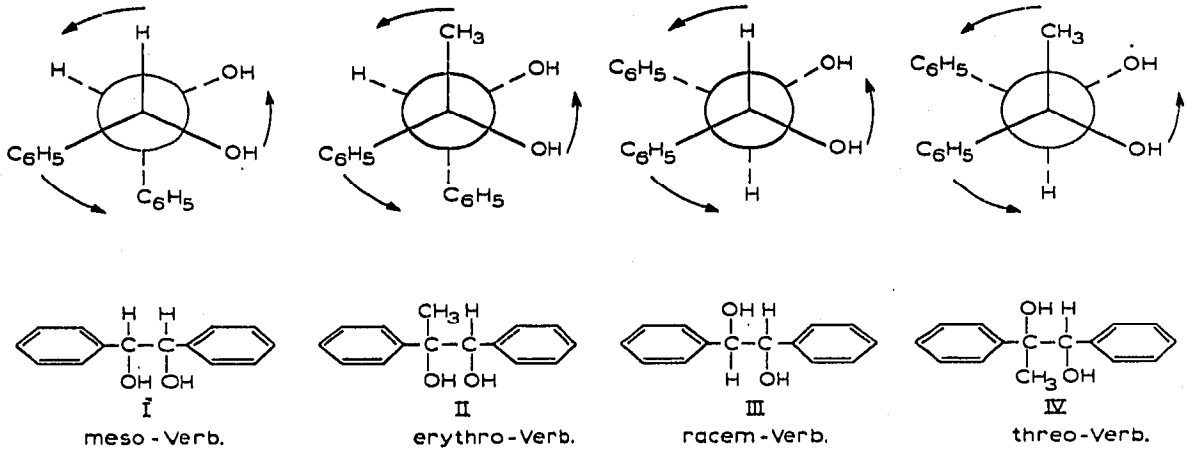

Fig. 1. Vergleich der Gruppenwechselwirkung von diastereomeren Hydrobenzoinen und α-Methylhydrobenzoinen.

lich waren und zu dicke Schichten bröckeln. Nachweis der Diole: Ammoniakalische $AgNO_3$-Lösung.

Papierchromatographie. Schleicher & Schüll-Papier 2043b wurde benutzt. Nur die absteigende Methode gab brauchbare Ergebnisse (Kammersättigung!). Detektion: Perjodat–Benzidin.

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Paper chromatography of oligogalacturonides*

Partial hydrolysis of polygalacturonic acid yields galacturonic acid and a homologous series of oligogalacturonides. The smaller oligogalacturonides have been separated by both paper[1–4] and anion-exchange[5,6] chromatography. This paper describes the separation of oligogalacturonides, with the degree of polymerization as high as 12. The relationship between chromatogram mobility and molecular size for this group of oligosaccharides is discussed.

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A partial enzymatic hydrolyzate of pectin was prepared in the following manner. Fifty grams of pectin were dissolved in 3 l hot water and, after cooling, 2 g pectinase (Nutritional Biochemicals Corp.) were added. The solution was incubated at room temperature until the viscosity decreased to that of water. The solution was then rapidly brought to boiling to inactivate the pectinase. After cooling, an extract of alfalfa (source of pectinesterase; 75 g alfalfa herbage blended in 150 ml 5 % saline) was added, and the solution was incubated for 24 h, with the addition of *N* NaOH to maintain the pH at 6.5. The solution was boiled again, and the process was repeated with a fresh extract of alfalfa. The oligogalacturonides were precipitated by the addition of 4 volumes 95 % ethanol, and lyophilized.

Chromatographic separations were made on Whatman No. 3 MM paper, using the descending technique. A Chromatocab (Research Specialties Co., Model A125), which accommodates 18 × 22-in. sheets, was used in a room maintained at 30°. The system (solvent I), ethyl acetate–acetic acid–water (10:5:6 v/v) effectively separated the smaller oligogalacturonides. Increasing the proportion of water in solvent I increased the mobilities of all the acids, with the greatest increases for the longer analogues.

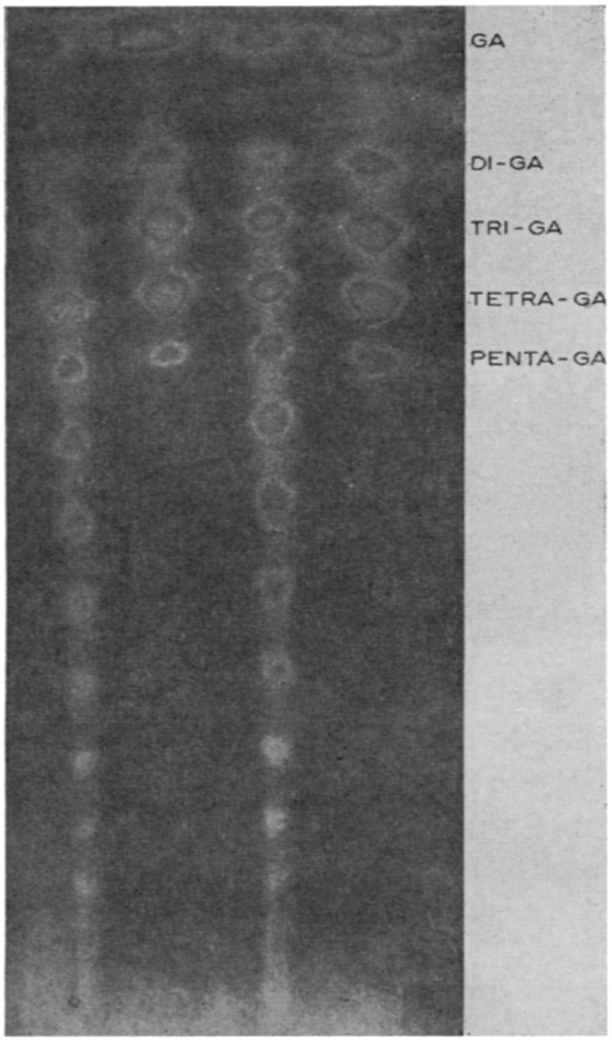

Fig. 1. Paper chromatogram of oligogalacturonides developed in ethyl acetate–acetic acid–water (10:5:9 v/v) and dipped in a mixed indicator solution.

Since the spots were not only shifted farther but also compressed on the sheet, a modification of solvent I, ethyl acetate–acetic acid–water (10:5:9 v/v) (solvent II), was used to separate the oligogalacturonides.

Fig. 1. shows the resolution of an enzymatic hydrolyzate of pectin, using solvent II with a multiple development technique. This chromatogram was obtained by five developments, each 12 h long. The reference compounds, galacturonic acid, di-, tri-, tetra- and penta-galacturonic acid, were prepared and purified by chromatography on Dowex-1 (formate). The oligogalacturonides appeared as red spots on a dark green background, when treated with a mixed indicator solution of 50 mg thymol blue, 250 mg methyl red and 600 mg bromthymol blue in 1 l 95 % ethanol. The pH of the dip solution was adjusted by the addition of *N* NaOH until a blue-green color was attained. Residual acetic acid on the chromatograms was removed by brief autoclaving and thoroughly drying prior to dipping. Separation of oligogalacturonides with the degree of polymerization as high as 12 was achieved with relative ease. However, it was considerably more difficult to separate the higher analogues.

The effect of the degree of polymerization on oligogalacturonide mobility is shown in Fig. 2. The R_{GA} (mobility relative to galacturonic acid) values represent data obtained from a series of chromatograms developed simultaneously by the continuous descending technique. Development time for individual chromatograms was varied from 15 to 75 h. When galacturonic acid moved off the sheet, R_{GA} was calculated from the mobility for tetra-galacturonic acid for which the R_{GA} value was well established. A plot of the logarithm of the R_{GA} values against the degree of polymerization disclosed a simple correlation between the mobility and the molecular size of the oligo-

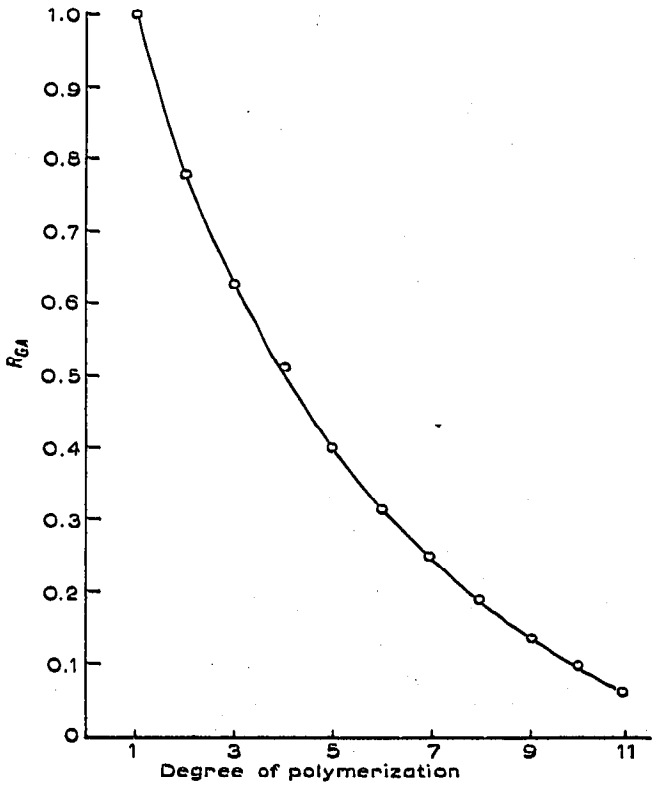

Fig. 2. Effect of the degree of polymerization on the migration of oligogalacturonides on paper chromatograms developed in solvent II.

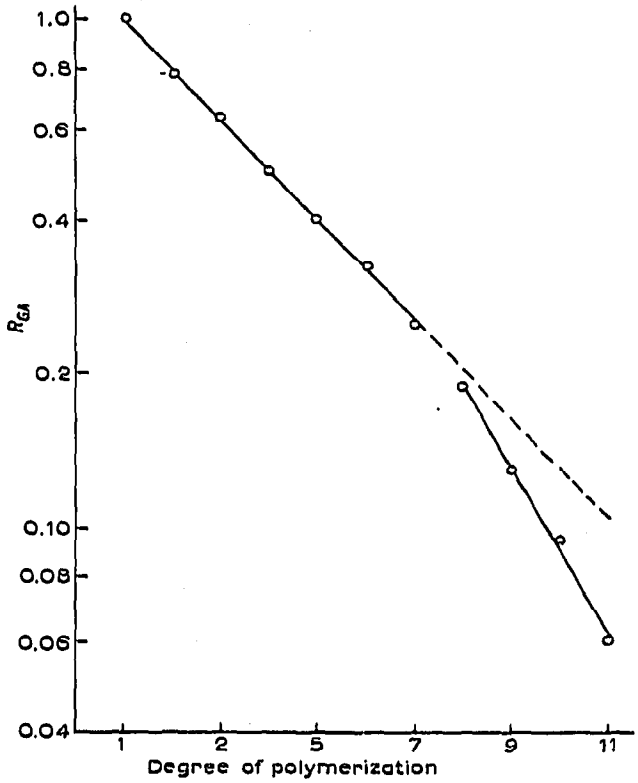

Fig. 3. A logarithmic plot of the R_{GA} values for oligogalacturonides in solvent II.

galacturonides as large as the heptamer (Fig. 3). As the degree of polymerization exceeded 7, a straight line with increased slope was obtained.

The relationship between chromatogram mobilities and molecular size of homologous oligosaccharide series has been reported[7,8]. On plotting the logarithm of R_F values against the molecular size for amylo-oligosaccharides, a straight line was obtained which included all except the lowest members of the series. Linear correlations were obtained for a number of homologous series by plotting molecular size against the logarithm of a partition function α', defined by the equation

$$\alpha' = R_F/(1 - R_F).$$

When a similar plot was made for our data, a straight line was obtained only for the oligogalacturonides possessing a degree of polymerization between 4 and 8.

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