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Carbon-13 Nuclear Magnetic Resonance Spectra of Alditol-Form Oligosaccharides Having the Fundamental Structural Units of the Malvaceae Plant Mucilages and a Related Polysaccharide

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The carbon-13 nuclear magnetic resonance spectra of five alditol-form oligosaccharides and a polysaccharide having the fundamental structural units of the Malvaceae plant mucilages were measured in aqueous solution and the signals were assigned.

Keywords——^{13}C-NMR; structural unit; Malvaceae; plant mucilage; alditol-form oligosaccharide

In our previous reports, the structural features of eight mucilages from plant sources in the Malvaceae family, *i.e.*, the roots of *Abelmoschus manihot*,[1] *Althaea officinalis*,[2] *Abelmoschus glutinotextilis*,[3] *Althaea rosea*,[4] and *Abelmoschus esculentus*,[5] the immature fruit of *Abelmoschus esculentus*,[6] and the leaves of *Althaea officinalis*[7] and *Althaea rosea*,[8] have been elucidated. The main part of most of them is made up of a component unit having the structure (1→4)-[*O*-β-(D-glucopyranosyluronic acid)-(1→3)]-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranose in common, while Okra-mucilage F[6] has no branch at the D-galacturonic acid residues in the main chain. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy is a useful tool for the structural determination of carbohydrates.[9-20] In this paper, the ^{13}C-NMR data for five oligosaccharides and a polysaccharide having the fundamental structural units of the above-mentioned plant mucilages are presented.

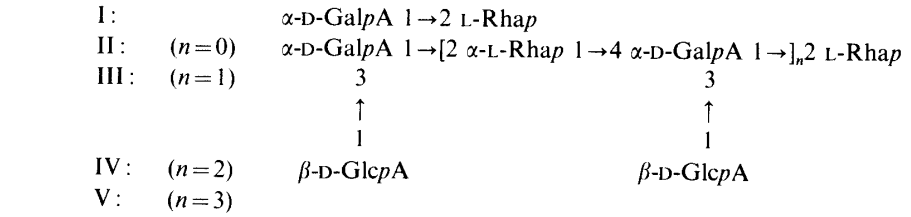

Chart 1. Structural Features of Oligosaccharides I, II, III, IV, and V

Rhap, rhamnopyranose; GalpA, galactopyranosyluronic acid; GlcpA, glucopyranosyluronic acid.

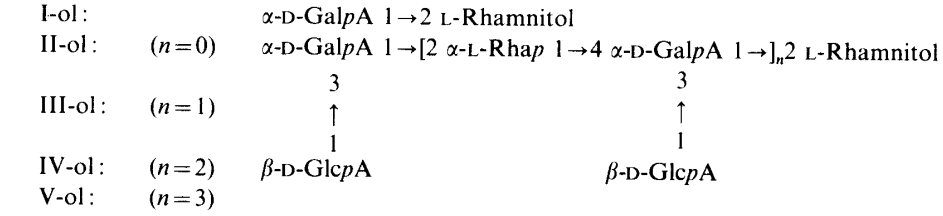

Chart 2. Structural Features of Reduced Oligosaccharides I-ol, II-ol, III-ol, IV-ol, and V-ol

Table I. ^{13}C-NMR Chemical Shift Data for L-Rhamnitol

Compound	C-1	C-2	C-3	C-4	C-5	C-6
L-Rhamnitol	65.72	73.75	72.13	75.92	69.80	21.47

Table II. ^{13}C-NMR Chemical Shift Data for Reducing Oligosaccharides

Compounds	C-1	C-2	C-3	C-4	C-5	C-6
I-ol						
Rhamnitol	62.45	80.97	71.75[a]	76.25	69.78[b]	21.37
Gal*p*A	100.57	70.63[b]	71.39[a]	73.94	72.83	175.72
II-ol						
Rhamnitol	62.44	81.10	71.41	76.27	69.63[c]	21.36
Gal*p*A	100.44	69.77[c]	81.48	73.41	72.41	175.01
Glc*p*A	106.39	75.64	77.77	73.98	77.23	175.41
III-ol						
Rhamnitol	62.47	81.05	71.99	76.23	69.78[d]	21.34
Gal*p*A (rs)	100.48	70.01[d]	81.59	78.58	72.59	175.68
Glc*p*A (rs)	106.81	75.96	78.09	74.38	77.88	176.47
Rha*p*	101.19	80.93	71.41	73.23	69.43	19.14
Gal*p*A (ns)	100.11	69.78[d]	81.59	73.91	72.59	174.95
Glc*p*A (ns)	106.42	75.73	78.09	74.17	77.88	175.76
IV-ol						
Rhamnitol	62.48	81.17	71.99	76.27	69.62[e]	21.32
Gal*p*A (rs)	100.57	69.86[e]	81.82	78.02	72.56	175.34
Glc*p*A (rs)	106.97	75.97	79.24	74.37	77.81	175.68
Rha*p* (c)	101.42	80.78	71.46	73.05	69.38	19.13
Gal*p*A (c)	100.50	69.78[e]	81.76	77.82	72.01	174.88
Glc*p*A (c)	106.97	75.92	79.23	74.05	77.39	174.88
Rha*p* (ns)	101.33	80.78	71.46	72.83	69.38	19.13
Gal*p*A (ns)	100.09	69.78[e]	81.53	73.13	72.01	174.64
Glc*p*A (ns)	106.46	75.68	78.60	73.82	77.28	174.88
V-ol						
Rhamnitol	62.46	81.14	71.99	76.26	69.76[f]	21.33
Gal*p*A (rs)	100.62	69.85[f]	81.80	78.02	72.58	175.42
Glc*p*A (rs)	106.99	75.99	79.32	74.37	77.82	175.86
Rha*p* (rsc)	101.40	80.78	71.43	73.07	69.39	19.12
Gal*p*A (rsc)	100.62	69.76[f]	81.80	78.02	72.58	174.95
Glc*p*A (rsc)	106.99	75.99	79.32	74.07	77.46	175.42
Rha*p* (nsc)	101.40	80.78	71.43	73.07	69.39	19.12
Gal*p*A (nsc)	100.50	69.76[f]	81.80	78.02	72.58	174.95
Glc*p*A (nsc)	106.99	75.99	79.32	74.07	77.46	175.42
Rha*p* (ns)	101.32	80.78	71.43	72.84	69.39	19.12
Gal*p*A (ns)	100.09	69.61[f]	81.55	73.07	72.58	174.72
Glc*p*A (ns)	106.46	75.68	78.62	73.83	77.32	174.95

Abbreviations: rs, reducing side; ns, non-reducing side; c, center. *a*—*f*) Assignments may be reversed.

The five oligosaccharides (I to V) having the structures shown in Chart 1 were obtained by partial hydrolysis of the mucilages, as described in a previous report.[2] To avoid the complexity of the spectra based on α- and β-anomeric forms of each reducing end unit, the oligosaccharides were treated with sodium borohydride to give the corresponding alditol forms (I-ol to V-ol in Chart 2). Assignments were made on the basis of comparison with the

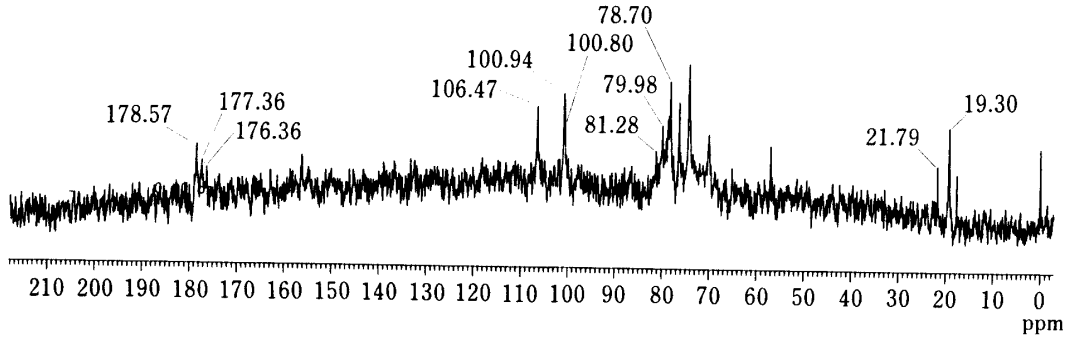

Fig. 1. ^{13}C-NMR Spectrum of Althaea-Mucilage OL

data for L-rhamnitol (Table I), methyl 2-*O*-α-L-rhamnopyranosyl-L-rhamnopyranoside,[21] 4-*O*-α-L-rhamnopyranosyl-D-galactopyranose,[22] methyl α-D-galactopyranosiduronic acid, and methyl β-D-glucopyranosiduronic acid,[23] and the results are shown in Table II.

The variations of chemical structure are reflected by the signals in the region of 77.82 to 106.99 ppm, which are mainly attributable to the anomeric carbons and the carbons involved in glycosidic linkages.

The C-1 signals for the glycosidic carbon of α-L-rhamnopyranose appeared at 101.19 to 101.42 ppm; the corresponding figures were 100.09 to 100.62 ppm for α-D-galactopyranosyluronic acid and 106.39 to 106.99 ppm for β-D-glucopyranosyluronic acid. Thus, the glycosidic carbon resonances are divided into three groups.

The downfield displacements of the C-2 signal of 2-*O*-α-(D-galactopyranosyluronic acid)-L-rhamnitol in I-ol and II-ol were 7.22 and 7.35 ppm, compared with the C-2 signal of L-rhamnitol, and those of 2-*O*-α-(D-galactopyranosyluronic acid)-L-rhamnose units in III-ol, IV-ol, and V-ol were 8.88 to 9.03 ppm, compared with α-L-rhamnopyranose. The downfield displacements of the C-3 signal of 3-*O*-β-(D-glucopyranosyluronic acid)-D-galacturonic acid units in II-ol, III-ol, IV-ol, and V-ol were 10.14 to 10.43 ppm, compared with the D-galacturonic acid unit in I-ol, and those of the C-4 signal of 4-*O*-α-L-rhamnopyranosyl-D-galacturonic acid units in III-ol, IV-ol, and V-ol were 4.41 to 5.17 ppm, compared with the D-galacturonic acid unit in II-ol.

The methyl signals (C-6) of rhamnitol and rhamnose were separately observed in the region of 21.34 to 21.37 ppm and at 19.14 ppm in I-ol, II-ol, and III-ol, and the carboxyl signals (C-6) of galacturonic acid were separately observed in the region of 174.95 to 175.72 ppm. Those of glucuronic acid were also separately observed in the region of 175.41 to 176.47 ppm in the same oligosaccharides. However, in the nonasaccharide (IV-ol) and dodecasaccharide (V-ol), some of the carbon signals having the same position numbers showed overlapping.

The ^{13}C-NMR spectrum of Althaea-mucilage OL from the leaves of *Althaea officinalis* is shown in Fig. 1. Structural studies[7] indicated that the mucilage is mainly composed of the fundamental trisaccharide (II) repeating unit with 1% acetyl groups and *ca.* 3% protein. The assignment of the signals was done by comparing the data with those of the oligosaccharides described above. The signals at 178.57 and 177.36 ppm are assigned to C-6 carbons of glucuronic acid and galacturonic acid residues, and the signals at 100.94 and 79.98 ppm are assigned to C-1 and C-2 carbons of α-1→2 linked L-rhamnose residues. The signals at 106.47 and 100.80 ppm are assigned to C-1 carbons of β-D-glucuronic acid and α-D-galacturonic acid residues, and those at 81.28 and 78.70 ppm to the C-3 and C-4 glycosyl-linked carbons in the α-D-galacturonic acid residues. In addition to the methyl signal of rhamnose at 19.30 ppm, the signals at 21.79 and 176.36 ppm are attributable to the methyl and carbonyl carbons in the acetyl groups.

The results described above indicate that ^{13}C-NMR spectroscopy can provide useful information about the structures of plant mucilages in the Malvaceae family if the mucilages show good solubility.

Experimental

Preparation of Samples——Oligosaccharides I to V were prepared by partial acid hydrolysis of the mucilages[1-5] from plants in the Malvaceae family. After purification by gel chromatography,[4] each oligosaccharide was dissolved in water, then reduced with sodium borohydride and treated with Dowex 50W-X8 (H^+) to obtain the corresponding alditol form.[1] After removal of boric acid by repeated addition and evaporation of methanol, the reduced oligosaccharides (I-ol to V-ol) were obtained. Althaea-mucilage OL was isolated from the leaves of *Althaea officinalis* as described previously.[7]

Measurement of ^{13}C-NMR Spectra——^{13}C-NMR spectra were recorded in sample tubes with an outside diameter of 5 mm on a JEOL JNM-GX 270 NMR spectrophotometer operating at 68 MHz in the pulsed Fourier-transform mode with complete proton decoupling at 27 C. Most of the oligosaccharides were dissolved in heavy water as 3% solutions, but the concentrations for the disaccharide and Althaea-mucilage OL were 0.4% and 1.4%, respectively. Acetone and 2,2-dimethyl-2-silapentane-5-sulfonate were used as internal standards for the oligosaccharides and the mucilage, respectively. Spectra were determined after 15000 (for the disaccharide) or 30000 (for the others) scans.

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