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LEAF ALKALOIDS OF *RAUWOLFIA VOMITORIA*

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Key Word Index—*Rauwolfia vomitoria*; Apocynaceae; leaf; indole alkaloids; E-*seco* indole; sarpagan; picrinine; akuammiline; heteroyohimbine; oxindole; yohimbine; indolenine.

Abstract—Nineteen indole alkaloids were isolated from Ghanaian *Rauwolfia vomitoria* leaves. The alkaloids comprised E-*seco* indole, sarpagan, picrinine, akuammiline, heteroyohimbine, oxindole, yohimbine and indolenine types. The biosynthetic relationship of the alkaloids is discussed.

INTRODUCTION

Previous investigations [1, 2] revealed the occurrence of at least 28 alkaloids in the roots and 22 alkaloids in the stem bark of the large shrub or small tree *Rauwolfia vomitoria* Afz., the swizzle-stick tree of southern Africa. Predominantly the roots yielded N_a-methyl-dihydroindole, heteroyohimbine and yohimbine-type alkaloids although the stems produced E-*seco* indole, sarpagan, N_a-demethyl-dihydroindole, heteroyohimbine and oxindole alkaloids.

Little work has been reported concerning the leaves although Pousset [3] reported ten alkaloids from leaves collected in Guinea and Nigeria and four alkaloids from leaves collected on the Ivory Coast. The present work records the occurrence of 19 alkaloids in a Ghanaian sample of *R. vomitoria* leaves.

RESULTS

Known *Rauwolfia* alkaloids were characterized using the analytical methods indicated in Table 1 and by fluorescence colours and chromogenic reactions with ferric chloride–perchloric acid and ceric sulphate reagents. The sole E-*seco* indole alkaloid identified was geissoschizol

Table 1. Alkaloids isolated from *R. vomitoria* leaves (8 kg)

	Alkaloid identified	Analytical methods	Yield (mg)
RVL1	Tetrahydroalstonine	mp, mmp, $[\alpha]_D$, UV, IR, MS, co-TLC	140
RVL2	Aricine	mp, mmp, $[\alpha]_D$, UV, IR, MS, co-TLC	500
RVL3	Isoreserpiline	mp, mmp, $[\alpha]_D$, UV, IR, MS, NMR, co-TLC	250
RVL5	Carapanaubine	mp, mmp, $[\alpha]_D$, UV, IR, MS, NMR, co-TLC	960
RVL6	Reserpiline	mp, mmp, $[\alpha]_D$, UV, IR, MS, co-TLC	120
RVL7	Isocarapanaubine	$[\alpha]_D$, UV, IR, MS, NMR, co-TLC	42
RVL8	Rauvoxine	mp, $[\alpha]_D$, UV, IR, MS, NMR	200
RVL9	Rauvoxinine	mp, $[\alpha]_D$, UV, IR, MS, NMR	66
RVL10	Geissoschizol	$[\alpha]_D$, UV, IR, MS, co-TLC	6
RVL11	Desacetyldesformoakuammiline	$[\alpha]_D$, UV, IR, MS	10
RVL12	Picrinine	mp, mmp, $[\alpha]_D$, UV, IR, MS, co-TLC	10
RVL13	Akuammiline	mp, mmp, $[\alpha]_D$, UV, IR, MS, NMR, co-TLC	22
RVL14	Deacetylakuammiline	$[\alpha]_D$, UV, IR, MS, NMR, co-TLC, acetyl derivative	25
RVL15	Perakine	$[\alpha]_D$, UV, IR, MS, NMR, co-TLC	15
RVL16	Normacusine B	$[\alpha]_D$, UV, IR, MS, co-TLC	8
RVL17	α-Yohimbine	mp, mmp, $[\alpha]_D$, UV, IR, MS, co-TLC	18
RVL18	Raucaffrinoline	$[\alpha]_D$, UV, IR, MS, NMR	20
RVL19	Peraksine	$[\alpha]_D$, UV, IR, MS, co-TLC	15

(RVL10). Five indolenine alkaloids were isolated and identified. The indolenines akuammiline (RVL13), deacetylakuammiline (RVL14) and desacetyl-desformo-akuammiline (RVL11) are characterized by a C-16 → C-7 linkage but perakine (RVL15) and raucaffrinoline (RVL18) possess a C-16 → C-5 linkage coupled with a C-17 → C-7 linkage, *O*-acetyl substitution at C-17 and CHO or CH_2OH groups respectively at C-20. The C-16 → C-7 linkage was also exhibited by picrinine (RVL12) but the indolenine double bond is displaced by a C-2 → C-5 oxygen bridge.

The sarpagan structure involves a C-16 → C-5 linkage and is represented in *R. vomitoria* leaves by normacusine B (RVL16). Peraksine (RVL19) resembles the sarpagans but additionally possessed a C-16 → C-20 heterocylic ring. The heteroyohimbine alkaloids identified were tetrahydroalstonine (RVL1), its 10-methoxy-congener aricine (RVL2), its 10,11-dimethoxy relative isoreserpiline (RVL3) and reserpiline (RVL6), the more stable isomer of isoreserpiline.

Derived from the dimethoxyheteroyohimbines were the oxindoles carapanaubine (RVL5), isocarapanaubine (RVL7), rauvoxine (RVL8), rauvoxinine (RVL9), and an oxindole not previously encountered in *Rauwolfia* species.

The alkaloid RVL4 demonstrated the UV characteristics and MS fragmentation pattern (*m/e* 223, 208, 180 and 69) typical of an oxindole. The relative intensity of the *m/e* 180 peak suggested an *allo* or *epi-allo* configuration with a C-19-α Me group [4]. The MW indicated by MS was 30 amu less than for the known *Rauwolfia* oxindoles e.g. carapanaubine, rauvoxine, and IR suggested monomethoxy substitution of the *ar*-ring. As such substitution was most probably in the C-10 or C-11 positions, synthetic oxindoles of aricine (10-methoxy, *allo*), reserpinine (11-methoxy, *allo*) and isoreserpinine (11-methoxy, *epi-allo*) were prepared by the method of Finch *et al.* [5]. RVL4 yielded identical analytical values to reserpinine oxindole ($[\alpha]_D$, UV, IR, MS, co-TLC). The sole yohimbine-type alkaloid found was α-yohimbine (RVL17).

DISCUSSION

The alkaloids of the leaves of *R. vomitoria* differ from those of stems and roots. Quantitatively the yield in the leaves is low (0.03%) in comparison with the reported yields in the stem bark (0.59%) [6], and the roots (1.93%) [7]. The heteroyohimbines and oxindoles comprised 41.51 and 52.36% respectively of the total alkaloids isolated and the dihydroindole alkaloids were conspicuously absent although important compounds in the stems [2] and roots [1].

The simplest alkaloid, isolated in trace amounts only, was the E-*seco* indole geissoschizol (**1**) which, by C-16 → C-5 linkage yields the sarpagan normacusine B (**2**), a precursor of the dihydroindole alkaloids. Inability to detect dihydroindole alkaloids suggests rapid translocation to the stems where N_a-demethyldihydroindoles, e.g. nortetraphyllicine, norajmaline, are found [2] and thence to the roots where N_a-methyl-dihydroindoles, e.g. tetraphyllicine, ajmaline, occur [1].

The indolenine compounds perakine (**3**) and raucaffrinoline (**4**) are probably sarpagan-derived with further C-17 → C-7 linkage to yield a cage-ring structure resembling the dihydroindole structure. It has been suggested that such compounds are missing intermediates between the corynantheine and sarpagan types and the indoline bases of the ajmaline type [8].

Peraksine (=vomifoline) (**5**) is also sarpagan-derived but its role has not been established and it may be a by-product of the main biosynthetic pathway. The C-16-carbomethoxy relative of geissoschizol, geissoschizine (**6**), was not detected although found in *R. vomitoria* stems (Iwu, M. M. and Court, W. E., personal communication) and in the leaves of *R. volkensii* Stapf [9]. Nevertheless geissoschizine has been shown by feeding experiments to be incorporated into the heteroyohimbine alkaloids [10, 11] which are represented in *R. vomitoria* leaves by tetrahydroalstonine (**7**), aricine (**8**), and isoreserpiline (**9**) which are characteristically *allo* (C3–Hα, C20–Hα) configuration compounds, and reserpiline (**10**), the more stable isomer of isoreserpiline, which is of the *epi-allo* (C3–Hβ, C20–Hα) configuration. Reserpiline has been reported as the major alkaloid in the stems [2] and the roots [12].

The occurrence of the akuammiline group of alkaloids (**11–13**) is not readily explained. Although such compounds are possibly derived from an E-*seco* compound by direct C-16 → C-7 linkage, it is significant that in the synthetic conversion of heteroyohimbine to oxindole a 7-acetoxyindolenine is involved [13] and could conceivably yield akuammiline-type compounds. Also akuammiline has so far only been found in *Rauwolfia* leaves yielding oxindoles and *allo/epi-allo* configuration heteroyohimbines [9].

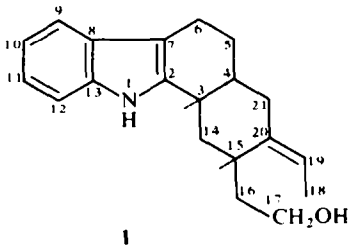
The significance of the related alkaloid picrinine (**14**) remains obscure although it has been found in *Rauwolfia* leaves not containing oxindoles and possessing dominantly *normal* (C3–Hα, C20–Hβ) configuration heteroyohimbines e.g. *R. cumminsii* Stapf [14].

Oxindoles have so far only been isolated from leaves of *Rauwolfia* species in which the heteroyohimbines present are dominantly 10,11-dimethoxy substituted and of the *allo* configuration. Thus, the leaves of *R. obscura* K. Schum. [15], *R. mombasiana* Stapf [16], *R. caffra* Sond. [18] and *R. cumminsii* [14], plants yielding *normal* configuration heteroyohimbines and especially ajmalicine, contained no detectable oxindoles. The oxindoles are believed to be derived from heteroyohimbine precursors [18]. The major oxindoles in *R. vomitoria* leaves were isomeric *ar*-dimethoxy compounds varying at the asymmetric centre C-7 which yields A and B oxindoles and at the centres at C-3 and C-20 providing the *allo* and *epi-allo* configurations. Thus isocarapanaubine (**15**) and carapanaubine (**16**) are *allo* A and *allo* B respectively and rauvoxinine (**17**) and rauvoxine (**18**) are *epi-allo* A and *epi-allo* B respectively. The configuration of the trace compound reserpinine oxindole (**19**) could not be established.

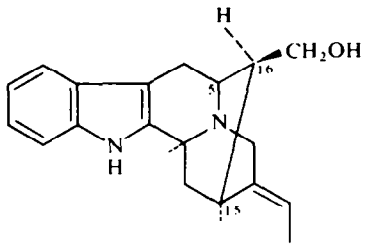
The alkaloidal pattern encountered in *R. vomitoria* leaves confirmed the absence of reserpine and thus the reason why the leaves are not used in the treatment of mental illnesses in African indigenous medicine. The occurrence of large amounts of oxindoles in the leaves, but not elsewhere in the plant, requires further investigation.

EXPERIMENTAL

Mps are uncorr. IR spectra were measured in KBr discs or $CHCl_3$. ^1H NMR spectra were determined in $CDCl_3$ or CD_3OD at 60 MHz. MS were obtained by direct inlet, 70 eV, 100 μA, 200–250°.



1



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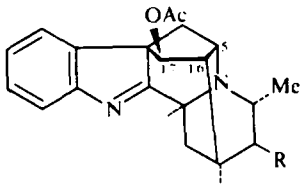
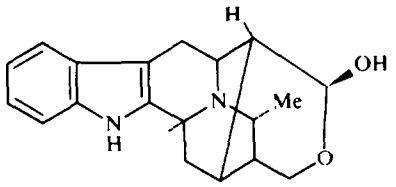


3 R = CHO
4 R = CH_2OH



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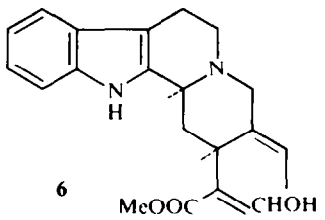
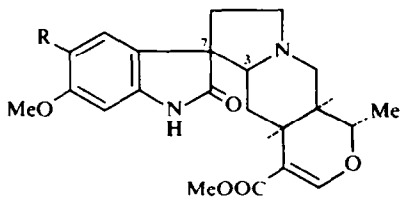
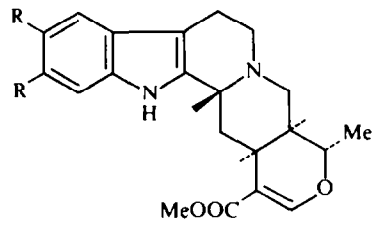


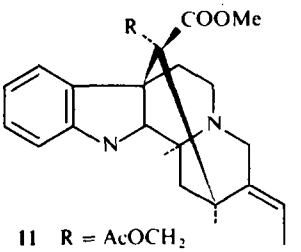
6



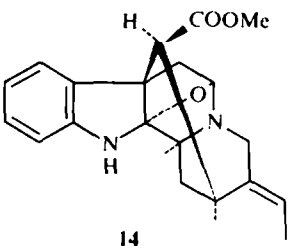
7 $R_1 = R_2 = H$
8 R_1 = MeO; R_2 = H
9 $R_1 = R_2$ = MeO



10 R = MeO



11 R = $AcOCH_2$
12 R = $HOCH_2$
13 R = H



14

15 C3-Hα; R = MeO; *allo* A
16 C3-Hα; R = MeO; *allo* B
17 C3-Hβ; R = MeO; *epi-allo* A
18 C3-Hβ; R = MeO; *epi-allo* B
19 C3-Hα; R = H

Plant material. Leaves of *R. vomitoria* Afz. were collected in Ghana by A. A. Enti, F.L.S. Reference sample No. RAU 109–781 was deposited with the collection of Materia Medica and Herbaria, University of Bradford.

Extraction and fractionation. Powdered leaves (8 kg) were defatted with petrol (40–60°). The dried marc was extracted by repeated overnight maceration with MeOH + 2% NH_4OH. Resultant extracts were evapd to dryness under red. pres. and bulked. The residue was dissolved in 2.5 l. N HCl, insoluble resinious matter being removed by filtration, and the aq. layer extracted with 4 × 200 ml $CHCl_3$. The evapd combined $CHCl_3$ fractions yielded the weakly basic fraction (17.5 g). The residual aq. layer rendered alkaline (pH 9, NH_3) was extracted with 4 × 200 ml $CHCl_3$. The evapd combined $CHCl_3$ fractions produced the intermediately basic fraction (7.2 g). The aq. layer further alkalinified (pH 12, NaOH), was extracted with 3 × 200 ml $CHCl_3$ to yield after evapn under red. pres. the strongly basic fraction (3.5 g).

Separation. The weakly basic fraction was adsorbed on a column (48 × 3.5 cm) of Al_2O_3 (400 g) and successively eluted with 500 ml vol. of C_6H_{14}: C_6H_{14}–EtOAc (10:1, 5:1, 3:1, 1:1, 2:3, 4.5, 1.3), EtOAc; EtOAc MeOH (50:1, 25:1, 10.1, 4:1, 2.1). Successive eluate samples (100 ml) were collected, screened by TLC and similar samples bulked to give fractions A–F. Fraction A separated by PLC on Si gel [Me_2CO petrol–CCl_4 *iso*-octane (5:6:6:3)] gave RVL1 (140 mg). Fraction B on PLC separation [$CHCl_3$ MeOH–petrol (5:1:4)] yielded RVL2 (500 mg) and RVL3 (250 mg). Fraction C separated by PLC [$CHCl_3$–MeOH petrol (7:1:2)] produced RVL4 (6 mg) and RVL5 (960 mg). Fraction D was separated by PLC [EtOAc–petrol MeOH *iso*-octane (86:1:5)] to yield one compound which was further purified by PLC [EtOAc Et_2O (1:1)] to give RVL6 (120 mg). Fraction E on PLC separation [EtOAc–petrol MeOH *iso*-octane (5:3:1:1)] yielded RVL7 (42 mg) and RVL8 (200 mg). Fraction F dissolved in MeOH yielded colourless crystals which were recrystallized from aq. MeOH as RVL9 (66 mg).

The intermediate and strongly basic fractions screened by TLC showed a similar pattern. Therefore they were combined and fractionated on a column (30 × 3.5 cm) of Al_2O_3 (250 g) using 500 ml vol. successively of EtOAc; EtOAc–MeOH (49:1, 19:1, 9:1, 17:3, 4:1, 7:3, 3:2, 1:1). Eluate samples (100 ml) were collected, screened by TLC and similar samples bulked and evapd to dryness to give fractions G–K. Fraction G separated by PLC [EtOAc–petrol–MeOH–*iso*-octane (10:4:1:5)] yielded RVL10 (6 mg) and RVL11 (10 mg). Fraction H on PLC separation [EtOAc–*iso*-PrOH NH_3 (890:17:3)] produced two major compounds RVL12 (10 mg) and RVL13 (22 mg) and a minor compound identical with RVL11. Fraction I was separated by PLC [$CHCl_3$ petrol–EtOAc–MeOH (5:2:2:1)] to yield RVL14 (25 mg), RVL15 (15 mg) and RVL16 (8 mg). Fraction J separated by PLC [EtOAc *iso*-PrOH–NH_3 (16:3:1)] gave RVL17 (18 mg) and RVL18 (20 mg). Fraction K on separation by PLC [Me_2CO petrol–EtOAc–NH_3 (6:2:1:1)] yielded RVL19 (15 mg).

Characterization. Alkaloids RVL 3 and RVLS 19 were identified by the methods stated in Table 1 either by comparison with authentic compounds or with published data [19]. RVL4, *reserpinine oxindole*, off-white amorphous powder; $[\alpha]_D^{21}$ − 22° ($CHCl_3$, c = 0.1); UV λ_{max}^{MeOH} nm: 218 (log ε 4.54), 248 *sh* (4.21), 287 (3.68), 294 (3.65); IR ν_{max}^{KBr} cm^{-1}: 3420 *s*, 2950 *s*, 2820 *w*, 2800 *w*, 1720 *s*, 1630 *s*, 1510 *s*, 1470 *s*, 1320 *s*, 1200 *s*, 1140 *m*, 1000 *w*, 850 *m*, 820 *w*, 770 *s*; MS *m/e* (rel. int.). 398 (100), 383 (9), 381 (12), 368 (13), 367 (14), 339 (6), 337 (6), 297 (10), 296 (7), 269 (8), 267 (7), 243 (10), 231 (13), 229 (8), 224 (20), 223 (98), 222 (25), 218 (9), 209 (9), 208 (29), 189 (20), 180 (25), 179 (17), 176 (12), 175 (15), 174 (17), 164 (14), 160 (11), 69 (90); dirty pink colour with $FeCl_3$–$HClO_4$; faint pink colour with $Ce(SO_4)_2$.

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