an oil: IR (film) 1765, 3400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37–6.77 (m, 4 H, Ph), 4.28 (t, J = 6.0 Hz, 2 H, CH_2), 3.85 (s, 3 H, CH_3), 3.75–3.5 (m, 2 H, CH_2), 2.03–1.20 (m, 7 H, 3 CH_2, OH). Anal. Calcd for $C_{13}H_{18}O_5$: C, 61.41; H, 7.14. Found: C, 61.43; H, 7.15.

2-[[(2-Methoxy-4-formylphenoxy)carbonyl]oxy]-4-pentanol (8). The synthesis of 8 was conducted on a 0.02-mol scale using the conditions described for 1, starting with the aryl chloroformate (derived from 2-methoxy-4-formylphenol) and 2,4-pentanediol. NMR, IR, and TLC data indicate the product is formed. However, a pure sample could not be isolated due to contamination by the phenolic and cyclic carbonate components. The inability to isolate a pure sample indicates rapid decomposition during isolation.

1-[(Phenoxycarbonyl)oxy]glycerol (9). To 25 mL of 10% acetic acid was added 1.5 g (0.006 mol) of 1-*O*-(phenoxycarbonyl)-2,3-*O*-isopropylideneglycerol (Pfeiffer, 1970). The emulsion was stirred vigorously and heated to 60 °C for 2 h. The solution was cooled to room temperature and extracted several times with petroleum ether and then ethyl ether. The ethyl ether layer was washed with aqueous saturated sodium bicarbonate and dried over anhydrous $MgSO_4$, followed by evaporation of the solvent under reduced pressure to yield a liquid. NMR, IR, and TLC data indicate the product was formed, however, like 8, was unstable.

1-[[(2-Methoxy-4-methylphenoxy)carbonyl]oxy]-glycerol (10). The synthesis of 10 was conducted on a 5-mmol scale using the conditions described for 9, starting with 1-*O*-[(2-methoxy-4-methylphenoxy)carbonyl]-2,3-*O*-isopropylideneglycerol (Pfeiffer, 1970). NMR, IR, and TLC data indicate the product was formed, however, like 8, was unstable.

1-[[(2-Methoxyphenoxy)carbonyl]oxy]-2-ethanol (11). The synthesis of 11 was conducted on a 0.01-mol scale using the conditions described for 1, starting with the aryl chloroformate (derived from 2-methoxyphenol) and 1,2-ethanediol. NMR, IR, and TLC data indicate the product was formed, however, like 8, was unstable.

Thermolysis of the Aryl Hydroxyalkyl Carbonates (1–7). A 10–50-mg sample of each of the aryl hydroxyalkyl carbonates was thermolyzed in a sealed tube under the specific conditions listed in Table I. The yield of released phenolic component in each case was determined by GC quantitation with an authentic sample.

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Registry No. 1, 100899-45-4; 2, 100899-46-5; 3, 100899-47-6; 4, 100899-48-7; 5, 100899-49-8; 6, 100899-50-1; 7, 100899-51-2; 8, 100899-52-3; 9, 100899-53-4; 10, 100899-54-5; 11, 100899-55-6; *o*-$MeOC_6H_4OH$, 90-05-1; PhOH, 108-95-2; $HOCH_2CH(OH)CH_2OH$, 56-81-5; $HOCH_2CMe_2CH_2OH$, 126-30-7; $HOCH(Me)CH_2CH(Me)OH$, 625-69-4; $HO(CH_2)_4OH$, 110-63-4; $HO(CH_2)_5OH$, 111-29-5; $HO(CH_2)_2OH$, 107-21-1; $HO(CH_2)_3OH$, 504-63-2; 2-methoxy-4-methylphenol, 93-51-6; phenyl chloroformate, 1885-14-9; 2-methoxy-4-methylphenyl chloroformate, 94192-20-8; 2-methoxyphenyl chloroformate, 2293-75-6; 2-methoxy-4-formylphenyl chloroformate, 94192-21-9; 2-methoxy-4-formylphenol, 121-33-5; 1-*O*-[(2-methoxy-4-methylphenoxy)carbonyl]-2,3-*O*-isopropylideneglycerol, 100899-57-8; 1-*O*-(phenoxycarbonyl)-2,3-*O*-isopropylideneglycerol, 100899-56-7.

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Pungent Compounds of Ginger (*Zingiber officinale* Roscoe) Extracted by Liquid Carbon Dioxide

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The pungent principles of ginger (*Zingiber officinale* Roscoe) were extracted by liquid carbon dioxide (600–700 psi). Individual pungent component was isolated by thin-layer chromatography (TLC) followed by preparative high-performance liquid chromatography (HPLC). Identification of pungent compounds was conducted by analytical HPLC and mass spectrometry (MS). 6-Gingerol (11.88%, w/w) was the most abundant pungent compound identified in the liquid carbon dioxide extract (CO_2(l) extract); other homologues of gingerols identified were 8-gingerol (1.67%) and 10-gingerol (2.38%). Only a trace amount of 6-shogaol was identified in the CO_2(l) extract.

INTRODUCTION

The pungent principle of ginger (*Zingiber officinale* Roscoe) has long been recognized as an important character related to the quality. To extract the pungent compounds, organic solvents such as acetone and dichloromethane can be used; the final product is dark and viscous oleoresin (Krukonis, 1984).

The knowledge of using carbon dioxide to extract plant materials has been known for 50 years; however, only recently has it been received with incresaing attentions (Moyler, 1984). It has been tried to extract the flavoring materials of vegetable or fruit juices (Schultz and Randall, 1970) and many other natural products (Caragay, 1981; Moyler 1984). This method shows great potential in replacing the conventional methods such as solvent extraction and steam distillation (Gardner, 1982; Meyer-Warnod, 1984).

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Quantitation of the pungent compounds in ginger has been done by thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Connell and Sutherland, 1969; Connell and McLachlan, 1972; Raghuveer and Govindarajan, 1978; Govindarajan, 1979; Chou et al., 1981; Harvey, 1981; Steinegger and Stucki, 1982; Baranowski, 1985). However, neither the TLC nor GC method is ideal because of poor resolution or incomplete retro-aldol degradation. HPLC turns out to be a good alternative to quantify the pungent compounds in ginger (Steinegger and Stucki, 1982; Baranowski, 1985).

This paper presents the studies on the pungent compounds of ginger extracted by liquid carbon dioxide.

EXPERIMENTAL SECTION

Sample Preparation. Mature ginger (*Z. officinale* Roscoe) rhizomes were purchased from the supplier near Hsinchu, Taiwan. The rhizomes were washed, sliced, freeze-dried, grounded, and sieved (200 mesh). About 10 g of ginger powder was placed in a glass Soxhlet extractor that was installed inside a commercial high-pressure CO_2(l) extractor (J&W Scientific Inc.). To start the extraction, the tightly closed apparatus was placed in a 45 ± 2 °C water bath. At equilibrium, the pressure inside was about 600–700 psi. The extraction was continued for 24 h. After extraction, the whole apparatus was immersed in a −70 °C cooling medium (alcohol) to lower the inside pressure. CO_2(l) extract of ginger was oilylike with brown to golden brown color. The extraction was repeated several times to obtain more sample. Essential oil of ginger was distilled according to the AOAC method (AOAC, 1980). Oleoresin of ginger was obtained by concentrating the acetone extract under reduced pressure.

Thin-Layer Chromatography. For analytical purpose, the samples were applied as spots on a 5 × 20 cm plate (silica gel 60 F-254, E. Merck). For preparative purpose, the sampls were applied as a strip on a 20 × 20 cm plate (silica gel 60 F-254, E. Merck). Glass-distilled *n*-hexane/diethyl ether (3/7) (E. Merck) was used as developing solvent. The developed plate was examined under a UV lamp (254 nm, Ultraviolet Product Model R-52). For sensory assessing of pungency regions in the TLC plate, those UV absorption zones were scraped, desorbed by methanol (glass distilled, E. Merck), filtered, and then tasted organoleptically by using a testing blotter.

Syntheses of Shogaols. The pungent gingerol region (R_f 0.15–2.22) on the TLC plate was scraped, desorbed by methanol, concentrated to dryness, dissolved in diluted sulfuric acid solution (pH 1.5), refluxed for 12 h, and then neutralized with diluted NaOH solution. The synthetic mixture (gingerols, shogaols) was extracted twice by *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).

Synthesis of Zingerone. The pungent gingerols were isolated as described above, dissolved in 2 N NaOH solution, refluxed overnight, and then neutralized with diluted HCl solution. The crude zingerone was extracted twice by *n*-hexane/diethyl ether (4/6), washed with distilled water, dried over anhydrous sodium sulfate, and concentrated to minimal volume. Zingerone was isolated by redeveloping on a 20 × 20 cm TLC plate (R_f 0.24–0.27).

High-Performance Liquid Chromatography. The chromatography was conducted on a Waters Associates (Milford, MA) system that included two Model M-6000A pumps, a Model 660 programmer, and a U6K injector. A

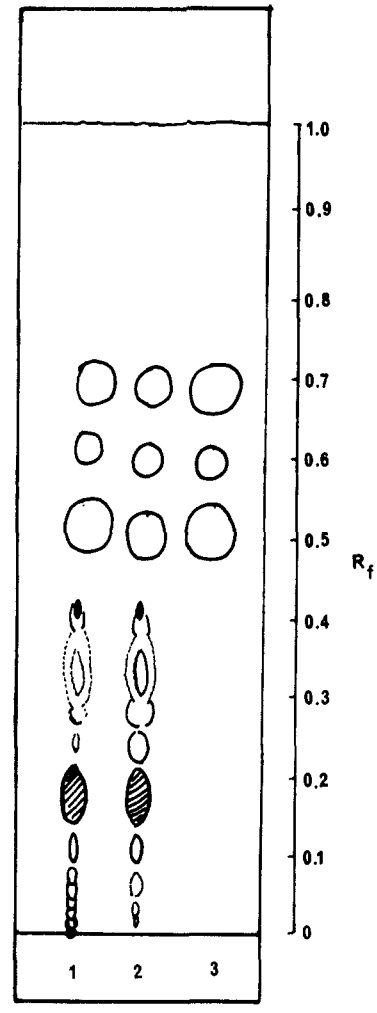

Figure 1. Thin-layer chromatographic separations of (1) oleoresin, (2) CO_2(l) extract, and (3) steam-distilled oil of ginger. Shaded areas are pungent when assessed organoleptically.

Varian (Walnut Creek, CA) Model 2050 variable UV detector and a Varian Model 4270 integrator were also used. A linear gradient started from methanol/water (65/35) to methanol (100) was adopted in all HPLC analyses. Detection was based on UV absorption at 282 nm. For preparative HPLC, two stainless-steel columns (60 cm × 8 mm) packed with reversed-phase absorbent (LiChroprep RP-18, 25–40 μm, E. Merck), were connected in series. The analysis time was 150 min, with the first 100 min in gradient elution. The mobile phase flow was 2 mL/min. Analytical HPLC was conducted on a Varian MicroPak SP-C18 reversed-phase column (15 cm × 4.6 mm, 5 μm). The analysis time was 50 min, with the first 40 min in gradient elution. The mobile phase flow was 1 mL/min. Vanillin (99%, Aldrich) was added as internal standard.

Mass Spectrometry. Molecular ion determinations of isolated gingerols and shogaols from preparative HPLC were conducted on a VG 7070 EQ (VG Analytical, Manchester, U.K.) mass spectrometer using the technique of fast atom bombardment (FAB). Molecular ion determination of zingerone was conducted in the same instrument using chemical ionization (CI) technique; isobutane was used as reactant gas. The fast-atom gun (Ion Tech, Teddington, U.K.) was operated at 8 kV with currents of between 1.0 and 1.5 mA. Xenon gas was used to bombard the sample. Samples were dissolved in glycerol and deposited on the target probe.

RESULTS AND DISCUSSION

In this study, the freeze-dried ginger powder was ex-

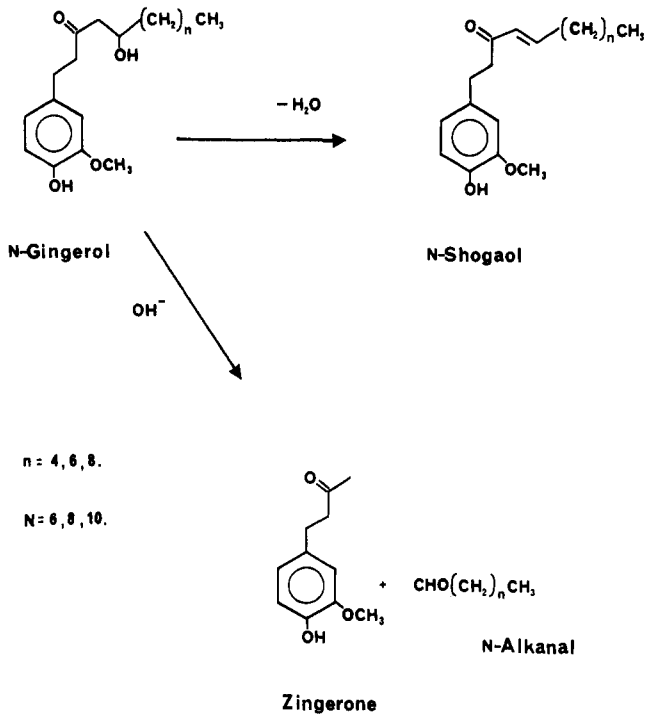

Figure 2. Structure of gingerols, shogaols, and zingerone and the relation during derivation.

tracted by liquid carbon dioxide (CO_2(l), 600–700 psi). The oily material obtained showed brown to golden brown color, with the aroma reminiscent that of fresh ginger and strongly pungent when tasted organoleptically.

Figure 1 shows the results of TLC analyses of (1) oleoresin, (2) CO_2(l) extract, and (3) essential oil of freeze-dried ginger. The plate was visualized under UV light (254 nm), areas showing absorption were spotted and tasted to determine pungency. Two shaded areas, one with R_f 0.42 and another with R_f 0.15–0.22, showed pungent taste. The separation profile of oleoresin is similar to those reported previously (Connell and Sutherland, 1969; Connell and McLachlan, 1972; Govindarajan, 1979; Steinegger and Stucki, 1982) except shows slightly pungency in the shogaol region (R_f 0.42). Instead, the intense pungency at R_f 0.15–0.22 indicates that gingerols are responsible for the pungency in both CO_2(l) extract and oleoresin of ginger. The higher proportion of gingerols also indicates the freshness of the sample (Steinegger and Stucki, 1982; Baranowski, 1985). The separation of essential oil shows three major spots (R_f 0.47–0.72), which coincides well with CO_2(l) extract at the same region. The similarity clearly indicates that liquid CO_2 has the ability to extract volatile compounds of ginger as cited (Moyler, 1984). The separation profile of CO_2(l) extract is similar to that of oleoresin except in the lower R_f region (0.1 and less) the nonspecific extraction by acetone will extract compounds (waxy materials).

The pungent gingerol fraction isolated from TLC was further fractionated by preparative HPLC with reversed-phase packing into three major gingerol compounds. Molecular ion determinations of isolated gingerols were

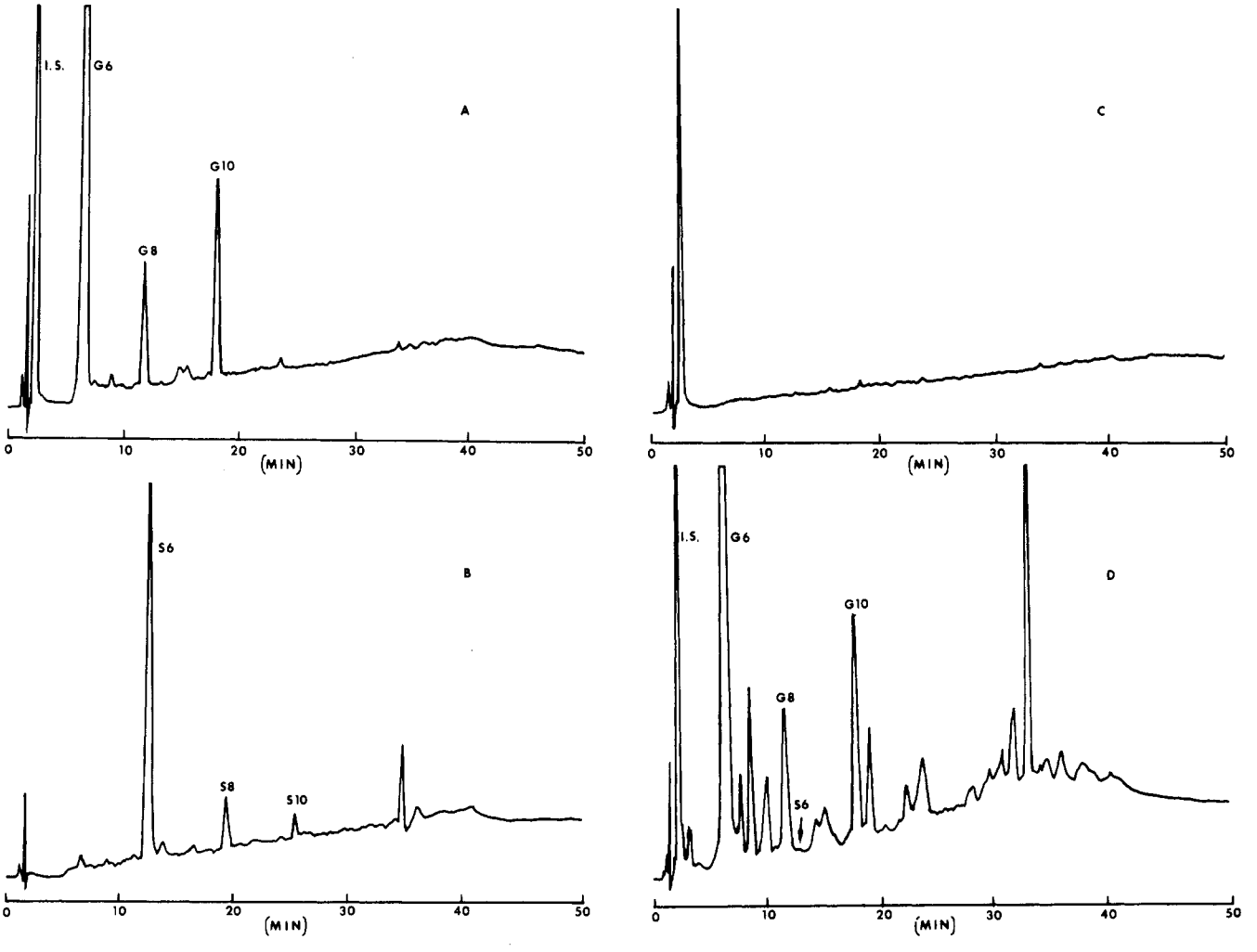

Figure 3. HPLC separations: (A) isolated gingerols; (B) shogaols derived from gingerols; (C) zingerone derived from gingerols; (D) CO_2(l) extract of ginger. Vanillin was added as internal standard in chromatograms A and D.

Table I. Quantitative Results of Pungent Compounds of Ginger Extracted by Liquid Carbon Dioxide and Analyzed by HPLC

compd	ret time, min	M_r[a]	% (w/w)[b]
zingerone	1.9	194	c
6-gingerol	6.2	294	11.88
8-gingerol	11.9	322	1.67
6-shogaol	12.5	276	0.08
10-gingerol	18.5	350	2.38
8-shogaol	19.7	304	c
10-shogaol	24.6	332	c

[a] Molecular ions determined by MS-FAB except zingerone which was determined by MS-CI. [b] Using vanillin as internal standard. [c] Does not exist.

conducted by FAB-MS, in order to minimize decomposition during identification (Barber et al., 1982). Molecular weights of 294, 322, and 350, which corresponded to 6-gingerol, 8-gingerol, and 10-gingerol, respectively, were confirmed.

Shogaols and zingerone were previously identified as two other categories of pungent compounds in aged or thermal-processed ginger samples (Connell and Sutherland, 1969; Govindarajan, 1979). Instead, only minute amounts of shogaols were reported in fresh ginger samples (Steineggerand Stucki, 1982; Baranowski, 1985). It is therefore impractical to isolate the minor constituents directly from CO_2(l) extract for identification. Figure 2 shows the scheme of deriving shogaols and zingerone from gingerols in this study. Shogaols derived from gingerols were isolated by TLC, fractionated by preparative HPLC, and then identified by FAB-MS. The molecular weights of 276, 304, and 332, which corresponded to 6-shogaol, 8-shogaol, and 10-shogaol, respectively, were confirmed. The molecular weight of zingerone (194), which was derived from basic degradation of gingerols, was confirmed by CI-MS. The derived shogaols and zingerone were used as authentic samples for identification.

Figure 3 shows the chromatograms of analytical HPLC of (A) isolated gingerols, (B) shogaols derived from gingerols, (C) zingerone derived from gingerols, and (D) CO_2(l) extract of ginger. Vanillin was added as internal standard in chromatograms A and D. Chromatographic separations of gingerols and shogaols are similar to previous report (Steinegger and Stucki, 1982). It is worth noting that the relative percentage of gingerols and shogaols derived from gingerols, as analyzed by HPLC, is not the same (G6/G8/G10 75.94/9.85/14.20 vs. S6/S8/S10 = 84.91/9.97/5.11). The disproportional ratio indicates that the rate of deriving 10-shogaol is slower than the rate of which 6-shogaol is derived; that is, the β-hydroxyl group of gingerol (Figure 2) during acidic dehydration may be stabilized by the longer alkyl side chain, as is the ratio of 10-gingerol vs. 6-gingerol.

The results of Figure 3 and Table I show that 6-gingerol, 8-gingerol, and 10-gingerol are the dominant gingerol compounds identified in the CO_2(l) extract. Only a trace amount of 6-shogaol (0.08%) can be identified in the CO_2(l) extract; this coincides well with the TLC result of this study. Zingerone could not be detected in the CO_2(l) extract.

The pungency analyses of gingerols and shogaols by Govindarajan (1979) indicate that 6-gingerol and 6-shogaol are the two most important components responsible for the pungency of ginger products. It is interesting to note that although 6-shogaol is derived from 6-gingerol during thermal processing or aging, it is reported to be even more pungent than 6-gingerol (1.5×10^5 vs. 0.8×10^5 SU (Scoville unit)) (Govindarajan, 1979). Other gingerols or shogaols are less important to contribute any pungency. For example, the Scovill unit (SU) of either 8-gingerol or 10-gingerol is less than 0.1×10^5. In this study, 6-gingerol accounts for about 75% of the gingerol compounds identified in the CO_2(l) extract; it is therefore considered to be the most important component responsible for the pungency of CO_2(l) extract of ginger.

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Registry No. 6-Gingerol, 23513-14-6; 8-gingerol, 23513-08-8; 6-shogaol, 555-66-8; 10-gingerol, 23513-15-7.

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