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WAX ESTERS AND TRITERPENE METHYL ETHERS FROM THE EXOCARP OF *ELAEIS GUINEENSIS*

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Key Word Index—*Elaeis guineensis*; Palmae; oil palm fruit; wax esters; triterpene methyl ethers; cylindrin; fatty acids and fatty alcohols.

Abstract—The wax esters isolated from the exocarp extract of oil palm fruit *Elaeis guineensis* were shown by GC and GC-MS analysis to be composed of mainly even numbered homologous esters between C_{40} and C_{60}. Methanolysis revealed that the fatty acid and fatty alcohols of these wax esters have carbon chain length ranging from C_{16} to C_{28} and C_{16} to C_{34}, respectively, as determined by GC and GC-MS. Two pentacyclic triterpene methyl ethers, viz. cylindrin and its Δ^{12}-isomer, were also isolated, the former being the dominant triterpenoid component. Traces of wax esters (5 ppm) in crude palm oil were also detectable.

INTRODUCTION

The commercial oil palm *Elaeis guineensis* is a monocotyledon in the family Palmae. It has three main fruit forms (or more loosely, three varieties) viz. pisifera, dura and tenera. Tenera, a hybrid between dura and pisifera, is the best in terms of oil yield and the most popular form for cultivation. The fruit is a drupe which is borne on a large compact bunch. The pericarp of the fruit consists of the outer exocarp, the fleshy mesocarp (oil bearing tissue) and the endocarp (shell).

Wax esters have not been reported in palm oil but the fatty alcohols of the unsaponifiables have been reported to be as high as 160 ppm [1]. In other oils, such as those from linseed, corn, soybean and sunflower [2], waxes were reported to be derived from the seed coat and if present in sufficient quantities, they can cause turbidity to the refined oil as was reported for sunflowerseed oil [3].

This paper reports on the composition and structures of wax esters and triterpene methyl ethers, isolated from the outer exocarp of the oil palm fruit.

RESULTS AND DISCUSSION

Extraction of the outer exocarp of fresh palm fruits (2.6 kg) and chromatographic separations gave 4.2 mg of pure wax esters. This represents only *ca* 1.6 ppm of the fresh weight of the palm fruits which is an extremely low level when compared to those of other plant waxes [4]. The amount of wax esters in crude palm oil (CPO) was separately estimated to be 5 ppm of the weight by GC. The wax esters isolated together with triterpene components from palm fruit exocarp are intact molecules and thus can be analysed by mass spectrometry to provide their molecular formulae. The mass spectrum of the wax esters revealed that they comprise an homologous series with carbon chain lengths ranging from C_{40} to C_{60} and that the even carbon homologues form the major components with only minor amounts of homologues with an odd carbon number. Table 1 lists the wax esters present and the percentage composition as estimated by GC. The molecular ions $[M]^+$ derived from the mass spectrum indicated that the wax esters have the general molecular formula of $C_nH_{2n}O_2$ with $n = 40$–60. Thus, it is concluded that these wax esters are esters of both saturated fatty acids and fatty alcohols. This fact is also verified by ^{1}H NMR where olefinic protons were not detectable. The ^{1}H NMR data of the wax esters show triplets at δ4.05 ppm and 2.29 ppm which clearly indicate the presence of ester linkage with the former triplet attributed to $-CH_2OCO-$ protons and the latter to $-CH_2COO-$ protons. The mass spectrum of the wax ester mixtures also shows an intense fragment ion of m/z 257 which is characteristic of a C_{16} (palmitic) acid derivative attribu-

Table 1. Composition of wax esters of palm fruit exocarp

C	$[M]^+$	Peak (%)* area	C	$[M]^+$	Peak (%)* area
40	592	1	50	732	11
42	620	3	52	760	10
44	648	6	54	788	9
46	676	28	56	816	8
48	704	18	58	844	6

*$[M^+]$ by MS. Peak areas by GC. Minor odd-carbon homologues C_{43} to C_{55} are detectable in trace amounts ($< 1\%$).

table to the $[C_{15}H_{31}CO_2H_2]^+$ fragment ion. The observation of this type of fragment ion also indicated that the alcohol portion of the ester forms the larger part of the weight of the wax ester molecules [5]. From the mass spectrum, the wax esters are concluded to be of a straight-chain type as no fragment ion due to the cleavage of branched-chains was observed. Table 2 lists the composition of fatty acids and *n*-alkanols of the wax ester mixture after methanolysis. The fatty acids have carbon chain length of C_{16}–C_{28}. The C_{16} acid is the major acid component of the wax esters which supports the observation of the intense *m/z* 257 fragment ion in the mass spectrum of the intact wax esters. The *n*-alkanols possess a much wider carbon chain length which ranged from C_{16} to C_{34}; C_{16} and the even-carbon components between C_{28} and C_{34} dominated the fatty alcohol composition which again was verified by the mass spectrum of the intact wax esters.

Triterpenoids were co-extracted together with the wax esters and the major components, triterpene methyl ethers, **1** and **2** were isolated by preparative TLC. The ^{13}C NMR data of **1** (Table 3) showed good agreement with those values reported for cylindrin [6]. The mass spectrum of **1** showed a strong $[M]^+$ at *m/z* 440 and also other characteristic fragment ions at *m/z* 425 and 408 due to the loss of methyl and methanol groups from the molecular ion, respectively (Scheme 1).

A second triterpene methyl ether **2** was also isolated in small quantities for which only mass spectrometric and ^{1}H NMR data were obtained. The mass spectral fragmentation pattern of **2** was quite similar to that of cyclindrin except for the observation of a base peak at *m/z* 218. The $[M]^+$ peak was also found at *m/z* 440 with characteristic fragment ions at *m/z* 393 $[M-15-32]^+$ and 408 $[M-32]^+$. The $[M-32]^+$ fragment ion indicates that a methoxy group is present and this is confirmed by the observation of the methoxy singlet at δ3.36 ppm in the ^{1}H NMR spectrum. Scheme 1 illustrates the formation of the fragment ions at *m/z* 218 and 175. This revealed that a Δ^{12}-double bond was present in the molecule as shown by the characteristic retro-Diels–Alder fragmentation of triterpene having this skeleton. Thus a tentative structure for **2** was proposed based on the ^{1}H NMR and mass spectral data.

Table 2. Composition of *n*-acids and *n*-alkanols obtained by methanolysis of wax esters of palm fruit exocarp*

C	Fatty acid (%)	*n*-Alkanol (%)
16	42	21
18	4	—
20	5	—
22	32	3
24	9	3
26	2	4
28	1	9
30	—	13
32	—	16
34	—	16
Others	5†	15‡

*% obtained by GC.
†C_{17}–C_{27} odd carbon number fatty acids.
‡C_{23}–C_{35} odd carbon number alkanols.

Table 3. ^{13}C NMR spectral data of **1** ($CDCl_3$ δppm) and cylindrin [6]

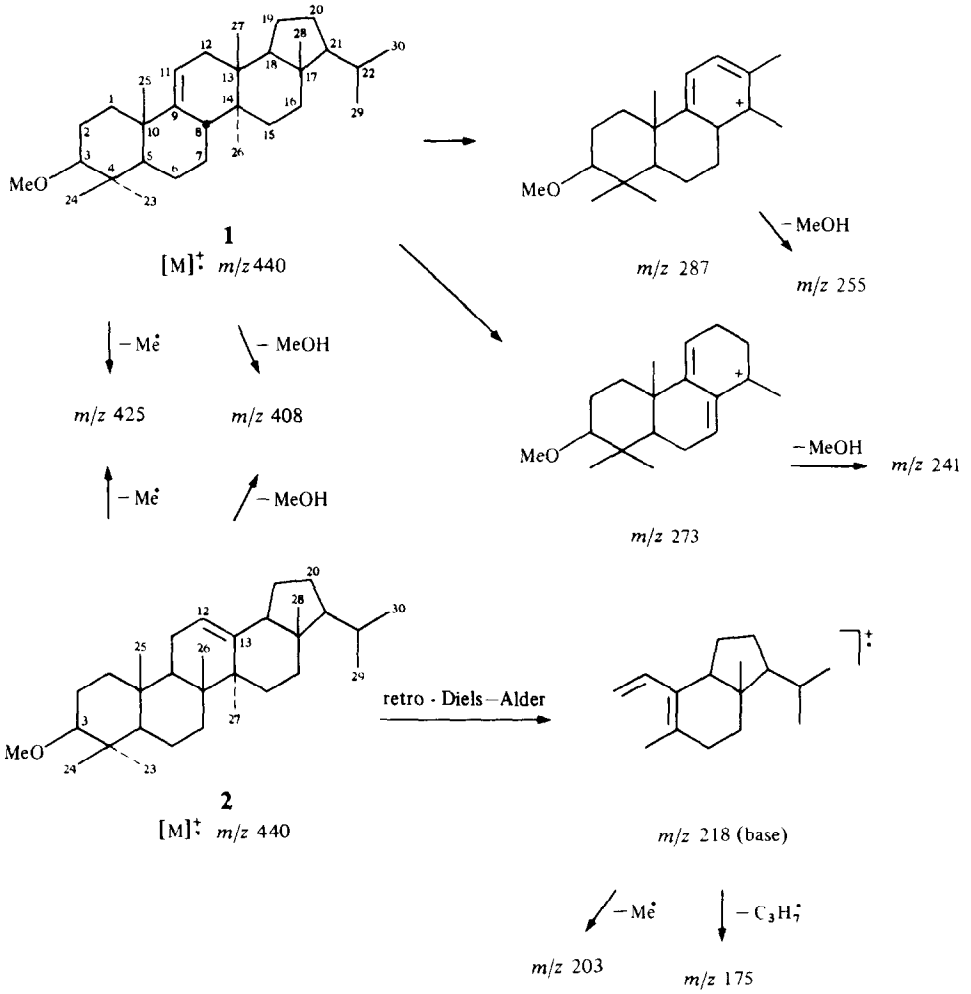
C	1	Cylindrin
1	36.0	36.0
2	22.6	22.6
3	88.7	88.7
4	39.0	39.1
5	52.9	52.9
6	21.4	21.4
7	28.3	28.3
8	41.1	41.1
9	146.9	149.1
10	39.0	39.7
11	114.2	114.2
12	36.1	36.1
13	36.8	36.8
14	38.2	38.3
15	29.7	29.7
16	36.0	36.0
17	42.9	42.9
18	52.2	52.2
19	20.2	20.2
20	28.3	28.3
21	59.7	59.7
22	30.8	30.8
23	28.3	28.3
24	16.4	16.4
25	22.1	22.2
26	17.0	17.1
27	15.3	15.3
28	14.0	14.0
29	22.1	22.2
30	23.0	23.0
OMe	57.5	57.5

EXPERIMENTAL

Materials. Fresh oil palm fruits (*E. guineensis*, Tenera hybrid) were 20-week-old fruits obtained from the Palm Oil Research Institute of Malaysia (PORIM) Research Station, Serdang, Selangor. The CPO sample was obtained from Chemara Research Station, Kumpulan Guthrie Sdn. Bhd., Seremban, Negeri Sembilan. TLC materials, silica gel (Merck 9385) and basic Al_2O_3 for CC were purchased from Merck. Fatty alcohol standards were purchased from Poly Science Corporation, U.S.A. A standard wax ester mixt. was synthesized by reacting the acid chlorides of a mixture of palmitic and oleic acids with cetyl alcohol. Other chemicals were of analytical or reagent grades and were used without further purification. Unless otherwise stated, all solvents were redist. before use.

^{1}H and ^{13}C NMR were recorded at 100 and 25 MHz, respectively, in $CDCl_3$ soln with TMS as int. std. Chemical shifts are reported as δ ppm downfield from TMS. GC was performed on a chromatograph equipped with a FID detector. GC-MS or direct probe EIMS were performed on a Kratos AEI MS3074 MS with a DS55 data system.

Wax extraction and fractionation. Whole palm fruits (2.6 kg) were carefully stripped from the fruit bunches and soaked in $CHCl_3$ for *ca* 3 min. The $CHCl_3$ ext was decanted, dried (Na_2SO_4) and concd by rotary evapn. The crude exocarp ext (0.9 g) obtained was chromatographed by CC on silica gel (30 g, 18 × 2 cm). This column was eluted with *n*-hexane, a mixt. of wax



Scheme 1.

esters and triterpene Me ethers was eluted immediately after the elution of the coloured non-polar carotenoids from the column; TLC with std wax esters was used to monitor the elution. In the isolation of wax esters from CPO, a preliminary sepn by basic Al_2O_3 CC was performed before chromatography over silica gel to exclude a large amount of triglycerides and fatty acids.

Further sepn of wax esters and triterpene Me ethers into two separate fractions was achieved by prep. TLC (20 cm × 20 cm, 2 mm thickness, silica gel $60F_{254}$) using petrol (bp 60–80°)–Et_2O–HOAc (90:10:1). Wax esters were detected as a dark blue spot by spraying with 6% molybdophosphoric acid in ethanol and heated to 180° for 5 min. Triterpene Me ethers were detected with I_2 vapour.

Analysis. Wax esters were analysed by GC on a 0.5 m × 2 mm i.d. glass column packed with 1% SP-2100, the temp. prog. from 180 to 320° at 4°/min and held at 320° for 15 min. The [M^+] of the wax ester mixt were obtained from the EIMS of the sample using the direct probe. Methanolysis of wax esters was carried out by NaOMe as described in ref. [7]. The fatty acid Me esters and fatty alcohols obtained by methanolysis were sepd into two components by prep. TLC and analysed by GC on a 1.5 m × 2 mm i.d. glass column packed with 3% OV-101 and temp. prog. from 120 to 320° at 4°/min. The composition of the fatty acid Me esters and fatty alcohols were determined by GC and GC-MS methods; identification was performed by co-chromatography or comparison of the MS spectral patterns with those of authentic samples. Quantitation of the wax esters of CPO was by GC using the synthetic esters as standards.

Triterpene Me ethers were analysed by MS and ^{1}H NMR spectrometry; the ^{13}C NMR data of **1** and cylindrin are given in Table 3.

Wax ester homologues. GC-MS (70 eV), *m/z* (rel. int): 746 (0.1), 732 (1.1), 718 (0.2), 704 (1.8), 690 (0.2), 676 (3.2), 662 (0.4), 648 (3.6), 634 (0.1), 620 (0.6), 592 (0.6), 420 (1.9), 392 (2.7), 341 (5.1), 313 (2.5), 285 (4.1), 257 (66.8), 256 (16.1), 239 (5.4), 125 (13.6), 111 (20.1), 97 (46.2), 83 (54.7), 71 (61.1), 69 (51.9), 57 (100), 55 (49.5), 43 (79.9). The M_r of higher mass wax esters were obtained by EIMS (probe): [$M]^+$, 872 (7), 844 (5), 816 (24), 802 (16), 788 (62), 774 (18), 760 (80), 746 (22), 732 (100), 718 (14), 704 (90), 690 (14), 676 (86), 662 (9), 648 (77), 634 (6), 620 (12), 592 (14).

Triterpene Me ethers. **1**, ($C_{31}H_{52}O$), EIMS, 70 eV, 440.40 (74.6, [$M]^+$), 425 (66.3), 408 (2.8), 393 (33.4), 273 (51.4), 255 (13.6), 241 (20.3). ^{1}H NMR (partial, Me region): δppm 0.74 (*br s*, C-27 and C-28, 6H), 0.79 (*s*, C-24 and C-26, 6H), 0.82 (*d*, J = 6.0 Hz, C-29 or C-30, 3H), 0.88 (*d*, J = 6.0 Hz, C-29 or C-30, 3H), 0.95 (*s*, C-23), 1.02 (*s*, C-25), 3.36 (*s*, OMe, 3H).

2, ($C_{31}H_{52}O$), EIMS, 70 eV, 440.38 (3.4, [$M]^+$), 425 (0.6), 408 (0.7), 393 (1.0), 257 (1.8), 229 (2.3), 218 (100), 175 (42.5). ^{1}H NMR (partial, Me region): δ ppm 0.74 (*s*, C-27, 3H), 0.79 (*s*, C-24, 3H), 0.84 (*s*, C-23, C-25 and C-28, 9H), 0.88 (*d*, J = 6.4 Hz, C-29 or

C-30, 3H), 0.95 (*d*, $J = 6.4$ Hz, C-29 or C-30, 3H), 1.12 (*s*, C-26, 3H), 3.36 (*s*, OMe, 3H).

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