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STRUCTURAL FEATURES OF AN ARABINOGALACTAN FROM THE SEEDS OF *BECIUM FILAMENTOSUM*

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Key Word Index—*Becium filamentosum*; *Ocimum*; Labiatae; mucilage; polysaccharide; L-arabino-D-galactan; chemotaxonomy.

Abstract—The Cetavlon non-precipitable fraction of *Becium filamentosum* seed mucilage on DEAE-cellulose column chromatography yielded three fractions. The major polysaccharide was composed of L-rhamnose, D-galactose and L-arabinose (1:2:2). Structural analysis revealed a (1 → 4)-linked D-galactopyranose backbone with occasional side chains at O-6 of (1 → 5)-linked L-arabinofuranose terminating in rhamnopyranosyl residues.

INTRODUCTION

The structural features of the major acidic polysaccharides from three *Ocimum* species, viz. *O. canum* Sims, [1], *O. basilicum* L. [2], *O. gratissimum* L. [3], and *Becium filamentosum* (Forsskal) Chiov. [4, 5] have been investigated. The first three are composed of a (1 → 4)-linked D-xylan backbone, with slight differences in the nature of the side chains, consisting of L-Ara*f*, D-Gal*p*, L-Rha*p* and D-Gal*p*A. That of *B. filamentosum* has a backbone of → 4)-D-Gal*p*A-(1 → 2)-L-Rha*p*-(1 →, two-thirds of the rhamnose being further substituted at O-4 by D-Gal*p* residues. Interestingly, the latter mucilage did not contain any xylose. The seeds of the first three species are black and oval in shape while those of *B. filamentosum* are brown and spherical. This paper describes the structural features of a neutral polysaccharide obtained from *B. filamentosum*.

RESULTS AND DISCUSSION

From the EDTA-soluble fraction of the polysaccharide complex of *B. filamentosum* seeds, a Cetavlon non-precipitable fraction was obtained in ~ 20% yield [4]. Acid hydrolysis of the fraction revealed L-rhamnose, L-arabinose and D-galactose in the ratio 1:3:2. This fraction on a DEAE-cellulose (CO_3^{2-} form) column gave three fractions when eluted with water (NP_1), 0.25 M sodium hydroxide (NP_2) and 0.5 M sodium hydroxide (NP_3). They are composed of L-arabinose and D-galactose (1:0:5); L-rhamnose, L-arabinose and D-galactose (1:2:2); and L-rhamnose, L-arabinose and D-galactose (0.5:3:1), respectively. Of these, NP_2 was homogeneous on microzone electrophoresis [6]. Hakomori methylation [7] of NP_2, followed by complete hydrolysis, derivatization into alditol (1-$^2H_1$) acetates and GC/MS analysis [8] revealed 2,3,4-tri-*O*-methylrhamnose (2.5 mol), 2,3-di-*O*-methylarabinose (6 mol), 2,3,6-tri-*O*-methylgalactose (4 mol) and 2,3-di-*O*-methylgalactose (2 mol). The formation of 2,3,6-tri-*O*-methyl- and 2,3-di-*O*-methylgalactose indicates that the polysaccharide contains a backbone of (1 → 4)-linked D-galactopyranosyl residues having branches through O-6. The presence of 2,3-di-*O*-methylarabinose indicates that the side chain residues are (1 → 5)-linked and are terminated by L-rhamnopyranosyl units.

The polysaccharide consumed 1.1 mol of periodate [4] and released 0.15 mol of formic acid per anhydro sugar residue. Smith degradation [4] products were glycerol and erythritol. These results are consistent with the methylation data.

Arabinogalactans, either in native form or associated with proteins (as proteoglycans), are widely distributed in plant tissues and are major components of gum exudates [9]. They have also been identified in pectic complexes of seeds, leaves, etc. and as a general hemicellulosic constituent of coniferous woods [9]. Three structural types, viz. the arabino-4-galactans, the arabino-3-6-galactans and polysaccharides having arabinose and galactose side chains, have so far been identified. Basically, the one from *B. filamentosum* mucilage belongs to type I. L-Rhamnopyranose, as a terminal residue, is not frequently reported as a constituent of arabino-4-galactans [9].

With regard to the seed characteristics, mucilage composition [3, 10, 11] and main structural features of the major acidic and neutral polysaccharides, the recent taxonomic reclassification of this plant, which was originally placed in the genus *Ocimum*, as *O. adscendens*, is in agreement.

EXPERIMENTAL

General methods. Most of the experimental methods have already been reported [4, 12]. Polysaccharides were hydrolysed with 0.5–1.0 M TFA at 100° for 4–6 hr.

Isolation and purification of the polysaccharide. From locally available *B. filamentosum* plants at fruiting stage, seeds were collected during December–January. Up to 1983 the plant was

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known as *O. adscendens* Willd., but it was later reclassified as *Becium filamentosum* (Forsskal) Chiov [5]. The crude polysaccharide complex (10 g), isolated from *B. filamentosum* seeds [4], was extracted with aq. EDTA (2 %, 1 l.) to give the EDTA-soluble fraction (1 g); an aq. soln (1 %) was treated with aq. Cetavlon (6.8 %) to obtain a precipitable (660 mg) and a non-precipitable (200 mg) fraction. The latter was dissolved in a minimum vol. of H_2O and applied to a DEAE-cellulose column (CO_3^{2-} form, 32.5 × 2.5 cm), which was eluted sequentially with H_2O, 0.25 M NaOH and 0.5 M NaOH, to obtain NP_1 (40 mg), NP_2 (80 mg) and NP_3 (35 mg), respectively. Microzone cell electrophoresis [6] indicated NP_2 to be homogeneous.

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TWO ACETYLENIC COMPOUNDS FROM *ECHINACEA PALLIDA* ROOTS

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Key Word Index—*Echinacea pallida*; *Echinacea angustifolia*; Asteraceae; 8-hydroxypentadecenediynone; 8-hydroxypentadecadieneynone; NMR.

Abstract—Two acetylenic compounds were identified as artifacts in stored roots of *Echinacea pallida*. Their structures were determined by ^{13}C and ^1H NMR spectra as 8-hydroxypentadeca-9*E*-ene-11,13-diyn-2-one and 8-hydroxypentadeca-9*E*,13*Z*-diene-11-yn-2-one.

INTRODUCTION

In our search for immunologically active compounds from *Echinacea* species we recently isolated two polysaccharides which have immunostimulating activity [1]. Four new sesquiterpene esters, which we have found in commercial *E. purpurea* roots [2–4], turned out to be constituents of *Parthenium integrifolium* L. which is frequently used as adulterant [5, 6]. Here we report the isolation and structural elucidation of two new acetylenic compounds **1** and **2** from the stored root powder of *E. pallida* Nutt. as well as from commercial *E. angustifolia* roots.

RESULTS AND DISCUSSION

In the HPL chromatogram of the chloroform extract of *Echinacea pallida* roots, which had been ground several days before extraction, two major peaks at retention time 5.8 min and 7.2 min were found, which could not be detected in fresh roots. On the other hand these constituents could also be found in the chloroform extract of commercial samples of *Echinacea angustifolia* roots [3].

The second peak, representing compound **1**, was isolated together with **2** (R_t 7.7 min) by preparative HPLC as a pale yellow oil. Compound **1** was separated from **2** by fractional crystallization in $CHCl_3$ under N_2. The IR