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CHARACTERIZATION OF THE LIPIDS OF SOME ORCHIDS

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Abstract—The lipids from leaves, spikes, pseudobulbs and roots from hybrid *Phalaenopsis*, *Cattleya* and *Cymbidium* have been characterized The fatty acids from total lipid extracts revealed the presence of significant proportions of odd chain saturated acids ranging from C_{15} to C_{23} in all three genera Leaf hydrocarbons of *Phalaenopsis* had high proportions of 31 and 33 carbon normal paraffins, whereas the major hydrocarbons from roots were 25, 27, 29 and 31 carbon normal paraffins Steroid esters from *Phalaenopsis* contained principally long chain saturated acids Analysis of phospholipids, fatty acids, triglycerides and steroid esters from *Phalaenopsis* revealed the presence of more than 30% saturated acids in each class. Leaf cuticle from *Phalaenopsis* was degraded and found to contain steroid residues, aromatic residues and principally saturated fatty acids ranging from C_{13} to C_{26}

INTRODUCTION

ORCHIDS are epiphytic monocotyledenous plants which have an unusual ability to store water. The reason for such ability to retain water, even in a dry atmosphere, is not known, but the waxy external surface may act as a barrier to moisture loss. A survey of the literature revealed only one reference to lipids of orchids and this described the oil secreted by the flowers of certain species of *Dendrobium*.[1]

Lipid analyses have been conducted by TLC upon the total lipids extracted from three genera of orchids, and the fatty acids isolated from total lipids were analyzed by GLC. Some of the major lipid classes of one variety have been further characterized by GLC analysis of their component fatty acids.

RESULTS

Lipid and Fatty Acid Composition of Phalaenopsis, Cattleya *and* Cymbidium

The content of extractable lipid was found to be, *Phalaenopsis* leaf 0·72%, root 0·73%, spike 1·30%; *Cattleya* leaf 0·7%, root 0·68%, pseudobulb 0·47%; and *Cymbidium* leaf 1·06%, root 0·64%.

The fatty acid compositions of the total lipid extracts of the three varieties of orchids are summarized in Table 1. As might be expected 16 0, 18 2 and 18 3 were the most abundant fatty acids. However, in contrast to most plants, the orchids contained odd chain fatty acids in significant amounts. Also striking was the presence of saturated fatty acids with chains longer than 20 carbon atoms.

Lipid fractionation revealed the expected hydrocarbons, steroid esters, triglycerides, free fatty acids, diglycerides, monoglycerides, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol.

[1] L. MÜLLER, *Ber. Deut Botan. Ges* **53**, 349 (1935)

TABLE 1 FATTY ACID COMPOSITION OF TOTAL LIPIDS FROM MAJOR PARTS OF THREE TYPES OF ORCHIDS

Fatty† acid	*Phalaenopsis*			*Cattleya*			*Cymbidium*	
	Leaves	Roots	Spikes	Leaves	Roots	Pseudo bulbs	Leaves and bulbs	Roots
15 0*	2 4 ± 0 5	4 9 ± 0 7	2 0 ± 0 2	1 8 ± 0 01	3 4 ± 0 1	2 0 ± 0 5	2 1 ± 0 4	3 2 ± 0 6
16 0	16 5 ± 3 4	14 4 ± 1 2	17 0 ± 9 0	13 4 ± 2 4	11 4 ± 1 6	16 7 ± 2 0	20 6 ± 8 4	16 9 ± 2 3
16 1		1 4 ± 0 4			1 2 ± 0 1	1 0 ± 0 3		
17 0	1 9 ± 0 1	2 2 ± 0 2	2 5 ± 0 2	1 8 ± 0 3	2 6 ± 0 4	2 2 ± 0 5	2 0 ± 0 7	2 5 ± 0 5
18 0	4 4 ± 0 8	6 0 ± 1 6	5 1 ± 0 3	8 9 ± 0 5	6 5 ± 1 2	6 6 ± 0 8	4 9 ± 1 6	4 2 ± 1 0
18 1	6 2 ± 1 5	8 8 ± 1 5	7 9 ± 1 9	4 0 ± 0 3	2 9 ± 0 3	4 5 ± 0 7	4 2 ± 1 4	3 3 ± 1 1
19 0		0 9 ± 0 5		0 3 ± 0 1	1 0 ± 0 2	0 5 ± 0 2		0 3 ± 0 2
18 2	12 9 ± 0 8	28 1 ± 8 7	27 4 ± 1 0	25 0 ± 1 3	34 7 ± 5 7	36 5 ± 3 2	25 1 ± 1 9	51 1 ± 7 3
20 0	0 9 ± 0 2	3 7 ± 1 3	0 8 ± 0 1	1 1 ± 0 2	10 1 ± 1 1	3 2 ± 0 9	0 7 ± 0 1	2 2 ± 0 8
18 3	48 7 ± 5 8	12 9 ± 2 0	28 2 ± 3 5	38 7 ± 2 2	13 1 ± 2 6	19 7 ± 3 9	26 6 ± 9 8	4 8 ± 1 9
21 0		0 5 ± 0 2			0 9 ± 0 1	0 3 ± 0 1	0 3 ± 0 1	0 3 ± 0 1
20 2	0 1 ± 0 1			0 1 ± 0 1	0 6 ± 0 04	0 8 ± 0 3	0 6 ± 0 1	0 4 ± 0 1
22 0	0 4 ± 0 1	4 4 ± 2 3	1 5 ± 1 7	0 4 ± 0 1	4 5 ± 0 4	1 4 ± 0 2	2 1 ± 0 4	1 8 ± 0 7
23 0	0 6 ± 0 2	1 4 ± 0 5	0 8 ± 0 1	0 2 ± 0 02	1 2 ± 0 03	0 7 ± 0 1	0 6 ± 0 1	1 0 ± 0 4
24 0	1 3 ± 0 2	6 4 ± 1 0	2 9 ± 1 8	0 6 ± 0 1	3 4 ± 0 3	1 4 ± 0 2	5 3 ± 2 1	3 0 ± 1 0

* The first number indicates carbon chain length, the second the number of double bonds

† Acids are listed in order of emergence from the GLC column Minor unidentified components are not listed Values are averages of analyses of 3 specimens and are % of total fatty acids

Analyses of Lipid Classes of Phalaenopsis

The hydrocarbons ranged from C_{18} to C_{34} (see Table 2) Analysis of a blank extract using comparable quantities of solvents gave no evidence of such compounds The peculiarity of the patterns of hydrocarbons and the differences observed between parts of the plants also suggest that contamination by petroleum hydrocarbons possibly present in solvents was minimal The major hydrocarbons of roots were C_{25}, C_{27}, C_{29} and C_{31}, however, the major hydrocarbons from leaves and spikes were C_{31} and C_{33}

The leaf hydrocarbons formed an homologous series of unit equivalent chain length (ECL) values in gas chromatographic analysis, suggesting that most of the hydrocarbons were normal paraffins The structures of the hydrocarbons of ECL value 26·5, 30 2 and 33 4 could not be elucidated because their abundances were too low for recording of useful mass spectra Mass spectra of the major components confirmed their identification to be normal paraffins

The substance having the same R_f as a steroid ester standard underwent color changes expected of steroids when it was sprayed with sulfuric acid and heated The mass spectrum of the composite steroid esters revealed acyl ions (RCO^+) corresponding to each major fatty acid in the GLC analysis In addition, a spectrum characteristic of a steroid was observed, and the prominent ions at *m/e* 396, 278, 255 and 213 suggest that the major steroid residue in the esters is a sitosterol isomer

The fatty acid composition of four major lipid classes was determined in the case of *Phalaenopsis* (Table 3) All odd chain saturated homologs from 13 0 to 25 0 were found, and odd chain acids were found in all lipid classes analyzed The total odd chain acids amounted to 9 8% for root phospholipids, 12 0% for root free fatty acids, 8·7% for root triglycerides and 10 2% for root steroid esters The presence of odd chain acids in root

TABLE 2 HYDROCARBONS OF *Phalaenopsis Mariposa*

Carbon atoms (ECL*)	Leaf (%)	Root (%)	Spike (%)
18		0 6 ± 0 8	1 4 ± 1 4
19		0 7 ± 0 4	0 7 ± 0 5
20		5 6 ± 7 9	4 0 ± 3 6
21		3 3 ± 3 0	0 8 ± 0 7
22	0 09 ± 0 08	0 7 ± 0 5	0 7 ± 0 5
23	0 08 ± 0 03	4 3 ± 1 0	0 4 ± 0 4
24	0 14 ± 0 04	2 8 ± 0 7	0 8 ± 0 6
25	0 27 ± 0 06	23 9 ± 7 5	1 6 ± 1 0
26	0 18 ± 0 05	4 3 ± 0 1	2 1 ± 1 6
26 5	0 04 ± 0 03	1 1 ± 0 9	0 2 ± 0 3
27	0 6 ± 0 05	20 1 ± 4 7	3 7 ± 1 8
28	0 3 ± 0 04	3 0 ± 2 0	3 5 ± 2 8
29	3 5 ± 0 3	6 0 ± 3 2	9 2 ± 4 6
30	2 9 ± 0 2	5 7 ± 5 1	2 0 ± 0 9
30 2	0 7 ± 1 0		
31	52 0 ± 3 0	9 4 ± 7 4	38 2 ± 5 8
32	9 3 ± 0 1	0 8 ± 1 1	9 7 ± 2 5
33	28 9 ± 2 5	0 5 ± 0 7	13 2 ± 1 7
33 4	0 3 ± 0 5		0 8 ± 1 2
34	0 3 ± 0 5		
> 34	Not detectable even in overloaded GLC		

* Equivalent chain length

lipids might be attributable to contamination of the roots with microorganisms which are known to be rich in odd chain acids. However, the presence of these acids in other plant tissues (12 8% in leaf steroid esters) suggests that they have either been produced metabolically by the plant or were absorbed by the roots and translocated Some orchids are known to be permeated by symbiotic fungi, and this might explain the unusual content of odd chain acids (see also Table 1)

The contents of saturated fatty acids in the four lipid classes analyzed were found to be 30% or more of the total fatty acids. In fact, the fatty acids present in the steroid esters of roots and leaves were 65 8 and 56 7% saturated acids, and the average chain length of saturated acids was longer in the steroid esters than in the other lipid classes

Cuticle from Phalaenopsis *Leaves*

Mass spectral analysis of all 13 fractions was attempted The mass spectrum of the most mobile fraction was found to be that of an unidentified steroid. The second fraction was methyl esters of predominantly saturated fatty acids Gas chromatographic analysis gave the following composition 13 0, 2 8%, 14 0, 3 8, 15 0, 0 6, 16 0, 9·1, 17 0, 1·6, 18 0, 5 1, 18 1, 3 7; 19 0, 0 9, 20 0, 17·5, 22 0, 6 6, 23 0, 7 9, 24 0, 22 2, 25 0, 3 6, 26 0, 11·8. This composition differs considerably from the analyses made of cuticle of other plant families,[2] in which the dominant fatty acids are of shorter chain length and of greater unsaturation. None of the remaining fractions had spectra consistent with the methylated

[2] G EGLINTON and D H HUNNEMAN, *Phytochem* 7, 313 (1968)

TABLE 3 FATTY ACID COMPOSITION OF LIPID CLASSES OF *Phalaenopsis mariposa*

	12 0	13 0	14 0	15 0	16 0	16 1	17 0	16 2	18 0	18 1
Phospholipids										
Leaf mean			0 2	1 7	18 4	0 1	1 9	0 1	5 0	6 2
± SD			± 0 01	± 0 4	± 3 2	± 0 1	± 0 1	± 0 02	± 1 2	± 1 5
Root mean	0 2	0 1	0 3	5 0	15 6	1 1	2 4	0 3	6 7	9 3
± S D	± 0 1	± 0 1	± 0 1	± 0 7	± 1 3	± 0 1	± 0 9	± 0 2	± 0 9	± 1 9
Spike mean		0 2	0 3	1 6	18 3	0 4	2 6	0 5	6 5	7 5
± S D		± 0 2	± 0 3	± 0 3	± 6 4	± 0 6	± 0 6	± 0 3	± 1 8	± 1 0
Fatty acids										
Leaf mean		1 6	1 5	2 2	15 9	1 4	2 3		6 5	6 8
± S D		± 0 6	± 0 8	± 0 6	± 1 9	± 0 4	± 0 2		± 0 9	± 0 6
Root mean		0 7	1 2	4 2	13 1	2 0	2 2	0 5	6 0	11 7
± S D		± 0 6	± 0 4	± 0 4	± 2 7	± 0 4	± 0 2	± 0 4	± 1 4	± 3 8
Spike mean	0 7	0 2	1 4	2 2	19 9	1 2	2 6	0 3	6 4	8 6
± S D	± 0 4	± 0 1	± 0 5	± 0 7	± 4 6	± 0 5	± 0 8	± 0 1	± 1 4	± 0 7
Triglycerides										
Leaf mean		0 2	0 1	2 5	16 9	1 9	2 5	0 2	7 5	14 3
± S D		± 0 2	± 0 1	± 0 4	± 2 6	± 0 2	± 0 4	± 0 2	± 0 3	± 2 3
Root mean		1 0	2 0	2 5	10 8	3 4	1 1	0 4	6 0	11 4
± S D		± 1 3	± 0 8	± 1 0	± 3 2	± 2 0	± 0 1	± 0 2	± 3 2	± 3 7
Spike mean		0 2	3 0	2 3	18 0	3 3	1 9	0 3	7 7	11 3
+ S D		± 0 3	± 0 3	± 1 1	± 0 8	± 0 1	± 0 1	± 0 2	± 0 7	± 2 7
Steroid esters										
Leaf mean	8 3	1 2	3 6	2 5	10 5	1 2	0 9	1 6	4 7	8 9
± S D	± 2 0	± 1 1	± 1 1	± 0 2	± 1 5	± 0 3	± 0 6	± 0 4	± 1 9	± 0 8
Root mean	1 9	0 2	1 6	1 9	7 0	2 5	0 7	0 9	8 5	5 5
± S D	± 1 9	± 0 2	± 0 4	± 0 3	± 2 8	± 0 7	± 0 3	± 0 5	± 5 8	± 1 3
Spike mean	2 2	T	1 3	1 4	17 8	2 1	1 1	0 9	3 1	8 5
± S D	± 1 6		± 0 2	± 0 5	± 6 1	± 2 0	± 0 9	± 0 2	± 1 1	± 1 5

	19 0	18 2	20 0	18 3	21 0	20 2	22 0	23 0	24 0	25 0
Phospholipids										
Leaf mean	0 1	11 0	0 1	52 6	0 5	0 2	0 3	0 4	0 8	0 3
± S D	± 0 04	± 1 3	± 0 1	± 1 2		± 0 1	± 0 2	± 0 2	± 0 03	± 0 3
Root mean	0 6	37 7	0 9	13 2	0 2	0 6	1 2	1 5	2 9	
± S D	± 0 1	± 5 2	± 0 1	± 3 7	± 0 1	± 0 01	± 0 4	± 0 3	± 0 2	
Spike mean	0 3	26 0	0 5	29 0	0 8	1 0	0 2	0 2	1 5	1 3
± S D	± 0 1	± 1 9	± 0 2	± 4 0	± 0 6	± 0 6	± 0 2	± 0 1	± 0 3	± 0 9
Fatty acids										
Leaf mean		12 3	1 8	42 8	0 3	0 3	1 1	0 5	1 9	
± S D		± 1 1	± 1 1	± 3 4	± 0 3	± 0 4	± 0 8	± 0 6	± 1 8	
Root mean	0 9	25 2	3 8	8 3	0 6	0 4	5 6	2 3	6 1	1 1
± S D	± 0 2	± 6 9	± 2 1	± 2 1	± 0 4	± 0 1	± 2 0	± 0 7	± 1 7	± 0 8
Spike mean	0 3	24 8	0 7	23 9	0 1	0 5	1 1	1 6	2 3	
± S D	± 0 0	± 3 6	± 0 2	± 3 3	± 0 1	± 0 1	± 0 1	± 0 5	± 0 5	
Triglycerides										
Leaf mean	0 7	14 8	1 5	32 2	0 3	0 4	0 7	0 6	0 8	
± S D	± 0 2	± 3 4	± 0 1	± 2 2	± 0 04	± 0 1	± 0 1	± 0 2	± 0 2	
Root mean	1 7	33 4	2 6	16 2	0 6	0 4	1 9	1 8	1 4	
± S D	± 1 5	± 9 8	± 1 5	± 7 1	± 0 4	± 0 2	± 2 0	± 1 8	± 1 1	
Spike mean	0 3	22 6	1 2	17 6	0 7	0 6	1 6	2 5	1 6	
± S D	± 0 3	± 0 8	± 0 7	± 2 8	± 1 0	± 0 5	± 0 1	± 1 0	± 1 0	
Steroid esters										
Leaf mean	0 7	13 9	7 6	14 2	1 9		3 6	3 6	5 6	2 0
± S D	± 0 1	± 4 3	± 0 9	± 3 8	± 0 8		± 1 1	± 1 3	± 1 8	± 2 8
Root mean	3 5	17 5	29 0	5 3	1 1	0 3	3 8	2 1	3 8	0 7
± S D	± 1 2	± 2 8	± 6 9	± 1 6	± 0 4	± 0 1	± 0 6	± 0 5	± 1 0	± 1 0
Spike mean	0 6	32 1	4 6	12 4	0 8		3 8	4 2	3 4	
± S D	± 0 4	± 3 8	± 1 2	± 0 7	± 0 6		± 0 8	± 1 9	± 2 1	

* Acids are listed in the order of emergence from the gas chromatograph Minor unidentified components are deleted Values are averages of analyses of 3 specimens and are % of total fatty acids

hydroxy acid structures which might be expected from a cross-linked ester polymer [3] Two fractions had mass spectra indicating the presence of aromatic nuclei

EXPERIMENTAL

Three specimens each of *Phalaenopsis* Mariposa (Goleta × Grace Palm), *Cattleya* Paula Hausermann (*C* Ethel leder × *C*. Bow Bells) and *Cymbidium* Tom Thumb (*Cym pumilum* × *Cym* Coronado) were obtained from Hausermann's Orchids, Elmhurst, Illinois The *Phalaenopsis* plants were seedlings in bud, and the *Cymbidium* and *Cattleya* were meristem propagations approaching flowering stage Hybrids were used to assure that replicates were as close to identical as possible

The entire *Phalaenopsis* plants were used for analysis, whereas backbulbs of the *Cattleya* and *Cymbidium* were eliminated Each plant was divided and the parts were weighed and analyzed separately The tissues were extracted with $CHCl_3$–MeOH (2 1) and with MeOH–$CHCl_3$ (2 1) in an Omnimixer. The combined extracts were washed with H_2O, and the H_2O phase was extracted with $CHCl_3$ The combined $CHCl_3$ phases were evaporated under reduced pressure at less than 40° The extracts were weighed and taken up in $CHCl_3$ and stored at − 20° until used The total lipid extracts were converted to methyl esters, which were analyzed by GLC for their component fatty acids

The total lipid extracts from *Phalaenopsis* were separated into their component lipid classes by TLC. Light petroleum (b p 30–60°)–Et_2O–HOAc (80 20 1) was used to separate low polarity lipids, free fatty acids, diglycerides, monoglycerides and high polarity lipids The low polarity lipids were rechromatographed using light petroleum–Et_2O (95 5) twice to separate hydrocarbons, steroid esters and triglycerides. The high polarity lipids were rechromatographed with Et_2O alone, to isolate the phospholipids The phospholipids were analyzed by two dimensional TLC using $CHCl_3$–MeOH–28% aq NH_3 (65 35 5) and using $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (100 40 20 20 10)

Leaf cuticle was prepared as follows Three leaves of *Phalaenopsis* were homogenized 3 × in H_2O, and the H_2O extracts discarded The pulp was extracted with $CHCl_3$–MeOH (2 1), followed by (1 2) to remove all extractable lipids The dried pulp was treated with BF_3–MeOH at 90° for 4 hr to effect a methanolysis of bound or polymerized lipids The released lipids were extracted with light petroleum followed by $CHCl_3$, and the combined extracts were washed with H_2O The components were separated on TLC using two developments with petroleum–Et_2O (95 5) to yield five main fractions

The material remaining at the origin was separated on TLC using light petroleum–Et_2O–HOAc (70 30 1) yielding five additional components, and with light petroleum–Et_2O–HOAc (20 80 1) yielding three more components

Methanolysis (BF_3–MeOH) of the total lipids, steroid esters, triglycerides, free acids and total phospholipids yielded methyl esters of their component fatty acids Hydrocarbons and methyl esters were analyzed by GLC using a 200 cm × 6 mm o d aluminum column packed with 20% ethylene glycol succinate on Anachrom ABS, 80–100 mesh, and operated at 204°.

Mass spectra were recorded using an Hitachi-RMU6D single-focusing magnetic scanning mass spectrometer at 70 eV, using either the direct sample insertion system or the gas chromatographic inlet. In the latter case, the column was 137 cm × 6 mm, packed with 10% SP 1000 on acid washed Gas Chrom W, 80–100 mesh, or 100 cm × 6 mm packed with 1% SP1000 on Gas Chrom P, 60–80 mesh

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[3] P E KOLATTUKUDY, *Biochem Biophys Res Commun* **41**, 299 (1970)

Key Word Index—*Phalaenopsis*, *Cattlega*, *Cymbidium*, Orchidaceae, orchids; lipids; odd-chain fatty acids, hydrocarbons