Journal of Chromatography, 360 (1986) 163–173
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 545

CHROMATOGRAPHIC ANALYSES OF GINGEROL COMPOUNDS IN GINGER (*ZINGIBER OFFICINALE* ROSCOE) EXTRACTED BY LIQUID CARBON DIOXIDE

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(Received January 20th, 1986)

SUMMARY

The gingerol compounds of freeze-dried ginger (*Zingiber officinale* Roscoe) were extracted by liquid carbon dioxide (600–700 p.s.i.), isolated by thin-layer chromatography and fractionated into individual gingerol compounds by preparative high-performance liquid chromatography. Two homologous series, 6-, 8-, 10-, 12-, 14-gingerols and methyl-6-, methyl-8-, methyl-10-, methyl-12-gingerols were identified by the combined results of (1) high-performance liquid chromatographic retention in analytical column; (2) molecular ion determination of isolated gingerol compounds by fast atom bombardment-mass spectrometry; (3) gas chromatography and gas chromatography–mass spectrometry analyses of aldehydes and ketones formed from the thermal degradation of the corresponding gingerol compounds. This is the first report of the presence in ginger of 14-gingerol and gingerols with methyl side-chains.

INTRODUCTION

Pungency is an important characteristic of ginger (*Zingiber officinale* Roscoe); compounds responsible for it are: gingerol homologues (6-, 8- and 10-gingerols), shogaol homologues (6-, 8- and 10-shogaols) and zingerone (Fig. 1)[1–5]. The homologues of gingerols are known to be the major constituents in fresh ginger[6–8]. Shogaols and zingerone are derived from gingerol on thermal processing or long-term storage[1–5]. It has been noted that 6-gingerol and 6-shogaol were two most pungent compound in ginger[3–5].

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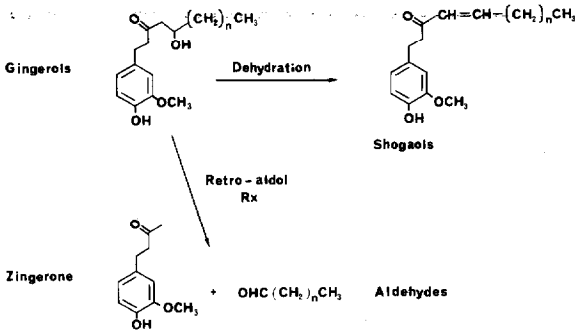

Fig. 1. Relationships between the pungent compounds of ginger.

Thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used to analyse the pungent compounds in ginger products[1–10]. Gingerols and shogaols can be separated as groups by TLC on silica gel plates, but individual components of gingerols and shogaols could not be well resolved[1,3]. On injection into a gas chromatograph, gingerols would spontaneously undergo retro-aldol degradation and thermal dehydration[1,3]. Aldehydes (C_6, C_8 and C_{10}) and zingerone are formed by the retro-aldol reaction, and shogaols are formed by thermal dehydration. Fig. 1 shows the general scheme of these reactions. Since gingerols are thermally unstable, the aldehydes formed can be correlated with the presence of individual gingerol compounds[1,3]. However, for direct analysis of either gingerols or shogaols, HPLC conducted on a reversed-phase column (RP-18) turned out to be the best method[6–8,11].

In recent years, the use of carbon dioxide, either in supercritical conditions or undercritical conditions (liquid form), as an extractant, has received considerable attention[12–17]. For the extraction of flavor compounds in ginger, liquid carbon dioxide is better than supercritical carbon dioxide for its selectivity for the desired constituents (essential oil and pungent compounds)[8,15,17,18]. Our previous report showed that the pungent compounds in ginger could be effectively extracted by liquid carbon dioxide[8,18].

This paper presents the further chromatographic analyses and identification of gingerol compounds in liquid carbon dioxide extract by TLC, HPLC, fast atom bombardment-mass spectrometry (FAB-MS), GC and GC–MS.

MATERIALS AND METHODS

Materials and chemicals

Mature ginger (*Zingiber officinale* Roscoe) rhizomes were purchased from a supplier near Hsinchu (Taiwan). The rhizomes were washed, sliced, freeze-dried, ground and sieved (200 mesh). Dry ice was also obtained from a supplier near Hsinchu. 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. *n*-Aldehydes (C_4–C_{14}) were obtained from Polyscience (Niles, IL, U.S.A.). 2-Heptanone, 2-nonanone and 2-undecanone were obtained from Aldrich (Milwaukee, WI, U.S.A.). 2-Tridecanone

was obtained from Caro (Tokyo, Japan). *n*-Alkanes (C_7–C_{25}) were obtained from Alltech Assoc. (Deerfield, IL, U.S.A.). Unless otherwise stated, all other chemicals were of reagent grade.

Liquid carbon dioxide extraction

About 110 g of freeze-dried ginger powder were placed in a glass Soxhlet extractor, which was installed inside a stainless-steel cylinder as described previously[8]. Crushed dry ice (10 kg) was poured into the cylinder. The cylinder was tightly closed and then heated by propylene glycol (45 ± 2°C) circulating in the outside jacket. The pressure inside the cylinder increased and stabilized at *ca.* 600–700 p.s.i. The extraction time was 48 h. After extraction, the cylinder was cooled to −60°C by circulating pre-cooled alcohol (95%) in the outside jacket. A golden-brown oily material (3.44% w/w, dry weight basis) was obtained after the evaporation of carbon dioxide.

TLC isolation of gingerol fraction

The oil extracted by the liquid carbon dioxide 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 and isolated.

Preparative HPLC separation of gingerol compounds

A Waters HPLC system (Waters Assoc., Milford, U.S.A.), consisting of two M-6000A pumps, a M-660 solvent programmer and an U6K injector, was used. 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 gingerol compounds were obtained during analyses using the stop-flow method.

FAB-MS

Molecular ion determinations of isolated gingerols 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.

GC

For GC analyses of aldehydes and ketones formed by the retro-aldol reaction, 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 gas velocity, 12 cm/s; nitrogen make-up gas flow-rate, 30 ml/min; hydrogen detector gas flow-rate, 30 ml/min; detector air flow-rate, 300 ml/min; temperature program, 50°C to 250°C at 4°C/min and isothermal at 250°C for 110 min. Linear retention indices of aldehydes and ketones formed were calculated using *n*-alkane (C_7–C_{25}, Alltech) as references[19]. Aliphatic aldehydes (C_6–C_{14}, Polyscience), 2-alkanones and zingerone[8,18] were used as authentic standards. For analysing the linear retention indices on a polar column, a fused-silica capillary column (50 m × 0.22 mm I.D., CP-WAX 57CB, equivalent to Carbowax 20M, Chrompack, The Netherlands) was also used. The temperature program was 50°C to 200°C at 2°C/min, held at 200°C for 50 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; accelerating 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

Isolation of gingerols from liquid carbon dioxide extract

The liquid carbon dioxide extract of ginger was a golden brown to brown oil. It had both the pungent and the aromatic characteristics of fresh ginger[8]. The pungent gingerol fraction of the liquid carbon dioxide extract could be easily separated by TLC[18]. The gingerol fraction was further fractionated into nine fractions by preparative HPLC on the reversed-phase column.

Molecular ion determinations of gingerols by FAB-MS

Fig. 2 shows the FAB-MS spectra of (A) 6-gingerol (MW 294), (B) 8-gingerol (MW 322), (C) 10-gingerol (MW 350) and (D) 12-gingerol (MW 378) as isolated by preparative HPLC. Since FAB-MS is performed at ambient temperature, the molec-

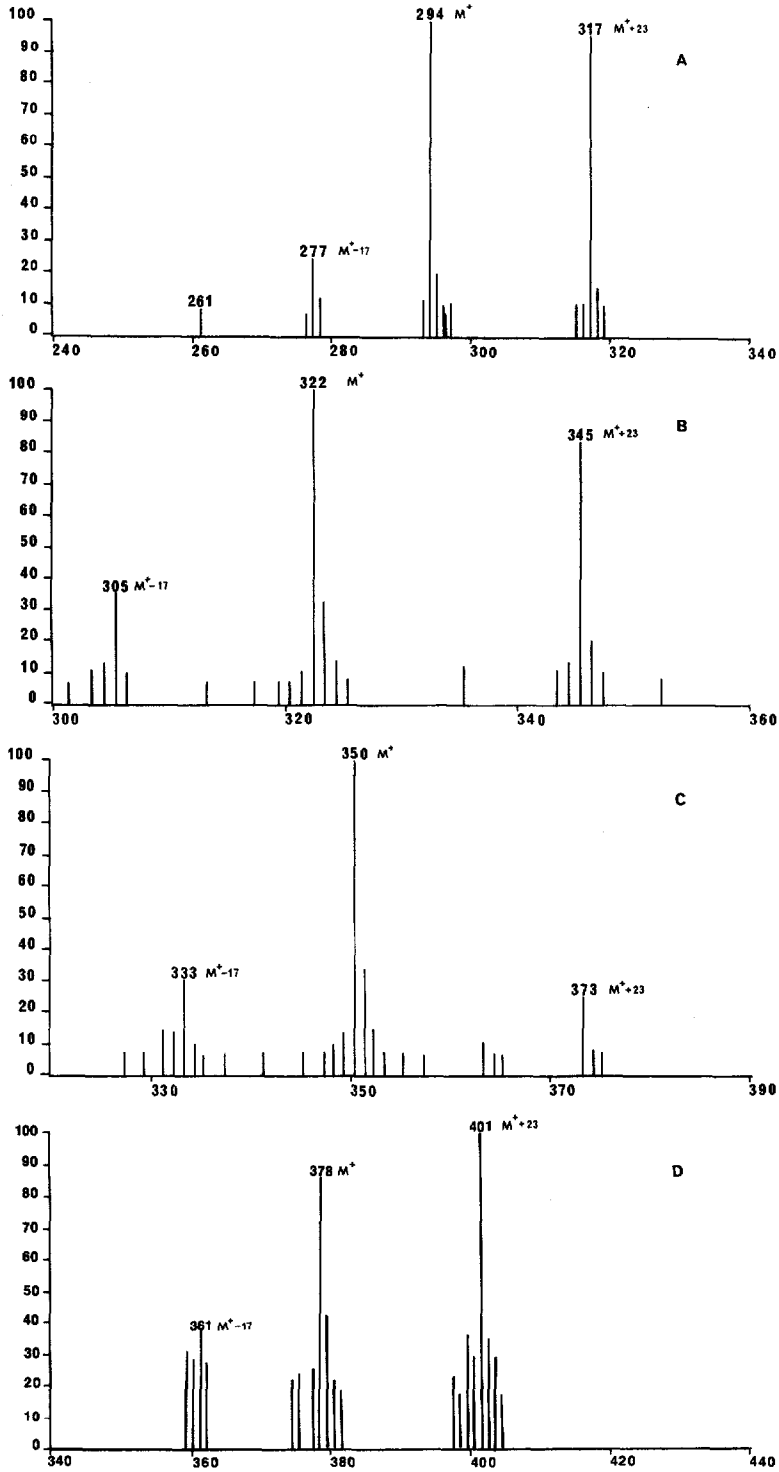

Fig. 2. FAB-MS spectra of isolated gingerols. (A) 6-Gingerol; (B) 8-gingerol; (C) 10-gingerol; (D) 12-gingerol.

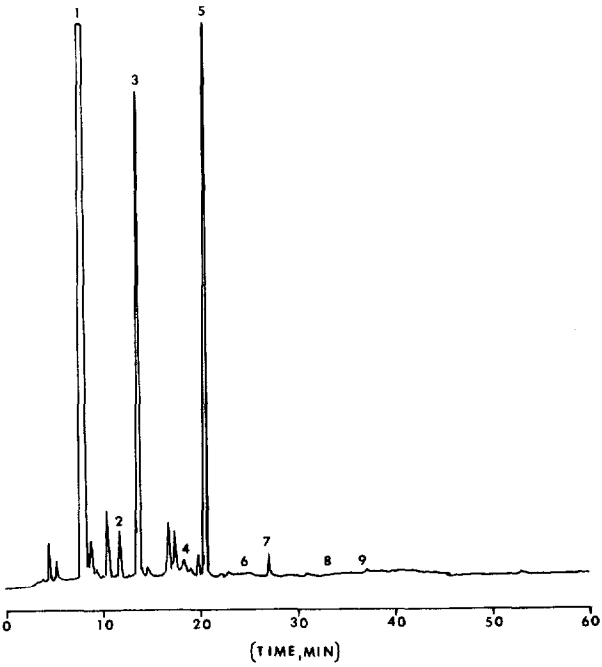

Fig. 3. Analytical HPLC separation of gingerols isolated from TLC.

ular ions of thermally labile compounds such as gingerols could thus be identified by using this technique. The primary ions used for molecular weight determination by FAB-MS were $M^{+\cdot}$, $[M - OH]^{+\cdot}$ and $[M + Na]^{+}$.

The presence of 6-, 8- and 10-gingerol in ginger has been previously reported by several groups[1–7]. 12-Gingerol was found in GC–MS analyses of tetramethylsilyl derivatives of ginger[10].

In this study, novel gingerol compounds with MW 308, 336 and 364 were positively identified by FAB-MS. These compounds appeared in consecutive order as shown in analytical HPLC elution (peaks 2, 4 and 6 in Fig. 3). The molecular weight of these compounds were one methylene unit more than the preceding gingerols (peaks 1, 3 and 5 in Fig. 3). However, the absence of $[M - OH]^{+\cdot}$ fragment in the FAB mass spectra indicates that these novel gingerols might not have the same structures as 6-, 8- and 10-gingerols. Another two gingerol compounds (MW 392 and 406) were tentatively identified by FAB-MS. Table I shows the FAB-MS fragmentations of gingerols as isolated by preparative HPLC. The percentage distribution of major gingerols in the liquid carbon dioxide extract was in good accordance with the previous report[18].

Fig. 4 shows the UV scanning spectra (200–400 nm) of 6-, 8- and 10-gingerol. The stop-flow technique during HPLC elution was used. All these gingerols showed a UV absorption maximum at 282 nm, the same value as previously reported for 6-gingerol[4].

From the results of HPLC retentions and FAB-MS data, it can be concluded

TABLE I

MAJOR IONS OF ISOLATED GINGEROL COMPOUNDS BY FAB-MS

*Peak No.**	*Compound*	*Percentage***	*$[M - OH]^{+\cdot}$*	*$M^{+\cdot}$*	*$[M + Na]^{+}$*
1	6-Gingerol	74.95	277(30)***	294(100)	317(95)
2	Methyl 6-gingerol	0.90	§	308(60)	331(100)
3	8-Gingerol	7.92	305(35)	322(100)	345(85)
4	Methyl 8-gingerol	0.10	§	336(42)	359(100)
5	10-Gingerol	10.86	333(33)	350(10)	373(30)
6	Methyl 10-gingerol	0.42	§	364 (35)	387 (100)
7	12-Gingerol	0.69	361(40)	378(86)	401(100)
8	Methyl 12-gingerol§§	§§§	§	392 (+)	415 (+)
9	14-Gingerol§§	§§§	§	406 (+)	429(+)

* Numbers refer to Fig. 3.

** Average of three determinations of gingerol fraction isolated by TLC, analysed by analytical HPLC.

*** Values in brackets indicate relative intensities.

§ Not determined.

§§ Tentatively identified by FAB-MS.

§§§ Percentage less than 0.1%.

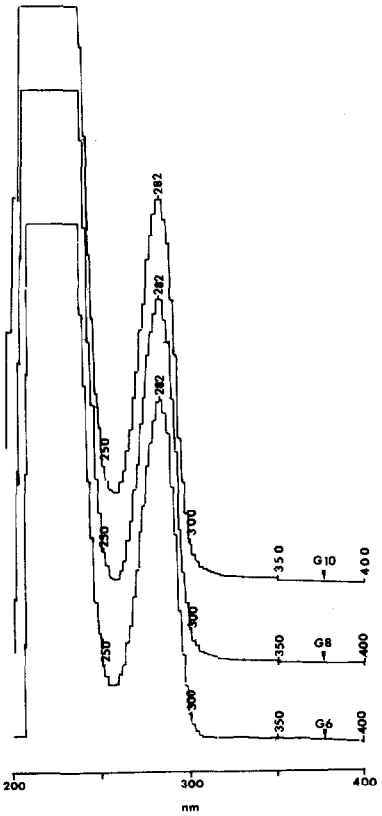

Fig. 4. UV scanning spectra of 6-, 8-, and 10-gingerol, from 200 to 400 nm at 2 nm per interval.

TABLE II

GC AND GC–MS DATA OF ALDEHYDES AND KETONES FORMED BY THERMAL DEGRADATION OF THE CORRESPONDING GINGEROL COMPOUNDS

Peak No.[*]	*Compound*	*MW*	*Percentage*[**]	I_k[***] *(CW-20M)*		I_k[***] *(OV-1)*		*Scan No.*[§]	*MS fragments*[§§]
				R	*S*	*R*	*S*		
1	Hexanal	100	77.58	1073	1072	780	779	65	83(100), 101(58), 99(15), 71(12)
2	2-Heptanone	114	0.85	1175	1172	867	865	93	115(100), 83(10), 69(6), 71(6)
3	Octanal	128	8.31	1276	1275	980	979	148	69(100), 111(92), 129(55), 83(15)
4	2-Nonanone	142	0.11	1382	1380	1071	1062	201	143(100), 69(45), 71(37), 83(35)
5	Decanal	156	11.76	1485	1483	1186	1185	284	83(100), 157(50), 69(40), 97(38)
6	2-Undecanone	170	0.49	1591	1586	1274	1274	343	171(100), 69(24), 71(22), 83(19)
7	Dodecanal	184	0.43	1696	1696	1388	1388	423	69(100), 83(93), 97(83), 185(54)
8	2-Tridecanone	198	0.11	1802	1803	1476	1475	482	69(100), 67(92), 71(90), 199(40)
10	Tetradecanal	212	0.35	1910	1911	1592	1590	532	69(100), 67(88), 71(80), 213(20)

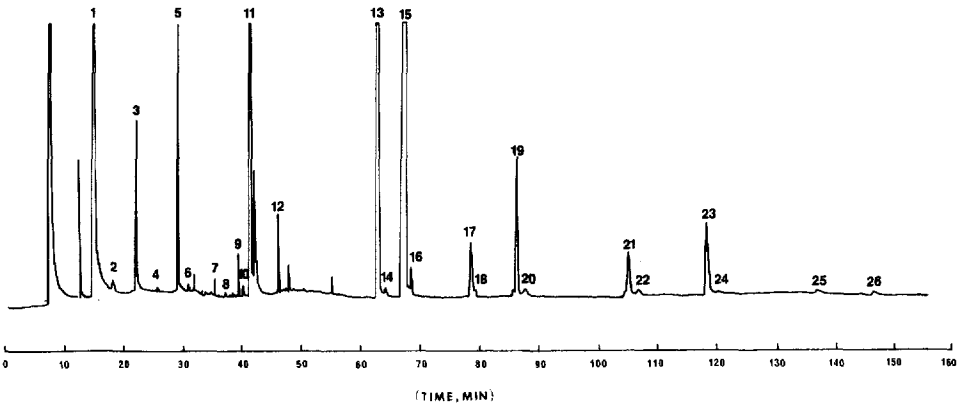

Fig. 5. Capillary GC separation of gingerols isolated from TLC. Peaks 1–10 (except peak 9, homologue of zingerone) are aldehydes; peak 11 is zingerone, peak 12 is dehydrozingerone, and peaks 13–26 are *cis* and *trans* isomers of shogaols.

that all consecutive gingerols in HPLC elution show difference of one methylene unit, although details about the structure are not clear.

GC and GC–MS analyses of retro-aldol decomposition products of gingerols

The use of GC to analyse gingerols was first proposed by Connell and McLachlan[2]. The β-hydroxyketone group of gingerol would undergo retro-aldol decomposition in the GC injector if the temperature is greater than 200°C; aliphatic aldehydes and zingerone were products observed. Raghuveer and Govindarajan[9] proposed that, during temperature-programmed GC, the pyrolytic breakdown of gingerols would produce aliphatic aldehydes, zingerone and shogaols. It was also suggested that the ratio of aldehydes formed could be correlated to the ratio of gingerols injected.

Fig. 5 shows the capillary GC separation of the pyrolytic products of gingerols isolated from TLC. Two types of capillary column (OV-1 and Carbowax 20M) were used for analyses. Aldehydes, ketones and zingerone, the retro-aldol decomposition products of gingerols, could be well separated in both types of column. Shogaols (*cis* and *trans* isomers, peaks 13–26 in Fig. 5), the thermal dehydration products of gingerols, could only be separated on OV-1 column. The isolation and identification of these isomeric shogaols will be discussed[20].

The results of GC and GC–MS (CI) analyses of aldehydes and ketones formed by retro-aldol decomposition are shown in Table II. The aldehydes were identified by comparing the retention indices and the mass spectra with those of authentic aliphatic aldehydes (C_4–C_{14}). The retention index values were also compared with published data[21]. The retention indices of C_6, C_8, C_{10} and C_{12} aldehydes formed by pyrolytic reaction matched well those of authentic aliphatic aldehydes in two types of column. A C_{14} aldehyde, formed by pyrolytic reaction, also matched well an authentic C_{14} aldehyde (tetradecanal). The presence of 14-gingerol could only be tentatively identified by FAB-MS. The EI (electron ionization) mass spectra of peaks 1, 3, 5, 7 and 10 in Fig. 5 were identical with those of C_6, C_8, C_{10}, C_{12} and C_{14} aldehydes.

The retention indices of peaks 2, 4, 6 and 8 in Table II did not match well with

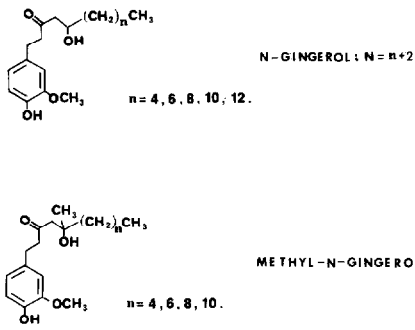

Fig. 6. Proposed structures of *n*-gingerols (6-, 8-, 10-, 12-, and 14-gingerol) and methyl-*n*-gingerols (methyl-6-, methyl-8-, methyl-10- and methyl-12-gingerol).

those of standard aliphatic aldehydes (C_7, C_9, C_{11} and C_{13} aldehydes), although the CI mass spectra showed the same molecular weights for both groups. Instead, the retention indices matched well with authentic 2-alkanone compounds (2-heptanone, 2-nonanone, 2-undecanone and 2-tridecanone) in two types of columns (Table II). The EI mass spectra of peaks 2, 4, 6 and 8 all showed base peaks at *m/z* 58, which indicates a keto group at the C_2 position. Mass spectra of 2-alkanones identified were identical to those of published spectra[22].

Fig. 6 shows the structures of two groups of gingerols identified in this study. FAB-MS confirmed the molecular weights of these compounds, and details of the structures were further confirmed by GC retention data and mass spectra in both the EI and CI modes. This is the first report of the presence in ginger of gingerols with a methyl side-chain. It is also the first report of the presence of 14-gingerol in ginger.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Chung-May Wu, Ms. May-Chien Kuo, Ms. Su-Er Liu, Ms. Lii-Yuen Lin and Mr. Ming-Ching Wang of Food Industry R&D Institute, Hsinchu, Taiwan, for technical assistance. C.-C. Chen acknowledges the financial support of the Council of Agriculture, Taiwan and FIRDI. The authors also acknowledge valuable discussions with Dr. M. Chien of Givaudan Coporation. New Jersey Agricultural Experiment Station Publication No. D-10205-2-86 supported by Hatch Regional Fund NE-116 and State Funds.

REFERENCES

1 D. W. Connell and M. D. Sutherland, *Aust. J. Chem.*, 22 (1969) 1033.
2 D. W. Connell and R. McLachlan, *J. Chromatogr.*, 67 (1972) 29.
3 V. S. Govindarajan, *Amer. Chem. Soc., Symp. Ser.*, 115 (1979) 53.
4 V. S. Govindarajan, *CRC Crit. Rev. Food Sci. Nutr.*, 17 (1982) 1.
5 V. S. Govindarajan, *CRC Crit. Rev. Food Sci. Nutr.*, 17 (1982) 189.
6 V. E. Steinegger and K. Stucki, *Pharm. Acta Helv.*, 57 (1982) 66.
7 J. D. Baranowski, *J. Chromatogr.*, 319 (1985) 471.
8 C.-C. Chen, M.-C. Kuo, C.-M. Wu and C.-T. Ho, *Abstracts American Chemical Society Meeting, Chicago, IL, Sept. 1985*, American Chemical Society, Washington, DC, 1985, paper 156.
9 K. G. Raghuveer and V. S. Govindarahan, *J. Food Qual.*, 2 (1978) 41.

10 D. J. Harvey, *J. Chromatogr.*, 212 (1981) 75.
11 C.-C. Chou, J. L.-B. Wu, M.-H. Chen and C.-M. Wu, in G. Charalambous (Editor), *The Quality of Foods and Beverages*, Academic Press, New York, 1981, p. 119.
12 E. G. Schultz and J. N. Randal, *Food Technol.*, 24 (1970) 94.
13 A. B. Caragay, *Perfum. Flavor.*, 6 (1981) 43.
14 D. S. Gardner, *Chem. Ind.*, 19 (1982) 402.
15 V. J. Krukonis, *Abstracts American Chemical Society Meeting, Philadelphia, August, 1984*, American Chemical Society, Washington, DC, 1984, paper 53.
16 B. Meyer-Warnod, *Perfum. Flavor.*, 9 (1984) 93.
17 D. A. Moyler, *Perfum. Flavor.*, 9 (1984) 109.
18 C.-C. Chen, M.-C. Kuo, C.-M. Wu and C.-T. Ho, *J. Agric. Food Chem.*, (1986) in press.
19 P. Majlát, Z. Erdös and J. Takács, *J. Chromatogr.*, 91 (1974) 89.
20 C.-C. Chen, R. T. Rosen and C.-T. Ho, *J. Chromatogr.*, 360 (1986) 175.
21 W. Jennings and T. Shibamoto, *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*, Academic Press, New York, 1980.
22 *EPA/NIH Mass Spectral Data Base*, U.S. Department of Commerce, Washington, DC, 1980.