COMPOSITION OF LIPIDS OF THE GRAIN OF OPAQUE-2 MAIZE

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It has been established that the change in the composition of the total lipids and the fall in the fatty acid content depend on the initial maize genotype. A decrease in the amount of unsaponifiable substances in the grain of mutants is accompanied by qualitative changes in the composition regardless of the nature of the genotype.

One of the directions for improving the quality of maize grain is connected with the creation of lysine-rich forms using the mutant gene opaque-2. The biochemical characteristics of opaque maize are connected mainly with a change in the processes involved in the biosynthesis of proteins and amino acids, since features of the lipid composition of the grain of the mutant forms have not been fully elucidated. In spite of the fact that the composition of the lipids of maize grain has been studied in fairly great detail [1], information on the accumulation of lipids in the grain of maize transformed by opaque-2 is contradictory [2-4]. The differences in the fatty acid compositions of the grain of the ordinary and mutant forms amount to slight changes in the levels of linoleic and oleic acids [2, 3].

It must be mentioned that the papers cited considered the composition of the lipids isolated by nonpolar solvents, which, as is well known do not lead to exhaustive extraction. Furthermore, there papers contain no information on the identification of the group composition of the lipids of the opaque maize grain with the use of spectral methods, the informativeness of which has been confirmed in a study of individual groups of plant lipids [5-7].

We have investigated the qualitative changes in the composition of maize grain lipids under the influence of the mutant gene by the methods of TLC and GLC and of IR and UV spectroscopy.

As TLC showed, the total lipids of the grain of the initial and mutant lines did not differ qualitatively in group composition, and they contained phospholipids, tri- and diglycerides, free fatty acids, sterols, and sterol esters.

In the IR spectra of the total lipids of the initial and mutant forms of maize it was possible to observe a number of characteristic absorption bands. In the region of C-H stretching vibrations several types of absorption were observed: vibration at a C=C double bond (3010 cm^{-1}), and a group of intense absorption bands of methyl and methylene groups (2920, 2850, 2870 cm^{-1}). The vibrations of associated OH groups were represented by a broad shoulder on the main band at about 3300 cm^{-1} and by a weak band at 2660-2675 cm^{-1} [5, 8].

The region of carbonyl absorption permits the identification of the ester groups of glycerides (1744 cm^{-1}) and the carboxy groups of fatty acids (1710 cm^{-1}). A band at 1054 cm^{-1} must be assigned to the vibrations of the P-O-C groupings of phospholipids [5,8]. The relative intensity of a group of bands in the 1120-1350 cm^{-1} region changes with the genotype of the maize. The absorption observed is connected with vibrations of the P=O bonds of the phospholipids, the C—O bond of ester groups, and a progression of fatty acid bands due to the deformation vibrations of methylene groups in the hydrocarbon chain [1, 9]. An isolated band of wagging CH vibrations at 720 cm^{-1} indicates the presence of long structures with a definite type of crystal packing [7, 10]. The OH deformation vibrations of carboxy groups of fatty acids appear in the form of a broadened band at 925 cm^{-1} [8].

The action of the opaque-2 gene on the lipid composition of maize grain depends on the genotype of the line investigated. Thus, the nature of the absorption of the total lipids of the initial and mutant lines W 155 and A 204 scarcely changed, while for the lipids of the

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TABLE 1. Amounts of Fatty Acids in Maize Grain, g per 100 g of Flour

Acid	Maize line											
	A 204			W 64 A			W 155			Wf 9		
	init. form	mutant form	mut./init.	init.	mutant	mut./init.	init.	mutant	mut./init.	init.	mutant	mut./init.
16:0	0,250	0,150	0,60	0,569	0,365	0,64	0,340	0,263	0,77	0,427	0,347	0,81
18:0	0,060	0,030	0,50	0,016	0,014	0,87	0,023	0,015	0,75	0,018	0,012	0,67
18:1	0,561	0,511	0,91	1,283	0,769	0,60	1,126	0,857	0,75	0,721	0,600	0,83
18:2	0,950	0,900	0,95	3,015	1,971	0,65	2,600	2,151	0,87	1,564	1,510	0,96
18:3	0,012	0,008	0,67	0,070	0,060	0,85	0,019	0,012	0,63	0,022	0,030	1,36
Σ	1,833	1,599	0,87	4,953	3,179	0,64	4,108	3,298	0,80	2,752	2,499	0,91

lysine-rich forms of the lines Wf 9 and W 64 A a rise in the relative intensity of the bands of O-H and C=C stretching vibrations (3300 cm^{-1} and 1655 cm^{-1}, respectively) and of C—H deformation vibrations (1465 cm^{-1}) and a broadening of the group of bands of C—H stretching vibrations and of carbonyl absorption was observed.

The nature of the absorption of the total lipids in the UV region is determined by the genotype of the maize. Thus, absorption bands were observed at 228, 284, and 320 nm in the UV spectrum of the initial line W 64 A, while for the lipids of the initial line W 155 bands were located at 233, 259, 278, and 303 nm.

In the grain of the mutant maize lines the amount of fatty acids had decreased (Table 1) and this tendency depended on the genotype. While for the A 204 and Wf 9 lines the fall in the amount of fatty acids was only 9%, for the W 64 A and W 15 lines it was 35 and 20%, respectively. The main effect of the decrease was achieved through a reduction of the biosynthesis of oleic and linoleic acids, which amount to more than 80% of the total amount of fatty acids.

The results that we obtained differ from those published previously [2, 4], according to which the amount of fatty acids in the grain of opaque maize is higher than in the initial maize. In our opinion, this fact is explained by the different methods of extracting the total lipids. In the isolation of oil from grain by nonpolar solvents only the nonpolar lipids, mainly glycerides, are extracted, while by the methods used in the present work [5] about 95% of lipids, including polar lipids (with strongly bound lipids as an exception) are extracted. As has recently been shown [10], it is just in the group of polar lipids that a change in the ratio of fatty acids under the influence of the gene opaque-2 is observed.

In view of the fact that the influence of the mutant gene on the qualitative composition of the fraction of unsaponifiable substances of maize grain has not hitherto been studied, we have made a comparative investigation of this group of lipids by spectroscopic methods and GLC. It has been established that in the grain of the mutant lines of maize the amount of unsaponifiable substances had fallen by 20-50% (Table 2).

The chromatographic spectra of the methylated unsaponifiable substances of the mutants were considerably impoverished. Thus, the unsaponifiable substances of the grain from the opaque maize lacked certain components, and the quantitative ratio between other components had changed.

The qualitative changes in the composition of the unsaponifiable substances of the grain of the mutant line A 204 agreed with the nature of the absorption in the IR region. Thus, for the unsaponifiable substances of the grain of the initial line A 204, in comparison with the mutant analog, in addition to a group of typical bands of vibrations of C—H at a double bond (3004 cm^{-1}) and of CH_2 and CH_3 groups (2954, 2920, 2854, 1462, and 1376 cm^{-1}), an increase in the intensity of the band of the C=O vibrations at 1740 cm^{-1} in relation to the carbonyl absorption at 1712 cm^{-1} was observed, and strong bands also appeared at 1262, 1090, 1066, 1016, and 796 cm^{-1}. The UV spectra of hexane solutions of the fraction of unsaponfiable substances were characterized by an absorption maximum in the 250-264 nm region (Table 3), which was represented by a broad band having a fine structure in a number of cases.

The differences established in the quantitative amounts and qualitative changes in the fraction of unsaponifiable substances for the mutant lines are of interest in connection with available information on the influence of the nature of the maize genotype on the structure of chlorophylls [11] and on the tocopherols content [12].

TABLE 2. Amounts of Unsaponifiable Substances in Maize Grain, g per 100 g of Flour

Maize line	Initial form	Mutant form	Mutant form / initial form
A 2B4	2,45 ± 0,12	1,96 ± 0,21	0,80
W64 A	2,50 ± 0,16	1,98 ± 0,14	0,79
W155	2,02 ± 0,18	1,02 ± 0,11	0,50
Wf9	2,40 ± 0,15	1,65 ± 0,15	0,69

TABLE 3. Characteristics of the UV Spectra of Hexane Solutions of the Fraction of Unsaponifiable Substances from Maize Grain

Line	Initial form		Mutant form	
	λ_{max}, nm	lg ε 1%	λ_{max}, nm	lg ε 1%
A 204	250	0,96	250	1,07
	259	0,96	259	1,06
	264	0,97	264	1,07
W 64A	253	1,11	261	0,89
	261	1,13	263	0,81
Wf 9	263	0,98	251	1,28
			250	1,28
			263	1,28

EXPERIMENTAL

The grain of initial and mutant forms of maize of the lines A 204, W 64 A, W 155, and Wf 9 in the phase of complete ripeness was investigated. The total lipids were extracted by the methods of Bligh and Dyer [5]. The fatty acids and the unsaponifiable substances were isolated after hydrolysis of the total lipids.

The IR spectra of the total lipids and of the fraction of unsaponifiable substance were investigated on a Specord IR instrument in the form of films on potassium bromide optical glass. UV spectra were obtained on a specord M-40 spectrometer in a 0.2-, 0.5-, or 1-centimeter cell in solution in diethyl ether (total lipids) or in hexane (fraction of unsaponifiable substances), the concentrations of the solutions being 0.013-0.3%.

TLC of the total lipids was carried out in accordance with [13], and GLC analysis on a Chrom-5 gas chromatograph. The methyl esters of the fatty acids and of the unsaponifiable substances were obtained by methylation with diazomethane in ethereal solution. Conditions of GLC for the fatty acid methyl esters; glass column (0.4 cm × 2 m) filled with 10% of poly(ethylene succinate) on Chromaton N-Super 80-100 mesh, isothermal regime (200°C), temperature of injection of the sample 260°C; rate of flow of carrier gas 40 ml/min; for the unsaponifiable substances — glass column (0.4 cm × 2 m), filled with 5% of SP-2100 on Chromaton N-Super 80-100 mesh; temperature regime: from 70 to 280°C at the rate of 5 deg/min, temperature of injection of the sample 150°C; rate of flow of carrier gas 30 ml/min [14].

SUMMARY

It has been established that the changes in the composition of the total lipids and the degree of decrease in the fatty acid content in the grain after the action of a mutant gene depends on the initial maize genotype.

A decrease in the amount of unsaponifiable substances in the grain of the mutants is accompanied by the qualitative changes in the composition, regardless of the nature of the genotype.

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TRITERPENOIDS OF Abies SPECIES.

V. STRUCTURE AND SPECTRAL PROPERTIES OF THE MAIN 9βH-LANOSTANE ACIDS OF SIBERIAN FIR NEEDLES

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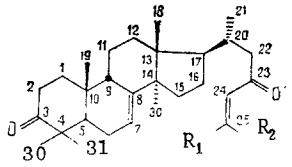
A four-stage conversion into a ketolactone has confirmed the structure proposed previously for two 3-ketolanostane acids isolated from an extract of Siberian fir needles. Features of the mass, PMR, and CD spectra of the methyl esters of these acids and of triterpenoids related to them are explained and discussed.

In [1], two new stereoisomeric acids isolated from the needles of the Siberian fir (Abies sibirica Ledeb), in which they are the main representatives of the 9βH-lanostanoids [2], were described. On the basis of spectral characteristics and biogenetic considerations, the probable structures expressed by formulas (I) and (II) were proposed for their methyl esters. A proof of the correctness of the proposed structures would be a basis for the futher chemical modification of the compounds under investigation and their conversion into other, still little studied, representatives of this peculiar group of triterpenoids found in Abies species [3, 4] and in marine organisms [5].



	R_1	R_2
I.	$COOCH_3$	CH_3
II.	CH_3	$COOCH_3$

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