CHAPTER II
EXPERIMENTAL

2.1 Instruments and Equipments

Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 F_{254}) (Merck KgaA, Darmstadt, Germany). Column chromatography was performed in silica gel (Merck Kieselgel 60 G) (Merck KgaA, Darmstadt, Germany). Melting points were determined with an Electrothermal 9100 melting point apparatus (American Instrument Exchange, Inc., MA, USA). Also, skin tests of studied compounds were performed on test plaster, Finn chamber (Epitest Ltd Oy., Tuusula, Finland). For UV irradiation, a Daavlin Psorawand UV lamp: PW-UVB-220 was used (Daavlin, OH, USA).

The FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared spectrophotometer: Impact 410 (Nicolet Instrument Technologies, Inc. WI, USA). Solid samples were incorporated into a pallet of potassium bromide. Liquid samples were dropped on sodium chloride cell. The ^{1}H- and ^{13}C-MNR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal reference on a Bruker (Germany): ACF 200 spectrometer which operated at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei, and 300.00 MHz for ^{1}H and 75.00 MHz for ^{13}C nuclei (Varian). Mass spectra (70 eV) were acquired from a Fissons Instrument mass spectrometer (Fissons Instruments, UK) and Matrix Assisted Laser Desorption/Ionozation-Time of Flight (MALDI-TOF MS) (Bruker, Germany). UV spectra were obtained with the aid of HP 8453 UV/VIS spectro-photometer (Agilent Technologies, CA, USA). The UV absorbance curves were recorded using a sample in the 1 cm quartz cell.

2.2 Chemicals

Solvents used in synthesis and spectroscopic techniques were reagent or analytical grades purchased from Labscan (Bangkok, Thailand). Solvents used in column chromatography were purified from commercial grade solvents prior to use by

distillation. The reagents used for synthesizing were purchased from Fluka Chemical Company (Buchs, Switzerland):

- 2,3,4-trimethoxy benzaldehyde
- 2,4,5-trimethoxy benzaldehyde
- 2,4,6-trimethoxy benzaldehyde
- 4-methoxy benzaldehyde
- pyridine
- 2-ethylhexanol
- 1-hexanol
- malonic acid

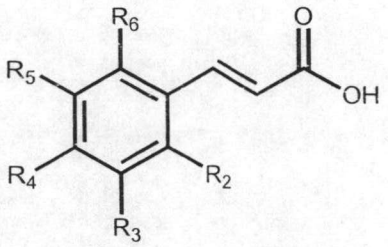
Piperidine was purchased from Sigma (Sigma Chemical Co., Steinheirg, Germany). Standard OMC was obtained from Merck Co. Ltd. (Bangkok, Thailand).

2.3 Synthesis of Substituted *trans*-cinnamic acids

General Procedure[26]

Malonic acid (2.08 g, 0.02 mol) was dissolved in 5 ml of pyridine and 0.01 mol substituted benzaldehyde and 0.15 mL piperidine were added. The mixture was refluxed for 4.5 hours at 70-75°C. After being cooled the reaction mixture was poured into a beaker containing 40 mL of cold water. The mixture was acidified by slowly adding with 5 mL of concentrated hydrochloric acid. The solid was separated by suction filtration, washed with cold water and recrystallized with ethanol.

Four substituted cinnamic acids were synthesized and ten substituted cinnamic acids structures are displayed in Fig 2.1.



Cpds	R_2	R_3	R_4	R_5	R_6
C1	H	H	OCH_3	H	H
C2[a]	OCH_3	OCH_3	H	H	H
C3[a]	OCH_3	H	OCH_3	H	H
C4[a]	OCH_3	H	H	OCH_3	H
C5[a]	H	OCH_3	OCH_3	H	H
C6[a]	H	OCH_3	H	OCH_3	H
C7	OCH_3	OCH_3	OCH_3	H	H
C8	OCH_3	H	OCH_3	OCH_3	H
C9[a]	H	OCH_3	OCH_3	OCH_3	H
C10	OCH_3	H	OCH_3	H	OCH_3

Figure 2.1 Structure of synthesized cinnamic acids

Methoxy Cinnamic Acids

4-methoxycinnamic acid (**C1**): White solid (54%), m.p. 171-173°C (lit.[27] 172-175 °C), R_f 0.5 (65% EtOAc/Hex), IR (KBr, cm^{-1}) 2588-2287, 2935, 2542, 1685, 1623, 1600, 1510, 1444, 1312, 1254, 1219 and 1171; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.76-7.68 (d, *J*=16.1 Hz, 1H, Ar-C**H**=), 7.51-7.47 (d, *J*=8.9 Hz, 2H, Ar-**H**), 6.93-6.88 (d, *J*=8.7 Hz, 2H, Ar-**H**), 6.34-6.26 (d, *J*=15.8 Hz, 1H, =C**H**-COOH) and 3.79 (s, 3H, O**CH**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 167.8 (-**C**OOH), 160.0, 129.9 (2×1C), 126.8, 144.3 (2×1C) (aromatic carbons), 143.7 (Ar-**C**H=), 116.4 (=**C**H-COOH) and 55.3 (O**C**H$_3$).

[a] These compounds were obtained from Sujittra Deesamer. *Synthesis and Herbicidal Activity of Cinnamic Acid and Related Compounds*. Master's Thesis, Department of Chemistry, Graduate School, Chulalongkorn University, 2000.

2,3,4-trimethoxycinnamic acid (**C7**): White mirror-like needle crystal (55%), m.p. 168-170°C, R_f 0.4 (65% EtOAc/Hex), IR (KBr, cm^{-1}) 3603-3343, 2974, 2939, 2827, 1693, 1619, 1584, 1495, 1460, 1266 and 1099; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.01-7.93 (d, *J*=16.11 Hz, 1H, Ar-C**H**=), 7.30-7.26 (d, *J*=8.85 Hz, 1H, Ar-**H**), 6.71-6.67 (d, *J*=8.80 Hz, 1H, Ar-**H**), 6.46-6.38 (d, *J*=16.06 Hz, 1H, =C**H**-COOH) and 3.90, 3.88, 3.86 (s, 9H, 3×OC**H**$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.1 (-**C**OOH), 155.9, 153.5, 142.3, 123.7, 121.1, 107.6 (aromatic carbons), 141.8 (Ar-**C**H=), 116.1 (=**C**H-COOH), 61.5, 60.9 and 56.02 (3×O**C**H$_3$).

2,4,5-trimethoxycinnamic acid (**C8**): Yellow solid (88%), m.p. 165-167°C (lit.[28]164-166°C), R_f 0.37 (65% EtOAc/Hex), IR (KBr, cm^{-1}) 3631-3335, 3009, 2935, 2823, 1685, 1600, 1514, 1464, 1433, 1401, 1299 and 1196; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.01-8.09 (d, *J*=16.0 Hz, 1H, Ar-C**H**=), 7.02, 6.85 (s, 2H, Ar-**H**), 6.41-6.32 (d, *J*=16.0 Hz, 1H, =C**H**-COOH), 3.92, 3.87 and 3.86 (s, 9H, 3×OC**H**$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.3 (-**C**OOH), 154.2, 152.5, 143.2, 114.5, 110.9 and 96.67 (aromatic carbons), 141.9 (Ar-**C**H=), 114.6 (=**C**H-COOH), 56.4-56.1 (3×O**C**H$_3$).

2,4,6-trimethoxycinnamic acid (**C10**); Pale yellow solid (65%), m.p. 224-226°C, R_f 0.47 (65% EtOAc/Hex), IR (KBr, cm^{-1}) 3623-3335, 3009, 2935, 2831, 1685, 1596, 1510, 1471, 1289, 1211 and 1025; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.89-7.81 (d, *J*=16.2 Hz, 1H, Ar-C**H**=), 6.58-6.50 (d, *J*=16.2 Hz, 1H, =C**H**-COOH), 6.27 (s, 2H, Ar-**H**), 3.43, 3.38 and 3.30 (s, 9H, 3×OC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 169.1 (-**C**OOH), 162.7, 160.7 (2×1C), 104.3, 90.82 (2×1C) (aromatic carbons), 134.74 (Ar-**C**H=), 117.43 (=**C**H-COOH), 65.4, 55.84 and 55.4 (3×O**C**H$_3$).

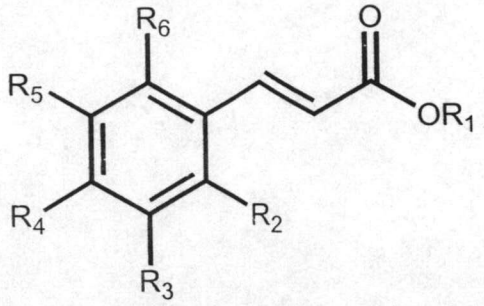
2.4 Synthesis of *trans*-Cinnamate Esters

General Procedure[29,30]

A mixture of 0.01 mol of substituted cinnamic acid and 1.19 g (0.01 mol) thionyl chloride was refluxed in toluene at 110 °C. To the top of the condenser is attached an exit tube leading to a gas-absorption trap (K_2CO_3). A mixture was heated until no further evolution of hydrogen chloride (45-60 minutes). An aspirator vacuum was applied to the top of the condenser to remove any remaining thionyl chloride before 0.01 mol of alcohol

was added. The mixture was again refluxed on water bath at 80 °C for 1 hour. It was then placed on a sand bath and refluxed at 110 °C in order to complete the reaction (1.5 hours, checked by TLC). The reaction mixture was cooled and washed with 100 mL of 2% sodium bicarbonate solution.

Three cinnamate esters were synthesized and their structures are displayed in Fig 2.2.



Cpds	R_2	R_3	R_4	R_5	R_6	R_1
E1	H	H	OCH_3	H	H	$CH_2CH(CH_2CH_3)C_4H_9$
E2	OCH_3	H	OCH_3	OCH_3	H	$CH_2CH(CH_2CH_3)C_4H_9$
E3	OCH_3	H	OCH_3	H	OCH_3	$CH_2CH(CH_2CH_3)C_4H_9$

Figure 2.2 Structure of synthesized cinnamate esters

Methoxy Cinnamate Esters

2-ethylhexyl-4-methoxycinnamate (**E1**): colorless oil (78%), R_f 0.5 (20% EtOAc/Hex), IR (neat, cm^{-1}) 2959, 2928, 2875, 1743, 1710, 1635, 1607, 1507, 1460, 1312, 1254 and 1167; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.64-7.56 (d, *J*=16.0 Hz, 1H, Ar-C**H**=), 7.48-7.44 (d, *J*= 8.6 HZ, 2H, Ar-**H**), 6.90-6.89 (d, *J*= 8.8 HZ, 2H, Ar-**H**), 6.33-6.25 (d, *J*=16.0 Hz, 1H, =C**H**-$COOR_1$), 3.85 (s, 3H, OCH_3), 4.10-4.07 (d, *J*=5.7 Hz, 2H, -OCH_2) and 1.63-0.85 (m, 15H, -C_7H_{15}); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.6 (-COOR), 161.3, 161.1, 129.7 (2×1C) and 114.3 (2×1C) (aromatic carbons), 144.1 (Ar-CH=), 115.8 (=CH-$COOR_1$), 66.8 (-OCH_3), 55.4, 38.9, 30.5, 28.9, 23.9, 23.0, 14.1 and 11.0 (alkyl carbons).

2-ethylhexyl-2,4,5-trimethoxycinnamate (**E8**): yellowish oil (66%), R_f 0.2 (20% EtOAc/Hex), IR (neat, cm^{-1}) 2931, 1858, 2631, 1701, 1611, 1508, 1461, 1293 and 1161; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.99-7.91 (d, *J*=16.1 Hz, 1H, Ar-C**H**=), 7.24, 6.48 (s, 2H), 6.38-6.30 (d, *J*=16.0 Hz, 1H, =C**H**-COOR), 4.10-4.07 (d, *J*= 5.8, 2H, -OC**H**$_2$), 3.95-3.82 (s, 9H, 3×-OC**H**$_3$) and 1.63-0.87 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.8 (-**C**OOR$_1$), 153.7, 151.9, 143.1, 114.9, 110.6 and 96.8 (aromatic carbons), 139.2 (Ar-**C**H=) and 115.7 (=**C**H-COOR$_1$), 56.3, 56.2 and 55.9 (3×-O**C**H$_3$), 66.6, 38.8, 30.4, 28.8, 23.8, 22.9, 13.9 and 10.9 (alkyl carbons).

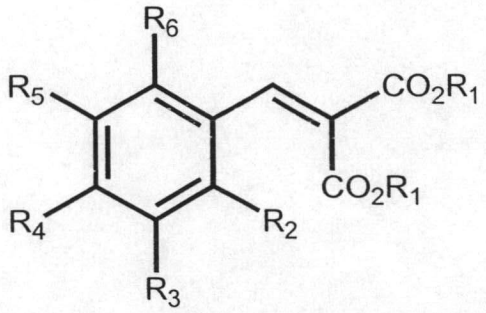
2-ethylhexyl-2,4,6-trimethoxycinnamate (**E10**): pale yellow solid (13%), m.p. 64-65°C, R_f 0.23 (20% EtOAc/Hex), IR (KBr, cm^{-1}); 2951, 2924, 1685, 1603, 1561, 1460, 1266, 1207, 1153 and 1114; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.11-8.03 (d, *J*=16.2 Hz, 1H, Ar-C**H**=), 6.75-6.67 (d, *J*=16.2 Hz, 1H, =C**H**-COOR), 6.09 (s, 2H), 4.09-4.06 (d, *J*= 5.7, 2H), 3.85-3.80 (s, 9H, 3×-OC**H**$_3$), 1.63-0.86 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 180.5 (-**C**OOR$_1$), 154.8, 154.6, 109.8, 109.6, 85.8 (aromatic carbons), 136.9 (Ar-**C**H=) and 125.2 (=**C**H-COOR$_1$), 75.0 (2×1C), 74.7 (-O**C**H$_3$ carbons), 58.2, 49.83, 48.3, 43.2, 42.3, 33.4 and 30.4 (alkyl carbons).

2.5 Synthesis of Benzalmalonate Derivatives

General Procedure[26, 31]

Malonate ester 0.02 mol is dissolved in 5 ml pyridine and 0.02 mol substituted benzaldehyde and 0.15 mL piperidine were added. The mixture was refluxed for 4.5 hours at 70-75 °C. After the mixture had been cooled, the solution was washed with two 10 mL portions of water, with two 10 mL portions of 1 *N* hydrochloric acid, and then with 10 mL of saturated sodium bicarbonate. The organic solution was dried with anhydrous sodium sulfate.

Six benzalmalonate derivatives were synthesized and their structures are displayed in Fig 2.3



Cpds.	R_1	R_2	R_3	R_4	R_5	R_6
BM1	CH_2CH_3	OCH_3	H	OCH_3	OCH_3	H
BM2	$CH_2CH(CH_2CH_3)C_4H_9$	OCH_3	H	OCH_3	OCH_3	H
BM3	$(CH_2)_5CH_3$	OCH_3	H	OCH_3	OCH_3	H
BM4	CH_2CH_3	H	H	OCH_3	H	H
BM5	$CH_2CH(CH_2CH_3)C_4H_9$	H	H	OCH_3	H	H
BM6	C_6H_{13}	H	H	OCH_3	H	H

Figure 2.3 Structure of synthesized benzalmalonate esters

Benzalmalonate esters

Diethyl-2',4',5'-trimethoxybenzalmalonate (**BM8-1**): pale yellow solid (74%), m.p. 98-99°C, R_f (20% EtOAc/Hex), IR (KBr, cm^{-1}); 2974, 2928, 2846, 1704, 1600, 1518, 1460, 1413, 1250, 1207, 1121 and 1021; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.04 (s, 1H, Ar-C**H**=), 6.98 (s, Ar-**H**, 1H), 6.46 (s, Ar-**H**, 1H), 4.34-4.21 (q, 4H, 2×-OC**H**$_2$-), 3.91, 3.86 and 3.78 (s, 9H, 3×–OC**H**$_3$), 1.39-4.19 (t, 6H, 2×-C**H**$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.4, 171.8 (2×-**C**OOR), 162.2, 156.5, 156.3, 142.5, 131.0 and 130.8 (aromatic carbons), 132.6 (Ar-**C**H=), 115.7 (=**C**($COOR_1$)$_2$), 75.6, 75.5 and 75.3 (-O**C**H$_3$), 80.7, 80.6, 33.5 and 33.3 (alkyl carbons).

Di-(2-ethylhexyl)-2',4',5'-trimethoxybenzalmalonate (**BM8-2**): yellowish oil (64%), R_f 0.3 (80% EtOAc/Hex), IR (neat, cm^{-1}): 2950, 2862, 1715, 1602, 1512, 1461, 1250, 1211 and 1130; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.03 (s, 1H, Ar-C**H**=), 6.95 (s, 1H, Ar-**H**), 6.45 (s, 1H, Ar-**H**), 4.13-4.12 and 4.09-4.08 (m, 4H, 2×-OC**H**$_2$-), 3.89, 3.82 and 3.76 (s, 9H, 3×-OC**H**$_3$), 1.38-0.75 (m, 30H, 2×-C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.8,

164.7 (2×-COOR), 154.0, 152.4, 142.9, 123.4, 111.5 and 96.4 (aromatic carbons), 137.1 (Ar-**C**H=), 113.5 (=**C**($COOR_1$)$_2$), 56.3, 56.2 and 55.9 (-O**C**H_3), 68.1, 67.4, 38.7, 38.5, 30.3, 30.2, 28.8, 28.7, 23.7, 23.5, 22.9, 22.8, 14.0, 13.9, 10.9 and 10.8 (alkyl carbons).

Dilhexyl-2',4',5'-trimethoxybenzalmalonate (**BM8-3**): pale yellow solid (73%), m.p. 62-63°C, R_f 0.2 (80% EtOAc/Hex), IR (KBr, cm^{-1}): 2955, 2928, 2854, 1712, 1607, 1518, 1464, 1238, 1200 and 1029; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.05 (s, 1H, Ar-C**H**=), 6.97 (s, 1H, Ar-**H**), 6.46 (s, 1H, Ar-**H**), 4.20-4.12 (t, 4H, 2×-OC**H**$_2$-), 3.91, 3.85 and 3.78 (s, 9H, 3×-OC**H**$_3$), 1.66-0.81 (m, 22H, 2×-C_5**H**$_{11}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 176.6, 173.6 (2×-**C**OOR), 163.0, 161.5, 151.8, 145.9, 122.2, 120.4 (aromatic carbons), 132.1(Ar-**C**H=), 105.3 (=**C**($COOR_1$)$_2$), 74.5, 74.2 and 65.1 (-O**C**H_3), 40.3, 37.5, 37.2, 34.5, 31.5, 31.4, 22.9 (alkyl carbons).

Diethyl-4'-trimethoxybenzalmalonate (**BM1-1**): colorless oil (10%), R_f 0.4 (75% Hexanes/EtOAc), IR (neat, cm^{-1}): 2982, 2932, 1720, 1600, 1510, 1456, 1386, 1258, 1207, 1176 and 1064; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.66 (s, 1H, Ar-C**H**=), 7.43-7.40 (d, *J*= 8.9 Hz, 2H, Ar-**H**), 6.89-6.86 (d, *J*= 8.8 Hz, 2H, Ar-**H**), 4.39-4.24 (q, 4H, 2×-OC**H**$_2$-), 3.80 (s, 3H, -OC**H**$_3$), 1.34-1.29 (t, 6H, 2×-C**H**$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 166.9, 164.2 (2×-**C**OOR), 161.4, 123.4, 141.5(2×1C) and 131.5 (2×1C) and 133.54 (2×1C) (aromatic carbons), 131.5 (Ar-**C**H=), 114.1 (=**C**($COOR_1$)$_2$), 55.1 (-O**C**H_3), 61.4, 61.2, 13.9 and 13.7 (alkyl carbons).

Di-(2-ethylhexyl)- 4'-trimethoxybenzalmalonate (**BM1-2**): pale yellowish oil (83%) R_f 0.6 (75% Hexanes/EtOAc), IR (neat, cm^{-1}): 2955, 2862, 1739, 1603, 1514, 1454, 1382, 1312, 1254, 1172 and 1064; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.63 (s, 1H, Ar-C**H**=), 7.40-7.24 (d, *J*= 8.9 Hz, 2H, Ar-**H**), 6.87-6.83 (d, *J*= 8.8 Hz, 2H, Ar-**H**), 4.16-4.01 (m, 4H, 2×-OC**H**$_2$-), 3.86 (s, 3H, OC**H**$_3$), 1.61-0.83 (m, 30H, 2×-C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.5 and 167.2 (2×-**C**OOR), 164.4, 161.4, 141.4 (2×1C) and 131.3 (2×1C) (aromatic carbons), 125.3 (Ar-**C**H=), 114.1 (=**C**($COOR_1$)$_2$), 55.1 (OCH_3), 67.8, 67.6, 55.1, 49.3, 38.6, 38.4, 30.2, 30.1, 28.7, 28.6, 23.6, 23.4, 22.8, 22.7, 13.8, 10.8 and 10.7 (alkyl carbons).

Dihexyl- 4'-trimethoxybenzalmalonate (**BM1-3**): pale yellowish oil (52%), R_f 0.5 (75% Hexanes/EtOAc), IR (neat, cm^{-1}): 2940, 2856, 1738, 1602, 1508, 1465, 1382, 1262,

1172 and 1071; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.62 (s, 1H, Ar-C**H**=), 7.40-7.35 (d, *J*= 8.9 Hz, 2H, Ar-**H**), 6.86-6.82 (d, *J*= 8.8 Hz, 2H, Ar-**H**), 4.26-4.21 and 4.19-4.14 (t, 4H, 2× -O**C**H_2-), 3.79 (s, 3H, O**C**H_3), 2.17-0.79 (m, 22H, 2×-C_5**H**$_{11}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.1, 164.3 (2×-**C**OOR), 164.1, 123.6, 141.5(2×1C) and 131.3 (2×1C) (aromatic carbons), 125.3 (Ar-**C**H=), 114.1 (=**C**($COOR_1$)$_2$), 55.1 (-O**C**H_3), 65.6, 65.3, 31.2, 28.4, 28.2, 25.4, 25.3, 22.4, 22.3 and 13.8 (alkyl carbons).

2.6 General Procedure for Molar Absorptivity Measurements [11, 32]

A stock solution of each compound was prepared in a 100 mL volumetric flask. The resulting stock solution was then diluted to five final concentrations. The UV absorbance of each final dilution was recorded by scanning wavelengths between 200 and 800 nm. The molar absorptivity (ε) was calculated for each compound at the wavelengths of maximum absorbance (λ_{max}) using Beer's law:

$$A = \varepsilon bc$$

Where *A* is absorbance of each concentration

b is the cell path length (cm)

and *c* is the concentration of the absorbing species in mole per litre

2.7 General Procedure for Photostability Test[11]

The photostability of the synthetic UV-filters was tested in methanol and hexanes. Stock solution of each compound was prepared in a 100 ml volumetric flask. The resulting 0.0536 mM solutions in methanol and hexanes were divided into two parts. One part was kept covered with foil at room temperature (dark sample) while the other part was irradiated by artificial UV lamp at room temperature (irradiated sample). Then UV absorption profile of each sample was analyzed on UV/VIS spectrometer. The absorbances of irradiated sample at various irradiant times were compared to those of dark samples.

The calculation of percent relative absorbance of each irradiated sample is given by:

$$\text{Percent of relative absorbance} = \left(\frac{\text{Absorbance of irradiated sample at time X}}{\text{Absorbance of dark sample at time X}} \right) \times 100$$

2.8 General Procedure for Irritation Test[33]

The 7.5% of test compounds in white petrolatum are applied to plasters, Finn Chamber as shown in figure 2.4. The two parallel series of Finn Chambers were placed on the skin at the back of volunteers for 24 hours. After removal of a test plaster, the skin area in question was evaluated. One column of the opened spots was then irradiated with 10 J/cm^2 of UVA light. After 24 hours both test area columns were inspected again.

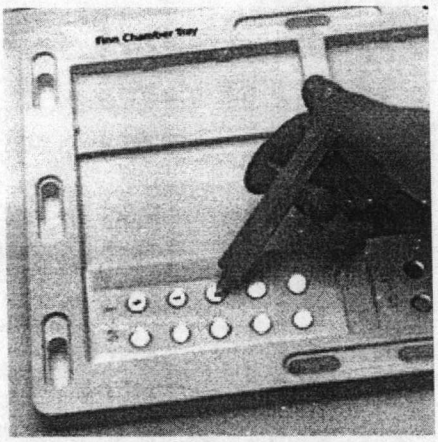


Figure 2.4 Applying of the test compounds to plaster, Finn Chamber