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

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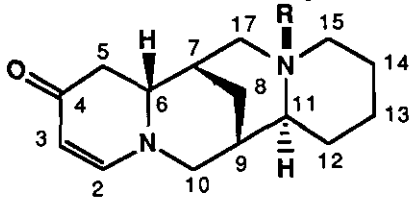
Abstract- A new lupin alkaloid, (-)-multiflorine *N*-oxide (**1**) was isolated from the seedlings of *Lupinus hirsutus* together with twelve known alkaloids. The structure of **1** was determined by spectroscopic methods and by chemical transformations.

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*.[1] We also described the alkaloidal components in the earlier stages of the seedlings and the change of alkaloidal pattern with germination.[2] 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**), (-)-13α-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'-α-L-rhamnosyloxycinnamoyl)epilupinine (**12**), and (-)-(*cis*-4'-α-L-rhamnosyloxycinnamoyl)epilupinine (**13**).

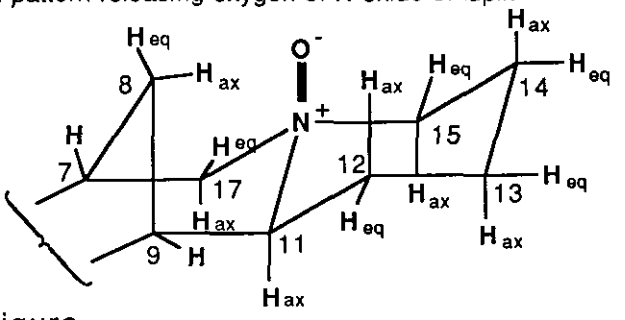
Compound **1** was isolated as a colorless oil in a yield of 0.0002 % from the fresh seedlings by repeated chromatography. The molecular formula of **1** was determined as $C_{15}H_{22}N_2O_2$ by the

in-beam hrms. The peak at *m/z* 246 corresponds to the fragment loosing 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}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}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.[3-8] Other peakes at *m/z* 134, 110, 97, 83, 55 and 41 were similar to those of **2**. The ir absorption at 980 cm^{-1} of **1** showed the presence of an *N*-oxide bond. In the ^{13}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}H nmr spectrum of **1** (Table 2), the protons at C-11, C-15

and C-17 were also appeared in downfield region between δ 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.[3-8] 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 ^{1}H nmr data (Table 2, Figure). The final confirmation of the structure of **1** including the absolute configration 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 configration of **1** was confirmed as *6R, 7S, 9S, 11S*, identical to that of (-)-multiflorine (**2**).[9] So far, we have isolated a few *N*-oxides of lupin alkaloids from leguminous plants.[1-7] Some *N*-oxides of lupin alkaloids were also reported in the literature,[10,11] 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.[10,11]

EXPERIMENTAL

^{1}H Nmr and ^{13}C nmr spectra were recorded at 500 and 125.65 MHz, respectively. TMS was used as an internal standard in $CDCl_3$. The complete assignments of protons and carbons were made by use of 2D-nmr experiments. Tlc was performed on silica gel plates ($60F_{254}$, 0.25 mm, Merck) in CH_2Cl_2-MeOH-28% NH_4OH (90:9:1). Analytical hplc was carried out as described previously.[12] Preparative hplc was performed on Licrosorb Si-60 5μm, (ø 4.6 x 150 mm) column with solvent system of 50% MeOH in ether-5% NH_4OH (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.[1] The total base (12.0 g) was subjected to a silica gel column usinig a solvent system of CH_2Cl_2-MeOH-28% NH_4OH as reported in the previous paper.[1] The **1**-rich fractions (20 mg) were eluted with the solvent system of CH_2Cl_2-MeOH-28% NH_4OH (100:20:3). The purification of these rich fractions by use of preparative hplc gave pure **1** (11.1 mg), as a colorless oil, $[\alpha]_D^{23}$ -145.8° (c=0.069, EtOH): uv λ_{max} (MeOH) nm (log ε) 316 (4.10), 229 (3.25 sh.); cd (c=2.8 x 10^{-4}, MeOH) $[\theta]_{326}$ -13300, $[\theta]_{296}$ +2700, $[\theta]_{227}$ -1100; hrms (in-beam) *m/z* (%) 262.1680 (M^+, calcd for $C_{15}H_{22}N_2O_2$, 262.1680, 30), 246 (39), 134 (100), 110 (24), 97 (12), 83 (28), 55 (14), 41 (28); ir ν_{max} ($CHCl_3$) 2930, 2850, 2770 (C-H), 1640 (conjugated C=O), 1590 (conjugated C=C), 980 (N^+-O^-) cm^{-1}; ^{13}C and ^1H nmr chemical shifts were shown in Tables 1 and 2, respectively, ^1H nmr δ 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-$H_{ax.}$), 3.82 (1H, d, J=12.9 Hz, 17-$H_{eq.}$), 3.64 (1H, br d, J=11.0 Hz, 15-$H_{eq.}$), 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-$H_{ax.}$), 3.11 (1H, dd, J=12.1 and 3.0 Hz, 10-$H_{eq.}$), 3.09 (1H, 15-$H_{ax.}$, overlapped with the signal of 10-$H_{ax.}$ and 10-$H_{eq.}$), 3.07 (1H, dd, J=12.1 and 2.5 Hz, 10-$H_{ax.}$), 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-$H_{ax.}$), 2.45 (1H, t, J=16.3 Hz, 5-$H_{ax.}$), 2.39 (2H, ddd, J=14.0, 13.2, and 4.1 Hz, 12-$H_{ax.}$ and 7-H), 2.27 (1H, ddd, J=16.3, 5.5, and 0.7 Hz, 5-$H_{eq.}$), 1.88 (1H, d, J=2.2 Hz, 9-H), 1.86 (1H, dd, J=15.7 and 1.9 Hz, 13-$H_{eq.}$), 1.62 (1H, br d, J=14.3 Hz, 14-$H_{eq.}$), 1.58 (1H, m, 12-$H_{eq.}$), 1.42 (1H, dddd, J=15.7, 13.2, 4.2, and 4.2 Hz, 13-$H_{ax.}$), 1.35 (1H, d, J=12.6 Hz, 8-$H_{eq.}$).

Reduction of 1 to 2.

Compound (**1**) (2 mg) was dissolved in 2 ml of MeOH and reduced with SO_2 gas for 10 min at 0 °C. The reaction mixture was analyzed on hplc.[11] 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.[3] Compound (**2**) (20 mg) was oxidized with *m*-chloroperoxybenzoic acid (21 mg) in 5 ml of CH_2Cl_2. The reacting species was purified by preparative hplc. The pure **1**, $[\alpha]_D^{24}$ -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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Received, 13th March, 1991