Surface Waxes from Grain, Leaves, and Husks of Maize (*Zea mays* L.)

G. BIANCHI and P. AVATO, Universita di Pavia, Dipartimento di Chimica Organica, 27100 Pavia, Italy; and F. SALAMINI, Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, 24100 Bergamo, Italy

Cereal Chem. 61(1):45-47

ABSTRACT

Kernels, husks, and leaves of adult maize plants are covered by waxes comprising long-chain alkanes, esters, aldehydes, alcohols, acids, and sterols. Sterols were absent in the previously studied epicuticular wax of maize seedlings. Kernel and husk waxes contain high percentages of esters, but the former plant organs lack aldehydes, and the latter have no alcohols.

Although a great deal of data on epicuticular wax of maize seedlings is available (Bianchi et al 1975, 1977, 1978, 1979, 1982), the literature contains no reports on the presence of waxy material on the corn grain surface. Although the composition of surface substances on the kernel is important because kernels are used directly for feeding animals, the surface lipids of corn leaves and husks of the adult plant are also important because they are a component of maize silage.

The present work was undertaken to compare the chemical composition of the corn grain wax with the plant. Furthermore, it seemed worthwhile to gather data to make a comparison among the surface waxes of two important seed grains, corn grain and sorghum grain, which are used without any industrial treatment (Bianchi et al 1979). The study should also contribute to an understanding of the biochemical pathways governing the synthesis of surface lipids in different tissues of the maize plant.

MATERIALS AND METHODS

Husks and mature leaves of the maize inbred WF9 (*Zea mays* L.), a line commonly cultivated to produce hybrids, were from plants grown at the farm of the Istituto Sperimentale per la Cerealicoltura, Bergamo, in 1980, and were harvested from mature plants 15 days after silk extrusion of the main ear. Mature ears were from the same inbred; 20 untouched ears were extracted by dipping in chloroform for 30 sec at room temperature. The husks were similarly treated to obtain the surface wax. The yields of wax recovered from the ears after evaporation of the solvent were 2.7 mg from the grains and 9.6 mg from the husks.

The leaves to be subjected to wax extraction were taken from five plants. The oldest three leaves of the plants were discarded, and the chloroform extraction was performed on the remaining nine by washing the leaves in cold chloroform for 30 sec. The yield was 21.0 mg of wax per leaf.

Composition of the waxes was determined by thin-layer chromatography (TLC) as previously described (Bianchi et al 1982). Column separations were done by gradient elution on silica gel 60/H (Merck). Carbon tetrachloride eluted *n*-alkanes, esters, and aldehydes in that order. Chloroform afforded alcohols and sterols, and chloroform containing 1% acetic acid gave free acids. Alcohols and sterols were often unresolved from column chromatography. In such cases, a further separation was performed on TLC plates, type $60F_{254}$ (Merck), using $CHCl_3$ containing a variable amount of EtOH as eluent. The fractions of sterols were made visible on TLC plates by the Libermann-Burchard test.

Gas chromatography (GC) analyses were performed by using an OV1 capillary column with 15-m, 0.1–0.15-mm film thickness, on an HRGC Carlo ERBA gas chromatograph with flame-ionization detector. Isothermal and programmed chromatograms were run using appropriate column temperature. Hydrogen flow was adjusted at 0.3 kg/cm^2. Alkanes and aldehydes were analyzed as such by gas chromatography. Alcohols were transformed into the corresponding acetates, and free acids into methyl esters. Composition of the esters was determined by analyzing combined acids and alcohol acetates from esters-transesterification followed by acetylation (Bianchi et al 1982). Esters as such were also subjected to GC analysis. Conditions for esters analysis were: start temperature 150°C, 40°C/min to 270°C, then hold at 270°C for 1 min, 7°/min to 355°C, then hold at 355°C for 15 min.

RESULTS

Definite chemical patterns of wax composition exist according to the part of the plant and the growth stage considered.

Two classes of compounds characterized by the hydroxylic function (alcohols and sterols) differ among the waxes from leaves, husks, and seedlings (Table I).

Alcohols are, in fact, absent in husks, whereas sterols have not been detected in the seedlings wax. Furthermore, most relevant features are found in the percentage composition: alcohols are dominant in seedlings, whereas esters are the prevailing class of compounds in leaves and husks (42 and 64%, respectively). The latter also contain a high amount of sterols (21%).

Although free acids are found only in trace amounts on seedlings, 8 and 14% are present on husks and leaves, respectively. Surprisingly, husk wax contains aldehydes (3%) but no alcohols. A similar case was also found in a maize mutant wax, *gl5*, which was characterized by a large amount of aldehydes (83.5%) with only a small quantity of alcohols (8.7%) (Bianchi et al 1978).

Kernel wax was characterized by the highest percentage of esters (76%) and, like husks, by very small amounts of the two biosynthetically related classes of compounds, aldehydes and alcohols (Kolattukudy 1976). The former were absent, and the latter amount to only 2%.

Tables II-V show the homologue compositions of the various classes of compounds. Alkanes (Table II) present two major features. In husks and kernels, *n*-alkanes are accompanied by a second series of homologues to which we attribute the structures either of isoalkanes or alkenes. Furthermore, although mature leaves have four dominant homologues, ie, C_{27}, C_{29}, C_{31}, and C_{33}, the spectrum is limited to the three chains C_{27}, C_{29}, and C_{31} in the other plant organs and in the seedlings.

Inspection of free alcohols and aldehydes of leaves and seedlings (Table II) shows a good resemblance between the two classes of

TABLE I
Chemical Composition of Surface Waxes of Different Organs of Maize Inbred WF9

Plant Part	Classes of Compounds (%)[a]					
	Alkanes	Aldehydes	Alcohols	Acids	Esters	Sterols
Leaf	17	9	14	14	42	4
Husk	4	3	ND[b]	8	64	21
Kernel	6	ND[b]	2	11	76	5
Whole seedling[c]	1	20	63	t	16	ND[b]

[a] Percentages are rounded off. t = Trace (less than 0.5%).
[b] Not detected.
[c] From Bianchi et al 1982.

compounds. In seedlings, however, there is higher chain-length specificity than in leaves, C_{32} being the dominant homologue. Moreover, whereas husk aldehydes are made up of only four even chains C_{26}–C_{32} present in similar percentages, kernel alcohols show a broad spectrum of homologues C_{18}–C_{32}, with the presence of some odd chains.

No particularly significant patterns are noticeable in free acid fractions, except the relatively narrow range presented by those of kernels.

The composition of esters is presented in Table III. Leaves and husks are characterized by a sole, dominant group of homologues in the range C_{44}–C_{48}, whereas two major groups of chains appear in kernels and seedlings. In esters from kernels, in addition to the two dominant homologues C_{46} (21%) and C_{48} (22%), there is a second minor group represented by C_{52} (10%) and C_{54} (17%). Two groups of homologues also occur in seedling esters, the first comprising the even C_{42}–C_{48} chains, and the second having 54–56 carbon atoms.

The composition of the acid and alcohol moieties of esters is shown in Table IV. The C_{32} alcohol represents 100% of this moiety in the case of seedlings, whereas larger spectra of homologues are found for the esterified alcohols of the various parts of the adult maize plant. The dominant homologues found were C_{22}, C_{24}, and C_{26}. The distribution of ester acids from the various sources indicates a loose chain-length specificity, with the most important chain length being C_{20}, C_{22}, C_{24}, and C_{26} in various order of prominence.

The results in Table V show the sterol composition of the three organs studied. Leaf and husk sterol fractions are made up of the three common phytosterols campesterol, stigmasterol, and β-sitosterol. An unidentified sterol (29%) is present in leaves. Kernel surface sterols appear to be a more complex mixture. We have identified campesterol, stigmasterol, and β-sitosterol, which represent 18% of the total. The remaining 82% is a mixture of at least nine unidentified sterols, as shown by GLC analysis.

DISCUSSION

Wax was present on all three organs of the adult plant, which suggests that in corn, wax is produced in plants from seedling stage to maturity. Because the surfaces of kernel, leaf, and husk are intrinsically different, evaluation of amount of wax per unit area was not workable. Therefore, this discussion is of the variations on chemical composition of the waxes from the three organs, taking into account our previous results related to maize wax on the seedlings of the normal plant and mutants (Bianchi et al 1975, 1977, 1979, 1982).

The data of Table I show that waxes of adult plant leaves, kernels and husks are altogether different from those of seedlings (Bianchi and Salamini 1975, Bianchi et al 1982). In fact, whereas the bulk of epicuticular wax of seedlings is made up of aldehydes and to a greater extent, alcohols, the esters in waxes of the adult plant organs are by far the dominant class of compounds. The compositional pattern of the latter waxes is reminiscent of those mutant maize seedlings homozygous for mutant alleles such as *gl1, gl2, gl3, gl4, gl8,* and *gl18*, in which esters represented one of the major classes of compounds (Bianchi et al 1975, 1977, 1979).

On the basis of experimental evidence gained from those studies, we advanced the hypothesis that long-chain molecules of maize plant wax are synthesized in two distinct complexes of enzymes called elongation-decarboxylation (ED)-I and ED-II, respectively. The ED-I controls the production of alkanes, aldehydes, and alcohols. ED-II is mainly responsible for the synthesis of esters. Furthermore, ED-I and ED-II determine the homologue composition within each class of compounds. The broad spectra of homologues, especially noticeable in the alcohols and aldehydes of the organs of the adult plant compared with the high chain-length specificity of the same compounds of seedlings (Table II), represents further similarity between the wax biochemistry in adult corn plant and in several maize mutants, whose wax is characterized by a loose chain-length specificity.

An early transmission electron microscopy study on epicuticular wax of maize (Bianchi and Marchesi 1960) showed that normal maize seedlings produce observable amounts of wax up to the fifth leaf-growth stage. Then, the appearance of the leaf surface of the older normal plant is indistinguishable from that of mutant seedlings. This finding further reinforces the hypothesis of similar wax biochemistry in maize adult plant organs and mutant seedlings.

TABLE II
Composition (%)[a] of Fractions from Waxes from Leaves (L), Husks (H), Kernels (K), and Seedlings (S) of Maize Inbred WF9

Carbon Chain Length	Alkanes						Aldehydes				Alcohols				Acids			
		H		K														
	L	A[b]	B[c]	A[b]	B[c]	S	L	H	K	S	L	H	K	S	L[d]	H	K	S
16	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...	13	25
17	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
18	...	...	...	...	...	...	...	...	...	...	...	...	3	...	2	22[e]	21[g]	13
19	...	...	...	...	...	...	...	...	...	...	...	...	5	...	t	t	...	...
20	...	...	...	...	...	...	...	...	...	...	...	...	7	...	3	8	1	4
21	...	...	...	...	...	...	...	...	...	...	...	...	1	...	t	t	...	...
22	...	t	...	2	...	...	...	...	...	...	1	...	8	...	5	29[f]	20	6
23	1	1	...	4	...	2	2	...	...	...	t	...	4	...	1	1	2	...
24	1	t	...	4	...	t	1	...	...	...	3	...	18	...	8	10	38	14
25	3	2	...	7	...	5	2	...	...	...	1	...	2	...	t	...	1	...
26	1	t	t	4	...	t	9	25	...	...	14	...	1	...	10	3	2	22
27	9	18	1	20	...	13	3	...	...	...	3	...	2	...	2	1	2	...
28	2	t	4	5	...	t	24	23	...	1	18	...	6	1	19	12	...	12
29	30	54	27	31	25	29	4	...	...	...	t	...	...	...	2	...	...	...
30	2	1	t	3	t	t	32	23	...	3	25	...	8	6	24	5	...	t
31	30	22	58	15	51	49	3	...	...	...	3	...	...	...	2	...	...	...
32	t	t	t	1	...	t	18	29	...	96	28	...	29	93	16	6	...	4
33	18	2	10	3	18	2	t	...	...	...	1	...	...	...	t	...	...	...
34	...	...	...	t	...	...	2	...	...	...	3	...	6	...	4	3	...	...
35	3	...	...	1	6	...	...	...	...	...	...	...	...	...	t	...	...	...

[a] Percentages are rounded off. t = Trace (less than 0.5%).
[b] *n*-Alkanes.
[c] Isoalkanes or alkenes, 18% and 10% of the total hydrocarbon fractions of husks and kernels, respectively.
[d] Two peaks, amounting to 2% of the total, are present in the gas chromatogram with retention time smaller than those of C_{18} and C_{22}.
[e] Mixture of saturated (11%) and unsaturated (11%).
[f] Mixture of saturated (17%) and unsaturated (12%).
[g] Mixture of saturated (9%) and unsaturated (12%).

TABLE III
Composition of Esters from Leaves (L), Husks (H), Kernels (K), and Seedlings (S) of Maize Inbred WF9

Carbon Chain Length	Composition (%)[a]			
	L	H	K	S
38	...	t	...	2
40	t	1	...	8
41	t	t	...	t
42	7	7	1	11
43	1	1	t	t
44	30	21	4	14
45	2	2	1	t
46	23	29	21	10
47	1	2	1	t
48	11	13	22	14
49	1	1	1	t
50	6	4	9	4
51	t	t	t	t
52	4	2	10	4
53	1	1	1	t
54	4	1	17	15
55	3	1	1	t
56	2	7	8	15
57	2	t	t	t
58	1	5	3	3
59	1	t	t	t
60	...	2	t	t

[a] Percentages are rounded off. t = Trace (less than 0.5%).

TABLE IV
Composition (%)[a] of Alcohol and Acid Moieties from Esters of Leaves (L), Husks (H), Kernels (K), and Seedlings (S) of Maize Inbred WF9

Carbon Chain Length	Esterified Alcohols				Esterified Acids			
	L	H	K	S	L	H	K	S
16	...	...	2	...	...	...	1	...
17	...	...	t	...	...	...	1	...
18	...	1	1	...	...	9	1	...
19	...	...	2	...	...	...	t	...
20	1	5	1	...	42	22	4	1
21	...	...	2	...	...	...	1	...
22	9	14	16	...	30	54	48	15
23	...	1	1	...	1	2	2	...
24	55	51	30	...	14	13	37	49
25	...	2	2	...	...	...	1	...
26	27	18	10	...	8	...	2	27
27	...	t	1	...	...	...	t	...
28	5	4	6	...	2	...	1	6
29	...	...	t	...	...	...	t	...
30	...	...	8	t	2	...	1	2
31	...	...	t	...	...	...	t	...
32	3	4	15	100	1	...	t	t
33	...	...	...	...	...	...	...	...
34	...	...	2	...	...	...	...	...
35	...	...	...	...	...	...	...	...
36	...	...	1	...	...	...	...	...

[a] Percentages are rounded off. t = Trace (less than 0.5%).

The foregoing results suggest that, although both ED systems are active in the maize adult plant, ED-II is clearly more efficient than ED-I.

The presence of free and esterified sterols in the shoots, roots, and seeds of maize was previously reported (Kemp and Mercier 1968, Kemp et al 1968, Rohmer 1972, Davis and Poneliet 1975, Sheid and Benveniste 1979, Douglas and Paleg 1981, Itoh et al 1981). In the cited papers, however, the sterols were obtained by extraction of the whole part of plant material, appropriately homogenated so that a large fraction of them came from the internal parts of the plant organs. Sterols reported in this article

TABLE V
Composition of Sterols from Leaves (L), Husks (H), and Kernels (K) of Maize Inbred WF9

Sterols	Composition (%)[a]		
	L	H	K
Campesterol	14	34	2
Stigmasterol	16	24	4
β-sitosterol	41	42	12
Unidentified sterols	29	...	82

[a] Percentages are rounded off.

were of external origin. This is supported by the composition of the kernel wax collected by our method of extraction. In fact, this wax is not contaminated by triglycerides, which are the most abundant internal lipids of the maize grain.

Finally, comparison of the chemical composition of maize grain wax with that of sorghum grain (alkanes, 1.3%; aldehydes, 31.9%; alcohols, 33.7%; esters, 4.0%; acids, 24.4%; unidentified, 4.7%) shows that the two waxy materials are quite different. The reason for this dramatic difference is not obvious, but anatomical structure and position of the two seed-bearing organs may be responsible.

ACKNOWLEDGMENT

This work was supported by Consiglio Nazionale delle Ricerche (Rome).

LITERATURE CITED

BIANCHI, A., and MARCHESI, G. 1960. The surfaces of the leaf in normal and glossy maize seedlings. Z. Vererbungsl. 91:214.

BIANCHI, G., and SALAMINI, F. 1975. Glossy mutants of maize. IV. Chemical composition of normal epicuticular waxes. Maydica 20:1.

BIANCHI, G., AVATO, P., and MARIANI, G. 1979. Composition of surface wax from sorghum grain. Cereal Chem. 56:491.

BIANCHI, G., AVATO, P., and SALAMINI, F. 1975. Glossy mutants of maize. VI. Chemical constituents of glossy-2 epicuticular waxes. Maydica 20:165.

BIANCHI, G., AVATO, P., and SALAMINI, F. 1977. Glossy mutants of maize. VII. Chemistry of glossy-1, glossy-3, and glossy-7 epicuticular waxes. Maydica 22:9.

BIANCHI, G., AVATO, P., and SALAMINI, F. 1978. Glossy mutants of maize. VIII. Accumulation of fatty aldehydes in surface waxes of gl5 maize seedlings. Biochem. Genet. 16:1015.

BIANCHI, G., AVATO, P., and SALAMINI, F. 1979. Glossy mutants of maize. IX. Chemistry of glossy-4, glossy-8, glossy-15, and glossy-18 surface waxes. Heredity 42:391.

BIANCHI, G., AVATO, P., and SALAMINI, F. 1982. Epicuticular waxes of albino maize. Phytochemistry 21:129.

DAVIS, D. L., and PONELEIT, C. G. 1975. Sterols in developing seed from low and high oil *Zea mays* strains. Phytochemistry 14:1201.

DOUGLAS, M. J., and PALEG, L. G. 1981. Lipid composition of *Zea mays* seedlings and water stress-induced changes. J. Exp. Bot. 32:499.

ITOH, T., SHIMIZU, N., TAMURA, T., and MATSUMOTO, T. 1981. 24-Methyl-E-23-dehydrolophenol, a new sterol and two other 24-methyl-E-Δ23-sterols in *Zea mays* germ oil. Phytochemistry 20:1353.

KEMP, R. J., and MERCIER, E. I. 1968. Studies on the sterols and sterols esters of the intracellular organelles of maize shoots. Biochem. J. 110:119.

KEMP, R. J., HAMMAN, A. S. A., GOAD, L. J., and GOODWIN, T. W. 1968. Studies on phytosterols biosynthesis: observations on the esterified sterols of higher plants. Phytochemistry 7:447.

KOLATTUKUDY, P. E., CROTEAU, R., and BUCKNER, J. S. 1976. Page 289 in: Chemistry and Biochemistry of Natural Waxes. P. E. Kolattukudy, ed. Elsevier, Amsterdam.

ROHMER, M., OURISSON, G., and BRANDT, R. D. 1972. Hydrosoluble complexes of sterols, sterols esters and their precursors from *Zea mays* L. Eur. J. Biochem. 31:172.

SCHEID, F., and BENVENISTE, P. 1979. Ergosta-5, 23-dien-3β-ol and ergosta-7,23-dien-3β-ol, two new sterols from *Zea mays* etiolated coleoptiles. Phytochemistry 18:1207.

[Received April 29, 1983. Accepted August 17, 1983]