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^{31}P-N.m.r. spectroscopy in wood chemistry‡. Phosphite derivatives of carbohydrates

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ABSTRACT

Selected acyclic and cyclic polyols, monosaccharides, disaccharides, higher oligosaccharides, and model compounds for lignin–carbohydrate complexes were reacted with 1,3,2-dioxaphospholanyl chloride, and the ^{31}P-n.m.r. spectra of the derivatives were obtained in order to correlate the signals with the structural details of the compounds. The derivatives of most of the carbohydrates exhibited spectra containing well resolved signals in the range 138–132 p.p.m., and the number of lines accorded with the number of hydroxyl groups in the respective compounds. The chemical shift values of the phosphorus atoms at C-1 in derivatives of monosaccharides were characteristic for the anomer involved. Since completely resolved spectra with signals characteristic for the sugars and the lignin-related parts, respectively, of relevant model compounds were obtained, the method seems very suitable for the structural elucidation of molecules derived from lignin–carbohydrate complexes. The cyclodextrins (cyclomalto-hexa-, -hepta-, and -octaose) were derivatized, and on examination gave spectra dramatically differing from each other.

INTRODUCTION

In our earlier work, the ^{31}P-n.m.r. chemical shifts of lignin model compounds derivatized with 1,3,2-dioxaphospholanyl chloride (**1**, Scheme 1) were found to be sensitive to the chemical environment of the phosphitylated centre[1]. These phenols, alcohols, and simple carboxylic acids gave derivatives whose ^{31}P-n.m.r. signals appeared in regions sufficiently well separated to permit distinguishing among them. Furthermore, the method of derivatization with **1** showed promise for determining fine-structural details in lignin model compounds. For example, it could distinguish among primary, secondary, and tertiary alcohols, as well as *erythro* and *threo* forms in arylglycerol β-aryl ethers. Furthermore, for a variety of cyclic, aromatic, and alicyclic compounds that contained hydroxyl groups on adjacent carbon atoms, the ^{31}P-n.m.r. chemical shifts of their derivatives with **1** were downfield compared to the shifts of their

‡ Part II. For Part I see ref. 1.
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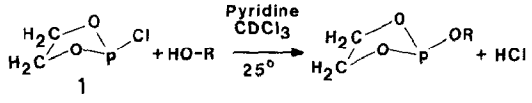
monofunctional analogues. Derivatives of **1** with symmetrical diols having hydroxyls attached to adjacent carbons gave only one ^{31}P signal. Examples include ethylene glycol, 1,4-dioxane-2,3-diol, *cis*-cyclohexane-1,2-diol, and *trans*-cycloheptane-1,2-diol (Table I). The ^{31}P-signals of these derivatives were shifted downfield by about 0.7 p.p.m. from the signal expected of their monosubstituted relatives. Such downfield shifts were more pronounced among glycols having a substituent at C-2, as shown by phenylethane-1,2-diol [$C_6H_5CH(OH)CH_2(OH)$] (ref. 1). For these glycols the downfield shifts of the phosphorus atoms at their primary centers were about 1.5 p.p.m., somewhat less for the secondary centers.

These observations gave the primary indication that the structures of carbohydrate molecules might also be investigated by derivatization with **1**, since it was known that carbohydrates formed stable compounds with reagents based on trivalent phosphorus[2]. In this work, the ^{31}P chemical shifts of derivatives of **1** with simple monosaccharides, oligosaccharides, and lignin–carbohydrate model compounds were examined in order to correlate the results with structural details of the carbohydrate moieties usually found in lignins.



1

R=Carbohydrate residue

Scheme 1. The derivatization reaction used throughout this work, *i.e.*, the reaction of 1,3,2-dioxaphospholanyl chloride (**1**) with hydroxyl groups in carbohydrates.

EXPERIMENTAL

All solvents and chemicals used were of reagent or analytical grade. The derivatizing reagent **1** was synthesized and purified as described previously[1]. The commercially

TABLE I

^{31}P-N.m.r. chemical shifts observed on derivatization of alcohols structurally related to carbohydrates[a]

Alcohol	^{31}P *Chemical shift (p.p.m.)*
Cyclohexanecarboxylic acid	127.2
cis-1,4-Cyclohexanedimethanol	132.3
Cyclohexylmethanol	132.5
Ethylene glycol	132.8
trans-1,3-Cyclohexanediol	133.3
cis- And *trans*-1,4-cyclohexanediol	133.7
cis-1,3-Cyclohexanediol	133.8
trans-1,2-Cycloheptanediol	134.2
cis-1,2-Cyclohexanediol	134.6
trans-1,2-Cyclohexanediol	134.1

[a] From ref. 1.

available reagent (Fluka Chemical Co.) was purified by vacuum distillation before use.

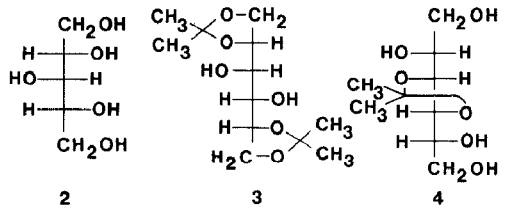
Carbohydrate samples were derivatized in 4-mL vials equipped with small magnetic stirring bars. The sample (10–15 mg) was covered with 200 μL of anhydrous pyridine and, after thorough mixing for 10–15 min, 200 μL of deuterated chloroform was added. Then, 150 μL of 1,3,2-dioxaphospholanyl chloride was added with vigorous stirring, and stirring was continued until the suspended carbohydrate particles were completely dissolved. Stirring the mixture during derivatization was found to be essential, otherwise incomplete phosphitylation was apparent in the spectra.

Aliquots of the reaction mixture were placed in 10- or 5-mm sample tubes, and the ^{31}P spectra were obtained on a Varian XL-300 n.m.r. spectrometer, operating at 121.5 MHz, by applying an inverse gated decoupling pulse sequence. The internal lock was provided by the deuterium atoms of the deuterochloroform used as the solvent. The external standard was 85% H_3PO_4, and a sweep width of 10 000 Hz was employed. The signal at 121.1 p.p.m., from the reaction product of **1** with water, was taken as the internal standard[1]. For qualitative studies, a pulse delay of 0.5 s, with a pulse width corresponding to a 45° flip angle, and a total of 128 transients were sufficient. For quantitative work, a pulse delay of 8 s and a pulse width of 90° were used. All shifts downfield from H_3PO_4 are considered positive. ^{1}H–^{31}P Heterocorrelation spectra were obtained for a variety of derivatized sugars in order to resolve assignment ambiguities. Spin lattice relaxation times were determined by the standard inversion–recovery method.

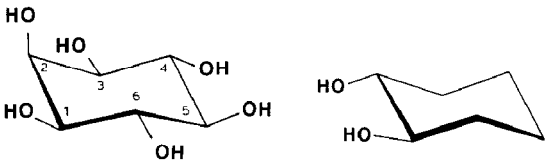
RESULTS AND DISCUSSION

Acyclic and cyclic polyols. — The ^{31}P-n.m.r. spectra of some cyclic and acyclic polyols derivatized with **1** were initially examined. Xylitol (**2**), an acyclic analogue of the simple sugars containing five hydroxyls, on phosphitylation with **1** gave only three ^{31}P-n.m.r. signals, at 132.6, 135.7, and 137.4 p.p.m., with relative intensities 2:2:1. The strong signals were assigned to the phosphorus atoms at C-1 and C-5, and C-2 and C-4, respectively, while the weaker signal was assigned to the phosphorus at C-3. This assignment was made by comparison with the spectrum of the derivative of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**3**) with **1**. This molecule, having its 1,2,5, and 6 hydroxyls blocked, gave a single ^{31}P-n.m.r. signal at 137.2 p.p.m. for the phosphorus atoms at C-3 and C-4. Furthermore, the derivative of 3,4-*O*-isopropylidene-D-mannitol (**4**), in which the hydroxyls at C-3 and C-4 are blocked, gave two ^{31}P signals, at 136.0 and 133.4 p.p.m. Thus, it is likely that the upfield signals from derivatives of **2** and **4** are due to the terminal, primary phosphite groups, in accord with our previous findings[1].

Mannitol (six hydroxyl groups) when derivatized with **1** gave five ^{31}P signals, at 139.1, 138.9, 136.4, 136.2, and 133.4 p.p.m. The intensity of the upfield signal at 133.4 p.p.m. was twice that of all others. This signal is likely due to the terminal, primary phosphite groups of the molecule, at C-1 and C-6, as its value is identical to that obtained from the same groups in the derivative of **4**. The signals at 136.2 and 136.4 p.p.m. were assigned to the C-2 and C-5 phosphites by comparison with the value



2 3 4



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obtained for the same positions in **4**. The remaining signals in the spectrum of the mannitol derivative, at 138.9 and at 139.1 p.p.m., were then attributed to the phosphorus atoms at C-3 and C-4. These chemical shifts are about 2 p.p.m. downfield from that of the derivative of **3**. The splitting of the signals for P-2 and P-5, and P-3 and P-4, which appear to be in identical environments, may be due to the presence of two different conformers of the mannitol phosphite.

It has been observed that in deuterium oxide solution the distribution of *eclipsed–gauche–trans* conformers of the hydroxymethyl groups in xylitol is 52:29:19, while in mannitol it is 54:46:0 (ref. 3). It is likely that the absence of the *trans* conformer in mannitol allows the phosphorus atoms attached to the primary centers to be more deshielded than those of the xylitol derivative.

myo-Inositol (**5**) was chosen as an example of a cyclic polyol having six hydroxyl groups. Its most stable conformer is the chair form having one axial and five equatorial hydroxyls[4–6]. The ^{31}P-n.m.r. spectrum of derivatized *myo*-inositol was rather simple, containing four signals: 137.8, 136.7, 135.6, and 134.7 p.p.m., with relative intensities of 1:1:2:2. Evidently the larger upfield signals were derived from the pairs of phosphite groups at C-4 and C-6, and at C-1 and C-3. It is likely that the signal at 134.7 p.p.m. is due to the C-4 and C-6 phosphites, since both groups are surrounded by equatorial functions. This conclusion arises from the observation that the phosphite from *trans*-1,2-cyclohexanediol (**6**), in which both substituents are equatorial, gave a signal at 134.1 p.p.m., while that from *cis*-1,2-cyclohexanediol, in which one substituent is axial, gave a signal at 134.6 p.p.m. (Table I). Hence the *myo*-inositol signal at 135.6 p.p.m. may be attributed to C-1 and C-3 phosphite groups. It is likely, therefore, that the presence of an axial substituent moves the ^{31}P chemical shift of the adjacent phosphites about 0.5–1.0 p.p.m. downfield. Accordingly, the signal at 137.8 p.p.m. may be tentatively assigned to the phosphorus atom at C-2, since it is attached to an axial oxygen. The remaining signal, at 136.7 p.p.m., may be due to the C-5 phosphite group which, however, is downfield-shifted by about 2 p.p.m. compared to its almost identical analogues at C-4 and C-6.

Monosaccharides and their derivatives. — Almost all sugars examined gave a number of weak signals sometimes overlapping with the main signals. For this reason an attempt was made to examine predominantly isolated forms of sugars.

The derivative of α-D-glucose, which has five hydroxyl groups, gave a ^{31}P spectrum containing five well separated lines of similar intensity in the range 132.4–137.8 p.p.m. (Fig. 1a and Table II). A number of small signals also appeared in the spectrum. When, however, L-glucose (mixture of α and β anomers) was examined, ten signals appeared with most of the extra lines corresponding to the weak signals of the spectrum of α-D-glucose (Fig. 1b).

To further assign the various ^{31}P resonances from the glucoses and other sugar molecules a number of substituted sugars were derivatized with **1** and their ^{31}P spectra were studied (Table II). Substitution of the C-1 hydroxyl in α-D-glucose by a methoxy group (methyl α-D-glucoside) resulted in the elimination of the signal at 133.4 p.p.m., otherwise present in the spectrum of α-D-glucose. It is likely that the C-1 phosphite group in most sugars gives rise to such an upfield signal, possibly followed by the C-6 primary phosphite.

All hydroxyl groups of β-glucose are of the equatorial conformation, while in α-glucose the anomeric hydroxyl (C-1) is in the axial conformation. Assuming that the original conformation is retained after phosphitylation with **1**, one may investigate the effect on the ^{31}P chemical shift of the inversion from equatorial to axial at C-1. An examination of the data in Table II for α-L-glucose and β-L-glucose shows that the signal for P-1 in the alpha anomer appears about 1.0 p.p.m. downfield from its beta counter-

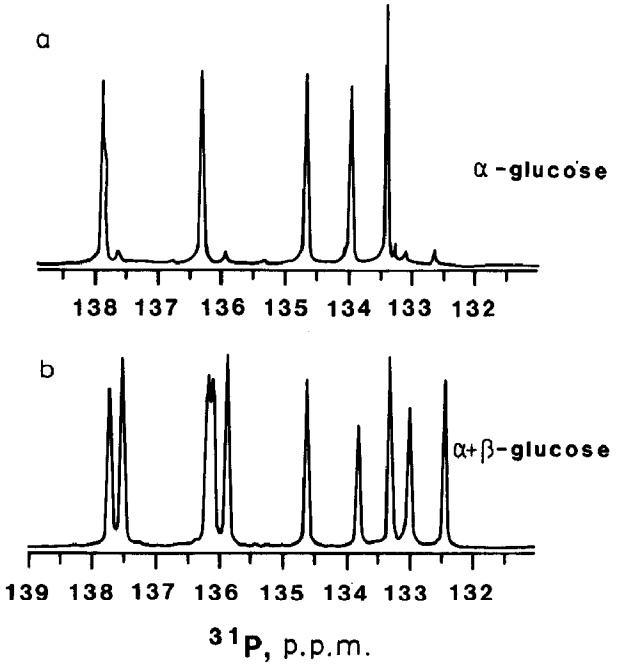


Fig. 1. ^{31}P-N.m.r. spectra of a, α-D-glucose, and b, α,β-L-glucose derivatized with **1**.

TABLE II

^{31}P-N.m.r. chemical shifts of sugars and substituted sugars derivatized with 1,3,2-dioxaphospholanyl chloride

Sugar	*Chemical shifts (p.p.m.)*				
	O-1-P	*O-2-P*	*O-3-P*	*O-4-P*	*O-6-P*
α-D-Glucose	133.4	134.4	136.3	137.8	134.0
α-L-Glucose	133.3	134.6	136.2	137.7	133.8
β-L-Glucose	132.4	135.9	136.1	137.5	133.0
Methyl α-D-glucopyranoside	subst.[a]	134.4	136.3	137.7	134.3
2-Deoxy-D-glucose	132.7	–	134.1	137.2	132.9
3-O-Me-α-D-glucose	132.7	135.1	subst.	137.3	133.5
4,6-*O*-Ethylidene-α-D-glucopyranose	133.9	135.1	135.1	subst.	subst.
Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside	subst.	135.0	135.2	subst.	subst.
D-Galactose	132.4	134.4	135.2	138.3	133.1
2-Deoxy-D-galactose	132.2	–	134.1	138.2	133.1
1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose	subst.	subst.	subst.	subst.	132.7
Methyl α-D-mannopyranoside	subst.	133.7	136.6	135.7	134.5
D-Xylose	133.5	134.4	135.7	133.8	–
L-Xylose	133.5	134.4	135.8	134.0	–
Methyl β-D-xylopyranoside	subst.	135.7	135.9	133.8	–
Methyl β-D-arabinopyranoside	subst.	134.9	136.2	134.3	
D-Lyxose	133.1	134.8	136.6	134.2	–

[a] Position not derivatizable because of substitution in the parent compound.

part. In our previous observations on *myo*-inositol and *cis*- and *trans*-1,2-cyclohexanediol it was noted that the presence of an axial substituent moves the ^{31}P chemical shift of the adjacent phosphite group about 0.5–1.0 p.p.m. downfield. This shift should also be reflected in the n.m.r. data of α- and β-glucoses, and it is for the phosphite group at C-6: the signal appeared at 133.8 p.p.m. in the α-spectrum, and 133.0 p.p.m. in the β-spectrum. It is not known, however, why the opposite effect was observed for the substituent at C-2.

The ^{31}P spectrum of the 2-deoxy-D-*arabino*-hexose (2-deoxy-D-glucose) derivative showed four clear signals, at 132.7, 134.1, 137.2, and 132.9 p.p.m., for its four phosphitylated hydroxyl groups. Since the signals at 132.9 and 132.7 are rather close to those observed for P-6 and P-1 in the β anomer of L-glucose, it is likely that the sample examined was the β form. The signal at 137.2 p.p.m. was assigned to the phosphorus atom at C-4 since it is farthest from the C-2 position, which is unsubstituted in this molecule. The C-4 position should be least affected by the deoxygenation, and the chemical shift should be closest to that observed for P-4 in β-L-glucose. The remaining signal, at 134.1 p.p.m., was assigned to the phosphorus atom at C-3, which is closest to the unsubstituted C-2 centre and thus greatly affected.

When the C-4 and C-6 hydroxyls were blocked, as in 4,6-*O*-ethylidene-α-D-glucopyranose, the remaining three derivatized hydroxyls gave two ^{31}P signals, with an

intensity ratio of 1:2. The upfield signal at 133.9 p.p.m. was assigned to the phosphite group at C-1. The downfield signal at 135.1 p.p.m. was assigned to the C-2 and C-3 phosphite groups, because the spectrum of derivatized methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, which contains only C-2 and C-3 hydroxyls, gave corresponding signals at 135.0 and at 135.2 p.p.m.

Substitution of the C-1 hydroxyl with a methyl group (as in methyl α-D-glucoside) resulted in almost no changes, as compared to α-D-glucose, in the ^{31}P chemical shifts of the phosphitylated derivative. However, a 3-*O*-methyl group (3-*O*-methyl-α-D-glucose), which unlike the terminally located 1-*O*-methyl has a central position in the molecule, had significant effects on the ^{31}P chemical shifts of the surrounding phosphite groups.

Further evidence for the assignments of the chemical shifts of phosphorus atoms attached at the various positions of monosaccharides was obtained by determining some ^{31}P spin-lattice relaxation times (T_1) of the phosphitylated anomers of L-glucose (Table III). The longest T_1 values were those of the ^{31}P atoms attached at C-6. Since these are isolated and free-rotating this result agrees with theory. The next longest relaxation times were those of the ^{31}P atoms at C-1, since they have only one adjacent phosphite group. The phosphorus at C-4 exhibited the shortest relaxation time.

The spectrum of derivatized D-xylose consisted of only four signals, at 133.5, 134.4, 135.7, and 133.8 p.p.m., indicating that the compound was predominantly in one anomeric form. In the light of the spectra of α-D- and α, β-L-glucose (Fig. 1) it is likely that the xylose sample was the α anomer, because its C-1 phosphite group gave a signal at 133.5 p.p.m., which is very close to that of the C-1 phosphite in derivatized α-D-glucose (133.4 p.p.m.). The β anomer of L-glucose gave a signal for the C-1 phosphite further upfield, at 132.4 p.p.m. Since xylose contains no hydroxymethyl group at C-5, the effect of this (derivatized) group on the chemical shifts of the phosphite groups at the remaining positions may be examined by comparing the spectrum of the derivative with that from α-D-glucose. No real differences in the chemical shifts of the phosphite groups at C-1 and C-2 resulted from the removal of the hydroxymethyl group at C-5, but the signals of the groups at C-3 and C-4 were greatly affected. In the 5-unsubstituted xylose derivative, both signals were shifted upfield.

D-Galactose and D-glucose are epimers, differing in their stereochemistry at C-4. The spectrum of derivatized D-galactose indicated that the sample was the β anomer, since the signals at 132.4 and 133.1 p.p.m. were closest to the signals of P-1 and P-6, respectively, of the β anomer of derivatized L-glucose (Table II). Significant differences between the two epimers were observed in the P-2, P-3, and P-4 signals. The signal at 134.4 p.p.m. in the D-galactose spectrum was attributed to the C-2 phosphite group, since this signal was eliminated when 2-deoxy-D-*lyxo*-hexose (2-deoxy-D-galactose) was examined. A downfield shift of about 0.8 p.p.m. is apparent for the C-4 phosphite when comparing the spectra of derivatized D-galactose and β-L-glucose.

Investigations of methyl β-D-xylopyranosides and methyl β-D-arabinopyranoside revealed significant effects of steric inversion at the C-2 and C-3 positions. Since the P-4 signals from derivatized D- and L-xylose were assigned as those adjacent to and downfield from the P-1 signals (which were the most upfield), the most upfield signals

TABLE III

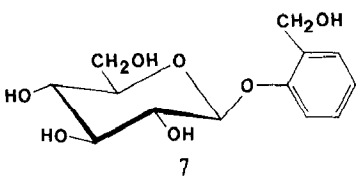
^{31}P Spin-lattice relaxation times (T_1) of anomeric L-glucose phosphites

^{31}P Location	*Anomer*	*Shift (p.p.m.)*	*Relaxation time (s)*
O-6	α	133.8	3.20
O-6	β	133.0	3.40
O-1	α	133.3	2.51
O-1	β	132.4	2.60
O-4	α	137.7	1.68
O-4	β	137.5	1.56

from derivatized methyl β-D-xylopyranoside and methyl β-D-arabinopyranoside, at 133.8 and 134.3 p.p.m., respectively, were also assigned to their C-4 phosphite groups. Similarly, the most downfield signals were assigned to the C-3 phosphites, based on observations on D- and L-xylose. The remaining signals in the methyl β-D-xylopyranoside and methyl β-D-arabinopyranoside spectra, at 135.7 and 134.9 p.p.m., respectively, were assigned to the C-2 phosphite groups.

Fully substituted sugar monomers or polymers such as D-glucose pentaacetate and tri-*O*-methylcellulose gave no ^{31}P signals after treatment with **1**.

Model compounds for lignin-carbohydrate complexes. — Among the prevailing questions in lignin chemistry is how cellulose and hemicelluloses are bound to lignin. Therefore, it was thought appropriate to examine the potential of the present method to reveal structural details in three relevant model compounds: salicin (**7**), rhapontin (**8**), and phloridzin (**9**). All three compounds were easily derivatized with **1**, and gave ^{31}P spectra in which the number of signals was equal to the number of hydroxyl groups in the compound (Table IV).



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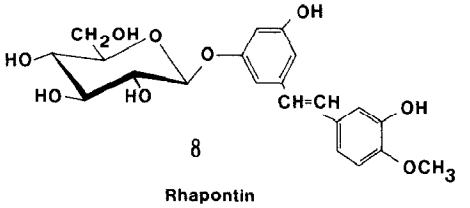
Salicin



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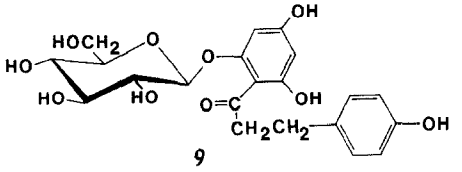
Rhapontin



9
Phloridzin

Salicin (**7**), containing one benzylic and four β-glucose hydroxyl groups, gave five signals, of which four were characteristic of the β-glucose moiety (Table II), and one was characteristic of benzylic alcohols[1]. The signal of the C-3 phosphite group in the sugar part of this molecule was shifted downfield by about 0.8 p.p.m. compared to that of the free sugar (β-L-glucose P-3, 136.1 p.p.m.). Furthermore, the signal at 131.4 p.p.m., assigned to the benzylic phosphite group of salicin, is shifted 1.4 p.p.m. upfield of that of 1,4-bezenedimethanol (132.8 p.p.m.)[1].

Rhapontin (**8**) contains two aromatic hydroxyls, one unhindered and one of the guaiacol type. It gave two distinct signals, characteristic of these two environments, at 130.2 and 128.3 p.p.m. This accords with our earlier work, in which the signal for the unhindered group appeared at 128.1 p.p.m., and the guaiacol signal at 130.1 p.p.m.[1] Similarly phloridzin (**9**), containing three aromatic hydroxyls, gave three distinct signals, at 129.5, 128.4, and 128.0 p.p.m. The signal at 129.5 p.p.m. may be assigned to the phosphite group that has a neighbouring *ortho* substituent. The other two signals are characteristic of unhindered phosphites[1].

The phosphite groups in the sugar moieties of rhapontin (**8**) and phloridzin (**9**) exhibited signals similar to those of salicin, thus confirming that in all three molecules the glucose units are in their β form.

Oligosaccharides. — Disaccharides and higher oligosaccharides were rapidly derivatized with **1**. The ^{31}P-n.m.r. spectra of these derivatives contained signals in accord with their structural features. Because of the number of signals in each spectrum many of the signals were very close to each other. However, complete assignment was accomplished with the aid of ^{1}H–^{31}P heterocorrelation data, permitting several observations to be made from the results, shown in Table V. The wide range covered by the chemical shifts (138.3–131.9 p.p.m.) can be divided into four distinct regions according to the environments of the various phosphite groups, as deduced from the spectra of the monosaccharide derivatives (Table II).

The most upfield signals, between 131.9 and 132.7 p.p.m., seem characteristic of the anomeric phosphite groups. The anomeric phosphite signal of α-D-lactose was

TABLE IV

^{31}P-N.m.r. chemical shifts of lignin–carbohydrate model compounds derivatized with 1,3,2-dioxaphospholanyl chloride

Compound	*Chemical shifts (p.p.m.)*						
	Glucose unit				*Lignin-like unit*		
	O-2-P	*O-3-P*	*O-4-P*	*O-6-P*	*Benzylic OH*	*Guaiacolic OH*[a]	*Phenolic OH*
Salicin	136.1	136.9	137.4	133.4	131.4	–	–
Rhapontin	136.4	136.6	137.4	133.2	–	130.2	128.3
Phloridzin	136.0	136.4	137.5	132.9	–	129.5	128.4

[a] Phenolic OH *ortho* to another substituent.

TABLE V

^{31}P-N.m.r. chemical shifts of oligosaccharides derivatized with 1,3,2-dioxaphospholanyl chloride

Compound	*Chemical shifts (p.p.m.)*				
	O-1-P, O-1'-P	*O-2-P and O-3-P,*[a] *O-2'-P and O-3'-P*[a]		*O-4-P, O-4'-P*	*O-6-P, O-6'-P*
β-Cellobiose	131.9	135.6	136.2	subst.	133.8
	subst.[b]	136.6	137.1	138.3	133.4
α-Lactose	132.7	134.4	134.9	subst.	132.7
	subst.	135.1	137.1	137.8	133.6
β-Lactose	132.1	134.8	135.4	subst.	132.9
	subst.	136.0	137.1	137.9	133.3
Maltose	132.1	134.9	136.4	subst.	132.2
	subst.	137.0	137.0	137.6	134.5
Maltotriose	132.0	135.0	135.3	subst.	134.4
	subst.	135.4	136.5	subst.	133.5
	subst.	136.9	136.9	137.7	133.1
α-Cyclodextrin	subst.	135.3[c]	135.3[c]	subst.	133.6[c]
β-Cyclodextrin	subst.	134.9	134.9	subst.	133.3
γ-Cyclodextrin	subst.	135.2	135.8	subst.	133.5
Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin	subst.	subst.	133.9	subst.	subst.

[a] Individual assignments at these positions not possible. [b] Position not derivatizable because of substitution in the parent sugar. [c] Value given is maximum of a broad signal.

about 0.6 p.p.m. downfield from that of its β counterpart. This downfield shift is considerably less than the 1.0 p.p.m. observed among monosaccharides.

The signals between 132.9 and 133.6 p.p.m. may be assigned to the C-6 phosphite groups of the disaccharides, while the most downfield signals, between 137.6 and 138.3 p.p.m., may be assigned to the C-4 phosphites.

The signals of the phosphorus atoms at positions 2 and 3 (*i.e.*, O-2-P and O-3-P) cannot be distinguished clearly, but they seem to have values between 134.4 and 137.1 p.p.m. The wider range of chemical shifts shown by the C-2 and C-3 phosphite groups reflects an influence of the number of units comprising the oligosaccharide. The most important factor appears to be the position of the sugar unit in the chain, whether internal or terminal. Accordingly, the ^{31}P-n.m.r. spectra of derivatized oligosaccharides should become simpler as the chain length increases. Furthermore, the relative intensity of the signals from the phosphite groups at C-1 and C-4 would be expected to decrease with increasing chain length since the relative abundance of end groups would decrease.

The ^{31}P spectra of three cyclodextrins derivatized with **1** are shown in Fig. 2. The cyclodextrins are cyclic oligosaccharides that do not contain C-1 and C-4 hydroxyls — only C-2, C-3, and C-6 hydroxyls — and accordingly exhibit no signals in the regions 131.9–132.7 and 137.6–138.3 p.p.m.

Cyclomalto-octaose (γ-cyclodextrin), composed of eight sugar units, gave rise to three distinct signals, at 135.2, 135.8, and 133.5 p.p.m., whose integrations were approximately similar, although the signal at 135.2 p.p.m. was of slightly lower in-

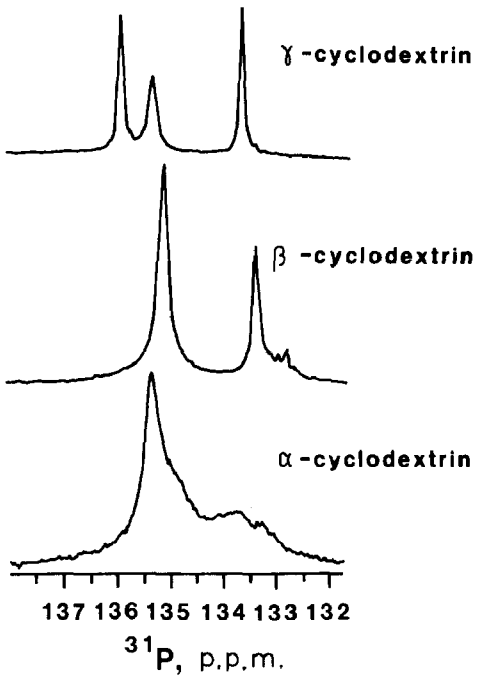


Fig. 2. ^{31}P-N.m.r. spectra of α-, β-, and γ-cyclodextrin derivatized with **1**.

tensity. The two downfield signals could be assigned to the phosphite groups at C-2 and C-3, while the upfield signal, at 133.5 p.p.m., was assigned to the C-6 phosphites. The ^{31}P spectrum of derivatized cyclomaltoheptaose (β-cyclodextrin), which is composed of seven sugar units, contained only two signals, at 134.9 and 133.3 p.p.m., with an integration ratio of 2:1. The signals arising from the phosphitylated hydroxyls at C-2 and C-3 seemed to have merged into a single and somewhat broader signal, while the signal of the phosphite groups at C-6, for the reasons discussed, remained at almost the same position (133.3 p.p.m.) and was of similar shape as in the spectrum of γ-cyclodextrin. Significant changes in the ^{31}P spectra are thus apparent when the size of the ring in these molecules is reduced by one unit. When the size was reduced by another sugar unit, as in cyclomaltohexaose (α-cyclodextrin, six sugar units), the spectrum of the derivative consisted of rather broad signals ranging between 132.5 and 136.5 p.p.m. It is possible that as the cyclodextrin ring becomes smaller in diameter the interactions between the dioxaphospholane substituents become more extensive, thus giving rise to broader signals.

Another β-cyclodextrin derivative examined was phosphitylated heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose (Fig. 3). In this compound all hydroxyl groups are blocked except that at C-3. One clear signal, at 133.9 p.p.m., was obtained from the derivatized molecule, together with a series of weaker signals at 134.2, 134.1, 133.9, and 133.7 p.p.m. Since the range covered by all signals in this spectrum is rather narrow (0.5 p.p.m.) it is unlikely that the weaker signals are due to phosphites of remaining

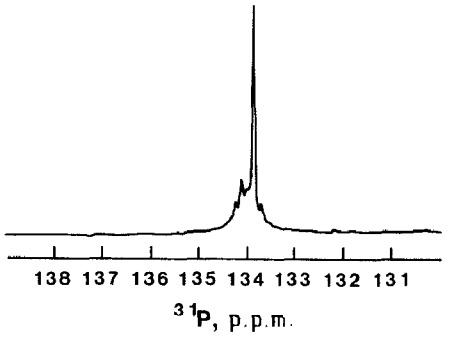


Fig. 3. ^{31}P-N.m.r. spectrum of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin derivatized with **1**.

unsubstituted hydroxyls at different positions. Because the cyclodextrin contains only one species of free hydroxyl, the weaker signals may indicate the various nonequivalent environments surrounding this hydroxyl group.

These results indicate that the ^{31}P-n.m.r. spectra of phosphitylated disaccharides and oligosaccharides may reveal information not only on the structures of the component sugars, but also on the structure of the polymeric chain itself.

CONCLUSIONS

1. Carbohydrates derivatized with 1,3,2-dioxaphospholanyl chloride gave rise to ^{31}P-n.m.r. spectra containing well resolved signals in the range 138–132 p.p.m., and the number of signals agreed with the number of hydroxyl groups in the respective molecules.

2. The primary hydroxyl groups of carbohydrates gave rise to ^{31}P signals upfield of the signals of their secondary counterparts.

3. The ^{31}P chemical shifts of the phosphite groups at C-1 in derivatives of monosaccharides were characteristic for the anomer involved. The signals of the C-1 phosphites of the α anomers were shifted downfield by about 1.0 p.p.m. from those of their β counterparts. The anomeric (P-1) signals usually appeared at the far upfield side of the spectra. The presence of an axial anomeric phosphite caused the ^{31}P chemical shift of the adjacent phosphite group to move downfield ~0.5–1.0 p.p.m.

4. Epimeric monosaccharides may also be distinguished by the ^{31}P-n.m.r. spectra of their derivatives with **1**.

5. The method described seems very suitable for the structural elucidation of lignin–carbohydrate complexes. For model compounds completely resolved spectra were obtained, with signals characteristic of the carbohydrate and lignin-related moieties.

6. The ^{31}P spectra of derivatives of disaccharides and higher oligosaccharides contained signals characteristic of hydroxyl groups in various positions in the parent compounds: 131.9–132.7 p.p.m., C-1; 132.9–133.6 p.p.m., C-6; and 137.6–138.3 p.p.m., C-4. The region 134.4–137.1 was assigned to C-2 and/or C-3, but the groups at these positions could not be distinguished individually.

7. Dramatically different ^{31}P spectra were obtained when α-, β-, and γ-cyclodextrin were derivatized with **1**. The reagent seems highly suitable for the characterization of such materials.

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