CHAPTER 3

RESULTS AND DISCUSSION

3.1 Chemistry

3.1.1 Synthesis of 4-Hydroxycoumarins

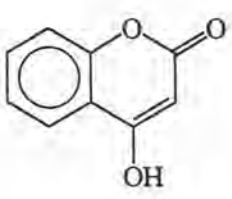
From literature review, there are various routes to synthesize 4-hydroxycoumarins. Condensation of phenols with malonic acids in the presence of zinc chloride and phosphorus oxychloride (method I) and condensation of phenols with malonyl chlorides in the presence of aluminum chloride (method II) are two examples among those described in Chapter 1. In this research, when method I was selected for the synthesis of 4-hydroxy-6-methyl-2*H*-1-benzopyran-2-one (**2**), the product was attained in moderate yield (56 %). For condensation of 2-hydroxyacetophenones with diethyl carbonate in the presence of an alkali metal (method III), the use of sodium was compared with that of sodium hydride. It was found that the trend when using sodium provided 4-hydroxycoumarin in a little lower yield (40-90 %) than that of sodium hydride (79-94 %). All of chosen methods provided the amount of the desired product, 4-hydroxycoumarin corresponded to those described in literature. Generally employing method I, 4-hydroxycoumarins were obtained in moderate yield (about 30-60% yield), whereas utilizing method III gave much better yield than that of method I especially using NaH (80-90% by using Na and 90-91% by using NaH).

In condensation of phenols with carbon suboxide and aluminum chloride (method IV) and C-carbonylation of 2-hydroxyacetophenone with carbon monoxide in the presence of sulfur and base or selenium without base (method V) were not studied. A main reason not to employ those methods is because carbon suboxide in carbon disulfide at -44°C under nitrogen atmosphere (method IV) and carbon monoxide (method V) were toxic and not facile to prepare. The condensation of fluorinated

phenols with aluminum chloride, followed by decarboxylation and deacetylation with 90% sulfuric acid (method VI) was also not examined.

In this research, all 4-hydroxycoumarins were synthesized by means of condensation of 2-hydroxyacetophenones with diethyl carbonate in the presence of sodium or sodium hydride. There are three steps for this methodological route using phenols as starting materials. The first step, phenols were acetylated by refluxing with acetic anhydride in the presence of pyridine. Resulting phenyl acetates were general attained in high yield (84-99%). Next, phenyl acetates were transformed to the corresponding 2-hydroxyacetophenones utilizing Fries rearrangement. The Fries rearrangement is usually the most suitable process for this regard since it is easy to perform and good yields are normally obtained.[45] Due to all products derived from the rearrangement of 4-substituted phenyl acetates, the product attained is the only one major product isomer of 2-hydroxyacetophenones. The final step is the condensation of 2-hydroxyacetophenones with diethyl carbonate in the presence of sodium or sodium hydride. As mentioned earlier, this classical synthesis method was used to synthesize 4-hydroxycoumarins and products were often obtained in high yield.

Eight 4-hydroxycoumarins were synthesized as aforementioned. Seven of them are known compounds (**1**-**7**). The other (**8**), to our best knowledge has never been reported in chemical literature; thus, it is a new compound. The comparative results of the synthesis of 4-hydroxycoumarins are tabulated in Table 3.1 and the structures are shown below.



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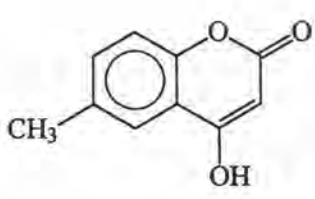

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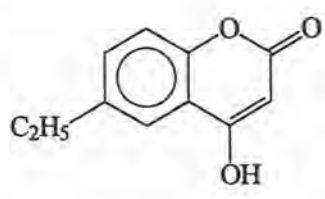

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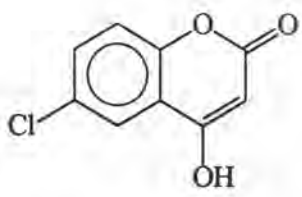

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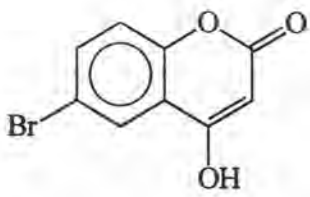

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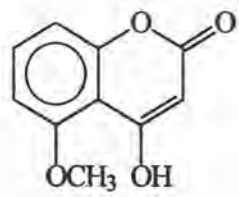

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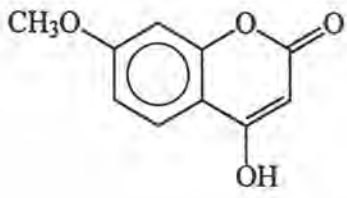

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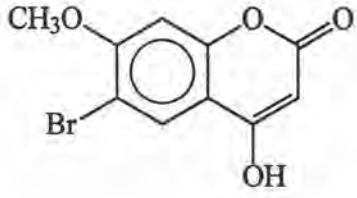


8

Table 3.1 The physical properties and % yield of synthesized 4-hydroxycoumarins

Cpd	Physical Properties		% Yield	References
	Appearance	m.p. (°C)		
1	pale yellow amorphous solid	209 - 211	40[A], 94[B]	13a, 17a, 26, 27
2	pale yellow amorphous solid	246 - 248	85[A], 56[C]	16, 17a, 27
3	white powder	211 - 214	27[B]	27
4	pale yellow needle	247 - 249 (dec)	90[A]	27
5	white needle	266 - 268	84[B]	27
6	pale yellow needle	147 - 149	90[B]	15b, 27
7	white platelet	249 - 251 (dec)	79[B]	14b, 17a, 27
8	pale brown needle	285 - 286 (dec)	79[B]	new compound

For synthesis method (see Chapter 2)

A : Method III (with Na), B : Method III (with NaH), C : Method I

All 4-hydroxycoumarins are solid. Their melting points (or decomposition points) are over 200°C, except for that of **6** (147-149°C). It was observed during data collection and should be noted here that 4-hydroxycoumarins with larger molecular

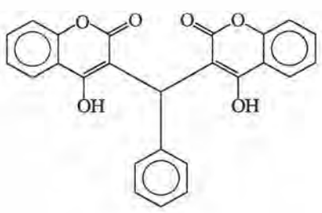
weight and with more dipole moment had a trend to have higher melting point than others. Examples for this regard can be illustrated as follows: 4-hydroxycoumarins with different molecular weight are in order from the highest to the lowest as: **8** (m.p. = 285-286°C, MW = 271) > **5** (m.p. = 266-268°C, MW = 241) > **4** (m.p. = 247-249°C, MW = 196.5) and **2, 3** (m.p. = 211-248°C, MW = 176-190) > **1** (m.p. = 209-211°C, MW = 162). The melting points of 4-hydroxycoumarins with different dipole moment could also be in order from the highest to the lowest as: **4, 5, 8** (247-286°C, halogen group) > **7** (249-251°C, methoxy group) > **3, 2** (211-214°C, alkyl group).

3.1.2 Synthesis of Dicoumarols

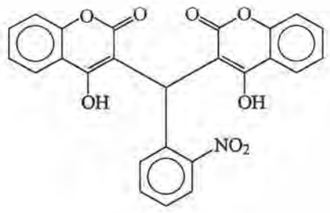
Methodology used for the synthesis of dicoumarols was condensation of 4-hydroxycoumarins with interested aromatic aldehydes in hot ethanol (method VII). Most dicoumarols were obtained in excellent yields (78-99 %), except for **D2** and **D5** which were attained in 62 and 67 %, respectively.

In general, 2 mol equivalents of 4-hydroxycoumarins were condensed with 1 mol equivalent of interested aromatic aldehydes in ethanol. This reaction is a common nucleophilic addition of aromatic aldehyde with a nucleophile at C-3 of benzopyrane ring. The reactivity arose from the electrophilic carbonyl carbon. It can become a strong electrophile by protonated in a polar solvent such as ethanol.[46]

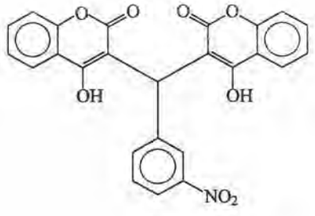
Twenty one dicoumarols were synthesized. Seventeen of them (**D1-D6, D8-D10, D12, D14-D17** and **D19-D21**) are known compounds. The others (**D7, D11, D13** and **D18**) are new compounds based upon no report of those compounds available in chemical literature. The structures of twenty one synthesized compounds were well-characterized by using various spectroscopic techniques (IR, ^{1}H- and ^{13}C-NMR) and elemental analysis. All spectroscopic evidence will be discussed in the following topic. The comparative results of all dicoumarols are tabulated in Table 3.2 and the structures are shown below.



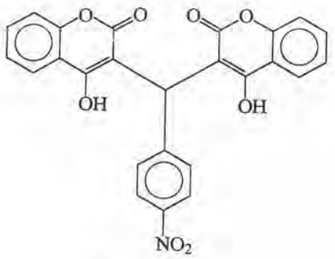
D1



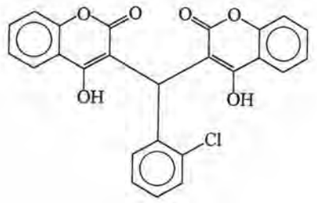
D2



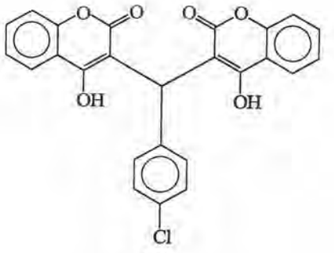
D3



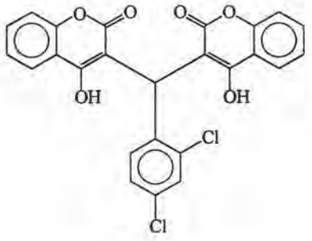
D4



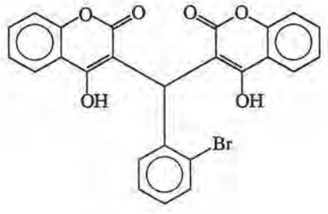
D5



D6



D7



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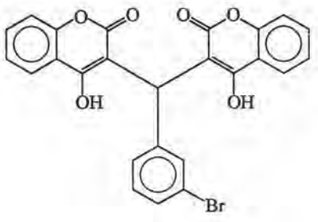
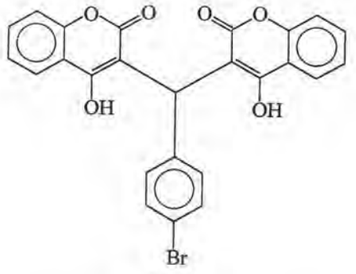
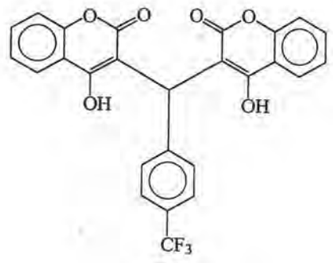


D9



D10



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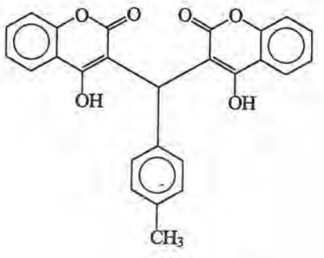

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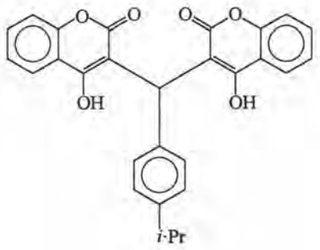
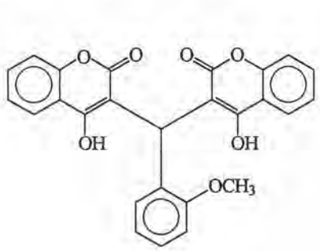


D13



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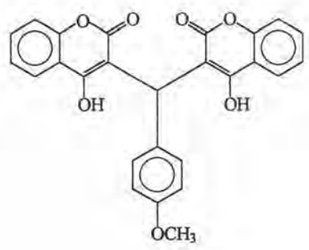

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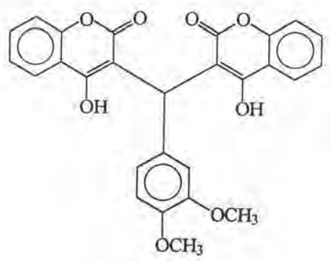
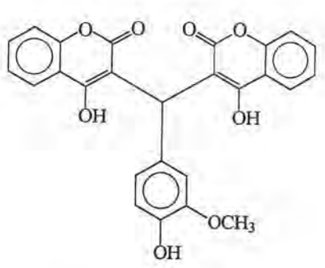
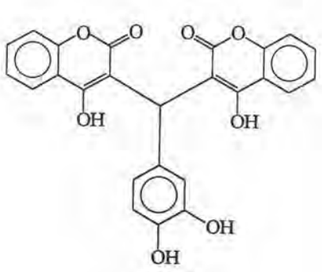


D16



D17



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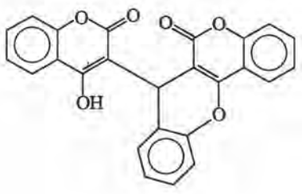
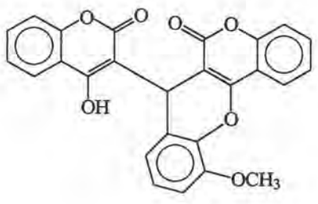
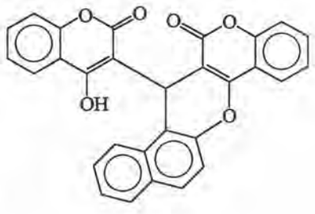


D19



D20



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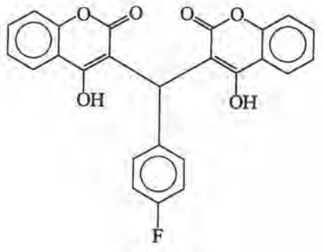
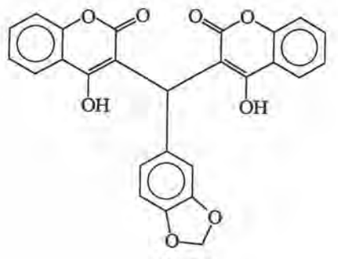


D22*



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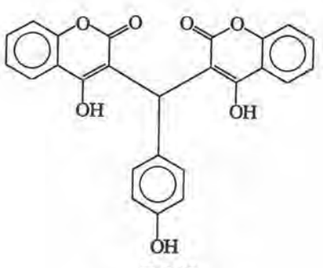


D24*

* kindly provided by Deesamer, S. and Tipnoysnga, J.

Table 3.2 The physical properties and % yield of dicoumarols

Cpd	Physical Properties		% Yield	References
	Appearance	m.p. (°C)		
D1	white crystal	232 - 234	95	8d, 8e, 28
D2	pale yellow powder	211 - 212 (dec)	62	8e, 29
D3	white amorphous solid	222 - 224	99	29
D4	small yellow crystal	235 - 237	78	8d, 8e, 29, 30
D5	white-mirror crystal	203 - 205	67	8e
D6	white-mirror crystal	248 - 250 (dec)	76	8d, 8e
D7	white powder	179 - 180	84	new compound
D8	white-mirror crystal	219 - 221	81	31
D9	white amorphous solid	223 - 225	96	31
D10	white powder	266 - 268	88	31
D11	small white-mirror crystal	282 - 283	97	new compound
D12	white amorphous solid	254 - 257 (dec)	94	8d
D13	white-mirror crystal	255 - 257	97	new compound
D14	white amorphous solid	212 - 214 (dec)	98	8e, 32
D15	white amorphous solid	250 - 251 (dec)	97	31
D16	white amorphous solid	264 - 266 (dec)	81	47
D17	pale yellow amorphous solid	215 - 217 (dec)	94	8e, 28, 33
D18	brown crystal	174 - 178 (dec)	83	new compound
D19	small white crystal	252 - 253	85	28, 34
D20	white powder	274 - 275 (dec)	91	8e
D21	brown crystal	244 - 245	96	48
D22	white powder	-[a]	-[a]	49
D23	pale pink powder	250 - 256 (dec)[b,c]	-[c]	8e, 33
D24	pale yellow mirror powder	212 - 215[b]	-[b]	8e

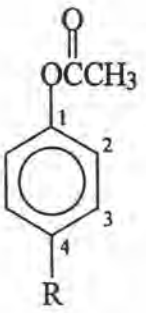
a) see ref. 49, b) see ref. 8e, c) see ref. 33

The results attained from Table 3.2 exhibited some informative data. In this research, dicoumarols were normally prepared in 1-2 replicates from the condensation of 4-hydroxycoumarins and various interested aromatic aldehydes. The desired product, dicoumarol, was generally obtained in high yield (85-99 %). The aromatic aldehydes bearing electron donating groups (alkyl, methoxy and hydroxy) tends to provide more yield of the corresponding dicoumarols than those having electron withdrawing substituents (nitro, chloro and bromo). With the same substituent, the *meta-*, *para-* and *ortho* positions relatively provided the amount of the desired product in order from the highest to the lowest. All synthesized dicoumarols are solid. Most of their m.p.'s are over 200°C, except for **D7** and **D18**. Among all dicoumarols studied, their m.p.'s were found to be of relationship with the position on a benzylidene ring as follows: *para* > *meta* > *ortho*. The substituent at *para* position on a benzylidene ring of dicoumarol tends to give a good symmetry. They should therefore well-arranged and have the highest melting points. *Meta*-isomers have a middle symmetry and have slightly lower melting point. *Ortho*-isomers, on the other hand, possessed the lowest symmetry and therefore should relatively have the lowest melting point.

3.1.3 Spectroscopic Data

Infrared Spectroscopy (IR)

Phenyl acetates

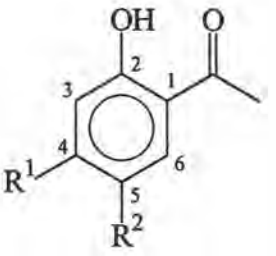


The FT-IR spectra of phenyl acetates normally revealed the absorption band of C-H aromatic stretching vibration at 3040-3100 cm^{-1} (w), C-H stretching vibration of $-CH_3$ at 2870-2950 cm^{-1} (w) and the C=C ring stretching vibration at 1490-1500 cm^{-1} (s). The strong absorption band at 1750-1775 cm^{-1} corresponded to the C=O stretching vibration of esters. Other absorption peaks belonging to C-O stretching vibration were detected at 1190-1200 and 1220 cm^{-1} (s). The FT-IR absorption band assignments of phenyl acetates are tabulated in Table 3.3.

Table 3.3 The FT-IR absorption band assignments of phenyl acetates

Cpd	wave number (cm^{-1})				
	Ar-H	$-CH_3$, $-CH_2$	C=O	Benzene	C-O
2a	3050 (w)	2880-2990 (m)	1775 (s)	1500 (s)	1190, 1220 (s)
3a	3040 (w)	2890-2970 (m)	1770 (s)	1500 (s)	1190, 1220 (s)
4a	3100 (w)	2870, 2950 (w)	1770 (s)	1490 (s)	1200, 1220 (s)
5a	3090 (w)	2870, 2930 (w)	1750 (s)	1490 (s)	1200, 1220 (s)

2-Hydroxyacetophenones

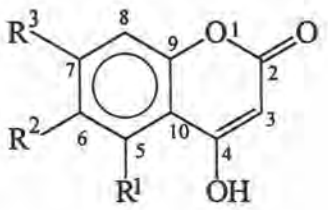


The FT-IR spectra of 2-hydroxyacetophenones showed the presence of O-H stretching vibration in the range of 2600-3300 cm^{-1} (br, s). The C-H stretching vibration of aromatic around 3010-3070 cm^{-1} (w) and absorption peak of C=C of aromatic ring stretching approximately 1480-1500 cm^{-1} (s) were observed. The absorption band due to C=O stretching vibration of ketone presented at 1650 cm^{-1}. Other absorption peaks of C-O stretching vibration were found at 1010-1260 cm^{-1}. The FT-IR absorption band assignments of 2-hydroxyacetophenones are tabulated in Table 3.4.

Table 3.4 The FT-IR absorption band assignments of 2-hydroxyacetophenones

Cpd	wave number (cm^{-1})					
	O-H	Ar-H	$-CH_3$, $-CH_2$	C=O	Benzene	C-O
2b	2600-3300 (s)	3010-3070 (w)	2850-2990 (m)	1650 (s)	1500 (s)	1050-1200 (s)
3b	2600-3300 (s)	3010-3060 (w)	2870-2970 (m)	1650 (s)	1490 (s)	1010-1220 (s)
4b	2650-3300 (s)	3010-3070 (w)	2880-2970 (w)	1650 (s)	1480 (s)	1010-1200 (s)
5b	2700-3300 (s)	3010-3070 (w)	2870-2950 (w)	1650 (s)	1490 (s)	1020-1200 (s)
8a	2700-3300 (s)	3070 (w)	2860-2980 (m)	1650 (s)	1490 (s)	1050-1260 (s)

4-Hydroxycoumarins



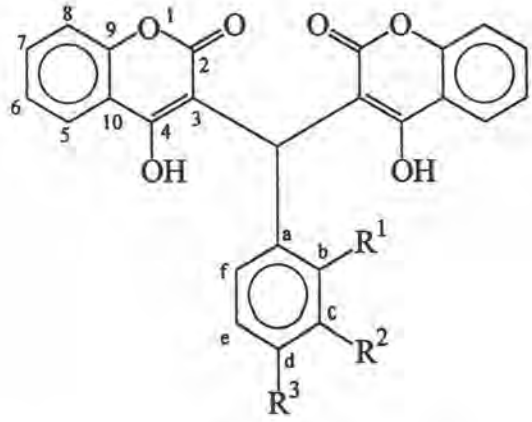
The FT-IR spectra of 4-hydroxycoumarins showed the presence of O-H stretching vibration around 3300-3570 cm^{-1} (br, w) and a very broad absorption band in the range of 2400-3090 cm^{-1} (s). The C-H stretching vibration of aromatic was observed at 3005-3090 cm^{-1} (w), whereas absorption peak of C-H bending vibration was detected at 2570-2830 cm^{-1}. The absorption band corresponded to C=O stretching vibration of pyrane ring was found approximately 1680-1730 cm^{-1} (s). Other absorption peaks of C-O stretching vibration were detected around 1020-1220 cm^{-1}. The FT-IR absorption band assignments of 4-hydroxycoumarins are tabulated in Table 3.5.

Table 3.5 The FT-IR absorption band assignments of 4-hydroxycoumarins

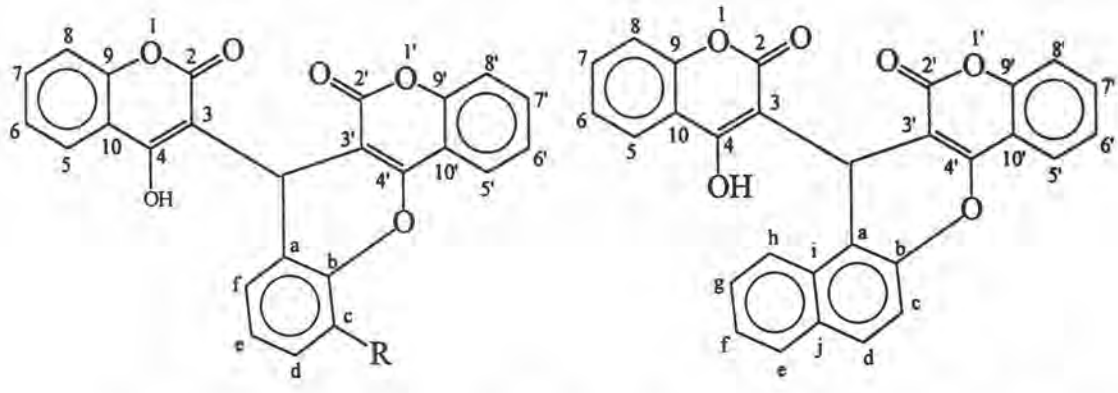
Cpd	wave number (cm^{-1})				
	O-H	Ar-H	C-H str.	C=O	C-O
1	2500-3650 (s)	3010-3070 (w)	2570-2830 (w)	1680 (s)	1100-1200 (s)
2	2450-3700 (s)	3010-3090(w)	2600-2820 (w)	1700 (s)	1100-1220 (s)
3	2450-3700 (s)	3085 (w)	2580-2800 (w)	1680 (s)	1100-1210 (s)
4	2450-3700 (s)	3010-3100 (w)	2600-2780 (w)	1700 (s)	1120-1200 (s)
5	2450-3400 (s)	3010-3070 (w)	2580-2720 (w)	1650 (s)	1120-1200 (s)
6	3150-3700 (s)	3005-3120 (w)	-	1730 (s)	1090-1180 (s)
7	2500-3700 (s)	3050-3130 (w)	2750-2590 (w)	1690 (s)	1030-1160 (s)
8	2500-3700 (s)	3120 (w)	2750-2590 (w)	1700 (s)	1050-1180 (s)

- : not assigned

Dicoumarols



The FT-IR spectra of dicoumarols gave common characteristic absorption peaks. To illustrate this, they showed the absorption pattern of O-H stretching vibration at 3350-3700 cm^{-1} (br, w) and a very broad absorption band at 2300-3300 cm^{-1} (s). The C-H stretching vibration of aromatic at 3010-3100 cm^{-1} (w), aliphatic C-H stretching vibration at 2850-2990 (w) and C=C ring stretching at 1490-1520 cm^{-1} were also detected. The absorption band belonging to C=O stretching vibration of a pyrane ring at 1650-1690 cm^{-1} and other absorption peaks of C-O stretching vibration at 1010-1300 cm^{-1} were also found.



The FT-IR characteristic absorption peaks of dicoumarols with a fused benzylidene ring and a fused naphthalidene ring were found very little different from those of the other aforementioned dicoumarols observed. The FT-IR absorption band assignments of both common dicoumarols and dicoumarols with a fused benzylidene ring and a fused naphthalidene ring are tabulated in Tables 3.6 and 3.7, respectively.

Table 3.6 The FT-IR absorption band assignments of dicoumarols

Cpd	wave number (cm^{-1})					
	O-H	Ar-H	C-H str.	C=O	benzo	C-O
D1	2400-3400 (s)	-	-	1650 (s)	1500 (m)	1100 (s)
D2	2400-3300 (s)	3090 (w)	2880-2980 (w)	1650 (s)	1500 (m)	1100 (s)
D3	2450-3650 (s)	3100 (w)	2970 (w)	1650 (s)	1500 (m)	1100 (s)
D4	2400-3300 (s)	3090 (w)	2950 (w)	1650 (s)	1500 (m)	1100 (s)
D5	2450-3700 (s)	3050 (w)	2900-2980 (w)	1650 (s)	1500 (m)	1100 (s)
D6	2550-3700 (s)	3080 (w)	2900-2990 (w)	1675 (s)	1500 (m)	1100 (s)
D7	2500-3700 (s)	3070 (w)	2850-2970 (w)	1650 (s)	1500 (m)	1110 (s)
D8	2450-3350 (s)	3050 (w)	2880-2950 (w)	1660 (s)	1500 (m)	1110 (s)
D9	2450-3650 (s)	3070 (w)	2890-2980 (w)	1670 (s)	1500 (m)	1100 (s)
D10	2450-3650 (s)	3080 (w)	2970 (w)	1680 (s)	1490 (m)	1100 (s)
D11	2450-3650 (s)	3060 (w)	2850-2975 (w)	1680 (s)	1510 (m)	1120 (s)
D12	2450-3350 (s)	3080 (w)	2890-2990 (w)	1680 (s)	1500 (m)	1100 (s)
D13	2500-3650 (s)	3050-3010 (w)	2890-2960 (w)	1680 (s)	1510 (m)	1100 (s)
D14	2300-3300 (s)	3080 (w)	2950 (w)	1660 (s)	1500 (m)	1100 (s)
D15	2450-3750 (s)	3100-3020 (w)	2900-2980 (w)	1690 (s)	1510 (m)	1100 (s)
D16	2500-3650(s)	3090-3010 (w)	2850-2980 (w)	1680 (s)	1510 (m)	1110 (s)
D17	2450-3650 (s)	3070 (w)	2850-2980 (w)	1680 (s)	1510 (m)	1100 (s)
D18	2350-3680 (s)	3080 (w)	2930 (w)	1660 (s)	1520 (m)	1100 (s)

- : not assigned

Table 3.7 The FT-IR absorption band assignments of dicoumarols with a fused benzylidene ring and a fused naphthalidene ring

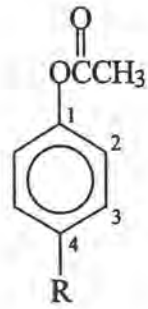
Cpd	wave number (cm^{-1})					
	O-H	Ar-H	C-H str.	C=O	benzo	C-O
D19	2450-3700 (s)	3090 (w)	2900-2970 (w)	1610-1700 (s)	1490 (m)	1110 (s)
D20	2550-3700 (s)	3010-3070 (w)	2850-2990 (w)	1610-1730 (s)	1490 (m)	1110 (s)
D21	3150-3650 (w)	3075 (m)	-	1610-1705 (s)	1500 (m)	1100 (s)

- : not assigned

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR

Phenyl acetates

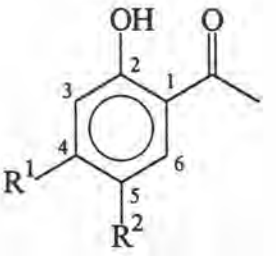


The ^{1}H-NMR spectra of phenyl acetates generally exhibited a singlet signal with 3H integration around 2.24-2.29 ppm which was methyl protons. Another signal with 4H integration detected approximately 6.94-7.49 ppm (d, J = 8.24-8.85 Hz) could be assigned for aromatic protons. The ^{1}H-NMR spectral assignments of phenyl acetates are presented in Table 3.8.

Table 3.8 The ^{1}H-NMR spectral assignments of phenyl acetates

Cpd	R	chemical shift (ppm)			
		H-2	H-3	CH_3-COO	H of R
2a	CH_3	6.94 (d)	7.14 (d)	2.24 (s)	2.31 (s)
3a	C_2H_5	7.01 (d)	7.20 (dd)	2.27 (s)	2.65 (q), 1.24 (t)
4a	Cl	7.03 (d)	7.33 (d)	2.29 (s)	-
5a	Br	6.89 (d)	7.49 (d)	2.29 (s)	-

2-Hydroxyacetophenones

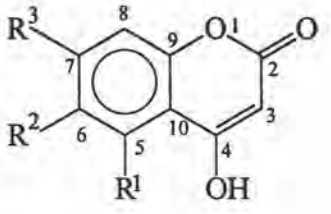


The ^{1}H-NMR spectra of 2-hydroxyacetophenones normally showed 3 H integration of the methyl proton signal around 2.56-2.63 ppm and the aromatic protons 4 H integration at 6.48-7.85 ppm (d, J = 8.24-8.85 Hz). The remaining 1H integration singlet signal at 12.07-12.66 ppm could be ascribed for a hydroxy proton. The ^{1}H-NMR spectral assignments of 2-hydroxyacetophenones are recorded in Table 3.9.

Table 3.9 The ^{1}H-NMR spectral assignments of 2-hydroxyacetophenones

	R		chemical shift (ppm)					
Cpd	R^1	R^2	H-3	H-4	H-6	$CH_3C=O$	-OH	H of R
2b	H	CH_3	6.89 (d)	7.29 (dd)	7.51 (s)	2.62 (s)	12.07 (s)	2.32 (s)
3b	H	C_2H_5	6.87 (d)	7.29 (dd)	7.49 (d)	2.59 (s)	12.11 (s)	2.55 (q), 1.19 (t)
4b	H	Cl	6.94 (d)	7.42 (dd)	7.69 (d)	2.63 (s)	12.13 (s)	-
5b	H	Br	6.89 (d)	7.55 (d)	7.83 (s)	2.62 (s)	12.15 (s)	-
8a	OCH_3	Br	6.45 (s)	-	7.85 (s)	2.56 (s)	12.66 (s)	3.92 (s)

4-Hydroxycoumarins

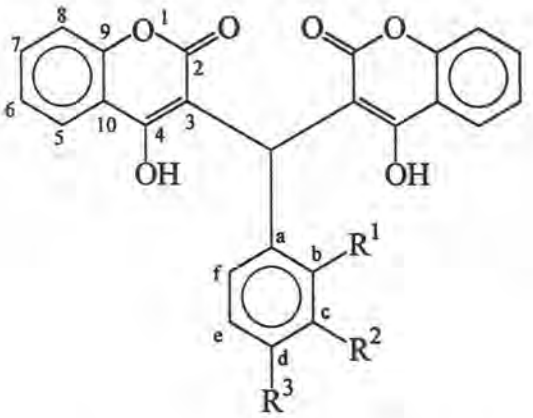


The ^{1}H-NMR spectra of 4-hydroxycoumarins exhibited 1 H integration singlet signal of an olefinic proton (H-3) around 5.44-5.66 ppm. The aromatic protons with 2-4 H integration were detected as singlet, doublet and triplet with various coupling constants at 6.91-7.85 ppm depending upon the number and the kind of substituent(s). The remaining 1 H integration singlet signal at 11.27-12.50 ppm was a hydroxy proton. The ^{1}H-NMR spectral assignments of 4-hydroxycoumarins are displayed in Table 3.10.

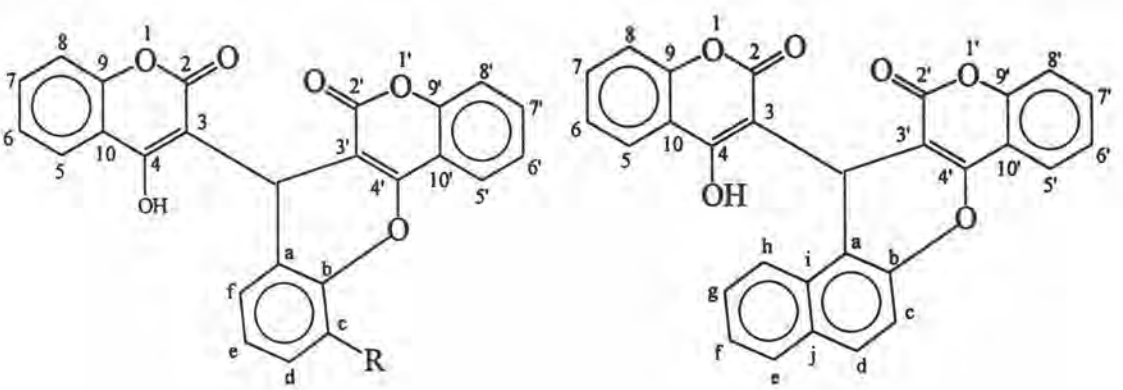
Table 3.10 The ^{1}H-NMR spectral assignments of 4-hydroxycoumarins

	R			chemical shift (ppm)						
Cpd	R^1	R^2	R^3	H-3	H-5	H-6	H-7	H-8	-OH	H-R
1	H	H	H	5.62(s)	7.84(dd)	7.35(dt)	7.65(dt)	7.37(d)	12.52(s)	-
2	H	CH_3	H	5.56(s)	7.57(s)	-	7.42(dd)	7.22(d)	12.49(s)	2.34(s)
3	H	C_2H_5	H	5.56(s)	7.61(d)	-	7.47(dd)	7.26(d)	12.40(s)	1.18(t),2.66(q)
4	H	Cl	H	5.66(s)	7.72(d)	-	7.64(dd)	7.38(d)	12.75(s)	-
5	H	Br	H	5.59(s)	7.85(d)	6.92(d)	7.75(s)	7.32(s)	12.69(s)	-
6	OCH_3	H	H	5.50(s)	-	6.91(dd)	7.54(t)	6.92(d)	11.27(s)	3.88(s)
7	H	H	OCH_3	5.44(s)	7.70(d)	-	-	6.83(s)	12.32(s)	3.83(s)
8	H	OCH_3	Br	5.48(s)	7.89(s)	-	-	7.13(s)	12.54(s)	3.95(s)

Dicoumarols



The ^{1}H-NMR spectra of dicoumarols were found to be very close to those of 4-hydroxycoumarins. The typical 1 H integration singlet signal at 6.00-6.62 ppm of CH-Ar was normally found. The aromatic protons with 3-5 H integration on benzylidene were detected as singlet, doublet and triplet at 6.60-7.63 ppm. The overlapped 4 H integration with 2 H of H-6 was observed at 7.31-7.41 ppm (br, t, J = 7.16-8.24 Hz). The other 2 H integration of H-7 exhibited at 7.58-7.66 ppm (t, J = 7.80-8.24 Hz). The broad doublet signal centered at 7.88-8.08 ppm with J = 7.09 Hz was 2 H integration of H-5. The remaining two broad singlet signals around 10.91-11.62 ppm were hydroxy protons (on a benzopyran ring).



The feature and pattern of signals in the ^{1}H-NMR spectra of dicoumarols with a fused benzylidene ring and a fused naphthalidene ring and those of common dicoumarols were alike. The ^{1}H-NMR spectral assignments of common dicoumarols and dicoumarols with a fused benzylidene ring are tabulated in Tables 3.11 and 3.12, respectively.

Table 3.11 The ^{1}H-NMR spectral assignments of dicoumarols

Cpd	R			chemical shift (ppm)											
	R^1	R^2	R^3	C<u>H</u>-Ar	H-5	H-6	H-7	H-8	-OH	H-b	H-c	H-d	H-e	H-f	H-R
D1	H	H	H	6.10(s)	8.03(dd)	7.37(br)	7.62(dt)	7.41(d)	11.29, 11.52(s)	7.22(d)	7.32(t)	7.27(t)	-	-	-
D2	NO_2	H	H	6.62(s)	8.02(d)	7.38(br)	7.62(t)	7.39(d)	11.20, 11.50 (s)	-	7.63(d)	7.55(t)	7.44(t)	7.45(d)	-
D3	H	NO_2	H	6.13(s)	8.04(d)	7.39(t)	7.66(t)	7.43(dd)	11.37, 11.56(s)	8.07(s)	-	8.14(d)	7.51(t)	7.58(d)	-
D4	H	H	NO_2	6.11(s)	8.03(d)	7.41(br)	7.66(t)	7.42(d)	11.35, 11.55(s)	7.40(dd)	8.17(d)	-	-	-	-
D5	Cl	H	H	6.14(s)	8.03(d)	7.37(br)	7.61(dt)	7.39(d)	10.91, 11.62(s)	-	7.46(d)	7.35(dd)	7.27(dt)	7.24(dt)	-
D6	H	H	Cl	6.04(s)	8.03(d)	7.37(t)	7.63(dt)	7.41(d)	11.29, 11.52(s)	7.15(d)	7.29(d)	-	-	-	-
D7	Cl	H	Cl	6.09(s)	8.03(s)	7.38(br)	7.62(dt)	7.39(d)	11.65(s)	-	7.37(d)	-	7.24(dd)	7.38(dd)	-
D8	Br	H	H	6.06(s)	8.01(d)	7.32(br)	7.58(dt)	7.46(d)	10.90, 11.56(s)	-	7.38(d)	7.30(dt)	7.15(dt)	7.38(d)	-
D9	H	Br	H	6.04(s)	8.03(d)	7.37(dt)	7.63(dt)	7.40(d)	11.28, 11.55(s)	*	*	*	*	*	-
D10	H	H	Br	6.00(s)	8.02(dd)	7.38(br)	7.62(dt)	7.39(d)	11.29, 11.52(s)	7.08(dd)	7.42(d)	-	-	-	-
D11	H	H	CF_3	6.10(s)	8.04(br)	7.39(t)	7.63(dt)	7.42(dd)	11.33, 11.54(s)	7.33(d)	7.58(d)	-	-	-	-
D12	H	H	CH_3	6.05(s)	8.02(d)	7.38(br)	7.61(dt)	7.38(d)	11.35, 11.50(s)	7.10(d)	7.10(d)	-	-	-	2.32(s)
D13	H	H	*i*-Pr	6.07(s)	8.03(d)	7.38(t)	7.62(dt)	7.38(t)	11.48(s)	7.13(dd)	7.17(d)	-	-	-	1.23(s), 1.25(s), 2.89(hep)
D14	OCH_3	H	H	6.08(s)	8.01(d)	7.35(dt)	7.58(dt)	7.37(d)	11.20(s)	-	7.27(d)	6.93(dt)	7.26(t)	6.84(dd)	3.56(s)
D15	H	H	OCH_3	6.04(s)	8.03(d)	7.38(br)	7.62(dt)	7.40(d)	11.28, 11.49(s)	6.84(d)	7.12(d)	-	-	-	3.79(s)
D16	H	OCH_3	OCH_3	6.07(s)	8.04(d)	7.37(t)	7.63(dt)	7.41(d)	11.28, 11.51(s)	6.71(s)	-	-	6.82(d)	6.77(d)	3.73(R^2), 3.87(R^3)
D17	H	OCH_3	OH	6.05(s)	8.01(d)	7.37(t)	7.60(dt)	7.38(d)	11.28, 11.48(s)	6.67(s)	-	-	6.71(dd)	6.84(d)	3.73(R^2), 5.59(R^3)
D18	H	OH	OH	6.03(s)	8.01(dd)	7.47(t)	7.73(dt)	7.46(d)	11.46(s)	6.76(s)	-	-	6.64(d)	6.78(d)	7.74(s), 7.74(s)

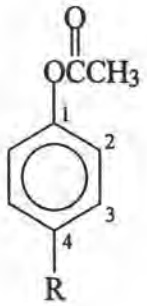
Table 3.12 The ^{1}H-NMR spectral assignments of dicoumarols with a fused benzylidene ring

Cpd	R^2	chemical shift (ppm)										
		C<u>H</u>-Ar	H-5	H-6	H-7	H-8	-OH	H-c	H-d	H-e	H-f	H-R
D19	H	5.76(s)	8.05(br), 8.11(dd)	7.79, 7.15(dt)	7.60, 7.70(dt)	7.22, 7.45(d)	*	*	*	*	*	-
D20	OCH_3	5.36(s)	8.02, 8.22(dd)	7.28, 7.43(dt)	7.47, 7.62(dt)	7.18, 7.39(d)	10.32(s)	-	6.70(d)	7.03(t)	6.89(dd)	3.99(s)

* : not assigned

^{13}C-NMR

Phenyl acetates



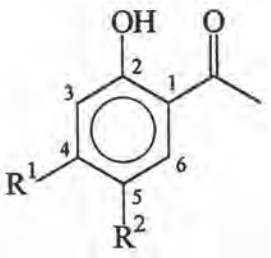
The ^{13}C-NMR spectra of phenyl acetates generally exhibited a methyl carbon adjacent to an ester functional group at 20.9-21.1 ppm, an ester C at 169.1-169.7 ppm and carbon(s) of alkyl side chain appearing at 15.6-28.3 ppm. Another set of signal assigned to aromatic carbons as C-1 (1C), C-2 (2C), C-3 (2C) and C-4 (1C) detected in the range of 148.5-149.7, 121.2-123.3, 128.7-132.4 118.9-141.7 ppm, respectively. The chemical shifts of alkyl groups on the side chain were observed around 15.7-28.3 ppm. The ^{13}C-NMR spectral assignments of phenyl acetates are exhibited in Table 3.13.

Table 3.13 The ^{13}C-NMR spectral assignments of phenyl acetates

Cpd	R	chemical shift (ppm)						
		C-1	C-2	C-3	C-4	C(O)O-	$\underline{C}H_3$-COO-	$\underline{C}$ of R
2a	CH_3	148.5	121.2	129.8	135.3	169.5	20.9*	20.7*
3a	C_2H_5	148.7	121.3	128.7	141.7	169.7	21.1	15.6,28.3
4a	Cl	149.1	122.9	129.4	131.2	169.2	21.0	-
5a	Br	149.7	123.3	132.4	118.9	169.1	21.0	-

* = could be interchangeable

2-Hydroxyacetophenones

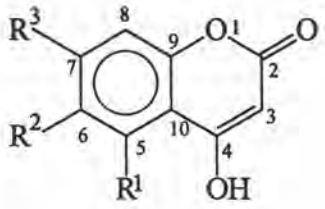


The ^{13}C-NMR spectra of a main skeleton of 2-hydroxyacetophenones revealed total of eight signals. The methyl carbon showed a typical signal at 26.2-26.7 ppm, while the carbonyl carbon showed a signal at 201.9-204.5 ppm. The remaining 6 C signals were aromatic carbons as C-1, C-2, C-3, C-4, C-5 and C-6 (1 C each) detected at 114.6-120.9, 160.3-161.8, 102.6-120.5, 136.3-164.3, 101.1-134.5 and 129.3-134.7 ppm, respectively. The alkyl and methoxy on the side chain were observed around 15.7-56.6 ppm. The ^{13}C-NMR spectral assignments of 2-hydroxyacetophenones are tabulated in Table 3.14.

Table 3.14 The ^{13}C-NMR spectral assignments of 2-hydroxyacetophenones

	R		chemical shift (ppm)								
Cpd	R^1	R^2	C-1	C-2	C-3	C-4	C-5	C-6	-C(O)-	CH_3	C of R
2b	H	CH_3	119.4	160.3	118.2	137.5	128.0	130.4	204.4	26.6	20.5
3b	H	C_2H_5	119.4	160.4	118.2	136.3	134.5	129.3	204.5	26.5	15.7,27.9
4b	H	Cl	120.3	160.9	120.1	136.3	123.5	129.9	203.6	26.7	-
5b	H	Br	120.9	161.3	120.5	139.1	110.4	132.9	203.5	26.7	-
8b	OCH_3	Br	114.6	161.8	102.6	164.3	101.1	134.7	201.9	26.2	56.6

4-Hydroxycoumarins

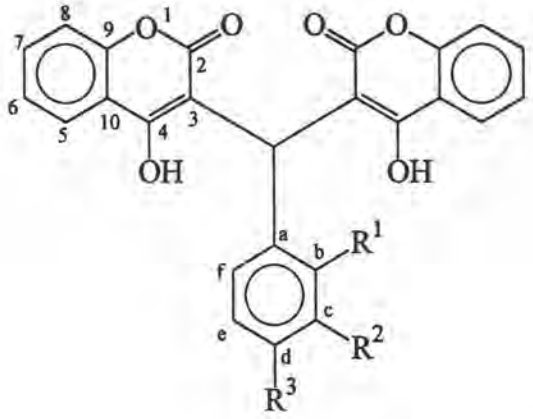


The signals belonging to alkyl and methoxy carbons in the ^{13}C-NMR spectra of 4-hydroxycoumarins were detected around 15.6-57.0 ppm. The remaining 9 C above 80 ppm (at 88.4-167.2 ppm) were carbons of benzopyrane ring as C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9 and C-10 (1 C each) observed at 161.3-163.1, 88.4-91.7, 164.4-167.2, 121.5-157.3, 106.0-139.4, 132.3-162.3, 100.5-118.7, 151.8-155.4 and 105.0-117.3 ppm, respectively. The ^{13}C-NMR spectral assignments of 4-hydroxycoumarins are shown in Table 3.15.

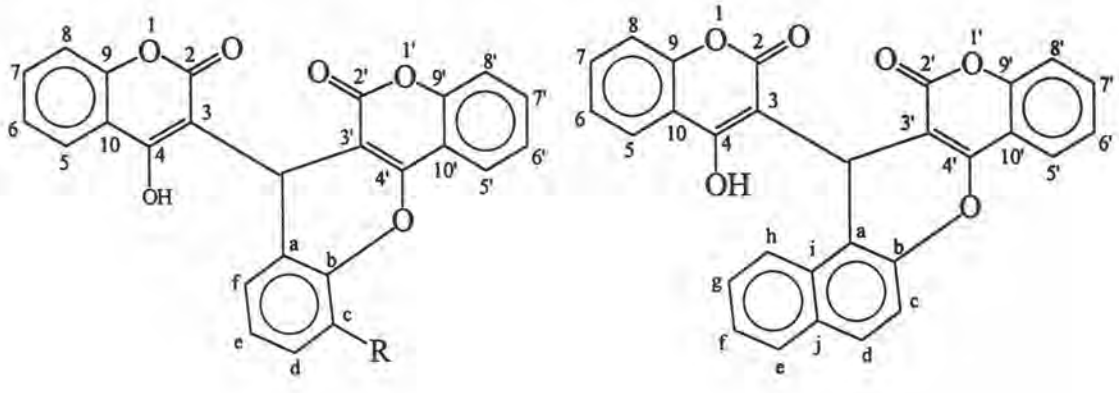
Table 3.15 The ^{13}C-NMR spectral assignments of 4-hydroxycoumarins

	R			chemical shift (ppm)									
Cpd	R^1	R^2	R^4	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C of R
1	H	H	H	161.9	91.0	165.6	123.2	123.8	132.6	116.3	153.5	115.8	-
2	H	CH_3	H	163.1	91.5	166.7	123.5	134.0	134.3	116.8	152.6	116.2	20.4
3	H	C_2H_5	H	162.0	90.9	165.6	121.5	139.4	132.4	116.2	151.8	115.5	15.6,27.4
4	H	Cl	H	161.4	91.7	164.5	122.3	127.9	132.3	118.5	152.1	117.3	-
5	H	Br	H	161.3	91.7	164.4	125.3	117.7	135.1	118.7	152.5	115.6	-
6	OCH_3	H	H	161.5	90.8	167.2	157.3	109.2	133.0	106.7	155.1	105.0	-
7	H	H	OCH_3	162.9	88.4	166.0	124.3	111.9	162.3	100.5	155.4	108.9	-
8	H	OCH_3	Br	161.8	89.1	164.9	126.4	106.0	158.5	100.7	154.5	109.9	57.0

Dicoumarols



The ^{13}C-NMR spectra of common dicoumarols exhibited the peaks of main structure similar to those of 4-hydroxycoumarins. The signals belonging to alkyl and methoxy carbons spectra were detected at 20.9-56.1 ppm and Ar-CH was observed around 33.8-37.6 ppm. The remaining 24 C above 100 ppm (at 103.2-169.3 ppm) were aromatic carbons of 2 benzopyrane ring (18 C) as C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9 and C-10 (2 C each) at 164.4-166.5, 103.2-106.1, 166.4-169.3, 124.2-124.7, 124.6-125.6, 131.8-133.7, 116.3-117.3, 152.1-153.3 and 116.2-117.6 ppm, respectively. Other six carbons as aromatic carbons of benzylidene ring were detected in the range of 109.4-158.4 ppm.



The ^{13}C-NMR spectra of dicoumarols with a fused benzylidene ring and a fused naphthalidene ring were found to be similar to those of common dicoumarols. However, those of a fused benzopyran ring normally occurred at lower chemical shifts, except for the C-3′ signal. The ^{13}C-NMR spectral assignments of dicoumarols and dicoumarols with a fused benzylidene ring are presented in Tables 3.15 and 3.16, respectively.

Table 3.16 The ^{13}C-NMR spectral assignments of dicoumarols

Cdp	R			chemical shift (ppm)															
	R^1	R^2	R^3	CH-Ar	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-a	C-b	C-c	C-d	C-e	C-f	C(R)
D1	H	H	H	36.2	164.6, 165.8	103.9, 105.6	166.8, 169.3	124.4	124.8	132.8	116.6	152.3, 152.5	116.8	135.2	126.4	128.6	126.8	-	-	-
D2	NO_2	H	H	33.8	164.4	103.8	166.4, 167.2	124.5	125.0	131.8	116.6	152.3, 152.4	116.8, 116.9	133.2	149.8	128.1	129.4	129.4	129.3	-
D3	H	NO_2	H	36.2	164.8, 166.5	103.2, 104.6	166.9, 169.1	124.5	125.2	133.3	116.3	152.3, 152.6	116.7, 116.8	138.0	122.1	148.7	121.7	129.5	132.7	-
D4	H	H	NO_2	36.5	164.7, 166.3	103.2, 104.7	166.9, 169.0	124.4	125.1	133.3	116.7	152.3, 152.5	116.2	143.3	127.5	123.8	146.8	-	-	-
D5	Cl	H	H	35.7	164.5, 165.1	104.4, 105.6	167.2, 168.7	124.4	124.9	132.8	116.6	152.1, 152.4	116.6	133.5	133.5	129.2	128.6	126.7	130.8	-
D6	H	H	Cl	35.8	164.6, 166.0	103.7, 105.3	166.8, 169.2	124.4	125.0	133.0	116.7	152.3, 152.5	116.6	133.9	128.8	128	132.7	-	-	-
D7	Cl	H	Cl	35.4	164.6, 165.3	104.2, 105.3	167.2, 168.7	124.4	125.0	133.0	116.6	152.3, 152.4	116.2, 116.3	132.3	133.7	130.5	134.2	127.0	130.2	-
D8	Br	H	H	37.6	164.8	105.1	167.5	124.4	124.9	132.9	116.6	152.3	116.6	135.1	123.4	134.3	127.3	128.8	129.4	-
D9	H	Br	H	36.0	164.7, 166.1	103.4, 105.1	166.8, 169.2	124.5	125.0	133.1	116.7	152.3	116.7	137.8	130.1	122.9	129.5	130.1	125.2	-
D10	H	H	Br	35.9	164.6, 166.0	103.7, 105.2	166.8, 169.2	124.4	125.0	133.0	116.7	152.3, 152.4	116.7	134.4	-	-	-	-	-	-
D11	H	H	CF_3	36.3	164.7	103.5, 105.1	166.9, 169.2	124.4	125.0	133.1	116.7	152.3, 152.6	116.3	139.7	126.9	125.6	116.7	-	-	-
D12	H	H	CH_3	35.8	164.5, 165.6	104.0, 105.7	166.8, 169.2	124.3	124.8	132.7	116.5	152.3	116.5	132.0	126.3	129.3	136.4	-	-	20.9
D13	H	H	*i*-Pr	35.9	164.5, 165.6	104.0, 105.8	166.8, 169.2	124.4	124.8	132.7	116.6	152.3, 152.5	116.8, 116.9	132.4	126.4	126.7	147.4	-	-	23.9, 33.6
D14	OCH_3	H	H	33.4	163.7	105.8	168	124.2	124.6	132.4	116.4	152.1	116.8	123.6	157.6	111.0	128.2	120.4	128.4	55.5
D15	H	H	OCH_3	35.5	164.5, 165.6	104.2, 105.8	166.8, 169.2	124.3	124.8	132.8	116.6	152.3	116.6	126.9	127.6	114.0	158.4	-	-	55.2
D16	H	OCH_3	OCH_3	35.8	164.6, 165.6	149.2	166.7, 169.3	124.3	124.8	132.8	116.7	152.3, 152.4	116.6	127.6	110.4	149.2	148.1	118.9	111.3	55.9, 56.1
D17	H	OCH_3	OH	35.8	164.6, 165.7	104.2, 105.7	166.8, 169.1	124.3	124.9	132.8	116.6	152.4	116.6	126.8	109.4	146.7	144.6	114.5	119.5	56.1
D18	H	OH	OH	36.4	165.3	106.1	168.4	124.7	125.6	133.7	117.3	153.3	117.6	128.0	114.8	145.9	144.7	116.1	118.9	-

Table 3.17 The ^{13}C-NMR spectral assignments of dicoumarols with a fused benzylidene ring

Cpd	R^2	chemical shift (ppm)																	
		CH-Ar	C-2, C-2′	C-3, C-3′	C-4, C-4′	C-5, C-5′	C-6, C-6′	C-7, C-7′	C-8, C-8′	C-9, C-9′	C-10, C-10′	C-a	C-b	C-c	C-d	C-e	C-f	C(R)
D19	H	28.7	160.4, 160.7	100.6, 107.4	161.3, 149.2	123.9, 122.6	125.3, 124.5	132.5, 132.2	116.4, 116.2	156.2, 152.0	116.1, 113.8	123.8	156.2	116.2	128.6	122.2	128.3	-
D20	OCH_3	30.1	116.3, 161.1	100.0, 108.7	166.2, 158.6	123.8, 122.4	125.0, 124.4	132.7, 131.8	116.8, 116.2	153.2, 152.1	117.0, 111.9	125.3	141.0	148.0	114.9	123.8	120.0	56.4

3.2 Biology

One of the major goals of this research is to find out the relationship between structures of 4-hydroxycoumarins and dicoumarols and the interested biological activity. Two bioassays : insect antifeedant against *Galleria mellonella* L. and growth inhibition of *Mimosa pigra* L. have been conducted. In addition, the results derived from this work were compared with those obtained when commercially available insecticides in the former case and commercially available herbicides in the latter case were employed.

3.2.1 Insect Antifeedant Activity against *Galleria mellonella*

Feeding deterrent bioassays usually depend on the ability to measure the amount of material consumed or not consumed by the insect. This assay might be divided into 2 parts: (a) a fecal pellet bioassay and (b) a choice test.[50] In this research, a choice test was chosen for treating 4-hydroxycoumarins, dicoumarols and commercially available insecticides against the greater wax moth larvae.

4-Hydroxycoumarins

Stemmed from previous reports of the insecticidal activity of natural occurring 4-hydroxycoumarins, for instance, 3-alkyl-4-hydroxycoumarins,[51] eight synthesized 4-hydroxycoumarins were subjected to antifeedant activity test against *Galleria mellonella* Linn. The results are presented as shown in Fig 3.1.

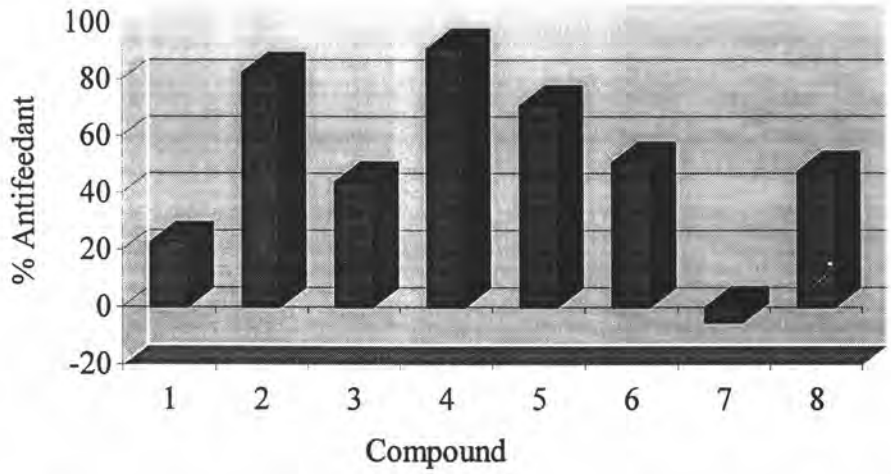


Fig 3.1 Percent antifeedant of eight synthesized 4-hydroxycoumarins against *G. mellonella*

Using 4-hydroxycoumarin (**1**) as a reference compound, it was observed that all prepared coumarins except for (7) displayed a better activity. Substituents at position 6 on a benzopyran ring revealed a difference in reactivity. To simplify the data attained, the comparison of the structure and antifeedant activity is presented as shown in Table 3.17.

Table 3.18 The comparative results of % antifeedant of synthesized 4-hydroxycoumarins

% Antifeedant	Cpd	Positions of substituents on a benzopyran ring		
		5	6	7
90	**4**	H	Cl	H
80	**2**	H	CH_3	H
70	**5**	H	Br	H
60				
50	**6**	OCH_3	H	H
	3, 8	H, H	C_2H_5, Br	H, OCH_3
40				
30				
20	**1**	H	H	H
10				
0				
	7	H	H	OCH_3

Based upon the results obtained, it could be seen that two major parameters may influence the antifeedant activity: a type and a position of a substituent(s). For instance, among methoxy substituents, the variation of the positions of a methoxy group greatly affected the activity. To illustrate this, a methoxy group at position 5 increased the reactivity, while that at position 7 made the activity worse comparing with a reference compound. A variety of substituents at position 6 also provided informative results. All compounds that have a substituent at position 6 enhanced the antifeedant activity. Among them, 4-hydroxycoumarin bearing a chloro substituent (**4**) gave a better result than a bromo derivative (**5**) with % antifeedant 91 and 71 %, respectively. In addition, a smaller group of an alkyl chain (say, methyl group (**2**)) also provided a better activity than a larger one (an ethyl group (**3**)) as much as 82 and 44 %, respectively.

From the rudiment consideration, the obtained information implied that % antifeedant of 4-hydroxycoumarins was largely structural dependent. 5-Methoxy and all 6-substituted 4-hydroxycoumarins enhanced the activity, whereas 7-methoxy substituent decreased the activity. Among substituents at position 6 of 4-hydroxycoumarins, halogen substituents generally gave a better result than alkyl groups. It should also be noted that a small group provided a better activity than a large one.

The three best 4-hydroxycoumarins considering from the % antifeedant attained above are **2**, **4** and **5**.

Dicoumarols

Even though dicoumarols have well-recognized to be of pharmacological properties such as treatment and prophylaxis of thromboembolic disorders in veins and arteries, enzyme inhibition and antimicrobial action,[5,8b] the uses as agrochemicals have never been extensively studied. Since the structures of dicoumarols are closely related to these of 4-hydroxycoumarins, the structure - activity relationship of this class is therefore worth considering to investigate. Twenty one synthesized dicoumarols and three kindly provided dicoumarols[a] were subjected to antifeedant activity test against *Galleria mellonella* Linn. The results are presented as shown in Fig 3.2.

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a) kindly provided by Deesamer, S. and Tipnoysnga, J.

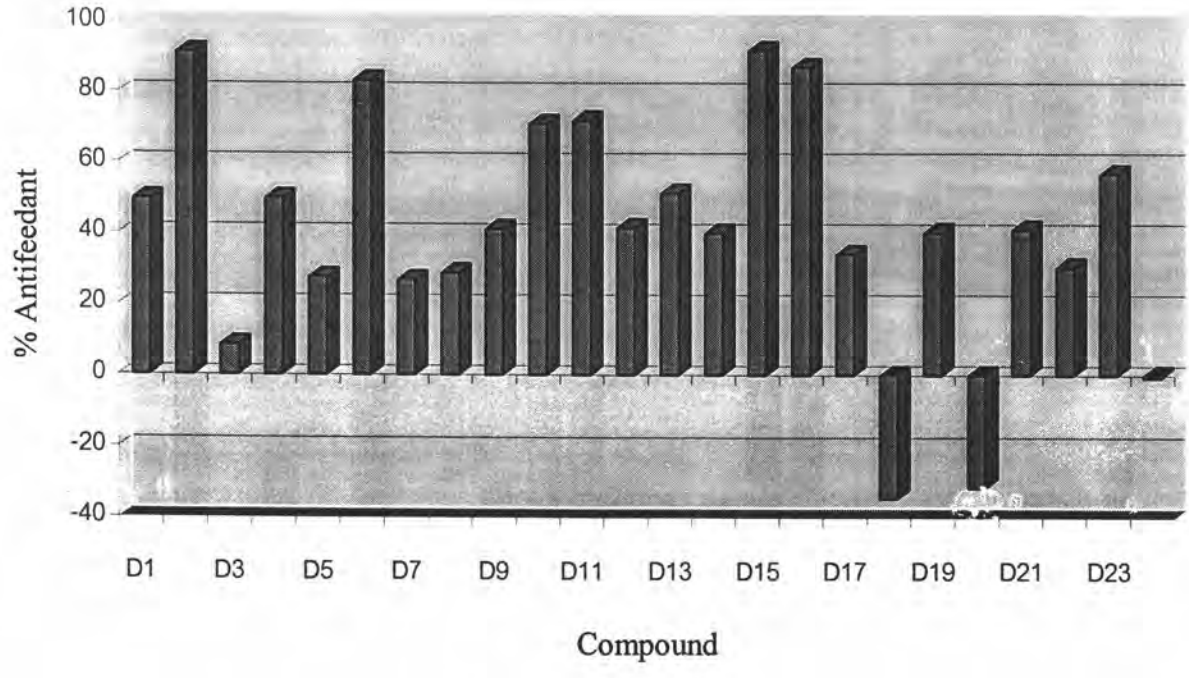


Fig 3.2 Percent antifeedant of twenty four dicoumarols against *G. mellonella*

It was observed that among prepared dicoumarols, there are six dicoumarols **D2**, **D6**, **D10**, **D11**, **D15** and **D16** revealed high activity (over 70 % antifeedant). Three dicoumarols **D4**, **D13** and **D23** showed a little more activity over that of **D1**. The rest fourteen dicoumarols **D3**, **D5**, **D7-D9**, **D12**, **D14**, **D17-D22** and **D24** exhibited lower activity than that of **D1**. The best one **D15** showed 92 % antifeedant activity, while the lowest activity of -35 % antifeedant is **D18**. To simplify the data accumulated, the comparison of the structure and antifeedant activity is presented as shown in Table 3.18.

Table 3.19 The comparative results of % antifeedant of dicoumarols

Anti-feedant	Not Fused Ring					Fused Ring
	Nitro	Halogen	Alkyl	Methoxy	Hydroxy	
90	**D2**			**D15**		
		D6		**D16**		
80						
70		**D10**	**D11**			
60						
				D23		
50	**D4**		**D13**	**D1**		
40		**D9**	**D12**	**D14**		**D19,D21**
					D17	
30		**D5,D8,D22**				
		D7				
20						
10	**D3**					
0					**D24**	
-10						
-20						
-30						**D20**
					D18	
-40						

Based upon the results obtained, it could be found that the antifeedant activity was influenced by the structural deviation of these compounds. All dicoumarol structures are different from each another at only a substituent on a benzylidene ring, except for three dicoumarols with fused benzylidene ring and fused naphthalidene ring (**D19-D21**). Therefore, they could be classified according to the antifeedant activity whether the structures were fallen into two types as three dicoumarols with fused aromatic ring or twenty one common dicoumarols.

Considering **D1** as a reference compound of common dicoumarols (**D1-D18** and **D22-D24**) and **D19** as a parent compound of fused dicoumarols (**D19-D21**), the relationship between their structures and activity could be summarized as:

A Common Dicoumarols

There are twenty one dicoumarols in this category. The structure of **D1** is composed of two units of 4-hydroxycoumarins and a unit equivalent of benzaldehyde. Surprisingly, **D1** (with 50 % antifeedant) showed activity about twice % antifeedant of **1** (as 4-hydroxycoumarin 1 unit, with 22 % antifeedant). This group can be classified into 5 subgroups based upon the difference in a main substituent on a benzylidene ring as follows: (i) nitro, (ii) halogen, (iii) alkyl, (iv) methoxy and (v) hydroxy groups.

(i) Nitro group as **D2** (at C-b), **D3** (at C-c) and **D4** (at C-d)

Among three variable nitro substituents tested, there is only one sample that revealed an outstanding activity **D2** (91 %). The next one is **D4** displaying antifeedant activity at 50 % with equal activity of **D1**. The other, **D3** had no activity with this bioassay.

(ii) Halogen group

This subgroup can be divided into three units based on the difference in a kind of halogen atom.

a. Fluoro group as **D22** (C-d)

This compound showed low antifeedant activity (30 %).

b. Chloro group(s) as **D5** (at C-b), **D6** (at C-d) and **D7** (at C-b and C-d)

D6 exhibited the highest activity (83%) in this subgroup, whereas **D5** (28 %) and **D7** (27 %) did not show any difference in activity.

c. Bromo group as **D8** (C-b), **D9** (C-c) and **D10** (C-d)

D10 (71 %) revealed the highest activity in this subgroup, followed by **D9** (41 %) and **D8** (29 %), respectively.

Considering all compounds in this subgroup, **D6** and **D10** are two compounds that revealed an interesting activity. Both of them have mono *para* substituent (chloro and bromo group, respectively). Even though **D22** has fluoro as mono *para*

substituent group like **D6** and **D10**, **D22** and the others did not exhibit much difference in this activity. Dicoumarols in this subgroup with halogen atom at *para* position are always the best ones, while those at *meta* positions showed medium activity and those at *ortho* positions revealed low activity.

(iii) Alkyl group at C-d as **D12** (with CH_3) and **D13** (with *i*-Pr)

D13 (51 %) with a larger alkyl group displayed a little more activity than that of **D12** (41 %). Both of them showed a comparable to that of **D1**.

(iv) Methoxy group as **D14** (at C-b), **D15** (at C-c) and **D16** (at C-c and C-d)

While **D14** (40 %) expressed a little lower activity than **D1**, **D15** (92 %) and **D16** (87 %) revealed quite high activity, especially **D15** is the best one among all studied dicoumarols.

(v) Hydroxy group(s) as **D18** (at C-c and C-d) and **D24** (at C-d)

Both **D18** and **D24** did not show any antifeedant activity (-35 and -1 % antifeedant activity, respectively).

Amazing contrast result of antifeedant activity of dicoumarols in subgroups (i) and (iv) could be rationalized. Whereas dicoumarol with a nitro group at *ortho* position exposed outstanding high activity, the other isomers exhibited low to medium activity. While dicoumarols with a methoxy group at *ortho* position revealed low activity, the others in this subgroup displayed high activity. Comparing nitro and methoxy groups with hydroxy and halogen substituents, they are larger and more bulky. The first two are strongest electrostatically influent substituent of all studied dicoumarols. This electrostatic influence descents from electron resonance of substituent group. Some steric structures might induce changeable structure in 3 dimensions by resonance with different from *meta* and *para* isomer. Nitro and methoxy groups have many effect on benzylidene alike, except for the fact that a nitro is an electron withdrawing group, whereas a methoxy is a strong electron donating group. It might be implied that strongly electrostatic influence substituent group increased activity. This trend was also found when comparing **D11** (72 %) with **D12**

(41 %), trifluoromethyl group had a larger influence than a methyl group and in the halogen atom series; **D6** (83 %) and **D10** (71 %), a chloro atom has higher electronegativity value than that of a bromo atom.

Another interesting point is the dicoumarol with the same type of substituent; the larger group trends to possess the better antifeedant activity than the smaller ones. This tendency was found by comparing **D6** and **D10** with **D22** and **D12** with **D13**. Chloro and bromo atoms are bigger than fluoro and *iso*-propyl group is larger than methyl group.

From this consideration, it will imply that the % antifeedant activity of common dicoumarols greatly relies on substituent(s) on a benzylidene ring which could be summarized as:

1) A strong electron withdrawing group such as a nitro group showed high activity at only *ortho* position
2) A strong electron donating group such as a methoxy group showed high activity at *para* position, and dimethoxy groups at *meta* and *para* positions
3) A benzylidene portion bearing a halogen atom at *para* position seemed to provide a better result than that at *meta* and *ortho* positions, respectively
4) A larger substituent had a tendency to increase more activity than the smaller ones

B Dicoumarols with fused aromatic ring

Since there are only three synthesized dicoumarols in this group, the evaluation of the outcome may not be fully covered. The structures of **D19** and **D21** are different at only the fused ring with benzylidene and naphthalidene moieties. Both dicoumarols displayed the activity with no noticeable distinct (40 and 41 %, respectively). While **D20** which was resemble to **D19** with methoxy substituent at C-c, exhibited -32 % antifeedant activity.

Unfortunately, these two compounds **D19** and **D21** showed medium antifeedant activity, a little lower than that of **D1** and **D20** had no activity with this interest bioassay.

From this consideration, it could be concluded that the % antifeedant of dicoumarols with fused aromatic ring may be influenced by:

1) dicoumarols with fused aromatic ring showed little lower activity than common dicoumarols
2) fused ring with benzylidene and naphthalidene moieties exhibited no different activity
3) a methoxy group at C-c decreased the antifeedant activity

In general considering, dicoumarols with a substituent on a benzylidene ring at *para* position always displayed the highest activity, whereas those at *meta* and *ortho* positions showed the medium and low activity, respectively, except for *o*-nitro substituent that showed the highest activity in the nitro series. The outstanding activity-promoting group on a benzylidene ring of common dicoumarols is a methoxy group. Nitro and halogen substituents are in the second rank and an alkyl group is in the following order. On the other hand, a hydroxy group declines an activity. All of fused ring dicoumarols showed activity lower than **D1**.

Considering the results of three commercially available insecticides **P1** - **P3**, whereas **P1** had no activity (-48 % antifeedant), **P2** and **P3** exhibited high antifeedant activity of 76 and 94 %, respectively. The interesting point is **P1** composed of naturally chemical substances from very well-known repellent plants (*Azadirachta indica* var. siamensis Valenton., *Alpinia nigra* (Gaertn.) B.L. Burtt and *Cymbopogon nardus* (Linn.) Rendle) against many kinds of insects,[52] but it could not control feeding behavior of the greater wax moth larvae. The combination formulae of **P2** and **P3** are the synthesized chemicals that used as insecticides. When comparing the results derived from these commercially available insecticides with those obtained from the synthesized 4-hydroxycoumarins and dicoumarols, it was found that among those prepared compounds, there are two 4-hydroxycoumarins: **2** and **4**, and four dicoumarols: **D2, D6, D15** and **D16** that showed high activity (82-92 % antifeedant) comparable to **P2** and **P3** (76-94 % antifeedant). Therefore, 4-hydroxycoumarins bearing a 6-methyl and 6-chloro substituents, and dicoumarols with a nitro substituent at *ortho*, methoxy at *meta*, dimethoxy at *meta* and *para*, and chloro at *para* positions

substituted on a benzylidene ring gave satisfied results. The rest five 4-hydroxycoumarins and seventeen dicoumarols exhibited lower activity than **P2**.

The comparison of % antifeedant activity of two potent 4-hydroxycoumarins (**2** and **4**) and four potent dicoumarols (**D2**, **D6**, **D15** and **D16**) with commercial insecticides (**P2** and **P3**) is presented as shown in Fig. 3.3.

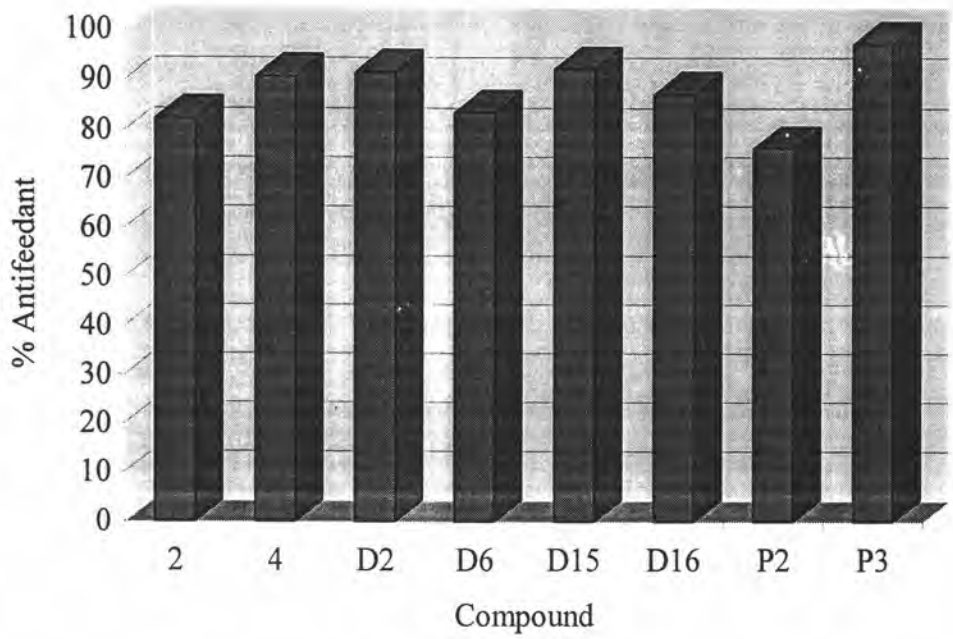


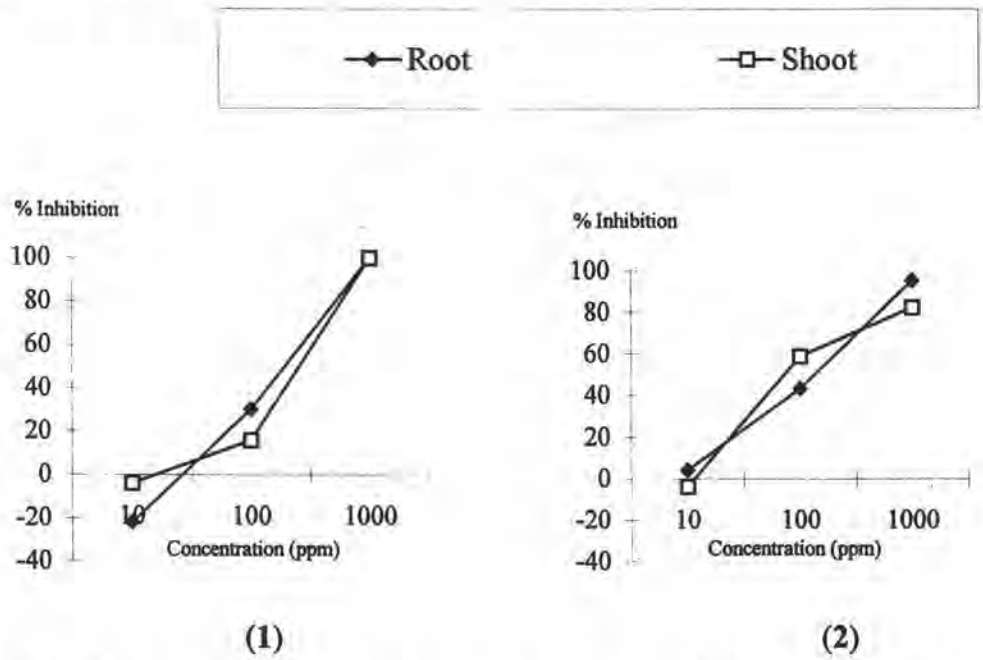
Fig 3.3 Percent antifeedant of potent 4-hydroxycoumarins and dicoumarols, and some commercial insecticides against *G. mellonella*

3.2.2 Weed Growth Inhibition against *Mimosa pigra* Linn.

The weed selected for bioassay in this study was *Mimosa pigra* Linn. (giant sensitive plant) which is a noxious woody weed distributed in the northern part of Thailand. It does not only play an important role only in agriculture, forestry and horticulture, but also in succession of ecosystem. Weed growth inhibition test is a kind of chemical methods, which is one of three efficient methods for controlling *M. pigra*: mechanical, chemical and biological methods. This method was chosen for treating 4-hydroxycoumarins, dicoumarols and commercially available herbicides against the giant mimosa.

4-Hydroxycoumarins

Even though the biological activity test of 4-hydroxycoumarins against giant mimosa has never been reported in chemical literature, they in fact showed inhibitory effect on growth of *Mimosa pigra*. The degree of inhibition/stimulation depended on the difference in structures of eight 4-hydroxycoumarins. The results are presented as shown in Fig 3.4.



(1) (2)

Fig 3.4 Percent root and shoot growing inhibition of 4-hydroxycoumarins against *M. pigra*

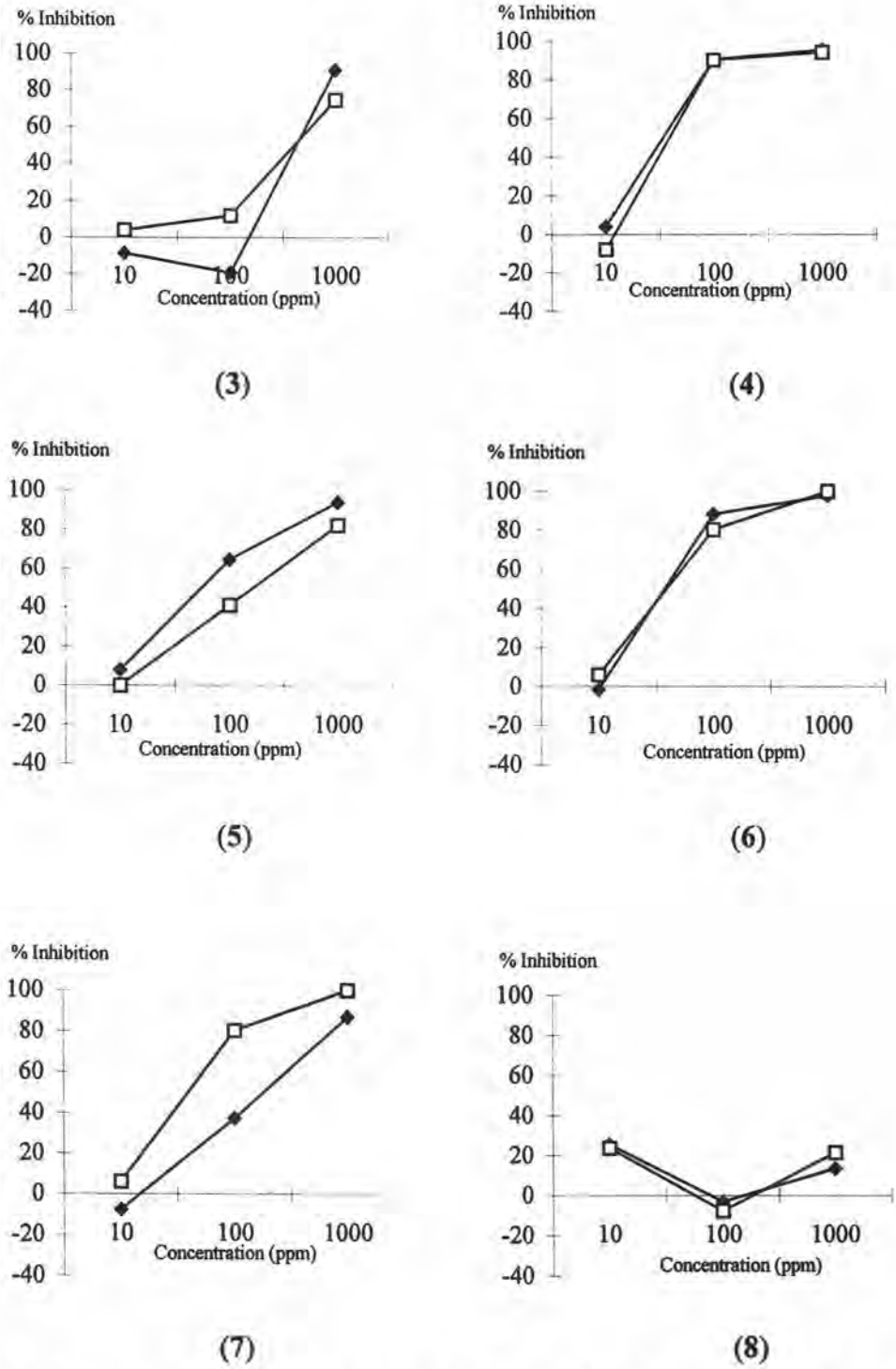


Fig 3.4 Percent root and shoot growing inhibition of 4-hydroxycoumarins against *M. pigra* (Cont.)

The graphs reveal the correlation between % root and shoot inhibitions and concentration of tested 4-hydroxycoumarins in the range of 10-1000 ppm. The substance possessing high activity should give the slope as increasing function in semi-logarithm graph (higher activity of each increases 1 unit of logarithm concentration of dose) and high % root inhibition at 100 ppm. The % inhibition of root is thought to be more essential for considering than that of % shoot inhibition. That is because the roots are directly contacted to the tested chemical in cellulose, while the shoot growth is contributed from root and attributed accumulated food from seed. Therefore, the profound effect of tested substance in this plant part might be misinterpreted. Percent root inhibition at 100 ppm was selected to be mainly contemplated. On the ground of low root inhibition always revealed by substance with low concentration (10 ppm) (*e.g.*, some common herbicides with low level of dose were grossly encountered to be plant promoters) and substance with high concentration (in the range of 1000-10000 ppm) always desisted herb growth at high level (high % root inhibition). The consideration at such conditions, the difference of inhibition activity of each tested substance would probably vanish. As follows, the general interpretations of these results are intensively concentrated at the first view on the slope of graph and % root inhibition at dose level 100 ppm.

Using 4-hydroxycoumarin (**1**) as a reference compound, it was observed that there are four prepared 4-hydroxycoumarins (**2**, **4**, **5** and **6**) showed higher activity. To simplify the data attained, the comparison of the structures and antigrowth activity (% root inhibition at dose level 100 ppm) is presented as shown in Table 3.20. From these results, it revealed that among eight 4-hydroxycoumarins studied, **8** did not give the good tendency of inhibition as those good herbicides should behave. Therefore, **8** was considered as it had no activity according to the above described criterion.

Table 3.20 The comparative results of % antigrowth of synthesized 4-hydroxycoumarins against *M. pigra*

% Inhibition	Code	Substituents on Benzopyran ring		
		5	6	7
90	**4**, **6**	H, OCH_3	Cl, H	H, H
80				
70				
	5	H	Br	H
60				
50				
	2	H	CH_3	H
40	**7**	H	H	OCH_3
30	**1**	H	H	H
20				
10				
0				
-10				
-20	**3**	H	C_2H_5	H

Based upon the results obtained, it could see that two major parameters may influence the antigrowth activity: a type and a position of substituent. For instance, among methoxy substituents, the variation of the positions of methoxy group greatly affected the activity. To illustrate this, a methoxy group at position 5 (**6**) increased the activity, while that at position 7 (**7**) did not have any influence on the activity comparing with a reference compound. In addition, a variety of substituents at position 6 also provided informative results. Among them, 4-hydroxycoumarin bearing a chloro substituent (**4**) gave a better result than a bromo derivative (**5**) with % antigrowth 91 and 65 %, respectively. Moreover, a smaller group of an alkyl chain

such as a methyl group (**2**) also provided a better activity than a later one (**3**) as much as % antigrowth 45 % inhibition and no activity, respectively.

From the above consideration, the obtained information implied that % antigrowth of 4-hydroxycoumarins was largely structure dependent. The methoxy substituent at positions 5- and 7-, all halogens and small alkyl group (methyl group) at position 6 enhanced the activity, whereas the other ones bearing a bromine atom at C-6 and a methoxy group at C-7, and large alkyl group (say, ethyl group) decreased activity. Among substituents at position 6 of 4-hydroxycoumarins, halogens generally provided better activity than alkyl groups. It should also be noted that a small group gave better activity than a large one.

The two best 4-hydroxycoumarins considering from the highest % antigrowth are **4** and **6** (with 90 % antigrowth) and a moderate one is **5** (with 65 %).

Dicoumarols

The biological activity test of twenty three dicoumarols against giant mimosa has also never been reported in chemical literature. This group of compound displayed different inhibitory on the growth of *Mimosa pigra*. The degree of inhibition depended on the difference in the structures of twenty three dicoumarols. The results are presented as shown in Fig 3.5.

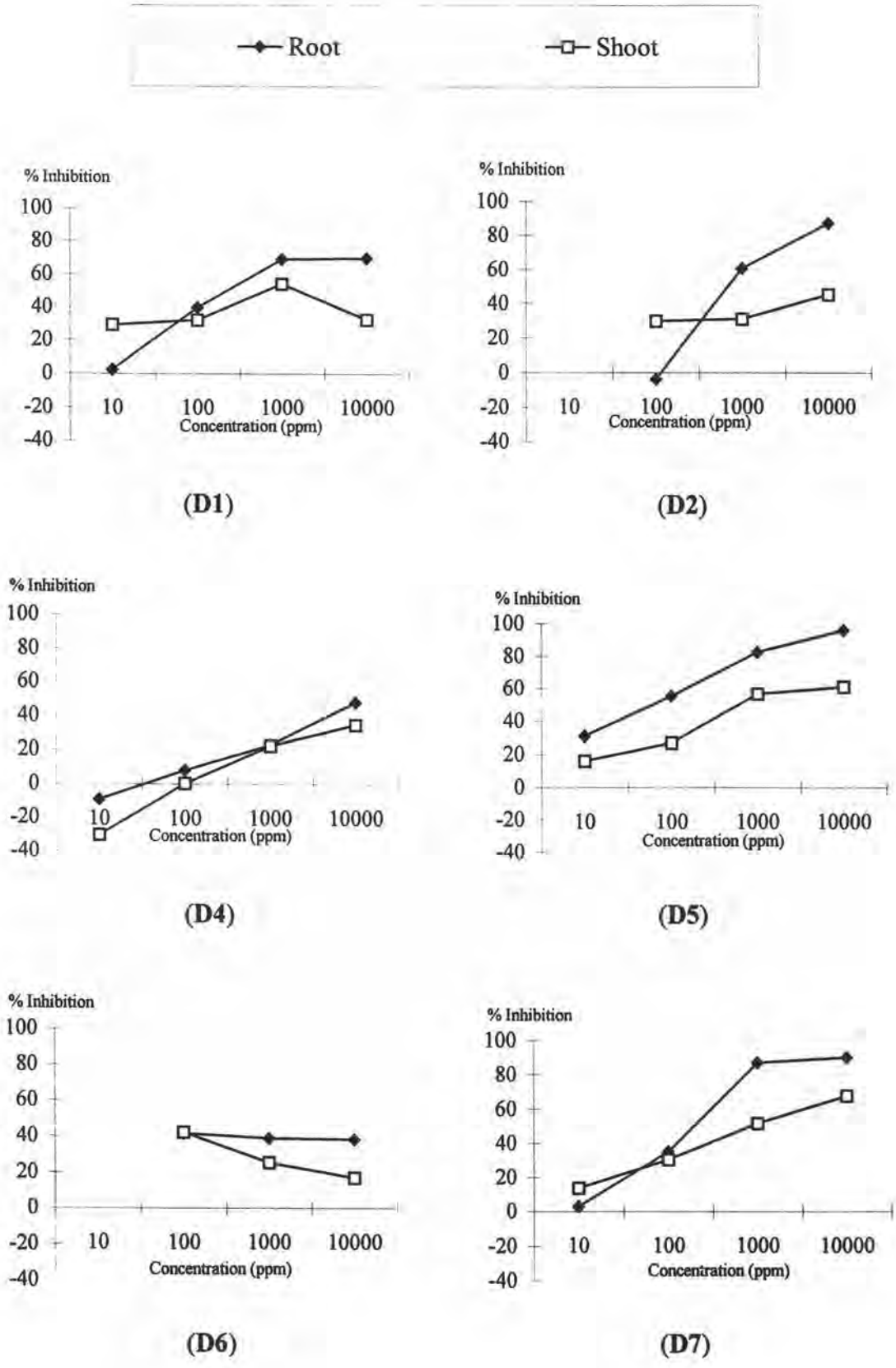
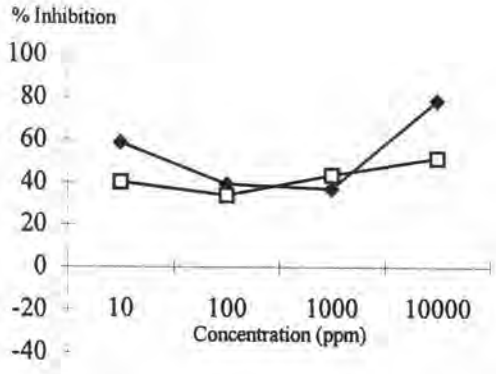
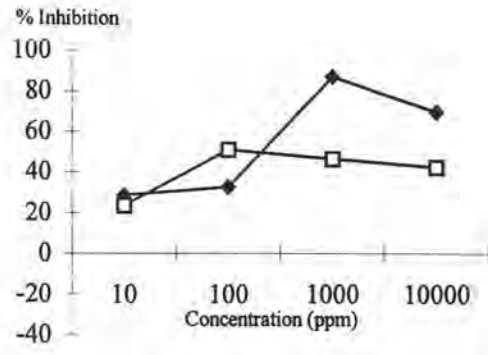


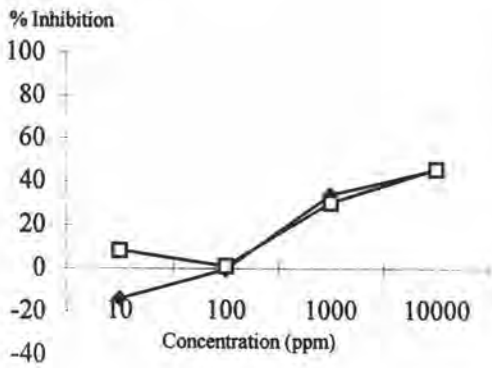
Fig 3.5 Percent root and shoot growing inhibition of dicoumarols against *M. pigra*



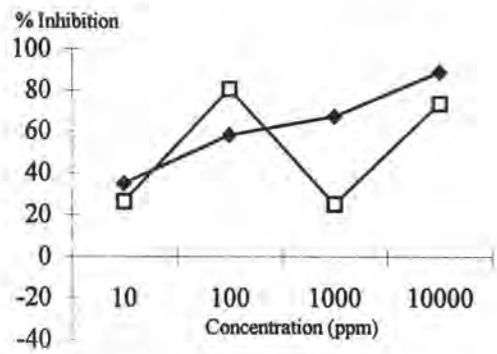
(D8)



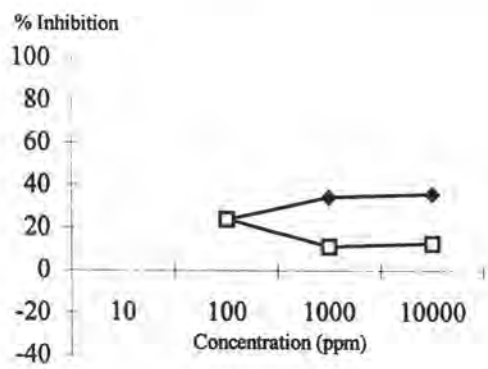
(D9)



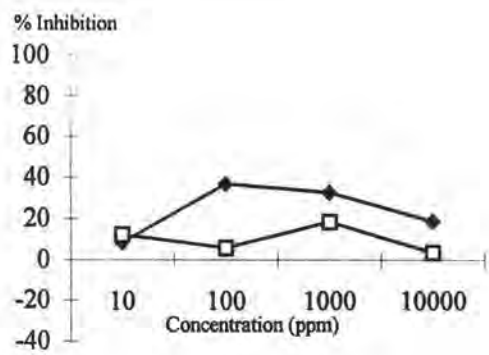
(D10)



(D11)

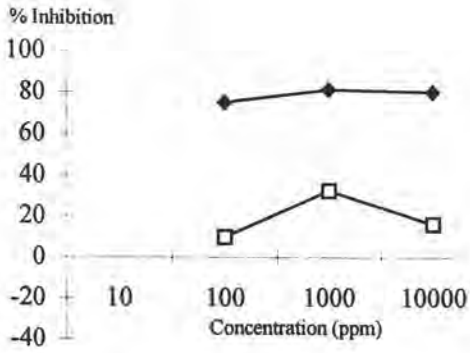


(D12)

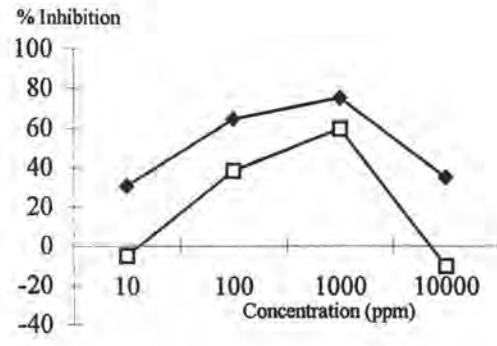


(D13)

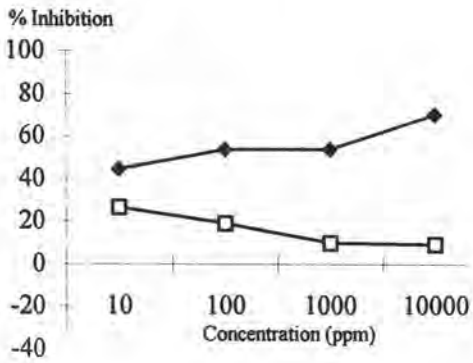
Fig 3.5 Percent root and shoot growing inhibition of dicoumarols against *M. pigra* (Cont.)



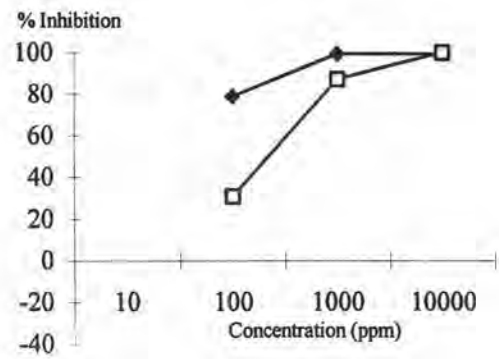
(D14)



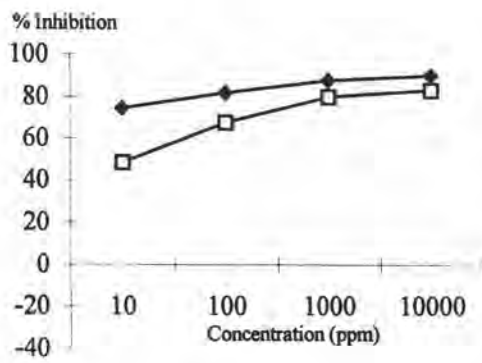
(D15)



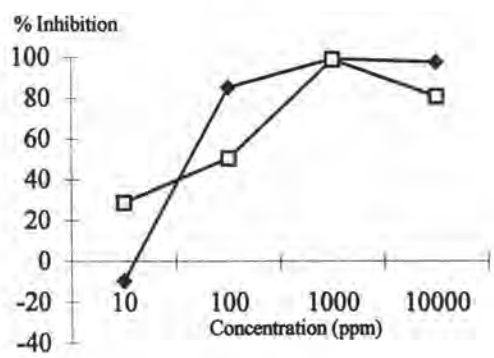
(D16)



(D17)

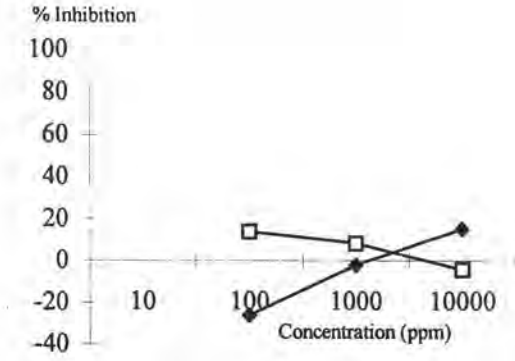


(D18)

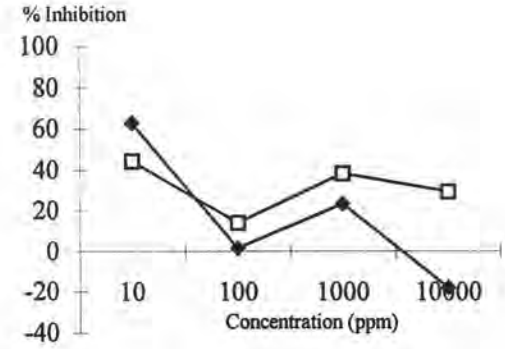


(D19)

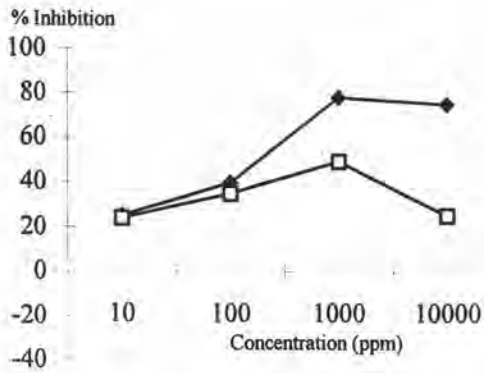
Fig 3.5 Percent root and shoot growing inhibition of dicoumarols against *M. pigra* (Cont.)



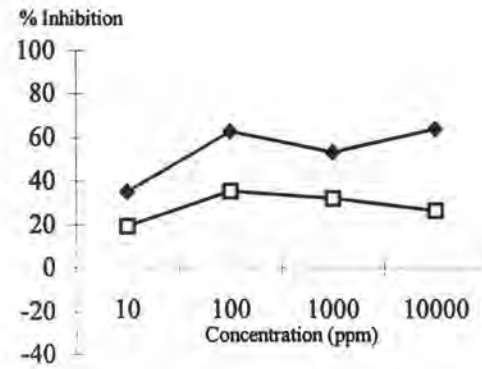
(D20)



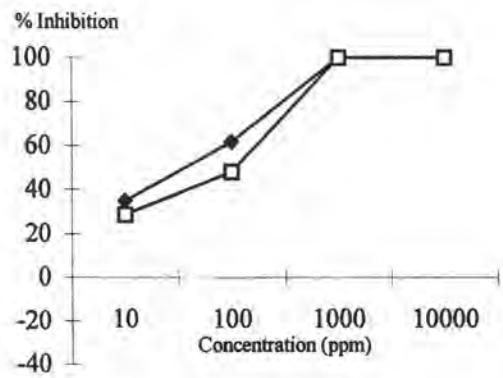
(D21)



(D22)



(D23)



(D24)

Fig 3.5 Percent root and shoot growing inhibition of dicoumarols against *M. pigra*

(Cont.)

The criterion used for interpretation of these derived results from a dicoumarol group was the same as those used for 4-hydroxycoumarins. Dicoumarols **1** and **19** were selected as reference compounds. To make an approached understanding from the data attained, the comparison of the structure and antigrowth activity (% root inhibition at dose level 100 ppm) is presented as shown in Table 3.21. Among the biological activity results derived from all dicoumarols tested, those of **D9, D13, D15, D21** and **D23** did not show the good correlation between the concentration used and % growth inhibition observed. Therefore, their results should be excluded from this examination.

Table 3.21 The comparative results of % antigrowth of dicoumarols against *M. pigra*

% Inhibition	Not Fused Ring					Fused Ring
	Nitro	Halogen	Alkyl	Methoxy	Hydroxy	
90						**D19**
80					**D17** **D18**	
70				**D14**		
60		**D11**			**D24**	
50		**D5**		**D16**		
40		**D6,D8,D22** **D7**	**D1**			
30						
20			**D12**			
10	**D4**					
0		**D10**				
-10	**D2**					
-20						
-30						**D20**

Based upon the results obtained, it was clearly supported the previous assumption that two major parameters influence the antigrowing activity: a type and a position of substituent(s). From the above table, the obvious relationship between the structures of dicoumarols and antigrowth activity could be rationally seen by considering types and positions of substituent(s) on a benzylidene ring. The relationship of not fused ring dicoumarol structure and activity can also be illustrated as follows:

1) The types of substituent: hydroxy > methoxy > alkyl > halogen > nitro.

Among a variety of substituents studied, a hydroxy group showed the highest activity (62-82 %), a little more than methoxy group (54-75 %) and alkyl group (25-35 %), respectively. The halogen atom (0-55 %) had a little effect on % antigrowth, while the nitro group displayed the lowest activity. All activities derived from the nitro derivatives were found to be lower than that of **D1**. This may imply that a nitro substituent decreased activity.

2. The positions of a substituent on a benzylidene ring could be arranged according to the tendency of activity and positions of substituent on a benzylidene ring from the highest to the lowest as: *ortho-*, *meta-* and *para-*, respectively.

However, dicoumarols which possessed a fused aromatic ring exhibited the major difference in activity. The activity of the best one (**D19**) showed the highest and that of the worst one (**D20**) displayed the lowest activity among all twenty three dicoumarols studied. It might be analyzed that dicoumarols with fused aromatic ring without any substituent on a benzylidene ring was the best one, while the presence of a methoxy on benzylidene and naphthalidene rings showed no activity.

In general consideration, the % growing inhibition of dicoumarols bearing various substiuents at different positions on a benzylidene ring might be arranged in order from the highest to the lowest as:

Substituent group : hydroxy > methoxy > alkyl > halogen > nitro

Position : *ortho* > *meta* > *para*

Considering the results of four commercially available herbicides **H1 - H4, H1** and **H3** exhibited high growth inhibition activity of 80 and 85 %, respectively. When comparing the results derived from these commercially available herbicides with those

obtained from the synthesized 4-hydroxycoumarins and dicoumarols, it was found that among those prepared compounds, there are two 4-hydroxycoumarins: **4** and **6**, and four dicoumarols: **D14**, **D17**, **D18** and **D19** that showed high activity (75-85 % antigrowth) comparable to **H1** and **H3** (80-85 % antigrowth). Therefore, 4-hydroxycoumarins bearing a 6-chloro and 5-methoxy substituents, and dicoumarols with electron donating groups (say hydroxy and methoxy) on a benzylidene ring and with a fused benzylidene ring gave pleased results.

The comparison of % growth inhibition activity of two potent 4-hydroxycoumarins (**4** and **6**) and four potent dicoumarols (**D14**, **D17**, **D18** and **D19**) with commercial herbicides (**H1** and **H3**) is presented as shown in Fig. 3.6.

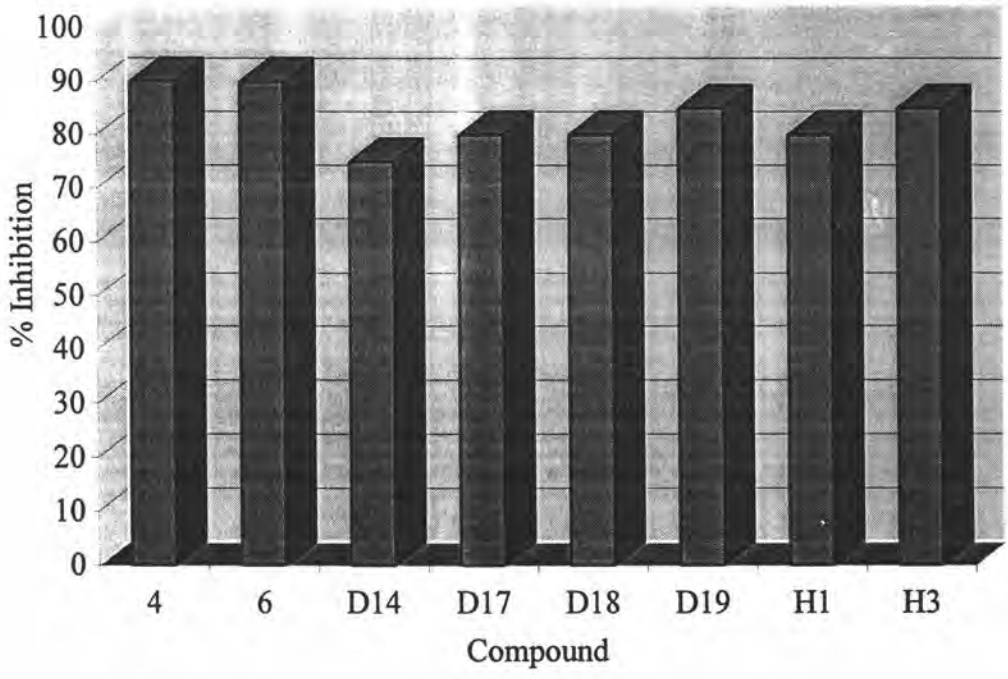


Fig 3.6 Percent growth inhibition of potent 4-hydroxycoumarins and dicoumarols, and some commercial herbicides against *M. pigra*

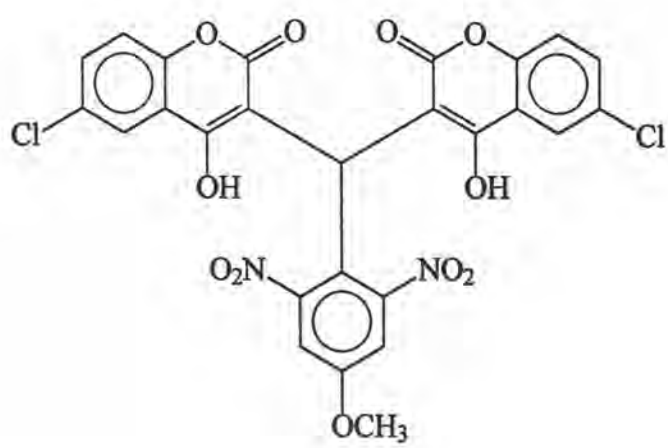
3.2.3 The Comparative Studies on Biological Activities of Studied Compounds

The results of the insect antifeedant activity of three 4-hydroxycoumarins (**4**, **2** and **5**) against *G. mellonella* were compared which those of commercially available insecticides (with 70 % antifeedant). It was found that the chloro, methyl and bromo substituents at C-6 were the best ones.

In the case of dicoumarols, six potent dicoumarols (**D2**, **D6**, **D10**, **D11**, **D15** and **D16**) were also comparatively tested to commercial insecticides (with 70 % antifeedant). It should be noted here that **D1** displayed twice as much as activity of **1**. The structure of dicoumarol (**D1**) composed of 4-hydroxycoumarin 2 units. This result might imply that more units of 4-hydroxycoumarin driven more activity. Dicoumarols with a methoxy group at *para* position and a nitro group at *ortho* position on a benzylidene ring showed the best antifeedant activity.

Regarding to the structure-insect antifeedant activity relationship of 4-hydroxycoumarins and dicoumarols, it was fortunately disclosed that **4**, **D2** and **D15** exhibited very good insect antifeedant activity comparable to that obtained utilizing commercially available insecticide, monocrotophos (94 %).

Based upon all information attained, the promising structure in the group of 4-hydroxycoumarin and its analogue for insect antifeedant activity could be drawn as shown below.



Focusing on the growth inhibition activity against *M. pigra*, it was found that the best two 4-hydroxycoumarins (**4** and **6**) possessed this particular activity should bear a chloro and a methoxy substituent at C-6 and C-5 positions, respectively, in a coumarin moiety.

In the case of dicoumarols, four synthesized dicoumarols (**D14, D17, D18** and **D19**) revealed the activity comparable to those of commercially available herbicides (with 70 % antigrowth). It was also found that **D1** displayed the activity relatively closed to that of **1**. This observation might be implied that the number of 4-hydroxycoumarin unit does not have any influence for this activity. Dicoumarols with a fused benzylidene ring bearing dihydroxy groups at *meta* and *para* positions, however exhibited the best growing inhibition activity.

Realizing to the structure-weed growth inhibition activity relationship of 4-hydroxycoumarins and dicoumarols, **4, 6** and **D19** were discovered to be comparable to those obtained utilizing commercially available herbicides, Mets and Broom (80 and 85 %, respectively)

Therefore, the potential structure of 4-hydroxycoumarin derivative could be suggested for the predication with the best growth inhibition activity against *M. pigra* as: