Carbohydrate Research, 81 (1980) 341–344

Note

Comparative structural studies of the D-mannans from a rosé wine and *Saccharomyces uvarum*

JEAN C. VILLETTAZ, RENATO AMADO, HANS NEUKOM*,
Swiss Federal Institute of Technology, Department of Food Science, CH-8092 Zurich (Switzerland)
MARC HORISBERGER, AND IAN HORMAN
Nestlé Products Technical Asisstance Co. Ltd., Research Department, CH-1814 La Tour-de-Peilz (Switzerland)

(Received August 27th, 1979; accepted for publication, November 2nd, 1979)

Wine contains a small amount of a high-molecular weight fraction consisting of various polysaccharides and proteins. The polysaccharides contain D-mannose as the main constituent[1–3]. It was of interest to purify and characterize the D-mannose-containing polysaccharide to determine its origin. D-Mannans may be released either from the grape or the yeast[1,2] cell-walls during processing and fermentation. The present work describes the isolation and structural features of a D-mannan isolated from a rosé wine. This D-mannan was compared to the D-mannan from the wine-producing yeast *Saccharomyces uvarum* (*carlsbergensis*).

TABLE I

APPROXIMATE COMPOSITION (%) OF D-MANNAN HYDROLYZATES

Component	*Rosé wine*		S. uvarum	
	Ethanol precipitate	*Purified D-mannan*	*Ethanol precipitate*	*Purified D-mannan*
Arabinose	9		0.2	
Xylose			2.2	
Rhamnose	2.1		[a]	
Glucose	6.6		3.2	
Galactose	17			
Mannose	65	100	93	100

[a]Fucose 1.4.

*To whom correspondence should be addressed.

TABLE II

MOLAR DISTRIBUTION OF HYDROLYSIS PRODUCTS FROM METHYLATED D-MANNANS

O-Methylmannitol	*Linkages present*	*Molar proportion of linkages in D-mannan from*	
		Rosé wine	S. uvarum
2,3,4,6-Tetra-	End group	1	1
3,4,6-Tri-	(1→2)	1.26[a]	1.16
2,4,6-Tri-	(1→3)		
2,3,4-Tri-	(1→6)	0.11	0.15
2,3-Di-	(1→4, →6)	0.07	0.08
3,4-Di-	(1→2, →6)	0.87	0.92
3-		trace	trace

[a]A complete separation of 3,4,6- and 2,4,6-tri-*O*-methylmannitol could not be achieved with the columns used.

TABLE III

PRODUCTS OF PERIODATE OXIDATION OF D-MANNANS[a]

Polyol	D-*Mannan from*	
	Rosé wine	*Yeast*
Glycerol	73	70
Erythritol	0.1	0.1
Mannitol	27	30

[a]Molar proportion.

From 10 L of a rosé wine, 10 g of material could be precipitated by ethanol. The mixture consisted mainly of a polysaccharide having D-mannose as the main component and of a small proportion (14%) of protein. The polysaccharide was purified by precipitation as a copper complex[4] to give a purified compound {$[\alpha]_D^{22}$ +76° (*c* 1, water)} containing only D-mannose. By use of the same isolation method, a mannan was prepared from *S. uvarum* {$[\alpha]_D^{22}$ +75° (*c* 1, water)} (Table I). The positive optical rotation indicates that both mannans have the α-D configuration, the values being in good agreement with those reported for yeast D-mannan isolated from *S. cerevisiae*[5].

Analysis of the cleavage products after methylation of the D-mannans (Table II) indicated that both polysaccharides had an identical structure, consisting of α-D-(1→2)- and -(1→3)-linked side-chains, attached to an α-D-(1→6)-linked backbone. The presence of only a trace of mono-*O*-methylmannitol indicated that both polysaccharides were fully methylated. The ratio of (1→2)- to (1→3)-linkages could not be established by methylation, since it was not possible to obtain a complete

separation of 3,4,6- and 2,4,6-tri-*O*-methylmannitol. However, from gas chromatographic evidence it was obvious that 2,4,6-tri-*O*-methylmannitol was the major component, indicating that the bulk of the side chains were (1→3)-linked.

Further evidence for the similarity of the structures of both D-mannans was obtained from the analysis of the periodate-degradation products (Table III). Since mannitol is only formed from (1→3)-linked D-mannose residues, the approximate ratio of these linkages may be deduced from the molar proportions given in Table III, considered with the methylation data from Table II. For every (1→2)-linkage in the side chains of the D-mannans, ~6 (1→3)-linkages are present. In contrast, the D-mannan from *S. cerevisiae* contains a higher percentage of (1→2)-linkages.

The results of the present study indicate that the structure of the highly branched D-mannan from rosé wine is essentially identical to that of the D-mannan from *S. uvarum*, which is used in the preparation of the wine. The D-mannan found in the wine is, therefore, most probably released from autolyzed yeast-cells during fermentation. The possibility that the D-mannan be derived from the hemicellulose fraction from the grape is excluded also by the observation that plant materials contain only linear (1→4)-β-D-mannans[5].

EXPERIMENTAL

General methods. — Uronic acid content was determined by the carbazole method[6]. The protein content was calculated as the sum of the various amino acids after hydrolysis of the ethanol precipitates; hydrolysis was performed with 6M hydrochloric acid for 16 h at 110°, in sealed tubes under nitrogen. Neutral sugars were determined by g.l.c. of the aldonitrile acetates; the samples were hydrolyzed with 2M trifluoracetic acid for 1 h at 120°, in sealed tubes under nitrogen, and the liberated monosaccharides were converted into the corresponding aldonitrile acetates by the method of Mergenthaler and Scherz[7]. Gas chromatographic separations were performed on a glass column (200 × 0.2 cm) packed with Chromosorb G-80 coated with 5% Carbowax 20 M; the oven temperature was programmed from 200 to 220° at 2°/min.

Isolation of D-mannans. — The rosé wine used was a "Pinot noir" wine, which was obtained from the Swiss Federal Agricultural Research Station in Wädenswil, Switzerland. It had been fermented with *Saccharomyces uvarum* (*carlsbergensis*), strain Nr. 18 (var. Fendant). The wine (10 L) was first concentrated under reduced pressure at 40° in a rotary evaporator, and then dialyzed against distilled water during 3 days at 2°. The nondialyzable material was centrifuged at 18,000*g* for 20 min, and to the supernatant was added 96% ethanol (5 vol.). The precipitate formed was left overnight at 2°, and then centrifuged (18,000*g*, 20 min). The pellet was dissolved in water and centrifuged, and the supernatant was freeze-dried (yield: 10 g).

A portion of the water-soluble residue (5 g) was dissolved in water (200 mL), the pH of the solution was adjusted to 9.0 by addition of 2M sodium hydroxide, and the solution was titrated with Fehling solution (Fehling I and II in equal amounts)[4].

The copper complex was centrifuged, re-suspended in distilled water (100 mL), and dissolved by addition of a few drops of concentrated hydrochloric acid. The D-mannan was precipitated by addition of 96% ethanol (500 mL). The precipitate was centrifuged and washed with 80% ethanol until no chloride ions were detectable. Finally, the D-mannan was dissolved in distilled water, dialyzed for 3 days at 2°, and freeze-dried (yield: 1.75 g).

The D-mannan of *S. uvarum* was isolated according to the method described by Peat *et al.*[8].

Methylation of the polysaccharides. — The polysaccharides were methylated by the method of Hakomori[9], as described by Sandford and Conrad[10]. The methylated polysaccharides were hydrolyzed by the formic acid–sulfuric acid method[11], and the acids were then neutralized by addition of barium carbonate. The resulting methyl sugars were identified and measured, as their alditol acetates, by g.l.c.[12] isothermally at 180°, on a glass column (200 × 0.3 cm) packed with Chromosorb Q coated with 3% OV-225. 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[13]. The identity of all *O*-acetyl-*O*-methylmannitols was confirmed by g.l.c.–m.s., using an OV-225–Scott column with a temperature program from 150 to 230° (5°/min.), coupled to an AEI MS 20 mass-spectrometer.

Periodate oxidation. — A sample of D-mannan (30 mg) was oxidized with 0.05M sodium metaperiodate (15 mL) at 4° in the dark. The periodate consumption was periodically determined by the iodometric method of Rankin and Jeanes[14]. After completion of the reaction (3 days), the excess of periodate was eliminated by addition of 1,2-ethanediol. The mixture was dialyzed, overnight at 4°, against distilled water, and concentrated to one third of the original volume. The oxidized D-mannan was reduced with sodium borohydride. The polyol was hydrolyzed with 0.5M sulfuric acid for 4 h at 100°, and the acid was neutralized with barium carbonate. The hydrolysis products were analyzed by g.l.c. as alditol acetates.

REFERENCES

1 W. Buechi and H. Deuel, *Helv. Chim. Acta*, 37 (1954) 1392–1398.
2 L. Usseglio-Tomasset and M. Castino, *Riv. Vitiv. Enol.*, 28 (1975) 328–339; *ibid.*, 28 (1975) 374–391; *ibid.*, 28 (1975) 401–402.
3 V. J. Zinchenko, F. L. Minchuk, and V. F. Andronov, *Prikl. Biokhim. Mikrobiol.*, 12 (1976) 772–779.
4 H. Deuel and H. Neukom, *Makromol. Chem.*, 4 (1949) 97–100.
5 G. O. Aspinall, *Polysaccharides*, Pergamon Press, New York, 1970, pp. 89–92.
6 T. Bitter and H. Muir, *Anal. Biochem.*, 4 (1962) 330–334.
7 E. Mergenthaler and H. Scherz, *Z. Lebensm.-Untersuch. Forsch.*, 162 (1976) 25–29.
8 S. Peat, W. J. Whelan, and T. E. Edwards, *J. Chem. Soc.*, (1961) 29–34.
9 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
10 P. A. Sandford and H. E. Conrad, *Biochemistry*, 5 (1966) 1508–1517.
11 O. H. Bouveng and B. Lindberg, *Methods Carbohydr. Chem.*, 5 (1965) 296–298.
12 J. Lönngren and Å. Pilotti, *Acta Chem. Scand.*, 25 (1971) 1144–1145.
13 C. G. Hellerqvist, B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg, *Carbohydr. Res.*, 8 (1968) 43–55.
14 J. C. Rankin and A. Jeanes, *J. Am. Chem. Soc.*, 76 (1954) 4435–4441.