CHAPTER 5

EXPERIMENTAL

5.0 EXPERIMENTAL

General

Solvents

The solvents used in this work were petroleum ether (40-60°C), petroleum ether (60-80°C), hexane, ethyl acetate, dichloromethane and methanol. Other chemicals were ammonium solution, hydrochloric acid and sodium sulphate anhydrous.

Instruments

A Kratos MS 30 mass spectrometer (GC-MS) and the PE SCIEX API 100 liquid chromatography mass spectrometer (LC-MS) were used to obtain the mass spectra.

The NMR spectra were recorded on JNM-LA 400Hz spectrometer using deuterated chloroform ($CDCl_3$) as solvent. The chemical shift values were reported in δ (ppm) units.

The UV absorption spectra were observed on a Shimadzu UV-160A spectrophotometer with MeOH as the solvent. The IR spectra were determined on a Perkin Elmer 1600 series FT spectrophotometer in chloroform. Melting points were measured on Fischer John melting point apparatus and were uncorrected.

Analytical tlc were carried out on silica gel (ALUGRAM® SIL G/UV $_{254}$) with fluorescent indicator. Silica gel 60 F_{254}Merck® was used for column (70-230 mesh ASTM, 230-400 mesh ASTM) chromatography and preparative (230-400 mesh ASTM) tlc.

Reagents

Preliminary screening of alkaloid was done on the plant samples (bark and leaves). In this case Mayer's reagent was used to test the presence of alkaloids in the plant. The Dragendorff reagent was used to detect the presence of alkaloids on tlc plates.

Mayer's Reagent

This reagent is a mixture of solution of 1.4 g mercuric iodide in 60ml of distilled water with a solution of 5.0g potassium iodide in 10ml of distilled water. The mixture was then made up to a 100ml solution. A positive test is indicated by the formation of a white precipitate when the aqueous layer (acidified) is treated with 2 to 3 drops of Mayer's Reagent.

Dragendorff Reagent

This reagent consists of a mixture of solution A and solution B. The solution A is made up from bismuth (III) nitrate (0.85g) which is added in a mixture of glacial acetic acid (10ml) and distilled water (40ml). While the solution B is 8.0g of potassium iodide dissolved in 20ml of distilled water. From there, an equal solution A and B is mixed as the stock solution.

Spray Reagent

Then the stock solution (20ml) was diluted in a mixture of acetic acid (20ml) and distilled water (60ml). A positive result is indicated by the formation of orange, yellow or red spots.

Ultraviolet Viewing Lamp[39]

The UV lamp is the common way to determine flavonoids that is through the color of the spot under the UV light. The spot color has the relationship with the flavonoids structure. Below is the summary of spot appearance of different flavonoids (table 5).

Table 5: The relationships between spot colour and flavonoids structure

UV light	Flavonoids type
Deep purple	(a) Usually flavones with 5-OH and 4'OH or 3-OH substituted flavonols with 5-OH and 4'OH (b) Some 5-OH flavanones and 4`-OH chalcones lacking B-ring hydroxyl groups (c) Flavones or flavonols with 5-OH but with the 4`-OH absent substituted (d) Isoflavones, dihydro flavonols and some flavanones with 5-OH (e) Chalcones with 2`- or 6`-OH but without a free 2- or 4-OH (f) Some 5-OH flavanones (g) Chalcones with a free 2- or/and 4-OH
Fluorescent light blue	(a) Flavones and flavanones lacking light blue a free 5-OH (b) Flavonols lacking free 5-OH but with the 3-OH substituted (c) Isoflavones lacking a free 5-OH
Invisible	Isoflavones lacking a free 5-OH
Dull yellow and yellow or orange fluorescent	Flavonols with a free 3-OH and with or without a free 5-OH
Fluorescent yellow, yellow green, blue-green or green	Aurones with a free 4`-OH and some 2- or 4-OH chalcones (a) Aurones lacking a free 4`-OH and flavanones lacking a free 5-OH (b) Flavonols with a free 3-OH and with or without a free 5-OH
Pale yellow	Dihydroflavonols lacking a free 5-OH

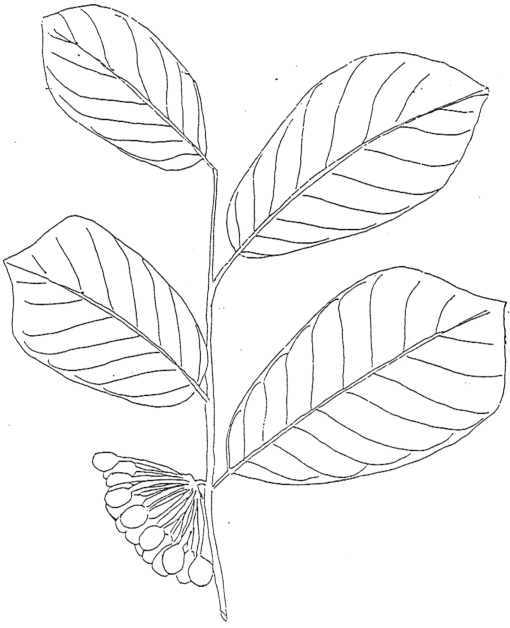

Figure 7: *Desmos dumosus*

Plant material

Desmos dumosus (Figure 7) is known locally as pisang-pisang padi, pisang-pisang pipit, akar kinching juhu or akar kenanga. It is a liana found in Assam, Myanmar and in the Peninsular of Malaysia southwards to Singapore.

This plant consists of flowers, leaves and seeds. It is a large climber or straggling shrub with young tomentose branches.

The flowers are solitary, opposed or extra-axillary with long greenish yellow on pedicels about 2 cm long and also with minute sub-median bract tomentose. The sepals are cordate or ovate acute tomentose. The petals are obovate to spathulate to broadly ovate lanceolate acuminate at both ends, densely pubescent at first.

The leave are membranous with broadly ovate to oblong ovate with rounded base. They are sparsely pubescent on the sunken midrib and nerves 10 to 12 pairs of the upper surface. The tomentose are purplish on both sides when young and glabrescent when adult. The length of the leaves is between 6-17cm, with breadth 4-7cm. The petioles are 7 mm to 2 cm long with rufous tomentose.

The ripe carpels are from 2 to 3 cm long, numerous, glabrous and constricted between the 2 to 3 ovoid joints. The color turns from yellow to dark purple. The stalks are from 1 to 1.5cm long and pubescent. The seeds are shining with the medium of brown testa.

Extraction of the plant material

Dried, ground leaves or barks of *Desmos dumosus* (1 kg) were soaked in petroleum ether (60-80°C) for three days at room temperature then filtered.

After being dried, the leaves or barks were rinsed with 10% of ammonia solution and again were soaked with dichloromethane (CH_2Cl_2) solvent for another three days. The CH_2Cl_2 extracts were concentrated to about 500ml and then extracted with 5% hydrochloric acid and repeatedly until Mayer's test was negative.

The hydrochloric extracts were basified with concentrated ammonia solution (28%) to pH 11 and extracted with CH_2Cl_2 in order to get alkaloids.

The CH_2Cl_2 extracts (dark brownish) were dried with anhydrous Na_2SO_4 and evaporated to dryness to give a crude alkaloid while the neutral part of the extraction was dried and evaporated to dryness for the other chemical constituents.

Separation and purification of compounds from the leaves

2.0g of CH_2Cl_2 soluble crude alkaloid of leaves was dissolved in CH_2Cl_2 and chromatographied over silica gel with the solvent systems of CH_2Cl_2 (100%), CH_2Cl_2: MeOH (99:1, 98:2, 97:3, 95:5) and finally 100% MeOH.

This yielded seven fractions in which four of them were subjected to extensive column chromatography and preparative tlc plates, after being monitored by tlc.

From the fraction of 99:1 (CH_2Cl_2: MeOH) of the solvent system, an oxoaporphines alkaloid was isolated. After further purification, the compound was confirmed to be *O*-methylmoschatoline **46**.

The next fraction was from the solvent system of 98:2 (CH_2Cl_2: MeOH). This fraction provided two compounds; lysicamine **45** and liriodenine **44** which were also of the oxoaporphines type.

Then, further isolations were done on the fraction 97:3 (CH_2Cl_2: MeOH) followed by the 95:5 solvent systems.

After several purifications, six compounds were isolated and their structures determined. They were noraporphine and proaporphines type of alkaloids namely nornuciferine **40** (from fraction 97:3), (-)-3-hydroxynornuciferine **41** (97:3), norlirioferine **42** (97:3), asimilobine **43** (97:3), stepharine 39 (95:5) and pronuciferine 37 (95:5).

While from the neutral part of the leaves extract, two flavonoids were isolated; 5-hydroxy-6,7-dimethoxyflavone **47** and 5-hydroxyl-7,8-dimethoxyflavone **48** which were purified from 100% CH_2Cl_2 solvent system in column chromatography.

Tlc of the fraction indicated the Dragendorff reagent-positive spots, suggested the presence of alkaloids, but it was latter shown that such coloration was due to flavonoids[50].

Below is the yield in percentage of the chemical constituents isolated from the leaves (Table 6). It was found that *O*-methylmoschatoline **46** was the major compound of the

leaves of *Desmos dumosus*, followed by liriodenine **44** and lysicamine **45**. These three compounds belonged to the oxoaporphines type of alkaloids.

The least compounds found in this part of the plant were the proaporphines. They were pronuciferine **37** and stepharine **39**. The small amount of these proaporphines may be due to the fact that they were indeed intermediates in the biogenesis of isoquinoline alkaloids and were seldom isolated from many plants[26].

Table 6: The yield of chemical constituents of the leaves

Compounds	Yield %
pronuciferine **37**	0.5
stepharine **39**	0.2
nornuciferine **40**	1.0
(-)-3-hydroxynornuciferine **41**	1.0
norlirioferine **42**	2.0
asimilobine **43**	0.8
liriodenine **44**	2.5
lysicamine **45**	2.5
O-methylmoschatoline **46**	7.5
5-hydroxy-6,7-dimethoxyflavone **47**	1.0
5-hydroxy-7,8-dimethoxyflavone **48**	0.8

Separation and purification of compounds isolated from the barks

2.0g of CH_2Cl_2 extract was chromatographied on a column of silica gel with solvent systems of 100% CH_2Cl_2 and CH_2Cl_2: MeOH of 99:1, 98:2, and 97:3. From the fraction of 99:1 solvent system, an oxoaporphine named *O*-methylmoschatoline **46** was isolated. In addition, two flavonoids; 5-hydroxy-6,7-dimethoxyflavone **47** and 5-hydroxyl-7,8-dimethoxyflavone **48** were also afforded.

An increment of the polarity of the solvent system to 98:2 (CH_2Cl_2: MeOH) afforded a tetrahydroprotoberberine compound together with an oxoaporphines. They were discretamine **50** and lysicamine **45** respectively. Finally, an aporphine named *O*-methylisopiline **49** was discovered from the fraction 97:3. According to the table below, as in the leaves, compound *O*-methylmoschatoline **46** represented the major alkaloid component in this plant, followed lysicamine **45**. Hence, it could be concluded that oxoaporphines alkaloids were the major alkaloid component of *Desmos dumosus*.

Table 7: The yield of chemical constituents of the bark

Compounds	Yield %
O-methylisopiline **49**	0.2
lysicamine **45**	1.0
O-methylmoschatoline **46**	2.5
discretamine **50**	0.4
5-hydroxy-6,7-dimethoxyflavone **47**	0.8
5-hydroxy-7,8-dimethoxyflavone **48**	0.3

Spectral data of compounds isolated from *Desmos dumosus*

pronuciferine **37**

m.p. 127-129°C

UV λ_{max} (MeOH), nm: 230(log ε 4.46), 280 (log ε 3.47)

IR ($CHCl_3$), cm^{-1}: 3445, 2850, 1662, 1488, 1455, 1438, 1375, 1295, 1265, 1000, 850

MS *m/z* (rel. int.): 311 [M^+] (100), 310 (53), 294 (2.6), 282 (56), 268 (36), 253 (13), 225 (15.6), 209 (9), 195 (3.8), 165 (11.6)

^{1}H NMR ($CDCl_3$), ppm:

δ 6.28-6.42 (2H, 2xd, J=10.00Hz, J'=1.80Hz, H-9(α'), H-11(α))

6.87-7.01 (2H, 2xd, J=10.00Hz, J'=1.80Hz, H-8(β'), H-12(β))

6.64 (1H, s, H-3), 2.04 (3H, s, N-CH_3), 3.60 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 2.20-3.00 (6H, m, 2xH-4, 2xH-5, 2xH-7)

stepharine **39**

UV λ_{max} (MeOH), nm: 230(log ε 4.44), 280 (log ε 3.45)

IR ($CHCl_3$), cm^{-1}: 3500, 2820, 1665, 1500, 1450, 1433, 1368, 1253, 1105, 1000, 850

MS *m/z* (rel. int.): 297[M^+] (100), 296(53), 268 (67.6), 253 (14.9), 238(10.8), 225(14.2), 209(9.4), 195(3.4), 165 (10.8)

nornuciferine **40**

m.p. 245-246°C

UV λ_{max} (MeOH), nm: 216(log ε 4.69), 272 (3.70)

IR ($CHCl_3$), cm^{-1}: 3688, 2895, 1596, 1460, 1420, 1370, 1190, 1150, 1105,1000, 965, 920, 850, 815

MS *m/z* (rel. int.): 281[M^+] (61.8), 280 (100), 266 (22.3), 250 (27.6), 236 (9.2), 221 (19.7), 165 (19.7), 152 (6.6)

^{1}H NMR ($CDCl_3$), ppm:

δ 6.58 (1H, s, H-3), 8.30 (1H, d, J=8.90Hz, H-11), 7.20 (3H, m, H-8, H-9, H-10), 3.60 (3H, s, OCH_3-1), 3.67 (3H, s, OCH_3-2), 3.25 (1H, m, H-6a), 2.98 (1H, m, H-5'), 2.59-2.81 (5H, m, 2xH-4, H-5, 2xH-7)

(-)-3-hydroxynornuciferine **41**

m.p. 163-164 °C

UV λ_{max} (MeOH), nm: 219(log ε 4.39), 240 (log ε 3.90), 280 (log ε 4.14), 292 (log ε 4.04)

IR ($CHCl_3$), cm^{-1}: 3521, 2942, 2800, 1480, 1360, 1040, 940, 925, 850

MS *m/z* (rel. int.): 297[M^+] (84.6), 296 (100), 280 (30.7)

^{1}H NMR ($CDCl_3$), ppm:

δ 7.16-7.30 (3H, m, H-8, H-9, H-10), 8.28 (1H, dd, J=7.80Hz, H-11), 3.72 (3H, s, OCH_3-1), 3.98 (3H, s, OCH_3-2), 3.42 (1H, m, H-6a), 2.92 (1H, m, H-5'), 2.62-2.78 (5H, m, 2xH-4, H-5, 2xH-7)

norlirioferine **42**

m.p. 94-96 °C

UV λ_{max} (MeOH), nm: 226(log ε 4.30), 281 (log ε 3.83), 310 (log ε 3.89),

IR ($CHCl_3$), cm^{-1}: 3617, 2950, 2820, 1603, 1585, 1150, 1000, 960, 925, 890

MS *m/z* (rel. int.): 327[M^+] (68.40), 326 (100), 312 (19.70), 310 (6.49), 298 (5.19), 295 (9.09), 283 (13.00), 281 (15.60), 267 (15.60)

^{1}H NMR ($CDCl_3$), ppm:

δ 8.08 (1H, s, H-11), 6.80 (1H, s, H-8), 6.59 (1H, s, H-3), 3.89 (3H, s, OCH_3-9), 3.88 (3H, s, OCH_3-2), 3.66 (3H, s, OCH_3-1), 2.04 (1H, s, N-H), 3.42 (1H, m, H-6a), 3.05 (2H, m, 2xH-5), 2.65-2.75 (4H, m, 2xH-4, 2xH-7)

^{13}C NMR($CDCl_3$), ppm:

δ 144.08 (C-1), 126.63 (C-1a), 127.93 (C-1b), 152.11 (C-2), 110.85 (C-3), 128.85 (C-3a), 29.20 (C-4), 43.17 (C-5), 55.76 (C-6a), 36.70 (C-7), 129.91 (C-7a), 111.3 (C-8), 145.25 (C-9), 144.87 (C-10), 113.83 (C-11), 124.07(C-11a), 60.19 (C-1, OCH_3), 55.86 (C-2, OCH_3), 56.06 (C-9, OCH_3).

asimilobine **43**

UV λ_{max} (MeOH), nm: 274(log ε 4.08), 229 (log ε 4.08)

IR ($CHCl_3$), cm^{-1}: 3521, 2940, 2830, 1600, 1580, 1360, 1105, 1080, 950, 920, 890

MS *m/z* (rel. int.): 267 [M^+] (58.7), 266 (100), 251 (30.7), 236 (17.3), 223 (7.3), 206 (8.0), 194 (8.0), 194 (6.7), 178 (16.0), 165 (14.7)

^{1}H NMR ($CDCl_3$), ppm:

δ 6.71 (1H, s, H-3), 7.16-7.30 (3H, m, H-8, H-9, H-10), 8.29 (1H, d, J=8.30Hz, H-11), 3.58 (3H, s, OCH_3-1), 3.42 (1H, m, H-6a), 3.05 (1H, m, H-5'), 2.52-2.68 (5H, m, 2xH-4, H-5, 2xH-7)

liriodenine **44**

UV λ_{max} (MeOH), nm: 237(log ε 4.10), 270 (log ε 3.80), 313(log ε 3.41), 410 (log ε 3.42)
IR ($CHCl_3$), cm^{-1}: 1662, 1420, 1357, 1255, 1120, 969, 958
MS *m/z* (rel. int.): 275[M^+] (100), 248 (5), 247 (18), 246 (10)
^{1}H NMR ($CDCl_3$), ppm:
δ 7.24 (1H, s, H-3), 6.40 (2H, s, OCH_2O), 7.82 (1H, d, J=5.00Hz, H-4), 8.92 (1H, d, J=5.00Hz, H-5), 7.61 (1H, m, H-10), 7.79 (1H, m, H-9), 8.62 (1H, dd, J=7.80Hz, J'=1.10Hz, H-8), 8.72 (1H, dd, J=8.70Hz, J,=1.80Hz, H-11)

lysicamine **45**

UV λ_{max} (MeOH), nm: 236(log ε 3.77), 267(log ε 3.69), 310(log ε 3.23), 395(log ε 3.04),
IR ($CHCl_3$), cm^{-1}: 1665, 1490, 1416, 1355, 1257, 1125, 1040, 955
MS *m/z* (rel. int.): 292[M+1], 276, 184, 122, 87
^{1}H NMR ($CDCl_3$), ppm:
δ 7.24 (1H, s, H-3), 4.02 (3H, s, OCH_3-1), 4.11 (3H, s, OCH_3-2), 7.80 (1H, d, J=5.30Hz, H-4), 8.80 (1H, d, J=5.20Hz, H-5), 9.17 (1H, dd, J=8.50Hz, J'=1.10Hz, H-11), 8.60 (1H, dd, J=7.80Hz, J'=1.70Hz, H-8), 7.78 (1H, m, H-9), 7.58 (1H, m, H-10)

O-methylmoschatoline **46**

m.p. 182-184°C
UV λ_{max} (MeOH), nm: 274(log ε 4.34), 315 (log ε 3.67), 436 (log ε 3.50)
IR ($CHCl_3$), cm^{-1}: 1662 (conjugated C=O), 1480, 1400, 1350,1260, 950
MS *m/z* (rel. int.): 322[M+1], 293, 235, 149, 89
^{1}H NMR ($CDCl_3$), ppm:
δ 8.91 (1H, d, J=5.40Hz, H-5), 8.16 (1H, d, J=5.40Hz, H-4), 9.10 (1H, dd, J=8.30Hz, J'=1.20Hz, H-11), 8.52 (1H, dd, J=7.80Hz, J'=1.80Hz, H-8), 7.54-7.75 (2H, m, H-9, H-10), 4.08 (3H, s, OCH_3-1), 4.10 (3H, s, OCH_3-2), 4.19 (3H, s, OCH_3-3)

^{13}C NMR ($CDCl_3$), ppm:

δ 61.10 (OCH_3-1), 61.57 (OCH_3-2), 61.91 (OCH_3-3), 140.30 (C-1), 145.90 (C-2), 144.50 (C-3), 119.27 (C-4), 144.66 (C-5), 182.75 (C-7), 127.76 (C-11), 128.28 (C-8), 129.04 (C-9), 134.48 (C-10), 134.65 (C-11a)

5-hydroxy-6,7-dimethoxyflavone **47**

m.p. 205-207°C

UV λ_{max} (MeOH), nm: 221(log ε 4.33), 274 (log ε 4.74), 313(log ε 4.27)

IR ($CHCl_3$), cm^{-1}: 3160, 1665, 1585, 1515, 1355, 1255, 1250,1230, 1170, 1075, 830, 820

MS *m/z* (rel. int.): 299 [M+1], 292, 276, 241, 187, 135, 87

^{1}H NMR ($CDCl_3$), ppm:

δ 6.50 (1H, s, H-8), 6.65 (1H, s, H-3), 12.61 (1H, s, OH), 3.90 (3H, s, OCH_3-6), 3.86 (3H, s, OCH_3-7), 7.48 (3H, m, H-3', H-4', H-5'), 7.83 (2H, m, H-2', H-6')

5-hydroxy-7,8-dimethoxyflavone **48**

m.p. 210-212°C

UV λ_{max} (MeOH), nm: 216(log ε 4.18), 273 (log ε 4.18), 320(log ε 3.91)

IR ($CHCl_3$), cm^{-1}: 3140, 1665, 1610, 1585, 1510, 1360, 1295, 1220, 1180, 1075, 830, 820

MS *m/z* (rel. int.): 299[M+1], 292, 276, 241, 187

^{1}H NMR ($CDCl_3$), ppm:

δ 6.42 (1H, s, H-6), 6.60 (1H, s, H-3), 12.09 (1H, s, OH), 3.93 (3H, s, OCH_3-7), 3.93 (3H, s, OCH_3-8), 7.52 (3H, m, H-3', H-4', H-5'), 7.88 (2H, m, H-2', H-6')

^{13}C NMR($CDCl_3$), ppm:

δ 56.34 (C-7, OCH_3), 61.67 (C-8, OCH_3), 95.85 (C-6), 105.35 (C-3), 126.32 (C-4'), 129.14 (C-3'), 131.92 (C-5'), 131.35 (C-2), 157.58 (C-2'), 158.74 (C-6'), 163.94 (C-OH), 182.73 (C=O)

O-methylisopiline **49**

UV λ_{max} (MeOH), nm: 213(log ε 3.97), 272 (log ε 3.76)

IR ($CHCl_3$), cm^{-1}: 2940, 2830, 1605, 1585, 1000, 960, 890

MS *m/z* (rel. int.): 312 [M+1], 210, 156, 74

^{1}H NMR ($CDCl_3$), ppm:

δ 3.71 (3H, s, OCH_3-1), 3.92 (3H, s, OCH_3-2), 3.89 (3H, s, OCH_3-3), 7.14-7.28 (3H, m, H-8, H-9, H-10), 8.25 (1H, d, J=7.80Hz, H-11), 3.42 (1H, m, H-6a), 2.98 (1H, m, H-5'), 2.52-2.96 (5H, m, 2xH-4, H-5, 2xH-7)

discretamine **50**

m.p. 209-211°C

UV λ_{max} (MeOH), nm: 207(log ε 3.49), 273(log ε 3.44)

IR ($CHCl_3$), cm^{-1}: 3246, 3000, 2850, 2960, 2920, 1455, 1340, 1240, 1190, 1125, 1025

MS *m/z* (rel. int.): 328[M+1], 256, 203, 148, 87

^{1}H NMR ($CDCl_3$), ppm:

δ 6.68 (1H, s, H-1), 6.71 (1H, s, H-4), 6.82 (1H, d, J=8.79Hz, H-12), 6.81 (1H, d, J=8.79Hz, H-11), 3.82 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.18 (1H, d, J=16.00Hz, H-8)