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Isoquinoline alkaloids from stem bark of *Colubrina decipiens* (Baill.) Capuron

Raymond François Fienena, Léa Herilala Rasoanaivo, Anne Wadouachi, Manitriniaina Rajemiarimiraho, Johnson Koleana Ramahambimanana and Amélie Raharisololalao

Abstract
From the stem bark of a Madagascar endemic plant, *Colubrina decipiens* Baill. Capuron, one new isoquinoline 9-hydroxy-2, 3, 10-trimethoxynoraprophine named Decipine 3 and four known isoquinolines, Magnococline 1 Atheroline 2, Nornantenine 4 and Stepholidine 5 were isolated and their structures were established by spectroscopic methods. These compounds are described for the first time for this plant.

Keywords: *Colubrina decipiens* Baill. Capuron, tetrahydroisoquinoline, stepholidine, atheroline, nornantenine, noraporphine, magnococline

1. Introduction
The *Colubrina* genus of the Rhamnaceae family includes about thirty species found in the hot parts of the globe. Six species are represented in Madagascar, of which *Colubrina decipiens* (Baill.) Capuron syn. *Macrorhamnus decipiens* Baill [1] is endemic to western Madagascar and popularly known as "tratraborondreo". This wood is used for construction, parquet flooring, joinery, interior trim, railway sleepers and furniture. This bark rubbed in water is used as a soap substitute [2]. However, some species of Rhamnaceae family showed diuretics and laxatives [3], antiprotozoan, cytotoxic and antiproliferative activities [4], antirheumatism, skin diseases and facilitate childbirth [5]. Chemical investigation of *Colubrina* species has led to the isolation of alkaloids and polyphenolic compounds [6, 7], saponoside [8], triterpenoid saponins [9, 10]. *Colubrina decipiens* (Baill.) Capuron has not previously been investigated phytochemically. In this paper we report the isolation and identification of one new isoquinoline, Decipine 3, and four known isoquinolines, Magnococline 1 Atheroline 2, Nornantenine 4 and Stepholidine 5 (figure 1) from alkaloid extract of the stem bark of *Colubrina decipiens.*

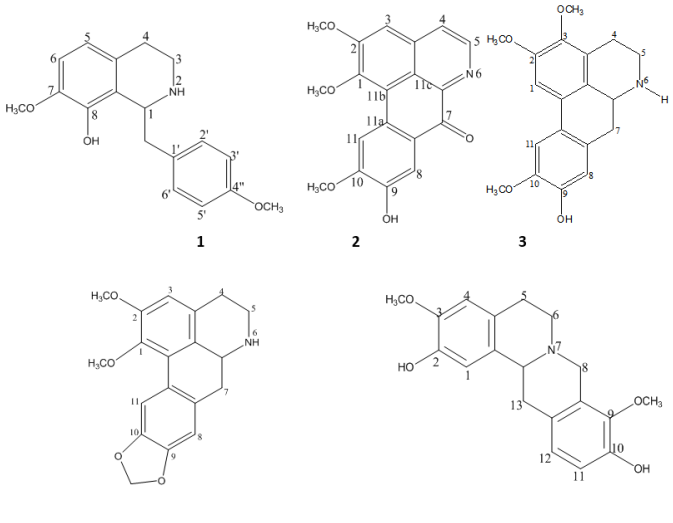


Fig 1: Structure of isoquinolines alkaloids isolated from stem bark of *Colubrina decipiens*

2. Materials and methods

2.1 Plant material

Colubrina decipiens (Baill) Capuron, collected in January 2010 from the Antsiranana, SAVA's Region, Madagascar, was identified to the herbarium references at Botanical and Zoological Park Tsimbazaza (PBZT, Antananarivo Madagascar) and a voucher specimen has been deposited in the "Laboratoire de Chimie des Substances Naturelles et Chimie Organique et Biologique" (LCSN/COB).

2.2 General experimental procedures

1D (^{1}H, ^{13}C, DEPT) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) NMR spectra were recorded on a Bruker Avance III 500 NMR operating at 500.19/125.78 MHz using $CDCl_3$ or CD_3OD as solvent and TMS as an internal standard. Column chromatography (CC) was carried out on silica gel F_{254} (Merck) or activated alumina III in glass blades. Thin layer chromatography was performed on precoated TLC plates (Merck, silica 60F254) and visualized by UV light and by spraying with Dragendorff reagent.

2.3 Extraction and fractionation

The air-dried, milled stem bark (500 g) was extracted for three hours in a Soxhlet with 3 L of hexane. The degreased powders, alkalinized with ammonium hydroxyde (25%) humections during 30 mn, were exhausted in a Soxhlet with dichloromethane until Mayer reaction negative. The dichloromethane extract was concentrated under reduced pressure. The concentrated solution was extracted with an HCl solution (5%). The aqueous phase containing the alkaloid salts are alkalinized with ammonium hydroxyde (25%) until pH = 9. The liberated alkaloids are then recovered by washing several times with dichloromethane (0.5 L). The obtained organic phase is then dried over anhydrous sodium sulphate. The filtration and evaporation of this phase was provided 2.3 g total alkaloids. The alkaloids extract (574 mg) was chromatographed over an activated alumina III column (60 g), eluting successively with a gradient solvent system of *n*-hexane-ethyl acetate (100:0 → 0:100) and a gradient solvent system of ethyl acetate-methanol (100:0 → 0:100) by adding 3 drops of ammonium hydroxide in 100 ml of each eluent, to give 18 fractions which two fractions F_2 [22.3 mg, hexane/AcO_2Et (90/10)] and F_4 [86.2 mg, hexane/AcOEt (30/70)] reacted by TLC with the Dragendorff reagent.

Fraction F_2 (22.3 mg) was submitted to CC over silica gel (25 g), eluted with a gradient solvent system of DCM-MeOH (98:2 → 80:20), to give three subfractions F_{21}, F_{22} and F_{23}. The subfraction F_{21} [8.2 mg, DCM-MeOH (98:2 → 95:5)] was then chromatographed with twice migration on preparative thin layer chromatography (silica gel) using the solvent system toluene-AcOEt (75:25), to obtain compound 1 (R_f= 0.25, amorphous solid, 2.1 mg) and an amorphous solid (R_f =0.3, amorphous solid 4.1 mg) identified as a mixture of compounds 2 and 3.

Fraction F_4 (86.2 mg) was subjected to a silica gel column (20 g), eluting with a gradient solvent system of DCM-MeOH (95:5 → 70:30), to give four subfractions F_{41}, F_{42}, F_{43} and F_{44}. The subfraction F_{43} [20.6 mg, DCM-MeOH(80:20)] was purified by preparative thin layer chromatography (silica gel) using the solvent system DCM-MeOH (90:10) adding 3 drops of ammonium hydroxide, to obtain compounds 4 (R_f =0.15, white crystals, 14.2 mg) and 5 (R_f =0.3, amorphous solid, 2.6 mg).

3. Results and Discussion

Structures were assigned by analysis of the ^{1}H, ^{13}C and 2D NMR spectra and by comparison with literature values.

By concerted use of one and two dimensional NMR spectroscopy, compound 1 was identified as magnococline [11, 12]. However, the assignments of methylene protons 3, 4 and 9 were not similar to those previously reported. Therefore, we revised the chemical shifts for H-3 (δ_H 2.82 and 3.22), H-4 (δ_H 2.65 and 2.84) and H-9 (δ_H 2.84 and 3.26) of magnococline 1 (Table 1).

Table 1: ^{1}H- (500 MHz, δ ppm, J in Hz), ^{13}C-NMR (125 MHz, δ ppm), COSY and HMBC spectroscopic data for compound 1 in methanol-d4.

Position	δc	δ_H (H, m, J in Hz)	COSY	HMBC (H→C)
1	54.4	4.31 (1H, dd; 1.8 and 8)	-	1, 8a, 8, 1'
2	-	-	-	-
3	38.1	3.22 (1H, dd; 1.8 and 8) 2.82 (1H, dd; 1.8 and 8)	4	1, 5, 9, 8a
4	29.3	2.65 1H, dd (1.8 and 8) 2.84, 1H, dd (1.8 and 8)	3	3, 4a, 5, 8a
4a	128.5	-		-
5	120.4	6.58 (1H, d; 8.8)	6	1, 4, 4a, 6, 7, 8, 8a
6	111.1	6.80 (1H, d; 8.8)	5	4a, 7, 8, 8a
7	146.7	-	-	-
8	144.1	-	-	-
8a	126.1	-	-	-
9	38.4	3.22 (1H, dd ;1.8 and 8) 2.82 (1H, dd ;1.8 and 8)	1'	1, 1', 2', 8a,
1'	133.0	-	-	-
2'	131.3	7.21 (1H, d ; 8.8)	3'	1', 3', 4', 6'
3'	115.1	6.89 (1H, d ; 8.8)	2'	1', 2', 4', 5'
4'	159.9	-	-	-
5'	115.1	6.89 (1H, d; 8.8)	6'	1', 3', 4', 6'
6'	131.3	7.21 (1H, d ; 8.8)	5'	1', 2', 4', 5'
H_3CO	55.7	3.78 (3H, s)	-	7
H_3CO	56.6	3.86 (3H, s)	-	4'

Compound 3 exhibited the proton spectrum (Table 2) characteristic of aporphine alkaloid [13] at 2.70-2.75 (H-4, m), 3.25-3.35 (H-5, m), 2.84-3.09 (H-7, m), 4.28 (H-6a, m), 6.73 (1H, s, Ar-H), 6.77(1H, s, Ar-H) and 7.97 (1H, s, Ar-H). The ^{1}H NMR spectrum also revealed the presence of three distinct methoxyl peaks at δ 3.65, 3.86 and 3.87.HMBC spectra showed correlations of the proton aromatic at δ_H 6.77 to carbons at δ_C 146.2, 121.8 and weak correlation to carbon at δ_C155.0.

The correlations of Ar-H at 7.97 (δ_C 113.3) to 147.2, 128.2, 127.6, 124.3, Ar-H at 6.73 (δ_C115.9) to 147.3, 124.3, H-3 at 3.87 (δ_C 56.4) to carbon at 147.3, and H-7 (δ_C34.1) to carbons 127.6, 124.3, 121.3, 115.9, 54.5 showed that the ring D (113.4, 124.0, 127.6, 115.9, 147.2, 148.2) is a 1, 2, 4, 5-tetrasubstituted benzene and the locations of these Ar-H at 6.73, 7.97 and the methoxyl group (δ_H 3.87,δ_C 56.4) were at C-8, C-11 and C-10 respectively. The chemical shift of the C-9 at δ_C 147.2 was characteristic of a hydroxyl group. Hence, the correlation of H-3 at 3.86 (δ_C56.4) to carbon at 154.3 permitted the location of this methoxyl group at C-2 (δ_C 154.3). Thus, compound 3 is 9-hydroxy-2, 3, 10-trimethoxynoraporphine.

Table 2: ^{1}H- (500 MHz, δ ppm, J in Hz), ^{13}C-NMR (125 MHz, δ ppm) and HMBC spectroscopic data for compound **3** in methanol-d4.

Position	δc	δ_H (H, m)	HMBC (H→C)
1	111.6	6.77 (1H, s)	2, 3, 11c
2	155.0	-	-
3	146.2		
3a	128.1	-	-
4	26.3	3.00 (2H, m)	
5	42.6	3.35 (1H, m) 3.65 (1H, m)	
6		-	-
6a	54.5	4.28 (1H, m)	
7	34.1	2.80 (1H, m) 2.90 (1H, m)	
7a	127.6	-	-
8	115.9	6.73 (1H, s)	10, 11a
9	147.2	-	-
10	148.2	-	-
11	113.4	7.97 (1H, s)	7a, 9, 11a
11a	124.0	-	-
11b	122.0	-	-
11c	121.8	-	-
OCH_3	60.5	3.65 (3H,s)	3
OCH_3	56.4	3.86 (3H,s)	2
OCH_3	56.4	3.87 (3H,s)	10

By concerted use of one and two dimensional NMR spectroscopy, compound 2 was identified as atheroline [14], compound 4 as nornantenine [15], and compound 5 as stepholidne [16]. Therefore, we assigned the chemical shifts for O-CH_2-O (δ_H 5.97 and δ_C 101.1), C-10 (δ_C 146.8), C-9 (δ_C 146.7) of nornantenine 4 [15]**.** In the same we revised the chemical shifts for C-1 (δ_C 125.4), C-12 (δ_C 113.1) of stepholidne 5 [16].

Compound 4 : ^{1}H NMR (CD_3OD, 500 MHz) δ(ppm): 7.95(H-11,s), 6.73(H-8,s), 6.60(H-3, s), 5.97(O-CH_2-O, s), 3.87(H_3CO-2), 3.66(H_3CO-1), 3.85(H-6a,m), 3.40(H-5β, t), 3.14(H-4β, t), 3.06(H-5α, t), 2.85(H-7β,d), 2.74(H-7α,d), 2.71(H-4α, t).

^{13}C NMR (125 MHz, CD_3OD) δ (ppm):152.5(C-2), 146.8(C-10), 146.7(C-9), 144.9(C-1), 130.1(C-7a) 128.4(C-11c), 126.9(C-3a), 126.9(C-11a), 125.6(C-11b), 111.2(C-3), 109.1(C-11), 108.4(C-8), 101.1(O-CH_2-O), 60.4(H_3CO-1), 56.1(H_3CO-2), 53.7(C-6a), 42.9(C-5), 37.1 (C-7), 28.6(C-4).

Compound 5 : ^{1}H NMR (CD_3OD, 500 MHz) δ(ppm): 6.81(H-1, s), 6.75(H-12, d, J = 8 Hz), 6.73(H-11, d, J = 8 Hz), 6.67(H-4, s), 4.24(H-8β,d), 3.82(H_3CO-9), 3.81(H_3CO-3), 3.63(H-13a, t), 3.60(H-8α,d), 3.35(H-13β, t), 3.28 (H-6β, t), 3.09(H-5β, t), 2.75(H-13α, t), 2,71(H-6α, s), 2.71(H-5α, t).

^{13}C NMR (125 MHz, CD_3OD) δ(ppm):148,0(C-9), 146.2(C-10), 146.2(C-2), 145.1(C-3), 130.2 (C-1a), 128.1(C-4), 126.0(C-12a), 125.4(C-1), 125.4(C-8a), 116.6(C-11), 113.1(C-12), 112.5(C-4), 60.8(C-13a), 60.4(H_3CO-3), 56.4(H_3CO-9), 54.7(C-8), 52.8(C-6), 32.3C-13).

The proton (δ_H) and carbon (δ_C) chemical shifts of compound 2 have not been reported previously and are reported here in Table 3 for the first time [14].

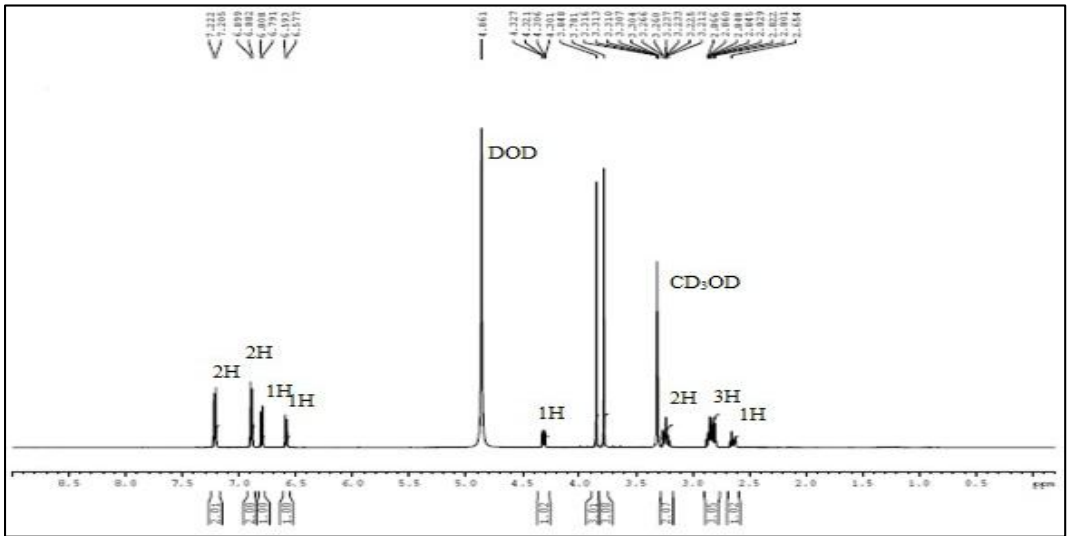
Table 3: ^{1}H- (600 MHz, δ ppm, J in Hz), ^{13}C-NMR (150 MHz, δ ppm) and HMBC spectroscopic data for compound **2** in methanol-d4.

Position	δc	δ_H (H, m, J in Hz)	HMBC (H→C)
1	152.5	-	-
2	158.5	-	-
3	107.4	7.23 (1H, s)	1, 4, 11c
3a	137.0	-	-
4	125.5	7.79 (1H, d, 8)	3, 11c
5	144.1	8.55 (1H, d, 8)	3a, 6a
6	-	-	-
6a	144.4	4.29 (1H, m)	7, 7a, 11b, 11c
7	182.0		
7a	127.5	-	-
8	114.5	7.57 (1H, s)	10, 11a
9	148.0	-	-
10	153.5	-	-
11	111.8	8.49 (1H, s)	7a, 9, 10, 11a, 11b
11a	129.2	-	-
11b	119.8	-	-
11c	122.3	-	-
OCH_3	60.9	3.96 (3H,s)	1
OCH_3	56.6	4.00 (3H,s)	10
OCH_3	56.7	4.03 (3H,s)	2

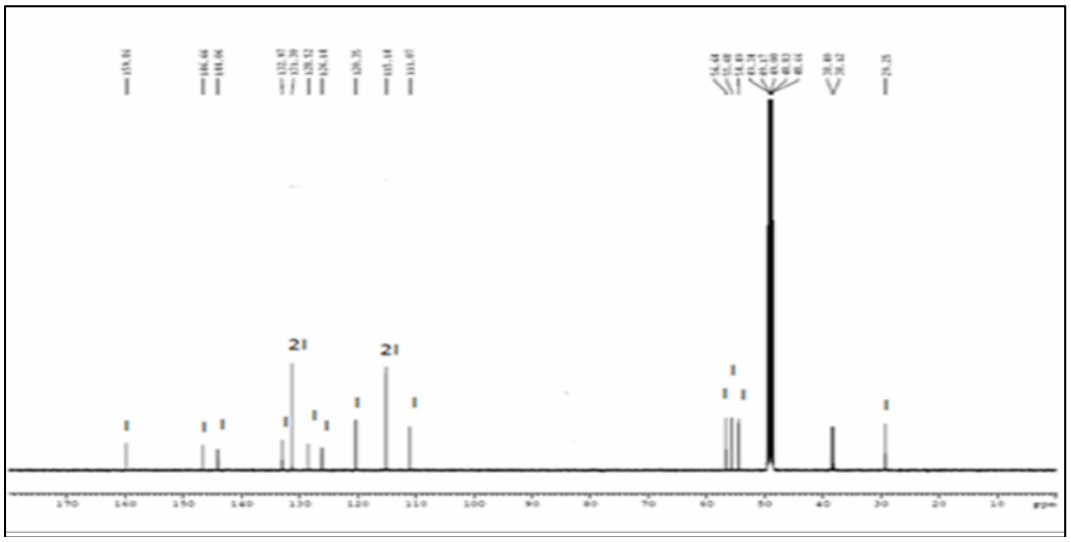
4. Conclusion

This work has demonstrated that stem bark of *Colubrina decipiens* Baillon is rich in isoquinoline alkaloids such the other species of *Colubrina* genus. The present study reports to the isolation and identification of five isoquinoline alkaloids one oxoaporphine (atheroline), two aporphines (nornantenine and a new decipine), one benzyl tetrahydroisoquinoline (magnococline) and one tetrahydroprotoberberine (stepholidine).

"**Isoquinoline alkaloids from stem bark of *Colubrina decipiens* (Baill.) Capuron" Magnococline**



1H NMR Spectra of magnococline



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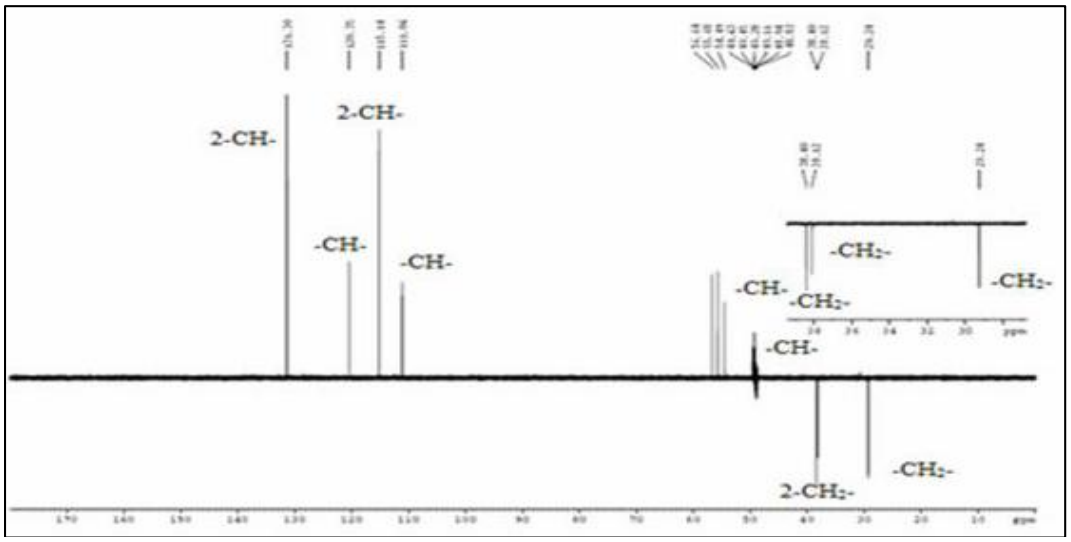
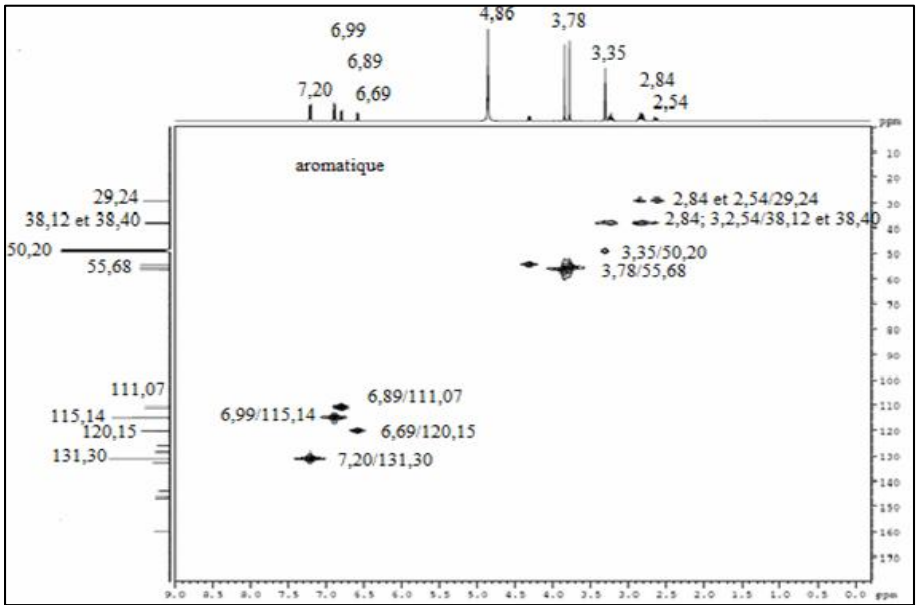
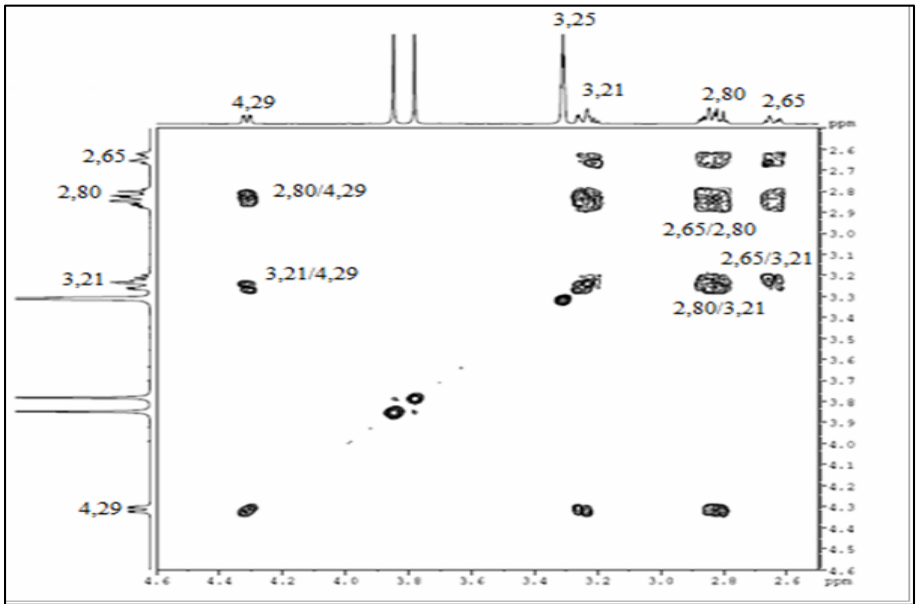


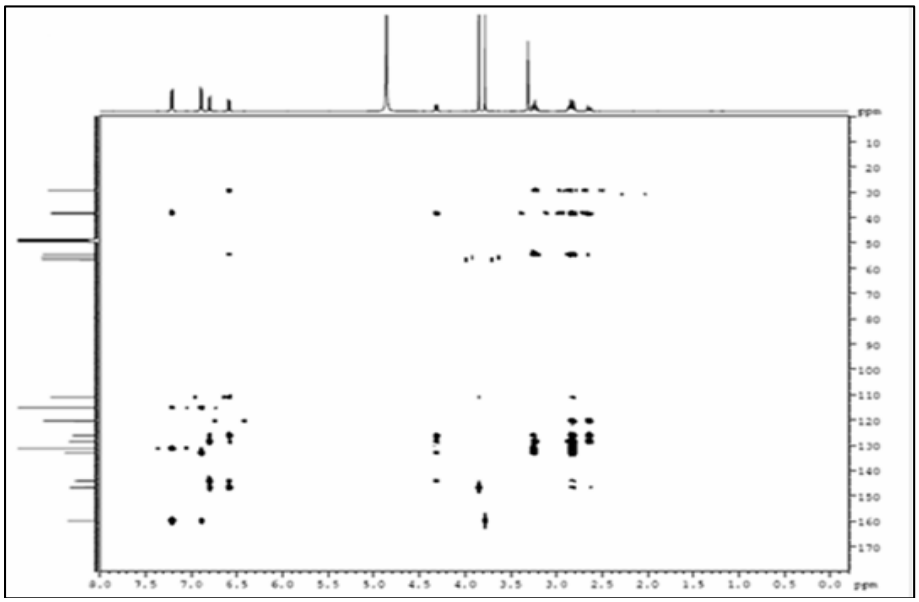
DEPT 135 spectra of magnococline



1H-13 C HSQC Spectra of magnococline

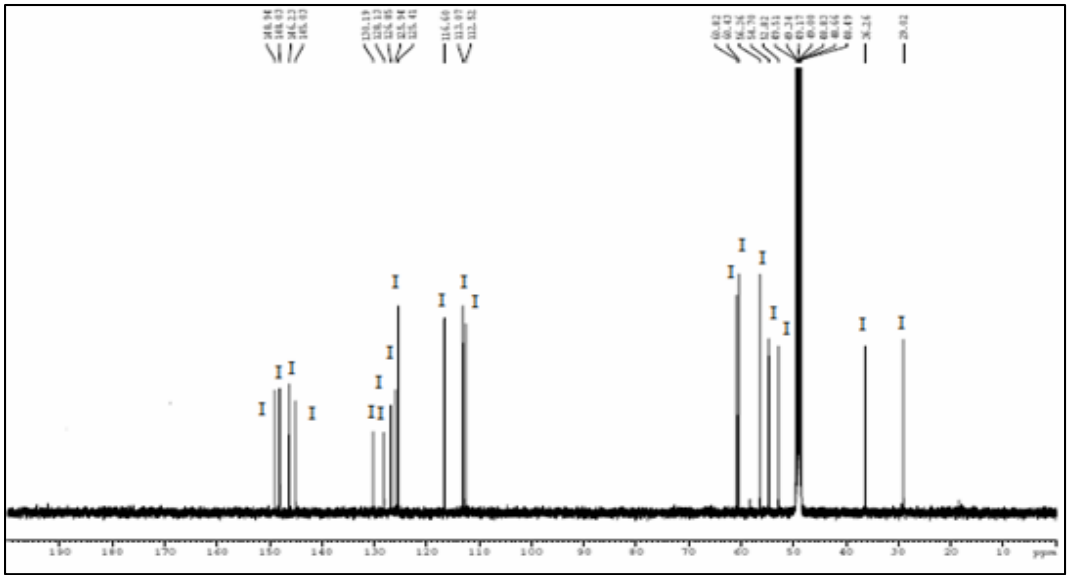


1H-1 H COSY Spectra of magnococline

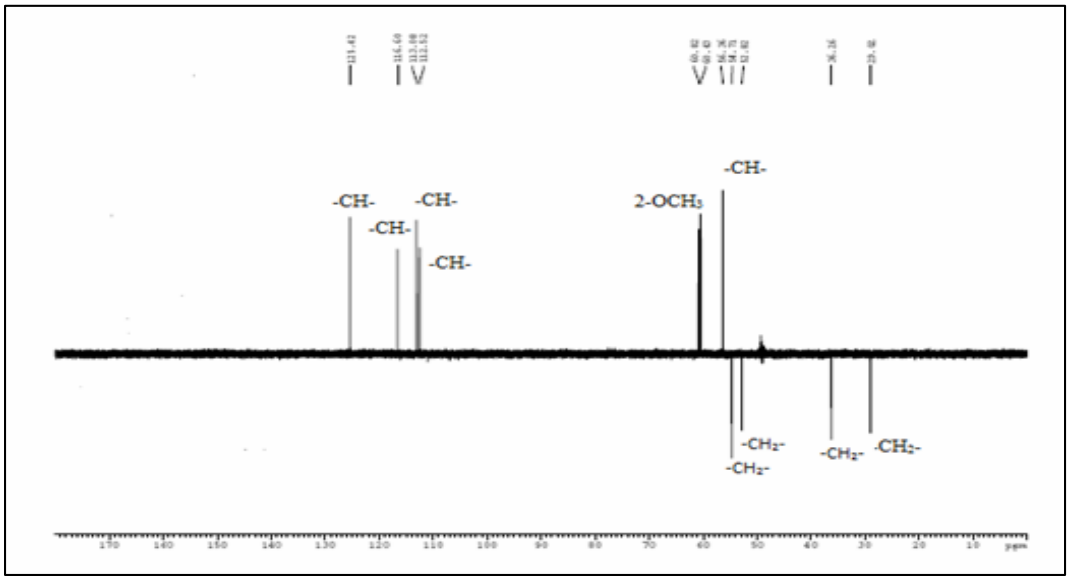


1H-13 C HMBC Spectra of magnococline

Stepholidine



13C NMR Spectra of stepholidine



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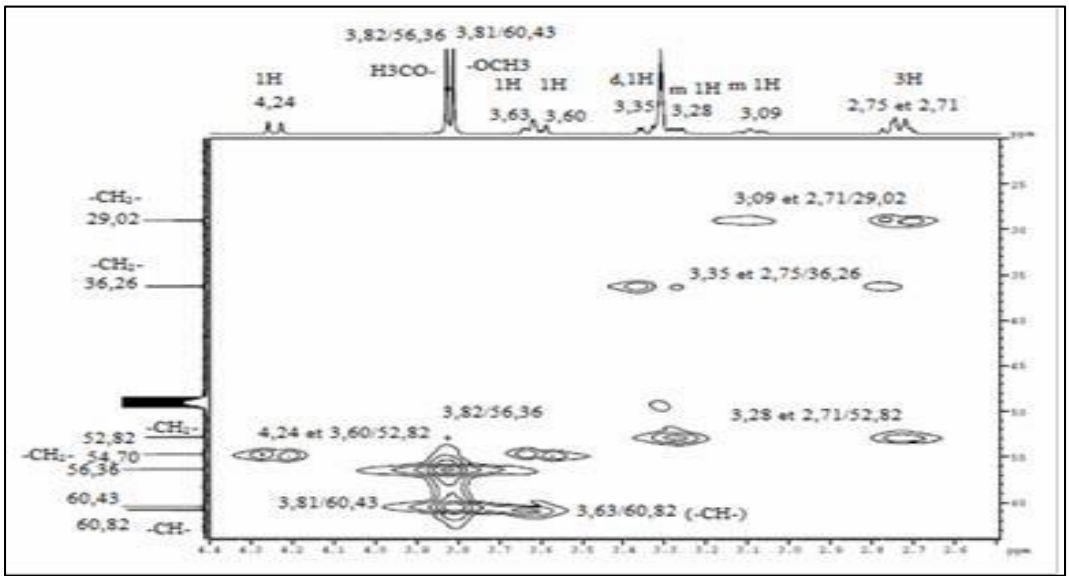
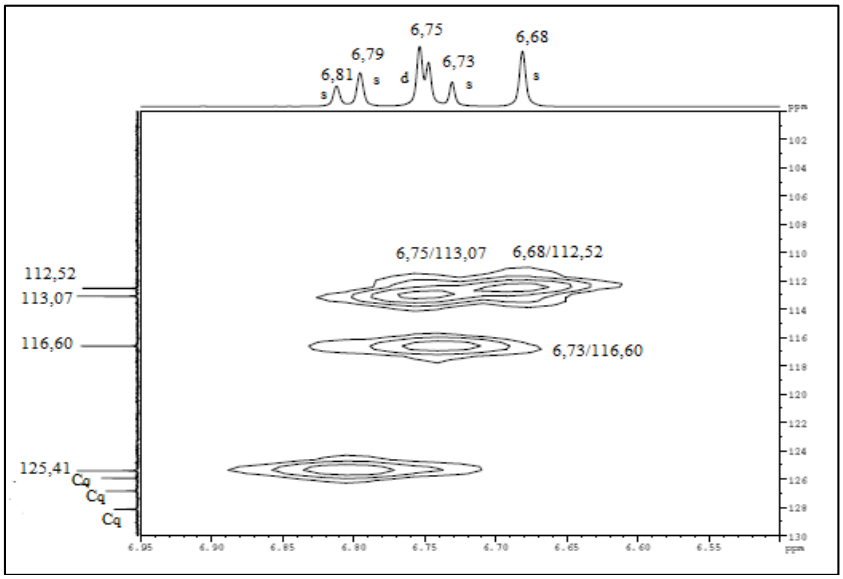
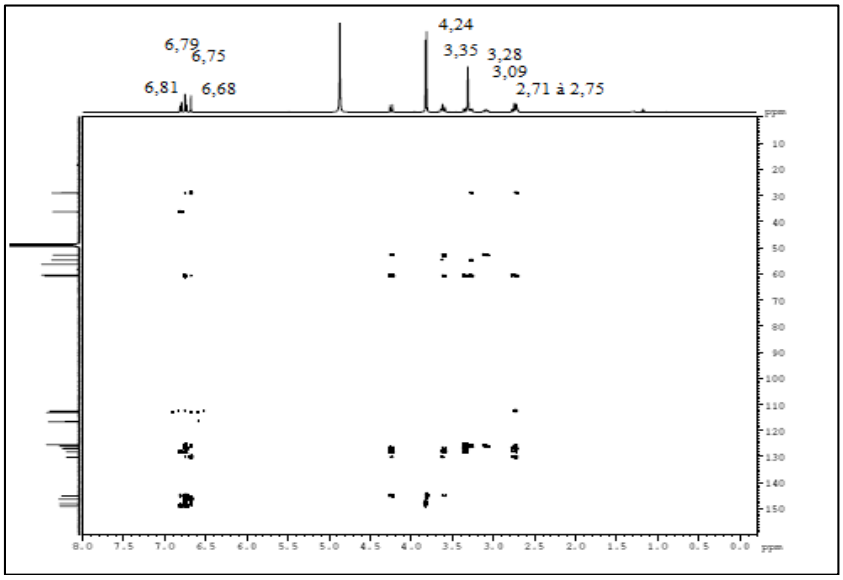


1H-13 C HSQC Spectra of stepholidine

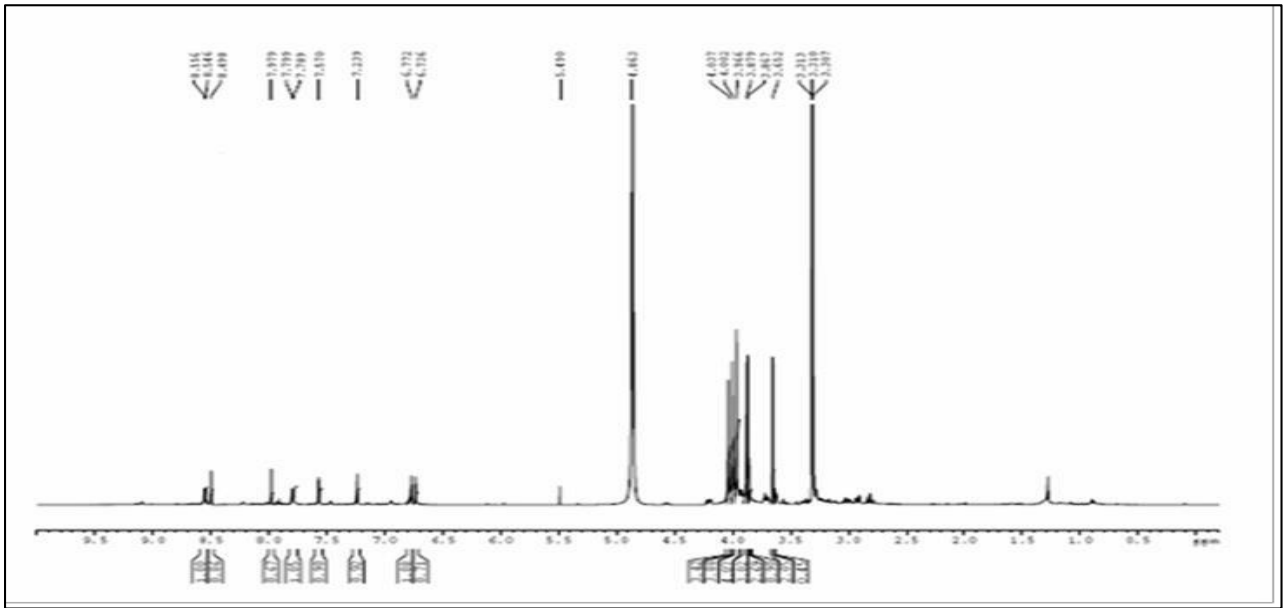


1H-13 C HSQC Spectra of stepholidine

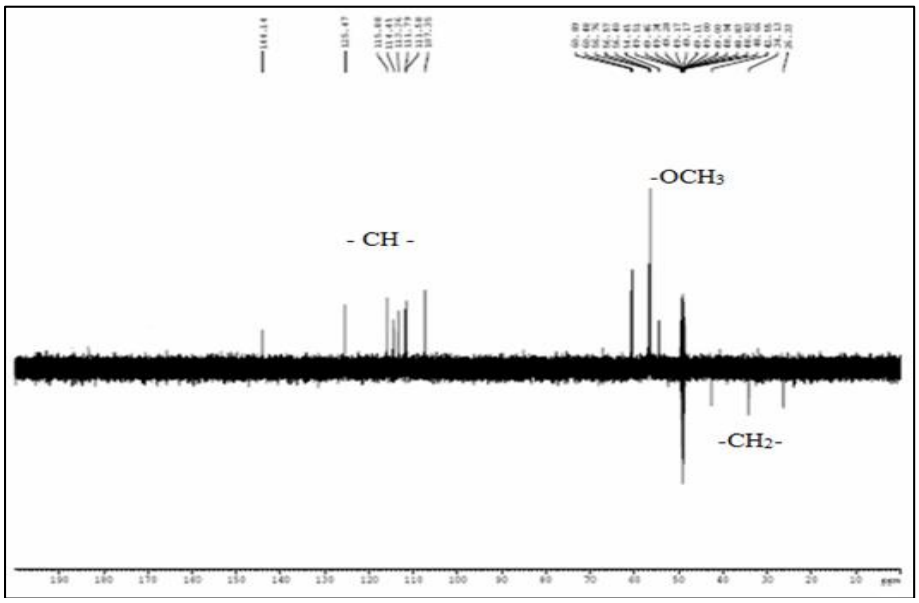


1H-13 C HMBC Spectra of stepholidine

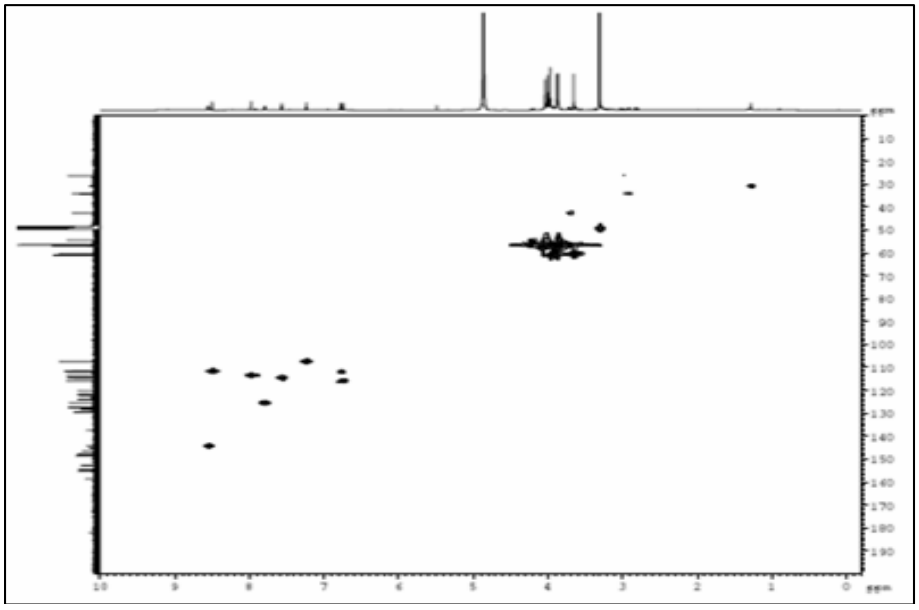
Athéroline and Decipine



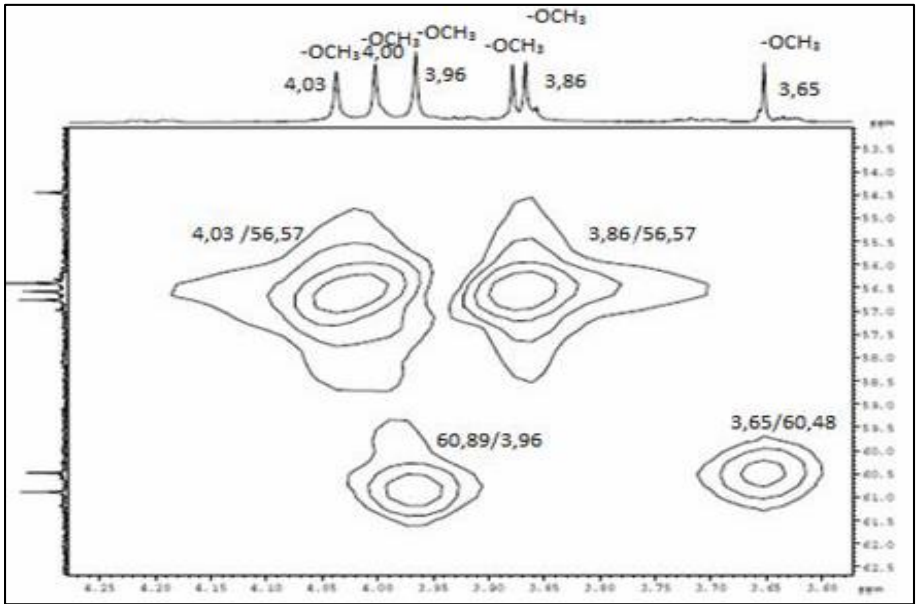
1H NMR Spectra of mixture of Atheroline and Decipine



DEPT 135 Spectra of a mixture of Atheroline and Decipine



1H-13 C HSQC Spectra of a mixture of Atheroline and Decipine



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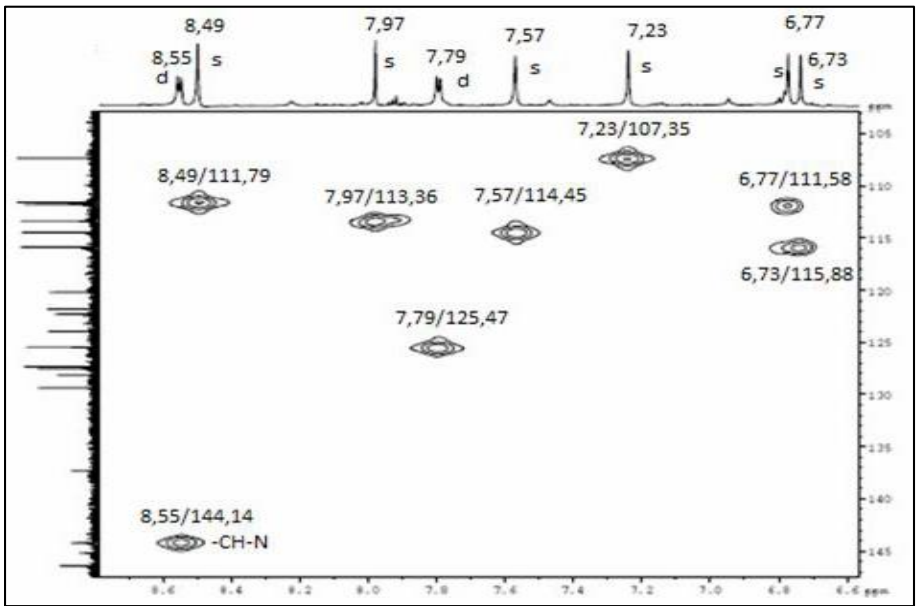
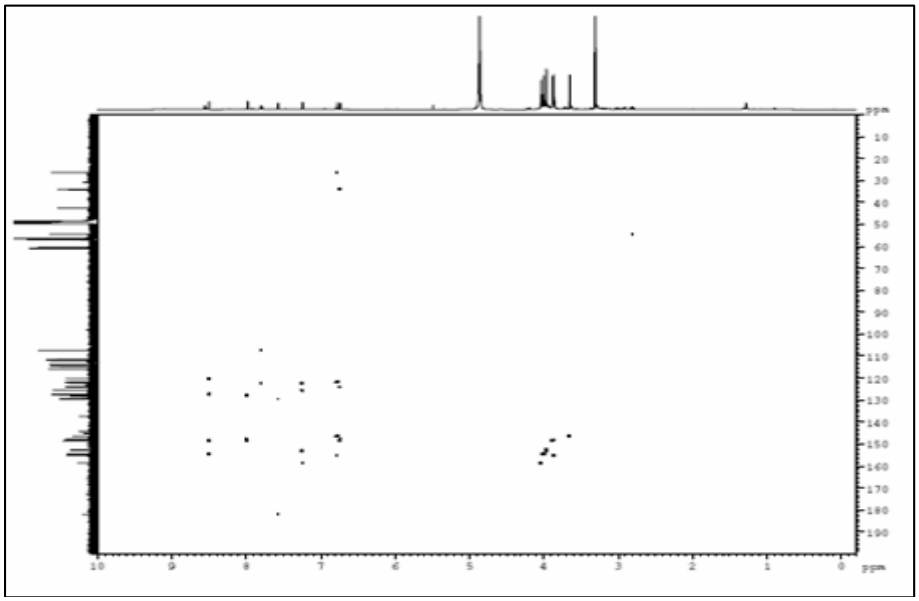
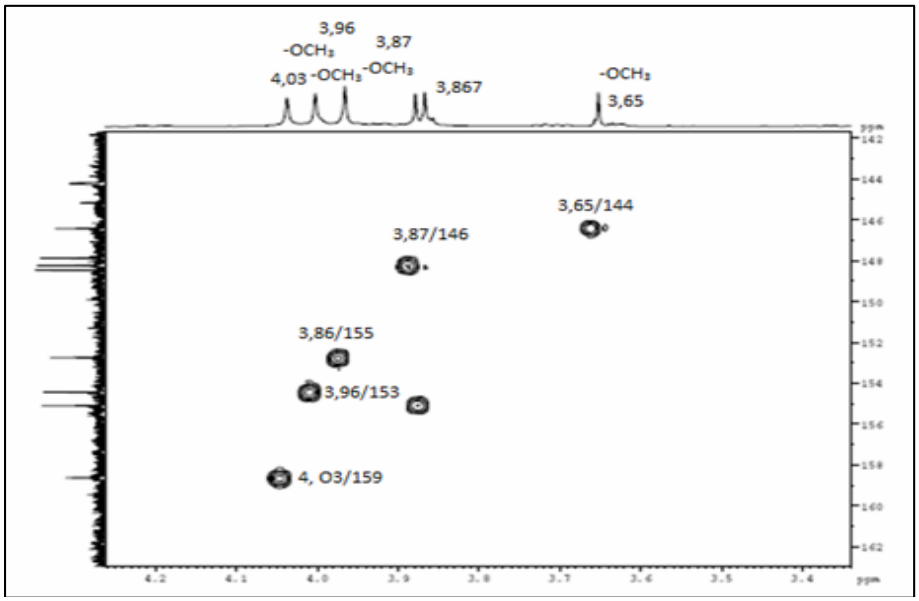


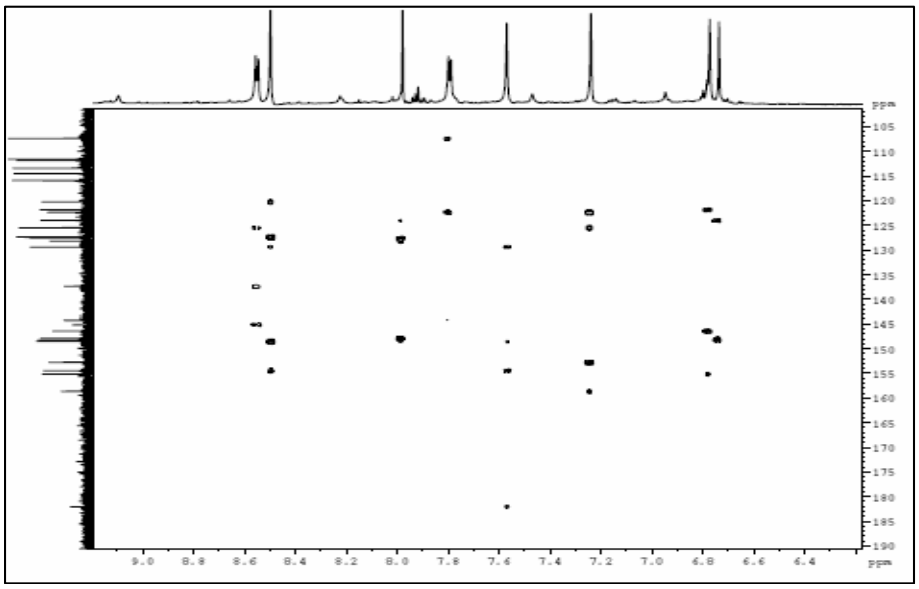
1H-13 C HSQC spectra of a mixture of Atheroline and Decipine



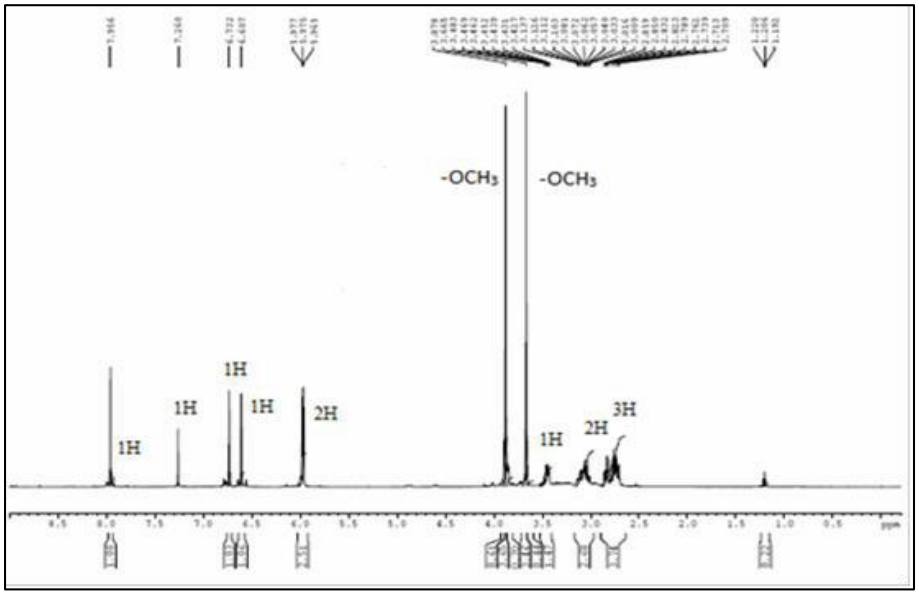
1H-13 C HMBC spectra of a mixture of Atheroline and Decipine



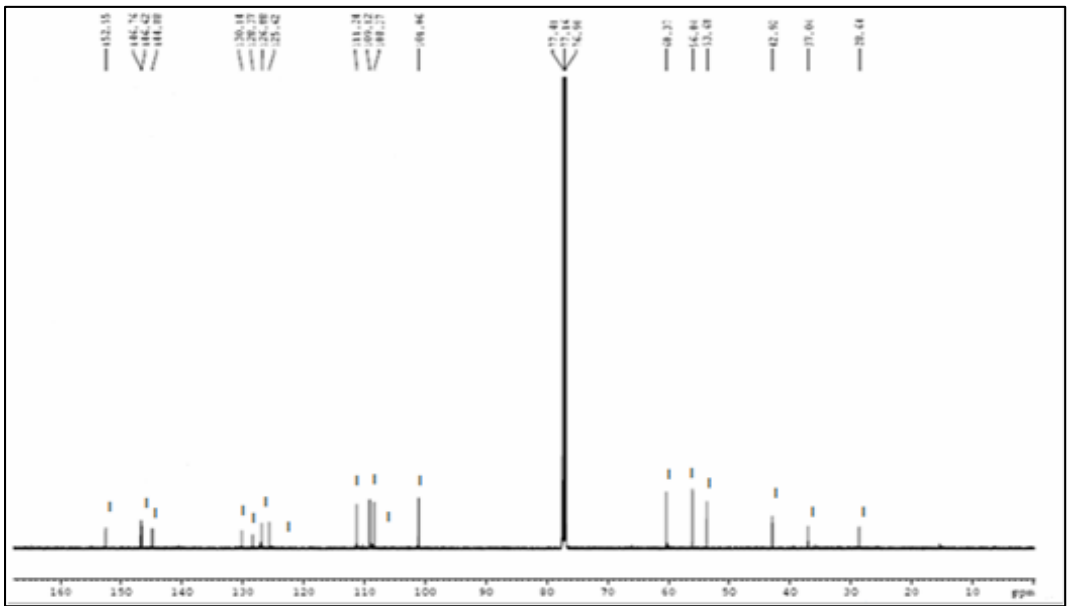
1H-13 C HMBC spectra of a mixture of Atheroline and Decipine



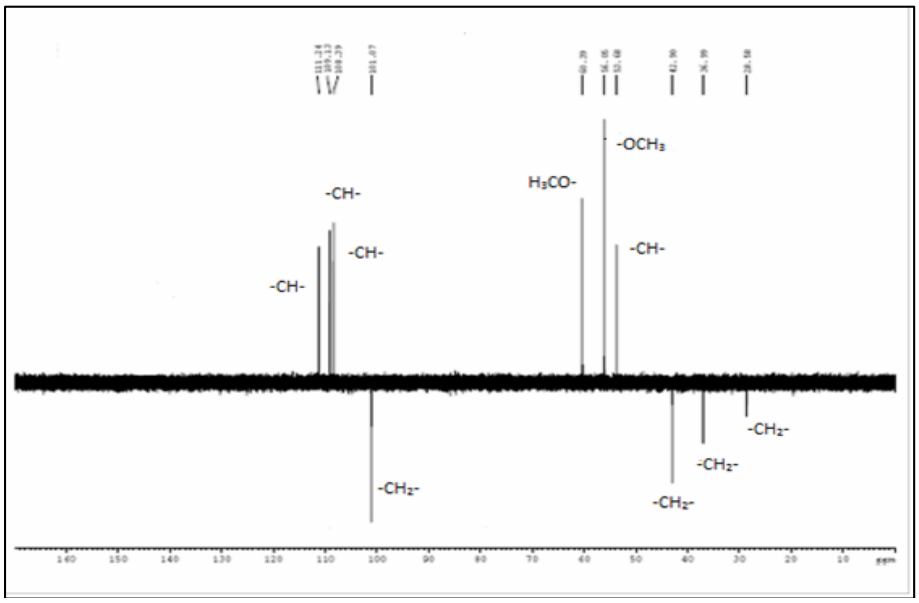
1H-13 C HMBC Spectra of a mixture of Atheroline and Decipine Nornantenine



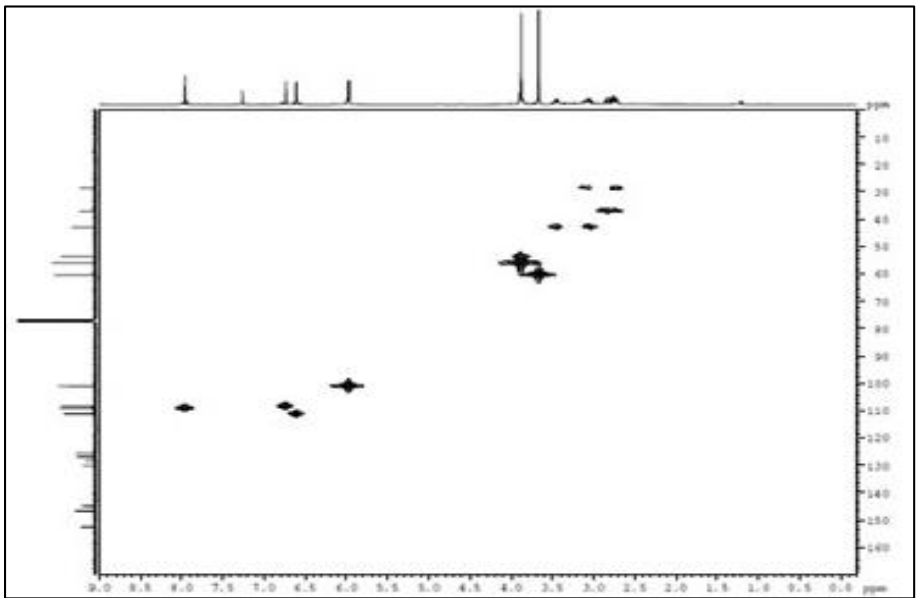
1H NMR Spectra of nornantenine



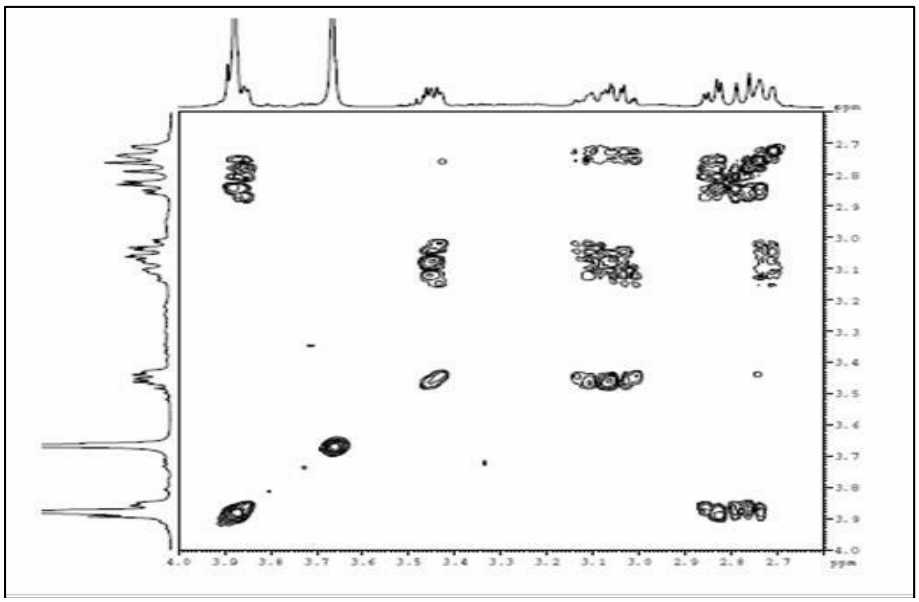
13C NMR Spectra of nornantenine



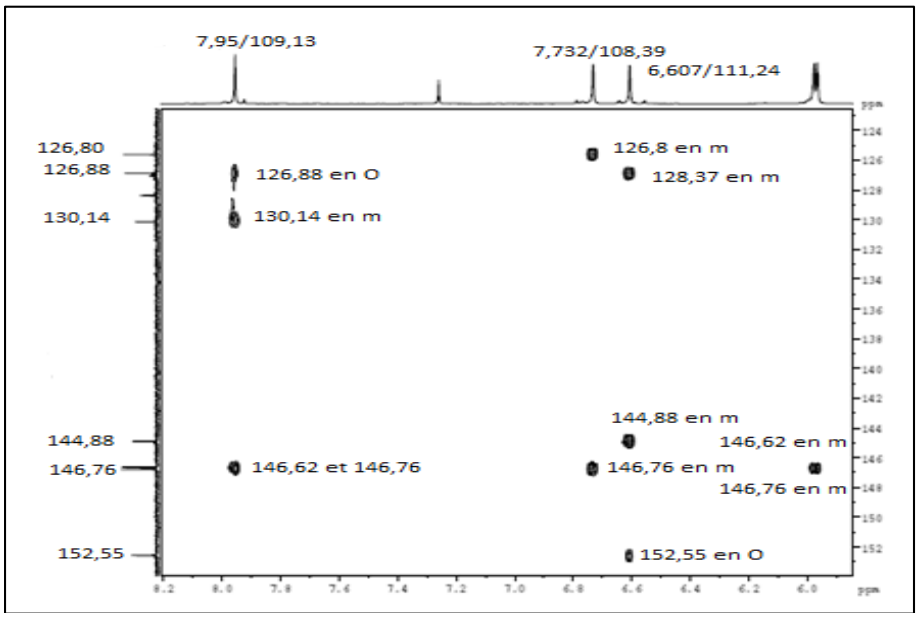
DEPT 135 NMR Spectra of nornantenine



1H-13 C HSQC Spectra of nornantenine



1H-1H COSY Spectra of nornantenine



1H-13C HMBC Spectra of nornantenine

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