COMPOSITION OF EPICUTICULAR WAXES ON BARLEY SPIKES

by

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Key words: eceriferum *mutants, secondary alcohols,* β-diketones, esterified alkan-2-ols, tissue specificity

Hydrocarbons, alkan-1-ol and alkan-2-ol containing esters, β-diketones, aldehydes, primary alcohols, hydroxy-β-diketones, free fatty acids and sometimes secondary alcohols are major lipid classes of epicuticular waxes on the spikes of the three wild type barley varieties Bonus, Foma and Carlsberg-II and many of the 40 *eceriferum* (*cer*) mutants examined. Division of the spikes into two parts, the awns and the spike minus awns, before wax isolation reveals that (i) β-diketones, hydroxy-β-diketones and esterified alkan-2-ols occur only on the spike minus awns, (ii) secondary alcohols are present only on the awns of Foma and its mutants and (iii) the other lipid classes are found on both parts of the spike. The chain length distributions of the lipid classes common to both parts of the spike are in some cases similar and in others different.

1. INTRODUCTION

The *eceriferum* (*cer*) loci control the synthesis and deposition of waxes on the cuticular surfaces of barley (20,23). Thus far 998 *cer* mutants located at 59 loci have been identified in the field by their effect on the wax coatings of the leaf blades, uppermost leaf sheaths plus internodes and/or spikes (see 22). The phenotypic classification of the spike wax is in essence a measure of the appearance of the lemmas which form the largest flat cuticular surfaces of the spike. Other parts of the spike include the awns, paleas and glumes, for example. The question may be asked whether or not all tissues of the

spike indeed synthesize the same wax lipids. As a first step we have determined the composition of the wax present on the awns versus that on the remainder of the spike, the spike minus awns. For this study we have choosen the mutant *cer-u*[69] as it has already been used in investigations on the mode of biosynthesis of the β-diketones (27, 41). The spike and leaf sheath plus internode waxes of this mutant differ from those of the wild type in lacking hydroxy-β-diketones and having a compensatory increased amount of β-diketones (37).

2. MATERIALS AND METHODS

Seeds of the barley (*Hordeum vulgare* L.) cvs. Bonus, Foma and Carlsberg II as well as of the *eceriferum* mutants *cer-e*[8], *-h*[13], *-i*[16], *-r*[19], *-n*[20], *-o*[28], *-c*[36], *-p*[37], *-zb*[38], *-q*[42], *-t*[46], *-w*[48], *-v*[49], *-x*[60], *-zc*[65], *-u*[69], *-zj*[78], *-cu*[108], *-z*[113], *-yh*[116], *-yc*[135], *-yd*[139], *-zn*[362], *-qu*[510] and *-yg*[1014] (20, 21, 23) were planted and grown to heading under optimum conditions for vegetative growth (6, 7) in the Phytotron at the Royal College of Forestry, Stockholm (35). In addition, seeds of Bonus, Foma and the mutants *cer-d*[5], *-a*[6], *-f*[9], *-m*[15], *-s*[31], *-k*[39], *-*[150], *-zr*[260], *-*[271], *-*[383], *-*[423], *-*[481], *-*[578], *-*[868] and *-*[1058] (20, 23, LUNDQVIST unpublished) were grown in a Percial PWG-108 growth chamber under environmental conditions matched as closely as possible to those used in the Phytotron (26). The epicuticular wax was isolated separately from the leaf blades, leaf sheaths plus internodes, total spikes, spikes minus awns and awns as described previously (36, 42). When only the lipid class composition of the leaf sheath plus internode wax was to be determined, collection of the required small amount of material was carried out using swab sticks (26).

The lipid class composition of a wax sample was determined from thin layer chromatograms (TLC) of the wax and standards run in two different solvent systems (42). Wax classes were isolated as follows: With the aid of column chromatography, the free fatty acids and when present the β-diketones and hydroxy-β-diketones were removed from a wax sample by complex formation and then recovered as described previously (27, 38). From the remainder of the wax sample the hydrocarbons, esters, aldehydes, primary alcohols and secondary alcohols were isolated via preparative TLC (36).

To determine the chain length compositions of the hydrocarbons, esters, β-diketones, primary alcohols and free fatty acids, they and/or their appropriate derivatives were subjected to gas liquid chromatography (GLC) (36, 37, 38, 42). The parameters used in the GLC procedure and the sources of internal standards have been detailed in the above references. In the present analyses, however, two 152.4 x 0.22 (internal diameter) cm stainless steel columns containing 5% SE-30 on 100/120 mesh Anakrom ABS (Analabs, Conn. USA) were employed.

Secondary alcohols were acetylated by the same method used to prepare primary alcohol acetates for GLC (36). Secondary alcohols isolated from *Brassica oleracea* (25, 30, 31) were used as standards. The instrument was a Varian Aerograph model 1700 gas chromatograph fitted with flame ionization detectors and coupled to an Infotronics model CRS-100 digital electronic computer. Two 152.4 x 0.22 (internal diameter) cm stainless steel columns containing 3% OV-1 on 100/120 mesh Anakrom ABS (Analabs) were employed. Isothermal chromatograms were run at 230°C. Detector and injector temperatures as well as nitrogen, hydrogen and air flows were adjusted to yield optimum sensitivities.

To verify the chain lengths and to determine the predominant isomers composing each secondary alcohol, the alcohols were oxidized, by a method based on that of BROWN and GARG (4), to ketones. This was accomplished by adding to the dry secondary alcohols 1 ml of diethyl ether, 1 ml of a saturated solution of $K_2Cr_2O_7$ and 20 drops of 10 *N* H_2SO_4. The reaction was allowed to proceed for 24 hours at room temperature with gentle shaking. Then the ether layer was removed and any remaining ketones extracted with two additional aliquots of diethyl ether. The combined diethyl ether extracts were washed with water and dried over anhydrous Na_2SO_4. TLC demonstrated that the oxidation of the secondary alcohols to ketones was complete. The resulting ketones were subjected to gas liquid chromatography-mass spectrometry (GLC-MS). For the latter an LKB type 9000 instrument (LKB Produkter AB, Stockholm, Sweden) was used with an ionizing potential of 70 ev, ionizing current of 60 μA. The ketones

were separated on a 100 x 0.22 (internal diameter) cm column containing 3% OV-1 at 235°C.

3. RESULTS

3.1. Lipid Class Composition of Spike Waxes

The epicuticular wax present on the spikes and uppermost leaf sheaths and internodes of Bonus barley has been shown to consist primarily of hydrocarbons, esters, β-diketones, aldehydes, primary alcohols, hydroxy-β-diketones and free fatty acids (36, 37). These seven lipid classes are also present on the same organs of Foma barley. In the wax from Foma spikes, an additional important lipid class is present which co-chromatographs in both TLC systems with the 29 carbon secondary alcohols isolated from *Brassica oleracea* (25, 30, 31). Of the three peaks present upon GLC of the Foma secondary alcohols, the middle one co-chromatographs with the *Brassica* standard. The chain length distribution is C_{27} = 4%, C_{29} = 46% and C_{31} = 50% (weight %). Confirmation of these results was obtained by oxidizing the Foma and *Brassica* secondary alcohols, and subjecting the resulting ketones to GLC-MS. Molecular ions for the three peaks were at m/e = 394, 422 and 450, respectively. A major pair of fragments formed during breakdown of a ketone in the mass spectrometer arises from homolytic fissions on either side of the carbonyl group. The identification of these α-cleavage peaks thus reveals the position of the carbonyl group(s) (2, 25, 32, 43). An examination of the mass spectra of the three ketone peaks indicates that the C_{27} secondary alcohol in Foma is predominantly heptacosan-9-ol (m/e = 141 plus 281). The two predominant structural isomers of the C_{29} secondary alcohol are, in order of decreasing importance as reflected by the relative sizes of the α-cleavage peaks, nonacosan-11 and -10-ol (m/e = 169 plus 281 and 155 plus 295, respectively). In the C_{31} secondary alcohol four structural isomers are important, namely hentriacontan-11, -13, -12 and -10-ol (m/e = 169 plus 309, 197 plus 281, 183 plus 295 and 155 plus 323, respectively).

To date, the lipid class composition has been determined for the spike waxes from the three wild types Bonus, Foma and Carlsberg-II and 40 *cer* mutants of which 30 were induced in Bonus, 8 in Foma and 2 in Carlsberg-II. Of these only Foma and its mutants *cer-zr*^{260}, -^{271}, *-zn*^{362}, -^{383}, -^{423}, -^{481}, *-qu*^{510} and -^{578} have secondary alcohols. The latter lipid class was not present in the leaf sheath and internode waxes from Foma or the six Foma mutants *cer-zr*^{260}, -^{271}, -^{383}, -^{423}, -^{481} and -^{578} which were examined.

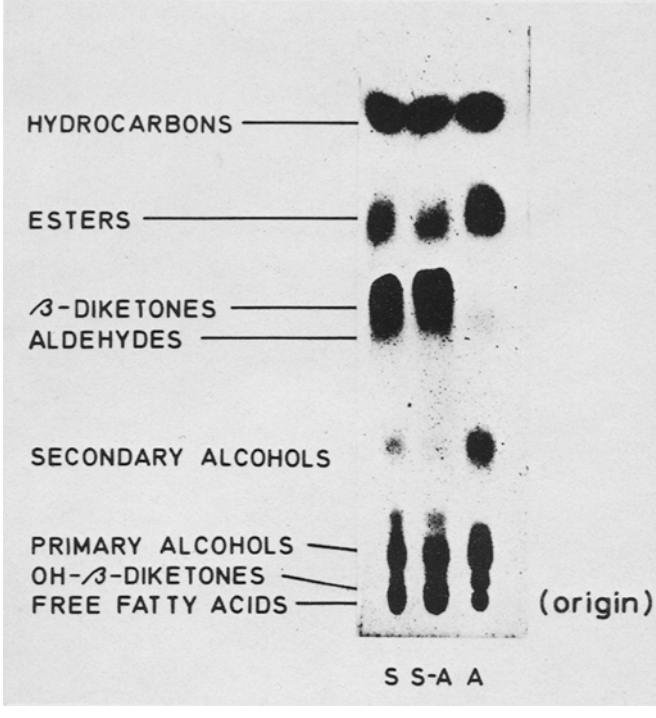

Figure 1. *Composition of the epicuticular waxes present on spikes of cer-*^{271}.
Lipid classes were separated on a Silica Gel H thin layer plate by development with benzene, and visualized by spraying with 50% H_2SO_4 followed by charring at 180°C. S = whole spikes; S-A = spike minus awns; A = awns

The composition of the epicuticular wax present on the spikes of *cer-*^{271} is shown in Fig. 1. Division of the spike before wax extraction into two parts, namely the awns and the spike minus awns, reveals that three of the major wax classes are specific for given parts of the spike (Fig. 1). The β-diketones and hydroxy-β-diketones are present only in the wax from the spike minus awns, whereas the secondary alcohols are found in the wax from the awns. Although in Fig. 1 no

trace of β-diketones and hydroxy-β-diktones can be seen in the awn wax, a trace of secondary alcohols is visible in the spike minus awn wax. Two interpretations of the latter observation are possible; either small amounts of secondary alcohols are indeed characteristic for spike minus awn wax, or the awns were not completely removed from the remainder of the spike when the spike was divided into two parts. Since no distinct gross morphological difference occurs to demarcate the boundary between a lemma and its awn, the latter is very likely to have happened.

3.2 Chain Length Distributions of the Lipid Classes on *cer-u*[69] Spikes

The chain length distributions of the hydrocarbons found on the awns and spike minus awns are similar in that the two dominating members have 31 and 29 carbons (Table I). The larger of these is the C_{31} homologue which amounts to 77% of the awn hydrocarbons and to 56% of those on the spike minus awns.

Two chain lengths are prominent components of the normal aldehydes, namely those having 32 and 30 carbons (Table II). In the total spike spectrum, however, the C_{28} and C_{26} homologues are present in greater quantities than expected. That is, the total spike aldehyde distribution should be some intermediate between that of its two parts, the awns and the spike minus awns. To verify these observations aldehydes were isolated from two additional sets of plants with the same results. In addition to the normal aldehydes, a second homologous series of unknown structure accounts for 18% of the aldehydes isolated from the awns (Table II). The longest members of this series, whose retention times were slightly greater than normal C_{32} and C_{34} aldehydes, were also those present in the largest quantities. This result was highly repeatable. By contrast the unknown series represents only 4% of the spike minus

Table I

Composition of hydrocarbons found on different parts of *cer-u*[69] spikes (weight %).

Number of Carbons[a]	Total Spike	Awn	Spike Minus Awns
21	0.6		1.2
22	tr		0.2
X			0.3
23	2.5	0.6	5.8
24	tr		0.1
X	0.2		0.7
25	1.9	1.0	3.7
26			tr
X	0.7	tr	1.6
27	2.4	2.0	3.2
28	0.3	0.2	1.0
X	2.5	2.0	3.5
29	14.8	11.4	18.2
30	1.7	1.6	2.2
31[b]	69.5	77.1	55.6
32	0.2	0.6	0.7
33	2.7	3.3	1.9

a. X represents a member of a second homologous series whose retention times are slightly less than the following normal hydrocarbon.

b. Includes the longest member of the X series which was too small to be resolved from the C_{31} hydrocarbon under the GLC conditions used.

tr = trace (<0,1%)

Table II

Composition of aldehydes found on different parts of *cer-u*[69] spikes (weight %).

Number of Carbons[a]	Total Spike	Awn	Spike Minus Awns
26	9.5	2.2	tr
Y	tr	tr	
28	13.8	4.9	2.5
Y	1.5	3.9	4.0
30	31.0	19.0	23.3
Y	tr	2.1	tr
31	tr	0.4	0.9
32	44.2	54.9	68.0
Y	tr	5.1	tr
34		tr	tr
Y		7.1	
Unknowns	tr	0.3	1.2

a. Y represents a member of a second homologous series whose retention times are slightly greater than those of the preceding normal aldehyde.

tr = trace (< 0.1%).

awn aldehydes and 2% of those from the total spikes (Table II). In repeat experiments, however, these values were as high as 20 and 18%, respectively, with the most prominent member having a retention time slightly greater than the normal C_{28} aldehyde.

A number of different factors may be contributing to the variability in the amount of the unknown aldehydes and to the non-intermediate spectrum of the total spike aldehydes. Although they account for less than 5% of the total spike wax, evidence is accumulating that aldehydes are important intermediates in the biosynthesis of several of the other wax classes (5, 8, MIKKELSEN and VON WETTSTEIN-KNOWLES unpublished). Slight differences in the age of the spikes at the time of wax harvest may therefore be significant. The spike minus awn epicuticular aldehydes may also be contaminated by internal aldehydes during wax extraction since it is impossible to avoid bringing cut surfaces into contact with the chloroform. And finally, to remove the β-diketones from the total spike and spike minus, awn wax, the wax is passed through a column containing copper acetate (26) which may result in some oxidation of the aldehydes. This step was omitted when the wax classes from the awns were being isolated and purified.

In the chain length distribution of the total spike free acids, the C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} chain lengths are all important homologues (Table III). By comparison the awn free acid spectrum is characterized by increased amounts of the shorter C_{16}, C_{18} and C_{20} homologues. This implies that the spike minus awn free acids must contain, relative to the total spike free acid spectrum, larger proportions of the longer C_{28}, C_{30} and C_{32} chain lengths. Reliable data for the spike minus awn free acids are not obtainable since contamination by internal fatty acids, especially C_{16} and C_{18}, occurs during the wax extraction procedure. The spectra of the ester acids differ markedly from those of the free acids (Table III) in consisting primarily of the shorter C_{16}, C_{18}, C_{20}, and C_{22} chain lengths of which the C_{20} homologue is the largest. The C_{24} and longer chains are minor constituents of the ester acids on the spike minus awns, and even on the awns they total only 8% of the ester acids.

The C_{26} chain length is the dominant member of

the free alcohols, alkan-1-ols, on both parts of the spike (Table IV). The complete spectra of the alcohols from the two sources differ, however. That is, the C_{24} and C_{26} homologues account for 74% of the spike minus awn alcohols but only 49% of those on the awns. On the other hand, the C_{30} and C_{32} free alcohols are relatively important on the awns (28%) compared to on the spike minus awns (7%). That greater amounts of the longer chain free alcohols are present on the awns compared to the spike minus awns (Table IV) is the reverse of what was observed for the free acids (Table III). The dominance of the C_{26} chain length is also characteristic for the leaf free primary alcohols (8, 36, 38) where it may account for as much as 90% of this wax class. When the importance of the C_{26} chain length is reduced in leaf waxes, however, either via a gene mutation or the environmental growth conditions, the shorter C_{22} and C_{24} homologues become prominent (8, 9, 36, 38) and not the longer C_{28}, C_{30} and C_{32} chain lengths as in the spike waxes.

Esterified alkan-1-ols are also present on both parts of the spike (Table IV). In the esters from the spike minus awns but not from those on the awns an additional type of alcohol is found. The latter, alkan-2-ols, do not occur free in the wax. The presence versus absence of the esterified alkan-2-ols is one of the major differences between the waxes on the two parts of the spike (Table IV). The chain lengths of the two types of ester alcohols are quite different. The alkan-2-ols are short, mainly C_{13} and C_{15}, whereas the

Table III

Composition of free and ester acids found on different parts of *cer-u*[69] spikes (weight % as methyl esters).

Number of Carbons	Free[a]		Ester		
	Total Spike	Awn	Total Spike	Awn	Spike Minus Awns
14		0.4		0.6	
15		0.1		tr	
16	1.4	5.6	12.4	12.6	9.1
17		0.1	0.3	tr	tr
18	2.6	5.9	13.4	13.3	11.2
19		tr	tr	tr	tr
20	11.9	26.3	50.8	36.4	53.6
21	0.3	0.5	0.5	tr	0.7
22	20.2	18.6	19.4	16.5	21.5
23	0.9	1.0	tr	tr	tr
24	21.4	21.1	2.0	7.0	2.0
25	0.4	0.6	tr	tr	tr
26	14.4	9.6		1.2	
27	0.2	tr			
28	13.6	3.1			
29	0.3				
30	8.2	0.7			
32	3.8	0.7			
Unknowns	0.4	5.6	1.2	12,4[b]	1.7

a. Data for free acids from the spike minus awns not available as they cannot be extracted without being contaminated by internal free acids.

b. Primarily a single unknown (= 11.5%) which co-chromatographs under the GLC conditions used with an iso-C_{18} acid.

tr = trace (< 0.1%).

Table IV

Composition of free and ester alcohols found on different parts of *cer-u*[69] spikes (weight % as acetates).

Number of Carbons	Free			Ester		
	Total Spike	Awn	Spike Minus Awns	Total Spike	Awn	Spike Minus Awns
Alkan-2-ols						
13				2.1		7.8
15				9.8		21.8
17				1.9		tr
Alkan-1-ols						
20	2.2	tr	0.2	2.2	1.6	1.6
21				tr		tr
22	2.6	2.2	3.3	23.7	21.4	20.5
23	tr	tr	0.1	tr	tr	0.4
24	9.7	4.6	13.8	22.2	20.8	23.7
25	0.6	0.1	0.9	tr	tr	0.5
26	57.5	43.6	60.1	27.5	48.6	11.1
27	0.4	tr	0.5	tr	tr	tr
28	14.7	15.9	12.7	2.3	5.8	0.9
29	0.3	tr	0.3			
30	5.5	11.2	4.5	tr	1.0	tr
31	tr	tr	tr			
32	3.0	16.9	1.7			
Unknowns[a]	3.6	5.3	1.9	8.3	0.8	11.5

a. Two unknowns occur in the free alcohols; the retention time of the first is slightly greater than the normal C_{21} alkan-1-ol and that of the second is slightly greater than the normal C_{30} alkan-1-ol. The single unknown present in the ester alcohols has a retention time slightly greater than the normal C_{21} alkan 1-ol.

tr = trace (< 0.1%).

alkan-1-ols are long, mainly C_{22}, C_{24}, and C_{26}. Because of their relatively low boiling points, the alkan-2-ols are quite susceptible to loss during their preparation for GLC. An analysis of the ester alcohol composition using GLC-MS gave a 43:57 ratio (weight %) of the alkan-2-ols to alkan-1-ols (42) whereas in the GLC analysis of the alcohol moieties released via saponification from the same spike minus awn ester sample (Table IV) the ratio is 33:67, respectively. The data presented in Table IV thus underestimate the amounts of the esterified alkan-2-ols, especially of the C_{13} homologue.

The spectrum of the esterified alkan-1-ols on the awns is similar to that of the free alcohols in being dominated by the C_{26} chain length. However, whereas the C_{28} and longer chain lengths are next in importance in the awn free alcohols, the C_{22} and C_{24} homologues claim this distinction in the awn ester alcohols. The large reduction in importance of the C_{26} ester alcohol in the spectrum from the spike minus awns appears to be counter balanced by the presence of the alkan-2-ols.

The unknown ester alcohol (Table IV) which has a slightly longer retention time than the normal C_{21} alkan-1-ol occurs in somewhat variable amounts in repeat experiments. It may be identical to the shortest member of an unknown homologous ester alcohol series reported on barley seedling leaves that are grown under some environmental conditions (8). Experiments are in progress to determine the structure of this unknown.

Table V presents the composition of the esters on the different parts of the spike. Only one

Table V

Composition of esters found on different parts of *cer-u*[69] spikes (weight %).

Number of Carbons	Total Spike	Awn		Spike Minus Awns
		Observed	Calculated[b]	
Alkan-2-ol esters				
31	1.5			1.6
33	12.0			19.4
35	18.2			31.6
37[a]	3.1			4.5
Alkan-1-ol esters				
36[a]	0.2		0.4	0.3
38	4.4	1.7	3.5	4.0
40	9.3	7.6	7.3	7.4
42	17.3	20.7	19.5	12.1
44	17.2	25.8	21.2	13.4
46	13.8	31.7	27.1	5.6
48	3.0	9.6	13.7	tr
50	tr	2.9	5.7	
52			1.3	
54			0.2	

a. On the GLC columns used a 37 carbon alkan-2-ol containing ester and a 36 carbon alkan-1-ol containing ester have the same retention times. The relative proportions of these two esters in this single GLC peak were estimated from a mass spectum of the peak (see 42).

b. The expected ester distribution was calculated assuming that the normal ester alcohols (Table IV, column 5) and normal ester acids (Table III, column 4) were esterified randomly.

tr = trace (< 0.1%).

type of ester, namely those having alkan-1-ols as their alcohol moiety, is present on the awns. In the observed awn ester distribution, the most significant chain lengths are those having 46, 44 and 42 carbons. A rather similar distribution is generated assuming that the esterification of the normal ester alcohols with the normal ester acids is independent of the chain lengths of the two moieties. Both better and worse fits of observed to calculated ester distributions have been reported and claimed to support the hypothesis that the esterification mechanism is a random one (14, 16, 28, 29, 33). While the presebt data do not strongly support the random esterification hypothesis, they also do not imply that the esterification enzyme (s) have a marked chain length specificity. On the spike minus awns, the alkan-1ol containing esters form only 43% of this wax class (Table V). The other 53% of the esters contain alkan-2-ols. In the latter type of ester, the most important homologues have 33 and 35 carbons, approximately eight carbons less than the dominating members of the alkan-1-ol containing esters. The longer the alkan-1-ol containing ester, the greater its prominence in the awn ester spectrum compared to in the spike minus awn and total ester spectra. A detailed comparison of the observed spike minus awn ester distribution with that expected if esterification was random has been presented in connection with the structural identification of the esterified alkan-2-ols (42).

4. DISCUSSION

Chemically, epicuticular waxes present on the (i) leaf blades, (ii) uppermost leaf sheaths plus

internodes and (iii) spikes of barley are easily distinguishable from one another (36, 37). The greatest difference is the absence of the two types of β-diketones and the alkan-2-ol containing esters in the leaf blade wax (36, 37, 42). In the present analyses the awn wax was found to be analogous to that of the leaf blades in also lacking these lipids. Awn wax of certain genotypes, however, contains prominent amounts of secondary alcohols which are absent in their leaf blade waxes. The chain length distributions of the other awn lipid classes, such as the hydrocarbons, may be quite similar to those found for the respective class from the spike minus awns. On the other hand, the distributions of the free primary alcohols from the two analyzed parts of the spike differ. The composition of wax isolated from intact barley spikes, thus, represents a composite of at least two different mixtures of lipids. Most likely separate analyses of the wax from other spike parts, such as the lemmas or paleas, will uncover additional tissue specificities. The excised whole spike has proven to be very active in the biosynthesis of wax lipids and useful for labelling experiments. The tissue specificity of wax composition may make it necessary to harvest the wax of the different parts of the spike separately or to use tissue slices of the individual parts for precursor incorporation. Thus, awns are useful for studies of the biosynthesis of the secondary alcohols but can be excluded in the analysis of β-diketone synthesis (41).

The presence of secondary alcohols on the awns of Foma and their absence in Bonus is a prominent difference between the epicuticular waxes of these two closely related barley varieties. Foma was derived from the progeny of a cross between Ymer, a sister variety of Bonus, and Morgenroth (10, 11). As in many other plant waxes (see 43), the chain length distribution of the secondary alcohols in Foma is similar to that of the awn hydrocarbons. That is, in both wax classes the 29 and 31 carbon chains are the most important, although the relative proportions of the two chain lengths differ. Presumably in barley hydrocarbons are precursors of the secondary alcohols as is the case in broccoli, *Brassica oleracea* (17, 18), and the grasshopper *Melanoplus sanguinipes* (3). The specificity of the barley hydroxylation enzyme(s) must be different from that of the *Brassica* enzyme(s). Whereas in both kale and broccoli two isomers of a single chain length are found (18, 25), in barley as many as four prominent isomers of a single chain length occur and three different chain lengths are hydroxylated. Insertion of hydroxyl group(s) into preformed β-diketone molecules is also known. In barley the locus *cer-u* is involved in the specific hydroxylation of carbon-25 of hentriacontan-14,16-dione (37, 40). To account for the different types of hydroxylated β-diketones which have been identified (see 34), the existence of a corresponding number of enzymes with the corresponding specificities are inferred (34, 40).

The boundaries for changes in wax composition in barley follow closely the morphological boundaries of different organs. In other plants such a detailed correlation does not hold. For instance, in wheat the β-diketones extend from the leaf sheath onto the basal 1-2 cm of the abaxial flag leaf surface (26). One may ask the question whether wax composition is determined as a tissue or cell specific differentiation. Within a leaf marked variation in wax composition is known. For example, only tubes, indicative of the presence of β-diketones, are present over the mid-rib of *Eucalyptus polyanthemos* leaves, but they are absent over most of the leaf lamina which is covered with wax plates (13). A short transition zone with both tubes and plates occurs adjacent to the mid-rib (see 24). In barley the leaf sheaths of the mutant *cer-zw*^{286} have a zebra-like appearance, that is, they have alternating rings of grey and green color (21). These tube and non-tube wax zones reflect the presence and absence of β-diketones, respectively (VON WETTSTEIN-KNOWLES unpublished). This intimates that wax synthesis and deposition in barley is cell specifically determined. In *Zea mays* the gene gl_1 is thought to act by specifically inhibiting structural wax formation over all leaf epidermal cells excepting the accessory cells of the stomates (19).

Even on the surface of a single cell, the wax structures may vary at different sites. Long thin tubes and highly lobed plates, rising as thin

sheets perpendicular to the surface, are contiguous on the lemmas of *cer-u*[69] (37). A strong reduction in β-diketone content in subglaucous mutants of wheat results in a few isolated groups of long tubes rather than an even distribution of very short tubes on the flag leaf sheaths (26). Such occurrences suggest that the exuding wax does not have a uniform composition (39). One of us (39) has concluded earlier that the organ and cell specific morphology of the wax bodies, often called crystals (see 15), depends on the chemical composition of the exudate, as well as on the way and rate by which the wax is exuded through the cuticle. This is supported by studies on the *in vitro* formation of wax bodies (crystals) from waxes dissolved in organic solvents. In JEFFREE *et al*'s (15) experiments, the epicuticular wax solution was moved with the aid of a wick through pores of discs or membranes, and in several instances formed upon contact with air wax bodies very similar to those found *in vivo*. Apart from the chemical composition, in these cases the amount and rate of movement of the wax solution through the pores was critical for the attainment of the proper wax morphology, whereas the size and distribution of the pores did not seem to matter. Most interestingly, a mixture of the epicuticular wax lipids could not be used to duplicate the specific wax morphologies found on Brussel sprout, *Brassica oleracea*, leaves. If the individual wax classes, however, were delivered successively through the pores, a morphological diversity of wax bodies resembling those on the leaf could be attained. ARMSTRONG (1) had earlier approached the problem in another way. He varied the rate at which the solvent was dispersed from solutions of waxes obtained from the surfaces of rape *Brassica napus* leaves. In contrast to JEFFREE *et al* (15), he obtained wax structures very closely resembling those present *in vivo*, given certain rates of solvent dispersal. In conclusion, studies of the cell and organ specific deposition of epicuticular waxes in wild type barley and the *cer* mutants have substantiated the importance of the chemical composition for wax morphology, as first pointed out by HALL *et al* (12). However, the processes of wax exudation are also involved in controlling wax morphology on the cuticular surfaces.

ACKNOWLEDGEMENTS

We are indebted to JUDY NIELSEN for technical assistance, to the personnel of the Phytotron at the Royal College of Forestry, Stockholm, for growing the plants, and to ROLF SERVIN of the Mass Spectrometry Laboratory, Chemistry Centrum, University of Lund, Sweden, for taking the mass spectra. This work was supported by grant GM-22051 of the National Institutes of Health, U.S. Public Health Service, the Carlsberg Foundation and Euratom Contract No. 124-74-1 B 10 DK. AGN gratefully acknowledges postdoctoral fellowships from the Carlsberg Foundation and the Danish Natural Science Research Council.

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