

**(-)-ARMEPAVINE: GRADIENT-ENHANCED 2D NMR STUDY AND X-RAY CRYSTAL STRUCTURE DETERMINATION\***Radek MAREK<sup>a1</sup>, Jaromir MAREK<sup>a2</sup>, Jiri DOSTAL<sup>b,\*\*</sup> and Jiri SLAVIK<sup>b</sup><sup>a</sup> *Laboratory of Biomolecular Structure and Dynamics, Faculty of Science, Masaryk University, 611 37 Brno, Czech Republic; e-mail: <sup>1</sup> rmarek@chemi.muni.cz, <sup>2</sup> marek@chemi.muni.cz*<sup>b</sup> *Department of Biochemistry, Faculty of Medicine, Masaryk University, 662 43 Brno, Czech Republic; e-mail: jrdostal@med.muni.cz*

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(-)-Armepravine, a benzyloisoquinoline alkaloid isolated from *Papaver caucasicum*, was examined by gradient-enhanced 2D NMR spectroscopy and X-ray diffraction analysis. Unambiguous <sup>1</sup>H, <sup>13</sup>C, and <sup>15</sup>N NMR assignment of the title alkaloid is reported.

**Key words:** (-)-Armepravine; Isoquinoline alkaloids; *Papaver caucasicum*; Gradient-enhanced 2D NMR spectroscopy; X-Ray crystallography.

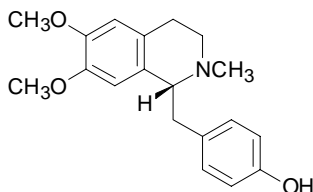
(-)-Armepravine, (1*R*)-1-(4'-hydroxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (**1**), was isolated for the first time from *Papaver armeniacum* (L.) DC. (*Papaveraceae*) by Konovalova *et al.*<sup>2</sup> in 1935 and its structure was solved by the same authors<sup>3</sup>.

(-)-Armepravine was later reported in *P. caucasicum* MARSCH.-BIEB.<sup>4,5</sup> and in several other related *Papaver* species (*cf.* ref.<sup>6</sup>). Enantiomeric (+)-(*S*)-armepravine occurs in *Argemone turnerae* A.M. POWELL (*Papaveraceae*)<sup>7</sup>. (-)-(*R*)-Armepravine was also sporadically found in some plant species of the *Celastraceae*, *Magnoliaceae*, *Nymphaeaceae* (in the racemic form) and *Rhamnaceae* families. A goniometric study of armepravine crystals has been conducted by Uzbek authors<sup>8</sup> in 1962. However, no relevant geometric parameters were reported. Steric correlation of (+)-*O*-methylarmepravine and (+)-laudanidine showed that the stereogenic carbon atom in natural (-)-armepravine has the *R* configuration<sup>9</sup>. It is known for a long time that in the benzyloisoquinoline series, the levorotatory and dextrorotatory alkaloids have the *R* and *S* configurations, respectively<sup>10,11</sup>.

\* Part XCVII in the series Alkaloids of the *Papaveraceae*; Part XCVI: see ref.<sup>1</sup>.

\*\*The author to whom correspondence should be addressed.

Recently, arnepavine oxalate and methiodide have been tested on antiplatelet activity and stated that they exhibited weak but significant inhibition of platelet aggregation<sup>12</sup>. In this paper, we report a detailed account of 2D NMR and crystal structure determination of the title alkaloid.



## EXPERIMENTAL

(-)-Arnepavine, m.p. 147–148 °C,  $[\alpha]_D^{22} -119 \pm 2^\circ$  (*c* 0.5 M, CHCl<sub>3</sub>) was isolated from *Papaver caucasicum* MARSCH.-BIEB. as described in ref.<sup>5</sup>. Colourless crystals were obtained from diethyl ether. <sup>1</sup>H, <sup>13</sup>C, and 2D NMR spectra were recorded on a Bruker Avance DRX 500 (500/125 MHz) spectrometer in CDCl<sub>3</sub> at 303 K,  $\delta$  values are in ppm. Signals were referenced to TMS as an internal standard. DQF-COSY (ref.<sup>13</sup>), NOESY (ref.<sup>14</sup>), HSQC (ref.<sup>15</sup>) and HMBC (refs<sup>16–18</sup>) spectra were recorded using a 5 mm triple-resonance probe head equipped with a gradient coil with following parameters: DQF-COSY spectra: relaxation 2.2 s, gradient ratio 15 : 30 G/cm, MC2 States-TPPI; NOESY spectra: relaxation delay 2.3 s, mixing time 600–800 ms, MC2 States-TPPI; HSQC spectra: relaxation delay 2.3 s, delay for evolution of <sup>1</sup>H-<sup>13</sup>C coupling constants 3.33 ms (optimized for <sup>1</sup>J(H,C) coupling of 150 Hz), gradient ratio 12 : 36 :  $\pm 6$  G/cm, GARP decoupling during acquisition, MC2 echo-antiecho; HMBC spectra: sequence<sup>18</sup> D1-90°(<sup>1</sup>H)-D6-90°(<sup>13</sup>C)-t<sub>1</sub>/2-G1-D16-180°(<sup>1</sup>H)-G2-D16-t<sub>1</sub>/2-90°(<sup>13</sup>C)-G3-D16-ACQ(t<sub>2</sub>), D16 = 100  $\mu$ s, G = 1 ms, MC2 QF; <sup>1</sup>H-<sup>13</sup>C correlation: D1 = 2.7 s, D6 = 66.6 ms, G1 : G2 : G3 = 30 : 18 : 24 G/cm; <sup>1</sup>H-<sup>15</sup>N correlation: D1 = 3.5 s, D6 = 100 ms, G1 : G2 : G3 = 42 : 18 : 30 G/cm.

The diffraction data were collected on a KUMA KM-4 four-circle single crystal diffractometer using the  $\omega$ -2 $\theta$  scan mode. The structure was solved by the direct method using SHELXS86 program<sup>19</sup>. All 24 non-hydrogen atoms were refined anisotropically by the weighted full-matrix least-squares procedure on  $F^2$  with weight  $w = 1/[\sigma^2(F_o^2) + (0.045P)^2 + 0.25P]$ , where  $P = (F_o^2 + 2F_c^2)/3$ , using the SHELXL93 program package<sup>20</sup>. All hydrogen atoms were localized from difference Fourier map and refined isotropically. Our choice of the *R* enantiomer was induced by the fact that we had the authentic (-)-*R*-arnepavine isolated from plant material<sup>9–11</sup>. The molecule was drawn by ORTEP (ref.<sup>21</sup>). Details about measurements and refinement are summarized in Table III.

## RESULTS AND DISCUSSION

The <sup>1</sup>H and <sup>13</sup>C chemical shifts are summarized in Table I. The standard <sup>1</sup>H NMR spectrum of (-)-arnepavine<sup>22,23</sup> in CDCl<sub>3</sub> displayed distinct singlets of the NMe, 6-OMe, and 7-OMe groups. The assignments of <sup>1</sup>H multiplets between 2.6 and 3.8 ppm was based on the gradient-enhanced phase-sensitive DQF-COSY experiment<sup>13</sup> where clear interactions of H-1 with H-11x and H-11y were found. DQF-COSY also indicated

interactions between the H-2' (H-6') and H-3' (H-5') hydrogen atoms. There was also observed a correlation between H-3x and H-3y, H-4x and H-4y and the interaction of the H-3 signal with H-4. The NOESY experiment<sup>14</sup> enabled us to distinguish between H-11x and H-11y, H-3x and H-3y, and to assign the signals of H-5, H-8, H-9, and H-10. The NOESY interactions of H-10 vs H-8 (C-10...H-8 2.50 Å) and H-9 vs H-5 (C-9...H-5 2.50 Å) were found. Considering the conformation in the solution and in the crystal (Fig. 1) the H-11x atom is close to H-8 (H-11x...H-8 3.54 Å vs H-11y...H-8 3.94 Å), H-11y reaches the proximity of NMe (H-11y...C-12 2.50 Å). A weak NOESY interaction of the H-3y signal with the H-11 atoms signal was also observed. The aromatic H-atoms resonated within the range of 5.9–6.9 ppm. The most up-field shifted is the H-8 atom ( $\delta$  5.98) which is near H-11x and probably under influence of the benzene ring anisotropy. Similarly, the 7-OMe group is slightly shifted (3.53 ppm) compared to 6-OMe (3.82 ppm). For an assignment of carbon atoms bound to the hydrogen atoms the gradient-enhanced HSQC pulse sequence<sup>15</sup> was applied. The pulsed-field-gradient<sup>16–18</sup> HMBC record led to the unambiguous assignment of quaternary carbon atoms. The signal of the H-9 hydrogen atoms showed a three-bond scalar interaction with C-6. On the other hand the H-atoms at C-10 correlate with the signal of C-7. Heteronuclear multiple bond interactions were also detected by gradient-enhanced phase-sensitive HSQC (GSQMBC, ref.<sup>24</sup>). All observed scalar as well as dipolar interactions are displayed in Table II. The gradient-enhanced <sup>1</sup>H-<sup>15</sup>N HMBC (refs.<sup>25,26</sup>) spectrum indicated the interactions of H-12, H-11, H-3, and H-4 with the nitrogen atom N-2 at 32.70 ppm.

The X-ray analysis of (-)-armepavine (Tables III and IV, Fig. 1) showed that both the aromatic rings do not deviate significantly from planarity. The average deviation of the atoms C1'–C2'–C3'–C4'–C5'–C6' from the least square plane through the atoms<sup>27</sup> is 0.0047

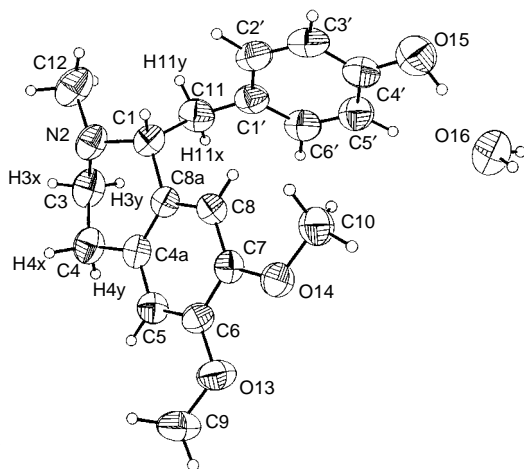


FIG. 1

A perspective view of (-)-*R*-armepavine with atom numbering

Å or  $0.5 \sigma/\delta$ , respectively, the average deviation of the atoms C4a–C5–C6–C7–C8–C8a from the plane is  $0.0027 \text{ Å}$  or  $0.2 \sigma/\delta$ , respectively. The angle between both the least square planes is  $59.3(4)^\circ$ . The adjacent oxygen atoms in the 6-OMe, 7-OMe, and 4'-OH groups are in the aromatic ring planes as indicated by corresponding torsion angles (Table IV). The hydrogenated part of isoquinoline adopts a distorted half-chair confor-

TABLE I  
 $^1\text{H}$  and  $^{13}\text{C}$  NMR data ( $\delta$ , ppm) of (-)-armepavine<sup>a</sup>

Atom	$^1\text{H}^b$	$^{13}\text{C}$	$^1J(\text{H,C})$ , Hz
1	3.72 (5.1,8.3)	64.81	137
3	–	45.93	136
3x	2.83 <sup>c</sup>	–	
3y	3.26 <sup>c</sup>	–	
4	–	24.51	127
4x	2.88 <sup>c</sup>	–	
4y	2.63 <sup>c</sup>	–	
4a	–	125.14	
5	6.56 s	111.23	156
6	–	147.33	
7	–	146.21	
8	5.98 s	111.27	156
8a	–	128.62	
9 (6-OMe)	3.82 s	55.70	144
10 (7-OMe)	3.53 s	55.43	144
11	–	40.26	127
11x	2.73 (8.3,13.6)	–	
11y	3.14 (5.1,13.6)	–	
12 (NMe)	2.51 s	41.95	134
1'	–	130.59	
2',6'	6.89 d (8.5) <sup>d</sup>	130.69	156
3',5'	6.64 d (8.5) <sup>d</sup>	115.38	157
4'	–	154.95	
4'-OH	5.40 br s	–	

<sup>a</sup> For numbering the atoms, see Fig. 1. <sup>b</sup> Coupling constants (in Hz) are given in parentheses. <sup>c</sup> Coupling constants not determined due to complexity of signals. <sup>d</sup> A,A',X,X' spin system.

TABLE II  
Scalar and dipolar interactions in (-)-arnepavine

	1	2	3x	3y	4x	4y	4a	5	6	7	8	8a	9	10	11x	11y	12	1'	2',6'	3',5'	4'	
1	c		d	d	d	d	d	d			b,d	d			a,b,d	a,b,d	b,d	d	d			
2	-	e	e	e																		
3x	d	e	c	a,b,c	a,b,d	a,b,d	d															
3y	d	e	a,b,c	c	a,b,d	a,b,d	d															
4x	e	a,b,d	a,b,d	c	a,b,c	d	d	b,d			d	d										
4y		a,b,d	a,b,d	a,b,c	c	d	d	b,d			d	d										
4a	d	d	d	d	-	d	-	d			d	-										
5	d	d	b,d	b,d	d	c	d	c	d	d	d	d	b									
6						d	-	d			d	d										
7						d	-	d			d											
8	b,d		d	d	d	d	d	d	d	d	c	d	b									
8a	d		d	d	d	d	d	d	d	d	d	-										
9						b	d						c									
10											d	b		c								
11x	a,b,d	e									b	d			c	c		d				
11y	a,b,d	e										d			c	c		d				
12	b,d	e	b,d	b,d													c					
1'	d														d	d		-		d	d	
2',6'	b,d										b		b		d	d		d		c	a,d	d
3',5'																		d		a,d	c	d
4'																		d		d	d	-

a, DQF-COSY; b, NOESY; c,  $^1\text{H}$ - $^{13}\text{C}$  HSQC ( $^1J(\text{H,C}) = 150$  Hz); d,  $^1\text{H}$ - $^{13}\text{C}$  HMBC ( $^2J(\text{H,C}) = 7.5$  Hz); e,  $^1\text{H}$ - $^{15}\text{N}$  HMBC.

mation with the C-3, N-2, and C-4 atoms significantly deviated from the best mean plane of isoquinoline by 0.309(3) Å, 0.448(2) Å, and 0.44(3) Å, respectively, as in other 1,2,3,4-tetrahydroisoquinoline systems<sup>28,29</sup>. Puckering parameters<sup>27</sup> of the C1–N2–C3–C4–C4a–C8a heterocyclic ring are  $Q = 0.511(14)$  Å,  $\phi = 88(2)^\circ$  and  $\theta = 48.5(12)^\circ$ . Armejavine crystallized with one molecule of water which is bound by a hydrogen bond to the phenolic hydroxy group. The length of the hydrogen bond H-15...O-16 is 1.75(4) Å. The O-15...O-16 distance is 2.65(3) Å. The mean value of the three C–N–C bond angles is  $112.4(2)^\circ$  indicating an  $sp^3$  hybridization of the N atom.

TABLE III  
Crystal data and structure refinement for (–)-armepavine

Diffractometer	KUMA KM-4
Formula, m.w.	$C_{19}H_{23}NO_3 \cdot H_2O$ , 331.4
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	$a = 6.2909(4)$ Å, $b = 10.822(2)$ Å, $c = 25.823(6)$ Å
Volume	$1\ 758.0(5)$ Å <sup>3</sup>
Z	4
Density (calculated)	$1.252$ Mg/m <sup>3</sup>
Absorption coefficient	$0.087$ mm <sup>-1</sup>
$F(000)$	712
Crystal size	$0.70 \times 0.60 \times 0.50$ mm
$\theta$ range for data collection	$2.04$ – $26.57^\circ$
Index ranges	$-7 \leq h \leq 1$ , $0 \leq k \leq 13$ , $0 \leq l \leq 32$
Reflections collected	2 189
Independent reflections	2 157 [ $R(\text{int}) = 0.0090$ ]
Refinement method	full matrix least-squares on $F^2$
Data; restraints; parameters	2 157; 0; 317
Goodness-off-fit on $F^2$	0.975
Final $R$ indices [ $I > 2\sigma(I)$ ]	$R1 = 0.0324$ , $wR2 = 0.0790$
$R$ indices (all data)	$R1 = 0.0441$ , $wR2 = 0.0849$
Largest different peak and hole	$0.150$ and $-0.127$ e Å <sup>-3</sup>

TABLE IV  
Bond lengths (Å), bond angles and selected dihedral angles (°) in (-)-armepavine

Atoms	Distances	Atoms	Distances
C1–N2	1.478(3)	C7–O14	1.376(2)
C1–C8a	1.514(3)	C8–C8a	1.405(3)
C1–C11	1.546(3)	C9–O13	1.430(3)
N2–C12	1.464(3)	C10–O14	1.433(3)
N2–C3	1.464(4)	C11–C1'	1.499(4)
C3–C4	1.507(4)	O15–C4'	1.360(3)
C4–C4a	1.505(3)	C1'–C6'	1.377(4)
C4a–C8a	1.384(3)	C1'–C2'	1.402(4)
C4a–C5	1.393(3)	C2'–C3'	1.371(4)
C5–C6	1.374(3)	C3'–C4'	1.384(4)
C6–O13	1.364(2)	C4'–C5'	1.381(4)
C6–C7	1.403(3)	C5'–C6'	1.377(4)
C7–C8	1.371(3)		
Atoms	Angles	Atoms	Angles
N2–C1–C8a	109.5(2)	C7–C8–C8a	121.3(2)
N2–C1–C11	114.5(2)	C4a–C8a–C8	118.7(2)
C8a–C1–C11	112.4(2)	C4a–C8a–C1	122.1(2)
C12–N2–C3	111.5(2)	C8–C8a–C1	119.2(2)
C12–N2–C1	113.5(2)	C1'–C11–C1	113.6(2)
C3–N2–C1	112.1(2)	C6–O13–C9	116.1(2)
N2–C3–C4	109.3(2)	C7–O14–C10	117.2(2)
C4a–C4–C3	111.5(2)	C6'–C1'–C2'	116.7(2)
C8a–C4a–C5	119.7(2)	C6'–C1'–C11	121.7(2)
C8a–C4a–C4	121.3(2)	C2'–C1'–C11	121.6(2)
C5–C4a–C4	119.0(2)	C3'–C2'–C1'	121.3(3)
C6–C5–C4a	121.6(2)	C2'–C3'–C4'	120.9(3)
O13–C6–C5	125.3(2)	O15–C4'–C5'	123.2(3)
O13–C6–C7	115.9(2)	O15–C4'–C3'	118.3(2)
C5–C6–C7	118.8(2)	C5'–C4'–C3'	118.5(3)
C8–C7–O14	124.7(2)	C6'–C5'–C4'	120.2(3)
C8–C7–C6	119.9(2)	C5'–C6'–C1'	122.5(2)
O14–C7–C6	115.4(2)		
Atoms	Dihedral angles	Atoms	Dihedral angles
C4a–C5–C6–O13	178.0(14)	O14–C7–C8–C8a	-179.9(13)
C5–C6–C7–O14	179.9(13)	C2'–C3'–C4'–O15	178(2)
O13–C6–C7–O14	1(2)	O15–C4'–C5'–C6'	-179(2)
O13–C6–C7–C8	-177.5(13)		

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