CHAPTER IV

RESULT AND DISCUSSION

The present work, using chromatographic techniques, has led to the isolation of four flavanone, (2*S*)- 5, 2', 6'-trihydroxy-8-lavandulyl-7-methoxyflavanone (exiguaflavanone B) from the $CHCl_3$: Hexane (1:1) extract of *Sophora exigua* Craib roots, (2*S*)- 5, 7, 2', 6'-tetrahydroxy-8-lavandulylflavanone (exiguaflavanone A), 5, 7, 2'-trihydroxy-8-lavandulylflavanone (kushenol A), and 5, 7, 2', 4', 6'-pentahydroxy-8-lavandulylflavanone from the $CHCl_3$ extract of *Sophora exigua* Craib roots. The structure elucidations were based on the data from UV, IR, MS, and NMR spectra.

1.The Structure Elucidation of the Isolated Compounds

1.1 Compound SE-1

Compound SE-1 was obtained as yellow viscous oil from F-018 by chromatographic techniques using silica gel column (10% petroleum ether in chloroform) and silica gel column (10% methanol in benzene). It yielded 30 mg. This compound was positive with Shinoda's test and $FeCl_3$ TS reagent.

The EIMS spectrum (Fig. 11) showed the molecular ion peak at m/z 438 (3%) and established the proposed molecular formula of $C_{26}H_{30}O_6$. The UV spectra of SE-1 in methanol (Fig. 12) showed absorption maxima at 289 and 340 nm indicated the characteristic of a flavanone chromophore. The bathochromic shift (24 nm) of band II in the UV spectrum with the presence of $AlCl_3$/ HCL(Fig. 12) were effected from the acid stable complex of $AlCl_3$ with the C-4 carbonyl and the C-5 hydroxy group of the flavanone derivative. The IR spectrum of SE-1 (Fig. 13) showed the presence of hydroxyl groups (3455 cm^{-1}) and a conjugated carbonyl group (1635 cm^{-1}).

Compound SE-1 was assigned as a known flavanone, exiguaflavanone B (65) (5, 2', 6'-trihydroxy-8-lavandulyl-7-methoxyflavanone) , by the analyses of ^{1}H and ^{13}C-nmr spectra. The ^{1}H-nmr spectrum (Fig. 14) provided the signals of 8 aliphatic protons, 7 olefenic protons, 3 hydroxyl groups, 1 methoxy group, and 3 methyl groups. The ^{13}C-nmr (Fig. 16) showed the signals of 1 carbonyl carbon, 5 sp^3 carbons, 7 sp^2 carbons, 8 quaternary carbons, 1 methoxy carbon and 3 methyl groups.

The signals in ^{1}H-nmr spectrum of SE-1 (FIg. 14 and 15) showed signal due to chelated hydroxyl proton (δ: 12.25 ppm). This signal shifts to down field when compared with other phenolic hydroxyl groups because of hydrogen bonding between hydroxyl group of C-5 to carbonyl function of C-4. Three-one proton doublets of doublets (Fig. 15) at δ 2.83 ppm (1H, dd, *J* = 17, 3 Hz, H-3*Z*) , 3.22 ppm (1H, dd, *J* = 14,17 Hz ,H-3*E*) , 5.95 ppm (1H, dd, *J* =14,3 Hz, H-2) were preferably assigned to H-2 and H-3 of the flavanone. The ^{1}H-nmr also showed the presence of the lavandulyl group (5-methyl-2-isoproprenyl-hex-4-enyl). The signals at δ : 1.46, 1.58, 1.63 ppm (3H, each, s, CH_3) assigned to positions 7", 6", 10" , respectively, 2.01 ppm (1H, dt, *J* = 20 , 8.3 Hz , H_a-3") overlapped with the signals at 2.02 ppm (1H , dt, *J* = 20 , 6.7 Hz , H_b-3"). These two signals due to germinal coupling between H_a and H_b of H-3" and coupling to H-2" and H-4" with the coupling constant of 8.3 and 6.7 Hz , respectively. The signal at 2.24 (1H , tt , *J* = 8.3 ,6.1 Hz , H-2") coupling to H_a-3" and H_a-1" with coupling constant of 8.3 Hz and coupling to H_b-3" and H_b-1" with coupling constant of 6.1 Hz. The signals at 2.55 ppm (1H, dd, *J* = 13,8.3 Hz, H_a-1") , 2.65 ppm (1H, dd, *J* =13,6.1 Hz, H_b-1"). These signals due to germinal coupling of Ha-1"and Hb-1" with the coupling constant of 13 Hz and coupling to one proton of H-2" with the coupling constant of 8.3 and 6.1 Hz , respectively. The signal at 4.48 ppm (1H, brd, *J* = 1.1 Hz , H_a-9") caused by long-range coupling with one proton of H-2" via zigzag coupling and the signal at 4.64 ppm (1H, p, H_b-9") caused by long-range coupling via a zigzag path between three protons of 10"-CH_3 and one proton of H-2" by zig-zag coupling. The signal at 4.99 ppm (1H, brt, *J*=7 Hz, H-4").coupling with methylene protons of H-3".

The two dimensional ^{1}H-^{1}H COSY nmr spectrum (Fig. 17) showed the correlation of the signal at δ 1.63 ppm to the signal at C (9")-H (δ : 4.84 ppm and 4.64 ppm) , was due to the methyl protons at C-(8") , and the ones at δ 1.46 and 1.58 ppm which showed the correlation to the signal of protons at C (4") (δ : 4.99 ppm) and C (3") (δ : 2.01-2.02 ppm) , were due to the protons of two methyl groups at C (5").

A one-proton singlet (δ : 6.15 ppm , s, H-6) was the proton of 5,7-dihydroxyflavanone nucleus because of its correlation to C-10 at δ 102.99 ppm in the COLOC spectrum (8 Hz). Three aromatic protons [δ : 6.45 ppm (2H, d, J = 8.3 Hz, H-3' and H-5') , 7.09 ppm (1H, J =8.3 Hz, H-4') showed the presence of 2', 6'-dioxygenated flavanone. These two signals were due to the *ortho* coupling of the phenyl proton at C (4'), C (3'), and C (5') respectively. Two hydroxyl protons singlet (δ : 6.24, s, OH-2', 6') were the hydroxyl proton of 2',6'-dihydroxylflavanone. A three proton singlet (δ : 3.84 ppm) were the protons of methoxy group which attached to position C-7 because of its correlation to H-6 in ^{1}H-^{1}H COSY nmr spectrum (Fig. 17).

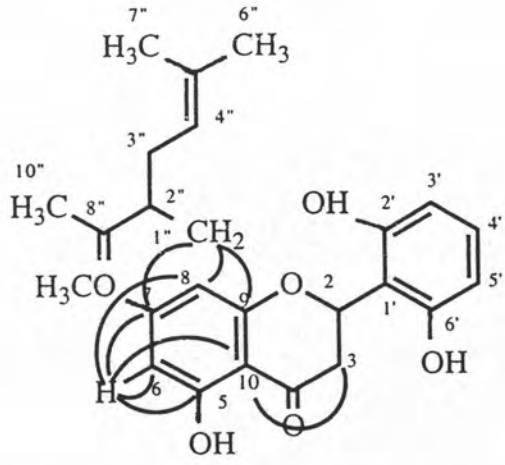
The ^{13}C-nmr spectrum of compound SE-1 confirmed the proposed structure. Their assignment was mainly based on the ^{13}C-^{1}H COSY nmr spectrum (Fig. 18). One carbonyl carbon at δ 196.90 ppm is assigned as C-4 of flavanone. The protonated sp^3 carbon of flavanone nucleus are C-2 at δ 75.54 ppm and C-3 at 41.28 ppm. The protonated sp^2 carbon of flavanone nucleus are C-6 at δ 93.54 ppm, C-3'and C-5' at 108.92 ppm, and C-4' at 130.03 ppm. The protonated sp^3 carbon of lavandulyl group are C-1" at δ 26.76 ppm, C-2" at 47.60 ppm, and C-3" at 31.48 ppm. The protonated sp^2 carbon of lavandulyl group are C-4" at δ 122.95 ppm, and C-9" at 110.93 ppm.

From the COLOC spectrum (8 Hz) can assinged the quaternary carbons of flavanone nucleus which were C-10 at δ 102.99 ppm, C-5 at 163.05 ppm, C-7 at 106.05 ppm, C-8 at 109.41 ppm, C-9 at 157.96 ppm, C-1' at 110.53 ppm, and C-2' and C-6' at 154.59 ppm. The quaternary carbons of lavandulyl group were C-5" at δ 131.99 ppm, and C-8" at 148.02 ppm.

The remaining signals in the upfield region are the signals of methyl group in lavandulyl side chain. The signal at δ 18.80 ppm showed the correlation to the signal at δ 1.63 ppm. This signal could be assigned as proton at C-(10"). Two methyl groups at C-(5") could not be assigned by ^{13}C-^{1}H COSY nmr spectrum, which must be determined by comparing their ^{13}C chemical shift. Because of the steric effect (or γ effect) due to C-3" at δ : 31.48 ppm, the high field signal at δ 17.71 ppm should be assigned to *Z*-C-(7"), and the lower field signal at 26.75 ppm to *E*-C-(6") So these methyl protons could be assigned from the correlation in the ^{13}C-^{1}H COSY spectrum,

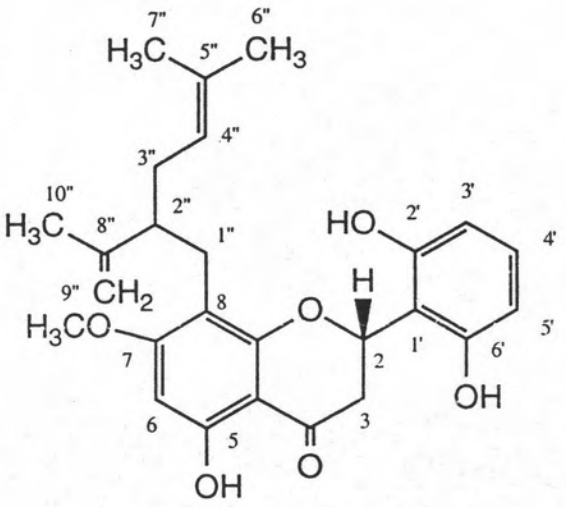
as the signal at δ 1.46 ppm was due to 7"-CH_3 and the one at δ 1.58 ppm was due to 6"-CH_3.

These spectral data indicated that SE-1 was 5, 2', 6'-trihydroxy-7-methoxyflavanone with a lavandulyl group at either C-6 or C-8. The COLOC spectrum (8 Hz) (Fig. 19) showed that the position of the lavandulyl group was at C-8 because a proton assigned at δ 6.15 ppm caused a cross peak with the carbon signals of C-5 at 163.06 ppm), C-7 at 166.05 ppm, C-8 at 109.41 ppm , C-10 at 102.93 ppm and at δ : 93.54 ppm which unambiguously assigned to C-6. And the signal at δ 2.55 , 2.65 ppm which were to H-1" of lavandulyl side chain caused a cross peak with a carbon signal of C-9 at δ 157.96 ppm , C-7 at δ 166.05 ppm and a signal at 109.42 ppm that can assigned to C-8. From these data indicated that a lavandulyl group attached to C-8.



The structure was confirmed by the mass fragmentation (Fig. 20) , a fragment, m/z 315 (M^+-123), suggested the presence of lavandulyl group. The fragment at m/z 179 suggested that the A-ring had one methoxy and one hydroxy groups.

From all of these informations, SE-1 was characterized as 5, 2', 6'-trihydroxy-8-lavandulyl-7-methoxyflavanone, which previously reported by Ruangrungsi *et al* (1991). This report showed that configuration at C-2 is 2*S*. Consequently, SE-1 is (2*S*) 5, 2', 6'-trihydroxy-8-lavandulyl-7-methoxyflavanone, named exiguaflavanone B (65). The structure of which is shown below.



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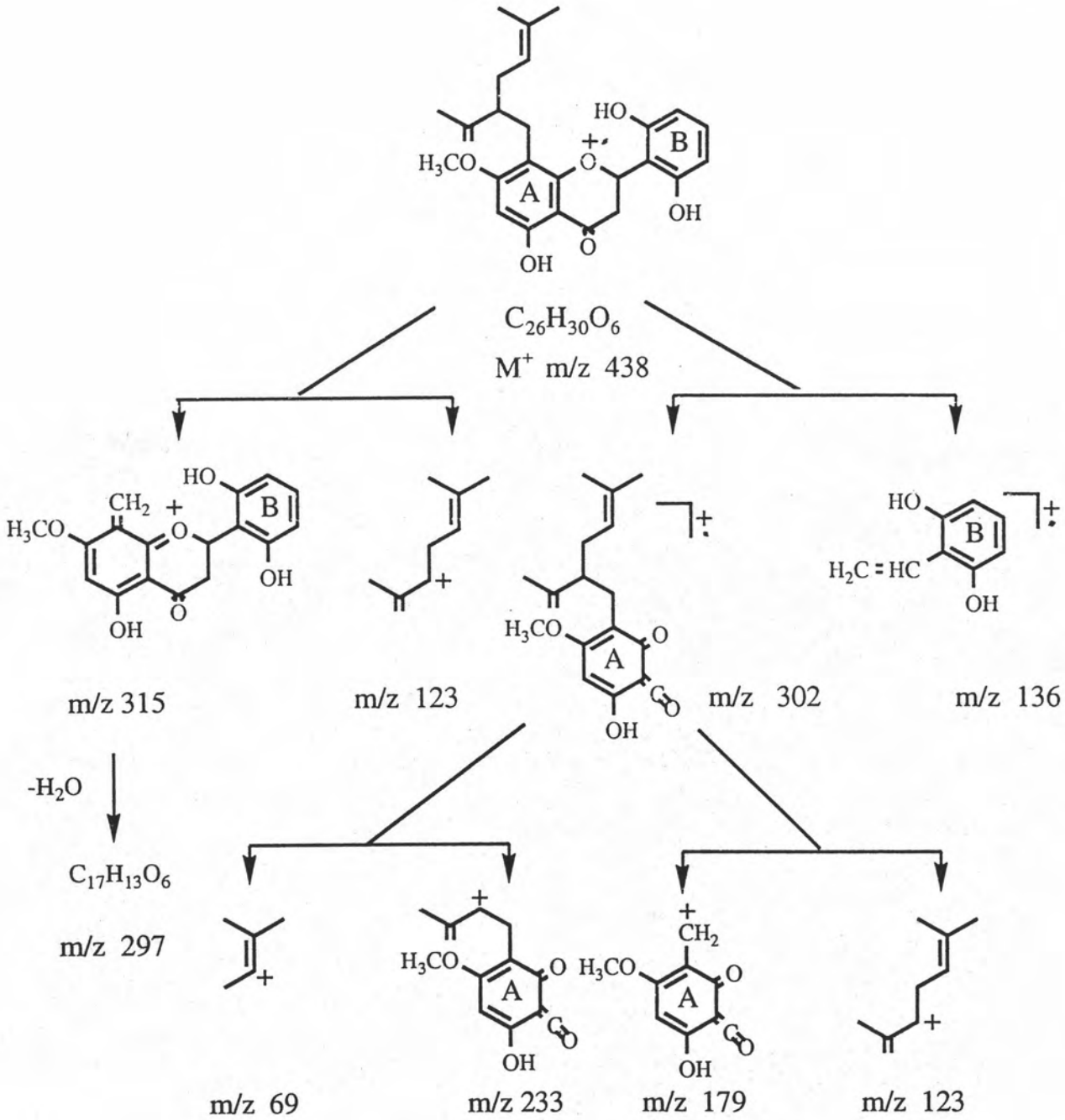


Figure 20 Mass fragmentation of SE-1

1.2 Compound SE-2

Compound SE-2 was obtained as white needles from F-002 by chromatographic techniques using silica gel column (40% acetone in chloroform) and RP-18 column (70% acetonitrile in water). It yielded 200 mg. This compound was positive with Shinoda's test and $FeCl_3$ TS reagent.

The EIMS spectrum (Fig. 21) showed the molecular ion peak at m/z 424 (3%) and established the proposed molecular formula of $C_{25}H_{28}O_6$. The UV spectra of SE-2 in methanol (Fig. 22) showed absorption maxima at 291 and 342 nm indicated the characteristic of a flavanone chromophore. The bathochromic shift (23 nm) of band II in the UV spectrum with the presence of $AlCl_3$/ HCL(Fig. 22) were effected from the acid stable $AlCl_3$ complex. From these data, SE-2 was considered to be 5-hydroxyflavanone derivative. The IR spectrum of SE-2 (Fig. 23) showed the presence of hydroxyl groups (3,550, 3,180 cm^{-1}) and a conjugated carbonyl group (1,650 cm^{-1}).

Compound SE-2 was assigned as a known flavanone, exiguaflavanone A (66) (5, 7, 2', 6'-tetrahydroxy-8-lavandulyl flavanone) , by the analysis of ^1H and ^{13}C-nmr-spectra. The ^1H-nmr spectrum (Fig. 24) provided the signals of 8 aliphatic protons, 7 olefinic protons, 1 hydroxyl group, and 3 methyl groups. The ^{13}C-nmr (Fig. 26) showed the signals of 1 carbonyl carbon, 5 sp^3 carbons, 8 sp^2 carbons, 8 quaternary carbons, 3 methyl groups.

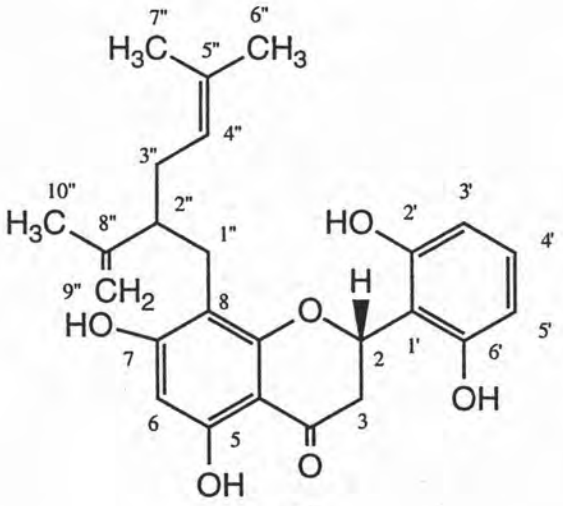
The signals in ^1H-nmr spectrum of SE-2 showed signal due to hydrogen-bonded hydroxyl proton (δ: 12.26 ppm). Three-one proton doublets of doublet (Fig. 25) at δ 2.53 ppm (1H, dd, J = 17, 4 Hz) , 3.85 ppm (1H, dd, J = 17,14 Hz) , 6.15 ppm (1H, dd, J = 14,4 Hz) were preferable assigned to H-3*Z*, H-3*E*, and H-2 of the flavanone , respectively. The ^1H-nmr also showed the presence of the lavandulyl group [δ : 1.48, 1.54, 1.61ppm (3H, each, s, CH_3), assigned to positions 7", 6", 10" respectively), 2.06 ppm (2H, m, H-3") , 2.57 ppm (1H, m, H-2"), 2.88 ppm (2H, d, J = 15 Hz, H-1") , 4.55 ppm (2H, m, H-9"), and 4.96 ppm (1H, brt, J = 5.5 Hz, H-4")].

A one-proton singlet (δ : 6.02 ppm , s, H-6) , it was the proton of 5,7-dihydroxyflavanone nucleus. Three aromatic protons [δ : 6.98 ppm (2H, d, J = 9 Hz, H-3' and H-5') , 7.03 ppm (1H, J = 9 Hz, H-4') show the presence of 2', 6'-dioxygenated flavanone. These two signals were due to the *ortho* coupling of the phenyl proton at C (4'), C (3'), and C (5') respectively.

The ^{13}C-nmr spectrum of compound SE-2 confirmed the proposed structure. The ^{13}C-nmr spectrum was almost super-imposable on that of SE-1 (see in Table 9) except for the presence of a methoxy group at δ 55.83 ppm.

The mass spectrum (MS) of SE-2, a fragment, m/z 301 (M^+-123), suggested the presence of lavandulyl group, a fragment, m/z 136, due to the retro-Diels-Alder cleavage of the flavanone, indicated that the B-ring of SE-2 has two hydroxy groups. The fragment at m/z 288 (M^+-136) suggested that the A-ring has one lavandulyl and two hydroxyl groups (Fig. 27).

From all of these data supported that compound SE-2 has four hydroxyl groups at C-5, C-7, C-2', and C-6' although the ^{1}H-nmr of SE-2 showed only one hydroxyl group at δ 12.26ppm. The assignments of protons and carbons of compound SE-2 were confirmed by comparison with the data of exiguaflavanone A (66) (5, 7, 2', 6'-tetrahydroxy-8-lavandulylflavanone) previously reported by Ruangrungsi *et al* (1991), which also reported that the configuration of C-2 is *S* . Compound SE-2 is, therefore, (2*S*)-5, 7, 2', 6'-tetrahydroxy-8-lavandulylflavanone. The assignments are summarized in Table 8 and Table 9. The structure of SE-2 is shown below :



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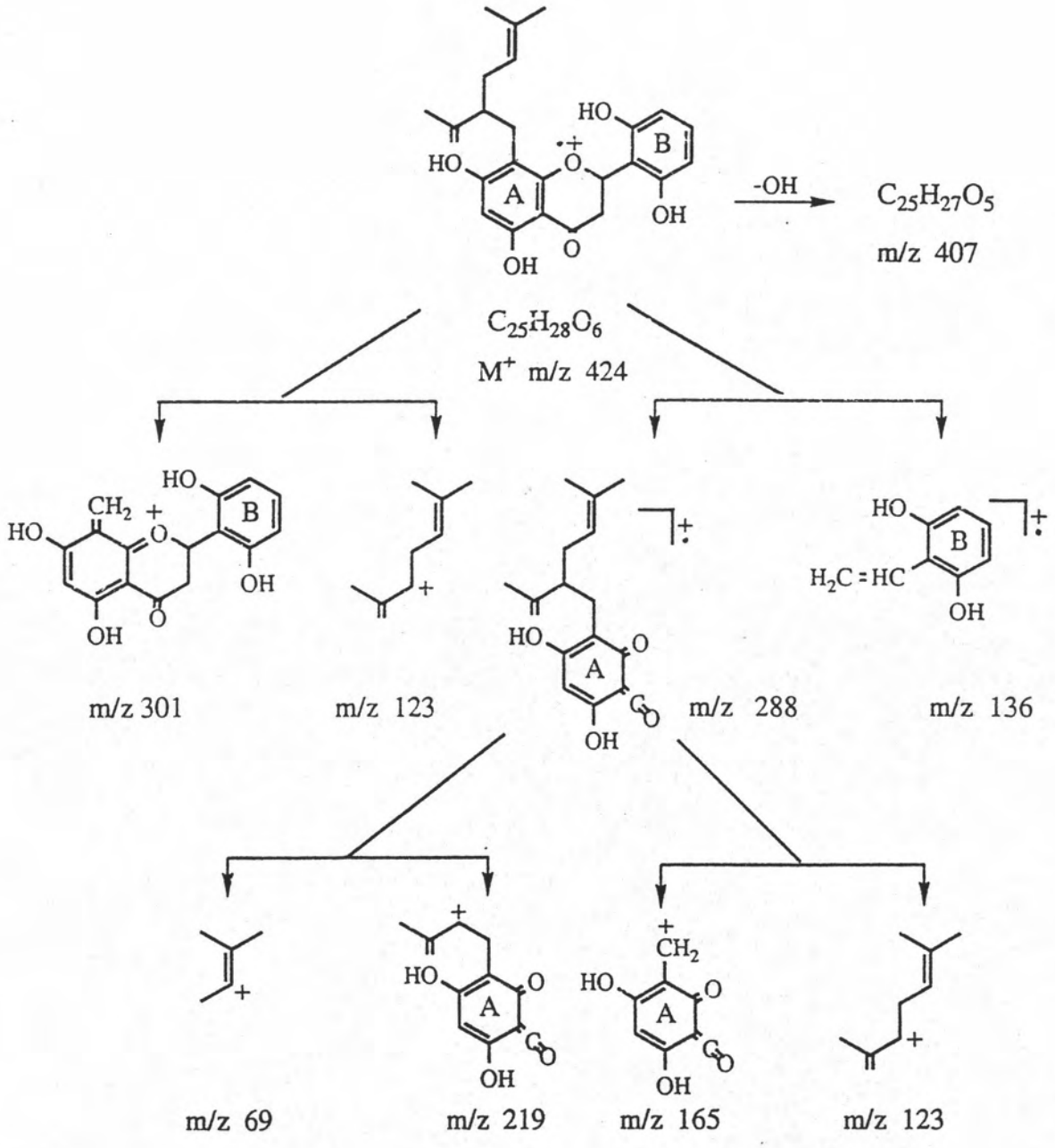


Figure 27 Mass fragmentation of SE-2

1.3 Compound SE-3

Compound SE-3 was obtained as white needles from F-002 by chromatographic techniques using silica gel column (40% acetone in chloroform) and RP-18 column (70% Acetonitrile in water). It yielded 90 mg. This compound was positive with Shinoda's test and $FeCl_3$ TS reagent.

The EIMS spectrum (Fig. 28) showed the molecular ion peak at m/z 408 (11.7%) and established the proposed molecular formula of $C_{25}H_{28}O_5$. The UV spectra of SE-3 in methanol (Fig. 29) showed absorption maxima at 291 and 342 nm indicated the characteristic of a flavanone chromophore. The bathochromic shift (24 nm) of band II in the UV spectrum with the presence of $AlCl_3$/HCl (Fig. 29) is effected from the acid stable $AlCl_3$. From these data, SE-3 was considered to be 5-hydroxyflavanone derivative. The IR spectrum of SE-3 (Fig. 30) showed the presence of hydroxyl groups (3,625, 3,200 cm^{-1}) and a conjugated carbonyl group (1,640 cm^{-1})

Compound SE-3 was assigned as a known flavanone, 5, 7, 2'-trihydroxy-8-lavandulylflavanone (67), by the analyses of ^1H and ^{13}C-nmr-spectra. The ^1H-nmr spectrum (Fig. 31) provided the signals of 8 aliphatic protons, 8 olefenic protons, 1 hydroxyl group, and 3 methyl groups. The ^{13}C-nmr (Fig. 33) shows the signals of 1 carbonyl carbon, 5 sp^3 carbons, 8 sp^2 carbons, 8 quaternary carbons, 3 methyl groups.

The ^1H-nmr spectrum showed signal due to hydrogen-bonded hydroxyl group (δ : 12.16 ppm). Three-one proton doublets of doublet (Fig 32) at δ 2.90 ppm (1H, dd, *J* = 17, 3 Hz) , 3.30 ppm (1H, dd, *J* = 17,14 Hz) , 5.74 ppm (1H, dd, *J* = 14, 3 Hz) were preferable assigned to H-3*Z*, H-3*E*, and H-2 of the flavanone , respectively. The ^1H-nmr also showed the presence of the lavandulyl group [δ : 1.47 ppm and 1.56 ppm (3H , each , d ,1.2 Hz) , assigned to positions 6"-CH_3 , 7"-CH_3., respectively , 1.66 ppm (3H, dd, *J* = 1 Hz , 10"-CH_3) , 2.07 ppm (2H, m , H-3") , 2.54 ppm (1H , m , H-2") , 2.66 ppm (2H, dd , *J* = 14, 6 Hz) , 4.57 ppm (1H, brd , *J* = 1 Hz , H_a-9") , 4.61 ppm (1H, p , *J* = 1 Hz , H_b-9") , and 5.00 ppm (1H, triplets of heptet , *J* = 6.7, 1.2 Hz , H-4").

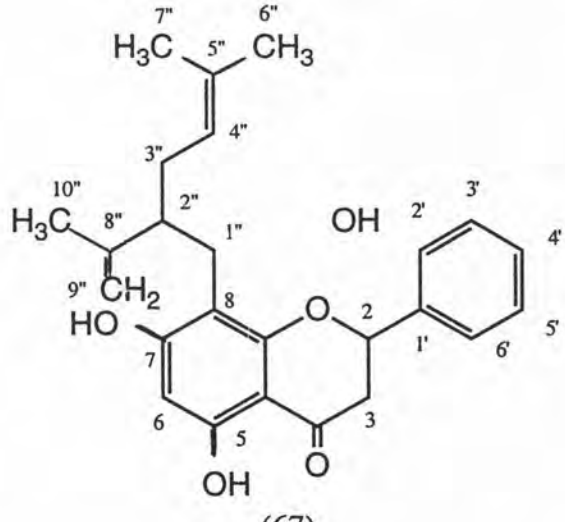
The signals of four aromatic protons at [δ 6.95 ppm (dd, *J* = 7.5 , 1 Hz, H-3'), 6.96 ppm (ddd, *J* = 7.5, 7.5, 1, H-5') , 7.22 ppm (ddd, *J* = 7.5, 7.5, 1.8, H-4') and 7.62 ppm (dd, *J* = 7.5 , 1.8 Hz, H-6'). Their splitting pattern and coupling constant

represented that there were one proton of the hydroxy group and four adjacent protons on the aromatic B-ring. A one proton singlet (δ : 6.02 ppm , s, H-6) was the proton of 5,7-dihydroxyflavanone nucleus.

The ^{13}C-nmr spectrum of compound SE-3 confirmed the proposed structure. The ^{13}C-nmr spectrum was almost superimposable on that of SE-2 (see in Table 9) except those due to C-1', C-3', C-5', and C-6'. The signal of C-1', C-3', and C-5' shift to their lower field region at δ 126.77 ppm, 116.18 ppm, and 120.69 ppm respectively. These shift resulted from resonance effect of hydroxy group at C (2'). The signal of C-6' shift to its higher field region , when compared with chemical shift of SE-2. Because there have not inductive effect due to hydroxyl group at C-6'.

The mass spectrum (MS) of SE-3, a fragment, m/z 285 (M^+-123), suggested the presence of lavandulyl group, a fragment, m/z 120, due to the retro-Diels-Alder cleavage of the flavanone, indicated that the B-ring of SE-3 has one hydroxyl group. The fragment at m/z 288 (M^+-120) suggested that the A-ring has one lavandulyl and two hydroxy groups (Fig. 34). These data supported that compound SE-3 has three hydroxy groups at C-5, C-7, and C-2' although the ^{1}H-nmr of SE-3 showed only one hydroxyl group at δ 12.16ppm.

The assignments of protons and carbons of compound SE-3 are comfirmed by comparison with the data of kushenol A (67) (5, 7, 2'-trihydroxy-8-lavandulylflavanone) previously reported by Wu *et al* (1984). The assignments are summarized in Table 8 and Table 9. The structure of SE-3 is shown below :



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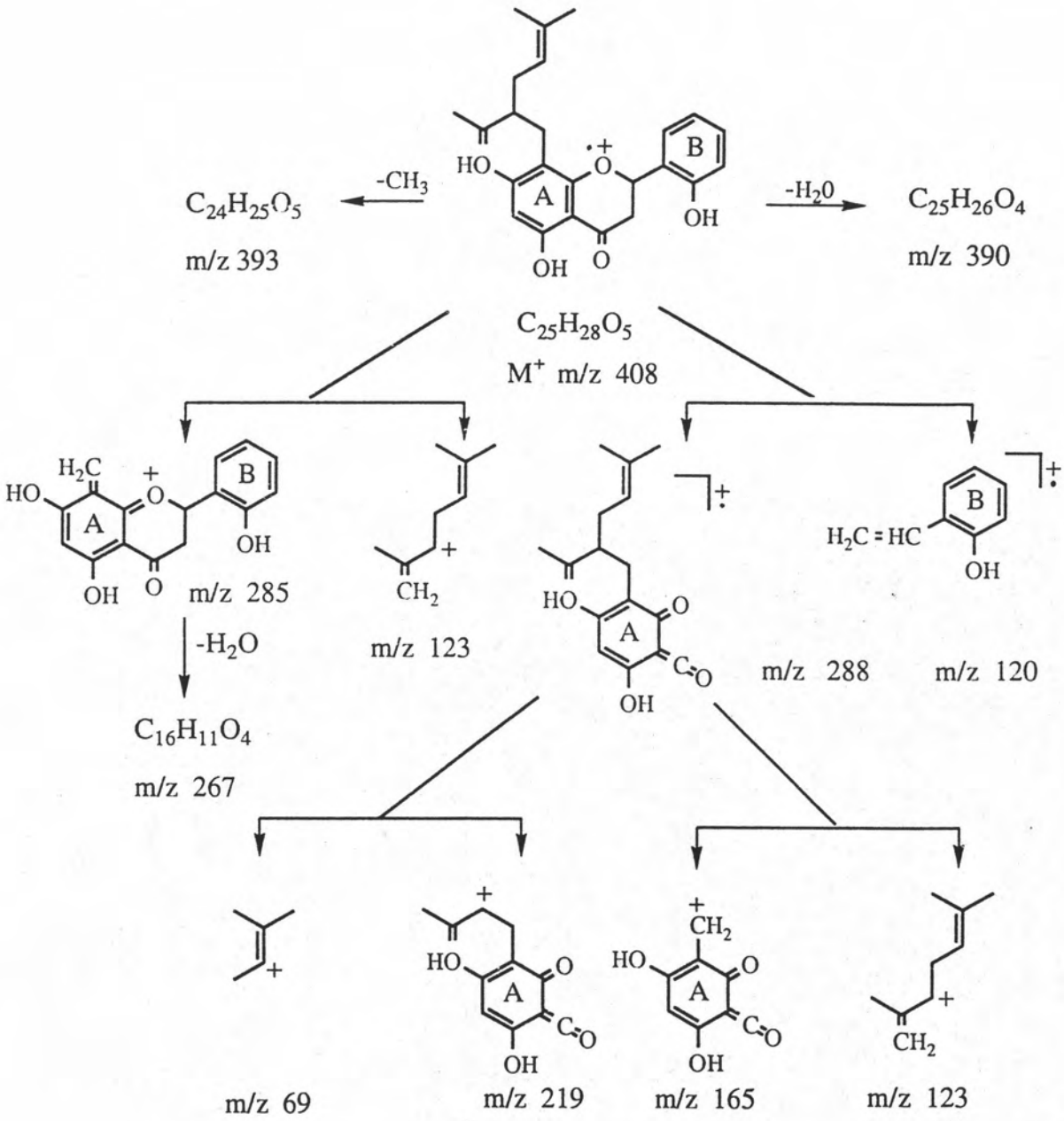


Figure 34 Mass fragmentation of SE-3

1.4 Compound SE-4

Compound SE-4 was obtained as amorphous powder from F-041 by chromatographic techniques using silica gel column (20% acetone in hexane) and silica gel column (10% ethyl acetate in hexane). It yielded 20 mg. This compound was positive with Shinoda's test and $FeCl_3$ TS reagent.

The EIMS spectrum (Fig. 35) showed the molecular ion peak at m/z 440 (4 %) and established the proposed molecular formula of $C_{25}H_{28}O_7$. The UV spectra of SE-4 in methanol (Fig. 37) showed absorption maxima at 291 and 340 nm.indicated the characteristic of a flavanone chromophore. The bathochromic shift (24 nm) of band II in the UV spectrum with the presence of $AlCl_3$/HCl (Fig. 37) was effected from the acid stable $AlCl_3$. From these data, SE-4 was considered to have 5-hydroxyflavanone moiety. The IR spectrum of SE-4 (Fig. 36) showed the presence of hydroxyl groups (3,350, 3,402 cm^{-1}) and a conjugated carbonyl group (1,600 cm^{-1}).

Compound SE-4 was assigned as a known flavanone, 5, 7, 2', 4', 6'-pentahydroxy-8-lavandulylflavanone (68), by the analysis of ^1H and ^{13}C-nmr-spectra. The ^1H-nmr spectrum (Fig. 38) provided the signals of 8 aliphatic protons, 6 olefenic protons, 5 hydroxyl groups, and 3 methyl groups. The ^{13}C-nmr (Fig. 41) shows the signals 1 carbonyl carbon, 5 sp^3 carbons, 5 sp^2 carbons, 10 quaternary carbons , and 3 methyl carbons.

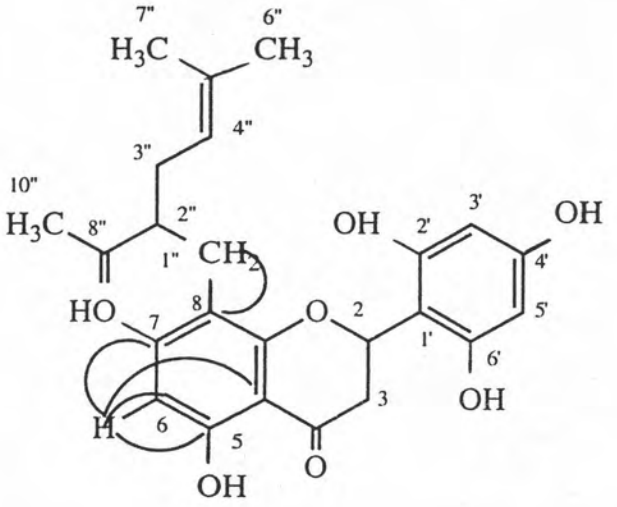
The signals in ^1H-nmr spectrum of SE-4 showed signal due to chelated hydroxy proton (δ 12.28 ppm). Three one proton signals at δ 2.47 ppm (1H, dd, J = 18, 3 Hz), 3.93 ppm (1H, dd, J = 18,14), 5.90 ppm (1H, dd, J = 14,3 Hz) were assigned to H-3*Z*, H-3*E*, and H-2 of the flavanone , respectively. These three signals (Fig. 39 and Fig 40) were due to one proton of C-2 together with two protons at C-3. The ^1H-nmr showed the presence of the lavandulyl group [δ 1.56 ppm (3H, d, J = 1.2 Hz) , 1.62 (3H, d, J = 1.2 Hz) , 1.71 (3H, dd, J = 1.2,1 Hz), assigned to position 7", 6", 10" , respectively, 2.08 (2H, m, H-3"), 2.59 (1H, m, H-2"), 2.65 (1H , m , H_b-1") , 2.68 (1H, dd , J = 15, 8 , H_a-1") , 4.57 (1H , brd , J = 1.2 Hz , H_a-9") , 4.61 (1H, dq, J = 1.2, 1.1 Hz, H_b-9") , 5.05 (1H,triplets of heptet, J = 7, 1.2 Hz).

The two dimensional ^{1}H-^{1}H COSY nmr spectrum (Fig. 42) showed the correlation of signal at δ 1.71 ppm to the signal at C (9")-H (δ : 4.57-4.61 ppm), was due to the methyl protons at C (8") , and the ones at δ 1.56 and 1.62 ppm which showed the correlation to the signal of C (4")-H (δ : 5.05 ppm) and C (3")-H (δ : 2.08 ppm), were due to the protons of two methyl groups at C-(5").

Two aromatic protons at δ 6.03 ppm (2H, s, H-3', and H-5') showed the presence of 2', 4', 6'-trioxygenatedflavanone. This signal was due to two symmetric protons (H-3', H-5') of the aromatic B-ring. The ^{1}H-nmr also showed the presence of four hydroxyl groups at δ 8.33 ppm (1-OH, s, 4'-OH), 8.49 ppm(2-OH, s, 2',6'-OH), and 9.59 ppm (1-OH, s, 7-OH).

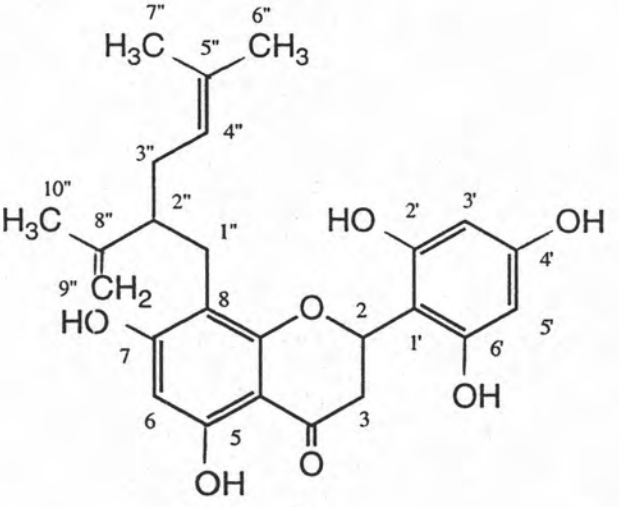
The ^{13}C-nmr spectrum of compound SE-4 confirms the proposed structure. Their assigned was mainly based on the ^{13}C-^{1}H COSY nmr spectrum (Fig. 43) and the COLOC spectrum (8 Hz) (Fig. 44). .The ^{13}C-nmr spectrum was almost superimposable on that of SE-3 (see in Table 9) except those due to C -1', C-3', C-5', and C-4'. The signal of C-1', C-3', and C-5' shift to their higher field region at δ 162.80 ppm, 103.93 ppm, and 96.12 ppm respectively. These shift resulted from the shielding effect of the hydroxy group adding to C-4'. The signal of C-4' shift to its lower field region at 162.80 ppm because of an inductive effect due to hydroxyl group adding to C-4'.

These spectral data indicated that SE-4 was 5, 7, 2', 4', 6'-pentahydroxyflavanone with a lavandulyl group at either C-6 or C-8. The COLOC spectrum (8 Hz) (Fig. 44) showed that the position of the lavandulyl group was at C-8 because a proton at δ 5.07 cause a cross peak with a carbon signal at C -5 at 163.11 ppm), C-7 at 165.12 ppm, C-10 at 102.94 ppm and at 95.14 ppm which can assigned to C-6. So this proton was assigned to H-6 of aromatic ring-A. And the COLOC spectrum also showed that a proton signal at δ 2.68 ppm cause a cross peak with a carbon at 108.19 ppm which assigned to C-8. So this protons assigned to H-1" of lavandulyl side chain. .



The mass spectrum (MS) of SE-4, a fragment, m/z 317 (M^+-123), suggested the presence of lavandulyl group, a fragment, m/z 152 due to the retro-Diels-Alder cleavage of the flavanone, indicated that the B-ring of SE-4 has three hydroxy group. The fragment at m/z 288 (M^+-152) suggested that the A-ring has one lavandulyl and two hydroxy groups (Fig. 45).

From all of these informations, SE-4 can be indentified a new member of the flavanones,named as 5, 7, 2', 4', 6'-pentahydroxy-8-lavandulylflavanone (68). The assignments are summarized in Table 8 and Table 9. The structure of SE-4 is shown below :



(68)

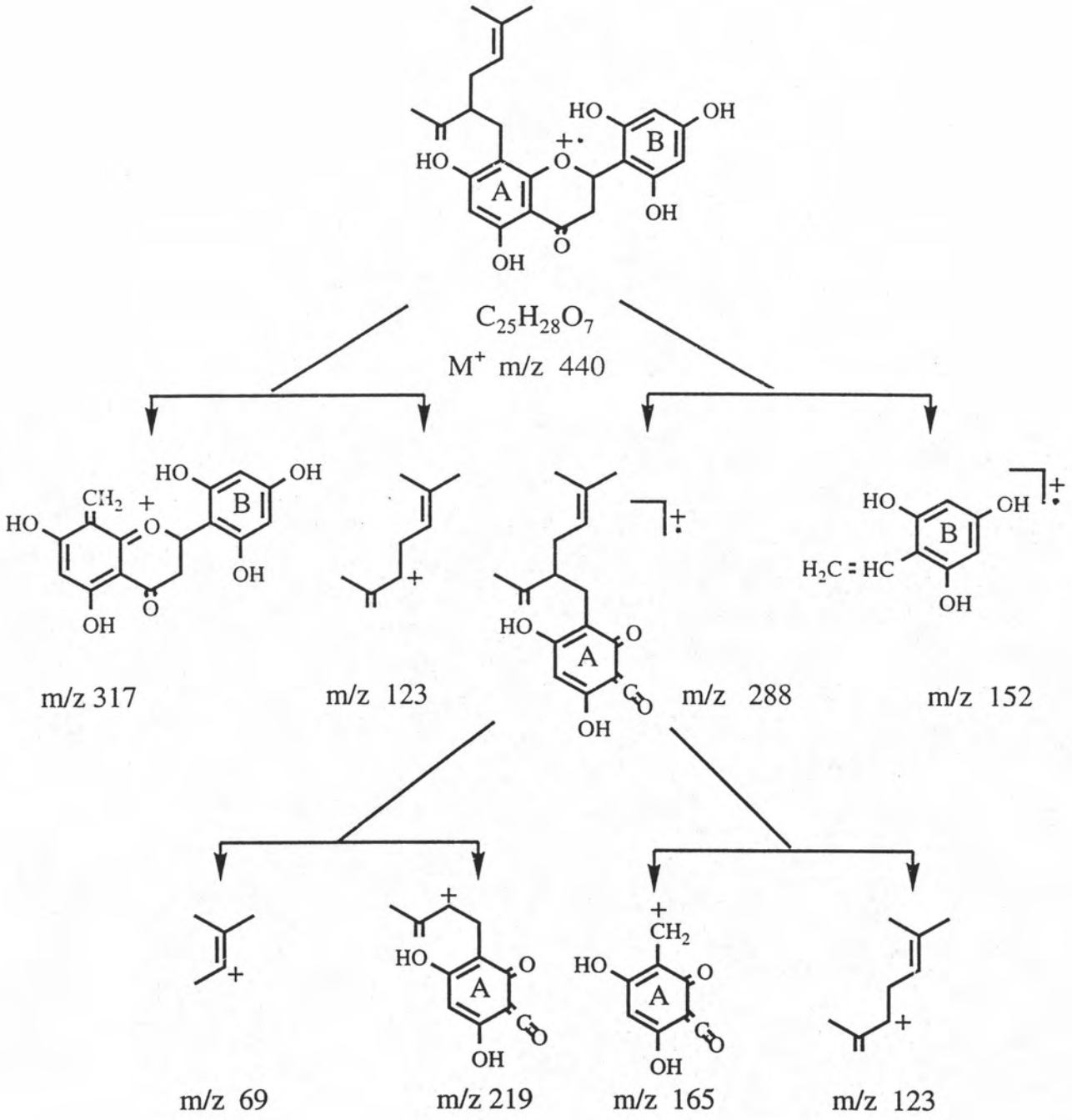


Figure 45 Mass fragmentation of SE-4

Table 8 ^{1}H NMR spectral Data of compound SE-1, SE-2, SE-3, and SE-4 (δ ppm, Hz)

H	SE-1*	SE-2**	SE-3**	SE-4**
2	5.95 (dd, *J*= 14, 3)	6.15 (dd, *J*=14, 3)	5.74 (dd, *J*=14, 3)	5.90(dd,*J*=14, 3)
3*Z*	2.83 (dd, *J*= 17, 3)	2.53 (dd, *J*=15,.3)	2.90 (dd, *J*= 17,.3)	2.47(dd,*J*=18, 3)
3*E*	3.22(dd, *J*=17, 14)	3.85 (dd, *J*=17, 14)	3.30(dd, *J*=17,14)	3.93 (dd *J*=18, 14)
5	12.25 (OH, S)	12.26 (OH, S)	12.16 (OH, S)	12.28 (OH, S)
6	6.15 (s)	6.02 (s)	6.05 (s)	5.07 (s)
7	-	- -	9.59 (s)	
2'	-	- -	8.49 (s)	
3'	6.45 (d, *J* = 8.3)	6.49 (d, *J* = 9)	6.95 (dd, *J*=7.5, 1)	6.03 (s)
4'	7.09 (t, *J* = 8.3)	7.03 (t, *J* = 9)	7.22 (ddd, *J* = 7.5, 7.5, 1.8)	8.33 (s)
5'	6.45 (d, *J* = 8.3)	6.49 (d, *J* = 9)	6.96 (ddd, *J*=7.5, 7.5, 1)	6.03 (s)
6'	-	-	7.62(dd,*J*=7.5,1.8)	8.49 (s)
1"	2.65 (dd, *J* = 14, 8.3, Ha), 2.55 (dd, *J* = 14, 6.1, Hb)	2.88 (d, *J* =6.2)	2.66 (dd, *J* = 14,6)	2.68 (dd, *J* =15 , 8, Ha), 2.65(m, Hb)
2"	2.24 (tt, *J*=8.3,6.1)	2.57 (m)	2.54 (m)	2.59 (m)
3"	2.01 (dt, *J*=20,6.7, Hb, *J*=20,8.3, Ha)	2.06 (m)	2.07 (m)	2.08 (m)
4"	4.99 (brt, *J* = 7)	4.96 (brt, *J* = 5.5)	5.00(th, *J*=6.7,1.2)	5.05 (th,*J*=7, 1.2)
6"	1.58 (s)	1.54 (s)	1.56 (d, *J*=1.2)	1.62 (d, *J*= 1.2)
7"	1.46 (s)	1.48 (s)	1.47 (d, *J*=1.2)	1.56(d, *J*=1.2)
9"	4.48 (brd,*J*=1.1) 4.64 (p, *J*=1.1)	4.55 (m)	4.57 (brd,*J*=1) 4.61(p, *J*=1)	4.57 (brs) , 4.61 (dq, *J*= 1.3,1.2)
10"	1.63 (s)	1.61 (s)	1.66 (dd, *J*=1)	1.71 (dd, *J*=1.2, 1.1)
OCH_3	3.84 (s)	-	-	-

* Measured in deuterated chloroform ** Measured in deuterated acetone

Table 9 ^{13}C NMR spectral data of compound SE-1, SE-2, SE-3, and SE-4 (δ ppm)

C	SE-1*	SE-2**	SE-3**	SE-4**
2	75.54	73.76	75.46	76.4 4
3	41.28	40.65	42.78	41.19
4	196.90	198.62	197.79	198.95
5	163.06	163.14	163.08	163.1
6	93.54	96.29	96.32	95.14
7	166.05	165.07	165.42	165.12
8	109.42	107.88	107.92	108.19
9	157.96	162.25	161.85	163.06
10	102.99	103.27	103.22	102.94
1'	110.54	111.12	126.77	103.93
2'	154.59	157.69	154.83	158.60
3'	108.92	108.45	116.18	96.12
4'	130.04	130.80	130.01	162.80
5'	108.92	108.45	116.18	96.12
6'	154.59	157.69	127.40	158.60
1"	26.76	27.73	27.80	27.42
2"	47.60	47.85	47.86	47.59
3"	31.48	31.93	31.87	32.15
4"	122.96	124.46	124.40	124.56
5"	132.00	131.59	131.70	131.54
6"	25.61	25.83	25.81	25.89
7"	17.71	17.84	17.84	17.93
8"	148.03	149.17	149.15	149.10
9"	110.93	110.89	111.21	111.20
10"	18.80	19.07	19.21	18.94
OMe	55.83	-	-	-

* Measured in deuterated chloroform

** Measured in deuterated acetone

All of isolated flavonoids in this investigation have a lavandulyl group substitution. This agree with a few chemotaxonomic points of view. The first one is that most of flavonoids with C5 or C10 side chain were found in leguminous plants. From the data survey indicated that, *Sophora* is one of the genus which enriched of various kind of flavanone. In addition, it can be notified that the lavandulyl group may be one of the chemotaxonomic marker of the genus *Sophora.* So, this recent work support the classification of genus *Sophora* in family Leguminosae and the chemotaxonomic significance agree with the morphological classification.

In the present study, the isolated flavanone which have the substitution pattern at C-2' and C-6' in the B-ring of flavanone is rare in nature. Such flavanones are only known previously from *Lonchocarpus orotinus* Benth (Leguminosae) (orotonin and orotonin 5-methyl ether). (+)-5, 2'-Dihydroxy-7, 8, 6'-trihydroxyflavanone, and (+) 5, 2'-dihydroxy-7, 8, 6'-trimethoxyflavanone, from the roots of *Scutellaria discolor* Colebr. (Labiatae), 5, 7, 2', 6'-tetrahydroxyflavanone and its 5-methylether from *S. baicalensis* (Kimura *et al*, 1982 ; Timimori *et al*, 1985 ; Waterman *et al*, 1987).

2.Biological Activities of the Isolated Compounds

The activities from the cardiovascular screening (RBC CA^{2+}-ATPase and Mg^{2+}-ATPase activity assay) determine a potential medicinal use for isolated compounds. The results are reported about % inhibition of Ca^{2+}ATPase and shown in Table 10.

Table 10 Red Blood Cell Ca^{2+}-ATPase Assay

Compound/Conc. μM	% Inhibition of Ca^{2+}-ATPase
SE-1	
200	100
100	100
50	61
25	1
10	0
SE-2	
200	100
100	100
50	100
25	64
10	20
SE-3	
200	100
100	100
50	100
25	46
10	0

The compounds SE-2 and SE-3 was found to be a moderately strong inhibitor with an IC_{50} of 20 μM, 26.5 μM respectively. Compound SE-1 has much lower activity (IC_{50} = 45.3 μM).

The test showed that SE-2 was the most active inhibitors of the three compounds tested. The results for brine shrimp toxicity of this compound. Lethal concentrations giving 50% deaths (LC_{50}) were determine that SE-2 LC_{50} was greater than 100 μM (17% deaths at 100 μM). The test indicated that all three compounds showed low toxicity towards brine shrimp.