CHAPTER IV

DISCUSSION

1. The alkaloidal content of *Uncaria* species

Uncaria is a large genus comprising of as many as 120 specific names in the Index Kewensis which might be resulted from the difficulties in distinguishing between the species. It was then thought that a knowledge of the alkaloids present might help to distinguish these species and also to confirm identifications made on the basis of morphological and anatomical characters. In view of the recent findings by Phillipson *et al.* (1978), it is considered that alkaloidal content would be a useful character to be included in the parameters to be selected for a taxonomic study of an alkaloid - containing genus. The majority of samples they examined were collected either while flowering or in fruit, therefore, to that extent they are comparable. Among the now recognised seven groups (Ridsdale, 1978), one group in particular, group IV, showed little variation in alkaloidal content between its species or within individual species. Some species from other groups were also relatively constant in their alkaloidal content and in the main, showed little variation, e.g., *U. macrophylla* Wall. and *U. barbata* Merr. (group I). However, their results showed that in particular instances there were constant differences in alkaloid composition between some species of *Uncaria*. In addition, some species

showed considerable variation in their alkaloidal content, e.g., *U. africana* G. Don (group VII), *U. orientalis* Guill. (group I), and *U. sessilifructus* Roxb. (group V). *U. attenuata* Korth. itself is not uniform in its alkaloidal constituents and the alkaloids exhibit features which appear to be rare within the genus. Phillipson *et al.* (1978) reported that it yielded mainly unsubstituted tetracyclic heteroyohimbines and oxindoles but some samples contained alkaloids with 9-hydroxy substitution (e.g. speciofoline), and some of the pentacyclic alkaloids possessed C(19) - CH_3 substituents with β configuration. These differences reveal that caution must be taken when taxonomic proposals are being made from chemical observations and clearly show that as wide a range of samples as possible should be examined.

Heteroyohimbine, both with closed and open E rings, and the corresponding oxindole are the major groups of alkaloid present in *Uncaria* species. They occur both in the form of parent bases and also together with their N-oxides. The minor groups include pyridino-indolo-quinolizidinone, roxburghine, β-carboline, yohimbine, yohimbine oxindole and gambirtannine. Geissoschizine methyl ether and dihydrocorynantheine pseudoindoxyl were separately reported from *U. rhynchophylla* (Miq.) Miq. ex Havil. and *U. attenuata* Korth. respectively.

Heteroyohimbines and oxindoles reportedly present in *Uncaria* species are mostly unsubstituted ones. The 9-hydroxy substituted alkaloids are rarely found, those reported so far are gambirine, rotundifoline, isorotundifoline and speciofoline. There are only four species reported as having the 9-hydroxy substituted alkaloids, i.e.

Uncaria attenuata Korth., *U. callophylla* Bl. ex Korth., *U. elliptica* R. Br. ex G. Don and *U. tomentosa* (Willd.) DC.

No 9-methoxy substituted alkaloid was reportedly present in this genus. The only literature stated the presence of one 9-methoxy substituted *allo* open E ring heteroyohimbine with ethyl group as R', mitragynine, was in the Alkaloid - Bearing Plants and Their Contained Alkaloids by Willaman and Schubert (1961) who referred to a book named Chemistry of alkaloids written in 1955 by A.P. Orekhov. By the time that book was written the separation and characterisation technique together with the knowledge of configuration and conformation of individual alkaloids were not well achieved. He might have taken by misidentification or misunderstanding as being mitragynine. This alkaloid was first reported as being found in *Mitragyna speciosa* Korth. and named in 1921, although Hooper had actually isolated it in 1904 as "an alkaloid" from "Kratom". Following this quite an amount of work on *Mitragyna* species has been undertaken especially from 1964 up to the present time. All ten species of this genus have been investigated throughly using modern techniques of separation and characterisation. Many plant parts were examined, collected in different season and ages and also at monthly intervals throughout a year. The results reveal that this particular alkaloid, mitragynine, is very specific for one species, *Mitragyna speciosa* Korth., where it occurs as major alkaloid and can be considered as characteristic of this species.

Within the last ten years, many workers have independently reported the presence of alkaloids in *Uncaria* species. None of them has stated mitragynine as being found. The most recent investigation reported

in 1978 by Phillipson *et al.* who investigated 400 samples of plant specimens from a wide geographical range absolutely confirmed this situation.

Willaman and Schubert (1961) reported that the stem of *Uncaria formosana* (Matsum.) Hayata contained mitragynine, gambirine, mitraphylline, uncarine B, rhynchophylline, rotundifoline and isorotundifoline. Saxton (1960) reported only uncarine B in the stem. Although no recent investigation has been reported regarding the alkaloidal content of the stem, the leaves and flowers were separately examined and reported by Phillipson *et al.*, in 1978. They found isomitraphylline and its N-oxide, mitraphylline and its N-oxide, uncarines A and B in the leaves. The same oxindoles without their N-oxides were detected in the flowers with the addition of the corresponding *pseudo* heteroyohombine, 3-isoajmalicine. In their investigation on *Uncaria* species, they noticed that the differences in alkaloidal content noted between leaves, stem, fruits, and flowers of samples taken from the same plant at the same time were slight and usually quantitative rather than qualitative. Hence, it might be assumed that the leaves of the plant they examined would have similar alkaloidal content and that no mitragynine would be found.

Willaman and Schubert (1961) also reported the presence of mitragynine (9-methoxy substituted), rotundifoline, isorotundifoline (both are 9-hydroxy subsituted), and mitraversine in the stem of *U. rhynchophylla* (Miq.) Miq. ex Havil. Phillipson *et al.* (1978) examined the stems and roots of the same species and found only

unsubstituted alkaloids, i.e. dihydrocorynantheine, corynantheine, hirsutine, hirsuteine, isorhynchophylline, rhynchophylline, isocorynoxeine and corynoxeine. In addition, Aimi *et al.* (1972, 1977) reported the alkaloidal content of the whole plant of this species and no such alkaloids as reported by Willaman and Schubert were included. In contrast, they were the same alkaloids as reported by Phillipson *et al.*, excluding isorhynchophylline and rhynchophylline but having akuammigine and geissoschizine methyl ether, also unsubstituted alkaloids, as additional constituents. The leaves of this plant have also been investigated by many workers from 1958 to 1978 but no mitragynine was revealed. The alkaloids reportedly present in the leaves are isorhynchophylline, rhynchophylline and its N-oxide, isocorynoxeine, corynoxeine, angustine, angustoline and angustidine.

The pentacyclic heteroyohimbine and oxindole alkaloids having the C(19) - CH_3 β configuration are present in some *Uncaria* species although in relatively minor proportions compared with their C(19) - CH_3 α analogues. Seven species are known to contain such alkaloids, i.e. *U. attenuata* Korth., *U. orientalis* Guill., *U. gambir* (Hunt.) Roxb., *U. sessilifructus* Roxb., *U. laevigata* Wall. ex G. Don, *U. hirsuta* Havil. and *U. africana* G. Don ssp. *africana* G. Don. The C(19) - CH_3 β heteroyohimbines previously reported were 19-epi-ajmalicine and 19-epi-3-isoajmallcine. The oxindole with such configurations found in *Uncaria* were uncarines A and B.

In *U. attenuata* Korth. itself there is a broad variation in the alkaloidal content. Phillipson and Hemingway (1975b) examined eight samples of this species and found that the heteroyohimbines in those

samples varied from closed E ring, i.e. 3-isoajmalicine, 19-epi-3-iso-ajmalicine and akuammigine to the open E ring isomers, i.e. dihydrocorynantheine, hirsutine, hirsuteine and corynantheine. Four samples were found to have only one heteroyohimbine while the other two contained three and another sample yielded two heteroyohimbines. In one last sample, no heteroyohimbine was found.

For the oxindoles, only two samples were reported to have the closed E ring isomers. There was speciophylline in one sample and isomitraphylline and its N-oxide, mitraphylline and its N-oxide, and uncarines A and B in another. The other six samples contained open E ring isomers and they were isorhynchophylline and its N-oxide, rhynchophylline and its N-oxide, isocorynoxeine, corynoxeine, rotundifoline, isorotundifoline, corynoxine B and speciofoline. Harmane, dihydrocorynantheine pseudoindoxyl and yohimbine isomers were found to be separately present in three samples.

They further investigated nine other samples but no other alkaloids than those previously reported were found (Phillipson *et al.*, 1978). Six of these nine samples contained only oxindoles while both oxindoles and heteroyohimbines were found in the other three samples. Where stems were also examined, the same alkaloids as in the leaves were reported.

This present investigation reveals further interesting variation in *Uncaria attenuata* Korth. The *allo* closed E ring unsubstituted heteroyohimbine with C(19) - CH_3 α, i.e. tetrahydroalstonine which has never been found in this species before was isolated. In addition, its

C(19) - CH_3 β analogue, rauniticine, which has only been reported from *Rauvolfia nitida* Jacq. of Apocynaceae in 1961 by Salkin *et al.* and not from any species of *Uncaria* before was obtained as major alkaloid. Furthermore, a totally new heteroyohimbine was isolated out and characterised as 14-hydroxy-3-isorauniticine, the *epiallo* isomer with C(14) - hydroxy substituent. This alkaloid has not previously been reported or obtained anywhere either naturally or synthetically.

Two oxindoles (TS_4, TS_5) were obtained in small quantities resulting in the impossibility of full identification. Only their hR_f values on several systems and mass spectra could be determined. The colours developed with ferric chloride in perchloric acid reagent shown them to be oxindoles. The mass spectra of both TS_4 and TS_5 show molecular molecular ion peaks at 368. From hR_f values, TS_5 seems to have similar values as those of authentic isopteropodine which also has molecular weight of 368. The hR_f values of TS_4 do not correspond with any of the known oxindoles. The mass spectra of both compounds revealed high percentage relative abundance of peaks at *m/e* 222 suggesting them to be pseudoindoxyl type of oxindoles (Phillipson and Hemingway, 1975b). TS_4 yielded peaks at *m/e* 368 (100 %), 223 (73 %) and 222 (40 %) while that of TS_5 also showed peaks at the same mass units, with only slight differences in percentage relative abundance, i.e. *m/e* 368 (100 %), 223 (88 %) and 222 (72 %). From their molecular weight, they might be pseudoindoxyls of unsubstituted heteroyohimbines of ajmalicine - type. Phillipson and Hemingway (1975b) characterised a new oxindole as dihydrocorynantheine pseudoindoxyl in the sample where dihydrocorynantheine

was found as major alkaloid. There is the possibility that one of the oxindoles obtained is the pseudoindoxyl of rauniticine which is the major alkaloid in the sample of *Uncaria attenuata* Korth. Further large scale extraction is required to obtain these alkaloids in sufficient amount to be fully characterised. The oxidation of the heteroyohimbines to their pseudoindoxyls to compare would confirm the identity of the naturally occurring ones.

The third oxindole was observed in one fraction (T_a) and the amount was even smaller than those of the two previously mentioned ones. The hR_f values of this alkaloid do not correspond with any of the known *Uncaria* and *Mitragyna* alkaloids so far compared. The same situation with that of the two pseudoindoxyls is encountered, i.e. large scale extraction is needed.

This present findings reveal new point in alkaloidal content of this particular species. It was previously known to contain *pseudo* closed E ring heteroyohimbines, both with C(19) - CH_3 α and β configurations and the *epiallo* isomer with only C(19) - CH_3 α. Also reportedly present were the *normal* and *pseudo* open E ring heteroyohimbines, together with the isomer with vinyl analogue of the latter, and the *epiallo* isomer with vinyl as R'. In this investigation, *allo* closed E ring analogous to the reported *epiallo* isomer and its C(19) - CH_3 β analogue were isolated and identified. However, no heteroyohimbine with open E ring was found. The overall pattern of the heteroyohimbines in this species so far reported could then be represented as follows :-

Closed E ring

	C(19) - CH_3 α	C(19) - CH_3 β
normal	-	-
pseudo	3-isoajmalicine	19-epi-3-isoajmalicine
allo	tetrahydroalstonine	rauniticine
epiallo	akuammigine	-

This sample of *Uncaria attenuate* Korth. exhibits different feature from those of the other samples of the same species and also of the majority of both *Uncaria* and the closely related genus *Mitragyna*. With the exception of *M. speciosa* Korth. which contains mitragynine as major alkaloid, most of the species of these two genera contain oxindoles as dominant alkaloids and heteroyohimbines are present only as minor constituents both qualitatively and quantitatively. In some samples, there were even shown to contain no heteroyohimbine at all. In contrast, in this sample heteroyohimbines were found as being major constituents although mature leaves were examined and also collected while flowering.

Considering the alkaloidal content of this sample of *Uncaria attenuata* Korth. as compare to that of *U. salaccensis* Bakh. f. nom provis, also from Thailand, it will be seen that they are similar in one aspect and that is both alkaloids with C(19) - CH_3 α and β configurations are present together, although the types of alkaloids are opposite, i.e. heteroyohimbine in the former and oxindole in the latter. *Uncaria* species of Thailand so far reported, i.e. *U. homomalla* Miq., *U. quadrangularis* Geddes and *U. salaccensis* Bakh. f. nom provis yielded

only oxindoles. Only one recent examination of the leaves of *Uncaria homomalla* Miq. collected regularly at monthly intervals throughout a year revealed the presence of tetrahydroalstonine in some month samples (Vitayanatpaisan, 1979). However one sample of this particular species, from Sumatra, Indonesia was reported to have 3-isoajmalicine, *pseudo* heteroyohimbine, in the leaves while other samples were found to contain only oxindole alkaloids.

2. The structure of the new naturally occurring heteroyohimbine alkaloid

The totally new naturally occurring heteroyohimbine alkaloid characterised as 14-hydroxy-3-isorauniticine had its structure elucidated from the CD, ultraviolet, infrared, nuclear magnetic resonance including the ^{13}C-nuclear magnetic resonance and mass spectra.

The arguments for the elucidation of the structure of TS_3, characterised as 14-hydroxy-3-isorauniticine are as follows :-

The ultraviolet spectrum of TS_3 (in methyl alcohol, λ_{max} 244, 270, 279, 290 nm; in methyl alcohol and hydrochloric acid, λ_{max} 229, 270, 280, 290 nm; reversible with alkali) indicates that the alkaloid is of heteroyohimbine type with ester vinyl ether system.

The mass spectrum of TS_3 shows typical fragmentations for tetrahydro-β-carboline with strong M^+-1 peak which is due to the loss of the C(3) - hydrogen and is characteristic for aromatic - unsubstituted closed E ring alkaloids of the ajmalicine-type. It loses 18 mass units, i.e. H_2O, hence does not seem to be an N-oxide but to possess a secondary alcohol function. The strong molecular ion at 368 mass units suggest it to be the alkaloid of ajmalicine-type with additional hydroxyl group instead of one of the hydrogen atoms in the basic structure. The

N.M.R. spectrum having three-proton doublet at δ 1.42 and one-proton quartet at δ 4.40 showing the presence of one methyl group in adjacent to one methylidyne group, hence further confirm the possibility of closed E ring as that of ajmallcine - type alkaloids which has molecular weight of 352. The hydroxyl group giving the additional 16 mass units does not seem to be in the aromatic ring A otherwise the fragments in the mass spectrum would be *m/e* 186, 199, 200, 214 and 255 instead of those seen at *m/e* 156, 169, 170, 184 and 225 which represent the aromatic - unsubstituted indole nucleus. The accurate mass measurements yield $C_{21}H_{24}N_2O_4$.

The infrared spectrum shows an imino group at 3440 cm^{-1}. There are strong Bohlmann bands at 2850, 2810 and 2750 cm^{-1} and the major methylidyne group absorption band at 2920 cm^{-1} suggesting TS_3 to have C(3) - H *trans* to the N (b) and that, therefore, the C/D ring junction is *trans*. The 1665 cm^{-1} band is a low value for ester carbonyl which might be due to the mentioned group bonded to a hydrogen atom. The C(16) - C(17) double bond is represented by the band at 1620 cm^{-1}. In addition, there is a broad band at 3350 cm^{-1} indicating the presence of a hydroxyl group.

The CD curve shows negative Cotton effect at 285 nm, therefore the C(3) - H configuration in TS_3 is β. The spectrum is identical with that of 3-isorauniticine prepared from the isolated rauniticine (TS_2), hence the C(19) - H is likely to be α as in 3-isorauniticine.

In the mass spectrum of TS_3, the relative abundance of the fragment ions are in agreement with being *allo* or *epiallo* isomers, i.e.

that of *m/e* 184 is of lower abundance than the *m/e* 169 and 170 ions and that of *m/e* 209 is also lower than *m/e* 223 (Beckett *et al.*, 1969).

In the N.M.R. spectrum, there is a three-proton doublet at δ 1.42, exactly the same value quoted for those alkaloids having *cis* D/E rings and *epiallo* configurations (Shamma and Moss, 1962). This is the signal for C(18) - methyl β. The signal at δ 4.40 indicates the C(19) - H / C(20) - H *cis*. The suggest that there is a methyl group adjacent to methylidyne group is confirmed by the double decoupling. Irradiation at δ 4.39 led to the collapse of doublet at δ 1.42 to give a singlet and irradiation at δ 1.35 led to the collapse of quartet at δ 4.40 and gave a doublet. In addition, other signals indicate four aromatic protons, i.e. four-proton multiplet at δ 7.25; imino of indole nucleus, i.e. well downfield at δ 9.35 as one-proton broad singlet which disappears on deuteration; methoxyl at δ 3.80 as three-proton singlet and olefinic proton at δ 7.75 as one-proton singlet. There is one-proton broad singlet signal at δ 5.95 which is considered to be the signal for hydroxyl group.

The position of the additional hydroxyl group is ascertained by the ^{13}C-N.M.R. spectrum from which each carbon atom is assigned by the presence of its signal. The δ values of TS_3 were compared with those of ajmallicine - type alkaloids reported by Wenkert *et al.* (1976). In the spectrum of TS_3, there is no signal at δ 30 - 40 for the C(14) which should appear as a triplet. However, there is a doublet signal instead at δ 76, i.e. deshield, therefore downfield and adjacent to one and not two protons. Furthermore, the signal for C(3) - H appear

as doublet at δ 65 instead of between δ 52 and 60, the C(15) - H signal is at δ 38 instead of δ 24 - 30 and the carbonyl signal is at δ 176 instead of δ 167. This establishes the position of hydroxyl group as being at C(14), having the β equatorial configuration. This is also supported by the use of Dreiding models.

The structure and conformation of this new alkaloid, 14-hydroxy-3-isorauniticine, can thus be assigned as shown below :-