6. P. Langcake and R. Y. Pryce, Experientia, 33, 151 (1977).
7. P. Langcake and R. Y. Pryce, Phytochemistry, 16, 1193 (1977).
8. R. Y. Pryce and P. Langcake, Phytochemistry, 16, 1452 (1977).
9. K. Nakajima, H. Taguchi, T. Endo, and I. Yosioka, Chem. Pharm. Bull., 26, 3050 (1978).
10. R. G. Powell, R. Bajaj, and J. L. McLaughlin, J. Nat. Prod., 50, 293 (1987).
11. K. Suzuki, T. Shimizu, J. Kawabata, and J. Mizutani, Agric. Biol. Chem., 51, 1003 (1987).
12. H. Kurihara, J. Kawabata, S. Ichikawa, and J. Mizutani, Agric. Biol. Chem., 54, 1097 (1990).
13. J. Kawabata, S. Ichikawa, H. Kurihara, et al., Tetrahedron Lett., 30, 3785 (1989).
14. P. Coggon, N. F. Janes, F. E. King, T. J. King, et al., J. Chem. Soc., Chem. Commun., 406 (1965).
15. M. U. S. Sultanbawa, S. Surendrakumar, P. Bladon, J. Chem. Soc., Chem. Commun., 619 (1980).
16. U. Samaraweera, S. Sotheeswaran, and M. U. Sultanbawa, Phytochemistry, 21, 2585 (1982).
17. J. A. Geewananda, P. Guanawardena, S. Sotheeswaran, et al., Phytochemistry, 25, 1499 (1986).
18. S. Sotheeswaran, M. U. Sultanbawa, S. Surendrakumar, and J. Bladon, J. Chem. Soc., Perkin Trans. I, 699 (1983).
19. S. Sotheeswaran, M. U. Sultanbawa, S. Surendrakumar, et al., J. Chem. Soc., Perkin Trans., I, 159 (1985).
20. A. P. Lins, M. Yoshida, O. R. Gottlieb, H. E. Gottlieb, and K. Kubitzki, Bull. Soc. Belg., 95, 737 (1986).

EFFECT OF A WILT INFECTION ON THE NEUTRAL LIPIDS OF COTTON LEAVES

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

UDC 677.21:581.45:547.915

The composition of the neutral lipids of leaves of a cotton plant of the wilt-resistant variety 175-F, the set of components in the fatty acid composition of the lipid classes, and the partial structures of the triacylglycerides have been determined. The changes taking place in the composition and structure of these lipids on artificial infection of the plant by the fungal pathogen Verticillium dahliae have been elucidated.

We have previously reported the qualitative composition of the cell lipids of young cotton leaves of the wilt-resistant variety 175-F [1]. The object of the present investigation was a comparative study of the changes in the neutral lipids (NLs) of the leaves of this variety on artificial infection of the plant with the pathogenic fungus Verticillium dahliae Kleb.

For analysis we used leaves gathered from healthy (I) and infected (II) plants. First the surface lipids were eliminated from the leaves, and then the cell lipids were extracted and these were separated by the CC method into NLs and glyco- and phospholipids. The yield of NLs was 30.0 mg/g of dry leaf tissue for (I) and 30.2 mg/g for (II) or, in relation to the weight of total lipids 42.8% (I), and 51.1% (II).

The neutral lipids were separated by a combination of CC and preparative TLC into individual classes the identification of which was made by spectral and chemical methods. The chlorophylls and carotenoids coextracted with the NLs underwent partial change during the process of extraction of the NLs and the amount was therefore determined from the weight of the native and modified forms isolated on CC.

Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 477-483, September-October, 1992. Original article submitted December 30, 1991.

 0009-3130/92/2805-0414$12.50

TABLE 1. Composition of the Neutral Lipids of Healthy (I) and Infected (II) Cotton Leaves

Class of lipid	Sample I		Sample II	
	mg/100g of dry tissue	% on the weight of the lipids	mg/100 g of dry tissue	% on the weight of lipids
Hydrocarbons:				
a) n-alkanes, n-alkenes, and alkyl- and alkenylbenzenes	140	4,6	100	3,3
b) n-alkenes and arylcycloalkenes (?)	60	2,0	50	1,7
c) squalene, alkyl- and alkenylbenzenes	70	2,3	50	1,7
d) carotenoids	110	3,6	130	4,3
Fatty acid esters with				
a) alkan-1-ols	70	2 3	70	2,3
b) cyclic alcohola	120	4,0	50	1,7
Plastoquinones	50	1,7	50	1,7
Tocopherols	50	1,7	20	0,7
Triacylglycerols	120	4.0	60	1,9
Polyprenols	280	9,2	300	9,9
Free fatty acids and xanthophylls	250	8,3	260	8,6
Alkan-1-ols	110	3,6	70	2,3
Triterpenols + 4-monomethylsteroids	Tr.	—	Tr.	
Phytosterols	210	6,9	200	6,6
Diacylglycerols	140	4,6	160	5,3
Fatty hydroxy acids and hydroxyacylacyl-glycerols	70	2,3	130	4,3
Monoacylglyerols	40	1,3	50	1,7
Chlorophyll pigments	1140	37,6	1270	42 0
Σ-lipids	3030	100	3020	100

TABLE 2. Composition of the Hydrocarbons of the Leaves of a Healthy Cotton Plant According to Mass Spectrum

Fraction	General formula	M+, m/z	Composition	Main component
1	C_nH_{2n+2}	240—338	C_{17}—C_{23}	C_{17}
	C_nH_{2n}	210—336	C_{15}—C_{23}	C_{15}
	C_nH_{2n-6}	204—0	C_{15}—C_{23}	C_{15}
	C_nH_{2n-8}	230—328	C_{17}—C_{23}	C_{17}
2	C_nH_{2n}	238—364	C_{17}—C_{25}	C_{17}
	C_nH_{2n-10}	242—340	C_{17}—C_{25}	C_{17}
3	C_nH_{2n-6}	246—400	C_{19}—C_{25}	C_{20}, C_{21}
	C_nH_{2n-8}	286—342	C_{21}—C_{25}	C_{22}
	C_nH_{2n-10}	410	C_{30}	

It can be seen from Table 1 that the infection of the leaves with the pathogen did not affect the qualitative set of components of the NLs. More than one third of the weight of the NLs (I) consisted of chlorophyllic and carotenoid pigments, and their proportion in (II) was greater. An increase in the amount of pigments in the photosynthetic tissues in response to fungal and viral infection have been reported for resistant varieties of plants [3]. When the pigments were left out of account, the amount of NLs proper in sample (I) was 17.8 mg/g of dry tissue and in (II) 16.2 mg/g of dry tissue, and, thus, damage by wilt somewhat lowers the level of NLs in cotton leaves. In the NLs of sample (I) polyprenols, hydrocarbons, and fatty acids (FAs) predominated, and in the NLs of (II), polyprenols and FAs. The infected leaves contained a considerably smaller amount than the healthy leaves of triacylglycerols (TAGs), tocopherols, and FA esters with sterols and triterpenols but almost twice as much hydroxy lipids.

According to UV and mass spectroscopies, the hydrocarbons of the two samples consisted of mixtures of n-alkane, n-alkenes, alkyl- and alkenylbenzenes, and squalene.

The mass numbers, M^+, and main components of the hydrocarbons are given in Table 2. According to results (Tables 1 and 2), in the NLs of (I) and (II) aliphatic and aromatic hydrocarbons with 15-17 carbon atoms predominated. Under the influence of infection, the level of hydrocarbons in the NLs fell.

TABLE 3. Fatty Acid Composition of the Lipids of Healthy (I) and Infected (II) Cotton Leaves (GLC, %)

Acid	Neutral lipids		Esters		Triacyl-glycerols		2-Monoacyl-clycerols		Free fatty acids		Monoacyl-glycerols	
	I	II	I	II	I	II	I	II	I	II	I	II
12:0	1,0	1,3	1,2	1,5	—	0,7	—	—	1,1	1,5	—	—
14:0	3,4	4,8	3,5	5,8	1,9	3,1	2,6	2,8	3,3	5,8	0,2	0,09
15:0	0,3	0,9	Tr.**	5,4	0,3	0,3	0,8	0,2	0,9	1,0	—	—
16:0	22,2	44,6	22,0	39,9	24,4	22,1	23,8	32,0	41,8	55,1	30,6	47,7
16:1	2,6	2,2	2,6	3,0	1,6	2,4	3,5	3,6	6,1	2,0	3,4	3,4
17:0	0,7	0,3	Tr.	Tr.	Tr.	0,5	2,9	1,6	Tr.	Tr.	—	—
18:0	3,6	4,9	4,7	11,3	8,1	4,7	9,6	8,8	6,5	5,2	Tr.	Tr.
18:1	13,8	14,0	17,0	16,4	28,5	21,6	28,5	27,0	28,5	13,8	27,2	22,6
18:2	32,5	11,1	27,6	2,6	15,4	13,5	23,7	12,0	8,6	6,7	16,4	8,8
18:3	19,9	15,9	15,4	10,7	19,8	31,1	4,6	12,0	3,2	8,9	22,2	16,6
20:0	Tr.	Tr.	6,0	3,4	—	—	—	—	Tr.	Tr.	—	—
Σ_{sat}	31,2	55,8	37,4	67,3	34,7	31,4	39,7	45,4	53,6	68,6	30,8	48,6
Σ_{unsat}	68,8	43,2	62,6	32,7	65,3	68,6	60,3	54,6	46,4	31,4	69,2	51,4

*Traces of the 21:0–26:0 fatty acids (mass spectrum).
**Traces.

The ester fraction was recrystallized from diethyl ether with cooling, giving a precipitate of wax esters and esters of FAs with cyclic alcohols.

According to the results of mass-spectrometric analysis, the main components of the wax esters of the NLs of the healthy leaves were esters of C_{26}–C_{32} n-alkanols with the 16:0 and 18:0 FAs, while in the NAs of the infected leaves they were esters of the same ethanols with the 16:0 acids.

Esters of FAs with cyclic alcohols were investigated by mass spectrometry and GLC in the form of the initial fraction and the products of its alkaline hydrolysis. It was found that these esters consisted mainly of 4-demethylsterols (phytosterols), 4,4-dimethylsterols (triterpenols), and 4-monomethylsterols (see the Experimental part) and the FAs shown in Table 3. The mass spectrum showed that in the esters 24-methylenecycloartanol was esterified selectively with the 16:0 and 18:3 FAs (sample I) or with the 16:0 FA (sample II). Such selectivity of esterification of the FAs was not observed in the other cyclic alcohols of this fraction.

According to Tables 1 and 3, infection of the cotton plant with wilt did not affect the amount and composition of the wax esters of the NLs of the leaves but caused a more than twofold fall in the level of esters of FAs with cyclic alcohols, which was accompanied simultaneously by a sharp decrease (10-fold) in the level of the 18:2 component of their acid fraction and a rise in that of the saturated acids, except for the 20:0 FA.

The sets of free alkan-1-ols and phytosterols of the two samples determined with the aid of mass spectrometry did not differ from that detected in the esters.

Triacylglycerols. The fatty acids of the two samples obtained after alkaline hydrolysis of the TAGs had compositions (Table 3) showing that the infection of the plant was accompanied by a rise of the 18:3-containing species of TAGs and a fall in the species with 18:1 in the leaves.

The structure and compositions of the TAGs were determined by the method of pancreatic hydrolysis, which gave the set of the FAs of 2-monoacylglycerols shown in Table 3. Table 4 gives the position-species composition of the TAGs calculated from the results of enzymatic hydrolysis, where P is the sum of the saturated acids, O that of the monoenoic FAs, and L represents the 18:2 and Le the 18:3 acids.

It can be seen from Table 4 that the main molecular species of the TAGs from sample (I) consisted of approximately equal proportions of species with the 16:0, 18:1, and 18:3 acids in the sn-2 position [16.6, 15.2, and 14.2 (mol. %), respectively]. In sample (II) the amounts of the analogous TAG species with the 16:0 and, particularly, with the 18:1 acid had fallen, and those with the 18:3 acid had doubled. Common for the two samples was the absence of appreciable amounts of TAGs with the 18:2 acid in the central position of the molecule, while such molecular species are characteristic for the TAGs of the seeds of higher plants [4].

TABLE 4. Position-Species Composition of the Triacylglycerols of Healthy (I) and Infected (II) Leaves of the Cotton Plant (mol. %)

Main species	I	Main Species	II
PPO	5.9	LeLeLe	6.7
PPLe	5.6	PPLe	4.8
POO	5.4	LLeLe	4.7
POLe	5.2	POLe	4.1
OPLe	5.1	OPLe	4.1
PLeO	5.1	PLeO	4.1
PLeLe	4.8	PLeLe	8.3
OOLe	4.6	LePle	4.1
OLeLe	4.3	OLeLe	6.8
	46.0		47.7

Others:

for sample (I):

22 species – 1-3.0 each
3 species – <1.0 each

for sample (II):

21 species – 1-3.5 each
7 species – <1.0 each

The distribution of the 16:0 acid in the sn-2 position is considered a feature of the biosyntheses of other glycerolipids (phospho- and glycolipids) in the leaves of higher plants and, namely, as an indication of the prokaryotic route of their formation. The species with unsaturated C_{18} FAs in the sn-2 position are biosynthesized by a mechanism characteristic of eukaryotic cells [5]. Our results show that this may also be valid for the biosynthesis of TAGs and, consequently, in sample (I) there was a higher proportion of TAG species of the prokaryotic type, and in (II) of those of the eukaryotic type.

The polyprenols were investigated by IR and mass spectrometry. In both samples a mixture of nine isoprenols with numbers of isoprene units n = 6-14 and a predominance of undeca- and dodecaprenols was found. As minor components there were seven with n = 8-14, which were assigned to dolichol and its lower isoprenologues. Appreciable quality differences were detected in the compositions of polyprenols of the two samples.

The free fatty acids of the two samples were, according to the results of GLC analysis, enriched with the 16:0 acid, the level of which rose under the influence of infection (Table 3). Of the unsaturated components in the free FAs the amount of the 18:1 acid had fallen considerably (2-fold), while the amount of the 18:3 acid had risen.

Monoacylglycerols. As for the other acyl-containing classes, a higher degree of total saturation of the acyl residues was characteristic for the monoacylglycerols of sample (II) than for those of sample (I) (Table 3).

The compositions and structures of the diacylglycerols and hydroxylipids of the two samples have been described previously [17].

The pigments detected in the NLs (I) and (II) by UV spectroscopy were chlorophyll "a" and β-carotene, and the products of their transformations.

Thus, in young healthy leaves of a cotton plant of the wilt-resistant variety a quarter of the weight of the total lipids consisted of NLs (without taking the pigments into account), in which polyprenols, hydrocarbons, and free FAs predominated. For the acyl-containing NLs, with the exception of the wax esters and free FAs, a high degree of unsaturation was characteristic. The main unsaturated residue in the esters was that of the 18:2 acid, while in the TAGs, the monoacylglycerols, and the free FAs it was the 18:1 variety. The free FAs were enriched with the 16:0 acid, which was dominant among the saturated acyls in all the lipid classes of the leaves.

A feature of the molecular structures of TAGs of the leaves of the cotton plant is the presence of species with the 16:0, 18:1, and 18:3 acids in the sn-2 position of the molecule.

Infection of the leaves by the pathogenic fungus V. dahliae caused a fall in their content of NLs, particularly TAGs, tocopherols, and FA esters with sterols and triterpenols, but at the same time the level of photosynthetic pigments and hydroxy lipids rose and the ratio of the acyls and acyl residues within the lipid classes changed.

The quantitative changes in the FAs in the individual classes bore a complex nature. In response to infection stress, the level of the 16:0 acid rose in the free FAs, the FA esters, and the monoacylglycerols, while in the TAGs it fell somewhat. Simultaneously, the level of the 18:2 acid in the FA esters and monoacylglycerols and that of the 18:1 acid in the TAGs and free FAs fell appreciably, while the level of the 18:3 acid in the two latter classes actually increased. These changes showed different roles of the individual lipids in the formation of the resistance of the plant to fungal infection.

EXPERIMENTAL

The conditions for taking the spectra have been described in [6]. Thin-layer chromatography was conducted on Silufol plates and 15 × 8 cm glass plates coated with L 5/40 silica gel (Czechoslovakia) with the aid of 6.5% of gypsum in the following solvent systems: 1) hexane; 2) hexane–diethyl ether (99:1); 3) (95:5); 4) (90:10); and 5) (7:3); and 6) hexane–acetone–benzene–isopropanol (69.5:25:4:1.5).

GLC analysis was carried out as described in [7]. The cotton plants were grown under artificial climate conditions and were infected as described in [8]. The surface lipids were eliminated from the leaves by a known method [1], the enzymes having been previously inactivated with boiling isopropanol [9]. The cell lipids were extracted from the comminuted leaves with chloroform–methanol (2:1, v/v) according to Folch. The CC of the lipids was conducted on silica gel L 100/160 (Czechoslovakia), the NLs being eluted with chloroform and then being separated by this method under the conditions described in [10].

The hydrolysis of the TAGs by pancreatic lipase was conducted by the method described in [11].

Hydrocarbons were eluted from the column with hexane. On TLC in system 1 they were separated into three fractions, with R_f 0.95, 0.7, and 0.4. The substances of all the fractions were stained with I_2 vapor, while fractions 2 and 3 gave a pink coloration on treatment with 50% H_2SO_4 and heating.

Hydrocarbons (1) (R_f 0.95) had weak adsorption in the UV spectrum in the 240-260 and 280-300 nm regions. Their mass spectrum contained M^+ and fragments from the breakdown under electron impact of n-alkanes and n-alkenes [12], and also peaks with m/z 147, 148, 133, 134, 119, 105, and 91 which are typical for substituted alkylbenzenes [12, 13]. The M^+ values of the latter corresponded to arylalkanes and arylalkenes (or arylcycloalkanes).

Hydrocarbons (2) (R_f 0.7) absorbed in UV light at λ_{max}^{hexane} 231, 256 nm; the mass spectrum revealed the above-mentioned diagnostic fragments of aromatic hydrocarbons and the M^+ peaks of, presumably, arylcycloalkenes; there were weak peaks of n-alkenes.

Hydrocarbons (3) (R_f 0.4), λ_{max}^{hexane} 236, 241, 252, 259, 273, 276, 282, 288, and 294 nm. Their spectrum contained as the main peaks those with m/z 410 (M^+), 395 $(M - 15)^+$, 367 $(M - 43)^+$, 341 $(M - 69)^+$, 273, 205, 137, 69 from squalene [7], and also the peaks of ions of aromatic hydrocarbons.

The wax esters (R_f 0.8-0.87), system (3), consisted, according to mass-spectrometric results, of 19 components, with M^+ 508-760 ($C_{34}H_{68}O_2$–$C_{52}H_{104}O_2$), and in sample (I) the strongest peaks were those of M^+ with m/z 648 ($C_{44}H_{88}O_2$), 676 ($C_{46}H_{92}O_2$), 704 ($C_{48}H_{96}O_2$), and 732 ($C_{50}H_{100}O_2$), and in (II) the same esters except for the C_{50} and C_{52} varieties.

According to the m/z values of the fragments $[R' - 1]^+$ [14], the alcoholic moieties of the wax esters were represented by a set of alkan-1-ols of the even series from C_{24} to C_{32}. The $C_{24:1}$–$C_{32:1}$ alken-1-ols were detected as impurities.

Acid moieties, determined by the m/z values of the fragments $[RCO_2H]^+$ and $[RCO_2H_2]^+$ consisted of saturated fatty acids of the even series from 12:0 to 26:0 with the 15:0-25:0 varieties as impurities. In the spectrum of sample (I) fragments of the 16:0 and 18:0 acyls predominated, and in (II) the 16:0 acyl.

The esters of the FAs with cyclic alcohols (R_f 0.82-0.85, system 3) were analyzed in the forms of the initial fraction and of the products of its alkaline hydrolysis. The hydrolysis products were separated by the TLC method in system 5 into alcoholic and acidic parts. The cyclic alcohols were identified from their mass spectra, while the FAs were converted into their methyl esters (MEs) and these were investigated by GLC (Table 3) and mass spectrometry.

According to the analytical results, the esters of the two samples consisted of mixtures of substances with M^+ 650, 652, 660-664, 672-678, 682-690, and 700, in the alcoholic part of which 4-demethylsterols predominated and in the acidic fraction residues of the 18:2, 16:0, 18:1, and 18:3 FAs (sample I) or the 16:0 and 18:1 acids (sample II).

Of the 4,4-dimethylsterols we identified β-sitosterol (M^+ 414) and stigmasterol (M^+ 412) and, as impurities, campesterol (M^+ 400) and cholesterol (M^+ 386) [15]. No alcohols with M^+ 442, 438, 428, and 424 (samples I and II) and 444 (sample II) were identified.

Of 4,4-dimethylsterols we detected α- and/or β-amyrin (M^+ 426, characteristic ion with m/z 218), cycloartenol (M^+ 426, m/z 286), and 24-cycloartanol (M^+ 440, m/z 300), 615, [15, 16], and of 4-monomethylsterols, presumably, citrostadienol (M^+ 426, m/z 397) and obtusifoliol (M^+ 426, m/z 327, 309) [17].

The plastoquinones (R_f 0.88, system 4) absorbed in UV at $\lambda_{max}^{CH_3OH}$ 255 nm, with a bathochromic shift of the 4,4-dimethylsterols we $\lambda_{max}^{CH_3OH}$ 287 nm region on the addition of a 20% alcoholic solution of $NaBH_4$, on the basis of which plastoquinone-9 was identified [18].

The tocopherols (R_f 0.6 system 5) were revealed on TLC in the form of a lilac-green spot after the plate had been sprayed with 50% H_2SO_4 and heated; they adsorbed in the UV spectrum at $\lambda_{max}^{ethanol}$ 292 nm [18]. In the mass spectrum we observed the peaks of M^+ with m/z 430 (100%) and of fragments formed as the result of the splitting out of alkyl substituents and the breakdown of the dihydropyran ring of α-tocopherol with m/z 205 (20), 176 (12), 166 (18), 165 (84), and 164 (25). A peak with m/z 416 (M^+, 0.9) showed the presence of β- and/or γ-tocopherol as impurity.

The triacylglycerols (TAGs, R_f 0.5, system 2) were transparent in UV light. Saponification led to the FAs, which were converted by treatment with diazomethane into the MEs, and these were analyzed by GLC.

The polyprenols (R_f 0.4, system 5) appeared on a chromatogram as a yellow-brown spot after it had been sprayed with 50% H_2SO_4 and heated; they were transparent in UV light.

The IR spectrum of the fraction corresponded to that of known polyprenols with unsaturated (1005 cm^{-1}; C–O of an allyl primary alcohol) and saturated (1058 cm^{-1}) "OH ends" of the molecules [19]. The spectrum contained bands at 899 and 890, with an inflection at 795 cm^{-1}, which are usually observed in trans-isoprenoids of the solanesol family, and also bands of medium intensity at 1330, 1295, 1225, 1135, and 1089 cm^{-1} of cis- forms (dolichol family).

The mass spectrum, with the ions M^+, $(M - 18)^+$, $(M - 18 - 69)^+$, and $(M - 87 - n68)^+$ was typical for the breakdown of polyprenols [19]. The strongest peaks of the $(M - 18)^+$ and M^+ ions with m/z 426-970 corresponded to isoprenologues with n = 6-14; the polyprenols with M^+ 766 ($C_{55}H_{90}O$) and 834 ($C_{60}H_{98}O$) predominated in the mixture. The spectrum also contained the ions of other isoprenologues with M^+ 564-972, which, according to [20], belonged to polyprenols of the dolichol family.

The monoacylglycerols (R_f 0.1, system 5) had bands in the IR spectrum at ν_{max}^{film} (cm^{-1}) 3100-3600 (OH...O), 1740, 1250, 1170 (OCO), and, after alkaline hydrolysis, FAs with the composition shown in Table 3 were found in the products isolated by extraction with diethyl ether.

The pigments of the chlorophyll group were eluted from the column together with the polar components of the NLs. According to the results of TLC in system 6 sample (I) contained three groups of pigments with R_f 0.45, 0.53, and 0.73, and sample (II), additionally pigments with R_f 0.49 and 0.56. The UV spectrum of the pigments isolated (TLC, system 6) showed absorption bands of chlorophyll "a" at $\lambda_{max}^{acetone}$ (nm) 430 and 662 (R_f 0.53) and the products of its decomposition at $\lambda_{max}^{acetone}$ 407, 534, 667 (R_f 0.73); $\lambda_{max}^{acetone}$ 410, 504, 534, 666 (R_f 0.56); $\lambda_{max}^{acetone}$ 412, 432, 520, 560, 658 (R_f 0.49); and $\lambda_{max}^{acetone}$ 406, 426, 660 nm (R_f 0.45) [21].

The carotenoids (R_f 0.6, system 2) were identified from their yellow-orange coloration and their UV spectrum, which showed bands at $\lambda_{max}^{acetone}$ (nm) 425, 455, 480, corresponding to β-carotene [22].

The xanthophylls (R_f 0.34, system 6) absorbed in the UV spectrum with $\lambda_{max}^{acetone}$ (nm) 440, 465, 477, on the basis of which they were assigned conjecturally to modification products of β-carotene [22].

LITERATURE CITED

1. S. G. Yunusova, S. D. Gusakova, Kh. T. Mirzaazimova, A. I. Glushenkova, S. A. Usmanov, and Yu. Ikramov, Khim. Prir. Soedin., 285 (1989).
2. H. H. Strain, J. Sherma, and M. Grandolfo, Anal. Chem., No. 8, 926 (1967).
3. M. Bounias, C. Coulomb, Y. Lizzi, and P. J. Coulomb, Chem. Abstr., 111, No. 11, 94014 (1989).
4. C. Litchfield, Fette, Seifen, Anstrichmittel, No. 4, 223 (1973).
5. G. Roughan and R. Slack, Trends Biochem. Sci., No. 9, 383 (1984).
6. S. D. Gusakova, S. G. Yunusova, and A. I. Glushenkova, Khim. Prir. Soedin., 31 (1992).
7. S. D. Gusakova, I. I. Vinokurov, and A. U. Umarov, Khim. Prir. Soedin., 288 (1981).
8. Yu. I. Ikramov, D. A. Musaev, S. M. Berdiev, S. A. Usmanov, Methodological Instructions for an Express-Temperature Method for the Early Evaluation and Selection of Cotton Plants for Resistance to Verticillium Wilt [in Russian], Ministry of Agriculture of the Uzbek SSR, Tashkent (1981).
9. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972).
10. S. G. Yunusova, I. P. Nazarova, S. D. Gusakova, and A. I. Glushenkova, Khim. Prir. Soedin., 319 (1980).
11. T. V. Khimova, D. T. Asilbekova, S. D. Gusakova, and A. U. Umarov, Khim. Prir. Soedin., 279 (1983).
12. I. G. Zenkevich and B. V. Ioffe, The Interpretation of the Mass Spectra of Organic Compounds [in Russian], Leningrad (1986), p. 152.
13. R. P. Eganhouse, E. C. Ruth, and I. R. Kaplan, Anal. Chem., 55, No. 13, 2120 (1983).
14. A. J. Aasen, H. H. Hofstetter, B. T. Iyengar, and R. T. Holman, Lipids, 6, No. 7, 502 (1971).
15. A. Kornfeldt and L.-B. Croon, Lipids, No. 5, 306 (1981).
16. T. Itoh, T. Tamura, and T. Matsumoto, Lipids, No. 3, 173 (1974).
17. T. Itoh, T. Tamura, and T. Matsumoto, J. Am. Oil Chemists' Soc., No. 8, 300 (1973).
18. H. K. Lichtenthaler, in: Lipids and Lipid Polymers in Higher Plants, M. Tevini and H. K. Lichtenthaler (eds.), Springer, New York (1977), p. 231.
19. A. R. Wellburn, J. Stevenson, F. W. Hemming, and R. A. Morton, Biochem. J., 102, 313 (1967).
20. J. W. Rip, C. A. Rupar, K. Ravi, and K. K. Carroll, Prog. Lipid Res., No. 4, 270 (1985).
21. T. Watanabe, A. Hongu, K. Honda, M. Nakazato, M. Konno, and S. Saitoh, Anal. Chem., 56, No. 2, 251 (1984).
22. F. H. Foppen, Chromatographic Rev., No. 3, 133 (1971).