

CHAPTER IV

DISCUSSION

In this present work , the chloroform extract of dried powdered stems of *Myriopteron extensum* Schum. (Periplocaceae) was separated by chromatographic technique and afforded an isolated compound "ME-1" as a major component. The structure elucidation of the compound was based on data from UV, IR, NMR, and Mass spectra, confirmed by comparison with those data reported in the literature (Smalberger et al, 1974). Conclusively, "ME-1" was identified as "lupinifolin", the known flavanone from *Tephrosia lupinifolia* Burch (DC) (Leguminosae).

ME-1 was obtained as a yellow needle crystal . A positive Shinoda test and the feature of UV spectrum (Figure 3.6 , page 93) showed the characteristic of a flavanone chromophor . The strong coloration with alcoholic ferric chloride indicated the presence of one or more phenolic OH groups in the molecule. The IR spectrum (Figure 3.7 ,page 94) showed OH absorption at 3250 cm^{-1} ; the band at 1620 cm^{-1} was assigned to the chelated flavanone CO group.

The nature of the substituents in the compound was indicated by its ^1H NMR spectrum (Table 3.2 , Figure 3.8-3.11). The doublets at δ 6.63 ($J=10.1\text{ Hz}$) and 5.50 ($J=10.1\text{ Hz}$), each equivalent to one proton, and the singlet at δ 1.43

and 1.45, together integrating for six protons, are characteristic of the *cis* double bond and *gem*-dimethyl group of a 2,2-dimethyl chromene moiety. The presence of a γ, γ -dimethylallyl sidechain was inferred from the singlet at δ 1.65 (6H), due to the *gem*-dimethyl group, and the doublet at δ 3.20 (2H, $J=7.4$ Hz) with the triplet at δ 5.14 (1H, $J=7.4$ Hz) of three protons in the allyl group.

The *para* disubstitution of the aromatic B-ring was deduced from signals due to four remaining A_2B_2 protons which were discernible as the two equivalent sets of *ortho* coupled doublets at δ 6.87 (2H, $J=8.5$ Hz) and δ 7.33 (2H, $J=8.5$ Hz). The C_2 and C_3 protons of a flavanone were considered as an ABX system; the C_2 proton, the X part, appears as a double doublet at δ 5.33 ($J_{AX} = 12.7$, $J_{BX} = 3.0$ Hz) while the C_3 protons, the AB part, appear at δ 3.03 ($J_{AX} = 12.7$, $J_{AB} = 17.0$ Hz) and δ 2.79 ($J_{BX} = 3.0$, $J_{AB} = 17.0$ Hz). The two signals of C_3 protons were assigned, according to the J -values, for the *trans*-(axial) and *cis*-(equatorial) protons respectively. The presence of a chelated C_5 -OH was evident from the signal of a phenolic proton which appeared as a singlet at low field position (δ 12.24), whereas the singlet at δ 4.85 was attributed to the other phenolic proton at C-4' position.

The mass spectrum (Figure 3.18 , page 105) showed the base peak at m/z 391 (M-15) which resulted by the loss of a methyl group of a dimethylchromene moiety. Fragment at

m/z 351 (M-55) confirmed the presence of a dimethylallyl group; it might have resulted by the rupture of the bond at the benzylic carbon adjacent to the A-ring as shown in Figure 4.1.

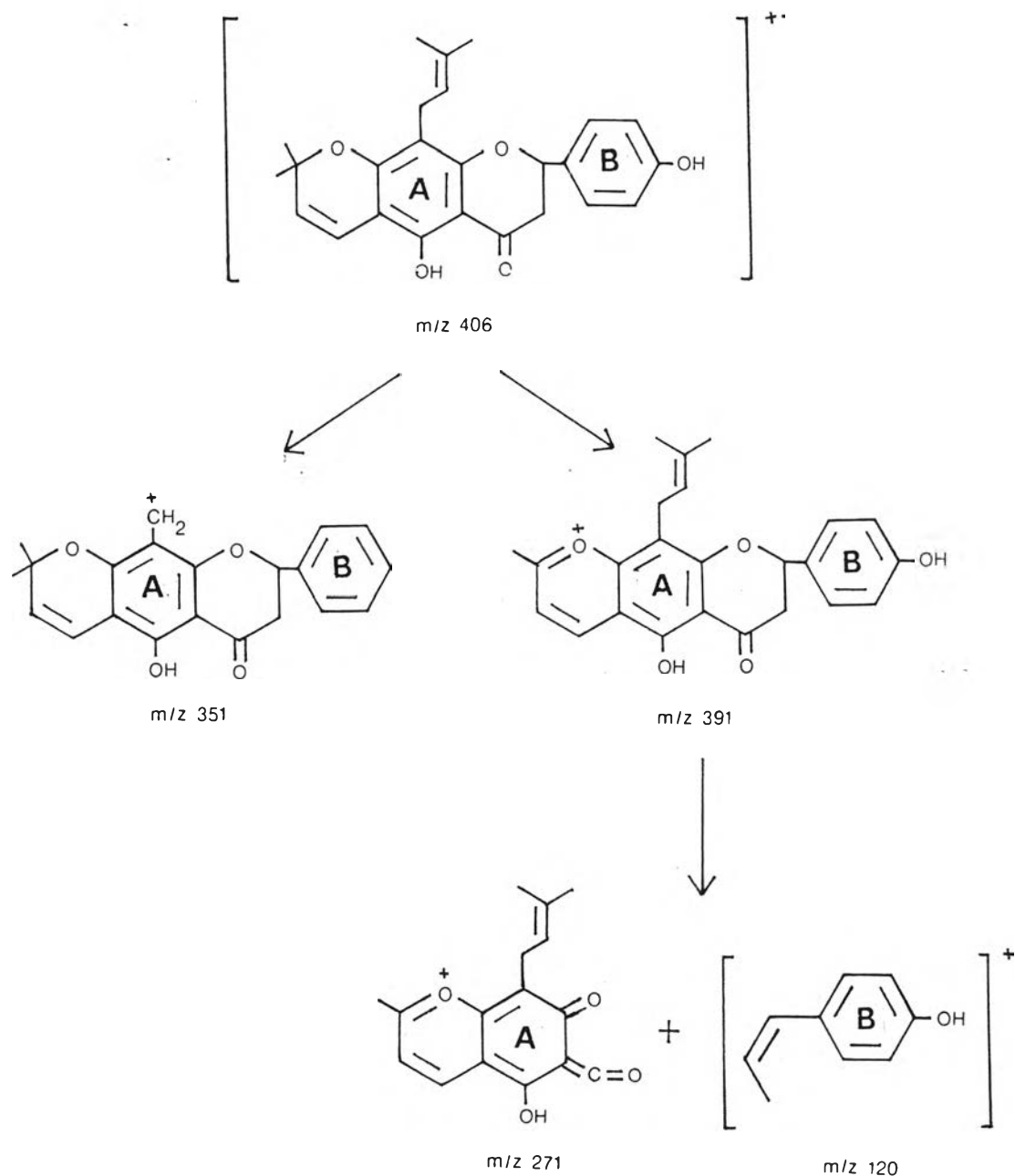


Figure 4.1 Mass Fragmentation of ME-1

The retro Diels-Alder fragments at m/z 271 and m/z 120 could be rationalized only if the non-chelated OH and the dimethylallyl sidechain were assigned to the B- and A-rings respectively (Figure 4.1). The non-chelated OH group is thus located at C-4' in agreement with NMR data while the dimethylallyl side chain could be located at either C-6 or C-8..

Treatment of ME-1 with formic acid yielded the product which gave positive ferric reaction, indicating that the dimethylallyl group is located at C-8 (Smalberger et al,1974). This point could be confirmed by using Nuclear Overhauser Effect Spectroscopy (NOESY). From the two dimensional NOESY spectrum which was given in Figure 3.14 (page 101), at point A, where the intersection occurred, showed the correlation between proton at C-4" position and phenolic proton at C-5 position. This means that the proton at C-4" position should be lie close in space to the 5-OH group.

According to the previous report of lupinifolin, only the ^1H -NMR assignment had been provided. In this experiment the ^{13}C -NMR assignment was added. The reported data were based on the interpretation of spectra obtained from various ^{13}C -NMR techniques which included the proton decoupling, the heteronuclear ^{13}C - ^1H correlation (^{13}C - ^1H HETCOR) and the selective INEPT experiment.

The proton decoupled spectrum (Figure 3.15 , page 102) showed signals of all carbons in the molecule. The position of a signal correlated to the type of carbon represented (i.e. carbonyl, aromatic, aliphatic, etc.). The confirmation was provided by the selective INEPT spectra (Figure 3.16, page 103) which pointed out the distinguishment between signals of carbons with different number of attached proton.

The assignment was mainly based on the $^{13}\text{C}-^1\text{H}$ HETCOR spectrum. From this spectrum all signals of carbons possessing attached proton could be assigned in accordance with the ^1H -NMR assignment. Those of 11 quarternary carbons in the molecule of lupinifolin were thus further investigated to assign all carbons they represented. Such carbons included C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-1', C-4', C-2'' and C-3''' . Among these 11 signals, one of the most high field and the most low field positions appeared at δ 78.14 and δ 196.51. The former was in the region typical for an aliphatic carbon and the latter was in the carbonyl range. The two signals were therefore assigned to the aliphatic C-2'' and the carbonyl C-4 respectively.

The other unassigned signals were related to 8 aromatic and 1 olefinic carbons. These carbons could be divided into two groups: four of which oxygenated type (C-5, C-7, C-9, C-4') and five of non-oxygenated ones (C-6, C-8, C-10, C-1', C-3'''). Owing to the deshielding effect, signals of the former group were more downfield than those of the

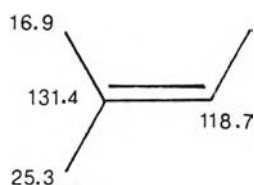
latter. Therefore four signals appearing in the comparative low field at δ 159.90, 159.36, 156.58, and 155.86 were attributed to the four oxygenated carbons. The first two signals were assigned to C-9 and C-7 (or might be interchanged) because the very close chemical shifts of the two signals was in agreement with the similar environment of the two carbons. The third signal was assigned to C-4' based on the comparison with resonated value of C-4' in the molecule of naringenin which was 157.4 ppm (using DMSO-d₆ as a solvent; Harborne et al, 1982). The last signal of the group should be assigned to C-5.

The rest of unassigned signals were due to non-oxygenated carbons. The resonated value of carbon at position 1' in the molecule of naringenin which was 128.9 ppm (using DMSO-d₆ as a solvent; Harborne et al, 1982) suggested that the possible position of the signal due to C-1' might be either δ 130.09 or δ 131.11. These two values were also in the range of non-oxygenated carbons without *ortho/para*-oxygenation (Harborne et al, 1982), the other carbon which related to either of them was thus C-3''' of the dimethylallyl sidechain.

The remainder were three signals in the range of non-oxygenated carbon with *ortho/para*-oxygenation (90-125 ppm; Harborne et al, 1982). The two closely located signals at δ 102.66 and 102.64 were assigned to C-6 and C-10 (or might be interchanged) owing to the similar environment of the two carbons. Thus the last signal of the group was arbi-

trary assigned to C-8.

An interesting point in C^{13} -NMR assignment is to note that the resonated values of two methyl carbons in the dimethylallyl sidechain are distinguishably different. From the two dimensional HETCOR spectrum (Figure 3.17, page 104), the signals of proton in 3^{'''} methyl groups showed the correlation with two signals of carbons at δ 17.84 and 25.82. The lowfield signal was assigned to a carbon of the methyl group which lied in the *cis*-form with the olefenic proton at position 2^{'''} (Z-CH₃); the highfield signal was assigned to that of the other methyl group (E-CH₃). The deshielding of E-methyl carbon could be attributed to the γ effect of the 1^{'''} methylene carbon. The assignment of these two methyl carbons was based on the resonated values of 2-methyl-2-butene (Silverstein, 1981) as shown below:-



For the aqueous portion, the presence of free sugar was determined by using the method of osazone formation. The product obtained was taken to run the IR spectrum in order to compare with those of osazone products of authentic mono and disaccharides. The result (Figure 3.19-3.20 , page 106-107) showed that the free sugar in the aqueous portion was sucrose.