CHAPTER IV

DISCUSSION

The Alkaloidal Patterns of *Gelsemium*, *Mostuea* and *Gardneria*

All of the alkaloids obtained from the species of *Gelsemium* can be divided into two main groups, the indole and oxindole alkaloids. In the indole group, there are three types of structures, the β-carboline anhydronium base sempervirine- , koumine- , and sarpagine-types, but in the indole group of *Gardneria* alkaloids only one type, tetrahydro-β-carboline-type, exists. Basic structure of sarpagine-type *Gelsemium* alkaloids is the same as that of tetrahydro-β-carboline-type *Gardneria* alkaloids. The differences are that all of *Gardneria* tetrahydro-β-carboline-type alkaloids possess methoxy substitutions on the aromatic ring at C(11), and some of them possess ether linkage between C(6) and C(17). These are not found in the sarpagine-type of the *Gelsemium* alkaloids. Configuration at C(19) of the *Gardneria* tetrahydro-β-carboline-type alkaloids is *E*, while those of the *Gelsemium* sarpagine-type alkaloids can be either *E* or *Z*.

Comparison between the oxindole group, those of the *Gardneria* alkaloids possess bonds between C(3) and N(b), while these bonds of the *Gelsemium* alkaloids are broken, and form ether linkage between C(3) and C(17).

There are three methoxy groups substitute at C(9), C(10) and C(12) on the aromatic ring and also substitutions at C(16) in the *Gardneria* oxindole alkaloids. In the *Gelsemium* oxindole alkaloids , there are methoxy substitutions at C(11) on the aromatic ring and an unusual feature in some of the compounds, i.e. the presence of N(a)-methoxy function. Alkaloid of imino-ether group has not been found in *Gelsemium* species. Besides mostueine, the rest of *Mostuea* alkaloids are those found in *Gelsemium*.

According to Sakai (1976) the plants in the genus *Gardneria* seem to be divided morphologically into two groups, A and B (A consists of *Gardneria nutans* Sieb. et Zucc. and *G. insularis* Nakai; B consists of *Gardneria multiflora* Makino, *G. shimadai* Hayata and *G. liukiuensis* Hatsushima). The plants of the group A bear one to three flowers separately on top of a flower system, while those of the group B bear flowers congestedly (three to ten). It is interesting to note that a distinct difference exists in the alkaloidal constituents of the two groups. The constituents of the plants of the group A possess the indole and the imino-ether group, and no oxindole or dimeric alkaloids be found in this group of plants. The tetrahydro-β-carboline-type indoles possessing a methoxy group at C(11) on their aromatic rings are characteristic to these plants of the group A. The constituents of the plants of the group B are the oxindole and the imino-ether alkaloids, no tetrahydro-β-carboline-type indole alkaloid has been found in this group.

The variable types of *Gelsemium* alkaloids can be indicated by colors produced with ferric chloride in perchloric acid spray reagent on TLC. The sarpagine-type alkaloids show olive green to grey spots while that of koumine show yellowish-brown spot. Humantenine-type alkaloids give yellowish-pink spot while gelsemine- and gelsedine-types produce pink and purple spots, respectively.

According to Chi, Lee and Lee (1938), leaves of *Gelsemium elegans* Benth. contained the highest quantity of crude alkaloid when compared with its stems and roots. They found that the yields of crude alkaloids from different parts of the plant were : root = 29 g (0.41 %) , stems = 20 g (0.29 %), leaves = 30 g (0.43 %) (7 kg of the material being used for the extraction in all three cases). From this investigation, 21.41 g of crude alkaloidal extract was obtained from 4.7 kg of dried leaves resulting in 0.46 % yield. The value corresponds well with that previously reported. The crude alkaloidal content in the stems of *Gelsemium sempervirens* (L.) Jaume St.-Hilaire was 1.17 % wt/wt (Schun and Cordell, 1985 a) and in the stems of *G. rankinii* Small was 0.83 % wt/wt (Schun *et al.*, 1986). Thus, the stems of *G. sempervirens* (L.) Jaume St.-Hilaire contains the highest quantity of crude alkaloidal content and that of *G. elegans* Benth. contains the lowest quantity. The main alkaloid in the stems of *G. sempervirens* (L.) Jaume St.-Hilaire and *G. elegans* Benth. are gelsemine but that of *G. rankinii* Small is gelsevirine (Schwarz and Marion, 1953; Schun *et al.*, 1986).

The Patterns of the New Naturally Occurring Alkaloids

1. GE-1

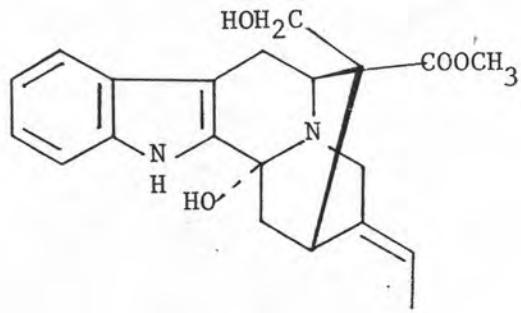
The color produced with ferric chloride in perchloric acid spray reagent on TLC plate shows that the nucleus of GE-1 is of indole group. This is confirmed by the pattern of absorption in UV spectrum which shows λ_{max} at 226 and 282 nm and λ_{min} at 246 nm. The spectrum indicated that the chromophore of GE-1 is indole nucleus with no substitution on the aromatic ring (Verpoorte, 1986). From the IR spectrum, GE-1 exhibits hydroxy group at 3380 cm^{-1}, carbonyl group at 1725 cm^{-1}, methylene and methyl groups at 1455 cm^{-1}, and 1440 cm^{-1}, respectively, also ester group at 1100 and 1025 cm^{-1}.

In the ^{1}H-NMR spectrum, GE-1 shows aromatic protons at 7.04-6.86 ppm and proton of nitrogen at 8.22 ppm. Three protons at 3.69 ppm and 1.57 ppm are the protons of the methoxy and methyl groups, respectively. Two low field protons at 4.18 and 3.28 ppm are the protons of alcohol group, and these two protons must be attached to the same carbon, since they possess high value of coupling constant.

The pattern of fragmentations of GE-1 in mass spectrum almost corresponds to that of voacarpine. It produces peaks at *m/e* 351, 338, 309 309, 265, 185, 184 and 130 as does voacarpine, and the molecular ion peaks are at the same position. However, the base peak of GE-1 is at *m/e* 184 while that of voacarpine shows at *m/e* 368, which is the molecular ion peak of the compound (Denayer-Tournay *et al.*, 1965). The peak at *m/e* 337 in

voacarpine possesses relative abundance of about 26.5 % and the peak at *m/e* 338 possesses low relative abundance. In contrast, these are altered in GE-1 : the former peak disappeared and the latter peak possesses relative abundance of about 28 %. From these informations, GE-1 may be an isomer, probably the 16-epimer, of voacarpine.

From the ^{1}H-NMR, the chemical shift of C(5)-H of GE-1 (4.44 ppm) is higher than that of akuammidine (3.08 ppm) and the value is near to that of polyneuridine (4.32 ppm), the 16-epimer of akuammidine. In akuammidine, the chemical shift of the proton is shielded by the methoxycarbonyl group (Lounassma *et al.*, 1985). From the chemical shift of C(5)-H in GE-1 and other mentioned informations, it can be concluded that GE-1 is 16-epi-voacarpine, the structure of which is shown below:-



2. GE-2

With ferric chloride in perchloric acid spray reagent on TLC plate, GE-2 gave the same color as gelsenicine. UV spectrum shows absorption at λ_{max} 208.6 and 255.2 nm and λ_{min} at 242.4 nm. These indicated that the chromophore of GE-2 is oxindole and the structural nucleus is the same as that of gelsenicine.

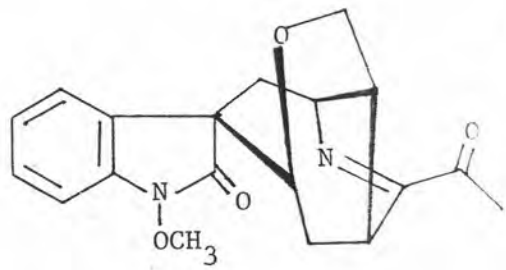
The IR spectrum shows two carbonyl groups at 1725 cm^{-1} and 1695 cm^{-1}, one is the carbonyl group of the oxindole nucleus and the other is located on the other side of the molecule. The methylene group is noted at 1460 cm^{-1} as a broad peak in which the methyl group may be hidden.

Coupling pattern of variable protons in ^{1}H-NMR spectrum is very similar to those of gelsenicine, except no coupling pattern of ethyl group is observed in the spectrum of GE-2 and chemical shift of the protons of methyl group is observed at lower field (2.66 ppm) than that of the corresponding protons of gelsenicine (1.28 ppm) (Yang and Chen, 1983). It shows aromatic protons at 7.54-6.89 ppm, and protons of methoxy group at 3.93 ppm. Two low field proton at 4.30 ppm are the protons of C(17) which formed ether linkage with C(3).

The fragmentation in mass spectrum is similar to that of gelsenicine, but the molecular ion peak is higher than that of the latter by 14 mass units, and the integration of the total protons from ^{1}H-NMR spectrum indicating 18 protons. These concluded that GE-2 possesses one oxygen atom more than gelsenicine, having carbonyl function. This corresponds to the IR indications, i.e. GE-2 possesses another additional

carbonyl group in the molecule. The high intensity peak at *m/e* 43 indicating $O\overset{+}{\equiv}C\text{-}CH_3$ group, also confirms the above evidence.

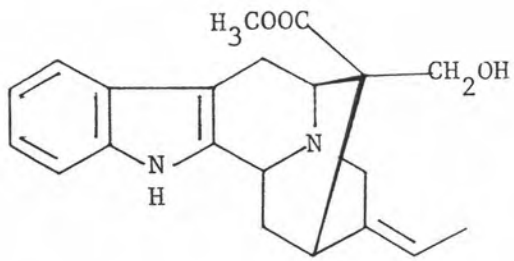
From the above informations, it can be concluded that the carbonyl function is at C(19). This is confirmed by the chemical shift of C(19) in ^{13}C-NMR which was observed in very low field (197.6 ppm). From all of these informations, GE-2 is characterized as 19-oxogelsenicine, the structure of which is shown below :-



3. GE-5

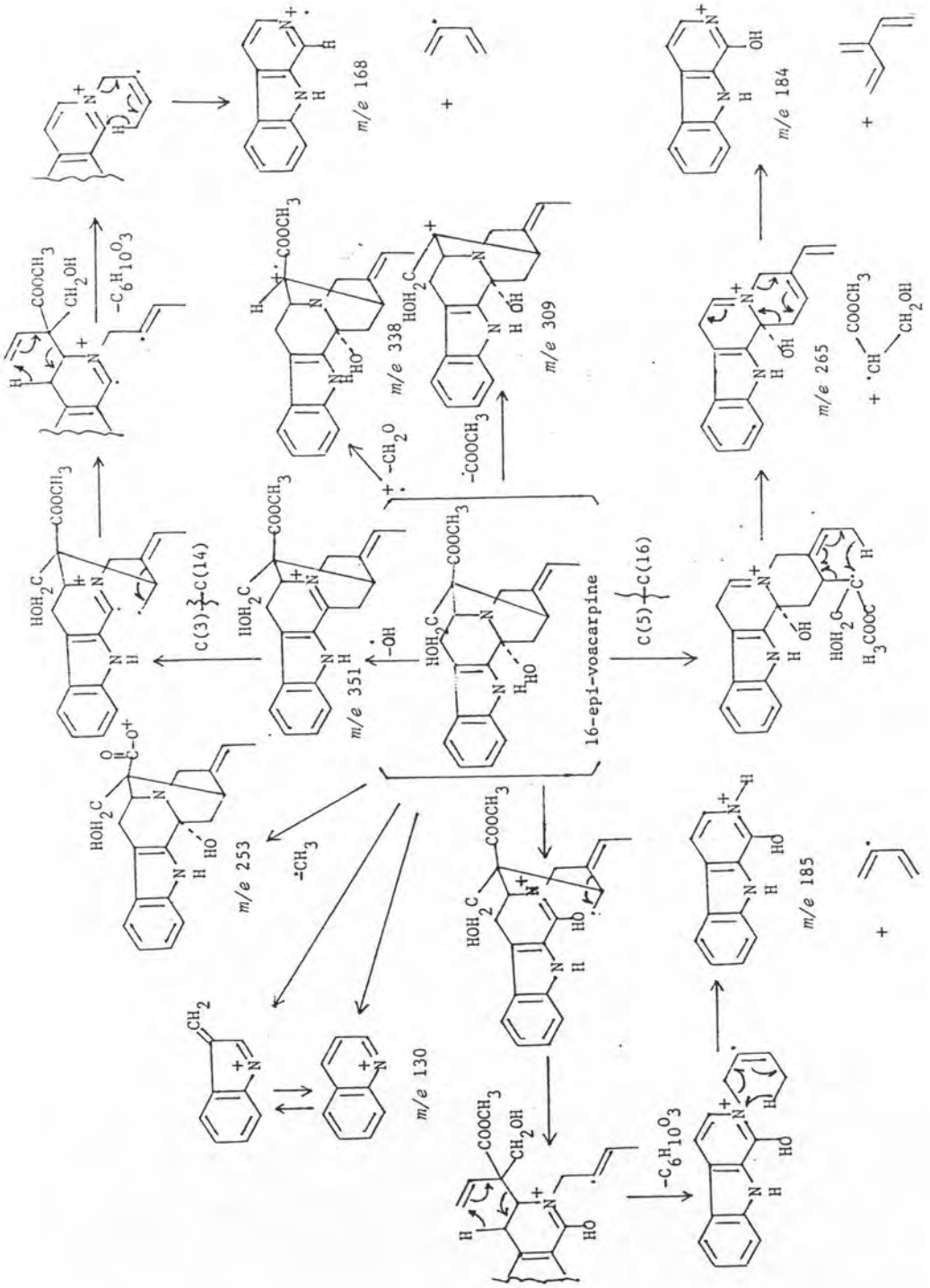
The UV and IR spectra of GE-5 correspond to those of akuammidine. In ^{1}H-NMR spectrum the coupling pattern and the values of the chemical shifts of variable protons in GE-5 also correspond to those of akuammidine (Lounasmaa *et al.*, 1985). The EIMS patterns of these two compounds are also similar, except that the base peak of akuammidine is at *m/e* 249 (Ohashi *et al.*, 1963) but that of GE-5 is at the molecular ion peak. The chemical shift of C(15) in ^{13}C-NMR spectrum is observed at

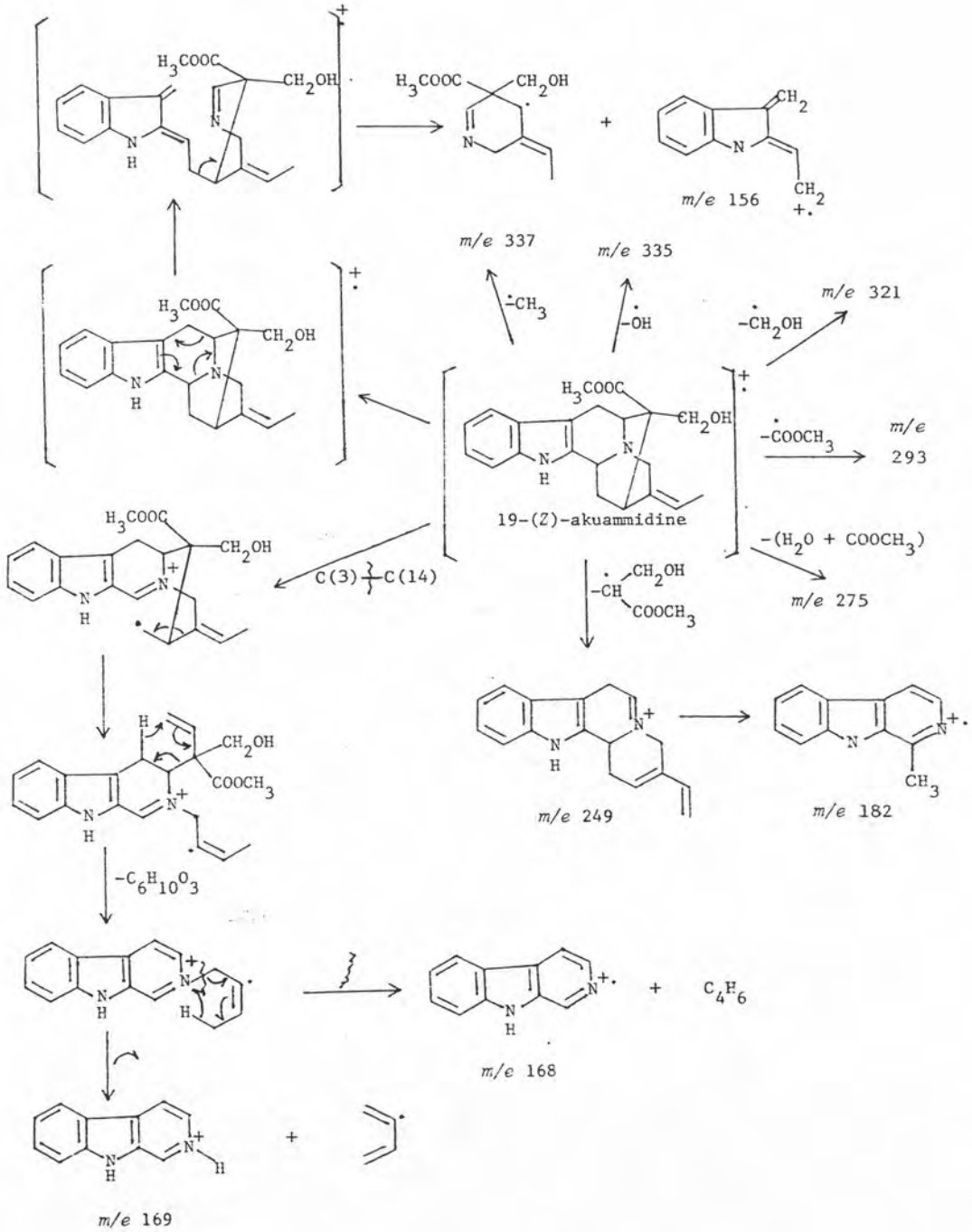
lower field (37.2 ppm) than that of the corresponding carbon of akuammidine (31.1 ppm) (S. Sakai, E. Yamanaka, M. Kitajima, M. Yokota, N. Aimi, S. Wongseripipatana and D. Ponglux, in press). This suggests that C(15) is not shielded by methyl group at C(19), thus the configuration of C(19) in GE-5 should be *Z* (Aimi *et al.*, 1978). From these informations, it can be concluded that GE-5 is 19-(*Z*)-akuammidine, the structure of which is shown below :-



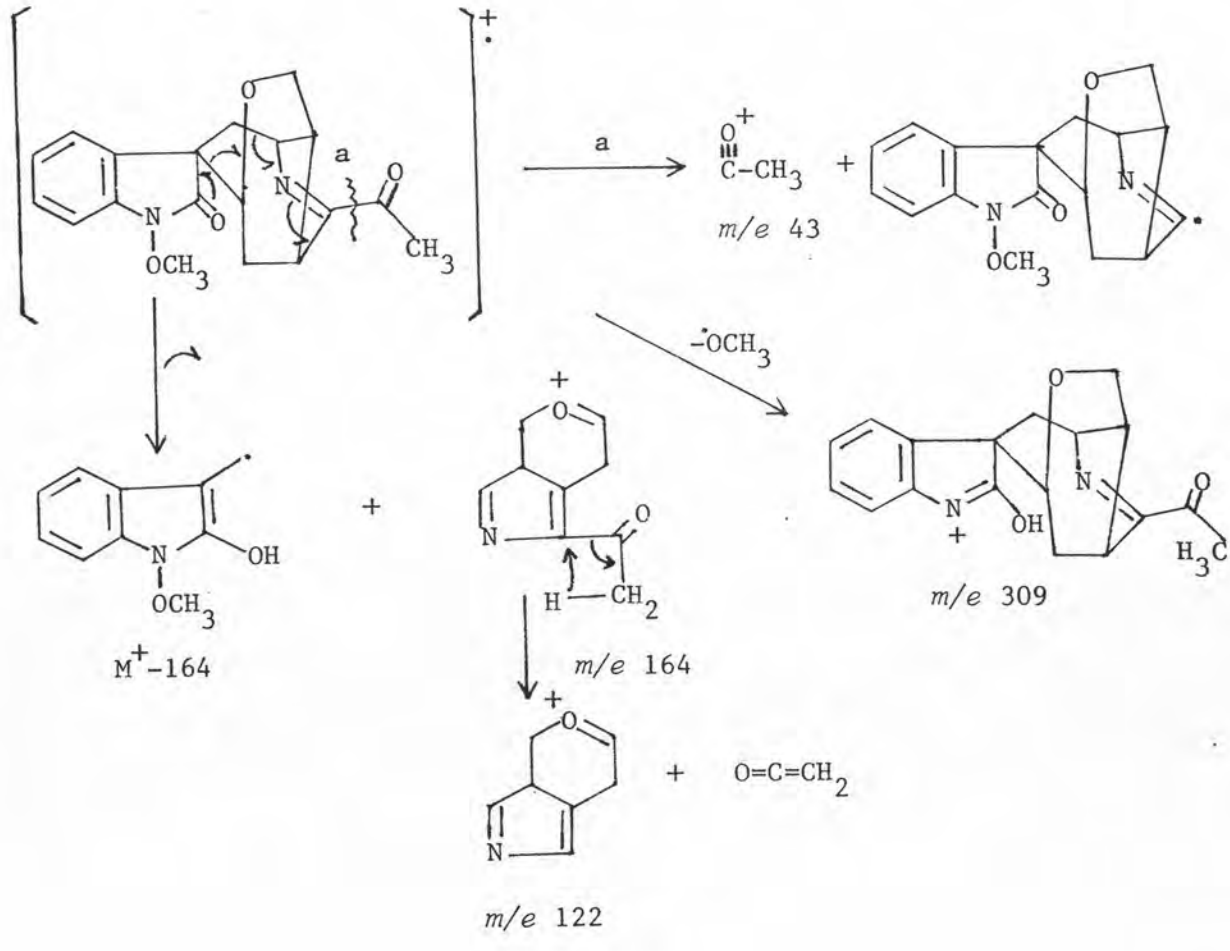
EIMS Patterns of the Isolated Alkaloids

The pattern of fragmentation of 16-epi-voacarpine in mass spectrum is very similar to that of 19-(Z)-akuammidine, both spectra show peaks at *m/e* M^+-CH_3, M^+-OH, M^+-CH_2OH, $M^+-COOCH_3$, $M^+-(CH_2OH-CH-COOCH_3)$ and β-carbolinium cation or its derivatives being substituted by hydroxy and methoxy groups in 16-epi-voacarpine and 19-(Z)-akuammidine, respectively. The patterns of fragmentation of the two compounds are shown as follows (Ohashi *et al.*, 1963; Denayer-Tournay *et al.*, 1965; and Xu, 1982) :-

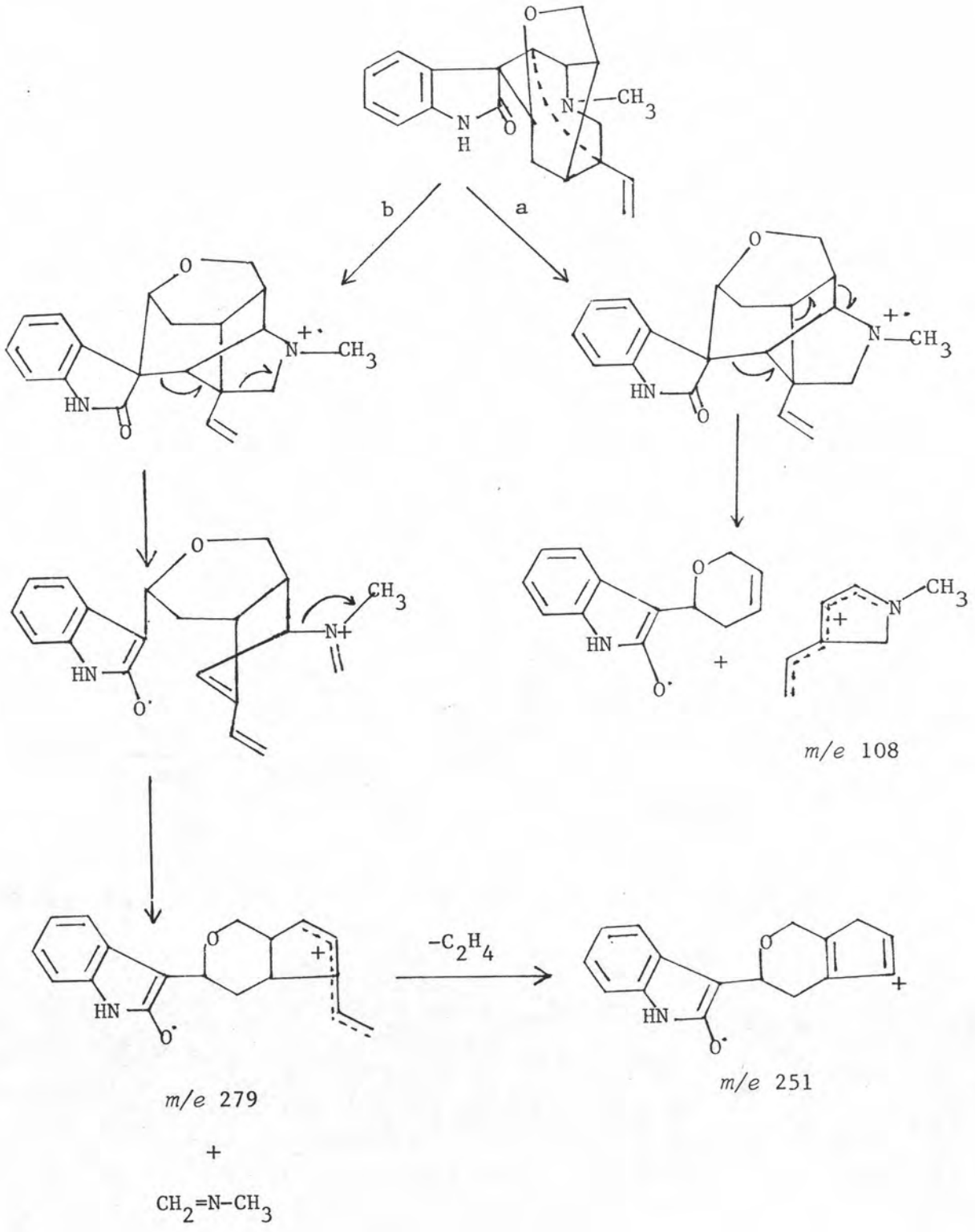

16-epi-voacarpine
m/e 351
m/e 338
m/e 309
m/e 253
m/e 168
m/e 184
m/e 265
m/e 185
m/e 130
C(3)–C(14)
C(5)–C(16)
-·OH
-·CH3O
-·COOCH3
-·CH3
-C6H10O3


m/e 337
m/e 335
m/e 156
m/e 321
m/e 293
m/e 275
19-(Z)-akuammidine
C(3)–C(14)
m/e 249
m/e 182
m/e 168
m/e 169

The mass spectrum of 19-oxogelsenicine is similar to that of gelsenicine, both spectra show peaks at *m/e* M^+-OCH_3, M^+-176 and 122. However, peaks at *m/e* M^+-176 show different intensities. It is the base peak in the latter (Du *et al.*, 1982; Yang and Chen, 1983), but is only high intense peak in the former. The base peak of 19-oxogelsenicine is the molecular ion peak, and additional high intensity peak at *m/e* 43 is $\overset{+}{O}\equiv C-CH_3$ group, occurring from bond broken between C(19)-C(20). The pattern of fragmentation is shown as follows :-



The molecule of gelsemine in mass spectrum is found to undergo fragmentation by two principle pathways upon electron impact. The most intense ion in the gelsemine spectrum occurring at *m/e* 108 (M^+-214) is characteristic of the fragmentation pathway (a) while a second mode of fragmentation (b) results in the ion at *m/e* 279, probably by extrusion of N(b) as CH_2=N-CH_3. A further loss of elements of ethylene emanating from the *m/e* 279 peak give rise to the ion at *m/e* 251 and is confirmed by a metastable peak at *m/e* 225.8 (Bindra, 1973). A peak at *m/e* 120 is attributed to the formation of 1-methyl-3-vinyl pyridinium ion.


b
a
m/e 108
m/e 279
+
CH2=N-CH3
-C2H4
m/e 251

The pattern of fragmentation of koumine in mass spectrum is shown as follows:-