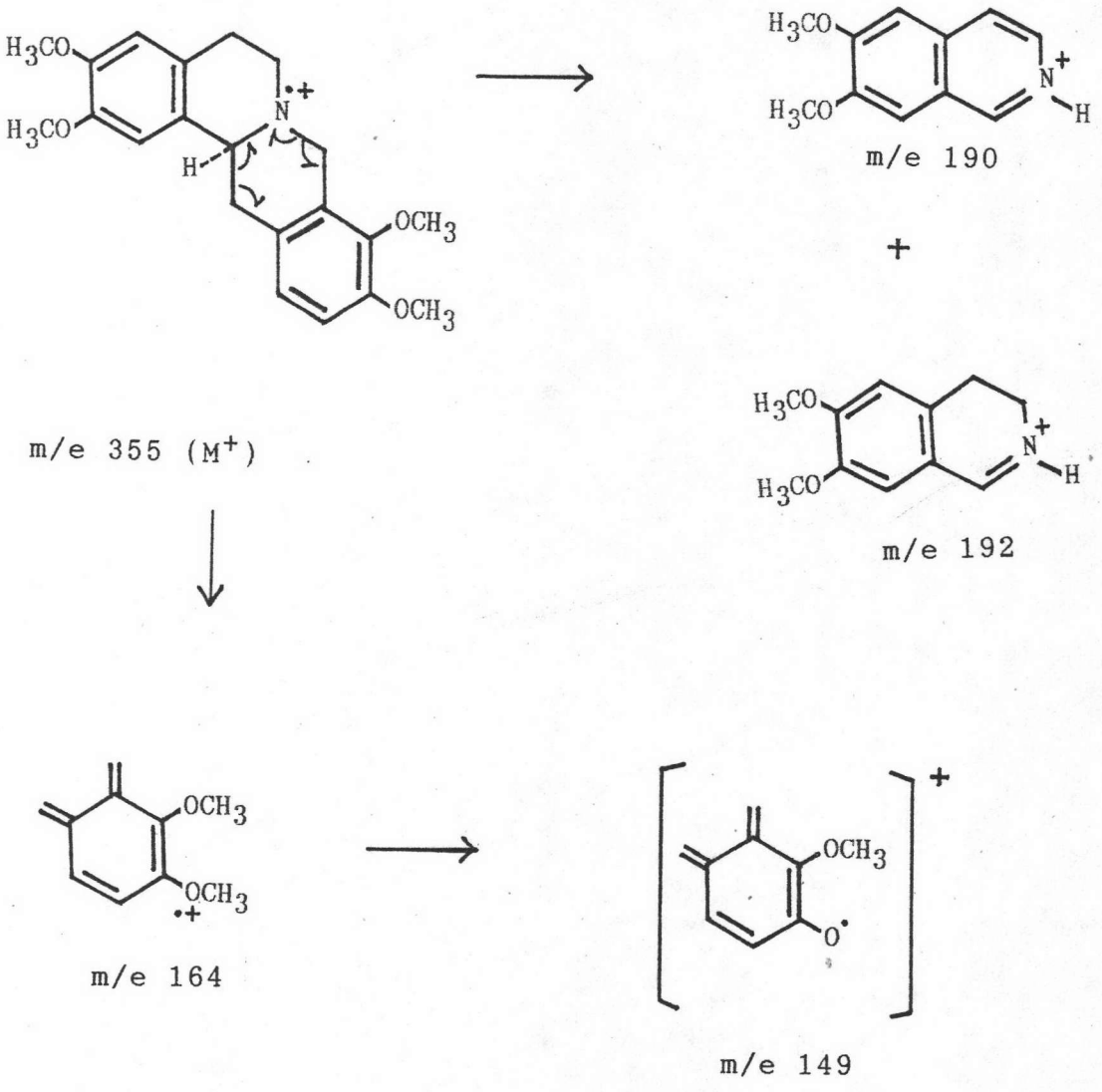
CHAPTER IV

DISCUSSION

Menispermaceae is the alkaloid-rich family especially isoquinoline alkaloids. In the view of chemotaxonomy, all isoquinoline alkaloids isolated from the genus *Coscinium* are members of either protoberberine or aporphine alkaloids. This present work has led to the isolation of four alkaloids from the stems of *Coscinium fenestratum* (Gaertn.) Colebr. All of them are known alkaloids which are identified by physical properties, spectroscopic data and direct comparison with authentic alkaloids.

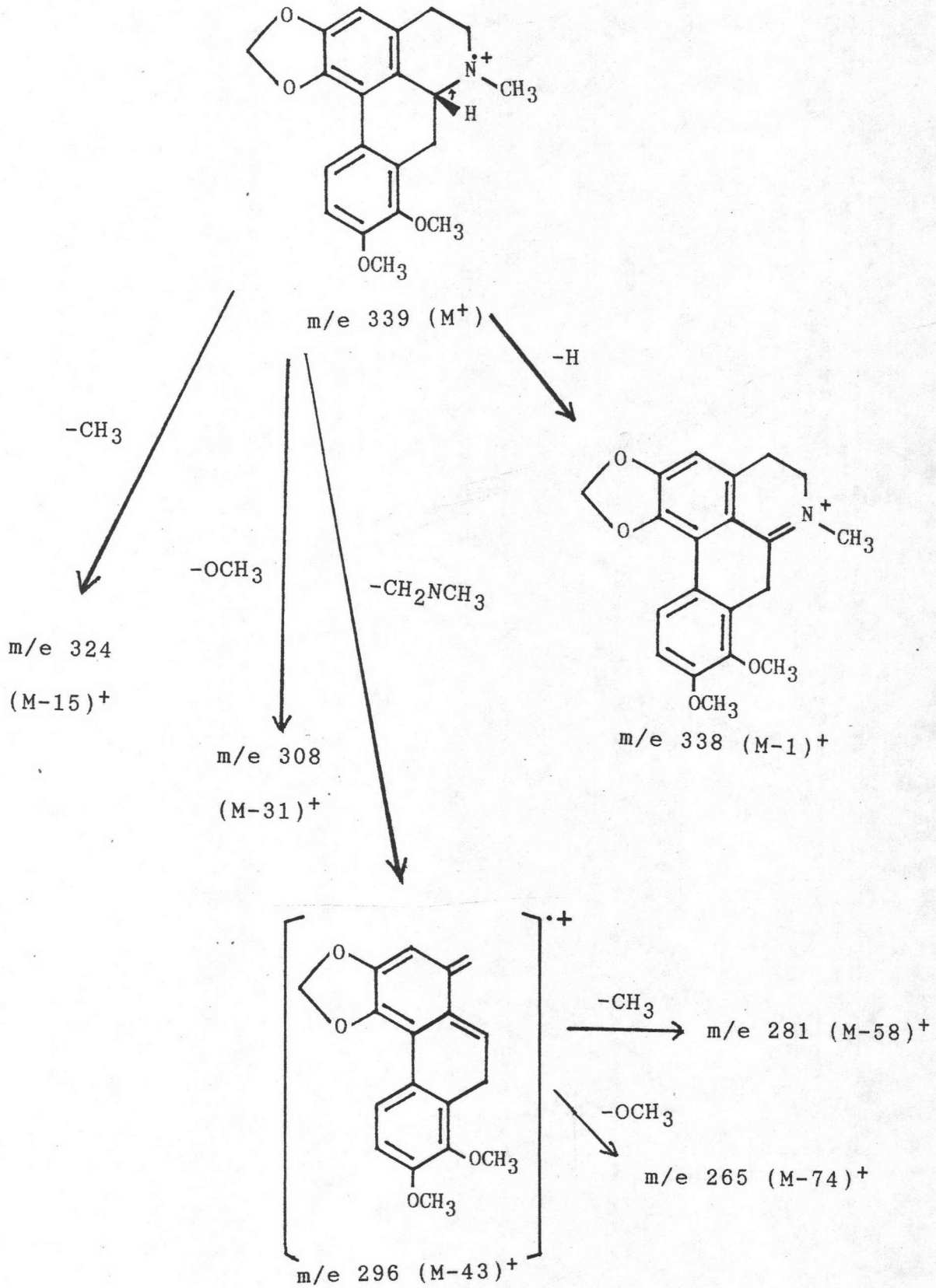
Three of the four isolated alkaloids are protoberberines, namely berberine, jatrorrhizine and tetrahydropalmatine. The remaining one belongs to aporphine alkaloids identified as crebanine. The important evidence used to distinguish the isolated protoberberines and aporphines is provided by mass spectral fragmentation patterns. The chief diagnostic ion in the spectrum of crebanine is $(M-1)^+$ peak occured at m/e 338 (basepeak) due to the loss of the hydrogen atom attached to C(6a). While the protoberberine alkaloids undergo facile fission at the two benzylic bonds that clearly shown in the spectrum of tetrahydropalmatine (Scheme 12). It is notable that the peak at m/e 164 indicats the two methoxyl substitutions on

the lower aromatic ring (ring D) of these three protoberberine alkaloids.



Scheme 12 The clevage of tetrahydropalmatine

The main fragmentation pathway of crebanine was outlined in scheme 13.



Scheme 13 The clevage of crebanine

The 200 MHz ^{1}H-NMR assignments of the isolated alkaloids especially the protoberberines based on the natures that they differ from one another in the type, number and placement of various oxygen functions (usually -OH or $-OCH_3$ and occasionally $-OCH_2O-$) on the two aromatic rings, A and D. The substitutions are usually present at C(2) and C(3) and either at C(9) and C(10) or at C(10) and C(11). Less frequent is substitution at C(1), C(5), C(8) and C(12) or C(13) (Shamma, 1972; Ohiri *et al*, 1983).

All of the three isolated protoberberines are 2, 3, 9, 10- series with different substituents. The oxygenation of ring D was determined by the multiplicity of the aromatic protons. In the case of 2,3,9,10-substitution, the C(1)-H and C(4)-H signals can readily be recognized as two 1-proton singlets while the *ortho*-coupling signals of C(11)-H and C(12)-H appear as doublets. This assignment is hampered in the case of 2,3,10,11-substitution owing to the fact that all aromatic protons in ring A and ring D show up as singlets. Furthermore, in the case of tetrahydroprotoberberine alkaloids, the multiplicity of geminal coupling of the C(8)-methylene group is of importance. When the $-OCH_3$ at C(9) position, the C(8)-axial proton is shifted upfield to become a doublet (J = 16 Hz) near 3.65 ppm and the doublet of the C (8)-equatorial proton is deshielded by the aromatic ring D to about 4.35 ppm. This result is shown with a negligible variation in tetrahydropalmatine (4.25 and 3.55 ppm for C(8)-H equitorial and C(8)-H axial, respectively).

The ^{1}H-NMR chemical shift assignment of the tetrahydroprotoberberine alkaloids, tetrahydropalmatine, and those of quaternary alkaloids, berberine and jatrorrhizine, can easily be distinguished by the downfield shifts. (1-proton singlet) of C(8)-H and C(13)-H as shown in berberine and jatrorrhizine. This deshielding effect indicated the presence of quaternary nitrogen atom. The signals of methoxyl substitution can readily be assigned as 3-proton singlet appeared in the region 3.86-4.11 ppm.

The determination for each proton of berberine and jatrorrhizine is straightforward. The difference of these two alkaloids is present by the substitutions at C(2) and C(3). The methylenedioxy protons of berberine showed a 2-proton singlet at 6.19 ppm whereas the C(2)-OCH_3 of jatrorrhizine was observed at 3.86 ppm. Furthermore, the signal of ^{13}C-NMR spectrum at 102.12 ppm also indicated the existence of methylenedioxy group as shown in berberine. Although the signal of C(3)-OH of jatrorrhizine disappeared from the ^{1}H-NMR spectrum due to its exchangeability and hydrogen bonding but the presence of phenolic function in the molecule was suggested by the absorption at 3450 cm^{-1} in the IR spectrum.

In order to settle conclusively the location of the hydroxyl and methoxyl functions on ring A, the ^{1}H-NMR spectrum of the tetrahydro derivative of jatrorrhizine is of necessity. Among tetrahydroprotoberberine alkaloids the C(2)-OH, C(3)-OCH_3 can easily be distinguished from the C(2)-OCH_3, C(3)-OH substitution because in the latter group the

chemical shifts for C(1)-H and C(4)-H differ by 0.05 ppm whereas in the former a difference of at least 0.20 ppm is observed (Ohiri, Verpoorte, and Svendsen, 1983). the signals of C(1)-H and C(4)-H of tetrahydro derivative obtained from reduction of jatrorrhizine appeared at 6.68 and 6.64 ppm respectively. This result used to locate the oxygenated substituents of jatrorrhizine.

Special features of the 200 MHz ^{1}H-NMR spectrum of tetrahydropalmatine included the signals of 3-proton singlet at chemical shifts 3.90, 3.88 and 3.86 (integrated as 12 protons) due to the four methoxyl groups and the signals at 6.74 and 6.63 ppm for the two aromatic protons in ring A which suggested that they were *para* to each other, i.e C(1) -H and C(4)-H, respectively and thus the two methoxyl groups were at C(2) and C(3). The two doublets at 6.89 and 6.79 ppm (J = 8.3 Hz) indicated the *ortho* coupling of C(12)-H and C(11)-H, respectively which determined the two methoxyl substitutions at C(9) and C(10). The assignment for each proton and ^{13}C-NMR spectrum of tetrahydropalmatine established in this present work clearly supported by the previous suggestions (Patra *et al*, 1980; Ruangrungsi *et al*, 1986; Hussain *et al*, 1989).

The structure determination of crebanine has characteristic pattern due to its aporphinoid skeleton. The furthest downfield aromatic proton, C(11)-H, is observed between 7.6-8.2 ppm. The C(3)-H is almost invariably the most shielded and typically appears as a singlet in the range 6.5-6.6 ppm. The other aromatic protons appear in the

region 6.7-7.4 ppm. (Shamma, 1972; Cordell, 1981) This result was shown exactly in those of crebanine which chemical shifts of C(11)-H, C(10)-H and C(3)-H were 7.72, 7.02 and 6.62 ppm, respectively. The two 1-proton doublets (J = 8.7 Hz) of C(11)-H and C(10)-H indicated the location of two methoxyl substitutions at C(8) and C(9). The assignment for an N-methyl function at 2.5 ppm, two methoxyl groups at 3.84 and 3.72 ppm and two protons of the methylenedioxy group at 6.12 and 5.95 ppm is straightforward. The characteristic signals of ^{13}C-NMR spectrum at 43.58 and 100.58 ppm provided further evidence supporting the presence of N-methyl function and methylenedioxy group, respectively. The proton assignment of crebanine in this present work is in agreement with those previously reported (Pharadai *et al*, 1981). The Complete assignment for each carbon atom of crebanine, as shown below, is established from the normal ^{13}C-NMR spectrum (proton noise decoupling spectrum) with the aids of distortionless enhancement by polarization transfer technique (DEPT) and comparison with the published data of stephanine, xylopinine and anonaine (Guinaudeau, 1983). For the exact ^{13}C-NMR assignment, other experiments should be carried out such as long range C-H COSY.

From the present work it is marked that the main alkaloids of this species are quaternary protoberberine alkaloids, berberine and jatrorrhizine. This result is in agreement with those of the Indonesian species previously reported by Siwon *et al* in 1980.

The important point to note is that tetrahydropalmatine and crebanine have never been reported to be found in this species before and this is the first report of their occurrences in *Coscinium fenestratum* Colebr.