

CHAPTER III

RESULTS AND DISCUSSION

3.1 Structural Elucidation of Compound I

Compound I was a bright white needle-like crystals with the melting point of 137-138°C and this compound was suggested the presence of a steroidal moiety because it gave a green colour (positive test) with Liebermann-Burchard's reagent. Its IR spectrum (Fig A.1) indicated the presence of O-H stretching at 3500-3430 cm^{-1} and C-O stretching at 1050 and 1020 cm^{-1} . The absorption band at 1650 cm^{-1} revealed the presence of C=C stretching vibration. The band at 2960 and 2840 cm^{-1} corresponded to the C-H stretching vibration of $-\text{CH}_3$ and $-\text{CH}_2-$ and the C-H bending vibration of them were shown at 1460 and 1380 cm^{-1} . The absorption band at 840 and 800 cm^{-1} were contributed to Δ -3 β -hydroxy steroids [59] (C-H out of plane bending). The principle IR absorption bands can be assigned as shown in Table 3.1.

Table 3.1 : The infrared absorption band assignments of Compound I

Wavenumber(cm^{-1})	Band type	Tentative assignment
3500-3430	strong, broad	O-H stretching vibration of R-OH
2960, 2840	strong	C-H stretching vibration of $-\text{CH}_3$, $-\text{CH}_2-$
1650	weak	C=C stretching vibration
1460, 1380	strong	C-H bending vibration of $-\text{CH}_3$, $-\text{CH}_2-$
1050, 1020	strong, medium	C-O stretching vibration
840, 800	weak	C-H out of plane bending vibration of $\text{R}_1\text{R}_2\text{C}=\text{CHR}_3$

The ^1H -NMR spectrum (Fig.A.3) showed signals of proton at 0.62-2.26 ppm which were compatible with the signals of methyl, methylene and methine protons. The chemical shift at 3.50 ppm corresponding to the signal of the proton attached to a carbon bearing a hydroxyl group (CH-OH) and the signal at 5.33 ppm exhibited the presence of the olefinic proton (-CH=C-).

The ^{13}C -NMR spectrum (Fig.A.4) in the range of 11.85-56.75 ppm indicated the sp^3 CH_3 , CH_2 and CH. The signals at 121.72 and 140.73 due to the olefinic carbon, $\text{CH}=\text{C}$ and $\text{CH}=\text{C}$, respectively.

All of the colour test, IR spectrum and NMR spectrum suggested that Compound I could be a steroidal compound which has a hydroxyl group, and was confirmed by GLC technique. Comparison the chromatogram of Compound I with that of the authentic samples of cholesterol, campesterol, stigmasterol and β -sitosterol (Fig.A.7) is shown one peak at the retention time 20.84 which were corresponded to β -sitosterol. EI mass spectra (Fig.A.2) exhibited a molecular ion peak at $M^+ = 414$ was compatible with the molecular formula, $\text{C}_{29}\text{H}_{48}\text{O}$, of β -sitosterol and Table 3.2 shows the comparison with the authentic sample [60].

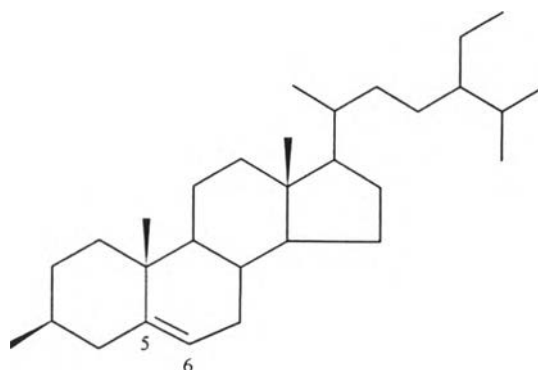
Table 3.2 : Comparison of the ^{13}C -NMR spectrum of Compound I and β -sitosterol

Position of carbon	β -sitosterol, δ (ppm)	Compound I, δ (ppm)
1	37.40	37.23
2	31.80	31.61
3	71.90	71.81
4	42.40	42.24
5	140.90	140.73
6	121.80	121.72
7	32.00	31.89
8	32.00	31.89

Table 3.2 (continue) : Comparison of the ^{13}C -NMR spectrum of Compound I and β -sitosterol

Position of carbon	β -sitosterol, δ (ppm)	Compound I, δ (ppm)
9	50.30	50.11
10	36.60	36.49
11	21.10	21.07
12	39.90	39.76
13	42.40	42.24
14	56.80	56.75
15	24.30	24.29
16	28.20	28.23
17	56.20	56.04
18	11.90	11.97
19	19.40	19.39
20	36.20	36.13
21	19.10	19.02
22	34.00	33.93
23	29.30	29.13
24	50.30	50.11
25	26.20	26.05
26	18.80	18.76
27	19.80	19.81
28	23.10	23.05
29	11.90	11.85

From the data aboved, these results suggested that Compound I was β -sitosterol.



β -sitosterol

3.2 Structural Elucidation of Compound II

Compound II was a yellow needle-like crystals and the melting point was 142-143°C. The IR absorption spectrum (Fig.A.8) exhibited the absorption band of C-H stretching of C-H aromatic at 3010 cm^{-1} . The C-H stretching and bending vibration of $-\text{CH}_3$ and $-\text{CH}_2-$ were shown at 2960 and 2910, 1470 and 1380 cm^{-1} , respectively. A strong peak at 1720 cm^{-1} was compatible with C=O stretching vibration of aliphatic ester [59] and the bands at 1520 and 1510 cm^{-1} revealed the C-H stretching of $-\text{O}-\text{CH}_3$ (C-O bond of ester group). The C=C stretching of $-\text{C}=\text{C}-\text{CO}$ and $-\text{C}=\text{C}-\text{Ph}$ were presented at 1630 and 1620 cm^{-1} , respectively. The absorption bands at 1445 cm^{-1} due to C-H stretching of $-\text{CH}_2-\text{O}-\text{Ph}$ and the bands at 1285 and 1260 cm^{-1} were compatible with C-O-C bonding (C-O bond of ester group). The results of infrared absorption bands were summarized in Table 3.3.

Table 3.3 : The infrared absorption band assignments of Compound II

Wavenumber(cm^{-1})	Band type	Tentative assignment
3010	weak	C-H stretching vibration of C-H aromatic
2960, 2910	weak	C-H stretching vibration of $-\text{CH}_3, -\text{CH}_2-$
1720	strong	C=O stretching vibration of ester group
1630	strong	C=C stretching vibration of $-\text{C}=\text{C}-\text{CO}$
1620	strong	C=C stretching vibration of $-\text{C}=\text{C}-\text{Ph}$
1520, 1510	strong	C-H stretching vibration of $-\text{O}-\text{CH}_3$
1445	strong	C-H stretching vibration of $-\text{CH}_2-\text{O}-\text{Ph}$
1285, 1260	strong	C-O-C bending vibration of ester group

The ^1H -NMR spectrum (Fig.A.10) showed the signal with 3H of methoxy group at 3.74 ppm and the signal at 5.95 ppm revealed the $\text{O}-\text{CH}_2-\text{O}$. The signals with 1H each at 6.46, 6.73, 6.82 and 7.39 ppm corresponding to $-\text{CH}=\text{alkene}$ and each at 6.75, 6.88 and 6.96 corresponding to $-\text{CH}=\text{aromatic}$.

From ^{13}C -NMR spectrum (Fig.A.11), ^{13}C -DEPT 90 (Fig.A.12) and ^{13}C -DEPT 135 (Fig.A.13), signal at 51.50 ppm was compatible with $-\text{OCH}_3$ (methoxy group) and the signal at 101.47 ppm corresponding to $\text{O}-\text{CH}_2-\text{O}$. The signals of $-\text{CH}=\text{(alkene)}$ were presented at 106.01, 108.62, 120.44 and 123.10 ppm and the signals of $-\text{CH}=\text{(aromatic)}$ were shown at 124.98, 140.73 and 145.02 ppm. The signals at 130.68, 148.89 and 149.00 ppm were assigned for $-\text{C}=\text{C}$ of aromatic and the signal of carbonyl of aliphatic ester ($\text{C}=\text{O}$) was inhibited at 167.94 ppm.

From the above mentioned NMR data, this compound was suggested that it was corresponded to methyl piperate [44,47] and the comparison of ^1H and ^{13}C -NMR signals were shown in Tables 3.4 and 3.5.

Table 3.4 : Comparison of the ^1H -NMR spectrum of Compound II and methyl piperate

Position of proton	Methyl piperate δ (ppm)	Compound II δ (ppm)	No. of hydrogen, peak type
2	6.75	6.73	1H, d
3	7.40	7.39	1H, dd
4	6.46	6.46	1H, dd
5	6.80	6.82	1H, d
7	6.97	6.96	1H, d
10	6.76	6.75	1H, d
11	6.89	6.88	1H, dd
-OCH ₂ O-	5.95	5.95	2H, s
-OCH ₃	3.85	3.74	3H, s

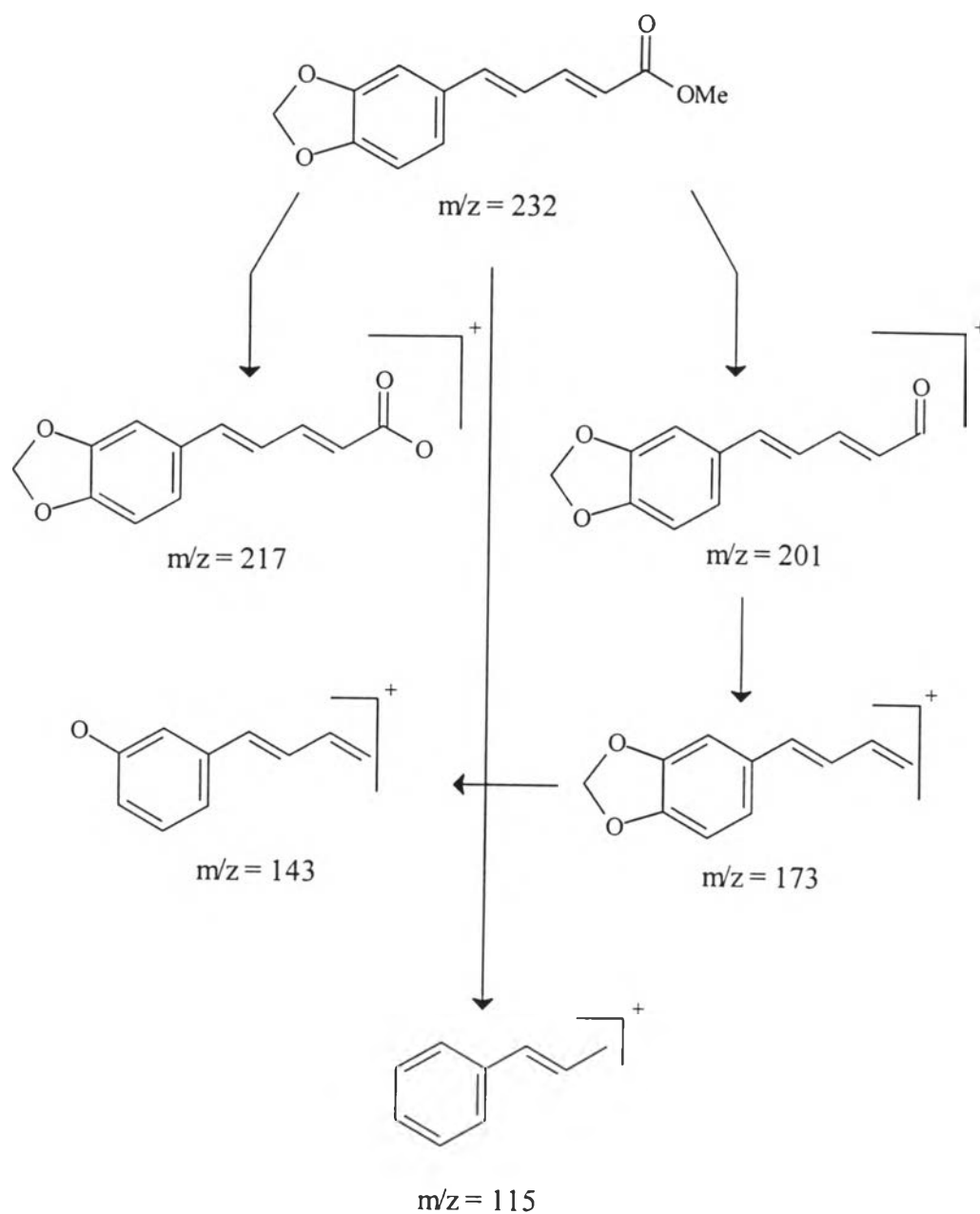
Table 3.5 : Comparison of the ^{13}C -NMR spectrum of Compound II and methyl piperate

Position of carbon	Methyl piperate, δ (ppm)	Compound II, δ (ppm)
1	165.70	167.94
2	121.60	123.10
3	107.20	108.62
4	104.70	106.01
5	118.70	120.44
6	129.30	130.68
7	123.30	124.98
8	147.30	149.00
9	147.10	148.89
10	139.60	140.73

Table 3.5 (continue) : Comparison of the ^{13}C -NMR spectrum of Compound II and methyl piperate

Position of carbon	Methyl piperate, δ (ppm)	Compound II, δ (ppm)
11	143.50	145.02
-OCH ₂ O-	100.10	101.47
-OCH ₃	49.90	51.50

From mass spectrum (Fig.A.9), the molecular ion was displayed at $M^+ = 232$ and other important fragmentations at 217 ($M^+ - \text{CH}_3$), 201 ($M^+ - \text{OCH}_3$), 173 ($M^+ - \text{OCH}_3 - \text{CO}$), 143 ($M^+ - \text{C}_3\text{H}_5\text{O}_3$) and 115 ($M^+ - \text{C}_3\text{H}_5\text{O}_3 - \text{CO}$). It indicated that Compound II had a similar fragmentation to methyl piperate (Scheme 3.1).

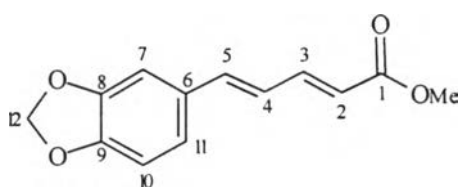


Scheme 3.1 : The fragmentation of Compound II

^1H - ^1H COSY (Fig.A.14) and ^1H - ^1H NOESY (Fig.A.15) presented the signal of proton at 6.73 ppm (H-2) coupled with the signal of proton at 7.39 ppm (H-3) and coupled long range with the signal at 6.46 ppm (H-4). The signal of proton at 6.46 ppm (H-4) coupled with both of the signals at 7.39 ppm (H-3) and 6.82 ppm

(H-5), but the signals of protons at 3.85 ppm (OMe), 5.95 ppm (OCH₂O) and 6.97 ppm (H-7) not coupled with the others.

From the above spectral data and comparison of Compound II with methyl piperate, this compound can be determined that it was a methyl-5-(3',4'-methylenedioxyphenyl)penta-2,4-dienoate which its common name is methyl piperate, C₁₃H₁₂O₄, and the structure of Compound II is shown below.



methyl piperate

3.3 Structural Elucidation of Compound III

Compound III was a bright colourless needle crystals or rectangular prism which were recrystallized from a mixture of chloroform-hexane and ethyl acetate-hexane, respectively. Its melting point was 158-159°C and R_f value was 0.61(SiO₂ : 40% ethyl acetate in hexane). The IR spectrum (Fig.A.16) showed a broad absorption band of hydroxyl group (O-H) at 3600-3200 cm⁻¹, C-H stretching of -CH aromatic at 3075 cm⁻¹ and the band at 840 cm⁻¹ revealed p-substitution of hexane ring. The absorption band at 1680 cm⁻¹ indicated the presence of carbonyl group. Chemical reaction between Compound III and NaHCO₃ gave no carbon dioxide (negative test) which was carried out to further confirm that Compound III was ester. The C-O bending absorption peaks were displayed at 1190 and 1170 cm⁻¹. The absorption bands at 1630 and 1600 cm⁻¹ revealed alkene group (C=C) while the bands at 1515 and 1450 cm⁻¹ showed the presence of C=C stretching vibration of C=C aromatic. The absorption bands at 1370 and 1330 cm⁻¹

corresponding to $\text{CH}_3\text{-C-CH}_3$ and the infrared spectrum assignments of this compound is presented in Table 3.6.

Table 3.6 : The infrared absorption band assignment of Compound III

Wavenumber(cm^{-1})	Band type	Tentative assignment
3600-3200	strong,broad	O-H stretching vibration of phenolic
3075	weak	C-H stretching vibration of -CH aromatic
2960, 2880	strong,medium	C-H stretching vibration of $-\text{CH}_3,-\text{CH}_2-$
1680	strong	C=O stretching vibration of ester
1630, 1600	medium,strong	C=C stretching vibration of C=C aliphatic
1515, 1450	strong,medium	C=C stretching vibration of C=C aromatic
1370, 1330	medium	C-H stretching vibration of $\text{CH}_3\text{-C-CH}_3$
1190, 1170	strong	C-O bending vibration
830	medium	p-substitution of benzene ring

The $^1\text{H-NMR}$ spectrum (Fig.A.18) showed the protons of $-\text{CH}_3$ (methyl group) at 0.86, 0.88 and 0.91 ppm and O-CH-CH_2 at 5.00 ppm. The signals at 6.32 and 7.61 ppm corresponding to $-\text{CH=CH-}$ alkene while the signals at 6.85(2H) and 7.42(2H) ppm were compatible with $-\text{CH=}$ aromatic and the signal of $-\text{OH}$ phenolic was shown at 6.31 ppm (broad).

The $^{13}\text{C-NMR}$ spectrum (Fig.A.19), $^{13}\text{C-NMR DEPT 90}$ (Fig.A.20) and $^{13}\text{C-NMR DEPT 135}$ (Fig.A.21) indicated the carbon of $-\text{CH}_3$ at 13.58, 18.95 and 19.72 ppm, $-\text{CH}_2-$ at 27.23, 28.06 and 36.85 ppm, $-\text{CH}$ aliphatic at 44.93 ppm, $-\text{O-CH}$ aliphatic at 80.41 ppm, $-\text{CH}$ alkene at 115.51 and 144.78 ppm, $-\text{CH=}$ aromatic at 115.98(2C) and 130.06(2C) ppm, tertiary-carbon aliphatic at 47.86 and 48.97 ppm, $-\text{C=}$ aromatic at 126.75 ppm, $-\text{O-C=}$ aromatic at 158.41 ppm and C=O at 168.72 ppm. The above NMR spectral data of Compound III were similar to the

NMR spectrum of borneol p-coumarate [43] as presented in Tables 3.7 and Table 3.8.

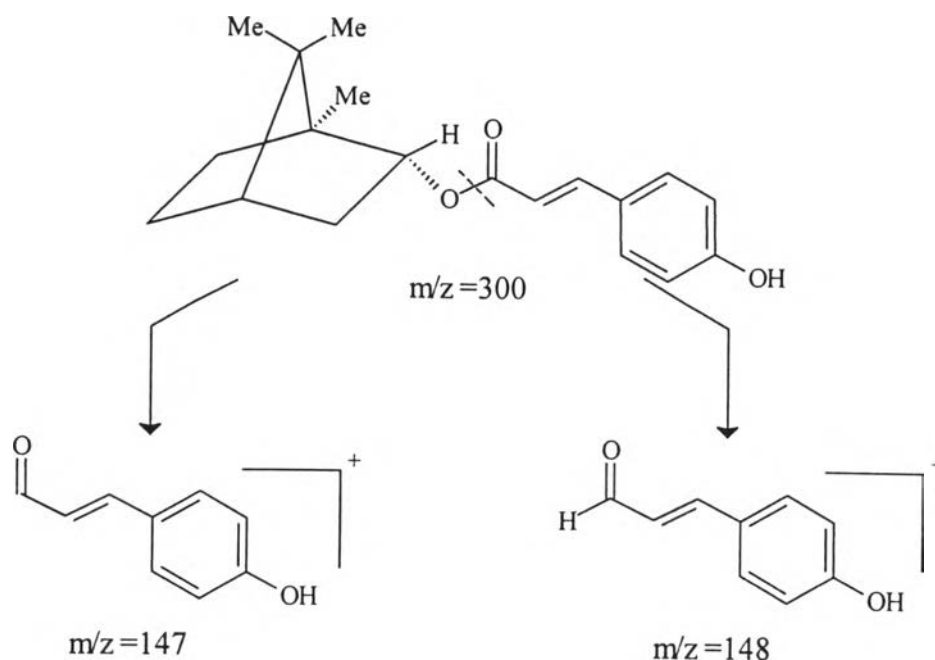
Table 3.7 : Comparison of the ^1H -MNR spectrum of Compound III and borneol p-coumarate

Position of proton	Borneol p-coumarate δ (ppm)	Compound III δ (ppm)	No. of hydrogen, peak type
2	5.01	5.00	1H, dt
3 α	1.05	1.04	1H, dd
3 β	2.43	2.40	1H, m
4	1.71	1.69	1H, t
5 α	1.28	1.25	1H, m
5 β	1.78	1.75	1H, m
6 α	2.05	2.00	1H, m
6 β	1.36	1.34	1H, m
8	0.90	0.88	3H, s
9	0.94	0.91	3H, s
10	0.88	0.86	3H, s
2'	6.33	6.32	1H, d
3'	7.62	7.61	1H, d
5', 9'	6.85	6.85	2H, br d
6', 8'	7.45	7.42	2H, br d
-OH	5.50	6.31	1H, br s

Table 3.8 : Comparison of the ^{13}C -NMR spectrum of Compound III and borneol p-coumarate

Position of carbon	Borneol p-coumarate, δ (ppm)	Compound III, δ (ppm)
1'	168.70	168.72
2'	115.10	115.51
3'	144.80	144.78
4'	126.40	126.75
5', 9'	115.80	115.98
6', 8'	129.90	130.06
7'	158.50	158.41
1	48.90	48.97
2	80.40	80.41
3	36.80	36.85
4	44.90	44.93
5	28.00	28.06
6	27.20	27.23
7	47.80	47.86
8	19.70	19.72
9	18.80	18.95
10	13.60	13.58

The mass spectrum of Compound III (Fig.A.17) showed the molecular ion peak at $M^+ = 300$ and the other important peaks were 147 and 148 (Scheme 3.2).



Scheme 3.2 : The fragmentation of Compound III

From ^1H - ^1H COSY (Fig.A.22) and ^1H - ^1H NOESY (Fig.A.23) the signal of proton at 6.32 ppm (H-2') coupled with the signal of proton at 7.61 ppm (H-3'), the signal of proton at 6.85 ppm (H-5', H-9') coupled with the signal of proton at 7.42 ppm (H-6', H-8') and the signal of proton at 5.00 ppm (H-2) coupled with the signal of proton at 1.05 ppm (H-3 α), 2.40 ppm (H-3 β) and 1.34 ppm (H-6 β) but not coupled with H-5 β (1.75 ppm) and H-6 α (2.00 ppm), respectively.

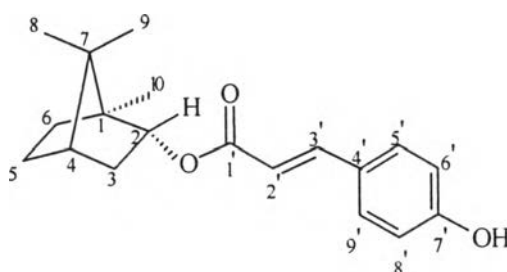
One bond C-H correlation (HMQC) and C-H long range coupling (HMBC) were carried out to further confirm the structure as presented in Fig.A.24 and Fig.A.25. From HMQC data showed the protons at 1.04 and 2.40 ppm were attached to the carbon at 36.85 ppm, the protons at 1.69 ppm was attached to the carbon at 44.93 ppm, the protons at 1.25 and 1.75 ppm were attached to the carbon at 28.06 ppm, and the protons at 1.34 and 2.00 were attached to the carbon at 27.23 ppm. In addition, the protons at 6.85 and 7.42 ppm were attached to the carbon at

115.98 and 130.06 ppm, respectively. The results of HMQC and HMBC techniques are summarized in Table 3.9.

Table 3.9 : The One Bond and Multiple Bond Correlation of Compound III

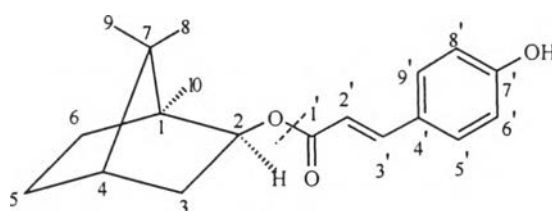
Carbon position	Proton (ppm)	One bond correlations Attached carbon (ppm)	Multiple bond correlations Attached carbon (ppm)
1	-	48.97	-
2	5.00	80.41	-
3	1.04,2.40	36.85	18.95,19.72
4	1.69	44.93	48.97,80.41
5	1.25,1.75	28.06	-
6	2.00,1.34	27.23	-
7	-	47.86	-
8	0.88	19.72	18.95,27.23,44.93,47.86, 48.97
9	0.91	18.95	19.72,27.23,44.93,47.86, 48.97
10	0.86	13.58	27.23,47.86,48.97,80.41
1'	-	168.72	-
2'	6.32	115.51	126.75,168.72
3'	7.61	144.78	115.51,115.98,130.06, 168.72
4'	-	126.75	-
5',9'	6.85	115.98	47.86,126.75,158.41
6',8'	7.45	130.06	47.86,144.78,158.41
7'	-	158.41	-
hydroxy in C-7'	6.31	-	115.51,115.98,158.41

From these above mentioned data, it can be determined that Compound III was borneol p-coumarate ($C_{19}H_{24}O_3$) and the structure is shown below.



borneol p-coumarate

Since the spectral data of Compound III (borneol p-coumarate) are shown as described above. It led to an important discovery that the previous report of *Piper ribesoides* Wall. [61] had been found the new compound from natural source which is isoborneol p-coumarate, $C_{19}H_{24}O_3$. It is an isomer of borneol p-coumarate and its structure is presented as follow:



isoborneol p-coumarate

Tables 3.10 and 3.11 showed the comparison of the 1H -NMR and ^{13}C -NMR data of these compounds.

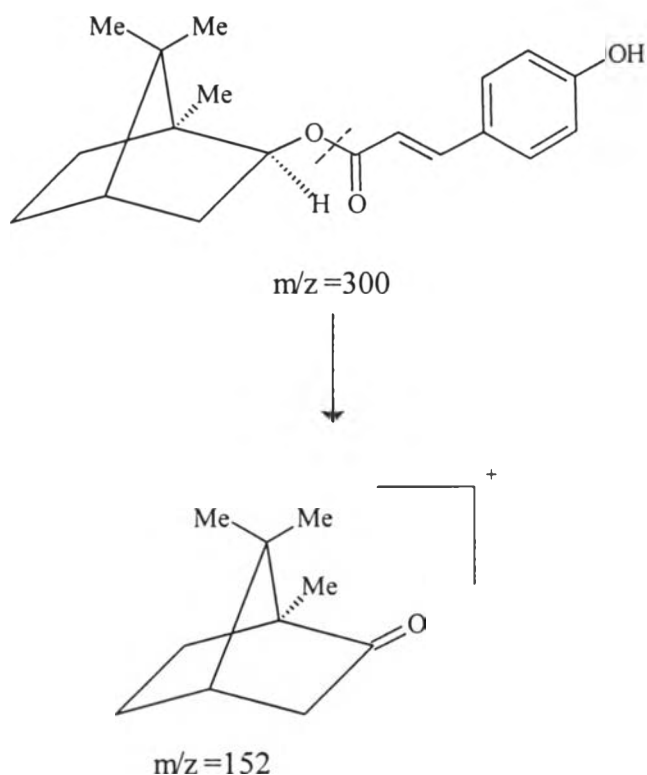
Table 3.10 : Comparison of the ^1H -MNR spectrum of borneol p-coumarate and isoborneol p-coumarate

Position of proton	Borneol p-coumarate δ (ppm), peak type	Isoborneol p-coumarate δ (ppm), peak type	No. of hydrogen
2	5.00, dt	4.81, t	1H
3 α	1.04, dd	1.21-1.83, m	1H
3 β	2.40, m	1.21-1.83, m	1H
4	1.69, t	1.21-1.83, m	1H
5 α	1.25, m	1.21-1.83, m	1H
5 β	1.75, m	1.21-1.83, m	1H
6 α	2.00, m	1.21-1.83, m	1H
6 β	1.34, m	1.21-1.83, m	1H
8	0.88, s	0.90, s	3H
9	0.91, s	0.87, s	3H
10	0.86, s	1.06, s	3H
2'	6.32, d	6.27, d	1H
3'	7.61, d	7.58, d	1H
5', 9'	6.85, br d	6.85, d	2H
6', 8'	7.42, br d	7.42, d	2H
-OH	6.31, br s	6.23, br s	1H

Table 3.11 : Comparison of the ^{13}C -NMR spectrum of borneol p-coumarate and isoborneol p-coumarate

Position of carbon	Borneol p-coumarate, δ (ppm)	Isoborneol p-coumarate, δ (ppm)
1'	168.72	167.70
2'	115.51	115.60
3'	144.78	144.60
4'	126.75	126.80
5', 9'	115.98	116.00
6', 8'	130.06	130.00
7'	158.41	158.30
1	48.97	49.00
2	80.41	81.50
3	36.85	39.00
4	44.93	45.00
5	28.06	27.00
6	27.23	33.70
7	47.86	47.70
8	19.72	20.10
9	18.95	20.11
10	13.58	11.50

The mass spectrum of isoborneol p-coumarate [61] presented the molecular ion peak at $M^+ = 302$ and the fragmentation pattern is 152 (Scheme 3.3).



Scheme 3.3 : The fragmentation of isoborneol p-coumarate

3.4 Structural Elucidation of Compound IV

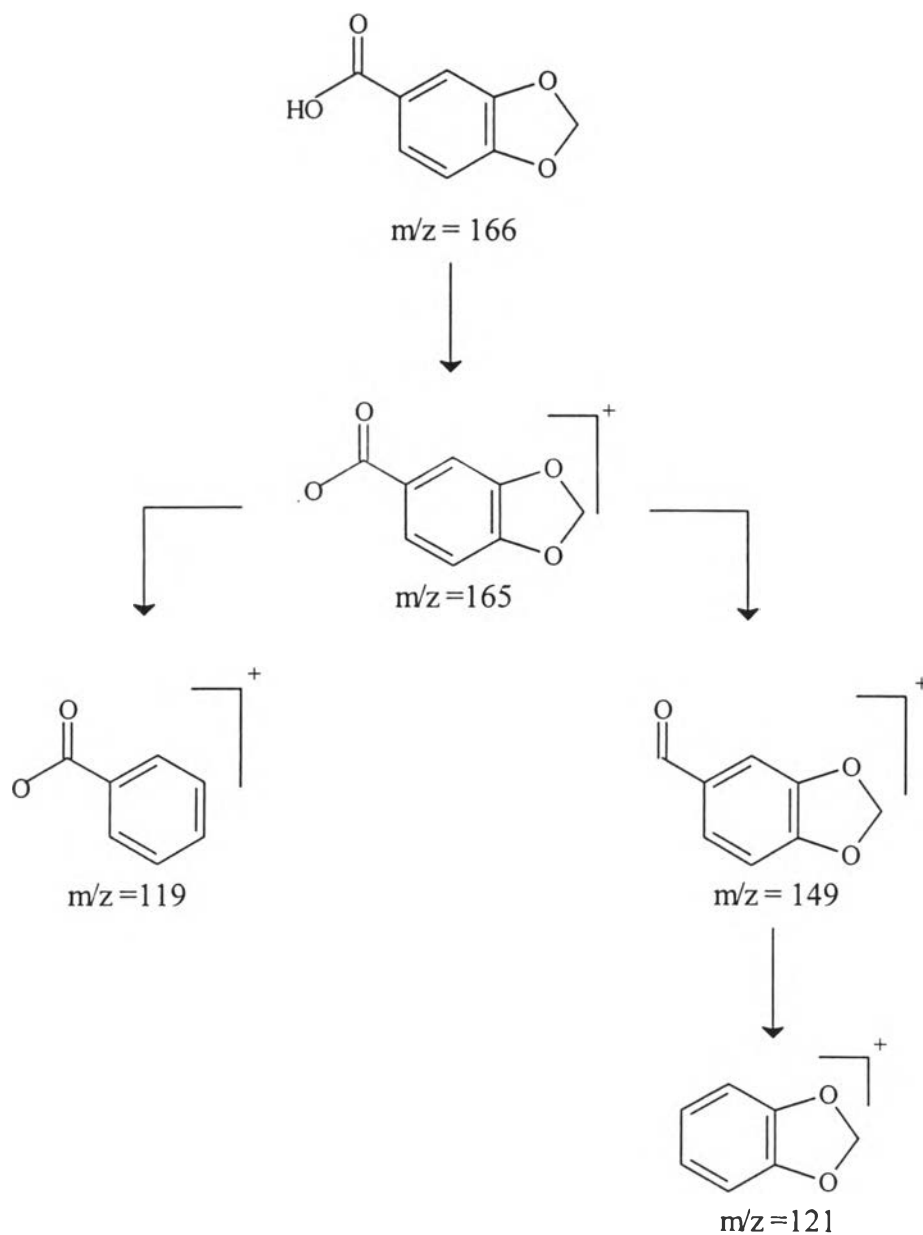
Compound IV was white amorphous solid (trace) which its IR spectrum (Fig.A.26) indicated the presence of O-H stretching vibration of carboxylic acid at $3300-2500\text{ cm}^{-1}$ and O-H bending at 1304 cm^{-1} . Conjugated C=O stretching was displayed at 1675 cm^{-1} and C-O stretching at 1265 cm^{-1} . The absorption band at 2920 cm^{-1} revealed the C-H stretching of O-CH₂-O and C=C stretching of aromatic moiety were shown at 1620 and 1455 cm^{-1} . The principal infrared absorption bands are presented in Table 3.12.

Table 3.12 : The infrared absorption band assignments of Compound IV

Wavenumber(cm^{-1})	Band type	Tentative assignment
3300-2500	broad	O-H stretching vibration of carboxylic acid
2920	weak	C-H stretching vibration of $-\text{CH}_2-$
1675	strong	C=O stretching vibration of carboxylic acid
1620, 1455	medium	C=C stretching vibration of aromatic
1304	medium	O-H bending vibration of carboxylic acid
1265	medium	C-O stretching vibration of carboxylic acid

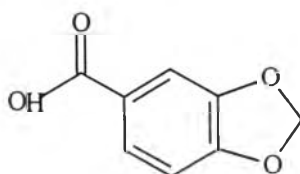
The $^1\text{H-NMR}$ spectrum (Fig.A.29) showed the proton of $\text{O-CH}_2\text{-O}$ at 6.05 ppm and the proton of $-\text{CH=}$ aromatic displayed at 6.85 ppm (H-7), 7.50 ppm (H-4) and 7.71 ppm (H-6).

EI mass spectra (Fig.A.27) exhibited molecular ion peak at $M^+ = 166$ and the other fragmentations (Scheme 3.4) are $165(M^+ - \text{H})$, $149(M^+ - \text{OH})$, $121(M^+ - \text{CHO}_2)$ and $119(M^+ - \text{CH}_3\text{O})$.



Scheme 3.4 : The fragmentation of Compound IV

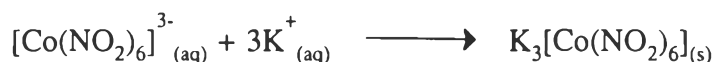
From the library search, the fragmentation pattern of Compound IV corresponding to the molecular formula, $C_8H_6O_4$, of 1,3-benzodioxole-5-carboxylic acid (Fig.A.28) and the structure of this compound as showed below.



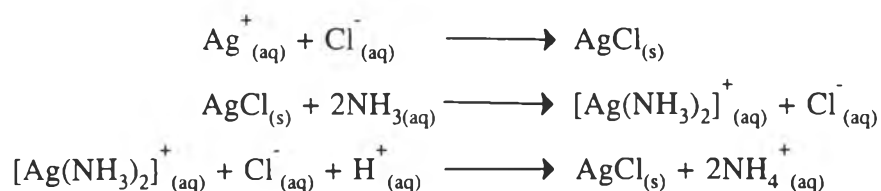
1,3-benzodioxole-5-carboxylic acid

3.5 Structural Elucidation of Compound V

Compound V was colourless cubical crystals and its melting point was over than 270°C which suggested that Compound V could not be an organic substance. This compound was soluble in water but insoluble in organic solvents, and presented a yellow precipitate of potassium cobaltinitrite when it was reacted with sodium cobaltinitrite ($\text{Na}_3[\text{Co}(\text{NO}_2)_6]$). This precipitate was insoluble in dilute acetic acid, as shown in the equation below. [62]



The flame test gave purple colour which supported that Compound V had K^+ as its cation. Further confirmation was reaction of this compound with silver nitrate solution (AgNO_3) gave white precipitate of silver chloride (AgCl) and it was soluble in dilute ammonia solution and precipitated back on the addition of dilute nitric acid. These results determined that Compound V had Cl^- as its anion, the equation was presented as follows:



It can be concluded that Compound V was KCl from the chemical analysis.