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Phenyl-terminated fatty acids in seeds of various aroids

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Abstract

A series of homologous ω-phenylalkanoic acids and ω-phenylalkenoic acids were isolated from seed lipids of various genera of the subfamily Aroideae of Araceae (the Jack-in-the-Pulpit family) and characterized. Besides the major acids, 11-phenylundecanoic acid, 13-phenyltridecanoic acid and 15-phenylpentadecanoic acid, all other homologous odd carbon number ω-phenylalkanoic acids from C_7 to C_{23} were detected in trace amounts. Additionally, one even carbon number acid, 12-phenyldodecanoic acid was found in several specimens in trace amounts. Similarly, two series of homologous odd carbon number monounsaturated ω-phenylalkenoic acids were found and characterized using dimethyl disulfide derivatization to locate the positions of their double bonds. In five acids from C_{11} to C_{19}, the double bond is located at the same distance, Δ^7, from the phenyl ring. In the other two acids of C_{13} and C_{15} chain length, the double bond is located at Δ^5 from the phenyl ring.
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Keywords: Aroids; Aroideae; Araceae; *Arisaema*; *Arum*; *Typhonium*; ω-Phenylalkanoic acids; ω-Phenylalkenoic acids; GC/TOF-MS

1. Introduction

Early in this ongoing systematic study of the acids of the seed lipids of Araceae, the Jack-in-the-Pulpit family, it was found that seeds of species of various genera in the subfamily Aroideae contained 13-phenyltridecanoic acid (Ph-13:0) as a major component (detected as methylester, **5**), comprising 5–16% of total seed fatty acids. Schmid et al. reported finding evidence, but with very little characterization, of various other phenyl-terminated, saturated and unsaturated, fatty acids of 11 and 15 carbon chain lengths, in addition to the principal Ph-13:0 (Schmid et al., 1997). Additional studies (to be reported elsewhere) encompassing all genera of Aroideae, showed that the phenyl-terminated fatty acids seemed to be limited only to certain genera as summarized in Table 1, suggesting a fundamental phylogenetic difference. The apparent anomaly in *Typhonium* has been also investigated by DNA studies (Renner and Zhang, 2004). However, this question is outside of the scope of this paper. The results reported here stem from the use of newly developed high resolution GC/TOF-MS instrumentation which enabled us to re-examine a randomly chosen group of specimens from the subfamily Aroideae previously analyzed by conventional GC/FID and GC/MS. These specimens were known to contain phenyl-terminated fatty acids.

Short chain length ω-phenylalkanoic acids have long been known to occur in natural systems. Benzoic, phenylacetic, 3-phenylpropanoic and 3-phenylpropenoic (cinnamic) acids are found quite commonly. For example, 3-phenylpropanoic acid is found in various propolis and mammalian exocrine secretions (Markham et al., 1996; Eisner et al., 1977) while cinnamic acid is a common constituent of floral fragrances. Eisner et al. reported the finding of the 3-, 5-, and 7-phenylalkanoic

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Table 1
13-Phenyltridecanoic acid (detected as methylester, **5**) in the seed oil of plants from subfamily Aroideae (Araceae)

Tribe	Genus
Ambrosineae	• *Ambrosina*
Areae	• *Arum*
	• *Biarum*
	• *Dracunculus*
	• *Eminium*
	• *Helicodiceros*
	• *Theriophonum*
	•○ *Typhonium*
Ariopsideae	○ *Ariopsis*
Arisaemateae	• *Arisaema*
Arisareae	• *Arisarum*
Cryptocoryneae	○ *Cryptocoryne*
	○ *Lagenandra*
Pinellieae	• *Pinellia*
Pistieae	○ *Pistia*
Thompsonieae	○ *Amorphophallus*
	○ *Pseudodracontium*

(•) Ph-13:0 present as a major component of fatty acids.
(○) Ph-13:0 low level or not detected.

acids as constituents of the stink of stinkpot turtles (Eisner et al., 1977). In 1996 Pupo et al. (1996) and in 1997 Schmid et al. (1997) independently reported the first finding of a long chain ω-phenylalkanoic acid as a naturally occurring material in the leaves of *Trichilia claussenii* and in the seed oil of Araceae plants. In the meantime, Carballeira et al. (1997) reported finding C_{10}, C_{12}, C_{14} and C_{16} ω-phenylalkanoic acids in bacteria. With the findings presented in this report, to date, all odd carbon chain length ω-phenylalkanoic acids from C_1 through C_{23} and even carbon chain length ω-phenylalkanoic acids of C_{10}, C_{12}, C_{14}, and C_{16}, have been reported to be found in nature. The phenyl-terminated fatty acids known to occur in nature are summarized in Table 2.

2. Results and discussion

Identification of phenyl-terminated fatty acids in this work was ultimately made by means of high resolution mass spectrometry after their conversion into methylesters. Most acids found in seed lipids are saturated and unsaturated straight chain fatty acids, with those of C_{16} and C_{18} chain length predominating. The profile of abundances of phenyl-terminated fatty acids from seeds of *A. korolkowii* is shown in Fig. 1. Due to their very low levels, most phenyl-terminated fatty acids cannot be detected in the total ion chromatogram (Fig. 1(a)) and post-acquisition selected ion accurate mass chromatograms were generated to detect minor species as shown in Fig. 1(b). Here the $[M-CH_3OH]^{\cdot+}$ ions were chosen to visualize the phenyl-terminated acids.

2.1. Mass spectral characterization of ω-phenylalkanoic acids

Electron impact mass spectra of ω-phenylalkanoic acid methylesters are characterized by the presence of the molecular ion and the loss of methanol, CH_3OH, for all the species encountered (see Fig. 2(a)). The abundance of the $[M-CH_3OH]^{\cdot+}$ fragment always exceeds that of the molecular ion, with the loss of CH_3OH more pronounced for longer chain ω-phenylalkanoic acids. For short chain ω-phenylalkanoic acids (such as C_7) $[M]^{\cdot+}$ is about 70% of $[M-CH_3OH]^{\cdot+}$ and then sigmoidally decreases approaching about 10% of $[M-CH_3OH]^{+}$ for C_{17}–C_{23} (data not shown). This is

Table 2
Natural occurrence of non-substituted ω-phenylalkanoic and alkenoic acids

Source	C_1	C_3	C_5	C_7	C_9	C_{11}	C_{13}	C_{15}	C_{17}	C_{19}	C_{21}	C_{23}	Reference
Seed oil of Araceae *sp.*				•	•	• ○	• ○○ □	• ○○	• ○	• ○	•	•	this work
						•	• ○	• ○					Schmid et al., 1997; Chen et al., 1997
Leaves of *Trichilia claussenii*						•	• ○	• ○					Pupo et al., 1996
Vibrio alginolyticus						•	•	•	•				Carballeira et al., 1997
Halophilic Bacillus						•	•	•					Carballeira et al., 2001
New Zeland propolis		•	• □										Markham et al., 1996
Stink of stinkpot turtle		•	•	•									Eisner et al., 1977
Commonly found	•	•	• ○										

(•) Saturated ω-phenylalkanoic acids; (○) monounsaturated ω-phenylalkanoic acids; (□) diunsaturated ω-phenylalkanoic acids

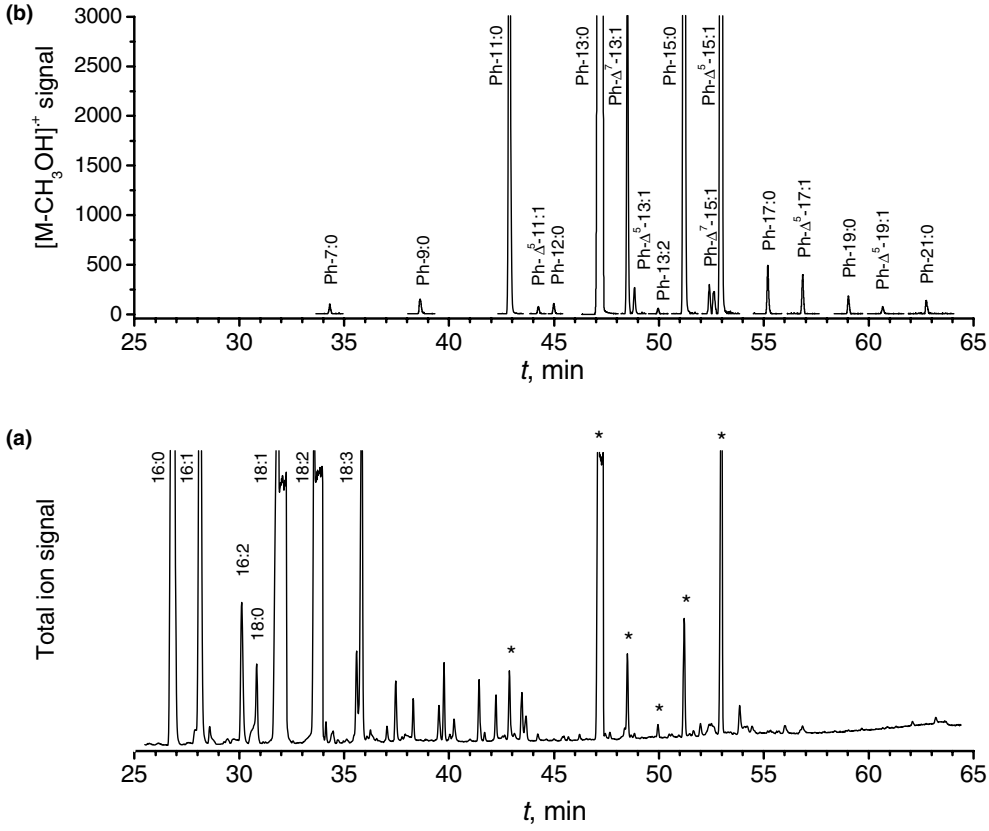


Fig. 1. Total ion chromatogram of the seed oil extracts from *Arum korolkowii* (a) and reconstructed $[M-CH_3OH]^{\cdot+}$ ion chromatograms (±0.040 u window) of the homologous ω-phenylalkanoic (**1**–**9**) and ω-phenylalkenoic acid (**11**–**18**) methylesters (b). ω-Phenylacid peaks that are seen in the total ion chromatogram (a) are marked with an asterisk (*).

an important detail for selected ion monitoring of trace amounts of long chain ω-phenylalkanoic acids as the monitoring of $[M-CH_3OH]^{\cdot+}$ instead of $[M]^{\cdot+}$ can improve detection levels by about an order of magnitude. Major peaks of alkylbenzene fingerprints at $m/z=91$ and 92 (tropylium ion C_7H_7, and C_7H_8) and $m/z=104$ and 105 (C_8H_8 and C_8H_9) are evident for medium sized phenyl-terminated fatty acids with C_{11}–C_{15} but are not abundant either for short chain (C_7, C_9) or long chain (C_{17}–C_{23}) fatty acid methylesters. Thus, while monitoring of $m/z=91$ or 104 can be efficiently used for selective screening of the major phenyl-terminated fatty acids, see Scheme 1, for structures **1**–**18** such as Ph-11:0 (**3**), Ph-13:0 (**5**), Ph-15:0 (**6**), Ph-13:1 (**16**) or Ph-15:1 (**13**) (as done previously by Schmid et al., 1997), it is not suitable for detection of minor phenyl-terminated fatty acids as discovered in this study.

The major acids of seed lipids are known to be straight chain compounds rather than branched. Chain branching produces characteristic changes in the MS fragmentation pattern and the observed mass spectra of the phenyl-terminated fatty acid methylesters show no evidence of branching. Chen et al. have characterized the major 13-phenyltridecanoic acid methylester (**5**) isolated (after esterification) from *Typhonium flagelliforme* by ^1H NMR and ^{13}C NMR spectroscopy, as unambiguously corresponding to a phenyl-terminated straight-chain fatty acid rather than a branched chain isomer (Chen et al., 1997).

2.2. Time-of-flight mass analysis for trace level speciation

The inherent advantage of time-of-flight (TOF) mass analysis for structure determination speciation studies at trace levels is that full mass scanning is accomplished without sacrificing sensitivity. High mass measurement precision is achieved by constant leaking of a reference compound directly into the ionization source (internal standard) and ions arising from the reference compound are efficiently eliminated from the recorded mass spectra of any eluting species by simply subtracting the background information. It is important to note that GC/TOF-MS instrumentation provides both high mass resolution and low level detection capability. A 0.001–0.002 u mass accuracy usually is achieved which permits one to assign the elemental composition for the measured mass signal. Besides that, under high mass accuracy and precision, post-acquisition selected ion monitoring is a powerful method of locating homologous species, for which the molecular formulae can be postulated a

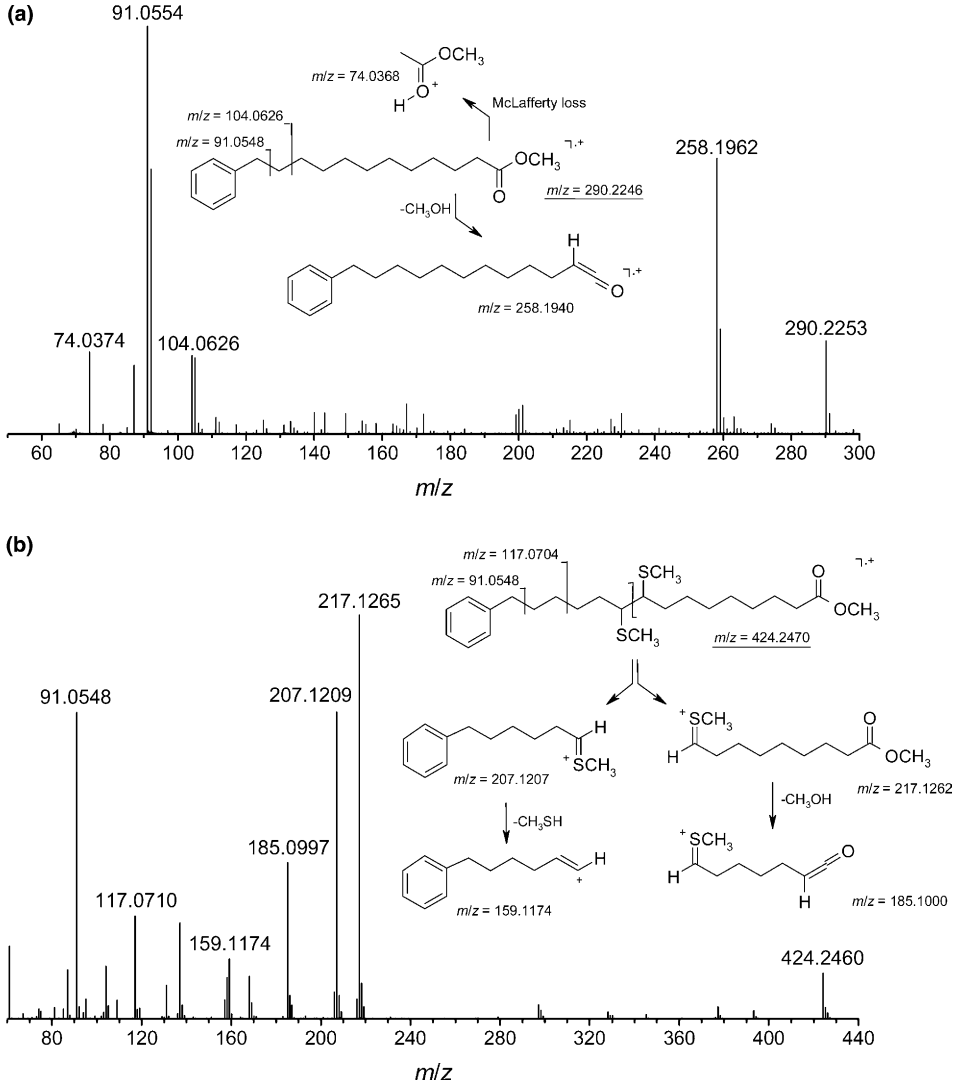


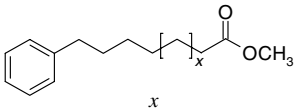
Fig. 2. High resolution GC/TOF-MS mass spectra of 12-phenyldodecanoic acid methylester (**4**) from seeds of *Arisaema wilsonii* (a) and α,β-*bis*(methylthio) derivative (**17a**) of 15-phenyl-pentadec-9-enoic acid methylester (**17**) from seeds of *Arum korolkowii* (b).

priori (for example, $C_{8+n}H_{8+2n}O_2$ in case of ω-phenylalkanoic acid methylesters). This feature (as utilized in the current study) allows for specific monitoring of molecular ions of the homologous species of interest.

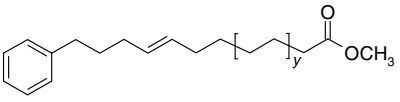
2.3. Characterization of ω-phenylalkenoic acids

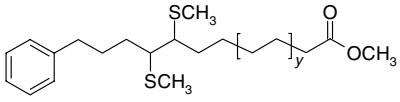
Determination of the positions of carbon–carbon double bonds in naturally occurring unsaturated fatty acids has been a classical problem. It arises due to the ability of the double bond to migrate in a charged molecular ion. One of the most frequently used methods to overcome the problem entails the addition of dimethyl disulfide across the double bond in the presence of iodine to form α,β-*bis*(methylthio)derivatives (Scribe et al., 1988). In electron impact mass spectrometry, these derivatives primarily undergo rupture of the C–C bond between the methylthio groups. This diagnostic feature allows for rapid identification of the fatty acid derivatives as illustrated in Fig. 2(b) with the example of the Ph-15:1 dimethyl disulfide derivative (**17a**).

The chromatogram of the α,β-*bis*(methylthio)derivatives of ω-phenylalkenoic acid methylesters (**11a**–**17a**) isolated from the seed oil of *A. korolkowii* (Fig. 3) shows the presence of two monounsaturated acid series. In five acids C_{11}, C_{13}, C_{15}, C_{17} and C_{19}, the double bond is located at the same distance, Δ^5, from the phenyl ring while in the other two acids of C_{13} and C_{15} chain length, the double bond is located at Δ^7 from the phenyl ring as shown in Scheme 1. Analysis of α,β-*bis*(methylthio)derivative electron impact mass spectra (see Table 3) showed that the pairs of Ph-13:1 (**16**) and Ph-15:1 (**13**) monounsaturated acids are remarkable for their respective amounts. The major Ph-13:1 (**16**) is Δ^7 while the major Ph-15:1 (**13**) is Δ^5. Minor Ph- Δ^5-13:1 (**12**), if present, is usually about 7% of the major Ph-Δ^7-13:1 (**16**) and

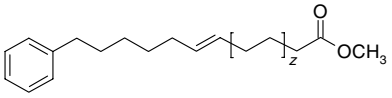


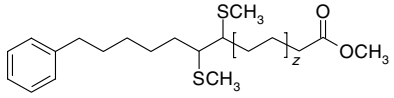
	x	
1	1	Ph-7:0
2	3	Ph-9:0
3	5	Ph-11:0
4	6	Ph-12:0
5	7	Ph-13:0
6	9	Ph-15:0
7	11	Ph-17:0
8	13	Ph-19:0
9	15	Ph-21:0
10	17	Ph-23:0



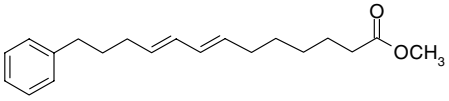


	y			y	
11	1	Ph-Δ^5-11:1	**11a**	1	Ph-Δ^5-11:1
12	2	Ph-Δ^5-13:1	**12a**	2	Ph-Δ^5-13:1
13	3	Ph-Δ^5-15:1	**13a**	3	Ph-Δ^5-15:1
14	4	Ph-Δ^5-17:1	**14a**	4	Ph-Δ^5-17:1
15	5	Ph-Δ^5-19:1	**15a**	5	Ph-Δ^5-19:1





	z			z	
16	2	Ph-Δ^7-13:1	**16a**	2	Ph-Δ^7-13:1
17	3	Ph-Δ^7-15:1	**17a**	3	Ph-Δ^7-15:1



18 Ph-13:2 (possibly)

Scheme 1. Structures of the ω-phenylalkanoic and -alkenoic acid methylesters (along with their *bis*-methylthio derivatives) found and identified in seed oil of subfamily Aroideae of Araceae.

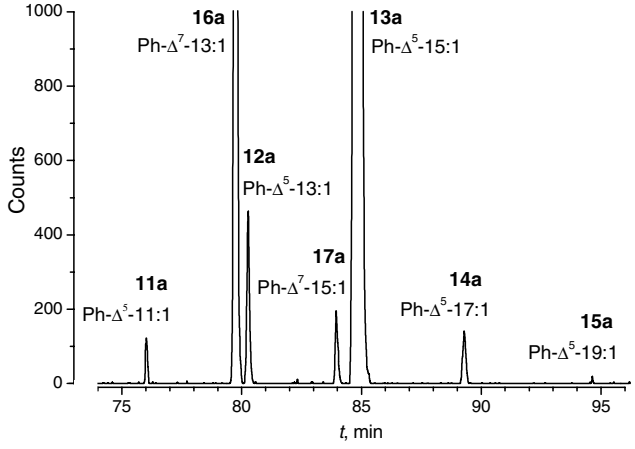


Fig. 3. Reconstructed molecular ion chromatogram of α,β-*bis*(methylthio) derivatives of methyl esters of monounsaturated ω-phenylalkenoic acids (**11a**–**17a**) isolated from *Arum korolkowii*.

minor Ph-Δ^7-15:1 (**17**) is only about 1% of the major Ph-Δ^5-15:1 (**13**) The assigned structures of monounsaturated acids are also consistent with their retention behavior, since the Δ^5 acid methyl esters and methyl ester *bis*(methylthio) derivatives always elute after the Δ^7 isomers.

Pupo et al. have reported the presence of Ph-14:1 and Ph-15:1 from *Trichilia clausenii* and to our knowledge this is the only report providing some information regarding the double bond position (Pupo et al., 1996). Ph-14:1 and Ph-15:1 olefinic protons in ^{1}H NMR experiments showed as two triplets at $\delta = 5.34$ ppm. Such a chemical shift along with the reported benzyl and α-carbonyl proton shifts is consistent with a double bond occupying a position anywhere from Δ^4 to Δ^{10-11} from the phenyl ring. Thus, while their work does not provide a definite location for the double bond position, it brackets the possible location which is consistent with

Table 3
High resolution mass spectral characterization of ω-phenyl-alkanoic and -alkenoic acids found in seed lipids of various genera of subfamily Aroideae of Araceae

Species	Aroid source[a]	Methylester EI+ mass spectra[b]	Disulfide derivative EI+ mass spectra[c]
Ph-7:0 (**1**)	*Arisaema wilsonii* *Arisaema utile*	$M^{\cdot+}$: 220.1477 u (+1.4 mu) $[M-CH_3OH]^{+}$: 188.1174 u (−2.7 mu)	–
Ph-9:0 (**2**) *Arisaema wilsonii*	*Arisaema utile* *Typhonium diversifolium*	$M^{\cdot+}$: 248.1766 u (−1.0 mu) $[M-CH_3OH]^{+}$: 216.1482 u (−3.2 mu)	–
Ph-11:0 (**3**)	*Arum elongatum* *Arum korolkowii* *Arum cylindraceum* *Arisaema utile* *Typhonium gigantium* *Typhonium diversifolium*	$M^{\cdot+}$: 276.2092 u (+0.3 mu) $[M-CH_3OH]^{+}$: 244.1836 u (+0.9 mu)	–
Ph-D^5-11:1 (**11**)	*Arum korolkowii* *Arum cylindraceum*	$M^{\cdot+}$: 274.1887 u (−3.6 mu) $[M-CH_3OH]^{+}$: 242.1659 u (−1.2 mu)	$M^{\cdot+}$: 368.1829 u (−1.5 mu) A^{+} : 161.0642 u (+0.6 mu) B^{+} : 129.0417 u (+4.3 mu) C^{+} : 207.1213 u (+0.6 mu)
Ph-12:0 (**4**)	*Arisaema wilsonii* *Arisaema utile* *Arum korolkowii* *Typhonium gigantium*	$M^{\cdot+}$: 290.2253 u (+0.7 mu) $[M-CH_3OH]^{+}$: 258.1962 u (+2.2 mu)	–
Ph-13:0 (**5**)	Abundant in all	$M^{\cdot+}$: 304.2403 u (+0.1 mu) $[M-CH_3OH]^{+}$: 272.2113 u (−2.7 mu)	–
Ph-D^5-13:1 (**12**) minor	*Arum elongatum* *Arum korolkowii* *Arisaema utile* *Arum cylindraceum*	$M^{\cdot+}$: 302.2261 u (+1.5 mu) $[M-CH_3OH]^{+}$: 270.2003 u (+1.9 mu)	$M^{\cdot+}$: 396.2137 u (−2.0 mu) A^{+} : 217.1250 u (−1.2 mu) B^{+} : 185.1001 u (+0.1 mu) $[C^{+}$ - H]: 178.0814 u (−0.2 mu)
Ph-D^7-13:1 (**16**) major	*Arum elongatum* *Arum korolkowii* *Arisaema utile*	$M^{\cdot+}$: 302.2241 u (−0.5 mu) $[M-CH_3OH]^{+}$: 270.2000 u (+1.6 mu)	$M^{\cdot+}$: 396.2142 u (−1.5 mu) A^{+} : 189.0952 u (+0.3 mu) B^{+} : 157.0716 u (+2.9 mu) C^{+} : 207.1222 u (+1.5 mu)
Ph-13:2 (**18**)	*Arum elongatum* *Arum korolkowii* *Arisaema utile*	$M^{\cdot+}$: 300.2087 u (−0.2 mu) $[M-CH_3OH]^{+}$: 268.1754 u (−7.3 mu) 143.0875 u ($C_{11}H_{11}$), 129.0714 u ($C_{10}H_9$), 115.0553 u (C_9H_7)	No results obtained
Ph-15:0 (**6**)	*Arum korolkowii* *Arum elongatum* *Arisaema utile* *Typhonium diversifolium*	$M^{\cdot+}$: 332.2740 u (+2.5 mu) $[M-CH_3OH]^{+}$: 300.2435 u (−1.8 mu)	–
Ph-D^5-15:1 (**13**) major	*Arum korolkowii*	$M^{\cdot+}$: 330.2559 u (+0.0 mu) $[M-CH_3OH]^{+}$:. 298.2268 u (−2.9 mu)	$M^{\cdot+}$: 424.2500 u (+3.0 mu) A^{+} : 245.1583 u (+0.8 mu) B^{+} : 213.1308 u (−0.5 mu) $[C^{+}$ - H]: 178.0837 u (+2.1 mu)
Ph-D^7-15:1 (**17**) minor	*Arum elongatum* *Arum korolkowii* *Arum cylindraceum*	$M^{\cdot+}$: 330.2568 u (+0.9 mu) $[M-CH_3OH]^{+}$: 298.2250 u (−4.7 mu)	$M^{\cdot+}$: 424.2460 u (−1.0 mu) A^{+} : 217.1265 u (+0.3 mu) B^{+} : 185.0997 u (−0.3 mu) C^{+} : 207.1209 u (+0.2 mu)
Ph-17:0 (**7**)	*Arum korolkowii* *Arisaema utile* *Typhonium diversifolium*	$M^{\cdot+}$: 360.3044 u (+1.6 mu) $[M-CH_3OH]^{+}$: 328.2738 u (−2.8 mu)	–

Table 3 (*continued*)

Species	Aroid source[a]	Methylester EI+ mass spectra[b]	Disulfide derivative EI+ mass spectra[c]
Ph-D^5-17:1 (**14**)	*Arum korolkowii*	$M^{\cdot+}$: 358.2886 u (+1.4 mu) $[M-CH_3OH]^{\cdot+}$: 326.2556 u (−5.4 mu)	$M^{\cdot+}$: 452.2790 u (+0.7 mu) A^+ : 245.1574 u (−0.1 mu) B^+ : 213.1335 u (+2.2 mu) C^+ : 207.1213 u (+0.6 mu)
Ph-19:0 (**8**)	*Arum korolkowii* *Arisaema utile*	$M^{\cdot+}$: 388.3311 u (−3.0 mu) $[M-CH_3OH]^{\cdot+}$: 356.3051 u (−2.8 mu)	–
Ph-D^5-19:1 (**15**)	*Arum korolkowii*	$M^{\cdot+}$: 386.3159 u (−2.6) $[M-CH_3OH]^{\cdot+}$: 354.2859 u (−6.4 mu)	$M^{\cdot+}$: 480.3157 u (+6.1 mu) A^+ : 273.1941 u (+5.3 mu) B^+ : 241.1579 u (+4.7 mu) C^+ : not detected
Ph-21:0 (**9**)	*Arum korolkowii* *Arisaema utile*	$M^{\cdot+}$: 416.3651 u (−0.3 mu) $[M-CH_3OH]^{\cdot+}$: 384.3353 u (−3.9 mu)	–
Ph-23:0 (**10**)	*Arisaema utile*	$M^{\cdot+}$: 444.3893 u (−7.4 mu) $[M-CH_3OH]^{\cdot+}$: 412.3694 u (−1.1 mu)	–

[a] Only the most representative specimens are listed.
[b] The difference between the experimental and the predicted theoretical mass is shown in parentheses (in milli-mass units).
[c] $A^+ = CH_3SCH\text{-}(CH_2)_nC(O)OCH_3^+$; $B^+ = A^+ - CH_3OH$; $C^+ = Ph\text{-}(CH_2)_n\text{-}CHSCH_3^+$.

our exact locations of double bond position of Δ^5 and Δ^7 for the two series of homologous acids.

Analysis of a few specimens, such as *Arum elongatum* and *A. korolkowii*, showed the presence of an ω-phenylalkanoic acid with a signal at $m/z = 300$ u. As the initial analyses of the seed oils were carried out using a quadrupole GC/MS instrument, this could be either a molecular ion of doubly unsaturated Ph-13:2 (**18**) or $[M-CH_3OH]^{\cdot+}$ ion of Ph-15:0 (**6**). High mass resolution analysis revealed this acid to be ω-tridecanedienoic acid (**18**). Position of the two double bonds could not be determined from the EI+ mass spectrum and disulfide derivatization failed to provide any additional information possibly due to the low volatility of the di-(*bis*methylthio) derivative (where disulfide moieties are added to both C–C double bonds). However, we speculate that Ph-13:2 (**18**) might be a Δ^5, Δ^7-dienoic acid (as suggested in Scheme 1), thus sharing the features of both monounsaturated acid homologue series Δ^5 and Δ^7.

Monounsaturated minor Ph-Δ^7-15:1 (**17**) showed the presence of two equally abundant isomers (Fig. 1, two minor peaks eluting at 52.5 min). This feature could be ascribed either to the structure (position of the double bond) or to geometric (*E, Z*) isomers. However, only one minor Ph-15:1 species is observed after the disulfide derivatization (Fig. 3, peak eluting at 84.0 min), suggesting the presence of *E* and *Z* isomers of Ph-Δ^7-15:1 (**17**).

Scheme 1 summarizes the structures of the phenyl-terminated fatty acids identified in this study. To our knowledge this is the first study to report the occurrence of saturated long-chain phenyl-terminated fatty acids Ph-17:0 (**7**), Ph-19:0 (**8**), Ph-21:0 (**9**) and Ph-23:0 (**10**) plus the short chain Ph-9:0 (**2**). Also identification of Ph-Δ^5-11:1 (**11**), Ph-Δ^5-13:1 (**12**), Ph-Δ^5-15:1 (**13**), Ph-Δ^5-17:1 (**14**), Ph-Δ^5-19:1 (**15**), and Ph-Δ^7-13:1 (**16**), Ph-Δ^7-15:1 (**17**) and an uncharacterized Ph-13:2 (**18**) are reported for the first time.

2.4. Retention time confirmation

Besides the accurate measurement of the molecular ion and its main fragments for all the phenyl-terminated fatty acid methylester homologues, the additivity of the retention times under constant temperature ramp conditions further validates the presence of an homologous series (Fig. 4). Retention times for ω-phenylalkanoic acid methyl esters increase incrementally with the increase of carbon atom chain length. Similar trends were

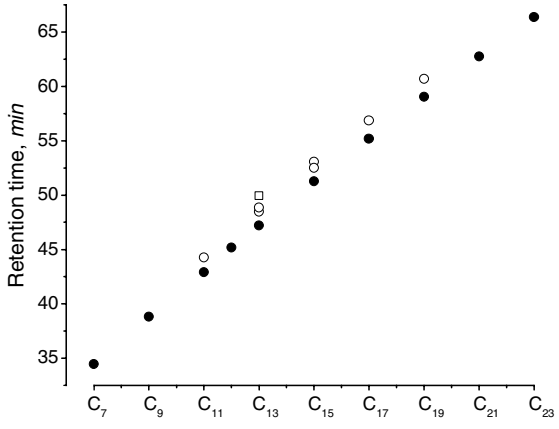


Fig. 4. Relationship of GC retention times and length of carbon atom chain for saturated (●), monounsaturated (○), and diunsaturated (□), phenyl-terminated fatty acid methylester homologues.

also observed for the monounsaturated acid methylesters (see Fig. 4). This feature was successfully used to predict and/or confirm retention times for all the novel phenyl-terminated fatty acids identified in this study.

The scarcity of aroid seeds led to analyses occasionally being carried out on four or fewer seeds. Nevertheless, the use of TOF-MS enabled the detection and characterization of extended series of homologous acids, with some present at very low levels. For example, both phenyl-acid characterization and disulfide derivatization shown in Figs. 1–3 were performed having only four seeds of *A. korolkowii*.

Phenyl-terminated fatty acids present in various genera of subfamily Aroideae have rather similar abundance profiles. The 13-phenyltridecanoic acid (**5**) is always present in the highest amount while Ph-11:0 (**3**) and Ph-15:0 (**6**) are found usually at levels about 100 times lower. Furthermore, as the C_{13} chain length is reduced or increased, the abundance of the phenyl-terminated acids is significantly reduced even to four orders of magnitude compared to Ph-13:0 (**5**). Of the mono-unsaturated phenyl-terminated acids, Ph-Δ^7-13:1 (**16**) and Ph-Δ^5-15:1 (**13**), are usually present in the highest amounts.

Plants of the family Araceae, usually referred to informally as Aroids, are used widely as foods after removal of toxic materials and as medicinals. The seeds of several species of genera in subfamily Aroideae are used in treatments for hemorrhoids and other problems in Turkey and in the Middle East (Habib-ur-Rehman et al., 1996; Sagalik et al., 2002a,b). Perhaps the efficacy, if any, of such treatments is due to the presence of the various ω-phenylalkanoic and ω-phenylalkenoic acids. Curiously, these acids seem to be absent from all other parts of the plants, including the berry tissues surrounding the seeds. The function of the phenyl-terminated fatty acids in the seeds of these plants remains an enigma.

3. Concluding remarks

More than half the genera in subfamily Aroideae (Grayum, 1990), contain ω-phenyl-alkanoic and-alkenoic acids in their seed lipids. The genera divide into two groups: one that has a sizable amount (5–16%) of 13-phenyltridecanoic acid (such as *Arisaema*) and the other which has none, or at most only a trace of it or of any of the other ω-phenyl-alkanoic or-alkenoic acids (such as *Ariopsis*). We report the finding of all the odd carbon homologous ω-phenylalkanoic acids from C_7 to C_{23}, along with one even carbon homologue, C_{12} (**4**). We also report the finding of two series of monounsaturated ω-phenylalkenoic acids; one of odd carbon homologues from C_{11} to C_{19} with the double bond situated at Δ^5 from the phenyl group (**11**–**15**), and a second, represented by only two acids, C_{13} and C_{15}, with the double bond at Δ^7 from the phenyl group (**16**–**17**). A C_{13} doubly unsaturated acid (**18**) has also been found but remains uncharacterized.

4. Experimental

4.1. Reagents and standards

All reagents were of analytical purity and used without further purification. Dimethyl disulfide was obtained from Fluka (Milwaukee, WI, USA). Standard fatty acid methyl esters were obtained from Supelco (Bellefonte, PA, USA).

4.2. Instrumentation

An Agilent 6890N gas chromatograph was utilized in this work (Agilent Technologies; Palo Alto, CA, USA), with a splitless injection mode employed and an injector temperature 250 °C. The column oven was initially at a temperature of 45 °C and was immediately ramped at 10 °C min^{-1} to a temperature of 220 °C. Helium was used as carrier gas and the column flow was set at a constant value of 1.0 mL min^{-1}. A nonbonded *bis*(cyanopropyl) polysiloxane fused silica capillary column SP-2560 (100 m, 0.25 mm i.d., 0.2 μm film thickness) was used for separation of fatty acid methylesters (Supelco; Bellefonte, PA, USA).

A Micromass GCT^{TM} orthogonal time-of-flight mass spectrometer (Micromass, Manchester, UK) coupled to the GC was used for mass spectral characterization of the species. Heptacosafluorotributylamine was used for mass calibration. The average mass accuracy was usually within 0.001–0.002 u. Estimated mass resolution of the instrument was $m/\Delta m = 3700$. All measurements were conducted using positive electron impact ionization at 70 eV.

4.3. Methods

Cleaned seeds, free of all berry tissues, were comminuted and treated with BF_3–MeOH at 60–80 °C to convert acyl seed lipid acids to their methylesters as described previously (Soukup and Holman, 1987). The sample size varied from two to four seeds depending on seed availability. The samples were washed with water, dried and diluted with 50–400 μL of heptane for GC/MS analyses. Dimethyl disulfide derivatization was carried out from the heptane extract according to the procedure of Scribe et al. (Scribe et al., 1988).

4.4. Seed sources

Arisaema wilsonii Eng. [American Rock Garden Society (ARGS)]; *Arisaema utile* Hook. f. ex Schott (ARGS); *Arum cylindraceum* Gasp. [B&T World Seeds (B&T)];

A. elongatum Steven (Antoine Hoog, A.M.D. Hoog Authentic Plants); *A. korolkowii* Regel (B&T); *Typhonium diversifolium* Wall. Ex Schott (E. Hornig, Seneca Hills Nursery); *Typhonium gigantium* Eng. (J. Bogner, Munich Botanical Garden).

4.5. Mass spectral identification of species

ω-Phenylalkanoic acid methylesters and their *bis*(methylthio) derivatives were identified from their high resolution GC/TOF-MS electron impact mass spectra and Table 3 summarizes the mass spectral information used for identification of all the ω-phenylalkanoic acids encountered.

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