New Ester Alkaloids from Lupins (Genus Lupinus)

P. Mühlbauer[1], L. Witte[2], and M. Wink[1,3]

Received: September 4, 1987

Abstract: Esters of 13-hydroxylupanine and 4-hydroxylupanine with acetic, propionic, butyric, isobutyric, valeric, isovaleric, tiglic, benzoic, and *trans*-cinnamic acid have been synthesized and characterized by capillary gas-liquid chromatography and mass spectrometry (EI-MS, CI-MS). In *Lupinus polyphyllus, L. albus, L. angustifolius*, and *L. mutabilis* we could identify new ester alkaloids (e.g. 13-propyloxylupanine, 13-butyryloxylupanine, 13-isobutyryloxylupanine, and 4-tigloyloxylupanine) besides the known esters, i.e. 13-acetoxylupanine, 13-isovaleroyloxylupanine, 13-angeloyloxylupanine, 13-tigloyloxylupanine, 13-benzoyloxylupanine, 13-*cis*-cinnamoyloxylupanine nine, and 13-*trans*-cinnamoyloxylupanine.

Introduction

Quinolizidine alkaloids (QA) are characteristic secondary metabolites of the Fabaceae (1, 2). More than 150 QA have been described [for review see (2)]. The hydroxylated QA of the lupanine-, lupinine-, and anagyrine-skeletons are often present in plants as esters with aliphatic and aromatic acids (2–5).

When examining the alkaloid pattern of more than 60 legume species (6), we detected a number of new ester alkaloids in *Lupinus polyphyllus, L. albus, L. angustifolius*, and *L. mutabilis*. In order to identify these alkaloids, we have synthesized a series of esters of 13-hydroxylupanine (**1**) and of 4-hydroxylupanine (**13**) with aliphatic and aromatic acids and have characterized them by capillary GLC and mass spectrometry [electron impact MS (EI-MS), chemical ionization MS (CI-MS)].

Materials and Methods

Plant material

Plants of *Lupinus polyphyllus, L. albus, L. angustifolius*, and *L. mutabilis* were grown in an experimental garden at Braunschweig. Lupins were harvested when they were full in flowers and they were stored at −20 °C until further processing.

Alkaloid extraction

In order to avoid the hydrolysis of alkaloid esters, a mild extraction protocoll was followed: frozen plant material was homogenized in 0.5 M HCl and left standing at room temperature for 30 min. After centrifugation of the homogenate (10 min; 10000 × g), the supernatant was made alkaline with 25 % ammonia and immediately applied to Extrelute columns (Merck, Darmstadt, FRG) or chemelute columns (ICT, Frankfurt). The alkaloids were eluted with CH_2Cl_2 and the solvent was evaporated *in vacuo*.

In order to obtain pure 4-hydroxylupanine (**13**) and 13-hydroxylupanine (**1**), crude alkaloid extracts, obtained from seeds of *L. mutabilis*, were separated by liquid chromatography on silica gel columns (122 × 12 cm; 70–230 mesh) with diethyl ether/methanol/ammonia (100/30/1) as eluent. 13-hydroxylupanine (**1**) was analyzed by ^{13}C-NMR ($CDCl_3$): δ (ppm) = 32.9 (2C); 19.1 (3C); 26.4 (4C); 60.8 (5C); 33.9 (6C); 27.6 (7C); 31.3 (8C); 46.6 (9C); 31.9 (10C); 57.2 (11C); 39.6 (12C); 63.9 (13C); 32.0 (14C); 49.2 (15C). As compared to (9) the compound was 13-*alpha*-hydroxylupanine (**1**). According to (2,4) the 4-hydroxylupanine was 4β-hydroxylupanine (**13**) (common name "nuttaline").

Alkaloid analysis

Capillary GLC and GLC-MS was performed under standard conditions as described by Wink et al. (3–7) employing flame ionisation and nitrogen detectors. For CI-MS either isobutane or NH_3 was employed as a reactant gas.

Synthesis of alkaloid esters

Acid chlorides for esterification were purchased from Aldrich or were obtained from the corresponding acids on treatment with oxalylic chloride in benzene for 12 h at room temperature as described in (3).

Esterification of 13-hydroxylupanine (**1**) and of 4-hydroxylupanine (**13**) with the respective carboxylic acid chlorides, given in excess, was accomplished in benzene/pyridine (10 : 1) (see 3). After 12 h at room temperature the solvent was evaporated. The ester alkaloids were obtained from the reaction mixture after purification on chemelute columns (s. "Alkaloid extraction"). These extracts usually contained some residual hydroxylupanine and the respective ester as the only nitrogen-containing compounds, as judged from GLC-analysis employing a nitrogen-specific detector. Typical yields of ester alkaloids were between 43–96 %. The identity of the synthesized alkaloids was confirmed by EI-MS, CI-MS and GLC-MS (Table I). Due to the small amounts of available hydroxylupanine, all reactions were performed on a μmol-scale.

Results and Discussion

In order to identify the ester alkaloids in extracts from shoots of *Lupinus polyphyllus, L. albus, L. angustifolius* and *L. mutabilis*, we have synthesized a series of esters of 13-*alpha*-hydroxylupanine (**1**) and of 4-*beta*-hydroxylupanine (**13**), (which are the main hydroxylated alkaloids of these species) with various aliphatic and aromatic acids. The characterization of these ester alkaloids (many of them constitute new derivatives) by capillary GLC and mass spectrometry is documented in Table I. Capillary GLC is a powerful method for the analysis of complex alkaloid mixtures and was shown to separate most of the naturally occurring stereoisomers (see 2, 7, 10). Fig. 1 illustrates the efficiency of alkaloid separation. Electron impact mass spectrometry is useful for the distinction of esters of the 4- and the 13-hydroxylupanine type in that m/z = 134 is the base peak of the 4-hydroxylupanine (**13**) series and m/z = 246 that of 13-hydroxylupanine (**1**) esters. CI-MS is especially helpful to establish the molecular ion of the respective compounds.

[1] Genzentrum der Universität München, Institut für Pharmazeutische Biologie, Karlstr. 29, D-8000 München 2, Federal Republic of Germany.

[2] Technische Universität Braunschweig, Institut für Pharmazeutische Biologie, Mendelssohnstr. 1, D-3300 Braunschweig, Federal Republic of Germany.

[3] Address for correspondence.

Table I. Characterization of ester alkaloids by capillary GLC and mass spectrometry.

Alkaloid	RI	Mass spectral data [*m/z* (intensity %)] EI-MS					CI-MS		
13-Hydroxylupanine (**1**)	2400	264 (35)	246 (43)	165 (40)	152 (100)	134 (40)	265 (100)	245 (15)	152 (5)
13-Acetoxylupanine (**2**)	2475	306 (12)	246 (100)	148 (35)	134 (65)	112 (43)	307 (100)	245 (8)	
13-Propyloxylupanine (**3**)	2565	320 (10)	246 (100)	148 (30)	134 (60)	112 (37)	321 (100)	245 (10)	
13-Isobutyryloxylupanine (**4**)	2598	334 (10)	246 (100)	148 (30)	134 (60)	112 (40)	335 (100)	247 (10)	145 (10)
13-Butyryloxylupanine (**5**)	2650	334 (10)	246 (100)	148 (30)	134 (60)	112 (37)	335 (100)	247 (12)	245 (12)
13-Isovaleroyloxylupanine (**6**)	2690	348 (5)	246 (100)	148 (25)	134 (50)	112 (30)	349 (100)	247 (12)	245 (15)
13-Valeroyloxylupanine (**7**)	2745	348 (5)	246 (100)	148 (30)	134 (60)	112 (40)	349 (100)	247 (20)	245 (22)
13-Angeloyloxylupanine (**8**)	2765	346 (10)	246 (100)	148 (20)	134 (40)	112 (25)	347 (100)	247 (30)	245 (40)
13-Tigloyloxylupanine (**9**)	2780	346 (10)	246 (100)	148 (20)	134 (40)	112 (30)	347 (100)	247 (20)	245 (20)
13-Benzoyloxylupanine (**10**)	3115	368 (3)	246 (100)	148 (20)	134 (40)	112 (20)	369 (100)	249 (40)	245 (30)
13-*trans*-Cinnamoyloxylupanine (**12**)	3415	394 (1)	246 (100)	148 (15)	134 (35)	112 (22)	395 (35)	249 (40)	245 (55)
4-Hydroxylupanine (**13**)	2270	264 (70)	247 (15)	165 (15)	150 (40)	136 (100)		265 (100)	136 (5)
4-Acetoxylupanine (**14**)	2490	305 (2)	246 (12)	148 (20)	134 (100)		307 (100)	249 (15)	134 (5)
4-Propyloxylupanine (**15**)	2590	320 (5)	246 (12)	148 (20)	134 (100)		321 (100)	249 (20)	134 (5)
4-Isobutyroyloxylupanine (**16**)	2620	334 (2)	246 (12)	148 (20)	134 (100)		335 (100)	249 (30)	247 (12)
4-Butyryloxylupanine (**17**)	2687	334 (2)	246 (12)	148 (20)	134 (100)		335 (100)	249 (40)	247 (20)
4-Isovaleroyloxylupanine (**18**)	2738	348 (2)	246 (12)	148 (20)	134 (100)		349 (100)	249 (50)	247 (20)
4-Valeroyloxylupanine (**19**)	2795	348 (2)	246 (10)	148 (20)	134 (100)		349 (100)	249 (70)	247 (30)
4-Tigloyloxylupanine (**20**)	2867	346 (1)	246 (20)	148 (20)	134 (100)		347 (100)	249 (20)	247 (10)
4-Benzoyloxylupanine (**21**)	3230	368 (2)	246 (20)	148 (20)	134 (100)		369 (25)	249 (100)	247 (50)

GLC Conditions (GC 8500 Perkin Elmer): Oven: 150 °C, 2 min isothermal; then 150–320 °C with 10 °C/min; column (DB-1): 25 m × 0.3 mm; injector: split, 250 °C, detector: 320 °C, flame ionization and nitrogen-specific detectors; carrier gas: helium 0.9 bar.

Table II. Patterns of ester alkaloids in aerial parts of *Lupinus polyphyllus, L. mutabillis, L. albus*, and *L. angustifolius*.

Species	Alkaloid composition (Total alkaloid = 100 %)													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Lupinus polyphyllus[a]	5.3	0.4	0.5	0.2	0.1	0.4	–	2.5	10.2	0.1	2.2	1.6	1.8	0.1
L. mutabilis[b]	2.5	0.6	2.2	0.2	0.7	0.4	–	2.8	7.3	1.9	1.6	0.3	+	+
L. angustifolius[c]	23.4	–	0.3	0.2	0.1	0.3	–	0.3	9.3	1.1	16.2	11.5	5.3	2.4
L. albus[d]	0.9	0.7	0.2	0.1	0.4	1.1	0.01	0.4	6.1	0.1	–	–	1.7	+

[a] = leaves, [b] = shoots, [c] = young plant, [d] = green pods.
Alkaloid extracts were analyzed by capillary GLC and GLC-MS (EI-, CI-MS).
1 = 13-hydroxylupanine, **2** = 13-acetoxylupanine, **3** = 13-propyloxylupanine, **4** = 13-isobutyryloxylupanine, **5** = 13-butyryloxylupanine, **6** = Isovaleroyloxylupanine, **7** = 13-valeroyloxylupanine, **8** = 13-angeloyloxylupanine, **9** = 13-tigloyloxylupanine, **10** = 13-benzoyloxylupanine, **11** = 13-*cis*-cinnamoyloxylupanine, **12** = 13-*trans*-cinnamoyloxylupanine, **13** = 4-hydroxylupanine, **14** = 4-tigloyloxylupanine.
+ = traces; – = not detected.

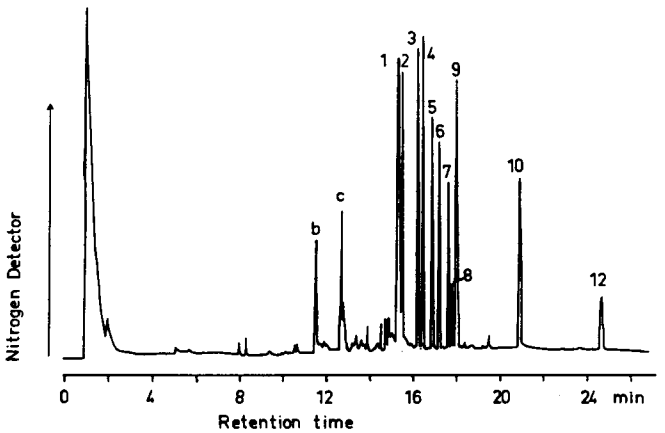
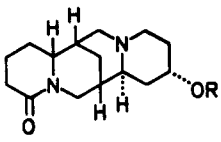
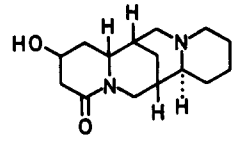


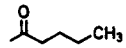
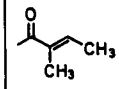
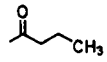
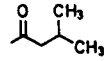
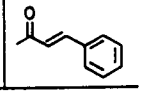
Fig. 1. Separation of a test mixture of quinolizidine alkaloids by capillary gas-liquid chromatography. [Mixture of synthetic esters of 13-hydroxylupanine (**1**). GLC-conditions as in Table I; peak numbers refer to the numbering in Table I. Additional peaks are b: tetrahydrorhombifoline, c: lupanine.

Up to 40 % of the total alkaloid fraction of the aerial green parts of lupins can be present as ester alkaloids, whereas esters are usually only minor alkaloids in lupin seeds (3, 6, 7; Table II). Analyzing the alkaloid extracts isolated from roots, stems, leaves, green fruits, and seeds of *L. polyphyllus, L. albus, L. angustifolius*, and *L. mutabilis*, we could unequivocally identify 12 ester alkaloids, of which 4 constitute new alkaloids, by capillary GLC (Retention times, peak matching) and capillary GLC-MS (EI, CI). Table II gives the quanitiative composition of the ester alkaloid fraction of these species. Those plant parts which were especially rich in ester alkaloids were tabulated here for comparison [A full account of all alkaloids present in the different plant parts of 15 lupin species will be published in a separate paper (11)]. In addition, 3–7 other ester alkaloids/species were detected as minor components, which differed in their alkaloid skeleton, but whose structure could yet not be elucidated unambiguously.





13

	R		R
1	H	7	
2		8	
3		9	
4		10	
5		11	
6		12	

13-Tigloyloxylupanine (**9**) generally figures as the most abundant ester alkaloid of the aerial green parts of lupins (6, 11), which is always accompanied by the *trans*-isomer 13-angeloyloxylupanine (**8**) and most of the other esters. We have characterized a tigloyl-CoA-13-hydroxylupanine *O*-tigloyl transferase from *L. albus* seedlings, which is responsible for the formation of the tiglic acid ester (8). Whether it is the same enzyme which catalyzes the formation of the other ester alkaloids seems to be unlikely (8) and needs to be studied in more detail.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft for grants and a Heisenberg fellowship (to M. W.), and the Fonds der Chemischen Industrie for support.

References

(1) Mothes, K., Schütte, H. R. (1969) Biosynthese der Alkaloide. VEB-Berlin.
(2) Kinghorn, A. D., Balandrin, M. F. (1984) in: Alkaloids: Chemical and biological perspectives (Pelletier, W. S., ed.), p. 105, Wiley, New York.
(3) Wink, M., Schiebel, H. M., Witte, L., Hartmann, T. (1982) Planta Med. 44, 15.
(4) Hatzold, T., Elmadfa, I., Gross, R., Wink, M., Hartmann, T., Witte, L. (1983) J. Agr. Food Chem. 31, 934.
(5) Wink, M., Hartmann, T., Witte, L., Schiebel, H. M. (1981) J. Nat. Prod. 44, 14.
(6) Wink, M. (1984) Stoffwechsel und Funktion von Chinolizidinalkaloiden in Pflanzen und pflanzlichen Zellkulturen. Habilitation-Thesis, Technische Universität Braunschweig.
(7) Wink, M., Witte, L., Hartmann, T., Theuring, C., Volz, V. (1983) Planta Med. 48, 253.
(8) Wink, M., Hartmann, T. (1982) Planta 156, 560.
(9) Bohlmann, F., Zeisberg, R. (1975) Chem. Ber. 108, 1043.
(10) Wink, M. (1987) Planta Med. 53, 509–514.
(11) Wink, M., Witte, L. (submitted for publication).

New Chromone Alkaloids from the Stem Bark of *Schumanniophyton magnificum*

Peter J. Houghton[1]

Received: October 19, 1987

Abstract: Fractionation of a methanolic extract of the stem bark of *Schumanniophyton magnificum* yielded large quantities of mannitol. In addition, noreugenin and ten related chromone alkaloids were isolated. Seven of these alkaloids had been isolated previously from *S. magnificum* and one other from *S.problematicum* but two of the alkaloids were novel, one was hydroxy-*N*-methylschumannificine and the other was the acetate of *N*-demethylrohitukine. The structures of the two alkaloids have been deduced from their spectral features.

Introduction

Several alkaloids whose structure is based on the chromone noreugenin have recently been reported from the root bark of *Schumanniophyton magnificum* Harms. (Rubiaceae) (1–4). The juice from the stem bark of this tree is reputedly used as an antidote to snake venom and the active principle has recently been identified as an alkaloid (5). This paper describes the alkaloidal content of the stem bark and also of the leaves of this plant.

[1] Pharmacognosy Research Laboratories, Chelsea Department of Pharmacy, King's College London, Manresa Road, London SW3 6LX, U.K.