Polysaccharides in Germination. Physical Characterization of the Pectic Araban of White Mustard Cotyledons*

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ABSTRACT: The molecular weight of mustard seed araban, determined by sedimentation equilibrium and vapor pressure osmometry, corresponds to a molecule containing about 45 sugar units. Arabans extracted at different pH values, extracted from different batches of resting seeds, and extracted from cotyledons after germination are all homogeneous and similar in the ultracentrifuge and on free solution electrophoresis in borate. These findings confirm that mustard seed araban is not a degradation artifact of a large heteropolysaccharide, and show that the change in araban structure which occurs with germination cannot take place by the simple addition or simple partial removal of arabinose units from a polysaccharide subfraction.

Mustard cotyledons contain an araban which is considered to be a natural component of the plant cell wall, quite separate and distinct from the side chains of similar structure attached to the acidic backbone of a co-occurring larger polysaccharide (Hirst *et al.*, 1965; Rees and Richardson, 1966; Gould *et al.*, 1965). The araban diminishes in amount during germination, and undergoes changes in molecular structure (Rees and Richardson, 1966). In this paper, we report physical results which throw some further light on the changes during germination and which confirm that the araban is not a degradation artifact. The results make an interesting comparison with physical data published for arabans that are thought to be artifacts.

Materials and Methods

Most of the general methods have been described already (Hirst *et al.*, 1965; Rees and Richardson, 1966). A sample of araban was extracted from commercial "White Mustard Seed Germ" (J. and J. Colman, Ltd., Carrow Works, Norwich, U. K.) using EDTA at pH 4.9, and purified by fractional precipitation with ethanol, acetone, and cetyltrimethylammonium hydroxide (Hirst *et al.*, 1965). After purification on a column of Sephadex G-15 and freeze-drying, the yield (2.5 g/kg) was only slightly less than had been obtained by extraction at pH 7.5 (3.5 g/kg). The difference probably arose because extraction of other wall polysaccharides and of protein is more efficient at pH 7.5 and there is therefore better breakdown of the tissue which results in more efficient extraction of the araban. The product contained 100% arabinose and had $[\alpha]_D$ −173° (water). Only arabinose was detectable after hydrolysis and paper chromatography.

Other araban specimens and the methylated arabans were those used earlier (Rees and Richardson, 1966). Ultracentrifugation was with the Spinco Model E. Free solution electrophoresis was in sodium tetraborate solution (0.05 M, pH 9.2) using the Beckman H apparatus. A vapor pressure osmometer (Model 301A, Mechrolab Inc., Mountainview, Calif.) was used for determination of the molecular weight of the methylated araban in benzene solution.

Results

Molecular Weights. The short-column technique in the ultracentrifuge (Yphantis, 1960) was used to determine the molecular weight in aqueous solution of the araban from resting seeds ("sample 1"). The value for $1 - \bar{V}\rho$ (where $\bar{V}$ is the partial specific volume of the araban, ρ is the density of the solution) was 0.38. The molecular weights determined at 0.8, 1.0, and 1.2% concentrations were 6100, 5700, and 5800, respectively (essentially weight-average values). A number-average determination on the di-*O*-methylaraban (also "sample 1") by vapor pressure osmometry over a concentration range of 1–6% followed by extrapolation to infinite dilution gave a value of 7500, corresponding to a value of about 6200 for the parent araban.

Ultracentrifugation and Electrophoresis. The arabans isolated previously from different batches of resting seeds and from germinated cotyledons by extraction at pH 7.5 (Rees and Richardson, 1966) and the product of extraction with EDTA at pH 4.9 were similar in the ultracentrifuge and on free solution electrophoresis. Typical diagrams are shown in Figure 1, and the results of some measurements are listed in Table I. Most of the small differences in sedimentation coefficients may be related to the fact that samples 2 and 4 were

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TABLE I: Physical Constants of Mustard Arabans.

Sample[a]	$[\alpha]_D$ (water), Deg	Electrophoretic Mobility[b]	Sedimentation Coefficient[c]
1	−176	2.2	1.32
2	−180	2.3	1.23
3	−176	2.2	1.41
4	−173	1.9	1.24

[a] Samples 1–3 correspond to the sample numbers defined earlier (Rees and Richardson, 1966, Table IV). Sample 4 is the araban whose isolation by extraction at pH 4.9 is described in this paper. [b] $\mu \times 10^5$ cm^2 v^{-1} sec^{-1} using 0.5% solution of araban in 0.05 M sodium tetraborate (pH 9.2) at 6.5 v/cm (current 16 ma). [c] Apparent s_{20} with a 1% solution of araban in 0.15 M NaCl at 334,000*g*.

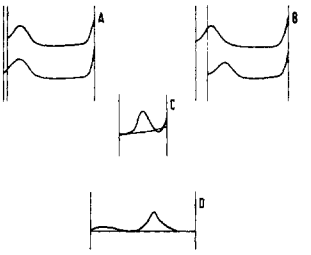

FIGURE 1: Sedimentation and electrophoresis diagrams for mustard arabans. (A) Sample 2 (upper) and sample 4 (lower); photograph after 98 min at 334,000*g* (1% araban solution in 0.15 M NaCl in 12-mm aluminium cell at 20°); bar angle 65°. (B) Sample 1 (upper) and sample 4 (lower); conditions as in A. (C) Ultracentrifugation of araban sample 4 under similar conditions to A and C but in a synthetic boundary cell (upper solution 0.15 M NaCl); photograph after 15 min; araban samples 1–3 behaved similarly to this. (D) Free boundary electrophoresis of 0.5% solution of araban sample 4 in 0.05 M sodium tetraborate (pH 9.2); photograph is of the ascending limb after 178 min at 6.5 v/cm (current 16 ma). The araban travels from left to right as a negatively charged borate complex; araban samples 1–3 gave peaks that were exactly alike in shape (for mobilities see Table I). Sample numbers are defined in Table I.

derived from one batch of seeds and samples 1 and 3 from another.

Discussion

The araban molecule is evidently quite small (it contains about 45 sugar units) and evidence from the sedimentation and electrophoresis diagrams, from the correspondence between the number-average and weight-average molecular weights, and from criteria used earlier (Hirst *et al.*, 1965) would suggest that it is not grossly heterogeneous. Chemical evidence has been given previously that the araban does not contain a linear component (Rees and Richardson, 1966). The apparent similarity of the arabans isolated before and after germination (Table I), however, shows that, as criteria of homogeneity, the sedimentation and electrophoresis diagrams are not sensitive to differences in degree of branching of the araban because it has been shown that these polysaccharide preparations differ quite markedly in this respect. It would therefore be unsafe to assume that individual molecules in a given araban preparation are nearly identical with respect to the degree of branching.

Arabans which occur naturally as side chains in larger molecules and which are released as elimination products during extraction at alkaline pH appear to remain attached to segments of the original main chain (Rees and Richardson, 1966; Hullar, 1963; Barrett and Northcote, 1965). The proportion of sugars other than arabinose would therefore depend on the size of the side chain. The molecular weight of mustard seed araban is low enough for such a main chain segment to have been detected, even had it consisted of only one unit. This molecular weight determination therefore provides further evidence that mustard seed araban is not a degradation artifact. Barrett and Northcote (1965) suggested that the parent pectins from which "araban artifacts" may be formed by eliminative degradation during extraction may have higher proportions of covalently bound neutral sugars associated with certain regions of the main chain. It is therefore likely that "araban side chains" closely situated to each other would be released in attachment to the same main chain segment. This would account for the wide difference between the molecular weight of mustard seed araban (6×10^3) and that of Barrett and Northcote's araban formed by degradation of apple pectin (2×10^5 or more). The conditions for release of the araban in Barrett and Northcote's work (sodium phosphate buffer, pH 6.8) would have caused little degradation subsequent to the elimination reaction. The much harsher lime water treatment which is used in the preparation of beet araban would, however, be expected to cause further degradation of the main chain segment by "peeling" (Whistler and BeMiller, 1958). Partial or complete separation of the "araban side chains" from each other would occur and a product with a lower molecular weight would be expected. Reported molecular weights for arabans isolated by this and similar methods are in the range $1–7 \times 10^4$ (Ingleman, 1945; Andrews *et al.*, 1959; Hullar, 1963; Tomimatsu and Palmer, 1963; Sengupta *et al.*, 1965). Comparison of our electrophoresis dia-

gram (Figure 1) with that published by Hullar (1963), suggests that beet araban is more heterogeneous than mustard seed araban, as might be expected on the basis of this discussion.

Our genuine arabans differ in optical rotation from arabans that have been isolated with the aid of harsh methods. Our specific rotations are in the range -173 to $-180°$, compared with the range -55 to $-100°$ for several samples that have been shown to contain other sugars (Andrews *et al.*, 1959; Hullar, 1963; Sengupta *et al.*, 1965).

When mustard seeds germinate, the araban in the cotyledons diminishes in amount and the degree of branching decreases (Rees and Richardson, 1966). The molecular weight and the molecular weight distribution would appear from the sedimentation diagrams to be little altered during this process. No partly degraded araban was detected after germination. It follows that if the change in branching is brought about by simple addition or subtraction of arabinose units, then all, or nearly all, of the araban molecules in the cotyledons must be affected equally to give a small average shift in molecular weight. The most likely possibility is perhaps that the structural changes result from turnover of some or all of the araban molecules, rather than simple modification. It is also possible that the araban in the resting seeds is heterogeneous with respect to degree of branching and that the most densely branched molecules are selectively and completely metabolized during germination.

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References

Andrews, P., Hough, L., Powell, D. B., and Woods, B. M. (1959), *J. Chem. Soc.*, 774.

Barrett, A. J., and Northcote, D. H. (1965), *Biochem. J. 94*, 617.

Gould, S. E. B., Rees, D. A., Richardson, N. G., and Steele, I. W. (1965), *Nature 208*, 876.

Hirst, E. L., Rees, D. A., and Richardson, N. G. (1965), *Biochem J. 95*, 453.

Hullar, T. L. (1963), Ph.D. Thesis, University of Minnesota, St. Paul, Minn.

Ingleman, B. (1945), *Communications, Swedish Paper Corp. 1*, 179; *Chem. Abstr. 39*, 5525.

Rees, D. A., and Richardson, N. G. (1966), *Biochemistry 5*, 3009 (this issue; preceding paper).

Sengupta, U. K., Mukherjee, A. K., and Rao, C. V. N. (1965), *Australian J. Chem. 18*, 851.

Tomimatsu, Y., and Palmer, K. J. (1963), *J. Polymer Sci. A1*, 1005.

Whistler, R. L., and BeMiller, J. N. (1958), *Advan. Carbohydrate Chem. 13*, 289.

Yphantis, D. A. (1960), *Ann. N. Y. Acad. Sci. 88*, 586.