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QUINOLIZIDINE/INDOLIZIDINE ALKALOIDS FROM THE SEED OF *CAMOENSIA BREVICALYX*

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Key Word Index—*Camoensia brevicalyx*; Leguminosae–Papilionoideae; quinolizidine/indolizidine alkaloids; camoensine; camoensidine; 12-α-hydroxycamoensine; 12-hydroxy-16-methoxy-11:12,13:14-tetradehydrocamoensine; ^{13}C NMR; chemical taxonomy.

Abstract—The seeds of *Camoensia brevicalyx* have yielded five quinolizidine/indolizidine alkaloids. The known alkaloids camoensine and camoensidine and the novel 12-α-hydroxycamoensine and 12-hydroxy-16-methoxy-11:12,13:14-tetradehydrocamoensine were characterized on the basis of their spectral data. A third novel alkaloid was tentatively identified as 12-hydroxycamoensidine. The taxonomic significance of the alkaloids is discussed briefly.

INTRODUCTION

The genus *Camoensia* Welw. *ex* Benth. (Leguminosae–Papilionoideae), which occurs in west tropical Africa [1], consists of two species of clambering shrubs, *C. brevicalyx* Benth. and *C. maxima* Benth. [2, 3]. Although most authorities have been consistent in placing it in the Papilionoideae, usually in the tribe Sophoreae, Yakolev [4] assigned it to the Caesalpinoideae.

A study of the roots of *C. maxima* [5] revealed the presence of three alkaloids, leontidine (**1**), camoensine (**2**) and camoensidine (**3**). Leontidine had previously been reported from two species of *Leontice* (Berberidaceae) [6]. These alkaloids, whilst obviously closely allied to the typical quinolizidine alkaloids of the Papilionoideae [7], differ from the normal alkaloids of that group in their possession of an indolizidine C/D ring system. Despite their atypical structure the occurrence of these alkaloids is considered to offer strong support for the majority opinion that *Camoensia* belongs to the Papilionoideae rather than the Caesalpinoideae.

In this paper we report the isolation and characterization or partial characterization of five quinolizidine/indolizidine alkaloids from the seeds of *C. brevicalyx*. The chemotaxonomic significance of the *Camoensia* alkaloids is discussed in terms of recent proposals regarding the distribution of quinolizidine alkaloids in the Papilionoideae [7].

RESULTS

Extraction of the defatted seeds of *C. brevicalyx* with $CHCl_3$ gave three alkaloids. The major component was separated by CC over alumina. The two minor components were obtained from the column as a mixture and subsequently separated by preparative TLC.

The major alkaloid analysed for $C_{14}H_{18}N_2O$ and gave UV and IR spectra identical to those recorded for leontidine (**1**) and camoensine (**2**) [5]. The ^{1}H NMR spectrum showed signals for H-3, H-4 and H-5 of an α-pyridone and double doublets for the H-10 protons which form an ABX system with H-9. The EIMS gave ions for fission through the C-ring: *m/z* 160 (**4**) and 146 (**5**) for A/B rings and *m/z* 96 (**6**) and 84 (**7**) for the D-ring [8]. These data are compatible with either **1** or **2**. ^{13}C NMR shifts were assigned (Table 1) by comparison with published data for anagyrine (**8**) [9]. The shift value observed for C-8 was 21.4 ppm. C-8 is only shielded to this extent when it is brought into close proximity to N-15, as occurs in quinolizidines in which H-9 and H-11 are *trans* [9, 10] [e.g. camoensine (**2**)]. By contrast in H-9/H-11 *cis* quinolizidines [e.g. leontidine (**1**)] C-8 resonates at values greater than 30 ppm.

One of the minor alkaloids analysed for $C_{14}H_{22}N_2O$. It gave no UV spectrum and lacked α-pyridone protons in the ^{1}H NMR spectrum. The EIMS was more complex than that of **2** but the occurrence of an ion at *m/z* 84 (**7**)

Table 1. ^{13}C chemical shift values for **2**, **3**, **8**, **9** and **11** (ppm)

Carbon No.	**2**	**8** [11]	**11**	**3**	**9** [11]
2	164.1 *s*	163.5 *s*	164.7 *s*	175.2 *s*	
3	117.2 *d*	116.6 *d*	117.0 *d*	33.4 *t*	33.0 *t*
4	139.2 *d*	138.6 *d*	139.5 *d*	20.6 *t*	19.6 *t*
5	105.2 *d*	104.3 *d*	106.1 *d*	30.1 *t*	26.7 *t*
6	151.4 *s*	151.1 *s*	152.6 *s*	62.0 *d*	61.7 *d*
7	35.2 *d*	35.6 *d*	34.4 *d*	33.8 *d*	34.9 *d*
8	21.4 *t*	22.6 *t*	22.9 *t*	23.8 *t*	27.3 *t*
9	29.2 *d*	32.7 *d*	27.2 *d*	29.7 *d*	32.4 *d*
10	55.1 *t*	51.6 *t*	52.1 *t*	43.3 *t*	46.6 *t*
11	66.3 *d*	63.3 *d*	74.7 *d*	64.8 *d*	63.8 *d*
12	25.4 *t*	25.7 *t*	68.7 *d*	30.5 *t*	33.5 *t*
13	20.9 *t*	19.2 *t*	34.0 *t*	25.8 *t*	24.5 *t*
(14)*		20.8 *t*			25.3 *t*
14 (15)*	51.9 *t*	53.0 *t*	52.1 *t*	55.7 *t*	55.3 *t*
16 (17)*	55.3 *t*	54.4 *t*	57.9 *t*	60.5 *t*	57.4 *t*

* Numbers in brackets refer to the extra carbon in the D-ring of **8** and **9**.

Spectra were run in $CDCl_3$.

was indicative of the pyrrolidine D-ring. Ions **4** and **5** were displaced to a position 4 MU higher. These data are consistent with a quinolizidine/indolizidine with a reduced A-ring; either **3** or tetrahydroleontidine. The ^{13}C NMR spectrum (Table 1) was comparable with that published for lupanine (**9**) [9] and showed C-8 resonating at 23.7 ppm thus confirming the alkaloid as camoensidine (**3**).

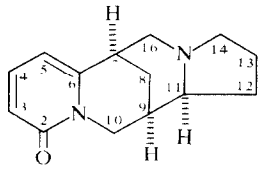
The second minor alkaloid analysed for $C_{14}H_{22}N_2O_2$. Unlike **2** and **3** it gave a sharp mp and the IR spectrum revealed an OH substituent. The EIMS gave ions for the A/B rings identical to **3**. The major ion for ring-D was observed at *m/z* 100 and included an oxygen. This suggested that the alkaloid was similar to **3** but substituted with a hydroxyl in ring-D or at C-16. Insufficient material was available for further analysis but in view of the occurrence of 12-α-hydroxycamoensine (**11**) in the MeOH extract (see below) it seems probable that this alkaloid is 12-hydroxycamoensidine (**10**).

CC of an aliquot of the MeOH extract over alumina gave **2** and **3** together with a further alkaloid which analysed for $C_{14}H_{18}N_2O_2$. Its UV spectrum was identical to **2**. The IR spectrum showed a hydroxyl substituent. EIMS gave ions **4** and **5** for the A/B rings and at *m/z* 112 (**6** + *Q*) and 100 (**7** + *Q*) for the D-ring. ^1H NMR showed the presence of the α-pyridone and gave double doublets for the H-10 protons, all in close agreement with **2**. A multiplet centred at δ 4.35 (1 H) was assigned to a CH–OH proton and a double doublet at δ 3.30 to H-11. In view of the occurrence of H-11 as a sharp double doublet, coupling to H-9, and one proton at H-12, the hydroxyl substituent was tentatively assigned to C-12.

A ^{13}C NMR spectrum (Table 1) gave signals for C-2 to C-10 and C-14 and C-16 in close agreement with **2**. Resonances for C-11 and C-13 showed deshielding of about 10 ppm, typical of carbons *ortho* to a hydroxyl bearing carbon and confirming the position of the hydroxyl group at C-12. The resonance position of C-8 again confirmed *trans* stereochemistry for H-9 and H-11.

The remaining problem was to assign the stereochemistry of the hydroxyl group at C-12. It has been shown [11, 12] that the broadening of the signal for the CH-OH proton in 1-hydroxy-indolizidine (**12**) differs widely between H-1/H-9 *cis* (*ca* 11 Hz) and *trans* (*ca* 21 Hz). In this case signal width for H-12 was 11 Hz, thus requiring H-12 to be *cis* to H-11 and identifying the alkaloid as the novel 12-α-hydroxycamoensine (**11**).

CC of the remainder of the MeOH extract over silica gel yielded traces of a fifth alkaloid (R_f = 0 on alumina) which analysed for $C_{15}H_{16}N_2O_3$. The IR spectrum indicated a hydroxyl substituent. EIMS gave ions **4** and **5** but none of the normal D-ring ions. The ^1H NMR spectrum showed the presence of the α-pyridone ring together with an additional AB quartet in the aromatic region. The highly deshielded position of one part of the AB quartet (δ 7.90) requires its assignment to H-14 with



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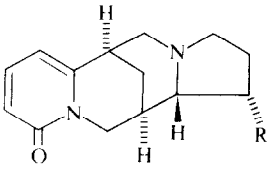
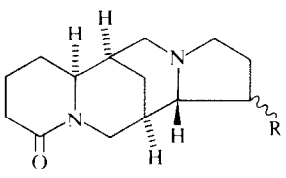
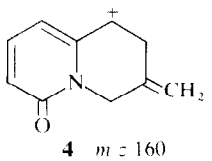


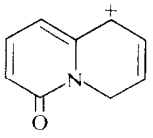
2 R = H
11 R = OH



3 R = H
10 R = OH



4 *m* = 160



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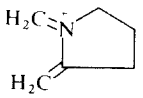

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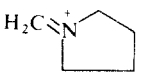
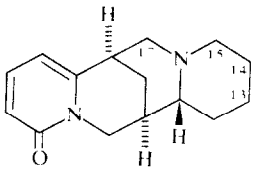


7 *m* = 84



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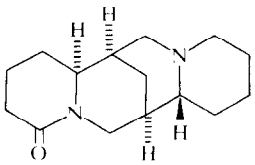

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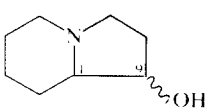

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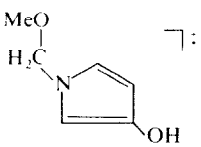
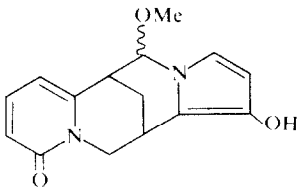


13 *m* = 127



14

the upfield portion at H-13. A sharp singlet at δ 3.15 (3 H) was assigned to a methoxyl group, confirmed by ions for $M^+ - Me$, $M^+ - MeO$ and $M^+ - MeCO$ in the EIMS.

These data suggested that the alkaloid was a quinolizidine/indolizidine with a typical A/B ring system, a double bond at C-13/C-14, hydroxyl and methoxyl substituents and a further double bond. As the ^1H NMR spectrum shows no further coupling to H-13 then C-12 must be fully substituted by either a hydroxyl or methoxyl group and by being part of the second double bond. Placement of the hydroxyl group at C-12 is favoured by the absence of an $M^+ - H_2O$ ion in the EIMS (cf. **11**), leaving the methoxyl group to be placed at C-16. This hypothesis is sustained by the occurrence of *m/z* 115 (**13**) with dependent ions for loss of CH_3, OCH_3 and CH_3CO. On the basis of the above the alkaloid is tentatively assigned the structure 12-hydroxy-16-methoxy-11:12,13:14-tetradehydrocamoensine (**14**). This is the first record of a quinolizidine alkaloid with a pyrrole D-ring occurring naturally although tetradehydroleontidine has been prepared synthetically [6].

DISCUSSION

The alkaloids isolated from *C. brevicalyx* are either identical to those of *C. maxima* or oxidation products thereof. *Camoensia* is, to date, the only genus of Leguminosae known to produce quinolizidine/indolizidine alkaloids. Their occurrence in both species of *Camoensia* strongly supports placement of *Camoensia* in the Papilionoideae, where closely allied tetracyclic quinolizidine alkaloids are widespread [7], rather than in the Caesalpinoideae, where quinolizidine alkaloids have not been recorded. Salatino and Gottlieb [7] have proposed a model for the evolution of quinolizidine alkaloids in the Papilionoideae and their systematic significance. *Camoensia* and its alkaloids were not included in that study but appear to belong to the cytisine/*para*-cytisine type which suggest an affinity to the tribes Sophoreae and Genisteae. The occurrence of 12-hydroxylation may also have some systematic significance [7].

EXPERIMENTAL

UV spectra were run in EtOH and IR spectra as KCl discs unless otherwise stated. ^1H NMR spectra were run at 90 MHz using TMS as int. standard. ^{13}C NMR spectra were run at 25.1 MHz in $CDCl_3$ using the FT mode and TMS as int. standard. EIMS were recorded at 70 eV. Mps are uncorr.

Plant material. Seeds of *Camoensia brevicalyx* Benth. were collected in the Douala-Edea Forest Reserve, west Cameroon, in the summer of 1976. A voucher, D. McKey and J. S. Gartlan 208, has been deposited at the Herbarium of the Royal Botanic Gardens, Kew.

Isolation of compounds. The ground seeds (130 g) were extracted with petrol (bp 40–60°), then $CHCl_3$, then MeOH. The $CHCl_3$ extract was concd and chromatographed over a column of alumina. Elution with $CHCl_3$ gave a mixture which, on standing, yielded **10** (4 mg). Prep. TLC of the supernatant on alumina (solvent: $CHCl_3$–MeOH, 49:1) gave **3** (10 mg). Continued elution of the column with $CHCl_3$–MeOH (99:1) gave **2** (150 mg). Identical CC of an aliquot of the MeOH extract gave traces of **3** and **10** followed by **2** (198 mg). Further elution with $CHCl_3$–MeOH (99:1) gave **11** (147 mg). CC of a second aliquot of the MeOH extract over Si gel gave, on elution with $CHCl_3$–MeOH (99:1) **14** (20 mg). Further elution with $CHCl_3$–MeOH (49:1) gave **2** and **11**.

Identification of isolated compounds. Camoensine (**2**). Brown, amorphous solid. Found: M^+ 230.1400; $C_{14}H_{18}N_2O$ requires 230.1419. $[\alpha]_D^{20}$ −108° (*c* 1.0, $CHCl_3$) (lit. [5] −186°). UV λ_{max} nm: 230, 308; (+HCl) 230, 297. IR ν_{max} cm^{-1}: 1650 (C=O), 1570, 1560. ^1H NMR ($CDCl_3$): δ 7.31 (1 H, *dd*, $J_1 = 9$ Hz, $J_2 = 7$ Hz, H-4), 6.43 (1 H, *dd*, $J_1 = 9$ Hz, $J_2 = 1$ Hz, H-5), 6.03 (1 H, *dd*, $J_1 = 7$ Hz, $J_2 = 1$ Hz, H-3), 4.20 (1 H, ABX, $J_{AB} = 14$ Hz, $J_{AX} = 1$ Hz, H-10a), 3.96 (1 H, ABX, $J_{AB} = 14$ Hz, $J_{BX} = 7$ Hz, H-10e). ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 230 $[M]^+$ (41), 160 (6), 146 (14), 96 (7), 84 (100).

Camoensidine (**3**). Brown amorphous solid. Found: M^+ 234.1721; $C_{14}H_{22}N_2O$ requires 234.1732. IR ν_{max} cm^{-1}: 1670 (C=O). ^1H NMR ($CDCl_3$): δ 4.40–3.50 (2 H, *m*, H-10). ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 234 $[M]^+$ (72), 164 (7), 150 (100), 136 (92), 122 (27), 84 (91).

? 12-*Hydroxycamoensidine* (**10**). Needles from MeOH, mp 200°. Found: M^+ 250.1692; $C_{14}H_{22}N_2O_2$ requires 250.1681. $[\alpha]_D^{20}$ −50° (*c* 0.1, MeOH). IR ν_{max} cm^{-1}: 3250 (OH), 1660 (C=O). EIMS *m/z* (rel. int.): 250 $[M]^+$ (100), 164 (1), 150 (12), 138 (45), 136 (25), 122 (18), 100 (20).

12-α-*Hydroxycamoensine* (**11**). Brown amorphous solid. Found: M^+ 246.1362; $C_{14}H_{18}N_2O$ requires 246.1368. $[\alpha]_D^{20}$ −115° (*c* 1.0, MeOH). UV λ_{max} nm: 230, 309; (+HCl) 230, 297. IR ν_{max} cm^{-1}: 3300 (OH), 1645 (C=O), 1560, 1550. ^1H NMR ($CDCl_3$): δ 7.31 (1 H, *dd*, $J_1 = 9$ Hz, $J_2 = 7$ Hz, H-4), 6.43 (1 H, *dd*, $J_1 = 9$ Hz, $J_2 = 1$ Hz, H-5), 6.03 (1 H, *dd*, $J_1 = 7$ Hz, $J_2 = 1$ Hz, H-3), 4.35 (1 H, *m*, $W = 11$ Hz, H-12), 4.16 (1 H, ABX, $J_{AB} = 16$ Hz, $J_{AX} = 1$ Hz, H-10a), 3.73 (1 H, ABX, $J_{AB} = 16$ Hz, $J_{BX} = 6$ Hz, H-10e), 3.30 (1 H, *dd*, $J_1 = 10$ Hz, $J_2 = 5$ Hz, H-11). ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 246 $[M]^+$ (64), 229 (5), 228 (7), 160 (27), 146 (33), 112 (21), 100 (100).

12-*Hydroxy*-16-*methoxy*-11:12,13:14-*tetradehydrocamoensine* (**14**). Brown amorphous solid. Found: M^+ 272.1153; $C_{15}H_{16}N_2O_3$ requires 272.1161. UV λ_{max} nm: 235, 315; (+HCl) 235, 309. IR ν_{max} cm^{-1}: 3400 (OH), 1650 (C=O). ^1H NMR (DMSO-d_6): δ 7.90 (1 H, *d*, $J = 5$ Hz, H-14), 7.22 (1 H, *dd*, $J_1 = 9$ Hz, $J_2 = 7$ Hz, H-4), 6.20 (2 H, *m*, H-3 and H-5), 4.86 (1 H, *d*, $J = 5$ Hz, H-13), 3.15 (3 H, s, 16-OCH_3). EIMS *m/z* (rel. int.): 272 $[M]^+$ (100), 257 $[M - CH_3]^+$ (39), 241 $[M - CH_3O]^+$ (8), 229 $[M - CH_3CO]^+$ (30), 160 (17), 146 (65), 127 (47), 112 (1), 96 (2), 82 (1).

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