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ISOLATION OF ALKALOIDS FROM *Alseodaphne pendulifolia* Gamb. (LAURACEAE) AND THEIR BIOLOGICAL ACTIVITIES

MUHAMMAD HAFIZ HUSNA BIN HASNAN

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I ASSOC. PROF. DR. MOHD AZLAN NAFIAH (SUPERVISOR'S NAME) hereby certifies that the work entitled ISOLATION OF ALKALOIDS FROM ALSEODAPHNE PENDULIFOLIA GAMB. (LAULACEAE) AND THEIR BIOLOGICAL ACTIVITIES (TITLE) was prepared by the above named student, and was submitted to the Institute of Graduate Studies as a * partial/full fulfillment for the conferment of MASTER OF SCIENCE (NATURAL PRODUCTS) (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

20/8/2014.

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ABSTRACT

The purpose of this study is to extract, isolate and elucidate the structure of alkaloid constituents from barks of *Alseodaphne pendulifolia* Gamb. (Lauraceae) and to determine the biological activities (antiplasmodial, cytotoxicity, antibacterial and antioxidant) against the isolated alkaloids. The samples were collected from Sg. Temau Forest Reserve, Kuala Lipis, Pahang and Sg. Tekam Forest Reserve, Jerantut, Pahang, Malaysia. The isolation and purification of alkaloids were done using various chromatographic techniques including chromatotron, column, preparative thin layer and thin layer chromatography. The structure elucidations of the isolated alkaloids were established by NMR spectral data using various 1D-NMR (^{1}H, ^{13}C, and DEPT) and 2D-NMR (COSY, HMQC/HSQC, HMBC, H2BC, HSQC-TOCSY and NOESY) techniques, aided via UV, IR, MS data and also by comparison with data from the literature. Further isolation process of the plants yielded four types of isoquinoline alkaloid; aporphine, aporphine *N*-oxide, oxoaporphine and bisbenzylisoquinoline. A new aporphine *N*-oxide; norisocorydine *N*-oxide **166** with four aporphines; corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123,** were successfully isolated from samples that collected from Sg. Temau Reserve Forest. Samples from Sg. Tekam Reserve Forest, yielded four aporphines; corydine **124**, *N*-methylhernagine **169**, hernagine **123** and hernovine **170**, an oxoaporphine; oxohernagine **122** was isolated together with a bisbenzylisoquinoline; costaricine **126**. Antiplasmodial assay against *Plasmodium falciparum* on the bark crude extract exhibited active antiplasmodial activity with IC_{50} value 0.804 µg/ml, while the isolated pure compounds corydine **124**, oxohernagine **122** and hernagine **123** showed the active antimalaria activity IC_{50} values at 1.184, 2.189 and 3.494 µM, respectively. The new discovered compound, norisocorydine *N*-oxide showed a moderate effect on cytotoxic assay using leukaemia cell lines HL-60 with IC_{50} value 11 µg/ml. Norisocorydine *N*-oxide **166**, corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123** exhibited very weak activity with IC_{50} values more than 500µg/mL against free radical scavenging effect of antioxidant DPPH assay and were not active against gram positive bacteria; *Bacillus subtilis* (B145). This is the first report for phytochemical and biological activity studies from barks of *Alseodaphne pendulifolia* Gamb., which has potential in determination of other various chemical constituents for new drug discovery.

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PEMENCILAN ALKALOID DARIPADA *Alseodaphne pendulifolia* Gamb. (LAURACEAE) DAN AKTIVITI-AKTIVITI BIOLOGI

ABSTRAK

Kajian ini bertujuan untuk mengekstrak, memencil dan menentukan struktur sebatian alkaloid daripada kulit batang *Alseodaphne pendulifolia* Gamb. (Lauraceae) dan menentukan aktiviti biologi (antiplasmodial, sitotoksisiti, antibakteria, dan antioksidan) terhadap sebatian alkaloid yang telah dipencilkan. Sampel diperoleh dari Hutan Simpan Sg. Temau, Kuala Lipis, Pahang dan Hutan Simpan Sg. Tekam, Jerantut, Pahang, Malaysia. Proses pemencilan dan penulenan alkaloid menggunakan pelbagai kaedah kromatografi termasuk kromatotron, kromatografi turus, kromatografi plat dan kromatografi lapisan nipis. Pengenalpastian struktur alkaloid yang telah dipencil dan ditentukan melalui data spektrum NMR menggunakan pelbagai teknik 1D-NMR (^{1}H, ^{13}C, dan DEPT) dan 2D-NMR (COSY, HMQC/HSQC, HMBC, H2BC, HSQC-TOCSY dan NOESY), serta disokong oleh data daripada UV, IR, MS, dan juga melalui perbandingan data kajian literatur. Proses pemencilan sebatian kimia terhadap tumbuhan ini telah berjaya memperoleh empat alkaloid jenis isokuinolina; aporfina, aporfina *N*-oksida, oxoaporfina dan bisbenzilisokuinolina. Sebatian alkaloid aporfina *N*-oksida yang baru ditemui; norisokoridina *N*-oksida **166** dan empat lagi aporfina; koridina **124**, *N*-metilhernagina **169**, oxohernagina **122** dan hernagina **123** telah berjaya dipencilkan daripada sampel yang diperoleh dari Hutan Simpan Sg. Temau. Bagi sampel yang diperolehi dari Hutan Simpan Sg. Tekam, empat aporfina telah berjaya dipencilkan; koridina **124**, *N*-metilhernagina **169**, hernagina **123** dan hernovina **170**, satu oxoaporfina; oxohernagina **122** juga dipencilkan bersama-sama dengan satu sebatian bisbenzilisokuinolina; kostaricina **126.** Ujian antiplasmodial *Plasmodium falciparum* ke atas ekstrak mentah menunjukkan aktiviti antimalaria yang aktif dengan nilai IC_{50} sebanyak 0.804 μg/ml, sementara untuk sebatian yang dipencilkan koridina **124**, oxohernagina **122** dan hernagina **123** menunjukkan aktiviti yang aktif iaitu nilai IC_{50} masing-masing sebanyak 1.184, 2.189 and 3.494 μM. Ujian ketoksikdan terdahap sel leukimia HL-60, mendapati sebatian baru; norisokoridina *N*-oxide **166** menunjukkan kesan sederhana dengan nilai IC_{50} 11 μg/ml. Sebatian norisokoridina *N*-oksida **166**, koridina **124**, *N*-metilhernagina **169**, oxohernagina **122** dan hernagina **123** menunjukkan aktiviti yang sangat lemah dengan nilai IC_{50} melebihi 500 μg/mL terhadap kesan penjerapan radikal bebas dalam ujian antioksida DPPH dan juga menunjukkan kesan tidak aktif melawan bakteria gram positif: *Bacillus subtilis* (B 145). Ini merupakan laporan pertama mengenai kajian fitokimia dan aktiviti-aktiviti biologi terhadap kulit batang *Alseodaphne pendulifolia* Gamb. yang mana berpotensi untuk menemukan pelbagai jenis sebatian kimia yang lain untuk penghasilan ubat-ubatan baru.

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CONTENTS

		PAGE
DECLARATION		ii
ACKNOWLEDGEMENT		iii
ABSTRACT		iv
ABSTRAK		v
CONTENTS		vi
LIST OF TABLES		ix
LIST OF FIGURES		xi
LIST OF SCHEMES		xiv
LIST OF CHEMICAL COMPOUND STRUCTURE		xv
ABBREVIATIONS		xix
CHAPTER 1 INTRODUCTION		1
	1.1 General	1
	1.2 Problem Statements	4
	1.3 Objectives of Study	7
	1.4 Family of Lauraceae	8
	1.5 Genus of *Alseodaphne*	12
	1.6 Species of *Alseodaphne pendulifolia* Gamb.	17
CHAPTER 2 GENERAL CHEMICAL ASPECTS		19
	2.1 General	19
	2.2 Alkaloid	21

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi
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CONTENTS (cont.)

2.3	Classifications of Alkaloids		24
	2.3.1	True Alkaloids	24
	2.3.2	Protoalkaloids	25
	2.3.3	Pseudoalkaloids	25
2.4	Isoquinoline Alkaloids		28
2.5	Aporphine Alkaloid		31
2.6	Bisbenzylisoquinoline Alkaloid		36
2.7	Chemical Constituent from Genus of Alseodaphne		43
CHAPTER 3	**MATERIAL AND EXPERIMENTAL**		58
3.1	General		58
3.2	Isolation and Structure Determination of Alkaloids		58
	3.2.1	Instruments	59
	3.2.2	Reagents	60
	3.2.3	Plants Materials	61
	3.2.4	Alkaloids Plants Screening	61
	3.2.5	Extraction of Plant Materials	62
	3.2.6	Separation and Purification of Alkaloids	63
	3.2.7	Physical and Spectral Data of Isolated Alkaloids	66
3.3	Biological Activity Assay		73
	3.3.1	*In-vitro* Antiplasmodial Assay	73
	3.3.2	Cell Culture and MTT Cytotoxicity Assay	74

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

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CONTENTS (cont.)

		3.3.3 Bacterial Culture and Disc Diffusion Assay	75
		3.3.4 DPPH Free Radical Scavenging Activity Assay	76
CHAPTER 4	**RESULTS AND DISCUSSION**		78
	4.1 Isolation of Alkaloids from Barks of *Alseodaphne pendulifolia* Gamb.		78
	4.2 Structure Elucidation of Isolated Alkaloid from Barks of *Alseodaphne pendulifolia* Gamb.		82
		4.2.1 APB 1, Norisocorydine *N*-oxide **166**	82
		4.2.2 APB 2, Corydine **124**	93
		4.2.3 APB 3, *N*-methylhernagine **169**	101
		4.2.4 APB 4, Oxohernagine **122**	109
		4.2.5 APB 5, Hernagine **123**	116
		4.2.6 APB 6, Hernovine **170**	123
		4.2.7 APB 7, Costaricine **126**	131
	4.3 Biological Activity of Alkaloid Compounds from Barks of *Alseodaphne pendulifolia*		139
CHAPTER 5	**CONCLUSION**		142
REFERENCES			144
APPENDIXES 1 Articles in Proceeding/Presented at Conferences/Seminar			158
APPENDIXES 2 Antiplasmodial Results Details			181

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LIST OF TABLES

Table	Title	Page
1.1.1	Specific Plants Parts in Certain Plant Species Used in Phytotherapy.	4
1.3.1	Uses of Species from Family of Lauraceae	11
1.4.1	List of Species in Genus of *Alseodaphne* (Lauraceae)	14
2.4.1	Examples of Alkaloids from Isoquinoline Subgroups and Their Source.	29
2.6.1	Groups and Subgroups for Classification of Bisbenzylisoquinoline Alkaloids and Their Examples	39
2.8.1	Chemical Constituents Isolated from Genus of *Alseodaphne*	43
3.2.4.1	Indicator for Mayer's Test on Alkaloid	62
3.2.5.1	Yield of Crude Alkaloids Extracted from Barks of *Alseodaphne pendulifolia* Gamb. (Lauraceae)	63
4.2.1.1	^{1}H NMR Spectral Comparison of APB 1 with Isocorydine **94** and Norisocorydine **95**	86
4.2.1.2	^{1}H NMR Spectral Comparison between Corydine *N*-oxide **167** toward Corydine **124** (Ferreira et al., 2010)	86
4.2.1.3	1D (^{1}H, ^{13}C and DEPT-135), 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 1	87
4.2.2.1	1D (^{1}H and ^{13}C) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 2	99
4.2.2.2	^{1}H And ^{13}C NMR Spectral Data Comparison of APB 2, with Corydine **124** from Literature	100
4.2.3.1	1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 3	107
4.2.3.2	^{1}H NMR Spectral Data Comparison of APB 3, with *N*-methylhernagine **169** from Literature (Guinaudeau et al., 1983)	108
4.2.4.1	1D (^{1}H, ^{13}C And DEPT-135) And 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 4	114

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LIST OF TABLES (cont.)

4.2.4.2	^{1}H NMR Spectral Data Comparison of APB 4, with Oxohernagine **122** from Literature (Chen Et Al., 1996)	115
4.2.5.1	1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 5	119
4.2.5.2	^{13}C NMR Spectral Data Comparison of APB 5, with Hernagine **123** in Literature Data (Phan et al., 1994)	122
4.2.6.1	1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 6	129
4.2.6.2	^{13}C NMR Spectral Data Comparison of APB 6, with Hernovine **170** in Literature Data (Phan et al., 1994)	130
4.2.7.1	1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 7	137
4.2.5.2	^{13}C NMR Spectral Data Comparison of APB 7, with Costaricine **126** from Literature (Phan et al., 1994)	138
4.3.1	Biological Activities (*In-Vitro* Antiplasmodial HRP2, Cytotoxic, Antibacterial and Antioxidant) of Alkaloids Against Alkaloids Crude Extract and Isolated Aporphine Alkaloids from Barks of *Alseodaphne pendulifolia* Gamb.	140

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LIST OF FIGURES

Figures	Title	Page
1.5.1	Fruits of *Alseodaphne pendulifolia*	18
1.5.2	Barks of *Alseodaphne pendulifolia*	18
1.5.3	Leaves of *Alseodaphne pendulifolia*	18
2.5.1	Numbering of Aporphine Skeleton	33
2.5.2	Configuration of Aporphine; Left – A Configuration of Aporphine; Right – B Configuration of Aporphine	33
2.6.1	Numbering system of Bisbenzylisoquinoline Alkaloids	37
4.2.1.1	^{1}H NMR Spectrum of APB 1	85
4.2.1.2	^{13}C NMR Spectrum of APB 1	88
4.2.1.3	DEPT-135 NMR Spectrum of APB 1	88
4.2.1.4	COSY NMR Spectrum of APB 1	89
4.2.1.5	HSQC NMR Spectrum of APB 1	89
4.2.1.6	HMBC NMR Spectrum of APB 1	90
4.2.1.7	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC Spectrum of APB 1	90
4.2.1.8	NOESY NMR Spectrum of APB 1	91
4.2.1.9	H2BC NMR Spectrum of APB 1	91
4.2.1.10	HSQC-TOCSY NMR Spectrum of APB 1	92
4.2.2.1	^{1}H NMR Spectrum of APB 2	96
4.2.2.2	^{13}C NMR Spectrum of APB 2	97
4.2.2.3	COSY NMR Spectrum of APB 2	97
4.2.2.4	HMQC NMR Spectrum of APB 2	98

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LIST OF FIGURES (cont.)

4.2.2.5	HMBC NMR Spectrum of APB 2	98
4.2.2.6	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 2	99
4.2.3.1	^{1}H NMR Spectrum of APB 3	103
4.2.3.2	^{13}C NMR Spectrum of APB 3	104
4.2.3.3	DEPT-135 NMR Spectrum of APB 3	104
4.2.3.4	COSY NMR Spectrum of APB 3	105
4.2.3.5	HSQC NMR Spectrum of APB 3	105
4.2.3.6	HMBC NMR Spectrum of APB 3	106
4.2.3.7	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 3	106
4.2.4.1	^{1}H NMR Spectrum of APB 4	111
4.2.4.2	^{13}C NMR Spectrum of APB 4	111
4.2.4.3	DEPT-135 NMR Spectrum of APB 4	112
4.2.4.4	COSY NMR Spectrum of APB 4	112
4.2.4.5	HSQC NMR Spectrum of APB 4	113
4.2.4.6	HMBC NMR Spectrum of APB 4	113
4.2.4.7	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 4	114
4.2.5.1	^{1}H NMR Spectrum of APB 5	118
4.2.5.2	^{13}C NMR Spectrum of APB 5	118
4.2.5.3	DEPT-135 NMR Spectrum of APB 5	120
4.2.5.4	HMQC NMR Spectrum of APB 5	120
4.2.5.5	COSY NMR Spectrum of APB 5	121

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LIST OF FIGURES (cont.)

4.2.5.6	HMBC NMR Spectrum of APB 5	121
4.2.5.7	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 5	122
4.2.6.1	^{1}H NMR Spectrum of APB 6	125
4.2.6.2	^{13}C NMR Spectrum of APB 6	126
4.2.6.3	Dept-135 NMR Spectrum of APB 6	126
4.2.6.4	COSY NMR Spectrum of APB 6	127
4.2.6.5	HMQC NMR Spectrum of APB 6	127
4.2.6.6	HMBC NMR Spectrum of APB 6	128
4.2.6.7	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 6	128
4.2.7.1	^{1}H NMR Spectrum of APB 7	133
4.2.7.2	^{13}C NMR Spectrum of APB 7	134
4.2.7.3	COSY NMR Spectrum of APB 7	134
4.2.7.4	HMQC NMR Spectrum of APB 7	135
4.2.7.5	HMBC NMR Spectrum of APB 7	135
4.2.7.6	Dept-135 NMR Spectrum of APB 7	136
4.3.7.7	COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 7	136

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LIST OF SCHEMES

Scheme	Title	Page
2.5.1	The Various Groups of Benzylisoquinoline Derived from *(S)*-Norcoclaurine **59**	34
2.5.2	Scheme: The Biogenetic Pathway from **49** to Corytuberine **60**, Isoboldine **61**, Glucine **62** and Bulbocapnine **63**	35
2.6.1	The Biogenetic Pathway of Berbamunine	38
4.1.1	Isolation of Alkaloid from the Barks of *Alseodaphne pendulifolia* Gamb. (KL 5250)	80
4.1.2	Isolation of Alkaloid from the Barks of *Alseodaphne pendulifolia* Gamb. (KL 5732)	81

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LIST OF CHEMICAL COMPOUNDS STRUCTURE

No.	Chemical compounds structure name	Page
1	Citric acid	5
2	Tartaric acid	5
3	Malic acid	5
4	Cysteine	5
5	Tryptophan	5
6	Histidine	5
7	Serine	5
8	Alanine	5
9	Glutamic acid	6
10	Vanillin	6
11	Anthocyanins	6
12	Resveratrol	6
13	Morphine	6
14	Aspirin	6
15	Etoposide	6
16	Pyrethrins	6
17	Allethrins	6
18	Rotenone	7
19	Gibberellins	7
20	Cytokinins	7
21	Abscisic acid	7
22	Ethylene	7
23	Paclitaxel	20
24	Vitamin A	20
25	Vitamin D	21
26	Cantharidin	21
27	Doxorubicin	21
28	Tubocurarine	22
29	Cocaine	23
30	Codeine	23
31	Quinidine	23
32	Quinine	23
33	Vinblastine	23
34	L-ornithine	26
35	L-lysine	26
36	L-tyrosine	26
37	L-tryptophan	26
38	L-histidine	26
39	Usambarensine	26
40	Hordenine	26
41	Mescaline	26
42	Coniine	26
43	Capsaicin	27
44	Ephedrine	27
45	Solanidine	27
46	Caffeine	27

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LIST OF CHEMICAL COMPOUNDS STRUCTURE (conc.)

47	Theobromine	27
48	Pinidine	27
49	(*S*)-Reticuline	29
50	Norglaucine	29
51	Palmatine	30
52	Buesgeniine	30
53	Allocryptopine	30
54	Thebaine	30
55	Emetine	30
56	(+)-Stephasubine	30
57	Dopamine	34
58	4-hydrophenylacetadehyde	34
59	(S)-Norcoclurine	34
60	Corytuberine	35
61	Isoboldine	35
62	Glaucine	35
63	Bulbocapnine	35
64	Papaverine	34
65	Berberine	34
66	Sanguinarine	34
67	Erythraline	34
68	Argemonine	34
69	(S)-coclaurine	38
70	*N*-methylcoclaurine	38
71	Berbamunine	38
72	Dauricine	40
73	Isoliensinine	40
74	Pisopowiaridine	40
75	Imine cordobimine	40
76	Stebisimine	41
77	Sciadoferine	41
78	Nortiliacorinine A	41
79	Cissampareine	41
80	1,2-Dehydromicranthine	42
81	Pseudorepanduline	42
82	Insulanoline	42
83	Dihydoisoobtusilactone	46
84	Dihydroobtusilactone	46
85	3-Epilitsenolide D_1	46
86	3-Epilitsenolide D_2	46
87	Alseodafuranone	46
88	Sanjoinine	46
89	(-)-*N*-norarmepavine	46
90	(+)-Reticuline	46
91	(+)-Coclaurine	47
92	(-)-Coclaurine	47
93	Lirioferine	47

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LIST OF CHEMICAL STRUCTURE (conc.)

94	Isocorydine	47
95	Norisocorydine	47
96	Obamegine	47
97	*N*-methyllaurotetanine	48
98	2-Norobamegine	48
99	Laurotetanine	48
100	(+)-2'-Noroxyacanthine	48
101	(+)-Seeperine	48
102	(-)-Gyrolidine	49
103	(+)-Norstephasubine	49
104	(+)-2-Norobaberine	49
105	*O*-Methyllimacusine	49
106	Norboldine	50
107	(+)-3',4'-Dihydrostephasubine	50
108	*O*-Methylrepandine	50
109	Armepavine	50
110	Doryafranine	50
111	Xylopinine	51
112	1-(4-Methoxybenzyl)-6-7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline	51
113	4-Hydroxy, 3-methoxy benzoic acid	51
114	Neolignan eusiderin-A	51
115	Norlirioferine	51
116	Lindcarpine	51
117	Norpredicentrine	51
118	α'-oxoperakensimine	52
119	Boldine	52
120	*N*-(2-hydroxypropyl)-pendulifoline A	52
121	*N*-(2-hydroxypropyl)-pendulifoline B	52
122	Oxohernagine	52
123	Hernagine	52
124	Corydine	52
125	Isocorytuberine	53
126	Costaricine	53
127	Sebiferine	53
128	*N*-(2-hydroxypropyl)-norboldine A	53
129	*N*-(2-hydroxypropyl)-norboldine B	53
130	*N*-cyanomethyl-norboldine	53
131	α'-oxoperakensimine A	54
132	α'-oxoperakensimine B	54
133	α'-oxoperakensimine C	54
134	*N*-methyl-2, 3, 6-trimethoxy morphinandien-7-one *N*-oxide	54
135	N-methyl-2, 3, 6-trimethoxymorphinandien- 7-one	54
136	Perakensol (7-hydroxy-2, 3, 6- trimethoxyphenanthrene)	55
137	Srilankine	55
138	1,1-Diethoxy-ethane	55
139	1-Methyl-1H-pyrrole	55

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LIST OF CHEMICAL STRUCTURE (conc.)

140	1,1-Diethoxy-2-methyl-propane	55
141	2-Methoxy-phenol	55
142	4-One,2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran	55
143	2-Methoxy-5-methylphenol	55
144	2-Methoxy-4-vinylphenol	55
145	D-allose	55
146	2,6-Dimethoxy-phenol	55
147	*N*-(2-morpholinoethyl)benzamide	55
148	1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane	55
149	Sucrose	56
150	1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-isoquinoline	56
151	2-Methoxy-4-(1-propenyl)-phenol	56
152	3,4,5-Trimethoxy-phenol	56
153	3,5-Dimethyl-2-nitro-phenol	56
154	2-Nonadecanone	56
155	2,2-Dimethyl–cyclohexanol	56
156	Tridecanoic acid methyl ester	56
157	3',5'-Dimethoxyacetophenone	56
158	(*E*)-3-Eicosene	56
159	2-Benzoyl-4-methoxymethyl-6-methyl-furo[2,3-b]pyridin-3-amine	56
160	2-Oxo-hexadecanoic acid, methyl ester	56
161	5-Hydroxy-2,3,3–trimethyl–2-(3-methyl-buta-1,3-dienyl) cyclohexanone	57
162	Di(3,4,methylenedioxy)benzylamine acetate	57
163	1-Hexyl-2-nitrocyclohexane	57
164	2-Methyl-3-(1-methylethenyl)-,acetate,(1à,2à,3à)-cyclohexanol	57
165	(-)-1,2,3,4-Tetrahydro isoquinolin-6-ol-1-carboxylic acid, 7-methoxy-1-methyl-methyl ester	57
166	Norisocorydine *N*-oxide	82
167	Corydine *N*-oxide	85
168	Isocorydine *N*-oxide	85
169	*N*-methylhernagine	101
170	Hernovine	123

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ABBREVIATIONS

^{13}C NMR	13-Carbon NMR
1D-NMR	One Dimension Nuclear Magnetic Resonance
^{1}H NMR	Proton NMR
2D-NMR	Two Dimension Nuclear Magnetic Resonance
CC	Column Chromatography
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CH_3	Methyl group
$CHCl_3$	Chloroform
cm^{-1}	Per centimetre
COSY	^{1}H-^{1}H Correlation Spectroscopy
D	Doublet
Dd	Doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulphoxide
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
G	Gram
HCl	Hydrogen chloride
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HREIMS	High Resolution Electron Spray Ionization Mass Spectroscopy

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ABBREVIATIONS (conc.)

HRP2	Histidine-rich Protein 2
HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
J	Coupling constant
kg	Kilogram
LC/MS	Liquid Chromatography – Mass Spectrometer
M	Molar
M	Meter
m	Multiplet
MeOH	Methanol
MHz	Mega Hertz
Ml	Milliliter
mM	Milimolar
NH_3	Ammonia
NMR	Nuclear Magnetic Resonance
OCH_3	Methoxyl group
OH	Hydroxyl group
pH	Power of Hydrogen
Ppm	Parts per million
PTLC	Preparative Thin Layer Chromatography
s	Singlet

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ABBREVIATIONS (conc.)

t	Triplet
TLC	Thin Layer Chromatography
α	Alpha
β	Beta
δ	Chemical shift
λ	Maximum wave length

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CHAPTER 1

INTRODUCTION

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1.1 General

Every single organism are made up by hundreds of thousands different chemical and each species producing its own characteristic mixture. Chemical composition in organism differ one to others, for example taste, scents, and colour of durian are totally different from apple because of its distinctive chemical composition. In spite of hundred or thousand of chemicals in the organism, each entities has their own ability of function either it was yet discovery or not by our modern scientists. But some different substances could share same properties (e.g., taste from citric acid **1** from citrus, tartaric acid **2** from grapes, and malic acid **3** from apples are perceived as sour by human tongue).

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In daily lives, besides of the varieties of plants in the world, delicious taste of fruits are beneficial to human body in various ways due to their distinctive chemical composition. In the Holy Quran, a number of fruits; banana (al-Waqi'ah [56]:28-33), dates (ar-Ra'd [13]:4), olive (an-Nahl [16]:10-11), pomegranate (al-'An'am [6]:141), fig (at-Tin [95]:1) and grapes (al-Mu'minun [23]:19) have been proven by modern science have their own nutritious to prevent or cure various of disease (Al-Muammar & Khan, 2012; Biglari, AlKarkhi, & Easa, 2008; Bitler et al., 2007; Dueñas, Pérez-Alonso, Santos-Buelga, & Escribano-Bailón, 2008; Leifert & Abeywardena, 2008; Scarminio, Fruet, Witaicenis, Rall, & Di Stasi, 2012). In verses of al-'An'am ([6]:99), Allah S.W.T. commands human to observe and recount the variety and the beauty of His amazing creation with the obligation to protect sustainable of environment (Azlan & Azad, 2012; *Holy Quran: English Translation by Abdullah Yusuf Ali*, 2000).

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وَهُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخْرَجْنَا بِهِۦ نَبَاتَ كُلِّ شَىْءٍ
فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ ٱلنَّخْلِ
مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّٰتٍ مِّنْ أَعْنَابٍ وَٱلزَّيْتُونَ وَٱلرُّمَّانَ
مُشْتَبِهًا وَغَيْرَ مُتَشَٰبِهٍ ۗ ٱنظُرُوٓا۟ إِلَىٰ ثَمَرِهِۦٓ إِذَآ أَثْمَرَ وَيَنْعِهِۦٓ ۚ إِنَّ فِى
ذَٰلِكُمْ لَـَٔايَٰتٍ لِّقَوْمٍ يُؤْمِنُونَ ﴿٩٩﴾

It is He Who sendeth down rain from the skies: with it We produce vegetation of all kinds: from some We produce green (crops), out of which We produce grain, heaped up (at harvest); out of the date-palm and its sheaths (or spathes) (come) clusters of dates hanging low and

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> *near: and (then there are) gardens of grapes, and olives, and pomegranates, each similar (in kind) yet different (in variety): when they begin to bear fruit, feast your eyes with the fruit and the ripeness thereof. Behold! in these things there are signs for people who believe* **(**al-An'am [6]:99).

Each specific part of certain plant parts are produces numerous beneficial drugs products for mankind usage. Higher plants contribute about 25% of the total drugs in clinical use in the world. The specific plants organs that usually used likes herba or aerial parts (herba), leaf (folium), flower (flos), fruits (fructus), bark (cortex), root (radix), rhizome (rhizoma), and bulb (bulbus). The examples of variety plants parts that used as phytotherapeutic products are listed in Table 1.1.1(Skalicka-Wozniak, Widelski, & Glowniak, 2008).

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Demand on natural products such as α-amino acid demands over two million tons per year to produce various products that important in industries such as cysteine **4**, tryptophan **5**, and histidine **6** as antioxidants; serine **7** and alanine **8** are used in cosmetics; and the widely used as flavor enhancer; glutamic acid **9** or commonly known as monosodium glutamate (MSG) (Ahluwalia, Kumar, & Kumar, 2006). Nowadays, natural products were not only restricted to food industries, but they are widely applied in other field of usage such as beverages (vanillin **10**, anthocyanins **11**, and resveratrol **12**), pharmaceuticals (morphine **13**, aspirin **14**, and etoposide **15**), pesticides (pyrethrins **16**, allethrins **17**, and rotenone **18**) and plant growth regulators (gibberellins **19**, cytokinins **20**, abscisic acid **21** and ethylene **22**)(Chinou, 2008).

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Table 1.1.1

Specific Plants Parts in Certain Plants Species that Used in Phytotherapy.

Plants parts	Plants
Fruits and seeds	Caraway (*Carum carvi*) Fennel (*Foeniculum vulgare*) Saw palmetto (*Serenoa repens*) Horse chestnut (*Aesculus hipppcastanum*)
Leaf	Balm (*Melissa officinalis*) Deadly nightshade (*Atropa belladonna*) Ginkgo (*Ginkgo biloba*) Peppermint (*Mentha piperita*) Bearberry (*Arctostaphylos uva-ursi*)
Flowers	Chamomile (*Chamomilla recutita*) Calendula (*Calendula officinalis*) Arnica (*Arnica Montana*)
Stem	Ephedra (*Ephedra sinica*) Hawthorn (*Crataegus monogyna*) Passion flower (*Passiflora incarnata*) Wormwood (*Arthemisia absynthium*)
Bark	Frangula (*Rhamnus frangula*) Willow (*Salix alba*)
Rhizome and root	Devil's claw (*Harpagophytum procubens*) Tormentill (*Potentilla erecta*) Rhubarb (*Rheum palmatum*) Kava-kava (*Piper methysticum*)

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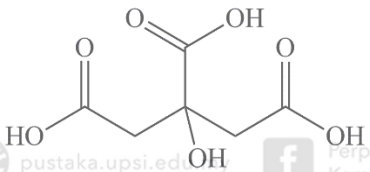
1.2 Problem Statements

Malaysia is a tropical rain forest country which has been blessed with plethora of natural resources. Stand by its own, North Borneo indicated 3500 species of endemic plant which exhibiting high levels vascular plant species richness and endemism, while the other 2400 species thrives in Malaysian Peninsula (Ahmad et al., 2010; J.

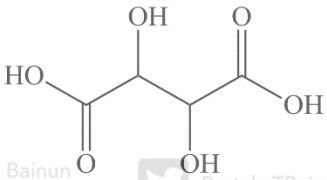
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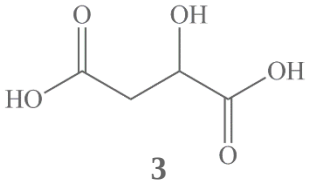
Williams et al., 2001). Today, illegal logging activities for agriculture practice in our forest was endangered the flora and the might be extinct within time before our phytochemist and pharmacist could discover their chemical constituents and potential as our new drugs discovery. A fast action must be taken to overcome this problem. This study will provide a new literature about alkaloid constituents and their biological activities from *Alseodaphne pendulifolia* Gamb. that collected from two different locations. This is the first report that study of alkaloid constituents from bark of this species. From the literature review, not much papers are reported about chemical constituents from genus of *Alseodaphne* (Thakur et al., 2012).



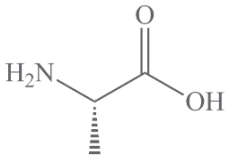
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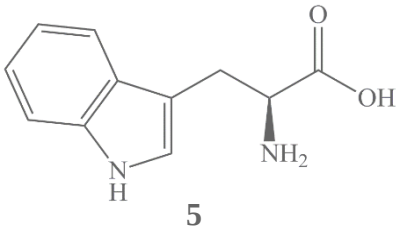
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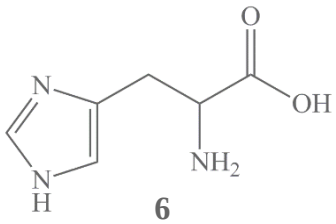
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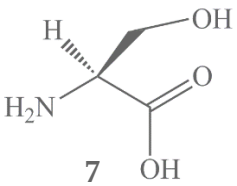
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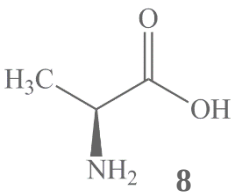
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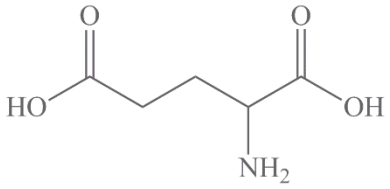
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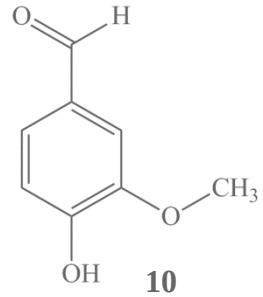
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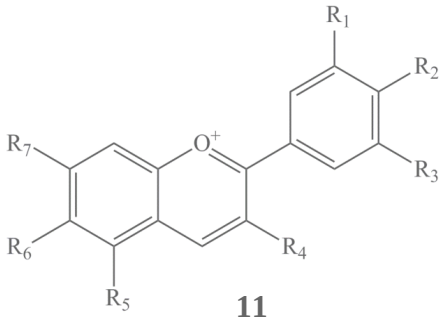
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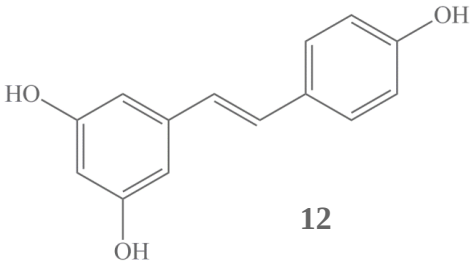
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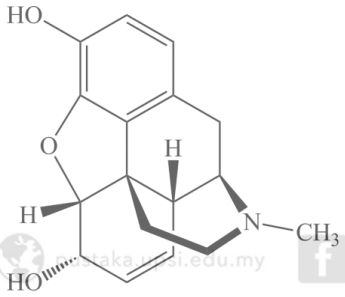
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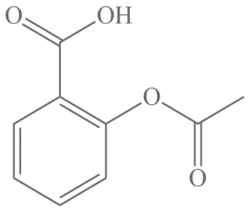
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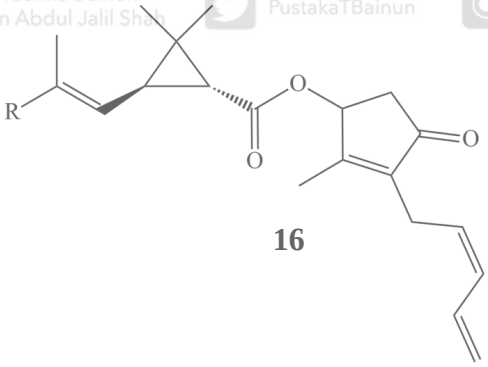
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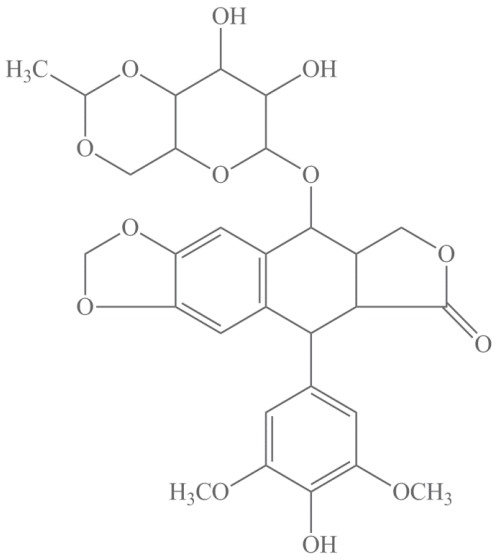
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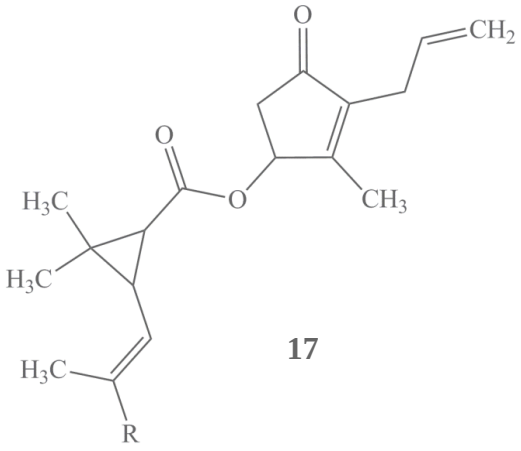
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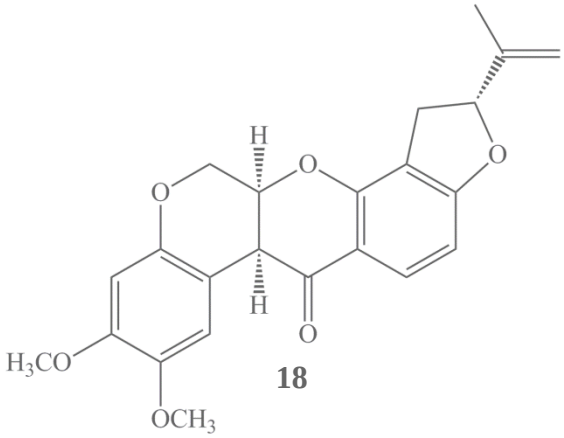
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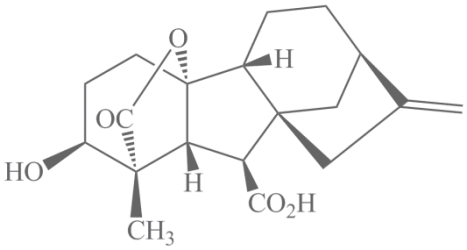
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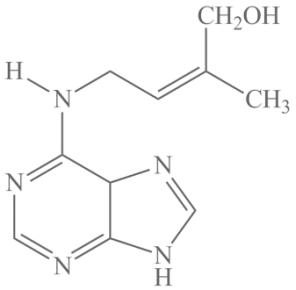
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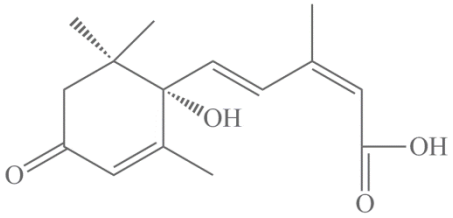
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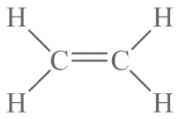
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1.3 Objectives of Study

The objectives of the study are as follows:

- To extract, isolate and purify alkaloid constituents from barks of *Alseodaphne pendulifolia* Gamb.
- To identify and elucidate the structure of the isolated alkaloids constituent using modern spectroscopy methods.
- To determine the antiplasmodial, cytotoxicity, antibacterial and antioxidant activities against the isolated alkaloids from the barks of *Alseodaphne pendulifolia* Gamb.

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1.4 Family of Lauraceae

The Lauraceae are predominantly arboreous family, a large pantropical family mainly composed of large trees or shrubs that mostly found in warm, tropical, humid subtropical and mild temperate regions of northern and southern hemispheres (Joshi, Verma, & Mathela, 2010; Simie et al., 2004). Sixty eight genera and more than two thousand species have been reported under this family (Drewes, Horn, & Mavi, 1997; Kumar, Rawat, Rahuja, Srivastava, & Maurya, 2009; Little, Stockey, & Penner, 2009; Oh et al., 2012; "The plant list, a working list of all known plant species.," 2010). The taxonomic hierarchy of Lauraceae family have been enlisted below:

Domain : Eukaryota
Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Laurales
Family : Lauraceae
Genera :

1. Actinodaphne
2. Aiouea
3. Alseodaphne
4. Aniba
5. Apollonias
6. Aspidostemon
7. Beilschmiedia
8. Camphora
9. Caryodaphnopsis
10. Cassytha
11. Chlorocardium
12. Cinnadenia

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13. Cinnamomum
14. Cryptocarya
15. Dehaasia
16. Dicypellium
17. Dodecadenia
18. Endiandra
19. Endlicheria
20. Eusideroxylon
21. Gamanthera
22. Hufelandia
23. Hypodaphnis
24. Iteadaphne
25. Kubitzkia
26. Laurus
27. Licaria
28. Lindera
29. Litsea
30. Machilus
31. Malapoenna
32. Mespilodaphne
33. Mezilaurus
34. Mocinnodaphne
35. Mutisiopersea
36. Nectandra
37. Neocinnamomum
38. Neolitsea
39. Nesodaphne
40. Notaphoebe
41. Nothaphoebe
42. Ocotea
43. Oreodaphne
44. Paraia
45. Parasassafras
46. Parthenoxylon
47. Persea
48. Phoebe
49. Phyllostemonodaphne
50. Pleurothyrium
51. Polyadenia
52. Potameia
53. Potoxylon
54. Povedadaphne
55. Ravensara
56. Rhodostemonodaphne
57. Sassafras
58. Schauera
59. Sextonia
60. Sinopora
61. Sinosassafras
62. Syndiclis

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63. Systemonodaphne
64. Tetranthera
65. Umbellularia
66. Urbanodendron
67. Williamodendron
68. Yasunia

This family is largely characterized by trimerous flowers, bi- or tetrasporangiate anthers with apical valvate dehiscence, and a unicarpellate gynoecium containing a single anatropous, apically attached ovule. Fruits of the family are fleshy, typically black to almost black at maturity and usually borne on enlarged receptacles that can form cupulate to fully enclosing structures. Fruits of Lauraceae are typically considered drupes, but are better classified as a berry since the "endocarp" is only made up of a thin, single-celled sclerified endodermis. Oil or mucilage idioblasts occur throughout the plant tissues, including the wood (Little et al., 2009).

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Name of Lauraceae itself is derived from the prominent member, the Grecian laurel, *Laurus nobilis*, is characterized by plants which have prominent oil cells in the leaves, wood and fruits. These oils are mostly aromatic hence provide a number of flavouring materials and spices (Schroeder, 1975). Recorded showed that the lauraceous plants have widespread use in both oriental medicine and occidental medicine (Shimonura, Sashida, Mimaki, Oohara, & Fukai, 1988). Some plants in this family have been employed in folk medicine for their interesting bioactivities (Rachmatiah, Mukhtar, Nafiah, Hanafi, Awang, et al., 2009; Rachmatiah, Mukhtar, Nafiah, Hanafi, Kosela, et al., 2009). *Cinnamomum camphora (L.)* Presl is a major source of camphor, which can be made into camphor oil and mothballs. In addition, camphor is taken orally to calm hysteria, nervousness, neuralgia and to treat serious diarrhoea. Camphor is also known to be effective in treating colds and chills (Lee et

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al., 2006). The extracts from *C. Cassia* have been claimed to reduce inflammation (Lee & Shibamoto, 2002) and to decrease serum glucose, total cholesterol and platelet counts (Khan, Safdar, Ali-Khan, Khattak, & Anderson, 2003). Table 1.3.1 showed uses of species from family of Lauraceae.

Table 1.3.1

Uses of Species from Family of Lauraceae

Uses	Species of Lauraceae	References
Spices	*Cinnamomum cassia* *Cinnamomum subavenium* *Cinnamomum zeylanicum* *Laurus nobilis* *Sassafras albidum*	(Gottlieb, 1972; Kumar et al., 2009; Perera & Rajapakse, 1991)
Nutritious fruits	*Persea americana* *Persea gratissima*	(Gottlieb, 1972; Kumar et al., 2009; Perera & Rajapakse, 1991)
Chinese folk medicines	*Cinnamomum cassia* *Lindera aggregate*	(Kumar et al., 2009)
Timber	*Eusideroxylon zwageri* *Mezilaurus itauba,* *Mezilaurus navalium* *Ocotea bullata* *Persea nanmu*	(Gottlieb, 1972)
Seed fat	*Laurus nobilis* *Litsea sebifera*	(Gottlieb, 1972)
Perfume oils	*Aniba duckei* *Aniba rosaeodora* *Ocotea pretiosa*	(Gottlieb, 1972)

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1.5 Genus of *Alseodaphne*

Genus of *Alseodaphne* consist more than fifty species (Table 1.4.1), distributed around Malaysia, China, Cambodia, Vietnam, Laos, Indonesia, Sri Lanka, Myanmar, Philippines, Thailand and India. This genus locally known as medang or tejur in Malaysia (International Seed Testing Association, 2013; Omar, Nafiah, Mukhtar, Awang, & Hadi, 2009), Wewarana in Sri Langka (Ariyadasa, 2002), Gemur in Indonesia (Joosten, Tapio-Bistrom, & Tol, 2012; Kalimantan Forests and Climate Partnership, 2009) and Nelthare in Tamil Arambamaram, Kanaippirandai (Alex, Charles, Leo, Joseph, & Mani, 2012; Charles, Stanly, Joseph, & Ramani, 2011).

From more than sixty species, only a few from this species were reported their usage. The trees of *Alseodaphne semecarfolia* was reported used as nest by Hornbill (Mudappa & Kannan, 1997; Velho, Datta, & Isvaran, 2009) and fruits was eaten as one of their food sources in Sg. Tekam Forest Reserve (Andrew D. Johns, 1987) but for *Alseodphne perakensis,* aborigines of the Sakai tribe claimed that the fruit from this plant is poisonous (Lajis, Mahmud, Din, & Toia, 1989). The versatile and aesthetic qualities hardwood of *Alseodaphne hainanensis, A.oblanceolata* and *A. perakensis,* are highly demand in specific market of "luxury" applications including buildings, furniture, ships and ornaments due to their quality of hue, grain, texture, strength and durability (Huang, 2011; Lajis et al., 1989; Schroeder, 1982). In construction sector, the durability of *A. dura* and *A. longipes* is leveraged as seawater piles (Schroeder, 1982).

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In Sumatra, Indonesia, *A. coriacea* is a livelihood sources for some Sumatran. It was harvested in deep forest for their valuable bark that rich with pyrethrins **16**, a compound that used in variety of insecticides especially in production of mosquito coil. Besides insecticides, the bark of this species also used in production of joss sticks (dupa cina), surfactant for recycling engine oil and some in cosmetics products (Janudianto, Mulyoutami, Joshi, Wardell, & Noordwijk, 2011; Joosten et al., 2012; Kristedi & Kieft, 2010; Lyons, 2003). In folk medicine, *A. semicarpifolia* was used as treatment of some illness likes cholera-like illness, rinderpest and dysentery diseases in cattle and also bark juice is applied externally for leach bite or chest pain (Alex et al., 2012; *Basic Medical Sciences; Intramural Research: An Annual Report*, 2012; Charles, Joseph, & Ramani, 2012; Charles et al., 2011; Harsha, Shripathi, & Hegde, 2005; Karuppusamy, 2007; Parinitha, Harish, Vivek, Mahesh, & Shivanna, 2004)

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Alseodaphne are classified as evergreen trees. The leaves were alternate, always clustered near apex of branchlet, pinninerved, often turning black when dry. Inflorescence axillary, paniculate or racemose; bracts and bracteoles deciduous. Flowers are bisexual and trimerous. Perianth tube short; perianth lobes, subequal or outer is smaller, slightly dilated after anthesis but absent in fruit. Fertile stamens, in three whorls; filaments of first and second whorls glandless, those of third whorl each with two glands at base; anthers four-celled; cells of first and second whorls introrse, those of third whorl extrorse or upper two lateral and lower two extrorse. Staminodes of innermost whorl, very small, nearly sagittate. Ovary partly immersed into shallow perianth tube; style often as long as ovary; stigma small, inconspicuous, discoid. Fruit black or purplish black when mature, ovoid, oblong, or subglobose; fruit stalk red,

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green, or yellow, sometimes nearly cylindric, fleshy, pulpy, always warty, truncate at apex (Shu, Hsi-wen, Jie, & Werff, 2008).

Table 1.4.1

List of Species in Genus of Alseodaphne (Lauraceae)

No.	Species	References
1	*A. albifrons*	**I,II,III**
2	*A. andersonii*	**II,IV,V**
3	*A. archboldiana*	**VI**
4	*A. bancana*	**II,VII,VIII**
5	*A. birmanica*	**II**
6	*A. borneensis*	**II**
7	*A. cavaleriei*	**II**
8	*A. ceratoxylon*	**IX,X**
9	*A. changchangensis*	**IV**
10	*A. coriacea*	**I,XI,XII**
11	*A. corneri*	**II,XIII**
12	*A. costalis*	**VIII**
13	*A. cuneata*	**X**
14	*A. dura*	**II,XIV**
15	*A. elmeri*	**II**
16	*A. elongata*	**II**
17	*A. falcate*	**X**
18	*A. foxiana*	**II**

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19	*A. garciniicarpa*	**II**
20	*A. gigaphylla*	**IV**
21	*A. glauciflora*	**II**
22	*A. glaucina*	**II**
23	*A. gracilis*	**II,V**
24	*A. grandis*	**VIII**
25	*A. griffithii*	**II**
26	*A. habrotricha*	**II**
27	*A. hainanensis*	**II,IV,V,XV,XVI,XVII**
28	*A. havilandii*	**X**
29	*A. himalayana*	**II**
30	*A. hokouensis*	**II,IV,V**
31	*A. huanglianshanensis*	**II,IV,V**
32	*A. insignis*	**I,II,IX,X,XVIII**
33	*A. intermedia*	**I,II,VII,XIX**
34	*A. khasyana*	**II**
35	*A. kochummenii*	**II**
36	*A. langugenosa*	**XIV**
37	*A. lanuginosa*	**II**
38	*A. longipes*	**II,XIV,XVIII**
39	*A. marlipoensis*	**II,IV,V**
40	*A. medogensis*	**II**
41	*A. micrantha*	**II**
42	*A. montana*	**II**

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43	*A. nigrescens*	**II,IV,VII,XX**
44	*A. oblanceolata*	**II,XIV,XIX**
45	*A. obovata*	**I**
46	*A. owdenii*	**II**
47	*A. paludosa*	**II,X**
48	*A. peduncularis*	**I,II**
49	*A. pendulifolia*	**I,II**
50	*A. perakensis*	**II,XIII,XIX,XXI,XXII,XXIII,XXIV**
51	*A. petiolaris*	**II,IV,V,XXV,XXVI,XXVII**
52	*A. philippinensis*	**II**
53	*A. ramosii*	**II**
54	*A. rhododendropsis*	**II**
55	*A. ridleyi*	**I,II**
56	*A. rubriflora*	**II**
57	*A. rubrolignea*	**II**
58	*A. rugosa*	**II,IV,V,XXVIII**
59	*A. semecarpifolia*	**II,IV,XXI,XXIX,XXX,XXXI,XXXII,XXXIII,XXXIV,XXXV,XXXVI,XXXVII,XXXVIII,XXXIX,XL,XLI**
60	*A. siamensis*	**II**
61	*A. sichourensis*	**II,IV,V**
62	*A. subopppositifolia*	**II**
78	*A. sulcata*	**II**
64	*A. tomentosa*	**II**
65	*A. tonkinensis*	**II**

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66	*A. utilis*	**II**
67	*A. umbaliflora*	**XVIII,XLII**
68	*A. wrayi*	**II**
69	*A. yunnanensis*	**II,V**

References : **I** (International Seed Testing Association, 2013), **II** (Thakur et al., 2012), **III** (Zulkifly, Kim, Majid, & Merican, 2011), **IV** (Li et al., 2011), **V** (Shu et al., 2008), **VI** (Bick, 1996), **VII** (Chong, Tan, & Corlett, 2009), **VIII** (Wong et al., 2013), **IX** (Kartawinata et al., 2006b), **X** (Kartawinata et al., 2006a), **XI** (Joosten et al., 2012), **XII** (Kalimantan Forests and Climate Partnership, 2009), **XIII** (Sulaiman et al., 2011), **XIV** (Schroeder, 1982), **XV** (Huang, 2011), **XVI** (Ortega et al., 2010), **XVII** (Zhang & Ma, 2008), **XVIII** (Hidayat & Simpson, 1994), **XIX** (Ya'akub, Kudus, Nazre, Noor, & Hanum, 2013), **XX** (Tata, Noordwijk, & Werger, 2008), **XXI** (Lajis et al., 1989), **XXII** (Mahmud, 1989), **XXIII** (Nafiah et al., 2011), **XXIV** (Okechukwu, 2005), **XXV** (Lan, Zhu, & Cao, 2012), **XXVI** (Majumdar, Datta, & Shankar, 2012), **XXVII** (Rout, Sajem, & Nath, 2012), **XXVIII** (Koskela, Hong, & Rao, 2002), **XXIX** (Alex et al., 2012), **XXX** (Ariyadasa, 2002), **XXXI** (Chanderbali, Werff, & Renner, 2001), **XXXII** (Charles & Alex, 2011), **XXXIII** (Charles et al., 2012), **XXXIV** (Charles et al., 2011), **XXXV** (Harsha et al., 2005), **XXXVI** (Jayakumar, Arockiasamy, & Britto, 2000), **XXXVII** (Karuppusamy, 2007), **XXXVIII** (Mahadkar, Valvi, & Jadhav, 2013), **XXXIX** (Mudappa & Kannan, 1997), **XL** (Parinitha et al., 2004), **XLI** (Parthasarathy, 1999), and **XLII** (Adinugroho, Suryadiputra, Saharjo, & Siboro, 2011)

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1.6 Species of *Alseodaphne pendulifolia* Gamb.

Alseodaphne pendulifolia Gamb. (Figure 1.5.1 – 1.5.3) is a medium to large sized tree with approximately 120 cm trunk circumference, height up to 18 m tall, that are rarely found in lowland forests around Perak, Terengganu, Pahang, Selangor, and Borneo Island. This plant has pale yellow flowers in auxiliary panicles, with perianth glabrous outside and outer whorl smaller than inner. The shape of fruits are obvoid with 1.5 x 1 cm in sized with about 15 cm long perianth tube which slightly enlarged (Nafiah, 2009; Ng, 1989; Thakur et al., 2012).

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The colour of the bark is brownish, smooth to lenticels parts and the inner bark are yellow same as sapwood. The twigs are thick with prominent leaf scars. The leaves are clustered at the ends of the twigs, dropping down with stalk about 2 - 4 cm along and blade leathery, slightly glacious below, obovate or oblanceolote shape. The apex is blunt or pointed and the base is narrowed. Midrib rose above and the secondary nerves are about 15-22 pairs, curving and joining near margin, which is visible on above side (Nafiah, 2009; Ng, 1989; Thakur et al., 2012).



Figure 1.5.1: Fruits of *Alseodaphne pendulifolia* Gamb.



Figure 1.5.2: Barks of *Alseodaphne pendulifolia* Gamb.



Figure 1.5.3: Leaves of *Alseodaphne pendulifolia* Gamb.

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CHAPTER 2

GENERAL CHEMICAL ASPECTS

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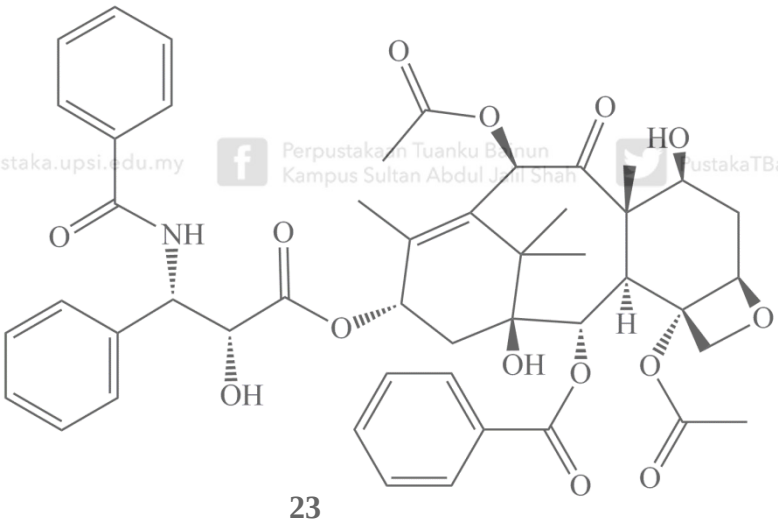
2.1 General

Term ‘natural products’ is defined as the products from natural origin including entire organism (a plant, an animal, an insert or a microorganism), part of an organism (barks or leaves of plants or specific animals/insects organs), an extract of an organism or part of organism, exudates and pure compounds such as alkaloids, terpenoids, flavonoids and tannin that isolated from plants (paclitaxel **23** from *Taxus brevifolia*), animals (vitamins A **24** and vitamin D **25** from cod liver oil), inserts (cantharidin **26** from *Blister beetles*) or microorganism (doxorubicin **27** from *Streptomyces peucetius*) from any terrestrial or marine source (Ratcliffe, Mello, Garcia, Butt, & Azambuja, 2011).

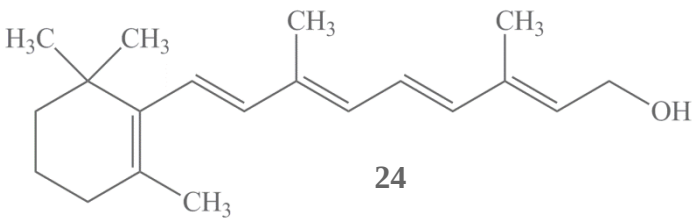
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In the most cases in natural products chemistry, the term ‘natural products’ refers to secondary metabolites of small molecules (molecular weight less than 2000 amu) produced by an organism that are not strictly necessary for the survival of the organism. Natural products especially plant-based medicines used since ancients era by primordial man in the healing process. History recorded that Hippocrates himself was used approximately 400 different plant species in therapeutic therapy for medicinal purposes. In year of 2005, statistic from World Health Organization was reported that 75 % of people still rely on plant-based traditional medicines for primary health care globally (Sarker, Latif, & Gray, 2006).



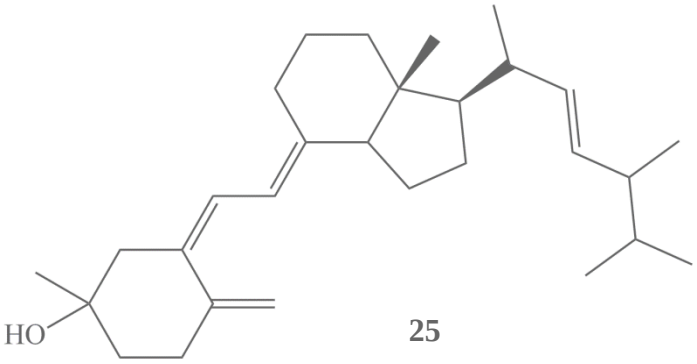
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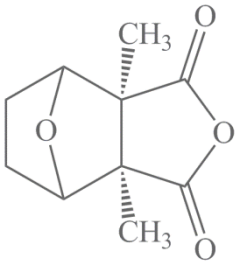
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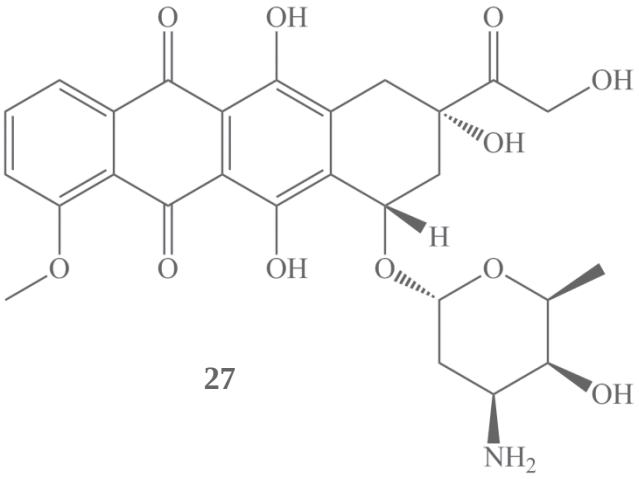
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2.2 Alkaloid

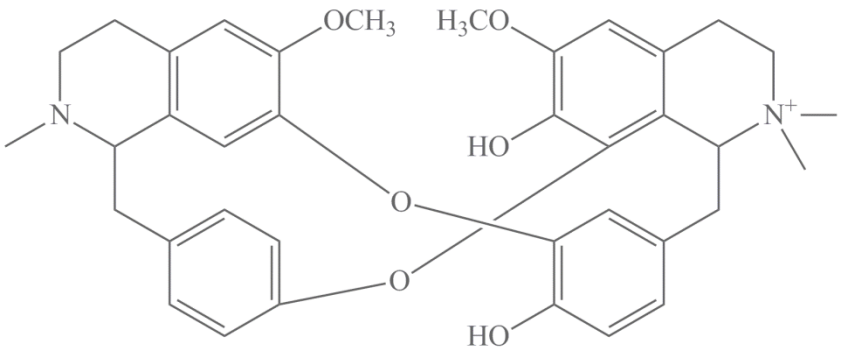
Alkaloids means alkali likes (alkali = base, oid = like sub), one of the largest and the most diverse classes of secondary metabolites of natural products that represent over 12,000 defined structures around the globe, ranging from simple structures to very complex ones. They can be recognized as nitrogen-containing bases, with their nitrogen atom, as a part of heterocyclic system, most of which possessing significant pharmacological properties and derived from amino acids or derivatives from which they are biosynthesized. Alkaloids are only slightly soluble in water but soluble in ethanol, benzene, ether and chloroform (Aniszewski, 2007).

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Mostly, they were isolated from plants but research of the last decades shown that are also have been found in bacteria, fungi and animals (Wink, 2000). The first alkaloid was isolated in Europe in early 19th century and the interest of study about their properties, function and usefulness was increase until today. Most of them are beneficial as drugs such as tubocurarine **28**, cocaine **29**, morphine **13**, codeine **30**, quinidine **31**, quinine **32** and vinblastine **33**. In the earlier century, scientists believed that alkaloids were waste products from plants but nowadays their general function in plants and animals seems to be as defence chemicals against predators (herbivores, carnivores and to a lesser degree against bacteria, fungi and viruses) that play an important role in the ecology of plants or animals producing them (Bick, 1996).

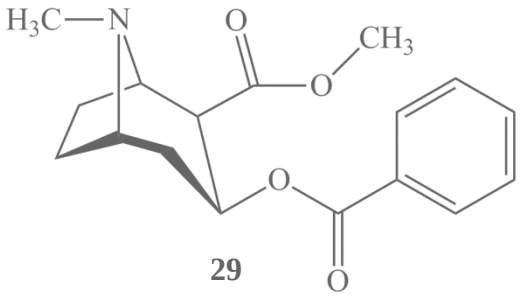
It has been estimated that more than 20% of all plant species contain alkaloids, especially members of the families Annonaceae, Ranunculaceae, Berberidaceae, Papaveraceae, Buxaceae, Cactaceae, Apocynaceae, Menispermaceae, Rutaceae, Solanaceae, Boraginaceae, Leguminosae and Lauraceae. Main classes of alkaloids groups in plants kingdom are pyridine, tropine, quinoline, isoquinoline, and indole alkaloids (Chinou, 2008).



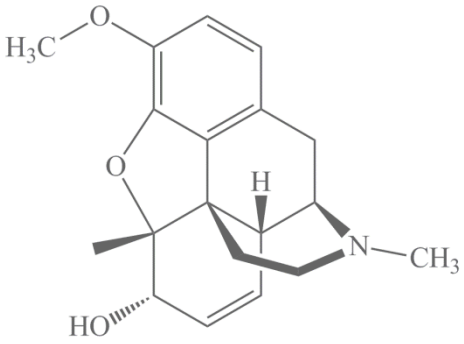
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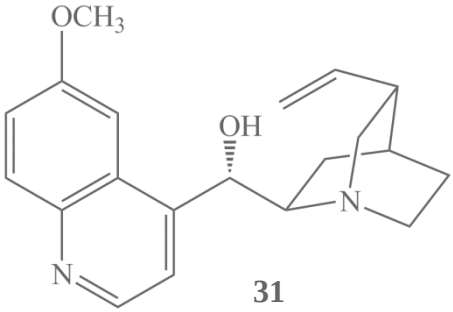
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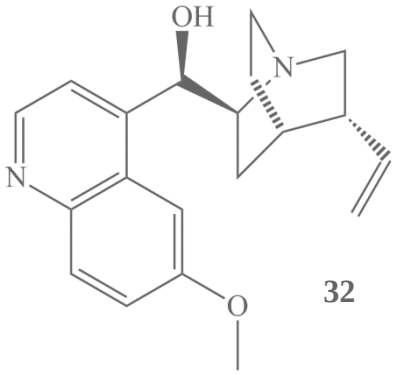
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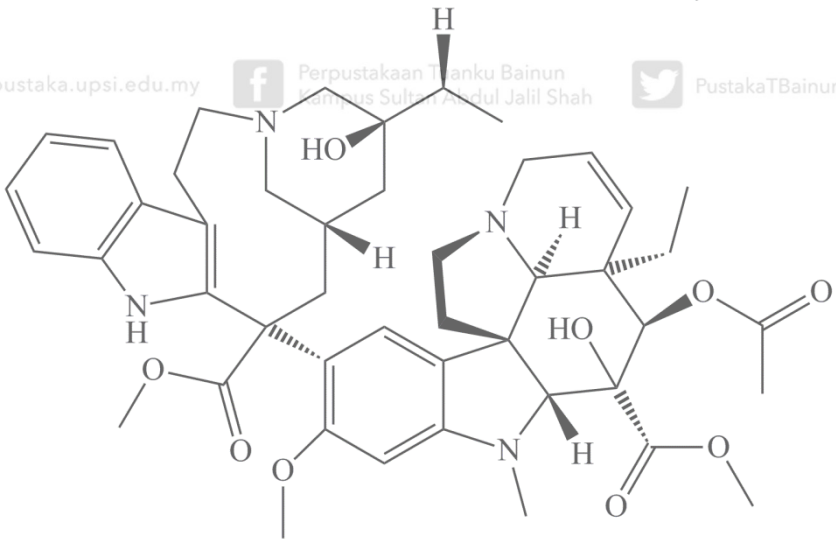
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2.3 Classification of Alkaloids

Alkaloids are divided according to their skeletons and origins, there are three main types of alkaloids: true alkaloids, protoalkaloids and pseudoalkaloids. True alkaloids and protoalkaloids are derived from amino acids, whereas pseudoalkaloids are not derived from these compounds.

2.3.1 True Alkaloids

True alkaloids derive from amino acid and they share a heterocyclic ring with nitrogen. These alkaloids are highly reactive substances with biological activity even in low doses. All true alkaloids have a bitter taste and appear as a white solid, with the exception of nicotine which has a brown liquid. True alkaloids form water-soluble salts. Moreover, most of them are well-defined crystalline substances which unite with acids to form salts. True alkaloids may occur in plants in the free state, as salts and as *N*-oxides. These alkaloids occur in a limited number of species and families, and are those compounds in which decarboxylated amino acids are condensed with a non-nitrogenous structural moiety. The primary precursors of true alkaloids are such amino acids as L-ornithine **34**, L-lysine **35**, L-tyrosine **36**, L-tryptophan **37** and L-histidine **38**. Examples of true alkaloids include biologically active alkaloids as cocaine **29**, quinine **32**, morphine **13** and usambarensine **39** (Aniszewski, 2007).

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2.3.2 Protoalkaloids

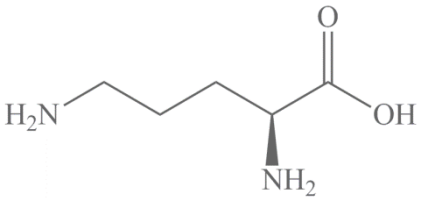
Protoalkaloids are compounds, in which the *N* atom derived from an amino acid is not a part of the heterocyclic. Such alkaloid include compounds derived from L-tyrosine **36** and L-tryptophan **37**. Protoalkaloids are those with a closed ring, being perfect but structurally simple alkaloids. They form a minority of all alkaloids. Hordenine **40** and mescaline **41** are good examples of is kind of alkaloid (Aniszewski, 2007).

2.3.3 Pseudoalkaloids

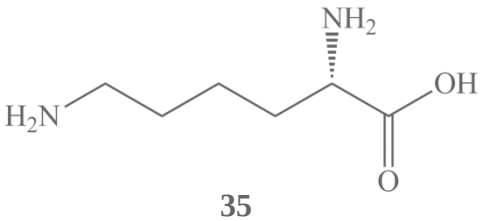
Pseudoalkaloids are compounds, which the basic carbon skeletons of which are not derived from amino acids. In reality, pseudoalkaloids are connected with amino acid pathways. They are derived from the precursors or postcursors (derivatives the indegradation process) of amino acids. They can also result from the amination and transamination reactions of the different pathways connected with precursors or postcursors of amino acids. These alkaloids can also be derived from non-amino acid precursors. The *N* atom is inserted into the molecule at a relatively late stage, for example, in the case of steroidal or terpenoid skeletons. Certainly, the *N* atom can also be donated by an amino acid source across a transamination reaction, if there is a suitable aldehyde or ketone. Pseudoalkaloids can be acetate and phenylalanine derived or terpenoid, as well as steroidal alkaloids. Examples of pseudoalkaloids include such compounds as coniine **42**, capsaicin **43**, ephedrine **44**, solanidine **45**, caffeine **46**, theobromine **47** and pinidine **48** (Aniszewski, 2007).

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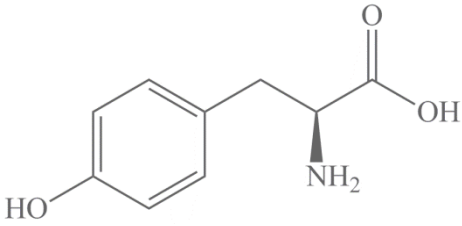
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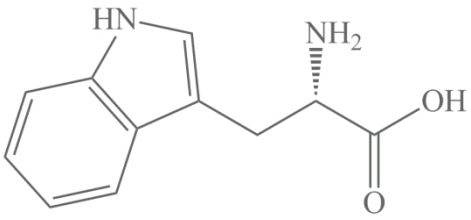
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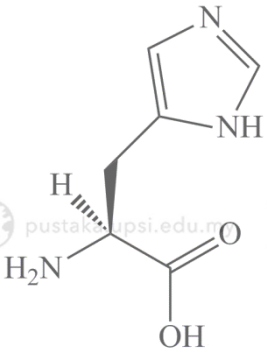
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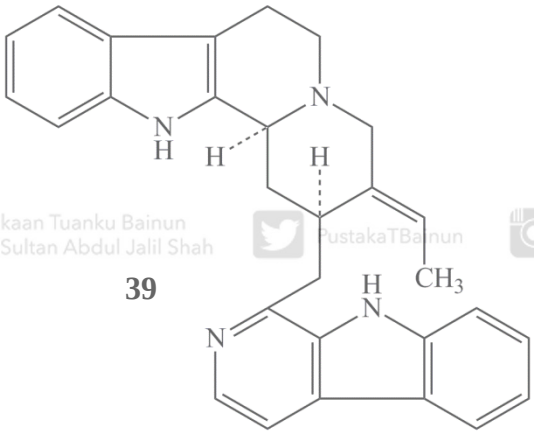
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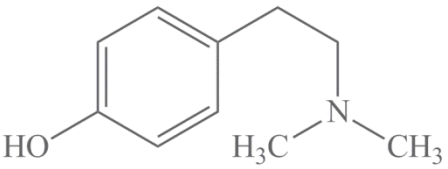
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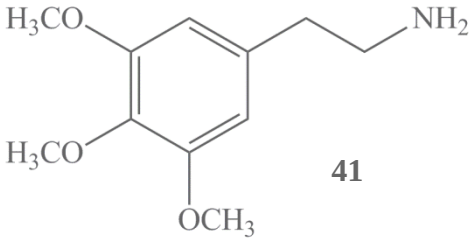
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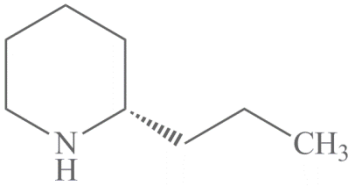
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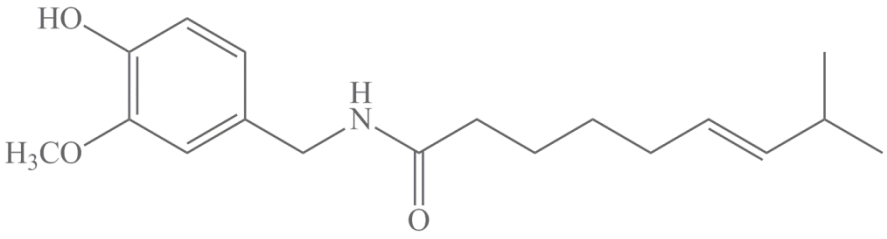
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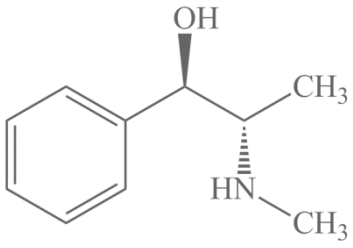
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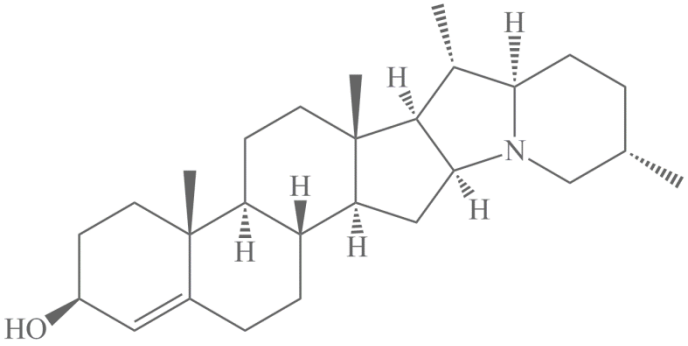
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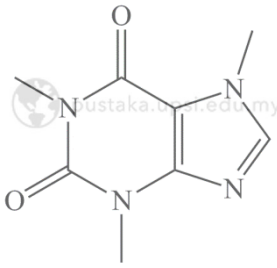
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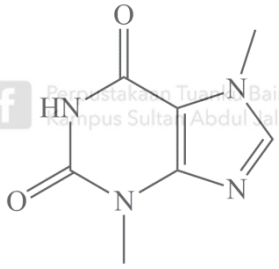
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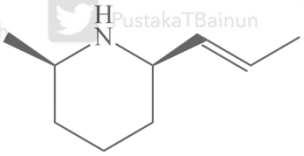
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2.4 Isoquinoline Alkaloids

Isoquinoline group is divided to eight main subgroups; benzylisoquinoline and benzyltetraisoquinoline, aporphine, protoberberine, benzophenantridine, protopine, phthalideisoquinoline, morphine, and emetine. Bisbenzylisoquinoline is another special subgroup of isoquinoline alkaloids because of its dimeric structure.

Biogenetically isoquinoline alkaloids are classified into phenylalanine group or its hydroxyderivative – tyrosine **36**. The precursors take part in synthesis of alkaloid molecule. Subgroup of benzylisoquinoline are synthesized from phenylalanine and phenylacetaldehyde, where tyrosine is the precursor of all opium alkaloids. However, alkaloids of type aporphine, protoberberine and morphinane arise from the benzylisoquinoline and benzyltetraisoquinoline alkaloids by appropriate condensations.

Isoquinoline alkaloids was isolated mainly in the following plant order: *Ranunculales*, *Papaverales*, *Geraniales*, *Retales*, *Plumboginales*, *Myrtiflorae* and *Rosales* (Waksmundzka-Hajnos & Petruczynik, 2008). Table 2.4.1 showed examples of alkaloids from isoquinoline subgroups and their source.

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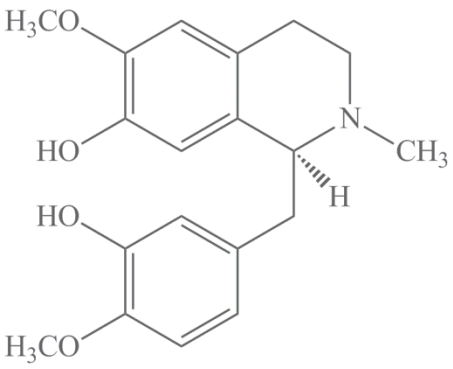
Table 2.4.1

Examples of Alkaloids from Isoquinoline Subgroups and Their Source.

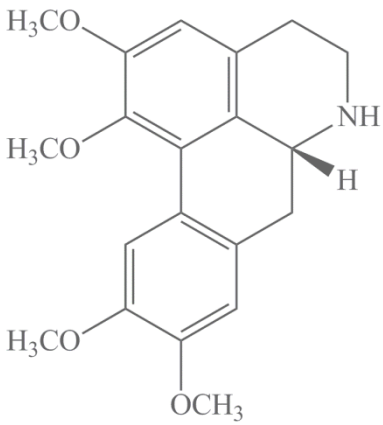
Isoquinoline subgroup	Example of Alkaloid	Sources	References*
Benzylisoquinoline	(*s*)-reticuline **49**	*Corydalis ternata*	I
Aporphine	norglaucine **50**	*Liriodendron tulipifera*	II
Protoberberine	palmatine **51**	*Rhizoma Coptidis*	III
Benzophenanthridine	buesgeniine **52**	*Zanthoxylum buesgenii*	IV
Protopine	allocryptopine **53**	*Bocconia cordava*	V
Morphine	thebaine **54**	*Papaver somniferum*	VI
Emetine	emetine **55**	*Carapichea ipecacuanha*	VII
Bisbenzylisoquinoline	(+)-stephasubine **56**	*Stephania hernandifolia*	VIII

*References: I-(Kim et al., 2010), II-(Graziose et al., 2011), III-(Ding, Chen, Lu, & Li, 2012), IV-(Tane, Wabo, & Connolly, 2005), V-(Iwasa, Okada, & Takao, 1983), VI -(S. Lee et al., 2011), VII -(Junior, Cruz, Santos, & Medeiros, 2012), VIII-(Patra, Mandal, Mukhopadhyay, & Ranu, 1987)

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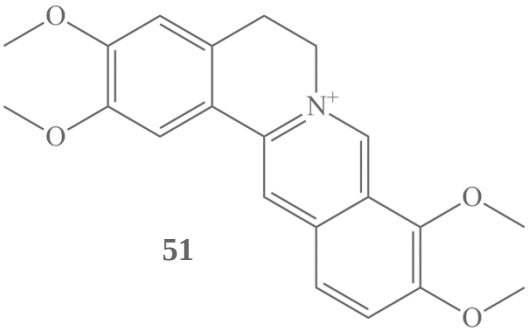
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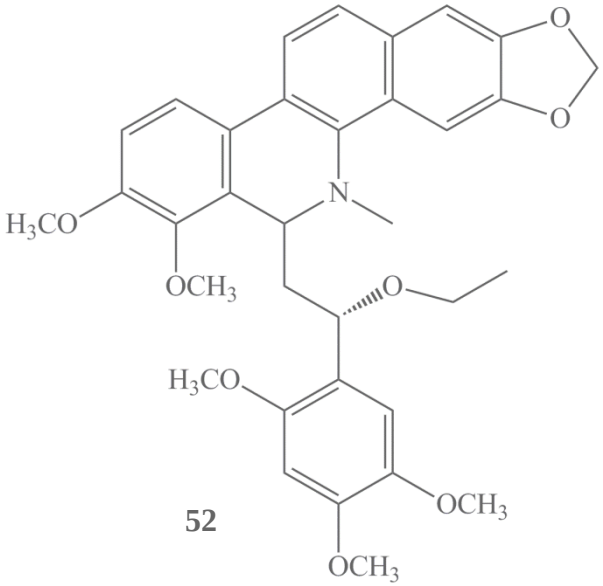
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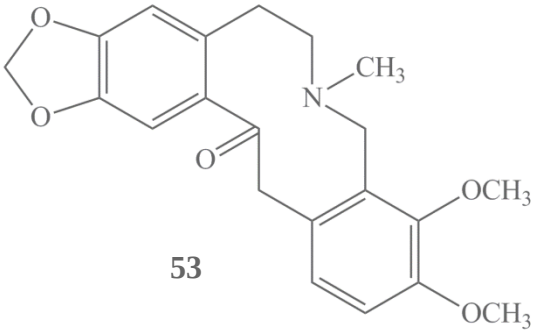
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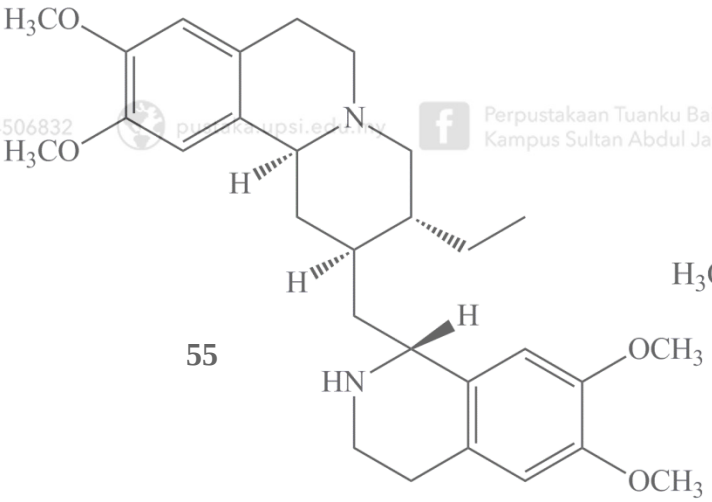
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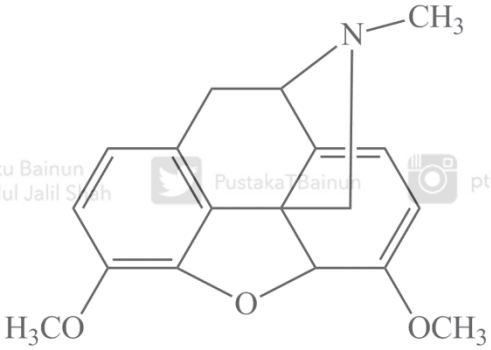
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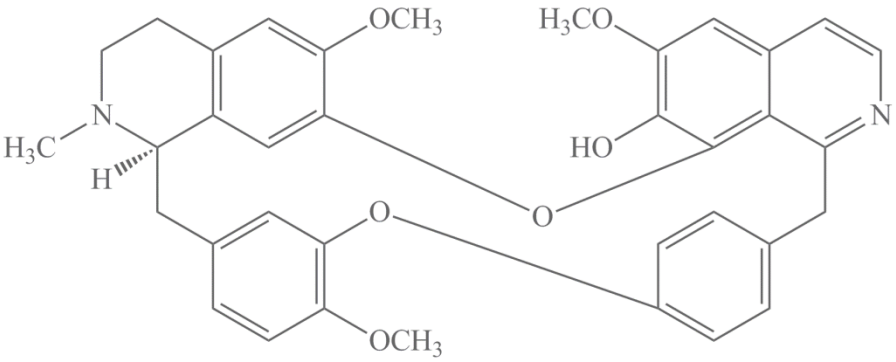
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2.5 Aporphine Alkaloids

Aporphine alkaloid make up a large group of isoquinoline alkaloids about 700 alkaloids isolated from more than twenty families of plants. The numbering of the skeleton is according to the accepted ruling and all aporphine alkaloids are based on the skeleton as shown in Figure 2.5.1. They consist of di-, tri-, tetra-, penta- and hexa-substituted derivatives, the substituents being hydroxyl, methoxy or methylenedioxy groups that can be situated over all four rings. The most widely distributed in nature are 1, 2, 9, 10- and 1,2,10,11-tetrasubstituted bases and 1,2,3,3,10-pentasubstituted ones. The disubstituted aporphines have substituents in the 1- and 2-position. *N*-oxides of aporphine alkaloids have also been isolated. The N atom in aporphine can be secondary, tertiary, or quaternary (Azimova & Yunusov, 2013; Israilov, Karimova, Yunusov, & Yunusov, 1980).

Oxoaporphine alkaloids with a completely dehydrogenated system and a C-7 ketone are widely distributed. These optically inactive colored bases are poorly soluble in organic solvents. Nonphenolic oxoaporphine are yellow and red; phenolic ones (with a C-1 or C-11 hydroxyl) are green. Ultraviolet-, mass- and nuclear magnetic resonance-spectra provide good information about the mutual positioning of the substituents in aporphine. Unsubstituent or monosubstituent (ring D) compounds have UV absorption maxima at 270-280 nm and a shoulder at 310-320 while, 1,2,9,10-substituted at 280 and 305-310 nm and 1,2,10,11-substituted at 270 and 305-310 nm. Hydroxyls in ring A are weaker phenolic bases than those in ring D (Azimova & Yunusov, 2013).

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Aporphine alkaloids have a twisted biphenyl system and an asymmetric H atom on C-6a. It has been demonstrated that the absolute configuration of the asymmetric center is *S* in dextro-rotating alkaloids with the A configuration; for levo-rotating, *R* (B configuration) as shown in Figure 2.5.2. This alkaloids cannot be diastereoisomers. Oxidation of phenolic alkaloids begins with the core containing the hydroxyl. Methylenedioxy in aporphine alkaloids most often located in the 1,2-position. Pharmacological investigations showed that aporphine alkaloids are slightly toxic compound. Many of the posses hypotensive and spasmolytic activity (Azimova & Yunusov, 2013).

Biosynthesis of aporphine alkaloid starts by condensation between dopamine **57** and 4-hydrophenylacetaldehyde **58** yielded the first alkaloid intermediate (S)-norcoclurine **59**, which mark the first and the central intermediate of isoquinoline alkaloids. The nitrogen atom in the ring B is often substituted with H or methyl while carbon at position 6 and 7 are usually oxygenated. This can be showed condensation to yielded (S)-reticuline **29**, where compound (S)-norcoclurine **59** are methylated on the carbon 6 of the phenol and the nitrogen atom before being hydroxylated at carbon 12 and finally methylated to (S)-reticuline **29** (Scheme 2.5.1).

Thus, the phenolic oxidative coupling generates the bond between the aporphine rings A and D shown by the transformation of reticuline **29** into corytuberine **60**. Here, corytuberine **60** are formed by ortho-ortho coupling of reticuline **29**. On the other hand, ortho-para coupling of reticuline **29** would give the isoboldine **61** which by further methylation could plausibly give rise to the glaucine

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62. This suggestion was comfirm by the feeding conversion of radio-active reticuline **29** into bulbocapnine **78** (Scheme 2.5.2).

Aporphine alkaloids have been reported from family of Annonaceae, Aristolochiaceae, Berberidaceae, Euphorbiaceae, Eupomatiaceae, Fumariaceae, Hernandiaceae, Hypecoaceae, Lauraceae, Magnoliaceae, Menispermaceae, Monimiaceae, Nelumbonaceae, Nympheaceae, Papaveraceae, Piperaceae, Ranunculaceae, Rhamnaceae, Rutaceae, and Saururaceae. Annonaceae and Lauraceae families (Guinaudeau, Leboeuf, & Cave, 1979, 1983, 1988, 1994).

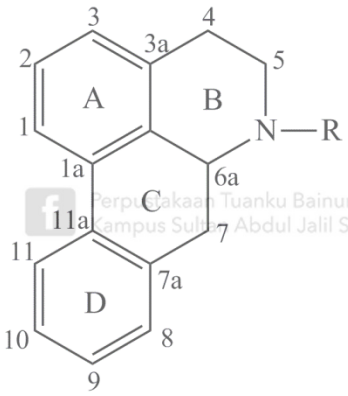


Figure 2.5.1: Numbering of Aporphine Skeleton

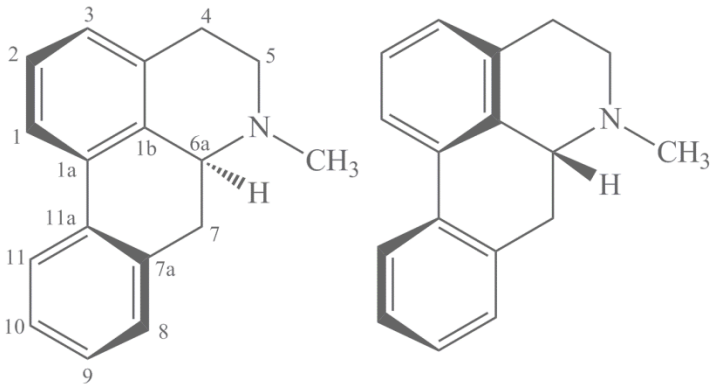
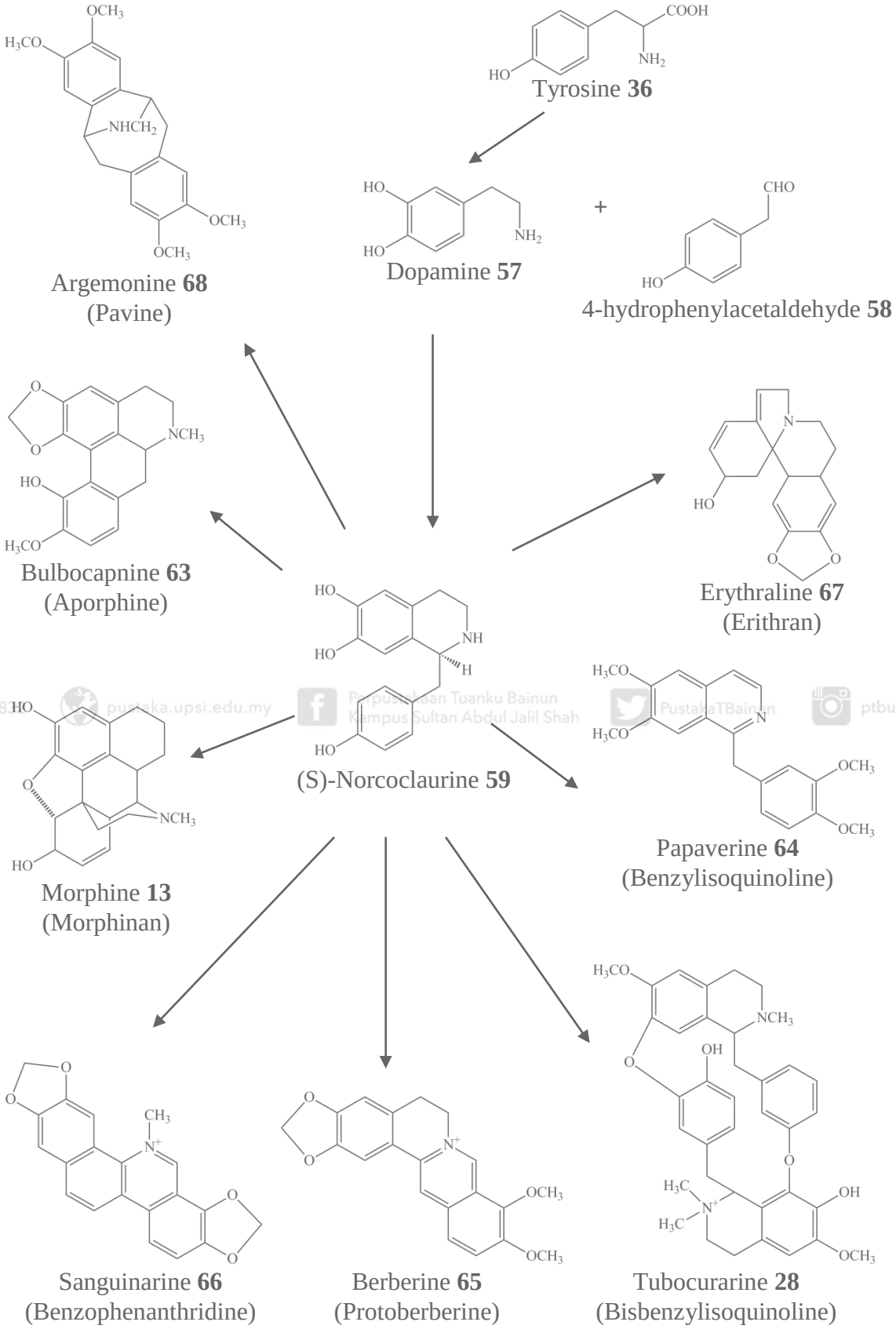


Figure 2.5.2: Configuration of Aporphine; Left – A configuration of aporphine; right– B configuration of aporphine

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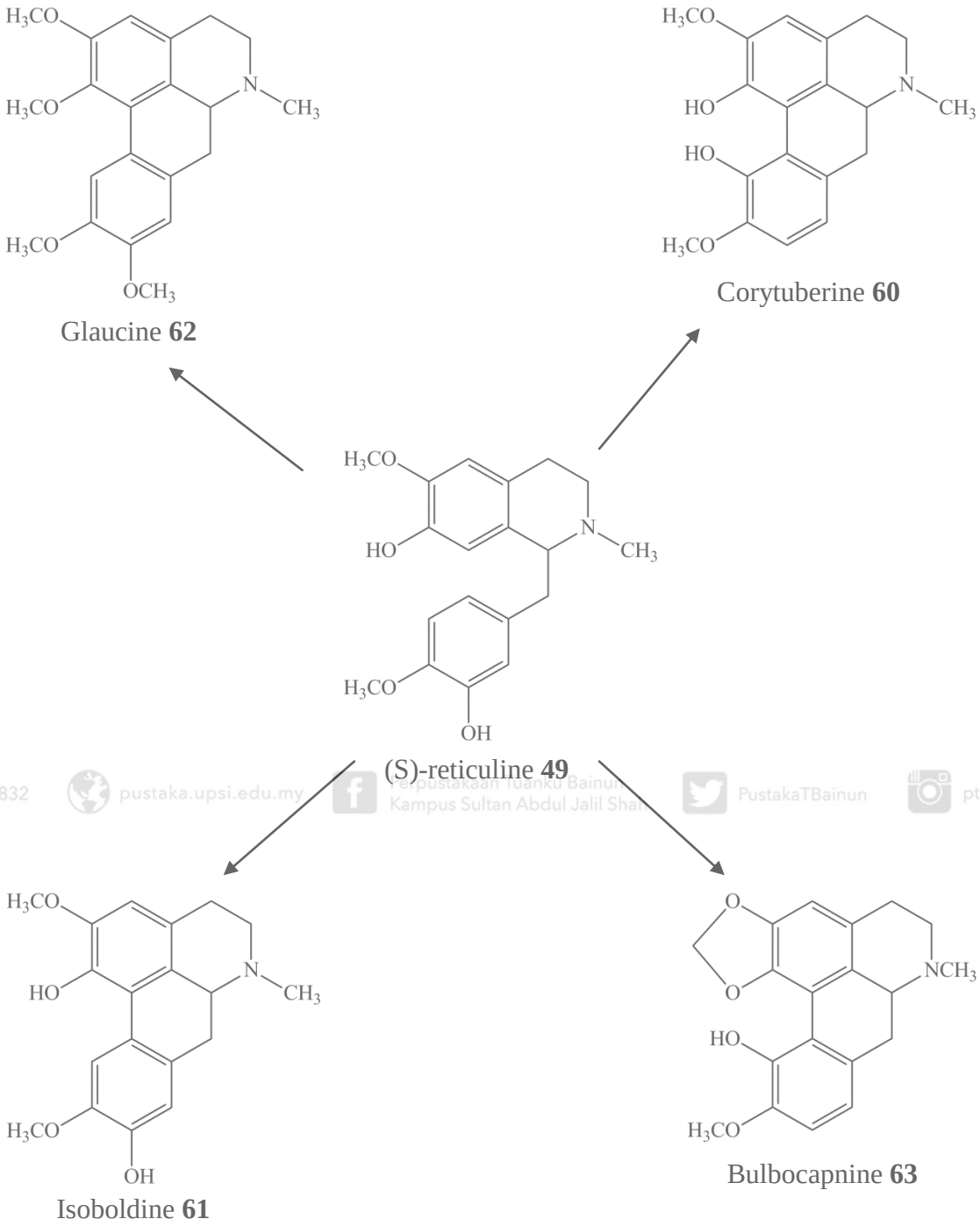
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Scheme 2.5.1: The various groups of benzylisoquinoline derived from *(S)*-norcoclaurine **59**

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Scheme 2.5.2: The Biogenetic Pathway from **49** to Corytuberine **60**, Isoboldine **61**, Glaucine **62** and Bulbocapnine **78**

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2.6 Bisbenzylisoquinoline Alkaloid

Bisbenzylisoquinolines are a large and diverse group of natural alkaloids that occur in many plant species. Many of the plants contain of these compounds enjoy a folkloric reputation as medicinal in various cultures. Recently, bisbenzylisoquinoline alkaloids have been widely demonstrated to possess a number of interesting and potent biological activities, including cytotoxicity and/or antiplasmodial activity.

The two moieties of bisbenzylisoquinoline are usually bound by one diaryl ether bridge or more, although carbon-carbon bridges or a methyleneoxy bridge may be present. These alkaloids are classified according to the nature, the number, and the attachment point of the bridges. In each subgroup, the alkaloids differ by the nature of their oxygenated substituents, the degree of unsaturation of the heterocyclic rings, and the stereochemistry of their two chiral centers, C-1 and C-1′.

The numbering system in bisbenzylisoquinoline is described as follow. First, for the half dimer, the lower aromatic ring (ring C) is always numbered so as to assign the smallest numbers to the substituent on that ring. Second, each half of the dimer is described based on its oxygenation pattern. The more highly oxygenated bisbenzylisoquinoline half constitutes the left hand side of the dimer while the prime numbering is given for the right hand side, the numbering system of bisbenzylisoquinoline alkaloids is shown in Figure 2.6.1. Due to large diverse group of bisbenzylisoquinoline, eleven main group and 44 subgroup had been created to classified them as shown in Table 2.6.1 (Schiff, 1999). Biosynthesis of bisbenzylisoquinoline (berbamunine) is shown in Scheme 2.6.1.

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Bisbenzylisoquinoline alkaloid has been reported from family of Annonaceae, Aristolochiaceae, Berberidaceae, Buxaceae, Euphorbiaceae, Hernandiaceae, Lauraceae, Magnoliaceae, Menispermaceae, Monimiaceae, Nymphaceae, Ranunculaceae, Rhamnaceae and Umbelliferae. In Lauraceae, it has been obtained from the genera Alseodaphne, Aniba, Crypto- carya, Litsea, Machilus, Mezilaurus, Nectandra, Neolitsea and Phoebe. (Schiff, 1987, 1999; Silva, Barbosa-Filho, Silva, Lacerda, & Da-Chunha, 2002).

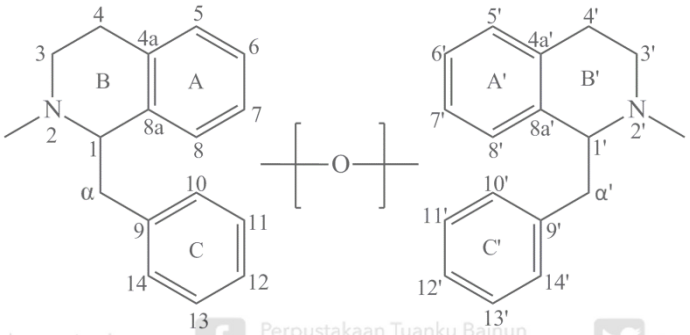
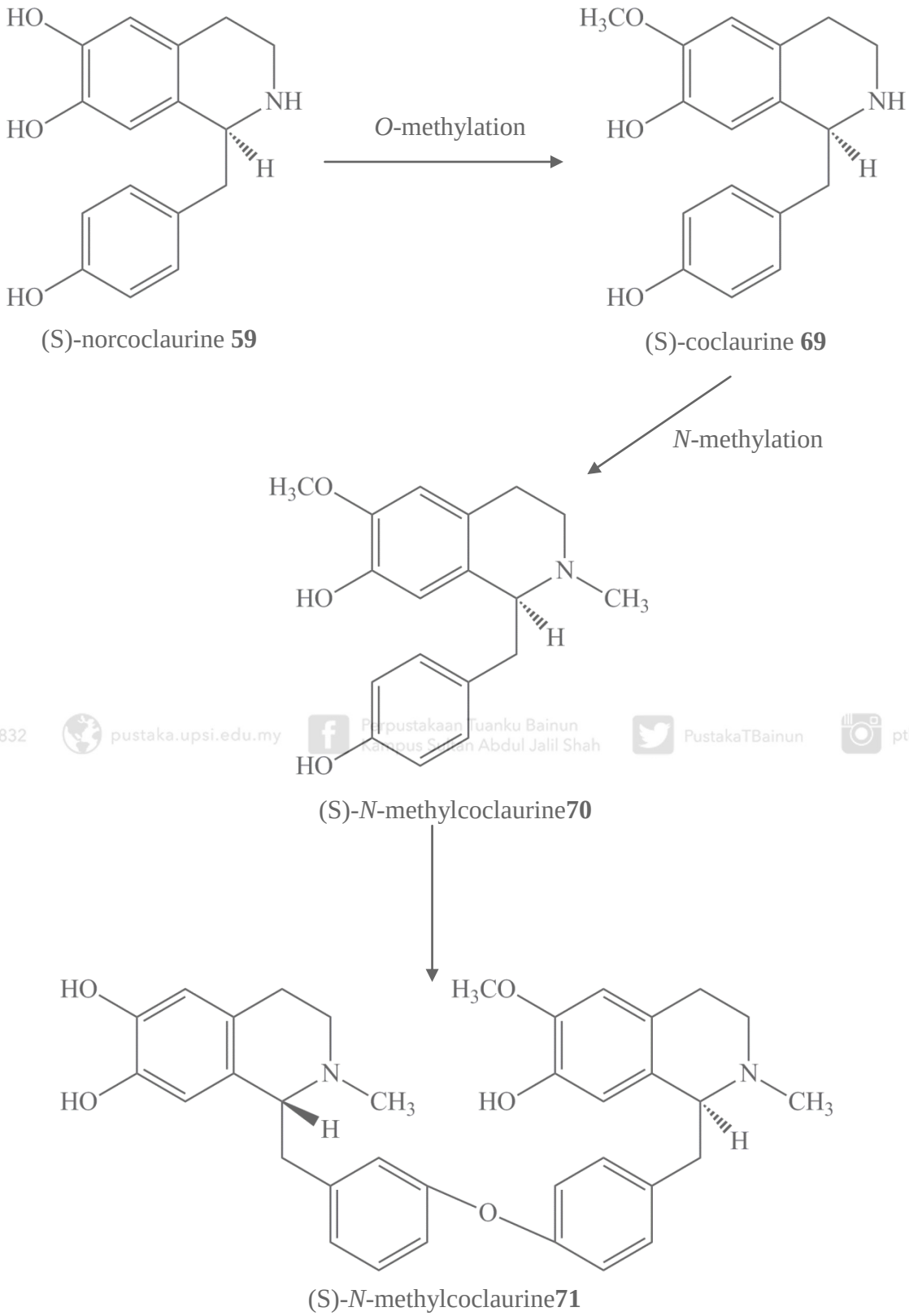


Figure 2.6.1: Numbering System of Bisbenzylisoquinoline Alkaloids

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(S)-norcoclaurine **59**

(S)-coclaurine **69**

(S)-*N*-methylcoclaurine**70**

(S)-*N*-methylcoclaurine**71**

Scheme 2.6.1: The Biogenetic Pathway of Berbamunine

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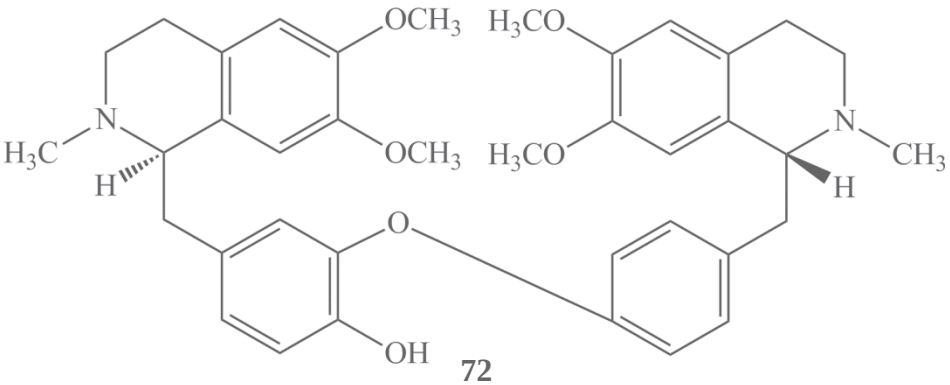
Table 2.6.1

Groups and Subgroups for Classification of Bisbenzylisoquinoline Alkaloids and Their Examples

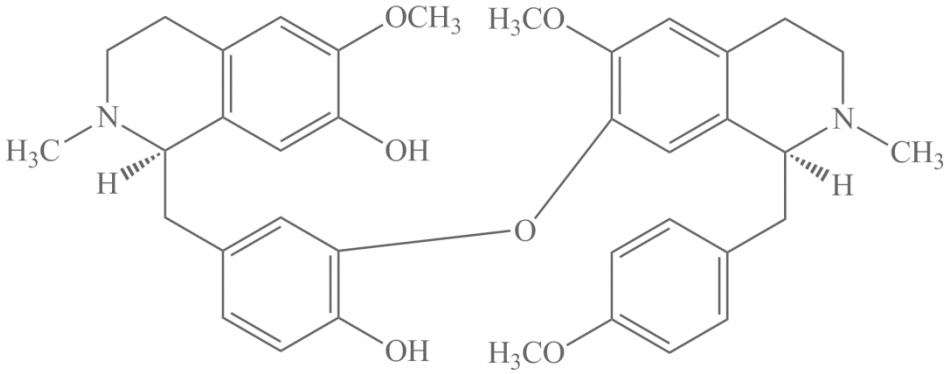
Group	Lingkage/s	Subgroup	Example
One diphenyl ether linkage	1. Tail to tail	I, Ia, Ib, II, IIa, IIb & III	Dauricine **72**
	2. Head to tail	V, Va,Vb, Vc & Vd	Isoliensinine **73**
One diphenyl linkage	1. Tail to tail	XXVII	Pisopowiaridine **74**
One diphenyl ether and one diphenyl linkage	1. Head to head & tail to tail, respectively	IV	Imine cordobimine **75**
Two diphenyl ether linkage	1. Head to head & tail to tail, respectively	VI, VIa, VII, VIII, IX, X, Xa, Xb, XI, XII, XIIa, XIII, XIV, XIVa, XV, XVI & XVII	Stebisimine **76**
	2. Head to tail & head to tail, respectively	XX & XXI	Sciadoferine **77**
Two diphenyl ether and one diphenyl linkages	1. Head to head, head to head & tail to tail, respectively	XVIII, XIX & XIXa	Nortiliacorinine A **78**
One diphenyl ether and one benzylphenyl ether linkage	1. Head to tail & head to tail, respectively	XXII & XXIIa	Cissampareine **79**
Two diphenyl ether and one diphenyl ether linkages	1. Head to head, head to head & tail to tail, respectively	XXIII, XXIIIa, XXIIIb and XXVIII	1,2-Dehydromicranthine **80**
One diphenyl ether, one benzylphenyl ether and one diphenyl ether linkage	1. Head to head, head to head & tail to tail, , respectively	XXV	Pseudorepanduline **81**
	2. Head to tail, head to tail & head to tail, respectively	XXVI	Insulanoline **82**

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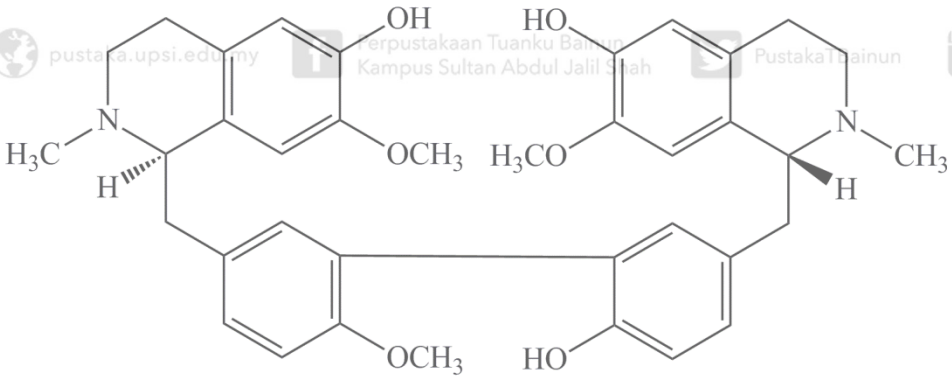
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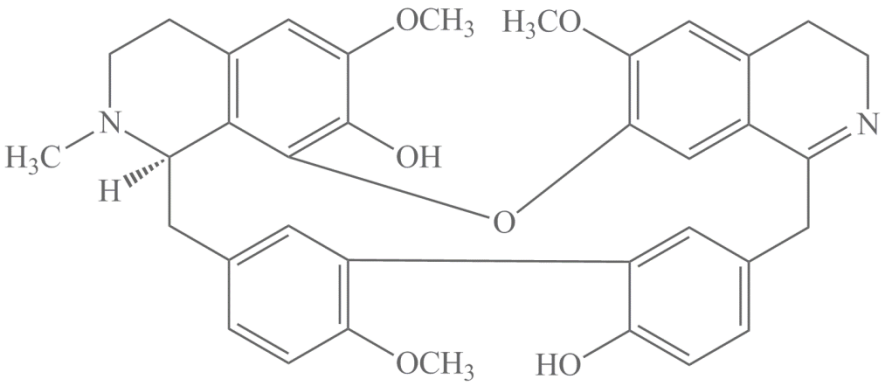
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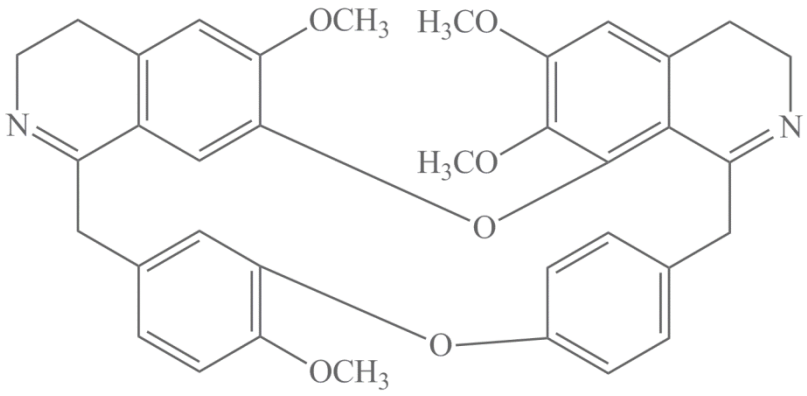
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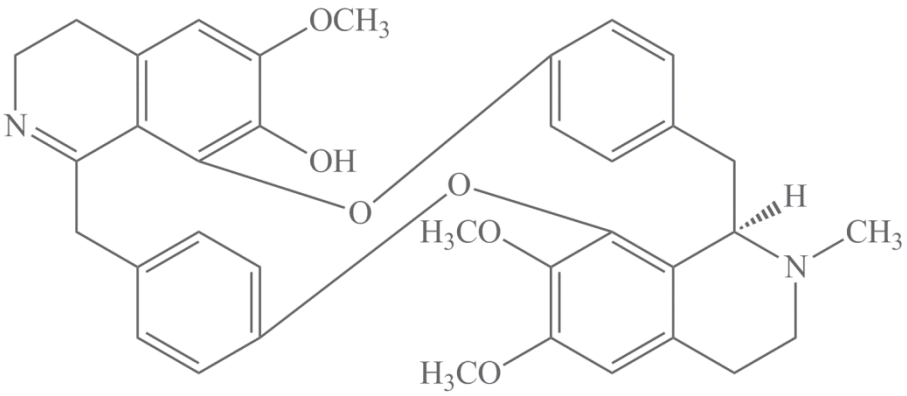
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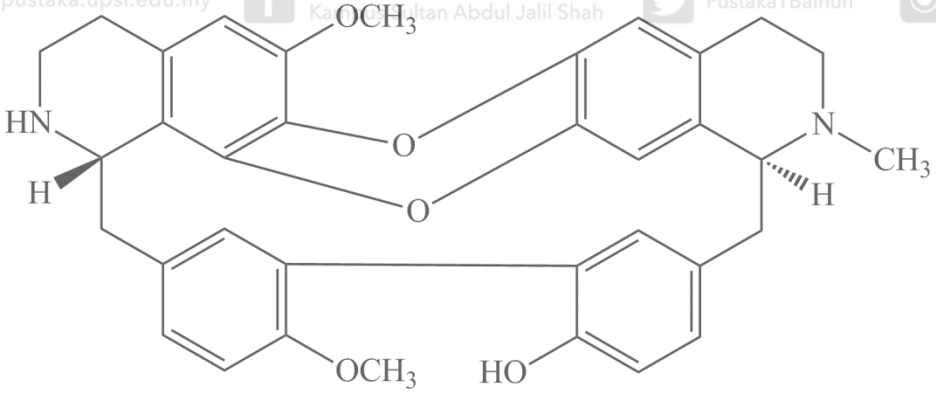


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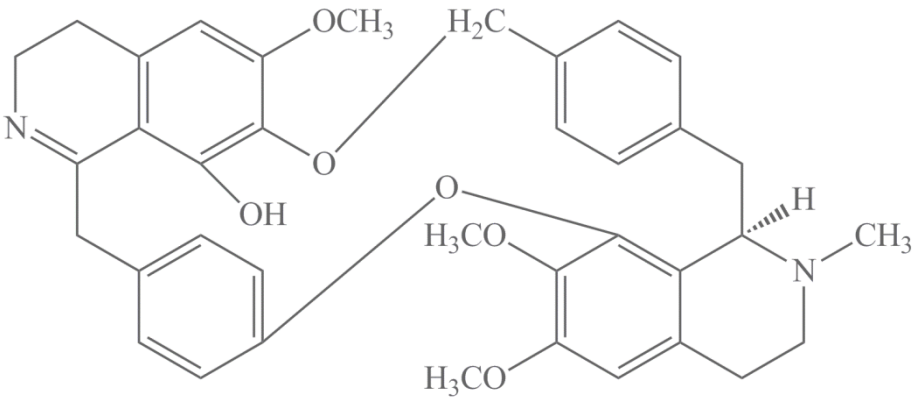


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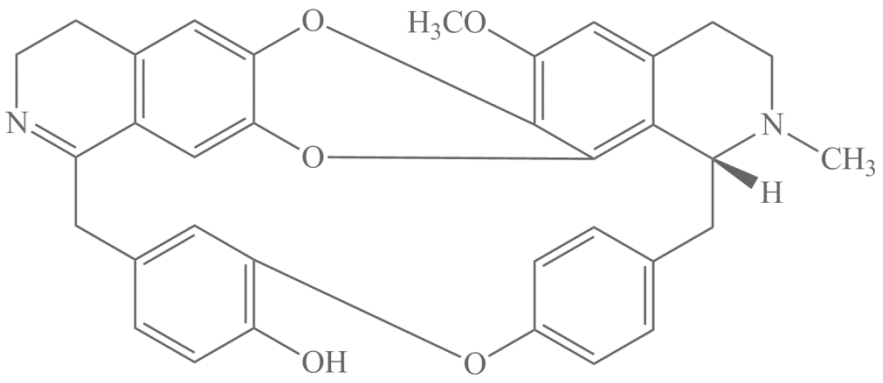
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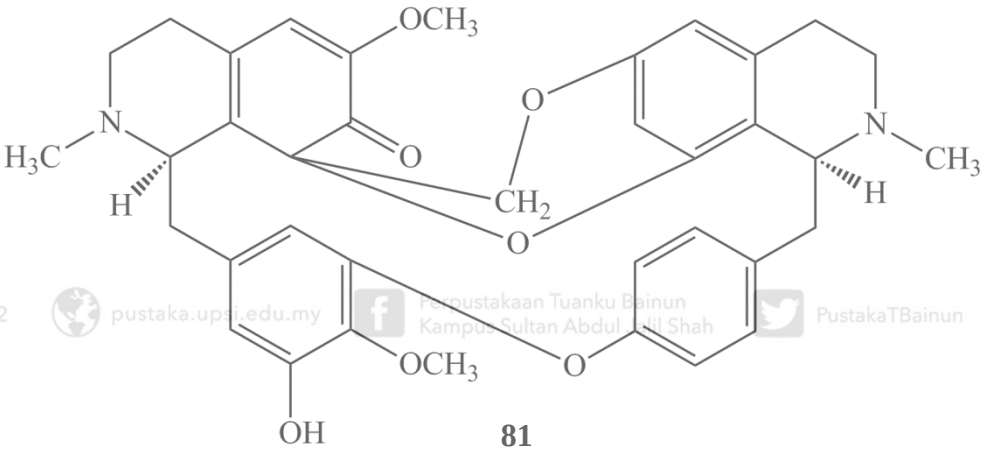
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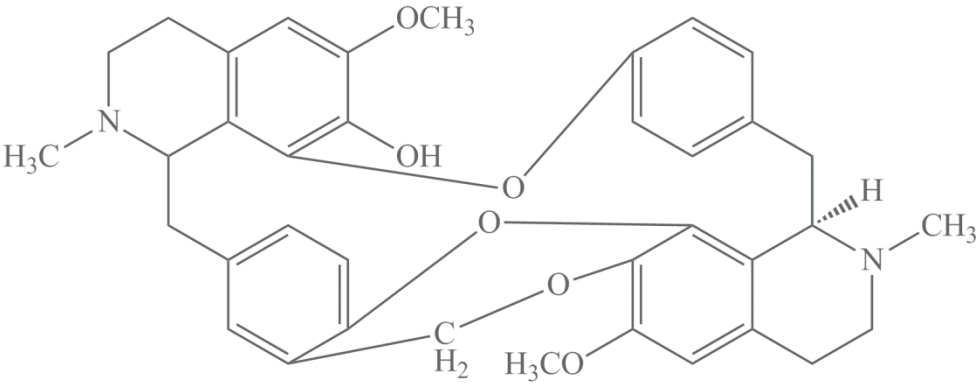
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2.7 Chemical Constituent from Genus of *Alseodaphne*

There are over 60 species from genus of *Alseodaphne*, still much work to phytochemist and pharmacologist because research and information about this species is still rare. Based on literature review, only eight *Alseodaphne* species have been reported for their chemical constituent, namely *A. andersonii, A. archboldiana, A. corneri, A. hainanensis, A. peduncularis, A. pendulifolia, A. perakensis* and *A. semicarpifolia*. The main alkaloidal constituent from *Alseodaphne* were isoquinoline subgroups, namely aporphines, benzylisoquinolines, bisbenzylisoquinolines, morphinandienones and protoamines. Table 2.8.1 shows the chemical constituents that have been identified in this genus based on their species.

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Table 2.8.1:

Chemical Constituents Isolated from Genus of Alseodaphne

Alseodaphne species	Isolated Alkaloids	References*
A. andersonii	Dihydoisoobtusilactone **83**	I
	Dihydroobtusilactone **84**	I
	3-Epilitsenolide D_1 **85**	I
	3-Epilitsenolide D_2 **86**	I
	Alseodafuranone **87**	I
A. archboldiana	Sanjoinine **88**	II,III
	(-)-*N*-Norarmepavine **89**	I,II,III
	(+)-Reticuline **90**	I,II,III
	(+)-Coclaurine **91**	I,II,III
	(-)-Coclaurine **92**	I,II,III
A. corneri	Lirioferine **93**	IV
	Isocorydine **94**	I,IV,V
	Norisocorydine **95**	I,IV,V
	Obamegine **96**	I,IV,V

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	N-Methyllaurotetanine **97**	I,IV,V
	2-Norobamegine **98**	I,V
	Laurotetanine **99**	I,V
	(+)-2'-Noroxyacanthine **100**	I,V
	(+)-Seeperine **101**	I,V
	(-)-Gyrolidine **102**	I,IV,V,VI,VII
	(+)-Norstephasubine **103**	I,V,VI,VII
	(+)-2-Norobaberine **104**	I,V
	(+)-Stephasubine **56**	I,IV,V,VI
	O-Methyllimacusine **105**	I,V,VI
	Norboldine **106**	I,V
	(+)-3',4'-Dihydrostephasubine **107**	I,V,VII
	O-Methylrepandine **108**	I,V
A. hainanensis	Armepavine **109**	I,VIII
	Doryafranine **110**	I,VIII
	Xylopinine **111**	I,VIII
	1-(4-Methoxybenzyl)-6-7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline **112**	I,VIII
	4-Hydroxy, 3-methoxy benzoic acid **113**	I
	Neolignan eusiderin-A **114**	I
A. peduncularis	Norlirioferine **115**	I,V
	Lindcarpine **116**	I,V
	Norpredicentrine **117**	I,V
	Laurotetanine **99**	I,V
	α'-Oxoperakensimine **118**	I,V
	Boldine **119**	I,V
A. pendulifolia	*N*-(2-Hydroxypropyl)-pendulifoline A **120**	I,V,IX
	N-(2-Hydroxypropyl)-pendulifoline B **121**	I,V,IX
	Oxohernagine **122**	I,V
	Hernagine **123**	I,V
	Corydine **124**	I,V
	Isocorytuberine **125**	I,V
	Costaricine **126**	I,V
A. perakensis	Sebiferine **127**	X,XI
	N-(2-Hydroxypropyl)-norboldine A **128**	V
	N-(2-Hydroxypropyl)-norboldine B **129**	V
	N-Methyllaurotetanine **82**	V,XII
	N-Cyanomethyl-norboldine **130**	V,XII
	α'-Oxoperakensimine A **131**	I,V,VII,XIII
	α'-Oxoperakensimine B **132**	I,V,VII,XIII
	α'-Oxoperakensimine C **133**	I,V,VII,XIII
	Norboldine **106**	I,V,XII
	N-Methyl-2, 3, 6-trimethoxy morphinandien-7-one *N*-oxide **134**	I

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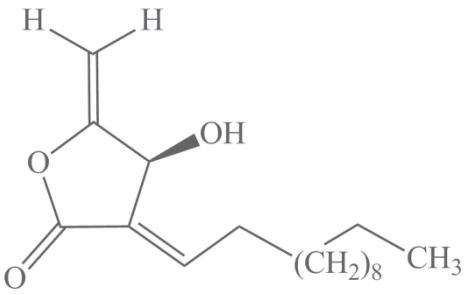
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	N-Methyl-2, 3, 6-trimethoxymorphinandien- 7-one **135**	I
	Perakensol (7-hydroxy-2, 3, 6-trimethoxyphenanthrene) **136**	I
A. semicarpifolia	Srilankine **137**	I
	1,1-Diethoxy-ethane **138**	I
	1-Methyl-1H-pyrrole **139**	I
	1,1-Diethoxy-2-methyl-propane **140**	I
	2-Methoxy-phenol **141**	I
	4-One,2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran **142**	I
	2-Methoxy-5-methylphenol **143**	I
	2-Methoxy-4-vinylphenol **144**	I
	D-Allose **145**	I
	2,6-Dimethoxy-phenol **146**	I
	N-(2-Morpholinoethyl)benzamide **147**	I
	1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane **148**	I
	Sucrose **149**	I
	1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-isoquinoline **150**	I
	2-Methoxy-4-(1-propenyl)-phenol **151**	I
	3,4,5-Trimethoxy-phenol **152**	I
	3,5-Dimethyl-2-nitro-phenol **153**	I
	2-Nonadecanone **154**	I
	2,2-Dimethyl–cyclohexanol **155**	I
	Tridecanoic acid methyl ester **156**	I
	3',5'-Dimethoxyacetophenone **157**	I
	(*E*)-3-Eicosene **158**	I
	2-Benzoyl-4-methoxymethyl-6-methyl-furo[2,3-b]pyridin-3-amine **159**	I
	2-Oxo-hexadecanoic acid, methyl ester **160**	I
	5-Hydroxy-2,3,3–trimethyl–2-(3-methyl-buta-1,3-dienyl) cyclohexanone **161**	I
	Di(3,4,methylenedioxy)benzylamine acetate **162**	I
	1-Hexyl-2-nitrocyclohexane **178**	I
	2-Methyl-3-(1-methylethenyl)-acetate,(1à,2à,3à)-cyclohexanol **164**	I
	(-)-1,2,3,4-Tetrahydro isoquinolin-6-ol-1-carboxylic acid, 7-methoxy-1-methyl-methyl ester **165**	I

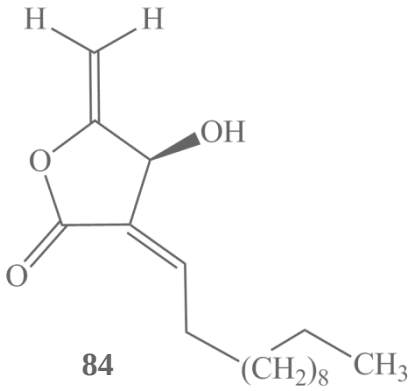
*References:- I (Thakur et al., 2012), II (Bick, 1996), III (Johns, Lamberton, & Sioumis, 1967), IV (Zahari, 2010), V (Nafiah, 2009), VI (Nafiah et al., 2010), VII (Mukhtar, Zahari, et al., 2009), VIII (F. Zhang, Liu, Li, Mai, & Lu, 1988), IX (Hadi et al., 2010), X (Shamma & Moniot, 1976), XI (Guha, Mukherjee, & Mukherjee, 1979), XII (Nafiah et al., 2011) and XIII (Mukhtar, Nafiah, et al., 2009).

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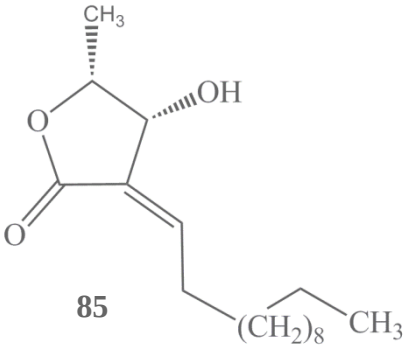
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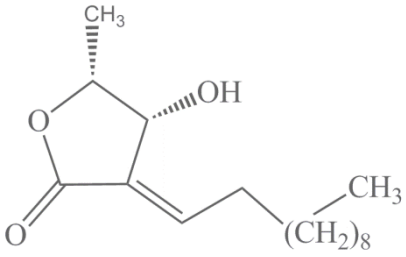
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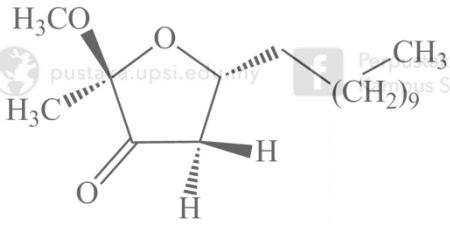
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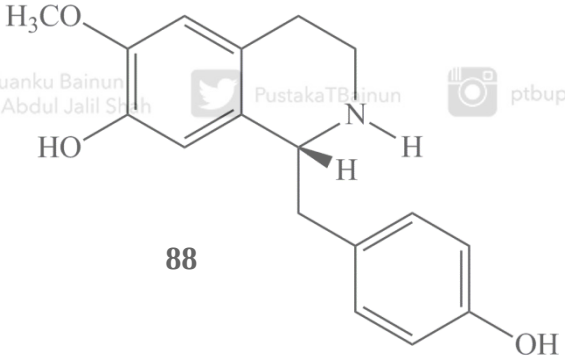
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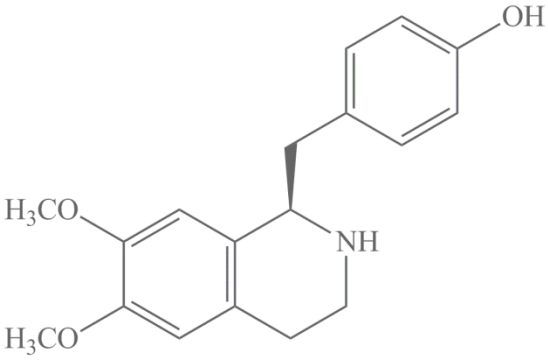
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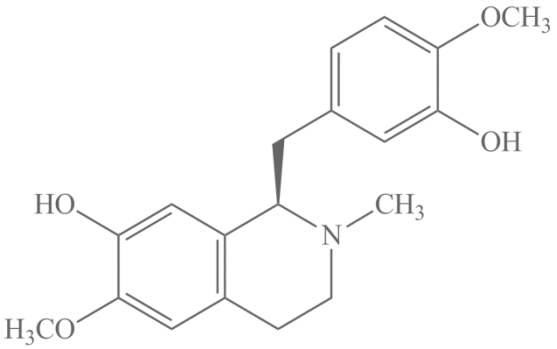
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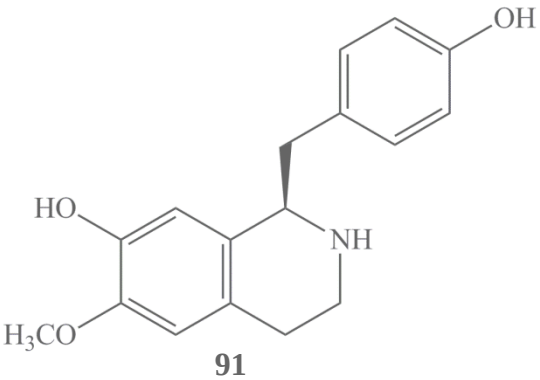
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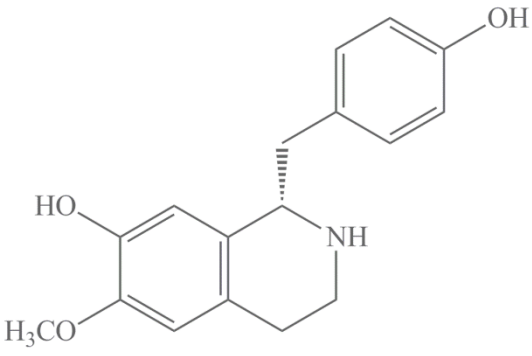
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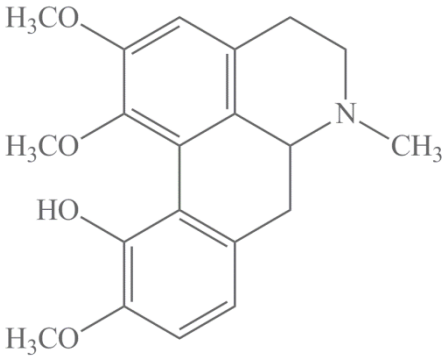
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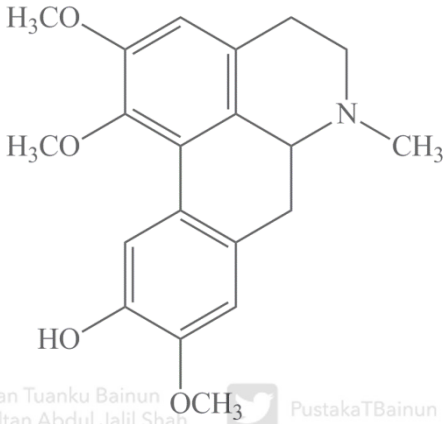
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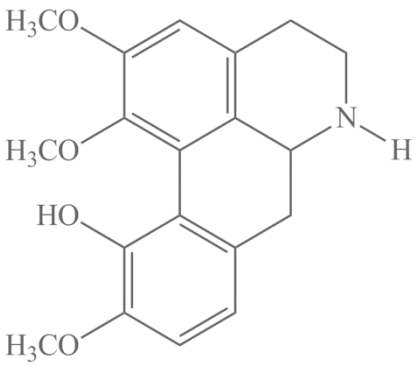


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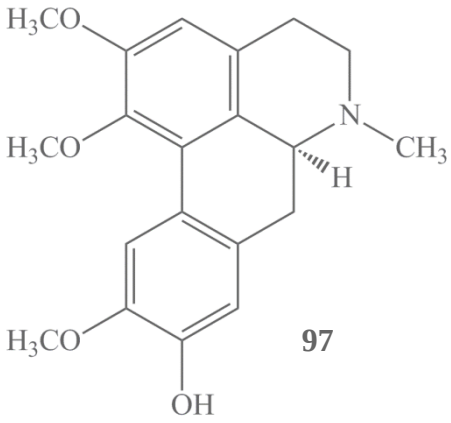


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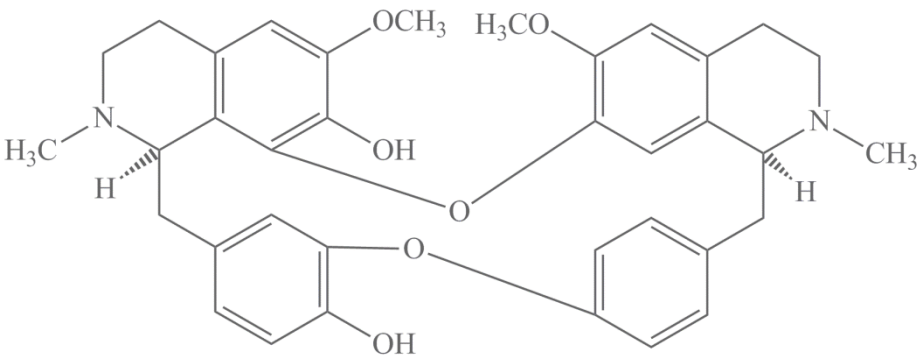
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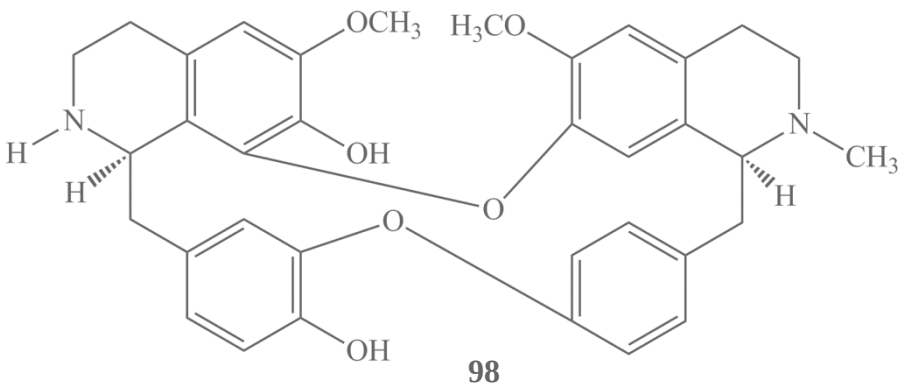
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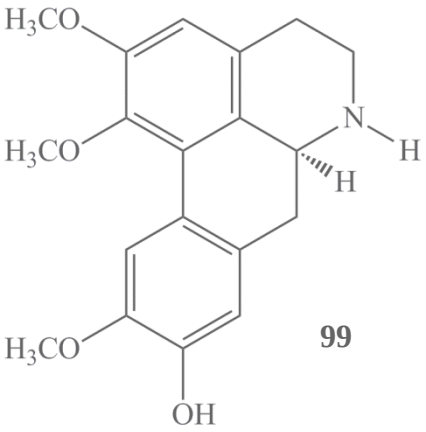
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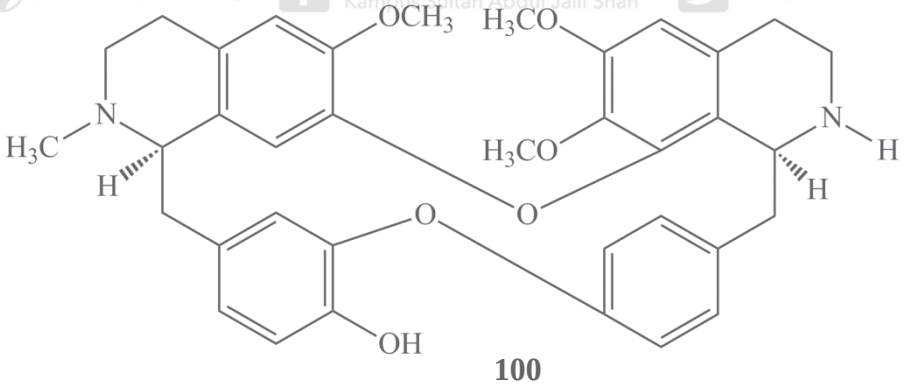


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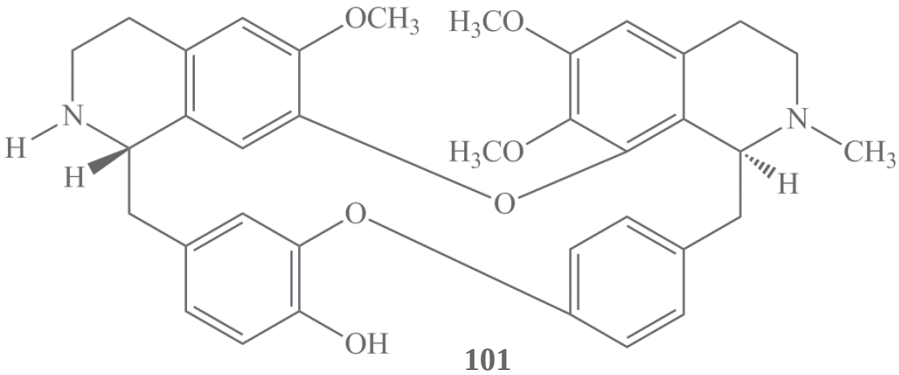


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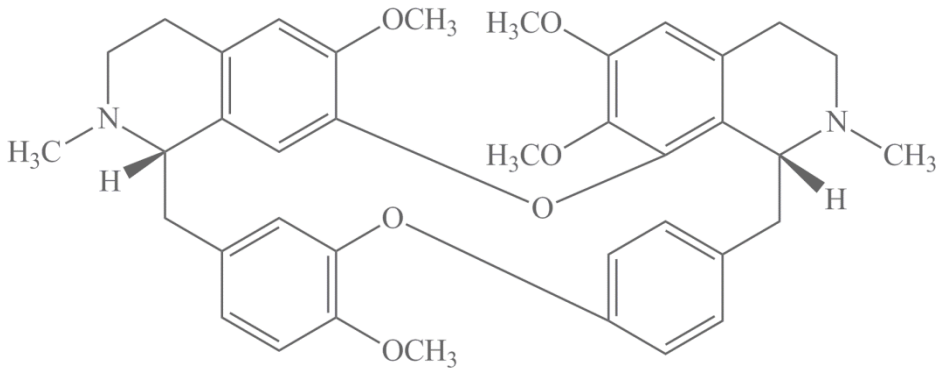
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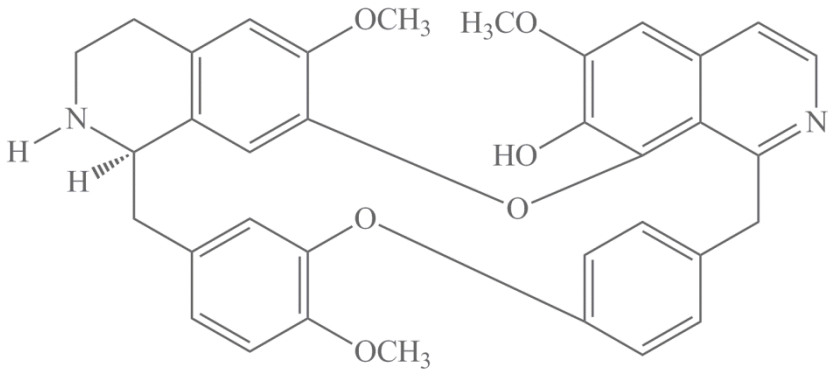
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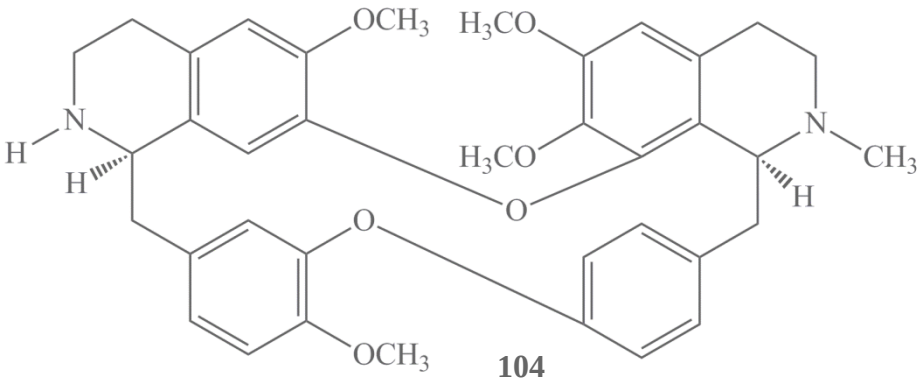
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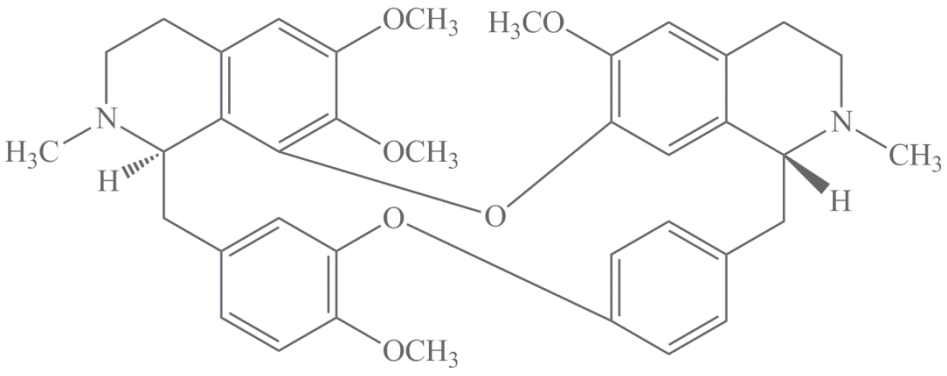
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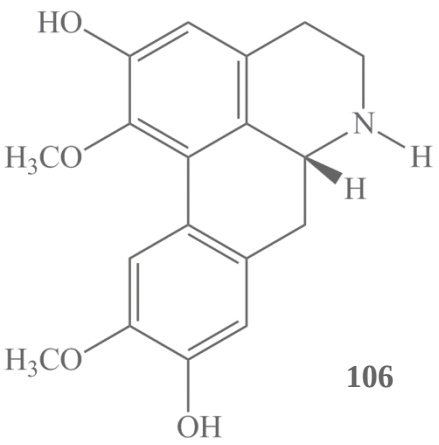
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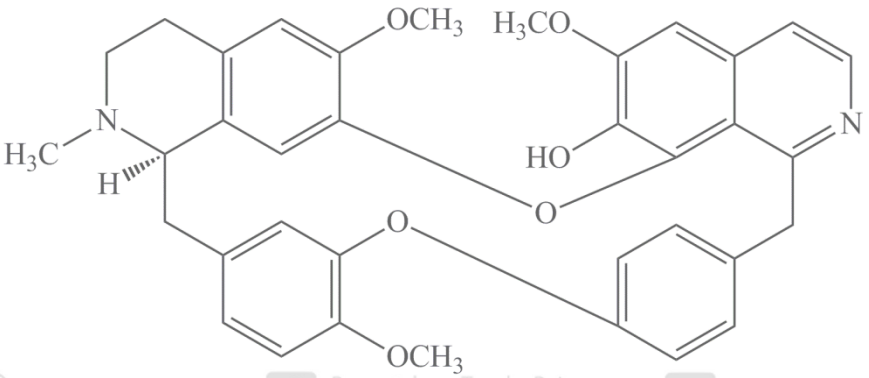
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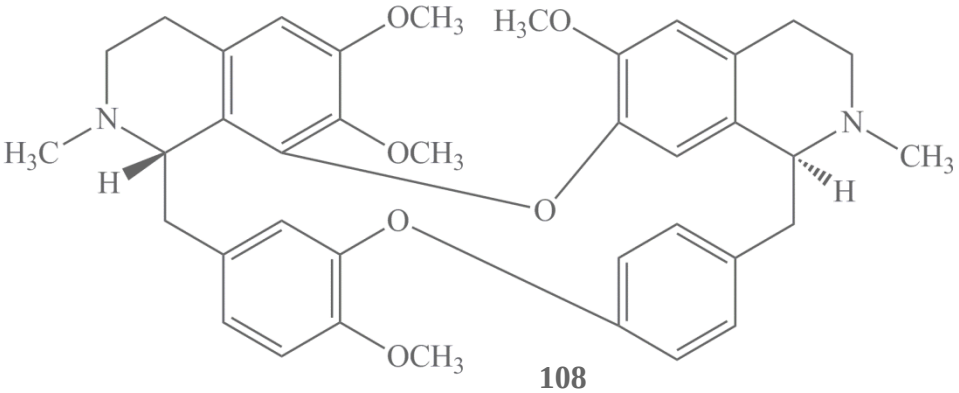
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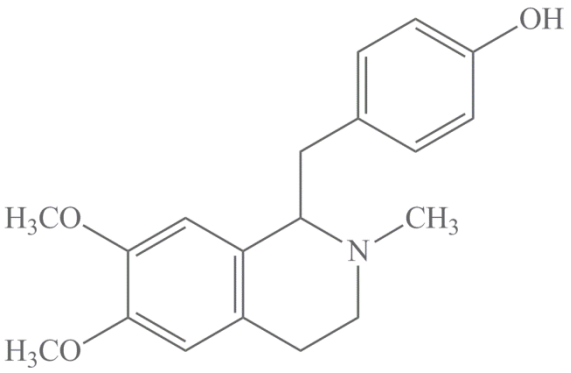
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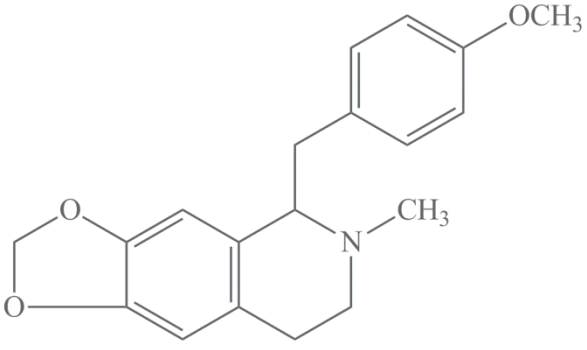
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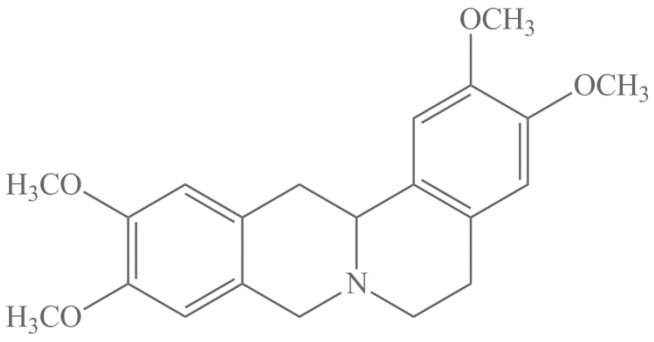
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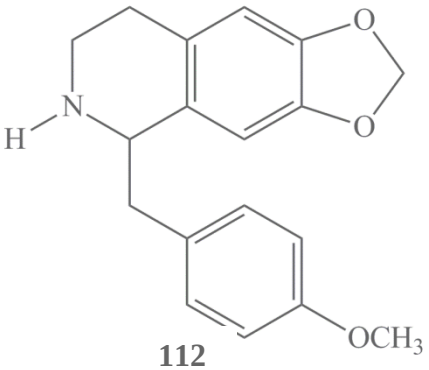
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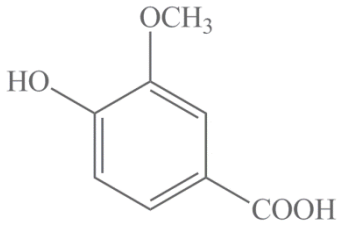
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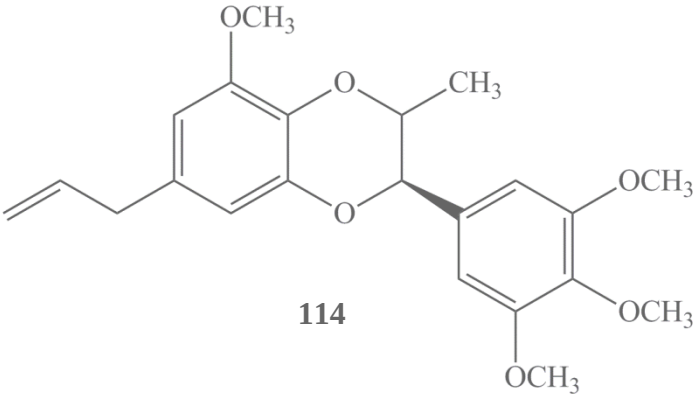
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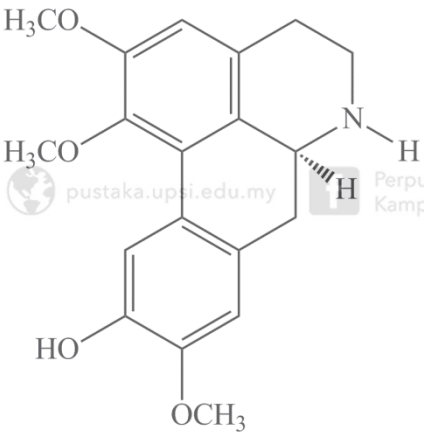
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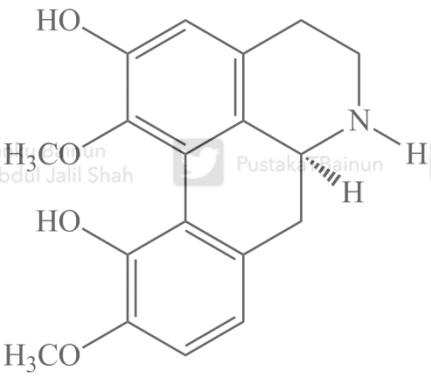
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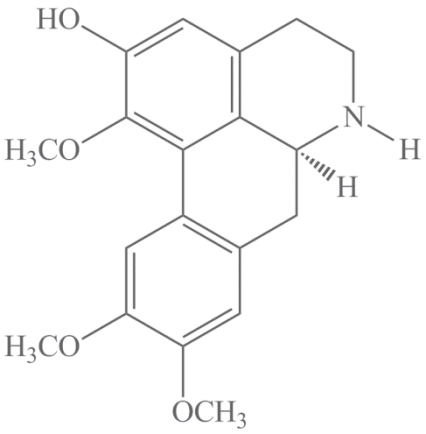
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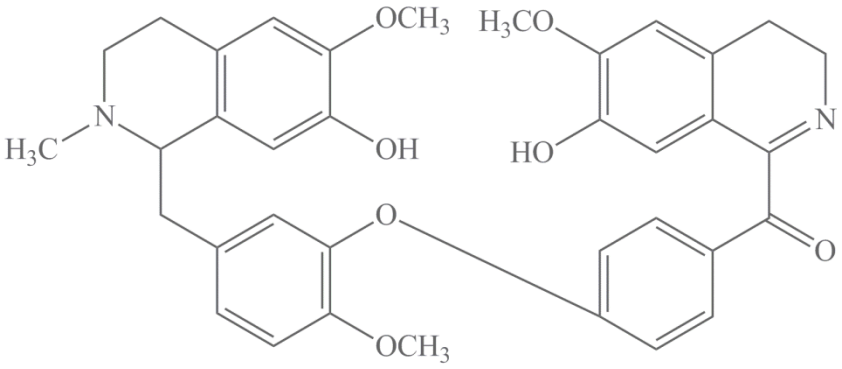
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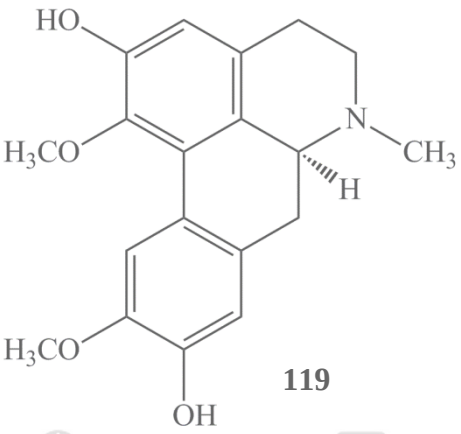
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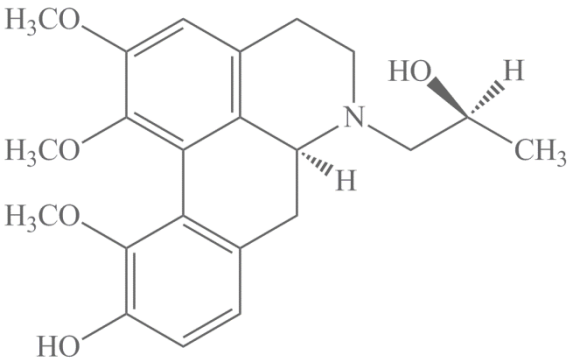
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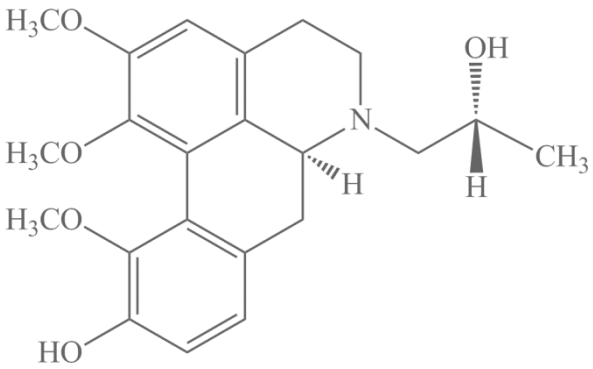


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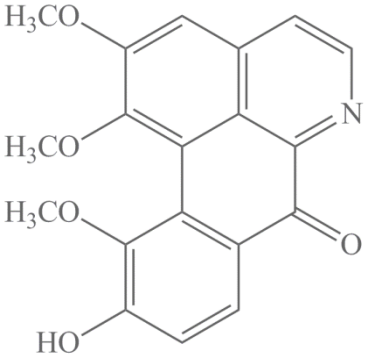


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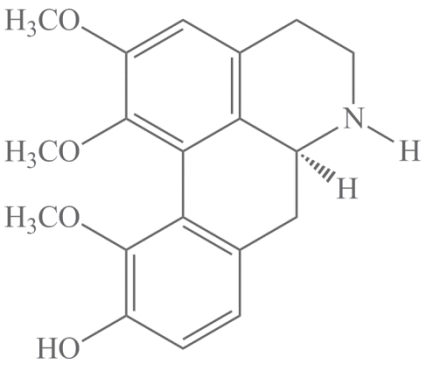
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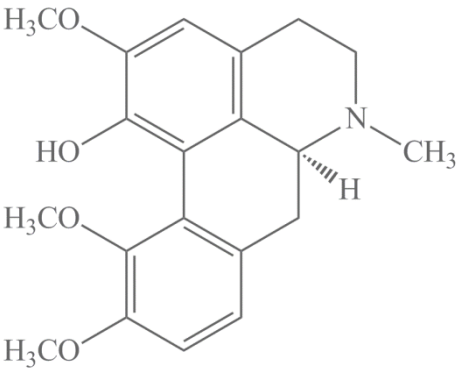
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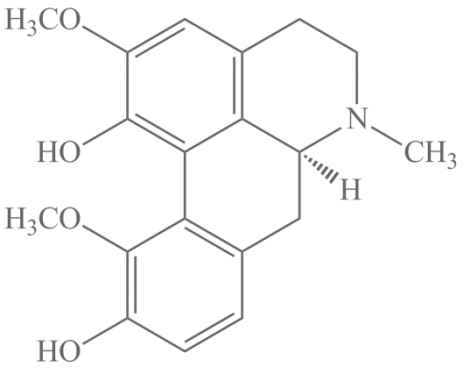
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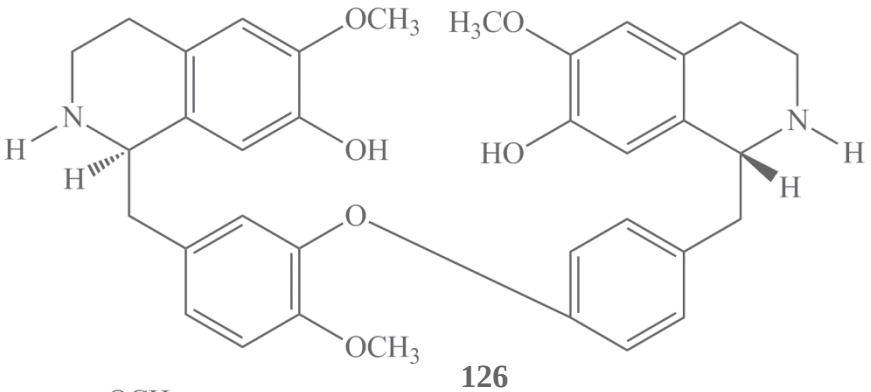
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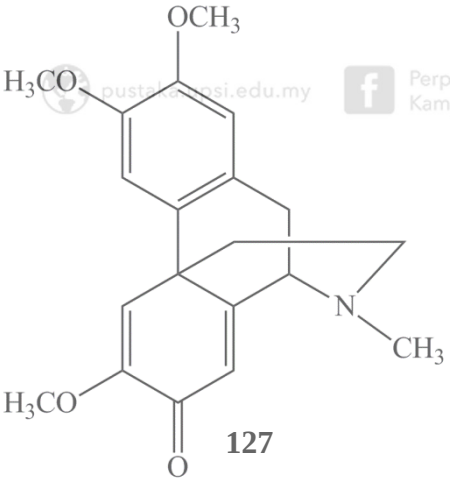
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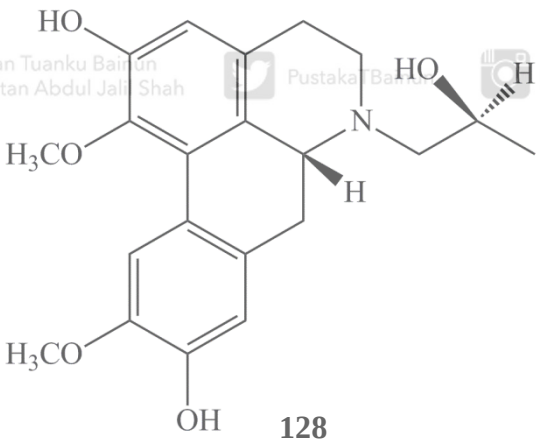
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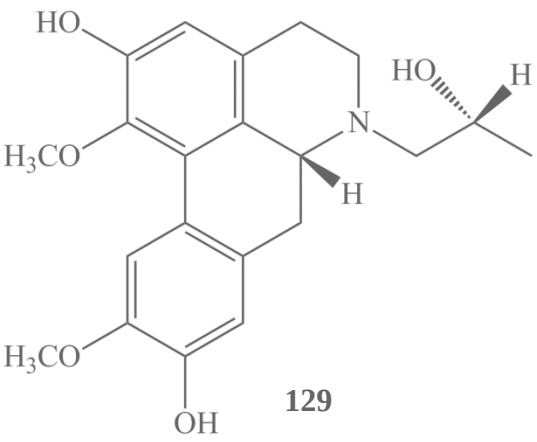
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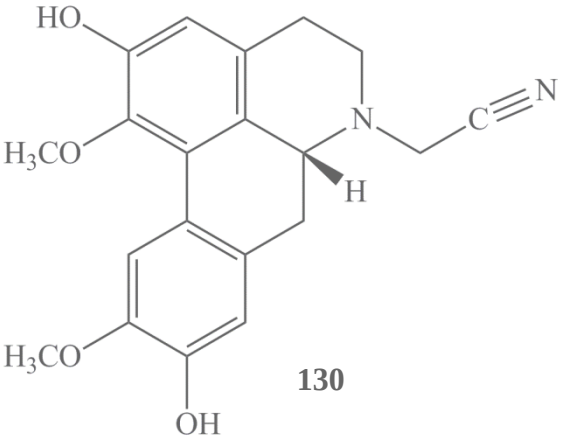
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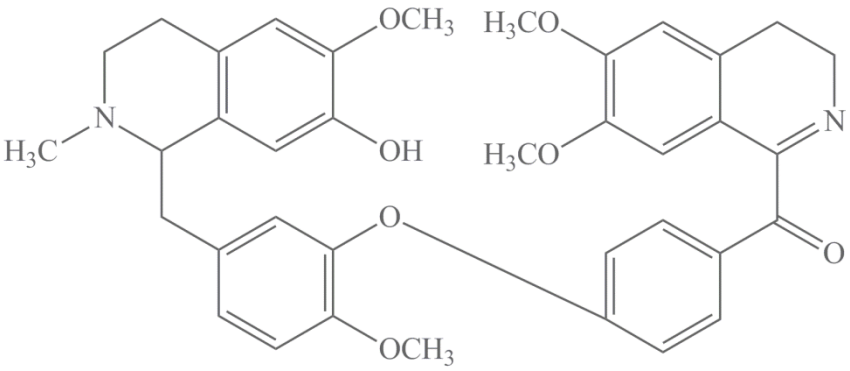
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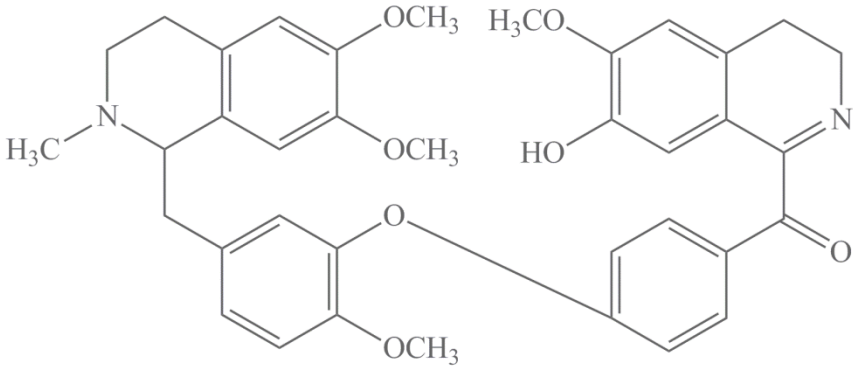
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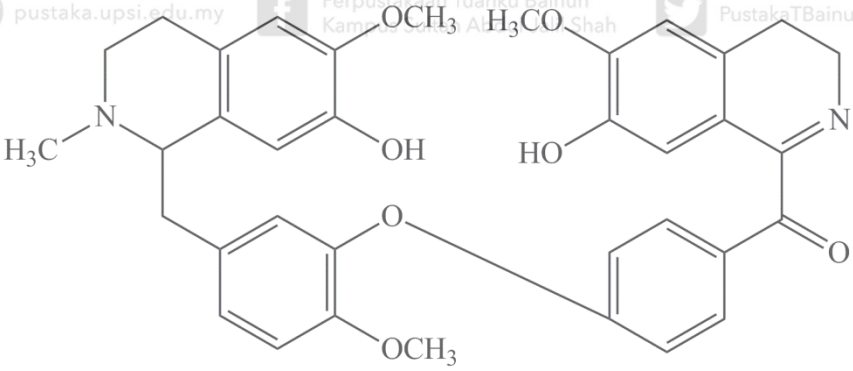


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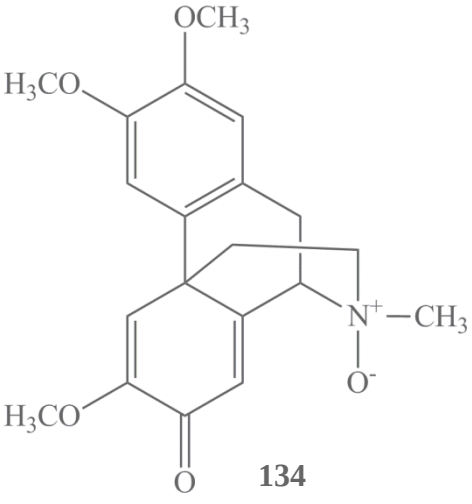


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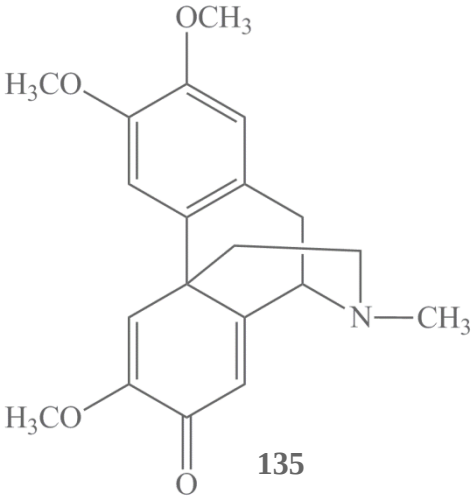
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133



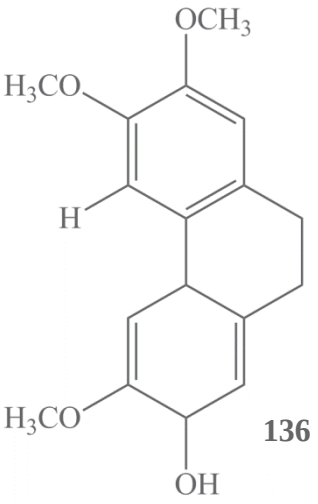
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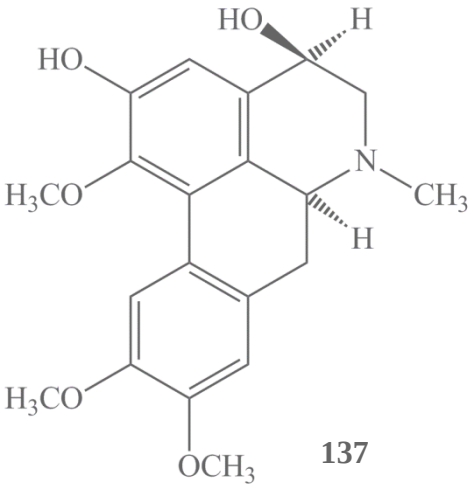
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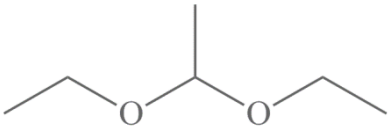
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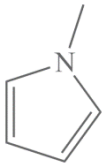
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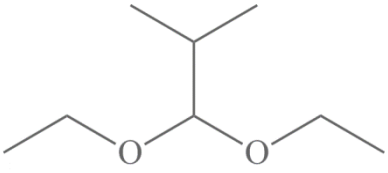
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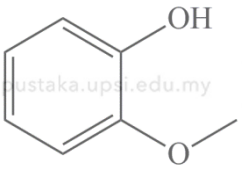
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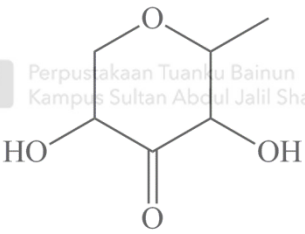
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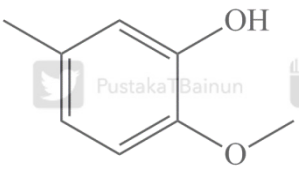
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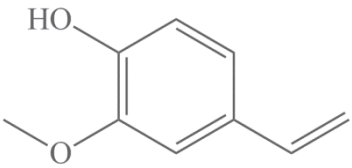
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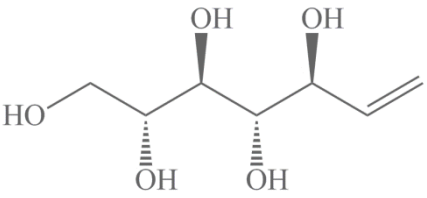
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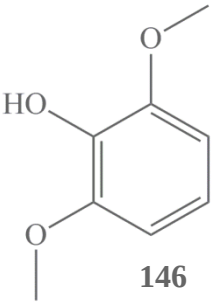
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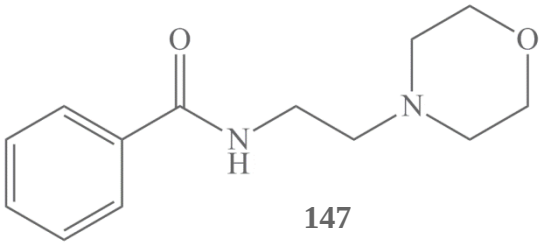
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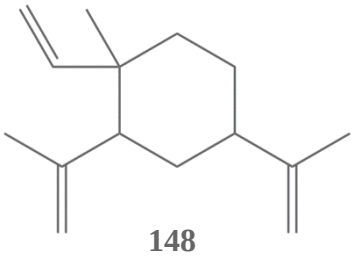
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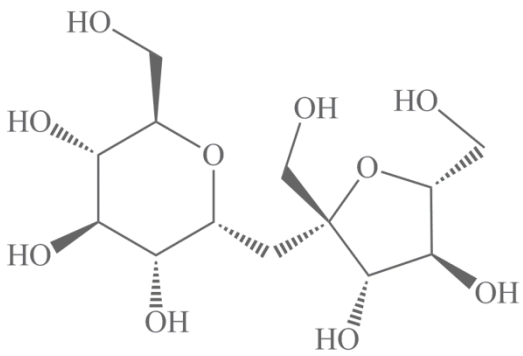
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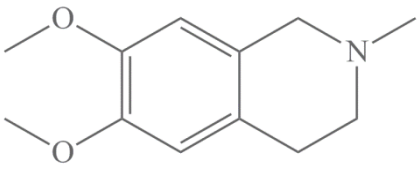
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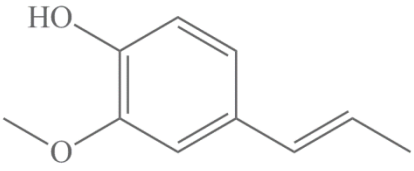
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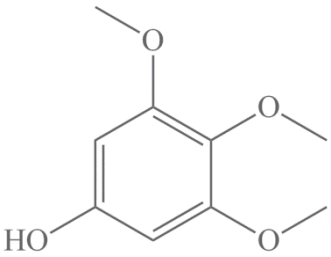
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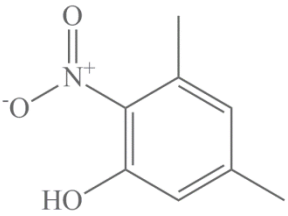
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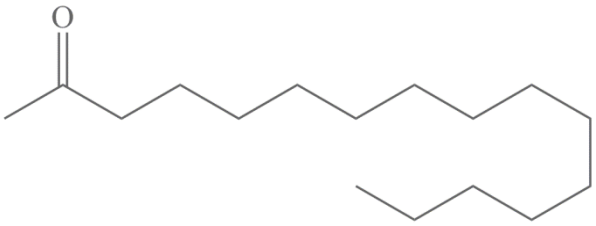
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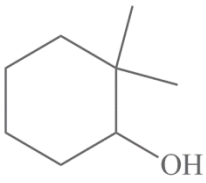
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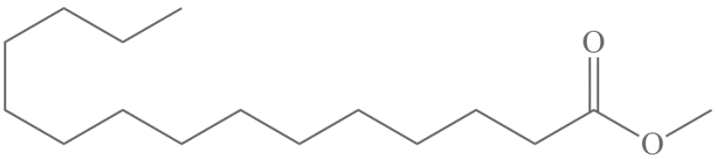


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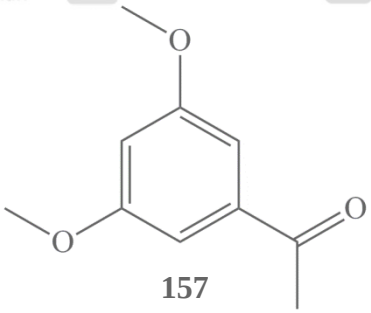


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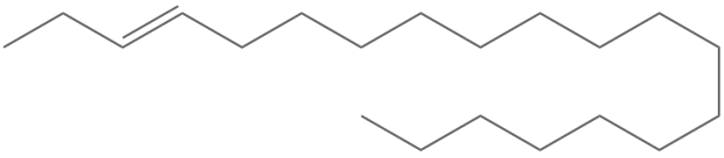
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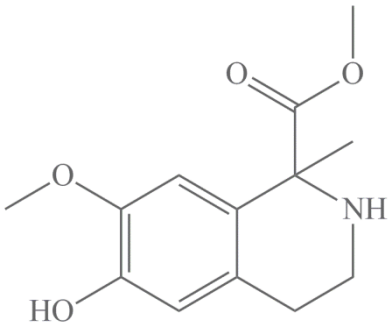
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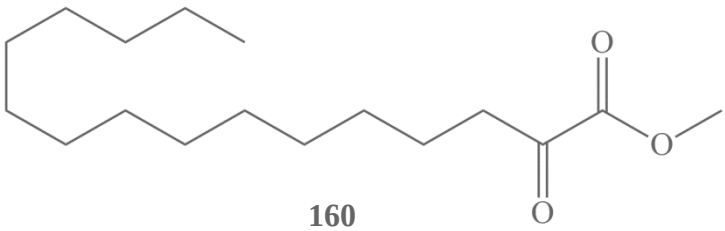
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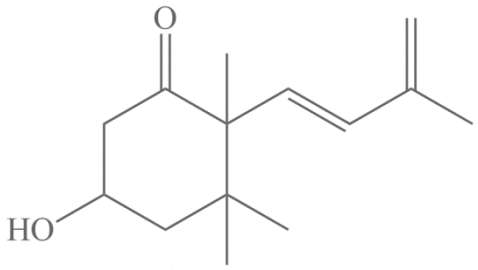
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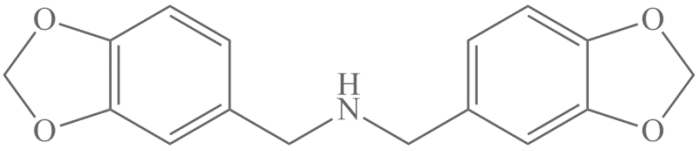
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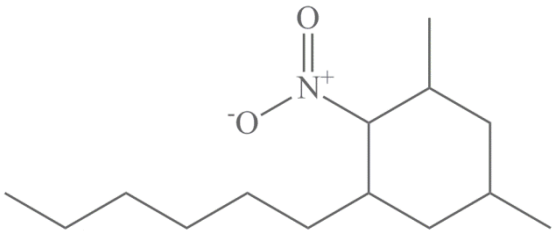
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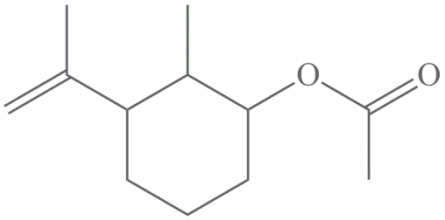
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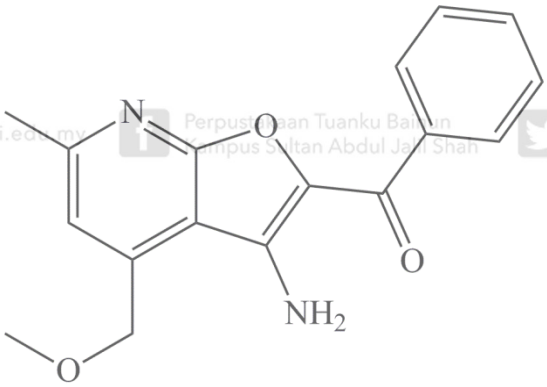
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178



164



165

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CHAPTER 3

MATERIAL AND EXPERIMENTAL

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3.1 General

The solvents used were hexane, dichloromethane, chloroform and methanol. All solvents are from AR grade except those that are used for bulk extractions were distilled prior to use. Other chemicals were hydrochloric acid, ammonia solution and sodium sulphate anhydrous.

3.2 Isolation and Structure Determination of Alkaloids

This section will describe about procedures for extraction, isolation and structural elucidation of alkaloids from *Alseodaphne pendulifolia* Gamb. barks.

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3.2.1 Instruments

Structural elucidation of alkaloidal constituents from barks of *Alseodaphne pendulifolia* Gamb. were aided by four scientific instruments namely ultra violet spectra (UV), infrared spectra (IR), mass spectra (MS), and nuclear magnetic resonance (NMR).

Ultra Violet Spectra (UV)

UV spectra were obtained using Perkin Elmer UV-Visible spectrophotometer with methanol as solvent.

Infrared Spectra (IR)

IR spectra were recorded on Nicolet 6700 FTIR spectrophotometer with chloroform as solvent.

Mass Spectra (MS)

HRESIMS are obtained on an Agilent 6530 Accurate-Mass Q-TOF LC/MS instrument.

Nuclear Magnetic Resonance (NMR)

1D (^{1}H, ^{13}C & DEPT) and 2D-NMR (COSY, HSQC/HMQC, HMBC, NOESY, H2BC, HSQC-TOCSY) spectra were recorded in deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OH) on JEOL JNM-FX 400MHz and JEOL ECX 500 MHz spectrometer. Chemical shifts (δ) were expressed in ppm and the coupling constants (*J*) are given in Hz.

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3.2.2 Reagents

In alkaloids plants screening, Mayer's Reagent (Potassium Mercuric Iodide) was used to detect the alkaloids content in plants, while Dragendorff's reagent was used to detects alkaloids spot in Thin Layer Chromatography (TLC) and Preparative Thin Layer Chromatography (PTLC).

Mayer's Reagent (Potassium Mercuric Iodide)

A solution of mercury (II) chloride (1.4 g) in distilled water (60 mL) was poured into a solution of potassium iodide (5.0 g) in distilled water (10 mL). The mixture was then made up to 150 mL. A positive result is indicated by the formation of white precipitate under acidic condition.

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Dragendorff's Reagent

Dragendroff's reagent was prepared by mixing 1mL of stock solution with 2 mL of glacial acetic acid and 10 mL of distilled water. Stock solution was prepared by mixing equal volumes of solution A and solution B as describe below:

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.

Orange spots on the developed TLC plates indicate the presence of alkaloids.

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3.2.3 Plants Materials

The plants material, barks of *Alseodaphne pendulifolia* Gamb. were collected from two different locations. First sample was collected from Sg. Temau Reserve Forest, Kuala Lipis, Pahang (KL 5250) and the second samples were collected from Sg. Tekam Reserve Forest, Jerantut, Pahang (KL 5732). This species were identified by Mr. Teo, botanist from Chemistry Department, collected by Herbarium Group and the voucher specimens are deposited at Herbarium, Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia.

3.2.4 Alkaloids plants screening

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About 5 g of dry plant sample was moistened with ammonia solution to agitate the alkaloids constituent. Then 100 mL of DCM was added to the sample and heated for 5 minutes. An amount (5 - 7 mL) of 5 % HCl solution was used to re-extract the basic constituent from 5 mL of the DCM extracts. The aqueous phases then was added 2 - 3 drop of Mayer's reagent (potassium mercuric iodide) to test the present of alkaloids in the sample. Table 3.2.4.1 categorise the stage of alkaloid contents by observation.

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Table 3.2.4.1:

Indicator for Mayer's Test on Alkaloid

Categories	Observation	Alkaloids content
0	clear extract solution	Nil
1+ and 2+	turbidity was weak and clear	Low
3+	white precipitate	High
4+	dense precipitate	Highest

3.2.5 Extraction of Plant Materials

Exhaustive extraction technique was used in this study. Collected fresh barks of *Alseodaphne pendulifolia* Gamb. were placed under room temperature until completely dried. First, the dried, ground samples were defatted with hexane for 14 hours using Soxhlet extractor to remove fat, non-polar organic compounds and waxes. The plant materials was dried and moistened with 25% ammonia solution for two hours to aggregate the nitrogen containing compound in the sample before re-extracted with dichloromethane (DCM) for another 14 hours.

The DCM extract was dried using rotary evaporator. The extract then dissolved in 500 mL DCM to re-extract with 5 % hydrochloric acid until negative Mayer's test was obtained. The combined extract was basified with 25 % ammonia solution until pH 11 and re-extracted with DCM until negative result was obtained with Mayer's reagent. Finally, the combined extracts were dried over anhydrous

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sodium sulphate (Na_2SO_4) and evaporated to give alkaloid crude extract. The percentage of yield alkaloids crude of both sample are shown in Table 3.2.5.1.

Table 3.2.5.1:

Yield of Crude Alkaloids Extracted from Barks of Alseodaphne pendulifolia Gamb. (Lauraceae)

Sample code	Place collected	Weight of crude alkaloid obtained (g)	Percentage of yields (%)
KL 5250 (3.0 kg)	Sg. Temau Reserve Forest, Kuala Lipis, Pahang	37.00	1.23
KL 5732 (3.0 kg)	Sg. Tekam Reserve Forest, Jerantut, Pahang	42.50	1.42

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3.2.6 Separation and Purification of Alkaloids

The crude extract was subjected to column chromatography with solvent mixture of increasing polarity as eluent (Hex, Hex/DCM, DCM, DCM/MeOH and MeOH). The fractions collected were pooled into several major fractions based on TLC pattern. Further isolation was continuously subjected to various chromatography methods; extensive column chromatography (CC), preparative thin layer chromatography (PTLC) and preparative centrifugal thin layer chromatography (PCTLC) to obtain the pure alkaloid.

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Column Chromatography

Column chromatography was performed using Merck Silica gel 60, 70-230 mesh ASTM (1.07734.1000) and 200-400 mesh ASTM (1.09385.1000). The silica gel was made into slurry with hexane before it was packed into appropriate column size and was allowed to equilibrate for at least an hour before the sample was introduced. The solvent systems used were combination of hexane, dichloromethane and methanol in increasing polarity at a certain flow rate. For the polar fractions, Sephadex^{TM} LH-20 purchased from GE Healthcare was used. The gel was made into slurry with DCM and MeOH; 1:1 ratio before it packed into column. Ratio 1:1; DCM and MeOH were also used as isocratic eluant. Fraction was monitored by thin layer chromatography (TLC) and appropriate fractions were combined and where necessary subjected to re-column chromatography, preparative TLC or preparative centrifugal TLC.

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Thin Layer Chromatography

Thin layer chromatography pattern form was used as indicator to combine the series of fraction from column chromatography and preparative centrifugal thin layer chromatography and also to determine the pure compound before confirm with ^{1}H NMR analysis. The TLC plate was spotted with the sample using a handmade fine glass capillary tube. The developed chromatographic tanks were saturated with various suitable solvent systems from mixture of hexane, dichloromethane and methanol. The saturated environment with ammonia also used in development of TLC give the better separation on alkaloid. The alkaloidal spots were visually examined under UV light (254 nm), followed by slightly dip into Dragondorff's reagent. Merck 1.05554.0001 Aluminium sheets TLC silica gel 60 F_{254} was used in this study.

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Preparative Thin Layer Chromatography

Preparative thin layer chromatography plates were prepared using Merck 1.07749.1000 Silica gel 60 PF_{254} containing gypsum. The 20 X 20 cm glass plate was prepared using plate leveller of 0.5 mm thick and the slurry was made in ratio 1:2 ; silica gel : cold distilled water was used. The plate was dried in oven at temperature 50°C until completely dried. Before use, all plates were activated with hexane.

Preparative Centrifugal Thin Layer Chromatography

Preparative centrifugal thin layer chromatography (PCTLC) (Harrison Research Chromatotron®) was used using a round glass plate with 24 cm in diameter. The prepared plate then dry in oven at 50°C until completely dry. The sample was dissolve in minimum volume of suitable solvent and loaded at the centre of the plate while spinning to create a thin band. Elution is carried out with gradient or isocratic solvent systems which ever appropriate.

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3.2.7 Physical and Spectral Data of Isolated Alkaloids

APB 1, Norisocorydine *N*-oxide 166

Molecular formula	:	$C_{19}H_{20}NO_5^+$
Physical appearance	:	Dark Brown Amorphous
$UV_{\lambda\ max}$ (MeOH), nm (log ε)	:	269 (4.13)
$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1}	:	3254, 3020, 1595, and 1372
Mass spectrum, m/z	:	342.16977 $[M]^+$

^{1}H NMR (400 MHz, $CDCl_3$), δ :

3.49 (3H, *s*, 1-OCH_3), 3.88 (3H, *s*, 2-OCH_3), 6.71 (1H, *s*, H-3), 3.41 (1H, *m*, H_{ax}-4), 2.87 (1H, *dd*, *J*= 6.5 and 16.8 Hz, H_{eq}-4), 3.78 (1H, *dd*, *J*= 3.7 and 13.2 Hz, H_{ax}-5), 3.18 (1H, *m*, H_{eq}-5), 4.04 (1H, *dd*, *J*= 3.7 and 13.2 Hz, H-6a), 3.21 (1H, *m*, H_{ax}-7), 2.97 (1H, *t*, *J*= 13.6 Hz, H_{eq}-7), 6.92 (1H, *d*, *J*= 8.2 Hz, H-8), 6.89 (1H, *d*, *J*=8.1 Hz, H-9), 3.46 (3H, *s*, 10-OCH_3)

^{13}C NMR (100 MHz, $CDCl_3$), δ :

146.3 (C-1), 61.0 (1-OCH_3), 124.8 (C-1a), 123.6 (C-1b), 152.9 (C-2), 56.1 (2-OCH_3), 111.8 (C-3), 125.4 (C-3a), 25.7 (C-4), 41.5 (C-5), 53.4 (C-6a), 35.1 (C-7), 126.8 (C-7a), 123.1 (C-8), 114.5 (C-9), 145.7 (C-10), 60.7 (10-OCH_3), 148.7 (C-11), 122.5 (C-11a)

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APB 2, Corydine 124

Molecular formula	:	$C_{20}H_{23}NO_4$
Physical apprearance	:	Dark Brown Amorphous
$UV_{\lambda\ max}$ (MeOH), nm (log ε)	:	219 (4.50), 271 (4.11), 307 (3.84)
$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1}	:	3529 and 3019
Mass spectrum, m/z	:	342.17272 $[M+H]^+$
$[\alpha]_D^{25}$ (Literature)	:	+204° (*c* = 0.6, EtOH) (Shamma & Salgar, 1973)

^{1}H NMR (400MHz, $CDCl_3$), δ :

8.70 (1H, br, *s*, 1-OH), 3.38 (3H, *s*, 2-OCH_3), 6.67 (1H, *s*, H-3), 3.16 (1H, *m*, H-4_{ax}), 2.66 (1H, *dd*, *J*= 2.9 and 15.5 Hz, H-4_{eq}), 3.04 (1H, *m*, H-5_{ax}), 2.53 (1H, *dd*, *J*= 4.0 and 23.5 Hz, H-5_{eq}), 2.54 (3H, *s*, *N*-CH_3), 2.96 (1H, *d*, *J*= 13.2 Hz, H-6a), 3.03 (1H, *dd*, *J*= 3.5 and 13.8 Hz, H-7_{ax}), 2.42 (1H, *t*, *J*= 13.2 Hz, H-7_{eq}), 7.07 (1H, *d*, *J*= 8.0 Hz, H-8), 6.86 (1H, *d*, *J*= 8.0Hz, H-9), 3.89 (3H, *s*, 10-OCH_3), 3.71 (3H, *s*,11-OCH_3)

^{13}C NMR (100 MHz, $CDCl_3$), δ :

142.3 (C-1), 123.8 (C-1a), 127.8 (C-1b), 149.2 (C-2), 56.0 (2-OCH_3), 111.3 (C-3), 119.2 (C-3a), 28.8 (C-4), 52.7 (C-5), 43.8 (*N*-CH_3), 62.0 (C-6a), 35.4 (C-7), 130.6 (C-7a), 124.4 (C-8), 110.9 (C-9), 151.8 (C-10), 56.0 (10-OCH_3), 143.8 (C-11), 62.7 (11-OCH_3), 126.4 (C-11a)

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APB 3, *N*-methylhernagine 169

Molecular formula	:	$C_{20}H_{23}NO_4$
Physical appearance	:	Brown amorphous
$UV_{\lambda\ max}$ (MeOH), nm (log ε)	:	222 (4.50), 270 (4.13), 301 (3.75)
$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1}	:	3515, 3018, 1464 and 1213
Mass spectrum, m/z	:	342.17122 $[M+H]^+$
$[\alpha]_D^{25}$ (Literature)	:	+144° ($CHCl_3$) (Yakushijin, Sugiyama, Murata, & Furukawa, 1980)

^{1}H NMR (500 MHz, $CDCl_3$), δ :

3.49 (3H, *s*, 1-OCH_3), 3.87 (3H, *s*, 2-OCH_3), 6.68 (1H, *s*, H-3), 3.15 (1H, *m*, H-4_{ax}), 2.70 (1H, *dd*, *J*= 3.5 and 16.1 Hz, H-4_{eq}), 3.04 (1H, *d*, *J*= 6.3 Hz, H-5_{ax}), 2.52 (1H, *m*, H-5_{eq}), 2.53 (1H, *s*, *N*-CH_3), 2.90 (1H, *dd*, *J*= 1.2 and 12.6 Hz, H-6a), 3.01 (1H, *dd*, *J*= 4.0 and 13.8 Hz, H-7_{ax}), 2.37 (1H, *t*, *J*= 13.2 Hz, H-7_{eq}), 6.90 (1H, *d*, *J*= 10.0 Hz, H-8), 6.86 (1H, *d*, *J*=10.0 Hz, H-9), 3.52 (3H, *s*, 11-OCH_3)

^{13}C NMR (125 MHz, $CDCl_3$), δ :

145.4 (C-1), 60.5 (1-OCH_3), 125.0 (C-1a), 128.8 (C-1b), 151.5 (C-2), 56.1 (2-OCH_3), 111.7 (C-3), 127.7 (C-3a), 28.9 (C-4), 53.0 (C-5), 44.0 (*N*-CH_3), 78.1 (C-6a), 35.6 (C-7), 129.8 (C-7a), 122.4 (C-8), 113.5 (C-9), 148.0 (C-10), 145.5 (C-11), 60.9 (11-OCH_3), 123.0 (C-11a)

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APB 4, Oxohernagine 122

Molecular formula	:	$C_{19}H_{15}NO_5$
Physical appearance	:	Brownish amorphous
$UV_{\lambda\ max}$ (MeOH), nm (log ε)	:	272 (4.43), 358 (4.01)
$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1}	:	3476, 3019, 1660 and 1521
Mass spectrum, m/z	:	338.10771 $[M+H]^+$
$[\alpha]_D^{24}$ (Literature)	:	±0° (*c* = 0.08, $CHCl_3$) (Chen, Tsai, & Chen, 1996)

^{1}H NMR (400 MHz, $CDCl_3$), δ :

4.09 (3H, *s*, 1-OCH_3), 3.75 (3H, *s*, 2-OCH_3), 7.20 (1H, *s*, H-3), 7.75 (1H, *d*, *J*= 4.0 Hz, H-4), 8.83 (1H, *d*, *J*= 4.0 Hz, H-5), 8.26 (1H, *d*, *J*= 8.0 Hz, H-8), 7.21 (1H, *d*, *J*= 8.0 Hz, H-9), 3.53 (3H, *s*, 11-OCH_3)

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^{13}C NMR (100 MHz, $CDCl_3$), δ :

157.0 (C-1), 56.5 (1-OCH_3), 115.2 (C-1a), 122.3 (C-1b), 152.0 (C-2), 62.4 (2-OCH_3), 105.7 (C-3), 134.6 (C-3a), 122.7 (C-4), 144.4 (C-5), 91.4 (C-6a), 181.6 (C-7), 144.9 (C-7a), 126.6 (C-8), 115.8 (C-9), 154.9 (C-10), 144.6 (C-11), 60.7 (11-OCH_3), 126.3 (C-11a)

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APB 5, Hernagine 123

Molecular formula : $C_{19}H_{21}NO_4$

Physical appearance : Light brown powder

$UV_{\lambda\ max}$ (MeOH), nm (log ε) : 223 (4.50), 270 (4.32), 301 (3.93)

$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1} : 3338, 3018, 1462 and 1213

Mass spectrum, m/z : 328.15543 $[M+H]^+$

$[\alpha]_D^{26}$ (Literature) : +252° (*c* = 1.3, MeOH) (Nafiah, 2009)

^{1}H NMR (500 MHz, $CDCl_3$), δ :

3.54 (3H, *s*, 1-OCH_3), 3.88 (3H, *s*, 2-OCH_3), 6.70 (1H, *s*, H-3), 3.03 (1H, *s*, H-4_{ax}), 2.72 (1H, *d*, *J*= 12.0 Hz, H-4_{eq}), 3.40 (1H, *d*, *J*= 6.9 Hz, H-5_{ax}), 3.01 (1H, *s*, H-5_{eq}), 3.73 (1H, *dd*, *J*= 3.5 and 12.6 Hz, H-6a), 2.80 (1H, *dd*, *J*= 4.0 and 13.2 Hz, H-7_{ax}), 2.52 (1H, *m*, *J*= 13.2 Hz, H-7_{eq}), 6.88 (1H, *d*, *J*= 8.0 Hz, H-8), 6.86 (1H, *d*, *J*= 8.0 Hz, H-9), 3.49 (3H, *s*, 11-OCH_3)

^{13}C NMR (125 MHz, $CDCl_3$), δ :

145.5 (C-1), 61.0 (1-OCH_3), 124.7 (C-1a), 129.9 (C-1b), 151.7 (C-2), 56.2 (2-OCH_3), 112.2 (C-3), 127.9 (C-3a), 28.8 (C-4), 42.9 (C-5), 54.1 (C-6a), 37.9 (C-7), 129.5 (C-7a), 122.5 (C-8), 113.7 (C-9), 148.2 (C-10), 145.5 (C-11), 60.5 (11-OCH_3), 123.2 (C-11a)

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APB 6, Hernovine 170

Molecular formula	:	$C_{18}H_{19}NO_4$
Physical appearance	:	Brown amorphous
$UV_{\lambda\ max}$ (MeOH), nm (log ε)	:	271 (3.60)
$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1}	:	3476.9, 3312.8, 3019.8 and 1475.2
Mass spectrum, m/z	:	314.13881 $[M+H]^+$
$[\alpha]_D^{18}$ (Literature)	:	+142° (*c*= 0.52, C_5H_5N) (Stuart & Chambers, 1967)

^{1}H NMR (500 MHz, $CDCl_3$), δ :

3.45 (3H, *s*, 1-OCH_3), 6.77 (1H, *s*, H-3), 3.00 (1H, *d*, *J*= 3.4 Hz, H-4_{ax}), 2.69 (1H, *d*, *J*= 12.6 Hz, H-4_{eq}), 3.36 (1H, *m*, H-5_{ax}), 2.99 (1H, *d*, *J*= 2.3 Hz, H-5_{eq}), 3.70 (1H, *dd*, *J*= 4.0 and 12.6 Hz, H-6a), 2.80 (1H, *dd*, *J*= 4.0 and 13.7 Hz, H-7_{ax}), 2.52 (1H, *t*, *J*= 13.7 Hz, H-7_{eq}), 6.93 (1H, *d*, *J*= 8.0 Hz, H-8), 6.88 (1H, *d*, *J*=8.0 Hz, H-9), 3.49 (3H, *s*, 11-OCH_3)

^{13}C NMR (125 MHz, $CDCl_3$), δ :

143.0 (C-1), 61.0 (1-OCH_3), 123.2 (C-1a), 129.1 (C-1b), 147.9 (C-2), 114.4 (C-3), 129.4 (C-3a), 28.5 (C-4), 42.9 (C-5), 54.1 (C-6a), 37.7 (C-7), 128.8 (C-7a), 123.0 (C-8), 113.8 (C-9), 148.1 (C-10), 144.8 (C-11), 60.8 (11-OCH_3), 122.6 (C-11a)

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APB 7, Costaricine 126

Molecular formula	:	$C_{35}H_{38}N_2O_6$
Physical appearance	:	Brown amorphous
$UV_{\lambda\ max}$ (MeOH), nm (log ε)	:	284 (4.12)
$IR_{\nu\ max}$ ($CHCl_3$), cm^{-1}	:	3418.5, 3296.8, 3019.7 and 1212.9
Mass spectrum, m/z	:	583.28929 $[M+H]^+$
$[\alpha]_D^{26}$ (Literature)	:	+46.4° (*c* = 0.248, $CHCl_3$) (Angerhofer, Guinaudeau, Wongpanich, Pezzuto, & Cordell, 1999)

^{1}H NMR (500 MHz, $CDCl_3$), δ :

4.05 (1H, *dd*, 4.0 and 9.2 Hz, H-1), 3.16 (1H, *m*, H-3_{ax}), 2.65 (1H, *m*, H-3_{eq}), 2.90 (1H, *m*, H-4_{ax}), 2.64 (1H, *m*, H-4_{eq}), 6.54 (1H, *s*, H-5), 3.79 (3H, *s*, 6-OCH_3), 6.70 (1H, *s*, H-8), 3.03 (1H, *dd*, *J*= 4.0 and 13.8 Hz, H-α_{ax}), 2.68 (1H, m, H-α_{eq}), 6.73 (1H, *d*, *J*=1.7 Hz, H-10), 3.83(3H, *s*, 12-OCH_3), 6.91 (1H, *d*, *J*= 8.0 Hz, H-13), 6.95 (1H, *dd*, *J*= 1.8 and 8.1 Hz, H-14), 4.08 (1H, *dd*, *J*= 3.5 and 9.8 Hz, H-1′), 3.23 (1H, *m*, H-$3'_{ax}$), 2.72 (1H, *m*, H-$3'_{eq}$), 2.92 (1H, *m*, H-$4'_{ax}$), 2.78 (1H, *m*, H-$4'_{eq}$), 6.49 (1H, *s*, H-5′), 3.78 (3H, *s*, 6′-OCH_3), 6.66 (1H, *s*, H-8′), 3.13 (1H, *m*, H-α'_{ax}), 2.80 (1H, *m*, H-α'_{eq}), 7.12 (1H, *d*, *J*= 8.6 Hz, H-10′), 6.86 (1H, *d*, *J*= 8.6 Hz, H-11′), 6.86 (1H, *d*, *J*= 8.6 Hz, H-13′), 7.12 (1H, *d*, *J*= 8.6Hz, H-14′)

^{13}C NMR (125 MHz, $CDCl_3$), δ :

56.8 (C-1), 41.0 (C-3), 29.2 (C-4), 130.1 (C-4a), 111.2 (C-5), 145.7 (C-6), 55.8 (6-OCH_3), 144.1 (C-7), 112.7 (C-8), 126.3 (C-8a), 41.2 (C-α), 131.4 (C-9), 121.0 (C-10), 145.7 (C-11), 149.7 (C-12), 56.1 (12-OCH_3), 112.5 (C-13), 125.2 (C-14), 56.5 (C-1′), 40.7 (C-3′), 29.1 (C-4′), 130.4 (C-4a′), 111.2 (C-5′), 145.6 (C-6′), 55.8 (6′-OCH_3), 144.0 (C-7′), 112.7 (C-8′), 126.1 (C-8a′), 41.8 (C-α′), 133.1 (C-9′), 130.5 (C-10′), 118.1 (C-11′), 156.0 (C-12′), 118.1 (C-13′), 130.5 (C-14′)

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3.3 Biological Activity Assay

The alkaloid crude extract and isolated compounds from barks of *Alseodaphne pendulifolia* Gamb. were assayed to various biological activities such as antiplasmodial, cytotoxic, antibacterial and antioxidant assays.

3.3.1 *In-vitro* Antiplasmodial Assay

The antiplasmodial activity of the alkaloid crude extracts and isolated alkaloids were determined against the chloroquine-resistant (K1) strains of *Plasmodium falciparum* that were cultured according to the methods described by Trager and Jensen with slight modifications (Makler & Hinrichs, 1993; Makler, Ries, Williams, & Bancroft, 1993; Trager & Jensen, 1976).

Microtitration techniques were used to measure the activity of samples over a wide range of concentrations. A solution of chloroquine diphosphate and artemisinin served as positive control in all experiments. All tests were performed in duplicate. Crude extract was dissolved in DMSO to produce a stock solution of 20 mg/ml. The stock solutions were subsequently diluted with deionized water at 20 concentrations of two-fold dilutions into two 96-well microtiter plates. 10µl of each concentration was transferred into another 96-well microtiter plates. 190µl of parasitised red blood cell suspensions (1% parasitaemia) were next added to each well.

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For the infected control, parasitised red blood cells were devoid of plant extracts whereas only non-parasitised red blood cells were prepared for the non-infected control plates were incubated for 24 hours at 37°C in a candle jar and were subsequently cooled at −20°C to lyse the red blood cells. The plates were allowed to reach room temperature, and 20µl of the blood suspension was dispensed into a new microtiter plate containing 100µL MALSTAT^{TM} reagent and 20µl nitroblue tetrazolium and phenazine ethosulfate mixture. Absorbance was measured with an ELISA plate reader at 780 nm. The percentage inhibition at each concentration was determined and the mean of IC_{50} values of parasite viability was calculated using probit analysis (Chan, Choo, Abdullah, & Ismail, 2004).

3.3.2 Cell Culture and MTT Cytotoxicity Assay

The HL-60 cells that were used in this study were obtained from the American Type Cell Collection (ATCC). The cells were cultured using RPMI 1640 culturing media (PAA, Leverkusen, Germany). Cells were trypsinized and counted using hemocytometer and plated in a microtiter plate of 96-wells. After an overnight incubation to allow cell attachment, the medium was changed and 0.2 mL of new supplemented medium was added into each well.

Cells were then treated with the different drug concentrations and incubated at 37 °C, 5% CO_2 for 24 h. Each concentration of the samples was assayed in triplicate. The colorimetric assays were performed at an absorbance of 570 nm. Results were expressed as a percentage of control giving a certain percentage of cell viability after

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24 hours exposure to the test agent. The potency of cell growth inhibition for test agent was expressed as an IC_{50} value.

3.3.3 Bacterial Cultures and Disc Diffusion Assay

The *in-vitro* antibacterial activity of the alkaloids was evaluated against pathogenic microorganism, Gram positive bacteria: *Bacillus subtilis* (B145). The strain was stored in the appropriate medium before use. Antibacterial activity of the isolated compounds were determined by the disc diffusion method (Hashim et al., 2012) with slight modifications in terms of sample concentration, volume of sample loaded and use of paper discs. The bacteria were cultured at 37 °C for overnight in nutrient broth and potato dextrose broth, respectively. The concentrations of the cultures were adjusted turbidometrically at a wavelength of 600 nm which gave 10^5–10^6 colony forming units (CFU) per mL.

The compounds to be tested were dissolved in dimethyl sulphoxide (DMSO) at concentration of 10 mg/mL. About 10 µL of each sample solution was loaded on Whatman No. 1 filter paper disc (Ø6 mm). The disc was placed on the surface of the agar plate (nutrient agar or potato dextrose agar) previously inoculated with bacteria. The agar plates were then inverted and incubated for 24 h at 37 °C. The antimicrobial activity was recorded by measuring the inhibition zone (IZ) in mm around each disc, against the test organisms. The experiments were repeated in triplicate and the results were expressed as average values. The antibiotic streptomycin sulfate (10 µg/disc), was used as positive control and DMSO as negative control in the assay.

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3.3.4 DPPH Free Radical Scavenging Activity Assay

The standard 1,1-diphenyl-1-picryhydrazyl (DPPH) assay was used with slight modification. In this technique, 96 micro-well flat bottom plates were used instead of cuvettes to consume minimal amounts of sample, vehicle and DPPH solutions. Stock solutions of the compounds were prepared at 1 mg/mL in DMSO. Each well was filled in with 100 µL of diluent (DMSO). The first row (row A) of the 96 wells was filled in with 100 µL of diluted sample at 1 mg/mL.

Concentration in the first row was 500 µg/mL of sample for each well and was serially diluted (two fold dilution) in 96 micro-well plates to varying concentrations, topping from 500 µg/mL down to the lowest 7.8 µg/mL. Then, 5 µL of the DPPH solution (2.5 mg/mL in DMSO) was added to each well. The DPPH solution kept in the dark at 4 °C. The plate was shaken to ensure thorough mixing before placed in the dark. After 30 minutes, the optical density of each well was read using ELISA Reader (EL340 Biokinetic Reader, Bio-Tek Instrumentation) at wavelength 517 nm. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 1 - \text{OD(DPPH + Sample)}/\text{OD(DPPH)} \times 100$$

A graph of percentage inhibition of free radical activity was plotted against concentration of crude extract and 50% inhibition concentration (IC_{50}) was obtained from the graph. The IC_{50} value was determined as the concentration of each sample required or able to scavenge 50% of the DPPH. All tests and analyses were run in triplicate and averaged. Antioxidant assay using DPPH method was used to assess the

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capacity of the compounds to scavenge stable free radical DPPH at different concentration. The radical scavenging effect was examined and compared with natural antioxidants α-tocopherol (Sigma, USA), ascorbic acid (Sigma USA), and quercetin (Sigma, USA) were used as positive controls.

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Isolation of Alkaloids from Barks of *Alseodaphne pendulifolia* Gamb.

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The barks of *Alseodaphne pendulifolia* Gamb. (Lauraceae) were collected from two different locations; Sg. Temau Reserve Forest, Kuala Lipis, Pahang and Sg. Tekam Reserve Forest, Jerantut, Pahang, Malaysia (later will be known as plant A and plant B, respectively) were selected to studied their alkaloid constituent. Mayer test resulted dense white precipitate that indicated positive result of alkaloid for both sample: plant A and plant B. Acid-base extraction of compounds, plant A and plant B was yield 1.23% and 1.42% of crude alkaloid, respectively.

Further isolation process was afforded four type of isoquinoline alkaloids; aporphine, aporphine *N*-oxide, oxoaporphine and bisbenzylisoquinoline. A new aporphine *N*-oxide alkaloids; norisocorydine *N*-oxide **166** with four known aporphine alkaloids; corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123**

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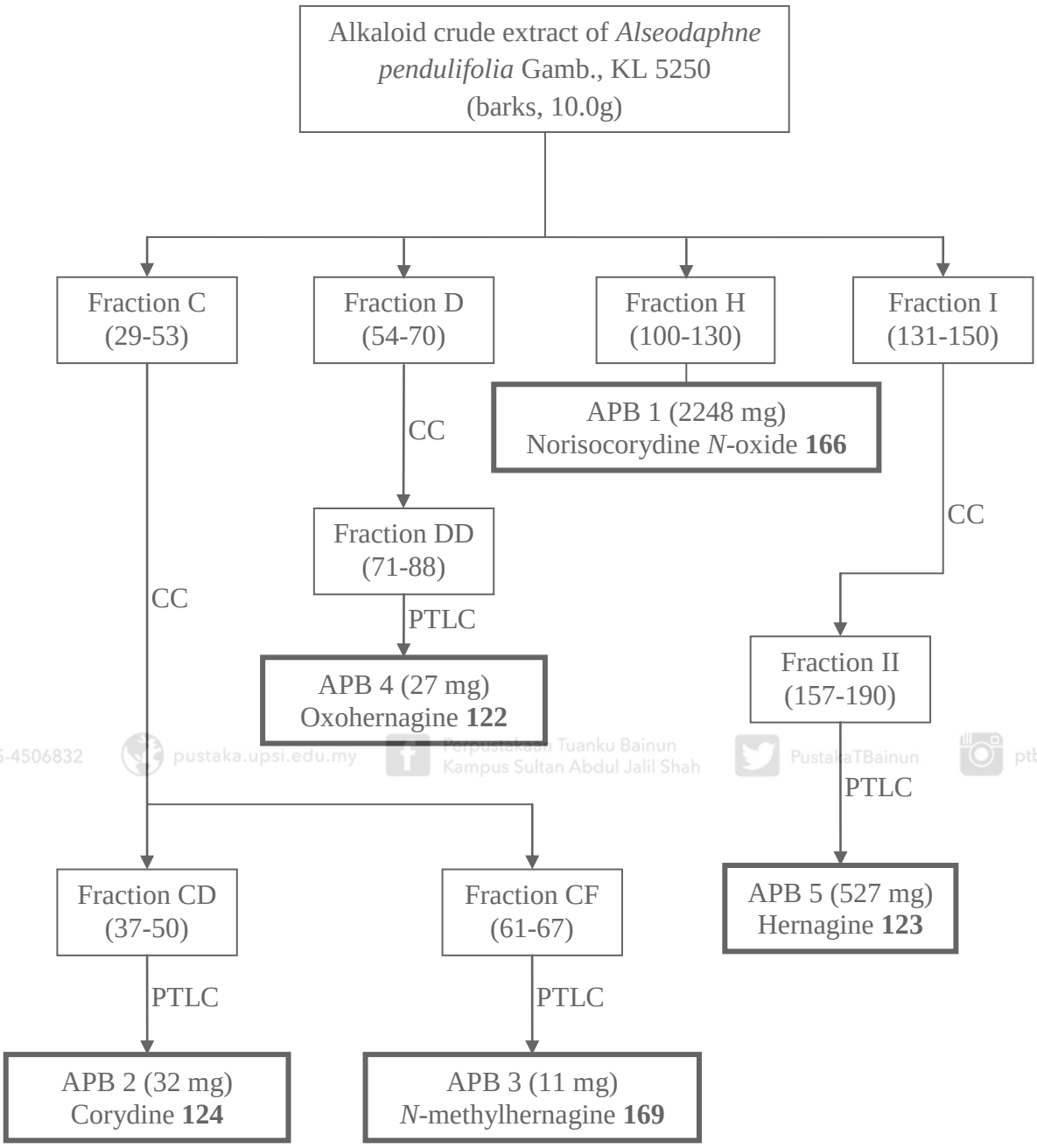
were successfully isolated from plant A. While from plant B, four aporphines; corydine **124**, *N*-methylhernagine **169**, hernagine **123** and hernovine **170**, a oxoaporphine; oxohernagine **122** was successfully isolated together with a bisbenzylisoquinoline alkaloids; costaricine **126**. The acronym of the sample code was used as APB is referring to *Alseodaphne pendulifolia*'s bark. Scheme 4.1.1 and 4.1.2 shows the alkaloids isolation process from barks of *Alseodaphne pendulifolia* Gamb. for both sample.

The major compound in plant A, norisocorydine *N*-oxide was not yield in plant B may cause by sample handling technique and the sample degrade while isolation process due to direct light or high temperature so it would not appear in isolated compounds in the alkaloid crude extract from the sample that collected from Sg. Tekam Reserved Forest, Jerantut, Pahang.

Classic extraction method in Soxhlet extractor is chosen due to its effectiveness to extract more sample mass than the other methods (Castro & Garcia-Ayuso, 1988). This method allows the samples to contact with fresh portion of the solvent (distillation). The isolation works were achieved by various chromatographic techniques as describe in Chapter 3 and the structure elucidation was performed via spectral method; namely 1D and 2D NMR, MS, IR, UV and also by comparison with previous literature. This is the first report about the isolation of alkaloid from barks of *Alseodaphne pendulifolia* Gamb.

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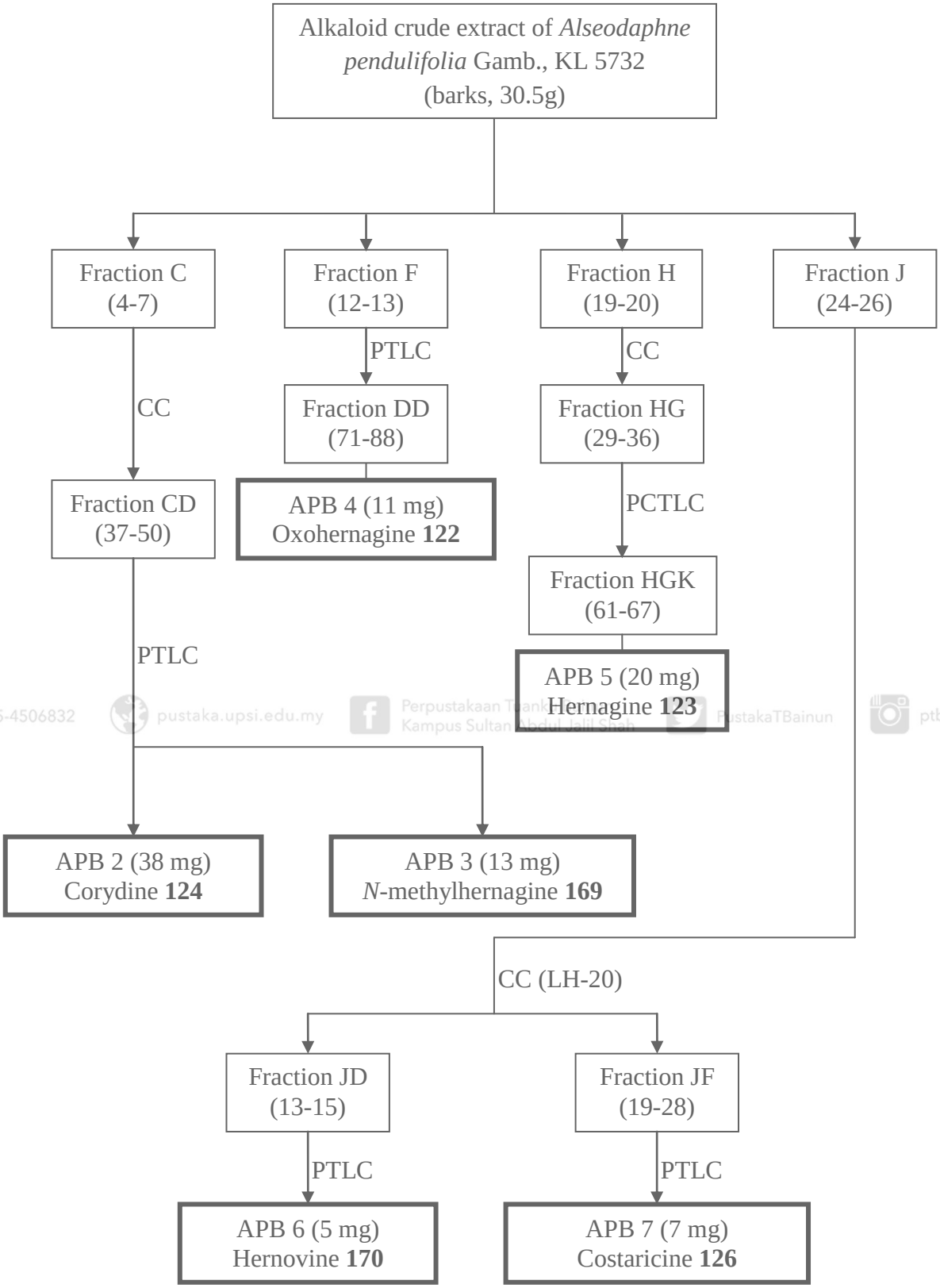
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Scheme 4.1.1: Isolation of Alkaloid from the Barks of *Alseodaphne pendulifolia* Gamb. (KL 5250)

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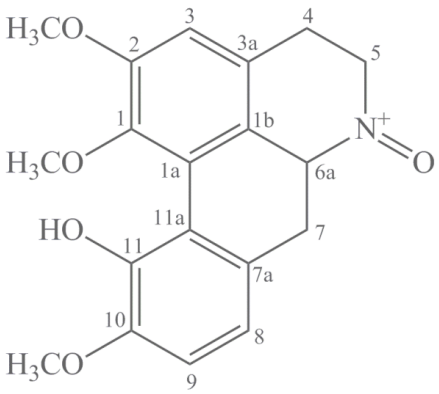
Scheme 4.1.2: Isolation of Alkaloid from the Barks of *Alseodaphne pendulifolia* Gamb. (KL 5732)

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4.2 Structural Elucidation of Isolated Alkaloid from Barks of *Alseodaphne pendulifolia* Gamb.

4.2.1 APB 1, Norisocorydine *N*-oxide 166



166

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Alkaloids APB 1 was isolated as major compound in barks of *Alseodaphne pendulifolia* Gamb. that collected from Sg. Temau Reserved Forest, Pahang. The appearance of these alkaloids was first afforded as a dark brown amorphous and slowly changes to black amorphous within times, due to decomposition of the compounds. The IR spectrum showed the absorption peaks of nitro aliphatic characteristic at 1595 and 1372 cm^{-1}, while the peak at 3254 and 3020 cm^{-1} is due to the presence of hydroxyl group and stretching C-H aromatic in the compound, respectively (Silverstein & Webster, 1998; Williams & Fleming, 1989). The UV spectrum exhibited an intense peak at 269 nm (log ε = 4.13). The HRESIMS of APB 1 gives a pseudo-molecular peak at m/z 342 corresponding to the molecular formula of $C_{19}H_{20}NO_5^+$ (m/z 342.16977 $[M]^+$; calc. 342.13415).

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The ^{1}H-NMR spectrum (Figure 4.2.1.1) revealed three methoxyl groups at δ 3.49, 3.55, and 3.88 as singlet which corresponding to proton attached to C-10, C-1 and C-2, respectively. The signal of aromatic proton appeared as a singlet at δ 6.71 was assigned to H-3. Another two aromatic proton at C-8 and C-9 were observed as doublet at δ 6.92 and 6.89 (*J*=8.0 Hz). In this compound, the signal at δ 4.10 (*J=dd*, 2.9, 12.6 Hz) was assigned to H-6a in view of the shielding effect of neighboring negative oxygenous ion compare to isocorydine **94** and norisocorydine **95** (Table 4.2.1.1) (Dang, Gong, Liu, & Yu, 2009). This situation spontaneously effected proton at position C-5 to appeared at δ 3.78 (*J=dd*, 5.8, 12.6 Hz). The nearly same conditions are occurred in corydine *N*-oxide **167** isolated from *Aristolochia lagesiana* var. intermedia (Ferreira, de Pascoli, Nascimento, Zukerman-Schpector, & Lopes, 2010) toward corydine **124** compounds have nearly same skeleton with norisocorydine *N*-oxide **166** toward norisocorydine **95**, the conditions affected the neighbouring protons of oxygenated nitrogen at C-6a and C-5 to shift more downfield as shown in Table 4.2.1.2 and the other aporphine *N*-oxide that successfully isolated recorded in *Berberis integerrima*; isocorydine *N*-oxide **168** (Azimova & Yunusov, 2013). Another six methylene proton signals appeared between δ 2.91 – 3.55 which are assigned for protons at C-4, C-5 and C-7, respectively (Table 4.2.1.3).

The ^{13}C and DEPT-NMR data (Figure 4.2.1.2 and 4.2.1.3) indicated the presence of 19 carbon signals; three aromatics at δ 111.8 (C-3), δ 123.1 (C-8) and δ 114.5 (C-9); three methoxyls at δ 61.0 (1-OCH_3), δ 56.1 (2-OCH_3) and δ 60.7 (10-OCH_3); three methylenes at δ 25.7 (C-4), δ 41.5 (C-5) and δ 35.1 (C-7); one methine at δ 53.4 (C-6a) and the rest of the signals are nine quaternary carbons were appeared

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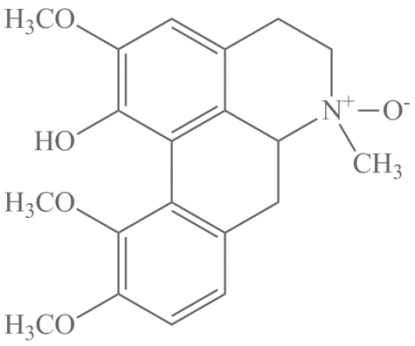
at δ 146.3 (C-1), δ 124.8 (C-1a), δ 123.6 (C-1b), δ 152.9 (C-2), δ 125.4 (C-3a), δ 126.8 (C-7a), δ 145.7 (C-10), δ 148.7 (C-11) and δ 122.5 (C-11a).

The positions of these carbons resonance were further supported by ^{1}H-^{1}H COSY (Figure 4.2.1.4), HSQC (Figure 4.2.1.5) and the long range ^{1}H-^{13}C correlations observed in the HMBC (Figure 4.2.1.6) spectra. The ^{1}H-^{1}H COSY spectrum suggested the fragments of C-4 to C-5, C-6a to C-7 and C-8 to C-9. In HMBC, the exact position of methoxyl groups were confirmed to be at C-1, C-2 and C-10 through long-range correlation from the proton at 1-OCH_3 to C-1, 2-OCH_3 to C-2 and 10-OCH_3 to C-10, HMBC data also important to confirm the position of quaternary carbons in ring A; from proton at C-3 to C-1, C-2, C-3a & C-1b and also in ring C; from proton at C-7 to C-7a. Other correlations were observed for proton at C-4 to C-5 & C-3a, C-5 to C-6a & C-3a, C-8 to C-7a & C-10, C-7 to C-6a & C-8 and C-9 to C-11 were shown in Figure 4.2.1.7, which confirmed all the ^{13}C signals of compounds. The NOESY correlation between H-3 and 2-OMe reconfirmed the assignment of 2-OCH_3, another correlation can be found between H-3 & H-4 and H-8 & H-7 (Figure 4.2.1.8). Aided by NOESY assignment was showed that proton at position C-6a of was align with proton H-7β and suggested that this compound possessed a beta spatial orientation. H2BC spectrum (Figure 4.2.1.9) revealed the following fragment C-4 to C-5, C-6a to C-7 and C-8 to C-9. Further analysis on HSQC-TOCSY experiment (Figure 4.2.1.10) was reconfirmed data from HSQC and COSY spectrum that support the position of C-4, C-5, C-6a, C-7, C-8 and C-9.

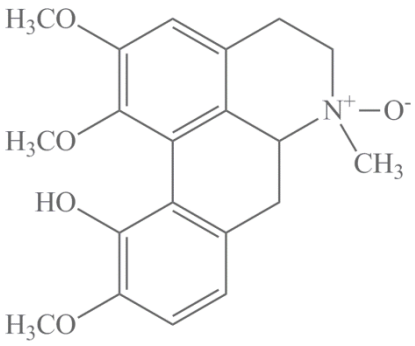
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Detailed analysis of the spectral data from ^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC, NOESY, H2BC dan HSQC-TOCSY NMR obtained led to the conclusion that compound APB **1** is a new aporphine alkaloid, named norisocorydine *N*-oxide **166**.



167



168

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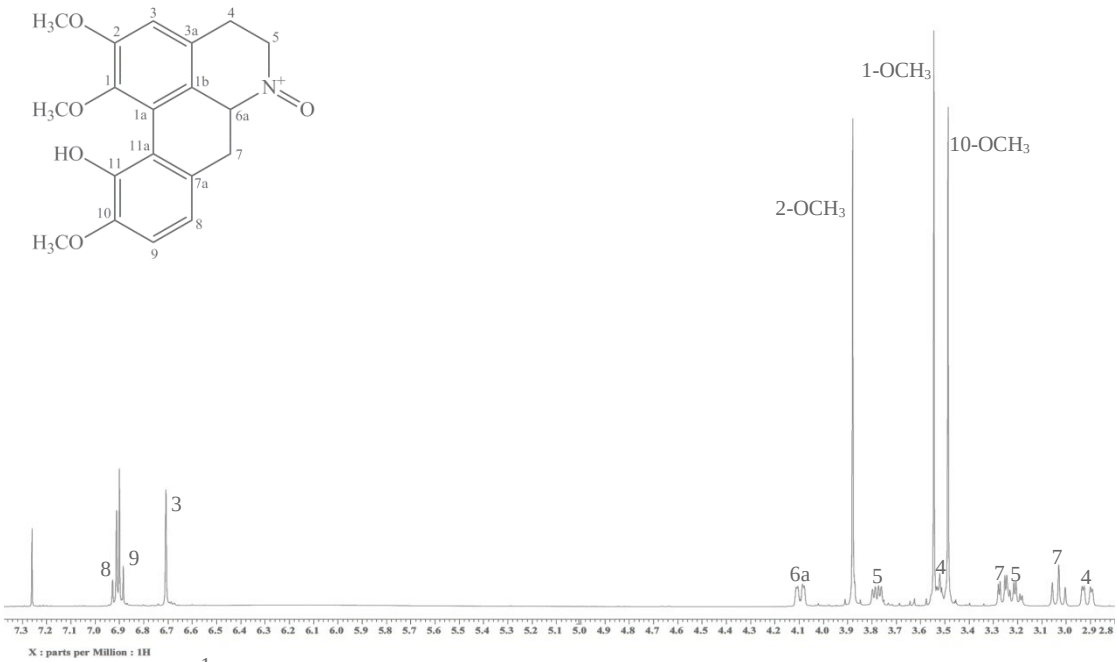


Figure 4.2.1.1: ^{1}H NMR Spectrum of APB 1

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Table 4.2.1.1:

*^{1}H NMR Spectral Comparison of APB 1 with Isocorydine **94** and Norisocorydine **95***

	^{1}H, δ, $CDCl_3$ (*J*,Hz)		
		(Ferreira et al., 2010)	
Position	**APB 1**	**94**	**95**
1-OCH_3	3.55 (*s*)	3.73 (*s*)	3.66 (*s*)
2-OCH_3	3.88 (*s*)	3.93 (*s*)	3.84 (*s*)
3	6.71 (*s*)	6.74 (*s*)	3.66 (*s*)
4	3.52 (*m*)	3.59-3.66 (*m*)	3.35 (*ddd*,5.0,11.7,17.5)
	2.91 (*dd*,4.0,17.2)	2.94 (*dd*,3.5,17.5)	2.80 (*dd*,3.8,17.5)
5	3.78 (*dd*,5.8,12.6)	4.03-4.10 (*m*)	3.59 (*dd*,5.0,12.3)
	3.21 (*dt*,4.6,12.6,17.2)	3.77-3.84 (*m*)	3.02 (*ddd*,3.8,11.7,12.3)
N-CH_3	Nil	3.09-3.13 (*br*,*s*)	Nil
6a	4.10 (*dd*,2.9,12.6)	3.70-3.77 (*m*)	3.85 (*m*)
7	3.26 (*dd*,4.0,13.8)	3.54-3.61 (*m*)	3.07 (*dd*,4.0,13.0)
	3.03 (*t*,13.2)	3.17 (*dd*,3.5,13.0)	2.90 (*dd*,13.013.5)
8	6.92 (*d*,8.0)	6.86 (*dd*,1.0,8.3)	6.76 (*d*,8.0)
9	6.89 (*d*,8.0)	6.89 (*d*,8.3)	6.79 (*d*,8.0)
10-OCH_3	3.49 (*s*)	3.92 (*s*)	3.84 (*s*)

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Table 4.2.1.2:

*^{1}H NMR Spectral Comparison Between Corydine N-oxide **167** Toward Corydine **124** (Ferreira et al., 2010)*

	^{1}H, δ, $CDCl_3$ (*J*,Hz)	
Position	**167**	**124**
1-OH	8.77 (*s*)	8.61 (*s*)
2-OCH_3	3.85 (*s*)	3.84 (*s*)
3	6.64 (*s*)	6.61 (*s*)
4	3.03 (*dd*,18.0,6.0)	3.08 (*ddd*,16.0,12.0,6.0)
	3.24 (*ddd*,18.0,12.5,6.0)	2.59 (*dd*,16.0,3.9)
5	3.82 (*m*)	2.93 (*ddd*,11.2,6.0,1.3)
	4.07 (*dd*,12.5,6.0)	2.43 (*ddd*,12.0,11.2,3.9)
N-CH_3	3.14 (*s*)	2.46 (*s*)
6a	4.47 (*dd*,13.0,3.5)	2.85 (*ddd*,13.7,3.5,1.3)
7	2.78 (*ddd*,13.0,12.5,1.0)	2.96 (*dd*,12.8,3.5)
	3.53 (*dd*,12.5,3.5)	2.33 (*ddd*,13.7,12.8,1.0)
8	7.12 (*dd*,8.0,1.0)	7.01 (*dd*,8.0,1.0)
9	6.86 (*d*,8.0)	6.80 (*d*,8.0)
10-OCH_3	3.85 (*s*)	3.83 (*s*)
11-OCH_3	3.66 (*s*)	3.66 (*s*)

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Table 4.2.1.3:

1D (^{1}H, ^{13}C and DEPT-135), 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 1

Position	^{1}H δ, $CDCl_3$ (*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HSQC	HMBC	DEPT-135
1		146.3				C_q
1-OCH_3	3.55 (*s*)	60.98		$H_{1\text{-OMe}}$	C_1	CH_3
1a		124.8				C_q
1b		123.6				C_q
2		152.9				C_q
2-OCH_3	3.88 (*s*)	56.1		$H_{2\text{-OMe}}$	C_2	CH_3
3	6.71 (*s*)	111.8		H_3	C_4,C_{1b}, C_1,C_2	CH
3a		125.4				C_q
4	3.52 (*m*)	25.7	H_4, H_5	H_4	C_5,C_{3a}	CH_2
	2.91 (*dd*,4.0,17.2)			H_4		
5	3.78 (*dd*,5.8,12.6)	41.5	H_5, H_4	H_5	C_{6a},C_{3a}	CH_2
	3.21 (*dt*,4.6,12.6,17.2)			H_5	C_{3a}	
6a	4.10 (*dd*,2.9,12.6)	53.4	H_7	H_{6a}		CH
7	3.26 (*dd*,4.0,13.8)	35.1	H_{6a}	H_7	C_{6a},C_8,	CH_2
	3.03 (*t*,13.2)			H_7	C_{7a}	
7a		126.8				C_q
8	6.92 (*d*,8.0)	123.1	H_9	H_8	C_{7a},C_{10}	CH
9	6.89 (*d*,8.0)	114.5	H_8	H_9	C_{11}	CH
10		145.7				C_q
10-OCH_3	3.49 (*s*)	60.7		$H_{10\text{-OMe}}$	C_{10}	CH_3
11		148.7				C_q
11a		122.5				C_q

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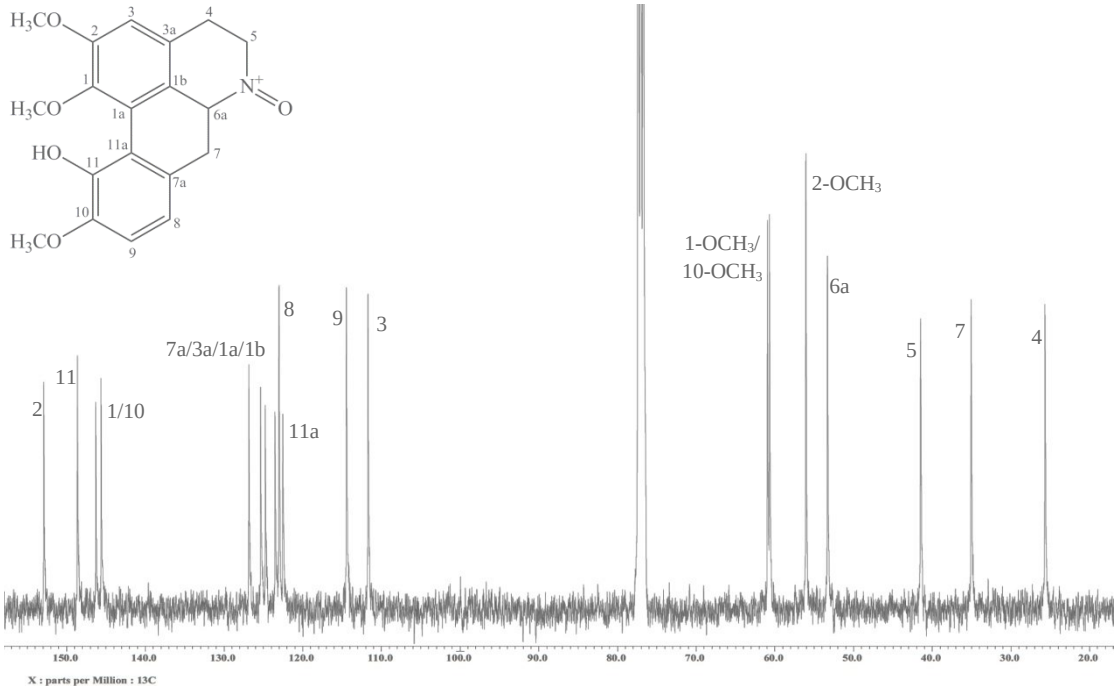


Figure 4.2.1.2: ^{13}C NMR Spectrum of APB 1

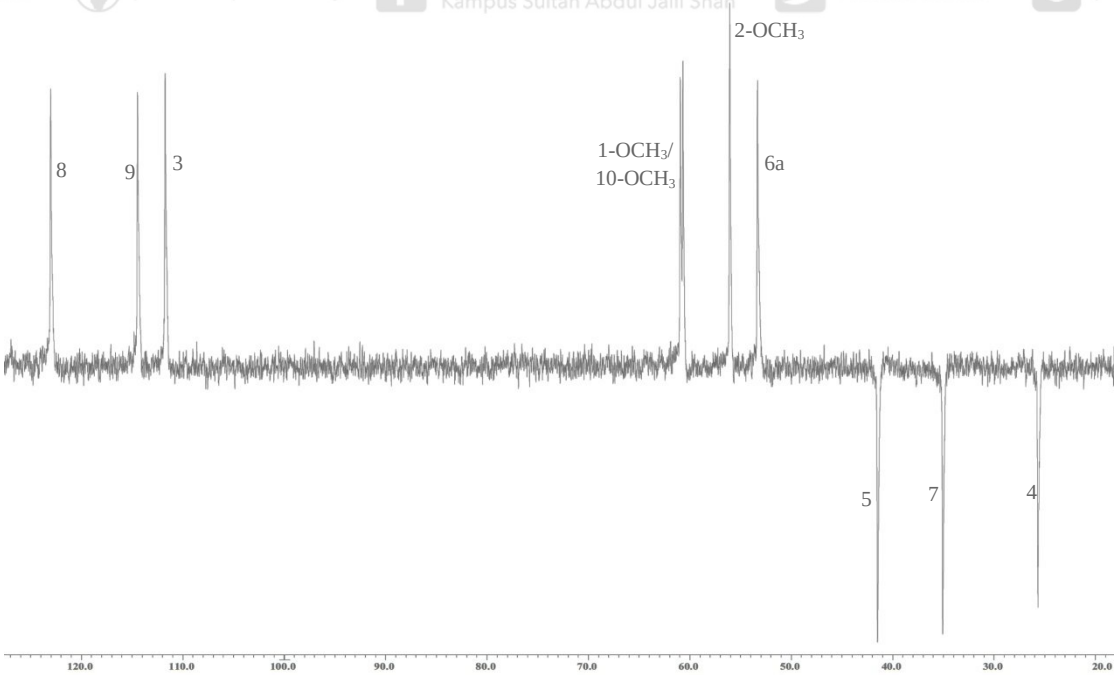


Figure 4.2.1.3: DEPT-135 NMR Spectrum of APB 1

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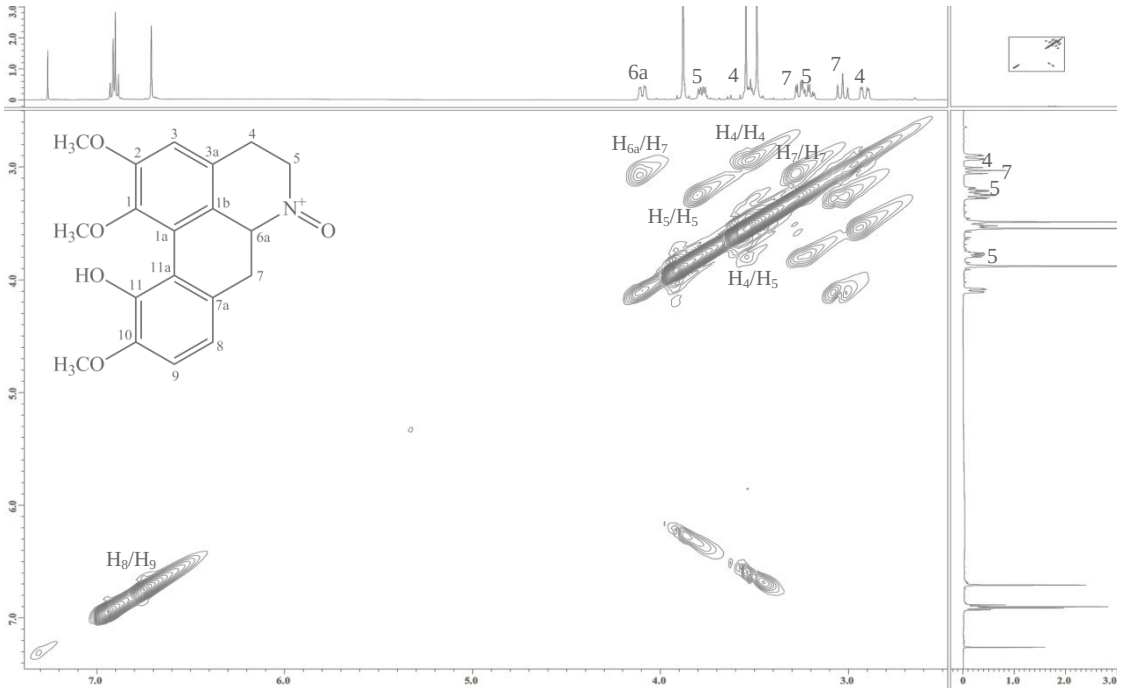


Figure 4.2.1.4: COSY NMR Spectrum of APB 1

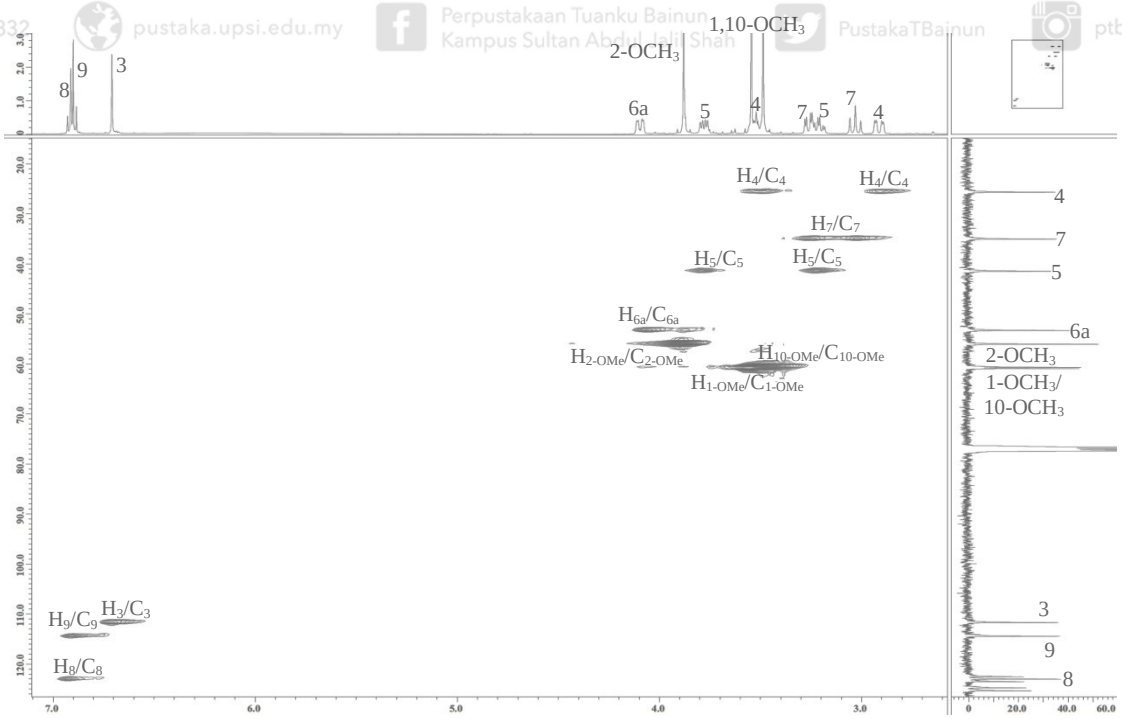


Figure 4.2.1.5: HSQC NMR Spectrum of APB 1

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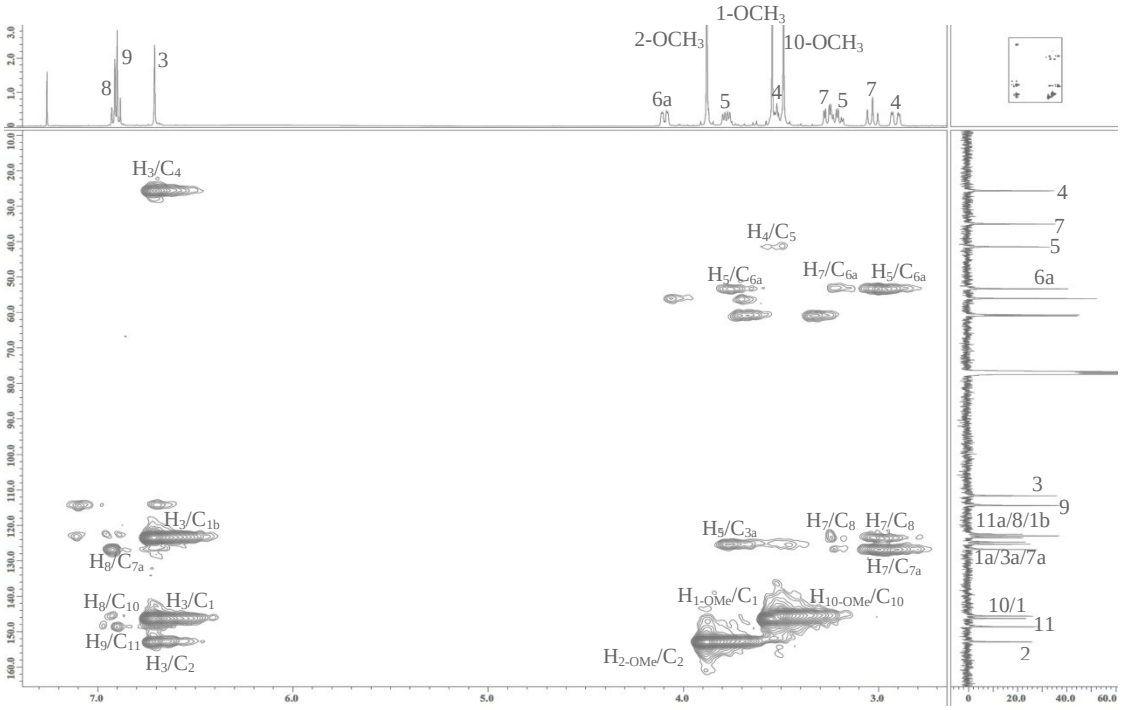


Figure 4.2.1.6: HMBC NMR Spectrum of APB 1

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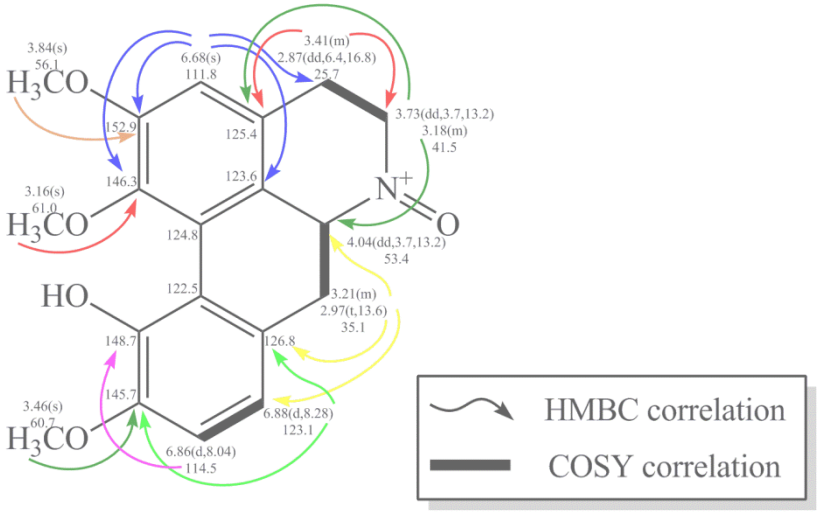


Figure 4.2.1.7: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC Spectrum of APB 1

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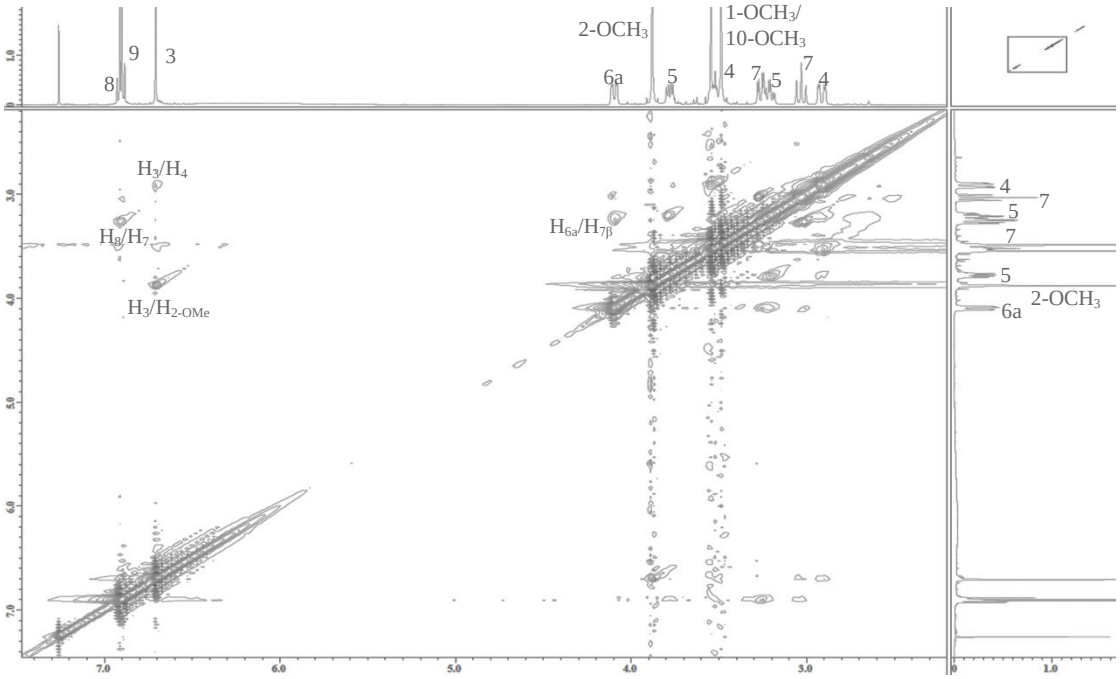


Figure 4.2.1.8: NOESY NMR Spectrum of APB 1

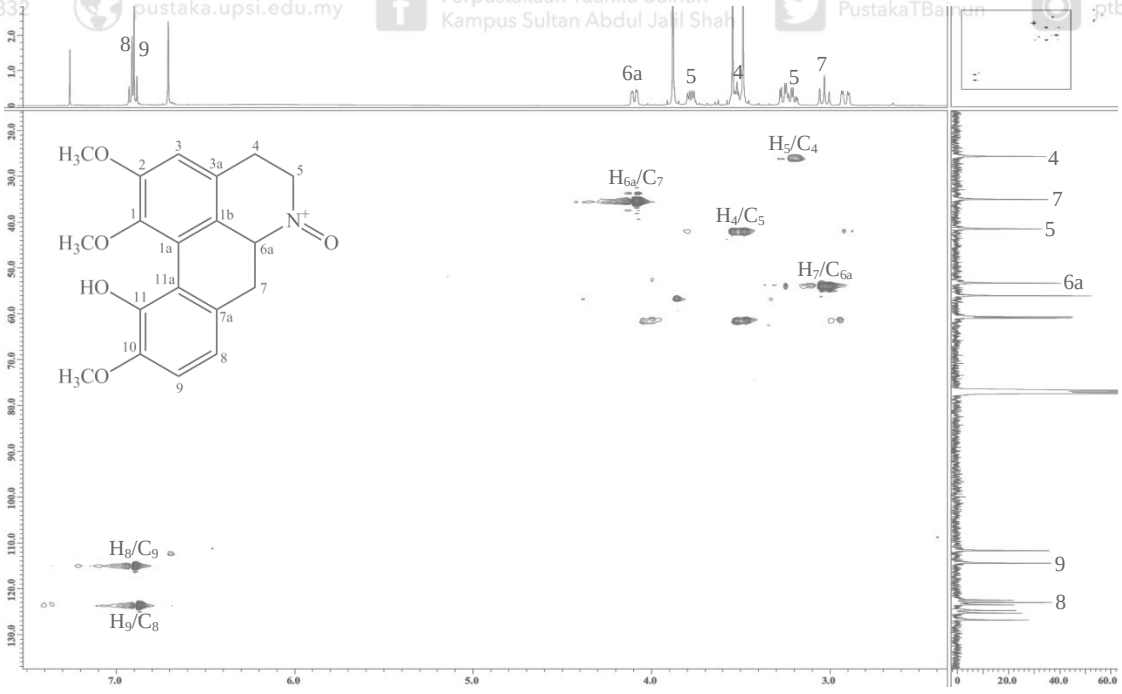


Figure 4.2.1.9: H2BC NMR Spectrum of APB 1

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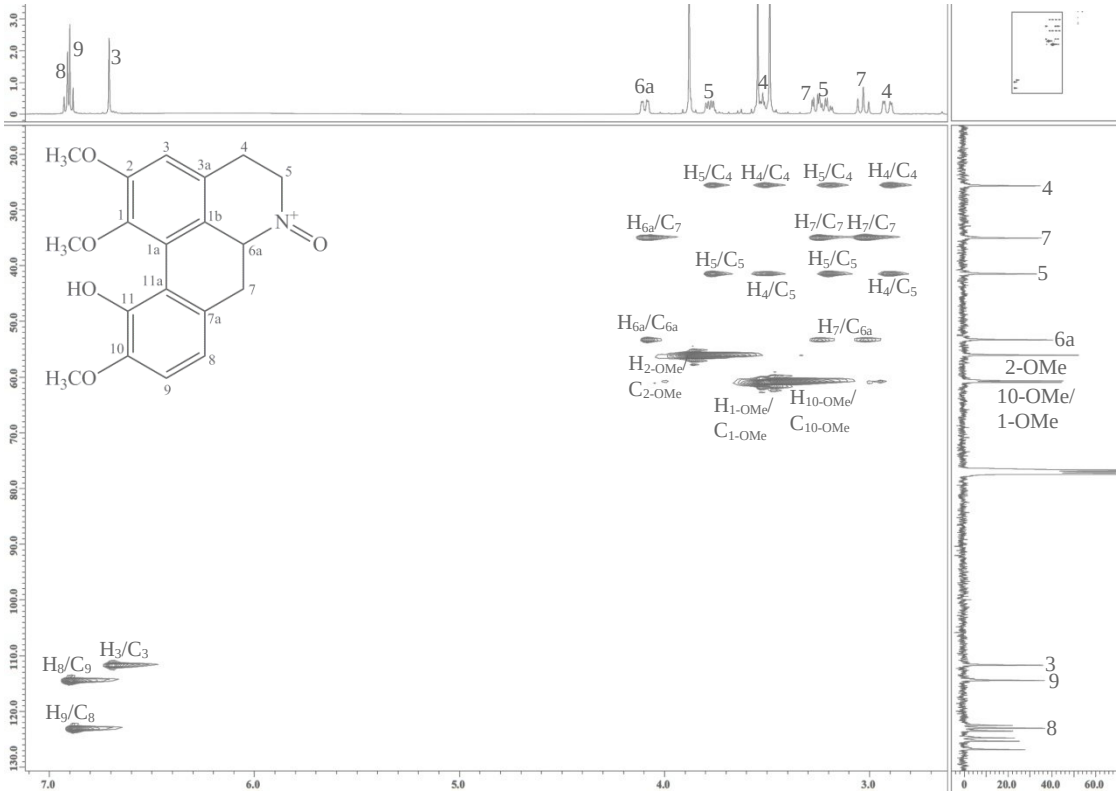
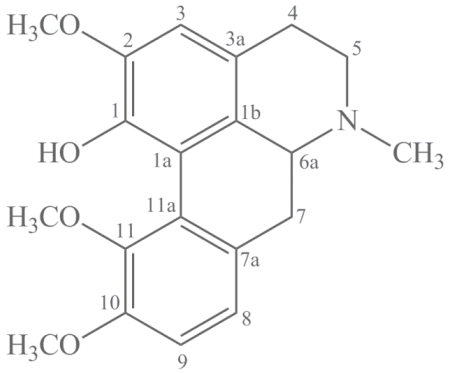


Figure 4.2.1.10: HSQC-TOCSY NMR Spectrum of APB 1

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4.2.2 APB 2, Corydine 124



124

Alkaloids APB 2 was obtained as a dark brown amorphous with $[\alpha]_D^{25}$ +204° (*c* = 0.6, EtOH) (Shamma & Salgar, 1973). The IR spectrum showed a peak at 3529.1 cm^{-1} typical of the stretching of a hydroxyl group. Another significant peak was also observed at 3019.1 cm^{-1} which indicated the presence of the C-H aromatic in the compound (Duddley & Ian, 1989; Silverstein & Webster, 1998). The UV spectrum display maxima absorption at 219, 271 and 307 nm (log ε = 4.50, 4.11 and 3.84, respectively) thus suggesting a 1,2,10,11-tetrasubstituted aporphine skeleton (Pyo, Yun-Choi, & Hong, 2003). The HRESIMS showed molecular ion $[M+H]^+$ peak at m/z 342.17272 (calc. 342.17053) corresponding to the molecular formula of $C_{20}H_{23}NO_4$.

A total of 15 protons signals were observed in ^1H NMR spectrum (Figure 4.2.2.1). Six methylene protons signals appeared between δ 3.16 – 2.42 which were assigned to the protons at C-4, C-5 and C-7, respectively. The signal for one methine protons at δ 2.96 (*d, J*=13.2 Hz) was deduced to be H-6a. Three strong signals were observed at δ 3.38, δ 3.89 and δ 3.71 corresponding to three methoxyl signals of 2-

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OCH_3, 10-OCH_3 and 11-OCH_3, respectively. Another strong singlet signal at high field; δ 2.54 attributed to *N*-methyl proton. H-8 and H-9 resonated as a pair of doublets at δ 7.07 and δ 6.86 with the vicinal coupling constant of 8.0 Hz and other one singlet aromatic proton signals at δ 6.67 was assigned to H-3. The most deshielded signals belongs to hydroxyl proton (1-OH) at δ 8.70 (*br*, *s*).

According to ^{13}C-NMR spectrum (Figure 4.2.2.2), indicated the presence of 20 carbon resonances; nine quaternary carbons: δ 142.3 (C-1), δ 123.8 (C-1a), δ 127.8 (C-1b), δ 149.2 (C-2), δ 119.2 (C-3a), δ 130.6 (C-7a), δ 151.8 (C-10), δ 143.8 (C-11) and δ 126.4 (C-11a); three aromatic carbons; δ 111.3 (C-3), δ 124.4 (C-8) and δ 110.9 (C-9); three methoxyl carbons: δ 56.0 (2-OCH_3), δ 56.0 (10-OCH_3) and δ 62.7 (11-OCH_3); three methylene carbons δ 28.8 (C-4), δ 52.7 (C-5) and δ 35.4 (C-7); a *N*-methyl carbon: δ 43.8 (*N*-CH_3) and one methine carbon δ 62.0 (C-6a).

The position of these carbon resonances were supported by ^{1}H-^{1}H COSY (Figure 4.2.2.3), HMQC (Figure 4.2.2.4) and HMBC (Figure 4.2.2.5). The COSY spectrum suggested fragments of C-8 – C-9, C-4 – C-5 and C-6a – C-7. The HMBC spectrum showed long-range correlations between the following protons and carbons: H-3 with C-4, C-3a, C-1b, C-1 and C-2; H-4 with C-5, C-3 and C-1b; H-7 with C-6a, C-8, C-1b and C-7a; H-8 with C-9, C-11a, C-10, C-6a, C-7; H-9 with C-7a, C-11, C-10; H-6a with C-5. The positions of methoxyl groups were determined to be at C-2, C-10 and C-11 through long-range correlations from protons at 2-OCH_3 to C-2, proton at 10-OCH_3 to C-10 and proton at 11-OCH_3 to C11. Other correlation were also observed (Figured 4.2.2.6), which determined the assignment of all the ^{13}C signals of compound APB 2 and full NMR assignments were shown in Table 4.2.2.1. Detailed

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analysis of the spectral data obtained led to the conclusion that compound APB 2 is aporphine type alkaloid; corydine **124**, based on comparison with literature review (Table 4.2.2.2).

Corydine **124** is a well known aporphine alkaloids which showed significant inhibitory activity against acetylcholinesterase and butyrylcholinesterase (Adsersen, Kjølbye, Dall, & Jäger, 2007), inhibit human drug metabolizing cytochrome P450 enzymes, selective inhibitors for CYP3A4 (Salminen et al., 2011), antiamoebic activity (Ramírez-Galicia, Martínez-Pacheco, Garduño-Juárez, & Deeb, 2012), anticancer activity (Bhakuni, Tewari, & Dhar, 1972) and antiplasmodial activity (Kaur, Jain, Kaur, & Jain, 2009), but it inactive for fungitoxic activity (Ma, Fukushi, Tahara, & Osawa, 2000).

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This compound reported to be found in over 20 plant species; *Aconitum orientale* (Ulubelen, Mericli, Mericli, & Yilmaz, 1996), *Annona squamosa* (Bhakuni et al., 1972; Leboeuf, Cave, Bhaumik, Mukherjee, & Mukherjee, 1982), *Aristolochia lagesiana* (Ferreira et al., 2010), *Berberis actinacantha* (Valencia, Patra, Freyer, & Shamma, 1984), *Cissampelos fasciculate* (Galinis, Wiemer, & Cazin, 1993), *Corydalis cava* (Adsersen et al., 2007; Meyer & Imming, 2008; Salminen et al., 2011; Sturm, Seger, Godejohann, Spraul, & Stuppner, 2007; Sturm, Seger, & Stuppner, 2007), *Dactylicapnos scandens* (Wang et al., 2011), *Dicentra spectabilis* (Ma et al., 2000), *Dicranostigma leptopodum* (Dang et al., 2009), *Fagara tinguassoiba* (Baarschers & Pachler, 1965), *Glaucium corniculatum* (Al-wakeel, Moubasher, & Roberts, 1995), *Glaucium flavum* (Daskalova, Iskrenova, Kiryakov, & Evstatieva, 1988), *Glaucium grandiflorum* (Morteza-Semnani, Saeedi, & Hamidian, 2004;

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Morteza-Semnani, Saeedi, Hamidian, Vafamehr, & Dehpour, 2002), *Glaucium oxylobum* (Fishbein & Falk, 1969), *Lindera myrrha* (Phan, Seguin, Tillequin, & Koch, 1994), *Monodora crispate* (Kablan et al., 2013), *Ocotea Vellosiana* (Garcez, Yoshida, & Gottlieb, 1995), *Stephania dinklagei* (Fishbein & Falk, 1969; Semwal et al., 2010; Thornber, 1970), *Stephania yunnanensis* (Dai, Hu, Sun, & Pan, 2012; Hu, Dai, Lu, & Pan, 2010), *Thalictrum dioicum* (Shamma & Salgar, 1973), *Xylopia danguyella* (Leboeuf et al., 1982) and *Zanthoxylum oxyphyllum* (Tiwari & Masood, 1978). Corydine **124** also can be found in family of Annonaceae (genus Xylopia), Papaveraceae (genus Corydalis, Dicranostigma and Glaucium), Ranunculaceae (genus Thalictrum) and Rutaceae (genus Zanthoxylum) (Guinaudeau et al., 1979)

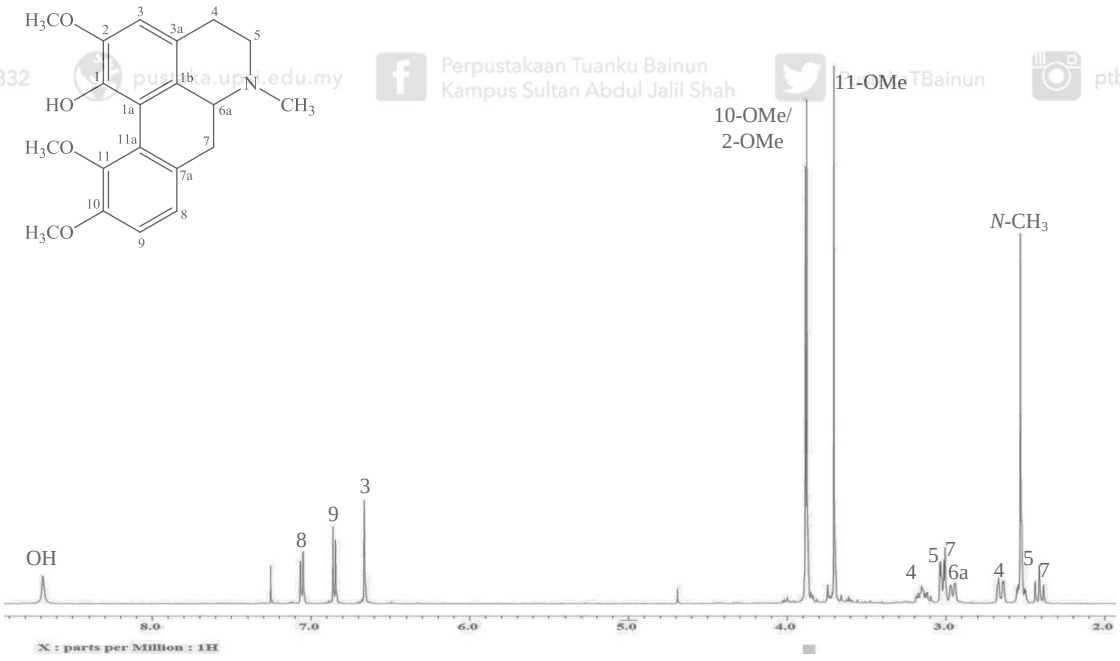


Figure 4.2.2.1: ^{1}H NMR Spectrum of APB 2

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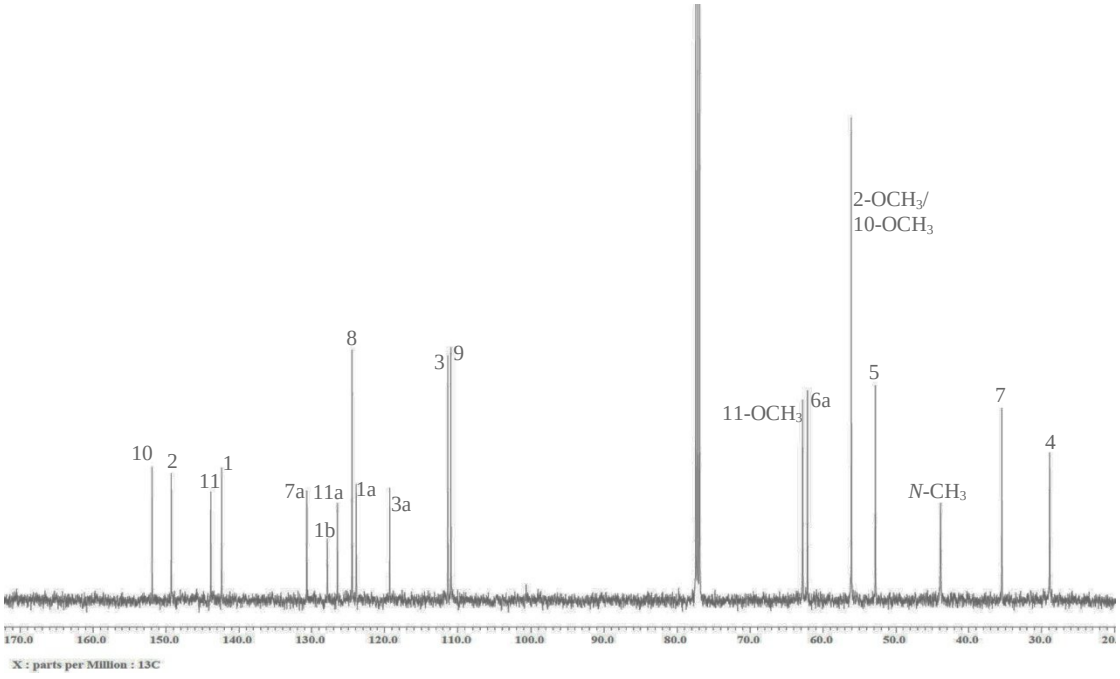


Figure 4.2.2.2: ^{13}C NMR Spectrum of APB 2

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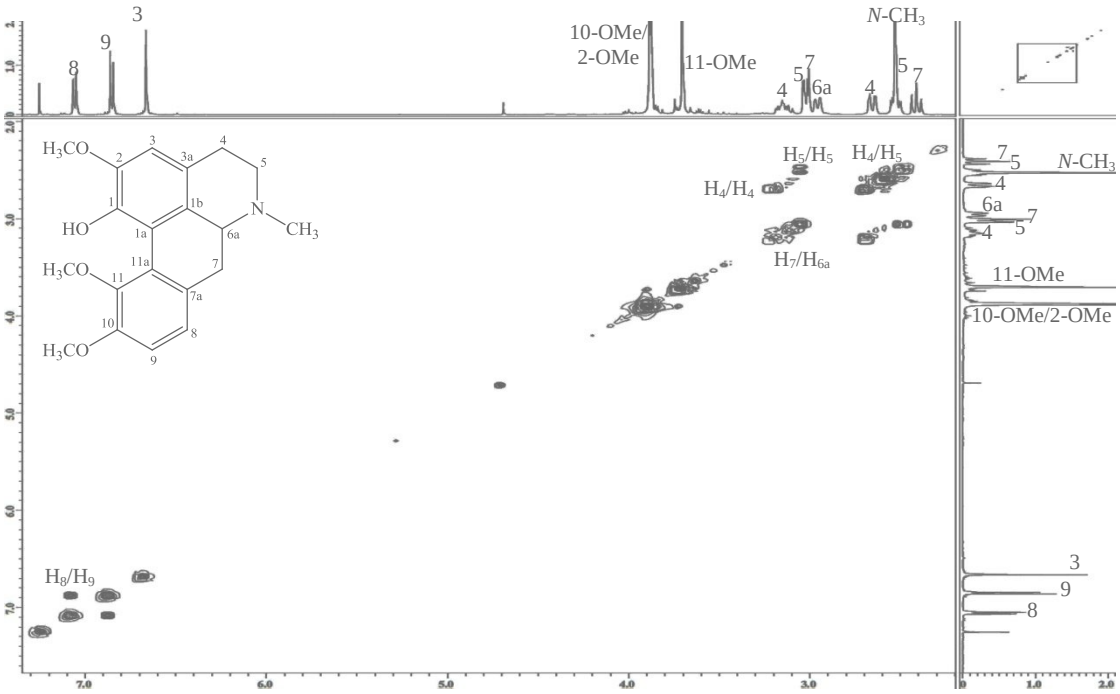


Figure 4.2.2.3: COSY NMR Spectrum of APB 2

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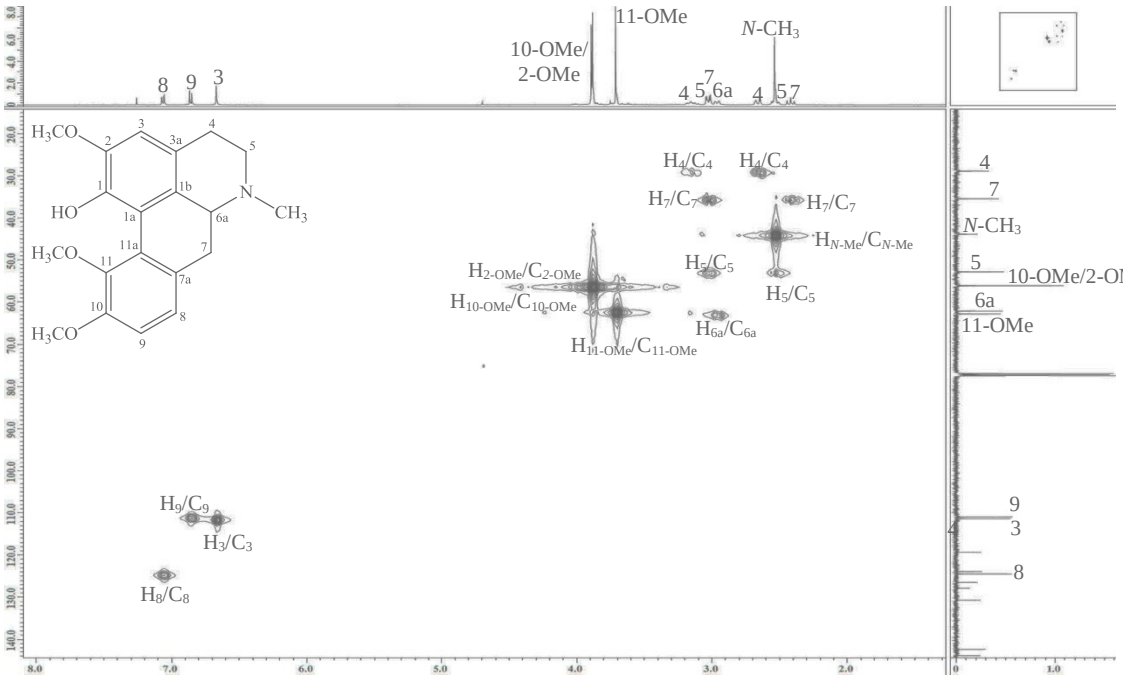


Figure 4.2.2.4: HMQC NMR Spectrum of APB 2

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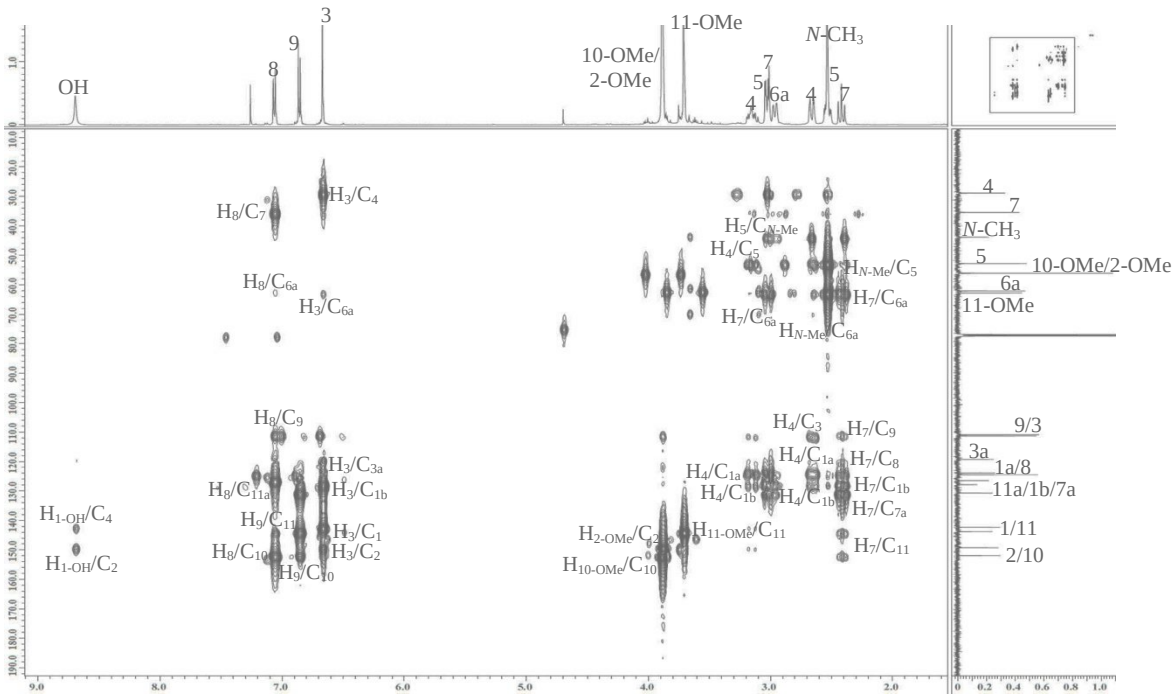


Figure 4.2.2.5: HMBC NMR Spectrum of APB 2

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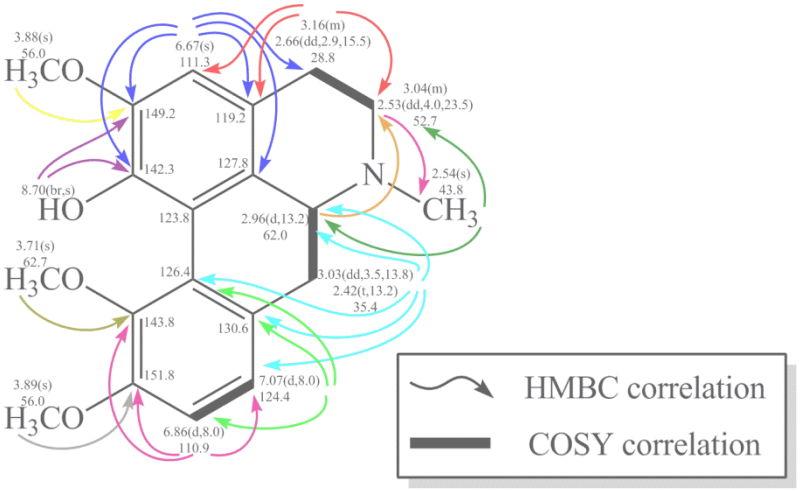


Figure 4.2.2.6: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 2

Table 4.2.2.1:

1D (^{1}H and ^{13}C) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 2

Position	^{1}H δ, $CDCl_3$ (*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HMQC	HMBC
1		142.3			
1-OH	8.70 (*br,s*)			$H_{1\text{-OH}}$	C_4,C_2
1a		123.8			
1b		127.8			
2		149.2			
2-OCH_3	3.88 (*s*)	56.0		$3H_{2\text{-OMe}}$	C_2
3	6.67 (*s*)	111.3		H_3	C_4,C_{6a},C_{3a},C_{1b},C_1,C_2
3a		119.2			
4	3.16 (*m*) 2.66 (*dd*,2.9,15.5)	28.8	H_4, H_5	H_4	C_5,C_3,C_{1b},C_{1a}
5	3.04 (*m*) 2.53 (*dd*,4.0,23.5)	52.7	H_5, H_4	H_5	$C_{N\text{-Me}}$
N-CH_3	2.54 (*s*)	43.8		$3H_{N\text{-Me}}$	C_5,C_{6a}
6a	2.96 (*d*,13.2)	62.0	7	H_{6a}	C_5
7	3.03 (*dd*,3.5,13.8) 2.42 (*t*,13.2)	35.4	6a	H_7	C_{6a},C_8,C_{1b}, C_{7a},C_9,C_{11}
7a		130.6			
8	7.07 (*d*,8.0)	124.4	H_9	H_8	C_9,C_{11a},C_{10},C_{6a},C_7
9	6.86 (*d*,8.0)	110.9	H_8	H_9	C_{7a},C_{11},C_{10}
10		151.8			
10-OCH_3	3.89 (*s*)	56.0		$3H_{10\text{-OMe}}$	C_{10}
11		143.8			
11-OCH_3	3.71 (*s*)	62.7		$3H_{11\text{-OMe}}$	C_{11}
11a		126.4			

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Table 4.2.2.2:

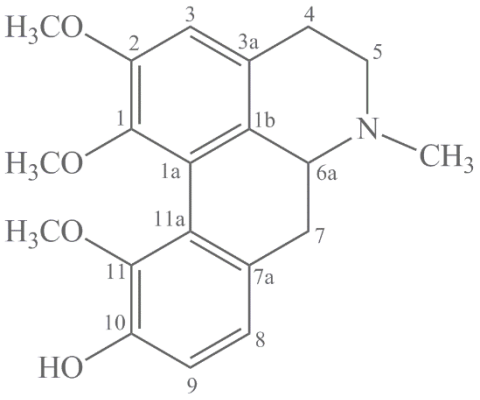
*^{1}H and ^{13}C NMR Spectral Data Comparison of APB 2, with Corydine **124** from Literature*

	^{1}H δ, $CDCl_3$ (*J*,Hz)		^{13}C (δ,$CDCl_3$)	
Position	APB 2	Corydine **124** (Ferreira et al., 2010)	APB 2	Corydine **124** (Ferreira et al., 2010)
1			142.3	142.3
1-OH	8.70 (*br*,*s*)	8.61 (*s*)		
1a			123.8	126.5
1b			127.8	128.2
2			149.2	149.1
2-OCH_3	3.88 (*s*)	3.84 (*s*)	56.0	56.0
3	6.67 (*s*)	6.61 (*s*)	111.3	111.3
3a			119.2	124.0
4	3.16 (*m*)	3.08 (*ddd*,16.0,12.0,6.0)	28.8	29.1
	2.66 (*dd*,2.9,15.5)	2.59 (*dd*,16.0,3.9)		
5	3.04 (*m*)	2.93 (*ddd*,11.2,6.0,1.3)	52.7	52.8
	2.53 (*dd*,4.0,23.5)	2.43 (*ddd*,12.0,11.2,3.9)		
N-CH_3	2.54 (*s*)	2.46 (*s*)	43.8	44.0
6a	2.96 (*d*,13.2)	2.85 (*ddd*,13.7,3.5,1.3)	62.0	62.8
7	3.03 (*dd*,3.5,13.8)	2.96 (*dd*,12.8, 3.5)	35.4	35.6
	2.42 (*t*,13.2)	2.33 (*ddd*,13.7,12.8,1.0)		
7a			130.6	130.9
8	7.07 (*d*,8.0)	7.01 (*d*,8.0)	124.4	124.3
9	6.86 (*d*,8.0)	6.80 (*d*,8.0)	110.9	110.8
10			151.8	151.8
10-OCH_3	3.89 (*s*)	3.83 (*s*)	56.0	56.0
11			143.8	143.8
11-OCH_3	3.71 (*s*)	3.66 (*s*)	62.7	62.0
11a			126.4	119.3

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4.2.3 APB 3, *N*-methylhernagine 169



169

Alkaloids APB 3 was isolated as brown amorphous solid with $[\alpha]_D^{25}$ +144° ($CHCl_3$) (Yakushijin et al., 1980). The alkaloid has the molecular formula of $C_{20}H_{23}NO_4$ as deduced by HRESIMS (m/z 342.17122 $[M+H]^+$; calc. 342.17053). The UV spectrum showed the absorption maxima at 222, 270 and 301nm (log ε = 4.50, 4.13 and 3.75, respectively) indicating the presence of 1, 2, 10, 11 substituted aporphine (Nozaka et al., 1990). IR spectrum revealed absorption at 3018.5 and 1464.8 cm^{-1} due to C-H aromatic stretching and aromatic system. The other absorption peaks at 3515.7 and 1213.0 cm^{-1} indicated the presence of hydroxyl group and C-O-C stretching in the structure (Donald, Gary, & George, 2001; Silverstein & Webster, 1998).

The ^{1}H NMR spectrum (Figure 4.2.3.1) showed a *N*-methyl singlet at δ 2.53 due to *N*-CH_3 protons. The signals for three methoxyl singlet appeared at δ 3.49, 3.87 and 3.52 corresponding to H-1, H-2 and H-11, respectively. In addition, H-4 and H-5 appeared as multiplet signals at δ 3.15 and 2.52, respectively. While a pair of doublet signals appeared in aromatic region at δ 6.90 and 6.86 with the vicinal coupling

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constant of 10.0 Hz corresponding to adjacent proton at H-8 and H-9, while another doublet signal for H-4 aliphatic at δ 3.04 (*J*=6.3Hz). A singlet signal also appeared at δ 6.68 corresponding to aromatic proton at position C-3, while a triplet signal at position C-7 was appeared at δ 2.37 (*J*=13.2 Hz). The rest of aliphatic signals appeared as doublet of doublet at δ 2.70 (*J*=3.5, 16.1 Hz), δ 2.90 (*J*=1.2, 12.6 Hz) and δ 3.01(*J*=4.0,13.8 Hz) that attached at position C-4, C-6a and C-7 respectively.

The ^{13}C spectrum (Figure 4.2.3.2) and DEPT-135 NMR spectrum (Figure 4.2.3.3) revealed a *N*-methyl carbons signals at δ 44.0 and three methoxyl carbons signals appeared at δ 60.5, 56.1 and 60.9 coressponding to 1-OCH_3, 2-OCH_3 and 11-OCH_3, respectively. The spectrum exhibited signals of three methylenes, one methine and nine quaternary carbons. The methylene carbons signals at δ 28.9, 53.0 and 35.6 were assigned to C-4, C-5 and C-7 respectively, while a methine signal at δ 78.1 was corresponded to C-6a. The aromatic carbon signals for C-3, C-8 and C-9 were appeared at δ 111.7, 122.4 and 113.5, respectively. In addition, the rest of carbon signals are quaternary carbon positioned at C-2, C-10, C-11, C-1, C-1b, C-7a, C-3a, C-1a and C-11a appeared at δ 123.0 – 151.5.

The assignments of these carbon resonances were further supported by ^{1}H-^{1}H COSY (Figure 4.2.3.4), HMQC (Figure 4.2.3.5) and the long range ^{1}H-^{13}C correlations were observed in the HMBC spectra (Figure 4.2.3.6). The ^{1}H-^{1}H COSY spectrum suggested the following fragments: C-4 – C-5, C-6a – C-7 and C-8 – C-9. In HMBC, the exact position of methoxyl groups were confirmed to be at C-1, C-2 and C-11 through long-range correlation from the proton at 1-OCH_3 to C-1, 2-OCH_3 to C-2 and 11-OCH_3 to C-11. HMBC data also important to confirm the position of

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quaternary carbons in ring A; from proton; H-3 to C-1, C-2, and C-1b and also in ring C; from proton H-7 to C-7a, C-3a, C-11 and C-11a. Several other correlations were also observed (Figure 4.2.3.7 and Table 4.2.3.1), which confirm the position of all the ^{13}C signals of compound.

The assignment of the structure of alkaloid APB 3 was confirmed by comparison of ^{1}H NMR data with literature (Table 4.2.3.2) (Guinaudeau et al., 1983). First discovery of this alkaloid from modification of hernagine **123** with formaldehyde and $NaBH_4$ (Yakushijin et al., 1980) while the first isolated *N*-methylhernagine **169** from plants was obtain from Lauraceae family: *Parabenzoin praecox* on 1984 (Guinaudeau et al., 1988).

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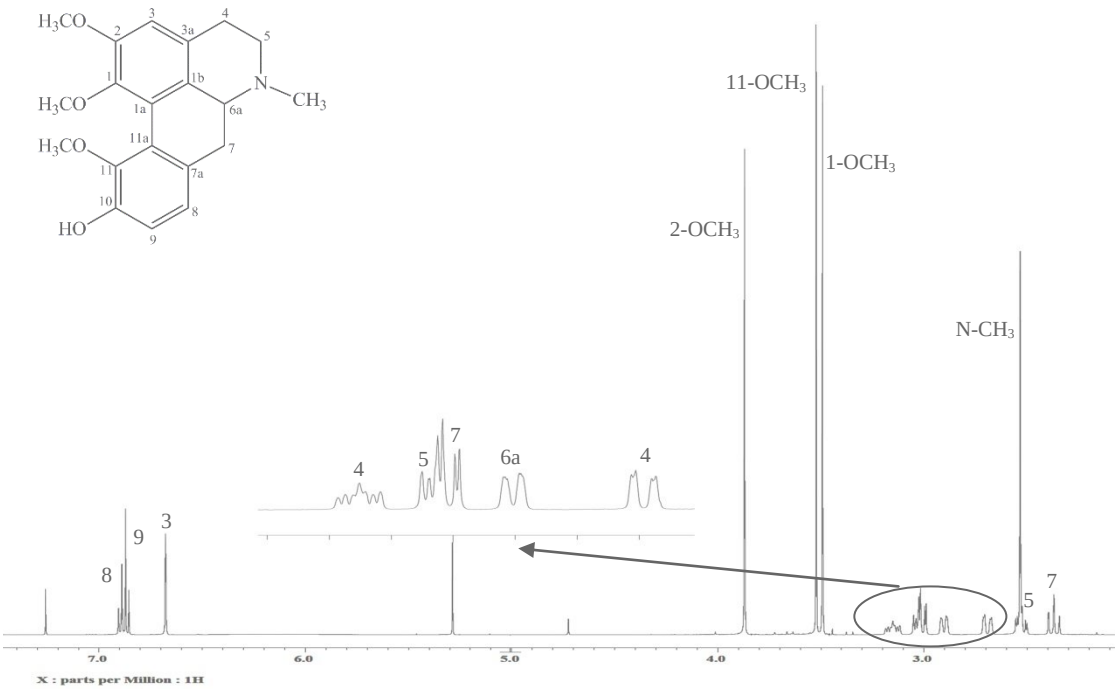


Figure 4.2.3.1: ^{1}H NMR Spectrum of APB 3

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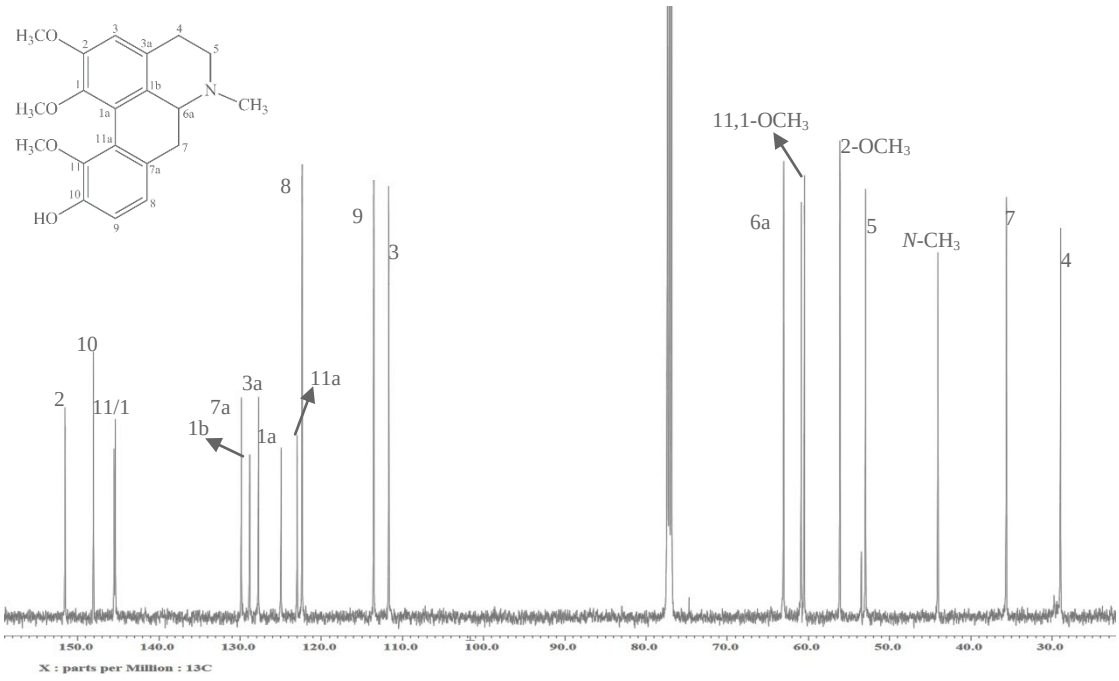


Figure 4.2.3.2: ^{13}C NMR Spectrum of APB 3

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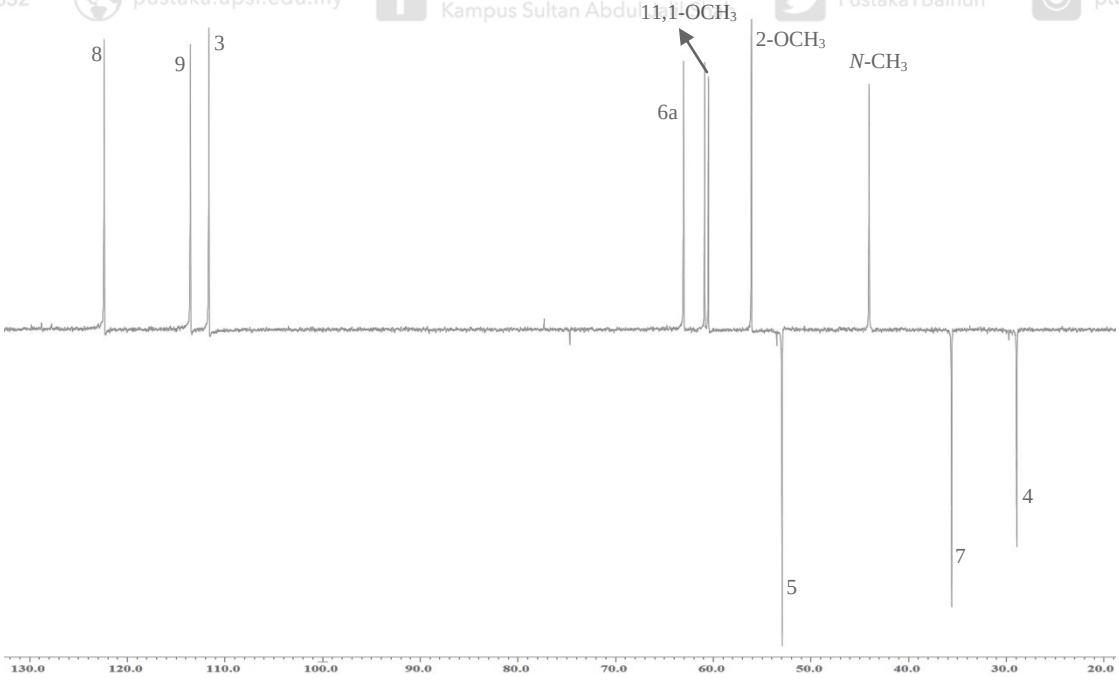


Figure 4.2.3.3: DEPT-135 NMR Spectrum of APB 3

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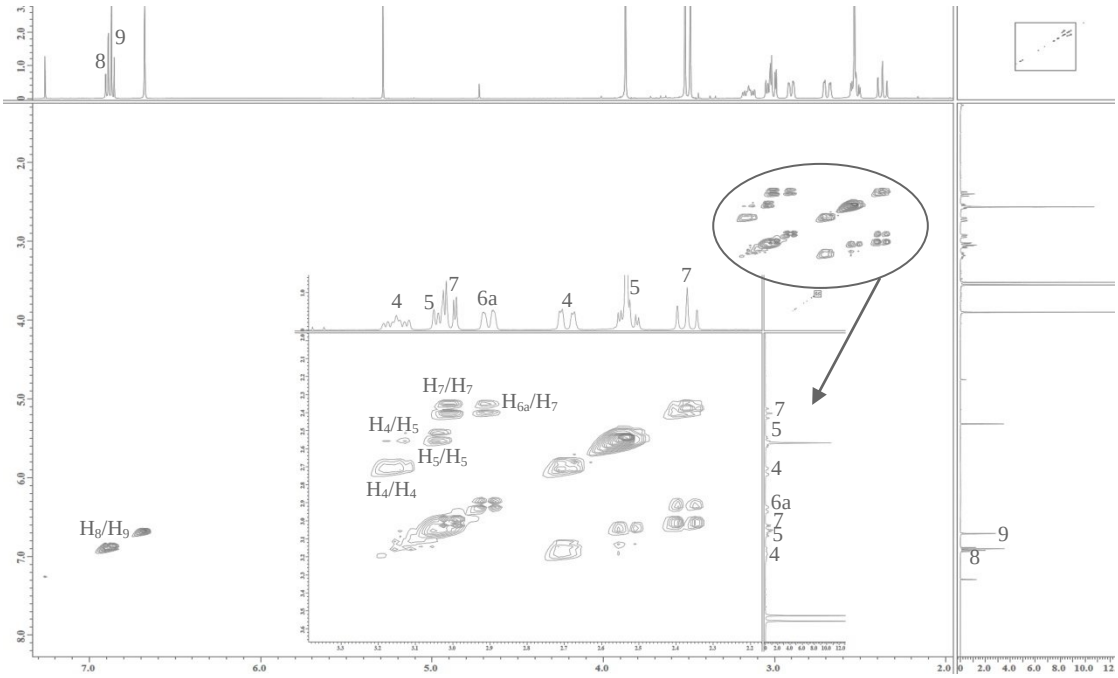


Figure 4.2.3.4: COSY NMR Spectrum of APB 3

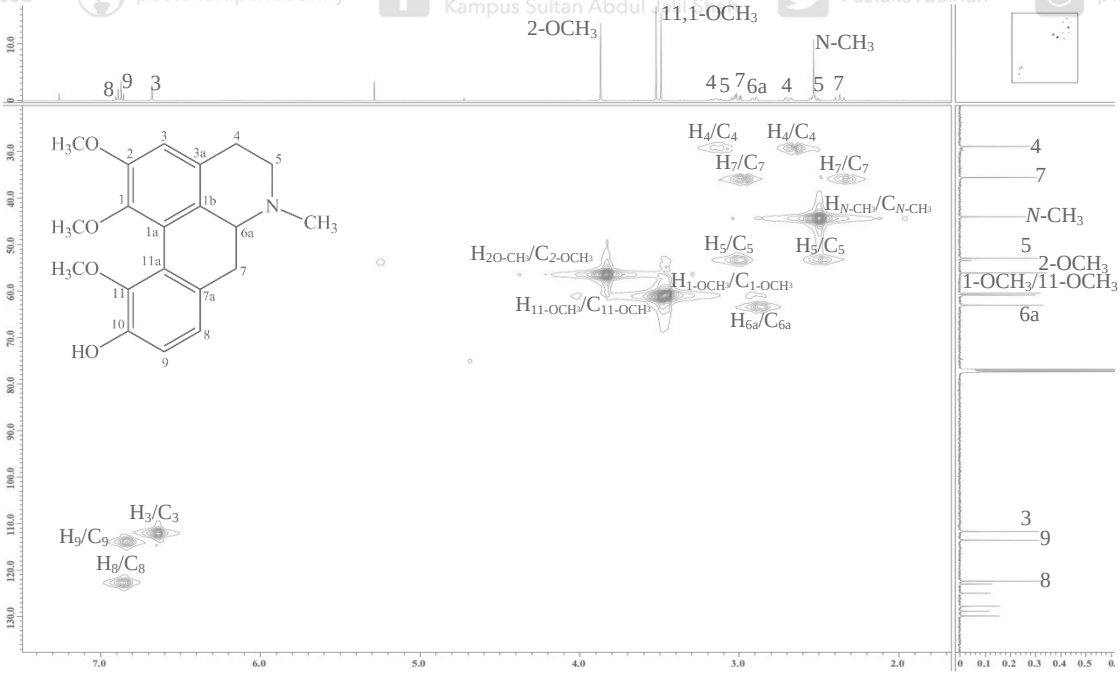


Figure 4.2.3.5: HSQC NMR Spectrum of APB 3

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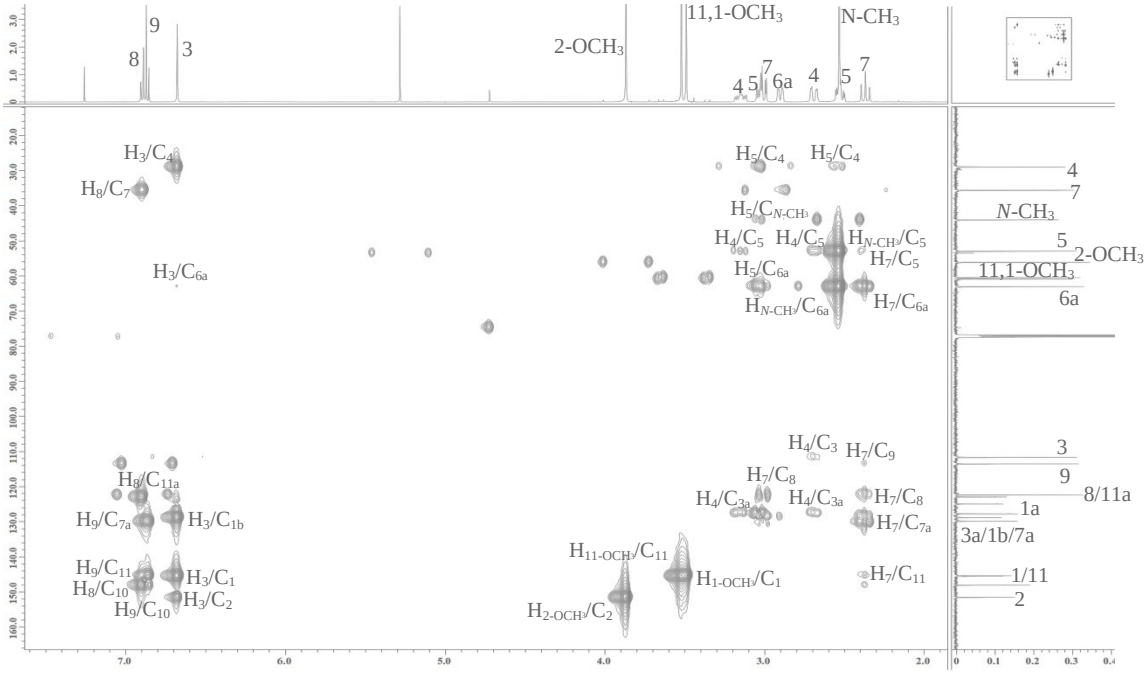


Figure 4.2.3.6: HMBC NMR Spectrum of APB 3

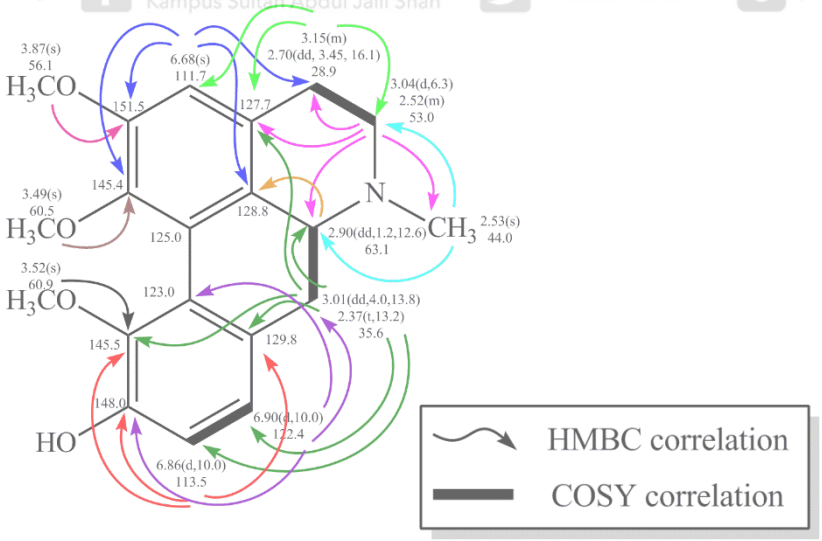


Figure 4.2.3.7: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 3

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Table 4.2.3.1:

1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 3

Position	^{1}H δ, $CDCl_3$ (*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HMQC	HMBC	DEPT-135
1		145.4				C_q
1-OCH_3	3.49(*s*)	60.5		$3H_{1\text{-OMe}}$	C_1	CH_3
1a		125.0				C_q
1b		128.8				C_q
2		151.5				C_q
2-OCH_3	3.87 (*s*)	56.1		$3H_{2\text{-OMe}}$	C_2	CH_3
3	6.68 (*s*)	111.7		H_3	C_1, C_{1b}, C_2, C_4	CH
3a		127.7				C_q
4	3.15 (*m*) 2.70 (*dd*, 3.5,16.1)	28.9	H_4, H_5	H_4	C_3, C_{3a}, C_5	CH_2
5	3.04 (*d*,6.3) 2.52 (*m*)	53.0	H_5, H_4	H_5	C_{3a}, C_4, $C_{N\text{-Me}}$, C_{6a}	CH_2
N-CH_3	2.53 (*s*)	44.0		$3H_{N\text{-Me}}$	C_5, C_{6a}	CH_3
6a	2.90 (*dd*,1.2,12.6)	78.1	H_7	H_{6a}	C_{1b}	CH
7	3.01 (*dd*,4.0,13.8) 2.37 (*t*,13.2)	35.6	H_7, H_{6a}	H_7	C_{3a}, C_{6a}, C_{7a}, C_8, C_9, C_{11}	CH_2
7a		129.8				C_q
8	6.90 (*d*,10.0)	122.4	H_9	H_8	C_7, C_{10}, C_{11a}	CH
9	6.86 (*d*,10.0)	113.5	H_8	H_9	C_{7a}, C_{10}, C_{11}	CH
10		148.0				C_q
11		145.5				C_q
11-OCH_3	3.52 (*s*)	60.9		$3H_{11\text{-OMe}}$	C_{11}	CH_3
11a		123.0				C_q

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Table 4.2.3.2:

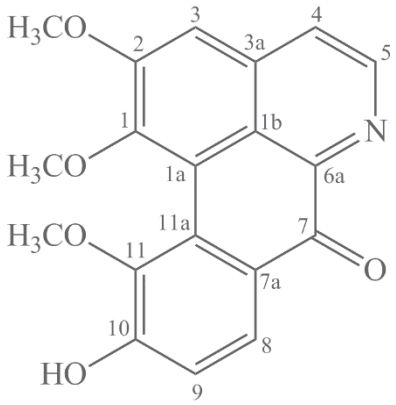
*^{1}H NMR Spectral Data Comparison of APB 3, with N-methylhernagine **169** from Literature (Guinaudeau et al., 1983)*

	^{1}H δ, $CDCl_3$ (*J*,Hz)	
Position	APB 3	*N*-methylhernagine **169** (Guinaudeau et al., 1983)
1-OCH_3	3.49 (*s*)	3.50 (*s*)
2-OCH_3	3.87 (*s*)	3.85 (*s*)
3	6.68 (*s*)	6.65 (*s*)
N-CH_3	2.53 (*s*)	2.55 (*s*)
9	6.86 (*d*,10.0)	6.85 (*s*)
11-OCH_3	3.52 (*s*)	3.51 (*s*)

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4.2.4 APB 4, Oxohernagine 122



122

Alkaloids APB 4 was isolated as a brownish amorphous with $[\alpha]_D^{24}$ ±0° (c = 0.08, $CHCl_3$) (Chen et al., 1996). The UV spectrum revealed absorbane band at 272 and 358 nm (log ε = 4.43 and 4.01, respectively), while the IR spectrum exhibited absorption for C-H aromatic, ketone and aromatic ring at 3019, 1660 and 1521 cm^{-1}, respectively (Duddley & Ian, 1989; Williams & Fleming, 1989). Another significant peak was also observed at 3476 cm^{-1} corresponding to the hydroxyl group in the structure. The HREIMS revealed $[M+H]^+$ peak at m/z 338.10771 (calc. 338.10285) thus suggesting a molecular formula of $C_{19}H_{15}NO_5$.

The ^1H NMR (Figure 4.2.4.1) spectrum showed a singlet at δ 7.20 corresponding to H-3. It also showed two doublet signals at δ 7.21 and 8.26 with the coupling constant of 8.0 Hz corresponding to the proton at aromatic region. The former was due to H-9 and the latter was assigned to H-8, respectively. For the H-4 and H-5, it gave a characteristic AB doublet at δ 7.75 and 8.83 with the coupling

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constant of 4.0 Hz. The small *J* value is due to the adjacent to the electronegative atom, nitrogen. It also showed three singlet signals attributed to three methoxyl groups at δ 4.09, 3.75 and 3.53 which were attached to C-1, C-2 and C-11, respectively. Besides that in APB 4 there are no signals for the aliphatic proton.

Aiding by ^{13}C (Figure 4.2.4.2) and DEPT NMR (Figure 4.2.4.3) spectra data, the data revealed three methoxyl signals at δ 56.5, 62.4 and 60.7 corresponding to methoxyl groups that attach to carbons C-1, C-2 and C-11. A signal at most downfielded positions δ 181.6 reconfirm the oxoaporphine skeleton and its proven that position C-7 was carbonyl group. That data also suggested that the presents of nineteen carbons with eleven quaternary carbons, three methoxyl and the rest are sp^2 carbons, indicating that it is was unsaturated and fully aromatic structure.

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The positions of these carbons resonances were further supported by COSY (Figure 4.2.4.4), HSQC (Figure 4.2.4.5) and HMBC (Figure 4.2.4.6) spectra. The COSY spectrum was made possible for the assignment of group of aromatic protons at position C-4 – C-5 and C-8 – C-9. The HSQC spectrum revealed the corresponding short range heteronuclear coupling between each of carbon atom and protons attached to it. On the other hand, the HMBC spectrum indicated long-range coupling between carbons and its surrounding proton atoms. The H-8 showed interaction with C-7, C-7a, C-10 and C-11a, while H-3 coupled with C-1, C-2 and C-4. The complete ^{13}C and ^{1}H coupling patterns of compound APB 4 was summarized in Table 4.2.4.1 and some examples of such interaction is showed in Figure 4.2.4.7.

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On the basis of above spectral data and comparison with literature data (Table 4.2.4.2), APB 4 was assigned as oxohernagine **122** previously isolated from *Hernandia nymphaeifolia* (Chen et al., 1996).

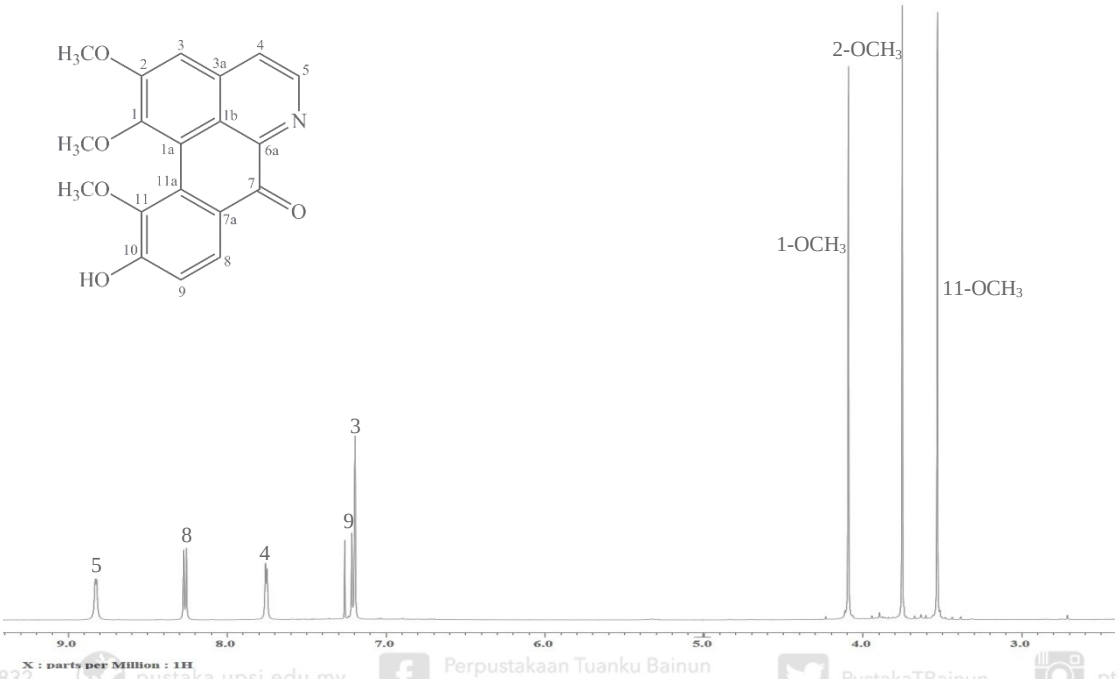


Figure 4.2.4.1: ^{1}H NMR Spectrum of APB 4

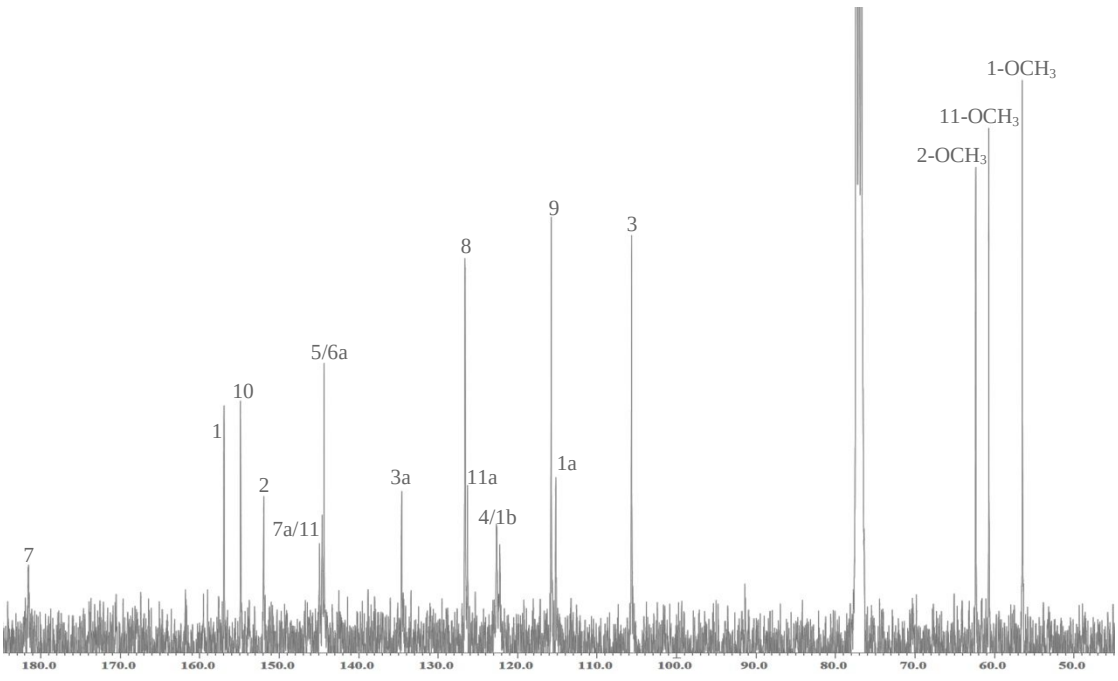


Figure 4.2.4.2: ^{13}C NMR Spectrum of APB 4

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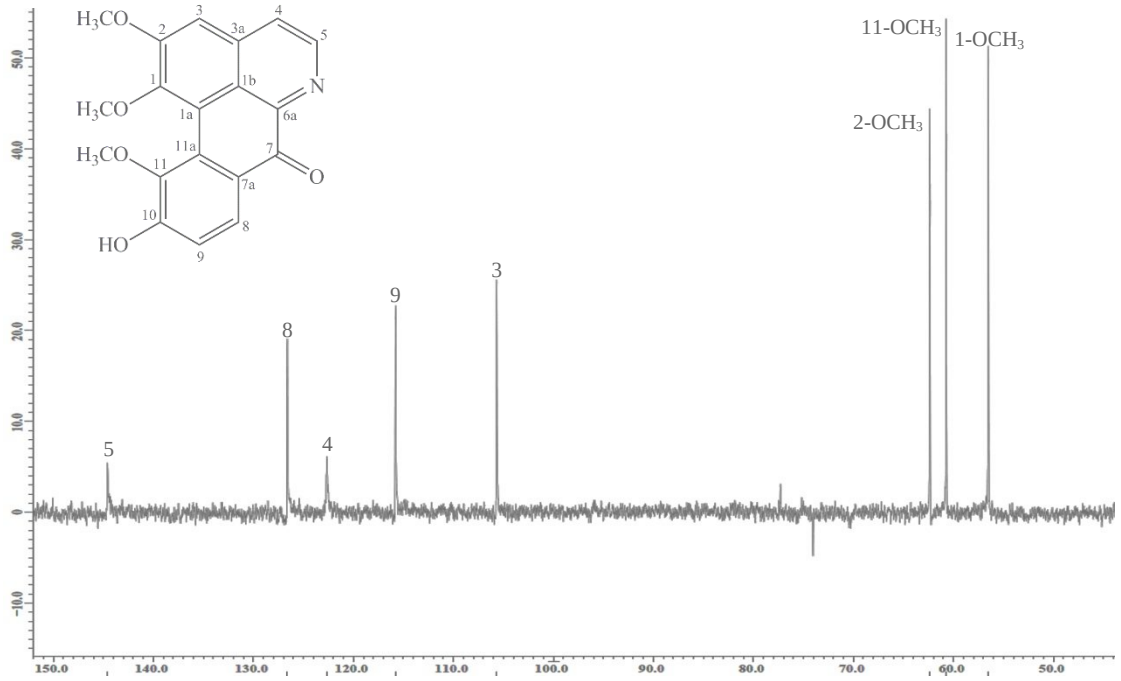


Figure 4.2.4.3: DEPT-135 NMR Spectrum of APB 4

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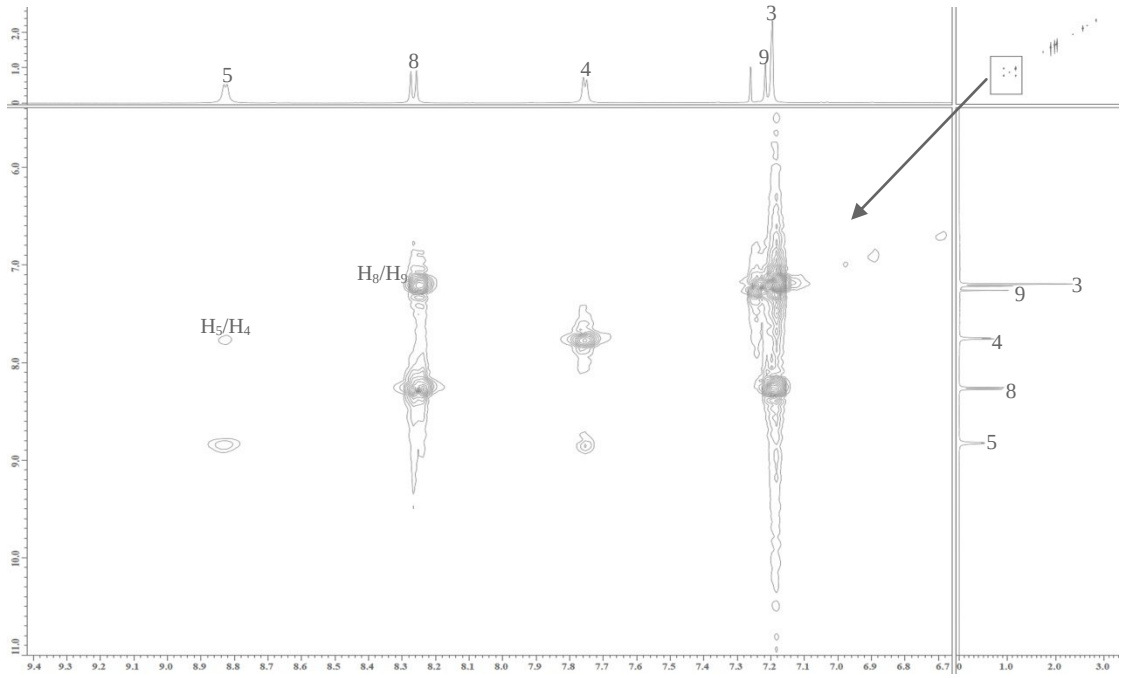


Figure 4.2.4.4: COSY NMR Spectrum of APB 4

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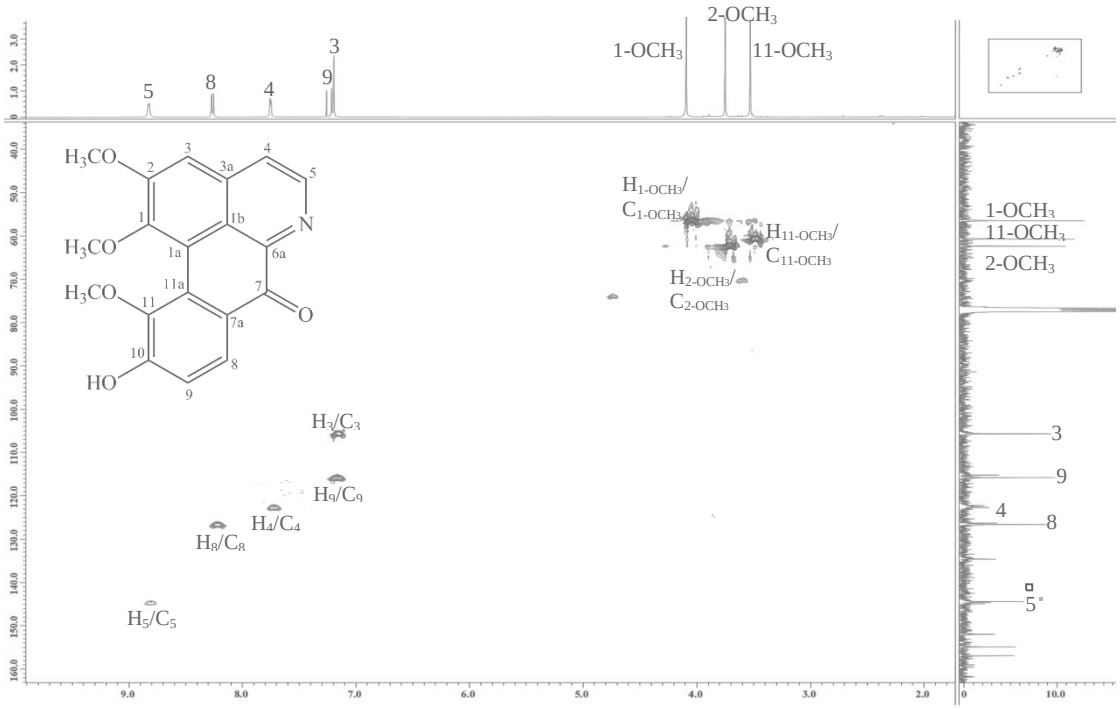


Figure 4.2.4.5: HSQC NMR Spectrum of APB 4

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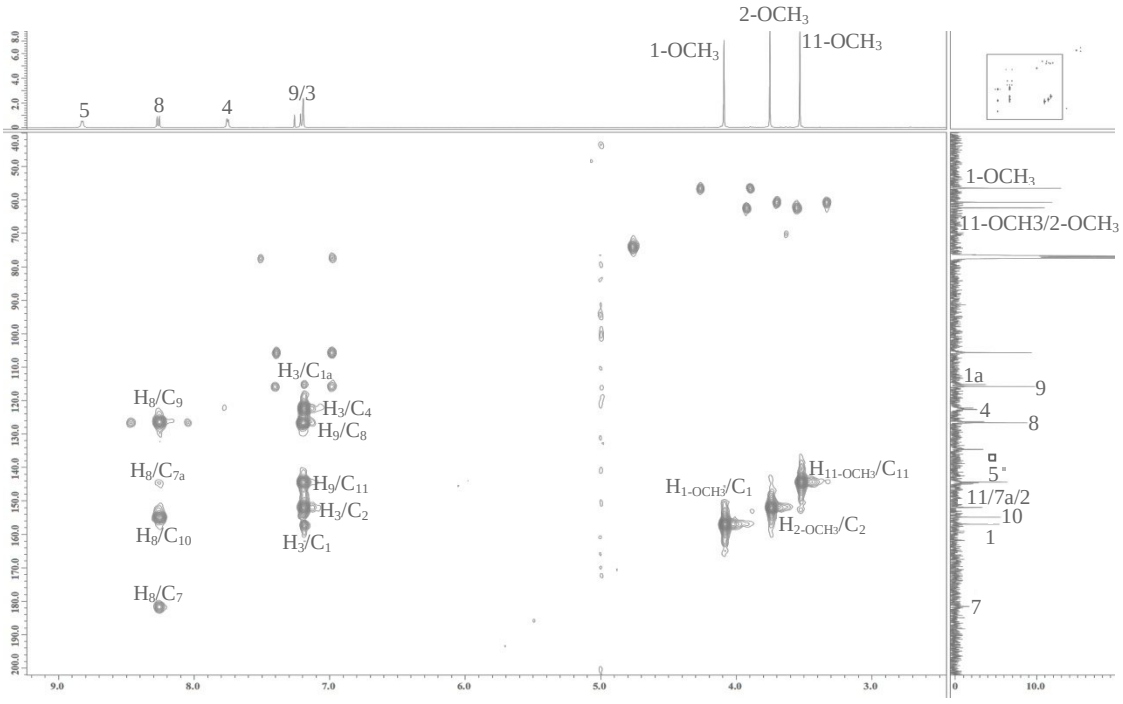


Figure 4.2.4.6: HMBC NMR Spectrum of APB 4

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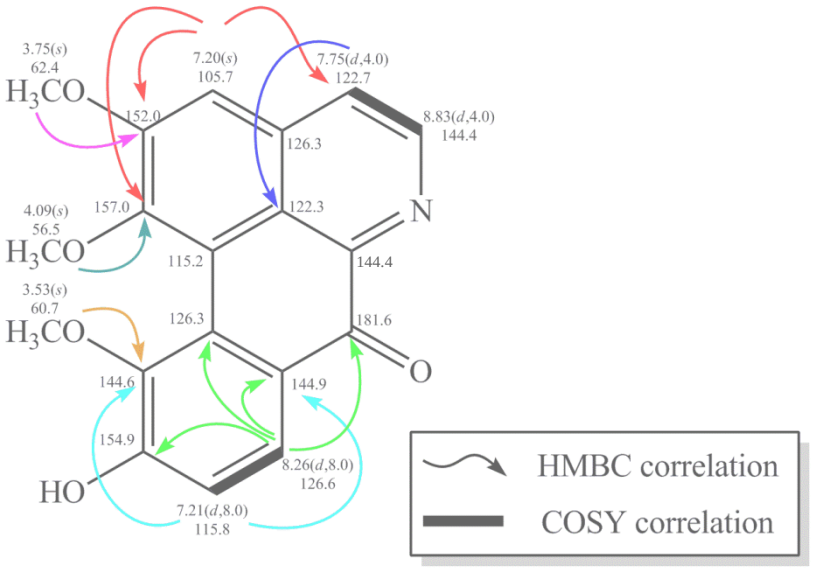


Figure 4.2.4.7: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 4

Table 4.2.4.1:

1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 4

Position	^{1}H δ, $CDCl_3$ (*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HSQC	HMBC	DEPT-135
1		157.0				C_q
1-OCH_3	4.09 (*s*)	56.5		$3H_{1\text{-OMe}}$	C_1	CH_3
1a		115.2				C_q
1b		122.3				C_q
2		152.0				C_q
2-OCH_3	3.75 (*s*)	62.4		$3H_{2\text{-OMe}}$	C_2	CH_3
3	7.20 (*s*)	105.7		H_3	C_1,C_2,C_4	CH
3a		134.6				C_q
4	7.75 (*d*,4.0)	122.7	H_5	H_4	C_{1b}	CH
5	8.83 (*d*,4.0)	144.4	H_4	H_5		CH
6a		144.4				C_q
7		181.6				C_q
7a		144.9				C_q
8	8.26 (*d*,8.0)	126.6	H_9	H_8	C_7,C_{7a},C_{10},C_{11a}	CH
9	7.21 (*d*,8.0)	115.8	H_8	H_9	C_8,C_{11}	CH
10		154.9				C_q
11		144.6				C_q
11-OCH_3	3.53 (*s*)	60.7		$3H_{11\text{-OMe}}$	C_{11}	CH_3
11a		126.3				C_q

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Table 4.2.4.2:

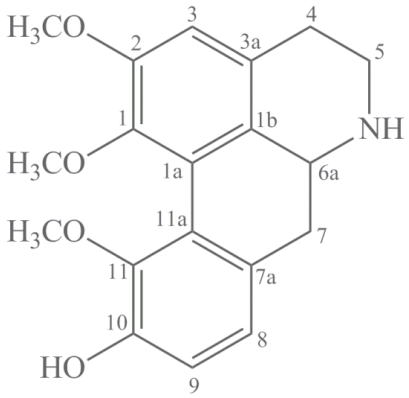
*^{1}H NMR Spectral Data Comparison of APB 4, with Oxohernagine **122** in Literature Data (Chen et al., 1996)*

	^{1}H δ, $CDCl_3$ (*J*,Hz)	
Position	APB 4	Oxohernagine **122** (Chen et al., 1996)
1-OCH_3	4.09 (*s*)	4.11 (*s*)
2-OCH_3	3.75 (*s*)	3.76 (*s*)
3	7.20 (*s*)	7.21 (*s*)
4	7.75 (*d*,4.0)	7.77 (*d*,5.3)
5	8.83 (*d*,4.0)	8.87 (*d*,5.3)
8	8.26 (*d*,8.0)	8.31 (*d*,8.5)
9	7.21 (*d*,8.0)	7.22 (*d*,8.5)
11-OCH_3	3.53 (*s*)	3.54 (*s*)

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4.2.5 APB 5, Hernagine 123



123

Alkaloids APB 5 was afforded as a light brown powder with $[\alpha]_D^{26}$ +252° (*c* = 1.3, MeOH) (Nafiah, 2009). The UV spectrum gave absorptions at 223 (log ε = 4.50), 270 (4.32) and 301 (3.75) nm thus suggesting a 1,2,10,11-tetrasubstituted aporphine skeleton (Cava & Bessho, 1966). The IR spectrum showed a strong absorption at 3338 cm^{-1} which indicated the presence of a conjugated hydroxyl group and the others absorption peaks revealed at position 3018 and 1462 cm^{-1} indicated the presence of aromatic system (Donald et al., 2001; Silverstein & Webster, 1998). The mass spectrum revealed a molecular ion peak at m/z 328.15543 $[M+H]^+$ (calc. 328.15488) corresponding to the molecular formula of $C_{19}H_{21}NO_4$.

The ^1H NMR spectrum (Figure 4.2.5.1) revealed three distinct methoxyl singlet signals at δ 3.54, 3.88 and 3.49, attached to C-1, C-2 and C-11 respectively. Three aromatic protons; a pair of doublet were observed at δ 6.88 and 6.86 with *J*-coupling = 8.0 Hz were assigned to two neighbouring protons H-8 and H-9, respectively; while another aromatic singlet signal appeared at δ 6.70 corresponding to

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H-3. The most deshielded aliphatic proton was H-6a, appeared as doublet of doublet at δ 3.73 (*J*= 3.5, 12.6). The other aliphatic protons signals can be observed between δ 3.40 to 2.52 (Table 4.2.5.1).

The ^{13}C NMR spectrum (Figure 4.2.5.2) showed the presence of 19 carbons, which consist of three aromatic carbons: C-8, C-9 and C-3; three methoxy carbons: 1-OCH_3, 2-OCH_3 and 11-OCH_3; three methylene carbons: C-5, C-7 and C-4; a methine carbon C-6a and the other were quaternary carbons: C-1, C-1a, C-1b, C-2, C-3a, C-7a, C-10, C-11 and C-11a. DEPT-135 NMR spectrum (Figure 4.2.5.3) proved the presence of three sp^3 carbons: C-5, C-4 and C-7; and three sp^2 carbons: C-3, C-8 and C-9. The positions of these carbon resonances were further supported by HMQC NMR spectrum (Figure 4.2.5.4).

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Further analysis by COSY NMR spectrum (Figure 4.2.5.5) suggested the following fragments: C-4–C-5, C-8–C-9 and C-6a–C-7. The HMBC spectrum (Figure 4.2.5.6) showed long-range correlations between the following protons and carbons: H-3 with C-2, C-1, C-1b and C-4; H-7 with C-1b, C-7a, C-8, C-9 and C-11; H-5 with C-3a, C-6a and C-4; H-8 with C-10, C-11a and C-7 and the other correlation were summarised in Figure 4.2.5.7, which determined the attribution of all the ^{13}C NMR signals of compound APB 5.

Comparison of the spectral data with the literature values (Table 4.2.5.2) (Phan et al., 1994) confirmed that alkaloid APB 5 was hernagine **123** which was first isolated from leaves of *Hernandia nymphaefolia* (Presl) or knowns as Kubitzki

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(Japanese name, Hasunoha-giri) collected from Iriomote Island, Japan (Yakushijin et al., 1980).

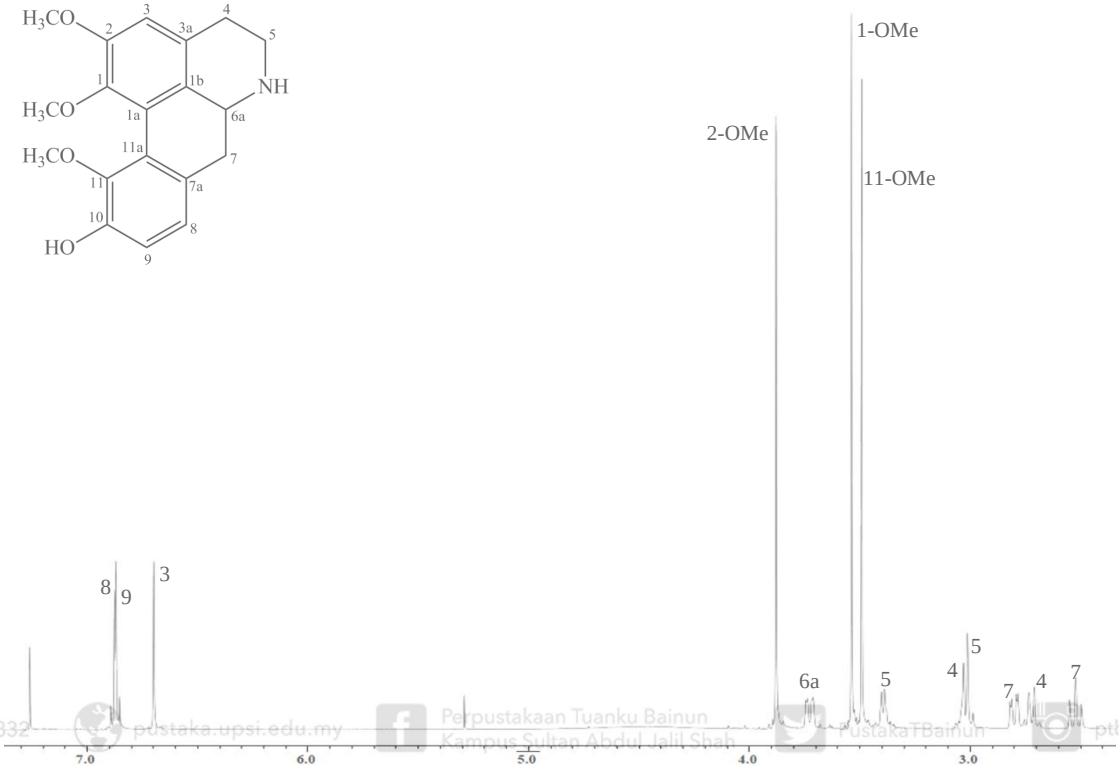


Figure 4.2.5.1: ^{1}H NMR Spectrum of APB 5

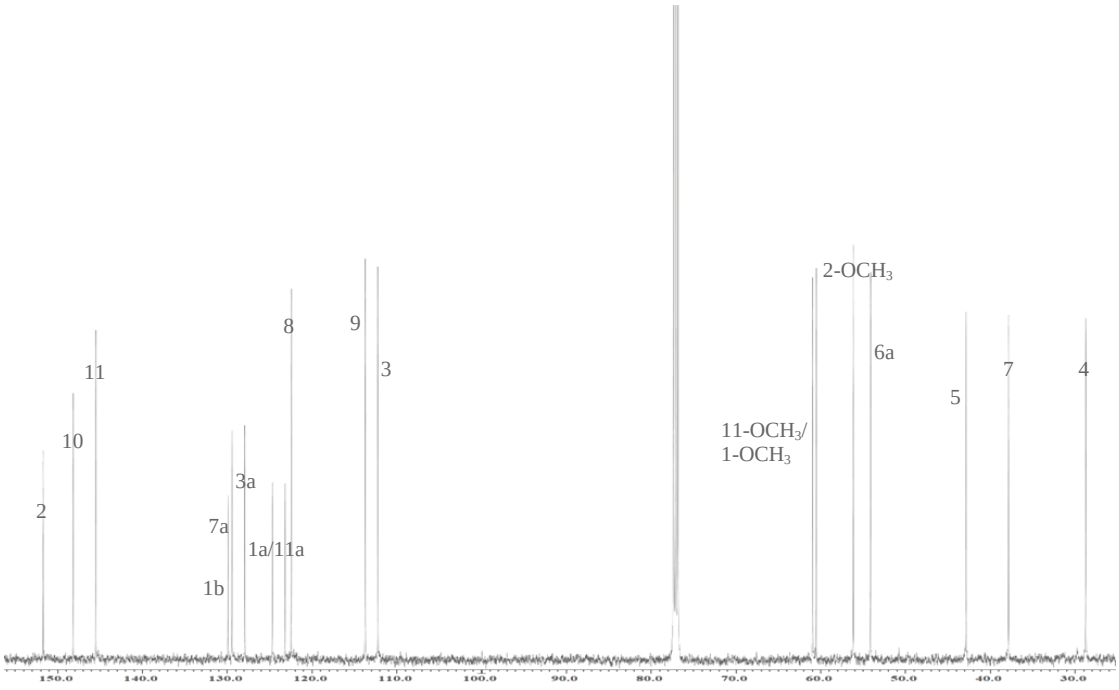


Figure 4.2.5.2: ^{13}C NMR Spectrum of APB 5

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Table 4.2.5.1:

1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 5

Position	^{1}H δ, $CDCl_3$ (*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HMQC	HMBC	DEPT-135
1		145.5				C_q
1-OCH_3	3.54 (*s*)	61.0		$3H_{1\text{-OMe}}$	C_1	CH_3
1a		124.7				C_q
1b		129.9				C_q
2		151.7				C_q
2-OCH_3	3.88 (*s*)	56.2		$3H_{2\text{-OMe}}$	C_2	CH_3
3	6.70 (*s*)	112.2		H_3	C_2,C_1,C_{1b}, C_{6a},C_4	CH
3a		127.9				C_q
4	3.03 (*s*) 2.72 (*d*,12.0)	28.8	H_5	H_4	C_{3a}, C_3	CH_2
5	3.40 (*d*,6.9) 3.01 (*s*)	42.9	H_4,H_5	H_5	C_{3a}, C_{6a},C_4	CH_2
6a	3.73 (*dd*,3.5,12.6)	54.1	H_7	H_{6a}	C_{7a}	CH
7	2.80 (*dd*,4.0,13.2) 2.52 (*m*,13.2)	37.9	H_{6a}	H_7	C_{1b}, C_{7a}, C_8, C_9, C_{6a}, C_{11}	CH_2
7a		129.5				C_q
8	6.88 (*d*,8.0)	122.5	H_9	H_8	C_{10}, C_{11a},C_7	CH
9	6.86 (*d*,8.0)	113.7	H_8	H_9	C_{11}, C_{7a}	CH
10		148.2				C_q
11		145.5				C_q
11-OCH_3	3.49 (*s*)	60.5		$3H_{11\text{-OMe}}$	C_{11}	CH_3
11a		123.2				C_q

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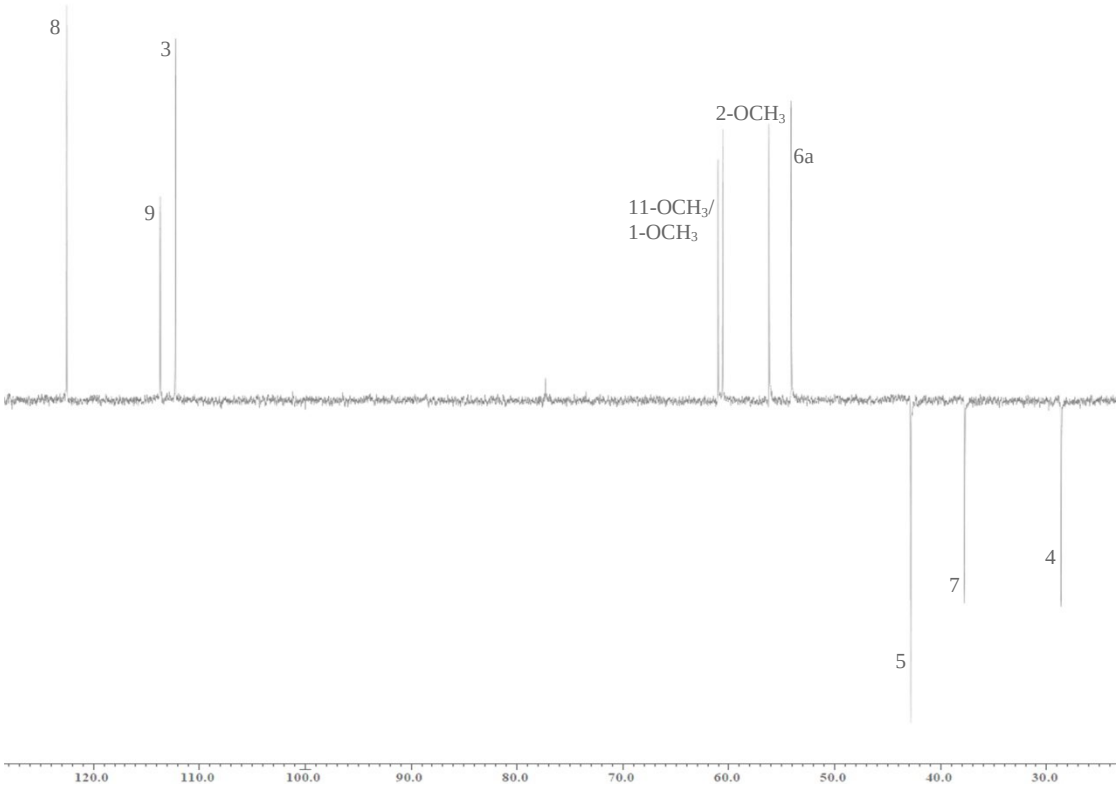


Figure 4.2.5.3: DEPT-135 NMR Spectrum of APB 5

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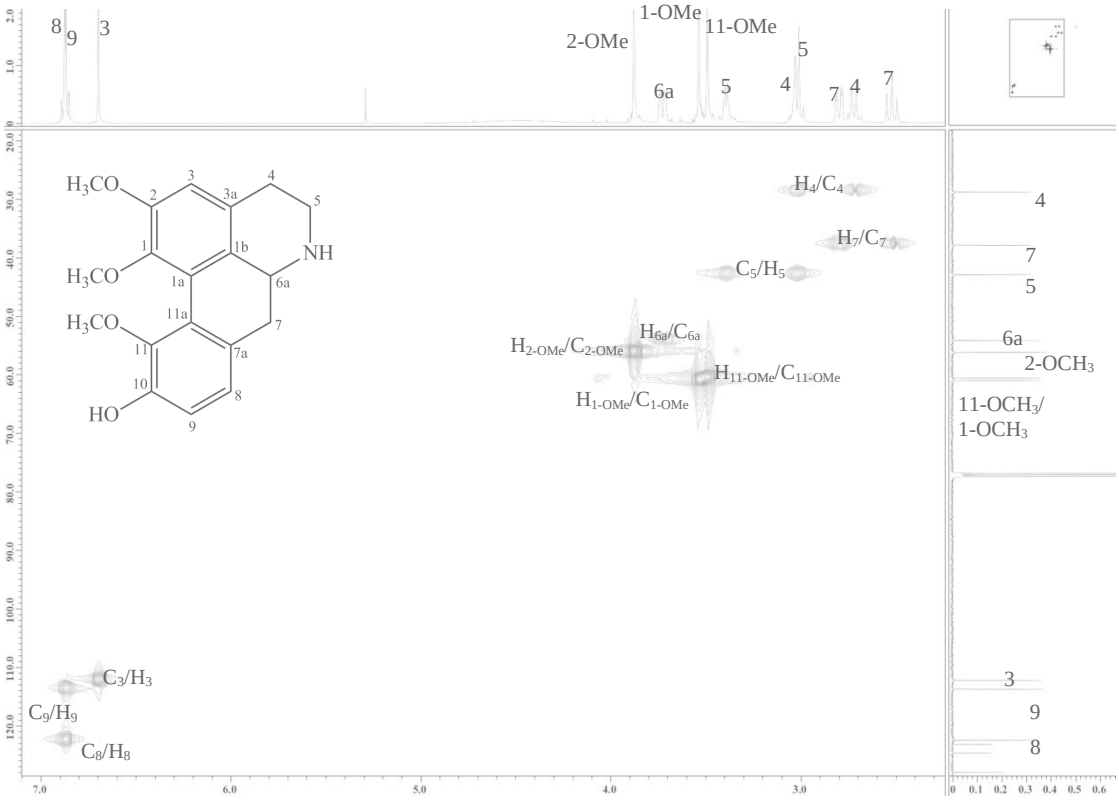


Figure 4.2.5.4: HMQC NMR Spectrum of APB 5

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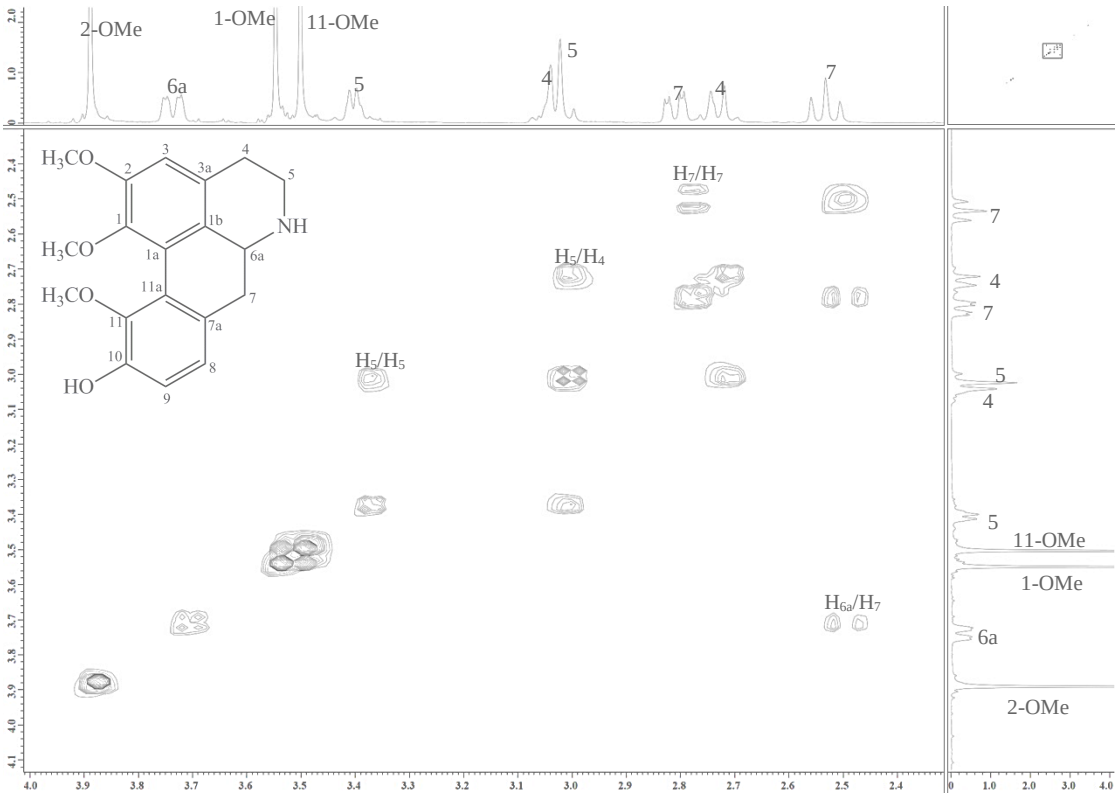


Figure 4.2.5.5: COSY NMR Spectrum of APB 5

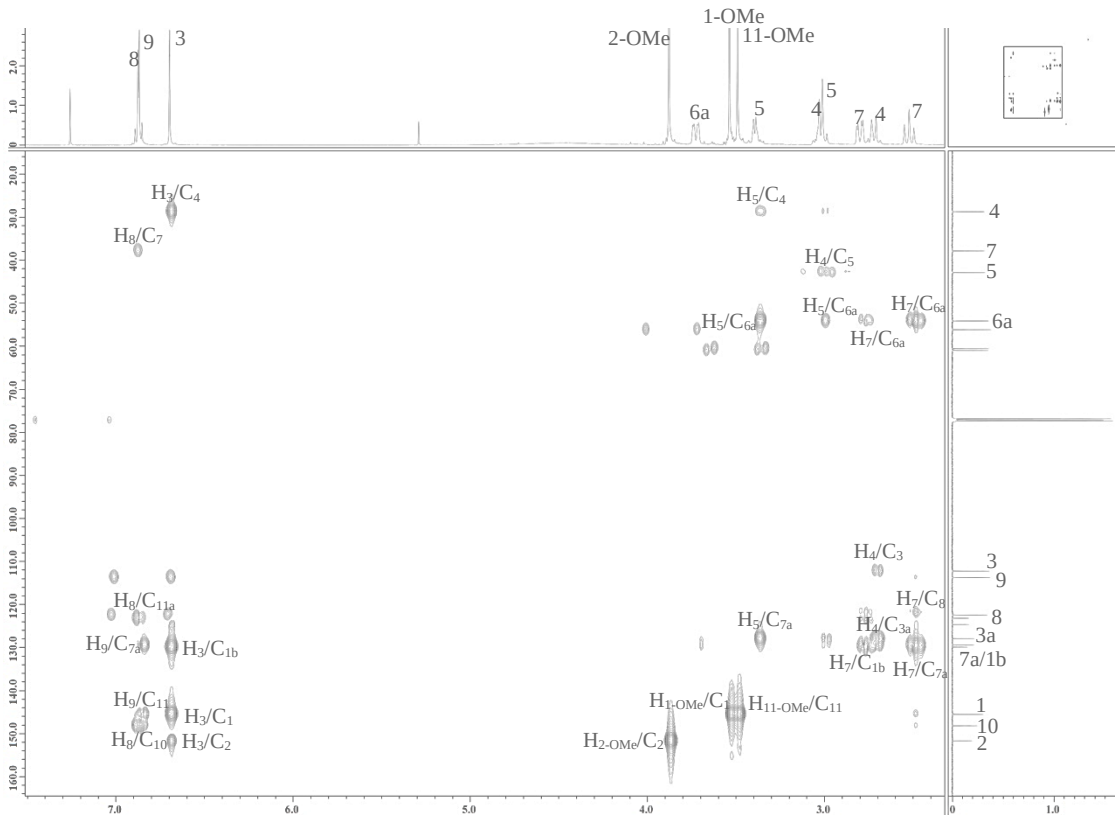


Figure 4.2.5.6: HMBC NMR Spectrum of APB 5

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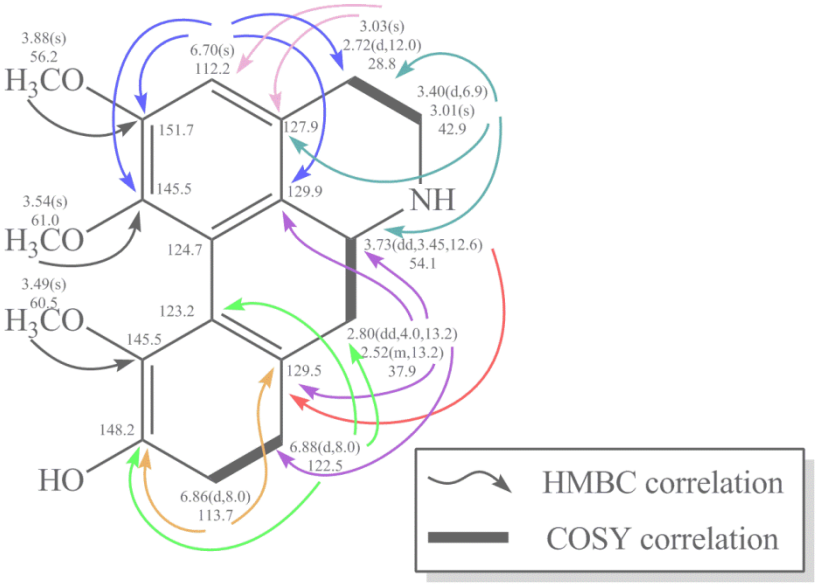


Figure 4.2.5.7: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 5

Table 4.2.5.2:

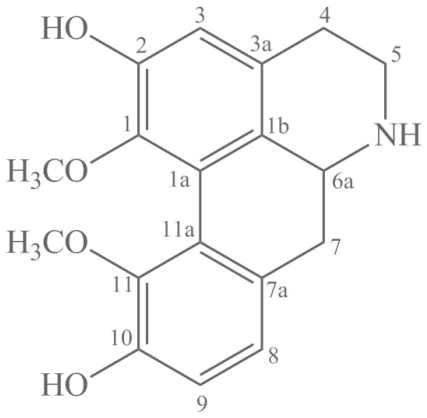
*^{13}C NMR Spectral Data Comparison of APB 5, with Hernagine **123** from Literature (Phan et al., 1994)*

	^{13}C (δ,$CDCl_3$)	
Position	APB 5	Hernagine **123** (Phan et al., 1994)
1	145.5	148.2
1-OCH_3	61.0	60.8
1a	124.7	124.6
1b	129.9	127.9
2	151.7	151.6
2-OCH_3	56.2	56.0
3	112.2	113.8
3a	127.9	130.0
4	28.8	28.7
5	42.9	42.8
6a	54.1	54.1
7	37.9	37.8
7a	129.5	129.3
8	122.5	122.4
9	113.7	113.8
10	148.2	148.2
11	145.5	145.4
11-OCH_3	60.5	60.4
11a	123.2	123.3

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4.2.6 APB 6, Hernovine 170



170

Alkaloid APB 6 was obtained as brown amorphous solid $[\alpha]_D^{18}$ +142° (*c*= 0.52, C_5H_5N) (Stuart & Chambers, 1967) . The UV spectrum exhibited maxima absorbance at 271 nm (log ε = 3.60), while the IR spectrum exhibited absorptions at 3476 cm^{-1} (NH), 3312 cm^{-1} (OH), 3019 cm^{-1} (stretching C-H aromatic) and 1475 cm^{-1} (aromatic system) (Duddley & Ian, 1989; Williams & Fleming, 1989). The mass spectrum exhibited of strong molecular ion peak at m/z 314.13881 $[M+H]^+$ (Calc. 314.13923) suggesting a molecular formula of $C_{18}H_{19}NO_4$.

The ^1H NMR spectrum data (Figure 4.2.6.1) of APB 6 were quite similar to APB 5 except the replacement of methoxyl to hydroxyl group at position C-2. Two signals appeared as singlet at δ 3.45 and δ 3.49 indicated the presence of two methoxyl groups attached to C-1 and C-11 at ring A and ring D, respectively. The most deshielded singlet signal corresponding to aromatic protons revealed at δ 6.77 which assigned to aromatic proton H-3. Another two aromatic proton; H-8 and H-9 appeared as doublet at δ 6.93 and δ 6.88 has same *J*-coupling = 8.0 Hz were assigned to two neighbouring protons, respectively. In addition, at δ 3.70 (*J*= 4.0 and 12.6 Hz)

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a doublet of doublet signal appeared corresponding to a methine proton at H-6a and rest of the signals are aliphatic protons.

The combination of ^{13}C and DEPT-135 NMR spectrum (Figure 4.2.6.2 and 4.2.6.3) revealed a total of 18 carbons signal; nine quaternary carbons (δ 148.1, 147.9, 144.8, 143.0, 129.4, 129.1, 128.8, 123.2 and 122.6 assign to C-10, C-2, C-11, C-1, C-3a, C-1b, C-7a, C-1a, and C-11a, respectively), four methine carbons (δ 123.0, 114.4, 113.8 and 54.1 assign to C-8, C-3, C-9 and C-6a, respectively), three methylenes (δ 42.9, 37.7 and 28.5 was assigned to C-5, C-7 and C-4, respectively) and two methoxyl carbon (δ 61.0 and 60.8 assign 1-OCH_3 and 11-OCH_3, respectively).

The ^{1}H-^{1}H COSY (Figure 4.2.6.4), HMQC (Figure 4.2.6.5) and the long range ^{1}H-^{13}C correlations were observed in the HMBC (Figure 4.2.6.6) spectra were supported the positions of these carbons resonance. The ^{1}H-^{1}H COSY spectrum suggested the fragments of C-4 to C-5, C-6a to C-7 and C-8 to C-9. The HMBC spectrum showed the cross peaks between H-3 with C-1, C-1b, C-2 & C-4, H-4 with C-1b, C-5, C-3a & C-3, H-5 with C-3a, C-4 & C-6a and H-7 with C-1b, C-6a, C-11a & C-7a revealed the structure of aporphine skeleton. The exact position of methoxyl groups were also confirmed through HMBC spectra to be at C-1 and C-11 through long-range correlation from the proton at 1-OCH_3 to C-1 and 11-OCH_3 to C-11. Another several correlations were also observed was shown in Figure 4.2.6.7 and Table 4.2.6.1, which confirm the position of all the ^{13}C signals of compounds.

The comparison of observed data with literature data (Table 4.2.6.2), the isolated compound was confirm that APB 6 is hernovine **170** (Phan et al., 1994)**.** The

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literature data slightly different for carbon values in position C-10 and C-11 but the exact position was clearly proved in HMBC spectral. Previously, hernovine **170** was isolated from various plant such as Formosan plant (Yaiwanese) (Chia, Chen, Chang, Teng, & Wu, 1999), *Croton wilsonii* Griseb.(Stuart & Chambers, 1967), *Hernandia ovigera* L. (Bick, 1996; Cava, Bessho, Douglas, Markey, & Weisbach, 1966; Ito & Furukawa, 1970; Leitão et al., 1999), *Lindera Myrrha* (Phan et al., 1994), *Hernandia catalpifolia* Plum (Leitão et al., 1999) and *Hernandia peltata* (Bick, 1996).

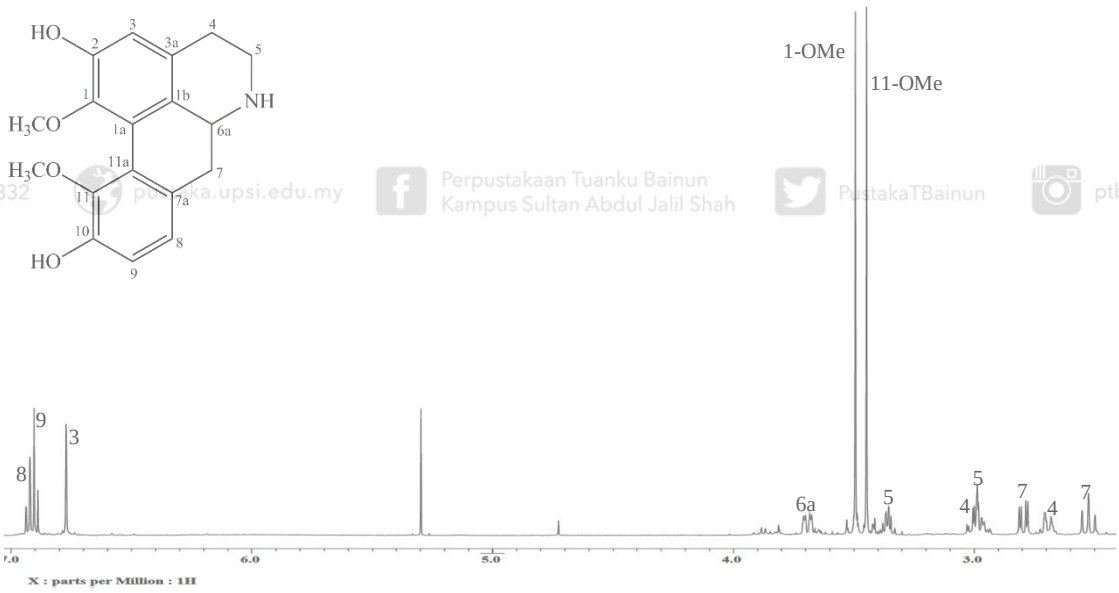


Figure 4.2.6.1: ^{1}H NMR Spectrum of APB 6

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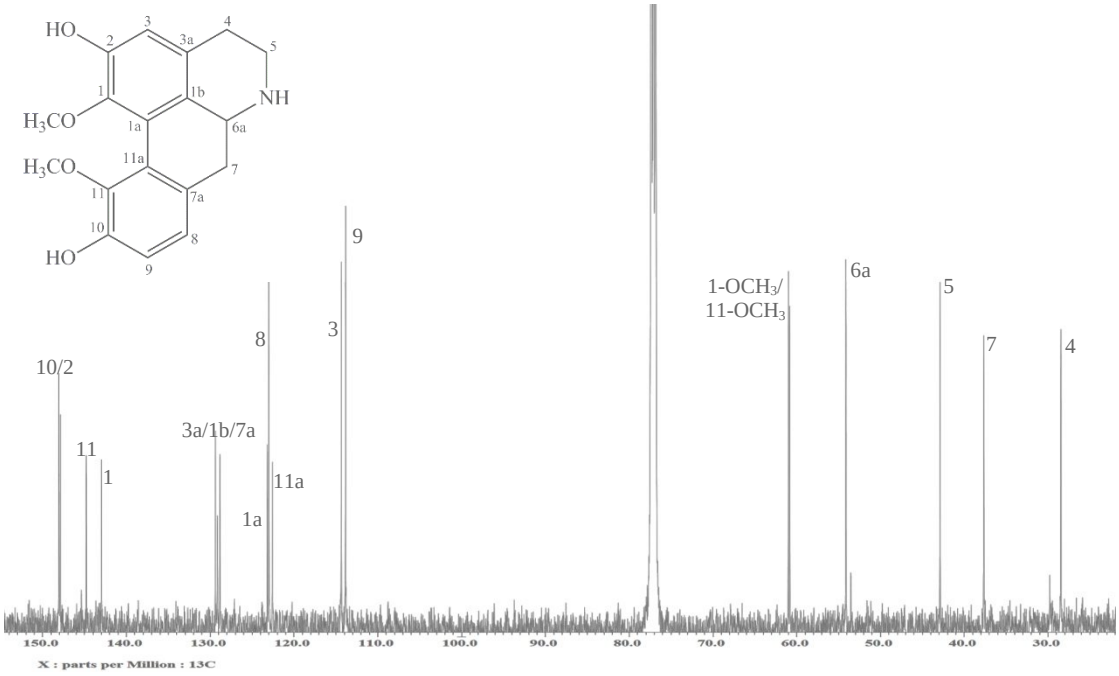


Figure 4.2.6.2: ^{13}C NMR Spectrum of APB 6

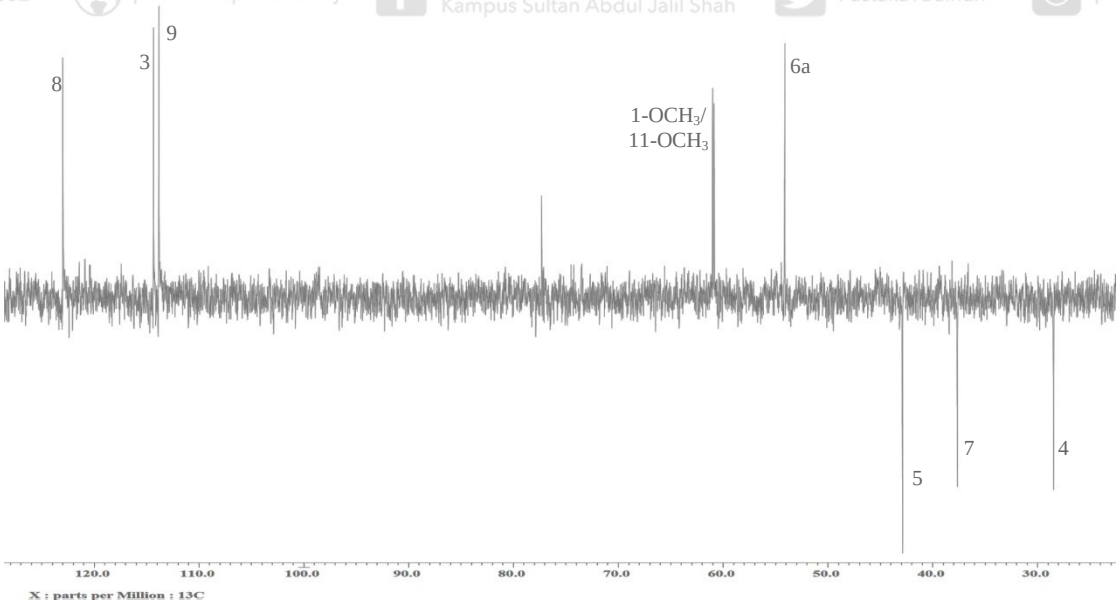


Figure 4.2.6.3: DEPT-135 NMR Spectrum of APB 6

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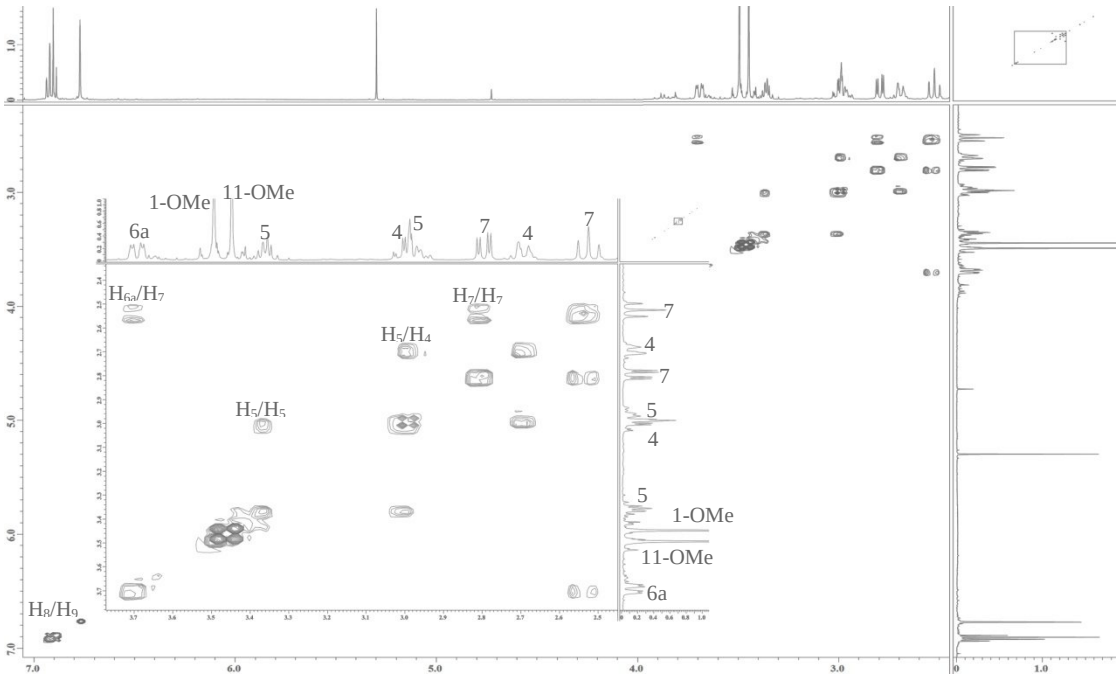


Figure 4.2.6.4: COSY NMR Spectrum of APB 6

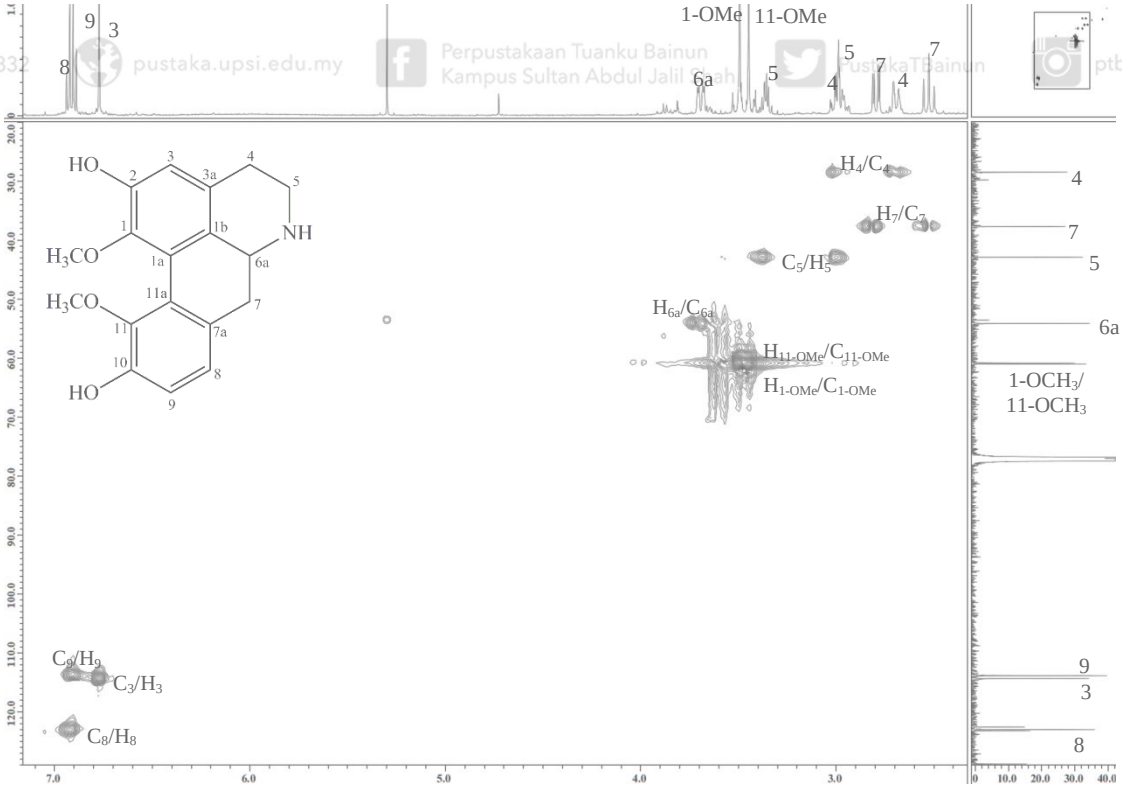


Figure 4.2.6.5: HMQC NMR Spectrum of APB 6

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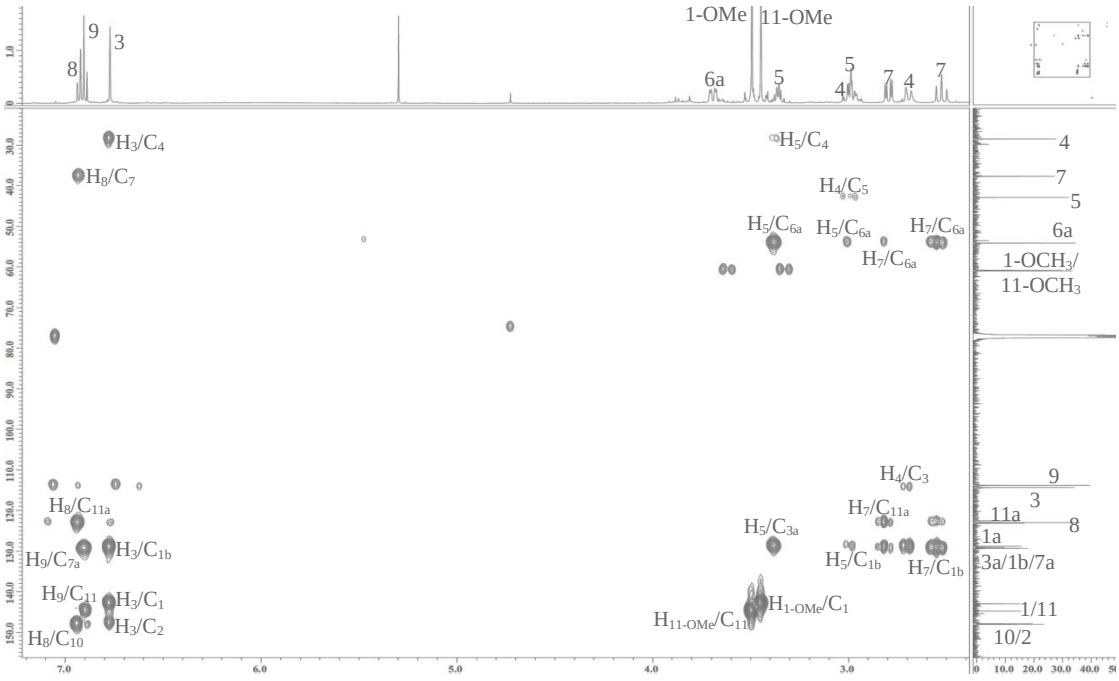


Figure 4.2.6.6: HMBC NMR Spectrum of APB 6

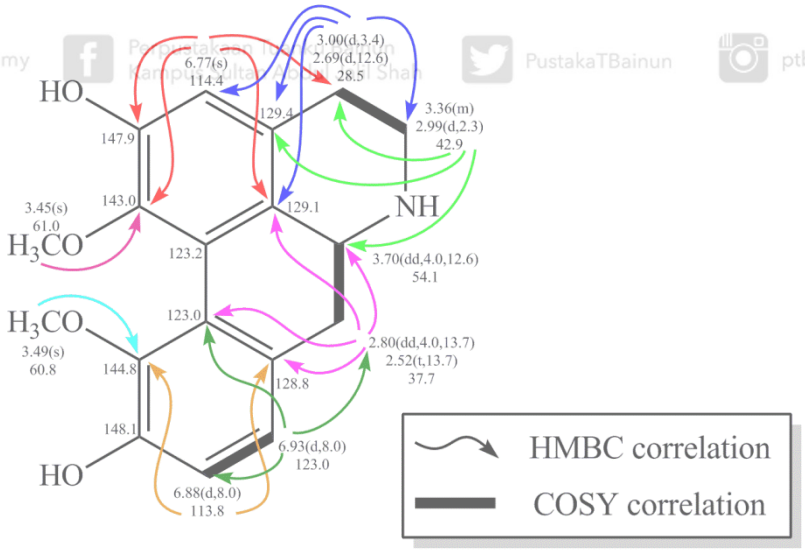


Figure 4.2.6.7: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 6

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Table 4.2.6.1:

1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 6

Position	^{1}H δ, $CDCl_3$ (*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HMQC	HMBC	DEPT -135
1		143.0				C_q
1-OCH_3	3.45 (*s*)	61.0		$3H_{1\text{-OMe}}$	C_1	CH_3
1a		123.2				C_q
1b		129.1				C_q
2		147.9				C_q
3	6.77 (*s*)	114.4		H_3	C_1, C_{1b}, C_2, C_4	CH
3a		129.4				C_q
4	3.00 (*d*,3.4) 2.69 (*d*,12.6)	28.5	H_5, H_4	H_4	C_{1b}, C_5 C_3, C_{3a}	CH_2
5	3.36 (*m*) 2.99 (*d*,2.3)	42.9	H_4	H_5	C_{3a}, C_4, C_{6a}	CH_2
6a	3.70 (*dd*,4.0,12.6)	54.1	H_7	H_{6a}		CH
7	2.80 (*dd*,4.0,13.7) 2.52 (*t*,13.7)	37.7	H_{6a}, H_7	H_7	C_{1b}, C_{6a}, C_{11a}, C_{7a},	CH_2
7a		128.8				C_q
8	6.93 (*d*,8.0)	123.0	H_9	H_8	H_7, H_{11a}, C_{10}	CH
9	6.88 (*d*,8.0)	113.8	H_8	H_9	C_{7a}, C_{11}	CH
10		148.1				C_q
11		144.8				C_q
11-OCH_3	3.49 (*s*)	60.8		$3H_{11\text{-OMe}}$	C_{11}	CH_3

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Table 4.2.6.2:

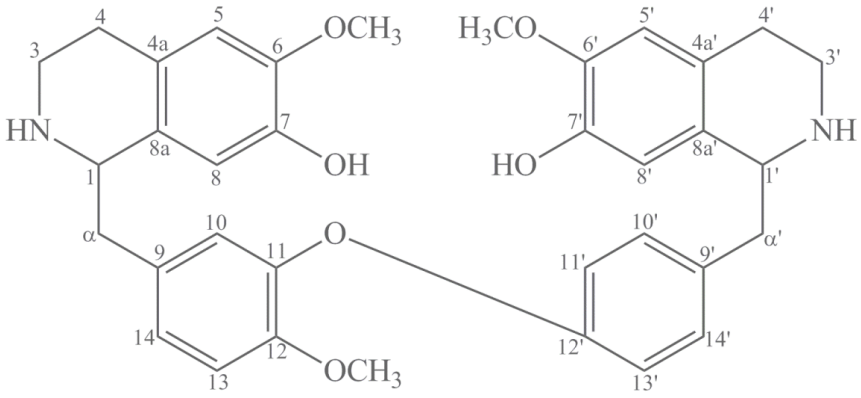
*^{13}C NMR Spectral Data Comparison of APB 6 , with Hernovine **170** from Literature (Phan et al., 1994)*

	^{13}C (δ,$CDCl_3$)	
Position	APB 6	Hernovine **170** (Phan et al., 1994)
1	143.0	147.0
1-OCH_3	61.0	60.8
1a	123.2	126.1
1b	129.1	129.8
2	147.9	149.6
3	114.4	116.4
3a	129.4	130.3
4	28.5	29.2
5	42.9	43.5
6a	54.1	55.3
7	37.7	38.7
7a	128.8	129.1
8	123.0	122.9
9	113.8	116.0
10	148.1	144.9
11	144.8	149.9
11-OCH_3	60.8	60.8
11a	122.6	125.1

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4.2.7 APB 7, Costaricine 126



126

APB 7 was isolated as brown amorphous solid with $[\alpha]_D^{26}$ +46.4° (*c* = 0.248, $CHCl_3$) (Angerhofer et al., 1999). It showed a molecular ion $[M+H]^+$ peak at m/z 583.28929 (Calc. 583.28081) in HRESIMS corresponding to the molecular formula of $C_{35}H_{38}N_2O_6$. The UV spectrum showed absorption maxima peak at 284 nm (log ε = 4.12) whichmindicate the presence of hydroxyl groups on a bisbenzylisoquinoline structure (Mahiou, Roblot, Fournet, & Hocquemiller, 2000). The IR spectrum exhibited peaks at 3418.5, 3296.8, 3019.7 and 1212.9 cm^{-1} corresponding to NH group, highly conjugated hydroxyl group, C-H aromatic and C-O-C bending; diphenyl ether group respectively (Donald et al., 2001; Duddley & Ian, 1989).

The ^{1}H NMR spectrum (Figure 4.2.7.1) revealed that APB 7 has no *N*-methyl group in the structure due to absent of 3H signal appeared in vicinity of δ 2.50. Three proton signal at δ 6.73 (H-10, *d, J*=1.7 Hz), δ 6.91 (H-13, *d, J*=8.0 Hz) and δ 6.95 (H-14, *d, J*=8.0 Hz) complete the trisubstituted pattern of ortho-para benzene ring in ring C, while in another benzene ring (ring C′) the resonance of protons *para* disubstituted

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(AA'BB' system) were appeared as two set of doublet signal (2H, each) at δ 7.12 (H-10′ and H-14′, *d*, *J*=8.6 Hz) and δ 6.86 (H-11′ and H-13′, *d*, *J*=8.6). In addition, another four singlet signal in aromatic region were attribute to H-5, H-8, H-5′ and H-8′ at δ 6.54, 6.70, 6.49 and 6.66, respectively. The spectrum also showed three methoxyl group singlets at δ 3.79, 3.83 and 3.78 assigned to 6-OCH_3, 12-OCH_3 and 6′-OCH_3, respectively. Two methines proton appeared at δ 4.05 (H-1, *dd*, *J*=4.0 and 9.2 Hz) and δ 4.08 (H-1′, *dd*, *J*= 3.5 and 9.8 Hz) and the rest of the signals are methylene protons.

The ^{13}C and DEPT NMR spectra (Figure 4.2.7.2 and Figure 4.2.7.3) confirmed the presence of thirty five carbons; thirteen sp^2 quarternary carbons at δ 130.1 (C-4a), δ 145.7 (C-6), δ 144.1 (C-7), δ 126.3 (C-8a), δ 131.4 (C-9), δ 145.7 (C-11), δ 149.7 (C-12), δ 130.4 (C-4a′), δ 145.6 (C-6′), δ 144.0(C-7′), δ 126.1(C-8a′), δ 133.1 (C-9′) and δ 156.0(C-12′), eleven sp^2 methines carbons at δ 111.2 (C-5), δ 112.7 (C-8), δ 121.0 (C-10), δ 112.5 (C-13), δ 125.2 (C-14), δ 111.2 (C-5′), δ 112.7 (C-8′), δ 130.5 (C-10′), δ 118.1 (C-11′), δ 118.1 (C-13′) and δ 130.5 (C-14′), two sp^3 methine carbons at δ 56.8 (C-1) and δ 56.5 (C-1′), six sp^3 methylenes carbons at δ 41.0 (C-3), δ 29.2 (C-4), δ 41.2 (C-α), δ 40.7 (C-3′), δ 29.1 (C-4′) and δ 41.8 (C-α′) and three methoxyl groups at δ 55.8 (6-OCH_3), δ 56.1 (12-OCH_3) and δ 56.8 (6′-OCH_3).

The complete assignment of the proton and carbon resonance was established using COSY (Figure 4.2.7.4), HMQC (Figure 4.2.7.5) and HMBC spectra (Figure 4.2.7.6). Further analysis by COSY NMR spectrum suggested the following fragments C-3–C-4, C-1–C-α, C-3′–C-4′, C-1–C-α, C-10′–C-11′ and C-13′–C-14′. In the HMBC spectrum, H-α showed correlation with C-10, C-14, C-9 and C-1. Meanwhile, H-α′

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revealed correlation with C-8a′, C-14′, C-9′, C-3′ and C-1′. Other significant correlations were shown in Figure 4.2.7.7. Complete spectral data for ^{1}H and ^{13}C NMR were summarised in Table 4.2.7.1.

The comparison between observed data and literature value confirmed that APB 7 is costaricine **126** (Table 4.2.7.2). This bisbenzylisoquinoline alkaloid was first isolated from trunk bark of *Nectandra salicifolia* which was collected from Costa Rica (Böhlke et al., 1996).

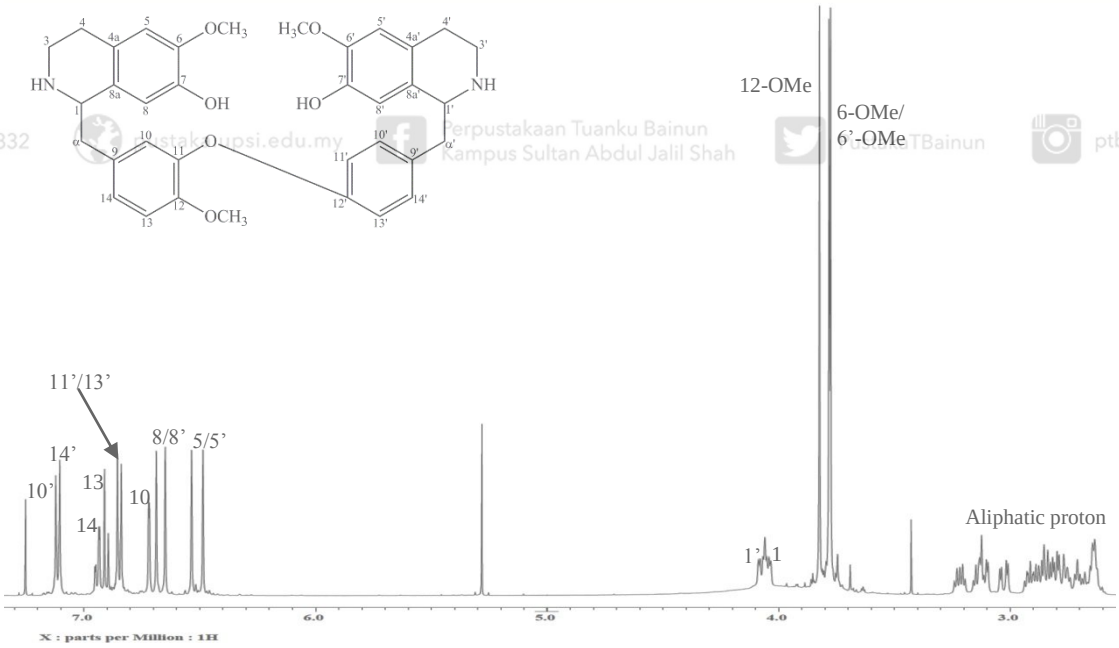


Figure 4.2.7.1: ^{1}H NMR Spectrum of APB 7

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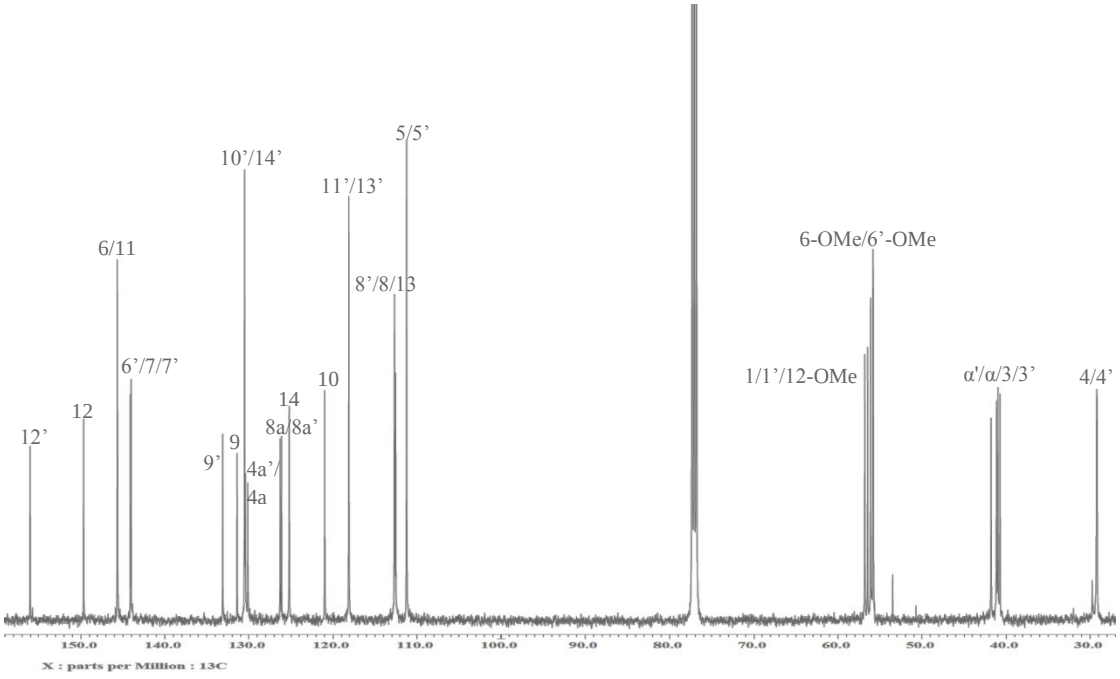


Figure 4.2.7.2: ^{13}C NMR Spectrum of APB 7

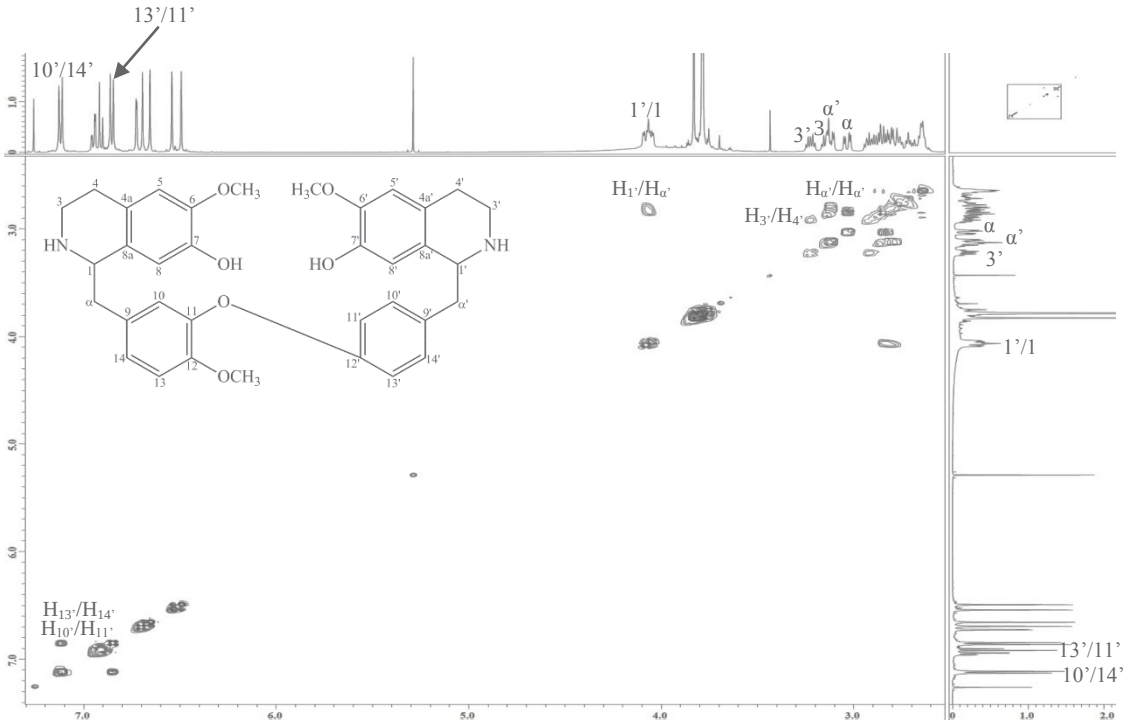


Figure 4.2.7.3: COSY NMR Spectrum of APB 7

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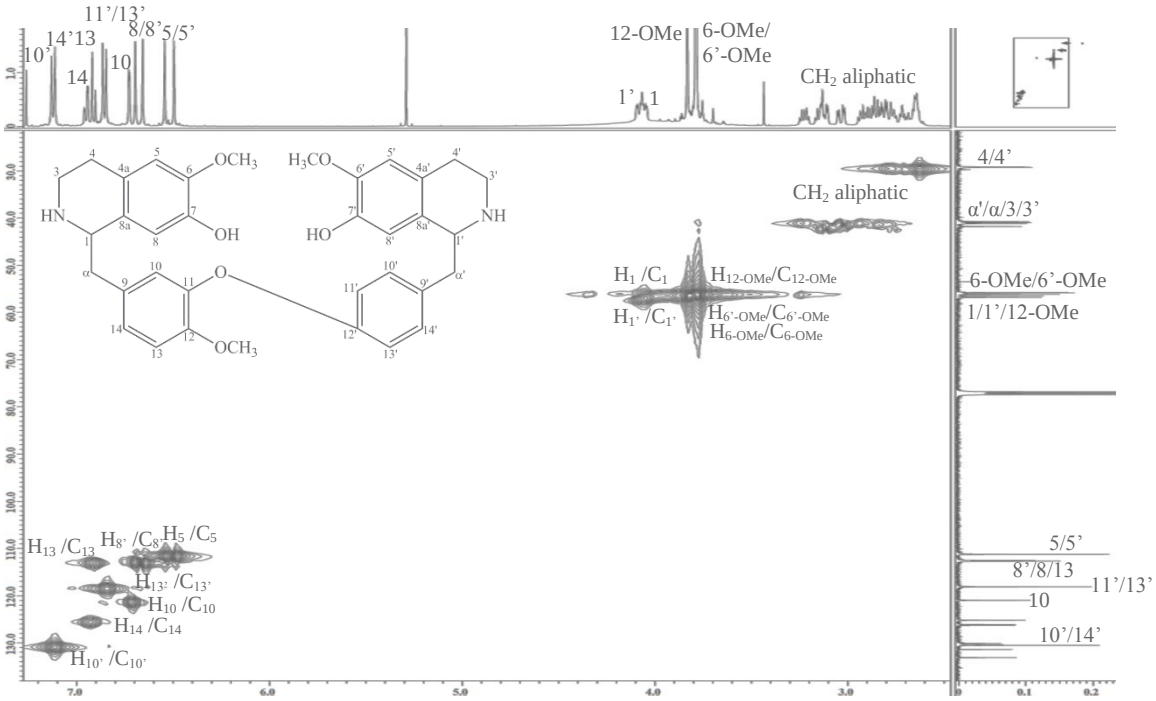


Figure 4.2.7.4: HMQC NMR Spectrum of APB 7

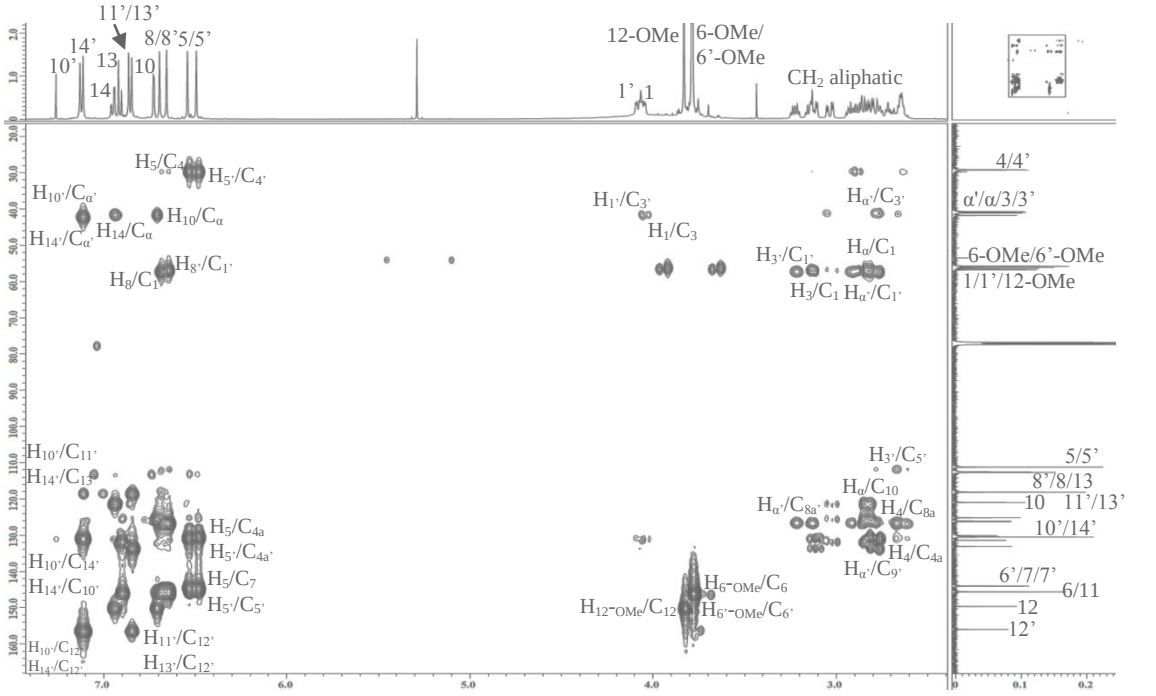


Figure 4.2.7.5: HMBC NMR Spectrum of APB 7

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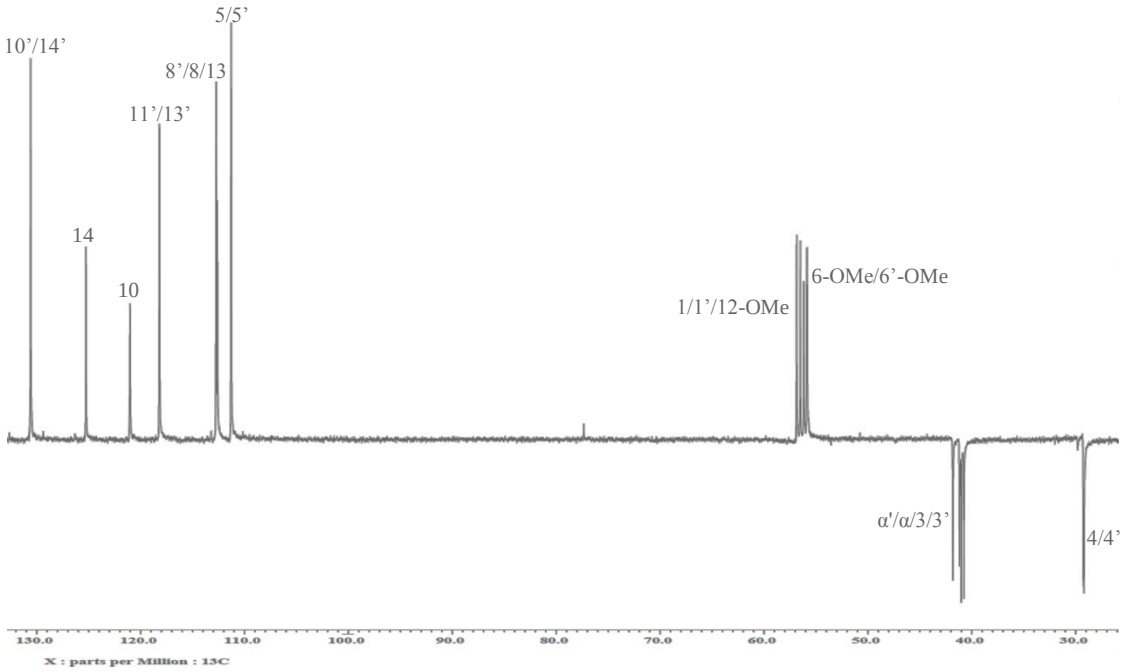


Figure 4.2.7.6: DEPT-135 NMR Spectrum of APB 7

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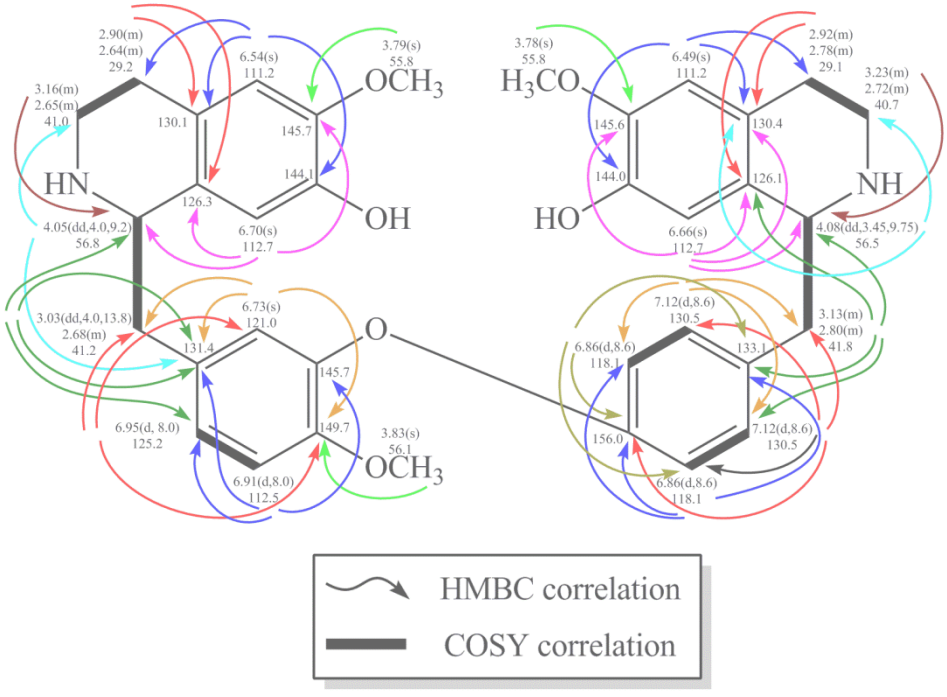


Figure 4.3.7.7: COSY and ^{1}H-^{13}C Coupling Pattern Observed in HMBC NMR Spectrum of APB 7

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Table 4.2.7.1:

1D (^{1}H, ^{13}C and DEPT-135) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of APB 7

Position	^{1}H δ,$CDCl_3$(*J*,Hz)	^{13}C (δ,$CDCl_3$)	COSY	HMQC	HMBC	DEPT-135
1	4.05 (*dd*,4.0,9.2)	56.8	$H_α$	H_1	C_3,C_9	CH
3	3.16 (*m*) 2.65 (*m*)	41.0	H_3,H_4	H_3 H_3	C_1	CH_2
4	2.90 (*m*) 2.64 (*m*)	29.2	H_3,H_4	H_4 H_4	C_{4a},C_{8a}	CH_2
4a		130.1				C_q
5	6.54 (*s*)	111.2		H_5	C_4,C_{4a},C_7	CH
6		145.7				C_q
6-OCH_3	3.79 (*s*)	55.8		$3H_{6\text{-OMe}}$	C_6	CH_3
7		144.1				C_q
8	6.70 (*s*)	112.7		H_8	C_1,C_6,C_{8a}	CH
8a		126.3				C_q
α	3.03 (*dd*,4.0,13.8) 2.68 (*m*)	41.2	H_1,$H_α$	$H_α$ $H_α$	C_{10},C_{14},C_9,C_1	CH_2
9		131.4				C_q
10	6.73 (*s*)	121.0		H_{10}	$C_α$,C_{12},C_9	CH
11		145.7				C_q
12		149.7				C_q
12-OCH_3	3.83 (*s*)	56.1		$3H_{12\text{-OMe}}$	C_{12}	CH_3
13	6.91 (*d*,8.0)	112.5		H_{13}	C_9,C_{14},C_{11}	CH
14	6.95 (*d*,8.0)	125.2		H_{14}	$C_α$,C_{10},C_{12}	CH
1′	4.08 (*dd*,3.5,9.8)	56.5	$H_{α'}$	$H_{1'}$	$C_{3'}$,$C_{4a'}$	CH
3′	3.23 (*m*) 2.72 (*m*)	40.7	$H_{4'}$	$H_{3'}$ $H_{3'}$	$C_{1'}$,$C_{5'}$	CH_2
4′	2.92 (*m*) 2.78 (*m*)	29.1	$H_{3'}$	$H_{4'}$ $H_{4'}$	$C_{8'a}$,$C_{4a'}$	CH_2
4a′		130.4				C_q
5′	6.49 (*s*)	111.2		$H_{5'}$	$C_{4'}$,$C_{4a'}$,$C_{7'}$	CH
6′		145.6				C_q
6′-OCH_3	3.78 (*s*)	55.8		$3H_{6'\text{-OMe}}$	$C_{6'}$	CH
7′		144.0				C_q
8′	6.66 (*s*)	112.7		$H_{8'}$	$C_{1'}$,$C_{4a'}$,$C_{8a'}$,$C_{6'}$	CH
8a′		126.1				C_q
α′	3.13 (*m*) 2.80 (*m*)	41.8	$H_{1'}$,$H_{α'}$	$H_{α'}$ $H_{α'}$	$C_{8a'}$,$C_{14'}$,$C_{9'}$,$C_{3'}$, $C_{1'}$,	CH_2
9′		133.1				C_q
10′	7.12 (*d*,8.6)	130.5	$H_{11'}$	$H_{10'}$	$C_{α'}$,$C_{11'}$,$C_{14'}$	CH
11′	6.86 (*d*,8.6)	118.1	$H_{10'}$	$H_{11'}$	$C_{13'}$,$C_{9'}$,$C_{12'}$	CH
12′		156.0				C_q
13′	6.86 (*d*,8.6)	118.1	$H_{14'}$	$H_{13'}$	$C_{11'}$,$C_{9'}$,$C_{12'}$	CH
14′	7.12 (*d*,8.6)	130.5	$H_{13'}$	$H_{14'}$	$C_{α'}$,$C_{13'}$,$C_{10'}$,$C_{12'}$	CH

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Table 4.2.5.2:

*^{13}C NMR Spectral Data Comparison of APB 7 , with Costaricine **126** in Literature Data (Phan et al., 1994)*

	^{1}H δ,$CDCl_3$(*J*,Hz)		^{13}C (δ,$CDCl_3$)	
Position	APB 7	Costaricine **126** (Phan et al., 1994)	APB 7	Costaricine **126** (Phan et al., 1994)
1	4.05 (*dd*,4.0,9.2)	4.05 (*dd*,3.5,9.0)	56.8	56.4
3	3.16 (*m*) 2.65 (*m*)	3.16 (*m*) 2.64 (*m*)	41.0	40.9
4	2.90 (*m*) 2.64 (*m*)	2.88 (*m*) 2.78 (*m*)	29.2	29.3
4a			130.1	126.3
5	6.54 (*s*)	6.51 (*s*)	111.2	111.2
6			145.7	145.4
6-OCH_3	3.79 (*s*)	3.81 (*s*)	55.8	55.7
7			144.1	144.0
8	6.70 (*s*)	6.69 (*s*)	112.7	112.6
8a			126.3	130.3
A	3.03 (*dd*,4.0,13.8) 2.68 (*m*)	3.04 (*dd*,3.5,14.0) 2.85 (*m*)	41.2	41.1
9			131.4	131.4
10	6.73 (*d*,1.7)	6.73 (*d*,2.0)	121.0	120.9
11			145.7	145.4
12			149.7	149.6
12-OCH_3	3.83 (*s*)	3.84 (*s*)	56.1	56.0
13	6.91 (*d*,8.0)	6.92 (*d*,8.5)	112.5	112.6
14	6.95 (*dd*,1.8,8.1)	6.96 (*dd*,2.0,8.5)	125.2	125.1
1′	4.08 (*dd*,3.5,9.8)	4.08 (*dd*,3.7,10.0)	56.5	56.7
3′	3.23 (*m*) 2.72 (*m*)	3.23 (*m*) 2.72 (*m*)	40.7	40.6
4′	2.92 (*m*) 2.78 (*m*)	2.92 (*m*) 2.76 (*m*)	29.1	29.2
4a′			130.4	126.1
5′	6.49 (*s*)	6.56 (*s*)	111.2	111.2
6′			145.6	145.5
6′-OCH_3	3.78 (*s*)	3.82 (*s*)	55.8	55.7
7′			144.0	143.9
8′	6.66 (*s*)	6.73 (*s*)	112.7	112.5
8a′			126.1	130.5
α′	3.13 (*m*) 2.80 (*m*)	3.14 (*m*) 2.80 (*dd*,3.7,13.5)	41.8	41.7
9′			133.1	133.1
10′	7.12 (*d*,8.6)	7.13 (*d*,8.5)	130.5	130.4
11′	6.86 (*d*,8.6)	6.86 (*d*,8.5)	118.1	117.9
12′			156.0	155.9
13′	6.86 (*d*,8.6)	6.86 (*d*,8.5)	118.1	117.9
14′	7.12 (*d*,8.6)	7.13 (*d*,8.5)	130.5	130.4

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4.3 Biological Activities of Alkaloid from Barks of *Alseodaphne pendulifolia*

For the bioassay, only norisocorydine *N*-oxide **166**, corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123** were tested for their biological activities, another two compound; hernovine **170** and costaricine **126** were not tested due to insufficient amount of the compounds to run the assays. The biological activities assays were in-vitro antiplasmodial assay against chloroquine-resistant (K1) strains of *Plasmodium falciparum*, MTT cytotoxicity assay against human promyelocytic leukemia cells (HL-60), *in-vitro* antibacterial activity against gram positive bacteria: *Bacillus subtilis* (B145) and antioxidant DPPH free radical scavenging activity assay. The biological activities were summarised in Table 4.3.1.

Preliminary screening on alkaloid crude extract from barks of *Alseodaphne pendulifolia* Gamb. (KL 5250) collected from Sg. Temau Reserved Forest was exhibited active antiplasmodial activity with IC_{50} 0.804µg/ml against *Plasmodium falciparum*, K1 isolate (resistant strain) for HRP2 assay. The isolated compounds; corydine **124**, oxohernagine **122** and hernagine **123** shows potent results 1.184, 2.189 and 3.494 µM $IC_{50,}$ respectively. While, norisocorydine *N*-oxide **151** and *N*-methylhernagine **169** showed moderate activity with IC_{50} value 6.454 and 5.313 µM, respectively.

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Table 4.3.1:

Biological Activities (In-vitro Antiplasmodial HRP2, Cytotoxic, Antibacterial and Antioxidant) of Alkaloids Against Alkaloids Crude Extract and Isolated Aporphine Alkaloids from Barks of Alseodaphne pendulifolia Gamb.

No.	Sample	Antiplasmodial (HRP2)		Cytotoxic (HL 60)	Antibacterial (*B. subtilis*)	Antioxidant (DPPH)
		IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)	Zone of inhibition (mm ± SD)	IC_{50} (µg/ml)
1.	Alkaloid Crude extract, *Alseodphne pendulifolia* (plant A)	0.804		NT	NT	NT
2.	Norisocorydine *N*-oxide **166**	2.208	6.454	11	-	>500
3.	Corydine **124**	0.404	1.184	48	-	>500
4.	*N*-methylhernagine **169**	1.814	5.313	38	-	>500
5.	Oxohernagine **122**	0.738	2.189	31	-	>500
6	Hernagine **123**	1.143	3.494	42	-	>500

* Upper limit indication for antiplasmodial HRP2 good activity, crude extract IC_{50} is < 10 µg/ml, compound IC_{50} is < 5 µM, standard drugs are chloroquine, quinine, mefloquine and artemisinine.
* In antibacterial assay doses of the samples were 1 mg/mL per disc, for positive control, streptomycin sulfate was used 10 µg/mL per disc and inhibit 21.00 ± 0.00 mm as positive control.
* Positive controls for antioxidant (DPPH assays) are ascorbic acid, α-tocopherol and quercetin with IC_{50} 20 ± 1.2, 60 ± 2.9 and 40 ± 5.8 µg/ml, respectively.
* Note: (-) No inhibition, (NT) Not tested.

Five isolated compounds from barks of *Alseodaphne pendulifolia* Gamb. were subjected for cytotoxic activity on leukaemia cell lines (HL-60), norisocorydine *N*-oxide **166** showed the moderate effect on HL-60 cell lines with IC_{50} value, 11 µg/ml while the other four compounds; corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123** show the weak activities with IC_{50} values 48, 38, 31 and 42 µg/ml, respectively. According to range of activity (inhibition), compounds which demonstrated the IC_{50} value in range of 20 - 100 µg/ml will be considered weak cytotoxic activities, compounds with IC_{50} values less than 5 µg/ml were considered

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very strong activity, while the intermediate values 5 - 10 µg/ml are strong activities and IC_{50} 10-20 µg/ml were considered moderate active (Sufian, Ramasamy, Ahmat, Zakaria, & Yusof, 2013; Wibowo et al., 2011).

Antibacterial assay of the compounds were evaluated using disc diffusion method. The preliminary screening for antibacterial activities was using gram positive bacterial species (*Bacillus subtilis*) and the activities were indicated by a clear zone of inhabitation. The results indicate that all the compounds tested were not active towards tested pathogenic bacteria since no appreciable zones of inhibition were observed.

Antioxidant assay by using DPPH method was used to assess the capacity of the tested compounds to scavenge stable free radical DPPH at different concentrations. In this technique, 96 micro-well flat bottom plates were used instead of cuvettes to consume minimal amounts of sample, vehicle and DPPH solution. The entire tested sample exhibited very weak activity with IC_{50} values of more than 500µg/mL against free radical scavenging effect of DPPH. This effect displayed suggested that these compounds were not able to neutralize the radicals formed during the peroxidation.

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CHAPTER 5

CONCLUSION

The first part of the study was aimed at an investigation of the alkaloidal contents from barks of *Alseodaphne pendulifolia* Gamb (Lauraceae family) collected in two different places. The samples were collected in Sg. Temau Forest Reserve, Kuala Lipis, Pahang and Sg. Tekam Reserve Forest, Jerantut, Pahang, Malaysia. Further isolation process of both plants yielded four type of isoquinoline alkaloids; aporphine, aporphine *N*-oxide, oxoaporphine and bisbenzylisoquinoline.

A new aporphine *N*-oxide alkaloids; norisocorydine *N*-oxide **166** with four known aporphine alkaloids; corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123** were successfully isolated from samples that collected in Sg. Temau Reserve Forest, while in sample that collected from Sg. Tekam Reserve Forest, four aporphine alkaloids; corydine **124**, *N*-methylhernagine **169**, hernagine **123** and

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hernovine **170**, a oxoaporphine; oxohernagine **122** was successfully isolated together with a bisbenzylisoquinoline alkaloids; costaricine **126**.

Four biological activities assays were used on isolated compounds: in-vitro antiplasmodial assay against chloroquine-resistant (K1) strains of *Plasmodium falciparum*, MTT cytotoxicity assay against human promyelocytic leukemia cells (HL-60), in vitro antibacterial activity against gram positive bacteria: *Bacillus subtilis* (B145) and antioxidant DPPH free radical scavenging activity assay.

For antimalaria HRP2 assay, the alkaloids crude extract of *Alseodaphne pendulifolia* Gamb. exhibited significant activity with IC_{50} value of 0.804 µg/ml against *Plasmodium falciparum*, K1 isolate (resistant strain). While the isolated compounds corydine **124**, oxohernagine **122** and hernagine **123** show the potent results (active activity) 1.184, 2.189 and 3.494 µM $IC_{50,}$ respectively. The others compounds; norisocorydine *N*-oxide **166** and *N*-methylhernagine **169** revealed moderate activity with IC_{50} value over 5 µM. The results of cytotoxic activity againts leukaemia cell lines (HL-60), only norisocorydine *N*-oxide **166** showed the moderate activity with IC_{50} value 11 µg/ml while the other compounds corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123** revealed the weak activities. Antibacterial activity on gram positive bacterial *Bacillus subtilis* (B 145) showed all the tested compounds were no activity against the tested pathogenic bacteria. Finally, all tested compounds exhibited very weak activity with IC_{50} values more than 500µg/mL against free radical scavenging effect of DPPH.

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APPENDIX 1

Articles in Proceeding/Presented at Conferences/Seminar

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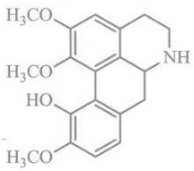
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STUDIES OF ALKALOID FROM BARK OF *ALSEODAPHNE PENDULIFOLIA* GAMB.

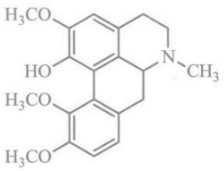
Muhammad Hafiz Husna Hasnan[1], Kartini Ahmad[1], Mohamad Nurul Azmi Mohamad Taib[2], Khalijah Awang[2], Mohd Azlan Nafiah[1]

[1]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia.
[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Alseodaphne pendulifolia Gamb. (Lauraceae Family) known as Medang or Tejur in common name was collected from Sg. Temau Forest Reserved, Kuala Lipis, Pahang. This phytochemical study from this plant, focus on plant's barks involve defatted process with hexane, extraction with dichloromethane and methanol, acid–base extraction, separations by using various chromatographic methods and structural determination by spectroscopic techniques such as UV, IR, NMR including 1D-NMR (^{1}H, ^{13}C, and DEPT), 2D-NMR (COSY, HSQC, and HMBC) and Mass Spectrometry test. Isolation works yielded two aporphine alkaloid; Norisocorydine (1) and Corydine (2).



(1)



(2)

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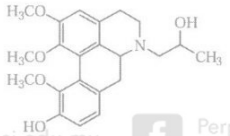
Alkaloid from leaves of *Alseodaphne pendulifolia* gamb.

Muhammad Hafiz Husna Hasnan[1], Kartini Ahmad[1], A. Hamid A. Hadi[2], Khalijah Awang[2], Mat Ropi Mukhtar[2], Mohd Azlan Nafiah[1]

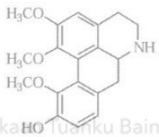
[1]Department Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia.
[2]Department Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

The phytochemical study on leaves of *Alseodaphne pendulifolia* Gamb. (Lauraceae family) collected from Sg. Temau Forest Reserved, Kuala Lipis, Pahang, Malaysia involve extraction, separations by using various chromatographic methods and structural determination by spectroscopic techniques such as UV, IR, NMR including 1D-NMR (^{1}H, ^{13}C, and DEPT), 2D-NMR (COSY, NOE-D, NOESY, HMQC/HSQC, and HMBC) and MS (LCMS and HRFABMS). Isolation works yielded one new aporphine alkaloid; *N*-(2-hydroxypropyl)-pendulifoline (**1**) with other known compounds; hernagine (**2**), corydine (**3**), isocorytuberine (**4**), oxohernagine (**5**) and costaricine (**6**).

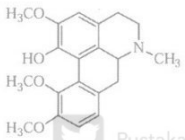
Keywords: *Alseodaphne pendulifolia* Gamb, Lauraceae



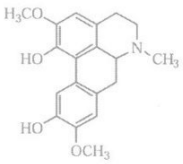
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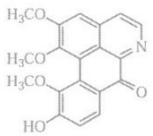
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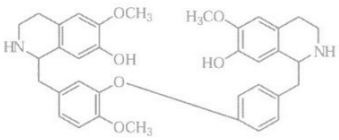
(3)



(4)



(5)



(6)

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ISOLATION OF STIGMASTEROL FROM THE LEAVES OF *KOPSIA SINGAPURENSIS* RIDL.

Lee Yean Shan[1], Kartini Ahmad[2], Khalijah Awang[3], Tan Siow Ping[4], Mohd Azlan Nafiah[5]
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Abstract

The isolation and purification of the leaves of *Kopsia singapurensis* Ridl. (Apocynaceae) revealed the presence of steroids, terpenes, and alkaloids. Stigmasterol **1** was isolated from the hexane crude extract of the leaves of this species. This phytochemical study involves extraction, separation by using various chromatographic methods and structural determination by spectroscopic technique such as IR, NMR including 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (COSY, NOESY, HMQC/HSQC, and HMBC) and GCMS, involving also comparison with data from the literature.

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CORYDINE AND OXOHERNAGINE FROM BARKS OF ALSEODAPHNE PENDULIFOLIA GAMB.

Muhammad Hafiz Husna Hasnan[1], Kartini Ahmad[2], Mohamad Azmi Mohamad Taib[3], Khalijah Awang[4], Mohd Azlan Nafiah[5]
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[1]hafiz_husna87@yahoo.com, [2]kartini@fsmt.upsi.edu.com.my, [3]Mohamad.Azmi@icsn.cnrs-gif.fr, [4]khalijah@um.edu.my, [5]azlan@fsmt.upsi.edu.my

Abstract

The species of *Alseodaphne pendulifolia* Gamb. (Lauraceae family) is locally known as *medang* or *tejur* commonly found in lowland forest in Perak, Terengganu, Pahang, Selangor, Johor, and Borneo. A phytochemical study on this species yielded two type of alkaloids that aporphine; Corydine **1** and oxoaporphine; Oxohernagine **2**. These two compounds was isolated from the acid-base crude extract from barks of this plant that subjected to extensive column chromatography (CC) and preparative thin layer chromatography (PTLC). The structural elucidation was performed via spectral methods, notably 1D- and 2D-NMR, UV, IR, and MS.

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NPMC 34P

Alkaloids Isolated from the Bark of *Alseodaphne perakensis*

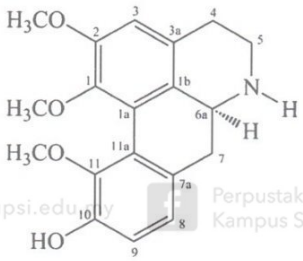
Mahfuzah Md Nazimuddin [1]*, Muhammad Hafiz Husna Hasnan[1], Kartini Ahmad [1], Khalijah Awang [2], A. Hamid A. Hadi [2], and Mohd Azlan Nafiah [1].

[1]*Department of Chemistry, Faculty of Science and Mathematics, University of Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak, Malaysia.*

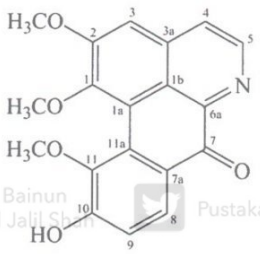
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zaf141dekly@gmail.com

The phytochemical study of the stem bark of *Alseodaphne perakensis* (Lauraceae) had been carried out. The dichloromethane extract produced two alkaloids namely hernagine **1** and oxohernagine **2**. The isolation and purification of the alkaloids were achieved using CC and PTLC techniques. The structural elucidations were performed by spectral methods mainly UV, IR, NMR including 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HMQC/HSQC, and HMBC).



1



2

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OR-20

A New Fistularin-3 derivative from an Australian Sponge *Pseudoceratina sp.*

Pinus Jumaryatno[1,4], Tina Skinner-Adams[2,3], Katherine T. Andrews[2,3], Rohan A. Davis[2], Joanne T. Blanchfield[4] and Mary J. Garson*[4]

[1]Pharmacy Department, Faculty of Mathematic and Natural Sciences, Universitas Islam Indonesia, Yogyakarta 55584, Indonesia; [2]Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia; [3]Queensland Institute of Medical Research, Locked Bag 2000, Herston 4029, Australia; [4]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia.
Email: m.garson@uq.edu.au

Chemical investigation of an Australian marine sponge, *Pseudoceratina sp.*, collected from Mooloolaba South-East Queensland has provided a series of bromotyrosine metabolites from a simple modified tyrosine to more elaborate linear compounds. Six known compounds, (+)-aeroplysinin-1 (1), a mixture of 19-deoxyfistularin-3 (2) and 11-deoxyfistularin-3 (3), 11,19-dideoxyfistularin-3 (4), aerothionin (5) and homoaerothionin (6) have been isolated together with a new fistularin-3 derivative, 11-deoxy-19-ketofistularin-3 (7). The structures of all compounds were characterised using 1D and 2D NMR techniques, MS and by comparison with literature data. Two selected fistularin-3 derivative metabolites 4 and 7 were screened for *in vitro* activity against chloroquine-sensitive (3D7) *P. falciparum* malaria parasites and showed antimalarial activity with IC_{50} values of 5.4 and 2.8 μM, respectively.

Keywords: *Pseudoceratina sp.*; bromotyrosine; fistularin-3; antimalarial

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OR-21

Antiplasmodial Active Compound from Hernagine Type of Aporphine Alkaloids

Muhammad Hafiz Husna Hasnan[1], Kartini Ahmad[1], Mohamad Nurul Azmi Mohamad Taib[2], Khalijah Awang[2], A. Hamid A. Hadi[2] and Mohd Azlan Nafiah[1]

[1]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia; [2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
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Phytochemistry investigation on alkaloids from *Alseodaphne pendulifolia* Gamb. yielded 3 types of aporphines; Hernagine **1**, N-methylhernagine **2**, and Oxohernagine **3**. Preliminary screening on alkaloids crude extract from this species exhibited an antiplasmodial activity (0.804 μg/ml IC_{50}) against *Plasmodium falciparum*, K1 isolate (resistant strain). Further investigation on isolated compound was found to possess antiplasmodial activity (0.738 - 1.814 μg/ml IC_{50}). This study involves extraction, separation by using various chromatography methods and structural elucidation by spectroscopic technique such as IR, NMR including 1D, 2D-NMR and MS.

Keywords: Aporphine; Alkaloid; Hernagine; *Alseodaphne pendulifolia*; Antiplasmodial

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OR-22

A Natural Soft Polymer used as Humidity Barrier as well as to Compact Ancient Raw Materials

Manuel Navarrete, Trinidad Martínez, Miguel A. Zúñiga

Faculty of Chemistry, National University of Mexico, CU, 04510 Mexico City, Mexico.
Email: jmnat33@unam.mx

Humidity coming from underground as well as environment goes into antique raw materials by capilarity, and it is one, if not the most important factor, for damaging the cultural goods all over the world, mainly when those so appreciated monuments are, for example, either cave or mural paintings, not to mention sculptures made out from materials with a certain degree of porosity. This paper describes how to obtain a warm polymer solution, which if it is sprinkled on the surface of treated materials penetrates through them by the same capilarity, origin of its damage, to create a solid barrier against humidity, coming either from underground or environment, and compacting the loose material at same time that it becomes cool and solid. This sort of porosity plug is not at all rigid and it does not form a definite bound between treated and untreated material portions, which creates

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Objective: To investigate the anticancer effect of Orthosiphon stamineus against human OSCC *in vitro.*

Methods: The proliferative activity of the OSCC was investigated by the doubling time assay. Proliferation essay was done to assess the anti-proliferative effect of *Orthosiphon stamineus* aqueous extract by treating the human OSCC with 0 – 300 µg/ml of the aqueous extract. The efficacy of *Orthosiphon stamineus* to inhibit the recovery of the OSCC colonies was measured by clonogenic assay. Caspase-3 activity was investigated using colorimetric assay.

Results: The results obtained from the human tumor colony-forming assay showed that *Orthosiphon stamineus* has suppressed the recovery of OSCC colonies in dose-dependant manner. *Orthosiphon stamineus* inhibits the clonogenic potential of OSCC with minimal cell surviving fraction of 0.0015. The surviving percentage of the proliferating OSCC decreases by increasing the concentration of the extract. Caspase activity was also enhanced by Orthosiphon stamineus.

Conclusion: The use of *Orthosiphon stamineus* may be beneficial for the suppression of the human OSCC and it may serve as a chemopreventive or therapeutic agent for the oral cancer.

Keywords: *Orthosiphon stamineus*; OSCC; Anti-cancer

P-122

Phytochemical Study on Alkaloids from Bark of *Alseodaphne perakensis* (Lauraceae) and Their Bioactivity

Mahfuzah Md Nazimuddin*[1], Muhammad Hafiz Husna Hasnan[1], Kartini Ahmad[1], Khalijah Awang[2], A. Hamid A. Hadi[2] and Mohd Azlan Nafiah[1]

[1]Department of Chemistry, Faculty of Science and Mathematics, University of Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia; [2]Centre of Natural Products and Drug Discovery, Block D, Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
Email: zaf141dekly@gmail.com

The purpose of this study was to isolate and identify compounds from the bark of *Alseodaphne perakensis* (Lauraceae) which commonly known as Medang to validate and evaluate the use of this species in antimalaria activity. The comprising compounds from the dichloromethane crude extract were separated using column chromatography and the structures of the compounds were elucidated using spectroscopic analysis. The identified compounds were aporphine alkaloids; corydine 1 and oxohernagine 2. These isolated compounds show IC_{50} values 0.404 µg/ml and 0.738 µg/ml, respectively against *Plasmodium falciparum,* K1 isolate (resistant strain).

Keywords: Lauraceae; *Alseodaphne perakensis*; aporphine alkaloid; corydine; oxohernagine

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P-123

Evaluation of the Acute, Sub-Chronic Toxicity and the Antioxidant Effect of Ellagic Acid in Smoker and Non Smoker an Experimental Study in Rats.

Mazen.M.Jamil, Fouad Hussain Al-Bayaty, Farha Ariffin, Rami Al-Batran

Faculty of Dentistry, Universiti Teknologi MARA, 40450 Shah Alam, Malaysia.
Email: fouad@salam.uitm.edu.my

Objective: The aim of this study is to investigate the possible protective antioxidant role of the Ellagic acid (EA) on smoker and non Smoker rats using biochemical and antioxidant approaches and to evaluate the acute and sub chronic toxicity test of Ellagic Acid.

Materials and Methods:Twenty four Adult male Sprague-dawley (SD) rats were randomly divided in four groups (6 rats each group). Group 1 control, Group 2 received Ellagic Acid, groups 3 and 4 were injected subcutaneously with (0.5 mg/kg) Nicotine (Ni) for one month to convert the animals into smoker. Then, group 4 was gavaged with EA (50 mg/kg) for 30 days using gastric tube. Animal sacrificed after 30 days. Antioxidants enzyme parameters, Superoxide Dismutase (SOD), Malondialdehyde (MDA), Catalze (CTA), Nitric oxide (NO) levels were evaluated in serum and liver, kidney, heart homogenate. Cotinine level was measured in serum.

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P-009

PHYTOCHEMICAL STUDY ON NORAPORPHINE ALKALOIDS ISOLATED FROM BARKS OF *Alseodaphne pendulifolia* Gamb

Muhammad Hafiz Husna Hasnan[1], Aina Farahiyah Abdul Manan[2], Kartini Ahmad[3], Khalijah Awang[4], A. Hamid A. Hadi[5] and Mohd Azlan Nafiah[6]
[1,2,3,6]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia
[4,5]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

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Malaysia is one of most biodiversity country with the oldest rain forest in the world. Among the 15,000 species of flowering plant thrives here, *Alseodaphne pendulifolia* Gamb is one of the species that contribute to this statistic which belong to Lauraceae family. A phytochemical studies on barks of this species involved extraction and isolation of alkaloid by using various chromatographic method including column chromatography, preparative thin layer chromatography and chromatotron. Structural determination was elucidated via spectroscopic method such as 1D and 2D NMR, UV, IR and MS. Two alkaloids were successfully isolated from this species; hernagine and hernovine, which classified as noraporphine alkaloid from aporphine skeleton.

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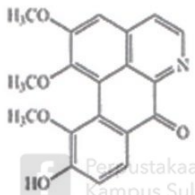
OR-018

HERNAGINE TYPE OF APORPHINE ALKALOIDS FROM *ALSEODAPHNE PERAKENSIS*

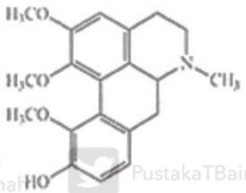
Mahfuzah Md Nazimuddin[1], Muhammad Hafiz Husna Hasnan[1], Mohd Azlan Nafiah[1], Khalijah Awang[2] and Kartini Ahmad[5]

[1]Chemistry Department, Faculty of Science and Mathematics, Universiti PendidikanSultan Idris, 35900 Tanjong Malim, Perak, Malaysia.
[2]Centre for Natural Products and Drug Discovery, Block D, Department of Chemistry, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail: zaf141deklv@gmail.com

The phytochemical study of the stem bark of *Alseodaphne perakensis* (Lauraceae) had been carried out The dichloromethane extract produced two hernagine type of aporphine alkaloids namely oxohernagine **1** and *N*-methylhernagine **2**. The isolation and purification of the alkaloids were achieved using CC and PTLC techniques. The structural elucidations were performed by spectral methods mainly NMR including 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (COSY, HMQC/HSQC, and HMBC).



(1)



(2)

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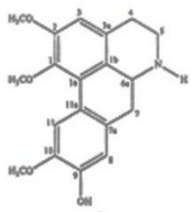
OR-006

APORPHINE ALKALOIDS FROM LEAVES OF *ALSEODAPHNE PEDUNCULARIS*

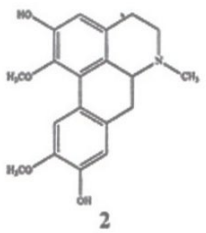
Ulil Amna, Muhammad Hafiz Husna Hasnan, Siti Nor Fadzilah Mohammad, Kartini Ahmad, Mohd Azlan Nafiah

Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjung Malim, Perak, Malaysia.
E-mail: ulil_amna@yahoo.com

The aim of this study was to isolate and identify alkaloid compounds from the leaves of *Alseodaphne peduncularis*. Two aporphines alkaloid have been afforded and identified as Laurotetanine **1** and Boldine **2**. This phytochemical study involved extraction, separation and purification by using various chromatography techniques. The structural elucidation performed by using spectoscopic techniques such as UV, IR, and NMR including 1D NMR (^{1}H, ^{13}C, and DEPT) and 2D NMR (HMQC, HMBC, and COSY).



1



2

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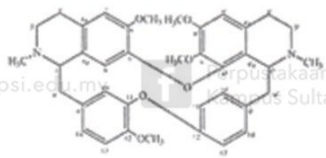
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P-005

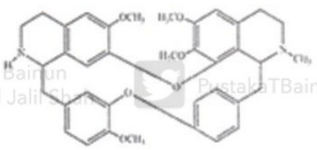
ALKALOIDS FROM ROOTS OF *Alseodaphne Corneri* Kosterm (Lauraceae)

Siti Nor Fadzilah Mohammad[1], Kartini Ahmad[1], Muhammad Hafiz Husna Hasnan[1]
Jennie Khoo Hui Chen[1], A. Hamid A. Hadi[2], Khalijah Awang[2], Mohd Azlan Nafiah[1]
[1]Dept. of Chemistry, Faculty of Sciences and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak
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Lauraceae distributed in the lowland and becoming more abundant in the mountains between 1200 and 1600 m altitude. Major producing states in Peninsular Malaysia including Kelantan, Perak, Terengganu, Negeri Sembilan and Kedah (Gan & Lim, 2004) *Alseodaphne corneri* Kosterm belongs to Lauraceae family. The *Alseodaphne* genus is well known for their alkaloid bearing plants that have the isoquinoline structures. A phytochemical study on the roots of *Alseodaphne corneri* had been carried out. The roots were air dried, grinded and extracted with dichloromethane. The extract was then proposed to further isolation and separation process such as column chromatography and preparative thin layer chromatography to obtain pure alkaloid compound. Two bisbenzylisoquinoline alkaloids were successfully isolated from the roots of *Alseodaphne corneri* namely gyrolidine (**1**) and 2-norobaberine (**2**). The structural elucidation was performed by spectral methods mainly NMR, UV, IR and MS. Finally, determination of compounds was further confirmed by comparison with previous works.



1



2

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P-008

ISOLATION OF APORPHINE ALKALOID FROM THE LEAVES OF *Alseodaphne corneri* Kosterm

Jennie Khoo Hui Chen[1], Kartini Ahmad[1], Muhammad Hafiz Husna bin Hasnan[1], Siti Nor Fadzilah Mohammad [1], Khalijah Awang[2], A. Hamid A. Hadi[2], Mohd Azlan Nafiah[1]
[1]Department of Chemistry, Faculty of Science and Mathematics, Sultan Idris Education University, 35900 Tanjong Malim, Perak.
[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur.
* azlan@fsmt.upsi.edu.my (Corresponding author)

The isolation and purification of the leaves of *Alseodaphne corneri* Kosterm yielded two aporphines; norlirioferine **1** and lirioferine **2**. This phytochemical study involves extraction, separation by using various chromatographic methods and structural determination by spectroscopic technique such as UV, IR, NMR including 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (COSY, NOESY, HMQC/HSQC, and HMBC) and MS.

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APPENDIX 2

Antiplasmodial Results Detail

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HN-NonLin

ANALYSIS OF MALARIA IN VITRO DRUG SENSITIVITY DATA (HN-NonLin V 1.1)

HOME
HELP

CALCULATE
RESET

EXPERIMENT NAME HELP: HRP 2 test

DATE: 10/10/2012

COLUMN LABELS HELP

	1495	1496	1497	1498	1499	1500	1501	1502	1503	1504	11	12

PLATE RESULTS HELP

	1495	1496	1497	1498	1499	1500	1501	1502	1503	1504	11	12
A	1.81	1.81	1.81	1.81	1.81	1.81	1.81	1.81	1.81	1.81		
B	1.69	1.22	1.51	1.47	1.73	1.57	1.87	1.91	1.73	1.65		
C	1.75	1.08	1.56	1.49	1.63	1.48	2.08	1.94	2.12	1.53		
D	1.54	0.96	1.61	1.32	1.40	1.32	1.47	1.69	1.87	1.29		
E	1.28	0.75	1.44	1.19	1.07	0.99	1.79	1.76	1.56	1.03		
F	1.15	0.53	1.13	1.02	0.96	0.85	1.09	1.22	0.71	0.71		
G	0.91	0.54	1.22	1.09	0.85	0.86	0.95	1.06	0.75	0.77		
H	0.79	0.64	1.00	0.91	0.92	0.98	1.05	1.02	0.78	0.83		

DRUG CONCENTRATIONS for serial dilution (x2) enter highest concentration only! HELP

	1495	1496	1497	1498	1499	1500	1501	1502	1503	1504	11	12
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0
C	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.0	0.0
D	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0
E	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0	0.0
F	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	0.0	0.0
G	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	0.0	0.0
H	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7		

PAGE HEADER HELP: Malaria in vitro drug sensitivity

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Malaria in vitro drug sensitivity

Plate Readings and ECs

HRP 2 test **DATE: 10/10/2012**

HN-NonLin V1.1

PLATE RESULTS HELP

	1495	1496	1497	1498	1499	1500	1501	1502	1503	1504	11	12
A	1.81467	1.81467	1.81467	1.81467	1.81467	1.81467	1.81467	1.81467	1.81467	1.81467	0	0
B	1.6885	1.2185	1.509	1.465	1.734	1.565	1.8715	1.9065	1.728	1.6475	0	0
C	1.7465	1.0845	1.5645	1.4865	1.6345	1.483	2.0775	1.9415	2.12	1.528	0	0
D	1.538	0.958	1.613	1.3235	1.4035	1.317	1.4715	1.687	1.87	1.293	0	0
E	1.2765	0.7545	1.4375	1.1925	1.073	0.991	1.794	1.763	1.555	1.033	0	0
F	1.1525	0.526	1.131	1.0215	0.958	0.8475	1.094	1.2215	0.7075	0.7085	0	0
G	0.908	0.535	1.218	1.089	0.85	0.858	0.948	1.0565	0.748	0.773	0	0
H	0.789	0.6435	1	0.9145	0.919	0.9785	1.0505	1.018	0.7845	0.8255	0	0

DRUG CONCENTRATIONS HELP

	1495	1496	1497	1498	1499	1500	1501	1502	1503	1504	11	12
Max	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	0.0	0.0

REGRESSION PARAMETERS HELP

	Polynome	EC_{50}	EC_{90}	EC_{95}	EC_{99}	R^2
1495	3	2.208	8.576	10.930	14.300	0.9853
1496	3	0.404	3.396	3.659	3.844	0.9307
1497	3	1.814	10.715	13.656	16.685	0.8555
1498	3	0.738	11.537	13.927	15.596	0.9335
1499	3	1.143	3.515	4.332	5.657	0.9922
1500	3	0.804	2.528	3.037	3.617	0.9773
1501	3	2.135	5.086	5.847	6.764	0.8037
1502	3	2.786	6.052	6.809	7.598	0.9508
1503	3	2.228	3.551	3.738	3.892	0.9593
1504	3	1.067	3.204	3.558	3.847	0.9978
11	3					
12	3					

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INDIVIDUAL REGRESSION PARAMETERS 10/10/2012

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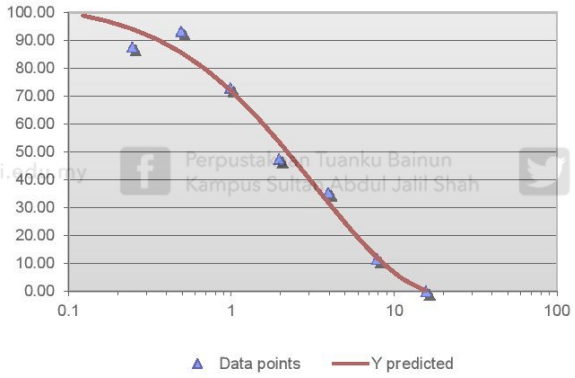
HN-NonLin V1.1

DRUG CONC	log (CONC+1)	Y obs	Y %	Y% pred
0.00	0.00	1.815	100.00	98.91
0.25	0.10	1.689	87.70	94.12
0.49	0.17	1.747	93.35	85.69
0.98	0.30	1.538	73.03	72.24
1.96	0.47	1.277	47.53	53.57
3.93	0.69	1.153	35.44	31.94
7.85	0.95	0.908	11.60	12.10
15.70	1.22	0.789	0.00	0.09

EC	Conc
EC50	2.208
EC90	8.576
EC95	10.930
EC99	14.300

Correl	
R^2	0.9853
Polynome	3

Enter order (1-7) of polynome ▲



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INDIVIDUAL REGRESSION PARAMETERS 10/10/2012

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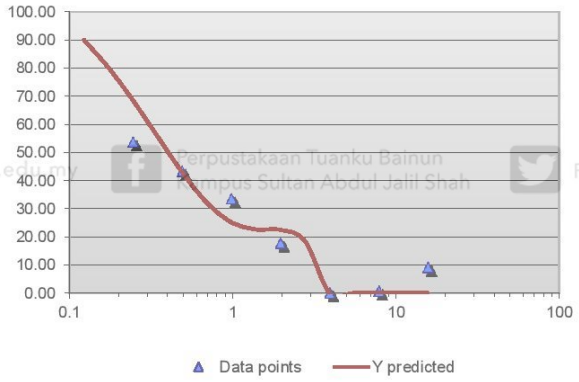
HN-NonLin V1.1

DRUG CONC	log (CONC+1)	Y obs	Y %	Y% pred
0.00	0.00	1.815	100.00	90.06
0.25	0.10	1.219	53.74	68.53
0.49	0.17	1.085	43.34	42.96
0.98	0.30	0.958	33.52	25.16
1.96	0.47	0.755	17.73	22.48
3.93	0.69	0.526	0.00	0.00
7.85	0.95	0.535	0.70	0.00
15.70	1.22	0.644	9.12	0.00

EC	Conc
EC50	0.404
EC90	3.396
EC95	3.659
EC99	3.844

Correl	
R^2	0.9307
Polynome	3

Enter order (1-7) of polynome ▲



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INDIVIDUAL REGRESSION PARAMETERS 10/10/2012

HRP 2 test **Column: 1497**

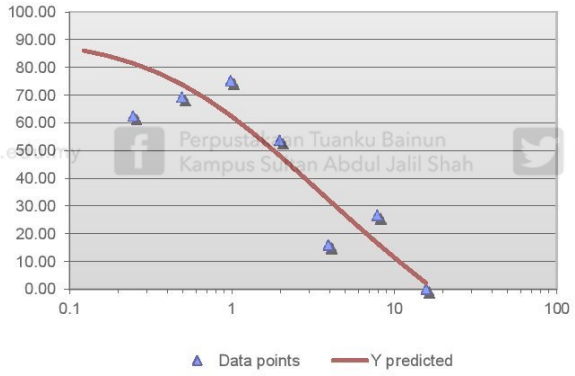
HN-NonLin V1.1

DRUG CONC	log (CONC+1)	Y obs	Y %	Y% pred
0.00	0.00	1.815	100.00	86.06
0.25	0.10	1.509	62.48	81.49
0.49	0.17	1.565	69.29	73.89
0.98	0.30	1.613	75.25	62.64
1.96	0.47	1.438	53.70	48.25
3.93	0.69	1.131	16.08	32.35
7.85	0.95	1.218	26.76	16.67
15.70	1.22	1.000	0.00	2.20

EC	Conc
EC50	1.814
EC90	10.715
EC95	13.656
EC99	16.685

Correl	
R^2	0.8555
Polynome	3

Enter order (1-7) of polynome ▲



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INDIVIDUAL REGRESSION PARAMETERS 10/10/2012

HRP 2 test **Column: 1498**

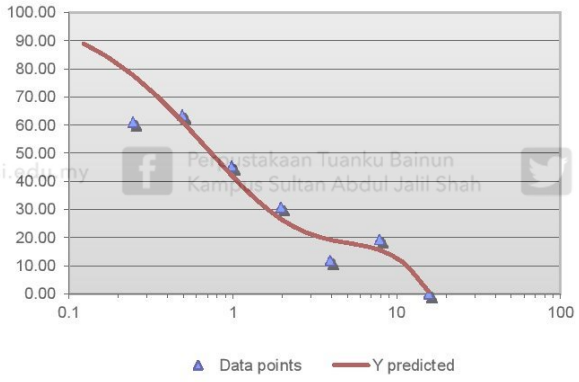
HN-NonLin V1.1

DRUG CONC	log (CONC+1)	Y obs	Y %	Y% pred
0.00	0.00	1.815	100.00	88.97
0.25	0.10	1.465	61.16	77.74
0.49	0.17	1.487	63.54	61.29
0.98	0.30	1.324	45.44	42.18
1.96	0.47	1.193	30.88	26.62
3.93	0.69	1.022	11.89	19.20
7.85	0.95	1.089	19.39	15.56
15.70	1.22	0.915	0.00	0.74

EC	Conc
EC50	0.738
EC90	11.537
EC95	13.927
EC99	15.596

Correl	
R^2	0.9335
Polynome	3

Enter order (1-7) of polynome ▲



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INDIVIDUAL REGRESSION PARAMETERS 10/10/2012

HRP 2 test **Column: 1499**

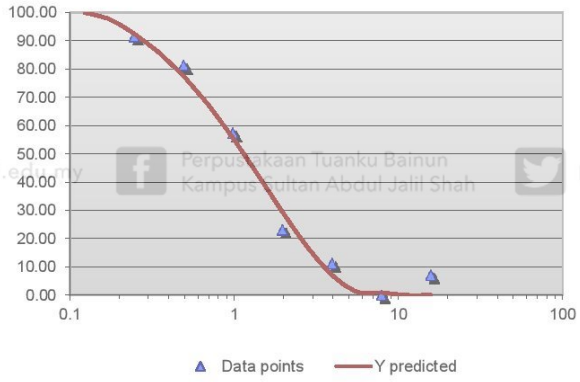
HN-NonLin V1.1

DRUG CONC	log CONC+1	Y obs	Y %	Y% pred
0.00	0.05	1.815	100.00	100.00
0.25	0.10	1.734	91.64	92.53
0.49	0.17	1.635	81.32	77.53
0.98	0.30	1.404	57.38	55.59
1.96	0.47	1.073	23.12	29.46
3.93	0.69	0.958	11.20	7.18
7.85	0.95	0.850	0.00	0.86
15.70	1.22	0.919	7.15	0.00

EC	Conc
EC50	1.143
EC90	3.515
EC95	4.332
EC99	5.657

Correl	
R^2	0.9922
Polynome	3

Enter order (1-7) of polynome ▲



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INDIVIDUAL REGRESSION PARAMETERS 10/10/2012

HRP 2 test **Column: 1500**

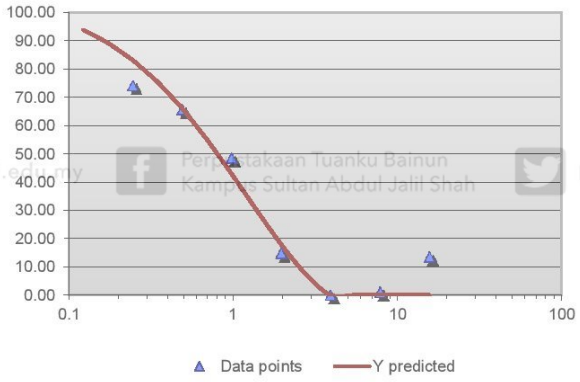
HN-NonLin V1.1

DRUG CONC	log CONC+1	Y obs	Y %	Y% pred
0.00	0.05	1.815	100.00	93.84
0.25	0.10	1.565	74.19	83.12
0.49	0.17	1.483	65.71	66.04
0.98	0.30	1.317	48.54	42.86
1.96	0.47	0.991	14.84	17.98
3.93	0.69	0.848	0.00	0.00
7.85	0.95	0.858	1.09	0.00
15.70	1.22	0.979	13.54	0.00

EC	Conc
EC50	0.804
EC90	2.528
EC95	3.037
EC99	3.617

Correl	
R^2	0.9773
Polynome	3

Enter order (1-7) of polynome ▲



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