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Note

3-*O*-Methyl-L-rhamnose from a *Rhizobium* capsular polysaccharide

LINDA K. JACKSON, MOREY E. SLODKI, MARTIN C. CADMUS, KERMIT A. BURTON, AND RONALD D. PLATTNER

Northern Regional Research Center, Agricultural Research, Science, and Education Administration, U. S. Department of Agriculture, Peoria, Illinois 61604 (U.S.A.)*

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Strains of *Rhizobium* isolated from the root nodules of legumes are designated as either fast- or slow-growing. The fast-growing *Rhizobia* contain the species *R. leguminosarum*, *R. meliloti*, *R. phaseoli*, and *R. trifolii*. Their acidic, extracellular, and capsular polysaccharides contain pyruvic acetal and *O*-acetyl substituents[1–5]. Polysaccharides containing pyruvic acid are also produced by certain strains of *R. japonicum*[6], a member of the slow-growing group. Other strains of this species[6], of *R. lupini*, and of isolates from the cowpea miscellany[7–10] form acidic polysaccharides that lack pyruvic acid but may contain 6-deoxyhexoses and mono-*O*-methyl sugars. The list of *O*-methylated sugars reported thus far as occurring in polysaccharides from the slow-growing group includes 4-*O*-methyl-D-glucuronic acid[11] and -D-galactose[6] from strains of *R. japonicum*; 4-*O*-methyl-D-glucose and -D-galactose[8] and 3-*O*-methyl-D-ribose[9] and -D-glucose[10], as well as 6-*O*-methyl-D-galactose[10], have been identified in polysaccharides from strains of the cowpea group. We now describe the identification of 3-*O*-methyl-L-rhamnose in the capsular polysaccharide of a *Rhizobium* obtained from the root nodules of *Acacia decurrens*. Occurrence of this sugar in bacteria has been reported for *Mycobacterium avium*[12], *M. marinum*[13], *Rhodopseudomonas capsulata*[14], and *Anabaena variabilis*[15].

Paper and thin-layer chromatography (t.l.c.) of hydrolyzates of the polysaccharide revealed an apparent 6-deoxyhexose of mobility substantially greater than that of L-rhamnose, which was also present, along with D-glucose, D-mannose, and D-galacturonic acid. In g.l.c. of the neutral monosaccharides as their per-*O*-acetylated aldononitriles (PAAN), the derivative of the unknown compound emerged from the column immediately ahead of the rhamnose derivative (T_{Rha} 0.92), and the area of its peak was approximately half that of the latter. Ion-impact mass spectrometry of the PAAN gave major fragment ions (*m/e* 101, 117, 142, 143, 159, and 203)

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characteristic of a 6-deoxy-3-*O*-methylhexose; the fragmentation path accords with those previously described[16] for PAAN derivatives from partially methylated sugars. This result was confirmed by g.l.c.–m.s. of the neutral sugars as their per(trideuteriomethyl)ated alditols. The 6-deoxyhexitol derivatives emerged as a single peak. Mass-spectral fragment-ions *m/e* 48, 63, 95, and 109 were contributed by both derivatives of rhamnitol in the mixture. Ions *m/e* 107, 142, 154, 189, and 203 were derived from the major, per(trideuteriomethyl) component; corresponding, primary fragment-ions of three fewer mass units (*m/e* 104, 139, 151, 186, and 200) arose, as expected, from the 3-*O*-methyl component. These fragmentations are analogous to those of per-methylated hexitols[17].

The 3-*O*-methyl sugar was isolated from a de-ionized hydrolyzate by column chromatography on cellulose, and crystallized from acetone–ether: m.p. (uncorr.) 115.5–117°, $[\alpha]_D^{25}$ +31° (*c* 1.99, water); lit. for 3-*O*-methyl-L-rhamnose, m.p. range 110 to 115° (refs. 18–22), $[\alpha]_D^{25}$ range +32 to +39° (refs. 13, 18–22). Demethylation with[23] BCl_3 in CH_2Cl_2 afforded a deoxyhexose having the t.l.c. and g.l.c. characteristics of L-rhamnose.

Carboxyl-reduction[24] of the polysaccharide converted the D-galacturonic acid residues into D-galactose and, after acid hydrolysis, permitted quantitative estimation of the sugar components as their PAAN derivatives. The molar ratios of 3-*O*-methyl-L-rhamnose:L-rhamnose:D-mannose:D-glucose:D-galactose were 1:2:2:4:1. Aside from the methylated rhamnose, the sugars were identified by the relative retention-times of their respective PAAN derivative in g.l.c., and according to their mobilities and color reactions on paper and thin-layer chromatograms. D-Glucose and D-galactose were further characterized by reaction with their specific D-hexose:O_2 oxidoreductases.

In all, five cultures of *Rhizobium* isolated from *A. decurrens* were kindly provided by Dr. Joe C. Burton of the Nitragin Company, Milwaukee, Wisconsin. Some of these strains had originally been acquired from the NifTAL Collection, Paia, Hawaii. All produce capsular polysaccharides that contain the same sugars in similar molar ratios of total 6-deoxyhexose:mannose:glucose. The ratio of L-rhamnose to its monomethyl ether is variable, but this might reflect differences in the rate of growth, as concurrent studies showed that the extent of 3-*O*-methylation increases with the age of the culture.

EXPERIMENTAL

Polysaccharide hydrolyzates (2M HCl for 1 h at 100°) were made neutral with Ag_2CO_3, the suspension was filtered, and the filtrate treated with the acid form of a cation-exchange resin. Descending chromatograms on Whatman No. 1 paper were irrigated with the upper phase of 1:2:2 pyridine–ethyl acetate–water[25]. Use of 2:5:5 pyridine–ethyl acetate–water gives better resolution of neutral hexoses in t.l.c. on cellulose precoated (0.10 mm) on plastic sheets (E. Merck, Darmstadt). Sugars were detected by spraying with a *p*-anisidine phthalate reagent, and heating at 100°.

The 6-deoxy-3-*O*-methylhexose had R_{Rha} 1.4 in both p.c. and t.l.c. with the aforementioned solvents, and R_{Rha} 1.2 when 5:5:1:3 pyridine–ethyl acetate–acetic acid–water[26] was employed as the t.l.c. solvent. Preparation of per-*O*-acetylaldononitriles and their analysis by g.l.c.–m.s. have been described[16,27]. The Hakomori procedure[28] was used for permethylation with CD_3I. For chromatographic separation of sugars on a column of microcrystalline cellulose, 32:1 1-butanol–water[29] was employed.

For production of capsular polysaccharides, the rhizobia were incubated for 8 days at 25° in reciprocally shaken (100 5-cm strokes/min) Fernbach flasks (2.8 L) containing 0.5 L of culture medium per flask. The medium (pH 6.8) consisted of (per L): yeast extract, 1 g; D-mannitol, 10 g; and soil extract[30] (0.2 L).

To isolate capsular polysaccharides, the cultures were treated with 95% ethanol (5 vol.) containing aqueous KCl (1%). The precipitates were taken up in 0.1M KOH, and the cells were removed by centrifugation. Cell-free polysaccharide was twice precipitated with 3 vol. of 95% ethanol containing KCl. Fractionation of this product by precipitation of acidic polysaccharide as the cetyltrimethylammonium salt[31] removed two minor, neutral components, namely, a glucomannan and an insoluble glucan (1 and 3%, weight basis, respectively). The overall yield of acidic capsular polysaccharide from the D-mannitol was 17–20%.

REFERENCES

1 W. F. Dudman and M. Heidelberger, *Science*, 164 (1969) 954–955.
2 L. T. P. M. Zevenhuizen, *J. Gen. Microbiol.*, 68 (1971) 239–243.
3 H. Björndal, C. Erbing, B. Lindberg, G. Fahraeus, and H. Ljunggren, *Acta Chem. Scand.*, 25 (1971) 1281–1286.
4 C. M. Hepper, *Antonie van Leeuwenhoek J. Microbiol. Serol.*, 38 (1972) 437–445.
5 R. Sømme, *Carbohydr. Res.*, 33 (1974) 89–96.
6 W. F. Dudman, *Carbohydr. Res.*, 46 (1976) 97–110.
7 R. W. Bailey, R. M. Greenwood, and A. Craig, *J. Gen. Microbiol.*, 65 (1971) 315–324.
8 L. D. Kennedy and R. W. Bailey, *Carbohydr. Res.*, 49 (1976) 451–454.
9 L. D. Kennedy, *Carbohydr. Res.*, 52 (1976) 259–261.
10 L. D. Kennedy, *Carbohydr. Res.*, 61 (1978) 217–221.
11 W. F. Dudman, *Carbohydr. Res.*, 66 (1978) 9–23.
12 A. P. Maclennan, *Biochem. J.*, 82 (1962) 394–400.
13 C. Villé and M. Gastambide-Odier, *Carbohydr. Res.*, 12 (1970) 97–107.
14 J. Weckesser, H. Mayer, and G. Drews, *Eur. J. Biochem.*, 16 (1970) 158–160.
15 J. Weckesser, A. Katz, G. Drews, H. Mayer, and I. Fromme, *J. Bacteriol.*, 120 (1974) 672–678.
16 F. R. Seymour, R. D. Plattner, and M. E. Slodki, *Carbohydr. Res.*, 44 (1975) 181–198.
17 L. S. Golovkina, N. S. Vul'fson, and O. S. Chizhov, *Zh. Org. Khim.*, 4 (1968) 737–743.
18 E. L. Hirst and S. Dunstan, *J. Chem. Soc.*, (1953) 2332–2337.
19 H. Muhr, A. Hunger, and T. Reichstein, *Helv. Chim. Acta*, 37 (1954) 403–427.
20 A. R. N. Gorrod and J. K. N. Jones, *J. Chem. Soc.*, (1954) 2522–2525.
21 T. Reichstein and E. Weiss, *Adv. Carbohydr. Chem.*, 17 (1962) 65–120.
22 J. R. Turvey and L. M. Griffiths, *Phytochemistry*, 12 (1973) 2901–2907.
23 T. G. Bonner, E. J. Bourne, and S. McNally, *J. Chem. Soc.*, (1960) 2929–2934.
24 R. L. Taylor and H. E. Conrad, *Biochemistry*, 11 (1972) 1383–1388.
25 M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, 44 (1949) 402–407.
26 F. G. Fischer and H. Dörfel, *Hoppe-Seyler's Z. Physiol. Chem.*, 301 (1955) 224–234.

27 B. A. DMITRIEV, L. V. BACKINOWSKY, O. S. CHIZHOV, B. M. ZOLOTAREV, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 19 (1971) 432–435.
28 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
29 P. A. J. GORIN AND J. F. T. SPENCER, *Can. J. Chem.*, 45 (1967) 1543–1549.
30 R. E. GORDON, W. C. HAYNES, AND C. HOR-NAY PANG, *The Genus* Bacillus, Agriculture Handbook No. 427, USDA, Washington, DC, 1973, pp. 100–101.
31 J. E. SCOTT, *Chem. Ind. (London)*, (1955) 168–169.