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ALKALOIDS OF ETHIOPIAN *CALPURNIA AUREA* SUBSP. *AUREA*

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Key Word Index—*Calpurnia aurea*; Leguminosae; quinolizidine alkaloids; novel alkaloids; 3β,4α,13α-trihydroxylupanine; 3β,4α-dihydroxy 13α-*O*-(2′-pyrrolylcarbonyl)-lupanine (calpaurine).

Abstract—Two novel alkaloids 3β,4α,13α-trihydroxylupanine and 3β,4α-dihydroxy 13α-*O*-(2′-pyrrolylcarbonyl)-lupanine (calpaurine) have been isolated from the leaves of Ethiopian *Calpurnia aurea* subsp. *aurea*. In addition, lupinine and epilupinine (both new for the genus), calpurmenine and calpurmenine pyrrolecarboxylic acid ester (previously found in subsp. *sylvatica* but not in subsp. *aurea*) have been isolated together with 13-hydroxylupanine, its tiglate and pyrrolecarboxylic acid esters (calpurnine), virgiline and virgiline pyrrolecarboxylic acid ester, alkaloids which have been reported previously from subsp. *aurea*.

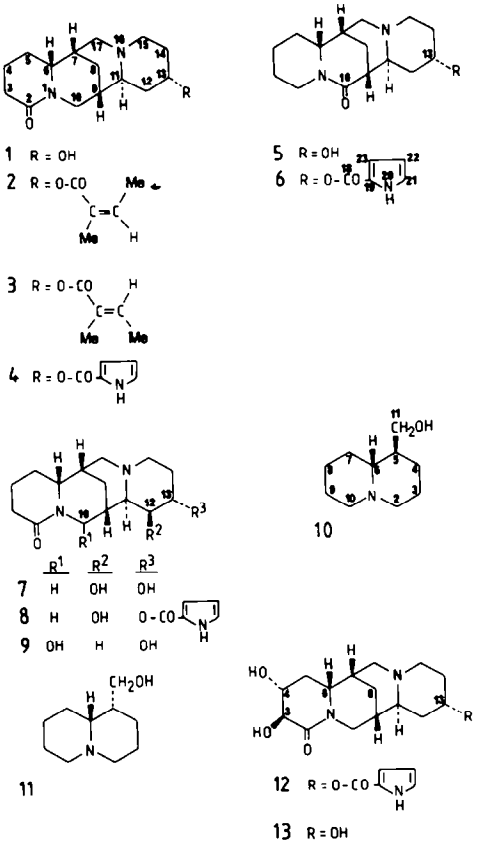
INTRODUCTION

The genus *Calpurnia* (Leguminosae) comprises some six or seven species which have their centre of distribution in South Africa [1]. *Bolusanthus* and *Virgilia* are closely related genera and, formerly, species of *Calpurnia* were included in the latter genus. *Calpurnia aurea* (Ait.) Benth. is a yellow-flowered small tree or shrub (Natal Laburnum) widely distributed in Africa from Cape Province to Eritrea and which also occurs in southern India. Three subspecies of *C. aurea* are recognised, namely subsp. *aurea* which occurs in Ethiopia but is distributed widely through Zaire, Zimbabwe, Angola and W. Africa, subsp. *sylvatica* (Burch.) Brummtt (*Sophora sylvatica* Burch.) which is found in Cape Province and subsp. *indica* Brummtt (*Virgilia aurea sensu* Wight and Walker-Arnott) which occurs in India [1]. Intermediates between subsp. *aurea* and *sylvatica* are known.

Subsp. *aurea* from Ethiopia is known locally as 'Digitta' and its extracts are used in indigenous medicine as insecticides as well as for the treatment of scabies, amoebic dysentry and diarrhoea in animals [2]. The leaves, in particular, are used for killing head lice in humans and ticks in cattle.

Previous chemical investigations of *C. aurea* have resulted in the isolation of a series of quinolizidine alkaloids. The leaves and twigs of Ethiopian *C. aurea* yielded 13-hydroxylupanine (**1**), a mixture of its angelate and tiglate esters (**2** + **3**) and of its 13-pyrrolecarboxylic acid ester (calpurnine, **4**) together with virgiline (**5**) and its pyrrolecarboxylic acid ester (**6**) [3]. Alkaloids **1**, **4**, **5** and **6** were isolated also from the leaves and twigs of S. African *C. aurea* subsp. *sylvatica* but this subspecies did appear to be somewhat different from subsp. *aurea* in that 12β,13α-dihydroxylupanine (calpurmenine, **7**) and its 13α-pyrrolecarboxylic acid ester (**8**) were also isolated [4]. The pods of subsp. *sylvatica* yielded alkaloids **1**, **4**, **6**, **7** and **8** together with 10,13-dihydroxylupanine (**9**) [4].

In the present investigation, leaves of Ethiopian *C. aurea* have been re-investigated for the presence of their alkaloidal constituents.



RESULTS AND DISCUSSION

TLC examination of the crude alkaloidal extract of Ethiopian *C. aurea* leaves indicated the presence of at least

13 alkaloids. Eleven of these alkaloids have been isolated and characterized. Four of them proved to be identical with those alkaloids previously isolated from the leaves and twigs of Ethiopian *C. aurea* [3] and the presence of 13-hydroxylupanine (**1**), calpurnine (**4**), virgiline (**5**) and virgilinepyrrolecarboxylic acid ester (**6**) were confirmed. These alkaloids were identified on the basis of their UV, IR, mass and ^{13}C NMR spectra (Experimental and Table 1). Previously, the presence of the mixed angelate/tiglate esters of 13-hydroxylupanine (**2** + **3**) had been reported but in the present work, only the tiglate ester (**3**) was obtained. The identification was made on the basis of UV, IR and mass spectral data and was confirmed by hydrolysis to 13-hydroxylupanine (co-TLC, IR, mass spectrum) and tiglic acid (^{1}H NMR) [5]. In addition, the presence of calpurmenine (**7**) and the 13α-pyrrolecarboxylic acid ester of calpurmenine (**8**), previously isolated from the S. African subsp. *sylvatica* [4], was established in the Ethiopian subsp. *aurea*. The identity of these two latter alkaloids was established by means of their UV, IR, mass and ^{13}C NMR spectral characteristics (Experimental and Table 1).

The four remaining alkaloids isolated from the leaves of Ethiopian subsp. *aurea* have not been described previously from the genus. Two were identified as epilupinine (**10**) and lupinine (**11**) on the basis of their mass, IR and ^{13}C NMR spectra (Experimental and Table 1). The two other alkaloids which were isolated proved to be novel compounds.

The structure of the first novel alkaloid, named calpaurine in a brief communication [6] was established as **12** by means of spectroscopic data which included mono- and bi-dimensional NMR techniques (Fig. 1). The IR spectrum had absorption bands at 2800–2700 (*trans*-quinolizidine), 3300–3200 (hydroxyls), 1640 and 1690 cm^{-1} (lactam and ester carbonyls). Evidence for a close similarity in structure to calpurnine (**4**) was obtained from mass spectral fragmentation. The presence of an ion fragment at *m/z* 278 was indicative of the loss of a pyrrolecarboxylic acid moiety. The $[M]^+$ at *m/z* 389 and the base peak at *m/z* 278 occurred at 32 mu higher than the corresponding ion signals in the spectrum of calpurnine indicating that the calpaurine molecule contained an additional two oxygen atoms.

These findings were substantiated by means of ^{13}C NMR measurements since the ^{13}C multiplicity of calpurnine (**4**) and calpaurine (**12**), revealed by DEPT spectra showed that the ratio of methine carbons to methylene carbons was 8:9 for calpurnine and 10:7 for the new alkaloid calpaurine. Hence, two additional substituents were indicated in the lupanine part of calpaurine and these must be hydroxyl groups as indicated by mass spectrometry. The assignments of the ^{13}C NMR spectra for calpaurine (**12**) and the closely related alkaloids which were isolated are given in Table 1.

Overcrowding in the upfield region of the ^{1}H NMR spectrum (Fig. 1) meant that it was impossible to make a complete assignment by the conventional monodimensional technique. Hence, a proton–proton two dimensional chemical shift correlation was used in order to assign the spectrum. In this type of experiment, the connectivities between protons in a given molecule are built up by observations of the ^{1}H–^{1}H couplings. These connectivities are revealed in a contour map and are seen as cross-peaks laying off the diagonal (Fig. 1). Even so, it was still not possible to complete all of the assignments and hence the information obtained was integrated with that gained from the analysis of a second ^{1}H–^{1}H chemical shift correlation spectrum of lupanine. A combination of these two experiments resulted in a complete assignment

Table 1. ^{13}C NMR chemical shifts of alkaloids isolated from Ethiopian *Calpurnia aurea**

C	**1**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[c]	**8**[d]	**10**[b]	**11**[b]	**12**[b]	**13**[c]
2	171.9	171.6	42.7	42.6	172.7	178.6	56.3	56.7	171.8	173.8
3	32.9	33.1	25.1	24.8	32.8	34.5	24.3	23.1	74.3	75.6
4	19.4	19.5	24.8	22.6	19.2	20.7	28.7	30.1	68.0	69.6
5	26.3	26.6	29.1	26.0	26.8	25.3	42.9	37.6	26.5	27.1
6	60.7	60.7	59.4	59.2	61.1	65.3	65.3	64.8	57.7	59.3
7	33.8	34.2	32.4	32.4	31.7	32.5	27.8	28.1	33.4	35.3
8	27.3	27.3	22.6	22.6	27.3	27.8	24.2	24.2	27.5	31.8
9	31.9	32.6	43.2	43.1	30.8	32.3	24.9	24.9	32.2	33.4
10	46.6	46.9	172.9	172.0	47.1	49.1	56.9	56.7	48.3	49.9
11	57.4	57.6	52.0	52.6	60.9	63.1	65.3	64.8	57.4	58.7
12	39.5	36.1	29.3	29.1	72.8	70.5	—	—	34.6	39.9
13	63.7	68.0	65.3	69.0	66.9	69.8	—	—	68.4	65.3
14	31.2	28.7	25.1	25.1	27.1	28.8	—	—	32.4	34.2
15	49.4	49.9	47.8	48.3	49.4	52.3	—	—	49.4	50.3
17	52.2	52.1	46.1	45.9	52.3	53.4	—	—	50.5	53.0
18	—	160.2	—	160.5	—	163.3	—	—	160.8	—
19	—	122.9	—	122.8	—	123.5	—	—	122.9	—
21	—	123.4	—	123.2	—	128.2	—	—	123.4	—
22	—	110.3	—	110.1	—	113.1	—	—	110.3	—
23	—	116.1	—	115.7	—	119.7	—	—	116.1	—

Solvents used: [a]$CDCl_3$; [b]CD_3OD; [c]D_2O + DCl.

*The chemical shifts for compounds **1**, **10** and **11** are closely similar to those reported previously [12].

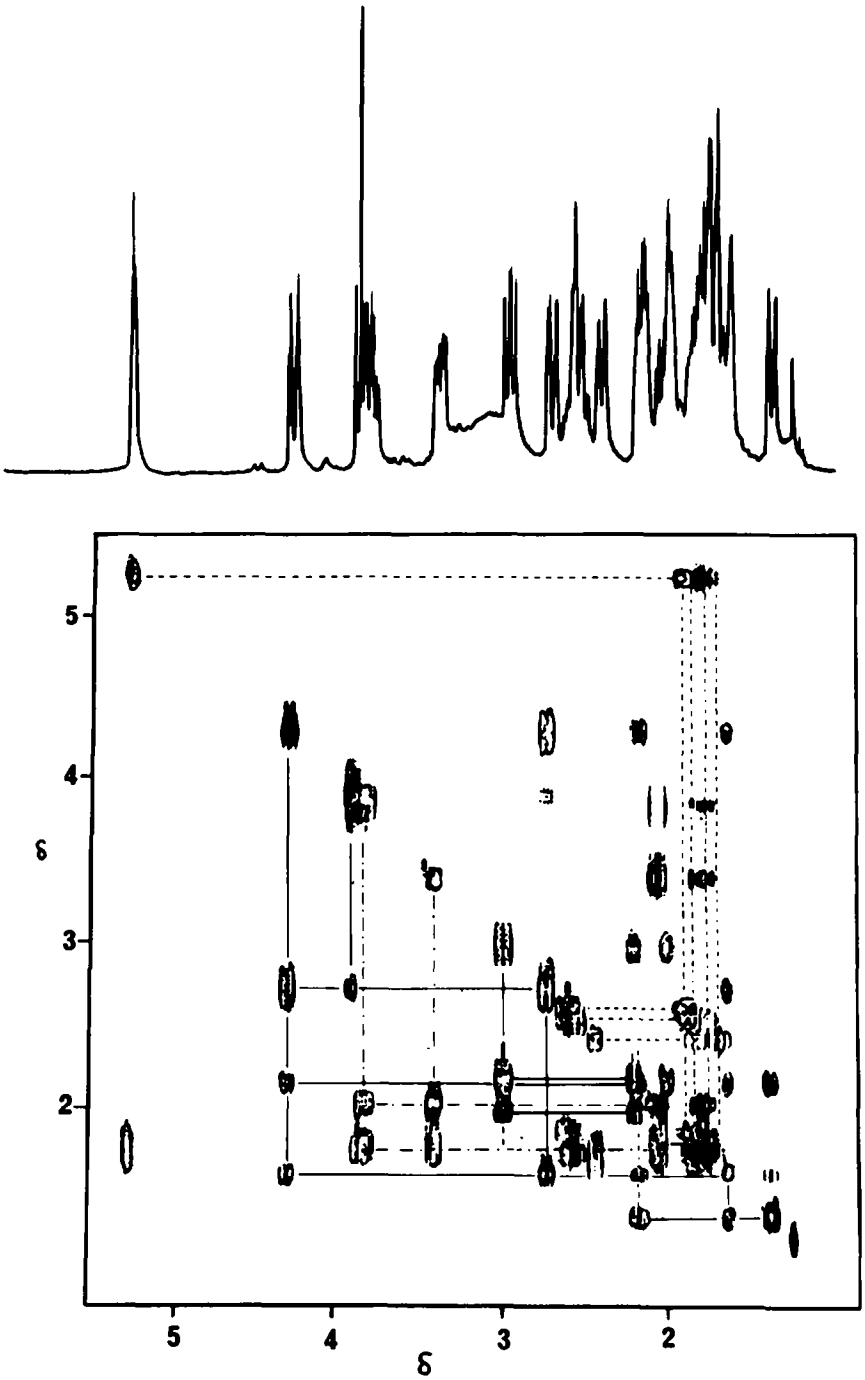


Fig. 1. ^{1}H NMR spectrum and ^{1}H–^{1}H two dimensional NMR spectrum of calpaurine (**12**).

of the ^{1}H spectrum and the following important findings were made for calpaurine. (1) The position of hydroxyl substitution must be at C-3 and C-4 and (2) the position of the ester linkage is at C-13.

The conformations, and hence the configurations of the hydrogens at C-3 and C-4, were established by NOE measurements and coupling constant considerations. From a study of molecular models, it can be predicted that if H-4 is axial and β, then in addition to the NOEs at H-8a, H-10a, H-5e and H-7, there would be enhancement of the H-4 signal on irradiation of the H-6 signal; there would be no such enhancement if H-4 were equatorial. The experimental findings (Fig. 2) clearly indicated that H-4 possesses an axial conformation and that C-4 is above the planar part of the ring, consistent with the conformation seen in lupanine-type alkaloids [7]. The $^3J_{H\text{-}4\text{-}H\text{-}3}$ of 10 Hz is indicative of a dihedral angle of 140° which is consistent with an axial conformation for H-3. From this data,

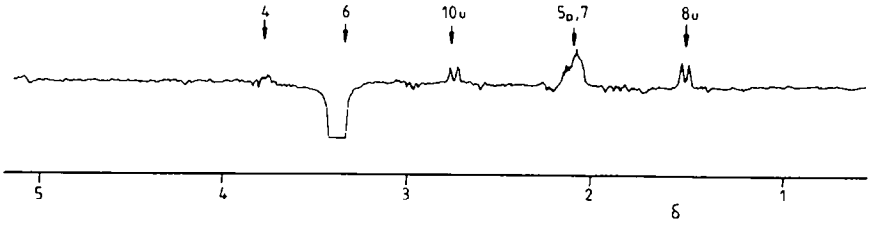


Fig. 2. Nuclear Overhauser difference spectrum on irradiation of the H-6 signal of calpaurine.

structure **12** is proposed for calpaurine. Full details of NMR experiments will be published elsewhere [8].

The second novel alkaloid (**13**) was the most polar of the alkaloids isolated and was a minor component isolated in a yield of 0.003%. The EI mass spectrum showed the presence of an $[M]^+$ at *m/z* 296 (measured 296.1738; $C_{15}H_{24}N_2O_4$ calculates for 296.1736) and the fragmentation pattern was indicative of a lupanine-type structure with three additional oxygen substituents [9]. The fragment peak at *m/z* 166 (measured 166.1235; $C_{10}H_{16}NO$ calculates for 166.1232), together with the base peak at *m/z* 152 (measured 152.1075; $C_9H_{14}NO$ calculates for 152.1076) were characteristic of a lupanine-type structure with one hydroxyl substituent in ring D. The mass spectral fragmentation pointed to the presence of two hydroxyl substituents in ring A. confirmation of the structure as 3β,4α,13α-trihydroxylupanine (**13**) was obtained by hydrolysis of calpaurine (**12**) which furnished pyrrole-2-carboxylic acid and an amino alcohol which was identical (TLC, mass spectrum) with the new minor alkaloid.

The two major alkaloids of Ethiopian *C. aurea* subsp. *aurea* were calpurnine (0.50%) and virgilinepyrrole carboxylic acid (0.55%). The present findings indicate that subsp. *aurea* and subsp. *sylvatica* have seven alkaloids which are common to each of the subspecies.

EXPERIMENTAL

Analytical TLC was carried out on silica gel GF_{254} (Merck) using the following solvent systems: (A) $CHCl_3$–MeOH–28% NH_4OH (90:9:1); (B) *iso*-PrOH–EtOAc–$CHCl_3$–28% NH_4OH (11:4:4:1); (C) $CHCl_3$–Et_2NH (9:1); (D) Et_2O–MeOH–28% NH_4OH (44:5:1). MS were recorded at 70 eV. ^1H and ^{13}C NMR spectra were taken at 300 MHz and 75 MHz, respectively, in $CDCl_3$, MeOH or D_2O solns with TMS as int. ref. The ^1H–^1H correlation spectra [10] were obtained using 256 increments of 2K points each. A 90° pulse was used for the detection of the signals after the evolution period.

The plant material was collected in January 1983 from the Shoa region 100 km north of Addis Ababa, Ethiopia and identified by Dr C. Stirton, Royal Botanic Gardens, Kew.

Extraction and isolation of alkaloids. Dried, powdered leaves (500 g) were defatted with *n*-hexane in a Soxhlet apparatus for 24 hr and the marc further extracted with MeOH for 48 hr. The dark green residue remaining, after removal of MeOH under red. pres., was taken up in 2% H_2SO_4 (100 ml) and filtered. The acidic aq. extract was washed with Et_2O until the washings were colourless, basified with conc. NH_4OH (pH 9) and extracted with Et_2O (8 × 100 ml). The combined Et_2O extracts were dried (Na_2SO_4), filtered and concd *in vacuo* to give a light brown semi solid (7.53 g, 1.5%). The alkaline aq. phase was further extracted with $CHCl_3$ (3 × 100 ml) which was dried (Na_2SO_4), filtered and concd to dryness under red. pres. to yield a reddish brown semi-solid (0.75 g, 0.15%). Prep. TLC revealed the presence of five and eight alkaloids in the Et_2O and $CHCl_3$ soluble fractions, respectively. Repeated prep. TLC using systems A, B and C afforded 11 alkaloids, 10 of which were characterized by their ^{13}C NMR spectral properties (Table 1) and as follows.

13-Hydroxylupanine (**1**). R_f 0.36 (system A); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–3200 (OH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1640 (lactam CO); MS *m/z* (rel. int.): 264 $[M]^+$ (47), 247 (30), 246 (47), 166 (30), 165 (42), 152 (100), 134 (28), 114 (23), 113 (28) and 112 (33) [11].

Tiglate ester of 13-hydroxylupanine (**3**). R_f 0.63 (system A); IR $\nu_{max}^{CHCl_3}$ 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1690 (ester CO), 1620 (lactam CO) cm^{-1}; MS *m/z* (rel. int.) 346 $[M]^+$ (8), 331 (12), 299 (8), 279 (18), 264 (21), 246 (100), 166 (29), 152 (19), 134 (33).

Hydrolysis. Alkaloid **3** (4 mg) was dissolved in 2% NaOH (4 ml) containing EtOH (0.5 ml) and heated at 60° for 4 hr. The hydrolysate was extracted with $CHCl_3$ (3 × 3 ml), dried (Na_2SO_4) and filtered. Removal of solvent gave a product (2.1 mg) which was identical with authentic 13-hydroxylupanine (co-TLC, IR, MS). The remaining aq. alkaline hydrolysate was acidified and extracted with $CHCl_3$ (2 × 3 ml) to yield tiglic acid (0.8 mg) which was distinguished from its isomers angelic acid and senecic acid by the chemical shifts of its olefinic proton (δ6.95, 1H, *m*) and methyl protons (δ1.76, 6H, *m*) [5].

Calpurnine (**4**). R_f 0.60 (system A); UV λ_{max}^{EtOH} 266 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1690 (ester CO), 1620 (lactam CO); MS *m/z* (rel. int.): 357 $[M]^+$ (2), 263 (2), 246 (100), 231 (5), 148 (13), 134 (25), 112 (18), 94 (17); identical co-TLC and MS with authentic sample.

Virgiline (**5**). R_f 0.41 (system A); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–3200 (OH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1620 (lactam CO); MS *m/z* (rel. int.): 264 $[M]^+$ (32), 248 (19), 247 (26), 236 (38), 193 (26), 152 (100), 147 (24), 146 (37), 134 (17), 112 (35), 94 (25), 84 (37); identical co-TLC and MS with authentic sample.

Virgilinepyrrolecarboxylic acid (**6**). R_f 0.71 (system A); UV λ_{max}^{MeOH}: 266 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1690 (ester CO), 1620 (lactam CO); MS *m/z* (rel. int.): 357 $[M]^+$ (2), 329 (4), 263 (4), 246 (100), 245 (25), 134 (17), 112 (9), 94 (12), 84 (17) [3]; identical with authentic virgilinepyrrolecarboxylic acid (co-TLC, IR, MS).

Calpurmenine (**7**). R_f 0.21 (system A); IR ν_{max}^{KBr} cm^{-1}: 3300–3200 (OH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1620 (lactam CO); MS *m/z* (rel. int.): 280 $[M]^+$ (30), 263 (17), 262 (43), 245 (20), 168 (100), 150 (61), 134 (32), 132 (18), 112 (26) [4].

Calpurmeninepyrrolecarboxylic acid (**8**). R_f 0.50 (system A); UV λ_{max}^{EtOH}: 266 nm; IR ν_{max}^{KBr} cm^{-1}: 3450 (NH), 3300–3200 (OH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1690 (ester CO), 1620 (lactam CO); MS *m/z* (rel. int.): 373 $[M]^+$ (11), 279 (11), 263 (57), 262 (100), 245 (32), 233 (5), 205 (11), 148 (32), 134

(42), 112 (50) [4]; identical co-TLC and MS with authentic sample.

Epilupinine (**10**). R_f 0.31 (system A); 0.46 (system D); MS *m/z* (rel. int.): 169 $[M]^+$ (48), 168 (39), 152 (88), 138 (64), 124 (22), 111 (38), 110 (40), 98 (32), 97 (63), 96 (38), 84 (31), 83 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3680 (free OH), 2810–2700 (*trans*-quinolizidine, Bohlmann bands).

Lupinine (**11**). R_f 0.31 (system A), 0.55 (system D); MS *m/z* (rel. int.): 169 ($[M]^+$, 47), 168 (39), 152 (88), 138 (64), 135 (22), 111 (38), 110 (40), 98 (37), 97 (63), 96 (32), 84 (31), 83 (100), 82 (35), 51 (57); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3680 (free OH), 3380 (broad OH), 2860–2700 (*trans*-quinolizidine, Bohlmann bands).

Calpaurine (**12**). R_f 0.25 (system A); UV λ_{max}^{EtOH}: 266 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 3300–3200 (OH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1690 (ester CO), 1640 (lactam CO). MS *m/z* (rel. int.): 389.1955 ($[M]^+$ 11; $C_{20}H_{27}N_3O_5$ for 389.1951), 371 (11), 295 (9), 278.1627 (100; $C_{15}H_{22}N_2O_3$ calc. for 278.1630), 261.1604 (57; $C_{15}H_{21}N_2O_2$ calc. for 261.1603), 245 (16), 237 (21), 207 (11), 181 (16), 148 (50), 134.0973 (66; $C_9H_{12}N$ calc. for 134.0790). ^1H and ^1H–^1H NMR spectral data: see Fig. 1.

Hydrolysis. Calpaurine (4 mg) was dissolved in 0.2 N NaOH in EtOH (5 ml) and heated at 60°. After complete hydrolysis as indicated by TLC ($CHCl_3$–MeOH–28% NH_4OH, 70:30:1), the soln was evapd (to 0.5 ml) and applied as a streak to a prep. TLC plate of silica gel GF_{254} and developed with the same solvent system. The major alkaloidal band was eluted with $CHCl_3$–MeOH (1:1) which was evapd to yield a semisolid (1.4 mg) which proved to be identical with 3β,4α,13α-trihydroxylupanine (**13**) (TLC, MS). The base line portion of the prep. TLC was eluted with 0.2 N HCl and extracted with Et_2O (4 × 4 ml). The combined Et_2O extracts were dried (Na_2SO_4), filtered and concd to a solid (0.8 mg) which was identified as pyrrole-2-carboxylic acid [co-TLC and identical ^1H NMR to an authentic sample (Sigma Ltd)].

3β,4α,13α-Trihydroxylupanine (**13**). R_f 0.06 (system A); IR ν_{max}^{KBr} cm^{-1}: 3300–3200 (OH), 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1640 (lactam CO); MS *m/z* (rel. int.): 296 $[M]^+$ (41), 280 (72), 279 (33), 278 (77), 262 (16), 182 (16), 166 (27), 165 (25), 152 (100), 150 (27), 134 (16), 126 (27), 114 (27).

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