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CONFIGURATION OF THE ACETAL CARBON ATOM OF PYRUVIC ACID ACETALS IN SOME BACTERIAL POLYSACCHARIDES

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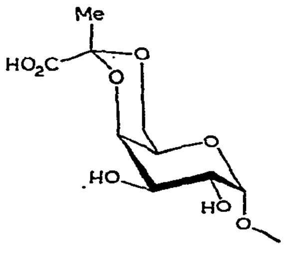
ABSTRACT

The configuration at the acetal carbon atom of pyruvic acid acetals present in some extracellular bacterial polysaccharides has been investigated. Assignment of the absolute configuration was made by comparing signals in the ^{13}C- and ^{1}H-n.m.r. spectra of the polysaccharides with those of model substances. The *S*-configuration was demonstrated in eight polysaccharides in which pyruvic acid is linked to O-4 and O-6 of D-glucopyranosyl or D-mannopyranosyl residues. The *R*-configuration was demonstrated in four polysaccharides in which pyruvic acid is linked to O-4 and O-6 of D-galactopyranosyl residues. Consequently, in each of these acetals, which form 1,3-dioxane rings, the methyl group is equatorial and the carboxyl group axial. The *S*-form was further demonstrated in four polysaccharides in which the pyruvic acid is linked to O-3 and O-4 of D-galactopyranosyl groups.

INTRODUCTION

Pyruvic acid, linked as a cyclic acetal to a glycosyl residue, is a common component in extracellular bacterial polysaccharides[1]. It is most often linked to O-4 and O-6 of D-glucopyranosyl, D-mannopyranosyl, or D-galactopyranosyl residues, which may be terminal or part of a chain. It has also been found linked to vicinal positions, namely to O-3 and O-4 of D-galactopyranosyl or L-rhamnopyranosyl residues and to O-2 and O-3 of D-galactopyranosyl and D-glucopyranosyluronic acid residues[1].

The acetal carbon of the pyruvic acid moiety is chiral and its absolute configuration, in two polysaccharides, has been determined by Gorin and co-workers[2,3]. It has the *R*-configuration in the extracellular polysaccharide from *Corynebacterium insidiosum*[2], in which it is linked to O-4 and O-6 of an α-D-galactopyranosyl group (**1**) and the *S*-configuration in the extracellular polysaccharide from *Xanthomonas* *campestris*[3], in which it is linked[4,5] to O-4 and O-6 of a β-D-mannopyranosyl group (**2**). The methyl group thus occupies the equatorial position in both acetals.



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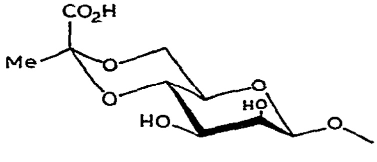

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The extracellular bacterial polysaccharides are often immunologically active and the pyruvic acid acetal may be an important part of an immunodominant region. The determination of its absolute configuration is therefore important. In order to study this configuration in the most common types of pyruvic acid acetals in polysaccharides, we have synthesised analogous acetals of methyl glycosides and characterized them by n.m.r. spectroscopy[6]. Using the information thus obtained, we have now determined the absolute configuration of the pyruvic acid moiety in some extracellular bacterial polysaccharides.

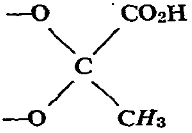
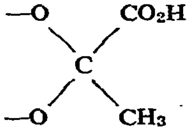
RESULTS AND DISCUSSION

The chemical shifts of the acetal methyl group in ^{1}H- and ^{13}C-n.m.r. spectra for acetals in which pyruvic acid is linked to O-4 and O-6 of methyl glucopyranosides and methyl galactopyranosides are given in Table I. The chemical shifts of the acetal carbon atom, the carboxyl carbon, and the anomeric carbon are also given. The chemical shift of the acetal carbon is in the same region as that of C-1 in the α-glycopyranosides. The effect of the pyruvic acid acetal upon the chemical shifts of anomeric carbons and protons is small, and not significantly different for the *R*- and *S*-forms. The chemical shifts for the methyl groups of the pyruvic acid acetal differ significantly and depend upon whether these groups are axial or equatorial. The difference is especially pronounced in ^{13}C-n.m.r. spectra, the chemical shifts being ~18 p.p.m. for the axial methyl groups and ~26 p.p.m. for the equatorial.

In Table II, the corresponding chemical shifts of methyl 3,4-*O*-hydroxyisopropylidene-D-galactopyranosides are given. These substances were prepared in order to study polysaccharides in which pyruvic acid is linked to O-3 and O-4 in D-galactopyranosyl residues. It was considered more convenient to perform a carboxyl-reduction of the polysaccharides than to oxidize the model substances to the corresponding 3,4-*O*-carboxyethylidene derivatives. As seen from Table II, the chemical shifts of the anomeric carbons are not significantly affected by the acetal group. However, the chemical shifts given by the acetal methyl groups are significantly

TABLE I

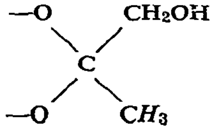
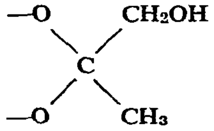
SELECTED CHEMICAL SHIFTS OF METHYL 4,6-*O*-(1-CARBOXYETHYLIDENE)GLYCOPYRANOSIDES

Pyranoside configuration	*Absolute configuration at the acetal carbon atom*	δ ^1H 	δ ^{13}C *C-1*	δ ^{13}C 		
				—*C*H$_3$	*C*	*C*=O
α-D-*gluco*-	*R*	1.68	101.0	17.5	99.0	173.9
	S	1.48	100.9	25.5	101.5	175.1
β-D-*gluco*-	*R*	1.65	104.9	17.7	99.2	174.3
	S	1.50	104.7	25.5	101.3	174.7
α-D-*galacto*-	*R*	1.46	100.8	26.1	n.d.[a]	175.8
	S	1.66	100.7	17.2	n.d.	173.5
β-D-*galacto*-	*R*	1.52	104.2	26.0	99.8	174.3

[a] n.d. = Not determined.

TABLE II

SELECTED CHEMICAL SHIFTS OF METHYL 3,4-*O*-HYDROXYISOPROPYLIDENE-D-GALACTOPYRANOSIDES

Anomeric configuration	*Absolute configuration at the acetal carbon atom*	δ ^1H 	δ ^{13}C *C-1*	δ ^{13}C 		
				—*C*H$_3$	*C*	—*C*H$_2$—
α	*S*	1.45	100.1	23.5	110.7	65.7
	R	1.30	100.1	21.8	110.9	66.5
β	*S*	1.45	103.6	23.5	111.3	65.8
	R	1.31	103.5	21.8	111.5	66.5

different for the *R*- and *S*-forms. In the ^{1}H-n.m.r. spectra, these signals appear at δ 1.45 for the *S*-forms and at δ 1.30 for the *R*-forms.

All of the polysaccharides investigated are composed of oligosaccharide repeating-units, and for most of them the structure of this repeating unit has been determined. These regular polysaccharides give simpler n.m.r. spectra than those having a random structure. Thus, for each polysaccharide studied, a single signal for the acetal methyl group was observed. The chemical shift of this signal, for

TABLE III

^{13}C-N.M.R. CHEMICAL SHIFTS FOR THE METHYL CARBON IN PYRUVIC ACID ACETALS LINKED TO O-4 AND O-6 OF GLYCOPYRANOSYL RESIDUES IN EXTRACELLULAR BACTERIAL POLYSACCHARIDES

Bacterium	*Lit.*[a]	*Sugar residue*	$\delta\,^{13}C$	*Absolute configuration at the acetal carbon atom*
Rhizobium meliloti	11	β-D-Glc*p*-(1→	26.8	*S*
Klebsiella K 31	25	β-D-Glc*p*-(1→	26.9	*S*
Klebsiella K 36	13	β-D-Glc*p*-(1→	26.7	*S*
Klebsiella K 56	14	→3)-β-D-Glc*p*-(1→	26.6	*S*
Rhizobium trifolii	15,16	→3)-β-D-Glc*p*-(1→	26.6[b]	*S*
Xanthomonas campestris[c]	4,5	β-D-Man*p*-(1→	26.8	*S*
Klebsiella K 5	17	→3)-β-D-Man*p*-(1→	26.7	*S*
Klebsiella K 6	18	→3)-β-D-Man*p*-(1→	26.8	*S*
Corynebacterium insidiosum	19	α-D-Gal*p*-(1→	27.1	*R*
Rhizobium trifolii	15,16	β-D-Gal*p*-(1→	27.1[b]	*R*
Klebsiella K 21	20	α-D-Gal*p*-(1→	27.2	*R*
Klebsiella K 35	12	→3)-α-D-Gal*p*-(1→	27.1	*R*

[a]Publication in which the structure of the polysaccharide is discussed. [b]This polysaccharide contains pyruvic acid linked both to D-glucopyranosyl and D-galactopyranosyl residues, and the assignments may be reversed. [c]Partially degraded material; see Experimental.

TABLE IV

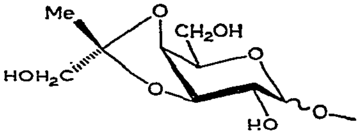
^{1}H- AND ^{13}C-N.M.R. CHEMICAL SHIFTS FOR THE METHYL GROUP IN PYRUVIC ACID ACETALS AND HYDROXYISOPROPYLIDENE ACETALS LINKED TO O-3 AND O-4 OF D-GALACTOPYRANOSYL GROUPS IN EXTRACELLULAR BACTERIAL POLYSACCHARIDES

Bacterium	*Lit.*[a]	*Sugar residue*	$\delta\,^{13}C$[b]	$\delta\,^{13}C$[c]	$\delta\,^{1}H$[b]	$\delta\,^{1}H$[c]	*Absolute configuration at the acetal carbon atom*
Klebsiella K 13	21	β-D-Gal*p*-(1→	25.2	—	1.41	1.46	*S*
Klebsiella K 33	22	β-D-Gal*p*-(1→	25.5	24.3	1.60	1.46	*S*
Klebsiella K 30	23	β-D-Gal*p*-(1→	24.6	24.3	1.59	1.45	*S*
Salmonella typhimurium 395 MRO, M2, M-antigen	24	β-D-Gal*p*-(1→	—	24.2	1.44	1.42	*S*

[a]See Table III, note *a*. [b]Original polysaccharide. [c]Carboxyl-reduced polysaccharide.

polysaccharides in which pyruvic acid is acetalically linked to O-4 and O-6 in D-glucopyranosyl, D-mannopyranosyl, or D-galactopyranosyl residues, is given in Table III. As is evident from Table III, all of these signals appear at 26–27 p.p.m., indicating the *S*-configuration when pyruvic acid is linked to D-glucopyranosyl or D-mannopyranosyl residues, and the *R*-configuration when it is linked to D-galactopyranosyl residues. The methyl group of the pyruvic acid acetal in each of the polysaccharides therefore occupies an equatorial, and the carboxyl group an axial, position, as in **1** and **2**.

Only four polysaccharides having pyruvic acid linked to O-3 and O-4 of D-galactopyranosyl groups were available. These were carboxyl-reduced and, from the chemical shifts of the acetal methyl group (Table IV), it is evident that the configuration at the acetal carbon atom is *S* in all four polysaccharides, as in **3**. Table IV also gives the chemical shifts of the acetal methyl group in the original polysaccharides.



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In the biosynthesis of pyruvic acid acetals of sugars, the first step most probably is the reaction of a hydroxyl group with phosphoenolpyruvate to give an enol ether. This product, on reaction with a neighbouring hydroxyl group, will give a cyclic acetal of pyruvic acid. The enol ether could also be reduced, giving an ether of lactic acid. Several sugars etherified with lactic acid are components of bacterial polysaccharides, *e.g.*, 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose[7] and the corresponding *R*-isomer[8]. The reduction of the intermediate enol ether may therefore occur from either side of the double bond. In principle, the reaction with a hydroxyl group, giving a pyruvic acid acetal, could also occur from either side of the double bond of the enol ether, giving acetals with different configurations. For each of the three types studied, namely, pyruvic acid linked to O-4 and O-6 of D-glucopyranosyl or D-mannopyranosyl residues, to O-4 and O-6 of D-galactopyranosyl residues, and to O-3 and O-4 of D-galactopyranosyl residues, only one of the possible isomers has been observed. This may reflect the stereochemical conditions during the biosynthesis, *e.g.*, a preferred conformation of the enol ether moiety. However, the available information is limited and does not justify more-detailed speculation.

EXPERIMENTAL

N.m.r. spectra were recorded with a JEOL FX-100 spectrometer. Spectra were recorded for solutions in D_2O at pD $\simeq$7. For ^{1}H-n.m.r. spectra, the chemical shifts are given in p.p.m. relative to internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate, and for ^{13}C-n.m.r. spectra, the chemical shifts are given in p.p.m. relative to external tetramethylsilane. Spectra of polysaccharides were recorded at 85°, whereas spectra of the reference compounds were determined at ambient temperature. In separate experiments, it was determined that the chemical shifts of the methyl groups in the pyruvic acid acetals varied by <0.03 p.p.m. (^{1}H) and <0.1 p.p.m. (^{13}C), respectively, in the temperature range 30–85°.

For carboxyl-reduction of polysaccharides, the procedure of Conrad and Taylor was used[9].

The polysaccharide from *Xanthomonas campestris* had to be depolymerized, in order to give less-viscous solutions and well-resolved spectra. In order to achieve random depolymerization, the polysaccharide (300 mg) in water (300 mL) was treated with bromine (0.1 mL) at pH 7 for 5 h at room temperature[10]. Sodium hydroxide was added to pH 11 and the solution kept at 100° for 1 h. The polysaccharide was recovered in almost quantitative yield by dialysis and freeze-drying.

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