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LEAF WAX OF DURUM WHEAT*

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Abstract—Leaf waxes from durum wheat varieties Pelissier and Stewart 63 contained hydrocarbons (9%), esters (9%), alcohols (17%), acids (3%), β-diketone (36%), hydroxy β-diketone (9%) and unidentified material (17%). The major hydrocarbon was nonacosane, the C_{36}–C_{56} esters were esters of C_{20}–C_{28} alcohols with C_{16}–C_{30} saturated acids and *trans* 2-docosenoic and 2-tetracosenoic acids, and the principal alcohol was octacosanol. The β-diketone was hentriacontane-14,16-dione and the hydroxy β-diketone was 25(S)-hydroxyhentriacontane-14,16-dione.

INTRODUCTION

THE COMPOSITIONS of the leaf waxes of varieties of plants important in agriculture have not been accurately determined until very recently, though the waxes probably affect water retention and wettability by herbicide and pesticide sprays.[1] Leaf wax of Little Club wheat was analysed previously, unusual components being the β-diketone, hentriacontane-14, 16-dione, and the mixture of hydroxy β-diketones, 8- and 9-hydroxyhentriacontane-14,16-diones.[2]

In a preliminary examination of leaf waxes from a number of other types of wheat, β-diketones were estimated by u.v. spectroscopy[3] and the other components by TLC.[2] The composition of waxes from two varieties of durum wheat (*Triticum durum* Desf.-Gramineae), Pelissier and Stewart 63, have now been determined and compared. These two varieties were chosen because they are not related by breeding and Stewart 63 is the predominant variety of durum wheat grown in Canada.

RESULTS

The yield of wax (0·4 per cent) was the same for each (Table 1) and similar to that of wax from Little Club wheat. Comparison by TLC with a mixture containing β-diketone and hydroxy β-diketone from Little Club wax (Fig. 1) showed a noticeable difference in the hydroxy β-diketone region, durum wheat components having a higher R_f.

Wax components were separated by silicic acid column chromatography but the previous procedure[2] was modified to obtain free acids more easily. Components were eluted with hexane containing increasing amounts of chloroform, free acids and hydroxy β-diketone were eluted together, fatty acids converted to methyl esters with diazomethane and separated by rechromatography. β-Diketone and hydroxy β-diketone were finally purified as the copper complexes.[3]

The compositions of the two leaf waxes were almost the same (Table 1) but differed appreciably from that of Little Club wax in that the percentages of β-diketone were considerably greater and the percentages of esters and free alcohols were correspondingly

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[1] J. H. TROUGHTON and D. M. HALL, *Australian J. Biol. Sci.* **20**, 509 (1967).
[2] A. P. TULLOCH and R. O. WEENINK, *Can. J. Chem.* **47**, 3119 (1969).
[3] D. H. S. HORN, Z. H. KRANZ and J. A. LAMBERTON, *Australian J. Chem.* **17**, 464 (1964).

TABLE 1. COMPOSITION (%)* AND YIELDS OF LEAF WAXES OF DURUM WHEATS

Component	Variety	
	Stewart 63	Pelissier
Hydrocarbons	9	7
Esters	9	10
Free alcohols	17	16
Free acids	3	3
β-Diketone	36	35
Hydroxy β-diketone	9	8
Unidentified	17	21
Yield (% of dry wt.)	0·4	0·4
$E_1^{1\%}$cm (isooctane)	145	135

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

TABLE 2. COMPOSITION* OF WAX FRACTIONS FROM STEWART 63 WHEAT

No. of C atoms	Hydrocarbons	Esters	Hydrolysis Products of esters		Free Acids	Free Alcohols
			Acids	Alcohols		
14	—	—	—	—	1·8	—
16	—	—	11·0	—	6·8	—
18	—	—	6·0	1·3	1·6	—
20	—	—	19·7	4·6	1·2	—
22	—	—	25·8	17·6	10·1	5·3
Trans 22:1	—	—	3·3	—	4·6	—
23	0·4	—	—	—	—	—
24	—	—	10·1	11·2	11·3	4·7
Trans 24:1	—	—	6·9	—	8·0	—
25	1·6	—	—	—	—	—
26	—	—	4·8	5·7	13·5	6·2
27	10·1	—	1·1	1·0	—	—
28	—	—	7·3	53·8	25·8	77·5
29	59·3	—	—	1·5	—	—
30	—	—	1·2	2·3	11·3	2·6
31	20·3	—	—	—	—	—
32	—	—	0·4	—	2·8	0·7
33	7·8	—	—	—	—	—
34	—	4·3	—	—	1·2	—
35	0·5	—	—	—	—	—
36	—	3·1	—	—	—	—
38	—	3·4	—	—	—	—
40	—	5·6	—	—	—	—
42	—	9·1	—	—	—	—
44	—	24·4	—	—	—	—
46	—	14·1	—	—	—	—
48	—	12·2	—	—	—	—
50	—	13·2	—	—	—	—
52	—	4·6	—	—	—	—
54	—	2·4	—	—	—	—
56	—	3·2	—	—	—	—
58	—	0·4	—	—	—	—
Unidentified	—	—	2·4	1·0	—	3·0

* In percent by weight, obtained by GLC (peaks were triangulated and areas converted to percentages).

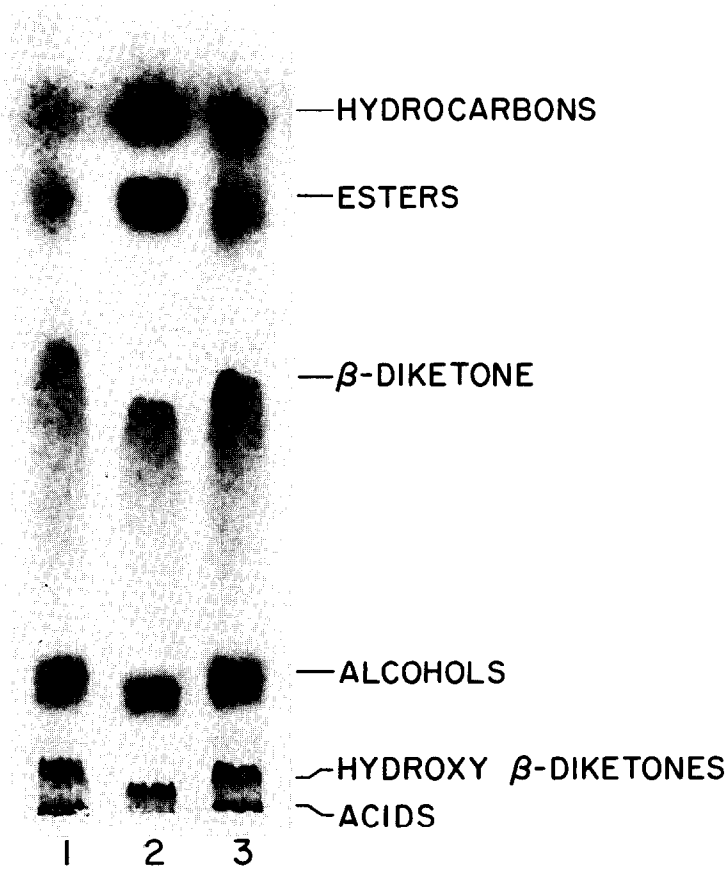

FIG. 1. TLC OF WHEAT LEAF WAXES.

1, Pelissier leaf wax; 2, mixture of: tetracosane, docosanol docosanoate, hentriacontane-14,16-dione, tetracosanol, 8- and 9-hydroxyhentriacontane-14,16-diones and tetracosanoic acid; 3, Stewart 63 leaf wax; in $CHCl_3$ containing 1%, v/v EtOH.

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lower. Percentages of hydrocarbons, free acids and hydroxy β-diketone were not very different. Hydrocarbons, esters and their hydrolysis products, free acids and free alcohols were analysed by GLC and compared in Tables 2 and 3.

The fractions from the two waxes had very similar compositions and contained the same components as were found in the corresponding fractions from Little Club wax, though there were differences in the relative amounts. Hydrocarbons showed the greatest difference with C_{29} the major component (almost 60 per cent) whereas in Little Club wax there were equal amounts of C_{29} and C_{31}. Hydrocarbon composition has been used a number of times in comparisons of plant species.[4]

When Little Club wax was analysed, ester fractions containing esters of *trans* 2-docosenoic and tetracosenoic acids were removed from the main ester fraction and analysed

TABLE 3. COMPOSITION* OF WAX FRACTIONS FROM PELISSIER WHEAT

No. of C atoms	Hydrocarbons	Esters	Hydrolysis Products of esters		Free Acids	Free Alcohols
			Acids	Alcohols		
14	—	—	1·0	—	—	—
16	—	—	9·9	—	9·2	—
18	—	—	2·6	—	3·3	—
20	—	—	16·3	4·5	1·5	—
22	—	—	28·0	10·9	11·2	0·6
Trans 22:1	—	—	3·9	—	5·4	—
23	1·8	—	—	—	—	—
24	—	—	10·2	12·0	10·4	7.2
Trans 24:1	—	—	9·9	—	9·3	—
25	2·4	—	—	—	—	—
26	—	—	4·7	4·6	11·6	4·2
27	8·8	—	—	2·1	0·7	1·0
28	—	—	7·6	64·3	22·8	83·7
29	56·5	—	—	—	0·8	—
30	—	—	2·2	1·6	11·0	2·7
31	23·4	—	—	—	—	—
32	—	—	—	—	2·1	0·6
33	6·3	—	—	—	—	—
34	—	2·0	—	—	0·7	—
36	—	2·8	—	—	—	—
38	—	2·2	—	—	—	—
40	—	2·7	—	—	—	—
42	—	7·4	—	—	—	—
44	—	23·3	—	—	—	—
46	—	14·0	—	—	—	—
48	—	10·4	—	—	—	—
50	—	14·7	—	—	—	—
52	—	6·1	—	—	—	—
54	—	3·1	—	—	—	—
56	—	7·7	—	—	—	—
58	—	2·7	—	—	—	—
60	—	0·9	—	—	—	—
Unidentified	0·3	—	3·7	—	—	—

* In percent by weight, obtained by GLC (peaks were triangulated and areas converted to percentages).

[4] G. EGLINTON and R. J. HAMILTON, *Science* **156**, 1322 (1967).

separately, but in the present investigation esters were not fractionated in this way. Taking this change of method into account, there is not much difference in composition of esters from the durum wheats and esters from Little Club; they are mainly C_{28}, with some C_{20}–C_{26}, alcohol esters of C_{16}–C_{30} acids. Free acids contain less C_{16} and more C_{28} components; free alcohols are similar to those obtained before with octacosanol the major component.

The β-diketone, hentriacontane-14,16-dione, was the same as that found in Little Club wax but the hydroxy β-diketone had a different structure. It was *25-hydroxyhentriacontane-14,16-dione*, and was the only one found. The 25-hydroxy compound had a higher R_f (TLC) and a smaller specific rotation than the 8- and 9-hydroxy isomers.

Alkaline hydrolysis of the hydroxy β-diketone gave mainly C_{14} acid, together with about 20% C_{16} and C_{18} acids, 10-hydroxyhexadecanoic acid, pentadecan-2-one and *11-hydroxyheptadecan-2-one*. Since only one methyl ketone was isolated, the C_{16} and C_{18} acids were probably derived from an unidentified impurity in the sample of hydroxy β-diketone used in hydrolysis experiments. The structure of 10-hydroxyhexadecanoic acid followed from the formation of C_9 and C_{10} dicarboxylic acids on oxidative cleavage,[5] and of 10-oxohexadecanoic acid on mild oxidation. *Methyl 10-hydroxyhexadecanoic* acid had a small positive rotation (in $CHCl_3$), very similar to that of methyl 9-L-hydroxyoctadecanoate, indicating the L-configuration.[6] Recently 10-D-hydroxyhexadecanoic acid was obtained by microbial hydration of palmitoleic acid and the specific rotation of the methyl ester reported for a methanol solution;[7] the rotations of the L- and D-isomers ($CHCl_3$ solutions) are compared in Table 4.

TABLE 4. SPECIFIC ROTATIONS OF METHYL 10-L- AND 10-D-HYDROXYHEXADECANOATE IN CHLOROFORM

Wave length (mμ)	Compound	
	Methyl 10-L-hydroxyhexadecanoate from wax of durum wheat (c, 4·6)	Methyl 10-D-hydroxyhexadecanoate from microbial hydration of palmitoleic acid (c, 4·3)
589	+0·4	−0·3
546	+0·5	−0·3
436	+0·7	−0·6
365	+1·1	−0·9

Thus the principal difference between wax of durum wheat and that of Little Club wheat is in the structures of the hydroxy β-diketones. From a biosynthetic point of view the occurrence of only one hydroxy β-diketone in durum wheat and of two, with hydroxyls on adjacent carbon atoms, in Little Club wheat is of considerable interest. It is not known whether β-diketones are formed by condensation of smaller (e.g. C_{16}) molecules or by modification of a long chain (e.g. C_{31}) compound. In the case of monoketones, such as occur in cabbage wax, the head to head condensation theory now seems unlikely.[8]

[5] A. P. TULLOCH, *Can. J. Chem.* **43**, 415 (1965).
[6] A. P. TULLOCH, *Can. J. Chem.* **46**, 3727 (1968).
[7] E. N. DAVIS, L. L. WALLEN, J. C. GOODWIN, W. K. ROHWEDDER and R. A. RHODES, *Lipids* **4**, 356 (1969).
[8] P. E. KOLATTUKUDY, *Lipids* **5**, 259 (1970).

The specific rotations of the hydroxy β-diketones and their hydrolysis products, indicated that they were optically pure but the occurrence of two hydroxy β-diketones in equal amounts in Little Club wax suggested that the introduction of the hydroxyl group was not positionally specific. This might occur by addition of water to a double bond with the hydroxyl becoming attached to each carbon to the same extent or by opening of an epoxide ring on each side of the oxygen atom.[9] However, the isolation of one isomer only from durum wheat wax suggests introduction of the hydroxyl group by stereospecific hydroxylation of a methylene group as occurs in formation of ricinoleic acid in the castor bean[10] or in formation of hydroxy acids by species of *Torulopsis*.[11]

Since long chain aldehydes have been reported in wheat waxes,[12] material remaining after conversion of β-diketones to copper complexes and fractions eluted from the column after β-diketone were examined by NMR for the characteristic aldehyde triplet at δ 9·76.[13] Only traces of aldehydes appeared to be present but it is possible that they decomposed at some stage in the working up procedure.

These fractions formed only 1–2 per cent of the wax but a much larger unidentified fraction (15–20 per cent) was eluted along with or just after the hydroxy β-diketone and was obtained as a residue after copper complex formation. The ratio of the signals for CH_2 α to carbonyl to those of CH_2O- of ester in the NMR spectrum suggested the presence of carbonyl groups and ester linkage in the ratio 3:1 but TLC indicated that it was not at all homogeneous. Ethanolysis gave some ethyl esters but no other definite components.

EXPERIMENTAL

Chromatography

Silicic acid column chromatography and GLC were carried out as described previously.[2] Silica gel G was used for TLC. $CHCl_3$ containing 1%, v/v EtOH was the solvent giving the following R_fs: hydrocarbons 0·84; docosanol docosanoate 0·73; methyl tetracosanoate 0·54; β-diketone 0·51; tetracosanol 0·14; 25-hydroxyhentriacontane-14,16-dione 0·046; 8- and 9-hydroxyhentriacontane-14,16-diones 0·033; tetracosanoic acid 0·00. The resolution between esters and hydrocarbons and between hydroxy β-diketones and alcohols was lost when $CHCl_3$ containing 2%, v/v EtOH[14] was used (some commercial $CHCl_3$ samples contained 2%).

Collection of Wax

Wheat was cut when the last leaf was completely developed and the heads were appearing. Wax was extracted as previously described.[2]

Separation of Components

Wax (10 g), from Stewart wheat, was applied to a silicic acid column (400 g). Hydrocarbons (0·82 g) were eluted with hexane (1·5 l.), esters and β-diketones (5·15 g) with hexane–$CHCl_3$ (4:1, 4 l.), and unidentified fraction (0·08 g) with hexane–$CHCl_3$ (1:1, 1 l.) and alcohols (1·2 g) with the same solvent. A mixture of alcohols, free acids, hydroxy β-diketone and gum were finally eluted with $CHCl_3$ (2 l.). The last fraction was treated with CH_2N_2 in CH_2Cl_2 and rechromatographed. Elution with hexane–$CHCl_3$ (4:1) gave methyl esters of free acids (0·31 g) followed by alcohols (0·38 g). Further elution with hexane–$CHCl_3$ (7:4) gave crude hydroxy β-diketone (1·9 g) and gum (0·6 g) was eluted with $CHCl_3$.

β-Diketones (3·35 g) were removed from the mixture with esters as the insoluble Cu complex[3] and rechromatography as above of the residue from the mother liquors gave wax esters (0·86), alcohols (0·04 g) and several unidentified fractions (0·3 g). Hydroxy β-diketone (0·85 g) was also isolated from the crude material, as the Cu complex, leaving a brown gum (1·00 g) in the mother liquors.

Analysis and identification of hydrocarbons, esters, ethanolysis of esters, separation of ethyl esters and alcohols and identification of acids and alcohols were carried out as previously described.[2]

[9] E. J. McKenna and R. E. Kallio, *Ann. Rev. Microbiol.* **19**, 183 (1965).
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[11] E. Heinz, A. P. Tulloch and J. F. T. Spencer, *J. Biol. Chem.* **244**, 882 (1969).
[12] H. N. Barber and A. G. Netting, *Phytochem.* **7**, 2089 (1968).
[13] J. A. Lamberton, *Australian J. Chem.* **18**, 911 (1965).
[14] A. P. Tulloch, *Chem. Phys. Lipids* (in press).

Hentriacontane-14,16-dione

β-Diketone from both waxes had m.p. 57·5–59°, not depressed by admixture with hentriacontane-14,16-dione from Little Club wax[2]; λ_{max} (isooctane) 273 nm (ε 12,500). β-Diketone from Stewart wheat gave, on alkaline hydrolysis, the same mixture of C_{14} and C_{16} acids and C_{15} and C_{17} ketones obtained before.[2]

25-Hydroxyhentriacontane-14,16-dione

Hydroxy β-diketone from Stewart wax was crystallised from EtOAc, m.p. 77·0–77·5°; λ_{max} (isooctane) 273 nm (ε 12,700); $[\alpha]_D^{25}$ + 0·38°, $[\alpha]_{546}^{25}$ + 0·43°, $[\alpha]_{436}^{25}$ + 0·90°, (c, 2·2 in $CHCl_3$) (measured with a Perkin–Elmer model 141 polarimeter); NMR (CCl_4): δ 2·2 (triplet, CH_2 α to carbonyl, 4 protons); δ 3·45 (multiplet, CH of CHOH, 1 proton); δ 5·32 (singlet, CH of enol form, 1 proton). (Found: C, 77·36; H, 12·61. $C_{31}H_{60}O_3$ required: C, 77·44; H, 12·58%.) Reduction to hydrocarbons[15] gave only hentriacontane. Hydroxy β-diketone (0·86 g) was refluxed for 18 hr in 6% ethanolic KOH (40 ml) and worked up and separated into acids and neutral ketones[2] (reaction was followed and products were examined by TLC in hexane–ether, 1:1). Acids were converted to methyl esters (CH_2N_2) and separated (silicic acid column) into esters (0·22 g) (elution with hexane–ether, 19:1) and hydroxy ester (0·23 g) (elution with hexane–acetone, 19:1). Methyl esters had the composition (GLC): C_{14}, 77%; C_{16}, 19%; C_{18}, 4%; C_{14} ester was isolated (preparative GLC), saponified and myristic acid identified by comparison with an authentic sample.

The hydroxy esters contained only one component (GLC), methyl 10-L-hydroxyhexadecanoate; leaflets from hexane: m.p. 44–45°; $[\alpha]_D^{25}$ in Table 4: (Found: C, 71·42; H, 12·11. $C_{17}H_{34}O_3$ required: C, 71·28; H, 11·98%.) Oxidation with chromic acid at 100°[5] gave C_9 and C_{10} dicarboxylic acids (GLC[16]) and oxidation at 25°[5] gave methyl 10-oxohexadecanoate, large leaflets from hexane; m.p. 36·5–37°; the m.p. was not depressed by admixture with methyl 10-oxohexadecanoate prepared by oxidation of methyl 10-D-hydroxyhexadecanoate.

The neutral ketone fraction was separated (silicic acid column) into ketone (0·15 g) and hydroxyketone (0·22 g). Pentadecan-2-one, m.p. and mixed m.p. with authentic ketone[2], 37–37·5°, was the only component of the ketone fraction (GLC). The hydroxy ketone was almost entirely one component (GLC), 11-hydroxyheptadecan-2-one; needles from hexane; m.p. 64–65°; $[\alpha]_D^{25}$ + 0·4°, $[\alpha]_{546}^{25}$ + 0·5°, $[\alpha]_{436}^{25}$ + 0·8°, $[\alpha]_{365}^{25}$ + 1·2° (c, 5·9 in $CHCl_3$); (Found: C, 75·22; H, 12·75. $C_{17}H_{34}O_2$ required: C, 75·50; H, 12·67%). Hydroxy β-diketone from Pelissier wax had the same m.p. as that from Stewart wax, the mixed m.p. was not depressed, and X-ray powder photographs were indistinguishable.

Unidentified Fractions

Minor fractions (1–2 per cent) were eluted after β-diketones; NMR (CCl_4): δ 2·2 (triplet CH_2 α to carbonyl); δ 9·76 (triplet, aldehydic proton, very weak signal). Major fraction (15 per cent) which was a soft gummy material, was eluted with and after hydroxy β-diketone; NMR (CCl_4): δ 2·2 (triplet CH_2 α to carbonyl); δ 4·0 (triplet, CH_2OCOR); ratio of first signal to second was about 4:1. Ethanolysis of this fraction (0·20 g) gave ethyl esters (0·06 g) (mainly ethyl stearate) and unidentified, more polar material (0·12 g), which gave almost no GLC response. TLC (benzene–EtOAc, 17:3), both before and after ethanolysis, gave streaks without sign of distinct components (other than ethyl esters). No alcohols or hydroxy esters could be detected.

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