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ACIDIC XYLAN FROM OLIVE PULP

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Key Word Index—*Olea europaea* var. *arolensis*; fruit pulp; polysaccharide; xylan.

Abstract—A purified hemicellulose A, isolated from olive pulp ('hojiblanca' variety) contained xylose and 4-*O*-methylglucuronic acid in the molar ratio 24:1. The molecular weight was estimated to be 9500 by GPC. From the results of partial hydrolysis, enzymatic hydrolysis, methylation analysis and identification of an aldobiuronic acid, it was concluded that hemicellulose A is a polymer of $\beta(1 \rightarrow 4)$-linked xylopyranosyl residues, having branches of 4-*O*-methylglucuronic acid groups on the O-2 atoms of the main chain.

INTRODUCTION

The hemicellulosic material from olive pulp has been studied in relation to its dietary fibre composition and fruit softening [1–6]. In previous papers, the isolation, purification and structures of various hemicelluloses from olive pulp of the 'gordal' variety were reported [7–10]. We now report the isolation, purification and structure of a xylan from pulp of the 'hojiblanca' olive variety.

RESULTS AND DISCUSSION

The hemicellulose A was isolated and purified by complexing with Fehling's solution. The purified polysaccharide had $[\alpha]_D^{21} - 51°$ (10% NaOH; *c* 0.5) and contained xylose (62.9%), 4-*O*-methylglucuronic acid (3.7%), glucose (1.1%), galactose (0.8%), mannose (0.7%) and arabinose (0.3%). The M_r of this polysaccharide, determined by GPC was 9500.

The hemicellulose A was incubated with β-xylosidase and the hydrolysate examinated by PC. Only xylose could be detected. Partial hydrolysis with acid gave 4-*O*-methylglucuronic acid (R_{xyl} 1.32), xylose and four oligosaccharides by PC. A linear relationship existed between the degree of polymerization (DP) of the oligosaccharides and their log α' ($\alpha' = R_f/1 - R_f$), $\log \alpha' = 0.076 - 0.444$ DP; $r = 0.999$. Similar results were obtained from the partial hydrolysis of a $\beta(1 \rightarrow 4)$ xylan used as a reference. These results indicated that partial hydrolysis of olive hemicellulose A yielded a homologous series of $\beta(1 \rightarrow 4)$-linked xylo-oligosaccharides [11].

Permethylation analysis of the hemicellulose A followed by GC and GC/MS gave 2,3,4-tri-, 2,3-di- and 3-mono-*O*-methylxylose, the molar ratio of the last two being 23.1:1.0. These results clearly show that this xylan contains chains of $(1 \rightarrow 4)$-linked xylopyranosyl residues having branches at O-2. Moreover, the values obtained for the molar ratios xylose/4-*O*-methylglucuronic acid (24.0) and 2,3-di-*O*-methylxylose + 3-*O*-methylxylose/3-*O*-methylxylose (24.1) suggest that the non-reducing end units are 4-*O*-methylglucuronic acid residues.

An aldobiuronic acid was isolated by preparative PC from the hydrolysate of the xylan, converted into the acetate of the methyl ester of its methyl glycoside and examined by GC/MS. The aldobiuronic acid derivative was identified as methyl 3,4-di-*O*-acetyl-2-*O*-(methyl-2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α-D-xylopyranoside [12].

From the results of these experiments it is clear that olive hemicellulose A is essentially a $(1 \rightarrow 4)$-linked β-D-xylan having a 4-*O*-methylglucopyranosyluronic acid group attached to O-2 xylose residues.

EXPERIMENTAL

General. All general methods were conducted as described earlier [7, 8]. The M_r was determined by GPC on Sephacryl S-300. Neutral sugars analysis was performed by GC of the alditol acetates after hydrolysis with 72% v/v H_2SO_4. The xylan was partially hydrolysed with 0.25 N H_2SO_4 for 90 min at 100°. Enzymatic hydrolysis was performed with β-xylosidase for 48 hr at 30°.

The polysaccharide was permethylated [13], hydrolysed, reduced and acetylated before identification by GC and GC/MS. The aldobiuronic acid was isolated by prep. PC from the hydrolysate of the xylan with 72% H_2SO_4. The aldobiuronic acid (1 mg) in dry MeOH (2 ml) was boiled under reflux for 24 hr in the presence of Dowex 50W X-8 resin. The Me ester of the Me glycoside was acetylated and analysed by GC/MS.

Isolation and purification of hemicellulose. Olive pulp of the 'hojiblanca' variety (4.4 kg) was triturated in a mixer, stabilized with hot 96% EtOH (30 l.) for 11 hr and extracted with $CHCl_3$–MeOH (1:1) (16 l.). The residual material (100 g) was suspended in 0.25 M NaOMe (1 l.) for 27 hr at room temp. [14] with stirring. The alkaline suspension was filtered and the insoluble material extracted × 3 with H_2O (1 l.) at room temp. The remaining solid was delignified by treatment with aq. NaOCl in the presence of HOAc [15]. The resulting holocellulose was extracted with 10% NaOH (1 l.) for 24 hr at room temp. under N_2 with stirring [16]. The alkaline extract was made neutral with 50% HOAc and then dialysed against H_2O for 3 days. The soln was concentrated and acidified with HOAc to give crude hemicellulose A (1.5 g). This crude product (1 g) was purified through the formation of its Cu complex by treatment with Fehling's soln, dissociation of the complex with 5% HCl in EtOH, soln in H_2O and precipitation with EtOH (yield 440 mg).

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(*S*)-(−)-3,4-DIHYDROXYBUTANOIC ACID γ-LACTONE FROM PUERTO RICAN *LYNGBYA MAJUSCULA*

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Key Word Index—*Lyngbya majuscula*; Oscillatoriaceae; 4(*S*)-hydroxy-2(3*H*)-dihydrofuranone.

Abstract—(*S*)-(−)-3,4-Dihydroxybutanoic acid γ-lactone, which has the opposite configuration of the lactone generated from hydrolysis of oscillatoxin A, is a metabolite of Puerto Rican *Lyngbya majuscula*.

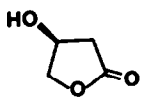
INTRODUCTION

Debromoaplysiatoxin is a potent tumour promoter which is found in certain varieties of the marine blue–green alga *Lyngbya majuscula* from the Hawaiian Islands [1], Enewetak Atoll [2], and Okinawa [3]. The 31-nor compound, oscillatoxin A, which is also a potent tumour promoter [4], is present along with debromoaplysiatoxin in a mixture of two blue–green algae found on the seaward side of Enewetak Island, viz. *Schizothrix calcicola* and *Oscillatoria nigroviridis* [5, 6]. Both debromoaplysiatoxin and oscillatoxin A have the same absolute stereochemistry [6]. The configuration at C-29 in both toxins is *R*. We report here the isolation and identification of (*S*)-(−)-3,4-dihydroxybutanoic acid γ-lactone (**1**) from a non-toxic variety of *L. majuscula* found abundantly at Mayaguez, Puerto Rico. This γ-lactone has the opposite absolute stereochemistry from the one obtained from chemical degradation of oscillatoxin A.

RESULTS AND DISCUSSION

Fractionation of the algal extract led to the isolation of compound **1**. ^{1}H NMR spectral analysis of the γ-lactone indicated that its gross structure was identical with that of the acid hydrolysis product from oscillatoxin A; its optical rotation, however, was opposite in sign with that of the degradation product [7], but matched that of the γ-lactone synthesized from (*S*)-(−)-malic acid [6, 7].

No debromoaplysiatoxin or oscillatoxin A-type compounds were found in the extract. The extract of this Puerto Rican *L. majuscula* showed no tumour promoting



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