ALKALOIDS OF *Stephania Hernandifolia*

VI. HERNANDIFOLINE

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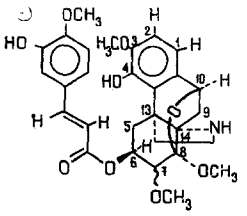
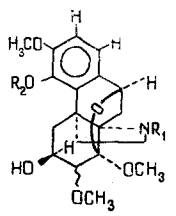
Counting an investigation of the composition of the alkaloids of the epigeal part of Stephania hernandifolia grown in theBlack Sea littoral of Caucasia [1-3] we have isolated a new base with the composition $C_{29}H_{33}O_9N$, which we have called hernandifoline. Structural formula I is proposed for it on the basis of chemical and physical investigations.

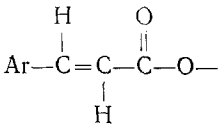


I



II $R_1=R_2=H$
III $R_1=CH_3, R_2=H$
IV $R_1=R_2=CH_3$

The alkaloid contains four methoxy groups, as is shown by the NMR spectrum (Fig 1, table), a



grouping [band at 1700 cm^{-1} in the IR spectrum of (I) (Fig. 2), and two doublets at 5.28 and 7.04 ppm (J = 15.6 Hz) in the NMR spectrum]. On treatment with acetic anhydride in pyridine, (I) gives a diacetyl derivative with the composition $C_{33}H_{37}O_{11}N$.

The alkaline hydrolysis of (I) gave a base (II) with the composition $C_{19}H_{25}O_6N$ and an acid with the composition $C_{10}H_{10}O_4$, which was identical with synthetic hesperetic acid [11].

On the basis of the NMR spectrum (Figs. 1 and 3 and Table 1), it was established that compound (II) contains three methoxy groups, one of which is aromatic (δ = 3.67 ppm). In the IR spectrum of (II) there are two narrow bands at 3565 and 3315 cm^{-1} and a broad band with a maximum at about 3100 cm^{-1} in the region of the O–H and N–H stretching vibrations. The number and nature of the mobile hydrogen atoms was determined by successive methylation. The methylation of (II) with methyl iodide in methanol, and also the hydrolysis of the N-methyl derivative of (I), gave a substance (III) with the composition $C_{20}H_{27}O_6N$. The NMR spectrum of the latter showed the signal of a $N-CH_3$ group, and the IR spectrum had two bands in the 3000 cm^{-1} region: a narrow band at 3525 cm^{-1} and a broadened band at 3250 cm^{-1} The further methylation of (III) with diazomethane gave a compound (IV) in the IR spectrum of which a single band remained in the high-frequency region at 3580 cm^{-1} (in chloroform), while in the NMR spectrum an additional signal of an aromatic methoxy group had appeared (δ = 3.84 ppm, singlet, 3H).

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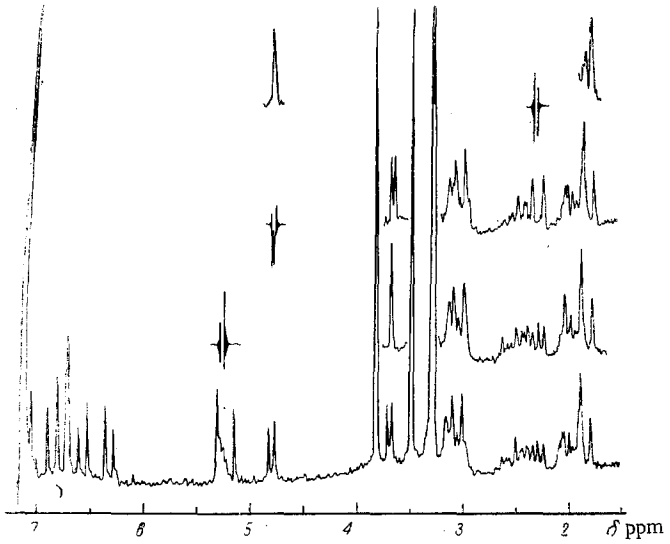


Fig. 1. NMR spectrum of hernandifoline (I) (in the upper positions are given the fragments of the double resonance spectrum, the positions of the beats coinciding with the positions of the irradiated signal).

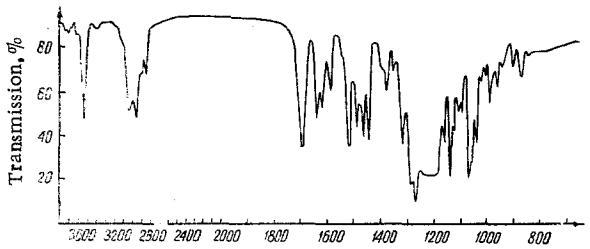
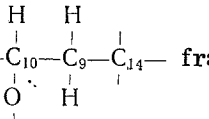


Fig. 2. IR spectrum of (I) ($CHCl_3$).

The protons of the  fragment form an AMX system. The chemical shifts and coupling constants of the protons of this system were determined by the double-resonance method (see Figs. 1 and 3 and Table 1). The assignment of the signal from H_{10} (Table 1) was made on the basis of literature information on the spectra of model compounds [4-7]. The structure of this fragment was confirmed by observing the nuclear Overhauser effect (NOE) [8] between the H_1 and H_{10} protons in the spectra of (I) and (II) [on irradiation of the H_1 signal, the integral intensity of the H_{10} signal rose by 17% in (I) and by 15% in (II)]. The presence of a methoxyl at C_3 follows from the finding of a NOE between the protons of one of the methoxy groups [in (I) δ = 3.31 ppm, and in (II) δ = 3.67 ppm] and the aromatic proton at C_2 [in (I), the integral intensity of the signal of the aromatic proton δ = 6.39 ppm increased by 12% on irradiation of the signal of the methoxyl, and in (II) the integral intensity of the singlet δ = 6.49 ppm rose by 13%]. The unusual shift in the signal of the aromatic methoxyl in the spectrum of (I) as compared with (II) by 0.26 ppm upfield is apparently connected with the screening influence of the acid residue on this group.

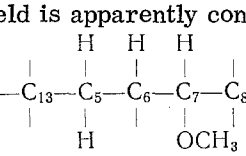
The fragment  in (I)-(IV) is shown by the NMR spectra (see Figs. 1 and 3 and Table 1). The chemical shifts and coupling constants of the protons were determined by the double-

TABLE 1. Characteristics of the NMR Spectra of Hernandifoline and Its Derivatives (δ ppm, Multiplicity,* J Hz)

Compound	H_1	H_2	H_5^a	H_5^e	H_6	H_7	$H-C_9-H$	H_{10}	C_6-OH	C_3-OMe	C_7-OMe	C_8-OMe	C_4-OMe	$N-CH_3$
I	6,64 d 8,0	6,39 d 8,0	1,98 q 15; 2,2	3,08	5,32 m	3,74 d 4,4	1,85 d 10,5	4,85 d 5,8	—	3,31 s	3,32 s	3,51 s	—	—
II	6,49 s		1,85 q 14,9; 2,8	3,04 q 14,9; 3,5	4,07 m	3,62 d 4,0	1,80 d 10,8	4,76 d 5,8	2,13 d 10,0	3,67 s	3,38 s	3,50 s	—	—
III	6,50 s		1,93 q q 14,8; 2,9	3,00 q 14,8; 3,4	4,05 m	3,62 d 4,1	1,45 d 10,8	4,81 d 6,2	2,24 d 9,8	3,72 s	3,38 s	3,48 s	—	2,48 s
IV	6,58 d 6,67 d		2,01 q 14,5; 3	2,72 q 14,5; 3,5	4,05 m	3,58 d 4	1,42 d 10,7	4,81 d 6	2,02 d 9,5	3,75 s	3,37 s	3,47 s	3,84 s	2,47 s

*s – singlet; d – doublet; m – multiplet; q – quartet.

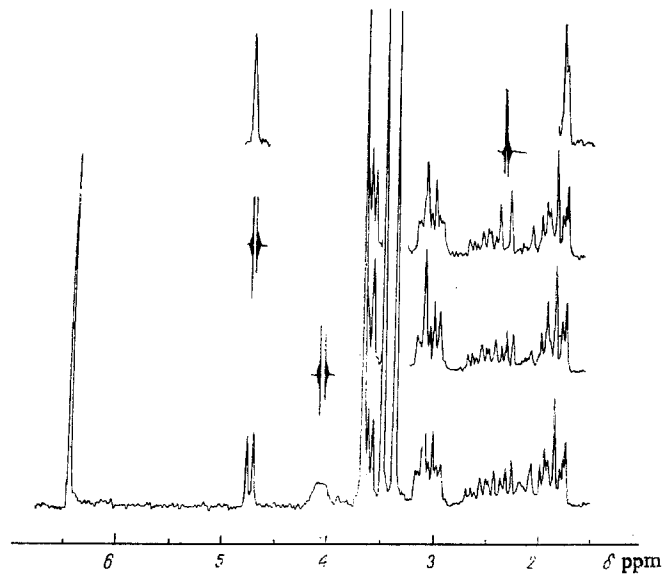


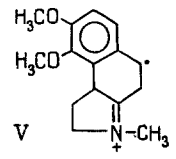
Fig. 3. NMR spectrum of compound (II) (fragments of the double-resonance spectrum given in the upper positions).

resonance method. The shift of the multiplet from H_6 in the upfield direction by 1.25 ppm on passing from (I) to (II) shows that the hesperetic residue acid is attached at C_6. Doublets at 2.13 ppm (J = 10 Hz), 2.24 ppm J = 9.8 Hz), and 2.02 ppm (J = 9.5 Hz) in the spectra of (II)-(IV) are assigned to the hydroxyl proton, since in the spectrum of (IV) in $CDCl_3$ with the addition of CH_3OD the doublet at 2.02 ppm disappears.

The signals at 3.08 and 3.04 ppm in the spectra of (I) and (II) are assigned to an equatorial proton at C_5 as a consequence of the fact that they are spatially close to the aromatic nucleus and the hydroxyl in position 4, which have a descreening effect on it. The H_5^a signals in the spectra of (I) and (II) appear in a stronger field (δ = 1.98 and 1.85 ppm, respectively). A paramagnetic shift of the signal of one of the C_5 methylene protons of this group has also been found previously in the spectrum of hernandoline [1, 2]. The combination of all the results obtained permits the conclusion that hernandifoline belongs to the group of hasubanan alkaloids and has the structure (I).

The mass spectrum of (II) also shows the pattern characteristic for the hasubanan alkaloids [9]: molecular peak m/e 363 (5%, maximum peak of the spectrum m/e 217, peaks with m/e 215 (32%), 216 (46%), and 218 (16%), and peaks with m/e 202 (13%) and 186 (15%) with mass numbers 15 and 31 units less than the mass number of the main peak of the spectrum. Structure (V) has been proposed for the ion corresponding to the maximum peak in the spectra of hasubanan alkaloids with methoxyls in positions 3 and 4 and a $N-CH_3$ group [9].

The mass number of the maximum peak in the spectrum of (II) confirms the presence of one phenolic hydroxyl and a secondary amino group in the molecule.



V

The hydroxyl at C_6 is axial. This is shown by the value of the coupling constant of the geminal proton with the protons at C_5 (Table 1). The observed splitting of the signal of the hydroxyl proton is apparently due to the fact that when the hydroxyl is in the axial position exchange is hindered by steric factors, as follows from a consideration of a model. The orientation of the substituent at C_7 remains obscure.

Only one example of a hasubanan alkaloid with an ester group has been described in the literature – stephavanine, isolated from Stephania abyssinica [10].

EXPERIMENTAL

The IR spectra were taken on a UR-10 instrument (paraffin oil and chloroform), and the NMR spectra on a HA-100D instrument in $CDCl_3$ (concentration of the solutions 5-10%, internal standard hexamethyldisiloxane). The double resonance and the investigation of the Overhauser effect were performed by using a GZ-18 generator. The preparation of the samples and the measurement of the integral intensities of the signals were done as described previously [8]. The mass spectrum of (II) was taken by Yu. S. Nekrasov on a MKh-1303 instrument (U_{ion} = 30B, t_{ev} = 180° C). The analytical results for all the compounds corresponded to the calculated figures.

Hernandifoline (I). The dry comminuted herb Stephania hernandifolia (10 kg) was moistened with 10% ammonia and exhaustively extracted with dichloroethane. The extract was treated with 10% sulfuric acid. The sulfuric acid solution was neutralized with ammonia to pH 6.5-7, and the alkaloids hernandoline and hernandolinol were exhaustively extracted with ether [1-3], and then the pH was raised to 9 and extraction was continued with chloroform. The chloroform extract was dried with sodium sulfate, concentrated to the state of a viscous sirup, and passed through a column of alumina (activity grade II). the chloroform eluate yielded 2.3 g of a mixture of three alkaloids, and after this mixture had been recrystallized three times from chloroform 1.2 g of hernandifoline was obtained in the form of the chloroform adduct, mp 227-227.5° C (Kofler).

Found %: C 62,58; 62.62; H 6.17; 6.13; N 2,49; 2,48; Cl 4,00. $C_{29}H_{33}O_9N$ 1/5$CHCl_3$.
Calculated %: C 62,29; H 5,89; N 2,48; Cl 3,65.

A suspension of the adduct of (I) in water was made alkaline with ammonia and treated with ether. This gave amorphous (I) with mp 128-129° C (Kofler), $[\alpha]_D^{20}$ −25° (*c* 2; ethanol).

Found %: C 63.44; 63,48; H 6,67; 6,65; N 2,56; 2,54. $C_{23}H_{33}O_9N \cdot 0{,}5\ H_2O$.
Calculated %: C 63,50; H 6,20; N 2,55.

Diacetylhernandifoline. A solution of 0.15 g of (I) in 1 ml of pyridine and 0.5 ml of acetic anhydride was left at room temperature for 48 h. The solvent was distilled off, an excess of water was added, and the reaction product was made alkaline with 25% ammonia and extracted with cloroform. The chloroform extract was dried with sodium sulfate, concentrated to the stage of a viscous sirup, and passed through a column of alumina. Elution was performed first with chloroform and then with methanol. The chloroform fraction yielded 0.015 g of a mixture of two substances. The methanolic solution gave 0.12 g of diacetylhernandifoline, $C_{33}H_{37}O_{11}N$, mp 171-171.5° C (benzene–ether) (Kofler).

N-Methylhernandifoline. A solution of 0.28 g of (I) in 5 ml of methanol containing 3 ml of methyl iodide was heated in the water bath for 3 h. The dry residue after the evaporation of the solvent was suspended in 10% ammonia and extracted first with ether and then with chloroform. The ether fraction yielded 0.26 g of amorphous N-methylhernandifoline. The chloroform extract yielded 0.03 g of unchanged (I).

Hydrolysis of (I). A mixture of 0.27 g of (I) and 5 ml of 0.5 N methanolic caustic soda was heated at 60° C for 2.5 h. The solvent was evaporated off and the dry residue was dissolved in 5% sulfuric acid

and extracted with ether, after which the acid solution was made alkaline with ammonia and the extraction was continued with chloroform. The ethereal extract yielded 0.10 g of hesperetic acid, $C_{10}H_{10}O_4$, mp 228-229° C (from ethanol).

The chloroformic extract yielded 0.16 g of the amino alcohol (II), which was purified by passage through a column of alumina in benzene. After recrystallization, the mp of (II) was 225-226° C (ethanol–ether).

Found %: C 63,07; 62,83; H 7,02; 7,13; N 3,80; 3,95 · $C_{18}H_{25}O_6N$ *M* 363 (mass spectrometric). Calculated %: C 62,39; H 6,99; N 3,90; *M* 363.

The N-Methyl Amino Alcohol (III). A solution of 0.12 g of (II) in 2.5 ml of methanol and 1 ml of methyl iodide was heated for 3 h. The solvent was distilled off and the dry residue was suspended in 10% ammonia and treated with chloroform. This gave 0.1 g of the N-methyl amino alcohol $C_{20}H_{27}O_6N$, mp 154-155° C (ether–methanol), $[\alpha]_D^{20} + 125°$ (*c* 0.4; ethanol).

Hydrolysis of N-Methylhernandifoline. A mixture of 0.26 g of N-methyl-(I) in 10 ml of 0.25 N methanolic caustic soda was heated for 3 h. The solvent was evaporated off and the dry residue was dissolved in 5% sulfuric acid and treated with ether, after which the solution was made alkaline with 25% ammonia and it was extracted with chloroform. The ethereal extract yielded 0.09 g of hesperetic acid and the chloroform extract yielded 0.16 g of (III).

The N, O-Dimethyl amino Alcohol (IV). A solution of 0.08 g of (III) in ether was treated with diazomethane (from 0.5 g of nitrosomethylurea) in 10 ml of ether. The mixture was left for a day and the solvent was distilled off. Substance (IV) was obtained with mp 124-125° C (ether–methanol).

SUMMARY

A new hasubanan alkaloid with the composition $C_{29}H_{33}O_9N$, mp 128-129° C (from ether), $[\alpha]_D^{20} - 25°$ (*c* 2; ethanol) containing an ester group has been isolated from the herb Stephania hernandifolia and has been called hernandifoline.

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