ISOLATION OF SECOISOLARICIRESINOL DIGLUCOSIDE FROM LIGNAN-CONTAINING EXTRACT OF *Linum usitatissimum* SEEDS

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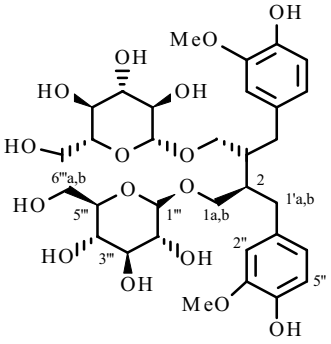
An effective method for obtaining secoisolariciresinol diglucoside (SDG) purified of lignan from extract of Linum usitatissimum *seeds was proposed. The lignan-containing concentrate obtained by aqueous ethanol extraction with microwave irradiation was purified using column chromatography. The structure of the compound was confirmed using IR, mass, and NMR spectra.*

Key words: lignans, *Linum usitatissimum* seeds, secoisolariciresinol diglucoside, preparative chromatography, HPLC, NMR.

Flax seeds (*Linum usitatissimum*) are a rich source of lignans with a broad spectrum of biological activity including powerful antioxidants, cancer-cell growth inhibitors, and bactericides and antivirals [1]. Flax seeds contain a large amount of (+)-[2*R*,2′*R*]-bis[(4-hydroxy-3-methoxyphenyl)methyl]-1,4-butanediylbis(β-glucopyranoside) (SDG) compared with other minor lignans [2].



The specific content of SDG in seeds of certain flax varieties is 1-2% whereas it is less than 0.002 and 0.001% in seeds of soy and grain, respectively [3].

The process for producing SDG includes successive extraction to give a mixture enriched with this compound and its subsequent separation using preparative ion-exchange and reversed-phase chromatography.

The extract was produced using the method developed by us that includes successive defatting of ground flax seed, hydrolysis with subsequent neutralization, and concentration of the resulting extract. The extraction was carried out together with alkaline hydrolysis of the aqueous ethanol mixture and microwave irradiation. This shortened significantly the time for preparing the initial extract and increased its yield to 23.2% with a SDG content of 61.2 mg/g calculated for dry extract. Ecologically safe solvents were used for this.

The ion-exchange sorbent Diaion HP-20 was used for purification of the lignan-containing extract. SDG was eluted by aqueous ethanol solutions. The final purification of the SDG-enriched fraction was carried out over reversed-phase silica gel C_{18}, which also enabled the separation to be performed using aqueous ethanol as the mobile phase. Elution curves were constructed for Diaion HP-20 sorbent and silica gel C_{18} (Fig. 1).

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TABLE 1. Isolation Procedure of SDG from Lignan-Containing Extract of *Linum usitatissimum* Seeds

Purification step	Sample mass, g	Sample yield relative to initial extract, %	Sample purity, %
Initial extract	23.2	100.0	6.12
Chromatography over Diaion HP-20	4.0	17.2	30.0
Chromatography over Diaion HP-20	0.8	3.4	65.0
Chromatography over reversed-phase silica gel C_{18}	0.24	1.03	95.3

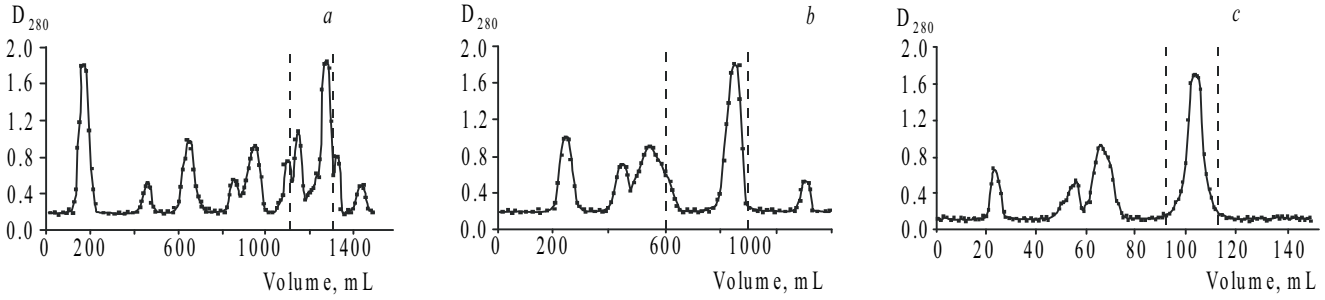

Fig. 1. Elution profiles of lignan-containing extract over Diaion HP-20 sorbent (a, b) and silica gel C_{18} (c). Dashed lines define bands corresponding to SDG-containing fractions.

A group of peaks (Fig. 1a and b) and a peak (Fig. 1c) corresponding to SDG-containing fractions that were concentrated and analyzed by HPLC can be seen in each of the elution profiles. Table 1 lists data for the yields and purities at each stage of SDG separation from the lignan-containing extract.

Chromatography over Diaion HP-20 could separate a rather large amount of extract, separate salts and polymers from the glucosides, and produce a sufficient yield of SDG-enriched fraction for its subsequent final purification over reversed-phase silica gel C_{18}.

Thus, an effective method for producing SDG purified of lignan from extract of flax seeds was developed. It includes preparative double separation of lignan-containing extract over ion-exchange sorbent Diaion HP-20 and final purification over reversed-phase silica gel C_{18}. The proposed method made it possible to isolate lignan and SDG with a purity of 95.3% in 1.03% yield relative to the initial extract.

EXPERIMENTAL

PMR spectrum of isolated SDG in D_2O was obtained on a Bruker Avance instrument (operating frequency 400 MHz) with CH_3CN internal standard (CH_3CN δ 2.06 ppm). The IR spectrum was recorded on a Nexus spectrometer (Thermo Nicolet).

Column chromatography was used to separate the lignan-containing fraction. Optical density of fractions eluted from the columns was measured at 280 nm on an SF-26 spectrophotometer. TLC monitoring was carried out on Kiselgel 60 F254 plates (Merck, USA) using water:isopropanol:aqueous ammonia (1:8:1) with detection using transillumination with radiation maximized at 254 nm.

The composition of SDG-containing fractions was analyzed by HPLC using a Waters Micromass ZQ 2000 GC—MS (Water, USA) and a BDS Hypersil C_{18} (250 × 4.6 mm) column. The elution was performed with a linear gradient using CH_3CN (solution A) and water with formic acid (0.1%) (solution B) (A:B; 0-5 min, 30:70; 20-30 min, 70:30; 50-65 min, 100:0) with 0.7 mL/min flow rate and detection by a diode-matrix detector at 280 nm and mass spectrometry with electrospray ionization.

Dried seeds of *L. usitatissimum* (120 g, Gold Flax) supplied by the Central Botanical Garden of the Belarus NAS were ground and defatted with hexane. The resulting solid (100 g) was treated with aqueous ethanol (50%, 2000 mL) and aqueous NaOH solution (80 mL, 4 M). The suspension was irradiated with microwaves (150 W) for 2 min six times with 1 min between irradiations. The extract was separated from the extracted solid and acidified with HCl (6 M) until the pH was 3-4. Evaporation of the aqueous ethanol extract in vacuo at reduced pressure in a rotary evaporator gave dry solid (23.2 g).

The concentrate was dissolved in water and separated by chromatography over a column (46 × 2.5 cm) of ion-exchanger Diaion HP-20 with elution by a stepwise gradient of aqueous ethanol (10, 15, 20, 40%) at flow rate 5 mL/min. SDG was eluted

by ethanol (40%), collected, concentrated, and rechromatographed. The separation was repeated over a column (40 × 1.15 cm) packed with the same ion-exchanger using an analogous stepwise gradient. The purest fractions containing SDG were eluted by ethanol (20%), collected, concentrated, and purified finally over a column (45 × 2 cm) of reversed-phase silica gel C_{18} with elution by a stepwise gradient of aqueous ethanol (10, 25, 50%) at flow rate 0.7 mL/min. Purified SDG was eluted from the column by aqueous ethanol (25%).

Secoisolariciresinol Diglucoside. $C_{32}H_{46}O_{16}$, colorless amorphous compound. (+)-Mass spectrum (ESI, *m*/*z*, I_{rel}, %): 345.77 (100) $[M - 2Glu + H_2O + H]^+$, 363.79 (92.9) $[M - 2Glu + 2H_2O + H]^+$, 327.77 (80.6) $[M - 2Glu + H]^+$, 704.83 (40.6) $[M + H_2O + H]^+$, 525.81 (32.3) $[M - Glu + H_2O + H]^+$, 687.84 (23.2) $[M + H]^+$, 709.78 (22.9) $[M + Na]^+$.

(–)-Mass spectrum (ESI, *m*/*z*, I_{rel}, %): 731.77 (100) $[M + 2Na - H]^-$, 721.85 (24.5) $[M + 2H_2O - H]^-$, 685.82 (9.8) $[M - H]^-$.

IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 2923, 2880, 2846 (CH_3–O–Ar), 1604, 1517, 1452, 1430, 1370 (C_{Ar}–O), 1274, 1155, 1125, 1075 (C–O–C), 1029, 885 (Ar), 817 (Ar), 798, 635, 563.

PMR spectrum (400 MHz, D_2O, δ, ppm, J/Hz): 1.99 (2H, m, H-2), 2.40 (2H, dd, J = 13.7, 11.2, H-1′a), 2.81 (2H, dd, J = 13.7, 11.2, H-1′b), 3.25-3.53 (8H, m, H-2‴, H-3‴, H-4‴, H-5‴), 3.55 (2H, dd, J = 10.2, 9.3, H-1a), 3.65 (6H, s, OCH_3), 3.72 (2H, dd, J = 11.8, 2.1, H-6‴a), 3.76 (2H, dd, J = 11.8, 4.2, H-6‴b), 4.08 (2H, dd, J = 10.2, 2.5, H-1b), 4.35 (2H, d, J = 7.8, H-1‴), 6.49 (2H, s, H-2″), 6.61 (2H, d, J = 7.8, H-6″), 6.78 (2H, d, J = 7.8, H-5″). The PMR spectrum agreed with that reported in the literature [4].

HPLC: retention time t_R = 16.09 min; product purity, 95.3%.

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