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EPICUTICULAR WAXES OF *SECALE CEREALE* AND *TRITICALE HEXAPLOIDE* LEAVES

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Key Word Index—*Secale cereale*; *Triticale hexaploide*; Gramineae; rye; leaf wax; β-diketones; 25 (S)-hydroxyhentriacontane-14,16-dione.

Abstract—Wax on leaves of rye and of hexaploid *Triticale* (60–70-day-old plants) contains hydrocarbons (6–8%), esters (10%), free alcohols (14–8%), free acids (3%), hentriacontane-14,16-dione (39–45%), 25 (S)-hydroxyhentriacontane-14,16-dione (13–11%) and unidentified (14–15%). Diesters (1–3%) are also present in rye wax. Compositions of hydrocarbons (C_{27}–C_{33}) and esters (C_{28}–C_{58}) are similar for both waxes. Free and combined alcohols of rye wax are mainly hexacosanol but alcohols of *Triticale* wax are mainly octacosanol. The composition of *Triticale* wax is close to that of its wheat parent *Triticum durum* (cv. Stewart 63). Esters of wax from ripe rye contain 58% of *trans* 2,3-unsaturated esters.

INTRODUCTION

WAXES on the leaf surfaces of several types of wheat[1–3] and of oats[4] have been investigated and analysed to find out whether any particular composition can be associated with drought resistance and also to determine the presence of possibly useful compounds. Other particularly glaucous and waxy-looking cereals are barley, the leaf wax of which was investigated by Jackson,[5] and rye. Leaf wax of a spring rye, *Secale cereale* L. (Gramineae) (cv. Prolific, commonly grown in Western Canada) has now been analysed.

Further it was of considerable interest to compare wax of this rye variety with that of the synthetic species *Triticale hexaploide* (Lart.) Strain 6A190 of *Triticale* which is the amphiploid obtained by crossing durum wheat (*Triticum durum*, cv. Stewart 63) with rye (cv. Prolific)[6] was used since wax of Stewart 63 wheat had already been analysed.[2]

RESULTS

Composition and yield of wax from rye, *Triticale* and durum wheat[2] (wax collected at 60–70 days after germination when leaf area was greatest) are compared in Table 1. Considering that plants were grown in different years and that the relative amounts of β-diketone and alcohols vary with the stage of growth,[7] the compositions are quite similar. β-Diketones are the major components and are most probably responsible for glaucousness of stem and leaf sheath.

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[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**, 3171.
[3] TULLOCH, A. P. and HOFFMAN, L. L. (1973) *Phytochemistry* **12**, 2217.
[4] TULLOCH, A. P. and HOFFMAN, L. L. (1973) *Lipids* **8**, 617.
[5] JACKSON, L. L. (1971) *Phytochemistry* **10**, 487.
[6] DEDIO, W., KALTSIKES, P. J. and LARTER, E. N. (1969) *Can. J. Botany* **47**, 1589.
[7] TULLOCH, A. P. (1973) *Phytochemistry* **12**, 2225.

TABLE 1. COMPOSITION* AND YIELDS OF LEAF WAXES FROM RYE, *Triticale* AND DURUM WHEAT (STEWART 63)†

Component	Rye	Triticale	Stewart 63[2]
Hydrocarbons	6	8	9
Esters	10	10	9
Free alcohols	14	8	17
Free acids	3	3	3
β-Diketone	39	45	36
Hydroxy β-diketone	13	11	9
Diesters	1	—	—
Unidentified	14	15	17
Yield (% of dry weight)	0·6	0·6	0·4
$E^{1\%}_{1cm}$(isooctane)	140	147	145

* Calculated from weights of components obtained by silicic acid column chromatography.
† 60–70-Day-old plants.

Wax from rye and *Triticale* collected after 30 days contains over 50% of alcohols and very little β-diketone showing that, as in the case of wheat[7] and oats,[4] β-diketone content increases with lengthening of the internodes and formation of the leaf sheath. In wax from ripe rye, alcohols had further decreased to 6%, β-diketone had risen to 49% and hydroxy β-diketone to 15%. Yield of wax is appreciably higher for rye and *Triticale* than for durum wheat though for ripe rye it is only 0·35%.

Hydrocarbon compositions (Table 2) are similar with C_{29} and C_{31} the major components. Compositions of free fatty acids and free alcohols show distinctive differences (Table 3), the principal alcohol in rye wax is hexacosanol but is octacosanol in *Triticale* and also in durum wheat wax.[2] Free acid compositions of *Triticale* and durum waxes are also similar with C_{28} the largest component, whereas in rye wax acids C_{26} is the largest component. As in previous investigations,[1–4] the range of chain length of the acids (C_{14}–C_{36}) is much greater than that of the alcohols (C_{22}–C_{32}).

TABLE 2. COMPOSITION OF HYDROCARBONS FROM WAXES FROM RYE, *Triticale* AND STEWART 63 WHEAT

Number of carbon atoms	Rye	Triticale	Stewart 63[2]
23	2	2	1
25	5	3	2
27	15	11	10
28	1	1	—
29	37	45	59
30	1	1	—
31	28	26	20
33	11	10	8
35	—	1	—

Long chain esters have similar compositions (Table 4); small percentages of shorter esters, C_{28}–C_{36}, and of esters of *trans* 2,3-unsaturated acids appear in all three waxes. Unsaturated esters form 58% of esters of wax from ripe rye. As would be expected from the compositions of free acids and alcohols, combined acids and alcohols of the esters are also different (Table 5). In esters from *Triticale* and durum wheat waxes the principal component is octacosanol and in rye esters it is hexacosanol. Combined acids show less difference but those of *Triticale* are closer to those of durum wheat than they are to those of

rye. Combined acids differ from free acids in having a larger percentage of shorter acids particularly C_{20} and C_{22}. Unusually short alcohols (C_{12} and C_{14}) also appear and their esters with shorter acids presumably form the C_{28}–C_{38} esters mentioned above.

TABLE 3. COMPOSITION OF FREE ACIDS AND FREE ALCOHOLS FROM WAXES FROM RYE, *Triticale* AND STEWART 63 WHEAT

	Nmber of carbon atoms	Rye		*Triticale*		Stewart 63 wheat	
		acid	alcohol	acid	alcohol	acid	alcohol
	14	3	—	8	—	2	—
	16	14	—	15	—	7	—
	18	2	—	2	—	2	—
	20	4	—	3	—	1	—
	22	8	1	5	—	10	5
Trans	22:1	—	—	—	—	5	—
	24	17	6	8	4	11	5
Trans	24:1	—	—	—	—	8	—
	26	24	91	15	11	13	6
	28	13	2	28	80	26	80
	30	6	—	14	4	11	3
	32	4	—	2	1	3	1
	34	3	—	—	—	1	—
	36	2	—	—	—	—	—

TABLE 4. COMPOSITION OF ESTERS FROM WAXES FROM RYE, *Triticale* AND STEWART 63 WHEAT

	Number of carbon atoms	Rye	*Triticale*	Stewart 63 wheat*
	28	1	1	1
	30	3	2	1
	32	5	4	2
	34	4	4	4
	36	2	4	3
	38	2	3	4
	40	6	5	6
Trans	40:1	1	1	1
	42	13	10	7
Trans	42:1	2	1	2
	44	16	18	20
Trans	44:1	2	1	4
	46	17	11	10
Trans	46:1	2	1	3
	48	8	13	10
Trans	48:1	—	1	—
	50	4	9	12
	52	7	4	4
	54	3	3	2
	56	1	3	3
	58	1	1	1

* Original esters[2] were reanalysed using Dexsil 300 column.

β-Diketone of rye and *Triticale* is the same, hentriacontane-14,16-dione, as previously found in waxes of cereals.[1–5] It is conveniently identified by MS[5] but Dexsil 300 columns, which had been used for high temperature GLC for 9 months, gave the same response for β-diketone as for C_{36} ester. GLC can, therefore, be used to verify chain length, look

for homologues and ester or alcohol impurities etc.; hydroxy β-diketone, however, still gave a poor response.

TABLE 5. COMPOSITION OF ACIDS AND ALCOHOLS PRODUCED BY HYDROLYSIS OF ESTERS FROM WAXES FROM RYE, *Triticale* AND STEWART 63 WHEAT

Number of carbon atoms		Rye		*Triticale*		Stewart 63 wheat	
		acid	alcohol	acid	alcohol	acid	alcohol
	12	—	3	—	2	—	1
	14	2	6	1	5	—	3
	16	9	—	15	1	11	—
	18	8	1	7	1	6	1
	20	28	3	33	2	20	4
	22	9	10	22	10	26	14
Trans	22:1	2	—	—	—	3	—
	24	10	23	7	15	10	13
Trans	24:1	5	—	—	—	7	—
	26	10	49	6	14	5	6
	28	4	3	7	47	7	56
	30	2	2	2	3	1	2
	32	1	—	—	—	—	—
Unidentified			—	—	—	4(3)	—

Rye and *Triticale* waxes both contain the same single hydroxy β-diketone, 25-hydroxyhentriacontane-14,16-dione (with the same optical configuration) as does durum wheat wax.[2] Wax from ripe rye contains 15% hydroxy β-diketone and only 6% free alcohol (which can interfere with isolation of the former component) and would be convenient material for biosynthetic investigation of this hydroxy β-diketone.

Diesters of diols, previously found in wax of spring wheat[3,7,8] are also present in rye wax, particularly, in wax of ripe rye. Acids of the diesters are mainly *trans* 2,3-docosenoic and tetracosenoic acids, as found earlier,[8] but chain lengths of the diols are one carbon shorter than those from spring wheat,[8] C_8–C_{12} compared to C_9–C_{12}. Wax from ripe durum wheat contains several percent of diesters[7] but the composition of the component diols has not been determined as yet.

Thus waxes of rye and durum wheat differ in the chain length of the major alcohol (both free and esterified) and when wax of *Triticale* is compared, it is clear that it resembles its wheat parent more closely than its rye parent. A similar result was found when phenolic components of *Triticale* leaves were compared with those of the rye and wheat parents.[6] It was suggested that "the effects of the duplicated genes derived from wheat override the effect of the single rye gene",[6] a large supply of enzymes synthesizing wheat components was postulated. Also, as in the present study, no new components not found in either of the parents was detected.

The formation of trans α,β-unsaturated esters in some of the waxes can be usefully considered further. Though ripe rye wax contained no more esters than did wax of 60-day-old plants, the esters contained 58% of C_{40}–C_{46} unsaturated esters, an increase from 7% (Table 4). Wax of ripe *Triticale* showed very little increase in unsaturated esters and esters of wax of ripe Stewart 63 durum wheat contained only 15% of unsaturated esters.[7] This variation is probably to some extent due to the effect of growing conditions since the rye, *Triticale*

[8] TULLOCH, A. P. (1971) *Lipids* **6**, 641.

and durum wheat were grown in different years. Also wax of a perennial grass has shown large variations in α,β-unsaturated ester content from year to year (A. P. Tulloch and L. L. Hoffman unpublished work). However, the extent to which α,β-unsaturated esters are formed under suitable growing conditions is most likely determined by the genetic make up of the plant.

In forming a large percentage of α,β-unsaturated esters rye resembles the Selkirk variety of spring wheat, esters from wax of which contained over 80% unsaturated esters.[7] Since C_{40}–C_{46} unsaturated esters have a lower melting point and a greater solubility than C_{40}–C_{56} saturated esters, physical properties of the wax coating of the leaf are probably changed by their presence. It seems not unreasonable to speculate that the ability to produce large amounts of unsaturated wax esters is a desirable feature which may enable ripening plants of some varieties to survive adverse conditions, such as drought, more easily.

EXPERIMENTAL

Collection of wax. Rye was grown outside in 1972 and *Triticale*, strain 6A190, in 1973. Some plants were cut after 30 days (from germination), some after 60–70 days, when the flag leaf was fully developed, and some when they were ripe (about 100 days). Plants were extracted as previously described,[1] the heads being excluded.

Chromatography. TLC analyses were made as described previously[2] and GLC analyses were carried out as before.[7]

Mass spectroscopy. Mass spectra were measured at the Department of Chemistry, University of Saskatchewan using an MS 12 mass spectrometer with a direct inlet. Spectra were recorded at 70 eV, with an accelerating voltage of 3 kV and an ion source temp. of 100°.

Column chromatography. Previously wax was separated on a silicic acid column by elution with hexane containing increasing proportions of $CHCl_3$.[1–4] $CHCl_3$ was used because relatively large percentages of poorly soluble octacosanol were present, even though some of the separations were not completely satisfactory (perhaps due to varying amounts of ethanol stabilizer in the $CHCl_3$). Waxes of rye and *Triticale*, however, contain much smaller proportions of free alcohols and are better separated using hexane–Et_2O mixtures. The method is illustrated by the separation of rye wax collected at 60 days. Wax (2·42 g) was applied to silicic acid (200 g, Biosil A) and hydrocarbons (0·14 g) were eluted with hexane (1 l.). Esters and β-diketone (1·2 g) were eluted together with hexane Et_2O (99:1, 21.) and this solvent (51.) also eluted unidentified material (0·04 g). A mixture (0·04 g) of unidentified components and diesters (50%) was eluted by hexane–Et_2O (98:2, 21.). After elution of unidentified material (0·03 g) by hexane–Et_2O (97:3, 31.), elution with hexane–Et_2O (96:4, 31.) gave free acids (0·08 g) and with the same solvent (7 l.) gave crude alcohols (0·33 g). Unidentified material (0·02 g) was eluted by hexane–Et_2O (93:7, 21.) and hydroxy β-diketone (0·31 g) by hexane–Et_2O (92:8, 31.). Finally unidentified components (0·27 g) were eluted by hexane–E_2O–EtOH (70:25:5, 31.). Fractions were examined by TLC and GLC and hydrocarbons, free acids and free alcohols identified as previously described.[1]

Esters and β-diketones. Since difficulty was previously experienced in obtaining esters completely free from β-diketones, even after repeated chromatography, the following procedure in which residual β-diketone is separated as semicarbazone was adopted. As much of the β-diketone (0·60 g) as could be removed by one copper acetate treatment[9] was separated. Remaining mixture (0·60 g) was dissolved in hot EtOH (20 ml) and added to semicarbazide HCl (0·45 g) dissolved in EtOH (8 ml) and pyridine (8 ml) and the solution refluxed for 15 min. The mixture was kept overnight, poured into water and esters and semicarbazones extracted with $CHCl_3$. Pure esters (0·25 g) were isolated by column chromatography on silicic acid (elution with hexane–Et_2O (99:1) and semicarbazones were eluted with $CHCl_3$:EtOH (90:10). Part of the esters was converted to methyl esters and alcohols by methanolysis with 5% methanolic hydrogen chloride and benzene (1:1) and esters and alcohols were separated as described previously. β-Diketone, isolated as the copper complex, had m.p. 59·5–60·0°, after crystallization from EtOAc. The structure was confirmed as hentriacontane-14,16-dione by the mass spectrum: *m/e* 464, 446, 296, 281, 278, 268, 253, 239, 220, 211, 192, 138, 113, 100 (base peak) 95, 69, 57, 43.[5] The same β-diketone was obtained from *Triticale* wax.

Hydroxy β-diketone. Material obtained by column chromatography was pure according to TLC. After crystallization from ethyl acetate the m.p. was 76·5–77·0°, undepressed by admixture with 25-hydroxyhentriacontane-14,16-dione from wax of Stewart 63 durum wheat;[2] $[\alpha]_D^{25}$ +0·30°. $[\alpha]_{546}^{25}$ +0·42°, $[\alpha]_{436}^{25}$ +0·57°, $[\alpha]_{365}^{25}$ +0·97°. MS: *m/e* 480, 462 (M $-H_2O$), 444 (M $-2H_2O$), 395 (35% of base peak; 25,26-cleavage, 366 (24,25-cleavage + H).

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

268, 253, 211, 100, 71, 69, 57, 55, 43 (base peak), 41. Hydroxy β-diketone from Stewart 63 wheat and from *Triticale* had the same mass spectra.

Diesters. Wax from ripe rye gave a better yield of almost pure diesters (3%) than did wax from rye cut at 60 days. Methanolysis and GLC analysis of the products was carried out as previously described;[8] diols had the composition C_8 8%, C_9 30%, C_{10} 35%, C_{11} 23%, C_{12} 4%, acids (as methyl esters) had the composition C_{14} 1%, C_{16} 2%, C_{20} 1%, C_{22} 2%, *trans* $C_{22:1}$ 40%, C_{24} 3%, *trans* $C_{24:1}$ 39%, C_{26} 2%, C_{28} 2%, C_{30} 2%, unidentified 6%.

Unidentified material. Complex mixtures of unidentified compounds eluted from the chromatographic column at various stages up to the elution of hydroxy β-diketone totalled 5%. Unidentified material eluted after hydroxy β-diketone totalled 9%, rechromatography after acetylation indicated that hydroxy esters and possibly free diols were present but the proportions were too small for isolation and characterisation.

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Note added in proof. Recently wax from rye straw was analyzed and had a composition very similar to that reported here (Streibl, M., Konečný, K., Trka, A., Ubik, K. and Pazlar, M. (1974) *Collection Czech. Chem. Comm.* **39,** 475). Also the same hydroxy β-diketone was isolated from rye wax (Dierickx, P. J. and Compernolle, F. (1974) *Phytochemistry* **13,** 682).