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SYNTHESIS AND ANTIESTROGENIC EFFECT OF SECOISOLARICIRESINOL DIGLUCOSIDE DERIVATIVES

Secoisolariciresinol diglucoside (SDG) is an important dietary lignan that is found at very high levels in flaxseed (*Linum usitatissimum*). Lignans are the polyphenolic compounds derived from the combination of two phenylpropanoid units. Flaxseed lignans have received much research interest in recent years because of reported phytoestrogenic and anticarcinogenic effects. The mechanism of action of these compounds is not well understood but may involve influence of different activities.

The evaluation of antiestrogenic effect of structural analogs of natural lignan SDG against MCF-7 breast cancer cells can be useful for understanding the biochemical basis of anticarcinogenic effect of lignans.

Synthesis of SDG derivatives and evaluation of their antiestrogenic effect against MCF-7 breast cancer cell line was the goal of the research work.

Firstly, the natural lignan SDG was isolated from flaxseed. The process of isolation of SDG from flaxseed was in detail described in our earlier work [1]. In generally, it includes the extraction SDG from defatted flaxseed with 50% aqueous ethanol and purification by column chromatography using Diaion HP-20 ion-exchange sorbent and C18 reversed-phase silica gel.

The transformation of SDG **1** (fig.1) into secoisolariciresinol (**2**) was carried out by means of acid hydrolysis. Aqueous solution of SDG (8 ml, 0,15 mmol) was hydrolyzed with hydrochloric acid (4 ml, 6M) at 98°C in water bath for 4 h. The received mixture was allowed to stay during 24 h at room temperature. The secoisolariciresinol was crystallized and washed with ice water to give 0,027 g ((51%) yield) of off-white powder with melting point 109,7–110,2 °C.

The synthesis of diacylated secoisolariciresinol by phenolic hydroxyls (**3**) was effected by the reaction with excess of acetyl chloride. The secoisolariciresinol (0,1005 g, 0,15 mmol) was added to a solution of acetyl chloride (0,9 ml, 12,7 mmol).

The reaction mixture was stirred at room temperature for 12 h. The suspension was concentrated under reduced pressure and was purified by preparative TLC Silica Gel G (Analtech Uniplate^{TM}, USA) with ethyl ace-

tate/petroleum ether (2:3 v/v). The fraction corresponding to secoisolariciresinol-4′,4″-diacetate was cut and extracted with chloroform after filtered and concentrated to give 0,042 g (63 % yield) of glassy solid.

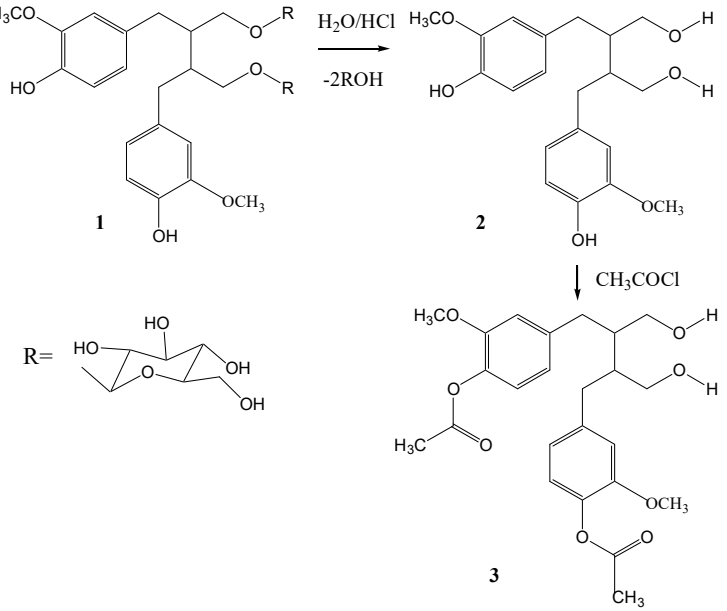

Fig. 1 Synthesis of SDG (1) derivatives 2 and 3

The physicochemical properties and purity of the compounds were assessed by TLC, analytical HPLC and ^{1}H, ^{13}C NMR, mass, IR spectral data.

The IR spectra of **1–3** showed the presence of hydroxyl groups (3350–3437cm^{-1}), aromatic ring (801–903 cm^{-1}) connected with methoxy group (2846–2851 cm^{-1}). The IR spectra of secoisolariciresinol-4′,4″-diacetate indicated the presence of carbonyl group of ester (1764 cm^{-1}). The ^{1}H NMR spectra of **1–3** compounds indicated the presence of threesubstituted aromatic ring at δ 6,49–6,92 with methoxy group at δ 3,65–3,80. In addition, signals of the aliphatic aglycone moiety were observed for two sets of methylene protons at δ 2,40–2,58 and 2,57–2,81; 3,53–3,55 and 3,92–4,08 suggesting the presence of benzylic and –CH_2-O-R groups, respectively. From value of the coupling constant of anomeric proton signal at δ 4,35 (2H, d, J=7,8 Hz), compound **1** was deduced to be composed of β-glucopyranoside. There was a signal at δ 5,73 (2H, s) corresponding to phenolic hydroxyls in compound **2**. The acetyl group signal of the compound **3** at 2,30 (6H, s) was correlated with diacylated derivative only by phenolic hydroxyls.

Estrogen receptor alpha (ERα) is expressed in about 70% of breast cancers. This makes hormone therapy possible in most breast cancer patients. Considering that ERα is expressed at a high level in MCF-7 cells and is a key proliferation driver, we have analyzed the effects of **2** and **3** on the

activity of this factor. For ERE-Luc assay, cells are transfected with the plasmids containing the luciferase reporter gene controlled by the promoter with estrogen responsive elements (ERE-Luc). Cells were treated with a physiological receptor ligand, 17β-estradiol, and lignan derivatives. Figure 2 shows that **2** and **3** inhibit the 17β-estradiol-induced activity of ERE-Luc.

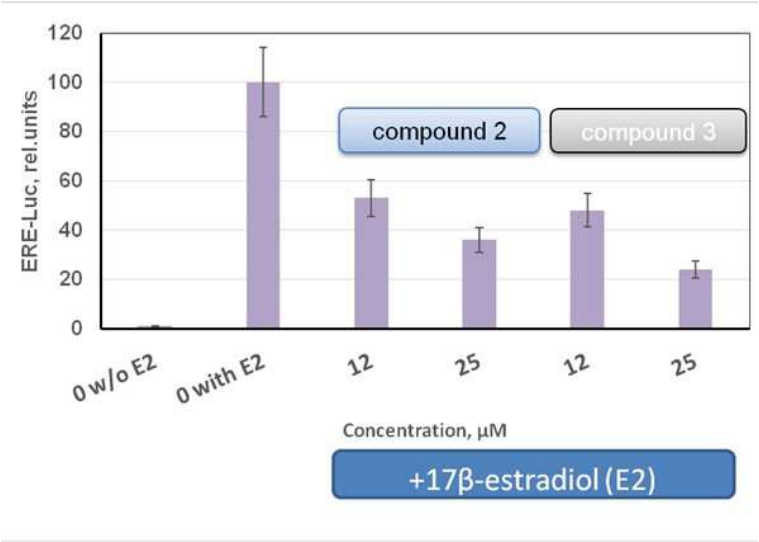

Fig. 2 Evaluation of ERα (ERE-Luc) activity in MCF-7 cells after treatment with compounds 2 and 3 (ERE-Luc – the luciferase controlled by the promoter with estrogen responsive elements, the luciferase was activated by 10 nM 17β-estradiol (E2))

Thus, the SDG derivatives **2** and **3** showed antiestrogenic potency. Natural lignan **1** did not affect the activity of Erα and it could be explained by the presence of glucose moiety that makes the molecule SDG more hydrophilic, so, this factor influenced greatly on the penetration of this molecule through the lipophilic membrane of the cells. The explanation of thy effect of secoisolariciresinol and secoisolariciresinol-4′,4″-diacetate arises from their structural similarity with estrogen. The derivatives of SDG can be considered for further development, including for new approaches in anticancer therapy.

LITERATURE

1. Stasevich O.V. Isolation of secoisolariciresinol diglucoside from lignan-containing extract of Linum usitatissimum seeds / Mikhalenok S.G., Kurchenko V.P. // Chem. Nat. Compd. – 2009. – Vol. 45. – P. 21–23.