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Commercially processed dry ginger (*Zingiber officinale*): Composition and effects on LPS-stimulated PGE_2 production

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Abstract

Using techniques previously employed to identify ginger constituents in fresh organically grown Hawaiian white and yellow ginger varieties, partially purified fractions derived from the silica gel column chromatography and HPLC of a methylene chloride extract of commercially processed dry ginger, *Zingiber officinale* Roscoe, Zingiberaceae, which demonstrated remarkable anti-inflammatory activity, were investigated by gas chromatography-mass spectrometry. In all, 115 compounds were identified, 88 with retention times (R_t) >21 min and 27 with <21 min. Of those 88 compounds, 45 were previously reported by us from fresh ginger, 12 are cited elsewhere in the literature and the rest (31) are new: methyl [8]-paradol, methyl [6]-isogingerol, methyl [4]-shogaol, [6]-isoshogaol, two 6-hydroxy-[*n*]-shogaols ($n = 8$ and 10), 6-dehydro-[6]-gingerol, three 5-methoxy-[*n*]-gingerols ($n = 4$, 8 and 10), 3-acetoxy-[4]-gingerdiol, 5-acetoxy-[6]-gingerdiol (stereoisomer), diacetoxy-[8]-gingerdiol, methyl diacetoxy-[8]-gingerdiol, 6-(4′-hydroxy-3′-methoxyphenyl)-2-nonyl-2-hydroxytetrahydropyran, 3-acetoxydihydro-[6]-paradol methyl ether, 1-(4′-hydroxy-3′-methoxyphenyl)-2-nonadecen-1-one and its methyl ether derivative, 1,7-*bis*-(4′-hydroxy-3′-methoxyphenyl)-5-methoxyheptan-3-one, 1,7-bis-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxy-5-acetoxyheptane, acetoxy-3-dihydrodemethoxy-[6]-shogaol, 5-acetoxy-3-deoxy-[6]-gingerol, 1-hydroxy-[6]-paradol, (2*E*)-geranial acetals of [4]- and [6]-gingerdiols, (2*Z*)-neral acetal of [6]-gingerdiol, acetaldehyde acetal of [6]-gingerdiol, 1-(4-hydroxy-3-methoxyphenyl)-2,4-dehydro-6-decanone and the cyclic methyl orthoesters of [6]- and [10]-gingerdiols. Of the 27 $R_t < 21$ min compounds, we had found 5 from fresh ginger, 20 others were found elsewhere in the literature, and two are new: 5-(4′-hydroxy-3′-methoxyphenyl)-pent-2-en-1-al and 5-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxy-1-pentanal. Most of the short R_t compounds are probably formed by thermal degradation during GC (which mimics cooking) and/or commercial drying. The concentrations of gingerols, the major constituents of fresh ginger, were reduced slightly in dry ginger, while the concentrations of shogaols, the major gingerol dehydration products, increased.

Keywords: *Zingiber officinale*; Zingiberaceae; Ginger; Rhizomes; Ginger derivatives

1. Introduction

In our previous report on the gas chromatography-mass spectrometry (GC-MS) analysis of partially purified fractions from two organically grown fresh white and yellow ginger varieties, *Zingiber officinale* Roscoe

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(Zingiberaceae) from Hawaii, we described the identification of 63 compounds including 31 compounds previously reported as ginger constituents, 25 new gingerol derivatives and 7 thermal degradation products of gingerols (Jolad et al., 2004). In this paper we report similar findings from the GC-MS analysis of biologically active column chromatography (CC) fractions from the CH_2Cl_2 extract of commercially processed dry ginger powder with special emphasis on their activity in inhibiting in vitro PGE_2 production.

Chronic inflammation has been associated with a number of human diseases including chronic obstructive pulmonary disease, asthma and rheumatoid arthritis. While "conventional" treatments have met with some success, patients suffering from diseases with associated chronic inflammation are turning to alternatives for relief of their symptoms or as prophylactic treatments. These alternatives include dietary supplements that are purported to have anti-inflammatory actions. However, the efficacy and potency of these supplements have not been studied in great detail. Plants (or supplements derived from the plants) that have received attention as being useful for chronic inflammation include ginger.

Inflammation is associated with a large range of mediators that initiate the inflammatory response, recruit and activate other cells to the site of inflammation and subsequently resolve the inflammation (Gallin and Snyderman, 1999). In general, the chemical mediators can be divided into two large classes: cytotoxins and arachidonic acid metabolites. Products produced by the metabolism of arachidonic acid include both cyclooxygenase products (prostaglandins, thromboxanes) and lipooxygenase products (leukotrienes). Products such as LTB4 and PGE_2 that are representative of these two pathways can initiate polymorphonuclear (PMN) leukocytes recruitment and changes in vascular tone and blood flow. Increased production of prostaglandins during an inflammatory response is achieved by induction of cyclooxygenase 2 (COX-2).

Several studies have indicated that compounds found in ginger are effective in relief of symptoms from chronic inflammatory diseases. Administration of ginger has resulted in patients relating decreased symptoms of rheumatoid arthritis (Srivastava and Mustafa, 1992). Gingerol (a major component of ginger) has been reported to have anti-inflammatory actions. For gingerol these include suppression of both cyclooxygenase and lipooxygenase metabolites and arachidonic acid (Kiuchi et al., 1992; Srivas, 1984; Tjendraputra et al., 2001). Our own research has found that organic extracts from ginger rhizomes or standards containing gingerols were able to inhibit LPS-induced PGE_2 ($IC50 < 0.1$ μg/ml) production in U937 cells. Extracts containing either predominantly gingerols or shogaols (identified by HPLC) were both highly active at inhibiting LPS-induced PGE_2 production ($IC50 < 0.1$ μg/ml), while extracts that contained unknown compounds were less effective ($IC_{50} < 3.2$ μg/ml). Extracts or standards containing predominantly gingerols were capable of inhibiting COX-2 expression while shogaol containing extracts had no effect on COX-2 expression (Lantz et al., 2005).

2. Results and discussion

Of the 10 final CC fractions analyzed for biological activity (Table 1), the initial two lipophilic fractions (X/1 and X/2) and the final polar fractions (X/9 and X/10), representing 9.0 (22.5%) and 11.2 g (28%), respectively, of the isolate, had insignificant activity and were, therefore, excluded from further analysis. The remaining six fractions (X/3–8), which exhibited

Table 1
Summary of column chromatography fractionation profile of CH_2Cl_2 extract (X, 40 g) of commercial dry ginger powder

Extract/fraction	Yield (g)	PGE_2 IC_{50} (μg/ml)	Cytotoxic dose (μg/ml)	Compounds detected
Fresh white ginger	–	0.051	10	Original
Fresh yellow ginger	–	0.072	50	Original
Dry ginger (X)	–	0.055	50	Original
X/1	8.96	0.411	50	[a]
X/2	0.06	0.255	>50	[a]
X/3	8.68	0.052	0.1	**1, 3–5, 9, 15, 19, 20, 22, 25, 26, 28–32, 34, 62, 63, 77, 78**
X/4	1.72	0.060	50	**10, 15, 16, 19, 20, 25, 36, 51, 54, 55, 59**
X/5	2.11	0.053	50	**9, 11, 19, 22, 25, 26, 36**
X/6	5.96	0.053	50	**9, 11, 18, 22, 25**
X/7	0.69	0.064	5	**6, 9, 19, 25, 49, 51, 65, 85**
X/8	0.70	0.060	50	**9, 18–20, 22, 25, 27, 43–45, 65, 70, 71, 86, 87**
X/9	4.77	0.430	50	[a]
X/10	6.44	25.388	50	[a]

Fractions (X/1–10), yields, activity in inhibiting in vitro PGE_2 production and compounds detected by GC-MS in the active fractions.
In vitro PGE_2 assay data for two fresh ginger extracts are included in the table for comparison.
[a] Not subjected to GC-MS analysis.

high anti-inflammatory activity, were analyzed by GC-MS to detect and identify the constituents present in them. The chromatographic separation profile, the biological activity data and the compounds identified in the biologically active fractions are summarized in Table 1. The structures, names, molecular ions $[M]^+$ and base peaks of the compounds identified in these active fractions in the retention time (R_t) >21 min region are listed in Table 2. The full scale gas chromatogram (R_t 12–35 min region) of the original crude CH_2Cl_2 extract of dry ginger, which exhibited pronounced anti-inflammatory activity, is reproduced in Fig. 1. The peaks are labeled with compound numbers from Table 2. Compounds identified in the $R_t < 21$ min region are listed separately in Table 3. Most of the peaks in this region were due to sesquiterpenes, which contribute to ginger's aroma. Most of these sesquiterpenes exhibited $[M]^+$ peaks at m/z 204, 218, 220 or 222; they were not identified in this study. Most of the thermal decomposition products were detected in HPLC fractions. The gas chromatogram of one such fraction (Y/*b*), in which most of the compounds in Table 3 were detected, is reproduced in Fig. 2 with peaks labeled with compound numbers from that table. The gas chromatograms of the six PGE_2-active fractions (X/3–8) are reproduced in Fig. 3 to demonstrate the further separation of the ginger constituents, and again the peaks identified are labeled by compound numbers from Table 2.

Several PGE_2-active CC fractions (X/*a–m* and Z1–Z4) and HPLC fractions (Y/1–55 and Y/*a–l*) generated during method development study for the large scale isolation of a fraction containing the three major gingerols ([6, 8,10]) were also subjected to GC-MS analyses and new compounds detected were included in this study.

A complicating factor in these direct GC-MS analyses was that some of the ginger compounds partially or completely thermally decomposed before they reached their retention time. The injection port temperature was 250 °C and the column temperature was raised from 80 to 280 °C between 5 and 25 min after injection. Although some of these decomposition products may not be natural products, all are likely produced when ginger is used in cooking at high temperatures and subsequently eaten; this should increase interest in their presence in this study. Fortunately, only a few major types of thermal decomposition occur with ginger compounds. Predominant are the thermal reverse aldol condensation of β-hydroxyketones (gingerols **6–12**, isogingerols **13** and **14** and certain diarylheptanoids) to give aldehydes and methyl ketones, many of which are shown in Table 3. Less important are dehydration of β-hydroxyketones (e.g., gingerols to shogaols) and loss of acetic acid from β-acetoxyketones (e.g., acetoxy-gingerols to shogaols). The corresponding HPLC–UV spectra run on the same fractions, while only showing the chromophores of UV-absorbing compounds, were run at room temperature and thus were very helpful in showing the relative amounts of certain compounds, e.g., the gingerols. The gingerol comparisons show that [8]-gingerol (**11**) is largely decomposed in GC by its R_t of 25.5 min and [10]-gingerol (**12**) is almost completely decomposed before its R_t. The large hump at about 23.5 min in the GC trace in Fig. 1 is mainly caused by the decomposition of gingerols; the hump drops precipitously after the remaining undecomposed main gingerol, [6]-gingerol (**9**), comes off at 23.8 min. The compounds coming off from about 20–24 min show increasing amounts of zingerone (**T17**), the major aromatic thermal decomposition product of all of the gingerols. Before we recognized that large amounts of zingerone (**T17**) from this decomposition were contaminating the compounds in this region, we misassigned samples of (*E*)-[4]-, -[6]- and -[8]-shogaols (**15**, **19** and **22**) as 1-(4′-hydroxy-3′-methoxyphenyl)-7-octen-3-one, -decen-3-one, and -dodecene-3-one, structures which would undergo McLafferty rearrangement (MLR) to give **T17** (Jolad et al., 2004).

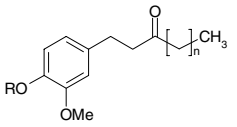
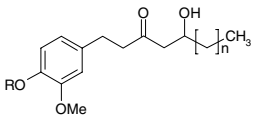
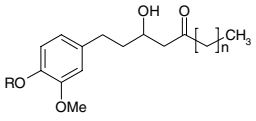
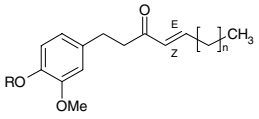
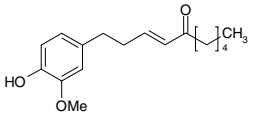
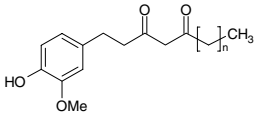
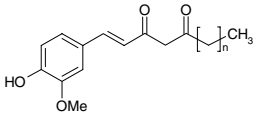
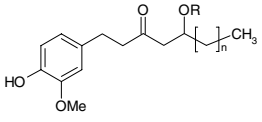
The results of the quantitative analyses of major components [6]-, [8]- and [10]- gingerols (**9, 11** and **12**) and [6]-shogaol (**19**) in the CH_2Cl_2 extract of dry ginger powder versus previously analyzed for the same compounds in fresh white and yellow ginger extracts (Jolad et al., 2004) are summarized in Table 4. [6]-Gingerol (**9**) predominated in all cases. The increase in the concentration of [6]-shogaol (**19**) in dry ginger to 3.3% from its concentration of 0.35% in fresh gingers signifies that while shogaols naturally co-occur with gingerols as minor constituents of ginger, in ginger powder they are derived largely from gingerols via dehydration during the drying process.

Known compounds found in dry ginger were identified by comparison of their R_t's and mass spectra with those reported for the same compounds from fresh ginger varieties (Jolad et al., 2004) and are not discussed here. New compounds and known compounds detected for the first time in ginger are discussed and illustrated with mass spectra.

2.1. Paradols (*1–5*)

All the paradols in Table 2 except **4** were previously detected in fresh ginger (Jolad et al., 2004). From the shifting of the diagnostic ions of [8]-paradol (**3**) by 14 mass units in its MS (m/z 320 $[M]^+$, 208, 193, 165, 164 and 151 (base)), new compound **4** (from fraction Z4/4, R_t 24.32 min) was identified as the homolog of methyl [6]-paradol (**2**) with two more CH_2 units in the carbon chain. Compound **4** was previously known only by synthesis (Locksley et al., 1972).

Table 2
Compounds identified by GC-MS in the column chromatography and HPLC fractions derived from the dichloromethane extract of commercial ginger powder

Structure	Cpd	*n*	R	$[M]^+$	Base	Name
	**1**	6	H	278	137	[6]-Paradol
	2	6	Me	292	151	Methyl [6]-paradol
	3	8	H	306	137	[8]-Paradol
	4	8	Me	320	151	Methyl [8]-paradol
	5	10	H	334	137	[10]-Paradol
	**6**	2	H	266	137	[4]-Gingerol
	7	2	Me	280	151	Methyl [4]-gingerol
	8	3	H	280	137	[5]-Gingerol
	9	4	H	294	137	[6]-Gingerol
	10	4	Me	308	151	Methyl [6]-gingerol
	11	6	H	322	137	[8]-Gingerol
	12	8	H	350	137	[10]-Gingerol
	**13**	2	H	266	137	[4]-Isogingerol
	14	4	Me	308	151	Methyl [6]-isogingerol
	**15**	2	H	248	137	(*E*)-[4]-Shogaol
	16	2	Me	262	151	Methyl (*E*)-[4]-shogaol
	17	3	H	262	137	(*E*)-[5]-Shogaol
	18	4	H	276	137	(*Z*)-[6]-Shogaol
	19	4	H	276	137	(*E*)-[6]-Shogaol
	20	4	Me	290	151	Methyl (*E*)-[6]-shogaol
	21	6	H	304	137	(*Z*)-[8]-Shogaol
	22	6	H	304	137	(*E*)-[8]-Shogaol
	23	6	Me	318	151	Methyl (*E*)-[8]-shogaol
	24	8	H	332	137	(*Z*)-[10]-Shogaol
	25	8	H	332	137	(*E*)-[10]-Shogaol
	26	10	H	360	137	(*E*)-[12]-Shogaol
	**27**	–	–	276	137	[6]-Isoshogaol
	**28**	4	–	292	137	[6]-Gingerdione
	29	6	–	320	137	[8]-Gingerdione
	30	8	–	348	137	[10]-Gingerdione
	31	10	–	376	137	[12]-Gingerdione
	**32**	4	–	290	177	1-Dehydro-[6]-gingerdione
	33	6	–	318	177	1-Dehydro-[8]-gingerdione
	34	8	–	346	177	1-Dehydro-[10]-gingerdione
	**35**	2	Ac	308	137	Acetoxy-[4]-gingerol
	36	4	Ac	336	137	Acetoxy-[6]-gingerol
	37	8	Ac	392	137	Acetoxy-[10]-gingerol
	38	2	Me	280	137	Methoxy-[4]-gingerol
	39	4	Me	308	137	Methoxy-[6]-gingerol
	40	6	Me	336	137	Methoxy-[8]-gingerol
	41	8	Me	364	137	Methoxy-[10]-gingerol

(*continued on next page*)

Table 2 (*continued*)

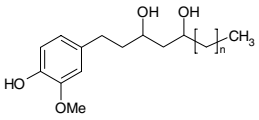
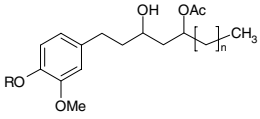
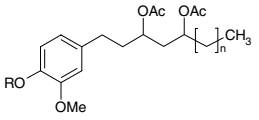
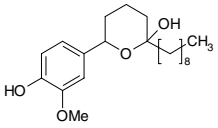
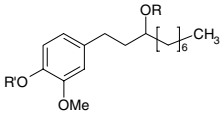
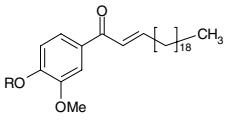
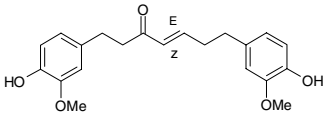
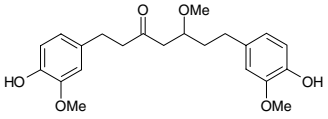
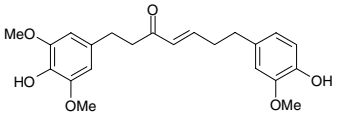
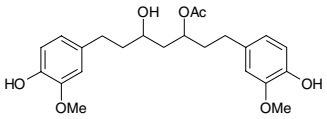
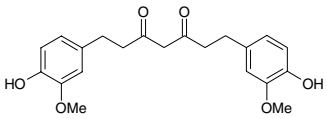
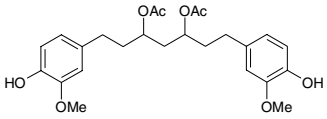
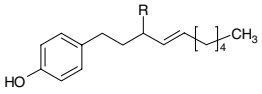
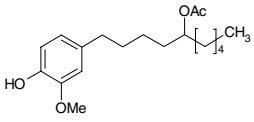
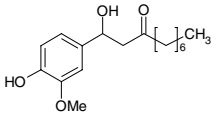
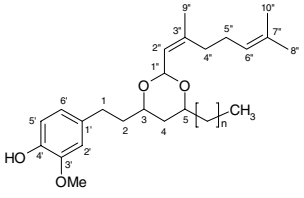
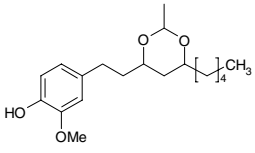
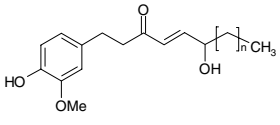
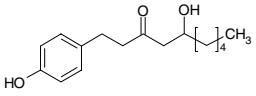
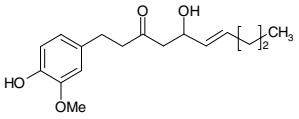
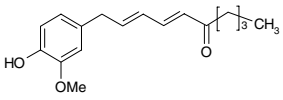
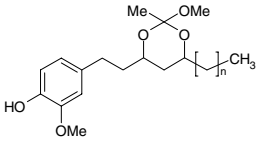
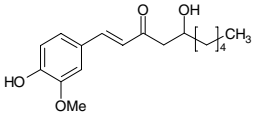
Structure	Cpd	*n*	R	$[M]^+$	Base	Name
	**42**	2	–	268	137	[4]-Gingerdiol
	43	4	–	296	137	[6]-Gingerdiol
	44	6	–	324	137	[8]-Gingerdiol
	45	8	–	352	137	[10]-Gingerdiol
	**46**	2	H	310	137	5-Acetoxy-[4]-gingerdiol
	47	2	Me	324	151	Methyl 5-acetoxy-[4]-gingerdiol
	48	2	H	310	137	3-Acetoxy-[4]-gingerdiol
	49	4	H	338	137	5-Acetoxy-[6]-gingerdiol
	50	4	H	338	137	Stereoisomer of **49**
	51	4	Me	352	151	Methyl 5-acetoxy-[6]-gingerdiol
	**52**	2	H	352	137	Diacetoxy-[4]-gingerdiol
	53	2	Me	366	151	Methyl diacetoxy-[4]-gingerdiol
	54	4	H	380	137	Diacetoxy-[6]-gingerdiol
	55	4	Me	394	151	Methyl diacetoxy-[6]-gingerdiol
	56	6	H	408	137	Diacetoxy-[8]-gingerdiol
	57	6	Me	422	151	Methyl diacetoxy-[8]-gingerdiol
	**58**	–	H	350	150	6-(4′-Hydroxy-3′-methoxyphenyl)-2-nonyl-2-hydroxytetrahydropyran
	**59**	–	H R′ = H	280	137/138	Dihydro-[6]-paradol
	60	–	Ac R′ = H	322	131	Acetoxydihydro-[6]-paradol
	61	–	Ac R′ = Me	336	177	Methyl ether derivative of **60**
	**62**	–	H	416	416	1-(4′-Hydroxy-3′-methoxyphenyl)-2-nonadecen-1-one
	63	–	Me	430	165	1-(3′,4′-Dimethoxyphenyl)-2-nonadecen-1-one
	**64**	–	–	356	137	(*Z*)-1,7-bis-(4′-Hydroxy-3′-methoxyphenyl)-4-hepten-3-one
	65	–	–	356	137	(*E*)-1,7-bis-(4′-Hydroxy-3′-methoxyphenyl)-4-hepten-3-one
	**66**	–	–	388	137	1,7-bis-(4′-Hydroxy-3′-methoxyphenyl)-5-methoxyheptan-3-one
	**67**	–	–	386	137	1-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-7-(4′-hydroxy-3′-methoxyphenyl)-4-hepten-3-one

Table 2 (*continued*)

Structure	Cpd	*n*	R	$[M]^+$	Base	Name
	**68**	–	–	418	137	1,7-bis-(4′-Hydroxy-3′-methoxyphenyl)-3-hydroxy-5-acetoxyheptane
	**69**	–	–	372	137	1,7-bis-(4′-Hydroxy-3′-methoxyphenyl)-3,5-heptadione
	**70**	–	–	460	137	*meso* and (3*S*,5*S*)-3,5-Diacetoxy-1,7-bis-(4′-hydroxy-3′-methoxyphenyl)heptane
	71	–	–	460	137	
	**72**	–	=O	246	175	Demethoxy-[6]-shogaol
	73	–	OAc	290	120	3-Acetoxy-3-dihydrodemethoxy-[6]-shogaol
	**74**	–	–	322	137	5-Acetoxy-3-deoxy-[6]-gingerol
	**75**	–	–	294	151	1-Hydroxy-[6]-paradol
	**76**	2	–	402	137	(2*E*)-Geranial acetal of **42**
	77	4	–	430	137	(2*Z*)-Neral acetal of **43**
	78	4	–	430	137	(2*E*)-Geranial acetal of **43**
	**79**	–	–	322	137	Acetaldehyde acetal of **43**
	**80**	3	–	292	137	6-Hydroxy-[6]-shogaol
	81	5	–	320	205	6-Hydroxy-[8]-shogaol
	82	7	–	348	205	6-Hydroxy-[10]-shogaol

(*continued on next page*)

Table 2 (*continued*)

Structure	Cpd	*n*	R	$[M]^+$	Base	Name
	**83**	–	–	264	107	Demethoxy-[6]-gingerol
	**84**	–	–	292	137	6-Dehydro-[6]-gingerol
	**85**	–	–	274	137	1-(4-Hydroxy-3-methoxyphenyl)-2,4-dehydro-6-decanone
	**86**	4	–	352	137	[6]-Gingerdiol, cyclic methyl orthoester
	87	8	–	408	137	[10]-Gingerdiol, cyclic methyl orthoester
	**88**	–	–	292	137	1-Dehydro-[6]-gingerol

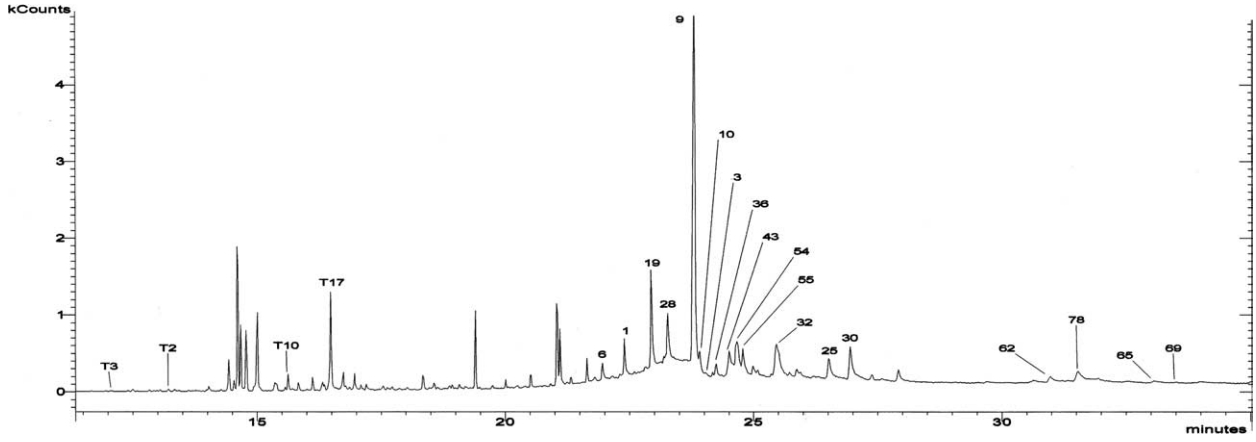


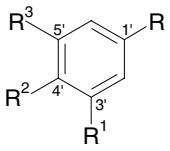
Fig. 1. GC chromatogram of original crude dichloromethane extract (X) of dry commercial ginger. Numbers refer to Tables 2 and 3.

2.2. Gingerols (*6–12*) and isogingerols (*13* and *14*)

Seven gingerols (**6**–**12**) and two isogingerols (**13**–**14**) were detected in this study. All but **8** and **14** were found in fresh Hawaiian ginger (Jolad et al., 2004) and compound **8** was reported from Japanese ginger (Yoshiro et al., 1973). Compound **14** is new.

Compound **8** co-occurred with **6** and **7** in Y/18 and had a retention time of 22.86 min, longer than those of **6** (21.94 min) and **7** (22.14 min). The mass spectrum of **8** [*m*/*z* 280 ($[M]^+$, 24%), 262 (15), 205 (11), 194 (17), 179 (10), 177 (8), 151 (21), 150 (50) and 137 (100)] was virtually identical to that of **6** [*m*/*z* 266 ($[M]^+$, 33%), 248 (16), 205 (10), 194 (12), 179 (10), 177 (6), 151 (16),

Table 3
Compounds with R_t less than 21 min detected by GC-MS in the dichloromethane extract of commercially processed dry ginger



	R^1	R^2	R^3	Ar
A	H	OH	H	Phenol
B	OCH_3	OH	H	Guaiacol
C	OCH_3	OCH_3	H	Veratrol
D	OH	OH	OCH_3	Pyrogallol 1-methyl ether
E	OCH_3	OH	OCH_3	Syringol
F	OH	OCH_3	OCH_3	Pyrogallol 1,2-dimethyl ether
G	OCH_3	OCH_3	OCH_3	Pyrogallol trimethyl ether

Cpd	Ar–R	R_t	$[M]^+$	Base	Name
T1	**A**–CHO	12.66	122	121	*p*-Hydroxybenzaldehyde
T2[a]	**B**–CHO	13.25	152	151	Vanillin
T3[a]	**B**–CH=CH_2	11.96	150	135	4-Vinylguaiacol
T4	**D**–CH=CH_2	14.50	166	151	4-Vinylpyrogallol monomethyl ether
T5	**E**–CH=CH_2	15.38	180	180	4-Vinylsyringol
T6	**B**–$COCH_3$	14.42	166	151	Acetovanillone
T7	**B**–$COOCH_3$	14.80	182	151	Methyl vanillate
T8[d]	**B**–$(CH_2)_2OAc$	16.75	210	137	2-(4′Hydroxy-3′-methoxyphenyl)ethyl acetate
T9	**A**–$(CH_2)_2CHO$	14.29	150	107	3-(4′-Hydroxyphenyl)-1-propanal
T10[a]	**B**–$(CH_2)_2CHO$	15.54	180	137	3-(4′-Hydroxy-3′-methoxyphenyl)-1-propanal
T11[b]	**D**–$(CH_2)_2CHO$	17.62	196	153	3-(3′,4′-Dihydroxy-5′-methoxyphenyl)-1-propanal
T12	**E**–$(CH_2)_2CHO$	17.22	210	167	3-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-1-propanal
T13[b]	**F**–$(CH_2)_2CHO$	18.27	210	167	3-(3′-Hydroxy-4′,5′-dimethoxyphenyl)-1-propanal
T14	**C**–CH=CHCHO	18.37	192	145	2-Dehydro-3-(3′,4′-dimethoxyphenyl)-1-propanal
T15	**G**–CH=CHCHO	20.73	222	175	2-Dehydro-3-(3′,4′,5′-trimethoxyphenyl)-1-propanal
T16[a]	**A**–$(CH_2)_2COCH_3$	15.25	164	107	4-(4′-Hydroxyphenyl)-2-butanone
T17[a]	**B**–$(CH_2)_2COCH_3$	16.40	194	137	Zingerone ([0]-paradol)
T18[a]	**C**–$(CH_2)_2COCH_3$	16.96	208	151	Zingerone methyl ether
T19[b]	**D**–$(CH_2)_2COCH_3$	18.41	210	167	4-(3′,4′-Dihydroxy-5′-methoxyphenyl)-2-butanone
T20	**E**–$(CH_2)_2COCH_3$	18.14	224	181	4-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-2-butanone
T21[b]	**F**–$(CH_2)_2COCH_3$	19.07	224	167	4-(3′-Hydroxy-4′,5′-dimethoxyphenyl)-2-butanone
T22	**G**–$(CH_2)_2COCH_3$	18.38	238	195	4-(3′,4′,5′-Trimethoxyphenyl)-2-butanone
T23[c]	**B**–$(CH_2)_2COCH_2CH_3$	17.47	208	137	[1]-Paradol
T24	**B**–CH=CHCOOH	27.38	194	194	Ferulic acid
T25[b]	**B**–$(CH_2)_2CH$=CHCHO	18.58	206	137	5-(4′-Hydroxy-3′-methoxyphenyl)-pent-2-en-1-al
T26[b]	**B**–$(CH_2)_2CH(OH)CH_2CHO$	19.71	224	137	5-(4′-Hydroxy-3′-methoxyphenyl)-3-hydroxy-1-pentanal
T27[a]	**B**–$(CH_2)_2CH(OH)CH_3$	16.70	196	138	Zingerol

[a] Detected in fresh ginger as well.
[b] Not reported in the literature.
[c] Reported by synthesis only.
[d] No reference cited in the literature.

150 (57) and 137 (100)] except that the $[M]^+$ and dehydration peaks were shifted upward by 14 mass units in **8** to *m/z* 280 $[M]^+$ and 262. Thus the additional 14 mass units in **7** [*m/z* 280 ($[M]^+$, 38%), 262 (13), 219 (6), 208 (13), 193 (11), 191 (6), 165 (23), 164 (33) and 151 (100)] come from methylation of the phenolic OH group and in **8**, from an additional CH_2 unit in the carbon chain.

Compound **14** (R_t 23.94 min), appearing in HPLC fraction Y/*f*, gave a GC shoulder peak for methyl [6]-gingerol (**10**, 23.81 min). Its mass spectrum [*m/z* 308 ([M]+, 3%), 290 (24), 219 (15), 194 (13), 177 (8), 165 (7), 164 (5) and 151 (100)] was similar to that of **10** [*m/z* 308 ($[M]^+$, 9%), 290 (18), 219 (12), 208 (36), 177 (3), 165 (28), 164 (11) and 151 (100)] with $[M]^+$ and base peaks at *m/z* 308 and 151, respectively, but in **14**, the MLR peak was at *m/z* 194 rather than at *m/z* 208. Thus **14** is the isomer of **10** in which the positions of the keto and OH groups at C-3 and C-5 are reversed.

2.3. Shogaols (*15–26*) and [6]-isoshogaol (*27*)

Twelve shogaols (**15–26**) were detected in this study, including two not previously reported from ginger: methyl (*E*)-[4]-shogaol (**16**) and (*E*)-[5]-shogaol (**17**). It is not clear how many of these are natural products, since they are well known to be formed by thermal dehydration of gingerols (Chen et al., 1986a,b) and also would be formed from acetoxy- and methoxy-gingerols. The most abundant shogaol, (*E*)-[6]-shogaol (**19**), was detected by HPLC at room temperature in our study, but might have been formed from [6]-gingerol (**9**) during commercial drying of the ginger.

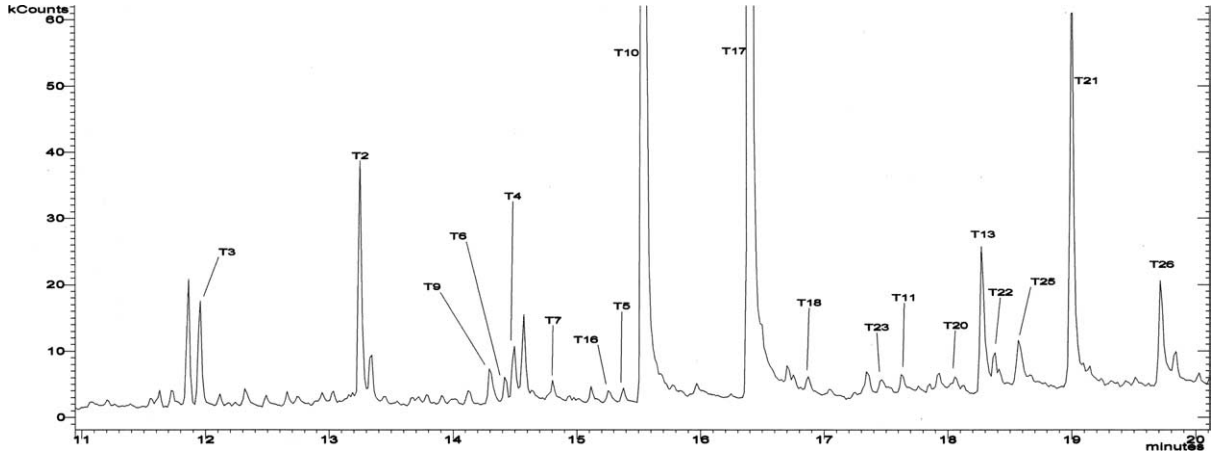


Fig. 2. GC chromatogram ($R_t < 20$ min region) of HPLC fraction Y/*b* from the original dichloromethane extract (X). Numbers refer to Table 3.

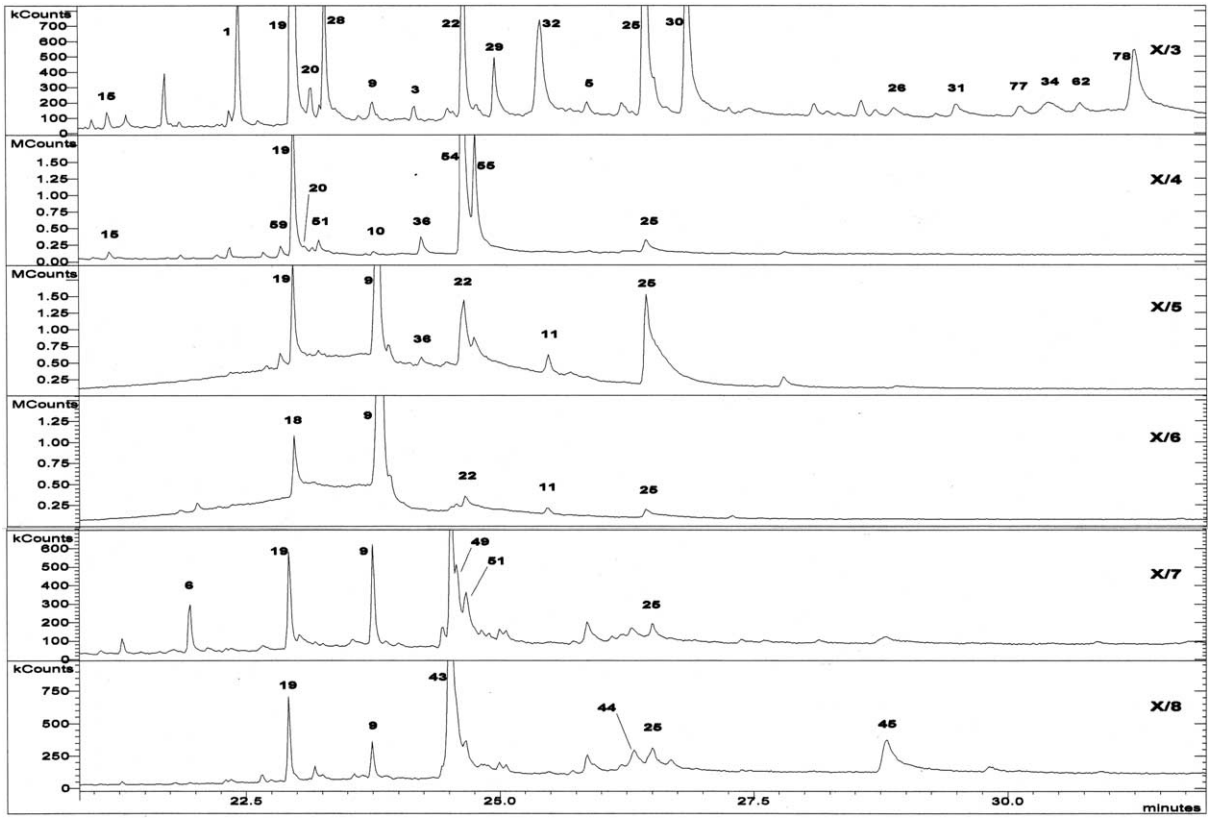


Fig. 3. GC chromatograms of PGE_2 active fractions (X/3–8).

Table 4
Summary of quantification of [6]-, [8]-, and [10]-gingerols (**9**, **11** and **12**) and [6]-shogaol (**19**) in the CH_2Cl_2 extracts of commercial dry and two fresh white and fresh yellow varieties of ginger

Ginger extract	Sample composition (%)			
	[6]-Gingerol (**9**)	[8]-Gingerol (**11**)	[10]-Gingerol (**12**)	[6]-Shogaol (**19**)
Dry	11.38 ± 0.04	2.17 ± 0.01	3.44 ± 0.01	3.29 ± 0.04
Fresh white	27.56 ± 0.04	3.20 ± 0.04	5.38 ± 0.00	0.36 ± 0.02
Fresh yellow	33.96 ± 0.17	4.64 ± 0.10	7.91 ± 0.19	0.35 ± 0.02

The new shogaols **16** and **17** both had $[M]^+$ peaks at *m*/*z* 262 (Fig. 4). The latter (**17**) was readily identified as (*E*)-[5]-shogaol from its mass spectrum which included strong peaks at *m*/*z* 137 (base) and 205, and a GC retention time (21.35 min) halfway between those of (*E*)-[4]-shogaol (**15**) and (*E*)-[6]-shogaol (**19**); it was previously known only from synthesis (Kim and Kim, 2004). The corresponding peaks in the mass spectrum of **16** were at *m*/*z* 151 and 219, indicating that it had two methoxyl groups on the ring and one less methylene group in the alkyl chain than **17**. That it was methyl (*E*)-[4]-shogaol was consistent with its GC retention time of 21.35, 0.27 min longer than that of (*E*)-[4]-shogaol (**15**). These structures would be expected to arise from dehydration of the corresponding gingerols, **7** and **8**, both of which were found in this study.

Using mass chromatography, Chen et al. (1986a,b) detected among other ginger components homologous series of methyl [6]-, [8]-, [10]- and [12]-gingerols and methyl (*Z*)- and (*E*)-[6]-, -[8]- and -[10]-shogaols with GC retention times slightly longer than those of the usual gingerols and shogaols, and proposed the added methyl groups to be at C-5 on the carbon chain, although their mass spectral [M + H] values did not indicate the site for the added methyl group. Since our EIMS results mentioned above clearly show that methylation is on the aromatic ring in our methylated gingerols (**7** and **10**) and shogaols (**16**, **20** and **23**), and since from similar sources and GC results we seem to have some of the same compounds they found, we propose that their compounds were also methylated on the phenolic hydroxyl group rather than at C-5.

The structure of new compound [6]-isoshogaol (**27**, R_t 22.22 min), found in fraction X/*m* just before (*Z*)-[6]-shogaol (**18**), was based on its MS (Fig. 4), which showed a strong $[M]^+$ peak *m*/*z* 276, a 137 peak (base), no *m*/*z* 205 or 119 peaks (characteristic of C=O at C-3), and peaks at *m*/*z* 122 and 94 coming from the loss of methyl and then CO from the 137 species. Small amounts of isoshogaols are expected in ginger from dehydration of isogingerols.

2.4. Gingerdiones (*28*–*31*)

No gingerdiones were previously reported in fresh white and yellow gingers but in dry ginger, four gingerdiones, [6] (**28**), [8] (**29**), [10] (**30**) and [12] (**31**), were detected in the X/3 fraction. The homologous relationship among these gingerdiones was evident from their mass spectra. In the mass spectra, the gingerdiones fragmented much the same way as the paradols, but several small differences showed which family they belong to, especially the larger *m*/*z* 150 peaks (derived from MLR) in the gingerdione mass spectra and larger *m*/*z* 179 peaks for the paradols. Reexamination of the mass spectra of paradols showed the compounds

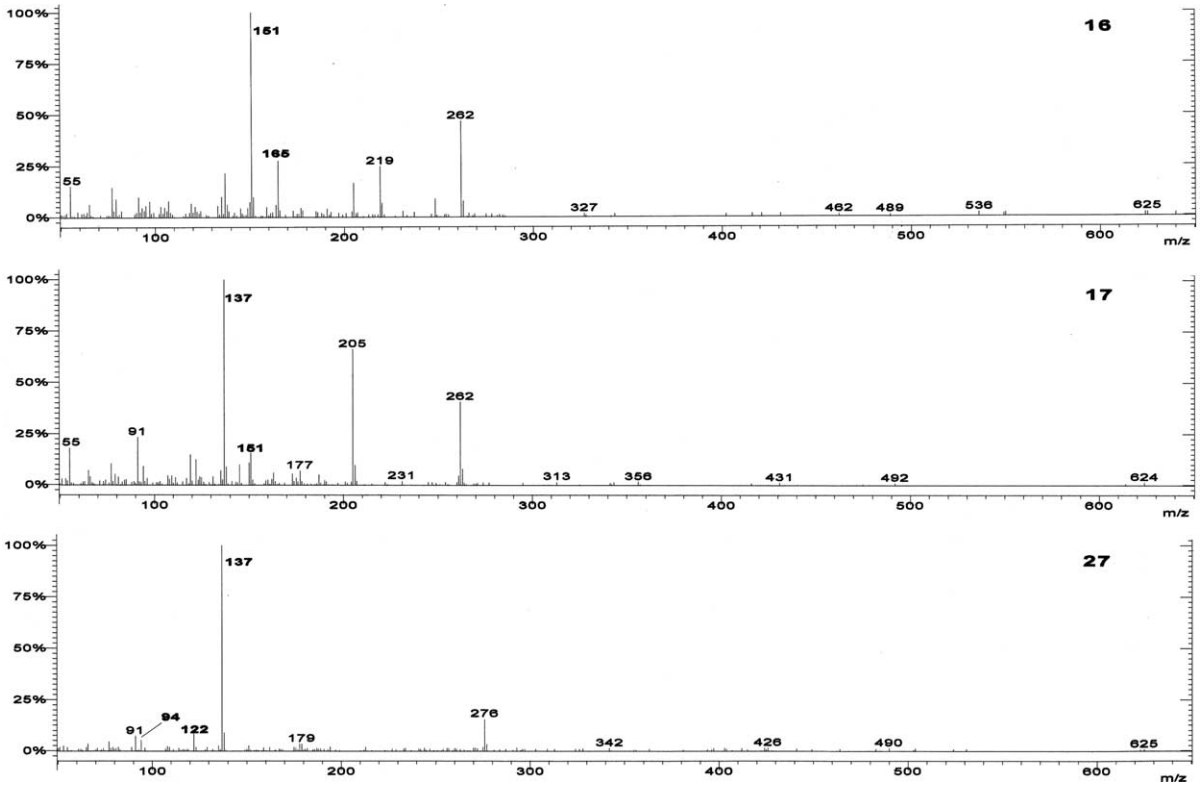


Fig. 4. Mass spectra of compounds **16**, **17** and **27** listed in Table 2.

assigned as odd-numbered paradols ([7], [9], [11] and [13]) in our earlier publication (Jolad et al., 2004) to actually be [6]-, [8]-, [10]- and [12]-gingerdiones, which have molecular ions with the same masses and nearly the same R_t's expected for the odd-numbered paradols. The mass spectral data for [6]-gingerdione (**28**) [*m*/*z* 292 ($[M]^+$, 30%), 221 (2), 193 (2), 179 (6), 151 (5), 150 (18) and 137 (100)] and [6]-paradol (**1**) [*m*/*z* 278 ($[M]^+$, 54%), 194 (4), 179 (32), 151 (22), 150 (14) and 137 (100)] are given to illustrate these points. Gingerdiones were known to occur in *Z. officinale* (Harvey, 1981a).

2.5. Methoxy-gingerols (*38*–*41*)

From fresh ginger, we reported only one member (**39**) of the homologous series, methoxy-[4]-, -[6]-, -[8]- and -[10]-gingerols (**38**–**41**). In dry ginger, all four members were detected in a single fraction (Z1/3). Their mass spectra showed the $[M]^+$ peaks of shogaol homologs **15**, **19**, **22** and **25**, due to the elimination of 32 mass units (MeOH), and their spectra below the M − MeOH peak nearly superimpose on the spectra of the corresponding shogaols. This conversion of methoxy-gingerols to the corresponding shogaols via elimination of methanol parallels the conversion of gingerols and acetoxygingerols to the corresponding shogaols via elimination of water and acetic acid, respectively. The methoxy-[4]-, -[8]- and -[10]-gingerols (**38**, **40** and **41**) are new compounds.

2.6. Mono- and di-acetoxy-gingerdiols (*46*–*57*)

Six monoacetoxy-gingerdiols (**46**–**51**) and six diacetoxy-gingerdiols (**52**–**57**) were detected in this study. All except **48**, **50**, **56** and **57**, which are new, were previously reported from fresh ginger (Jolad et al., 2004).

Compound **48** had a retention time (23.00 min) slightly longer than that of **46** (22.95 min) but exhibited a very similar mass spectrum with $[M]^+$ and base peaks at *m*/*z* 310 and 137, respectively. The most significant difference between their spectra was a peak at *m*/*z* 207 (56%) for **48**, absent for **46**. The isomeric structure **48** with the acetyl group at C-3 rather than at C-5 as in **46** gives this ion by elimination of acetic acid to give a 3,4-double bond followed by cleavage of the 5,6 bond.

Compound **50** appeared in an overlapping peak with **49** in fraction X/*m* and had an R_t (24.43 min) slightly lower than that of **49** (24.48 min). Their mass spectra were nearly identical except for the peak heights. Most notably, the M − HOAc peak at *m*/*z* 278 was larger in **50** (75%) than in **49** (22%), while the M − (HOAc + H_2O) peak at *m*/*z* 260 was more abundant in **48** (32% versus 7%). Compounds **49** and **50** were therefore considered to be stereoisomers. Compound **56** was identified as the higher homolog of **54** by two methylene units. Compound **57** (*m*/*z* 422 $[M]^+$) was recognized as the methyl ether of **56**.

2.7. Compound *58*

This new compound, detected in HPLC fraction X/*g*, had an R_t (26.89 min) about a minute longer than that of [10]-shogaol (**25**, 25.83 min). Its mass spectrum (Fig. 5) had peaks at *m*/*z* 350 $[M]^+$ (same as [10]-gingerol (**12**)), 332 (M − H_2O, same as $[M]^+$ of **25**), 205, 179 and a base peak unusually at *m*/*z* 150. The absence of a peak at *m*/*z* 137 or 151 suggested substitution at C-1. Structure **58** is proposed with routes to major fragment ions given in Scheme 1.

2.8. Dihydro-[6]-paradols (*59*–*61*)

Along with dihydro-[6]-paradol (**59**) and acetoxydihydro-[6]-paradol (**60**), previously detected in fresh ginger (Jolad et al., 2004), new compound methyl acetoxydihydro-[6]-paradol (**61**) was found in the CC (Z4/4) and HPLC (Y/*k*) fractions. The structure of **61**was based on its mass spectral peaks (Fig. 5) at *m*/*z* 336 ($[M]^+$, 33%), 276 (M − HOAc, 100), 177 (98), 164 (29) and 151 (92), all shifted upward by 14 mass units from **60**, and its R_t of 23.28 min, slightly longer than that of **60** (23.11 min).

2.9. Compounds *62* and *63*

These new compounds, with R_t 30.95 min (**62**) and 32.49 min (**63**), displayed mass spectra with only four pronounced peaks for **62** at *m*/*z* 416 ($[M]^+$, base), 191, 151 and 123, all shifted upward by 14 mass units for **63**. These features suggest that **62** and **63** contain the same carbon chain but are differentiated by **62** having a guaiacol grouping which is methylated to a veratrol grouping in **63**. The fragmentations proposed for **62** and **63** are shown in Scheme 2.

2.10. Diarylheptanoids (*64*–*71*)

Eight diarylheptanoids (**64**–**71**) were identified in dry ginger. Compounds **64** (Harvey, 1981b), **65**/**69** (Jolad et al., 2004), **67** (Endo et al., 1990), **70** (Kikuzaki et al., 1991) and **71** (Ma et al., 2004) were previously reported from ginger. Compounds **64** and **65** were the major components of certain HPLC fractions (Y/11 and Y/18). Their GC retention times were 31.79 (**64**) and 33.06 min (**65**), and their relative intensities were in the ratio of 1:4. Both displayed $[M]^+$ peaks at *m*/*z* 356, base peaks at *m*/*z* 137 and similar fragmentation patterns, with *m*/*z* 219/179 stronger in **64** and 206/163/162 stronger in **65** (Fig. 6). These data coupled with our experience with (*Z*)- and (*E*)-shogaols indicated that **64** is (*Z*)-gingerenone A and **65** is (*E*)-gin-

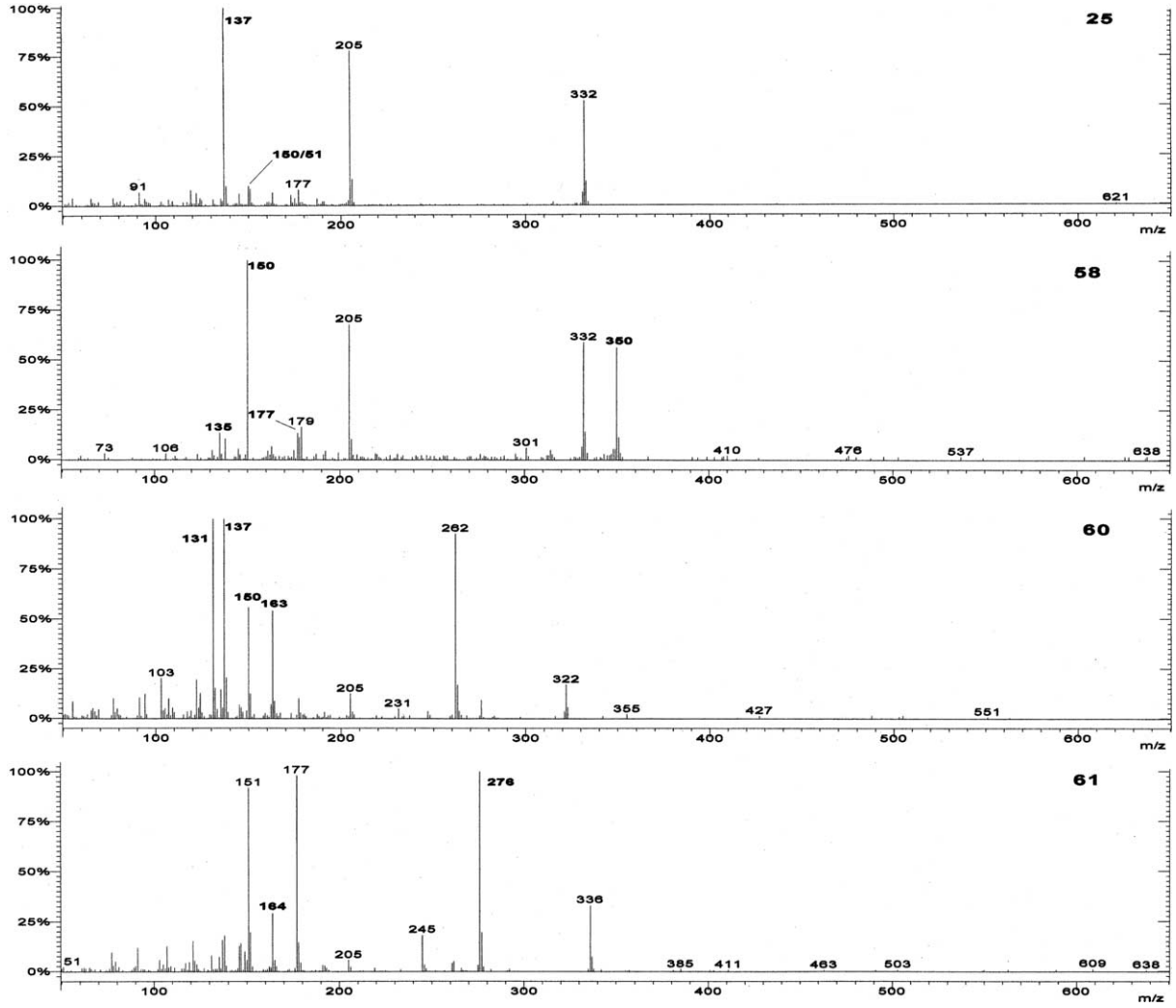
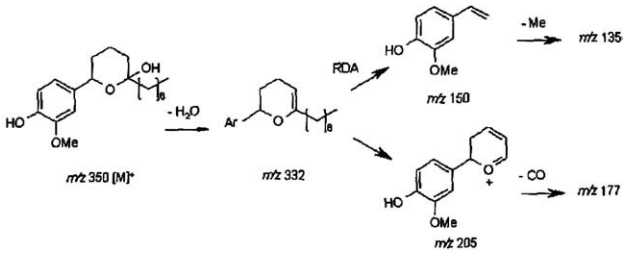
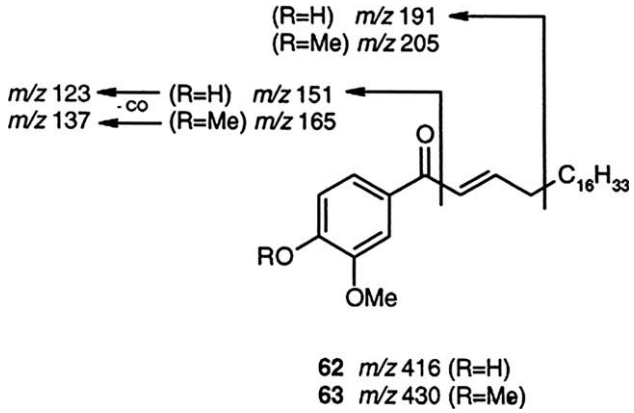


Fig. 5. Mass spectra of compounds **25**, **58**, **60** and **61** listed in Table 2.



Scheme 1. Major fragment ions in the mass spectrum of **58**.



Scheme 2. Major fragment in the mass spectra of **62** and **63**.

gerenone A. The *Z* isomer (**64**) was previously found in GC-MS analyses of tetramethylsilane derivatives of ginger (Harvey, 1981b).

The structure of new compound **66** (R_t 34.03 min) found in fraction Z1/8 was evident from its mass spectrum (Fig. 6), with *m/z* 388 ($[M]^+$, 356 [M – MeOH, the $[M]^+$ peak of (*Z*)- and (*E*)-gingerenone A (**64/65**)), and further peaks resembling those of a mixture of (*Z*)- and (*E*)-gingerenone A (**64/65**). Diarylheptanoids containing a 5-methoxyl group and a 3-keto group have been identified in *Zingiberaceae* species, *Alpinia officinarum* (Hideji et al., 1985) and *A. blepharocalyx* (Kadota et al., 2003).

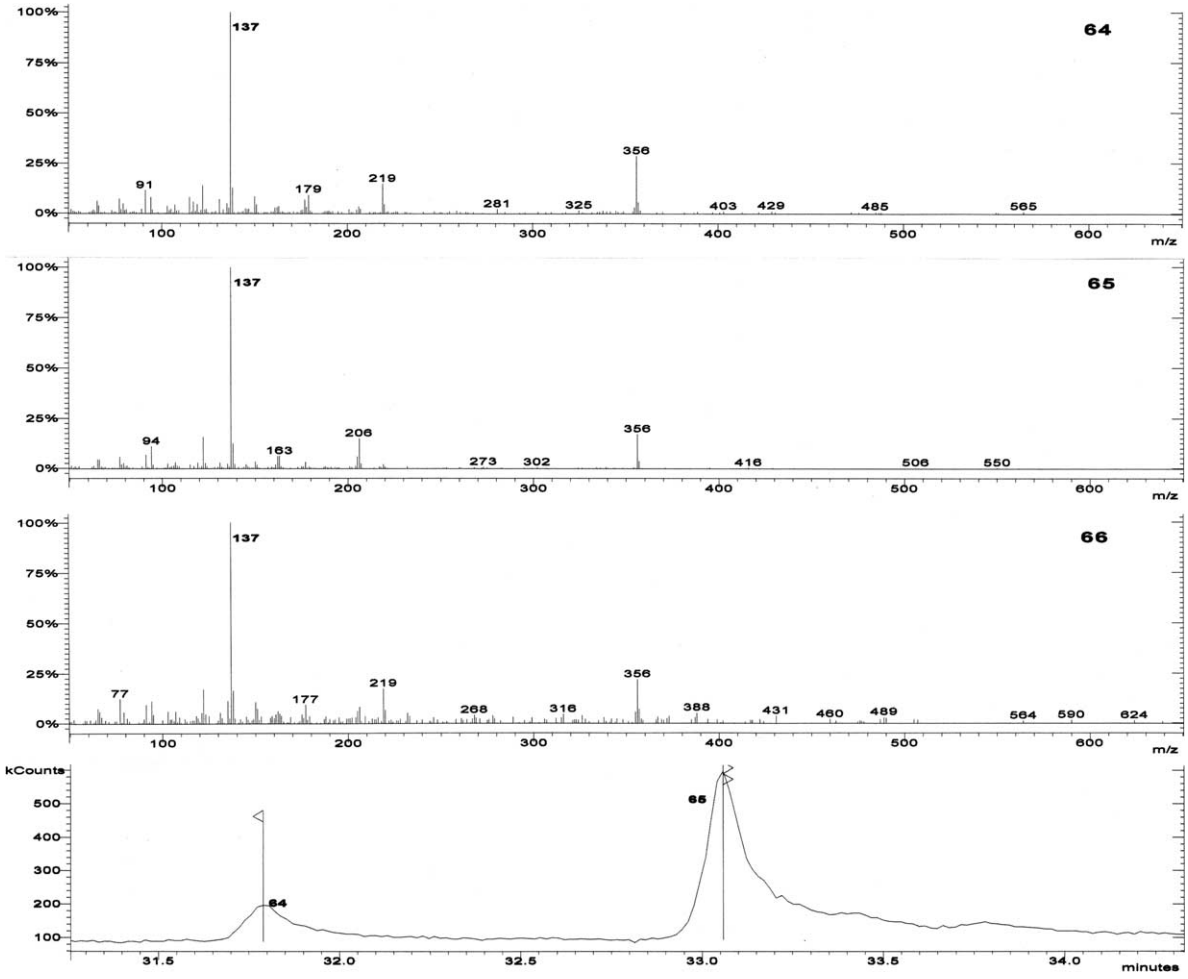


Fig. 6. Mass spectra of compounds **64**, **65** and **66** listed in Table 2. GC chromatogram of compounds **64** and **65**.

Compound **67**, detected in HPLC fraction Y/18 with the very long GC retention time of 38.14 min, was recognized as gingerinone B from its mass spectrum which exhibited an $[M]^+$ peak at *m/z* 386 (26%) and fragment peaks at 167 (syringyl-CH_2^+, 66%) and 137 (guaiacyl-CH_2^+, 100%). It was previously reported from ginger in a mixture of gingerinone B and isogingerinone B (Endo et al., 1990).

New diarylheptanoid **68** exhibited an $[M]^+$ peak at *m/z* 418 and peaks at *m/z* 358 (M – HOAc), 340 (358-H_2O), 204, 203, 190, 163, 151, 150 and 137 (base) which were reminiscent of the fragmentation peaks of 5-acetoxy-gingerdiols (**46**–**51**).

Compounds **70** and **71** were detected in CC fraction X/8. In GC, they appeared in overlapping peaks with retention times of 37.46 (**70**) and 37.77 min (**71**). Their mass spectra were nearly identical, with $[M]^+$ peaks at *m/z* 460 followed by pronounced peaks at *m/z* 400 (M – HOAc) and 340 (400-HOAc) and peaks in the lower mass region at *m/z* 204, 203, 190, 189, 177, 176, 175, 163, 150 and 137 (base) which were reminiscent of the fragmentation pattern of diacetoxy-gingerdiols (**52**–**57**). These spectral data were in accord with the data reported for (*meso*)-diacetoxy-1,7-bis-(4′-hydroxy-3′-methoxyphenyl)-heptane (Kikuzaki et al., 1991). Evidently one is the *meso* form and the other is the 3*S*,5*S* stereoisomer, recently reported from Chinese ginger (Ma et al., 2004). This is the first report of **70** and **71** in ginger by GC-MS.

2.11. Compound 72

Compound **72** (R_t 21.67 min), found in CC fractions X/*g*–*h*, was identified as (*E*)-demethoxy-[6]-shogaol by comparison of its mass spectrum (Fig. 7) with that of (*E*)-[6]-shogaol (**19**, 22.93 min), which showed diagnostic peaks of **19** to be shifted downward by 30 mass units to *m/z* 246 $[M]^+$, 175 (base), 147 and 107 in **72**. In support of this structure, their R_t difference was similar to that between **T16** and **T17** (Table 3). It was previously reported from ginger rhizomes (Kikuzaki et al., 1994).

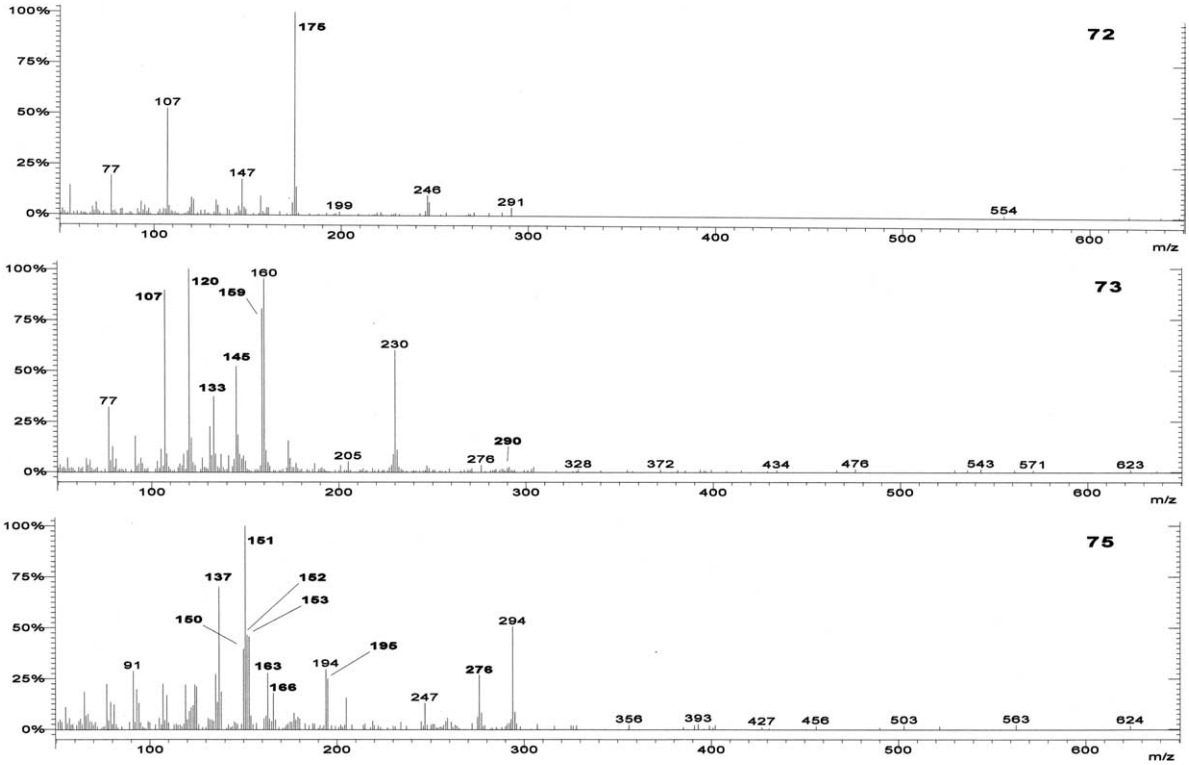


Fig. 7. Mass spectra of compounds **72**, **73** and **75** listed in Table 2.

2.12. Compound 73

The structure of new compound **73** (R_t 24.02 min) was based on its comparison of its mass spectrum (Fig. 7) with that of 3-dihydro-[6]-shogaol (R_t 23.69 min), detected in fresh ginger (Jolad et al., 2004). Except for the difference of 42 mass units between the $[M]^+$ peaks of **73** (m/z 290) and the shogaol derivative (m/z 248), both produced similar peaks, intensified in **73**.

2.13. Compound 74

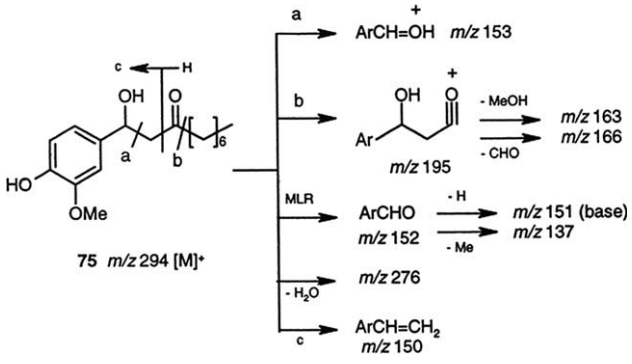
This new compound **74** (R_t 23.37 min), co-occuring with acetoxydihydro-[6]-paradol (**60**, R_t 23.11 min) in fraction Z4/4 and having very similar MS peaks (m/z 322 $[M]^+$, 262 (M − HOAc), 163, 150, 137 (base) and 131), was apparently a regioisomer of **60**. The other peaks in **74** were much weaker relative to the base peak at m/z 137, indicating that the acetoxy group was farther out on the chain than in **60**. On the basis that the usual other oxygenation position of gingerols and derivatives is C-5, **74** is proposed to be 5-acetoxy-3-deoxy-[6]-gingerol.

2.14. Compound 75

This new compound (R_t 23.46 min) was found in CC fraction X/k along with [6]-gingerol (**9**, R_t 23.65 min). In its mass spectrum (Fig. 7), the $[M]^+$ (m/z 294) and dehydration (m/z 276) peaks were the same as for **9** but the base peak was shifted upward from m/z 137 in **9** to 151 in **75**. This gain of 14 mass units suggested the methyl [5]-gingerol structure, but this was ruled out by the absence of peaks at m/z 208 and 219. Instead, **75** had peaks at m/z 195, 166, 163, 153 and 152. To account for these peaks, we propose the 1-hydroxy-[6]-paradol structure **75** with fragmentation as shown in Scheme 3.

2.15. Compounds 76–78

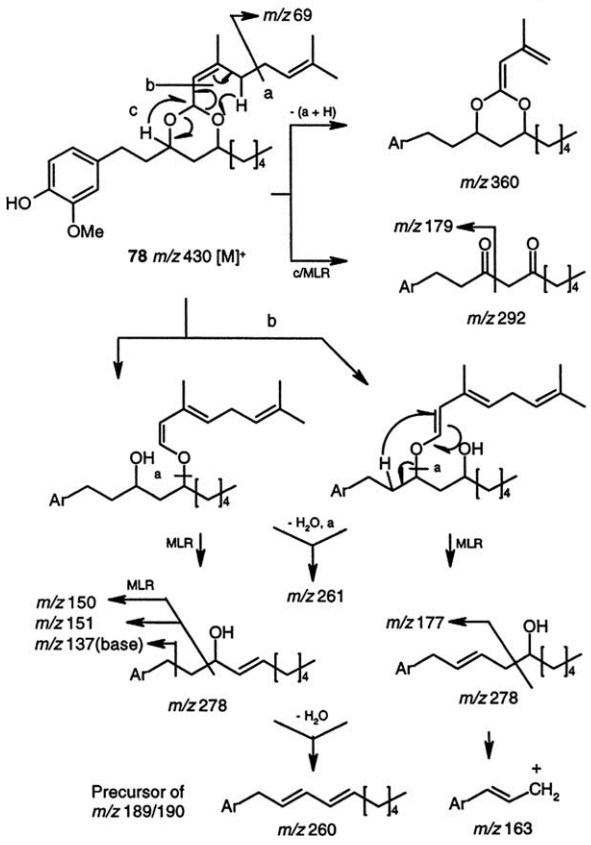
Two of these new compounds (**77** and **78**) were found in main CC fraction X/3 but all four were in purified fraction Z4/3 of CC fraction Z3/5, with **76** (R_t 28.41



Scheme 3. Major fragment ions in the mass spectrum of **75**.

min) and **79** (23.38 min) giving minor peaks and **77** (30.35 min) and **78** (31.55 min) equally strong major peaks. Compounds **77** and **78** gave hardly differentiable mass spectra (Fig. 8) with $[M]^+$ at *m*/*z* 430 and 137 as the base peak. In the low mass region, besides the base peak at *m*/*z* 137, peaks at *m*/*z* 190, 189, 177, 163, 151, 150 and 131 suggested them to be gingerdiol derivatives. In the mid mass region, prominent peaks at *m*/*z* 292 (M-138), 278 (M-152), 261 (M-169) and 260 (278-H_2O) were consistent with this view. The high mass region showed no peak for the loss of water, but instead for loss of 43 and 70 mass units. The loss from M of 70 mass units was related to a strong peak at *m*/*z* 69 in the low mass region. These fragments (Scheme 4) and NMR data (Section 4.2.1) on **78** indicate **77** and **78** to be stereoisomeric geranial and neral acetals of [6]-gingerdiol as shown. Geranial and neral are among several monoterpenes which have been found in ginger (Sharma et al., 2002; Gurib-Fakim et al., 2002). Minor compound **76** gave an $[M]^+$ peak at *m*/*z* 402, 28 mass units less than those of **77**/**78**. Its other peaks with *m*/*z* >250 were also shifted downward by 28 mass units, but the lower mass region was unchanged. Thus **76** was identified as the geranial acetal of [4]-gingerdiol (**42**).

Compound **79**, like **77**–**78**, exhibited peaks (*m*/*z* 278, 261, 260, 190, 189, 179, 177, 163, 151, 150 and 137 (base)) for all the structural elements of [6]-gingerdiol (**43**) in addition to strong peaks at *m*/*z* 322 $[M]^+$ and 262. The low mass $[M]^+$ peak together with losses from



Scheme 4. Major fragment ions in the mass spectrum of **78**.

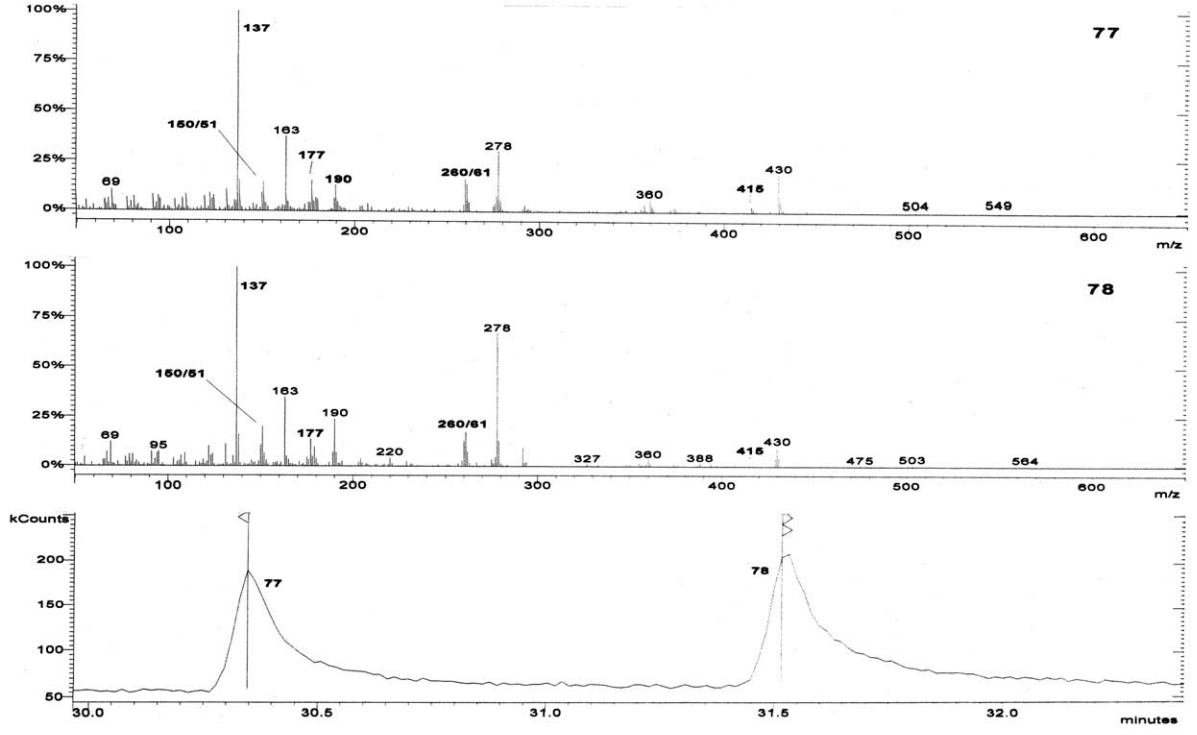


Fig. 8. Mass spectra and GC chromatograms of compounds **77** and **78** listed in Table 2.

M of 44 (*m*/*z* 278) and of 60 (*m*/*z* 262) mass units (equivalent to the elements of acetaldehyde and acetic acid, respectively) indicated **79** to be the acetaldehyde acetal of [6]-gingerdiol (**43**) as shown.

2.16. Compounds 80–82

These new compounds **80** (Y/19, R_t 24.50 min), **81** (X/*j*, R_t 26.13 min) and **82** (X/*j*, R_t 28.44 min) displayed mass spectra differentiable only from their $[M]^+$ peaks, separated by 28 mass units at *m*/*z* 292 (**80**), 320 (**81**) and 348 (**82**), suggesting homology. Their mass spectra below *m*/*z* 206 were very similar to those of shogaol homologs, e.g., [10]-shogaol (**25**, Fig. 9). The peaks at *m*/*z* 206 were much stronger for **80**–**82** (60% versus 18% in **25**). Other distinguishing peaks were at *m*/*z* 231 and M − H_2O for **80**–**82**. The molecular ion peaks of **80**–**82** were 16 mass units higher than those of the corresponding shogaols (**19**, **22** and **25**), suggesting that they are shogaols with an additional OH group on the chain. We favor the 6-position, with fragmentation routes as shown in Scheme 5 (Jolad et al., 2004). Compound **80** was reported from *Z. officinale* (Nakatani and Kikuzaki, 1992). Compounds **81** and **82** are new. The mass spectrum of **82**, reproduced in Fig. 9, illustrates the fragmentation pattern of this type of compound.

2.17. Compound 83

In the mass spectrum of **83**, R_t 23.03 min, from CC fraction Z1/9, the diagnostic peaks of [6]-gingerol (**9**)

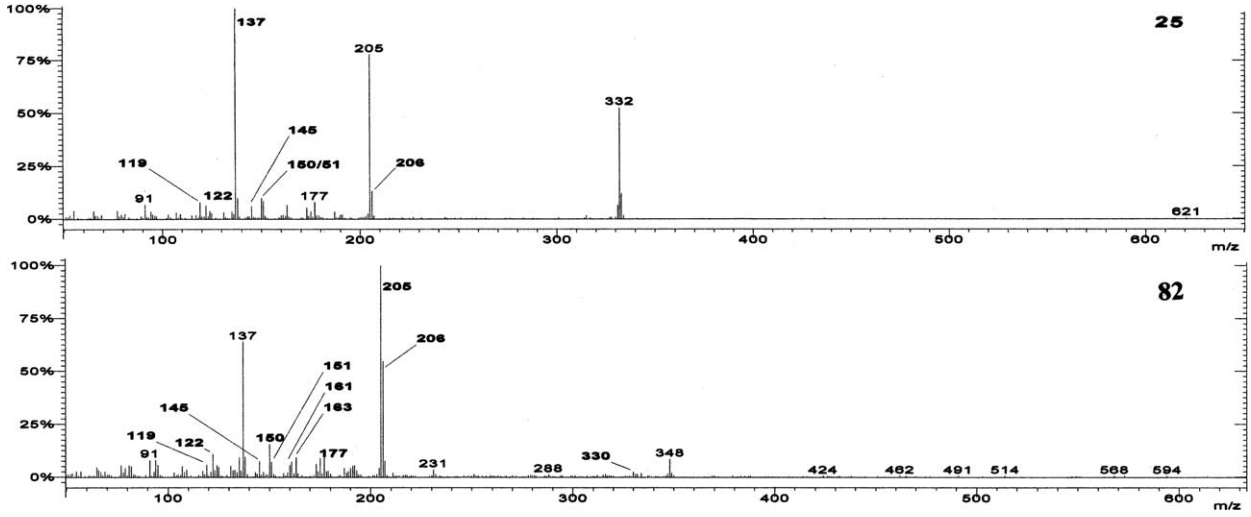
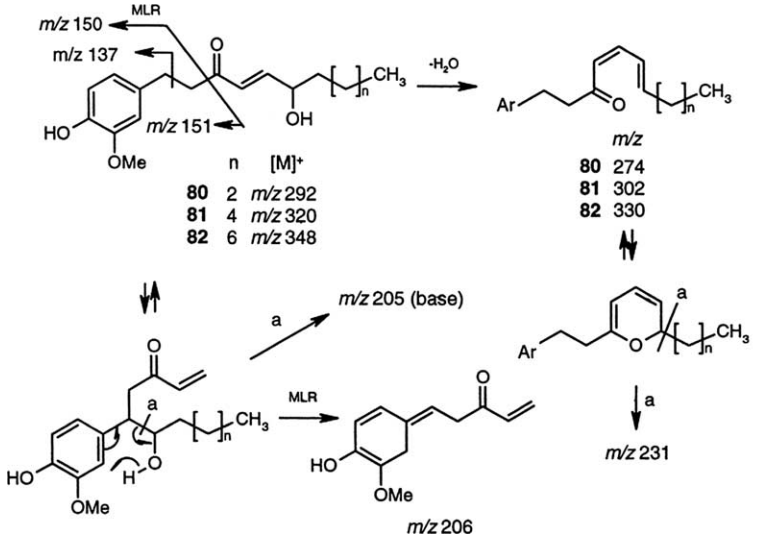


Fig. 9. Mass spectra of compounds **25** and **82** listed in Table 2.



Scheme 5. Fragment ions in the mass spectra of **80**–**82**.

were shifted downward by 30 mass units to *m/z* 264 $[M]^+$, 246 ($M - H_2O$), 175, 164 (MLR), 149, 147, 121, 120 and 107 (base), indicating the guaiacol grouping in **9** was replaced by a phenol grouping in **83**. Compound **83** was thus demethoxy-[6]-gingerol, previously reported from ginger rhizomes (Kikuzaki et al., 1994), and the likely precursor of demethoxy-[6]-shogaol (**72**). This is the first report of **83** from ginger by GC-MS.

2.18. Compound *84*

Found in fraction Y/19, new compound **84** had R_t 23.49 min, $[M]^+$ at *m/z* 292 (two less than [6]-gingerol (**9**)), and like **9** gave a strong dehydration peak at *m/z* 274 and peaks at *m/z* 179, 151 (weak), 150 (strong) and 137 (base) (Fig. 10). However, it lacked an MLR peak at *m/z* 194, probably because the proposed allylic alcohol structure **84** dehydrates so readily. Its fragmentation, rationalized in Scheme 6 (Jolad et al., 2004), shows routes to the strong *m/z* 81 and 217 peaks.

2.19. Compound *85*

Occurring in HPLC fraction Y/19 and CC fraction X/7, new compound **85** (R_t 21.27 min) exhibited no peaks between the $[M]^+$ (*m/z* 274) and base (*m/z* 137) peaks (Fig. 10). Peaks below *m/z* 137 at *m/z* 122, 107, 94 and 81, the daughter ions of the usual *m/z* 137 structure, support its presence. Structure **85**, which gives the additional peak at *m/z* 95, is proposed, with fragmentations as shown in Scheme 7 (Jolad and Hoffmann, 1990).

2.20. Compounds *86* and *87*

Detected in CC fraction X/8 where gingerdiols **42**–**45** were found, these compounds had retention times 23.18 (**86**) and 26.68 min (**87**). Their $[M]^+$ peaks [*m/z* 352 (**86**) and 408 (**87**)] were separated by 56 mass units but their coherent fragmentation leading to common ions (*m/z* 190, 189, 175, 163, 157, 150, 138 and 137 (base)) indicated them to be homologous. There were no dehydration or deacetylation peaks either in **86** or **87**, yet the peaks in

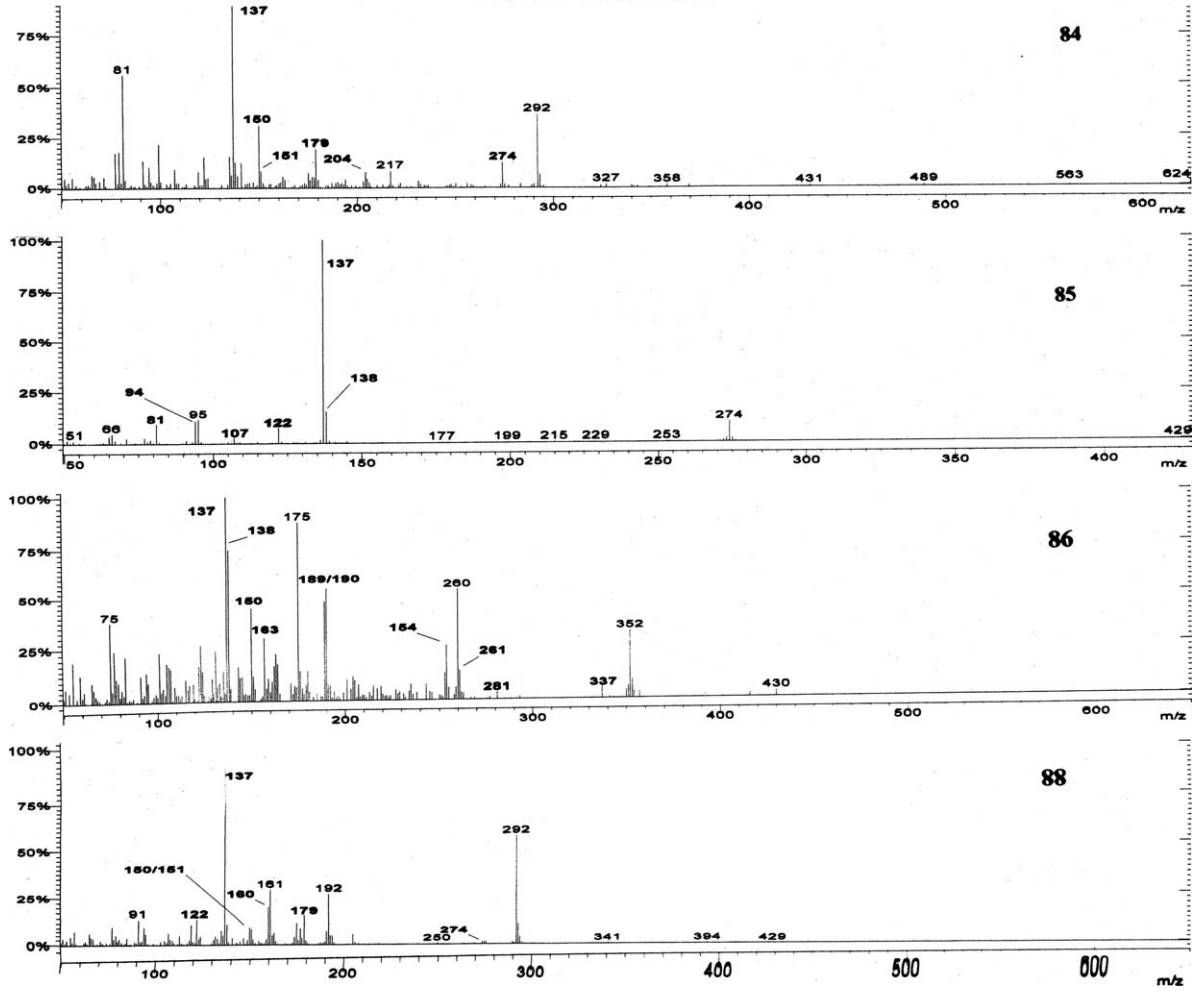
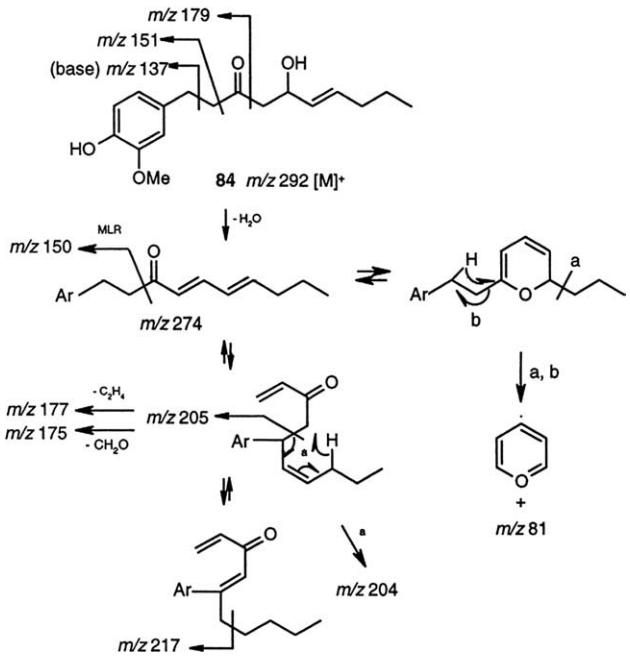
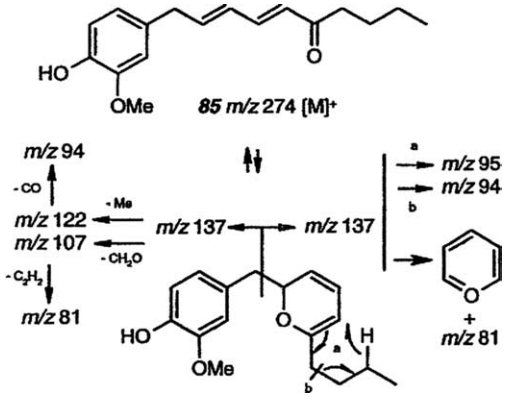


Fig. 10. Mass spectra of compounds **84**–**86** and **88** listed in Table 2.



Scheme 6. Fragment ions in the mass spectrum of **84**.



Scheme 7. Fragment ions in the mass spectrum of **85**.

the region below *m/z* 205 were essentially identical to those observed in the mass spectra of gingerdiols **42**–**45** and diacetoxy-gingerdiols **52**, **54** and **56** following the expulsion of substituents at C-3 and C-5. In the high mass region, the losses from $[M]^+$ of 71 (*m/z* 281), 91 (*m/z* 261), 92 (*m/z* 260) and 98 (*m/z* 254) mass units in **86**, which parallel the losses from $[M]^+$ of 127 (*m/z* 281), 91 (*m/z* 317), 92 (*m/z* 316) and 154 (*m/z* 254) mass units in **87**, are rationalized in Scheme 8 from the proposed structures. The mass spectrum of **86** is reproduced in Fig. 10.

2.21. Compound *88*

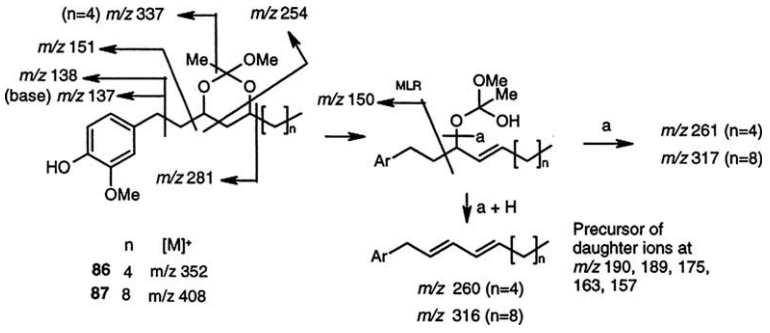
Found in the HPLC fraction Y/19 containing **80**, compound **88** (R_t 23.73 min, shorter than that of **80**) had the same $[M]^+$ (*m/z* 292) and base (*m/z* 137) peaks as **80** but no strong *m/z* 206 peak (Fig. 10). Instead, a strong MLR peak at *m/z* 192 was seen indicating **88** to be 1-dehydro-[6]-gingerol. Its fragmentation is shown in Scheme 9. Although this is the first report of detection of **88** from a natural source, it was synthesized (Denniff and Whiting, 1976) and shown to be an intermediate in the biosynthesis of [6]-gingerol (**9**) in *Z. officinale* (Macleod and Whiting, 1979).

2.22. Compounds with R_t less than 21 min

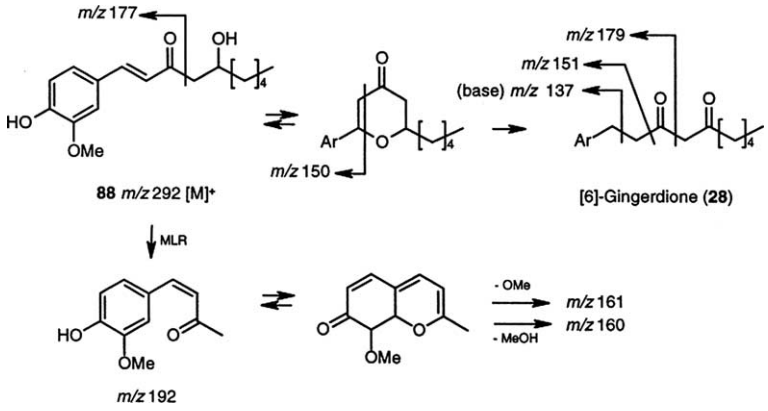
Of the 27 compounds (**T1**–**T27**) listed in Table 3, **T10** and **T17** were found in many fractions of dry and fresh gingers. In the CC fractions, **T24** was detected in X/*k*. All others were found in two HPLC fractions, Y/11 and Y/*b*. Only seven (**T2**, **T3**, **T10**, **T16**–**T18** and **T27**) of the 27 compounds were detected in fresh ginger. Most of the ginger constituents in Table 2 contain a guaiacol (**B**) moiety, some contain a veratrol (**C**) ring, two a phenol (**A**) ring, one a syringol (**E**) ring but none **D, F** or **G** rings. Finding seven products (**T4**, **T11**, **T13**, **T15**, **T19, T21** and **T22**) in Table 3 containing **D, F** or **G** rings suggests the presence of additional gingerols and gingerol derivatives containing those heavier groups which underwent thermal breakdown under GC conditions.

2.22.1. Compounds *T1* and *T2*

In the GC-MS of **T1**, the major peaks of **T2** (*m/z* 152 $[M]^+$, 151 (base) and 123; identified as vanillin using a



Scheme 8. Fragment ions in the mass spectra of **86** and **87**.



Scheme 9. Fragment ions in the mass spectrum of compound **88**.

reference standard) were shifted downward by 30 mass units, indicating ring **B** in **T2** was replaced by ring **A** in **T1**.

*2.22.2. Compounds **T3–T5***

T3 was recognized as 4-vinylguaiacol, a characteristic fragment ion seen in the MS of gingerols and shogaols, from its peaks at *m/z* 150 $[M]^+$, 135 (base), 107, 79 and 77. **T4**, 4-vinylpyrogallol monomethyl ether, had its main peaks shifted upward by 16 mass units to *m/z* 166 ($[M]^+$ (base), 151 (97%) and 123 (82%), and in **T5**, 4-vinylsyringol, these peaks were 14 mass units higher at *m/z* 180 ($[M]^+$, base), 165 (69%) and 137 (53%).

*2.22.3. Compounds **T6** and **T7***

T6 was identified as acetovanillone using a reference standard. **T7**, with a similar fragmentation pattern to **T6** after the loss of OMe to give the base peak at *m/z* 151, was identified as methyl vanillate.

*2.22.4. Compound **T8***

T8 displayed an $[M]^+$ peak at *m/z* 210 (like aldehydes **T12** and **T13** and ketone **T19**) but none of the characteristic daughter ions of the aldehydes and ketone. Instead, an unusually strong M − HOAc peak at *m/z* 150, shown to be **T3** by its further fragmentation, was seen. With the base peak at *m/z* 137, the 2-(4′-hydroxy-3′-methoxyphenyl)-ethyl acetate structure **T8** seems more likely than the biogenetically favored 1-(4′-hydroxy-3′-methoxyphenyl)-ethyl acetate.

*2.22.5. Compounds **T9, T11** and **T13***

All of the aldehydes **T9–T13** had base peaks at M-43 from substituted benzyl cations. Other generally strong peaks were from the loss of one and two CO's at M-28 and M-56. It was helpful to compare the fragmentation pattern with that of the corresponding methyl ketone (**T16–T22**) when that was available. Following the loss of H in **T9** and Me in **T16**, both gave peaks at *m/z* 149, 121, 107 (base), 94, 91 and 77. **T11** and its methyl ketone counterpart **T19** both gave fragments at *m/z* 153, 138, 135, 125, 110, 107 and 77. **T13** fragmented in much the same way as **T11** but with the major peaks shifted upward by 14 mass units to *m/z* 210 ($[M]^+$), 182, 167 (base) and 154. **T13** was assigned as the 3′-hydroxyl compound and the isomeric **T12** as the 4′-hydroxyl compound partly because the longer R_t (by one minute) fits better with the less hindered hydroxyl group.

*2.22.6. Compounds **T14** and **T15***

T15 was readily recognized from its having all of the main peaks of **T14** shifted upward by 30 mass units to *m/z* 222 $[M]^+$, 221, 207, 175 (base), 147 and 119.

*2.22.7. Compounds **T19–T22***

Methyl ketones **T16–T22** all gave strong $[M]^+$, M-43 and M-57 peaks. **T19–T21** also had strong M-75 peaks (75%, 30%, and 70%, respectively), probably from the further loss of MeOH from the M-43 cations. The presence of an OH group in the aromatic ring appears to be requisite for the elimination of MeOH from these cations since **T22**, which does not contain an OH group, gave no M-75 peak. It appears that MeOH is lost more readily from compounds with OH meta to the methoxyl being lost, since **T17** and **T20** give weak M-75 peaks and **T19** and **T21** give strong ones. As with **T12/T13**, the **T20/T21** assignment was also supported by the longer R_t of **T21**.

*2.22.8. Compounds **T23** and **T24***

T23 was assigned as [1]-paradol because it gave all the fragmentation peaks of zingerone (**T17**) but had $[M]^+$14 mass units higher at *m/z* 208. In addition, it gave peaks at M-29 and M-30. **T23** was known only by synthesis (Clark et al., 1977). **T24** was identified as ferulic acid from its MS peaks at *m/z* 194 ($[M]^+$ and base), 177 (M − OH), 145 (177-MeOH) and 117 (145-CO).

2.22.9. Compounds *T25* and *T26*

T25, previously unreported, had an MS nearly matching that of diarylheptanoid **65** below the **T25** $[M]^+$ at *m/z* 206: 163, 162, 138, 137 (base), 122 and 94, and thus apparently had the structure shown. **T26** has all of the peaks of **T25** plus all of the peaks of **T10** and an $[M]^+$ at *m/z* 224; the proposed structure gives **T25** by dehydration and **T10** by MLR.

2.23. Summary

It is evident that commercially processed dry ginger, like fresh ginger, contains [6]-, [8]- and [10]-gingerols (**9**, **11** and **12**, Table 4) as the major pungent principles with [6]-gingerol (**9**) as the dominant ginger constituent and trace quantities of [4]- and [5]-gingerols (**6** and **8**). Some reduction in the concentration of [6]-, [8]- and [10]-gingerols (**12**, **14** and **15**) occurs during the commercial drying process, mainly due to dehydration to shogaols (notably **19**). The GC chromatograms of fresh and dry gingers (Fig. 1) also showed slight variations in the minor constituents. The high level of in vitro anti-inflammatory activity observed in the partially purified fractions of fresh ginger containing non-volatile pungent components (gingerols and/or gingerol analogs) was preserved in the fractions derived similarly from dry ginger (Table 1). The high anti-inflammatory activity exhibited by many minor non-volatile pungent constituents of ginger (gingerol analogs) is apparently comparable to that demonstrated by the three major gingerol homologs **9**, **11** and **12**.

3. PGE_2 assay results

The original dry ginger CH_2Cl_2 extract (X) and 6 (X/3–X/8) out of its 10 CC fractions (X/1–X/10) had high anti-inflammatory activities similar to those exhibited by fresh ginger extract and its CC fractions. As can be seen in Table 1, fractions containing gingerols (X/5 and X/6) and gingerol analogs (X/3, X/4, X/7 and X/8) showed potent inhibition of LPS-stimulated PGE_2 production ($IC_{50} = 0.05$ 0.08 μg/ml), comparable to IC_{50} for indomethacin in our assay system. Gingerols and ginger analogs have been proposed to have different mechanisms of action in PGE_2 inhibition, as gingerols inhibit COX-2 mRNA induction while gingerol analogs do not (Lantz et al., 2005).

4. Experimental

4.1. Plant material

Ginger powder (*Z. officinale*) was purchased from San Francisco Herb and Natural Food.

4.2. Extraction and fractionation

Ginger powder (2500 g) was extracted with CH_2Cl_2 (7500 ml) for 36 h at 25 °C. After filtration, washing and work-up, a 40 g portion of the total CH_2Cl_2 extract (X, 159 g, 6.4%) was subjected to column chromatography (CC) on silica gel following the methodology developed in our laboratory for the separation of gingerol fractions in gram quantities for in vivo assays. The CC fractions were pooled into 10 fractions (Table 1, X/1–10) based on their TLC and HPLC profiles and analyzed for anti-inflammatory activity (in vitro PGE_2 assay). In another experiment, a scaled-down CC was carried out with the collection of smaller refined fractions, pooled into 13 fractions (X/*a–m*) and analyzed as above. In another study, a single PGE_2 active fraction (Y, 23.5 g) was isolated by repeating the above CC with another 40.0 g lot of X and subjected to preparative HPLC. Two sets of time-based fractions were collected: one set of 55 fractions at half-minute intervals (Y/1–55) and another set of 12 fractions at 5-min intervals (Y/*a–l*). PGE2 active CC fractions collected during method development study (Z1/1–11, Z2/1–18, Z3/1–11 and Z4/1–7 (sub-fractions of Z3/5)) were also examined by GC-MS and new compounds detected were included.

4.2.1. Isolation of compound *78*

A 40 g portion of CH_2Cl_2 extract was separated by CC into 11 fractions (Z3/1–11) as described for fractions X/1–10. Further separation of sub-fraction Z3/5 (0.97 g) by rechromatography as above into 7 fractions (Z4/1–7) followed by prep. reversed-phase HPLC of fraction Z4/2 (79 mg) on a Phenomenex Luna C18 (2), 5 μm, 250 × 4.6 mm column with MeCN–H_2O system (flow rate: 1 ml/min) gave pure **78** (8 mg). ^1H NMR (500 MHz, Bruker Instrument, $CDCl_3$): 0.88*t* (6.5 Hz, 3H, H10), 1.50*dt* (13 and 2 Hz, 1H, H4eq), 1.60s (3H, H10″), 1.68*s* (3H, H8″), 1.74*s* (3H, H9″), 2.62*dt* (15 and 7.5 Hz, 1H, H1), 2.72*ddd* (15, 9 and 5.5 Hz, 1H, H1), 3.62*m* (2H, H3 and H5), 3.86*s* (3H, OMe), 5.11*br t* (6.5 Hz, 1H, H6″), 5.17*d* (6 Hz, 1H, H1″), 5.35*br d* (6 Hz, 1H, H2″), 6.67*br d* (8 Hz, 1H, H6′), 6.70*br s* (1H, H2′), 6.82*d* (8 Hz, 1H, H5′); ^{13}C NMR: 14.1 (C10), 17.4 (C9″), 17.7 (C10″), 22.6 (C9), 24.7 (C7), 25.7 (C8″), 26.2 (C5″), 30.9, 35.9, 36.9, 37.8 (C1), 39.3 (C4″), 55.8 (OMe), 75.4/76.5 (C3/C5), 98.6 (C1″), 111.0/114.1 (C2′/C5′), 121.0 (C6′), 122.5 (C2″), 123.9 (C6″), 131.7 (C7″), 133.9 (C1′), 142.3 (C3″), 143.6 (C4′), 146.3 (C3′).

4.3. Cell culture and assay

HL-60 cells were cultured and stored at −80 °C until assayed for human PGE_2 and cytotoxicity as previously described (Jolad et al., 2004).

4.4. Chromatographic analysis

Instrumentation, operating conditions and sample preparation procedure used in this investigation to analyze samples by gas chromatography-mass spectrometry (GC-MS) and high-pressure liquid chromatography (HPLC) were described previously (Jolad et al., 2004).

4.5. Preparative HPLC

System: Agilent 1100 Series Purification System and Agilent 220 Microplate Sampler with a diode array detector. Operating conditions: column: Phenomenex Luna C18 (2) [5 μm, 250 × 21.2 mm]; guard column: Phenomenex C18 [60.0 × 21.2 mm]; mobile phase A: nanopure water; mobile phase B: HPLC grade methanol; flow rate: 21.2 ml/min; detection λ: 210, 230 and 280 nm; temperature: ambient; gradient program:

Time (min):	0	8	32	65	68
B (%):	55	60	60	100	100

Sample preparation/collection: Y was dissolved in methanol (2 ml/g); injection volume: 12 × 400 μl [55 fractions collected at half-min intervals (Y/1–55)] and 6 × 1.0 ml [12 fractions collected at 5 min intervals (Y/*a*–*l*)].

4.6. Quantification

The HPLC quantification of [6]-, [8]- and [10]-gingerols (**9**, **11** and **12**, Table 2) and [6]-shogaol (**19**) in the dry ginger extract (X) was carried out as reported previously for fresh ginger extracts (Jolad et al., 2004). Stock solutions of the four compounds were prepared from reference standards (Dalton Chemical Laboratories, Canada). Seven different levels of working standards were prepared freshly by combining and diluting the stock standard solution appropriately. The percentage composition values for each ginger extract shown in Table 4 are the average values of three injections of different concentrations.

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