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Note

Structural analyses of L-idurono-D-glucuronans (phallic acids) isolated from fungi belonging to the Phallales*

HIROSHI TSUCHIHASHI**, TOSHIRO YADOMAE, AND TOSHIO MIYAZAKI***

Department of Microbial Chemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03 (Japan)

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From the results of a survey of Ascomycotina and Basidiomycotina, glycuronans were found to be widely distributed in the taxon Phallales, and these glycuronans were named phallic acids[1].

One of the phallic acids, protuberic acid, has been isolated from *Kobayasia nipponica*[2] and is composed of (1→4)-linked α-L-iduronic acid (IdoA) and β-D-glucuronic acid (GlcA) residues[3,4] in the ratio 1:2. Protuberic acid has mainly a trisaccharide repeating-unit[5]. Furthermore, we found that three other phallic acids from *Aseroe arachnoidea*[6], *Phallus impudicus*[7], and *Pseudocolus fusiformis*[6] are composed of (1→4)-linked α-L-IdoA and β-D-GlcA residues in the ratios 1:2, 1:2, and 1:3, respectively.

We now describe the structures of six species of phallic acids isolated from *Dictyophora rubrovolvata*, *Ileodictyon gracile*, *Linderia bicolumnata*, *Lysurus mokusin*, *Mutinus bambusinus*, and *Phallus rugulosus*, and summarise the analytical data for ten species of phallic acids.

The phallic acids were eluted with ~0.3M NaCl during ion-exchange chromatography on DEAE-Sephadex A-25 (Cl^- form) as described[6], and the properties of the purified acids are summarised in Table I. The phallic acids contained no nitrogen, phosphorus, or sulphate.

The alditol acetates derived from carboxyl-reduced phallic acids were identified as 2,3,4-tri-*O*-acetyl-1,6-anhydroidose, hexa-*O*-acetyliditol, and hexa-*O*-acetylglucitol. The glucose–idose ratios are shown in Table I. The chemical shifts

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**Present address: Department of Pharmacology, Niigata College of Pharmacy, 5829 Kamishinei-cho, Niigata 950-21, Japan.

***To whom inquiries should be addressed.

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TABLE I

PROPERTIES OF PHALLIC ACIDS (L-IDURONO-D-GLUCURONANS)

	Yield[a] *(%)*	*Uronic acid content (%)*	*C/O ratio*	*Glucose–idose*[b] *ratio*	$[\alpha]_D$ *(degrees)*	*Molecular weight*
Aseroe arachnoidea[c]	0.17	95.4	0.80	1.5	−74	240,000
Dictyophora rubrovolvata	0.14	94.6	0.81	2.1	−74	74,000
Ileodictyon gracile	0.46	98.5	0.86	2.7	−75	130,000
Kobayasia nipponica[d]	0.25	94.5	0.79	2.3	−76	170,000
Linderia bicolumnata	0.36	98.2	0.72	1.9	−71.5	53,000
Lysurus mokusin	0.31	95.2	0.82	1.7	−74	40,000
Molinus bambusinus	0.30	94.3	0.77	1.8	−74	50,000
Phallus impudicus[e]	0.65	92.5	0.82	2.3	−74	150,000
Phallus rugulosus	0.77	94.0	0.80	2.0	−70	80,000
Pseudocolus fusiformis[c]	0.16	94.4	0.88	3.1	−72	45,000

[a]Calculated from each fruiting body. [b]Obtained by g.l.c. analysis of the alditol acetates derived from the reduced phallic acid. [c]From ref. 6. [d]From refs. 2, 3, and 4 [e]From ref. 7.

of the n.m.r. signals for H-1 and the carbon atoms were assigned as shown in Table II. The chemical shifts of the signals for the six newly isolated phallic acids were identical with those of the glycuronans isolated from *A. arachnoidea*, *K. nipponica*, *Ph. impudicus*, and *Ps. fusiformis*. These results indicate that the phallic acids consist of (1→4)-linked α-L-IdoA and β-D-GlcA residues, and that the glucose–idose ratios (Table I) and D-GlcA/L-IdoA ratios (Table II) are 2 or 3. In the ^{13}C-n.m.r. spectrum of the phallic acid from *I. gracile* (Table II), which was similar to that of the acid from *Ps. fusiformis*, some signals showed an ~1.5–2.0-fold increase in intensity compared with those of protuberic acid[5], and there was one additional signal. These signals were identical with those of the acid from *Mucor mucedo*. Furthermore, signals for adjacent iduronic acid residues were not found in the spectra of all phallic acids. Therefore, these results suggest the possibility that the structure of phallic acids having a D-GlcA–L-IdoA ratio of 1:2 is identical with that of protuberic acid except for molecular size, and that the structures of phallic acids having a D-GlcA–L-IdoA ratio of 1:3 possess a tetrasaccharide repeating-unit, in which one D-GlcA residue is added to the trisaccharide repeating-unit of protuberic acid.

EXPERIMENTAL

Materials and methods. — All fungi were collected from natural environments. Uronic acid, phosphorus, nitrogen, and sulphate contents, carbazole–orcinol (C/O) ratios, and identification of uronic acids by t.l.c. and g.l.c. were carried out by the methods described previously[3–7]. Optical rotations were measured with semimicro tubes at 20° and a JASCO DIP-Digital polarimeter.

Preparation of phallic acids. — The fresh fruiting-bodies of six species of

TABLE II

N M R DATA FOR PHALLIC ACIDS

	Chemical shifts (p p m.)[a]							*Assignment*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*H-1 (J, Hz) Integral*	
Aseroe arachnoidea[6]	104 3	74 4	75.5	82 2	76 5	176 1	4 57(7 2)2 2	β-GlcA
	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
Dictyophora rubrovolvata	104 3	74.4	75 5	82.2	76.6	176.1	4.58(7 7)2.2	β-GlcA
	103 6	74 0	75 5	80.6	77 1	175 7		β-GlcA
	102 6	71.6	71 0	80.6	71.0	175 5	5 02(3 0)1 0	α-IdoA
Ileodictyon gracile	104 4	74.4	75.4	82.2	76 5	176 1		β-GlcA
	103 5	74.1	75 4	80.5	77.3	175.8	4 59(7.6)3.1	β-GlcA
	103 5*	74.0*	75.4*	82 2*	76.5*	175 8*		β GlcA
	102.6	71.7	71.0	80.5	71.0	175 6	5 03(3 0)1 0	α-IdoA
Kobayasia nipponica[5]	104.3	74 5	75 4	82.2	76.6	176 1	4.55(7 3)2 3	β-GlcA
	103 5	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
Linderia bicolumnata	104.2	74.4	75.5	82 1	76 6	176 1	4 56(7 8)2 0	β-GlcA
	103 5	74 0	75 3	80 5	77 1	175.8		β-GlcA
	102 5	71 7	71.0	80.3	71 0	175 8	4 98(3.0)1 0	α-IdoA
Lysurus mokusin	104 3	74.4	75 5	82.2	76.5	176.1	4 56(7 7)2 1	β-GlcA
	103 5	74 2	75.5	80.5	77 1	175 8		β-GlcA
	102.6	71.6	70.4	80.5	71 0	175 6	4 97(3 0)1 0	α-IdoA
Mutinus bambusinus	104 3	74.4	75 5	82.1	76 5	176 1	4 59(7.8)1.9	β-GlcA
	103.6	74 0	75 5	80.4	77 2	175.8		β-GlcA
	102 6	71.6	70.8	80 2	71.0	175 7	5.01(3.0)1.0	α-IdoA
Phallus impudicus[7]	104.1	74.4	75.3	82.2	76 7	176 1	4 57(7 8)2 3	β-GlcA
	103.4	74.1	75 3	80.2	77.3	175 9		β-GlcA
	102.5	71 3	70.8	80.2	71 0	175.6	4 98(3 2)1 0	α-IdoA
Phallus rugulosus	104.3	74 4	75 5	82.1	76 5	176.1	4 60(7.8)2 1	β-GlcA
	103.6	74 0	75.5	80 4	77 2	175 8		β-GlcA
	102 6	71 7	71 0	80.4	71 0	175.7	5.07(3 0)1 0	α-IdoA
Pseudocolus fusiformis[6]	104 2	74 4	75.4	82 1	76 6	176 1		β-GlcA
	103 5	74 1	75 4	80.5	77 1	175 8	4 59(7 8)3.0	β-GlcA
	103 5*	74 1*	75 4*	82.1*	76 6*	175 8*		β-GlcA
	102.6	71.6	69 6	80 5	70.9	175 5	5 00(3 0)1.0	α-IdoA
Mucor mucedo[9]	103 5	74 0	75 5	82 0	76 7	175 9	4 64(8.0)—	β-GlcA

[a]The signals marked * had high intensity or were new signals in comparison with those of protuberic acid from *Kobayasia nipponica*.

fungi were extracted three times with distilled water at 4° for 24 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 solutions (50 mg/5 mL) of dried 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 0–1M NaCl. Fractions (4 mL) were collected at 20–25 mL/h. Phallic acids 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 each lyophilised phallic acid was eluted from a column (1.4 × 120 cm) of Sepharose 4B, equilibrated and eluted with 0.2M NaCl. Molecular size was estimated with 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 phallic acid fractions were dialysed against distilled water for 3 days, concentrated, decationised with Dowex 50 (H^+) resin, neutralised with 0.1M NaOH, and lyophilised.

Reduction of phallic acids and identification of the components. — Phallic acids were reduced by the method of Taylor and Conrad[8], and the products were hydrolysed with 2M trifluoroacetic acid at 100° for 2 h and then reduced with $NaBH_4$. The reduced products were converted[3] 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 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 mode with complete proton decoupling. The chemical shifts were expressed as p.p.m. downfield from that of tetramethylsilane using internal TSP. Proton-decoupled Fourier-transform spectra were measured by using a repetition time of 2.0 s, a pulsed width of 7 μs (45°), 8k real data points, a sweep width of 5,000 Hz, and, typically, 20,000–100,000 scans. The glycuronans isolated from *A. arachnoidea*[6], *K. nipponica*[5], *Mucor mucedo*[9], *Ph. impudicus*[7], and *Ps. fusiformis*[6] were used as reference materials for assigning the ^{13}C-chemical shifts.

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