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# EPICUTICULAR WAX OF *TRITICUM AESTIVUM* DEMAR 4

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**Abstract**—The composition of epicuticular wax from plants of bread wheat (Demar 4 variety) at 3 stages of growth was studied. After germination for 30 and 130 days the components were hydrocarbons, esters, aldehydes and alcohols, the latter class of compounds, comprising mainly octacosanol, being dominant. From 130 to 190 days after germination, plants began to synthesize β-diketones and hydroxy-β-diketones whilst aldehydes disappeared.

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## INTRODUCTION

An investigation of the role of the genotype in determining the synthesis and deposition of epicuticular wax in cereals has been initiated [1]. In this paper the composition of the epicuticular waxes of common wheat (variety Demar 4) at three development stages, 30 (I), 130 (II) and 190 (III) days after germination, is presented.

## RESULTS AND DISCUSSION

Wax was extracted from whole plants. The percentages of the wax (Table 1) ranged from 0.87 to 0.12% (dry wt) showing a decrease with development of the plants. This variation is higher than that reported for other *Triticum* species which ranged from 0.7 to 0.3% [2]. The classes of compounds present in the waxes are the same as those reported for other varieties of wheat [2–5] except for diesters and free acids which were not found in the waxes examined in this work. Column chromatography was found to be the most reliable means for quantitative analysis of the wax classes. The classes of compounds and the homologues within each class were identified by TLC, IR, GLC and MS as previously described [1].

Hydrocarbons formed an average of 11% of the wax of the 3 stages of growth examined (Table 1) and the homologue compositions are almost the same with major $C_{27}$, $C_{29}$, $C_{31}$ and $C_{33}$ components (Table 2). These results are similar to the hydrocarbon composition of wax from Selkirk wheat (*Triticum aestivum*) [2].

Table 1. Composition (%) and yields of wheat wax samples I, II and III

| Date of harvest | I<br>25.11.75 | II<br>4.3.76 | III<br>3.5.76 |
|---|---|---|---|
| Yield (% dry wt) | 0.87 | 0.61 | 0.12 |
| Hydrocarbons | 9.4 | 17.8 | 6.9 |
| Esters | 15.3 | 19.4 | 12.8 |
| Aldehydes | 14.1 | 16.0 | — |
| Primary alcohols | 61.2 | 46.8 | 42.5 |
| β-Diketones | — | — | 12.2 |
| Hydroxy-β-diketones | — | — | 25.6 |

Methanolysis of the esters showed that *n*-octacosanol was the major component of the esterified alcohols but other shorter chain homologues were also present, especially from stages of growth II and III. The chain length range for the fatty acids was $C_{16}$–$C_{28}$ where $C_{16}$, $C_{18}$, $C_{20}$ and $C_{22}$ were the largest components (Table 2).

The free alcohol content of the wax of each growth stage, was high (42–61%) and *n*-octacosanol was always the major component (Tables 1 and 2). Aldehydes were found only at the earlier stages of growth (I and II) and were mainly *n*-octacosanal (99%). Only small differences in the composition of the waxes of stages I and II were observed. Such results are not unexpected because the period between 30–130 days (I and II) is winter time in N. Italy when plant growth is negligible. With the spring, development is resumed and at 190 days (III) the flag leaf is completed and the spike is emerging from the sheath. This stage is characterized by the appearance in the wax of two new classes of compounds, β-diketones and hydroxy-β-diketones which together then constitute *ca* 38% of the total wax.

β-Diketones were composed essentially of two homologues, $C_{31}$ (95%) and $C_{29}$ (5%); hydroxy-β-diketones comprised two $C_{31}$ positional isomers. Both hydroxy-β-diketones and β-diketones gave a strong purple–red colouration with $FeCl_3$ and showed a strong UV absorption maximum at 277 nm in *iso*-octane with $E^{1\%}_{1\,\mathrm{cm}}$ (see Experimental) similar to previous data reported in the literature [5, 6].

MS analysis [6] established the structures of the β-diketones as hentriacontane-14,16-dione and nonacosane-12,14-dione and 8- and 9-hydroxyhentriacontane-14,16-dione for the hydroxy-β-diketones.

As already reported [2] and also found during the present study, β-diketones and hydroxy-β-diketones are present in different parts of the plant such as blades, sheaths and heads (a study on the subject is in progress [7]).

With the appearance of the β-dicarbonyl compounds there is a drastic decrease in the amount of wax produced

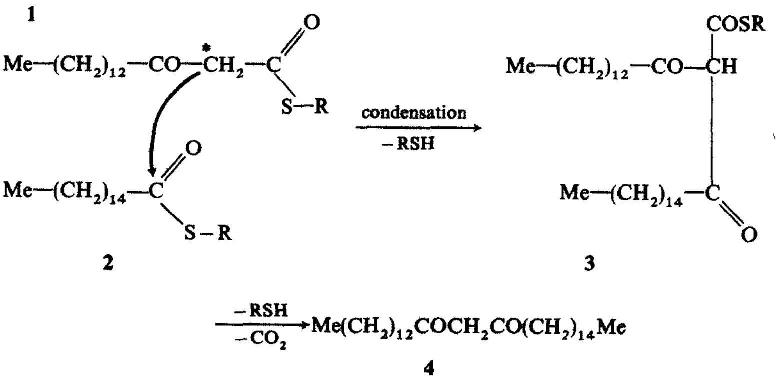
Table 2. Homologue composition of wax fractions of wheat (%)

| Numbers of C atoms | Alkanes | | | Aldehydes | | | Primary alcohols | | | Esterified alcohols | | | Esterified fatty acids | | | $\beta$-Diketones | | | Hydroxy-$\beta$-diketones | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III |
| 14 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 15 | — | — | — | — | — | — | — | | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 16 | — | — | — | — | — | — | — | — | — | 1.4 | 9.6 | — | 26.9 | 21.7 | 19.7 | — | — | — | — | — | — |
| 17 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 18 | — | — | — | — | — | — | — | — | — | 2.4 | 16.0 | 2.5 | 17.9 | 19.0 | 12.7 | — | — | — | — | — | — |
| 19 | 0.1 | 1.6 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 20 | 0.3 | 0.4 | 0.5 | — | — | — | — | — | — | 0.3 | — | 3.4 | 16.8 | 16.2 | 23.9 | — | — | — | — | — | — |
| 21 | 0.4 | 4.7 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 22 | 0.4 | 0.2 | tr | — | — | — | tr | 9.0 | — | 2.7 | 4.0 | 11.9 | 23.9 | 27.7 | 34.6 | — | — | — | — | — | — |
| 23 | 1.5 | 1.1 | 0.5 | — | — | — | — | 0.2 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 24 | 0.3 | 0.4 | tr | — | — | — | — | 3.3 | tr | 0.3 | tr | 7.6 | 6.4 | 3.7 | 7.9 | — | — | — | — | — | — |
| 25 | 2.7 | 3.9 | 1.1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 26 | 0.7 | 0.4 | — | — | — | — | tr | 1.6 | tr | tr | tr | tr | 3.6 | 1.5 | tr | — | — | — | — | — | — |
| 27 | 17.4 | 19.9 | 14.5 | — | — | — | — | 0.5 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 28 | 0.8 | 0.1 | — | 99 | 99 | tr | 99 | 83.9 | 99 | 92.9 | 68.4 | 74.6 | 4.5 | 7.2 | 1.2 | — | — | — | — | — | — |
| 29 | 17.1 | 15,0 | 37.5 | — | — | — | — | — | — | — | — | — | — | — | — | — | | 5 | — | — | — |
| 30 | — | — | — | — | — | — | tr | 0.6 | — | — | — | — | — | — | — | — | — | | — | — | |
| 31 | 34.1 | 31.6 | 27.7 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 95 | — | — | 100 |
| 32 | — | — | — | — | — | — | — | 0.9 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 33 | 24.2 | 12.1 | 17.7 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| others | — | 8.7 | — | 1.0 | 1.0 | — | — | — | — | — | 2.0 | — | — | 3.0 | — | — | — | — | — | — | — |

(0.61 to 0.12% dry wt) with the disappearance of the aldehydes together with a marked decrease in hydrocarbons accompanied, to a minor extent, by esters and alcohols (Table 1).

While the mechanism of formation of hydrocarbon , esters, aldehydes and alcohols is well known and almost universally accepted, the biosynthesis of $\beta$-diketones is still an open question. According to Kolattukudy [8], $\beta$-diketones are formed by condensation of two acid units with malonate followed by decarboxylation. This mechanism, however, was criticized by Tulloch [2] and, more recently, by Netting and von Wettstein–Knowles [9] on the basis that such a route is feasible only for symmetrical $\beta$-diketones while compounds such as hentriacontane-14,16-dione, hentriacontane-10,12-dione and the other asymmetrical diones found in waxes would require participation of two different C units, $C_{14}$, $C_{16}$ and $C_{10}$, $C_{20}$, respectively.

Netting and von Wettstein-Knowles [9], making use of labelled acetate, advanced an alternative mechanism for the formation of $\beta$-diketones. According to these authors, hentriacontane-14,16-dione found in barley is formed through an elongation mechanism where $C_2$ units are added to a mixture of short chain precursors to give the complete molecule of hentriacontane, which in turn is transformed into the $\beta$-diketone. The way in which the $\beta$-diketone is formed from the hydrocarbon, has still to be elucidated. As stated by the authors, this mechanism needs to be further substantiated. At this point we propose an alternate hypothesis for the synthesis of $\beta$-diketones taking into account our results. In fact, as already pointed out above, with the appearance of $\beta$-



Scheme 1. A possible mechanism for the synthesis of hentriacontane-14,16-dione 4 in Demar 4 bread wheat. *R* may be either CoA or ACP or both.

diketones and their hydroxy derivatives, the wheat plant shows a clear decrease in the formation of the other classes of compounds present in the wax (Table 1). These findings indicate that at later stages of growth the enzyme systems responsible for the synthesis of alkanes, esters, aldehydes and alcohols are less effective while new biosynthetic pathways become dominant.

We believe that in the biosynthetic pools where $\beta$-diketones are formed, the reduction enzymes which transform $\beta$-ketoesters into esters, according to the elongation mechanism, are only partially operating and that instead other enzymes catalyze the formation of the $\beta$-dicarbonyl compounds. According to this hypothesis, the precursor $C_{14}$ acid is elongated to the $C_{16}$ $\beta$-ketoester **1** which in turn is only partially reduced by the appropriate enzyme to the corresponding $C_{16}$ ester **2** (Scheme 1). At this point a highly specific condensation system brings about condensation to hentriacontane-14,16-dione, seemingly through a nucleophilic attack by the carbanoid carbon of **1** (indicated by *) onto the ester carbonyl of **2** to give **3** on elimination of RSH. In the final step **3** → **4**, the thiol ester is hydrolyzed and, after elimination of $CO_2$, hentriacontane-14,16-dione **4** is formed.

Another important point is the biosynthetic significance of the presence of the 8– and 9–hydroxyhentriacontane-14,16-diones, undoubtedly deriving from the unsubstituted $\beta$-diketone. These compounds have been found in all the wheat and related species varieties so far studied [3–5, 10–12]. Cereal species can thus be classified into two groups according to the position of the OH. The first group comprising *Triticum compactum* [3], *T. aestivium*: Selkirk, Manitou [5] and Demar 4 [present work] is characterized by the presence in the wax of the 8- and 9-OH $C_{31}$ isomers whilst the species of the second group namely *T. durum* (Pelissier, Stewart) [4], *Secale cereale*, *Triticale hexaploide* [10], *Agropyron intermedium*, *Agropyron Smithii* [11, 12] syhthesize either one or both of the 25- and 26-OH isomers.

## EXPERIMENTAL

Plants of the common winter wheat variety Demar 4 were grown in the field in the Po valley near Milan during 1975–76. A number of plants were collected at 3 stages (Table 1), swirled in $CHCl_3$ for *ca* 1 min and the extract filtered and evaporated to dryness. GLC analyses were performed as previously described [1]. MS were measured with a direct inlet system at 70 eV with an ion source temp. of 220°. IR spectra were measured in KBr discs and UV spectra in *iso*-octane solns. Mps of the waxes extracted were 65–78° sample (I), 64–75° sample (II) and 62–72° for sample (III). The wax samples had in addition to C–H absorption, the following characteristic peaks in their IR spectra ($cm^{-1}$): (I) 3380 (OH alcoholic), 1738 (C=O ester), 1715 (weak peak, aldehyde); (II) the same peaks as (I); (III) 3380–3400 (OH alcoholic and enolic), 1738 (ester), 1600 (shoulder) and 1640 ($\beta$-diketones and hydroxy-$\beta$-diketones). A typical column chromatography separation was carried out on a 500 g Kieselgel H column which was exhaustively washed with 500 ml of $CCl_4$ and then loaded with 0.5 g of wax. The following order of elution with $CCl_4$ was observed: hydrocarbons, esters, aldehydes and $\beta$-diketones. The solvent was then changed to $CHCl_3$ and alcohols and hydroxy-$\beta$-diketones respectively were then eluted. The purity of all fractions was checked by TLC. For the identification of the homologues in the hydrocarbon, free alcohol, aldehyde and ester fractions GLC was used (Table 2). Alkyl esters were transesterified to fatty alcohols and Me esters by refluxing 20 mg of esters with 5 ml of 10% HCl–MeOH and 5 ml of $C_6H_6$ for 2 hr. Identification of the products was made by GLC comparison and in some instances, by coinjection with authentic compounds. Pure $\beta$-diketones after column chromatography separation had mp 55–59°; IR: 1740 shoulder, 1640, 1465, 1418, 905, 815, 785, 770, 730, 720 $cm^{-1}$; UV: $\lambda_{max} = 277$ nm; $E^{1\%}_{1\,cm} = 168.8$. MS and GLC showed the presence of two components hentriacontane-14,16-dione ($\geqslant 95\%$); MS *m/e* (rel. intensity): 464 (25) $M^+$, 446 (19), 309 (18), 296 (25), 281 (41), 278 (21), 268 (28), 253 (34), 250 (20), 239 (43), 220 (19), 211 (45), 192 (19), 100 (100), and nonacosane-12,14-dione ($\leqslant 5\%$) MS *m/e*: 436 (8) $M^+$, 418 (6), 309 (18), 296 (25), 281 (41), 278 (21), 253 (34), 240 (9), 239 (43), 225 (14), 222 (3), 220 (19), 183 (16), 169 (16), 100 (100). Hydroxy-$\beta$-diketones had a mp 67.5–70.5°; IR, 1720 and 1695 shoulders, 1635, 1470, 1412, 1130–1140, 1090, 785, 770, 720 $cm^{-1}$; UV: $\lambda_{max} = 277$ nm; $E^{1\%}_{1cm} = 223.5$; $\varepsilon = 10.727$. MS, *m/e* (rel. intensity): 480 (8) $M^+$, 462 (24), 444 (12), 381 (16), 367 (10), 352 (4), 309 (23), 297 (6), 296 (12), 284 (14), 281 (37), 269 (22), 266 (31), 251 (36), 239 (48), 227 (7), 209 (20), 43 (100). Using the two ions *m/e* 381 and 367. characteristic of the 8- and 9-OH isomers, respectively, the hydroxy-$\beta$-diketones were shown to be a mixture of 8-hydroxyhentriacontane-14,16-dione (61%) and 9-hydroxyhentriacontane-14,16-dione (39%).

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