Phytochemistry, 1968, Vol. 7, pp. 2089 to 2093. Pergamon Press. Printed in England

CHEMICAL GENETICS OF β-DIKETONE FORMATION IN WHEAT

H. NEWTON BARBER and A. G. NETTING

Botany Department, University of New South Wales, Kensington, N.S.W., Australia

(*Received* 24 *April* 1968)

Abstract—Glaucous lines of wheat are characterized by the presence of β-diketones in the wax on non-vegetative organs. Green (non-glaucous) lines possess, at most, only a trace of β-diketone. Comparison of a glaucous line with a green line, deficient for a chromosome arm carrying a wax-producing gene, shows that suppression of β-diketone formation is correlated with the appearance of normal primary alcohols, which are absent from the glaucous plants. It is suggested that the primary alcohols are formed from a precursor of the β-diketones.

INTRODUCTION

GLAUCOUSNESS, due to a superficial deposit of light-scattering crystallites of wax, is largely restricted in wheat, to the flagleaf-sheath, the abaxial surface of the flagleaf, the peduncle and the ear. The degree of glaucousness of the adaxial surface of the flagleaf is variable, depending on the variety, but is less than that of the flagleaf-sheath.[1] Macey[2] has shown that mutants of *Brassica oleracea* and *Pisum sativum*, which are non-glaucous, have wax of a different chemical composition to that from normal glaucous plants. He interpreted some of these changes in terms of genetic blocks in the presumed biosynthetic pathways. We have carried out similar analyses of the wax from glaucous and non-glaucous wheats with a similar end in view.

RESULTS AND DISCUSSION

The lines of wheat used, their phenotypes and genetic constitutions are given in Table 1. They include four glaucous lines and four non-glaucous lines, and are all hexaploids with the exceptions of line 3 and line 8. The non-glaucous lines all involve a change in chromosome 2B. They include: (1) A line deficient for the non-standard arm of chromosome 2B: the missing arm contains a gene for the production of glaucousness.[3] (2) A hexaploid line which possesses a dominant inhibitor of glaucousness located on the non-standard arm of chromosome 2B.[4,5] (3) A tetraploid line from which the inhibitor in the hexaploid was derived by hybridization.[6] (4) A line which possesses a recessive mutation, producing non-glaucousness, again on the non-standard arm of chromosome 2B.[7] Driscoll[5] has located the

[1] J. H. TROUGHTON and D. M. HALL, *Australian J. Biol. Sci.* **20**, 509 (1967).
[2] M. J. K. MACEY, Chemistry and Genetics of Plant Cuticle Waxes, Ph.D. Thesis, University of New South Wales (1967).
[3] C. J. DRISCOLL, *Ann. Meeting Am. Soc. Agron. Abstracts*, p. 65 (1964).
[4] C. J. DRISCOLL and N. F. JENSEN, *Can. J. Gen. Cytol.* **6**, 324 (1964).
[5] C. J. DRISCOLL, *Genetics* **54**, 131 (1966).
[6] N. F. JENSEN and C. J. DRISCOLL, *Crop Sci.* **2**, 504 (1962).
[7] J. STUCKEY, personal communication.

TABLE 1. GENETIC STOCKS

Line	Description	$E^{1\%}_{272nm}$
	Non-glaucous lines	
1	"Chinese Spring" deficient for the non-standard arm of chromosome 2B	14·8
2	A line heterozygous for a dominant inhibitor	3·5
3	"Long Kernel" (tetraploid AABB), homozygous for the same dominant inhibitor as in line 2	4·2
4	"Chinese Spring" homozygous for a recessive mutation	8·4
	Glaucuous lines	
5	"Chinese Spring" (two replicates)	75·3 77·5
6	"Chines Spring" tetrasomic 2B	68·0
7	"Bearded Yalta"	54·2
8	*T. durum* Desf. cv. mindum (tetraploid AABB), very glaucous	145

All lines used, except lines 3 and 8, are taxonomically *Triticum aestivum* L. emend. Thell. Line 3 is a hybrid derived from *T. dicoccoides* Körn. and *T. polonicum* L.

dominant inhibitor at 42 to 50 map units from the centromere. However, the allelic relationships of the three genes (dominant inhibitor, recessive mutation and normal wax-producing gene) are unknown.

Figure 1 shows a thin-layer chromatogram of the waxes from the eight lines of wheat listed in Table 1, as well as cauliflower leaf wax, the components of which have been identified by Macey.[2] From a comparison of the chromatograms, wheat-wax contains hydrocarbons,

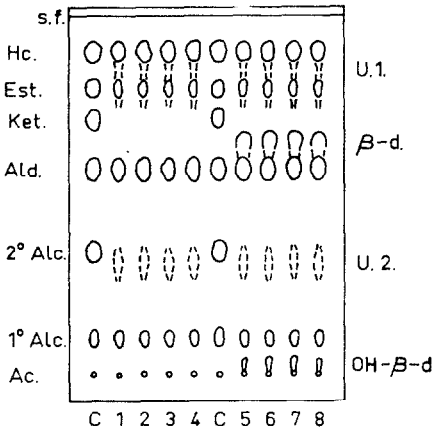

FIG. 1. THIN-LAYER CHROMATOGRAM OF WAXES EXTRACTED FROM CAULIFLOWER LEAVES AND WHEAT PLANTS BY DIPPING INTO BOILING LIGHT PETROL. FOR 20 sec.

Layer material: Silica gel G. Layer dimensions: 0·3 × 100 × 100 mm. Developing solvent: benzene. Detection of spots: conc. H_2SO_4 + charring.

Samples; C Cauliflower leaf wax; 1 to 8 waxes from eight lines of wheat (see Table 1). *Cauliflower leaf wax components;* Left side of chromatogram, Hc. = Hydrocarbons, Est. = Esters, Ket. = Ketones, Ald. = Aldehydes, 2° Alc. = Secondary Alcohols, 1° Alc. = Primary Alcohols, Ac. = Acids. s.f. = solvent front. *Wheat wax components;* Right side of chromatogram (only those not also found in cauliflower leaf wax are named). U.1 and U.2 = unidentified components of low concentration. β-d. = β-diketones, OH-β-d = Hydroxy-β-diketones.

esters, aldehydes, primary alcohols and acids in common with cauliflower leaf wax. These identifications have been confirmed on lines 1 and 7 by i.r. spectrophotometry of fractions obtained by column chromatography. The dashed spots in Fig. 1 represent two unidentified components of low concentration in the wheat-waxes.

The most interesting feature of Fig. 1 is that the waxes from the glaucous lines contain two components not present in the waxes from the non-glaucous lines. The component which runs in front of the aldehydes has been identified as β-diketone. The complete wax, extracted from line 5, gave a distinctive band between 1650 and 1600 cm^{-1} (KBr crystal) in the i.r. *Eucalyptus* waxes, rich in β-diketones, have a strong band at 1607^{-1} (CCl_4).[8] This band was absent from the i.r. spectra of waxes from non-glaucous plants and was also lost when the presumed β-diketone was removed by complexing with copper, by the method of Horn, Kranz and Lamberton.[8] The extracted copper-complex had absorption bands at 1562 and 1518 cm^{-1} (KBr crystal). The copper-complex prepared from *Eucalyptus* wax had absorption bands at 1562 and 1523 cm^{-1} (nujol).[8] The whole waxes from lines 2, 7 and 8 had absorption maxima in their u.v. spectra (very weak for line 2) at 272 nm; *Eucalyptus* waxes have maxima at 273 nm, which again is due to the presence of β-diketones.[8]

Attempts to determine the structure of the β-diketone have only been partially successful. Tulloch and Weenink[9] state that in *Triticum compactum* the β-diketone fraction contains principally hentriacontan-14, 16-dione. Our results agree with this.

The other component characteristic of the glaucous lines runs between the origin and the primary alcohols (Fig. 1). It is probably hydroxy-β-diketone, which occurs together with the β-diketones in *T. compactum*.[9] We have partially purified this component from line 7. Its i.r. spectrum and the fact that it complexes with copper are in accord with this suggestion.

The relative amounts of β-diketone, including the hydroxy-β-diketone, present in each wax were estimated by measuring the absorption of a 1 per cent solution of the wax in hexane at 272 nm.[8] The method is approximate only, as Horn *et al.* point out, since it assumes that all the β-diketones (and in this case, hydroxy-β-diketones) have similar molecular weights. They also note that other wax components interfere, giving high results, particularly when the β-diketone content is small. The results are given in Table 1. All glaucous lines gave $E^{1\%}_{272nm}$ values greater than 54, while all non-glaucous lines gave values less than 15. Line 8, which was phenotypically extremely glaucous, gave the maximum value of 145. This strict correlation suggests that the β-diketones, together with the hydroxy-β-diketones, are responsible for glaucousness in wheat. Since Troughton and Hall[1] found that glaucousness was correlated with the proportion of wax rodlets to platelets, on the leaf surface, these rodlets may consist predominantly of β-diketones and hydroxy-β-diketones. Hall *et al.*[10] have shown that a similar correlation occurs between the form of the wax crystallites and the amount of β-diketones in clinal forms of *Eucalyptus urnigera* and the grass *Poa colensoi*. In all three genera the forms richest in β-diketone are the most glaucous.

As regards the biosynthesis of the β-diketones, detailed chemical comparisons are available only for lines 7 (glaucous) and 1 (non-glaucous). Table 2 gives the analysis of the waxes into their major components. Thus, concurrently with the loss of β-diketone in line 1, there is an increase in the proportion of primary alcohols and to a lesser extent of esters. The esters have not been investigated further. However gas–liquid chromatography of the primary alcohols shows the interesting situation in that the non-glaucous line, which is deficient

[8] D. H. S. Horn, Z. H. Kranz and J. A. Lamberton, *Australian J. Chem.* **17**, 464 (1964).
[9] A. P. Tulloch and R. O. Weenink, *Chem. Commun.* No. 8, 225 (1966).
[10] D. M. Hall, A. I. Matus, J. A. Lamberton and H. N. Barber, *Australian J. Biol. Sci.* **18**, 323 (1965).

for a chromosome arm, possesses an additional homologous series of primary alcohols absent from the glaucous line. This additional series agrees in retention times with the normal alcohols. The other series, which has not been characterized completely, is very similar chromatographically in both glaucous and non-glaucous lines. It has weak carbonyl peaks in the i.r. spectrum and may be a keto-alcohol.

TABLE 2. APPROX. % COMPOSITION OF WAX FROM LINE 7 AND LINE 1

Component	Line 7	Line 1
Hydrocarbon	6	6
Unknown 1	2	4
Esters	10	17
Aldehydes	3	5
Primary alcohols	15	34
β-Diketones and hydroxy-β-diketones	18	Trace
Polar components (by subtraction)	46	34

The formation of the series of primary alcohols in plants deficient for the chromosome arm carrying the gene(s) responsible for β-diketone formation, suggests immediately that the primary alcohols are either a precursor, or a derivative of the precursors, of the β-diketones. There is evidence[2] that the primary alcohols of leaf-waxes are formed by reduction of normal acids. In β-diketone-rich waxes it seems more likely that the acids rather than the alcohols would be the precursors of the β-diketones. However, before speculating in detail on the biochemical possibilities, it will be advisable to characterize both the free acids and the esters in the waxes from normal and chromosomally deficient plants.

It is noteworthy that the composition of the hydrocarbons is unchanged in the glaucous and deficient lines. They are normal with carbon numbers from 18 to 33 in both types. Thus, it appears that the synthesis of hydrocarbons is unconnected with the synthesis of β-diketones. In eucalypts, a somewhat different situation exists. Here suppression of β-diketone formation leads to an increase in percentage hydrocarbon with a different carbon number distribution in green and glaucous forms.[10] Again it is difficult to assume that the extra hydrocarbon is a direct precursor of the β-diketone. On the other hand it may be a product derived from the precursors of the β-diketones.

In wheat, gene(s) other than those situated on chromosome 2B are involved in the production of β-diketone. For example, there is evidence that gene(s) on chromosome 6B are involved.[4] It will be interesting to see how the chemistry of wax from plants deficient for chromosome 6B differ from those deficient for chromosome 2B.

EXPERIMENTAL

All plant material, except line 7 which was grown at Narrabri, N.S.W., was grown in the Botany Department glasshouses or gardens. It was harvested about 2 weeks after ear emergence on the primary tillers. Extraction was effected by dipping the shoots into boiling light petrol (b.p. 60–80°) for approximately 20 sec. Evaporation of the solvent on the water bath gave the desired wax sample.

The absorbant for TLC was a 0·3 mm layer of silica gel G activated at 110° for 30 min. Approximately 4 μl of a 1 per cent solution of the wax in $CHCl_3$ was applied to the origin and the chromatogram developed in benzene. Spots were detected by spraying with 18 N H_2SO_4 followed by charring at 170°.

Column separations of lines 1 and 7 were made by gradient elution on florisil. The following solvents were used in successive pairs: light petrol. (b.p. 60–80°), CCl_4, benzene, $CHCl_3$, ethyl acetate, acetone, ethanol,

methanol. The fractions collected were evaporated to dryness in a stream of O_2-free N_2 and taken up in $CHCl_3$ and a sample applied to a thin-layer plate. Fractions giving the same distribution of spots in TLC were pooled for subsequent work.

I.r. spectra were obtained using a KBr crystal with a Perkin Elmer Model 337. Identification of the peaks was made by comparison with the correlations listed in Bellamy.[11]

The hydrocarbons and primary alcohols from lines 1 and 7 were analysed by GLC. A 5 ft × ⅛ in. stainless-steel column packed with 15 per cent S.E. 30 on Chromosorb W, 100–200 mesh, was used. The column temperature was 260°. The flow rate of the carrier gas, nitrogen, was 25 ml/min. The conditions used for the primary alcohols were similar except that the column temperature was 273°.

Acknowledgement—We wish to thank the Australian Research Grants Committee for financial support, and Dr. C. J. Driscoll and Professor I. A. Watson for the plant material.

[11] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules*, Wiley, New York (1958)