CHAPTER 2

EXPERIMENTAL

2.1 ***Instruments and Chemicals.***

Melting point was recorded in ^{o}C and was measured on an Electrothermal Melting Point Apparatus. Infrared spectra were recorded using FTS FT-IR spectrophotometer and major bands (υ) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU) and principle bands (λmax) were recorded as wavelengths (nm) and log ε in chloroform solution. Nuclear magnetic resonance spectra were recorded on a FTNMR Bruker Ultra Shield^{TM} 300 MHz at Department of Chemistry, Faculty of Science, Prince of Songkla University or UNITY INOVA spectrometer 500 MHz at Central Instrument Facilities, Prince of Songkla University. X-ray ORTEP diagram was recorded using SMART APEX CCD X-ray Diffractometer. Spectra were recorded in deuterochloroform, deuteroacetone and DMSO-d6 and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Optical rotation was measured in chloroform solution with sodium D line (590 nm) on an AUTOPOL^{R} II automatic polarimeter. Solvent for extraction and chromatography were distillated at their boiling point ranges prior to use except diethyl ether was analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063-0.200). Flash Column chromatography was performed on silica gel (Merck) type 60 (0.040-0.063). Precoated plated of silica gel 60 GF_{254} or reversed-phase C-18 were used for analytical purposes.

2.2 Plant material

Leaves of *U. purpurea* Blume. were collected from Pattalung Province, Thailand. The plant was identified by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University.

2.3 Extraction

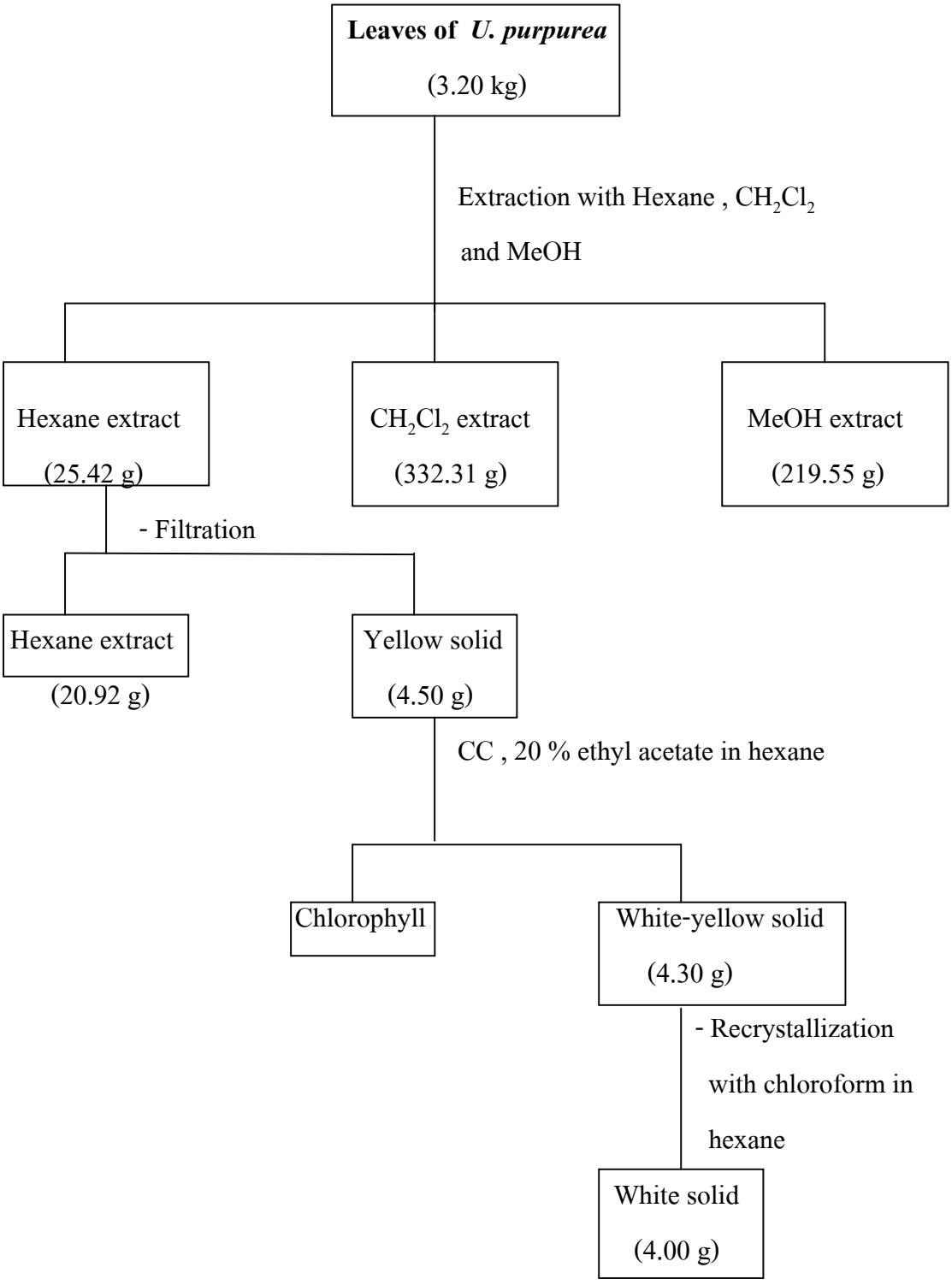
Air-dried leaves (3.2 kg) of *U. purpurea* Blume were extracted with hexane, methylene chloride and methanol, successively, at room temperature. The mixtures were filtered and concentrated under reduced pressure to give crude hexane, (25.42 g) methylene chloride (332.31 g) and methanol extracts (219.55 g). Some yellow solid (4.5 g) precipitated from hexane extract was filtered. The filtrate was further evaporated to dryness to afford crude hexane extract (20.92 g) as a yellow-green viscous liquid.

2.4 Isolation and Chemical Investigation

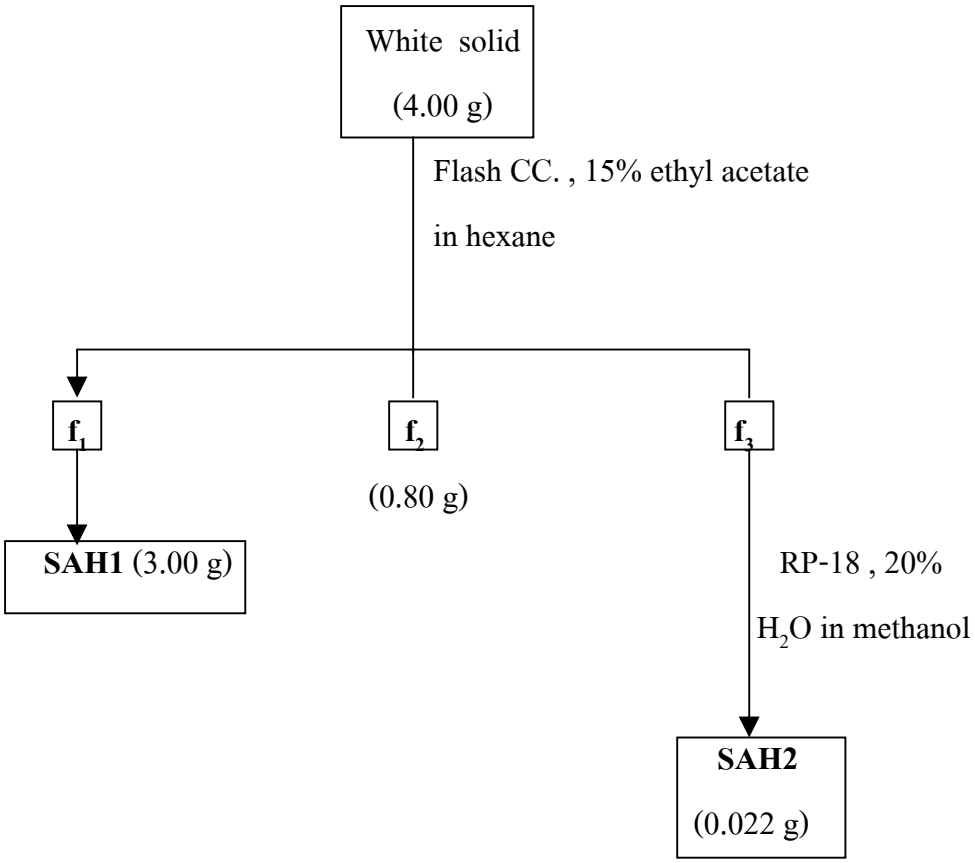
2.4.1 Investigation of the crude hexane extract from the leaves of *U. purpurea* Blume

The chlorophyll contamination in the yellow solid from the hexane extract (4.50 g) was removed by column chromatography on silica gel using 20% ethyl acetate in hexane as eluent to yield white-yellow solid (4.30 g) after removal of solvents under reduced pressure. The white-yellow solid was recrystallized from chloroform in hexane to afford white solid (4.00 g). The white solid was further purified by flash

column chromatography on silica gel using hexane as eluent and increasing polarity with ethyl acetate to give 3 fractions.



Scheme 6 Extraction and isolation of crude extract from the leaves of *U. purpurea* Blume



Scheme 7 Extraction and isolation of compounds **SAH1** and **SAH2** from the leaves of *U. purpurea* Blume

Fraction f_1 was obtained as a white solid (3.00 g, R_f = 0.15, 20% ethyl acetate : hexane), compound **SAH1**, nuclear magnetic resonance spectroscopic data corresponded to **pipoxide** (Joshi *et al*, 1979)

Fraction f_2 was obtained as a white solid. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate : hexane showed two UV-active spots with R_f value 0.15 and 0.13. No further purification was performed because this fraction was a mixture of **fraction f_1** and **fraction f_3** in ratio 1:1.

Fraction f_3 was obtained as a white solid. Chromatogram characteristic on normal phase TLC with 20% ethyl acetate : hexane showed one UV-active spot but two spots on RP-18 TLC with 20% H_2O : methanol. Further separation of **fraction f_3** applied as a dilute solution (less than 4.0 mg/sheet) using RP-18 sheets with 20% H_2O : methanol as a mobile phase (5 runs) gave compound **SAH2** (0.022 g , R_f = 0.13 , 20% ethyl acetate : hexane) and compound **SAH1**.

Compound SAH1 (White solid)

$[\alpha]_D^{28}$ **:** $+ 51.99^{o}$ ($c = 0.327$, $CHCl_3$)

mp : 149-150 ^{o}C

UV (MeOH) λ_{max} (nm) (log ε): 273 (3.20), 228 (4.27) and 202 (4.07)

IR (KBr) ν (cm^{-1}) : 3441 (OH stretching), 1728 and 1723 (C=O stretching), 1682 (C=C stretching), 1279 (C-O stretching), 711 (C-H bending)

^{1}H NMR($CDCl_3$) (δ ppm) (500 MHz) : 8.06 (4H, *m*), 7.58 (2H, *m*), 7.45 (4H, *m*), 6.10 (1H, *ddd*, *J* = 10, 4, 2 Hz), 5.91 (1H, *dt*, *J* = 10, 2 Hz), 5.67 (1H , *ddd*, *J* = 8, 3, 2 Hz), 5.00 (1H, *d* (AB), *J* = 12.5 Hz), 4.48 (1H, *d* (AB), *J* = 12.5 Hz), 4.33 (1H, *dd*, *J* = 8, 6 Hz), 3.60 (1H, *dd*, *J* = 4, 2 Hz), 3.17 (1H, *d*, *J*, = 6 Hz)

^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz) : 166.87, 166.20, 133.44, 133.38, 132.96, 129.84, 129.78, 129.44, 129.40, 128.47, 128.43, 124.73, 74.82, 71.06, 62.91, 59.49, 54.20

DEPT–135° ($CDCl_3$)

CH : 133.44, 133.38 ,132.96, 129.84, 129.78, 128.47, 128.43, 124.73 74.82, 71.06, 59.49, 54.20

CH_2 : 62.91

Compound SAH2 (White solid)

$[\alpha]_D^{27.3}$ **:** $+50^{o}$ ($c = 0.04$, $CHCl_3$)

mp. : 132-134°C

UV (MeOH) λ_{max} (nm) (log Ɛ): 276 (3.49), 235 (3.31), 222 (3.56) and 205 (3.55)

IR (neat) ν (cm^{-1}) : 3839 (OH stretching), 1716 (C=O stretching), 1631 (C=C stretching), 1273 (C-O stretching), 711 (C-H bending)

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) : 8.06 (2H, *m*) ,7.76 (1H, *d*, *J* = 15.9 Hz), 7.59 (1H , *m*), 7.53 (2H, *m*), 7.46 (2H, *m*), 7.40 (2H, *m*), 7.39 (1H, *m*) , 6.47 (1H, *d*, *J* = 15.9 Hz), 6.08 (1H, *ddd*, *J* = 9.9, 3.6, 2.7 Hz), 5.86 (1H, *dt*, *J* = 9.9, 1.8 Hz), 5.56 (1H, *dt*, *J* = 8.1, 2.1 Hz), 5.00 (1H, *d* (AB), *J* = 12 Hz), 4.48 (1H, *d* (AB), *J* = 12 Hz), 4.25 (1H, *d*, *J* = 8.1 Hz), 3.59 (1H, *dd*, *J* = 3.9, 1.8 Hz)

^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz) : 167.32, 166.20, 146.31, 134.11, 133.43, 133.12, 130.66, 129.83, 129.51, 128.97, 128.51, 128.25, 124.64, 117.21, 74.40, 71.10, 62.92, 59.55, 54.25

DEPT 135^{o} ($CDCl_3$)

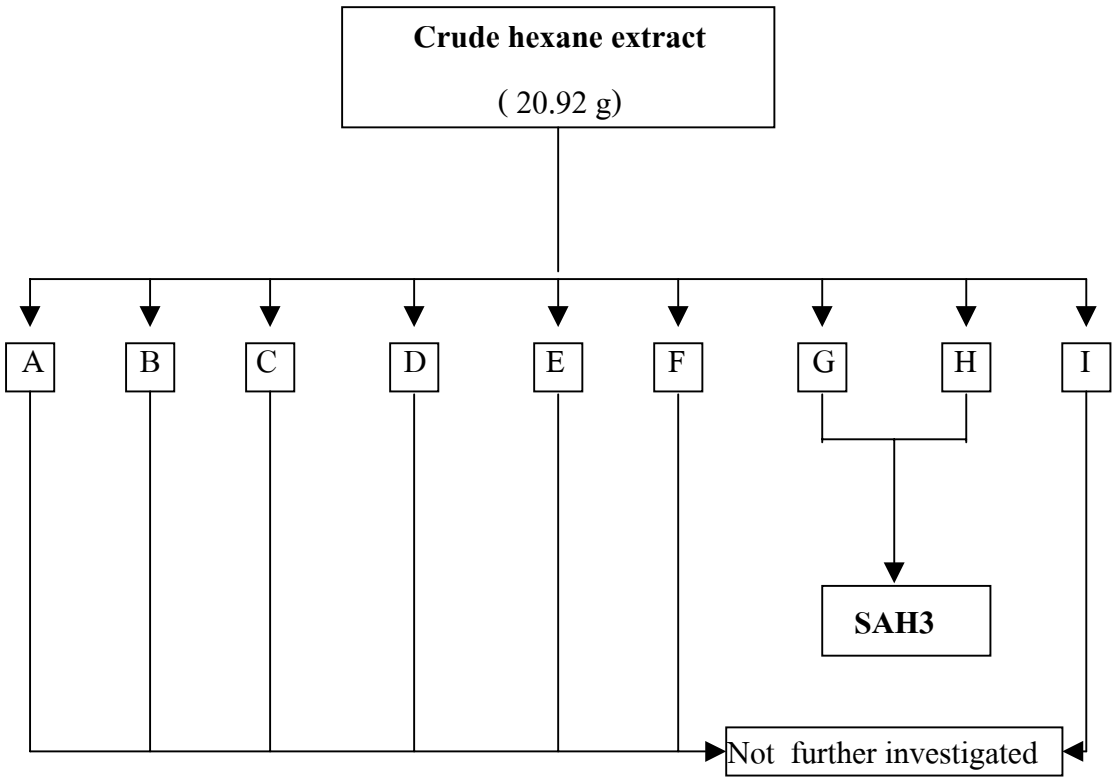
CH : 146.31, 133.43, 133.11,130.66, 129.82 (2xC), 128.96 (2xC), 128.50 (2xC), 128.24 (2xC), 124.62, 117.17, 74.37, 71.07, 54.24

CH_2 : 62.89

HRMS (observed) *(m/z)* : 393.1334 [M^++1]

HRMS (calculate) *(m/z)* : 393.1338 [M^++1]

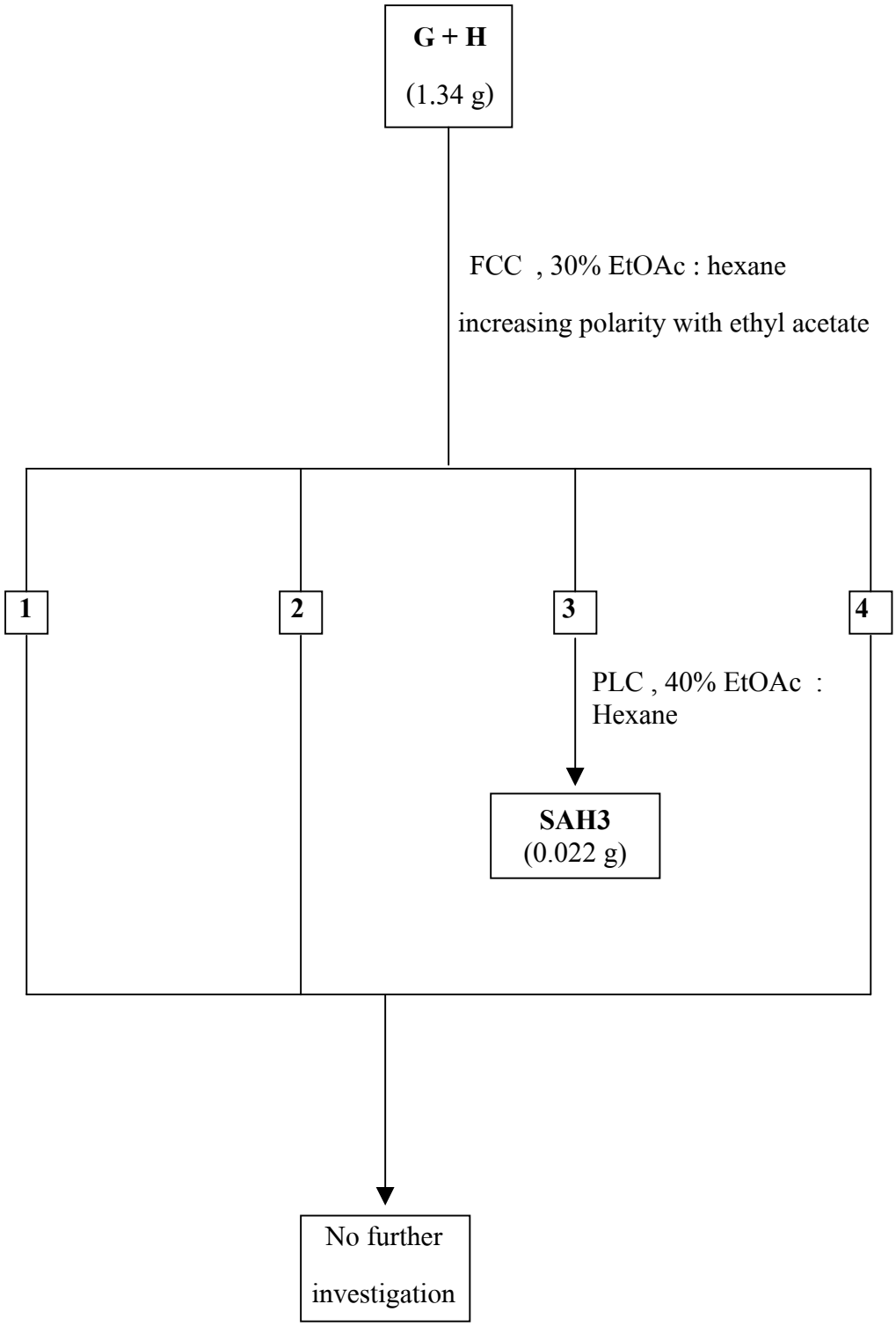
The hexane extract of leaves *of U. purpurea* Blume (yellow-green viscous liquid, 20.92 g) was subjected to quick column chromatography on silica gel (QCC) using hexane as eluent and increasing polarity with ethyl acetate and methanol, respectively, to give 9 fractions, as shown in **scheme 8**.



Scheme 8 Isolation of compound **SAH3**

Fraction A, **B**, **C**, **D**, **E**, **F** and **I** were obtained as a viscous oil. Chromatogram characteristics on normal phase TLC with 50 % EtOAc : hexane showed none of well-separated spots under UV. Therefore, no further purification was performed.

Fraction G and H (1.34 g), as green viscous liquid, were subjected to repeated chromatography (FCC and PLC) to give compound **SAH3** (0.022 g) as a white solid, as shown in **scheme 9**. Chromatogram characteristic on normal phase TLC with 70% EtOAc : hexane showed one UV – active spot with R_f value of 0.39.



Scheme 9 Isolation of compound **SAH3**

Compound SAH3 (White solid)

$[\alpha]_D^{25.9}$ **:** - 90.90° (c = 0.011, $CHCl_3$)

mp. : 110–112 °C

UV (MeOH) λ_{max} (nm) (log ε) : 230 (4.56), 211 (4.12) and 202 (4.43)

IR (neat) ν (cm^{-1}) : 3444 (OH stretching), 1703 (C=O stretching), 1601 (C=C stretching), 1277 (C-O stretching) and 709 (C-H bending of monosubstituted phenyl ring)

^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz) : 7.99 (2H, *m*), 7.94 (2H, *m*), 7.52 (2H, *m*), 7.37 (4H, *m*), 5.99 (1H, *ddd*, *J* = 10, 4, 1.5 Hz), 5.84 (1H, *ddd*, *J* = 10, 2.5, 1 Hz), 5.70 (1H, *m*), 4.87 and 4.72 (2H, *d* (AB system), *J* = 12 Hz), 4.34 (1H, *d*, *J* = 4 Hz), 4.24 (1H, *d*, *J* = 6 Hz), 3.46 (1H, *br s*), 3.43 (1H, *br s*) , 3.17 (1H, *br s*)

^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz) : 167.78, 167.09, 133.42, 133.37, 129.81, 129.78, 129.76, 129.43, 129.23, 128.41, 128.38, 126.71, 75.90, 74.10, 70.79, 68.68, 66.67

DEPT–135° ($CDCl_3$)

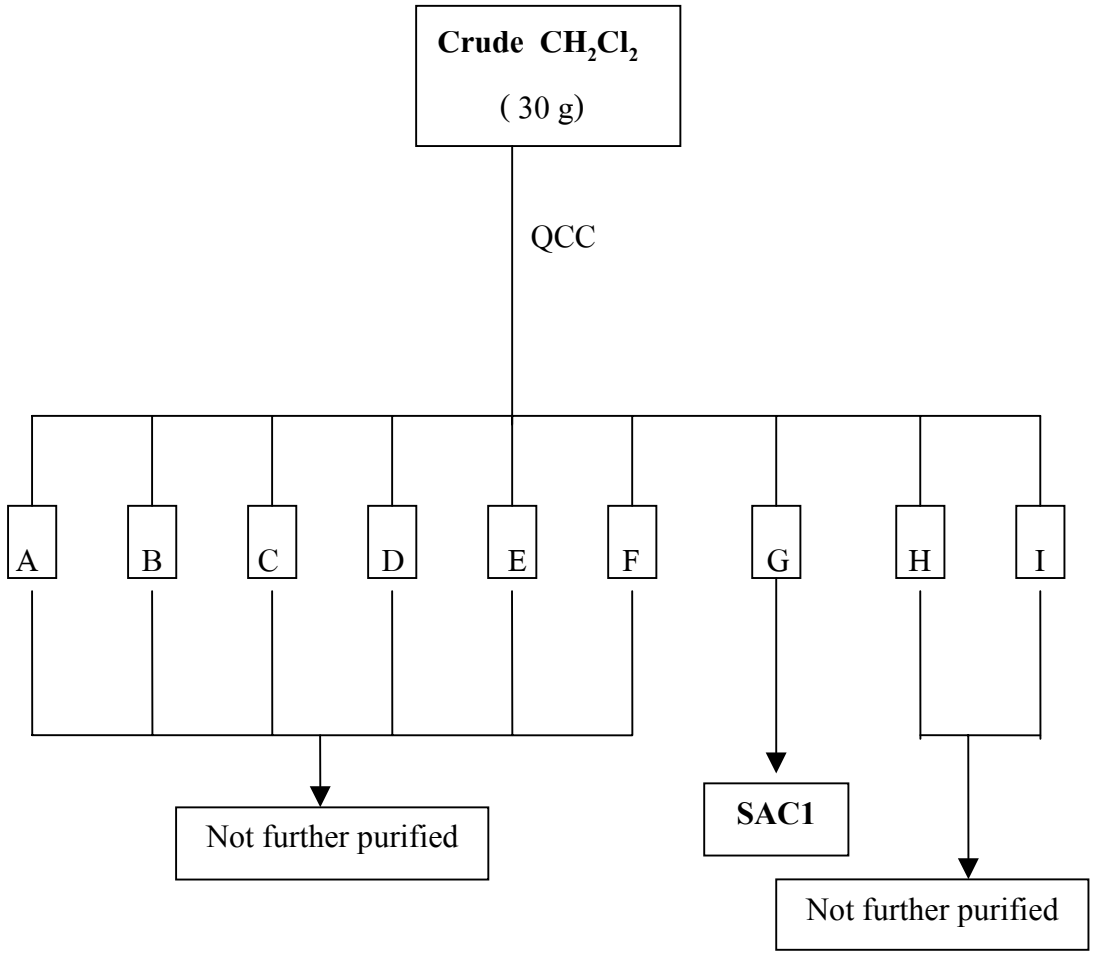
CH : 133.42, 133.37, 129.81, 129.78, 129.76, 128.41, 128.38, 126.71, 74.10, 70.79, 68.68

CH_2 : 66.67

HRMS *(m/z)* : 385.1287 [M^++1]

2.4.2 Investigation of the crude methylene chloride extract from the leaves of *U. purpurea* Blume.

The methylene chloride extract (30 g) was chromatographed on silica gel (QCC) using hexane as eluent and increasing polarity with ethyl acetate to give 9 fractions. **Fractions G** was further purified by chromatography. **Fractions A**, **B**, **C**, **D**, **E**, **F**, **H** and **I** were not further investigated , as shown below.

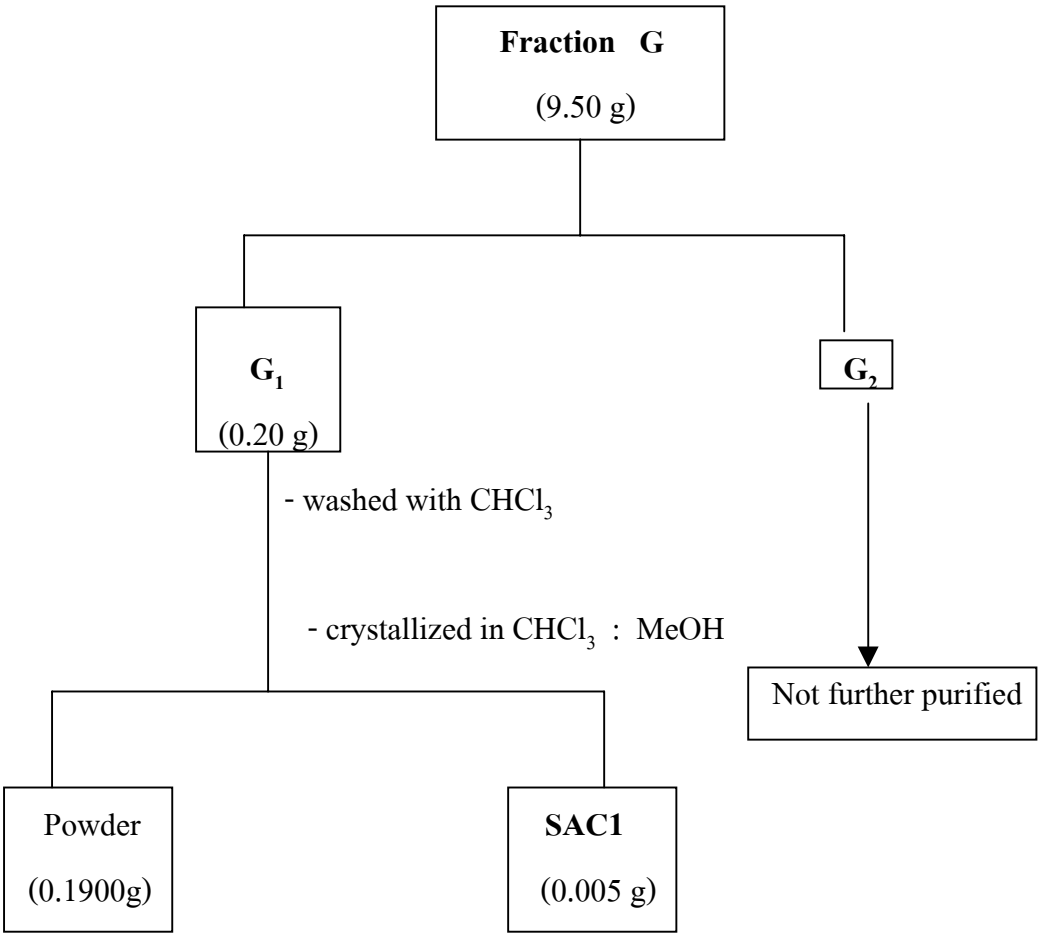


Scheme 10 Isolation of compounds **SAC1** from leaves of *U. purpurea* Blume

Fraction A (1.70 g) was rechromatographed on silica gel using 2 % EtOAc : hexane as eluent to give long chain hydrocarbons and fatty acid derivative. It was not further investigated.

Fraction B, **C**, **D**, **E,** and **F** were obtained as viscous liquid. Chromatogram characteristics on normal phase TLC showed many unseparated UV-active spots. Therefore, no further purification was performed.

Fraction G (9.50 g) as a yellow solid suspended in dark green viscous liquid was filtered and washed with chloroform to give subfraction $\mathbf{G_1}$ and $\mathbf{G_2}$. Subfraction $\mathbf{G_1}$ was further purified by crystallization. Subfraction $\mathbf{G_2}$ was not further investigated as shown in **Scheme 11**.



Scheme 11 Isolation of compound **SAC1**

Subfraction **G_1** (0.20 g) was washed with chloroform several times and crystallized from $CHCl_3$: MeOH for several days to give a powder (0.1900 g) concomitant with white crystallized needles. Efforts to collect only the crystals using the forceps and looking under microscope gave the white needles, compound **SAC1** (0.005 g). Chromatogram characteristic on normal phase TLC with acetone : hexane (2:1, v/v) showed one UV-active spot with R_f value 0.5.

Compound SAC1 (White solid)

$[\alpha]_D^{25.9}$ **:** - 111.11^o (c = 0.018, $CHCl_3$)

mp. : 149-151 ^{o}C

UV (MeOH) λ_{max} (nm) (log Ɛ) : 230 (4.72), 211 (4.21) and 203 (4.50)

IR (neat) ν (cm^{-1}) : 3453 (O-H stretching), 1702 (C=O stretching), 1273 (C-O stretching), 709 (C-H bending of monosubstituted phenyl ring)

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) : 8.03 (2H, *dd*, *J* = 7.8, 0.6 Hz), 7.96 (2H, *dd*, *J* = 7.8, 0.6 Hz), 7.57 (1H, *m*), 7.53 (1H, *m*), 7.42 (2H, *m*), 7.37 (2H, *m*), 5.91 (1H, *dd, J* = 10.5, 2.1 Hz), 5.75 (1H, *dd*, *J* = 10.2, 2.1 Hz), 5.33 (1H, *d*, *J* = 10.5 Hz), 4.51 (1H, *d* (AB), *J* = 11.7 Hz), 4.46 (1H, *d* (AB), *J* = 11.7 Hz), 4.41 (1H, *ddd*, *J* = 10.2, 4.5, 2.1 Hz), 4.16 (1H, *ddd*, *J* = 10.5, 7.5, 4.2 Hz), 3.80 (1H, *s*), 2.65 (1H, *br d* , *J* = 4.5 Hz), 2.56 (1H, *br d*, *J*= 4.2 Hz)

^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz) : 133.91, 133.31, 130.23, 129.99, 129.69, 129.37, 128.78, 128.65, 128.48, 79.64, 74.31, 73.95, 72.24, 66.97

DEPT-135^{o} ($CDCl_3$)

CH : 133.90, 133.30, 130.22, 129.98, 129.68, 129.35, 128.64, 128.47, 79.63, 73.94, 72.22

CH_2 : 66.96

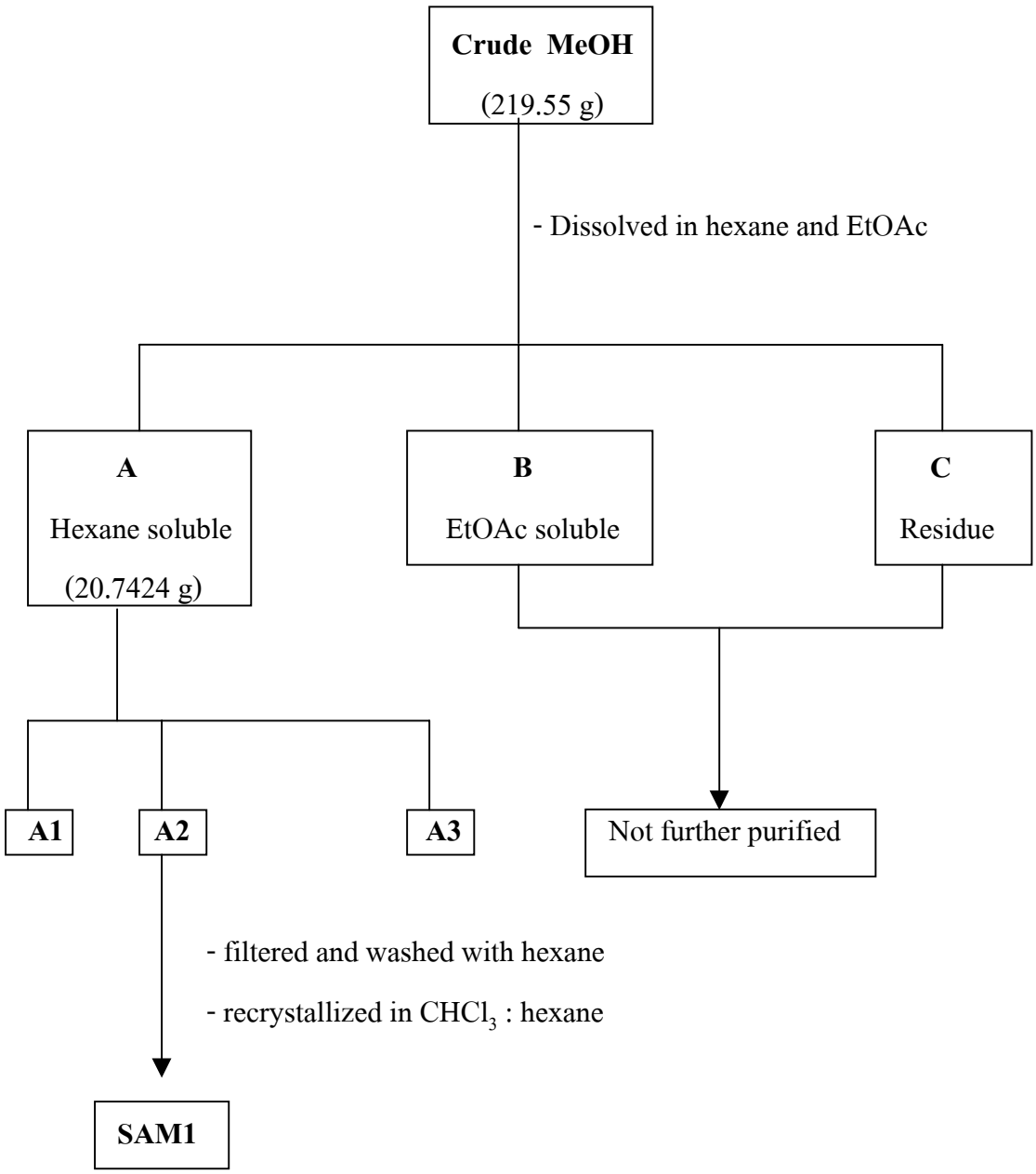
EIMS (observed) (*m/z*) : 249 $[M-135]^+$, 231 $[M-153]^+$, 122 $[M-262]^+$, 77 $[M-307]^+$

Fraction H and **I** (0.4069g) were rechromatographed (CC) on silica gel using 30 % EtOAc : hexane and together purified by PLC, chromatogram characteristic on normal phase TLC with 40 % acetone : hexane showed one UV-active spot. ^{1}H NMR spectrum indicated a mixture of many compounds. This mixture was obtained in low quantity, so it was not further investigated.

2.4.3 Investigation of the crude methanol extract from the leaves of *U. purpurea* Blume.

The methanol extract (219.55 g) was dissolved in hexane and ethyl acetate, successively and then evaporated under reduced pressure to give a dark brown viscous liquid and dark green viscous liquid of hexane soluble and EtOAc soluble,

respectively. The EtOAc soluble and the residue were not further investigated **(Scheme 12)**.



Scheme 12 Solubilization of crude methanol extract from the leaves of *U. purpurea* Blume with hexane and EtOAc

Fraction A (20.7424 g) was chromatographed on silica gel (QCC) using hexane as eluent and increasing polarity with ethyl acetate and methanol to give 3 fractions, $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$. **Subfraction A2** as a white solid suspended in green viscous liquid was filtered and washed with hexane and then recrystallized from $CHCl_3$: hexane to afford compound **SAM1** (0.100 g, white solid). Chromatogram characteristic on normal phase TLC with acetone : hexane (2:1, v/v) showed one UV-active spot with R_f value of 0.58. From ^{1}H NMR 300 MHz spectroscopic data, it was found to be a benzoic acid. It was not further investigated, as shown in **scheme 12**.

Compound SAM1 (White solid)

mp. : 117 – 118 ^{o}C

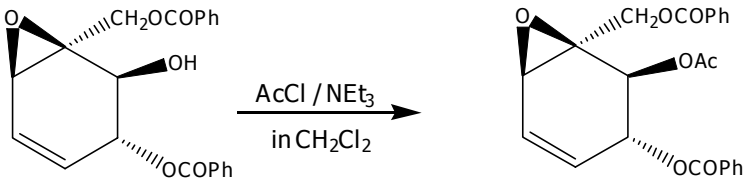
UV (MeOH) λ_{max} (nm) (log ε) : 227 (4.14), 211 (3.81) and 202 (3.99)

IR (KBr) ν (cm^{-1}) : 3500-2500 (O-H stretching), 1686 (C=O stretching), 1292 (C-O stretching) and 705 (C-H bending of monosubstituted phenyl ring)

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) : 8.13 (2H, *dd*, *J* = 7.8, 1.5 Hz), 7.64 (1H, *tt*, *J* = 7.5, 1.2 Hz), 7.48 (2H, *t*, *J* = 7.8 Hz)

2.5 *Synthesis of Pipoxide Derivatives*

2.5.1 Acetylation of Compound SAH1 (pipoxide)



SAH1 **ST1**

Compound **SAH1,** a pipoxide, (0.052 g, 0.142 mmole) was dissolved in methylene chloride (5 ml), then dry triethylamine (0.0792 ml, 0.568 mmole) and acetyl chloride (0.0304 ml, 0.426 mmole) were added into a pipoxide solution under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 40 hrs., then poured into ice water and extracted 5 times with methylene chloride (5x10 ml). The combined organic extracts were washed 2 times with saturated sodium hydrogen carbonate (2x10 ml) and 3 times with water (3x10 ml) . The organic layer was dried over anhydrous Na_2SO_4 , filtered and evaporated to give the crude mixture which was purified by PLC on silica gel with 25% EtOAc : hexane as the developing solvent to afford compound **ST1**, R_f value 0.40 in 30 % EtOAC : hexane (36 mg, 62.07 %)

Compound ST1 (Colorless viscous liquid)

$[\alpha]_D^{25.9}$: + 14.08° (c = 0.071, $CHCl_3$)

UV (MeOH) λ_{max} (nm) (log Ɛ) : 230 (4.52), 212 (4.17), 203 (4.39)

IR (neat) ν (cm^{-1}) : 1749 and 1720 (C=O stretching of ester), 1598 (C=C stretching of phenyl ring), 1266 (C-O stretching), 705 and 700 (C-H bending of phenyl ring)

^{1}H NMR ($CDCl_3$) (δ pp m) (300 MHz) : 8.03 (4H, *m*), 7.58 (2H, *m*), 7.45 (4H, *m*), 6.12 (1H, *dt*, *J* = 9.9, 3.3 Hz), 5.95 (1H, *dt*, *J* = 9.9, 1.8 Hz), 5.89 (1H, *d*, *J* = 8.4 Hz), 5.77 (1H, *dt*, *J* = 8.4, 2.1 Hz), 4.68 and 4.42 (2H, *d* (AB system), *J* = 12 Hz), 3.63 (1H, *dd*, *J* = 3.9, 1.8 Hz), 2.08 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ pp m) (300 MHz) : 170.19, 165.83, 133.55, 133.44, 133.39, 129.82, 129.79, 129.40, 129.30, 128.53, 124.26, 72.13, 71.02, 62.20, 58.37, 54.54, 20.74

DEPT-135° ($CDCl_3$)

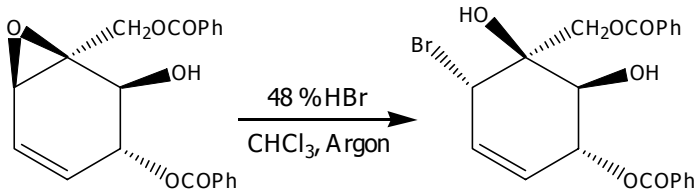
CH : 133.55, 133.44, 133.39, 129.79, 129.83, 128.53, 124.27, 71.02, 72.13, 54.54

CH_2 : 62.20

CH_3 : 20.74

ESITOFMS (*m/z*) : 409 [M^++1]

2.5.2 Hydrobromination of Compound SAH1



SAH1 **ST2**

Compound **SAH1** (0.100 g, 0.273 mmole) was dissolved in chloroform (2ml) and 48 % HBr (0.040 ml, 0.355 mmole) was added into the mixture. After the mixture was kept and stirred at 0 ^{o}C under argon atmosphere for 3 hrs, the resulting precipitate was then filtered and washed with a mixture of 90 % $CHCl_3$: hexane to give compound **ST2** as a white solid, mp. = 198-200 ^{o}C, R_f value 0.40 in 1% MeOH : $CHCl_3$ (0.1208 g, 99.18 %).

Compound ST2 (White solid)

mp. **:** 198-200 ^{o}C

UV (MeOH) λ_{max} **(nm) :** 231 (4.42), 214 (4.06) and 203 (4.36)

IR (KBr) ν **(cm^{-1}) :** 3512 (O-H stretching), 1694 (C=O stretching), 1281 (C-O stretching) and 709 (C-H bending of monosubstituted phenyl ring)

^{1}H NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (300 MHz) : 8.11 (4H, *m*), 7.64 (2H, *m*), 7.51 (4H, *m*), 6.07 (1H, *ddd*, *J* = 9.9, 4.5, 1.8 Hz), 5.92 (1H, *br d*, *J* = 8.1), 5.78 (1H, *dd*, *J* = 9.9, 2.4 Hz), 4.93 (1H, *d*, *J* = 4.5 Hz), 4.84 and 4.71 (2H, *d* (AB system), *J* = 12 Hz), 4.40 (1H, *d*, *J* = 8.1 Hz).

^{13}C NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (75 MHz) : 166.48, 166.43, 133.11, 133.04, 130.01, 129.72, 129.64, 128.85, 128.35, 128.31, 126.95, 75.41, 74.09, 70.05, 68.87, 48.94.

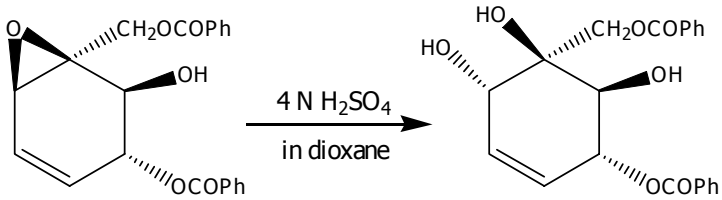
DEPT-135^{o} ($CDCl_3$)

CH : 132.73, 132.69, 129.22, 129.20, 128.41, 128.02, 127.98, 126.72, 73.77, 68.94, 48.92

CH_2 : 68.62

EIMS *(m/z)* : 447 [M^++1] and 449 [M^++3] in ratio 1:1

2.5.3 Hydrolysis of Compound SAH1 with sulfuric acid



SAH1 **ST3**

Compound **SAH1** (0.100 g, 0.273 mmole) was dissolved in dioxane (15 ml), and 4N sulfuric acid (1.5 ml, 28.1 mmole) was added into the solution mixture. The solution mixture was stirred at room temperature for 22 hrs, poured into water then extracted 5 times with chloroform (5x10 ml). The organic extract was washed with water, dried over anhydrous Na_2SO_4 and then evaporated to give crude mixture which was purified by PLC (silica gel) with 70 % EtOAc : hexane as the developing solvent to give compound **ST3** as a colorless viscous liquid, R_f value 0.52 in 60 % acetone : hexane (10.8 mg, 10.30 %) and a mixture of compound **SAH1** and **ST3** (18.6 mg, 17.74 %).

Compound ST3 (Colorless viscous liquid)

$[\alpha]_D^{26.6}$ **:** - 78.95° (c = 0.038, $CHCl_3$)

UV (MeOH) λ_{max} **(nm) (log Ɛ) :** 230 (4.30), 212 (3.92) and 202 (4.21)

IR (Neat) ν **(cm^{-1}) :** 3438 (OH stretching), 1704 (C=O stretching), 1278 (C-O stretching) and 709 (C-H bending of monosubstituted phenyl ring).

^{1}H NMR ($CDCl_3$) (δ pp m) (300 MHz) : 8.03 (2H, *m*), 7.98 (2H, *m*), 7.56 (2H, *m)*, 7.40 (4H, *m*), 6.02 (1H, *ddd*, *J* = 10.2, 3.9, 1.5 Hz), 5.88 (1H, *ddd*, *J* = 10.2, 3, 1 Hz), 5.70 (1H, *m*), 4.89 and 4.75 (2H, *d* (AB system), *J* = 12.3 Hz), 4.33 (1H, *br d*, *J* = 3.9 Hz), 4.24 (1H, *d*, *J* = 6 Hz).

^{13}C NMR ($CDCl_3$) (δ pp m) (75 MHz) : 167.82, 133.48, 133.41, 130.88, 129.82, 129.54, 129.26, 128.45, 126.87, 75.92, 74.30, 70.86, 68.56, 66.76

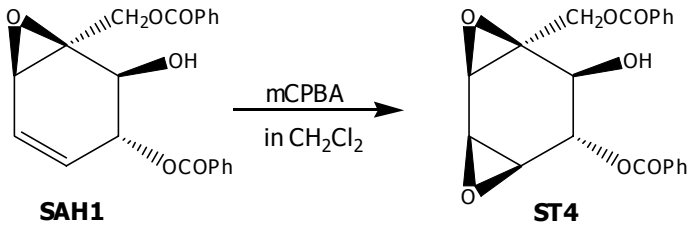
DEPT-135^{o} ($CDCl_3$)

CH : 133.51, 129.84, 129.53, 128.47, 126.88, 74.31, 70.86, 68.55

CH_2 : 66.76

ESITOFMS *(m/z)* : 407 [M^{+}+Na]

2.5.4 Epoxidation of Compound SAH1



SAH1 **ST4**

Compound **SAH1** (0.0553 g, 0.151 mmole), was dissolved in methylene chloride (1.5 ml) at -10 ^{o}C (ice + $CaCl_2$), then a solution of *m*-chloroperbenzoic acid (0.0322 g, 0.1865 mmole) in methylene chloride (4.0 ml) was added dropwise into the above solution. The mixture was stirred at room temperature for 23 hrs under nitrogen

atmosphere. Then, the mixture was poured into ice-water and extracted several times with chloroform, followed by washing with water and dried over anhydrous Na_2SO_4. After filtration, the chloroform was removed by rotary evaporator under reduced pressure. The obtained crude mixture was purified by PLC (silica gel) with 30 % EtOAc : hexane as a developing solvent to give compound **ST4** (26.4 mg, 47.74 %, R_f = 0.19).

Compound ST4 (Colorless viscous liquid)

$[\alpha]_D^{27.0}$: - 61.2° (*c* = 0.049, $CHCl_3$)

UV (MeOH) λ_{max} (nm) (log ε) : 274 (2.54), 229 (3.64) and 203 (3.40)

IR (Neat) ν (cm^{-1}) : 3482 (OH stretching), 1719 (C=O stretching), 1272 (C-O stretching) and 709 (C-H bending of monosubstituted phenyl ring)

^{1}H NMR ($CDCl_3$) (δ pp m) (300 MHz) : 8.08 (4H, *m*), 7.58 (2H, *m*), 7.45 (2H, *m*), 5.51 (1H, *dd*, *J* = 8.1, 0.6 Hz), 4.91 and 4.32 (2H, *d* (AB system), *J* = 12 Hz), 4.42 (1H, *d*, *J* = 8.1 Hz), 3.78 (1H, *d*, *J* = 2.4 Hz), 3.65 (1H, *dd*, *J* = 4.5, 2.4 Hz), 3.50 (1H, *br d*, *J* = 4.5 Hz).

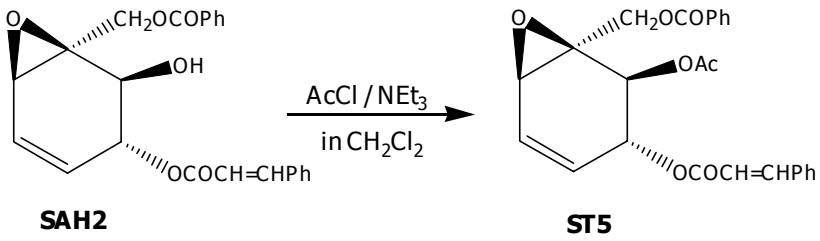
^{13}C NMR ($CDCl_3$) (δ pp m) (75 MHz) : 166.74, 166.20, 133.56, 133.49, 129.99, 129.84, 129.30, 128.52, 128.48, 73.82, 68.01, 62.55, 61.10, 57.40, 53.73, 52.06

DEPT-135° ($CDCl_3$)

CH : 133.57, 133.50, 129.99, 129.84, 128.53, 128.48, 73.80, 68.00, 57.40, 53.75, 52.07

CH_2 : 62.55

2.5.5 Acetylation of Compound SAH2



SAH2 **ST5**

Compound **SAH2** (0.0101 g, 0.0255 mmole) was dissolved in methylene chloride (3 ml) and then dry triethylamine (28.6 μl) and acetyl chloride (14 μl, 0.1962 mmole) were added into the above solution under nitrogen atmosphere. The mixture was stirred at room temperature for 6 days then poured into ice-water and extracted 3 times with methylene chloride (3x10 ml). The combined extracts were washed twice with saturated sodium hydrogen carbonate (2x10 ml), 2 times with water (2x10 ml), dried over anhydrous Na_2SO_4 and filtered. After the organic solvent was removed, the crude mixture was purified by PLC (silica gel) with 30 % EtOAc : hexane as a developing solvent to give compound **ST5** (3.4 mg, 33.66 %, R_f= 0.26)

Compound ST5 (Colorless viscous liquid)

$[\alpha]_D^{26.3}$: - 83.30° (c = 0.012, $CHCl_3$)

UV (MeOH) λ_{max} (nm) (log ε) : 276 (3.57), 235 (3.37), 222 (3.61) and 205 (3.60)

IR (Neat) ν (cm^{-1}) : 1724 and 1719 (C=O stretching), 1636 (C=C stretching), 1270 (C-O stretching) and 711 (C-H bending of monosubstituted phenyl ring)

^{1}H NMR ($CDCl_3$) (δ pp m) (500 MHz) : 8.06 (2H, *dd*, *J* = 8.5, 1.5 Hz), 7.70 (1H, *d*, *J* = 16 Hz), 7.58 (1H, *m*), 7.52 (2H, *d*, *J* = 6.5, 2.5 Hz), 7.47 (2H, *m*), 7.40 (2H, *m*), 7.39 (1H, *m*), 6.41 (1H, *d*, *J* = 16 Hz), 6.09 (1H, *ddd*, *J* = 10, 4, 2.5 Hz), 5.89 (1H, *dt*, *J* = 10, 2 Hz), 5.78 (1H, *d*, *J* = 8.5 Hz), 5.70 (1H, *ddd*, *J* = 8.5, 2.5, 1.5 Hz), 4.65 and 4.41 (2H, *d* (AB system), *J* = 12 Hz), 3.61 (1H, *dd*, *J* = 4, 2 Hz), 2.12 (3H, *s*)

^{13}C NMR ($CDCl_3$) (δ pp m) (125 MHz) : 170.24, 166.06, 146.05, 134.08, 133.63, 133.42, 130.60, 129.82, 128.91, 128.51, 128.25, 124.11, 117.06, 71.42, 71.18, 62.23, 58.35, 54.50, 20.79

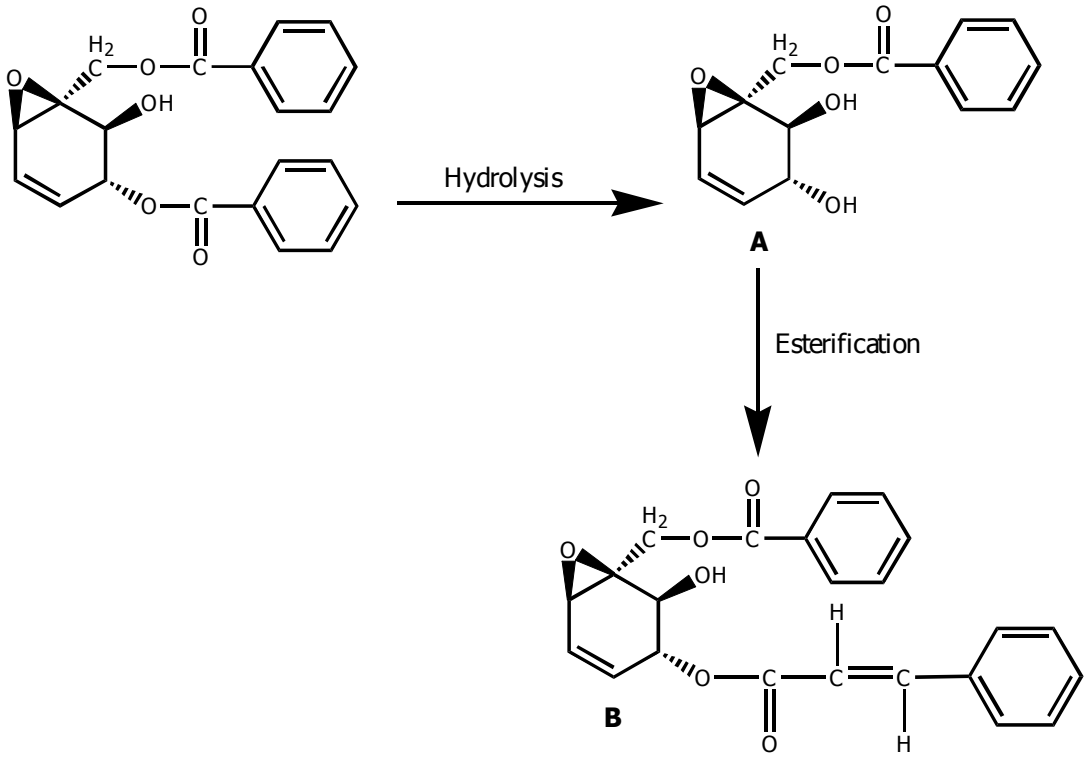
DEPT-135° ($CDCl_3$)

CH : 146.05, 133.63, 133.41, 130.95, 129.81, 128.91, 128.52, 128.25, 124.11, 117.05, 71.42, 71.18, 54.49

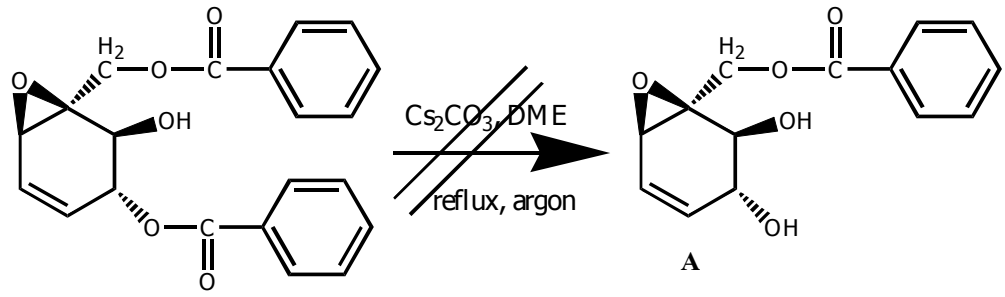
CH_2 : 62.23

CH_3 : 20.78

2.6 The synthetic confirmed of compound SAH2 was approached in two steps using pipoxide as starting material : partial selective hydrolysis of an ester at C-3 and followed by acylation of the corresponding hydroxyl group. The hydrolyzed product (A) should be more polar than a starting material and visualized as a brown spot with $K_2Cr_2O_7$-H_2SO_4 spray reagent. ^{1}H NMR spectrum of oxymethine proton at position 3 should move upfield from δ5. 67 to δ3. 5-4.5 ppm.



2.6.1



A

Pipoxide (0.0508 g, 0.1387 mmole) was dissolved in dimethoxyethane (5 ml) then Cs_2CO_3 (0.0904 g) was added into the pipoxide solution under argon atmosphere. The reaction mixture was refluxed at 75-80^{o}C for 7 days. The dimethoxyethane was removed by evaporation under reduced pressure then ice-water and 3N HCl (1 drop) was added into the mixture and extracted 3 times with ethyl acetate (3x20 ml). The organic extract was washed with 2N $KHCO_3$ (20 ml) and with saturated sodium chloride (20 ml), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by PLC on silica gel with 40 % EtOAc : hexane as the developing solvent to afford 4 bands.

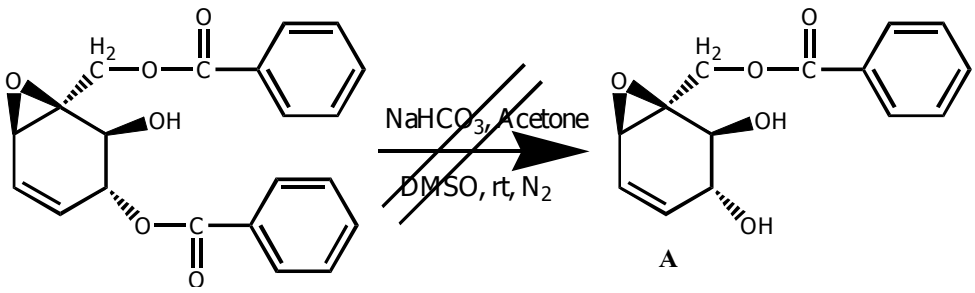
Band 1 as a white solid (7.1 mg), chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot of unreacted starting material.

Band 2 as a colorless viscous liquid (18.1 mg), chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot. ^{1}H NMR spectrum indicated a mixture of three non-target compounds. No further investigation was performed.

Band 3 as a colorless viscous liquid (9.7 mg), chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot. ^{1}H NMR spectrum indicated a mixture of two non-target compounds. It was therefore not further investigated.

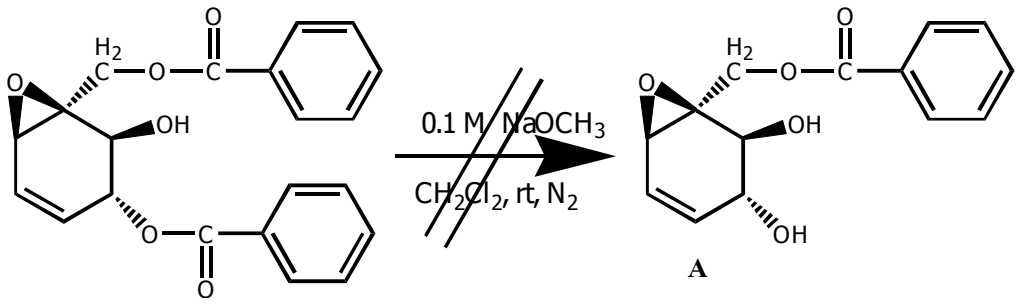
Band 4 as a colorless viscous liquid (15.2 mg), chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot. ^{1}H NMR spectrum indicated a mixture of two non-target compounds, no further purification was performed.

2.6.2



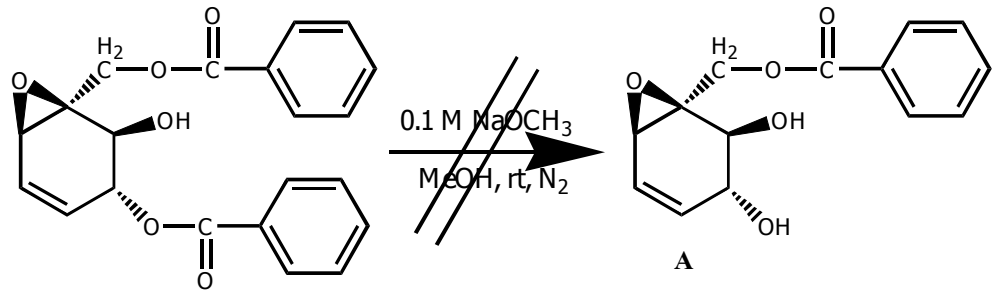
Pipoxide (0.1004 g, 0.2743 mmole) was dissolved in acetone (1 ml), and dimethylsulfoxide (1 ml), then sodium hydrogen carbonate (0.0231 g) was added into the solution. The mixture was left stirring at room temperature for 6 hrs under nitrogen atmosphere. Then the mixture was filtered and the filtrate was extracted 4 times with chloroform (4x15 ml), followed by washing with water (2x5 ml) and drying over anhydrous Na_2SO_4. After filtration, the chloroform was removed by evaporation under reduced pressure. The crude mixture was purified by PLC on silica gel with 15% EtOAc : hexane as a developing solvent to give 4 bands. ^{1}H NMR spectrum indicated these compounds were not the hydrolyzed products. Therefore, no further investigation was performed.

2.6.3



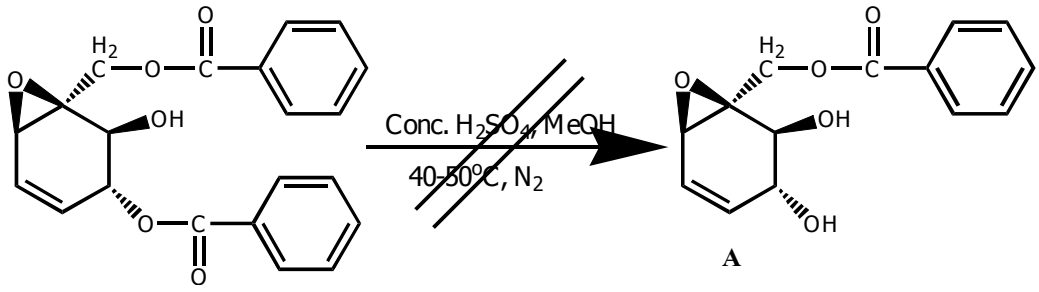
Pipoxide (0.1002 g, 0.2737 mmole) was dissolved in methylene chloride (1 ml), then 0.1 M $NaOCH_3$ (2.8 ml) was added. The solution was stirred at room temperature under nitrogen atmosphere for 20 minutes and then added 0.1 M Na_2HPO_4. H_2O (3 ml) for stopping the reaction. The mixture was evaporated under reduced pressure and extracted with methylene chloride (30 ml), followed by washing with water (2x5 ml) and drying over anhydrous Na_2SO_4. After filtration, the methylene chloride was removed by evaporation under reduced pressure. This mixture gave one UV-active spot on normal phase TLC with 30 % EtOAc : hexane which was less polar than starting material. Thus, it was not further investigated.

2.6.4



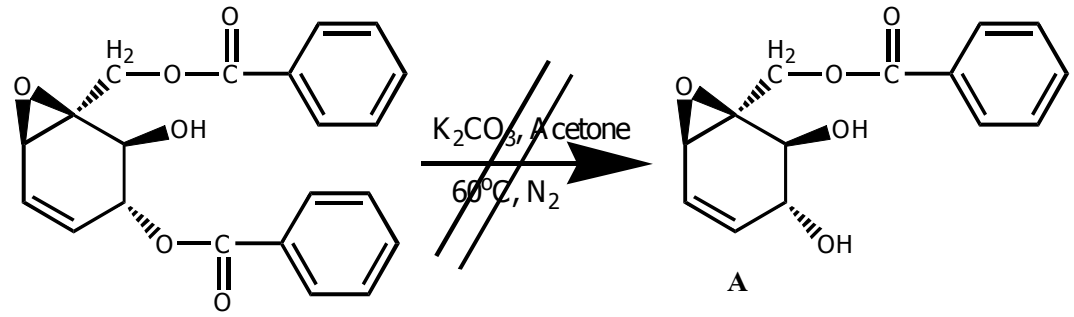
Pipoxide (0.0503 g, 0.137 mmole) was dissolved in methanol (3 ml), then 0.1 M sodium methoxide (1.4 ml) was added under nitrogen atmosphere at room temperature and the solution was stirred for 20 minutes. The reaction was stopped by adding $Na_2HPO_4.H_2O$ (1.4 ml) into the mixture. After filtration, and the methanol was removed by evaporation under reduced pressure, the mixture was dissolved in methylene chloride (15 ml) and water (5 ml), then the aqueous layer was extracted 4 times with methylene chloride (4x15 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered, and methylene chloride was removed by evaporation under reduced pressure. Chromatogram characteristic on normal phase TLC with 20 % EtOAc : hexane showed one UV-active spot and it was less polar than starting material. No further investigation was performed.

2.6.5



Pipoxide (0.050 g, 0.137 mmole) was dissolved in methanol (10 ml), then the mixture was refluxed at 40-50°C under nitrogen atmosphere for 5 minutes. After that, conc. H_2SO_4 (4 drops) was added and the solution was left stirring for 15 minutes. The saturated $NaHCO_3$ (1 ml) was added into the mixture for stopping the reaction. The methanol was removed by evaporation under reduced pressure. The residue was extracted several times with methylene chloride. The methylene chloride layer was washed with water, dried over anhydrous Na_2SO_4, and filtered. Methylene chloride was removed by evaporation under reduced pressure to yield a crude mixture (51.3 mg) which was purified by PLC on silica gel with 40 % EtOAc : hexane to give 4 bands. ^{1}H NMR spectrum indicated a mixture of non-target compounds.

2.6.6



Pipoxide (0.100 g, 0.273 mmole) was dissolved in dry acetone (5 ml), then potassium carbonate (75.5 mg) was added into pipoxide solution. The mixture was stirred under nitrogen atmosphere at 60°C for 5 days. After filtration, the yellow solution was evaporated to remove acetone, then the yellow viscous liquid was purified by PLC on silica gel with 40 % EtOAc : hexane as a developing solvent to afford 5 bands.

Band 1 (3.1 mg), as a colorless viscous liquid, chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot and it was less polar than starting material. No further investigation was performed.

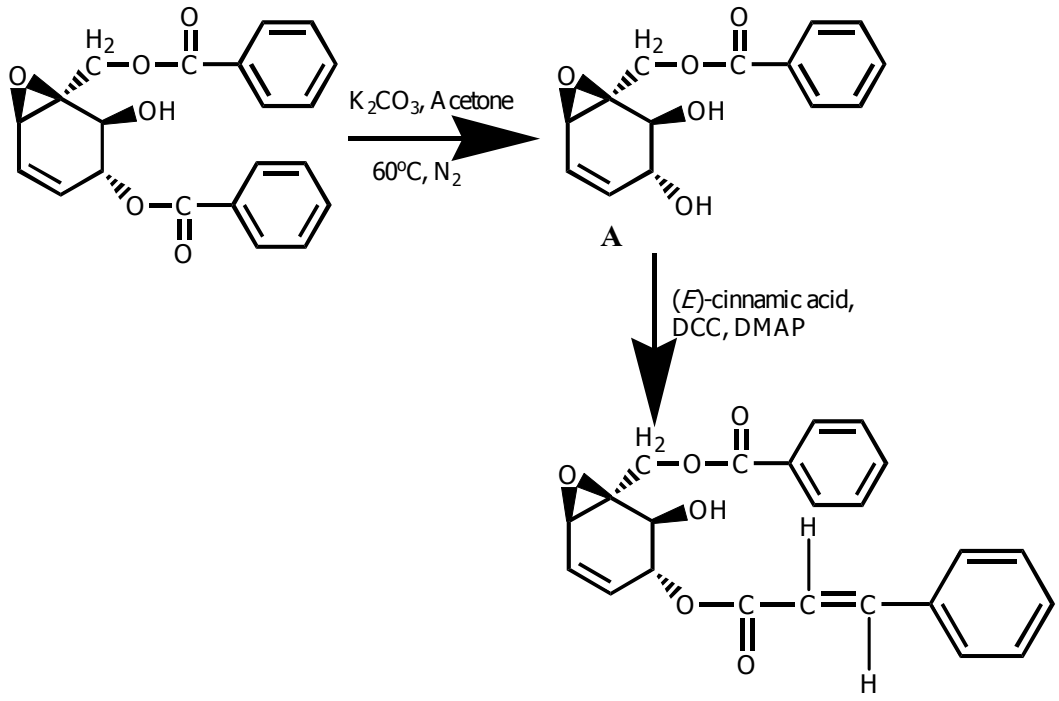
Band 2 (10.9 mg), as a white solid, chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot, it was unreacted starting material. No further investigation was performed.

Band 3 (4.5 mg), as a yellow viscous liquid, chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed two UV-active spots and it was not visualized as a brown spot with $K_2Cr_2O_7$-H_2SO_4 spray reagent. Thus, no further investigation was performed.

Band 4 (2.4 mg), as a colorless viscous liquid, chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed two UV-active spot. It was visualized as a brown spot with $K_2Cr_2O_7$-H_2SO_4 spray reagent. It was obtained in low quantity, it was not further investigated.

Band 5 (24.1 mg), as a yellow viscous liquid, chromatogram characteristic on normal phase TLC with 40 % EtOAc : hexane showed one UV-active spot and showed the characteristic of 2°-alcohol by visualizing as a brown spot with $K_2Cr_2O_7$ reagent. The ^1H NMR spectrum of this band indicated a mixture of two compounds of non-target compounds.

2.6.7



Pipoxide (0.050 g, 0.137 mmole) was dissolved in dry acetone (5 ml), then potassium carbonate (38.0 mg) was added. The mixture was stirred under nitrogen atmosphere at 60°C for 5 days. After filtration, the yellow solution was evaporated to remove acetone which resulted in an orange viscous liquid (64.4 mg). The solution of (*E*)-cinnamic acid (0.0317 g) in methylene chloride (4 ml) was stirred under nitrogen atmosphere at room temperature. The resulting solution was cooled to 0°C while continued with stirring, followed by addition of DCC (0.0912 g) and DMAP

(0.0029 g), successively. Then the solution of orange viscous liquid in 2 ml methylene chloride was added into (*E*)-cinnamic acid-DCC-DMAP solution and the resulting mixture was kept stirring at 0^{o}C for 3 hrs, followed by stirring at room temperature for another 2 days. The mixture obtained was purified by PLC on silica gel with 30 % ether : hexane as a developing solvent, after removing of methylene chloride by evaporation under reduced pressure to give 2 bands.

Band 1 as a colorless viscous liquid, chromatogram characteristic on normal phase TLC with 40 % ether : hexane showed one UV-active spot. ^{1}H NMR spectrum indicated a rearomatized-compound, so it was not further investigated.

Band 2 as a colorless viscous liquid, chromatogram characteristic on normal phase TLC with 40 % ether : hexane showed one UV-active spot. ^{1}H NMR spectrum indicated a mixture of many non-target compounds. No further investigation was performed.

Effort to transform pipoxide to compound **A** via partial selective hydrolysis under various basic or acidic conditions was unsuccessful. Compound **A** may be less stable, due to a high tendency to lose water to form undesirable aromatic compounds.