Lignan Analysis in Seed Oils from Four *Sesamum* Species: Comparison of Different Chromatographic Methods

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Different chromatographic methods, thin-layer chromatography (TLC), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS) and normal- and reversed-phase high-performance liquid chromatography (HPLC), were compared for their ability to separate the different lignans present in four *Sesamum* species, *viz., S. indicum* Linn., *S. alatum Thonn., S. radiatum* Schum & Thonn. and *S. angustifolium* (Oliv.) Engl. The advantages and limitations of each method are discussed, and a combination of methods is suggested for qualitative analyses. Two-dimensional TLC was found to be a valuable qualitative technique and one-dimensional TLC is useful for preparative purposes. GC is a good supplement for qualitative analysis, but it had many limitations as a quantitative tool—it involves many preparative steps, no suitable internal standard was found to be commercially available and the various lignans had markedly different response factors. GC/MS is a necessary technique to confirm the identity of the lignans present. HPLC is a one-step technique suitable for quantitative analyses, and is fast and simple because it involves direct injection of oil solutions. Reversed-phase HPLC was unable to separate sesamolin and sesangolin, but a normal-phase silica column provided satisfactory separation for these two lignans. 2-Episesalatin of *S. alatum*, however, did not elute from the normal-phase column. Once lignans are identified, a relevant HPLC method can be used for quantitative analyses. Sesamin was present in large amounts in *S. radiatum*, in considerable amounts in *S. indicum* and *S. angustifolium*, and in small amounts in *S. alatum*. Sesamolin occurred in considerable amounts in *S. indicum* and *S. angustifolium*, but only in small amounts in the other two wild species studied. *Sesamum alatum* was characterized by high amounts of 2-episesalatin, and *S. angustifolium* was characterized by high levels of sesangolin.

KEY WORDS: 2-Episesalatin, GC, GC/MS, HPLC, lignans, seed oils, sesame, sesamin, sesamolin, *Sesamum*, sesangolin, TLC.

Two lipid-soluble furofuran lignans, sesamin and sesamolin, are the main components of the unsaponifiable fraction in the seed oils from *Sesamum indicum* Linn. (1,2). These two compounds have been linked to many of the unique chemical and physiological properties of sesame seed oil (2). Two phenolic antioxidant factors, sesamol and sesaminol, are present in sesame seed oil in trace amounts (3), but are liberated from sesamolin by acid clay bleaching during the refining of unroasted sesame seed oil (4). Sesamol was also reported to be liberated from sesamolin during frying, especially when refined oil from roasted seeds was used (5). Sesamolin, although not having any antioxidative properties in itself, thus is an important precursor to the phenolic antioxidants, and its presence in the oil could be of great value.

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Improving the oil content of the seeds, modifying the fatty acid composition and increasing the content of antioxidants in sesame oils are important objectives of current sesame plant breeding (6,7). Intensified collection and investigation of the wild species of the genus *Sesamum* were recommended (7,8). The seed oils from three related wild *Sesamum* species from Sudan, *viz., S. alatum* Thonn., *S. radiatum* Schum & Thonn. and *S. angustifolium* (Oliv) Engl., were studied for their fatty acid and triacylglycerol compositions (9), for their des-, mono- and di-methyl sterols (10), and for the patterns of their unsaponifiable fractions in two-dimensional thin-layer chromatography (TLC) (11).

The structures of sesamin and its related compounds in *Sesamum* oils are shown in Figure 1. 2-Episesalatin, a furofuran lignan related to sesamin, was isolated from the seed oil of *S. alatum* (12). Another related lignan, sesangolin, has been isolated from the seed oil of *S. angolense* (13). Preliminary reports on the presence of sesamin-type lignans in some wild *Sesamum* species have been published (14,15). Bedigian *et al.* (14) screened the seed oils from eight *Sesamum* species for sesamin and sesamolin by TLC. They reported that sesamin and sesamolin occurred in the seed oils of *S. indicum, S. angolense, S. angustifolium* and *S. calycinum*. Oils from *S. latifolium* and *S. radiatum* contained sesamin but not sesamolin, whereas, oils from *S. alatum* and *S. capense* were void of both lignans. Fukuda *et al.* (15) used high-performance liquid chromatography (HPLC) to provide quantitative data on the levels of sesamin and sesamolin in the three wild species—*S. radiatum, S. schinzianum* and an unknown sample from Malaysia.

This paper describes and compares different chromatographic methods [TLC, gas chromatography (GC), GC/mass spectrometry (GC/MS) and HPLC] developed in our laboratory or modified from published works to identify the major lignans in the seed oils of *S. indicum, S. alatum, S. radiatum* and *S. angustifolium*.

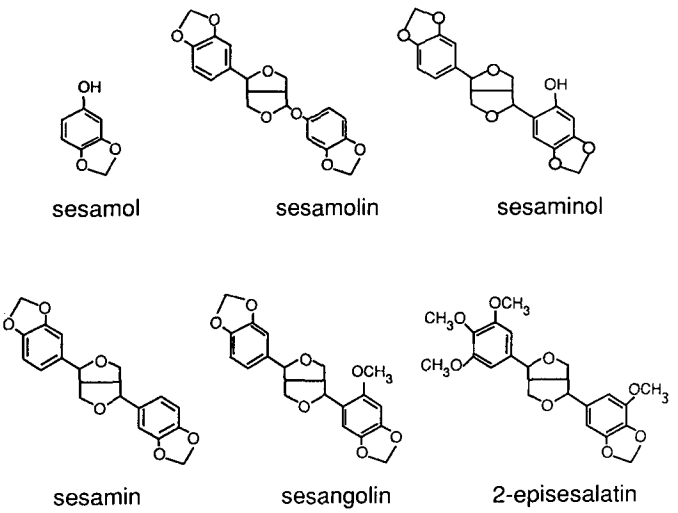

FIG. 1. The chemical structures of sesamin and related compounds in *Sesamum* oils.

MATERIALS AND METHODS

Seeds and reagents. Seeds from different cultivars of *S. indicum* Linn. and from different collections of the three related wild species, *S. alatum* Thonn., *S. radiatum* Schum & Thonn. and *S. angustifolium* (Oliv.) Engl., were collected from different locations in Sudan, as presented elsewhere (9).

All solvents were of analytical grade (E. Merck, Darmstadt, Germany) and were used without further purification. Pre-coated Silica-gel 60 TLC plates (20 × 20 cm, 0.25 mm layer thickness) were also purchased from Merck. Purified sesamol, sesamin and sesamolin standards were donated by Dr. Yasuko Fukuda (Ichimura Gakuen Junior College, Aichi, Japan). Sitosterol was purchased from Sigma Chemical Co. (St. Louis, MO), and cycloartenol was a gift from Prof. Takashi Kaneda (Faculty of Home Economics, Kohriyama Womens College, Fukushima, Japan). Tri-Sil (Pierce Chemical Co., Rockford, IL) was used for the preparation of the trimethylsilyl (TMS) derivatives of the unsaponifiables. Tocopherol standards (DL α-, β-, γ- and δ-tocopherol) were purchased as an isomer kit from Merck. α-Tocotrienol was a gift from Roche Products AB (Skärholmen, Sweden).

Oil extraction and saponification. Oven-dried (103°C, 4 h) sesame seeds (5 g) were extracted in a Soxhlet apparatus for 6 h with 150 mL *n*-hexane. The oils obtained were saponified by reflux with ethanolic potassium hydroxide (50 mL, 1 M, 1 h). The unsaponifiables were extracted twice with 40 mL diethyl ether. The ether extracts were washed with water and dried over anhydrous sodium sulfate according to IUPAC (16). The ether was evaporated *in vacuo* at *ca.* 25°C, and the unsaponifiables were kept in chloroform/diethyl ether (4:1, vol/vol) solutions at −20°C for further analyses.

Isolation of 2-episesalatin and sesamin. Upon cold storage (4°C, 3 d) of the *n*-hexane extracts of *S. alatum* and *S. radiatum*, 2-episesalatin precipitated as white, crystalline solid from *S. alatum* extracts (12), and sesamin precipitated from *S. radiatum* extracts. These compounds are present in high concentrations in these two species. The purity of the compounds was checked by TLC and ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR.

TLC. Portions of the unsaponifiables (*ca.* 1.5 mg) were applied as 1.5-cm bands by means of an autoapplicator, Linomat-3 (CAMAG, Muttenz, Switzerland). One-dimensional TLC (1D-TLC) was performed with the following mobile phases: petroleum ether/diethyl ether/acetic acid (PDEA; 70:30:1, vol/vol/vol), chloroform/benzene/methanol (CBM; 60:40:1, vol/vol/vol) and chloroform/diethyl ether (CDE; 90:10, vol/vol). Two-dimensional TLC (2D-TLC) was performed with hexane/diethyl ether (HDE; 70:30, vol/vol) and CDE (90:10, vol/vol) in the first and second dimensions, respectively, as previously described (11). The plates were visualized by spraying with 10% phosphomolybdic acid in ethanol/diethyl ether (1:1, vol/vol) and heating at 110°C for 5–10 min. Spots were identified against the authentic standards (sitosterol, cycloartenol, sesamol, sesamin, sesamolin, 2-episesalatin and γ-tocopherol) applied as reference spots on both sides of each plate. Identification of sesangolin was achieved by GC/MS analysis of extracts from preparative TLC.

For preparative TLC separations, the CDE system (10) was used as the mobile phase. The developed chromatograms were allowed to dry for a few seconds. The plates were then covered with other glass plates, leaving the reference zone exposed. Reference standards were visualized by exposing the plate to iodine vapor. The region with R_f 0.47–0.69 was scraped and eluted twice with chloroform/methanol (4:1, vol/vol).

Preparation of samples for GC and GC/MS. A standard mixture of known amounts of γ-tocopherol, sesamin, sesamolin and 2-episesalatin was prepared. A small portion of the fraction obtained from preparative TLC (R_f 0.47–0.69, CDE) of the unsaponifiables of the oil of *S. angustifolium* was added to provide sesangolin. The standard mixture was used in GC and GC/MS analyses to determine the relative retention time (RRT) values of these standards, their response factors (RF) and their mass spectra.

Fractions obtained from preparative TLC (R_f 0.47–0.69, CDE) or portions of the total unsaponifiables of each species were placed in glass-stoppered test tubes. The solvents were evaporated under nitrogen, and the TMS ether derivatives of the mixture were prepared by adding 100 μL of the Tri-Sil reagent. The tubes were shaken in a Vortex mixer to dissolve the samples in the reagent and then heated at *ca.* 60°C for 30 min. The TMS reagent was then removed under nitrogen, and the residue was dissolved in *n*-hexane and stored at −20°C until injected into GC.

GC. Samples (1–2 μL) were injected into a cross-linked methyl silicone capillary column (HP-1, 25 m × 0.32 mm i.d., film thickness 0.52 μm; Hewlett-Packard, Avondale, PA), which was fitted into a Varian 3700 gas chromatograph (Varian Analytical Instruments, Palo Alto, CA) equipped with a flame-ionization detector and a falling-needle injector (Chrompack, Middelburg, The Netherlands). The column and detector temperatures were 245 and 330°C, respectively, and helium was used as carrier gas at a flow rate of 2.2 mL/min. Peaks were recorded and peak areas computed on an HP 3390 A integrator (Hewlett-Packard).

GC/MS. GC/MS analyses were performed on a Finnigan 9610 gas chromatograph (Finnigan Corp., Cincinnati, OH) coupled to a Finnigan 4000 mass spectrometer with an Incos data system (San Jose, CA). The TMS ether derivatives of the standard mixture and the unsaponifiables were injected into a CP Sil 5 column (25 m × 0.25 mm i.d.; Chrompack) by on-column injection. The column temperature was initially 90°C for 2 min, then programmed to 260°C at 20°C/min and finally kept at 260°C for 40 min. Helium was used as the carrier gas at a flow rate of 1.8 mL/min. The mass spectra were recorded at an electron energy of 70 eV, and the ion source temperature was 250°C. The spectra were scanned in the range of *m/e*; 100–500 at 1.1 scans/s.

HPLC. HPLC analyses were performed on an SP 8700 HPLC system with SP 8750 organizer module (Spectra Physics, San Jose, CA). The oils were dissolved in hexane/chloroform (2:1, vol/vol), and 10-μL samples were used for injections.

Reversed-phase separations were performed with two Chrom Spher C18 (10 cm × 3 mm i.d., particle size 5 μm; Chrompack) columns connected in series. The mobile phase was 70% methanol in water (4) at a flow rate of 0.4 mL/min. Peaks were detected at 290 nm with a Lambdamax model 480 U.V. detector (Waters, Milford, MA) and were recorded with an HP 3390 A integrator.

The samples were also analyzed for the tocopherols and lignans by normal-phase HPLC on two Chrom Spher (10 cm × 3 mm i.d., particle size 5 μm; Chrompack) silica columns connected in series. The mobile phase was 6% diethyl ether in *n*-heptane at a flow rate of 0.7 mL/min for 15 min and then 1.5 mL/min for 25 min. Peaks were detected with a Perkin-Elmer LS-2 filter fluorimeter (Perkin-Elmer, Norwalk, CT) at an excitation wavelength of 295 nm and an emission wavelength of 320 nm. Peaks were recorded with an HP 3390 A integrator and were identified by comparison with standards and quantitated against β-tocopherol as internal standard.

RESULTS AND DISCUSSION

TLC. 1D-TLC for the separation of the unsaponifiables from the four *Sesamum* species with the two mobile systems-(PDEA) (70:30:1, vol/vol/vol) and CBM (60:40:1, vol/vol/vol) previously used by Bedigian *et al.* (14), are presented in Figure 2, and the results from these separations are summarized in Table 1.

The separation provided by the PDEA system (Fig. 2, top) was comparable to that of the HDE (80:20, vol/vol) system previously used by Itoh *et al.* (17). In the PDEA system, sesamin extended from the desmethyl sterols to the monomethyl sterols, depending on its concentration. Sesamolin overlapped slightly with the dimethyl sterols. *Sesamum indicum* gave large spots for both sesamin (R_f 0.25–0.33) and sesamolin (R_f 0.39–0.43). *Sesamum alatum* had only minor spots for sesamin and sesamolin but a large spot for 2-episesalatin (R_f 0.01–0.08). *Sesamum radiatum* showed a large sesamin/desmethyl sterols spot and no sesamolin spot, while *S. angustifolium* showed a large spot for sesangolin plus sesamin plus desmethyl sterols (R_f 0.10–0.32) and a minor sesamolin spot.

The CBM (60:40:1, vol/vol/vol) system, on the other hand, gave TLC patterns similar to those obtained by the CDE system previously described (11). In the CBM system (Fig. 2, bottom), sesamin and sesamolin were close to γ-tocopherol (R_f 0.55–0.60) in region c (R_f 0.44–0.69, CBM), while 2-episesalatin, the major lignan in *S. alatum*, extended between the desmethyl- and monomethyl-sterols in another region, b (R_f 0.24–0.44, CBM). In this system, *S. indicum* had moderate-sized spots for both sesamin (R_f 0.47–0.54) and sesamolin (R_f 0.61–0.68). Comparatively, *S. alatum* showed two minor spots at R_f values corresponding to sesamin and sesamolin. *Sesamum radiatum* had a large spot extending over the whole area of sesamin and γ-tocopherol, and had no sesamolin spot. Large amounts of sesamin were present in this species and could also be estimated by weighing the sesamin precipitated upon cold storage of the hexane-extracted oil (*ca.* 2–2.5%). *Sesamum angustifolium* gave a large spot at R_f 0.45–0.67. When this spot was scraped, eluted and analyzed by HPLC and GC/MS, it was found to be a mixture of γ-tocopherol, sesamin, sesamolin and a large amount of sesangolin.

2D-TLC of the unsaponifiables of *S. radiatum* and *S. angustifolium* are presented in Figure 3. Those of *S. indicum* and *S. alatum* were presented in a previous paper (11), where *S. alatum* had minor spots for both sesamin and sesamolin. In this separation, *S. radiatum* displayed a large spot for sesamin. In *S. angustifolium*, the joint sesangolin/sesamin spot in the CDE direction separated into two spots in the HDE direction. Faint elongated spots were observed in both species. The elongated spot in *S. angustifolium* corresponds to sesamolin, but that in *S. radiatum* is closer to the sesamin spot and could not be assigned to sesamolin. Some minor unknown spots were also observed in the 2D-TLC, especially in *S. alatum* and *S. angustifolium.*

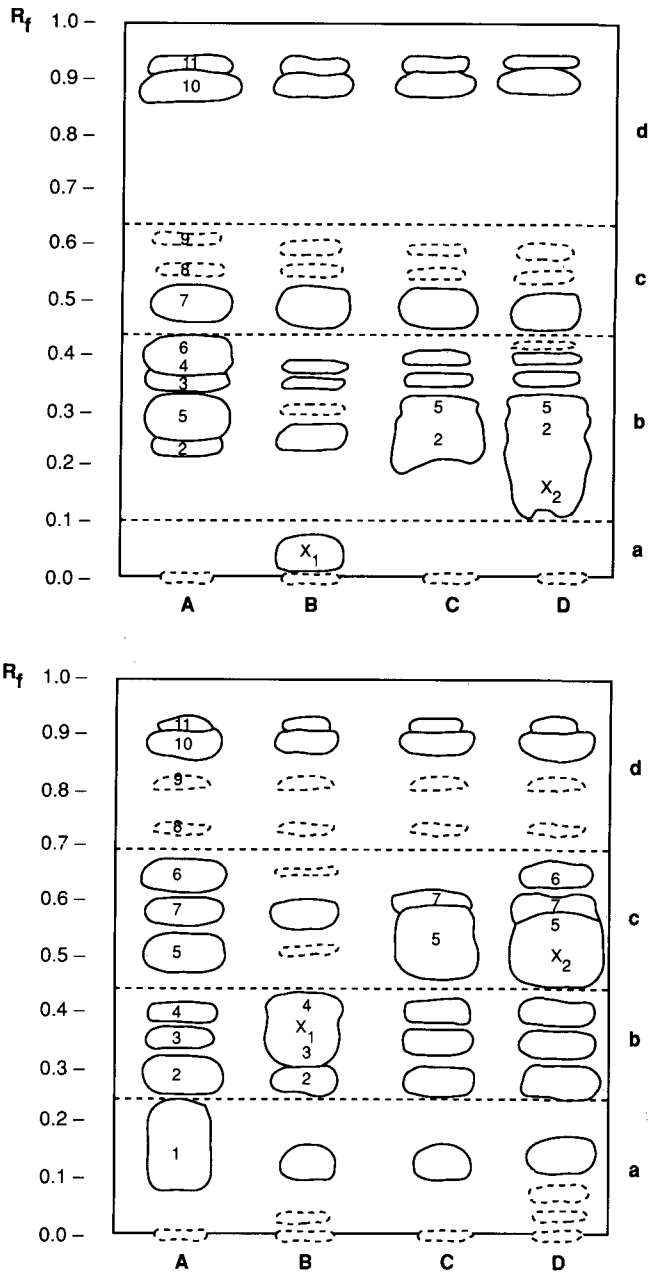

FIG. 2. One-dimensional thin-layer chromatography separations of the unsaponifiables of A, *Sesamum indicum*, B, *S. alatum*, C, *S. radiatum* and D, *S. angustifolium* on Silica-gel 60 plates with the solvents: top panel: petroleum ether/diethyl ether/acetic acid (70:30:1, vol/vol/vol); and bottom panel, chloroform/benzene/methanol (60:40:1, vol/vol/vol). Spots; (2) desmethylsterols, (3) monomethylsterols, (4) dimethylsterols, (5) sesamin, (6) sesamolin, (7) γ-tocopherol, (X_1) 2-episesalatin, (X_2) sesangolin, and (1, 8–11) unknowns.

TABLE 1

Levels of Sesamin and Sesamolin in the Four *Sesamum* Species as Assayed by Thin-Layer Chromatography[a]

	Sesamin		Sesamolin	
Species	CBM 0.52^b	PDEA 0.31^b	CBM 0.58^b	PDEA 0.41^b
Sesamum indicum	++	++	++	++
S. alatum[c]	trace	trace	trace	n.d.
S. radiatum	++	+++	n.d.	n.d.
S. angustifolium[d]	++++	+	+	n.d.

[a]CBM, chloroform/benzene/methanol (60:40:1, vol/vol/vol); PDEA, petroleum ether/diethylether/acetic acid (70:30:1, vol/vol/vol); (+to++++) represents visual assessment of color intensities after spraying with phosphomolybdic acid; trace, intensity <<+; n.d., not detected.

[b]Numbers are solvent R_f.

[c]Also large spots (++++) of 2-episesalatin (R_f 0.37 in CBM, and R_f 0.05 in PDEA).

[d]A mixture of sesamin and sesangolin in the CBM system, and an additional spot (+++++) of sesangolin appeared in PDEA (centered at R_f 0.19).

Different TLC systems were thus used to screen the four species for the separation patterns of their unsaponifiables. Our findings are in good agreement with the results of Bedigian *et al.* (14) on the presence of sesamin and sesamolin in the seed oils of *S. indicum* and *S. angustifolium* and the presence of sesamin only in *S. radiatum*. However, in this study we observed faint spots for both sesamin and sesamolin in the oil from *S. alatum*, in contrast to the findings Bedigian *et al.* (14). The reason may be related to the different samples analyzed or to the fact that Bedigian *et al.* (14) applied the total oil on TLC plates, whereas we used the unsaponifiables, where these lignans are more concentrated. In addition, use of TLC alone prevented the observation of spots for 2-episesalatin and sesangolin in *S. alatum* and *S. angustifolium*, respectively (14).

TLC was also used as a preparative step for GC. Based on results from different TLC separations, the CDE system (region with R_f 0.47–0.69) is suggested as a preparative TLC step for the separation of the major lignans (sesamin, sesamolin and sesangolin) from *S. indicum, S. radiatum, S. angustifolium* and species with similar TLC patterns. However, this region is not adequate for *S. alatum* and closely related species where there might also be some other major or minor lignans similar to 2-episesalatin in another region (R_f 0.25–0.47, CDE). The entire unsaponifiable fraction should be used in such cases.

GC. Figure 4 shows the gas-chromatographic separation of a mixture of authentic samples of γ-tocopherol, sesamin, sesamolin, 2-episesalatin and extracts of *S. angustifolium* (from preparative TLC, CDE) to provide sesangolin. The identification of the various compounds in the different seed oil samples was made by comparison of their retention times (Fig. 4) and their MS fragmentation patterns (Table 2) with those of the standards. Sesangolin was identified entirely from the GC/MS fragmentation pattern (on theoretical basis) due to lack of a standard sample.

A major drawback of the GC method is the considerable difference in the response factors of the different compounds. Sesamin (retention time, R_t, 11.85 min; RRT 1.00) had a detection limit of *ca.* 1 ng/injection. Sesamolin (RRT 1.14) was exceptional in having a very high response factor (RF 4.00, detection limit *ca.* 4 ng/injection) as compared to sesamin (RF 1.00). 2-Episesalatin (RRT 2.47) had a response factor of 0.40. The response factor of sesangolin (RRT 1.56) was not determined due to lack of an authentic sample. The high response factor of sesamolin made it difficult for this system to detect small amounts of this lignan.

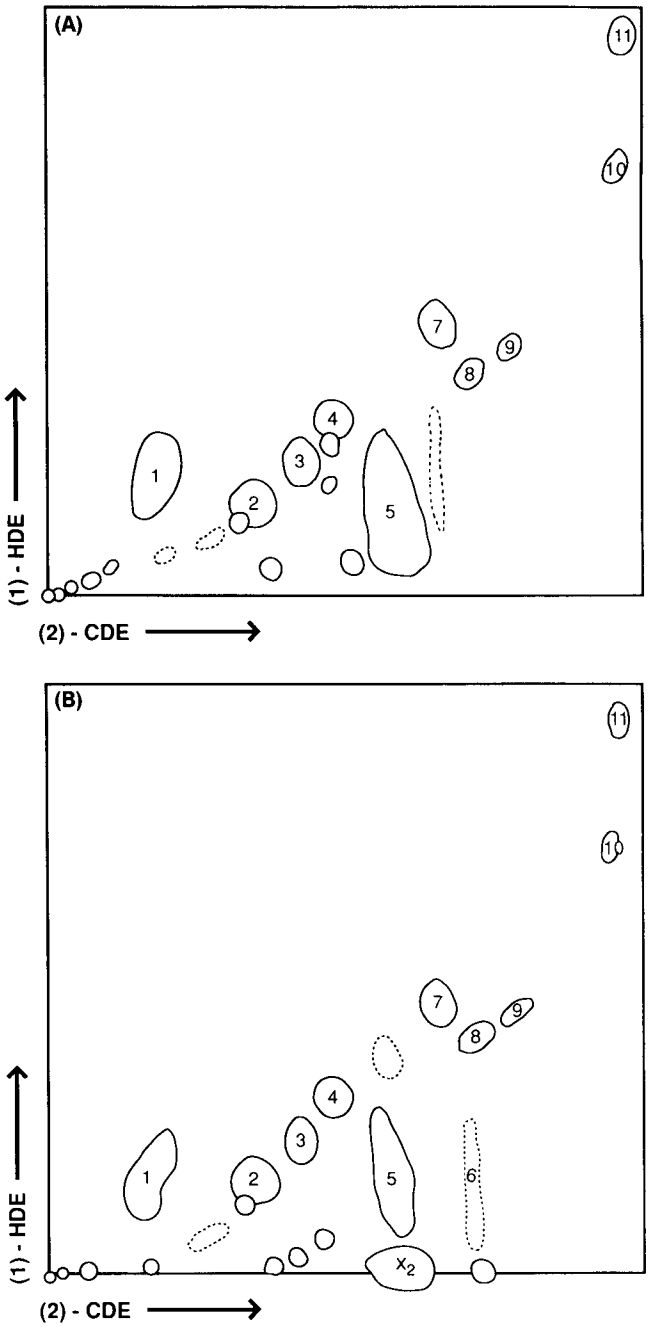

FIG. 3. Two-dimensional thin-layer chromatography separations of the unsaponifiables of: A, *Sesamum radiatum*; and B, *S. angustifolium* on Silica-gel 60 plates with the solvents: (1) HDE, *n*-hexane/diethyl ether (7:3, vol/vol) and (2) CDE, chloroform/diethyl ether (9:1, vol/vol). Spots: (2) desmethylsterols, (3) monomethylsterols, (4) dimethylsterols, (5) sesamin, (6) sesamolin, (7) γ-tocopherol, (X_2) sesangolin and (1,8–11) unknowns.

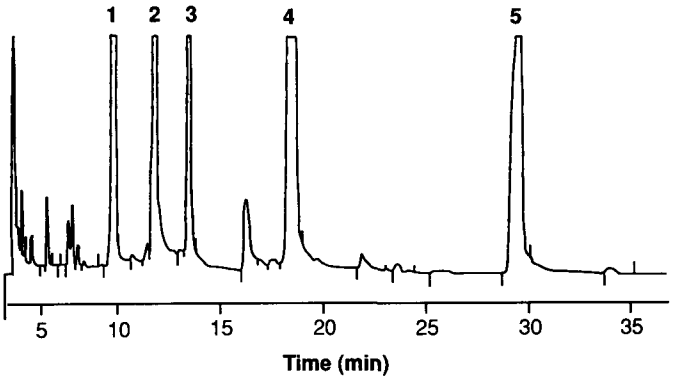

FIG. 4. Gas chromatography separation of a standard mixture of 1, γ-tocopherol, 2, sesamin; 3, sesamolin; 4, sesangolin and 5, 2-episesalatin. Column: HP-1, 245°C, carrier gas: He, 2.2 mL/min detector, flame-ionization detection, 330°C.

GC and GC/MS analyses of the extracts from preparative TLC showed sesamin to be present in considerable amounts in *S. indicum* and *S. angustifolium*, and in large amounts in *S. radiatum*, but only in small amounts in *S. alatum*. Sesamolin was detected in considerable amounts in *S. indicum*, and only small peaks for this compound were observed in the chromatograms of *S. alatum* and *S. angustifolium*. The major lignan constituent of *S. angustifolium* was sesangolin, the lignan previously isolated from *S. angolense* (13), and 2-episesalatin was the major lignan in *S. alatum*. Chromatograms from GC and GC/MS analyses showed minor peaks for other possible sesamin analogues in the three wild species (*m/z*: 149, 135, together with other relevant ions, data not shown).

When the TMS ether derivatives of the total unsaponifiables were directly analyzed in the same GC system (HP-1 column, 245°C), satisfactory separations were obtained for sesamin and sesamolin, which eluted early in the chromatogram before the sterols. Sesangolin co-eluted with sitosterol, the major sterol constituent, and 2-episesalatin co-eluted with cycloartenol and cycloeucalenol, two minor sterol constituents (unpublished observations).

If the present method could be optimized by temperature programming, or another GC column could provide a better separation, direct GC analysis of the unsaponifiables may be a promising tool in the qualitative analysis of lignan-type compounds in *Sesamum* oils. The large differences in response factors and the high response factor of sesamolin may, however, still be a factor limiting the applicability of GC to quantitative analysis.

HPLC. Lignans in the oils from the four *Sesamum* species were analyzed by a reversed-phase HPLC system comparable to that used by Fukuda *et al.* (4). This system gave good separation of 2-episesalatin (RRT 0.66) and sesamin (R_t 6.66 min, RRT 1.00). The separation of sesamolin (RRT 1.32) and sesangolin (RRT 1.22) by this HPLC method is, however, difficult and seemed to give a twin peak when either of them is present in large amounts, as is the case in *S. angustifolium* with high levels of sesangolin (Fig. 5).

Normal-phase HPLC (Fig. 6) was able to provide a better separation for sesamol, sesamin, sesamolin and sesangolin, and also separated the tocopherols (α-, β-, γ-, δ-tocopherols and α-tocotrienol), thereby making it a good system for co-analysis of these components. This observation was made "incidentally" in the analysis of tocopherols, which are normally quantitated by a fluorescence detector. If only sesamin-type compounds are to be analyzed by normal-phase HPLC, an ultraviolet detector is quite adequate. 2-Episesalatin, however, did not elute under the conditions used for normal-phase HPLC analysis. Thus, this HPLC system is needed for qualitative and quantitative analysis of sesamolin and sesangolin in *S. angustifolium*, but is not the system of choice for the analysis of the lignans of *S. alatum*, or lignans as polar as or more polar than 2-episesalatin.

HPLC analyses indicated that sesamin is present in very high amounts in *S. radiatum*, in high amounts in *S. indicum* and *S. angustifolium*, and in low amounts in *S. alatum*. Sesamolin was found to occur in high amounts in *S. indicum* and *S. angustifolium*, but only in low amounts in the other two wild species studied. *Sesamum alatum* was characterized by high amounts of 2-episesalatin, and *S. angustifolium* was characterized by high levels

TABLE 2

Main Fragmentation Ions Used in the Identification of the Lignans

Lignan	Sesamin	Sesamolin	Sesangolin	2-Episesalatin
M^+ (%)	354(5)	370(1)	384(10)	430(23)
M-ArCHO$^+$	204(−)	220(−)	234(−)	250(1)
	—	204(1)	204(1)	234(5)
M-ArCHO-H$^+$	203(8)	219(−)	233(−)	249(3)
	—	203(8)	203(8)	233(8)
Ar.CH=CH.CH$_2^+$	161(25)	177(−)	191(20)	207(31)
	—	161(1)	161(22)	191(25)
Ar.CHO$^+$	150(42)	166(−)	180(16)	196(19)
	—	150(5)	150(22)	180(32)
Ar.CO$^+$	149(100)	165(−)	179(30)	195(19)
	—	149(10)	149(100)	179(100)
Ar.CH$_2^+$	135(41)	151(5)	165(27)	181(59)
	—	135(100)	135(29)	165(84)
Ar$^+$	121(19)	137(20)	151(7)	167(10)
	—	121(3)	121(12)	151(27)
Others	—	233(5)	—	197(36)
	—	—	—	169(57)
	—	—	—	166(30)

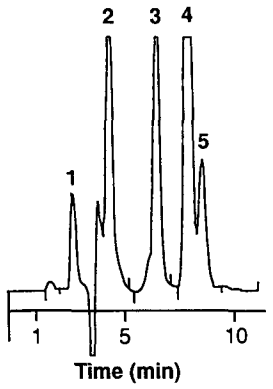

FIG. 5. High-performance liquid chromatography separation of a standard mixture of: 1, sesamol; 2, 2-episesalatin; 3, sesamin; 4, sesangolin and 5, sesamolin. Column: Chrom Spher C18, Mobile phase, methanol/water (7:3, vol/vol); 0.4 mL/min); detector, UV, 290 nm.

of sesangolin. These results are in agreement with those from GC analyses. Quantitative data on the levels of the different lignans are reported in the companion paper (18). Fukuda *et al.* (15) used only reversed-phase HPLC for quantitative determinations and reported the following levels (in mg/kg oil) of sesamin and sesamolin, respectively, in three wild species: *Sesamum radiatum* from India (256, 36), *S. schinzianum* from Indonesia (546, 108) and an unknown sample from Malaysia (1152, 1361). Based on our observations on reversed-phase HPLC separations, it is also possible that the HPLC system of Fukuda *et al.* (15) had given a twin peak for sesamolin and sesangolin and might have caused confusion in the interpretation of the peaks obtained for two compounds in the wild species studied. Because sesamolin is a known precursor of two antioxidants, sesamol and sesaminol (4,5), the presence of either sesamolin or sesangolin in the wild species has to be confirmed by other methods (2D-TLC, GC, normal-phase HPLC or GC/MS).

2D-TLC is a valuable technique for the qualitative analysis of the various lignans in the four *Sesamum* species. Due to its simplicity, it is recommended for screening work, notably in less advanced laboratories. 1D-TLC with CDE (90:10, vol/vol) is useful for preparative TLC separations of the lignans from the unsaponifiables of *S. indicum, S. radiatum* and *S. angustifolium* (but not those of *S. alatum*) for further GC separations.

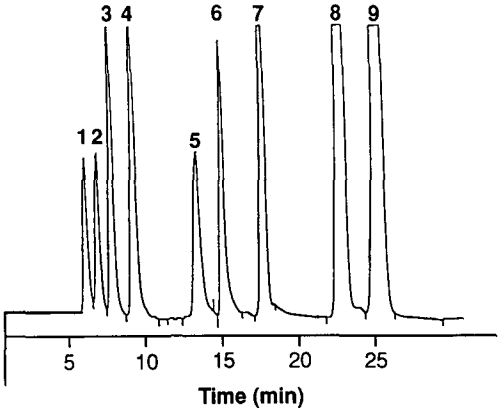

FIG. 6. High-performance liquid chromatography separation of a standard mixture of: 1, α-tocopherol; 2, α-tocotrienol; 3, β-tocopherol; 4, γ-tocopherol; 5, δ-tocopherol; 6, sesamol; 7, sesamolin, 8, sesamin and 9, sesangolin. Column: Chrom Spher silica; mobile phase, diethyl ether/heptane (6:94, vol/vol); detector fluorescene, excitation 295 nm, emission 320 nm.

GC and GC/MS analyses are necessary to establish the identity of the lignans present. A major advantage of GC is the availability of MS as a reliable detector for the identification of the compounds present in addition to retention data. However, GC was not useful to quantitate the lignans for a number of reasons. It involves many preparative steps (saponification, TLC and silylation). In addition, considerable efforts to find a suitable commercially available internal standard (*viz.*, a lignan of comparable structure and chromatographic retention data) to account for losses during these steps were unsuccessful. Also, the system responded differently to the various compounds, and sesamolin had a high response factor.

The HPLC systems used are simple and fast, as they involve direct injection of oil solutions. HPLC, however, generally lacks specific detectors because the availability of HPLC/MS is quite exclusive. The reversed-phase HPLC system did not provide satisfactory separation of sesamolin and sesangolin, whereas 2-episesalatin did not elute from the normal-phase HPLC system. Hence, the reversed-phase system could be used for the analysis of the seed oil lignans of *S. indicum, S. alatum* and *S. radiatum*, but not *S. angustifolium*. The normal-phase system, on the other hand, is suitable for the analysis of the lignans of *S. indicum, S. angustifolium* and *S. radiatum*, but not *S. alatum*..

A combination of methods seemed to be optimal, at present with 2D-TLC, GC, GC/MS and/or different HPLC systems to establish the identity of the lignans present. Then the appropriate HPLC method should be applied for quantitative analysis. This combination of methods will be useful for genetic, phytochemical or chemotaxonomic studies of the unexplored wild species of the genus *Sesamum*. This may be quite interesting because the identity and levels of the lignans in the different *Sesamum* species seem to be genetically controlled with only marginal contribution from year or locality of production (18). Additional efforts to develop HPLC and/or GC system(s) for better separations of the sesamin-related compounds in the different *Sesamum* species are desirable.

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REFERENCES

1. Tashiro, T., Y. Kukuda, T. Osawa and M. Namiki, *J. Am. Oil Chem. Soc. 67*:508 (1990).
2. Budowski, P., and K.S. Markely, *Chem. Revs. 48*:125 (1951).

3. Fukuda, Y., and T. Osawa, *Agric. Biol. Chem. 49*:301 (1985).
4. Fukuda, Y., M. Nagata, T. Osawa and M. Namiki, *J. Am. Oil Chem. Soc. 63*:1027 (1986).
5. Fukuda, Y., M. Nagata, T. Osawa and M. Namiki, *Ibid. 50*:857 (1986).
6. Ashri, A., in *Oil Crops of the World*, edited by G. Röbbelen, R.K. Downey and A. Ashri, McGraw-Hill, Inc., New York, 1989, pp. 375–387.
7. Anonymous, in *Sesame: Status and Improvements*, edited by A. Ashri, FAO Plant Production and Protection, Paper 29, 1981, p. 192.
8. Anonymous, in *Sesame and Safflower: Status and Potentials*, edited by A. Ashri, FAO Plant Production and Protection, Paper 66.
9. Kamal-Eldin, A., G. Yousif, L.Å. Appelqvist, and G.M. Iskander, *J. Fat Sci. and Technol. 94*:254 (1992).
10. Kamal-Eldin, A., L.Å. Appelqvist, G. Yousif and G.M. Iskander, *J. Sci. Food and Agric. 59*:327 (1992).
11. Kamal-Eldin, A., G. Yousif and L.Å. Appelqvist, *J. Am. Oil Chem. Soc. 68*:844 (1991).
12. Kamal-Eldin, A., and G. Yousif, *Phytochemistry 31*:2911 (1992).
13. Jones, W.A., M. Beroza and E.D. Becker, *J. Org. Chem. 27*:3232 (1962).
14. Bedigian, D., D.S. Seigler and J.R. Harlan, *Biochem. System. and Ecology 13*:133 (1985).
15. Fukuda, Y., T. Osawa, S. Kawagishi and M. Namiki, *J. Japanese Soc. Food Sci. and Technol. 35*:483 (1988).
16. *IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn., edited by C. Paquot, and A. Hautfenne, Blackwell Sci. Publications, 1987, pp. 157–161.
17. Itoh, T., T. Tamura and T. Matsumoto, *J. Am. Oil Chem. Soc. 50*:300 (1973).
18. Kamal-Eldin, A., and L.Å. Appelqvist, *Ibid.*, 71:149 (1994).

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