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LIPID COMPLEX OF THE KERNELS OF SEEDS OF COTTON PLANTS OF THE WILT-RESISTANT VARIETY 175-F

Kh. T. Mirzaazimova, S. G. Yunusova, S. D. Gusakova, and A. I. Glushenkova

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The amount, qualitative and class compositions, and total fatty acid composition of the free, bound, and strongly bound lipids of kernels of the seeds of cotton plant of variety 175-F have been determined, and with the aid of enzymatic hydrolysis the strongly bound lipids have been isolated from the cottonseed kernels in the native form. It has been established that more than half the weight of the lipids comprises neutral varieties and the remainder are polar components. The lipids of the more stable lipid-protein complexes possess a higher degree of saturation.

As is well known, the polar lipids of plants are integral components of cell membranes and, in a complex with protein and other compounds, determine their structure and properties. In order to break down the lipid-protein components and to extract the lipids it has been proposed to use ethanol [1] or alcohol-containing extractants [2]. By these solvents, lipids bound to proteins by Van der Waals hydrophobic interaction or hydrogen bonds are isolated from the plant tissues. However, there is a group of lipids strongly bound with protein by covalent bonds which are not extracted by organic solvents. They can be extracted only by breaking down the complex with the aid of acid or alkaline hydrolysis [1], during which the strongly bound lipids (SBLs) lose their native state. Their approximate composition and amount must therefore be judged from the fatty acids (FAs) isolated from alkaline hydrolysates. The SBLs have been isolated from cottonseed kernels [3] and from rice grains [4] in this way.

In recent years, it has been proposed to use proteolytic enzymes to cleave the lipid-protein bonds [5]. After enzymatic hydrolysis, the lipids liberated from the complexes, which have, in the main, retained their native form, are readily extracted by solvents. The glycolipids strongly bound with the protein in sunflowerseed kernels have been isolated by this method [6]. Among the glycolipids, the authors concerned found seven classes, the predominating ones being monogalactosyldiacylglycerols (MGDGs), digalactosyldiaclyglycerols (DGDGs), (acylmonogalactosyl)diacylgycerols (AMDGs), and sulfoquinovosyldiacylglycerols (SQDGs).

There is no information on the native SBLs of the cotton plant.

We have made a comparative study of the bound lipids (BLs) and SBLs of the seed kernels of a cotton plant of the variety 175-F isolated by two methods. The first method [I] was the official method [1] and consisted in the successive extraction from the ground kernels first of the free lipids (FLs) with hexane in a Soxhlet apparatus, then of the SBLs by breaking down the lipoproteins with boiling ethanol followed by extraction with diethyl ether in a Soxhlet apparatus, and then of the SBLs by severe alkaline hydrolysis in the form of FAs.

In the second method (II) the BLs were isolated by exhaustive extraction with a mixture of chloroform and methanol in a Soxhlet apparatus, and the SBLs by enzymatic hydrolysis.

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TABLE 1. Yields of Lipids from the Kernels of a Cotton Plant of Variety 175-F

Lipids and accompanying substances	Method I		Method II	
	% on the weight of the kernel	% on the weight of the lipids	% on the weight of the kernel	% on the weight of the lipids
Free	36,4	95,0	35,9	91,9
Bound	1,8	3,3	3,6	6,5
Strongly bound:	0,9	1,7	0,8	1,1
a) nonlipid	—	—	0,1	0,1
b) lipids	—	—	0,7	1,0
Residual	—	—	0,03	0,5
a) nonlipid	—	—	0,02	0,3
b) lipids	—	—	0,01	0,2
Total yield	39,1	100	40,33	100

For enzymatic hydrolysis we used previously defatted protosubtilisin having its optimum action under alkaline conditions, since it is under these conditions that it is possible to isolate from cottonseed kernels in the native form not only the SBLs but also polyphenols, including gossypol [7]. To evaluate the completeness of the extraction of the SBLs (II), the residue after enzymatic hydrolysis was subjected to severe alkaline hydrolysis with the subsequent isolation of the FAs. The yields of the SBLs (II) and of the residual lipid were determined after the separation of nonlipid impurities. All the extracts were brown in color.

Table 1 gives the yields of lipids with the extractable accompanying components obtained by the two methods.

As can be seen from Table 1, the degree of extraction of the BLs by method II was twice as high as by method I, while the yield of SBLs (II) was comparable with that of the SBLs (I). Only 0.2% of the lipid complex could not be extracted from the cottonseed kernels by enzymatic hydrolysis. However, the total yield of lipids and accompanying substances in the case of method II was 1.23% higher than by the method (I) usually adopted.

The quantitative compositions of the lipids of the two samples were found by the GLC method in systems 1-6. According to the results of analysis, the FLs and BLs (I and II) of this variety consisted of the same three components of neutral lipids (NLs), glycolipids (GLs), and phospholipids (PLs) as the analogous groups of lipids of the other species [8] and the other varieties of the cotton plant [3, 9] that have been studied. All the groups of lipids contained polyphenolic pigments and, according to TLC in system 3, the FLs, the BLs (II) and the SBLs (II) gave – in addition to a brown spot of an unidentified pigment (R_f 0.51) – a spot (R_f 0.67) corresponding in its mobility and qualitative reaction with 50% H_2SO_4 to standard gossypol. The SBLs (I) were found to contain hydrocarbons, sterols, free fatty acids, and polyphenolic pigments, while the BSLs (II) contained the whole set of components of the neutral and polar lipids characteristic for the FLs and BLs. The residual lipids (II) were found to contain the same components as the SBLs (I).

To determine the ratio of the individual groups of lipids freed from impurities, the SBLs (II) were separated by preparative TLC in system 7 into neutral and polar lipids, the amounts of which were 56.6 and 43.4% (on the weight of the SBLs), respectively, i.e., more than half the SBLs of the kernels of a cotton plant of this variety consisted of nonpolar and weakly polar components.

The fatty acid composition of the lipids according to GLC results is shown in Table 2. Since the composition of the acids of the FLs of two samples were the same, the table gives only one of them. Apart from the acids shown in Table 2, according to the mass spectrum the FAs of the SBLs I and II contained minor amounts of the 21:0-24:0 high-molecular-mass acids. The compositions of the FSAs of the BLs and the SBLs depended on the method for their isolation. Considerable differences were observed in the amounts of individual FAs, which affected the total degree of unsaturation. For all the groups of lipids isolated by the two methods, except for the BLs II, there was a regular increase in the total degree of unsaturation of the lipids with an increase in the strength of their binding with protein. However, the BLs II were more unsaturated, and the SBLs II more saturated, than the analagous lipids (I).

TABLE 2. Fatty Acid Composition of the Lipids of the Kernels of a Cotton Plant of Variety 175-F (% GLC).

Lipid	Acids									
	14:0	16:0	16:1	17:0	18:0	18:1	18:2	20:0	Σ_{sat}	Σ_{unsat}
Free	Сл.	22,0	0,8	—	3,5	16,3	57,4	—	25,5	74,5
Bound										
method I	0,1	28,4	1,5	—	1,3	25,2	43,5	—	29,8	70,2
method II	0,1	21,1	0,4	—	3,6	22,4	52,4	—	24,8	75,2
Strongly bound:										
method I	0,2	27,0	1,3	0,4	5,1	23,2	42,8	—	32,7	67,3
method II	1,0	32,6	4,1	1,2	3,5	19,2	38,4	Сл.	38,3	61,7
Remainder II*	1,5	31,5	1,4	—	6,0	17,3	39,3	0,8	42,0	58,0

*12:0-2.2%.

EXPERIMENTAL

The mass spectra were taken on a MKh-1310 instrument with a SVP-5 system for the direct introduction of the sample at an ionizing voltage of 70 V, a collector current of 80 μA, a temperature of the ionization chamber of 130-150°C, and a temperature of the evaporating ampul of 130-140°C. GLC was performed as described in [8]. Analytical TLC was conducted on Silufol in the following solvent systems: 1) hexane–diethyl ether (9:1); 2) hexane–diethyl ether (7:3); and 3) benzene–methanol (20:5); and on silica gel L-5/40 (Czechoslovakia) in the following systems: 4) acetone–benzene–water (91:30:8); 5) chloroform–methanol–25% ammonia (65:25:5); 6) direction I: chloroform–methanol–25% ammonia (8:3:1); direction II: chloroform–methanol–acetic acid–water (8:3:1:1); and 7) chloroform. The plates were visualized with iodine vapor and by spraying with 50% H_2SO_4 followed by heating, while the GLs and PLs were revealed with specific reagents [2].

The seeds of a cotton plant of variety 175-F were supplied by S. A. Usmanov and Yu. Ikramov of the V. S. Zaitsev Institute of Cotton-Plant Selection and Seed Production. The seeds were first freed from husks, and the kernels were ground in a coffee mill.

The extraction of the FLs, BLs, and SBLs by method I and of the residual lipids was carried out by the procedure of [1]; the BLs II were extracted with a mixture of chloroform and methanol (2:1, v/v) in a Soxhlet apparatus for 46 h. The enzyme preparation protosubtilin G 10X was defatted by three extractions with diethyl ether. Enzymatic hydrolysis was carried out in accordance with [7]. To eliminate nonlipid components from the SBLs II, the extracts of the products of enzymatic hydrolysis were treated twice with a 0.04% solution of $CaCl_2$ [10]. The residual lipids were separated from the nonlipid components by reextraction with hexane.

Mass spectra of the methyl esters of the FAs, m/z: 242, 268, 270, 284, 294, 296, 298, 326, 340, 354, 368, 382 (M^+); 241, 267, 269, 283, 293, 295, 297, 325, 339, 353, 367, 381 $(M - 1)^+$; 211, 237, 239, 253, 263, 265, 267, 295, 309, 323, 337, 351 $(M - 31)^+$; 210, 236, 238, 252, 262, 264, 266, 294, 308, 322, 336, 350 $(M - 32)^+$.

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A NEW GUAIANOLIDE – OPOFERZIN FROM THE ROOTS OF Ferula oopoda

S. V. Serkerov, U. Rikhlevska, A. N. Aleskerova, and N. F. Mir-Babaev

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A new guaianolide with the composition $C_{20}H_{22}O_5$, mp 150-152°C, has been isolated from the resin of the roots of Ferula oopoda, (Boiss. et Buhse) Boiss. It has been established that it corresponds to the structure of 11α-angeloyloxy-2-oxo-5βH, 6αH-guaia-1(10), 3,7- trien-6,12-olide.

Six natural and four artefactual sesquiterpene lactones isolated from the resin of the roots of Ferula oopoda (Boiss. et Buhse) Boiss. have been reported previously [1, 2]. In the present paper we give the results of an investigation of the structure of a new guainolide from this plant material which has the composition $C_{20}H_{22}O_5$, mp. 150-152°C, and has been called opoferzin.

The IR spectrum of opoferzin has absorption of a γ-lactone ring (1770 cm^{-1}), of an α,β-saturated group (700 cm^{-1}), of a conjugated ketone group in a five membered ring (1660 cm^{-1}), and of double bonds in conjugation (1660, 1630 cm^{-1}).

The presence and nature of the ester group was found both by saponification and from the NMR spectrum. On saponification, a distillable acid with mp. 44-45°C was obtained which was identified from its IR spectrum as angelic acid. The NMR spectrum of opoferzin had signals at (ppm) 1.90 (d, J = 7 Hz, Ch_3–CH=, each component being also split into a doublet with J = 1.4 Hz), 2.03 (d, J = 1.4 Hz, CH_3–C=), and 6.14 (m, –CH=), which are characteristic for an angelic acid residue.

The three-proton singlet at 2.33 and 2.43 ppm and a one-proton singlet at 6.23 ppm in the spectrum indicated the presence in the opoferzin molecule of the cyclopentadiene structural fragment that is characteristic for the sesquiterpene lactams badkhyzin [1], talassins A and B [3], diversolide [4], olgin, and olgoferin [5].

Thus, of the three methyl groups of the guaiane hydrocarbon skeleton two are vinyl methyls (at C_4 and C_{10}). The third methyl group (at C_{11}) appeared in the NMR spectrum in the form of doublet at 1.80 ppm (J = 1.4 Hz). The nature of the splitting and the value of the chemical shift of the latter indicated the methyl of a gem-ester group [3, 5].

As the PMR spectrum showed, the opoferzin molecule contains four double bonds. One of them is tetrasubstituted and two are trisubstituted, these being located at C_1–C_{10}, C_3–C_4, and C_{17}–C_{18}, respectively. The fourth double bond, also trisubstituted, is located at C_7–C_8. This was shown by the one-proton signals of an olefinic proton at 6.08 ppm (d, J = 6 Hz) and of the protons of a vicinal CH_2 group (q, 2.82 ppm, J = 6, J = 14.6 Hz; and d, 2.65 ppm, J = 14.57 Hz), which are characteristic for an ABX system.

The lactone proton appeared in the spectrum in the form of a quartet at 4.56 ppm (J = 10.5; J = 1.4 Hz). As can be seen from the spin-spin coupling constants, the last-mentioned proton interacts with one vicinal proton (H-5), the signal of which is represented in the spectrum by a doublet at 3.13 ppm (J = 10.5 Hz). These facts showed the presence of a ring at C_6–C_7.

The configurations of the C_5 and C_6 asymmetric centers followed from the spin-spin coupling constants of H-5 (10.5 Hz), and also from the results of an x-ray study of sesqui-

V. L. Komarov Institute of Botany, Academy of Sciences of the Azerbaidzhan SSR, Baku.
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