SUMMARY

1. Umbelliferone 7-O-[O-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside, 8-(2',3'-dihydroxy-3'-methylbutyl)-7-β-D-glucopyranosyloxycoumarin, and diosmetin 7-O-β-D-glucopyranoside have been isolated from the epigeal parts of Phlojodicarpus villosus and Ph. sibiricus.

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A NEW ISOFLAVONOSTILBENE FROM THE HEARTWOOD OF Maackia amurensis

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UDC 547.636+547.972

A new isoflavonostilbene which has been called maackiasin has been isolated from an ethanolic extract of the heartwood of Maackia amurensis and its structure has been established by spectral methods. The maackiasin molecule is constructed of fragments of the isoflavone retusin and of 3,3',4',5-tetrahydroxystilbene linked by a diether bond. The dioxane grouping connecting the two fragments is constructed from the hydroxyls at C-7 and C-8 of the isoflavone and the vinyl grouping of the stilbene. Maackiasin is the first representative of a new type of natural polyphenols.

We have previously reported the isolation of three isoflavones – formononetin, genistein, and retusin – and two stilbenes – resveratrol and 3,3',4',5-tetrahydroxystilbene – from the ethyl acetate fraction of an ethanolic extract of the wood of maackia (Maackia amurensis Rupr. et Maxim) growing in Maritime Territory [1].

Among the phenolic compounds isolated there was also a substance M-6 corresponding on its elementary composition to the formula $C_{30}H_{22}O_9$ and issuing from a chromatographic column after the substances mentioned above. In the present paper we give the results of the determination of the structure of this compound.

Compound M-6 was optically inactive. No fluorescence was observed under UV, which indicated the absence of a stilbene structure in the molecule of this compound. The ^{13}C NMR spectrum (Table 1) contained the signals of 30 carbon atoms, which correspond to the formula

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Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 39-42, January-February, 1986. Original article submitted March 18, 1985.

0009-3130/86/2201-0035$12.50

TABLE 1. Chemical Shifts in the ^{13}C NMR Spectrum of Compound M-6

Carbon atom	Chemical shift, ppm	Carbon atom	Chemical shift, ppm
2	153,1	1″	132,8
3	124,0	2″	106,4
4	174,7	3″	158,1
5	123,3	4″	103,0
6	118,4	5″	158,1
7	145,8	6″	106,4
8	137,7	1‴	119,6
9	146,0	2‴	115,3*
10	114,9	3‴	145,0†
1′	126,6	4‴	147,6†
2′	130,2	5‴	115,7*
3′	113,7	6‴	116,9
4′	159,1	CH	79,7
5′	113,7		
		CH	79,7
6′	130,2	OCH_3	55,7

*and †) Assignment of the chemical shifts ambiguous.

Note. The assignment of the signals was made on the basis of literature information [2, 3].

TABLE 2. Mass Spectra of Retusin (M-4), of 3,3',4',5-Tetrahydroxystilbene (M-5), and of Compound M-6

Compound	Ion													
M-4	284	283	269		—	241	—	—	—	—	152	132	124	117
%	100	25,5	11,3			8,2					38,8	24,5	7,7	16,0
M-5	—	—	—	245	244	243	227	226	198	197	173	—	—	—
%	—	—	—	14,7	100,0	8,4	10,0	6,3	12,6	18,4		—	—	—
M-6	284	283	269	245	244	241	227	198	197	173	152	132	124	117
%	100,0	18,2	11,7	3,0	23,9	4,5	23,0	3,0	4,5	3,1	48,4	28,8	5,9	7,6

calculated from the results of elementary analysis. The molecular ion was not detected in the mass spectrum of M-6 but there were peaks characteristic for the fragmentation of the isoflavone retusin, together with peaks corresponding to the fragments of a tetrahydroxystilbene (Table 2). When the ionizing voltage was lowered from 70 to 12 V, only two ions, with m/z 244 and 284, remained in the mass spectrum.

A comparison of the PMR spectra of retusin, 3,3',4',5-tetrahydroxystilbene, and the substance under investigation likewise showed the presence of resonance maxima of the first two compounds in the spectrum of substance M-6. A difference from the total identity of the positions and splittings of the signals of the protons in the PMR spectra of the compounds mentioned was a shift of the protons of the stilbene vinyl group upfield from 6.80 ppm (d, J = 16 Hz) and 6.99 ppm (d, J = 16 Hz) to 5.20 and 5.25 ppm, and also a shift of the signals for C-5 and C-6 of ring A of the isoflavone downfield by 0.17 and 0.18 ppm (Table 3). GLC of the reaction mixture obtained on the silylation of compound M-6 (TMCS—HMDS, 1:1), showed the presence of a single peak issuing from the chromatographic column at 290°C; the PMR spectrum of the substance isolated showed a signal at δ 0.24 ppm with an integral intensity corresponding to the presence of four TMS groups. The acetylation of M-6 [acetic anhydride—pyridine (1:1)] also gave a single product, with mp 238°C (from ethanol), having the mass spectrum m/z: 694 (M^+), 652 (M^+-CH_2CO), 610, 568, 526, 412, 370, 328, 286, 294, 269, 245, 244, 227, 226, 198, 152, 132. The subsequent splitting off of four molecules of ketene both from the molecular ion and from the ion with m/z 412, corresponding to an acetylated stilbene fragment, indicated that the stilbene hydroxyls were retained in the composition of the substance M-6 and did not participate in the formation of the bond with the isoflavone mol-

TABLE 3. Chemical Shifts in the PMR Spectra of Retusin (M-4), of Tetrahydroxystilbene (M-5), and of Compound M-6

Proton	M-4			M-5			M-6		
	δ, ppm	multiplicity	J,Hz	δ, ppm	multiplicity	J, Hz	δ, ppm	multiplicity	J, Hz
H-2	8,5	s	—				8,55	s	—
H-5	7,60	d	9				7,77	d	9
H-6	7,08	d	9				7,26	d	9
H-2′ H-6′	7,64	dd	2;9				7,64	dd	2;8
H-3′ H-5′	7,12	dd	2;9				7,15	dd	2;8
OCH_3	3,92	s	—				3,91	s	—
H-α(β)				6,80	d	16	5.20	d	8
H-β(α)				6,99	d	16	5,25	d	8
H-2″				6,50	d	2	6,25*	d	2
H-4″				6,23	t	2	6,27	t	2
H-6″				6,50	f	2	6,28*	d	2
OH-3″ OH-5″				9,35	br.s	—	9,43	br.s	—
H-2‴				7,05	d	2	6,83	d	2
H-5‴				6,82	d	8	6,76	d	8
H-6‴				6 93	dd	2;8	6,68	dd	2;8
OH-3‴				9,05†	br.c	—	9,05†	br.s	—
OH-4‴				9,25†	br.c	--	9,25†	br.s	—

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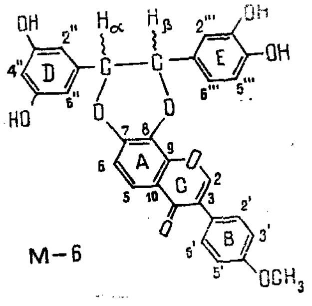
*and †) Assignment of the signals ambiguous. Solvent DMSO-d_6; TMS) 0.

Abbrevation: s) singlet; d) doublet; dd) doublet of doublets; t) triplet.

Note. The numbering of the protons of the compounds is given in the formula of M-6 shown.

ecule. The PMR spectrum of M-6 (see Table 3) contained proton signals identical in the values of their chemical shifts with the signals of the nuclear protons and the methoxyl of ring B of the isoflavone and the proton at C-2 of ring C. Consequently, these fragments were also retained in the composition of the substance M-6. Thus, on the formation of the molecule under consideration from the presumed precursors — the isoflavone and the stilbene — the bond between them could be realized with the participation of the two hydroxyls at C-7 and C-8 of the isoflavone and the carbon atoms of the vinyl group of the stilbene, i.e., through the formation of a diether bond with the loss of two hydrogen atoms. An experimental proof of the presence of a diether bond was obtained by cleaving substance M-6 with boron bromide, which led to the isolation of retusin and the product of its demethylation.



M-6

To establish the complete structure of substance M-6, which we propose to call maackiasin, it remained to determine the locations of the differently substituted aromatic rings D and E in relation to positions 7 and 8 of ring A, and also the spatial configuration of the molecule. With this aim we performed an x-ray structural investigation of a single crystal of maackiasin, the results of which will be reported later.

Dioxane structures similar to that which has been found in maackiasin have been detected with increasing frequency among plant polyphenols in recent years. They have been found in silybin and its analogs [4, 5], in hydnocarpin [6], in rhodiolin [7], etc. However, between these compounds — the so-called flavonolignans — and maackiasin there is a substantial difference consisting in the fact that in place of the C_6—C_3 fragment (usually coniferyl alcohol), an isoflavone and a polyhydroxylated stilbene participate in the formation of the substance that we have detected. Maackiasin can be considered as the first representative of a new type of natural compound compound among polyphenols.

EXPERIMENTAL

The characteristics of the initial plant material, the conditions of isolating the substance M-6 and the instrumental technique have been described in [1].

Compound M-6 formed white acicular crystals with mp 243.5°C (dioxane). In TLC on Silufol, it gave a spot stained yellow-green by diazotide sulfanilic acid and violet by $FeCl_3$. Its elementary composition was 67.99% C; 4.49 H. Calculated for $C_{30}H_{22}O_9$, 68.40% C and 4.18% H. The UV spectrum in methanol contained the maximum at 259 mm that is characteristic for isoflavones and also a weak broad maximum at 290 nm which experienced a bathochromic shift of 6 nm on the addition of $AlCl_3$. IR spectrum, cm^{-1}: ν_{max}^{KBr} 3400-3250, 1624, 1607, 1569. In the carbonyl region of the spectrum taken in DMSO a maximum appeared at 1641 cm^{-1}, which indicated the participation of a CO group in the formation of an intermolecular hydrogen bond which was cleaved under the action of DMSO.

To confirm the presence of the diether bond in the structure of the compound under investigation, 52 mg of M-6 in 20 ml of CH_2Cl_2 was cooled to −20°C and a solution of 0.6 ml of BBr_3 in 5 ml of CH_2Cl_2 was added. The temperature was brought to that of the room, and after 16 h the mixture was diluted with water. The products were extracted with methyl ethyl ketone and the extract was dried over Na_2SO_4, and it was separated on a column of Sephadex LH-20 [10 × 240 nm; $CHCl_3$—methyl ethyl ketone (9:1)]. This yielded retusin (TLC, melting point, mass spectrum), and 4,7,8-trihydroxyisoflavone (demethylated retusin) with Rf 0.17 [Silufol, $CHCl_3$—methyl ethyl ketone (3:1)]; mass spectrum: m/z 270 (M^+) 271, 269, 153, 152, 124, 118. The second fragment of the M-6 molecule could not be detected under these conditions; other than the isoflavones mentioned above, only a brown polymeric product was isolated.

SUMMARY

A new isoflavonostilbene has been isolated from the heartwood of Maackia amurensis and has been called maackiasin; it is constructed of fragments of the isoflavone retusin and of 3,3',4',5-tetrahydroxystilbene linked by a diether bond.

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