N-ETHOXYCARBONYL-L-PROLINAMIDE, A NEW ALKALOID FROM THE LEAVES OF *Arnica montana* L.*

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From the chloroform extract of the leaves of *Arnica montana* L. the as yet undescribed N-ethoxycarbonyl-L-prolinamide (*V*) was isolated in addition to other substances. Its structure was elucidated on the basis of mass, infrared and ^{1}H-NMR spectroscopy and confirmed by synthesis.

Several years ago we studied the compounds of the light petroleum extract of the leaves of *Arnica montana* L. (*Compositae*, tribe *Senecioneae*)[1]. We also investigated the substances which we obtained by chloroform extraction from a material previously extracted with light petroleum. The chloroform extract was worked up similarly as the ethanolic extract obtained during the isolation of salonitenolide[2], and we isolated from it both substances which were also present in the light petroleum extract, such as arnicolide A (*I*), tetrahydrohelenalin (*II*) and dihydrohelenalin (*III*), and also loliolide (*IV*) described in anothe paper[3] and, finally, an alkaloid (*V*) of the composition $C_8H_{14}N_2O_3$ with two active hydrogens. The infrared spectrum of substance V contained absorption bands at 3390, 3205, 1675 and 1628 cm^{-1}, corresponding to the amide group, and bands at 1683 and 1184, belonging to the ethoxycarbonyl group.

The structure of the substance under investigation was deduced from the following facts: Its elemental analysis agrees with the high resolution mass spectrometric determination of the molecular peak. The integral of the ^{1}H-NMR spectrum corresponds to 12 protons where 2 protons, unobserved in the spectrum, are exchangeable, as indicated by the number of active hydrogens. The loss of CH_2NO_2 from M^+ and the composition of the base-peak in the mass spectrum (C_4H_8N) (Scheme 1) indicates the presence of a carboxamide group and a pyrrolidine ring in the investigated molecule. Hence, one of the alternative structure *V* and *VI* might belong to the

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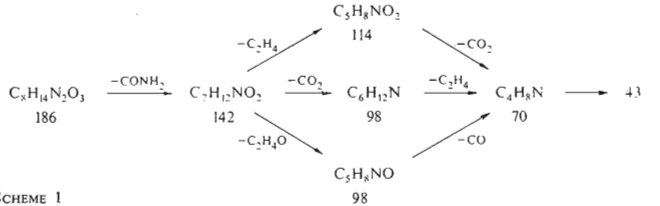
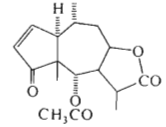
native substance. In view of the fact that 1,2-disubstituted pyrrolidine derivatives lose on electron impact primarily substituents in the position α to the nitrogen atom[4,5], and in view of the fact that the fragmentation of the substance investigated agrees with the fragmentation of N-ethoxycarbonyl-L-prolinol, that begins with the ion m/e 132 (ref.[5]), we consider the structures *V* as more probable.

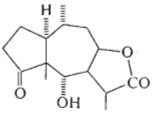


SCHEME 1

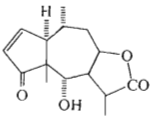
A definite decision as to which of the two structures is correct was brought by the synthesis of compound *V* from L-proline. Applying a known procedure[6] we synthesized the amide of L-proline and submitted it to the reaction with ethyl chloroformate which gave N-ethoxycarbonyl-L-prolinamide (*V*). The latter was identical with the native substance in all respects. Simultaneously the synthesis of compound *V* from L-proline also proves its absolute configuration.



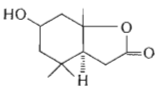
I



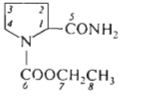
II



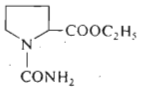
III



IV



V



VI

Up to now, N-ethoxycarbonyl-L-prolinamide (*V*) has not been found in natural material. In view of its structure this compound may be classified among the relatively scarce pyrrolidine alkaloids[7,8], from which it differs, however, by the substitution

with the ethoxycarbonyl group on the nitrogen atom of the pyrrolidine ring, considering that such a substitution is not common even in alkaloids of other types with an amide group.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. For column chromatography silica gel according to Pitra and Štěrba[9] (30–60 μ, deactivated by the addition of 11% of water) was used. For thin-layer chromatography silica gel G Merck was used. The IR spectra were measured on a Unicam SP 200 and on a Zeiss UR-10 (Jena) spectrophotometer. ^{1}H-NMR spectra were measured on a Tesla BS 487 (80 MHz) instrument. The mass spectra were measured on a Varian MAT-311 spectrograph. Optical rotation was determined with an objective polarimeter in methanol. Circular dichroism was measured on a Roussel-Jouan Dichrographe CD 185 in methanol.

Working up of the Chloroform Extract

Dry ground leaves of *A. montana* L. (16 kg) were extracted with light petroleum[1] and then with chloroform. The chloroform extract was evaporated and the residue partitioned between light petroleum and 60% aqueous ethanol as described earlier[2]. A residue (14·1 g) was obtained which was dissolved in benzene and then chromatographed on silica gel (300 g). The course of chromatography is summarized in Table I.

Arnicolide A (I): From fraction 1 (Table I) arnicolide A (*I*) was obtained by chromatography on silica gel. It was identical in all respects with a sample of arnicolide A (*I*) isolated from the light petroleum extract[1].

Loliolide (IV): From fraction 2 (Table I) loliolide (*IV*) was isolated by chromatography on silica gel, as described earlier[3].

Tetrahydrohelenalin (II) *and dihydrohelenalin* (III): From fraction 2 (Table I) tetrahydrohelenalin (*II*) and dihydrohelenalin (*III*) were isolated by chromatography on silica gel; both substances were identical in all respects with those isolated from the light petroleum extract[1].

TABLE I
Chromatography of the Chloroform Extract

Fraction	Solvent	Volume, l	Residue, g	Substance
1	benzene–ether 4 : 1	1·5	1·9	*I*
2	benzene–ether 4 : 1	4·4	2·0	*II*–*IV*
3	benzene–ether 1 : 1	2·5	1·1	–
4	ether–methanol 9 : 1	2·0	3·9	*V*

N-Ethoxycarbonyl-L-prolinamide (*V*)

a) Fraction 4 (Table I; 3·8 g) was rechromatographed on silica gel (200 g). Using light petroleum, acetone and ether (4 : 3 : 3) for elution alkaloid *V* was isolated, m.p. 103—103·5°C (ether), $[\alpha]_D^{20}$ −55·8° (*c* 0·12). ORD spectrum: 350 nm, [*Φ*] −583; 233 nm, [*Φ*] −3457; 225 nm, [*Φ*] −2684 (through). CD spectrum: 215 nm, $\Delta\varepsilon$ −1·69. IR spectrum (cm^{-1}): 3390, 3205, 1675, 1628 ($CONH_2$), 1685, 1184 ($COOC_2H_5$). Mass spectrum (*m/e*, relative intensity, composition): 186, 1·4, $C_8H_{14}N_2O_3$ (M^+); 168, 0·6; 142, 81·9, $C_7H_{12}NO_2$; 114, 11·8, $C_5H_8NO_2$; 98, 20·5, $C_6H_{12}N$, C_5H_8NO; 70, 100, C_4H_8N; 69, 15·7, C_4H_7N; 68, 26·8, C_4H_6N; 55, 6·4; 44, 22·8, CO_2, $CONH_2$, 43, 29·1; 42, 12·6; 41, 27·6. ^{1}H-NMR spectrum (chemical shifts in δ-scale, p.p.m.): 1·31, t, 3 H_8; J = 7·5 Hz; 1·85—2·45, m, 4 H_2 + H_3; 3·57, t. 2 H_4, J = 5·5 Hz; 4·25, q, 2 H_7, J = 7·5 Hz, 4·46 q, 1 H_1, J_1 = 5, J_2 = 7. For $C_8H_{14}N_2O_3$ (186·2) calculated: 51·60% C, 7·58% H, 15·05% N; 1·08% H act.; found: 51·68% C, 7·58% H, 15·13% N, 1·12% H act.

b) Ethyl chloroformate (1·0 ml) was added in several portions to an ice-cooled solution of the amide of L-proline[6] (1·0 g) in 5 ml 50% aqueous acetone and 1·4 ml of triethylamide, under stirring, and the mixture was stirred for an additional 3 hours at room temperature. The mixture was acidified with dilute HCl and extracted with chloroform; the combined chloroform extracts were worked up to give 430 mg of the product from which pure amide *V* (200 mg) was obtained by crystallization. M.p. 102—103°C, $[\alpha]_D^{20}$ −58·1° (*c* 0·45). The mixed melting point of the synthetic and native N-ethoxycarbonyl-L-prolinamide was undepressed. The identity is further confirmed by the identity of all physical constants measured, as well as the analytical data given under *a*).

We are grateful to Dr K. Jošt for his valuable advice and help during the synthetic work. The elemental analyses were carried out in the analytical department of our Institute (under the direction of Dr J. Horáček) by Mrs V. Rusová and Mr V. Štěrba. The infrared spectra were measured by Mrs S. Holubová, Mrs K. Matoušková and Mr P. Formánek. Optical rotation values were determined by Mrs Z. Ledvinová and the circular dichroism and optical rotation dispersion data by Dr S. Vašíčková. We thank all those mentioned, as well as Mrs B. Panáčková and Miss M. Heranová for technical assistance.

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