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STRUCTURAL INVESTIGATIONS OF AN ARABINAN FROM GRAPE JUICE

J. C. VILLETTAZ†, R. AMADO‡ & H. NEUKOM

Swiss Federal Institute of Technology, Department of Food Science, 2 Universitätstrasse, CH-8092 Zürich, Switzerland

ABSTRACT

An arabinan has been isolated from grape juice and purified by chromatography on polyamide and repeated ethanol precipitations. The structural identity of the arabinan was established by enzymatic degradation of the polysaccharide with a purified α-L-arabinofuranosidase and methylation analysis. The results obtained suggest that the arabinan consists of an α(1 → 5)-linked backbone of L-arabinofuranosyl residues to which sidechains of L-arabinose are attached in the 3-position.

1. INTRODUCTION

Only few genuine arabinans from different plant sources have been isolated and their structures elucidated (Roudier & Eberhard, 1965; Rees & Richardson, 1966; Stevens & Selvendran, 1980). They all show a highly-branched structure and are mostly considered to be associated with the pectic substances in the plant cell walls (Kindl & Woeber, 1975).

During a study on the polysaccharides of grape juice and wine, an arabinan, soluble in 85% ethanol was obtained (Villettaz, 1979). This paper describes the isolation and structural investigation of the arabinan from grape juice.

2. EXPERIMENTAL

2.1. *General Methods*

Uronic acid content was determined by the modified carbazole method (Bitter & Muir, 1962). The protein content was calculated as the sum of the various amino

† Present address: Swiss Ferment Co. Ltd, Mülhauserstr. 70, CH-4056 Basel, Switzerland.
‡ To whom all correspondence should be addressed.

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acids after hydrolysis with 6 M hydrochloric acid for 16 h at 110°C, in sealed tubes under nitrogen. Neutral sugars were determined by glc; the samples were hydrolysed with 2 M trifluoroacetic acid for 1 h at 120°C in sealed tubes under nitrogen, and the liberated monosaccharides converted into the corresponding aldonitrile acetates by the method of Mergenthaler & Scherz (1976). Gas chromatographic separations were performed on a glass column (200 x 0·2 cm) packed with Chromosorb G-80 coated with 5% Carbowax 20 M; the oven temperature was programmed from 200 to 220°C at a rate of 2°C/min. Quantitative measurements were done with a calculating integrator system (SP 4000, Spectra-Physics, Darmstadt, GFR) using D-xylonitrile-tetra-acetate as internal standard.

Thin layer chromatography of neutral sugars was performed on cellulose F 254 plates (Merck AG, Darmstadt, GFR) using ethyl acetate : pyridine : glacial acetic acid : water (36 : 36 : 7 : 21 by vol) as the solvent system and anilinphthalate as the spraying agent as described by Stahl (1967).

2.2. *Isolation of the L-Arabinan*

The grape juice prepared from 'Pinot noir' grapes was obtained from the Swiss Federal Agricultural Research Station at Waedenswil, Switzerland. Grape juice (50 litres) was first concentrated under reduced pressure at 40°C in a rotatory evaporator, and dialysed against distilled water for 3 days at 2°C. The non-dialysable material was centrifuged at 18 000 x g for 20 min, and poured into five volumes of 96% ethanol. The precipitate was left overnight at 2°C and centrifuged at 18 000 x g for 20 min. The supernatant was adjusted to pH 7·0 with sodium hydroxide solution, concentrated under reduced pressure at 40°C in a rotary evaporator, dialysed against distilled water and freeze dried (yield: 3590 mg of crude arabinan).

2.3. *Purification of the Crude L-Arabinan*

A portion of the ethanol-soluble preparation was dissolved in water and decolorised on a polyamide column (Polyamid Woelm, Eschwege, GFR) using water as the eluant. Ethanol was added to the clear percolate to a final concentration of 85% (v/v). After 12 h at 2°C the precipitate formed was centrifuged off. This step was repeated several times, the final supernatant was centrifuged, dialysed and freeze-dried to give a white purified L-arabinan.

2.4. *Degradation of the Pure Arabinan by α-L-Arabinofuranosidase*

Arabinan (20 mg) was dissolved in 10 ml distilled water; 2 ml of α-L-arabinofuranosidase (isolated and purified by the method of Gremli (1968) at our department) solution in McIlvaine buffer (pH 4·0) were then added and the mixture incubated at room temperature. After different times (30 min to 8 h) samples were withdrawn and analysed by tlc for liberated sugars as described above.

2.5. *Methylation of the L-Arabinan*

Arabinan (10 mg) was dried at 100°C for 5 h at 10 Torr in a drying pistol over sulphuric acid. The dry sample was dissolved in 1 ml dimethylsulphoxide and methylated by the method of Hakomori (1964) as described by Sanford & Conrad (1966). The methylated polysaccharide was hydrolysed by the formic acid–sulphuric acid method (Bouveng & Lindberg, 1965) and neutralised by addition of barium carbonate. The resulting methylated sugars were identified and measured, as their alditol acetates, by glc isothermally at 180°C on a glass column (200 x 0·3 cm) packed with Chromosorb Q coated with 3% OV-225 (Lönngren & Pilotti, 1971).

The relative concentration of the individual sugars was calculated by assuming the response factors to be proportional to the molecular weights of the acetylated methylalditols (Hellerqvist *et al.*, 1968). The identity of all O-acetyl-O-methyl-arabinitols was confirmed by glc-ms, using an OV-225-Scott column with a temperature programme from 150 to 230°C (5°C/min), coupled to an AEI MS 20 mass spectrometer.

3. RESULTS AND DISCUSSION

The non-dialysable material obtained from grape juice was separated into ethanol-insoluble and ethanol-soluble fractions, the yields were 252 mg/litre and 71·8 mg/litre of grape juice, respectively. The ethanol-soluble (crude L-arabinan) material contained 72% of neutral sugars, the remainder consisted mostly of colouring substances (anthocyanins). Purification on a polyamide column yielded a colourless product. Repeated precipitations at 85% ethanol concentration removed the polysaccharide fractions containing sugars other than arabinose (Table 1). Evaporation of the fraction soluble in 85% ethanol gave a pure L-arabinan consisting of arabinose only (Table 1).

The solubility in 85% ethanol indicated a highly-branched structure and low molecular weight of the isolated L-arabinan. The structure of the arabinan was established by enzyme degradation and methylation respectively. Incubation of the arabinan with α-L-arabinofuranosidase, an exo-enzyme, for 2 h gave arabinose only. The arabinose residues therefore must be present in the α-L-furanose configuration.

TABLE 1
Composition of the Ethanol-soluble Fraction of Grape Juice Polysaccharides Before and After Purification (Percentage of Total Neutral Sugar)

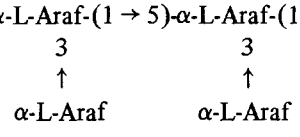
	Crude arabinan	*Purified arabinan*
Rhamnose	1·7	–
Fucose	–	–
Arabinose	65·5	100
Mannose	14·7	–
Glucose	10·6	–
Galactose	7·4	–

TABLE 2
Molar Distribution of Hydrolysis Products from Methylated Arabinan

O-Methylarabinitol	*Linkages present*	*Molar proportion of linkages in arabinan*
2,3,5-Tri	Araf-(1→	1·0
2,5-Di	→1)-Araf-(3→	Trace
2,3-Di	→1)-Araf-(5→	2·0
2-	→1)-Araf-(5→ ↓ 3	1·4
3-	→1)-Araf-(5→ ↓ 2	Trace

Methylation analysis confirmed the furanose configuration and furnished additional information on the type of linkage.

Analysis of the cleavage products after methylation of the arabinan (Table 2) indicated that the polysaccharide consists mainly of (1→3) and (1→5) linked arabinofuranosyl residues and that about 30% of the arabinose residues are tri-substituted (2,3,5-tri-O-methyl-arabinitol) and therefore occur as nonreducing end groups. The (1→5)-glycosidic linkage type predominates in the grape juice arabinan. Therefore, an arabinan built according to the general scheme suggested by Aspinall (1970) can be assumed. The identification of only traces of 3-O-methylarabinitol in the degradation products indicates the absence of 2-linked arabinose residues. The methylation data therefore suggest the following repeating units and structural features of the arabinan from grape juice:



This structure differs from the arabinans isolated from soybean and mustard seed (Aspinall & Cottrell, 1971), rape seed (Siddiqui & Wood, 1974) and cabbage (Stevens & Selvendran, 1980) in which arabinitol in appreciable amounts was identified as a degradation product. The structure of the isolated arabinan is, however, very similar to the so-called pectic arabinans and hence it is assumed that this arabinan is related to the pectic substances in the grape.

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