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13-PHENYLTRIDECANOIC ACID IN SEED LIPIDS OF SOME AROIDS

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Key Word Index—*Arum*; *Arisaema*; Araceae; seed lipids; 13-phenyltridecanoic acid; ω-phenylalkanoic acids; ω-phenylalkenoic acids.

Abstract—Several closely related long-chain ω-phenylalkanoic and ω-phenylalkenoic acids occur in the seed lipids of genera of the subfamily Aroideae of the Araceae. One, 13-phenyltridecanoic acid, is a major component. This is the first report of these acids in plant lipids. Their presence in only one subfamily may indicate that the Araceae is diphyletic.

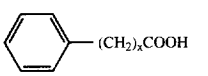
INTRODUCTION

The phylogeny of the Araceae (the 'Jack-in-the-Pulpit' or Aroid family), with 2500 species in 107 genera, is currently in a state of flux with various taxonomists realigning genera within subfamilies based on new data or on reinterpretations of old data. Grayum [1], in a comprehensive review of the family, has discussed the problems while proposing some new alignments. Of considerable importance is the question of whether the Araceae are monophyletic or polyphyletic. In the Haemodoraceae, the presence of unique compounds in all genera of the family has been cited as evidence that this family is monophyletic [2, 3]. As reported here, an apparently unique acid present in the seeds of some genera of the Araceae, but not in others, may be evidence of a diphyletic origin.

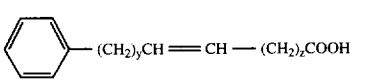
RESULTS AND DISCUSSION

We began a study of the fatty acid profiles of the seed lipids of the Araceae by analysing the seeds of five *Arisaema* and one *Arum* species. The acids of the seed lipids were converted to methyl esters and analysed by GC [4]. Comparisons between peak retention times with those of authentic standards resulted in the identification of most of the major acids as saturated or unsaturated straight-chain fatty acids common in plant and animal tissues. However, one major compound remained unidentified. It comprised *ca* 6–18% of the total fatty acids in the six species of the two genera that were examined.

Herein, we report the identification of the unknown acid as 13-phenyltridecanoic acid (13PTDA) (**1b**). This phenyl-substituted fatty acid is an unexpected component of plant lipids and this would appear to be the first report of its natural occurrence. 13PTDA is accompanied by small accounts (less than 0.1%) of the corresponding saturated 11-phenylundecanoic (**1a**) and 15-phenylpentadecanoic (**1c**) acids, as well as the monounsaturated 13-phenyltridecenoic (**2a**) and 15-phenylpentadecenoic (**2b**) acids.



1_a x = 10 11-phenylundecanoic acid
1_b x = 12 13-phenyltridecanoic acid
1_c x = 14 15-phenylpentadecanoic acid



2_a y + z = 10 13-phenyltridecenoic acid
2_b y + z = 12 15-phenylpentadecenoic acid

Identification of the new acids was carried out by means of GC-mass spectrometry. A methyl ester sample prepared as described [4] from *Arum maculatum* was used because of its known high content of these compounds. Mass spectrometric data indicated that the principal acid had an aromatic phenyl ring, one carboxyl group and a M_r of 290. This along with the fragmentation pattern and the retention times compared with saturated and unsaturated fatty acids on columns of different polarities, led to the assignment of the structure shown in **1b**. Structures of the minor acids, **1a**, **1c**, **2a**, and **2b**, were deduced in a similar manner. The newly reported acids thus have phenyl-terminated saturated straight chains of 11, 13, and 15 carbon atoms, and monounsaturated straight chains

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of 13 and 15 carbon atoms. However, the position of the double bond in the two unsaturated acids was not determined.

The seeds of a total of 54 species representing 20 genera of Araceae have now been analysed. These genera, which constitute almost 19% of the total genera in the family, include representatives of all five of Grayum's [1] subfamilies of Araceae: seven from the Aroideae, five from the Calloideae, four from the Lasioideae, two from the Pothoideae and two from the Colocasioideae. 13-PTDA has thus far been found only in species of these seven genera of the subfamily Aroideae (*Amorphophallus*, *Arisaema*, *Arisarum*, *Arum*, *Biarum*, *Dracunculus* and *Pinellia*) which have been analysed. The amount present, expressed as a percentage of the total acids of the seed lipids, varies from almost 20% in *Arum* where nine of the 24 known species have been analysed, to *ca* 0.2% in *Amorphophallus*, where only one of the more than 100 known species has been analysed. Data are summarised in Table 1.

The presence of 13PTDA and the other newly reported acids in only the one subfamily (Aroideae) has a potential bearing on the phylogeny of the family. The presence of phenalenone pigments in all genera of the Haemodoraceae is cited as evidence that the family is monophyletic [2, 3]. The phenalenones are found singly or in various combinations in only this one family of higher plants and in some lower plants, four genera of Hyphomycetes and one genus of Discomycetes. In the Araceae, the apparently unique occurrence of 13PTDA in only one subfamily may constitute evidence, as one possibility, that the family is diphyletic. However, since this is an ongoing study, it is conceivable that future work may identify the new acids in genera of another subfamily or even in a closely related family.

EXPERIMENTAL

Materials. Mature fruits and/or seeds of various sp. of Araceae were acquired and stored in glass vials after wetting with a small amount of MeOH to prevent degradation until analysis could be carried out. They came from plants under cultivation in the experimental garden of V. G. Soukup, from plants maintained in other research collections or from collections in the wild by V. G. Soukup or other specialists in the Araceae. Seeds of the following taxa were analysed in this study: *Arisaema triphyllum*, *A. dracontium*, *A. stewardsonii*, *A. sikokianum*, *A. serratum v. serratum*, *A. ringens*, *f. sieboldii*, *A. angustata v. peninsulae*, *A. amurense*, *A. robustum*, *A. heterophyllum*, *A. erubescens*, *A. echinatum*, *A. yunnanense*, *A. aridum*, *A. auriculatum*, *A. flavum*, *A. jacquemontii*, *A. tortuosum*; *Arum italicum*, *A. maculatum*, *A. creticum*, *A. nigrum*, *A. alpinum*, *A. orientale*, *A. cyrenaicum*, *A. dioscoridis*, *A. pictum*; *Arisarum vulgare*; *Pinellia ternata*, *P. tripartita*, *P. pedatisecta*; *Dracunculus canariensis*, *D. vulgaris*; *Biarum dispar*; *Amorphophalus konjac*; *Anthurium barclayanum*, *A. acaule*, *A. wagenerianum*; *Peltandra virginica*, *P. sagittaefolia*; *Symplocarpus foetidus*; *Zantedeschia albomarginata*, *Z. rhemanii*; *Allocasia macrorhiza*, *A. odora*; *Colocasia gigantea*; *Calla palustris*; *Lysichiton americanum*; *Orontium aquaticum*; *Cyrtosperma ferox*; *Culcasia liberica*; *Nephthytis poisonii*; *Spathiphyllum phryniifolium*; *Epipremnum pinnatum*. Voucher specimens are deposited at CINC or the herbaria of other major universities or botanic gardens.

Table 1. Content of 13PTDA in seed oils from genera of the subfamily Aroideae (Araceae)

Genus	Number of species analysed	% total fatty acids Average	Range
Arum	9	18.3	20.3–12.2
Dracunculus	2	13.9	17.2–10.6
Arisaema	18	11.2	24.1–6.2
Pinellia	3	5.9	5.9–5.9
Biarum	1	5.7	6.8–4.5
Arisarum	1	2.8	4.1–2.0
Amorphophallus	1	0.2	0.2–0.2

Extraction and analysis. All traces of outer fruit tissues whether fleshy (as in *Arum*, *Arisaema*, etc.) or dry and papery (as in *Pinellia*) were removed and stored for separate analyses. Cleaned seeds were comminuted and then treated with BF_3–MeOH to convert acyl seed lipid acids to their corresponding Me esters which were analysed by GC as previously described [2].

GC-MS analyses of the Me esters from *Arum maculatum* were carried out using Me esters, TMSi esters and, after hydrolysis, the free acids. The GC-MS equipment had electronic pressure control and mass-selective detection. A 30 m HP-5 MS capillary column was programmed from 180–270° at 15° min^{-1} with an initial hold of 1 min and a final hold of 5 min. The carrier flow rate was 0.9 ml min^{-1} with split ratio of 80:1. The injection port was held at 230°, and the detector at 280°. The chromatogram was scanned from 40–500 mu at a rate of 3 scans sec^{-1} after a delay of 2.5 min. The Me ester of 13PTDA had a R_t of 7.3 min, compared with R_ts of 4.9 and 6.1 min for Me hexadecanoate and Me octadecanoate, respectively. The MS of 13PTDOMe had a base peak at m/z 91 (tropylium ion) and other prominent peaks at m/z 74 and 87. An apparent $[M]^+$ was found at 304. The corresponding TMSi ester had a R_t of 8.0 min. Again the base peak occurred at m/z 91, with a prominent $[M-15]^+$ ion at m/z 347 and a $[M]^+$ at m/z 362.

The free acid had a R_t of 7.5 min. The base peak was at m/z 91 with a large fragment at m/z 92; the apparent $[M]^+$ was at m/z 290. All of the spectra had a prominent ion at m/z 272, representing $[M-32]^+$ for the Me ester, $[M-90]^+$ for the TMSi ester and $[M-18]^+$ for the free acid. These data indicate a compound with a benzenoid ring, one carboxyl and a M_r of 290. 13PTDA (**1b**) fits these criteria and the struc-

ture fits the general long-chain nature of most plant lipid acids. When the chromatograms of the Me and TMSi esters were examined for other peaks with a major ion at m/z 91, small amounts of four homologues were found. These were identified, on the basis of R_ts relative to 13PTDA, the $[M]^+$ of the Me esters and $[M-15]^+$ ions of the TMSi esters as **1a**, **1c**, **2a** and **2b**.

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REFERENCES

1. Grayum, M. H., *Annals of the Missouri Botanical Gardens*, 1990, **77**, 628.
2. Cooke, R. G. and Edwards, J. M., *Fortschritte der Chemie Organischer Naturstoffe*, 1981, **40**, 158.
3. Simpson, M. G., *Annals of the Missouri Botanical Gardens*, 1990, **77**, 724.
4. Soukup, V. G. and Holman, R. T., *Phytochemistry*, 1987, **26**, 1015.