Phytochemistry, 1976, Vol. 15, pp. 733–735. Pergamon Press. Printed in England.

STEM ALKALOIDS OF *RAUWOLFIA OBSCURA*

PETER TIMMINS and WILLIAM E. COURT
Postgraduate School of Studies in Pharmacy, University of Bradford, West Yorkshire BD7 1DP

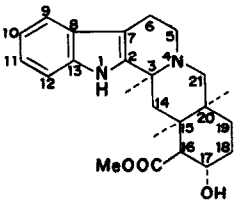
(*Revised received* 4 *September* 1975)

Key Word Index—*Rauwolfia obscura*; Apocynaceae; stem alkaloids; tetrahydroalstonine; vomalidine; alstonine; α-yohimbine; 10-methoxygeissoschizol; 19,20-dehydroyohimbine; choline.

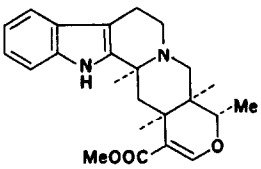
Abstract—The indole alkaloids alstonine, 10-methoxygeissoschizol, tetrahydroalstonine, vomalidine, α-yohimbine and 19, 20-dehydroyohimbine, an unidentified anhydronium-like base and choline were isolated from *Rauwolfia obscura* stems. The diester alkaloids reserpine and rescinnamine, which occur in the roots, were not detected. The origin and inter-relationship of the detected alkaloids are discussed.

INTRODUCTION

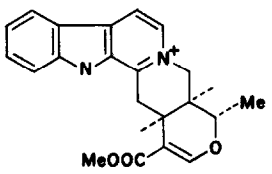
Earlier investigations established the occurrence of dihydroindole and indole alkaloids in the root bark of *Rauwolfia obscura* and usambarine-type alkaloids in the leaves [1, 2]. The presence of six tertiary indole and dihydroindole alkaloids, a water-soluble indole alkaloid and choline in the stems is now reported and their possible inter-relationships discussed.



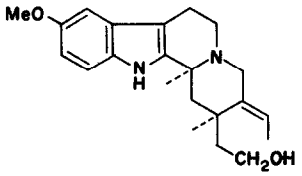
(1)



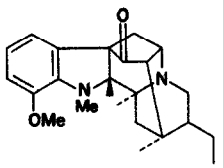
(2)



(3)



(4)



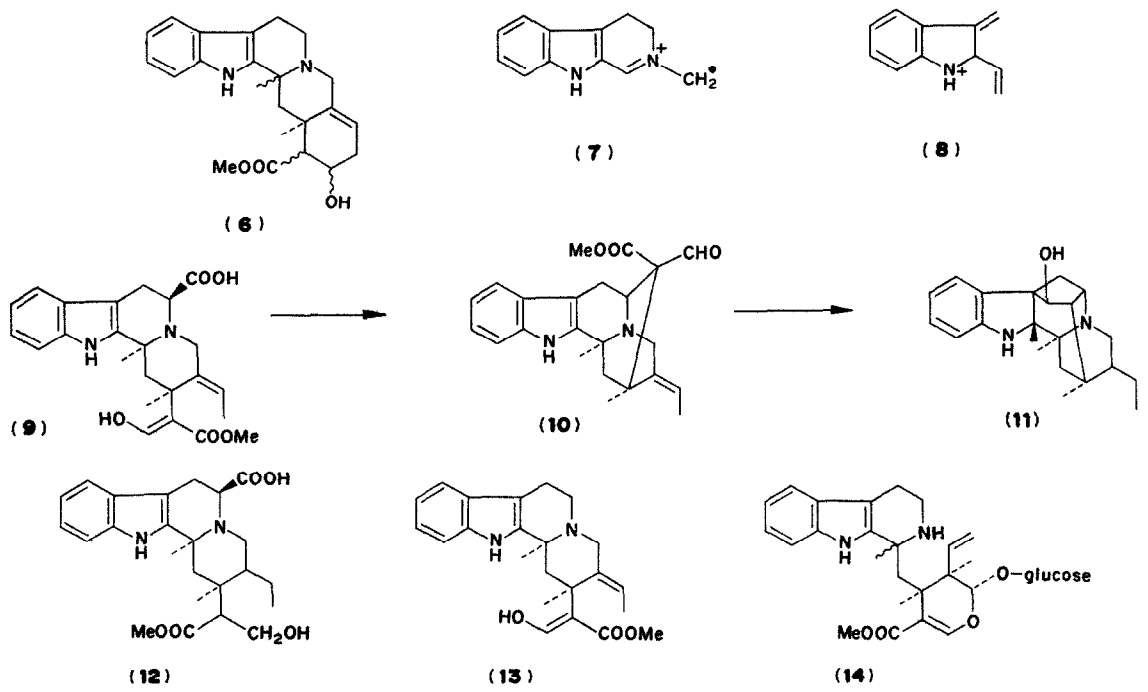
(5)

* *Nomenclature of yohimban compounds.* All known compounds of this type possess C-15H; four configurations are thus possible and are named as follows: *normal* (C-3Hα, C-20Hβ); *pseudo* (C-3Hβ, C-20Hβ); *allo* (C-3Hα, C-20Hα); *epi-allo* (C-3Hβ, C-20Hα).

RESULTS

The indole alkaloids α-yohimbine (**1**), tetrahydroalstonine (**2**), alstonine (**3**) and 10-methoxygeissoschizol (**4**) and the dihydroindole alkaloid vomalidine (**5**) were isolated and identified by comparison of their properties (UV, IR, MS, mp, co-TLC, as quantities isolated permitted) with authentic samples or published data. A dehydroyohimbine alkaloid of unknown stereochemistry (ROSB5) was also obtained; its structure is discussed below. The water-soluble alkaloids were recovered from their precipitated reineckates as chloride salts by ion exchange and two compounds were separated by PLC, choline and an alkaloid whose UV spectral properties and fluorescence colours on TLC plates were similar to those of alstonine and serpentine. Unfortunately this compound apparently underwent thermal decomposition in the mass spectrometer and was not available in sufficient amounts for further investigation.

UV and IR data indicated that alkaloid ROSB5 was closely related to the yohimbines. It showed an unsubstituted indolic absorption spectrum at λ_{max} 225, 282, 289 nm and the IR spectrum demonstrated the presence of a carbomethoxy group (1720 cm^{-1}). MS gave M^+ at *m/e* 352 (100% relative intensity) indicating two hydrogen atoms less than in the unsubstituted yohimbines (M^+ 354). Other fragments in the MS at *m/e* 351 ($M^+ - 1$, 91), 184(15), 170(35), 169(42) and 156(65) were typical of yohimbinoid alkaloids [3]. The *m/e* 156 peak was much more intense relative to the *m/e* 184 peak than in typical yohimbines e.g. yohimbine gives *m/e* 156 (12%) and *m/e* 184 (18%). This can be explained in terms of a double bond in the C-19, C-20 position (**6**), such a bond inhibiting formation of the species *m/e* 184 (**7**) and favouring formation of species *m/e* 156 (**8**) according to the proposed fragmentation pathway for yohimbines [3]. Comparable observations were reported concerning the MS of 19,20-dehydroyohimbine (*normal* configuration*) isolated from *Aspidosperma pyricollum* [4]. Insufficient material was isolated to enable elucidation of its stereochemistry but as α-yohimbine is the predominant yohimbine occurring in *R. obscura*, it is probable that the compound is 19,20-dehydro-α-yohimbine (*allo* configuration).



DISCUSSION

The occurrence of the E-seco alkaloid 10-methoxygeissoschizol (**4**) in the stems and leaves, but not in the roots, suggests that this compound is only synthesised in the aerial parts of the plant.

As the dihydroindole alkaloids occur almost exclusively in the roots, it is reasonable to assume that the dihydroindoles (**11**) are derived from an E-seco compound with implication of a sarpagine-type compound (**10**) although the latter has not as yet been detected in *R. obscura*. Accumulation of the sarpagine-type intermediate to detectable levels may not occur.

Vomalidine and ajmaline-type dihydroindoles were detected also in methanolic extracts of stem bases but not in extracts of the thinner, green twig-bark (co-TLC and chromogenic reactions). This suggests that conversion to dihydroindoles occurs in the stem base region.

Vann Tamelen *et al.* [5] have indicated that an alkaloid of the type **9** (5-carboxy-geissoschizine) could undergo oxidative decarboxylation to give rise to the ajmaline–sarpagine group. The only naturally-occurring 5-carboxy-geissoschizine so far isolated [6] is adirubine (**12**) from *Adina rubescens*. Geissoschizine (**13**) is considered [7] to be one of the first formed alkaloids in *Catharanthus roseus*, being derived from the glycosidic alkaloids vincoside and iso-vincoside (isomers of **14**). Assuming a similar pathway to operate in *Rauwolfia* species, geissoschizine could be metabolised to 10-methoxygeissoschizol in the aerial parts of *R. obscura*, this being probably converted to the usambarine-type alkaloids of the leaves [2]. Geissoschizine, vincoside and isovincoside have yet to be isolated from *R. obscura*, or any other *Rauwolfia* species. Such isolation may prove difficult as these intermediates are probably trace components of the alkaloid pool.

The co-occurrence of the *allo*-compounds tetrahydroalstonine (**2**) and alstonine (**3**) is not surprising; they are readily chemically interconvertible and the anhydronium base is possibly derived from the indole alkaloid in the plant. The occurrence of the related *normal* configuration alkaloid pair, serpentine and ajmalicine, has been previously noted in *R. macrophylla* [8]. The heteroyohimbines are considered to be amongst the first alkaloids formed from the nitrogenous glycosides [7].

It has not yet been established if the yohimbine alkaloids are biosynthesised from a secologanin precursor [7] but if this is so, then the C-3H configuration is possibly established at this stage. Only the C-3Hβ epimer vincoside has been incorporated into indole alkaloids in *C. roseus* [9] and thus an isomerisation process is necessary to yield the *allo* and *normal* series alkaloids (C-3Hα). The nature of such a process has not been elucidated and it may be that vincoside does not have this unique status in all indole-alkaloid yielding species.

The plant may therefore have the potential to produce α-yohimbine, 3-epi-α-yohimbine, reserpine and *iso*-reserpine. No 3-epi-α-yohimbine was detected and presumably its precursor is metabolised to reserpine, whereas α-yohimbine, for stereochemical reasons, is not so metabolised and no *iso*-reserpine was detected, α-yohimbine occurring predominently. *Iso*-reserpine has not been confirmed as a natural product, having been isolated only from the mother liquors of industrial reserpine production [10].

Further evidence must be obtained before the interrelationships of the *Rauwolfia* alkaloids can be fully explained, although the co-occurrence of the dihydroindoles, E-seco alkaloids, heteroyohimbines and yohimbines can be related to current biogenetic theory.

EXPERIMENTAL

Mps are uncorrected. IR spectra were measured in KBr discs. MS were obtained by direct inlet, 70 eV, 100 μA, 200–250°.

Plant material. Stems of *R. obscura* K. Schum. 0.2–1.5 cm dia. were collected in Zaire (voucher No. RAU 108-702 deposited with the Collection of Materia Medica and Herbaria, University of Bradford). The fibrous stem bark was easily separated from the stem wood using a scalpel.

Extraction and separation. Stem bark. 500 g powdered stem bark was extracted by maceration with MeOH in portions over 5 days. The bulked macerates (7 l) were evaporated by red pres to a viscous residue which was taken up in 1.0 N HCl (2 l). After overnight refrigeration and filtration to remove resinous material, the extract was defatted (2.5 l. hexane in portions) and fractionated into weakly and strongly basic fractions [11]. The residual aq soln was treated with excess ammonium reineckate soln and the ppt. collected (pH12 fraction). Acidification to pH6 and pH2 respectively with the addition of ammonium reineckate soln yielded ppts of pH6 and pH2 quaternary fractions. The alkaloids were separated by TLC (Si gel GF_{254}, 250 μm layers) or PLC (500 μm layers) and detected by fluorescence (254 and 366 nm) and spraying with Dragendorff's reagent or 0.2 M $FeCl_3$ in 35% aq. $HClO_4$ with heating to yield coloured spots.

Characterisation. The weakly basic fraction (148 mg) revealed traces of 2 alkaloids by TLC (Me_2CO–petrol–CCl_4–isooctane, 35:30:20:15); PLC, same solvent system, facilitated isolation (ROSB1, least polar, 0.5 mg; ROSB2, 1 mg). The strongly basic fraction (473 mg) showed 4 alkaloids by TLC ($CHCl_3$–MeOH, 9:1 with NH_3 vapour). The main base, ROSB3, was recovered as the hydrochloride (10 mg) by direct crystallisation. The remaining 3 bases were isolated by PLC ($CHCl_3$–MeOH, 9:1 with NH_3 vapour), ROSB4, 4 mg; ROSB5, 2 mg; ROSB6, 1.5 mg). The 3 quaternary fractions were regenerated as chlorides by stirring Me_2CO solns with Zerolit FFIP resin (chloride form) for 1.5 hr. The pH2 fraction yielded virtually no alkaloid. The pH6 fraction yielded a single alkaloid (PLC: eluant MeOH: 880 NH_3, 95:5) ROSB7, 0.8 mg. The pH 12 fraction yielded a single Dragendorff-positive compound by PPC (Whatman 3MM, eluent *n*-BuOH–HCl–H_2O, 4:1:5, upper phase), ROSB8, 0.3 mg as chloride.

Stem wood. 2.5 kg treated similarly yielded 0.45 g weakly basic fraction from which no alkaloids were isolated. The strongly basic fraction yielded ROSW1, 9.5 mg; ROSW2, 0.4 mg; ROSW3, 0.4 mg; ROSW4, 0.3 mg. The H_2O-sol alkaloids were pptd at pH2 with ammonium reineckate and regenerated as before; ROSW5, 0.5 mg; ROSW5, 3 mg.

Identification of alkaloids. ROSB1, *Tetrahydroalstonine*, yellow powder, co-TLC with an authentic sample (6 systems), chromogenic reactions, fluorescence colours, UV. ROSB2, *Vomalidine*, cream powder, co-TLC with an authentic sample (6 systems) chromogenic reactions, fluorescence colours, UV, MS. ROSB3=ROSW1, *Alstonine* HCl, yellow–brown crystals, mp. 279–283° dec., co-TLC, chromogenic reactions, fluorescence colours, UV, IR, MS. MS showed little fragmentation; characteristics resembled serpentine but TLC yielded slightly higher values than for serpentine. Reduction product shown to be *tetrahydroalstonine* (so-TLC, UV, IR, MS). Therefore ROSB3 was confirmed as *alstonine*. ROSB4=ROSW2, *α-Yohimbine*, white powder, co-TLC, with an authentic sample (6 systems), chromogenic reactions, fluorescence colours, UV, IR, MS, mp. ROSB5=ROSW3, *19,20-dehydroyohimbine*, cream powder; UV: λ_{max}^{MeOH} nm: 225, 282, 289 λ_{min} 248 nm. IR γ_{max}^{KBr} cm^{-1}: 3400, 2950, 1720, 1455, 1440, 1320. MS *m/e* (rel. int.): 352(M^+, 100), 351(91), 249(18), 184(15), 170(25), 169(31), 156(37), 144(13). For 19, 20-dehydroyohimbine from *Aspidosperma pyricollum* [4], UV: λ_{max}^{EtOH} nm: 226, 283, 293. IR γ_{max} cm^{-1} 3448 (NH), 1724; MS *m/e* (rel. int.): 352(100), 351(93), 184(11), 170(39), 169(56), 156(70). ROSB6=ROSW4, *10-methoxygeossoschizol*, cream powder, co-TLC, UV, IR, MS. Identical with 10-methoxygeissoschizol isolated from *R. obscura* leaves [2]. ROSB7=ROSW5, unidentified water-soluble base, yellow powder: UV: λ_{max}^{MeOH} nm: 230, 252, 307; $\lambda_{max}^{MeOH:NaOH}$ nm: 229, 277*sh*, 283, 328. ROSB8=ROSW6, *choline chloride*, off-white crystals; co-TLC (6 systems), co-PC (4 systems), IR.

Acknowledgements—We wish to thank Dr. P. W. LeQuesne and Dr. R. C. Elderfield for an authentic sample of tetrahydroalstonine. One of us (PT) acknowledges receipt of a Science Research Council Research Studentship.

REFERENCES

1. Timmins, P. and Court, W. E. (1974) *Phytochemistry* **13**, 1997.
2. Timmins, P. and Court, W. E. (1975) *Planta Med.* **27**, 105.
3. Antonaccio, L. D., Pereira, N. A., Gilbert, B., Vorbrueggen, H., Budzikiewicz, H., Wilson, J. M., Durham, L. J. and Djerassi, C. (1962) *J. Am. Chem. Soc.* **84**, 2161.
4. Arndt, R. R. and Djerassi, C. (1965) *Experientia* **21**, 566.
5. Van Tamelen, E. E., Haarstad, V. R. and Orvis, R. L. (1968) *Tetrahedron* **24**, 687.
6. Brown, R. T., Chapple, C. L. and Lee, G. K. (1972) *Chem. Commun.* 1007.
7. Cordell, G. A. (1974) *Lloydia* **37**, 219.
8. Timmins, P. and Court, W. E. (1974) *Phytochemistry* **13**, 281.
9. Battersby, A. R., Burnett, A. R., Hall, E. S. and Parsons, P. G. (1968). *Chem. Commun.* 1582: Battersby, A. R., Burnett, A. R. and Parsons, P. G. (1969) *J. Chem. Soc. C.* 1193.
10. Müller, J. M. (1962) *Swiss Pat.* 361,814; *Chem. Abstr.* **59**: 1159.
11. Court, W. E., Evans, W. C. and Trease, G. E. (1958) *J. Pharm. Pharmac.* **10**, 380.