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Characterization of gingerol-related compounds in ginger rhizome (*Zingiber officinale* Rosc.) by high-performance liquid chromatography/electrospray ionization mass spectrometry

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This study sought to determine the utility of liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) coupled with diode array detection in identifying gingerol-related compounds from crude extracts of ginger rhizome. The fragmentation behaviors of compounds in both (−)- and (+)ESI-MS/MS were used to infer and confirm the chemical structures of several groups of compounds, including the gingerols, methylgingerols, gingerol acetates, shogaols, paradols, gingerdiols, mono- and diacetyl gingerdiols, and dehydrogingerdiones. Diode array detection at different wavelengths was used to confirm MS/MS-based identification. In total, 31 gingerol-related compounds were identified from the methanolic crude extracts of fresh ginger rhizome in this study. Three of these compounds were found to be new compounds. This study demonstrated that LC/ESI-MS/MS is a powerful on-line tool for identification of gingerol-related compounds, especially for thermally labile compounds that cannot be readily detected by GC/MS analysis. Copyright © 2005 John Wiley & Sons, Ltd.

The rhizome of ginger (*Zingiber officinale*, Rosc.) Zingiberaceae has long served culinary and medicinal uses.[1] Two major groups of compounds including gingerol-related compounds and diarylheptanoids have been reported as bioactive components from this plant.[2,3] Gingerol-related compounds, comprising distinct groups (homologous series) that are differentiated by the length of their unbranched alkyl chains, have recently gained attention in a variety of biological activity studies.[4–7] Analytical tools are therefore needed to characterize this group of compounds from diverse sources including plant material or processed products.

Many analytical methods including gas chromatography coupled with mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC), and its coupling to mass spectrometry (LC/MS), nuclear magnetic resonance (NMR), thin layer chromatography (TLC) and capillary electrophoresis (CE), have been used to characterize gingerol-related compounds in ginger.[8–12] Among these methods, GC/MS has been used quite often to analyze ginger samples. Nevertheless, gingerol-related compounds with relatively long side chains are not easily detected by this method, due to their low volatility and thermal lability. LC/MS has been shown to be an effective method for on-line analysis of these types of compounds.[10] Single-dimension MS analysis, however, cannot provide sufficient information to accurately identify all known, let alone unknown, compounds. In contrast, our initial investigation using LC/electrospray ionization (ESI) tandem mass spectrometry (MS/MS) to characterize and analyze three authentic gingerol standards suggested that this technique could be employed successfully as a powerful and specific tool for on-line analysis of gingerol-related compounds.

This study sought to use LC/ESI-MS/MS to identify known and unknown gingerol-related compounds in ginger rhizome. Both negative and positive ionization ESI-MS/MS were used to obtain fragmentation data, which was used to characterize the structures of this group of compounds. UV spectra were also obtained by an in-line diode array detector. By comparison of their mass spectra, UV spectra, and chromatographic characteristics with those of authentic standard compounds and/or against each other,

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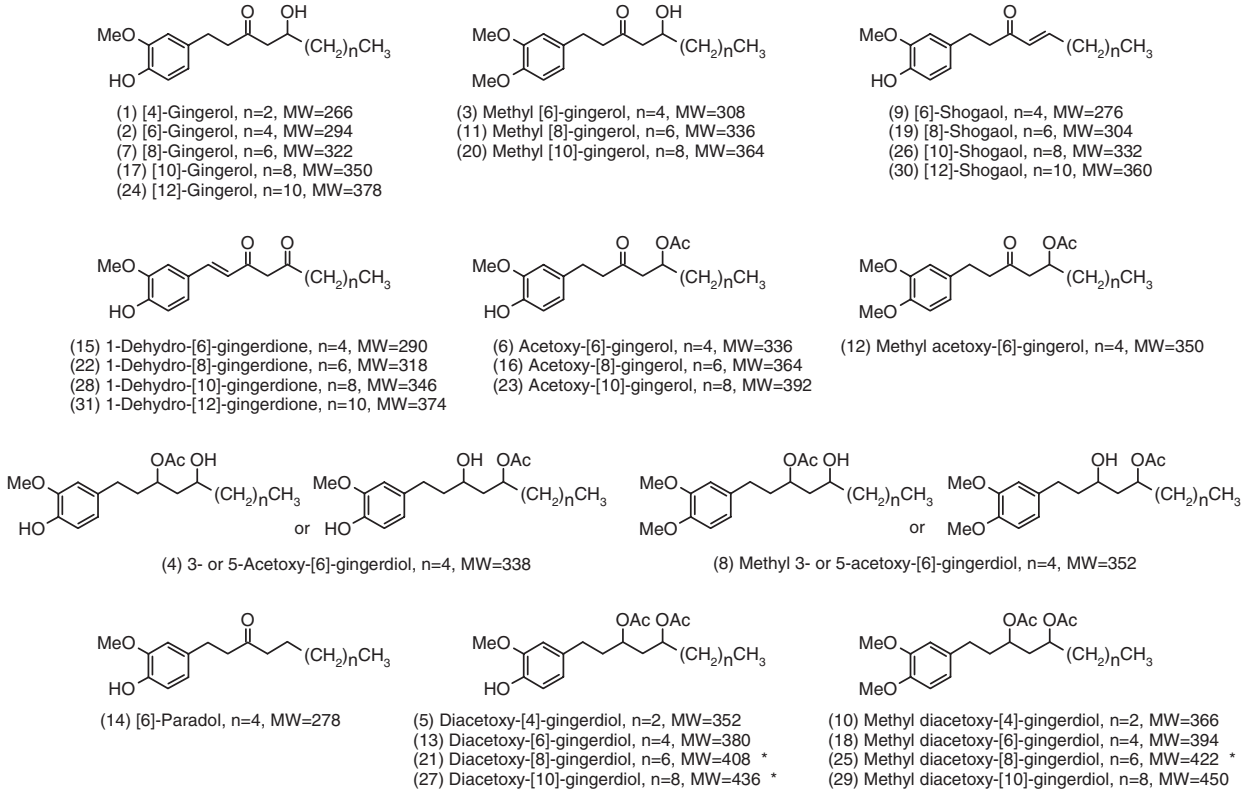


Figure 1. The chemical structures and molecular weights of gingerol-related compounds identified in extracts from ginger rhizome. Note: * indicates new compound.

31 gingerol-related compounds were identified (see Fig. 1). Three of these were new compounds.

EXPERIMENTAL

Chemicals and reagents

HPLC-grade acetonitrile and methanol were from Burdick & Jackson (Muskegon, MI, USA). Formic acid was from J. T. Baker (Mallinkrodt Baker, Inc., Phillipsburg, NJ, USA). Ammonium formate was from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was re-distilled. Authentic standards of [6]-shogaol and [6]-, [8]-, and [10]-gingerols were purchased from ChromaDex, Inc. (Santa Ana, CA, USA).

Plant material and sample preparation

Fresh ginger rhizomes were collected from plants grown in a greenhouse at the University of Arizona, frozen in liquid nitrogen, and kept at −80°C until analysis, as described elsewhere.[13] Methanolic extracts for LC/ESI-MS/MS analyses were produced from fresh frozen ginger rhizome samples as described elsewhere,[13] with overnight extraction at room temperature and shaking at 200 rpm.

LC/ESI-MS/MS analysis of ginger extracts

LC/ESI-MS/MS analyses of ginger extracts were performed on an Agilent 1100 HPLC system coupled to an in-line diode array detector (DAD) and an Agilent LC-MSD-Trap-SL ion trap mass spectrometer (Palo Alto, CA, USA).

LC separation of gingerol-related compounds

Column: Discovery® HS C18, 3 μm, 15 cm × 2.1 mm (Supelco, Bellefonte, PA, USA); guard column: Discovery® HS C18, 3 μm, 2 cm × 2.1 mm (Supelco); mobile phase: (A) buffer (5 mM ammonium formate, 0.1% formic acid, in ddH_2O) and (B) acetonitrile; gradient (in buffer A): 0–2 min, 5% B; 2–57 min, 5–100% B; 57–60 min, 100% B; 60–65 min, 100–5% B; 65–75 min, 5% B. Flow rate: 0.25 mL/min; temperature, 40°C; injection volume, 5 μL.

Diode array detection

The DAD was set at 425 nm (for signal A), 280 nm (for signal B), and 230 nm (for signal C), at 4 nm bandwidth individually, with 550 nm reference wavelength, at 50 nm bandwidth. Full spectral scanning was also performed from 200–600 nm, with range steps of 2 nm.

MS and MS/MS parameters for the Agilent LC-MSD-Trap-SL

The acquisition parameters for positive and negative modes were: drying N_2 temperature, 350°C; flow rate 10 L/min; nebulizer pressure 60 psi; HV capillary 4500 V; HV end plate offset −500 V; capillary current 65.9 nA (positive mode), 62.3 (negative mode); end plate current 1482.7 nA (positive mode), 1378.7 (negative mode); capillary exit RF amplitude 99.3 V (positive mode), −99.3 V (negative mode); skimmer 40.0 V (positive mode), −40.0 V (negative mode); mass range measured *m/z* 50–900. These were the optimized parameters for the maximum transmission of the gingerol-derived ions.

RESULTS AND DISCUSSION

General LC/MS/MS approach

Crude methanolic extracts, produced from fresh-frozen ginger rhizomes, were used directly for LC/ESI-MS/MS analyses, without further sample cleanup. This analysis

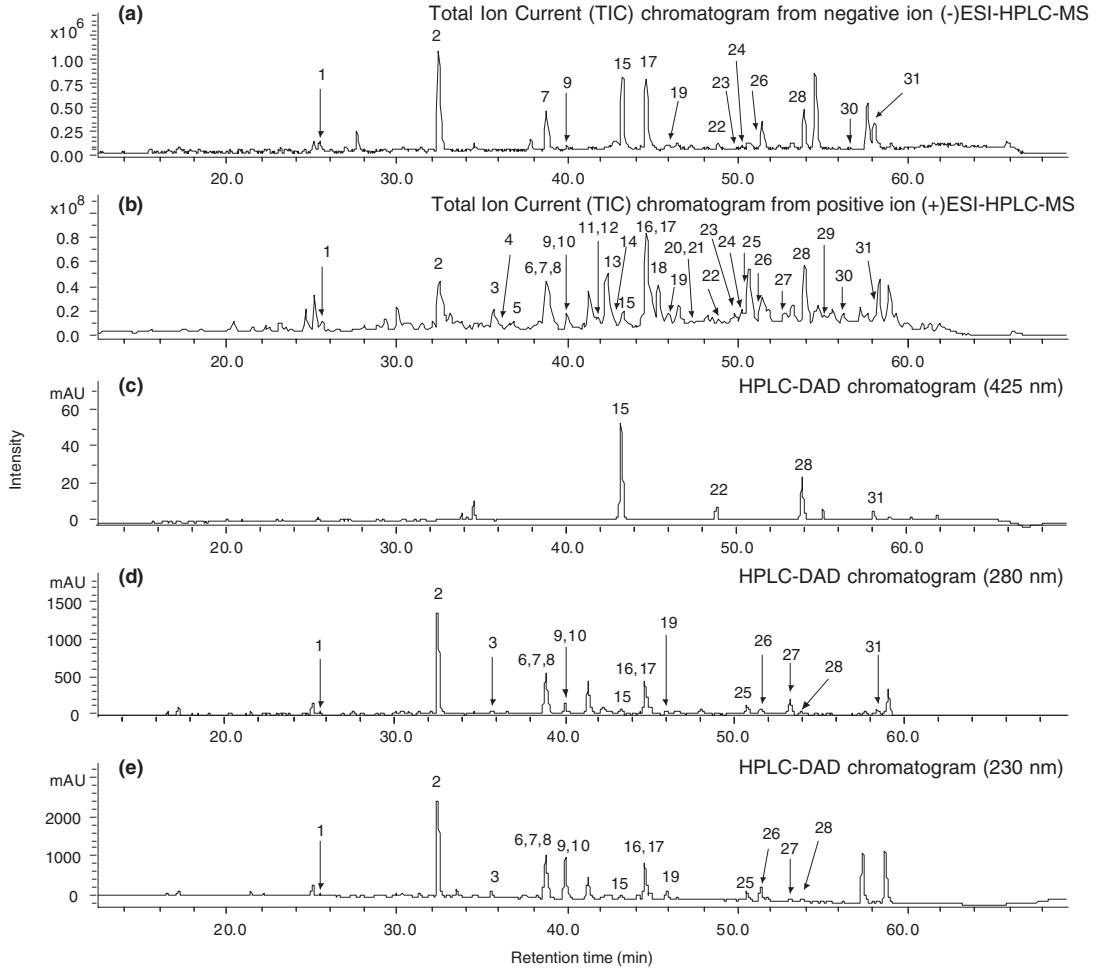


Figure 2. LC/MS analysis of ginger rhizome extracts. (a) and (b) are total ion current (TIC) chromatograms from negative ion (−)ESI-HPLC-MS and positive ion (+)ESI-HPLC-MS, respectively; (c), (d), and (e) are HPLC-DAD chromatograms set at 425, 280, and 230 nm, respectively, of the crude ginger rhizome extract.

also included in-line diode array detection. As shown in Fig. 2, both negative and positive ionization ESI-MS were used to detect gingerol-related compounds. Obviously, (+)ESI-MS is more sensitive than (−)ESI-MS in detecting this group of compounds under the condition used for our analysis (Fig. 2 and Table 1). Among the 31 identified compounds, 14 were detected after deprotonation in (−)ESI-MS and only 8 of these provided (−)ESI-MS/MS spectra (summarized in Table 1). In contrast, all 31 compounds were detected as protonated molecular ions, and/or ammonium/sodium adduct ions in (+)ESI-MS. In addition, (+)ESI-MS/MS spectra were obtained for all 31 compounds (summarized in Table 1). For the 14 compounds detected in both (−)- and (+)ESI-MS analyses, their molecular weights (MWs) were characterized by deprotonated and protonated molecular ions/adduct ions, respectively. For those compounds only detected in (+)ESI, their MWs were suggested by protonated molecular ions and/or their corresponding ammonium/sodium adduct ions. Their fragmentation behaviors in (−)- and/or (+)ESI-MS/MS were used to confirm their molecular structures. In-line diode array detection set at 425, 280, and 230 nm were also helpful in providing structural confirmation. The gingerols show a characteristic UV absorption maximum at 280 nm and a shoulder at 230 nm.[10] In addition to these wavelengths, compounds were observed with absorption maxima at ~425 nm, suggesting the presence of an extended conjugation system. In addition to UV absorption, retention times (R_t) in reversed-phase (RP)-HPLC were also found to be useful in the structure confirmation and especially for compounds belonging to homologous series.

Characterization of compounds 1, 2, 7, 17, 24, 3, 11, and 20 (gingerols and methylgingerols)

Compounds 1, 2, 7, 17, and 24 were observed in both negative and positive mode LC/ESI-MS analyses. Based on deprotonated molecular ions in (−)ESI-MS and their corresponding adduct ions in (+)ESI-MS (Table 1), the MWs of these compounds, differentiated by units of 28 Da ($-C_2H_4-$), were confirmed. The MWs of compounds 1, 2, 7, 17, and 24 matched those of [4]-, [6]-, [8]-, [10]-, and [12]-gingerols, respectively. The retention times for all five compounds in RP-HPLC increased by about 6 min for each additional $-C_2H_2-$ unit (see Table 1). This observation further supported that they belonged to a homologous series of increasing hydrophobicity due to increased alkyl chain length. Compounds 1, 2, 7, and 17 were observed in the HPLC-DAD chromatograms at 280 and 230 nm, characteristic absorption for the gingerols, supporting our tentative identification (Fig. 2). Compound

Table 1. Chromatographic and mass spectral characteristics of gingerol-related compounds detected by LC-ESI-MS in extracts from ginger rhizome

	Negative ESI		Positive ESI			
t_R (min)	(−)-ESI-MS (*m/z*)	(−) ESI-MS/MS (*m/z*)[a]	(+) ESI-MS (*m/z*)	(+) ESI-MS/MS (*m/z*)[a]	Compound	Compound name
25.6	265 $[M-H]^-$	N/D[c]	249 $[M+H-H_2O]^+$	177	1	[4]-Gingerol
			284 $[M+NH_4]^+$	249, 267		
			289 $[M+Na]^+$	N/D		
32.5	293 $[M-H]^-$	193, 99, 275, 178	277 $[M+H-H_2O]^+$	177	2	[6]-Gingerol
			312 $[M+NH_4]^+$	277, 295, 177		
			317 $[M+Na]^+$	217		
			611 $[2M+Na]^+$	317		
35.7	N/D	N/D	291 $[M+H-H_2O]^+$	191	3	Methyl [6]-gingerol
			326 $[M+NH_4]^+$	291, 309		
			331 $[M+Na]^+$	231		
36.1	N/D		321 $[M+H-H_2O]^+$	261, 163, 137	4	3- or 5-Acetoxy-[6]-gingerdiol
			356 $[M+NH_4]^+$	321, 339		
			361 $[M+Na]^+$	301, 203		
36.7	N/D		370 $[M+NH_4]^+$	293, 233, 163, 353	5	Diacetoxy-[4]-gingerdiol
			375 $[M+Na]^+$	N/D		
			293 $[M+H-AcOH]^+$	233, 163, 137, 275		
38.6	N/D		354 $[M+NH_4]^+$	277, 337, 259, 137	6	Acetoxy-[6]-gingerol
			359 $[M+Na]^+$	N/D		
38.7	321 $[M-H]^-$	193, 127, 303, 178	305 $[M+H-H_2O]^+$	177	7	[8]-Gingerol
			340 $[M+NH_4]^+$	305, 323		
			345 $[M+Na]^+$	217		
39.1	N/D		370 $[M+NH_4]^+$	275, 335, 353, 177	8	Methyl 3- or 5-acetoxy-[6]-gingerdiol
			335 $[M+H-H_2O]^+$	275, 177, 151		
39.9	275 $[M-H]^-$	N/D	277 $[M+H]^+$	137	9	[6]-Shogaol
40.0	N/D		384 $[M+NH_4]^+$	307, 247, 177, 367	10	Methyl diacetoxy-[4]-gingerdiol
41.8	N/D		319 $[M+H-H_2O]^+$	191	11	Methyl [8]-gingerol
			354 $[M+NH_4]^+$	319, 337		
			359 $[M+Na]^+$	N/D		
41.9	N/D		368 $[M+NH_4]^+$	291, 351, 273, 151	12	Methyl acetoxy-[6]-gingerol
			373 $[M+Na]^+$	N/D		
42.2	N/D		398 $[M+NH_4]^+$	321, 261, 163, 381	13	Diacetoxy-[6]-gingerdiol
			403 $[M+Na]^+$	343, 163, 261, 137, 321, 283		
			321 $[M+H-AcOH]^+$	261, 163, 137, 303		
42.8	N/D		279	137	14	[6]-Paradol
43.3	289 $[M-H]^-$	149, 134, 139	291 $[M+H]^+$	177	15	1-Dehydro-[6]-gingerdione
			313 $[M+Na]^+$	N/D		
44.4	N/D		382 $[M+NH_4]^+$	305, 365, 287, 137	16	Acetoxy-[8]-gingerol
			387 $[M+Na]^+$	N/D		
44.6	349 $[M-H]^-$	193, 155, 178, 331	333 $[M+H-H_2O]^+$	177	17	[10]-Gingerol
			368 $[M+NH_4]^+$	333, 351		
			373 $[M+Na]^+$	217		
45.2	N/D		412 $[M+NH_4]^+$	335, 275, 177, 395	18	Methyl diacetoxy-[6]-gingerdiol
			417 $[M+Na]^+$	357, 177, 275, 151, 297, 335		
			335 $[M+H-AcOH]^+$	275, 177, 151, 317		
45.9	303 $[M-H]^-$	N/D	305 $[M+H]^+$	137	19	[8]-Shogaol
47.4	N/D	N/D	347 $[M+H-H_2O]^+$	191	20	Methyl [10]-gingerol
			382 $[M+NH_4]^+$	347, 365		
			387 $[M+Na]^+$	231		
47.6	N/D		426 $[M+NH_4]^+$	349, 289, 163, 409	21[b]	Diacetoxy-[8]-gingerdiol
			431 $[M+Na]^+$	N/D		
			349 $[M+H-AcOH]^+$	331, 289, 163, 187		
48.8	317 $[M-H]^-$	149, 134, 167	319 $[M+H]^+$	177	22	1-Dehydro-[8]-gingerdione
49.8	391 $[M-H]^-$	N/D	410 $[M+NH_4]^+$	333, 393, 137, 315	23	Acetoxy-[10]-gingerol
			415 $[M+Na]^+$	355		
50.2	377 $[M-H]^-$	193, 183, 178, 361	361 $[M+H-H_2O]^+$	177	24	[12]-Gingerol
			401 $[M+Na]^+$	217		
50.5	N/D		440 $[M+NH_4]^+$	363, 303, 177, 423	25[b]	Methyl diacetoxyl-[8]-gingerdiol
51.4	331 $[M-H]^-$	N/D	333 $[M+H]^+$	137	26	[10]-Shogaol
52.8	N/D		454 $[M+NH_4]^+$	377, 317, 163, 437	27[b]	Diacetoxy-[10]-gingerdiol
			459 $[M+Na]^+$	N/D		
53.9	345 $[M-H]^-$	149, 134, 195	347 $[M+H]^+$	177	28	1-Dehydro-[10]-gingerdione
55.2	N/D		468 $[M+NH_4]^+$	391, 331, 177, 451	29	Methyl diacetoxyl-[10]-gingerdiol
56.3	359 $[M-H]^-$	N/D	361 $[M+H]^+$	137	30	[12]-Shogaol
58.0	373 $[M-H]^-$	149, 223, 134	375 $[M+H]^+$	177	31	1-Dehydro-[12]-gingerdione

[a] Product ions shown in each row are given in the order of their relative abundance: the first ion, in each case, is the most abundant.
[b] Compound was tentatively identified as a new compound.
[c] N/D indicates that the precursor ion in ESI-MS and/or product ions in ESI-MS/MS was/were not detectable.

24 was not observed in the HPLC-DAD chromatograms due to its very low abundance in the crude extract.

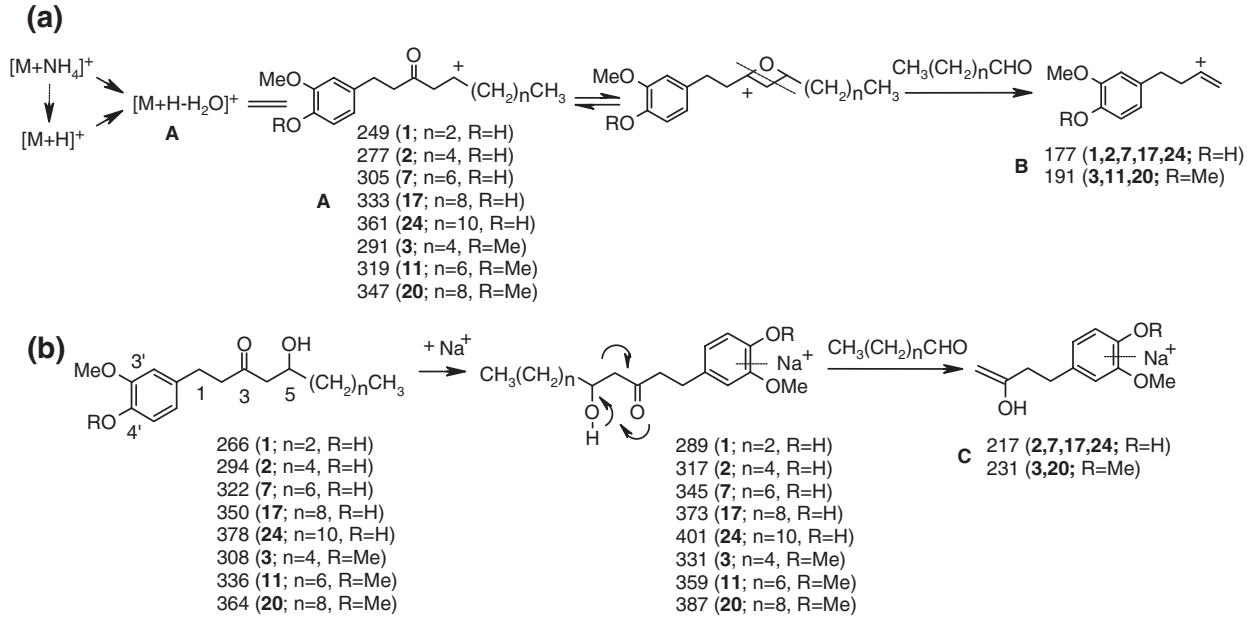
In addition, all five compounds demonstrated consistent fragmentation behaviors in both (−)- and (+)ESI-MS/MS analyses, which further supported their identities. Compounds 2, 7, and 17 were confirmed as [6]-, [8]-, and [10]-gingerols by comparison of their fragmentation behaviors and retention times in RP-HPLC with those of authentic standards. Compound 24 was identified as [12]-gingerol because its MW and fragmentation behavior in both (−)-and (+)ESI-MS/MS were consistent with the other gingerols. Although compound 1 was detected in both (−)- and (+)ESI-MS analyses, its (−)ESI-MS/MS spectrum was not available due to its low abundance in crude ginger rhizome extracts. Based on its MW and fragmentation behavior in (+)ESI-MS/MS (Table 1 and Scheme 1), compound 1 was tentatively identified as [4]-gingerol.

In (+)ESI-MS, the protonated molecular ions of the gingerols were not observable. Instead, ion A $[M+H-H_2O]^+$ was detected as a highly abundant peak in (+)ESI-MS due to the keto (site of protonation) and hydroxy (loss of water) groups on the alkyl chain. In contrast, the ammonium and sodium adduct ions of the gingerols were detectable (Table 1). In addition, the fragmentation behaviors in (+)ESI-MS/MS of the ammonium and sodium adduct ions of the gingerols were different (Scheme 1). For ammonium adduct ions, ion A $[M+H-H_2O]^+$ was formed as the major product ion by the loss of one H_2O and one NH_3. Ion A was further fragmented by the loss of a neutral alkyl moiety and a rearrangement (Scheme 1), leading to the formation of a predominant ion B at *m/z* 177. In contrast, ion C at *m/z* 217 was produced as the base peak in (+)ESI-MS/MS analysis of sodium adduct ions, which could only be rationalized by a McLafferty rearrangement and the loss of a neutral alkyl moiety (Scheme 1). No ions formed by loss of H_2O were detected from the (+)ESI-MS/MS of the sodium adduct ions. This observation suggested that Na^+ and NH_4^+ may associate with different functional groups of the gingerol molecules during the ESI process, with Na^+ likely associating with the phenolic hydroxyl group and NH_4^+ associating with the alkyl hydroxyl group.

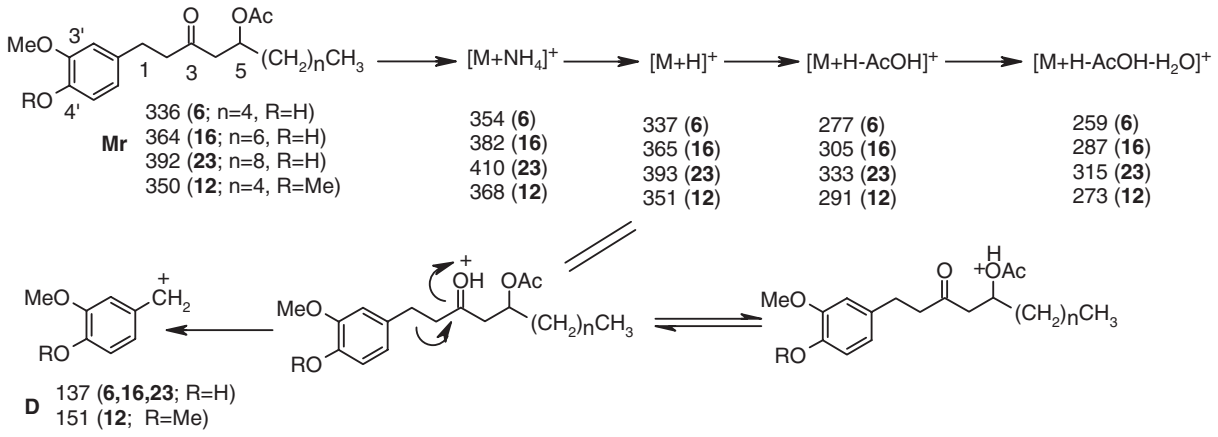
Compared to compounds 2, 7, and 17, the NH_4^+ and Na^+ adducts of compounds 3, 11, and 20 showed an increase of 14 Da (Table 1), respectively, suggesting an additional CH_3 (Me) group instead of a H atom. Thus, these three compounds could be methyl [6]-gingerol, methyl [8]-gingerol, and methyl [10]-gingerol or [7]-gingerol, [9]-gingerol, and [11]-gingerol. Their characteristic product ions B at *m/z* 191 and C at *m/z* 231 (Table 1 and Scheme 1), which also demonstrated an increase of 14 Da, suggested that the additional Me group was attached to the phenolic hydroxyl group. Therefore, compounds 3, 11, and 20 were identified as methyl [6]-gingerol, methyl [8]-gingerol, and methyl [10]-gingerol. Their retention times, differentiated by about 6 min in sequence, further supported that they were a series of homologs (Table 1). However, support from HPLC-DAD analysis was only available for compound 3 due to the low abundance of compounds 11 and 20 in the crude extract (Fig. 2). These three compounds are not likely to be artifacts of the extraction procedure, which did employ methanol (extractions performed with shaking at room temperature), because methanolic or aqueous methanolic solutions of the pure gingerols, whether relatively diluted or concentrated, never show contamination by these compounds, even if stored for prolonged periods of time (months) (data not shown).

Characterization of compounds 6, 16, 23, and 12 (gingerol acetates)

The ammonium and sodium adduct ions of compounds 6, 16, and 23 observed in (+)ESI-MS showed an increase of 42 Da



Scheme 1. (a) (+)ESI-MS/MS fragmentation of gingerols and methylgingerols 1, 2, 7, 17, 24, 3, 11, and 20 (protonated ions and ammonium adducts). (b) (+)ESI-MS/MS fragmentation of gingerols and methylgingerols 1, 2, 7, 17, 24, 3, 11, and 20 (sodium adducts).



Scheme 2. (+)ESI-MS/MS fragmentation of gingerol acetates 6, 16, 23, and 12.

when compared to compounds 2, 7, and 17 (Table 1). In addition, the base peak in (+)ESI-MS/MS spectra of these compounds was produced by the loss of 60 Da (AcOH). This information suggested that an acetoxy group instead of a hydroxy group was present on the aliphatic side chain of compounds 6, 16, and 23. Therefore, the structures of acetoxy-[6]-gingerol, acetoxy-[8]-gingerol, and acetoxy-[10]-gingerol were suggested for compounds 6, 16, and 23, respectively. These proposed structures of compounds 6, 16, and 23 were further supported by the formation of product ion D at *m/z* 137, revealing the presence of the aromatic moiety of these molecules (Scheme 2).

Compared to compound 6, the ammonium adduct ion and all of the corresponding product ions of compound 12 showed an increase of 14 Da (Table 1), suggesting that these two compounds are homologs differentiated only by one CH_2 group (Me instead of H). Because of its product ion D (*m/z* 151 instead of 137), compound 12 appeared to be methyl acetoxy-[6]-gingerol (Scheme 2).

Characterization of compounds 4 and 8 (monoacetyl gingerdiols)

Compared to compound 6, compound 4 showed an increase of 2 Da for its corresponding ammonium and sodium adduct ions in (+)ESI-MS (Table 1). The ion $[M+H-H_2O]^+$ at *m/z* 321, resulting from the loss of one H_2O and NH_3 from the molecule, was the base peak in the (+)ESI-MS/MS of its ammonium adduct ion $[M+NH_4]^+$ at *m/z* 356 (Table 1). This observation suggested that compound 4 could be 3- or 5-acetoxy-[6]-gingerdiol with a second aliphatic hydroxy group instead of a keto group on the alkyl chain. The relatively lower retention time in RP-HPLC for this compound when compared to compound 6 also supported the presence of a hydroxy group instead of a keto group. The formation of product ions E at *m/z* 261 and F at *m/z* 163 supported our tentative identification of compound 4 as 3- or 5-acetoxy-[6]-gingerdiol (Scheme 3). However, further evidence to determine the position of the acetyl group on either the 3- or 5-hydroxy was not available. In the exact same manner, compound 8 was identified as methyl 3- or 5-acetoxy-[6]-gingerdiol when compared to compound 12.

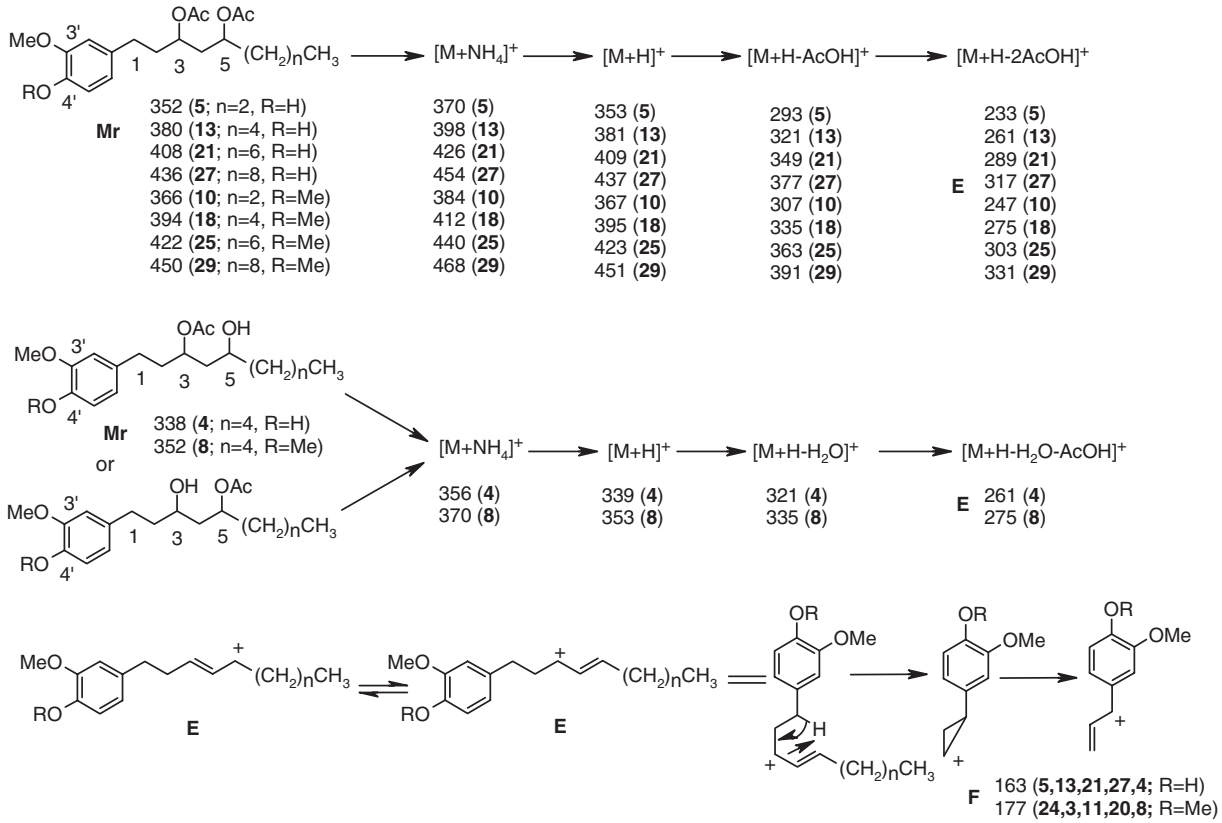
Characterization of compounds 5, 13, 21, 27, 10, 18, 25, and 29 (diacetyl gingerdiols)

Compounds 5, 13, 21, and 27, respectively, differentiated by 28 Da ($-C_2H_4-$) for their corresponding ammonium and sodium adduct ions in (+)ESI-MS, showed retention time increases of ~5.2 min in the RP-HPLC chromatograms, suggesting that these four compounds were homologs (Table 1). The formation of product ions $[M+H-AcOH]^+$ and $[M+H-2AcOH]^+$ in the (+)ESI-MS/MS analysis of ammonium adduct ions from these compounds suggested two acetoxy groups on the alkyl chain. In addition, compared to compound 4 (see above), the ammonium and sodium adduct ions of compound 13 showed an increase of 42 Da, revealing that the hydroxy group on the alkyl chain of compound 4 was substituted by one acetoxy group in compound 13. Therefore, compound 13 was tentatively identified as diacetoxy-[6]-gingerdiol. The formation of product ions E at *m/z* 261 and F at *m/z* 163 in (+)ESI-MS/MS for compounds 4 and 13 further supported our tentative identification for compound 13 (Scheme 3). By comparison to compound 13, the other three homologs, compounds 5, 21, and 27, were tentatively identified as diacetoxy-[4]-gingerdiol, diacetoxy-[8]-gingerdiol, and diacetoxy-[10]-gingerdiol, respectively (Table 1 and Scheme 3). Among these, diacetoxy-[8]-gingerdiol and diacetoxy-[10]-gingerdiol were new compounds.

Compared to compounds 5, 13, 21, and 27, the ammonium and sodium adduct ions in (+)ESI-MS and all their corresponding product ions in (+)ESI-MS/MS of compounds 10, 18, 25, and 29 demonstrated an increase of 14 Da ($-CH_2$), respectively (Table 1 and Scheme 3). This observation suggested that compounds 10, 18, 25, and 29 were methyl diacetoxy-[4]-gingerdiol, methyl diacetoxy-[6]-gingerdiol, methyl diacetoxy-[8]-gingerdiol, and methyl diacetoxy-[10]-gingerdiol, respectively. Among these, methyl diacetoxy-[8]-gingerdiol was a new compound.

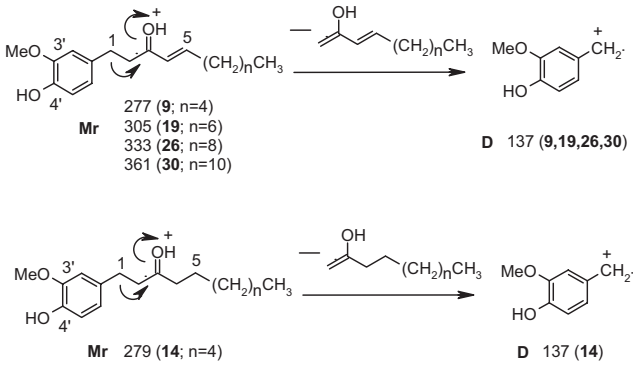
Characterization of compounds 9, 19, 26, 30, and 14 (shogaols and paradols)

Deprotonated molecular ions in (−)ESI-MS and protonated molecular ions in (+)ESI-MS, differentiated by 28 Da ($-C_2H_4-$), were detected for compounds 9, 19, 26, and 30



Scheme 3. (+)ESI-MS/MS fragmentation of mono- and diacetyl gingerdiols 4, 5, 8, 13, 21, 27, 10, 18, 25, and 29.

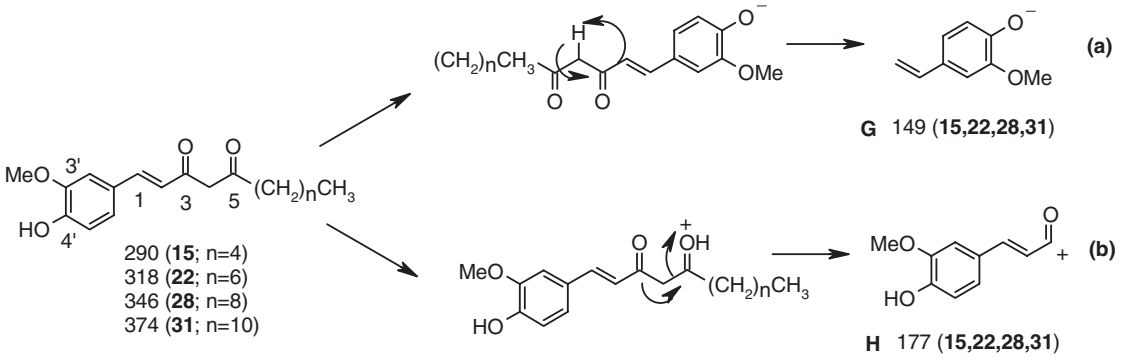
(Table 1), supporting the MWs of these compounds. [6]-, [8]-, [10], and [12]-Shogaol, a series of homologs which have been previously isolated from ginger, matched the MWs of compounds 9, 19, 26, and 30, respectively. In addition, these compounds showed retention time increases of ~5.6 min in RP-HPLC analysis (Table 1), supporting the indication that they were homologs. In the (+)ESI-MS/MS spectra of these compounds, the product ion D at *m/z* 137 was the only major peak observed (Scheme 4). This observation is reasonable because the keto group on the alkyl chain is the only group that causes fragmentation in (+)ESI-MS/MS of these protonated molecules. By comparing the chromatographic (retention time) and spectral data (ESI-MS/MS) with an authentic standard compound, compound 9 was confirmed to be [6]-shogaol. Compounds 19, 26, and 30 were tentatively identified as [8]-shogaol, [10]-shogaol, and [12]-shogaol by comparing their MWs, fragmentation behaviors in (+)ESI-MS/MS and retention times in RP-HPLC to those of compound 9. Further support from (−)ESI-MS/MS was not available for these compounds due to their low abundance in the crude extract of fresh ginger rhizomes. Compared to compound 9, compound 14 showed an increase of 2 Da of its corresponding protonated ions in (+)ESI-MS (Table 1). This suggested that compound 14 might be [6]-paradol, with the lack of the double bond between carbons 4 and 5. Its relative later retention time when compared to [6]-shogaol in RP-HPLC also supported this hypothesis. In addition, the formation of only major product ion D at *m/z* 137 in (+)ESI-MS/MS supported the assignment of compound 14 as [6]-paradol (Scheme 3).



Scheme 4. (+)ESI-MS/MS fragmentation of shogaols and paradols 9, 19, 26, 30, and 14.

Characterization of compounds 15, 22, 28, and 31 (dehydrogingerdiones)

Compounds 15, 22, 28, and 31 were detected in both (−)- and (+)ESI-MS (Table 1). Their corresponding MWs, differentiated by 28 Da ($-C_2H_4-$), were thereby obtained. A series of homologs, 1-dehydro-[6]-gingerdione, 1-dehydro-[8]-gingerdione, 1-dehydro-[10]-gingerdione, and 1-dehydro-[12]-gingerdione (Fig. 1), respectively, which have been previously reported from ginger, were suggested for compounds 15, 22, 28, and 31 because of matching MWs. In addition, all four compounds were observed in HPLC-DAD at 425 nm (Fig. 2), suggesting the presence of an extended conjugation system. This observation supported the presence of a double bond between carbons 1 and 2 of



Scheme 5. (a) (−)ESI-MS/MS fragmentation of dehydrogingerdiones 15, 22, 28, and 31. (b) (+)ESI-MS/MS fragmentation of dehydrogingerdiones 15, 22, 28, and 31.

these homologs. Compared to the corresponding gingerols with alkyl chains of the same length, the dehydrogingerdiones showed relatively long retention times in RP-HPLC, due to the lack of an aliphatic hydroxy group (Table 1). Moreover, the base peak (ion G) at *m/z* 149 was produced by a *β*-H shift to the double bond in (−)ESI-MS/MS, leading to the loss of a neutral moiety (Scheme 5). This rearrangement reaction was also observed for the curcuminoids in (−)ESI-MS/MS (H. Jiang and D. R. Gang, unpublished), closely related compounds which also possess a *β*-diketone group. Furthermore, the formation of the major product ion H at *m/z* 177 in (+)ESI-MS/MS also supported our tentative identification of these compounds as dehydrogingerdiones (Scheme 5).

CONCLUSIONS

A total of 31 gingerol-related compounds, belonging to different homologous series and differentiated by structural differences on the alkyl chain and the aromatic ring, were identified in methanolic crude extracts from fresh-frozen ginger rhizome by LC/ESI-MS/MS coupled to diode array detection. Interestingly, many of the identified compounds were only detected by the MS detector, therefore, suggesting that the LC/MS analysis is not only more specific, but also more sensitive than diode array analysis for this group of compounds. Another advantage offered by MS detection is that compounds with very close retention times in RP-HPLC can be distinguished and identified by selective (extracted) ion chromatograms from full MS and MS/MS analysis. This technique is important for the analysis of crude extracts of ginger rhizomes by HPLC without prior fractionation. Nevertheless, many gingerol-related compounds, especially those that lacked available phenolic hydroxy groups for deprotonation, were not detectable by (−)ESI-MS analysis. Diode array detection was, however, very helpful for structural distinction and confirmation and especially for compounds with UV absorption at specific wavelengths. For example, the 1-dehydrogingerdiones were easily distinguished from other gingerol-related compounds in the HPLC-DAD chromatogram at 425 nm, due to their extended conjugation systems (Fig. 2). Therefore, negative and positive mode HPLC/ESI-MS/MS analysis coupled to diode array detection was found to be a powerful and fast on-line tool for the identification of this group of compounds with relatively complete coverage.

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