

**STEREOSTRUCTURE OF (-)-MULTIFLORINE N-OXIDE:  
A NEW LUPIN ALKALOID FROM *LUPINUS HIRSUTUS***

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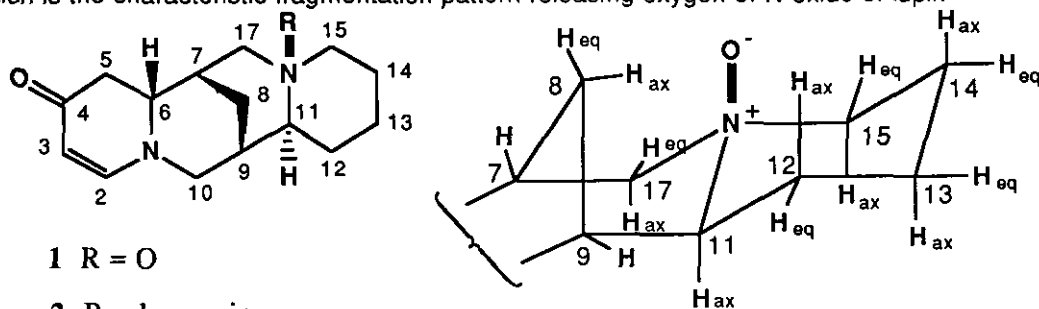
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**Abstract-** A new lupin alkaloid, (-)-multiflorine *N*-oxide (**1**) was isolated from the seedlings of *Lupinus hirsutus* together with twelve known alkaloids. The structure of **1** was determined by spectroscopic methods and by chemical transformations.

*Lupinus hirsutus* Linn. (Leguminosae) is a herbaceous annual plant containing lupin alkaloids. We have already reported the isolation of nine lupin alkaloids from the aerial parts of *L. hirsutus*.<sup>1</sup> We also described the alkaloidal components in the earlier stages of the seedlings and the change of alkaloidal pattern with germination.<sup>2</sup> In the present paper, we describe the structural determination of (-)-multiflorine *N*-oxide (**1**) isolated from the seedlings of this plant together with twelve known alkaloids, (-)-multiflorine (**2**), (-)-13 $\alpha$ -tigloyloxymultiflorine (**3**), (-)-5,6-dehydromultiflorine (**4**), (+)-epilupinine (**5**), (+)-epilupinine *N*-oxide (**6**), (+)-epilupinine acetate *N*-oxide (**7**), (+)-(*trans*-4'-hydroxy-3'-methoxycinnamoyl)epilupinine (**8**), (+)-(*trans*-4'-hydroxycinnamoyl)epilupinine (**9**), (+)-(*cis*-4'-hydroxycinnamoyl)epilupinine (**10**), (+)-(*trans*-4'-acetoxycinnamoyl)epilupinine (**11**), (-)-(*trans*-4'- $\alpha$ -L-rhamnosyloxycinnamoyl)epilupinine (**12**), and (-)-(*cis*-4'- $\alpha$ -L-rhamnosyloxycinnamoyl)epilupinine (**13**).

Compound **1** was isolated as a colorless oil in a yield of 0.0002 % from the fresh seedlings by repeated chromatography. The molecular formula of **1** was determined as C<sub>15</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub> by the

in-beam hrms. The peak at  $m/z$  246 corresponds to the fragment losing 16 mass unit from  $M^+$ , which is the characteristic fragmentation pattern releasing oxygen of *N*-oxide of lupin



1 R = O

2 R = lone pair

Figure

Table 1.  $^{13}\text{C}$  Nmr Data of 1 and 2

C	1	2	1-2
2 (d)	155.8	155.6	+0.2
3 (d)	101.9	98.9	+3.0
4 (s)	191.9	192.5	-0.6
5 (t)	39.9	39.3	+0.6
6 (d)	61.6	60.3	+1.3
7 (d)	30.4	31.1	-0.7
8 (t)	24.5	25.8	-1.3
9 (d)	33.5	34.5	-1.0
10 (t)	57.5	57.5	0
11 (d)	70.8	63.6	+7.2
12 (t)	27.8	31.5	-3.7
13 (t)	23.0	24.8	-1.8
14 (t)	20.3	23.7	-3.4
15 (t)	69.8	55.2	+14.6
17 (t)	65.1	51.1	+14.0

Table 2.  $^1\text{H}$  Nmr Data of 1 and 2

H	1	2	1-2
2	6.85	6.84	+0.01
3	5.60	4.96	+0.64
5ax	2.45	2.16	+0.29
5eq	2.27	2.68	-0.41
6	3.47	3.46	+0.01
7	2.39	2.03	+0.36
8ax	3.85	2.20	+1.65
8eq	1.35	1.28	+0.07
9	1.88	1.65	+0.23
10ax	3.07	3.17	-0.07
10eq	3.11	3.19	-0.08
11	2.91	2.06	+0.85
12ax	2.39	1.47	+0.92
12eq	1.58	1.58	0
13ax	1.42	1.32	+0.10
13eq	1.86	1.78	+0.08
14ax	2.53	1.51	+1.02
14eq	1.62	1.60	+0.02
15ax	3.09	2.17	+0.92
15eq	3.64	2.81	+0.83
17ax	3.33	2.37	+0.96
17eq	3.82	2.92	+0.90

alkaloids.<sup>3-8</sup> Other peaks at  $m/z$  134, 110, 97, 83, 55 and 41 were similar to those of 2. The ir absorption at  $980\text{ cm}^{-1}$  of 1 showed the presence of an *N*-oxide bond. In the  $^{13}\text{C}$  nmr spectrum of 1 (Table 1), the signals of C-11, C-15 and C-17 were shifted downfield in the range of 7-15 ppm compared to those of 2. In the  $^1\text{H}$  nmr spectrum of 1 (Table 2), the protons at C-11, C-15

and C-17 were also appeared in downfield region between  $\delta$  2.9 and 3.8 compared to those of **2**. The shifts of these signals of the carbons and protons adjacent to the tertiary nitrogen atom were in good agreement with the substituent effects of *N*-oxide reported in other lupin alkaloids.<sup>3-8</sup> The signals of axial protons at C-8, C-12 and C-14 were observed in downfield range compared to those of **2**, due to the anisotropic effects of the axial *N*-oxide bond at N-16. Consequently, rings C and D in the structure of **1** were assumed to have boat and chair conformations, respectively, from these <sup>1</sup>H nmr data (Table 2, Figure). The final confirmation of the structure of **1** including the absolute configuration was performed by chemical interconversions between **1** and **2**. The compound(**1**) was reduced by sulphur dioxide to give **2**. Furthermore, **1** was synthesized from **2** by oxidation with *m*-chloroperoxybenzoic acid. In the cd spectrum, **1** showed negative Cotton effects at 326 nm ( $[\theta]_{326} -13300$ ) and at 227 nm ( $[\theta]_{227} -1100$ ) and a positive effect at 296 nm ( $[\theta]_{296} +2700$ ). These are similar Cotton effects to those of **2** ( $[\theta]_{328} -6400$ ,  $[\theta]_{297} +780$ ,  $[\theta]_{224} -2700$ ). The synthetic **1** from **2** showed the same Cotton effects as those of natural **1**. Therefore, the absolute configuration of **1** was confirmed as 6*R*, 7*S*, 9*S*, 11*S*, identical to that of (-)-multiflorine (**2**).<sup>9</sup> So far, we have isolated a few *N*-oxides of lupin alkaloids from leguminous plants.<sup>1-7</sup> Some *N*-oxides of lupin alkaloids were also reported in the literature,<sup>10,11</sup> but the *N*-oxides were not common in the nature. The *N*-oxidation of alkaloids may occur with specific enzymes in plants considering the role of alkaloidal *N*-oxide.<sup>10,11</sup>

## EXPERIMENTAL

<sup>1</sup>H Nmr and <sup>13</sup>C nmr spectra were recorded at 500 and 125.65 MHz, respectively. TMS was used as an internal standard in CDCl<sub>3</sub>. The complete assignments of protons and carbons were made by use of 2D-nmr experiments. Tlc was performed on silica gel plates (60F254, 0.25 mm, Merck) in CH<sub>2</sub>Cl<sub>2</sub>-MeOH-28% NH<sub>4</sub>OH (90:9:1). Analytical hplc was carried out as described previously.<sup>12</sup> Preparative hplc was performed on Licrosorb Si-60 5 $\mu$ m, ( $\phi$  4.6 x 150 mm) column with solvent system of 50% MeOH in ether-5% NH<sub>4</sub>OH (500:20) at 220 nm.

## Plant materials

The seeds of *L. hirsutus* were purchased from Sakata Seeds Co. Ltd., Yokohama, Japan. The seedlings were grown in moistened vermiculite under daylight for 7-10 days at 25°C.

### Extraction and Isolation

The total alkaloidal fraction from the 75% EtOH (12 l) extracts of the fresh seedlings (5 kg) was obtained in a yield of 0.24% of the fresh weight as described previously.<sup>1</sup> The total base (12.0 g) was subjected to a silica gel column using a solvent system of CH<sub>2</sub>Cl<sub>2</sub>-MeOH-28% NH<sub>4</sub>OH as reported in the previous paper.<sup>1</sup> The 1-rich fractions (20 mg) were eluted with the solvent system of CH<sub>2</sub>Cl<sub>2</sub>-MeOH-28% NH<sub>4</sub>OH (100:20:3). The purification of these rich fractions by use of preparative hplc gave pure 1 (11.1 mg), as a colorless oil, [ $\alpha$ ]<sub>D</sub><sup>23</sup> -145.8° (c=0.069, EtOH); uv  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ) 316 (4.10), 229 (3.25 sh.); cd (c=2.8 x 10<sup>-4</sup>, MeOH) [ $\theta$ ]<sub>326</sub> -13300, [ $\theta$ ]<sub>296</sub> +2700, [ $\theta$ ]<sub>227</sub> -1100; hrms (in-beam) *m/z* (%) 262.1680 (M<sup>+</sup>, calcd for C<sub>15</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>, 262.1680, 30), 246 (39), 134 (100), 110 (24), 97 (12), 83 (28), 55 (14), 41 (28); ir  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 2930, 2850, 2770 (C-H), 1640 (conjugated C=O), 1590 (conjugated C=C), 980 (N<sup>+</sup>-O<sup>-</sup>) cm<sup>-1</sup>; <sup>13</sup>C and <sup>1</sup>H nmr chemical shifts were shown in Tables 1 and 2, respectively, <sup>1</sup>H nmr  $\delta$  6.85 (1H, d, J=7.7 Hz, 2-H), 5.60 (1H, d, J=7.7 Hz, 3-H), 3.85 (1H, m, 8-H<sub>ax.</sub>), 3.82 (1H, d, J=12.9 Hz, 17-H<sub>eq.</sub>), 3.64 (1H, br d, J=11.0 Hz, 15-H<sub>eq.</sub>), 3.47 (1H, ddd, J=14.9, 5.0, and 2.5 Hz, 6-H), 3.33 (1H, dd, J=12.9 and 2.5 Hz, 17-H<sub>ax.</sub>), 3.11 (1H, dd, J=12.1 and 3.0 Hz, 10-H<sub>eq.</sub>), 3.09 (1H, 15-H<sub>ax.</sub>, overlapped with the signal of 10-H<sub>ax.</sub> and 10-H<sub>eq.</sub>), 3.07 (1H, dd, J=12.1 and 2.5 Hz, 10-H<sub>ax.</sub>), 2.91 (1H, ddd, J=12.9, 3.6, and 3.6 Hz, 11-H), 2.53 (1H, ddd, J=14.3, 13.5, and 4.1 Hz, 14-H<sub>ax.</sub>), 2.45 (1H, t, J=16.3 Hz, 5-H<sub>ax.</sub>), 2.39 (2H, ddd, J=14.0, 13.2, and 4.1 Hz, 12-H<sub>ax.</sub> and 7-H), 2.27 (1H, ddd, J=16.3, 5.5, and 0.7 Hz, 5-H<sub>eq.</sub>), 1.88 (1H, d, J=2.2 Hz, 9-H), 1.86 (1H, dd, J=15.7 and 1.9 Hz, 13-H<sub>eq.</sub>), 1.62 (1H, br d, J=14.3 Hz, 14-H<sub>eq.</sub>), 1.58 (1H, m, 12-H<sub>eq.</sub>), 1.42 (1H, dddd, J=15.7, 13.2, 4.2, and 4.2 Hz, 13-H<sub>ax.</sub>), 1.35 (1H, d, J=12.6 Hz, 8-H<sub>eq.</sub>).

### Reduction of 1 to 2.

Compound (1) (2 mg) was dissolved in 2 ml of MeOH and reduced with SO<sub>2</sub> gas for 10 min at 0 °C. The reaction mixture was analyzed on hplc.<sup>11</sup> Compound (2) was identified by direct comparison with an authentic sample on hplc.

### Synthesis of 1 from 2.

The compound (1) was synthesized according to the method reported previously.<sup>3</sup> Compound (2) (20 mg) was oxidized with *m*-chloroperoxybenzoic acid (21 mg) in 5 ml of CH<sub>2</sub>Cl<sub>2</sub>. The reacting species was purified by preparative hplc. The pure 1, [ $\alpha$ ]<sub>D</sub><sup>24</sup> -144.4° (c=0.16, EtOH), was obtained in a yield of 75% (16 mg). The structure of synthetic products was identified by ir spectrum and by co-hplc as compound (1).

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