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# CHROMATOGRAPHIC ANALYSES OF ISOMERIC SHOGAOL COMPOUNDS DERIVED FROM ISOLATED GINGEROL COMPOUNDS OF GINGER (*ZINGIBER OFFICINALE* ROSCOE)

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## SUMMARY

Isomeric shogaols were derived by thermal dehydration of isolated gingerols of ginger (*Zingiber officinale* Rescoe), isolated by thin-layer chromatography and fractionated by preparative high-performance liquid chromatography. Two homologous series of isomeric shogaol compounds, *cis*- and *trans*,-6-shogaol, 8-shogaol, 10-shogaol, 12-shogaol and *syn*- and *anti*-methyl-6-shogaol, methyl-8-shogaol, methyl-10-shogaol, were identified by the combined results of analytical high-performance liquid chromatography, fast atom bombardment-mass spectrometry, gas chromatography, gas chromatography–mass spectrometry and $^1$H NMR spectroscopy. This is the first report of the presence of *cis* and *trans* isomers of shogaols and shogaols with methyl side-chains.

## INTRODUCTION

The pungent principles of fresh ginger (*Zingiber officinale* Roscoe) are composed primarily of gingerol compounds[1–9]. However, during storage or thermal processing, shogaol compounds, the dehydration products of gingerols, are formed and play an important role in the final product[1,3,6,10]. 6-Shogaol was first isolated and identified by Nomura and Tsurami[11–13]. The homologues of 6-, 8- and 10-shogaol were later identified by Connell and co-workers[1,2], and thermal dehydration under acidic condition was proposed for the conversion of gingerols into shogaols[10]. When

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the gingerols are injected into a gas chromatograph, a retro-aldol reaction generates zingerone and aldehydes with different chain lengths[1,2]. It was also found that a certain proportion of gingerols is thermally dehydrated and converted into shogaols[14]. Structural determinations of isolated shogaols showed that *trans* conformations were predominant[1,15].

It is worth noting that although 6-shogaol is derived from 6-gingerol, a pungency study indicated that 6-shogaol is much more pungent than 6-gingerol ($1.5 \cdot 10^5$ *vs.* $0.8 \cdot 10^5$ Scoville units)[3]. 6-Gingerol and 6-shogaol are the two most important pungent compounds of ginger products; other gingerols and shogaols are less important. For example, the pungencies of 8- and 10-gingerols and shogaols are less than $0.1 \cdot 10^5$ Scoville units[3].

Previous gas chromatographic (GC) analyses of isolated gingerols had shown that, besides the *trans*-shogaols that would be generated as cited[14], the corresponding *cis*-shogaols are also formed[9]. This report presents the further chromatographic analyses and identifications of isomeric shogaols by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), fast atom bombardment-mass spectrometry (FAB-MS), GC, GC–MS and $^1$H NMR spectroscopy.

## EXPERIMENTAL

### *Materials and chemicals*

Mature ginger (*Z. officinale* Roscoe) rhizomes were purchased from a supplier near Hsinchu (Taiwan). The rhizomes were washed, sliced, freeze-dried, ground and sieved (200 mesh). Solvents for TLC analyses (*n*-hexane, diethyl ether and methanol) were reagent grade (Merck) and glass-distilled. Solvents for HPLC analyses (methanol and water, HPLC-grade, Merck) were filtered (0.23 μm, Fluoropore, Millipore). TLC plates (20 cm × 20 cm, silica gel F-254) were obtained from Merck. Unless otherwise stated, all other chemicals were of reagent grade.

### *TLC isolation of shogaol fraction*

The oily liquid carbon dioxide extract was applied as a stripe on a silica gel plate (20 × 20 cm). Diethyl ether–*n*-hexane (7:3) was used as developing solvent. After development 16 cm from the origin, the plate was removed and examined under a UV lamp (254 nm). The blue zone under UV light with $R_F = 0.15$–$0.22$ was scraped off, desorbed by methanol, concentrated to dryness, dispersed in dilute sulphuric acid (1.5 *N*) and refluxed for 12 h, then neutralized with dilute sodium hydroxide. The synthetic mixture (gingerols and shogaols) was extracted twice with *n*-hexane–diethyl ether (4:6), washed with distilled water, dehydrated over anhydrous sodium sulfate and concentrated to minimal volume. The pungent shogaols were isolated by redeveloping on a 20 × 20 cm TLC plate ($R_F$ 0.40–0.45).

### *Preparative HPLC separation of shogaol compounds*

A Waters HPLC system was used (Waters Assoc., Milford, U.S.A.), which included two M-6000A pumps, a M-660 solvent programmer and a U6K injector. A Varian (Walnut Creek, CA, U.S.A.) Model 2050 UV detector and a Varian Model 4270 integrator were also used. Two stainless-steel columns (60 cm × 8 mm I.D.) connected in series were packed with reversed-phase absorbent (LiChroprep RP-18,

25–40 μm, Merck). A linear gradient from methanol–water (65:35) to pure methanol was used. The flow-rate was 2 ml/min. The time of each separation was 150 min with the first 100 min in gradient elution. Detection was based on UV absorption at 282 nm. Peaks eluted from 50 min to 120 min were collected, pooled and concentrated.

*Analytical HPLC*

A Hewlett-Packard (Palo Alto, CA, U.S.A.) 1084B HPLC system was used, equipped with two pumps, an adjustable auto-injector, a variable-wavelength UV detector, a built-in integrator and a reversed-phase column (RP-18, 20 cm × 4.6 mm I.D., 5 μm, Hewlett-Packard). A linear gradient from methanol–water (65:35) to pure methanol was used. The time of each analysis was 60 min with the first 50 min in gradient elution. The flow-rate was 1 ml/min. Detection was based on UV absorption at 282 nm. Scanning spectra (200–400 nm) of major shogaol compounds were obtained during analyses using the stop-flow method.

*FAB-MS*

Molecular ion determinations of isolated shogaols from preparative HPLC were conducted on a VG (VG Analytical, Manchester, U.K.) Model 7070 EQ mass spectrometer. The instrument was equipped with a FAB ion source. The fast atom gun (Ion Tech, Teddington, U.K.) was operated at 8 kV with currents of 1.0–1.5 mA. Xenon gas was used to bombard the sample. Samples were dissolved in glycerol and deposited on the target probe.

*NMR*

$^1$H NMR spectra were recorded at 400 MHz on a Bruker WH-400 NMR spectrometer in perdeuteromethane (Aldrich). Chemical shifts were referenced to tetramethylsilane (Aldrich) as internal standard.

*GC*

A Shimadzu (Tokyo, Japan) GC-8APTF gas chromatograph was equipped with a flame ionization detector, a capillary injection system (CLH-800, Shimadzu) and a fused-silica capillary column (50 m × 0.2 mm I.D., cross-linked OV-1, Hewlett-Packard). The operating conditions were as follows: injector and detector temperatures, 250°C; hydrogen carrier velocity, 12 cm/s; make-up nitrogen flow-rate, 30 ml/min; detector hydrogen flow-rate, 30 ml/min; detector air flow-rate, 300 ml/min; temperature program, 50°C to 250°C at 4°C/min, held at 250°C for 110 min.

*GC–MS*

For GC–MS analyses in the electron impact (EI) mode, a Hewlett-Packard 5985B GC–MS system was used. A Hewlett-Packard 5840A gas chromatograph equipped with a fused-silica capillary column (50 m × 0.22 mm I.D., CP-SIL 5 CB, equivalent to OV-1, Chrompack) was connected directly to the mass spectrometer. The operating conditions were as follows: injector temperature, 250°C; temperature program, 50°C to 250°C at 4°C/min, held at 250°C for 110 min; helium carrier gas velocity, 14 cm/s; temperature of ion source and all connection parts, 200°C; electron energy, 70 eV; electron multiplier voltage, 2600 V.

For GC–MS analyses in the chemical ionization (CI) mode, a Hewlett-Packard

5790 gas chromatograph was coupled to the VG 7070 EQ mass spectrometer. The column and operating conditions for GC were the same as above. The reactant gas was isobutane (0.3–0.5 Torr).

## RESULTS AND DISCUSSION

Shogaols isolated from TLC were fractionated by preparative HPLC into seven fractions. FAB-MS determination of molecular ions indicated the presence of molecular weights of 276, 290, 304, 318, and 332 (Table I), the final two fractions failed to show any significant spectra. The primary ions used for molecular weight determination of shogaols were $M^{+\cdot}$ and $[M + Na]^+$. The difference of molecular weight between consecutive fractions in HPLC elution was one methylene unit. The molecular weights of 276, 304 and 332, which correspond to 6-, 8- and 10-shogaol, respectively, (ignoring *cis* and *trans* isomers) were confirmed by comparison with the published data[3,6,8,16]. Fig. 1 shows the HPLC chromatogram of isolated shogaols. Compared with previous reports[6–8], a better separation of *cis* and *trans* shogaols has been achieved. Fig. 2 shows the $^1H$ NMR (400 MHz) spectrum of fraction 1 obtained from preparative HPLC (which actually contains three components, *i.e.* peaks 1, 2 and 3, as shown in Fig. 1). This spectrum was interpreted as a mixture of *cis*- and *trans*-6-shogaol. The approximate ratio of *cis*- to *trans*-6-shogaol in this fraction could be estimated by the ratio of protons ($H_a$ and $H_b$) attached to the unsaturated double bond (region f, $\delta$ = 6.0–7.0 in Fig. 2). In Fig. 3, the ratio of *cis*-$H_b$ to *trans*-$H_a$ estimated from the integrated value was 0.20–0.25. This value showed that *trans*-6-shogaol is the dominant compound in the mixture. The sequence of *cis*-6-shogaol

TABLE I

FAB-MS AND HPLC ANALYSES OF ISOLATED SHOGAOL COMPOUNDS

| *Peak No.** | *Compound* | *Percentage*** | *$[M]^{+\cdot}$ (rel. %)* | *$[M+Na]^+$ (rel. %)* |
|---|---|---|---|---|
| 1 | *cis*-6-Shogaol | 24.83 | 276(100) | 299(30) |
| 2 | *syn*-Methyl-6-shogaol | 0.64 | 290(100) | 313(46) |
| 3 | *trans*-6-Shogaol | 63.35 | 276(100) | 299(30) |
| 4 | *anti*-Methyl-6-shogaol | 1.57 | 290(100) | 313(46) |
| 5 | *cis*-8-Shogaol | 1.72 | 304(71) | 327(100) |
| 6 | *syn*-Methyl-8-shogaol | *** | 318(40) | 341(100) |
| 7 | *trans*-8-Shogaol | 4.37 | 304(71) | 327(100) |
| 8 | *anti*-Methyl-8-shogaol | 0.14 | 318(40) | 341(100) |
| 9 | *cis*-10-shogaol | 1.03 | 322(68) | 355(100) |
| 10 | *syn*-Methyl-10-shogaol | *** | 346(?)§ | |
| 11 | *trans*-10-Shogaol | 2.31 | 332(68) | 355(100) |
| 12 | *anti*-Methyl-10-shogaol | *** | 346(?)§ | |
| 13 | *cis*-12-Shogaol | *** | 360(?)§ | |
| 14 | *trans*-12-Shogaol | *** | 360(?)§ | |

* Numbers refer to Fig. 1.

** Average of three determinations by analytical HPLC.

*** Percentage less than 0.10%.

§ Not detected.

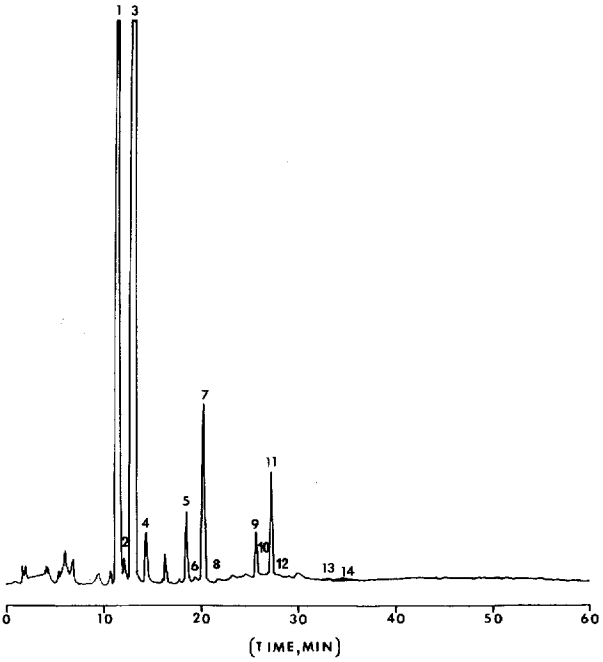


Fig. 1. Analytical HPLC separation of isomeric shogaols isolated from TLC.

(peak 1) and *trans*-6-shogaol (peak 3) in HPLC elution agrees well with their relative polarities. Other isomers of shogaols identified in Table I also follow the similar elution pattern. The ratio of *cis*- to *trans*-6-shogaol estimated from NMR spectra can be correlated well with that from HPLC; however, HPLC turns out to be a better method for quantitation of *cis* and *trans* isomers.

In our previous study[9], we detected novel gingerols with methyl side-chains in the liquid carbon dioxide extract of ginger. It was therefore reasonable to assume that the corresponding shogaols derived from thermal dehydration could also be identified. Shogaol compounds with molecular weights of 290 and 318, which correspond to *syn*- and *anti*-methyl-6-shogaol and methyl-8-shogaol, respectively, were identified by FAB-MS (Table I). The higher carbon number homologues were not detected by FAB-MS.

Fig. 4 shows the UV scanning spectra (200–400 nm) of *trans*-6-, 8- and 10-shogaol. The stop-flow technique during HPLC elution was used. Two absorption maxima, one at 226–232 nm and the other at 282–286 nm, were observed. The absorption maximum shifted to longer wavelength as the chain length increased. The absorption maxima of 6-, 8- and 10-shogaol reported by Govindarajan[4] were 227, 282 and 287 nm.

The use of GC to separate shogaol compounds was first reported by Raghuveer and Govindarajan[14]. In this study, a better separation of isomeric shogaol compounds has been achieved. Fig. 5 shows the capillary GC separation of isomeric shogaol compounds isolated by TLC. A total of fourteen compounds were detected. The data from GC and GC–MS (EI and CI) analyses of isomeric shogaols are shown

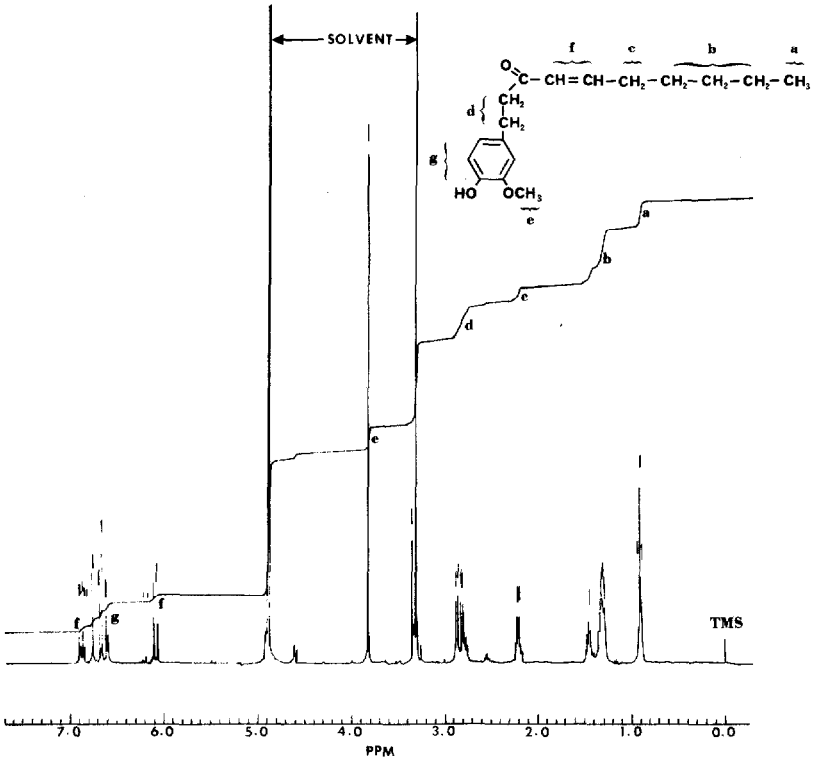


Fig. 2. $^1$H NMR (400 Hz) spectrum of a mixture of *cis*- and *trans*-6-shogaol. a = 0.90 ppm (t, 3H); b1 = 1.31 ppm (m, 4H); b2 = 1.45 ppm (m, 2H); c = 2.20 ppm (m, 2H); d1 = 2.80 ppm (t, 2H); d2 = 2.85 ppm (t, Bz, 2H); e = 3.81 ppm (s, 3H); f = 6.10 ppm, 6.90 ppm (m, 1H); g = 6.61–6.76 ppm (m, Ar, 3H). Abbreviations: s = singlet; d = doublet; t = triplet; m = multiplet; Bz = benzylic methylene; Ar = aromatic. Solvent impurities at 3.30 ppm and 4.88 ppm; both singlets.

in Table II and Fig. 6. The isomers of 6-, 8- and 10-shogaol all showed very similar mass spectra (EI and CI), the only significant differences observed were in the retention times and the relative intensities of the ions. The EI mass spectra of isomers of 12-shogaol derived from ginger (Fig. 6) have not previously been reported.

Owing to their minute concentrations, the identification of novel isomeric shogaols with methyl side-chain was accomplished by detecting the molecular ions. The technique of mass chromatography was used in order to enhance the detection sensitivity[17]. Fig. 7 shows the mass chromatography of isomers of methyl-6-, methyl-8- and methyl-10-shogaol.

In our previous study[9], methyl-12-gingerol and 14-gingerol were also identified as constituents in the liquid carbon dioxide extract of ginger; however, the corresponding isomers of shogaols derived from thermal dehydration were not detected in this study. The finding in chromatograms of relatively low intensities of higher-carbon-number shogaols indicates that the $\beta$-hydroxyl group of gingerols may be stabilized during dehydration by the longer alkyl side-chain[8].

Fig. 8 shows the structures of the isomeric shogaols identified in this study. This is the first report of the use of chromatographic methods (HPLC and GC) to

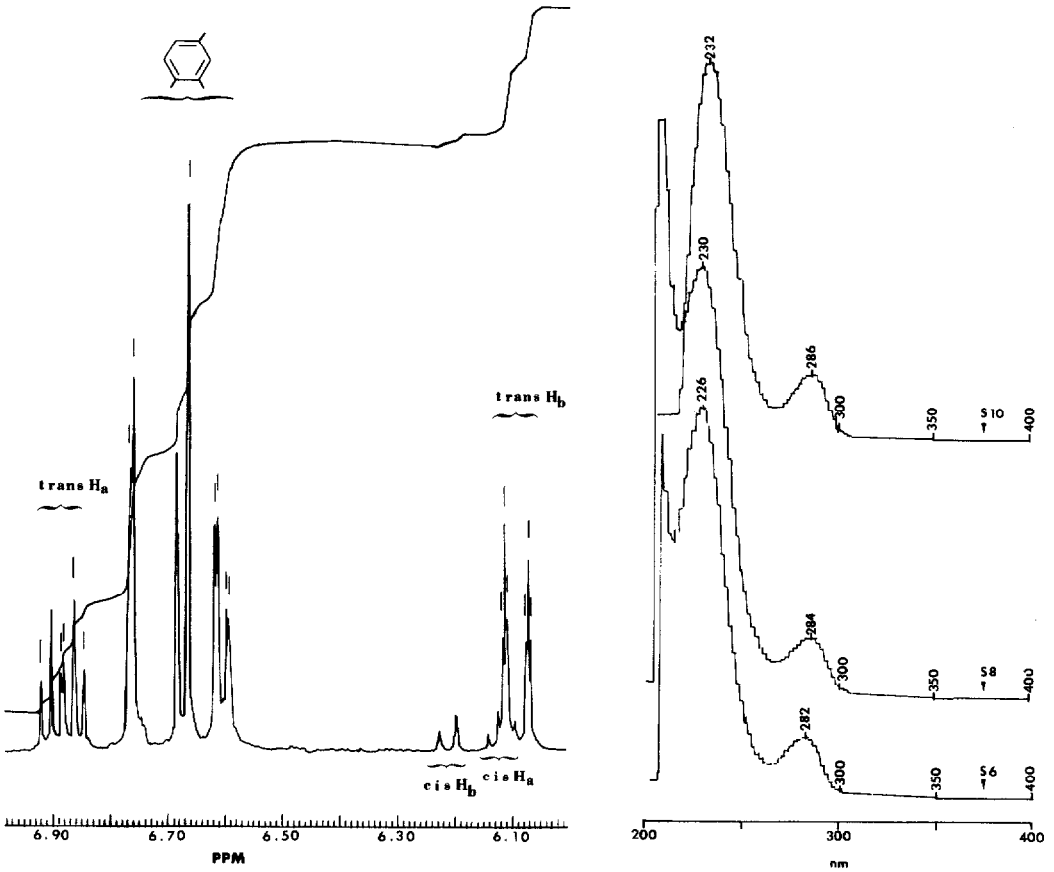


Fig. 3. Enlarged NMR spectrum at double bond region of isomers of 6-shogaol. *trans* $H_b$, 6.08–6.12 ppm, $J$ = 16 Hz; *cis* $H_a$, 6.095–6.12 ppm, $J$ = 10 Hz; *cis* $H_b$, 6.20–6.225 ppm, $J$ = 10 Hz; *trans* $H_a$, 6.86–6.90 ppm, $J$ = 16 Hz.

Fig. 4. UV scanning spectra of *trans*-6-, 8- and 10-shogaol, from 200 to 400 nm at 2 nm per interval.

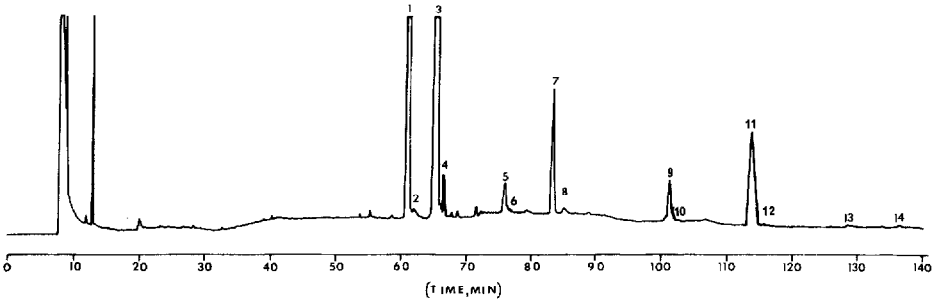


Fig. 5. Capillary GC separation of isomeric shogaols isolated from TLC.

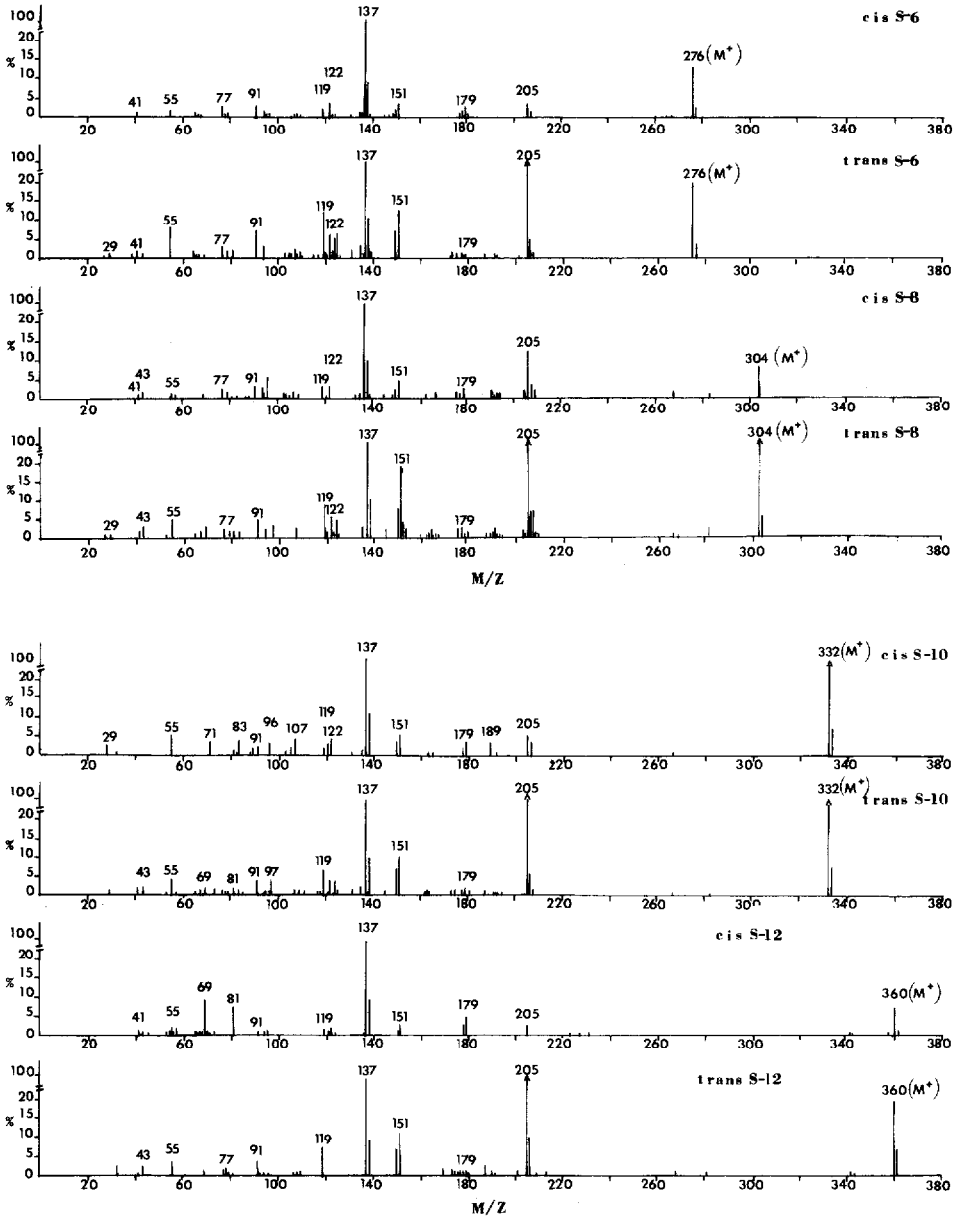


Fig. 6. Mass spectra (EI) isomers of 6-, 8- and 12-shogaol.

separate isomers of shogaol compounds. The results obtained from HPLC and GC are in good agreement; however, in view of the time needed for separation and the operation temperatures, HPLC is the better method.

TABLE II

GC AND GC–MS ANALYSES OF ISOLATED SHOGAOL COMPOUNDS

| *Peak No.** | *Compound* | *MW* | *Percentage*** | *CI–MS data**** |
|---|---|---|---|---|
| 1 | *cis*-6-Shogaol | 276 | 25.14 | 137(100), 277(20), 177(19), 276(15). |
| 2 | *syn*-Methyl-6-shogaol | 290 | 0.48 | 291(+)§ |
| 3 | *trans*-6-Shogaol | 276 | 57.38 | 137(100), 277(35), 277(24), 177(15) |
| 4 | *anti*-methyl-6-shogaol | 290 | 0.89 | 291(+)§ |
| 5 | *cis*-8-Shogaol | 304 | 2.37 | 137(100), 305(17), 177(14), 179(12) |
| 6 | *syn*-Methyl-8-shogaol | 318 | 0.28 | 319(+)§ |
| 7 | *trans*-8-Shogaol | 304 | 5.08 | 137(100), 305(28), 304(16), 177(14) |
| 8 | *anti*-Methyl-8-shogaol | 318 | 0.72 | 319(+)§ |
| 9 | *cis*-10-Shogaol | 332 | 2.97 | 137(100), 333(20), 179(18), 177(10) |
| 10 | *syn*-Methyl-10-shogaol | 346 | + | –§§ |
| 11 | *trans*-10-Shogaol | 332 | 4.59 | 137(100), 333(30), 332(17), 177(14) |
| 12 | *anti*-Methyl-10-shogaol | 346 | + | 347(+)§ |
| 13 | *cis*-12-Shogaol | 360 | + | –§§ |
| 14 | *trans*-12-Shogaol | 360 | + | 361(+)§ |

* Numbers refer to Fig. 5.

** Average of three determinations by capillary GC.

*** Value in parentheses indicates relative percentage; those underlined are $[M+H]^+$.

§ Molecular ion determined by mass chromatography.

§§ Not present.

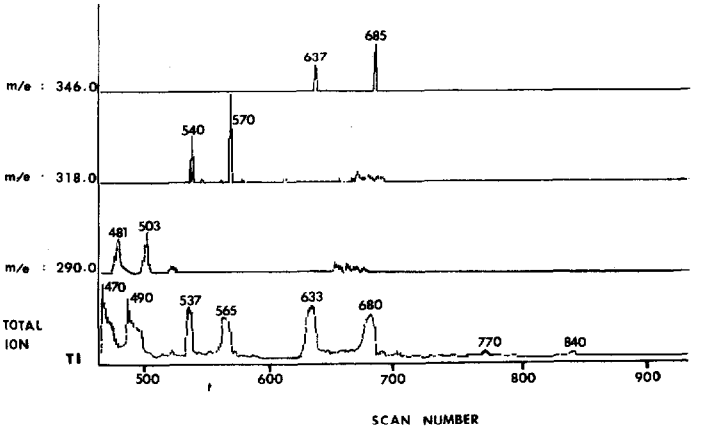


Fig. 7. Mass chromatography of isomers of methyl-6-, methyl-8- and methyl-10-shogaol. The bottom row indicates the total ion chromatography of GC–MS. 470 = *cis*-6-shogaol; 481 = *syn*-methyl-6-shogaol; 490 = *trans*-6-shogaol; 503 = *anti*-methyl-6-shogaol; 537 = *cis*-8-shogaol; 540 = *syn*-methyl-8-shogaol; 565 = *trans*-8-shogaol; 570 = *anti*-methyl-8-shogaol; 633 = *cis*-10-shogaol; 637 = *syn*-methyl-10-shogaol; 680 = *trans*-10-shogaol; 685 = *anti*-methyl-10-shogaol; 770 = *cis*-12-shogaol; 840 = *trans*-12-shogaol.

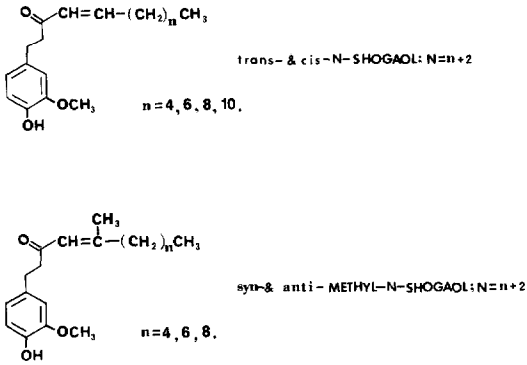


Fig. 8. Proposed structure of isomers of N-shogaols (6-, 8-, 10- and 12-shogaol) and methyl-N-shogaols (methyl-6-, methyl-8- and methyl-10-shogaol).

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