*, 108 (1975) 361–364.
20 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
21 P. ÅMAN, M. MCNEIL, L.-E. FRANZÉN, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 95 (1981) 263–282.