

Lipids in Carrot Roots

I. The Purification of Crude Lipids and the Composition of the Neutral Lipids

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Filtration through a Sephadex LH-20 gel column was found to be an effective method for the removal of contaminating sugars, phosphorus compounds and amino acids from lipids extracted from carrot root.

At the end of the growth season, the lipids in carrot root amount on average to 0.23 % of the fresh weight, and 1.8 % of the dry weight. Neutral lipids represent 63 %, glycolipids 21 %, and phospholipids 16 % of total lipids.

When the neutral lipids were fractionated by chromatography on a silicic acid column, the largest fractions consisted of triglycerides, sterol esters, sterol, and free fatty acids which amounted to 36, 31, 10, and 6 %, respectively, of the neutral lipids, and 23, 20, 6, and 4 % of the total lipids.

Most non-photosynthetic plant tissues contain very little lipids.¹ Despite their low contents, the lipids are important in many respects. As is well known, they are precursors of numerous aroma compounds and their oxidation may shorten the shelf-life of many food products.^{2,3} Plants like the carrot which contain very little lipids, but much water pose great analytical problems. Especially when their lipids are extracted, abundant non-lipid components accompany the lipids.⁴ Promising results in the purification of plant extracts have been obtained by gel filtration.⁵ For this reason we undertook to study whether filtration through Sephadex LH-20 gel can be used to purify lipids extracted from carrots.

Lipids extracted from carrot roots were resolved into neutral lipid, glycolipid, and phospholipid fractions by chromatography on a silicic acid column and the neutral lipids were separated into subclasses. Before fractionation of the purified lipids on the silicic acid column, they were passed through a column of a chelating resin in the sodium form to ensure a definite chromatographic elution sequence of the anionic phospholipids and glycolipids.⁶

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EXPERIMENTAL

Materials. Carrots (*Daucus carota*), variety Feonia AH, were obtained from a farm in Köyliö, South-western Finland. The carrots were harvested in October, 1970. They were washed with water, the tips and short green upper sections of the roots were removed, and the roots were cut into small cubes.

The reagents and solvents were of analytical grade. The solvents were dried and distilled before use.

Extraction of lipids. One hundred grams of the freshly cut carrot cubes were extracted with 600 ml of a 2 : 1 (v/v) chloroform-methanol mixture at 7°C for 2 min in a Serval Omni-Mixer rotating at 5000 r.p.m. The solids remaining after filtration of the homogenate were extracted again with 100 ml of the same solvent mixture. The final residue was washed with four 50 ml portions of chloroform on a Büchner funnel. The water that separated from the combined extract and wash mixture was washed with chloroform, which was then added to the organic phase. The extracts were dried with a mixture of anhydrous sodium sulphate and sodium bicarbonate and the solvents were removed in a rotating evaporator at 25°C. The crude lipids that remained were weighed and dissolved in chloroform and the solution in chloroform was stored at -20°C.

Column chromatography on Sephadex LH-20 gel. The crude lipids were purified by eluting them through a column of Sephadex LH-20 gel (AB Pharmacia, Uppsala, Sweden) as proposed by Maxwell and Williams.⁵ The Sephadex LH-20 gel had been washed with water but dried with acetone instead of air. The loading was 5–50 mg of crude lipids per gram of dry Sephadex LH-20. The total bed volume of the column was about 14 ml. The lipids were eluted first with a 3 : 1 (v/v) chloroform-methanol mixture and then with a 1 : 1 (v/v) methanol-water mixture. To determine how the lipid and non-lipid sugars and phosphorus compounds migrated during the fractionation, 27 eluate fractions with the volumes given in Figs. 1 and 2 were collected during the elution of a sample of the crude lipids. In routine Sephadex gel chromatography of the crude lipids, the following fractions were collected on elution of the column with the chloroform-methanol mixture: a 7 ml forerun, fraction A, which preceded a pigment band, a 9 ml lipid fraction B containing coloured matter, and a 32 ml fraction C. A 32 ml fraction D was collected on elution of the column with the methanol-water mixture.

Ion-exchange column chromatography was used to replace calcium and magnesium of the lipids by sodium. The resin (Chelex, 100, 100–200 mesh, sodium form, Bio-Rad Laboratories, Richmond, California) was prepared and the aqueous solvent was replaced by the used solvent mixture as described by Carter and Weber⁶ with the exception that the pH of the resin slurry was 7–7.5 as proposed by Renkonen.⁷ The bed volume of the resin in a chromatography column 15 cm long and 1.6 cm in diameter was 30 ml. The loading was at most 10 mg of lipids per ml of bed volume. The lipids were eluted as one fraction from the column with six bed volumes of a 5 : 4 : 1 (v/v/v) chloroform-methanol-water mixture.

Chromatography on a silicic acid column was employed to fractionate the lipids after the ion-exchange chromatography into neutral lipids, glycolipids, and phospholipids. Silicic acid (100 mesh, AR, Mallinckrodt Chemical Works, St. Louis, Missouri) was activated by heating at 115°C for 20 h. The column and the eluting solvents and their volumes were identical with those used by Rouser *et al.*⁸ The loading was 10–60 mg of lipids per gram of silicic acid.

Column chromatography on silicic acid was used also to resolve the neutral lipids into subclasses. 15 g of silicic acid was added to a glass tube, 1.6 cm in inside diameter, whereupon a column, 15 cm long, resulted. The loading was 10–15 mg of lipids per gram of silicic acid. The lipids were eluted with the same solvents as Barron and Hanahan⁹ employed to resolve neutral lipids from rat liver, but the volumes of the solvents were only one fourth of those used by Barron and Hanahan. Ninety 15 ml samples were collected during the fractionation.

Thin-layer chromatography. Glass plates covered with a layer of silica gel G (for thin-layer chromatography, E. Merck AG), 0.25 mm thick, were heated at 110°C for 60 min to activate the gel. The chromatograms were developed by one-dimensional chromatography in glass jars lined with filter paper using a 65 : 25 : 4 (v/v/v) chloroform-methanol-water mixture for glyco- and phospholipids, and a 90 : 10 : 1 (v/v/v) hexane-diethyl ether-glacial acetic acid mixture for neutral lipids. In two-dimensional chromatography, the

plates were developed in the first run with the mentioned chloroform-methanol-water mixture and in the second run with a 80 : 50 : 10 (v/v/v) di-isobutyl ketone-glacial acetic acid-water mixture. The spots containing components were rendered visible by exposing the plates to iodine vapour, by spraying the plates with a solution of ninhydrin in butyl alcohol, Dragendorff's reagent,¹⁰ or periodate-Schiff¹¹ reagent, or by spraying the plates with 30 % sulfuric acid and heating them at about 120°C.

Analysis of eluted fractions. All the fractions collected by column chromatography were weighed after evaporation of the solvent. In addition, the contents of total sugars, water-soluble sugars, and total phosphorus in the effluent fractions collected during gel filtration were determined. The sugars were determined with the anthrone reagent¹² as described by Maxwell and Williams.⁵ The sugar contents of the lipid fractions were obtained by subtracting the water-soluble sugar contents from the total sugar contents. Total phosphorus was determined by the method of Chen *et al.*¹³ except that the residues remaining after the samples had been heated with perchloric acid were neutralized by adding first 6 N potassium hydroxide and then dilute aqueous potassium carbonate solution. During the column chromatography of neutral lipids on silicic acid, the Liebermann-Burchard reaction was used to follow the elution of sterol esters and sterols. The free fatty acids were determined by titration of the samples in ethanol with 0.01 N methanolic sodium hydroxide.

RESULTS AND DISCUSSION

The contents of crude lipids found in different extractions of carrot roots varied slightly, from 250 to 300 mg/100 g of fresh plant tissue. The results are in satisfactory agreement with those of Dalgarno and Birt.¹

Preliminary experiments show that a significant proportion of the impurities in crude lipids extracted from carrot roots are sugars and phosphates. A

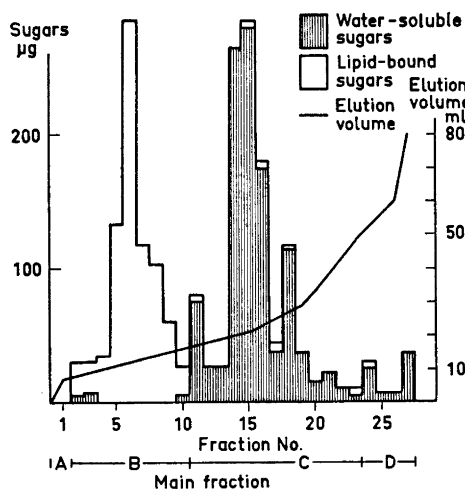


Fig. 1. Distribution of lipid-bound sugars and water-soluble sugars in eluate fractions when crude lipids extracted from carrot root were eluted from a Sephadex LH-20 column. Fractions 1–23 were eluted with a 3 : 1 chloroform-methanol mixture and fractions 24–27 with a 1 : 1 methanol-water mixture. The main fractions A–D are described in Methods.

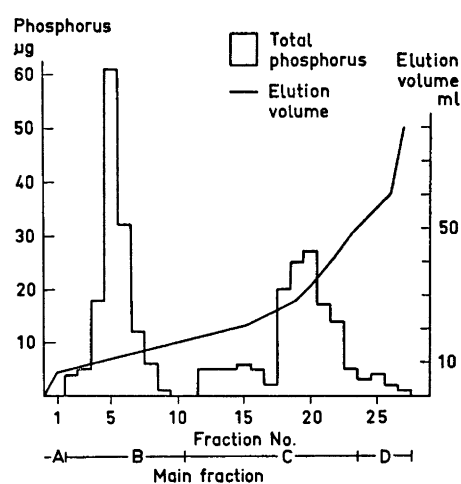


Fig. 2. Distribution of total phosphorus in eluate fractions when crude lipids extracted from carrot root were eluted from a Sephadex LH-20 column. Fractions 1–23 were eluted with a 3 : 1 chloroform-methanol mixture and fractions 24–27 with a 1 : 1 methanol-water mixture. The main fractions A–D are described in Methods.

detailed picture of the elution of sugars when crude lipids were purified by gel filtration is provided by the sugar contents of 27 eluate fractions that are plotted in Fig. 1. The results show that the lipid-bound sugars were eluted first in a rather narrow band (fractions 2–10) followed by the non-lipid sugars in a much wider band (fractions 11–27). The separation of lipid and non-lipid sugars was effective although the weight of crude lipids taken varied from 4 to 80 mg/g of Sephadex LH-20.

In the elution pattern of total phosphorus presented in Fig. 2, there was a narrow band in nearly the same position as that of lipid-bound sugars. Another broader phosphorus band emerged in the fractions 12–27; about half of the total phosphorus was eluted with this band. Thus phosphorus was eluted in two separate zones. Thin-layer chromatographic analyses revealed that the first narrow band contained phospholipids whereas the latter broad band, where the phosphorus was also in bound form, did not contain phospholipids. It was not possible to detect in the fractions 12–27 degradation products of phosphatides other than orthophosphate by the hydrolysis and alcoholysis series of Dawson.¹⁴

When the fractionation on the Sephadex LH-20 column was followed by thin-layer chromatography, all the lipids were found to be eluted in fractions 2–10. The thin-layer chromatographic analyses revealed also that amino acids were eluted with fractions 11–20 with a peak in the fractions 15–16 and were well separated from lipid components. Pigments were visible in lipid fractions 2–10.

On the basis of the results presented above, the elution program described in Methods was chosen for the purification of the crude lipids of carrot roots routinely. Four main fractions A–D were collected, of which fraction B contained the lipids (fractions 2–10 in Figs. 1 and 2).

When the crude lipids were purified by gel filtration, the weights of recovered lipids (fraction B) varied from 213 to 259 mg, average 232 mg, per 100 g of fresh carrot roots. This corresponds on average to 1.8 % of the dry weight of roots. The weights of solids in the fraction C was on average 27 mg, and the weights of solids in the fraction D was on average 3 mg/100 g of fresh carrot tissue. The forerun fraction A contained no solids. The recovery of total solids in the fractionation was 99.2 %. The crude lipids of carrot root contained from 8 to 14 %, average 12 %, non-lipid compounds; comparable data are not available in the literature. This shows that careful purification of extracted lipids is necessary before any attempt is made to determine the composition of the lipids.

The recovery of carrot root lipids after passage through the column of Chelex ion-exchange resin in the sodium form was on average 99.8 %. The fractionation of the lipids so treated on a silicic acid column showed that they contained 63 % neutral lipids, 21 % glycolipids, and 16 % phospholipids, the total recovery being about 98 %. One hundred grams of fresh carrot roots contained 143 mg of neutral lipids, 40 mg of glycolipids, and 35 mg of phospholipids. The respective percentages on a dry weight basis were 1.15, 0.38, and 0.28. When the course of the fractionation was followed by weighing the eluted solutes and by thin-layer chromatography employing specific colour reagents, the lipid classes were found to be eluted in the order stated by Rouser *et al.*⁸

Table 1. Composition of neutral lipids of carrot root determined by silicic acid column chromatography.

| Lipid fraction in order of elution | % of neutral lipids | % of total lipids | mg per 100 g of fresh roots | mg per 100 g of dry roots |
|---------------------------------------|---------------------------|-------------------------|-----------------------------------|---------------------------------|
| Hydrocarbons and pigments | 5.1 | 3.3 | 7.4 | 59 |
| Sterol esters | 30.9 | 19.5 | 45.1 | 362 |
| Triglycerides and free fatty acids | 41.8 | 26.3 | 61.1 | 489 |
| Free fatty acids ^a | 5.9 | 3.7 | 8.6 | 69 |
| Sterols | 9.7 | 6.1 | 14.1 | 113 |
| Diglycerides | 3.0 | 1.9 | 4.3 | 34 |
| Unidentified | 2.3 | 1.5 | 3.4 | 27 |
| Monoglycerides | 3.0 | 1.9 | 4.4 | 35 |
| Other unidentified components | 6.5 | 4.1 | 9.6 | 75 |
| Total | 100.0 | 63.0 | 145.8 | 1167 |

^a Determined by titration assuming a mean molecular weight of 280.

and that the employed volumes of solvents were appropriate for the fractionation of carrot root lipids.

The results presented for the fractions in Table 1 in the order of elution were obtained when the carrot root neutral lipids were separated into subclasses by silicic acid column chromatography. It was confirmed by thin-layer chromatography and using special reagents that the lipid classes were separated as presented in the elution scheme of Barron and Hanahan.⁹ The main components of the neutral lipids were triglycerides, which represented about 36 %, and sterol esters, which represented about 31 % of the neutral lipids. Sterols amounted to about 10 % and free fatty acids, which were present in the triglyceride fraction, to about 6 % of the neutral lipids. These four fractions together amounted to 83 % of the neutral lipids.

Comparatively little information is available on the compositions of the lipids of plants and especially their nonphotosynthetic tissues.¹⁵ Carrot root lipids contain as much glycolipids as potato lipids but relatively more neutral lipids and relatively less phospholipids.¹⁶ Particularly high, 26 %, is the proportion of steroids in carrot root lipids; in potato lipids the percentage is about 9. Also the percentage of triglycerides is higher in carrot roots (over 20 % of the lipids) than in potatoes (15 % of the lipids).

Further investigation on the composition of glycolipids and phospholipids is in progress.

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Received September 15, 1972.