CHAPTER 3

EXPERIMENTAL

3.1 SOURCE AND AUTHENTICATION OF PLANT MATERIALS

The plants were collected from various areas in Peninsular Malaysia as summarized in Table 3.1 and before any chemical analysis was carried out, voucher specimens were prepared for proper documentation. The herbarium specimens were given a number and the location and date of collection of the plant specimens were also recorded. Voucher specimens are deposited at the herbarium, Chemistry Department, University of Malaya, the herbarium, Forest Research Institute Malaysia, and in some cases at the Rijksherbarium, Leiden.

The specimens were identified by Dr. C.E. Risdale (CER) of the Rijksherbarium, Leiden and K.M. Wong (WKM) and K.M. Kochummen (KMK) of the Forest Research Institute Malaysia, as summarized in Table 3.2

Table 3.1 : Collection of *Uncaria* Samples in Peninsular Malaysia

Place	Date	No. of samples collected & screened
Northern Region		
Pulau Langkawi	June 89	1
Batu Gajah	June 89	8

Table 3.1 continued ....

Place	Date	No. of samples collected & screened
West Coast		
Puchong	June 87	10
	Aug. 87	10
	Nov. 87	6
	Feb. 88	4
	May 88	15
	June 88	17
	Nov. 88	8
	Jan. 89	6
	Apr. 89	2
Kuala Lumpur	Feb. 89	3
Pasoh	July 87	6
Port Dickson	Aug. 88	3
Southern Region		
Kota Tinggi	Nov. 88	18
Kluang	Nov. 88	12
Mersing	June 89	14
Desaru	May 90	6

Table 3.1 continued ....

Place	Date	No. of samples collected & screened
Central Region		
Lanchang	Oct. 87	11
Genting Highlands	Sept. 87	15
	Dec. 97	9
	Jan. 88	8
	Mac. 88	3
	Feb. 89	3
Gombak	Sept. 87	2
	Dec. 87	6
	May 89	1
East Coast		
Bukit Besi	Aug. 88	19
Bukit Kesing	June 87	25
	Aug. 88	52
	Feb. 90	17
Dungun	Apr. 87	8

Table 3.2 : Source and Authentication Of Plant Materials

Herbarium Specimen No.	Date	Locality	Species	Identification
GK 124	May 1988	Puchong	*U. callophylla*	CER
GK 361	Nov 1988	Puchong	*U. borneensis*	CER
GK 022	June 1987	Bukit Kesing	*U. longiflora* var. *longiflora*	WKM
GK 037	June 1987	Bukit Kesing	*U. longiflora* var. *pteropoda*	WKM
GK 076	Sept 1987	Gombak	*U. lanosa* var. *glabrata*	WKM
GK 186	July 1988	Puchong	*U. lanosa* var. *glabrata*	KMK
GK 042	June 1987	Bukit Kesing	*U. lanosa* var. *ferrea*	KMK
GK 043	June 1987	Bukit Kesing	*U. cordata* var. *cordata* f. *sundaica*	WKM
GK 032	June 1987	Bukit Kesing	*U. cordata* var. *cordata* f. *cordata*	WKM
GK 014	June 1987	Puchong	*U. cordata* var. *ferruginea* f. *ferruginea*	WKM
GK 023	June 1987	Bukit Kesing	*U. elliptica*	CER
GK 190	July 1988	Bukit Kesing	*U. elliptica*	CER
GK 269	Aug 1988	Bukit Kesing	*U. elliptica*	CER
GK 063	Sept 1987	Genting Highlands	*U. acida*	CER
GK 074	Sept 1987	Gombak	*U. gambir*	CER
GK 024	June 1987	Bukit Kesing	*Uncaria sp. A*	WKM
GK 033	June 1987	Bukit Kesing	*Uncaria sp. B*	WKM
GK 044	June 1987	Bukit Kesing	*Uncaria sp. C*	WKM
GK 262	Aug 1988	Bukit Kesing	*Uncaria sp. D*	KMK

3.2 GENERAL

Melting points were recorded on a hot stage Leitz Wetzler melting point apparatus and were uncorrected. ^1H and ^{13}C NMR were recorded using a JEOL FX 100 Spectrometer in $CDCl_3$ solution with TMS as internal standard at 100 and 25 MHz respectively. Chemical shifts were reported as ppm downfield from TMS. Direct probe EIMS (70eV) were performed on a Kratos AEI MS 3074 Mass Spectrometer with a DS 55 data system. UV spectra were recorded on a Shimadzu UV-160A spectrometer in 95% ethanol. IR spectra were recorded in $CHCl_3$ solutions on a Beckmann Acculab Infrared Spectrometer. All solvents were distilled before use except for diethyl ether, which was passed through activated alumina prior to use.

300 MHz ^1H NMR and 2D NMR spectra were obtained courtesy of Prof. C. K. Sha, National Tsing Hua University, Taiwan and Dr. C. H. Chuah, University of Malaya, Malaysia respectively. EIMS and FABMS were obtained courtesy of Prof H. H. Lee, National University of Singapore, and Prof. C. K. Sha, National Tsing Hua University, Taiwan respectively.

3.3 CHROMATOGRAPHIC METHODS

3.3.1 Column Chromatography

Flash chromatography was performed using Merck silica gel 9385 (230-400 Mesh ASTM). The ratio of silica gel to sample was approximately 30:1 for crude samples and 100:1 for semi pure

fractions. The gel was made into a slurry with chloroform before it was packed into the column and was allowed to equilibrate for at least an hour before use. When diethyl ether was used as eluting solvent, the column was packed by the dry packing method. The solvent systems normally used to elute the columns were chloroform with increasing methanol gradient or diethyl ether with increasing ethyl acetate gradient followed by chloroform with methanol gradient. Fractions were monitored by t.l.c. and appropriate fractions were combined and where necessary subjected to futher separation by rechromatography or preparative t.l.c.

3.3.2 Thin Layer Chromatography (t.l.c.)

Thin layer chromatography (t.l.c.) was routinely used to detect and separate the various alkaloids. The crude alkaloid extracts, fractions from column chromatography and isolated pure alkaloids were examined by t.l.c. using precoated 5x10 cm glass plates, 0.25 mm thickness, silica gel 60 F_{254} (Merck, Darmstadt, G.F.R.). The t.l.c. plates were spotted with a piece of fine glass capillary tube and then developed in saturated chromatographic tanks with various solvent systems at room temperature (28 ^{o}C). The alkaloidal spots were visualised by examination of the t.l.c. plates under u.v. light (254 and 365 nm), followed by spraying with Dragendorff's reagent which formed orange spots on a pink background.

The hR_f values are tabulated in Table 3.3. (The values given are the average of two separate determinations).

Table 3.3 : **The hR_f Values Of Alkaloids Isolated From Malaysian *Uncaria* Species**

Alkaloid	Solvent System*					
	a	b	c	d	e	f
Dihydrocorynantheine [20]	39	32	62	58	64	76
Gambirine [22]	19	10	44	32	33	56
Isogambirine [86]	14	7	40	30	32	54
Gambireine [87]	26	16	56	41	40	61
Isopteropodine [34]	30	30	52	54	65	73
Pteropodine [35]	23	24	47	46	63	68
Isorhynchophylline [38]	28	29	57	59	67	75
Rhynchophylline [39]	5	4	19	35	52	69
Isocorynoxeine [40]	28	29	57	59	67	75
Corynoxeine [41]	5	4	19	35	52	69
Rotundifoline [42]	27	26	58	61	69	78
Yohimbine [47]	8	3	22	22	36	52
β-yohimbine [49]	5	2	9	20	25	48
α-yohimbine [54]	22	11	42	38	44	58
Alloyohimbine [53]	0	0	0	7	29	47
Pseudoyohimbine [50]	0	0	2	3	21	37
3-epi-β-yohimbine [52]	1	0	2	3	16	29
Callophylline [88]	2	0	13	17	43	58
Callophylline A [89]	2	0	10	12	25	38
Callophylline B [90]	0	0	4	6	29	44

*a - diethyl ether

b - ether : hexane (1:1, v/v)

c - ether : ethyl acetate (1:1, v/v)

d - chloroform : acetone (5:4, v/v)

e - chloroform : methanol (10:1, v/v)

f - chloroform : methanol (10:2, v/v)

3.3.3 Preparative Thin Layer Chromatography

Preparative t.l.c. was carried out on silica gel 60 F_{254} (Merck 7730) on 20 x 20 cm glass plates of layer thickness 0.75 mm (15 g silica gel in 27 ml distilled water per plate). The plates were air dried for an hour and then activated overnight in an oven at 110 °C before use.

Preparative t.l.c. was used routinely for the isolation as well as purification of the compounds. The weight of sample applied to each plate was approximately 15 mg. Samples were introduced onto the plates as a continous streak using fine capillary tubes and then developed in saturated chromatographic tanks at room temperature (28 °C).

The solvent systems used were:

(a) Diethyl ether

(b) Ethyl acetate : diethyl ether (1:1)

(c) Methanol : chloroform (1:10)

(d) Hexane : ethyl acetate (1:1)

The developed plates were visualised under ultra violet light and the required bands were scrapped off and extracted exhaustively with absolute ethanol. The ethanol extracts were evaporated to dryness and the alkaloids were then taken into chloroform.

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Rhynchophylline [39]	5	4	19	35	52	69
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Corynoxeine [41]	5	4	19	35	52	69
Rotundifoline [42]	27	26	58	61	69	78
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β-yohimbine [49]	5	2	9	20	25	48
α-yohimbine [54]	22	11	42	38	44	58
Alloyohimbine [53]	0	0	0	7	29	47
Pseudoyohimbine [50]	0	0	2	3	21	37
3-epi-β-yohimbine [52]	1	0	2	3	16	29
Callophylline [88]	2	0	13	17	43	58
Callophylline A [89]	2	0	10	12	25	38
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b - ether : hexane (1:1, v/v)

c - ether : ethyl acetate (1:1, v/v)

d - chloroform : acetone (5:4, v/v)

e - chloroform : methanol (10:1, v/v)

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The solvent systems used were:

(a) Diethyl ether

(b) Ethyl acetate : diethyl ether (1:1)

(c) Methanol : chloroform (1:10)

(d) Hexane : ethyl acetate (1:1)

The developed plates were visualised under ultra violet light and the required bands were scrapped off and extracted exhaustively with absolute ethanol. The ethanol extracts were evaporated to dryness and the alkaloids were then taken into chloroform.

3.3.4 Spray Reagent

Dragendorff's Reagent

Solution A: 0.85 g of bismuth nitrate was dissolved in a mixture of 10 ml glacial acetic acid and 40 ml of distilled water.

Solution B: 8 g of potassium iodide was dissolved in 20 ml of distilled water.

A stock solution was prepared by mixing equal volumes of solution A and B. Dragendorff's reagent was made by mixing 1 ml of stock solution with 2 ml of glacial acetic acid and 10 ml of distilled water. Orange spots on the developed t.l.c. plates indicate the presence of alkaloids.

3.4 EXTRACTION OF ALKALOIDS

The dried and coarsely ground plant materials were extracted with distilled ethanol over 2/3 days. The ethanol extract was filtered off and the residue was then resoaked with a fresh portion of ethanol. This procedure was repeated 3/4 times.

The combined ethanol extract was concentrated to about 1/20 of its original volume under reduced pressure and then added slowly into a large excess of 5% hydrochloric acid solution with constant stirring. The acidic solution was filterred through kieselghur to remove the non-alkaloidal components. The filtrate was defatted witn n-hexane or ether and then basified

with concentrated ammonia solution. The liberated alkaloids were extracted exhaustively with chloroform. The chloroform extract was then washed with distilled water and dried over anhydrous sodium sulphate. Removal of the solvent by evaporation under reduced pressure furnished the crude alkaloids.

Repurification of the crude alkaloid extracts by repetition of this cycle was found to be necessary especially for leaf extracts in order to obtain a cleaner crude alkaloidal mixture.

3.5 ISOLATION OF ALKALOIDS

3.5.1 Isolation of Alkaloids From *U. callophylla*

In the earlier part of the work, extraction was carried out using 1 kg of leaves which gave 1.7 g of the crude mixture. Chromatography of this mixture showed that in addition to the three major alkaloids *viz.*, gambirine, dihydrocorynantheine and callophylline, other minor alkaloids were also present. Subsequently 5 kg of leaves were extracted to give 4.2 g of the crude alkaloidal mixture. Chromatography of this mixture allowed the detection and isolation of some of the minor alkaloids *viz.*, gambireine, yohimbine, pseudoyohimbine, α-yohimbine, β-yohimbine, callophylline A and callophylline B. In order to obtained sufficent amounts of these minor alkaloids for complete characterization, extraction was eventually carried out on 10 kg of leaves. The procedure for the isolation of the alkaloids is described below for the largest

scale extraction. The essential procedure is summarized in the flow diagram shown in Figure 3.1.

Extraction of 10 kg of leaves followed by partitioning into acid and the usual workup gave 6.8 g of crude alkaloidal mixture designated as T1. The crude alkaloidal mixture obtained was shown by t.l.c. to consist of a complex mixture of alkaloids with three major components. This mixture was initially dissolved in $CHCl_3$ (50 ml) and allowed to stand in the cold for 12 hours. A white solid was formed which was collected by filtration and washed with cold chlcroform. The white solid, (2.04 g) designated as T2, was shown to be a mixture of two major alkaloids UC1 (Dihydrocorynantheine) and UC3 (Gambirine). The mother liquour was collected and evaporated to dryness to yield T3 (4.76 g). T3 was initially chromatographed over silica gel 60 using chloroform with increasing methanol gradient to give essentially three fractions containing partially resolved mixtures of alkaloids.

Fraction 1 : was subjected to further separation by preparative t.l.c. (silica gel 60, diethyl ether) to furnish UC1 (Dihydrocorynantheine) and UC2 (Rotundifoline).

Fraction 2 : contained a complex mixture of alkaloids. It was rechromatographed over silica gel using diethyl ether with increasing ethyl acetate gradient followed by $CHCl_3$ with increasing methanol gradient to give essentially 8 fractions. Extensive preparative t.l.c. and column chromatography of these fractions yielded the pure alkaloids, viz., UC1 - UC12.

The procedure for the isolation of the alkaloids is summarized in Figure 3.1.

Fraction 3 : contained minor polar and baseline alkaloids. No further study was carried out.

The following alkaloids were isolated:

UC1, **Dihydrocorynantheine** was recrystallized from diethyl ether, m.p. 104°C (lit.[48] 105 °C). R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 0.85 (3H, t, $CH_2\underline{CH_3}$), 1.04-3.50 (13H, overlapping m), 3.69 (3H, s, COOMe), 3.73 (3H, s, OMe), 7.01-7.48 (4H, m, aromatic), 7.27 (1H, s, H-17), and 8.16 (1H, br s, NH). EIMS, m/z (rel. int.): 368(100, M^+), 367 (80.4, M^+-1), 353 (49.9, M^+-15), 239 (11.8), 225 (29.1), 184 (94.0), 170 (39.0) 169 (34.3) and 156 (36.9). Calcd. for $C_{22}H_{28}N_2O_3$ 368.210. Found: 368.305. UV: λ max (EtOH): 227, 250 (sh), 283, and 291 nm. IR (KBr): 3500, 2900, 2340, 2300, 2740, 1700, 1630 cm^{-1}
The ^{13}C NMR data are presented in Table 2.3.

UC2, **Rotundifoline** was purified using preparative t.l.c. (ether) R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 0.80 (3H, t, $CH_2\underline{CH_3}$), 0.5-3.2 (13H, overlapping m), 3.58 (3H, s, COOMe), 3.69 (3H, s, OMe), 6.36 (1H, d, J = 8 Hz, H-10), 6.57 (1H, d, J = 8 Hz, H-12), 7.04 (1H, t, J = 8 Hz, H-11), 7.22 (1H, s, H-17) and 8.30 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.9.

UC3, **α-Yohimbine** was isolated and purified by preparative t.l.c. (diethyl ether : ethyl acetate, 1 : 1, v/v). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 1.4-3.5 (*ca.* 15H, overlapping m), 3.84 (3H, s, COOMe), 3.99 (1H, br s, H-17), 7.1-7.5 (4H, m, aromatic H) and 7.75 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.18.

UC4, **Callophylline** was isolated and purified by preparative t.l.c. (diethyl ether : ethyl acetate, 1:1 followed by $CHCl_3$: MeOH, 10 : 1) and obtained as an amorphous powder m.p. >330 °C dec. (lit.[12] 330 °C). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 300 MHz) : δ 0.87 (3H, t, $CH_2\underline{CH_3}$), 1.0-4.1 (*ca.* 29H, overlapping m), 3.56 (1H, d, *J* = 9 Hz, H-21'), 3.70 (3H, s, OMe), 3.70 (3H, s, COOMe), 3.82 (3H, s, COOMe'), 4.17 (1H, br s, H-17'), 4.70 (1H, br s, H-3'), 6.58 (1H, d, *J* = 8 Hz, H-11), 6.69 (1H, d, *J* = 8 Hz, H-12), 7.12 (1H, ddd, *J* = 7, 7, 1 Hz, H-10'), 7.19 (1H, ddd, *J* = 7, 7, 1 Hz, H-11'), 7.37 (1H, s, H-17), 7.45 (1H, dd, *J* = 7 Hz, H-12'), 7.48 (1H, dd, *J* = 7, 1 Hz, H-9'), 7.69 (1H, br s, NH), and 8.29 (1H, br s, NH). FABMS, m/z : 737.4 (MH^+), calcd. for $C_{43}H_{52}N_4O_7$ + H 737.3914. IR (KBr) : 3795, 3200-3445, 2935, 2915, 2880, 2820, 1850, 1705, 1690 and 1640cm^{-1}. UV : λ_{max} (EtOH) : 228, 232.5, 275, 295 and 300 nm. The ^{13}C NMR data are presented in Table 2.11. The ^{13}C NMR DEPT Spectrum is presented in Figure 2.8. The ^1H-^1H COSY and ^1H-^{13}C Correlation Spectra are presented in Figure 2.9 and 2.10.

UC5, **Gambireine** was purified using preparative t.l.c. ($CHCl_3$: MeOH, 10 : 1, v/v). R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 1.80-3.20 (12H, overlapping m), 3.68 (3H, s, COOMe), 3.75 (3H, s, OMe), 5.3-5.7 (1H, m, -<u>CH</u>=CH_2), 4.8-5.0 (2H, m, -CH=<u>CH_2</u>), 6.38 (1H, dd, J = 8.0, 2.0 Hz, H-12), 6.78-6.91 (2H, m, H-10 & H-11), 7.32 (1H, s, H-17), and 7.74 (1H, br s, NH) EIMS, m/z (rel. int.): 382 (100, M^+), 381 (56.2, M^+-1), 367 (45.9, M^+-15), 253 (23.6) 239 (25.3), 200 (93.1), 186 (8.6), 185 (37.3), and 172 (50.6). Calcd. for $C_{22}H_{26}N_2O_3$ 382.1892. Found: 382.1868. UV: λmax (EtOH): 227, 250 (sh), 286, and 295 nm. The ^{13}C NMR data are presented in Table 2.3.

UC6, **Gambirine** was recrystallized from diethyl ether, m.p. 165 °C (lit.[48] 163-165 °C). R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz) : δ 0.88 (3H, t, J = 7, 7 Hz, CH_2<u>CH_3</u>), 1.8-3.2 (13H, overlapping m), 3.69 (3H, s, COOMe), 3.77 (3H, s, OMe), 6.40 (1H, dd, J = 7.5, 2.0 Hz, H-12), 6.79-7.02 (2H, m, H-10 & H-11), 7.36 (1H, s, H-17), and 7.67 (1H, br s, NH). EIMS, m/z (rel. int.) : 384 (100, M^+), 383 (43.0, M^+-1), 369 (30.4, M^+-15), 255 (11.0), 241 (13.0), 200 (70.0), 186 (32.8), 185 (22.6), and 172 (26.6). Calcd. for $C_{22}H_{28}N_2O_3$ 384.205. Found: 384.200. UV: λmax (EtOH): 227, 250 (sh), 286, and 295 nm. IR (KBr): 3350-3200, 2920, 2880, 2820, 2760, 1700 and 1630 cm^{-1} The ^{13}C NMR data are presented in Table 2.3.

UC7, **Isogambirine** was purified using preparative t.l.c. ($CHCl_3$: MeOH, 10 : 1, v/v). R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 0.85 (3H, t, $CH_2\underline{CH_3}$), 1.80-3.20 (13H, overlapping m), 3.69 (3H, s, COOMe), 3.75 (3H, s, OMe), 6.84 (1H, d, J = 2.4 Hz), 6.77 (1H, dd, J = 8.5, 2.4 Hz), 7.09 (1H, d, J = 8.5 Hz), 7.36 (1H, s, H-17) and 7.54 (1H, br s, NH). EIMS, m/z (rel. int.): 384 (100, M^+), 383 (93.5, M^+-1), 369 (48.4, M^+-15), 255 (20.7), 241 (25.0), 200 (74.5), 186 (12.5), 185 (21.2) and 172 (20.6). Calcd. for $C_{22}H_{28}N_2O_3$ 384.2049. Found : 384.2052. UV: λmax (EtOH) : 226, 240 and 279 nm. The ^{13}C NMR data are presented in Table 2.3.

UC8, **Callophylline B** was isolated and purified by preparative t.l.c. ($CHCl_3$: MeOH, 10 : 1, v/v) and obtained as an amorphous powder. The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 300 Hz) : δ 0.86 (3H, t, $CH_2\underline{CH_3}$), 1.0-4.1 (*ca.* 29H, overlapping m), 3.60 (1H, d, J = 9 Hz, H-21'), 3.73 (3H, s, OMe), 3.73 (3H, s, COOMe), 3.81 (3H, s, COOMe'), 4.16 (1H, br s, H-17'), 4.66 (1H, br s, H-3'), 6.48 (1H, dd, J = 8, 2 Hz, H-12'), 6.6-6.7 (2H, m, H-10 & H-12), 6.9-7.1 (2H, m, H-10' & H-11'), 7.37 (1H, s, H-17), 7.62 (1H, br s, NH), and 8.24 (1H, br s, NH). FABMS, m/z : 753.4763 (MH^+), cacld. for $C_{43}H_{52}N_4O_8$ + H 753.3863. UV : λmax (EtOH) : 227, 290 and 300 nm. The ^{13}C NMR data are presented in Table 2.16.

UC9, Callophylline A was isolated as a white amorphous powder by preparative t.l.c. ($CHCl_3$: MeOH, 10 : 1, v/v). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 300 Hz) : δ 0.87 (3H, t, CH_2CH_3), 1.0-4.1 (*ca.* 29H, overlapping m), 3.57 (1H, d, *J* = 9 Hz, H-21'), 3.75 (3H, s, OMe), 3.75 (3H, s, COOMe), 3.86 (3H, s, COOMe'), 4.69 (1H, br s, H-3'), 6.54 (1H, d, *J* = 8 Hz, H-11), 6.69 (1H, d, *J* = 8 Hz, H-12), 7.12 (1H, dd, *J* = 7, 7 Hz, H-10'), 7.19 (1H, dd, *J* = 7, 7 Hz, H-11'), 7.37 (1H, s, H-17), 7.46 (1H, d, *J* = 7 Hz, H-12'), 7.51 (1H, d, *J* = 7 Hz, H-9'), 7.62 (1H, br s, NH), and 8.02 (1H, br s, NH). FABMS, m/z : 737.4562 (MH^+), cacld. for $C_{43}H_{52}N_4O_7$ + H 737.3914. UV : λmax (EtOH) : 228, 230, 285, 295 and 300 nm. The ^{13}C NMR data are presented in Table 2.14.

UC10, Yohimbine was isolated and purified by preparative t.l.c. (diethyl ether : ethyl acetate, 1:1, v/v). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 1.4-3.5 (*ca.*15H, overlapping m), 3.81 (3H, s, COOMe), 4.22 (1H, br s, H-17), 7.1-7.5 (4H, m, aromatic H) and 7.80 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.18.

UC11, β-Yohimbine was isolated and purified using preparative t.l.c. ($CHCl_3$: MeOH 10 : 1, v/v). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 1.4-3.4 (*ca.*15H, overlapping m), 3.83 (3H, s, COOMe), 7.0-7.5 (4H, m, aromatic H)

and 7.81 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.18.

UC12, Pseudoyohimbine was recrystallized from chloroform, m.p. 268 ^{o}C (lit.[12] 268 ^{o}C). The R_f values are given in Table 3.1. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.3-3.4 (*ca.* 15H, overlapping m), 3.78 (3H, s, COOMe), 4.25 (1H, br s, H-17), 4.45 (1H, br s, H-3), 7.1-7.5 (4H, m, aromatic) and 7.95 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.18.

Extraction of 500 g of stem gave 0.31 g of crude alkaloids, and t.l.c. indicated the presence of three major alkaloids and some baseline alkaloids. The crude alkaloids were subjected to chromatography with silica gel ($CHCl_3$ with increasing MeOH gradient) to yield the three major alkaloids, viz., dihydrocorynantheine, yohimbine and pseudoyohimbine. No further work was carried out on the baseline alkaloids.

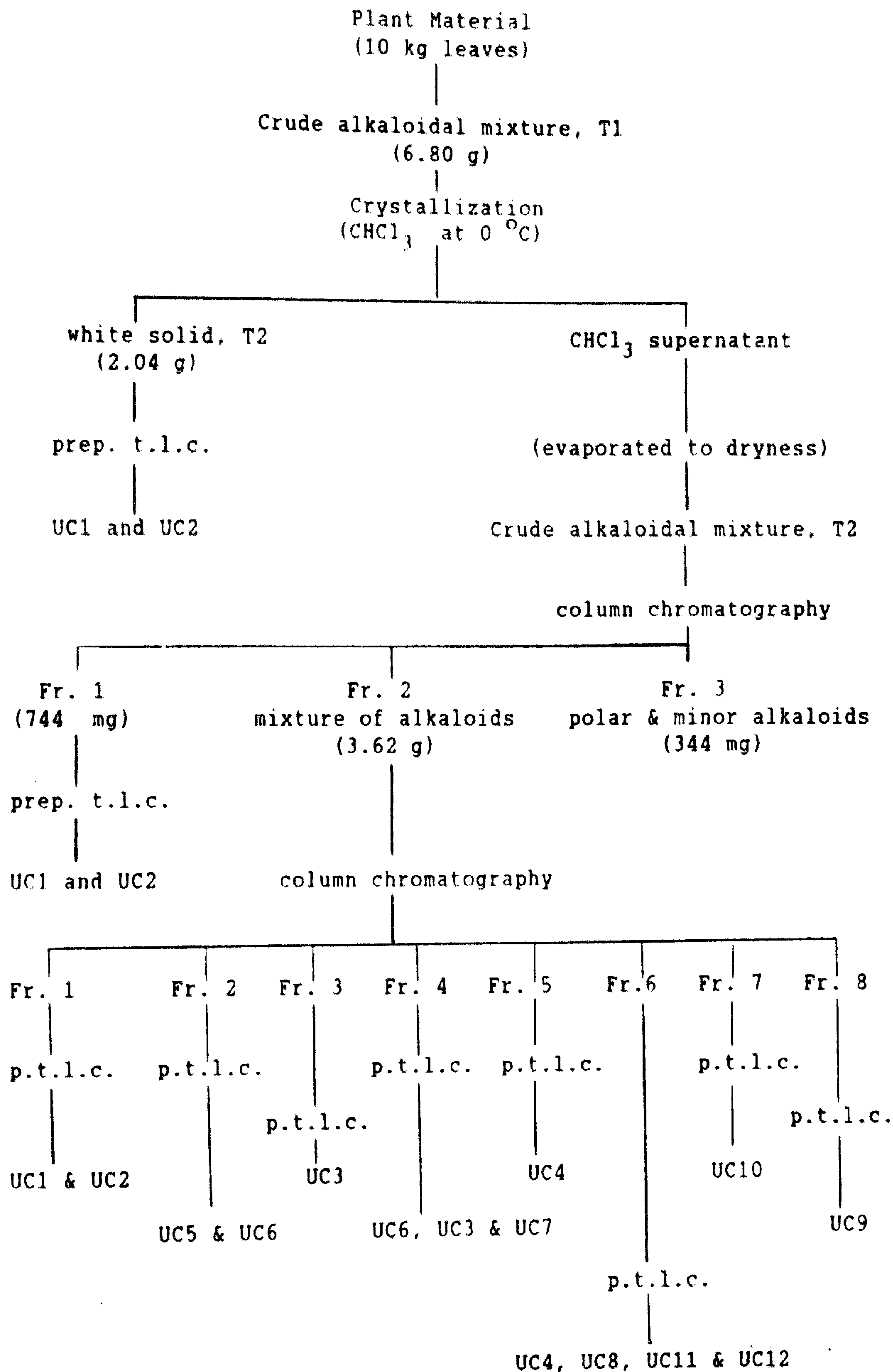

Fig. 3.1 : Isolation of alkaloids from *U. callophylla*

3.5.2 Isolation of alkaloids from *U. borneensis*

Extraction of 1.2 kg. of leaves followed by partitioning into acid and the usual workup gave 952 mg of crude alkaloidal mixture. The crude alkaloidal mixture was shown to consist of three major alkaloids accompained by three or four minor alkaloids.

This mixture was initially chromatographed over silica gel (chloroform with increasing methanol gradient) to give 5 fractions containing partially resolved mixtures of alkaloids. Repeated column chromatography and preparative t.l.c. of these fractions yielded the alkaloids UB1-UB6. The essential procedure is summarized in the flow diagram shown in Figure 3.2.

Fraction 1 yielded one major alkoloid , designated as UB1. Fraction 2 was shown to contain the three major alkaloids, viz., UB1, UB2 and UB3. UB1 was isolated from the mixture by column chromatography (silica gel, diethyl ether with increasing ethyl acetate gradient). UB2 and UB3 eluted together but separation was accomplished by preparative t.l.c. ($CHCl_3$: MeOH, 10:1, 2x development). Fraction 3 contained one major alkaloid, UB4 and several minor alkaloids. Only UB4 was succesfully isolated by preparative t.l.c. (ethyl acetate : diethyl ether, 1:1 followed by $CHCl_3$: MeOH, 10:1). Fraction 4 contained only one major alkaloid, UB5 which was obtained by preparative t.l.c. ($CHCl_3$: MeOH, 10:1; 2x development). Fraction 5 also contained one major alkaloid, UB6, which was isolated by preparative t.l.c. ($CHCl_3$: MeOH, 10:1; 2x development).

UB1 was shown to be actually a mixture of two alkaloids from its ^{1}H and ^{13}C NMR spectra. This mixture proved resistant to further resolution as attempts to separate the alkaloids by t.l.c. in various solvent systems and fractional crystallization were unsuccessful.

The following alkaloids were isolated:

UB1a, Isocorynoxeine was isolated as a mixture with UB1b (isorhychophylline) and its identity was established from ^{13}C NMR spectral data. R_f values are given in Table 3.1. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 0.7-3.5 (11H, overlapping m), 3.57 (3H, s, COOMe), 3.68 (3H, s, OMe), 5.30-5.70 (1H, m, $C\underline{H}=CH_2$), 4.85-5.02 (2H, m, $CH=C\underline{H}_2$), 6.84-7.48 (4H, m, aromatic H), 7.23 (1H, s, H -17), and 8.89 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.9.

UB1b, Isorhynchophylline was isolated as a mixture with UB1a (isocorynoxeine) and its identity was established from ^{13}C NMR data spectral data. R_f values are given in Table 3.1. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 0.4-3.4 (16H, overlapping m) 0.79 (3H, t, $CH_2C\underline{H}_3$), 3.52 (3H, s, $COO\underline{Me}$), 3.60 (3H, s, OMe), 6.80-7.20 (4H, m, aromatic), 7.14 (1H, s, H-17) and 8.98 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.9.

UB2, Corynoxeine was obtained as pale yellow crystals from ether, m.p. 205-206 °C (lit.[61] 212-214 °C). Rf values are given in Table 3.1. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 0.72-3.46 (11H, overlapping m), 3.60 (3H, s, CO_2CH_3), 3.70 (3H, s, OMe), 5.35-

5.69 (1H, m, $-\underline{CH}=CH_2$), 4.86-5.02 (2H, m, $-CH=\underline{CH_2}$), 6.88-7.27 (4H, m, aromatic), 7.22 (1H, s, H-17), and 8.83 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.9.

UB3, Rhynchophylline was obtained as yellowish crystals from benzene, m.p. 206-208 °C (lit.[52] 212-213 °C). R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 0.44-3.45 (16H, overlapping m) 0.77 (3H, t, $CH_2\underline{CH_3}$), 3.55 (3H, s, $COO\underline{Me}$), 3.62 (3H, s, OMe), 6.80-7.20 (4H, m, aromatic), 7.18 (1H, s, H-17) and 8.92 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.9.

UB4, Alloyohimbine was isolated and purified by preparative t.l.c. (ethyl acetate : diethyl ether, 1:1, followed by $CHCl_3$: MeOH, 10:1). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 1.4-3.5 (*ca.* 15H, overlapping m), 3.77 (3H, s, $COO\underline{Me}$), 3.90 (1H, br s, H-17), 7.1-7.5 (4H, m, aromatic H) and 7.82 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.18.

UB5, Pseudoyohimbine was isolated and purified by preparative t.l.c. ($CHCl_3$: MeOH, 10:1, 2x development). Its spectral data are as described previously for *U. callophylla*.

UB6, 3-Epi-β-yohimbine was isolated and purified by preparative t.l.c. ($CHCl_3$: MeOH, 10 : 1, 2x development). The R_f values are given in Table 3.1. ^1H NMR ($CDCl_3$, 100 MHz): δ 1.4-3.5 (*ca.*

15H, overlapping m), 3.82 (3H, s, COOMe), 4.44 (1H, br s, H-3), 7.1-7.5 (4H, m, aromatic) and 8.01 (1H, br s, NH). EIMS, m/z rel. int.) : 354 (84.2, M^+), 353 (100, M^+-1), 295 (6), 223 (4), 197 (3), 184 (10), 170 (8), 169 (13.4) and 156 (9). Cacld. for $C_{21}H_{26}N_2O_3$ 354.1943. Found: 354.1821. The ^{13}C NMR data are presented in Table 2.18.

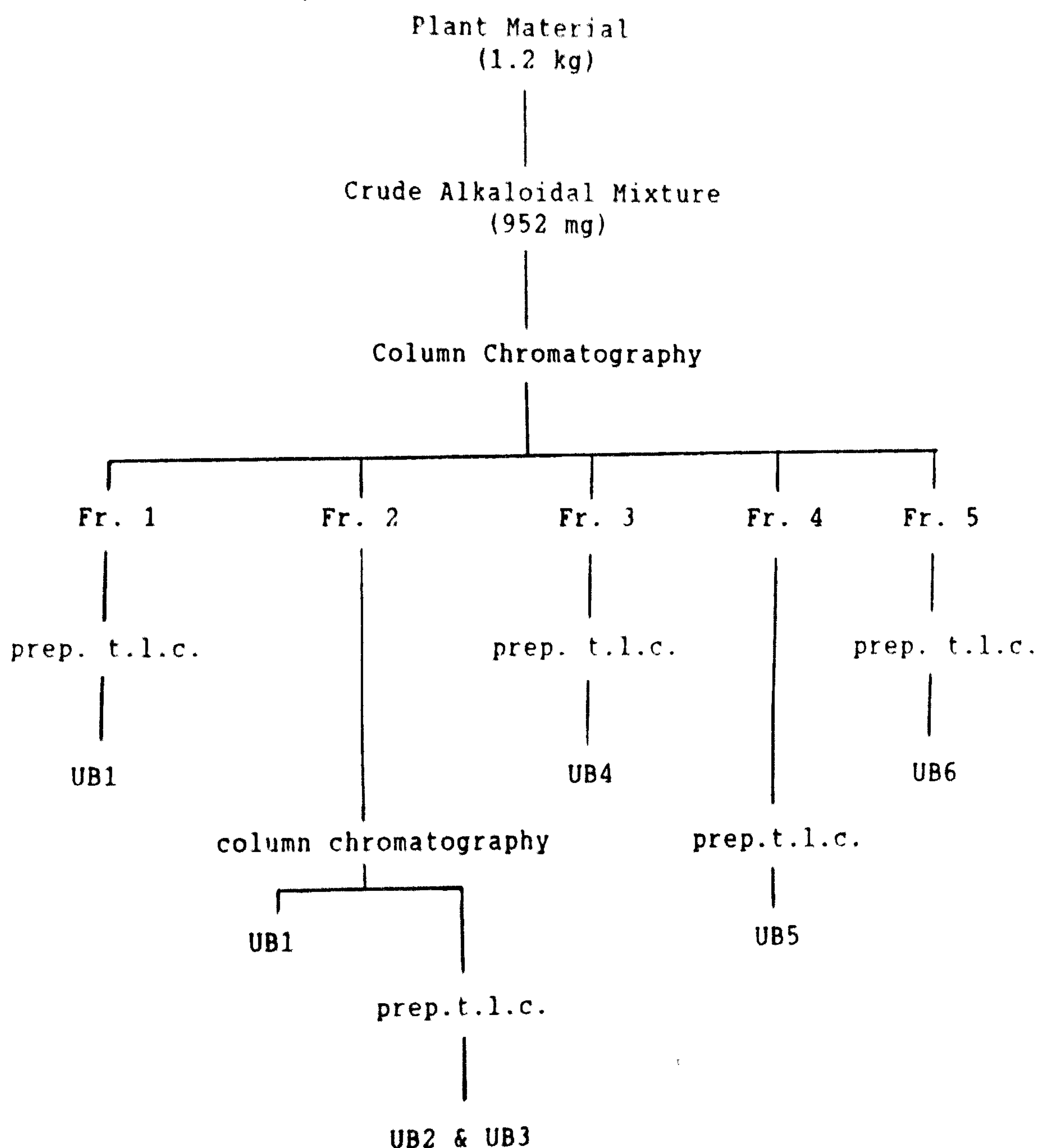

Fig. 3.2 : Isolation of alkaloids from *U. borneensis*

3.5.3 Isolation of alkaloids from *U. longiflora var. longiflora*

Extraction of 250 g of leaves gave 0.96 g of crude alkaloids. The essential procedure is summarized in the flow diagram shown in Fig. 3.3. The crude alkaloidal mixture was chromatographed over silica gel using chloroform with increasing methanol gradient to give three fractions. Fraction 1 had one major alkaloid, designated as UL1. Fraction 2 which contained the major alkaloids was rechromatographed using chloroform with increasing methanol gradient and preparative t.l.c. to give UL1, UL2 and UL3. Fraction 3 contained a mixture of minor polar alkaloids. No further work was carried out on this fraction. UL1 was actually a mixture of two alkaloids which proved resistant to further resolution. Attempts to separate these alkaloids by t.l.c. in various solvent systems and fractional crystallization were not sucessful.

The following alkaloids were isolated:

UL1a, isocorynoxeine and **UL1b, isorhynchophylline** were isolated as a mixture with UL1a as the major component. Their identity were established from ^{13}C NMR data .

UL2, corynoxeine and **UL3, rhynchophylline** were identified by their spectral data (^{1}H & ^{13}C NMR) as described previously for *U. borneensis.*

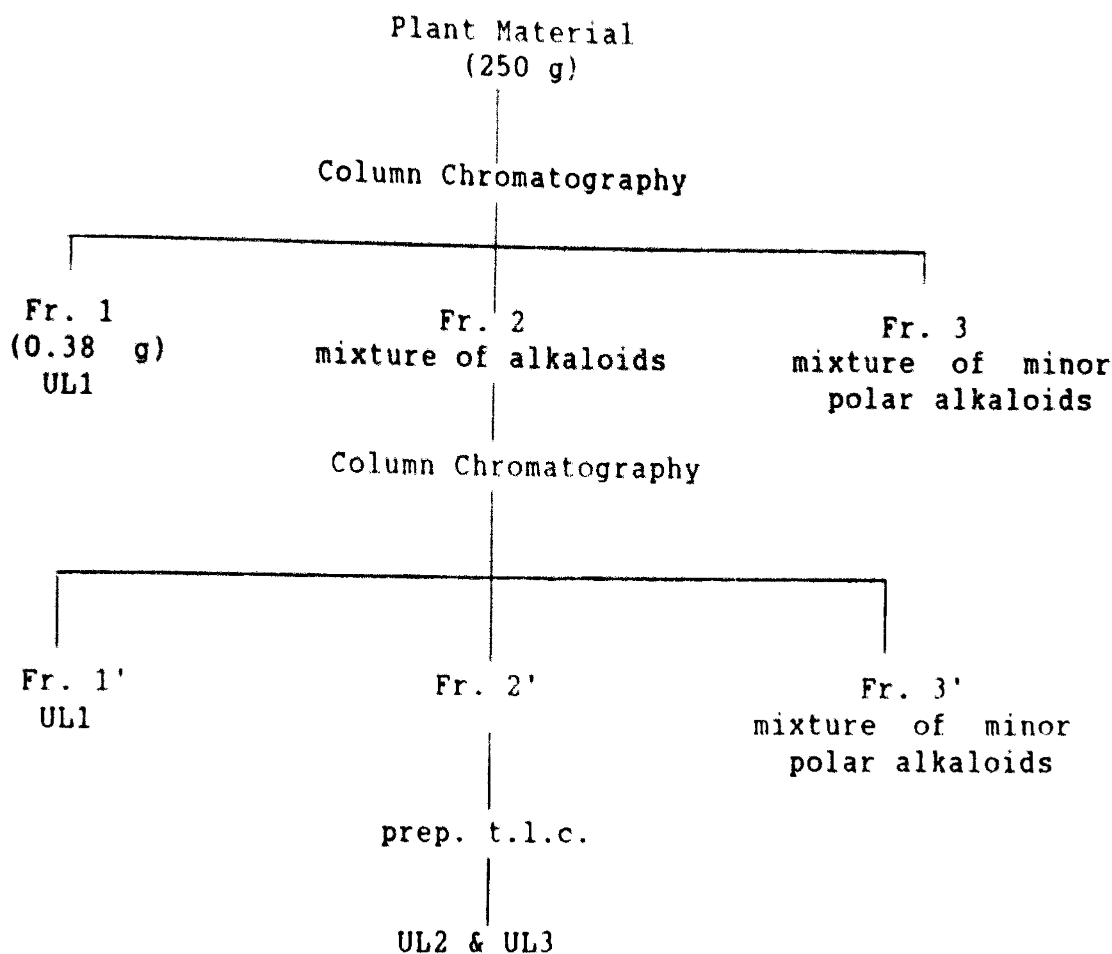

Fig. 3.3: Isolation of alkaloids from *U. longiflora v. longiflora*

3.5.4 Isolation of alkaloids from *U. longiflora var. pteropoda*

Extraction of 500 g of leaves gave 0.5 g of crude alkaloidal extract. The crude alkaloidal extract was initially examined by t.l.c. which showed the presence of two major relatively non-polar components with a third more polar minor component. The crude mixture was chromatographed over silica gel and eluted with dichloromethane with increasing methanol gradient. The two major

components eluted together and were not separated. Separation of the two major alkaloids was accomplished by preparative t.l.c. using ethyl acetate : cyclohexane, 1:1 as a developing solvent, and with two develpoments of the plates. This resulted in the isolation of UP1 and UP2 . The third component, UP3, was obtained in trace amount which was insuffîcent for spectral characterization. UP1 and UP2 were identified as isopteropodine and pteropodine respectively based on their spectral data.

UP1, Isopteropodine was crystallized from benzene as white needles, m.p. 207-209 °C (lit.[27] 209-211 °C). R_f values are given in Table 3.1. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 0.68-3.45 (11H, overlapping m) 1.41 (3H, d, J = 7 Hz, C19-Me) 3.60 (3H, s, COOMe), 4.2-4.5 (1H, m, H-19), 6.80-7.30 (4H, m, aromatic H), 7.41 (1H, s, H-17) and 8.31 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.5.

UP2, Pteropodine was crystallized from benzene as pale yellow rods, m.p. 211-212 °C (lit.[27] 217-219 °C). R_f values are given in Table 3.1. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 0.80-3.50 (11H, overlapping m) 1.33 (3H, d, J = 7 Hz, C19-Me) 3.53 (3H, s, COOMe), 4.3-4.7 (1H, m, C19-H), 6.80-7.20 (4H, m, aromatic H), 7.41 (1H, s, H-17) and 8.40 (1H, br s, NH). The ^{13}C NMR data are presented in Table 2.5.

Extraction of the stem gave a lower yield of alkaloids (0.06 g from 500 g of stem), which was shown to be of similar composition as the leaf.

3.5.5 Isolation of alkaloids from *U. lanosa var. glabrata*

Extraction of the leaves (600 g) gave a brownish gummy alkaloid extract (270 mg). Chromatography of this crude extract followed by preparative t.l.c. (silica gel 60, ethyl acetate : hexane, 1:1, 2x development) afforded two alkaloids, *viz.*, **isopteropodine** and **pteropodine**. One minor alkaloid was also isolated but was insufficient for spectral characterization.

3.5.6 Isolation of alkaloids from *U. lanosa var. ferrea*

Extraction of 500 g of leaves gave 0.4 g of crude alkaloids. Chromatography of this crude extract (silica gel, chloroform with increasing methanol gradient) followed by prepararive t.l.c. (silica gel, ethyl acetate : hexane, 1:.1, 2x development) afforded the two major alkaloids UF1 and UF2.

UF1 and **UF2** were identified as **isopteropodine** and **pteropodine**. from their spectral physical data. The third minor component UF3 was isolated in insufficient amount for spectral characterization.

3.5.7 Isolation of alkaloids from *U. cordata var. cordata f. sundaica*

Extraction of 200 g of leaf and 350 g of stem yielded 210 mg and 126 mg of crude alkaloids respectively. The t.l.c. of both the leaf and stem extracts showed the presence of only one major

alkaloid. Column chromatography of the crude alkaloid (silica gel 60, chloroform with increasing methanol gradient) followed by preparative t.l.c. ($CHCl_3$:MeOH, 10.1) yielded **dihydrocorynantheine.**

3.5.8 Isolation of alkaloids from *U. acida*

Extraction of several samples of *U. acida* collected from different locations consistently tested negative for alkaloids. This is quite consistent with the results previously reported for *U. acida var acida* where either no alkaloids or very small amounts were detected.

3.5.9 Isolation of alkaloids from *U. gambir*

Extraction of 1 kg of leaves and 500 g of stem yielded only 40 mg and 37 mg of crude alkaloids respectively. The t.l.c. chromatogram of both the leaf and stem extacts showed the presence of minor polar alkaloids. The crude alkaloids at this stage contained signficant non-alkaloidal material and the actual yield of alkaloids is estimated to be about 0.00001% Therefore, further separation of these extracts were not carried out and attempts to recollect this species for further study were unsuccessful.

3.5.10 Isolation of alkaloids from *U. elliptica*

Several samples of leaves were screened and the extracts contained a complex mixture of alkaloids as shown by t.l.c. The alkaloid pattern appeared to be consistent between samples. An extraction of 220 g of leaves gave 0.75 g of crude alkaloidal mixture. Chromatography (column chromatography and preparative t.l.c.) of this mixture gave several alkaloids in very small amounts. No further study was carried out due to insufficient quantities of plant material.

3.6 DETERMINATION OF RUTIN CONTENT IN MALAYSIAN *UNCARIA*

Dried plant material (10 g) was extracted with hot 70% ethanol (100 ml) for 30 min and the extract concentrated to dryness. The dried extract was dissolved in hot MeOH (10 ml) and a known volume (0.1-0.5 ml) of the solution was then used for the isolation of rutin by preparative t.l.c. (silica gel, 20 x 20 cm x 0.75 mm, $CHCl_3$/ MeOH / AcOH, 8:2:1). Authentic rutin was applied as reference at one corner of the t.l.c. plates. After development of the plates, the required rutin 'band' was visualised in UV light, scrapped off and then extracted with MeOH (10 ml x 2). The MeOH solution was filtered and evaporated to dryness after which the residue was taken up into 95% EtOH and the UV spectrum obtained. A few drops of 25% ammonia solution was added to induce a bathochromic shift which improved the

spectrum and enhanced the rutin absorption bands. The concentration of rutin was obtained by the standard addition method and the results are shown in Table 3.4.

Table 3.4 : Rutin Content[*] in Malaysian *Uncaria*

Species	% Rutin[#]
U. callophylla	2.6
U. acida	0.6
U. lanosa var. ferrea	0.8
U. gambir	0.4
U. cordata var. cordata f. cordata	0.08
U. cordata var. cordata f. sundaica	-
U. cordata var. ferruginea f. leiantha	0.10
U. elliptica	0.08
U. borneensis	-
U. lanosa var. glabrata	-
U. longiflora var. pteropoda	-
U. longiflora var. longiflora	-

[*] Leaf extracts

[#] % yield based on dry wt.; - not detected