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STEM ALKALOIDS OF *RAUWOLFIA VOMITORIA*

NAWAL N. SABRI and WILLIAM E. COURT
Postgraduate School of Studies in Pharmacy, University of Bradford, Bradford, BD7 1DP, U.K.

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Key Word Index—*Rauwolfia vomitoria*; Apocynaceae; stem indole alkaloids; E-seco heteroyohimbine; sarpagan; dihydroindole; yohimbine; heteroyohimbine; oxindole.

Abstract—22 indole alkaloids were isolated from the stem bark of Nigerian *Rauwolfia vomitoria* and 20 characterized. The alkaloids comprised E-seco heteroyohimbine, sarpagan, dihydroindole, yohimbine and heteroyohimbine types. The biosynthetic relationship of the alkaloids is discussed.

INTRODUCTION

Earlier investigations established the occurrence in the roots of *Rauwolfia vomitoria* Afz. of 28 alkaloids comprising indole, dihydroindole, ψ-indoxyl and oxindole alkaloids [1]. Study of leaf samples yielded 10 alkaloids including indole, E-seco heteroyohimbine and oxindole types [2]. The presence of alkaloids in the stems including indole, dihydroindole, E-seco heteroyohimbine, oxindole, ψ-indoxyl and indolenine types is now reported and the possible interrelationships discussed.

RESULTS

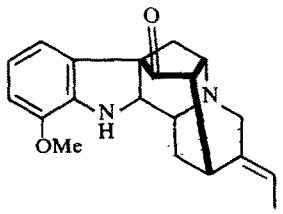
The indole alkaloids 10-methoxy-geissoschizol (**1**), 10-hydroxy-geissoschizol (**2**), sarpagine (**3**) and normacusine B (**4**) were isolated and identified. The dihydroindole alkaloids purpeline, norpurpeline and norseredamine were accompanied by two related alkaloids RA and RB. UV and IR data suggested that both compounds were indoline alkaloids, RA demonstrating a 1,2-disubstituted indole pattern and RB a 1,2,4-trisubstituted indole pattern. This was confirmed by the chromogenic reaction with $FeCl_3/HClO_4$ reagent (RA orange-red, RB red-violet). MS fragmentation patterns revealed a nordihydroindole structure (*m/e* 169, 168, 143, 130, 117) for RA and an hydroxy-nor-dihydroindole structure (*m/e* 185, 184, 159, 146, 133) for RB. In both alkaloids MS fragments at M − 31 (M^+-CH_2OH) and M − 45 (M^+-CH_2CH_2OH) suggested the breakdown of a 5-membered ring with an hydroxyl group in the C-17 position. This was confirmed by the absence of carbonyl groups (no 1735 cm^{-1} peaks in IR). PMR confirmed the presence of the exocyclic ethylidene sidechain in both cases (δ5.30, *dq*, H and 1.65, *d*, 3H). RB demonstrated a 10 nm shift of the UV spectrum in alkali. It was therefore concluded that RA was nortetraphyllicine (1-demethyl-19,20-didehydro-ajmalan-17-ol) and RB 10-hydroxy-nortetraphyllicine (1-demethyl-10-hydroxy-19,20-didehydroajmalan-17-ol).

The yohimbine type alkaloids detected were yohimbine, methyl reserpate and RC. For RC the UV spectrum and MS fragmentation pattern (*m/e* 184, 170, 169, 156, 144, 143) suggested a yohimbinoid structure with no substitution of the aromatic ring (confirmed by IR). Presence of a carbomethoxy group was indicated by IR (1720 cm^{-1}) and PMR (δ3.81, *s*, 3H). RC was therefore identified as an hydroxy-yohimbine [3]. The hydroxy group was not phenolic (no shift of UV maxima in alkali). Although insufficient material was available to confirm identity, it was considered, bearing in mind other yohimbine derivatives in *Rauwolfia* spp., that RC was probably 18-hydroxy-yohimbine.

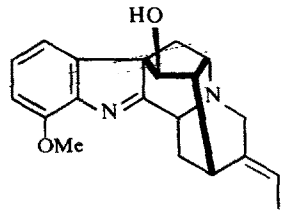
The most prominent alkaloid in *R. vomitoria* roots and leaves would appear to be the heteroyohimbine compound reserpiline [1, 4] and this was also true in the stem bark. Related alkaloids found in the stem bark included isoreserpiline and RD, a compound readily converted to reserpiline by hydrogenation (UV, IR, MS, co-TLC). The MS fragmentation pattern of RD suggested a β-carboline structure with dimethoxy substitution at 10, 11 in the A ring (IR). Presence of the $CH_3COO—C{=}C—O—$ grouping was indicated by UV (inflection at 252 nm) and IR (1700 *s* and 1640 *m* cm^{-1}) spectra. As the strong M^+peak at *m/e* 410 for RD was readily converted to *m/e* 412 by hydrogenation, it was concluded that RD was a dehydroreserpiline. MS revealed that the *m/e* 216 peak was more intense than the *m/e* 244 peak suggesting a 19–20 double bond which would inhibit formation of the *m/e* 244 fragment and favour formation of the *m/e* 216 peak. Thus RD was probably 19,20-dehydroreserpiline.

Other alkaloids derived from the dimethoxy-heteroyohimbine structure were isoreserpiline-ψ-indoxyl, the oxindoles carapanaubine, isocarapanaubine and rauvoxine and two other pseudoindoxyls RE and RF which occurred in trace amounts and could not be identified satisfactorily.

The alkaloid RG demonstrated an indoline struture (UV 253 nm with shift of 17 nm in strong acid, IR 1590 cm^{-1}) with 1, 2, 3-trisubstitution of the indole aromatic ring (IR 780 *m*, 760 *m*, 735 *m* cm^{-1}) and methoxy substitution of the aromatic ring (IR 1450 cm^{-1} PMR δ3.78, *s*, 3H and violet colour with $FeCl_3/HClO_4$ reagent). This was confirmed by the MS fragmentation pattern (*m/e* 199, 198, 173, 160) and M^+ − 43 and M^+ − 29 peaks suggesting the presence of a 5-membered ring. No carbonyl group was indicated (absence of IR 1730 cm^{-1} peak) but an exocyclic ethylidene sidechain was found (PMR δ5.35, *m*, 1H and 1.66, *d*, 3H). High resolution



(A)



(B)

MS gave the molecular formula $C_{20}H_{22}N_2O_2$. Acetylation of RG was attempted and proved difficult yielding *ca* 10% acetyl derivative; silylation with TMS proved equally difficult. Two possible formulae are presented. Compound **A**, norpurpeline, has already been isolated from the stem of *R. cumminsii* Stapf [5] and was shown to differ from RG by co-TLC. Therefore, the indolenine formula **B** is considered more probable. The close proximity of the β-hydroxy-group to the indolenine double bond could account for the slow chemical reactivity. It was concluded that the most probable structure was **B**.

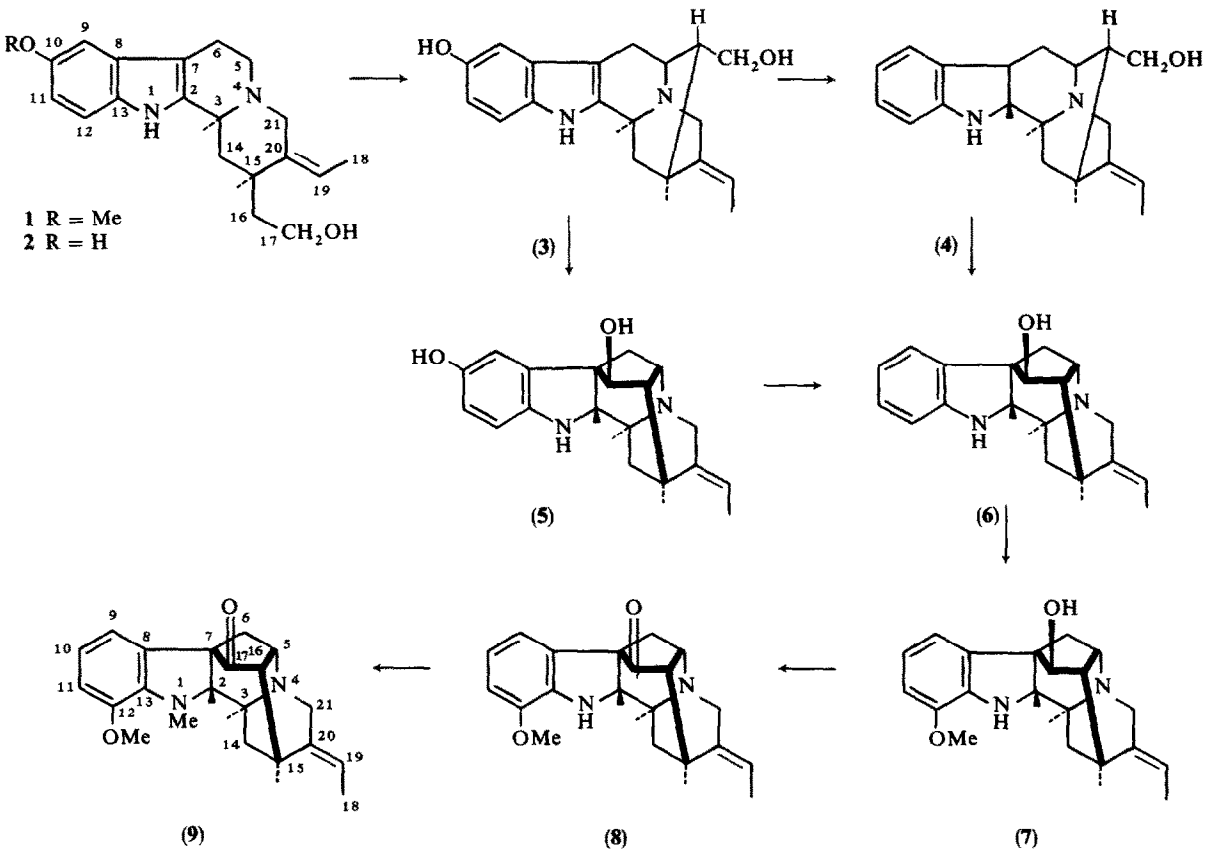
Yield of alkaloids

From 1.5 kg stem bark the principal alkaloids obtained were reserpiline (600 mg), isoreserpiline (100 mg), hydroxyyohimbine (35 mg), yohimbine (30 mg), purpeline (25 mg), norpurpeline (24 mg), norseredamine (24 mg), nortetraphyllicine (20 mg), carapanaubine (18 mg) and indolenine RG (18 mg).

DISCUSSION

The marked difference between the alkaloids occurring at differing levels of the plant axis has been observed in *R. caffra* Sond. [6, 7] and *R. obscura* K. Schum. [8, 9]. *R. vomitoria* presents a similar pattern. Thus 10 alkaloids were found in the leaves [2], 20 in the stems (this work) and 28 in the roots [1]. It is significant that E-seco indole alkaloids were only detected in the leaves and stem and the greatest diversity of dihydroindole alkaloids occurred in the roots suggesting an increasing complexity in the alkaloidal mixture as the axis is descended. As in *R. obscura* [8], it is therefore reasonable to assume that the dihydroindoles are derived from E-seco heteroyohimbine compounds via sarpagine-type intermediates [10].

The interrelationship of the dihydroindoles cannot readily be determined. It is possible that 10-methoxygeissoschizol (**1**) is demethylated to yield 10-hydroxygeissoschizol (**2**) which is, in turn, converted to sarpagine (**3**). Dehydroxylation yields normacusine B (**4**) which



undergoes ring closure to form nortetraphyllicine (**6**), an intermediate postulated in earlier work [11]. Alternatively sarpagine could be converted via 10-hydroxy-nor-tetraphyllicine (**5**) to nortetraphyllicine. Nortetraphyllicine can be readily converted to norseredamine (**7**) by methoxylation of the aromatic ring; such methoxylation of an indole aromatic ring by bacteria has already been demonstrated [12]. Dehydrogenation would yield norpurpeline (**8**) and subsequent N_a-methylation purpeline (**9**). Significantly the N_a-methylated derivatives tetraphyllicine and seredamine together with mitoridine, the 12-hydroxy variant of seredamine, occur in the roots [1] thus suggesting that such compounds are derived by N_a-methylation and not from N_a-methylated precursors. Ajmaline (ajmalan-17, 21-diol), an alkaloid commonly found in *Rauwoifia* roots, was not detected, nor were its derivatives 12-methoxy-ajmaline, isoajmaline, vomalidine, sandwicine and iso-sandwicine, compounds characterized by a C-20 ethyl sidechain and a C-17 hydroxyl group and all known to occur in the roots [17].

The heteroyohimbine alkaloids, aricine and tetrahydroalstonine, have been reported in both leaves [2] and roots [1] but we were unable to detect them in our stem material. Alstonine, the anhydronium base corresponding with tetrahydroalstonine, was likewise not detected, but the related *allo* (C-3Hα, C-20Hα) heteroyohimbine compound isoreserpiline and the *epi-allo* (C-3Hβ, C-20Hα) compound reserpiline were found and occur also in leaves and roots.

18-Hydroxy-yohimbine and methyl reserpate co-occurring in the stem suggests a route towards the pharmacologically important alkaloids reserpine and rescinnamine, the 18-hydroxy benzoic and cinnamic esters, respectively, which occur in reasonable yield in the roots [4] but have not been detected in the leaves or stems.

The significance of the 19, 20-dehydroreserpiline and the indolenine alkaloid RG in the stem has yet to be established.

Considering our previous work on the alkaloids of *Rauwolfia* species *R. caffra* [6, 7], *R. cambodiana* Pierre ex Pitard [13], *R. cumminsii* [5, 14, 15], *R. macrophylla* Stapf [16], *R. obscura* [8, 9, 17, 18], *R. oreogiton* Mgf. [19] and *R. vomitoria* [1] it is apparent that the alkaloids characterizing the genus *Rauwolfia* comprise 5 groups: E-seco heteroyohimbine, sarpagan, dihydroindole, yohimbine and heteroyohimbine (including the ψ-indoxyls, oxindoles and anhydronium bases).

EXPERIMENTAL

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

Plant material. Stem bark of *R. vomitoria* Afz., *ca* 3 mm thick was collected in Nigeria. Reference sample, No. RAU 107–761, was deposited with the collection of Materia Medica and Herbaria, University of Bradford.

Extraction and fractionation. Powdered stem bark (1.5 kg) was macerated overnight with MeOH and 2% NH_4OH. The filtered extract was evapd to dryness under red. pres., dissolved in 1 l. N HCl and extracted with 5 × 100 ml $CHCl_3$. The evapd combined $CHCl_3$ fractions yielded the weakly basic fraction. The aq. acidic layer rendered alkaline (pH 8, NH_3) was extracted with 5 × 100 ml $CHCl_3$. The evapd combined $CHCl_3$ fractions yielded the intermediately basic fractions. The residual aq. layer was made more alkaline (pH 10, NH_3) and the strongly basic fraction recovered in $CHCl_3$.

Separation. The weakly basic fraction was adsorbed on an Al_2O_3 column (50 × 1.5 cm) and successively eluted with C_6H_{14}; C_6H_{14}–EtOAc (8:1, 4:1, 2:1, 1:1, 1:2, 1:3); EtOAc; EtOAc–MeOH (9:1, 8:1, 4:1, 1:1), 100 ml eluate fractions were collected and concd under red. pres.before separation of the alkaloids by PLC on Si gel GF 254 layers (250 nm thick) using solvent systems Me_2CO–petrol (40–60°)–CCl_4–*iso*octane (7:6:4:3), *n*-BuOH–EtOAc–$C_2H_2Cl_2$ (1:3:6) and EtOAc–*iso*PrOH–NH_3 (16:13:1). Eluted alkaloids were purified by rechromatography using $CHCl_3$–MeOH (9:1). The intermediately basic fraction was dissolved in Et_2O and separated by PLC on Si gel layers using Me_2CO–Petrol–Et_2NH (2:7:1) and purified by rechromatography using $CHCl_3$–MeOH (9:1). The strongly basic fraction was separated on Si gel layers using Me_2CO–MeOH–HOAc (14:25:1) and repurification with $CHCl_3$–MeOH (7:3).

Identification of alkaloids. Weak base fraction. Purpeline, reserpiline, isoreserpiline, isoreserpiline-ψ-indoxyl, isocarapanaubine and *carapanaubine* were identified by comparison with published data [20] and authentic samples, (UV, IR, NMR, MS, mp, chromogenic reactions, fluorescence colours and co-TLC, 6 systems). *Rauvoxine* was identified by comparison with published data [21, 22] (UV, IR, MS, TLC). *RE*, yellowish amorphous powder; UV λ_{max}^{MeOH} nm: 225 (log ε 2.93), 250 (2.97), 283 (2.59), 400 (2.16), λ_{min}^{MeOH} nm: 236, 271, 340; IR ν_{max}^{KCl} cm^{-1}: 3400 *s*, 2950 *s*, 2825 *m*, 1700 *m*, 1680–1660 tr *s*, 1620 *s*, 1585 *m*, 1500 *s*, 1465 *w*, 1440 *s*, 1380 *s*, 1280 *s*, 1250 *m*, 1200 *s*, 1130 *s*, 1085 *s*, 1060 *w*, 1020 *s*, 995 *w*, 935 *m*, 860 *m*, 830 *m*, 800 *w*, 790 *m*, 770 *s*, 750 *m*; MS *m/e* (rel. int.): 428 (54), 412 (8), 411 (24), 397 (5), 224 (7), 223 (49), 222 (100), 219 (5), 218 (5), 208 (6), 206 (24), 205 (6), 190 (6), 180 (8), 149 (5), 69 (23); no colour reaction with $FeCl_3$/$HClO_4$; bright green fluorescence. *RF*, yellowish amorphous powder; UV λ_{max}^{MeOH} nm: 227 (log ε 3.09), 292 (2.54), 303 (2.51), 400 (1.77), λ_{max}^{MeOH} nm: 269; IR ν_{max}^{KCl} cm^{-1}: 3400 *m*, 2900 *m*, 1690 *s*, 1615 *s*, 1580 *w*, 1490 *m*, 1445 *m*, 1385 *w*, 1295 *s*, 1250 *w*, 1195 *s*, 1150 *m*, 1090 *s*, 1010 *m*, 950 *w*, 835 *w*, 800 *w*, 790 *w*, 780 *w*, 750 *w*; MS *m/e* (rel. int.): 428 (30), 412 (65), 411 (50), 397 (30), 336 (30), 311 (15), 307 (10), 283 (25), 269 (8), 257 (10), 244 (20), 223 (35), 222 (100), 216 (25), 206 (25), 187 (10), 174 (20), 149 (40), 69 (55); yellowish reaction with $FeCl_3$/$HClO_4$; bright green fluorescence.

Intermediate base fraction. Norseredamine, 10-methoxy-geissoschizol and *yohimbine* were identified by comparison with published data [20] and authentic samples (UV, IR, NMR, MS, mp, chromogenic reactions, fluorescence colours, and co-TLC). *Norpurpeline* was identified by comparison with published data [5, 20]. *RA*, colourless needle crystals; mp 280° (dec); UV λ_{max}^{MeOH} nm: 210, 240, 288, λ_{min}^{MeOH} nm: 222, 262; IR ν_{max}^{KCl} cm^{-1}: 3400 *s*, 2950 *m*, 1580 *s*, 1470 *m*, 1440 *m*, 1420 *m*, 1310 *w*, 1260 *w*, 1235 *w*, 1100 *m*, 1050 *w*, 1020 *w*, 960 *w*, 935 *w*, 815 *w*, 770 *w*, 750 *m*; MS *m/e* (rel. int.): 294.17464 (M^+, 100; $C_{19}H_{22}N_2O_2$ calculated as 294.173204, error 0.001436), 293 (60), 277 (10), 263 (16), 249 (10), 184 (10), 170 (18), 169 (78), 168 (42), 144 (16), 143 (26), 130 (28), 117 (16); PMR (CD_3OD): δ7.3–6.6 (*m*, 4H), 5.3 (*dq*, 1H), 3.8 (*s*, 1H), 3.55 (*m*, 2H), 3.1 (*d*, 2H), 2.0 (*s*, 1H), 1.87 (*s*, 1H), 1.65 (*d*, 3H), 1.25 (*m*, 3H); orange-red colour with $FeCl_3$/$HClO_4$. *RC* yellowish amorphous powder; UV λ_{max}^{MeOH} nm: 226, (log ε 3.51), 283 (2.81), 290 (2.75), λ_{min}^{MeOH} nm: 247, 287; IR ν_{max}^{KCl} cm^{-1}: 3450–3300 *s*, 2950 *s*, 1720 *s*, 1580–1560 *s*, 1450 *s*, 1360 *w*, 1320 *w*, 1285 *m*, 740 *s*; MS *m/e* (rel. int.): 370 (100), 369 (100), 355 (5), 353 (6), 352 (5), 311 (6), 309 (6), 224 (5), 233 (5), 221 (7), 184 (9), 170 (14), 169 (16), 168 (6), 156 (12), 144 (9), 143 (7); PMR (CD_3OD): δ7.4–6.9 (*m*, 4H), 3.81 (*s*, 3H), 1.91 (*s*, 2H), 1.8–1.2 (*m*, 6H), 1.16 (*s*, 1H); dark green colour with $FeCl_3$/$HClO_4$. *RG*, off-white amorphous powder; UV λ_{max}^{MeOH} nm: 220, 253, 285, 290, λ_{min}^{MeOH} nm: 244, 275; IR ν_{max}^{KCl} cm^{-1}: 3410 *m*, 2970 *s*, 2850 *w*, 1590 *s*, 1490 *m*, 1460 *s*, 1420 *w*, 1290 *w*, 1225 *s*, 1130 *m*, 1105 *s*, 1090 *s*, 975 *m*, 950 *w*, 840 *m*, 780 *m*, 760 *m*; MS *m/e* (rel. int.): 322.16883 (M^+, 100; $C_{20}H_{22}N_2O_2$ calculated as 322.16811, error 0.00072), 321 (13), 294 (20), 293 (53), 279 (20), 200 (13), 199 (35), 198 (33), 173 (27), 160 (40), 134 (20), 130 (16), 109 (20), 108 (33); PMR (CD_3OD): δ7.87–6.81 (*m*, 3H), 5.35 (*m*, 1H), 3.78 (*s*, 3H), 3.58 (*bs*, 1H), 3.08 (*d*, 3H), 1.88 (*s*, 1H), 1.66 (*d*, 3H), 1.2 (*bs*, 3H); violet colour with $FeCl_3$/$HClO_4$.

Strong base fraction. Sarpagine and *normacusine B* were identified by comparison with published data [20] and authentic samples (UV, IR, MS, mp, chromogenic reactions and co-TLC). *RB*, greyish-white amorphous powder; UV λ_{max}^{MeOH} nm: 215, 222 sh, 245 sh, 270, 280, 290, λ_{min}^{MeOH} nm: 258; IR ν_{max}^{KCl} cm^{-1}: 3370 *s*, 2950 *s*, 1580 *s*, 1480 *m*, 1460 *s*, 1440 *s*, 1420 *s*, 1310 *m*, 1260 *m*, 1240 *m*, 1185 *w*, 1100 *s*, 1050 *w*, 1040 *w*, 960 *w*, 880 *w*, 825 *w*, 780 *m*, 740 *m*; MS *m/e* (rel. int.) 311 (25), 310 (100), 309 (33), 279 (33), 256 (6), 200 (5), 199 (5), 186 (12), 185 (53), 184 (25), 164 (10), 159 (12), 146 (14), 113 (22) 71 (53), 69 (26); red-violet colour with $FeCl_3/HClO_4$. *RD*. yellow powder with bright yellow fluorescence; UV λ_{max}^{MeOH} nm: 218 sh, 252 sh, 400; IR ν_{max}^{KCl} cm^{-1}: 3500–3450 *s*, 3010 *m*, 2950 *m*, 1700 *s*, 1640 *s*, 1580–70 *s*, 1480 *m*, 1420 *s*, 1340 *w*, 1270–50 *m*, 1215 *m*, 1120 *m*, 1090 *w*, 1050 *m*, 1020 *m*, 930 *w*, 820 *m*, 800 *m*, 765 *m*; MS *m/e* (rel. int.) 412 (30), 411 (44), 410 (M^+, 100), 409 (30), 397 (12), 395 (30), 381 (14), 367 (15), 354 (27), 353 (33), 351 (15), 323 (12), 283 (15), 269 (15), 258 (12), 257 (47), 244 (7), 230 (2), 229 (4), 222 (7), 216 (17). 10-*hydroxygeissoschizol* and *methyl reserpate* were identified by comparison (UV, IR, MS, chromogenic reactions) with published data [20].

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