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STRUCTURE OF L-IDURONO-D-GLUCURONANS FROM THE FUNGI *Aseroe arachnoidea* AND *Pseudocolus fusiformis**

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ABSTRACT

L-Idurono-D-glucuronans have been isolated from the fungi *Aseroe arachnoidea* and *Pseudocolus fusiformis*. Methylation analysis of the reduced glycuronans and ^{1}H- and ^{13}C-n.m.r. spectroscopy of the glycuronans indicated a linear structure containing (1→4)-linked α-L-iduronic acid and β-D-glucuronic acid residues in the molar ratios 1:2 (*A. arachnoidea*) and 1:3 (*P. fusiformis*).

INTRODUCTION

Uronic acid-containing polysaccharides are widely distributed in Nature, and those composed entirely of uronic acid residues are alginic acid[1,2], pectic acid[3], mucoric acid[4], and protuberic acid[5] (PA).

PA has been isolated from *Kobayasia nipponica*, which belongs to the Gasteromycetes[5], and is composed of (1→4)-linked α-L-iduronic acid and β-D-glucuronic acid residues[6,7] in the ratio 1:2. The structure of PA is mainly the trisaccharide repeating-unit[8] →4)-β-D-Glc*p*A-(1→4)-α-L-Ido*p*A-(1→4)-β-D-Glc*p*A-(1→.

In certain instances, the polysaccharides of various species have been used by taxonomists as an aid in classification of the parent organisms, such as Lypomyces sp.[9,10] or yeasts[11,12]. We now discuss whether the structure of the glycuronans from *A. arachnoidea* and *P. fusiformis*, which belong to the Gasteromycetes, is similar to that of PA from *K. nipponica* or not.

EXPERIMENTAL

Materials and methods. — *Pseudocolus fusiformis* was collected at Hachioji, a surburb of Tokyo, in September. *Aseroe arachnoidea* was collected at Okazaki, a surburb of the Aichi prefecture of Japan, and was kindly supplied by Mr. Sumio Honda.

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Uronic acid was determined by the orcinol[13] and carbazole[14] methods. Carbazole/orcinol (C/O) ratios were determined by the method of Hoffman *et al.*[15]. Phosphorus[16], nitrogen[17], and sulfate[18] were determined by literature procedures. Identification of uronic acids by t.l.c. and g.l.c. was carried out as previously described[6]. Optical rotations were measured for solutions in semimicro tubes at 20° with a JASCO DIP-Digital polarimeter.

Purification of the glycuronans from A. arachnoidea *and* P. fusiformis. — The fresh fruiting-bodies were extracted thrice with distilled water at 4° for 16 h and the filtered extracts were added to ethanol (3 vol.). The precipitates were collected, washed successively with ethanol, acetone, and ether, and dried *in vacuo*.

Aqueous 10% solutions of the precipitates were added to aqueous 10% cetyltrimethylammonium bromide until precipitation was complete. The precipitates were collected by centrifugation and washed with cold water, and solutions in aqueous 10% sodium chloride were poured into ethanol (3 vol.) with stirring. The resulting precipitates were collected, and solutions in water were dialysed against distilled water for 3 days and then lyophilised.

The aqueous solutions (50 mg/5 mL) of lyophilised powder were fractionated on a column (1.6 × 20 cm) of DEAE-Sephadex A-25 (Cl^- form), equilibrated with 0.01M HCl, by gradient elution with 0 → M NaCl. Fractions (4 mL) were collected at 20–25 mL/h. The glycuronans were eluted with ~0.3M NaCl. The fractions were dialysed against distilled water for 3 days, concentrated, and lyophilised.

An aqueous solution (30 mg/mL) of the lyophilised glycuronan was eluted from a column (1.4 × 120 cm) of Sepharose 4B, equilibrated and eluted with 0.2M NaCl. Molecular size was estimated by using the following standards, Dextran T-500 (mol. wt. 500,000), T-250 (250,000), T-110 (110,000), T-70 (70,000), and T-40 (40,000). Fractions (2.5 mL) were collected at 10–15 mL/h. The glycuronan fractions were dialysed against distilled water for 3 days, concentrated, decationised with Dowex 50(H^+) resin, neutralised with 0.1M NaOH, and lyophilised. The purified glycuronans are designated as AA from *A. arachnoidea* and as PF from *P. fusiformis*.

Reduction of the glycuronan and identification of the components. — Reduced glycuronans (R-AA and R-PF) were prepared by the method of Taylor and Conrad[19], hydrolysed with 2M trifluoroacetic acid at 105° for 2 h, and then reduced with $NaBH_4$. The reduced products were converted[7] into alditol acetates, and subjected to g.l.c. at 210° (injector 270°), using a glass column (0.3 × 200 cm) packed with 3% of OV-225 on Uniport KS (60/80 mesh), with nitrogen as the carrier gas at 60 mL/min.

Methylation analyses of R-AA and R-PF. — R-AA and R-PF were methylated[7], and then hydrolysed with 90% formic acid at 100° for 5 h followed by M trifluoroacetic acid at 100° for 2 h. Each hydrolysate was concentrated, and the syrupy residue was reduced and acetylated. The methylated alditol acetates were then subjected to g.l.c. at 188° (injector 230°), using a glass column (0.3 × 300 cm) packed with 3% of ECNSS-M on Gas Chrom Q (100/120 mesh), with nitrogen as the carrier gas at 60 mL/min.

N.m.r. spectroscopy. — ^{1}H-N.m.r. spectra were recorded at 70° for solutions

in D_2O (internal TSP) with a JEOL-PS-100 spectrometer. ^{13}C-N.m.r. spectra (25 MHz) were recorded at 70° for solutions in D_2O with a JEOL-FX-100 spectrometer in the pulsed Fourier-transform (F.t.) mode with complete proton-decoupling. The chemical shifts were expressed as p.p.m. downfield from that of Me_4Si by using internal 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate (TSP). Proton-decoupled F.t. spectra were measured by using a repetition time of 2.0 μs, a pulsed width of 7 μs (45°), 8K real data points, a sweep width of 5,000 Hz, and, typically, 20,000–40,000 scans. PA isolated from *K. nipponica* was used as the reference material for assigning the ^{13}C-chemical shifts.

RESULTS

Properties of AA and PF. — The yields and uronic acid contents during the purification steps (see Experimental) are shown in Table I, and a typical elution from

TABLE I

PURIFICATION OF GLYCURONANS

Purification step	A. arachnoidea *Yield*[a]			*Uronic acid*[b] (%)	P. fusiformis *Yield*[a]			*Uronic acid*[b] (%)
	A (mg)	B (%)	C (%)		A (mg)	B (%)	C (%)	
EtOH precipitation	690	0.46	100	49.6	657	0.44	100	45.9
Cetavlon precipitation	258	0.17	37.4	89.7	245	0.16	37.3	91.9
DEAE-Sephadex A-25 form (Cl^-)	212	0.14	30.7	93.5	186	0.12	28.3	93.8
Sepharose 4B	186	0.12	27.0	95.4	167	0.11	25.4	94.4

[a]*A*, From fresh fruiting-bodies (150 g). *B*, Based on the fruiting bodies. *C*, Based on the ethanol precipitate. [b]Determined by the method of Bitter and Muir[14].

TABLE II

PROPERTIES OF THE GLYCURONANS

Glycuronan[a]	$[\alpha]_D$ (degrees)	*Uronic acid (%)*		*C/O ratio*	*Molecular weight*	*Component*
		Orcinol	*Carbazole*			
AA	−73.6	101.5	95.4	0.80	~240,000	D-GlcA L-IdoA
PF	−72.4	100.4	94.4	0.88	~45,000	D-GlcA L-IdoA
PA	−76.3	102.1	92.3	0.79	~170,000	D-GlcA L-IdoA

[a]Sodium salts.

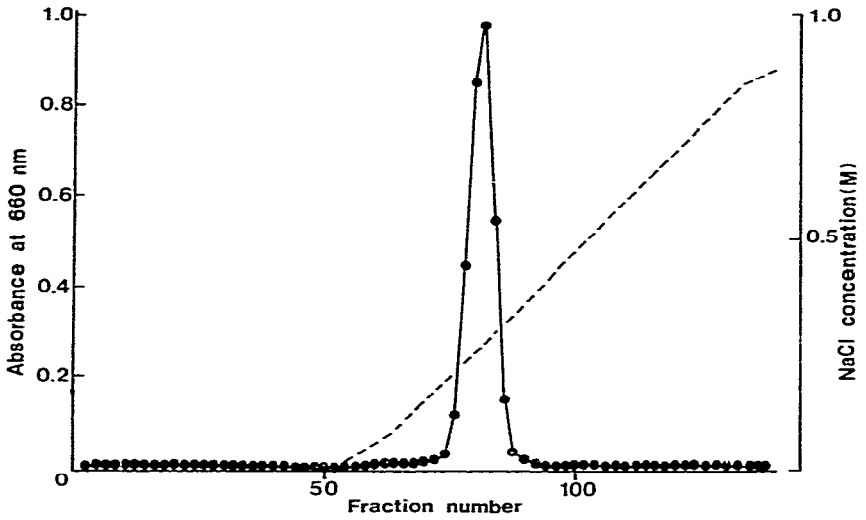

Fig. 1. Elution profile of PF on DEAE-Sephadex A-25 (Cl^- form): the NaCl concentration was measured by refractometry.

DEAE-Sephadex is shown in Fig. 1. The properties of the purified glycuronans are shown in Table II. The glycuronans contained D-glucuronic acid and L-iduronic acid residues, but no nitrogen, phosphorus, or sulfate.

Methylation analysis of R-AA and R-PF. — The alditol acetates derived from R-AA and R-PF were identified as 2,3,4-tri-*O*-acetyl-1,6-anhydroidose, hexa-*O*-acetylliditol, and hexa-*O*-acetylglucitol. The glucose–idose ratios were 1.5:1 (for R-AA) and 3.1:1 (for R-PF).

The results of methylation analysis for R-AA and R-PF are shown in Table III. The glucose–idose ratios were 2.1:1 (for R-AA) and 3.1:1 (for R-PF).

N.m.r. spectroscopy. — The chemical shifts of H-1 and carbon atoms were assigned, and are shown in Table IV. The chemical shifts of the signals for AA and PF were identical with those of PA, which possesses a trisaccharide repeating-unit[8]. These results indicated that the glycuronans AA and PF consist of (1→4)-linked α-L-iduronic acid and β-D-glucuronic acid residues.

TABLE III

G.L.C. DATA FOR METHYLATED ALDITOL ACETATES DERIVED FROM R-AA AND R-PF

	T[a]	*PF*	*AA*
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol	1.00	1.0	1.0
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol	2.51	19.2	56.4
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylliditol	2.72	6.5	27.8
Glucose/Idose ratio	—	3.1	2.1

[a]Retention time relative to that (9.6 min) of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.

TABLE IV

N.M.R. DATA FOR THE GLYCURONANS

Glycuronan[a]	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*H-1 Shift (Hz)*	*Ratio*	*Assignment*
	104.2	74.4	75.4	82.1	76.6	176.1	4.59(7.8)	3.0	β-GlcA
PF	103.5	74.1	75.4	80.5	77.1	175.8			β-GlcA
	102.6	71.6	69.6	80.5	70.9	175.5	5.00(3.0)	1.[illegible]	α-IdoA
	104.3	74.4	75.5	82.2	76.5	176.1	4.57(7.2)	2.2	β-GlcA
AA	103.6	74.1	75.5	80.6	77.2	175.8			β-GlcA
	102.6	71.6	69.4	80.6	71.0	175.6	4.97(3.0)	1.0	α-IdoA
	104.3	74.5	75.4	82.2	76.6	176.1	4.55(7.3)	2.3	β-GlcA
PA	103.6	74.2	75.4	80.6	77.3	175.8			β-GlcA
	102.6	71.6	70.4	80.6	71.0	175.6	4.94(3.1)	1.0	α-IdoA

[a]Sodium salts.

DISCUSSION

The aqueous extracts of the fungi *A. arachnoidea* and *P. fusiformis*, after precipitation with ethanol, contained ~40% of glycuronan, and the addition of cetyltrimethylammonium bromide precipitated most of this. The glycuronans were further purified, to remove paramagnetic molecules that would interfere with n.m.r. spectroscopy. The properties of the purified glycuronans were similar to those of PA except for molecular weight (Table II). The molecular weights of AA and PF were 240,000 and 45,000 (*cf.* 170,000 for PA). These results were supported by the methylation analysis data. The ratios of tri-*O*-methyl to tetra-*O*-methyl sugars were 84.2:1 for R-AA, 25.7:1 for R-PF (Table III), and 44.6:1 for R-PA[7], respectively. The linkages in the glycuronans were shown by methylation analysis and ^{13}C-n.m.r. spectroscopy to be (1→4) for both the L-iduronic acid and D-glucuronic acid residues (Tables III and IV). The n.m.r. data also indicated the configurations to be α-L-iduronic acid and β-D-glucuronic acid (Table IV). The ratios of glucuronic acid to iduronic acid were shown to be 2:1 in AA and 3:1 in PF by examination of alditol acetates, methylated alditol acetates, and ^{1}H-n.m.r. data (Tables III and IV).

The assignment of chemical shifts in the ^{13}C-n.m.r. spectrum of PA was determined from those of the oligosaccharides, *O*-(β-D-glucopyranosyluronic acid)-(1→4)-D-glucuronic acid, *O*-(α-L-idopyranosyluronic acid)-(1→4)-D-glucuronic acid, *O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-D-glucuronic acid, and *O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-D-glucuronic acid, obtained by partial hydrolysis[8] (0.01M H_2SO_4, 100°, 5.5 h). The chemical shifts of the signals for PF and AA were identical with those of PA, and the structure of AA was identical with that of PA except for the molecular weight. Signals for adjacent iduronic acid residues were not found in the spectrum of PF. Therefore, the n.m.r.

data suggested that PF consists of a tetrasaccharide repeating-unit in which a D-glucuronic acid residue is incorporated in the trisaccharide repeating-unit of PA.

The finding that glycuronans composed of L-iduronic acid and D-glucuronic acid residues exist in fungi belonging to the Gasteromycetes is of interest from a taxonomic view-point.

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