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ALKALOIDS OF *RAUWOLFIA CUMMINSII* STEM

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Key Word Index—*Rauwolfia cumminsii*; Apocynaceae; indole alkaloids; *E-seco*; sarpagan; dihydroindole; yohimbine; heteroyohimbine; anhydronium bases.

Abstract—24 indole alkaloids were isolated from the stem bark of *Rauwolfia cumminsii* and 21 identified. The alkaloids comprised *E-seco*, sarpagan, dihydroindole, yohimbine, heteroyohimbine, 18-hydroxyyohimbine ester and anhydronium types together with peraksine and deacetylpicraline. The probable biosynthesis of the alkaloids is discussed.

INTRODUCTION

Investigation of the roots of *Rauwolfia cumminsii* Stapf [1] revealed the presence of at least 8 alkaloids which were identified as the heteroyohimbine compounds aricine and ajmalicine, the anhydronium bases serpentine and 19-episerpentine, the yohimbinoid alkaloids yohimbine and α-yohimbine and the 18-hydroxy-yohimbine ester alkaloids, reserpine and rescinnamine. As further studies [2] showed that the leaves yielded at least 12 different alkaloids including dihydroindole bases, a detailed examination of the stem bark was undertaken and resulted in the isolation of 21 alkaloids and 3 incompletely identified trace alkaloids.

RESULTS AND DISCUSSION

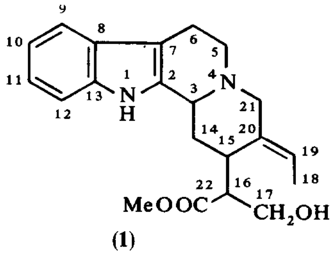
Known *Rauwolfia* alkaloids were characterized by analytical methods as indicated in Table 1 and by fluorescence colours and chromogenic reactions with ferric chloride–perchloric acid reagent. Physical data were also compared with published data [3].

The compound CMS11 produced a UV spectrum indicating an ar-unsubstituted indole chromophore. MS peaks at M-17, M-18, M-30 and M-31 normally associated with yohimbine were absent and the chromogenic reaction was consistent with an *E-seco* heteroyohimbine structure. IR data revealed the presence of OH (3400 cm^{-1}), NH (2950 cm^{-1}) and CH_3COO (1700 and 1240 cm^{-1}). MS peaks at M-59 and M-73 indicated CH_3COO and CH_2-CH_3COO groupings and peaks at *m/e* 184, 170, 169 and 156 corresponded with a β-carboline moiety. As the major peaks occurred at *m/e* 170, 169 and not *m/e* 169, 168, the structure was not a sarpagan pentacyclic ring system.

Table 1. Alkaloids isolated from *R. cumminsii* stems

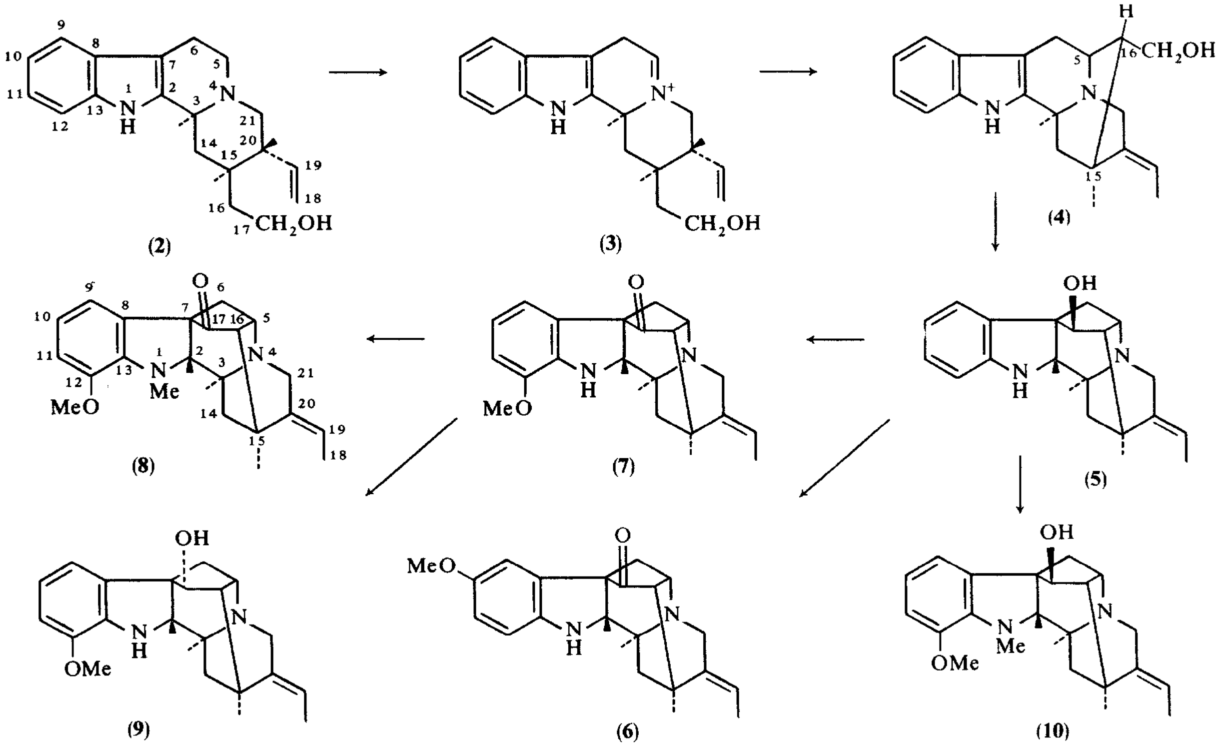
Compound	Identity	Analytical methods
Weakly basic fraction		
CMS 1	ajmalicine	UV, IR, MS co-TLC
CMS 2	corynantheol	UV, IR, MS, mp, $[\alpha]_D$
CMS 3	peraksine	UV, IR, MS, mp
CMS 4	aricine	UV, IR, MS, co-TLC
CMS 5	reserpine	UV, IR, MS, co-TLC
CMS 6	rescinnamine	UV, IR, MS, co-TLC
CMS 7	10,11-dimethoxyajmalicine	UV, IR, NMR, MS, co-TLC
CMS 8	norpurpeline	UV, IR, MS, co-TLC
CMS 9	dihydronorpurpeline	UV, IR, MS, co-TLC
CMS 10	endolobine	UV, IR, MS, co-TLC
Intermediate base fraction		
CMS 13	purpeline	UV, IR, MS, co-TLC
CMS 14	seredamine	UV, IR, MS, co-TLC
CMS 16	nortetraphyllicine	UV, IR, MS, co-TLC
CMS 17	normacusine B	UV, IR, MS, co-TLC
CMS 18	18-hydroxy-yohimbine	UV, IR, MS, co-TLC
CMS 20	deacetyl-picraline	UV, IR, MS, $[\alpha]_D$
Strongly basic fraction		
CMS 22	yohimbine	UV, IR, MS, co-TLC
CMS 23	serpentine	UV, IR, MS, co-TLC
CMS 24	19-epi-serpentine	UV, IR, MS, co-TLC

In the absence of PMR data due to insufficiency of isolated material, and bearing in mind the co-occurrence of corynantheine-type alkaloids in the plant, it was concluded that the most probable structure was (1) but the stereochemistry could not be deduced.



(1)

UV data suggested that compound CMS 12 was an ar-unsubstituted indole. This was confirmed by MS (*m/e* 223, 184, 170, 169, 156) but the compound differed from the typical ajmalicinoid structure by the presence of fragments at *m/e* 352 (M-18) and *m/e* 267. This indicated an OH group attached to the heterocyclic ring *E*. Chromatographic comparison revealed that CMS 12 was not an 18-hydroxy-yohimbine. The fragment *m/e* 267 (M-103) is equivalent to M-H + CHOH + CHCOOMe and suggests an ajmalicinine-type structure. Comparison with published data [4] indicated that CMS 12 was probably *ajmalicinine* (Me ester of (16α, 17β, 19α)-17-hydroxy-19-methyl-oxayohimban-16-carboxylic acid) and this was confirmed by treatment of CMS 12 with *p*-toluene sulphonyl chloride in pyridine which produced ajmalicine in good yield (UV, IR, MS, co-TLC) [4].



The UV spectrum of compound CMS 15 indicated a simple indole chromophore and the chromogenic reaction suggested a sarpagan-type structure. IR and MS data confirmed an unsubstituted indole structure (IR: 3400, 1595 and 750 cm^{-1} and MS: *m/e* 169, 168, 156, 144, 143); the MS revealed a similar fragmentation pattern to normacusine B but the M$^+$ was 14 amu higher and the fragmentation indicated no substitution in the indole portion. As the compound could not be readily acetylated, it was concluded that the CH_2 unit was associated with the C-16 primary alcoholic substituent. The *m/e* 169, 168 fragments are characteristic of sarpagan and *m/e* 263 represented M-CH_2.O.CH_3. Compound CMS 15 was therefore considered to be *normacusine B-O-methyl* (sarpagan-17-methoxy).

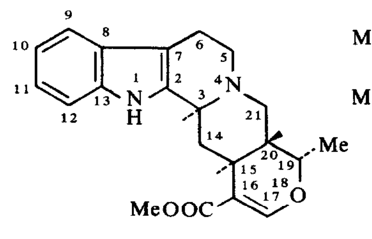
A simple indole type UV spectrum was also given by compound CMS 19 which was unaffected by acid or alkali. MS fragmentation resembled macusine B-*O*-methyl below *m/e* 308 [5] but the M$^+$ was 16 units higher at *m/e* 324. Considering the occurrence of MS peaks at M-16(O), M-31(CH_2OH) and M-45 (CH_2.CH_2OH) and the chromogenic reaction it was concluded that CMS 19 was normacusine *B-O-methyl-N-oxide* (sarpagan-17-methoxy-N_b-oxide). On reduction with sulphurous acid a compound identical (UV, IR, MS, co-TLC) with normacusine B-*O*-methyl was obtained.

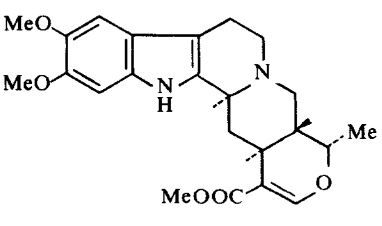
Compound CMS 21 was available in trace amounts only and could not be characterized but the presence of *m/e* 168 and 169 mass fragments and the chromogenic reaction suggested that a sarpagan unit was present.

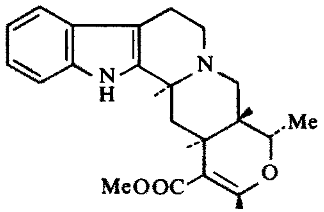
The generally accepted theory of indole alkaloid biogenesis [6] suggests that *E-seco* alkaloids of the corynantheine or geissoschizine type are converted to dihydroindole compounds via sarpagan-type intermediates. It is significant therefore, that *E-seco* compounds have been detected only in the leaves [2] and the stems of *R. cumminsii*.

Thus corynantheol (**2**) and possibly CMS 11, another *E*-seco compound, are converted via an imino-compound (**3**) and C-16 → C5 ring closure to the sarpagan normacusine B (**4**) which occurs also as its *O*-Me compound and N_b-oxide. A further ring closure (C17 → C7) leads to the dihydroindole compounds nortetraphyllicine (**5**), endolobine (**6**), norpurpeline (**7**), purpeline (**8**), dihydronorpurpeline (**9**) and seredamine (**10**). Alternative ring closures yield the minor alkaloids deacetyl-picraline (C16 → C6) and peraksine (= vomifoline) (C16 → C5; C20 → C16) which have also been detected in *R. vomitoria* Afz. leaves [7].

Yohimbines were not detected in the leaves [2] although reported in the roots [3]. Yohimbine, 18-hydroxy-yohimbine and the derived ester alkaloids


(**11**)


(**12**)



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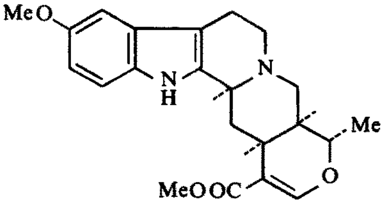

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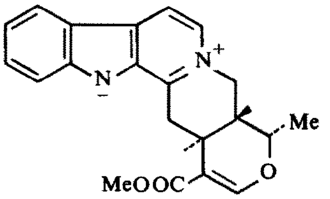

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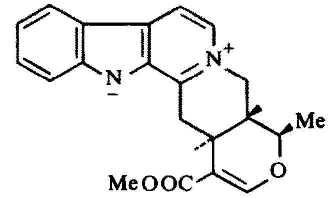

(16)

reserpine and rescinnamine were minor components of the alkaloid mixture of the stems.

The principal heteroyohimbine alkaloids, ajmalicine (**11**), 10, 11-dimethoxy-ajmalicine (**12**) and ajmalicinine (**13**) are *normal* configuration (C-3Hα, C-20Hβ) compounds, whereas the minor alkaloid aricine (**14**) has the *allo* configuration (C-3Hα, C-20Hβ). The dimethoxy-heteroyohimbine does not apparently give rise to oxindoles as in *R. vomitoria* [8] but the anhydronium bases serpentine (**15**) and 19-epi-serpentine (**16**) are derived from ajmalicine.

The occurrence of demethylajmalan dihydroindole alkaloids in the leaves and some related ajmalan dihydroindoles in the stems indicates that the demethyl compounds are probably formed first and subsequently methylated further down the axis. It is surprising therefore that N_a-methylated dihydroindoles have so far not been found in the roots, especially as other *Rauwolfia* species investigated in our laboratories have yielded significant amounts.

The isolation from a *Rauwolfia* species of ajmalicinine and 10, 11-dimethoxyajmalicine, alkaloids previously isolated from *Cabucala striolata* M. Pichon [4] is further evidence of the close relationship of the genera within the apocynaceous tribe Rauvolfieae. Deacetyl-picraline, an alkaloid isolated from *Alstonia scholaris* R. Br. [9] indicates also the near relationship with the tribe Alstonieae.

EXPERIMENTAL

Stem bark of *Rauwolfia cumminsii* Stapf was collected in Ghana and supplied by the Forest Herbarium, Kumasi, Ghana. Voucher specimen RAU 105-601 was deposited with the collection of Materia Medica and Herbaria, University of Bradford. Methods used for TLC, UV, IR, NMR, MS and fluorescence and chromogenic tests have been described previously [10, 11].

Extraction. Powdered stem bark (2 kg) was extracted by overnight maceration in 5 l. MeOH and further extracted with a further 5 l. and 2 × 2 l. for 8 hr. The combined MeOH extract was concentrated to *ca* 200 ml under red. pres. and poured into 500 ml H_2O. The resultant muddy-brown liquid was extracted with 5 × 1 l. EtOAc and the bulked EtOAc fractions evaporated to dryness. The extract was dissolved in 500 ml 0.1 N HCl and the soln filtered and extracted successively with 5 × 300 ml $CHCl_3$. The bulked $CHCl_3$ fractions were concd under red. pres. to yield the weakly basic fraction A.

The aq. layer was made alkaline (pH 9) with NH_3 and extracted with 5 × 300 ml $CHCl_3$. Evapn of the bulked $CHCl_3$ fractions under red pres yielded the intermediate basic fraction B.

The residual aq. layer was adjusted to pH 11 with NH_3 and NaOH to yield, after similar $CHCl_3$ extraction, the strongly basic fraction C.

Separation. Fraction A separated by TLC on Si gel (Me_2CO–petrol–CCl_4–isooctane, 7:6:4:3, and Me_2CO–$CHCl_3$, 4:5) yielded CMS 1–10. Fraction B was similarly separated (Me_2CO–petrol–Et_2NH, 2:7:1) to yield CMS 11–21. Fraction C was separated (EtOAc–*i*-PrOH–NH_3, 16:3:1) to yield 3 bands which, after elution and purification by TLC ($CHCl_3$–MeOH, 3:1), gave CMS 22–24.

Characterization. CMS 1–10, 13, 14, 16–18, 20, 22–24, see Table 1. CMS 11, off-white amorphous powder; UV: λ_{max}^{MeOH} nm: 226, 285, 291; IR ν_{max}^{KBr} cm^{-1}: 3400 *s*, 2950 *m*, 1700 *s*, 1585 *m*, 1410 *s*, 1240 *m*. 760 *s*; MS *m/e* (rel. int.): 354 (M^+, 85), 353 (100), 295 (29), 281 (20), 223 (8), 222 (5), 221 (60), 207 (25), 184 (18), 183 (5), 170 (20), 169 (35), 156 (12), 147 (42); greenish-brown colour with $FeCl_3$–$HClO_4$ reagent.

CMS 12, white crystalline powder: mp 165°; $[\alpha]_D^{20} = 0°$ ($CHCl_3$); UV: λ_{max}^{MeOH} nm: 225, 281, 290; IR ν_{max}^{KBr} cm^{-1}: 3400 *s*, 2950 *m*, 1725 *s*, 1645 *m*, 1430 *m*, 760 *s*, 740 *m*; MS *m/e* (rel. int.): 370 (M^+, 100), 369 (80), 352 (50), 337 (18), 267 (35), 223 (30), 184 (57), 170 (4), 169 (35), 168 (30), 156 (70), 143 (18); greenish-brown colour with $FeCl_3$–$HClO_4$ reagent.

CMS 15, off-white amorphous powder; UV: λ_{max}^{MeOH} nm: 225, 283, 289 (log ε 4.60, 3.85, 3.80); IR ν_{max}^{KBr} cm^{-1}: 3400 *s*, 2930 *m*, 1595 *s*, 1455 *m*, 1360 *m*, 1214 *m*, 1165 *s*, 750 *s*; MS *m/e* (rel. int.): 308 (M^+, 90), 307 (14), 294 (80), 293 (75), 279 (25), 277 (30), 263 (30), 185 (40), 184 (8), 183 (15), 182 (18), 169 (100), 168 (95), 167 (50), 156 (45), 154 (40), 144 (42), 143 (35), 130 (43), 129 (18); grey colour with $FeCl_3$–$HClO_4$ reagent.

CMS 19, cream amorphous powder; UV: λ_{max}^{MeOH} nm: 220, 273, 282 *sh*, 290; IR ν_{max}^{KBr} cm^{-1}: 3400 *s*, 2950 *m*, 1660 *s*, 1420 *w*, 1115 *m*, 1090 *s*, 840 *m*, 740 *s*; MS *m/e* (rel. int.): 324 (M^+, 25), 308 (45), 294 (8), 293 (20), 279 (25), 207 (18), 189 (30), 185 (20), 168 (100), 146 (30), 143 (25), 130 (30); indigo-blue, turning grey on heating, with $FeCl_3$–$HClO_4$ reagent.

CMS 21, off-white amorphous powder; UV: λ_{max}^{MeOH} nm: 226, 285, 293; IR ν_{max}^{KBr} cm^{-1}: 3400 *m*, 2950 *s*, 1740 *s*, 1400 *w*, 1100 *s*, 740 *m*; MS *m/e* (rel. int.): 530 (M^+, 20), 529 (13), 515 (15), 370 (16), 369 (14), 335 (10), 322 (20), 312 (14), 293 (14), 282 (24), 256 (4), 251 (10), 238 (30), 226 (20), 212 (12), 211 (10), 208 (50), 197 (34), 185 (30), 184 (100), 182 (20), 177 (25), 169 (25), 168 (60), 167 (30), 155 (30), 129 (35), 109 (20): steel grey colour with $FeCl_3$–$HClO_4$ reagent.

REFERENCES

1. Iwu, M. M. and Court, W. E. (1977) *Planta Med.* **32**, 158.

2. Iwu, M. M. and Court, W. E. (1978) *Planta Med.*, in press.
3. Gabetta, B. and Mustich, G. (1975) Spectral Data of Indole Alkaloids (Inverni Della Beffa, Milan, Italy.)
4. Bombardelli, E., Bonati, A., Danieli, B., Gabetta, B and Mustich, G. (1974). *Fitoterapia* **45**, 183.
5. Angenot, L. (1975) *Planta Med.* **27**, 34.
6. Cordell, G. A. (1974) *Lloydia* **37**, 219.
7. Pousset, J. L. and Poisson, J. (1965) *Ann. Pharm. Fr.* **23**, 733.
8. Pousset, J. L. (1967) *Trav. Lab. Matiere. Med. Pharm. Galenique, Fac. Pharm. Paris* **52**, 13.
9. Bartlett, M. E., Sklar, R., Smith, A. F. and Taylor, W. I. (1963) *J. Org. Chem.* **28**, 2197.
10. Habib, M. S. and Court, W. E. (1974) *Planta Med.* **25**, 331.
11. Court, W. E. and Timmins, P. (1975) *Planta Med.* **27**, 319