ISOLATION OF N-[N-(D-LYSERGYL)-L-VALYL]-CYCLO(L-PHENYLALANYL-D-PROLYL) FROM THE ERGOTOXINE TYPE OF FIELD ERGOT AND SOME OF ITS REACTIONS*

A.ČERNÝ[a], A.KRAJÍČEK[b], J.SPÁČIL[b], M.BERAN[a], B.KAKÁČ[a] and M.SEMONSKÝ[a]

[a] *Research Institute of Pharmacy and Biochemistry, 130 60 Prague 3 and*
[b] *Galena, Komárov u Opavy*

Received March 23rd, 1976

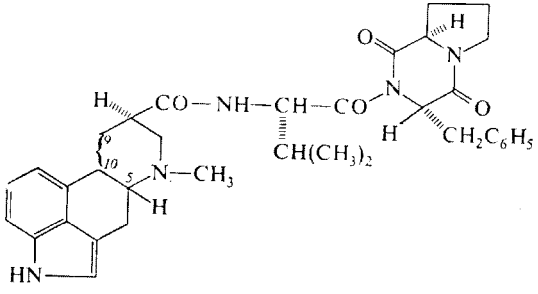
From a group of alkaloids extracted under gentle conditions from field ergot containing mainly ergocristine a new alkaloid was isolated and identified as N-[N-(D-lysergyl)-L-valyl]-cyclo(L-phenylalanyl-D-prolyl) (*I*). The structure of the alkaloid was confirmed by its conversion to the 9,10-dihydro derivative *II* which was methanolyzed to the methyl ester of N-(D-dihydrolysergyl)-L-valine (*IV*) and cyclo(L-phenylalanyl-D-prolyl) (*III*) and hydrazinolyzed to the hydrazide of N-(D-dihydrolysergyl)-L-valine (*V*). Alkaloid *I* is identical with the alkaloid isolated by Stütz and coworkers from lyophilized mycelia of *Claviceps purpurea* producing ergocristine.

During isolation of ergocristine from field ergot of ergotoxine type which produced mainly ergocristine, thin-layer chromatography of the crude set of bases detected the presence of a new alkaloid which was readily decomposed with alkalis and with methanol. To isolate the alkaloid the ergot was extracted at 4°C with a mixture of ether and 20% ethanol; the extract was freed of nonalkaloid and coloured substances and the mixture of bases was purified *via* tartrates and phosphates. The new alkaloid was concentrated in mother liquors after preparation of base phosphates from which it was isolated in a pure state by column chromatography on silica gel and crystallization from benzene. The properties and the physico-chemical constants of the alkaloid isolated here corresponded to N-[N-(D-lysergyl)-L-valyl]-cyclo(L-phenylalanyl-D-prolyl) (*I*) which was isolated by Stütz and coworkers[1] from lyophilized mycelia of ergocristine-producing *Claviceps purpurea* at the time when we were working on the structure of the alkaloid.

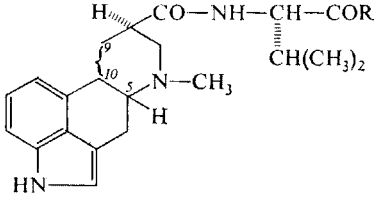
Alkaloid *I* was hydrogenated in aqueous dioxane, using Raney nickel as catalyst when (as indicated by spectral analysis) the double bond in position 9,10 of the ergolene part of I was saturated and a dihydro derivative was formed (*II*). The residue of D-dihydrolysergic acid in the molecules of *II* – *V* corresponds to D-dihydrolysergic-I acid. Methanolysis of *II* at 100°C yielded as principal products cyclo(L-phenylalanyl-

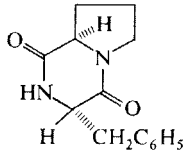
* Part XLVIII in the series Ergot Alkaloids; presented at the Conference on Medicinal Plants, Mariánské Lázně, April 21–25, 1975. Part XLVII: This Journal *41*, 2765 (1976).

-D-prolyl)[2] (*III*) and the methyl ester of D-dihydrolysergyl-L-valine[3] (*IV*); this last-named compound was identical with the authentic sample prepared by the azide method from the hydrazide of D-dihydrolysergic acid and L-valine methyl ester.



I, $C_{(9)}C_{(10)} = -CH{=}C<$
II, $C_{(9)}C_{(10)} = -CH_2-CH<$ ($H_{(5)}$, $H_{(10)}$ *trans*)





III

IV, *V*, $C_{(9)}C_{(10)} = -CH_2-CH<$, ($H_{(5)}$, $H_{(10)}$ *trans*)
IV, R = OCH_3
V, R = $NHNH_2$
VI, $C_{(9)}C_{(10)} = -CH{=}C<$, R = $NHNH_2$

The amide bond between the residue of D-lysergyl- or D-dihydrolysergyl-L-valine and the substituted 2,5-piperazinedione in alkaloids *I* and *II* is exceptionally sensitive to alkaline conditions. Thus, *e.g.*, upon treatment with hydrazine hydrate in aqueous dioxane at room temperature alkaloid *II* gives rise to hydrazide *V* which corresponds by its properties to literature data[3], while alkaloid *I* gives rise to hydrazide *VI*.

EXPERIMENTAL

The melting points were determined in Kofler's block and are not corrected. Samples of compounds for analysis were dried at 0·1 Torr at a temperature proportional to their melting point. The values of specific rotation were obtained in a Perkin-Elmer type 141 polarimeter and refer to compounds free of crystal solvent. The UV spectra were measured in an Optica Milano CF 4R

spectrophotometer, IR spectra in an Infrascan Hilger and Watts spectrophotometer. ^{1}H-NMR spectra were measured in a Tesla BSC 487 spectrometer, in deuteriochloroform, using tetramethylsilane as internal standard. The values are given in the δ p.p.m. scale. The homogeneity of the compounds was evaluated by paper chromatography in 1-butanol–acetic acid–water 4 : 1 : 5 (system A) or on a paper impregnated with formamide using benzene as the mobile phase (B) and by thin-layer chromatography on reflex silica gel plates with a luminescence indicator (Silufol UV_{254} by Kavalier) in chloroform–ethanol–triethylamine 90 : 10 : 5 (C) and on silica gel (Merck) in chloroform–benzene–ethanol 75 : 15 : 10 (D) and methanol–butyl acetate–10% acetic acid 5 : 5 : 1 (E). Compounds *I* and *VI* were detected under UV light at 366 nm, the hydrogenated alkaloids were detected after paper chromatography under UV light after previous illumination with sunlight, after thin-layer chromatography with a 0·5% solution of *p*-dimethylaminobenzaldehyde in cyclohexane and hydrogen chloride vapour. Amino acid analysis was done after an acid hydrolysis of samples carried out by a 10 h heating of the compound with 20% hydrochloric acid at 100°C.

N-[N-(D-Lysergyl)-L-valyl]-cyclo(L-phenylalanyl-D-prolyl) (*I*)

1000 g ground ergotoxine ergot of Czechoslovak origin known to produce mainly ergocristine was macerated with 2000 ml of a mixture of ether with ethanol (4 : 1) for 5 h at 4°C. Maceration with the same mixture (1200 ml) was repeated four times. The combined extracts were evaporated at reduced pressure, the residue (200 g) was stirred with 2000 ml light petroleum (b.p. 30—60°C). The petroleum-insoluble fraction was filtered, dissolved in acetone and bases were precipitated with excess light petroleum. The crude product was digested with 160 ml chloroform and, after separation of the insoluble fraction, the sample was chromatographed on a column of 40 g silica gel, using chloroform and then a mixture of chloroform and methanol (9 : 1) to elute the compounds. The alkaloid-containing fractions were freed of solvents by evaporation to yield 9·75 g of a mixture of alkaloids which was purified *via* tartrates and phosphates of the bases (see ref.[4]). Bases isolated from mother liquors after the above salts were combined (2·1 g) and chromatographed on a column of silica gel (200 g) using chloroform and 5% methanol for elution of substances. Fractions containing pure alkaloid *I* were pooled and the base was crystallized from benzene, m.p. 234—236°C (under decomposition), $[\alpha]_D^{20}$ +8° (*c* 0·5, chloroform), and $[\alpha]_D^{20}$ +41° (*c* 0·5, pyridine); R_F 0·7 (B), 0·65 (C), 0·55 (D). UV spectrum (methanol); λ_{max} 308 (3·957), 219 (4·404) nm (log ε). IR spectrum (KBr pellet): 3300 (indole NH), 1709 (amide-I), 1665 (cyclic tertiary amidic carbonyls), 1535 (amide-II), 790 (9, 10 double bond) cm^{-1}. ^{1}H-NMR spectrum: 0·78 (3 H, d), 1·00 (3 H, d) and 2·00 (1 H, m), H of the isopropyl group; 2·49 (1 H, t), hydrogen at $C_{(2)}$ of proline; 3·67 (3 H, s) hydrogens of the methyl group at $N_{(6)}$; 3·30 (1 H, m), hydrogen at $C_{(8)}$; 3·21 (2 H, d), 7·27 (5 H, s), hydrogens of the benzyl group; 5·25 (1 H, t), hydrogen at $C_{(2)}$ of phenylalanine; 5·68 (1 H, dd), hydrogen at $C_{(2)}$ of valine; 6·49 (1 H, mcd), 6·90 (1 H, s) and 7·00—7·30 (3 H, m), hydrogens of the lysergic acid residue; 8·45 (1 H, d), hydrogen of the amide group; 8·60 p.p.m. (1 H, bs, disappears after deuterization), hydrogen at $N_{(1)}$. Mass spectrum: 593 (M^+). For $C_{35}H_{39}N_5O_4$ (593·7) calculated: 70·81% C, 6·62% H, 11·79% N; found: 70·60% C, 6·80% H, 11·76% N. Titration equivalent (by titration with perchloric acid in anhydrous acetic acid): 596·2. Amino acid analysis: 1 valine, 1 phenylalanine, 1 proline. Alkaline hydrolysis (by 6 h boiling with 8% aqueous solution of potassium hydroxide according to[5]): thin-layer chromatography and comparison with a standard identified lysergic acid (E).

Dihydrogen phosphate of base I: m.p. 193—195°C under decomposition (from ethanol). For $C_{35}H_{42}N_5O_8P$ (691·7) calculated: 10·13% N, 4·47% P; found: 10·20% N, 4·33% P.

N-[N-(D-Dihydrolysergyl)-L-valyl]-cyclo(L-phenylalanyl-D-prolyl) (*II*)

A solution of alkaloid *I* (0·59 g, 1 mmol) in 90% aqueous dioxane (20 ml) was hydrogenated in the presence of 0·5 ml Raney nickel (suspension in water) under atmospheric pressure at 40 to 50°C until consumption of hydrogen ceased (some 2 h). The precipitated dihydro derivative was dissolved by heating the mixture, the catalyst was filtered while hot, the filtrate was concentrated at reduced pressure to about 1/10 the original volume and an equal volume of water was added. The precipitate was filtered; 0·64 g containing 13·5% crystal solvent, m.p. 249—251°C (90% aqueous dioxane), $[\alpha]_D^{20}$ +20·5° (*c* 0·5, pyridine), R_F 0·3 (B), 0·6 (C), 0·35 (D). UV spectrum (methanol): λ_{max} 289 (3·762), 279 (3·858), 273 (3·842) 222 (4·462) nm (log ε). IR spectrum (KBr pellet): 3 300 (indole NH), 1 709 (amide-I), 1 665 (cyclic tertiary amidic carbonyls), 1 535 (amide-II) cm^{-1}. NMR spectrum: 0·84 (3 H, d), 1·02 (3 H, d) and 2·00 (1 H, t), hydrogens of the isopropyl group; 323 (2 H, t) and 7·27 (5 H, s), hydrogens of the benzyl group; 2·5 (1 H, t) and 5·24 (1 H, t), hydrogens of dioxopiperazine; 6·80—7·30 (3 H, m), hydrogens of indole; 6·89 (1 H, s), hydrogen at $C_{(2)}$ of lysergic acid, 3·50 (3 H, s), hydrogens of the methyl group at $N_{(6)}$; 8·50 p.p.m. (1 H, bs, disappears after deuterization), hydrogen at $N_{(1)}$. Mass spectrum: 595 (M^+). For $C_{35}H_{41}N_5O_4$ (595·7) calculated: 70·57% C, 6·93% H, 11·75% N; found: 70·64% C, 7·04% H, 11·75% N.

Methyl Ester of N-(D-Dihydrolysergyl)-L-valine (*IV*)

a) *By methanolysis of alkaloid* II: Suspension of *II* (0·5 g) in methanol (10 ml) was heated for 8 h in a sealed tube on a boiling water bath. Methanol was distilled away at reduced pressure from the solution containing *II* and *IV* and 5—10% of another less polar compound (in systems B and C); the residue was dissolved in ether (100 ml) and the solution extracted with water (5 . 5 ml), into which the cyclodipeptide *III* shifted. Crude *IV* (0·4 g) was obtained from the ether fraction after distillation of the solvent. This was purified by chromatography on a column of silica gel (10 g), using a mixture of chloroform with methanol (9 : 1) for elution. Then its was recrystallized from acetone, m.p. 243—245°C (under decomposition), $[\alpha]_D^{20}$ —135° (*c* 0·2, pyridine). It is identical in its physico-chemical constants with the authentic sample (see below).

b) *From the hydrazide of* D-*dihydrolysergic*-I *acid*: A 1M solution of sodium nitrite (5 ml) was added to suspension of the hydrazide of D-dihydrolysergic-I acid (ref.[6,7]) (1·42 g, 5 mmol) in 0·2M hydrochloric acid (25 ml) at 0—5°C under stirring. This was followed by a dropwise addition of 30 ml 0·2M hydrochloric acid. The mixture was stirred for 10 min, made alkaline with 1M sodium hydrogen carbonate (10 ml) and azide was extracted with ether. The dried ether extract (K_2CO_3) was concentrated at 0°C to about 15 ml, the precipitated azide was dissolved by adding 15 ml dioxane and the solution was combined with L-valine methyl ester (1·31 g, 10 mmol). The mixture was left to stand for 24 h at 20°C in the dark, the solvents were evaporated at reduced pressure, the residue was dissolved a in mixture of chloroform with methanol (9 : 1) and the solution was shaken with water. After drying the chloroform fraction (Na_2SO_4) and evaporation of the solvents at reduced pressure, the product (1·61 g) which contained besides ester *IV* a substantial amount of a more polar compound (systems B,C), probably the corresponding urea, was chromatographed on a column of silica gel (32 g), using a mixture of chloroform with methanol (9 : 1) for elution. Fractions containing ester *IV* (1·18 g) were recrystallized from acetone to yield ester *IV* melting at 243—245°C (under decomposition), $[\alpha]_D^{20}$ —136° (*c* 0·2, pyridine)[3]. UV spectrum (methanol): λ_{max} 292 (3·72), 281 (3·80), 275 (3·78) nm (log ε).

Cyclo(L-phenylalanyl-D-prolyl) (*III*)

The aqueous extracts after processing the product of methanolysis of *II* (see above) were evaporated at reduced pressure and the residue (0·17 g) was recrystallized from ethyl acetate. The cyclo-

dipeptide obtained (*III*, 50 mg) corresponded in its properties to data from the literature[2] (m.p. 150—151°C, $[\alpha]_D^{20}$ +99° (*c* 0·23, water)).

Hydrazide of N-(D-Dihydrolysergyl)-L-valine (*V*)

Compound *II* (0·345 g containing 13·5% crystal solvent, 0·5 mmol) was dissolved at 80—90°C in 80% aqueous dioxane (14 ml), the solution was cooled to 25°C. Methanol (1·7 ml) and hydrazine hydrate (1·7 ml) were added and the mixture was left to stand for 3 days at 20°C. Precipitate was 0·147 g (80%) of hydrazide *V*, melting at 300—310°C (decomposition)[3], $[\alpha]_D^{20}$ −127·5° (*c* 0·2, 90% aqueous pyridine).

Hydrazide of N-(D-Lysergyl)-L-valine (*VI*)

Hydrazinolysis of alkaloid *I* (1·0 g) carried out as with *II* yielded the hydrazide *VI* (0·43 g; 67%). After recrystallization from 90% ethanol it melted at 250—300°C (decomposition); $[\alpha]_D^{20}$ −12·2° (*c* 0·2, 90% pyridine). Amino acid analysis: valine. For $C_{21}H_{27}N_5O_2$ calculated: 66·12% C, 7·13% H, 18·36% N; found: 66·03% C, 7·25% H, 18·52% N.

The analyses were done by Mrs J. Komancová, paper chromatography by Mrs M. Jelínková at the analytical department of this institute (headed by Dr J. Körbl). The molecular weights were determined by mass spectrometry by Dr M. Ryska, Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague.

REFERENCES

1. Stütz P., Brunner R., Stadler P. A.: Experientia *29*, 936 (1973).
2. Ott H., Frey A. J., Hofmann A.: Tetrahedron *19*, 1675 (1963).
3. Stoll A., Hofmann A., Petrzilka T.: Helv. Chim. Acta *34*, 1544 (1951).
4. Pitra J., Sapara V.: Česk. Farm. *5*, 585 (1956).
5. Jacobs W. A., Craig L. C.: J. Biol. Chem. *104*, 547 (1934).
6. Stoll A., Hofmann A., Petrzilka T.: Helv. Chim. Acta *29*, 635 (1946).
7. Stoll A., Hofmann A., Jucker E., Petrzilka T., Rutschmann O., Troxler F.: Helv. Chim. Acta *33*, 108 (1950).

Translated by A. Kotyk.