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STRUCTURES OF TWO NEW FLAVANONES FROM *Vexibia alopecuroides*

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Two new flavanones — vexibinol and vexibidin — have been isolated from the chloroform fraction of an ethanolic extract of the roots of *Vexibia alopecuroides* by column chromatography on silica gel. Their structures have been established on the basis of chemical and spectral characteristics. Their IR, UV, ^1H, and ^{13}C NMR, and mass spectra are given.

We have reported the isolation of four flavonoids from the roots of *Vexibia alopecuroides* (L) Yakovl. (*Sophora alopecuroides*) [1]. Continuing this investigation, from the chloroform fraction of an alcoholic extract we have isolated two new flavonoids which have been called vexibinol and vexibidin. Vexibinol, $C_{25}H_{28}O_6$ (I), M^+ 424, gives positive reactions with $FeCl_3$ solution and with magnesium in hydrochloric acid. Its IR spectrum has the absorption bands of hydroxy groups (3366 cm^{-1}), of a carbonyl group (1632 cm^{-1}), and of aromatic C=C bonds (1604, 1519 cm^{-1}). The UV spectrum of vexibinol ($\lambda_{max}^{C_2H_5OH}$ (nm) 293, 340 sh; log ε 4.23, 3.69) is characteristic for flavanones [2]. The results of a study of spectra taken in the presence of CH_3COONa, $AlCl_3$, and CH_3ONa showed the presence of phenolic hydroxy groups at C-7, C-5, and C-4'.

The acetylation of (I) with acetic anhydride in pyridine gave an acetyl derivative (II) the PMR spectrum of which showed the signals of the protons of the methyls of four Ar—$OCOCH_3$ groups (Table 1). Consequently, vexibinol contains four phenolic hydroxy groups, and the signals of their protons appeared at 9.37, 9.63, 10.67, and 12.13 ppm (C_5—OH) in the PMR spectrum of (I) taken in DMSO-d_6.

The presence of the characteristic signals of the H-2 and H-3 protons in the spectrum confirmed that (I) was flavanone [2, 3]. Furthermore, signals of four aromatic protons and the protons of the side chain were detected in the spectra (Table 1). According to the PMR and ^{13}C NMR spectra (Table 2), the side chain of (I) had the structure of 2-isopropenyl-5-methylhex-4-enyl. Flavanones and chalcones having such a side chain are rare but have been isolated from some species of plants of the genera *Sophora* and *Ammothamnus* [4-6]. In the mass spectrum of vexibinol there are the peaks of ions with m/z 163 and 136, which shows the presence of two phenolic hydroxy groups in ring B [3, 7].

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TABLE 1. Chemical Shifts of the Protons of Vexibinol (I), Vexibidin (III), and Their Acetyl Derivatives (δ scale, ppm)

	Solvent	H-2	H-3	Positions of the proton										
				H-8	H-3′	H-5′	H-6′	2H-1″	H-4″	2H-10″	H-2″; 2H-3″	=C–CH_3	–OCH_3	Ar–$OCOCH_3$
I.	DMSO –d_6	5,52 q (13; 3 Hz)	2,59 q (17,4; 3 Hz) 3,12 q (17,4; 13 Hz)	5,96 s	6,36 d (2,4 Hz)	6,28 q (8; 2,4 Hz)	7,23 d (8 Hz)	1,94 m	4,92 m	4,51 br.s 4,58 br.s	2,45 m	1,46; 1,55 1,59 br.s	—	—
I.	Py–d_5	6,00q (13; 5 Hz)	2,83–3,23 m	6,30 s	6,77 br.s	6,73 q (8,4; 2,5 Hz)	7,58 d (8,4 Hz)	2,23 m	5,10 m	4,67 br.s 4,77 br.s	2,92 m	1,43 (2×CH_3) 1,74 br.s	—	—
II.	$CDCl_3$	5,45q (12; 4,5 Hz)	2,42–2,95* m	6,45 s	6,91 br.s	6,98 q (8,5; 2,2 Hz)	7,57 d (8,5 Hz)	1,97 m	4,85 m	4,38 br.s 4,53 br.s	2,42– 2,95*	1,44 1,53 (2×CH_3)	—	2,23 (3×CH_3) 2,29
III.	DMSO –d_6	5,54 q (11,8; 3 Hz)	2,62 q (17,4; 3 Hz) 3,15 q (17,4; 11,8 Hz)	5,96 s	6,46 d (2,2 Hz)	6,36 q (8,2; 2,2 Hz)	7,32 d (8,2 Hz)	1,94 m	4,90 m	4,49 br.s 4,57 br.s	2 45 m	1,45; 1,54 1,59 br.s	3,73	
	Py–d_5	5,72 q (12; 3,7 Hz)	2,62–3,22 m	6,26 s	6,62 br.s	6,68 q (8,5; 2 Hz)	7,51 d (8,5 Hz)	2,19 m	5,07 m	4,65 br.s 4,71 br.s	2,86 m	1,42; 1,47 1,71 br.s	3,55	
IV.	$CDCl_3$	5,65	2,47–2,92* m	6,43 s	6,74 br.s	6,66 q (8,2; 2,4)	7,48 d (8,2 Hz)	1,97 m	4,81 m	4,42 br.s 4,53 br.s	2,47– 2,92*	1,42 1,56 (2×CH_3)	3,65	2,22 (2×CH_3) 2,29

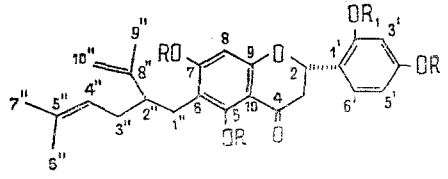
The signals denoted by asterisks in the horizontal rows are superposed upon one another. The signals of methyl groups have a singlet nature. The spectra in DMSO-d_6 were taken with the use of TMS as internal standard; in the other cases 0 – HMDS. Abbreviations: s) singlet; d) doublet; q) quartet; m) multiplet; br.s) broadened singlet.

TABLE 2. Characteristics of the ^{13}C NMR Spectra of the Flavanones in DMSO-d_6 (δ scale, ppm, 0 — TMS)

Carbon atom	Euchrestaflavanone B [11] (the signals of the ring carbon atoms are given)	Vexibinol	Vexibidin	2-Isopropenyl-5-hex-4-enol [12]	Multiplicity
C-2	74,1	73,7	73,4		*d*
C-3	41,6	41,4	41,2		*t*
C-4	197,1	194,4	196,5		*s*
C-5	161,2	160,8	160,9		*s*
C-6	106,9	106,2	106,2		*s*
C-7	164,3	164,4	164,5		*s*
C-8	95,1	94,9	95,0		*d*
C-9	160,2	160,5	160,4		*s*
C-10	101,8	101,4	101,4		*s*
C-1′	118,1	115,7	116,9		*s*
C-2′	153,2	155,2	157,2		*s*
C-3′	102,3	102,2	98 8		*d*
C-4′	155,5	158,0	158,7		*s*
C-5′	115,5(*S*)	106,1	106,8		*d*
C-6′	127,3	127,3	127,4		*d*
C-1″		30,7	30,7	64,0	*t*
C-2″		46,2	46,2	50,0	*d*
C-3″		26,5	26,5	28,6	*t*
C-4″		123,1	123,1	122,5	*d*
C-5″		130,4	130,3	132,2	*s*
C-6″		17,6	17,5	17,8	*q*
C-7″		25,7	25,4	25,7	*q*
C-8″		147,5	147,5	145,6	*s*
C-9″		18,6	18,5	19,7	*q*
C-10″		110,5	110,5	112,6	*t*
—OCH_3		—	55,1	—	*q*

The UV spectra and also the values of the chemical shifts and the nature of the splitting of the signals of the aromatic protons of ring B (H-3', H-5', and H-6') show that the hydroxy groups are located at C-2' and C-4'. This was confirmed by the formation of resorcinol as a result of the alkaline cleavage of vexibinol. The side chain could occupy the C-6 or the C-8 position in ring A, since the PMR spectrum of (I) showed a one-proton singlet at 5.96 ppm due to H-6 or H-8. An unambiguous assignment of this signal from the value of the chemical shift is difficult. In the ^{13}C NMR spectrum of vexibinol there are signals with δ 73.7 and 41.4 ppm due to the ^{13}C nuclei of C-2 and C-3 of the flavanone skeleton. The signals of the C-6 and C-8 carbons in the spectrum of naringenin appear at δ 96.3 and 95.4 ppm, respectively [8].

The values of the chemical shifts of the ^{13}C signals of C-6 (δ 106.1 ppm) and C-8 (δ 94.9 ppm) in the spectrum of vexibinol show the position of the side chain at C-6. A comparison of the ^{13}C NMR spectrum of (I) with that of sophoraflavanone B [9] and those of euchrestaflavanones A, B, and C [10, 11] confirmed the conclusion drawn. The assignment of the signals of the other carbon atoms of (I) was made on the basis of its spectra obtained under the conditions of complete and partial (off-resonance) decoupling from protons, and also by a comparison of them with spectra of aringenin, euchrestaflavanone B, and 2-isopropenyl-5-methylhex-4-enol [12]. The results of the assignment of the signals in the ^{13}C NMR spectra are given in Table 2.



I. $R = R_1 = H$ III. $R = H$, $R_1 = CH_3$

II. $R = R_1 = COCH_3$ IV. $R = COCH_3$, $R_1 = CH_3$

Since, according to the PMR spectrum, ring B occupies the equatorial position ($J_{H_2H_{ax}}$ = 13 Hz), the positive Cotton effect at 327 nm (Δε + 1.8) and the negative effect of 289 nm (Δε—17.3) in the circular dichroism spectrum show that (I) has the 2S configuration [13]. Thus, structure (I) describes vexibinol.

Vexibidin, $C_{26}H_{30}O_6$ (III), M^+ 438, also, according to its UV spectrum ($\lambda_{max}^{C_2H_5OH}$ (nm); 293, 339; log ε 4.33, 3.64) belongs to the flavanone group. Its IR spectrum contains strong

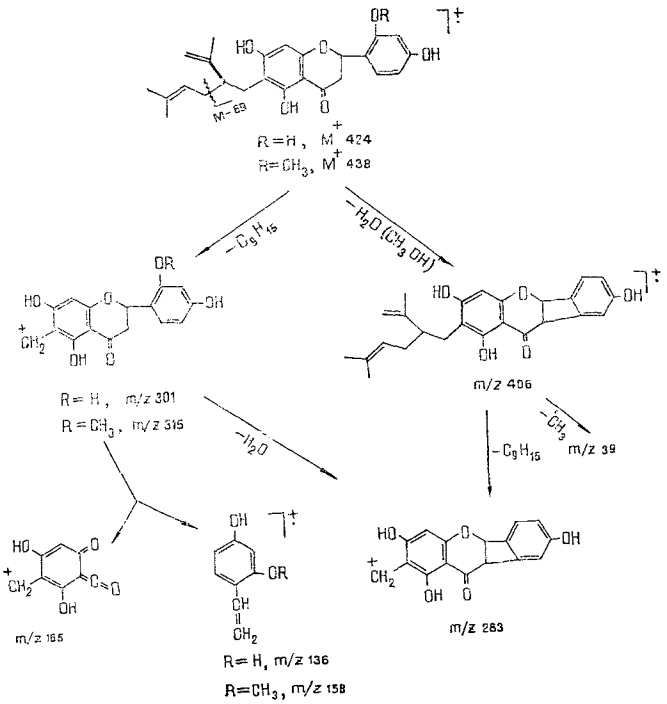
absorption bands at (cm^{-1}) 3384-3275 (OH groups), 1638 (C=O), and 1598 and 1510 (aromatic C=C bonds). Vexibidin gave a positive reaction with $FeCl_3$ solution and formed a triacetyl derivative (IV). The PMR spectrum of (III) confirmed that it belonged to the flavanone group and differed from the spectrum of (I) by the presence of an additional signal of the protons of a methoxy group (δ 3.73 ppm). This permitted the assumption that in vexibidin, unlike (I), one of the hydroxy groups is methylated. According to the mass spectrum of (III), containing the peaks of ions with m/z 177 and 150, the methoxy group is present in ring B.

The presence of phenolic OH groups at C-5, C-7, and C-4' was established by a study of the UV spectra of (III) taken in the presence of ionizing and complex-forming additives. Since there is a hydroxyl at C-4', the CH_3O group must occupy the C-2' position. This was confirmed by the results of a comparative study of the chemical shifts of the H-3', H-5', and H-6' protons in the PMR spectra (Table 1) and of the ^{13}C nuclei of C-1', C-2', and C-3' in the ^{13}C NMR spectra of vexibinol and vexibidin. As can be seen from Table 2, for (I) δ C-2' is 155.1 ppm, and for (III) it is 157.2 ppm. In view of the fact that the replacement of OH by OCH_3 leads to a paramagnetic shift of the α-carbon atom by approximately 2 ppm, it can be stated with confidence that the $-OCH_3$ group in vexibidin is present at C-2'. The side chain in the molecule of (III) is likewise located at C-6, since the signals of the ^{13}C nuclei of C-6 and C-8 appear at 106.3 and 95.0 ppm, respectively.

When (III) was subjected to Adams hydrogenation, tetrahydrovexibidin (V) was obtained with the composition $C_{26}H_{34}O_6$, M^+ 442. In the PMR spectrum of (V) the signals of three olefinic protons had disappeared and the signals of the protons of the methyls of two isopropyl groups and of methylene groups had appeared. These facts confirm the structure of the side chain of (III). The results of a study of the circular dichroism spectrum ($\Delta\varepsilon_{330}$ +1.8; $\Delta\varepsilon_{290}$ −19.07) showed that (III) also has the 2S absolute configuration [13]. Consequently, vexibidin has structure (III).

Position isomers of vexibinol and vexibidin — norkurarinone and isokurarinone — differing in the position of the side chain (at C-8 in the latter two) have been isolated from *Sophora angustifolia* Sieb. et Zucc. and *Sophora flavescens* Ait., respectively [4, 5]. This shows that vexibinol and norkurarinone, and also vexibidin and isokurarinone, are formed in plants as the result of the cyclization of the corresponding chalcones. Depending on which hydroxy groups participate in the cyclization reaction (C-2' or C-6' of the chalcone), either (I) and (III) or norkurarinone and isokurarinone are formed.

It is interesting to note that vexibinol and vexibidin have negative, and norkurarinone and isokurarinone, positive, specific rotations.



Fragmentation of vexibinol and vexibidin in mass spectrometry.

It has been established that the main direction of fragmentation of flavanones in mass spectrometry is retrodiene decomposition [3, 7]. However, as the results of a study of the mass spectra of (I) and (III) have shown, the first act in the fragmentation of these flavanones is the successive breakdown of the side chain with the formation of the ions M − 69 and M − 123. The subsequent retrodiene decomposition of the latter leads to ions with m/z 165, 150, and 136. The mass spectrum of (I), unlike that of (III) contains the strong peak of an ion with m/z 406 arising as the result of the splitting out of H_2O from the molecular ion, as is confirmed by the corresponding metastable peak.

The splitting out of water from the molecular ion in mass spectrometry is also characteristic for euchrestaflavanones B and C [11], each of which contains a hydroxy group at C-2'. In the spectrum of vexibidin, in which the above-mentioned hydroxy group is methylated, in place of the M − H_2O ion a M − CH_3OH ion appears with a low intensity. Consequently, the elimination of H_2O and of CH_3OH takes place at the expense of the C_2'—OH and C_2'—OCH_3 groups and one of the hydrogen atoms at C-3. The ion with m/z 406 so formed then passes with the ejection of a C_9H_5 fragment at the expense of the side chain into an ion with m/z 283. The latter can also be formed as the result of the ejection of H_2O by an ion with m/z 301, which is also confirmed by the corresponding metastable peak.

EXPERIMENTAL

General Remarks. For column chromatography we used 100/250 μ silica gel (Chemapol, Czechoslovakia). Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates. Solvents of the chloroform—methanol system in the following ratios were used: 9:1 (system 1); 19:1 (system 2); 96:4 (system 3); and 98:2 (system 4). The flavonoids were detected by spraying the plates with a 1% solution of vanillin in concentrated sulfuric acid. Mass spectra were recorded on an MKh-1310 instrument at an ionizing voltage of 50 V, IR spectra on a UR-20 spectrometer in KBr, and UV spectra on a Hitachi EPS-3T spectrometer. PMR spectra were taken on JNM-C60-H and JNM-4H-100/100 MHz instruments (0 — HMDS), and ^{13}C NMR spectra on a Varian XL-200 instrument in DMSO-d_6 (0 — TMS). The circular dichroism curve was measured on a Jasco-20 spectropolarimeter.

Isolation of the Flavonoids. The air-dry comminuted roots of *Vexibia alopecuroides* (10 kg) gathered in October 1981 in the Akdar'ya region of Samarkand province were exhaustively extracted with ethanol at room temperature. The extract was evaporated to 3 liters, diluted with water (1:1), and extracted successively with petroleum ether, chloroform, ethyl acetate, and butanol. The chloroform extract, after the solvent had been distilled off, yielded 290 g of combined extractives. Part of the dry residue (100 g) was chromatographed on a column of silica gel (1500 g). On elution with system 4, fractions 66-89 were combined and were rechromatographed on a polyamide column to give 0.91 g of glabrol, while fractions 90-95 yielded 1.20 g of vexibidin. Then 0.15 g of isobavachin (fractions 100-102), 3.39 g of vexibinol (fractions 111-117), and 0.065 g of ammothamnidin (fractions 131-140) were isolated. When the elution of the column was continued with system 3, 1.27 g of trifolirhizin was obtained.

Vexibinol (I). $C_{25}H_{28}O_6$, mp 174-176°C (from methanol), $[\alpha]_D^{20} - 36.5 \pm 2^\circ$ (c 1.1; CH_3OH); $\lambda_{max}^{C_2H_5OH}$ (nm): 293, 340 sh. (log ε 4.23, 3.69); +CH_3COONa: 293, 335; +$AlCl_3$: 241, 310; +CH_3ONa: 243, 333. Mass spectrum, *m/z* (%): M^+ 424 (15), 409 (3.5) 408 (3), 407 (6), 406 (19), 391 (7), 389 (3), 363 (7), 338 (4), 337 (11), 302 (18), 301 (76.5), 284 (24), 283 (100), 219 (13), 166 (5), 165 (37), 139 (5), 137 (4.5), 136 (6), 124 (7), 123 (9), 109 (12), 107 (5.5), 106 (9). Circular dichroism (c 0.11, methanol): Δε + 16.4 (220 nm), Δε − 17.3 (289 nm), Δε + 2.49 (312 nm) Δε + 1.8 (327 nm).

Vexibinol Tetraacetate (II). Vexibinol (40 mg) was acetylated with 1 ml of acetic anhydride in 0.5 ml of pyridine at room temperature for 2 h. The reaction mixture was poured into ice water, and the resulting precipitate was filtered off and recrystallized from acetone to give compound (II) with the composition $C_{33}H_{36}O_6$, mp 69-71°C, ν_{max}^{KBr} cm^{-1}: 1770, 1218-1188 (ester group); 1692 (C=O in a ring); 1607 (aromatic C=C bonds).

Alkaline Cleavage of (I). A solution of 400 mg of (I) in 100 ml of 50% caustic soda solution was heated in the water bath in an atmosphere of nitrogen for 3 h. The reaction mixture was cooled, diluted with water, acidified with 10% sulfuric acid, and extracted with ether. The ethereal extract was washed with 5% sodium carbonate solution and then with water and was dried with anhydrous sodium sulfate, and the solvent was evaporated off. The residue was separated on a column of silica gel with elution by chloroform. This gave a

substance with mp 108-109°C identical with resorcinol according to TLC and the absence of a depression of a mixed melting point.

Vexibidin (III), $C_{26}H_{30}O_6$, mp 157-158°C, $[\alpha]_D^{20}$ −43.6 ± 2° (*c* 1.07; CH_3OH), $\lambda_{max}^{C_2H_5OH}$ (nm): 293, 339 sh. (log ε 4.33, 3.64); +CH_3COONa: 294, 340; +$AlCl_3$: 316; +CH_3ONa: 243, 333. Mass spectrum, *m/z* (%): M^+ 438 (8,5), 315 (77,5), 285 (3), 219 (17), 203 (2), 191 (9), 177 (8), 166 (14,6), 165 (100), 151 (22.6), 150 (10,7), 137 (9), 135 (15), 123 (12), 121 (4). Circular dichroism (c 0.084, methanol): Δε +15.8 (226 nm); Δε −19.07 (290 nm), Δε +2.7 (313 nm); Δε +1.8 (330 nm).

Vexibidin Triacetate (IV). A solution of 40 mg of (III) in 1 ml of pyridine was treated with 2 ml of acetic anhydride and the mixture was left at room temperature for 24 h. After the usual working up, the triacetate (III), $C_{34}H_{36}O_9$, was obtained with mp 76-77°C; ν_{max}^{KBr}, cm^{-1}: 1772, 1218-1190 (ester group); 1691 (C=O in a ring); 1609 (aromatic C=C bonds).

Tetrahydrovexibidin (V). A solution of 70 mg of (III) in 15 ml of ethanol was treated with 25 mg of PtO_2 and the mixture was shaken in a current of hydrogen for 3 h. Then it was filtered, the filtrate was evaporated, and the residue was recrystallized from benzene. This gave compound (V) with the composition $C_{26}H_{34}O_6$, mp 168-170°C. Mass spectrum: *m/z* (%): M^+ 442(14), 315(24), 314(100), 290(13), 284(10), 190(10), 165(78,5), 150(16,5), 136(11), 134(7,5), 122 (6.5). PMR spectrum (Py-d_5, ppm): 0.71 (6 H, 5.5 Hz, $-CH(CH_3)_2$); 0.94 (6 H, d, 5 Hz, $-CH(CH_3)_2$); 1.22 (4 H, m, $-CH_2-CH_3$); 2.61-3.11 (4 H, m, $Ar-CH_2$, 2H-3); 3.58 (3 H, s, $-OCH_3$); 5.82 (1 H, q, 11.2 and 4.5 Hz, H-2); 6.39 (1 H, s, H-8); 6.75 (1 H, br.s, H-3'); 6.82 (1 H, q, 8.5 and 2.5 Hz, H-5'); 7.64 (1 H, d, 8.5 Hz, H-6').

SUMMARY

The new flavanones vexibinol and vexibidin have been isolated from the roots of *Vexibia alopecuroides* and their structures have been established on the basis of chemical and spectral characteristics.

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