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DIMERIC STILBENES OF THE WOOD OF Maackia amurensis

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UDC 547.636+547.972

Dimeric stilbenes have been isolated from the wood of Amur maackia for the first time. Two of them have been identified as the known scirpusins A and B. The structure of a new dimeric stilbene with a dioxane linkage, which has been called maackinin, has been established. The PMR and ^{13}C NMR spectra of the substances isolated have been studied in detail.

We have previously reported on the isolation from alcoholic extracts of the hardwood of Amur maackia Maackia amurensis Rupr. et Maxim. of isoflavonoids — formononetin, genistein, and retusin — and two stilbenes — resveratrol and piceatannol [1] and also of an isoflavonostilbene, which was called maackiasin [2]. The substances were designated as M-1, M-2, M-3, M-4, M-5, M-6, respectively.

We have continued the study of the chemical composition of an ethyl acetate fraction of the alcoholic extract of maackia wood and have isolated another three substances, M-7, M-8, and M-9. They proved to be related compounds, and on the basis of spectral properties and other physicochemical characteristics they have been identified as dimeric stilbenes. Oligomeric stilbenes have apparently not hitherto been detected in representatives of the genus Maackia not in the closely related genera Cladrastis and Sophora. At the same time, Japanese scientists have recently found two trimers of the stilbene resveratrol — (−)-α-viniferin and (+)-α-viniferin — in an extract of the roots of the plant Caragana chamalagu, a representative of another legume genus (Caragana) [3].

Since oligomeric hydroxylated stilbenes exhibit a high biological activity, their chemical structure is peculiar, and their functions in plants are not completely clear, we have found it necessary to preface an account of the experimental results with a short review of the literature relating to this group of natural polyphenols.

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Oligomeric stilbenes in the composition of the grape have been studied comparatively recently and in extremely great detail. It has been shown in a number of investigations that monomeric (resveratrol) and oligomeric stilbenes (α-, β-, γ-, and ξ-viniferins) fulfill the function of phytoalexins in the grape [4, 5].

In response to irradiation with ultraviolet, a synthesis from labeled precursors first of resveratrol, then of the dimer ξ-viniferin and, after that of the trimer α-viniferin took place in grapes [6, 7].

On the basis of the experimental facts it was shown that in Vitis vinifera the biosynthesis of stilbenes takes place by the same phenylalanyl polymalonate route as the formation of isoflavonoids and pterocarpans – common phytoalexins of plants of the legume family.

Nakajima et al. [9] isolated from the bulrush Scirpus flaviatilis (Torr.), family Cyperaceae, together with monomeric stilbenes – resveratrol and piceatannol – two new hydroxylated dimers, which they called scirpusins A and B. On the basis of NMR and other physicochemical characteristics, the authors established that the molecule of scirpusin A was constructed of two monomeric units: resveratrol and piceatannol, and that of scirpusin B of two piceatannol molecules. They showed that the substances isolated were not artefacts but were actually biosynthesized by the oxidative coupling of monomeric stilbenes, since the extraction of the plant material with cold methanol in the dark showed the presence of these substances in the extract. The same compounds, and also ξ-viniferin, were found by Powell et al. in the closely related plant Scirpus maritimus. They also found an antileukemic activity of alcohol extracts of the seeds. Bulrush finds use in Chinese folk medicine [10].

In later studies devoted to the investigation of plants of the family Cyperaceae the isolation was reported of – together with ξ-viniferin – a resveratrol trimer, miyabenol C, two tetramers, miyabenol A and B, and a tetramer, kobophenol A, possessing antimicrobial properties [11-13].

Plants of the family Dipterocarpacae are extremely rich in oligostilbenes. A trimer of resveratrol, hopeaphenol [14] has been obtained from Hopea odorata, and a new trimer of resveratrol, which has been called copalliferol, from Vateria copallifera [15]. These three compounds differ by the method of linkage of the resveratrol structural units.

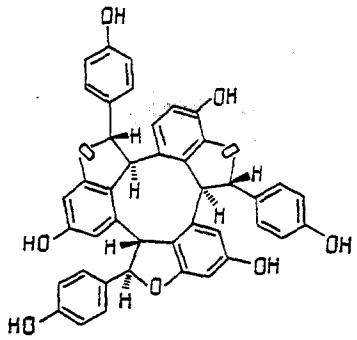
Subsequently, investigations of various plants of the family Dipterocarpaceae were continued, and new resveratrol trimers were isolated; copalliferol B and its isomer stemonoporol [16-18], and also vaticaffinol, constructed of two ξ-viniferin molecules [19].

We must also mention dimeric and trimeric derivatives of resveratrol – gnetins, F, G, H, and I – isolated from the plant Welwitshia mirabilis [20]. Just like gnetins C and E, they could be formed by the oxidative coupling of two and three molecules of resveratrol.

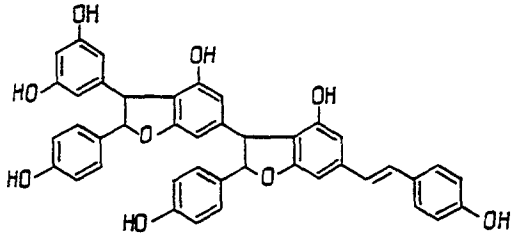
The molecules of the oligomeric stilbenes described in the above-mentioned literature (Fig. 1) are constructed by means of various types of linkages of the monomeric units: with the aid of furan and of 5-, 6-, 7-, and 9-membered carbon rings. At the same time, we have found no information on oligomeric stilbenes formed with the aid of a 1,4-dioxane linkage, which is characteristic for such plant polyphenols as the flavonolignans and neolignans, and the isoflavonostilbene maackiasin isolated from maackia.

Investigation of the Ethyl Acetate Fraction. The material was obtained from an alcoholic extract of maackia hardwood after the elimination of the lipid components with hexane, as described in [1]. In the lower, polar, section of the TLC of this fraction (below substance M-6), on treatment with ferric chloride and sulfuric acid, the spots of three substances were detected, and these have been designated as M-7, M-8, and M-9, in order of decreasing R_f value (Fig. 2). A mixture of these substances was obtained by column chromatography on silica gel using various solvents: from this mixture, by repeated separation, it was possible to isolate the most polar substance, M-9. After purification of the residue by gel chromatography on Sephadex LH-20, part of the material was separated by reversed-phase chromatography on silanized silica gel C-18 in aqueous ethanol, which led to the individual substances M-7 and M-8.

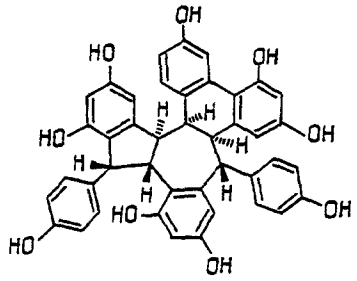
The UV spectra of all three substances practically coincided with the spectra of resveratrol and piceatannol. The mass spectra did not contain the peaks of the molecular ions, but the peak of an ion with m/z 244 showed the participation of a tetrahydroxystilbene



α-Viniferin



Gnetin E



Copalliferol A

Fig. 1

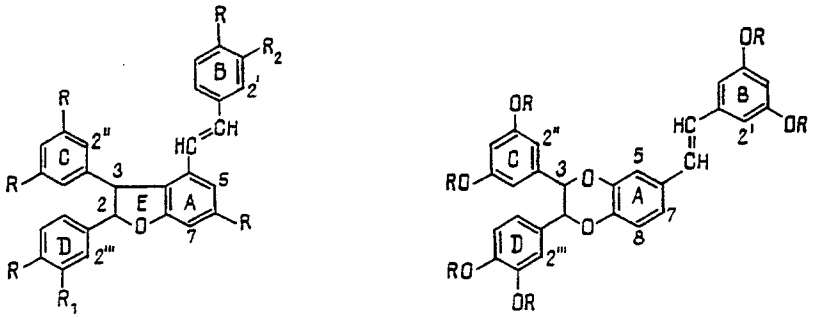
fragment in the structure of each of them. The presence of hydroxy groups was confirmed by their IR and PMR spectra. Hence, it was possible to assume that substances M-7, M-8, and M-9 were polyhydroxylated stilbenes.

To obtain more informative spectra, the substances were converted into their acetates. The mass fragmentation of the acetates of M-7 and M-9 coincided completely with the fragmentation of the acetates of scirpusins A and B [10], which enabled them to be regarded as dimeric stilbenes.

The PMR spectrum of compound M-7 showed the presence of two characteristic doublets at 4.37 and 5.26 ppm with an SSCC of 5.6 Hz, corresponding to two protons of a dihydrofuran ring at the carbon atoms in the third and second positions, and in the ^{13}C NMR spectrum of M-7 that of signals at 55.4 and 92.4 ppm, belonging to the carbon atoms of a dihydrofuran ring (C-3 and -2), which completely confirmed the dihydrofuran linkage of the monomeric units in the M-7 molecule.

Doublet signals in the PMR spectrum at 6.59 and 6.82 ppm with the SSCC 16 Hz corresponded to protons at a double bond present in the trans position relative to one another, and a singlet with an integral intensity corresponding to the three protons of ring C at 6.03 ppm agreed with a triplet at 6.94 ppm having an SSCC of 2 Hz (1 H) and a doublet at 7.02 ppm with an SSCC of 2 Hz (2 H) for the same protons in the acetate of M-7. Furthermore, a well-defined doublet at 6.23 ppm with an SSCC of 2 Hz and a doublet of doublets at 6.56 ppm with SSCCs of 2 and 8 Hz (1 H) related to the protons of ring D in positions 2 and 6.

In the 8.8-9.2 ppm interval of the PMR spectrum there were the signals of 6 protons of hydroxy groups. On the basis of the facts given, substance M-7 was identified as scirpusin A (Fig. 2).



I R=OH, $R_1=R_2=H$ ξ-Viniferin

II $R=R_1=OH$, $R_2=H$ M-7

III $R=R_1=OCOCH_3$, $R_2=H$

IV $R=R_1=R_2=OH$ M-9

VII $R=R_1=R_2=OCOCH_3$

V R=H M-8

VI $R=COCH_3$

Fig. 2

In the mass spectrum of the acetate of M-9 it was possible clearly to trace the splitting out of the three acetyl residues (one isolated one and two in the meta position) with the formation of an ion of mass 654, which then split into two large fragments with m/z 326 and 328 after which, for each of them, the two acetyl residues present in the ortho position to one another split out.

The PMR spectrum of M-9, just like the spectrum of M-7, contained the signals of the protons of a dihydrofuran ring at 4.34 and 5.25 ppm with a SSCC of 5.6 Hz, a singlet at 6.03 ppm corresponding to the three protons of ring C, a doublet at 6.51 ppm with a SSCC of 16 Hz (proton at a double bond), and, in the 8.8-9.2 ppm region, the signals of seven protons of hydroxy groups; the signals of eight aromatic protons and that of olefinic protons were represented by a multiplet.

The PMR spectrum of the acetate of M-9 was somewhat better resolved: doublets at 5.08 and 5.68 ppm belonging to the protons of a dihydrofuran ring, a triplet at 6.79 ppm (2 H), probably two degenerate doublets with a SSCC of 2 Hz, each of one proton, belonging to rings B and D; a triplet at 6.90 ppm (1 H) and a doublet at 7.01 ppm (2 H) — the protons of ring C. For ring A a single doublet was revealed at 7.80 ppm (1 H) with a characteristic SSCC of 1.7 Hz.

The PMR spectra obtained for M-7 and M-9 and those of their acetates agreed well with the PMR spectra of ξ-viniferin and of scirpusins A and B, the most fully interpreted being the spectra of ξ-viniferin while in the spectra of scirpusins A and B most of the signals of the aromatic protons appeared in the form of multiplets.

Compound M-8 was investigated both in the form of the native substance and in that of its acetyl derivative. The mass of the molecular ion of its acetate proved to be greater than the mass of the M-7 acetate by 16 units, i.e., by one oxygen atom, and less than the mass of the M-9 acetate by 42 units, i.e., by one acetate group.

These facts, and also the UV and IR spectra permitted the assumption that the M-8 molecule was constructed of two molecules of a tetrahydroxystilbene and contained six free hydroxy groups and two neutral oxygen atoms. Our hypotheses were confirmed fairly convincingly by the scheme of the fragmentation of the M-8 acetate. First the splitting out of four acetyl residues present in the meta positions in rings B and C took place with the formation of an ion with mass of 570 which then broke down into two fragments with masses of 328 and 242, with the subsequent splitting out of two acetyl groups present in the ortho position in the fragment with a mass of 328 and the formation of two ions with m/z 244 and 242 corresponding to a tetrahydroxystilbene ion.

The PMR and ^{13}C NMR spectral results also confirmed the presumed structure of the M-8. Its PMR spectrum differed from those of M-7 and M-9 by the absence of two doublets with SSCCs of 5.6 Hz, corresponding to the protons of a dihydrofuran ring, but at the same time two doublets appeared with close values of the chemical shifts, 4.87 and 4.95 ppm, and a SSCC of 8 Hz, which showed the dioxane linkage of the two tetrahydroxystilbene structural units.

TABLE 1. Chemical Shifts (δ) of the C Atoms in the ^{13}C NMR Spectra of ξ-Viniferin, of Compounds M-7, M-8, and M-9, and of their Acetylated Derivatives

Ring	Atom	Compound					
		I	II	III	IV	V	VI
A	1	135,8	134,7	134,6	134,8	130,6	130,8
	2	103,6	103,3	111,8	103,3	115,0	115,0
	3	157,0	158,5	151,8	158,2	138,2	138,2
	4	96,2	95,9	113,1	95,7	143,2	143,5
	5	161,8	161,6	159,7	160,6	116,6	116,4
	6	119,2	118,2	124,5	113,0	119,3	111,1
B	1	129,3	127,9	134,1	128,4	128,8	129,7
	2	128,1	127,5	127,5	113,6	114,5	117,0
	3	115,7	115,4	122,0	145,3	157,7	150,
	4	157,6	158,3	155,2	145,0	142,0	114,4
	5	115,7	115,4	122,0	115,4	157,7	150,7
	6	128,1	127,5	127,6	115,7	104,5	117,0
E	2	93,4	92,4	91,1	92,3	79,2	77,3
	3	56,6	55,4	53,9	55,2	79,5	74,1
—C=C—	1*	129,5	128,9	130,0	129,4	127	130,0
	2*	122,4	122,1	123,7	122,0	125,0	125,1
C	1	147,3	145,1	144,8	145,0	143,7	143,5
	2	105,4	105,5	118,5	115,2	105,1	118,8
	3	159,3	158,5	151,2	158,3	153,3	151,3
	4	101,5	101,1	114,9	101,0	142,6	117,4
	5	159,3	158,5	151,2	153,3	153,3	151,3
	6	106,4	105,5	118,5	105,2	106,1	118,8
D	1	133,2	132,5	138,9	132,6	127,2	134,5
	2	127,3	112,7	120,9	112,6	114,2	120,8
	3	115,5	145,1	142,0	145,8	144,7	142,3
	4	157,6	145,9	142,0	145,0	145,2	142,0
	5	115,5	115,4	124,0	115,4	115,1	123,6
	6	127,3	116,7	124,0	116,7	119,6	125,6

*Assignment of the signals uncertain.

The presence in the molecule of two benzene rings symmetrically substituted by two hydroxyls (rings B and C) could be judged from a singlet at 6.09 ppm (3 H), belonging to ring C and also a triplet at 6.13 ppm (1 H) and a doublet at 6.42 ppm (2 H), each with a SSCC of 2 Hz, corresponding to three protons of ring B.

Two doublets of doublets at 6.50 and 7.11 ppm with SSCCs of 2 and 8 Hz, and also doublets at 6.66 and 7.20 ppm with a SSCC of 2 Hz each related to the protons of rings A and D. Two aromatic and two olefinic protons were represented in the spectrum in the form of a multiplet.

The protonic spectrum of the acetate of M-8 was uninformative, since the bulk of the aromatic hydrogen atoms gave signals in the spectrum in the form of multiplets. The two protons of the dioxane ring were represented in the form of a broad singlet at 5.33 ppm.

The structures of substances M-7, M-8, and M-9 were also confirmed by their ^{13}C NMR spectra. This is the first time that the spectra of scirpusins A and B and their acetates, and also those of compound M-8 and its acetate, have been obtained.

As a basis we took the ^{13}C spectral characteristics of ξ-viniferin [6, 10] and calculated the theoretical parameters of the acetylated derivative and of the acetylated analogs of scirpusins A and B. Complete coincidence of the calculated values of the chemical shifts was observed for the acetates of scirpusins A and B and the acetates of M-7 and M-9, respectively. In the ^{13}C NMR spectrum of compounds M-7, 28 signals of C-atoms were found, of which four signals in the 145-162 ppm region of resonance corresponded to seven phenolic C atoms, while nine signals in the 95-140 ppm resonance region likewise belonged to aromatic carbon atoms, two doublet signals at 122.1 and 128.9 ppm corresponded to two olefinic C atoms, and doublet signals at 55.4 and 93.2 ppm corresponded to the aliphatic C-atoms of the dihydrofuran ring.

A comparison with the chemical shifts in the ^{13}C NMR spectra of ξ-viniferin and of compound M-7 (Table 1) showed a coincidence of the chemical shifts of the signals of the

C atoms of rings A, B, C, and E, while for ring D the difference was considerable. Ring D of ξ-viniferin had two doubled signals of C atoms at 115.5 and 127.3 ppm, which showed the symmetry of its substitution in positions 1 and 4. The spectrum of compound M-7 showed the signals of all six aromatic C-atoms of ring D, which indicated a disturbance of the symmetry of substitution (see Table 1). According to empirical rules of the influence of OH substituents, such values of the chemical shifts correspond to the signals of the C atoms of an aromatic ring having hydroxy groups in positions 3 and 4. This conclusion was confirmed by the ^{13}C NMR spectrum of the acetate of M-7 which permitted an unambiguous assignment to be made of the signals by using the difference in the influence of hydroxy and acetyl groups on the chemical shifts of the C atoms according to their positions in the aromatic ring.

In the ^{13}C NMR spectrum of compound M-9, the characteristic signals of C atoms of a dihydrofuran ring were observed at 92.3 and 55.2 ppm, the signals of the C-atoms of aromatic ring C substituted by hydroxy groups in positions 3 and 5 at 105.2 (2 C) and 101.0 ppm, and those of ring D substituted by hydroxyls in positions 3 and 4, at 112.6, 145.8, and 145.0 ppm, and also the signals of the C atoms of ring A condensed with a dihydrofuran ring at 95.7, 103.3, and 160.6 ppm. A comparison of the ^{13}C NMR spectra of compounds M-7 and M-9 revealed the presence of an additional signal of a phenolic C-atom and the absence from the spectrum of M-9 of signals characteristic for a 1,4-substituted aromatic ring. At the same time, characteristic signals appeared with chemical shifts of 113.6, 145.0, and 145.3 ppm corresponding, as shown above, to the signals of the C-atoms of ring D. The results obtained showed that compounds M-7 and M-9 differed only by an additional hydroxy ring in ring B, which confirmed the identity of M-9 and scirpusin B (Fig. 2).

In the ^{13}C NMR spectrum of compound M-8 (as also in those of M-7 and M-9), 28 signals of C atoms were observed. Thus, at the same time, the characteristic signals of the aliphatic C atoms of a dihydrofuran ring were absent and the signals of an aliphatic C-atom were observed at 79.2 and 79.5 ppm with a SSCC J_{C-H} = 155.3 ppm, which corresponded only to the C-atoms of a dioxane ring. Analysis of the spectrum permitted the identification of the signals of the C atoms of the two aromatic rings B and C, symmetrically substituted with hydroxyls in positions 3 and 5, at, respectively, 102.1 and 104.5 ppm (2 C) and at 102.6-106.1 ppm (2 C), and the signals of the C atoms of one ring (D) having hydroxyls in positions 3 and 4 with chemical shifts of 114.2, 145.2, and 144.7 ppm. Furthermore, the spectrum lacked the signal at 160 ppm of a phenol C atom of ring A participating in the formation of a dihydrofuran ring and characteristic for the spectra of compounds M-7 and M-9.

A comparison of the ^{13}C NMR spectra of the initial M-8 with that of its acetate enabled the signals of aromatic ring A to be singled out (130.6, 138.2, 143.2 ppm – singlets; 115.0, 116.6, and 119.3 ppm – doublets) and also confirmed the conclusion of the presence of two symmetrically substituted rings and one ring having hydroxyls in positions 3 and 4.

Two signals in a relatively strong magnetic field at 115.0 (d) and 116.6 (d) ppm and a signal at 130.6 ppm may correspond to a 1,3,4-substituted aromatic ring, as has been confirmed by calculation.

Thus, on the basis of the analysis performed, the structure in Fig. 2 is proposed for compound M-8, and the name maackinin.

The structures of the dimeric stilbenes of maackia determined on the basis of the spectral characteristics that we have obtained are not absolutely reliable. The facts used do not permit an unambiguous placement of rings C and D with respect to the dihydrofuran or the dioxane ring. We hope to eliminate this indeterminacy with the aid of an x-ray structural analysis of single crystals.

EXPERIMENTAL

Melting points were determined on a Boëtius stage (not corrected). UV spectra were taken on a Cary–Varian 219 instrument in ethanol, and IR spectra on a Specord R-75 instrument in KBr. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer in DMSO-d_6, using TMS as internal standard, at working frequencies of 250 MHz for ^{1}H and 62.9 MHz for ^{13}C, and mass spectra on a LKB-9000S instrument at ionizing energies of 15 and 70 eV.

For column chromatography we used L-type silica gel, and for TLC Silufol plates. In TLC we used the toluene–acetate–formic acid (10:8:2) system, the revealing reagents being a 10% solution of ferric chloride and sulfuric acid in ethanol.

The wood was gathered in 1989 (Southern Maritime Territory). For separation by column chromatography we used 130 g of the ethyl acetate fraction of an alcoholic extract of the wood.

M-7, $C_{28}H_{22}O_7$, gray-green precipitate, UV $\lambda_{max}^{ethanol}$, nm: 290 sh, 305 sh, 320; IR ν_{max}^{KBr}, cm^{-1}: 3388 (br. sing., OH), 1608, 1508, (arom. and –C=C–); ^1H NMR (δ, DMSO-d_6); 4.37 (1H, d, J = 5.6 Hz, C_3–H), 5.26 (1H, d, J = 5.6 Hz, C_2–H), 6.03 (3H, s, $C_{2'',4'',6''}$–H), 6.23 (1H, d, J = 2 Hz, $C_{2'''}$–H), 6.56 (1H, dd, J = 2 Hz, J = 8 Hz, $C_{6'''}$–H), 6.58 (1H, d, J = 16 Hz, $C_{\alpha(\beta)}$–H), 6.64-6.72 (4H, m., arom.–H), 6.82 (1H, J = 16 Hz, $C_{\beta(\alpha)}$–H), 7.13 (2H, d, J = 8 Hz, $C_{2',6'}$–H), 8.81-9.23 (6H, br. s., OH). Mass spectrum: m/z 244, 137.

The hexaacetate of M-7, $C_{40}H_{34}O_{13}$, colorless needles (alcohol–water), mp 173-175°C, UV-$\lambda_{max}^{ethanol}$, nm: 292, 311; ^1H NMR (δ, DMSO-d_6): 2.20-2.30 (18H, 6 × OAc), 5.08 (1H, d, J = 5.6 Hz, C_3–H), 5.77 (1H, d, J = 5.6 Hz, C_2–H), 6.78 (1H, d, J = 2 Hz, $C_{2'''}$–H), 6.88 (1H, d, J = 16 Hz, $C_{\alpha(\beta)}$–H), 6.94 (1H, t, J = 2 Hz, $C_{4''}$–H), 7.02 (2H, d, J = 2 Hz, $C_{2'',6''}$–H), 7.05 (1H, d, J = 16 Hz, $C_{\beta(\alpha)}$–H), 7.08 (2H, d, J = 8 Hz, $C_{3',5'}$–H). Mass spectrum: 722 (M^+), 680 ($M^+ - CH_2CO$), 638 (680 – CH_2CO), 596 (638 – CH_2CO), 554 (596 – CH_2CO), 328 (554 – 226), 286 (328 – CH_2CO), 244 (286 – CH_2CO), 215, 137, 123, 107, 43.

M-8, $C_{28}H_{22}O_8$, gray-green precipitate, UV $\lambda_{max}^{ethanol}$ nm: 290, 305 sh., 320; IR, ν_{max}^{KBr}, cm^{-1}: 3388 (br. sing., OH), 1608, 1508 (arom. and –C=C–); ^1H NMR (δ, DMSO-d_6): 4.87 (1H, d, J = 8 Hz, C_3–H), 4.93 (1H, d, J = 8 Hz, C_2–H), 6.09 (3H, s, $C_{2'',4'',6''}$–H), 6.13 (1H, t, J = 2 Hz, $C_{4'}$–H), 6.42 (2H, d, J = 2 Hz, $C_{2',6'}$–H), 6.50 (1H, dd, J = 2 Hz, J = 8 Hz, $C_{7(6''')}$–H), 6.66 (1H, d, J = 2 Hz, $C_{2'''(5)}$–H), 6.58-6.97 (5H, m, arom.–H), 7.11 (1H, dd, J = 2 Hz, J = 8 Hz, $C_{6'''(7)}$–H), 7.20 (1H, d, J = 2 Hz, $C_{5(*''')}$–H). Mass spectrum 244, 137.

Hexaacetate of M-8, $C_{40}H_{34}O_{14}$, colorless needles (alcohol–water); mp 92-94°C, UV $\lambda_{max}^{ethanol}$, nm: 290, 322; ^1H NMR (δ, DMSO-d_6): 2.20-2.30 (18H, 6 × OAc), 5.33 (2H, br. s., $C_{2,3}$–H), 6.87-7.32 (14H, m, arom.–H, $C_{\alpha,\beta}$–H). Mass spectra: 738 (M^+), 696 ($M^+ - CH_2CO$), 654 (696 – CH_2CO), 328 (654 – 326), 286 (328 – CH_2CO), 284 (326 – CH_2CO), 244 (286 – CH_2CO), 242 (282 – CH_2CO), 215, 137, 123, 107, 43.

M-9, $C_{28}H_{22}O_8$, gray-green precipitate, UV $\lambda_{max}^{ethanol}$, nm: 288, 306 sh., 328; IR λ_{max}^{KBr}, cm^{-1}: 3388 (br. sing., OH), 1608, 1508 (arom. and –C=C–); ^1H NMR (δ, DMSO-d_6): 4.34 (1H, d, J = 5.6 Hz, C_3–H), 5.25 (1H, d, J = 5.6 Hz, C_2–H), 6.03 (3H, s, $C_{2'',4'',6''}$–H), 6.25 (1H, d, J = 2 Hz, arom.–H), 6.33 (1H, d, J = 2 Hz, arom.–H), 6.51 (1H, d, J = 16 Hz, $C_{\alpha(\beta)}$–H), 6.55-7.00 (7H, m, arom.–H, $C_{\beta(*)}$–H), 8.81-9.23 (7H, OH). Mass spectrum: 244, 242, 137.

The heptaacetate of M-9, $C_{42}H_{36}O_{15}$, colorless needles (alcohol–water); mp 95-99°C, UV $\lambda_{max}^{ethanol}$, nm: 302, 310; ^1H NMR (δ, DMSO-d_6): 2.20-2.30 (21H, 7 × OAc), 5.08 (1H, d, J = 5.6 Hz, C_3–H), 5.68 (1H, d, J = 5.6 Hz, C_2–H), 6.79 (2H, t, J = 2 Hz, $C_{2',2'''}$–H), 6.84 (1H, d, J = 16 Hz, $C_{\alpha(\beta)}$–H), 6.90 (1H, d, J = 2 Hz, C^*_{m}–H), 7.01 (2H, d, J = 2 Hz, $C_{2'',6''}$–H), 7.08 (1H, d, J = 17 Hz, $C_5(*)$–H), 7.05-7.33 (7H, m, arom.–H, $C_{\beta(\alpha)}$–H). Mass spectrum: 780 (M^+), 738 ($M^+ - CH_2CO$), 696 (738 – CH_2CO), 654 (696 – CH_2CO), 328 (654 – 326), 286 (328 – CH_2CO), 284 (326 – CH_2CO), 244 (286 – CH_2CO), 242 (282 – CH_2CO), 215, 137, 123, 107, 43.

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

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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.

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