Phytochemistry, 1975, Vol. 14, pp. 1855–1863. Pergamon Press. Printed in England.

ALKALOIDS OF *UNCARIA ATTENUATA*, *U. ORIENTALIS* AND *U. CANESCENS**

J. DAVID PHILLIPSON and SARAH R. HEMINGWAY

Department of Pharmacognosy, The School of Pharmacy, (University of London), 29–39, Brunswick Square, London WC1N 1AX, England

(*Received* 7 *February* 1975)

Key Word Index—*Uncaria attenuata*, *U. orientalis*, *U. canescens*; Rubiaceae; heteroyohimbine, oxindole alkaloids; 3-*iso*-19-*epi*-ajmalicine; *epiallo*-corynantheine; dihydrocorynantheine pseudoindoxyl; yohimbine isomers; yohimbine oxindole.

Abstract—3-*Iso*-19-*epi*-ajmalicine, *epiallo*-corynantheine and dihydrocorynantheine pseudoindoxyl, not previously known as natural products, have been isolated from samples of *U. attenuata*. Akuammigine, dihydrocorynantheine, hirsutine, hirsuteine, mitraphylline, speciophylline, uncarines A and B, isorhynchophylline rhynchophylline, isocorynoxeine, corynoxeine, corynoxine B, rotundifoline, specioloine, two yohimbine isomers, a yohimbine oxindole and an unidentified indole alkaloid (M^+, *m/e* 347) have been obtained from samples of the same species. 3-*Iso*-ajmalicine, harmane, isopteropodine, pteropodine, uncarine F, speciophylline, isomitraphylline, mitraphylline and *N*-oxides of these six oxindole alkaloids have been isolated from samples of *U. orientalis*. Several samples of *U. canescens* have yielded harmane while one sample contained the four pteropodine isomers. The variation in the alkaloid content of these three species is discussed.

INTRODUCTION

During the screening of *Uncaria* species for the presence of alkaloids, our attention was drawn to a sample of Philippine leaves from a herbarium sheet originally labelled "*U. canescens* Korth". because an extract gave strong positive reactions for alkaloids. This result contrasted with the results from other samples of *U. canescens* which were mainly low in their alkaloid content. Our curiosity was aroused further when it was realized that this species, more correctly named *U. canescens* Korth. ssp. *canescens*, does not grow in the Philippines although *U. canescens* ssp. *velutina* (Havil.) Rids. does. The sample in question, Elmer 10733, shows affinity with *U. orientalis* Guill. and with *U. attenuata* Korth. but it matches most closely with Philippine material described by Elmer as *U. bulusanensis* [1]. Complete identification of this species is not possible because so few collections have been made but Ridsdale has proposed [1] that this sample be referred to *U. bulusanensis* Elm., now regarded as *U. attenuata* Korth. ssp. *bulusanensis* (Elm.) Rids., msc. [2]. Because difficulties obviously exist in the correct identification of these *Uncaria* species, it was thought that a knowledge of the alkaloids present might help to distinguish these species and to confirm identifications made on the basis of morphological and anatomical characters.

RESULTS AND DISCUSSION

The details of the separation and identification of the alkaloids from some leaf samples of *U. attenuata*, *U. orientalis* and *U. canescens* are given in the Experimental. The screening results presented in Table 1 were obtained from leaves collected over a wide geographical range and they were selected as being representative of a larger

* Part 4 in the series "Alkaloids from *Uncaria* species". For Part 3 see Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12**, 2795.

Table 1. Alkaloids isolated from different samples of *U. attenuata*, *U. orientalis* and *U. canescens* leaves

Sample screened	Alkaloid*	Structure
U. attenuata ssp. attenuata		
(a) Korthals s.n.	*hirsuteine*	**2**, *pseudo*, R = vinyl
	akuammigine	**1**, *epiallo*, C-19 Meα
	3-isoajmalicine	**1**, *pseudo*, C-19 Meα
	speciophylline	**11**, *epiallo* A, C-19, Meα
	harmane	**12**
(b) Ridsdale s.n.	*isorhynchophylline* and *N*-oxide	**10**, *normal* A, R = Et, R′ = H
	rhynchophylline and *N*-oxide	**10**, *normal* B, R = Et, R′ = H
	isocorynoxeine	**10**, *normal* A, R = vinyl, R′ = H
	corynoxeine	**10**, *normal* B, R = vinyl, R′ = H
	dihydrocorynantheine	**2**, *normal*, R = Et
	hirsutine	**2**, *pseudo*, R = Et
	hirsuteine	**2**, *pseudo*, R = vinyl
	pseudoyohimbine	**8**, *pseudo*, C-16Hβ, C-17Hβ
	yohimbine isomer	**8**
	a yohimbine oxindole	**9**
(c) van Oostrom 12565	isorhynchophylline	**10**, *normal* A, R = Et, R′ = H
	rhynchophylline	**10**, *normal* B, R = Et, R′ = H
	hirsutine	**2**, *pseudo*, R = Et
(d)—(Herb. L. 908. 221–905)	*dihydrocorynantheine*	**2**, *normal*, R = Et
	isorhynchophylline	**10**, *normal* A, R = Et, R′ = H
	rhynchophylline	**10**, *normal* B, R = Et, R′ = H
	rotundifoline	**10**, *normal* A, R = Et, R′ = OH
	isorotundifoline	**10**, *normal* B, R = Et, R′ = OH
	dihydrocorynantheine -pseudoindoxyl	**3**, R = H
(e) Wenzel 1038	uncarine A	**11**, *normal* A, C-19 Meβ
	uncarine B	**11**, *normal* B, C-19 Meβ
	mitraphylline and *N*-oxide	**11**, *normal* B, C-19 Meα
	isomitraphylline *N*-oxide	**11**, *normal* A, C-19 Meα
U. attenuata ssp. *bulusanensis*		
(a) Elmer 14917	*epiallo-corynantheine*	**2**, *epiallo*, R = vinyl
	dihydrocorynantheine	**2**, *normal*, R = Et
	rotundifoline	**10**, *normal* A, R = Et, R′ = OH
	isorotundifoline	**10**, *normal* B, R = Et, R′ = OH
(b) Elmer 10733	3-*iso*-19-*epi*-ajmalicine	**1**, *pseudo*, C-19 Meβ
(c) Kandern	*speciofoline*	**10**, *epiallo* B, R = Et, R′ = OH
	rhynchophylline	**10**, *normal* B, R = Et, R′ = H
	corynoxine B	**10**, *allo* B
	unidentified indole alkaloid	M^+ *m/e* 347
U. orientalis		
(a) Rutten 1932	harmane	**12**
	unidentified alkaloids†	
(b) Kalkman 4288	3-isoajmalicine	**1**, *pseudo*, C-19 Meα
(c) Ridsdale s.n.	*isopteropodine* and *N*-oxide	**11**, *allo* A, C-19 Meα
	pteropodine and *N*-oxide	**11**, *allo* B, C-19 Meα
	uncarine F and *N*-oxide	**11**, *epiallo*, C-19 Meα
	speciophylline and *N*-oxide	**11**, *epiallo* A, C-19 Meα
(d) Ridsdale s.n. (Markham bridge)	*isopteropodine* and *N*-oxide	**11**, *allo* A, C-19 Meα
	pteropodine and *N*-oxide	**11**, *allo* B, C-19 Meα
	uncarine F and *N*-oxide	**11**, *epiallo* B, C-19 Meα
	speciophylline and *N*-oxide	**11**, *epiallo* A, C-19 Meα

Table 1 (*contd*)

Sample screened	Alkaloid*	Structure
(e) Carr 12196	harmane	**12**
	unidentified alkaloids†	
(f) Kajewski 620	*isomitraphylline* and *N*-oxide	**11**, *normal* A, C-19 Meα
	mitraphylline and *N*-oxide	**11**, *normal* B, C-19 Meα
U. canescens ssp. canescens		
(a) Put 1173	harmane	**12**
	unidentified alkaloids†	
(b) Fri 3359	harmane	**12**
	unidentified alkaloids†	
(c) Korthals s.n.	harmane	**12**
	unidentified alkaloids†	
(d) Kostermans and Anta 144	harmane	**12**
(e) Wirawan 83	unidentified alkaloids†	
(f) P. W. Richards 1276	harmane	**12**
	unidentified alkaloids†	
U. canescens ssp. *velutina*		
(a) Elmer 8874	—	
(b) Wenzel 2575	*isopteropodine* and *N*-oxide	**11**, *allo* A, C-19 Meα
	pteropodine and *N*-oxide	**11**, *allo* B, C-19 Meα
	uncarine F and *N*-oxide	**11**, *epiallo* B, C-19 Meα
	speciophylline and *N*-oxide	**11**, *epiallo* A, C-19 Meα

* Major alkaloids in italics.
† Complex mixtures of Ehrlich^{+ve} spots (purple) on TLC.

series of samples examined. Table 2 lists the type of alkaloids found in all three species summarizing the results from the entire series examined, including those from additional samples not listed in the Experimental.

The alkaloids identified as being present in the herbarium samples of *U. attenuata*, *U. orientalis* and *U. canescens* are mainly of the heteroyohimbine and oxindole types (Tables 1 and 2). Identifications were made by TLC and GLC comparisons of the extracts with reference alkaloids, followed by elution of alkaloids from TLC plates for determination of UV and mass spectra. The unidentified alkaloids from *U. canescens* and from some samples of *U. orientalis* were complex mixtures of Ehrlich and Dragendorff positive substances which produced streaks on TLC. Their further characterization was not possible because only small amounts of leaf material were examined.

The major alkaloid from one of the samples of *U. attenuata* ssp. *bulusanensis* (Elmer 10733, originally labelled "*U. canescens* Korth.") was identified as a pentacyclic heteroyohimbine (**1**) by its UV and mass spectra (M^+, *m/e* 352). Peaks at *m/e* 209 (10%) and 225 (11%) were accompanied by others at *m/e* 223 (10%) and 251 (3%) suggesting that the configuration* is either *normal* (C-3 Hα, C-20 Hβ) or *pseudo* (C-3 Hβ, C-20 Hβ) [3]. TLC comparison with reference compounds indicated that it had R_f values intermediate between ajmalicine (**1**, *normal*, C-19 Meα) and 3-isoajmalicine (**1**, *pseudo*, C-19 Meα) and

* Four diastereoisomeric compounds are theoretically possible for heteroyohimbine alkaloids (**1, 2**). All known compounds of this type possess C-15 Hα configuration and the four possible configurations are defined as *normal* (C-3 Hα, C-20 Hβ), *pseudo* (C-3 Hβ, C-20 Hβ), *allo* (C-3 Hα, C-20 Hα) and *epiallo* (C-3 Hβ, C-20 Hα). For oxindole alkaloids (**10, 11**) eight diastereoisomers are theoretically possible since each of the above four configurations can exist as A or B isomers depending upon the configuration at C-7. In the A isomers the lactam carbonyl is below the plane of the C–D rings and in the B isomers it is above.

Table 2. Alkaloid-types present in samples of *U. attenuata*, *U. orientallis* and *U. canescens*

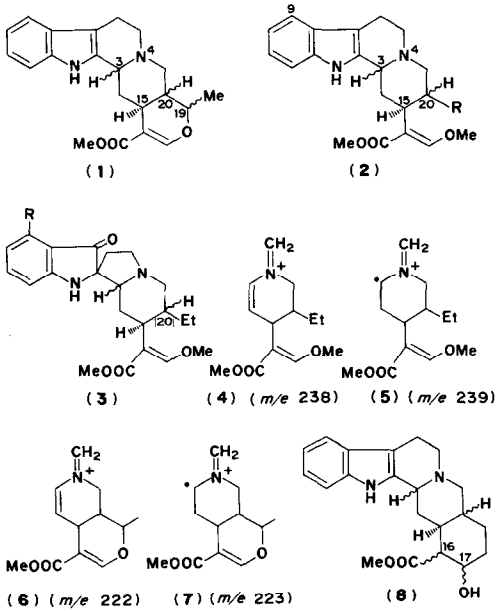
Species	No. of samples examnd.	No. of samples contg.	Closed E-ring		E-seco			Ehrlich^{+ve} streaks Unidentified alkaloids
			Oxindole	Heteroyohimbine	Oxindole	Heteroyohimbine	Harmane	
U. attenuata	11	4	–	–	+	–	–	–
ssp. *attenuata*		5	–	–	+	+	–	–
		1	+	+	–	+	+	–
		1	+	–	–	–	–	–
U. attenuata	3	1	–	+	–	–	–	–
ssp. *bulusanensis*		1	–	–	+	+	–	–
		1	–	–	+	–	–	+
U. orientalis	11	6	–	–	–	–	+	+
		4	+	–	–	–	–	–
		1	–	+	–	–	–	–
U. canescens	18	7	–	–	–	–	+	+
ssp. *canescens*		5	–	–	–	–	–	+
		6	–	–	–	–	–	–
U. canescens	2	1	–	–	–	–	–	–
ssp. *velutina*		1	+	–	–	–	–	–

also that it was not tetrahydroalstonine (**1**, *allo*, C-19 Meα), 19-*epi*-ajmalicine (**1**, *normal*, C-19 Meβ), rauniticine (**1**, *allo*, C-19 Meβ) or akuammigine (**1**, *epiallo*, C-19 Meα). Hence of the eight diastereoisomers of **1**, only two were not available for direct TLC comparison, isorauniticine (*epiallo*, C-19 Meβ) and 3-*iso*-19-*epi*-ajmalicine (*pseudo*, C-19 Meβ). On the basis of arguments previously advanced for relating configuration to TLC behaviour [4] and because of the MS data, it was thought that this alkaloid might be the *pseudo*, C-19 Meβ isomer of **1**. In order to confirm this deduction a small sample of synthetic 19-*epi*-ajmalicine [5] was oxidized with mercuric acetate to the Δ-3,4 compound which was then reduced to a mixture of the *normal* and *pseudo* isomers [6,7]. The *pseudo* isomer was separated from the reaction mixture by preparative TLC and its TLC, GLC, UV and MS properties shown to be identical with those of the alkaloid isolated from *U. attenuata* ssp. *bulusanensis*. 3-*Iso*-19-*epi*-ajmalicine has not previously been isolated as a natural product although it has been synthesized [8,9].*

An alkaloid separated by preparative TLC from another sample of *U. attenuata* ssp. *bulusanensis* (Elmer, 14917) was identified as a C-20 vinyl E-*seco* heteroyohimbine (**2**, R = vinyl) by means of its colour reactions on TLC, its UV and mass spectra. Since it had R_f values lower than those of dihydrocorynantheine (**2**, *normal*, R = ethyl) it was neither corynantheine (**2**, *normal*, R = vinyl) nor the C-20 vinyl analogue of corynantheidine (**2**, *allo*, R = vinyl) [4]. Direct TLC comparison showed that it was not hirsuteine (**2**, *pseudo*, R = vinyl) and hence the only remaining configuration possible is *epiallo*. The R_f values were higher than those of isocorynantheidine (**2**, *epiallo*, R = ethyl), behaviour consistent with its being the C-20 vinyl analogue [4]. Hence it is concluded that the alkaloid is the *epiallo* isomer of corynantheine and hirsuteine (**2**, R = vinyl), here named *epiallo*-corynantheine. There was insufficient alkaloid for CD or NMR measurements, for isomerization or for hydrogenation of the C-20 vinyl group so that confirmation of identity could not be obtained.

An alkaloid isolated from a sample of *U. attenuata* ssp. *attenuata* from Sabah, has been characterized as the new alkaloid dihydrocorynantheine pseudoindoxyl (**3**, R = H, C-20 Hβ). The MS of this yellow alkaloid showed a M^+ at *m/e* 384 and a fragmentation similar to that of the E-*seco* oxindole alkaloids except that the base peak was observed at *m/e* 238 (attributable to an ion of structure **4**) instead of *m/e* 239 (**5**).

* Subsequent to the compilation of this paper, a sample of Professor Winterfeldt's synthetic 3-*iso*-19-*epi*-ajmalicine was supplied to us by Dr. R. T. Brown. This synthetic alkaloid proved to have identical TLC hR_f values to the natural product.



The MS of mitragynine pseudoindoxyl (**3**, R = OMe, C-20 Hα) resembles that of dihydrocorynantheine pseudoindoxyl in that the base peak occurs at *m/e* 238 (**4**) although the M^+ (*m/e* 414) is 30 m.u. higher [10]. An analogous situation has been noted in the closed E ring alkaloids since the base peak of ajmalicine pseudoindoxyl occurs at *m/e* 222 (**6**) while that of the corresponding oxindoles (mitraphylline, isomitraphylline) occurs at *m/e* 223 (**7**) [11,12] but apart from this difference, many of the ions are common to both types of compound. Because the major alkaloid from this particular leaf sample was identified as dihydrocorynantheine, it seemed likely that the yellow alkaloid might be its pseudoindoxyl. This was confirmed by oxidation of dihydrocorynantheine (**2**, *normal*, R = ethyl) to its pseudoindoxyl [13] which was identical with the natural alkaloid (TLC, GLC and MS).

Prepared dihydrocorynantheine pseudoindoxyl (**3**, R = H, C-20 Hβ) retains the C-15 Hα and C-20 Hβ configurations. Theoretically, four isomers can be envisaged due to the asymmetry about the C-2/C-3 bond (**3**). MS and TLC indicated that only one isomer was formed and this result is consistent with previous findings that only one isomer of yohimbine pseudoindoxyl is formed, i.e. that possessing C-3 Hα and A configurations (C-7 CO below the plane of the C, D, E rings) [11]. Similar arguments have been applied to the pseudoindoxyls of ajmalicine and tetrahydroalstonine [11] and are here extended to one of the corresponding E-*seco* compounds (**3**, R = H, C-20 Hβ).

A chloroform solution of dihydrocorynantheine was exposed to light and air for several days; examination by TLC indicated that the pseudoindoxyl was not an artifact produced during the extraction procedure as previously suggested [14]. If this suggestion were true then it is surprising that pseudoindoxyls have not previously been isolated from *Uncaria* or from the related genus *Mitragyna*.* The isolation of dihydrocorynantheine together with the corresponding pseudoindoxyl and oxindole (rhynchophyllines) derivatives which can be obtained *in vitro* from the same intermediate (acetoxy- or hydroxyindolenine) suggests that such an intermediate might be present in the plant.

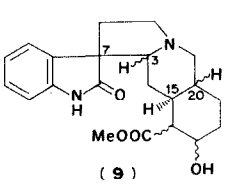
Four partially characterized alkaloids have been obtained in very small quantities during this investigation. The MS of two minor alkaloids isolated from *U. attenuata* ssp. *attenuata* (Ridsdale s.n.) indicate that they are yohimbine isomers (**8**) [15]. Co-chromatography with the available isomers, yohimbine, α-yohimbine and pseudoyohimbine, indicated that one corresponds to pseudoyohimbine. A third trace alkaloid isolated from the same source gave oxindole colour reactions on TLC with the ferric chloride–perchloric acid spray reagent. The MS of this alkaloid suggests that it is a yohimbine oxindole (**9**) [16]. Yohimbine and yohimbine oxindoles have not previously been identified from *Uncaria* species and such oxindoles have not been reported previously as natural products. The fourth alkaloid was obtained from *U. attenuata* ssp. *bulusanensis* (Kandern). Its UV and MS indicate that it is a tetrahydro-β-carboline derivative with a MW of 347 (M^+, $C_{21}H_{21}N_3O_2$ from accurate mass measurements). The UV spectrum shows that the tetrahydro-β-carboline is not conjugated with another chromophore. By analogy with alkaloids from related plants and in order to account for

* Mitragynine pseudoindoxyl has not been isolated from a *Mitragyna* sp. but by the biotransformation of mitragynine with a species of the fungus *Helminthosporum*; the pseudoindoxyl is 10 times more active as an analgesic than mitragynine [10].

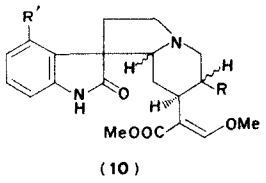
the number of rings and double bonds, it is probable that the third nitrogen is in a pyridinyl E-ring. Larger quantities of plant material will be required before these four alkaloids can be isolated in sufficient amounts for complete structure determinations.

U. attenuata, *U. orientalis* and *U. canescens* are species from a group of Malesian taxa which comprises the largest section of this pantropical genus [2]. Two subspecies of *U. attenuata* are recognized, ssp. *attenuata* which corresponds to the material originally described by Korthals and which is found in Malaya, Sumatra, Java and Borneo, and ssp. *bulusanensis* (Elm.) Rids. (syn. *U. bulusanensis* Elm.; *U. canescens* auct. non Korth., non Rids.; F.—Vill.) which includes material previously named *U. bulusanensis* and *U. attenuata* from the Philippines and Sulawesi. At present this latter subspecies is considered to be an insufficiently known entity and when more material becomes available it may well be considered to represent an extreme variant of *U. attenuata* ssp. *attenuata*. *U. orientalis* Guill. is generally similar to *U. attenuata* in its morphology and in cuticle characters, its range of distribution is from the Moluccas, New Guinea and the Solomon Islands to the New Hebrides. *U. canescens* comprises two subspecies of which one, ssp. *canescens* (syn. *U. canescens* Korth., *U. ovata* Hook f., *U. glaucescens* Craib.) is found in Malaya, Sumatra, Borneo and Java and is characterized by short trigonal calyx lobes, while the other, ssp. *velutina* (syn. *U. velutina* Havil., *U. clavisepala* Elm.), is found in the Philippines and is characterized by long spathulate calyx lobes.

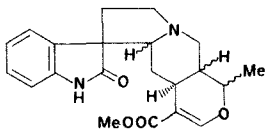
Samples of these three species have been screened for alkaloids and the results are summarized in Tables 1 and 2. The five samples of *U. attenuata* ssp. *attenuata* listed in Table 1 contain heteroyohimbine and/or oxindole alkaloids which may have E-*seco* (**2**, **10**) or E-cyclic (**1**, **11**) ring systems. The most frequently encountered alkaloids in this subspecies were of the E-*seco* oxindole type (Table 2). Of the three samples of *U. attenuata* ssp. *bulusanensis* available for screening, one gave an E-*seco* heteroyohimbine (**2**) as the major alkaloid, another an E-cyclic heteroyohimbine (**1**) and the third contains E-*seco* oxindole alkaloids (**10**) and an unidentified indole alkaloid (M^+, *m/e* 347). Although E-*seco* alkaloids



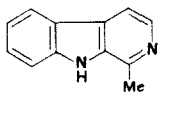
(9)



(10)



(11)



(12)

predominated in two out of the three samples of ssp. *bulusanensis*, the variability between the three samples shows that further material must be examined before affinities between the two ssp. can be established. Minority samples of ssp. *bulusanensis* and *attenuata* yielded closed E-ring alkaloids (**1**, **11**) possessing C-19 Meβ configuration and since such alkaloids seem to occur infrequently in the genus, they may represent a link between the two subspecies. The alkaloid composition of the *U. orientalis* samples examined shows that there are two distinct types, one having closed E-ring oxindoles or heteroyohimbines and the other containing harmane (**12**) with complex mixtures of unidentified alkaloids indicated by the Ehrlich positive streaks on TLC (Tables 1 and 2). Eighteen samples of *U. canescens* ssp. *canescens* ranging from Thailand, Malaya, Sumatra, Bangka and Borneo were screened. The samples all contained little alkaloid, consisting of harmane and Ehrlich positive streaks on TLC. No oxindoles or heteroyohimbines were detected in this subspecies which appears to be the most uniform of the three species in its alkaloid content. Only two samples of *U. canescens* ssp. *velutina* were available for screening and they differed from each other in that one contained the pteropodine isomers (**11**, *allo/epiallo*) while the other was alkaloid negative.

These results show that the individual alkaloids present within any sample from these three species may differ from those present in another sample of the same species. Thus, there does not appear to be any correlation between geographical distribution and alkaloid content. These infraspecific differences may be as great as the dif-

ferences between species and possibly reflect the general plasticity of the species. The differences which have been noted in the alkaloid content from samples of species of the related genus *Mitragyna*, have been related to the time of collection (e.g. heteroyohimbines may predominate in young leaves) [17]. The *Uncaria* samples screened would mainly have been collected during flowering or fruiting so that it is not anticipated that the time of collection would account for all the differences in alkaloid content. The alkaloids of some samples of *U. orientalis* resemble those of *U. canescens*, while others contain oxindole alkaloids and in this respect resemble *U. attenuata*. However the *U. orientalis* oxindoles differ in their stereochemistry and E-ring structure from the majority of those obtained from *U. attenuata*, although a small proportion of the *U. attenuata* oxindole alkaloids are of the *U. orientalis*-type.

Thus the observed morphological affinities between these two species are in part upheld by the types of alkaloid present. The results do show that *U. attenuata* and *U. canescens* differ markedly in their alkaloid content and that the Philippines sample (Elmer 10733) originally labelled "*U. canescens* Korth." and which yielded a heteroyohimbine alkaloid, has more affinity in its alkaloid content with *U. attenuata*, the species in which it is now classified.

EXPERIMENTAL

The plant material was supplied by the Rijksherbarium, Leiden, and some was collected by Dr. C. E. Ridsdale (B. A. Krukoff Botanist, Rijksherbarium). For the extraction procedures see Part 1 [18]. The TLC systems used were Si gel G/GF_{254} (2:1) with, A. $CHCl_3$–Me_2CO (5:4), B. $CHCl_3$–EtOH (95:5), C. Et_2O–EtOAc (1:1), D. EtOAc–*iso*PrOH–conc. NH_4OH (100:2:1), E. EtOAc–*iso*PrOH–conc. NH_4OH (80:15:5), F. EtOAc–*iso*PrOH–conc. NH_4OH (60:35:5), G. $CHCl_3$–MeOH (6:1). Details of detection on TLC, GLC, UV and MS conditions are described separately [19]. The alkaloids were separated by prep. TLC and identified by their R_f values and colour reactions on TLC, R_t values, UV and MS. *Samples examined for alkaloid content* (leaves extracted unless stated otherwise). *U. attenuata* ssp. *attenuata*. (a) *Korthals s.n.* (Herb. L. 908, 221–891), Sumatra, 1833–1836. 807 mg yielded 5 mg of total alkaloid (0·62%) which was separated on system A. The major alkaloid was identified as hirsuteine (TLC, GLC, UV, MS). Akuammigine, 3-isoajmalicine (TLC, GLC, UV, MS), speciophylline (TLC, GLC, MS) and harmane (TLC) were also present. (b) *Ridsdale s.n.*, Cult. Hort. Bog. XVIIC 27, W. Java, 1968. TLC and GLC indicated that there was no difference between the alkaloids of the leaves (10·67 g yielded 72 mg, 0·67%), stem bark (1·16 g yielded 5·6 mg, 0·48%) or stem wood (5·5 g yielded 8·7 mg, 0·16%). The major alkaloids were separated by prep. TLC (system B) and identified as isorhynchophylline, rhynchophylline, isocorynoxeine and corynoxeine (TLC, GLC, UV, MS). Minor alkaloids were separated by systems A, F or by Et_2O–diethylamine (95:5) and identified as hirsutine and hirsuteine (TLC, GLC, UV, MS) and the *N*-oxides of isorhynchophylline and rhynchophylline (TLC, GLC). Three other minor alkaloids were identified as follows: *Pseudoyohimbine*, hR_f values in systems B, 9; E, 56; G, 29. $FeCl_3/HClO_4$, grey–green turning brown; Ehrlich's reagent, purple; $Ce(SO_4)_2$–H_2SO_4, orange; R_t 14·7 min.

The R_f values, colour reactions and R_t value are identical to those of pseudoyohimbine. UV λ_{max} 225, 284, 291 nm. MS, *m/e* 354 (M^+, 97; found 354·1933, $C_{21}H_{26}N_2O_3$ requires 354·1943), 353 (100), 339 (2), 337 (2; found 337·1909, $C_{21}H_{25}N_2O_2$ requires 337·1916, M^+-OH), 325 (4), 323 (4), 232 (4), 223 (4), 209 (3), 184 (11), 170 (16), 169 (19), 156 (12), 144 (11), 143 (9). A *yohimbine isomer*, hR_f values in systems B, 2; D, 3; E, 34; G, 20. $FeCl_3$–$HClO_4$, grey–green turning brown; Ehrlich's reagent, purple; $Ce(SO_4)_2$–H_2SO_4, orange; R_t 15·6 min. The hR_f and R_t values show that the compound is not yohimbine, α-yohimbine or pseudoyohimbine. UV λ_{max}, 224, 275 sh, 284, 291 nm. MS *m/e* 354 (M^+, 96; found 354·1936, $C_{21}H_{26}N_2O_3$ requires 354·1943), 353 (100), 339 (1), 337 (2), 325 (4), 295 (8), 223 (4), 184 (13), 170 (15), 169 (17), 156 (13), 144 (10), 143 (8). A *yohimbine oxindole*, hR_f values in systems B, 5; E, 39; G, 44; $FeCl_3$–$HClO_4$, pink. MS, *m/e* 370 (M^+, 100; found 370·1921, $C_{21}H_{26}N_2O_4$ requires 370·1892), 355 (11), 353 (27), 339 (10; found 339·1638, $C_{20}H_{23}N_2O_3$ requires 339·1709, M^+-OMe), 337 (6), 335 (6), 225 (59; found 225·1335, $C_{12}H_{19}NO_3$ requires 225·1365), 212 (24), 199 (24), 130 (14). (c) *van Oostroom 12565*, Java, 29:1:1950. 1·0 g yielded 2·4 mg of total alkaloids (0·23%) identified as isorhynchophylline, rhynchophylline and hirsutine (TLC, GLC). (d) (*Herb. L., 908*, 221–905), Sabah, no date. This material, although mounted in 1908, was seen by Haviland and was probably collected during the mid-19th century. 1·0 g yielded 9·8 mg (0·98%, total alkaloid which was separated by prep. TLC (system B) into dihydrocorynantheine (major alkaloid), isorhynchophylline, rhynchophylline and rotundifoline (TLC, GLC, UV, MS), isorotundifoline (TLC) and dihydrocorynantheine pseudoindoxyl (TLC, GLC and MS identical with prepared alkaloid—see below). (e) *Wenzel 1038*. Leyte, Philippines, 1914. 439 mg yielded 5·9 mg of total alkaloids (1·2%) which was separated by prep. TLC (system D) into uncarine A, uncarine B and mitraphylline (TLC, GLC, UV, MS). The presence of mitraphylline and isomitraphylline *N*-oxides was indicated by TLC. *U. attenuata* ssp. *bulusanensis*. (a) *Elmer 14917*, Irosin, Luzon, Philippines, 11. 1915. 373 mg yielded 4·4 mg (1·18%) of total alkaloid which was separated by prep. TLC (system B). *Epiallo*-corynantheine (major alkaloid) hR_f values, A, 29; B, 22; D, 35; $FeCl_3$–$HClO_4$, grey–brown; Ehrlich's reagent, purple; UV λ_{max} 225, 245 sh, 284, 291; MS, *m/e* 366 (M^+, 100), 365 (57), 351 (44), 338 (16), 337 (59), 335 (27), 249 (21), 237 (34), 223 (14), 184 (17), 171 (18), 170 (29), 169 (39), 156 (29), 144 (14). Minor alkaloids, dihydrocorynantheine, rotundifoline and isorotundifoline (TLC, GLC, MS). (b) *Elmer 10733*, Mt. Apo, Todaya, Mindanao, Philippines, 6–10. 1909. 430 mg yielded 3 mg (0·7%) of total alkaloid which was separated by prep. TLC (system A) into 3-*iso*-19-*epi*-ajmalicine (TLC, GLC, UV, MS identical with prepared compound—see below). (c) *Kandern*, Goeroepaki, Sulawesi, 12.6.1917. 274 mg yielded 3·7 mg (1·35%) total alkaloid which was separated by prep. TLC (system A). Speciofoline, major alkaloid (TLC, UV, MS). Minor alkaloids, rhynchophylline

and corynoxine B (TLC, GLC) and an unidentified indole alkaloid, hR_f values, A, 32; B, 37; $FeCl_3$–$HClO_4$, grey; Ehrlich's reagent, purple; $Ce(SO_4)_2$–H_2SO_4, yellow; UV λ_{max} 223, 274, 282, 291 nm; MS, *m/e* 347 (M^+, 100; found 347·1626, $C_{21}H_{21}N_3O_2$ requires 347·1634), 346 (87), 332 (21; found 332·1378, $C_{20}H_{18}N_3O_2$ requires 332·1399, M^+-Me), 285 (12), 218 (24), 189 (22), 177 (20), 175 (20), 169 (40; found 169·0765, $C_{11}H_9N_2$ requires 169·0766), 147 (22), 144 (29), 143 (20). *U. orientalis*. (a) *Rutten 1932*, Ruta river, W. Seram, Moluccas, 25:1:1919. 1·9 g yielded 3·7 mg (0·19%) of total alkaloid which contained harmane (TLC) and an Ehrlich^{+vc} streak. (b) *Kalkman 4288* ("*U. attenuata* var. *papuana*"), Star Mts., New Guinea, 20:6:1959. 852 mg yielded 6·1 mg (0·72%) of total alkaloid from which 3-isoajmalicine (TLC, GLC, UV, MS) was separated by prep. TLC (system B). (c) *Ridsdale s.n.* New Guinea, 1968. 8·2 g yielded 126 mg (1·5%) of total alkaloid which was separated by prep. TLC (system C) into isopteropodine, pteropodine, uncarine F, speciophylline (TLC, GLC, UV, MS) and their respective *N*-oxides (TLC, reduction with H_2SO_3 and TLC). 1·0 g of stem bark yielded 10·4 mg (1%) and 3·6 g of stem wood yielded 6·1 mg (0·17%) of total alkaloid. TLC and GLC indicated that the alkaloids of leaf, stem bark and stem wood were identical. (d) *Ridsdale s.n.* Markham bridge, Lae, N.E. New Guinea, 1968. 1·6 g yielded 20 mg (1·27%) of total alkaloid which was separated by prep. TLC (system C) into isopteropodine, pteropodine, uncarine F, speciophylline (TLC, GLC, UV, MS) and their respective *N*-oxides (TLC, reduction with H_2SO_3 [18] and TLC). (e) *Carr 12196*, Koitake, Papua, New Guinea, 6. 1936. 847 mg yielded 5·8 mg (0·68%) total alkaloid which was separated by prep. TLC (system A) into harmane (TLC, UV) and an Ehrlich^{+vc} streak. (f) *Kajewski 620*, Santa Cruz Is., New Hebrides, no date. 1·6 g yielded 16·9 mg (1·0%) of total alkaloid which was separated by prep. TLC (system A) into isomitraphylline, mitraphylline (TLC, GLC, UV, MS) and their respective *N*-oxides (TLC, GLC; reduction with H_2SO_3 and TLC). *U. canescens* ssp. *canescens*. (a) *Put 1173*, Thailand, 6:11:1927. 1·7 g yielded 1·6 mg (0·09%) total alkaloid which contained harmane (TLC) and gave an Ehrlich^{+vc} streak. (b) *Fri 3359*, Pahang State, Malaya, 22:3:1967. 392 mg yielded 7·0 mg (1·8%) which contained harmane (TLC) and gave an Ehrlich^{+vc} streak. (c) *Korthals s.n.*, Sumatra, 6. 1833–7. 1936. 602 mg yielded 2·4 mg (0·4%) of total alkaloid which contained harmane (TLC) and gave an Ehrlich^{+vc} streak. (d) *Kostermans and Anta 144*, Bangka, 26:8;1939. 1·8 g yielded 3·7 mg (0·21%) total alkaloid which contained harmane (TLC) and gave an Ehrlich^{+vc} streak. (e) *Wirawan 83*, S. W. Java, 24:12:1963. 502 mg, alkaloid negative. (f) *P. W. Richards 1276* (*U. ovata* Hook f.), near Dulit Mts., Sarawak, 8.1932. 558 mg yielded 2·2 mg (0·39%) of total alkaloid which contained harmane (TLC) and gave an Ehrlich^{+vc} streak. *U. canescens* ssp. *velutina*. (a) *Elmer 8874*, Luzon, Philippines, 1907. 273 mg, alkaloid negative. (b) *Wenzel 2575*, Philippines, *c.* 1915. 572 mg, yielded 5·4 mg (0·94%) of total alkaloid which was separated by prep. TLC (system A) into isopteropodine, pteropodine, uncarine F, speciophylline (TLC, GLC, UV, MS) and their respective *N*-oxides (TLC).

3-Iso-19-epi-*ajmalicine*. 19-*Epi*-ajmalicine (*ca* 2 mg) was heated at 60° for 2 hr. with glacial HOAc (5 ml) and $Hg(OAc)_2$ (25 mg). Excess Hg^{2+} were removed by thioacetamide treatment and after centrifugation, the supernatant was treated with Zn and conc. HCl (1 drop) for 15 min. After filtering, the soln was made alkaline with NH_4OH and extracted into $CHCl_3$ which was washed, dried (Na_2SO_4) and evaporated to dryness. Prep. TLC (system D) yielded 19-*epi*-ajmalicine (0·9 mg) and 3-*iso*-19-*epi*-ajmalicine (0·2 mg). Both compounds gave identical UV spectra, λ_{max} (EtOH) 224, 240, 274, 282, 290 nm. 19-*Epi*-ajmalicine, hR_f values, A, 78; B, 63; D, 75; R_t 19·5 min. 3-*Iso*-19-*epi*-ajmalicine hR_f values identical with Professor Winterfeldt's synthetic alkaloid supplied by Dr. R. T. Brown, A, 20; B, 39; D, 27; R_t 12·7 min. MS, *m/e* 352 (M^+, 100), 351 (70), 337 (5), 225 (8), 223 (10), 184 (30), 170 (21), 169 (23), 156 (70).

Dihydrocorynantheine pseudoindoxyl. Dihydrocorynantheine (19 mg) was dissolved in DMSO (2 ml) and sodium methoxide (1 ml; 1·1 g Na in 15 ml of dry MeOH) added. O_2 was bubbled through the mixture for 45 min at 50° during which time a red colour developed. Cooled mixture was diluted with H_2O and extracted into EtOAc which was washed, dried (Na_2SO_4) and concentrated. The major component (4·3 mg, 22%) was separated by prep. TLC (system D). hR_f values, A, 43; B, 26; D, 37; $FeCl_3$–$HClO_4$, purple; $Ce(SO_4)_2$–H_2SO_4, yellow. R_t 10·2 min. MS, *m/e* 384 (M^+, 58), 367 (9), 353 (9), 240 (9), 239 (47), 238 (100), 224 (12), 210 (8), 209 (4), 208 (9), 130 (6), 75 (8), 69 (10).

Acknowledgements—We thank the Director of the Rijksherbarium, Leiden, and Dr. C. C. Berg, Department of Botany, State University of Utrecht, for herbarium samples, and Dr. C. E. Ridsdale, Rijksherbarium, Leiden, for additional plant material and for helpful discussions. We are grateful to the following for generously supplying alkaloids: Dr. R. Goutarel, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France (19-*epi*-ajmalicine); Dr. R. T. Brown, Department of Chemistry, University of Manchester (3-*iso*-19-*epi*-ajmalicine); Professor S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University Japan (uncarines A and B); Professor E. J. Shellard and Dr. P. J. Houghton, Chelsea College, University of London (corynoxeine and isocorynoxeine); Dr. W. E. Court, School of Pharmacy, University of Bradford (yohimbine hydrochloride, pseudoyohimbine, α-yohimbine). We thank Mr. D. Carter and Dr. B. J. Millard, Mass Spectrometry Service, The School of Pharmacy, University of London, for determining the mass spectra. We acknowledge the financial support (to S.R.H.) of The Burrough's Scholarship from The Pharmaceutical Society of Great Britain.

REFERENCES

1. Ridsdale, C. E. (1973) personal communication.
2. Ridsdale, C. E. (1972) Ph.D. Thesis, University of Bristol.
3. Haddock, R. E. (1970) Ph.D. Thesis, University of London.
4. Phillipson, J. D. and Shellard, E. J. (1967) *J. Chromatog.* **31**, 427.
5. Djakouré, L., Jarreau, F.-X., Goutarel, R. and Janot, M. M. (1972) *Compt. Rend. Acad. Sci. Paris* **274C**, 1520.
6. Wenkert, E. and Roychaudhuri, D. K. (1956) *J. Org. Chem.* **21**, 1315.
7. Weisenborn, F. L. and Diassi, P. A. (1956) *J. Am. Chem. Soc.* **78**, 2022.
8. Winterfeldt, E., Gaskell, A. J., Korth, T., Radunz, H. E. and Walkowiak, M. (1969) *Chem. Ber.* **102**, 3558.
9. Brown, R. T. and Chapple, C. L. (1974) *J. Chem. Soc. Chem. Commun.* 740.
10. Zarembo, J. E., Douglas, B., Valenta, J. and Weisbach, J. A. (1974) *J. Pharm. Sci.* **63**, 1407.
11. Finch, N., Gemenden, C. W., Hsu, I. H.-C, Kerr, A., Sim, G. A. and Taylor, W. I. (1965) *J. Am. Chem. Soc.* **87**, 2229.
12. Finch, N., Hsu, I. H.-C., Taylor, W. I., Budzikiewicz, H. and Djerassi, C. (1964) *J. Am. Chem. Soc.* **86**, 2620.
13. Merlini, L. and Nasini, G. (1973) personal communication.

14. Thomas, D. W. and Biemann, K. (1968) *Tetrahedron* **24**, 4223.
15. 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.* **24**, 2161.
16. Gilbert, B., Brissolese, J. A., Finch, N., Taylor, W. I., Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 1523.
17. Shellard, E. J. and Houghton, P. J. (1971) *Planta Medica* **20**, 82.
18. Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12**, 1481.
19. Phillipson, J. D. and Hemingway, S. R. (1975) *J. Chromatog.* **105**, 163.