New Lupine Alkaloids from the Seedlings of Lupinus hirsutus and Change of Alkaloid Pattern with Germination

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NEW LUPINE ALKALOIDS FROM THE SEEDLINGS OF *LUPINUS HIRSUTUS* AND CHANGE OF ALKALOID PATTERN WITH GERMINATION

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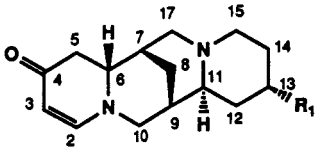
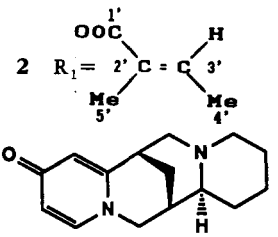
ABSTRACT.—Two new lupine alkaloids, (−)-13α-tigloyloxymultiflorine [**2**] and (+)-(*trans*-4′-acetoxycinnamoyl)epilupinine [**10**], were isolated from 7- to 10-day-old seedlings of *Lupinus hirsutus* (blue lupine) together with ten known alkaloids. The structures of the new alkaloids were confirmed by spectroscopic methods and by chemical transformations. We also clarified variations of the alkaloidal content at various stages of seedling growth.

Plants of the genus *Lupinus* (Leguminosae) are known to contain a variety of structural types of lupine alkaloids (1). They have been used as ornamental plants and as economic plants for fodder and for soil nitrogenation. We have already reported that certain members of the genus *Lupinus* are rich in esters of bicyclic quinolizidine alkaloids with the derivatives of cinnamic acid (2–8). We recently have reported the isolation and structural determination of two new glycosidic lupine alkaloids from the aerial parts of *Lupinus hirsutus* and the distribution of alkaloids in several organs of the mature plant (2). In the present paper, we describe the isolation of two new lupine alkaloids, (−)-13α-tigloyloxymultiflorine [**2**] and (+)-(*trans*-4′-acetoxycinnamoyl)epilupinine [**10**], from the seedlings of *L. hirsutus* together with ten known alkaloids: (−)-multiflorine [**1**], (−)-Δ^5-dehydromultiflorine [**3**], (+)-epilupinine [**4**], (+)-epilupinine *N*-oxide [**5**], (+)-epilupinine acetate *N*-oxide [**6**], (+)-(*trans*-4′-hydroxy-3′-methoxycinnamoyl)epilupinine [**7**], (+)-(*trans*-4′-hydroxycinnamoyl)epilupinine [**8**], (+)-(*cis*-4′-hydroxycinnamoyl)epilupinine [**9**], (−)-(*trans*-4′-α-L-rhamnosyloxycinnamoyl)epilupinine [**11**], and (−)-(*cis*-4′-α-L-rhamnosyloxycinnamoyl)epilupinine [**12**]. We

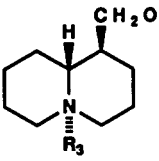


1 R_1=H

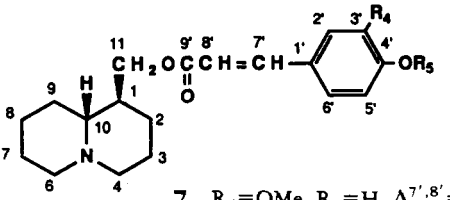


2 R_1=

3



4 R_2=H, R_3=lone pair
5 R_2=H, R_3=O
6 R_2=Ac, R_3=O



7 R_4=OMe, R_5=H, $\Delta^{7',8'}$=trans
8 R_4=R_5=H, $\Delta^{7',8'}$=trans
9 R_4=R_5=H, $\Delta^{7',8'}$=cis
10 R_4=H, R_5=Ac, $\Delta^{7',8'}$=trans
11 R_4=H, R_5=α-L-rha, $\Delta^{7',8'}$=trans
12 R_4=H, R_5=α-L-rha, $\Delta^{7',8'}$=cis

have also revealed variations of the alkaloidal contents in the growth time-course of *L. hirsutus* seedlings.

RESULTS AND DISCUSSION

From the 75% EtOH extract of the fresh 7- to 10-day-old seedlings of *L. hirsutus*, twelve alkaloids were isolated by repeated Si gel chromatography and by preparative hplc (Table 1). Ten of these alkaloids have been characterized in previous papers (2,9). These alkaloids were identified on the basis of their ms, uv, ir, $[\alpha]_D$, and ^1H- and ^{13}C-nmr data and were confirmed by chemical transformations as reported previously (2,9). (−)-Δ^5-dehydromultiflorine [**3**] has recently been isolated as a new compound from *Lupinus termis* Forsk. (10). The two remaining alkaloids proved to be novel compounds.

The first novel alkaloid, (−)-13α-tigloyloxymultiflorine [**2**], the least polar alkaloid by tlc, was isolated in a yield of 0.0008% of the plant fresh wt (Table 1). The

TABLE 1. Physical Constants and Contents of Lupine Alkaloids Isolated from the Seedlings of *Lupinus hirsutus*.

Alkaloid	mp	$[\alpha]_D$	(*c*, EtOH)	Yield (% fresh wt)
(−)-Multiflorine [**1**]	108–109°	−272°	(0.25)[a]	0.0157
(−)-13α-Tigloyloxymultiflorine [**2**]	oil	−292°	(0.10)	0.0008
(−)-Δ^5-Dehydromultiflorine [**3**]	oil	—[b]	—	0.00006
(+)-Epilupinine [**4**]	78°	+23.0°	(0.50)	0.0289
(+)-Epilupinine *N*-oxide [**5**]	210°	+31.0°	(0.70)	0.1003
(+)-Epilupinine acetate *N*-oxide [**6**]	188°	+12.0°	(0.26)	0.0006
(+)-(*trans*-4′-Hydroxy-3′-methoxy-cinnamoyl)epilupinine [**7**]	110°	+25.0°	(0.20)	0.0004
(+)-(*trans*-4′-Hydroxy-cinnamoyl)epilupinine [**8**]	162°	+23.0°	(0.13)	0.0003
(+)-(*cis*-4′-Hydroxy-cinnamoyl)epilupinine [**9**]	141–142°	+14.7°	(0.10)	0.0002
(+)-(*trans*-4′-Acetoxy-cinnamoyl)epilupinine [**10**]	oil	+20.5°	(0.12)	0.0002
(−)-(*trans*-4′-α-L-Rhamnosyl-oxycinnamoyl)epilupinine [**11**]	oil	−76.0°	(0.62)	0.0023
(−)-(*cis*-4′-α-L-Rhamnosyl-oxycinnamoyl)epilupinine [**12**]	oil	−52.8°	(0.99)	0.0020

[a]In MeOH.

[b]Cd was measured instead of $[\alpha]_D$ because of the small amounts of the samples.

molecular formula of **2** was determined as $C_{20}H_{28}N_2O_3$ by hrms. Evidence for a close similarity in structure to 13-tigloyloxylupanine was obtained from the fragmentation pattern in the eims (11). The presence of an ion fragment at *m/z* 244 $[M-100]^+$ in the eims of **2** was indicative of the loss of a tigloyl moiety from $[M]^+$. The ir spectrum of **2** showed absorption bands at 3000, 2930, 2850 cm^{-1} (Bohlmann bands), 1700, 1380, 1260 cm^{-1} (ester), 1640 cm^{-1} (α,β-unsaturated C=O), and 1590 cm^{-1} (conjugated C=C). The presence of a tigloyl moiety at C-13 was suggested by ^{13}C-nmr signals (Table 2) at δ 167.3 (s, C-1′), 129.2 (s, C-2′), 137.1 (d, C-3′), 14.4 (q, C-4′), 12.1 (q, C-5′), and 68.4 (d, C-13). The ^1H-nmr signals at δ 6.90 (1H, br d, $J=6.9$ Hz, H-3′), 1.83 (3H, d, $J=6.6$ Hz, Me-4′), 1.87 (3H, s, Me-5′) and 5.17 (1H, br s, H_{eq}-13β) also indicated the presence of a tigloyl group at position 13α (axial). These nmr data were partially coincident with those of (+)-13α-tigloyloxylupanine but not with those of (+)-13α-angeloyloxylupanine (12). Furthermore, a correlation peak between the H-3′ signal and the Me-4′ signal was observed in the NOESY spectrum. Thus, the side

TABLE 2. ^{13}C-nmr Chemical Shifts of **2**, **6**, and **10**.[a]

Carbon	Compound		
	2	**6**	**10**
C-1	—	35.5 (d)	40.8 (d)
C-2	155.1 (d)	27.3 (t)	29.2 (t)
C-3	98.8 (d)	19.7 (t)	25.0 (t)
C-4	192.5 (s)	68.7 (t)	56.4 (t)
C-5	39.6 (t)	—	—
C-6	60.1 (d)	69.2 (t)	56.6 (t)
C-7	31.4 (d)	20.0 (t)	24.2 (t)
C-8	25.7 (t)	23.1 (t)	24.3 (t)
C-9	33.8 (d)	23.4 (t)	28.3 (t)
C-10	57.5 (t)	73.1 (d)	64.8 (d)
C-11	57.3 (d)	65.3 (t)	65.9 (t)
C-12	33.7 (t)		
C-13	68.4 (d)		
*C*OMe	—	170.9 (s)	
CO*Me*	—	20.8 (q)	
C-14	26.9 (t)		
C-15	49.3 (t)		
C-17	49.7 (t)		
C-1′	167.3 (s)		132.1 (s)
C-2′	129.2 (s)		129.2 (d)
C-3′	137.1 (d)		122.2 (d)
C-4′	14.4 (q)		152.2 (s)
C-5′	12.1 (q)		122.2 (d)
C-6′			129.2 (d)
C-7′			143.8 (d)
C-8′			118.1 (d)
C-9′			166.8 (s)
*C*OMe			169.1 (s)
CO*Me*			21.1 (q)

[a]δ ppm from TMS in $CDCl_3$ (multiplicity).

chain was demonstrated to be an (*E*)-2-methyl-2-butenoyl (tigloyl) residue. Final confirmation of the structure of **2** was made by hydrolysis to (−)-13α-hydroxymultiflorine, which was determined by direct comparison to an authentic sample obtained from *L. termis* (10). From these results, the structure of **2** was fully confirmed as (−)-13α-tigloyloxymultiflorine [**2**].

The second novel alkaloid, (+)-(*trans*-4′-acetoxycinnamoyl)epilupinine [**10**], was a minor component isolated in a yield of 0.0002% of the plant fresh wt (Table 1). The molecular formula of **10** was determined as $C_{21}H_{27}NO_4$ by hrms. The fragment ions at *m/z* 315, 168, 152, 147, 136, 110, and 97 in the eims were characteristic of those in the spectrum of **8** (9). The presence of an acetyl group was suggested by the ^{13}C signals at δ 169.1 (s, C-10′) and 21.1 (q, C-11′) and by ^{1}H signal at δ 2.31 (3H, s, Me-11′). Therefore, the fragment ion at *m/z* 315 in the eims can be assigned to the peak corresponding to the loss of an acetyl group from $[M]^+$. The ir spectrum of **10** showed the presence of an ester group (1770, 1710, 1320, 1280, 1180 cm^{-1}) and an aromatic group (1610, 1590, 1510 cm^{-1}). The conformation of the double bond was determined as the trans form from the coupling constants of the signals at δ 7.66 (1H, d, J = 15.9 Hz, H-7′) and 6.40 (1H, d, J = 15.9 Hz, H-8′) in the ^{1}H nmr. The hydrolysis of **10** with 5% HCl gave **8** and a small amount of **4**. Conversely, **10** was synthesized from **8** by acetylation with Ac_2O in pyridine. Thus, the structure of **10** was determined as (+)-(*trans*-4′-acetoxycinnamoyl)epilupinine [**10**].

Variations of the alkaloidal content in the early stages of seedlings are shown in Figure 1. The concentrations of the ester-type alkaloids of (+)-epilupinine, such as **7**, **8**, and **11**, in the dry seeds were quite low. However, their concentrations increased rapidly during the first 3–10 days of growth of the seedlings. In the later stages, their concentrations diminished gradually. These phenomena were also observed in the change of the ester-type alkaloids of (−)-lupinine during the seedling growth of *L. luteus* (3–8).

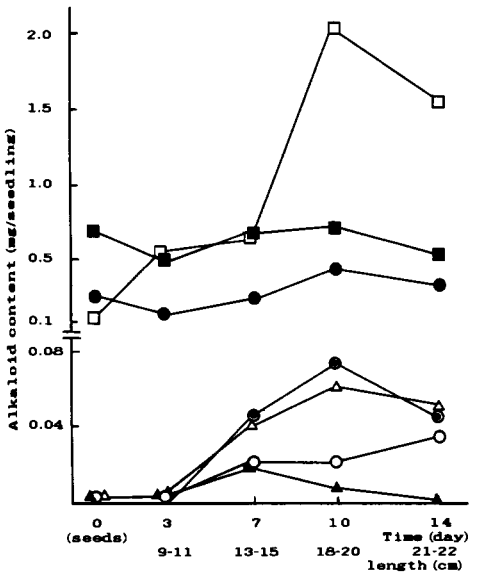


FIGURE 1. Variations of the alkaloid contents in the seedlings during the growth of *L. hirsutus*. Alkaloid content was estimated by hplc. Symbols: -●-compound **1**, -○-compound **2**, -■-compound **4**, -□-compound **5**, -▲-compound **7**, -△-compound **8**, -⊙-compound **11**.

The concentration of **2** increased concomitantly with development of the seedlings. A similar observation on the change of 13-tigloyloxylupanine was also reported previously (13).

Interestingly, 13α-hydroxymultiflorine, which may be considered to be the precursor of **2**, was detected neither in the seeds nor in the seedlings. Thus, the activity of acyltransferase responsible for the conversion of 13α-hydroxymultiflorine into its tiglate is possibly higher than that of the enzyme for conversion of multiflorine [**1**] into 13α-hydroxymultiflorine.

The concentration of (+)-epilupinine *N*-oxide [**5**] showed the same behavior as that of the ester-type alkaloids. A similar observation has been made on the *N*-oxides of pyrrolizidine alkaloids in *Crotalaria scassellstii* (14). So far, we have demonstrated the several enzymatic activities for biotransformation of alkaloids, acylation (8,15), and methylation (16), in the seedlings of several plants. These enzymatic activities tend to increase temporarily during the first 3 to 6 days after germination. In any case, the physiological significance of alkaloidal metabolism is not yet known in detail. Therefore, these observed changes of alkaloid pattern are interesting from the viewpoint of the metabolism and role of the lupine alkaloids in plants.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The high and low resolution eims were measured at 70 eV. ^{1}H- and ^{13}C-nmr spectra were recorded at 500 and 100.4 MHz, respectively. TMS was used as internal

standard in $CDCl_3$. Cd spectra were obtained on a JASCO J-500A spectropolarimeter. Tlc was carried out on 0.25 mm-precoated Si gel plates in a solvent system of CH_2Cl_2-MeOH-28% NH_4OH (90:9:1). Preparative tlc was conducted with 0.5-mm precoated Si gel plates in a solvent system of CH_2Cl_2-MeOH-28% NH_4OH (85:14:1). Analytical hplc was performed as described previously (17).

EXTRACTION AND ALKALOID ISOLATIONS.—The seedlings of *L. hirsutus* were grown in moistened vermiculite in daylight for 7–10 days at 25°. The total alkaloidal fraction from the 75% EtOH extracts of the fresh seedlings (5 kg) was obtained in a yield of 0.24% of the fresh wt as described previously (18). The total alkaloids (12.0 g) were subjected to Si gel cc with the solvent system of CH_2Cl_2/MeOH/28% NH_4OH as reported in a previous paper (2). The fractions rich in compound **2** (79 mg) were eluted in a solvent system of CH_2Cl_2-MeOH-28% NH_4OH (100:1:0.2). Preparative tlc separation of these rich fractions gave pure **2**. All other alkaloids were isolated by repeated Si gel chromatography as described previously (2) in a yield shown in Table 1.

(−)-13α-Tigloyloxymultiflorine [**2**].—Colorless oil, $[\alpha]^{23}D$ −292° (c = 0.10, EtOH); uv (MeOH) λ max 327 nm (log ε 4.03), 222 (4.08 sh); hrms *m/z* (%) $[M]^+$ 344.2218 (20) (calcd for $C_{20}H_{28}N_2O_3$, 344.2100), 244 (63), 164 (8), 149 (39), 134 (49), 132 (100), 110 (43), 96 (22), 83 (24), 82 (27), 55 (43), 41 (22); ir ν max ($CHCl_3$) cm^{-1} 3000, 2930, 2850 (Bohlmann bands), 1700, 1380, 1260 (ester), 1640 (α,β-unsaturated C=O), 1590 (conjugated C=C); ^1H nmr δ 6.90 (1H, br d, J = 6.9, H-3′), 6.86 (1H, d, J = 7.4, H-2), 5.17 (1H, br s, H_{eq}-13), 4.99 (1H, d, J = 7.4, H-3), 3.50 (1H, br d, J = 15.7, H-6), 3.26 (1H, d, J = 12.1, H_{ax}-17), 3.21 (1H, d, J = 12.1, H_{eq}-17), 3.00 (1H, t-like, H_{eq}-10), 2.52 (1H, br d, J = 11.3, H_{ax}-10), 1.87 (3H, s, Me-5′), 1.83 (3H, d, J = 6.6, Me-4′); ^{13}C nmr see Table 2.

HYDROLYSIS OF **2** TO (−)-13α-HYDROXYMULTIFLORINE.—Compound **2** (5 mg) was hydrolyzed with 1 N NaOH at room temperature for 1.0 h. The product was purified by use of preparative tlc in a yield of 79% (3 mg). The hydrolytic product was identified as (−)-13α-hydroxymultiflorine by eims (9) and by cd (c = 0.000187, MeOH) $[\theta]^{18}$ (nm) −21600 (330), −8100 (216) (negative maxima), +3000 (292) (positive maximum). The authentic sample, (−)-13α-hydroxymultiflorine, was obtained from the seeds of *L. termis*.

(+)-(trans-4′-Acetoxycinnamoyl)epilupinine [**10**].—Colorless oil, $[\alpha]^{23}D$ +20.5° (c = 0.12, EtOH); uv (MeOH) λ max 281 nm (log ε 4.66), 217 (4.54); hrms *m/z* (%) $[M]^+$ 357.1933 (9) (calcd for $C_{21}H_{27}NO_4$, 357.1935), 315 (2), 168 (4), 152 (100), 147 (7), 136 (6), 110 (7), 97 (11), 83 (15), 69 (26), 55 (16); ir ν max ($CHCl_3$) cm^{-1} 2950, 2810, 2760 (Bohlmann bands), 1770, 1710, 1320, 1280, 1180 (ester), 1640 (-CH=CH-), 1610, 1590, 1510 (aromatic); ^1H nmr δ 7.66 (1H, d, J = 15.9, H-7′, trans), 7.56 (2H, d, J = 8.5, H-2′, H-6′, aromatic), 7.13 (2H, d, J = 8.5, H-3′, H-5′, aromatic), 6.40 (1H, d, J = 15.9, H-8′, trans), 4.21 (1H, dd, J = 11.3, 3.9, H-11), 4.17 (1H, dd, J = 11.3, 5.4, H-11), 3.01 (2H, m, H_{eq}-4, H_{eq}-6), 2.31 (3H, s, Me-11′), 2.21 (2H, m, H_{ax}-4, H_{ax}-6); ^{13}C nmr see Table 2.

HYDROLYSIS OF **10** TO **8**.—Compound **10** (5 mg) was hydrolyzed with 3% HCl at 37° for 2.0 h. The acidic aqueous solution was alkalinized with 2.5% NH_4OH and extracted with CH_2Cl_2. Hplc analysis of the CH_2Cl_2 extracts gave **8** and a small amount of **4**.

SYNTHESIS OF **10** FROM **8**.—Compound **8** (47 mg) dissolved in 2 ml of pyridine was reacted with Ac_2O (1.5 ml) for 24 h at room temperature. The products were extracted and purified by use of cc on Si gel with the solvent system of CH_2Cl_2-MeOH-28% NH_4OH (100:1:0.2). Compound **10**, $[\alpha]^{23}D$ +20.0° (c = 0.28, EtOH), was obtained in a yield of 53% (28 mg).

(−)-Δ⁵-Dehydromultiflorine [**3**].—Colorless oil; cd (c = 0.000189, MeOH) $[\theta]^{18}$ (nm) −1900 (332), −5300 (261) (negative maxima). The cd spectra showed the same Cotton effects as those of **3** isolated from *L. termis* (10). Therefore, compound **3** was determined as the minus form. Other spectral data have been published previously (10).

(+)-Epilupinine acetate N-*oxide* [**6**].—Mp 188°; $[\alpha]^{23}D$ +12.0° (c = 0.26, EtOH) [lit. (9) mp 192°, $[\alpha]D$ +14°]; uv (MeOH) λ max 315 nm (log ε 2.54), 227 (2.44 sh); ^1H nmr δ 4.06 (1H, dd, J = 11.5, 4.4, H-11), 4.02 (1H, dd, J = 11.5, 3.6, H-11), 3.36 (2H, t, J = 13.5, H_{eq}-4, H_{eq}-6), 3.07 (2H, m, H_{ax}-4, H_{ax}-6), 2.86 (1H, dt, J = 11.4, 2.3, H_{ax}-10), 2.44 (1H, m, H_{ax}-1), 2.06 (3H, s, 13-Me). The ^{13}C-nmr data (Table 2) has not been reported previously. For ir and eims see Beck *et al.* (9).

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