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Note

Isolation of 3-*O*-methyl-D-ribose from a *Rhizobium* polysaccharide

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The extracellular polysaccharides produced in pure culture by *Rhizobium* bacteria that are classed as slow-growing, non-acid producers are markedly heterogeneous in composition, in contrast to polysaccharides from those classed as fast-growing, acid producers[1]. The slow-growing group includes strains that nodulate soya beans (*Rhizobium japonicum*), the Cowpea group of *Rhizobium*, and slow-growing strains nodulating *Lotus* and *Lupinus* species (*Rhizobium lupini*). An extensive survey[1] of the polysaccharides from slow-growing *Rhizobium* strains, mainly those associated with *Lotus* species, reported the presence of three unidentified sugars. Two of these have been shown[2] to be 4-*O*-methyl-D-galactose and 4-*O*-methyl-D-glucose; the former sugar has also been isolated[3] from the polysaccharides of some strains of *Rh. japonicum*. The identification of 3-*O*-methyl-D-ribose from the extracellular polysaccharide of a Cowpea strain of slow-growing *Rhizobium*, strain CB756, is now reported.

Paper chromatography (p.c.; solvents *1* or *2*) of acid hydrolysates of the polysaccharide gave a single aniline hydrogen phosphate-reactive[4] spot with R_{GLC} greater than that of rhamnose (together with previously reported[1], common components). In solvent *2*, the spot, previously designated[1] X_3, appeared to be homogeneous, gave the colour reaction of a hexose, and had R_{GLC} 1.55 (previously reported[1] as 2.15; the difference is attributed to the use of chromatography paper from a roll instead of sheets). In solvent *1*, the leading edge of the spot gave the colour reaction of a pentose, suggesting two components; the "hexose" and "pentose" components had R_{RHA} 1.55 and 1.7 (R_{GLC} 5.1 and 5.6), respectively, but streaked somewhat and could not be sufficiently separated to achieve purification of the individual compounds. For further experiments, the mixture was isolated by preparative p.c. (solvent *1*). Analytical p.c. and g.l.c. showed the mixture to be free of other sugars; in particular, ribose was shown to be absent.

After reduction of the mixture with $NaBH_4$ and acetylation of the products with acetic anhydride–pyridine[5], the resulting mixture of alditol acetates gave, on g.l.c., a single peak with T_{MAN} 0.20 (T_{RHA} 1.0), sometimes with a slight shoulder on the leading edge. Mass spectrometry of the initial portion of the peak gave a clean

spectrum with major fragments at *m/e* 43, 87, 129, and 189. When NaB^2H_4 was used to reduce the mixture, the major fragments were at *m/e* 43, 87, 88, 129, 130, 189, and 190, with fragments separated by one unit being of approximately equal intensity. These data show that the sugar emerging first on g.l.c. is a 3-*O*-methylpentose[6]. The later-emerging compound(s) remain(s) unidentified.

Treatment of the sugar mixture with boron tribromide[7] left the "hexose" component unchanged, but demethylated the *O*-methylpentose to give ribose, identified by p.c. (solvent *1*) and its colour reaction with aniline hydrogen phosphate, and by g.l.c.–m.s. of its alditol acetate. Solvent *1* readily separates the four aldopentoses, and the three pentitol acetates are separated on g.l.c. After preparative p.c. (solvent *1*) of the boron tribromide-treated mixture, ~2 mg of ribose were isolated; no other sugars were present, and crystals formed on desiccation of the solution. The optical rotation of the ribose was negative, which is indicative of the D configuration[8]; with the instrumentation available, there was insufficient material to allow accurate calculation of the specific optical rotation.

The sugar in question is therefore 3-*O*-methyl-D-ribose; to my knowledge, this is the first report of its occurrence as a natural product.

Rh. lupini[2] and *Rh. japonicum*[3] have previously been shown to have *O*-methyl sugars in their extracellular polysaccharides. The present work shows that one of the Cowpea group, strain CB756, also has this characteristic. Production of a diverse variety of extracellular polysaccharides, some of them including unusual sugars, is thus emerging more clearly as a characteristic of the slow-growing *Rhizobia*. The contrast[1] with fast-growing strains adds further support to the division of the genus into these two groups.

EXPERIMENTAL

The general methods used were as previously described[2], except that the reaction time for demethylation with boron tribromide was extended to 6 days. Solvents[1] used for descending p.c. on Whatman No. 1 or (for preparative p.c.) No. 3MM papers were: (*1*) butyl acetate–pyridine–ethanol–water (8:2:2:1), and (*2*) ethyl acetate–pyridine–water (12:5:4). Optical rotations were measured with a Bendix NPL type 143 automatic polarimeter. Growth of organisms and isolation of polysaccharide have been described previously[1]; for some experiments, the bacteria were grown in a synthetic medium[2] to exclude the possibility that the 3-*O*-methyl-D-ribose was derived from a component such as the yeast extract.

Rhizobium strain CB756 was from the Divisional collection maintained by R. M. Greenwood.

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