SUMMARY

The fatty acid compositions of the free lipids of the cell walls of three of the four strains studied of new species of the genus Flavobacterium have been determined by the GLC-MS method. The monosaccharide compositions of the LPSs of all the stains investigated have been determined by paper and gas-liquid chromatography. The chemical and serological heterogeneity of the LPSs of the microorganisms studied has been shown. The acidic nature of the polysaccharidic component of the LPSs has been revealed by analytical methods.

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STRUCTURE OF ERGOLIDE

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UDC 547.314+582.998

The epigeal part of Erigeron khorassanicus Boiss. has yielded a new sesquiterpene lactone of the pseudoguaiane type – ergolide $C_{17}H_{22}O_5$, mp 179-180°C (ethanol) $[\alpha]_D^{20}$ + 123° (c 4.88; ethanol). On the basis of chemical transformations and spectral characteristics, its structure and configuration have been established as 6-acetoxy-4-oxo-1,7αH,6,8,10βH-pseudoguai-11(13)-en-8,12-olide.

The isolation from Erigeron khorassanicus Boiss., family Asteraceae of the known lactone britanin [1, 2] and the new compounds erigerolide, $C_{19}H_{26}O_7$, mp 152-153°C (ethanol), $[\alpha]_D^{20}$ + 2° (c 6.36; ethanol) and ergolide (I), $C_{17}H_{22}O_5$ (corrected composition), mp 179-180°C (ethanol), $[\alpha]_D^{20}$ + 123° (c 4.88; ethanol) has been reported previously [2].

In the present paper we give information to establish the structure and stereochemistry of ergolide (I).

In the IR spectrum of (I), bands showing its α,β-unsaturated lactone nature are located at 1770 and 1670 cm^{-1}. At 1740 cm^{-1} the absorption bands of the carbonyl of a five-membered ring and the band of an ester carbonyl coincide.

Institute of the Chemistry of Plant Substances of the Uzbek Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 568-571, September-October, 1986. Original article submitted April 16, 1986.

0009-3130/86/2205-0000$12.50

TABLE 1. Chemical Shifts and Spin-Spin Coupling Constants of the Protons of Ergolide ($CDCl_3$, 0 – TMS)

Proton	Chemical shift (δ,ppm)	SSCC, Hz
H-1	2,28 m	
H-6	5,50 d	$^3J=7,8$
H-7	3,03 m	$^3J=10,3$; 7,8; $^4J=3,5$& 3,1
H-8	4,49 oc	$^3J=11,7$; 10,3& 2,8
H-9β	2,50 oc	$^2J=13,3$; $^3J=4,4$ & 2,8
H-10	1,86 m	
H-13	5,86 d	$^4J=3,1$
H-13	6,21 d	$^4J=3,5$
CH_3-14	1,12 d	$^3J=6,6$
CH_3-15	1,08 s	
CH_3OCO	1,98 s	

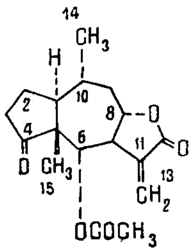
Symbols: s – singlet; d – doublet; oc – octet; m – multiplet.

TABLE 2. Chemical Shifts of the Carbon Atoms of Ergolide (in $CDCl_3$ relative to TMS)

C atom	δ, ppm	C atom	δ, ppm
1	46,6 d	9	44,7 t
2	24,5 t	10	30,0 d
3	38,1 t	11	139,0 s
4	214,6 s	12	169,4 s
5	56,2 s	13	120,6 t
6	75,3 d	14	19,8* q
7	52,3 d	15	18,4* q
8	76,4 d	OC=0 | CH_3	169,4 s 21,1 q

*The assignment of these signals may be interchanged.

Symbols: s – singlet; d – doublet; t – triplet; q – quartet.



In the PMR spectrum of (I) at 1.98 ppm there is a 3H singlet of the methyl of an acetyl group, and in the ^{13}C NMR spectrum, in addition to a signal corresponding to two ester carbonyl carbon atoms at 169.4 ppm, there is also a singlet at 214.6 ppm. From the value of its chemical shift, the latter corresponds to a carbonyl group in a cyclopentane ring [3], i.e., it confirms the deductions from the IR spectrum of (I).

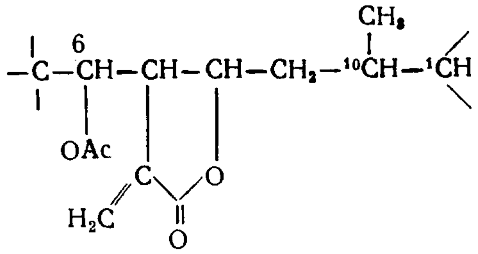
Thus, the five oxygen atoms in the ergolide molecule are represented in the form of an acetyl group, a γ-lactone ring, and a keto group in a cyclopentanone ring.

In the PMR spectrum of (I) a singlet at 1.08 ppm and a doublet ($^3J = 6.6$ Hz) at 1.12 ppm, each with an intensity of 3 H, correspond to the protons of two methyl groups. The first of them is, from the values of its chemical shift, the resonance line of an angular methyl group [4].

The spectrum also contains one-proton doublets at 5.86 ppm ($^4J = 3.1$ Hz) and 6.21 ppm ($^4J = 3.5$ Hz), which are characteristic for the protons of an exocyclic methylene group attached to a lactone ring.

A detailed analysis of the spectrum of (I) using the method of multifrequency resonance, beginning with the doublet signal at 5.50 ppm of the proton geminal to the acetyl group permitted a chain of protons linked to one another to be determined.

On this basis an assignment of the signals was made and the SSCCs between the protons were determined (Table 1). As the result of these experiments it was established that the structure of ergolide included the following fragment:



When (I) was dehydrogenated, an azulenic hydrocarbon was obtained.

The facts given above, in the light of the characteristics of the ^{13}C NMR spectrum, show that ergolide is a sesquiterpene lactone of the pseudoguaiane type.

The acetyl function is located at C-6 and has the α orientation, since the value of ^{3}J between H-6 and H-7 amounts to 7.8 Hz [4]. The secondary methyl group is attached at C-10. The lactone ring is trans-linked to the main hydrocarbon skeleton of the molecule at C-7 and C-8, as is shown by the values of the SSCCs between the H-6 and H-7 protons (7.8 Hz), between H-7 and H-8 (10.3 Hz), between H-8 and Hα-9 (11.7 Hz), and between H-8 and Hβ-9 (2.8 Hz).

In the PMR spectrum of (I), the H-10 signals appears in the form of a complex multiplet at 1.86 ppm. When the protons of the secondary methyl group at 1.12 ppm were irradiated with a strong radiofrequency field the nature of the splitting of this multiplet simplified to a considerable degree and it acquired the form of a sextet. From this structure of the splitting of the signal, due to the spin-spin interaction of H-10 with the vicinal protons, we find that the ^{3}J values between H-1 and H-10 and between Hα-9 and H-10 are equal to one another at 11 Hz, and that between Hβ-9 and H-10 is 4.4 Hz. These SSCC values show the α orientation of H-1 and of the methyl group at C-10.

The keto group may be located at C-2, C-3, or C-4, but the chemical shift of C-5 (56.2 ppm) in the ^{13}C NMR spectrum of (I) shows its position at C-4.

The stereochemistry of the linkage of the cyclopentane and cycloheptane rings was revealed by a comparative study of the CD spectra of ergolide and arnifolin. In the molecule of the latter, these rings are translinked to one another [5]. For both substances a positive Cotton effect was observed, at 298 nm (Δε + 1.26) and at 300 nm (Δε + 1.88), respectively. We find from this that in the ergolide molecule the trans-linkage of the rings is realized with the β orientation of the angular methyl group.

A product of the saponification of ergolide corresponded in its physicochemical constants to the lactone carpesiolin [6-8]; Bohlmann et al. called the same lactone 6α-hydroxy-2,3-dihydroaromaticin [9].

The preparation of the monoacetyl derivative of carpesiolin is known [6], and the structure of this derivative corresponds to ergolide.

Thus, ergolide is 6α-acetoxy-4-oxo-1,7αH,6,8,10βH-pseudoguai-11(13)-en-8,12-olide (I) and it is a new natural sesquiterpene lactone.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer (in KBr tablets), PMR spectra on a SC-300 (300 MHz) instrument (Varian), and ^{13}C NMR spectra on a CFT-20 instrument (Varian) in $CDCl_3$.

Isolation of Ergolide. The leaves and small stems of Erigeron khorassanicus (15 kg) were extracted with ethanol. The concentrated extract was dissolved in ethanol and the solution was diluted with water to give a 60% ethanolic solution. The resulting precipitate was separated off and the combined lactones were extracted from the solution with chloroform. By driving off the solvent, 186 g of purified extract was obtained, and this was chromatographed on silica gel of type KSK (in a ratio of 1:10) with successive elution by petroleum ether—benzene (4:1; 1:1; and 3:7), benzene, benzene—ethyl acetate (99:1; 97:3, 95:5; 9:1; and 1:1), and ethyl acetate. This gave 3.7 g of (I) (fractions 21-32, petroleum ether—benzene (1:1) eluate); mp 179-180°C (ethanol), $C_{17}H_{22}O_5$. R_f 0.46 (benzene—ethyl acetate (2:3));

revealing agent — a 1% solution of potassium permanganate in 1% sulfuric acid.

Dehydrogenation of Ergolide. A mixture of 100 mg of (I) and 100 mg of amorphous selenium was heated at 300-350°C for 50 min. The reaction product was extracted with petroleum ether and was chromatographed on alumina. This gave a blue-violet liquid which was identified as guaiazulene by TLC with a marker.

Hydrolysis of Ergolide. A solution of 100 mg of ergolide in 10 ml of ethanol was treated with 5 ml of 4% KOH solution. The mixture was heated in the water bath at 50-55°C for 0.5 h and was then diluted with water, acidified with 10% H_2SO_4 to pH 1.0, and extracted eight times with ethyl acetate, after which the extract was washed with $NaHCO_3$ solution and with water and the solvent was evaporated off. The total material obtained was chromatographed on silica gel. This gave a hydroxylactam with mp 123-124°C (ethanol), $C_{15}H_{20}O_4$, R_f 0.32 (benzene—ethyl acetate (1:1)); revealing agent: a 1% sulfuric acid.

SUMMARY

From *Erigeron khorassanicus* Boiss, a new natural sesquiterpene lactone of the pseudoguiane type — ergolide — has been isolated, and its structure has been established on the basis of chemical transformations and an analysis of ^1H and ^{13}C NMR spectra.

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