

CHAPTER 5

EXPERIMENTAL

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5.1 General Experimental Procedures

Solvents

The solvents used in this work were dichloromethane, methanol and hexane. All solvents are from AR grade except those that are used for folk extractions (distilled). Other chemicals were hydrochloric acid, ammonia solution and sodium sulphate anhydrous.

Instruments

The ^1H NMR and ^{13}C NMR spectra were recorded in chloroform-D, methanol-D and acetone-D on a JOEL JNM-FX400. Chemical shift were reported in ppm and the coupling constants were given in Hz.

The mass spectra were measured on a JMS 700 spectrometer using NBA as the matrix for FAB analysis. The Automass Thermofinnigan was used for HR ESI⁺ and ESI⁻ analysis. The EIMS spectra were obtained on Shimadzu GC-MS QP2000A spectrometer 70 eV.

The infrared spectra were obtained with chloroform as a solvent on a Perkin Elmer spectrum 2000-FTIR spectrometer. The UV spectra were measured on a UV visible recording spectrophotometer, Model Shimadzu UV-160A with ethanol as a solvent.

The industrial and analytical reagent grade solvent was used for extraction and column chromatography. Silica gel 60 and G-60 70-230 mesh ASTM (Merck 774) were

used for Column Chromatography. Aluminium and glass supported silica gel 60 F₂₅₄ were used for Thin Layer Chromatography and preparative TLC, respectively. The silica and plates were activated at 100°C for one hour and stored in a dessicator until needed. TLC spots were visualized under ultra-violet light (254 nm and 365 nm) followed by spraying with the required spotting reagent.

Reagents

a) Mayer's reagent

Mayer's reagent was used for alkaloid screening. A positive result was indicated by formation of white precipitate under acidic condition. The Mayer's reagent was made as follows:

A solution of mercury (II) chloride (1.4 g) in distill water (60 ml) was mixed into a solution of potassium iodide (5.0 g) in distilled water (10 ml). The mixture was then made up to 150 ml with distilled water.

b) Dragendroff's reagent

Dragendroff's reagent was used for spraying of alkaloids on TLC plate. Orange spots on the developed TLC plates indicated the presence of alkaloids. The Dragendroff's reagent was made as follows:

Solution A: Bismuth (III) nitrate (1.7 g) was dissolved in a mixture of 20 ml glacial acetic acid and 80 ml of distilled water.

Solution B: Potassium iodide (16 g) was dissolved in 40 ml of distilled water.

Spray reagent was made from a stock solution (40 ml) was diluted in a mixture of 40 ml glacial acetic acid and 120 ml distilled water.

5.2 Plant Material

Cryptocarya densiflora was collected at Tembat Ulu, Terengganu. The series number was given KL 5211. The species was identified by the phytochemical team, Chemistry Department, Faculty of Science, University of Malaya. The specimens were deposited at the Chemistry Herbarium, Faculty of Science, University of Malaya.

5.3 Extraction and Isolation of Plant Material

5.3.1 Extraction

The extraction of the plant materials (leaves and barks) were carried out by cold percolation or exhaustive extraction using the soxhlet extractor. The milled dried leaves and barks of the plant were first defatted with hexane for 3 days at room temperature. The n-hexane extract was then dried on the rotary evaporator. After being dried, the plant material was moistened with 28% NH₃ solution and left to soak 2 hours; this was to aggregate the nitrogen-containing compounds in the plant.

They were then successively re-extracted with dichloromethane and methanol and then check with a Mayer's reagent test after each extraction to make sure the extraction was completed. Dichloromethane extract were concentrated under reduced pressure to a volume of about 500 ml and tested for alkaloids content using TLC and spraying with Dragendroff's reagent. The dichloromethane extract were repeatly extracted with a solution of 5% hydrochloric acid until Mayer's test negative. Later it was basified with 10%

ammonia solution to about pH 11 and then re-extracted with dichloromethane. The crude of alkaloids fraction were dried with sodium sulphate anhydrous and evaporated under reduced pressure.

The methanol extract were concentrated under reduced pressure until dry and then acidified by 5% hydrochloric acid solution. The acid solution was then filtered and basified with 10% ammonia solution and then re-extracted with ethylacetate. The residue were dried with sodium sulphate anhydrous and evaporated under reduce pressure.

5.3.2 Isolation and purification

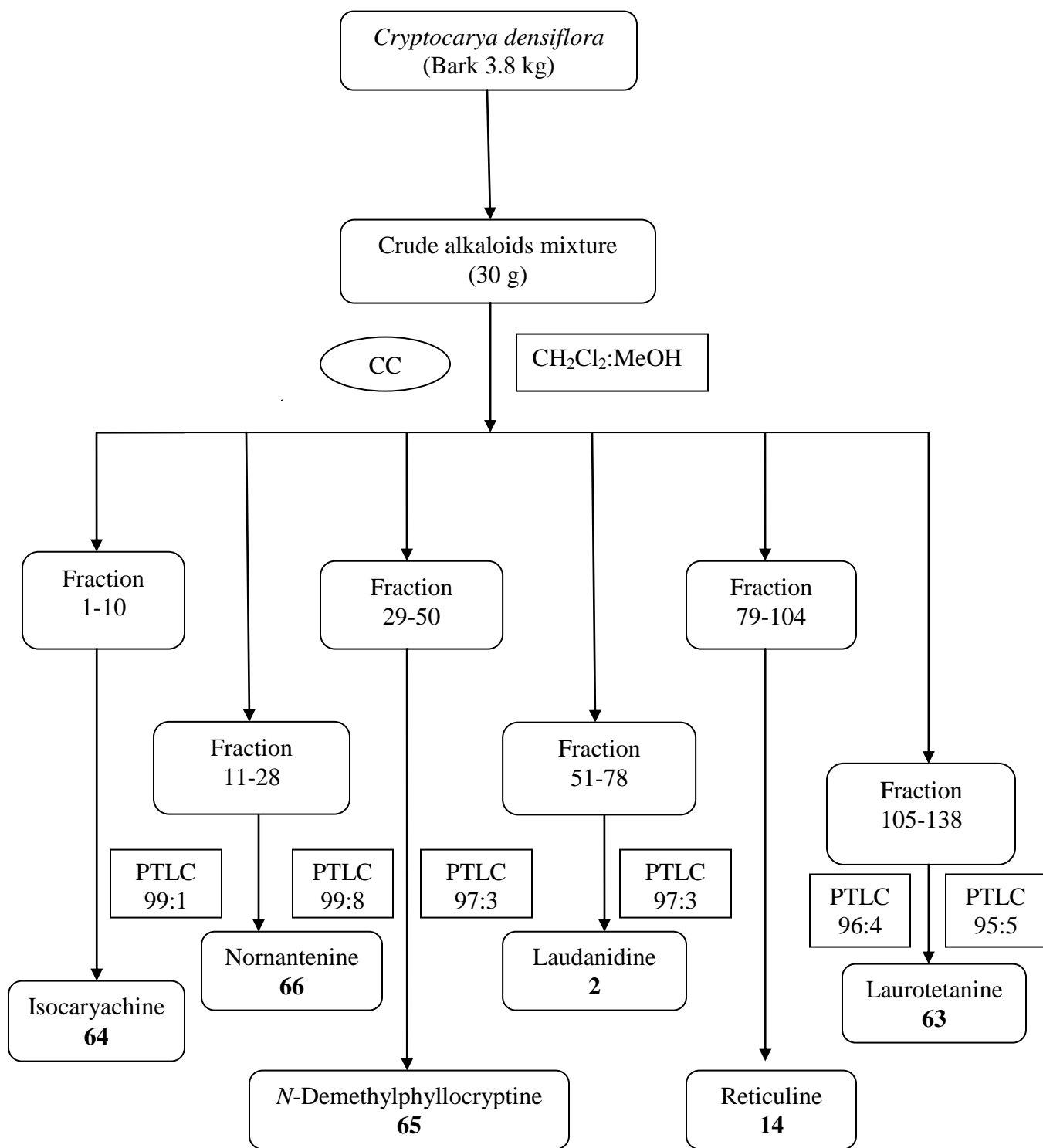
The crude alkaloid was subjected to column chromatography over silica gel 60 as stationary phase. The solvent system used for chromatography was dichloromethane with increasing portion of methanol (gradient elution system). The ratio of the solvent between CH_2Cl_2 and CH_3OH were (100:0; 99:1; 98:2 96:4; 93:7; 90:10; 85:15; 80:10 and 50:50) and finally 100% MeOH. Fractions were collected every 100 ml and each fraction was tested with aluminium TLC plate for their alkaloids. The alkaloid spots were first detected by UV light (254 and 366 nm) and confirmed by spraying with Dragendroff's reagent. Fraction having spots with the same R_f values and stains were combined and treated as a group. The combined groups were then treated separately to isolate and purify its alkaloid content either by extensive column chromatography or preparative TLC. The purified alkaloids were controlled by a single spot on the TLC. The isolation and purification procedures were summarized in the Scheme 5.1 and 5.2.

Table 5.1: Chromatography Results of the Alkaloidal of *Cryptocarya densiflora* (bark)

Compounds	Solvent system (CH ₂ Cl ₂ : MeOH)	Weight/mg
Isocaryachine 64	99:1	8.9 mg
Nornantenine 66	99:8	7.6 mg
<i>N</i> -Demethylphyllodyptine 65	97:3	6.8 mg
Laudanidine 2	97:3	5.3 mg
Reticuline 14	96:4	7.3 mg
Laurotetanine 63	95:5	9.6 mg

Table 5.2: Chromatography Results of the Alkaloidal of *Cryptocarya densiflora* (leave)

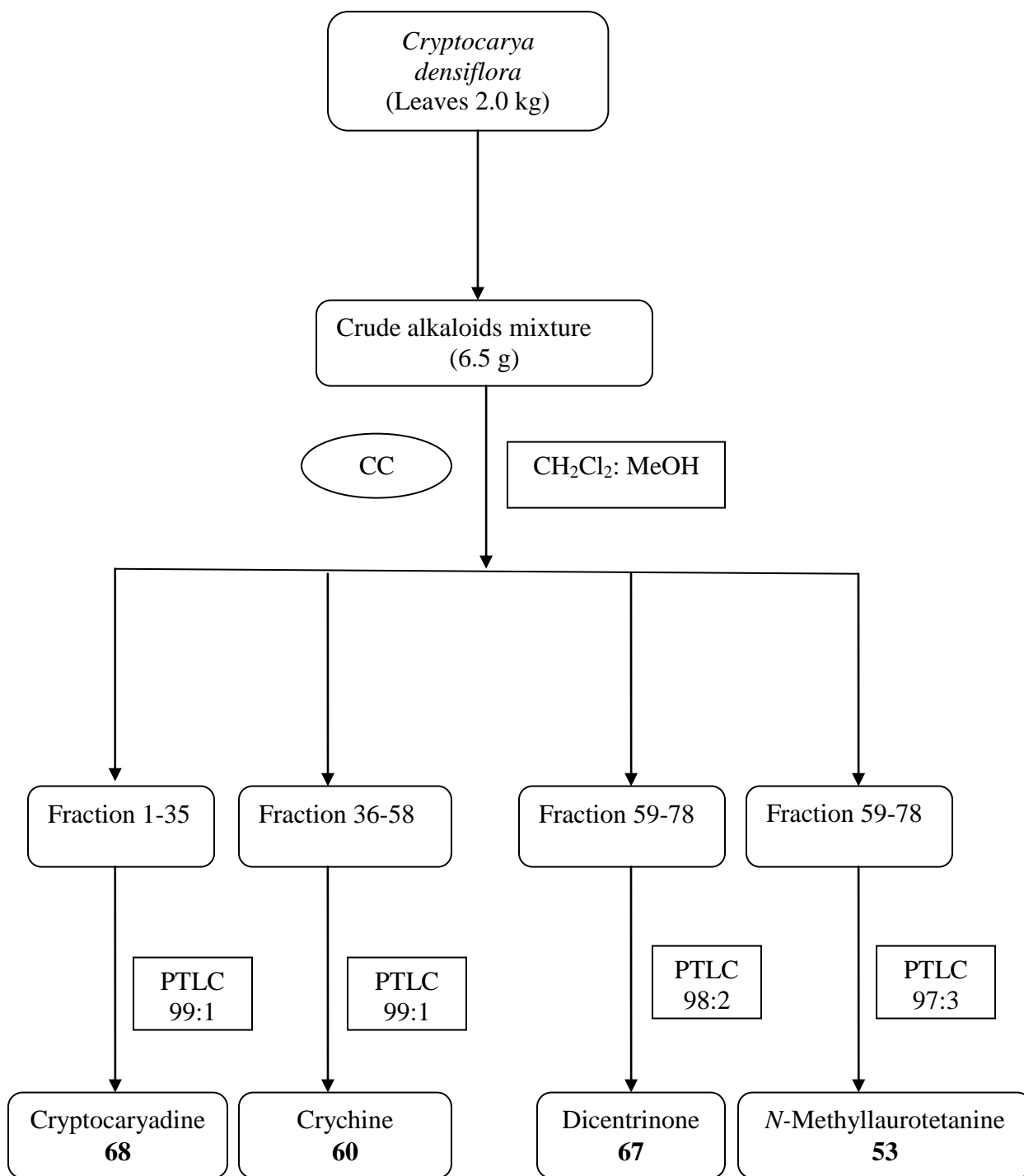
Compounds	Solvent system (CH ₂ Cl ₂ : MeOH)	Weight/mg
Cryptocaryadine 68	99:1	13.7 mg
Crychine 60	99:1	10.6 mg
Dicentrinone 67	98:2	5.3 mg
<i>N</i> -Methylaurotetanine 53	97:3	8.8 mg



CC: column chromatography

PTLC: preparative thin layer chromatography

Scheme 5.1: Isolation and purification of alkaloids from the bark of *Cryptocarya densiflora*.



CC: column chromatography

PTLC: preparative thin layer chromatography

Scheme 5.2: Isolation and purification of alkaloids from the leave of *Cryptocarya densiflora*.

5.4 Physical and Spectral Data of Isolated Compounds

5.4.1 *Cryptocaya densiflora*

Laurotetanine 63	: pale brownish amorphous solid
	: C ₁₉ H ₂₁ NO ₄
UV _{max} (MeOH), nm	: 220, 281, 302 and 312 nm
IR _{vmax} cm ⁻¹	: 3429.87 cm ⁻¹
Mass spectrum m/z	: 328.16
¹ H NMR (CDCl ₃) δ, ppm	: 6.57 (1H, <i>s</i> , H-3), 2.74 (1H, <i>dd</i> , <i>J</i> = 13.68, 4.64 Hz, H-4), 3.01(1H, <i>dd</i> , <i>J</i> = 12.92, 4.1 Hz, H-5 _α), 3.65 (1H, <i>m</i> , H-5 _β), 3.80 (1H, <i>dd</i> , <i>J</i> = 4.40, 13.20 Hz, H-6a), 2.64 (1H, <i>d</i> , <i>J</i> = 13.68, H-7), 6.77 (1H, <i>s</i> , H-8), 8.06 (1H, <i>s</i> , H-11), 3.64 (3H, <i>s</i> , 1-OMe), 3.86 (3H, <i>s</i> , 2-OMe), 3.87 (3H, <i>s</i> , 10-OMe).
¹³ C NMR (CDCl ₃) δ ppm	: 144.31 (C-1), 126.83 (C-1a), 127.42 (C-1b), 152.23 (C-2), 110.83 (C-3), 129.00 (C-3a), 29.09 (C-4), 43.14 (C-5), 53.78 (C-6a), 36.59 (C-7), 129.79 (C-7a), 113.97 (C-8), 145.37 (C-9), 144.98 (C-10), 111.36 (C-11), 124.05 (C-11a), 60.28 (1-OMe), 56.11 (2-OMe), 55.92 (10-OMe).

Isocaryachine 64	: brown amorphous
	: C ₁₉ H ₁₉ NO ₄
UV _{max} (MeOH), nm	: 280 and 219 nm
IR _{vmax} cm ⁻¹	: 3435.96 cm ⁻¹ 1229.50 and 925.10 cm ⁻¹
Mass spectrum m/z	: 326.10
¹ H NMR (CDCl ₃) δ, ppm	: 6.51 (1H, <i>d</i> , <i>J</i> = 4.16 Hz, H-1), 6.42 (1H, <i>s</i> , H-4), 2.49 (1H, <i>d</i> , <i>J</i> = 16.08, H-5 _α), 3.24-3.34 (1H, <i>m</i> , H-5 _β), 3.87 (1H, <i>d</i> , <i>J</i> = 8.32 Hz, H-6), 6.51 (1H, <i>d</i> , <i>J</i> = 4.16 Hz, H-7), 6.35 (1H, <i>s</i> , H-10), 2.49 (1H, <i>d</i> , <i>J</i> = 16.08 Hz, H-11 _α), 3.24-3.34 (1H, <i>m</i> , H-11 _β), 3.87 (1H, <i>d</i> , <i>J</i> = 8.32 Hz, H-12), 3.78 (3H, <i>s</i> , 8-OMe), 2.44 (3H, <i>s</i> , 6,12-NMe), 5.74 (2H, <i>d</i> , <i>J</i> = 1.2 Hz, 2,3-OCH ₂ O).
¹³ C NMR (CDCl ₃) δ ppm	: 109.05 (C-1), 130.90 (C-1a), 145.71 (C-2), 146.03 (C-3), 108.38 (C-4), 124.34 (C-4a), 34.18 (C-5), 56.50 (C-6), 128.97 (C-6a), 106.81 (C-7), 144.17 (C-8), 145.05 (C-9), 114.24 (C-10), 124.72 (C-10a), 32.94 (C-11), 56.13 (C-12), 55.71 (8-OMe), 40.59 (6,12-NMe), 100.31 (2,3-OCH ₂ O).

<i>N</i>-demethylphyllocryptine 65	: dark brown amorphous
	: C ₁₉ H ₂₁ NO ₄
UV _{max} (MeOH), nm	: 286 nm
IR _{vmax} cm ⁻¹	: 3419.45 cm ⁻¹
Mass spectrum m/z	: 328.15
¹ H NMR (CDCl ₃) δ, ppm	: 3.66 (1H, <i>t</i> , 6.12 Hz, H-1), 2.73-2.75 (1H, <i>m</i> , H-3 _α), 3.11-3.17 (1H, <i>m</i> , H-3 _β), 2.56 (1H, <i>m</i> , H-4 _α), 2.82 (<i>dd</i> , <i>J</i> = 5.92, 16.00 Hz, H-4 _β), 6.51 (1H, <i>s</i> , H-5), 6.22 (1H, <i>s</i> , H-8), 3.01 (1H, <i>dd</i> , <i>J</i> = 5.88, 13.92 Hz, H-α), 2.73 (1H, <i>d</i> , 6.88 Hz, H-1'), 6.74 (1H, <i>d</i> , 1.96 Hz, H-2'), 6.72 (1H, <i>d</i> , 8.08 Hz, H-5'), 6.56 (1H, <i>dd</i> , 1.92, 8.28 Hz, H-6'), 5.83 (2H, <i>d</i> , <i>J</i> = 1.44 Hz, 6,7-OCH ₂ O) 3.84 (3H, <i>s</i> , 4'-OMe), 2.45 (3H, <i>s</i> , NMe)
¹³ C NMR (CDCl ₃) δ ppm	: 65.11 (C-1), 46.37 (C-3), 25.41 (C-4), 126.95 (C-4a), 108.43 (C-5), 145.43 (C-6), 145.43 (C-7), 107.94 (C-8), 130.50 (C-8a), 41.07 (C-α), 133.03 (C-1'), 115.65 (C-2'), 145.43 (C-3'), 145.09 (C-4'), 110.51 (C-5'), 120.98 (C-6'), 100.61 (6,7-OCH ₂ O), 55.99 (4'-OMe), 42.38 (<i>N</i> -Me).

Nornantenine 66	: brownish amorphous solid
	: C ₁₉ H ₁₉ NO ₄
UV _{max} (MeOH), nm	: 218, 282, and 308 nm
IR _{vmax} cm ⁻¹	: 929 and 1040 cm ⁻¹
Mass spectrum m/z	: 326
¹ H NMR (CDCl ₃) δ, ppm	: 6.60 (1H, <i>s</i> , H-3), 2.70 (1H, <i>m</i> , H-4 _α), 3.01 (1H, <i>d</i> , <i>J</i> = 8.68Hz, H-4 _β), 3.01 (1H, <i>d</i> , 8.68 Hz, H-5 _α), 3.37 (H-1, <i>d</i> , 6.88 Hz, H-5 _β), 3.80 (1H, <i>dd</i> , 5.04, 13.28 Hz, H-6a), 2.70 (1H, <i>m</i> , H-7), 6.72 (1H, <i>s</i> , H-8), 7.95 (1H, <i>s</i> , H-11), 3.66 (3H, <i>s</i> , 1-OMe), 3.87 (3H, <i>s</i> , 2-OMe), 5.96 (2H, <i>d</i> , <i>J</i> = 3.64 Hz, 9,10-OCH ₂ O).
¹³ C NMR (CDCl ₃) δ ppm	: 144.94 (C-1), 126.76 (C-1a), 128.02 (C-1b), 152.37 (C-2), 111.23 (C-3), 128.81 (C-3a), 29.08 (C-4), 43.11 (C-5), 53.72 (C-6a), 37.47 (C-7), 130.55 (C-7a), 108.28 (C-8), 146.68 (C-9), 146.68 (C-10), 109.05 (C-11), 125.66 (C-11a), 60.32 (1-OMe), 55.98 (2-OMe), 100.96 (9,10-OCH ₂ O).

Dicentrinone 67 : yellow solid
 : $C_{19}H_{13}NO_5$
 UV_{max} (MeOH), nm : 295 nm
 IR_{vmax} cm^{-1} : 1262 and 983 cm^{-1}
 Mass spectrum m/z : 336.08
¹H NMR (CDCl₃) δ, ppm : 7.18, (1H, *s*, H-3), 7.76 (1H, *d*, *J* = 5.36 Hz),
 8.87 (1H, *d*, *J* = 5.36 Hz, H-5), 7.99 (1H, *s*,
 H-8), 8.67 (1H, *s*, H-11), 6.13 (2H, *s*, 1,2-
 OCH₂O), 3.99 (3H, *s*, 9-OMe), 4.08 (3H, *s*,
 10-OMe).

Cryptocaryadine 68 : pale brownish amorphous solid
 : $C_{22}H_{25}NO$
 UV_{max} (MeOH), nm : 302 nm
 IR_{vmax} cm^{-1} : 3448.18 cm^{-1}
 Mass spectrum m/z : 352.19
¹H NMR (CDCl₃) δ, ppm : 2.40 (1H, *d*, *J* = 10.52 Hz, H-1), 6.59 (1H, *s*,
 H-4), 6.43 (*d*, *J* = 1.36 Hz, H-5), 6.58 (*d*, *J* =
 1.84, H-8), 3.09 (*d*, *J* = 16.48 Hz, H- α') 3.84
 (1H, *d*, *J* = 16.48 Hz, H- α''), 6.82 (1H, *d*, *J* =
 8.68, H-2'), 6.60 (1H, *d*, *J* = 8.68, H-3'), 6.60
 (1H, *d*, *J* = 8.68, H-5'), 6.82 (*d*, *J* = 8.68, H-
 6'), 2.27 (1H, *dd*, *J* = 8.72, 17.88 Hz, H-7' α),
 3.29 (1H, *t*, 9.16 Hz, H-7' β), 1.59 (1H, *m*, H-

8'_α) 2.08 (1H, *m*, H-8'_β), 1.83 (1H, *m*, H-9'_α),
1.95 (1H, *m*, H-9'_β), 2.48 (1H, *m*, H-10'_α),
2.70 (1H, *d*, *J* = 16.0 Hz, H-10'_β), 3.48 (3H, *s*,
6-OMe), 3.75 (3H, *s*, 7-OMe).

¹³C NMR (CDCl₃) δ ppm : 60.70 (C-1), 132.4 (C-3), 120.8 (C-4), 134.8
(C-4a), 112.9 (C-5), 147.9 (C-6), 147.2 (C-7),
110.5 (C-8), 132.5 (C-8a), 57.8 (C-α), 132.3
(C-1'), 130.2 (C-2'), 115.3 (C-3'), 155.4 (C-
4'), 115.3 (C-5'), 130.2 (C-6'), 54.2 (C-7'),
30.5 (C-8'), 37.8 (C-9'), 21.4 (C-10'), 55.5
(6-OMe), 55.7 (7-OMe).

Crychine 60 : brownish amorphous solid

C₁₉H₁₇NO₄

UV_{max} (MeOH), nm

IR_{vmax} cm⁻¹ 935.85, 1039.51 cm⁻¹

Mass spectrum *m/z* : 324.12

¹H NMR (CDCl₃) δ, ppm : 6.55 (2H, *s*, H-1), 6.40 (2H, *s*, H-4), 2.52 (2H,
d, *J* = 16.60 Hz, H-5_α), 3.34 (2H, *dd*, *J* = 5.88,
16.12 Hz, H-5_β), 3.93 (1H, *d*, *J* = 5.6 Hz, H-
6), 6.55 (2H, *s*, H-7), 6.40 (2H, *s*, H-10), 2.52
(1H, *d*, *J* = 16.60 Hz, H-11_α), 3.34 (1H, *dd*, *J*
= 5.88, 16.12 Hz, H-11_β), 3.93 (2H, *d*, *J* = 5.6
Hz, H-12), 2.49 (3H, *s*, 6,12-NMe), 5.79 (2H,

	<i>d</i> , <i>J</i> = 1.20 Hz, 2/3-OCH ₂ O), 5.84 (2H, <i>d</i> , <i>J</i> = 1.20 Hz, 8/9-OCH ₂ O).
¹³ C NMR (CDCl ₃) δ ppm	: 107.09 (C-1), 130.87 (C-1a), 146.08 (C-2), 146.45 (C-3), 108.73 (C-4), 124.94 (C-4a), 34.13 (C-5), 56.74 (C-6), 130.87 (C-6a), 107.09 (C-7), 146.08 (C-8), 146.45 (C-9), 108.73 (C-10), 124.94 (C-10a), 34.13 (C-11), 56.74 (C-12), 40.87 (6,12- <i>N</i> -Me), 100.68 (2/3 and 8/9-OCH ₂ O).
Reticuline 14	: brownish amorphous solid
	: C ₁₉ H ₂₃ NO ₄
UV _{max} (MeOH), nm	: 293 nm
IR _{vmax} cm ⁻¹	: 3350 cm ⁻¹
Mass spectrum m/z	: 329
¹ H NMR (CDCl ₃) δ, ppm	: 3.63-3.66 (1H, <i>m</i> , H-1), 2.69-3.18 (2H, <i>m</i> , H-3), 2.53-2.82 (2H, <i>m</i> , H-4), 6.52 (1H, <i>s</i> , H-5), 6.38 (1H, <i>s</i> , H-8), 2.69-3.02 (2H, <i>m</i> , H-α), 6.75 (<i>d</i> , <i>J</i> = 1.96 Hz, H-2'), 6.71 (1H, <i>d</i> , <i>J</i> = 8.32 Hz, H-5'), 6.57 (1H, <i>dd</i> , <i>J</i> = 1.96, 8.04 Hz, H-6'), 3.83 (3H, <i>s</i> , 4'-OMe), 3.83 (3H, <i>s</i> , 6-OMe), 2.43 (3H, <i>s</i> , NMe).
¹³ C NMR (CDCl ₃) δ ppm	: 64.60 (C-1), 46.75 (C-3), 24.93 (C-4), 129.99

(C-4a), 110.50 (C-5), 145.10 (C-6), 143.45 (C-7), 113.74 (C-8), 125.15 (C-8a), 41.03 (C- α), 133.06 (C-1'), 115.65 (C-2'), 145.23 (C-3'), 145.36 (C-4'), 110.62 (C-5'), 121.01 (C-6'), 55.98 (4'-OMe), 55.98 (6-OMe), 42.43 (N-Me).

Laudanidine 2 : pale brownish amorphous solid

: $C_{20}H_{25}NO$

UV_{max} (MeOH), nm : 298 and 244 nm

IR_{vmax} cm^{-1} : 3390 cm^{-1}

Mass spectrum m/z : 344.18

1H NMR ($CDCl_3$) δ , ppm : 3.67 (1H, *dd*, $J = 5.2, 7.8$ Hz, H-1), 2.78-2.73 (1H, *m*, H-3_a), 3.20-3.14 (1H, *m*, H-3 _{β}), 2.86-2.80 (1H, *m*, H-4 _{α}), 4.5 (1H, *d*, $J = 16.0$ Hz, H-4 _{β}), 6.53 (1H, *s*, H-5), 6.03 (1H, *s*, H-8), 2.68 (1H, *dd*, $J = 7.9, 13.7$ Hz, H- α), 3.11 (1H, *dd*, $J = 5.2, 13.7$, H- α'), 6.75 (1H, *d*, $J = 2.07$ Hz, H-2'), 6.70 (1H, *d*, $J = 8.17$, H-5'), 6.50 (1H, *dd*, $J = 2.07, 8.17$ Hz, H-6'), 3.80 (3H, *s*, 6-OMe), 3.54 (3H, *s*, 7-OMe), 3.82 (3H, *s*, 4'-OMe), 2.49 (3H, *s*, NMe).

<i>N</i>-methyllaurotetanine 53	: brown amorphous
	: C ₂₀ H ₂₃ NO ₄
UV _{max} (MeOH), nm	: 303, 248 and 227 nm
IR _{vmax} cm ⁻¹	: 3465 and 2998 cm ⁻¹
Mass spectrum m/z	: 342.20
¹ H NMR (CDCl ₃) δ, ppm	: 6.58 (1H, <i>s</i> , H-3), 2.68 (<i>d</i> , <i>J</i> = 3.64, H-4 _α), 3.16 (1H, <i>m</i> , H-4 _β), 2.51 (1H, <i>d</i> , 3.64Hz, H- 5 _α), 3.03 (1H, <i>dd</i> , <i>J</i> = 5.96, 11.92 Hz, H-5 _β), 2.99 (1H, <i>m</i> , H-6a), 2.64 (1H, <i>d</i> , <i>J</i> = 3.2 Hz, H-7 _α), 2.95 (1H, <i>d</i> , <i>J</i> = 4.12 Hz, H-7 _β), 6.82 (1H, <i>s</i> , H-8), 8.06 (1H, <i>s</i> , H-11), 3.65 (3H, <i>s</i> , 1-OMe), 3.88 (3H, <i>s</i> , 2-OMe), 3.90 (3H, <i>s</i> , 10-OMe), 2.54 (3H, <i>s</i> , <i>N</i> -Me).
¹³ C NMR (CDCl ₃) δ ppm	: 144.22 (C-1), 127.10 (C-1a), 127.20 (C-1b), 152.00 (C-2), 110.31 (C-3), 128.93 (C-3a), 29.27 (C-4), 53.38 (C-5), 62.63 (C-6a), 34.32 (C-7), 130.18 (C-7a), 113.96 (C-8), 144.88 (C-9), 145.32 (C-10), 111.24 (C-11), 124.04 (C-11a), 60.24 (1-OMe), 55.85 (2-OMe), 56.13 (10-OMe), 44.04 (<i>N</i> -Me).