Chromatographic Separation of Glucopyranosyl Sinapate from Canola Meal

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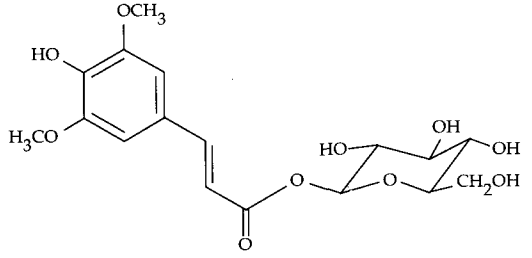
The compound 1-*O*-β-D glucopyranosyl sinapate (GPS), a phenolic glycoside, was separated from ethanolic extracts of defatted canola meal by a two-step chromatographic method. The first step involved Sephadex LH-20 chromatography with methanol as the eluting solvent. The solvent from the fraction containing GPS was evaporated, and glucopyranosyl sinapate was subsequently separated by a semi-preparative high-performance liquid chromatography method with an RP-18 column and a mobile phase consisting of water/acetonitrile/acetic acid (88:10:2, vol/vol/vol).

KEY WORDS: Antioxidant activity, canola meal, glucopyranosyl sinapate, HPLC, phenolics, Sephadex LH-20 chromatography.

Sinapic acid is the main phenolic acid present in rapeseed and canola meals (1,2). The content of sinapic acid, liberated from glycosides of eleven varieties of rapeseed flour, ranged from 6.7 to 11.2 mg/100 g meal. The content of phenolic acids in the glycoside form accounted for approximately 1% of the total content of these compounds (3).

The compound, 1-*O*-β-D glucopyranosyl sinapate (GPS), was identified in the ethanolic extracts of canola meal (4). Its structural identity (Scheme 1) has been confirmed by ultraviolet (UV), infrared (IR) mass spectrometry (MS) and nuclear magnetic resonance (NMR) techniques (4). Preparative thin-layer chromatography was used for isolation of GPS (4). This compound also has been isolated from cotyledons of *Raphanus sativus* (5) and from leaves of *Brassica oleracea* (6). These authors used column chromatography on Sephadex LH-20 (Pharmacia, Uppsala, Sweden) with methanol as a solvent and a polyamide CC 6 with methanol/water (6:4, vol/vol) (5) or a polyamide CC 6 with continuous water/methanol gradient (6). The present study reports on a facile semi-preparative high-performance liquid chromatography (HPLC) method for the separation of GPS from canola meal.



SCHEME 1

EXPERIMENTAL PROCEDURES

Canola seed (6.0 g) was ground and then defatted with hexane in a Soxhlet apparatus. Defatted meal was then extracted into 100 mL 95% (vol/vol) ethanol while heating in a 250-mL conical flask at 80°C for 20 min. The extraction was repeated twice, and the resultant meal was separated by centrifugation for 10 min at 5000 × *g*. Ethanolic extracts were then combined and evaporated to dryness under vacuum at 40°C. The yield of the crude extract was 12.9% of the defatted meal. The procedure was repeated four times to obtain sufficient amounts of canola extract.

One gram of dried canola extract was dissolved in 8 mL methanol and was applied onto a C16/100 column packed with Sephadex LH-20. Methanol was used for elution, and forty-eight 8-mL fractions were collected with a fraction collector. Absorbance values were measured at 330 nm, the characteristic wavelength absorption for GPS (4). A Hewlett-Packard 8452A diode array spectrophotometer (Palo Alto, CA) was used for this purpose. Fractions were also monitored by thin-layer chromatography (TLC) on silica gel plates (Sigma Chemical Co., St. Louis, MO) with a mixture of chloroform/methanol/water (65:35:10, vol/vol/vol, lower phase) (7) as the mobile phase. Plates were sprayed with a solution consisting of equal volumes of 1.0% (wt/vol) aqueous solutions of ferric chloride and potassium ferricyanide (8).

Fractions diagnostic for the presence of phenolic compounds [R_f = 0.50, characteristic for GPS (4)] were combined, and methanol was evaporated. GPS was then separated by a semi-preparative HPLC technique in a Shimadzu (Tokyo, Japan) chromatographic system, consisting of an LC-6A pump, SPD-6AV UV-VIS spectrophotometric detector, SCL-6B system controller and a CR 501 Chromatopac recorder/integrator. A Hibar prepacked column RT (10 × 250 mm) with Lichrosorb RP-18 (7 μmk) (Merck, Darmstadt, Germany) was used. The mobile phase consisted of a mixture of water/acetonitrile/acetic acid (88:10:2, vol/vol/vol) (3). The flow rate was 4 mL/min. An injection volume of 500 μL was used, and the absorbance values were read at 330 nm. The assignment of GSP was based on UV, MS, IR, ^{1}H NMR and ^{13}C {^{1}H} NMR techniques.

For rechromatography of pure compounds, the same basic system and a CSL analytical column (4.5 × 250 mm) with Spherisorb-ODS-2 (10 μm) (Chromatography Sciences Company Inc., Montreal, Canada) was used. A flow rate of 1 mL/min and an injection volume of 20 μL were used.

RESULTS AND DISCUSSION

The chromatograms (Fig. 1) of the eluated fractions from the Sephadex LH-20 column shows one large, three intermediate and two small peaks. TLC analysis of fractions indicated that GPS was present in tubes 22–27 (marked with arrows in Fig. 1), corresponding to the second intermediate peak. The fraction containing GPS constituted 14.6% of the total weight of the extract and exhibited strong antioxidant effects in a β-carotene–linoleate system (4). The weight of this separated fraction was 120 mg.

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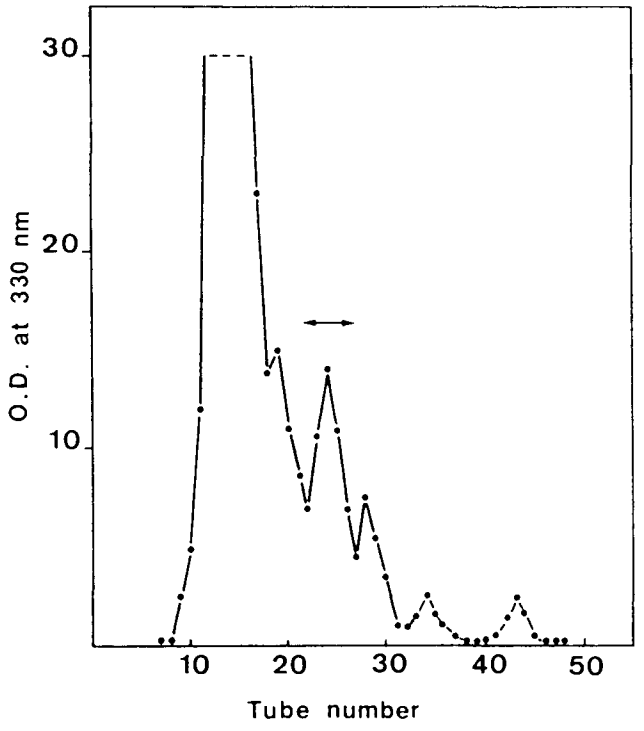

FIG. 1. Fractions from Sephadex LH-20 (Pharmacia, Uppsala, Sweden) chromatography of ethanolic extracts of canola meal by using methanol for elution to collect forty-eight 8-mL eluate fractions. O.D., optical density.

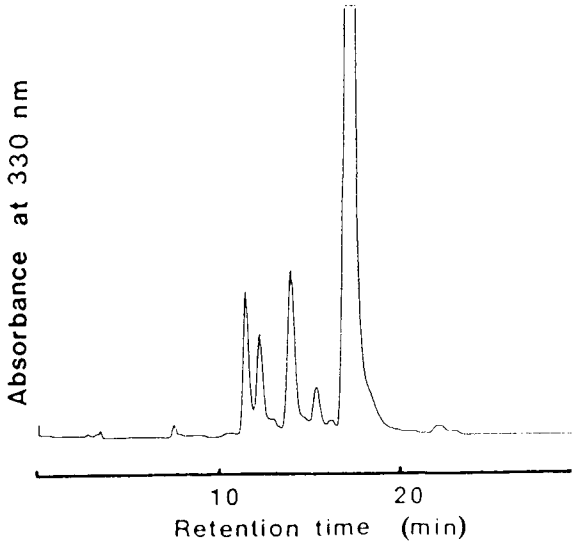

FIG. 2. Semi-preparative high-performance liquid chromatography separation of glucopyranosyl sinapate from the fraction separated on a Sephadex LH-20 column (Pharmacia, see Fig. 1). A pre-packed retention time (10 × 250 mm) column with Lichrosorb RP-18 (7 μm) (Merck, Darmstadt, Germany) was used and water/acetonitrile/acetic acid (88:10:2, vol/vol/vol) was employed as a mobile phase.

Semi-preparative HPLC (Fig. 2) of this fraction showed that GPS (retention time 17.29 min) was the main phenolic compound. The weight of the pure GPS was 12.3 mg. Chromatographic conditions that were employed assured good separation of GPS from four other phenolic compounds with retention times of 11.46, 12.29, 13.96 and 15.42 min. The GPS showed an absorption maximum at

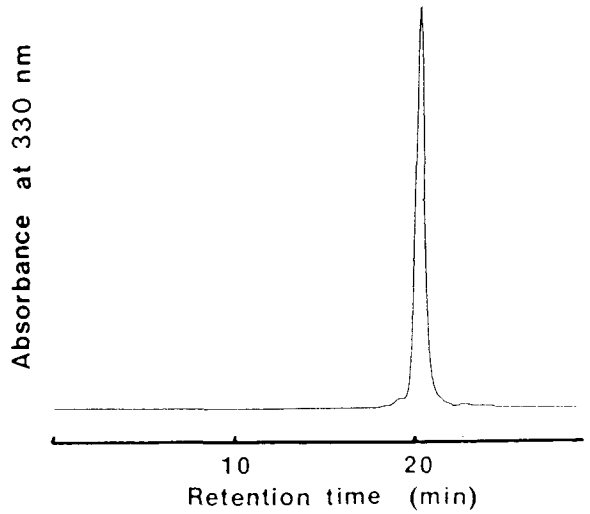

FIG. 3. Rechromatography of glucopyranosyl sinapate on a CSL analytical high-performance liquid chromatography column (4.5 × 250 mm) with Spherisorb-ODS-2 (10 μm).

330 nm, due to the sinapic acid portion of the molecule (1,2,9). The MS analysis indicated two major fragment ions with *m/z* of 180 and 206, corresponding to the glucose and sinapic acid moieties of GPS, respectively (4); however, the molecular ion did not appear. The IR spectrum of the compound further confirmed the presence of hydroxyl groups, an aromatic ring and an ester bond. The compound upon hydrolysis yielded sinapic acid and glucose as indicated by TLC by using a series of monosaccharides and phenolic acid standards (4).

Rechromatography of GPS on an analytical HPLC column (Fig. 3) confirmed that the purity of the isolated compound was >95%. The chromatographic method employed may therefore be used for isolation of the standard GPS. A large Sephadex column and multiple semi-preparative HPLC runs would allow isolation of sufficient quantities of GPS for investigation of its biological activity and its antioxidant properties.

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