NON-CYCLOL (LACTAME) ERGOT ALKALOIDS

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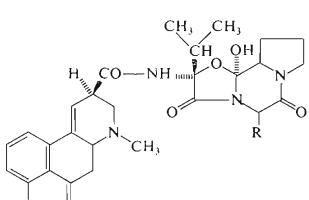
Two new alkaloids were isolated from the field ergot. Their structures, N-(D-lysergyl-L-valyl)-cyclo(L-valyl-D-prolyl) (*IV*) and N-(D-lysergyl-L-valyl)cyclo(L-leucyl-D-prolyl) (*V*), were assigned by mass, ^{1}H and ^{13}C NMR spectroscopy.

A representative of new type of ergot alkaloids[1] having the non-cyclol structure *II*, is the lactame analogue of ergocristine (*I*), described by Stütz and coworkers[2]. This compound was also found in ergocristine ergot of Czechoslovak origin[3]. A role of the compounds of this group in the biogenesis of the peptide part of ergot alkaloids has already been discussed by Floss and coworkers[4–6]. During the isolation of crude bases of the ergotoxine family from the ergot of Czechoslovak provenience, two new compounds were detected with chromatographic behaviour different from that of all other ergotoxine alkaloids and close to the properties of the compound *II*. This paper is devoted to the elucidation of their structure.



I, R = $CH_2C_6H_5$

Unlike to ergocristine (*I*) (ref.[7]), N-(D-lysergyl-L-valyl)-cyclo(L-phenylalanyl-D-prolyl) (*II*) exhibits in the electron-impact mass spectrum a molecular ion of sufficient intensity (*m/z* 593, $C_{35}H_{39}N_5O_4$). Characteristic fragments containing the ergoline part of the molecule, arise by the rupture of the bonds $C_{(3')}$—$N_{(4')}$ (*l*, *m/z* 349), $C_{(2')}$—$C_{(3')}$ (*m*, *m/z* 321) and $C_{(8)}$—$C_{(17)}$ (*n*, *m/z* 221) (Scheme 1). These

fragmentations require a transfer of one or two hydrogen atoms. The fragmentation series 349 (*l*) → 321 (*m*) → 221 (*n*) was proved by the registration of metastable ions. The ion *d*, formed by breaking the $C_{(3')}$—$C_{(4')}$ bond, is complementary to the ion *l*. This ion decomposes further by a known way [7] under formation of ions *m/z* 153 (*g*) → → 125 (*h*) → 70 (*i*), ion *k* (*m/z* 120) and ion *m/z* 91. On the contrary to *I*, the ion *b* (*m/z* 267) due to the rupture of the $C_{(17)}$—$N_{(1')}$ bond, is missing in the spectrum. The ^{1}H NMR spectrum of compound *II* differs from that of *I* in the multiplicity of the NH-protons (doublet + singlet *vs.* two singlets) and in the presence of the $H_{(2')}$ signal at 5·68 ppm. The latter proton is responsible for the doublet splitting of the amide proton signal (Table I). Greater is also the magnetic nonequivalence of the isopropyl methyls (0·24 ppm *vs.* 0·11 ppm).The triplet appearence of the $H_{(5')}$ signals indicates a CH_2 group in its vicinity. Marked differences are also evident in the ^{13}C NMR spectra (Table II). The signals of carbons —C(—)—O and O—C(—)—O

TABLE I

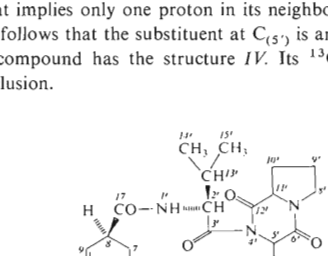
^{1}H NMR data of selected protons in lactame ergot alkaloids and model compounds (59·797 MHz, C^2HCl_3, 25°C, δ-scale, coupling constants in Hz given in parentheses below the chemical shifts)

Proton	*I*	*II*	*III*	*IV*	*V*
$N_{(1)}$—H	8·30 s	8·14 s	8·39 s	8·30 s	8·00 s
$H_{(2)}$	6·91 s	6·93 s	6·90 s	6·91 s	6·92 s
$N_{(6)}$—CH_3	6·38 dd (6·1, 1·2)	6·50 d (4·3)	6·60 d (6·1)	6·46 d (4·4)	6·47 d (4·9)
$N_{(1')}$—H	9·77 s	8·47 d (8·6)	9·09 d (8·6)	8·25 d (9·3)	8·16 d (8·6)
$H_{(2')}$	[a]	5·68 dd (8·6, 2·4)	5·65 dd (8·6, 2·4)	5·82 dd (9·3, 3·4)	5·75 dd (8·6, 2·5)
$H_{(5')}$	4·70 t (6·4)	5·27 t (4·3)	5·12 t (4·9)	4·89 d (9·3)	5·08 t (6·1)
$C_{(2')}$—R	0·89 d[b] (6·8) 1·00 d[b] (6·8)	0·76 d[b] (6·7) 1·00 d[b] (6·7)	0·85 d[b] (6·1) 1·05 d[b] (6·1)	0·76 d[b] (6·8) 1·10 d[b] (6·8)	0·73 d[b] (7·3) 1·18 d[b] (7·3)
$C_{(5')}$—R	7·19 s[c]	7·29 s[c]	7·28 s[c]	0·99 d[d] (6·4)	0·97 d[d] (6·1)

[a] Not observed; [b] 3 H; [c] 5 H; [d] 6 H.

($C_{(2')}$ and $C_{(12')}$) are missing and there four carbonyl carbon signals instead of three signals of this type in *I*. The chemical shifts of carbons close to the site of the structural change are also different. No significant changes are observed with the signals of the ergine moiety. All carbon signals in *II* and its 8-epimer *III* can be assigned without difficulties using single frequency off-resonance, noise off-resonance, selective decoupling and comparison with the spectra of other peptide alkaloids[8]. There is a good agreement between the spectra of peptide parts of *II* and *III*; only the carbons of the ergine D-ring exhibit some chemical shift differences.

More abundant new compound has the composition $C_{31}H_{39}N_5O_4$ (high resolution). Its molecular ion (similarly to that of *II*) produces ions m/z 349, 321, and 221 and further the ions m/z 154, 125, and 70. The diagnostic ion k (m/z 72) suggests the substitution at $C_{(5')}$ by C_3H_7 (ref.[7]). The ions b (m/z 267) and d (m/z 196) are missing in the spectrum. However, there is the ion f (m/z 154) arising from d by the elimination of C_3H_6 and its daugher ions g (m/z 125) and i (m/z 70). A doublet of the $N_{(1')}$—H proton at 8·25 ppm, doublet of doublets of the $H_{(2')}$ proton at 6·82 ppm in the ^{1}H NMR spectrum, the signals of four carbonyl carbons in the ^{13}C NMR spectrum (174·5, 173·8, 169·9 and 165·3 ppm) and the absence of the carbons of O—C— type together clearly indicate a lactame-type alkaloid. In comparison with the NMR spectra of *II*, the signals of the proton and carbons of the phenyl group are missing. In addition, there is a six-proton doublet (J = 6·4 Hz) of two secondary methyls at 0·99 ppm in the ^{1}H NMR spectrum. The proton $H_{(5')}$ gives rise to a doublet at 4·89 ppm that implies only one proton in its neighbourhood. From the above mentioned facts if follows that the substituent at $C_{(5')}$ is an isopropyl group. Therefore, the studied compound has the structure *IV*. Its ^{13}C NMR spectrum agrees well with this conclusion.



II, R = $CH_2C_6H_5$
IV, R = $CH(CH_3)_2$
V, R = $CH_2CH(CH_3)_2$

The second compound investigated has the elemental composition $C_{32}H_{41}N_5O_4$, *i.e.* it contains one CH_2 group more than *IV*. Its mass spectrum exhibits besides the molecular ion m/z 559 the fragmentation series 349 → 321 → 221 and 154 →

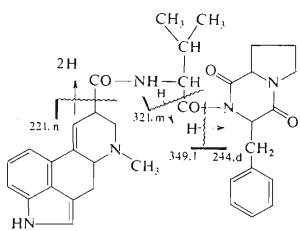
TABLE II

^{13}C chemical shifts of lactame alkaloids and model compounds (15·036 MHz, C^2HCl_3, 25°C, δ-scale)

Ergine part						Peptide part[a]					
Atom	*I*	*II*	*III*	*IV*	*V*	Atom	*I*	*II*	*III*	*IV*	*V*
2	119·2	119·9	118·5	119·7	119·8	2′	89·9	60·1	60·7	60·1	59·7
3	110·6	111·2	110·6	110·8	111·0	3′	165·7*	174·3	173·7	173·8	173·7
4	30·9	29·2	29·2	29·7	29·1	5′	56·8	59·0	58·9	64·3	58·1
5	59·3	60·1	62·9	60·5	60·4	6′	165·4*	164·5	164·5	165·3	165·6
7	48·2	50·0	55·2	50·7	50·8	8′	46·1	44·6	44·1	45·5	45·5
8	44·3	44·8	43·5	44·3	44·3	9′	21·7	22·0	22·0	22·8	22·9
9	118·8	118·9	118·5	119·0	118·8	10′	22·4	22·9	27·3	23·3	23·3
10	138·9	138·1	137·0	137·7	137·6	11′	64·3	59·0	58·9	58·2	58·4
11	129·6	129·9	128·0	129·4	129·4	12′	103·7	169·0	169·1	169·9	159·1
12	111·9	111·7	112·4	111·8	111·9	13′	34·3	29·5	29·9	30·3	30·3
13	123·3	123·3	123·2	123·3	123·5	14′	16·9**	20·3*	20·4*	20·2*	20·1*
14	110·2	109·8	109·9	109·9	109·8	15′	15·3**	15·9*	16·2*	16·2*	16·0*
15	133·8	133·9	134·0	134·0	133·9	16′	39·6	37·7	37·6	30·3	40·3
16	125·9	126·3	126·4	126·3	125·4	17′	139·2	135·5	134·0	19·4	25·1
17	176·2	174·5	174·3	174·5	174·5	18′	130·1[b]	130·3[b]	130·1[b]	19·4	23·2**
N—CH_3	40·9	41·6	44·8	41·8	41·8	19′	128·0[b]	128·8[b]	128·5[b]		21·6**
						20′	126·3	127·7	127·5		

[a] Values denoted by equal number of asterisks can be interchanged within each column; [b] 2 C.

→ 125 → 70. The ions *b* (*m*/*z* 267) and *d* (*m*/*z* 210) are missing in the spectrum. The ion *k* appears in this case at *m*/*z* 286 (a CH_2 difference with respect to *IV*). That suggests the substitution by C_4H_9 at $C_{(5')}$. The moiety NH—CH found in the ^1H NMR spectrum (Table I) and four carbonyls observed in the ^{13}C NMR spectrum (Table II) qualify this compound as an another lactame alkaloid. It contains four secondary methyl groups according to its ^1H NMR spectrum (Table I). The proton $H_{(5')}$ gives rise to a triplet what means that there is a methylene group next to it. Four carbon signals remains unassigned after assigning the carbons of the ergine part and the carbons $C_{(2')}$ to $C_{(15')}$ of the peptide moiety (Table II). They comprise one CH, one CH_2 and two CH_3. The only consistent interpretation is the attachment of an isobutyl side chain to $C_{(5')}$, leading to the structure *V*.



SCHEME 1

EXPERIMENTAL

Melting points were determined in the Kofler apparatus. UV spectra were measured in ethanol on a Unicam SP 1800 spectrophotometer. Optical rotation $[\alpha]_D$ was measured using a Metra instrument. Mass spectrometer Varian MAT 311 (70 eV energy of ionizing electrons, ionizing current 1 mA, direct inlet at 190°C, ion source temperature 200°C) was used for the study of the mass spectra. The elemental composition of ions was measured by a peak-matching technique (±5 ppm) with perfluorokerosene standard. The metastable ions were measured in the second field-free region of the spectrometer by scanning the electrostatic sector voltage. ^1H and ^{13}C NMR spectra were measured on a Jeol FX-60 spectrometer (59·797 and 15·036 MHz) at 25°C in deuteriochloroform containing tetramethylsilane as an internal standard. Chemical shifts were calculated from the digitally obtained address differences with the accuracy ±0·005 and ±0·06 ppm. The assignments of signals given in the Table II are based on the off-resonance and selective decoupling experiments and the comparison with the literature[8]. Semipreparative liquid chromatography was realized on an instrument built from the high-pressure micropump VCM 300 and a variable wavelength UV detector UVM-4 (Development Department of Czechoslovak Academy of Sciences, Prague, Czechoslovakia). The stationary phase was Lichrosorb-NH_2 particle size 10 μm, column dimensions 500 × 4 mm i.d.); the elution by diethyl ether–ethanol (9 : 1) was performed in the isocratic mode.

Isolation of Alkaloids

The work-up of ergocornin-ergocryptine field ergot of Czechoslovak provenience (500 kg) yielded 200 g of crystalline bases. These were converted into the dihydrogen phosphates of their laevorotatory forms by heating with phosphoric acid in ethanol. The bases were liberated by aqueous sodium hydrogen carbonate, extracted by ether and the solvent was then removed. The residue (135 g) was chromatographed on a fifty-fold excess of silica gel (eluent: chloroform); 35 g of 20% concentrate of the lactame alkaloids were obtained. Repeated chromatography on the hundred-fold excess of silica gel in the system chloroform–methanol (99 : 1) yielded an oil which was precipitated by ether. Further chromatographic purification and crystallization from benzene provided *IV* (400 mg) and *V* (200 mg). Twenty times repeated semipreparative cycle yielded *V* (17 mg) of HPLC purity. Both alkaloids give a positive van Urk reaction. Among the products of their acid hydrolysis were found valine, leucine, and proline (TLC on silica gel, phenol–water, detection by ninhydrine).

N-(D-*Lysergyl*-L-*valyl*)-*cyclo*(L-*phenylananyl*-D-*prolyl*) (II):

Physical properties identical with lit.[3]. Mass spectrum *m/z* (% of relative intensity, elemental composition, assignment): 593 (0·6, $C_{35}H_{39}N_5O_4$, M^+), 349 (16, $C_{21}H_{23}N_3O_2$), 321 (25, $C_{20}H_{23}N_3O$), 244 (40, $C_{14}H_{16}N_2O_2$, *d*), 221 (41, $C_{15}H_{13}N_2$), 153 (78, $C_7H_9N_2O_2$, *g*), 125 (100, $C_6H_9N_2O$, *h*), 120 (8, $C_8H_{10}N$, *k*), 91 (64, C_7H_7), 70 (81, C_4H_8N, *i*).

N-(D-*Lysegyl*-L-*valyl*)-*cyclo*(L-*valyl*-D-*prolyl*) (IV):

M.p. 196—198°C, $[\alpha]_D^{20}$ +57·8° (*c* 0·5%, pyridine). Mass spectrum: 545 (0·8, $C_{31}H_{39}N_5O_4$, M^+), 349 (3, $C_{21}H_{23}N_3O_2$), 321 (3, $C_{20}H_{23}N_3O$), 221 (7, $C_{15}H_{13}N_2$), 154 (54, $C_7H_{10}N_2O_2$, *f*), 125 (19, $C_6H_9N_2O$, *h*), 72 (13, $C_4H_{10}N$, *k*), 70 (100, C_4H_8N, *i*).

N-(D-*Lysergyl*-L-*valyl*)-*cyclo*(L-*leucyl*-D-*prolyl*) (*V*):

M.p. 108—110°C, $[\alpha]_D^{20}$ +31·1° (*c* 0·5%, pyridine). Mass spectrum: 559 (1, $C_{32}H_{41}N_5O_4$, M^+), 349 (4, $C_{21}H_{23}N_3O_2$), 321 (6, $C_{20}H_{23}N_3O$), 221 (15, $C_{15}H_{13}N_2$), 167 (8, $C_{12}H_9N$), 154 (79, $C_7H_{10}N_2O_2$, *f*), 125 (25, $C_6H_9N_2$, *h*), 86 (19, $C_5H_{12}N$, *k*), 70 (100, C_4H_8N, *i*).

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