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ALKALOIDS AND ISOFLAVONOIDS FROM *LUPINUS PUBESCENS* BENTH

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من الخلاصة الكحولية لنبات الليوبينس بيسنس بينث تم فصل خمسة من الأيزوفلافونيدات وهى جينستين ، لوتيون ، ٢-هيدروكسى جينستين ، جينستين-٧-أ-جلوكوزيد ، جينستين-٤'-أ-جلوكوزيد وذلك بالإضافة إلى بيتاسيتوستيرول جلوكوزيد. وقد تم كذلك فصل ثلاثة قلويدات وهى (+)١٧-أوكسوسبارتين ، (+)-١٧-أوكسوليوبانين ، ٥،٦-ديهيدروليوبانين.
وقد تم التعرف على هذه المركبات بإستخدام الطرق الطيفية المختلفة. بالإضافة إلى مقارنة القلويدات بعينات قياسية.

From the 75% alcohol extract of Lupinus pubescens Benth herb five isoflavonoids were isolated and identified as genistein, luteone, 2´-hydroxy genistein, genistein-7-O-ß-glucoside and genistein-4´-O-ß-glucoside, in addition to ß-sitosterol glucoside. More over three quinolizidine alkaloids were isolated from the herb of the plant namely (+)-17-oxosparteine, (+)-17-oxolupanine and 5,6-dehydrolupanine. The structure of the isolated compounds was established by spectroscopic methods, as well as comparison of the isolated alkaloids with authentic samples.

INTRODUCTION

The genus *Lupinus* (Leguminosae) is known to be a rich source of the lupin alkaloids[1] as well as isoflavonoids. Studies on the chemical constituents of the genus have carried out by many researchers,[2] and various non-polar isoflavonoids have been reported.[3-5] Lupin alkaloids have been shown to be of some use in establishing phytogenetic relationships at the generic and tribal levels in the Papilionoideae.[1-6] 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.[6-7]

Lupinus pubescens Benth is considered among the old world lupins growing in North America.[8,9] In a recent study, 22 alkaloids were detected by GC/MS in the leaves and seeds of the plant.[9]

This paper deals with the structural elucidation and identification of isoflavonoids and alkaloids from the leaves and stems of the title plant. From the 75% alcohol extract of *L. pubescens* herb five isoflavonoids were isolated and identified as genistein, luteone, 2´-hydroxy genistein, genistein-4´-O-ß-glucoside, and genistein-7-O-ß-glucoside in addition to ß-sitosterol glucoside. More over three quinolizidine alkaloids were isolated from the herb of the plant namely (+)-17-oxosparteine, (+)-17-oxolupanine and 5,6-dehydrolupanine. The separation and identification of the other alkaloids are in progress. The structure of the isolated compounds was established by spectroscopic methods, as well as comparison of the isolated alkaloids with authentic samples. This is the first report of the isolated compounds from titled plant.

EXPERIMENTAL

Melting points were uncorrected and determined by Koffler's hot stage microscope.

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IR spectra were taken in KBr for solid materials and $CHCl_3$ for oily substances using Unicam SP 1205 spectrophotometer. UV spectra were measured in MeOH and different ionizing and complexing agents using Unicam 1750 spectrophotometer. The low resolution EIMS were measured on a Hitachi M-60 spectrometer at 70 eV. ^{1}H-NMR spectra were recorded on JEOL GSX 400 spectrometer. TMS was used as internal standard in DMSO-d_6 and $CDCl_3$. Dragendorff's reagent was used for alkaloids detection. TLC was carried out on pre-coated silica gel plates (Kieselgel 60 F_{254} E. Merck) using solvent systems $CHCl_3$-MeOH-28% NH_4OH (85:15:1) as system I. $CHCl_3$-MeOH (95:5) as system II and $CHCl_3$-MeOH (90:10) as system III.

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 during fruiting. The voucher specimen was identified by Prof. A. Fayed (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt).

Extraction and isolation

The air-dried powdered aerial parts (stems and leaves) of *L. pubescens* Benth (1 Kg) were extracted with ethanol 75% at room temperature by maceration and percolation till exhaustion. The combined extracts were concentrated under reduced pressure to a syrupy consistency (80 g). The alcohol free concentrate was divided into two fractions. Fraction A (43 g) was diluted with distilled water and extracted successively with hexane and n-Bu.OH. The n-Bu.OH fraction (10 g) was fractionated on silica gel column chromatography using $CHCl_3$-MeOH gradients. Elution with $CHCl_3$-MeOH (97.5-2.5) afforded a mixture of compounds **1** and **2**. This mixture was subjected to PPC using 60% HOAc to afford pure **1** and **2**. Fractions eluted with $CHCl_3$-MeOH (96:4) afforded only compound **3**. Elution with $CHCl_3$-MeOH (9:1) afforded compound **4**. Elution with $CHCl_3$-MeOH (8:2) afforded compound **5** and further elution afforded **6**.

Fraction B (37 g) was suspended in 10 % acetic acid and the insoluble materials were filtered off. The acid extract was washed with Et_2O x 2, basified with 28 % NH_4OH and extracted with $CHCl_3$ repeatedly until it became negative to Dragendorff's reagent. The $CHCl_3$ extract was washed with distilled water (2 x 100 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to a semisolid residue (6 g).

The crude alkaloids (6 g) were sujected to silica gel column chromatography (E. Merck, type 60, 230 mesh, 300 g, 4x100 cm) and then gradiently eluted using $CHCl_3$-MeOH-28% NH_4OH to yield three alkaloids as follows: Fractions eluted with $CHCl_3$-MeOH-28% NH_4OH (99:1:0.05) afforded compound **7**, while those eluted with $CHCl_3$-MeOH-28% NH_4OH (98.5:1.5:0.05) afforded compound **8** and fractions eluted with $CHCl_3$-MeOH-28% NH_4OH (98:2:0.05) afforded compound **9**.

Genistein (1), Colourless needles (acetone) (50 mg), m.p 298-300°, eluted by 2.5% MeOH-$CHCl_3$, R_f value= 0.42 in system II. It has dark purple flourescence in UV light. EIMS: m/z (rel. int. %) 270 [M^+, 100%], 153 (12), 152 (23), 135 (15), 124 (9) and 108 (6). ^{1}H-NMR ($CDCl_3$) δ 6.20 (1H, d, J= 2 Hz, H-6), 6.36 (1H, d, J= 2 Hz, H-8), 6.81 (2H, dd, J= 8 & 2 Hz, H-3′, H-5′), 7.34 (2H, dd, J= 8 & 2 Hz, H-2′, H-6′), 8.29 (1H, s, H-2), 12.93 (1H, s, 5-OH). UV λ_{max}^{nm}; MeOH, 262, 297(sh), 327(sh), NaOAC, 271, 326, NaOAc/H_3BO_3, 262, 335(sh), $AlCl_3$, 273, 312 (sh), 376, $AlCl_3$+HCl, 273, 312 (sh), 376, NaOMe, 276, 327 (sh)

Luteone (2) Pale yellow needles (acetone) (25 mg), m.p 224-226°. Dark purple flourescence in UV light, eluted by 2.5% MeOH/$CHCl_3$, R_f value= 0.38 in system II. ^{1}H-NMR ($CDCl_3$) δ 8.15 (1H, s, H-2), 6.50 (1H, s, H-8), 6.45 (1H, d, J= 2.4 Hz, H-3′), 6.42 (1H, dd, J= 8.5 Hz

& 2.4 Hz, H-5´), 7.09 (1H, d, J= 8.5 Hz, H-6´), 3.36 (2H, m, H-1´´a, H-1´´b), 5.28 (1H, br.t, J= 7 Hz, H-2´´), 1.65 and 1.75 (each 3 H, s,Me-4´´, Me-5´´). UV λ_{max}^{nm}; MeOH, 268, 290 (sh), NaOAC, 271, 336, $NaOAc/H_3BO_3$, 268, 290 (sh), $AlCl_3$, 275, 316 (sh), $AlCl_3$+HCl, 275, 314(sh), NaOMe, 278, 336.

2´-hydroxy genistein (3) Colourless needles (methanol) (25 mg), m.p 270-272°, eluted by 4 % MeOH-$CHCl_3$, R_f value= 0.29 in system II. It has dark purple flourescence in UV light. EIMS: m/z (rel. int. %) 286 [M^+, 100%], 219 (10), 153 (80), 152 (25), 134 (40). ^{1}H-NMR ($CDCl_3$) δ 6.10 (1H, d, J= 2 Hz, H-6), 6.27 (1H, d, J= 2 Hz,H-8), 6.38 (1H, dd, J= 7. 8 & 2.4 Hz, H-5´), 6.36 (1H, d, J= 2.4 Hz, H-3´), 6.97(1H, d, J= 7.8 Hz, H-6´), 8.07 (1H, s, H-2). UV λ_{max}^{nm}; MeOH, 260, 286 (sh), 339, NaOAC, 270, 331, $NaOAc/H_3BO_3$, 260, 336 (sh), $AlCl_3$, 272, 308 (sh), 377, $AlCl_3$+HCl, 272, 308(sh), 377, NaOMe, 274, 322.

ß-sitosterol-3-O-ß-glucoside (4) white granular powder (MeOH), m.p 296-298°, R_f value= 0.25 in system III. ^{1}H-NMR (DMSO) δ 4.25 (1H, d, J= 7.5 Hz, H-1´), 5.35 ((1H, dd, J= 4.9 & 2 Hz, H-6), 2.91-3.42 (m, sugar protons), 3.65 (1H, t, J= 12 Hz, H-3), 0.63 (3H, s, 18-CH_3), 0.76 (3H, d, J= 6 Hz, 26-CH_3), 0.80 (3H, d, J= 6 Hz, 27-CH_3), 0.82 (3H, t, J= 6 Hz, 29-CH_3), 0.90 (3H, d, J= 6 Hz, 21-CH_3), 0.94 (3H, s, 19-CH_3). ^{13}C-NMR (100 MHz, DMSO) δ 36.81 (C-1), 29.24 (C-2), 76.76 (C-3), 38.30 (C-4), 140.25 (C-5), 121.16 (C-6), 31.42 (C-7), 31.36 (C-8), 49.50 (C-9), 36.19 (C-10), 20.58 (C-11), 40.33 (C-12), 41.84 (C-13), 56.17 (C-14), 23.84 (C-15), 27.76 (C-16), 55.42 (C-17), 11.64 (C-18), 18.92 (C-19), 35.45 (C-20), 18.60 (C-21), 33.35 (C-22), 25.47 (C-23), 45.15 (C-24), 28.71 (C-25), 19.07 (C-26), 19.68 (C-27), 22.60 (C-28), 11.76 (C-29), 100.78 (C-1´), 73.46 (C-2´), 76.25 (C-3´), 70.11 (C-4´), 76.94(C-5´), 61.96 (C-6´).

Genistein-7-O-ß-glucoside(Genistin) (5) white amorphous powder (65 mg). Deep purple flourescence in UV light, eluted with 20% MeOH in $CHCl_3$. $R_f$value= 0.1 in system III. ^{1}H-NMR (DMSO) δ 6.45 (1H, d, J= 2 Hz, H-6), 6.71 (1H, d, J= 2 Hz, H-8), 6.81 (2H, d, J= 8 Hz, H-3´, H-5´), 7.38 (2H, d, J= 8 Hz, H-2´, H-6´), 8.41 (1H, s, H-2), 13.05 (1H, s, 5-OH), 9.61 (1H, s, 4´-OH), 5.05 (1H, d, J= 7.5 Hz, H-1´´). UV λ_{max}^{nm}; MeOH, 262, 330 (sh), NaOAC, 262, 328, $NaOAc/H_3BO_3$, 262, 328 (sh), $AlCl_3$, 273, 310(sh), 373, $AlCl_3$+HCl, 273, 309 (sh), 372, NaOMe, 271, 353 (sh).

Genistein-4´-O-ß-glucoside (Sophoricoside)(6) white amorphous powder (65 mg). Deep purple flourescence in UV light, eluted with 20% MeOH in $CHCl_3$. R_f value= 0.08 in system III. ^{1}H-NMR (DMSO) δ 6.25 (1H, d, J= 2 Hz, H-6), 6.46 (1H, d, J= 2 Hz, H-8), 6.82 (2H, d, J= 8.5 Hz, H-3´, H-5´), 7.40 (2H, d, J= 8.5 Hz, H-2´, H-6´), 8.56 (1H, s, H-2), 12.93 (1H, s, 5-OH), 4.96 (1H, d, J= 7.5 Hz, H-1´´). UV λ_{max}^{nm}; MeOH, 262, 325 (sh), NaOAC, 272, 326, $NaOAc/H_3BO_3$, 262, 326 (sh), $AlCl_3$, 273, 312 (sh), 371, $AlCl_3$+HCl, 273, 311 (sh), 371, NaOMe, 250 (sh), 275, 328.

(+)-17-oxosparteine (7) colourless needles, m.p 82-84°. $[\alpha]_D^{20}$= +19.5 (C= O.1, Ethanol), R_f value= 0.76 in system I. IR, ν(cm^{-1}), 2850-2700 (trans quinolizidine band), 1630 (lactam carbonyl). ^{1}H-NMR ($CDCl_3$) δ 4.78 (1H, dt, J= 13.5, 2, 2.2 Hz, H-15α), 2.52 (1H, m, H-15ß). EIMS m/z (rel. int. %), 248 (M^+, 48), 97 (100), 247 (12), 220 (38), 191 (15), 110 (75), 98 (90).

(+)-17-oxolupanine (8) colourless needles, m.p 152-154°. $[\alpha]_D^{20}$= +139.5 (C= 0.1, Methanol), R_f value= 0.75 in system I. EIMS m/z (rel. int. %), 262 (M^+, 45), 150 (100), 151 (30), 136 (12), 112 (36), 110 (45), 97 (30).

(+) 5,6-dehydrolupanine (9) colourless oil. $[\alpha]_D^{20}$= +38.2 (C= 0.1, Ethanol), R_f value= 0.69 in system I. IR, ν (cm^{-1}), 2850-2700 (trans quinolizidine band), 1630 (lactam carbonyl). ^{1}H-NMR ($CDCl_3$) δ 4.94 (1H, t, J= 4 Hz, H-5), 4.05 (1H, d, J= 12.2 H-10α), 3.25 (1H, dd, J= 12.2, 5 Hz, H-10ß). EIMS m/z (rel. int. %),

246 (M^+, 48), 98 (100), 136 (12), 134 (8), 97 (36) and 84 (16).

Acid hydrolysis:

Each isolated glycoside (10 mg) was separately dissolved in 10 ml MeOH to which 10 ml of 0.05 M H_2SO_4 was added and the mixture was refluxed on a boiling water bath for 3 hours. The mixture was then cooled and the aglycone was extracted with $CHCl_3$, purified and subjected to TLC and spectral studies. The aqueous phase was neutralized with barium carbonate, filtered and the filtrate was examined by PC for the liberated sugar. The sugar moiety in the hydrolysate in all cases was identified as glucose, while the aglycone was identified as genistein for compounds **5** and **6** and ß-sitosterol for compound **4.**

RESULTS AND DISCUSSION

The vacuum dried alcohol 75% of *L. pubescens* Benth aerial parts was successively exhausted with n-hexane and n-butanol. From the n-butanol extract, five isoflavones **1-3,5,6** have been isolated (see experimental). The isoflavonoid nature of the isolated compounds was deduced from the UV spectra (λ_{max} at *ca* 262 and *ca* 290 (sh))[10,11] and from ^{1}H-NMR (characteristic low-field singlet assigned to an isoflavonoid C_2-H at *ca* δ 8.2).[12]

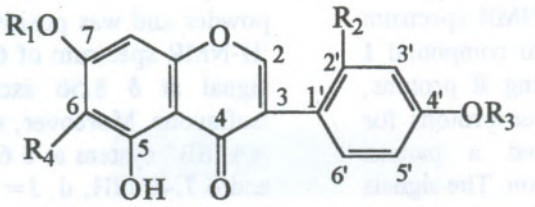
Compound 1 was obtained as colourless needles (acetone). The ^{1}H-NMR spectrum showed a characteristic signal at δ 8.29 ascribable to the H-2 signal of the isoflavone. Furthermore, the ^{1}H-NMR spectrum revealed signals due to an aromatic AA´XX´ system at δ 6.81 (2H, dd, J= 8 & 2 Hz) and δ 7.34 (2H, dd, J= 8 & 2 Hz) assigned to H-3´, H-5´ and H-2´, H-6´, respectively, and two meta coupled aromatic protons at δ 6.20 (1H, d, J= 2 Hz) and δ 6.36 (1H, d, J= 2 Hz) assigned to H-6 and H-8, respectively. The bathochromic shifts observed with NaOAc and $AlCl_3$ indicate the presence of free phenolic hydroxyl groups in positions 7 and 5 respectively. The MS spectrum of **1** revealed M^+ at m/z 270. The above mentioned data proved the structure of compound **1** as genistein.[13,14]

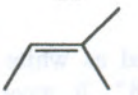
Compound 2 was obtained as pale yellow needles, m.p 224-226° and gave a violet colour with EtOH-$FeCl_3$ reagent. The ^{1}H-NMR spectrum of **2** showed a characteristic down field singlet at δ 8.15 which clearly indicated the isoflavone nature of the compound. The bathochromic shift of the UV maxima observed by adding $AlCl_3$ indicated the presence of a free OH group *peri* (C-5 position) to the carbonyl group.[14] A bathochromic shift of the maxima was also observed by adding NaOAc which suggested a free OH group at C-7.[14] The ^{1}H-NMR spectrum of **2** showed signals due to one aromatic proton as a singlet at δ 6.50 (1H, s, H-8), and an ABX system at δ 6.45, δ 6.42 and δ 7.09 for 2´, 4´-dihydroxyphenyl residue in compound **2.**[3]

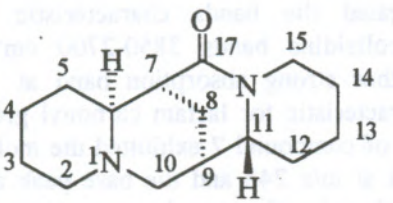
The ^{1}H-NMR spectrum of **2** also exhibited signals at δ 1.65 and 1.75 (3H each, s) assigned for non-equivalent terminal methyl groups, an olefinic proton at δ 5.28 (1H, br.t, J= 7 Hz) for H-2´´ and a signal at δ 3.36 attributed to the methylene (2H, m, H-1´´ a and H-1´´ b). These fragments are characteristic for 3,3-dimethyl allyl unit.[3]
Comparison of compound **2** with **3** clearly showed the absence of proton 6 in **2** and similar oxygenation pattern of ring B indicating the attachment of 3, 3-dimethyl allyl unit at C_6 of **2.**

The aforementioned data were superimposable with those reported for luteone[3,4] which was isolated from different species of *Lupinus*.[3,4]

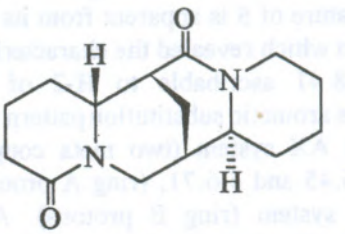
Compound 3 was found to have an UV and ^{1}H-NMR spectra consistent with an isoflavone structure.[10-12] Bathochromic shifts observed with NaOAc and $AlCl_3$ indicated that compound **3** was phenolic with free phenolic hydroxyl groups in positions 7 and 5 respectively.[14] The MS spectrum of **3** revealed M^+ at m/z 286 consistent with the molecular formula $C_{15}H_{10}O_6$ and exceeding by 16 a.m.u from compound **1** and also showed fragment ions at m/z 152 and 134 resulted from RDA fragments of an isoflavone



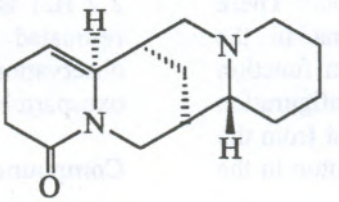
	R_1	R_2	R_3	R_4
Compound 1	H	H	H	H
Compound 2	H	OH	H	
Compound 3	H	OH	H	H
Compound 5	gluc.	H	H	H
Compound 6	H	H	gluc.	H



Compound 7



Compound 8



Compound 9

having two hydroxyls in both benzoyl and cinnamoyl moieties.[13,14] The ^{1}H-NMR spectrum of **3** showed a close similarity to compound **1** except the chemical shifts of ring B protons, where compound **3** revealed three protons for ring B. These protons formed a pattern consistent with 2´,4´-hydroxylation. The signals centered at δ 6.36, 6.38 and 6.97 were assigned to protons on C-3´, C-5´ and C-6´, respectively. The above mentioned data confirmed the identity of compound **3** as 2´-hydroxy genistein.[4,13]

Compound 4 was obtained as white granular powder melted at 296-298°. It gave positive tests for unsaturated sterols and/or triterpenes and carbohydrates and/or glycosides.

From the physical (m.p and mmp), chemical and spectral data (^{1}H-NMR and ^{13}C-NMR)[15], this compound was identified as ß-sitosterol-3-O-ß-glucoside.

Compound 5, white amorphous powder was positive to EtOH-$FeCl_3$ reagent. The isoflavonoid nature of **5** is apparent from its ^{1}H-NMR spectrum which revealed the characteristic singlet at δ 8.41 ascribable to H-2 of the isoflavone. The aromatic substitution pattern was defined by an AX system (two meta coupled doublets at δ 6.45 and δ 6.71, (ring A protons) and AA´BB´ system (ring B protons). Acid hydrolysis of **5** afforded the aglycone genistein and glucose.

Addition of $AlCl_3$, however, produced a bathochromic shift of the UV maxima, thereby, suggesting the presence of a free OH group *peri* (C-5 position) to the carbonyl function.[14] There was no change in the UV maxima in the presence of NaOAc, the C-7 oxygen function was, therefore, substituted.[14] The ß-configuration of the glucosyl moiety was determined from the coupling constant of the anomeric proton in the ^{1}H-NMR spectrum (J= 7.5 Hz).

From all the abovementioned data (^{1}H-NMR and UV) in addition to acid hydrolysis. This compound was identified as genistein-7-O-ß-glucopyranoside.[2]

Compound 6 was obtained as white amorphous powder and was positive to $FeCl_3$ reagent. The ^{1}H-NMR spectrum of **6** showed a characteristic signal at δ 8.56 ascribable to H-2 of the isoflavone. Moreover, signals due to an aromatic AA´BB´ system at δ 6.82 (2H, d, J= 8.5 Hz) and δ 7.40 (2H, d, J= 8.5 Hz) assigned for H-3´, H-5´ and H-2´, H-6´, respectively, an aromatic AX system at δ 6.25 (1H, d, J= 2 Hz) and δ 6.46 (1H, d, J= 2 Hz) assigned for H-6 and H-8, respectively and one anomeric proton at δ 4.96 (1H, d, J= 7.5 Hz). Acid hydrolysis of the compound 4 afforded glucose (PC) and genistein (co-chromatography with authentic sample). The Bathochromic shifts observed with NaOAc and $AlCl_3$ indicated that compound **6** was phenolic with free phenolic hydroxyl groups in positions 7 and 5 respectively.[14] Based on these observations **6** was persumed to be genistein-4´-O-ß-glucopyranoside.[2]

Compound 7 was isolated as white needles melted at 82-84°. It is soluble in benzene, $CHCl_3$, methanol and ethanol. Its IR spectrum revealed the bands characteristic for trans quinolizidine bands 2850-2700 cm^{-1},[16,17] and another strong absorption band at 1630 cm^{-1} characteristic for lactam carbonyl group.[18] The MS of compound **7** exhibited the molecular ion peak at m/z 248 and the base peak at m/z 97. Another significant peaks were also found at m/z 247, 220, 110 and 98. This fragmentation pattern is in accordance with the data reported for (+)-17-oxosparteine.[19,20] The 400 MHz ^{1}H-NMR spectrum exhibited an isolated signal resonated at δ 4.78 as dt (1H, dt, J= 13.5, 2, 2.2 Hz) assigned for H-15α, while H-15ß was resonated at δ 2.52 (1H, m). Based on these observations **7** was persumed to be (+)-17-oxosparteine.

Compound 9 was isolated as colourless oil (6 mg) and has $[\alpha]_D^{20}$ = +38.2 (C= 0.1, MeOH). The IR spectrum showed the bands characteristic for *trans* quinolizidine bands 2850-2700 cm^{-1},[16,17] and another strong absorption band at 1635 cm^{-1} characteristic for lactam carbonyl group. The MS of compound **9** exhibited the molecular ion

peak at m/z 246 and the base peak at m/z 98. Another significant peaks were also found at m/z 245, 136, 134, 110, 97 and 84. This fragmentation pattern is in accordance with the data reported for (+)-5,6-dehydrolupanine.[21] The 400 MHz ^{1}H-NMR spectrum revealed the triplet resonating at δ 4.94 (1H, t, J= 4 Hz), doublet resonating at δ 4.05 (1H, d, J= 12.2 Hz) and the double doublet at δ 3.25 (1H, dd, J= 12.2, 5 Hz) assigned for H-5, H-10α, and H-10ß, respectively. From the abovementioned data this compound was identified as(+)-5,6-dehydrolupanine.[21]

Compound **8** was isolated in a minor quantity (3 mg), and its identification as (+)-17-oxolupanine was based mainly upon comparison of its EIMS and $[\alpha]_D^{20}$ with that reported for (+)-17-oxolupanine.[19]

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