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Composition of the leaf wax of Little Club wheat¹

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Leaf wax of Little Club wheat is composed of: hydrocarbons (8–15%); esters (20–26%); alcohols (25–42%); acids (2%); β -diketone (6–10%); hydroxy β -diketones (9–16%); and unidentified material (9–12%). The esters are mainly octacosanol esters of C₁₄ to C₃₂ fatty acids but lesser amounts of esters of *trans* 2-tetracosenoic and *trans* 2-docosenoic acids also occur. The free alcohols are almost entirely octacosanol. The β -diketone is hentriacontane-14,16-dione and the hydroxy- β -diketones are a 1:1 mixture of 8- and 9-hydroxyhentriacontane-14,16-diones.

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During investigations of the spores of wheat stem rust, spores were collected by washing infected wheat plants with light petroleum (1). It was then found that a good yield of wheat leaf wax could be recovered from the petroleum washings. This extraction procedure was applied to a number of wheat varieties and the yield of wax varied from 0.2 to 0.45% of the dry weight depending on the variety and maturity of the plants.

Apparently only one previous investigation of wheat leaf wax has been made. Pollard *et al.* (2) extracted an unspecified variety of wheat and reported that the principal component of saponified wax was octacosanol. Since large amounts of straw are potentially available in Western Canada, it seemed worthwhile to determine the composition of the wax and to find out whether or not it contained any useful components.

Horn and Lamberton (3) showed that β -diketones are major components of a number of plant waxes and that they can be detected and estimated by infrared (i.r.) and ultraviolet (u.v.) spectroscopy. We have found that they can also be readily detected by thin-layer chromatography (t.l.c.) since they rapidly give yellow spots on exposure to iodine vapor. Hydrocarbons, esters, free alcohols, and other hydroxy compounds can also be detected by t.l.c.

Waxes from 6 varieties of wheat were examined spectroscopically and by t.l.c., and that of Little Club (*Triticum compactum* Host.) contained the most even distribution of the different components. Though Little Club is not a commercial wheat variety it was convenient to

examine wax from it, since most of the components could be isolated in reasonable yield, rather than waxes from some other varieties which contained major proportions of just one or two components.

The approximate composition of Little Club wax collected in 1964 and 1965 is shown in Table I along with the yields and u.v. absorption characteristics. The 1965 wax, though harvested at approximately the same stage of growth, contained more alcohols and less β -diketones than the 1964 wax. It has been suggested recently (4) that some varieties of wheat produce wax containing alcohols in place of β -diketones, but growing conditions and maturity apparently must also be taken into account.

Separation of Components

β -Diketones were removed from the wax (1965) as copper complexes (3, 5) but part of the alcohols crystallized at the same time. The resulting mixture of β -diketones, alcohols, and hydroxy- β -diketones was separated chromatographically. Free acids were lost, presumably as copper salts. However, they were isolated in earlier experiments with 1964 wax (6).

Wax remaining after the above removal of β -diketones and part of the alcohols was separated into 20 fractions by a combination of column chromatography and crystallization as shown in Fig. 1. Similar fractions were combined and examined by gas-liquid chromatography (g.l.c.). The composition of the hydrocarbons (fraction 1) is shown in Table II; the principal components were saturated C₂₇–C₃₃ hydrocarbons with an odd number of carbon atoms.

Esters, Free Acids, and Free Alcohols

Analysis, by g.l.c., of esters obtained by combining fractions 6, 13, and 18 (Fig. 1),

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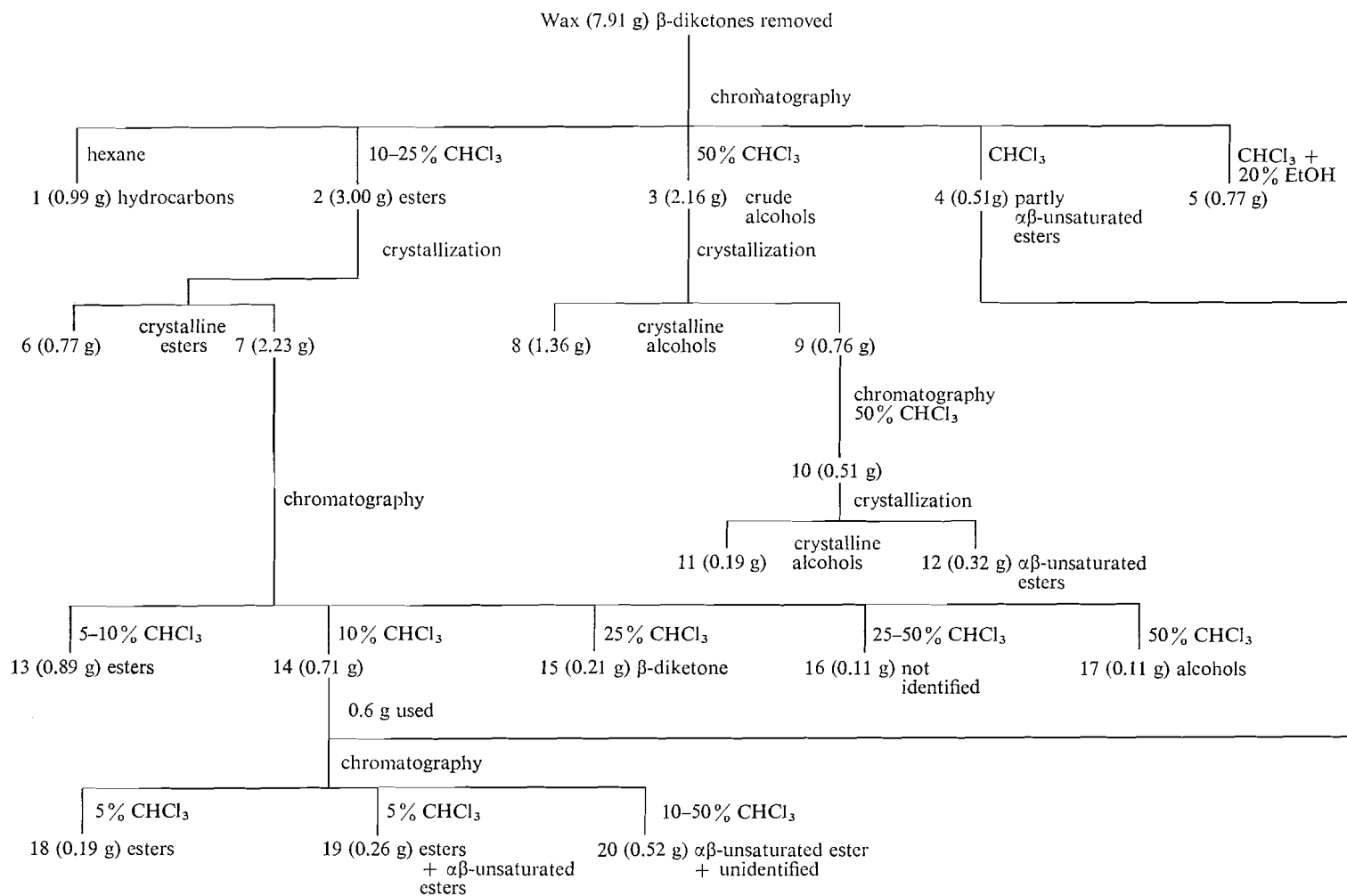


FIG. 1. Separation of wax after removal of β -diketones. Percentages refer to chloroform in hexane and crystallizations were from chloroform. Fractions were identified by thin-layer chromatography and nuclear magnetic resonance spectroscopy.

TABLE I
Approximate composition and yields of wax samples

Date of harvest	20-7-64	21-8-64	6-7-65
Yield (% of dry weight)	0.45	0.37	0.43
Ultraviolet absorption ($E_{1\text{cm}}^{1\%}$ at 273 m μ)	63	46	39
Hydrocarbons	15	*	8
Esters	20		26
Free alcohols	25		42
Free acids	2		†
β -Diketone	10		6
Hydroxy- β -diketone	16		9
Unidentified	12		9

*Composition of this sample was not determined.
†Could not be determined by the method used with this sample.

TABLE II
Composition of wax fractions

Number of C atoms	Hydrocarbons %	Esters %	Hydrolysis products of esters		Free* acids	Free alcohols
			Acids %	Alcohols %		
14	—	—	0.2	—	2.8	—
16	—	—	8.6	—	34.7	—
18	—	—	5.4	—	5.6	—
20	—	—	18.2	1.3	1.8	—
21	0.7	—	—	0.4	—	—
22	0.1	—	38.4†	5.7	21.4†	—
23	1.2	—	—	0.2	—	—
24	0.1	—	13.1‡	4.6	14.9‡	0.9
25	3.1	—	—	0.2	—	—
26	—	—	3.2	—	5.1	4.6
27	9.9	—	—	0.4	—	0.2
28	—	—	11.1	79.4	7.5	93.4
29	31.9	—	—	—	—	—
30	—	—	1.6	0.9	3.9	0.9
31	34.7	—	—	—	—	—
32	—	—	0.2	—	2.0	—
33	16.6	—	—	—	—	—
34	—	—	—	—	0.3	—
35	1.7	—	—	—	—	—
40	—	1.2	—	—	—	—
42	—	3.9	—	—	—	—
44	—	19.2	—	—	—	—
46	—	14.4	—	—	—	—
48	—	18.8	—	—	—	—
50	—	23.5	—	—	—	—
52	—	5.5	—	—	—	—
54	—	3.9	—	—	—	—
56	—	9.6	—	—	—	—

*From wax collected in 1964.
†Includes cf. 1% of *trans* 2-docosenoic acid.
‡Includes cf. 4.5 of *trans* 2-tetracosenoic acid.

showed that they consisted of C_{44} - C_{56} mono-esters. The major hydrolysis products were C_{16} - C_{30} fatty acids and octacosanol (Table II), showing that the original esters were octacosanol esters of these acids.

Small percentages of *trans* 2-tetracosenoic and

docosenoic acids were observed in the above acids during g.l.c. analysis, but much larger proportions of the two acids were present in the hydrolysis products of fractions 12, 19, and 20 (Table III). The structures of these acids were deduced from their g.l.c. retention times, the

TABLE III
Composition of hydrolysis products of fractions containing *trans* $\alpha\beta$ -unsaturated esters

Number of C atoms	Fraction 12		Fraction 19		Fraction 20	
	Acids %	Alcohols %	Acids %	Alcohols %	Acids %	Alcohols %
14	0.2	—	—	2.9	3.6	—
16	0.5	—	1.4	0.7	13.4	—
18	0.2	—	0.6	6.9	3.6	—
20	0.2	0.7	1.4	41.1	3.0	4.3
21	—	0.1	—	—	—	—
22	5.3	9.7	2.1	25.1	17.8	11.4
<i>trans</i> 22:1	39.6	—	18.0	—	15.2	—
23	—	0.1	—	—	—	—
24	4.5	24.9	3.0	7.7	6.5	5.7
<i>trans</i> 24:1	49.5	—	39.8	—	15.5	—
25	—	0.2	—	—	—	—
26	0.2	26.0	1.4	2.3	3.9	7.6
27	—	0.5	—	—	—	2.1
28	—	36.9	13.4	11.4	13.6	66.9
30	—	0.9	2.8	1.9	3.0	2.0
32	—	—	1.1	—	0.9	—
Unidentified	—	—	15.0(4)*	—	—	—
Yield on hydrolysis (as % of fraction weight)	45	36	47	15	60	12

*Number in parentheses refers to number of components.

nuclear magnetic resonance (n.m.r.) signals at 5.7 and 6.8 p.p.m., characteristic of acids with *trans* 2,3-unsaturation (7, 8), and the fact that oxidation with the permanganate-periodate reagent (9) yielded saturated C₂₀ and C₂₂ acids. *trans* 2-Tetracosenoic and docosenoic acids do not seem to have been isolated from natural sources before though a number of *trans* $\alpha\beta$ -unsaturated C₁₀ acids have been isolated from bees (7, 10).

Fractions 12, 19, and 20, which were eluted just after the monoesters or along with the alcohols (Fig. 1), appeared from t.l.c. to be mixtures of several components. The hydrolysis products are listed in Table III. Difunctional components such as diols or hydroxy acids, the presence of which could account for the low yields of alcohols, were not detected. The structures of the parent compounds could not be completely elucidated, but part probably consists of esters of *trans* 2-tetracosenoic and docosenoic acids with C₂₀-C₂₈ alcohols.

It is of interest that the *trans* $\alpha\beta$ -unsaturated acids are found only as C₂₂ and C₂₄ compounds whereas the acids of the other ester fractions range from C₁₄ to C₃₂. When all ester fractions are considered together, the approximate % of esters containing *trans* 2-docosenoic acid is 5%

and of esters containing *trans* 2-tetracosenoic acid is 10%.

The free fatty acids in Table II are similar to those of the esters except that palmitic acid is the major component. These acids were isolated from wax collected in 1964 whereas the other analyses were made on material collected in 1965. However, hydrolysis of the total ester fraction of the 1964 wax gave acids with the composition: C₁₄ 2.4; C₁₆ 9.8; C₁₈ 4.5; C₂₀ 9.9; C₂₂ 20.1; *trans* C_{22:1} 10.2; C₂₄ 8.4; *trans* C_{24:1} 15.5; C₂₆ 3.1; C₂₈ 8.4; C₃₀ 4.5; C₃₂ 2.8; C₃₄ 0.4%, which is not very different from that of the 1965 esters.

The composition of the free alcohols is similar to that of the alcohols of the esters but an even higher proportion of octacosanol is present (Table II).

β -Diketone

Only one β -diketone, hentriacontane-14,16-dione, was present. The chain length was established by reduction to hentriacontane (3). Alkaline hydrolysis gave equal amounts of pentadecan-2-one and heptadecan-2-one and of myristic and palmitic acids. This diketone has not previously been isolated in the pure state though it occurs as a minor component of the mixed β -diketones of leaf wax of two species of

Eucalyptus (5). Wax of the grass *Festuca glauca* contained mainly tritriacontane-12,14-dione (5).

Hydroxy- β -diketones

These components also gave only hentriacontane on reduction. The n.m.r. spectrum showed that a secondary hydroxyl group was present which was at least 4 methylene groups removed from the diketone group (11). The neutral fraction obtained by alkaline hydrolysis was a mixture of heptadecan-2-one and hydroxypentadecan-2-ones, which were separated on a silicic acid column. The acidic fraction was shown by g.l.c. to be a mixture of palmitic and hydroxymyristic acids, which as methyl esters, were also separated chromatographically.

Oxidation of the hydroxy esters with chromium trioxide at 25° (12) gave an oxomyristate which appeared to be a mixture of 6- and 7-oxomyristates (g.l.c. using QF-1 column (13)). Oxidation with chromium trioxide at 100° (12), when cleavage occurs on both sides of the oxygenated carbon atom, gave the monocarboxylic acids heptanoic, octanoic, and nonanoic approximately in the ratio 1:2:1, and the dicarboxylic acids glutaric, adipic, and pimelic also in the ratio 1:2:1, showing that an approximately equimolar mixture of 6- and 7-hydroxymyristates had been oxidized.

The n.m.r. spectrum of the hydroxymyristates in quinoline was indistinguishable from that of a 1:1 mixture of synthetic 6- and 7-hydroxymyristates. It was shown previously (11) that, using quinoline as solvent, the center of the α -CH₂ signal of 6-hydroxystearate appears at ca. 0.1 p.p.m. downfield from the corresponding signal of 7-hydroxystearate. The hydroxymyristates showed signals at both positions and the rest of the spectrum also agreed with the proposed structure. This was confirmed by the mass spectrum of the hydroxymyristates which showed peaks at m/e 145 (also shown by methyl 6-hydroxymyristate) and at m/e 159 (also shown by methyl 7-hydroxymyristate). Mass spectra of the corresponding hydroxystearates (14) show that the peak at m/e 145 is due to the ion $^+\text{CHOH}(\text{CH}_2)_4\text{CO}_2\text{CH}_3$ and at m/e 159 is due to the ion $^+\text{CHOH}(\text{CH}_2)_5\text{CO}_2\text{CH}_3$. The hydroxymyristates have a positive rotation suggesting the L-configuration (15, 16).

We conclude that the hydroxy- β -diketone fraction of Little Club wax consists of an

approximately 1:1 mixture of 8- and 9-hydroxyhentriacontane-14,16-diones.

Hydrolysis of the most polar wax fraction (5, Fig. 1) gave palmitic acid as 15% of the fraction and a brown gum from which no definite compounds could be isolated. No diols or hydroxy acids could be detected. The unidentified portion of the wax consists of material of indefinite composition from fraction 5 above and of materials not recovered from the chromatographic columns.

The compositions of the wax components have certain features of interest. The hydrocarbons are composed mainly of 4 chain lengths C₂₇–C₃₃, whereas the other components with an odd number of carbon atoms, the β -diketones, have a chain length of 31 carbons only. The lack of relation between chain length of hydrocarbons and β -diketones was also observed with waxes of *Eucalyptus* species (5). The compositions of the free acids and free alcohols are fairly similar to those of the acids and alcohols of the esters. In many studies of waxes these pairs of components have not been examined separately. However, the acids contain a wide variety of chain lengths but the alcohols are composed largely of octacosanol. These differences and also the finding of unsaturated C₂₂ and C₂₄ acids, suggest the operation of several different biosynthetic pathways. Diols and hydroxy acids, which are frequently isolated from plant waxes (17) are apparently absent. Hydroxy- β -diketones have not previously been found in waxes, though hydroxy-ketones are present in cabbage wax (18).

Experimental

Silicic acid (Bio-Sil A from Bio-Rad Laboratories) was used for column chromatography, after activation at 120° for 18 h, and silica gel G for t.l.c. Typical R values found by t.l.c. of representative compounds in benzene and chloroform respectively were: triacontane 0.86, 0.74; octadecanol octadecanoate 0.68, 0.70; methyl octacosanoate 0.40, 0.49; β -diketone 0.38, 0.45; octacosanol 0.07, 0.15; hydroxy- β -diketones 0.02, 0.06; octacosanoic acid 0.00, 0.00. Chloroform was the most satisfactory solvent for t.l.c. although it sometimes did not separate hydrocarbons and wax esters. Iodine vapor was used to detect β -diketones, which rapidly gave dark orange spots, and the other components were detected by spraying with 50% sulfuric acid and heating.

Nuclear magnetic resonance spectra were measured at 100 Mc.p.s. using a Varian HA-100 spectrometer. Chemical shifts are in parts per million (p.p.m.) from tetramethylsilane (internal standard). Carbon tetrachloride was used as solvent except where otherwise

stated. Specific rotations were measured at 25° using a Perkin Elmer model 141 polarimeter. Infrared spectra were recorded in carbon tetrachloride (5% solution) in a 0.1 mm cell. Mass spectra were determined by the Morgan-Schaffer Corporation, Montreal.

Gas-Liquid Chromatography

Gas-liquid chromatography (g.l.c.) was carried out using: (A) an F and M model 402 gas chromatograph with flame ionization detectors fitted with 3 ft × 1/4 in. glass columns packed with 20–30 mesh glass beads coated with 0.3% silicone SE 30 and having a flow rate of 45 ml of helium/min, with the temperature programmed at 2°/min from various temperatures between 100 and 200° to 325° depending on the samples being analyzed; (B) an F and M model 5750 gas chromatograph with a flame ionization detector fitted with an 8 ft × 1/4 in. stainless steel column packed with 5% w/w 1,3-propanediol succinate (prepared by Dr. B. M. Craig) on 60–80 mesh acid washed chromosorb W operated isothermally at 215° with a flow rate of 40 ml/min; and (C) a unit of conventional design with thermal conductivity detectors and a 3 ft × 1/4 in. copper column packed with 5% silicone SE 30 on 60–80 mesh acid washed celite.

When synthetic, even numbered long chain alcohol esters of long chain acids were analyzed by unit (A) and the temperature programmed from 200–325°, a plot of retention temperature against carbon number (19) was almost a straight line making possible the measurement of the carbon numbers of the wax esters. The results were confirmed by re-analysis after addition of synthetic esters of various chain lengths to the mixture. Also, analysis of mixtures of synthetic esters showed that correction factors were not required to calculate composition. Unit (A) programmed from 100 to 275° was used to analyze hydrocarbons, alcohols, and methyl esters. Hydrocarbons and methyl esters were also analyzed using units (B) and (C); alcohols and their acetates were also analyzed on unit (C); carbon numbers were determined from isothermal analyses.

Synthesis of Octacosanol and Other Model Compounds

13-Oxo-octacosanoic acid (19.8 g) was synthesized from 1-morpholino-1-cyclododecene (20) (17.5 g) and hexadecanoyl chloride (13.7 g) by the method previously used to prepare 13-oxodocosanoic acid (21). After crystallization from ethyl acetate, the m.p. was 100.5–101.5 (lit. (22), 99.5°). Wolf-Kishner reduction of the oxo acid (18 g) and crystallization of the product from chloroform gave octacosanoic acid (10 g), m.p. 89.5–90.5 (lit. (22), 90.2°). Reduction of octacosanoic acid with lithium aluminum hydride in tetrahydrofuran gave octacosanol in high yield. The alcohol was crystallized from chloroform, m.p. 82.5–83.5 (lit. (22), 82.5°); the acetate had a m.p. 64°.

Other long chain alcohols were prepared by reduction of the corresponding acids which were commercial products. Even-numbered hydrocarbons with 26 and 34 carbons were prepared by the method previously used to prepare tetracosane (23). The synthesis and properties of long chain wax-like esters will be described elsewhere.

Isolation and Properties of the Wax

Plants of *Triticum compactum* Host. var. Little Club

were grown outdoors and collected 4–6 weeks before maturity (generally just before the heads appeared and when green leaf formation was greatest). One sample was also collected from mature straw. The plants were swirled in light petroleum b.p. 40–60° for about 10 s (cf. 1) and the extract filtered and evaporated to dryness.

Further washings generally yielded only a small amount of wax with a composition similar to that of the first extract, though sometimes later extracts seemed to contain higher proportions of polar, pigmented material. Purdy and Truter (24) found that repeated extraction of cabbage leaves with different solvents gave wax samples which all had similar compositions. Some wax components, as pure compounds, were poorly soluble in light petroleum but, as mixtures in the wax, were quite soluble.

The melting point of the wax was 42–62°. The i.r. spectra had a broad peak at ca. 1600 cm⁻¹ (β -diketone), a small shoulder at 1655 cm⁻¹ (double bond of $\alpha\beta$ -unsaturated ester), broad carbonyl absorption at 1705–1730 cm⁻¹, and a small OH peak at 3670 cm⁻¹; the peak at 1655 cm⁻¹ was most pronounced in the spectrum of wax from mature straw. Thin layer chromatography indicated the presence of hydrocarbons, esters, β -diketone, alcohols, hydroxy β -diketones, and a trace of free acids.

Separation of Components

β -Diketones were separated from the wax (14.2 g, collected 6-7-65) as copper complexes, as described by Horn *et al.* (5), but some alcohols also crystallized with the complex. The mixture (4.6 g) obtained after decomposition of the complex was chromatographed on silicic acid giving β -diketone (0.73 g) (elution with hexane-chloroform 3:1), alcohols (1.61 g), and a mixture of alcohols and hydroxy- β -diketones (2.0 g) (elution with hexane-chloroform 1:1). The mixed fraction gave alcohols (0.3 g) on crystallization from chloroform and the mother liquors, after two further chromatographic separations, gave alcohols (0.64 g) and hydroxy- β -diketones (0.98 g).

When wax collected on 20-7-64 was separated on a column without removing diketones first (6), free acids were found in the alcohol fraction. Treatment with diazomethane and rechromatography on silicic acid (elution with hexane-chloroform 3:1) gave methyl esters of the free acids (0.044 g from 5.7 g wax).

Determination of Structure of Components of Wax

Hydrocarbons

Analysis of fraction 1 (Fig. 1) by g.l.c. and examination by n.m.r. spectroscopy showed that the principal components were saturated, straight chain compounds (25). Chromatography on a silver nitrate-silicic acid column (26) gave only a small fraction (4% of total hydrocarbons) which appeared (by n.m.r.) to be partly unsaturated hydrocarbons.

Saturated esters

Fractions 6, 13, and 18 (Fig. 1) were combined and analyzed by g.l.c. using unit (A) and programming from 250–325°. By adding eicosanol eicosanoate as internal standard (27), components found by g.l.c. were shown to compose 91% of the combined fractions. Esters (1.746 g) were refluxed for 22 h with ethanol (125 ml) containing 5% hydrogen chloride; the mixture was then poured into water, extracted with chloroform and dried over sodium

sulfate. The product (1.852 g) was examined by t.l.c. and showed only traces of unhydrolyzed material; there were no spots corresponding to diols or hydroxy esters.

The product was chromatographed on silicic acid (200 g) and elution with hexane - 10% chloroform gave hydrocarbons (0.057 g) and unhydrolyzed esters (0.068 g). Elution with hexane - 25% chloroform gave ethyl esters (0.685 g) and with hexane - 50% chloroform, first an unidentified component (0.065 g) with R_f 0.3 (chloroform) and then alcohols (0.956 g). Ethyl esters were converted to methyl esters by refluxing with methanolic hydrogen chloride (6%) for 20 h. Methyl esters and alcohols were analyzed by g.l.c. giving the results shown in Table II. A portion of the alcohols was acetylated and the relative intensities of the n.m.r. signals (in chloroform, with chloroform proton as lock signal) confirmed that they were straight chain, saturated, primary alcohols.

Fraction 5 was hydrolyzed, after ethanolsis, and gave acids (0.104 g) consisting mainly of palmitic acid but no other definite compounds were isolated.

$\alpha\beta$ -Unsaturated esters

Fraction 12

Analysis by t.l.c. showed a major spot with R_f 0.18 (benzene) and 0.55 (chloroform) and a minor spot in the free alcohol region. Analysis by g.l.c. showed small C_{22} to C_{28} alcohol peaks and a single very broad peak in the C_{52} to C_{62} ester region. The n.m.r. spectrum showed signals for free primary alcohols (triplet 3.5 p.p.m., c 1 proton), acylated primary alcohols (triplet 4.00 p.p.m., c 2 protons), and for 2 protons of a *trans* double bond $\alpha\beta$ to an ester group (H-2, doublet, 5.70 p.p.m.; $J = 16$ c.p.s.; H-3, 2 triplets, 6.82 p.p.m., $J = 16$ c.p.s.).

Fraction 19

Analysis by t.l.c. (benzene) showed a major spot at R_f 0.65 and a minor spot at 0.40; in chloroform there were spots at R_f 0.70 and 0.50. Analysis by g.l.c. showed peaks with retention times of C_{42} , C_{44} , and C_{46} esters. The n.m.r. spectrum was similar to that of fraction 12 except that the free alcohol $-\text{CH}_2\text{OH}$ signal was missing and the other signals were weaker and less well-defined.

Fraction 20

Analysis by t.l.c. (chloroform) showed 6 ill-defined spots, 4 of which had R_f 's intermediate between those of wax ester and of alcohol and the other two were slower. No peak was obtained by g.l.c. using unit (A) at 350°. The n.m.r. spectrum was similar to that of fraction 19.

On ethanolsis, as above, fraction 12 (0.280 g) gave esters (0.130 g) and alcohols (0.104). The i.r. spectrum of the methyl esters was very similar to that of methyl *trans* 2-octadecenoate. The carbon numbers of the two principal components of the methyl esters were 22.4 and 24.4 (silicone column) and 23.35 and 25.4 (polyester column). Methyl *trans* 2-octadecenoate had carbon numbers 18.4 and 19.35 using the same two columns.

Oxidation of the methyl esters of fraction 12 with the permanganate-periodate reagent (9, 28) gave a mixture of monocarboxylic acids with the composition C_{20} , 39.3%; C_{22} , 56.7%; and C_{24} , 4.0% (g.l.c. analysis as methyl esters using two different columns).

On ethanolsis, fraction 19 (0.250 g) gave esters (0.117 g) and alcohols (0.037 g) and an unidentified

product (0.042 g) (R_f 0.3 (CHCl_3)). Ethanolsis of fraction 20 (0.510 g) gave esters (0.300) and alcohols (0.060 g) and unidentified gum (0.104 g). The compositions of methyl ester and alcohol fractions are given in Table III.

β -Diketone

The β -diketone was crystallized from ethyl acetate and had m.p. 58-59° and u.v. absorption maximum at 273 μ (ϵ 12 125). The i.r. spectrum had a strong band at 1605 cm^{-1} . Nuclear magnetic resonance: terminal CH_3 0.87 (6 protons); methylene groups, 1.25; α - CH_2 (triplet, 2.19 (4 protons)); CH of enolic form (singlet, 5.31 (0.9 protons)).

Anal. Calcd. for $C_{31}H_{60}O_2$: C, 80.10; H, 13.01. Found: C, 79.92; H, 12.80.

β -Diketone (1.0 g) was refluxed with 4% ethanolic potassium hydroxide for 18 h when water was added; extraction with hexane gave neutral product (0.53 g). The aqueous layer was then acidified and the acidic product (0.52 g) extracted with ether. Analysis by g.l.c. (unit (C)) showed that the neutral product consisted of equal amounts of pentadecan-2-one and heptadecan-2-one and the acidic product of equal amounts of myristic and palmitic acids.

The ketones were separated in batches by g.l.c. (unit (C) at 180°) and gave equal weights of pentadecan-2-one, m.p. 37.5-38.5°, the mixed m.p. with synthetic ketone (m.p. 37.5-38.5, lit. (29) gives 39°) was 38.0-38.5°, and heptadecan-2-one, m.p. 47.5-48.5°, the mixed m.p. with synthetic ketone (m.p. 48-48.5°, lit. (29) gives 48°) was 47.5-48.5°. Authentic methyl ketones were synthesized by alkaline hydrolysis of methyl 3-oxohexadecanoate and 3-oxooctadecanoate (30, 31). The acids were converted to methyl esters and separated in the same way giving equal weights of methyl myristate and methyl palmitate. Hydrolysis yielded myristic acid, m.p. and mixed m.p. 51.5-53°, and palmitic acid, m.p. and mixed m.p. 62-63°.

Reduction of the β -diketone (3, 32) gave only hentriacontane (g.l.c. using unit (C) at 250°).

Free Alcohols

Fractions 8, 11, and 17 (Fig. 1) were combined with alcohols separated from the β -diketone fraction and analyzed by g.l.c.; a portion was also acetylated and reanalyzed. Crystallization from chloroform gave almost pure octacosanol (99% by g.l.c.), the m.p. was 81.5-82.5° and mixed m.p. with synthetic octacosanol (m.p. 82.5-83.5°) was 82-83°.

Hydroxy- β -diketones

Hydroxy- β -diketones were crystallized from ethyl acetate and had m.p. 70-72° and $[\alpha]_D + 0.99$, $[\alpha]_{546} + 1.19$, $[\alpha]_{436} + 1.94$, $[\alpha]_{365} + 2.91$ (c , 9.3 in chloroform). The u.v. absorption maximum was at 273 μ (ϵ 12 410) and the i.r. spectrum had bands at 1602 (s) and 3660 (w) cm^{-1} . Nuclear magnetic resonance: CH of CHOH (multiplet) 3.43, CH of enolic form (singlet) 5.30 and one hydroxylic proton removed by D_2O .

Anal. Calcd. for $C_{31}H_{60}O_3$: C, 77.44; H, 12.58. Found: C, 77.39; H, 12.47.

On hydrolysis as before, hydroxy- β -diketones (0.76) gave acidic (0.39 g) and neutral (0.40) fractions. After conversion of acids to methyl esters, g.l.c. analysis (unit (C), 220°) showed only one peak for each fraction

but after acetylation both fractions showed 2 approximately equal peaks differing by 2 carbon numbers (cf. 12 and 13).

The acidic fraction, as methyl esters, was chromatographed on a silic acid column. Elution with hexane - 4% ether gave methyl palmitate (0.19 g) which on hydrolysis gave palmitic acid, m.p. and mixed m.p. with authentic acid 61-62°. Elution with hexane - 5% acetone yielded methyl hydroxymyristates. After crystallization from hexane these esters had m.p. 31-33° and $[\alpha]_D +1.33$, $[\alpha]_{546} +1.51$, $[\alpha]_{436} +2.54$ and $[\alpha]_{365} +3.86$ (c, 7.4 in chloroform). Nuclear magnetic resonance (quinoline): the signal in the α CH₂ region contained peaks at 2.38 and 2.30 corresponding to the central portion of the α CH₂ signal in spectra of 6- and 7-hydroxymyristates respectively. The rest of the spectrum was indistinguishable from that of a 1:1 mixture of synthetic methyl 6- and 7-hydroxymyristates (13).

Anal. Calcd. for C₁₅H₃₀O₃: C, 69.72; H, 11.70. Found: C, 69.39; H, 11.47.

Hydrolysis gave hydroxymyristic acids which, after crystallization from hexane had m.p. 61.5-62.5 (neutralization equiv. 247; calcd. for C₁₄H₂₈O₃, 244).

Chromic acid oxidation at 25° (12) gave oxo acids with m.p. 53-53.5° which was unchanged by repeated crystallization from hexane (6-oxotetradecanoic acid has m.p. 70.5-71.5 and 7-oxotetradecanoic acid has m.p. 67.5-68° (13)). When analyzed by g.l.c. using a QF-1 column (conditions used with methyl oxooctadecanoates (13)), two partly resolved peaks of equal size with retention times of 21.4 and 22.3 min were obtained (methyl 6-oxo and 7-oxotetradecanoates had retention times of 21.5 and 22.3 min respectively).

The mixed oxo esters were oxidized with chromic oxide at 100° (12) and the product converted to methyl esters. Analysis by g.l.c. (QF-1 column) showed the presence of C₇, C₈, and C₉ monocarboxylic esters (retention times 1.65, 2.4, and 3.3 min) and C₅, C₆, and C₇-dicarboxylic esters (retention times 4.8, 7.0, and 9.9 min). The molar percentages were C₇ 26.7; C₈ 46.7; C₉ 26.6 and C₅ 25.0; C₆ 50.6; C₇ 24.4. When the oxidation product was saponified, steam distilled, and mono- and dicarboxylic acids analyzed separately, the same acids were found as before showing that decanoic and succinic acids, which have retention times (as methyl esters), close to those of glutaric and nonanoic acids respectively, were absent.

The neutral fraction isolated on hydrolysis of the hydroxy β -diketones was separated into two approximately equal fractions as above. The first, heptadecan-2-one, was crystallized from methanol and had m.p. and mixed m.p. 47-48°. The second, 7- and 8-hydroxypentadecan-2-ones, had m.p. 39-53°, and $[\alpha]_D +1.06^\circ$, $[\alpha]_{546} +1.22^\circ$, $[\alpha]_{436} +2.10^\circ$, $[\alpha]_{365} +3.12^\circ$ (c, 7.5 in chloroform).

Anal. Calcd. for C₁₅H₃₀O₂: C, 74.32; H, 12.48. Found: C, 74.56; H, 12.45.

Reduction of the hydroxy- β -diketones as before gave only hentriacontane.

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