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BIOSYNTHESIS OF ANTIOXIDANT LIGNANS IN *SESAMUM INDICUM* SEEDS

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Key Word Index—*Sesamum indicum*; Pedaliaceae; seeds; biosynthesis; lignans; pinoresinol; piperitol; sesamin; sesamolin; antioxidants.

Abstract—Sesame lignans, whose biosynthetic pathway is the subject of this study, have well-established antioxidant and health protecting properties. Using a combination of radio- and stable-isotopically labelled precursor administration experiments, it was demonstrated that *E*-coniferyl alcohol undergoes stereoselective coupling to afford (+)-pinoresinol in *Sesamum indicum* seeds. Only this enantiomer, and not its (-)-antipode, is metabolized further in maturing seeds to afford (+)-piperitol, (+)-sesamin, and (+)-sesamolin. Introduction of the methylene dioxy bridges occurs sequentially with piperitol first being formed, this being subsequently modified to afford sesamin. Copyright © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The potent antioxidant properties of seed extracts from *Sesamum indicum* and sesame oil are attributed mainly to the presence of the lignans, sesamin **1**, sesamolinol **2**, and sesaminol **3** [1-3]. The first two are natural products, whereas sesaminol **3** results from acid-catalyzed rearrangement of sesamolin **4** during industrial bleaching [4] (Fig. 1). Sesame lignans also have various biological activities, which include: synergistic effects with pyrethrum insecticides [5–7], and inhibitors of Δ-5 desaturases [8] in mammals. Sesame lignans, when provided in the diet, can also protect against ethanol and carbon tetrachloride induced liver damage [9], reduce serum cholesterol level [10] as well as increase vitamin E activities [11, 12] and the availability of γ-tocopherol *in vivo* [13].

Little is known about the biosynthetic pathways leading to the sesame lignans, except for one very preliminary study [14] which claimed that [D,L]-[1-^{14}C]tyrosine was incorporated into sesamin **1** when administered to cell suspension cultures of *S. indicum*. This is a surprising result, since all other studies have implicated phenylalanine [15, 16] as the precursor amino acid of the lignin–lignan metabolites, except for the grasses which can also utilize tyrosine [17]. Subsequent phenylpropanoid metabolism into the sesame lignan branch proper can be proposed to involve stereoselective coupling of two achiral molecules of *E*-coniferyl alcohol **5** to give (+)-pinoresinol **6a**, as previously established for lignan formation in *Forsythia sp.* [18–20]. In the latter case, a "dirigent" (Latin: to guide or align) protein was discovered as responsible for controlling this first example of bimolecular phenoxy radical coupling [20]. It has been purified to apparent homogeneity [20] and the encoding gene cloned [21].

If (+)-pinoresinol **6a** is formed in an analogous manner in *S. indicum*, then its resulting metabolism into the "oxygen-inserted" lignan (+)-sesamolin **4**, as well as into (+)-sesamin **1**, could be envisaged as occurring via either of the routes shown in Scheme 1. Clearly in the absence of metabolite turnover/time course data, it is not possible to distinguish between these possible pathways. In this investigation, we therefore sought to first establish if (+)-pinoresinol **6a** served as a precursor of the sesame lignans **1**, **2**, and **4**, and if possible to determine whether the oxygen insertion reaction preceded piperonyl group (aryl methylenedioxy) formation. To our knowledge, no description of piperonyl group formation in the lignans has been described, although there are two reports of cytochrome P-450/NADPH dependent microsomal preparations able to catalyse aryl methylenedioxy formation in the benzophenanthridine alkaloids [22, 23] and isoflavonoids [24], respectively.

RESULTS AND DISCUSSION

Mature seeds of *S. indicum* were first examined for their lignan contents and composition, as well as pro-

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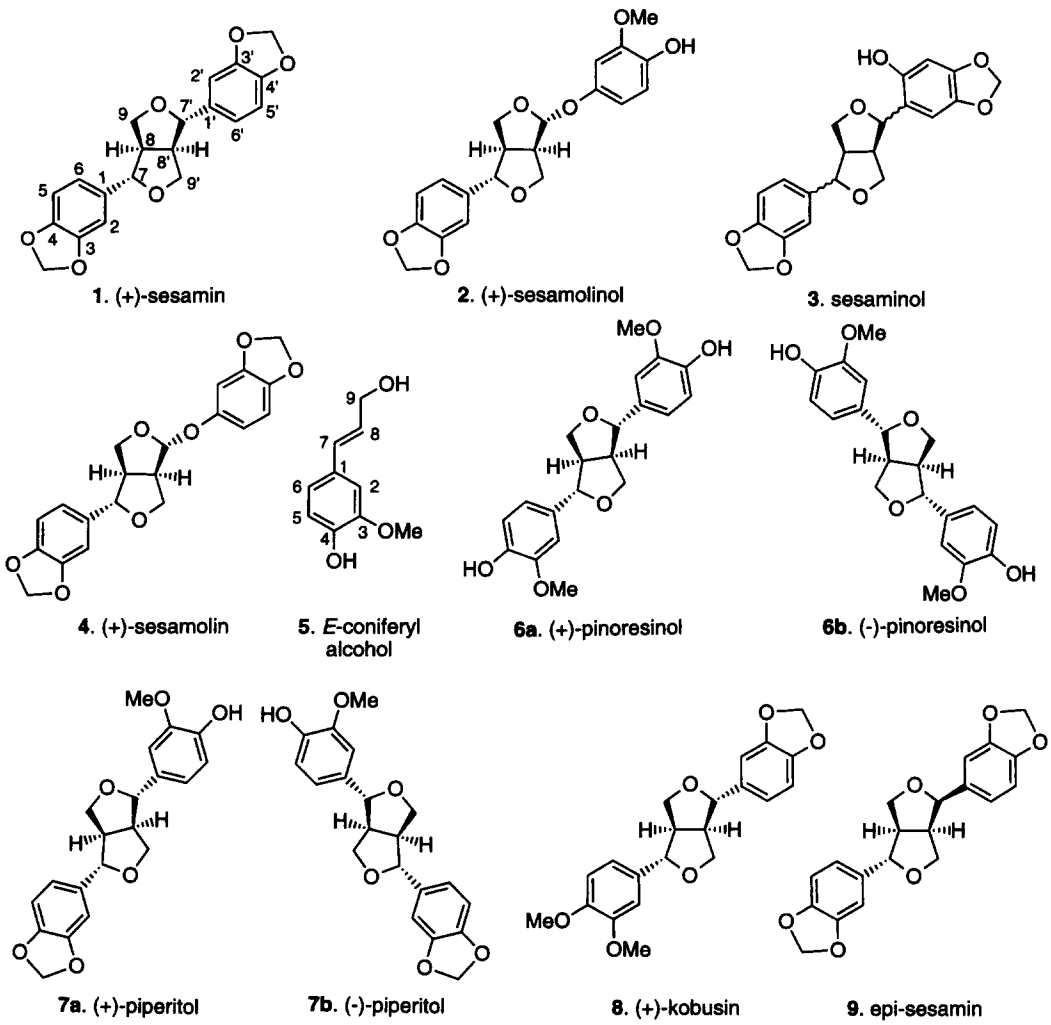


Fig. 1. Lignans from *Sesamum indicum* and Sesaminol 3.

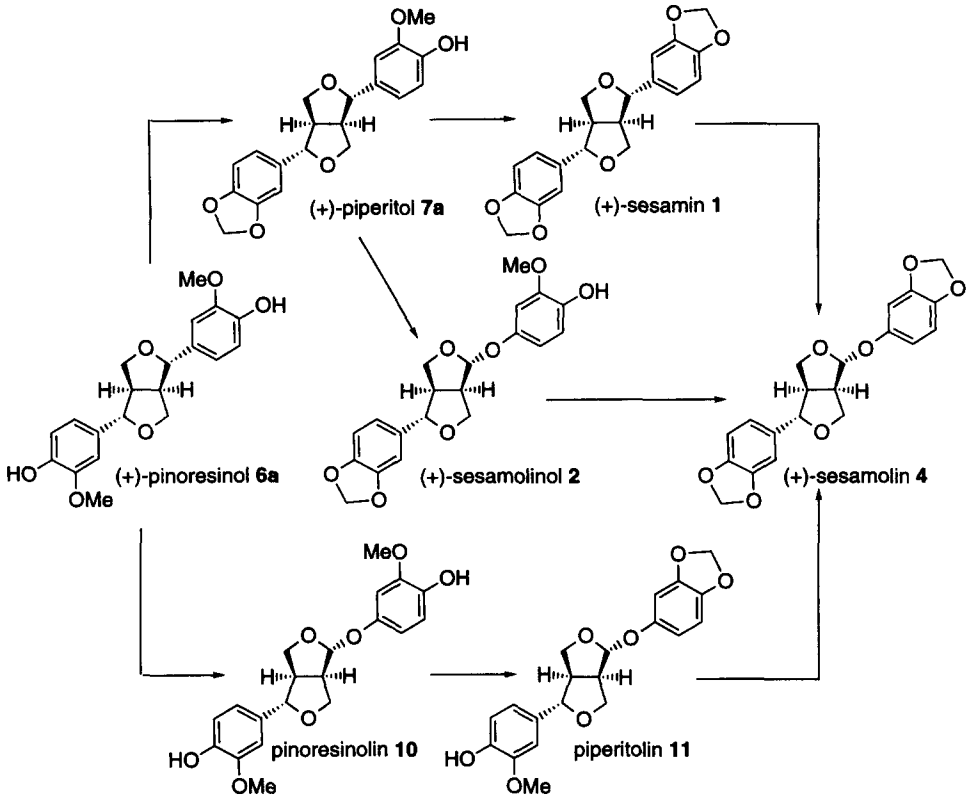
viding a source of authentic lignans. Thus, following extraction and multiple chromatographic steps [25], the known sesame lignans, (+)-sesamolin **4** [26, 27], (+)-sesamin **1** [27], (+)-pinoresinol **6a** [28], (+)-piperitol **7a** [1, 29], (+)-kobusin **8** [30], and (+)-epi-sesamin **9** [31] were isolated, together with ferulic acid.

It was instructive to ascertain whether active sesame lignan biosynthesis occurred in developing seeds, pods, seeds and pods together or in some other tissue. Thus, [U-^{14}C]Phe (19.6 kBq, 102.4 kBq μmol^{-1}) was first administered to intact seed-containing pods and seeds of *S. indicum*, under aseptic conditions, for 48 hr. As can be seen from Table 1, when [U-^{14}C]Phe was administered to the pods, this resulted in its small but

Table 1. Summary of results of administration of various putative precursors to *Sesamum indicum*

Precursor*	Total radioactivity administered (kBq)	Specific activity of precursor (kBq μmol^{-1})	Time of incorporation (hr)	Total incorporation (%) Pinoresinol **6**	Piperitol **7**	Sesamin **1**	Sesamolin **4**
[U-^{14}C]Phe	19.60	102.4	48	n.d.	n.d.	0.11	0.16
E-[8-^{14}C] Coniferyl alcohol **5**	1.76	3.39	12	3.18	2.26	1.42	0.72
(±)-[3,3'-O^{14}CH$_3$] Pinoresinols **6a/6b**	9.70	48.1	12	n.d.	6.96	10.58	2.13
(±)-[3-O^{14}CH$_3$]Piperitols **7a/7b**	1.25	1.15	8	n.d.	n.d.	5.22	0.87

*[U-^{14}C] Phe was administered to pods, whereas all other precursors were administered to seeds.
n.d. = not detected.



Scheme 1. Possible Biosynthetic Routes to the Sesame Lignans, (+)-Sesamin **1** and (+)-Sesamolin **4** from (+)-Pinoresinol **6a**.

significant metabolism into the lignans, (+)-sesamolin **4** and (+)-sesamin **1** (0.16 and 0.11%, respectively). On the other hand, it was not incorporated into these same metabolites when directly administered to seeds. In contrast, *E*-[8-^{14}C]coniferyl alcohol **5** (1.76 kBq, 3.39 kBq μmol^{-1}) was metabolized into pinoresinol **6** (3.18%), piperitol **7** (2.26%), sesamin **1** (1.42%) and sesamolin **4** (0.72%) when administered to seeds, but was not when supplied to the pods (Table 1). Formation of pinoresinol **6** was of particular interest, since this finding was in general agreement with our previous observations using *Forsythia* sp. that catalytic entry into the furanofuran lignans and derivatives thereof resulted from stereoselective coupling of *E*-coniferyl alcohol **5** [20, 32, 33].

Having defined the location of sesame lignan biosynthesis, attention was next directed to the sequence and enantiospecificity of their lignan biosynthetic transformations. This in turn necessitated synthesis of the racemic radiolabelled putative precursors, (±)-[3,3′-O^{14}CH$_3$]pinoresinols **6a/6b** and (±)-[3-O^{14}CH$_3$]piperitols **7a/7b**, as well as (+)-[7,7′-^{3}H$_2$] sesamin **1**. These were synthesized via modification of known strategies [34-36], i.e., (±)-[3,3′-O^{14}CH$_3$] pinoresinols **6a/6b** were obtained from the protected benzaldehyde **14**, its 1,3 dithiane **16** and butenolide as shown to generate **17** (Fig. 2). The synthon **17** was subsequently hydrolyzed to give **18**, then sequentially reduced to give **19** and **20**, with cyclization of the latter affording the required furofuran **21**. Next, *O*-methylation with ^{14}CH$_3$I and subsequent debenzylation of the resulting product afforded the required (±)-[3,3′-O^{14}CH$_3$]pinoresinols **6a/6b** (156 mg, 48.1 kBq μmol^{-1}). In an analogous manner, synthesis of (±)-[3-O^{14}CH$_3$]piperitols **7a/7b** (52 mg, 1.15 kBq μmol^{-1}) followed an identical strategy, except that the piperonyl analogue **22** was employed. The third putative precursor, (+)-[7,7′-^{3}H$_2$]-sesamin **1** (96 mg, 64.01 kBq μmol^{-1}) was obtained from naturally occurring (+)-sesamin **1**, via generation of its dianion with *n*-BuLi, and subsequent quenching with tritiated water.

With the potential precursors in hand, we next sought to ascertain whether (+)- and (−)-[3,3′-O^{14}CH$_3$] pinoresinols **6a/6b** served as precursors of piperitol **7**, sesamin **1** and sesamolin **4**. To address this question, racemic (±)-[3,3′-O^{14}CH$_3$]pinoresinols **6a/6b** (9.71 kBq, 48.1 kBq μmol^{-1}) were administered to the maturing pods and seeds, respectively. As previously noted for *E*-[8-^{14}C]coniferyl alcohol **5**, only metabolism into the lignans of interest occurred when the radiolabelled pinoresinols were administered to the seeds, and not to the pods. As shown in Table 1, its metabolism resulted in a significant conversion over a 24 hr period into piperitol **7** (6.96%), sesamin **1** (10.58%), and sesamolin **4** (2.13%). A subsequent time-course of metabolism of (±)-[3,3′-O^{14}CH$_3$] pinoresinols **6a/6b** in seeds at approximately the same

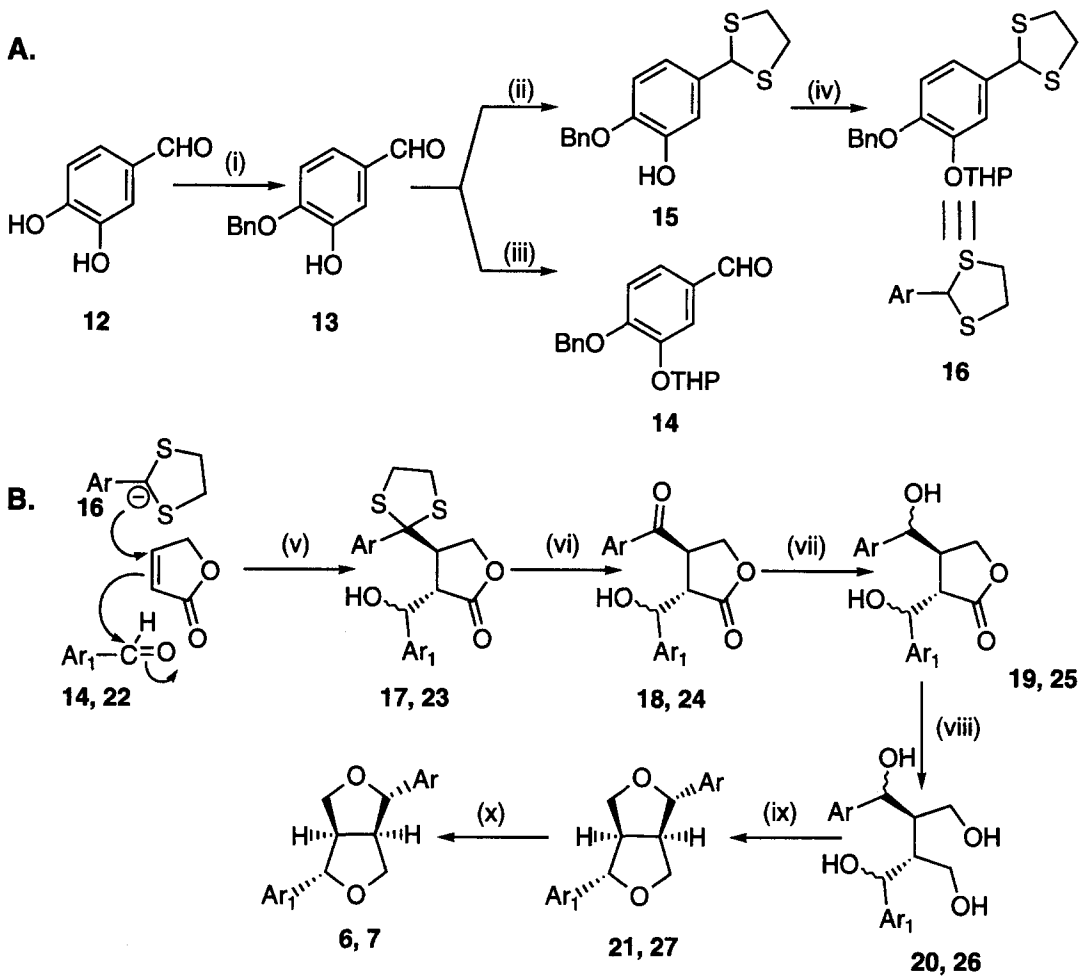


Fig. 2. Synthesis of (±)-[3,3′-O$^{14}CH_3$]pinoresinols **6a/6b** and (±)-[3-O$^{14}CH_3$]piperitols **7a/7b**. A. Formation of synthons **14** and **16**; B. Subsequent synthetic steps to **6a/6b** and **7a/7b**. Legend: (i): K_2CO_3, BnBr, DMF; (ii): HS-$(CH_2)_3$-SH, HCl, $ZnCl_2$, dioxane; (iii) and (iv): DHP, *p*TSA, CH_2Cl_2; (v): LDA, THF; (vi): HgO, BF_3-Et_2O, THF/H_2O; (vii): $NaBH_4$, THF/MeOH; (viii): $LiAlH_4$, THF; (ix): TMSBr, CH_2Cl_2; (x): $^{14}CH_3I$/CH_3I, K_2CO_3, DMF then H_2, Pd/C, EtOAc. For compound **17**: Ar = Ar_1 = 4-OBn-3-OTHP-phenyl; for compound **23**: Ar = 4-OBn-3-OTHP-phenyl, Ar_1 = 3,4-methylenedioxyphenyl; for compounds **18, 19, 20** and **21**: Ar = Ar_1 = 4-OBn-3-OH-phenyl; for compounds **24, 25, 26** and **27**: Ar = 4-OBn-3-OH-phenyl, Ar_1 = 3,4-methylenedioxyphenyl; for compound **6**: Ar = Ar_1 = 4-OH-3-OCH_3-phenyl; for compound **7**: Ar = 4-OH-3-OCH_3-phenyl, Ar_1 = 3,4-methylenedioxyphenyl.

stage of maturation (Figure 3) revealed that all three lignans, piperitol **7**, sesamin **1**, and sesamolin **4** were initially rapidly formed over a 10-15 hr period, with piperitol **7** being subsequently metabolized down to near background levels over 50 hr. On the other hand, the amounts of both sesamin **1** and sesamolin **4** gradually increased, reaching a near plateau level over this same time frame. Chiral column HPLC analysis of the remaining (unmetabolized) radiolabelled pinoresinol established that the (+)-antipode **6a** was being progressively depleted, relative to the (−) form **6b** which was not metabolized further into these products (data not shown). Accordingly, only (+)-piperitol **7a**, (+)-sesamin **1**, and (+)-sesamolin **4** were being biosynthesized.

Further support for these enzymatic transformations was established using (±)-[7,7′-$^2H_2$] pinoresinols **6a/6b** (mol. wt. 360, $M^+ + 2$) as precursor [32], this being administered to immature *S. indicum* seeds over various time intervals (1 and 48 hr). The piperitol **7** so formed was purified from the seeds following 1 hr metabolism, whereas both sesamin **1** and sesamolin **4** were isolated after 48 hr. The mass spectrum of piperitol **7** had a $[M^+]$ at *m/z* 358 $[M+2]^+$ (78%), 357 $[M+1]^+$ (10%), 356 $[M]^+$ (6%) indicating that (+)-[7,7′-$^2H_2$]pinoresinol **6a** had been intactly incorporated (data not shown). A somewhat analogous situation was observed for sesamin **1**, whose mass spectrum revealed an ion cluster at *m/z* 356 $[M+2]^+$ (30%), 355 $[M+1]^+$ (32%), and 354 $[M]^+$ (38%). In the case of sesamolin **4**, however, no detectable deuterium incorporation was observed in its mass

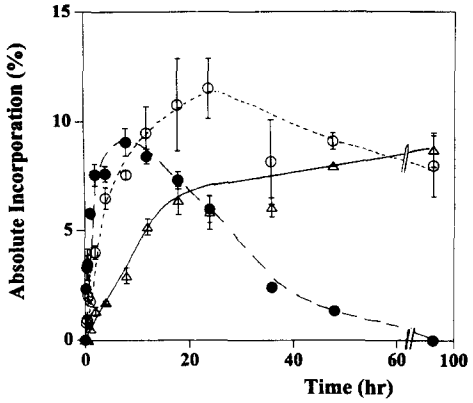


Fig. 3. Metabolism of (±)-[3,3′-O^{14}CH$_3$]Pinoresinols **6a/6b** into ● (+)-Piperitol **7a**, ○ (+)-Sesamin **1** and Δ (+)-Sesamolin **4**.

spectrum, a presumed consequence of the high endogenous levels of sesamolin **4** present in the seeds.

Lastly, the metabolism of (±)-[3-O^{14}CH$_3$]piperitols **7a/7b** (1.25 kBq, 1.15 kBq μmol^{-1}) and [7,7′-^{3}H$_2$]-sesamin **1** in *S. indicum* seeds was examined over a 8 and 24 hr period, respectively. Under these conditions, the radiolabelled (+)-piperitol **7a** was metabolized into (+)-sesamin **1** (5.22% incorporation) and sesamolin **4** (0.87% incorporation), respectively, whereas on the other hand (+)-[7,7′-^{3}H$_2$]sesamin **1** was apparently not converted any further.

Concluding Remarks

The sesame lignans have significant plant protective properties as antioxidants, as well as having important roles in health protection. In this study, it was established that sesame lignan formation, i.e., to afford (+)-piperitol **7a**, (+)-sesamin **1**, and (+)-sesamolin **4** occurs during seed maturation and ripening. Using both radio- and stable-isotopically labelled (±)-pinoresinols **6a/6b**, it was also demonstrated that (+)-pinoresinol **6a** was employed as precursor, with sequential stages of methylenedioxy group formation occurring to first give (+)-piperitol **7a** and then (+)-sesamin **1**. Future studies will be directed towards characterizing the proteins involved in sesame lignan formation, with particular emphasis being placed upon methylenedioxy bridge formation and the unusual oxygen insertion step, affording (+)-sesamolin **4**.

EXPERIMENTAL

Plant material. Sesamum indicum plants were grown in Washington State University greenhouse facilities.

Reagents. [^{14}C]-Methyl iodide (1.99 GBq mmol^{-1}), ^{3}H$_2$O (74 MBq mmol^{-1}), [2-^{14}C]malonic acid (17.5 GBq mmol^{-1}), and L-[U-^{14}C]phenylalanine (175 MBq mmol^{-1}) were purchased from Amersham.

Instrumentation and chromatographic materials. Silica gel TLC and CC were performed on Kieselgel 60 F_{254} (Merck, 20x20 cm, 2 mm) and silica gel-60 (EM Science, 230–400 mesh ASTM), respectively. All solvents and chemicals used were reagent or HPLC grade, unless otherwise stated. THF was distilled over potassium benzophenone ketyl immediately prior to use. Mps are uncorrected. Infrared and UV/VIS spectra were recorded on Perkin Elmer 1720-AFT-IR and Lambda UV/VIS spectrometers. ^{1}H NMR and ^{13}C NMR (300 MHz) spectra were recorded on a Brüker AMX spectrometer, using $CDCl_3$ as a solvent with chemical shifts (δ) reported downfield from TMS (internal standard). Mass spectra were performed on a VG 7070E (70 eV) spectrometer. Radioactive samples were analyzed in Ecolume (ICN Biomedicals) and measured using a liquid scintillation counter (Packard 2000 CA Tri-carb). HPLC instrumentation was as previously described [33]. HPLC separations were carried out using either reversed-phase (Waters, Novapak, 150 × 3.9 mm i.d., 5 μm) or chiral (Chiral Technologies, Chiralcel OD, 250 × 4.6 mm i.d.) columns with detection at 280 nm. For reversed phase HPLC, lignans were sepd at a flow rate of 0.9 ml min^{-1} using a linear gradient system: MeOH-H_2O (65:35) at t = 0 min to (30:70) at t = 40 min. For chiral HPLC sepns, the EtOH used was denatured with 2-PrOH (HPLC grade, Aldrich) and *n*-hexane was >95% pure (HPLC grade, Baker). Separation of (+)- and (−)-pinoresinols **6a/6b** employed EtOH-hexanes (1:1) at a flow rate of 0.8 ml min^{-1}, whereas (+)- and (−)-piperitols **7a/7b** used EtOH-hexanes (1:9) at a flow rate of 0.5 ml min^{-1}. All HPLC samples were filtered (ACRO LC3S disposable filter, 0.45 μm, Gelman Science) prior to analysis.

Chemical syntheses. E-[8-^{14}C]Coniferyl alcohol **5** (3.39 kBq μmol^{-1}) was prepared exactly as described [37]. (±)-[3,3′-O^{14}CH$_3$] pinoresinols **6a/6b** were synthesized using the strategy of Ziegler and Schwartz [34], as modified by Fujimoto *et al.* [35], and Katayama *et al.* [36] with additional modifications as described below.

4-*Benzyloxy*-3-*hydroxybenzaldehyde* **13**. 3,4-Dihydroxybenzaldehyde **12** (5.76 g, 41.7 mmol) in *N*,*N*′-dimethylformamide (40 ml), K_2CO_3 (5.52 g, 40 mmol) and benzylbromide (5.35 ml, 45 mmol) were stirred overnight under N_2 at room temp. The resulting suspension was next partitioned between H_2O (150 ml) and Et_2O (3 x 150 ml). The organic solubles were combined, washed with satd NaCl soln (2 x 80 ml), then dried (Na_2SO_4) and evapd *in vacuo* to dryness to give a solid (7.8 g), which was subsequently applied to a silica gel column eluted with CH_2Cl_2. Fractions containing the product were pooled and evapd to dryness *in vacuo* to afford the required aldehyde **13** (5.33 g, 56.1%). ^{1}H NMR ($CDCl_3$): δ 5.20 (2H, *s*, OCH_2), 6.10 (1H, *br s*, OH), 7.02 (1H, *d*, *J* = 8.2 Hz, H-5), 7.34-7.44 (7H, *m*, H-6, H-2, Ar-H), 9.82 (1H, *s*, CHO); EIMS *m/z* (rel. int.): 228 [M]$^+$ (9), 137 (3), 136 (1), 109 (5), 92 (24), 91 (100).

4-*Benzyloxy*-3-(*tetrahydropyran*-2′-*yloxy*)-*benzal*

dehyde **14.** To a soln of 4-benzyloxy-3-hydroxybenzaldehyde **13** (1.52 g, 6.7 mmol) in CH_2Cl_2 (30 ml) were added dihydropyran (3 ml, 5 eq) and *p*-TsOH (13 mg) under N_2 at 0°. After 40 min, the soln was neutralized with triethylamine and partitioned between CH_2Cl_2 and H_2O. The organic phase was next washed with satd NaClsoln, dried (Na_2SO_4) and concd *in vacuo* to afford the protected aldehyde **14.** (1.89 g, 90.4%). ^{1}H NMR ($CDCl_3$): δ 1.43-2.08 (7H, *m*, H-5′-H-3′, H-5), 2.11 (1H, *m*, Hβ-5 or Hα-5), 2.86 (2H, *m*, Hβ-4, Hα-6), 3.02 (2H, *m*, Hα-4, Hβ-6), 5.08 (2H, *s*, H-7′), 5.46 (1H, *t*, J = 2.9 Hz, H-2′), 6.88 (1H, *d*, J = 8.4 Hz, H-5), 7.05 (1H, *dd*, J = 2.2, 8.4 Hz, H-6), 7.23 (1H, *d*, J = 2.2 Hz, H-2), 7.28-7.43 (5H, *m*, Ar-H); EIMS *m/z* (rel. int.): 312 $[M]^+$ (2), 229 (6), 228 (9), 137 (6), 109 (7), 92 (34), 91 (100), 85 (100).

2-(3-*Hydroxy*-4-*benzyloxyphenyl*)-1,3-*dithian* **15**. To a soln of **13** (2.47 g, 10.83 mmol) in dioxane (25 ml) was added $ZnCl_2$ (387 mg, 2.8 mmol) and concd HCl (2.3 ml). The resulting suspension was cooled to 0°, following which 1,3-propanedithiol (1.1 ml, 11 mmol) was added. The suspension was stirred for an additional 20 min, then partitioned between H_2O and EtOAc. The organic solubles were washed with satd $NaHCO_3$, 1N NaOH, and satd NaCl soln, respectively, then dried (Na_2SO_4), and evapd *in vacuo* to give the dithian **15** which was crystallized from hexanes-EtOAc (3.20 g, 93%).

2-(3′-*Dihydropyran*-2′-*yloxy*-4′-*benzyloxyphenyl*)-1,3-*dithian* **16**. To a soln of dithian **15** (3.19 g, 10.03 mmol) in CH_2Cl_2 (30 ml), was added dihydropyran (4.57 ml, 50.15 mmol, 5 eq) and *p*-TsOH (30 mg, 0.16 mmol). After stirring under N_2 at 0° for 1 hr, triethylamine was added until the mixt. was neutralized, with the resulting soln then partitioned between H_2O and EtOAc. The organic layer was washed with satd NaCl soln, dried (Na_2SO_4) and evapd *in vacuo* to give, following recrystallization from Et_2O-hexanes, the protected 1,3-dithian **16** (3.12 g, 77.4%).

(±)-4,4′-*Dibenzyloxy*-3,3′-*bisdihydropyran*-7′-*hydroxy*-7α,7β-*propanedithio*-3,3′-*bis*-*demethylmatairesinols* **17.** To a stirred soln of dithian **16** (1.93 g, 4.80 mmol) in dry THF (35 ml), at -78° under Ar, was slowly added *n*-BuLi (3.3 ml of 1.6 M in hexanes, 1.1 eq) over 40 min. Following stirring for an additional 1 hr, butenolide (340 μl, 4.80 mmol, 1 eq) in dry THF (20 ml) was slowly added with the whole then stirred for a further 1 hr. A soln of the protected benzaldehyde **14** (1.47 g, 4.71 mmol) in dry THF (25 ml) was next added over 40 min, with stirring being maintained for an additional 1 hr, following which the temp. of the reaction was allowed to rise to room temp. The reaction mixt. was partitioned between EtOAc and satd NaCl soln, with the organic phase washed with satd NaCl soln until neutral. The resulting organic solubles were dried (Na_2SO_4) and evapd *in vacuo* to give a pale viscous oil (4.18 g). Following silica gel chromatography, via elution with a gradient of hexanes-EtOAc, fractions containing a mixt. of the epimeric alcohols **17** (1.94 g, 50.6%) were combined and used without further purification.

(±)-4,4′-*Dibenzyloxy*-7′-*hydroxy*-7-*oxo*-3,3′-*bis*-*demethylmatairesinols* **18.** To a stirred soln of epimeric alcohols **17** (1.94 g, 2.43 mmol) in 15% aqueous THF (50 ml), under N_2 at room temp., were added HgO (1.58 g, 7.29 mmol, 3 eq) and BF_3-Et_2O (1 ml, 7.89 mmol). After stirring for 1 hr, the reaction mixt. was extracted with CH_2Cl_2. The CH_2Cl_2 solubles were combined, successively washed with satd Na_2CO_3 and NaCl solns, then dried (Na_2SO_4), and evapd *in vacuo* to afford a mixt. of epimeric alcohols **18** (1.65 g, 96%) that were used without further purification.

(±)-4,4′-*Dibenzyloxy*-7,7′-*dihydroxy*-3,3′-*bisdemethylmatairesinols* **19**. To a stirred soln of epimeric alcohols **18** (1.65 g, 2.33 mmol) in THF-MeOH (1:5, 130 ml), at 0° under Ar, was added $NaBH_4$ (89 mg, 2.35 mmol). After 20 min, the reaction mixt. was partitioned between EtOAc and satd NaCl soln. The resulting organic solubles were dried (Na_2SO_4), and evapd *in vacuo* to afford the mixt. of diols **19** (1.53 g, 92.5%), which were used without further purification.

(±)-4,4′-*Dibenzyloxy*-7,7′-*dihydroxy*-3,3′-*bisdemethylsecoisolariciresinols* **20.** To a stirred suspension of $LiAlH_4$ (231 mg, 5.78 mmol, purity: 95%) in dry THF (40 ml), at 50° under Ar, was added dropwise the epimeric mixt. of diols **19** (1.51 g, 2.13 mmol) in dry THF (20 ml) over 30 min. The temp. was then reduced to 0°, following which excess $LiAlH_4$ was destroyed by addition of aqueous THF. The reaction mixt. was then partitioned between EtOAc and satd NaCl soln, with the resulting organic solubles dried (Na_2SO_4) and evapd *in vacuo* to afford the mixt. of tetraols **20** (1.34 g, 1.88 mmol, 88.3%).

(±)-4,4′-*Dibenzyloxy*-3,3′-*bisdemethylpinoresinols* **21**. To a stirred soln of the tetraols **20** (1.34 g, 1.87 mmol) in CH_2Cl_2 (40 ml), under Ar at room temp., was added Me_3SiBr (497 μl, 3.77 mmol, 2 eq). After 15 min, the soln was partitioned between 10% $NaHCO_3$ soln and CH_2Cl_2. The organic phase was next washed with satd NaCl soln, dried (Na_2SO_4) and concd *in vacuo* to yield crude material (0.95 g) which was further purified by silica gel chromatography eluted with CH_2Cl_2-Me_2CO (95:5). Fractions containing the desired product were combined and evapd to dryness *in vacuo* to afford **21** (449 mg, 47%); ^{1}H NMR ($CDCl_3$): δ 3.05 (2H, *m*, H-8, H-8′), 3.85 (2H, *dd*, J = 3.7, 9.2 Hz, Hα-9, Hα-9′), 4.22 (2H, *dd*, J = 7.0, 9.2 Hz, Hβ-9, Hβ-9′), 4.70 (2H, *d*, J = 4.3 Hz, H-7, H-7′), 5.67 (4H, *s*, 2 × OCH_2), 6.79 (2H, *dd*, J = 2.6, 8.3 Hz, H-6, H-6′), 6.87 (2H, *d*, J = 8.3 Hz, H-5, H-5′), 6.92 (2H, *d*, J = 2.6 Hz, H-2, H-2′), 7.34-7.40 (10 H, *m*, Ar-H); EI MS *m/z* (rel. int.): 510 $[M]^+$ (12), 419 (11), 290 (10), 220 (100), 190 (21), 149 (12), 121 (22).

(±)-[3,3′-*O*^{14}*CH*$_3$]*pinoresinols* **6a/6b**. To a soln of **21** (330 mg, 0.65 mmol) in dry DMF (10 ml) were added K_2CO_3 (350 mg, 2.54 mmol) and $^{14}CH_3I$ (28.7 MBq, 1.99 GBq mmol^{-1}). The resulting suspension was stirred for 12 hr under Ar at room temp. following which CH_3I (80.56 μl, 1.29 mmol) was added. After

stirring overnight, the whole was then partitioned between EtOAc and satd NaCl soln, with the resulting organic solubles dried (Na_2SO_4), and concd *in vacuo* to give the crude product (332 mg) which was used without further purification. This was then reconstituted in EtOAc-MeOH (7:3, 40 ml) to which (10%) Pd/C (150 mg) was next added. The resulting suspension was stirred for 1.5 hr under H_2 at room temp., following which EtOAc (20 ml) was added, with the whole then filtered through Celite (14 g). The EtOAc solubles were next evapd to dryness *in vacuo*, then reconstituted in a minimum amount of EtOAc and applied to a silica gel column eluted with EtOAc-hexanes (1:4). Fractions containing the desired racemic product were combined and evapd to dryness *in vacuo*. Recrystallization from Et_2O-hexanes afforded (±)-[3,3′-O^{14}CH$_3$]pinoresinols **6a/6b** (156 mg, 67%, 48.1 kBq μmol^{-1}).

(±)-4-*Benzyloxy*-3-*dihydropyran*-7′-*hydroxy*-7α, 7β-*propanedithio*-3,4-*bisdemethyl*-*bursehernin* **23**. To a stirred soln of dithian **16** (5.25 g, 13.06 mmol) in dry THF (75 ml) under Ar at -78° was added *n*-BuLi (8.8 ml of 1.6 M in hexanes, 14.14 mmol, 1.1 eq) over 40 min. Following stirring for an additional 1 hr, butenolide (955 μl, 13.46 mmol) in dry THF (12 ml) was slowly added to the reaction mixt. over 1 hr at the same temp. Then a soln of aldehyde **22** (2.15 g, 14.34 mmol) in dry THF (15 ml) was added over 40 min. Stirring was continued for an additional 12 hr, following which the temp. was allowed to rise to room temp. The reaction mixt. was next partitioned between EtOAc and satd NaCl soln, with the organic solubles washed with satd NaCl soln until neutralization, then dried (Na_2SO_4) and evapd *in vacuo* to give a pale viscous oil (9.81 g). This was next submitted to silica gel chromatography, eluted with EtOAc-hexanes, with fractions containing the desired 7′ epimeric alcohols **23** (6.52 g, 78.4%) combined and used without further purification.

(±)-4-*Benzyloxy*-7′-*hydroxy*-7-*oxo*-3,4-*bisdemethyl*-*bursehernin* **24**. To a stirred soln of **23** (2.51 g, 3.95 mmol) in 15% aqueous THF (70 ml) under N_2 at room temp. was added H_2O (2.56 g, 11.82 mmol) and BF_3-Et_2O (1.5 ml, 11.84 mmol). After stirring for 1 hr, the reaction mixt. was extracted with CH_2Cl_2. The CH_2Cl_2 solubles were combined, washed with satd Na_2CO_3 and satd NaCl soln, dried (Na_2SO_4), and evapd *in vacuo* to afford a mixt. of the epimeric alcohols **24** (1.6 g, 88%) that were used without further purification.

(±)-4-*Benzyloxy*-7,7′-*dihydroxy*-3,4-*bisdemethyl*-*bursehernin* **25**. To a stirred soln of **24** (2.09 g, 4.52 mmol) in THF-MeOH (1:5, 100 ml) was added $NaBH_4$ (189 mg, 4.97 mmol). After 20 min, the reaction mixt. was partitioned between EtOAc and satd NaCl soln. The combined organic solubles were subsequently dried (Na_2SO_4) and evapd *in vacuo* to afford a mixt. of diols **25** (1.98 g, 94.4%), which were used without further purification.

(±)-4-*Benzyloxy*-7,7′-*dihydroxy*-3-*demethyltetrahydropiperitols* **26**. To a stirred soln of $LiAlH_4$ (215 mg, 5.4 mmol) suspended in dry THF (60 ml) at 50° under Ar was added dropwise the soln of epimeric alcohols **25** (1.98 g, 4.26 mmol) in dry THF (25 ml). After decomposition of excess hydride by addition of aqueous THF at 0°, the reaction mixt. was partitioned between EtOAc and satd NaCl soln. The combined organic layer was dried (Na_2SO_4) and evapd *in vacuo* to afford a mixt. of the tetraols **26** (1.88 g, 94%) which were used without further purification.

(±)-4-*O*-*Benzyloxy*-3-*demethylpiperitols* **27**. To a stirred soln of **26** (1.88 g, 4.00 mmol) in CH_2Cl_2 (70 ml) under Ar at room temp. was added Me_3SiBr (1.05 ml, 8.00 mmol, 2 eq). After 15 min, the soln was partitioned between satd $NaHCO_3$ soln and CH_2Cl_2. The organic solubles were next washed with satd NaCl soln, dried (Na_2SO_4) and concd *in vacuo* to give the crude product (1.85 g) which was further purified by Si-gel chromatography eluted with EtOAc-hexanes (1:4). Fractions containing the product were combined and evapd to dryness *in vacuo* to afford pure **27** (670 mg, 37.5%); ^{1}H NMR ($CDCl_3$): δ 3.04 (2H, *m* H-8, H-8′), 3.83 (1H, *dd*, J = 2.0, 4.1 Hz, Hα-9′), 3.86 (1H, *dd*, J = 1.9, 4.3 Hz, Hα-9), 4.20 (1H, *dd*, J = 6.6, 9.1 Hz, Hβ-9 or Hβ-9′), 4.23 (1H, *dd* J = 6.8, 9.1 Hz, Hβ-9 or Hβ-9′), 4.68 (1H, *d*, J = 5.0 Hz, H-7′), 4.72 (1H, *d*, J = 4.7 Hz, H-7), 5.08 (2H, *s*, OCH_2), 5.74 (1H, *s*, OH), 5.93 (2H, *s*, OCH_2O), 6.80 (2H, *dd*, J = 2.0, 8.3 Hz, H-6, H-6′), 6.88 (2H, *d*, J = 8.3 Hz, H-5, H-5′), 6.93 (2H, *d*, J = 2.0 Hz, H-2, H-2′), 7.34-7.41 (10 H, *m*, Ar-H).

(±)-*Piperitols* **7a/7b.** To a soln. of **27** (28 mg, 0.063 mmol) in dry DMF (5 ml) were added K_2CO_3 (11 mg, 0.08 mmol) and CH_3I (436 μl, 0.07 mmol). The reaction mixt. was stirred for 12 hr under Ar at room temp. The resulting suspension was next partitioned between EtOAc and satd NaCl soln, with the organic solubles dried (Na_2SO_4) and concd *in vacuo* to afford crude *O*-benzyl piperitol (27 mg). This was then redissolved in MeOH (10 ml), to which was added 10% Pd-C (15 mg) with the resulting suspension stirred for 2 hr at room temp. under H_2. The reaction mixt. was diluted with EtOAc (20 ml), filtered (Celite, 7g), with the EtOAc solubles evapd to dryness *in vacuo*, then reconstituted in a minimum amount of EtOAc and applied to a preparative TLC silica gel plate eluted with EtOAc-hexanes (1:4). Fractions containing the desired lignan were combined and evapd to dryness *in vacuo* to afford (±)-piperitols **7a/7b** (15 mg, 67%).

(±)-[3-*O*^{14}*CH*$_3$]*Piperitols* **7a/7b.** To a soln of **27** (103 mg, 0.23 mmol) in dry DMF (7 ml) were added K_2CO_3 (0.72 mmol) and $^{14}CH_3I$ (8.3 MBq, 1.99 GBq mmol^{-1}). The reaction mixt. was stirred for 12 hr under Ar at room temp. following which CH_3I (25 μl, 0.24 mmol) was added and the whole was stirred overnight. The resulting suspension was partitioned between EtOAc and satd NaCl soln, with the organic solubles then dried (Na_2SO_4) and concd *in vacuo* and purified as before to afford the crude *O*-benzylated product (85 mg). This was redissolved in MeOH (10

ml), to which was added 10% Pd-C (45 mg), with the resulting suspension stirred for 2 hr at room temp. under H_2. The resulting suspension was diluted with EtOAc (20 ml), filtered (Celite, 9 g), with the EtOAc solubles evapd to dryness *in vacuo*, then reconstituted in a minimum amount of EtOAc and applied to prep-TLC silica gel plates eluted with EtOAc-hexanes (1:4). Fractions containing the required lignan were combined and evapd to dryness *in vacuo* to afford (±)-[3-$O^{14}CH_3$]piperitols **7a/7b** (52 mg, 63.5%, 1.15 kBq μmol^{-1}).

(+)-[7,7′-$^3H_2$]Sesamin **1.** To a soln of (+)-sesamin **1** (156 mg, 0.44 mmol) in dry THF (30 ml) was added *n*-BuLi (1.5 ml) at −75°, under Ar, and the suspension was allowed to stir for 30 min, following which addition of ^3H_2O (200 μl, 74 MBq $mmol^{-1}$) was added over 15 min. The soln was next allowed to rise to room temp. and partitioned between H_2O and EtOAc, then washed with satd NaCl soln, dried (Na_2SO_4) and concd *in vacuo* to dryness. The resulting solid material was suspended in a minimum amount of EtOAc and applied to a silica gel column eluted with CH_2Cl_2–Me_2CO (95:5). Fractions containing the required product were combined, dried and recrystallized to give (+)-[7,7′-$^3H_2$]sesamin **1** (96 mg, 64.01 kBq μmol^{-1}) as a white solid.

Administration of L-[*U*-^{14}C]*phenylalanine.* Freshly harvested, unripe pods of *S. indicum* were soaked in 200 μl of a soln containing L-[U-^{14}C]phenylalanine (31.7 μg, 19.6 kBq, 102.4 kBq μmol^{-1}) for 48 hr at 26° under constant white light. Four replicates were carried out. After absorption of the precursor soln, distilled water was added periodically to prevent dryness. After 48 hr, the pods and seeds were separated and individually freeze-dried. To the ground seeds was added 3,3′-dimethyl-dihydroguaiaretic acid (14 μg) as an internal standard, and the whole was extracted with CH_2Cl_2-EtOAc (5 ml, 1:1, X3) with aid of a sonicator for 15 min. The resulting organic solubles were taken to dryness *in vacuo*, then redissolved in hexanes-EtOAc (1.5 ml, 4:1), and chromatographed on silica gel (2.5 x 1 cm) successively eluted with hexanes-EtOAc (9:1, 2 ml) and EtOAc (4 ml). The EtOAc solubles were concd *in vacuo* to dryness and redissolved in MeOH (100 μl), with a 20% aliquot removed for scintillation counting. The remaining soln was filtered, and subjected to HPLC analysis. Fractions containing pinoresinol **6**, piperitol **7**, sesamin **1**, and sesamolin **4** were individually isolated and subjected to scintillation counting analysis (see Table 1).

Administration of E-[8-^{14}C]coniferyl *alcohol* **5** *to S. indicum seeds.* An aqueous soln of *E*-[8-^{14}C]coniferyl alcohol **5** (93 μg, 1.76 kBq, 3.39 kBq μmol^{-1}) was administered to approximately 400 mg of seeds (freshly removed from the pods). After absorption of the precursor, distilled H_2O was added to prevent dryness as above. After 12 hr metabolism, the seeds were harvested, freeze-dried, extracted, and the purified lignans **6**, **7**, **1**, and **4** analyzed as described above (see Table 1).

Administration of (±)-[3,3′-*O*$^{14}CH_3$]*pinoresinols* **6a/6b** *and* (±)-[3-*O*$^{14}CH_3$]*piperitols* **7a/7b** *to S. indicum seeds.* Aq. solns (5% DMSO, 5% Tween 80) of (±)-[3,3′- $O^{14}CH_3$]pinoresinols **6a/6b** (72.3 μg, 9.71 kBq, 48.1 kBq μmol^{-1}), and (±)-[3- $O^{14}CH_3$] piperitols **7a/7b** (387 μg, 1.25 kBq, 1.15 kBq μmol^{-1}) were individually administered to approximately 400 mg of seeds (freshly removed from the pods), for periods of 20 min to 96 hr and 8hr, respectively. After metabolism, the seeds were harvested, freeze-dried, extracted, with the lignans purified and analyzed as described above (see Table 1).

Administration of (±)-[7,7′-$^2H_2$]pinoresinols ***6a/6b*** *to S. indicum seeds.* (±)-[7,7′-$^2H_2$]Pinoresinols **6a/6b** (3 mg) in H_2O (1 ml) were administered to *S. indicum* seeds (10 g) over 2 and 48 hr, respectively. Following metabolism, the resulting lignan extracts were obtained as before and loaded onto silica gel columns (3 x 1 cm). The fractions containing piperitol **7**, sesamin **1**, and sesamolin **4** were collected and subjected to C-18 reversed-phase HPLC. The individual lignans were then analyzed by MS (EI mode): EI MS *m/z* (rel. int.): (+)-[7,7′-$^2H_2$]piperitol **7a**, 358 $[M+2]^+$ (78) 357 $[M+1]^+$ (10), 356 $[M]^+$ (6), 328 (56), 205 (14), 182 (13), 162 (10), 153 (22), 152 (87), 136 (31); (+)-[7,7′-$^2H_2$]-sesamin **1**: 356 $[M+2]^+$ (30), 355 $[M+1]^+$ (32), 354 $[M]^+$ (38), 323 (56), 219 (6), 204 (8), 203 (20), 162 (15), 161 (35), 151 (12), 150 (29), 149 (100), 137 (17), 136 (41), 122 (22), 121 (16).

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