APHORPINE ALKALOIDS FROM BARK OF *Cryptocarya ferrea*

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ABSTRACT

*Isolation, identification and characterization of the compounds isolated from the bark of Cryptocarya ferrea yielded three known aphorpine alkaloids. They are (-)-O-methylisopiline **1**, (+)-norlirioferine **2** and (+)-lirioferine **3**. Structural elucidation was established through several spectroscopic methods, such as 1D-NMR (^{1}H, ^{13}C, DEPT, NOE), 2D-NMR (COSY, NOESY, HMQC, HMBC), UV, IR, and MS and comparison with the published data.*

Keywords: *Cryptocarya ferrea; (-)-o-methylisopiline, (+)-norlirioferine, (+)-lirioferine*

INTRODUCTION

Plants of the Lauraceae family are known to contain a variety of alkaloids and other chemical constituents, some of which are reported to have interesting pharmacological properties. Amongst Lauraceae family, *C. ferrea* is the species that has never been studied for its alkaloid constituents. However, other species of *Cryptocarya* have been previously reported. Using combination of reverse-phase liquid chromatography using ion-pair solvent system techniques, (+)-(1*R*, 1a*R*)-1a-hydroxymagnocurarine, oblongine, methyloblongine and xanthoplanine were isolated from *Cryptocarya konishii* [1]. Two alkaloids, (±)-romneine**,** a benzylisoquinoline, and cryprochine, a proaphorpine, were isolated from the leaves and bark of *C*. *Chinesis* [2]. The Investigation and isolation of the leaves of *C. chinensis* yielded ten alkaloids, (-)-isocaryachine-*N*-oxide, Isoboldine-*β*-*N*-oxide**,** 1-hydroxycryprochine**,** (+)-isocaryachine**,** (+)-caryachine, (-)-caryachine, (-)-isocaryachine, isoboldine, (-)-munitagenine and bisnorargemonine [3].

Fifteen alkaloids were isolated from leaves, bark and root of *C*. *longifolia*, reticuline, *N*-methylcoclaurine, coclaurine, longifolidine, norisocorydine, laurotetanine, *N*-methyllaurotetanine, isoboldine, laurolistine, norargemonine, bisnorargemonine, scoulerine, longifolonine, cryptoleurospermine, and thalifoline [4]. Alkaloid reticuline was isolated from dried bark of *C*. *foveolata* [5]. From phytochemical investigation on the bark of *C*. *amygdalina* were isolated two olifinic acids, cryptocaryic acid and amygdalinic acid and two alkaloids, orientaline and laudanidine [6].

EXPERIMENTAL SECTION

Material

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. The silica and plates were activated at 100 °C in the oven before used.

Bark of *C. ferrea* were collected at Tekai, Jerantut, Pahang, Malaysia. The plant materials were collected and identified by the phytochemical team, Chemistry Department, Faculty of Science, University of Malaya. The specimens were deposited at the Chemistry Herbarium, Faculty of Science, University of Malaya.

Instruments

The 1-D and 2D-NMR spectra were recorded in chloroform-D on a JOEL JNM-FX400. 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 solvent. The optical rotation was obtained on Jasco P1010 with tungsten lamp. The UV spectra were measured on a UV visible recording spectrophotometer, Model Shimadzu UV-160A with methanol as a solvent.

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Procedure

Extraction

Extraction of the bark (2.3 kg) was carried out by maceration overnight. The milled dried sample was defatted with 15 L of n-hexane. The n-hexane 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 25 L of dichloromethane and 10 L of methanol and then check with a Mayer's reagent test after each extraction to make sure the extraction was completed.

Dichloromethane extract were concentrated under reduced pressure to a volume of about 500 mL and tested for alkaloids content using TLC and spraying with Dragendorff's reagent. The dichloromethane extract were extracted with a solution of 5% hydrochloric acid (10 L) until Mayer's test negative. The combined extract were then basified with 10% ammonia solution to about pH 11 and then re-extracted with dichloromethane. The crude of alkaloids fraction were dried with sodium sulphate anhydrous (500 g) and evaporated under reduced pressure.

Isolation

Crude of alkaloids (8 g) were isolated using Column Chromatography with silica gel 60 as stationary phase. The solvent system used for chromatography was dichloromethane with increasing portion of methanol (gradient elution system). The ratio of the solvent between CH_2Cl_2 and CH_3OH were (100:0; 99:1; 98:2; 96:4; 93:7; 90:10; 85:15; 80:10 and 50:50). Fractions were collected every 100 mL and each fraction was tested with aluminium TLC plate for their alkaloids. The alkaloid spots were first detected by UV light (254 and 366 nm) and confirmed by spraying with Dragendorff's reagent. Fraction having spots with the same R_f values and stains were combined and treated as a group. The combined groups were isolated again with CC or preparative TLC to purify the alkaloids.

RESULT AND DISCUSSION

(-)-*O*-methylisopiline 1

Alkaloid (-)-*o*-methylisopiline **1**, with $[\alpha]_D^{25}$ -27° (*c* = 0.015, MeOH) was obtained as a brownish amorphous solid. The UV spectrum exhibited maxima at 283 and 302 nm suggesting that compound is a noraporphine. The IR spectrum showed the presence of an NH group at 3424 cm^{-1}.

The EI mass spectrum revealed a molecular ion peak at m/z 311 which corresponded to a molecular formula of $C_{19}H_{21}NO_3$. The presence of the fragmentation at m/z 282 $[M-29]^+$ indicated that alkaloid was an *N*-unsubstituted (NH) aphorphine [7]. Other significant peaks were also observed at m/z 310 (base peak), indicated the loss of H^+, which was a characteristic of aporphine.

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

Position	δ_H , ppm (J in Hz)	δ_C (ppm)	HMQC	HMBC	δ_H , ppm (J in Hz) *o*-methylisopiline
1	-	150.14	-	-	-
1a	-	122.25	-	-	-
1b	-	131.84	-	-	-
2	-	150.68	-	-	-
3	-	145.46	-	-	-
3a	-	132.02	-	-	-
4	2.64-2.77, *m* 2.80-2.91, *m*	23.58	H-5	1b	
5	2.64-2.77, *m* 3.34-3.38, *m*	42.66	H-4	3a, 6a	
6a	3.73-3.78, *dd*, J_1 =13.68, J_2=4.64	53.80	H-6a	3a, 7,	
7	2.64-2.77, *m*	36.94	H-7	1b, 7a, 8, 11	
7a	-	135.40	-	-	-
8	7.10-7.26, *m*	127.78	H-8	-	7.3, *m*
9	7.10-7.26, *m*	126.95	H-9	-	7.3, *m*
10	7.10-7.26, *m*	128.98	H-10	-	7.3, *m*
11	8.20, *d*, *J* =7.80	127.73	H-11	1a, 7a, 9	8.31, *dd*, J_1=8, J_2=2.2
11a	-	122.75	-	-	-
OMe-1	3.66, *s*	60.65	-	1	3.68, *s*
OMe-2	3.84, *s*	60.37	-	2	3.87, *s*
OMe-3	3.88, *s*	60.94	-	3	3.87, *s*

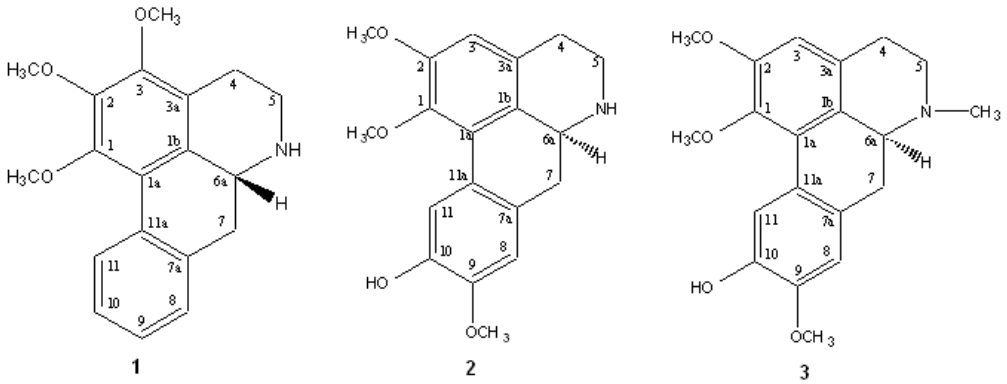

Figure 1. Structure of (-)-*O*-methylisopiline **1**, (+)-norlirioferine **2** and (+)-lirioferine **3**

The ^{1}H NMR spectrum showed the signals of three aromatic methoxyl groups at δ 3.66, δ 3.84 and δ 3.88, attached to C-1, C-2 and C-3, respectively. A downfield chemical shift was observed at δ 8.20 as a doublet (*J* = 7.80 Hz) and it is a typical of the hydrogen bonded H-11. Three aromatic protons were observed at δ 7.20-7.26 as a multiplet attached to C-8 and C-9, and C-10, respectively. The aliphatic protons gave a multiplet peaks between δ 2.64-3.78 ppm. The ^{13}C NMR spectrum gave a total of 19 carbon atoms. The DEPT spectrum revealed three methoxyls, three methylenes, five methines and eight quaternary carbon signals. The assignments of ^{13}C NMR is summarized in Table 1.

The HMBC experiment showed correlation of H-7 to C-1b, C-7a, C-8 and C-11. In addition, the signal of H-11 was correlated to C-1a, C-7a and C-9. While, proton OMe-1, OMe-2 and OMe-3 showed the long range correlations to the signal at C-1, C-2 and C-3, respectively.

Finally, assignment of all proton and carbon signals from DEPT, HMQC, COSY, HMBC and was identified by direct comparison with an authentic literature data [7-8] the alkaloid **1** was named *o*-methylisopiline.

(+)-Norlirioferine 2

(+)-Norlirioferine **2**, with $[\alpha]_D^{25}$ $+45^O$ (*c* = 0.02, MeOH) was isolated as a brownish amorphous solid. The UV spectrum indicated that the absorption at 314 nm is a typical of 1, 2, 9 and 10 tetrasubstituted aporphine [10]. These absorption peaks were due to the degree of resonance in the biphenyl system. The IR spectrum revealed absorptions at 3386 and 3317 cm^{-1} indicated the presence of hydroxyl and NH groups in the molecule.

The ESI$^+$ mass spectrum showed $[M+H]^+$ peak at m/z 328.2. The EI mass spectrum showed a molecular ion peak at m/z 327 corresponding to a molecular formula of $C_{19}H_{21}NO_4$. The peak at m/z 298 $[M-29]^+$ was due to the loss of methylene imine. The presence of fragmentation at m/z 296 $[M-31]^+$ suggested the C-1 was substituted by a methoxyl group. Moreover, the presence of fragmentation at m/z 298 $[M-29]^+$ due to retro Diels-Alder mechanism, indicated that alkaloid was an *N*-unsubstituted (NH) aporphine [7].

The ^{1}H NMR spectrum showed three aromatic proton signals appeared as singlet at δ 7.79 (*s*, H-11), 6.67 (*s*, H-8), and 6.52 (*s*, H-3). Three distinct methoxyl peaks at δ 3.58, 3.80 and 3.80 were assignable to attach to C-9, C-1 and C-2 respectively. The aliphatic protons appeared as multiplets at the region of δ 2.57-3.79.

The ^{13}C NMR spectrum showed the presence of 19 carbons and DEPT experiment showed that there are three methyls, three methylenes, four methines and nine quaternary carbons present in the molecule. The ^{1}H NMR and ^{13}C NMR data are shown in Table 2. Finally, comparison with the published data [11], confirmed that alkaloid is (+)-norlirioferine **2**.

(+)-Lirioferine 3

(+)-Lirioferine **3** was isolated as a pale brownish amorphous solid and $[\alpha]_D^{25}$ $+36^0$ (*c* = 0.098, MeOH). The UV spectrum showed absorption maxima at 313 (sh) and 245 nm suggesting that **3** was a noraporphine type of alkaloid. The IR spectrum showed absorption peak at 3376 cm^{-1} indicated the presence of hydroxyl group in the structure.

The ESI$^+$ mass spectrum exhibited an $[M+H]^+$ peak at m/z 342.3. The EIMS spectrum 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. Moreover, the presence of fragmentation at m/z 298 $[M-43]^+$ indicated that alkaloid was an *N*-substituted (*N*-CH_3) aporphine [7].

Table 2. ^{1}H NMR (in $CDCl_3$, 400 MHz) and ^{13}C NMR (in $CDCl_3$, 100 MHz) data of **2**

Position	δ_H , ppm (J in Hz) of **2**	δ_C (ppm) of **2**	δ_H (ppm), J (Hz) norlirioferine	δ_C (ppm) norlirioferine
1	-	144.23	-	144.08
1a	-	126.76	-	126.63
1b	-	127.42	-	127.93
2	-	152.15	-	151.91
3	6.61, *s*	110.68		110.69
3a	-	128.70	-	128.85
4	2.67-2.76, *m*	28.78		29.24
	3.03-3.08, *m*			
5	3.03-3.08, *m*	42.88		43.14
	3.39-3.44, *m*			
6a	3.87-3.89, *m*	53.61		53.76
7	2.67-2.76, *m*	36.25		36.66
7a	-	129.49	-	129.72
8	6.76, *s*	114.03	6.79, s	111.33
9	-	145.46	-	145.25
10	-	145.06	-	144.90
11	8.09, *s*	111.34	8.08, *s*	113.96
11a	-	123.75	-	123.77
OMe-1	3.68, *s*	60.12	3.66, *s*	60.13
OMe-2	3.90, *s*	55.79	3.88, *s*	55.86
OMe-9	3.90, *s*	55.94	3.89, *s*	55.98

Table 3. ^{1}H NMR (in $CDCl_3$, 400 MHz) and ^{13}C NMR (in $CDCl_3$, 100 MHz) data of **3**

Position	δ_H , ppm (J in Hz) **3**	δ_C (ppm) **3**	HMQC	HMBC	δ_C (ppm) lirioferine
1	-	144.21	-	-	144.0
1a	-	127.78	-	-	
1b	-	127.04	-	-	
2	-	152.02	-	-	151.4
3	6.50, *s*	110.22	H-3	1, 2, 1b, 4	110.5
3a	-	128.60	-	-	
4	2.54-2.59, *m*	28.81	H-4	-	
5	3.01-3.06, *m*	53.13	H-5	3a,4,NMe,6a	
6a	2.97-2.99, *m*	62.43	H-6a	3a,5, NMe,1a,7,7a	
7	2.88-2.92, *dd*, J_1=13.64,J_2=3.88	33.97	H-7	6a, 7a, 8, 11a	
7a	-	129.84	-	-	
8	6.74, *s*	113.93	H-8	7, 10, 11a	111.0
9	-	145.32	-	-	146.1
10	-	144.89	-	-	144.3
11	7.98, *s*	111.20	H-11	1a, 7a, 9	115.0
11a	-	123.84	-	-	
OMe-1	3.57, *s*	60.18	-	1	
OMe-2	3.80, *s*	56.02	-	2	
OMe-9	3.82, *s*	55.75	-	9	
N-Me	2.48, *s*	43.58	-	5, 6a	

The ^{1}H NMR spectrum of **3** (Table 3) exhibited three aromatic proton signals at δ 6.50, δ 6.74 and δ 7.98 assignable to H-3, H-8 and H-11, respectively. 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 [10,12-14], thus structure of **3**

was determined as illustrated and named (+)-Lirioferine.

CONCLUSION

Isolation, identification and characterization of the compounds isolated from the bark of *Cryptocarya ferrea* yielded three known aphorpine alkaloids. They are (-)-*O*-methylisopiline **1,** (+)-norlirioferine **2** and (+)-lirioferine **3**.

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