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AN ACIDIC XYLAN FROM EXTRACELLULAR POLYSACCHARIDES OF SUSPENSION-CULTURED CELLS OF *NICOTIANA TABACUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae, tobacco; cell culture, extracellular polysaccharide; acidic xylan.

Abstract—An acidic xylan was isolated from the extracellular polysaccharides of suspension-cultured tobacco cells. Its structure was investigated by methylation analysis and ^{13}C NMR spectroscopy and was shown to consist of a main chain of β-(1 → 4)-linked D-xylopyranosyl residues to which were attached as side-chains, α-D-glucuronic acid residues at O-2.

INTRODUCTION

Xylan, consisting of β-(1 → 4)-linked xylopyranosyl residues, has been recognized as one of the components of plant cell walls [1]. In tobacco, xylan and 4-*O*-methylglucuronoxylan have been isolated from stalk [2] and from midrib cell wall [3], respectively. As previously reported [4], it has been suggested that xylan-type polysaccharide is present in the extracellular polysaccharide (ECP) of suspension-cultured tobacco cells on the basis of methylation analyses of ECP and its subfractions. Here we report the isolation and structural investigation of the xylan.

RESULTS AND DISCUSSION

The ECP solution was fractionated using DEAE-Sephadex A-25 from which a xylan-containing fraction was eluted with the buffer containing 0.2 M sodium chloride. This fraction was considered to be a mixture of xylan and arabinogalactan-protein (AGP) [5], because sugar analysis indicated considerable amounts of arabinose and galactose residues and because this fraction reacted with β-galactosyl Yariv antigen [6], this reaction being one of the properties of AGP. Xylan was separated from AGP using cellulose column chromatography. The fraction eluted from the cellulose column with sodium hydroxide contained mainly xylose as the neutral sugar.

The material thus obtained seemed to be a homogeneous preparation from the results of zone electrophoresis and gel-filtration. Its MW was estimated to be *ca* 10 000. It had $[\alpha]_D$ −42° (*c* 0.1; water) and consisted of neutral sugar and uronic acid in a molar ratio of *ca* 5:1. The uronic acid was identified as glucuronic acid (not 4-*O*-methylglucuronic acid) by GLC [5]. The protein content was negligible.

The ^{13}C NMR spectrum of the acidic xylan was recorded to establish the anomeric configuration of the component sugars. The spectrum showed in the anomeric carbon region three signals at δ 103.80, 103.04 and 100.35. The first two signals were assigned to the β-D-xylopyranose residue and the third one to α-D-glucuronic acid residues, respectively, from the literature data [7, 8]. Thus the anomeric configurations of the D-xylopyranose and D-glucuronic acid residues in the acidic xylan were determined as β and α, respectively.

Methylation analysis of the acidic xylan revealed 2,3,4-tri-*O*-methyl-D-xylose (terminal), 2,3-di-*O*-methyl-D-xylose (4-linked) and 3-*O*-methyl-D-xylose (2,4-linked) in the molar ratio 4.7:81.3:14.0. The result of the methylation analysis indicated that the acidic xylan had a β-(1 → 4)-linked xylan backbone to which α-D-glucuronic acid residues were attached as side-chains on the O-2 of the D-xylopyranosyl residues.

Although Aspinall *et al.* [9] suggested the presence of a xylan-type chain in the ECP of sycamore, it was considered to be part of the xyloglucan and the xylan was not purified from the ECP. Thus, as far as we know, this is the first example of the xylan obtained from plant ECP.

EXPERIMENTAL

General. Concn was performed under red. pres. at 45° or less. Optical rotation was measured with a JASCO model DIP-181 polarimeter. Zone electrophoresis was performed on a Whatman GF/A glass microfibre paper at 1500 V for 30 min with 0.1 M sodium tetraborate (pH 9.2). After electrophoresis, the paper was cut into 1 cm strips, and the sugar on each strip was eluted with H_2O. The content of total sugar [10] and uronic acid [11] was measured colorimetrically. HPLC was conducted with a Waters Solvent Delivery System 6000 constant-flow pump and a Waters R-401 differential refractometer for monitoring the column effluent Separations were performed on a column of TOYO SODA TSK-Gel G3000SW, which was calibrated with Dextran T-series (Pharmacia) using 0.9% NaCl as the eluant. GLC was performed with FID and a column (glass capillary, 0.28 mm × 50 m) coated with OV-101 using N_2 as carrier gas at 1 ml/min. Neutral sugar analysis was performed by GLC of the alditol acetate derivatives [12] after hydrolysis in 2 M TFA at 120° for 1 hr. Methylation analysis was performed by the Hakomori method [13] as described in ref. [4]. Reduction was carried out using $NaBD_4$ instead of $NaBH_4$. GLC and GC/MS of the partially methylated alditol acetates were essentially as described in ref. [4]. The ^{13}C NMR spectrum (25.1 MHz) [14] was obtained using a 5 mm tube in D_2O at 70° with MeOH (49.8 ppm from TMS) as the internal standard. Protein was determined by the method of ref. [15].

Preparation of acidic xylan. ECP solution (1 l.) prepared as described in ref. [16] was applied to a column (2.5 × 15 cm) of DEAE-Sephadex A-25 equilibrated in 10 mM Tris–HCl buffer (pH 8). After washing with the buffer, the column was eluted with

the buffer containing 0.2 M NaCl to give the xylan-containing fraction Since the preliminary experiment revealed that the xylan became partly water-insoluble after freeze-drying, the isolation procedures were performed without lyophilization. The 0.2 M NaCl fraction gave, on complete acid hydrolysis, L-arabinose, D-xylose and D-galactose in the molar ratio 38 3 . 33.4 . 28.3 with trace amounts of D-mannose and D-glucose as neutral sugars. This fraction, after dialysis against H_2O, was applied to a column (2 3 × 12 cm) of cellulose powder (Whatman, CF 11), which was first washed with water to give crude AGP and then eluted with 1 M NaOH to give acidic xylan. The alkaline fraction was dialysed against H_2O and then centrifuged. The supernatant gave L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose in the molar ratio 4.0 : 89.5 : 1.3 : 3.1 : 2.1. The yield of the acidic xylan was *ca* 50 mg from 1 l of ECP soln.

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VOLATILE COMPONENTS IN CELL SUSPENSION CULTURES OF *CRYPTOMERIA JAPONICA*

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Key Word Index—*Cryptomeria japonica*; Taxodiaceae; cell suspension culture; volatiles; diterpenes; aldehydes; fatty acids and their esters.

Abstract—The cell suspension culture of *Cryptomeria japonica* contains volatile oils, the yield of which was 0.005–0.01 % of the fresh cells. In the volatiles, five aldehydes, ten fatty acids and their esters, and two diterpenes of abietatriene and ferruginol have been found. Of these, palmitic acid is present as the most predominant component, amounting to *ca* 40 % of the volatiles.

INTRODUCTION

Cryptomeria is a rich source of volatile oils. *C. japonica* (Japanese cedar) is known to yield *ca* 0.7 % of volatiles from the fresh leaves, in which diterpenes of kaurene, isokaurene, 9-ketoferruginol (sugiol) and the others have been found [1]. Recently, many callus tissues and suspension cells have also been found to produce mono- and sesquiterpenes [2, 3] As one of us recently succeeded in establishing the cell culture of Japanese cedar [4], we thus examined the volatile oil in the suspension cultured cells.

RESULTS AND DISCUSSION

The cell suspension culture of Japanese cedar was maintained in a modified liquid medium based on that of Schenk and Hildebrandt [6] From this, the 14th generation of suspension cells was harvested at a 14-day culture (the stationary growth phase) and subjected to extraction of volatiles. The yield of volatile oils obtained by a simultaneous distillation extraction [7] was 0.005–0.01 % of the fr. wt. The cells at this growth stage contained a

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