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LIPID AND STEROL COMPOSITION OF THE POLLEN OF THE WEST AFRICAN OILPALM, *ELAEIS GUINEENSIS*

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Key Word Index—*Elaeis guineensis*; Palmae; oilpalm; lipids; free fatty acid; sterols; steryl esters; hydrocarbons.

Abstract—The lipid classes, fatty acid methyl esters and the sterols of oilpalm pollen were analysed. The neutral lipid fraction consisted of triglycerides, esterified and free sterols and trace amounts of hydrocarbons. Monogalactosyl and digalactosyl diglycerides, phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine represented the polar lipids. The major fatty acids were linoleic, palmitic and linolenic acids together with small to trace amounts of oleic, stearic, arachidic, myristic, lauric, palmitoleic and margaric acids. Unsaturated fatty acids predominated over saturated ones in the ratio of 3:2. The 4-desmethyl sterols were the major phytosterols in the free form but they constituted a lower proportion of the sterols in the esterified state. 28-Isofucosterol was isolated and characterized as the principal sterol.

INTRODUCTION

The earliest reports on the lipid composition of pollens are those of Anderson [1, 2] after which, sporadic reports appeared which showed that pollens are rich in lipid components [3–11]. With the development of more precise chromatographic techniques for lipid analyses, less work of this nature has been reported [12–15] and instead attention has focussed on the sterols of pollens [16–22]. This seemingly one-sided investigation was almost entirely due to the finding of 24-methylenecholesterol [23, 24] and oestrone [25] in pollens. Interest in the chemistry and biochemistry of pollens has been sustained from four points of view. Pollen is important firstly as the carrier of the male genetic material, secondly as an essential food for honey bees, thirdly for man as the source of many serious allergies and fourthly for its medicinal value.

Studies [26] on the dynamics of the fats, starch and protein in sunflower pollen showed that these stored compounds were good diagonistic features of sterile and fertile forms of pollen. It is known that good fruit-set and high crop yield depend to a great extent on viable pollen. Germination, which is a good test of viability, depends upon, among other things, the food store of the tissue involved. During germination the stored food is mobilized for growth and development. This report describes part of a detailed study of the chemical composition of the pollen of the oilpalm, *Elaeis guineensis*, which it is intended will contribute to a better understanding of the problem of pollen viability in this plant.

RESULTS

TLC showed that the pollen neutral lipids of *Elaeis guineensis* consisted of triglycerides, free sterols, steryl esters and small amounts of hydrocarbons. Monogalactosyl and digalactosyl diglycerides, phosphatidyl inositol, phosphatidyl choline and phosphatidyl ethanolamine constituted the polar lipids. The fatty acid composition of both the total lipids and the individual lipid classes is shown in Table 1. The major fatty acids were linoleic, palmitic and linolenic acids, with small to trace amounts of oleic, stearic, arachidic, lauric, myristic, palmitoleic and margaric (C_{17})

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Table 1. Fatty acid composition of *Elaeis guineensis* pollen lipids

	Fatty acid									
	12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0
RR_t*	1	1·8	3·4	4·2	4·7	6·3	7·5	10·0	13·8	12·2
Triglycerides	t	0·6	29·9	0·9	t	12·3	7·7	25·8	18·1	4·3
Polar lipids†	0·6	0·8	41·3	t	t	5·5	10·8	23·5	16·1	0·8
Steryl esters		t	13·8		t	2·2	15·3	52·7	14·7	1·2
Total lipids	0·2	0·5	27·1	0·3	0·3	6·0	6·9	35·4	20·9	2·3

* On PEGS at 166°. † The polar lipids comprise phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, monogalactosyl and digalactosyl diglycerides.

acids. One notable feature was the large proportion of unsaturated fatty acids in the pollen lipids which thus differ from the palm mesocarp and kernel oils [27]. There was no significant difference in composition between the fatty acids of the triglycerides and those of the polar lipids. On the other hand, the fatty acid pattern of the steryl esters was markedly different from those of the other lipid fractions. Linoleic acid was the major fatty acid (up to 52%) and on the whole, over 80% of the fatty acids of this fraction were unsaturated. This high content of unsaturated acids certainly influenced the overall proportion of unsaturated fatty acids found in the total lipid extract.

The major free sterols were the 4-desmethyl sterols with smaller amounts of the 4,4-dimethyl and 4,α-methyl sterols. The composition of the 4-desmethyl sterols, after purification by TLC, is shown in Table 2. The identification of the principal component as 28-isofucosterol was based on GC-MS and the GLC RRt on OV-17. The MS had a parent ion at *m/e* 412 indicating a C_{29} phytosterol, and fragmentation ions at *m/e* 397 (M^+-Me), 394 (M^+-H_2O), 379 (M^+-Me − H_2O), 314 [M^+-part of side chain (C_7H_{14})], 299 (M^+-C_7H_{14} − Me), 296 (M^+-C_7H_{14} − H_2O). This is identical to the reported MS of 28-isofucosterol [28]. The other sterols (Table 2) were identified by co-chromatography on GLC with authentic sterol samples.

An unusually high concentration of esterified sterols was found in the lipid extract. On TLC these ran as a single band with the hydrocarbons near the solvent front. Following saponification the esterified sterols were found to contain more 4,4-dimethyl and 4,α-methyl sterols and less 4-desmethyl sterols than were present in the free sterols. The composition of the esterified 4-desmethyl sterols was clearly different from that of the free 4-desmethyl sterols (Table 2). Whereas 28-isofucosterol was the most abundant sterol in the free form, sitosterol was the major sterol in the esterified state. Similar relationships between the esterified and free sterols have been reported [29] for pea and maize seedlings and it was concluded that the free and esterified sterols do not occur in a state of simple equilibrum.

DISCUSSION

The amount of lipid in the pollen varies according to the plant family. Todd and Bretherick [3]

Table 2. Composition of the major 4-desmethyl sterols of *Elaeis guineensis* pollen

Sterols	RR_t*	Composition (%) Free sterols	Esterified sterols
Cholesterol	1·0	1·5	10·5
Campesterol	1·3	25·0	11·3
Stigmasterol	1·4		6·1
Sitosterol	1·6	13·8	37·3
28-*Iso*fucosterol	1·8	56·3	34·8
Unidentified	2·0	3·3	

* RR_t with respect to cholesterol on 3% OV-17.

reported that extracts of *Taraxacum dens leonis* (dandelion) pollen yielded 14% of total lipids and mustard species contained 8–10%. Sosa and Sosa-Bourdouil [7, 10] found a lipid content of 10% in hazel (*Coryllus avellana*) pollen and Standifer [12] reported 18·9% for dandelion pollen. In this study a comparative figure of 23·3% was found for the total lipid extract of oilpalm pollen. Sassan [30] studying the fine structure of germinated *Petunia* pollen reported large lipid droplets in the mature pollen grain which disappeared as germination progressed. Weight for weight, lipids yield more energy than either carbohydrates or proteins. It therefore seems logical that the highly reduced male gametophyte of flowering plants should possess lipids as a major storage product. It is only in this way that enough nutrient can be stored in a limited space to tide the pollen over the period between germination and fertilization.

Lipid has a function in metabolism and is involved in the production of membranes in rapidly growing cells. This could explain the presence in pollen of phospholipids and galactolipids which are membrane lipids.

The cyclitols are an interesting group of compounds that are related metabolically to sugars. Myoinositol frequently occurs in pollens as a major component or in a combined form as phosphatidyl inositol [31]. During germination increases in inositol occur as the phospholipid is hydrolysed [31]. In pollen, inositol can be incorporated into pectins during germination [32, 33].

Fatty acids are very common in pollen and on the whole they show a very high degree of unsaturation with linoleic or oleic, palmitic and linolenic acids as the major fatty acids [14, 15, 34]. As a storage compound, the functional value of pollen lipid is thus enhanced. The predominance of unsaturated fatty acids (more than 60%) over the saturated ones might serve to keep the pollen light which in turn could aid in dispersal. Secondly unsaturated fatty acids could act as potential precursors for the synthesis of aromatic compounds which are found as attractants in pollen [34, 35].

Analyses of the sterols from the pollen of different plants have so far revealed no one principal phytosterol in pollens. Pollen from thirteen species of plants showed a series of C_{27}, C_{28} and C_{29} phytosterols. 24-Methylenecholesterol was the major sterol in four, sitosterol in six, cholesterol in two and stigmasterol in one [17, 18, 22]. In this study 28-isofucosterol was found as the principal phytosterol of the oilpalm pollen. Biosynthetically the sterols mentioned above are related and correspond to successive methylations by *S*-adenosylmethionine [19, 36, 37]. In pollens the accumulation of one sterol rather than another must be dependent upon the varying importance of different metabolic pathways.

EXPERIMENTAL

Pollen analysed in this study was obtained from tenera and dura varieties of *Elaeis guineensis*, hand-collected and received vacuum-sealed from Nigeria. There was no significant difference in the lipid composition of the pollen from different varietal forms of this plant. Pollen samples were homogenised in a mortar and lipids extracted with $CHCl_3$–MeOH (2:1) at room temp.

TLC and PLC (0·5 mm) was on Si gel HF developed with Et_2O–petrol (3:7). Lipids were transmethylated [38] and the fatty acid methyl esters were analysed by GLC on 10% PEGS (2 m × 6 mm i.d.) at 166° and a N_2 flow rate of 60 ml/min. Sterols in the free form were analysed on 3% OV-17 at 266°. The presence of 28-isofucosterol was established by GC-MS at 25 eV and using a 3% OV-17 column.

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REFERENCES

1. Anderson, R. J. and Kulp, W. L. (1922) *J. Biol. Chem.* **50,** 433.
2. Anderson, R. J. (1923) *J. Biol. Chem.* **55,** 611.
3. Todd, F. E. and Bretherick, O. (1942) *J. Econ. Entomol.* **35,** 312.
4. Kiesel, A. and Rubin, B. (1929) *Z. Physiol. Chem.* **182,** 241.
5. Vivino, A. E. and Palmer, L. S. (1944) *Arch. Biochem.* **4,** 129.
6. Ridi, M. S. and Aboul-Wafa, M. H. (1950) *J. Roy. Egypt Med. Assoc.* **33,** 168.
7. Sosa-Bourdouil, C. and Sosa, A. (1954) *Bull. Soc. Chim. Biol.* **36,** 393.
8. Sekine, H. and Li, T. (1942) *Rept. Jap. Assoc. Adv. Sci.* **16,** 563.
9. Mariella, R. P., Bernstein, T. B. and Mosher, A. L. (1952) *Bol. Col. Quim. Puerto Rico* **9,** 15.
10. Sosa, A. and Sosa-Bourdouil, C. (1952) *C. R. Acad. Sci.* **235,** 971.
11. Avivi, E. and Leibowitz, J. (1960) *Bull. Res. Council Israel* **9A,** 157.
12. Standifer, L. N. (1966) *J. Apicult. Res.* **5,** 93.
13. Scott, R. W. and Strohl, M. J. (1962) *Phytochemistry* **1,** 189.
14. Ching, T. M. and Ching K. K. (1962) *Science* **138,** 890.
15. Standifer, L. N. (1966) *Ann. Entomol. Soc. Am.* **59,** 1005.
16. Barbier, M., Hugel, M. F. and Lederer, E. (1960) *Bull. Soc. Chim. Biol.* **42,** 91.

17. Hugel, M. F., Vetter, W., Audier, H., Barbier, M. and Lederer, E. (1964) *Phytochemistry* **3,** 7.
18. Devys, M. and Barbier, M. (1965) *Compt. Rend.* **261,** 4901.
19. Barbier, M. (1966) *Ann. Abeille* **9,** 243.
20. Devys, M. and Barbier, M. (1966) *Phytochemistry* **5,** 1031.
21. Devys, M. and Barbier, M. (1967) *Compt. Rend.* **264,** 504.
22. Standifer, L. N., Devys, M. and Barbier, M. (1968) *Phytochemistry* **7,** 1361.
23. Barbier, M. and Schindler, O. (1959) *Helv. Chim. Acta* **42,** 1998.
24. Barbier, M., Reichstein, T., Schindler, O. and Lederer, E. (1959) *Nature* **184,** 732.
25. Ridi, M. S., Mofty, A., Khalifa, K. and Soliman, L. (1960) *Z. Naturforsch.* **15B,** 45.
26. Pirev, M. N. (1966) *IZV Akad. Nauk. Mold SSR Ser. Biol. Khim. Nauk.* **11,** 91.
27. Hilditch, T. P. and Williams, P. N. (1964) *The Chemical Constitution of Natural Fats* 4th ed. Chapman and Hall, London.
28. Gibbons, G. F., Goad, L. J. and Goodwin, T. W. (1968) *Phytochemistry* **7,** 983.
29. Kemp, R. J., Hamman, A. S. A., Goad, L. J. and Goodwin, T. W. (1968) *Phytochemistry* **7,** 447.
30. Sassan, M. A. (1964) in *Pollen Physiology and Fertilization* (Linskens, H. F., ed.). North-Holland, Amsterdam.
31. Stanley, R. G. (1971 in *Pollen Development and Physiology* (Heslop-Harrison, J. ed.) Butterworths, London.
32. Stanley, R. G. and Loewus, F. L. (1964) in *Pollen Physiology and Fertilization* (Linskens, H. F. ed.). North-Holland, Amsterdam.
33. Kroh, M. and Loewus, F. L. (1968) *Science* N.Y. **160,** 1352.
34. Lepage, M. and Boch, R. (1968) *Lipids* **3,** 530.
35. Opute, F. I. (1975) *J. Exp. Botany* (In press).
36. Villanueva, V. R., Barbier, M. and Lederer, E. (1964) *Bull. Soc. Chim. Fr.* 1423.
37. Lederer, E. (1964) *Biochem. J.* **93,** 449.
38. Feldman, G. L., Johnson, H. T., Culp, T. W. and Gowan, R. H. (1962) *Poultry Science* **41,** 418.