PYRROLIZIDINE ALKALOIDS. XVI.*

ALKALOIDS FROM SOME PLANTS OF THE GENUS *Ligularia*** ***

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From *Ligularia elegans* CASS. (syn. *L. macrophylla* (LEDEB.) D. C.), *L. brachyphylla* HAND.-MAZZ. and *L. dentata* (A. GRAY) HARA, the alkaloid clivorine $C_{21}H_{27}NO_7 \cdot H_2O$ (*I*) was isolated. In all those plants, the newly described alkaloids ligularine $C_{23}H_{31}NO_9$ (*II*) (isolated from *L. elegans* and *L. dentata*) and ligudentine (isolated from *L. dentata* and *L. brachyphylla*) were detected. Thin-layer chromatography of *L. elegans* revealed a further alkaloid which could not be isolated. From all the plants, fumaric acid was isolated. On the basis of NMR spectral analysis, the structure *II* was proposed for ligularine and a partial structure for ligudentine. Furthermore, the structure *V* was suggested for the dimethylester of the dilactone of clivoric acid.

A few years back, we reported[1] the isolation of the pyrrolizidine alkaloid clivorine $C_{21}H_{29}NO_8$ from the plant *Ligularia clivorum* MAXIM. Later on, we assigned[2-4] the structure *I* ($C_{21}H_{27}NO_7 \cdot H_2O$) to this alkaloid. In this paper, the isolation of alkaloids from three other plants of the genus *Ligularia* (*Asteraceae*) has been described. All these plants are indigenous to East Asia, however, in Europe they are often cultivated as ornamental plants in parcs and green belts.

A work-up of the plant *L. elegans* CASS. (syn. *L. macrophylla* (LEDEB.) D. C.) yielded 0·15% per dry weight of a mixture of crude alkaloids, which is much less than that obtained from the closely related *L. clivorum*[1]. Crystallization of the crude mixture gave an alkaloid which, on the basis of the mixed melting point, infrared spectra and thin-layer chromatography was identical with clivorine (*I*). We divided the mother liquors into an alkaline ethereal and an alkaline chloroform extract. On crystallization, the alkaline ethereal extract afforded some more clivorine (*I*). Thin-layer chromatography (silica gel G) of the mother liquors showed along with

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clivorine (*I*) (hR_F 35) the presence of some other alkaloids of hR_F 75, 72 and 46. The same alkaloids were detected[3] in the chloroform extract of the solution of a mixture of crude alkaloids obtained from *L. clivorum* in 10% hydrochloric acid. Column chromatography on silica gel gave a pure uncrystallized substance of hR_F 46 which we named ligularine. Besides ligularine, this chromatography yielded again clivorine (*I*). We did not succeed to obtain the substances of hR_F 75 and 72 in pure form. By thin-layer chromatography of the alkaline chloroform extract, the presence of clivorine, traces of ligularine, and a substance of hR_F 75 were detected. On crystallization, this mixture gave pure clivorine (*I*).

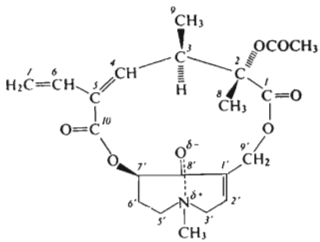
L. dentata (A. Gray) Hara is according to the seed-index of some botanical gardens identical with *L. clivorum*. In the available literature, Engler and Prantl[5] mention the additional designation *dentata* for one form of *L. clivorum*. An investigation of this plant revealed the presence of three alkaloids which were identical with those obtained from *L. clivorum*; the occurrence of the fourth alkaloid of hR_F 75 was not observed. On the basis of this finding it is impossible to decide whether the above mentioned plants are identical because the plants were collected in different years and localities. The total content of the mixture of crude alkaloids in this plant amounted to 0·29% per dry weight. The alkaline chloroform extract yielded pure clivorine; thin-layer chromatography of the mother liquors did not show the presence of any other alkaloid. The alkaline ethereal extract gave a mixture of three alkaloids which on crystallization afforded pure clivorine. By column chromatography of the mother liquors on silica gel, only clivorine could be isolated in crystalline form. Ligularine was again uncrystallized, thin-layer chromatography showed, however, that it was pure. The third alkaloid of hR_F 72 was obtained in form of a chromatographically pure amorphous substance which we named ligudentine.

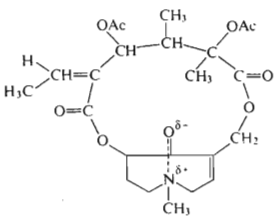
L. brachyphylla Hand.-Mazz. was worked up in the same manner as *L. dentata* to afford on the whole 0·26% of a mixture of crude alkaloids per dry weight. On crystallization, the alkaline chloroform extract gave pure clivorine; thin-layer chromatography of the mother liquors did not reveal the presence of any other alkaloid. The alkaline ethereal extract gave an uncrystallized residue which on thin-layer chromatography was found to consist of the same components as that obtained from the extract from *L. dentata*. Column chromatography on silica gel yielded the pure alkaloids clivorine, ligularine and ligudentine.

In an attempt to isolate alkaloids bound in form of N-oxides, we did not obtain a Dragendorff-positive substance from any of the studied plants of the genus *Ligularia* (the mixture isolated from *L. clivorum*[1] and designated as "alkaloids from N-oxides" was also Dragendorff-negative). It appears that in these plants the absence of alkaloids bound as N-oxides is characteristic. This finding could perhaps serve as one of the chemotaxonomical features when plants of the genus *Ligularia* have to be differentiated from those of the genus *Senecio* where the presence of N-oxides is common. The composition of the nitrogen-containing Dragendorff-negative sub-

stances which are found present in the portion obtained after reduction with zinc dust; in unknown the same.

The methanolic extracts of the three investigated plants gave fumaric acid (see Experimental). Fumaric acid was isolated earlier from *L. clivorum*[1], *Senecio aegyptius* and *S. desfontainei*[6]. Manske[7] has already drawn attention to the presence





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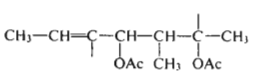

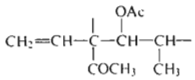

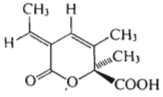

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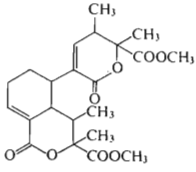

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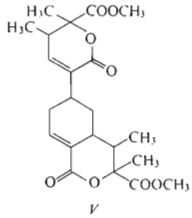

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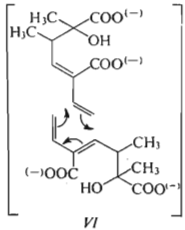

VI

of fumaric acid in some plants of the genus *Senecio*. It appears that the presence of this acid is characteristic for the plants of the genus *Senecio* and *Ligularia*.

THE STRUCTURE OF LIGULARINE AND LIGUDENTINE

The molecular weight (465) of ligularine was determined by mass spectrometry and corresponds to the formula $C_{23}H_{31}NO_9$. The infrared spectrum (Fig. 1) of ligularine in chloroform does not show absorption in the region of vibration of hydroxy groups. The broad absorption band at about 1600 cm^{-1}, similarly to that of clivorine[1], indicates the presence of a basic moiety with transannular interaction between the keto group and the tertiary nitrogen atom. Consequently, the basic moiety of ligularine is probably formed by othonecine or by a substance similar to it. This is in agreement with the course of the mass-spectrometric fragmentation of ligularine which is analogous to that of clivorine (*I*), however, different from that of pyrrolizidine alkaloids having bicyclic bases[8].

Its NMR spectrum (Fig. 2) exhibits one methyl doublet at 1·040 p.p.m. with $J = 7$ Hz, one methyl singlet at 1·475 p.p.m., two CH_3CO— singlets at 2·015 and 2·096 p.p.m., one broad N—CH_3 singlet at 2·145 p.p.m., and one methyl attached to the double bond which appears as doublet of doublets at 1·973 p.p.m. ($J = 7·3$ and 1·3 Hz). The chemical shift value (1·475 p.p.m.) of the tertiary methyl indicates that an oxygen atom is adjacent to the same carbon atom. The N—CH_3 signal was assigned using 0·555 p.p.m. downfield shift after tetradeuterioacetic acid treatment. There are eight protons in 3·0–6·3 p.p.m. region, five of them giving rise to an ABKXY system (5·247, 4·268, 6·260, 3·300, and 3·600 p.p.m.). Irradiation at 6·260 p.p.m. (an olefinic proton signal) perturbs both parts of the XY quartet and also sharpens the lines due to Hp A and B protons. The chemical shifts and $J_{AB} = 11·4$ Hz allow to assign these resonances to a —CH_2OCO— group. The value of $J_{XY} = 18·4$

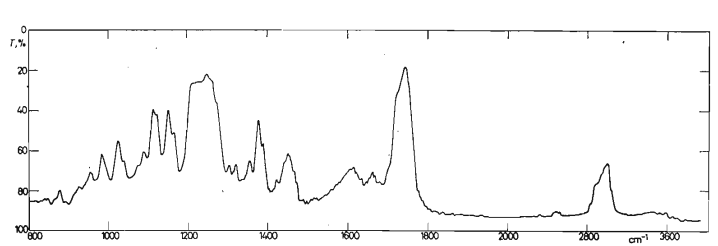

FIG. 1

Infrared Spectrum of Ligularine (*II*) in Chloroform

Hz suggests a geminal coupling. Since these signals moved 0·460 p.p.m. downfield in the solution acidified by tetradeuterioacetic acid, they are attributable to $—CH_2—N$ protons. The decoupling proved that these protons are responsible for the further broadening of the $—CH_2O—$ signals. Thus the following sequence is present in the molecule $—N—CH_2—CH=\overset{|}{C}—CH_2—OCO—$. The five lines multiplet at 4·935 p.p.m. is coupled to two protons located between 2·2 and 2·8 p.p.m. which in turn interact with other two protons in the 2·900 p.p.m. multiplet. The latter experience 0·400 p.p.m. downfield shift after tetradeuterioacetic acid addition. Therefore all these protons belong to the saturated ring system of othonecine. The $\sum J_7$, value equal to 6 Hz indicates[9] the *exo*-buckling of the saturated ring contrary to the unsaturated ring in the pyrrolizidine system. The remaining two protons in the 5·0 to 6·0 p.p.m. region are to be assigned to the protons of the acid component. The olefinic proton signal at 5·855 p.p.m. is a quartet of doublets (J = 7·3 and 0·8 Hz), the former being a geminal coupling to the olefinic methyl and the latter an allylic one to the 5·615 p.p.m. proton, which is responsible for the 1·3 Hz splitting of this peak. The values of these couplings suggest the location of this proton (5·615 p.p.m.) in an allylic position to the double bond. The acetoxy group bonded to this atom could account for the chemical shift value. It must be concluded that the 5·615 p.p.m. proton is also coupled to the proton near 2·025 p.p.m., and that proton is responsible for the 7 Hz interaction of methyl at 1·040 p.p.m. (revealed by Indor[10] experiment). The last signals are methyl at 1·475 p.p.m. and one acetyl group. They are most likely

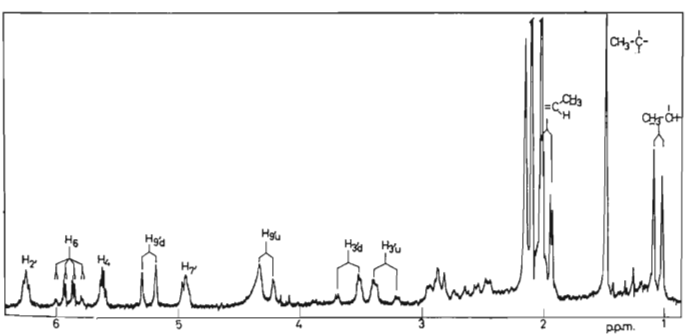

FIG. 2

NMR Spectrum of Ligularine (*II*) in Deuteriochloroform at 100 MHz

attached to the same carbon atom. Now the fragment $CH_3—CH=C(OAc)—CH(CH_3)—C(OAc)—CH_3$ can be drawn up and the formula *II* for the whole molecule is built from the partial structures given above. The acidic moiety of the molecule is an acetylated hygrophyllinecic acid[11] (absolute configuration 2*R*, 3*R*, 4*R*) or its stereoisomer, respectively. In view of the carboxyl group, the *cis*-arrangement of the methyl group at $C_{(6)}$ results from the value of the chemical shift of the olefinic proton at $C_{(6)}$ (5·885 p.p.m.)[8].

On mass spectrometry, ligudentine exhibits the highest peak at *m/e* 406 which, however, is not a molecular peak. The infrared spectrum of this substance in chloroform differs considerably from those of clivorine[1] and ligularine particularly due to the absence of the absorption band at c. 1600 cm^{-1}. Obviously, the basic moiety of ligudentine is of another type than that in clivorine or ligularine. The infrared spectra show that there are no hydroxyl groups present in the molecule.

The NMR spectrum of ligudentine (Fig. 3) shows two methyl singlets at 1·247 and 1·490 p.p.m., one methyl doublet at 1·140 p.p.m. with $J = 6{\cdot}5$ Hz and two acetyl singlets at 2·040 and 2·240 p.p.m. The last signal could represent also a N—CH_3 group. An ABX-type signal arising from a vinyl group (5·150, 5·060, and 6·255 p.p.m.,

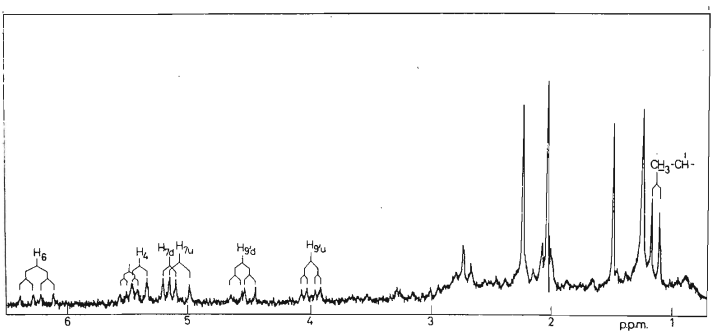

FIG. 3
NMR Spectrum of Ligudentine in Deuteriochloroform at 100 MHz

J = 0·8, 12·0, and 17·8 Hz) is found in the 5·0–6·0 p.p.m. region. The assignment of these protons was confirmed by tickling. The X-proton signal has a small long-range coupling to the doublet at 5·405 p.p.m. (J = 12 Hz). The latter coupling is removed on irradiating the signal of one proton at 2·910 p.p.m. which is also responsible for the 6·5 Hz splitting of the methyl at 1·140 p.p.m. These facts indicate that J = 12 Hz is a vicinal coupling. The 5·405 p.p.m. signal could be assigned to an olefinic proton and, therefore, the acidic moiety might be identical with that of clivorine (*I*). The four-line pattern due to one proton at 5·465 p.p.m. with splitting 5 and 9 Hz is an X-part of a complex system. The remaining protons are hidden in the 2·3–2·9 p.p.m. region. This is in agreement with the assumed —O—CH—CH_2— fragment whose —O—CH— proton can be assigned to $H_{(7')}$. The last two distinct protons form an AB part of an ABX system (δ_A = 4·565, δ_B = 4·00 p.p.m., J_{AB} = = 11·8 Hz, further splitting 9 and 5 Hz) and are ascribed to a —CH_2O— group. As there are no other olefinic protons, the basic moiety might be saturated. The whole structure could not be elucidated owing to the small amount of material available.

Structure of the Dilactone of Clivoric Acid

In the previous paper[3], we have described the alkaline hydrolysis of clivorine (*I*) which gives rise to clivonecic acid (*III*). Acidic hydrolysis of clivorine (*I*) (in 1M-H_2SO_4) affords[3] the dimeric dilactone of clivorinic acid $C_{20}H_{24}O_8$. On esterification with diazomethane it yields a dimethylester C_{22} . $H_{28}O_8$.

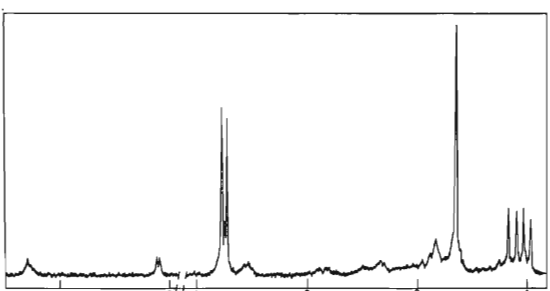

Fig. 4

NMR Spectrum of the Dimethyl Ester Dilactone of Clivorinic Acid (*V*) in Deuteriochloroform at 100 MHz

The nature of its NMR spectrum (Fig. 4) suggests that the molecule is unsymmetric. There are two methyl doublets at 1·145 and 1·000 p.p.m., $J = 7{\cdot}5$ and 6·5 Hz, one methyl singlet at 1·645 p.p.m. integrating for 6 hydrogens, two —$COOCH_3$ singlets at 3·740 and 3·785 p.p.m., one-proton quartet of doublets at 2·870 p.p.m., one distinct proton at 3·550 p.p.m. and two olefinic protons at 7·280 and 6·110 p.p.m. (doublet, $J = 3$ Hz). The lower field olefinic proton is coupled to the 3·550 and 2·870 p.p.m. protons; their interaction is shown by double resonance experiment. On irradiating the 2·870 p.p.m. multiplet, the 3 Hz splitting is removed and the signal is converted to singlet; in addition the 3·550 p.p.m. signal and methyl doublet at 1·145 p.p.m. are perturbed. The second methine proton responsible for the 6·5 Hz coupling of 1·000 p.p.m. doublet was located at 1·980 p.p.m. It has no interaction with olefinic protons. The 7·280 p.p.m. multiplet is coupled to two protons in 2·4—2·6 p.p.m. region. The chemical shift of remaining three protons (1·7—1·9 p.p.m.) points out their aliphatic nature.

The described NMR spectrum is compatible with structures *IV* and *V*. The genesis of these substances can be derived by application of Diels-Alder condensation of two intermediates *VI*. In our opinion, structure *V* appears to be more plausible.

EXPERIMENTAL

The melting points have been determined on the Kofler block and are uncorrected. Thin-layer chromatography was carried out on "silica gel CH" (Spolana, Neratovice) containing gypsum (10%) (in the text referred to as "silica gel G"), using the solvent system benzene–ethyl acetate–diethylamine 7 : 2 1, detection with Dragendorff reagent. The R_F values are expressed as hR_F-values ($R_F \times 100$). Column chromatography was carried out on silica gel, grain size 0·05—0·20 mm (Merck-Darmstadt). The solutions of all the substances in organic solvents were dried over anhydrous sodium sulphate. The infrared spectra were measured in chloroform solutions or in KBr tablets on a Hilger model Infrascan spectrophotometer, the mass spectra on a MCH 1303 (SSSR) and the NMR spectra on a Varian HA-100 instrument; the chemical shifts are expressed in δ values.

Isolation of Alkaloids from *L. elegans* Cass. (syn. *L. macrophylla* (Ledeb.) D. C.)

The dry ground plant (30 kg), collected in October 1967 at Karlova Studánka of Bruntál, Czechoslovakia, was continually extracted with a total of 360 liters of methanol. The extract was concentrated to a volume of 3 liters, diluted with water 1 : 1, acidified with a saturated solution of citric acid and washed several times with light petroleum to remove fatty substances and chlorophyll. Then the solution was extracted 5 times with 2 liters of ether (acidic ethereal extract), made alkaline with ammonia to pH 10·5, and extracted again 5 times with 2 liters of chloroform (alkaline chloroform extract). The aqueous layer was acidified with 15% hydrochloric acid to Congo red, zinc dust (50 g), was added, the mixture was allowed to stand for 20 hours and then filtered. The solution was made alkaline with ammonia, extracted with chloroform, dried, and the solvent removed by distillation to yield only 3·3 g of a residue which was not Dragendorff-positive. By kjehdalization of this fraction, 1·63% N were detected.

The acidic ethereal extract yielded 51·3 g of a residue which on crystallization from ethyl acetate gave 18 g of a substance of m.p. 284—286°C (sealed off capillary) which on the basis of the melting point and the infrared spectra was identified as fumaric acid.

The alkaline chloroform extract was dried, chloroform was removed by distillation to yield 44·8 g of a mixture of crude alkaloids. Crystallization of this mixture from ethyl acetate gave

9·5 g of a substance of m.p. 147—149°C. The mixed melting point with authentic clivorine (*I*) was undepressed and the infrared spectra of these two substances were also identical. The mother liquors were evaporated, the residue dissolved in 5% sulphuric acid, and the solution washed several times with ether. The aqueous solution was then filtered over a thin layer of activated coal, made alkaline with ammonia, and extracted at first 5 times with 200 ml ether (extract *A*) and then 5 times with 200 ml chloroform (extract *B*). The extract *A* was dried and the solvent removed by distillation to yield 3·35 g of a residue which on crystallization gave 1·35 g of clivorine (*I*), m.p. 148—149°C. By thin-layer chromatography (silica gel G) of the mother liquors (2·0 g), clivorine (hR_F 35) along with some other alkaloids of hR_F 46, 72 and 75 were detected. The mixture (2·0 g) was separated by column chromatography on silica gel (60 g) in a tube 28 mm i.d.; 5 ml fractions were collected using the solvent system benzene–ethyl acetate–diethylamine 7 : 2 : 1. On the basis of the results obtained from thin-layer chromatography, the individual fractions were combined, evaporated to dryness, the residue dissolved in 5% sulphuric acid, the solution washed with ether, made alkaline with ammonia, and extracted with chloroform. The chloroform extract was dried and distilled. After having collected a total of 105 fractions, the column was washed once more with 200 ml of ethanol. The fractions 1—63 yielded only an uncrystallized mixture of substances of hR_F 75, 72 and 46 which could not be separated owing to the small amount of material available. The fractions 64—96 yielded uncrystallized ligularine (*II*) (210 mg) of hR_F 46, $[\alpha]_D^{24} -34° \pm 3°$ (*c* 0·82 in chloroform). The molecular ion M^+ 465 in the mass spectrum corresponds to formula $C_{23}H_{31}NO_9$. The infrared spectrum is given in Fig. 1, the NMR spectrum in Fig. 2. The fractions 97—105 gave a mixture of clivorine (*I*) with ligularine (*II*). Crystallization of the ethanolic fraction from ethyl acetate afforded 300 mg of clivorine (*I*). The extract *B* was dried and the solvent was distilled off to yield 15·1 g of a residue which on thin-layer chromatography showed to contain besides clivorine (hR_F 35) traces of substances of hR_F 46 and 75. Crystallization from ethyl acetate gave 7·6 g of clivorine (*I*), m.p. 147—149°C. A further investigation of the mother liquors was not carried out.

Isolation of Alkaloids from *L. dentata* (A. Gray) Hara

The plant was cultivated on our experimental grounds in Olomouc from seeds obtained from the Botanical Gardens, Utrecht (The Netherlands); they were collected when flowering (biennials) in the first half of August 1968. The dry plant and its roots were ground (2337 g) and continually extracted with a total of 30 liters of methanol. The extract was concentrated *in vacuo* to 500 ml, diluted with water 1 : 1 and acidified with citric acid. Chlorophyll and fatty substances were removed with light petroleum, the aqueous layer was extracted 5 times with 200 ml of ether (acidic ethereal extract), made alkaline with ammonia to pH 10·5, and extracted at first 5 times with 200 ml of ether (alkaline ethereal extract) and then 5 times with 200 ml of chloroform (alkaline chloroform extract). After acidification with hydrochloric acid to Congo red, zinc dust (30 g) was added. After twelve hours the mixture was filtered, the solution made alkaline and extracted with chloroform. The yield gave 0·97 g of a residue which on thin-layer chromatography produced a slight Dragendorff-positive spot on the starting line. By kjehdalization of the dry material, 2·22% N were detected.

The acidic ethereal extract was dried and the solvent removed by distillation to yield 7·6 g of a residue. After addition of ethyl acetate, the product afforded 2·2 g of a substance which on the basis of the mixed melting point and infrared spectrum was identified as fumaric acid.

The alkaline ethereal extract yielded 3·0 g of a residue. It was purified in the same manner as that obtained from *L. elegans* and gave 1·9 g of a purified mixture of alkaloids which on crystallization from ethyl acetate afforded 0·7 g of clivorine (*I*), m.p. 147—149°C. The mother liquors (1·2 g) were subjected to column chromatography on alumina (80 g, activity II; Reanal, Hungary)

by using the solvent system benzene–chloroform 4 : 6. Thus, however, the substances could not be separated. Therefore, the mixture was separated in the same manner as that obtained from the extract *A* of *L. elegans* by using silica gel (50 g) in a column of 25 mm i.d. for 1·2 g of the mixture of alkaloids. Fractions amounting to 7 ml, on the whole 78 fractions, were collected and, finally, the column was washed once more with 100 ml of ethanol. The fractions 1—4 gave a Dragendorff-negative residue. From the fractions 5—10, 87 mg of a substance of hR_F 72 were obtained, slightly contaminated by ligularine (hR_F 46). The residue was dissolved in ethanol and filtered over a thin layer of activated coal. The filtrate was evaporated, dissolved in ether and filtered over a thin layer of alumina. After evaporation of the solvent, the filtrate gave 23 mg of glassy ligudentine which on thin-layer chromatography produced only one spot of hR_F 72. On mass spectrometric measurements, the substance exhibited the highest peak at m/e 406. The NMR spectrum is given in Fig. 3. The fractions 11—22 (130 mg) and 41—78 (95 mg) gave a mixture of ligularine and ligudentine, and ligularine and clivorine, respectively. A further investigation of these mixtures was not carried out. From the fractions 23—40, pure ligularine (*II*) (87 mg), $[\alpha]_D^{24} -32° \pm 3°$ (*c* 0·745 in chloroform) was obtained which on the basis of infrared spectral data (Fig. 1) is identical with the substance of hR_F 46 from *L. elegans*. On crystallization, the ethanolic fraction gave 140 mg of clivorine, m.p. 147—149°C, the mixed melting point with the authentic sample was undepressed.

The alkaline chloroform extract yielded 3·9 g of a residue which on the basis of thin-layer chromatography contained only clivorine (hR_F 35). Crystallization from ethyl acetate gave 1·3 g of pure clivorine, m.p. 147—149°C.

Isolation of Alkaloids from *L. brachyphylla* Hand.-Mazz.

The plant was cultivated on our experimental grounds in Olomouc from seeds obtained from the Botanical Gardens of the Justus Liebig University, Giessen, GFR, and the plants (biennials) were collected when flowering at the end of August 1968. The dry ground plant (1 180 g) was continually extracted with methanol (15 liters) and the extract investigated in the same manner as that from *L. dentata*. On thin-layer chromatography of the portion corresponding to alkaloids from N-oxides (0·86 g) produced only one slight Dragendorff-positive spot on the starting line. By kjehdalization of the residue, 1·47% N were detected. The acidic ethereal extract gave 7·7 g of a residue which on crystallization from ethyl acetate afforded 2·1 g of fumaric acid. The alkaline ethereal extract gave 1·2 g of a residue which on thin-layer chromatography showed to contain the alkaloids clivorine (*I*), ligularine (*II*), and ligudentine. The mixture was chromatographed on a column of silica gel in the same manner as that obtained from *L. dentata*. There were obtained 40 mg of ligudentine, 150 mg of ligularine and 350 mg of clivorine. The alkaline chloroform extract yielded 1·9 g of a residue which on thin-layer chromatography showed to contain only clivorine (hR_F 35). The residue was extracted 3 times with 20 ml of boiling ethyl acetate which on crystallization gave 800 mg of pure clivorine of m.p. 148—150°C.

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