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Note

An L-arabinan from apple-juice concentrates

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An L-arabinan obtained from a commercial sample of apple juice has proved to be of interest from the chemical point of view, and because of its mode of aggregation to form microcrystalline particles that are remarkably uniform in shape. Although arabinans are widespread in Nature, being found in seeds, vegetables, fruits, and bark tissue of many kinds, their isolation in undegraded form is rendered difficult by their acid-lability and their association with other polysaccharides of pectic type.

In the present instance, material precipitated from juice concentrates was readily purified to give a polysaccharide, of high, negative optical rotation, which contained L-arabinofuranosyl residues only; methylation analysis and n.m.r. spectroscopy of the polysaccharide showed that the arabinan is α-$(1\rightarrow5)$-linked and essentially linear. This molecular structure contrasts with the more usual, branched character of known plant arabinans[1], although the linkage mode is the one normally found in main chains thereof. The lack of branching is possibly the underlying reason for the formation of particles of relatively uniform size and shape as warm solutions of the arabinan are brought to a near-freezing temperature and kept thereat for some days; the process is reversed on warming to 60°. Regularity of structure is further indicated by the results of X-ray analysis, whereby diffraction patterns indicative of a high degree of order are obtained.

The possibility that the L-arabinan was produced by degradative, enzymic action from more-complex polysaccharide material is very real, in view of the nature of the industrial process employed.

EXPERIMENTAL

Isolation and aggregation of the arabinan. — Celite was added to concentrated juice from apples (Starking and Granny Smith varieties) in which a sediment had formed during storage, and the filter-cake obtained therefrom was washed repeatedly

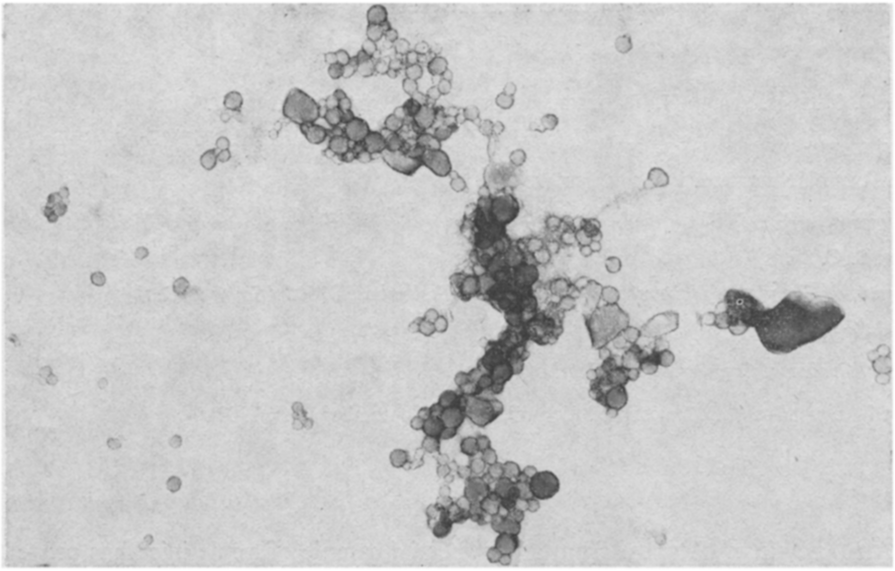

Fig. 1. Electron micrograph of the particles that formed during the initial, 24-h storage at 4°. [Positively contrast-stained with uranyl acetate. Particle size, 30 nm average.]

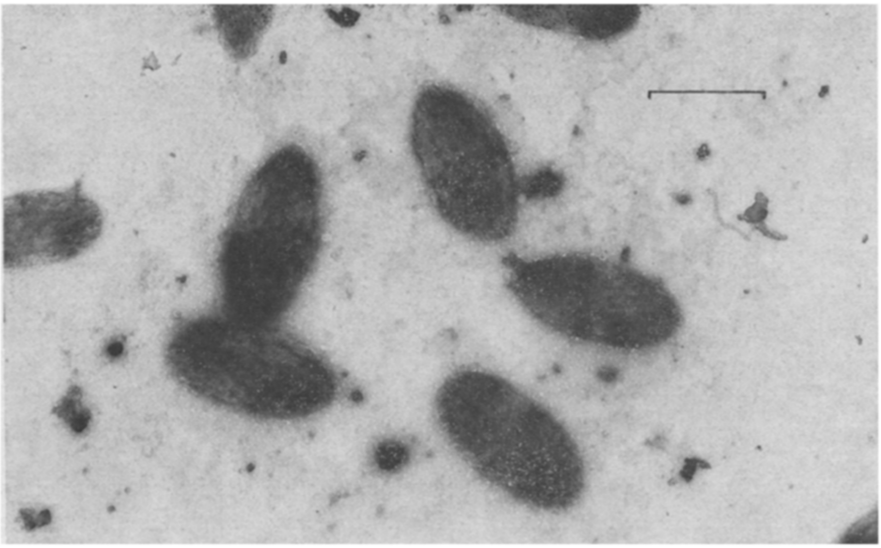

Fig. 2. Electron micrograph of finally washed and crystallized particles. [Positively contrast-stained with uranyl acetate. Particle dimensions, 1700 × 800 nm.]

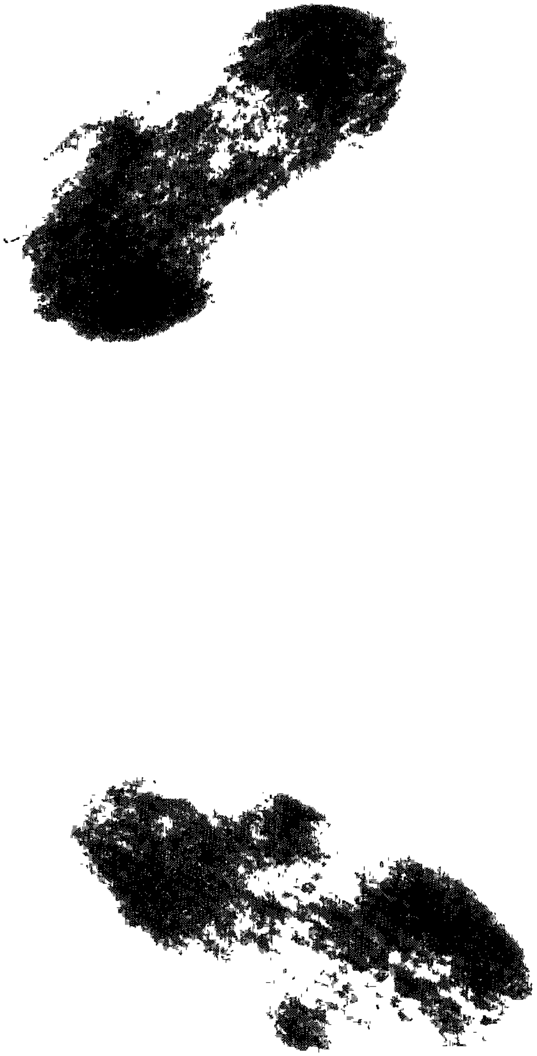

Fig. 3. Electron micrograph of finally washed and crystallized particles at higher magnification. [Negatively stained with phosphotungstate. Particle dimensions, 1800 × 700 nm.]

with cold, distilled water on a Büchner funnel, to remove all pigmented material, and then extracted with boiling water in small portions on the filter. After storage for 24 h at 4°, the filtrate became hazy, at which point an aliquot was removed and centrifuged at 12,000g for 60 min; the resulting pellet was then examined by electron microscopy as soon as practicable (see Fig. 1). The remaining fluid was kept for a further 7 days at 4°, during which time cloudiness developed, and a precipitate began to settle. This was collected in the form of pellets by centrifuging at 12,000g for 40 min, and purified by twice dispersing it in distilled water at 4°, and centrifuging as before. When the product was re-suspended in water and heated to 90°, a clear, pale-brown solution was obtained. On being stored at 4°, precipitation again occurred, and the pellets obtained by centrifugation were examined by electron microscopy (see Figs. 2 and 3).

From the electron micrographs, it could be seen (see Fig. 1) that the particles that formed during the initial period of storage for 24 h were of various sizes, with a mean diameter of 30 nm. Small particles were, however, absent from the purified material (see Figs. 2 and 3). The final product obtained after allowing crystallization to proceed for 7 days consisted of large, ellipsoidal aggregates measuring, on average, 1700 and 840 nm along the major and minor axes, respectively. These dimensions were interdependent; when the major axis was longer, the minor was shorter, which suggests that the volumes or masses of the particles were constant. Observation of a large number of the particles indicated a remarkable degree of similarity among them.

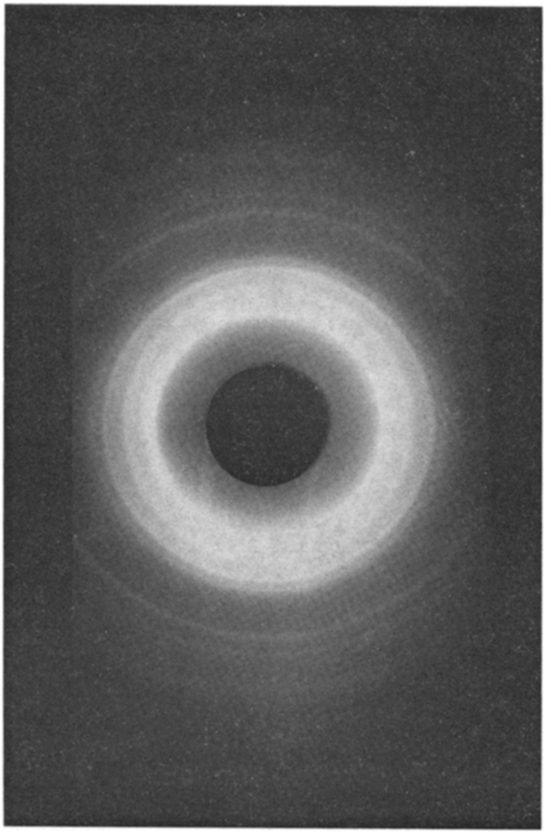

Fig. 4. X-Ray diffractogram of a specimen of powdered L-arabinan.

In general, their surfaces appeared to be fibrous in nature. The aggregates were prepared from three different batches of filter cake, by the same purification method, with reproducible results.

X-Ray crystallographic analysis of the powdered specimen of L-arabinan was performed on Kodak NS-392T film by the Debye–Scherrer method[2], using a Philips powder camera, *r* 28.65 mm, and Ni-filtered CuKα radiation. Exposure to the X-ray beam (30 kV, 20 mA) for 3–4 h was followed by development of the film for 5 min, immersion in a stop bath, and fixation for 5 min. The reflections recorded were measured with a linear comparator (light box–travelling microscope), and a set of intensities (*m*, medium; *s*, strong; *w*, weak) was assigned to each, as follows.

Interplanar spacing (pm)	510	427	377	352	277	256	225
Intensity	*s*	*vw*	*m*	*w*	*mw*	*w*	*vw*

The intensities and definition of the lines (see Fig. 4) indicated clearly that the molecular structure of the L-arabinan is ordered and repetitive, and the diffractogram should prove a useful diagnostic measure of the particular structure involved.

Particle-size determination: *gel-permeation chromatography*. — A sample (14 mg) of dried material was suspended in water (6 mL), and dissolved by warming at 60°, to produce the nonaggregated form. The solution remained clear for at least 24 h at room temperature. A portion (1 mL) of freshly prepared solution was transferred to a dextran-calibrated column (total volume 90 mL) of Bio-Gel P-10, and eluted with M sodium chloride at a flow rate of 20 mL/h. A single peak (assayed by the phenol–sulfuric acid method[3]), corresponding to $\overline{M}_w$ 10,000, was obtained for the nonaggregated particles.

Hydrolysis in acid. — The kinetics of hydrolysis of the solid material in trifluoroacetic acid solution at pH 2.0 and 100° was studied by polarimetry, samples being periodically removed during 14 h of heating. Initial $[\alpha]_D$ $-127.3°$, final $+103°$, and hydrolysis rate-constant $k = 154 \times 10^{-6}\ s^{-1}$ between 5 and 10 h, were consistent with a uniformly linked, α-L-arabinofuranan structure, support for which came from chemical tests and n.m.r. spectroscopy. Hydrolysis of the polysaccharide yielded L-arabinose only, as shown by paper chromatography, g.l.c.–m.s. of the alditol acetate, and the formation of the derived benzoylhydrazone (X-ray powder diagram identical with that of an authentic specimen). Traces of hexoses and uronic acid had been detected, together with the major sugar component (which was arabinose), in a hydrolyzate of the sediment as originally isolated (prior to the purification process).

Methylation analysis. — Purified polysaccharide (11 mg) was methylated once by the Hakomori method[4,5], and the product was analyzed by hydrolysis, conversion of the methylated sugars formed into their alditol acetates, and gas–liquid chromatography thereof (3% of OV-225 on Chromosorb W; 185→210° at 2°.min^{-1}). The proportions of sugars obtained (characterized by retention times and mass spectrometry, and estimated by using effective carbon-response factors[6]) were:

2,3,5-Me_3Ara (2), 2,3,4-Me_3Ara (3), 2,3-Me_2Ara (93), and 2-MeAra (3). Any branching present must, accordingly, be strictly limited in extent.

N.m.r. spectroscopy. — ^{1}H-N.m.r. spectroscopy of the polysaccharide in D_2O showed a broad spectrum at ambient temperature, but the complex spectrum recorded at 85° contained a clearly defined doublet at δ 5.1 ($J \sim 1$ Hz) similar in chemical shift and splitting to the anomeric-proton signal of methyl α-L-arabinofuranoside[7].

The ^{13}C-n.m.r. spectrum of the polysaccharide in Me_2SO-d_6 at 35° contained four lines: a doublet at δ 108.01 corresponding to the α-L-anomeric carbon atoms in the polymer[8,9] (for the β anomer, δ 102.2 would be expected), a triplet at highest field (δ 67.06) assigned to C-5, a strong doublet at δ 81.61 attributed to C-2 and C-3 (deshielded, due to H-bond formation between molecules), and a doublet at δ 77.35, assigned to C-4.

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REFERENCES

1 J. C. Villataz, R. Amado, and H. Neukom, *Carbohydr. Polym.*, 1 (1981) 101–105.
2 L. V. Azaroff and M. J. Buerger, *The Powder Method in X-Ray Crystallography*, McGraw-Hill, New York, 1958.
3 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
4 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
5 P. A. Sandford and H. E. Conrad, *Biochemistry*, 5 (1966) 1508–1517.
6 D. P. Sweet, R. H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, 40 (1975) 217–225.
7 B. Capon and D. Thacker, *Proc. Chem. Soc.*, (1964) 369.
8 E. Breitmaier and W. Voelter, *^{13}C-NMR Spectroscopy*, 2nd edn., Verlag Chemie, New York, 1978, p. 252.
9 J. B. Stothers, *Carbon-13 NMR Spectroscopy*, Academic Press, New York, 1972, p. 467.