Isolation and separation of mono- and digalactosyldiglycerides from spinach leaves with Sephadex LH-20

The usual methods for the isolation and separation of monogalactosyldiglyceride (GDG) and digalactosyldiglyceride (GGDG) are chromatography on Florisil, DEAE–cellulose and silicic acid[1] and treatment with acetone followed by chromatography on a silicic acid column as described by WEENINK[2–4], and ROUSER *et al.*[5]

This note describes the isolation and separation on a column of Sephadex LH-20 of GDG and GGDG from the supernatant remaining after removing the majority of the phospholipids by acetone precipitation of the total lipid extract.

Methods and materials

Extraction of total lipids. About 1 kg of spinach leaves (*Spinacia oleracea*) were washed with water and kept one minute at 95° to denature the enzymes. After cooling, the leaves were homogenized in a Waring blendor with 96% ethanol and the residue was filtered off on a Büchner funnel. The residue was then washed with 96% ethanol until colorless. The total filtrate was dried *in vacuo* to yield 14 g of solid material. This was dissolved in 600 ml diethyl ether and was washed with water. The ether phase was dried *in vacuo* to give a yield of 10.9 g. This residue was then dissolved in 220 ml *n*-heptane pre-equilibrated with 95% methanol and was distributed between *n*-heptane pre-equilibrated with 95% MeOH and 95% MeOH pre-equilibrated with *n*-heptane as described by CARTER, MCCLUER AND SLIFER[6]. After drying the MeOH-phase *in vacuo*, the phospholipids from the MeOH phase were removed by precipitation with acetone at −10°. The precipitate was filtered off and the filtrate was used after drying for Sephadex column chromatography.

Sephadex LH-20 chromatography. 100 g Sephadex LH-20 were allowed to swell for 2 h in chloroform and was then packed into a column with inner dimensions of 50 × 3 cm. The column was washed with 500 ml chloroform. The acetone soluble lipids dissolved in 4 ml chloroform were applied to the column. Successive elutions were made with 800 ml $CHCl_3$, 1000 ml $CHCl_3$–MeOH (10:1, v/v), and 575 ml MeOH. Fractions of 5 ml were collected.

Results and discussion

It was observed that not all phospholipids were removed from the acetone-soluble lipids by repeated precipitation. When the acetone-soluble lipids were chromatographed on TLC plates of silica gel with $CHCl_3$–MeOH (10:1, v/v) as solvent, a blue spot was found after colouring with the molybdenum blue reagent of DITTMER AND LESTER[7]. These remaining phospholipids came out of the column only after elution with 260 ml $CHCl_3$. The glycolipid GDG was eluted directly after the phospholipids in 200 ml. After elution with 400 ml $CHCl_3$–MeOH (10:1, v/v) the second glycolipid (GGDG) came through in the following 150 ml. The phospholipid GDG- and GGDG-fractions were concentrated and examined by TLC with $CHCl_3$–MeOH (10:1, v/v) and $CHCl_3$–MeOH–H_2O (65:25:4, v/v) as solvent systems. Detection was with rhodamine B–2′,7′-dichlorofluorescein[8]; anisaldehyde reagent[9] and molybdenum blue reagent.[7]

The GDG- and GGDG-fractions were hydrolysed with 0.1 *N* KOH in MeOH for 15 min at 37°. The reaction mixtures were neutralized with Amberlite IR 120 (H-form), filtered and the filtrate was applied to a paper chromatogram (Whatman No.

3MM). Elution was with phenol–water (3:1, w/w). After colouring with the periodate–Schiff reagent we found a single spot in each fraction. In the GDG-fraction, a spot with R_F 0.68 (= monogalactosyglycerol) was found and in the GGDG-fraction the spot had R_F 0.51 (= digalactosylglycerol).

This demonstrated that:

(1) The phospholipid fraction contained no glycolipids.

(2) The GDG-fraction contained no phospholipids or GGDG.

(3) The GGDG-fraction was free of GDG and phospholipids.

Most of the plant pigments were removed by this method. However, the glycolipid fractions contained some yellow-brown pigments.

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Partial resolution of mandelic acid with Sephadex gels

Racemic mandelic acid and other racemic substances have been resolved, at least partially, with optically inactive eluents and with starch[1–3] and cellulose[4–8] as the stationary phase. Since Sephadex is a crosslinked polymer of glucose, it seemed likely that it could also serve as the immobile phase in chromatographic resolutions.

Experimental

Sephadex G-10 and G-25 were obtained from Pharmacia Fine Chemicals and pretreated according to the company literature. Research grade DL-mandelic acid (Aldrich Chemical Co., Inc., Milwaukee 10, Wisc.) was found to be optically inactive and was used without further purification. De-ionized water was used. The other chemicals were of reagent grade and were used without further treatment.