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Separation and Identification of Diarylheptanoids in Supercritical Fluid Extract of *Alpinia Officinarum* by UPLC–MS–MS

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Abstract

In the present study, ultra-performance liquid chromatography (UPLC) coupled to electrospray ionization (ESI^+) tandem mass spectrometry (MS) was developed to identify and characterize the diarylheptanoids in the supercritical fluid extract (SFE) of *Alpinia officinarum*. The method established provides good reproducibility of UPLC and shows high precision with all the mass accuracy of less than 5 ppm. The ESI–MS–MS fragmentation behavior of every group and their appropriate characteristic pathways were proposed. On the basis of analyzing the fragmentation pathways, elemental composition provided by software Masslynx, mass data of the standard compounds and the information regarding polarity obtained from retention time data, in all, 23 diarylheptanods were characterized. All of them have been reported in *Alpinia officinarum*. They were classified into six distinct groups (homologous series). Compared to the references, the fragmentation pathways of the first and second group were detailed much more and complementary. Further more, the fragmentation pathways of the last four groups were firstly discussed. The fragmentation rules deduced and the data provided could aid in the characterization of other diarylheptanoids of these types and would be useful for the further research of diarylheptanoids in *Alpinia officinarum* or the other plants.

Introduction

As a traditional Chinese herb, the rhizome of *Alpinia officinarum* (Zingiberaceae) has been used in China for relieving stomach ache, treating colds, invigorating the circulatory system, and reducing swelling (1). Diarylheptanoids are the main active substances in *Alpinia officinarum*.

Diarylheptanoids belong to a class of natural products with a 1,7-diarylheptane skeleton possessing a variety of biological and pharmacological activities including anti-inflammatory, antioxidant, antiemetic, and antitumor activities (2).

Due to low volatility and thermally labile properties, diarylheptanoids cannot be analyzed by gas chromatography–mass spectrometry (3,4). A variety of analytical methods including high-performance liquid chromatography (HPLC) and its coupling to mass spectrometry (LC–MS), thin layer chromatography (TLC), and capillary electrophoresis (CE) have been applied to characterize diarylheptanoids in turmeric (5–6,8–12), ginger (13) and *Alpinia officinarum* (7). For this paper, ultra-performance liquid chromatography–tandem mass spectrometry was utilized. UPLC technique has been applied more and more in recent years due to its great separation and resolution. Quadrupole time-of-flight mass spectrometry (Q-TOF-MS) enables automated exact mass measurement of precursor and fragment ions to yield high confidence in structural elucidation. UPLC–Q-TOF-MS–MS offers high chromatographic resolution with exact mass measurement for both MS and MS–MS. It can help to discover minor constituents, which are difficult to be obtained by classical means. Diarylheptanoids are major class of biologically active natural products in *Alpinia officinarum*. However, no online analytical method has been reported to characterize and measure these compounds in *Alpinia officinarum*. In this paper, SFE technology was applied to extract diarylheptanoids. On the basis of analyzing the MS spectra data of diarylheptanoids and polar differences combining with the literatures, the fragmentation pathways of diarylheptanoids in *Alpinia officinarum* are discussed in detail. Compared with the references (11–13), the fragmentation pathways of the first and second group were more detailed and complementary much more. Further more, the fragmentation pathways of the last four groups were firstly discussed.

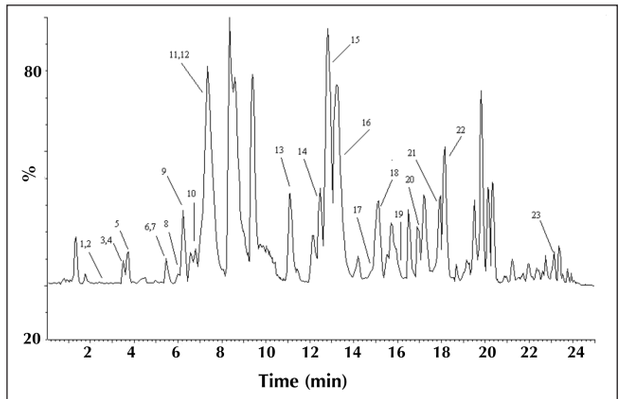

Figure 1. The base peak chromatogram from positive ion UPLC–ESI^+–Q-TOF–MS analysis.

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Experiments

Instruments and chemicals

A Waters UPLC was coupled to a single-wavelength UV detector and Q-TOF micro MS equipped with electrospray ionization ion source (ESI). The column used was an Acquity UPLC BEH C18, 1.7 µm, 2.1 × 50 mm, and a MassLynx 4.1 data processing system (Waters Technologies, Milford, MA) was also used. HA121-50-01 supercritical extraction apparatus was obtained from Hua'an Supercritical Extraction Limited Company (Jiangsu, China). HPLC-grade acetonitrile and methanol were purchased from Merck company (Darmstadt, Germany); formic acid was purchased from Dikma (Lake Forest, CA); deionized water was re-distilled. Carbon dioxide (99.9% purity) was obtained from Shiyuan gas company, (Guangzhou, China). The standard substances of 5-hydroxy-1-phenyl-7-(4- hydroxy-3-methoxylphenyl)-3-heptanon,5-hydroxy-1,7-disphenyl-3-heptanon,1,7-bisphenyl-4-en-3-heptanone (purity > 90%) were prepared in our laboratory and were identified by comparison of their physical data ([a]D, IR, 1H NMR, 13C NMR, and MS) with reported values (7,14). Lock mass: leusine enkephalin (Sigma-Aldrich, St. Louis, MO).

Plant material and sample preparation

Alpinia officinarum was purchased from Xuwen county in Guangdong province and was identified by Professor Wei-Min Li in Guangzhou University of Chinese Medicine. Dry *Alpinia officinarum* powder was extracted by SFE to obtain an SFE extract. The extract was dissolved in methanol to 8 mg/mL and filtered through 0.2-µm membranes before UPLC–MS–MS analysis. The standard substances of diarylheptanoids were dissolved in methanol to 0.1 mg/mL and filtered through 0.2-µm membranes

The experiment conditions of SFE were as follows: flow rate 22, L/h, the extraction pressure, 30Mpa; temperature, 32°C; releasing pressure 6Mpa; temperature, 36°C; extraction time, 4 h, with an extraction rate of 3%.

UPLC–MS–MS

The solvent of standard substances were injected into the MS system directly. The mobile phases used were as follows: (A) 0.1% formic acid–H_2O (B) acetonitrile; gradient: 0–6 min, 22%–36% B; 6–11.5 min, 36–42% B; 11.5–14.5 min, 42–50% B; 14.5–18 min, 50-80% B; 18–21 min, 80% B; 21–25 min, 80–100% B. The flow rate was 0.25 mL × min – 1; temperature 25°C; injection volume 2 µL.

High purity nitrogen was used as the nebulizer and auxiliary gas; argon was used as the collision gas. The mass spectrometer was operated in positive ion mode with a capillary voltage of 3 kV, sample cone voltage of 20 V, extraction cone voltage of 2 V, cone gas flow of 50 L/h, desolvation gas flow of 600 L/h, desolvation temperature of 350°C, source temperature of 100°C, collision energy of 10 eV (for all diarylheptanoids identified including the standard substances). Mass accuracy was maintained by using a lock spray with leucine enkephalin $(M+H)^+$ *m/z* 556.2771, concentration: 250 ng/µL, flow rate: 25 µL/min) as reference. The full mass scanning range was from *m/z* 50 to 800.

Reproducibility of UPLC and precision of MS

According to the requirement of qualitative analysis, UPLC reproducibility was measured as the relative standard deviation of retention time in UPLC chromatograms and MS precision as the error of molecular weight of $(M+H)^+$ in total ions chromatogram (TIC) from five consecutive injections of the same sample. Five peaks in UPLC chromatograms and 11 molecular weights of $(M+H)^+$ in TIC were chosen at random to check on the reproducibility of UPLC and the precision of MS.

Table I. The Reproductivity of UPLC

Peak	Rt1 (min)	Rt2 (min)	Rt3 (min)	Rt4 (min)	Rt5 (min)	RSD%
1	7.03	7.02	7.02	7.03	7.03	0.078
2	7.22	7.22	7.22	7.22	7.22	0.0
3	8.26	8.26	8.26	8.26	8.26	0.0
4	8.48	8.47	8.47	8.47	8.47	0.058
5	12.43	12.42	12.42	12.42	12.42	0.0045
6	12.72	12.72	12.72	12.72	12.72	0.0

Table II. The Precision of MS

Number	$(M+H)^+$ 1 (error ppm)	$(M+H)^+$ 2 (error ppm)	$(M+H)^+$ 3 (error ppm)	$(M+H)^+$ 4 (error ppm)	$(M+H)^+$ 5 (error ppm)
10	327.1587 (–2.8)	327.1583 (–4.0)	327.1605 (2.8)	327.1606 (3.1)	327.1607 (3.4)
11	299.1645 (–0.7)	299.1639 (–2.1)	299.1657 (4.0)	299.1652 (1.7)	299.1642 (–1.7)
12	329.1741 (–3.6)	329.1739 (–4.3)	329.1756 (0.9)	329.1758 (1.5)	329.1744 (–2.7)
14	281.1539 (–1.1)	281.1533 (–3.2)	281.1544 (0.7)	281.1554 (4.3)	281.1544 (0.7)
15	311.1635 (–3.9)	311.1638 (–2.9)	311.1650 (1.0)	311.1651 (1.3)	311.1650 (1.0)
17	297.1485 (–2.0)	297.1495 (1.3)	297.1502 (3.7)	297.1501 (3.4)	297.1498 (2.4)
19	325.1436 (–1.2)	325.1429 (–3.4)	325.1449 (2.8)	325.1445 (1.5)	325.1443 (0.9)
20	265.15931 (–0.4)	265.1585 (–2.6)	265.1599 (2.6)	265.1600 (3.0)	265.1595 (1.1)
21	281.1531 (–3.9)	281.1532 (–3.6)	281.1539 (–1.1)	281.1538 (–1.4)	281.1542 (0)
22	279.1378 (–2.5)	279.1389 (1.4)	279.1391 (2.1)	279.1379 (–2.1)	279.1380 (–1.8)
23	401.2829 (–3.7)	401.2835 (–2.2)	401.2831 (–3.2)	401.2839 (–1.2)	401.2838 (–1.5)

Results and Discussion

The optimal UPLC–Q-TOF-MS–MS method was applied to SFE extract. The total ion current chromatograms in positive ESI mode were shown in Figure 1. This method provided good reproducibility of UPLC (Table I) and showed high precision with all the mass accuracy of less than 5 ppm (Table II). For most of the constituents, $(M+H)^+$ ions were observed. These results provided helpful information for confirming molecular weight and structure of the constituents. All constituents were temporarily deduced from several aspects: mass data of the standard compounds, elemental composition of software Masslynx listed the possible molecular composition, the molecular composition would be determined by comparing with the literature data, and

MS–MS fragmentation pathways and the retention time would verify the results. A total of 23 diarylheptanoids were identified (Table III and Figure 1). We classified them into six structure skeletons (Figure 2). The identification of each specific group of diarylheptanoids was discussed in detail. In order to study the fragmentation pathways of diarylheptanoids in detail, the collision energy was set to 10 eV for all diarylheptanoids identified including the standard substances because more ions

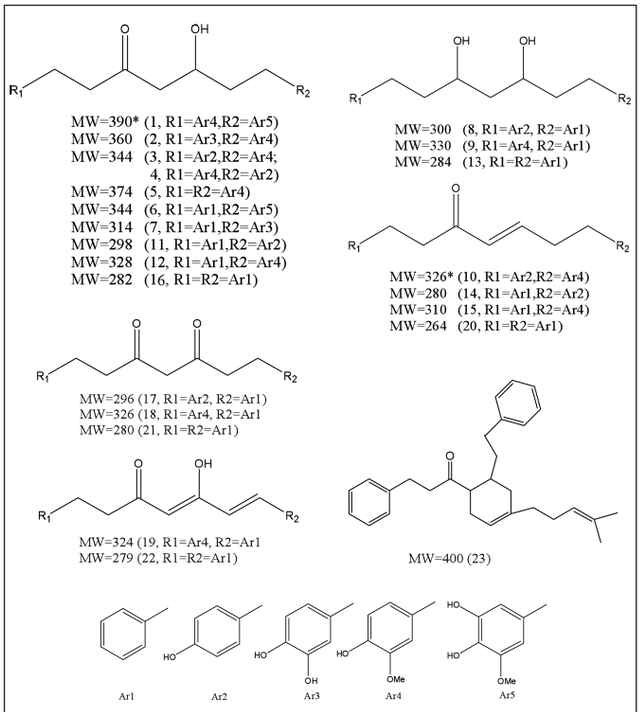

Figure 2. Chemical structures and molecular weights of diarylheptanoids identified. Note: the corresponding names of these compounds are provided in Table I.

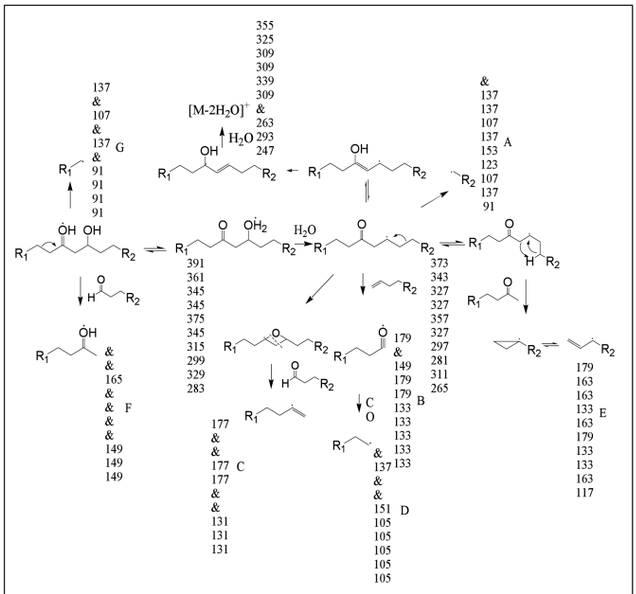

Figure 3. (A) ESI+ fragmentation of diarylheptanoids 1, 2, 3, 4, 5, 6, 7, 11, 12, and 16; & indicates the abundance of the corresponding ion was weak or the ion was not detected.

information in MS^2 spectra could be provided at this collision energy. Because of the structure differences and the fact that only one collision energy was set, some ions in the schemes were not detected or the abundance was weak.

Characterization of compounds 1–7, 11, 12, and 16

All the diarylheptanoids possess a common structural moiety, consisting of 5-hydroxy and 3-oxo groups on the heptane skeleton. The structural differences within this group lie in the pattern of substitution on the aromatic rings. The fragmentation pathway of this group is shown in Figure 3. The MS^2 spectra of standard diarylheptanoids 12 and 16 (Figure 4) were in accordance with the Figure 3. In order to describe the identification of these compounds clearly, they were classified into four subgroups, according to their approximate retention time (Table

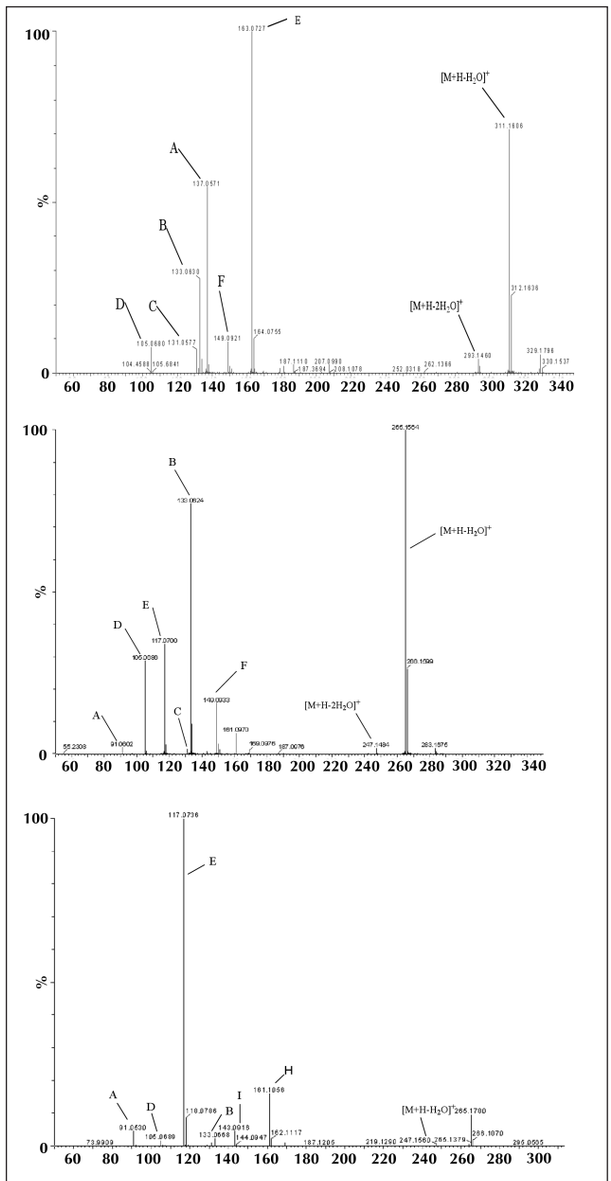

Figure 4. MS^2 spectra of standard substances of compounds 12, 16, and 20 in ESI+–MS–MS mode.

Table IIIA. Chromatographic and MS Characteristics of Ciarylheptanoids detected by UPLC–ESI$^+$–MS in SFE Extract of *Alpinia officinarum**

Number	t_R (min)	ΔAM (ppm)	(M+H)$^+$	Main fragment ions in MS^{2†}	Composition
1	2.43	–3.1	391.1745	179.0733, 355.1518, 137.0640, 373.1680, 177.0917	$C_{21}H_{26}O_6$
Identification: 5-hydroxy-1-(4-hydroxy-3-methoxylphenyl)-7-(3,4-dihydroxy-5-methoxylphenyl)-3-heptanone					
2	2.46	–3.0	361.1640	177.0977, 343.1463, 137.0569, 324.9547	$C_{20}H_{24}O_6$
Identification: 5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone					
3 4	3.51	3.5	345.1714	163.0777, 327.1584, 137.0636, 177.0948, 149.0651, 107.0548, 309.1436, 165.0957, 179.0737	$C_{20}H_{24}O_5$
Identification: 5-hydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxylphenyl)-3-heptanone					
Identification: 5-hydroxy-7-(4-hydroxyphenyl)-1-(4-hydroxy-3-methoxylphenyl)-3-heptanone					
5	3.72	2.1	375.1816	177.0934, 357.1690, 137.0635, 163.0796, 339.1626, 179.0752, 151.0799	$C_{21}H_{26}O_6$
Identification: 5-hydroxy-1,7-bis(4-hydroxy-3-methoxylphenyl)-3-heptanon					
6	5.557	–1.4	345.1697	179.0704, 327.1585, 133.0658, 153.0568, 309.1558, 105.0616	$C_{20}H_{24}O_5$
Identification: 5-hydroxy-1-phenyl -7-(3,4-dihydroxy-5-methoxylphenyl)-3-heptanone					
7	5.60	–3.8	315.1584	149.0592, 297.1519, 133.0655, 123.0484, 105.0656	$C_{19}H_{22}O_4$
Identification: 5-hydroxy-1-phenyl-7-(3,4-dihydroxyphenyl)-3-heptanone					
8	6.08	–1	301.1801	265.1602, 107.0528, 171.1237, 283.1740, 133.0667, 117.0738, 131.0910	$C_{19}H_{24}O_3$
Identification: 3,5-dishydroxy -1-(4-hydroxyphenyl)-7-phenylheptane					
9	6.219	–0.6	331.1907	295.1671, 137.0606, 171.1181, 163.0758, 313.1889, 117.0708, 131.0885	$C_{20}H_{26}O_4$
Identification: 3,5-dishydroxy-1-(4-hydroxy-3-methoxylphenyl)-7-phenylheptane					
10	6.682	–2.8	327.1587	137.0612, 163.0760, 309.1469, 177.0949	$C_{20}H_{22}O_4$
Identification: 1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxylphenyl)-4-en-3-heptanone					
11	7.129	–0.7	299.1645	133.0654, 281.1516, 149.0966, 107.0500, 105.0706, 263.1427, 131.0927,91.0553	$C_{19}H_{22}O_3$
Identification: 5-hydroxy-1-phenyl -7-(4-hydroxyphenyl) -3-heptanone					
12	7.322	–3.6	329.1741	163.0752, 311.1613, 137.0602, 133.0656, 149.0970, 293.1516, 131.0506, 105.0712, 91.0563	$C_{20}H_{24}O_4$
Identification: 5-hydroxy-1-phenyl-7-(4-hydroxy-3-methoxylphenyl)-3-heptanone					
13	11.112	0	285.1855	171.1192, 117.0713, 131.0879, 91.0570, 249.1690, 133.1086, 267.1681	$C_{19}H_{24}O_2$
Identification: 3,5-dihydroxy-1,7-bisphenylheptane					
14	12.482	0	281.1542	107.0499, 133.0657, 161.0981, 263.1429, 105.0737, 143.0872	$C_{19}H_{20}O_2$
Identification: 1-phenyl-7-(4-hydroxyphenyl)-4-en-3-heptanone					
15	12.861	–4.5	311.1633	163.0754, 137.0602, 293.1523, 105.0647, 133.0663, 161.0977	$C_{20}H_{22}O_3$
Identification: 1-phenyl-7-(4-hydroxy-3-methoxylphenyl)-4-en-3-heptanone					
16	13.225	3.2	283.1707	265.1575, 133.0659, 117.0712, 105.0706, 149.0970, 247.1481, 91.0561, 131.0883	$C_{19}H_{22}O_2$
Identification: 5-hydroxy-1,7-bisphenyl-3-heptanone					
17	14.828	–1.3	297.1487	107. 0498, 133.0649, 149.0611, 105.0716, 279.1373	$C_{19}H_{20}O_3$
Identification: 1-(4-hydroxyphenyl)-7-phenylhepta-3,5-dione					
18	15.383	–0.9	327.1593	309.1458, 137.0610, 179.0710, 105.0716, 133.0666	$C_{20}H_{22}O_4$
Identification: 1-(4-hydroxy-3-methoxylphenyl)-7-phenylhepta-3,5-dione					
19	15.83	–1.2	325.1436	131.0502, 307.1304, 137.0609, 179.0715	$C_{20}H_{20}O_4$
Identification: 5-hydroxy-1-(4-hydroxy-3-methoxylphenyl)-7-phenyl-4,6-dien-3-heptanone					

* Retention time (t_R); difference between measured and calculated accurate molecular weight in ESI$^+$ (ΔAM).
† Product ions shown in each row are given in the order of their relative abundance.

III). Subgroup 1 includes compounds 1 and 2, which possess three hydroxy groups on the aromatic rings. Subgroup 2 is composed of compound 3, 4, 5, 6, and 7, which possess two hydroxy groups on the aromatic rings. One or no hydroxy group is present on the aromatic rings of compound 11, 12, and 16 in subgroup 3.

In positive mode, precursor ions at *m/z* 391, 361, 345, 345, 375, 345, 315, 299, 329, and 283 (M+H)$^+$ in MS spectra were observed for compounds 1, 2, 3, 4, 5, 6, 7, 11, 12, and 16 respectively, suggesting M_r of 390, 360, 344, 344, 374, 344, 314, 298, 328, and 282. In MS^2 spectra, the corresponding ions at 373, 343, 327, 327, 357, 327, 297, 281, 311, and 263 (M–H_2O)$^+$ were also detected, suggesting the presence of an hydroxyl group on the heptane skeleton. The corresponding ions at 355, 325, 309, 309, 339, 309, 279, 263, 293, and 245 (M–$2H_2O$)$^+$ were also detected, but their abundances were much lower than that of (M–H_2O)$^+$; so, it was concluded that an oxo group exists on the heptane skeleton instead of an hydroxyl group, and the (M–$2H_2O$)$^+$ was formed by a rearrangement and loss of H_2O from the ion (M-H_2O)$^+$ (Figure 3). The ions A–F were produced by loss of a neutral moiety from the precursor ions.

In group 1, the MS and MS^2 spectra abundance of compared 1 and 2 were weak but main diagnostic ions can be seen. Compared to 1, compound 2 demonstrated a decrease of 30 Da (+OMe) for its precursor ion and some of its corresponding product ions (Table III), suggesting that it could be a homolog of compound 1. In addition, its similar retention time (Table III) also support this hypothesis. On the basis of analysis previously described and Figure 3, the proposal structure of compound 1 and 2 were tentatively confirmed.

In group 2, compounds 3 and 4 detected at the same retention time (Table III) had the same (M+H)$^+$ 345 in MS spectra and their MS^2 spectra were mixed together, indicating that their polarities were almost the same. Their structures differed from the substitution positions on the aromatic ring (R1 or R2) of the OH and OMe moieties (Table III). Compared to compound 3, compound 5 demonstrated an increase of 30 Da (+OMe) for its precursor ion and some of its corresponding product ions (Table III), suggesting that it could be a homolog of compound 3. In addition, their similar retention time (Table III) also supports this

hypothesis. Compound 6 also had the ion $(M+H)^+$ 345, similar to compounds 2 and 3, the substitution positions on the aromatic ring of the OH and OMe moieties were their structure features (Table III). Compared to compound 6, compound 7 demonstrated a decrease of 30 Da (+OMe) for its precursor ion and some of its corresponding product ions (Table III), suggesting that it might be a homolog of compound 6. Their similar retention time (Table III) also support this hypothesis. On the basis of analysis above and Figure 3, the proposal structures of this group were tentatively confirmed.

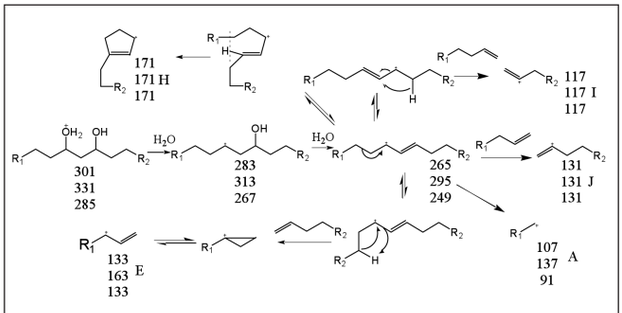

Figure 5. ESI^+ fragmentation of diarylheptanoids 8, 9, and 13.

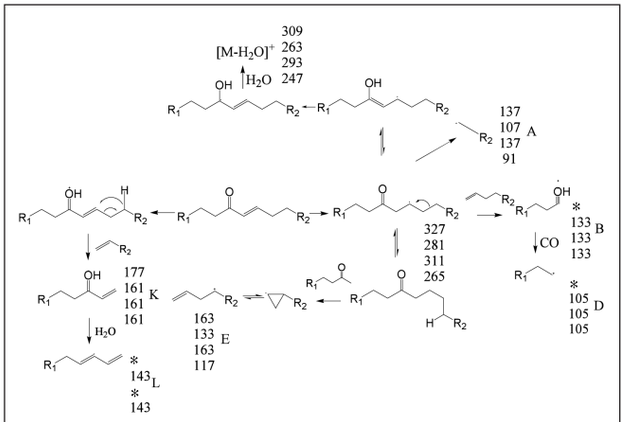

Figure 6. ESI^+ fragmentation of diarylheptanoids 10, 14, 15, and 20; * indicates the abundance of the corresponding ion was weak or the ion was not detected.

In group 3, compared to compound 16, the compound 11 demonstrated an increase of 16 Da (+OH) for its precursor ion and some of its corresponding product ions (Table III), suggesting that it could be a homolog of compound 16. Their retention time differences indicated their polarities differences, which supported this hypothesis. Compared to compound 11, compound 12 demonstrated an increase of 30 Da (+OMe) for its precursor ion and some of its corresponding product ions (Table III), suggesting that it could be a homolog of compound 11. In addition, their similar retention time (Table III) supports this hypothesis. On the basis of analysis above and Figure 3, the proposal structures of this group were tentatively confirmed.

Characterization of compounds 8, 9, and 13

This group of compounds possessed a common structural moiety of 3,5-dihydroxy on the heptane skeleton (Figure 1). This structural skeleton is also present in ginger, whose fragmentation mechanisms in ESI^+–MS–MS have been studied (13).

In positive mode, precursor ions at *m/z* 301, 331, and 285 $(M+H)^+$ in MS spectra were observed for compounds 8, 9, and 13, respectively, suggesting M_r of 300, 331, and 284. In MS^2 spectra, the corresponding ions at 283, 313, and 267 $(M-H_2O)^+$, and 267, 297, and 249 $(M-2H_2O)^+$ were detected. Furthermore, the abundances of $(M-2H_2O)^+$ were much higher than that of $(M-H_2O)^+$, differing from the previously mentioned group, suggesting the presence of 3,5-dishydroxy moiety on the heptane skeleton instead of on the aromatic rings. The ions A and E were produced by loss of a neutral moiety from the precursor ions in the same pathway as the above structural skeleton. When compared to compound 9, compound 8 showed a decrease of 30 Da (H instead of OMe) for its precursor ions and some of its corresponding product ions (Table III), indicating that it could be a homolog of compound 9, differing by lack of a methoxy group. In addition, the similar chromatographic behaviors of compounds 8 and 9 supported the hypothesis. Compared to compound 9, compound 13 demonstrated a decrease of 46 Da (OMe+OH) for its precursor ion and some of corresponding product ions (Table III) which could also be a homolog of compound 9. Their retention time differences indicate their polarities differences, which supported this hypothesis. On the basis of analysis previously mentioned, proposal structures of these compounds were confirmed in Figure 2.

Table IIIB. Chromatographic and MS Characteristics of Ciarylheptanoids detected by UPLC–ESI^+–MS in SFE Extract of *Alpinia officinarum

Number	t_R (min)	ΔAM (ppm)	$(M+H)^+$	Main fragment ions in $MS^{2\dagger}$	Composition
20	17.207	0.4	265.1593	117.0704, 161.0976, 143.0869, 133.0670, 91.0543, 247.1467	$C_{19}H_{20}O$
Identification: 1,7-bisphenyl-4-en-3-heptanone					
21	17.953	–2.8	281.1534	133.0659, 105.0710, 263.1441, 91.0507	$C_{19}H_{20}O_2$
Identification: 1,7-bisphenylhepta-3,5-dione					
22	18.225	–0.4	279.1384	131.0504, 105.0718, 133.0657, 91.0555, 261.1438	$C_{19}H_{18}O_2$
Identification: 5-hydroxy-1,7-bisphenyl-4, 6-dien-3-heptanone					
23	23.142	–3.7	401.2829	133.0665, 383.2751, 105.0726, 267.2114	$C_{29}H_{36}O$
Identification: Officinarumane C					

* Retention time (t_R); difference between measured and calculated accurate molecular weight in ESI^+ (ΔAM).

Characterization of compounds 10, 14, 15, and 20

All the diarylheptanoids in this group possess a common structural moiety, consisting of 5-ene and 3-oxo groups on the heptane skeleton (Figure 2). The MS^2 spectra of standard substance of compound 20 (Figure 4) were in accordance with the Figure 6.

In positive mode, ions at *m/z* 327,281, 311, and 265 $(M+H)^+$ in MS spectra were observed for compounds 10, 14, 15, and 20, respectively, suggesting M_r of 326, 280, 310, and 264. In MS^2 spectra, the ions A, B, and D were

formed by loss of a neutral moiety from the precursor ions in the same way as the first structural skeleton. The ions E and F were formed by loss of a neutral moiety from the precursor ions. When compared to compound 15, compound 14 showed a decrease of 30 Da (H instead of OMe) for its precursor ion and some of its corresponding product ions (Table III), indicating that it could be a homolog of compound 15, differing by lack of a methoxy group. Compared to compound 14, compound 20 demonstrated an decrease of 16 Da (OH) for its precursor ion and some of corresponding product ions (Table III), suggesting that its M_r was 264 Da and that it could also be a homolog of compound 15. Compared to compound 15, compound 10 showed a increase of 16 Da (OH) and their main product ions are same. Their remarkable differences of retention time indicate their remarkable difference of polarity. So the OH group was unambiguously on the benzene ring R1. Their proposal fragmentation pathways in ESI$^+$–MS–MS were shown in Figure 6, so they were tentatively identified in Table III.

Characterization of compounds 17, 18, and 21

All the diarylheptanoids in this group possess a common structural moiety, consisting of 3,5- dione group on the heptane skeleton (Figure 2).

In positive mode, ions at *m/z* 297, 327, and 281 $(M+H)^+$ in MS spectra were detected for compounds 17, 18, and 21 respectively, suggesting M_r of 296, 326, and 280. In MS^2 spectra, the ions A, B, and D were formed by loss of a neutral moiety from the precursor ions in the same way as the first structure skeleton. When compared to compound 18, compound 17 showed a decrease of 30 Da (H instead of OMe) for its precursor ion and some of its corresponding product ions (Table III), indicating that it could be a homolog of compound 18, differing by lack of a methoxy group. When compared to compound 17, compound 21 showed a decrease of 16 Da (H instead of OH) for its precursor ion and some of its corresponding product ions (Table III), indicating that it could be a homolog of compound 17. Their retention time differences indicate their polarities differences which supported this hypothesis. Their proposal fragmentation pathways in ESI$^+$–MS–MS were shown in Figure 7, which was simple and similar to the above pathways partially. They were tentatively identified in Table III.

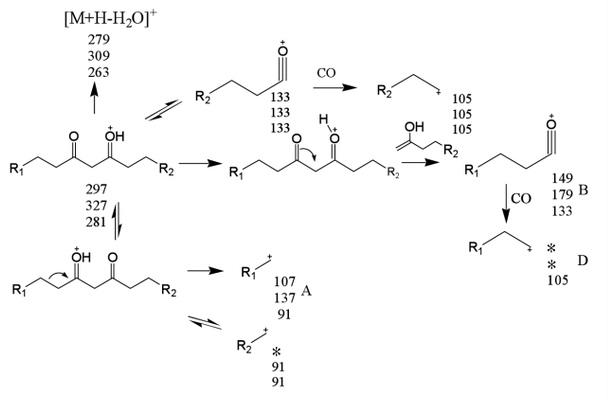

Figure 7. ESI$^+$ fragmetation of diarylheptanoids 17, 18, 21; * indicates the abundance of the corresponding ion was weak or the ion was not detected.

Characterization of compounds 19 and 22

All the diarylheptanoids in this group possess a common structural moiety shown in Figure 2. In positive mode, ions at *m/z* 279 and 325 $(M+H)^+$ in MS spectra were detected for compounds 19 and 22, respectively, suggesting M_r of 278 and 324. In MS^2 spectra, the ions A, B, and D were formed by the loss of a neutral moiety from the precursor ions in the same way as the first structure skeleton. The ion J at *m/z* 131 (base peak) was obtained by the loss of a neutral moiety from the precursor ions $(M+H)^+$. When compared to compound 22, compound 19 showed a decrease of 46 Da (H instead of OMe and OH) for its precursor ion and some of its corresponding product ions (Table III), indicating that it may be a homolog of compound 22. Their remarkable differences of retention time indicated their remarkable difference of polarity. In MS^2 spectra, the ion 131 of the two compounds were base peak which indicated that they have common structural moiety. The ions $(M+H–H_2O)^+$ 261 and 307 can be seen, but the abundances were low. Their proposal fragmentation pathways in ESI$^+$–MS–MS were shown in Figure 8, which was simple and similar to the above pathways partially. So they were tentatively identified in Table III.

Characterization of compound 23

The structure of this diarylheptanoid was not linear. Compound 23 displayed the $(M+H)^+$ ion at *m/z* 401 in MS spectra. The ions at *m/z* 133, 105, 283, and 267 were detected in MS^2 spectra. Its proposal fragmentation pathway was shown in

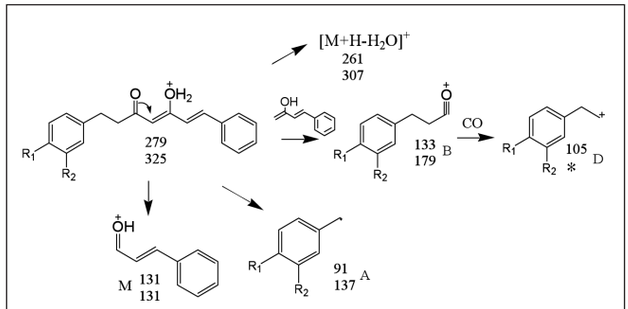

Figure 8. ESI$^+$ fragmentation of diarylheptanoids 19 and 22; * indicates the abundance of the corresponding ion was weak or the ion was not detected.

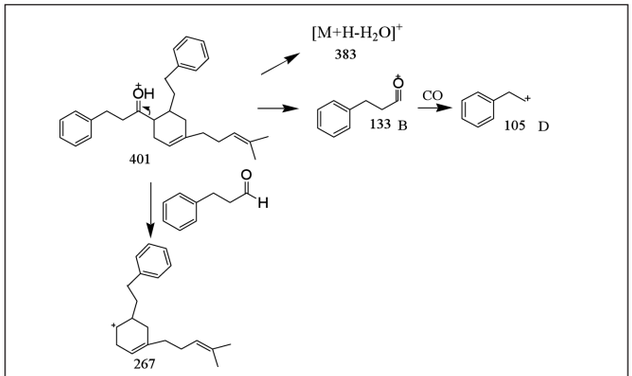

Figure 9. ESI$^+$ fragmentation of diarylheptanoids 23.

Figure 9, which was simple and similar to the above pathways partially. So it was tentatively identified as Officinarumane C.

Conclusions

Ultra-performance liquid chromatography–tandem mass spectrometry is a strong technique for the rapid identification of diarylheptanoids in *Alpinia officinarum*. This paper has established a method for rapid characterization and identification of diarylheptanoids in *Alpinia officinarum* by ultra performance liquid chromatography coupled with electrospray ionization mass spectrometry. This method could be used for the identification of the same type of diarylheptanoids in *Alpinia officinarum* or other plants.

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References

1. The State Pharmacopoeia Commission of the People's Republic of China. *Pharmacopoeia of the People's Republic of China*, Chemical Industry Press, Beijing, China, 2005, p. 202.
2. J. Luo, Y. Feng, and X. Ji. Advances in studies on naturally occurring linear diarylheptanoids. *Chin. Tradit. Herb. Drugs* **39(12):** 14–19 (2008).
3. R. Hiserodt, T.G. Hartman, C.T. Ho, and R.T. Rosen. characterization of podered turmeric by liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. *J. Chromatogr. A.* **740:** 51–63 (1996).
4. R. Richmond and E. PomboVillar. Gas chromatography-mass spectrometry coupled with pseudo-Sadtler retention indices, for the identification of components in the essential oil of *Curcuma longa* L. *J. Chromatogr. A* **760:** 303–308 (1997).
5. G.K. Jayaprakasha, L. Jagan Mohan Rao, and K.K. Sakariah. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. *J. Agric. Food Chem.* **50:** 3668–3672 (2002).
6. R. Bos, T. Windono, H.J. Woerdenbag, Y.L. Boersma, A. Koulman, and O. Kayser. HPLC-photodiode array detection analysis of curcuminoids in Curcuma species indigenous to Indonesia. *Phytochem. Anal.* **18(2):** 118–122 (2007).
7. Z. Liu, S. Sang, T. G. Hartman, C. Ho, and R.T. Rosen. Determination of Diarylheptanoids from Alpinia officinarum (Lesser Galangal) by HPLC with Photodiode Array and Electrochemical Detection. *Phytochem. Anal.* **16:** 252–256 (2005).
8. K. Inoue, S. Hamasaki, Y. Yoshimura, M.Yamada, M. Nakamura, Y. Ito, and H. Nakazawa. Validation of LC–electrospray–MS for determination of major curcuminoids in foods. *J. Liq. Chromatogr. Rel. Technol.* **26:** 53–62 (2003).
9. M. Lechtenberg, B. Quandt, and A. Nahrstedt. Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of Curcuma domestica Val. and Curcuma xanthorrhiza Roxb. by capillary electrophoresis. *Phytochem. Anal.* **15(3):** 152–158 (2004).
10. A.P. Gupta, M.M. Gupta, and S. Kumar. Simultaneous determination of curcumins in curcuma samples using high-performance thin-layer chromatography. *J. Liq. Chromatogr. Relat. Technol.* **22:** 1561–1569 (1999).
11. H. Jiang, B.N. Timmermann, and D.R. Gang. Use of liquid chromatography–electrospray ionization tandem mass spectrometry to identify diarylheptanoids in turmeric (*Curcuma longa* L.) rhizome. *J. Chromatogr. A* **1111:** 21–31 (2006).
12. H. Jiang, A. Somogyi, N.E. Jacobsen, B.N. Timmermann, and D.R. Gang. Analysis of curcuminoids by positive and negative electrospray ionization and tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **20:** 1001–1012 (2006).
13. H. Jiang, B.N. Timmermann, and D.R. Gang. Characterization and identification of diarylheptanoids in ginger (Zingiber officinale Rosc.) using high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **21:** 509–518 (2007).
14. F. Kiuchi, M. Shibuya, and U. Sankawa. Inhibitors of prostaglandin biosynthesis from *Alpinia officinarum*. *Chem. Pharm. Bull.* **30(6):** 2279–2282 (1982).

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