STRUCTURE OF A NEW CHALCONE AMMOTHAMNIDIN FROM *Ammothamnus lehmanni*

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A new chalcone ammothamnidin with the composition $C_{25}H_{28}O_4$, mp 112-114°C $[\alpha]_D^{20}$ +4.5° (methanol) has been isolated from the epigeal part and roots of *Ammothamnus lehmanni* Bge. On the basis of chemical transformations and IR, UV, ^1H and ^{13}C NMR, and mass spectra it has been shown that ammothamnidin has the structure of 2,2',4'-trihydroxy-3'-(2"-isopropenyl-5"-methylhex-4"-enyl)chalcone.

Ammothamnus lehmannii Bge. (family Fabaceae) is a shrub growing in the Karakumy and Kyzylkumy of Central Asia [1]. Decoctions of the roots are used in folk medicine for the treatment of rheumatism [2]. A number of quinolizidine alkaloids have been isolated from this plant, and it contains pigmentary substances [4] the nature of which has not so far been established.

From the chloroform fraction of an ethanolic extract of the roots by absorption chromatography on a column of silica gel a new crystalline substance of phenolic nature has been isolated with the composition $C_{25}H_{28}O_4$ (M^+ 392, 1987) having mp 112-114°C, $[\alpha]_D^{20}$ +4.5° (c 0.22; MeOH), which we have called ammothamnidin (I). The IR spectrum of (I) contains absorption bands at (cm^{-1}) 3150-3430 (OH groups), 1625 (conjugated C=O), 1615, 1557, and 1522 (C=C bonds of an aromatic system).

On the basis of qualitative reactions [5] and its UV spectrum [6] [$\lambda_{max}^{ethanol}$ (nm): 232 sh, 261 sh, 321 sh, 390 (log ε 4.18, 3.99, 4.01, 4.46)], we have assigned (I) to the chalcone derivatives.

The presence in the PMR spectrum of (I) (CD_3OD) of the signals of two trans-olefinic protons at 7.59 and 7.99 ppm (1 H each, doublet of doublets, J = 15.5 Hz) confirmed that the substance belonged to the chalcone group [7]. The spectrum lacked the signals of the protons of methoxy groups. On acetylation with acetic anhydride in the presence of pyridine, (I) formed a triacetyl derivative (II), in the PMR spectrum ($CDCl_3$) of which there were the signals of the protons of the methyls of three aromatic acetyl groups (δ 2.21, 2.23, and 2.25 ppm, each with an intensity of 3 H, s). Consequently, ammothamnidin contains three phenolic hydroxy groups.

The spectrum recorded in DMSO-d_6 shows the signal of the proton of a $C_{2'}$—OH group at 14.1 ppm (1 H, broadened singlet), and the signals of the protons of another two phenolic hydroxyls in the 9.70-10.30 ppm region. The presence of a hydroxy group at $C_{2'}$ was also confirmed by the bathochromic shift of the first band in the UV spectrum by 48 nm on the addition of $AlCl_3$. The long-wave shift of this band (Δλ 108) with an increase in its intensity when the UV spectrum was recorded in the presence of sodium methanolate shows the location of a hydroxy group in ring B.

To elucidate its structure, the chalcone was subjected to cleavage with 50% KOH solution in an atmosphere of nitrogen. A reaction product was isolated from the acid fraction and was identified by comparison with an authentic sample as salicylic acid.

Consequently, the second hydroxy group is present in position C-2 of the chalcone.

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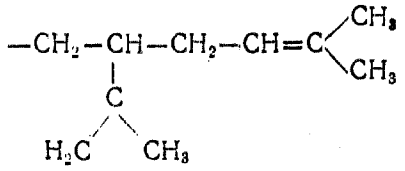
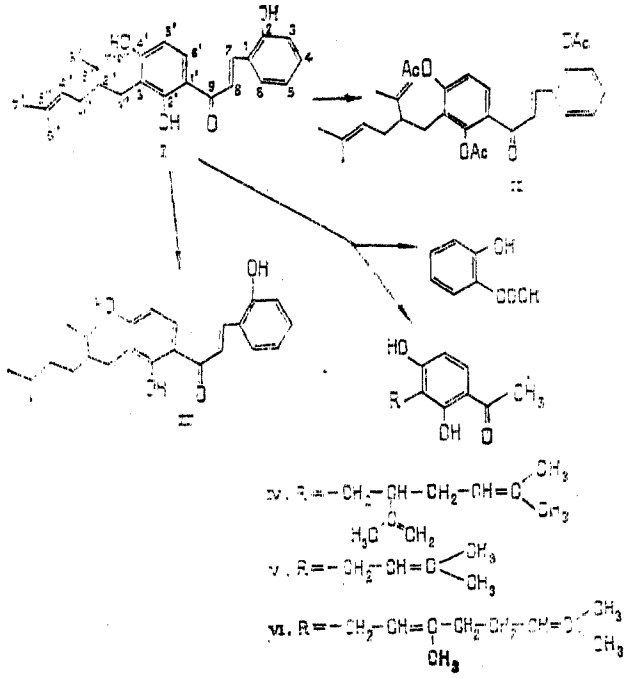
Judging from its composition and mass and PMR spectra, ammothamnidin must contain a side chain consisting of ten carbon atoms. The PMR spectrum of (II) shows the signals of the protons of three vinylmethyl groups at 1.49, 1.56, and 1.66 ppm (3 H and broadened singlet each), of a terminal methylene group (4.42 and 4.56 ppm, 1 H each, broadened singlets) of an olefinic proton (4.94 ppm, 1 H, multiplet), and of Ar—C<u>H</u>$_2$— (2.63 ppm, 2 H, doublet J = 6.5 Hz) and also a three-proton multiplet in the 1.85-2.18 ppm region. The presence of two double bonds in the side chain of (I) was confirmed by the formation of a hexahydro derivative on Adams hydrogenation.

The facts presented, together with the optical activity, show that (I) contains a lavandulyl side chain having the structure of 2-isopropenyl-5-methylhex-4-enyl:



Such a side chain is found among the chalcones and flavanones isolated from some species of *Sophora* [8, 9].



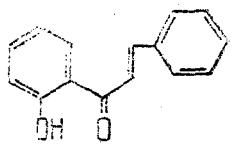
From the neutral fraction of the products of alkaline cleavage of (I) was isolated an acetophenone derivative (IV) with the composition $C_{18}H_{24}O_3$ (M^+ 288), λ_{max} (nm) 217, 235 sh, 289, 319 sh. Its PMR spectrum ($CDCl_3$) showed the signals of the protons of a Ar—CO<u>CH</u>$_3$ group (2.45 ppm, 3 H, singlet), of two ortho-aromatic protons at 6.22 and 7.35 ppm (1 H, d, each 8.8 Hz), and also the signals of the protons of the above-mentioned side chain. The characteristics of its UV and PMR spectra were close to those of compounds (V) and (VI) [10, 11] which were also obtained by the cleavage of chalcones. Thus, the product of the cleavage of (I) has the structure (IV).

Hence, the structure of ammothamnidine must be 2,2',4'-trihydroxy-3'-(2"-isopropenyl-5"-methylhex-4"-enyl)chalcone (I). The assumed structure is completely confirmed by the ^{13}C NMR spectrum.

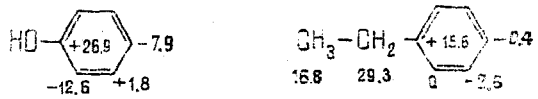
In the ^{13}C NMR spectrum there are 24 signals from 25 carbon atoms (two ^{13}C signals have coincident chemical shifts). The signals from 6 sp^3 hybridized carbon atoms of the side chain appear in the 17-46 ppm region, and between 102 and 191 ppm are observed the signals from 18

sp^2-hybridized carbon atoms, including those from three carbon atoms of a flavonoid skeleton bearing hydroxy groups at (ppm) 161.5 (s), 162.5 (s), and 164.1 (s), and from one carbonyl atom at 191.0 (s).

The literature [12] has information on the chemical shifts of a chalcone with the structure



Taking into account the contributions of the substituents OH and C_2H_5 to the chemical shifts of the ^{13}C nuclei of the aromatic ring

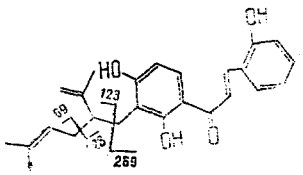


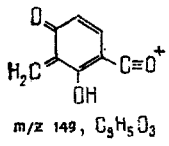
and using literature information for chalcones of similar structure [12-14] and of unsaturated hydrocarbons with a branched chain [15, 16], and also with the aid of calculations by an additive scheme of the influence of substituents on the chemical shifts of aromatic carbon atoms [17], an assignment has been made of the signals of all the carbon nuclei in the spectrum of ammothamnidin (in DMSO-d_6):

C	δ, ppm	C	δ, ppm	C	δ, ppm
1	113.9	1′	112.6	3″	26.9
2	161.5	2′	162.5	4″	123.4
3	107.0	3′	113.4	5″	130.2
4	130.5	4′	164.1	6″	17.6
5	108.0	5′	102.5	7″	25.5
6	129.3	6′	130.5	8″	147.7
7	139.7	1″	30.9	9″	18.5
8	115.7	2″	46.0	10″	110.7
9	191.0				

It is known that under the conditions of mass spectrometry chalcones isomerize into the corresponding flavanones, and their fragmentation takes place in two directions [18-20].

In the mass spectrum of ammothamnidin the peaks of ions corresponding to the usual fragmentation pathways of chalcones have a low intensity, which is probably connected with the presence of the long side chain. Ions with m/z 323 (M − 69), 269 (M − 123), and 268 (M − 124) are formed by the splitting out of the side chain from ammothamnidin.





m/z 149, $C_9H_5O_3$

Hydrocarbon ions with the composition C_8H_{13} (109.1035) and C_9H_{16} (124.1256) are formed from the side chain of (I).

Ammothamnidin has also been isolated from the epigeal part of *Ammothamnus lehmannii*.

EXPERIMENTAL

General Observations. For column chromatography we used silica gel of type L 100/150 μm. Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates in the solvent systems 1) acetone–hexane (1:1) and 2) chloroform–methanol (8:2). UV spectra were recorded on an

EPS-3T spectrophotometer, and IR spectra on a UR-20 instrument in KBr tablets. PMR and ^{13}C NMR spectra were obtained on a Varian XL-200 spectrometer in DMSO-d_6 (0 – TMS) and a JNM-4H-100 spectrometer with HMDS as internal standard. Mass spectra were recorded on a MKh-1303 instrument at an ionizing voltage of 40 eV and a temperature of 160-180°C. Elementary compositions were measured on a MKh-1310 mass spectrometer.

Isolation of Ammothamnidin. The comminuted and dried roots of *Ammothamnus lehmannii* (9.5 kg) were extracted eight times with ethanol at room temperature. The solvent was distilled off in vacuum. The concentrated ethanolic extract was diluted with distilled water (1:1) and was extracted successively with petroleum ether (yield of extract 38 g), chloroform (668 g), ethyl acetate (22 g), and butanol (52 g).

The chloroform extract (120 g) was chromatographed on a column of silica gel (1:15). The substances were eluted with hexane, with hexane–chloroform in various proportions, with chloroform, and with chloroform–methanol with increasing concentrations of the latter. The fraction obtained on elution with chloroform–methanol (95:5) was rechromatographed on a column of silica gel with elution by the acetone–hexane (1:2) and (1:1) system. The eluates were distilled and the residue was recrystallized from acetone–hexane (1:1). In this way 0.5 g of ammothamnidin was isolated.

Ammothamnidin (I), $C_{25}H_{28}O_4$, mp 112-114°C (from acetone–hexane); R_f 0.56 (system 1), $[\alpha]_D^{20}$ +4.5° (c 0.22; MeOH), $FeCl_3$ (+).

UV spectrum, $\lambda_{max}^{C_2H_5OH}$ nm: 232 sh., 261 sh., 321 sh., 390 (log ε 4.18, 3.99, 4.01, 4.46). IR spectrum, ν_{max}^{KBr} (cm^{-1}): 3510-3430 (OH), 1625 (conjugated C=O), 1615, 1557, 1522 (aromatic C=C bonds).

PMR spectrum (Py-d_5): 1.45; 1.50; 1.80 (3 H, br.s, each, 3 =C–CH_3); 2.12-2.37 (2 H, m –CH_2–CH=); 2.80-3.20 (3 H, m, Ar–CH_2–CH); 4.65 and 4.75 (1 H, br.s, each, >C=CH_2); 5.13 (1 H, =CH); 6.53 (1 H, d, 8 Hz, H-5'); 6.64 (3 H, m, H-3,4,5); 7.64 (1 H, d, 8 Hz, H-6); 7.87 (1 H, d, 9 Hz, H-6'); 8.00 (1 H, d, 16 Hz, H_α); 8.69 (1 H, d, 16 Hz, H_β).

PMR spectrum (CD_3OD): 1.49; 1.56; 1.66 (3 H, br.s, each, 3 =C–CH_3; 2.01 (2 H, m, >CH–CH_2–CH=); 2.62 (2 H, m, Ar–CH_2); 6.30 (4 H, m, H-3,4,5,5'); 7.40 (d, 9 Hz, H-6'), 7.63 (1 H, br.d, 8 Hz, H-6); 7.59 (1 H, d, 15.5 Hz, H_α); 7.99 (1 H, d, 15.5 Hz, H_β).

Mass spectrum, m/z (%): M^+ 392 (6), 323 (2), 269 (3), 268 (2), 149 (12), 135 (8), 125 (8), 124 (56), 110 (24), 109 (76), 96 (12), 94 (17), 91 (15), 82 (12), 81 (43), 79 (21), 71 (16), 69 (34), 67 (100), 57 (22), 55 (42), 53 (18), 41 (63).

Acetylation of (I) to Give (II). A solution of 50 mg of (I) in 1 ml of pyridine was treated with 2 ml of acetic anhydride and the mixture was left at room temperature. After 24 h, the acetyl derivative was isolated in the usual way; $C_{31}H_{34}O_7$, R_f 0.7 (system 1).

PMR spectrum ($CDCl_3$): 1.52 (3 H, br.s, =C–CH_3); 1.61 (6 H, br.s, 2 =C–CH_3); 2.21, 2.23, 2.25 (each 3 H, s, 3 Ar–OCOCH_3); 1.85-2.18 (3 H, m, >CH_2–CH); 2.63 (d, J = 6.5 Hz, Ar–CH_2); 4.42 and 4.56 (1 H, br.s, each, >C=CH_2); 4.94 (1 H, m, =CH); 7.00 (1 H, d, 16 Hz, H_α); 7.48 (1 H, d, 16 Hz, H_β); 6.90-7.05 (4 H); 7.36-7.62 (2H).

Hydrogenation of (I) to Give (III). In the presence of PtO_2 100 mg of (I) in 30 ml of ethanol was hydrogenated for 3 h, and the reaction product was dissolved in chloroform and chromatographed on a column of silica gel. Elution with carbon tetrachloride gave hexahydro-(I)(III) with the composition $C_{25}H_{34}O_4$, M^+ 398; R_f 0.65 (system 1), $FeCl_3$ (+).

Alkaline Cleavage of (I). A solution of 200 mg of (I) in 50% caustic potash solution was boiled in an atmosphere of nitrogen for 4 h. The reaction mixture was acidified with 5% sulfuric acid solution and extracted with ether. The ethereal extract was washed with 5% sodium bicarbonate solution. The soda extract was neutralized with 5% sulfuric acid and extracted with ether. The new ethereal extract was washed with distilled water, dried with anhydrous sodium sulfate, and filtered, and the solvent was distilled off. The residue was chromatographed on a column of cellulose. A substance was isolated with mp 156-158°C, R_f 0.73 (system 2), $FeCl_3$ (+) identical with salicylic acid (TLC, IR spectrum).

The residual ethereal solution was washed with 1% hydrochloric acid and with distilled water and was dried with anhydrous sodium sulfate and filtered, and the solvent was distilled off. The residue was recrystallized from hexane, giving substance (IV), $C_{18}H_{22}O_3$, mp 47-49°C, R_f 0.65 (system 2), $FeCl_3$ (+).

UV spectrum: λ_{max} (ethanol), nm: 217, 235 sh., 289, 319. PMR spectrum ($CDCl_3$): 1.50, 1.59, 1.63 (3 H, br.s, each, 3 =C–CH_3); 2.06 (2 H, t, >CH–CH_2–); 2.45 (3 H, s, –$COCH_3$); 2.65 (2 H, d, 7 Hz, Ar–CH_2–CH–); 4.56 (2 H, m, >C=CH_2); 4.98 (1 H, m, =CH); 6.22 and 7.35 (1 H, d, each; J = 8.8 Hz, H-5 and H-6). Mass spectrum, m/z (%): M^+ 288 (21), 286 (M – H_2, 14), 273 (M – CH_3, 22), 271 (286 – CH_3, 10), 246 (7), 245 (22), 243 (6.5), 219 (M – 69, 26), 216 (7.5), 205 (7.5), 203 (12), 201 (7), 177 (9), 166 (22), 165 (100).

SUMMARY

A new chalcone, ammothamnidin, has been isolated from the roots and epigeal part of *Ammothamnus lehmannii* Bge. On the basis of chemical transformations and the results of a study of IR, UV, mass ^1H and ^{13}C NMR spectra, its structure has been established as 2,2',4'-trihydroxy-3'-(2" -isopropenyl-5" -methylhex-4" -enyl)chalcone.

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