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ALKALOIDS OF SOUTH AFRICAN SAMPLES OF *CALPURNIA AUREA* SUBSP. *SYLVATICA*

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Key Word Index—*Calpurnia aurea*; Leguminosae; quinolizidine alkaloids; 10,13-dihydroxylupanine; calpurmenine; 12β,13α-dihydroxylupanine; 13-(2′-pyrrolylcarboxyl)calpurmenine; 12-hydroxy-13-*O*-(2′-pyrrolylcarbonyloxy)lupanine.

Abstract—Two new alkaloids, calpurmenine (12β,13α-dihydroxylupanine) and its 13α-pyrrolylcarboxylic acid ester have been isolated from a South African sample of *Calpurnia aurea*. The alkaloid 10,13-dihydroxylupanine, earlier found in *Cadia purpurea* but absent from Ethiopian material of *Calpurnia aurea*, was also identified.

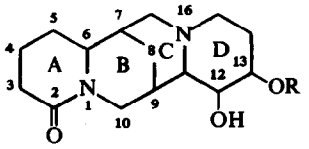
INTRODUCTION

While investigating the alkaloids of *Calpurnia aurea* collected in Ethiopia [1], our attention was drawn to the alkaloids of the same shrub growing in South Africa. The Botanical Research Institute at Pretoria supplied us with an amount of dried *Calpurnia aurea* (Ait.) Benth. (voucher No. 3206), collected in its botanical garden and checked by Mr. D. Fourie. The Ethiopian and South African samples were compared by Mr. Ir. J. J. Bos (Laboratorium voor Plantensystematiek en -geografie, Wageningen, The Netherlands) and were found to be *Calpurnia aurea* subsp. *aurea* and *Calpurnia aurea* subsp. *sylvatica*, respectively, according to the description by Brummitt [2].

When extracts of the Ethiopian and South African samples were compared by TLC, two major alkaloid spots were noticed in the latter which did not appear in the former. Consequently, we isolated the two compounds of the South African *Calpurnia* from an extract of leaves (together with small twigs) and one of pods, and report the results herein.

RESULTS AND DISCUSSION

The South African sample yielded the well known alkaloids, i.e. hydroxylupanine, calpurnine, virgiline and its pyrrolylcarboxylic acid ester, as earlier found in the Ethiopian sample [1]. These were isolated and identified by TLC and MS. In addition, the alkaloid 10,13-dihydroxylupanine was found in the CH_2Cl_2 extract of the pods. This compound, having a MW of 280 and also occurring in *Cadia purpurea* [3], was absent from the Ethiopian sample. The two alkaloids, specifically present in the South African material were isolated from the extracts of leaves and pods. One of them, alkaloid **1**, had a MW of 280 and the other, alkaloid **2**, appeared to be a pyrrolylcarboxylic acid ester of **1**. The MS of the first alkaloid showed, apart from the usual peaks belonging to the lupanine skeleton, the presence of two OH groups; there was, however, quite a difference from 10,13-dihydroxylupanine with regard to ion abundances and fragmentation pattern. The ready loss of H_2O was rather significant. MS produced the following abundant peaks: *m/e* 280, 263, 262, 245, 168, 150, 132 and 112. According to the fragment ions *m/e* 168, 150, 132 and 112, it could be deduced that the second OH group was situated in either ring C or ring D. The IR spectrum of methylated alkaloid **1** confirmed this assumption because of a slight alteration in the surroundings of the N atom (at 2700–2800 cm^{-1}) with regard to the unmethylated compound.



1 R = H
2 R = COC_4H_4N

With the help of X-ray diffraction it was possible to determine the exact position of the second OH group at C(12) and at the same time the whole conformation of alkaloid **2**. We concluded that rings C and D are *trans*-connected via the bridge C(11)–N(16); the carbonyl oxygen atom O(2) is connected to C(2) in ring A; the hydroxyl oxygen atoms O(12) and O(13) are connected in *axial b* orientations to carbon atoms C(12) and C(13) in ring D; N(16) has the normal pyramidal hybridization, with its lone pair in axial orientation, but N(1) has a nearly planar trigonal hybridization. The trigonal hybridization of N(1) as well as C(2) induces the *sofa* conformation of ring A with C(4) at 0.6 Å *outside* the planar configuration of atoms N(1), C(2), O(2), C(3), C(5) and C(6). Ring C has a nearly ideal *boat* conformation with C(8) as prow and N(16) as stern. The full details of the diffraction analysis and the conformation description are reported elsewhere [4].

Since neither of the two alkaloids in question have been described before, we propose to call alkaloid **1** calpurmenine and, consequently, alkaloid **2** is 13-(2′-pyrrolylcarboxyl)calpurmenine.

EXPERIMENTAL

TLC was carried out on Al_2O_3 (Merck No. 5575). Three solvent systems (MeCN–MeOH (10:3, system a), CH_2Cl_2–MeOH (19:1, system b) and C_6H_5Me–Me_2CO–MeOH (34:3:3, system c)) were used. $R_{s1} = R_f$ compound:R_f13-hydroxylupanine.

(A) An $EtOH_{96\%}$ – HOAc (99:1) extract of 19 g dried and ground pods was extracted with C_6H_5Me to discard most of the chlorophyll. After basification with Na_2CO_3, the residue (5.1 g) was extracted with Et_2O, CH_2Cl_2 and EtOH successively. These three fractions were chromatographed on Al_2O_3 (Merck No. 1077). The Et_2O fraction (1.50 g) yielded calpurnine, 13-hydroxylupanine and alkaloid Cp_I (4.3 mg; R_{st} 0.97 system a, R_{st} 0.71 system b, R_{st} 0.88 system c); the EtOH fraction (1.39 g) yielded alkaloid Cp_{III} (5 mg; MW 246; R_{st} 1.28 system a, R_{st} 1.83 system b, R_{st} 2.58 system c). The CH_2Cl_2 fraction (620 mg) appeared to contain the alkaloids **1** and **2**. This fraction was chromatographed on an Al_2O_3 column (∅ 25 mm) by absorbing with CH_2Cl_2–MeOH (19:1) to a height of 170 mm. Following chromatography the column was divided into 6 sections, from which 7 alkaloids were isolated, viz. 13-hydroxylupanine, 10,13-dihydroxylupanine, calpurmenine and its pyrrolylcarboxylic acid ester, calpurnine, virgilinepyrrolylcarboxylic acid ester and alkaloid Cp_{II} (5 mg; MW 278; R_{st} 0.22 system a, R_{st} 0.00 system b, R_{st} 0.00 system c).

(B) Dried and ground leaves and small twigs (47 g) were continuously extracted in a Soxhlet with $EtOH_{96\%}$–HOAc (99:1) for 3 days. After evapn of the solvent, the resulting gummy extract (15.55 g) was suspended in 50% EtOH (800 ml), the ppt. was filtered off and the EtOH evapd. The slightly acid soln was then treated in a liquid–liquid extractor with Et_2O, CH_2Cl_2 and, after addition of NH_4OH, again with CH_2Cl_2 successively. The Et_2O extract (290 mg) gave calpurnine, 13-hydroxylupanine, calpurmenine, calpurmeninepyrrolylcarboxylic acid ester and alkaloid Cp_{IV} (3.5 mg; MW 362; R_{st} 1.11 system a, R_{st} 1.76 system b, R_{st} 1.18 system c). The acid CH_2Cl_2 extract (917 mg) was further chromatographed on Al_2O_3 with CH_2Cl_2 containing increasing concns of MeOH as solvent. Elution with CH_2Cl_2–MeOH (94:6) and CH_2Cl_2–MeOH (96:4–0:100) yielded 100 mg crude alkaloid **2** and 35 mg crude alkaloid **1**, respectively. The ammoniacal CH_2Cl_2 extract (247 mg) was also chromatographed on Al_2O_3 (Merck No. 1077) and eluted with Et_2O first, then with CH_2Cl_2 containing increasing concns MeOH. These fractions afforded (besides a small amount of **1** and of **2**) the alkaloids 13-hydroxylupanine, virgiline, virgilinepyrrolylcarboxylic acid ester, alkaloid Cp_V (3 mg; R_{st} 1.31 system a, R_{st} 1.80 system b, R_{st} 2.37 system c) and alkaloid Cp_{VI} (8 mg; R_{st} 0.67 system 1, R_{st} 0.44 system b, R_{st} 0.32 system c). The isolated quantities of all numbered Cp alkaloids were too small to allow further investigation.

Alkaloid **1** = *calpurmenine* = *12β,13α-dihydroxylupanine.* Several fractions containing calpurmenine (56 mg) were purified on alumina with Et_2O and CH_2Cl_2. The yield was 30 mg. We succeeded in crystallizing this compound from MeOH–EtOAc–Et_2O. MS *m/e* 280 (M^+), 263, 262, 245, 168, 150, 132, 112, etc. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1620 (CO).

Alkaloid **2** = 13-O-(*2'-pyrrolylcarbonyl*)*calpurmenine.* A 100 mg crude **2** fraction was repeatedly crystallized by dissolving in MeOH (weakly acidified with 2 N HOAc) and adding Et_2O–CH_2Cl (1:1). Only crystals of this compound were suitable for X-ray diffraction. MS: *m/e* 373 (M^+), 355, 344, 279, 263, 245, 233, 206, 193, 164, 150, 112, etc. IR ν_{max}^{KBr} cm^{-1}: 3270 (OH), 3190 (OH), 1690 (CO ester), 1610 (CO keto) and below 1400 a number of peaks characteristic for the pyrrolylcarboxylic acid group.

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REFERENCES

1. van Eijk, J. L. and Radema, M. H. (1977) *Planta Med.* **32**, 275.
2. Brummitt, R. K. (1967) *Kirkia* **6**, 123.
3. van Eijk, J. L., Radema, M. H. and Versluis, C. (1976) *Tetrahedron Letters* **24**, 2053.
4. Vermin, W., de Kok, A. J., Romers, C., Radema, M. H. and van Eijk, J. L. (1979) *Acta Crystallogr. Sect. B* **35**, in press.