CHAPTER IV

EXPERIMENTAL

Source of Plant Material

The plant material of Strychnos ignatii Berg. was collected from Ao Luk District, Krabi Province, Southern Southern Thailand during March 1984, and was anthenticated by comparision with the herbarium specimens Collection NO. Kerr 18582 March 1930, at Botany section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

A voucher specimen of the plant material has been deposited in the Department of Pharmaceutical Botany, the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

General Techniques

1. Chromatographic Techniques

1.1 Routine Thin-Layer Chromatography (TLC)

Technique	: one way ascending
Adsorbent	: silica gel G (E. Merk) 30 g in 60 ml distilled water
Plate sizes	: 10 cm x 20 cm or 20 cm x 20 cm
Layer thickness	: 0.25 mm

Activation : air-dried for 15 minutes and then warmed in hot aired oven at 110°C for 15 min

Solvent system : 1) $CHCl_3$:EtOAc:MeOH:Conc.NH_4OH (80:10:10:1)

2) EtOAc:Isopropanol:Conc.NH_4OH (80:15:5)

3) $CHCl_3$:MeOH:Conc.NH_4OH (90:10:1)

4) EtOAc:Isopropanol:Conc.NH_4OH (45:35:5)

5) $CHCl_3$:MeOH (1:3)

6) EtOAc:$CHCl_3$:MeOH (6:1:1)

Distance : 15 cm

Laboratory temperature : 28°-35°C

Development : The plates were developed in a Chromatographic tank lined with filter paper.

Detection : 1) Dragendorff's spray reagent

2) Ferric chloride - perchloric acid spray reagent

1.2 Preparative Thin-Layer Chromatography (PLC)

Technique	: one way ascending
Adsorbent	: mixture of silica gel G (E.Merck) and silica gel GF$_{254}$ (E.Merck) (3:1)
Plate sizes	: 20 cm x 20 cm and 20 cm x 40 cm
Layer thickness	: 0.50 mm
Solvent system	: 1) $CHCl_3$:EtOAc:MeOH:Conc.NH_4OH (80:10:10:1) 2) EtOAc:Isopropanol:Conc.NH_4OH (80:15:5)
Distance	: 20 cm
Laboratory temperature	: 28°-30°C
Development	: The plates were developed in a Chromatographic tank lined with filter paper
Detection	: 1) Short wavelength UV light 2) Dragendorff's spray reagent 3) Ferric chloride-Perchloric acid spray reagent
Substance Recovering	: The scraped off zones were warmed with a mixture of $CHCl_3$: MeOH (1:1), and filtered. After removal of the solvent, the residues were taken up in $CHCl_3$ and filtered.

1.3 Spraying Reagent

1) Dragendorff's spray reagent

This reagent was used as a general alkaloid detecting reagent which characterized the alkaloids by giving orange colour.

The stock solution consisting of a mixture of bismuth oxynitrate 1.7 g glacial acetic acid 20 ml, distilled water 80 ml and 5% aqueous potassium iodide 100 ml

The working solution was made by mixing 10 ml of stock solution with 20 ml glacial acetic acid and 70 ml distilled water.

2) Ferric chloride-perchloric acid spray reagent

This reagent was made by mixing 1 ml 0.5 M ferric chloride solution with 100 ml 35% aqueous perchloric acid solution.

The reagent gave a range of colours depending on the nature of the substitution patterns in the aromatic part of the N-acylindoline nucleus and also on the difference type of alkaloid sketetons. The colours would either develop immediately after spraying or only after heating the chromatographic plates at 90 ^{0} C for 5 to 30 min .

1.4 Column Chromatography (CC)

Column sizes : The glass columns 3 cm-10 cm in diameter were used depending on the quantity of sample to be seperated.

Adsorbent : 1) silica gel 60 (E. Merck)
2) neutral alumina activity I(E. Merck)

Packing method : wet packing

Solvent : Several solvents were used for difference materials and the details were demonstrated later.

2. Reference Compounds

The reference compounds, strychnine 54 and brucine 55 were available in the laboratory of the Department of Pharmacognosy, the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3. Melting point

Melting points were determined on a Gallenkamp Melting point apparatus Model MFB 595.

The melting points are uncorrected.

4. Spectroscopy

4.1 Ultraviolet (UV) Spectroscopy

Ultraviolet absorption spectra were determined on a Bausch & Lomb spectrophotometer 2000.

4.2 Infrared (IR) Spectroscopy

Infrared absorption spectrum were recorded as KBr disc. on a Perkin Elmer Model 283 Spectrophotometer. The absorption bands were reported in wave number (cm^{-1})

4.3 Nuclear Magnetic Resonances (NMR) Spectroscopy

The ^{1}H NMR and ^{13}C NMR spectra were obtained with 3 different instruments :

4.3.1) Nuclear Magnetic Resonance spectrometer Model FX 900 (JEOL) at 90 MHz for ^{1}H NMR and ^{13}C NMR of S1 and S2

4.3.2) Nuclear Magnetic Resonance spectrometer Model GX 270 (JEOL) at 270 MHz for ^{1}H NMR and ^{13}C NMR of G1

4.3.3) Nuclear Magnetic Resonance spectrometer Model IBM 270 at 270 MHz for ^{1}H NMR of S1, S2, G1 and G2

Tetramethylsilane was used as an internal standard (δ = 0.00 ppm).

4.4 Mass Spectroscopy

The low resolution mass spectra were obtained on a Mass spectrometer Model DX 300 (JEOL). A direct inlet system operating at 70 eV with the temperature range between 150^{0} to 300^{0} was used.

5. Solvents

The solvents of commercial grade were redistilled before used.

Extraction Procedure

Air-dried and ground stem bark 800 g was exhaustively extracted in a Soxhlet apparatus with (10x4 litre) methanol. The methanolic extracts were concentrated under reduced pressure to dryness (176.3 g) and then ground with Kieselguhr and exhaustively eluted with chloroform and followed by 2% conc. ammonium hydroxide in methonol.

The chloroform eluate was concentrated under reduced pressure to obtain about 100 ml of concentrated extract. The extract was basified with conc. ammonium hydroxide and then partitioned with (2x20 ml) distilled water. The chloroform portion was finally evaporated under reduced pressure to dryness to yield crude tertiary alkaloid 39.5 g (4.94%), coded as CA.

The basic methanolic eluate was dried under reduced pressure to yield a residue, coded as MA.

The Soxhlet-extracted marc was soaked with 200 ml, 2% conc. ammonium hydroxide for overnight and extracted with methanol in a Soxhlet apparatus for 30 hours. The methanolic extract was concentrated under reduced pressure to yield about 200 ml of dark viscous liquid and was combined with MA. The combined extract was basified to pH 9 with conc. ammonium hydroxide and extracted with (5x500 ml) chloroform. The chloroform extract was evaporated under reduced pressure to dryness

to yield crude tertiary alkaloid 32.8 g (4.1%), coded as CB. The remaining aqueous portion was concentrated under reduced pressure to yield a residue of tertiary and quaternary alkaloids 152.8 g (19.78%), coded as PA.

TLC of the CA fraction in solvent system 1 showed the presence of 4 spots of alkaloids.

TLC of the CB fraction in solvent system 1 showed the presence of at least 9 spots of alkaloids. Five of which gave blue colour with the ferric chloride-perchloric acid spray reagent.

TLC of the PA fraction in solvent system 1 showed many spots of alkaloids near the base line. Some of which gave blue colour with the ferric chloride-perchloric acid spray reagent. This alkaloid fraction was not further investigated.

Isolation of Individual Alkaloids

1. Isolation of S1 and S2 from Crude CA

1.1 Isolation of S1

The crude CA (39.5 g) was dissolved in ethyl acetate and filtered. The ethyl acetate insoluble precipitate (15.3 g) and the filtrate were collected separately.

The precipitate was further crystallized in the mixture of chloroform and methanol to yield prismatic crystals of S1 (10.3318 g). S1 gave a pink colour with the spray reagent 2.

The ethyl acetate portion was evaporated under reduced pressure to yield a residue A (23.4 g). Residue A was later subjected to colomn chromatography for the isolation of alkaloid S2.

1.2 Isolation of S2

The alkaloid S2 was isolated by column and preparative thin-layer chromatography techniques.

Column chromatography

First column

The alkaloid mixture (13.0 g) was fractionated under gravity on an alumina column. The flat bottom glass column (6.5 cm in diameter) contained neutral alumina activity I (180 g) and the subsequently eluents ; benzene : chloroform (1:1), chloroform , chloroform : methanol (1:1) and methanol were used. The (120x100 ml) fractions were collected as shown in the following table :-

Fraction	Eluent
1 - 9	
10 - 15	Benzene:$CHCl_3$ (1:1)
16 - 70	
71 - 98	$CHCl_3$
99 -102	$CHCl_3$:MeOH (1:1)
103 -120	MeOH

Combined fraction 16-70 (9.3581 g) showed the presence of a pink and a yellow colour spots on checked tlc in system 1 (spray reagent 2). The fraction was dissolved in the mixture of ethyl acetate and methanol and filtered. The insoluble material S1 (2.4992 g) was collected.

The filtrate was evaporated under reduced pressure to give an alkaloidal residue B (6.8589 g). This residue was further investigated by using second column chromatography.

Second Column

The residue B (6.8589 g) was subjected to silica gel column. The column (6.5 cm in diameter) was packed with (200 g) silica gel 60 and eluted with the solvent chloroform: ethyl acetate:methanol (80:10:10). The (30x100 ml) fractions were collected as shown in the following table :-

Fraction	Eluent
1 - 5	
6 - 16	$CHCl_3$: EtOAc: MeOH (80:10:10)
17 - 28	
29 - 30	

Checked tlc on solvent system 1 (spray reagent 2) indicated that the major alkaloid of the fraction 6-16 was S1 so it was not further investigated.

The combined fraction 17-28 gave at least 4 spots of alkaloids and was further subjected to the third column chromatography as follows :-

Third column

The material from the combined fraction 17-28 was separated through a silica gel column (5 cm in diameter) which was previously packed with silica gel 60 (60 g). The subsequent elution was carried out by using ethyl acetate, ethyl acetate:methanol (7:3) and methanol, respectively. As a result, the (150x25 ml) fractions were collected and combined in accordance with their tlc properties as shown in the following Table (page 111) :-

Fraction	Eluent
1 - 12	EtOAc
13 - 25	
26 - 41	
42 - 51	
52 - 56	EtOAc : MeOH
57 - 85	(7:3)
86 - 94	
95 -120	
121 -150	MeOH

Checked tlc in solvent system 1 (spray reagent 2), indicated that the combined fraction 57-85 (0.9061 g) contained a large yellow colour spot of alkaloid, however the crystallization of this alkaloid was not successful. This material was further purified by subjected to preparative tlc as follows, while the other fractions were not further investigated.

Preparative Thin-Layer Chromatography

The combined fraction (0.9061 g) was separated by preparative thin layer chromatrography using solvent system 2 to give 2 zones of alkaloids. The upper zone was further purified by passing through a small column of silica gel and eluted with chloroform:methanol (1:1). The eluate was brought to dryness followed by crystallization in acetone - diethyl ether mixture to give an aggregrate of prism crystals (0.7743 g) of S2.

The lower zone from the preparative tlc was not investigated.

2. Isolation of the Alkaloids from Crude CB

The crude CB (32.8 g) contained at least 9 spots of alkaloids on checked tlc in system 1. The chromatogram on spraying with spray reagent system 2 indicated the presence of alkaloids S1, S2 and other 5 blue colour spots of alkaloids. The CB material was divided to 3 equal portions and each portion was subjected to column chromatography in the same manner.

Column Chromatrography of CB

Each lot of CB was separated through a (400 g) neutral alumina activity I column (10 cm in diameter). The column was subsequently eluted with hexane:chloroform (1:1); hexane:chloroform (3:7); chloroform; and methanol. The overall fractions (150x100 ml) were collected and combined (see Table page 114) in accordance with the tlc information.

Eluent	Fraction	Combined Portion	Remarked
Hexane:$CHCl_3$ (1:1)	1 - 15	A	alkaloids S1 and S2
	16 - 30		
	31 - 34	B	alkaloids S1 and S2 which overlapped with two other blue coloured spots.
Hexane:$CHCl_3$ (3:7)	35 - 90	C	alkaloids S1 and S2 together with five other blue coloured spots and one grey coloured spot.
	91 -120	D	one alkaloid with green coloured spot and other four blue coloured spots
$CHCl_3$	121 -130	E	At least three polar alkaloids which gave blue coloured spots
MeOH	131 -150	F	Dirty mixture as severe tailing

The combined portion A-F were investigated as follows :-

Portion A	The material was crystallized from methanol to give 7.5816 g of S1
Portion B	The material was chromatographed through column chromatography and some fractions were combined with portion C
Portion C	This material was subjected to column chromatography and several fractions were investigated by preparative tlc which yield alkaloids G2, B1 and B2
Portion D	This material was separated by column chromatography to give alkaloid G1
Portion E and F	These materials were not investigated.

2.1 Isolation of G1 from Portion D of CB

Column Chromatography

The material of portion D (0.8577 g) was divided into two equal portions. Each portion (about 0.4 g) was separated through a silica gel column. The column (4 cm in diameter) was packed with (20 g) silica gel 60 and eluted with chloroform:methanol (1:3) and methanol: conc. ammonium hydroxide (4:1), respectivety. The (40 x 10 ml) fractions were collected and those which contained similar compositions were combined as shown in the following table:-

Fraction	Eluent
1 - 2	$CHCl_3$:MeOH (1:3)
3	
4 - 5	
6 - 8	
9 -12	
13 -40	
41	MeOH:conc NH_4OH (4:1)

Checked tlc in system 1 (spray reagent 2) indicated that fraction 3 (0.1965 gm) gave single green coloured spot and the material was further purified by column chromatography as follow:

The fraction 3 material was separated through a (10 g) silica gel 60 column (3 cm in diameter). The column was eluted with chloroform : methanol (1:3) and the (10x100 ml) fractions were collected as shown in the following table :-

Fraction	Eluant
1	
2	CHCl :MeOH (1:1)
3 - 10	3

Checked tlc of fraction 2 in solvent system 1 (spray reagent 2) indicated the presence of single spot which gave a green colour. It was crystallized from absolute alcohol as colourless needles (44.3 mg) of G1

2.2 Isolation of the Alkaloids from Portion B of CB

Column Chromatography

The material 2.2963 g of portion B was separated through a (80 g) neutral alumina activity I column (7 cm in diameter). The successive elution (100 x 100 ml fractions) of the column was carried out as shown in the following Table:

Fraction	Eluent
1 - 20	Benzene
21 - 35	Benzene:$CHCl_3$ (9:1)
36 - 50	Benzene:$CHCl_3$ (7:3)
51 - 70	Benzene:$CHCl_3$ (1:1)
71 - 85	Benzene:$CHCl_3$ (1:9)
86 - 90	$CHCl_3$
91 -100	MeOH

Checked tlc in solvent system 2 (spray reagent 2) of the combined fraction (71-100) indicated the presence of two blue coloured spots which were overlapped by alkaloids S1 and S2. However, this fraction (0.0630 g) was further subjected to another column chromatography as follow.

The mentioned material was separated through a (70 g) neutral alumina activity I column (5 cm in diameter). The column was eluted with benzene:chloroform (7:3), chloroform and methanol. The fractions (120x25 ml) were collected as shown in the following Table :-

Fraction	Eluent
1 - 15	
16 - 30	Benzene:$CHCl_3$ (7:3)
31 - 81	
82 -100	$CHCl_3$
101 -120	MeOH

Checked tlc in solvent system 1 (spray reagent 2) of the combined fraction (31-100) indicated the presence of 3 blue coloured spots, a grey coloured spot and probably alkaloid S2. Since only small quantity was obtained, this material was later combined with some material from the processed portion C.

2.3 Isolation of G2, B1 and B2 from Portion C of CB

On checked tlc, the alkaloidal portion C (0.5989) gave 5 spots of blue colour together with the several alkaloids, two of which were corresponding to alkaloids S1 and S2. The material was subjected to column chromatography followed by preparative tlc as follows :

Column Chromatography

The material of portion C (0.5989 g) was separated through a 100 g neutral alumina activity I column (5 cm in diameter). The elution (60 x 100 ml) of the column was carried out as shown in the following table :-

Fraction	Eluent
1 - 10	Benzene
11 - 15	⊤ Benzene:$CHCl_3$ (1:9) ⊥
16 - 50	
51 - 56	$CHCl_3$
57 - 60	MeOH

Checked tlc in system 1 (spray reagent 2) indicated that each combined fraction contained the alkaloids as follows :-

Fraction	1 - 15	contained S1 and S2
Fraction	16 - 60	contained a grey coloured spot accompanied by 3 blue coloured spots. This material was corresponded with the fraction 31-100 of the alkaloids portion B and they were combined.

Preparative Thin-Layer Chromatography

The mentioned combined fractions from portion B and portion C was subjected to the preparative tlc in solvent system 1

The chromatogram gave 9 bands as coded BB 1 to BB 9 in series of decreasing Rf values.

BB 1 The highest zone of chromatogram contained only a little amount of alkaloids and the material was not further investigated.

BB 2 This material gave 3 spots, one of which gave grey colour while the rests gave blue colour on spraying with spray reagent 2

BB 3 This material contained 2 blue coloured spots as same as those of the band BB 2

BB 4 This material gave impurity mixture of alkaloids and was not further investigated.

BB 5 This material contained 3 spots which gave blue colour with spray reagent 2 and another compound corresponded to S2

BB 6 Checked tlc revealed that this material contained one blue coloured spot, one yellow coloured spot corresponded to S2, and one violet-blue coloured spot.

BB 7 This material contained several alkaloids but it was not further investigated.

BB 8 This material gave 2 spots of blue colour but the material was not further investigated since the alkaloid content was very low and they decomposed readily.

BB 9 This material was not further investigated because the small quantity was available.

2.3.1 Isolation of G2

The material BB 2 (64.9 mg) was further purified by preparative tlc in system 1 which gave 3 bands of alkaloids. They were coded in series of decreasing Rf values as BB 21, BB 22 and BB 23, respectively.

All bands of alkaloids were removed from the silica gel zones by washing with the mixture of chloroform:methanol (1:1) and finally taken up in chloroform. Checked tlc (sprayed reagent 2) was carried out and the results were as follows :

BB 21 This material gave one spot of blue colour which was not further investigated due to its small quantity.

BB 22 This material gave one spot of grey colour. It was crystallized from methanol to gave aggregate of needle crystals (12.7 mg) coded as G2.

BB 23 This material 1.3 mg gave one of blue coloured spot which was not further investigated.

2.3.2 Isolation of B1

The material BB 5 (35.6 mg) was subjected to preparative tlc in system 1 which gave 3 bands of alkaloids coded as BB 51, BB 52 and BB 53, respectively, in series of decreasing Rf values.

BB 51 This material gave a blue coloured spot with the spray reagent 2. The material was further purified twice by preparative tlc in system 2 which gave alkaloid fraction B1 (8.4 mg). The crystallization of B1 was not successful.

BB 52 This material was not clean and was not further investigated.

BB 53 This material gave a compound corresponded to the alkaloid S2 and was not further investigated.

2.3.3 Isolation of B2

The material BB 6 (25.2 mg) was subjected to preparative tlc system 1 which gave 2 bands of alkaloids. The upper zone coded as BB 61 and the lower zone coded as BB 62

The colour reaction of the alkaloid zones with spray reagent 2 were used for the differentiation between the two alkaloidal zones as follow :-

BB 61 This material gave yellow coloured spot with the spray reagent 2 and was not further investigated.

BB 62 This material gave one spot of violet-blue colour with the spray reagent 2. Purification of this alkaloid was made by further preparative tlc in the solvent system 2 and 1, respectively. This purified alkaloid (1.2 mg) was coded as B2.

Characterization and Identification of the Individual Alkaloid.

Six alkaloids were isolated from the plant as demonstrated in Table 4 :-

Table 4

Isolated Alkaloids from the Stem Bark of Strychnos ignatii Berg.

Laboratory Code	Alkaloid
S1	Strychnine 54
S2	Brucine 55
* G1	Geissoschizol 161
* G2	Polyneuridine 24
B1	Longicaudatine 119
** B2	Dihydrolongicaudatine 163-165

* Alkaloids were first isolated from this plant

** Incompletely characterized alkaloid

The chemical and physical characteristic properties of the individual alkaloids are following described.

S1 Strychnine 54

The base crystallized from chloroform-methanol as colourless prismatic crystals and a gave pink coloured spot with the ferric chloride - perchloric acid spray reagent.

TLC system (hRf) : 1 (58) 2 (44)
3 (83) 4 (93)

Melting point : 280 °C

UV (MeOH) [see Figure 17 page 218]
λ_{max} (nm) : 210, 253, 277, 280

IR (KBr disc) [see Figure 18 page 219]
ν_{max} (cm^{-1}) : 1665, 1600, 1480, 1390, 760

MS (EIMS, 290 °C) [see Figure 19 page 220]
m/z 334 (M^+, $C_{21}H_{22}N_2O_2$, 100 %), 333 (13.3)
319 (5.8) , 306 (5.7) , 305 (4.8) , 172 (5.3) ,
169 (6.0) , 167 (8.4) , 163 (9.2) , 162 (19.3) ,
161 (14.5) , 144 (16.2) , 143 (15.3) , 136 (8.8) ,
135 (6.4) , 134 (13.4) , 132 (7.2) , 130 (15.7) ,
121 (10.9) , 120 (20.3) , 119 (9.8) , 115 (7.1) ,
108 (9.4) , 107 (15.8)

^{1}H NMR (in $CDCl_3$ at 270 MHz) [see Figure 20 page 221]

Chemical shift δ (ppm)	Proton[a]	Multiplicity
8.08	1H ; H-12	d ($J_{12,11}$ = 8 Hz)
7.27-7.22	1H ; H-9	m
7.21-7.05	2H ; H-10, H-11	m
5.89	1H ; H-19	ill-defined t
4.27	1H ; H-17	m
4.18-4.01	2H ; H-18 α , H-18 β	m
3.92	1H ; H-3	br s
3.84	1H ; H-2	d($J_{2,16}$ = 10.5 Hz)
3.69	1H ; H-21 β	d($J_{21\beta,21\alpha}$ =14.8 Hz)
3.20-3.07	3H ; H-23 α , H-15, H-5 β	m
2.85	1H ; H-5 α	q
2.77	1H ; H-21 α	d($J_{21\alpha,21\beta}$ =14.8 Hz)
2.64	1H ; H-23 β	dd($J_{23\beta,23\alpha}$ = 17Hz) ($J_{23,17}$ = 3.2 Hz)
2.29-2.38	1H ; H-14 β	dt($J_{14\beta,14\alpha}$ =14.3Hz)
1.89-1.84	2H ; H-6α, H-6 β	m
1.44	1H ; H-14 α	d($J_{14\alpha,14\beta}$ =14.3 Hz)
1.24-1.29	1H ; H-16	dt($J_{16,2}$ = 10.5 Hz)

[a] The assignments are set out by comparision with the published spectral data of strychnine 54 (34,101)

^{13}C NMR (in $CDCl_3$, 90 MHz) [see Figure 21 page 222]

Carbon[b]	Chemical shift δ(ppm)
C- 2	60.09
C- 3	60.09
C- 5	50.33
C- 6	42.86
C- 7	51.91
C- 8	132.74
C- 9	122.23
C-10	124.13
C-11	128.46
C-12	116.16
C-13	142.17
C-14	26.82
C-15	31.59
C-16	48.22
C-17	77.59
C-18	64.58
C-19	127.16
C-20	140.55
C-21	52.66
C-22	169.26
C-23	42.48

[b] The assignments are set out by comparision with the published spectral data of strychnine 54 (102).

S2 Brucine 55

The base crystallized from acetone-diethyl ether as aggregate of prisms and gave a yellow coloured spot with the ferric chloride - perchloric acid reagent.

TLC system (hRf) : 1 (46) , 2 (27)
3 (79) , 4 (85)

Melting point : 178°-179° C

UV (MeOH) [see Figure 22 page 223]

λ_{max} (nm) : 218, 261,298

IR (KBr disc) [see Figure 23 page 224]

ν_{max} (cm^{-1}) : 1659, 1500, 1464, 1450, 1400, 1290, 1280, 1100, 850

MS (EIMS, 190° C) [see Figure 24 page 225]

m/z 394 (M^+, $C_{23}H_{26}N_2O_4$, 100%), 393 (3), 380 (6.7) , 379 (25.9) , 365 (2.2) , 264 (2.5) , 204 (4.4) , 203 (7.1) , 197 (4.8) , 190 (4.7) , 188 (3.0) , 162 (7.4) , 161 (5.3) , 160 (3.2) , 146 (4.1) , 144 (3.5) , 138 (3.9) , 136 (4.8) , 134 (5.7) , 132 (3.2) , 130 (3.6) , 122 (3.9) , 121 (5.3) , 120 (11.2) , 108 (5.5) , 107 (11.0)

^{1}H NMR (in $CDCl_3$ at 270 MHz) [see Figure 25 page 226]

Chemical shift δ (ppm)	Proton[a]	Multiplicity
7.78	1H ; H-12	s
6.65	1H ; H-9	s
5.87	1H ; H-19	ill-defined triplet
4.37	1H ; H-17	dt
4.17-4.00	2H ; H-18 α , H-18 β	m (eight lines)
3.89	3H ; OCH_3	s
3.84	3H ; OCH_3	s
3.83-3.79	1H ; H-2	d ($J_{2,16}$ = 10.5 Hz)
3.68	1H ; H-21 β	dd($J_{21\beta,21\alpha}$ = 14.8) ($J_{21\beta,19}$ =1.2 Hz)
3.19-3.04	3H ; H-5 β , H-15, H-23 α	m
2.88-2.77	1H ; H-5 α	m
2.69	1H ; H-21 α	d($J_{21\alpha,21\beta}$ =14.8 Hz)
2.67-2.60	1H ; H-23 β	dd($J_{23\beta,23\alpha}$ =17.5Hz) ($J_{23\beta,17}$ = 3.3 Hz)
2.38-2.29	1H ; H-14 β	dt($J_{14\beta,14\alpha}$ =14.3Hz)
1.94-1.81	2H ; H-6 α , H-6 β	m
1.45	1H ; H-14 α	d($J_{14\alpha,14\beta}$ = 14.3Hz)
1.28-1.22	1H ; H-16	dt ($J_{16,2}$ = 10.5 Hz)

[a] The assignments are set out by comparision with the published spectral data of brucine 55 (34).

^{13}C NMR (in $CDCl_3$, 90 MHz) [see Figure 26 page 227]

Carbon	Chemical shift δ (ppm)
C- 2	60.29
C- 3	59.86
C- 5	50.11
C- 6	42.31
C- 7	51.84
C- 8	123.46
C- 9	105.48
C-10	146.11
C-11	149.08
C-12	100.93
C-13	135.87
C-14	26.76
C-15	31.47
C-16	48.16
C-17	77.68
C-18	64.52
C-19	127.25
C-20	140.42
C-21	52.60
C-22	168.91
C-23	42.31
2 X OCH_3	56.12, 56.31

[b]

The assignments are set out by comparision with the published spectral data of brucine 55 (102).

G1 Geissoschizol 161

The base crystallized from absolute alcohol as colourless needles and gave a green colour spot with the ferric chloride - perchloric acid reagent.

TLC system (hRf) : 1 (43) , 2 (67)
3 (93) , 5 (65)

Melting point : 216 °C

UV (MeOH) [see Figure 27 page 228]

λ_{max} (nm) : 224, 280, 283

IR (KBr disc) [see Figure 28 page 229]

ν_{max} (cm^{-1}) : 3200, 2930, 2840, 2720, 1424, 1110, 740

MS (EIMS, 150 °C) [see Figure 29 page 230]

m/z 297 (21.2) , 296 (M^+, $C_{19}H_{24}N_2O$, 100 %)
295 (99.7) , 279 (7.8) , 265 (12.9) , 252 (14.3) ,
251 (33.8) , 249 (12.4) , 237 (15.3) , 223 (12.2) ,
184 (8.0) , 182 (10.3) , 171 (19.8) , 170 (26.9) ,
169 (53.8) , 168 (13.7) , 156 (22.4) , 144 (12.8) ,
143 (9.23) , 130 (6.38)

^{1}H NMR (in DMSO-D_6 at 270 MHz) [see Figure 30 page 231]

Chemical shift δ (ppm)	Proton[a]	Multiplicity
10.72	1H ; NH	s
7.33	1H ; H-9	d ($J_{9,10}$ = 7.4 Hz)
7.27	1H ; H-12	d ($J_{12,11}$ = 7.0 Hz)
7.02-6.89	2H ; H-11, H-10	m
5.34	1H ; H-19	q
4.27	1H ; H-3	t ($J_{3,14}$ = 5 Hz)
3.91	1H ; OH	t (J = 5.2 Hz)
3.45	1H ; H-21α	d ($J_{21\alpha, 21\beta}$ =11.9 Hz)
3.30-3.24	2H ; H-17α ,H-17β	dd (overlapped by moisture signal
3.09-2.98	1H ; H-5β[b]	m
2.87-2.72	4H ; H-5α[b] ,H-6β H-15, H-21β	m
2.46	1H ; H-6α	d (overlapped by solvent peak)
2.18-2.08	1H ; H-14α[c]	m
1.98-1.89	1H ; H-14β[c]	m
1.58	3H ; H-18	d (d)($J_{18,19}$ =6.7Hz)
1.50-1.22	2H ; H-16α ,H-16β	m

[a] The assignments are set out by comparision with the published spectral data of geissochizol 161 (103).

[b,c] The assignments may be interchanged by Feng, X.Z. et al. (103).

^{13}C NMR (in DMSO-D6, 270 MHz) [see Figure 31 page 232]

Carbon[b]	Chemical shift δ (ppm)
C- 2	135.8
C- 3	53.6
C- 5	50.7
C- 6	18.5
C- 7	105.5
C- 8	126.9
C- 9	117.4
C-10	118.5
C-11	120.2
C-12	110.9
C-13	137.8
C-14	32.8
C-15	31.1
C-16	36.4
C-17	58.8
C-18	12.8
C-19	118.2
C-20	135.6
C-21	54.9

[b] The assignments set out by comparision with the spectral data of geissoschizine 1 (35) and its congeners (104).

G2 Polyneuridine 24

The base crystallized from methanol as aggregate of needles crystals. The alkaloid gave a grey colour spot with the ferric chloride - perchloric acid reagent

TLC system (hRf) : 1 (0.78) , 2 (0.72)
3 (0.95) , 6 (0.34)

Melting point : 230°-232.5° C

UV (MeOH) [see Figure 32 page 233]

λ_{max} (nm) : 225, 272, 279, 288

IR (KBr disc) [see Figure 33 page 234]

ν_{max} (cm-1) : 3600, 3400, 3300, 3000, 1720, 1460, 1215, 1090, 720

MS (EIMS, 190° C) [see Figure 34 page 235]

m/z 353 (23.8), 352 (M^+, $C_{21}H_{24}N_2O_2$, 100 %),
351 (49.2) , 337 (17.7) , 335 (14.5) , 322 (16.3) ,
321 (55.3) , 293 (21.1) , 250 (18.5) , 249 (96.6) ,
236 (22.2) , 235 (10.9) , 221 (12.9) , 183 (11.6) ,
182 (21.8) , 170 (17.5) , 169 (92.7) , 168 (70.6) ,
167 (15.1) , 156 (19.8) , 143 (12.9) , 130 (12.8) ,
129 (13.5) , 128 (10.6) , 115 (16.0) , 107 (12.6)

^{1}H NMR (in $CDCl_3$ at 270 MHz) [see Figure 35 page 236] The signal due to the OH-group is omitted.

Chemical shift δ (ppm)	Proton[a]	Multiplicity
8.05	1H ; NH	s
7.48	1H ; H-9	d ($J_{9,10}$ = 7 Hz)
7.33	1H ; H-12	d (d)($J_{12,11}$ =6.7Hz)
7.15	1H ; H-11, H-10	m
5.28	1H ; H-19	q
4.28	1H ; H-5	d ($J_{5,6}$ = 5.68 Hz)
4.07	1H ; H-3	dd ($J_{3,14\alpha}$ = 9.4 Hz, $J_{3,14\beta}$ = 4.5 Hz)
3.72	3H ; $COOCH_3$	s
3.21	1H ; H-15	br m
3.10	1H ; H-6 α	dd($J_{16\alpha,16\beta}$ =16.5Hz)
2.95	1H ; H-6 β	d(d)($J_{16\beta,16\alpha}$ 16Hz)
1.89	1H ; H-14	m
1.59	1H ; H-18	d(d)($J_{18,19}$ = 6.9Hz) overlapped by moisture signal

[a] The assignments are set out by comparision with the published spectral data of polyneuridine 24 (105).

B1 Longicaudatine 119

The base gave a blue colour spot with the ferric chloride - perchloric acid reagent.

TLC system (hRf) : 1 (53) , 2 (39)
3 (86) , 4 (94)

MS (EIMS, 210°C) [see Figure 36 page 237]

m/z 568 (M^+, $C_{38}H_{40}N_4O$, 100 %), 566 (43.2), 565 (20.8) , 564 (35.4) , 563 (28.9) , 539 (13.6) , 538 (9.8) , 537 (10.1) , 397 (17.7) , 350 (21.2) , 331 (21.2) , 286 (29.0) , 284 (33.9) , 259 (41.8) , 251 (46.7) , 250 (89.8) , 249 (73.6) , 234 (33.1) , 221 (26.9) , 177 (39.6) , 171 (56.6) , 170 (41.1) , 169 (38.2) , 144 (57.9) , 135 (47.5) , 133 (100) , 130 (42.5)

B2 Dihydrolongicaudatine 163-165

The base gave a purple-blue colour spot with the ferric chloride - perchloric acid reagent.

TLC system (hRf) : 1 (45) , 2 (26)
3 (75) , 4 (91)

MS (EIMS, 250 °C) [see Figure 37 page 238]

m/z 571 (27.5), 570 (M^+, $C_{38}H_{42}N_4O$, 70.3),
569 (43.8) , 568 (65.6) , 567 (22.0) , 565 (7.3) ,
450 (8.7) , 448 (9.1) , 436 (7.7) , 331 (15.7) ,
285 (23.2) , 284 (18.7) , 252 (14.4) , 251 (47.8) ,
250 (19.0) , 249 (27.1) , 247 (10.0) , 237 (12.4) ,
235 (10.5) , 225 (29.3) , 224 (88.7) , 223 (40.5) ,
222 (10.4) , 221 (15.0) , 218 (11.2) , 209 (12.3) ,
199 (11.0) , 198 (12.5) , 197 (25.6) , 185 (11.0) ,
184 (21.6) , 183 (10.1) , 182 (12.2) , 171 (20.8) ,
170 (100 % base peak) , 169 (49.7) , 168 (16.8) ,
167 (10.7) , 166 (19.7) , 144 (24.2) , 143 (13.6) ,
135 (12.5) , 130 (10.8) , 129 (14.9) , 123 (14.2) ,
121 (11.6)