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IDENTIFICATION AND QUANTITATIVE DETERMINATION OF D-*ARABINO*-HEXULOSONIC ACID IN *CYTTARIA* SPECIES

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Key Word Index—*Cyttaria harioti*; *C. johowii*; Compositae; D-*arabino*-hexulosonic acid identification.

Abstract—D-*arabino*-hexulosonic acid has been identified by GLC in an aqueous extract from *Cyttaria harioti* and *Cyttaria johowii* and the amount present (1 and 0·05% respectively) determined.

INTRODUCTION

In previous papers [1, 2] we have reported the occurrence of D-*arabino*-hexulosonic acid as constituent of a polysaccharide from *Cyttaria harioti* Fischer. We now describe the identification of the keto acid in aq. extracts of *Cyttaria harioti* Fischer and *C. johowii* Espinosa by GLC and its determination by quantitative PC. This is the first report of the presence of a free hexulosonic acid in fungi.

RESULTS

After precipitation of the polysaccharides from an aqueous extract of the stroma by addition of ethanol, the filtrate was evaporated *in vacuo* and MeOH was added to the residue to give a soluble and an insoluble fraction. PC of the insoluble fraction showed the presence of a product with the same chromatographic properties as D-*arabino*-hexulosonic acid [1, 2], another compound which is under investigation and a low proportion of oligosaccharides and glucose. The acid is easily recognized by the pink colour it gives with *p*-anisidine hydrochloride [3]. Pentoses give the same colour but they have much higher R_f values. Identification of the acid was effected by GLC of the TMS derivative (Table 1). The keto acid was also analyzed by GLC of the TMS derivatives of the γ-glucono and γ-mannono lactones obtained by reduction of the hemicalcium salt, followed by decationization and lactonization. GLC was not used for the quantitative determination of D-*arabino*-hexulosonic acid because a poor correlation between concentration and response was reported for another hexulosonic acid (L-*xylo*-hexulosonic) [4].

Table 1. Relative retention times for TMS derivatives of D-*arabino*-hexulosonic acid and related compounds

	3% OV-17* (183 × 0·3 cm)	10% NPGS† (183 × 0·3 cm)
D-*arabino*-hexulosonic acid	1·58 and 1·68	2·20
arabitol	1·00 (7 min)	1·00 (3 min)
D-glucono-1,4-lactone	2·02	3·60
D-mannono-1,4-lactone	2·34	5·46

* T_{inj} 290°, T_d 320°, T_c programmed from 120 to 200° (4°/min), N_2, 30 ml/min.

† T_{inj} 250°, T_d 300°, T_c 3 min at 150° and then programmed from 150–185° (10°/min), N_2, 30 ml/min.

We adapted the PC method used for the determination of reducing sugars with *p*-anisidine hydrochloride; the spectrum of the stain produced showed a max at 510 nm in accordance with the value for aldopentoses and hexuronic acids [3]. To avoid decomposition of the acid, the H_2O extract

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from the stroma was concentrated by freeze-drying. A 1% concentration was found in *Cyttaria harioti* and 0·05% in *C. johowii*.

EXPERIMENTAL

General. PC was performed on Whatman No. 1 paper, using (A) *n*-BuOH–C_5H_5N–H_2O (6:4:3), (B) C_5H_5N–EtOAc–HOAc–H_2O (5:5:1:3), (C) *n*-BuOH–EtOH–H_2O (5:2:2). Detection was effected with: (a) $AgNO_3$–NaOH [5], (b) *p*-anisidine hydrochloride [3], (c) hydroxylamine–$FeCl_3$ [6]. GLC was carried out on stainless steel columns (183 × 0·3 cm) packed with : 1) 3% OV-17 on Chromosorb W (HP), 80–100 mesh or 2) 10% NPGS on Chromosorb G, with N_2 at a flow rate of 30 ml/min. The samples were trimethyl-silylated before GLC. D-*arabino*-hexulosonic acid hemi-calcium salt was used as standard for the quantitative determination and its purity determined as 98% by decarboxylation according to the method described by Barker *et al.* [7] for uronic acids.

Extraction of D-*arabino-hexulosonic acid.* Powdered dried fruit bodies (10 g) collected in Villa Angostura (Neuquen, Argentina) were macerated in 400 ml H_2O at room temp. The insoluble residue was removed by centrifugation, the polysaccharides precipitated by adding EtOH and separated by centrifugation. The supernatant was evaporated under reduced pressure and the residue taken into MeOH. PC of the insoluble fraction showed the presence of a compound with the same mobilities as an authentic sample of D-*arabino*-hexulosonic acid, R_g 0·23 (solvent A), 0·60 (solvent B) and 0·41 (solvent C); another non-reducing acid component with similar mobilities was the main component and is under investigation. The mixture was separated by preparative PC to afford a fraction with a higher concn of the keto acid.

GLC. Samples were dissolved in H_2O and decationized by stirring with Dowex 50 (H^+). After filtration, the soln was freeze-dried, silylated and subjected to GLC (for details, see Table 1).

Identification of D-*arabino-hexulosonic acid by borohydride reduction.* D-*arabino*-hexulosonate (20 mg) in H_2O (2 ml) was reduced with $NaBH_4$ (150 mg). After 1 hr the excess $NaBH_4$ was destroyed, the soln was evaporated to dryness and the boric acid was removed by several distillations with MeOH. The syrup was treated with 0·5 ml conc HCl and taken to dryness, heating at 100° *in vacuo* (oil pump) to aid lactonization. PC (solvents A and B, reagent c) showed 2 spots corresponding to D-glucono-1,4-lactone and D-mannono-1,4-lactone. The fractions from *Cyttaria* separated by PC were reduced and lactonized by the same treatment. Both samples were trimethylsilylated and analyzed by GLC (see Table 1).

Quantitative determination of D-*arabino-hexulosonic acid.* The calibration curve was prepared from D-*arabino*-hexulosonic acid hemicalcium salt (0·2095 g) dissolved in H_2O (10 ml). From 2 to 10 λ of the solution was spotted on 2 papers and developed with solvent B during 18 hr. The papers were air dried and immersed into *p*-anisidine hydrochloride reagent solution. After air-drying the papers were heated at 130° for 10 min. The individual pink spots were cut out in rectangular areas and each of them eluted for 10 min with 4 ml $SnCl_2$ [3]. The absorbances were determined at 510 nm. D-*Arabino*-hexulosonic acid concn in *Cyttaria harioti* Fischer was determined in a H_2O extract (10 g extracted with 500 ml H_2O) concentrated by freeze-drying. The aq. extract from *Cyttaria johowii* was previously treated with EtOH to precipitate polysaccharides, centrifugated, the EtOH evaporated *in vacuo* at 30° and the aq. soln freeze-dried.

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