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Note

Polysaccharides from the roots of the marsh mallow (*Althaea officinalis* L., var. Rhobusta): dianhydrides of oligosaccharides of the aldose type

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Partial hydrolysis[1] of the heteropolysaccharide complex from the roots of the marsh mallow (*Althaea officinalis* L., var. Rhobusta) (a medicinal plant), which is composed of D-galacturonic acid, L-rhamnose, D-glucuronic acid, and D-galactose in the molar ratios 1:1:1:1.2, afforded, in addition to reducing oligosaccharides, two other oligosaccharides (**1** and **2**). Oligosaccharide **1** had $[\alpha]_D$ +76.5° (*c* 1, water) and was composed of D-galacturonic acid and L-rhamnose; oligosaccharide **2**, which had $[\alpha]_D$ +51.6° (*c* 1, water), contained D-galacturonic acid, L-rhamnose, and D-glucuronic acid in equimolar ratios. These compounds did not reduce Fehling's solution or picric acid and were unaffected by sodium borohydride, indicating that all of the anomeric carbons were involved in linkages.

A non-reducing oligosaccharide containing D-galacturonic acid and L-rhamnose has been described[2,3], which was shown[3] to be a 1,2′:2,1′-dianhydride of α-D-galactopyranuronic acid and β-L-rhamnopyranose. According to our knowledge, a non-reducing oligosaccharide containing D-galacturonic acid, L-rhamnose, and D-glucuronic acid has not been described hitherto. We now report elucidation of the structures of **1** and **2**, using ^{1}H- and ^{13}C-n.m.r. spectroscopy.

The ^{1}H-n.m.r. data of **1** and **2** are listed in Table I. Comparison of the ^{1}H-n.m.r. data of **1** with those for L-rhamnopyranose[4] and D-galactopyranuronic[5] acid revealed similar coupling constants. The $J_{1',2'}$ value of 3.7 Hz (*cf.* 3.0 and 7.5 Hz for α- and β-D-galacturonic acid[5], respectively) indicated the D-galacturonic acid residue to be α. The $J_{1,2}$ value of 1.3 Hz for the L-rhamnose residue indicated[4] it to be β. In

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TABLE I

^{1}H-N.M.R. DATA FOR **1** AND **2**

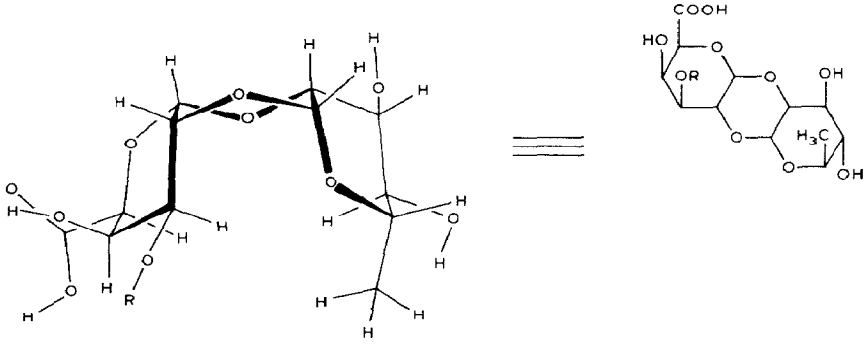
Compound	*Unit*	*Chemical shifts* (δ, p.p.m.)						Coupling constants *(Hz)*				
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
1	D-GalA	5.29	3.92	4.78	4.39	4.77		3.7	10.4	3.4	1.4	
	L-Rha	4.89	4.25	3.77	3.49[a]	3.38[a]	1.36[a]	1.3	3.8	9.6[a]	9.4[a]	4.0[a]
	D-GlcA	5.03	3.37[b]	3.56[b]	3.61[b]	3.97		7.9	[c]	[c]	9.5[a]	
2	D-GalA	5.28	4.07	4.94	4.54	4.78		3.7	10.3	3.4	1.3	
	L-Rha	4.89	4.24	3.76	3.46[b]	3.37[b]	1.37	1.3	3.8	9.6[a]	[c]	4.0[a]

[a]Approximation to first order. [b]Assigned on the basis of a 2D-hetero-correlated experiment. [c]Overlapping multiplets.

spite of non-reducing properties of 1, the chemical shifts for H-1 of the D-galacturonic acid (5.29 p.p.m.) and L-rhamnose residues (4.89 p.p.m.) differed only slightly from those of H-1 in α-D-galactopyranuronic acid (5.31 p.p.m.) and β-L-rhamnopyranose (4.86 p.p.m.). However, the H-3′ signal was shifted markedly to low field (4.78 p.p.m.) in comparison with that (3.84 p.pm.) of H-3 for α-D-galacturonic acid[5], and H-2 of the L-rhamnose residue was deshielded (0.31 p.p.m.) compared to the corresponding proton in L-rhamnose. Fujiwara and Arai[3] observed a similar large deshielding of H-3′ in the 1,2′:2,1′-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranuronate compared to that in methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate)-β-L-rhamnopyranoside. Therefore, the deshielding of H-3 is indicative of the presence of a 1,4-dioxane grouping between the D-galacturonic acid and L-rhamnopyranose residues in **1**. Confirmation of the presence of two glycosidic bonds between the two units in **1** was provided by ^{13}C-n.m.r. spectroscopy. Starting from the known ^{1}H chemical shifts of **1**, the ^{13}C chemical shifts (Table II) were assigned by the result of a 2D-heterocorrelated experiment[6]. The presence of two pairs of heteronuclear three-bond couplings through glycosidic bonds was then established. Using the selective transfer of polarisation[7] from H-1, H-2′, H-1′, and H-2, the signals of C-2′, C-1, C-2, and C-1′, respectively, appeared in the individual spectra (some other carbon signals of the respective saccharide units were also oberved). These experiments confirmed the existence of (1→2′)- and (1′→2)-glycosidic linkages in **1**.

The 1,4-dioxane grouping in **1** significantly affected the chemical shifts of the resonances for C-1 and C-2 in each unit. These nuclei were shielded (> 5 p.p.m.) in comparison to the respective carbons in β-L-Rha-(1→3)-α-D-Gal[8] and β-D-Gal-(1→2)-β-L-Rha[9] (reference compounds for the L-rhamnose moiety), and α-D-Gal-(1→2)-α-D-ManOR[10] and α-L-Fuc-(1→2)-α-D-Gal[11] (reference compounds for the D-galacturonic acid moiety). Thus, **1** is identified as α-D-galactopyranuronic acid β-L-rhamnopyranose 1,2′:2,1′-dianhydride.

The ^{1}H-n.m.r. data (Table I) for the D-galacturonic acid and L-rhamnose



1 R = H

2 R = D-Glc*p*A

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS[a] FOR **1** AND **2**

Compound	*Unit*	*Chemical shifts* (δ, p.p.m.)					
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1	D-GalA	95.9	71.6	69.7	71.5	73.9	173.2
	L-Rha	92.6	77.0	72.1	72.8	73.2	18.2
	D-GlcA	103.8	74.0	76.3	72.4	75.6	174.4
2	D-GalA	95.6	70.6	77.3	71.1	73.3	172.9
	L-Rha	92.4	76.8	72.0	72.6	73.1	18.3

[a]For solutions in D_2O (internal MeOH).

residues in **2** are similar to those in **1**, indicating the presence of two glycosidic linkages between the D-galacturonic acid and L-rhamnose residues.

In the region for anomeric protons, there was a signal at 5.03 p.p.m. (J 7.9 Hz) consistent with a non-reducing β-D-glucopyranuronic acid residue[12]. The ^{13}C signals for **2** were assigned by the results of a 2D-heterocorrelated experiment[6]. Based on the known ^{1}H chemical shifts, most ^{13}C resonances were assigned unambiguously. The signals of C-2″, C-3″, and C-4″ (of D-glucuronic acid) and C-4 and C-5 (of L-rhamnose) could not be assigned in this way due to overlapping of the respective ^{1}H signals. The assignment of these resonances was aided by comparison with chemical shifts of the ^{13}C resonances for methyl β-D-glucopyranosiduronic acid[13] and **1**. The ^{13}C chemical shifts (Table II) for the signals of the corresponding carbon atoms of the D-galacturonic acid and L-rhamnose residues of **1** and **2** were similar except for that of the resonance of C-3′ of the D-galacturonic acid residue (**1**, 69.7 p.p.m.; **2**, 77.3 p.p.m.), reflecting 3′-substitution in **2**. The chemical shifts of the remaining signals for **2** were similar to those of the corresponding carbon reso-

TABLE III

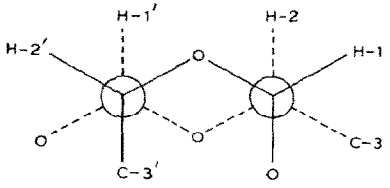
$^3J_{H,C}$ VALUES FOR **1**[a]

Coupling constant	*Value (Hz)*	*Dihedral angle*[d] *(degrees)*
$J_{H\text{-}2,C\text{-}1}$	6.2[b]	−170
$J_{H\text{-}1,C\text{-}2'}$	1.8[c]	+55
$J_{H\text{-}1',C\text{-}2}$	6.2[b]	+170
$J_{H\text{-}2',C\text{-}1}$	2.5[c]	−50

[a]Accurate to ±0.1 Hz. [b]By selective 2D INEPT[16]. [c]By a selective 2D J-resolved experiment[17]. [d]Determined from the Karplus-type dependence[15]; negative sign represents anticlockwise rotation.

nances for methyl β-D-glucopyranosiduronic acid[13]. Considering the similarity of the ^{1}H- and ^{13}C-n.m.r. data for the D-galacturonic acid and L-rhamnose residues in **2** and **1**, and the data for the D-glucuronic acid residue, **2** is identified as 3-*O*-(β-D-glucopyranosyluronic acid)-α-D-galactopyranuronic acid β-L-rhamnopyranose 1,2′:2,1′-dianhydride.

The stereochemistry of the central dioxane ring in **1** and **2** was determined on the basis of the appropriate $^3J_{H,C}$ values, which show Karplus-type dependence[14,15] on dihedral angle and were measured in a 2D *J*-resolved experiment[16,17]. The results obtained are summarised in Table III. The dihedral angles found correspond to a chair conformation (**3**) of the central 1,4-dioxane ring. The resulting $^1C_4$ and $^4C_1$ conformations of the L-rhamnopyranose and D-galacturonic acid residues, respectively, accord with those found by Fujiwara and Arai[3].



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The dianhydride **1** has been isolated also from an acid hydrolysate of Karaya gum[2] and by derivatisation of 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose[3]. The formation of **1** and **2**, as well as of dianhyrides of higher oligosaccharides, occurred[1] on acid hydrolysis of a heteropolysaccharide complex, the main chain of which is composed of (1→4)-linked D-galacturonic acid and (1→2)-linked L-rhamnose residues. It appears that the intermediate C-1 carbocation of L-rhamnose, formed on splitting of the (1→2) linkage between L-rhamnose and D-galacturonic acid, may approach and react with HO-2 of the D-galacturonic acid due to rotation around the C-1–C-2 bond, so that a 1,4-dioxane ring is formed between the saccharide units, the chair conformation of which is determined by the $^4C_1$ and $^1C_4$ conformations of D-galacturonic acid and L-rhamnose, respectively.

The formation of dianhydrides of the aldose type under conditions of acid hydrolysis is being studied further.

EXPERIMENTAL

Oligosaccharides **1** and **2** were obtained from a partial acid hydrolysate of the heteropolysaccharide complex[1], applying the separation method described by Larsson[2].

N.m.r. spectra were recorded on 4% solutions in D_2O (^{1}H, internal DSS; ^{13}C, internal MeOH) with a Bruker AM-300 spectrometer.

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