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Phytosterols and Lignans from the Sesame Dregs of *Sesamum indicum*

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Abstract - Phytochemical investigation of the sesame dregs of *Sesamum indicum* was conducted by open column and prep-HPLC chromatography. Two phytosterols (**1** and **2**) and two lignans (**3** and **4**) were isolated from the MeOH extracts of sesame dregs, and identified as β-sitosterol (**1**), daucosterol (**2**), sesamin (**3**), and sesamolin (**4**) by spectral analysis. Although these compounds were already isolated from sesame, it is important that they were still main phytochemical components in the sesame dregs.

Key words - Daucosterol, Sesame dregs, Sesamin, Sesamolin, *Sesamum indicum*, β-Sitosterol

Introduction

Sesame, one of the most important crops throughout the world, is cultivated in Korea, Japan, China, and other East Asian countries. Likewise sesame seeds have been utilized for a long time as edible oilseeds and food materials (Heywood, 1991). There are many phytochemical studies on sesame seeds which have been noted with lignans (Bedigian *et al*., 1985; Osawa *et al*., 1985), lignan glucosides (Katsuzaki *et al*., 1994; Moazzami *et al*., 2006) and glucoses (Hatanaka, 1959; Suzuki *et al*., 1993). Naphthoquinones (Ogasawara *et al*., 1993; Hasan *et al*., 2000, 2001) and anthraquinones (Ogasawara *et al*., 1993; Furumoto *et al*., 2003, 2006) were also isolated from the roots and hairy root cultures of *S. indicum*. Also it was reported that contents of lignan type compounds in the sesame seeds and oils were analyzed by HPLC (Fukuda *et al*., 1986; Kim *et al*., 2006; Moazzami *et al*., 2006).

Sesame seeds are used as protection for weakened liver and improve/maintain kidney function in traditional medicine. Aside from this medical function, a dose of sesame seed extract administered to a rat was found to help lower the level of blood sugar, and increase the liver level and glycogen content in the muscles. However, if used in excess, the level of glycogen content in the muscles will be reduced (Park and Sung, 2007). The extracts from *S. indicum* and seed oil were attributed mainly to the existence of the lignan type compounds that have various biological activities such as antioxidant properties (Fukuda *et al*., 1985; Osawa *et al*., 1985) and synergistic effects with pyrethrum insecticides (Haller *et al*., 1942; Casida, 1970).

In the process, many of the sesame dregs used in the food industry was left after extracting the oil from sesame seeds, and they are important agricultural waste. Generally, sesame dregs have been used as a feedstuff as well as a fertilizer. Yoo *et al*. (2004) measured the antioxidant activity of separation of brown substances from defatted sesame dregs. Not many researches, however, were reported to have performed on the subject of sesame dregs. In the course of searching for the new materials from important agricultural waste, phytochemical components from sesame dregs were isolated using open column and prep-HPLC chromatography, and were identified using spectral analysis.

Materials and Methods

Plant materials

White sesame (*Sesamum indicum* L.) was grown in Sinan, Jeonnam Province, 2005, and sesame dregs were obtained

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from OKCHUN Food Co. Ltd., Gyeonggi Province, 2006, Korea. It was a by-product after making sesame oil in this company.

General instruments

EI- and FAB-MS spectral data were measured with a JEOL JMS-600W and JMS-AX505WA (Japan) mass spectrometer. IR spectra were obtained on a SHIMADZU FT/IR-8400S instrument (Japan) on a KBr disc. NMR spectra were recorded with a Bruker AVANCE 300 NMR (Germany) spectrometer in $CDCl_3$ using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz. Evaporation was conducted by EYELA rotary evaporator system (Japan) under reflux *in vacuo*. Thin layer chromatography (TLC) was conducted with Kiesel gel 60 F_{254} (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10% H_2SO_4 in MeOH followed by heating at 100℃. Silica gel (200-400 mesh ASTM; Merck Co., Germany) was used for an open column chromatography.

Extraction and isolation

Sesame dregs were coarsely ground and maintained in an oven at 45℃. Sesame dregs (5.195 kg) were extracted several times with MeOH and MeOH : *n*-hexane (70 : 30, v/v) under reflux for 3 hr. After the removal of the solvent *in vacuo*, the extracts (496.6 g) were suspended in H_2O (45℃) and then partitioned in turn using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. Among them, the *n*-hexane soluble fraction was concentrated under reduced pressure to obtain the *n*-hexane fraction (285.3 g), which took the *n*-hexane fraction (5.6 g) out of the whole *n*-hexane fraction from sesame dregs of *S. indicum*. It was subjected to a silica gel open column chromatography (6 × 80 cm, No. 7734), with a gradient of *n*-hexane-EtOAc and EtOAc-MeOH to yield 21 subfractions. One of these, subfraction 2, was yielded on *n*-hexane : EtOAc (99 : 1) condition, and this subfraction using recrystallization led to the isolation of compound **1** (26.2 mg). The subfraction 7, obtained by 15% EtOAc in *n*-hexane, yielded compound **3** (132.4 mg). The subfraction 6 was separated by recycling preparative HPLC. The recycling process was performed

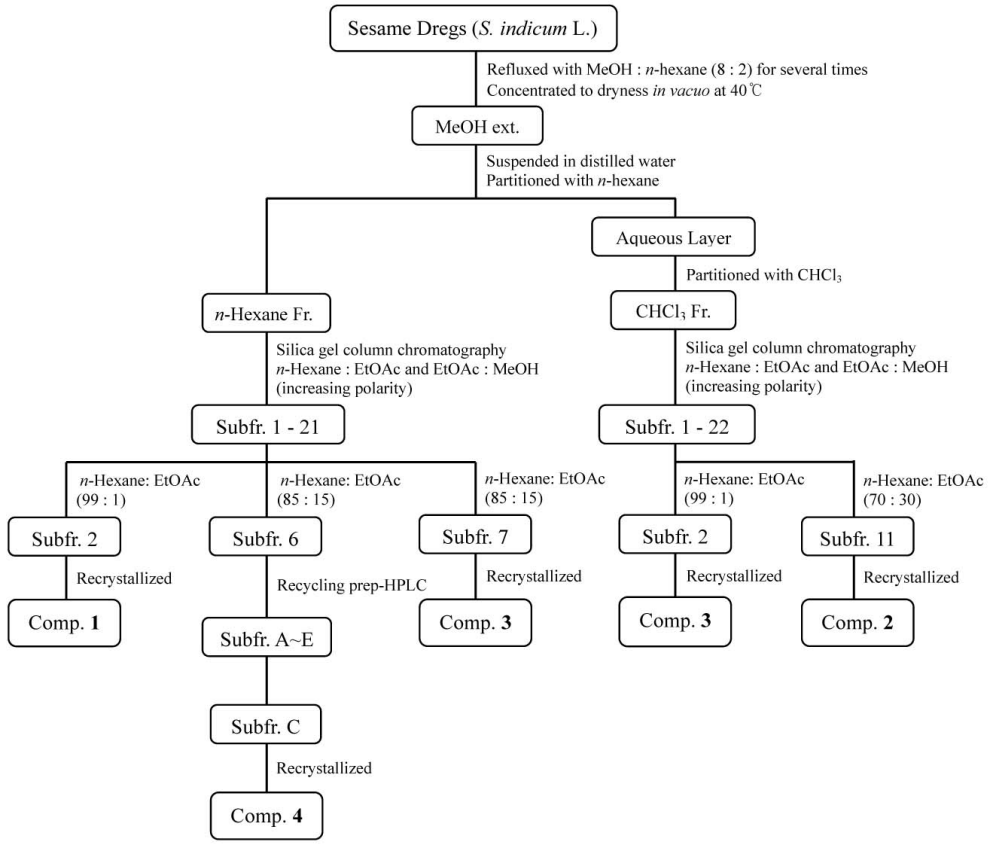


Fig. 1. Isolation scheme of sesame dregs.

three times and then the last peaks were classified into five sections: A ~ E. Among them, the C section was recrystallized to obtain compound **4** (7.8 mg). A portion of the chloroform fraction (11.3 g) was further purified on a silica gel column chromatography (6 × 80 cm, No. 7734). The subfrations 2 (*n*-hexane : EtOAc = 99 : 1) and 11 (*n*-hexane : EtOAc = 70 : 30) were recrystallized from MeOH to obtain compounds **3** (91.2 mg) and **2** (16.2 mg), respectively. The isolation scheme was shown at Fig. 1.

β-Sitosterol (1)

White crystals; IR (KBr): 3436 (OH), 1468 (C=C) cm^{-1}; EI-MS (rel. int., %): *m/z* 414 $[M]^+$ (44.8), 396 (60.3), 381 (22.6), 329 (14.3), 303 (19.5), 273 (13.8), 255 (35.5), 213 (34.9), 159 (30.2), 145 (43.8); ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): see Table 1.

Daucosterol (2)

White powders; IR (KBr): 3409 (OH), 1074 (C-O) cm^{-1}; FAB-MS: *m/z* 577 $[M + H]^+$; ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): see Table 1.

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compounds **1** and **2** in $CDCl_3$

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		37.4		36.8
2		29.8		29.3
3	3.52 (tt, 5.7, 9.0)	72.0	3.54 (tt, 8.0, 14.0)	78.7
4		39.9		38.3
5		141.1		140.4
6	5.35 (br d, 5.1)	122.2	5.34 (br d, 4.6)	121.2
7		32.0		31.4
8		31.8		31.3
9		50.3		49.6
10		36.6		36.2
11		21.2		20.6
12		40.7		40.1
13		42.4		41.8
14		56.9		56.2
15		24.4		23.9
16		28.4		27.8
17		56.2		55.1
18	0.70 (s)	11.9	0.65 (s)	11.7
19	1.00 (s)	19.1	0.98 (s)	19.1
20		36.3		35.5
21	0.92 (d, 6.3)	18.9	0.90 (d, 4.8)	18.6
22		34.1		33.3
23		26.2		25.4
24		46.0		45.1
25		29.3		28.7
26	0.84 (d, 6.3)	19.9	0.78 (d, 5.1)	18.9
27	0.87 (d, 6.6)	19.5	0.81 (d, 5.4)	19.7
28		23.2		22.6
29	0.79 (t, 6.0)	12.1	0.82 (t, 7.8)	11.8
1′			4.22 (d, 7.8)	100.8
2′				73.5
3′				76.9
4′				70.1
5′				76.7
6′				61.1

Sesamin (3)

Colorless crystals; IR (KBr): 1035 (C-O) cm^{-1}; EI-MS (rel. int., %): *m/z* 354 $[M]^+$ (74.1), 323 (9.6), 219 (5.1), 203 (35.6), 161 (49.7), 149 (100), 135 (65.6), 103 (16.5); ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): see Table 2.

Sesamolin (4)

Colorless crystals; IR (KBr): 1041 (C-O) cm^{-1}; EI-MS (rel. int., %): *m/z* 370 $[M]^+$ (10.3), 233 (10.4), 203 (14.1), 149 (8.4), 135 (100), 115 (5.3), 81 (8.4); ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): see Table 2.

Results and Discussion

Phytochemical investigation of sesame dregs was conducted by a repeated column chromatography. The *n*-hexane and chloroform fractions of the MeOH extracts from sesame dregs of *S. indicum* have led to the isolation of compounds **1** - **4** in the course of searching for the new materials from agricultural waste.

Compound **1** was isolated as white crystals from the *n*-hexane fraction of sesame dregs. In the EI-MS spectrum of **1**, molecular peak showed at *m/z* 414 and IR spectrum of **1** exhibited absorption bands for hydroxyl at 3436 cm^{-1}. In the ^{1}H-NMR spectrum of **1**, two angular methyl singlet two signals of 18- and 19-Me at δ 0.70 and 1.00, and the doublet of 21-, 26-, and 27-Me at δ 0.92, 0.84, and 0.87 were observed, respectively. The broad doublet at δ 5.35 showed H-6. The ^{13}C-NMR spectrum of **1** showed 27 resonances, and C-5 and -6 signals were noticed at δ 141 and 122, respectively. It was compared to NMR data in the literatures. Accordingly, the structure of **1** was elucidated as β-sitosterol by spectral data analysis with an authentic sample as described in the literature (Chang *et al*., 1981; Rubinstein *et al*., 1976; Umlauf *et al*., 2004). β-Sitosterol has an anti-inflammatory, anti-tumor, and anti-microbial activities (Park *et al*., 2001; Yuk *et al*., 2007). Also β-sitosterol inhibits the growth of several specific types of tumor cells *in vitro* and decreases the size and the extent of tumor metastases *in vivo* (Awad *et al*., 2007).

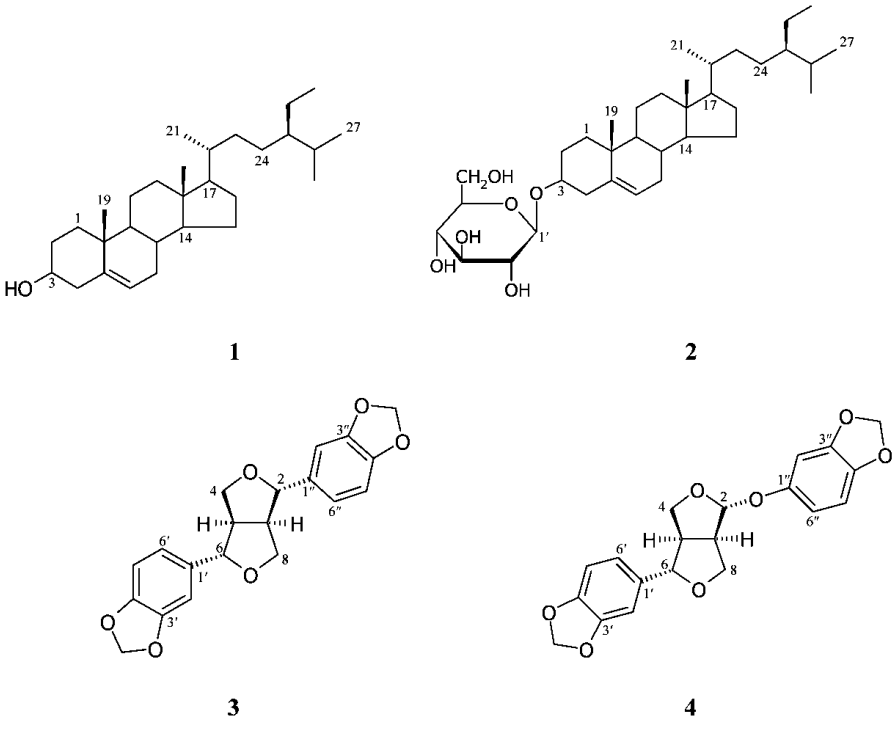
Table 2. ^{1}H- and ^{13}C-NMR spectral data for compounds **3** and **4** in $CDCl_3$

No.	**3**		**4**	
	δ_H	δ_C	δ_H	δ_C
1	3.05 (q, 6.3)	54.5	2.95 (q, 9.0)	53.4
2	4.72 (d, 4.5)	86.0	5.50 (s)	107.1
4eq	4.24 (dd, 6.9, 9.0)	71.9	4.13 (dd, 6.0, 9.3)	69.9
4ax	3.87 (dd, 3.8, 9.2)		3.96 (d, 9.3)	
5	3.05 (q, 6.3)	54.5	3.31 (q, 7.5)	52.9
6	4.72 (d, 4.5)	86.0	4.40 (d, 7.5)	87.2
8eq	4.24 (dd, 6.9, 9.0)	71.9	4.43 (t, 9.0)	71.4
8ax	3.87 (dd, 3.8, 9.2)		3.64 (dd, 7.5, 9.0)	
1′		135.3		134.6
2′	6.85 (d, 2.1)	106.7	6.88 (d, 1.7)	106.8
3′		148.3		148.4
4′		147.4		147.7
5′	6.78 (d, 7.7)	108.4	6.78 (d, 7.5)	108.4
6′	6.79 (dd, 2.1, 7.7)	119.6	6.83 (dd, 1.7, 7.5)	119.9
-OCH_2O-	5.92 (s)	101.3	5.92 (s)	101.5
1″		135.3		152.1
2″	6.85 (d, 2.1)	106.7	6.63 (d, 2.4)	100.4
3″		148.3		148.3
4″		147.4		143.0
5″	6.78 (d, 7.7)	108.4	6.71 (d, 8.4)	108.4
6″	6.79 (dd, 2.1, 7.7)	119.6	6.50 (dd, 2.4, 8.4)	109.2
-OCH_2O-	5.92 (s)	101.3	5.96 (s)	101.3

Compound **2** was isolated as white powder from the chloroform fraction of sesame dregs and IR spectrum of **2** showed absorption bands for hydroxyl at 3409 cm^{-1} and glycosidic C-O at 1074 and 1024 cm^{-1}. In the FAB-MS spectrum of **2**, $[M + H]^+$ peak showed at *m/z* 577 matching to the molecular formula $C_{35}H_{60}O_6$. The NMR spectra showed sterol moiety that was found to be similar to that of **1**. Comparing the NMR data of **2** with that of **1** exhibited replacement of a hydroxyl group of **1** by the sugar moiety. The broad doublet at δ 5.34 showed H-6 and the signals of δ 3.00 ~ 5.00 showed glycoside. Due to the chemical shift of C-3 of β-sitosterol changed from δ 72.0 to 78.7 and the anomeric proton of glucose showed at δ 4.22 (d, *J* = 7.8 Hz), glucose position was at C-3 (β-linkage) of aglycone. Accordingly, the structure of **2** was elucidated as daucosterol by spectral data analysis with an authentic sample as described in the literature (Chang *et al*., 1981). Daucosterol has an immunomodulating activity (Lee *et al*., 2007) and decreased vascular permeability (Sugiyama and Seki, 1991).

Compound **3** was recrystallized from MeOH and obtained as colorless crystal from the *n*-hexane and chloroform fractions of sesame dregs. The EI-MS spectrum of **3** showed at *m/z* 354 which correspond to the molecular formula of $C_{20}H_{18}O_6$. The IR spectrum in KBr exhibited absorption band for C-O at 1035 cm^{-1}. The ^{1}H-NMR spectrum of **3** exhibited aromatic protons signal, ABX splitting pattern, at δ 6.85 (d, *J* = 2.1 Hz), 6.79 (dd, *J* = 7.7, 2.1 Hz), and 6.78 (d, *J* = 7.7 Hz) appeared. Also the signal at δ 5.92 indicated the methylenedioxy signal in the structure. The ^{13}C-NMR spectrum of **3** showed 10 resonances, this means that **3** had symmetric structure as compared to the EI-MS data. The ^{13}C-NMR spectrum data showed methylenedioxy signal at δ 101. In Fig. 2, it is suggested that **3** is a lignan named as sesamin by EI-MS, ^{1}H- and ^{13}C-NMR spectral data analysis. This is confirmed with the results based on literatures (Mohamed and Awatif, 1998; Kim *et al*., 2006; Wu, 2007). The effect of this compound, a major lignans in sesame seed, decreased fatty acid synthesis in rat liver accompanying the down-regulation of sterol regulatory element binding protein-1 (Ide *et al*., 2001). Also it has multiple functions such as cholesterol-lowering and anti- hypertensive activities (Tsuruoka *et al*., 2005).

Compound **4** was isolated as colorless crystals from the *n*-hexane fraction of sesame dregs. The EI-MS spectrum of **4**



1 **2**

3 **4**

Fig. 2. Structures of compounds **1** – **4**.

exhibited a $[M]^+$ peak at *m/z* 370 and the IR spectrum of **4** in KBr exhibited the presence of C-O (1041 cm^{-1}) function. The ^{1}H-NMR spectrum of **4** showed quartet signals assigned to two methine protons at δ 2.95 and 3.31. Two doublets for one of each *o*- and *m*-coupled proton at δ 6.71 (*J* = 8.4 Hz) and 6.63 (*J* = 2.4 Hz), and double doublets at δ 6.50 (*J* = 8.4, 2.4 Hz) were obtained. Also these were ABX splitting proton signals. Same with the proton resonances at δ 6.88 (d, *J* = 1.7 Hz), 6.83 (dd, *J* = 7.5, 1.7 Hz), and 6.78 (d, *J* = 7.5 Hz). The δ 6.63 and 6.50 signals were long-range coupled with signal at δ 4.40 (d, *J* = 7.5 Hz). Compound **4** has the resemblance proton chemical shifts for the methylenedioxy signal (δ 5.96 and 5.92). The ^{13}C-NMR spectrum of **4** showed 20 resonances, and the signal of furofuran ring (δ 107.1) and the signal of piperonyl group (δ 152.1) were chemical shifted by +20~30 ppm to down field and thus, showed that ether (-O-) group have been existing between furofuran ring (δ 87.2) and piperonyl group (δ 134.6). From these data, it is suggested that **4** is a lignan, named sesamolin by spectral data analysis. It was compared with spectral data in the literature (Kang *et al*., 1995). This compound showed inhibitory effect on the microsomal system (Grafoorunissa and Rao, 2004).

Recent years have witnessed that development of processed foods using oil and fat has increased. Industrial by-products such as grape wastes of wine industry, olive oil residues, and soybean milk residues are important agricultural wastes. Nevertheless, the variety studies of these by-products have been published. The analyzable parameters were reported to estimate the solid by-products and residues from the winery (Bustamante *et al*., 2008), pigment production by *Monascus purpureus* growth using grape wastes of wine industry (Silveira *et al*., 2008), and compositions of soybean milk & olive oil residues (Yang, 2005; Sellami *et al*., 2008). Lots of sesame dregs left after extracting the oil from sesame seeds are important agricultural waste, food materials and new natural resources for the future. In the course of searching new constituents from sesame dregs of *S. indicum*, two phytosterols (**1**: β -sitosterol, and **2**: daucosterol) and two lignans (**3**: sesamin, and **4**: sesamolin) were isolated by a repeated column chromatography (Fig. 2). These compounds were reported for the first time from the sesame dregs of *S. indicum*.

Based on these results, we found out that the sesame dregs still contained phytochemical components, although they are byproducts of the manufacturing process. This shows the usefulness of the sesame dregs and the need for the development of new agricultural materials. In this regard, further studies should be focused on the industrial applications for the development of new natural products.

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