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Complexes of sodium vanadate(V) with methyl α-D-mannopyranoside, methyl α- and β-D-galactopyranoside, and selected *O*-methyl derivatives: a ^{51}V and ^{13}C NMR study

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Abstract

The hydroxyl group stereochemistry of complexation of sodium vanadate(V) with Me α-Man*p*, Me α- and β-Gal*p* and selected *O*-methyl derivatives in D_2O was determined by ^{51}V, 1D and 2D ^{13}C NMR spectroscopy at pD 7.8. The ^{51}V approach served to show the extent of complexation and the minimum number of esters formed. That of Me α-Man*p* gave rise mainly to a ^{51}V signal at δ −515, identical with that of its 4,6-di-*O*-methyl derivative, which had only a 2,3-*cis*-diol exposed. The ^{13}C NMR spectra contained much weaker signals of the complexes, but both glycosides showed strong C-2 and C-3 α-shifts of +17.3 and +10.8 ppm, respectively. As expected, Me 2,3-Me_2-α-Man*p*, which contains a 4,6-diol, did not complex. Me Gal*p* anomers and their derivatives showed more diversity in the structure of its oxyvanadium derivatives. Me α-Gal*p*, with its 3,4-*cis*-diol, complexed to give rise to ^{51}V signals at δ −495 (9%), −508 (10%), and −534 (4%). These shifts and proportions were maintained with Me β-Gal*p* and Me 6Me-α-Gal*p*. ^{51}V NMR spectroscopy showed that Me 3Me-β-Gal*p*, with its possibly available 4,6-diol, did not complex. Similarly, Me α-Gal*p*+vanadate gave a ^{13}C DEPT spectrum that did not contain an inverted signal at δ >71.4, as would be expected of a C-6 resonance suffering a strong downfield α-shift. Me 2,6-Me_2-α-Gal*p*, with a 3,4-*cis*-diol group, gave rise to two ^{51}V signals of complexes at δ −492 (9%) and −508 (9%), showing more than one structure of oxyvanadium derivatives.

Keywords: Me α-Man*p*; Me α- and β-Gal*p*; OMe ethers; VO_3(V) complexes; Structures; ^{51}V, ^{13}C NMR spectroscopy

1. Introduction

Our interest in vanadate complexes of carbohydrates arose since many biochemical and physiological functions have been suggested for vanadium, although it does not yet have a clearly defined role in higher organisms.[1] Recently a complex of oxyvanadium with a lichen galactomannan showed activity related to peritoneal macrophages and leishmanicidal activity.[2] In order to determine the monosaccharide units capable of forming vanadate complexes, model methyl α-D-mannopyranoside, methyl α- and β-D-galactopyranoside, and selected *O*-methyl derivatives are examined by ^{51}V and ^{13}C NMR spectroscopy. The solvent employed was deuterium oxide, which is analogous to water present in physiological systems.

The most pertinent investigation on complexation in aqueous systems is by Tracey and Gresser[3] who used ^{51}V NMR spectroscopy to investigate the stereospecificity of complexation of vanadate(V) with cyclohexanediols and methyl glycosides. They found that *cis*-cyclohexane-1,2-diol complexed more strongly than the trans-isomer at pH 8.5, as shown by the NMR signals of the complexes, which appeared downfield from those of $NaVO_3$ oligomers. Configurational restraints of hydroxyl groups would be expected with Me α- and β-Glc*p*, which contain trans-vicinal OH and 4,6-OH groups, and these complexed less strongly than Me β-Man*p*, with a *cis*-2,3-OH, and α- and β-isomers of Me Gal*p*, with *cis*-3,4-OH groups. It was suggested that its proximity of

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OH-6 was sufficient for involvement in complexation.[3] This possibility is now investigated using a combination of ^{51}V and ^{13}C NMR spectroscopy, each with its peculiar application in structural investigations.

2. Results

A preliminary ^{51}V NMR experiment was carried out on a D_2O solution of sodium vanadate(V) alone (pD 7.8), and this gave a mixture of signals at δ −549 (1%), −565 (2%), −570 (78%), and −578 (19%), all attributed to vanadate oligomers.[3,4]

2.1. Complexation of vanadate with methyl α-D-mannopyranoside and its *O*-methyl derivatives

2.1.1. Methyl α-D-mannopyranoside + sodium vanadate. On admixture of $NaVO_3$ with an excess of Me α-Man*p* (1:3 molar ratio), complexation occurred as previously observed with the β anomer to give rise to ^{51}V signals at lower field.[3] These were at δ −515 (16%), −506 (2%) and −499 (1%) (Fig. 1(A)), although on adjusting the pD to 7.0, complexation was more pronounced, with the main signal at δ −515 (20%) being accompanied by minor ones in greater proportions at δ −499 (12%) and −492 (5%) (spectrum not shown). As the complexation at pD 7.8 promised to be more specific, it was maintained in the following experiments.

The ^{13}C NMR spectrum of complexed Me α-Man*p* (Fig. 2(A)) contained signals less intense, when compared with those of the ^{51}V spectrum. However, they served to elucidate the substitution positions. The ^{13}C signals of the glycoside were compared with those of the complex and identified by a combination of ^{1}H(obsd)^{13}C HMQC and ^{1}H TOCSY (correlation was not obtained with COSY). The H-1 signal of the complex at δ 5.43 correlated with H-2 at δ 4.20 and in turn with H-3 at δ 3.61. HMQC showed respective correlations with C-2 (δ 87.4; α-shift +17.3 ppm) and C-3 (δ 80.0; α-shift +10.8 ppm, respectively).

Other signals readily identified were at δ 98.9 (C-1; β-shift −1.1 ppm) and 54.1 (OCH_3-1; shift +0.2 ppm). A minor DEPT-inverted C-6 signal appeared at δ 65.3, although its shift was low and could not arise from direct vanadate esterification at OH-6.

2.1.2. Methyl 4,6-di-*O*-methyl-α-D-mannopyranoside + sodium vanadate. ^{51}V NMR spectrometry provided signals of vanadate pentamers at δ −578 (23%) and tetramers at δ −570 (68%), and that of a complex at δ −517 (23%) (Fig. 1(B)), whose shift corresponds to that of the main signal formed with Me α-Man*p* (Fig. 1(A)).

The ^{13}C signals of the glycoside and minor ones of its complex (not shown) were compared, those of the latter appearing at δ 99.3 (C-1; β-shift −1.1 ppm), 87.8 (C-2; α-shift +18.1 ppm), 80.2 (C-3; α-shift +10.5 ppm). These were identified by the above approach using signals of H-1 (δ 5.44) with TOCSY-identified H-2 (δ 4.19), and H-3 (δ 4.10).

2.1.3. Methyl 2,3-di-*O*-methyl-α-D-mannopyranoside + sodium vanadate. Complexation of the 4,6-diol group did not occur, since ^{51}V signals did not appear in the $\delta > -549$ region (not shown).

2.1.4. Methyl 6-*O*-methyl α-D-mannopyranoside + sodium vanadate. Its *cis*-2,3-diol group gave rise to an expected ^{51}V NMR signal at δ −515 (12%) and others at δ −507 (4%) and −491 (2%) (Fig. 1(C)).

In its ^{13}C NMR spectrum (not shown), minor signals appeared at δ 99.4 (C-1; β-shift −2.0 ppm), 87.7 (C-2; α-shift +17.4 ppm), and 80.4 (C-3; α-shift +9.7 ppm)

2.1.5. Methyl 2-*O*- and 3-*O*-methyl-α-D-mannopyranosides, each + sodium vanadate. These derivatives, with only the unfavorable 4,6-diol geometry available, showed zero and only 1% complexation (signal at δ −506) in their ^{51}V NMR spectra, respectively (not shown).

2.2. Complexation of methyl α- and β-D-galactopyranoside and their *O*-methyl derivatives with vanadate

2.2.1. Methyl α- and β-D-galactopyranosides, each + sodium vanadate. As previously shown,[3] the galactosides readily complexed to give a variety of products, which could depend on the substitution positions and the structure of the oxyvanadate complex. At pH 7.8, we found that Me α-Gal*p* formed three ^{51}V signals of complexes at δ −495 (9%), −508 (10%), and −535 (4%), with those of unreacted vanadate at δ −570 (59%) and −578 (18%) (Fig. 1(D)), which is similar to the β anomer (Fig. 1(E)) that gave rise to three signals at δ −493 (10%), −509 (9%), and −534 (3%) (plus δ −570 (58%), and −578 (20%)).

The complexes of Me β-Gal*p* gave rise to small ^{13}C signals at δ 83.2 and 89.1 (Fig. 2(B)), which did not lend themselves to the COSY–TOCSY–HMQC approach for identification. However, comparison of their relative shifts with those of uncomplexed Me β-Gal*p* at δ 69.7 (C-4) and 73.0 (C-3), suggested the assignments shown in Table 1. Other smaller signals of the complex were at δ 78.9 and 80.3, but were not identified.

^{13}C signals of vanadate esters of Me α-Gal*p* appeared at δ 86.3, and 83.4 (Fig. 2(C)), which again could not be assigned by the COSY–TOCSY–HMQC approach. Other smaller signals were present at δ 80.3, and 79.3. Inverted DEPT signals of complexed C-6 were not detected at $\delta > 71.4$

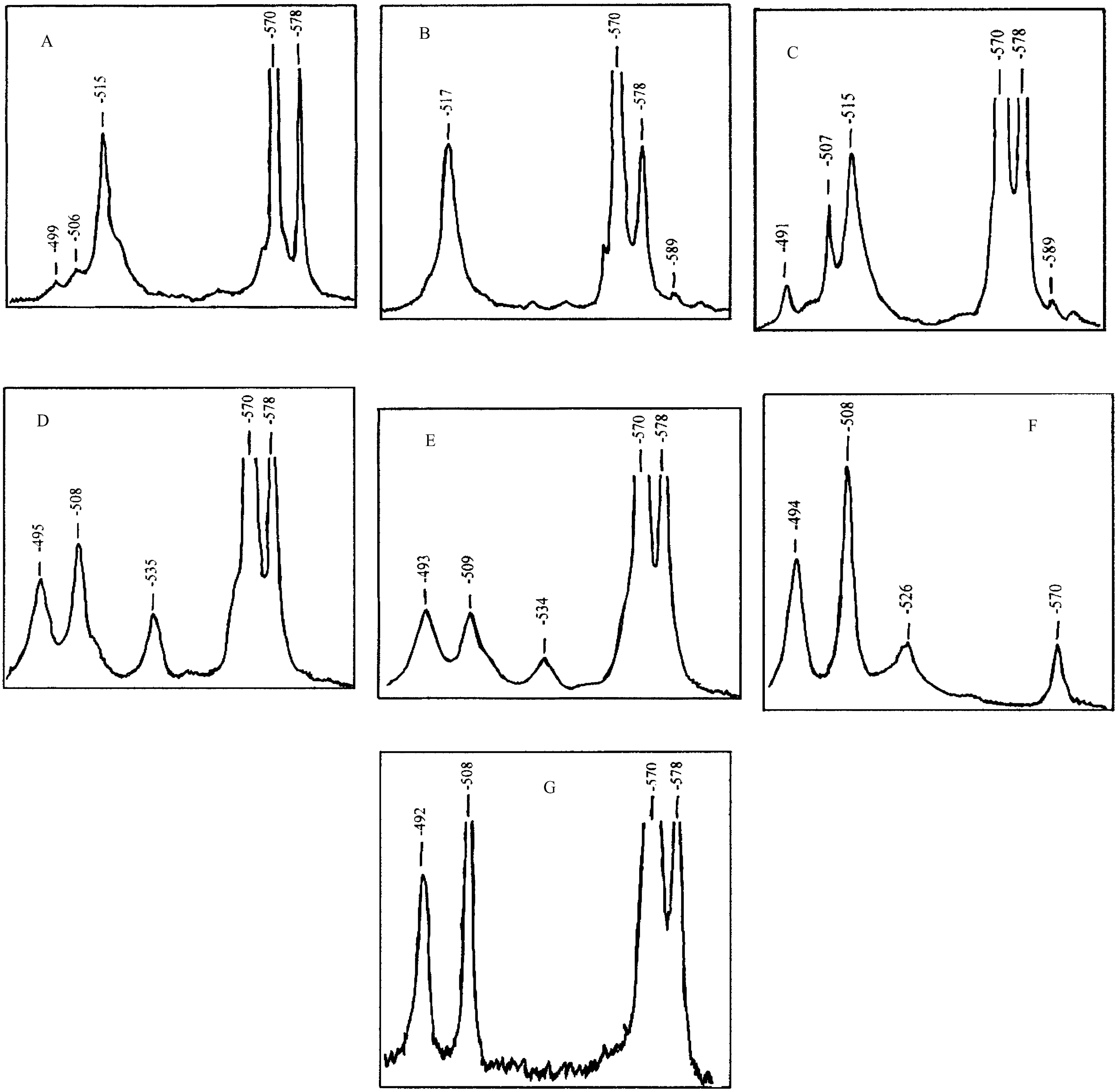

Fig. 1. ^{51}V NMR spectra of complexes of sodium vanadate(V) with Me α-Man*p* (A), Me 4,6-Me$_2$-α-Man*p* (B), Me 6Me-α-Manp (C), Me α-Gal*p* (D), Me β-Gal*p* (E), Me 6Me-α-Gal*p* (F), and Me 2,6-Me$_2$-α-Gal*p* (G).

2.2.2. Methyl 3-*O*-methyl-β-D-galactopyranoside + sodium vanadate. The ^{51}V NMR spectrum did not contain signals of a complex (not shown), showing that the 4,6-OH group did not esterify.

2.2.3. Methyl 6-*O*-methyl-α-D-galactopyranoside + sodium vanadate. The ^{51}V spectrum (Fig. 1(F)) contained signals at −494 (33%), −508 (37%), and −526 (7%), which is a similar relative proportion to that obtained for complexes of α and β anomers of Me Gal*p* and Me 6Me-α-Gal*p*. The ^{13}C NMR spectrum (not shown) contained minor signals at δ 83.3 and 89.4.

2.2.4. Methyl 2,6-di-*O*-methyl-α-D-galactopyranoside + sodium vanadate. The ^{51}V spectrum (Fig. 1(G)) contained signals at δ −492 (9%) and −508 (9%) showing that its 3,4-*cis*-diol was substituted by different forms of vanadate adduct. Signals of the complex were present at δ 80.2 and 88.5 in the ^{13}C spectrum (not shown).

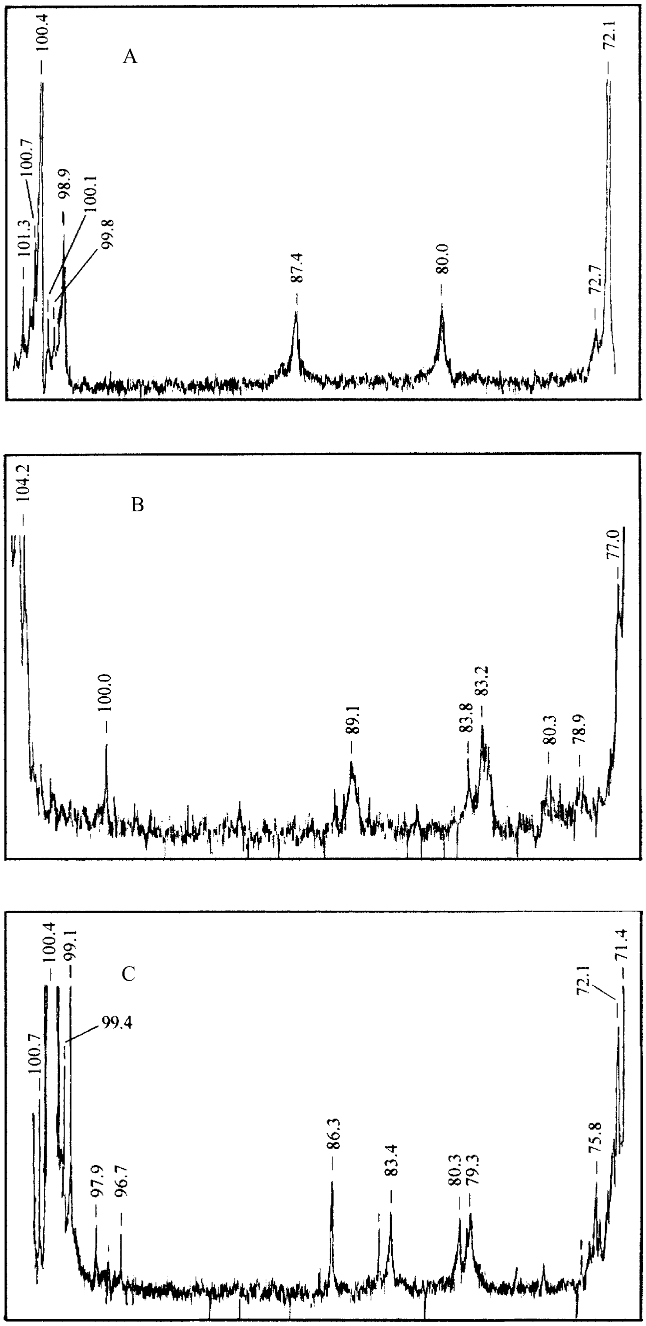

Fig. 2. Partial ^{13}C NMR spectra of complexes of sodium vanadate(V) with Me α-Man*p* (A), Me β-Gal*p* (B), and Me α-Gal*p* (C).

3. Discussion

The ^{51}V NMR spectrum of sodium vanadate+Me α-Man*p* in D_2O solution at pD 7.8 showed complexation with one principal signal at δ −515 (Fig. 1(A), Table 1), being downfield from those of vanadate oligomers. The spectrum of the 4,6-di-*O*-methyl derivative of Me α-Man*p*, with its isolated 2,3-*cis*-diol, also contained this signal, as did its 6-*O*-methyl derivative, although a small proportion of a signal at δ −505 also appeared (Table 1, Fig. 1(B)). The absence of ^{51}V signals of complexes with 2-*O*-, 3-*O*-, and 2,3-di-*O*-methyl derivatives showed, as expected,[3] that little or no 4,6-esterification occurred.

Table 1
Chemical shifts of ^{51}V and ^{13}C NMR signals formed by sodium vanadate complexes

Glycoside [a]	^{51}V NMR signals [b,c]	^{13}C NMR signals:assignments [c]
Me α-Man*p*	−499 (1%), −506 (2%), −515 (16%)	98.9 (C-1), 80.0 (C-3), 87.4 (C-2)
Me 6Me-α-Man*p*	−491 (2%), −507 (4%), −515 (12%)	99.4 (C-1), 87.7 (C-2), 80.4 (C-3)
Me 4,6-Me$_2$-α-Man*p*	−517 (23%)	99.3 (C-1), 87.8 (C-2), 80.2 (C-3)
Me α-Gal*p*	−495 (9%), −508 (10%), −535 (4%)	86.3, 83.4, 80.3, 79.3
Me β-Gal*p*	−493 (10%), −509 (9%), −534 (3%)	89.1 (C-3), 83.2 (C-4), 80.3, 78.9
Me 6Me-α-Gal*p*	−494 (33%), −508 (37%), −526 (7%)	82.3, 89.4
Me 2,6-Me$_2$-α-Gal*p*	−492 (9%) −508 (9%)	80.2, 88.5

[a] According to their ^{51}V spectra (not shown), Me 2Me-α-Man*p*, Me 3Me-α-Man*p*, Me 2,3-Me$_2$-α-Man*p*, and Me 3Me-β-Gal*p* did not form complexes.

[b] Percentage values when taken into account those of inorganic vanadate oligomers.

[c] Shifts in δ (ppm).

The ^{13}C NMR spectrum of Me α-Man*p* plus sodium vanadate (Fig. 2(A)) contained much smaller signals of the complexes than would be expected from the ^{51}V data (compare with Fig. 1(A)), perhaps explaining why they have not been previously investigated in aqueous solutions. This property is not due to long T_1 values, as their intensity was not increased on extending the delay time from 2 to 5 s, leaving the explanation that the exchange rate in the equilibrium of complex formation is more rapid on the ^{13}C time scale. The TOCSY–HMQC approach showed complexation at OH-2,3 resulting in strong α-shifts of C-2 and C-3 signals of +17.3 and +10.8 ppm, respectively. These shifts are close to those of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside when compared with its $[NBu^n_4][H_2VO_4]$ derivative in CD_3CN, which were +17.4 and +9.7 ppm.[5] Similar shifts were now found with vanadate complexes of Me 4Me-α-Man*p* and Me 4,6-Me$_2$-α-Man*p*, which contain 2,3-*cis*-diols.

Three ^{51}V signals of complexes were formed with Me α- and Me β-Gal*p* (Fig. 1(D and E); Table 1), in agreement with the previous observation that a greater number of complexed species are formed with the α and β anomers of Me Gal*p* than with Me β-Man*p*.[3] Complexation with the 3,4-*cis*-diol of Me 2,6-Me$_2$-α-Gal*p* took place, since two ^{51}V signals were detected

(Table 1, Fig. 1(G)): the two signals were due to the presence of vanadium(V) complexes with different structures, since Verchère and coworkers[6] in their review, indicated that although *cis*-diols form predominant 2:2 carbohydrate-to-vanadate complexes, those with 1:1 and 1:2 ratios have also been observed. Tracey and Gresser[3] observed that the OH-6 group of Me Gal*p* could be sufficiently close to the *cis*-OH-3,4 groups to be involved in complexation. However, formation of a complex with a 4,6-diol was not now detected with Me 3Me-β-Gal*p*. A lack of involvement is also consistent with the similarity of the ^{51}V spectrum of Me 6Me-α-Gal*p* (Fig. 1(F), Table 1) with that of Me α-Gal*p* (Fig. 1(D), Table 1).

Examination of the ^{13}C NMR spectra of complexes of Me β-Gal*p* showed four nuclei of complexed species (Fig. 2(B)), whereas five appeared with Me α-Gal*p* (Fig. 2(C)). None of these could be assigned. The DEPT spectrum of Me α-Gal*p* did not contain any inverted signals at $\delta > 71.4$ from involvement of OH-6 in complexation, which would result in a strong downfield α-shift of the C-6 resonance from δ 62.5.

Geraldes and Castro,[4] observed the complexation of reducing monosaccharides with ammonium vanadate at pH 7. ^{51}V NMR spectroscopy showed that glucose complexed via its *cis*-1,2-α-diol. In the case of mannose, bidentate complexes might also have been formed, although these were not observed in the proton spectrum. A tridentate complex, encompassing the consecutive *cis*-1,2,3-triol groups having an a–e–a geometry in a $^{1}C_4$ conformation, was proposed. However, in our glycosides such a hydroxyl group arrangement is not possible.

It can be concluded that our galactomannan, which showed biological activity when mixed with vanadate,[2] formed esters in units that were not glycosidically substituted on the *cis*-4,6-diol of galactopyranose or the *cis*-2,3-diol of mannopyranose.

4. Experimental

4.1. Preparation of partially *O*-methylated methylglycosides

Me 2,3-Man$_2$-Man*p*[7] and Me 4,6-Me$_2$-α-Man*p*[8,9] were prepared according to the literature.

Me 6Me-α-Man*p* was prepared from Me α-Man*p* by successive tritylation, benzylation with benzyl bromide–Ag$_2$O in DMF, detritylation with 80% HOAc–H$_2$O at 100 °C, methylation,[10] and debenzylation with Pd/H$_2$ in HOAc.

Me 2Me-α-Man*p* and Me 3Me-α-Man*p* were prepared by the action of refluxing 3% MeOH–HCl on 2Me-Man[11] and 3Me-Man,[12] respectively.

Me 3Me-β-Gal*p* was obtained by successive methylation[10] and methanolysis with 3% MeOH–HCl under reflux, of 1,2-5,6-di-*O*-isopropylidene α-galactofuranose.[13] The product gave rise to a C-1 signal of the β anomer at δ 104.5.

Me 6Me-α-Gal*p* was obtained starting from 1,2-3,4-di-*O*-isopropylidene-α-D-galactopyranose (0.40 g),[14] which was methylated[10] and the product treated with MeOH (20 mL) containing *p*-toluenesulfonic acid (0.20 g) under reflux for 2 h. The solution was then added to excess water, which was deionized and evaporated to give a product (0.32 g), which was the α anomer of Me 6MeGal*p* with a C-1 signal at δ 98.6.

Me 2,6-Me$_2$-α-Gal*p* was prepared starting from methyl α-D-galactopyranoside (2.0 g), which was stirred with acetone (200 mL) containing ZnCl$_2$ (2.0 g) and H$_2$SO$_4$ for 3 h. This method was used as previously cited methods did not function.[15] Pyridine (10 mL) was then added, and the suspension was evaporated to a residue that was partitioned between EtOAc and H$_2$O. The former was washed with H$_2$O and evaporated to a syrup (0.95 g). A portion (0.33 g) was methylated,[10] and the product was partly hydrolyzed with aq H$_2$SO$_4$ at pH 2.0 at 100 °C for 2 h. The solution was neutralized (BaCO$_3$) and filtered, and the filtrate was extracted with hexane. The aqueous layer was evaporated to a syrup (0.24 g).

4.2. Preparation of solutions of methyl glycosides with sodium vanadate

Sodium vanadate (20 mg) was dissolved in D$_2$O (0.2 mL) by heating at 100 °C in a 5-mm NMR tube. After cooling, a solution of 3 molar equiv of methyl glycoside in D$_2$O (0.3 mL) was then added.

4.3. NMR spectroscopy

This was carried out using a Bruker DRX-400 Avance spectrometer. ^{13}C spectra were obtained at 100.6 MHz with 1 s delay and 0.6 s acquisition times, and 80,000 transients, necessary to detect the minor signals of vanadate complexes. ^{1}H,^{1}H TOCSY and COSY and ^{1}H(obsd),^{13}C HMQC procedures were as those described in the Bruker Manual. Chemical shifts are expressed as δ (ppm) relative to that of Me$_4$Si ($\delta = 0$).

^{51}V spectra were obtained at 105.2 MHz, with 0.5 s delay and 0.10 s acquisition times, and 1000 transients. Chemical shifts are in δ (ppm) relative to that of VOCl$_3$ ($\delta = 0$).

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