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Note

Isolation of an α-(1→4)-linked (galactosyluronic acid)galactose*

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It has already been shown that an α-(1→4)-linked (galactosyluronic acid)-galactose is present in the water-soluble polysaccharide of *Phellodendron amurense* Ruprecht[2]. Aldobiouronic acids may be readily obtained by prolonged, acid hydrolysis of appropriate substrates, and many reports have been published thereon[3–5]. Several aldobiouronic acids containing galacturonic acid, such as 2-*O*-(α-D-galactosyluronic acid)-L-rhamnose[5,6] and 4-*O*-(α-D-galactosyluronic acid)-D-xylose[7], have been obtained crystalline. Studies on 4-*O*-(α,β-D-glucosyluronic acid)-D-galactose[8,9] have also been published. However, no report on a (1→4)-linked (galactosyluronic acid)-galactose is available at present. Therefore, isolation, and further study of the structure, of such a compound are now described.

EXPERIMENTAL

Fraction E_5 (1 g) of the water-soluble polysaccharide of *P. amurense*[10] was separated into three fractions by chromatography on a column of Dowex-1 X-8 with a linear gradient of acetic acid. The acidic oligosaccharides eluted with 1.2–1.4M acetic acid were collected, and the eluates were evaporated to dryness (231 mg). The residue in 2.5% methanolic hydrogen chloride[11] was boiled for 4 h under reflux. The solution was evaporated, with repeated addition of methanol, the residue acetylated with 1:1 acetic anhydride–pyridine, the ester extracted with chloroform, the extract evaporated, and the product purified on a column of magnesol–Celite. Crystallization, and recrystallization, from ethanol afforded the peracetate (**1**) of the methyl ester methyl glycoside of the title compound (72.3 mg); m.p. 228.7–229.4°,

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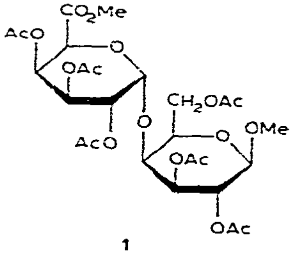
*Studies on the Water-soluble Polysaccharide of *Phellodendron amurense* Ruprecht, Part IV. For Part III, see ref. 1.

$[\alpha]_D^{22.5}$ +95.89° (*c* 0.73, chloroform); t.l.c.: R_F 0.39 in 4:1 benzene–acetone; ν_{max}^{KBr} 3000–2900 (w, C–H), 1755 (vs, C=O), 1438 (m, CH_3 of methyl ester), 1370 (s, CH_3 of acetoxyl), 1250–1200 (vs, C–O), 1090 (s, C–O–C), 1050 (vs, C–O–C), 938 (m, C–O–C), 859 (m, type 2A), and 795 cm^{-1} (w, type 3); *m/z* 604 (M^+ − 60). For detailed analyses of the e.i. and c.i. mass spectra, see ref. 12.

Compound **1** was deacetylated, and the product reduced with sodium borohydride; a solution of the reduction product in 1% methanolic hydrogen chloride in a sealed tube was heated for 4 h at 90°. G.l.c. of the per(trimethylsilyl)ated methanolyzate showed only galactose, indicating that the product of **1** consisted of one galacturonic acid and one galactose residue.



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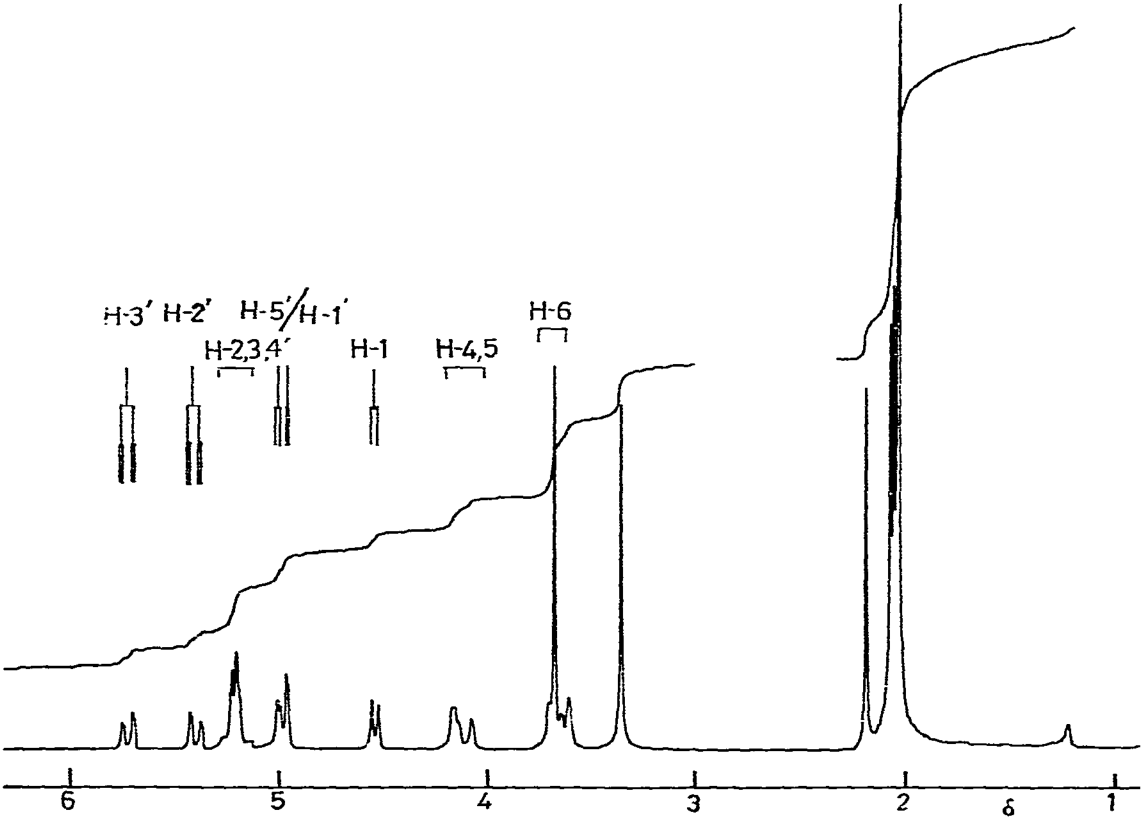

Fig. 1. 200-MHz, ^{1}H-n.m.r. spectrum of compound **1**.

Reduced compound **1** was methylated with methylsulfinyl carbanion and methyl iodide according to the method of Sandford and Conrad[13]. The product was hydrolyzed, and the sugars were converted into the alditol acetates. G.l.c. thereof showed two peaks ($R_{\mathrm{Me_4Glc}}$ 1.20 and 2.46) of about equal size, the former giving *m/z* 45, 117, 161, and 205 ions, but lacking 189 ions, showing that it was 2,3,4,6-tetra-*O*-methylgalactopyranose, and the latter giving *m/z* 45, 117, and 233 ions, but lacking *m/z* 161, 189, and 203 ions, showing it to be 2,3,6-tri-*O*-methylgalactopyranose. Therefore, **1** was the peracetate of a methyl 4-*O*-(methyl galactopyranosyluronate)-galactopyranoside.

Further structural studies on **1** were made by ^{1}H-n.m.r. spectroscopy. As every signal in the 90-MHz spectrum could not be resolved, the 200-MHz spectrum was recorded and measured (see Fig. 1). Each peak was assigned by proton–proton decoupling and INDOR methods. Two sharp singlets, at 3.36 and 3.68 p.p.m., showed that **1** was the peracetate of a methyl glycoside methyl ester. Among six acetoxyl signals at ~2 p.p.m., only one was shifted toward low magnetic field, showing that only one *axially* oriented acetoxyl group was present in **1**. The H-4 signal was found at 4.08–4.18 p.p.m. and the $J_{1,2}$ and $J_{1',2'}$ values were 5.82 and 1.61 Hz, respectively. These results strongly supported formulation of **1** as methyl 2,3,6-tri-*O*-acetyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate)-β-D-galactopyranoside, because previous work[2] had revealed that both the galactose and the galacturonic acid in the water-soluble polysaccharide of *P. amurense* are D sugars. The $J_{1,2}$ (5.83 Hz), $J_{2',3'}$ (5.40 Hz), $J_{3',4'}$ (1.61 Hz), and $J_{4',5'}$ (2.91 Hz) values showed that both residues in **1** are in the $^4C_1$(D) conformation.

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