

## LEPTOCARPINE, A NEW PROTOPINE ALKALOID

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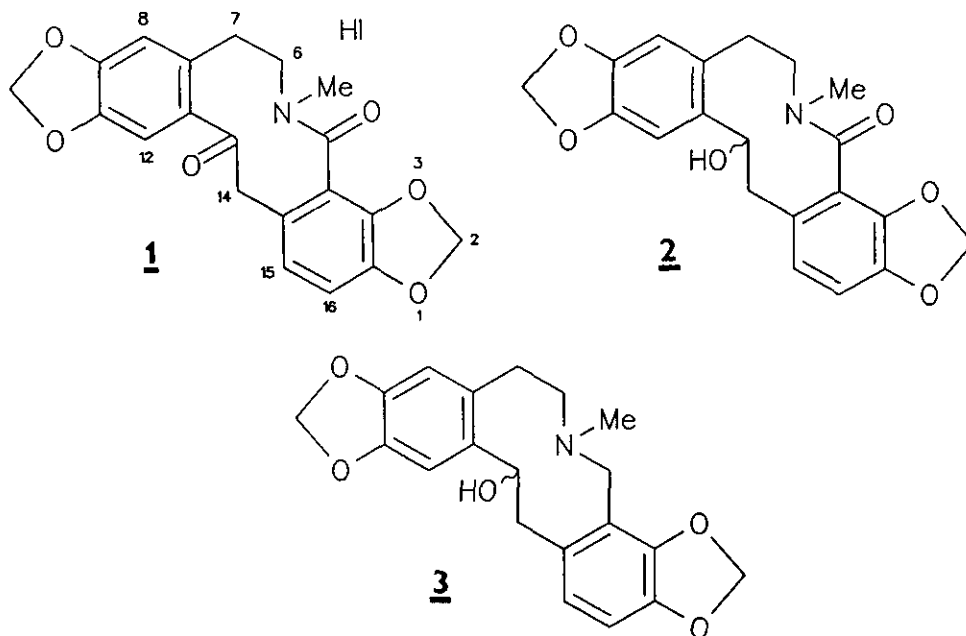
*Abstract* - The structure of leptocarpine (1) a new protopine alkaloid isolated from the whole plant of *Hypocoum leptocarpum* was elucidated on the basis of spectral analysis, including 2D nmr spectroscopy and chemical transformation.

*Dedicated to Prof. Arnold Brossi on the Occasion of his 70th Birthday.*

In our previous work, the alkaloid leptocarpine (formerly denoted as HL1) (1) was isolated from *Hypocoum leptocarpum* HOOK.F. et THOMS.<sup>1</sup> The structure of the optically inactive compound, isolated as a HI adduct from quaternary alkaloid fraction, was not hitherto elucidated. The molecular formula of alkaloid (1) was determined to be C<sub>20</sub>H<sub>17</sub>NO<sub>6</sub> (M<sup>+</sup> 367.1052, calcd 367.1055) by high resolution mass spectrometry (HREIMS) together with elemental analysis. According to <sup>1</sup>H nmr (Table 1), this compound contains two benzene rings (substitution pattern 1,2,4,5- and 1,2,3,4-), two -OCH<sub>2</sub>O- groups, -CH<sub>2</sub>CH<sub>2</sub>-, isolated CH<sub>2</sub>, and CH<sub>3</sub>-N<sup>+</sup>. <sup>13</sup>C Nmr adds to the above mentioned features ten quaternary sp<sup>2</sup>-hybridised carbons consisting of four normal, four bonded to oxygen, and two C=O (Table 2). All protons are attached to carbons. Two -OCH<sub>2</sub>O- groups and

two carbonyls account for the all six oxygen atoms present in the molecule. Two absorptions of compound (1) in ir at 1672 and 1636  $\text{cm}^{-1}$  were observed in the solid state. The former was assigned to the free carbonyl group and the latter indicates the carbonyl group of the amide type.

Reduction of 1 with  $\text{NaBH}_4$  in MeOH afforded optically inactive alcohol (2) in 82 % yield as the only reduction product. The molecular formula  $\text{C}_{20}\text{H}_{19}\text{NO}_6$  ( $M^+$  369.1216, calcd 369.1212) was determined by HREIMS. The CHOH group flanked by a methylene (Table 1) was formed at the expense of one carbonyl (Table 2). The ir spectrum of compound (2) displayed the absorption at 3550  $\text{cm}^{-1}$  (OH) and an amide carbonyl group absorption at 1610  $\text{cm}^{-1}$ .



Further reduction of 2 with  $\text{LiAlH}_4$  in THF removes the remaining carbonyl and gives the key compound (3) (Figure 1),  $\text{C}_{20}\text{H}_{21}\text{NO}_5$  from HREIMS ( $M^+$  355.1415, calcd 355.1419). The connectivity pattern required for structure elucidation was derived by 2D nmr spectroscopy (COSY, delayed

COSY, NOESY, HETCOR,<sup>2</sup> and HETCOR optimized for  $^2J$  and  $^3J$  of 5 and 10 Hz). The ambiguity of the attachment of methylenedioxy group to the second aromatic ring arising from the long-range H,H-couplings was removed by the last mentioned experiment (Figure 1). Thus, the established structure for **3** means that the parent compound (**1**) belongs to the protopine family and has a carbonyl at C-4.

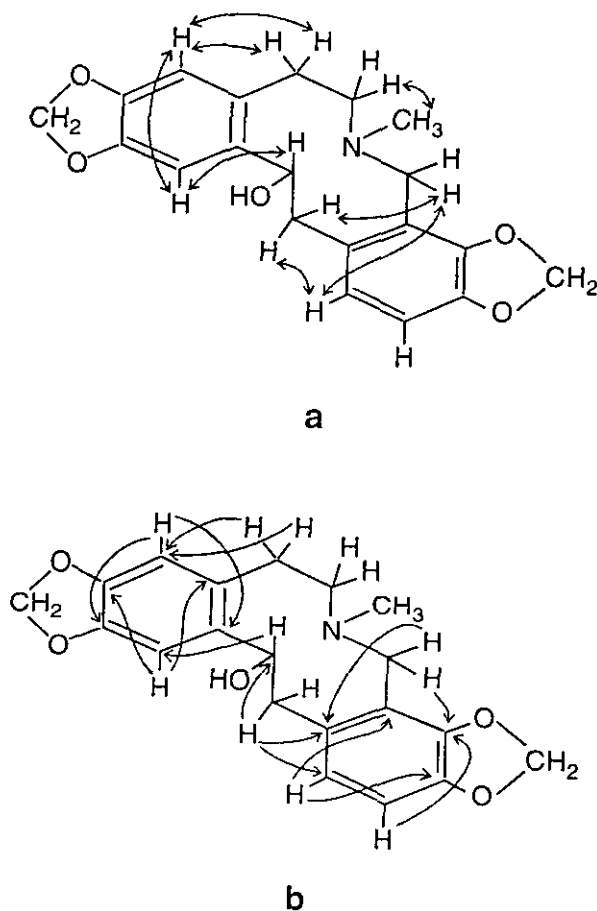


Figure 1. Connectivities Determined by Delayed COSY (a) and Long-range  $^1\text{H}, ^{13}\text{C}$ -Correlated Spectrum (b) for Compound (**3**)

Table 1.  $^1\text{H}$  Nmr Spectral Data for Compounds (1), (2), and (3).

Position	<u>1</u> <sup>a</sup>	<u>2</u> <sup>b</sup>	<u>3</u> <sup>b</sup>
4			4.49 d 11.8 <sup>d</sup> 4.62 d 11.8 <sup>d</sup>
6	4.17 t <sup>c</sup> 7.9 <sup>d</sup>	2.90 m 3.28 m	2.68 m 3.10 m
7	3.22 t <sup>c</sup> 7.9 <sup>d</sup>	2.78 m <sup>c</sup>	2.47 m 2.72 m
8	6.85 s	6.62 s	6.55 s
12	7.11 s	6.72 s	6.71 s
13		4.27 dd 7.9, 4.3 <sup>d</sup>	3.58 dd 8.3, 3.1 <sup>d</sup>
14	3.41 s <sup>c</sup>	3.23 dd 14.5, 7.9 <sup>d</sup> 3.29 dd 14.5, 4.3 <sup>d</sup>	2.98 dd 14.2, 8.3 <sup>d</sup> 2.91 dd 14.2, 3.1 <sup>d</sup>
15	6.58 d 8.0 <sup>d</sup>	6.69 d 7.9 <sup>d</sup>	6.63 d 8.0 <sup>d</sup>
16	6.92 d 8.0 <sup>d</sup>	6.46 d 7.9 <sup>d</sup>	6.70 d 8.0 <sup>d</sup>
N-CH <sub>3</sub>	3.81 s	2.63 s	2.23 s
OCH <sub>2</sub> O	6.10 s <sup>c</sup>	5.98 d 1.3 <sup>d</sup> 5.99 d 1.3 <sup>d</sup>	5.93 d 1.5 <sup>d</sup> 5.94 d 1.5 <sup>d</sup>
OCH <sub>2</sub> O	6.12 s <sup>c</sup>	6.01 d 1.3 <sup>d</sup> 6.02 d 1.3 <sup>d</sup>	5.97 d 1.5 <sup>d</sup> 6.01 d 1.5 <sup>d</sup>

<sup>a</sup>Measured in CDCl<sub>3</sub> + CD<sub>3</sub>OD. <sup>b</sup>Measured in CDCl<sub>3</sub>. <sup>c</sup>Two protons.

<sup>d</sup>Coupling constant(s) in Hz.

Table 2.  $^{13}\text{C}$  Nmr Spectral Data for Compounds (1), (2), and (3).

Carbon	<u>1</u> <sup>a</sup>	<u>2</u> <sup>b</sup>	<u>3</u> <sup>b</sup>
3a	147.12	144.8	146.11
3b	113.56	123.1	123.04
4	166.13	166.7	54.98
6	52.32	48.9	47.49
7	25.14	24.3	24.62
7a	135.93	127.0	127.69
8	110.70	108.3	108.17
8a	153.57	146.2	146.20
11a	148.13	146.4	146.33
12	109.07	107.9	107.68
12a	126.51	127.0	129.57
13	174.12	65.4	66.60
14	34.13	40.4	41.19
14a	120.39	126.7	132.70
15	121.93	124.3	123.36
16	108.28	107.3	107.73
16a	147.35	146.6	145.68
OCH <sub>2</sub> O	102.13	100.8	100.81
OCH <sub>2</sub> O	103.01	100.8	101.03
N-CH <sub>3</sub>	44.70	42.9	43.12

<sup>a</sup>Measured in DMSO. <sup>b</sup>Measured in CDCl<sub>3</sub>.

## EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Ir spectra were measured on a Perkin-Elmer PE-567 spectrophotometer. Uv spectra were recorded by a Perkin-Elmer PE-552 spectrophotometer. Nmr spectra were measured on a Varian VXR-400 spectrometer (400 MHz for  $^1\text{H}$ , 100 MHz for  $^{13}\text{C}$  spectra) at 25 °C. Chemical shifts in ppm are given with respect to TMS, coupling constants

are expressed in Hz. Carbon signal multiplicity were determined by Attached Proton Test (APT) or DEPT experiments. 2D Nmr experiments - COSY, delayed COSY, NOESY,  $^1\text{H}$ ,  $^{13}\text{C}$  - COSY (HETCOR), and  $^1\text{H}$ ,  $^{13}\text{C}$  - COSY optimized for the detection of  $^3\text{J}$  and  $^2\text{J}$  (5 and 10 Hz) - were performed using manufacturer's software. Mass spectra were measured on a Finnigan MAT-90 spectrometer (ei 70 eV, ci  $\text{NH}_3$ ). Elemental analyses were performed with a Perkin-Elmer PE-D240 apparatus. Tlc values were reported previously<sup>1</sup>.

6,7,14-Trihydro-5-methyl-bis[1,3]benzodioxolo[4,5-c:5',6'-g]azecin-4,13(5H)-dione (1) as hydroiodide associate:

Leptocarpine (1) was isolated in the form of hydroiodide as previously described.<sup>1</sup> Yellow needles; mp 246-248°C; Anal. Calcd  $\text{C}_{20}\text{H}_{18}\text{NO}_6\text{I}$ : C,48.30; H,3.68; N,2.85; I,25.63. Found: C,48.36; H,3.85; N,2.62; I,24.92. EIms, m/z (% rel.int.): 367 ( $\text{M}^+\text{-HI}$ , 10), 336 (20), 335 (84), 322 (21), 320 (30), 142 (100), 128 (64), 127 (37); HREIms, ( $\text{M}^+\text{-HI}$ ): 367.1052; CIms, 368 ( $\text{M}^++1$ , 100), 356 (7), 336 (92), 324 (28); uv (MeOH)  $\lambda_{\text{max}}$  223 nm (log  $\epsilon$  4.32), 247 (4.21), 304 (3.85), 365 (3.92); ir (KBr)  $\nu_{\text{max}}$  1672, 1636  $\text{cm}^{-1}$ .

6,7,14-Trihydro-13-hydroxy-5-methyl-bis[1,3]benzodioxolo[4,5-c:5',6'-g]-azecin-4(5H)-one (2), as monohydrate:

Leptocarpine (1) (30 mg) was dissolved in 50 ml of methanol/water (1:1) and excess of  $\text{NaBH}_4$  was added while stirring. Reaction mixture was stirred for 2 h, pH adjusted to 7 with 5% sulfuric acid, and methanol was evaporated under reduced pressure. Solution was basified with conc. ammonia, product (2) was extracted with chloroform and recrystallized from methanol/water. Colorless crystalline solid (24.7 mg, 82%); mp 212°C; Anal. Calcd for  $\text{C}_{20}\text{H}_{19}\text{NO}_6\cdot\text{H}_2\text{O}$ : C,61.99; H,5.42; N,3.61. Found: C,61.75; H,5.61; N,3.64. EIms, m/z (% rel. int.): 190 (100), 162 (19);

HREIms, ( $M^+$ ): 369.1216; CIms 370 ( $M^++1$ , 24), 190 (100); uv (MeOH)  $\lambda_{\max}$  218 nm (log  $\epsilon$  3.95), 295 (3.66); ir (KBr)  $\nu_{\max}$  3550, 1610  $\text{cm}^{-1}$ .

4,6,7,14-Tetrahydro-13-hydroxy-5-methyl-bis[1,3]benzodioxolo-[4,5-*c*:5',6'-*g*](5*H*)azecin (3) as monohydrate:

(a) Leptocarpine (1) (120 mg, 0.24 mmol) was dissolved in 100 ml of dry THF and 0.3 g (7.9 mmol) of  $\text{LiAlH}_4$  were slowly added while stirring. Reaction mixture was heated under reflux for 2 h, then 50 ml of water were added and pH adjusted to 7 with 5% sulfuric acid. THF was evaporated under reduced pressure and solution was basified with conc. ammonia; product (3) extracted with chloroform and recrystallized from ethanol. Yield 37.5 mg (44%). (b) Compound (2) (20 mg) was reduced as in (a). Yield 14.3 mg (74%). Both reduction products were identical. Colorless needles; mp 143-144°C; Anal. Calcd for  $\text{C}_{20}\text{H}_{21}\text{NO}_5$ : C, 67.66; H, 5.97; N, 3.94. Found: C, 67.67; H, 6.07; N, 4.10. EIms,  $m/z$  (% rel.int.): 355 ( $M^+$ , 14), 190 (100); HREIms, ( $M^+$ ): 355.1415; CIms 356 ( $M^++1$ , 100), 338 (40), 190 (60); uv (MeOH)  $\lambda_{\max}$  225 nm (log  $\epsilon$  3.85), 238sh (3.74), 294 (3.75); ir (KBr)  $\nu_{\max}$  3480  $\text{cm}^{-1}$ , no peaks in carbonyl frequency region.

#### ACKNOWLEDGEMENT

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#### REFERENCES

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