Phytochemistry, Vol. 21, No. 1, pp. 129–131, 1982.
Printed in Great Britain.

0031-9422/82/010129-03 $03.00/0

EPICUTICULAR WAXES OF ALBINO MAIZE

GIORGIO BIANCHI, PINAROSA AVATO and FRANCESCO SALAMINI*

Istituto di Chimica Organica, Viale Taramelli 10, 27100 Pavia, Italy; * Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, via Stezzano 24, 24100 Bergamo, Italy

(*Received* 6 *February* 1981)

Key Word Index—*Zea mays*; Gramineae; maize; albino seedlings; epicuticular wax composition; biosynthesis.

Abstract—Epicuticular wax of albino maize seedlings contains alkanes (9.5 %), esters (23.3 %), aldehydes (14.1 %) and free alcohols (53.1 %). The wax composition resembles that of a normal plant as regards free alcohols and aldehydes. The alkanes, however, contain a high percentage of even-chain homologues and the esters are made up of alcohols and acids different from those of the normal plant. The biosynthetic aspects of wax formation in a plant devoid of chloroplasts are discussed.

INTRODUCTION

The biosynthesis of the commonest fatty acids found in internal lipids, namely C_{16} and C_{18}, has been the subject of several studies in recent years (see [1]). These studies have shown that fatty acid synthesis in plants is mainly accomplished by two biosynthetic pathways. The first requires the presence of a *de novo* fatty acid synthetase system made up of a collection of enzymes and acyl carrier protein (ACP) which convert acetyl CoA and malonyl CoA to palmityl ACP. The second depends upon an enzymatic system completely distinct from the first in which, in brief, palmityl ACP elongation to stearyl ACP requires malonyl ACP and NADPH. Both biosynthetic pathways have been found to operate in chloroplasts and consequently they must be strictly dependent on the photosynthetic activity of the plant [1]. The long-chain (C_{20}–C_{32}) compounds found in the epicuticular waxes, according to the most widely accepted theory, are produced by an elongation process from C_{16} and C_{18} chains by the addition of C_2 units from malonyl CoA [2, 3]. This process takes place in the epidermal tissue, a tissue which usually contains no chloroplasts other than in the guard cells. This tissue, moreover, is also capable of a *de novo* synthesis of C_{16} and C_{18} molecules, as found by Cassagne and Lessire for *Allium porrum* [4]. The question arises as to whether or not the photosynthetic production of wax precursors such as C_{16} and C_{18} fatty acyl chains by the mesophyll cells of the leaves can in some way influence the amount and the composition of the wax extruded by the epidermis. The first clear distinction between the functions of mesophyll and epidermal cells in the synthesis of lipid acyl chains has been provided recently by von Wettstein–Knowles and coworkers who studied the waxes from green and yellow basal segments of maize leaves [5].

As a continuation of our studies on the chemical genetics of the waxes of normal and mutant maize lines ([6], in this reference are the definitive data of the normal plant wax), we considered it worth examining the relationship between photosynthetic and epidermal synthesis of C_{16} and C_{18} precursors by comparison of normal maize seedlings with albino mutant seedlings which contain no trace of chlorophyll. This paper reports on epicuticular wax of albino maize, its composition and biochemical implications.

RESULTS AND DISCUSSION

Table 1 shows the composition of normal and albino waxes. The albino plants appear to be endowed with more wax than the normal plants. This is considered to be due to the fact that the albino leaves are much less expanded and thinner than those of the normal plants. So, if wax production is of comparable level in the two types of plants, the smaller plant will have more waxy material both in terms of unit surface area and unit weight. Inspection of the relative percentages of the classes of compounds found in the waxes, indicate that alkanes are present in much higher amounts in albino than in normal wax. The other wax constituents (esters, aldehydes and alcohols) do not differ significantly in the two genotypes. The homologue compositions of the compounds are given in Table 2. The relevant features are: (1) the aldehydes and primary alcohols are practically the same in both lines; (2) the albino *n*-alkane mixture is characterized by a biosynthetic block at the $C_{30} \rightarrow C_{32}$ step and contains an unusually high percentage (37 %) of even-chain alkanes; (3) the range of esterified alcohols and acids are quite different in the two lines.

Table 1. Composition of normal and albino waxes

	% Wax		mg/kg fr. wt	
Components	Albino	Normal*	Albino	Normal*
Alkanes	9.5	1.4	136.2	8.1
Esters	23.3	15.5	334.1	89.9
Aldehydes	14.1	20.4	202.2	118.3
Alcohols	53.1	62.7	761.3	363.7
Total			1433.8	580.0

* Data from [6].

Table 2. Relative composition (%) of fractions from normal and albino maize

No. of C. atoms	Alkanes		Aldehydes		Free primary alcohols		Esterified primary alcohols		Esterified fatty acids	
	Albino	Normal*	Albino	Normal*	Albino	Normal*	Albino	Normal*	Albino	Normal*
16							0.3		21.8	
17										
18							1.5		20.9	
19	0.3									
20	0.9						5.5		18.3	0.8
21	2.5									
22	5.6						5.9		23.0	14.8
23	8.8	1.8								
24	10.8	tr†			0.2		15.1		9.2	48.6
25	12.1	5.3								
26	9.5	tr	tr		1.3		9.0		3.9	27.6
27	12.8	12.5								
28	6.2	tr	3.0	0.8	2.1		26.4		2.9	5.9
29	17.5	28.9								
30	2.9	tr	3.6	2.7	3.7	0.8	11.3	tr		2.3
31	8.6	49.1								
32	0.9	tr	93.4	96.5	92.7	99.2	25.0	100.0		tr
33	0.6	2.4								
34			tr							

* Data from [6].
† tr, trace.

Now, let us consider the data in the light of our previous pertinent results from normal maize and mutants. For the sake of clarity, we present here an outline of the two elongation processes advanced for the synthesis of the long-chain molecules of maize wax [6, 10]: (1) the elongation–decarboxylation I (ED-I) governing formation of the longest (C_{28}–C_{32}) alkanes, aldehydes and free alcohols; (2) the elongation–decarboxylation II (ED-II), responsible mainly for the synthesis of shorter chains of esters. Albino ED-I is apparently indistinguishable from that of the normal plant as regards aldehydes and free alcohols, but the alkanes increase in percentage (1.4–9.5) and also the homologue distribution is affected with, as already cited, the appearance of remarkable amount of even-chain forms. The situation for ED-II is quite different. Thus while normal plant esters contain only *n*-dotriacontanol (C_{32}), a product of ED-I activity, in albino esters there are nine homologous alcohols with C_{32} forming only 25% of the total. This situation resembles that found in glossy mutants known to be blocked in wax biosynthetic pathway at specific steps [6]. However, the effects of the albino mutation cannot be straightforwardly related to any specific elongation pathway active in wax synthesis. The modification exerted by the mutation on the esterified primary alcohols seems more attributable to secondary effects than to a direct involvement of the mutation in the process of elongation of acyl chains. As far as the acid moieties of the esters are concerned, the principal chains of the albino esters are in the range C_{16}–C_{22} whereas in the normal plant the major chains are C_{22}, C_{24} and C_{26}.

Wettstein-Knowles *et al.* [5], in their study on the composition of wax from yellow and green leaf segments of normal maize, obtained results of direct relevance for the present study. They found that the homologue distribution of aldehydes and free alcohols from the two types of tissue are very similar, while a prominence of shorter homologues is observed in the alkanes and ester components from yellow tissue. The resemblance between albino and yellow tissue wax is striking but not unexpected considering that both are characterized by a low level or lack of chloroplast activity in the mesophyll cells.

Furthermore, the presence in albino maize of significant quantities of two acids, palmitic (21.8%) and stearic (20.9%), which are absent in normal wax are of relevance to the ED-II complex activity. The results reported elsewhere [6, 10, 11] on glossy mutants sustain the conclusion that any mutation that reduces the production of wax by interfering with the main elongation system (ED-I), causes an accumulation of precursors that, in turn, can influence the synthesis of the shorter alcoholic moieties of esters resulting from the ED-II activity. Thus, activation of ED-II by the presence in the substrate pool of abundant short chains (C_{16} and C_{18}) which are underused by a ED-I operating at a low level, may provide a simple explanation of the previous findings concerning the abnormal composition of esters of glossies 2, 3 and 4. However, in the case of albino maize, where ED-I is unaffected by the mutation, it is challenging to explain the high percentage of short-chain alcohol moieties in the esters as due to the presence of significant amounts of palmitic and stearic acids. For albino maize, we find it more reasonable to advance the hypothesis that the higher than normal levels of C_{16} and C_{18} chains in the wax classes is the result of an altered physiology of the cells

underneath the mesophyll. Also the shorter than normal alkanes of albino maize confirm the activated state of ED-II in this genotype.

In conclusion, the present study indicates that the epidermis has a great capacity for synthesizing C_{16} and C_{18} fatty acyl chains, the necessary building blocks for wax production, and supports also the hypothesis that palmityl and stearyl chains are synthesized in the same or directly connected enzymatic pools in which the wax classes are produced. Furthermore, this study confirms that photosynthetic activity is not a direct determinant on wax formation.

EXPERIMENTAL

A strain of the inbred WF9 was available that segregates normal and albino seedlings in the ratio 3:1. The albino mutation carried in the heterozygous state was of unknown cytogenetic location. In the segregating population, the albino seedlings were always devoid of chlorophyll. The strain was grown in a greenhouse in Jan. 1980.

Albino seedlings were collected at the third–fourth leaf stage of growth. At this stage the albino plants were smaller (about 1/2) than the normal seedlings. Waxes were extrd by dipping the seedlings in cold $CHCl_3$ for 45–60 sec. Evaporation of the solvent in a rotary evaporator under red. pres. yielded the wax sample. The wax was fractionated by CC by gradient elution on Si gel 60 H (Merck). CCl_4 eluted *n*-alkanes, esters and aldehydes in that order; $CHCl_3$ afforded the alcohols. The fractions obtained were checked for the identification of the various wax classes by TLC as previously described [7]. TLC detection of aldehydes was by the Purpald test [8]. IR and MS data of individual wax classes have already been reported [9]. GLC was carried out on a dual FID instrument using glass columns (2 m × 4 mm) packed with 1% OV 1 on 100–120 mesh GCPS. Isothermal and temp. programmed analyses were run from 160 to 280° at 5°/min; N_2 and H_2 were adjusted to yield optimum sensitivity.

Peak areas were determined by the triangulation method. The free fatty alcohols (10–25 mg) were acetylated for GLC with Ac_2O (5 ml) and pyridine (a few drops) overnight at room temp. Esters were converted into methyl esters and alcohols by refluxing in 20% BF_3. MeOH for 48 hr.

Combined acids and alcohols of esters were treated with Ac_2O and pyridine to obtain alcohol acetates. The mixture was then analysed by GLC. Alkanes were subjected also to capillary GLC using a glass column (25 m × 0.3 mm) packed with SE52. Temp. was programmed from 150 to 300° at 5°/min.

Authentic samples of each class of compounds were used as standards for TLC and GLC.

Acknowledgement—We wish to thank CNR (Rome) for financial aid.

REFERENCES

1. Stumpf, P. K. (1975) in *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Galliard, T. and Mercer, E. I., eds.) pp. 95–113. Academic Press, New York.
2. Kolattukudy, P. E., Croteau, R. and Buckner, J. S. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) pp. 289–317. Elsevier, Amsterdam.
3. Wettstein-Knowles, P. von (1979) in *Advances in the Biochemistry and Physiology of Plant Lipids* (Appelqvist, L. A. and Liljenberg, C., eds.) pp. 1–26. Elsevier–North-Holland Biomedical Press, Amsterdam.
4. Cassagne, C. and Lessire, R. (1974) *Physiol. Vég.* **12**, 149.
5. Wettstein-Knowles, P. von, Avato, P. and Mikkelsen, J. D. (1980) in *Biogenesis and Function of Plant Lipids* (Mazliak, P., ed.) pp. 271–274. Elsevier–North-Holland, Amsterdam.
6. Bianchi, G., Avato, P. and Salamini, F. (1979) *Heredity* **42**, 391.
7. Bianchi, G., Avato, P. and Salamini, F. (1975) *Maydica* **20**, 165.
8. Dupont Durst, H. and Gokel, G. W. (1978) *J. Chem. Edu.* **55**, 206.
9. Bianchi, G. and Salamini, F. (1975) *Maydica* **20**, 1.
10. Bianchi, G., Avato, P. and Salamini, F. (1977) *Maydica* **22**, 9.
11. Bianchi, G., Avato, P. and Salamini, F. (1978) *Biochem. Genet.* **16**, 1015.