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Note

High-performance anion-exchange chromatography of saturated and unsaturated oligogalacturonic acids

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Pectic substances are a group of complex natural acidic polysaccharides that are not yet fully characterized. They play an important role in the food industry as gelling agents.

The partial enzymic hydrolysis of the polygalacturonic acid regions in pectic substances releases a series of oligogalacturonic acids. Depending on the enzyme used, saturated or unsaturated (1 → 4)-linked α-D-galactopyranosyluronic oligomers* can be obtained[1]. The unsaturated function is located at C-4 of the galactopyranosyluronic acid chain. These degradation products have been reported to act as pharmaceutical agents[2]; furthermore, they allow a view inside the fine structure of the various pectic substances[3].

An analytical method that gives separation and determination of the galacturonic acid oligomers would facilitate research on their properties. At present they are analyzed to an increasing extent by high-performance liquid chromatography. Various techniques (reviewed by Hicks[4]) have been used to achieve the best separation. Recently the analytical determination of saturated oligogalacturonic acids by high-performance anion-exchange chromatography (HPAEC) was revealed to be a powerful tool[5–7].

This article describes the separation of both saturated and unsaturated oligomers by HPAEC with either ultraviolet (UV) or pulsed amperometric detection (PAD).

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* Systematic name of the unsaturated ring system: 4-deoxy-β-L-*threo*-hex-4,5-enopyranosyluronic acid.

EXPERIMENTAL

Materials.—Saturated and unsaturated oligogalacturonic acids with degrees of polymerization (dp) from 2 to 10 and from 2 to 7, respectively, were obtained by the method of Hotchkiss et al.[8], which includes enzymic breakdown of pectic acid (Serva Feinbiochemica, Heidelberg, Germany) with polygalacturonase (Röhm GmbH, Darmstadt, Germany) and pectate lyase (Serva). The molecular weights to dp 6 were confirmed by fast atom bombardment mass spectroscopy. The dp values of higher oligomers were assigned by retention time extrapolation. Galacturonic acid monohydrate was purchased from E. Merck, Darmstadt.

Chromatography.—The experimental system was equipped with a GMP pump, a CarboPac PA1 (25 mm × 3 mm i.d.) precolumn, a CarboPac PA1 (250 mm × 4 mm i.d.) column, a type II PAD with a gold working electrode (Dionex GmbH, Idstein, Germany) and a Hitachi D-2000 integrator (E. Merck). The electrode potential was maintained at +0.05 V for 480 ms, +0.6 V for 120 ms and −0.6 V for 60 ms (output range, 1 μA), the voltages measured against a silver/silver hydroxide electrode. Additionally for the unsaturated oligomers a UV 432 detector (Kontron, Eching, Germany) at 235 nm was used. The isocratic eluent was 0.1 N NaOH with 0.5 M NaOAc at 22°C and a flow rate of 1 $\mathrm{mL \cdot min^{-1}}$. For the separation of unsaturated and saturated oligomers in one analytical run, a linear gradient constructed of 0.1 N NaOH in 0.4 M NaOAc (A) and 0.1 N NaOH in 0.5 M NaOAc (B) was used. The gradient went from all A to all B over the first 10 min, followed by all B to 25 min.

RESULTS AND DISCUSSION

For the determination of capacity factors, mixtures of the first ten saturated or the first six unsaturated galacturonic acid oligomers were chromatographed under isocratic conditions on a strong base anion-exchange resin.

Fig. 1 shows the separation of several saturated and unsaturated oligomers in one chromatographic run. The occurrence of the various dp's is typical for the enzymic breakdown of acid-soluble pectic acid by pectate lyase[9].

Effect of chain length on capacity factors.—When neutral carbohydrates are chromatographed under alkaline conditions (0.1 N NaOH) on a strong-base anion-exchange resin, different retention behaviour is caused by differences in the pK_a values of their hydroxyl groups, differences in their chain lengths, and variations in their structures. For instance, ln k' of the members of a homologous series of malto-oligosaccharides is linearly correlated with chain length[10]. This work shows that the same correlation is possible with oligogalacturonic acids. Due to the low pK_a of the uronic acid carboxylic group, it is necessary to add sodium acetate to the eluent to force faster elution of these strongly retained materials. Values of ln k_i of members of the homologous series of saturated and unsaturated oligomers are plotted vs. chain length (Fig. 2). Both series yield straight lines, but

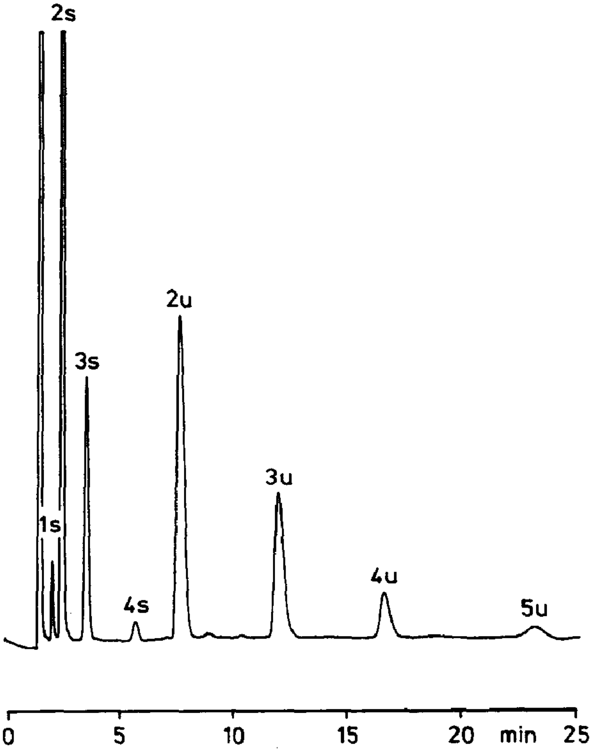

Fig. 1. Separation of saturated and unsaturated oligogalacturonic acids. The dp value is indicated above each peak (s = saturated, u = unsaturated). Column, Dionex CarboPac PA1; flow rate, 1 mL·min^{-1}; eluent *A*, 0.1 N NaOH in 0.4 M NaOAc; *B*, 0.1 N NaOH in 0.5 M NaOAc, gradient, 0.4 → 0.5 M NaOAc (0–10 min), 0.5 M NaOAc (10–25 min); sample, degradation products of acid-soluble pectic acids, 20–200 mg·L^{-1} each; sample volume, 20 μL; detection PAD.

the members of the unsaturated series have much higher capacity factors than those of the saturated series, a phenomenon first noticed by Hotchkiss et al.[11], who used a potassium oxalate eluent at pH 6. The slopes of the two lines are very similar: 0.481 (saturated) vs. 0.437 (unsaturated), suggesting that the difference in chromatographic behaviour is almost completely localized in the unsaturated end. Analogous saturated and unsaturated uronic acids have such different capacity factors because of the conjugation of the double bond with the carboxyl group found in unsaturated uronic acids, which in other molecules containing similar conjugated double-bond systems leads to much lower pK_a values[12,13]. It can be expected that the unsaturated analogues are the stronger anions due to the induction effect of the double bond, leading to stronger retention on the anion-exchange resin.

Response of pulsed amperometric and ultraviolet detectors to unsaturated oligogalacturonic acids.—Both UV and PA detectors yielded closely related responses to the members of the unsaturated series (Table I). For the UV detector this is due to a quite uniform molar extinction coefficient within the series

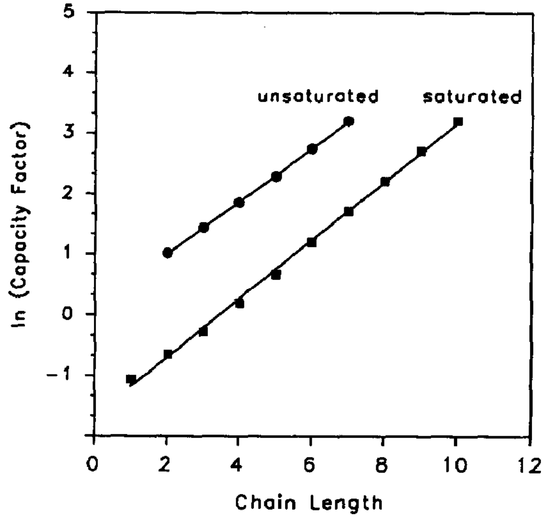

Fig. 2. Effect of chain length on capacity factors of members of homologous series of saturated and unsaturated galacturonic acid oligomers. Eluent flow rate, 1 mL·min^{-1}; isocratic eluent, 0.1 N NaOH in 0.5 M NaOAc; temperature, 22°C; sample volume, 20 μL; detector, PAD/gold working electrode.

TABLE I

Detector response of unsaturated oligogalacturonic acid [a]

dp	Response per μmol (10^7 area counts)	
	PAD	UV
2	53.1	277.5
3	57.6	278.5
4	53.5	275.5
5	55.0	276.5
6	54.5	279.5

[a] Column, Dionex CarboPac PA1; eluent flow rate, 1 mL·min^{-1}; isocratic eluent concentration, 0.1 N NaOH in 0.5 M NaOAc; temperature, 22°C; sample concentration, 50 mg·L^{-1}; sample volume, 20 μL; detectors, Dionex PAD with gold working electrode; pulse program, $E_1 = +0.05$ V, $E_2 = +0.6$ V, $E_3 = -0.6$ V, $t_1 = 480$ ms, $t_2 = 120$ ms, $t_3 = 60$ ms, output range, 1 μA or Kontron UV 432, 235 nm, range 0.05 AU.

($\epsilon \approx 4800$ L·mol^{-1}·cm^{-1})[14]. For the PAD this response behaviour had not previously been reported. In the case of saturated oligogalacturonic acids, the molar response factors are affected by dp and eluent composition[6].

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