CHAPTER 2

EXPERIMENTAL

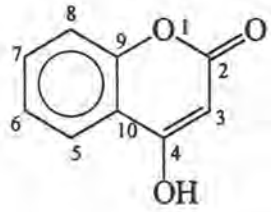
2.1 General Procedure

Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Thin layer chromatography (TLC)[25] was performed on aluminum sheets precoated with silica gel (Merck Kieselgel 60 PF_{254}). The FT-IR spectra were recorded on a Fourier Transformed Infrared Spectrophotometer model Impact 410: solid samples were incorporated to potassium bromide to form a pellet. The ^{1}H-NMR and ^{13}C-NMR spectra were performed in deuterated chloroform ($CDCl_3$), deuterated dimethylsulfoxide (DMSO-d_6) and deuterated acetone (CD_3COCD_3) with tetramethylsilane (TMS) as an internal reference on a Bruker model ACF 200 spectrometer and a Jeol, model JNM-A500 which operated at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei and 500.00 MHz for ^{1}H and 125.00 MHz for ^{13}C nuclei, respectively. The chemical shifts were assigned by comparison with residue solvent protons. Elemental analysis (EA) was carried out on a Perkin Elmer PE 2400 Series II: option CHN on.

All solvents used in this research were purified prior to use by standard methodology except for those which were reagent grades. The reagents used for synthesizing the precursors, 4-hydroxycoumarins and dicoumarols were purchased from Fluka Chemical Company or otherwise stated and were used without further purification.

2.2 Synthesis of 4-Hydroxycoumarins and Starting Materials

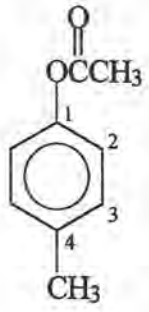
4-Hydroxy-2*H*-1-benzopyran-2-one (1)



Experiment a: 2-Hydroxyacetophenone 2.04 g (0.015 mol) was mixed with diethyl carbonate 20 mL, after that the pulverized sodium 3 g was added. After initial vigorous reaction had subsided, the reaction mixture was gently heated on the heating mantle for an hour. Ethanol was then added to destroy the excess of sodium and the excess of diethyl carbonate was removed with ether. The product (**1**) was obtained upon acidification, crystallized as pale yellow amorphous solid 0.97 g (40 %) from dilute ethanol, m.p. 209-211°C (lit[17a] m.p. 209-210°C); R_f 0.75 (ethanol); IR (KBr) 3650-2500, 3070-3010, 2900, 2740, 2550, 1680, 1550, 1250, 1200, 1120 and 1110 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.62 (1H, s), 7.35 (1H, dt, J = 7.56, 1.07 Hz), 7.37 (1H, d, J = 8.24 Hz), 7.65 (1H, dt, J = 7.86, 1.68 Hz), 7.84 (1H, dd, J = 7.78, 1.68 Hz) and 12.51 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 91.0 (1C, C-3), 115.8 (1C, C-10), 116.3 (1C, C-8), 123.2 (1C, C-5), 123.8 (1C, C-6), 132.6 (1C, C-7), 153.5 (1C, C-9), 161.9 (1C, C-2) and 165.6 (1C, C-4).

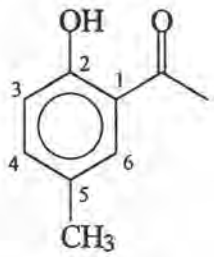
Experiment b: The reaction was performed as described above, except for the use of diethyl carbonate 150 mL and sodium hydride 1.8 g instead of sodium, yielding product (**1**) 2.28 g (94 %), identical with an authentic sample.

4-Methylphenyl acetate (2a)



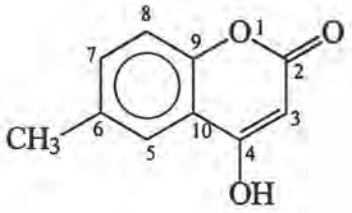
4-Methylphenol 21.99 g (0.20 mol) was mixed with acetic anhydride 40 mL in the presence of pyridine 14 mL. The reaction mixture was refluxed for 2 hours. The mixture was worked up by pouring into ice-water and extracted with dichloromethane twice (50 mL each). The combined organic layer was then extracted with 2N hydrochloric acid (25 mL) and 1N sodium hydroxide (2 x 30 mL), respectively and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to give the crude 4-methylphenyl acetate. The desired compound (**2a**) as pale yellow liquid 26.12 g (84 %) was received by distillation the crude product at 199-211°C, (lit[26] b.p. 212-213°C); R_f 0.93 (chloroform); IR (neat) 3050, 2990, 2920, 2880, 1775, 1500, 1375, 1200, 1100 and 1020 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.24 (3H, s), 2.31 (3H, s), 6.94 (2H, d, J = 8.24 Hz) and 7.14 (2H, d, J = 8.54 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.7 (1C, $\underline{C}H_3$-Ar), 20.9 (1C, $\underline{C}H_3$-COO-), 121.2 (2C, C-2), 129.8 (2C, C-3), 135.3 (1C, C-4), 148.5 (1C, C-1) and 169.5 (1C, C=O).

2-Hydroxy-5-methylacetophenone (2b)



4-Methylphenyl acetate (**2a**) 3.91 g (0.025 mol) was mixed with anhydrous $AlCl_3$ 6.67 g (0.050 mol). The reaction mixture was refluxed at 120°C for 2 hours. The mixture was worked up by slowly pouring into ice-water and then adding the cooled 2N hydrochloric acid (50 mL). The reaction mixture was stirred at 0°C for 30 minutes to precipitate the product. The crude product was filtered off and washed with cold water. The product (**2b**) as yellow crystal 2.60 g (67 %) was recrystallized from hexane, m.p. 47-48°C, (lit[26] m.p. 50°C); R_f 0.91 (chloroform); IR (KBr) 3300-2600, 3070-3010, 2990-2850, 1650, 1500, 1350, 1290, 1250, 1200 and 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.32 (3H, s), 2.62 (3H, s), 6.89 (1H, d, J = 8.24 Hz), 7.29 (1H, dd, J = 8.54, 2.14 Hz), 7.51 (1H, s) and 12.07 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.5 (1C, $\underline{C}H_3$-Ar), 26.6 (1C, $\underline{C}H_3$-C(O)-), 118.2 (1C, C-3), 119.4 (1C, C-1), 128.0 (1C, C-5), 130.4 (1C, C-6), 137.5 (1C, C-4), 160.3 (1C, C-2) and 204.4 (1C, C=O).

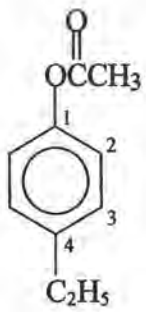
4-Hydroxy-6-methyl-2*H*-1-benzopyran-2-one (2)



Experiment a: 2-Hydroxy-5-methylacetophenone (**2b**) 2.26 g (0.015 mol) was mixed with diethyl carbonate 20 mL, after that the pulverized sodium 3 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**2**) was obtained upon acidification, crystallized as pale yellow amorphous solid 2.24 g (85 %) from dilute ethanol, m.p. 246-248°C (lit[16] m.p. 247-249°C); R_f 0.71 (ethanol); IR (KBr) 3700-3350, 3300-2450, 3090, 3010, 2940-2830, 2820-2600, 1700, 1480, 1320, 1220 and 1100 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.34 (3H, s), 5.56 (1H, s), 7.22 (1H, d, J = 8.43 Hz), 7.42 (1H, dd, J = 8.43, 1.77 Hz), 7.57 (1H, s) and 12.49 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 20.4 (1C, CH_3-), 91.5 (1C, C-3), 116.2 (1C, C-10), 116.8 (1C, C-8), 123.5 (1C, C-5), 134.0 (1C, C-6), 134.3 (1C, C-7), 152.6 (1C, C-9), 163.1 (1C, C-2) and 166.7 (1C, C-4).

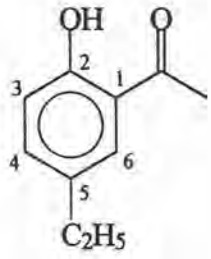
Experiment b: ρ-Cresol 2.43 g (0.022 mol) was mixed with phosphorus oxychloride 6.5 g and zinc chloride 10.0 g. The vigorous reaction mixture was cooled in ice-water bath, after that the reaction was gently refluxed at 60-65°C for 1.5 hours. The mixture was worked up by slowly pouring into ice-water. The crude product was dissolved with 10% sodium carbonate solution and filtered. The solution was acidified with dilute hydrochloric acid yielding product (**2**) 2.22 g (56 %), identical with an authentic sample.

4-Ethylphenyl acetate (3a)



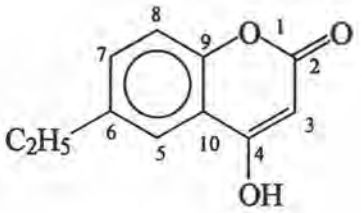
4-Ethylphenol 6.11 g (0.05 mol) was mixed with acetic anhydride 10 mL in the presence of pyridine 4 mL. The reaction mixture was refluxed for 2 hours. Other procedures were followed those used in the synthesis of **2a**. By removing the solvent, the product (**3a**) was derived as pale yellow liquid 8.14 g (99 %), b.p. 226-228°C (lit[26] b.p. 226-227°C / 750 mm Hg); R_f 0.66 (dichloromethane); IR (neat) 3050, 2990, 2920, 2880, 1775, 1500, 1380, 1220, 1190 and 1020 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.24 (3H, t, J = 7.56 Hz), 2.27 (3H, s), 2.65 (2H, q, J = 7.66 Hz), 7.01 (2H, dd, J = 8.54, 1.95 Hz) and 7.20 (2H, d, J = 8.52 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 15.6 (1C, $\underline{C}H_3$-CH_2-), 21.1 (1C, $\underline{C}H_3$-COO-), 28.3 (1C, CH_3-$\underline{C}H_2$-), 121.3 (2C, C-2), 128.7 (2C, C-3), 141.7 (1C, C-4), 148.7 (1C, C-1) and 169.7 (1C, C=O).

5-Ethyl-2-hydroxyacetophenone (3b)



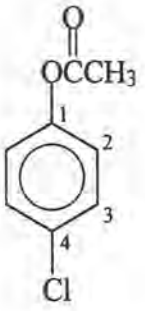
4-Ethylphenyl acetate (**3a**) 4.11 g (0.025 mol) was mixed with anhydrous $AlCl_3$ 6.67 g (0.050 mol). The reaction mixture was refluxed for 2 hours. Other steps were similarly performed as those used for the synthesis of **2b**. The product (**3b**) as orange liquid 3.79 g (92 %) was obtained by removing the solvent, b.p. 249-251°C; R_f 0.65 (dichloromethane); IR (neat) 3300-2600, 3060-3010, 2970, 2930, 2870, 2790, 2690, 1650, 1490, 1370, 1300, 1220, 1140 and 1010 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.19 (3H, t, J = 7.60 Hz), 2.55 (2H, q, J = 7.59 Hz), 2.59 (3H, s), 6.87 (1H, d, J = 8.53 Hz), 7.29 (1H, dd, J = 8.54, 2.15 Hz), 7.49 (1H, d, J = 2.10 Hz) and 12.11 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 15.7 (1C, $\underline{C}H_3$-CH_2-), 26.5 (1C, $\underline{C}H_3$-COO-), 27.9 (1C, CH_3-$\underline{C}H_2$-), 118.2 (1C, C-3), 119.4 (1C, C-1), 129.3 (1C, C-6), 134.5 (1C, C-5), 136.3 (1C, C-4), 160.4 (1C, C-2) and 204.5 (1C, C=O).

6-Ethyl-4-hydroxy-2*H*-1-benzopyran-2-one (3)



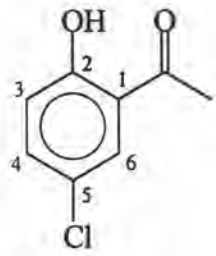
5-Ethyl-2-hydroxyacetophenone (**3b**) 3.36 g (0.020 mol) was mixed with diethyl carbonate 60 mL, after that sodium hydride 1.90 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**3**) was obtained upon acidification, crystallized as white powder 1.06 g (27 %) from dilute ethanol, m.p. 211-214°C (lit[27] m.p. 216-218°C); R_f 0.72 (methanol); IR (KBr) 3700-3350, 3300-2450, 3085, 2960, 2865, 2800, 2580, 1680, 1580, 1310, 1250, 1210 and 1100 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.18 (3H, t, J = 7.59 Hz), 2.66 (2H, q, J = 7.57 Hz), 5.56 (1H, s), 7.26 (1H, d, J = 8.47 Hz), 7.47 (1H, dd, J = 8.47, 2.15 Hz) and 12.40 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 15.6 (1C, $\underline{C}H_3$-CH_2-), 27.4 (1C, CH_3-$\underline{C}H_2$-), 90.9 (1C, C-3), 115.5 (1C, C-10), 116.2 (1C, C-8), 121.5 (1C, C-5), 132.4 (1C, C-7), 139.4 (1C, C-6), 151.8 (1C, C-9), 162.0 (1C, C-2) and 165.6 (1C, C-4).

4-Chlorophenyl acetate (4a)



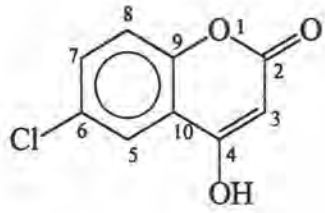
4-Chlorophenol 26.63 g (0.21 mol) was mixed with acetic anhydride 40 mL in the presence of pyridine 14 mL. The reaction mixture was refluxed for 2 hours. Other procedures were followed those used in the synthesis of **2a**. The crude product was distilled and the product (**4a**) as pale yellow liquid 30.20 g (85 %) was received at 79-81°C (7-8 mm Hg), (lit[26] b.p. 226-228°C); R_f 0.51 (chloroform); IR (neat) 3100, 2950, 2870, 1770, 1490, 1390, 1200, 1100 and 1010 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.29 (3H, s), 7.03 (2H, d, J = 8.85 Hz) and 7.33 (2H, d, J = 8.85 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (1C, $\underline{C}H_3$-COO-), 122.9 (2C, C-2), 129.4 (2C, C-3), 131.2 (1C, C-4), 149.1 (1C, C-1) and 169.2 (1C, C=O).

5-Chloro-2-hydroxyacetophenone (4b)



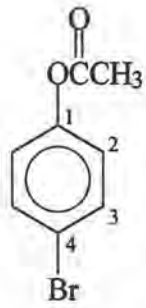
4-Chlorophenyl acetate (**4a**) 9.01 g (0.05 mol) was mixed with anhydrous $AlCl_3$ 13.33 g (0.10 mol). The reaction mixture was refluxed for 2 hours. Other steps were similarly performed as those used for the synthesis of **2b**. The product (**4b**) as yellow crystal 2.60 g (82 %) was obtained by recrystallization from hexane, m.p. 51-52°C, (lit[26] m.p. 55°C); R_f 0.53 (chloroform); IR (KBr) 3300-2650, 3070-3010, 2970-2880, 1650, 1480, 1350, 1320, 1280, 1200, 1090 and 1010 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.63 (3H, s), 6.94 (1H, d, J = 8.85 Hz), 7.42 (1H, dd, J = 8.85, 2.45 Hz), 7.69 (1H, d, J = 2.44 Hz) and 12.13 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.7 (1C, CH_3), 120.1 (1C, C-3), 120.3 (1C, C-1), 123.5 (1C, C-5), 129.9 (1C, C-6), 136.3 (1C, C-4), 160.9 (1C, C-2) and 203.6 (1C, C=O).

6-Chloro-4-hydroxy-2*H*-1-benzopyran-2-one (4)



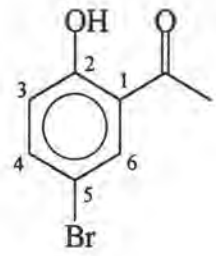
5-Chloro-2-hydroxyacetophenone (**4b**) 2.56 g (0.015 mol) was mixed with diethyl carbonate 20 mL, after that the pulverized sodium 3 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**4**) was obtained upon acidification, crystallized as pale yellow needle 2.66 g (90 %) from dilute ethanol, m.p. 247-249°C (dec) (lit[27] m.p. 266-268°C); R_f 0.73 (ethanol); IR (KBr) 3700-3350, 3300-2450, 3100, 3010, 2950, 2780, 2780, 2600, 1700, 1620, 1580, 1300, 1200 and 1120 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.66 (1H, s), 7.38 (1H, d, J = 8.76 Hz), 7.64 (1H, dd, J = 8.73, 2.52 Hz), 7.72 (1H, d, J = 2.52 Hz) and 12.75 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 91.7 (1C, C-3), 117.3 (1C, C-10), 118.5 (1C, C-8), 122.3 (1C, C-5), 127.9 (1C, C-6), 132.3 (1C, C-7), 152.1 (1C, C-9), 161.4 (1C, C-2) and 164.5 (1C, C-4).

4-Bromophenyl acetate (5a)



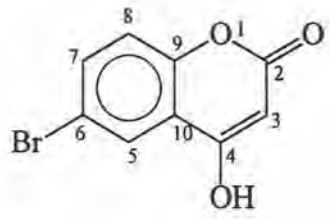
4-Bromophenol 38.85 g (0.22 mol) was mixed with acetic anhydride 40 mL in the presence of pyridine 14 mL. The reaction mixture was refluxed for 2 hours. Other procedures were carried out in the same manner as those employed in the synthesis of **2a**. The crude product was distilled and the product (**5a**) as pale yellow liquid 37.13 g (77 %) was obtained at 90-91°C (6-7 mm Hg), (lit[26] b.p. 235-240°C); R_f 0.52 (chloroform); IR (neat) 3090, 2930, 2870, 1750, 1490, 1350, 1200, 1060 and 1000 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.29 (3H, s), 6.98 (2H, d, J = 8.85 Hz) and 7.49 (2H, d, J = 8.85 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (1C, $\underline{C}H_3$-COO-), 118.9 (1C, C-4), 123.3 (2C, C-2), 132.4 (2C, C-3), 149.7 (1C, C-1) and 169.1 (1C, C=O).

5-Bromo-2-hydroxyacetophenone (5b)



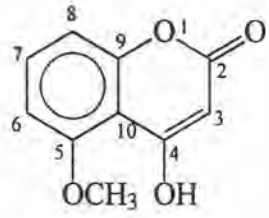
4-Bromophenyl acetate (**5a**) 10.33 g (0.05 mol) was mixed with anhydrous $AlCl_3$ 13.33 g (0.10 mol). The reaction mixture was refluxed for 2 hours. Other general steps were conducted by using the same methodology as those employed for the synthesis of **2b**. The product (**5b**) as pale orange rhombic crystal 9.10 g (88 %) was gained by recrystallization from hexane, m.p. 59-60°C, (lit[26] m.p. 62°C); R_f 0.53 (chloroform); IR (KBr) 3300-2700, 3070-3010, 2950-2870, 2400, 1850, 1650, 1490, 1200, 1090 and 1020 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.62 (3H, s), 6.89 (1H, d, J = 8.85 Hz), 7.55 (1H, dd, J = 8.85, 2.44 Hz), 7.55 (1H, d, J = 2.44 Hz) and 12.15 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.7 (1C, $\underline{C}H_3$-COO-), 110.4 (1C, C-5), 120.5 (1C, C-3), 120.9 (1C, C-1), 132.9 (1C, C-6), 139.1 (1C, C-4), 161.3 (1C, C-2) and 203.5 (1C, C=O).

6-Bromo-4-hydroxy-2*H*-1-benzopyran-2-one (5)



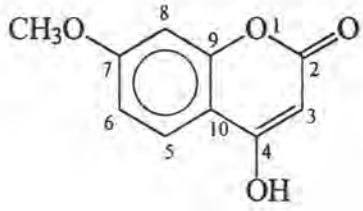
5-Bromo-2-hydroxyacetophenone (**5b**) 2.15 g (0.010 mol) was mixed with diethyl carbonate 100 mL, after that sodium hydride 1.85 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**5**) was obtained upon acidification, crystallized as white needle 2.03 g (84 %) from dilute ethanol, m.p. 266-268°C (lit[27] m.p. 275-277°C); R_f 0.73 (ethanol); IR (KBr) 3400-2450, 3070-3010, 2720, 2580, 1650, 1610, 1560, 1300, 1200 and 1120 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.59 (1H, s), 7.32 (1H, d, J = 8.75 Hz), 7.75 (1H, dd, J = 8.75, 2.43 Hz), 7.85 (1H, d, J = 2.43 Hz) and 12.69 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 91.7 (1C, C-3), 115.6 (1C, C-10), 117.7 (1C, C-6), 118.7 (1C, C-8), 125.3 (1C, C-5), 135.1 (1C, C-7), 152.5 (1C, C-9), 161 3 (1C, C-2) and 164.4 (1C, C-4).

4-Hydroxy-5-methoxy-2*H*-1-benzopyran-2-one (6)



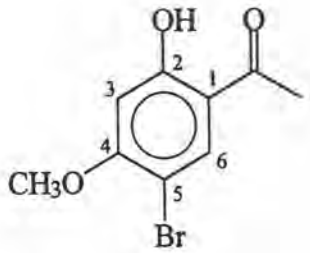
2-Hydroxy-6-methoxyacetophenone 1.66 g (0.010 mol) was mixed with diethyl carbonate 100 mL, after that sodium hydride 1.85 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**6**) was obtained upon acidification, crystallized as pale yellow needle 1.72 g (90 %) from dilute ethanol, m.p. 147-149°C (lit[15b] m.p. 155°C); R_f 0.61 (methanol); IR (KBr) 3700-3150, 3120-3005, 2980, 2850, 1730, 1650, 1410, 1180 and 1090 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.88 (3H, s), 5.50 (1H, s), 6.92 (2H, d, J = 8.34 Hz), 7.54 (1H, t, J = 8.34 Hz) and 11.27 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 56.5 (CH_3-), 90.8 (1C, C-3), 105.0 (1C, C-10), 106.7 (1C, C-8), 109.2 (1C, C-6), 133.0 (1C, C-7), 155.1 (1C, C-9), 157.3 (1C, C-5), 161.5 (1C, C-2) and 167.2 (1C, C-4).

4-Hydroxy-7-methoxy-2*H*-1-benzopyran-2-one (7)



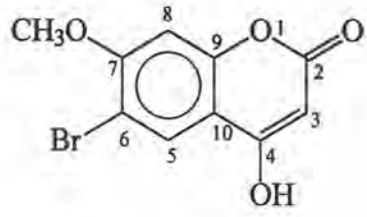
2-Hydroxy-4-methoxyacetophenone 2.65 g (0.016 mol) was mixed with diethyl carbonate 150 mL, after that sodium hydride 1.85 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**7**) was obtained upon acidification, crystallized as white platelet 2.41 g (79 %) from dilute ethanol, m.p. 249-251°C (dec) (lit[14b] m.p. 249-253°C (dec)); R_f 0.73 (methanol); IR (KBr) 3700-3350, 3300-2500, 3130-3050, 2995-2810, 2750, 2590, 1690, 1610, 1320, 1240, 1160 and 1030 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.83 (3H, s), 5.44 (1H, s), 6.83 (1H, s), 6.91 (1H, dd, J = 4.80, 2.41 Hz), 7.70 (1H, d, J = 9.44 Hz) and 12.32 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 55.8 ($\underline{C}H_3O$-), 88.4 (1C, C-3), 100.5 (1C, C-8), 108.9 (1C, C-10), 111.9 (1C, C-6), 124.3 (1C, C-5), 155.4 (1C, C-9), 162.3 (1C, C-7), 162.9 (1C, C-2) and 166.0 (1C, C-4).

5-Bromo-4-methoxy-2-hydroxyacetophenone (8a)



4-Methoxy-2-hydroxyacetophenone 1.66 g (0.010 mol) was dissolved in 80 % acetic acid, Br_2 1.60 g (0.010 mol) was then added. Upon stirring for a few moment, a white mass separated was filtered off. The product (**8a**) as colorless needle 2.12 g (86 %) was obtained by recrystallization with dichloromethane, m.p. 153-154°C; R_f 0.58 (dichloromethane); IR (KBr) 3300-2700, 3070, 2980, 2950, 2900, 2860, 1650, 1490, 1360, 1260 and 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.56 (3H, s), 3.92 (3H, s), 6.45 (1H, s), 7.85 (1H, s) and 12.66 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.2 (1C, $\underline{C}H_3$-C(O)-), 56.6 (1C, $\underline{C}H_3$-O-), 101.1 (1C, C-5), 102.6 (1C, C-3), 114.6 (1C, C-1), 134.7 (1C, C-6), 161.8 (1C, C-2), 164.3 (1C, C-4) and 201.9 (1C, C=O).

6-Bromo-4-hydroxy-7-methoxy-2*H*-1-benzopyran-2-one (8)

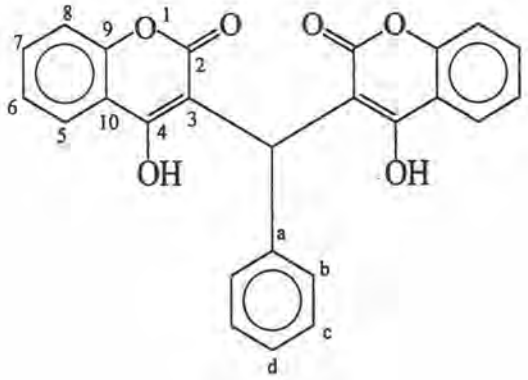


5-Bromo-4-methoxy-2-hydroxyacetophenone (**8a**) 1.84 g (0.0075 mol) was mixed with diethyl carbonate 75 mL, after that sodium hydride 1.85 g was added. The reaction mixture was refluxed for an hour. Other procedures were followed those used in the synthesis of **1**. The product (**8**) was obtained upon acidification, crystallized as small pale brown needle 1.55 g (79 %) from dilute ethanol, m.p. 285-286°C (dec); R_f 0.74 (methanol); IR (KBr) 3700-3350, 3300-2500, 3120, 2990, 2860, 2750, 2590, 1700, 1610, 1300, 1180, 1130 and 1050 cm^{-1}; ^{1}H-NMR (DMSO-d_6 and CD_3COCD_3) δ (ppm): 3.95 (3H, s), 5.48 (1H, s), 7.13 (1H, s), 7.89 (1H, s) and 12.54 (1H, br, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 57.1 ($\underline{C}H_3O$-), 89.9 (1C, C-3), 100.7 (1C, C-8), 106.1 (1C, C-6), 110.0 (1C, C-10), 126.5 (1C, C-5), 154.5 (1C, C-9), 158.6 (1C, C-7), 161.8 (1C, C-2) and 164.9 (1C, C-4). Elemental analysis found %C 44.30 and %H 2.56; calcd. for $C_{10}H_7O_4Br$ (MW 271.08): %C 44.31 and %H 2.60.

The FT-IR, ^{1}H-NMR and ^{13}C-NMR spectra of **8** are shown in Figs 1, 2 and 3, respectively.

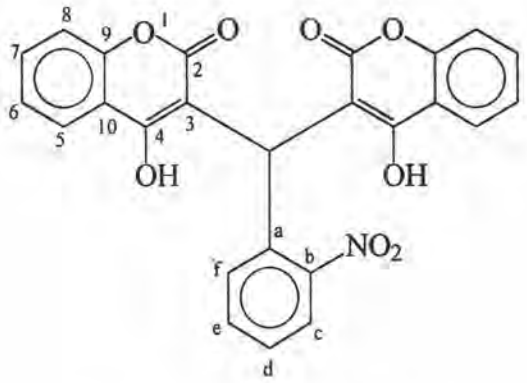
2.3 Synthesis of Dicoumarols

3,3′-(Benzylidene)*bis*-4-hydroxycoumarin (D1)



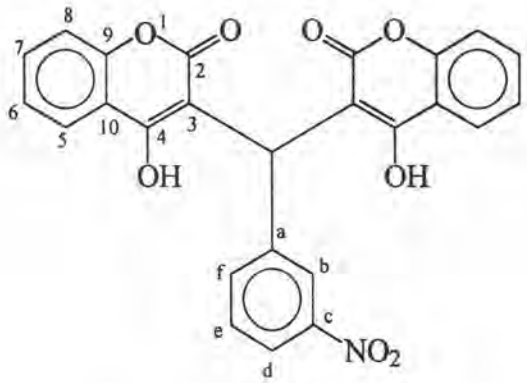
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 0.75 mL (7.5 mmol) of benzaldehyde was added. The solution was refluxed approximately 15 hours until the solid began to precipitate. The solution was kept at that temperature for another 0.5 hour. After that the reaction mixture was cooled down. The product (**D1**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give small white crystal 2.92 g (95 %), m.p. 232-234°C (lit[28] m.p. 227-229°C); R_f 0.69 (ethanol); IR (KBr) 3400-2400, 3020, 2850, 2750, 2600, 1650, 1500, 1460, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.10 (1H, s), 7.22 (2H, d, J = 8.24 Hz), 7.27 (1H, t, J = 7.17 Hz), 7.32 (2H, t, J = 7.63 Hz), 7.37 (2H, br, s), 7.41 (2H, d, J = 8.24 Hz), 7.62 (1H, dt, J = 7.78, 1.53 Hz), 8.03 (2H, br), 11.29 (1H, br, s) and 11.52 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.2 (1C, CH-Ar), 103.9, 105.6 (2 x 1C, C-3), 116.5 (1C, C-10), 116.6 (2C, C-8), 116.8 (1C, C-10), 124.4 (2C, C-5), 124.8 (2C, C-6), 126.4 (2C, C-b), 126.8 (1C, C-d), 128.6 (2C, C-c), 132.8 (2C, C-7), 135.2 (1C, C-a), 152.3, 152.5 (2 x 1C, C-9), 164.6, 166.8 (2 x 1C, C-2) and 166.8, 169.3 (2 x 1C, C-4).

3,3′-(2-Nitrobenzylidene)*bis*-4-hydroxycoumarin (D2)



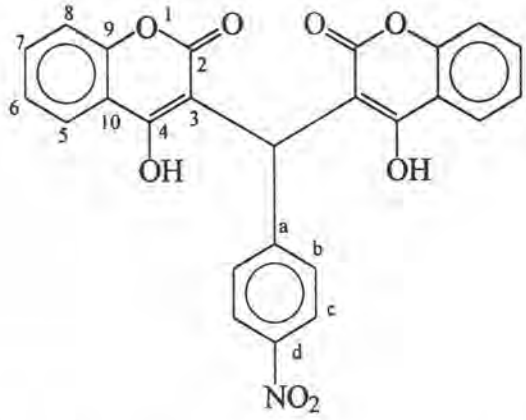
4-Hydroxycoumarin 2.42 g (15 mmol) was dissolved in 45 mL of ethanol and 1.11 g (7.5 mmol) of *o*-nitrobenzaldehyde was added. Other procedures were carried out in the same manner as those employed in the synthesis of **D1**. The product **(D2)** was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give pale yellow powder 2.08 g (62 %), m.p. 211-212°C (dec) (lit[8e] m.p. 183-184°C (dec)); R_f 0.66 (methanol); IR (KBr) 3300-2400, 3090, 2980, 2880, 2710, 2580, 1650, 1500, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.62 (1H, s), 7.38 (2H, br), 7.39 (2H, d, J = 8.24 Hz), 7.44 (1H, t, J = 7.63 Hz), 7.45 (1H, d, J = 7.94 Hz), 7.55 (1H, t, J = 7.70, 1.38 Hz), 7.62 (2H, t, J = 7.93 Hz), 7.63 (1H, d, J = 7.63 Hz), 8.02 (2H, br), 11.22 (1H, br, s) and 11.54 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 33.8 (1C, CH-Ar), 103.8, 116.4 (2 x 1C, C-3), 116.6 (2C, C-8), 116.8, 116.9 (2 x 1C, C-10), 124.5 (1C, C-5), 125.0 (1C, C-6), 128.1 (1C, C-c), 129.3 (C, C-f), 129.5 (1C, C-e), 132.7 (1C, C-f), 133.3 (2C, C-7), 138.0 (1C, C-a), 148.7 (1C, C-b), 152.3, 152.6 (2 x 1C, C-9), 164.8, 166.5 (2 x 1C, C-2) and 166.9, 169.1 (2 x 1C, C-4).

3,3'-(3-Nitrobenzylidene)*bis*-4-hydroxycoumarin (D3)



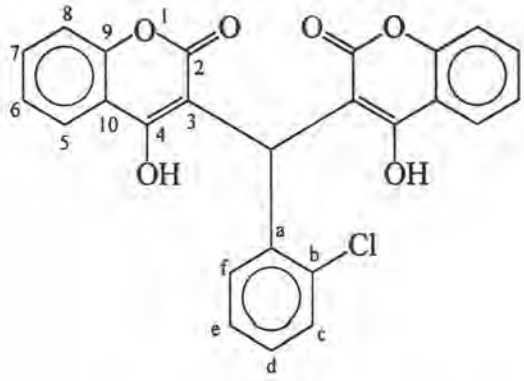
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.14 g (7.5 mmol) of *m*-nitrobenzaldehyde was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D3**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white amorphous solid 3.41 g (99 %), m.p. 222-224°C (lit[29] m.p. 231-232°C); R_f 0.74 (methanol); IR (KBr) 3640-3350, 3300-2450, 3100, 2970, 2730, 2600, 1650, 1500, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.13 (1H, s), 7.39 (2H, t, J = 7.63 Hz), 7.43 (2H, dd, J = 7.94, 3.66 Hz), 7.51 (1H, t, J = 7.93 Hz), 7.58 (1H, d, J = 7.93 Hz), 7.66 (2H, t, J = 7.78 Hz), 8.00 (1H, d, J = 7.63 Hz), 8.07 (1H, s), 8.09 (1H, d, J = 7.63 Hz), 8.14 (1H, d, J = 8.24 Hz), 11.37 (1H, s) and 11.56 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.2 (1C, CH-Ar), 103.2, 104.6 (2 x 1C, C-3), 116.3 (2C, C-10), 116.7, 116.8 (2 x 1C, C-8), 121.7 (1C, C-d), 122.1 (1C, C-b), 124.5 (2C, C-5), 125.2 (2C, C-6), 129.5 (1C, C-e), 132.7 (1C, C-f), 133.3 (2C, C-7), 138.0 (1C, C-a), 148.7 (1C, C-c), 152.3, 152.6 (2 x 1C, C-9), 164.8, 166.5 (2 x 1C, C-2) and 166.9, 169.1 (2 x 1C, C-4).

3,3'-(4-Nitrobenzylidene)*bis*-4-hydroxycoumarin (D4)



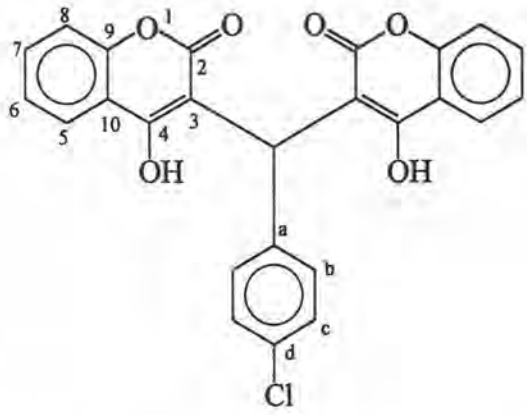
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.14 g (7.5 mmol) of *p*-nitrobenzaldehyde was added. Other steps were similarly performed as those used for the synthesis of **D1**. The product **(D4)** was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give small yellow crystal 2.67 g (78 %), m.p. 235-237°C (lit[30] m.p. 234-236°C); R_f 0.50 (methanol); IR (KBr) 3300-2400, 3090, 2950, 2720, 2590, 1650, 1500, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.11 (1H, s), 7.40 (2H, dd, J = 7.93, 1.07 Hz), 7.41 (2H, br), 7.42 (2H, br, d, J = 7.63 Hz), 7.66 (2H, t, J = 7.78 Hz), 7.99 (1H, d, J = 7.62 Hz), 8.08 (1H, d, J = 7.63 Hz), 8.17 (2H, d, J = 8.85 Hz), 11.35 (1H, s) and 11.50 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.5 (1C, CH-Ar), 103.2, 104.7 (2 x 1C, C-3), 116.2 (2C, C-10), 116.7, 116.7 (2 x 1C, C-8), 123.8 (2C, C-c), 124.4 (2C, C-5), 125.1 (2C, C-6), 127.5 (2C, C-b), 133.3 (2C, C-7), 143.3 (1C, C-a), 146.8 (1C, C-d), 152.3, 152.5 (2 x 1C, C-9), 164.7, 166.3 (2 x 1C, C-2) and 166.9, 169.0 (2 x 1C, C-4).

3,3'-(2-Chlorobenzylidene)*bis*-4-hydroxycoumarin (D5)



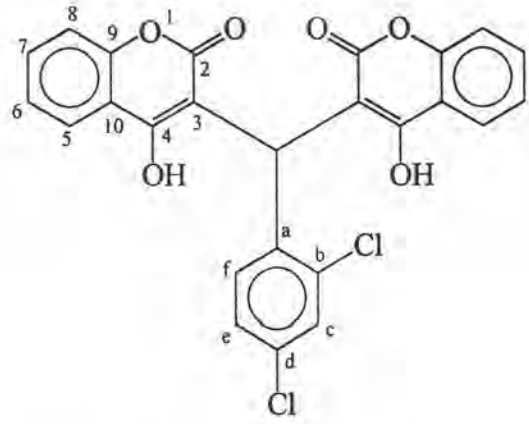
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.1 mL (7.5 mmol) of *o*-chlorobenzaldehyde was added. Other general steps were conducted by using the same methodology as those employed for the synthesis of **D1**. The product (**D5**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white-mirror crystal 2.36 g (67 %), m.p. 203-205°C (lit[8e] m.p. 183-184°C); R_f 0.74 (ethanol); IR (KBr) 3700-3350, 3300-2450, 3050, 2980, 2900, 2850, 2720, 2580, 1650, 1600, 1550, 1500, 1300 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.14 (1H, s), 7.24 (1H, dt, J = 7.63, 1.83 Hz), 7.27 (1H, dt, J = 7.63, 1.83 Hz), 7.35 (1H, dd, J = 7.63, 1.83 Hz), 7.38 (2H, br), 7.39 (2H, br, d, J = 8.24 Hz), 7.46 (1H, d, J = 7.33 Hz), 7.61 (2H, dt, J = 7.32, 1.68 Hz), 8.03 (2H, d, J = 7.93 Hz), 10.91 (1H, br, s) and 11.62 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.7 (1C, CH-Ar), 104.4,105.6 (2 x 1C, C-3), 116.6 (2C, C-10), 116.6 (2C, C-8), 124.4 (2C, C-5), 124.9 (2C, C-6), 126.7 (2C, C-e), 128.6 (1C, C-d), 129.2 (1C, C-c), 130.8 (1C, C-f), 132.8 (2C, C-7), 133.5 (1C, C-b), 133.5 (1C, C-a), 152.1, 152.4 (2 x 1C, C-9), 164.5, 165.1 (2 x 1C, C-2) and 167.2, 168.7 (2 x 1C, C-4).

3,3'-(4-Chlorobenzylidene)*bis*-4-hydroxycoumarin (D6)



4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.05 g (7.5 mmol) of *p*-chlorobenzaldehyde was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D6**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white-mirror crystal 2.54 g (76 %), m.p. 248-250°C (dec) (lit[8e] m.p. 250-252°C); R_f 0.83 (ethanol); IR (KBr) 3700-3350, 3300-2550, 3080, 2990, 2950, 2900, 2880, 2730, 2600, 1675, 1600, 1560, 1500, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.04 (1H, s), 7.15 (2H, dd, J = 8.85,1.22 Hz), 7.29 (2H, d, J = 8.54 Hz), 7.37 (2H, br, t, J = 8.24 Hz), 7.41 (2H, d, J = 8.24 Hz), 7.63 (2H, dt, J = 7.86, 1.68 Hz), 7.99 (1H, br, d, J = 7.33 Hz), 8.07 (1H, br, d, J = 7.63 Hz), 11.29 (1H, br, s) and 11.52 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.8 (1C, CH-Ar), 103.7, 105.3 (2 x 1C, C-3), 116.6 (2C, C-10), 116.7 (2C, C-8), 124.4 (2C, C-5), 125.0 (2C, C-6), 128.0 (2C, C-c), 128.8 (1C, C-b), 132.7 (1C, C-d), 133.0 (2C, C-7), 133.9 (1C, C-a), 152.3, 152.5 (2 x 1C, C-9), 164.6, 166.0 (2 x 1C, C-2) and 166.8, 169.2 (2 x 1C, C-4).

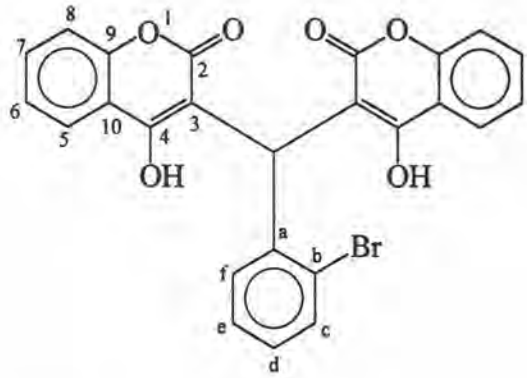
3,3'-(2,4-Dichlorobenzylidene)*bis*-4-hydroxycoumarin (D7)



4-Hydroxycoumarin 3.57 g (20 mmol) was dissolved in 66 mL of ethanol and 1.75 g (10 mmol) of 2,4-dichlorobenzaldehyde was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D7**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white powder 4.30 g (84 %), m.p. 179-180°C; R_f 0.82 (ethanol:dichloromethane [1:1]); IR (KBr) 3700-3350, 3300-2500, 3070, 3030, 2970, 2900, 2850, 2730, 2590, 1650, 1610, 1560, 1500, 1300 and 1110 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.09 (1H, s), 7.24 (1H, dd, J = 8.55, 2.14 Hz), 7.37 (1H, d, J = 2.13 Hz), 7.38 (1H, dd, J = 8.54, 0.92 Hz), 7.38 (2H, br), 7.39 (2H, d, J = 8.24 Hz), 7.62 (2H, dt, J = 7.93, 1.53 Hz), 8.03 (2H, br) and 11.65 (2H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.41 (1C, CH-Ar), 104.2, 105.3 (2 x 1C, C-3), 116.2, 116.3 (2 x 1C, C-10), 116.6 (2C, C-8), 124.4 (2C, C-5), 125.0 (2C, C-6), 127.0 (1C, C-e), 130.2 (1C, C-f), 130.5 (1C, C-c), 132.3 (2C, C-a), 133.0 (1C, C-7), 133.7 (1C, C-b), 134.2 (1C, C-d), 152.3, 152.4 (2 x 1C, C-9), 164.6, 165.3 (2 x 1C, C-2) and 167.2, 168.7 (2 x 1C, C-4). Elemental analysis found %C 62.21 and %H 3.21; calcd. for $C_{25}H_{14}O_6Cl_2$ (MW. 481.29): %C 62.39 and %H 2.93.

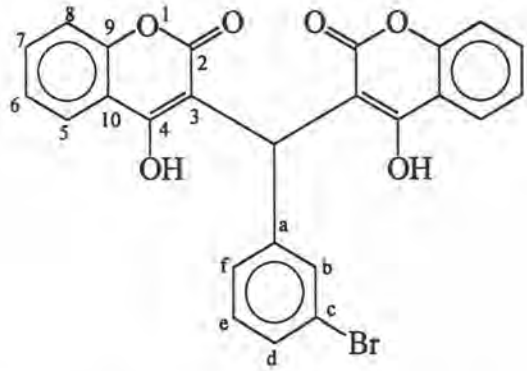
The FT-IR, ^{1}H-NMR and ^{13}C-NMR spectra of **D7** are shown in Figs 4, 5 and 6, respectively.

3,3'-(2-Bromobenzylidene)*bis*-4-hydroxycoumarin (D8)



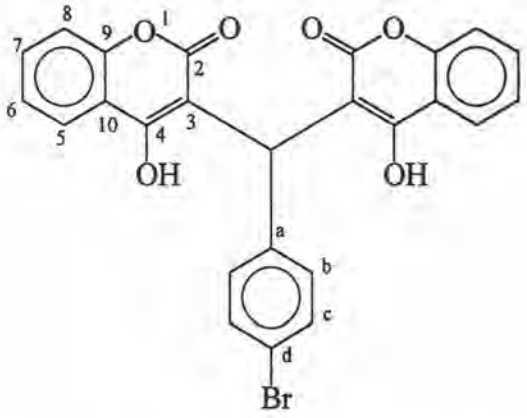
4-Hydroxycoumarin 4.86 g (30 mmol) was dissolved in 90 mL of ethanol and 1.74 mL (15 mmol) of *o*-bromobenzaldehyde was added. Other general steps were conducted by using the same methodology as those employed for the synthesis of **D1**. The product (**D8**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white-mirror crystal 6.31 g (81 %), m.p. 219-221°C (lit[31] m.p. 215-216°C); R_f 0.59 (ethanol:dichloromethane [1:1]); IR (KBr) 3350-2450, 3050, 2950, 2880, 2720, 2600, 1660, 1550, 1500, 1350 and 1110 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.06 (1H, s), 7.15 (1H, dt, J = 7.60, 1.53 Hz), 7.30 (1H, dt, J = 7.69, 1.21 Hz), 7.32 (2H, br), 7.38 (2H, d, J = 7.86 Hz), 7.46 (2H , br, d, J = 7.82 Hz), 7.58 (2H, dt, J = 6.53, 1.62 Hz), 8.01 (2H, d, J = 7.20 Hz), 10.90 (1H, br, s) and 11.56 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 37.6 (1C, CH-Ar), 105.1 (2C, C-3), 116.6 (2C, C-10), 116.6 (2C, C-8), 123.4 (1C, C-b), 124.4 (2C, C-5), 124.9 (2C, C-6), 127.3 (2C, C-d), 128.8 (1C, C-e), 129.4 (1C, C-f), 132.9 (2C, C-7), 134.3 (1C, C-c), 135.1 (1C, C-a), 152.3 (2C, C-9), 164.8 (2C, C-2) and 167.5 (2C, C-4).

3,3′-(3-Bromobenzylidene)*bis*-4-hydroxycoumarin (D9)



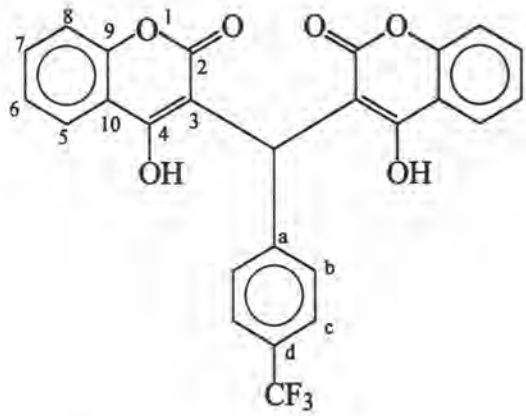
4-Hydroxycoumarin 3.57 g (20 mmol) was dissolved in 66 mL of ethanol and 1.85 g (10 mmol) of *m*-bromobenzaldehyde was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D9**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white amorphous solid 5.01 g (96 %), m.p. 223-225°C (lit[31] m.p. 227-228°C); R_f 0.66 (ethanol:dichloromethane [1:1]); IR (KBr) 3650-3350, 3300-2450, 3070, 2980, 2890, 2920, 2890, 2860, 2730, 2600, 1670, 1600, 1570, 1500, 1350, 1100 and 1090 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.04 (1H, s), 7.16 (1H, s), 7.34 (3H, br), 7.40 (2H, br), 7.40 (2H, d, J = 8.82 Hz), 7.63 (2H, dt, J = 6.96, 1.65 Hz), 8.03 (2H, d, J = 8.39 Hz), 11.28 (1H, s) and 11.55 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.0 (1C, CH-Ar), 103.4, 105.1 (2 x 1C, C-3), 116.7 (2C, C-10), 116.7 (2C, C-8), 122.9 (1C, C-c), 124.5 (2C, C-5), 125.0 (2C, C-6), 125.2 (1C, C-f), 129.5 (1C, C-d), 130.1 (2C, C-b, C-e), 133.1 (1C, C-7), 137.8 (1C, C-a), 152.3 (2C, C-9), 164.7, 166.1 (2 x 1C, C-2) and 166.8, 169.2 (2 x 1C, C-4).

3,3'-(4-Bromobenzylidene)*bis*-4-hydroxycoumarin (D10)



4-Hydroxycoumarin 3.57 g (20 mmol) was dissolved in 66 mL of ethanol and 1.85 g (10 mmol) of *p*-bromobenzaldehyde was added. Other steps were similarly performed as those used for the synthesis of **D1**. The product (**D10**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white powder 4.59 g (88 %), m.p. 282-284°C (lit[31] m.p. 265-266°C); R_f 0.84 (ethanol:dichloromethane [1:1]); IR (KBr) 3650-3350, 3300-2450, 3080, 2970, 2730, 2700, 2690, 2600, 1680, 1600, 1490, 1350, 1230, 1100 and 1040 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.00 (1H, s), 7.08 (2H, dd, J = 8.60, 1.12 Hz), 7.39 (2H, d, J = 7.17 Hz), 7.40 (2H, br), 7.42 (2H, d, J = 8.62 Hz), 7.62 (2H, dt, J = 7.83, 1.61 Hz), 8.02 (2H, br, s), 11.21 (1C, br, s) and 11.54 (1C, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.9 (1C, CH-Ar), 103.7, 105.2 (2 x 1C, C-3), 116.7 (2C, C-8), 116.7 (2C, C-10), 120.8 (1C, C-d), 124.4 (2C, C-5), 125.0 (2C, C-6), 128.3 (2C, C-b), 131.7 (2C, C-c), 133.0 (2C, C-7), 134.4 (1C, C-a), 152.3, 152.4 (2 x 1C, C-9), 164.6, 166.0 (2 x 1C, C-2) and 166.8, 169.1 (2 x 1C, C-4).

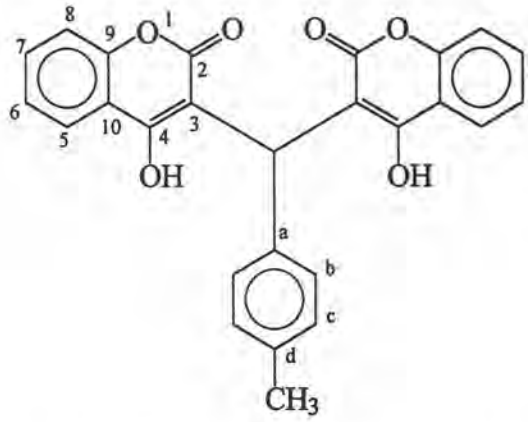
3,3'-(4-Trifluoromethylbenzylidene)*bis*-4-hydroxycoumarin (D11)



4-Hydroxycoumarin 3.57 g (20 mmol) was dissolved in 66 mL of ethanol and 1.47 g (10 mmol) of *p*-trifluoromethylbenzaldehyde was added. Other steps were similarly performed as those used for the synthesis of **D1**. The product (**D11**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give small white-mirror crystal 4.65 g (97 %), m.p. 282-283°C; R_f 0.74 (ethanol:dichloromethane [1:1]); IR (KBr) 3650-3350, 3300-2450, 3060, 2975, 2940, 2880, 2850, 2740, 2620, 1680, 1620, 1570, 1510, 1340 and 1120 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.10 (1H, s), 7.33 (2H, d, J = 8.24 Hz), 7.39 (2H, br, t, J = 7.63 Hz), 7.42 (2H, dd, J = 7.94, 3.67 Hz), 7.58 (2H, d, J = 8.24 Hz), 7.63 (2H, dt, J = 7.86, 1.68 Hz), 8.04 (2H, br), 11.33 (1H, br, s) and 11.54 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.3 (1C, CH-Ar), 103.5, 105.1 (2 x 1C, C-3), 116.3 (2C, C-10), 116.7 (3C, 2C-8, C-d), 124.4 (2C, C-5), 125.0 (2C, C-6), 125.6 (2C, C-c), 126.9 (C-b), 133.1 (2C, C-7), 139.7 (1C, CF_3-), 152.3, 152.6 (2 x 1C, C-9), 164.7, 166.2 (2 x 1C, C-2) and 166.9, 169.2 (2 x 1C, C-4). Elemental analysis found %C 65.05 and %H 3.26; calcd. for $C_{26}H_{15}O_6F_3$ (MW. 480.40): %C 65.01 and %H 3.15.

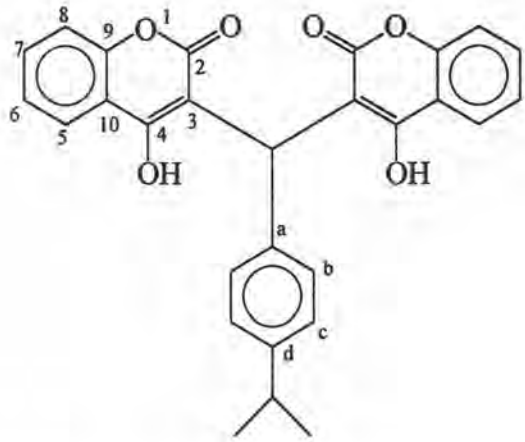
The FT-IR, ^{1}H-NMR and ^{13}C-NMR spectra of **D11** are presented in Figs 7, 8 and 9, respectively.

3,3'-(4-Methylbenzylidene)*bis*-4-hydroxycoumarin (D12)



4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 0.90 g (7.5 mmol) of *p*-tolualdehyde was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D12**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white amorphous solid 3.02 g (94 %), m.p. 254-257°C (dec) (lit[8d] m.p. 269-271°C); R_f 0.79 (methanol); IR (KBr) 3350-2450, 3080, 2990, 2890, 2730, 2600, 1680, 1600, 1550, 1500, 1350, 1300 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.32 (3H, s), 6.05 (1H, s), 7.08 (2H, dd, J = 8.85, 0.92 Hz), 7.11 (2H, d, J = 8.55 Hz), 7.37 (2H, br, t, J = 8.24 Hz), 7.39 (2H, br, d, J = 8.24 Hz), 7.61 (2H, dt, J = 7.32, 1.52 Hz), 8.02 (2H, br, s), 11.35 (1H, br, s) and 11.50 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.9 (1C, CH_3-), 35.8 (1C, CH-Ar), 104.0, 105.7 (2 x 1C, C-3), 116.5 (4C, 2C-8, 2C-10), 124.3 (2C, C-5), 124.8 (2C, C-6), 126.3 (2C, C-b), 129.3 (2C, C-c), 132.0 (1C, C-a), 132.7 (2C, C-7), 136.4 (1C, C-d), 152.3 (2C, C-9), 164.5, 165.6 (2 x 1C, C-2) and 166.8, 169.2 (2 x 1C, C-4).

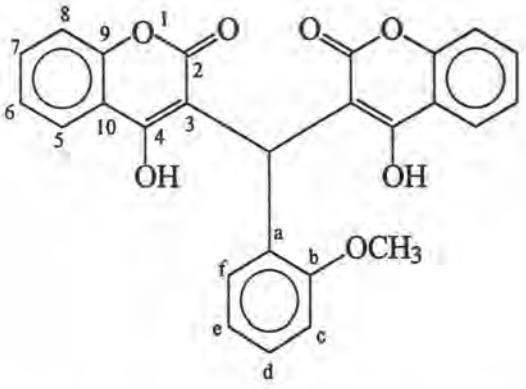
3,3'-(4-iso-Propylbenzylidene)*bis*-4-hydroxycoumarin (D13)



4-Hydroxycoumarin 3.57 g (20 mmol) was dissolved in 66 mL of ethanol and 1.66 mL (10 mmol) of *p*-isopropylbenzaldehyde was added. Other procedures were carried out in the same manner as those employed in the synthesis of **D1**. The product (**D13**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white-mirror crystal 4.73 g (97 %), m.p. 255-257°C; R_f 0.67 (ethanol:dichloromethane [1:1]); IR (KBr) 3650-3350, 3300-2500, 3050, 3010, 2960, 2890, 2740, 2600, 1680, 1600, 1560, 1510, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.23 (3H, s), 1.25 (3H, s), 2.89 (1H, hep, J = 6.92 Hz), 6.07 (1H, s), 7.13 (2H, dd, J = 7.63, 0.61 Hz), 7.17 (2H, d, J = 8.55 Hz), 7.38 (2H, br, t, J = 7.93 Hz), 7.40 (2H, d, J = 8.24 Hz), 7.62 (2H, dt, J = 7.86, 1.68 Hz), 8.03 (2H, br) and 11.48 (2H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 23.9 (2C, $\underline{C}H_3$-CH-), 33.6 (1C, CH_3-$\underline{C}$H-), 104.0, 105.8 (2 x 1C, C-3), 116.6 (2C, C-8), 116.8, 116.9 (2 x 1C, C-10), 124.4 (2C, C-5), 124.8 (2C, C-6), 126.4 (1C, C-b), 126.7 (2C, C-c), 132.4 (2C, C-a), 132.7 (2C, C-7), 147.4 (1C, C-d), 152.3, 152.5 (2 x 1C, C-9), 164.5, 165.6 (2 x 1C, C-2) and 166.9, 169.0 (2 x 1C, C-4). Elemental analysis found %C 73.97 and %H 5.19; calcd. for $C_{28}H_{22}O_6$ (MW. 454.48): %C 74.00 and %H 4.88.

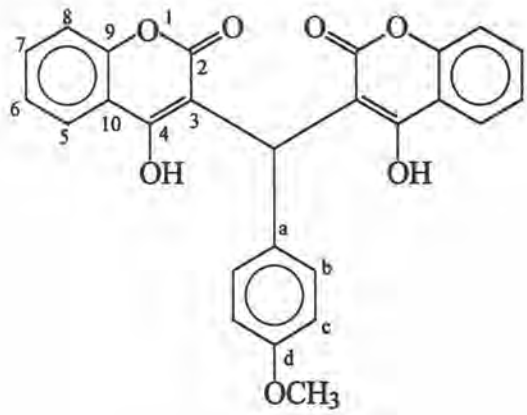
The FT-IR, ^{1}H-NMR and ^{13}C-NMR spectra of **D13** are displayed in Figs 10, 11 and 12, respectively.

3,3'-(2-Methoxybenzylidene)*bis*-4-hydroxycoumarin (D14)



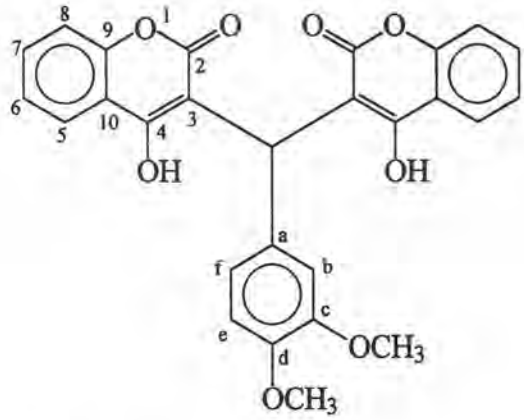
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.02 g (7.5 mmol) of *o*-methoxybenzaldehyde was added. Other steps were similarly performed as those used for the synthesis of **D1**. The product **(D14)** was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white amorphous solid 3.20 g (98 %), m.p. 212-214°C (dec) (lit[32] m.p. 218°C); R_f 0.84 (methanol); IR (KBr) 3300-2300, 3080, 2950, 2730, 2600, 1660, 1600, 1500, 1310, 1100 and 1020 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.56 (3H, s), 6.08 (1H, s), 6.84 (1H, dd, J = 7.94, 0.92 Hz), 6.93 (1H, dt, J = 7.56, 0.92 Hz), 7.26 (1H, t, J = 7.94 Hz), 7.27 (1H, d, J = 7.63 Hz), 7.35 (2H, dt, J = 7.63, 1.22 Hz), 7.37 (2H, br, d, J = 8.40 Hz), 7.58 (2H, dt, J = 7.86, 1.68 Hz), 8.01 (2H, dd, J = 7.94, 1.53 Hz) and 11.2 (2H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 33.4 (1C, C<u>H</u>-Ar), 55.5 (1C, <u>C</u>H_3O-), 105.8 (2C, C-3), 111.0 (1C, C-c), 116.4 (2C, C-8), 116.8 (2C, C-10), 120.4 (1C. C-e), 123.6 (1C, C-a), 124.2 (2C, C-5), 124.6 (2C, C-6), 128.2 (1C, C-d), 128.4 (2C, C-f), 132.4 (2C, C-7), 152.1 (2C, C-9), 157.6 (1C, C-b), 163.7 (2C, C-2) and 168.0 (2C, C-4).

3,3′-(4-Methoxybenzylidene)*bis*-4-hydroxycoumarin (D15)



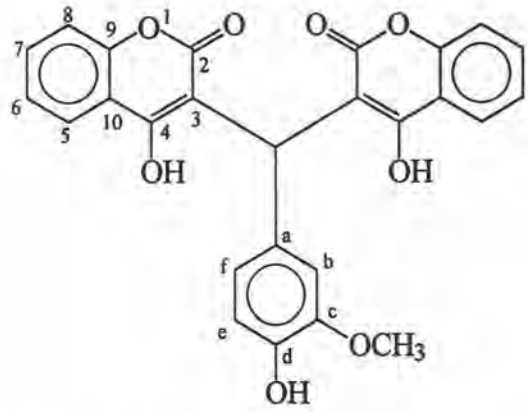
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.02 g (7.5 mmol) of anisaldehyde was added. Other procedures were carried out in the same manner as those employed in the synthesis of **D1**. The product (**D15**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white amorphous solid 3.23 g (97 %) from ethanol-dichloromethane, m.p. 250-251°C (dec) (lit[8d] m.p. 248-252°C); R_f 0.72 (ethanol); IR (KBr) 3700-3350, 3300-2450, 3100-3020, 2980, 2900, 2820, 2750, 2650, 2410, 1695, 1510, 1280 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.79 (3H, s), 6.04 (1H, s), 6.8[a] (2H, d, J = 8.85 Hz), 7.12 (2H, d, J = 8.85 Hz), 7.38 (2H, br), 7.40 (2H, d, J = 8.54 Hz), 7.62 (2H, dd, J = 7.38, 1.68 Hz), 8.00 (1H, br, s), 8.05 (1H, br, s), 11.28 (1H, br, s) and 11.49 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.5 (1C, C<u>H</u>-Ar), 55.2 (1C, <u>C</u>H$_3$O-), 104.2, 105.8 (2 x 1C, C-3), 114.0 (2C, C-c), 116.6 (4C, 2C-8, 2C-10), 124.3 (2C, C-5), 124.8 (2C, C-6), 126.9 (1C, C-a), 127.6 (2C, C-b), 132.8 (2C, C-7), 152.3 (2C, C-9), 164.5, 165.6 (2 x 1C, C-2) and 166.8, 169.2 (2 x 1C, C-4).

3,3'-(3,4-Dimethoxybenzylidene)*bis*-4-hydroxycoumarin (D16)



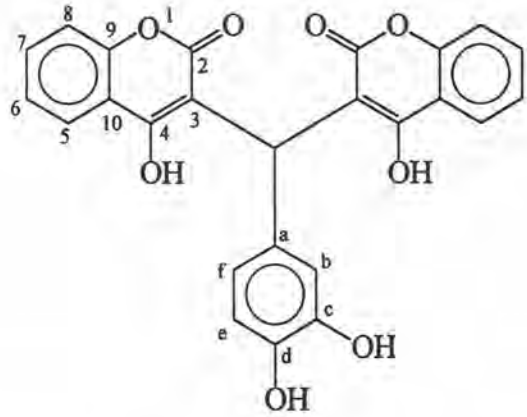
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.26 g (7.5 mmol) of verateraldehyde was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D16**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white amorphous solid 2.91 g (81 %), m.p. 264-266°C (dec); R_f 0.68 (ethanol); IR (KBr) 3650-3330, 3300-2500, 3090, 3010, 2980, 2940, 2900, 2850, 2740, 2610, 1675, 1610, 1560, 1510, 1350, 1320, 1260, 1250, 1140 and 1110 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.73 (3H, s), 3.87 (3H, s), 6.07 (1H, s), 6.71 (1H, br, s), 6.77 (1H, d, J = 8.24 Hz), 6.82 (1H, d, J = 8.24 Hz), 7.38 (2H, br, t, J = 7.63 Hz), 7.41 (2H, br, d, J = 8.24 Hz), 7.63 (2H, br, dt, J = 8.24, 1.63 Hz), 8.02 (1H, br, s), 8.06 (1H, br, s), 11.28 (1H, br, s) and 11.51 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.8 (1C, CH-Ar), 55.9, 56.1 (2 x 1C, CH_3O-), 104.2, 105.8 (2 x 1C, C-3), 110.4 (1C, C-b), 111.3 (1C, C-f), 116.6 (2C, C-10), 116.7 (2C, C-8), 118.9 (1C, C-e), 124.3 (2C, C-5), 124.8 (2C, C-6), 127.6 (1C, C-a), 132.8 (2C, C-7), 148.1 (1C, C-d), 149.2 (1C, C-c), 152.3, 152.4 (2 x 1C, C-9), 164.6, 165.6 (2 x 1C, C-2) and 166.7, 169.3 (2 x 1C, C-4).

3,3'-(4-Hydroxy-3-methoxybenzylidene)*bis*-4-hydroxycoumarin (D17)



4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.14 g (7.5 mmol) of vanillin was added. Other steps were similarly performed as those used for the synthesis of **D1**. The product (**D17**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give pale yellow amorphous solid 3.22 g (94 %), m.p. 215-217°C (dec) (lit[33] m.p. 213-215°C); R_f 0.89 (ethanol); IR (KBr) 3650-3350, 3300-2450, 3070, 2980, 2920, 2880, 2850, 2720, 2600, 1680, 1600, 1510, 1350, 1280, 1210 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.73 (3H, s), 5.59 (1H, s), 6.05 (1H, s), 6.67 (1H, s), 6.71 (1H, dd, J = 8.24, 2.11 Hz), 6.84 (1H, d, J = 8.15 Hz), 7.37 (2H, t, J = 7.16 Hz), 7.38 (2H, d, J = 7.88 Hz), 7.60 (2H, dt, J = 7.81, 1.64 Hz), 8.01 (2H, d, J = 7.09 Hz), 11.28 (1H, br, s) and 11.48 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.8 (1C, C<u>H</u>-Ar), 56.1 (1C, <u>C</u>H_3O-), 104.2, 105.7 (2 x 1C, C-3), 109.4 (1C, C-b), 114.5 (1C, C-e), 116.6 (4C, 2C-8, 2C-10), 119.5 (1C, C-f), 124.3 (2C, C-5), 124.9 (2C, C-6), 126.8 (1C, C-a), 132.8 (2C, C-7), 144.6 (1C, C-d), 146.7 (1C, C-c), 152.4 (2 x 1C, C-9), 164.6, 165.7 (2 x 1C, C-2) and 166.8, 169.1 (2 x 1C, C-4).

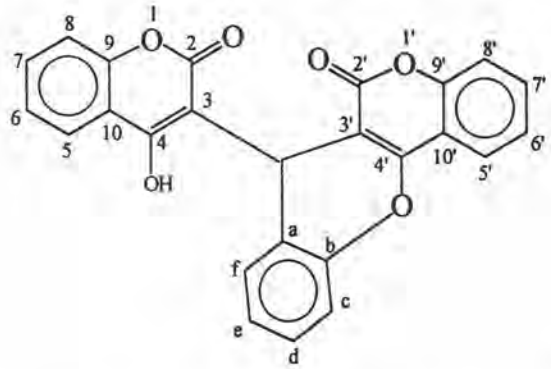
3,3'-(3,4-Dihydroxybenzylidene)*bis*-4-hydroxycoumarin (D18)



4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.04 g (7.5 mmol) of 3,4-dihydroxybenzaldehyde was added. Other procedures were carried out in the same manner as those employed in the synthesis of **D1**. The product (**D18**) was filtered, washed with cold ethanol and recrystallized with acetone to give white powder 2.76 g (83 %), m.p. 174-178°C (dec); R_f 0.77 (ethanol); IR (KBr) 3680-3350, 3300-2350, 2930, 2740, 2650, 2600, 1660, 1610, 1560, 1520, 1280, 1190, 1100 and 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.03 (1H, s), 6.64 (1H, d, J = 8.39 Hz), 6.76 (1H, s), 6.78 (1H, d, J = 8.24 Hz), 7.46 (2H, d, J = 7.94 Hz), 7.73 (2H, dt, J = 7.78, 1.53 Hz), 7.73 (2H, t, J = 7.78 Hz), 7.74 (2H, br), 8.01 (2H, dd, J = 8.24, 1.52 Hz) and 11.46 (2H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.4 (1C, CH-Ar), 106.1 (2C, C-3), 104.8 (1C, C-b), 116.1 (1C, C-e), 117.3 (2C, C-8), 117.6 (2C, C-10), 118.9 (1C, C-f), 124.7 (2C, C-5), 125.6 (2C, C-6), 128.0 (1C, C-a), 133.7 (2C, C-7), 144.7 (1C, C-d), 145.9 (1C, C-c), 153.3 (2 x 1C, C-9), 165.3 (2 x 1C, C-2) and 168.4 (2C, C-4).

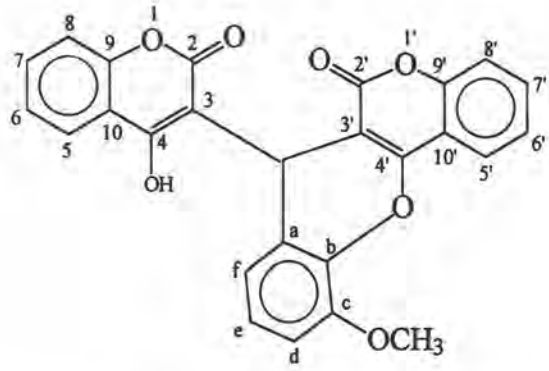
The FT-IR, ^{1}H-NMR and ^{13}C-NMR spectra of **D18** are shown in Figs 13, 14 and 15, respectively.

3-[6-Oxo(1)benzopyrano(4,3-*b*)-(1)benzopyran-7-yl]-4-hydroxycoumarin (D19)



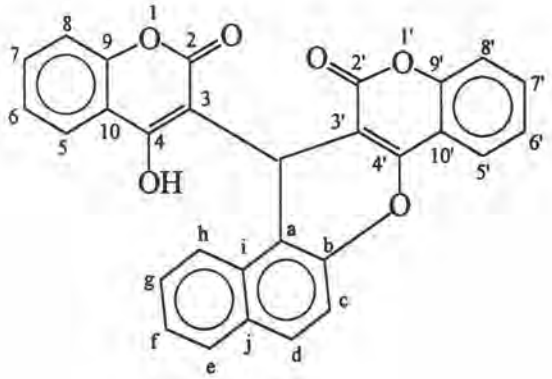
4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 0.96 g (7.5 mmol) of salicylaldehyde was added. Other procedures were carried out in the same manner as those employed in the synthesis of **D1**. The product (**D19**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give small white crystal 2.87 g (85 %), m.p. 252-253°C (lit[34] m.p. 252-254°C); R_f 0.89 (ethanol); IR (KBr) 3700-3350, 3300-2450, 3090, 2970, 2900, 2650, 2650, 2480, 1700, 1650, 1610, 1560, 1490, 1450, 1400, 1210, 1110, 1070 and 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.76 (1H, br, s), 7.15 (1H, dt, J = 7.02, 2.14 Hz), 7.22 (1H, br, d, J = 7.33 Hz), 7.34 (4H, m), 7.45 (1H, dd, J = 8.39, 0.77 Hz), 7.49 (1H, dt, J = 7.63, 0.92 Hz), 7.60 (1H, dt, J = 7.78, 1.53 Hz), 7.70 (1H, dt, J = 7.78, 1.53 Hz), 8.05 (1H, br, s) and 8.11 (1H, dd, J = 7.94, 1.53 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 28.7 (1C, CH-Ar), 100.6 (1C, C-3), 107.4 (1C, C-3′), 113.8 (1C, C-10′), 116.1 (1C, C-10), 116.2 (1C, C-8′), 116.2 (1C, C-c), 116.4 (1C, C-8), 122.2 (1C, C-e), 122.6 (1C, C-5′), 123.8 (2C, C-a), 123.9 (1C, C-5), 124.5 (1C, C-6′), 125.3 (1C, C-6), 128.3 (1C, C-f), 128.6 (1C, C-d), 132.2 (1C, C-7′), 132.5 (1C, C-7), 149.2 (1C, C-4′), 152.0 (1C, C-9′), 152.2 (1C, C-9), 156.2 (1C, C-b), 160.4 (1C, C-2′), 160.7 (1C, C-2) and 161.3 (1C, C-4).

3-[5-Methoxy-6-oxo(1)benzopyrano(4,3-*b*)-(1)benzopyran-7-yl]-4-hydroxy coumarin (D20)



4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.14 g (7.5 mmol) of *o*-vanillin was added. Other procedures were followed those used in the synthesis of **D1**. The product (**D20**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give white powder 3.12 g (91 %), m.p. 274-275°C (dec); R_f 0.84 (ethanol); IR (KBr) 3700-3350, 3300-2550, 3070, 3010, 2990, 2940, 2900, 2850, 2830, 2720, 2620, 2600, 2500, 1730, 1610, 1490, 1280, 1220 and 1110 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.99 (3H, s), 5.36 (1H, s), 6.70 (1H, d, J = 7.63 Hz), 6.89 (1H, dd, J = 8.27, 1.52 Hz), 7.03 (1H, t, J = 7.93 Hz), 7.18 (1H, dd, J = 8.24, 0.61 Hz), 7.28 (1H, dt, J = 7.63, 1.22 Hz), 7.39 (1H, dd, J = 8.24, 0.61 Hz), 7.43 (1H, dt, J = 7.77, 1.22 Hz), 7.47 (1H, dt, J = 7.78, 1.53 Hz), 7.62 (1H, dt, J = 8.24, 1.53 Hz), 8.02 (1H, dd, J = 7.94, 1.53 Hz), 8.22 (1H, dd, J = 7.94, 1.53 Hz) and 10.32 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 30.1 (1C, C<u>H</u>-Ar), 56.4 (1C, <u>C</u>H_3O-), 100.0 (1C, C-3), 108.7 (1C, C-3′), 111.9 (1C, C-10′), 114.9 (1C, C-d), 116.2 (1C, C-8′), 116.8 (1C, C-8), 117.0 (1C, C-10), 120.0 (1C, C-f), 122.4 (1C, C-5′), 123.8 (1C, C-5), 123.8 (1C, C-e), 124.4 (1C, C-6′), 125.0 (1C, C-6), 125.3 (1C, C-a), 131.8 (1C, C-7′), 132.7 (1C, C-7), 141.0 (1C, C-b), 148.0 (1C, C-c), 152.1 (1C, C-9′), 153.2 (1C, C-9), 158.6 (1C, C-4′), 161.1 (1C, C-2′), 161.3 (1C, C-2) and 166.2 (1C, C-4).

3-[8-Oxo(1)naphthopyrano(4,3-*b*)-(1)benzopyran-9-yl]-4-hydroxy coumarin (D21)



4-Hydroxycoumarin 2.43 g (15 mmol) was dissolved in 45 mL of ethanol and 1.29 g (7.5 mmol) of 2-hydroxynaphthaldehyde was added. Other steps were similarly performed as those used for the synthesis of **D1**. The product (**D21**) was filtered, washed with cold ethanol and recrystallized with dichloromethane-ethanol to give yellow needle 2.76 g (83 %), m.p. 244-245°C; R_f 0.77 (ethanol); IR (KBr) 3650-3150, 3075, 1705, 1610, 1575, 1500, 1230 and 1100 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.91 (1H, dt, J = 7.32, 1.22 Hz), 7.09 (1H, dd, J = 8.55, 0.62 Hz), 7.52-7.74 (9H, m), 7.97 (1H, dt, J = 8.24, 0.61 Hz), 8.25 (1H, d, J = 8.85 Hz), 8.78 (1H, s) and 11.79 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 54.5 (1C, CH-Ar), 112.6-160.5 (28C, aromatic carbons).

2.4 Bioassay Experiments

As mentioned earlier, the goal of this research is the study of the relationship of 4-hydroxycoumarins and insect antifeedant against *Galleria mellonella* Linn. larvae and weed growth inhibition of *Mimosa pigra* Linn. The outcome from this study may apply for agricultural purposes. The following bioassay experiments were therefore performed.

2.4.1 Insect Antifeedant against *Galleria mellonella* Linn. Larvae

The greater wax moth larvae, *Galleria mellonella* Linn. larvae, has been recognized as a pest in honey bee, *Apis mellifera* L. The moth larvae often destroy unprotected combs in storage facilities or in colonies that become weaken or die. Each female adult lay eggs on the breakage of bee wax, developing larvae feed and burrow through that comb. The feeding damage makes the comb either unusable or reconstructed by bee. Losses due to this pest are considerable and have been estimated to about $ 4 million per year in United States, as a combination of labor costs wax replacement, and lost revenues due to the carbohydrate consumption requirement of bees rebuilding combs.[35] Moreover in other warm areas, larvae of the greater wax moth cause extensive loss because they may develop continuously throughout the year.[36]

Greater wax moth passes through four distinct stages: the egg or ovum (embryo stage), the caterpillar or larvae (growing stage), the chrysalis or pupae (transition stage), and finally the adult or imago (reproduction stage). This four-stage development in insect is known as complete metamorphosis.[37]

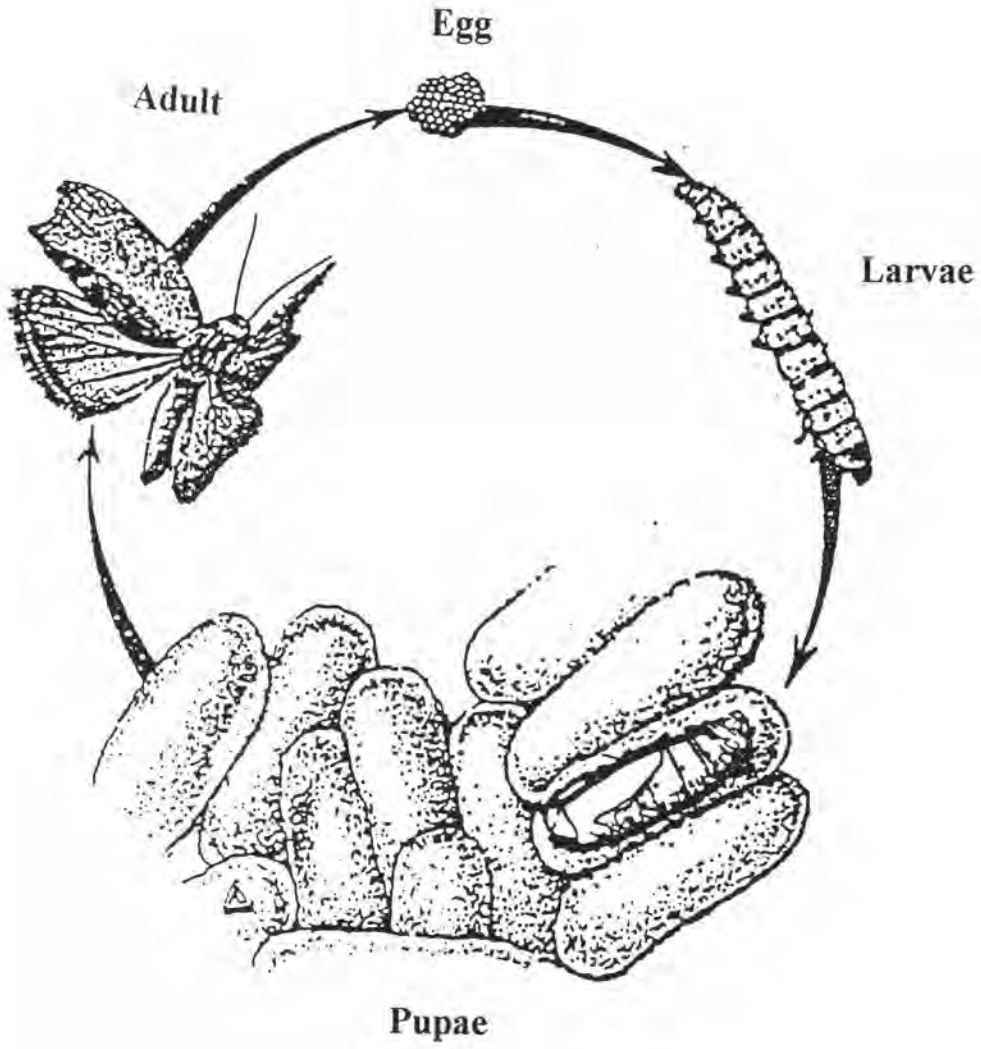


Fig 2.1 Life cycle of *Galleria mellonella* Linn.

CLASSIFICATION[38]

Kingdom Animalia

Phylum Arthropoda

Class Insecta

Order Lepidoptera

Family Pyralidae

Genus Galleria

Species *Galleria mellonella* Linn.

Methods of Controlling *Galleria mellonella* Larvae

Several methods of wax moth control that are currently used as well as several methods that have been proposed for use could be summarized as follows:

1. Chemical controlling method: this method is fuming gases, such as *p*-dichlorobenzene, methylbromide and phosphine; fumigation with carbon dioxide or ethylene oxide.[39]

2. Physical controlling: it is possible to kill all life stages of the wax moth by exposing them to 0-5°F for as little as 2 hours. It is also possible to kill this insect with high temperature. Exposure to 115-120°F will produce 100% mortality in as little as one and a half hours.[40]

3. Biological controlling: this method gets rid of worm by the use of pest of wax moth such as: a braconid wasp, *Apanteles galleriae* Wilkinson, various species of predaceous ants, especially the red imported fire ant, *Solenopsis invicta* Buren, and an entomorphilous bacterium, *Bacillus thuringiensis* Berliner.[40]

4. Control of the greater wax moth by beekeeper: it is simply a matter of proper colony management favoring strong populations.[41]

General Procedure for Antifeedant Activity Test

Three grams of larvae food[a] were put in glassware. The treatment solution[b] 3 mL was dripped into and mixed homogeneously, labeled as "treatment". The controlled food was prepared by dripping the same quantity of the same solvent and labeled as "control". The solvent was allowed to evaporate from each food glassware at room temperature by air drying about 3 hours. After that each food about 0.5000 g was put in a weighed aluminum foil bowl (cup bowl of size: diameter at the bottom =

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a) The larvae food was consisted of children supplementary food (cerelac) : bee pollen : liquid mixture = 3 : 1 : 1 (wt by wt). The liquid mixture was consisted of honey : distilled water : glycerol = 1 : 1.5 : 1.1 (volume by volume).

b) The treatment compound 0.0075 g was dissolved in 3.0 mL of a suitable solvent. The solvent for each compound which provided good solubility and rapidly volatile such as acetone and dichloromethane were preferred. This ratio was used to test in 6 replicates and 0.5000 g of food was used for each replicate (concentration of treatment in food is 0.25 % wt by wt).

2.5 cm and at the rim = 4.5 cm). Then each bowl was placed pair-wise (treatment & controlled) in the plastic box. Ten third-forth instar[c] (at the same size, 0.5-0.6 cm length) larvae were placed in the same box, after being starved for 3 hours. It was then placed in an incubator at 34-36°C. After 48 hours, the worms were counted and both bowls were weighed to determine the weight loss of treatment and controlled food by comparing to weight loss of control of control food bowl.[d]

$$\% \text{ Antifeedant} = \{ (C-T) / (C+T) \} \times 100 \%$$

where 'T' is the weight loss of food in treatment bowl and 'C' is the weight loss of food in controlled bowl.

Antifeedant of 100 % represents total inhibition of feeding activity.

The Results of Antifeedant Activity against *Galleria mellonella* Linn. Larvae

The results of antifeedant activity of 4-hydroxycoumarins, dicoumarols and commercially available insecticides against *Galleria mellonella* larvae are tabulated in Tables 2.1-2.3, respectively.

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c) The greater wax moth larvae were obtained from the damage combs of Bee Research Unit of Chulalongkorn University, Tambol Bangkhantak, Amphur Muang, Samutsongkhram Province, Thailand. The wax moth adult larvae were reared on artificial diet and life stages were kept in environmental chambers.

d) The control of control food bowl consists of controlled food that was done like control food, but there was no worm in the box. It was compared the different weight of food from other factors, such as loss moisture. It was mandatory to perform in order to calculate % antifeedant.

Table 2.1 The results of antifeedant activity of 4-hydroxycoumarins against *G. mellonella* larvae

Entry	Compound	% Antifeedant
1	**1**	22.42
2	**2**	82.38
3	**3**	43.60
4	**4**	90.70
5	**5**	70.72
6	**6**	50.53
7	**7**	-5.48
8	**8**	47.44

Table 2.2 The results of antifeedant activity of dicoumarols against *G. mellonella* larvae

Entry	Compound	% Antifeedant
1	**D1**	49.68
2	**D2**	91.45
3	**D3**	8.48
4	**D4**	49.80
5	**D5**	27.57
6	**D6**	83.49
7	**D7**	26.85
8	**D8**	28.79
9	**D9**	41.19
10	**D10**	71.03
11	**D11**	72.02
12	**D12**	41.44
13	**D13**	51.33
14	**D14**	39.95
15	**D15**	92.00
16	**D16**	87.12
17	**D17**	34.07
18	**D18**	-34.73
19	**D19**	40.30
20	**D20**	-31.66
21	**D21**	41.08
22	**D22**	30.46
23	**D23**	56.87
24	**D24**	-0.70

Table 2.3 The results of antifeedant activity of commercially available insecticides against *G. mellonella* larvae

Entry	Insecticide*	% Antifeedant
1	**P1**	-48.38
2	**P2**	76.20
3	**P3**	94.04

* see appendices

2.4.2 Weed Growth Inhibition against *Mimosa pigra* Linn.

M. pigra Linn., in English known variously as the Giant Sensitive Plant, the Thorny Sensitive Plant, commonly called giant mimosa. The name for *M. pigra*, in Thai is Maiyarap Yak. It is a source and host for insects and diseases and broom around the agricultural land, in dam and beside the high way road. This therefore causes serious problems for irrigation and transportation.[42]

Giant mimosa is a medium tree and has been recognized as a weed in both of water and land.

Fig 2.2 *Mimosa pigra* Linn. (Giant mimosa)

Fig 2.2 *Mimosa pigra* Linn. (Giant mimosa) (Cont.)

CLASSIFICATION[43]

Kingdom Plantae (Metaphyta)
Division Tracheophyta
Subdivision Pteropsida
Class Angiospermae
Subclass Monocotyledoneae
Order Rosales
Family Leguminosae
Genus Mimosa
Species *Mimosa pigra* Linn.

Methods of Controlling *Mimosa pigra*

The methods of giant mimosa growing control could be classified into 2 types as follows:[44]

1. Chemical controlling method: chemical control includes all control techniques involving the use of chemical agents (herbicides) to kill *M. pigra*.

2. Biological controlling method: biological control is defined as "the action of parasites, predators and pathogens in maintaining another organism's density at a lower level than would occur in their absence.

General Procedure for Weed Growth Inhibition Test

Tested compound was dissolved in a proper solvent[a] at concentration of 10000, 1000, 100 and 10 ppm. The 3.0 mL of solution was poured in a glass tube (diameter 30 mm and length 120 mm) which contained 1.5 g cellulose powder. The controlled tube was prepared by the same solvent using the same methodology. All tubes were covered with aluminum foil, dried up and heated at 50°C in vacuum oven for 6-12 hours, followed by the addition of 4.5 mL of distilled water to each tube and then cellulose powder was well-mixed. Three seedling of giant mimosa with radical root length 1-2 mm (seed was previously grown 3 days) were transplanted in each tube, 3 tubes for each concentration. The tubes were sealed with transparent vinyl film and kept in growth chamber at 30°C, 24 hours daylight. After 7 days, The seedlings were cleared from artificial food, both lengths of root and shoot of both treatment and controlled plants were measured and compared.[b]

$$\% \text{ Growth Inhibition} = \{ 1 - (T / C) \} \times 100 \%$$

where 'T' is root (or shoot) length of treated seedling and 'C' is root (or shoot) length of treated seedling.

Growth inhibition of 100 % represents total inhibition of growing.

The Results of Weed Growth Inhibition against *Mimosa pigra* Linn.

The results of weed growth inhibition of 4-hydroxycoumarins, dicoumarols and commercially available herbicides against *Mimosa pigra* are displayed in Tables 2.4-2.6, respectively.

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a) ethanol for 4-hydroxycoumarins and dichloromethane for most dicoumarols (the others: **D18** and **D24** used ethanol).

b) All of these results were kindly provided from Weed Science sub. Division Botany of Weed Science Division, Department of Agriculture, Ministry of Agriculture and Cooperatives.

Table 2.4 The results of weed growth inhibition of 4-hydroxycoumarins against *M. pigra*

Cpd	% Growing Inhibition at (ppm)					
	Root			Shoot		
	10	100	1000	10	100	1000
1	-21.88	30.19	100.00	-3.86	15.74	100.00
2	4.15	43.20	95.27	-3.86	58.85	82.36
3	-8.86	-19.51	90.53	3.98	11.82	74.52
4	4.15	90.53	95.27	-7.78	90.20	94.12
5	7.70	64.50	94.08	0.06	41.21	82.36
6	-1.76	88.17	97.63	5.94	80.40	100.00
7	-7.68	37.29	86.98	11.82	19.66	70.61
8	25.45	-2.95	13.62	23.57	-7.78	21.61

Table 2.5 The results of weed growth inhibition of dicoumarols against *M. pigra*

Cpd	% Growing Inhibition at (ppm)							
	Root				Shoot			
	10	100	1000	10000	10	100	1000	10000
D1	2.28	39.70	69.11	69.64	29.31	32.16	53.99	32.49
D2	-	-3.70	60.64	87.04	-	29.58	30.99	45.07
D4	-9.09	8.05	22.86	47.69	-30.13	0.26	22.08	34.55
D5	31.06	55.32	82.53	95.97	16.09	26.92	56.93	61.19
D6	-	41.56	38.88	38.39	-	42.25	25.35	16.90
D7	2.93	35.28	87.15	90.24	13.79	30.46	51.72	67.82
D8	58.54	39.19	37.24	78.70	40.23	33.91	43.68	51.72
D9	28.62	32.68	87.18	69.76	23.56	51.15	46.55	42.53
D10	-14.15	-0.49	33.98	45.69	8.05	1.15	30.46	45.98
D11	34.96	58.32	67.23	88.91	26.21	80.65	25.00	73.39
D12	-	23.72	34.23	35.94	-	23.94	11.27	12.68
D13	8.29	36.91	32.52	18.70	12.07	5.75	18.39	3.45
D14	-	75.31	81.42	80.32	-	9.86	32.39	16.20
D15	30.59	64.54	75.13	34.96	-4.84	38.31	59.68	-10.08
D16	44.71	53.91	53.77	70.15	26.61	19.04	9.78	9.19
D17	-	78.97	99.27	99.27	-	30.99	87.32	100.00
D18	74.62	81.84	87.69	90.00	48.46	67.69	80.00	83.08
D19	-9.76	85.07	99.07	97.40	28.72	50.20	98.59	80.62
D20	-	-25.90	-2.20	15.16	-	14.08	8.45	-4.20
D21	63.03	1.51	23.19	-17.98	44.35	14.11	38.31	29.44
D22	25.20	39.84	78.05	74.96	24.14	35.06	49.48	25.29
D23	35.13	62.69	53.11	63.87	19.35	35.48	32.26	26.61
D24	34.96	61.95	100.00	100.00	28.74	48.28	100.00	100.00

- : no test

Table 2.6 The results of weed growth inhibition of commercially available herbicides against *M. pigra*

Herbicide*	% Growing Inhibition at (ppm)							
	Root				Shoot			
	10	100	1000	10000	10	100	1000	10000
H1	45.67	80.39	80.71	89.39	40.01	18.76	10.01	30.01
H2	19.30	-1.59	83.92	92.93	26.26	42.51	50.01	66.25
H3	85.85	85.85	89.71	95.82	33.76	41.26	52.51	67.50
H4	-1.92	52.74	81.67	96.78	1.26	46.26	52.51	81.25

* see appendices