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Sesamolinol Glucoside, Disaminyl Ether, and Other Lignans from Sesame Seeds

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ABSTRACT: The application of a procedure based on XAD-4 adsorption resin permitted the obtainment of an enriched polyphenolic extract from *Sesamum indicum* seeds. Chemical analysis of the obtained extract led to the identification of 12 lignans. Among them, 2 lignans, (+)-sesamolinol-4′-*O*-*β*-D-glucoside and disaminyl ether, are reported for the first time as natural compounds. Their structure has been determined by spectroscopic methods, mainly by the application of one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) techniques [heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and nuclear Overhauser effect spectrometry (NOESY)] and mass spectroscopy. The isolated compounds were evaluated for their antimutagenic activity. Among the tested lignans, the most active lignan was found to be sesamolin, followed by sesamolinol and samin, against H_2O_2. Additionally, some of the tested lignans showed desmutagenic activity against benzo[*a*]pyrene (BaP).

KEYWORDS: *Sesamum indicum, lignans, (+)-sesamolinol-4′-O-β-D-glucoside, XAD-4 resin adsorption, antimutagenic activity*

INTRODUCTION

Sesame (*Sesamum indicum*, Pedaliaceae), one of the most important oilseed crops, with a worldwide production reaching 3.3 megatons annually,[1] is a rich source of lignans, such as sesamine, sesamolin, and sesaminol.[2] Sesame seeds, sesame oil, and their constituents possess well-identified antioxidant activities,[2−5] as well as protective activity against DNA damage.[6] In a previous work,[7] we reported the promising antimutagenic properties of the methanolic extract of the sesame coat, broadening the potential health protective effects of sesame-based diet products.

The discovery of naturally occurring chemical compounds present in the human diet with antimutagenic and anticarcinogenic potency is of great importance because of the unwanted consequences of an increased rate of mutations and the related elevated risk of cancer development. The previously reported antimutagenic activity of the sesame coat prompted us to extend our study to the constituents of the dehulled sesame seed, which is the mainly used edible part of sesame. To investigate the ability of the dehulled sesame seed lignans to protect against mutations, we studied a polyphenol-enriched extract of sesame seeds using a methodology based on XAD-4 resin adsorption, which had been previously successfully applied during our work on the sesame coat.[8] The applied methodology led to the isolation of one new lignan, one lignan reported for the first time from a natural source, and several known lignans and permitted their preliminary evaluation as antimutagenic agents.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. The infrared (IR) spectra were obtained on a Perkin-Elmer Paragon 500 instrument. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers [^{1}H (400 and 200 MHz) and ^{13}C (50 MHz)]. The ^{1}H−^{1}H and ^{1}H−^{13}C NMR experiments were performed using standard Bruker microprograms. Electrospray mass spectra were recorded with a Q-TOF1 Micromass apparatus equipped with a ESI-Z spray source [electrospray ionization mass spectrometry (ESI−MS); V_c = 30 V]. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing Si gel 60 Merck (20−40 μm). Thin layer chromatography (TLC) was performed on plates coated with Si gel 60 F_{254} Merck, 0.25 mm. Resin adsorption experiments were performed using XAD-4 (Rohm and Haas). Fast centrifugal partition chromatography (FCPC) were performed on a Kromaton instrument using a 200 mL capacity rotor.

Plant Material. Dehulled sesame seeds were purchased from Haitoglou SA (Thessaloniki, Greece).

Extraction and Isolation. Ground seeds of *S. indicum* L. (4 kg) were exhaustively extracted at room temperature with solvents of increasing polarity: cyclohexane (3 × 4 L), CH_2Cl_2 (3 × 4 L), and MeOH (3 × 4 L). The dried methanol extract (60.0 g) was diluted with water (5 L) and filtered. The filtrate was passed through a column containing 0.3 kg of XAD-4 resin. The resin was rinsed with water (2 L). Elution with MeOH (3 L), followed by evaporation under reduced pressure, afforded an enriched polyphenolic extract (6.22 g).

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FCPC performed on the enriched polyphenolic extract permitted the isolation of 10 known lignans, with 1 lignan isolated for the first time from a natural source, and a new lignan. The known compounds were identified on the basis of their physical and spectral data and compared to literature values. The structures of the new compounds were established using NMR spectroscopy and mass spectrometry (MS).

A portion of the enriched polyphenolic extract (4.00 g of 6.22 g) was divided into 3 approximately equal parts. Each part was submitted to FCPC using a C_6H_{12}/EtOAc/MeOH/H_2O (2:3:2:3) biphasic solvent system, beginning with the organic phase as the mobile phase. The dual mode was activated after collection of 1 L of mobile phase into 50 tubes of 20 mL each. Then, another 1 L of the new mobile phase, the aqueous phase, was collected into another 50 tubes of 20 mL. The tubes were pooled on the basis of TLC data analysis (migration solvent 95:5 CH_2Cl_2/MeOH) to afford 20 fractions. Fraction 2 (419 mg) was rechromatographed on silica gel 60 Merck (40−65 μm) with cyclohexane/acetone (from 100:0 to 50:50 gradient), to afford successively (+)-disaminyl ether (**1**)[9] (28 mg), (+)-sesamin[10] (170 mg), and (+)-sesamolin[11] (19 mg). Fraction 3 (296 mg) was rechromatographed on silica gel 60 Merck (40−65 μm) with cyclohexane/acetone (from 100:0 to 50:50 gradient), to afford (+)-piperitol[12] (22 mg) and (+)-sesaminol[13] (145 mg). Fraction 4 afforded (+)-sesamolinol[14] (23 mg). Fraction 5 afforded (+)-episesaminone[9] (41 mg). Fraction 6 afforded (+)-pinoresinol (179 mg).[15] Fraction 7 afforded (+)-samine[16] (18 mg). Fractions 12 and 13 were pooled and submitted to a new FCPC using a C_6H_{12}/EtOAc/MeOH/H_2O (1:9:1:9) biphasic solvent system, leading to the isolation of (+)-sesaminol-2-*O*-β-D-glucopyranoside,[17] (+)-sesamolinol-4′-*O*-β-D-glucoside (**2**) (34 mg), and (+)-sesaminol-2′-*O*-β-D-glucopyranosyl(1 → 2)-*O*-β-D-glucopyranoside[17] (97 mg) (Figure 1).

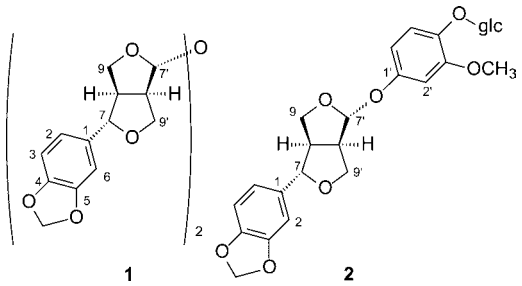

Figure 1. Structures of compounds **1** and **2**.

(+)-Sesamolinol-4′-*O*-β-D-glucoside (2). White amorphous solid, $[\alpha]_D$ +89° (CH_3OH, *c* 0.2). ^{1}H NMR (CD_3OD) δ: 2.96 (1H, m, H-8), 3.32 (1H, overlapped, H-5″), 3.34 (1H, overlapped, H-8′), 3.34 (1H, overlapped, H-4″), 3.46 (1H, overlapped, H-2″), 3.48 (1H, overlapped, H-3″), 3.63 (1H, dd, *J* = 9 and 7.5 Hz, H-9′a), 3.69 (1H, dd, *J* = 11.5 and 4.5 Hz, H-6″a), 3.83 (3H, s, O−CH_3), 3.87 (1H, dd, *J* = 11.5 and 1.5 Hz, H-6″b), 3.98 (1H, dd, *J* = 9 and 1.5 Hz, H-9a), 4.10 (1H, dd, *J* = 9 and 6 Hz, H-9b), 4.41 (1H, overlapped, H-9′b), 4.41 (1H, d, *J* = 8 Hz, H-7), 4.77 (1H, d, *J* = 7 Hz, H-1″), 5.65 (1H, br s, H-7′), 5.94 (2H, s, O−CH_2−O), 6.62 (1H, dd, *J* = 8.5 and 3 Hz, H-6′), 6.72 (1H, d, *J* = 3 Hz, H-2′), 6.79 (1H, d, *J* = 8 Hz, H-5), 6.86 (1H, dd, *J* = 8 and 2 Hz, H-6), 6.90 (1H, d, *J* = 2 Hz, H-2), 7.10 (1H, d, *J* = 8.5 Hz, H-5′). ^{13}C NMR (CD_3OD) δ: 52.8 (C-8), 53.3 (C-8′), 55.6 (O−CH_3), 61.2 (C-6″), 69.6 (C-9), 70.6 (C-4″), 72.0 (C-9′), 77.2 (C-5″), 77.2 (C-3″), 74.3 (C2″), 87.9 (C-7), 102.1 (O−CH_2−O), 103.4 (C-2′), 103.4 (C-1″), 107.1 (C-2), 107.2 (C-7′), 109.0 (C-5), 109.0 (C-6′), 119.3 (C-5′), 120.8 (C-6), 135.1 (C-1), 142.4 (C-4′), 148.0 (C-4), 148.8 (C-3), 151.3 (C-3′), 153.2 (C-1′). High-resolution (HR)-ESI−MS: *m*/*z* 535.1821 $[M + H]^+$, $[C_{26}H_{30}O_{12} + H]^+$; calculated 535.1816.

Ames' Test. *Preparation of Bacterial Assays.* *Salmonella typhimurium* TA100 as well as rat liver S9 mix were purchased from Moltox, Inc., Boone, NC. The strain was checked routinely for ampicillin resistance, ultraviolet sensitivity, and spontaneous revertants.

Antimutagenicity Test. The antimutagenicity test was performed according to the method by Ames et al.[18] and as modified by Lazarou et al.[7] More specifically, 0.1 mL of dimethylsulfoxide (DMSO) or 0.1 mL of tested compound solution in DMSO (containing 50 μg of tested compound), 0.1 mL of bacterial suspension, and 0.1 mL of H_2O_2 were added to molten top agar (2 mL final volume). The reagent mix was briefly shaken by hand and poured onto a GM agar plate. The plates were incubated at 37 °C for 3 days, and then the number of revertant colonies was counted.

Desmutagenic Test against Activated Benzo[a]pyrene (BaP). The desmutagenicity test was according to the method described by Watanabe et al.[19] More specifically, 0.1 mL of tested compound solution in DMSO (containing 50 μg of tested compound) was preincubated with 0.5 mL of S9 mix for 30 min at 37 °C. Subsequently, 0.1 mL of activated by means of S9 BaP (10 μg) and 0.1 mL of bacterial suspension were added to the above mixture and incubated for 20 min at 37 °C. After the addition of top agar (2 mL final volume), the mixture was poured onto the GM agar plate, as described before.

Alternatively, 0.1 mL of tested compound solution in DMSO (containing 50 μg of tested compound) was preincubated with 0.1 mL of activated by means of S9 BaP (10 μg) for 30 min at 37 °C. Subsequently, 0.1 mL of bacterial suspension were added to the above mixture and incubated for 20 min at 37 °C. After the addition of top agar (2 mL final volume), the mixture was poured onto the GM agar plate, as described before.

Calculation of the Antimutagenic Activities. All of the antimutagenicity assays were carried out in triplicate and on three separate experiments. Results are expressed as the mean ± standard deviation (SD) of His^+ revertants per plate. Negative and positive controls were included in each assay. The shown His^+ values have been obtained after subtraction of the background levels of His^+ revertants (negative control). No growth inhibition or any bactericidal effects were observed at the tested levels of lignans. The percent inhibition of mutagenicity was calculated as

$$\text{percent inhibition of mutagenicity} = (\text{His}^+ \text{ revertants induced/plate} - \text{His}^+ \text{ revertants induced in the presence of tested lignans/plate}) / \text{His}^+ \text{ revertants induced/plate}$$

RESULTS AND DISCUSSION

The basic target of this study was to apply to sesame seeds the methodology previously developed by our team for the obtainment of enriched polyphenolic extracts from the sesame coat[8] and then to evaluate the potential antimutagenic activities of some of the isolated compounds. The first step in this procedure was the extraction with cyclohexane and CH_2Cl_2 to remove efficiently the contained oil (about 50%). In a second step, the plant material was extracted with methanol and the dried extract was diluted in water. The solution was filtered, and the filtrate was submitted to an adsorption process on XAD-4 resin to enrich the polyphenol concentration. Finally, the polyphenolic compounds were recovered by elution with MeOH, giving an enriched extract. It is noticeable that this procedure (Figure 2) afforded a mixture almost free from sugars and polar lipids, which where successfully removed by application of the adsorption resin methodology.

This extract, submitted to several chromatographic separations, afforded 10 known lignans, 1 lignan isolated for the first time from a natural source (**1**), and a new lignan, (+)-sesamolinol-4′-*O*-β-D-glucoside (**2**).

Compound **1** was identified as disaminyl ether. This compound presented identical spectroscopic data, with a

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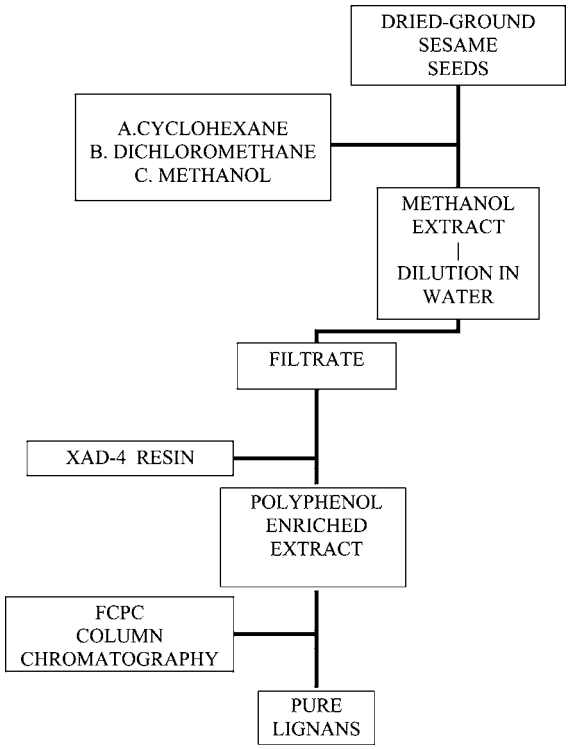

Figure 2. Schematic overview of the procedure for obtaining enriched polyphenolic extract from sesame seeds.

product resulting from the hydrolytic procedure of (+)-sesamolin in H_2SO_4–EtOH.[9] However, this is the first report of the isolation of this compound as a naturally occurring product.

Compound **2** was obtained as a colorless amorphous solid, whose molecular formula was determined as $C_{26}H_{30}O_{12}$ by HR-ESI–MS. The ^{1}H NMR spectrum recorded in CD_3OD exhibited a broad singlet at δ 5.65 and a doublet at δ 4.41 (J = 8 Hz), characteristic of the H-7 and H-7′ of sesamolinol-type lignans. The presence of six aromatic protons organized in two ABX systems, associated with other signals accounting for two aliphatic methines (H-8 and H-8′), two oxygenated methylenes (H-9 and H-9′), one methylenedioxy group, and one methoxy group attached to an aromatic ring, were also noticeable. The above signals were closely related to those previously described for sesamolin,[11] in terms of both chemical shifts and coupling constants. In addition, the presence of a set of resonance at 4.77 (d, J = 7 Hz), 3.32, 3.34, 3.46, and 3.48 ppm and a methylene at 3.69 (dd, J = 11.5 and 4.5 Hz) and 3.87 ppm (dd, J = 11.5 and 1.5 Hz) were characteristic of a sugar moiety. The only possible linkage position for an O-glucoside on a sesamolinol structure is 4′. This was also further confirmed by the heteronuclear multiple-bond correlation (HMBC) of H-1″ with C-4′, permitting the structure of compound **2** to be determined as (+)-sesamolinol-4′-O-β-D-glucoside.

Finally, prolonged enzymatic hydrolysis of compound **2** with β-D-glucosidase afforded, although in low yield, (+)-sesamolinol, confirming the structure of compound **2** as (+)-sesamolinol-4′-O-β-D-glucoside.

In summary, the use of XAD-4 adsorption resin permitted the obtainment of an efficiently enriched polyphenolic extract from sesame seeds. A new lignan, (+)-sesamolinol-4′-O-β-D-glucoside (**2**), and a samin dimer, disaminyl ether (**1**), hitherto known as a hydrolytic procedure byproduct, have been isolated from the extracts.

Antimutagenic Activity. Concerning the antimutagenic activity of the isolated lignans, it was clear that all of the tested lignans showed protective activity against H_2O_2 (Table 1) at

Table 1. Antimutational Properties of Tested Lignans (at 25 μg/mL) against H_2O_2

lignan	percent inhibition (%)
sesamolinol-4′-O-β-D-glucoside	26 ± 5.2
sesamolinol	50 ± 4.6
samine	40 ± 4.8
sesaminol-2-O-β-D-glucopyranoside	38 ± 5.5
sesamolin	51 ± 6.5
episesaminone	33 ± 4.7
piperitol	20 ± 3.1

variable degrees. Among the tested lignans, the most active lignan was found to be sesamolin, followed by sesamolinol and samin.[20] Additionally, some of the tested lignans showed desmutagenic activity against activated BaP. The activity was increased after preincubation of the tested lignans with BaP activated by the S9 fraction, with piperitol showing the highest activity (Table 2). Although there is a previous report on the

Table 2. Antimutational Properties of Isolated Lignans (at 25 μg/mL) against the Mutational Activity of BaP[a]

	percent inhibition (%)	
lignan	A	B
sesamolinol	8.6 ± 1.3	19 ± 3.2
samine	9.2 ± 1.8	21 ± 5.4
sesamolin	6.4 ± 1.0	28 ± 4.5
episesaminone	0	19 ± 2.8
piperitol	1.5 ± 0.6	29 ± 4.2

[a]In the first case (column A), each lignan was preincubated for 20 min with the S9 mix, before the simultaneous addition of bacterium strain TA100 and BaP activated by the S9 mix. In the second case (column B), each lignan was preincubated with BaP activated by the S9 mix, before the addition of the bacterium strain TA100. The results are shown as percent inhibition of mutagenicity.

antimutagenic activity of sesamol,[21] a sesamine degradation product, and another report related to the non-mutagenicity of sesamin,[22] to our knowledge, these preliminary results are the first report on the antimutagenic properties of pure sesame lignans.

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