Carbohydrate Research, 77 (1979) 281–284

Note

Detection and identification of L-iduronic acid in the glycuronan "protuberic acid" from *Kobayasia nipponica**

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(Received December 28th, 1978; accepted for publication, April 19th, 1979)

The water-soluble glycuronan "protuberic acid" (PA)[1] has been isolated from *Kobayasia nipponica*, a fungus of Rhizopogonaceae, and postulated to consist mainly of a linear chain of (1→4)-linked D-glucuronic acid residues. However, a significant proportion of an unidentified substance was detected in the acid hydrolysate of methylated reduced-PA, in addition to 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose, and the uronic acid content of PA was 91% when determined by the method of Bitter and Muir with D-glucuronic acid as the standard. We now report further on the components of PA.

The identification of iduronic acid is usually based on specific optical rotation[2,3], colorimetric analysis using the carbazole-to-orcinol ratio[4,5], g.l.c.[5–9], and ion-exchange chromatography[10,11]. The presence of L-iduronic acid residues in heparin, heparan sulphate, and dermatan sulphate[3,4,12–14] and in the capsular polysaccharide[5] of *Clostridium perfringens* Hobbs 10 has been proposed, but there is no report of its occurrence in fungi.

The colorimetric determinations of uronic acid for decationised PA, shown in Table I, suggest that PA contains other uronic acids in addition to glucuronic acid. PA contained no nitrogen, phosphorus, and sulphate.

TABLE I

COLORIMETRIC DETERMINATIONS ON DECATIONISED PA (D-PA)

Methods	*Percentage (w/w)*		
	D-PA	*Glucuronic acid*	*Iduronic acid*
Orcinol–HCl (O)	104.4	100[a]	130[a]
Carbazole (C)	78.7	100[a]	30[a]
Bitter–Muir	94.5	100[b]	83[b]
C/O ratio	0.75	1.00[a]	0.22[a]

[a]From Ref. 4. [b]From Ref. 27.

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When PA was hydrolysed with 0.5M H_2SO_4 at 100° for 3 h, four products were detected by t.l.c. with $R_{Glucuronolactone}$ values corresponding to iduronolactone (1.17), glucuronolactone (1.00), iduronic acid (0.71), and glucuronic acid (0.53). However, when the hydrolysis was prolonged to 12 h, only glucuronic acid could be detected in the hydrolysate.

In g.l.c.–m.s., the trimethylsilyl derivatives of the hexuronolactones prepared from the hydrolysate of PA gave characteristic fragment ions of *m/e* 392, 377, and 287, corresponding to M^+, $M^+ - 15$, and $M^+ - 15 - 90$, respectively, and the retention times were identical with those of authentic iduronolactone and glucuronolactone, respectively (Table II). Also, products with fragment ions (*m/e* 466, 451, and 361, corresponding to M^+, $M^+ - 15$, and $M^+ - 15 - 90$) characteristic of aldono-1,4-lactones derived from the uronic acids were detected. The g.l.c. data (Table III) indicated them to be idono-1,4-lactone and gulono-1,4-lactone.

TABLE II

G.L.C. DATA[a] FOR TRIMETHYLSILYLATED URONOLACTONES

Parent	T	
	SE-30	*OV-17*
D-Glucuronolactone	0.46	1.66
D-Mannuronolactone	0.58	1.95
L-Iduronolactone	0.34, 0.36	0.99, 1.09
Hydrolysate component	0.34, 0.37	0.99, 1.09

[a]Obtained at 150° on a glass column (0.3 × 200 cm) of Gas Chrom Q (60–80 mesh) coated with 1% of SE-30 or 1% of OV-17 at 150°; nitrogen flow-rate, 50 ml/min. Retention time (*T*) relative to that of trimethylsilylated mannitol (SE-30, 21.91 min; OV-17, 8.83 min).

TABLE III

G.L.C. DATA[a] FOR TRIMETHYLSILYLATED ALDONO-1,4-LACTONES

Parent	T	
	SE-30	*OV-17*
L-Gulono-1,4-lactone	0.79	1.82
D-Mannono-1,4-lactone	1.06	2.39
L-Idono-1,4-lactone	0.82	1.76
L-Galactono-1,4-lactone	0.70	1.47
Hydrolysate component	0.82	1.76

[a]Obtained at 160° using the conditions described in Table II (nitrogen flow-rate for SE-30, 30 ml/min): trimethylsilylated mannitol, *T* 17.98 (SE-30) and 5.29 min (OV-17).

The carbazole-to-orcinol ratio of the iduronic acid, isolated from the hydrolysate of PA, was 0.32 and the $[\alpha]_D$ value was $+32°$ (c 0.3, water; after 6 h). These data agree with those of L-iduronic acid[2,3,5].

Thus, the components of PA are D-glucuronic acid and L-iduronic acid, and this is the first report of the latter acid in the fungal kingdom. The biosynthesis of PA may resemble that of alginic acid[15,16], which contains guluronic acid and its 5-epimer, mannuronic acid. The former component is formed by the action of GDP-mannuronic acid-5-epimerase[17,18] and polymannuronic acid-5-epimerase[19,20]. Iduronic acid in PA may be synthesised by UDP-glucuronic acid-5-epimerase as for heparin[21,22].

EXPERIMENTAL

PA was isolated from the fungus *Kobayasia nipponica*, as previously described[1]. T.l.c. and p.c. of uronic acids were performed by the ascending method on cellulose-coated plastic sheets (Merck) and Toyo Roshi No. 50 paper, respectively, using ethyl acetate–acetic acid–water (3:1:1) and detection by alkaline silver nitrate[23] and *p*-anisidine hydrochloride[24]. Uronic acid was estimated by the carbazole[25], orcinol[26], and Bitter–Muir[27] methods with D-glucuronic acid as the standard. Nitrogen, phosphorus, and sulphate were determined by elemental analysis and the methods of Fiske and SubbaRow[28], and Dodgson and Price[29]. PA was decationised by using a Dowex-50(H^+) resin. Aldonic acids were prepared by the method of Perry *et al.*[30].

Iduronic acid standard was obtained from a hydrolysate of dermatan sulphate. Dermatan sulphate (Seikagaku Kogyo Co. Ltd.) was hydrolysed with 0.5M H_2SO_4 in a sealed tube at 100° for 3 h. The hydrolysate was neutralised, passed through a column of Dowex-50(H^+) resin, and subjected to p.p.c. Similarly, fractions corresponding to glucuronic acid, glucuronolactone, iduronic acid, and iduronolactone were isolated from a hydrolysate of PA.

G.l.c. of trimethylsilyl derivatives (internal mannitol standard) was performed on a Shimadzu GC-6A instrument with flame-ionization detectors. Uronic and aldonic acids were trimethylsilylated by using acetonitrile (100 μl), chlorotrimethylsilane (50 μl), and trifluorobis(trimethylsilyl)acetamide (80 μl) at 60° for 10 min.

G.l.c.–m.s. was performed on a Hitachi K-53 gas chromatograph coupled to a Hitachi RMU-7L double-focussing mass spectrometer. A glass column packed with 1.5% of SE-30 on Chromosorb W was used at 163° (injector 180°) with helium as the carrier gas at 2 kg/cm^2. The mass range, chamber voltage, and chamber temperature were 750, 70 eV, and 200°, respectively.

ACKNOWLEDGMENTS

The authors thank Mr. Y. Shida for the mass-spectrometry measurements, Miss F. Chokki for technical assistance, and Professor B. Lindberg (Stockholm University) for advice.

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