PERGAMON

Phytochemistry 51 (1999) 5–9

PHYTOCHEMISTRY

# Glycosidic alkaloids from *Lupinus varius*

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Received 8 October 1998

**Abstract**

A new glycosidic alkaloid, (−)-(*trans*-3′-methoxy-4′-α-L-rhamnosyloxy cinnamoyl) epilupinine, was isolated from the aerial parts of *Lupinus varius*, together with the eight known alkaloids, (−)-(*trans*-4′-α-L-rhamnosyloxycinnamoyl) epilupinine, (+)-(*trans*-4′-hydroxy-3′-methoxy-cinnamoyl) epilupinine, (+)-epilupinine, (+)-epilupinine-*N*-oxide, (−)-multiflorine, (−)-$\delta^5$-dehydromultiflorine, (+)-ammodendrine and (−)-sparteine. Benzoyl epilupinine was identified by GC–mass spectral analysis. The structure of the new glycosidic alkaloid was determined by chemical and spectroscopic methods. 

*Keywords: Lupinus varius*; Fabaceae; Aerial parts; Quinolizidine alkaloid; Lupin alkaloid; (−)-(*trans*-3′-Methoxy-4′-α-L-rhamnosyloxy cinnamoyl) epilupinine; Alkaloid content

## 1. Introduction

Plants of the genus *Lupinus* are known to contain a variety of structural types of lupin alkaloids (Kinghorm, Salim, & Smolenski, 1980). They have been used as ornamental plants and as economic plants for fodder and for soil nitrogenation. *Lupinus varius* is an annual herb growing wild in north Africa, south Europe, west Syria, Palestine and Sinai (Tackholm, 1974; Jafri & El-Gaadi, 1980). Previous investigations of this species revealed the presence of several quinolizidine, as well as dipiperidine, alkaloids (Crow & Michael, 1957; Peterson, 1963; Wink, Meibner, & Witte, 1995; Mohamed & Hassanean, 1997).

In the course of our studies on lupin alkaloids in leguminous plants (Abdel-Haleim et al., 1992; Abdel-Haleim et al., 1995; Abdel-Haleim, 1995; Abdel-Haleim, Abdel-Fatteh, Halim, & Murakoshi, 1997), we report in the present paper the isolation and structural elucidation of a new alkaloid, (−)-(*trans*-3′-methoxy-4′-α-L-rhamnosyloxycinnamoyl) epilupinine (**1**) and eight known alkaloids, two of which were isolated for the first time from the aerial parts of *L. varius* growing in Libya, viz. (+)-(*trans*-4′-hydroxy-3′-methoxy-cinnamoyl) epilupinine (**2**), (−)-(*trans*-4′-α-L-rhamnosyloxycinnamoyl) epilupinine (**3**), (+)-epilupinine (**4**), (+)-epilupinine-*N*-oxide (**5**), (−)-multiflorine (**6**), (−)-$\delta^5$-dehydromultiflorine (**7**), (+)-ammodendrine (**8**) and (−)-sparteine (**9**). Benzoyl epilupinine was also detected as a minor compound for the first time from nature and identified on the basis of GC–mass spectrometric analysis.

## 2. Results and discussion

From the 75% ethanol extract of the dry aerial parts, nine alkaloids (**1**–**9**) were isolated by repeated silica gel CC and prep. TLC. Amongst these (**2**, **3**) two were isolated for the first time from the aerial parts of *L. varius*, while six alkaloids were reported in the seeds of *L. varius* growing in Sinai in Egypt (Mohamed & Hassanean, 1997). All these alkaloids were identified by their physicochemical properties (m.p., $[\alpha]_D$, IR, mass spectra, $^1$H NMR and $^{13}$C NMR) and chromatographic behavior (HPLC and GC).

A new lupin alkaloid (**1**) was isolated as an amorphous solid in a yield of 0.006% of the dry weight, after repeated silica gel chromatography. The EI showed a $[M]^+$ at $m/z$ 491. The fragment ion observed at $m/z$ 345 can be accounted for by $[M\text{-rhamnosyl}]^+$. These peaks are characteristic of lupinine and epilupinine derivatives containing a rhamnosyl residue (Murakoshi et al., 1977; Takamatu et al., 1990). The fragmentation pattern below at $m/z$ 345 closely resembles that of (+)-(*trans*-4′-hydroxy-3′-methoxycinnamoyl) epilupinine (**2**) which coexists with **1** in the same plant. Hydrolysis of **1** with 3% HCl

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0031-9422/99/$ - see front matter 
PII: S0031-9422(98)00722-5

gave L-rhamnose, which was identified by chromatography on TLC and the aglycone was also confirmed as **2** by mass spectrometry, $^1$H NMR and co-TLC.

The IR spectrum of **1** showed the presence of ester (1700 and 1242 $cm^{-1}$), aromatic (1605 and 1510 $cm^{-1}$) and hydroxyl (3370 $cm^1$) groups, as well as *trans*-quinolizidine bands at 2945, 2805 and 2765 $cm^{-1}$ (Bohlmann, 1958). The compound also displayed characteristic UV absorption of a cinnamoyl moiety at 226 and 296 nm. The $^1$H NMR spectrum (Table 1) revealed the presence of the methyl group of the rhamnosyl moiety at $\delta$ 1.29 (3H, d, $J=6.2$ Hz), the anomeric proton at $\delta$ 5.54 (1H, d, $J=1.5$ Hz) and a singlet of three protons for a methoxyl group at $\delta$ 3.92. In addition, this spectrum exhibited two *ortho*-coupled protons at $\delta$ 7.14 (d, $J=8.3$ Hz) and $\delta$ 7.08 (dd, $J=8.3$, 1.1 Hz), as well as *meta*-coupled proton at $\delta$ 7.5 (d, $J=1.1$ Hz), which were assigned to C-5′-, C-6′ and C-2′ protons of the aromatic ring of the cinnamoyl moiety, respectively. The configuration of the double bond in the cinnamoyl moiety was determined as *trans*, from the coupling constants of the signals at $\delta$ 7.6 (1H, d, $J=15.8$ Hz, H-7′) and $\delta$ 6.31 (1 H, d, $J=15.8$ Hz, H-8′).

In the $^{13}$C NMR spectrum of **1** (Table 2), the signals of the methyl carbon at $\delta$ 71.9 and an anomeric carbon at $\delta$ 99.2 also indicated the presence of a rhamnosyl moiety. Assignments, including all protons and carbons, were confirmed by $^1$H–$^1$H correlation spectroscopy (COSY) and HMQC. Furthermore, HMBC measurements (Fig. 1) helped in the unambiguous confirmation of the sites of attachment of certain cinnamoyl moieties to the epilupinine nucleus and to the rhamnosyl moiety. For example, glycosylation occurred at C-4′ as observed from coupling between H-1″ of rhamnose ($\delta$ 5.54) and C-4′ of the agylcone moiety ($\delta$ 147.9). Also, CH-long range coupling was observed between H-11 of epilupinine ($\delta$ 4.18) and C-9′ of cinnamoyl moiety ($\delta$ 167.9). From these results, it can therefore be established that, the new glycosidic alkaloid is (−)-(*trans*-3′-methoxy-4′-α-L-rhamnosyloxycinnamoyl) epilupinine.

Benzoyl epilupinine, a minor compound, that has not been previously characterized as a natural product, was identified by GC–mass spectrometry of the ethanol extracts of *L. varius*. The mass spectrum of alkaloid displayed a $[M]^+$ at $m/z$ 273 together with fragment ions at $m/z$ 168 and 152 (base peak), which are characteristic for epilupinine-type alkaloid (Takamatu et al., 1990). According to these data and the fragment ion at $m/z$ 168, which could be due to the elimination of a $C_6H_5$–CO moiety, it was deduced that we were dealing with an epilupinine skeleton, having a side chain of mass 105 (benzoyl portion). Thus, this compound could be ten-

Table 1
$^1$H NMR (500 MHz $CDCl_3$) data of compounds **1**–**3**

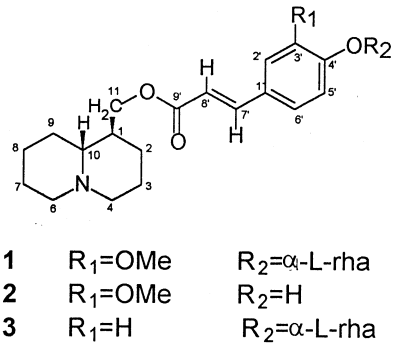
| H | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 1.87, m, H-1α | 1.87, m, H-1α | 1.78, m, H-1α |
| 2 | 1.34, m, H-2α, 2β | 1.31, m, H-2α, 2β | 1.33, m, H-2α, 2β |
| 3 | 1.37–1.77, m, H-3α, 3β | 1.71, m, H-3α, 3β | 1.67, m, H-3α, 3β |
| 4 | 3.06, m, H-4α, 6α | 3.0, m, H-4α, 6α | 2.94, m, H-4α, 6α |
| 6 | 2.22, m, H-4β, 6β | 2.19, m, H-4β, 6β | 2.15, m, H-4β, 6β |
| 7 | 1.44, m, H-7α, 7β | 1.37, m, H-7α, 7β | 1.40, m, H-7α, 7β |
| 8 | 1.84, m, H-8α, 8β | 1.84, m, H-8α, 8β | 1.77, m, H-8α, 8β |
| 9 | 1.37, m, H-9a | 1.36, m, H-9a | 1.3, m, H-9a |
| 10 | 1.9–2.0, m, H-9b, 10β | 2.19, m, H-9b, 10β | 1.79–1.89, m, H-9b, 10β |
| 11 | 4.14, dd, (11.2, 3.5) H-11a | 4.19, m, H-11 α, 11β | 4.17, m, H-11a |
| | 4.17, dd, (11.2, 5.3) H-11b | | 4.12, m, H-11b |
| 2′ | 7.5, d (1.1), H-2′ | 7.0, d (1.2), H-2′ | 7.43, d (8.6), H-2′,6′ |
| 3′ | – | – | 7.03, d (8.6), H-3′,5′ |
| 5′ | 7.14, d (8.3), H-5′ | 6.94, d (8.3), H-5′ | 7.43, d (8.3), H-2′-6′ |
| 6′ | 7.08, d (8.3), H-6′ | 7.10, d (8.3), H-6′ | 7.03, d (8.6), H-3′-5′ |
| 7′ | 7.6, d (15.8), H-7′ | 7.63, d (16.0), H-7′ | 7.6, d (16.0), H-7′ |
| 8′ | 6.31, d (15.8), H-8′ | 6.30, d (16.0), H-8′ | 6.30, d (16.0), H-8′ |
| 3′-OMe | 3.86, s, 3′-OMe | 3.95, s, 3′-OMe | – |
| *Rha* | | | |
| 1″ | 5.54, d(1.5), H-1″ | – | 5.5, d(1.6), H-1″ |
| 2″ | 4.18, m, H-2″ | – | 4.15, m, H-3″ |
| 3″ | 4.0, m, H-3″ | – | 3.99, dd (9.3,2.5), H-3″ |
| 4″ | 3.6, m, H-4″ | – | 3.6, t (9.3), H-4″ |
| 5″ | 3.8, m, H-5″ | | 3.7, m, H-5″ |
| 6″ | 1.29, d(6.2), Me | 1.28, d(6.0), Me | |

Coupling constants in Hz in parenthesis.

Table 2
$^{13}C$ NMR data of compounds **1**–**3** (125 MHz, $CDCl_3$)

| Position | 1 | 2[a] | 3[a] |
|---|---|---|---|
| 1 | 40.9d | 40.7d | 41.2d |
| 2 | 29.7t | 29.6t | 29.3t |
| 3 | 25.6t | 24.7t | 25.4t |
| 4 | 56.5t | 56.3t | 56.8t |
| 6 | 56.6t | 56.6t | 57.1t |
| 7 | 24.3t | 24.0t | 24.1t |
| 8 | 24.4t | 24.1t | 24.5t |
| 9 | 28.4t | 28.5t | 28.6t |
| 10 | 65.2d | 65.0d | 65.4d |
| 11 | 66.0t | 65.6t | 66.2t |
| 1′ | 129.6s | 128.8s | 128.8s |
| 2′ | 111.5d | 109.5d | 130.1d |
| 3′ | 150.7s | 148.2s | 116.5d |
| 4′ | 147.9s | 146.9s | 158.3s |
| 5′ | 116.7d | 114.8d | 116.5d |
| 6′ | 122.5d | 123.1d | 130.1d |
| 7′ | 145.1 | 145.1d | 144.7d |
| 8′ | 114.2d | 115.1d | 116.3d |
| 9′ | 167.2s | 167.2s | 167.5s |
| 3′-OMe | 56.4q | 55.9q | – |
| *Rha* | | | |
| 1″ | 99.2d | – | 98.1d |
| 2″ | 71.0d | – | 71.0d |
| 3″ | 71.9d | – | 71.9d |
| 4″ | 73.3d | – | 73.3d |
| 5″ | 69.5d | – | 69.5d |
| 6″ | 17.9q | – | 17.9q |

[13]C NMR chemical shifts have been reported previously Takamatu et al., 1990 and the values given here are close to the published values.



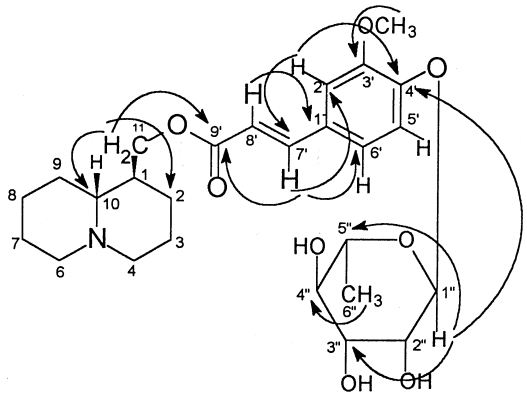




Fig. 1

tatively identified as benzoyl epilupinine. This is the first report of the detection of a benzoyl aster of an epilupinine-type alkaloid. However, the benzoyl ester of a lupanine-type alkaloid was previously detected in *L. polyphyllus* and *Genista cinerea* (Faugeras & Paris, 1970; Muhlbauer, Witte, & Wink, 1988).

It is worth noting that previous work (Wink et al., 1995; Mohamed & Hassanean, 1997) repeated that the seeds of *L. varius* are free from the ester-type of alkaloids of (+)-epilupinine. Meanwhile, our study has demonstrated that its aerial parts accumulate large quantities of ester-type alkaloids and their corresponding glycosides; the same phenomenon was reported in *L. luteus* (Murakoshi, Sugimoto, Haginwa, Ohmiya, & Otomasu, 1975; Murakoshi et al., 1977; Murakoshi, Toriizuka, Haginiwa, Ohmiya, & Otomasu, 1979) and *L. hirsutus* (Takamatu, Saito, & Murakoshi, 1991).

## 3. Experimental

### 3.1. General

M.p.'s uncorr. IR: thin films of $CHCl_3$ and KBr. Optical rotations: 10 cm path length in solvent stated. $^1H$ and $^{13}C$ NMR: 500 and 125 MHz, respectively, with TMS used as int. standard in $CDCl_3$ and $C_5D_5N$. EIMS: 70 eV. TLC: silica gel (Kieselgel 60, F254) in $CH_2Cl_2$–MeOH–28% $NH_4OH$ (43:6:1)[a], $Et_2O$–MeOH–28% $NH_4OH$ (17:2:1)[b] and *n*-BuOH–HoAc–$H_2O$ (3:1:1)[c]. Chromatograms of alkaloids were visualized by spraying with Dragendorff's and iodoplatinate reagents. GC–MS was performed using a glass column (2 m × 3 mm i.d.) packed with 2% OV-17 on Gas Chrom Q, using He as carrier gas. The column was programmed from 220 to 280°C at 5°C $min^{-1}$ and then held isothermally. The chromatograph was interfaced through a jet separator with a mass-spectrometer operated at 70 eV.

### 3.2. Extraction and isolation of alkaloids

Aerial parts of *L. varius* L. ssp. *orientalis* Franco et Silva (= *L. digitalis* Frossk., *L. pilosus* L.) were collected from Tripoli (Libya) in the flowering season. A voucher specimen, identified by Professor Dr. El-Gady, Faculty of Science, El-Faateh University, Tripoli is deposited in the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. The total alkaloid fr. from the 75% EtOH extract of dry aerial parts (1 kg) was obtained in yields of 1.1% of the dry wt (Ohmiya,

Otomasu, Murakoshi, & Haginiwa, 1974). The mixt. of bases (10 g) was chromatographed on a silica gel column (Merck, type 60, 230–400 mesh, 300 g, 2.5 × 60 cm) and gradient elution using MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1) (Saito, Kobayashi, Ohmiya, Otomasu, & Murakoshi, 1989; Ohmiya, Kubo, Otomasu, Saito, & Murakoshi, 1990). Frs enriched in **1** (65 mg) were eluted with 13% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1), together with **3**. Pure **1** (28 mg) was obtained from these frs by further purification using prep. TLC (silica gel, Kiselgel 60, F254, 1 mm layer thickness) in $Et_2O$–MeOH–28% $NH_4OH$ (17:2:1).

(−)-(*trans*-3′-Methoxy-4′-α-L-rhamnosyloxy cinnamoyl) epilupinine (**1**). Amorphous solid. $[\alpha]_D^{24}$ −80°(EtOH, *c* 0.28). UV $\lambda_{max}^{MeOH}$ nm: 230, 295. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3370 (OH), 2945, 2805, 2765 (*trans*-quinolizidine bands), 1700, 1242 (ester), 1635 (C=C), 1605, 1515 (aromatic). EIMS *m*/*z* (rel. int.): 491 ($[M]^+$, 10), 345 (20, [M-rhamnose moiety]$^+$), 168 (13), 152 (100), 136 (8), 111 (5), 96 (8). $^1H$ ($CDCl_3$, 500 MHz) and $^{13}C$ NMR ($CDCl_3$, 125 MHz): Tables 1 and 2.

The other known alkaloids were eluted in the following order.

(+)-(*trans*-4′-hydroxy-3′-methoxy-cinnamoyl) epilupinine (**2**): yield: 22 mg. Needles ($C_6H_6$), m.p. 118°. $[\alpha]_D^{24}$ +34° (EtOH, *c* 0.2). Eluted by 4% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1), UV, IR and MS as reported in the literature (Takamatu et al., 1990; Ohmiya, Saito, & Murakoshi, 1995). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

(−)-Multiflorine (**4**): yield: 11 mg. Oil. $[\alpha]_D^{24}$ −300° (EtOH, *c* 0.1). Eluted by 5% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1). UV, IR and MS and $^1H$ NMR as reported in the literature (Cordell, Saxton, Shamma, & Smith, 1989; Takamatu, Saito, Sekine, Ohmiya, & Murakoshi, 1991).

(+)-Epilupinine (**5**): yield: 450 mg. Needles (hexane), m.p. 79° $[\alpha]_D^{24}$ +31° (EtOH, *c* 0.2). Eluted by 8% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1). UV, IR and MS, $^1H$ and $^{13}C$ NMR as reported in the literature (Cordell et al., 1989; Ohmiya et al., 1995).

(−)-$\delta^5$-Dehydromultiflorine (**6**): yield: 17 mg. Oil. $[\alpha]_D^{24}$ −94° (EtOH, *c* 0.1). Eluted by 9% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1), UV, IR and MS, $^1H$ and $^{13}C$ NMR as reported in the literature (Mohamed et al., 1990).

(+)-Ammodendrine (**7**): yield: 25 mg. Oil. $[\alpha]_D^{24}$ +7.5° (EtOH, *c* 0.22). Eluted by 10% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1). UV, IR and MS, $^1H$ and $^{13}C$ NMR as reported in the literature (Ohmiya et al., 1995; Abdel-Haleim et al., 1997).

(+)-Epilupinine *N*-oxide (**8**): yield 21 mg. Needles ($C_6H_6$), m.p. 210° $[\alpha]_D^{24}$ +31° (EtOH, *c* 0.1). Eluted by 11% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1). UV, IR and MS, $^1H$ and $^{13}C$ NMR as reported in the literature (Takamatu et al., 1990).

(−)-(*trans*-4′-α-L-Rhamnosyloxy cinnamoyl) epilupinine (**3**): yield: 22 mg. Amorphous solid. $[\alpha]_D^{24}$ −76.1° (EtOH, *c* 0.21). Eluted by 13% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1) and purified by prep. TLC in $Et_2O$–MeOH–28% $NH_4OH$ (17:2:1). UV, IR and MS as reported in the literature (Takamatu et al., 1990), $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

(−)-Sparteine (**9**): yield: 15 mg. Oil. $[\alpha]_D^{24}$ −16.5° (EtOH, *c* 0.1). Eluted by 14% MeOH in $CH_2Cl_2$–28% $NH_4OH$ (500:1). UV, IR and MS, $^1H$ and $^{13}C$ NMR as reported in the literature (Bohlmann & Zeisberg, 1975; Ares, Phillipson, & Mascagni, 1986).

### 3.3. Hydrolysis of compound 1

**1** (5 mg) was hydrolyzed with 3% HCl at 37°C for 2 h. The acidic aq. soln was made alkaline with $NH_4OH$ and extracted with $CH_2Cl_2$. After drying, the $CH_2Cl_2$ extracts gave a base which was identified as **2** (co-chromatography, MS and $^1H$ NMR). The mother liquor was adjusted to pH 7 with 5% HCl and then passed through an Amberlite MB-3 column. The salt-free soln was concd and examined chromatographically by TLC using solvent system c. The sugar was identified as L-rhamnose.

## Acknowledgements

The authors are greatly indebted to Dr. Fumio Ikegami, Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan, for carrying out the GC–MS analysis.

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