Phytochemistry, Vol. 22, No. 10, pp. 2297–2300, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

LEAF ALKALOIDS OF *RAUWOLFIA CAFFRA*

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(*Received* 29 *November* 1982)

Key Word Index—*Rauwolfia caffra*; Apocynaceae; leaves; indole alkaloids; akuammicine; corynane; indolenine; macroline; norharman; peraksine and sarpagan bases.

Abstract—Eighteen alkaloids were isolated from South African *Rauwolfia caffra* leaves and comprised corynane, sarpagan, peraksine, akuammicine, macroline, indolenine and harman types. Heteroyohimbines and dihydroindoles were not detected. The principal alkaloids were the indolenine compounds raucaffrinoline, perakine and vomilenine and the indole alkaloids peraksine and dihydroperaksine.

INTRODUCTION

The pan-African species *Rauwolfia caffra* Sond. occurs as a large tree widely distributed, but not abundantly, in secondary fringing forest in high rainfall areas [1]. The tree has been known by several synonymous names, viz. *R. natalensis* Sond., *R. inebrians* K. Schum., *R. obliquenervis* Stapf, *R. goetzei* Stapf, *R. welwitschii* Stapf, *R. ochrosioides* K. Schum. and *R. oxyphylla* Stapf [2]. In indigenous medicine the leaves have been used in the treatment of gonorrhoea and skin diseases, and as emetics. The leaves are also used to poison small animals [3].

Six indole alkaloids were earlier reported in the leaves of *R. caffra* [4] and the current work has been undertaken in order to find related compounds.

RESULTS

Known apocynaceous alkaloids were identified by physical and spectroscopic analysis, by reference to published data [5] as indicated in Table 1 and by chromogenic reactions [6].

For compound RCL 2, the UV spectrum indicated an indolenine-type chromophore and the IR spectrum revealed a strong carbonyl band at 1740 cm^{-1} and the absence of aromatic substitution. EIMS peaks at *m/z* 182, 181, 180, 169, 168 and 156 were consistent with an indolenine structure with substitution on the C-ring. Fragments at $[M-43]^+$ and $[M-59]^+$ suggested loss of COMe and OCOMe groups, respectively, the former being confirmed by high resolution measurement. The peaks at *m/z* 263 and 249 suggested a compound possessing an ethylidene side chain at C-20 but insufficient material was available for NMR confirmation. Nevertheless, as vomilenine also occurs in large amounts in the leaves, and accurate EIMS measurement indicated the molecular formula $C_{21}H_{22}N_2O_2$, it was concluded that compound RCL 2 was 21-deoxyvomilenine.

The UV spectrum of compound RCLM 1 suggested an indolenine chromophore and the IR spectrum revealed the presence of NH (3350 cm^{-1}) and two ester groups (1740 and 1720 cm^{-1}), and the absence of aromatic substitution. Careful inspection of the EIMS confirmed the suggested indolenine structure, the lower region of the

Table 1. Identification of apocynaceous alkaloids

Compound	Identity	Analytical methods	Yield (mg)
RCL 1	19,20-Dihydroakuammicine	UV, IR, EIMS	4
RCL 3	Vomilenine	$[\alpha]_D$, UV, IR, EIMS, NMR	70
RCL 4	Akuammicine	UV, IR, EIMS, co-TLC	2
RCL 5	Akuammicine-N_b-oxide	UV, IR, EIMS, co-TLC	4
RCLM 2	Strictamine	$[\alpha]_D$, UV, IR, EIMS	5
RCLM 3	Sitsirikine	$[\alpha]_D$, UV, IR, EIMS	4
RCLM 4	Akuammidine	UV, IR, EIMS	3
RCLM 5	Perakine	$[\alpha]_D$, UV, IR, EIMS, NMR	300
RCLM 6	Norharman	UV, IR, EIMS	5
RCLM 7	Raucaffrinoline	$[\alpha]_D$, UV, IR, EIMS, NMR	570
RCLM 8	Normacusine B	$[\alpha]_D$, UV, IR, EIMS, NMR, co-TLC	40
RCLM 9	Peraksine	$[\alpha]_D$, UV, IR, EIMS, NMR, co-TLC	90
RCLM 10	Macrophylline	$[\alpha]_D$, UV, IR, EIMS, NMR, co-TLC	50
RCLM 11	Sarpagine	$[\alpha]_D$, UV, IR, EIMS, NMR, co-TLC	20
RCLM 13	Dihydroperaksine	Mp, $[\alpha]_D$, UV, IR, EIMS, NMR	80

spectrum resembling spectra of the known *Rauwolfia* indolenine compounds perakine, raucaffrinoline and vomilenine. Chromogenic reactions [6] eliminated the presence of a dihydroindole nucleus. High resolution EIMS indicated the empirical formula $C_{23}H_{26}N_2O_4$ and the $[M]^+$ peak at *m/z* 394 might therefore be attributed to akuammiline, an alkaloid previously isolated from *Rauwolfia* species [7], but co-TLC with an authentic sample yielded different R_f values. EIMS in the upper region showed peaks at *m/z* 365, 351, 335, 322, 321, 292, 275 and 263 attributable to the loss of Et, Ac, OAc, CHOAc, CH_2OAc, OAc + Ac, HOAc + OAc and CHOAc + OAc groups, respectively. As vomilenine co-occurred in the leaves and the difference between RCLM 1 and vomilenine was 45 amu, this was considered to be due to an added OAc group at C-21 and an Et side chain instead of the ethylidene group at C-20. Despite the lack of NMR data, RCLM 1 was identified provisionally as 21-acetyl-19,20-dihydro-vomilenine.

The UV spectrum of compound RCLM 12 indicated an unsubstituted indole chromophore which was confirmed by the IR spectrum (740 cm^{-1}). A well-defined band at 3350 cm^{-1} suggested an OH group. The EIMS parent peak at *m/z* 305 pointed to a compound of the macroline type, the high resolution measurement indicating the empirical formula $C_{19}H_{19}N_3O$. Although the compound resembled suaveoline, the two extra amu were attributed to the presence of an OH group in place of the terminal Me group. Investigation of the lower region of the EIMS indicated an unsubstituted tetrahydro-β-carboline moiety. The upper region revealed fragments at *m/z* 288, 287, 273, 272, 260, 259, 245 and 243, which can be related to the loss of OH, H_2O, OH + Me, H_2O + Me, CHOH + Me, CH_2OH + Me, CH_2OH + NMe and CH_2OH + NH_2Me fragments, respectively. Peaks at *m/z* 178 and 177 were considered to be due to the rupture of the C-5 → C-6 and C-2 → C-3 linkages, respectively, although this is not the favoured route of fragmentation in suaveoline [8]. The presence of Bohlmann's bands in the IR spectrum indicated that C-3H was probably α. The ^1H NMR spectrum showed the occurrence of an N_b-Me group (δ3.04) but other peaks were almost identical with those reported for suaveoline [8]. The signal at δ2.1 was attributed to the C-19H_2 protons. Therefore, compound RCLM 12 was identified as 18-demethyl-19-hydroxy-N_a-demethyl-N_b-methyl-suaveoline.

DISCUSSION

The alkaloidal mixture in the leaves of *R. caffra* differs from earlier reported data for the leaves of the African species *R. vomitoria* Afz. [7], *R. obscura* K. Schum. [9], *R. cumminsii* Stapf [10], and *R. mombasiana* Stapf [11]. In particular the occurrence of indolenine compounds in high yield is noteworthy. Such alkaloids comprised 72% of the total alkaloid yield, the major indolenines being raucaffrinoline, perakine and vomilenine (60.2, 31.7 and 7.4%, respectively, of the total indolenines present). Also significant was the absence of the nordihydroindole alkaloids, although their precursor, normacusine B, was detected.

Current theories of the biosynthesis of indole alkaloids indicate a route from the nitrogenous glycoside strictosidine to the heteroyohimbine alkaloids which involves 4,21-dehydrocorynantheine aldehyde, 4,21-dehydrogeissoschizine and cathenamine [12]. Although geissoschizines were not detected in the present sample of *R. caffra* leaves, the corynane compound sitsirikine (**1**), which occurred in trace amounts only, can be related to 4,21-dehydrocorynantheine aldehyde as a probable derivative of the main biosynthetic pathway to the heteroyohimbine alkaloids [13]. Derived from corynane compounds by C-16 → C-5 linkage are the sarpagan compounds sarpagine (**2**), akuammidine (**3**) and normacusine B (**4**). Akuammidine is considered to precede normacusine B and its 10-hydroxy congener sarpagine.

The indolenine compounds have been regarded as intermediate between the sarpagan and ajmalan groups of alkaloids [14, 15]. Ajmalan compounds were conspicuously absent from the current batch of *R. caffra* leaves although found in abundance in the roots [16, 17]. Indolenine alkaloids must be considered as important intermediates which, by C-17-deacetylation and saturation of the C-1 → C-2 double bond, are convertible to other more stable compounds. Significantly 21-deoxy-vomilenine relates in this manner to the nordihydroindole alkaloid nortetraphyllicine, and 21-*O*-acetyl-19,20-dihydro-vomilenine to the nordihydroindole alkaloid norajmaline, compounds occurring elsewhere in the plant [18].

The suaveolines macrophylline (**5**) and 18-demethyl-19-hydroxy-N_a-demethyl-N_b-methyl-suaveoline (**6**) represent a group of macroline compounds which were suggested by Kompis *et al.* [19] as intermediates between strictosidine and the peraksines. It is significant, therefore, that peraksine (**7**) and its dihydro congener (**8**) are present in fair yield in *R. caffra* leaves. Alternatively peraksine, perakine (**9**) and raucaffrinoline (**10**) can be regarded as sarpagan-derived [19], in which event the suaveolines must be considered as by-products of the pathway.

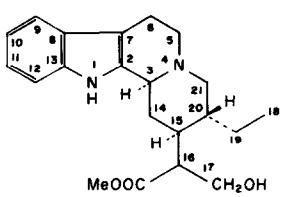
Strictamine (**11**) is corynane-derived by the C-16 → C-7 linkage and can be related to the occurrence of akuammicine (**12**) and its 19,20-dihydro and N_b-oxide derivatives. Also related to strictamine are the akuammiline compounds which were isolated from the leaves of other *Rauwolfia* species, e.g. *R. oreogiton* Mgf. [20], *R. volkensii* Stapf [21] and *R. vomitoria* [7], but not from the current sample of *R. caffra* leaves.

Norharman (β-carboline) (**13**) has not previously been detected in *Rauwolfia* species although the known *Rauwolfia* alkaloids are β-carboline derivatives.

The occurrence of alkaloids in *R. caffra* leaves agrees with the general pattern of akuammiline, corynane, macroline, peraksine and sarpagan compounds found in the leaves of other African *Rauwolfia* species. The absence of pleiocarpamine, N_a-demethyl-dihydroindole and heteroyohimbine-type alkaloids may be a seasonal variation as some such compounds were reported in an earlier communication [4]. Likewise the occurrence of indolenine compounds may be seasonal and warrants further investigation, although some indolenine alkaloids have been isolated from freshly harvested, undried roots [22, 23].

EXPERIMENTAL

Leaves of *R. caffra* Sond. were collected and supplied in 1977 by the Department of Forestry, Pretoria, South Africa. Voucher specimens (RAU 101–773) have been lodged with the Pharmaceutical Society of Great Britain Collection of Materia Medica and Herbaria, University of Bradford, U.K. The macro-



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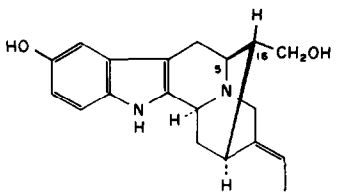

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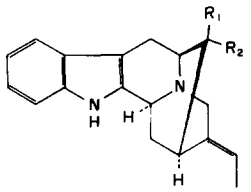
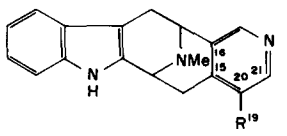
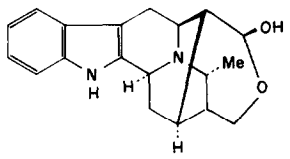


3 R_1 = COOMe, R_2 = CH_2OH
4 R_1 = H, R_2 = CH_2OH



5 R = CH_2CH_2OH
6 R = CH_2OH



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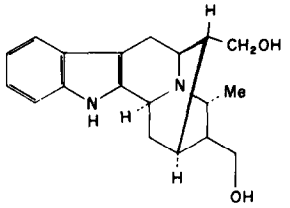

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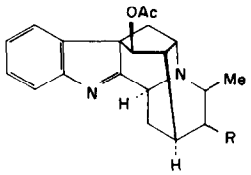
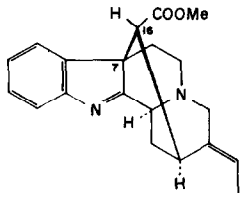


9 R = CHO
10 R = CH_2OH



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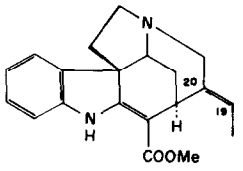

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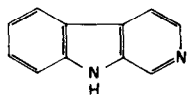

13

scopical and microscopical characters of the leaf samples agreed with published data [1].

Analytical methods. The methods employed for TLC, UV, IR, ^{1}H NMR, EIMS and fluorogenic and chromogenic tests have been described earlier [6, 24, 25].

Extraction. Powdered leaves (8 kg) were extracted by the method described earlier for *R. volkensii* leaves [21], yielding 16.5 g crude weak base fraction, 9.5 g crude intermediate base fraction and 4.0 g crude strong base fraction.

Separation. The total weak base fraction (16.5 g) was separated by column chromatography (CC) using a 53 × 3.5 cm glass column packed with Al_2O_3 (activated neutral Brockmann grade I). The column was eluted successively with 500 ml vols. of C_6H_{14}–EtOAc (7:13, 1:4, 3:17, 1:19), EtOAc, EtOAc–MeOH

(19:1, 9:1, 17:3, 3:1, 13:7, 11:9, 9:11, 7:13 and 1:3) and MeOH. Seventy-five 100 ml fractions were collected and representative concentrates were monitored by TLC using Si gel layers, solvent systems $C_2H_4Cl_2$–EtOAc–MeOH (6:3:1), EtOAc–petrol (bp 40–60°)–MeOH (7:1:2) and EtOAc–*iso*-PrOH–MeOH (7:2:1 and 13:4:3) and $FeCl_3$–$HClO_4$ and $Ce(SO_4)_2$ spray reagents. Chromatographically similar fractions were bulked to yield three fractions (A, B and C). Fraction A separated by prep. TLC (EtOAc–$C_2H_4Cl_2$–MeOH, 3:6:1) yielded compound RCL 1 (4 mg). Fraction B similarly separated (EtOAc–MeOH-*iso*-PrOH–petrol, 4:4:3:1) to give compound RCL 2 (3 mg), and fraction C likewise (EtOAc–*iso*-PrOH–MeOH, 13:4:3) produced compounds RCL 3 (70 mg), RCL 4 (2 mg) and RCL 5 (4 mg).

As representative concentrates of the intermediate and strong base fractions revealed chromatographic similarity on TLC examination, the fractions were bulked and fractionated as before by CC using successive 500 ml quantities of C_6H_{14}–EtOAc (1:1), EtOAc, EtOAc–MeOH (4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4) and MeOH. Fractions (75 ml) were collected and monitored by TLC as before; similar fractions were bulked to yield fractions A–F. Fraction A yielded alkaloids RCLM 1 (3 mg) and RCLM 2 (5 mg) when separated on Si gel layers using $C_2H_4Cl_2$–EtOAc–EtOH–petrol (12:6:2:1). Fraction B with $C_2H_4Cl_2$–EtOAc–MeOH-*iso*-octane (8:8:3:1) produced compounds RCLM 3 (4 mg), RCLM 4 (3 mg), RCLM 5 (300 mg), RCLM 6 (5 mg) and RCLM 7 (570 mg). Using $CHCl_3$–MeOH (9:1) in NH_3 atmosphere, fraction C gave alkaloids RCLM 8 (40 mg) and RCLM 9 (90 mg). Fraction D yielded compounds RCLM 10 (50 mg) and RCLM 11 (20 mg) with $CHCl_3$–MeOH–petrol (7:2:1). With EtOAc–Me_2CO–*iso*-PrOH–MeOH–petrol–NH_3 (12:3:2:2:1:1) fraction E yielded compound RCLM 12 (50 mg) and similarly fraction F produced alkaloid RCLM 13 (80 mg).

Identification of alkaloids. Known apocynaceous alkaloids were identified as indicated in Table 1.

RCL 2, 21-*deoxyvomilenine*, yellow amorphous powder; UV λ_{max}^{MeOH} nm (log ε): 220 (3.73), 227 (sh), 257 (3.28); λ_{max}^{MeOH} nm: 238; IR ν_{max}^{KBr} cm^{-1}: 3400 m, 2925 s, 1740 s, 1660 w, 1590 s, 1450 m, 1405 s, 1375 m, 1230 s, 1100 m, 1035 m, 770 w, 740 s; EIMS (probe) 70 eV, *m/z* (rel. int.): 334.16898 $[M]^+$ (66; $C_{21}H_{22}N_2O_2$ calculated as 334.168118, error 0.00087), 309 (3), 291.15035 (100; $C_{19}H_{19}N_2O$ calculated as 291.149730, error 0.00062), 275 (55), 263 (17), 261 (3), 249 (10), 247 (10), 235 (12), 182 (24), 181 (1), 180 (14), 169 (58), 168 (65), 156 (17), 144 (7), 143 (5), 130 (7), 115 (7); chromogenic reactions, buff with $Ce(SO_4)_2$ reagent, no colour with $FeCl_3$–$HClO_4$ reagent.

RCLM 1, 17-*acetyl*-19,20-*dihydro-vomilenine*, off-white amorphous powder; UV λ_{max}^{MeOH} nm (log ε): 220 (4.88), 227 sh (4.26), 257 (3.85); λ_{min}^{MeOH} nm: 245; IR ν_{max}^{KBr} cm^{-1}: 3350 m, 2925 s, 2850 m, 1740 s, 1720 w, 1590 s, 1450 s, 1385 s, 1235 s, 1100 w, 1030 m, 950 w, 900 w, 820 w, 775 s, 750 s; EIMS (probe) 70 eV, *m/z* (rel. int.): 394.1898 $[M]^+$ (20; $C_{23}H_{26}N_2O_4$ calculated as 394.1892, error 0.0006), 365 (6), 351 (7), 335 (100), 322 (5), 321 (7), 292 (43), 291 (8), 275 (6), 263 (5), 249 (6), 225 (16), 221 (8), 206 (14), 196 (20), 182 (16), 181 (12), 180 (14), 169 (37), 168 (45), 156 (10), 144 (10), 143 (10), 130 (10), 115 (22); chromogenic reactions, buff colour with $Ce(SO_4)_2$ reagent, very faint pink colour with $FeCl_3$–$HClO_4$ reagent.

RCLM 12, 19-*hydroxy*-N_a-*demethyl*-N_b-*methyl-suaveoline*, white amorphous powder; $[\alpha]_D^{22}$ +33.8° (*c* 0.3, MeOH); UV λ_{max}^{MeOH} nm (log ε): 225 (4.32), 273 (3.77), 283 (3.75), 291 (3.64); λ_{min}^{MeOH} nm: 252; IR ν_{max}^{KBr} cm^{-1}: 3350 s, 3125 w, 2960 w, 2925 s, 2850 w, 1660 m, 1590 s, 1500 w, 1450 s, 1420 w, 1380 s, 1330 m, 1300 w, 1220 w, 1160 w, 1140 w, 1110 s, 1060 w, 1020 m, 810 w, 740 s; EIMS (probe) 70 eV, *m/z* (rel. int.): 305.1527 $[M]^+$ (100; $C_{19}H_{19}N_3O$ calculated as 305.1528, error 0.0001), 288 (23), 287 (18), 273 (15), 272 (21), 260 (9), 259 (9), 245 (9), 243 (11), 197 (12), 183 (22), 182 (16), 178 (27), 177 (27), 170 (18), 169 (82), 156 (18), 144 (41), 143 (27), 130 (13); ^1H NMR (CD_3COCD_3): δ8.7 (*s*, 1H), 8.32 (*s*, 1H), 7.5–6.82 (*m*, 4H), 4.58 (*d*, 1H), 4.4 (*d*, 1H), 3.04 (*s*, 3H), 2.1 (*s*, 2H); chromogenic reaction, greyish-black with $FeCl_3$–$HClO_4$ reagent.

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