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LEAF WAX OF *TRITICUM AESTIVUM**

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Key Word Index—*Tritium aestivum*; Gramineae; Spring wheat; leaf wax; *trans* 2-docosen-1-ol; hydroxy-β-diketones.

Abstract—Leaf waxes from spring wheat varieties Selkirk and Manitou contain hydrocarbons (6%, 10%), long chain esters (14%, 13%), free acids (5%, 8%), free alcohols (19%, 21%), β-diketone (16%, 20%), hydroxy β-diketones (8%, 10%), unidentified gum (29%, 16·5%) and minor amounts of diol diesters, glycerides and aldehydes. The major hydrocarbon is nonacosane and major esters are octacosyl esters of C_{14}–C_{32} acids but C_{20} and C_{22} alcohol esters of *trans* 2-docosenoic and tetracosenoic acids are also present (Selkirk 20%, Manitou 10% of total esters). Previously unknown *trans* 2-docosen-1-ol is present as an ester (Selkirk 5%, Manitou 2·5% of total esters). Free acids are C_{14}–C_{32} acids and *trans* 2-docosenoic and tetracosenoic acids (Selkirk 30%, Manitou 9% of free acids). Octacosanol is the principal free alcohol. Hentriacontane-14,16-dione is the β-diketone and the hydroxy β-diketones are a 1:1 mixture of 8- and 9-hydroxyhentriacontane-14,16-diones.

INTRODUCTION

LEAF waxes of a club wheat[1] and of two durum wheats[2] were analysed previously. The wheat varieties most commonly grown in Canada, however, are varieties of *Triticum aestivum* L. (Gramineae) having the spring growth habit. Accordingly, in continuation of an investigation of differences between waxes of species and varieties of plants important in agriculture, the composition of the waxes of two varieties of spring wheat have been determined. The varieties Selkirk and Manitou were examined because they are not closely related by breeding, having only about half their parents in common.

Since one objective of these investigations was isolation and identification of possibly useful wax components, plants were extracted at the stage which gave the greatest yield of wax (when the flag leaf was completely formed). This paper describes the separation, analysis and identification of the wax components; variation in composition with growth stage is reported in the following paper.[3]

RESULTS

The two waxes have similar compositions (Table 1), though Manitou contains slightly more free acids and β-diketones. The larger proportion of β-diketones is also indicated by the more intense UV absorption at 273 nm shown by Manitou wax (see Ref. 4). Major wax components were separated on a silicic acid column as before[2] and analysed by GLC. The compositions are given in Tables 2 and 3.

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* Issued as NRCC No. 13174.

[1] TULLOCH, A. P. and WEENINK, R. O. (1969) *Can. J. Chem.* **47**, 3119.

[2] TULLOCH, A. P. and HOFFMAN, L. L. (1971) *Phytochemistry* **10**, 871.

[3] TULLOCH, A. P. and HOFFMAN, L. L. (1973) *Phytochemistry* **12**, 2225.

[4] HORN, D. H. S., KRANZ, Z. H. and LAMBERTON, J. A. (1964) *Australian J. Chem.* **17**, 464.

Hydrocarbons are similar with nonacosane the major component. Esters range from C_{38} to C_{60} and unsaturated components, mainly C_{42}, C_{44} and C_{46} were detected by examining the esters before and after hydrogenation. Because these components have emergence temperatures sufficiently greater than those of the corresponding saturated esters that partial resolution is obtained using a silicone column, they are most probably esters of *trans* 2-docosenoic and *trans* 2-tetracosenoic acids. (Methyl esters of these acids have greater retention times than C_{22} and C_{24} saturated esters and are completely resolved from these esters by GLC[1].) The presence of these unsaturated esters was confirmed by the appearance of appreciable amounts of unsaturated C_{22} and C_{24} ethyl esters in the ethanolysis products. Long chain unsaturated esters formed almost 20% of the esters of Selkirk wax but only about 10% of Manitou wax esters. Since the esters are C_{42}–C_{46} and the component acids presumably C_{22} and C_{24} they must be selectively esterified by C_{20}–C_{24} alcohols and not with octacosanol which is the major alcohol obtained on hydrolysis. Fractions rich in α,β-unsaturated esters were previously obtained from Little Club wax and gave mainly the shorter alcohols on ethanolysis.[1] The longer chain C_{48}–C_{56} esters are probably mainly octacosyl esters of C_{20}–C_{28} acids.

TABLE 1. COMPOSITION (%)* AND YIELDS OF LEAF WAXES OF SPRING WHEAT

Component	Variety Selkirk	 Manitou
Hydrocarbons	6	10
Esters	14	13
Free Alcohols	19	21
Free Acids	5	8
β-Diketone	16	20
Hydroxy β-diketones	8	10
Diol diesters	3	1
Glycerides	?	1
Aldehydes	—	0·5
Unidentified fractions		
Eluted between β-diketones and alcohols	3	4
Eluted after hydroxy β-diketones	21	12·5
Held on column	5	—
Yield (% of dry wt)	0·5	0·5
$E^{1\%}_{1\,\mathrm{cm}}$ at λ_{max} 273 nm (isooctane)	61	75

* Calculated from the weights of components obtained by silicic acid chromatography.

In addition long chain esters of *trans* 2-docosen-1-ol are most probably present, forming about 5% of Selkirk esters and 2·5% of Manitou esters. This alcohol was detected by the appearance of what was presumably 3-ethoxy-1-docosene in the ethyl esters isolated after acid ethanolysis of the long chain esters. The ether is no doubt formed during ethanolysis by allylic rearrangement and solvolysis of the 2,3-unsaturated alcohol.[5] The ether had a carbon number of 20·5 using a silicone column and 20·4 using a 1,3-propanediol succinate column. Ethyl *trans* 2-eicosenoate (with which the ether might be confused) would have a similar carbon number on the silicone column but a carbon number of 21·4 on the polyester column.[1]

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[5] BRAUDE, E. A. (1950) *Quart. Rev.* (*London*) **4**, 404.

TABLE 2. COMPOSITION* OF WAX FRACTIONS FROM SELKIRK WHEAT

No. of C atoms	Hydrocarbons	Esters	Hydrolysis products of esters		Free acids	Free alcohols
			Acids	Alcohols		
14	—	—	0·5	—	1·1	—
16	—	—	5·5	—	9·4	—
18	—	—	5·2	1·5	2·3	—
20	—	—	12·9	10·6	1·4	—
22	—	—	21·4	15·6	14·7	1·5
Trans 22:1	—	—	13·0	10·0	14·5	—
23	2·4	—	—	—	—	—
24	—	—	7·7	6·8	9·8	3·2
Trans 24:1	—	—	19·4	—	18·0	—
25	3·6	—	—	—	—	—
26	—	—	3·4	3·2	3·8	3·3
27	12·0	—	—	0·9	—	0·3
28	—	—	6·6	47·6	14·9	90·7
29	45·8	—	—	1·4	—	—
30	—	—	1·0	1·4	5·1	1·0
31	23·8	—	—	—	—	—
32	—	—	—	—	1·4	—
33	6·1	—	—	—	—	—
34	—	—	—	—	0·6	—
38	—	2·4	—	—	—	—
Trans 38:1	—	1·1	—	—	—	—
40	—	3·7	—	—	—	—
Trans 40:1	—	1·4	—	—	—	—
42	—	5·0	—	—	—	—
Trans 42:1	—	5·1	—	—	—	—
44	—	13·5	—	—	—	—
Trans 44:1	—	8·3	—	—	—	—
46	—	9·3	—	—	—	—
Trans 46:1	—	4·0	—	—	—	—
48	—	9·9	—	—	—	—
50	—	12·2	—	—	—	—
52	—	4·3	—	—	—	—
54	—	3·1	—	—	—	—
56	—	11·9	—	—	—	—
58	—	3·7	—	—	—	—
60	—	1·1	—	—	—	—
Unidentified	6·3	—	3·4	1·0	3·0	—

* In % by wt, obtained by GLC.

Proof of the presence of *trans* 2-docosen-1-ol was obtained using esters from wax of ripe Selkirk wheat. Fractionation of these esters on a silicic acid column gave a fraction almost entirely composed of C_{42}, C_{44} and C_{46} unsaturated esters.[3] Acid ethanolysis gave the ether, eicosanol and docosanol in the approximate ratio 1:2:1 in addition to C_{22} and C_{24} unsaturated esters. When the esters were first hydrogenated and then subjected to ethanolysis, eicosanol and docosanol (1:1) were formed, no ether was produced but a few % of hydrogenolysis product, presumably docosane, appeared. Oxidation of the esters with permanganate-periodate followed by acid methanolysis and analysis of the products by GLC[6] showed eicosanol and docosanol (2:1) and also methyl eicosanoate in the amount to be

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[6] TULLOCH, A. P. and CRAIG, B. M. (1964) *J. Am. Oil Chemists' Soc.* **41**, 322.

expected if about half the C_{22} alcohol originally present in the esters contained a 2,3-double bond. Methyl esters of C_{20} and C_{22} acids also resulted from oxidation of the unsaturated C_{22} and C_{24} acids in the proportions expected from the ethanolysis experiments.

TABLE 3. COMPOSITION *OF WAX FRACTIONS FROM MANITOU WHEAT

			Hydrolysis products of esters			
No. of C atoms	Hydrocarbons	Esters	Acids	Alcohols	Free acids	Free alcohols
14	—	—	1·5	—	6·5	—
16	—	—	13·9	—	19·8	—
18	—	—	8·6	0·9	5·1	—
20	—	—	15·6	3·0	1·6	—
22	—	—	27·3	10·8	13·2	1·1
Trans 22:1	—	—	3·8	5·0	4·0	—
23	1·2	—	—	—	—	—
24	—	—	7·6	7·3	8·6	2·5
Trans 24:1	—	—	8·3	—	4·7	—
25	2·9	—	—	—	—	—
26	—	—	2·5	5·8	8·2	5·7
27	15·0	—	—	—	—	—
28	—	—	8·4	61·4	19·9	88·6
29	52·2	—	—	—	—	—
30	—	—	0·9	1·8	5·6	2·1
31	19·6	—	—	—	—	—
32	—	—	0·4	—	1·3	—
33	2·3	—	—	—	—	—
34	—	—	—	—	0·5	—
38	—	2·5	—	—	—	—
Trans 38:1	—	0·2	—	—	—	—
40	—	4·6	—	—	—	—
Trans 40:1	—	0·5	—	—	—	—
42	—	6·8	—	—	—	—
Trans 42:1	—	3·1	—	—	—	—
44	—	15·8	—	—	—	—
Trans 44:1	—	3·8	—	—	—	—
46	—	11·0	—	—	—	—
Trans 46:1	—	2·6	—	—	—	—
48	—	11·9	—	—	—	—
50	—	17·9	—	—	—	—
52	—	4·5	—	—	—	—
54	—	3·9	—	—	—	—
56	—	8·0	—	—	—	—
58	—	1·9	—	—	—	—
60	—	1·0	—	—	—	—
Unidentified	6·8	—	1·2	4·0	1·0	—

* In % by wt, obtained by GLC.

To establish the geometric configuration of the unsaturated C_{22} alcohol the esters were hydrolysed with alkali and the alcohol fraction isolated. Column chromatography yielded part of the unsaturated alcohol in pure form, the IR spectrum had a strong band at 965 cm^{-1} showing that the double bond has the *trans* configuration The NMR spectrum (CCl_4) showed a 2 proton doublet at 3·96 ppm, confirming the $-CH{=}CH.CH_2OH$ structure, the 2 proton multiplet at 5·55 ppm for the double bond protons does not, however, clearly distinguish between *cis* and *trans* double bonds.[7]

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[7] OMURA, S., NAKAGAWA, A., SEKIKAWA, D., OTAMI, M. and HATA, T. (1969) *Chem. Pharm. Bull.* **17**, 2361.

trans-2-Docosen-1-ol was synthesized by reduction of methyl *trans*-2-docosenoate with diisobutyl aluminium hydride (a number of 2,3-unsaturated alcohols up to C_{20} have been synthesized in this way[8]) and was indistinguishable from the natural product. Unlike esters of *trans* 2,3-unsaturated acids which have greater retention times, using non-polar columns, than saturated esters, the acetate of *trans* 2-docosen-1-ol had the same retention time as docosyl acetate. It can be assumed, therefore, that GLC does not distinguish between *trans* 2,3-unsaturated esters of the unsaturated alcohol and esters of the same acids with the corresponding saturated alcohol. Short chain (C_6–C_{10}) *trans* 2,3-unsaturated alcohols and their esters have been found in the scent of insects[9,10] but longer chain compounds do not seem to have been reported in natural products.

Free acids also contained C_{22} and C_{24} unsaturated acids, 31% in Selkirk and 9% in Manitou. Comparisons of free and combined acids and alcohols are interesting. The acids are fairly similar though there is a marked difference in the proportion of eicosanoic acid, 11–15% in the combined acids and 1–2% in the free acids. Free alcohols are almost entirely octacosanol but combined alcohols have much more C_{20}–C_{24} which apparently occur mainly as esters of unsaturated C_{22} and C_{24} acids.

The β-diketone was hentriacontane-14,16-dione, the same as that from club wheat[1] and durum wheat.[2] The hydroxy β-diketone fraction was the same 1:1 mixture of 8- and 9-hydroxyhentriacontane-14,16-diones previously obtained from club wheat wax.[1]

Several minor components were identified. The structure of the diol diesters of Selkirk has already been described[11] and very similar compounds were obtained from Manitou wax, but in smaller quantity. A mixture of glycerides (1%) was obtained from Manitou wax and GLC analysis suggested that the fatty acids of the major component totalled 34 carbon atoms, but only C_{14} and C_{16} acids were isolated on hydrolysis. The structure could not be established further because of the small quantity available. Lesser amounts of glycerides might also be present in Selkirk wax but were not isolated in pure form. Glycerides have previously been found in cuticle wax of *Phragmites communis*[12] but do not seem to have been reported in other leaf waxes. A very small percentage of aldehydes (mostly C_{28}) was present in Manitou wax but none was detected in Selkirk wax.

The other major fraction consisted of gummy material eluted from the silicic acid column after hydroxy β-diketone. Like similar material from other wheat waxes it yielded a complex mixture of indefinite composition on ethanolysis.

Thus the principal differences between the waxes of these two varieties of spring wheat are the greater proportions of unsaturated esters and free acids in Selkirk and of β-diketones in Manitou. These waxes are also more like wax of club wheat than that of durum wheat. Hydroxy β-diketones in particular are the same mixture of 8- and 9-hydroxyhentriacontane-14,16-diones in spring and club wheats,[1] but durum wheat contains 25-hydroxyhentriacontane-14,16-dione.[2] A relatively close resemblance to club wheat is perhaps to be expected since this wheat and spring wheat are hexaploid with 42 chromosomes, but durum wheat is tetraploid with 28 chromosomes.

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[8] Falk, F. and Mainas, C. (1968) *Fette, Seifen, Anstrichmittel* **70**, 709.

[9] Gilby, A. R. and Waterhouse, D. F. (1965) *Proc. Roy. Soc.* **162B**, 105.

[10] Devakul, V. and Maarse, H. (1964) *Anal. Biochem.* **7**, 269.

[11] Tulloch, A. P. (1971) *Lipids* **6**, 641.

[12] Simionescu, C., Diaconescu, E. and Feldman, D. (1960) *Rev. Chim. Akad. Rep. Pop. Roumaine* **5**, 57.

EXPERIMENTAL

Chromatography. TLC was carried out as previously described.[2] GLC analyses were performed using a Hewlett-Packard model 402 gas chromatograph with flame ionization detectors, fitted with a 1 m × 3 mm stainless steel column packed with 80–100 mesh, acid washed, silanized Chromosorb W coated with 2% silicone SE-30. He flow rate was 45 ml/min.

Wax collection. Wheat was cut about 65 days after germination when the flag leaf was fully developed; some heads had appeared but were excluded from the extraction as far as possible. Wax was extracted as before.[1]

Separation of wax components. Waxes of both varieties (10·6 g) were separated on silicic acid (Biosil A), methyl esters of free acids isolated by re-chromatography and major fractions purified in the same way as reported for durum wheat wax.[2] Fractions were identified by TLC and NMR spectroscopy (Varian HA-100 spectrometer). Methods of analysis and identification of hydrocarbons, esters, β-diketone, free acids as methyl esters, free alcohols and ethanolysis of long chain esters were described earlier.[1]

Unsaturated Esters. These compounds had emergence temps slightly greater than saturated C_{42}, C_{44} and C_{46} esters ($C_{42\cdot3}$, $C_{44\cdot3}$ and $C_{46\cdot3}$). When a portion was hydrogenated over 5% Pd–C in EtOAc at 50° (product recovered by extracting the catalyst with $CHCl_3$) unsaturated esters disappeared and saturated C_{42}–C_{46} components increased by the expected amounts.

Isolation of trans *2-docosen-*1-ol.Esters isolated from wax of ripe Selkirk wheat[3] were used. The NMR spectrum (CCl_4) showed that they consisted mainly of normal and 2,3-unsaturated alcohols esterified by *trans* 2,3-unsaturated acids: δ 4·02 (*t*, $CH_2O.COR$); δ 4·45 (*d*, $CH_2O.COR$ α to double bond); δ 5·55 (*m*, protons of double bond α to $-CH_2O-$); δ 5·70 (*d*, H-2 of α,β-unsaturated ester (high field portions of doublet are over-lapped by preceding multiplet); δ 6·80 (H-3 of α,β-unsaturated esters). The relative intensities of the doublet at δ 4·45 and the triplet at δ 4·02 showed that 2,3-unsaturated alcohols made up about 35% of the alcohols of the esters. A portion (0·45 g) was refluxed with 5% KOH in 90% MeOH (15 ml) and C_6H_6 (15 ml) for 5 hr. H_2O (100 ml) was added, alcohols were extracted with Et_2O and the extract washed and dried (Na_2SO_4). Alcohols so obtained (0·226 g) were chromatographed on a silicic acid column. The first fraction eluted with hexane–Et_2O (23:2) contained only unsaturated C_{22} alcohol (0·037 g). Crystallization from hexane gave *trans* 2-docosen-1-ol, m.p. 61–62°. The IR spectrum (CS_2) had a strong band at 965 cm^{-1}; NMR (CCl_4): δ 2·00 (unresolved *m*, CH_2 α to double bond, 2 protons); δ 3·96 (*d*, *J* 4 Hz, CH_2OH α to double bond, 2 protons); δ 5·55 (*m*, 2 protons of double bond).

Synthesis of trans *2-docosen-*1*-ol.* A soln of methyl *trans* 2-docosenoate (0·39 g)[11] in C_6H_6 (4 ml) was added to a soln of di-isobutyl aluminium hydride (20% in hexane, 4 ml) at 2°. The mixture was heated at 65° for 1 hr, cooled, decomposed with 0·5 N H_2SO_4, extracted with Et_2O and the extract washed and dried. Distillation gave pure *trans* 2-docosen-1-ol, b.p./0·05 mm 145° (bath); after hexane crystallization, m.p., and m.m.p. with natural alcohol, were 61–62°. The NMR spectrum (CCl_4) was indistinguishable from that of natural alcohol. GLC examination, using Dexsil 300, showed that acetates of synthetic and natural alcohol and docosyl acetate all had the same emergence temps. Found: C, 81·30; H, 13·66. $C_{22}H_{44}O$ required: C, 81·41; H, 13·66%.

Hydroxy β-diketone. Crude hydroxy β-diketone fraction (1·38 g) from Selkirk wax gave hydroxy β-diketone (0·77 g) after purification as the copper complex.[3] The m.p. was 73–74° and $[\alpha]_D^{25}$ +0·88°; $[\alpha]_{546}^{25}$ + 1·17 and $[\alpha]_{436}^{25}$ + 2·06°. $E_{1cm}^{1\%}$ at λ_{max} 273 nm was 261. The X-ray powder photograph was indistinguishable from that of hydroxy β-diketone from Little Club wax, and alkaline hydrolysis gave the same products as before. The NMR spectrum (quinoline) of the hydroxy tetradecanoate obtained on hydrolysis showed that approximately equal amounts of 6- and 7-hydroxy C_{14} ester were present proving that the parent hydroxy β-diketone was again a 1:1 mixture of 8- and 9-hydroxyhentriacontane-14,16-dione.[1] Hydroxy β-diketone from Manitou wax was identified in the same way.

Diol diesters. Isolation and identification of these components from Selkirk wax was reported earlier,[11] Manitou wax yielded a smaller amount of diesters which GLC analysis showed to have a similar composition and which gave the same products on ethanolysis.

Aldehydes. The complex mixture (0·6 g) eluted just after the main β-diketone fraction during chromatography of Maintou wax was freed from β-diketone (0·3 g) by copper complex formation and separated by preparative TLC (in hexane–methylene chloride (1:1)) into 5 fractions. One fraction (R_f similar to that of β-diketone) was shown by NMR spectroscopy (CCl_4) to be an approximately 1:1 mixture of aldehyde (*t* at δ 9·76) and β-diketone (*s* at δ 5·30). Reduction with lithium aluminium hydride gave a mixture of alcohols the main component of which was octacosanol. No aldehydes were detected in similar fractions from Selkirk wax.

Glycerides. A fraction (0·26 g) eluted (hexane–$CHCl_3$ (3:1)) during re-chromatography of the more polar fractions of Manitou wax (to isolate methyl esters and hydroxy β-diketone) had strong NMR signals characteristic of glycerides.[13] Purification by preparative TLC ($CHCl_3$ containing 1% EtOH) gave nearly pure

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[13] HOPKINS, C. Y. (1965) in *Progress in the Chemistry of Fats and Other Lipids* (HOLMAN, R. T., ed.), Vol. VIII, p. 213, Pergamon Press, Oxford.

glycerides (0·11 g), the only other identifiable fraction was a mixture of alcohols (0·05 g). GLC analysis indicated that the major component was a C_{34} glyceride (compared to synthetic trilaurin as C_{36}). Common glycerides did not seem to be present as there were only very small peaks in the C_{46}–C_{54} range. The fraction was refluxed with 5% methanolic HCl, $CHCl_3$ added, neutralized with $AgCO_3$, MeOH taken off and fatty esters extracted with hexane. The residue was acetylated and shown to contain triacetin by GLC. The fatty esters contained mainly C_{14} and C_{16} esters, but a short (e.g. C_4) ester could have been lost during the working up process. The portion of the crude fraction from Selkirk wax which remained after removal of hydroxy β-diketone as the copper complex appeared to contain some glycerides (NMR) but as at least five other components were present (TLC) they were not isolated.

Unidentified fractions. The major unidentified fraction was eluted from the silicic acid column after hydroxy β-diketone. Part of this material from Selkirk wax (0·49 g) was subjected to ethanolysis. The product was chromatographed on silicic acid and gave ethyl esters (0·04 g) with the percentage composition: C_{12},4; C_{14},2; C_{16},27; C_{18},3; C_{20},4; C_{22},7; t-C_{22}:1,8; C_{24},5; t-C_{24}:1,11; C_{26},4; C_{28},7; C_{30},3; unidentified 15%. Five other fractions were isolated from the column but no components were identified.

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