ON THE LONG CRYSTAL-SPACINGS IN WAX ESTERS AND THEIR VALUE IN MICRO-ANALYSIS OF PLANT CUTICLE WAXES

by

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I. INTRODUCTION

Normal aliphatic long-chain compounds crystallise as piles of unimolecular layers with the carbon chains in parallel, upright or tilted position. Their X-ray powder diagrams show two sets of interferences, one at normal and one at small diffraction angles. The former depend on the side spacings between the chains, the latter on the thickness of the layers and, hence, on the length and nature of the chains and their angle with the basal plane. The latter interferences are, therefore, of great importance in the identification of *n*-aliphatic long-chain products, in particular for accurate chain length determinations. In the field of natural waxes they have led to a better definition of products isolated from the waxes, as may appear for example from the extensive researches on plant- and insect-waxes by CHIBNALL, PIPER *et al.*[1].

An X-ray diffraction study of waxes scraped from plant surfaces[2] has shown that many of these waxes, without any chemical pretreatment, produce X-ray interferences at similar small diffraction angles. Fairly accurate estimates of nature and chain length of the main long-chain constituents of such waxes could be made from these interferences. The value of this result lies in the fact that taking an X-ray diagram is a rather simple procedure which requires no more material than a fraction of a milligram. Chemical analysis of such small quantities of wax would be impossible, and if a sufficiently large quantity were available the procedure would still be complicated.

The long spacings in the plant waxes mentioned above normally indicated either a long-chain primary alcohol, a paraffin or a ketone resp. secondary alcohol as the main constituent of a wax. Evidence was produced that these compounds are present in the waxes in the rhombic modification, *i.e.* with the carbon chains perpendicular to the lattice planes with long spacing.

With two of the waxes studied, however, none of the above-mentioned types of long-chain compounds could fit the long spacings observed. It was, therefore, suggested that in these cases an ester is the main constituent of the wax. This was supported by the fact that a chain length could be deduced which indicated a possible wax ester. However, there were two uncertainties in these calculations. First it was presumed that with esters in waxy mixtures the packing of the chains is also rhombic. Then, we were forced to use long spacings of rhombic wax esters which were based on theoretical estimations checked only with some empirical data on the long spacings of certain ethyl esters[3]. No empirical data were available on the long spacings of true wax esters (C_{40}–C_{64}) and very few on the long-chain esters of the lengths C_{24}–C_{40} (SHEARER[4], KOHLHAAS[5]).

The purpose of the present work was, therefore, threefold.

In the first place we wished to determine the relation of the long spacing to the chain length in the esters of saturated long-chain alcohols and acids. This could be done by synthesising a number of these esters and checking their measured long spacings with the calculated ones. As available time was limited, it was impossible to synthesise the long-chain alcohols and acids longer than C_{22} which are required for the preparation of true wax esters. Therefore, only esters from shorter alcohols and acids, most of which were commercially available, and esters from the alcohol resp. acid fractions of a natural wax with some of the shorter acids resp. alcohols were prepared.

In the second place we wished to find out whether in natural waxes with a high ester value the ester component is present in the rhombic modification and whether long spacings observed in such waxes would enable us to determine the chain length of the ester component. This could be done by first calculating the chain length from the observed long spacing, using the relation between these values as determined for ester chains in rhombic packing in the first part of the work. Then this chain length had to be compared with the sum of the chain lengths of the alcohol and acid fraction obtained by saponification of the wax. A reasonable harmony would be indicative of a rhombic ester packing and, at the same time, would confirm the analytical value of the long spacings. In view of this end we examined the long spacings of four plant waxes with a high ester content, as well as the long spacings of their saponification products.

Finally, on the basis of the experience gained in the preceding work, we have made an attempt to define the ester fraction in some waxes with a supposed high ester content from their X-ray long-chain interferences alone.

The chemical work was mainly done by C. SCHAMHART.

II. CALCULATION OF CHAIN LENGTH – LONG SPACING RELATIONS

In order to deduce a chain length from the long spacing measured in a *n*-aliphatic long-chain compound it is desirable to have a general formula available for the chain length–long spacing relation in the homologous series in question. In the present study we need such formulae primarily for the wax alcohols, fatty acids and esters. For the alcohols and acids they can be deduced from empirical long spacings given in the literature; for the esters they can be calculated from the relations found for the foregoing compounds.

Of particular importance are the relations for chains placed in vertical position with reference to the lattice planes with long spacing, because long-chain alcohols from natural waxes, unless very pure, crystallise in the rhombic modification. Probably, the same may be said of the wax esters, although, the ethyl esters normally crystallise in a monoclinic form, *i.e.* with tilted chains[3]. The wax acids also show the rhombic packing, at least if obtained by saponification from a wax ester as the non-fractionated acid compound, which will be demonstrated. Pure fatty acids are known to accept a monoclinic form. Under certain conditions, however, rhombic spacings have also been observed in some pure members of this series[6].

The formulae required for deducing chain lengths can be evolved as follows.

For long-chain compounds in which there is a single layer of molecules between the lattice planes with long spacing (*e.g.* paraffins, ketones and sec. alcohols), the long spacing may be represented by the following general formula

$$d = ma + b \qquad (1)$$

in which m is the number of carbon atoms of the compound in question, a is the C–C distance in the chain direction, and b is the distance between the terminal C atoms of adjoining chains in that direction; *cf.* Fig. 1a*.

For primary alcohols and fatty acids, which crystallise in double-layers we get respectively:

$$d = 2ma + b + c \qquad (2)$$

$$\text{and} \quad d = 2ma + b + e \qquad (3)$$

in which c and e represent the distances between the C atoms at respectively the CH_2OH and COOH ends of the chains; *cf.* Fig. 1b.

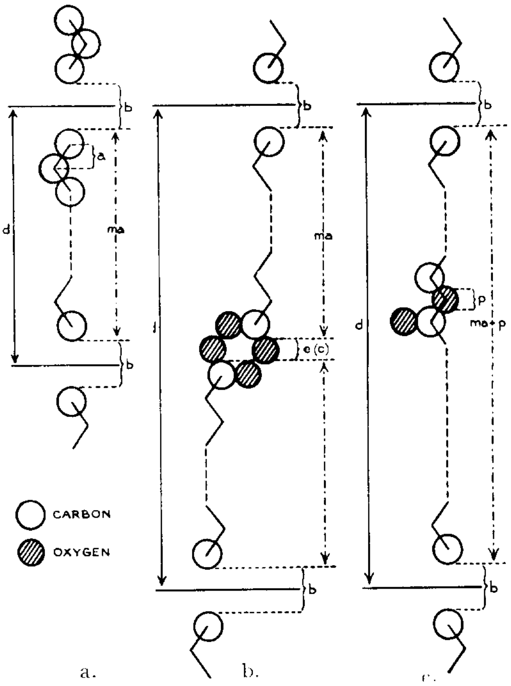

Fig 1. Diagrams of n-aliphatic long chain molecules in vertical position between the net planes with long spacing. a. odd-number paraffins, b. even-number fatty acids, c. even-number esters.

The long spacing for esters may be represented by

$$d = ma + b + p \qquad (4)$$

if it is presumed that the oxygen atom that forms the ester linkage is built in like a carbon atom of the chain, as is shown in Fig. 1c, and p is the part contributed to the spacing by this oxygen atom. The values of a, b, $b + c$, $b + e$ and $b + p$ have now to be calculated from empirical data.

The value of a, or the C–C distance in the chain direction, is normally given as 1.26 A in the literature. Other values are: 1.27 A (MÜLLER[8], KOHLHAAS[5]), 1.3 A (MALKIN[9]). We have determined the distance from the empirical long spacings for a number of long-chain paraffins[10] and from the long spacings for primary alcohols[10, 11]. These long spacings are mentioned in Table I. If the paraffin spacings are plotted in a graph against the number of C-atoms, a line corresponding to an increment of 1.27 A per C-atom appears to fit these plots best. If the alcohol spacings are plotted in a graph a line corresponding to 2.54 A increment of the spacing per C-atom is in best correspondence with the position of the plots. Since the alcohols crystallise in double layers the latter value corresponds to twice the increment of the chain

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* d in Fig. 1a and c does not correspond to the $c \sin \beta$ value of the unit cell, as the length of the unit cell in odd-number paraffins and even-number esters comprises two molecules[8, 5].

length per carbon atom, and, therefore, 1.27 A is shown again as the value of *a*. Therefore, we have based our further calculations on this value.

TABLE I

RHOMBIC LONG SPACINGS OF PARAFFINS (a), PRIMARY ALCOHOLS (b) AND FATTY ACIDS (c) WITH DATA ON THE INTERCHAIN DISTANCES IN THE CHAIN DIRECTION

a				*b*				*c*			
m	d	$m \times 1.27$	$d-m \times 1.27$	m	d	$2m \times 1.27$	$d-2m \times 1.27$	m	d	$2m \times 1.27$	d $2m \times 1$..
11	15.9	14.0	1.9	12	34.8	30.5	4.3	11	30.1	27.9	2.2*
15	21.0	19.05	1.95	14	39.7	35.6	4.1	13	35.5	33.0	2.5*
17	23.6	21.6	2.0	16	44.9	40.6	4.3	14	37	35.6	1.4*
18	25.3	22.9	2.4*	18	50.2	45.7	4.5	15	40.0	38.1	1.9*
19	26.2	24.1	2.1	19	52.8	48.2	4.6	16	41.2	40.6	0.6
20	27.4	25.4	2.0	21	57.4	53.3	4.1	17	43.2	43.2	0*
21	28.7	26.7	2.0	25	68.5	63.4	5.1*	18	46.6	45.7	0.9
23	31.0	29.2	1.8*	26	70.5**	66.0	4.5	25	63.0	63.4	0.4*
24	32.6	30.5	2.1	27	73.0**	68.6	4.4	27	69.0	68.6	0.4*
26	35.2	33.0	2.2	28	75.4**	71.1	4.3	31	80.4	78.7	1.7*
27	37.1	34.3	2.8*	29	78.0**	73.6	4.4	32	82.0	81.3	0.7
29	38.8	36.8	2.0				43.5 av.: *4.35*				2.1 av.: *0.7*
30	40.0	38.1	1.9								
31	41.6	39.4	2.2								
32	42.7	40.6	2.1								
34	45.3	43.2	2.1								
35	46.7	44.5	2.2								
60	78.2	76.2	2.0								
			30.75 av.: *2.05*								

m indicates number of carbon atoms in the chain.
d indicates long spacing in A.
* values not used in the determination of averages.
** spacings taken from PIPER *et al.*[11].

The distance *b* between the terminal carbon atoms at the CH_3 ends of the molecules under consideration is assumed to be the same for the several types of molecules. It may be calculated from column 4 in Table Ia. In this column the *b* value for each of the members of the paraffin series is mentioned. These *b* values are found as the differences between $m \times 1.27$ and the empirical spacings from column 2. The average, 2.05 A, is taken as the correct value for *b*.

Inserting *a* and *b*, as determined above, in (1) yields for the rhombic

paraffins: $$d_{\text{rh}} = m \times 1.27 + 2.05 \qquad (1a)$$

In the same manner as *b* in (1), $(b + c)$ in (2) is calculated from column 4 in Table Ib. Thus we find for the

alcohols: $$d_{\text{rh}} = 2m \times 1.27 + 4.35 \qquad (2a)$$

Rhombic long spacings for certain long-chain fatty acids are given in Table Ic. They have been taken from a table with long spacings of fatty acids given by TRILLAT[10]. The value $(b + e)$ in (3) is calculated again in the same manner as for the paraffins from column 4 in Table Ic. The dispersion of the values in this column is fairly wide. The values for the even-number acids C_{16}, C_{18} and C_{32}, however, agree very well. We have, therefore, taken these as representative. Further, we are justified in doing this as only even-number acids are found in natural waxes. Thus we find for the

fatty acids: $d_{rh} = 2m \times 1.27 + 0.7$ (3a)

It is remarkable that $b + e$ is smaller than b. e must, therefore, be negative. This means that the carboxyl ends of adjoining chains in the COOH double layer must be very densely packed.

With the data evolved from the above it is now possible to calculate a theoretical chain length–long spacing relation for the esters, since the values of a and b in the esters may neither be expected to be different from those in the paraffins and p may be calculated from the atomic diameter of oxygen. The atomic diameters of C and O are 1.54 A and 1.32 A respectively[12]. If the angle between the oxygen bonds is assumed to be nearly equal to that between the carbon bonds in the chain, we find $p = 1.32\ a/1.54 = 1.09$ A. Thus we find for the

esters: $d_{rh} = m \times 1.27 + 3.15$ (4a)

The only data available of rhombic long-chain esters are the spacings of the ethyl esters with 19, 20, 21, 22 and 23 C atoms which, after melting on a glass strip, were maintained at a temperature of a few degrees below the m.p.[3]. These spacings and those calculated for the same esters from (4a) are compared in Table II, where it can be stated that the correspondence is very satisfactory. There is no reason to suspect that true wax esters, if in the rhombic form, will show a structure essentially different from that of the ethyl esters, and therefore it is presumed that (4a) will also apply to the former. In the next section this is investigated.

TABLE II

EXPERIMENTAL AND THEORETICAL RHOMBIC LONG SPACINGS IN CERTAIN ETHYL ESTERS

m	*19*	*20*	*21*	*22*	*23*
$d_{\cdot exp.}$	27.1	28.6	29.8	31.3	32.4
$d_{\cdot calc.}$	27.3	28.5^5	29.8^5	31.1	32.3^5

The ethyl esters normally occur in the so-called β form with tilted chains[3]. The form with vertical chains changes into the β form on further cooling.

It seemed interesting to investigate whether the long-chain esters prepared by us, if not showing vertical-chain spacings, would possess spacings corresponding to a same angle of tilt as that of the ethyl esters in the β form. Therefore, we determined the chain length–long spacing relation in the β ethyl esters. The spacings underlying this determination are those given by MALKIN[9] which, in part, have been taken from FRANCIS *et al.*[7]. They are mentioned in Table III, column 2. If they are plotted against the carbon content it appears that a line corresponding to an increment of 1.13 A per C atom is closest to these spacings. (Some dispersion, however, exists.) For the even ethyl esters the mean value of $b + p$ is 2.9 A and for the odd ones 3.2 A, as is seen from column 4, Table III. Thus we find for the

even-number ethyl esters: $d_{\beta} = m \times 1.13 + 2.9$ (5a)

and for the

odd-number ethyl esters: $d_{\beta} = m \times 1.13 + 3.2$ (5b)

TABLE III

LONG SPACINGS OF β ETHYL ESTERS AND CALCULATION OF $b + p$

m	*d*	*m* × *1.13*	*d–m* × *1.13*		*m* × *1.08*	*d–m* × *1.08*	
			even	*odd*		*even*	*odd*
18	23.0	20.3	2.7		19.45	3.65	
19	24.8	21.5		3.3	20.5		4.3
20	25.8	22.6	3.2		21.6	4.2	
21	27.0	23.7		3.3	22.7		4.3
22	27.9	24.8	3.1		23.8	4.1	
23	29.4	26.0		3.4	24.8		4.6
24	29.9	27.2	2.7		25.9	4.0	
25	31.5	28.3		3.2	27.0		4.5
26	32.2	29.4	2.8		28.1	4.1	
27	33.6	30.5		3.1	29.2		4.4
28	34.5	31.6	2.9		30.2	4.3	
29	35.7	32.8		2.9	31.3		4.4
30	36.7	33.9	2.8		32.4	4.3	
31	38.1	35.1		3.1	33.5		4.6
32	38.8	36.2	2.6		34.6	4.2	
34	41.1	38.4	2.7	av: *3.2*	36.7	4.4	
36	43.5	40.7	2.8		38.9	4.6	
38	46.3	42.9	3.4		41.1	5.2	
40	48.1	45.2	2.9		43.2	5.1	
48	57.5	54.2	3.3		51.8	5.7	
			av.: *2.9*				

The angle of tilt, β, is given by the C–C distance in the chain direction (1.27 A) and the increment of spacing per C atom for chains tilted at an angle β (1.13 A) as $\sin \beta = 1.13/1.27$. Hence, $\beta = 62.5°$.

It must be noted, that for the lower β-ethyl esters, *i.e.* those from C_{19}–C_{32}, when their spacings are plotted against the carbon content, the plots show better correspondence to a graph with an ascent of 1.08 A per C-atom, than to one with 1.13 A ascent per C-atom. The plots for the higher members of the series, however, show an increasing deviation from the extrapolation of this graph, as appears from Table III, column 7. This phenomenon suggests that in fact the chain length–long spacing relation in the ethyl esters under consideration is not exactly linear, *i.e.* in the lower members, β might be slightly smaller than 62.5° and in the higher members slightly greater.

III. THE LONG SPACINGS IN A SERIES OF SYNTHETIC LONG-CHAIN ESTERS

1. *Material*

Because of the difficulties of obtaining alcohols and acids longer than C_{22}, at first only esters with radicals up to C_{22} were prepared. Later other esters were prepared by combining certain of the above shorter products with the unfractionated alcohol and acid fraction from a natural wax. The esters of the first type will be discussed in the present section, the others in section IV.

The commercial products used were the C_{12}, C_{14}, C_{16} and C_{18} alcohols, obtained from I.C.I., Billingham and palmitic acid (85%) and stearic acid (87%) obtained from L. Light and Co. Ltd, Colmbrook. Furthermore, a sample of pure stearic acid was prepared from linseed oil and a sample of erucic acid from rape oil. The latter product was used as the basic substance for the preparation of behenic (C_{22}) acid and docosanol. Further heptadecanoic acid was prepared from C_{16} alcohol.

For these products the long spacings as measured from their diagrams and the theoretical long spacings calculated from the formulae derived in the preceding section or known from the literature, are given in Table IV. In addition, the melting points determined or known from the literature, are mentioned in this table. It will be seen that the melting points are much more sensitive to impurities than the long spacings. This is evident in particular on comparison of the data for the two samples of stearic acid. In only two of the other samples, namely hexadecanol and palmitic acid, there is a difference of some importance between the measured long spacing and that calculated or given in the literature.

TABLE IV

DATA OF PRODUCTS USED IN THE SYNTHESIS OF ESTERS

	*Long spacing in A**			*Melting point °C*	
	obs.	*calc.*	*lit.*	*obs.*	*lit.*
Dodecanol	34.7	34.85	34.8^{T}	24.2	23.8^{W}
Tetradecanol	39.9	39.95	39.7^{T}	35.2	37.6^{W}
Hexadecanol	45.4	44.95	44.9^{T}	48.6	49.3^{W}
Octadecanol	50.1 **	50.10	50.2^{T}	57.8	58.5^{W}
Docosanol	60.3	60.25		69.0	72.3^{W}
				Setting points	
Palmitic (C_{16}) acid	38.5	—	39.1^{F} (B)	55.7	62.5^{F}
Margaric (C_{17}) acid***	38.7§	—	38.6^{M} (C)	60.2	60.9^{R}
Stearic (C_{18}) acid	39.8		39.8^{F} (C)	65.0	69.3^{F}
Stearic acid***	39.8	—	39.8^{F} (C)	69.3	69.3^{F}
Behenic (C_{22}) acid***	48.3		48.3^{F} (C)	77.8	79.7^{R}

* The spacings are derived from the X-ray diagrams presented in Fig. 2 and 3.

** 3 weak reflections in the diagram, corresponding to 42.2 A (B spacing = 41.35 A^{T}), indicate that part of the alcohol is crystallised in the B form.

*** Samples prepared or isolated during present work.

( ) Letters in brackets denote crystal forms.

T: TRILLAT[10], F: FRANCIS, COLLINS AND PIPER[7], M: MALKIN[9], W: WARTH[19], R: RALSTON[20].

— The theoretical rhombic long spacings of the fatty acids have not been mentioned because apparently the acids had assumed the B or C form.

§ Two weak reflections in the diagram, corresponding to the 3rd and 5th order of a 42.25 A spacing, probably indicate that part of the C_{17} acid has assumed the rhombic form.

The X-ray diagrams of the alcohols and acids are shown in Figs. 2 and 3.

Esters were prepared (by way of the acid chloride, see below) from palmitic acid with ethyl alcohol and the C_{12}–C_{18} alcohols, and from stearic acid with the C_{12}–C_{18} and C_{22} alcohols. Further, the esters hexadecyl heptadecanoate (C_{16}–C_{17}) and octadecyl heptadecanoate (C_{18}–C_{17}) were prepared in the same manner. The tetradecyl stearate was prepared in two samples, one from the commercial and one from our own preparation of stearic acid, and the docosyl stearate from our own stearic acid. Further, ethyl stearate and a second sample of hexadecyl stearate were prepared in the direct manner.

A survey of these esters and their melting points may be found in Table V, columns 1–4. The melting points in column 5 are those found in the literature. For certain of the esters no m.p. was found in the literature. Considerable differences between the values in the columns 4 and 5 are observed only for ethyl palmitate and dodecyl stearate. It is supposed that in both cases our numbers are on the low side on account of impurity. On the other hand, by comparison with the other numbers, the value mentioned by WARTH[19] for dodecyl stearate seems to be too high.

TABLE V

MELTING POINTS AND LONG SPACINGS OF PREPARED ESTERS

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
			m.p. C				*Long spacings in A*		
$m_{alc.}$	$m_{ac.}$	$m_{est.}$	*obs.*	*lit.*	*n*	*obs.*	*calc. (A)*	*calc. (B)*	*diff.*
2	16	18	23	24.4^{F}	4	*23.05*	26.05	*23.2*	*−0.15*
					2	26.95			−0.9
12	16	28	39.4	41^{R}	6	38.8	38.7	*34.5*	+0.1
14	16	30	46.9	48^{R}	5	*37.0*	41.2	*36.8*	*+0.2*
16	16	32	51.0	51.6^{W} 54^{K}	5	*41.2*	44.3§	*39.4*§	*+1.8*
16	16	32^{b}			5	*40.7*	44.3§	*39.4*§	*+1.3*
18	16	34	57.3	55^{W}	8	46.1	46.4	*41.3*	0.3
2	18*	20	33.7	34.0^{F}	4	*25.3*	28.6	*25.5*	*−0.2*
12	18	30	45.3	49^{W}	6	*36.8*	41.2	*36.8*	*0*
14	18	32	50.0		8	43.5	43.7	*39.0*	−0.2
					3	*39.3*			*+0.3*
14	18*	32	52.0		8	43.5	43.7	*39.0*	−0.2
16	18	34	56.2	55^{W} 56.3^{W}	7	*41.4*	46.7§	*41.6*§	*−0.2*
16	18*,**	34	56.2		7	*41.8*	46.7§	*41.6*§	*+0.2*
18	18	36	60.3	58.5^{W} 62^{W}	7	*43.7*	48.7	*43.6*	*+0.1*
22	18*	40	64.5***		11	*50.2*	53.9	*48.1*	*+2.1*
					6	53.9			0
22	18*	40^{b}	67 ***		6	54.3	53.9	*48.1*	+0.4
					7	*50.7*			*+2.6*
16	17	33	56.3		7	*40.7*	45.0	*40.2*	*+0.5*
18	17	35	61.1		6	46.7	47.5	*42.4*	−0.7

* own preparation of stearic acid.
** direct esterification.
*** no sharp m.p. observed.
[b] recrystallised from benzene.
§ spacings increased above the theoretical value with 0.5 A (A form) and 0.3 A (B form), because the hexadecanol used had a slightly higher spacing than is normal; *cf.* Table IV.
For F, R and W *cf.* legend to Table IV; K: KOHLHAAS[5].
Numbers in italics have reference to tilted-chain spacings.
Further explanation in the text, sections III, 2 and 3.

It must finally be noted that we have not tried to ensure high purity of the basic materials nor of the preparations, because the long spacings are only slightly sensitive to impurities, as is indicated in Table IV.

2. *Experimental*

a. Preparation of non commercial products of Table IV

Stearic acid was isolated from hydrogenated linseed oil. The following procedure was recommended to us by Dr. J. UHLENBROEK of the Laboratory of Organic Chemistry of this University. 350 g hydrogenated linseed oil were heated for 20 hours on reflux with 4.5 litres absolute methanol in the presence of dry HCl. The methyl stearate was washed with NaCl and Na_2CO_3 solution, dried in benzenic solution over Na_2SO_4 and fractionated to a solidification point of 37.6° C. Yield 195 g. Saponification followed by addition of 160 ml 35% KOH and 160 ml (2 ×) 96% ethanol until the alcohol was evaporated. The fatty acid was liberated from the soap with 10% H_2SO_4, washed with distilled water and dried. Yield 175 g stearic acid with s.p. 69.2° C.

Erucic acid was prepared in essentially the same manner from rape oil. The yield from 3 litres rape oil was 425 g crude erucic acid. This was dissolved in 850 ml ethanol 96%. After cooling for 4 h a white crystalline mass of erucic acid had formed. The amount was *ca.* 200 g, and the s.p. 32.8° C. After 24 h a further quantity of 120 g with s.p. 32.2° C had crystallised out. The former fraction was recrystallised from ethanol and then yielded 180 g erucic acid with s.p. 33.2° C. The second fraction was purified according to the method of BERTRAM[15] with mercury acetate. The yield was 20 g erucic acid with s.p. 33.38° C (lit.: 33.5° C).

Behenic acid was prepared from erucic acid according to the directions given in VOGEL[13] by hydrogenation in the presence of Raney nickel catalyst.

The *docosanol* was kindly prepared for us in the Laboratory of Organic Chemistry of this University.

Heptadecanoic acid was prepared from hexadecanol according to the following principle:

$$6\,R_1OH + 2P + 3Br_2 \longrightarrow 6\,R_1Br + 2H_3PO_3$$

$$R_1Br + KCN \longrightarrow R_1CN + KBr$$

$$R_1CN + 2H_2O \xrightarrow{(KOH)} R_1COOH + NH_3\uparrow$$

The bromide was prepared as described by VOGEL[13], the acid according to the directions given by J. R. RUHOFF[14] for tridecanoic acid.

b. *Preparation of esters*

The esters were prepared according to the method of BRIGL and FUCHS[17] in which esterification is effected by the reaction of the acid chloride with the alcohol in the presence of quinoline.

The acid chlorides were prepared according to the directions given by VOGEL[13].

The esters were obtained by adding dropwise the acid chlorides (2–15 g) dissolved in chloroform (10–20 ml) to a chloroform solution (20 ml) containing the alcohol and quinoline, with stirring and cooling. The acid chloride and quinoline were added in excess of 40 mol %. After standing one to two days in a closed flask the chloroform was evaporated and the quinoline removed by adding 50 ml 4 *N* HCl. Melting twice in water transformed the excess acid chloride into acid. The acid was removed by dissolving in 50–200 ml 96% ethanol and boiling with Ca acetate for some time on a water bath. The alcohol was evaporated, and the ester extracted with chloroform from the Ca acetate and Ca soap of the excess acid. The esters were recrystallised twice from absolute alcohol.

Ethyl stearate was prepared by heating 20 g stearic acid (own preparation) with 50 ml absolute alcohol in the presence of dry HCl for 14 hours. The yield was 15 g ethyl stearate. A second sample of hexadecyl stearate was also prepared in the direct way.

c. *X-ray method*

The X-ray diagrams were made on flat film with a specimen-film distance adjusted at 80 mm. The collimator was a totally reflecting slit collimator with the slit width increasing in the direction of the specimen according to the principle described by LELY and VAN RIJSSEL[18]. We used two glass bars, 10 cm long and 4 × 3.5 mm cross section. One of the faces of 100 × 4 mm of each bar is carefully polished. The bars are mounted with the polished sides facing each other between two glass plates of the same length and *ca.* 1.5 × 7 mm cross section; the whole is mounted in a brass tube.

Variation of the slit width at both ends is a simple procedure. Slit widths of 125 μ at the narrow end and 200 μ at the wide were found to be very suitable for preliminary inspection, or obtaining a survey of a series of specimens. With a rotating target tube operated at 40 kV/100 mA the exposure was *ca.* 10′. A better resolution of closely spaced lines was obtained with the slit widths of the wedge adjusted at 25 μ and 145 μ. Then the exposure under the same conditions was *ca.* 35′. The diagrams represented in Figs. 2–6 are taken with the latter slit apertures.

A beam stop, 1 mm wide, was placed at 25 mm distance from the film. Specimens were 1 mm thick by 0.5 mm wide and 6 mm long. They were made by pressing a small sample of the material to be X-rayed into an oblong opening of the above dimensions in the specimen holder. This specimen holder is a small celluloid disc, 1 mm thick by *ca.* 7 mm diameter.

Before placing them in the specimen holder, some samples (the fatty acids and esters discussed in section III) were melted on a copper plate which was then rapidly cooled down on ice. This was necessary to obtain a sufficiently small crystallite size for producing homogeneously blackened diffraction bands. Despite this precaution some specimens gave rather coarsely speckled bands.

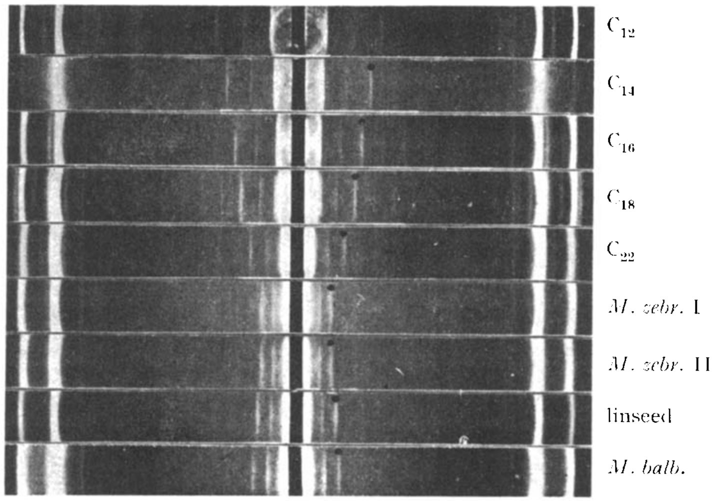

Fig. 2. X-ray diagrams of alcohols.
● for easier comparison, in Figs. 2–6 the third order interferences are marked with a black dot.

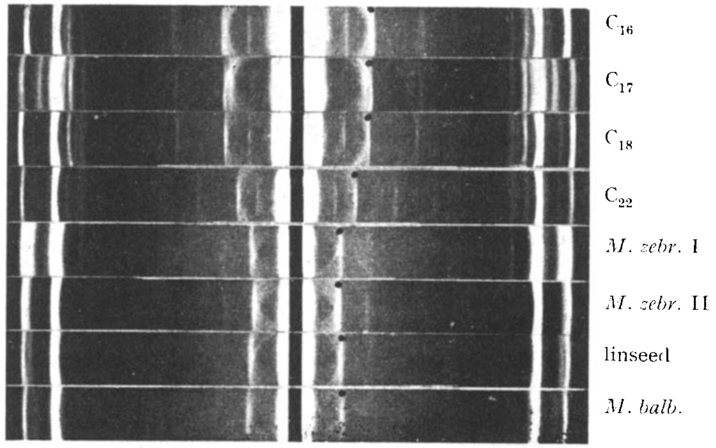

Fig. 3. X-ray diagrams of fatty acids.

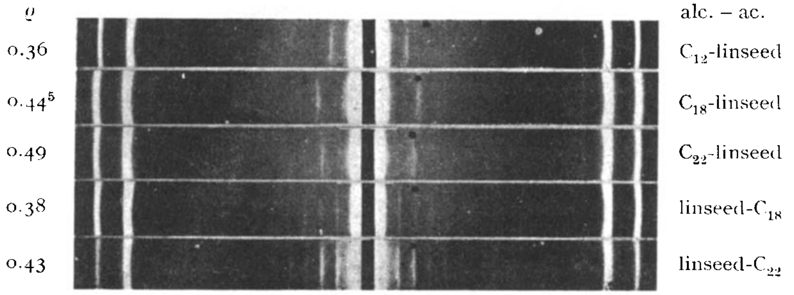

Fig. 5. X-ray diagrams of esters with an alcohol or acid radical longer than C_{22}.

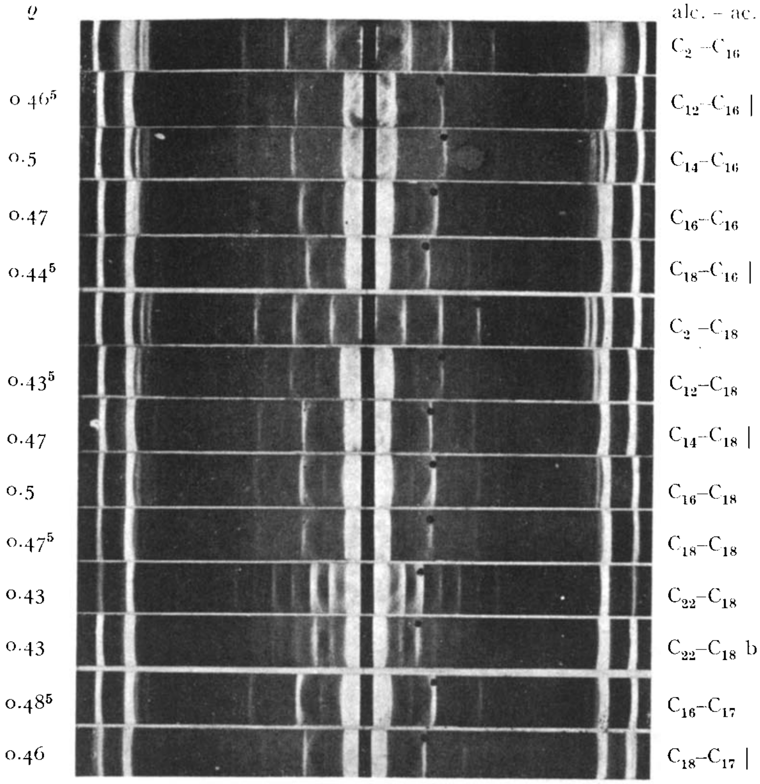

Fig. 4. X-ray diagrams of esters of short alcohols and acids.
| Diagrams of vertical chain esters are marked with a vertical line.

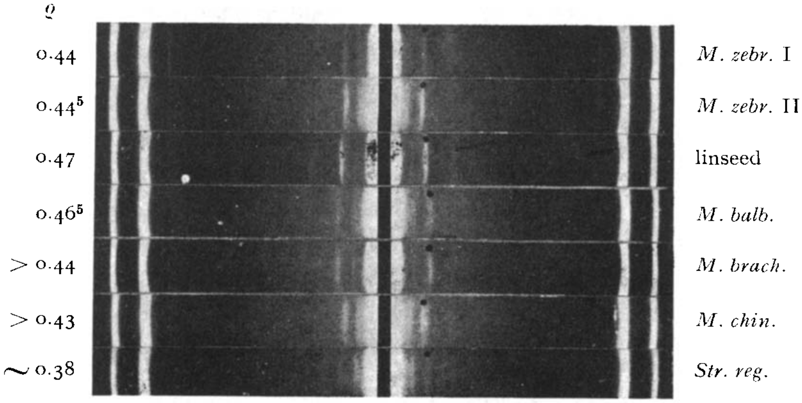

Fig. 6. X-ray diagrams of plant waxes examined.

The technique of X-ray photography usually applied in the study of long spacings of fatty and waxy materials, *i.e.* the technique in which X-rays fall at grazing incidence on a specimen pressed or melted onto a glass mount which is slowly rocked through a small angle, has certain advantages over the method we used. With the latter method, however, the side-spacing reflections stand out more clearly. Since we also wanted to observe possible variations of these and since the method applied sufficed for our purpose, we did not use apparatus with a rocking mechanism.

3. *Results*

In Fig. 4 the X-ray diagrams of the prepared esters are presented, and in Table V, column 7 the long spacings calculated from the diagrams are summed up. Further, in the next two columns of Table V, the theoretical long spacings as calculated from the relations (4a) and (5a), derived in section II for esters with vertical chains (A form) and esters with tilted chains (B form) are given. As seen from the diagrams, some esters exhibit a double set of reflections corresponding to two long spacings. In such cases the two spacings are mentioned in column 7, and a bracket denotes that they belong to the same ester preparation. The upper spacing is the one belonging to the strongest set of interferences. The numbers in column 7 are printed in italics if they correspond better with the theoretical B spacing than with the A spacing for the ester in question. The numbers in column 6 denote for every spacing the order number of the reflection of highest order visible in the original diagram. In column 10 the differences between the long spacings found and the theoretical long spacings are mentioned; differences between B spacings are printed in italics.

From a comparison of the observed long spacings in column 7 and the theoretical long spacings in columns 8 and 9 it is at once clear that in general there is close correspondence of the empirical spacing with either the calculated A or B spacing. With 14 of the 21 spacings in column 7 the difference does not exceed 1% of the empirical spacing, *cf.* column 10. It appears, therefore, that the esters under consideration may assume a form with vertical chains (A form) or one with tilted chains (B form). Both forms may occur together in the same specimen, although this is seldom observed. In the B form the tilt is the same as in the β-ethyl esters, as the theoretical B spacings are based on MALKIN's data for the β ethyl esters of long chain fatty acids (section II). Further, we may conclude that in most cases the B form is present, whereas only four esters show the rhombic form. There is no indication that the occurrence of the rhombic form is restricted to either the longer or the shorter chain lengths.

4. *Discussion*

The data recorded in Table V give rise to several questions and remarks, which will be briefly discussed in the following.

a. Is the appearance of the rhombic form in certain esters to be regarded as a feature of these particular esters or is it due to impurity, *i.e.* would it appear in any of the other esters by contamination with adjoining homologues as has been observed with *n*-long chain alcohols[11]? It is noted that the two samples of tetradecyl stearate, although prepared separately and from different stearic acid samples, both show the A form, and the two samples of hexadecyl stearate prepared in a different manner and from different stearic acid samples, both show the B spacing. This suggests that the first possibility is true.

We have tried to gain some further evidence concerning this problem by mixing

References p. 44.

some of the esters. The mixtures investigated and their spacings are summed up in Table VI. It appears from this table that (1) in none of the proportions applied did the mixtures of two long-chain esters both accepting the B form (*cf.* the mixtures of hexadecyl palmitate and hexadecyl stearate) accept the A form, (2) if dodecyl palmitate (A form) is added to dodecyl stearate (B form) in a proportion 1:8, the mixture still accepts the B form. The same is true if tetradecyl stearate (A form) is added to tetradecyl palmitate (B form) in a proportion 1:1, or octadecyl palmitate (A form) to octadecyl stearate (B form) in a proportion 1:1.

TABLE VI

LONG SPACINGS IN MIXTURES OF CERTAIN OF THE ESTERS MENTIONED IN TABLE V

Mixed esters and ratios	*Long spacings in A*		
	obs.	*calc. (A)*	*calc. (B)*
C_{12}–C_{16}	38.8*	38·7	*34·5*
4:1**	36.8	39·25	*35·0*
1:1**	37·8	40·05	*35·7*
1:8	*36.8*	41·05	*36.6*
C_{12}–C_{18}	*36.8*	41·2	*36.8*
C_{14}–C_{16}	*37·0*	41·2	*36.8*
4:1	*38.1*	41·75	*37·3*
1:1	*39·3*	42·55	*37·9*
1:9	43·3	43·55	*38·9*
C_{14}–C_{18}	43·5	43·7	*39·0*
C_{16}–C_{16}	*41·2*	44·3	*39·4*
4:1	*41.8*	44·75	*39·7*
1:1	*42·3*	45·4	*40·3*
1:9	*41·7*	46.1	*41.1*
C_{16}–C_{18}	*41.8*	46.7	*41.6*
C_{18}–C_{16}	*46.1*	46.4	*41·3*
4:1	*46.2*	46.85	*41·7*
1:1	*46.8*	47·6	*42·5*
1:9**	45·9	48.6	*43·4*
C_{18}–C_{18}	*43·7*	48·7	*43·6*

* Spacings of unmixed esters are the same as in Table V, and are repeated here for easier comparison with the spacings of the mixtures.

For simplicity the calculated spacings of the mixtures are determined by assuming the spacings to increase linearly with the mean chain length in the mixture. However, considering the data of PIPER *et al.*[11, 21] for mixtures of long chain paraffins and primary alcohols, this assumption is probably incorrect.

Observed spacings printed in italics correspond best with the B spacings, those in common type with the A spacings calculated for the mixtures in question.

** Denotes mixtures with long spacings slightly closer to the theoretical B than to the A spacing, but with side-spacing interferences which do not point to a B form.

These examples may show that even very strong contamination by an adjoining homologue normally crystallising in the A form does not necessarily give rise to the appearance of the A form in esters normally crystallising in the B form.

The spacings of the mixtures again suggest that the appearance of the vertical chain position in certain of the esters is a feature of these particular esters.

b. Why do two esters in Table V, namely hexadecyl palmitate and docosyl stearate show a difference between theoretical and empirical long spacing that is much higher than for the others? We have tried to reach a more nearly correct long spacing with these esters by slow crystallisation. They were dissolved in warm benzene, which then was slowly evaporated. After this treatment the hexadecyl palmitate had indeed a somewhat shorter spacing, but still 1.3 A longer than the theoretical B spacing. The docosyl stearate showed again two spacings, but they had become each *ca.* 0.5 A longer, and the set of interferences belonging to the A spacing had now become the stronger one (*cf.* Fig. 4). We have not been able to explain satisfactorily the rather great difference between experimental and theoretical long spacings in these two esters.

Nevertheless, one might think of the occurrence of a deviating tilt of the chains; the docosyl stearate exhibits 11 orders of sharp long-chain reflections, pointing to good crystallisation, and there is no reason for appreciable contamination by longer or shorter chains, because the basic substances were of reasonable purity. Further the value mentioned by Shearer[4] for the long spacing of hexadecyl palmitate (40.4 A) is also notably longer (1.3 A) than the theoretical value calculated from (5a) section II (39.1 A), while his values for ethyl palmitate, octyl palmitate and ethyl stearate are in satisfactory correspondence with the theoretical long spacings (differences respectively 0, +0.2 and −0.3). The long spacing measured for hexadecyl palmitate by Kohlhaas[5] (38.9 A) is close to the theoretical value. However, it refers to single crystals of the highest possible purity.

c. Finally it may be remarked that inspection of the side spacings in Fig. 4 reveals a difference between the esters with long spacings corresponding to the vertical chain position and those with long spacings corresponding to the tilted position. The latter almost always (the exception is docosyl stearate) give a more or less distinct reflection close inside the most intense side spacing reflection. Certain esters, *e.g.* ethyl palmitate and ethyl stearate, give two such reflections. The reflection, apparently, enables us to establish, without measuring the long spacing, that an ester is present in the B form, at least for the range of chain lengths considered.

The spacing corresponding to this reflection is 4.27 A (sin θ value = 0.1805). These values are liable to slight variation. Probably we are dealing here with (112). The reflection (112) was observed by Kohlhaas[5] on rotation photographs of a single crystal of hexadecyl palmitate as a strong spot with sin θ = 0.1800. It may be noted that a similar reflection also distinguishes the fatty acids and alcohols with tilted chains from those with vertical chains; *cf.* Fig. 3 and the diagram of octadecanol with a tilted chain fraction in Fig. 2.

We may conclude from this section that certain of the esters investigated, at least if not of the highest possible purity, crystallise with their chains in vertical position to the basal planes, whereas others show a tilted chain position. In both the form with vertical and that with tilted chains, the chain length–long spacing relation is the same as in the ethyl esters with either the vertical or tilted chain position. Generally, an ester with the tilted chain position requires the presence of a fairly high amount of an adjacent homologue with the vertical position before the mixture has vertical chains and *vice versa*. The esters with tilted chains may generally be recognised by one or more side spacing reflections that are not present in the diagrams of those with vertical chains.

IV. THE INVESTIGATION OF CERTAIN NATURAL WAXES WITH A HIGH ESTER CONTENT

1. *Material*

The two waxes with a supposed high ester content mentioned in the introduction, and which gave rise to the present research, had both been collected from species of *Musaceae*, namely *Strelitzia reginae* and a *Musa* spec. present in the botanical gardens of the University of Leiden, indicated as *Musa paradisiaca*. A redetermination of the latter species has revealed that this name was incorrect and that we were dealing with a specimen of *Musa brachycarpa* Backer.

Only small quantities of these two waxes were available and therefore we could not check our earlier X-ray results[2] with an X-ray investigation of the saponification products of the same waxes. However, three other *Musa* wax samples were available in sufficient quantity, as well as a wax sample with high ester content originating from linseed.

One of the *Musa* waxes was a sample of wax from *Musa zebrina* van Houtte, present in the collection of this laboratory. Another sample was kindly placed at our disposal by Dr. A. J. ULTÉE, Delft. The sample, in all probability, also belonged to *Musa zebrina*. In the following we will distinguish these samples as *Musa zebrina* I and II respectively. The third sample was collected from the stems of *Musa balbisiana*, which bear a thick wax layer. The species is present in the green houses of this laboratory. The wax layer was scraped from the stems.

The linseed wax was sent to us by the Unilever Research Laboratory, Zwijndrecht, by the kind collaboration of its director Dr. H. A. BOEKENOOGEN. This wax separates in small quantities in linseed oil and probably originates from the seed coat. Also the results of chemical analysis of the wax, the method of saponification and the saponification products were placed at our disposal*.

Esters were prepared from the fatty acid fraction of linseed wax with dodecanol, octadecanol and docosanol. The alcohol fraction of linseed wax was esterified with stearic acid and behenic acid.

The method of esterification was essentially the same as described in section III, 2b.

Further, we made improved diagrams of the two waxes from *Musaceae* studied earlier and examined the wax from *Musa chinensis*, present in the botanical gardens at Delft. The latter wax was not available in sufficient quantity to investigate the saponification products.

2. *Constants of the waxes and saponification procedures*

For the *Musa zebrina* waxes the following constants were found:

	M. zebrina I	*M. zebrina* II
acid value	1.9	2.8
ester value	85.1	85.2

The esters were saponified by heating them for 7 hours in a mixture of ethanol 96% and benzene 1:1 with alcoholic KOH solution. After dilution of the boiled mixture with warm water, the alcohol and acid were obtained in the usual manner from the separated benzene and water layers respectively. In the separation the formation of a soap emulsion gave some trouble, and no quantitative results were achieved. The alcohol and acid fractions were recrystallised from petroleum ether 60–80.

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* For this material and data, and for permission to publish the latter we are highly indebted to Dr. H. A. BOEKENOOGEN and his collaborators of the Unilever Research Laboratory.

The *Musa balbisiana* wax was saponified according to the method described below for linseed wax, but petroleum ether 60–80 was used instead of benzine and petroleum ether 40–60. Constants of the wax were not determined. 700 mg of wax was saponified in 14 ml 0.5 *N* KOH. The yield was *ca.* 300 mg fatty acid fraction and 320 mg alcohol fraction. On account of the comparatively diffuse long-chain interferences of the alcohol fraction it was supposed that the latter sample contained ester. Therefore, it was saponified for another 2.5 hours and treated further in the same manner. Yield: 265 mg alcohol fraction.

For the following data of the linseed wax and the method of saponification we are indebted to Mr. J. B. A. STROINK of the Unilever Research Laboratory, Zwijndrecht.

The wax was prepared from a concentrate in linseed oil by repeated crystallisation from petroleum ether 40–60.

Constants of the wax:		Yields after saponification and extraction:	
acid value	0.7	unsaponifiable matter	48.4 %
ester value	89.7	fatty acids	54.1 %
acetyl value	12.5	glycerol	1.95%
iodine value	0.35		

Constants of unsaponifiable matter:		Constants of fatty acids:	
acid value	1.5	acid value	157.8
acetyl value	134.5	acetyl value	0.0

These data yield the following rough analysis of the wax:

free fatty acids	0.5 %
free alcohols	8.0 %
glycerides	18.5 %
wax ester + hydrocarbons	73.0 %

The saponification value calculated from this analysis is 89.8 which corresponds satisfactorily with the experimental value of 90.4.

Method of saponification and extraction:
5 g of wax is saponified with 100 ml 0.5 *N* alcoholic KOH on reflux for 90′. Then the liquid is transfused into a separatory funnel with 100 ml warm benzine 60–90. The container is rinsed with warm ethanol and 100 ml warm benzine. These liquids and warm water are added to the contents of the separatory funnel. The ethanol-water layer is drawn off and the benzine layer brought into a second separatory funnel containing 100 ml warm water. The ethanol-water layer is shaken twice with 100 ml warm benzine and the wash benzine passed into the second separatory funnel. The benzine is then washed with 50 ml 0.5 *N* alkali in water and next with warm water containing some ethanol until the wash water is no longer alkaline with phenolphthalein.

The ethanol-water layer and all the wash water are collected in the first separatory funnel and hydrochloric acid is added in order to decompose the soaps. The fatty acids are extracted by three washings with petroleum ether 40–60. The collected petroleum ether solutions are washed with water to remove HCl.

By evaporation, resp. cooling, the benzine yields the wax alcohols with paraffins, the petroleum ether yields the fatty acids.

3. *Discussion of results with saponified and prepared waxes*

In Table VII columns 4–7 the X-ray data, obtained in the same manner as described in section III, 3, are compiled of respectively the *Musa* and linseed waxes, their saponification products, the esters prepared with the linseed alcohol and the linseed acid, and of the three wax samples that were not saponified and are discussed in the next section. The corresponding X-ray diagrams are presented in Figs. 2 (alcohols), 3 (fatty acids), 5 (prepared esters) and 6 (waxes).

Explanation of Table VII. In column 4 the order number of the highest order long-chain reflection visible on the original diagram is given. Column 5 shows the long spacing found from the long chain reflections. In some of the diagrams of prepared

TABLE VII

DATA OF NATIVE WAXES, OF THEIR SAPONIFICATION PRODUCTS, OF ESTERS PREPARED WITH SOME OF THE LATTER, AND DATA OF OTHER NATIVE WAXES THAT WERE NOT SAPONIFIED

Explanation is given in the text.

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Material	*Melting point °C*		*n*	*Long sp. A*	*m*	m_{alc+ac}	ϱ	*Alcohol* *Acid*
	obs.	*theor.*						
Wax *Musa zebrina* I			4	72.6	54.7	55.7	0.44	
alc.	80.3	86	5	82.1	30.6			30
ac.	72.0	80	5	64.5	25.1			24 + 26
Wax *Musa zebrina* II			3	73.8	55.6	56.6	0.44^5	
alc.	81.2	86	7	81.7	30.4			30
ac.	73.2	85	5	67.3	26.2			26
Wax linseed			5	66.7	50.1	51.8	0.47	
alc.	81.3	80.3	5	73.1	27.0			26 + 28
ac.	72.6	79	5	63.1	24.8			24 + 26
Wax *Musa balbisiana*			3	62.2	46.6	47.0	0.46^5	
alc.	67.5	76.0	5	65.3	24.2			24
ac.	69.0	76.0	5	58.5	22.8			22 + 24
Prepared esters:								↑ Predominating chain lengths suggested in alcohol and acid fractions of waxes ↓
C_{12} alc.-lins. acid	53	*ca.* 60	4	50.0	36.9	36.9	0.36	
C_{18} alc.-lins. acid	58.0	*ca.* 70	3	56.3	41.8	42.8	0.44^5	
C_{22} alc.-lins. acid	70	*ca.* 74	3	61.7	46.2	46.8	0.49	
lins. alc.-C_{18} acid	68.6	73	4	59.7	44.1	45.0	0.38	
lins. alc.-C_{22} acid	60?	*ca.* 76	11	60.6	51.0 (B)	49.0	0.43	
Waxes of:								
Musa brachicarpa			4	60.8	45.5		> 0.44	24 22
Musa chinensis			5	72.2	54.5		> 0.43	30–28 24–26
Strelitzia reginae			4	65.3	48.3		~ 0.38	30–28 18–20

esters there are weak long-chain reflections visible belonging to unidentified impurities; they are lost in reproduction.

In column 6 the number of carbon atoms in the chain is given as calculated from the measured long spacing using the rhombic relation $d_{\text{rh}} = m \times 1.27 + 3.15$ derived in section II and checked in section III. In one case, however, indicated with (B), the relation for tilted chains has been used because the former led to an improbable result. It is evident that the values found for *m* represent mean chain lengths because we are not dealing with pure compounds.

In column 7 the chain lengths of the esters are given as obtained by adding the numbers of carbon atoms of the component alcohol and acid mentioned in column 6 or—in the case of the C_{12}–C_{22} components of the prepared esters—known otherwise.

The theoretical melting points in column 3 are not given as such in the literature. As regards the alcohols and acids, the melting points mentioned are those of binary mixtures of *n*-primary alcohols and of *n*-fatty acids with the same mean chain lengths as found for our own products. They have been derived from the melting point graphs for binary mixtures of even-number adjacent homologues given by Piper *et al.*[11].

The theoretical melting points of the prepared esters are based on the melting points of wax esters given by WARTH[19]. However, the esters in question, with one exception, do not occur in WARTH's table. Therefore we have taken the melting points of esters with the same chain length but other alcohol and acid radicals or have estimated them by intrapolation.

Columns 8 and 9 will be explained later.

It is remarkable in Table VII that in most cases the chain lengths measured on the esters in column 6 are slightly smaller than those obtained by summation of the lengths of the component radicals in column 7. The difference might be due to the fact that in mixtures of long chain compounds consisting of adjacent homologues, there is no linearity between the long spacing and the mean chain length. As has been shown by PIPER *et al.*[11, 21] for binary mixtures of adjacent odd-number paraffins and even-number alcohols, the increment of the long spacing in mixtures with a proportion of the longer chain up to about 70% is more than proportional to the mean chain length. Beyond this proportion the mixture shows the long spacing of the longer chain, which is even exceeded between 90 and 100%. Therefore, calculation of the mean chain length of a mixture on the basis of linearity will generally result in a chain length that is too long, *i.e.* the proportion of the longer chains will be overestimated. This fault is doubled by summation of the chain lengths found for alcohol and acid fraction to obtain that of the ester. In the direct calculation of the chain lengths of the esters the fault is made only once.

In *Musa zebrina* I and II a further cause of the difference observed has probably to be sought in the purification of the saponification products by recrystallisation. In this procedure a greater proportion of the shorter chain lengths than of the longer ones remains dissolved in the solvent. Therefore, a higher proportion of the longer chain lengths will be found in the purified alcohol and acid fractions than in the non-purified fractions.

This was demonstrated as follows: 280 mg fatty acid obtained by saponification of 700 mg wax from *Musa zebrina* I for 4 hours, and treating further as indicated for linseed wax, was remelted three times over boiling water and then recrystallised twice from a solution *ca.* 1:100 by weight in petroleum ether 60–80 on standing at room temperature. Thus *ca.* 135 mg purified fatty acid was obtained, and 70 mg was obtained by evaporation of the remaining and combined petroleum ether portions.

This process was repeated using 280 mg fatty acid from *M. balbisiana*, from which two fractions of 115 mg were obtained. Melting points, long spacings and corresponding numbers of carbon atoms of the total fatty acids and the fractions are mentioned in Table VIII.

TABLE VIII

EFFECT OF PURIFICATION ON TOTAL FATTY ACID FRACTIONS

	M. zebrina I			*M. balbisiana*		
	m.p. °C	*l.s.*	*m*	*m.p.* °C	*l.s.*	*m*
Total fatty acid	71.3	62.5	24.3	68.5	58.5	22.8
Recrystallised fraction	72.7	63.8	24.8	70.0	60.0	23.3
Residue from evaporated solvent	69.8	61.0	23.7	67.3	57.3	22.3

For column headings see explanation of Table VII.

Taking into account the above considerations, Table VII shows a satisfactory correspondence between the chain lengths obtained for a wax by summation of the chain lengths found for the saponification products and the chain lengths measured on the whole wax. Also, the chain lengths of the prepared esters are in satisfactory agreement with the summed chain lengths of the component alcohol and acid fractions, except that of the ester of linseed alcohol with C_{22} acid.

The above general agreement is reached on the assumption that the chains are vertical to the basal planes. The conclusion is therefore justified that wax esters of the types in question, *i.e.* with both radicals or one radical longer than C_{22}, tend to crystallise in the rhombic form.

A further conclusion is that the fatty acid fractions of the *Musa* and linseed waxes have also assumed the rhombic form because if the long spacings of these are interpreted as spacings of forms with tilted chains, they yield a much longer chain length, and the summed chain lengths of alcohol and acid will then become too long. Fatty acids normally assume the tilted-chain B or C form.

We cannot explain the long spacing of the last of the prepared esters. Its deviating value is the more remarkable because the number of long chain reflections in the diagram is very high and, therefore, the ester appears to be very well crystallised. A similar case of an ester yielding a great number of long chain interferences in the diagram, but with a spacing too long to be regarded as a B spacing and too short for an A spacing, was encountered among the esters of Table V in the case of docosyl stearate. In both cases the long spacing lies nearer to the theoretical B than to the A spacing, though the side spacings do not point to the B form (*cf.* section III, 4).

None of the samples examined is of high purity, as may be seen from the generally considerable difference between the observed and theoretical melting points. That the long spacings nevertheless give good results as to the constitution of the esters, again demonstrates that the long spacing of a wax is a much more valuable constant than its melting point, even though for purity control the determination of the melting point is indispensable.

4. *Application of results from previous sections to diagrams of non-saponified waxes*

a. *Nature and chain length of main constituent*

In the following, using the results from the previous section, we discuss what details about the three non-saponified waxes result from their X-ray data. The types of compounds that might occur as main constituents are paraffins, ketones or secondary alcohols, primary alcohols or wax esters, these normally forming the main constituents of plant cuticle waxes.

As regards the *Musa* waxes, it is seen from Fig. 6 that the long chain interferences do not show a regular decrease in intensity from the lower to the higher orders as do those of the *n*-long chain paraffins. Therefore, paraffins have to be excluded as main constituents. The interferences of the *Strelitzia* wax show a rather regular decrease. But the long spacing, if belonging to paraffins, ketones or sec. alcohols, would correspond to a mixture of C_{35} and C_{37} chains, as may be calculated from (1a) in section II, taking into account that even-number members of the homologous series in question do not occur in plant waxes[1], or do occur but in small amounts[23]. These lengths have only once been observed in plant waxes[1]. Generally the chains are shorter. It is therefore very improbable that one of these products forms the main constituent of *Strelitzia* wax. It is still more improbable for the wax of *M. chinensis*, because its long spacing is still longer.

The long spacings of *M. brachycarpa* wax would correspond to a mixture of C_{33} and C_{35} chains if they belonged to a ketone or sec. alcohol. Evidence exists indeed for the occurrence of C_{33} ketone or sec. alcohol in certain waxes[2]. It is unlikely, however, that we are dealing with these substances in the above *Musa* waxes, firstly because there is no evidence of their presence in the other *Musa* waxes, and secondly because they are rarely found of this length. Paraffin, ketone and sec. alcohol are therefore excluded as main constituents in each of the three waxes.

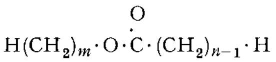
The remaining possibilities for the constitution of the three waxes are that they contain alcohols or esters as their main constituent. We suppose that in *M. brachycarpa* and *M. chinensis* wax the intensities of the second orders would be higher if mainly wax alcohol were present—*cf.* the diagrams of *Musa* and linseed alcohol fractions in Fig. 2—, while in *Strelitzia* wax the intensities are irreconcilable with the presence of alcohol as main constituent because the second order is stronger than the third. Most probably, therefore, the waxes consist of esters. This is supported by the fact that the *Musa* waxes investigated in more detail have been shown to consist mainly of wax ester. In that case the mean chain length of the esters, when calculated from (4a) in section 2, is as indicated in column 6 of Table VII.

b. *Position of the ester group in the chain*

Finally we want to examine whether the intensities of the long-chain reflections enable us to make suggestions concerning the chain lengths of alcohol and acid radical of the esters.

The intensity distribution among the successive orders of long chain reflections in ketones and wax esters is dependent on the position of the carbonyl group in the chain. The relation has been studied by SHEARER[22]. It was shown that if d is the long spacing and ϱd the distance of the centre of the carbonyl group from the nearest end of the molecule, the reflections with order numbers n/ϱ disappear. Hence, if ϱ is $\frac{1}{2}$ or $\frac{1}{3}$ or $\frac{1}{4}$ *etc.* the orders $2\,n$, or $3\,n$, or $4\,n$ *etc.* disappear.

It is noted that for simplicity, in the calculation of ϱ, the length in the chain direction occupied by the hydrogen atoms at the ends of the ester chain is taken as being equal to that of half a carbon atom. Hence, if m is the number of carbon atoms of the ester alcohol, n that of the ester acid, and the ester oxygen atom is taken as a carbon atom, ϱ is found as $\frac{m+2}{m+n+2}$, or as $\frac{n}{m+n+2}$. This is easily seen if the ester is written as follows:



$$H(CH_2)_m\cdot O\cdot \overset{O}{C}\cdot (CH_2)_{n-1}\cdot H$$

Then we find on the alcohol side of the centre of the carbonyl group a length $(\frac{1}{2} + m + 1 + \frac{1}{2})$C and on the acid side $(\frac{1}{2} + n - 1 + \frac{1}{2})$C. For $(m + 2) < n$ the first relation for ϱ has to be used, for $(m + 2) > n$ the second one.

The above effect is clearly demonstrated in our diagram of the ester of C_{12} alcohol with "linseed acid", where ϱ is about $\frac{1}{3}$ and, accordingly, the third order long chain reflection is very weak. It is further demonstrated in the diagram of tetradecyl palmitate in Fig. 4, in which $\varrho = 0.5$ and the even orders are almost extinguished. It will be observed, however, that also in dodecyl palmitate and tetradecyl and octadecyl stearate, with $\varrho = 0.47$, order 2 is very weak, whereas in hexadecyl stearate, again with $\varrho = 0.5$, it has a clearly higher intensity. From an extinguished second order we may not, therefore, conclude to a $C=O$ position exactly half way down the chain, and in case of a clearly visible second order this position can not be excluded. Consideration of the higher even orders is required for more conclusive evidence.

Since in our diagrams of native *Musa* waxes only the second and third order long chain reflections stand out sufficiently clear for a comparison of intensities, we have to confine ourselves to the intensity relation of these. In order to come, despite the above discrepancies, to an estimation of the position of $C=O$ on account of the intensities of these two reflections, we have been guided by the following reasoning. If order 2 is extinguished or weak and order 3 is strong for $\varrho = 0.50$, whereas the reverse is the case for $\varrho = 0.33$, they will be about equally strong at the intermediate value of ϱ, *i.e.* for $\varrho = 0.41^5$. On comparison with the intensity relation for $\varrho = 0.38$ in SHEARER's Fig. 6 (SHEARER, *loc. cit.*), this seems to be not far beyond the truth. Therefore, if order 2 is weaker than 3 we must select a value of $0.50 > \varrho > 0.41^5$ for the position of the carbonyl group and if order 3 is the weaker one a value of $0.41^5 > \varrho > 0.33$*.

This may also be checked on some of our own diagrams. Of particular importance in this respect are the diagrams of linseed alcohol stearate and linseed alcohol docosanoate, with resp. $\varrho = 0.38$ and $\varrho = 0.43$. It will be seen that in the former order 2 is stronger than 3, whereas in the latter (and in docosyl stearate, also with $\varrho = 0.43$) it is slightly weaker, in accordance with our assumption. It is further clear that in linseed wax the difference between orders 2 and 3 is greater than in the *Musa zebrina* waxes, because of the higher ϱ value of the former. On the other hand, in *M. balbisiana*, where the ϱ value is still higher, the difference is nearly the same as in the *M. zebrina* waxes. The latter case again demonstrates that not too much value should be attached to differences in the intensity relation of orders 2 and 3 when 2 is considerably weaker than 3.

It may be concluded that the ratio of the intensities of orders 2 and 3 of the long chain reflections in waxes with a high ester content permits at least a rough estimation of the position of the $C=O$ group in the chain: If order 2 is considerably weaker than 3, we find $\varrho > \sim 0.43$, if it is slightly weaker to slightly stronger, $\sim 0.43 > \varrho > \sim 0.38$, and if it is considerably stronger, $\varrho < \sim 0.38$. The chain lengths of alcohol and acid radical, however, remain unknown as long as no indication from other sources is available about which of the two is the longer.

In order to reach an estimation of the position of the $C=O$ group for the last three waxes of Table VII we have assigned a ϱ value to each of them on the basis of the ratio of the intensities of its second and third order interferences. On account of this value and considering that, according to the experience with the *Musa zebrina*, linseed and *Musa balbisiana* waxes, the alcohol will probably be the longer component, an approximate chain length composition for alcohol and acid may be suggested as mentioned in column 9.

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* For the sake of brevity values of $\varrho < 0.33$ are not taken into consideration since they do not occur among the prepared esters examined here and, because of the results with the saponified waxes, neither are they expected to occur in the non-saponified samples.

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SUMMARY

In order to investigate the relation of the chain length to the long crystal-spacing in wax esters, the X-ray long spacings have been studied in esters synthesised from some of the C_{16}–C_{22}, saturated *n*-fatty acids with *n*-primary alcohols in the range C_{12}–C_{22}, and in mixtures of such esters.

Certain of the esters were found to show a vertical chain position with reference to the lattice planes with long spacing, whereas in others the chains assume a tilted position. In the latter case, with few exceptions, the tilt was found to be the same as that known for the ethyl esters.

An ester assuming the tilted chain position requires a high contamination by one with vertical chains in order to obtain a mixture with vertical chains and *vice versa.*

Certain plant waxes with a high ester content as well as their saponification products have been examined in order to investigate whether analytical value may be attributed to the long spacings observed in such waxes.

Using data obtained by the study of the synthesised esters, evidence could be provided that the long spacings are indicative of the mean chain length of the ester component of the wax. The ester chains in the native waxes have the vertical position with reference to the lattice planes with long spacing.

Using the data and experience gained in the investigations mentioned above and applying SHEARER's data on the intensity distribution among the long chain interferences as connected with the position of the carbonyl group in ketone and ester chains, suggestions have been made concerning the constitution of the cuticle waxes from three *Musaceae* by evaluation of their X-ray diagrams alone.

RÉSUMÉ

Dans le but de rechercher la relation entre la longueur de chaîne et la grande périodicité cristalline des esters de cire, les grandes périodicités röntgenographiques d'esters synthétisés d'acides gras normaux saturées de C_{16} à C_{22} et d'alcools primaires normaux de C_{12} à C_{22} ont été étudiés, ainsi que les grandes périodicités de quelques mélanges de ces esters.

Pour certains de ces esters il résultait que les chaînes sont perpendiculaires aux plans réticulaires à grande périodicité, tandis que pour d'autres les chaînes sont obliques. Dans ce dernier cas, à quelques exceptions près, l'inclinaison observée est la même que celle connue pour les esters éthyliques.

Un ester possédant une position de chaîne oblique exige une contamination importante par un ester à chaînes verticales pour donner un mélange avec des chaînes verticales et *vice versa.*

Certaines cires végétales à teneur élevée en esters, ainsi que leurs produits de saponification, ont été examinées dans le but de rechercher quelle valeur analytique peut être attribuée aux grandes périodicités qu'on observe dans de telles cires.

A l'aide des résultats obtenus dans l'étude des esters synthétiques, les auteurs ont pu montrer que les grandes périodicités fournissent la longueur moyenne de chaîne des esters dans la cire. Les chaînes d'ester dans les cires natives sont verticales par rapport aux plans réticulaires à grandes périodicités.

Les auteurs ont recherché quelles conclusions relatives à la constitution des cires cuticulaires de trois *Musaceae* pouvaient être tirées de la seule étude de leurs diagrammes aux rayons X, en utilisant les résultats et l'expérience recueillis aux cours des recherches décrites plus haut et en appliquant les résultats de SHEARER sur la distribution d'intensité parmi les interférences de chaîne longue en relation avec la position du groupe carbonyle dans les chaînes de cétones et d'esters.

References p. 44.

ZUSAMMENFASSUNG

Um die Beziehungen zwischen der Kettenlänge und der langen Netzebenenabstand in Wachsestern festzustellen wurden die röntgenografisch erhaltenen langen Netzebenenabstände in synthetischen Estern, hergestellt aus einigen der gesättigten C_{16}-C_{22} *n*-Fettsäuren und *n*-primären C_{12}-C_{22} Alkoholen, untersucht und dieselben Abstände in Mischungen solcher Ester.

Bei einigen Estern wurde ein senkrechter Kettenstand in Bezug auf die Basisebene gefunden, während die Ketten anderer Ester eine schiefe Stellung einnehmen. In letzterem Falle wurde mit wenigen Ausnahmen festgestellt, dass die Neigung dieselbe war, als diejenige, die für Ethylester bekannt ist.

Einem Ester mit schiefer Kettenstellung muss eine grosse Menge eines Esters mit senkrechten Ketten beigemischt werden, um eine Mischung mit senkrechten Ketten zu erhalten und umgekehrt.

Es wurden bestimmte Pflanzenwachse mit hohem Estergehalt, sowie deren Verseifungsprodukte untersucht, um festzustellen, ob man den in solchen Wachsen beobachteten langen Netzebenenabstände irgendwelche analytische Bedeutung beimessen darf.

Auf Grund von Ergebnissen, welche beim Studium synthetischer Ester erhalten wurden, konnte gezeigt werden, dass die langen Netzebenenabstände der mittleren Kettenlänge der Esterkomponente des untersuchten Wachses entsprechen. Esterketten in nativen Wachsen nehmen eine vertikale Stellung in Bezug auf die Basisebenen ein.

Mit Rücksicht auf den Ergebnissen und Erfahrungen der obigen Studien, und durch Anwendung von SHEARER's Befunde bezüglich der Intensitätsverteilung bei Langketteninterferenzen im Zusammenhang mit der Lage der Karbonylgruppe in Keton und Esterketten, wurde untersucht, welche Angaben über die Zusammensetzung der Wachsüberzüge dreier *Musaceae* sich aus der ausschliesslichen Auswertung ihrer Röntgendiagramme ergeben.

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