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LUPIN ALKALOIDS FROM THE SEEDS OF *THERMOPSIS LUPINOIDES*

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Key Word Index—*Thermopsis lupinoides*, Leguminosae, seeds, lupin alkaloids, quinolizidine alkaloids, (+)-lupanine N_{16}-oxide, (+)-lupanine

Abstract—A new lupin alkaloid, (+)-lupanine *N*-oxide, was isolated from the seeds of *Thermopsis lupinoides* together with nine known alkaloids. The structure of the new compound was determined by spectroscopic methods and chemical transformations.

INTRODUCTION

Thermopsis lupinoides Link is a herbaceous plant containing a large amount of lupin alkaloids [1]. We have already reported the isolation of eight lupin alkaloids from the flowers, leaves, stems and roots of *T. lupinoides* [2]. In the present communication, we describe the isolation of a new alkaloid, (+)-lupanine *N*-oxide (**1**), from the seeds of *T. lupinoides* and the structural elucidation by spectroscopic methods and chemical transformations.

RESULTS AND DISCUSSION

From the 75% EtOH extract of the seeds of *T lupinoides*, a new alkaloid (**1**) was isolated in a yield of 0.005% of the fr. wt by repeated silica gel chromatography. We also isolated nine known lupin alkaloids, (+)-lupanine (**2**, main base), (−)-anagyrine, (−)-cytisine, (−)-*N*-methylcytisine, (−)-sparteine, (+)-17-oxolupanine, *N*-formylcytisine, baptifoline, and ammodendrine.

The in-beam HRMS spectrum of **1** indicated the molecular formula $C_{15}H_{24}N_2O_2$ (M^+, *m/z* 264.1838, calc. 264.1837). The fragment pattern of **1** was similar to that of (+)-lupanine (**2**). The IR spectrum showed the presence of an amido group (1640 cm^{-1}) and an *N*-oxide bond (930 cm^{-1}). 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, δ 7–14 compared to those of **2**. The up-field shifts, on the contrary, were observed in the signals of C-8, C-12 and C-14, because of the steric effects of axial *N*-oxide bond at *N*-16. These substituent effects of *N*-oxide group were also reported in other lupin alkaloids [3–5]. In the ^1H NMR spectrum of **1**, the signals in low field were assigned by decoupling experiments and comparison

Table 1 ^{13}C NMR chemical shifts of (+)-lupanine *N*-oxide (**1**) and (+)-lupanine (**2**) in $CDCl_3$

C	**1**	**2**	Δ(**1**–**2**)
2	172.1	171.3	+0.8
3	33.0	33.1	−0.1
4	19.4	19.7	−0.3
5	27.7	26.8	+0.9
6	61.8	60.9	+0.9
7	33.6	35.0	−1.4
8	22.7	27.4	−4.7
9	31.7	32.5	−0.8
10	47.0	46.8	+0.2
11	71.4	64.0	+7.4
12	27.7	33.6	−5.9
13	25.7	24.5	+1.2
14	20.3	25.3	−5.0
15	69.6	55.4	+14.2
17	65.2	52.9	+12.3

with the spectral data of **2** The 10α and 10β protons resonated at δ4.52 and 2.55, respectively. The protons at C-15 and C-17 appeared in low-field between δ2.9 and 3 7 compared with those of **2** because of the substituent effects of *N*-oxide [6] Thus, the structure of **1** was determined as (+)-lupanine *N*-oxide

To confirm the structure of **1**, chemical conversions between **1** and **2** were performed. Compound **1** was reduced by sulphur dioxide gas to give **2**. Furthermore, **1** was synthesized from **2** by oxidation with *m*-chloroperoxybenzoic acid.

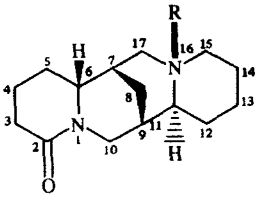
The *N*-oxides of lupin alkaloids are apparently rare in plants [7, 8]. Only seven *N*-oxides [8] and a few other examples [3, 4] are reported in the literature Many of the known lupin alkaloids are, however, oxygenated on carbons adjacent to nitrogen, e.g lupanine, 17-oxolupanine, and 6β-hydroxylupanine [9] The present study is the first isolation of (+)-lupanine *N*-oxide (**1**) from natural sources, although **1** had already been synthesized artificially [10, 11] It is interesting that we have also isolated (+)-lupanine and (+)-17-oxolupanine from the seeds of *T. lupinoides* Because it is possible that **1** is the biosynthetic intermediate from (+)-lupanine to (+)-17-oxolupanine [7]

EXPERIMENTAL

Extraction and isolation of alkaloids The seeds of *T lupinoides* were collected in July in Hokkaido, Japan 10 g of total alkaloidal fraction from 400 g of the seeds was prepared as described in ref [2] This fraction was dissolved in *n*-hexane The insoluble fraction was further purified on a silica gel column **1** was eluted by the solvent system of CH_2Cl_2–MeOH–28% NH_4OH (50 10 1) The 20 mg of **1** was recrystallized from C_6H_6

(+)-*Lupanine* N-*oxide* (**1**) Hygroscopic needle, mp 58° (C_6H_6), $[\alpha]_D^{24}$ +65 5° (EtOH) HRMS (in-beam) *m/z* (rel int) 264 1838 $[M]^+$ (16), 248 $[M-O]^+$ (58), 247 $[M-OH]^+$ (29), 150 (30), 149 (45), 148 (50), 136 (100), 110 (38), 98 (53), 97 (46). 84 (42), 41 (67) IR ν_{max}^{neat} cm^{-1} 1640 (C=O), 930 (N–O) ^{1}H NMR (400 MHz, $CDCl_3$) δ4 52 (1H, *d*, *J* = 13 0 Hz, 10α-H), 3 82 (1H, *m*, $W_{h/2}$ = 20 Hz, 6β-H), 3 66 (1H, *t*, *J* = 11 6 Hz, 17β-H), 3 40 (2H, *m*, $W_{h/2}$ = 21 Hz, 15β-H), 2 99 (1H, *t*, *J* = 12.0 Hz, 15α-H), 2 94 (1H, *dd*, *J* = 11 6 and 2 7 Hz, 17α-H), 2 55 (3H, *d* like, *J* = 13 0 Hz, 10β-H). ^{13}C NMR see the data in Table 1

Reduction of **1** *to* **2**. 10 mg of **1** was dissolved in 2 ml of MeOH and reduced with SO_2 gas for 15 min at 0° The purification by prep TLC using solvent system of CH_2Cl_2-MeOH–28% NH_4OH (980 20 1) gave 4 mg of pure **2**, $[\alpha]_D^{24}$ +65 0



1 R = O
2 R = lone pair

Synthesis of **1** *from* **2** (+)-Lupanine (**2**) (260 mg) was dissolved in 5 ml of CH_2Cl_2 *m*-Chloroperoxybenzoic acid (174 mg) in CH_2Cl_2 was added gradually to the former soln After stirring 6 hr at room temp the products were extracted and purified by CC on silica gel with the solvent system of CH_2Cl_2–MeOH–28% NH_4OH (200 8 1) **1**, $[\alpha]_D$ +65 3°, was obtained with a yield of 71% (190 mg)

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