CHAPTER 2

Experimental

2.1 Instruments and Chemicals

Melting points were measured with a Sanyo Gallenkamp melting point apparatus and were uncorrected. Optical rotation $[\alpha]_D$ values were measured with a Bellingham & Stanley APD440 polarimeter. The UV spectra were recorded with a Perkin-Elmer UV-vis spectrophotometer, whereas the IR spectra were obtained using Bruker Tensor FT-IR spectrophotometer. NMR spectroscopic data were obtained on a 400 MHz Bruker FT-NMR Ultra Shield and a 500 MHz Varian Unity INOVA spectrometers. Chemical shifts are recorded in parts per million (δ) in $CDCl_3$ (δ_H 7.26 and δ_C 77.0) and/or acetone-d_6 (δ_H 2.05 and δ_C (CO) 206.2 and (CH_3) 30.6), with TMS as an internal reference. Mass spectrometric data were obtained on a Micro TOF, Bruker Daltonics QTOF (ESI) mass spectrometers. Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was performed on Sephadex LH-20, and silica gel (Merck) type 100 (63-200 μm) and type 60 (5-40 μm for Quick column chromatography; QCC and 40-63 μm for Flash column chromatography; FCC). All solvents for extraction and chromatography were routinely distilled prior to use.

2.2 Plant Materials

2.2.1 The Leaves and Twigs of *D. yunnanense*

The leaves and twigs of *D. yunnanense* (Annonaceae) were collected from Doi Tung, Chiang Rai Province, Thailand in March 2013. The plant was identified by J.F. Maxwell from the CMUB Herbarium, Chiang Mai University, where a voucher specimen has been deposited (specimen no. Maxwell 07-351).

2.2.2 The Leaves and Twigs of *M. cuneata* Craib

The leaves and twigs of *M. cuneata* were collected from Doi Tung, Chiang Rai Province, Thailand in September 2013. The plant was identified by J.F. Maxwell from the CMUB Herbarium, Chiang Mai University, where a voucher specimen has been deposited (specimen no. Martin van de Bult 1328).

2.3 Extraction and Isolation

2.3.1 Extraction and Isolation of Compounds from the Twigs of *D. yunnanense*

The air-dried twigs of *D. yunnanense* (7.1 kg) were extracted with acetone (15 L × 3) over a period of 3 days at room temperature. Removal of the solvent under reduced pressure provided the acetone extract (120.80 g), a dark brown gum. This extract was separated by QCC over silica gel eluting with a gradient of hexanes:EtOAc:MeOH (19:1:0 to 0:4:1) to give five fractions (A–E). Fraction B (2.04 g) was further separated by Sephadex LH-20 with MeOH to afford four sub-fractions (B1-B4). Sub-fraction B2 (69.9 mg) was fractionated by CC over silica gel with hexanes:acetone (7:3) to afford three sub-fractions (B2A-B2C). Compound **DY12** (10.9 mg) was contained from the second sub-fraction (32.5 mg) as colorless crystals after washing with MeOH. Fraction D (4.04 g) was separated by Sephadex LH-20 with MeOH to afford eight sub-fractions (D1-D8). Sub-fraction D2 (285.8 mg) was washed with MeOH to give compound **DY2** (17.7 mg) as a light orange solid. Sub-fraction D4 (102.0 mg) was further purified by CC over silica gel with a gradient of CH_2Cl_2:MeOH (99:1 to 4:1) giving five sub-fractions (D4A-D4E). Compound **DY4** (1.6 mg) was obtained as a yellow solid and compound **DY3** (34.6 mg) as a white solid from sub-fractions D4B and D4D, respectively. Purification of sub-fraction D5 (1.14 g) by Sephadex LH-20 with MeOH gave three sub-fractions (D5A-D5C). Compounds **DY11** (2.1 mg), as yellow viscous oil and **DY1** (6.0 mg), as a blue solid, were obtained from sub-fraction D5B (352.3 mg) after purification by CC over silica gel using hexanes:acetone (3:2) as a mobile phase. Sub-fraction D6 (881.5 mg) was separated by silica gel CC with hexanes:acetone (7:3) to afford three sub-fractions (D6A-D6C). Sub-fraction D6B (73.0 mg) was further isolated by CC over silica gel with CH_2Cl_2:MeOH

(49:1) to provide three sub-fractions (D6B1-D6B3). The second sub-fraction (16.6 mg) was fractionated by CC over silica gel with hexanes:EtOAc (3:2) to provide compounds **DY6** (3.5 mg) as yellow needles, and **DY5** (2.5 mg) as yellow needles. Sub-fraction D7 (156.9 mg) was separated by CC over silica gel with a gradient of CH_2Cl_2:MeOH (49:1 to 19:1) to afford five sub-fractions (D7A-D7E). Purification of sub-fraction D7B (23.1 mg) by CC over silica gel with hexanes:EtOAc (3:2) gave compounds **DY10** (4.0 mg) as brownish-yellow needles and **DY8** (6.3 mg) as yellow needles. Compound **DY7** (9.0 mg) as light green needles, was isolated from sub-fraction D7D (19.1 mg) after purification by CC over silica gel with CH_2Cl_2:MeOH (49:1). Sub-fraction D7E (37.0 mg) was further purified by silica gel CC using hexanes:acetone (7:3) to give compound **DY9** (8.1 mg) as yellow needles (see **Figure 2.1**).

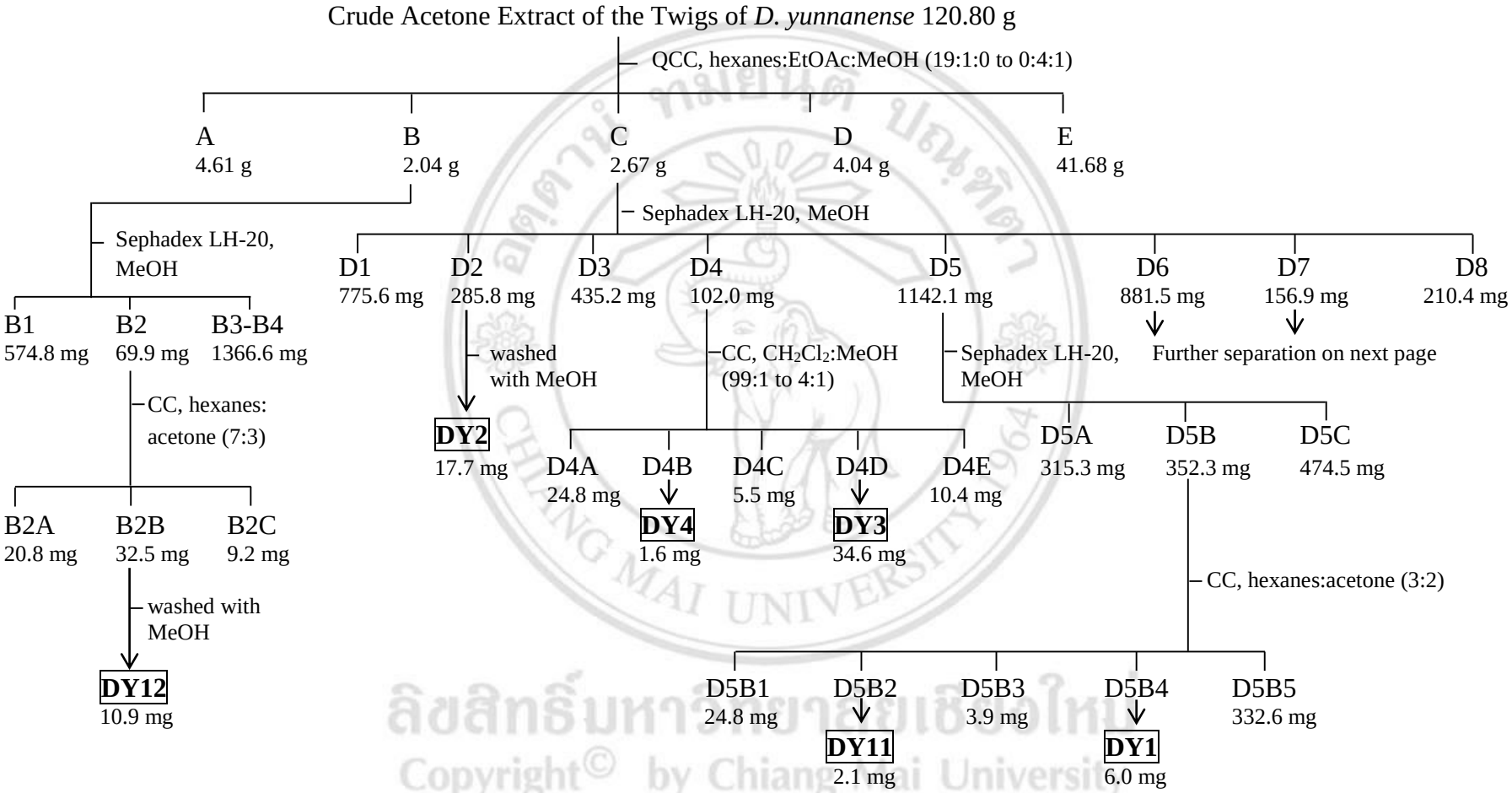

Figure 2.1 Isolation of Compounds from the Twigs of *D. yunnanense*

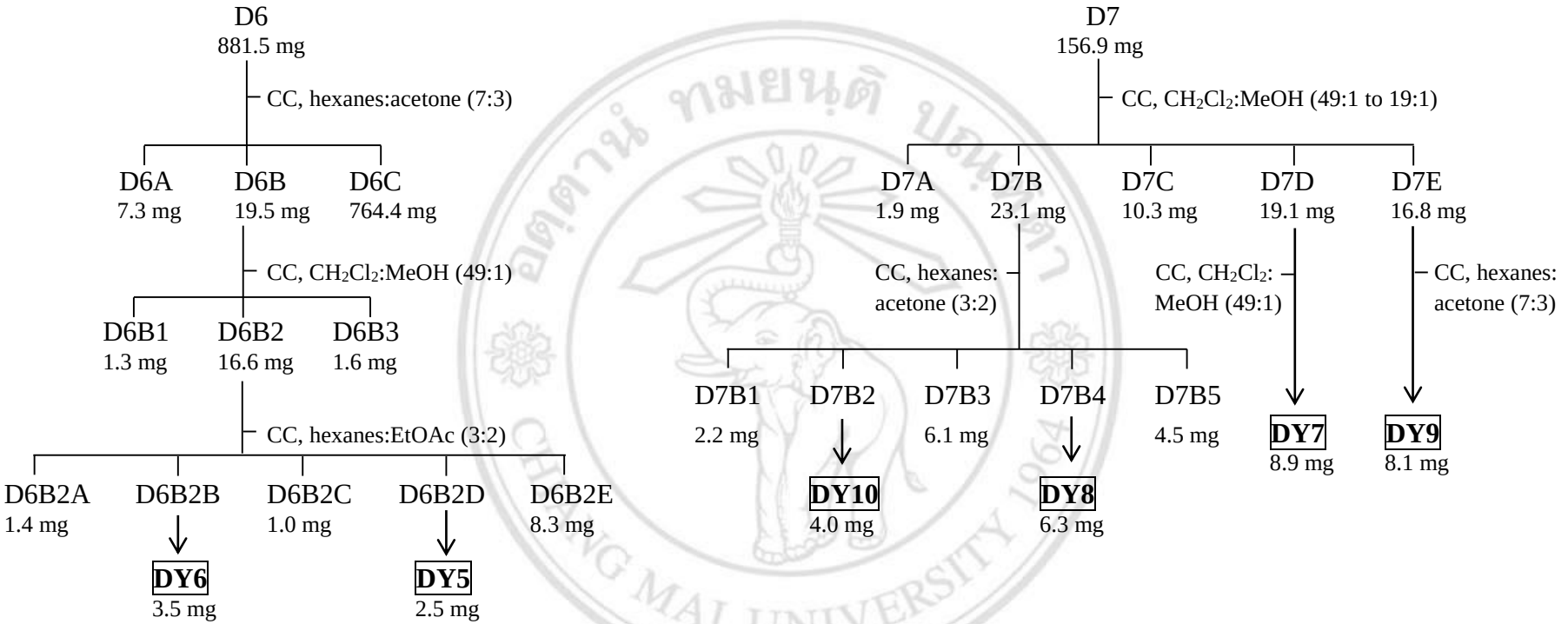

Figure 2.1 (Continued)

Compound **DY1** (Obtusipetadione): blue solid; mp 255–256 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 224 (4.01), 276 (3.83), 322 (3.84) and 590 (3.02); IR (neat) ν_{max} 1671, 1626, 1525, 1458, 1312, 1234, 1122 and 1056 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.1**; HRESIMS *m/z* 368.1124 $[M+H]^+$ (calcd for $C_{20}H_{18}NO_6$, 368.1134).

Compound **DY2** ((–)-Sinactine): light orange solid; mp 178–179 °C; $[\alpha]_D^{28}$ –205 (*c* 0.276, $CHCl_3$) (lit. $[\alpha]_D$ = –312 (*c* 1.0, $CHCl_3$), Goto and Kitasato, 1930); UV/VIS λ_{max} (MeOH) nm (log ε) 222 (4.0) and 286 (3.7); IR (neat) ν_{max} 1513, 1459, 1257, 1046 and 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.2**.

Compound **DY3** ((–)-Epicatechin): white solid; mp 248–249 °C; $[\alpha]_D^{28}$ –109 (*c* 0.080, MeOH) (lit. $[\alpha]_D^{26}$ –54 (*c* 0.100, acetone), Kashiwada *et al*., 1990); UV/VIS λ_{max} (MeOH) nm (log ε) 222 (3.9) and 281 (3.3); IR (neat) ν_{max} 3285, 1627, 1521, 1469, 1361, 1278, 1148, 1110, 1051 and 1015 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see **Table 3.11**.

Compound **DY4** (3'-*O*-Methyl-(–)-epicatechin): yellow solid; mp 198–200 °C; $[\alpha]_D^{28}$ –84 (*c* 0.018, MeOH) (lit. $[\alpha]_D$ –56 (*c* 0.800, MeOH), Mohamed, 2013); UV/VIS λ_{max} (MeOH) nm (log ε) 224 (4.2) and 279 (3.7); IR (neat) ν_{max} 3361, 1613, 1517, 1463, 1367, 1272, 1145, 1092 and 1036 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) data, see **Table 3.12**.

Compound **DY5** (Goniopedaline): yellow needles; mp 224–225 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 248 (3.3), 291 (3.1) and 390 (2.7); IR (neat) ν_{max} 3482, 1696, 1539, 1463, 1409 and 1070 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.4**.

Compound **DY6** (Aristolactam BII): yellow needles; mp > 256 °C (decompose); UV/VIS λ_{max} (MeOH) nm (log ε) 233 (4.0), 264 (3.9), 277 (3.9), 287 (3.9), 318 (3.4) and 388 (3.3); IR (neat) ν_{max} 3170, 1757, 1455, 1377, 1187, 1133 and 1096 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) data, see **Table 3.8**.

Compound **DY7** (Piperolactam A): light green needles; mp 280–282 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 235 (3.8), 264 (3.8), 277 (3.8), 287 (3.8), 366 (3.0) and 392 (3.1); IR (neat) ν_{max} 3441, 1638, 1420, 1356 and 1292 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see **Table 3.6**.

Compound **DY8** (Aristolactam AII): yellow needles; mp 270–272 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 225 (3.2), 277 (3.4) and 342 (2.8); IR (neat) ν_{max} 3472, 3185, 1682, 1660, 1450, 1454, 1379, 1279, 1125 and 1043 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) data, see **Table 3.5**.

Compound **DY9** (10-Amino-3,6-dihydroxy-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam): yellow needles; mp 195–196 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 240 (3.7) and 402 (3.0); IR (neat) ν_{max} 3357, 1674, 1536, 1417, 1231, 1154 and 1065 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) data, see **Table 3.10**.

Compound **DY10** (3,5-Dihydroxy-2,4-dimethoxyaristolactam): brownish-yellow needles; mp > 210 °C (decompose); UV/VIS λ_{max} (MeOH) nm (log ε) 257 (3.4) and 410 (2.8); IR (neat) ν_{max} 3223, 1689, 1540, 1450, 1420, 1269, 1233, 1156, 1099 and 1060 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see **Table 3.9**.

Compound **DY11** (Piperolactam C): yellow viscous oil; UV/VIS λ_{max} (MeOH) nm (log ε) 234 (4.3), 260 (4.3), 291 (4.2), 344 (3.7), 382 (3.6) and 402 (2.9); IR (neat) ν_{max} 3438, 1689, 1652, 1458, 1385, 1119 and 1085 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) data, see **Table 3.7**.

Compound **DY12** ((+)-Crotepoxide): colorless crystals; mp 151–152 °C; $[\alpha]_D^{28}$ +71 (*c* 0.128, $CHCl_3$) (lit. $[\alpha]_D^{25}$ + 70 (*c* 1.200, $CHCl_3$), Nazir *et al*., 2009); UV/VIS λ_{max} (MeOH) nm (log ε) 231 (3.9) and 275 (2.8); IR (neat) ν_{max} 1754, 1728, 1453, 1371, 1273, 1188, 1125 and 1046 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.13**.

2.3.2 Extraction and Isolation of Compounds from the Leaves of *D. yunnanense*

The air dried leaves of *D. yunnanense* (1.6 kg) were extracted with MeOH (15 L × 3) over a period of 3 days at room temperature. The MeOH extract was concentrated under reduced pressure to give MeOH extract (110.35 g) as a dark green gum. The crude extract was partitioned with EtOAc to obtain EtOAc-soluble fraction and then removal the solvent under reduced pressure provided the EtOAc extract (66.48 g) as a dark green gum. This crude extract was chromatographed by QCC over silica gel using hexanes as eluent and increasing the polarity with EtOAc to afford ten fractions (A-J). Fraction B (740.0 mg) was separated by Sephadex LH-20 with MeOH to provide three sub-fractions (B1-B3). The second sub-fraction (206.0 mg) was fractionated by CC over silica gel using a gradient of CH_2Cl_2:EtOAc (99:1 to 19:1) as a mobile phase to give three sub-fractions (B2A-B2C). The second sub-fraction (43.0 mg) was further separated by Sephadex LH-20 with MeOH to obtain two sub-fractions (B2B1 and B2B2). Purification of sub-fraction B2B2 (24.7 mg) by CC with hexanes:acetone (9:1) gave compound **DY14** (2.2 mg) as a colorless oil. Fraction D (2.30 g) was separated by CC with hexanes:EtOAc (7:3) to give three sub-fractions (D1-D3). The second sub-fraction, D2 (150.3 mg) was purified by Sephadex LH-20 using MeOH as eluent to obtain compound **DY15** (5.5 mg) as a yellow viscous oil. Compound **DY13** (3.2 mg) as a yellow gum was obtained from separation of fraction E (363.0 mg) by CC over silica gel using a gradient of hexanes:acetone:MeOH (9:1:0 to 0:9:1) as a mobile phase. Fraction G (1.20 g) was separated by Sephadex LH-20 with MeOH to provide three sub-fractions (G1-G3). Sub-fraction G2 (273.9 mg) was purified by FCC using hexanes:acetone (9:1 to 3:2) as eluent to afford compound **DY2** (3.7 mg) as a white-orange solid. Fraction I (5.80 g) was separated by QCC over silica gel and eluted with a gradient of CH_2Cl_2:EtOAc:MeOH (1:0:0 to 0:4:1) to give ten sub-fractions (I1-I10). Sub-fraction I2 (29.9 mg) was subjected to CC with a gradient of hexanes:acetone (7:3 to 1:1) to yield compound **DY12** (62.3 mg) as colorless needles. Sub-fraction I4 (35.0 mg) was separated by CC with CH_2Cl_2:MeOH (99:1) to afford three sub-fractions (I4A-I4C). The second sub-fraction, I4B (18.5 mg) was purified by Sephadex LH-20 with MeOH to achieve compound **DY16** (10.7 mg) as brownish-yellow gum. Sub-fraction

I5 (55.7 mg) was performed by CC with hexanes:EtOAc (3:2) and further purified by Sephadex LH-20 with MeOH to provide compound **DY5** (1.1 mg) as a yellow solid. Sub-fraction I6 (220.3 mg) was subjected to CC with CH_2Cl_2:acetone (9:1) to give three sub-fractions (I6A-16C). The second sub-fraction, I6B (20.6 mg) was further separated by Sephadex LH-20 with MeOH to yield five sub-fractions (I6B1-I6B5). Compound **DY10** (2.3 mg) as a yellow solid was obtained in the fourth sub-fraction while compound **DY21** (3.0 mg) as a yellow gum was isolated from sub-fraction I6B2 (15.0 mg) by CC with CH_2Cl_2:MeOH (49:1). Sub-fraction I7 (334.1 mg) was separated by CC with CH_2Cl_2:MeOH (49:1) to afford five sub-fractions (I7A-I7E). Sub-fraction I7B (139.1 mg) was fractionated by Sephadex LH-20 with MeOH to give three sub-fractions (I7B1-I7B3). The sub-fraction I7B2 (62.4 mg) was purified by CC using hexanes:acetone (7:3) as a mobile phase to afford compounds **DY17** (39.8 mg) as a colorless viscous oil and **DY18** (2.0 mg) as a colorless viscous oil. Purification of sub-fraction I7D (20.7 mg) by Sephadex LH-20 with MeOH gave compound **DY7** (2.2 mg) as a yellow solid. Sub-fraction I8 (489.6 mg) was performed by Sephadex LH-20 with MeOH to give three sub-fractions (I8A-I8C). The second sub-fraction, I8B (105.9 mg) was fractionated by CC with CH_2Cl_2:MeOH (24:1) to afford three sub-fractions (I8B1-I8B3). The second sub-fraction (58.9 mg) was separated by repeated CC with a gradient of hexanes:acetone (3:2 to 2:3) to give three sub-fractions (I8B2A-I8B2C). The sub-fraction I8B2B was purified by Sephadex LH-20 eluting with MeOH to achieve compound **DY19** (18.8 mg) as a yellow viscous oil. Fractionation of sub-fraction I9 (2.05 g) by QCC with a gradient of CH_2Cl_2:MeOH (49:1 to 9:1) provided three sub-fractions (I9A-I9C). The second sub-fractions, I9B (444.1 mg) was fractionated by Sephadex LH-20 with MeOH to provide three sub-fractions (I9B1-I9B3). The second sub-fraction, I9B2 (37.1 mg) was separated by CC with a gradient of hexanes:acetone (3:2 to 1:4) to obtain three sub-fraction (I9B2A-I9B2C). The second sub-fraction, 16.1 mg was purified by Sephadex LH-20 with CH_2Cl_2:MeOH (1:4) to afford compound **DY20** (6.5 mg) as an orange-yellow gum (see **Figure 2.2**).

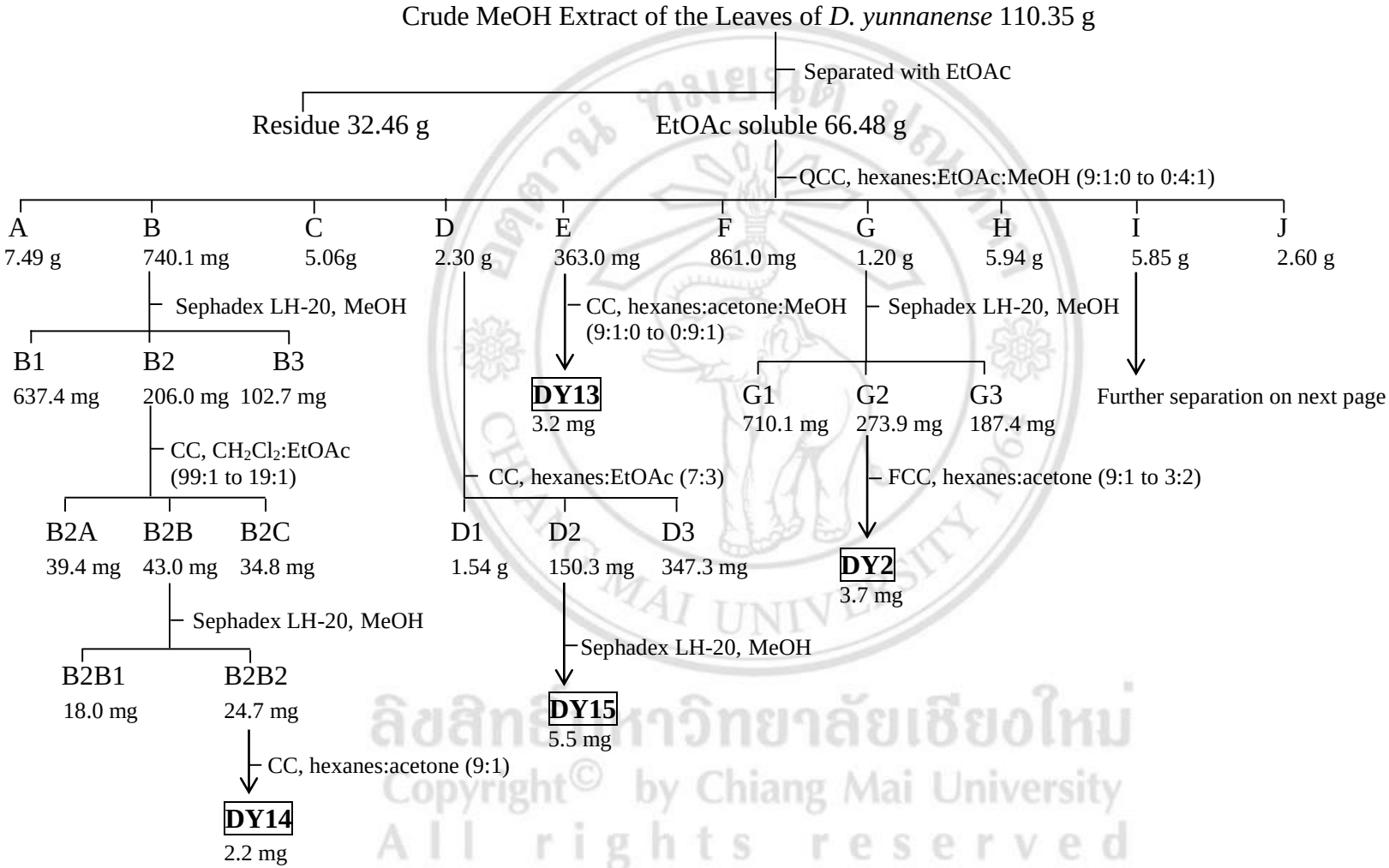

Figure 2.2 Isolation of Compounds from the Leaves of *D. yunnanense*

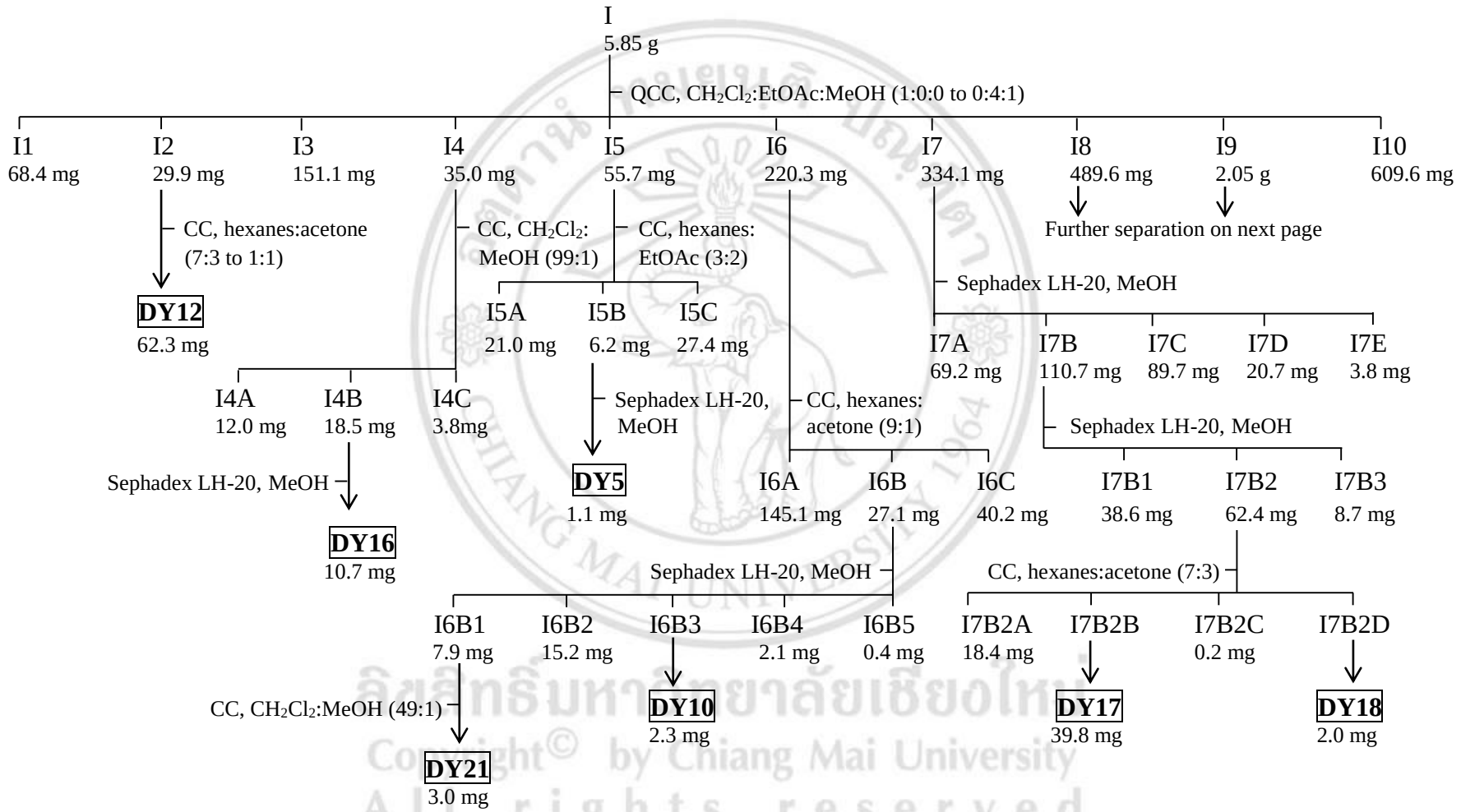

Figure 2.2 (Continued)

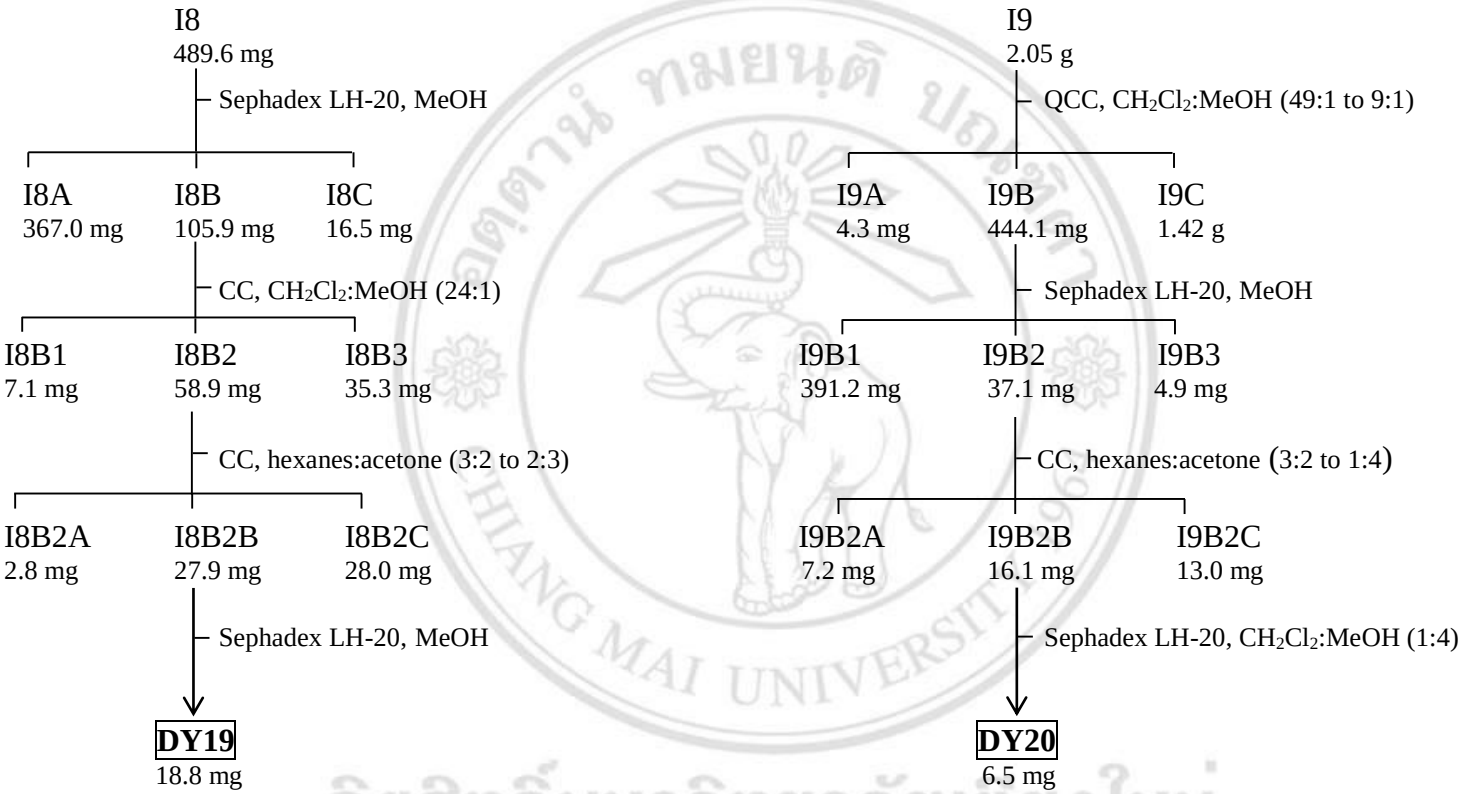

Figure 2.2 (Continued)

Compound **DY13** (Corydaldine): yellow gum; UV/VIS λ_{max} (MeOH) nm (log ε) 222 (4.2), 259 (3.7) and 296 (3.5); IR (neat) ν_{max} 3354, 2924, 2853, 1652, 1604, 1513, 1482, 1460, 1340, 1276, 1222, 1121 and 1051 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.19**.

Compound **DY14** (*trans*-5,6-Diacetoxy-1-(benzoyloxymethyl)-1,3-cyclohexa-diene): colorless oil; $[\alpha]_D^{28}$ –300 (*c* 0.026, $CHCl_3$) (lit. $[\alpha]_D^{28}$ –233 (*c* 0.380, $CHC1_3$), Kodpinid *et al*., 1983); UV/VIS λ_{max} (MeOH) nm (log ε) 231 (3.7) and 258 (3.4); IR (neat) ν_{max} 3386, 2923, 2853, 1725, 1739, 1459, 1373, 1271, 1238, 1111 and 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.14**.

Compound **DY15** ((–)-Desoxypipoxide): yellow viscous oil; $[\alpha]_D^{28}$ –273 (*c* 0.076, $CHCl_3$) (lit. $[\alpha]_D^{25}$ –276 (*c* 0.145, $CHCl_3$), Schulte *et al*., 1982); UV/VIS λ_{max} (MeOH) nm (log ε) 230 (4.4) and 267 (3.8); IR (neat) ν_{max} 3459, 2923, 2852, 1718, 1602, 1452, 1317, 1272, 1178, 1113, 1070 and 1027 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.15**.

Compound **DY16** ((–)-Arcabucoine): brownish-yellow gum; $[\alpha]_D^{28}$ –350 (*c* 0.058, MeOH) (lit. $[\alpha]_D^{20}$ –324 (*c* 0.330, MeOH), Corredor Barinas and Cuca Suárez, 2011); UV/VIS λ_{max} (MeOH) nm (log ε) 224 (4.5) and 283 (4.1); IR (neat) ν_{max} 3444, 2926, 1759, 1666, 1517, 1462, 1391, 1267, 1216, 1186, 1107 and 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.20**.

Compound **DY17** ((+)-Senediol): colorless viscous oil; $[\alpha]_D^{28}$ +142 (*c* 0.218, MeOH) (lit. $[\alpha]_D^{25}$ +198 (*c* 2.330, MeOH), Starks *et al*., 2012); UV/VIS λ_{max} (MeOH) nm (log ε) 230 (4.2) and 274 (3.2); IR (neat) ν_{max} 3447, 2924, 2852, 1723, 1739, 1453, 1373, 1245, 1116, 1044 and 1027 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.16**.

Compound **DY18** (1*S*,2*R*,3*R*,4*S*-2-[(Benzoyloxy)methyl]cyclohex-5-ene-1,2,3,4-tetrol, 4-acetate): colorless viscous oil; $[\alpha]_D^{28}$ +330 (*c* 0.018, MeOH) (lit. $[\alpha]_D^{25}$ +452 (*c* 0.089, MeOH), Starks *et al*., 2012); UV/VIS λ_{max} (MeOH) nm (log ε) 230 (3.6) and 273 (2.7); IR (neat) ν_{max} 3401, 2924, 2853, 1718, 1739, 1455, 1373, 1276, 1116 and 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.17**.

Compound **DY19** (Uvaribonol G): yellow viscous oil; $[\alpha]_D^{28}$ −165 (*c* 0.348, MeOH) (lit. $[\alpha]_D^{24}$ −120 (*c* 0.070, MeOH), Pan *et al*., 1998); UV/VIS λ_{max} (MeOH) nm (log ε) 231 (4.1) and 274 (3.0); IR (neat) ν_{max} 3445, 2924, 2853, 1723, 1743, 1453, 1375, 1319, 1245, 1094 and 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.18**.

Compound **DY20** ((−)-Corydalmine): yellow-orange gum; $[\alpha]_D^{28}$ −384 (*c* 0.106, EtOH) (lit. $[\alpha]_D^{20}$ −156.8 (*c* 1.000, EtOH), Blanchfield, 2003); UV/VIS λ_{max} (MeOH) nm (log ε) 220 (4.2), 281 (3.8), 345 (3.5) and 402 (3.0); IR (neat) ν_{max} 3386, 2925, 2853, 1747, 1609, 1514, 1460, 1368, 1303, 1260, 1231, 1131, 1101 and 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.22**.

Compound **DY21** (*trans*-*N*-Cinnamoyltyramine): yellow gum; UV/VIS λ_{max} (MeOH) nm (log ε) 218 (4.2), 223 (4.2) and 276 (4.2); IR (neat) ν_{max} 3313, 2925, 2854, 1656, 1613, 1514, 1450, 1340 and 1227 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) data, see **Table 3.24**.

2.3.3 Extraction and Isolation of Compounds from the Leaves of *M. cuneata*

The air-dried leaves (0.60 kg) of *M. cuneata* were extracted with acetone (15 L × 3) over a period of 3 days at room temperature. Removal of the solvent under reduced pressure provided the acetone extract (88.41 g) as a dark green gum. The crude extract was separated by QCC over silica gel, eluting with a gradient of hexanes and acetone (1:0 to 2:3) to give seven fractions (A-G). Fraction B (603.1 mg) was separated by Sephadex LH-20 with CH_2Cl_2:MeOH (1:4) to afford two sub-fractions (B1 and B2), the latter sub-fraction (31.6 mg) was further fractionated by Sephadex LH-20 with MeOH to provide three sub-fractions (B2A-B2C). The second sub-fraction (7.0 mg) was purified by CC over silica gel using hexanes:EtOAc (9:1) as a mobile phase achieved compound **MC6** (2.5 mg) as a brownish yellow solid. Fraction D (1.30 g) was washed with acetone and hexanes, respectively to give compound **MC7** (140.0 mg) as a yellow solid. Separation of fraction E (10.73 g) by QCC over silica gel with a gradient of CH_2Cl_2:MeOH (1:0 to 9:1) gave five sub-fractions (E1-E5). Purification of sub-fraction E2 (20.0 mg) over Sephadex LH-20 with MeOH gave compounds **MC5** (2.3 mg) as a yellow viscous oil and **MC2** (9.7 mg), as a yellow solid. Sub-fraction E4

(402.5 mg) was separated by Sephadex LH-20 with MeOH to achieve three sub-fractions (E4A-E4C). Sub-fraction E4B (286.5 mg) was further purified by CC with hexanes:acetone (7:3) to yield compound **MC9** (149.4 mg), as a yellow viscous oil. Fraction F (7.82 g) was separated by silica gel QCC with a gradient of CH_2Cl_2:MeOH (1:0 to 9:1) to afford three sub-fractions (F1-F3). The sub-fraction F2 (408.2 mg) was further separated by Sephadex LH-20 with MeOH to provide four sub-fractions (F2A-F2D). The second sub-fraction, F2B (18.6 mg) was further purified by Sephadex LH-20 with MeOH to afford compound **MC4** (4.4 mg) as a brownish-yellow gum. Sub-fraction F2D (28.0 mg) was further separated by CC over silica gel with hexanes:EtOAc (7:3) to provide compound **MC1** (8.1 mg) as a bright pink solid. The fraction G (5.96 g) was separated by silica gel QCC with a gradient of CH_2Cl_2:MeOH (1:0 to 9:1) to afford three sub-fractions (G1-G3). The sub-fraction G2 (111.4 mg) was purified over Sephadex LH-20 with MeOH to obtain compounds **MC8** (10.1 mg) as a yellow gum and **MC3** as a brownish-red gum (3.7 mg). Sub-fraction G2B (20.3 mg) was further purified by CC over silica gel using hexanes:EtOAc (2:3) as a mobile phase to afford compound **MC10** (2.8 mg) as yellow viscous oil (see **Figure 2.3**).

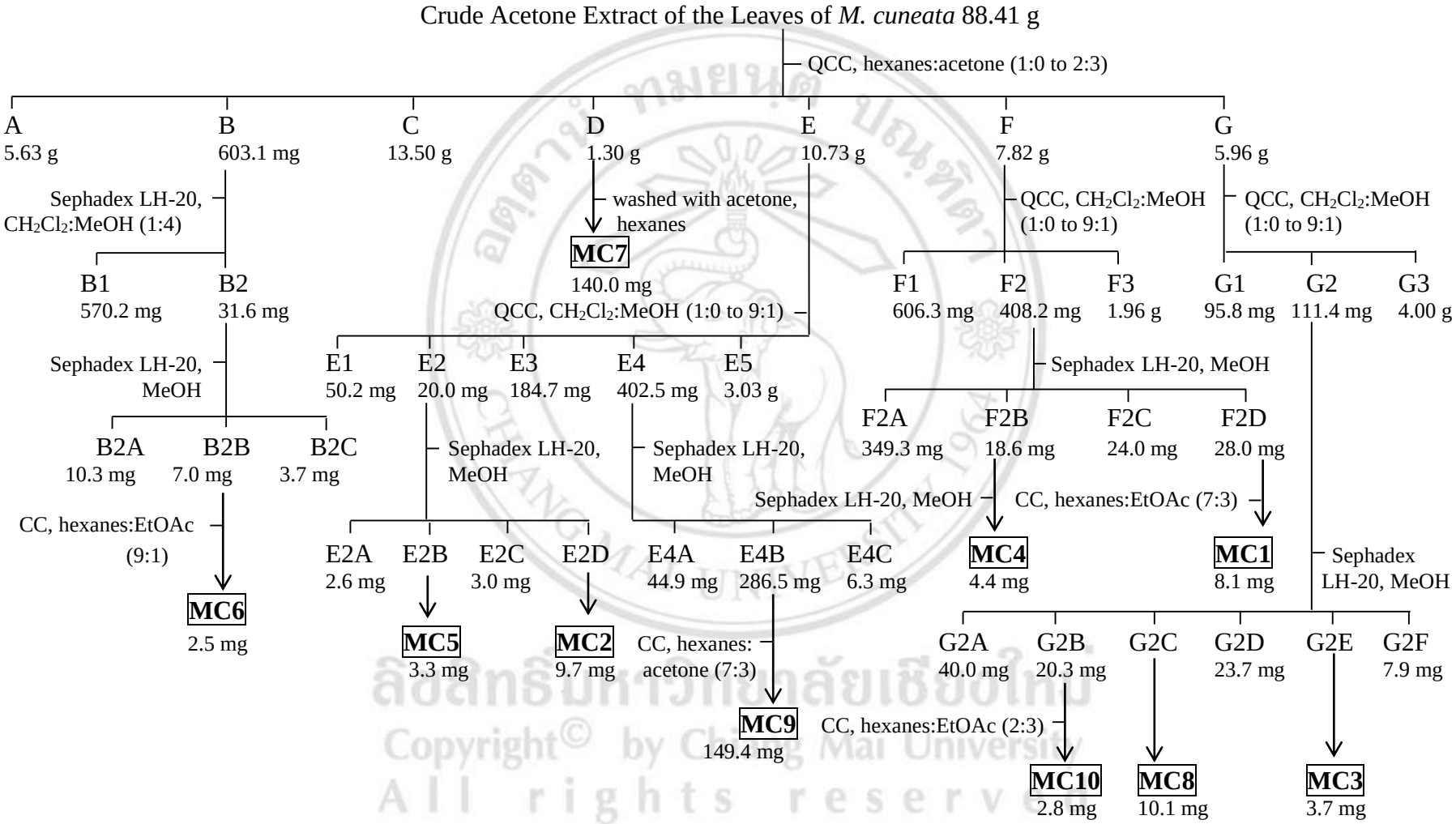

Figure 2.3 Isolation of Compounds from the Leaves of *M. cuneata*

Compound **MC1** (Miliusacunine A): bright pink solid; mp 218–220 °C; UV (MeOH) λ_{max} nm (log ε) 224 (4.5), 338 (4.2), 371 (4.1) and 389 (4.1); IR (neat) ν_{max} 3419, 1649, 1617, 1584, 1504, 1463, 1278 and 1126 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see **Table 3.29**; HRESIMS *m/z* 356.1136 $[M+H]^+$ (calcd for $C_{19}H_{18}NO_6$, 356.1134).

Compound **MC2** (Miliusacunine B): yellow solid; mp 217–218 °C; UV (MeOH) λ_{max} nm (log ε) 224 (4.6), 341 (4.2), 371 (4.2) and 389 (4.1); IR (neat) ν_{max} 3388, 1648, 1586, 1494, 1461, 1280 and 1032 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see **Table 3.29**; HRESIMS *m/z* 392.1112 $[M+Na]^+$ (calcd for $C_{20}H_{19}NO_6Na$, 392.1110).

Compound **MC3** (Miliusacunine C): brownish-red gum; UV (MeOH) λ_{max} nm (log ε) 225 (4.3), 340 (4.0), 372 (3.9) and 389 (3.9); IR (neat) ν_{max} 3421, 1648, 1611, 1583, 1502, 1463, 1278, 1126 and 1093 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.29**; HRESIMS *m/z* 378.0958 $[M+Na]^+$ (calcd for $C_{19}H_{17}NO_6Na$, 378.0954).

Compound **MC4** (Miliusacunine D): brownish-yellow gum; $[\alpha]_D^{28}$ –175 (*c* 0.074, MeOH); UV (MeOH) λ_{max} nm (log ε) 218 (4.5), 262 (4.0) and 341 (3.8); IR (neat) ν_{max} 3421, 1636, 1615, 1584, 1459, 1267, 1120 and 1091 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.30**; HRESIMS *m/z* 380.1104 $[M+Na]^+$ (calcd for $C_{19}H_{19}NO_6Na$, 380.1110).

Compound **MC5** (Miliusacunine E): yellow viscous oil; $[\alpha]_D^{28}$ –93 (*c* 0.024, MeOH); UV (MeOH) λ_{max} nm (log ε) 218 (4.4), 265 (3.8) and 325 (3.5); IR (neat) ν_{max} 3421, 1635, 1590, 1459, 1271, 1121, 1097 and 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.30**; HRESIMS *m/z* 372.1454 $[M+H]^+$ (calcd for $C_{20}H_{22}NO_6$, 372.1447).

Compound **MC6** (5-Hydroxy-3,7-dimethoxy-3′,4′-methylenedioxy flavone): brownish-yellow solid; mp 181–183 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 219 (4.4),

254 (4.4) and 351 (4.3); IR (neat) ν_{max} 3448, 1633, 1496, 1443, 1367, 1334, 1310, 1215, 1106 and 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.26**.

Compound **MC7** (Pachypodol): yellow solid; mp 173–174 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 219 (4.3), 254 (4.3) and 355 (4.3); IR (neat) ν_{max} 3247, 2924, 1661, 1592, 1497, 1462, 1377, 1342, 1309, 1242, 1213, 1166, 1126, 1035 and 1006 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.25**.

Compound **MC8** (4′-Hydroxy-3,5,7,3′-tetramethoxyflavone): yellow gum; UV/VIS λ_{max} (MeOH) nm (log ε) 220 (4.4), 248 (4.3) and 343 (4.2); IR (neat) ν_{max} 3432, 2927, 2854, 1610, 1516, 1460, 1345, 1283, 1217, 1164, 1114 and 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.27**.

Compound **MC9** ((+)-Miliusol): yellow viscous oil; $[\alpha]_D^{25}$ +72 (*c* 0.049, $CHCl_3$) (lit. $[\alpha]_D^{23}$ +38 (*c* 0.500, $CHCl_3$), Huong, 2004); UV/VIS λ_{max} (MeOH) nm (log ε) 220 (3.8); IR (neat) ν_{max} 3416, 2922, 1769, 1668, 1444, 1380, 1326, 1274, 1215, 1091 and 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.31**.

Compound **MC10** ((+)-Syringaresinol): yellow viscous oil; $[\alpha]_D^{28}$ +35 (*c* 0.034, $CHCl_3$) (lit. $[\alpha]_D^{30}$ +5.0 (*c* 0.460, MeOH), Sumioka *et al*., 2011); UV/VIS λ_{max} (MeOH) nm (log ε) 217 (4.5), 240 (4.2) and 272 (3.6); IR (neat) ν_{max} 3422, 2938, 2849, 1614, 1518, 1460, 1426, 1324, 1216 and 1114 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see **Table 3.32**.

2.3.4 Extraction and Isolation of Compounds from the Twigs of *M. cuneata*

Air-dried twigs (2.2 kg) of *M. cuneata* were macerated with acetone (15 L × 3) over a period of 3 days at room temperature and evaporated under reduced pressure to afford the acetone extract (35.01 g) as a dark brown gum. The crude extract was separated by QCC over silica gel and eluted with a gradient of hexanes:CH_2Cl_2:MeOH (1:0:0 to 0:4:1) to give ten fractions (A-J). Fraction B (860.0 mg) was separeted by CC over silica gel eluting with CH_2Cl_2 to give three sub-fractions (B1-B3). Sub-fraction B2 (46.0 mg) was purified by Sephadex LH-20 with MeOH to provide compound **MC6** (1.1 mg) as a brownish-yellow solid. Fraction D (2.79 g) was fractionated by QCC

using hexanes:EtOAc (7:3 to 1:1) as eluent to give three sub-fractions (D1-D3). Sub-fraction D2 (149.2 mg) was further purified by CC with CH_2Cl_2 to provide compound **MC7** (40.0 mg) as a yellow solid. Fraction F (556.3 mg) was purified by Sephadex LH-20 with MeOH to afford compound **MC11** as a brownish-red gum (1.2 mg). Fraction H (1.25 g) was fractionated by Sephadex LH-20 with MeOH to obtain three sub-fractions (H1-H3). The second sub-fraction, H2 (425.2 mg) was purified by CC with CH_2Cl_2:EtOAc (1:9) to give compound **MC12** as an orange gum (17.7 mg). Fraction I (1.40 g) was separated by Sephadex LH-20 with MeOH to give three sub-fractions (I1-I3). Sub-fraction I2 (194.6 mg) was further fractionated by CC with hexanes:acetone (7:3) to yield three sub-fractions (I2A-I2C). Separation of sub-fraction I2B (24.0 mg) by Sephadex LH-20 with MeOH provided compound **MC13** as an orange-yellow gum (2.7 mg). Purification of sub-fraction I2C (19.0 mg) by Sephadex LH-20 with MeOH obtained compound **MC14** (9.7 mg) as a white solid (see **Figure 2.4**).

Compound **MC11** (Chrysoplenetin): brownish-red gum; UV/VIS λ_{max} (MeOH) nm (log ε) 220 (4.1), 257 (4.0) and 350 (3.9); IR (neat) ν_{max} 3424, 2927, 2855, 1712, 1654, 1597, 1515, 1465, 1355, 1274, 1218 and 1004 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see **Table 3.28**.

Compound **MC12** (*trans*-*N*-Feruloyltyramine): orange gum; UV/VIS λ_{max} (MeOH) nm (log ε) 222 (4.3), 287 (4.1) and 317 (4.1); IR (neat) ν_{max} 3259, 2925, 2854, 1652, 1591, 1512, 1430, 1363, 1263-1123 and 1029 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) data, see **Table 3.23**.

Compound **MC13** (*trans*-*N*-Caffeoyltyramine): orange-yellow gum; UV/VIS λ_{max} (MeOH) nm (log ε) 222 (4.4), 287 (4.1) and 319 (4.1); IR (neat) ν_{max} 3416, 2926, 1651, 1603, 1517, 1447, 1367, 1280 and 1116 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data, see **Table 3.20**.

Compound **MC14** (*trans*-*N*-Coumaroyltyramine): white solid; mp 247–249 °C; UV/VIS λ_{max} (MeOH) nm (log ε) 224 (4.3) and 291 (4.3); IR (neat) ν_{max} 3428, 2920, 2851, 1657, 1606, 1525, 1451, 1375, 1244 and 1110 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) data, see **Table 3.22**.

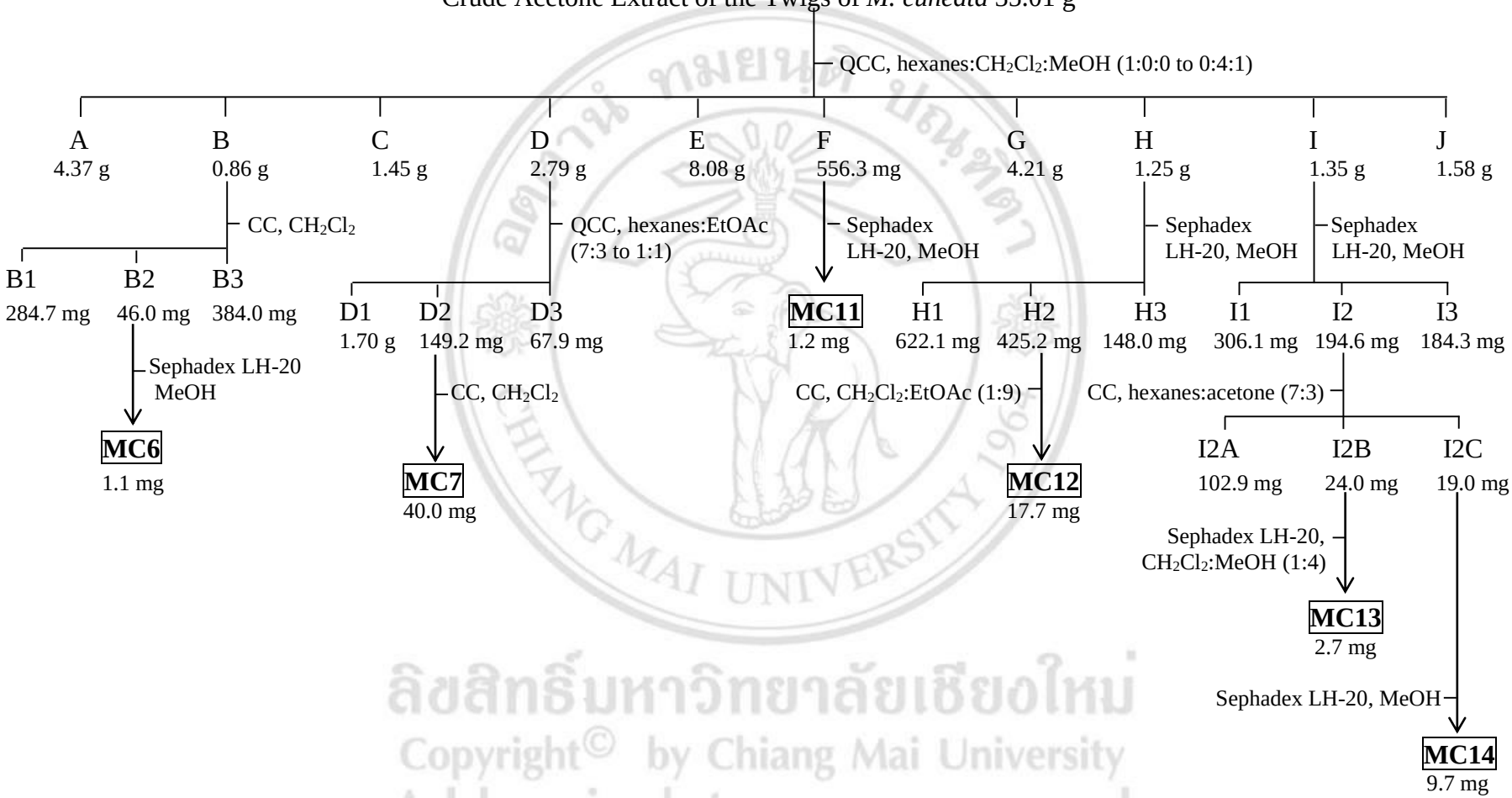

Figure 2.4 Isolation of Compounds from the Twigs of *M. cuneata*

2.4 Biological Assays

2.4.1 Antibacterial Assay

Antibacterial activity against Gram-positive bacteria (*Bacillus cereus* TISTR 688, *B.subtilis* TISTR 008, *Staphylococcus aureus* TISTR 1466, *S. epidermidis* ATCC 12229 and *Micrococcus luteus* TISTR884) and Gram-nagative bacteria (*Escherichia coli* TISTR 780, *Salmonella typhimurium* TISTR 292 and *Pseudomonas aeruginosa* TISTR 781) were performed employing Resazurin Microplate assay (REMA). The standard compounds were vancomycin and gentamycin and the test compounds were dissolved in DMSO. The lowest drug concentration effecting was considered as the minimum inhibitory concentration (MIC) by a two-fold serial dilution method (CLSI, 2002).

2.4.2 Antimalarial Assay

The *in vitro* antimalarial activity against *Plasmodium falciparum* (TM4 and K1, multidrug resistant strains) was carried out using as described by Wangchuk *et al*. (2011). The chloroquine, cycloguanil and pyrimethamine were used as the reference substances.

2.4.3 Cytotoxic Assay

The cytotoxicity assay of all pure compounds against KB (human oral cavity cancer cells), NCI-H187 (small cell lung cancer), MCF7 (breast cancer) cell lines and normal Vero cell from African green monkey kidney were evaluated using Sulforhodamine B (SRB) assay as described by Hunt *et al*. (1999). The tamoxifen, ellipticine and doxorubicine were used as the standard substances.