Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 4244–4246, July 1980
Immunology

Immunochemical determination of the configuration of a haptenic substituent

(organic chemistry/microbiology)

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ABSTRACT **Quantitative inhibition of specific immune precipitation, a rapid microanalytical technique requiring no expensive equipment, was used to determine the stereoconfiguration of pyruvyl groups attached as acetals to two hydroxyls of nonreducing lateral end groups of the capsular polysaccharides of *Klebsiella* serotypes K11 and K21. The *R* and *S* isomers of 4,6-*O*-pyruvyl-D-α-methylgalactoside were used as inhibitors with appropriate polysaccharide antigens and antisera to the two serotypes. The *R* isomer was a potent inhibitor of precipitation in both antisera, showing that the pyruvylgalactosyl residues in the polysaccharides of both K11 and K21 are in the *R* form, in which the methyl group of pyruvyl is equatorial to the plane of the acetal ring.**

Inhibition of specific immune precipitation by simple chemical substances of low molecular weight was heavily relied upon by Landsteiner and his coworkers in their classical studies on the chemical basis of immune specificity (1). The introduction of quantitative microanalytical methods for the estimation of precipitating antibodies (precipitins) (2, 3) provided far more exact data on the inhibition of precipitins by fractional units of immunogens and has been crucial in elucidating structural features of antigens (4) and of the combining sites of antibodies (5).

Pyruvic acid, $CH_3COCOOH$, linked through its C2 to a sugar residue as a cyclic acetal, occurs as a constituent of agar (6) and of extracellular polysaccharides elaborated by certain bacteria. The spatial configurations arising from the asymmetry of the linkage at C2 have been determined (7–9). Perhaps surprisingly, in view of the ubiquitous occurrence of pyruvic acid in living cells, pyruvyl-sugar residues are often important, even immunodominant, antigenic determinants (10–13).

Recently, the *R* and *S* forms of 4,6-*O*-pyruvyl-D-methylgalactoside and -glucoside were synthesized (14) to make possible the studies in ref. 9. In the *R* isomer the methyl group of the pyruvyl residue is equatorial to the plane of the acetal ring (Fig. 1), whereas in the *S* isomer the carboxyl group is equatorial. The two pyruvylgalactosides gave promise of being powerful reagents for simple quantitative inhibition tests to establish the configuration of the 4,6-pyruvylgalactosyl residues on the extracellular polysaccharides of *Klebsiella* serotypes K11 and K21, for which antisera were available. The successful outcome of such tests is described in the present paper.

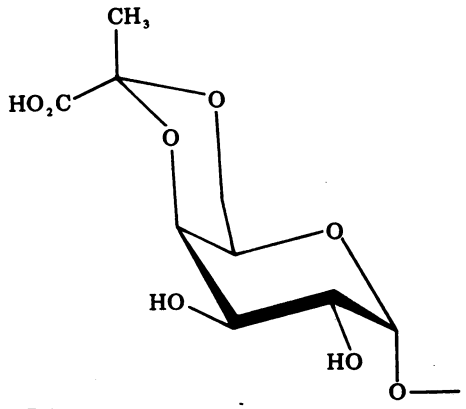


FIG. 1. *R* form of 4,6-*O*-pyruvyl-D-galactosyl. In the *S* isomer the positions of the CH_3 and COOH are interchanged.

In only one previous instance, to our knowledge, has the configuration of the pyruvyl residue been shown to be of immunological consequence. This was done by inhibition tests with analogs of the reactive group in the pneumococcal type XXVII system (10).

MATERIALS AND METHODS

Polysaccharides K11 and K21 were isolated (15) by one of us (W.N.); polysaccharide of *Rhizobium trifolii* was isolated by W.F.D.; the pyruvylgalactosides were prepared and separated by I.K. (14). Antisera to *Klebsiella* K11 and K21 were raised in rabbits by J.E. and the analyses were carried out as in ref. 4 by M.H.

Polysaccharide K11 has the structure (16):

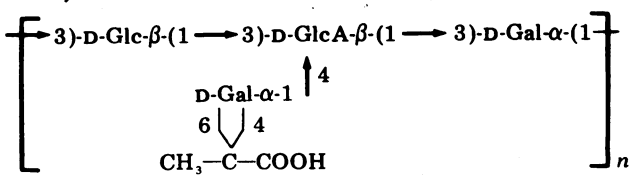


Table 1. Inhibition in the K11 anti-*Klebsiella* type 11 system by *R* and *S* isomers of neutralized 4,6-pyruvyl-α-methyl-D-galactoside

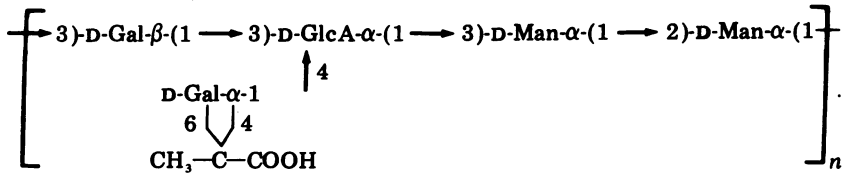
Isomer added, μM	Total vol., ml	Time at 0–1°C, days	Antibody N pptd., μg	Inhibition, %
None	0.45	4	33	—
5*R*	0.45	4	27	18
10*R*	0.45	4	24	27
5*S*	0.45	4	33	0
10*S*	0.45	4	34	0
None	0.55	13	30	—
20*R*	0.55	13	19	37
20*S*	0.55	13	29	3

Table 2. Inhibition by the pyruvylgalactosides of crossprecipitation of anti-*Klebsiella* K21 by polysaccharide of *Rhizobium trifolii* TA1

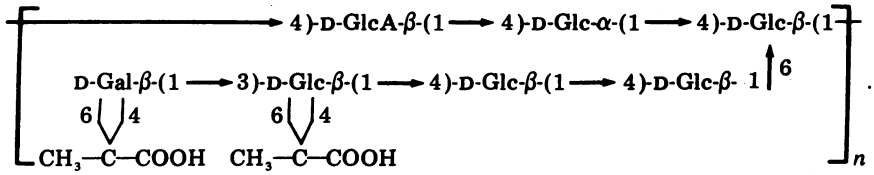
Isomer added, μM	Antibody N pptd., μg	Inhibition, %
None	24	—
5*R*	3	88
5*S*	21	12.5

Total volume was 0.33 ml; time at 0–1°C was 5 days.

Polysaccharide K21 has the structure (17, 18):



The polysaccharide of *Rhizobium trifolii* TA1 (19, 20) is



The *R*α-galactoside (70 mg) was dissolved in 3 ml of H_2O, neutralized to phenol red with dilute NaOH, and made up to 5.3 ml with 0.15 M NaCl; 50 mg of the *S* isomer was similarly treated in 2 ml of H_2O and made up to 3.8 ml with 0.15 M NaCl. Both solutions were calculated to contain 50 μmol/ml.

Duplicate tubes were set up at 0°C with an appropriate amount of antiserum and the quantity of saline or galactoside indicated in the tables. After 25–60 min, an amount of polysaccharide calculated to be at approximate equivalence was added. The tubes were allowed to stand as indicated and the analyses were completed in the usual way (4).

RESULTS AND DISCUSSION

The data given in Table 1 show that only the *R* isomer of 4,6-*O*-pyruvyl-α-methyl-D-galactoside is capable of inhibiting the precipitation of antiserum to *Klebsiella* type K11 by the purified extracellular polysaccharide isolated from the same serological (and chemical) type. This characterizes the *R* form as the one synthesized by the microorganism and demonstrates once more the reliability and simplicity of the immunochemical technique.

Because polysaccharides K11 and K21 precipitated nearly equal quantities of antibody from the antisera to both types, it was decided to use a less massive crossreaction (about 30%) for the inhibition tests with anti-K21. The data in Table 2 again show that the *R* isomer of the nonreducing lateral end group of the polysaccharide of K21 is the naturally occurring form. This is valid even for a cross reaction because it is the fit of the inhibitor into the combining site of the antibody that is measured. The result confirms that obtained by nuclear magnetic resonance (9).

The extensive inhibition achieved in this instance might be due to prevention, by the second pyruvyl residue of polysaccharide TA1, of any auxiliary binding to antibody that would be expected if the 1,3-linked D-glucose of TA1 were unsubstituted. It had previously been shown that D-mannose, which occurs in K21, is partially equivalent immunologically to D-glucose (21).

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