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FATTY ACID COMPOSITION AND THE CHARACTERIZATION OF A NOVEL DIOXO C_{18}-FATTY ACID IN THE LATEX OF *HEVEA BRASILIENSIS*[*]

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Key Word Index—*Hevea brasiliensis*; rubber latex; fatty acid composition; furanoid fatty acid; dioxo acid; 10,13-dioxo-11-methyloctadecanoic acid.

Abstract—The fatty acids of the triacylglycerol fraction of the latex of the rubber plant consists of 97 % of a C_{18} furanoid fatty acid, 10,13-epoxy-11-methyloctadeca-10,12-dienoic. The free fatty acid fraction is composed of a more equally distributed mixture of 16:0, 18:0, 18:1, 18:2 and the furanoid acid. A novel dioxo fatty acid, 10,13-dioxo-11-methyloctadecanoic, was also isolated and characterized.

INTRODUCTION

Long-chain fatty acids containing a furan system in the alkyl chain have been isolated as minor components from seed [1] and fish [2–6] oils. However, Hasma and Subramaniam [7] have reported the presence of over 90 % of 10,13-epoxy-11-methyloctadeca-10,12-dienoic acid (**1**) in the triacylglycerol fraction of the lipids of the latex of the rubber tree.

Our aim in this paper was to reassess the fatty acid composition of the triacylglycerols of this plant, to report on the composition of the free fatty acid fraction, and to search for novel fatty acids present in the latex.

RESULTS AND DISCUSSION

Our recent studies of the chemistry of furanoid fatty esters [8] revealed the ease of hydrolytic ring opening of the furan ring to give the corresponding dimethylene-interrupted dioxo derivatives, and the facile BF_3-catalysed cyclodehydration reaction of the dioxo derivatives to the furanoid esters [9]. It was also noted that treatment of dimethylene-interrupted dioxo fatty acids with H_2SO_4 in MeOH caused esterification of the carboxyl group only, without cyclodehydration of the dioxo function. With such selective esterification and cyclodehydration methods available, it was possible to isolate from rubber latex a novel dioxo C_{18}-fatty acid, which was characterized as 10,13-dioxo-11-methyloctadecanoic acid.

The freshly collected NH_3-stabilized latex was air-freighted from Malaysia to Hong Kong for extraction. A copious amount of Me_2CO was used to cause coagulation of the rubber material. The Me_2CO extract furnished a viscous material upon removal of the solvent. Silica gel column chromatographic separation of the polymeric material of the viscous Me_2CO extract gave a product composed of four major fractions detected by TLC, viz. non-polar hydrocarbons, triacylglycerols, free fatty acids and a polar fraction (Y).

The triacylglycerol and fatty acid fractions were isolated by preparative TLC and their fatty acid composition determined (Table 1) by GC. The triacylglycerols (constituting *ca* 0.6 % by wt of the latex) were composed essentially of the furanoid acid **1** with only traces of 16:0, 18:0, 18:1, 18:2 and 18:3 acids. Mass spectral analysis of the methyl ester derivative of **1** gave

Table 1. Fatty acid composition of triacylglycerols and free fatty acids in the latex of the rubber plant

	Components						
Fraction	16:0	16:1	18:0	18:1	18:2	18:3	Furanoid acid (1)
Triacylglycerols	0.2	—	0.3	0.2	0.4	1.8	97.1
Free fatty acids	15.5	2.4	19.9	16.7	26.3	—	19.2
Total fatty acids	2.6	0.3	4.0	1.7	6.3	1.4	83.7

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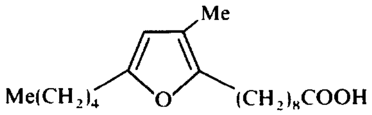
[*] Part XXIV in the series "Fatty Acids". For Part XXIII see Lie Ken Jie, M. S. F., Chan, H. M. W., Wai, J. S. M. and Sinha, S. (1981) *J. Am. Oil. Chem. Soc.* (in press).



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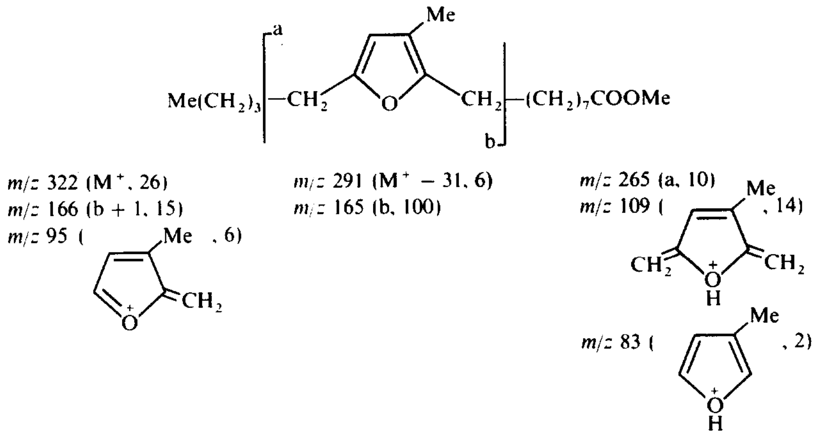


Scheme 1. Mass spectral fragmentation of methyl 10,13-epoxy-11-methyloctadeca-10,12-dienoate.

the fragments shown in Scheme 1. ^{13}C NMR of the methyl ester of (**1**) showed characteristic chemical shifts (ppm) (Table 2).

The free fatty acid fraction showed a markedly different fatty acid composition pattern with *ca* equal quantities of 16:0, 18:0, 18:1, 18:2 and acid **1**.

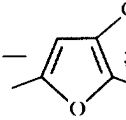
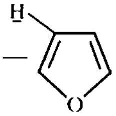
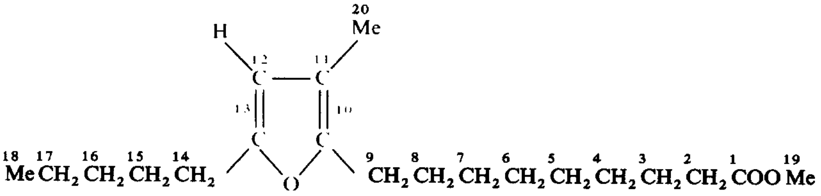
The polar fraction Y was composed of a relatively small quantity of triacylglycerols and a non-lipid material (mp 135°). The ^{1}H NMR spectrum of this mixture revealed chemical shifts for protons of a methyl-substituted furan nucleus (δ 1.9, *s*, — ; 5.74, *s*, — ), a triacylglycerol structure (4.25, *m*, $-C\underline{H}_2-O-CO-$) and a dimethylene-interrupted dioxo system (2.66, *s*, $-CO-C\underline{H}_2-C\underline{H}_2-CO-$), but no indication of free carboxyl acid functions. The IR spectrum showed a strong absorption at 1700 cm^{-1} due to νC=O stretching vibration. Fraction Y was saponified and the non-lipid material separated from the fatty acid components.

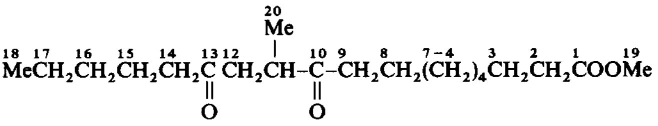
Table 2. ^{13}C NMR data for methyl 10,13-epoxy-11-methyloctadeca-10,12-dienoate



C(1)	174.2	C(8)	28.7	C(15)	27.9
C(2)	34.1	C(9)	25.9	C(16)	31.5
C(3)	25.0	C(10)	149.4*	C(17)	22.5
C(4)	29.2	C(11)	113.8	C(18)	14.0
C(5)	29.2	C(12)	107.7	C(19)	51.3
C(6)	29.2	C(13)	153.5*	C(20)	9.9
C(7)	29.2	C(14)	28.0		

* From ref. [11].

Table 3. ^{13}C NMR data for methyl 10,13-dioxo-11-methyloctadecanoate



$$\overset{18}{\mathrm{Me}}\overset{17}{\mathrm{CH_2}}\overset{16}{\mathrm{CH_2}}\overset{15}{\mathrm{CH_2}}\overset{14}{\mathrm{CH_2}}\underset{\underset{\mathrm{O}}{\|}}{\overset{13}{\mathrm{C}}}\;\overset{12}{\mathrm{CH_2}}\overset{\overset{\overset{20}{\mathrm{Me}}}{|}}{\mathrm{CH}}-\underset{\underset{\mathrm{O}}{\|}}{\overset{10}{\mathrm{C}}}-\overset{9}{\mathrm{CH_2}}\overset{8}{\mathrm{CH_2}}\overset{7-4}{\mathrm{(CH_2)_4}}\overset{3}{\mathrm{CH_2}}\overset{2}{\mathrm{CH_2}}\overset{1}{\mathrm{CO}}\overset{19}{\mathrm{OMe}}$$

C(1)	174.3	C(8)	23.5	C(15)	22.4
C(2)	34.1	C(9)	41.0	C(16)	31.4
C(3)	25.0	C(10)	213.6	C(17)	22.7
C(4)	29.2	C(11)	41.3	C(18)	13.9
C(5)	29.2	C(12)	45.6	C(19)	51.4
C(6)	29.2	C(13)	209.7	C(20)	16.8
C(7)	29.2	C(14)	42.9		

Esterification of the fatty acids with H_2SO_4–MeOH gave two spots on TLC. The less polar methyl ester fraction was composed of 18:0 (2.2 %), 18:1 (4.4 %), 18:2 (4.5 %) and acid **1** (88.9 %). The more polar methyl ester fraction was characterized as methyl 10,13-dioxo-11-methyloctadecanoate from ^{13}C NMR and mass spectral analyses (Table 3 and Scheme 2).

Treatment of the isolated methyl 10,13-dioxo-11-methyloctadecanoate with BF_3–MeOH gave the furanoid ester **1** with physical properties identical to those exhibited by the furanoid acid **1** obtained from the triacylglycerol fraction.

It is uncertain at this stage whether the novel fatty acid, 10,13-dioxo-11-methyloctadecanoic, is to be considered a precursor of the furanoid acid (**1**) or a product of a hydrolysis process involving the furanoid acid itself. The estimated amount of this novel dioxo acid is *ca* 0.03 % by wt of latex. The nature of the non-lipid solid component from fraction Y is currently under investigation.

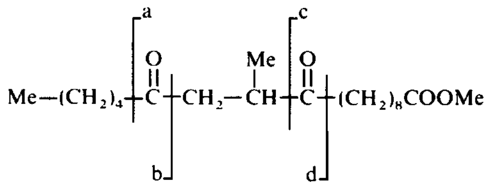
EXPERIMENTAL

General procedures. General (GR) grade of solvents was employed for extraction purposes and where required solvents were dried and distilled before use. Details of column, TLC and GC are described in ref. [10]. ^1H and ^{13}C NMR studies were carried out at 90 MHz, and MS were obtained by direct insertion at 70 eV.

Isolation of lipid fractions from latex. The NH_3-stabilized latex emulsion (220 g) was poured into a 800 ml beaker containing Me_2CO (400 ml) and the mixture stirred for 15 min. The coagulated rubber material was then continuously extracted with hot Me_2CO in a Soxhlet apparatus for 3 hr. The combined Me_2CO extracts were evapd under red. pres. to yield a viscous material (*ca.* 3 g). This material (10 g) was chromatographed on a Si gel G column (70 g) using Et_2O–petrol (3:7) (500 ml) as eluant. The eluate was evapd to give a mixture of compounds (8 g). TLC analysis of this mixture (Si gel G, Et_2O–petrol, 1:9) showed 4 spots, A–D (R_f = 0.95, 0.88, 0.55 and 0.27, respectively). The mixture (200 mg) was streaked on a prep. TLC plate (20 × 20 cm, 1 mm thick Si gel G) and developed with Et_2O–petrol (1:4). The plate was sprayed with 0.5 % of 2′,7′-dichlorofluorescein in EtOH and viewed under UV. Four bands corresponding to spots A–D were observed and each band was separated and the compounds were removed from the adsorbant using Et_2O–petrol (1:1). The yield of compounds obtained for A–D was *ca* 7, 40, 38 and 15 % (based on wt of mixture of compound streaked on plate).

Fraction A. The ^1H NMR of the fraction showed signals at δ 0.9 (–Me) and 1.2–1.6 ($-CH_2-$) only. The IR gave peaks at 2800–3000 cm^{-1} (νC–H) and peaks in the fingerprint region characteristic of hydrocarbons.

Fraction B. (500 mg) was refluxed with C_6H_6 (2 ml) and 0.5 M NaOMe (3 ml) in MeOH (10 ml) for 45 min. H_2O was added and the reaction mixture extracted with petrol (3 × 20 ml). The extract was washed with H_2O (2 × 20 ml) and dried (Na_2SO_4). Evapn of the solvent furnished a mixture of methyl esters



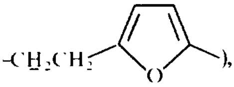
m/z 340 (M^+, 2)
m/z 237 (a − 32, 26)
m/z 169 (d, 56)
m/z 128 (?, 33)

m/z 322 (M^+ − 18, 44)
m/z 199 (c, 93)
m/z 139 (?, 66)
m/z 99 (b, 66)

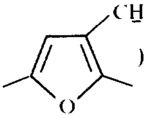
m/z 309 (M^+ − 31, 44)
m/z 184 (McLafferty rearrangement, 100)
m/z 144 ($\mathrm{Me(CH_2)_4\overset{{}^+OH}{\overset{\|}{C}}CH_2\overset{CH_2}{\overset{\|}{C}}H}$, 62)

Scheme 2. Mass spectral fragmentation of methyl 10,13-dioxo-11-methyloctadecanoate.

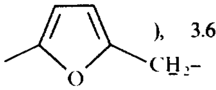
(470 mg). GC analysis of the mixture was carried out on DEGS and APL phases (Table 1). TLC R_f: 0.9 (Et_2O–petrol, 1:9); IR cm^{-1}: 3010 (νC–H, furan), 1740 (νC=O, ester), 1610, 1565, 1370 (νC=C, furan ring), 1010 (furan ring breathing); ^1H NMR: δ 0.88 (3 H, -Me), 1.25 (12 H, $-CH_2-$), 1.60 (6 H, $-C\underline{H}_2CH_2COOMe$,



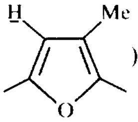
), 1.90 (3 H,



), 2.25 (2 H, $-C\underline{H}_2COOMe$), 2.50 (4 H,



), 3.64 (3 H, $-COOC\underline{H}_3$), 5.74 (1 H,



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Fraction C. (100 mg) was refluxed with 2% H_2SO_4 in dry MeOH (20 ml) for 1 hr. H_2O (50 ml) was added and the mixture extracted with Et_2O (3 × 20 ml). The extract was successively washed with H_2O (20 ml), 10% $NaHCO_3$ (20 ml), H_2O (20 ml) and dried (Na_2SO_4). The solvent was removed by distillation and the residue (90 mg) analysed by GC (Table 1).

Fraction D. (1 g) was refluxed with 2% NaOH in EtOH (50 ml) for 1 hr. H_2O (100 ml) was added and the reaction mixture extracted with Et_2O (2 × 100 ml). The aq. layer was isolated, acidified with 2 M HCl and extracted with Et_2O (3 × 50 ml). The Et_2O extract was washed with H_2O, dil. $NaHCO_3$ soln and dried (Na_2SO_4). The solvent was distilled and the residue refluxed with 2% H_2SO_4 in dry MeOH for 20 min. The product (methyl ester) was isolated in the usual way and streaked on a prep. TLC plate (20 × 20 cm, 1 mm thick Si gel G) and developed with Et_2O–petrol (3:7).

The more polar fraction (R_f = 0.28, 20 mg, 2% based on wt of fraction D) was isolated and subjected to spectroscopic and MS analyses. IR $\nu_{max}^{CS_2}$ cm^{-1}: 1740 (νC=O, ester), 1705 (νC=O, oxo); ^1H NMR: δ 0.88 (3 H, -Me), 1.08 (3 H, J = 7 Hz, $-CH(Me)-CO-$), 1.25 (12 H, $-CH_2-$), 1.6 (6 H, $-C\underline{H}_2-CH_2-CO-$), 2.3–2.6 (8 H, $-CH_2CO-$), 2.9 (1 H, $-C\underline{H}(Me)-CO-$), 3.64 (3 H, -COOMe). This fraction was then refluxed with 10% BF_3–MeOH (20 ml) for 20 min. H_2O (50 ml) was added and the mixture extracted with petrol (2 × 30 ml). The solvent was removed under red. pres. and the physical property of this isolated product was identical to those exhibited by the furanoid ester **1**.

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