Aphorpines and benzylisoquinoline alkaloids from bark of *Cryptocarya crassinervia*

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Abstract. Two aphorpine alkaloids, (+)-lirinine **1** and (+)-lirioferine **2** and two bezylisoquinoline alkaloids (+)-reticuline **3** and (-)-*N*-Methylisococlaurine **4** were isolated from bark of *Cryptocarya crassinervia*. The structures were elucidated by spectral analysis, including 1D-NMR (^{1}H, ^{13}C, DEPT), 2D-NMR (COSY, HMQC, HMBC), UV, IR, and MS and comparison with the published data.

Key words*:* Cryptocarya crassinervia, lauraceae, benzylisoquinoline, aphorpin, alkaloid

Introduction

Past study on the alkaloid content of the species *C. crassinervia* provided phenantrenes alkaloid compounds. Isolation of The bark of this species provided two new phenantrene alkaloids, 2-hydroxyatherosperminine and *N*-demethyl-2-methoxyatherosperminine (Awang, et al.., 2008). Genus *Cryptocarya* contained many types of compounds mainly alkaloids. In Indonesia and Malaysia this species is known as *medang*. The species is a medium sized tree to 20 m tall, rarely to 30 m tall and 125 cm girth. Bole is brownish, scaly. Inner bark is reddish brown, granular. Sapwood is pale yellow. The leaves, stalk 0.7-2.5 cm long, blade thickly leathery, elliptic to oblong or almost rounded, 12-32 x 8-15 cm, upper surface glabrous except for midrib which is covered with short velvety hairs, very rarely with a short up tip or notched. The secondary nerves is 5-8 pairs, curving and joining near margin, prominently, raised below. Tertiary nerves and reticulations raised below. The flowers are in terminal and axillary reddish hairy panicles. The fruit is greenish, oblong to ovate, 2.5 x 1.5 cm when dry, with faint ridges. The species occurrence from lowlands to hill forest up to 900 m and distributed in Malaysia and Indonesia (Ng, 1989).

Material and Methods

General Experimental Procedures

The 1-D and 2D-NMR spectra were recorded in chloroform-D and Acetone-D6 on a JEOL JNM-FX400. The mass spectra were measured on a JMS 700 spectrometer using NBA as the matrix for FAB analysis. The Automass Thermofinnigan was used for HR ESI^{+} and ESI^{-} analysis. The EIMS spectra were obtained on Shimadzu GC-MS QP2000A spectrometer 70 eV. The IR spectra were recorded on the Perkin Elmer 1600 Series FTIR using $CHCl_3$ as a solvent. The UV spectra were measured on a UV visible recording spectrophotometer, Model Shimadzu UV-160A with methanol as a solvent. The industrial and analytical reagent grade solvent was used for extraction and column chromatography. Silica gel 60 and G-60 70-230 mesh ASTM (Merck 774) were used for Column Chromatography. Aluminium and glass supported silica gel 60 F_{254} were used for Thin Layer Chromatography and preparative TLC, respectively.

Procedure

Extraction and isolation

Extraction of the bark was carried out by exhaustive extraction using soxhlet extractor. The milled dried sample was defatted with n-hexane and the extract was then dried on the rotary evaporator. The plants material was dried and moistened with 10% NH_3 and left overnight. They were then successively re-extracted with dichloromethane and then check with a Mayer's reagent test after each extraction to make sure the extraction was completed.

Dichloromethane extracts were concentrated under reduced pressure to a volume of about 500 ml and tested for alkaloids content using TLC and spraying with Dragendorff's reagent. The dichloromethane extracts were repeatedly extracted with a solution of 5% hydrochloric acid until Mayer's test negative. The combined extract were then basified with ammonia solution to about pH 11 and then re-extracted with dichloromethane. The crude of alkaloids fraction evaporated under reduced pressure.

Crude of dichloromethane (8 g) were isolated using column chromatography with silica gel 60 as stationery phase. The solvent system used for chromatography was dichloromethane with increasing portion of methanol (gradient elution system). The ratio of the solvent between CH_2Cl_2 and CH_3OH were (100:0; 99:1; 98:2; 97:3; 96:4; 95:5; 94:6). Every fraction was collected (100 mL) and each fraction was tested with TLC plate for their alkaloids. The alkaloid spots were confirmed by spraying with Dragendorff's reagent. The combined groups were isolated again with CC or preparative TLC to purify the alkaloids. Based on pattern of TLC they were divided into eight fractions (A-H). Fraction C (86 mg), CH_2Cl_2-CH_3OH; 98:2, was then purified with CC to gave (+)-lirinine **1** (22 mg). Fraction D (331 mg) eluted with CH_2Cl_2-CH_3OH (96:4) was further separated using CC to gave (+)-lirioferine **2** (63 mg). Fraction F (216 mg) eluted with CH_2Cl_2-CH_3OH (95:5) was further separated using CC to gave (+)-reticuline **3** (72 mg). Fraction G (112 mg) eluted with CH_2Cl_2-CH_3OH (94:6) were further separated using CC and PTLC to gave (-)-*N*-Methylisococlaurine **4** (55 mg).

(+)-lirinine 1

Brownish amorphous solid; $[\alpha]_D^{25}$ $+64^O$ (*c* = 0.015, MeOH); IR (KBr) ν_{max} cm^{-1} 3372, 2923, 2841, 1583; MS (EI, 70 eV), *m/z* 311, 310, 296, 280, 268; UV_{mx} (MeOH), nm 297; ^1H and ^{13}C NMR ($CDCl_3$) *δ*, ppm, see Table 1.

(+)-lirioferine 2

Brownish amorphous solid; $[\alpha]_D^{25}$ $+67^O$ (*c* = 0.02, MeOH); IR (KBr) ν_{max} cm^{-1} 3365, 2924, 2852, 1463; MS (EI, 70 eV), *m/z* 341, 340, 326, 310, 298, 283, 267; UV_{mx} (MeOH), nm 305. ^1H NMR ($CDCl_3$) δ, ppm 6.54 (1H, *s*, H-3), 2.61-2.70 (1H, *m*, H-4_a), 3.14-3.20 (1H, *m*, H-4_b), 2.50-2.57 (1H, *m*, H- H-5_a), 3.05-3.09 (1H, *dd,* J_1 = 11.24 Hz, J_2 = 5.64 Hz, H-5_b), 3.05-3.09 (1H, *dd,* J_1 = 11.24 Hz, J_2 = 5.64 Hz, H-6a), 2.56-2.59 (1H, m, H-7_a), 2.91-2.95 (1H, *dd,* J_1 = 13.92 Hz, J_2 = 4.16 Hz, H-7_b), 6.76 (1H, *s*, H-8), 8.02 (1H, *s*, H-11), 3.61 (3H, *s*, OMe-1), 3.86 (3H, *s*, OMe-2), 3.86 (3H, *s*, OMe-9), 2.53 (*N*-Me); ^{13}C NMR ($CDCl_3$), δ, ppm 144.18(C-1), 126.40 (C-1a), 127.02 C-1b), 152.01 (C-2), 110.80 (C-3), 128.44 (C-3a), 28.68 (C-4), 53.07 (C-5), 62.39 (C-6a), 33.84 (C-7), 129.70 (C-7a), 113.99 (C-8), 145.39 (C-9), 144.19 (C-10), 111.20 (C-11), 123.72 (C-11a), 60.06 (OMe-1), 55.72 (OMe-2), 55.94 (OMe-9), 43.47 (*N*-Me).

(+)-reticuline 3

Brownish amorphous solid; $[\alpha]_D^{25}$ $+29^O$ (*c* = 0.052, MeOH); IR (KBr) ν_{max} cm^{-1} 3400, 2936, 2842, 1593, 1514; MS (EI, 70 eV), *m/z* 329, 192, 177, 137, 122; UV_{mx} (MeOH), nm 296. ^1H NMR ($CDCl_3$) δ, ppm 3.70-3.73 (1H, *t*, H-1), 2.70-2.80 (1H, *m*, H-3), 2.57-2.61 (1H, *m*, H-4_a), 3.14-3.19 (1H, *m*, H-4_b), 6.51 (1H, *s*, H-5), 6.27 (1H, *s*, H-8), 2.70-2.80 (1H, *m*, H- α_a), 3.02-3.07 (1H, *dd*, J_1 = 14.00 Hz, J_2 = 5.60 Hz, H-α_b), 6.73 (1H, *d*, *J* = 2.00 Hz, H-2'), 6.68 (1H, *d*, *J* = 8.00 Hz, H-5'), δ 6.51-6.54 (1H, *dd*, J_1 = 8.40 Hz, J_2 = 2.00 Hz, H-6'), 2.45 (3H, *s*, *N*-Me), 3.80 (3H, *s*, OMe-6), 3.80 (3H, *s,* OMe-4'); ^{13}C NMR ($CDCl_3$), δ, ppm 64.29 (C-1), 46.18 (C-3), 24.46 (C-4), 129.14 (C-4a), 110.51 (C-5), 145.45 (C-6), 143.47 (C-7), 111.85 (C-8), 124.33 (C-8a), 40.71 (C- α), 132.48 (C-1'), 120.90 (C-2'), 145.19 (C-3'), 145.42 (C-4'), 110.58 (C-5'), 115.71 (C-6'), 41.78 (*N*-Me), 55.84 (OMe-6), 55.70 (OMe-4').

(-)-*N*-Methylisococlaurine 4

Brownish amorphous solid; $[\alpha]_D^{25}$ -100^O (c = 0.002, MeOH); IR (KBr) ν_{max} cm^{-1} 3378, 2922, 2851, 1597; MS (EI, 70 eV), *m/z*; 192, 177, 176, 148, 107, 92; UV_{mx} (MeOH), nm 292, 223. ^1H NMR (Acetone-D6), *δ*, ppm 3.57-3.61 (1H, *t*, H-1), 2.61-2.67 (2H, *m*, H-3), 2.43-2.50 (2H, *m*, H-4), 6.57 (1H, *s*, H-5), 6.49 (1H, *s*, H-8), 2.78-2.83 (2H, *m*, H-α), 6.95-6.97 (2H, *d*, *J* = 8.50 Hz, H-2' and H-6'), 6.65 (2H, *d*, *J* = 8.50 Hz, H-3' and H-5'), 2.39 (3H, *s*, *N*-Me), 3.76 (3H, *s*, OMe-7); ^{13}C NMR (Acetone-D6), *δ*, ppm 65.77 (C-1), 48.29 (C-3),

26.06 (C-4), 126.04 (C-4a), 112.26 (C-5), 146.90 (C-6), 145.35 (C-7), 115.08 (C-8), 130.83 (C-8a), 41.32 (C- α), 131.58 (C-1'), 131.56 (C-2' and C-6'), 115.54 (C-3' and C-5'), 43.02 (*N*-Me), 55.27 (OMe-7).

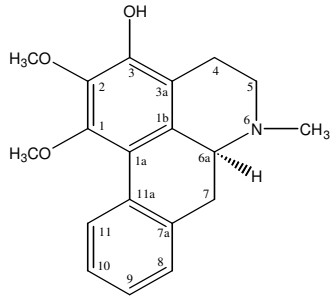
RESULTS AND DISCUSSION

(+)-lirinine 1

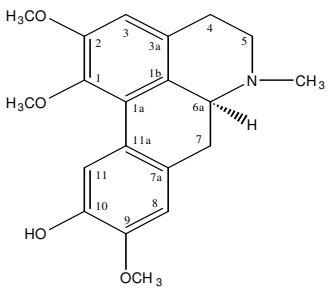
Alkaloid **1** with $[\alpha_D^{25}$ +64^O (*c* = 0.05, MeOH) was isolated as brownish amorphous solid. The UV spectrum exhibited maxima at 297 nm typical of a 1,2,3-trisubtituted aporphine (Chen and Chang, 1978). The IR spectrum revealed a strong absorption at 3372 cm^{-1} indicated the presence of hydroxyl in the structure. The ESI^+ mass spectrum exhibited a molecular ion peak at m/z 312.2 $[M+H]^+$, which correlated to a molecular formula of $C_{19}H_{21}NO_3$ whereas the EI mass spectrum showed a molecular ion peak at m/z 311 corresponding to a molecular formula of $C_{19}H_{21}NO_3$. The base peak at m/z 310 was due to the loss of H and the peak at m/z 296 $[M-15]^+$ was due to the loss of CH_3, respectively. Fragmentation ion at m/z 280 $[M-31]^+$ suggested the C-1 was substituted by a methoxyl group. Moreover, the presence of fragmentation at m/z 268 $[M-43]^+$ indicated that the alkaloid was an *N*-substituted (*N*-CH_3) aphorphine (Ohashi, et al..,1963).

The ^1H NMR spectrum (Table 3.31) revealed two methoxyl signals at δ 3.58 and δ 3.86 which were probably attached to C-1 and C-3, respectively. A downfield chemical shift was observed as a doublet (*J* = 8.00 Hz) at δ 8.18-8.20 and it is a typical resonating of H-11. Three aromatic protons were observed as a multiplet at δ 7.16-7.29 assigned to H-8, H-9, and H-10, respectively. One sharp peak attributed to the *N*-methyl resonated as a singlet at δ 2.56. The aliphatic protons gave a multiplet peak between δ 2.46 to 3.12. The ^{13}C NMR spectrum revealed the presence of 19 carbons and the DEPT experiment showed the presence of one *N*-methyl signal, two methoxyl groups, three methylenes, five methines and eight quaternary carbons.

The position of the methoxyl group was identified *via* NOE-diff. experiment and it showed that irradiation of OMe-1 resulted in 1.7% enhancement of H-11, thus confirmed the methoxyl group belonged to C-1. Finally, comparison with the literature values (Chen and Chang, 1978 and Hara, et al..,1981), it is confirmed that the alkaloid **1** was (+)-lirinine.



1



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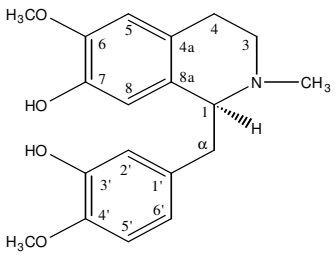

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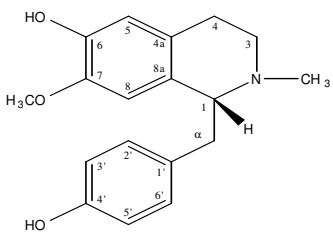

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Table 1. ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR (DCl_3, 100 MHz) data of **1**

Position	δ_H , ppm (J in Hz)	δ_C (ppm)	COSY	HMQC	HMBC
1	-	144.04	-	-	-
1a	-	121.52	-	-	-
1b	-	122.48	-	-	-
2	-	144.10	-	-	-
3	-	141.06	-	-	-
3a	-	126.40	-	-	-
4	2.81-2.84, *m* 2.93-2.94, *m*	23.14	H-4/H-5	H-4	1b, 3
5	2.46-2.52, *m* 3.12-3.15, *m*	52.85	H-4/H-5	H-5	3a, 6a
6a	3.05-3.12, *m*	62.19	H-6a/H-7	H-6a	3a, 7a, 1a
7	2.63-2.70, *m* 3.05-3.12, *m*	34.37	H-6a/H-7	H-7	1b, 8
7a	-	131.64	-	-	-
8	7.16-7.29, *m*	127.00	H-8/H-9	H-8	10, 11a
9	7.16-7.29, *m*	127.13	H-8/H-9, H-9/H-10	H-9	7a, 11
10	7.16-7.29, *m*	127.23	H-9/H-10, H-10/H-	H-10	8, 11a
11	8.18-8.20, *d*, *J*=8.00	127.96	11	H-11	1a, 7a, 9
11a	-	135.48	H-10/H-11	-	-
OMe-1	3.58, *s*	60.42	-	-	1
OMe-2	3.86, *s*	60.06	-	-	2
N-Me	2.56, *s*	43.35	-	-	5, 6a

(+)-lirioferine 2

Alkaloid **2**, with $[\alpha]_D^{25}$ $[\alpha]_D^{25}$ $+67^O$ (*c* = 0.02, MeOH) was isolated as a brownish amorphous solid. Spectrum of UV showed absorption maximum at 305 nm suggesting that **1** was a noraporphine type of alkaloid. Absorption peak at 3376 cm^{-1} indicated the presence of hydroxyl group in IR spectrum. Spectrum of EIMS showed a molecular ion peaks at m/z 341 corresponded to a molecular formula of $C_{20}H_{22}NO_4$. The base peak at m/z 340 was due to the loss of H and m/z 326 $[M-15]^+$ was due to the loss of CH_3, respectively. The fragmentation observed at m/z 310 $[M-31]^+$ suggested that C-1 was substituted by a methoxyl group. The presence of fragmentation at m/z 298 $[M-43]^+$ indicated that alkaloid was an *N*-substituted (*N*-CH_3) aporphine (Ohashi, et al..,1963).

Three aromatic proton signals at δ 6.54 (1H, *s*, H-3); 6.76 (1H, *s*, H-8) and 8.02 (1H, *s*, H-11) displayed in spectrum of ^{1}H NMR. Three methoxyl groups which appeared as a singlet at δ 3.57, δ 3.80 and δ 3.82 were attached to C-1, C-2 and C-9, respectively. One *N*-methyl singlet was observed at δ 2.48 and the aliphatic protons appeared as a multiplet in the region between δ 2.54 to 3.06. The ^{13}C NMR spectrum established the presence of 20 carbons. The DEPT experiment showed four methyls, three methylenes, four methines and nine quaternary carbon signals in the molecule. The structural elucidation was completed by the help of the 2D experiments (COSY, HMQC and HMBC). Comparison with the authentic sample and its data from literature values (Marsaioli, et al..,1979, Chen, et al..,1976, Smolnycki, et al..,1978, Shamma, 1960), confirmed that the alkaloid **2** is indeed (+)-lirioferine.

(+)-Reticuline 3

Compound **3** was isolated as a brownish amorphous solid. The UV spectrum showed absorption band at 293 nm. The IR spectrum showed absorption at 3392 cm^{-1} indicated the presence of hydroxyl group in the structure. This alkaloid exhibited an $[M+H]^+$ in the $(HRESI)^+$ mass spectrum at m/z 330.1691. The EI mass spectrum showed a molecular ion peak at m/z 329 corresponding to a molecular formula of $C_{19}H_{23}NO_4$. A base peak at m/z 192 $[M-137]^+$, was due to the loss of $[C_8H_9O_2]^+$, a characteristic of benzylisoquinoline (Dumontet, et al..,2001).

The ^{1}H NMR spectrum of **3** revealed two methoxyl groups overlapped to each other at *δ* 3.80 corresponding to 6-OMe and 4'-OMe. Five aromatic protons appeared at *δ* 6.73 (*d*, *J* = 2.00 Hz, 1H, H-2'), *δ* 6.68 (*d*, *J* = 8.00 Hz, 1H, H-5'), *δ* 6.51-6.54 (*dd*, J_1 = 8.40 Hz,

J_2 = 2.00 Hz, 1H, H-6′), *δ* 6.51 (*s*, 1H, H-5), *δ* 6.27 (*s*, 1H, H-8). Proton *N*-methyl resonated as a singlet at *δ* 2.45. The ^{13}C NMR spectrum showed there were 19 carbon resonances, which is in agreement with the molecular formula of reticuline. The DEPT spectrum showed the appearance of three methyls, three methylenes and six methines and seven quaternary carbons in the molecule skeleton. The assignment of carbon and hydrogen in the structure was further confirmed by HMBC and HMQC experiment. The reported values data of (Chowdhury, et al.., 1976, Richard, et al..,1990, Castro, et al.,1985 and Jendrzejewski, 1990), support the structure of alkaloid **3**.

(-)-N-methylisococlaurine 4

Alkaloid **4** was isolated as a brownish amorphous solid with $[\alpha]_D^{25}$ -100^O (c=0.02, MeOH). The IR spectrum showed the presence of hydroxyl group at 3378 cm^{-1}. The UV spectrum showed absorption maxima at 292, 223 nm. The molecular ions peak was absent in the EIMS spectrum. However, a fragmentation ion at m/z 192 appeared as a base peak thus, indicating that alkaloid was a benzylisoquinoline type with one methoxyl group in ring A.

The spectrum of ^{1}H-NMR showed one methoxyl proton signal at δ 3.76 appeared as a singlet which most probably attached to C-7. Protons H-5 and H-8 peaks appeared as a singlet at δ 6.57 and δ 6.49, respectively. Four aromatic protons resonated at δ 6.95-6.97 (2H, *d*, *J* = 8.50 Hz, H-2′ and H-6′), 6.65 (2H, *d*, *J* = 8.50 Hz, H-3′ and H-5′). Another signal presence as a singlet at δ 2.39 was belonged to *N*-methyl group. The aliphatic protons appeared as multiplets at the region of δ 2.43-3.61. The COSY spectrum indicated the correlation between H-3′/H-5′ and H-2′/ H-6′.

The ^{13}C NMR spectrum displayed 18 carbon signals in the molecule. The DEPT spectrum showed there are one methoxyl, one *N*-methyl, three methylenes, seven methines and six quaternary carbons. Assignment of all proton and carbon signals, DEPT, HMQC, COSY, NOE-Diff. and HMBC and by comparison with literature values (Bhakuni, et al..,1972 and Dasgupta and Ray, 1979), confirmed that **4** is *N*-methylisococlaurine.

Conclusion

Two aphorpine alkaloids, (+)-lirinine **1** and (+)-lirioferine **2** and two bezylisoquinoline alkaloids (+)-reticuline **3** and (-)-*N*-Methylisococlaurine **4** were isolated from bark of *Cryptocarya crassinervia*.

Acknowledgements

I would like to express my greatest thanks to Professor Dr. Abdul Hamid Abdul Hadi, Professor Dr. Khalijah Awang and Associate Professor Dr. Mat Ropi Mukhtar for their help and encouragement in this research work. I also grateful for the support of Technological and Professional Skill Development Sector Project, TPSDP, No. 465/J11/SPMU-TPSDP/2000 (Indonesian Government) and Vot F, No. F0142/2003B, PPF/FP092/2005C (Malaysian Government).

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