8-HYDROXYERGOTAMINE, A NEW ERGOT ALKALOID

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A new alkaloid was found in the mother liquors remaining after the isolation of ergotamine from the natural ergot. The structure of 8-hydroxyergotamine was assigned by the spectroscopic methods.

Improving the purity of the comercial ergotamine, we observed its contamination by a so far undescribed minor alkaloid. This compound accumulates in the mother liquors after the crystalization of ergotamine base. Since it does not undergo the isomerization, its separation from the other alkaloids (ergotamine, ergotoxine and ergostine) is easy. The subject of this communication is the description of the isolation and the structure elucidation of this compound.

RESULTS

The infrared spectrum of the new alkaloid is very similar to that of ergotamine. Its mass spectrum contains the fragmentation series m/z 314, 244, 153, 125 and 70, characteristic for the peptide moiety of ergotamine (*I*). Also the ions m/z 43, 91 and 120 originate from this part of the molecule[1]. The ion m/z 267 which corresponds with ergotamine to its ergoline part, is not present in the mass spectrum. Instead of it, an ion m/z 283, $C_{16}H_{17}N_3O_2$, occurs. It contains one oxygen atom more than the ergotamine. Since the ions m/z 283 and 314 are complementary, the summary formula $C_{33}H_{35}N_5O_6$ can be deduced for the molecule, in a good agreement with the results of the elemental analysis. The ^{13}C-NMR spectrum confirms the presence of 33 carbon atoms. Two pairs of the carbon atoms are magnetically equivalent. To confirm the presence of the peptide substructure, we used the procedure of Bremser and coworkers[2]. The signals of all sixteen carbon atoms of the peptide moiety were picked up in the ^{13}C-NMR spectrum (Table 1). Their chemical shift was within 1 ppm identical with the literature data[3] for ergotamine and ergotaminine and the corresponding signals also had the same degree of protonation (determined from the off-resonance multiplicity). The calculated values of the normalized similarity –

0·7344 and 0·9721 for the comparison with ergotamine and its peptide part, respectively – show clearly that both alkaloids have identical peptide moieties. Only several signals can be identified in the ^{1}H-NMR spectrum: the singlet of the tertiary methyl group at 1·32 ppm ($C_{2'}$-Me), N-methyl group singlet at 2·81 ppm (the position 6), one-proton triplet (J = 5 Hz) at 4·73 ppm (H-5″) one-proton doublet (J = 2 Hz) of an olefinic proton at 6·63 ppm, broad exchangeable one-proton singlet at 6·41 ppm ($C_{12'}$-OH), one proton singlet at 7·04 ppm (H−2), multiplet of eight aromatic protons in the region of 7·16−7·62 ppm and two exchangeable singlets of the NH protons at 8·76 and 9·89 ppm. From these data it follows that the arrangement of the A and B rings is unchanged, ring D contains one N-methyl group and there is one trisubstituted double bond in the ergoline moiety. The possible positions for this bond are between carbon atoms 4,5 or 8,9 or 9,10. The similarity of the UV spectrum to that of 9,10-ergolene compounds, however, does not allow to reject the alternative

TABLE I

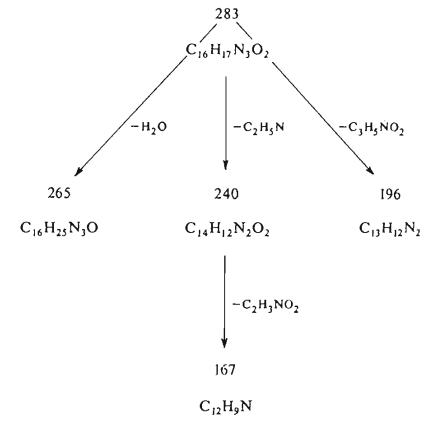
Comparison of ^{13}C-NMR Spectra [a] of Ergotamine (*I*) and 8-Hydroxyergotamine (*II*)

Peptide part				Ergoline part			
Assignment	chemical shift			Assignment	chemical shift		
	I [b]	*II*	difference		*I* [b]	*II*	difference
2′	86·4 s	86·8 s	0·1	2	120·3 d	120·6 d	−0·3
3′	166·7 s	166·9 s	0·2	3	109·2 s	109·5 s	0·3
5′	57·0 d	57·2 d	0·2	4	27·5 t	26·8 t	−0·7
6′	165·1 s	165·3 s	0·2	5	62·3 d	62·4 d	0·1
8′	46·7 t	46·8 t	0·1	7	56·0 t	62·9 t	6·9
9′	22·6 t	22·6 t	0·0	8	43·4 d	72·7 s	29·3
10′	26·8 t	26·8 t	0·0	9	119·2 d	121·5 d	2·3
11′	64·8 d	64·8 d	0·0	10	136·9 s	139·0 s	2·1
12′	103·7 s	103·7 s	0·0	11	128·0 s	127·1 s	−0·9
13′	24·7 q	24·8 q	0·1	12	111·9 d	112·3 d	0·4
14′	39·6 d	39·4 d	−0·2	13	123·1 d	123·2 d	0·1
15′	139·6 s	139·7 s	0·1	14	111·1 d	111·5 d	0·4
16′, 20′ [c]	130·8 d	130·7 d	−0·1	15	134·7 s	134·8 s	0·1
17′, 19′ [c]	128·6 d	128·6 d	0·0	16	126·8 s	127·1 s	0·3
18′	128·3 d	128·5 d	0·2	17	175·2 s	176·6 s	1·5
				N–Me	44·3 q	43·8 q	−0·5

[a] In hexadeuterodimethyl sulfoxide; chemical shifts given in the δ(C)-scale. Abbreviations for the off-resonance multiplicity: s singlet, d doublet, t triplet, q quartet; [b] ref.[3]; [c] Intensity corresponding to two carbons.

with double bond between C4 and C5. This possibility can be eliminated since the chemical shift of C4 in ergotamine and our compound is nearly the same (Table I). Therefore, the double bond is located between C9 and C10. The absence of the ion m/z 282, $C_{16}H_{16}N_3O_2$ in the mass spectrum is in agreement with this conclusions[4].

By comparison of the ^{13}C-NMR signals of the ergoline carbons (Table I), it becomes immediately apparent the difference in the $\delta(C) = 72{\cdot}7$ signal multiplicity (Table I) which is due to a quaternary sp^3- carbon attached to oxygen. This fact indicates that the excess oxygen atom (with respect to ergotamine) is a part of a tertiary hydroxyl group. Such a group can be located at the position 5 and 8 only. Considering the chemical shifts of C−4 and C−5 in II, the former possibility can be excluded. The observed chemical shift differences can now be satisfactory interpreted placing the hydroxyl group at C−8: the largest effect is observed at C−8, then at C its neighbour C−7 and transmitted through the double bond to C−9 and C−10.

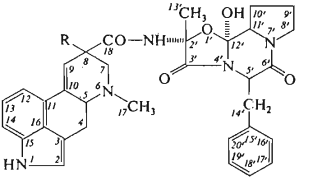


SCHEME 1

Detail of the Fragmentation of *II* (ion m/z 283)

Further arguments are provided by a detailed examination of fragmentation of the m/z 283 ion (Scheme 1). Retro–Diels-Alder fragmentation involving C−6 and C−7 (elimination of C_2H_5N) and the splitting involving C−7 and C−8 (loss of $C_3H_5.NO_2$) again confirm the presence of the C−9, C−10 double bond[4]. The elimination of $C_2H_3NO_2$ from the m/z 240 ion together with the two already mentioned fragmentation pathways is consistent with the localization of the hydroxyl group at C−8.

Thus, the new alkaloid is 8-hydroxy derivative of ergotamine and has the structure *II*.



I, R = H
II, R = OH

The work on establishing its absolute configuration at C–8 by a correlation with penniclavine and isopenniclavine[5] is in progress.

EXPERIMENTAL

The melting point was determined in a Kofler hot stage and was uncorrected. The UV spectrum was measured in ethanol on the spectrophotometer Unicam SP 1800. The infrared spectrum was measured on a Unicam SP 1 000 spectrometer in a KBr pellet. Optical rotations were measured on the instruments Opton. OLD 4 (546·1 nm) and Metra (α_D). Thin layer chromatography was performed on silica gel Merck 100 and CE cellulose Riedel de Häën AG. The following solvent systems were used: S 1 chloroform–ethanol 99 : 1; S2 chloroform–benzene 4 : 1; S3 chloroform–toluen–aceton–ethanol 25 : 15 : 10 : 10; S4 n-heptane–chloroform–aceton–ethanol 10 : 50 : : 10 : 5; S5 n-heptane–ethyl acetate–diethylamine 5 : 6 : 0·02. Mass spectrum was measured using the Varian MAT 311 apparatus; energy of ionizing electrons 70 eV, ionizing current 1 mA, direct inlet at 190°C, ion source temperature 200°C. The elemental composition of all mentioned ions was confirmed by high-resolution measurement (±5 ppm) and the fragmentations of the *m/z* 314 and 283 ions were proved by the DADI technique[6]. ^{1}H-NMR spectrum (CW) was measured in deuteriochloroform–hexadeuteriodimethyl sulfoxide mixture (1 : 1) on the Varian HA-100 instrument with hexamethyldisiloxane as an internal standard; δ(H) = 0·06. ^{13}C-NMR spectra were measured in the FT mode on a JEOL FX-60 (15·036 MHz) spectrometer in hexadeuteriodimethyl sulfoxide at 25°C. Its central signal δ(C) = 40·4 (ref.[7]) was used as the reference and the literature data[3,8] were appropriately corrected. The chemical shifts are given in the δ-scale with respect to tetramethylsilane and the positive axis in the direction of the decreasing magnetic field. ^{1}H chemical shifts (±0·005 ppm) were measured by an electronic counter; ^{13}C chemical shifts (±0·06 ppm) were calculated from the digitally obtained address differences.

8-Hydroxyergotamine

Grinded ergot (250 kg) from the field culture containing mainly ergotamine was extracted by 1 500 l of mixture ether with ethanol (1 : 1) at room temperature. The extracted alkaloids were then treated by 500 l of 1% tartaric acid. After neutralization by aqueous ammonia to pH 7·5, 1 100 g of crude alkaloid mixture was obtained. The peptide alkaloids were then transformed to their laevoratory forms by the reaction with 1N sulfuric acid in absolute ethanol and acetic acid. The

bases were prepared from the mixture of crude crystalline sulfates (1 200 g) by aqueous natrium hydrocarbonate and extracted by 100 l of ether. Ergotamine was removed by crystalization. The mother liquors were concentrated *in vacuo* at 40°C giving 22 g of the bases. Ergotamine, ergocrystine, and ergostine were by eight hours boiling in twenty-fold excess of methanol transformed into their poorly soluble dextrorotatory forms whose crystals were filtered off after cooling. The residue of the mother liquors (7 g) was subjected again to the same operation, after which procedure remained 4 g of the product. It was further separated by chromatography on 200 g silica gel in the system S1. The 10 ml fractions were collected. New alkaloid (1·9 g) was rechromatographed on silica gel in the system S2. The obtained preparation (1·3 g) was crystalized from hot ethyl acetate and then from hot chloroform. An amount of 600 mg of white crystals was obtained, m.p. 197°C, $[\alpha]_D^{20} = +14°$ (*c* 1% in pyridine), $[\alpha]_{546\cdot1}^{20}$ +32·9 (*c* 1% in pyridine). The compound is good soluble in dioxane, pyridine and dimethyl sulfoxide, less in methanol and chloroform. It gives a positive van Urk and Keller tests. Elemental analysis found 66·88% C, 6·0% H, 11·7% N; for $C_{33}H_{35}N_5O_6$ calculated 66·32% C, 5·90% H, 11·72% N. UV ethanol, λ_{max}, (log ε): 261 nm (3·95), 381 nm (3·57). Mass spectrum: *m/z* (% of relative intensity, composition): 314 (30, $C_{17}H_{18}N_2O_4$), 283 (13, $C_{16}H_{17}N_3O_2$), 265 (3, $C_{16}H_{15}N_3O$), 244 ($C_{14}H_{16}N_2O_2$), 240 (21, $C_{14}H_{12}N_2O_2$), 196 (10, $C_{13}H_{12}N_2$), 167 (22, $C_{12}H_9N$), 154 (36, $C_{11}H_8N$), 153 (78, $C_7H_9N_2O_2$), 125 (53, $C_6H_9N_2O$), 120 (7, $C_8H_{10}N$), 91 (50,C_7H_7), 70 (100, C_4H_8N), 43 (53, C_2H_3O). The comparison of chromatographic behavior of the new alkaloid and its congeners is given in the Table II.

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TABLE II

Thin-Layer Chromatography of 8-Hydroxyergotamine (*II*) and Its Congeners[a]

Alkaloid	Chromatographic system		
	S3[b]	S4[b]	S5[c]
Ergotamine (*I*)	35	24	15
Ergostine	48	34	54
8-Hydroxyergotamine (*II*)	53	36	60
Ergotoxine	66	45	68
Ergotaminine	83	56	70

[a] R_F . 100; [b] silica gel; [c] cellulose impregnated by formamide.

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