# CHAPTER IV

# DISCUSSION

## Spectral Properties of the Isolated Alkaloids

In the view of chemotaxonomy nearly all alkaloids isolated from *Mitragyna* species are of heteroyohimbine-type and the corresponding oxindole. This present work has led to the isolation of 9 alkaloids from the fresh leaves of *Mitragyna speciosa* (Korth.) Havil. All of them are known alkaloids which can be divided into two main groups, the heteroyohimbine and oxindole alkaloids. These are distinguished by the color produced with ferric chloride in perchloric acid spray reagent on TLC plate (Houghton and Shellard, 1974). This premise is further supported by the IR spectrum which shows a strong -NH absorption in both heteroyohimbines and oxindoles, and addition of two carbonyl absorptions (ester and amide) for oxindoles while only one (ester) is found in the heteroyohimbine group. The signal of $^{13}$C-NMR spectrum at 180-181 ppm (amide) also indicates the existence of oxindole nucleus.

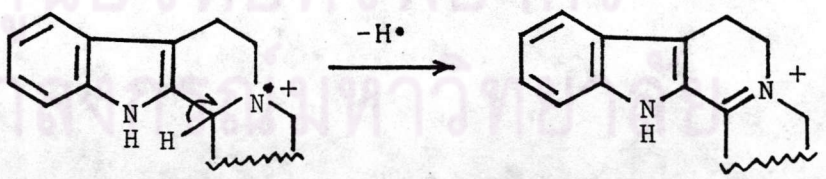
Six of the nine isolated alkaloids are heteroyohimbines, four of which are open E ring alkaloids, namely mitragynine, paynantheine, speciogynine, and mitraciliatine. The other two heteroyohimbines belong to

the closed E ring alkaloids, namely tetrahydroalstonine and ajmalicine. The remaining three being closed E ring oxindoles identified as isopteropodine and the two C(7) stereomers, mitraphylline and isomitraphylline. In the elucidation of the stereochemistry about C(7) in the oxindole alkaloids the chemical shifts of the C(9)-H and of the C(14)-Hβ are of importance (Crabb, 1978). Thus in the 500 MHz $^{1}$H-NMR spectrum of isomitraphylline (*normal* A) the C(9)-H absorbs to high frequency (7.35 ppm) of that in the *normal* B isomer, mitraphylline (7.19 ppm) as a result of its proximity to the N(4) atom in the oxindole A. In isomitraphylline, the signal of C(14)-Hβ appears at very upfield (0.61 ppm) as the result of sheilding by the aromatic system. This effect clearly contrasts to that of mitraphylline in which the C(14)-Hβ absorbs at 1.20 ppm. The similar pattern has also been noted in the spectrum of isopteropodine (*allo* A) in which signal of the C(14)-Hβ appears at 0.86 ppm.

The important evidence used to distinguish the isolated closed E ring heteroyohimbines and oxindoles is provided by mass spectral fragmentation patterns. The most intense ion in the spectra of mitraphylline and isomitraphylline occur at *m/e* 223 (base peak) and therefore must constitute the alicyclic portion of these alkaloids. Its genesis may be visualized through homolytic rupture of the C(5)-C(6) and C(3)-C(7) bonds,

yielding the neutral species and positive ion of *m/e* 223. This positive ion can be decomposed further in several ways. There are three important ions in the mass spectra of the closed E ring oxindoles (mitraphylline, isomitraphylline, and isopteropodine) arise from the cleavages as shown in Figure 16 (Shamma and Foley, 1967). However, no reliable assignment of stereochemistry can be made on the basis of the intensities of the peaks for the molecular ion, or for those at *m/e* 223, 208, and 69.

In the closed E ring heteroyohimbine, ajmalicine, one of the chief diagnostic features of its mass spectrum is a pronounced $M^+$-1 peak. This is predominantly due to the loss of the hydrogen atom attached to C(3) with formation of positive ion of *m/e* 251 (67.45 %) in which the positive charge is stabilized by conjugation with the aromatic system as well as by participation of the electron pair on N(4) (Budzikiewicz, Djerassi and Williams, 1964).




$M^+$ (*m/e* 252) $M^+$-1 (*m/e* 251)

This fragmentation pattern also extends to those of isolated open E ring heteroyohimbine alkaloids as shown

in mitragynine (*allo*) and speciogynine (*normal*) in which their relative abundances of $M^+$-1 peak (*m/e* 397) are of 79.07 % and 82.02 %, respectively.

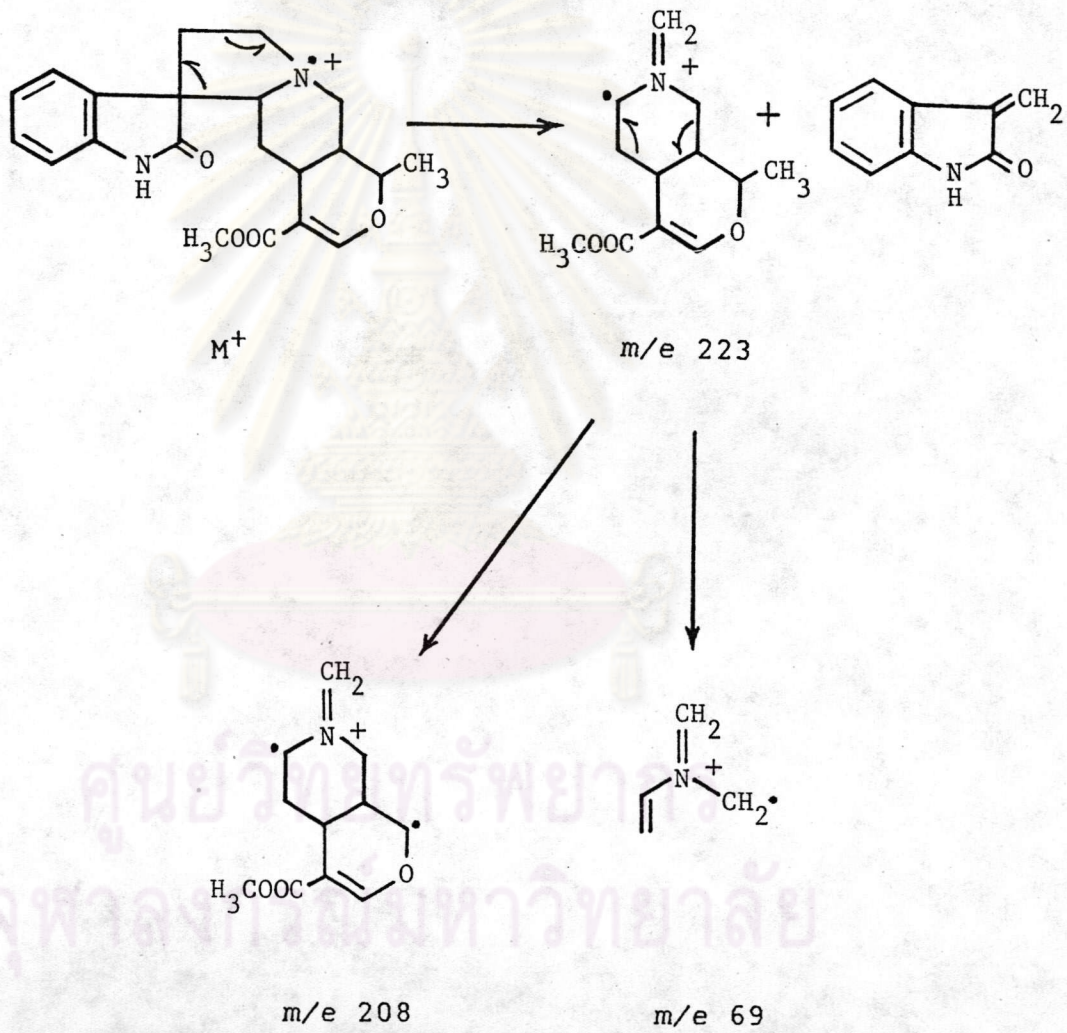



Figure 16 The cleavages of the closed E ring oxindole alkaloids

The increased resolution of the 500 MHz $^1$H-NMR leads to more complete proton assignment of 9 isolated alkaloids. For each 24 protons of tetrahydroalstonine, ajmalicine, mitraphylline, isomitraphylline, and isopteropodine the identification of 13 out of 24 protons, viz. C(9)-H, C910)-H, C(11)-H, C(12)-H, C(17)-H, C(18)-$CH_3$, C(19)-H, C(23)-$OCH_3$, and -NH is straight forward. The assignments of the 11 remaining protons are obtained by the aids of decoupling method and two dimensional NMR (COSY: proton-proton correlation spectroscopy). The coupling constants found and the dihedral angles in different H-C-C-H systems measured with the aids of Dreiding models for different configuration, clearly support with the previous stereochemical suggestions (Wenkert *et al.*, 1976; Lounasmaa and Kan, 1980; Martin, Sanduja and Alam, 1986).

All four of isolated open E ring heteroyohimbine alkaloids are of isomers with C(9)-methoxy. Three of which, mitragynine (*allo*), speciogynine (*normal*), and mitraciliatine (*pseudo*) are identical in UV absorption and mass spectral fragmentation pattern. For each of 30 protons of these three alkaloids the identification of 17 out of 30 protons, viz. C(9)-$OCH_3$, C(10)-H, C(11)-H, C(12)-H, C(17)-H, C(17)-$OCH_3$, C(18)-$CH_3$, C(23)-$OCH_3$, and -NH can be easily resolved by 500 MHz $^1$H-NMR. These 9 signals (17 protons) of mitragynine, speciogynine, and mitraciliatine are identical. Their differences have been

noted in the upfield signals of $^{1}$H-NMR which referred to their configurations. In $^{13}$C-NMR spectra, the chemical shift of C(3) and C(6) can be used to supported the configurational assignment. For the *normal* and *allo* heteroyohimbine alkaloids, the chemical shifts of C(3) and C(6) are 59-61 and 21-22 ppm, respectively (Wenkert et al., 1976). This result is shown exactly in those of ajmalicine which chemical shifts of C(3) and C(6) are 60.37 and 21.69 ppm, respectively, and shown with a negligible variation in mitragynine (61.28 and 23.95 ppm for C(3) and C(6), respectively).

The remaining one is paynantheine (*normal*). It's $^{1}$H-NMR spectrum is similar to that of mitragynine except that there is no three-proton triplet in the region of 0.87 ppm indicating the absence of a C(18)-methyl group in the E *seco* ring. The C(19)-H, C(18)-H (*trans*), and C(18)-H (*cis*) of paynantheine reveal $^{1}$H-NMR signals of characteristic field position and multiplicity at chemical shifts 5.58 ppm (ddd, J= 17.6, 10.2, 3.0 Hz), 5.00 ppm (dd, J= 17.3, 2.0 Hz), and 4.95 ppm (dd, J= 10.2, 2.0 Hz), respectively. This result indicates the presence of the $-C=CH_2$ (vinyl group) at C(20).

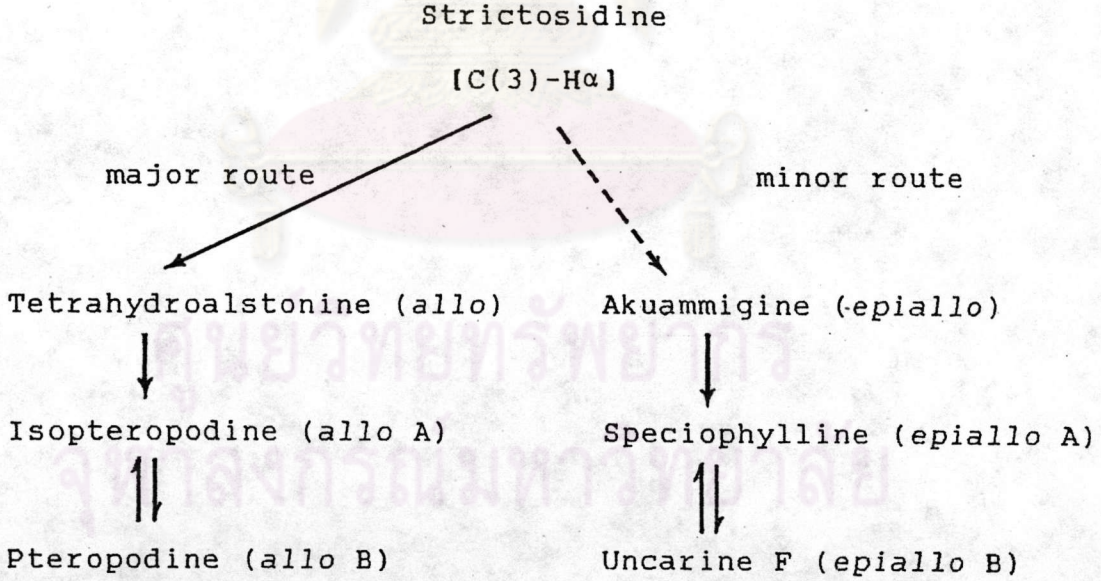
## Relationship of Alkaloids in the Plant

Based on the previous work of *Mitragyna* alkaloids there appears to be so close a relationship between the configuration of the indole and oxindole alkaloids when both are present in any *Mitragyna* species. Since there is no evidence of interconversion between the substituted and unsubstituted indole alkaloids in the plant (Shellard and Houghton, 1974a), there are 8 possible biosynthetic routes for the alkaloids found in species of *Mitragyna* as shown below :-

| Series | Open E ring | Closed E ring |
|---|---|---|
| *normal-pseudo* | 1. C(9)-H | 5. C(9)-H |
| | 2. C(9)-$OCH_3$ | 6. C(9)-$OCH_3$ |
| *allo-epiallo* | 3. C(9)-H | 7. C(9)-H |
| | 4. C(9)-$OCH_3$ | 8. C(9)-$OCH_3$ |

Only the *allo-epiallo* closed E ring C(9)-$OCH_3$ series (No. 8) have not been found in *Mitragyna* species. In *Mitragyna speciosa* (Korth.) Havil. alkaloids from six of them have been isolated, and series 3 and 4 (the *allo-epiallo* open E ring) alkaloids dominate the species. Apart from the series 8, it is series 6 (*normal-pseudo* C(9)-$OCH_3$ closed E ring) alkaloids which are not present (Shellard, Houghton and Resha, 1978b).

It is important to note that one of these series, the *allo-epiallo* closed E ring C(9)-H series (No. 7) is formerly considered to be doubtful in *Mitragyna speciosa* (Korth.) Havil. because of the lack of supporting evidence. Only traces of two *epiallo* alkaloids, akuammigine and its corresponding oxindole speciophylline have been isolated in previous work (Shellard, Houghton and Resha, 1978b,c). In the light of experimental evidence from this present work on isolation of the *allo* C(9)-H closed E ring, tetrahydroalstonine and its corresponding oxindole isopteropodine, the mentioned proposal is fully supported. It can be indicated diagrammatically as follows :-




However, until now the B oxindoles, pteropodine and uncarine F, have not yet been isolated from *Mitragyna speciosa* (Korth.) Havil. The absence of pteropodine in

this present work would depend upon the extent of isopteropodine-pteropodine interconversion.

It is probable that the absence of isopteropodine and tetrahydroalstonine in previous work which has been done using dried plant materials involves the specific enzyme system which still effected during drying process, but not in sudden inhibition by blending the fresh leaves with methanol in this investigation. Examination of thirteen monthly samples of the fresh leaves of the mature plant of this particular plant may reveal further evidence supporting the presence of tetrahydroalstonine and isopteropodine in the present work.

Another interesting point to note is that no substantial quantity of open E ring oxindoles has been isolated in this present work, while large amounts of closed E ring oxindoles, isomitraphylline (*normal* A), mitraphylline (*normal* B), and isopteropodine (*allo* A) have been isolated. This is in contrast with those reported by Shellard, Houghton and Resha (1978b) where the open E ring oxindoles corynoxine, mitrafoline, mitragynine oxindole B, and speciofoline are dominant oxindoles in this species. However, the isolation of ajmalicine, the *normal* C(9)-H closed E ring in this present work is a reasonable evidence supporting the presence of isomitraphylline and mitraphylline.

Further point to note is that like the previous work (Beckett, Shellard and Tackie, 1965; Shellard, Houghton and Resha, 1978b) mitragynine (*allo*) is obtained as the dominant alkaloid. The presence of mitragynine is also considered exclusive to *Mitragyna speciosa* (Korth.) Havil.(Shellard, 1974).

From the present work it is marked that the dominant alkaloids in mature plants of this species are those having C(3)-Hα, i.e. *normal* and *allo*. This result is in agreement with those reported by Shellard, Houghton and Resha (1978b). However, in the young plants (two years old) of this species the major indole alkaloids are those possessing C(3)-Hβ (Shellard, Houghton and Resha, 1978c). This has later been proposed by Shellard and his co-workers that in the young plant mitragynine (*allo*) is almost entirely converted to mitragynine oxindoles A and B of which is transformed to the *epiallo* heteroyohimbine, speciociliatine. While in the mature plants need a reservior of mitragynine, so there is no emphasis on the enzymatic conversion of mitragynine to the corresponding oxindoles (Shellard, Houghton and Resha, 1978c). This suggestion is a probable explanation for the absence of the open E ring oxindoles in this present work.

At this point the experimental proof indicates that variation in the quantities of the individual alkaloids present and their presence or absence amongst

different samples would depend upon the extent of the indole-oxindole conversion or upon the utilization of the alkaloids in the plants' metabolic processes in various stages of plant growth.