organisms is correct. It should be noted that in an analogous study, Nartey[12] demonstrated that the cyanide produced by fermenting cassava products was due, in part, to contamination by cyanogenic bacteria (and/or the catabolism of endogenous cyanogenic glycosides by extracellular enzymes excreted by non-cyanogenic fungi and bacteria).

A survey of the literature of fungal cyanogenesis suggested that the cyanide reported for several fungi may be bacterial in origin. Fungi that were possibly contaminated with cyanogenic bacteria include *Grifola frondosa* (Dickson ex Fr.) S. F. Gray (=*Polyporus*; Polyporaceae)[13], *Grifola umbellata* (Pers. ex Fr.) Pilát (=*Polypilus*; Polyporaceae)[13], *Melanoleuca cognata* (Fr.) Konr. and *Omphalina griseopallida* (Desm.) Quel (Tricholomataceae)[14]. According to the original reports, these species failed to liberate detectable quantities of cyanide for at least 24 hr and most generally required longer incubation periods. When positive, these species gave a weak cyanogenic response. We were unable to detect cyanide in fresh basidiocarps of *G. frondosa* with Feigl–Anger or picrate reagent. This observation confirmed Bach's[15] earlier work with the same fungus. Pending further investigation, the cyanogenic ability of the fungi listed above is questionable. It is likely that cyanogenic bacteria are responsible for other reports of cyanogenesis in fungi and/or plants. Unfortunately, few investigators cite the period of incubation required for a positive cyanide test, information that is critical for determining the potential for bacterial contamination.

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AN ARABINOXYLOGLUCAN FROM EXTRACELLULAR POLYSACCHARIDES OF SUSPENSION-CULTURED TOBACCO CELLS

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; cell culture; extracellular polysaccharide; arabinoxyloglucan.

Abstract—An arabinoxyloglucan (AXG) isolated from extracellular polysaccharide of suspension-cultured tobacco cells was investigated by methylation analysis, partial acid hydrolysis and ^{13}C NMR spectroscopy. It was found that the AXG is structurally similar to that isolated from the midrib of tobacco leaves.

INTRODUCTION

Xyloglucans have been known as one of the major polysaccharides of the cell walls of dicotyledonous plants [1]. Tobacco cell walls, however, contain arabinoxyloglucan (AXG), not xyloglucan, as one component of the hemicellulosic polysaccharides. Tobacco AXG has been isolated from the midrib of the leaves and structurally characterized [2–4].

Suspension-cultured cells of tobacco secrete into their growth medium extracellular polysaccharides (ECP). Methylation studies of the ECP [5] have suggested that AXG may also be present in ECP. This

Table 1. Sugar and methylation analyses of AXG and XG

	Glycosyl composition (mol %)		Glycosyl-linkage composition (mol %)				
	AXG	XG	RRt*	O–Me	Linkage	AXG	XG
Arabinosyl	13	2	0.77	2, 3, 5	Terminal	8	+
Xylosyl	27	27	0.82	2, 3, 4	Terminal	17	26
			0.95	3, 4,	2-	17	4
Glucosyl	60	71	1.17	2, 3, 6	4-	32	43
			1.40	2, 3	4, 6-	26	26

*Relative to that of 2, 3, 4, 6-tetra-*O*-methyl-D-glucitol diacetate.

paper describes the isolation and structural investigation of this AXG.

RESULTS AND DISCUSSION

When ECP solution was fractionated on a DEAE-Sephadex column, *ca* 50% of the sugars were recovered in buffer washing fraction (F-1). Paper electrophoresis of F-1 gave two spots, one of which was thought to be AXG, on the basis of sugar and methylation analysis [5]. Although it was not possible to separate these two polysaccharides in F-1 by chromatography on cellulose powder or on a Con A-Sepharose column, ammonium sulfate precipitation, rarely used for polysaccharide purification [6], enabled the separation of these polysaccharides. Material thus obtained which was insoluble in saturated ammonium sulfate was electrophoretically homogeneous and had $[\alpha]_D = +44°$ ($c = 1.0$, water). It turned dark brown to reddish brown, when mixed with iodine/potassium iodide solution. The polysaccharide contained 99% carbohydrate and gave, on total acid hydrolysis, L-arabinose, D-xylose and D-glucose in molar ratios of 13:27:60. Protein [7] was absent. Its MW was estimated to be 53000 by high-performance, gel-permeation chromatography.

The ^{13}C NMR spectrum of the polysaccharide showed, in anomeric carbon regions, three signals at δ 109.8, 103.2 and 99.6, which were assigned to α-L-arabinofuranosyl, β-D-glucopyranosyl and α-D-xylopyranosyl residues, respectively, from the literature data [4]. The signals of other carbon atoms appeared between δ 85 and 61. The spectrum was almost identical with that of the AXG isolated from the midrib of tobacco leaves [4], suggesting that the polysaccharide is structurally similar to tobacco midrib AXG.

The AXG was methylated, acid-hydrolysed, reduced with sodium borodeuteride and acetylated. GLC and GC/MS analyses of the products as the partially methylated alditol acetates gave terminal arabinofuranosyl, terminal and 2-linked xylopyranosyl and 4-linked and 4, 6-linked glucopyranosyl residues as shown in Table 1. The proportion of individual sugars obtained by methylation analysis confirmed the results obtained by acid hydrolysis with the exception of arabinose, whose proportion is lower. This was probably attributed to a loss of terminal arabinofuranose units during the methylation procedure.

Hydrolysis of the AXG under mild acid conditions removed almost all of the arabinosyl and a small proportion of the xylosyl residues to yield the degraded polysaccharide, xyloglucan (XG). This was insoluble in water, but soluble in sodium hydroxide. It had $[\alpha]_D = +51°$ ($c = 0.5$, 1 N NaOH) and gave, on total acid hydrolysis, L-arabinose, D-xylose and D-glucose in molar ratios of 2:27:71. Methylation analysis of the XG (Table 1) showed that, compared to AXG, the removal of the arabinofuranosyl residue resulted in an increase of terminal xylopyranosyl residues and a decrease of 2-linked xylopyranosyl residues, which still remained in small amounts in XG.

All the data above indicated that the AXG obtained from tobacco ECP could consist of a backbone of β-(1→4)-linked D-glucopyranosyl residues, some of which are attached at C-6 by α-D-xylopyranosyl, α-L-arabinofuranosyl-(1→2)-α-D-xylopyranosyl and α-D-xylopyranosyl-(1→2)-α-D-xylopyranosyl side-chains. The structural feature described above is very similar to that of the AXG of tobacco midrib [4].

EXPERIMENTAL

General. Concn was performed under red. pres. at 45° or less. Optical rotation was measured with a JASCO model DIP-181 polarimeter. Paper electrophoresis was performed on a Whatman GF/A glass microfibre paper at 1500 V for 30 min with 0.1 M Na tetraborate (pH 9.2). Carbohydrates were detected by heating with H_2SO_4. 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, 0.27 mm × 50 m) coated with 3% OV-101 using He as the carrier gas at 1 ml/min. Total carbohydrate was determined by the PhOH–H_2SO_4 method [8] using glucose as the standard. Neutral sugar analysis was performed by GLC of the diethyl dithioacetal TMSi derivatives [9, 10] after hydrolysis in 2 M TFA at 120° for 1 hr. Methylation analysis was performed by the method of ref. [11] as described in ref. [5]. Reduction was done using $NaBD_4$ instead of $NaBH_4$. GLC and GC/MS of the partially methylated alditol acetates were essentially as described [5]. ^{13}C NMR spectrum (25.1 MHz) [12] was obtained using a 5-mm tube in D_2O–0.1 M NaOD (50 mg/ml) at 70° with MeOH (δ 49.8 from TMS) as the int. standard.

Preparation of AXG and XG. Suspension culture of tobacco cells was carried out as described [10]. After filtration and centrifugation to remove cells, the ECP soln was

dialysed against 10 mM NaPi buffer (pH 6) for 24 hr. The clear soln was then applied to a column (15 × 2.5 cm) of DEAE-Sephadex A 25 equilibrated in the same buffer. The buffer washing fraction was collected, dialysed against H_2O and freeze-dried (F-1). F-1 (1 g) was dissolved in H_2O (200 ml) and $(NH_4)_2SO_4$ added to satn. The ppt was obtained by centrifugation and dissolved in H_2O. The above procedure was repeated once and the soln dialysed and freeze-dried to give AXG (475 mg). The AXG (30 mg) was treated with 0.05 M TFA (5 ml) at 100° for 2 hr and the degraded polysaccharide (XG) was recovered by EtOH (4 vol.) precipitation (yield, 23 mg). EtOH-soluble fraction contained L-arabinose and D-xylose in a molar ratio of 82:18.

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CONSTITUENTS OF ESSENTIAL OIL *OF BOSWELLIA FREREANA*

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Key Word Index—*Boswellia frereana*; Burseraceae; terpenes; essential oil.

Abstract—The composition of the essential oil of *Boswellia frereana* was investigated by GC/MS. Ten terpenes were identified and *p*-cymene was by far the most abundant component of the oil.

INTRODUCTION

Recently we started a program to investigate the chemical constituents of frankincense produced by *Boswellia frereana* (Somaliland name Meydi). Two triterpenes, lupeol and epilupeol, were previously isolated and identified from the neutral extract of frankincense produced by the same plant [1]. Here we describe the identification of several compounds contained in the essential oil of this gum-resin [2].

RESULTS AND DISCUSSION

Two procedures were utilized to isolate the essential oil. Procedure (a) made use of steam distillation on the residue obtained by solvent extraction of the gum-resin, and procedure (b) made use of steam distillation directly on the gum-resin. For several reasons, attributable to decomposition or alteration of the terpenes, we used procedure (b) for the identification of terpene constituents of the essential oil.

The terpenes were identified by their mass spectra obtained in the GC/MS analysis and, when possible, by comparison with authentic samples. The mixture obtained from steam distillation was extracted with *n*-hexane and the solvent removed by distillation at low temperature. The final oil was analysed by GC in the following monoterpene and diterpene hydrocarbons: α-pinene, sabinene, myrcene, α-terpinene, limonene, *p*-cymene, α-cubebene, terpinen-4-ol, cembrene, isocembrene [3] and two unknown monoterpenes $C_{10}H_{16}$ and $C_{10}H_{18}O$. *p*-Cymene was the most abundant component of the mixture.