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Short communication

Isolation of the lignan secoisolariciresinol diglucoside from flaxseed (*Linum usitatissimum* L.) by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography was successfully used for the isolation and purification of secoisolariciresinol diglucoside, a bioactive lignan from flaxseed (*Linum usitatissimum* L.). The solvent system consisted of *tert*.-butylmethyl ether–*n*-butanol–acetonitrile–water (1:3:1:5). The purity and identity of the isolated compound was checked by high-performance liquid chromatography analysis in combination with mass spectrometry and NMR measurements.

Keywords: *Linum usitatissimum*; Counter-current chromatography; Plant materials; Secoisolariciresinol diglucoside; Glucosides; Lignans; Phytoestrogens; Plant hormones

1. Introduction

Flaxseed is rich in α-linolenic acid and soluble and insoluble fibre and is therefore of considerable nutritional value [1]. Putative anticarcinogenic, estrogenic and antiestrogenic effects of flaxseed consumption have been shown to be due to the presence of so-called phytoestrogens [2,3]. In detail, lignan-type phytoestrogens have been detected in flaxseed with the main component being secoisolariciresinol diglucoside (SDG) [4,5]. Also present are a number of other lignans, i.e., matairesinol, isolariciresinol and pinoresinol [5].

Lignans are wide spread phenolic compounds in a variety of foods, particularly whole grains and legumes [2]. Recently, lignans were even detected in tea and coffee [6]. Secoisolariciresinol is the precursor of the major mammalian lignans enterodiol and enterolactone, which are found in the urine and faeces of humans after flaxseed intake [7,8]. Lignans, especially the mammalian lignans enterodiol and enterolactone, have been shown to exert anticarcinogenic properties. Moreover, in epidemiological studies, they have been associated with a reduced cancer risk [2,3]. Flaxseed supplementation has shown positive effects on the early markers of colon carcinogenesis [9], on the initial and promotional stages of mammary tumorigenesis [10,11] and could represent a potential treatment for renal disorders [12]. Whole flaxseed consumption also lowered serum low-density lipoprotein (LDL)-cholesterol concentrations in postmenopausal women [13]. As

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regards to the metabolism, recent studies have identified oxidative metabolites of the mammalian lignans [14,15] and additional precursors [16].

Conventional chromatographic methods use solid stationary phases. In contrast, high-speed counter-current chromatography (HSCCC), as an all-liquid chromatographic technique, avoids adsorption losses and the formation of artifacts due to the lack of active surfaces [17,18]. A great choice of solvent systems is readily available which renders the technique very versatile. In the present communication, the application of HSCCC to the isolation of SDG using a hydrophilic solvent system is described.

2. Experimental

2.1. Extraction of SDG and clean-up prior to separation

Flaxseed (ca. 200 g) was ground and defatted at room temperature in 400 ml of *n*-hexane. The defatted flaxseed was extracted at ambient temperature with 600 ml of methanol–water (70:30, v/v) over 2 h. The extract was filtered over a Buchner filter and evaporated to dryness in vacuo and freeze-dried to yield 9.8 g of a brown powder. The lyophilisate was dissolved and hydrolyzed at room temperature with 200 ml of 1 *M* NaOH over 3 h [4]. The solution was carefully neutralized with diluted hydrochloric acid and applied to an Amberlite XAD-2 column (70×4 cm). After rinsing with ca. 2 l of water, elution of a lignan-enriched fraction was carried out with 500 ml of methanol. The eluate was evaporated in vacuo and freeze-dried prior to HSCCC separation. The yield was 1300 mg of freeze-dried XAD-2 extract starting from 200 g of flaxseed. The isolation protocol is schematically depicted in Fig. 2.

2.2. HSCCC

A high-speed counter-current chromatograph manufactured by Pharma-Tech Research (Baltimore, MD, USA) was equipped with three coils (2.6 mm internal diameter of the tubing) to give a final volume of the liquid phase of 850 ml. The separations were run at a revolution speed of 1000 rpm.

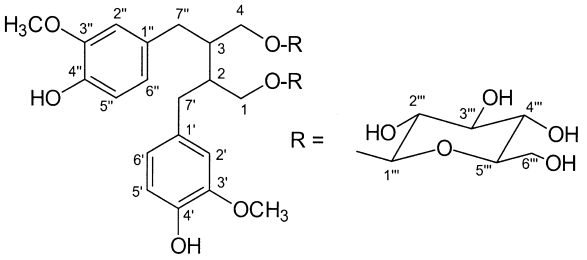

Fig. 1. Structure of secoisolariciresinol diglucoside (SDG).

The mobile phase was delivered by a Biotronik HPLC pump BT 3020 (Jasco, Groß-Umstadt, Germany). The sample (lyophilisate of XAD-2 extract from flaxseed) was dissolved in 20 ml of an 1:1 mixture of the light and heavy phases. The amount of sample injected was 800 mg. Elution was monitored with a Knauer UV–Vis detector (Berlin, Germany) at 280 nm. The solvent system used for the separation of SDG consisted of *tert*.-butylmethyl ether(TBME)–*n*-butanol–acetonitrile–water(1:3:1:5; the less dense layer as the stationary phase, flow-rate: 3.0 ml/min, head-to-tail mode; all chemicals were analytical-grade and obtained from local suppliers).

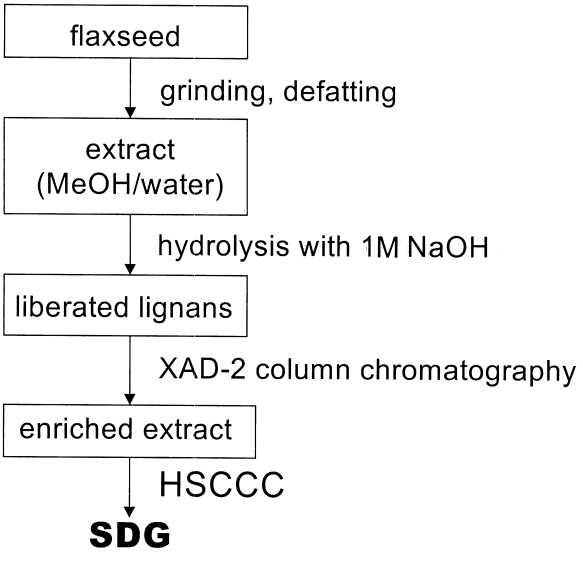

Fig. 2. Work-up protocol for the isolation of SDG.

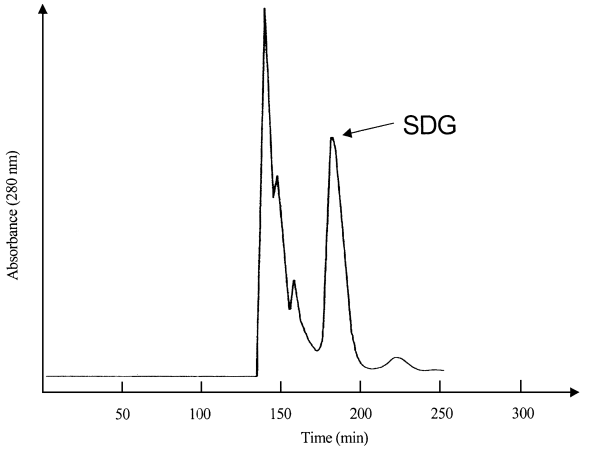

Fig. 3. HSCCC separation of SDG from the XAD-2 extract of flaxseed. Solvent system: *tert*.-butylmethyl ether–*n*-butanol–acetonitrile–water (1:3:1:5; flow-rate: 3.0 ml/min; the less dense layer as the stationary phase; retention of stationary phase: 58%).

2.3. HPLC analysis

A Jasco ternary gradient unit LG-980-02, with degasser and a MD-910 multiwavelength detector driven by Borwin chromatography software was used. The peak detection was carried out at 280 nm. The chromatographic separation was performed on a Hypersil RP_{18} column (250×4.6 mm) from Phenomenex (Aschaffenburg, Germany) at ambient temperature. The mobile phase was a linear gradient of 2% aqueous acetic acid (solvent A) and acetonitrile (solvent B). Conditions: initial A–B (95:5); in 45 min A–B (50:50); back to initial conditions; flow-rate: 0.9 ml/min.

2.4. Proton magnetic resonance spectroscopy (^{1}H NMR)

The experiments were performed on a Bruker

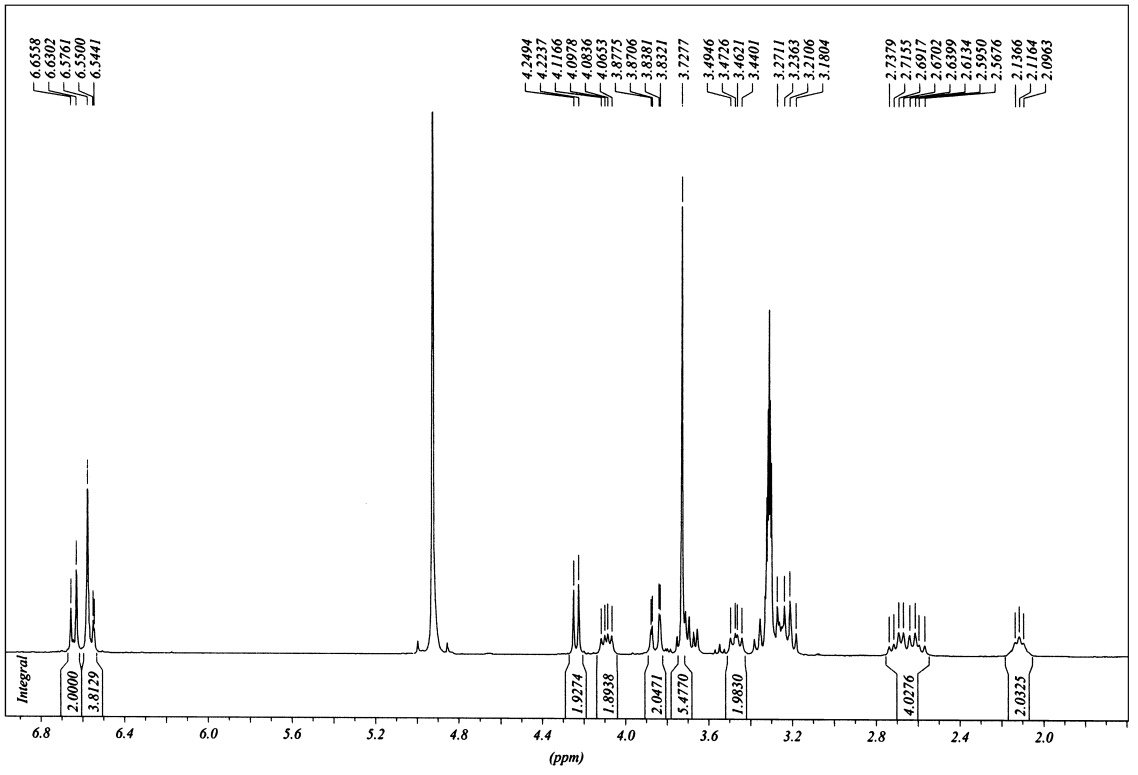

Fig. 4. ^{1}H NMR (300 MHz, $C^2H_3O^2H$) spectrum of isolated SDG.

AMX 300 spectrometer. ^{1}H NMR (300 MHz, $C^2H_3O^2H$, ppm): δ 2.12 (2H, m, H2/H3); 2.60 (2H, dd, J=14.0, 8.0 Hz, H7′a/H7″a); 2.70 (2H, dd, J=14.0, 6.5 Hz, H7′b/H7″b); 3.19 (2H, dd, J=9.0, 8.0 Hz, H2‴); 3.25 (2H, m, H5‴); other sugar resonances obscured by solvent signal; 3.47 (2H, m, H1/H4); 3.72 (6H, s, 2×OCH_3); 3.85 (2H, dd, J=12.0, 2.0 Hz, H6‴a); 4.08 (2H, dd, J=12.0, 6.0 Hz, H6‴b); 4.24 (2H, d, J=8.0 Hz, H1‴); 6.56 (2H, dd, J=8.0, 2.0 Hz, H6′/H6″); 6.58 (2H, d, J=2.0 Hz, H2′/H2″); 6.64 (2H, d, J=8.0 Hz, H5′/H5″).

3. Results and discussion

Flaxseed contains as much as 5.5 g/kg of lignans and represents an excellent source for the preparative isolation of SDG (for structure cf. Fig. 1) [2,4]. In the work-up process an hydrolysis step was carried out in order to liberate the lignans from their complex forms [4]. The yield of SDG without alkaline hydrolysis was found to be much lower, therefore treatment with sodium hydroxide solution is essential to enhance the yield of SDG (Fig. 2).

Fig. 3 shows the preparative HSCCC separation of 800 mg of an XAD-2 extract from flaxseed to yield 280 mg of the lignan SDG in one HSCCC run. In the search for a suitable solvent system, we found the addition of *n*-butanol necessary for optimum partitioning of the highly polar diglucoside in the organic stationary phase.

The identity of SDG was verified by electrospray ionization-MS and ^{1}H NMR (pseudomolecular ion at m/z 685=[M(686)–H]$^-$; MS–MS of 685: m/z 523 [M(686)–H-glucosyl]$^-$, m/z 361 [M(686)–H-2× glucosyl]$^-$. The purity, as determined by HPLC using peak areas at a detection wavelength of 280 nm, was above 93%. The ^{1}H NMR spectrum of SDG proving the purity of the isolated compound is shown in Fig. 4.

The SDG standard will be used for bioavailability and metabolic studies which, in the past, were often carried out with whole flaxseed, crude extracts or aglycones, but not with purified genuine SDG.

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