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A NEW DIALDOSE DIANHYDRIDE

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ABSTRACT

A new dialdose dianhydride derivative was obtained from an acid hydrolyzate of the water-soluble polysaccharide of wobaku wood by successive treatment with methanolic hydrogen chloride and acetic anhydride–pyridine. This compound was determined to be the 1,2′:1′,2-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranuronate.

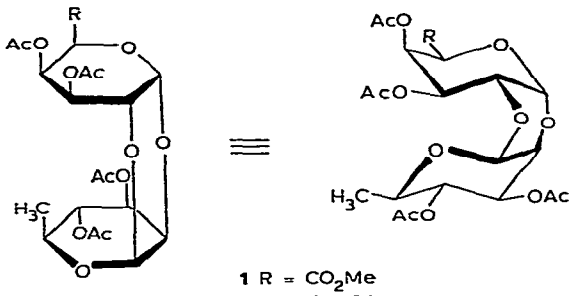
INTRODUCTION

Wobaku (*Phellodendron amurense* Rupr.) is a tree belonging to the Rutaceae; it contains in the bark the yellow alkaloid berberine, and this plays a main role as a dyestuff in dyeing[1], achieved by immersing cloth in an aqueous extract of the crushed bark. This extract contains, in addition to the berberine, a water-soluble polysaccharide having a high uronic acid content and a high viscosity[2]. As this polysaccharide has an effect in the yellow dyeing, a study of the polysaccharide has now been performed. During its course, a uronic acid derivative having a dianhydride structure was obtained by successively treating the products from the partial, acid hydrolysis of the polysaccharide with methanolic hydrogen chloride and acetic anhydride–pyridine.

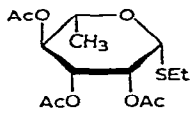
Several dianhydrides of sugars have been reported and studied[3–8]; six of them contain only D-fructose residues. Ketoses readily undergo self-condensation, with the formation of diketone dianhydrides, in concentrated, aqueous solution, or when treated with cold, concentrated acid[4]. A di-D-fructose dianhydride is also formed[5] by treating inulin with inulinase II. Similarly, di-D-ribofuranose 1,5′:1′,5-dianhydride[7,8] is readily prepared. We now describe a dianhydride containing one residue of L-rhamnose and one of D-galacturonic acid; this appears to be the first example of such a dianhydride containing residues of two different sugars.

RESULTS AND DISCUSSION

The water-soluble polysaccharide obtained from wobaku wood consists of arabinose (15.5%), rhamnose (3.8%), galacturonic acid (77.1%), and galactose (3.1%). The polysaccharide was partially hydrolyzed with 0.5M sulfuric acid, and the products were separated into four fractions by means of a carbon–Celite column.



1 R = CO_2Me
4 R = CH_2OAc



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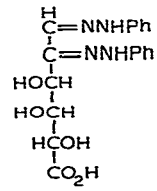

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By gel-permeation chromatography on Sephadex G-15, the fraction (Fraction E_5) eluted by 19:1 water–ethanol was found to contain only disaccharides. Complete hydrolysis of Fraction E_5 with acid gave arabinose (0.9%), rhamnose (40.0%), galacturonic acid (20.2%), and galactose (38.9%). As the yield of Fraction E_5 was ~7.2%, almost all of the rhamnose residues in the polysaccharide were present in combination with galacturonic acid or galactose. Fraction E_5 was treated with boiling, 2.5% methanolic hydrogen chloride under reflux, and the mixture of products was acetylated. From the acetylation products, compound **1** was separated in crystalline form.

Compound **1** was hydrolyzed with 2M trifluoroacetic acid, and the products were examined by color reactions and paper chromatography. The results of the Dische–Shettles method[9], the carbazole–sulfuric acid method[10], and paper chromatography indicated that **1** contained a rhamnose residue and a glycuronic acid residue in the ratio of 1:1. G.l.c. analysis of the hydrolyzate of **1** showed that it contained rhamnose and galacturonic acid in the molecular ratio of 1:1.

Compound **1** was completely hydrolyzed with 2M trifluoroacetic acid, and the product was separated into two fractions on a column of Dowex-1 X8 (OAc^-) resin. The unretained fraction was evaporated to dryness, the residue was treated with ethanethiol plus hydrochloric acid, and the product acetylated, to give acetate **2**. The n.m.r. spectrum of **2** suggested that it was ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside, and its melting point, specific rotation, and i.r. spectrum all agreed completely with those of authentic **2**. Therefore, the neutral-sugar residue in **1** is L-rhamnose. The acidic fraction was eluted with 0.2M acetic acid, and the eluate evaporated to a pale-yellow syrup; this was converted into its phenylosazone (**3**). The specific rotation, melting point, and i.r. spectrum of **3** agreed with those of authentic D-*lyxo*-hexos-2-uluronic acid 1,2-bis(phenylhydrazone) (D-galacturonic

TABLE I

RELATIVE RETENTION-TIMES OF PARTIALLY METHYLATED SUGARS IN THE FORM OF ALDITOL ACETATES

Position of Me	*Relative retention times*				
	Authentic D-*glucose*	*Reference value*[12]			
		D-*Glucose*	D-*Galactose*	L-*Rhamnose*	**4**
2,3	5.65	5.39		0.98	
3,4				0.92	0.93
2,3,4	2.49	2.48	3.41	0.46	
2,3,5			3.28		
2,3,6	2.52	2.50	2.42		
2,4,6			2.28		
2,5,6			3.25		
3,4,6			2.50		2.48
2,3,4,6	**1.00**	**1.00**	1.25		

acid phenylosazone), showing that C-3 to C-5 of the uronic acid residue in **1** have the D-*lyxo* configuration.

Compound **1** was next reduced with sodium borohydride, affording the corresponding hexose derivative, which was acetylated with acetic anhydride–pyridine to give the pentaacetate **4**. This was simultaneously deacetylated and methylated with methylsulfinyl carbanion[11], and the product was hydrolyzed with 90% formic acid and then with 0.25M sulfuric acid, and the resulting mixture converted into the alditol acetates. The partially methylated alditol acetates were analyzed by g.l.c. (see Table I). There was good agreement between the relative retention-times and the reference data[12] for the authentic sugars. There were two peaks (Peaks I and II) in the chromatogram of the methylated derivatives of **4**. Peak I had a relative retention-time of 0.93, and Peak II had 2.48. Reference values suggested that Peak I was the alditol acetate derived from 3,4-di-*O*-methylrhamnose, and that Peak II was that of that from 3,4,6-tri-*O*-methylgalactose, but the possibility that Peak I was the alditol acetate derivative from 2,3-di-*O*-methylrhamnose and that Peak II was that of that from 2,3,6-tri-*O*-methylgalactose was not completely excluded. In order to check this possibility, g.l.c.–mass spectra were measured (see Fig. 1). The absence of peaks at *m/e* 117 and 203, and the presence of *m/e* 131 and 189 in the spectrum of Peak I strongly indicated that Peak I was 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol. Similarly, the absence of peaks of *m/e* 117 and 233, and the presence of *m/e* 45 and 189 in the spectrum of Peak II showed that this was not the alditol acetate derivative from 2,3,4- but from 3,4,6-tri-*O*-methylgalactose. The ratio of the areas of Peaks I and II on the chromatogram was 0.9 : 1.0, showing that **4** consisted of rhamnose and galactose residues in the molecular ratio of 1 : 1. The important fact that the alditol acetate derivative of neither 2,3,4-tri-*O*-methylrhamnose nor 2,3,4,6-tetra-*O*-methylgalactose was found in the chromatogram showed that, in **4**, two bonds were present, between two hydroxyl groups on C-1 and C-2 of rhamnose and

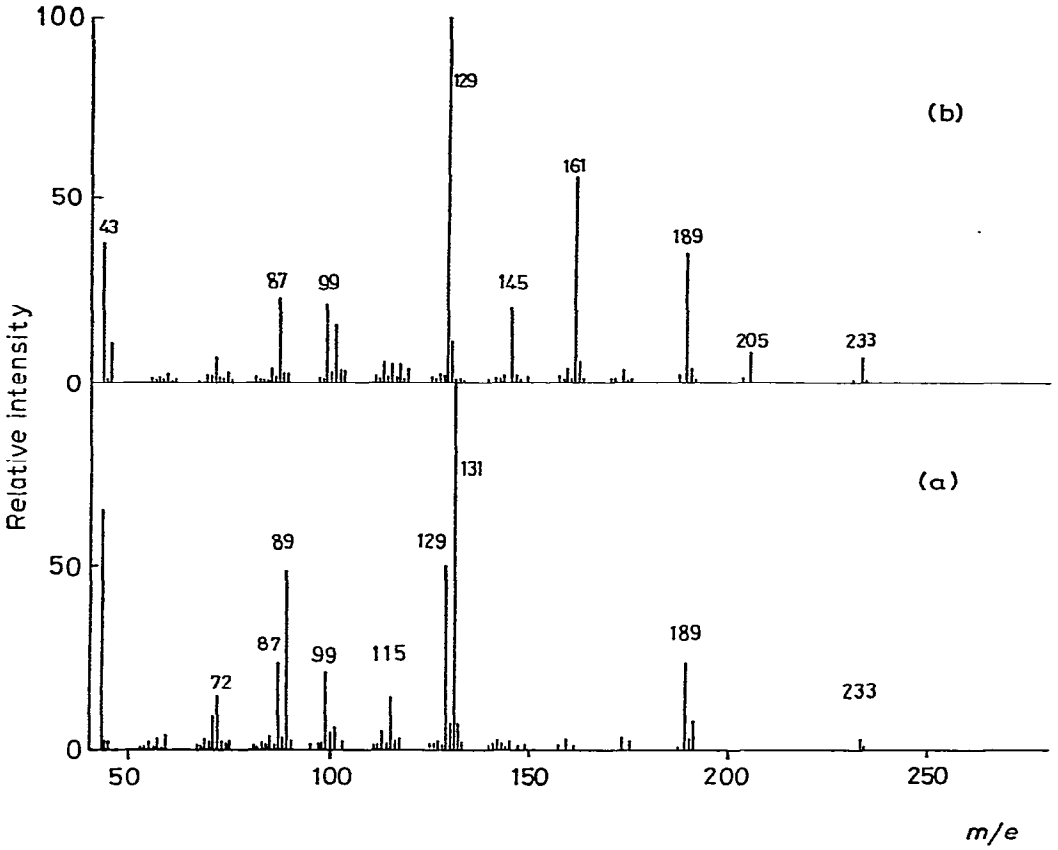

Fig. 1. Mass spectra of g.l.c. Peaks I (a) and II (b).

two hydroxyl groups on C-1 and C-2 of galactose. There were two possible arrangements for these two bonds, namely; (*a*) two glycosidic bonds (1,2′ and 1′,2), and (*b*) one glycosidic bond (1,1′) and one ether bond (2,2′). The formolysis employed to hydrolyze the methylation product of **4** would not have broken the ether bond, and so, possibility (*b*) was precluded. The fact that the partially methylated sugars were, respectively, a 3,4-di-*O*-methylrhamnose and a 3,4,6-tri-*O*-methylgalactose derivative showed that both residues were in the pyranose form. Therefore, the backbone structure of **4** was L-rhamnopyranose D-galactopyranose 1,2′:1′,2-dianhydride.

In order to study the structure in more detail, n.m.r. spectra were measured (see Fig. 2). All protons were assigned with the aid of a spin–spin decoupling technique (see Table II). The fact that a sharp, 3-proton singlet was observed at a relatively low magnetic field (3.73 p.p.m.) for **1**, but not for **4**, showed that this singlet was due to a methyl ester. Therefore, **1** was not a methyl glycoside of a substituted galacturonic acid, but its methyl ester. The anomeric protons and H-2 and H-2′ appeared at extremely high magnetic fields, in the spectra of both **1** and **4**, showing that four hydroxyl groups (on C-1, C-2, C-1′, and C-2′) were not acetylated. This fact agreed

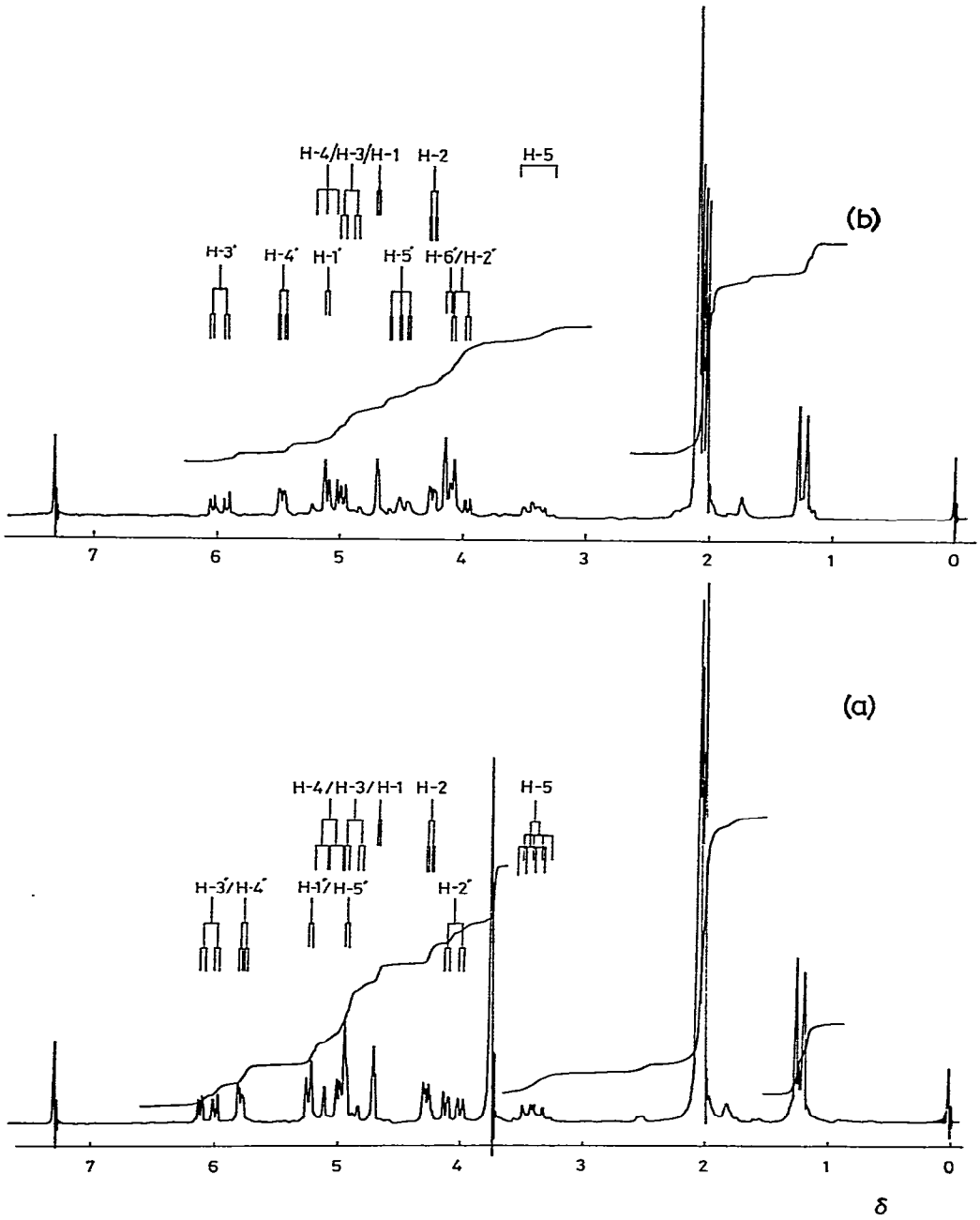

Fig. 2. N.m.r. spectra of compound **1** (a) and **4** (b).

well with the results of methylation analysis. Therefore, the rest of the hydroxyl groups must all have been acetylated, because the n.m.r. spectrum of **1** showed that it contained 4 acetyl groups, and treatment with D_2O had no effect on the spectrum. This conclusion was strongly supported by the fact that H-3, H-4, H-3′, and H-4′

TABLE II

N.M.R. PARAMETERS OF COMPOUNDS **1** AND **4**

Proton	*Chemical shifts (δ)*			*Coupling constants (Hz)*	
	1	**4**		**1**	**4**
H-1	4.70	4.70	$J_{1,2}$	1.4	1.4
H-2	4.27	4.26	$J_{2,3}$	3.4	3.4
H-3	4.89	4.92	$J_{3,4}$	10.3	9.5
H-4	5.09	5.16	$J_{4,5}$	8.5	9.5
H-5	3.40	3.40	$J_{5,6}$	6.2	6.1
H-6	1.21	1.24			
H-1′[a]	4.94	5.12	$J_{1',2'}$	3.5	3.5
H-2′	4.07	4.02	$J_{2',3'}$	10.7	11.0
H-3′	6.07	5.98	$J_{3',4'}$	3.2	3.2
H-4′	5.80	5.47	$J_{4',5'}$	1.5	1.5
H-5′	4.93	4.52	$J_{5',6'}$	—	6.5
H-6′	—	4.10			

[a]Primed numbers refer to the D-galacturonic acid residue.

appeared at low magnetic fields. The $J_{4,5}$ value (8.5 Hz) of the L-rhamnose residue showed that H-4 and H-5 had a *trans*-diaxial relationship, showing that the L-rhamnose residue was pyranoid and in the $^1C_4$(L) conformation. The $J_{2',3'}$ value (10.7 Hz) of the D-galacturonic acid residue showed that it was present in the $^4C_1$(D) conformation. The small $J_{4',5'}$ value (1.5 Hz) of the D-galacturonic acid residue was attributable to the fact that free rotation did not occur around the C-4′–C-5′ bond, showing that the D-galacturonic acid residue was pyranoid, and confirming the $^4C_1$(D) conformation. This result agreed with the results of the methylation analysis. The anomeric configuration of the D-galacturonic acid residue was determined to be α from the $J_{1',2'}$ value of 3.5 Hz. The anomeric configuration of the L-rhamnose residue could not be determined from the $J_{1,2}$ value, but, were the L-rhamnose in the α form, the substituents on C-1 and C-2 would be oriented *trans*-diaxially, and a dianhydride bond could not be formed; therefore, the L-rhamnose residue is in the β form. From these results, the structure of **1** was found to be that of the 1,2′:1′,2-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranuronate.

The n.m.r. spectrum of **4** showed a pattern similar to that of **1**. Successive reduction with sodium borohydride and acetylation gave a product displaying two new signals, at 4.10 and 4.52 p.p.m.; that at 4.10 p.p.m. was due to H-5′. The disappearance of the singlet at 3.75 p.p.m. and the presence of an extra acetyl-group signal showed that the methyl ester had been changed into the acetate of a primary alcohol. Therefore, the structure of **4** is that of the 1,2′:1′,2-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and 3,4,6-tri-*O*-acetyl-α-D-galacto-pyranose.

The H-3′ signals of **1** and **4** were shifted, extremely, toward low field, in com-

parison with that (5.28 p.p.m.) of methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranuronate)-β-L-rhamnopyranoside. This large, deshielding effect cannot be explained solely by the effect of the acetyl group present on O-4′. One of the main effects would be a magnetic anisotropy of the four oxygen atoms (O-1, O-1′, O-3, and the ring-oxygen atom of rhamnose residue) around H-3′.

It was confirmed by i.r. spectroscopy and g.l.c. that the 1,2′:1′,2-dianhydride of L-rhamnopyranose and methyl D-galactopyranuronate was formed from 2-*O*-(α-D-galactosyluronic acid)-β-L-rhamnopyranose, which was isolated from Fraction E_5 by treatment with methanolic hydrogen chloride.

EXPERIMENTAL

Complete hydrolysis. — Complete hydrolysis of each sample was achieved by heating it in 2M trifluoroacetic acid for 3 h at 100°.

Preparation of water-soluble polysaccharide from wobaku wood. — Water-soluble polysaccharide was extracted from the commercial bark of wobaku wood. Briefly ground bark (2 kg) was soaked in water (10 liters), and the mixture was kept for 24 h with occasional stirring. The resulting, viscous solution was squeezed through fine linen cloth, and the resulting solution was treated with ethanol (2 vol.). The precipitated polysaccharide was twice reprecipitated from an aqueous solution, and the final precipitate was soaked in ethanol, and dried at 80°, to give the polysaccharide (108 g).

Partial hydrololysis of the polysaccharide. — Powdered polysaccharide (105 g) was hydrolyzed with 0.5M sulfuric acid for 5 h at 100°. The acid was neutralized with barium carbonate, and the precipitate was removed by filtration. The filtrate was chromatographed on a column of 1:1 carbon–Celite with water–ethanol. The materials eluted with 5% ethanol were pooled, and evaporated, to give a pale-yellow, amorphous powder (Fraction E_5, 8.25 g).

1,2′:1′,2-Dianhydride (**1**) *of 3,4-di-*O*-acetyl-β-*L*-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-*D*-galactopyranuronate.* — Fraction E_5 (5 g) in 2.5% methanolic hydrogen chloride was boiled for 6 h under reflux. The solution was made neutral with silver carbonate, and evaporated to a light-brown syrup; this was acetylated with 1:1 acetic anhydride–pyridine for 3 days at room temperature. The solution was poured onto ice, and extracted with chloroform. The extract was successively washed with 10% hydrochloric acid, water, saturated sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated, to give a pale-yellow syrup. Crystallization from ethanol afforded crude **1**. Recrystallization from 1:1 ethanol–ether gave pure product, m.p. 250.5° (dec.), $[\alpha]_D^{17}$ +156.2° (*c* 2.43, chloroform); t.l.c., R_F 0.44 (1:24 methanol–benzene); for n.m.r. data, see Fig. 2 and Table II.

Anal. Calc. for $C_{21}H_{28}O_{14}$: C, 50.00; H, 5.60; mol. wt., 504. Found: C, 49.93; H, 5.49; mass spectrum 445 ($M^+ - 59$).

1,2′:1′,2-Dianhydride (**4**) *of 3,4-di-*O*-acetyl-β-*L*-rhamnopyranose and 3,4,6-tri-*O*-acetyl-α-*D*-galactopyranose.* — Compound **1** (100 mg) was reduced with sodium

borohydride (50 mg) in ethanol (10 ml) for 1 h. The product was treated with acetic acid, the mixture freed of sodium by chromatography on a column of Amberlite IR-120 (H^+) ion-exchange resin, the solution evaporated to a syrup, and the syrup acetylated with acetic anhydride–pyridine. The solution was extracted with chloroform, and the extract was processed as usual. Crystallization from ethanol gave **4**, m.p. 179–181°; $[\alpha]_D^{18}$ +134.2° (*c* 0.32, chloroform); t.l.c., R_F 0.52 (1:24 methanol–benzene); for n.m.r. data, see Fig. 2 and Table II.

Anal. Calc. for $C_{22}H_{30}O_{14}$: C, 50.97; H, 5.83; mol. wt., 518. Found: C, 50.49; H, 5.61; mass spectrum 459 ($M^+ - 59$).

Ethyl 2,3,4-tri-O-acetyl-1-thio-β-L-rhamnopyranoside (**2**) *and D-galacturonic acid phenylosazone* (**3**). — Compound **1** (150 mg) dissolved in ethanol (30 ml) was deacetylated with 0.1M sodium methoxide (0.5 ml) for 1 h at 0°. The solution was then passed through a column of Amberlite IR-120 (H^+) ion-exchange resin. The eluate was evaporated, with repeated addition of methanol, and the product completely hydrolyzed with 2M trifluoroacetic acid. The hydrolyzate was separated into two fractions by means of a column of Dowex-1 X8 (AcO^-) ion-exchange resin. The unretained fraction (rhamnose, 41.2 mg) was dissolved in conc. hydrochloric acid (0.5 ml), and ethanethiol (0.5 ml) was added slowly, the reaction temperature being kept below 30°. The mixture was stirred for 1 h, made neutral with conc. ammonium hydroxide, evaporated under diminished pressure, and the residue dried with ethanol, and acetylated with acetic anhydride–pyridine for 24 h at room temperature. The mixture was poured onto ice, and extracted with chloroform, and the extract was washed and dried as usual; it was then evaporated to a syrup, and this was crystallized from ethanol. Recrystallization from ethanol gave pure **2** (28.4 mg), m.p. 108–109°; $[\alpha]_D^{33}$ +57.9° (*c* 0.19, chloroform); n.m.r. data ($CDCl_3$): δ 1.16–1.40 (6 H, Rha-CH_3 and SCH_2CH_3), 1.97 and 2.05 (s, 6 H, 2 *eq*-OCOMe), 2.18 (s, 3 H, *ax*-OCOMe), 2.72 (q, 2 H, CH_2), 3.55 (o, 1 H, H-5), 4.74 (d, 1 H, H-1), 4.9–5.2 (2 H, H-3,4), 5.45 (q, 1 H, H-2), $J_{1,2}$ 1.4, $J_{2,3}$ 3.0, $J_{4,5}$ 9.8, and $J_{5,6}$ 6.

Anal. Calc. for $C_{14}H_{22}O_7S$: S, 9.86; mol. wt. 334. Found: S, 10.56; mass spectrum, 334 (M^+).

The acidic fraction [galacturonic acid, 27.5 mg; eluted from a column of Dowex-1 X8 (H^+) ion-exchange resin by 0.2M acetic acid], phenylhydrazine hydrochloride (50 mg), and sodium acetate (80 mg) were dissolved in water (2.5 ml), and the mixture was heated for 1 h in a boiling-water bath, and cooled. The resulting, yellow-brown crystals were collected, and recrystallized from ethanol by gradual addition of water, to give **3** (17.5 mg), m.p. 141.2–141.6°; $[\alpha]_D^{33}$ +32.5→+9.3° (24 h; *c* 0.215, methanol).

Anal. Calc. for $C_{19}H_{22}N_4O_5$: N, 15.05. Found: N, 14.81.

Analysis of sugar composition. — Analysis of the sugar composition was performed by the method of Perry and Hulyalkar[13]. The sugar sample was reduced with sodium borohydride and then lactonized by treatment with conc. hydrochloric acid. G.l.c. of the product was conducted with a column (2 m) of 5% of SE-30 on Chromosorb W at 180° at a rate of flow of carrier gas of 25 ml/min.

Methylation analysis. — Methylation was achieved by using methylsulfinyl carbanion[11]. Compound **4** (15 mg) was dissolved in dimethyl sulfoxide (10 ml), and methylated with methylsulfinyl carbanion and methyl iodide. The product was hydrolyzed with 90% formic acid for 2 h at 100° and then with 0.25M sulfuric acid for 18 h at 100°. The products in the hydrolyzate were converted into the alditol acetates by successive treatment with sodium borohydride and acetic anhydride–pyridine, and these were analyzed by g.l.c. on a column (2 m) of 3% of ECNSS-M on Gaschrom Q at 180° with a flow rate of carrier gas of 25 ml/min. G.l.c.–mass spectra were measured with a column (1 m) of 5% of OV-1 at 180° at a chamber voltage of 20 eV.

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