

## CHAPTER IV

### RESULT AND DISCUSSION

The 1.5 Kg of dried heartwood *A.lakoocha* Roxb. was macerated with petroleum ether and chloroform, respectively. From petroleum ether extract, AA-4 and BB-2 was obtained by column chromatography. The concentrated chloroform extract was dissolved in the mixture of water:ethanol (7:3) and then partition with hexane. From hexane part, BB-2 and BB-3.3 was obtained by column chromatography. During partition CC-1 was precipitated out.

The yield of AA-4, BB-2, BB-3.3 and CC-1 were  $4.8 \times 10^{-3}$  %,  $11.8 \times 10^{-3}$  %,  $2.47 \times 10^{-3}$  % and  $1.63 \times 10^{-1}$  %, respectively, based on dry weight of *A.lakoocha* Roxb. powder.

#### 1. Structure Elucidation of AA-4

Compound AA-4 was white plate that gave positive result with Libermann-Burchard reagent. Its melting point was 136-137°C. The melting of AA-4 after acetylation (AA-4Ac) was 124-125°C. The IR spectrum of AA-4 (Figure 2) showed the pattern of the functional group as table 8.

Table 8 The IR spectrum assignment of AA-4

Range of absorption (cm <sup>-1</sup> )	Assignment
3600-3100 (broad)	O-H stretching
2950-2800 (strong)	C-H stretching
1640 (weak)	C=C stretching
1460,1380 (medium)	C-H bending of CH <sub>3</sub>
1070 (medium)	C-O stretching
950,800 (weak)	C-H bending out of plane

The IR spectrum of AA-4Ac was shown in Figure 3. From two IR spectra, melting points and Libermann-Burchard reaction implied that AA-4 was steroid. The <sup>13</sup>C NMR spectrum of AA-4 was shown in Figure 4 and its chemical shift compared with standard  $\beta$ -sitosterol and standard stigmasterol shown in table 9 (Wright et al, 1978)

The MS spectrum of AA-4 and  $\beta$ -sitosterol shown in Figure 5 The fragment of AA-4 comprised characteristic fragment of  $\beta$ -sitosterol and stigmasterol. The peak at m/z 414 and 412 were molecular peak of  $\beta$ -sitosterol and stigmasterol, respectively. The scheme 6 showed the fragmentation pathway of  $\beta$ -sitosterol and stigmasterol (Biemann,1962).



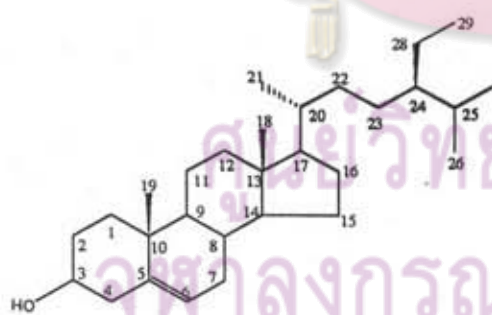
Table 9 The chemical shift of  $^{13}\text{C}$  NMR of  $\beta$ -sitosterol, stigmasterol and AA-4.

No. of carbon	$\delta$ (ppm) of $\beta$ - sitosterol	$\delta$ (ppm) of stigmasterol	$\delta$ (ppm) of AA-4
1	37.31	37.31	37.25
2	31.57	31.69	31.65
3	71.69	71.81	71.79
4	42.25	42.35	42.32
5	140.75	140.80	140.76
6	121.59	121.69	121.69
7	31.92	31.94	31.91
8	31.92	31.94	31.91
9	50.17	50.20	50.14
10	36.51	36.56	36.50
11	21.11	21.11	21.08
12	39.81	39.74	39.78
13	42.33	42.35	42.30
14	56.79	56.91	56.77
15	24.32	24.39	24.29
16	28.26	28.96	28.23
17	56.11	56.06	56.07
18	11.87	12.07*	11.85, 11.99*
19	19.40	19.42	19.38
20	36.17	40.54*	36.14, 39.78*

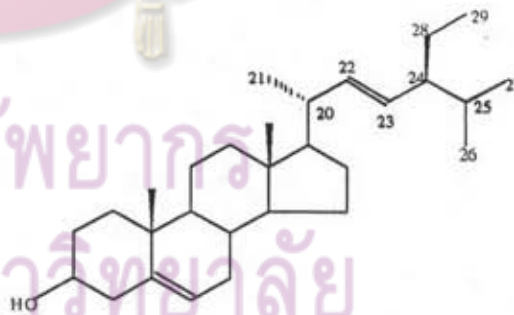
Table 9 continued.

No. of carbon	$\delta$ (ppm) of $\beta$ - sitosterol	$\delta$ (ppm) of stigmasterol	$\delta$ (ppm) of AA-4
21	18.82	21.11*	18.77, 21.08*
22	33.95	138.37*	33.96, 138.12*
23	26.13	129.32*	26.11, 129.28*
24	45.85	51.29*	45.85, 51.23*
25	29.18	31.94*	29.18, 31.91*
26	19.84	21.26*	19.80, 21.08*
27	19.07	19.02	19.03
28	23.09	25.44*	23.07, 25.38*
29	12.32	12.27	11.99

All data proposed that AA-4 was the mixture of  $\beta$ -sitosterol (2) and stigmasterol (3).

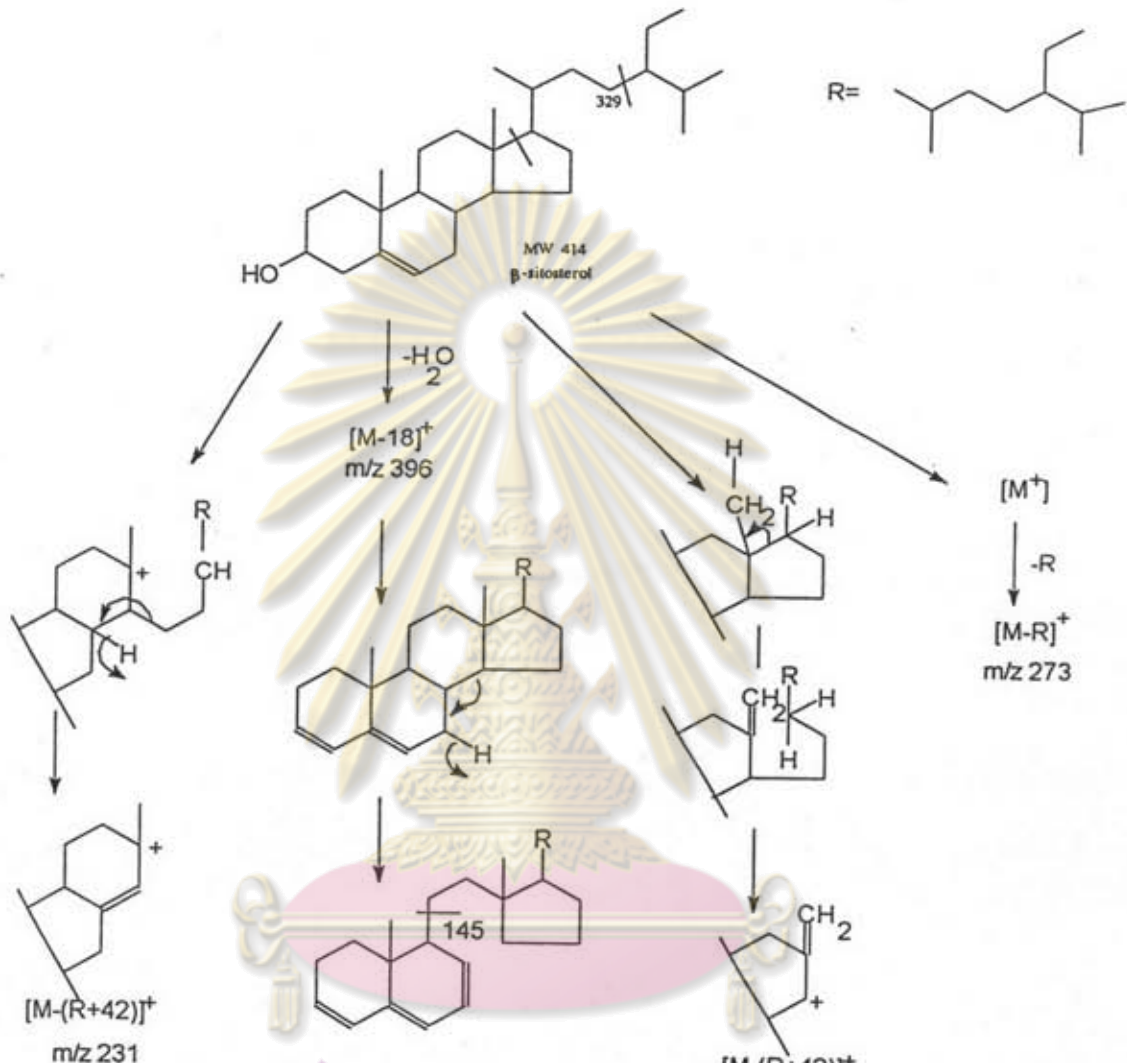


(2)



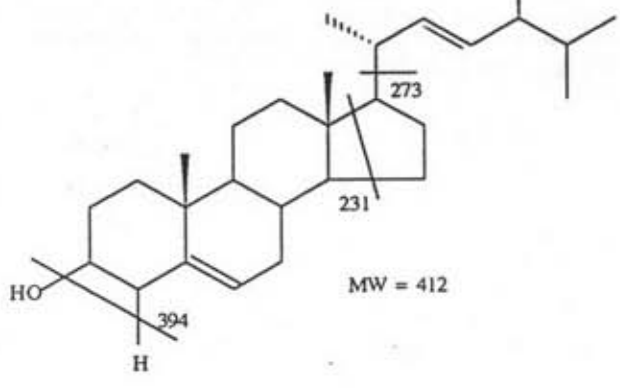
(3)





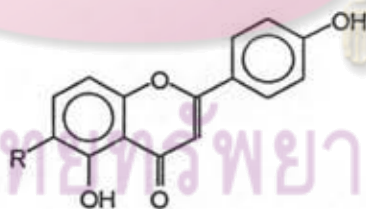
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## 2 Structure Elucidation of BB-2

Compound BB-2 was yellow needle crystal that melted at 259-261°C. When it reacted with 5% AlCl<sub>3</sub> solution in ethanol on TLC, giving fluorescent yellow spot in long wave UV light. The UV absorption spectrum (Figure 6) showed characteristic band II ( $\lambda_{\text{max}} = 292$ ) and band I ( $\lambda_{\text{max}} = 368$ ) of flavones. The high intensity absorption in long wavelength region (band I) was attributed to the maximum conjugation of B-ring with chromone ring because of the coplanarity of this two ring. These implied that BB-2 was flavonoid. The UV absorption of BB-2 with shift reagent was shown in Figure 7. When sodium methoxide was added to BB-2 solution (in MeOH), its spectra showed bathochromic shift. This was an indicator of 4'-OH of flavones and flavonols. And when BB-2 + AlCl<sub>3</sub> and BB-2 + AlCl<sub>3</sub> + HCl spectra were measured and compared with BB-2 spectrum. The three alike spectra revealed that BB-2 was possibly 5-OH with substitute group on C-6 of flavones and flavonols (Markham, 1982) (see (4)).



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(4)

The IR spectrum of BB-2 (Figure 8) showed the pattern of functional groups as table 10.

Table 10 The IR spectrum assignment of BB-2.

Range of absorption ( $\text{cm}^{-1}$ )	assignment
3450-3350 (strong)	O-H stretching
2939,2861 (medium)	C-H stretching
1651,1623 (strong)	C=O stretching
1580-1550, 1480-1440 (medium-strong)	C=C stretching
1210 (strong)	C-O stretching
855, 809 (weak)	C-H bending out of plane

The BB-2  $^1\text{H}$  NMR spectrum (Figure 9) showed sixteen signals of twenty-six protons. The protons were divided into seven aromatic or olefinic protons, seventeen  $\text{sp}^3$  protons and two protons bonding with oxygen. The position of proton was assigned by H-H COSY (Figure 10) as followed. Six protons at signal  $\delta$  1.1 ppm (dd,  $J=6.8, 1.5\text{Hz}$ ) could be assigned as the signal of 3H-19 and 3H-20 protons that vicinal coupled ( $J=6.8\text{Hz}$ ) with H-18 and geminal coupled ( $J=1.5\text{Hz}$ ) with their protons (Figure 11a). One  $\text{sp}^3$  proton signal at  $\delta$  2.47 ppm (m,  $J=7, 6.8, 1.1$ ) could be assigned as H-18 that vicinal coupled ( $J=7\text{Hz}$ ) with H-17 ( $\delta$  6.7 ppm, dd,  $J=16, 7\text{Hz}$ ); vicinal coupled ( $J=6.8\text{Hz}$ ) with 3H-19 and 3H-20 and long range coupled ( $J=1.1\text{Hz}$ ) with H-16 ( $\delta$  6.55 ppm, dd,  $J=16, 1.1\text{Hz}$ ) (Figure 11b). The H-16 and H-17 were coupled each other, their vicinal coupling constant equaled to 16 Hz (Figure 12). The signal at  $\delta$  5.48 ppm (dt,  $J=9.2, 1.2\text{Hz}$ ) could be assigned as H-12 that vicinal coupled with H-11 ( $\delta$  6.22 ppm, d,  $J=9.2\text{Hz}$ ) and long range coupled with two vinyl methyl proton H-14 ( $\delta$  1.9 ppm, d,  $J=1.2\text{Hz}$ ) and H-15 ( $\delta$  1.6 ppm, d,  $J=1.2\text{Hz}$ ) (Figure 11c). The singlet signal at  $\delta$  3.94 ppm was assigned as the methoxyl proton (7-OCH<sub>3</sub>). Its chemical shift was higher



than the other methyl group since it bounded to oxygen which had high electronegativity.

The aromatic proton signal at  $\delta$  6.57ppm was assigned as H-5' (dd,  $J=8.5,2.1\text{Hz}$ ) that ortho-coupled with H-6' ( $\delta$  7.63ppm,  $J=8.5\text{Hz}$ ) and meta-coupled with H-3' ( $\delta$  6.45ppm,  $J=2.1\text{Hz}$ ) (Figure 12). The singlet signal at  $\delta$  6.48ppm was assigned as the aromatic proton at ring A (H-8). The phenolic proton signals at  $\delta$  9.83ppm and 13.53ppm could be assigned as 4'-OH and 5-OH, respectively. The latter was downfield caused it had hydrogen bonded with carbonyl group at C-4.

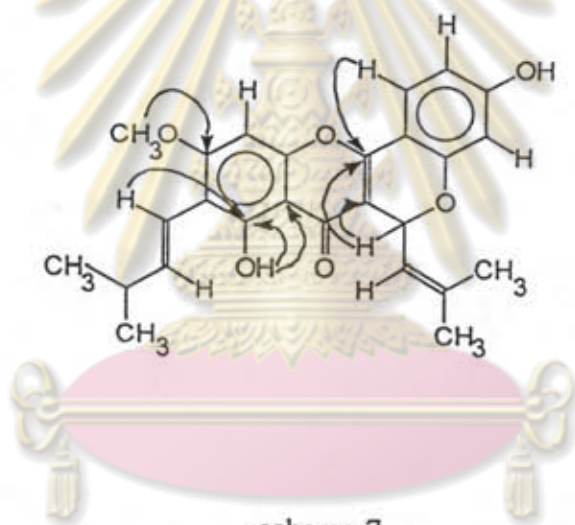
The BB-2  $^{13}\text{C}$  NMR spectrum (Figure 13) showed twenty-five signals of twenty-six carbons. There was an equal signal of carbon atom. They were divided into twelve quaternary carbons (all of them were  $\text{sp}^2$ ), methine carbons (seven  $\text{sp}^2$  and two  $\text{sp}^3$ ) and five methyl carbons (see DEPT, Figure 14). The methyl signal at  $\delta$  55.68ppm was characteristic of methoxyl signal. From C-H COSY (Figure 15), the methyl signal at  $\delta$  18.32ppm and 25.57ppm belonged to C-14 and C-15, respectively. The  $\delta$  22.38 ppm signal had high intensity thus it contained two equal methyl groups that assigned to C-19 and C-20. The aromatic carbon at C-8, C-3', C-5' and C-6' shown signal at  $\delta$  115.40, 104.11, 89.45 and 124.82ppm. The olefinic carbons at C-12, C-16 and C-17 showed signal at  $\delta$  120.89, 109.87 and 142.06ppm. The methine signal at  $\delta$  32.75 and 69.27ppm was C-18 and C-11, respectively. The latter signal was downfield because it bounded to oxygen atom.

From 4Hz, 8Hz and 12Hz COLOC spectrum (Figure 16, 17; 18, 19 and 20, 21), it confirmed the carbon position and defined the quaternary carbon as follow (see scheme 7, 8 and 9, respectively).



Table 11 The  $^1\text{H}$  ( $\delta$  ppm) correlated with  $^{13}\text{C}$  ( $\delta$  ppm) at 4Hz.

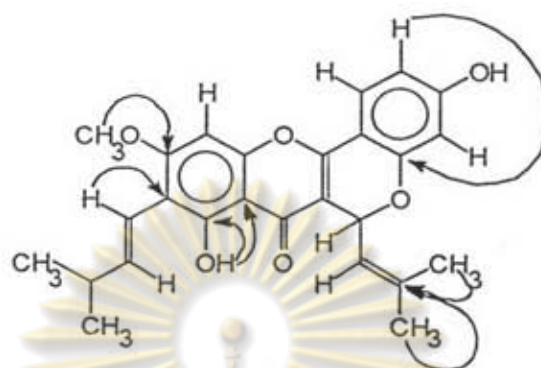
$^1\text{H}$ ( $\delta$ ppm)	$^{13}\text{C}$ ( $\delta$ ppm)
5-OH ( $\delta$ 13.53)	C-5( $\delta$ 158.68),C-10( $\delta$ 105.20)
$\text{CH}_3\text{O}$ ( $\delta$ 3.94)	C-7( $\delta$ 162.05)
H-11( $\delta$ 6.22)	C-2( $\delta$ 155.46),C-3( $\delta$ 109.03)
H-16( $\delta$ 6.55)	C-5( $\delta$ 158.68)
H-6'( $\delta$ 7.63)	C-2(155.46)



scheme 7

Table 12 The  $^1\text{H}$  ( $\delta$  ppm) correlated with  $^{13}\text{C}$  ( $\delta$  ppm) at 8Hz.

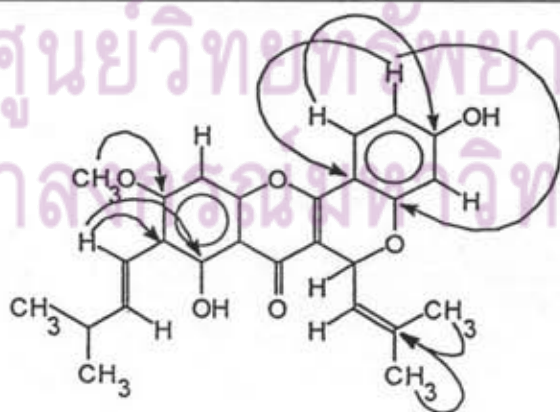
$^1\text{H}$ ( $\delta$ ppm)	$^{13}\text{C}$ ( $\delta$ ppm)
5-OH ( $\delta$ 13.53)	C-5( $\delta$ 158.68),C-10( $\delta$ 105.20)
$\text{CH}_3\text{O}$ ( $\delta$ 3.94)	C-7( $\delta$ 162.05)
H-14( $\delta$ 1.9)	C-13( $\delta$ 138.26)
H-15( $\delta$ 1.6)	C-13( $\delta$ 138.26)
H-16( $\delta$ 6.55)	C-6( $\delta$ 109.38)
H-5'( $\delta$ 6.57)	C-2'( $\delta$ 154.88)



scheme 8

Table 13 The  $^1\text{H}$  ( $\delta$  ppm) correlated with  $^{13}\text{C}$  ( $\delta$  ppm) at 12Hz.

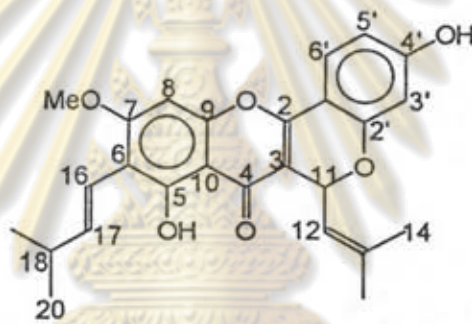
$^1\text{H}$ ( $\delta$ ppm)	$^{13}\text{C}$ ( $\delta$ ppm)
$\text{CH}_3\text{O}$ ( $\delta$ 3.94)	C-7 ( $\delta$ 162.05)
H-14( $\delta$ 1.9)	C-13( $\delta$ 138.26)
H-15( $\delta$ 1.6)	C-13( $\delta$ 138.26)
H-16( $\delta$ 6.55)	C-5( $\delta$ 158.68),C-6( $\delta$ 109.38)
H-5'( $\delta$ 6.57)	C-1'( $\delta$ 107.03),C-2'( $\delta$ 154.88)
H-6'( $\delta$ 7.63)	C-4'( $\delta$ 162.92)



scheme 9

The most downfield signal at  $\delta 178.14\text{ppm}$  was assigned as carbonyl carbon (C-4) and  $\delta 157.43\text{ppm}$  was assigned as C-9. All protons and carbons assignment of BB-2 were shown in table 14. The elucidated structure of BB-2 was confirmed by MS spectrum (Figure 22). It showed fragmentary peak at  $m/z$  434  $[M]^+$ ,  $m/z$  391  $[M-(\text{CH}_3)_2\text{CH}]^+$ ,  $m/z$  378  $[M-(\text{C}_4\text{H}_8)]^+$ ,  $m/z$  379  $[M-(\text{CH}_3)_2\text{C}=\text{CH}]^+$ ,  $m/z$  335  $[m/z$  391-H-( $\text{CH}_3)_2\text{C}=\text{CH}]^+$  (base peak) and  $m/z$  190, 162 from retro diel-alder (see scheme 10).

All data proposed that BB-2 was cycloartocarpin (5).



(5)

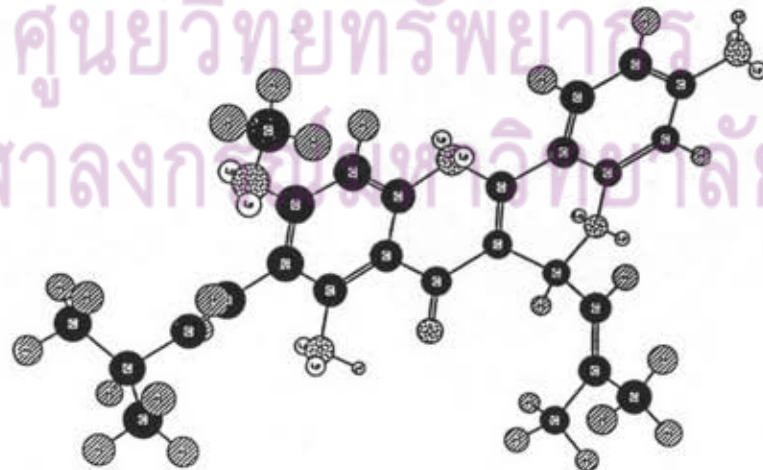




Table 14  $^1\text{H}$  and  $^{13}\text{C}$  assignment of BB-2.

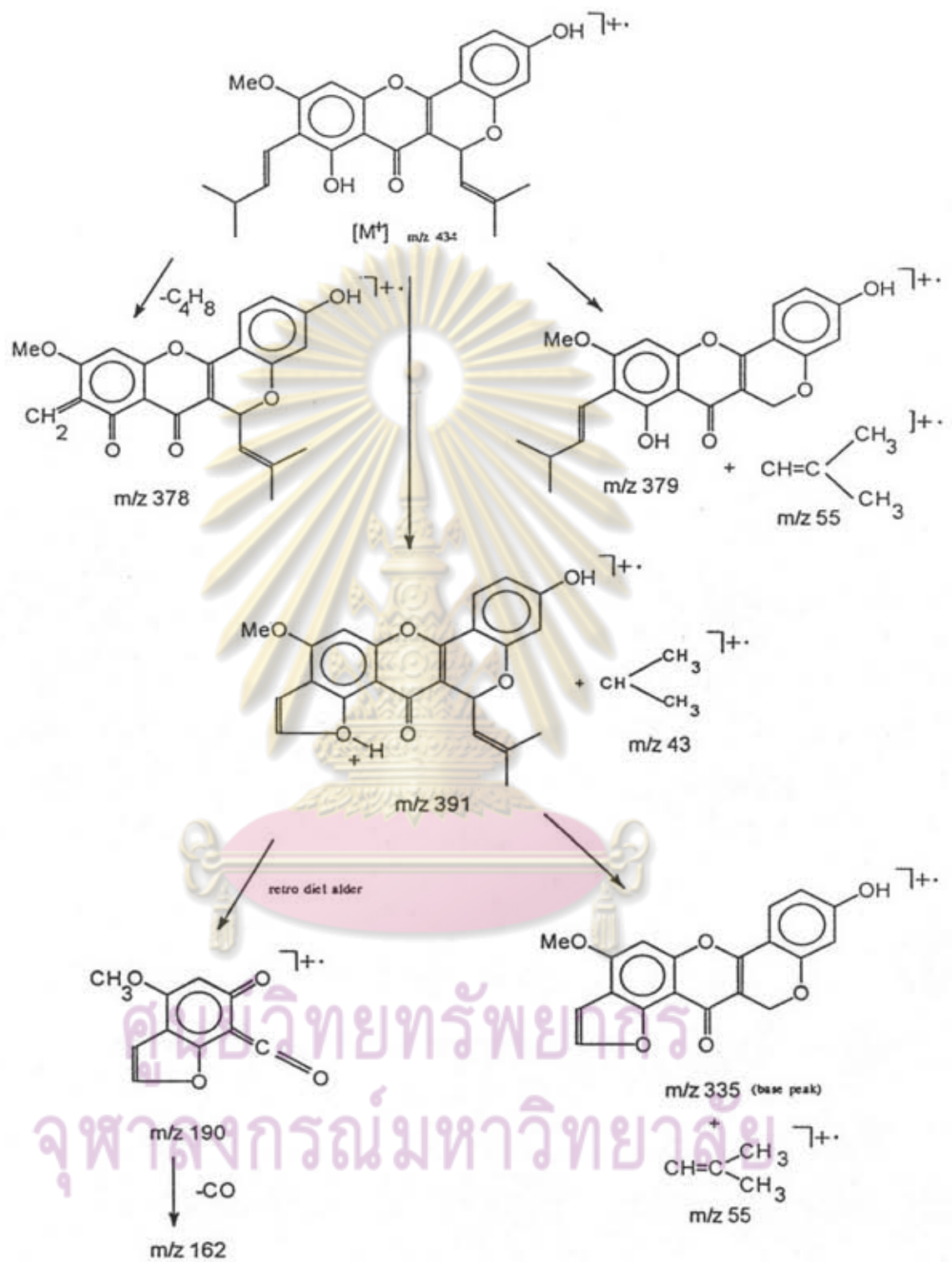
position	$\delta$ (ppm) of C	$\delta$ (ppm) of H (multiplicity, J Hz)	long range correlation from H to C		
			J=4Hz	J= 8 Hz	J= 12 Hz
1	-	oxygen	-	-	-
2	155.46	-	-	-	-
3	109.03	-	-	-	-
4	178.14	-	-	-	-
5	158.68	-	-	-	-
6	109.38	-	-	-	-
7	162.05	-	-	-	-
8	115.40	6.48 (s)	-	-	-
9	157.43	-	-	-	-
10	107.03	-	-	-	-
11	69.27	6.22(d, 9.2)	C-2,C-3	-	-
12	120.89	5.48(dt,9.2,1.2)	-	-	-
13	138.26	-	-	-	-
14	18.32	1.9 (d, 1.2)	-	C-12,C-13, C-15	C-12,C-13, C-14
15	25.57	1.6 (d, 1.2)	-	C-12,C-13, C-14	C-12,C-13
16	109.87	6.55(dd,16,1.1)	C-5	C-6, C-18	C-5,C-6,C-16
17	142.06	6.7 (dd,16,7)	-	-	-
18	32.75	2.47(m,7,6.8,1.1)	-	-	-
19	22.38	1.1 (dd,6.8,1.5)	C-19	C-19	C-17,C-19



Table 14 continued

position	$\delta$ (ppm) of C	$\delta$ (ppm) of H (multiplicity, J Hz)	long range correlation from H to C in COLOC		
			J= 4Hz	J= 8 Hz	J= 12 Hz
20	22.38	1.1 (dd,6.8,1.5)	-	C-20	C-20
1'	105.20	-	-	-	-
2'	154.88	-	-	-	-
3'	104.11	6.45 (d,2.1)	-	-	-
4'	162.92	-	-	-	-
5'	89.45	6.57 (dd,8.5,2.1)	C-3'	C-2',C-6'	C-1',C-2'
6'	124.82	7.63 (d,8.5)	C-2	-	C-1',C-4'
5-OH	-	13.53(s)	-	C-5	-
7-OCH <sub>3</sub>	55.68	3.94(s)	OCH <sub>3</sub>	C-7	C-7
4-OH	-	9.83(s)	-	-	-

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scheme 10



### 3 Structure Elucidation of BB-3.3

Copmpound BB-3.3 was yellow crystal. Its melting point was 133-134°C. The IR spectrum of BB-3.3 (Figure 23) shown the pattern of functional groups as table 15 and its UV spectrum was shown in Figure 24.

Table 15 The IR spectrum assignment of BB-3.3

Range of absorption ( $\text{cm}^{-1}$ )	assignment
3500-2500 (strong)	O-H stretching
1632 (strong)	C=O stretching
1620-1580,1490-1440(strong)	C=C stretching
1230 (strong)	C-O stretching
900-675 (strong)	C-H bending out of plane
720-670 (strong)	"

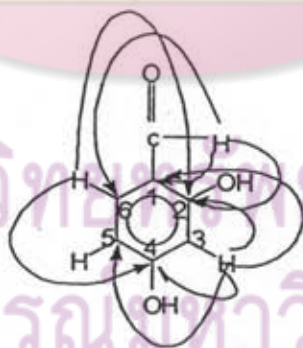
The BB-3.3  $^1\text{H}$ NMR spectrumn Figure 25,26,27,28showed four signals of four protons. The singlet signal at  $\delta$  9.69ppm (H-1,s) was assigned as proton of aldehyde. The 1H signal at  $\delta$  6.45ppm (dd, $J=8.56,2.29\text{Hz}$ ) could be assigned as H-5 that ortho-coupled with H-6 ( $\delta$  7.48ppm,d, $J=8.54\text{Hz}$ ) and meta coupled with H-3 ( $\delta$  6.28 ppm,d, $J=2.14\text{Hz}$ ). Due to using a deuterated protic solvent ( $\text{CD}_3\text{OD}$ ), the deuterium exchanged (scheme 11) phenomena occured and the signals of phenolic protons were disappear. But the signal of  $\text{CD}_3\text{OH}$  ( $\delta$  3.32ppm) and HDO ( $\delta$  4.85ppm) were detected. The signal at  $\delta$  5.50 and  $\delta$  7.11ppm were impure signal.



scheme 11

From DEPT 135 (Figure 29b), there were four signals of methine carbon (C-H) and three signals of quaternary carbon. From C-H COSY (Figure 30), it confirmed that  $\delta$  195.47ppm was carbonyl carbon of aldehyde. The carbon signals at  $\delta$  136.81, 109.94 and 103.30ppm were assigned to C-6, C-5 and C-3, respectively. This results from correlation with hydrogen signal at  $\delta$  7.48, 6.45 and 6.28ppm, respectively (see C-H COSY).

The quaternary carbons were assigned by 12Hz COLOC (Figure 31). The correlation of aldehydic proton at  $\delta$  9.64ppm to  $\delta$  116.23 and 165.60ppm confirmed that the former was C-1 and the latter was C-2. The H-3 and H-6 protons showed the correlation to quaternary carbon at  $\delta$  167.28ppm thus this carbon was assigned as C-4 carbon. The correlation pattern (H to C) of BB-3.3 was shown below.



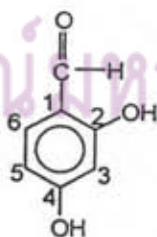
scheme 12

Table 16  $^1\text{H}$  and  $^{13}\text{C}$  assignment of BB-3.3.

Position	$\delta$ (ppm) of C	H (ppm) (multiplicity, J Hz)	long range correlation from H to C in COLOC (J = 12 Hz)
1	116.28	-	-
2	165.60	-	-
3	103.30	6.28 (d, 2.14)	C-1, C-2, C-4, C-5
4	167.28	-	-
5	109.94	6.45 (dd, 8.56, 2.14)	-
6	136.81	7.48 (d, 8.56)	C-2, C-4
1-CHO	195.47	9.69 (s)	C-1, C-2, C-6

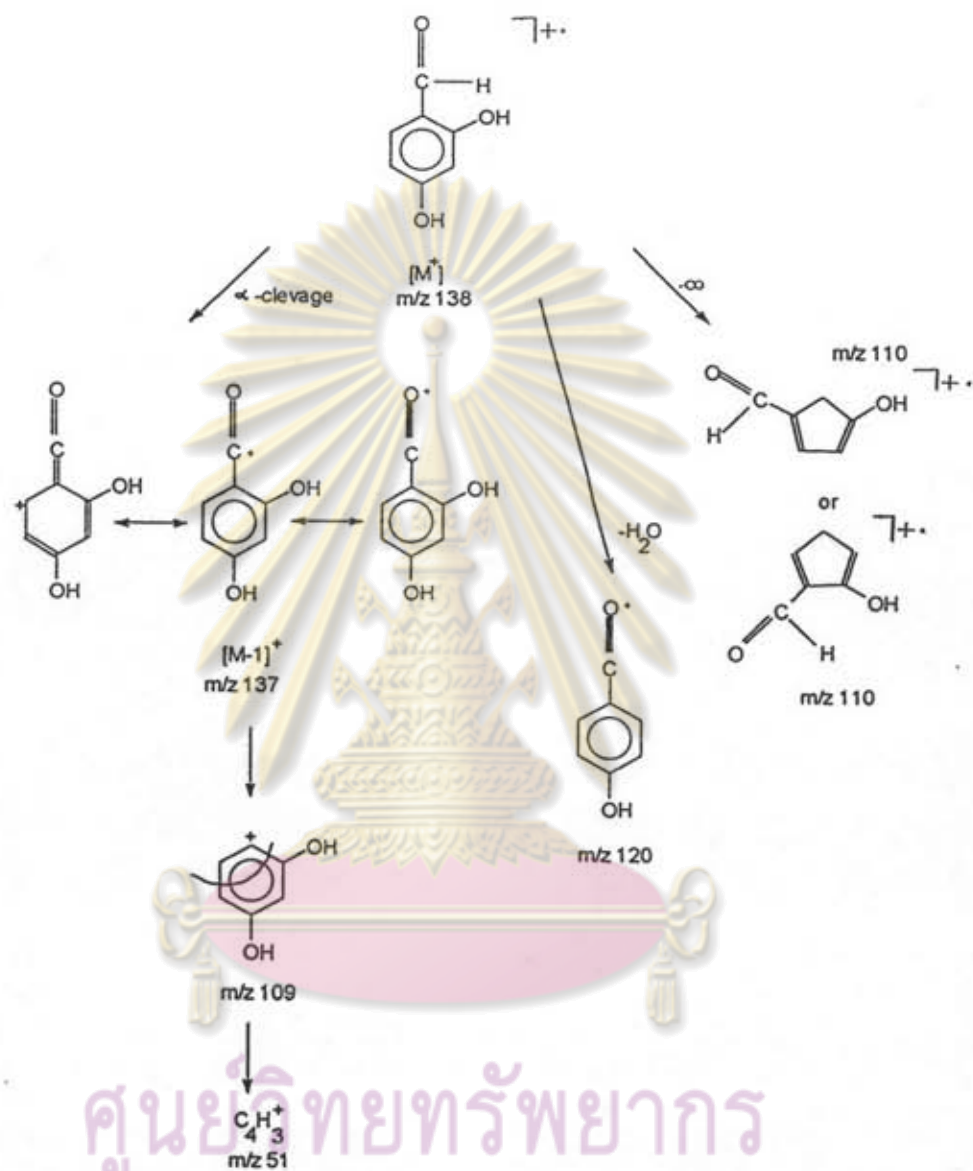
The MS spectrum (Figure 32) and the fragmentation pattern (Scheme 13) confirmed the structure of BB-3.3. The  $m/z$  138 fragment was molecular ion  $[\text{M}]^+$ . The  $\alpha$ -cleavage of  $[\text{M}]^+$  aldehyde resulted in stable fragment,  $m/z$  137  $[\text{M}-1]^+$ , that continued losing carbon monoxide ( $-\text{CO}$ ) giving  $m/z$  109. The dehydration of  $[\text{M}]^+$  gave  $m/z$  120 and gave  $m/z$  110, when it loss ( $-\text{CO}$ ).

All data proposed that BB-3.3 was 2,4-dihydroxybenzaldehyde (6).



(6)





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 scheme13

#### 4 Structure Elucidation of CC-1

Compound CC-1 was yellow needle. Its melting point was 203°C. The IR spectrum of CC-1 (Figure 33) shown the pattern of the functional groups as table 17.

Table 17 The IR spectrum assignment of CC-1

Range of absorption ( $\text{cm}^{-1}$ )	assignment
3500-3000 (strong)	O-H stretching
1660-1590,1500-1450 (strong)	C=C stretching
1200-1100 (strong)	C-O stretching
980-960 (strong)	C-H bending out of plane
827 (strong)	C-H "
720-670 (strong)	C-C "

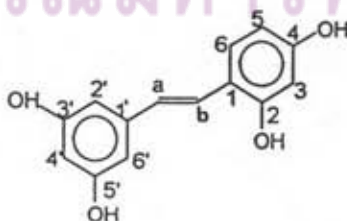
The CC-1  $^1\text{H}$  NMR spectrum (Figure 34,) showed ten signals of twelve protons. The singlet signal at  $\delta$  8.22ppm was assigned as two equal hydroxyl groups (3-OH and 5-OH). The two singlet signal at  $\delta$  8.42 and  $\delta$  8.60ppm could be assigned as 2'-OH or 4'-OH. The signal at  $\delta$  6.52ppm (d, $J$ =2.1Hz) was assigned as two equal protons at H-6 and H-2 that meta coupled with H-4 ( $\delta$  6.24ppm, t, $J$ =2.1Hz). The signal at  $\delta$  6.38ppm (dd, $J$ =8.3,2.4Hz) could be assigned as H-5' that ortho-coupled with H-6' ( $\delta$  7.39ppm,d, $J$ =8.3Hz) and meta-coupled with H-3' ( $\delta$  6.44ppm,d,  $J$ =2.4Hz). The signals at  $\delta$  7.33ppm was vicinal coupled with  $\delta$  6.88ppm equaling to 16.8 Hz. They were trans to each other because the vicinal coupling constant was high. The signal at  $\delta$  7.33 and 6.88ppm was assigned to be

Ha and Hb, respectively, by comparison with the model of sorocein B (Messana et al.,1991). The Ha was downfield by the resonance effect of hydroxyl groups at C-2' and/or C-4'. The signals at  $\delta$ 2.06 and 3.18 ppm were signals of acetone and H<sub>2</sub>O, respectively. The assignment of protons of CC-1 was shown in table 18.

Table 18 <sup>1</sup>H assignment of CC-1.

position	$\delta$ (ppm), (multiplicity, J= Hz)
1'	-
2'-OH	8.42 (s)
3'	6.44 (d, 2.4)
4'-OH	8.60 (s)
5'	6.38 (dd,8.3,2.4)
6'	7.39 (d, 8.3)
1	-
2,6	6.52 (d, 2.1)
5,3-diOH	8.22 (s)
4	6.24 (t, 2.1)
Ha	7.33 (d, 16.8)
Hb	6.88 (d, 16.8)

All data proposed that CC-1 was 3,5,2',4'-tetrahydroxystilbene (7).



(7)

