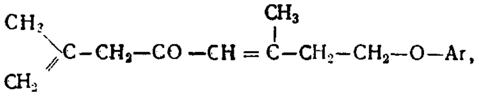
THE STRUCTURE OF DIVERSIN AND DIVERSININ – COUMARINS OF Ferula diversivittata

V. V. Kiseleva, G. K. Nikonov,
and M. O. Karryev

UDC 547.587.53

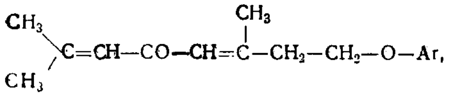
Ferula diversivittata Rgl. et Schmal., family Umbelliferae, is a perennial herbaceous plant growing in Central Asia [1].

From the roots of the plant growing in Kazakhstan we have previously isolated the coumarin diversin with the composition $C_{19}H_{20}O_4$, mp 97-98.5° C (I), which is the ether of umbelliferone and a monoterpene ketone, and we also put forward a hypothesis concerning the presence in diversin of its isomer (II) as an impurity [2]:



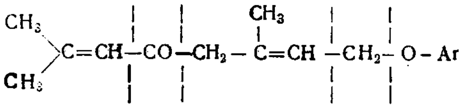
$$\begin{matrix}CH_3 & & \\ & \searrow & \\ & & C-CH_2-CO-CH=\overset{\overset{CH_3}{|}}{C}-CH_2-CH_2-O-Ar, \\ & /\!/ & \\ CH_2 & & \end{matrix}$$

I



$$\begin{matrix}CH_3 & & \\ & \searrow & \\ & & C=CH-CO-CH=\overset{\overset{CH_3}{|}}{C}-CH_2-CH_2-O-Ar, \\ & / & \\ CH_3 & & \end{matrix}$$

II



$$\begin{matrix}CH_3 & & \\ & \searrow & \\ & & C=CH-CO-CH_2-\overset{\overset{CH_3}{|}}{C}=CH-CH_2-O-Ar \\ & / & \\ CH_3 & & \end{matrix}$$

III

where Ar is coumarin-7-yl.

We have investigated the roots of this species of Ferula growing in Turkmenia. By column chromatography on KSK silica gel we have isolated two isomeric coumarins with the composition $C_{19}H_{20}O_4$; one with mp 97-98° C (from aqueous ethanol) (II) and the other with mp 55-57° C (from ethanol) (III). On treatment with a mixture of acetic and sulfuric acids, the substances underwent cleavage, forming umbelliferone, and, consequently, they are derivatives of 7-hydroxycoumarin and monoterpene alcohols of the composition $C_{10}H_{16}O_2$.

The catalytic hydrogenation of the first coumarin over PtO_2 (Adams method) gave a tetrahydro derivative with mp 37-38.5° C (M^+ 316). From the composition and constants of the substance itself and those of its derivative, the first coumarin corresponded to diversin. At the same time, we directed our attention to the fact that the integral intensities of the signals of the protons in the NMR spectrum of the substance were not given in the Brief Communication. This cast doubt on the correctness of the structure suggested for diversin and impelled us to perform additional investigations.

In the IR spectrum of diversin there are absorption bands at 1720 cm^{-1} (coumarin carbonyl), 1615 and 1570 cm^{-1} (aromatic nucleus), and 1380 and 1390 cm^{-1} (gem-dimethyl groups), while absorption bands of hydroxy groups are absent. Considerable broadening of the bands at 1705-1720 cm^{-1} and 1610-1635 cm^{-1} and, in particular, inflections at 1710 and 1625 cm^{-1} gave grounds for assuming the presence in the diversin molecules of a dienone grouping $-C=C-\underset{\underset{O}{\|}}{C}-C-C=$ [3]. The hypothesis of the presence of this chromophore

---

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Institute of Chemistry, Academy of Sciences of the Turkmen SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 344-349, May-June, 1975. Original article submitted March 13, 1974.

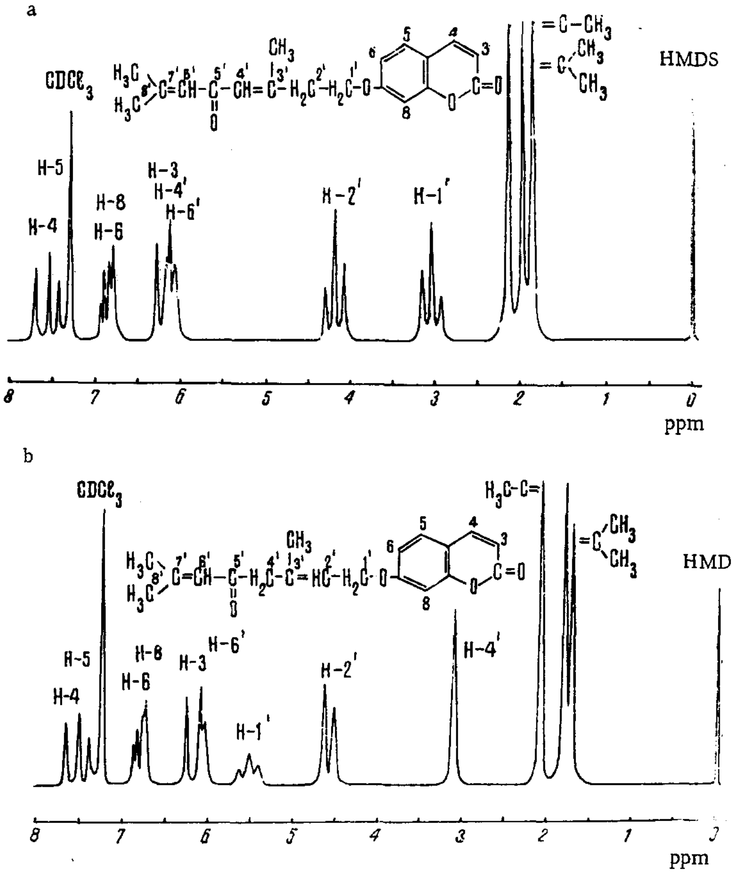


Fig. 1. NMR spectra of diversin (a) and diversinin (b).

was confirmed by the electronic spectrum in which, in addition to the long-wave maximum at λ_{max} 320 nm (log ε 4.29) characteristic of 7-hydroxycoumarin derivatives, there is absorption at 275 nm (log ε 4.43) and 250 nm (inflection) (log ε 4.32).

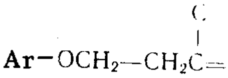
Diversin did not react under the usual conditions with 2,4-dinitrophenylhydrazine, which is characteristic for doubly unsaturated ketones, and on oxidation with permanganate it formed acetone, which was identified by the formation of dibenzylideneacetone.

Thus, diversin is an ether of umbelliferone and a monoterpene hydroxyketone with the composition $C_{10}H_{16}O_2$ containing the fragments $(CH_3)_2C{=}C$ and $-C{=}C-CO-C{=}C-$.

The integral intensities of all the signals in the NMR spectrum of the diversin that we had isolated (Fig. 1) have been given in the Brief Communication. The spectrum showed the signals of the protons of a 7-monosubstituted coumarin – doublets at 7.62 and 6.08 ppm, J = 10 Hz, and at 7.30 ppm, J = 8.5 Hz; quartet at 6.80 ppm, J_1 = 8.5 Hz, J_2 = 2 Hz; and a doublet at 6.73 ppm, J = 2.0 Hz (H-4, H-3, H-5, H-6, and H-8). In addition, three-proton singlets are observed at 2.10, 1.94, and 1.82 ppm, showing the presence of three methyl groups on double bonds; and at 4.15 and 3.05 ppm there are two three-proton triplets with J = 7 Hz of 2H each due to the protons of a $Ar-O-CH_2-CH_2-C{=}$grouping. It follows from these facts that the molecule of the substance has two double bonds located at the $C_{3'}$ and $C_{6'}$ carbon atoms to which methyl groups are attached. The double bond of the first isoprenoid unit is located between $C_{3'}$ and $C_{4'}$. In the 6.02 and 6.10 ppm regions there are two broadened singlets due to the olefinic protons in a $C{=}CH-\underset{\overset{\|}{O}}{C}-CH{=}C$ fragment. The broadening of these signals is due to the allyl interaction of the olefinic protons with the protons of the methyl and methylene groups. A similar chemical shift of olefinic protons in the grouping mentioned has been observed by Bohlmann in the case of the lactones of Laserpitium prutenicum [3]. On the basis of the facts presented, it follows that diversin has the structure (III).

N. P. Kir'yalov [2] has previously suggested that this plant contains a substance with the structure (II). Such a structure is confirmed by the results of the fragmentation of the substance under the influence of electron impact at the positions denoted by the broken lines. The mass spectrum of diversin shows the peak of the molecular ion M^+ 312, and also fragments with m/e 297 ($M^+ - 15$), 163 and 175 (protonated ion of umbelliferone and fragment formed by the α-cleavage of the ether bond with the transfer of hydrogen from the terpene part of the molecule to the coumarin part), 151 and 136 (terpenoid radical and section after the detachment of a methylene group from it), 258, 230, 85, and 57 (fragments formed by the α,β-cleavage of the ketone).

The second coumarin, which we called diversinin, is, according to its UV spectrum [λ_{max} 320 nm (log ε 4.32)] also an ether of umbelliferone, but its terpenoid chain does not contain a dienone grouping (the features of the UV and IR spectra observed for diversin are absent). Its NMR spectrum (Fig. 1, b) shows the signals characteristic for a 7-hydroxycoumarin nucleus: doublets at 7.58 and 6.18 ppm, J = 10 Hz, and at 7.30 ppm, J = 8 Hz, and quartet at 6.8 ppm, $J_1 = 8$ Hz, $J_2 = 2$ Hz, and also a doublet at 6.73 ppm, J = 2 Hz due to the H-4, H-3, H-5, H-6, and H-8 protons, respectively. The spectrum also contains three three-proton singlets at 2.10, 1.81, and 1.72 ppm caused by the protons of methylene groups on double bonds. Their presence shows that the side chain of diversinin has two double bonds present at $C_{2'}$ and $C_{6'}$.

The difference between the NMR spectra of diversin and diversinin consist in the fact that in the second substance there are no triplets due to the methylene protons in a  $Ar-OCH_2-CH_2\overset{\overset{C}{|}}{C}=C$ grouping. Apart from these, the spectrum of diversinin shows a doublet at 4.61 ppm, J = 7 Hz (2H) and a triplet at 5.51 ppm, J = 7 Hz, demonstrating the presence of the $Ar-OCH_2-CH=C$ grouping. Consequently, one of the double bonds in diversinin is located between carbon atoms 2' and 3'. In addition to this, in the spectrum under consideration there are a broadened singlet at 6.05 ppm (1H) corresponding to the olefinic proton in a $C=CH-C=O$ fragment and a two-proton singlet at 3.09 ppm due to the methylene protons in a $O=C-CH_2-C=C$ grouping. On the basis of these facts, it may be concluded that diversinin is a position isomer of diversin with the structure of 7-(3',7'-dimethyl-5'-oxoocta-2',6'-dienyloxy)coumarin (III). Its oxidation by potassium permanganate gave acetone, which was identified as dibenzylideneacetone with mp 92-95° C.

This structure is also well described by the nature of the fragmentation of the substance under the influence of electron impact.

Together with the coumarin mentioned, from the roots of the plant we isolated umbelliferone and β-sitosterol, which were identified by their IR spectra and mixed melting points.

EXPERIMENTAL METHOD

The NMR spectra were taken on a Jeol-60 MHz instrument (in solutions of the substances in $CDCl_3$, δ scale from the signal of HMDS taken as 0); the IR spectra on a UR-10 spectrometer (KBr); the UV spectra on a Hitachi spectrophotometer (solutions of the substances ethanol); and the mass spectra on an MKh-1303 spectrometer. For thin-layer chromatography (TLC) we used type KSK silica gel containing 5% of gypsum in the hexane–benzene–methanol (5 : 4 : 1) and petroleum ether–ethyl acetate (3 : 1) systems. The revealing agent was diazotized sulfanilic acid after the chromatograms had been sprayed with a 10% solution of caustic soda in methanol and 1% vanillin/sulfuric acid.

The results of elementary analysis corresponded to the calculated figures.

Isolation of Diversin. The dried and comminuted roots (5 kg) were exhaustively treated with ethanol (5 × 5 liters) and the extract was evaporated to a viscous residue (1.5 kg). This was diluted with a twofold amount of water and extracted with ether. Distillation of the extract yielded 650 g of substance, 100 g of which was deposited on a chromatographic column containing silica gel (h = 32, d = 9 cm) and was eluted with petroleum ether–benzene (9 : 1), 100-ml fractions being collected. Fractions 6-19 yielded 1.2 g of a light yellow crystalline substance $C_{19}H_{20}O_4$ with mp 98° C (from aqueous ethanol), R_f 0.43, readily soluble in chloroform, benzene, methanol, and acetone, less readily in ether, and insoluble in petroleum ether and water.

Acid Cleavage of Diversin. A solution of 0.2 g of diversin in 3 ml of CH_3COOH and 1 ml of H_2SO_4 was left to stand for 12 h. Then the liquid was diluted with water and made alkaline. An intense blue fluorescence appeared. When the liquid was acidified and extracted with ether, crystals were obtained with mp 288° C which showed no depression of the melting point in admixture with umbelliferone.

Hydrogenation of Diversin. The hydrogenation of 0.2 g of diversin was performed in ethanol in the presence of 70 mg of PtO_2 (Adams). About 30 ml of hydrogen was absorbed, corresponding to two double bonds. The reaction products, obtained in the usual way, contained several substances, and these were separated on a column (H = 25 cm, d = 1.5 cm) containing KSK silica gel with elution by petroleum ether–diethyl ether (1 : 1). This yielded 50 mg of tetrahydrodiversin with mp 37-39° C and umbelliferone with mp 228° C.

Oxidation of Diversin. A solution of 0.1 g of diversin in 8 ml of dioxane was treated with 1 ml of 5% $KMnO_4$ and the mixture was left to stand for 12 h. Then 1 ml of the liquid was distilled off and to it were added four drops of benzaldehyde and two drops of 10% NaOH, and the mixture was left to stand for 12 h. This gave yellowish crystals with mp 92-95° C showing no depression of the melting point in admixture with a sample of dibenzylideneacetone.

Isolation of Diversinin. Elution of the column with a mixture of petroleum ether and benzene (7 : 1) yielded 2 g of a crystalline substance, $C_{19}H_{20}O_4$, mp 55-57° C (from ethanol), R_f 0.36, readily soluble in chloroform, benzene, methanol, and acetone, less readily in ether, and insoluble in petroleum ether and water.

Oxidation of Diversinin. A solution of 0.1 g of diversinin in 8 ml of dioxane was treated with 1 ml of 5% $KMnO_4$ and the mixture was left for 12 h. Then 1 ml of the liquid was distilled off, and to it were added four drops of benzaldehyde and two drops of 10% NaOH. On standing, a precipitate of dibenzylideneacetone precipitated.

Isolation of Umbelliferone and β-Sitosterol. Elution of the column with benzene–chloroform (1 : 1) yielded 1.5 g of umbelliferone. Fractions 3-5 of a petroleum ether eluate yielded β-sitosterol.

SUMMARY

From the roots of the Ferula diversivittata Rgl. et Schmalh growing in Turkmenia we have isolated a new coumarin, $C_{19}H_{20}O_4$, mp 55-57° C, which we have called diversinin, and also coumarins known previously – diversin and umbelliferone – and β-sitosterol.

On the basis of spectral characteristics it has been established that diversin has the structure of 7-(3',7'-dimethyl-5'-oxoocta-3',6'-dienyloxy)coumarin, and diversinin is its $\Delta^{2'}$ position isomer.

LITERATURE CITED

1. Flora of Turkmenia [in Russian], Vol. V, Ashkhabad (1950), p. 241.
2. N. P. Kir'yalov, Khim. Prirodn. Soedin., 51 (1969).
3. F. Bohlmann and C. Zdero, Chem. Ber., 104, 1611 (1971).