Bull. Pharm. Sci., Assiut University, Vol. 23, Part 1, June 2000, pp. 79-83.

LUPIN ALKALOIDS FROM THE HERB OF *LUPINUS PUBESCENS* BENTH

M. A. Ramadan

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, 71526 Assiut, Egypt

من الخلاصة الكحولية لنبات الليوبينس بيبسنس بينث تم فصل ستة من قلويدات الليوبين وهى، (+)- تيتراهيدروروميبفولين ، (+)- ليوبانين ، (-)-ملتيفلورين ، (+)-١٣-ألفا-هيدروكسى ليوبانين ، (+)-أمودنديرين و(-)-سبارتين. وتم التعرف على هذه المركبات بواسطة الطرق الطيفية المختلفة والمقارنة بمواد قياسية.

From the 75% ethanol extract of Lupinus pubescens Benth herb, six lupin alkaloids; (+)-tetrahydrorhombifoline, (+)-lupanine, (-)-multiflorine, (+)-13α-hydroxylupanine, (+)-ammodendrine and (-)-sparteine were isolated for the first time from the plant. The structure of these alkaloids was established by spectroscopoic methods, as well as comparison with authentic samples.

INTRODUCTION

The plants of the genus *Lupinus* (Leguminosae) are known to be a rich source of the lupin alkaloids.[1] Lupin alkaloids have been shown to be of some use in establishing phytogenetic relationships at the generic and tribal levels in the Papilionoideae.[1-2] These compounds are also important because of their toxicity for humans and live stock as constituents of poisonous plants and because some of them exhibit potentially useful pharmacological activities.[2-3] *Lupinus pubescens* Benth is considered among the old world lupins growing in North America.[4] In a recent study,[4] 22 alkaloids were detected by GC/MS in the leaves and seeds of the plant, however, no isolation and further spectral studies were done on the seeds or the leaves of the plant cultivated in Egypt.

In the present study, six lupin-type quinolizidine alkaloids were isolated from the herb of the plant namely: (+)-tetrahydro-rhombifoline, (+)-lupanine, (-)-multiflorine, (+)-13α-hydroxylupanine, (+)-ammodendrine and (-)-sparteine.

EXPERIMENTAL

Melting points were uncorrected and determined by Koffler's hot stage microscope. IR spectra were taken in KBr for solid materials and $CHCl_3$ for oily substances using Unicam SP 1205 spectrophotometer. The low resolution EIMS were measured on a JEOL JM-60 spectrometer at 70 eV. ^1H NMR spectra were recorded on JEOL GSX 400 spectrometer. TMS was used as internal standard in DMSO-d_6 and $CDCl_3$. Dragendorff's was used as a reagent for alkaloids. TLC was carried out on pre-coated silica gel plates (Kieselgel 60 F_{254}, E. Merck) using solvent systems CH_2Cl_2-MeOH-28% NH_4OH (43:6:1) as system I and $CHCl_3$-MeOH-28% NH_4OH (85:15:1) as system II.

Plant materials

The seeds of *L. pubescens* Benth were supplied from Prof. Dr, H. B. C. Frenzel, Hohenheim University, Germany and cultivated at the Medicinal Plant Experimental Station of Al-Azhar University, Assiut in October 1995 and collected in May 1996 in fruiting stage. The voucher specimen was identified by Prof. Dr. A. Fayed (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt).

Extraction and isolation

The air-dried powdered aerial shoots (stems

Received in 20/11/1999 & Accepted in 27/1/2000

and leaves) of *L. pubescens* Benth (1.5 kg.) were extracted with ethanol 75% by maceration and percolation till complete exhaustion (5 L x 3). The extract was concentrated under reduced pressure to a syrupy consistency. The alcohol free concentrate was acidified with 5% HCl, refrigerated over night, filtered and the filtrate was washed with $CHCl_3$ (2x150 ml). The acidic solution was rendered alkaline with NH_4OH and extracted with successive portions of $CHCl_3$ (5x250 ml). The $CHCl_3$ extract was washed with distilled water (2x100 ml). The organic extract was evaporated under reduced pressure to a semi-solid residue (13 g). TLC screening of the basic fraction using silica gel G as adsorbent and systems I and II as developers, revealed the presence of at least 12 dragendorff's positive spots.

The basic fraction (13 g) was chromatographed on silica gel column (Merck, type 60, 230 mesh,750 g, 6x120 cm) and then gradiently eluted using $CHCl_3$-MeOH-28% NH_4OH to yield six alkaloids as follows:

(+)-Tetrahydrorhombifoline(1): Colourless oil, (7 mg),it is soluble in $CHCl_3$, MeOH and EtOH, and insoluble in benzene and ether. $[\alpha]_D^{25}$ = + 81°, (C = 0.1, EtOH) eluted by $CHCl_3$-MeOH-28% NH_4OH (98.5:1.5:0.05); R_f values = 0.82 and 0.61 in systems I and II, respectively. EIMS: m/z (rel. int.%) 248 [M^+, 3%], 208 (15), 207 (100), 112 (21), 108 (9) and 58 (88); IR ($CHCl_3$) ν cm^{-1}: 2850-2700 (Bohlmann's bands), 1620 (pyridone C=O) and 1580 (C=C); ^{1}H-NMR ($CDCl_3$) δ 5.61 (1H, m, H-16), 4.86 (2H, m, H_2-17), 3.6 (1H, m, H-6), 4.51 (1H, dt, J= 10 Hz, 2.5 Hz, H-10α).

(+)-Lupanine (2): Yellow oil, (100 mg), it is soluble in $CHCl_3$, MeOH, acetone and EtOH, and insoluble in benzene and ether $[\alpha]_D^{25}$ = +50° (C= 0.1, MeOH) eluted by $CHCl_3$-MeOH-28% NH_4OH (95:5:0.05); R_f values= 0.72 and 0.63 in systems I and II, respectively. EIMS m/z (rel. int.%), 248 (67), 247 (39), 219 (10), 151 (13), 150 (41), 149 (52), 136 (100), 134 (15), 110 (20), 98 (22), 55 (18), 41 (16); IR ($CHCl_3$) ν cm^{-1}: 2850-2700 (Bohlmann's bands), 1640 (amide C=O), ^{1}H-NMR (DMSO-d_6); δ 4.39 (1H, dt, J= 11.2 Hz, 2.5 Hz, H-10α), 2.99 (1H, m, H-6), 2.5 (1H, m, H-10ß), 2.7 (1H, m, H-15ß), 2.6 (1H, t, J= 11 Hz, H-17ß).

(-)-Multiflorine (3): Yellow oil, (19 mg), it is soluble in $CHCl_3$, MeOH and EtOH, and insoluble in petroleum ether and ether $[\alpha]_D^{25}$ = -295° (C= 0.1, MeOH), eluted by $CHCl_3$-MeOH-28% NH_4OH (93:7:0.05), R_f values= 0.61 and 0.35 in systems I and II, respectively. IR ($CHCl_3$); ν cm^{-1} 2850-2700 (Bohlmann's bands), 1640, 1580 (conjugated carbonyl, C=C); ^{1}H NMR ($CDCl_3$) δ 6.87 (1H, d, J= 7.7 Hz, H-2), 4.92 (1H, d, J= 7.7 Hz, H-3), 3.41 (1H, ddd, J= 15.9 Hz, 5.1 Hz, 2.3 Hz, H-6), 3.17 (1H, d, J= 12 Hz, H-10α), 3.13 (1H, dd, J= 12 Hz, 3 Hz, H-10ß), 2.90 (1H, dd, J= 12.8 Hz, 8.6 Hz, H-17ß), 2.35 (1H, dd, J= 12.8 Hz, 3.6 Hz, H-17α), 2.64 (1H, t, J= 16.2 Hz, H-5α); EIMS, m/z (rel. int.%) 246 (70), 191 (11), 150 (20), 149 (53), 134 (100).

(+)-Ammodendrine (4): Yellow oil, (15 mg), it is soluble in $CHCl_3$, MeOH and EtOH, and insoluble in benzene and ether $[\alpha]_D^{25}$ = +7.8° (C= 0.1, MeOH), eluted by $CHCl_3$-MeOH-28% NH_4OH (91:9:0.05), R_f values= 0.52 and 0.26 in systems I and II, respectively. EIMS; m/z (rel. int.%) 208 (39), 191 (29), 179 (52), 165 (100), 152 (53), 149 (16), 137 (42), 136 (58), 123 (65), 122 (37), 110 (66), 94 (31), 84 (31), 43 (37), IR ($CHCl_3$) cm^{-1}: 1630 (C=O), 1420 (C-N), ^{1}H-NMR ($CDCl_3$), δ 7.2 and 6.59 (1H, s, H-6), 3.6 (2H, m, H-2), 3.05 (2H, m, H-2`$_{eq}$, H-6`$_{eq}$), 2.60 (1H, m, H-6`$_{ax}$) 2.15-2.09 (3H, s, 8-Me).

(+)-13α-hydroxylupanine (5): Fine needles (65 mg) m.p 171-173°, it is soluble in $CHCl_3$, MeOH and EtOH, and insoluble in petroleum ether, benzene and ether. $[\alpha]_D^{25}$ = +43° (C= 0.15, $CHCl_3$), eluted with $CHCl_3$-MeOH-28% NH_4OH (90:10:0.05). R_f values= 0.42 and 0.24 in systems I and II, respectively. IR (KBr) ν cm^{-1} 3345 (OH), 2900-2750 (Bohlmann's bands),

1600 (amide C=O); ^{1}H-NMR ($CDCl_3$) δ 4.5 (1H, dt, J= 13.2 Hz, 2.3 Hz, H-10α), 4.1 (1H, t, J= 2.8 Hz, H-13ß), 3.3 (1H, m, H-6), 2.9 (1H, t, J= 10.5 Hz, H-17ß); EIMS, m/z (rel. int.%) 264 (70), 247 (35), 246 (55), 152 (100), 134 (30),126 (20), 113 (30), 108 (15) and 98 (15).

(-)-sparteine (6): Viscous colourless oil, (110 mg), it is soluble in $CHCl_3$, MeOH, acetone and EtOH, and insoluble in petroleum ether and ether $[\alpha]_D^{25}$ = -17° (C= 0.1, MeOH) eluted by $CHCl_3$-MeOH-28% NH_4OH (84:16:0.05), R_f values = 0.17 and 0.1 in systems I and II, respectively. IR ($CHCl_3$) ν cm^{-1}; 2850, 2730 (Bohlmann's Band), 1440, 1375, 1165, 1185. EIMS; m/z (rel. int.%) 234 (M^+, 43), 193 (26), 150 (20), 137 (100), 136 (42), 110 (27), 98 (85). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (1H, dt, J= 11 Hz, 2.5 Hz, H-10α), 2.50 (1H, m, H-17ß), 2.58 (1H, m, H-2α), 2.71 (1H, m, H-17α), 2.80 (1H, m, H-15α).

RESULTS AND DISCUSSION

Investigation of the herb of *L. pubescens* Benth led to the isolation and identification of six alkaloids from the 75% EtOH extract of the herb of *L. pubescens* by silica gel column chromatography.

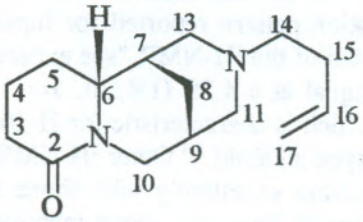
Compound **1** showed $[M]^+$ at m/z 248 and the base peak at m/z 207 in the EIMS. The IR spectrum of **1** showed characteristic *trans* quinolizidine bands (Bohlmann's bands) at 2850-2700 cm^{-1},[5,6] an amide carbonyl at 1620 cm^{-1} and characteristic absorption for C=C at 1580 cm^{-1}. The ^{1}H-NMR spectrum of **1** showed one proton as double triplet at δ 4.51 characteristic for the 10α proton, in addition to one olefinic proton multiplet at δ 5.61 assigned for H-16 and two olefinic methylene proton multiplet at δ 4.86 assigned for H_2-17. The above data suggested that compound **1** is tetrahydrorhombifoline.[2,4,7]

Compound **2** was isolated as yellow oily substance. Its IR spectrum revealed absorption bands at cm^{-1} 2850-2700 for the *trans* quinolizidine bands[5,6] and another absorption band at 1640 cm^{-1} for the lactam carbonyl group. The MS exhibited the molecular ion peak $[M]^+$ at m/z 248 and the base peak at m/z 136 with other prominent fragments at m/z 247, 150, 134 and 98 in accordance with the mass fragmentation pattern reported for lupanine.[2,4,8] Examination of the ^{1}H-NMR "see experimental" showed signal at δ 4.39 (1H, dt, J= 11.2 Hz, 2.5 Hz) which is characteristic for H-10α of the lupanine type alkaloid.[8,9] Other ^{1}H NMR signals were matching excellently with those reported for lupanine.[8,9] From the above mentioned data compound **2** could be assigned as (+)-lupanine which is reported frequently from many Leguminaceous plants.[8]

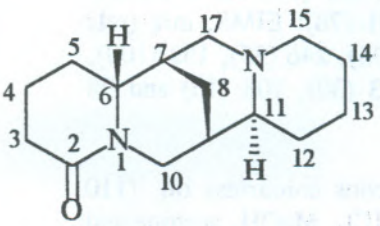
Compound **3** was obtained as oily material. Its IR exhibited absorption peaks typical for conjugated carbonyl and double bond[10] at 1640 and 1580 cm^{-1}, respectively, and bands for *trans* quinolizidine[5,6] at 2850-2700 cm^{-1}. The EIMS of compound **3** showed M^+ at m/z 246 and the base peak at 134 in accordance with the reported data of (-)-multiflorine.[2,4,11] The ^{1}H-NMR of **3** showed a downfield proton at δ 6.87 (1H, d, J= 7.7 Hz, H-2) coupled to H-3 proton at δ 4.92 (1H, d, J= 7.7 Hz) which indicated a multiflorine type alkaloid.[12] The physical, and spectroscopic data as well as co-chromatography with authentic sample confirmed the identity of compound **3** as (-)-multiflorine.[2,4,11,12]

Compound **4** was obtained as pale yellow oil. Its IR spectrum showed an absorption bands for amide carbonyl at 1630, and C-N stretching at 1420 cm^{-1}. The mass fragmentation showed $[M]^+$ at m/z 208 and the base peak at m/z 165 typical for ammodendrine.[2,4,13] The mass spectrum further showed peaks at m/z 191 and 43 corresponding to $[M-OH]^+$ and $[CH_3CO]^+$ indicating the presence of hydroxyl and N-acetyl groups, respectively. Some of the ^{1}H-NMR spectral signals are doubled e.g signals at δ 7.20 and 6.59 which were integrating for one proton (H-6) and the two singlets at δ 2.15 and 2.09 integrating for three protons (CO-$\underline{CH_3}$). This phenomena indicated the presence of two isomers due to restricted rotation about the N-Ac bond. The above physico-chemical properties and spectral data IR, MS and ^{1}H NMR confirmed that compound **4** ammodendrine,[14,2,4] which is dipipridyl alkaloid commonly found in Leguminous plants.[1]

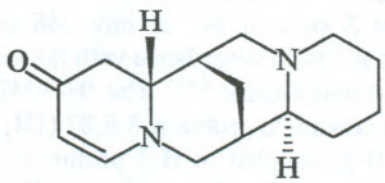
The IR spectrum of compound **5** showed



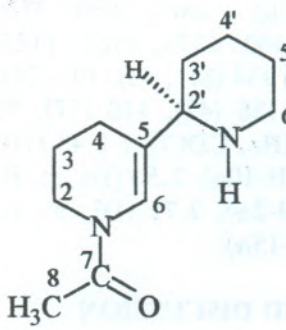
Compound 1



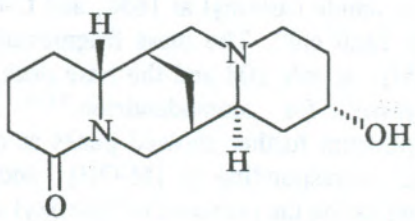
Compound 2



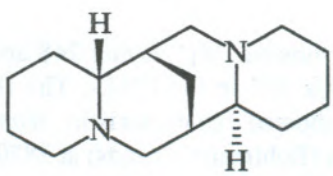
Compound 3



Compound 4



Compound 5



Compound 6

absorption bands characteristic for the *trans* quinolizidine alkaloids (2900-2750 cm^{-1}),[5,6] hydroxyl group at 3345 cm^{-1} and an amide carbonyl group at 1600 cm^{-1}.[10,15] The ^{1}H-NMR showed a downfield proton at δ 4.5 (1H, dt, J= 13.2, 2.3 Hz, H-10α) which indicated a lupanine type alkaloid[9] and another signal at δ 4.1 (1H, t, J= 2.8 Hz) assigned for H-13ß. The MS data showed a M^+ at 264 and other significant peaks at m/z 247 (M^+-OH), and 246 (M^+-H_2O) indicating the presence of a hydroxyl group which is confirmed by IR peak at 3345 cm^{-1}. The hydroxyl group was located at positon 13α from the chemical shift of H-13ß (δ 4.1 ppm) multiplicity (t) and the small coupling constant (2.8 Hz).[16-18] The above physical, chromatographic and spectroscopic data are identical to those reported for the alkaloid (+)-13α-hydroxy lupanine.

Compound **6** was obtained as oily substance having $[\alpha]_D^{25}$ = -17° (C= 0.1, MeOH). Its IR spectrum showed absorption bands at 2850 and 2730 characteristic for *trans* quinolizidine bands.[5,6] The mass spectrum revealed molecular ion peak M^+ at m/z 234 and base peak at m/z 137 characteristic for sparteine[17,8]. Co-chromatography of the isolated substance with authentic sample of sparteine in different solvent systems confirmed the identity of compound **6** as sparteine.

Confirmation of the identification of the isolated compounds **1-6** was also carried out by co-chromatography with authentic samples.

Acknowledgments

The author is grateful to Prof. Dr. H. B. C. Frenzel, Hohenheim University, Germany for providing the seeds of the plant and Dr. M. H. Mohamed, Dean of the Faculty of Pharmacy, Al-Azhar University, Assiut. For providing the authentic samples.

REFERENCES

1- J. A. Mears and T. J. Mabry, Chemotaxanomy of the Leguminosae (Harborne, J. B., Bouter, D, and Tuner, B. L., eds), p. 73 (1971).

2- A. D. Kinghorn and M. F. Balandrin, in "Alkaloids, Chemical and Biological Perspectives" (Pelletier, S. W., ed), Vol. 2 p. 105 (1984).

3- F. Galinovsky, Fortschr. Chem. Org. Naturst, **8**, 245, (1951).

4- M. Wink, C. Meißner and L. Witte, Phytochemistry, **38** (1), 139 (1995).

5- F. Bohlmann, Chem. Ber., **91**, 2157 (1958).

6- F. Bohlmann, Angew. Chem., **69**, 641 (1957).

7- M. F. Balandrin and A.D. Kinghorn, J. Nat. Prod., **44**, 495 (1981).

8- O. Ohmiya, K. Saito and I. Murakoshi, in "The Alkaloids", Academic Press. Inc., Vol. **47**, p. 1 (1995).

9- F. Bohlmann and D. Schumann, Tetrahedron Letters, **28**, 2435 (1965).

10- R. M. Silverstein, Spectrometric Identification of Organic Compounds, 3rd ed, John Willy, New York (1981) p. 112.

11- M. Wink, L. Witte and T. Hartmann, Planta Medica, **43**, 342 (1981).

12- M. H. Mohamed, "Phytochemical Studies on lupin Alkaloids in Some Egyptian Plants", Ph.D. Thesis, Faculty of Pharmacy, Al-Azhar University, Assiut (1991).

13- M. Wink, L. Witte and T. Hartmann C. Theuring and V. Volz, Planta Medica, **48**, 253 (1983).

14- O. B. Abdel-Halim, "A Phytochemical Study of Certain Plants Belonging To the Family Leguminosae", Ph.D. Thesis, Faculty of Pharmacy, El-Manuoura University, El-Mansoura (1992).

15- K. Nakanishi, "Infrared Absorption Spectroscopy Practical", Holden-Day, San Francisco and Nankodo Company Ltd, Tokyo, (1962).

16- M. P. Nasution, A. D. Kinghorn and R. J. Molyneux, Phytochemistry **32**, 1605 (1993).

17- T. Nakano, A. S. Spinelli and A. M. Mendez, J. Org. Chem. **39**, 3585 (1974).

18- G. Veen, C. Schmidt, L. Witte, V. Wary and F. C. Czygan, Phytochemistry, **31**, 4343 (1992).